Fish Passage on the Broad River: an assessment of the benefits to freshwater mussels

Completion Report to the Broad River Mitigation Fund

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General Inventory of Mussels in the Broad and Upper Congaree Rivers

We conducted searches of 60 sites on the Broad River, and 5 sites on selected tributaries. Study sites searched are shown on a map (Figure 1). Search methods differed based upon water depth and clarity and included visual searches with the naked eye, as well as searches involving snorkeling, SCUBA diving, and batiscopes (clear-bottomed view buckets). The amount of time spent at each site varied depending upon the amount of suitable habitat present, water clarity, and search effectiveness. Repeated trips were made to several of the highest density sites below the Columbia dam in search of gravid mussels to use in testing to determine suitable host fishes, and to sites at Parr Reservoir where SCUBA was used on subsequent trips. On the first trip to Parr Reservoir, mussels were located during periods of low water when sand bars are often exposed and mussels are either out of the water or in very shallow areas and can be located without the use of SCUBA equipment. On a subsequent visit, SCUBA was used to examine the deeper areas of the lake, particularly in deeper pockets surrounding the areas where shallow water mussels were found, because species composition may differ between shallow and deep areas. Because of the stringent rules regarding SCUBA diving activities of SCDNR staff and the lack of Freshwater Fisheries Section staff participating in SCDNR scientific diving program, all SCUBA diving was conducted by NC State University staff. The extremely low water levels particularly in the late summer and fall due to the severe drought minimized the need for SCUBA diving, so we were able to limit SCUBA activity to Parr Reservoir only. Some of the deeper parts of the river below the Columbia dam were searched again in the fall when even the center of the channel was wadeable and could be accessed using snorkeling gear.

We located 9 species below the Columbia Dam: *Elliptio complanata, E. congaraea*, *E. lanceolata complex, E. roanokensis, Lampsilis cariosa, L. radiata, Ligumia nasuta, Uniomerus carolinanus* (from shell material only), and *Villosa delumbis* (Table 1). The *Elliptio lanceolata* complex is not well resolved, though it does contain several currently recognized species known from South Carolina, *E. producta*, *E. folliculata*, and *E. angustata* as well as several other forms that are not currently recognized as distinct species or not thought to occur in South Carolina. Due to uncertainty regarding the distinctness of members of this complex and difficulty in distinguishing them, we have chosen to group members of this complex. A map of study sites where mussels (all species combined) were present or apparently absent is shown in Figure 1.

The section of river below Parr Reservoir and above the Columbia Dam contained some very dense populations of mussels, although the diversity was much lower than below the dam (Table 1). The habitat quality appeared to be excellent, although specific parameters were not measured. A wide variety of substrate types were present including gravel beds and large boulders, the substrate was very stable, and the water generally fairly clear. Abundant shoals and rapids were present which can help increase the availability of dissolved oxygen in the water. Four species were observed in this region, *Elliptio complanata*, *E. lanceolata* complex, *Uniomerus carolinanus*, and *Villosa delumbis*. All of the species from below Parr Reservoir were also found in the reservoir. The presence of one additional species, *Utterbackia imbecillis*, was identified from a single shell. Parr Reservoir has some unusual habitat characteristics. Each day water is pumped back and forth between Lake Monticello and Parr Reservoir, causing the reservoir to experience wide water level fluctuations averaging 4 feet per day but occasionally reaching as much as 9 feet in one day. Therefore, mussels that prefer the shallow and medium depths of the lake may often become exposed by the rapidly changing water levels. They also

experience a greater amount of flowing water than in most impoundments, which may explain why species composition was similar to that of the unimpounded sections of the River. Many impoundments in South Carolina are dominated by *Utterbackia imbecillis* and *Pyganodon cataracta* (personal observation), but that was not the case in Parr Reservoir.

Above Parr Reservoir, we found very few mussels relative to the lower sections of the river (Table 1). Although water quality parameters were not taken, the upper sections of the river were observed to be quite turbid, lower in substrate heterogeneity, and river bed substrates less stable. We were unable to find mussels at many of the sites above Parr Reservoir despite extensive effort. Typically, the sites at which we found a few mussels contained some gravel beds or at least a few boulders among the sand, apparently adding to the stability of the substrate. The mussels were most often found in these substrates rather than in long stretches of exposed sand.

Of the species found only below the Columbia Dam and not above, *L. cariosa* is of highest priority, *E. roanokensis*, *L. nasuta*, and *L. radiata*, are of high priority, and *E. congaraea* is of moderate priority as defined in South Carolina's Comprehensive Wildlife Conservation Strategy (Kohlsaat et al. 2005). *Elliptio complanata*, members of the *E. lanceolata* complex, and *V. delumbis*, all found above and below the dam, are classified as moderate priority (Kohlsaat et al. 2005).

Voucher specimens are stored at the NC Museum of Natural Sciences. Catalog numbers 43399, 43353, 46742, 46835, 46837, 46866, 46867 contain mussels that were collected during the course of the study.

Determination of the seasonality of reproduction

2007

In 2007, during the general inventory and when gravid mussels were collected as brood stock for host trials, we checked female mussels and mussels not exhibiting sexual dimorphism for reproductive status (i.e. gravid or not gravid), as time permitted. Due to the potential stress on the mussels associated with checking them for their status (shells were often chipped, and there is also a slight possibility of fatal injury to the anterior adductor muscle) checking the status of mussels was kept to a minimum. This preliminary data can be used to determine the general time of year in which mussels are reproducing and are likely to interact with potential fish hosts. In some cases, it was possible to determine if the stage of gravidity was early or late, but this is a subjective decision, and easier to determine in some cases than in others.

In the Broad River below the Columbia Dam, a few (3 out of 24) *E. roanokensis* in the early gravid state were found on May 15. On May 16 in the Congaree River just below its confluence with the Broad and Saluda Rivers, no gravid *E. roanokensis* were found despite the fact that 77 individuals across 3 sites were checked. Although more data is needed to test the statistical significance of this observation, it seems possible that the reproduction of this species is occurring later in the Congaree than the Broad. Gravid individuals were observed in the upper Congaree on May 31, June 20, June 21, and July 3, when 20 out of approximately 50 checked individuals were gravid.

No gravid individuals of *E. congaraea* were observed, but only 6 live individuals were found on May 15, May 16 and June 21. As this species is not sexually dimorphic, it is unknown how many, if any, of these individuals are female. Gravid *E. complanata* were observed on March 27 below Parr Reservoir, below Columbia Dam on May 15 (early stage gravid only), and May 31. Of eight individuals below Columbia Dam whose status was checked on June 20 and 21, none were observed to be gravid. Gravid individuals of the *E. lanceolata* complex were

observed on March 28 below Parr Reservoir, and on May 15 and May 31 below Columbia Dam. *Ligumia nasuta* was only found on May 15, but 12 out of a total of 16 individuals were gravid at this time. Both of the live female individuals of *Lampsilis radiata* were gravid and collected below the Columbia Dam on May 15. Gravid *Lampsilis cariosa* were observed on May 16 and May 31. Gravid *Uniomerus carolinanus* were found on March 27 below the Columbia dam. Gravid *Villosa delumbis* were observed below Parr Reservoir on March 28 and 29 and below Columbia Dam on May 15 and 31. No gravid individuals were observed when female *V. delumbis* were collected below the dam on June 20 or 21, or in Parr Reservoir on Septemeber 27.

2008

During 2008, a more extensive assessment of the seasonality of reproduction was conducted. Two locations below the Columbia Dam were selected for mark and recapture of individuals, so that the timing and duration of the brooding period could be assessed, and the approximate time when gravid mussels released glochidia could be determined. We chose the Congaree River at the Blossom Street Bridge (33.98708^{o}N, -81.04551^{o}W), and an area on the east side of an island complex forming the confluence of the Broad and Saluda rivers (34.00421^{o}N, -81.05748^{o}W), hereafter referred to as Riverfront Park, since the site was accessed by walking along the trail at Riverfront Park upstream from the Laurel St. entrance) as the two sites for repeated sampling, because they were the sites below the Columbia Dam examined in 2007 that were of highest density and diversity of mussels. Species diversity between the two sites overlaps, but each site contains species not found at the other site. Therefore, adding a second site allowed us to increase the number of species assessed. The site at Riverfront Park does not receive any water from the Saluda except possibly during extreme flooding events due

to the islands that separate the two rivers at this point. The lower site is in an area where the water from the two rivers has some ability to mix but is not yet well mixed. An aerial photograph of these two sites showing the differences in turbidity and separation of water from the two rivers is shown in Figure 2. A third site, in the Broad River, just upstream from I-126 was sampled three times. This site was chosen because of the abundance of *Ligumia nasuta*, a species found in low abundance at Riverfront Park, and not present at the Blossom St. Bridge. Because this site was less diverse than the other two sites, not as much effort was placed on visiting this site frequently, and mussels were not marked and recaptured.

We collected mussels from both sites from late March through late June. The target interval between sampling dates was two weeks, but this often had to be adjusted somewhat due to water levels and scheduling conflicts. By the end of June, most mussels at the upper site had released their glochidia, and very few gravid individuals were found. Although few individuals were gravid at this time at the Blossom St. Bridge, two additional visits to the Blossom St. Bridge were made in July because of the observation in 2007 that suggested that some mussels may reproduce later at this site than farther upstream and we wanted to know if the number of gravid individuals might increase in July. At each site visit, as many mussels as possible were collected, measured in the longest dimension, checked for the presence of glochidia on the outer gills, marked and released.

We collected mussels by snorkeling or a combination of SCUBA diving and snorkeling, depending upon the water depth on each sampling date. On the bank, we opened them by partially prying open the valves along the ventral margin, and checked their reproductive status by classifying them as gravid or not gravid. Swelling with tubes of glochidia was visible on the outer gills of gravid mussels. On each survey, all mussels found not gravid were etched with a

single digit number using a Dremel™ tool to indicate the survey date. This allowed us to document the reproductive history of non-gravid mussels should they later be found gravid, while still being able to quickly return them to the river to minimize desiccation and thermal stress. The first time a mussel was found to be gravid, a uniquely numbered flexible plastic 4mm x 8mm tag (Hallprint Pty Ltd., Victor Harbor, South Australia) was super-glued to the shell. The unique tag could later be used to track the reproductive status of the mussel through time. After all mussels were examined and marked accordingly, they were immediately returned to the river in small area (roughly 15 m^2) that provided good habitat but allowed the mussels to be relocated relatively easily. On each successive sampling date, we thoroughly searched this area to recapture mussels in addition to searching a broader area at the study site to locate additional individuals.

Sampling dates at Riverfront Park were March 27, April 17, May 2, May 14, May 30, June 4, June 17-18, and June 25. On 17 June 2008, a thunderstorm interrupted field work, and the examination and marking of mussels had to be completed on the following day. In the meantime, mussels not yet examined were kept in the river in collection bags with wide mesh that allowed adequate water flow. Sampling at Blossom St. Bridge was conducted either the same day or within 2 days depending upon time remaining when the first site was completed. Weather conditions and the amount of additional field assistance affected the time taken to sample each site. Dates for sampling at Blossom St. Bridge were March 27, April 16, May 1, May 15, May 30, June 4, June 17, June 27, July 14, and July 29. The start date of the sampling period was limited by cold water temperatures and higher flows earlier in the year which caused poor visibility and safety concerns for field work.

The sample size of *E. congaraea* was low, but it was found gravid only at the Riverfront Park site. The only individual found on March 27 and both individuals found on April 17 were gravid, while none of the 7 individuals found on March 27 or the 5 individuals found on April 16 at the Blossom St. Bridge were gravid. All gravid individuals were taken into the lab for the release of glochidia on March 27, to examine glochidial morphology and assist in identifying individuals found in drift nets. Gravid individuals collected on April 17, were used in the lab as brood stock for host tests. Several individuals were found at both sites later in the year, but none were gravid later than April 17. Due to low sample sizes, it is difficult to determine the extent of the brooding season for this species, though it may be restricted to the early spring.

Data on the dates various species of mussels were gravid are presented in Figures 3-9. Short term brooders, *E. complanata*, *E. congaraea*, *E. roanokensis*, *E. lanceolata* complex, and *Uniomerus carolinanus* release their entire brood at one time, and were found either with gills full of glochidia or completely empty. Only one individual of *Uniomerus carolinanus* was found gravid at Riverfront Park on March 27, so data is not presented for this species. Long-term brooders, *Ligumia nasuta*, *Lampsilis cariosa*, *Lampsilis radiata*, and *Villosa delumbis* all release a few glochidia when their lures are attacked by a potential host fish. Therefore, it was possible to classify individuals as fully gravid, partially released, or not gravid (Figures 6-9). Each of these four species exhibits sexually dimorphic shell shape, so data is only presented for females. In contrast, *Elliptio* species are not sexually dimorphic, so the data is presented for all individuals. For *E. complanata*, *E. lanceolata* complex, and *E. roanokensis*, there is sufficient data at both Riverfront Park and Blossom St. Bridge to compare the fraction of gravid mussels on various dates and determine if the timing of reproduction is similar at both sites. On dates where field work for both sites could not be completed within a day, paired dates within 1-2 days

of the other site were compared. A chi-squared test was conducted to determine if the fraction of gravid mussels significantly differed between sites. Stars on figures indicate dates or paired dates on which the fraction of gravid mussels at each site differed. Because the order of sampling of the two sites varied and paired sampling events were within 1-2 days of one another, we do not expect any systematic bias due to the fact that sampling sometimes took place on two different days.

The mark recapture technique we used allowed us to track the status of an individual over time. Several *Elliptio* species were documented to complete multiple broods. At Riverfront Park, two individuals of *E. complanata* and three *E. roanokensis* were documented with two separate broods. Four individuals of *E. lanceolata* complex were documented with two broods and four individuals with three broods. At Blossom St. Bridge, two individuals were documented with two broods, but gravid individuals of *E. complanata* and *E. lanceolata* complex were uncommon, and none were found with multiple broods.

2009

Although field work in 2009 was not part of the original plan for this project, we conducted some follow up work. A follow up host test was conducted in 2009 to further determine the compatability of *E. roanokensis* with white perch, and during the process of searching for brood stock over several days, some information on the presence of gravid individuals was gained.

At Riverfront Park on May 24, only one individual out of ten *E. roanokensis* found was gravid, on June 16 no individuals out of four found were gravid, and on June 23, four out of eight

found were gravid. This was a surprising result, since *E. roanokensis* appeared to be nearly completely finished brooding by mid June in 2008. Also at Riverfront Park on June 23, three out of four *E. complanata* were gravid, and one out of three *E. lanceolata* complex were gravid. Two of the gravid *E. roanokensis* and all three of the gravid *E. complanata* collected at this time were marked individuals that had also been gravid in 2008, indicating that these species are capable of reproducing in two adjacent years. At the Blossom St. Bridge, gravid *E. roanokensis* were abundant July 3, 2007, not abundant during July of 2008 (Figure 3), and on June 17, 2009 no individuals were gravid out of 16 checked. The sample sizes and number of dates examined in 2007 and 2009 are not sufficient to test this hypothesis, but it appears that the timing of reproduction may vary substantially from year-to-year. The higher water levels in 2009 and lack of SCUBA equipped assistance, as this was not part of the original study, prevented frequent checking of individuals, and collection of large sample sizes.

Drift Net collections

Glochidia were collected in drift nets (3 per site) at the lower end of mussel beds at Blossom St. Bridge and Riverfront Park as an additional measure of the timing of reproduction in mussels at both sites. The dates nets were set out was delayed slightly (April 11) relative to the dates of searching for gravid mussels due to a delay in receiving ordered nets. Afterwards, nets were placed on dates when gravid mussels were collected. Glochidia freely floating in the water column are recently released individuals that have a chance of attaching to fish if encountered promptly. The viability of glochidia after release varies, depending upon species, populations,

and water temperature, but ranges of 3 days to two weeks have been recorded (Zimmerman and Neves 2002, Akiyama and Iwakuma 2007).

Glochidia of different species differ in shape and size, but many species are difficult to distinguish from one another. We had intended to use genetic tools to distinguish between species, but there was difficulty in extracting viable DNA from the preserved drift net samples (See more information in the following section).

A graph of the total abundance of glochidia is presented per minute that each net was in the water (Figure 10). More glochidia were collected at Blossom St. Bridge than at Riverfront Park, particularly earlier in the season. Blossom St. Bridge is downstream of the Riverfront Park site. Although the pattern of glochidial abundance did not reflect the apparently delayed brooding season at Blossom St. Bridge, it is not surprising, since drift net samples are a collection of glochidia released upstream of the location of the nets. The distance glochidia can move before settling on the bottom was unknown, but depends upon the size of the glochidia, depth, and water velocity. *Villosa delumbis* had the largest and most distinctly shaped glochidia of any species. Because it was readily distinguishable from other glochidia by sight, a graph of the abundance of this species is presented (Figure 11). Overall it was low in abundance, probably because its large size may have caused it to settle out of the water column more quickly, and since adults are the smallest species present, overall brood size, is probably the smallest.

Use of genetic analysis to identify individuals

All genetic research was completed at the North Carolina Musem of Natural Sciences (NCSM). To identify samples collected, sorted, and preliminarily identified from drift net samples from the Broad River, attempts were made to isolate viable DNA for PCR & sequencing

from sorted glochidia. Two methods (a scaled-down version of the 5 Prime ArchivePure DNA purification kit, formerly PureGene by Gentra Systems, Inc., and the QuickExtract™ DNA Extraction Solution from Epicentre Biotechnologies) were used which had previously worked with other glochidia, but neither provided viable DNA for analysis. Reasons for these failures are currently unknown, but could be a result of staining or soaping the samples during sorting, or simply a by-product of the glochidia being recovered from the water column, an atypical situation for viable animals which might have resulted in death of the larvae and loss of tissues (and DNA) due to rapid natural degradation. Since the primary goal of this work was to be able to provide a PCR-RFLP identification guide for use with isolated glochidia, and efforts to isolate DNA from sampled glochidia have failed, we provide the information below which tentatively might prove to be useful once successful DNA isolations occur.

PCR-RFLP entails generating a commonly utilized PCR product, subjecting that product to restriction endonucleases to cut (or restrict) that product into pieces based on the actual DNA sequence of that product, and then electrophorese the fragments generated on an agarose-based gel to separate the pieces into size-based fragments. The goal of this is to be able to identify size-based fragment patterns unique to each species so that a reliable identification can be determined from these PCR products. As stated above, we were unsuccessful in generating usable DNA for this analysis, so we provide the following data based tentatively on information generated from previously sequenced individuals from the ncsm collection. Please note that due to the high level of DNA variability of *Elliptio* species in general, we provide information for only *E. Roanokensis*. Reliable genetic determination of other species will likely be impossible using common (current) DNA markers.

The mitochondrial gene, COI, is commonly used in molecular systematic studies of unionid taxa and was chosen as the PCR product used for these analyses. It is easily amplified and well characterized in the literature (Folmer et al. 1994; and a large body of literature since) as the "gene of choice" for many phylogenetic studies of unionid systematics. We have chosen two common restriction endonucleases (EcoRI and BamHI) for all primary restrictions. Secondary restrictions can be performed if ambiguous results are recovered using BsaI for most species or SspI for *Lampsilis* ssp. Please note that these results have not yet been empirically tested, but are predicted to work once viable DNA can be recovered from glochidia based on sequences from cataloged vouchers of adults collected during this study and housed in NCSM.

Species	Fragment(s) recovered (in base pair length)	Adding BsaI (when needed) **or SspI for *Lampsilis ssp.***
Elliptio congaraea	485 & 175 (occasionally 485, 139 & 36)	461, 139, 36, 24
Elliptio roanokensis	485 & 175	461, 175 & 24
Lampsilis cariosa	660 (uncut)	**622 & 38**
Lampsilis radiata	660 (uncut)	**606 & 54**
Lampsilis splendida	660 (uncut)	**660 (uncut)**
Ligumia nasuta	485 & 175	485 & 175
Villosa delumbis	625 & 35	625 & 35

Noteworthy Research Findings

1) *Lampsilis splendida*/*Lampsilis radiata*

    a. *L. splendida* has been recognized in SC from the Savannah, Pee Dee, and Santee-Cooper systems in SC. *L. radiata* is recognized from the Pee Dee & Santee-Cooper system in SC. These species are similar conchologically and have been confused in the literature with various authors ascribing non-overlapping distributions for the species. One question raised in this study was "which species is/are present in SC and what are their distributions?"

    b. Given our limited sampling of vouchered materials from the region, it would appear that *L. radiata* is documented as present in the Broad River, SC (NCSM 45688, L. sp.). It should be noted we are not concluding that *L. splendida* is absent from the system. *L. splendida* is documented, genetically, from the Saluda system (NCSM 45015, *L. splendida*) and we fully expect it to be possible that both species co-occur. Further sampling of vouchered materials will be required to verify this, but for now it should be noted that both species are likely to be present in the Santee-Cooper system. A similar conclusion is likely to be recovered in the Pee Dee as well.

Problems still remaining

Elliptio as a genus is genetically too variable to reliably define PCR-RFLP patterns for identifications. In most instances, species other than *E. roanokensis* & *E. lanceolata* are too genetically variable for reliable assignment to genetic groupings. The complexity of these species remain perplexing; alternative routes are favored to identifying the members of *Elliptio*, in general, than any application of genetic species determination might be at this time.

Determination of suitable fish host species for mussels found below Columbia dam

In order to determine if the species of fish travelling through the fish ladder at the Columbia Dam are the appropriate hosts for the freshwater mussels found below the dam but not above, we conducted host trials testing the compatibility of glochidia released from gravid mussels and various fish species present below the dam. Time and space constraints did not permit every species of fish present to be tested for every mussel, but we chose fish and mussel combinations based upon the most abundant fish species in the upper Congaree, and in the case of *Lampsilis* species, piscivorous fish species were chosen, because they were most likely to respond to the lures used by the mussels to attract fish (resembling minnows). Anadromous species were particularly difficult to keep alive during transportation and for long periods in the lab, but attempts were made and were sometimes successful. Because individual fish may vary in their ability to expel glochidia of various mussel species, when possible, we tested two or more individual fish in separate aquaria. Due to space constraints, it was not often possible to use large numbers of fish in tests, and in most cases we were limited to 1-3 individual fish per fish-mussel combination.

All laboratory host trials were conducted at the Freshwater Mussel Propagation Laboratory (FMPL) at the North Carolina State University College of Veterinary Medicine in Raleigh, NC. One exception was a follow up trial involving white perch and *E. roanokensis*. This follow up trial was conducted in a wet lab operated at the Baruch Institute for Marine and Coastal Sciences at the University of South Carolina in Columbia, SC. Fishes used in host trials were held in aquaria ranging from 8-380 liters in size depending upon the size and species of fish. Each fish was held in a separate aquarium and infested with the glochidia of only one mussel species to avoid any uncertainty in the identification of juvenile mussels at the time that

they were released from the host fish. The water used in the facility was municipal water from Raleigh, NC treated with a carbon filter and Ammo-Lock® (Aquatic Ecosystems, Apopka, FL) to remove chloramines. During holding, all fish were fed according to their preferences either feed pellets, frozen blood worms, live meal worms, nightcrawlers, Asian clams (*Corbicula fluminea*), or feeder fish.

The trial at the University of South Carolina used municipal water from the City of Columbia at a temperature of 21-22 ^{o}C. Chloramides were removed with Ammo-Lock®, and the water was filtered with a biological filtration system. In this system, water is pumped through a compartment containing plastic "bio-balls" as substrate for bacteria that break down ammonia.

We extracted glochidia from gravid mussels by flushing the marsupia with a water-filled syringe. All fish smaller than 15 cm were infected with glochidia using a batch infestation method. We aerated the tank vigorously to keep glochidia in suspension, allowing them to attach to the fish as the fish respired and passed the mussel larvae over their gills. After infestation was confirmed by visual examination of the gills, we separated the fish by species into various aquaria and maintained them at 20-23°C. All fish 15 cm or larger were anesthetized using tricaine methanosulfate (MS-222), and glochidia were pipetted on to their gills. After the fish recovered from anesthesia, they were separated into aquaria by species. Although no attempt was made to estimate the number of glochidia used to infest each fish, the typical fecundity rates of mussels (thousands per individual) permitted ample numbers of glochidia for one or two mussels to infest a large number of fish. After 12 days, we began siphoning the fish tanks routinely through a 150-μm mesh sieve to check for transformed juvenile mussels. Successful transformation to the juvenile stage was determined under a dissecting microscope by the presence of two adductor muscles or by foot movement.

Ligumia nasuta

Five gravid *Ligumia nasuta* were collected from the Broad River downstream of the Columbia Dam on 15 May 2007. On 17 May 2007, fish representing 23 species were collected by boat electrofishing in the Congaree River near Columbia, SC. Both fish and mussels were transported to the FMPL in Raleigh, NC and held at 20-23°C. On 21 May 2007, we extracted glochidia from two gravid *L. nasuta* by flushing the marsupia with a water-filled syringe. Three fish species collected for this host trial – yellow perch (*Perca flavescens*), American shad (*Alosa sapidissima*), and Threadfin shad (*Dorosoma petenense*) – did not survive in the laboratory until the host trial could begin. A fourth species – gizzard shad (*Dorosoma cepedanium*) – died one week into the host trial before any transformed juveniles could be obtained.

Two yellow perch (*Perca flavescens*) and two American eels (*Anguilla rostrata*) were tested in a second batch infestation on 13 August 2007 to replace the yellow perch that died and to add an additional species to the test. Unfortunately, not all anadromous species were available late in the season when the second set of tests was attempted. The yellow perch were collected from Jordan Lake in Chatham County, NC by angling on 3 August 2007. The eels were collected from the Santee River rediversion canal between Lakes Marion and Moultrie. Glochidia were extracted with a water-filled syringe from one of the remaining gravid female *L. nasuta* collected on 15 May 2007 that were held in the FMPL. We then combined the glochidia with the fish in 8 liters of water and aerated them vigorously for 20 minutes. Infestation was confirmed by visual examination of the gills of the yellow perch, and each fish was placed in a

separate aquarium and maintained at 21-24°C. Tanks were siphoned routinely to check for transformed juvenile mussels.

Of the fish that survived the host trials, largemouth bass (*Micropterus salmoides*), bluegill (*Lepomis macrochirus*), pumpkinseed (*Lepomis gibbosus*) and yellow perch (*Perca flavescens*) served as the most efficient hosts (Table 2). Redbreast sunfish (*Lepomis auritus*) and Redear sunfish (*Lepomis microlophus*) served as poor to moderate hosts, and no other fish species facilitated metamorphosis to the juvenile stage (Table 2). Juveniles were encysted on the fish from 11 to 24 days.

Elliptio roanokensis

Fish representing 19 species were collected by boat electrofishing in the Congaree River, and an additional species, blueback herring (*Alosa aestivalis*), was purchased from a bait shop in Columbia, SC. We transported the fish to the FMPL in Raleigh, NC and maintained them in various aquaria at 18-24°C. On 3 July 2007, we collected 20 gravid *Elliptio roanokensis* from the Congaree River just downstream of the Blossom Street Bridge in Columbia, SC. They were transported to the FMPL in Raleigh, NC and held in separate 8-liter aquaria in a recirculating system at 22-24°C. Each day they were monitored for release of glochidia into the aquaria. On 8 July 2007, one individual released its brood. The glochidia were determined to be viable and actively snapping by visual examination using a dissecting microscope and were subsequently collected for use in infestation of the fish.

Fish less than 15 cm were batch infested in approximately 12 liters of water for 30 minutes as described above, and fish greater than 15 cm were anesthetized and infested by hand.

We separated fish by species, maintained them in aquaria at 19-23°C and siphoned their tanks routinely to check for transformed juveniles.

Of the 20 species used in the host trial, one of them – the northern hogsucker (*Hypentelium nigricans*) – did not survive to the end of the trial and could not be assessed as a host. Three species – blueback herring (*Alosa aestivalis*), gizzard shad (*Dorosoma cepedanium*), and white perch (*Morone americana*) – served as successful hosts; however, only one of the two white perch tested served as a host (Table 3). Juveniles remained attached to fish from 10-16 days.

In order to determine if white perch is an effective host or only a marginally suitable host, a follow up trial in 2009 was planned using five replicates and testing the percent transformation rates of glochidia that initially attach to the fish. The number of attaching glochidia in a batch infestation is unknown, but siphoning and counting the number of transformed juveniles and untransformed glochidia coming off of a fish every 2-3 days was used to determine the number of attached glochidia after infestation. Higher water levels in the spring of 2009 made accessing the river difficult, since SCUBA assistance was only available during the main portion of the project in 2007 and 2008. The one gravid individual collected May 24 released non-viable glochidia in the lab, and we were unable to find additional gravid individuals until June 23. Three out of the four released glochidia on June 29, and they were used to infest the fish. Although only approximately one fourth of the glochidia appeared to be alive (they were observed snapping), none were released as unhatched eggs. The fourth individual released its brood on June 30, and was not used during the infestation. Its brood contained a mixture of unhatched eggs and viable glochidia.

Five white perch were infested, and of these only three yielded any juvenile mussels. Transformed juveniles were found from 16-18 days after infestation. Transformation rates (number of juvenile mussels divided by juvenile mussels plus dead glochidia that prematurely dropped off) are presented in Table 4.

Lampsilis cariosa

Fish representing 11 species were collected by boat electrofishing in the Congaree River near Columbia, SC and transported to the FMPL. Because of the lure display of *Lampsilis cariosa* and its tendency to attract piscivorous hosts, we eliminated suckers (Catostomidae), minnows (Cyprinidae) and darters (*Etheostoma* and *Percina*) from consideration in the host trials. We collected two yellow perch (*Perca flavescens*) by angling in Jordan Lake in Chatham County, NC. Three gravid *L. cariosa* were collected from the Broad and Congaree Rivers near Columbia, SC and maintained in the FMPL until the host trial could begin. On 6 August 2007, we extracted glochidia from two of the females and batch infested all fish in approximately 70 liters of water for 20 minutes. We then separated fish into separate aquaria by species and siphoned aquaria routinely to check for transformed juveniles.

The channel catfish (*Ictalurus punctatus*) collected for this host trial jumped out of their tank prior to infestation and were not tested as potential hosts. The white perch (*Morone americana*) used in the test did not survive long enough to produce juveniles and could also not be evaluated as potential hosts. The smallmouth bass (*Micropterus dolomieu*), largemouth bass (*Micropterus salmoides*), black crappie (*Pomoxis nigromaculatus*), and striped bass (*Morone saxatilis*) each served as efficient hosts (Table 5). Yellow perch (*Perca flavescens*) served as an

inefficient host producing only one glochidium from one of two fish. Juveniles remained attached to fish 14-22 days.

Lampsilis radiata

Fish representing 10 species were collected by boat electrofishing in the Congaree River near Columbia, SC and transported to the FMPL in Raleigh, NC. We also used backpack electrofishing to collect two yellow perch (*Perca flavescens*) from Morgan Creek (Cape Fear River Basin) in Chatham County, NC. Because of the lure display of *Lampsilis radiata* and its tendency to attract piscivorous hosts, we eliminated suckers (Catostomidae), minnows (Cyprinidae), and darters (*Etheostoma* and *Percina*) from consideration in the host trials. On 15 May 2007, we collected two gravid *L. radiata* from the Broad/Congaree River near the confluence with the Saluda at the Riverfront Park in Columbia, SC. These mussels were maintained at 15-17°C at the FMPL until the host trial began. On 6 August 2007, we extracted glochidia from both gravid females and combined with fish in approximately 70 liters of water for 25 minutes. Once infestation was confirmed by visual examination of the gills, fish were divided into separate aquaria and maintained at 21-24°C. We then siphoned tanks routinely to check for transformed juveniles.

All catfish (Ictaluridae) collected for this trial – channel catfish (*Ictalurus punctatus*), flathead catfish (*Pylodictis olivaris*), and flat bullhead (*Ameiurus platycephalus*) – jumped out of their tank and died prior to infestation. The black crappie (*Pomoxis nigromaculatus*) and white perch (*Morone americana*) died prior to transformation of juveniles and could not be assessed as potential hosts. Of the fish that successfully survived the trial, both largemouth bass

(*Micropterus salmoides*) and yellow perch (*Perca flavescens*) served as efficient hosts (Table 6). The bluegill (*Lepomis macrochirus*) and striped bass (*Morone saxatilis*) produced only one and two juveniles respectively, and none of the other species tested facilitated transformation. Juveniles remained encysted on the fish for 14-30 days.

Elliptio congaraea

We collected two gravid *Elliptio congarea* from the Broad River at Riverfront Park in Columbia, SC on 17 April 2008. Potential host fish (Table 7) were collected from the Congaree River in Columbia on 18 April. Fish and mussels were transported live in aerated coolers back to the Freshwater Mussel Propagation Facility at the NC State University College of Veterinary Medicine. On 21 April, one adult *E. congarea* released its brood, which mostly consisted of non-viable eggs. We then decided to wait on the other mussel to release before commencing with the infestation. On 25 April, the other adult released its brood into the aquarium in which it was being held. Again, approximately ¼ of the brood consisted of mature glochidia while the remainder where non-viable eggs (Fig. 1). We infested fish immediately upon discovering this released brood.

Larger fish were anesthetized using MS-222, and glochidia were pipetted directly onto the gills. Smaller fish were placed into 40 liters of water in a 100-liter round tank with the remainder of the brood and heavily aerated for 2 hours. The large volume of water was necessary to prevent overly stressing the threadin shad in a small tank. After infestation, fish were separated by species into different aquaria. We routinely siphoned the tanks 2-3 times per

week through a fine mesh sieve for 6 weeks following infestation searching for transformed juveniles.

It is unknown whether *Elliptio congarea* naturally has a small percentage of their brood that is fertilized and viable or if premature release was stimulated by handling stress. We found a wide range brood viability in *Elliptio roanokensis* observed in this study. In some cases, those relatives of *E. congarea* released only non-viable eggs, and in other cases, over 75% of the brood was viable. We have observed this same phenomenon in other short-term brooders we have attempted to propagate at NC State University.

We found no live transformed juveniles in any of the tanks and decided that the host test had failed six weeks after the infestation. If the test were successful, we should have recovered juveniles in only two to three weeks. Because no juveniles were recovered, we cannot rule out any of the species tested as potential hosts. These negative results may have been due to one of three possibilities. Perhaps we didn't have the actual host species in the laboratory. We did only have 14 species of fish, but they ranged across several families and were a basic representation of the fish community in the Congaree. Despite substantial collection efforts, no other species were found during electrofishing at the time gravid *E. congaraea* were collected. Another possibility is that we just didn't have enough glochidia to properly infest the fish. Glochidia in our batch infestation were far less concentrated than what we normally use due to the lack of viable glochidia and the need for a large tank to hold the threadfin shad. Certainly infestation rates were low, but we believe the fish were sufficiently exposed to produce at least a small degree of attachment. A third possibility is that even the glochidia that appeared viable were not healthy enough to attach and transform. We have seen this occur in other short-term brooders propagated at NC State University. Despite more surveys, no other gravid *E. congarea* were

found during the study period, so tests could not be repeated. Due to the rarity of *E. congarea* in the Broad and Congaree Rivers, future attempts to determine its host would likely have more success using mussels from other rivers where they are more abundant and where multiple healthy broods can be obtained.

Follow up assessment of mussel recolonization above the fish ladder

We had planned on making several trips to the section of the Broad River between the Columbia fish ladder and the Parr Reservoir dam in 2009 to look for species not previously found there that could have recolonized due to the fish passage's operations during the time that it has been operational. However, high water levels during the spring of 2009 and the lack of SCUBA divers made planning such efforts difficult. SCUBA divers were available for the main portion of the project, but not follow up efforts in 2009. One trip was made on May 21, 2009. Conditions were suitable for finding mussels only at shallow shoals around islands, and two sites were searched.

At one site, 34.19732 ^{o}N, -81.21883^{o}W, 10 *E. complanata*, 11 *E. lanceolata* complex, and 2 *V. delumbis* were found. Four people using snorkels and batiscopes expended an effort to 40 minutes each for a total of 2.67 person hours at the site, but only one of the four was experienced at searching for mussels, and found nearly all individuals encountered at the site. Therefore, the catch per unit effort at this site and date is not comparable to previous trips in which all or most individuals were experienced and were able to find many individuals. The second site was located at 34.19244^{o}N, -81.20799^{o}W. Using the same sampling crew as the first site, for 45 minutes, a total of 10 *E. complanata* and 2 *E. lanceolata* complex were found, all in

shallow channels with slower flowing water in between small islands within a large island complex. The searching conducted is nowhere near what is needed to adequately assess the post-fish passage diversity of mussels from Parr Reservior to the Columbia fish ladder.

Discussion and Recommendations

Sections of the Broad River below Parr Reservoir and above Columbia Dam appeared to be of high quality and were found to be able to support high densities of mussels. Therefore, we expect that the ability of mussels to pass through the Columbia Dam through the fish ladder is likely to benefit additional species found below the dam and not above. This is of particular conservation value, since the species found below the dam are, in general, of higher conservation priority.

The South Carolina portions of the Broad River above Parr Reservoir appear to be in poor condition, due to high turbidity levels, and unstable sediments. Previous studies (Bettinger et al. 2003) have noted that although riparian habitats throughout most sections of the SC portion of the Broad river are in good condition, some bank erosion problems are present in a 7 mile stretch above the highway 34 bridge crossing of the Parr Reservoir. Eighty seven percent of the riparian area was considered to be in good condition (> 50 m wide and composed of mature trees). Although much of the river contained healthy riparian areas, degradation including high turbidity, was observed above Parr Reservoir and it increased in intensity below sand mining operations (Bettinger et al. 2003). Past land use and the historic elimination of riparian forested areas prior to the re-establishment of forested buffers in recent years may have also contributed to heavy sediment loads that may still remain in stream and river channels (James 2007). Additional assessment to quantify the differences in habitat quality above and below this

impoundment and to explore potential restoration options is needed. These upper sections of the river may not support additional species of mussels even if fish passage opportunities made them accessible to mussels.

Reproduction of *Elliptio* species appeared to be both delayed and reduced at Blossom St. Bridge compared to Riverfront Park in 2008 (Figures 1-3). The reason for this is unknown, although cold water releases from the Saluda River, non-point source pollution in the Saluda watershed, a more highly urbanized system than the Broad River drainage, or pollution from a sewage spill on a tributary of the Saluda in 2008 are all possibilities. Mortality appeared to be extremely high at the Blossom St. Bridge, although it was not consistently measured, because the goal of sampling was to examine live individuals for reproductive status, and empty shells were frequently overlooked. Pollution could cause stress on the mussels and either cause them to cease reproduction or to die. Water quality data from SC Department of Health and Environmental control was examined to look for differences between the lower most sampling stations on the Broad and the Saluda Rivers, but no consistent differences long-term could be found in parameters expected to affect mussels. Sediment analysis showed the presence of some metals and polyaromatic hydrocarbons above detectible limits at the Blossom St. Bridge, which could be cause for concern. However, comparable data for the lower Saluda and lower Broad was not available, so it is unclear which watershed the impacts are coming from.

Temperature has been well-documented to affect mussel reproduction. Low temperatures are known to delay or inhibit the reproduction of freshwater mussels. Matteson (1948) noted that fewer *E. complanata* with mature glochidia could be found in 1945 when the mean water temperature from June 27 to July 7 was 20.9 ^{o}C. In comparison, mean water temperatures during this time in 1943 and 1944 were 22.5 and 24.0 ^{o}C respectively, when gravid mussels were more

abundant. The timing of reproduction in *Margaritifera margaritifera* in Scotland varies between years depending upon weather conditions, and a minimum of 3000 degree days occurs between annual episodes of glochidial release (Hastie and Young 2003). Heinricher and Layzer (1999) found that the washboard mussel, *Megalonaias nervosa*, had not reproduced in the Cumberland River for at least 20 years. When they relocated mussels from the river below two hypolimnetic dams where the water rarely reached a temperature of 20^{o} C, to the Tennessee River with a more natural thermal regime and a prolonged average monthly temperature ranging from approximately 26 to 28^{o} C from June through September, they began to reproduce.

Lellis and Johnson (1996) found that temperature and photoperiod controlled the release of glochidia in *Elliptio complanata*, in Pennsylvania. Glochidia were released between 16 and 19^{o} C during periods of rising temperature, and increasing photoperiod. When photoperiod was held constant at winter-like conditions, release of glochidia did not occur under rising temperatures, indicating that the interaction between temperature and photoperiod is important. Higher temperatures may be required to stimulate the release of glochidia in the summer than in the spring. The decrease in reproductive output of mussels at the Blossom St. Bridge relative to Riverfront Park early in the season is particularly puzzling, because water temperatures between the two rivers do not differ as much in the spring as in late summer. Kleinschmidt (add citation here) monitored temperature in the Congaree River at the Blossom St. Bridge in 2006 and 2007 and found that much of the time the east bank maintained temperatures close to those in the Broad River. In contrast the temperatures on the west side more closely matched those in the Saluda. However, the temperatures on the east bank did occasionally diverge from those in the Broad, particularly late in the summer, during water releases of high flow rates, and when flows were lower in the Broad River (Kleinschmidt xxxx). Matteson (1948) noted that glochidia died

when water temperature changed suddenly, and that gravid female mussels prematurely aborted glochidia when exposed to sudden changes in water temperature. Because so many potential factors may explain the differences in reproduction between the sites, controlled laboratory studies on these species are greatly needed. Studies manipulating temperature fluctuation and its effect on the release of glochidia and the development of gametes in females are recommended. Studies using water and sediment from each source in the lab and measuring the effects on reproduction may also assist in understanding the mechanisms.

The use of the fish ladder by various fish species was evaluated during the 2007 season from March 23-May 14. Fish were monitored two days per week either from 6:00 am to 10:00 am and 4:00 pm to 8:00 pm or from 10:00 am to 6:00 pm, for a total of 122 hours (Kleinschmidt 2007). Some fish species that we demonstrated to be successful host species for some mussels were observed moving through the fish ladder, but many were not. The numbers of individuals of many anadromous species, particularly threadfin shad were low considering the large number of threadfin shad seen schooling below the ladder and the large numbers of American shad (328,828) and blueback herring (49,343) noted to have passed through the St. Stephens fish lift downstream in the Santee drainage (Kleinschmidt 2007). Blueback herring was not observed using the Columbia fish ladder during the observation period, nor were several other species that we collected in the upper Congaree approximately 7-8 miles below the dam. While some of the species may have occasionally passed at times the fish ladder was open but not under observation, the numbers moving through the ladder were not likely to be very high if they were not observed during the 122 observation hours. Therefore, we expect that the ability of the Columbia fish ladder to effectively pass fish could be improved. In 2008, a much greater number of threadfin shad (4,209) were observed moving through the fish ladder using the same

sampling schedule as in 2007 for a total of 152 hours from March 13 to May 29, suggesting an improvement in the ability of the ladder to pass anadromous species (Kleinschmidt 2008). However, no blueback herring and relatively few (7 individuals) of American Shad were observed during this time. There were some differences in the species composition of fish observed moving through the ladder. Most notably, striped bass was observed in substantial numbers (53 individuals), while it was not observed at all in 2007. Low numbers of some host fishes and differences in movement patterns between years suggests that the ability of mussels of additional species to colonize in a particular year may be intermittent.

The only hosts that we found to be successful for *Ligumia nasuta* observed moving through in 2007 were largemouth bass (*Micropterus salmoides*) and redbreast sunfish (*Lepomis auritus*), which was only a marginal to moderately successful host. Both of these were observed moving through the ladder in low numbers; 17 largemouth bass, and 21 redbreast sunfish were observed. None of the four other hosts we determined to be successful or marginal for this mussel species, were observed moving through the ladder (Kleinschmidt, 2007). In 2008, two additional hosts, redear sunfish (*Lepomis microphus*) and Bluegill (*Lepomis macrochirus*) were observed moving through the ladder in low numbers, one and seven individuals, respectively. The only successful hosts for *E. roanokensis* observed in the fish ladder were gizzard shad (*Dorosoma cepedianum*), 742 individuals observed, white perch, only two individuals observed, a marginally effective host. Blueback herring was the only successful host we know of for *E. roanokensis* for which no individuals were observed moving across the ladder. For *L. cariosa*, the only known successful hosts which were observed moving through the fish ladder in 2007 were largemouth (*Micropterus salmoides*) and smallmouth bass (*Micropterus dolomieu*) (Kleinschmidt 2007), but striped bass (*Morone saxatilis*), an especially successful host, and

Bluegill, a marginal host, utilized the ladder in 2008 (Kleinschmidt 2008). Largemouth bass was the only successful host for *L. radiata* observed using the fish ladder in 2007 (Kleinschmidt 2007), but in 2008, bluegill, a marginally successful host was also observed (Kleinschmidt 2008).

In conclusion, the Columbia Dam fish ladder may have the potential to assist mussels in dispersing above the dam. Of the four mussel species for which fish host testing was conducted, at least one successful host species was also observed to be moving through the fish ladder, but it remains to be determined if the dates of operation are compatible with the timing of the mussels' reproduction. Since the rates at which fish passed through the ladder were generally low, and glochidial infestation rates in nature tend to be low (C. Eads, NC State U, personal comm.), mussels, if transported, are probably transported at a fairly low rate. Any changes to the fish ladder operations that could increase the volume and/or species diversity of fish passed may assist in the dispersal of mussels, since several highly successful hosts including white perch, white bass, and black crappie were not observed moving through the ladder in either year.

Assessment of the species diversity of mussels above the Columbia Dam several additional years after fish passage facilities are operational would be an informative future study. If fish passage successfully facilitates the transport of additional mussels upstream, the observed response is expected to be slow. Smith (1985) reported the range expansion of the mussel, *Anodonta implicata* above several dams on the Connecticut River following the establishment of fish passage facilities and/or the trucking of anadromous species. Although the sampling was not reported at regular intervals, the first date that the mussel was reported above each dam was a minimum of three years following the initiation of fish ladders or trucking and stocking of fish above the dam. McLain and Ross (2005) and Jones (2009) have reported a strong relationship

between the dispersal ability of host fishes and the speed of mussel dispersal. Though many of the fish in this study utilized wide ranging species such as bass, herring, and shad, if the fish passage facility transports only a few individuals of the successful host species through the ladder, dispersal is expected to be slower than in a free-flowing river where a greater number of fish are likely to move through the area. Effective transport of some mussel species may also be delayed until the fish passage operation schedule is extended.

Because most species were still releasing glochidia in June and in July in some cases, and several species (*Lampsilis cariosa, Lampsilis radiata,* and *Ligumia nasuta*) appeared to release most of their glochidia during or after late May, the original operation schedule (March-May) was not ideal in permitting the passage of all species of freshwater mussels. Catches of glochidia in drift nets actually increased from June to July, probably due to the releases of particular species and the fact that viable glochidia may remain in the water column for a few weeks after their release. In response to preliminary data from this study and requests from conservation agencies, South Carolina Electric & Gas on behalf of the City of Columbia (Licensee of the Columbia Hydroelectric Project (FERC Project No. 1895) has agreed to operate the fish passage year-round, except during extremely low inflow periods and during scheduled maintenance (South Carolina Electric & Gas, 2009). We commend South Carolina Electric & Gas and the City of Columbia on assisting in facilitating the passage of freshwater mussels. The year-round schedule is ideal, because even though the reproduction of mussels is concentrated in certain seasons, reproductive timing may vary from year to year.

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Table 1. Results of the general inventory of the main stem broad river. All dates are in 2007. CPUE= catch per unit effort in live mussels per person hour

site no.	latitude	longitude	date	person-hours	species	no. live	no. shells	CPUE
Upper Congaree River								
1	33.9688	-81.04007	5/31	0.4	*E. lanceolata* complex	1	0	2.5
					E. roanokensis	1	0	2.5
2	33.97004	-81.03893	5/31	0.5	*E. complanata*	2	0	4.0
					E. lanceolata complex	3	0	6.0
					E. roanokensis	2	0	4.0
					V. delumbis	1	0	2.0
3	33.97513	-81.04359	5/31	0.33	*E. lanceolata* complex	1	0	3.0
					E. roanokensis	5	0	15.0
					L. cariosa	1	0	3.0
4	33.97782	-81.04698	5/16	0.67	*E. roanokensis*	1	0	1.5
5	33.97812	-81.04536	5/16	1.67	*E. complanata*	5	0	3.0
					E. lanceolata complex	1	1	0.6
					E. roanokensis	26	0	15.6
					L. cariosa	2	0	1.2
					V. delumbis	1	0	0.6
6	33.98165	-81.04714	4/25	0.47	*E. complanata*	0	1	0.0
					E. lanceolata complex	1	0	2.1
7	33.98669	-81.04763	5/16	1.25	none	-	-	-
8	33.98708	-81.04551	5/16	3.75	*E. complanata*	9	0	2.4
					E. congaraea	1	0	0.3
					E. lanceolata complex	2	0	0.5
					E. roanokensis	73	0	19.5
					L. cariosa	1	0	0.3
					V. delumbis	1	0	0.3
			5/31	0.83	*E. complanata*	5	0	6.0
					E. lanceolata complex	3	0	3.6
					E. roanokensis	51	0	61.4

			8/14	1.5	*L. cariosa* *E. complanata* *E. lanceolata* complex *E. roanokensis* *L. cariosa* *V. delumbis*	1 1 3 12 4 1	0 0 0 0 0 0	1.2 0.7 2.0 8.0 2.7 1.2
9	33.996	-81.052	5/16	0.67	*E. complanata* *E. lanceolata* complex	1 1	0 1	1.5 1.5
10	33.99732	-81.05421	4/25	0.43	*E. complanata* *E. lanceolata* complex *E. roanokensis*	0 0 0	2 2 1	- - -
11	34.00077	-81.06044	4/25	0.17	None	-	-	-
12	34.00301	-81.05532	6/20	1.0	*E. complanata* *E. roanokensis*	1 1	0 0	1 1
13	34.00421	-81.05748	5/15	5.0	*E. complanata* *E. congaraea* *E. lanceolata* complex *E. roanokensis* *L. radiata* *L. nasuta* *Villosa delumbis*	8 3 21 22 2 1 14	0 0 1 0 0 0 1	1.6 0.6 4.2 4.4 0.4 0.2 2.8
Broad River below Parr Reservoir								
18	34.07909	-81.08981	3/27	1.5	*E. complanata* *E. lanceolata complex* *V. delumbis*	48 26 1	1 0 0	32 17.3 0.4
19	34.0934	-81.10606	3/27	1.17	*E. complanata* *E. lanceolata complex* *U. carolinanus*	27 1 10	6 14 0	23.1 0.9 8.5
20	34.13413	-81.13848	3/28	0.5	*E. complanata* *E. lanceolata complex*	37 14	0 0	74 28

21	34.15881	-81.15317	3/28	0.5	*E. complanata*	4	0	8
					E. lanceolata complex	4	0	8
22	34.16693	-81.16542	3/28	0.75	*E. complanata*	44	0	58.7
					E. lanceolata complex	4	0	5.3
					U. carolinanus	1	0	1.3
					V. delumbis	2	0	2.6
23	34.19955	-81.22483	3/28	1.33	*E. complanata*	3	0	2.3
					E. lanceolata complex	8	0	6.0
					U. carolinanus	38	0	28.5
					V. delumbis	7	0	5.3
24	missing	missing	3/29	0.75	*E. complanata*	13	0	17.3
					E. lanceolata complex	24	0	32.0
					V. delumbis	2	0	2.7
25	missing	missing	3/29	1.0	*E. complanata*	63	0	63.0
					E. lanceolata complex	35	0	35.0
					V. delumbis	11	0	11.0
Parr Reservoir								
26	34.28227	-81.34766	8/31	0.75	*E. complanata*	1	0	1.3
					E. lanceolata complex	47	16	62.7
					V. delumbis	3	0	4.0
			9/26	2.17	*E. complanata*	1	0	0.5
					E. lanceolata complex	25	9	11.5
					U. carolinanus	1	0	0.5
					V. delumbis	4	1	1.8
27	34.28503	-81.34099	9/26	2.33	none	0	0	-
28	34.2859	-81.33821	8/31	0.33	*E. lanceolata* complex	1	6	3.0
			9/26	2.0	*E. lanceolata* complex	4	4	2.0
					U. carolinanus	2	0	1.0

					U. imbecillis *V. delumbis*	0 1	1 0	- 0.5
29	34.29477	-81.34232	9/27	2.0	*E. lanceolata* complex *U. carolinanus* *V. delumbis*	16 2 2	7 0 0	8.0 1.0 1.0
30	34.30006	-81.34343	8/31 9/26	0.58 2.0	*E. complanata* *E. lanceolata* complex *E. lanceolata* complex *V. delumbis*	1 18 2 16	0 3 0 0	1.7 31.0 1.0 8.0
31	34.32524	-81.36617	9/7 9/27	0.5 2.0	*E. lanceolata* complex *V. delumbis* *E. lanceolata* complex	3 1 1	0 0 0	6.0 2.0 0.5
32	34.33614	-81.37004	9/7	0.5	*E. lanceolata* complex	0	2	4.0
Broad River above Parr Reservoir								
33	34.50299	-81.42056	4/26	0.27	none	0	0	-
34	34.54028	-81.42664	4/26	0.67	none	0	0	-
35	34.5933	-81.42075	7/16	1.33	*E. lanceolata* complex *V. delumbis*	11 1	0 0	8.3 0.8
36	34.60525	-81.4172	7/16	0.67	*E. lanceolata* complex	1	0	1.5
37	34.63086	-81.41812	7/16	0.67	*E. lanceolata* complex	1	0	1.5
38	34.65604	-81.44328	7/16	0.5	none	0	0	-
39	34.66316	-81.44566	7/16	0.33	none	0	0	-
40	34.72609	-81.46175	8/16	0.17	none	0	0	-
41	34.75092	-81.47244	8/16	0.5	none	0	0	-

42	34.76659	-81.45328	8/16	0.67	none	0	0	-
43	34.77276	-81.45538	8/16	0.67	none	0	0	-
44	34.77607	-81.45499	8/16	1.0	*E. lanceolata* complex	3	1	3.0
45	34.8766	-81.47118	8/22	1.0	*E. lanceolata* complex	2	0	2.0
46	34.91208	-81.47171	8/22	1.0	none	0	0	0.0
47	34.93425	-81.47374	8/22	1.67	*E. lanceolata* complex	5	1	3.0
48	34.94893	-81.49248	7/19	0.5	none	0	0	-
49	34.97158	-81.48045	7/19	0.33	none	0	0	-
50	35.00663	-81.48038	7/19	0.5	none	0	0	-
51	35.01047	-81.48329	7/19	0.57	none	0	0	-
52	35.02319	-81.21877	7/19	0.67	none	0	0	-
53	35.05651	-81.5395	9/13	0.83	none	0	0	-
54	35.05773	-81.54175	9/13	1.25	*E. lanceolata* complex	1	0	0.8
55	35.08725	-81.57247	9/5	0.5	*E. lanceolata* complex	3	0	6.0
56	35.09025	-81.57183	9/5	1.0	*E. complanata* *E. lanceolata* complex *E. roanokensis*	1 2 1	2 0 0	1.0 2.0 1.0
57	35.10257	-81.57387	9/5	0.83	*E. complanata* complex	0	1	-
58	35.11959	-81.58197	9/5	0.5	none	0	0	-
59	35.1335	-81.59599	9/5	0.33	none	0	0	-
60	35.1869	-81.6302	9/18	1.5	none	0	0	-

Selected tributaries of the Upper Broad								
Guyon Moore Creek	34.98664	-81.47167	10/9	1.0	none	0	0	-
Buffalo Creek	35.1275	-81.55068	10/9	1.33	none	0	0	-
Kings Creek	35.04171	-81.47832	10/9	1.5	none	0	0	-
Thickety Creek	34.92847	-81.52916	10/11	1.0	none	0	0	-
Pacolet River	34.8736	-81.53146	10/11	2.5	none	0	0	-

Table 2. results of host fish testing for *Ligumia nasuta*. Each replicate represents one individual fish kept in a separate tank. *=Infested 13 August 2007

FISH SPECIES	**Replicate**	**Transformed Juveniles Produced**
Anguillidae		
American eel (*Anguilla rostrata*)*	A	0
American eel (*Anguilla rostrata*)*	B	0
Catostomidae		
Quillback (*Carpiodes cyprinus*)	A	0
Northern hogsucker (*Hypentelium nigricans*)	A	0
Spotted sucker (*Minytremia melanops*)	A	0
Shorthead redhorse (*Moxostoma macrolepidotum*)	A	0
Centrarchidae		
Redbreast sunfish (*Lepomis auritus*)	A	2
Redbreast sunfish (*Lepomis auritus*)	B	28
Redbreast sunfish (*Lepomis auritus*)	C	5
Redbreast sunfish (*Lepomis auritus*)	D	9
Redbreast sunfish (*Lepomis auritus*)	E	1
Pumpkinseed (*Lepomis gibbosus*)	A	78
Bluegill (*Lepomis macrochirus*)	A	335
Bluegill (*Lepomis macrochirus*)	B	91
Bluegill (*Lepomis macrochirus*)	C	44
Redear sunfish (*Lepomis microlophus*)	A	4
Redear sunfish (*Lepomis microlophus*)	B	0
Largemouth bass (*Micropterus salmoides*)	A	91
Cyprinidae		
Whitefin Shiner (*Cyprinella nivea*)	A	0
Spottail shiner (*Notropis hudsonius*)	A	0
Coastal shiner (*Notropis petersoni*)	A	0
Ictaluridae		
Flat bullhead (*Ameiurus platycephalus*)	A	0
Channel catfish (*Ictalurus punctatus*)	A	0
Flathead catfish (*Pylodyctis olivaris*)	A	0
Moronidae		
White perch (*Morone americana*)	A	0
Striped bass (*Morone saxatilis*)	A	0

Percidae		
Tessellated darter (*Etheostoma olmstedi*)	A	0
Yellow perch (*Perca flavescens*)*	A	344
Yellow perch (*Perca flavescens*)*	B	258
Piedmont darter (*Percina crassa*)	A	0

Table 3. results of host fish testing for *Elliptio roanokensis*. Each replicate represents one individual fish kept in a separate tank.

Fish species	Replicate	Transformed Juveniles Produced
Anguillidae		
American eel (*Anguilla rostrata*)	A	0
Catostomidae		
Quillback (*Carpiodes cyprinus*)	A	0
Quillback (*Carpiodes cyprinus*)	B	0
Northern hogsucker (*Hypentelium nigricans*)	A	Died
Spotted sucker (*Minytremia melanops*)	A	0
Notchlip redhorse (*Moxostoma collapsum*)	A	0
Shorthead redhorse (*Moxostoma macrolepidotum*)	A	0
Centrarchidae		
Redbreast sunfish (*Lepomis auritus*)	A	0
Redbreast sunfish (*Lepomis auritus*)	B	0
Bluegill (*Lepomis macrochirus*)	A	0
Redear sunfish (*Lepomis microlophus*)	A	0
Redear sunfish (*Lepomis microlophus*)	B	0
Largemouth bass (*Micropterus salmoides*)	A	0
Largemouth bass (*Micropterus salmoides*)	B	0
Smallmouth bass (*Micropterus dolomieu*)	A	0
Smallmouth bass (*Micropterus dolomieu*)	B	0
Black crappie (*Pomoxis nigromaculatus*)		0
Clupeidae		
Blueback herring (*Alosa aestivalis*)	A	304
Gizzard shad (*Dorosoma cepedanium*)	A	24
Gizzard shad (*Dorosoma cepedanium*)	B	20
Cyprinidae		
Whitefin Shiner (*Cyprinella nivea*)	A	0
Whitefin Shiner (*Cyprinella nivea*)	B	0
Whitefin Shiner (*Cyprinella nivea*)	C	0
Ictaluridae		
Channel catfish (*Ictalurus punctatus*)	A	0
Flathead catfish (*Pylodyctis olivaris*)	A	0
Moronidae		

White perch (*Morone americana*)	A	0
White perch (*Morone americana*)	B	35
Striped bass (*Morone saxatilis*)	A	0
Percidae		
Yellow perch (*Perca flavescens*)	A	0

Table 4. results of follow up host trial to examine transformation rates of *E. roanokensis* on white perch.

Replicate	**Transformed Juveniles Produced**	**Number died**	**Transformation rate**
A	6	296	1.99%
B	1	524	0.19%
C	1	902	0.11%
D	0	857	0%
E	0	1758	0%

Table 5. results of host fish testing for *Lampsilis cariosa*. Each replicate represents one individual fish kept in a separate tank.

Fish species	Replicate	Transformed Juveniles Produced
Anguillidae		
American eel (*Anguilla rostrata*)	A	0
American eel (*Anguilla rostrata*)	B	0
Centrarchidae		
Redbreast sunfish (*Lepomis auritus*)	A	0
Redbreast sunfish (*Lepomis auritus*)	B	0
Bluegill (*Lepomis macrochirus*)	A	2
Bluegill (*Lepomis macrochirus*)	B	0
Redear sunfish (*Lepomis microlophus*)	A	0
Redear sunfish (*Lepomis microlophus*)	B	0
Smallmouth bass (*Micropterus dolomieu*)	A	57
Smallmouth bass (*Micropterus dolomieu*)	B	64
Largemouth bass (*Micropterus salmoides*)	A	423
Largemouth bass (*Micropterus salmoides*)	B	47
Largemouth bass (*Micropterus salmoides*)	C	0
White bass (*Morone chrysops*)	A	1276
Black crappie (*Pomoxis nigromaculatus*)	A	816
Moronidae		
White perch (*Morone americana*)	A	Died
White perch (*Morone americana*)	B	Died
Striped bass (*Morone saxatilis*)	A	4079
Percidae		
Yellow perch (*Perca flavescens*)	A	1
Yellow perch (*Perca flavescens*)	B	0

Table 6. results of host fish testing for *Lampsilis radiata*. Each replicate represents one individual fish kept in a separate tank.

Fish species	Replicate	Transformed Juveniles Produced
Anguillidae		
American eel (*Anguilla rostrata*)	A	0
American eel (*Anguilla rostrata*)	B	0
Centrarchidae		
Redbreast sunfish (*Lepomis auritus*)	A	0
Redbreast sunfish (*Lepomis auritus*)	B	0
Bluegill (*Lepomis macrochirus*)	A	1
Bluegill (*Lepomis macrochirus*)	B	0
Redear sunfish (*Lepomis microlophus*)	A	0
Redear sunfish (*Lepomis microlophus*)	B	0
Redear sunfish (*Lepomis microlophus*)	C	0
Smallmouth bass (*Micropterus dolomieu*)	A	0
Largemouth bass (*Micropterus salmoides*)	A	517
Largemouth bass (*Micropterus salmoides*)	B	314
Black crappie (*Pomoxis nigromaculatus*)	A	Died
Moronidae		
White perch (*Morone americana*)	A	Died
White perch (*Morone americana*)	B	Died
Striped bass (*Morone saxatilis*)	A	2
Striped bass (*Morone saxatilis*)	B	0
Percidae		
Yellow perch (*Perca flavescens*)	A	242
Yellow perch (*Perca flavescens*)	B	424

Table 7. Fish infested with *Elliptio congarea* glochidia. Replicates vary in the number of individuals

Common Name	Replicate	Number of Fish in replicate	Transformed Juveniles Produced
Catostomidae			
Channel catfish (*Ictalurus punctatus*)	A	1	0
Northern hogsucker (*Hypentelium nigricans*)	A	1	0
Quillback (*Carpiodes cyprinus*)	A	1	0
	B	2	0
Shorthead redhorse (*Moxostoma macrolepidotum*)	A	1	0
Spotted sucker (*Minytremia melanops*)	A	1	0
Centrarchidae			
Bluegill (*Lepomis macrochirus*)	A	1	0
	B	1	0
Redbreast sunfish (*Lepomis auritus*)	A	2	0
	B	2	0
Redear sunfish (*Lepomis microlophus*)	A	1	0
	B	1	0
Largemouth bass (*Micropterus salmoides*)	A	1	0
	B	1	0
Cyprinidae			
Whitefin shiners (*Cyprinella nivea*)	A	5	0
	B	5	0
Clupeidae			
Threadfin shad (*Dorosoma petenense*)	A	10	0
Lepisosteidae			
Longnose gar (*Lepisosteus osseus*)	A	1	0
Moronidae			
White perch (*Morone Americana*)	A	1	0
Percidae			
Yellow perch (*Perca flavescens*)	A	2	0
	B	1	0

Figure 1. Map of general inventory sites and dams on the Broad River. The red marks indicate dams on the river. The lower most dam is the Columbia Dam where the fish ladder had been installed. Green circles indicate study sites where mussels were found, while black circles indicate the absence of mussels.

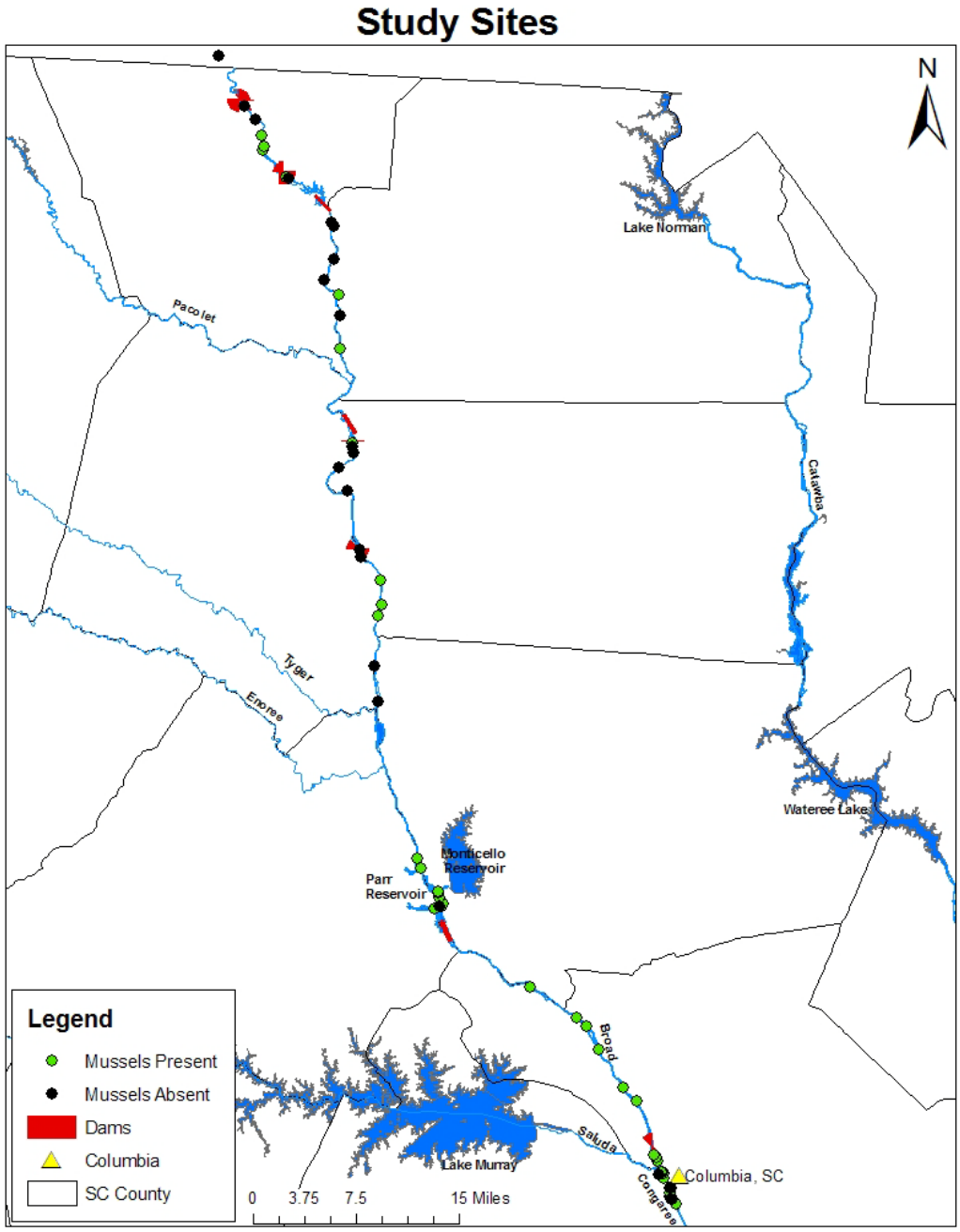

Figure 2. Repeat monitoring sites at Riverfront Park (upstream) and Blossom St. Bridge (downstream). Note the differences in the color of the water in the Broad (eastern) and Saluda (western) Rivers, indicating higher turbidity in the Broad. It is also apparent that while the right and left banks of the Congaree are not yet well mixed at Blossom St. Bridge, some slight mixing is evident when compared with stretches of river farther upstream.

Figure 3. Fraction of gravid *E. roanokensis* at both monitoring sites. Stars indicate dates on which the fraction of individuals located was significantly greater ($P \leq 0.05$) at Riverfront park than at Blossom Street Bridge. Major tick marks with month names represent the first of each month.

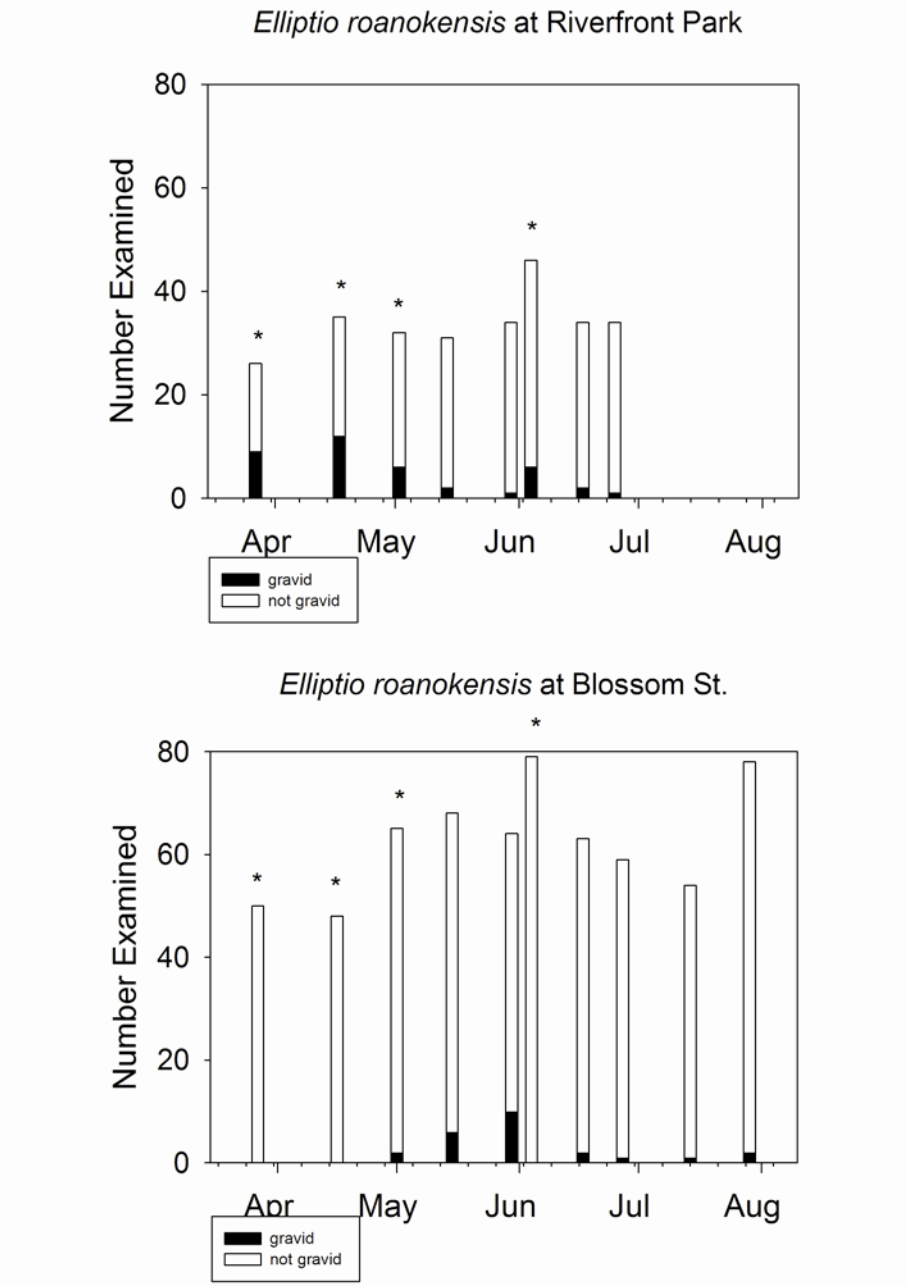

Figure 4. Fraction of gravid *E. complanata* at both monitoring sites. A star indicates dates on which the fraction of individuals located was significantly greater ($P \leq 0.05$) at Riverfront park than at Blossom Street Bridge. Major tick marks with month names represent the first of each month.

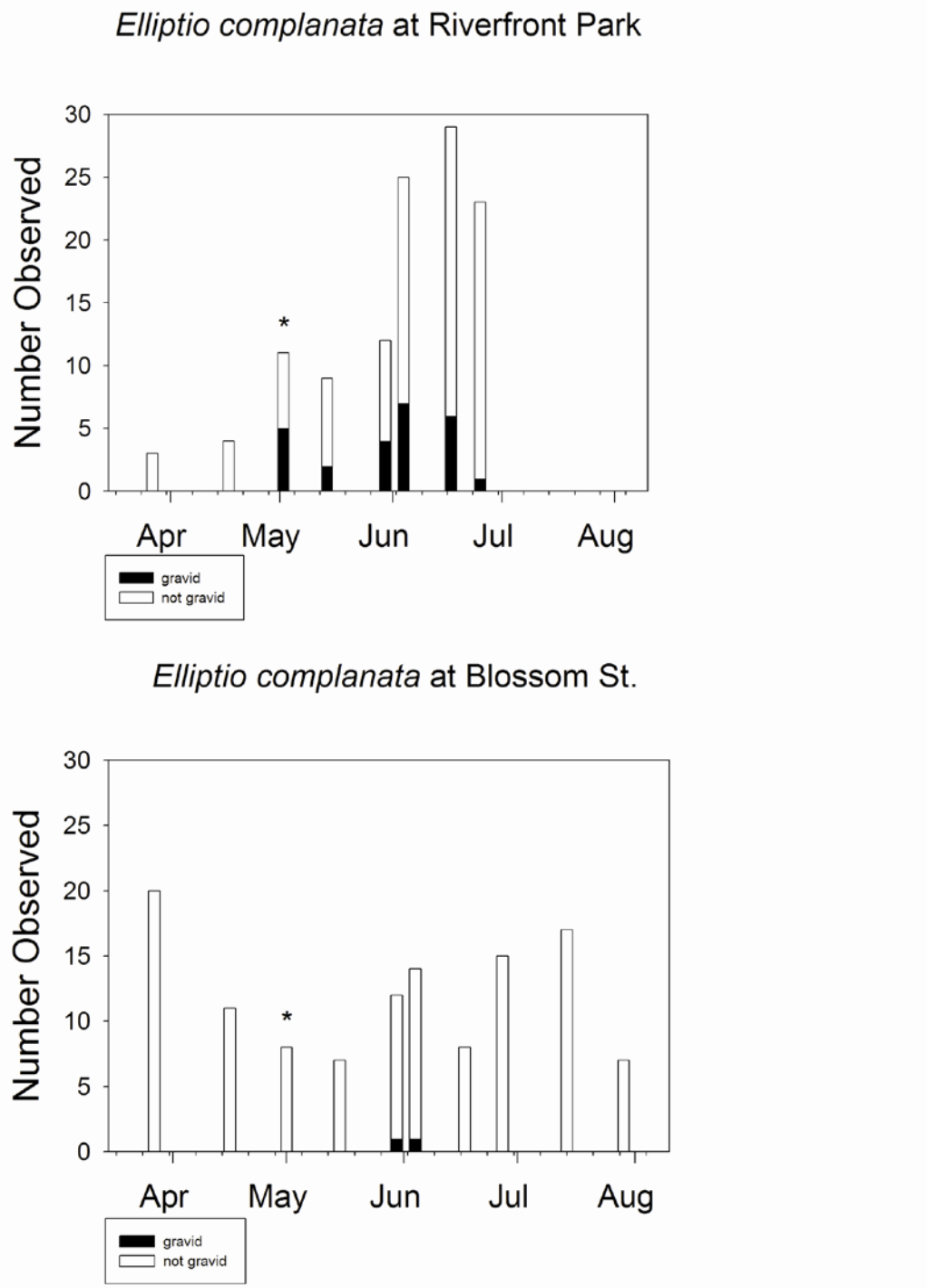

Figure 5. Fraction of gravid members of the *E. lanceolata* complex at both monitoring sites. A star indicates dates on which the fraction of individuals located was significantly greater ($P \leq 0.05$) at Riverfront park than at Blossom Street Bridge. Major tick marks with month names represent the first of each month.

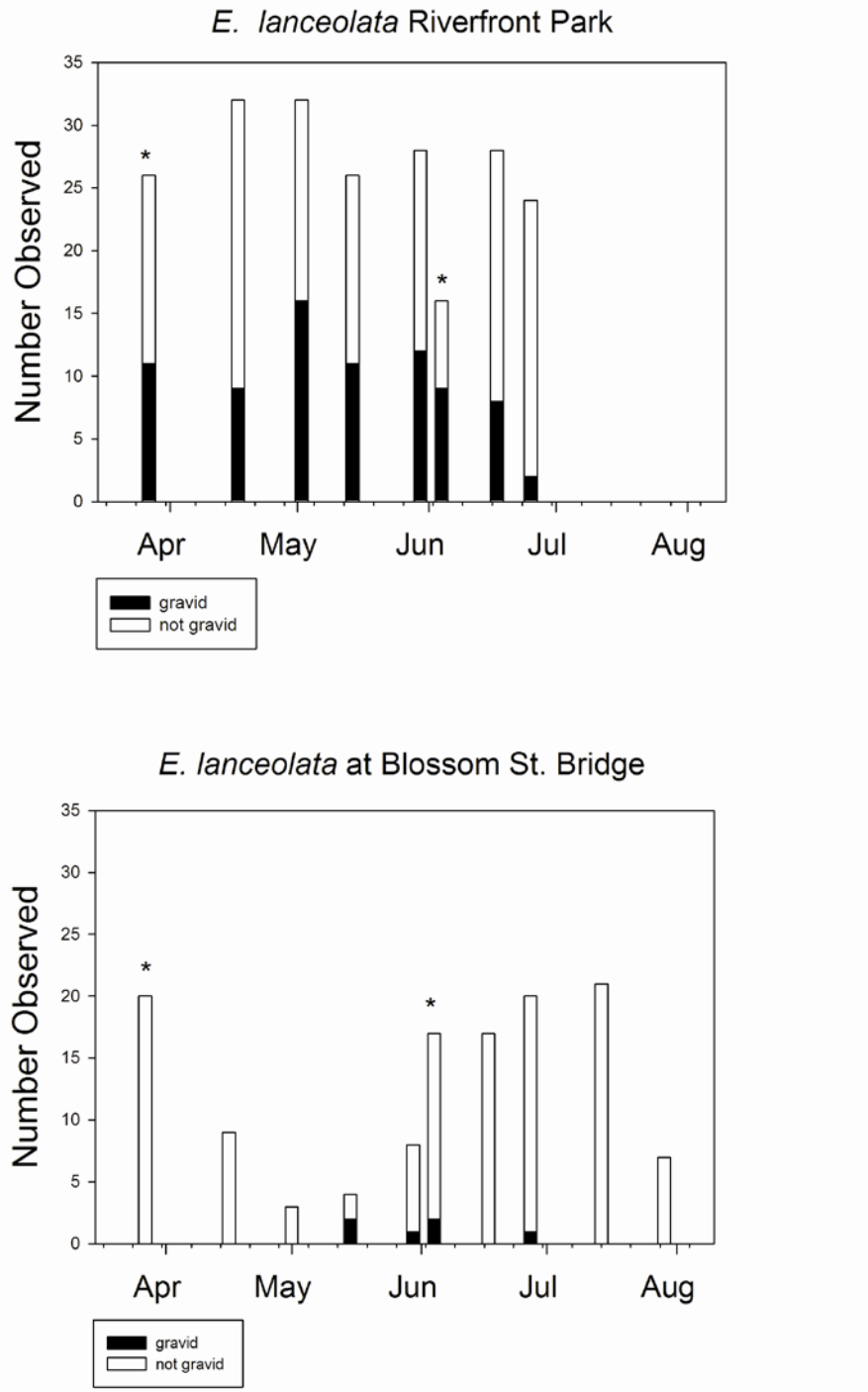

Figure 6. Fraction of gravid female *Ligumia nasuta* at the Broad River site above I-126 and at Riverfront Park. Black bars represent individuals that were gravid with gills completely full of glochidia. Grey bars represent gravid individuals that have released part of a brood, and clear bars represent individuals not gravid. Major tick marks with month names represent the first of each month.

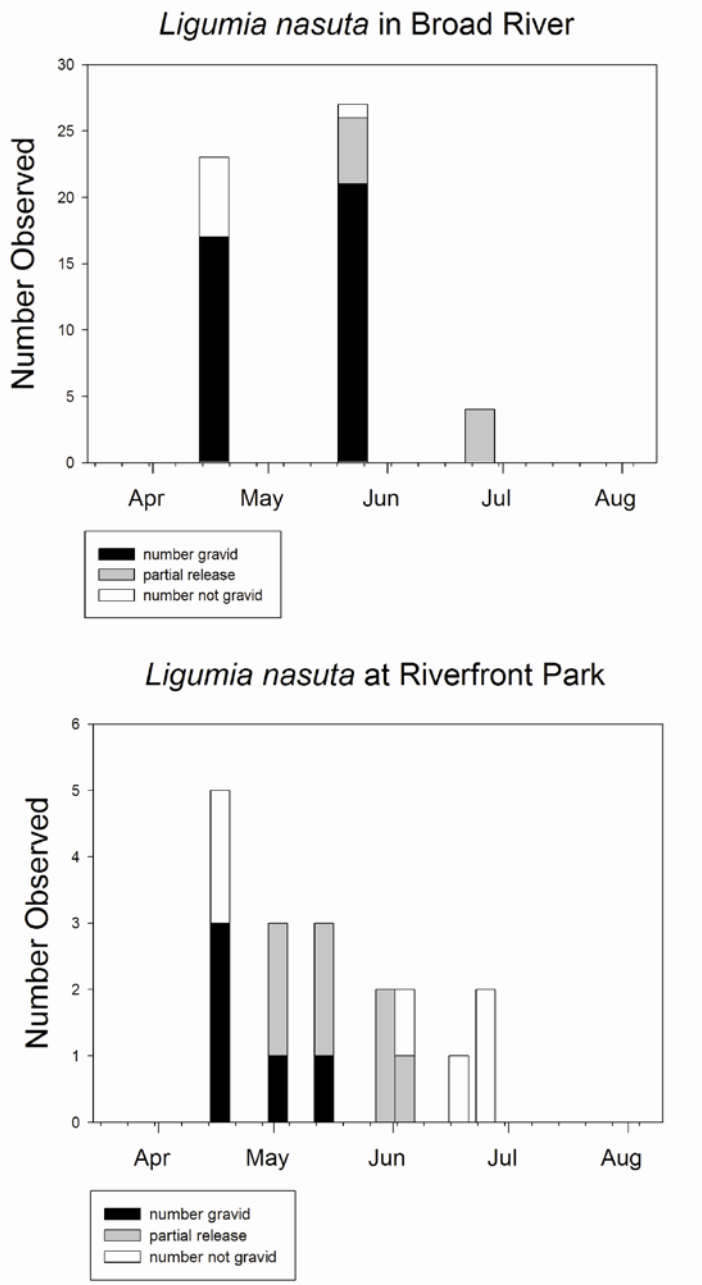

Figure 7. Fraction of gravid female *Lampsilis cariosa* at Blossom St. Bridge, the only repeated monitoring site at which the species was found. Black bars represent individuals that were gravid with gills completely full of glochidia. Grey bars represent gravid individuals that have released part of a brood, and clear bars represent individuals not gravid. Major tick marks with month names represent the first of each month.

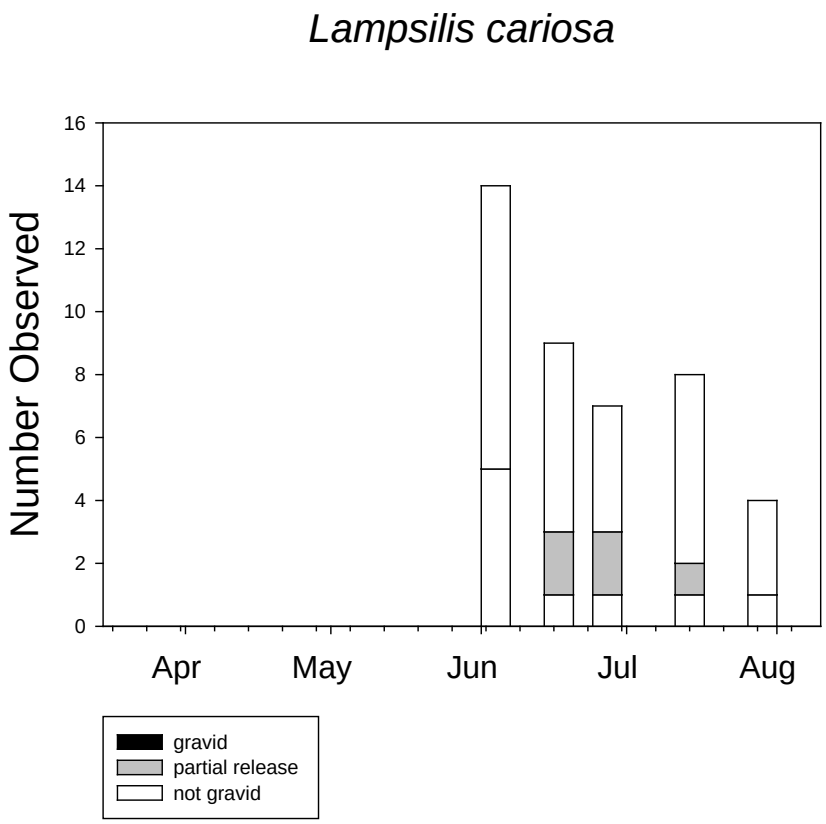

Figure 8. Fraction of gravid female *Lampsilis radiata* at Riverfront Park, the only repeated monitoring site at which multiple individuals were found. One individual was found gravid at Blossom St. Bridge, March 27, April 16 and May 1, but not recaptured afterwards. Black bars represent individuals that were gravid with gills completely full of glochidia. Grey bars represent gravid individuals that have released part of a brood, and clear bars represent individuals not gravid. Major tick marks with month names represent the first of each month.

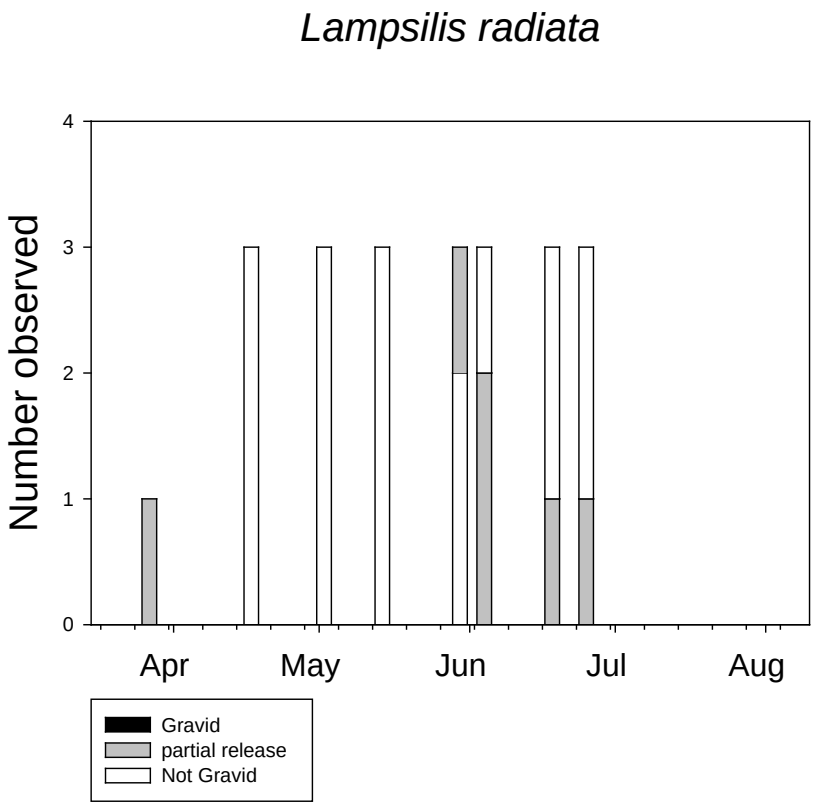

Figure 9. Fraction of gravid female *Villosa delumbis* at Riverfront Park, the only site at which gravid individuals were found in 2008. One female was found at the Blossom St. Bridge site on June 4, but it was not gravid. Black bars represent individuals that were gravid with gills completely full of glochidia. Grey bars represent gravid individuals that have released part of a brood, and clear bars represent individuals not gravid. Major tick marks with month names represent the first of each month.

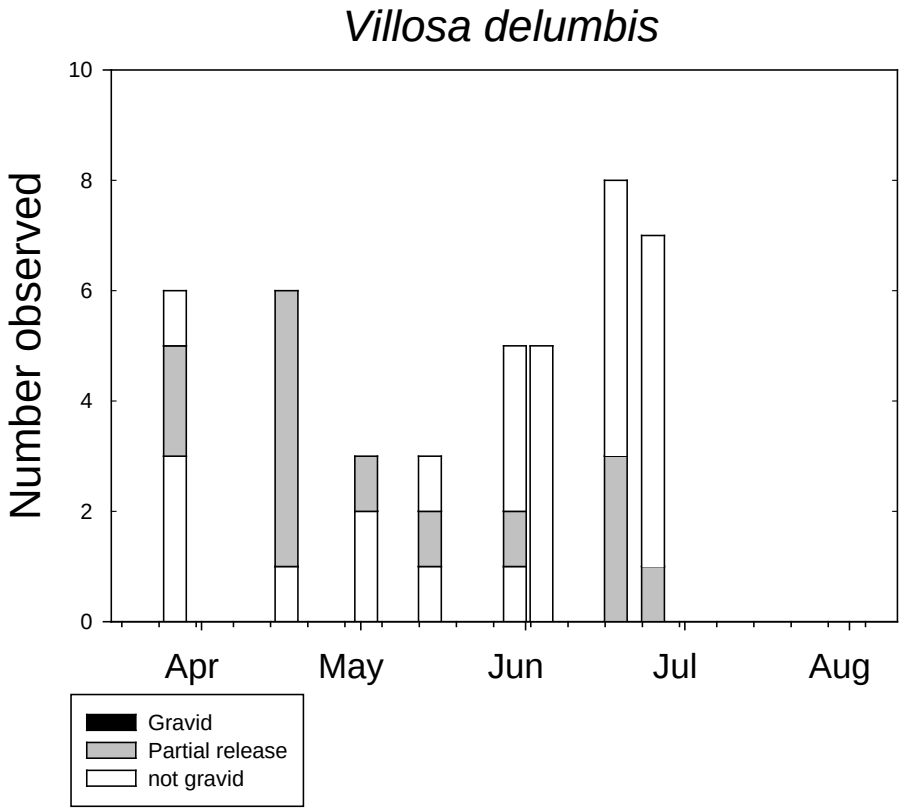

Figure 10. Glochidia collected in drift nets. All species have been combined.

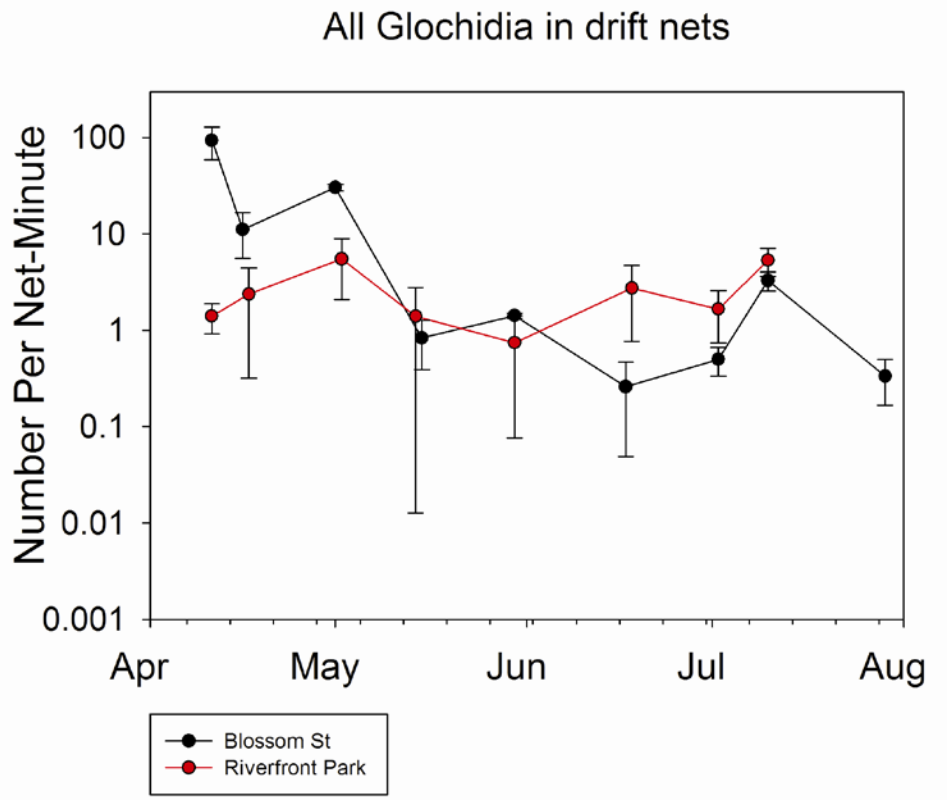

Figure 11. *Villosa delumbis* glochidia in drift nets