Restoration of the endangered Cumberland elktoe (*Alasmidonta atropurpurea*) and Cumberland bean (*Villosa trabalis*) (Bivalvia: Unionidae) in the Big South Fork National River and Recreation Area, Tennessee and Kentucky

by

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ABSTRACT

The Big South Fork National River and Recreation Area (NRRA), located in Tennessee and Kentucky, has prepared a management plan to include restoration of its mussel fauna to historic levels. Restoration activities include propagation of juvenile mussels and relocation of adults to suitable sites in the Big South Fork of the Cumberland River (BSF) and its tributaries. This study was conducted to identify host fish for Cumberland elktoe (*Alasmidonta atropurpurea*) and Cumberland bean (*Villosa trabalis*), to determine suitable juvenile culture conditions for *Epioblasma brevidens* and *V. trabalis*, and to locate sites important to future mussel restoration efforts in the NRRA.

Host fish identifications and propagation techniques were determined for two of the endangered species in the NRRA, Cumberland elktoe (*Alasmidonta atropurpurea*) and Cumberland bean (*Villosa trabalis*). Of seven host species tested, banded sculpin (*Cottus carolinae*) was the most suitable host fish for propagation of *A. atropurpurea*. Of five host species tested, fantail darters (*Etheostoma flabellare*) were the most suitable host fish for propagation of *V. trabalis*. Culture techniques to raise juvenile mussels in captivity were evaluated, using newly metamorphosed juveniles of *V. trabalis* and *E.*

brevidens in recirculating systems. No differences in juvenile growth or survival were detected among substrates used (fine sediment, coarse sand, and a mixture of the two). Recirculating system design seemed to affect juvenile growth and survival; however, variable condition of juveniles also seemed to affect results, making it difficult to determine effects from trial treatments.

Finally, an assessment of potential sites in the NRRA for restoration activities was conducted using spatial analysis in a geographic information system (GIS) and several measures of conservation value. Mussel restoration sites were assessed for potential threats from adjacent land uses that may negatively affect mussels, including coal mines, oil and gas wells, transportation corridors, agriculture and urban development. Sites were also evaluated on their current conservation value to designate which sites are most important to long-term maintenance of mussel fauna. Several sites were identified that contain relatively few land-use threats, and are appropriate for mussel restoration activities, including Big Island, Station Camp Creek, and Parchcorn Creek sites on the mainstem BSF, as well as sites on Clear Fork and North White Oak Creek. Many of these sites also have high conservation values. Other sites had relatively high land-use threats that need to be addressed before restoration activities take place. Such sites include Leatherwood Ford, Rough Shoals Branch, Blue Heron, and Yamacraw on the mainstem BSF. The dominant threat to most sites came from transportation corridors, whereas some sites in southern and eastern portions of the watershed also were threatened by coal mines, and oil and gas wells.

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General Introduction

Big South Fork of the Cumberland River

The Big South Fork of the Cumberland River (BSF) is located in north-central Tennessee and south-central Kentucky, in the Cumberland Plateau sub-section of the Appalachian Plateau Physiographic Province (Ahlstedt et al. 2005). Geology in the BSF watershed is characterized by Mississippian age limestone capped by Pennsylvanian age sandstone. Both of these formations have been deeply eroded by streams over time, forming steep valley walls around the stream channel (Bakaletz 1991). The BSF begins at the confluence of the Clear Fork and New rivers in Tennessee and flows 124 km northeast through Tennessee and Kentucky, joining the Cumberland River near Burnside, Kentucky. Several kilometers of the lower BSF are affected by the impoundment of the Cumberland River by Wolf Creek Dam, which now forms Lake Cumberland.

The watershed of the BSF is mostly rural, with low human population density (Ahlstedt et al. 2005). This land is very rugged and contains large tracts of forest and deposits of fossil fuels, such as coal, oil, and gas (NPS 2003). Resource extraction in the BSF watershed has been ongoing for almost a century. It reached its peak in the 1960s and 1970s, but continues at relatively low levels today (NPS 2003).

Historic mussel fauna

Historically, at least 55 freshwater mussel species were known from the BSF (NPS 2003). However, since the BSF was continuous with the rich mussel fauna of the Cumberland River, the BSF presumably contained as many as 70 mussel species (Gordon and Layzer 1989, Ahlstedt et al. 2005). A survey by Wilson and Clark (1914) of the entire Cumberland River system found 28 species at Parkers Lake Station near

Yamacraw, KY, and 32 species at the shoal above Burnside, KY, on the lower BSF. Shoup and Peyton (1940) surveyed 86 sites throughout the BSF watershed and found a total of 11 species. Effects from acid mine drainage and pollutants were evident at this time, especially in portions of the New River (Shoup and Peyton 1940). Although this was an extensive survey of the BSF and its tributaries, mussels were only one aspect of the study. Therefore, few conclusions can be drawn on the apparent decline of mussel species at that time since sites surveyed may not have occurred in prime mussel habitat (Shoup and Peyton 1940). Neel and Allen (1964) surveyed the Cumberland River system prior to its impoundment at Wolf Creek Dam; their study included the two BSF sites surveyed by Wilson and Clark (1914). At one site (Yamacraw), no mussels were found; at the shoal above Burnside, 16 species were found (Neel and Allen 1964). Again, effects of acid mine drainage were noted (Neel and Allen 1964).

Designation of National River and Recreation Area

In 1974, parts of the BSF watershed were designated as a National River and Recreation Area (NRRA). The free-flowing mainstem of the BSF and several tributaries are included in the NRRA, which is managed by the National Park Service (NPS). The NRRA was dedicated 'to conserve[e] and interpret[e] an area containing unique cultural, historical, geological, biological, archeological, scenic, and recreational resources, [and] preserving the Big South Fork of the Cumberland River as a natural free-flowing stream…'(NPS 1997). The conservation mandate of the NRRA is similar to that of a national park; however, extractive uses, such as hunting and natural resource extraction, are allowed. Other recreational activities in the NRRA include camping, hiking, horseback riding, birding, fishing, rock climbing, canoeing and whitewater rafting.

Current mussel fauna

After designation as a NRRA in 1974, the BSF was surveyed to assess aquatic biota. The impact of natural resource extraction was believed to be so severe that no mussels survived (Ahlstedt et al. 2005). Extraction of the area's natural resources increased pollutants in overland runoff, such as acid mine drainage and sediment, which are typically detrimental to mussel populations (Rikard et al. 1986). Surveys by Harker et al. (1979, 1980, 1981) and Schuster (1988) led to the discovery of about 20 species that remained. Bakaletz (1991) surveyed the BSF and several tributaries, and reported 22 resident species. Additional surveys of biota at specific sites in the BSF were conducted by Shute et al. (1999) and Dunn (2000), who found 16 and 18 species, respectively, at a total of 3 sites on the mainstem BSF. Ahlstedt et al. (2005) thoroughly surveyed the BSF and its tributaries for mussels from 1998 to 2002, reporting a total of 26 species. These surveys have shown that even though this river is isolated from the main channel of the Cumberland River by a reservoir, the BSF is one of the best remaining refugia for freshwater mussels in the Cumberland River system (Ahlstedt et al. 2005).

Species of conservation concern

Among the species found in the BSF are five federally endangered mussels: tan riffleshell (*Epioblasma florentina walkeri*), Cumberlandian combshell (*E. brevidens*), Cumberland bean (*Villosa trabalis*), Cumberland elktoe (*Alasmidonta atropurpurea*), and littlewing pearlymussel (*Pegias fabula*) (Ahlstedt et al. 2005). All of these endangered species also occur in portions of the Tennessee River system, except the Cumberland elktoe, which is a Cumberland River endemic. Populations of the five endangered mussels in the BSF represent some of the largest remaining in the Cumberland system

(Ahlstedt et al. 2005). No federally threatened species are known to occur in the BSF, but several species of special concern also have somewhat large populations in the BSF (Williams et al. 1993, Ahlstedt et al. 2005). These include pheasantshell (*Actinonaias pectorosa*), elktoe (*A. marginata*), slippershell mussel (*A. viridis*), plain pocketbook (*Lampsilis cardium*), black sandshell (*Ligumia recta*), Cumberland moccasinshell (*Medionidus conradicus*), and Tennessee clubshell (*Pleurobema oviforme*) (Williams et al. 1993).

BSF Mussel Recovery

The NRRA is one of the first NPS holdings to be actively involved in freshwater mussel recovery. Plans are in place to augment and reintroduce mussel populations in the NRRA, which involves relocating adults as well as propagating juveniles for release (Biggins et al. 2001). Broodstock for juvenile propagation will come from the BSF, but if no adults of target species can be found in the BSF, broodstock from either the Cumberland or Tennessee rivers will be used. Likewise, adult mussels may be relocated from relatively stable populations elsewhere in the Cumberland or Tennessee rivers (Biggins et al. 2001). To protect reproducing mussel populations, other management strategies are being taken such as designating areas for horse and trail crossings and providing information to the public on mussel recovery efforts.

The goal of my research is to aid in BSF mussel recovery efforts, with the specific objectives of 1) identifying suitable host fish for two of the endangered species in the NRRA, Cumberland elktoe (*Alasmidonta atropurpurea*) and Cumberland bean (*Villosa trabalis*), 2) determining suitable juvenile culture conditions for *Epioblasma brevidens* and *V. trabalis*, and 3) locating sites important to future mussel restoration efforts in the NRRA.

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Chapter 1: Determination of host fishes for *Alasmidonta atropurpurea* and *Villosa trabalis*.

Introduction

Freshwater mussels and their hosts

Freshwater mussels have a complex life history. Of the 297 species and subspecies that occur in North America (Williams et al. 1993), most have a larval development stage that requires parasitism on a host, usually a fish or rarely a salamander. The glochidia encyst on the host's gills or fins and use energy from the host to develop organs for the juvenile life stage. The parasitic transformation stage typically lasts from one to six weeks, depending on species and water temperature. After transformation, juvenile mussels excyst from the host and begin a free-living, benthic life stage where they grow to adults.

Mussels are selective in the species of fish that can serve as hosts (Coker et al. 1921, Zale and Neves 1982). Some mussels use only one or two fish species as hosts, while others appear to be more general in host selection, with successful transformation taking place on 10 or more host species (Parmalee and Bogan 1998). Although mussels may transform on several host fish, one or two may be most suitable, with a large number of successful transformations, whereas another species allows relatively few transformations (Bruenderman and Neves 1993, Watson 1999, Rogers et al. 2001, Jones and Neves 2002).

Suitable host species can be identified in the laboratory. Glochidia are directly exposed to fish to ensure that they come in contact with a host. With this direct exposure, more glochidia attach and transform into juveniles than would be expected in the wild (Neves and Widlak 1988), which thereby increases recruitment success. By exposing several potential host species to mussel glochidia and holding them in captivity, host species can be identified and compared to determine the most suitable hosts.

Two federally endangered mussel species for which optimal host(s) have yet to be identified are the Cumberland elktoe (*Alasmidonta atropurpurea*) and Cumberland bean (V*illosa trabalis*). Populations of *V. trabalis* and *A. atropurpurea* are localized, and restricted to relatively few streams and rivers in the upper Tennessee and Cumberland River systems. Both mussels are found in the Big South Fork (BSF) National River and Recreation Area (NRRA) in north-central Tennessee and south-central Kentucky, the study site for this project. To maintain and augment these populations, several agencies began a cooperative program to propagate juveniles for release (Biggins et al. 2001). Lists of host fish tested from previous studies are available (Layzer and Anderson 1991, 1992; Gordon and Layzer 1993; Mair et al. 2003), but none document comparisons of transformation success among species. The objective of this study was to determine the most suitable host species (i.e., the species allowing greatest transformation success in laboratory settings).

Description of *Alasmidonta atropurpurea*

The Cumberland elktoe, *Alasmidonta atropurpurea*, (subfamily Anodontinae), was first described by Rafinesque in 1831. It was federally listed as endangered in 1997, having undergone a significant reduction in total range and population density (USFWS 2004). This species, endemic to the upper Cumberland River drainage in northeastern Tennessee and southeastern Kentucky, has 12 currently known populations in this drainage (USFWS 2004). These populations typically occur in headwater streams, and are often the only mussel species present (Ahlstedt et al. 2005). The Cumberland elktoe is one of the few species found in the Cumberland River above Cumberland Falls.

Juveniles of *A. atropurpurea* typically have a yellowish-brown periostracum with dark green rays, while the adults are usually darker with less distinct rays. Parmalee and Bogan (1998) describe the adult shells as being subovate in shape, reaching a maximum length of 100 mm. The shells are thin, but not fragile, and the nacre color is usually shiny white, sometimes with bluish tints. The shell morphology most closely resembles that of the elktoe, *A. marginata*.

Information on stream habitat characteristics associated with *A. atropurpurea* is limited. The species has been found in the upper tributaries of the BSF that have slow current, an abundance of large cobbles, and a sand and mud substrate (Gordon and Layzer 1989). It typically occurs at depths of less than 1 m (Parmalee and Bogan 1998). S. Ahlstedt (personal communication, U.S.G.S.) and S. Bakaletz (personal communication, NPS) noted that this species inhabits the calm, flowing waters between pools and riffles, and that sand is an important component of the microhabitat in which it is found.

Little is known of the life history, longevity, and breeding cycle of *A. atropurpurea*. A single report by Gordon and Layzer (1993) lists results of a host fish study and observations on its period of gravidity. The glochidia were found encysted on 5 fish species: whitetail shiner (*Cyprinella galactura*), northern hogsucker (*Hypentelium nigricans*), rock bass (*Ambloplites rupestris*), longear sunfish (*Lepomis megalotis*), and rainbow darter (*Etheostoma caeruleum*) (Gordon and Layzer 1993). However, under laboratory conditions, *A. atropurpurea* glochidia transformed on only one of the 12 species tested, the northern hogsucker (Gordon and Layzer 1993). Females were found to be gravid from October to May, indicating that the species is a long-term brooder.

Description of *Villosa trabalis*

The Cumberland bean, *Villosa trabalis*, (subfamily Lampsilinae) was first described by T.A. Conrad in 1834, and was listed as federally endangered in 1976. Historically, *V. trabalis* was reported in several locations in both the upper Cumberland and upper Tennessee River systems. Its current distribution consists of 8 streams in Kentucky, Tennessee, and North Carolina, with no significant number of individuals at any site (USFWS 1984).

Shells of *V. trabalis* are solid and elongate with inflated, inequilateral, and irregularly oval valves. The ventral margin is evenly curved. Female shells reach a slightly larger size than males, attaining a maximum length of about 55 mm. The periostracum is olive green with numerous faint wavy green rays, whereas the nacre is a bluish white or white, with a bluish iridescence posteriorly (Parmalee and Bogan 1998).

Stream habitat features associated with *V. trabalis* seem to be somewhat specific. Gordon and Layzer (1989) reported that *V. trabalis* can be found in sand, gravel, and cobble substrates, in waters with moderate to swift currents and depths less than 1 m. Parmalee and Bogan (1998) further describe its typical habitat as small rivers and streams, in gravel or sand and gravel substrate with fast current in riffle areas.

Investigations into the life history of *V. trabalis* have shown that it is a bradytictic, or long-term brooder (Parmalee and Bogan 1998). Reported host fishes for *V. trabalis* are the rainbow darter (*Etheostoma caeruleum*), arrow darter (*E. sagitta*), barcheek darter (*E.*

obeyense), fantail darter (*E. flabellare*), Johnny darter (*E. nigrum*), snubnose darter (*E. simoterum atripinne*), sooty darter (*E. olivaceum*), striped darter (*E. virgatum*), and stripetail darter (*E. kennicotti*) (Layzer and Anderson 1991, 1992). Mair et al. (2003) reported successful juvenile transformation on black sculpin (*Cottus baileyi*), greenside darter (*E. blennioides*) and fantail darter (*E. flabellare*), and no transformation on rock bass (*Ambloplites rupestris*) or redbreast sunfish (*Lepomis auritus*). M. McGregor (personal communication, Ky. Dept. of Fish and Wildlife Resources) also reported that the striped darter (*E. virgatum*) was the most successful in transforming juveniles of *V. trabalis*. A comparison of the suitability of host species under identical conditions was needed.

Methods

Mussel collections

Gravid females of *A. atropurpurea* and *V. trabalis* were collected from reaches in the upper Cumberland drainage containing locally stable populations. These areas include Parchcorn Creek Shoal on the mainstem of the Big South Fork (BSF); North White Oak Creek, a tributary of the BSF in Tennessee; and Sinking Creek, a tributary of Rock Castle Gorge River in Kentucky. Five collection trips were made from spring 2004 through summer 2005. Upon collection, mussels were examined for gravidity by opening the shells wide enough to observe whether gill tissue was inflated. Gravid mussels were placed in containers with stream water and transported to the Aquatic Wildlife Conservation Center (AWCC) in Marion, Virginia. Females were held at AWCC until host fish trials began at Virginia Tech, Blacksburg, Virginia.

In April 2004, one partially gravid *V. trabalis* was collected from Parchcorn Creek Shoal. This individual was brought to AWCC for use in fish infestations; however glochidia were released prior to infestation. No infestations were made at that time. This individual was released back in Parchcorn Creek Shoal in fall 2004.

In November 2004, 4 gravid *A. atropurpurea* were collected from North White Oak Creek, Tennessee. Individuals were brought to AWCC and used to complete two host fish trials. Individuals were returned to the BSF in April 2005.

In February 2005, two gravid *V. trabalis* were collected from Sinking Creek, KY. Individuals were brought to AWCC and used to complete one host fish trial. Individuals were returned to Monte McGregor, Kentucky Department of Fish and Wildlife Resources, in March 2005.

Two additional collecting trips were made in April and May 2005, but we were unable to find gravid individuals of either *A. atropurpurea* or *V. trabalis*.

Fish species selection and collection

Of the 44 species of fish reported by O'Bara et al. (1982) to inhabit the BSF and its tributaries, few species were feasible to use in these trials. Most BSF species were difficult to collect due to long traveling distances and lack of knowledge of where to find them in sufficient abundances for host testing. Some fish species reported to occur in the Cumberland River system (Gordon and Layzer 1989) were tested as hosts, even though they were not documented in the BSF (O'Bara et al. 1982). Species used in these trials were selected because they had been cited previously as hosts, were closely related to previously identified host species (Neves et al. 1985), or were known hosts for a congener mussel, and were relatively abundant in nearby streams that allowed collection.

Potential host fish were collected from streams in Virginia or Kentucky that are known to contain few or no mussel species. This was done to avoid using fish which may have developed an acquired immunity to glochidia encystment from prior infestations (Arey 1923). Fish were collected with a Smith-Root battery-powered backpack electroshocker, dip nets, and/or a seine. Fish were transported in aerated coolers from collection sites to Virginia Tech's Freshwater Mollusk Conservation Center (FMCC). Fish were sorted to species and held in aquaria at FMCC and fed bloodworms for the duration of the experiments. Potential host fish tested for each species are listed in Tables 1.1 - 1.3.

Glochidial infestations

Two infestations of *A. atropurpurea* and one infestation of *V. trabalis* were completed from fall 2004 through spring 2005. Infestation methods generally followed those of Zale and Neves (1982), with some modifications. Gravid females were transported in a water-filled cooler from AWCC to FMCC. Mussels were opened slightly and held gaped while extracting glochidia. A sterile hypodermic needle was used to penetrate the gill marsupium, and water from the syringe was used to flush the glochidia from the gill. The glochidia and water were collected in a Petri dish. A sample of glochidia was tested for viability using a weak saline solution. If glochidia were fully developed and healthy, they snapped shut in this solution (Rogers et al. 2001). A sample of 20 glochidia was measured with an ocular micrometer. Glochidia and host fish were placed in a container with water agitated by air stones until fish were adequately infested, as they respired and swam in the container. Adult female mussels were returned to AWCC and held there until being returned to the natal stream.

After infestation, fish were placed in 38 L glass-bottomed aquaria by species. The aquaria were siphoned every other day until juveniles were recovered, and on a daily basis thereafter. Particles were filtered through 450 µm and 125 µm mesh sieves; the contents of the 125 µm sieve were examined with a dissecting microscope to locate transformed juveniles or sloughed glochidia. The number of juveniles or glochidia in the siphoned material, number of days after infestation, and number of fish in the aquarium were recorded. Shell length and width measurements of the transformed juveniles also were recorded. Juveniles were collected for use in later experiments.

Data analysis

Data analysis included calculating the number of juveniles produced per fish and the percent of encysted glochidia that transformed into juveniles for each fish species. Both provide an indication of juvenile transformation success. Fish were not held individually in aquaria due to space limitations, so one aquarium with multiple individuals of a species was treated as one sampling unit. Therefore, sample sizes indicate the number of aquaria for each species, not the number of individuals infested.

To calculate the number of juveniles transformed per fish in each aquarium, the total number of juvenile mussels per aquarium was recorded, then divided by the number of fish. Occasionally, fish died while juveniles were still transforming. A weighted average of the number of fish in the aquarium was then calculated and used to find the average number of juveniles per fish per aquarium. The weighted number of fish per aquarium was calculated by multiplying the number of fish alive by the proportion of days that number of fish was in the aquarium while juveniles were dropping off, and then summing those values. The percent of encysted glochidia that transformed into juveniles was calculated for each aquarium by dividing the number of juveniles collected from that aquarium by the total number of shells excysted from the fish in that aquarium.

Initial analyses revealed that data did not meet the assumptions of normality and homogeneity of variance for parametric tests. However, the data did satisfy the assumptions if log or square root transformations were performed. Using transformed data in normal theory analysis was preferable over non-parametric analysis of untransformed data because it was possible to obtain means and confidence intervals, which could not be done with non-parametric analysis (S. Aref, Virginia Tech Statistical Department, personal communication). Number of juveniles per fish was transformed using the square root of the data. The percent of glochidia successfully transformed to juveniles was transformed using the log of the data for *A. atropurpurea*, which best fit the data; the square root transformation was the best fit to transform percent data for *V. trabalis*. The data were back-transformed to provide means and confidence intervals. Number of juveniles per fish and percent of glochidia that transformed were analyzed using an analysis of variance (ANOVA) program and Tukey's Honestly Significant Difference for multiple comparisons. All statistics were computed using SAS statistical package software (SAS Institute 2002).

Results

Alasmidonta atropurpurea – Trial 1

Fish were infested on November 26, 2004, with glochidia from the gills of 2 females. Glochidia from these females had a mean length of 319 (±3.6) µm and a mean height of 351 (±3.3) µm. Of the 52 fish infested, 25 survived until the end of the trial.

Four fish species successfully transformed glochidia to juveniles (Table 1.1). These species included banded sculpin (*Cottus carolinae*), northern hogsucker (*Hypentelium nigricans*), redline darter (*Etheostoma rufilineatum*) and fantail darter (*E. flabellare*). Only banded sculpin and northern hogsuckers survived through the entire period of detachment. No encysted glochidia remained on these species at the end of the experiment. Redlines and fantails survived long enough for some juveniles to transform, but all eventually died with glochidia still encysted.

A total of 328 juveniles of *A. atropurpurea* were produced (Table 1.1). One aquarium per species was used in this trial, so only one datum per species is reported instead of a mean. Banded sculpins produced the highest number of juveniles per fish at 25.0, followed by northern hogsuckers at 8.4, redline darters at 5.0, and fantail darters at 4.8. A slightly higher percentage of attached glochidia transformed to juveniles on northern hogsuckers, 13.1%, than banded sculpins, 11.1%. Redline darters had a juvenile transformation rate of 0.6%, and fantail darters had a rate of 0.4%.

***Alasmidonta atropurpurea* – Trial 2**

Fish were infested on January 13, 2005, with glochidia from the gills of 2 females. Glochidia from these females had a mean length of 330 (±2.9) μm and a mean height of 338 (±3.4) μm. Of the 59 fish infested, 29 survived until transformed juveniles were collected, and 23 fish survived until the end of the trial. Mortality was again a problem since the one infested northern hogsucker and all redline darters died before any juveniles excysted. Several glochidia were found encysted on those species when they died. Banded sculpins and fantail darters were the species that survived long enough for juveniles to excyst (Table 1.2).

A total of 413 juveniles were produced in this trial (Table 1.2). In this trial, banded sculpin produced a mean of 44.2 juveniles per fish, while the mean number of juveniles per fantail darter was 5.4. The mean percent of shells that transformed into juveniles on banded sculpin was 8.3%, whereas the mean percent for fantail darters was 1.6%.

***Alasmidonta atropurpurea* – pooled**

The results from both trials were pooled. Since only one result was available for redline darters and northern hogsuckers, those species could not be statistically tested for differences in number of juveniles per fish and the percent of glochidia transformed to juveniles. The mean number of juveniles produced per banded sculpin (38.2) was higher than the mean number of juveniles produced per fantail darter (4.0) ($p<0.001$) (Figure 1.1). The mean percent of glochidia transforming into juveniles on banded sculpins (7.4%) was also higher than that on fantail darters (1.0%) ($p=0.011$) (Figure 1.2).

Villosa trabalis

Fish were infested with *V. trabalis* glochidia from 2 females on March 23, 2005. Of 119 fish infested, 101 survived until the end of the trial. All five fish species produced active, pedal-feeding juveniles (Table 1.3), for a total of 1,156 juveniles.

Fantail darters produced a mean of 25.5 juveniles per fish, followed by striped darters (14.6), banded sculpins (13.7), greenside darters (2.6), and redline darters (0.4). The number of juveniles produced by fantail darters was higher than striped darters ($p=0.072$) and banded sculpin ($p=0.052$), but at α of 0.05, those species grouped together. These three species produced a significantly greater number of juveniles per fish than redline darters and greenside darters ($p<0.01$) (Figure 1.3).

Fantail darters also had the highest mean percent of transformed juveniles (62.4%), followed by striped darters (38.8%), banded sculpins (30.1%), redline darters (4.7%) and greenside darters (2.9%) (Figure 1.4). Means of the percent of successfully transformed glochidia on fantail darters and striped darters were not different ($p=0.116$), but they were higher than the means for greenside darters and redline darters ($p<0.0001$) (Figure 1.4). Fantail darters had a higher mean percent transformed than banded sculpins ($p=0.016$). Banded sculpins had a higher mean percent than greenside darters and redline darters ($p<0.0005$).

Discussion

Alasmidonta atropurpurea

My results confirm the report of Gordon and Layzer (1993) that the northern hogsucker serves as a host for *A. atropurpurea*. Three additional species were identified as hosts, two of them (banded sculpin and fantail darter) confirmed in sequential trials. My results also identified the redline darter as a host in trial 1, but was not confirmed subsequently in trial 2. Mortality in captivity is not usually a problem with this species, so it seems that they were most likely over-infested and died due to secondary infections. Transformation rates were not published by Gordon and Layzer (1993), so no comparisons of host suitability of northern hogsuckers could be made.

The banded sculpin seemed to be the best host overall, even though statistical comparisons could only be made between two of the four host species. Banded sculpin produced significantly more juveniles per fish than the fantail darter, as well as having a higher percent of successful transformations. Interestingly, the banded sculpin had been tested by Gordon and Layzer (1993) and was not shown to be a successful host. Circumstances of that trial, such as number of fish surviving and actual number of glochidia attaching to each fish, were not available and could explain the negative result of their host trial.

Another hypothesis is that the conflicting results are due to immunological differences of fish and mussels collected in different drainages (Rogers et al. 2001). Fish were collected from the Roaring River basin in Tennessee in Gordon and Layzer (1993), where as I collected banded sculpins from Sinking Creek, Giles County, and from the North Fork Roanoke River, Montgomery County, Virginia. Based on comparisons of fantail darters collected from several drainages as hosts for the tan riffleshell (*Epioblasma florentina walkeri*), Rogers et al. (2001) hypothesized that fish are exposed to different pathogens in different drainages, and would have varying immunological responses to infestations of glochidia. Perhaps the immune systems of fishes in the source drainages for this study were more conducive for *A. atropurpurea* transformations than the fishes collected by Gordon and Layzer (1993). If there is evidence for inter-drainage immunological differences in fish that affect their suitabilities as hosts, the results of several host trials may need to be re-examined.

The two data points of transformation on northern hogsuckers seem to indicate that it may have similar host suitability as banded sculpins. Northern hogsuckers did not produce as many juveniles per fish as banded sculpins, but did have a higher percent of successful transformations, although not statistically different. However, two factors may have affected northern hogsucker results. Hove (1990) noted that during his host fish trials, sucker species tended to consume anything lying on the bottom of the substrate-free aquarium. When examining the material from the bottom of the hogsucker tank, I also noticed that there was less debris that would contain juveniles. When searching for food, it is possible that hogsuckers consumed juveniles and even empty, excysted shells. If that is the case, their host suitability may have been underestimated. Also, flatworms were present in the northern hogsucker aquarium, which were not present in aquaria of the other species. Flatworms are known predators of juvenile mussels (Zimmerman et al. 2003), and their presence would further underestimate the hogsuckers' suitability as hosts. Future tests with northern hogsuckers, which have been treated for flatworms and designed to eliminate host fish consumption, may indicate that the ability of the northern hogsucker to serve as a host for *A. atropurpurea* is comparable or perhaps better than that of the banded sculpin.

Of the species tested as hosts, the northern hogsucker was the most difficult to collect and maintain in captivity. Four attempts were made to collect northern hogsuckers of sizes that could be held in the aquaria available at the FMCC when *A. atropurpurea* was gravid. Northern hogsuckers <20 cm in length were difficult to find in winter and typically did not survive in aquaria for more than a few days, while fish larger than 20 cm did not fit in available aquaria. In the first trial when all northern hogsuckers survived, fish were being held in one large 208 L aquarium. Since that aquarium was not available for use in the next trial, hogsuckers were held in the smaller 38 L aquaria used for all other infested species. Of those held in the smaller aquaria, one remained alive long enough to infest, but died a few days later.

Collection and maintenance of the banded sculpin was easier than that of the northern hogsucker, and its host suitability was shown to be greater than that of the fantail darter. Therefore, the banded sculpin was the preferred host for captive propagation purposes of the fish species tested. It is unknown whether another species of darter or sucker that inhabits the BSF and its tributaries, but not tested here, may prove to be even more useful. However, if that is the case, the banded sculpin may still be preferred for propagation at FMCC because they can be collected and infested in high enough numbers to compensate for lower transformation rates.

Villosa trabalis

This study confirmed observations reported by Mair et al. (2003), McGregor (personal communication), and Layzer and Anderson (1991, 1992) that banded sculpins, striped darters, fantail darters, redline darters and greenside darters are successful hosts for *V. trabalis*. In this trial, fantail darters had the highest mean number of juveniles produced per fish, and the highest mean percent transformations. However, means of fantails darters were not significantly higher than means of striped darters, although further testing is required. Fantail darters did have a higher mean percent transformation than banded sculpins, but not a higher mean number of juveniles produced. All three species (banded sculpin, fantail darter, and striped darter) had a significantly higher mean percentage of successful transformations and mean number of juveniles per fish than redline and greenside darters.

These results corroborate those of Mair et al. (2003), who report similar numbers of juveniles per fish produced by fantail darters (18 and 43 juveniles/fish), greenside darters (3 juveniles/fish), and sculpins (15, 15, and 19 juveniles/fish) in infestations made in 2001 and 2002. My results are also similar to those of M. McGregor (Kentucky Dept. of Fish and Wildlife Resources, personal communication) who found that striped darters produced more juveniles per fish than every other species tested, when infested with *V. trabalis*.

For propagation of *V. trabalis* at FMCC, fantail darters are the preferred hosts. They produce high numbers of juveniles, have a high transformation success, are relatively abundant in nearby streams, and are easy to maintain in captivity. Striped darters produce similar numbers of juveniles per fish and percentage transformation success, but do not occur in any streams near FMCC and are more aggressive than fantail darters. Only one male and one female striped darter could be held in an aquarium, whereas fantail darters can be held at higher densities (up to 10 individuals per tank). By using fantail darters instead of striped darters, more juveniles can be produced using a smaller number of aquaria. Banded sculpins, although not as suitable as fantail darters, are another option to use as hosts since they can be collected easily and held in high densities.

Implications for natural recruitment

Although one or two host species may be preferred for captive propagation of a mussel species, it is important to assess all successful host species when considering mussel recruitment in the wild. The species that I found to be the preferred hosts for the two mussel species studied (banded sculpins and fantail darters) are not reported to occur in the BSF (O'Bara et al. 1982). Therefore, wild recruitment seems to depend on other host species versus those listed here as having high transformation success.

For *A. atropurpurea*, it seems that high numbers of marginal hosts may supplement recruitment that primarily takes place on one wild host species. The northern hogsucker, being a relatively abundant catostomid in the BSF system, may be the preferred host fish in this river. Gordon and Layzer (1993) showed that glochidial attachment takes place on this fish in the wild, and that it was the main species on which *A. atropurpurea* glochidia were consistently found. Glochidial transformation may also take place on darter species, although in lower numbers. Some of the more common darter species encountered by O'Bara et al. (1982) in the BSF include the rainbow darter (*E. caeruleum*), bluebreast darter (*E. camurum*), barcheek darter (*E. obeyense*), logperch (*Percina caprodes*) and blackside darter (*P. maculata*). These darters may have similar host suitability as fantail and redline darters and, combined with their abundance, may serve as hosts for *A. atropurpurea* recruitment in the river.

Villosa trabalis may use a similar strategy in its recruitment. Striped darters seem to be the preferred wild host fish. In streams with relatively large populations of *V. trabalis* in Kentucky, there are also large populations of striped darters (M. McGregor, Kentucky Dept. of Fish and Wildlife Resources, personal communication). Several darters are reported as being "marginal" hosts (J. Layzer, Tennessee Tech University, personal communication), but given their abundances in streams with *V. trabalis*, these darters may contribute to some recruitment of juveniles.

Recommendations for additional host fish testing

Only a small number of possible host species were tested in these trials, and there are several species that warrant consideration as hosts, especially for *A. atropurpurea*. There is evidence that related or congeneric fish species can serve as hosts for the same mussel species (Neves et al. 1985). Therefore, other sucker, darter, and sculpin species, especially those known to occur in the BSF and its tributaries, could serve as hosts for *A.*

atropurpurea. Likewise, other darter or sculpin species may serve as hosts for *V. trabalis*, in addition to the 10 known host species listed above.

Additional hosts for *A. atropurpurea* may be found by testing fishes of congeneric species, although not all mussel species in the same genus share similar hosts (Neves et al. 1985, Parmalee and Bogan 1998). Hosts of the elktoe (*A. marginata*), such as the white sucker (*Catostomus commersoni*), shorthead redhorse (*Moxostoma macrolepidotum*), and warmouth (*Lepomis gulosus*) (Howard and Anson 1922) could be tested with *A. atropurpurea*. Based on my results with the banded sculpin, it also may be worthwhile to retest the centrarchids and cyprinids that Gordon and Layzer (1993) reported as unsuitable hosts for *A. atropurpurea*.

Further host investigations could be made by comparing the suitability of hosts from different source drainages. In this study and in other host identification studies, it was assumed that host ability of a fish species does not vary greatly within species or among drainages. However, Rogers et al. (2001) report a slight decrease in host suitability using fish and mussels from source drainages at greater distances compared to fish and mussels from closer drainages. Possible inconsistencies in host suitability within species have not been fully researched, although assumptions are made that it is somewhat consistent. This is an area that deserves further evaluation.

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Table 1.1: Results of fish host identification tests using glochidia of *Alasmidonta atropurpurea* (trial 1). Results are based on the number of aquaria used, not number of fish infested. One aquarium was used per species in this trial; therefore, reported values are a single datum instead of mean values. Numbers of juveniles and fish are rounded to nearest whole number.

Fish species (no. of aquaria)	No. fish	No. alive	No. Days	No. glochidia encysted (total)[3]	No. Juveniles	No. juveniles per fish	% transformation success	Days to detachment (peak)	Mean temp. (°C) (range)
Cottidae									
Cottus carolinae (1)[1]	15	11	40	2,473	275	25	11.12	16(20-21)	17.2 (16-18.5)
Percidae									
Etheostoma blennioides (1) [2]	2	2	40	528	0	0	0	-	17.2(16-18.5)
E. flabellare (1) [1]	15	1	19	1,539	6	5	0.39	16(19)	17.2(16-18.5)
E. rufilineatum (1)[1]	8	1	18	890	5	5	0.56	16(16-18)	17.2(16-18.5)
Catostomidae									
Hypentelium nigricans (1) [2]	5	5	40	320	42	8	13.13	16(16-20)	18.4 (15-19)
Cyprinidae									
Luxilus albeolus (1)[1]	4	3	40	1281	0	0	0	-	17.2(16-18.5)
Cyprinella galactura (1)[2]	3	1	40	332	0	0	0	-	17.2(16-18.5)
Total juveniles					328				

Comments:

1. Banded sculpins, fantail darters, redline darters, and white shiners were infested together for 40 min with glochidia from 3 gills on November 26, 2004.
2. Greenside darters, northern hogsuckers, and whitetail shiners were infested together for 40 min with glochidia from 3 gills on November 26, 2004.
3. Total number of glochidia sloughed off before maturity, glochidia remaining encysted on dead fish, and juveniles that excysted.

Table 1.2: Results of fish host identification tests using glochidia of *Alasmidonta atropurpurea* (trial 2). Results are based on the number of aquaria used, not number of fish infested. Numbers of juveniles and fish are rounded to nearest whole number.

Fish species (no. of aquaria)	No. fish	No. alive	No. Days	No. glochidia encysted[2]	No. Juveniles	Mean juveniles per fish (95% CI)	Mean % transformation success (95% CI)	Days to detachment (peak)	Mean temp. (°C) (range)
Cottidae									
Cottus carolinae (3) [1]	18	6	40	3,945	301	44.2 (27.1-62.1)	8.3 (1.1-15.5)	12(16-17)	17.9 (16-18.5)
Percidae									
Etheostoma flabellare (5) [1]	34	19	40	7,198	112	5.4 (1.1-9.6)	1.6 (0.4-2.7)	12(14)	17.9 (16-18.5)
E. rufilineatum (2) [1]	6	0	10	442	0	0	-	-	17.7 (16-18.5)
Catostomidae									
Hypentelium nigricans (1) [1]	1	0	7	198	0	0	-	-	17.1 (16.5-17.5)
Total juveniles					413				

Comments:

1. All species were infested together for 30 min with glochidia from 3 gills from 2 adults on January 13, 2005.
2. Total number of glochidia sloughed off before maturity, glochidia remaining encysted on dead fish and juveniles that excysted.

Table 1.3: Results of fish host identification tests using glochidia of *Villosa trabalis* (trial 1). Results are based on the number of aquaria used, not number of fish infested. Numbers of juveniles and fish are rounded to nearest whole number.

Fish species (no. of aquaria)	No. fish	No. alive	No. Days	No. glochidia encysted[2]	No. Juveniles	Mean juveniles per fish (95% CI)	Mean % transformation success (95% CI)	Days to detachment (peak)	Mean temp. (°C) (range)
Cottidae									
Cottus carolinae (5)[1]	31	28	40	1,261	397	13.7 (9.4-18.7)	30.1 (20.7-41.4)	10(21)	18.8 (16.5-20)
Percidae									
Etheostoma flabellare (4)[1]	21	18	40	732	484	25.5 (18.9-33.0)	62.4 (46.9-80.2)	12(24)	18.8(16.5-20)
E. rufilineatum (5)[1]	32	25	40	157	11	0.4 (0-1.6)	4.7 (1.5-9.6)	20(25)	18.8(16.5-20)
E. virgatum (6)[1]	23	16	40	585	235	14.6 (10.6-19.3)	38.8 (28.8-50.3)	18(24)	18.8(16.5-20)
E. blennioides (3)[1]	12	12	40	410	29	2.6 (0.6-5.8)	2.9 (0.2-8.4)	12(18-21)	18.8(16.5-20)
Total juveniles					1156				

Comments:

1. All species were infested together for 30 min with glochidia from 4 gills from 2 adults on March 23, 2005.
2. Total number of glochidia sloughed off before maturity, glochidia remaining encysted on dead fish and juveniles that excysted.

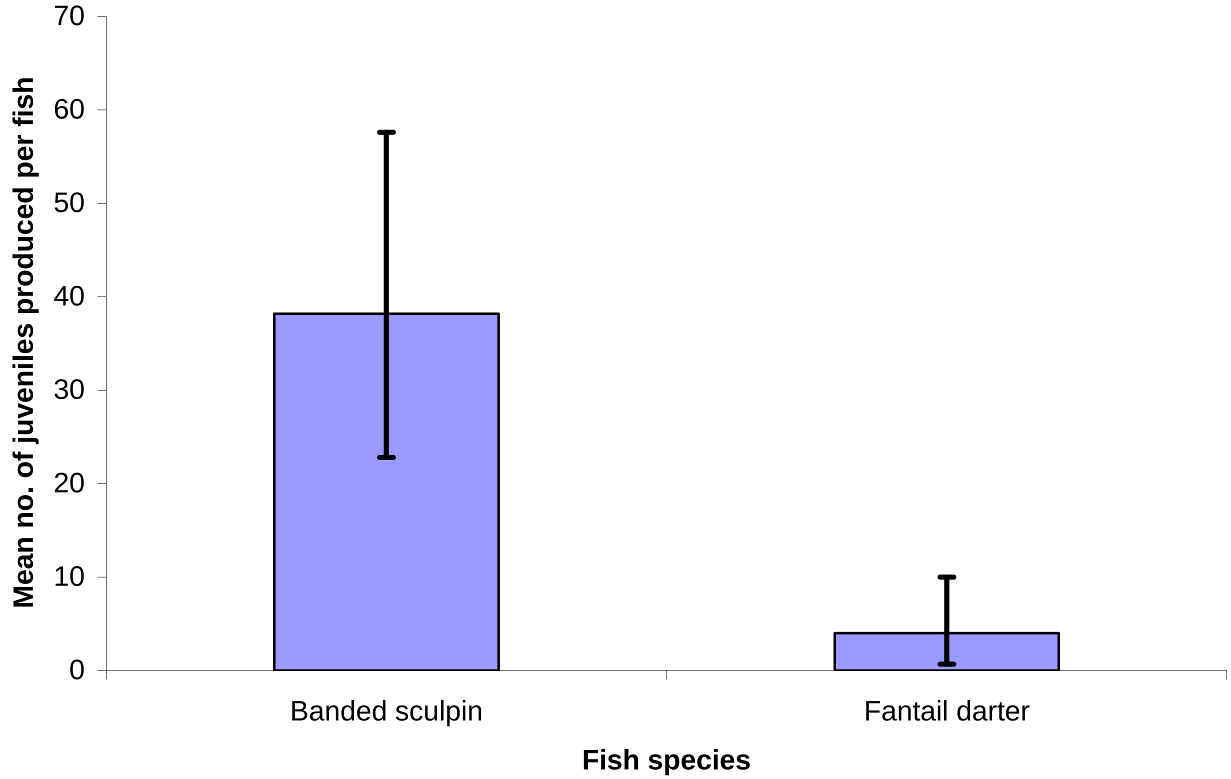

Figure 1.1: Comparison of mean number of juveniles per fish produced from banded sculpins (n=4) and fantail darters (n=6) infested with *Alasmidonta atropurpurea* glochidia. Data from trials 1 and 2 were pooled, error bars indicate 95% confidence intervals. Means are significantly different ($p<0.001$).

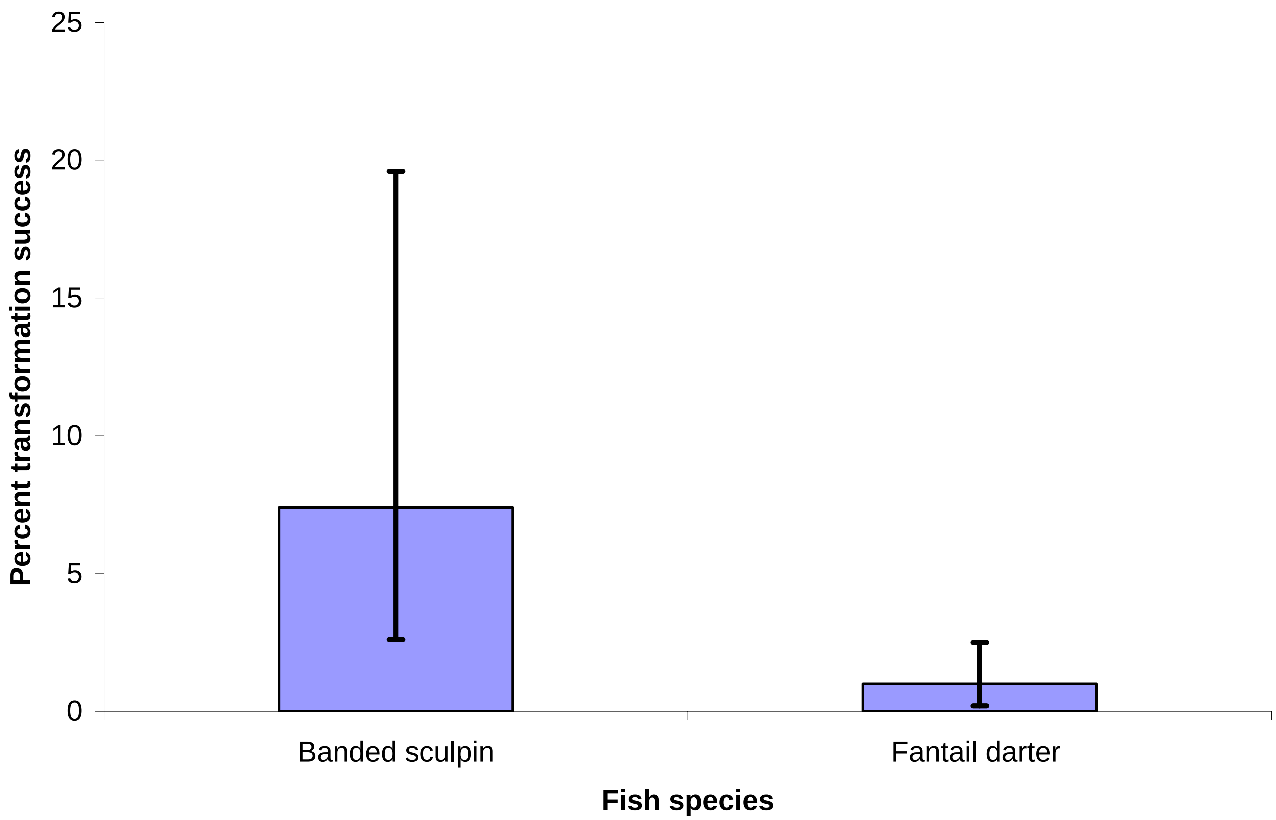

Figure 1.2: Comparison of mean percent of *Alasmidonta atropurpurea* glochidia transforming on banded sculpins (n=4) and fantail darters (n=6). Data from trials 1 and 2 were pooled, error bars indicate 95% confidence intervals. Means are significantly different ($p=0.011$).

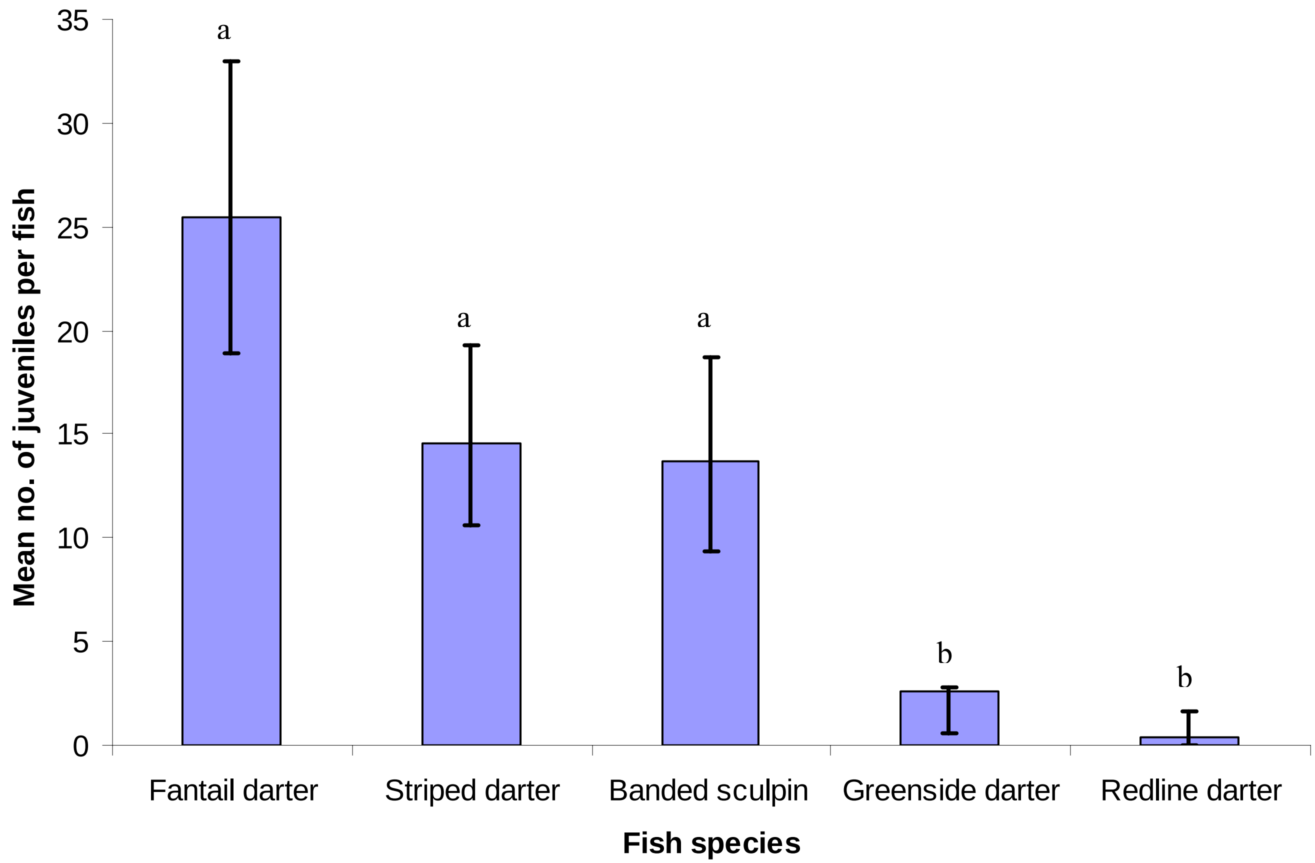

Figure 1.3: Comparison of mean number of juveniles per fish produced from fantail darters (n=4), striped darters (n=6), banded sculpins (n=5), greenside darters (n=3), and redline darters (n=5) infested with *Villosa trabalis* glochidia (error bars indicate 95% confidence intervals). Means of species labeled with same letter are not significantly different at α=0.05, using Tukey's HSD for multiple comparisons.

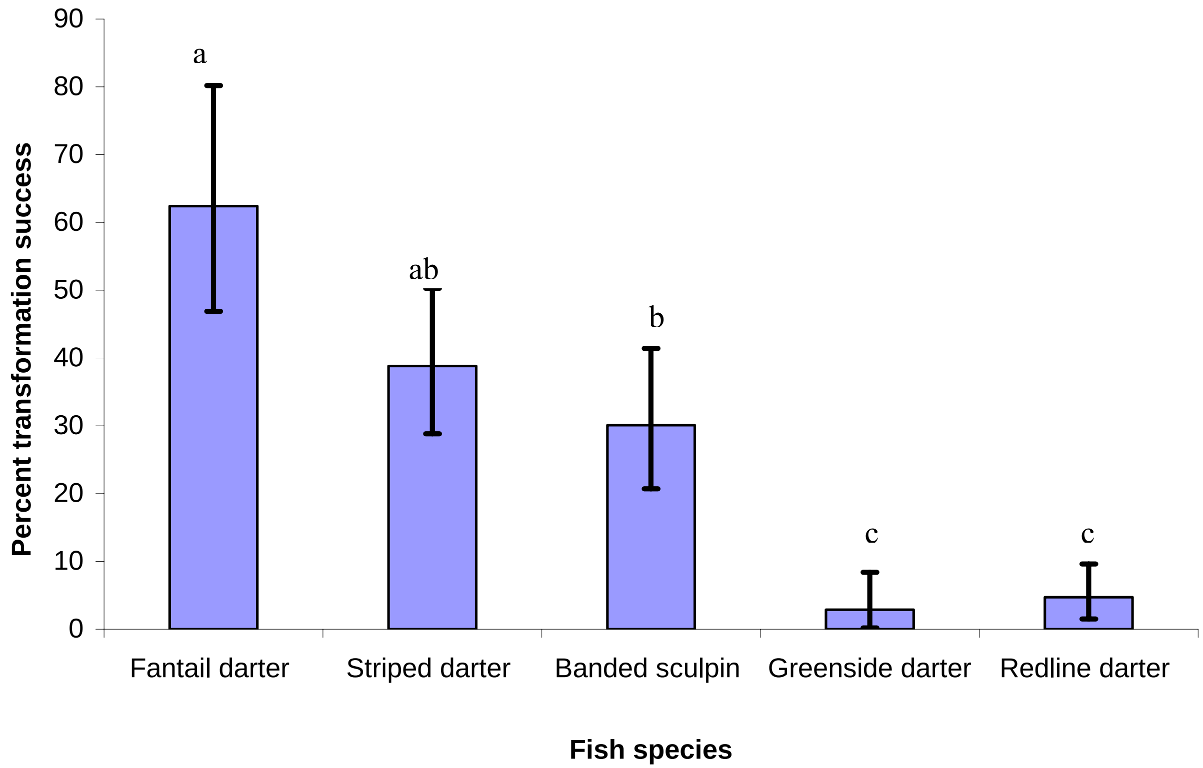

Figure 1.4: Comparison of mean percent of *Villosa trabalis* juveniles attached to and transforming on fantail darters (n=4), striped darters (n=6), banded sculpins (n=5), greenside darters (n=3), and redline darters (n=5) (error bars indicate 95% confidence intervals). Means of species labeled with same letter are not significantly different at α=0.05, using Tukey's HSD for multiple comparisons.

Chapter 2: Determination of suitable substrate for culturing juvenile freshwater mussels.

Introduction

Efforts to raise juvenile mussels in laboratory settings have been documented since the early 1900s (Lefevre and Curtis 1912, Coker et al. 1921, Howard 1922), but early experiments had limited success. Juveniles of some species were successfully cultured in cages held in rivers or ponds, or in troughs fed with river water. However, these methods worked for culture of only a few species (Coker et al. 1921, Howard 1922). Limited testing was done on laboratory mussel culture techniques between the 1920s and the passage of the Endangered Species Act (ESA) in 1973 (Jenkinson and Todd 1997). The recent effort to successfully raise juveniles in a laboratory setting was reported by Hudson and Isom (1984). Today, studies of juvenile culture typically result in much higher survival rates compared to early studies (O'Beirn et al. 1998, Hanlon 2000, Beaty and Neves 2004, Liberty 2004).

Substrate type can affect survival and growth of captive juvenile mussels (Jones et al. 2005), since juveniles use it as both a source of nutrition and as physical habitat (Yeager et al. 1994, Gatenby et al. 1997). In the first few weeks post-metamorphosis, juveniles have not yet developed the ability to suspension feed, so nutrition is obtained by collecting material on the surface of adjacent sediment particles via the ciliated foot (Yeager et al. 1994, Gatenby et al. 1997). Yeager et al. (1994) reported that food material at this time may include algae, bacteria, diatoms, detritus, and inorganic colloidal particles. Substrates that either directly provide food or that allow juveniles to easily obtain food are necessary for long-term culture of juveniles (Gatenby et al. 1997).

Efforts to identify suitable juvenile substrate in the wild have had little success. Juvenile mussels are difficult to find, and few habitat associations have been made with this life stage (Coker et al. 1921, Neves and Wildlak 1987). Laboratory experiments are typically performed to identify appropriate substrate composition for culture because of the difficulties of determining substrate preference in the wild (Coker et al. 1921). Optimal substrates are those which result in highest survival and growth of juveniles.

Determination of suitable substrate for laboratory culture of juveniles is especially important for federally endangered mussels. Endangered juveniles may be propagated during seasons that are not favorable for juvenile release (Hanlon 2000). By maintaining juveniles in captivity, release locations and dates can be controlled (Jones et al. 2005). Also, there is evidence that juveniles released at larger sizes may have higher survival than juveniles released at smaller sizes (Hanlon 2000, Jones et al. 2005). However, there is no consensus as to what substrate is optimal for juvenile mussels. At the Freshwater Mollusk Conservation Center (FMCC) at Virginia Tech, juveniles of the rainbow mussel (*Villosa iris*) and oyster mussel (*Epioblasma capsaeformis*) grew larger and had higher survival in fine sediment (<200 μm) than in sand (200–300 μm) (Jones et al. 2005). However, Rogers (1999), also performing juvenile rainbow mussel culture trials at FMCC, reported higher growth and survival in mixed substrates (>1400 μm) and in sand (500-800 μm) rather than in fine sediment (<120 μm). Beaty and Neves (2004) showed no difference in survival and growth of rainbow mussel juveniles reared in substrate <120 μm and 120-600 μm. Juvenile culture trials at the Aquatic Wildlife Conservation Center

(AWCC) in Marion, Virginia, have used sand (1000-2500 μm) with good results for the rainbow mussel and the wavyrayed lampmussel (*Lampsilis fasciola*) (Hanlon 2000, Zimmerman 2003, Liberty 2004). Rearing juveniles in some type of substrate does result in higher growth and survival than rearing them with no substrate (O'Beirn et al. 1998).

Few juvenile culture experiments have been conducted with endangered mussel species (Zimmerman 2003, Liberty 2004, Jones et al. 2005). Juveniles from common species (i.e., rainbow mussel and wavyrayed lampmussel) typically are used since juveniles of those species are easier to culture (Gatenby et al. 1997). However, using species that seem to be more tolerant of environmental conditions may not be ideal to determine needs of endangered species that may be less tolerant. Suitable substrate for growth and survival also may differ by species, genera, or subfamily.

Identifying a suitable substrate for culture of the two endangered species propagated in Chapter 1, Cumberland elktoe (*Alasmidonta atropurpurea*) and Cumberland bean (*Villosa trabalis*) was the objective of this study. However, problems arose in obtaining juveniles of *A. atropurpurea* and *V. trabalis*, so other mussel species were included in substrate evaluations; Cumberlandian combshell (*Epioblasma brevidens*) and fluted shell (*Lasmigona costata*). The Cumberlandian combshell is a federally endangered species found in the Big South Fork (BSF) River (Parmalee and Bogan 1998) and successfully propagated at the FMCC with some consistency (Mair et al. 2003). Few experiments have been conducted on the substrate preferences of juveniles of this species. The fluted shell is a common species found in the BSF and is in the same subfamily (Anodontinae) as *A. atropurpurea*. These anodontine species have similar juvenile morphology (Parmalee and Bogan 1998), and adults are found in similar substrates in the wild (Bakaletz 1991), so it is hypothesized that they may have similar juvenile culture preferences. Two substrate trials were conducted with *E. brevidens*, and one trial was conducted with *V. trabalis*. No formal trials were run with *A. atropurpurea*, or *L. costata*, but information on their culture is reported.

The methods used in these trials allowed for an additional comparison of two recirculating system designs. Several recirculating systems have been used for juvenile culture, ranging from modified livestock feed troughs, glass aquaria, and small individual containers with varying degrees of success (Gatenby et al. 1996, O'Beirn et al. 1998, Jones and Neves 2002). The basic system design incorporates containment of juveniles and substrate with constant recirculating water flow. As a secondary objective, two designs (modified livestock feed trough and individual recirculating dishes) were evaluated for success in juvenile culture. Trough systems have had good success in previous studies (O'Beirn et al. 1998, Jones et al. 2005), but replications of troughs require large amounts of space (O'Beirn et al. 1998). Systems with small individual containers are easy to sample and allow replication of substrate treatments (Liberty 2004). However, it also has been reported that juveniles confined in small containers may not grow as well (Hanlon 2000).

Methods

Trial 1

Substrate treatments

A total of 720 four-week old juveniles of *E. brevidens* produced in summer 2004 were used in this trial.

Two substrate sizes were evaluated, fine sediment (<200 μm) and sand (500-1000 μm). Both were supplemented with an equal weight of particulate organic material (POM), which may act directly as a food source for juveniles or indirectly as a food source for bacteria that juveniles consume. Therefore, the presence of POM may affect growth and survival. Most POM is sieved out when obtaining sand-only substrate sizes, but may remain in fine sediment substrate sizes (Jones et al. 2005). Particulate organic matter was added to both treatments to ensure results would not be biased by potential food sources present in one treatment and not in the other.

Sand substrate used in all trials was collected in June 2004 from Sinking Creek, a trout stream in Giles County, Virginia. Fine sediment used in all trials was collected in June 2004 from a diverse mussel assemblage location in the Clinch River, Tazewell County, Virginia. These sites were the most accessible to obtain the substrate sizes needed. The substrates were sorted using mesh sieves corresponding to the above size categories and boiled for 5 min to remove potential parasites (Zimmerman et al. 2003). After heat treatment, fine sediment was maintained under aeration to oxidize organics and reduce organic acids that result from decaying organic matter (Jones et al. 2005). POM was made by grinding dried maple leaves collected on the Virginia Tech campus in a food processor and sorting to sizes less than 1000 μm.

Recirculating system design

The design of the recirculating system was modeled on systems at the AWCC that produce good juvenile survival and growth rates (Liberty 2004). The individual containers used in this trial were round, clear plastic food storage bowls (9 cm high x 13 cm wide) with flat bottoms from Aero Housewares (Leominster, MA 01453) (Figure

2.1). Holes were cut in the bottom of the containers, and a 1.2 cm diameter PVC standpipe 3.5 cm tall was inserted and secured in the hole with a 1.2 cm diameter PVC male converter.

Approximately 25 ml of substrate was added to each container along with 1 g of POM, for a substrate depth of 1mm. With the standpipe inserted, the dishes held approximately 1 L of water each. Six dishes were placed over a 60x35x30 cm Rubbermaid clear plastic container acting as a reservoir, holding approximately 50 L of water (Figure 2.1). To recirculate water, three Mini-Jet multi-use adjustable pumps (MN404, Aquarium Systems, Mentor, OH 44060) were placed in the bottom of each reservoir. Plastic airline tubing extended from the pumps into the dishes through a hole in the side. Water exited the dishes through the standpipe, falling into the reservoir below. Flow was controlled by adjusting the intake valve on the pump and was set at 1L/min. The plastic tubing was secured to the side of the dish with a zip tie and was positioned to circulate the water in a counter-clockwise motion to provide current. Water in the reservoirs was aerated to maintain oxygen levels.

Three dishes in each reservoir contained sand/POM substrate while the other three had fine sediment/POM substrate (Figure 2.1). Three reservoirs were used, with a total of 9 replicates for each substrate treatment. Forty juveniles were placed in each of the 18 dishes. These juveniles were sampled after 14 d (juvenile age at this time was 42 d), but were not sampled again until 81 d (juvenile age at this time was 109 d). Water recirculating in the reservoirs was the standard FMCC 50:50 mixture of dechlorinated tap water and well water (Henley et al. 2001). When needed, the reservoirs were refilled with water to replace any lost by evaporation.

Trial 2

Substrate treatments

One thousand and fifty newly metamorphosed juveniles of *V. trabalis* produced in spring 2005 were used in this trial.

The presence of POM in dishes of trial 1 made sampling juveniles difficult and its use was discontinued. The concern for a deficiency in food availability in sand-only substrates was resolved by varying the levels of fine sediment in each treatment and continuing to supplement the juvenile diet with cultured algae. Both trials 2 and 3 used three treatments: (1) fine sediment only (fine), (2) a 50:50 mixture (by volume) of sand and fine sediment (mixed), and (3) a 90:10 mixture of sand and fine sediment (coarse). Substrate sizes and collection sites were the same as in trial 1.

Recirculating system design

Two types of recirculating systems were used in trial 2. The recirculating dishes from trial 1 were used with a few modifications, and a recirculating raceway trough also was used, similar to raceways used by O'Beirn et al. (1998) and Jones et al. (2005) (Figures 2.2 and 2.3). The trough was 140 cm long, 50 cm wide, and 30 cm high and had a 8 cm diameter PVC standpipe 18 cm high, holding 120 L water. Water drained through the standpipe and into a 70 L reservoir. A 1.65 amp magnetic pump (Little Giant Pump, Co., Oklahoma City, OK 73112) recirculated water from the reservoir to the trough and could be controlled using ball valves on the 2 cm diameter PVC pipe that connected the reservoir and trough. Flow was adjusted to a rate of 10 L/min. Three containers made of fine mesh (<105 μm), glued to a 6 cm high piece of 23 cm diameter PVC, were placed in the bottom of the trough. Fifty milliliters of each substrate treatment was placed in the containers for a substrate depth of about 1.5 mm.

Modifications to the dishes used in trial 1 were as follows: 5.5 cm standpipes were inserted, increasing the volume of water in the dishes to 1.5 L. Instead of three 50 L reservoirs, one large 200 L reservoir container was used (60x100x50 cm), and 15 dishes were placed above it. Mini-Jet pumps and plastic airline tubing were used to return water to the dishes. Water in the reservoirs was aerated to maintain oxygen levels.

Seventy five juveniles were placed in each of 12 dishes. Fifty juveniles were placed in each of the containers in the trough. Water used in the reservoir and trough was the standard FMCC 50:50 mixture of de-chlorinated tap water and well water. When needed, the reservoirs were refilled with similar water to replace any lost by evaporation.

Trial 3

Substrate treatments

One thousand and two hundred newly metamorphosed juveniles of *E. brevidens* produced in summer 2005 were used in this trial. Substrate treatments were the same as in trial 2, namely; fine sediment only (fine), 50:50 mixture of sand and fine sediment (mixed), and 90:10 mixture of sand and fine sediment (coarse).

Recirculating system design

Two types of recirculating systems were used in this trial, the trough system and a modified version of the dish system used in trial 2 (Figures 2.2 and 2.4). The dish system in this trial is similar to the juvenile system described by Henley et al. (2001). The dishes were round, white plastic Rubbermaid containers, 19 cm high with a diameter of 21cm (Figure 2.4). A 9 cm tall standpipe was secured in each dish allowing water to drain to the 70 L reservoir through 2.5 cm diameter PVC pipes. A 1.65 amp magnetic pump recirculated water through 2.5 cm diameter PVC pipes to the dishes. Flow to each dish was regulated by ball valves attached to the PVC piping. The dishes held 3 L of water, and flow was regulated to approximately 0.5 L/min. One hundred and fifty milliliters of substrate was put in each dish for a depth of 4 mm. One hundred juveniles were placed in each dish and each trough container. Water used in the reservoir and trough was predominantly the 50:50 mixed tap and well water. Some unmixed well water was added to the system when treated water was unavailable in order to replace water lost by evaporation.

Culture of *Alasmidonta atropurpurea* and *Lasmigona costata*

Juveniles of *A. atropurpurea* were produced in fall and winter of 2004-2005 as described in Chapter 1; number of juveniles produced in each trial was 328 and 413. Replicated grow-out trials were not performed with so few juveniles, but juveniles were cultured to evaluate possible substrates for use in the future. The first batch of juveniles was cultured in the main juvenile grow-out system at the FMCC. This system consists of four 38 L glass aquaria that drain into a common 200 L reservoir. A magnetic pump recirculated water through PVC pipes and could be adjusted with a ball valve. Water entered the aquaria at approximately 1 L/min. Two millimeters of fine sediment (<200 μm) was used as substrate. Juveniles were placed in one aquarium in Dec 2004 and were sampled once in Jan 2005.

The second batch of *A. atropurpurea* was raised in a container in the trough system (Figure 2.2), with a 50:50 mixture of sand and fine sediment approximately 2 mm deep. These juveniles were sampled every 14 d for 84 d, from January to April 2005.

Attempts were made to culture juveniles of *L. costata* as a surrogate for *A. atropurpurea* in spring 2005. However, only 100 juveniles were produced from a single infestation in spring 2005, and no gravid females of either species could be found to produce juveniles for a culture experiment at that time. The juveniles of *L. costata* were grown out in separate dishes in the dish system used for juveniles of *V. trabalis* in trial 2 (Figure 2.3). Approximately 33 juveniles were placed in each of 3 dishes containing one of the three substrate treatments (fine, mixed, or coarse). Juveniles were sampled every 14 d for 28 d, in May and June 2005.

Supplemental algae

In addition to detrital and bacterial food sources, juveniles were fed cultured algae to ensure that nutritional requirements were met. Wild algae from the FMCC pond was screened through a 35 μm-mesh sieve, and indoor alga monocultures were fed to juveniles in trial 1. Only mono-cultured algae were fed in the second and third trials and to juveniles of *L. costata* and *A. atropurpurea*. The dominant algal species fed was either *Nannochloropsis oculata* or *Neochloris oleoabundans*. Both are species of green algae that contain polyunsaturated fatty acids that are an important component of juvenile diets (Gatenby et al. 1997), and are appropriately sized for juvenile digestion (3-10 μm in size) (Beck and Neves 2003). Algae were circulated in the grow-out systems at a density of approximately 30,000 cells/ml (Jones et al. 2005).

Sampling

In all trials, the initial number of juveniles placed in each container and initial shell length measurements for a sub-set of 10 juveniles per container were recorded (Hanlon 2000, Zimmerman 2003). Every 14 d thereafter, juveniles were removed from the containers and measurements for length (representative of growth), and counts of surviving juveniles were made by separating substrate and juveniles with appropriately sized sieves. The juveniles were washed from the sieves into a Petri dish with enough water to keep them submerged. Live juveniles were counted with a dissecting scope to calculate survival for each container. The lengths of 10 randomly chosen live juveniles were measured to calculate mean length for each container. In some trials, no live juveniles were present so measurements were taken of empty shells remaining in the container. Presence of algae in the digestive tracts of the juveniles was noted. Sampling continued for 56 d, unless otherwise noted.

Water chemistry and temperature control

Water used in all treatments was the standard FMCC mix of city and well water (Henley et al. 2001). Mean hardness values for city and well water are 120 mg/L and 370 mg/L as $CaCO_3$, respectively. These waters were mixed in equal volume in a conditioning tank so that they were similar in hardness to the natal stream of mussels. The conditioned water was dechlorinated with sodium thiosulfate. During the culture trials the water continuously was recirculated through a UV sterilizer and aerated.

Water chemistry and temperature measurements were taken to monitor the environment to which juveniles were exposed. There are no published guidelines for water chemistry requirements in juvenile mussel culture, but Henley et al. (2001) summarized known tolerances for freshwater mussels and other sensitive aquatic organisms, including a miniumum pH of 6.0 and maximum ammonia at 0.09 mg/L. Beaty and Neves (2004) concluded that hardness in the range of 200-250 mg/L seems to be suitable for juveniles. Water chemistry of the systems generally reflected that of the

FMCC conditioning tanks, which where used to fill the reservoirs of the systems in the trials. Water temperature of the systems was influenced by ambient air temperature in the building where the systems were kept, except for the first batch of *A. atropurpurea*, which were grown in the temperature-controlled FMCC grow-out system. Water temperature was measured with a hand held thermometer at noon each day of the trial. Ammonia, pH, dissolved oxygen, alkalinity, and hardness were measured every 14 d when sampling occurred. Ammonia was measured with a Hach DR/2000 spectrophotometer. Titrations were performed to measure alkalinity and hardness. Dissolved oxygen (DO) was measured with a YSI meter.

Data analysis

Survival data were square root-transformed so that they satisfied the assumptions of normality and homogeneity of variance. This was done so that parametric statistical tests based on normal distribution could be performed. Repeated measures ANOVAs were then used to look for differences in survival between treatments and systems at each sampling event (SAS Institute 2002). Means and confidence intervals of survival reported here are back-transformed estimates. Length data were distributed normally and variances were homogeneous, so no transformations were needed for parametric tests. The mean lengths of juveniles in each container were used in ANOVAs to investigate differences between treatments and systems at each sampling event (SAS Institute 2002). The exception to comparing mean lengths for each container was in *A. atropurpurea* and *L. costata* culture, when individual juvenile lengths were compared using ANOVAs.

Comparisons between sampling events were used to evaluate whether trends were evident in the trials; however, comparisons at the last sampling event were the most important since overall survival and growth was the focus of the trials.

Results

Trial 1

Survival

Mean survival in the fine sediment treatment at 14 d (42 d of age) was 3.6 % (95 % confidence interval of 0.4-10.1 %) and was not different from mean survival in the sand treatment (3.6 %, 0.4-10.0) (p=0.99). At 81 d (109 d of age), no live individuals remained (Figure 2.5).

Growth

At day 0 (28 d of age), mean length of juveniles was 351.5 μm (95 % confidence interval of 348-355 μm). Lengths were not significantly different between treatments at this time (p=0.879). At 14 d, mean length of juveniles in the fine sediment/POM treatment (367.5 μm) was significantly greater than mean length of juveniles in the sand/POM treatment (351.2 μm) (p=0.015) (Figure 2.6). At 81 d (109 d of age), there were no live juveniles to compare, but lengths of empty shells that remained in the substrate were taken. There was no difference in the mean length of shells found in the fine treatment (375.3 μm) and the sand treatment (370.4 μm) at this time (p=0.77) (Figure 2.6). There were no differences in replications throughout the trial (p=0.71).

Mean lengths of juveniles had significantly increased in the fine sediment treatment between 0 d and 14 d (p=0.039), but did not increase significantly between 14 d and 81 d (p=0.52), suggesting that growth occurred during the first 14 d of the trial, but not thereafter. Mean lengths in the sand treatment did not increase significantly between 0 d and 14 d (p=0.99) but did increase significantly between 14 d and 81 d (p=0.0004). This suggests that the few juveniles that survived the first 14 d continued to grow before dying (Figure 2.6).

Water chemistry & temperature

Water chemistry measurements from the reservoirs were within the range considered suitable for juveniles (Table 2.1). Ammonia ranged from 0.02-0.05 mg/L, hardness from 260-270 mg/L, alkalinity from 215-230 mg/L, DO from 6.5-7.5 mg/L. At all three sampling events, pH registered 7.8. Mean water temperature in the reservoirs was 22.9°C, with a range from 20-24.5°C throughout the trial (Figure 2.7).

Trial 2

Survival

Mean juvenile survival among all treatments and systems at 14, 28, and 42 d was 46.3 % (95 % confidence interval of 41.4 – 51.6 %), 19.9 % (16.7 – 23.3 %), and 8.3 % (6.3 – 10.6 %), respectively. All juveniles died by 56 d. There were no differences in survival among any of the substrate treatments (p=0.168) (Figure 2.8). There also were no differences in survival between systems until 42 d, when more juveniles were alive in the trough (13.2 %) than in the dish system (4.6 %) (p=0.008) (Figure 2.9).

Growth

At day 0, mean juvenile length was 265 µm (95 % confidence interval of 260-269 µm). Lengths were not different among treatments or between systems at this time (p=1.0). Mean juvenile length among all treatments and systems at 14, 28, 42, and 56 d was 321 (316-325), 363 (358-368), 380 (376-385), and 388 (383-393) µm, respectively.

There were differences in lengths between each sampling event across all treatments ($p<0.0001$), indicating that juveniles were growing during that time. There was no difference in length between 42 d and 56 d ($p=0.095$), indicating that little growth had taken place during the last 2 wk of the trial.

There were no differences in lengths among any of the treatments ($p=0.62$) (Figure 2.10). However, there were differences in juvenile lengths between systems; juveniles held in the trough were significantly larger than juveniles held in the dish system at 28 d ($p=0.015$) and 56 d ($p=0.027$) (Figure 2.11).

Water chemistry

Water chemistry from the reservoirs of the two systems was within the suitable range for juveniles and was similar in both systems (Table 2.2 and 2.3). Ammonia ranged from 0.02-0.05 mg/L, hardness from 260-280 mg/L, alkalinity from 200-235 mg/L, pH from 7.7-7.9, and DO from 6.5-7.5 mg/L. However, temperatures between the systems differed (Figure 2.12). Mean water temperature in the trough (16.3°C) was higher than that in the dish system (15.5°C) ($p<0.0001$).

Trial 3

Survival

Mean juvenile survival among all treatments and systems at 14, 28, 42, and 56 d was 78.3 % (95 % confidence interval of 66.4 - 91.2 %), 38.8 % (30.5 - 48.0 %), 31.1 % (23.8 - 39.4 %), and 14.5 % (9.6 - 20.3 %), respectively. Survival was not different among any of the treatments ($p=0.98$) (Figure 2.13). There were differences in survival between systems at 28 d ($p=0.0005$), 42 d ($p=0.0003$), and 56 d ($p<0.0001$), as more juveniles were alive in the dish system than in the trough (Figure 2.14).

Growth

At 0 d, mean length of juveniles of *E. brevidens* was 251.5 μm (95 % confidence interval of 238-264). Lengths were not significantly different among treatments or between systems at this time (p=1.0). Mean juvenile length among all treatments and systems at 14, 28, 42, and 56 d were 388 (375-401) μm, 470.0 (457-483) μm, 549 (536-562) μm, and 606 (594-620) μm, respectively. There were differences in lengths between sampling events (p<0.0001 in each case), indicating that juvenile growth continued throughout the trial. There were no significant differences in lengths among any of the treatments (p=0.71) or either system (p=0.26) throughout the trial (Figures 2.15 and 2.16).

Water chemistry

Water chemistry measurements from the reservoirs of the two systems were within a suitable range for juveniles and similar in both systems (Table 2.4 and 2.5). Ammonia ranged from 0.01-0.05 mg/L, hardness from 295-350 mg/L, alkalinity from 190-405 mg/L, pH from 7.8-8.0, and DO from 6.5-7 mg/L. Temperatures between the systems differed (Figure 2.17). Mean temperature of water in the dish system (23.4°C) was higher than that in the trough system (22.3°C) (p=0.023).

Culture of *A. atropurpurea* and *L. costata*

The first batch of juveniles of *A. atropurpurea* reared in the FMCC culture system was sampled at 30 d. Survival at that time was 0 %. Lengths of empty shells recovered at 30 d (393.8 μm, 95 % confidence interval of 381-406 μm) were larger than shells of juveniles at 0 d (326.3 μm, 313-338 μm) (p<0.001) indicating that some growth occurred before all juveniles died.

The second batch of *A. atropurpurea* survived longer than the first. Juvenile survival at 14, 28, 42, 56, and 70 d was 45.8 %, 17.4 %, 9.2 %, 1.5 % and 1.0 %, respectively (Figure 2.18). At 0 d, mean juvenile length of *A. atropurpurea* was 326.3 (313-339) µm. This was not significantly different from the mean length at 0 d in batch 1 ($p=1.0$). Mean length at 14, 28, 42, 56, and 70 d was 396.3 (384-409) µm, 437.5 (425-450) µm, 438.8 (426-451) µm, 470.0 (457-483) µm and 525.0 (502-548) µm, respectively (Figure 2.19). There was an increase in length between each sampling event ($p<0.001$), except between 28 and 42 d ($p=0.88$). At 28 d, juveniles from batch 2 were larger than juveniles in batch 1 ($p<0.0001$), but juveniles at 14 d in batch 2 were of similar size to juveniles recovered on 28 d in batch 1 ($p=0.78$). This indicates that if growth rates were similar between batches, then the juveniles in batch 1 died somewhere around 14 d of age.

Survival of *L. costata* at 14 d was 33.3 % in the coarse treatment, 12.1 % in the mixed treatment, and 0 % in the fine treatment (Figure 2.20). By 28 d, no live juveniles remained in either treatment. Mean length among all treatments was 350.8 (344-358) µm at 0 d and 377.8 (368-387) µm at 14 d. At 28 d, no live juveniles were measured, but mean length of the empty shells was 412.5 (401-423) µm. Growth was inconsistent in the treatments; the only significant increases in length occurred between 14 and 28 d in the coarse treatment ($p=0.003$) and between 0 and 14 d in the mixed treatment ($p=0.026$).

Water chemistry

Water chemistry from the reservoirs of all systems was within the suitable range for juveniles (Table 2.2, 2.6 and 2.7). Ammonia ranged from 0.02-0.05 mg/L, hardness from 190-280 mg/L, alkalinity from 170-235 mg/L, pH from 7.7-8.2, and DO from 6.5-

7.5 mg/L. Temperature for batch 1 of juveniles of *A. atropurpurea* was constant at 19°C; mean water temperature for batch 2 was 16.3°C and ranged from 14.5-18°C (Figure 2.12). Mean temperature for *L. costata* was 15.8°C and ranged from 15-16.5°C (Figure 2.7).

Discussion

Trial 1

Fine sediment initially seemed to be a better substrate than sand for juveniles of *E. brevidens*. Neither substrate resulted in higher survival than the other, but juveniles in the fine sediment treatment exhibited more growth over the first 14 d of the trial. However, differences in growth were not apparent by the end of the trial, which indicates that substrate size did not affect growth of these juveniles.

Success in juvenile culture in this trial was negligible. According to Jones et al. (2005), success of juvenile culture depends on several factors, including condition of the parent mussel and glochidia. Other juveniles of *E. brevidens* produced from the same female mussels as those in trial 1 were raised in the main FMCC culture systems, but all died by 60 d of age as well (Mair et al. 2003). This seems to indicate that either the adult mussels or the glochidia were not in good condition, which translated into poor quality juveniles. Therefore, little information can be interpreted from these results on the appropriateness of substrates used.

Trial 2

Substrate composition did not affect survival and growth of juveniles of *V. trabalis* through 56 d, as all treatments had similar mean survival and growth. There were differences in growth and survival between systems, as the trough system had higher overall growth and higher survival at 42 d than the dish system.

Overall survival in this trial was poor, did not differ among substrates, and only differed between systems at 42 d. There was complete mortality by 56 d in all treatments and systems. Poor culture results have been reported for this species by Mair et al. (2003), indicating that perhaps juveniles of *V. trabalis* have culture needs yet to be defined. Another factor in their poor survival may have been the maturity of glochidia used to obtain juveniles. When *V. trabalis* glochidia were extracted for infestation, undeveloped egg material was present along with developed glochidia. Therefore survival and growth comparisons among substrates may not be representative of more developed and healthy juveniles of *V. trabalis*.

Substrate appeared to have no impact on juvenile growth, leading to the conclusion that all substrates provided similar physical habitat and food sources. If fine sediment is indeed an important juvenile food source (Gatenby et al. 1996), a small amount seems adequate to be beneficial to juveniles. However, since juveniles in this trial may have been affected by condition of glochidia, no conclusion is possible.

Differences in water temperature may account for the dissimilarity in growth between the systems. Beaty and Neves (2004) showed that juvenile growth is correlated to the number of days above 15°C. Mean daily temperature of the dish system was 15.5°C, with 8 d below 15°C; temperature in the trough system averaged 16.3°C and 0 d below 15°C. Warmer temperatures would have allowed juveniles to grow faster in the trough than in the dish system. The difference in temperature between systems most likely was caused by different locations within the building where the systems were kept.

Systems were exposed to the same ambient air temperature, but the reservoir of the dish system sat directly on the concrete floor, while the trough and its reservoir were elevated on cinder blocks above the floor. The trough also sat in front of the heater; the dish system sat near the door where temperatures were not as warm. The direct contact with the floor and location near the door seemed to keep the dish system from getting as warm as the trough system.

Another possible reason for the differences in growth between systems is the rate at which water is supplied to the culture chambers. Although water entered and exited the trough at a higher velocity (10 L/min) than the dishes (1 L/min), turnover times were longer in the trough (7 min) than in the dishes (1 min). The slower turnover in the trough may have allowed more algae to settle into the juvenile containers, and therefore more food would have been available to juveniles in the trough. The higher turnover rates in the dishes should have kept juveniles well supplied with fresh water and food, but it appears that perhaps the dish system did not allow algae to settle out of suspension as well as it did in the trough.

Trial 3

Substrate composition had no impact on survival or growth of *E. brevidens* in this trial. Survival was significantly higher in the dish system than the trough system, although there were no differences in growth between systems.

As in trial 2, substrate did not appear to impact juvenile survival and growth. All treatments appeared to provide juveniles with adequate food and/or physical habitat. A low amount of fine sediment, as in the coarse treatment, seems to be enough to provide nutritional benefits, along with supplemental algae. Conversely, a substrate made up completely of fine sediment, which is less time consuming to collect, prepare, and sample than a mixed fine sediment/sand substrate, does not seem to negatively affect juveniles, and so would be an appropriate substrate to use in future culture efforts of *E. brevidens*.

As in trial 2, differences in juvenile grow-out were evident between the dish system and the trough system. However, in this trial, the dish system resulted in much higher survival than the trough system. Water chemistry, amount of algae provided, and turnover times were similar in both systems, which should have provided nearly identical culture environments. Temperatures were different in the systems, with the dish system averaging about 1°C warmer than the trough. However, higher temperatures typically result in higher juvenile growth but lower survival (Beaty and Neves 2004). The dish system had higher survival than the trough but equal growth, so it does not seem likely that temperature would account for the differences in survival. One possibility would be that predation by flatworms and/or *dipteran* larvae was greater in the trough than in the dish system. These predators were present in both systems while sampling juveniles, despite using heat treated substrates, and may therefore have affected juvenile survival.

A. atropurpurea* and *L. costata

The first batch of juveniles of *A. atropurpurea* had poor survival, which may have resulted from immature glochidia. The host fish infestation took place in November, and there were no signs that glochidia were immature, such as presence of undeveloped egg material. However, the second batch of *A. atropurpurea* had more successful culture results, lending support to the speculation that batch 1 may have been immature. However, the batches were grown in different substrates and different systems, which makes it difficult to determine whether higher survival and growth were due to better substrate and/or system design or to more mature glochidia.

Results of juvenile survival of *L. costata* seem to indicate that survival in substrates of only fine sediment may be lower than that in substrates which contain larger particle sizes. This may be because juveniles of *L. costata* are larger than those of *V. trabalis* or *E. brevidens* and may require substrate with larger interstitial spaces in which to burrow and feed. Based on results in trials 2 and 3, the mixed and coarse substrate treatments would have provided the larger interstitial spaces while still providing adequate food from fine sediment. Growth showed no differences between treatments and was poor overall.

The juveniles of *L. costata* seemed to suffer from effects of poor adult or glochidial condition, so inferences of suitable culture techniques are speculative. Glochidia were extracted from adults that had been held in captivity for several months and were at the end of their release season. These glochidia were slow to respond to a saline solution, although some were able to attach and transform on fish. Also, these results are not conclusive since treatments were not replicated. Culture techniques for juveniles of *A. atropurpurea* and *L. costata* require further study.

General conclusions

These studies agree with the conclusions of Jones et al. (2005) that culture of juvenile freshwater mussels requires optimization of several factors to be successful. These factors include physiological condition of adult mussels, maturity of glochidia, species differences in juvenile stage, quality and quantity of food, water quality, predation, and substrate (Jones et al. 2005). Effects from maturity or condition of glochidia and/or adults were evident throughout these grow-out efforts, which made it difficult to draw conclusions on the suitability of different substrates and culture systems. This is well demonstrated in the difference in juvenile lengths of *E. brevidens* between trial 1 and 3. At 42 d of age, mean length of juveniles from trial 1 and 3 were 361 μm and 549 μm, respectively. The juveniles in these trials were produced in two different years, with the juveniles produced in 2005 (trial 3) of much better condition. Since there is variability in juvenile condition from year to year, several trials would need to be conducted to make solid conclusions on the suitability of different substrates and systems (Jones et al. 2005).

However, based on these limited results, temperature, flow, and predation seem to be the main factors determining how well juveniles survived or grew. Substrates did not appear to affect growth or survival of species; but there is one possible confounding effect. Substrates not only varied by size, but also by stream-source geology. Fine sediment was taken from the Clinch River, with geologically dominant sandstone, whereas sand was taken from Sinking Creek, with a mostly limestone geology. Both streams are supportive of healthy aquatic life, but slight chemical differences between substrates could remain after preparation and, therefore, affect juvenile growth and survival. It would be recommended for future studies to obtain substrates from similar sources in order to prevent the possibility of geological differences confounding effects of substrate size.

Assuming little to no effects from geological sources are present, fine sediment seems to be an appropriate substrate to culture juveniles of *E. brevidens* and *V. trabalis*. I would recommend using fine sediment because it is easier to collect, prepare, and sample than a substrate of mixed sizes. By using only fine sediment, the need to collect and prepare another substrate size is eliminated. Also, fine sediment (<200 µm in size) is easily separated from the juveniles (typically >200 µm in size) by washing the contents of the culture containers through a 200 µm mesh sieve. When using a mixed sand and fine sediment substrate, the fine sediment can be easily separated from the juveniles, while the sand requires extra effort to extract juveniles. Therefore, using a 100 % fine sediment substrate would be most time efficient for the researcher.

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Table 2.1: Water chemistry measurements taken from the experiment of *Epioblasma brevidens* culture in trial 1.

Date	Ammonia (mg/L)	Hardness (mg/L)	Alkalinity (mg/L)	pH	DO (mg/L)
7-Jul-04	0.02	270	215	7.8	7.5
21-Jul-04	0.04	270	220	7.8	6.5
26-Sep-04	0.05	260	230	7.8	7.0
MEAN	0.04	267	222	7.8	7.0

Table 2.2: Water chemistry measurements taken from the experiment of the dish system used in *Villosa trabalis* culture in trial 2. Juveniles of *Lasmigona costata* also were reared in this system beginning May 12, 2005.

Date	Ammonia (mg/L)	Hardness (mg/L)	Alkalinity (mg/L)	pH	DO (mg/L)
14-Apr-05	0.02	260	200	7.7	6.5
28-Apr-05	0.04	260	200	7.8	7.0
12-May-05	0.04	260	200	7.8	7.5
26-May-05	0.05	270	205	7.9	7.0
9-Jun-05	0.04	270	235	7.7	7.0
MEAN	0.04	264	208	7.8	7.0

Table 2.3: Water chemistry measurements taken from the experiment of the trough system used in *Villosa trabalis* culture in trial 2.

Date	Ammonia (mg/L)	Hardness (mg/L)	Alkalinity (mg/L)	pH	DO (mg/L)
14-Apr-05	0.02	260	200	7.7	7.0
28-Apr-05	0.04	260	200	7.8	7.0
12-May-05	0.04	270	200	7.7	7.0
26-May-05	0.04	270	205	7.8	7.5
9-Jun-05	0.04	280	230	7.8	7.0
MEAN	0.04	268	207	7.8	7.1

Table 2.4: Water chemistry measurements taken from the experiment of the dish system used in *Epioblasma brevidens* culture in trial 3.

Date	Ammonia (mg/L)	Hardness (mg/L)	Alkalinity (mg/L)	pH	DO (mg/L)
19-Jun-05	0.02	350	310	7.8	7.0
03-Jul-05	0.05	300	300	7.7	6.5
17-Jul-05	0.04	320	405	7.9	6.5
29-Jul-05	0.05	315	400	7.8	7.0
14-Aug-05	0.04	295	365	7.9	7.0
MEAN	0.04	316	356	7.8	6.8

Table 2.5: Water chemistry measurement taken from the experiment of the trough system used in *Epioblasma brevidens* culture in trial 3.

Date	Ammonia (mg/L)	Hardness (mg/L)	Alkalinity (mg/L)	pH	DO (mg/L)
19-Jun-05	0.01	300	200	7.8	7.0
03-Jul-05	0.04	300	190	7.7	6.5
17-Jul-05	0.04	315	200	7.8	6.5
29-Jul-05	0.04	310	350	7.8	7.0
14-Aug-05	0.05	300	350	8	7.0
MEAN	0.04	305	258	7.8	6.8

Table 2.6: Water chemistry measurements taken from the experiment of the FMCC system used to culture the first batch of *Alasmidonta atropurpurea* produced in 2004.

Date	Ammonia (mg/L)	Hardness (mg/L)	Alkalinity (mg/L)	pH	DO (mg/L)
16-Dec-04	0.02	190	170	8.2	7.5
16-Jan-05	0.02	230	180	7.9	7.0

Table 2.7: Water chemistry measurements taken from the experiment of the trough system used to culture the second batch of *Alasmidonta atropurpurea* produced in 2005.

Date	Ammonia (mg/L)	Hardness (mg/L)	Alkalinity (mg/L)	pH	DO (mg/L)
2-Feb-05	0.03	280	220	7.7	7.0
14-Feb-05	0.04	260	225	7.8	7.0
1-Mar-05	0.05	250	220	7.9	7.0
16-Mar-05	0.04	240	215	7.8	6.5
31-Mar-05	0.05	260	190	7.9	7.5
17-Apr-05	0.04	250	205	7.9	7.0
MEAN	0.04	257	213	7.8	7.0

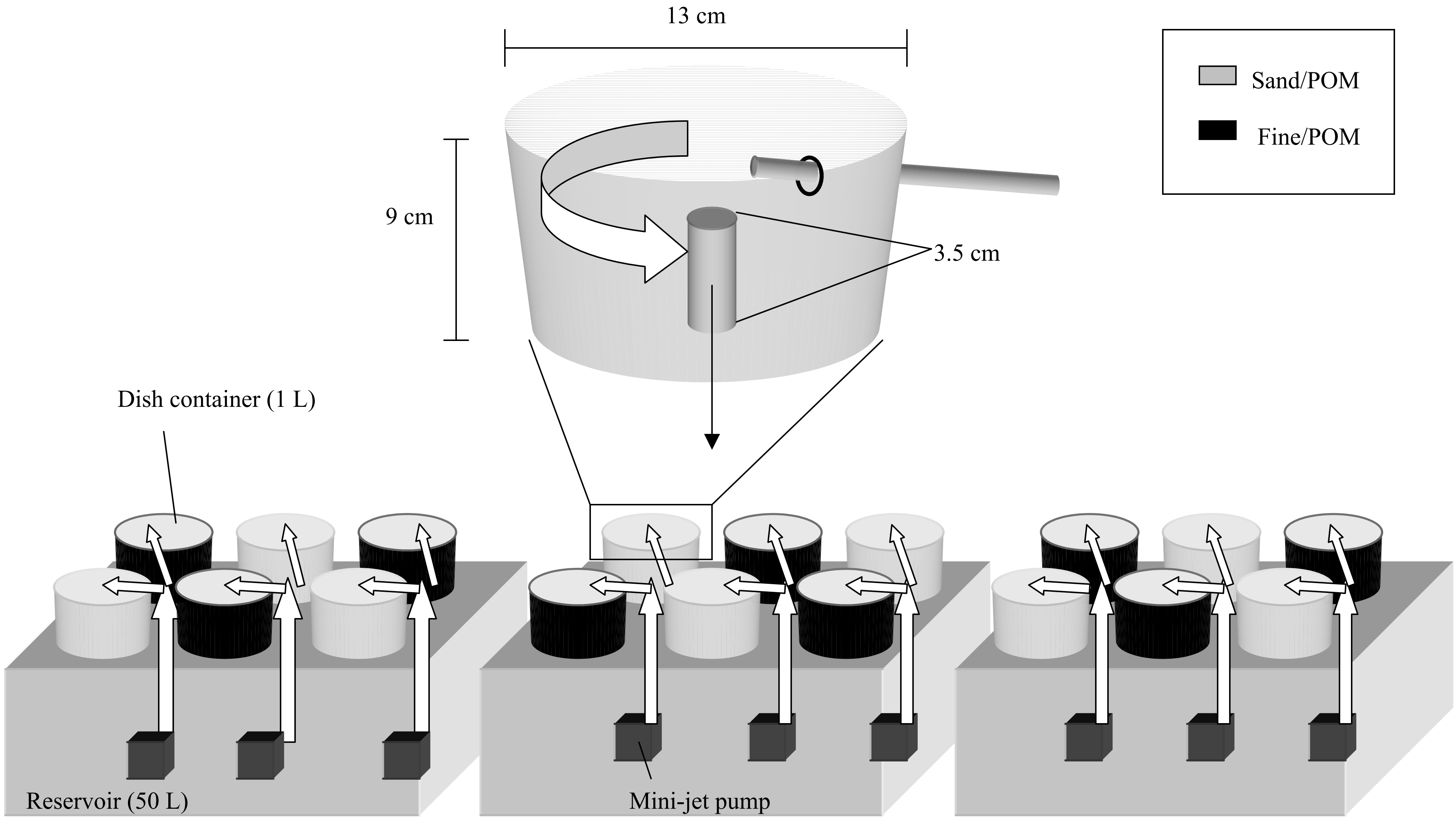

Figure 2.1: Recirculating system design used in the culture of juveniles of *Epioblasma brevidens* in trial 1.

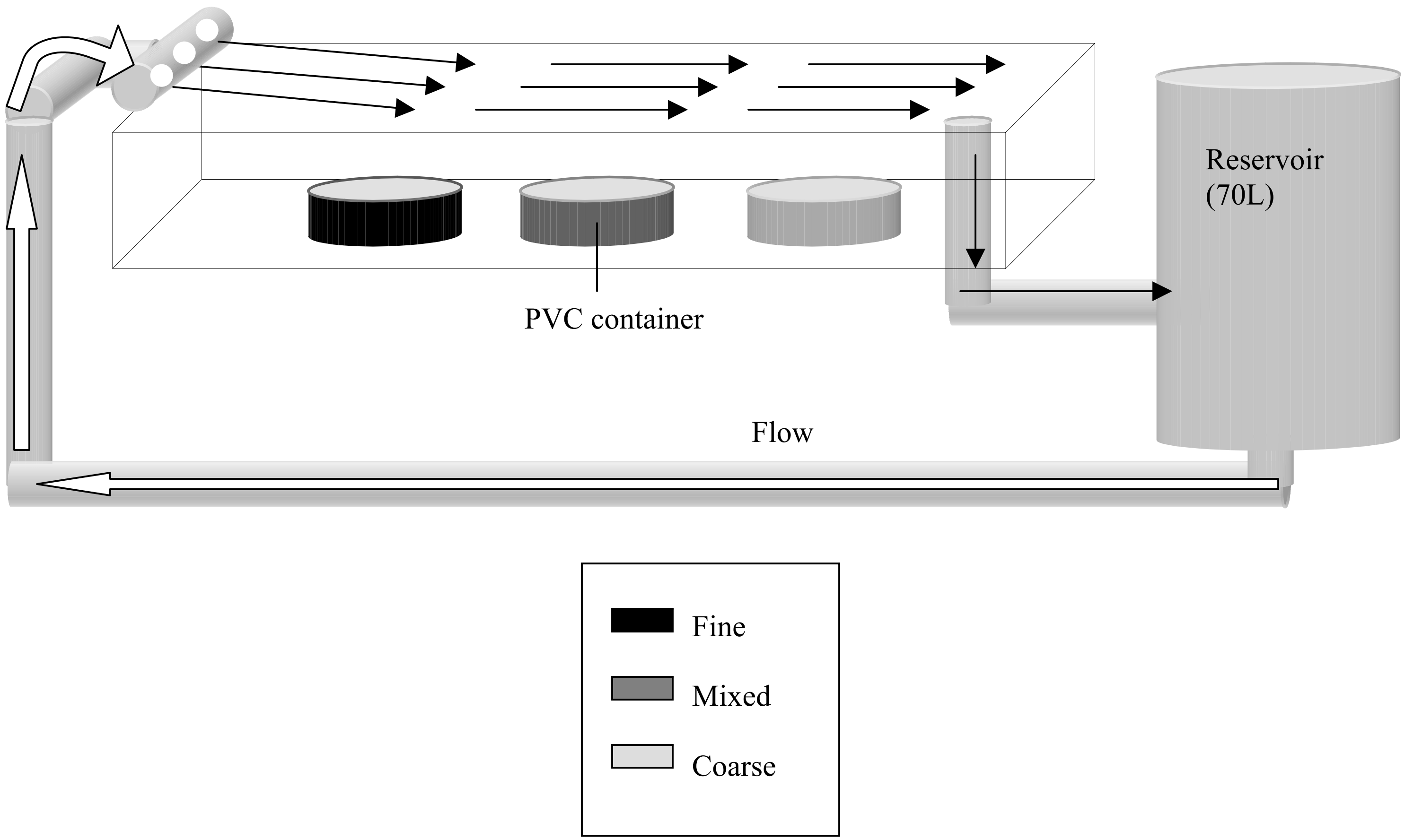

Figure 2.2: Recirculating system used in the culture of juveniles of *Villosa trabalis* and *Epioblasma brevidens* in trials 2 and 3. The same system with one dish was used for culture of *A. atropurpurea*.

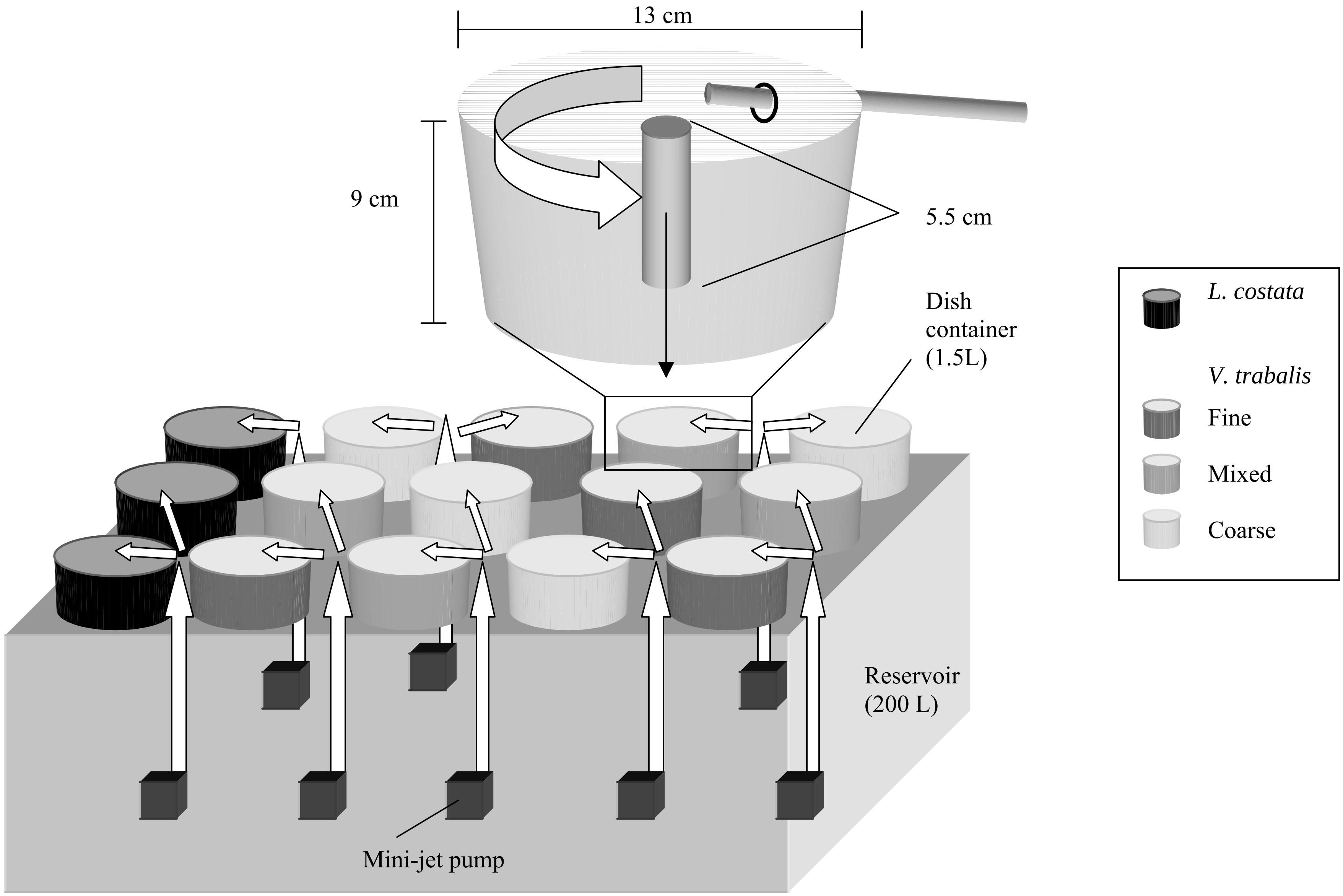

Figure 2.3: Recirculating system design used in culture of juveniles of *Villosa trabalis* in trial 2 and for culture of juveniles of *Lasmigona costata*.

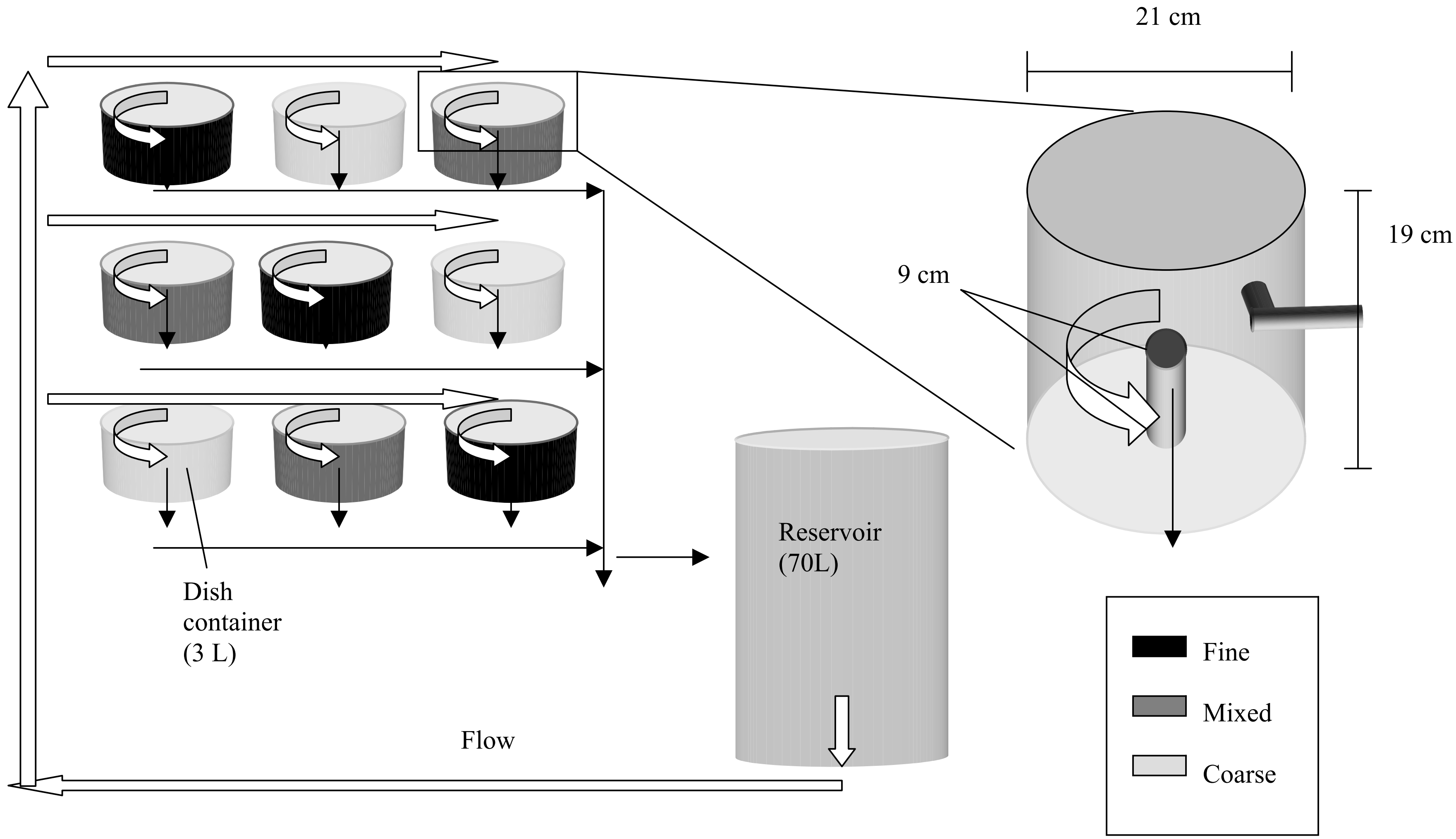

Figure 2.4: Recirculating system design used in culture of juveniles of *Epioblasma brevidens* in trial 3.

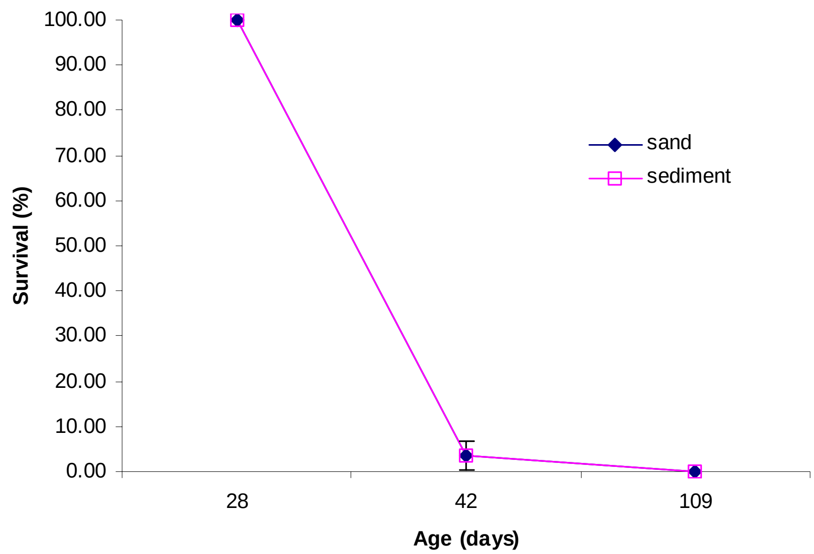

Figure 2.5: Mean survival of juveniles of *Epioblasma brevidens* in sand (n=9) and fine sediment (n=9) substrate treatments in trial 1. Error bars indicate 95 % confidence intervals and are representative of variation among containers. There were no significant differences in survival between treatments. Trial 1 was started with juveniles 28 d of age.

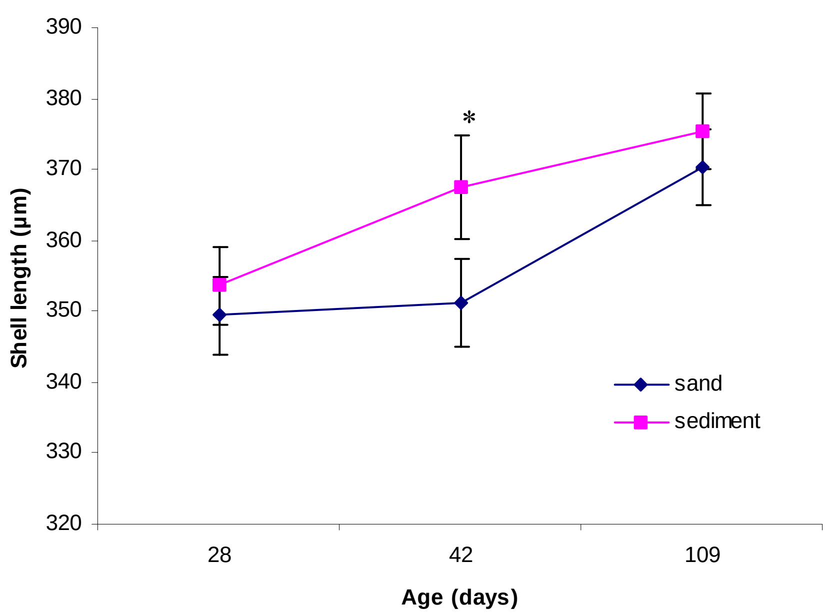

Figure 2.6: Mean shell lengths of juveniles of *Epioblasma brevidens* in sand (n=9) and fine sediment (n=9) substrate treatments in trial 1. Error bars indicate 95 % confidence intervals and are representative of variation among containers. * indicates a significant difference ($\alpha = 0.05$). Trial 1 was started with juveniles 28 d of age.

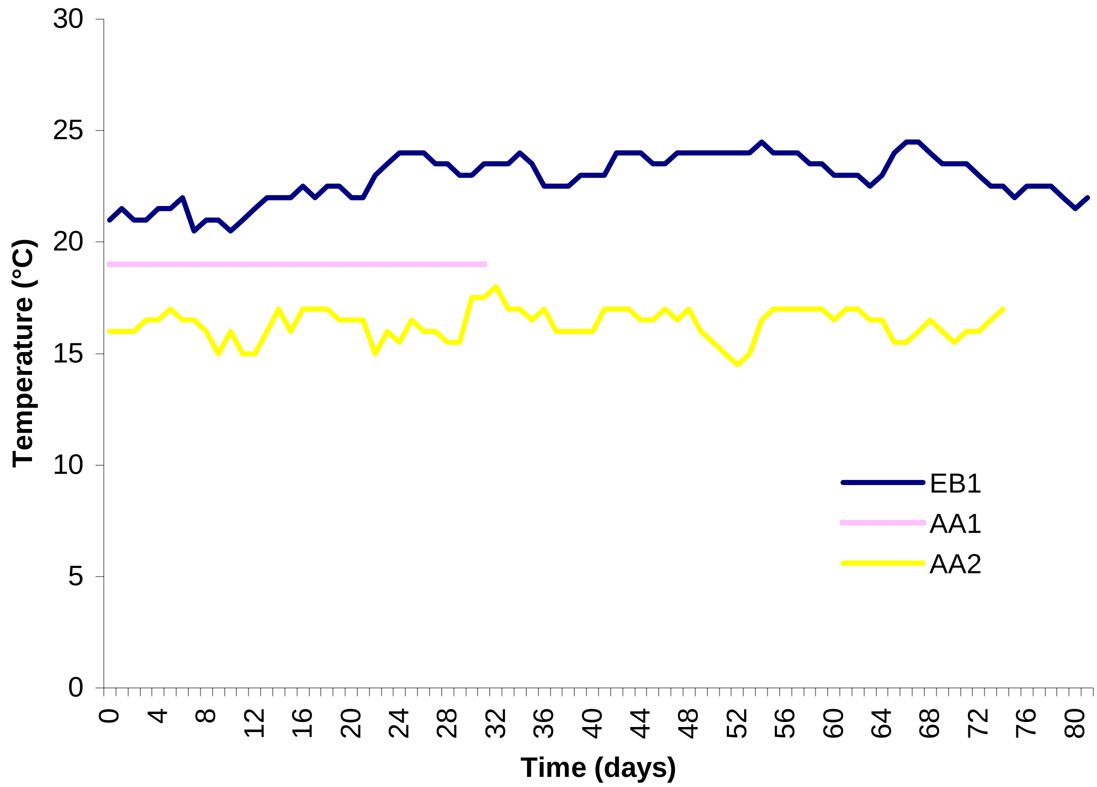

Figure 2.7: Water temperature in culture experiments with *Epioblasma brevidens* in trial 1 (EB1), and in *Alasmidonta atropurpurea* batch 1 (AA1) and batch 2 (AA2).

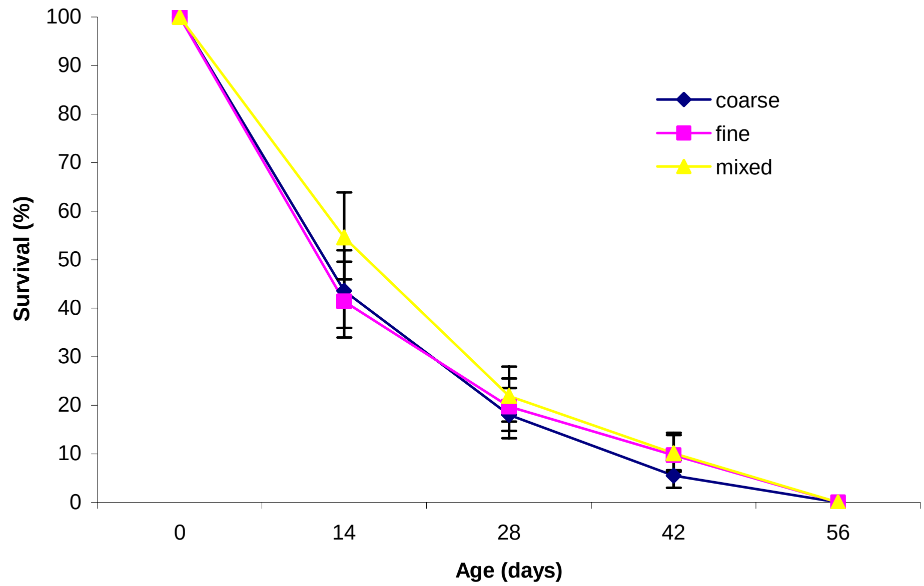

Figure 2.8: Mean survival of juveniles of *Villosa trabalis* in coarse (n=5), fine (n=5), and mixed (n=5) substrate treatments from trial 2. Error bars indicate 95 % confidence intervals and are representative of variation among containers. There were no significant differences in survival among treatments.

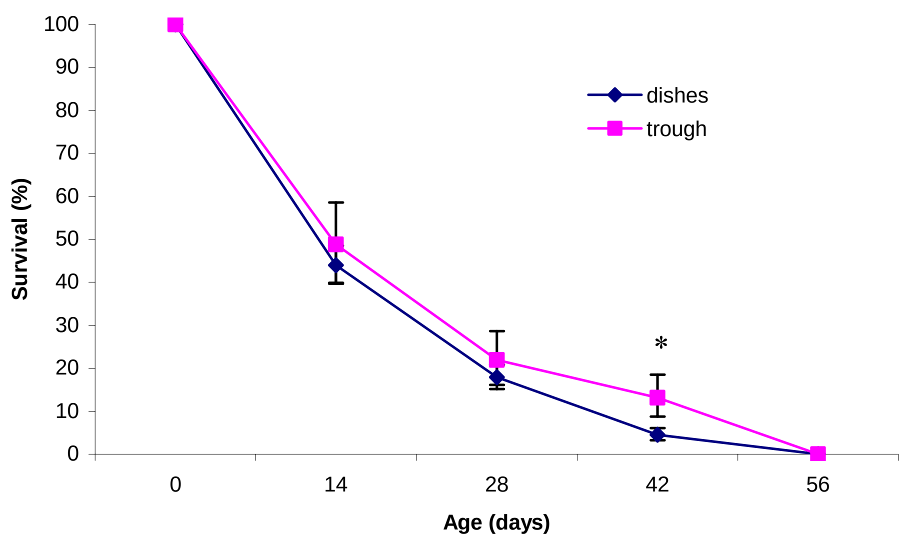

Figure 2.9: Mean survival of juveniles of *Villosa trabalis* in the dish (n=12) and trough (n=3) systems in trial 2. Error bars indicate 95 % confidence intervals and are representative of variation among containers. * indicates a significant difference (α = 0.05).

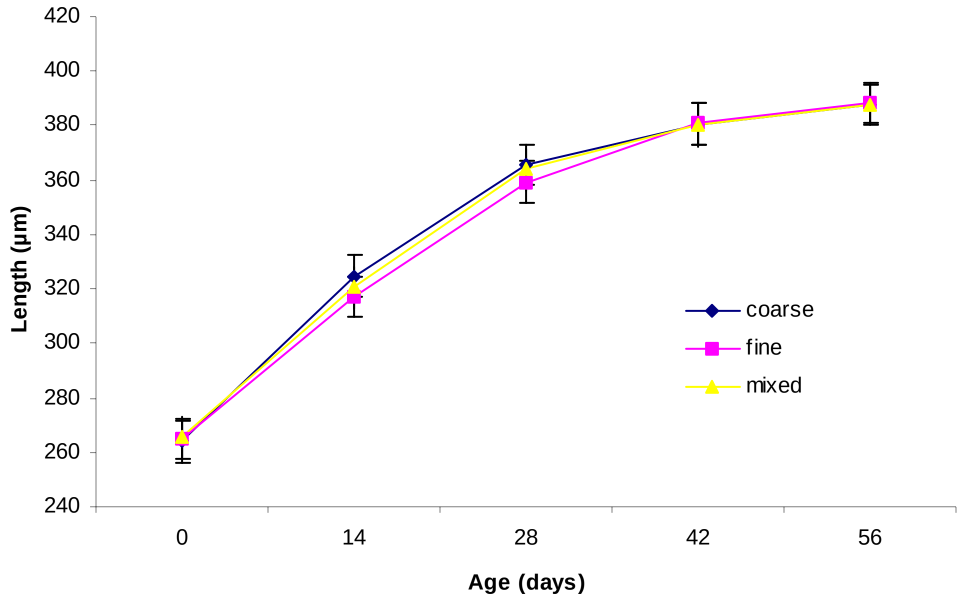

Figure 2.10: Mean lengths of juveniles of *Villosa trabalis* in coarse (n=5), fine (n=5), and mixed (n=5) substrate treatments in trial 2. Error bars indicate 95 % confidence intervals and are representative of variation among containers. There were no significant differences in lengths among treatments.

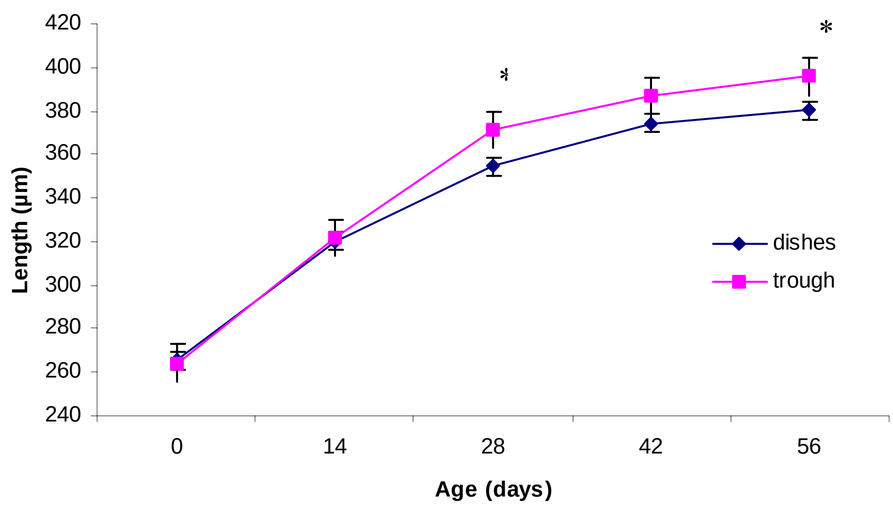

Figure 2.11: Mean lengths of juveniles of *Villosa trabalis* in dish (n=12) and trough systems (n=3) in trial 2. Error bars indicate 95 % confidence intervals and are representative of variation among containers. * indicates a significant difference (α = 0.05).

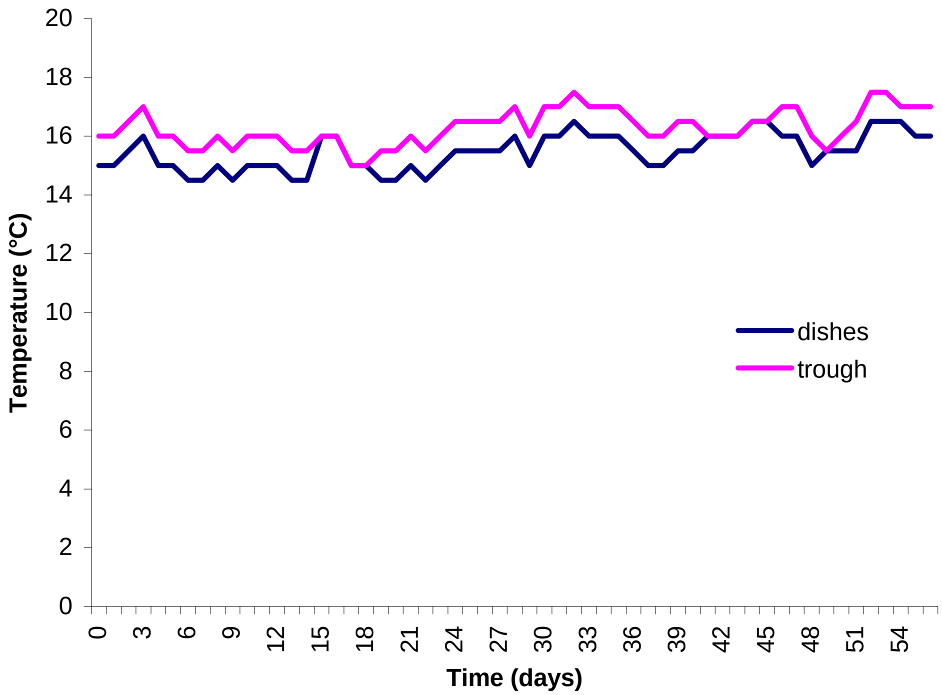

Figure 2.12: Water temperatures in the dish and trough system used in culture of juveniles of *Villosa trabalis* (trial 2). Juveniles of *Lasmigona costata* also were raised in the dish system beginning at 28 d.

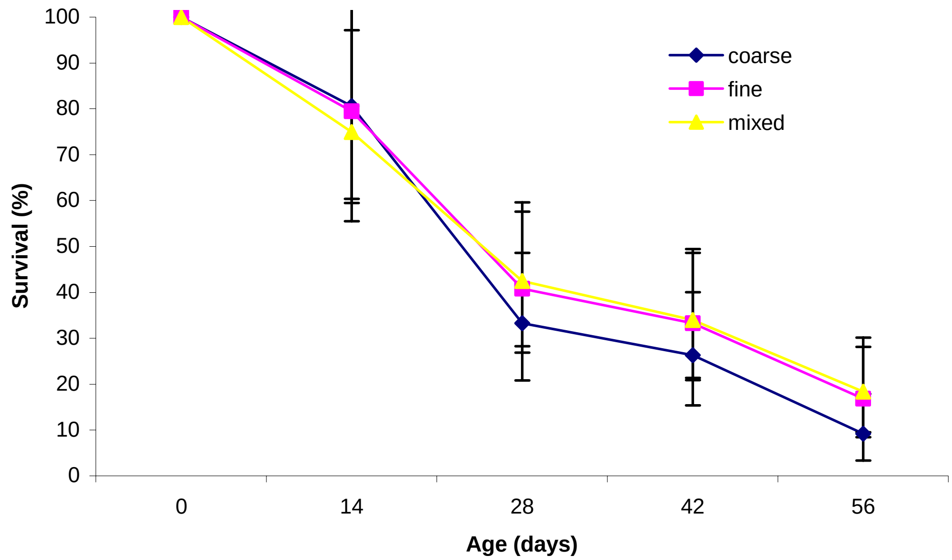

Figure 2.13: Mean survival of juveniles of *Epioblasma brevidens* in coarse (n=4), fine (n=4), and mixed (n=4) substrate treatments in trial 3. Error bars indicate 95 % confidence intervals and are representative of variation among containers. There were no differences in survival among treatments.

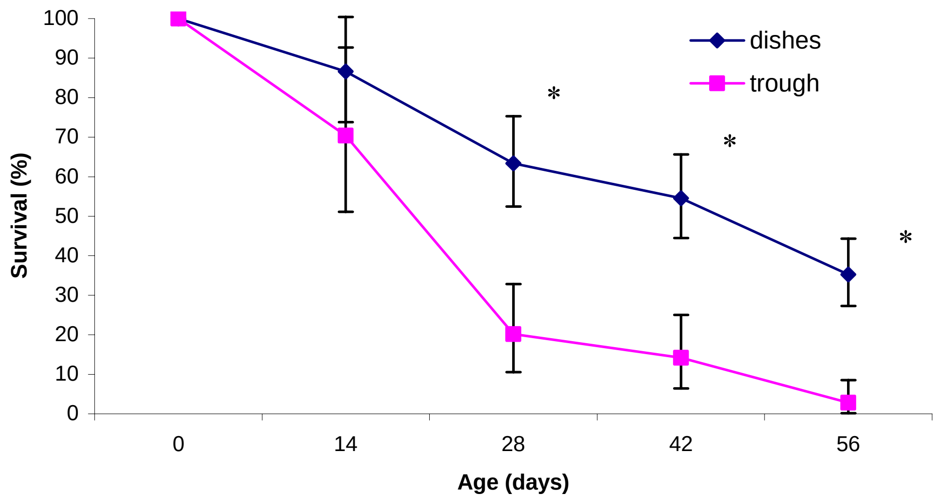

Figure 2.14: Mean survival of juveniles of *Epioblasma brevidens* in the dish (n=9) and trough (n=3) systems in trial 3. Error bars indicate 95 % confidence intervals and are representative of variation among containers. * indicates a significant difference (α = 0.05).

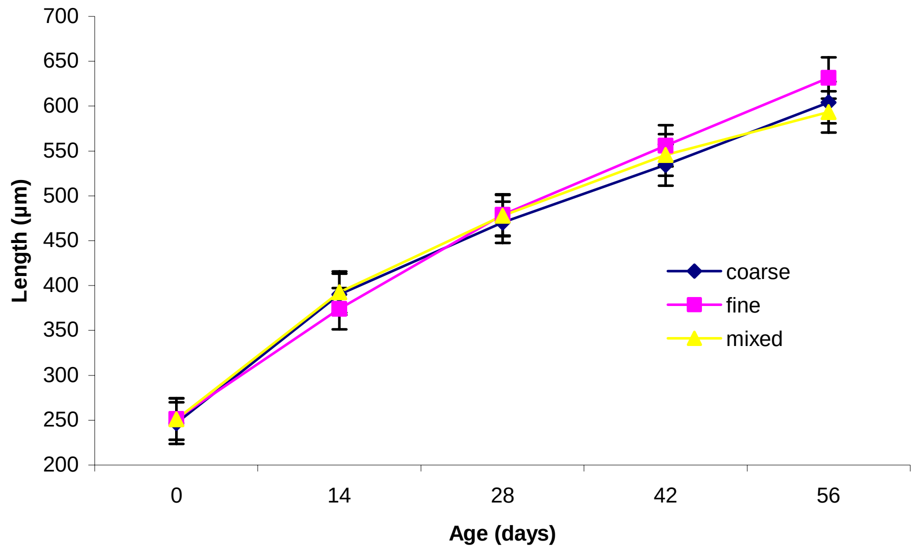

Figure 2.15: Mean lengths of juveniles of *Epioblasma brevidens* in coarse (n=4), fine (n=4), and mixed (n=4) substrate treatments in trial 3. Error bars indicate 95 % confidence intervals and are representative of variation among containers. There were no differences in length among treatments.

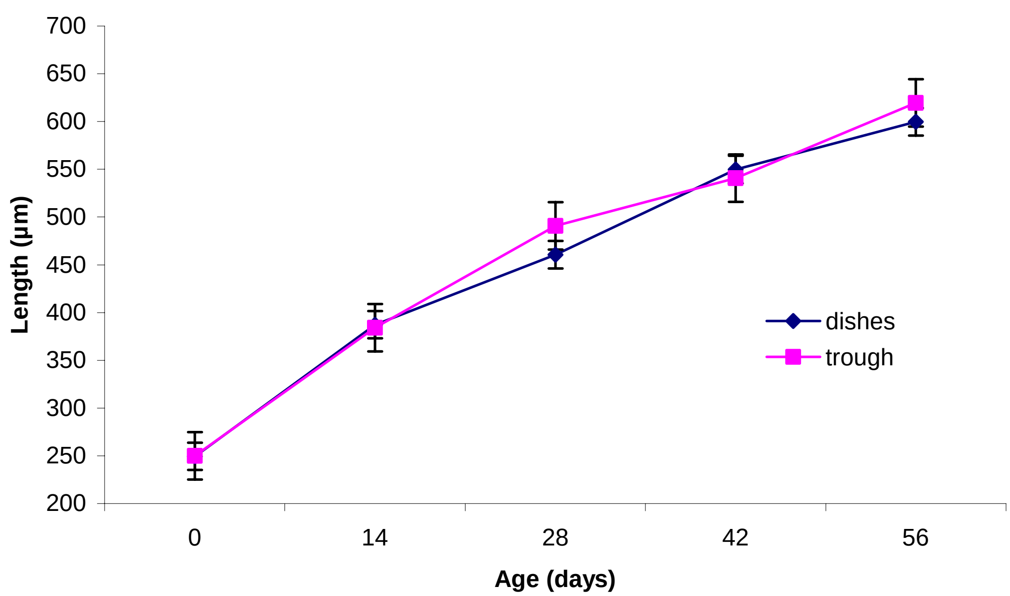

Figure 2.16: Mean lengths of juveniles of *Epioblasma brevidens* in the dish (n=9) and trough (n=3) systems in trial 3. Error bars indicate 95 % confidence intervals and are representative of variation among containers. There were no differences in lengths between systems.

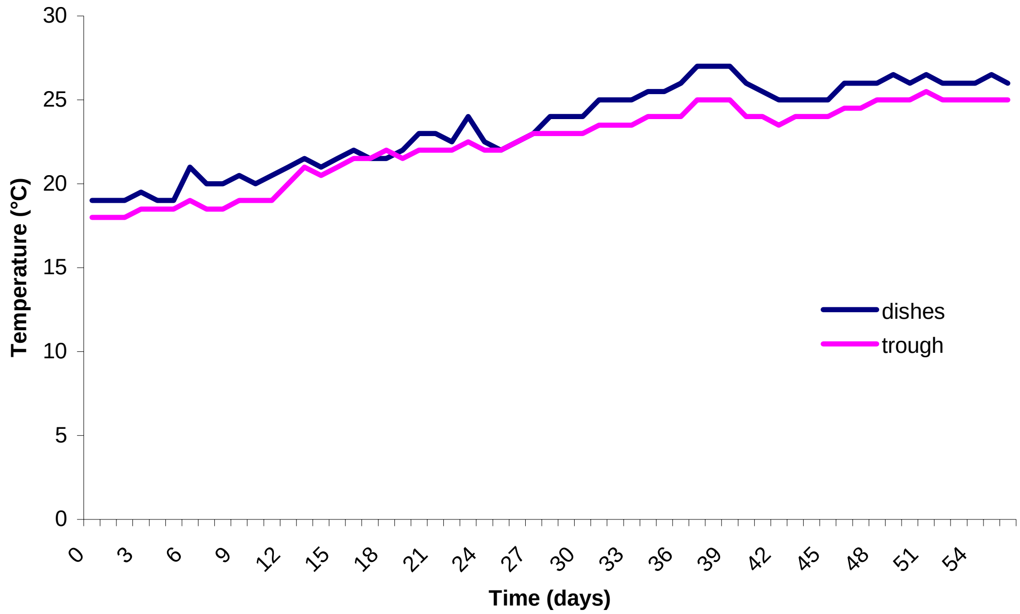

Figure 2.17: Water temperatures from the dish and trough systems used in juvenile culture of *Epioblasma brevidens* (trial 3).

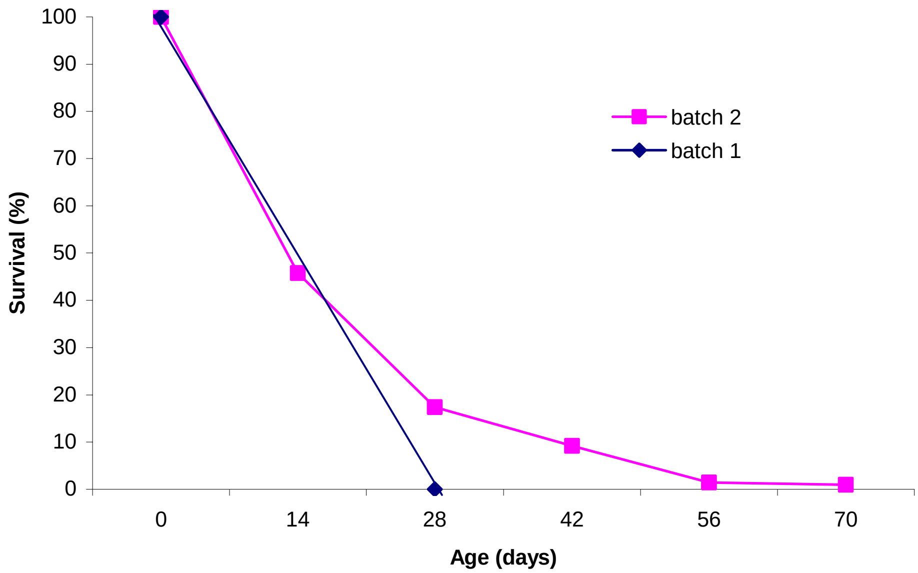

Figure 2.18: Juvenile survival of *Alasmidonta atropurpurea* from batch 1 (produced in Dec 2004, n=1) and batch 2 (produced in Feb 2005, n=1).

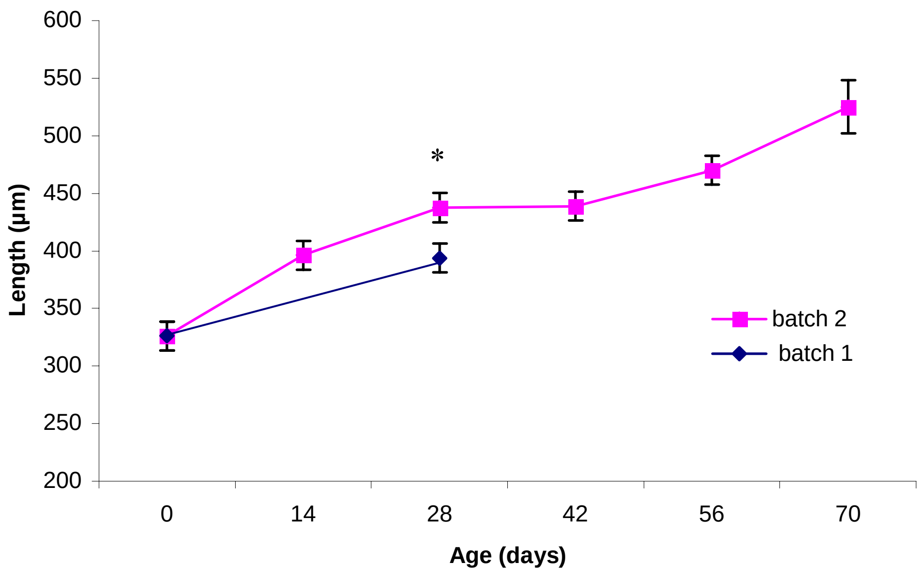

Figure 2.19: Mean lengths of juveniles of *Alasmidonta atropurpurea* of batch 1 (produced in Dec 2004) and 2 (produced in Feb 2005). Error bars indicate 95 % confidence intervals and are representative of variation within one container. * indicates a significant difference ($\alpha = 0.05$).

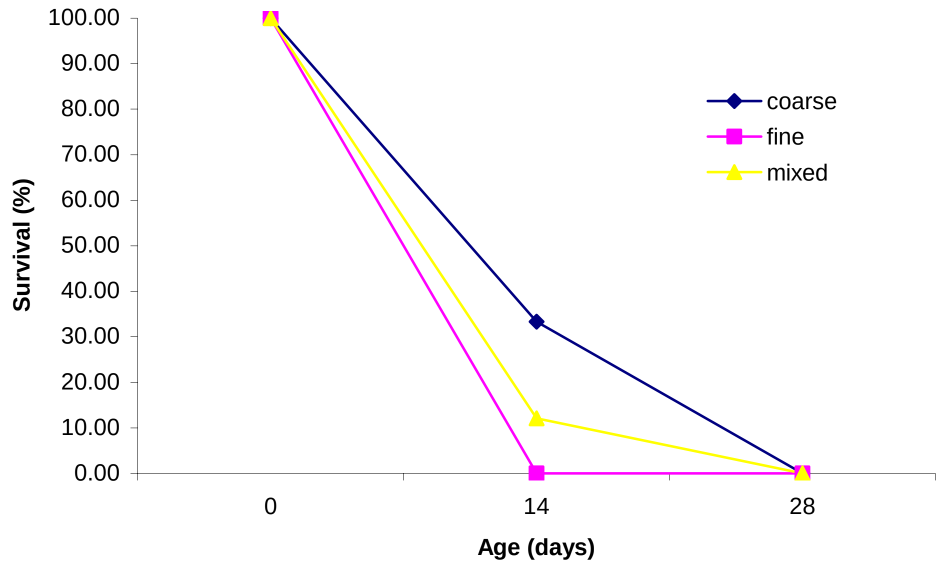

Figure 2.20: Juvenile survival of *Lasmigona costata* in coarse (n=1), fine (n=1), and mixed (n=1) substrate treatments.

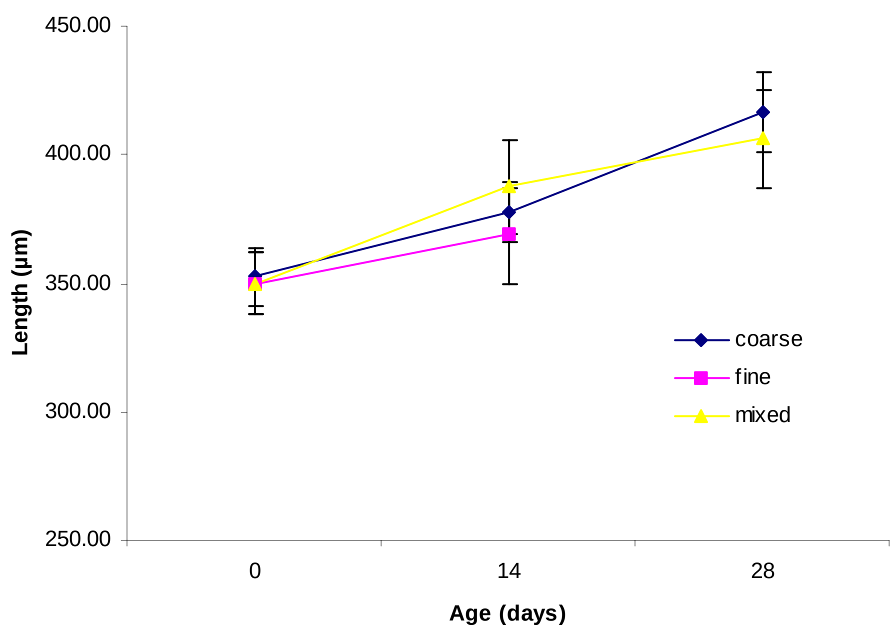

Figure 2.21: Mean lengths of juveniles of *Lasmigona costata* in coarse, fine and mixed substrate treatments. Error bars indicate 95 % confidence intervals and are representative of variation within each container. There were no significant differences among treatments.

Chapter 3: Assessment of threats and conservation value of sites in the Big South Fork National River and Recreation Area.

Introduction

The Big South Fork (BSF) of the Cumberland River is one of the best refugia for freshwater mussels in the Cumberland River system (Ahlstedt et al. 2005). The BSF and it tributaries contain at least 25 mussel species, five of which are federally endangered. Historically the BSF contained 55 species, about half of which apparently have been extirpated from the watershed (Ahlstedt et al. 2005). The main stem BSF is located within a National River and Recreation Area (NRRA), managed by the National Park Service (NPS). The NPS is working with the U.S. Fish and Wildlife Service (USFWS) to maintain and restore its mussel populations, to include relocation, augmentation through captive propagation, and monitoring (Biggins et al. 2001).

To successfully restore and conserve freshwater mussels in the BSF, specific sites need to be identified where restoration or conservation activities can occur. These activities should focus on sites that provide adequate mussel habitat, are of high conservation value, and remain relatively free from natural or anthropogenic threats, such as pollution and habitat alteration. This study focuses on evaluating potential conservation sites based on their prospective environmental suitability and minimal threat from anthropogenic land use on the adjacent landscape.

Threat assessment

Streams and their biota have been drastically altered by anthropogenic activities in the past century (Richter et al. 1997). Building of dams, channelization and dredging of stream beds, and large-scale changes in land use are some of the causes implicated in major changes in stream biota, especially freshwater mussels (Neves et al. 1997). These activities change the physical and chemical conditions of streams, making them unfit for biota that had previously been supported (Richter et al. 1997).

Streams within the NRRA boundaries are in little danger of being altered by dam construction, channelization, or dredging since the area was established 'to preserve the free-flowing sections of the BSF' (NPS 2003a). However, 90% of the BSF watershed is located outside of NRRA boundaries, and several land uses in the watershed are associated with negative impacts to mussels and other aquatic fauna, either through direct exposure to contaminants or through indirect alteration of habitat (Rikard et al. 1986, Diamond et al. 2002, Warren and Haag 2005). These land uses include coal, oil, and natural gas extraction, agricultural and urban development, and existence of numerous roads, railways, and trails (Ahlstedt et al. 2005).

Coal mining is associated with acid mine drainage (AMD) and high concentrations of sediment and dissolved heavy metals, which can severely impact mussels (O'Bara et al. 1982, Layzer and Anderson 1992). AMD can alter the water chemistry of streams, making environmental conditions intolerable for mussels or their fish hosts. Increased concentrations of dissolved heavy metal ions (Cd, Cu, Pb, Hg, Ni, Zn) are associated with AMD and can be toxic to mussels (Keller and Zam 1991). Ferric hydroxide (called 'yellow boy') can precipitate on stream bottoms in AMD-impacted areas, effectively smothering benthic biota (O'Bara et al. 1982). Increased sediment loads associated with mining can affect mussels and other aquatic fauna as well (Layzer and Anderson 1992, Houp 1993). The sediment may be coal fines from processing sites or surface soil that has been removed in strip mining. Downstream distances affected by coal mining vary depending on the stream, intensity and type of mining, and season (Rikard et al. 1986, Layzer and Anderson 1992). Most of the coal mining in Tennessee occurs in the BSF watershed, especially the New River drainage (NPS 2003a, Ahlstedt et al. 2005).

Gas and oil extraction also is prevalent in the BSF watershed (NPS 2003a), which can significantly impact mussels (Warren and Haag 2005). High chloride levels from brine wastes associated with oil extraction are present in the BSF (Rikard et al. 1986, Layzer and Anderson 1992) and are implicated in mussel declines in northern portions of the drainage (Warren and Haag 2005). Keller et al. (1998) observed mussel mortality after oil spills in streams, but stated that little is known of the long-term impacts of oil contaminants in sediments on mussels. Studies assessing the effects of oil spills on other stream invertebrates reported that increased levels of petroleum products were correlated with decreases in the abundance of sensitive taxa such as Ephemeroptera, Plecoptera, and Trichoptera (Poulton et al. 1997). The impacts of petroleum spills have been detected almost 100 km downstream of the spill site (Poulton et al. 1997). In 1994, 82% of Tennessee's total oil production and 60% of its total gas production came from Fentress and Morgan counties in the BSF watershed (NPS 2003a).

Roads, rails, and trails are another potential source of water pollution in the BSF (O'Bara et al. 1982). Sediment loads and contaminant concentrations in streams tend to increase below bridges (Maltby et al. 1995), which can interfere with normal mussel growth and reproduction. Common roadway contaminants include hydrocarbons and heavy metals (Maltby et al. 1995), and sediment especially from unpaved dirt roads (Rikard et al. 1986). Catastrophic toxic spills have extirpated mussels in other regions (Jones et al. 2001) and are a concern for mussel populations in the BSF. Equestrian trail use in the NRRA also is a known threat. The NRRA is well known for its horse trails, and horseback riding is a very popular activity. Horse trails cross the BSF at two designated locations, but there are several other locations on the BSF and its tributaries where crossings are known to occur. Horses can crush mussels as they cross the river, and, although this is not believed to be a major mortality factor, it is of concern to endangered mussel species within the NRRA (NPS 2003a).

Agriculture can affect mussels in several ways and is listed as a dominant threat to freshwater biota (Richter et al. 1997). Most of the land in the BSF watershed is currently forested; however, there are a few areas of agriculture that may affect mussel populations. Agriculture removes natural vegetation from the landscape, which often leads to changes in runoff patterns. Changes in drainage patterns, including flash floods and high sediment loads, can alter stream habitat (Poole and Downing 2004).

Urbanization also is detrimental to mussels. Land that is developed or urbanized usually is paved and impervious to water (Wheeler et al. 2005). Since water cannot infiltrate the surface, amount of runoff increases and can alter hydrologic patterns causing severe flash floods, bank erosion and low base flows, which alter stream habitat (Klein 1979, Wheeler et al. 2005). Urban land also may impact mussels through increased levels of contaminants, such as motor oil, industrial products, heavy metals, and excessive nutrients (Wang et al. 2001, Wheeler et al. 2005). No large cities are located within the BSF watershed, but a few industrial and residential areas are located near streams.

Other potential impacts to mussels in the BSF come from permitted point source discharges. Facilities with discharges listed in the National Pollutant Discharge Elimination System (NPDES) are found in the BSF watershed, most of which are associated with coal mining and wastewater treatment facilities. Goudreau et al. (1993) reported that wastewater treatment plants release chemicals that affect normal mussel growth and reproduction. These discharges can potentially affect restoration efforts.

For successful mussel restoration, it will be important to identify areas threatened by the above land uses. There are many methods to assess whether a site is affected by surrounding land uses, including water quality monitoring, benthic macroinvertebrate and/or stream fish sampling, and conducting a threat assessment based on data from remote sources, such as topographic maps and aerial photos (Bryce et al. 1999, Gergel et al. 2002). A threat assessment based on potential threats is a simple and inexpensive way to evaluate the status of a site without requiring repeated visits. Bryce et al. (1999) showed that results of threat assessments were comparable to results based on water quality and macroinvertebrate sampling. Zimmerman (2003) used a similar approach in conducting an ecological risk assessment to identify potential mussel release sites on the Clinch River in Virginia. She used field observations and data from geographic information system (GIS) layers to evaluate and rank river sections based on potential habitat and potential threats to mussels. My study used methods modified from Bryce et al. (1999) and Zimmerman (2003) for site evaluations in the BSF; proximity and intensity of threats in the watershed of each site were used to rank sites on a relative threat scale.

Conservation value

Despite decades of intense resource extraction and pollution, the BSF system supports a large number of species, some of which occur only infrequently elsewhere in the Cumberland River system (Ahlstedt et al. 2005). Management plans for the NRRA include restoration of mussel fauna to historic levels and monitoring of established populations. There are several sites throughout the NRRA that may be of importance to management goals for BSF mussel resources. Prioritizing sites based on conservation value is a way to identify which sites are most important to long-term preservation of freshwater mussels in the BSF (Winston and Angermeier 1995). By identifying areas with high conservation value, restoration efforts can be focused on areas in need of urgent protection (Angermeier and Winston 1997). Focusing on high priority sites also allows restoration efforts to be more cost-effective. Data on mussels in the BSF from Ahlstedt et al. (2005) were available and used to assess conservation value among sites.

There are several ways to measure conservation value, including simple metrics such as species richness or species diversity (Angermeier and Winston 1997). Other more complex metrics that account for species abundance, rarity, and endemic status can provide further information on which sites may best preserve aquatic fauna into the future (Winston and Angermeier 1995, Filipe et al. 2004). According to Angermeier and Winston (1997), different measures, i.e. species richness and Index of Center of Density (ICD), rate sites differently in their conservation value. By considering several measures of conservation value, managers can determine what their management goal is (i.e., preserving federally protected species, high species richness, or large, healthy populations) and which sites are most critical to meeting those goals.

Methods

Threat assessment

A threat assessment similar to those of Bryce et al. (1999) and Zimmerman (2003) was conducted on 39 sites in the BSF. These sites were surveyed for mussels by Ahlstedt et al. (2005) (Figure 3.1, Appendix 3-A). Instead of using habitat and threat variables as assessed in Zimmerman (2003), this study assessed only land use threats in the BSF watershed. All sites had in-stream habitat features that seemed favorable for mussels (Ahlstedt et al. 2005).

Land use threats were identified in the BSF watershed that may affect mussel survival, growth, or recruitment, to include coal mining, oil and gas extraction, roads, rails, trails, agriculture, and urban land (Ahlstedt et al. 2005). NPDES sites also are potential threats to mussels, but exact coordinates of NPDES sites were not accessible. Threats from NPDES sites were usually accounted for in percent urban land and coal mining threat variables and were not included explicitly in this assessment. Table 3.1 lists known impacts to aquatic fauna, effective threat levels or thresholds, and corresponding literature for the threats used in this assessment. Data for each threat were obtained from sources listed in Table 3.2., and were entered into a Geographic Information System (GIS) spatial analysis program (ArcGIS, ESRI Technologies, California). An elevation raster was used to create a stream network. Site locations from Ahlstedt et al. (2005) were plotted and, if needed, were adjusted so that they resided on a stream grid cell. Watershed boundaries were calculated upstream from each site location using the elevation raster. Watershed surface flow length was used in measuring distance to nearest threat and level of threat at each site, instead of using a straight-line distance. Measurements at each site included distance from site to the nearest coal mine, oil or gas well, paved road crossing and unpaved road crossing, and the number of coal mines and oil or gas wells, paved road density and unpaved road density, and percent of agriculture or urban land within a 2 km upstream area of each site. By limiting the upstream evaluation area, watershed sizes were comparable in both tributary and mainstem sites (1.2-3.9 km^2). Also, the majority of site watershed areas overlapped since some tributary and all mainstem sites were located in a nested fashion. By evaluating a smaller portion of the watershed, sites were more independent of each other. A distance of 2 km was used based on work by Wang et al. (2001) and Diamond et al. (2002), who found that macroinvertebrates, fish, and in some cases mussel fauna were associated most strongly with land uses approximately 2 km upstream of a site.

Distinctions were made between paved and unpaved roads because of the differences in the types of threats they present to mussels. The greatest threat from paved roads was believed to catastrophic highway spills that could potentially impact mussels in large sections of nearby streams. Contaminants from roadway runoff, including petroleum products, herbicides, heavy metals, etc., also were a potential threat to nearby mussels. Paved roads included any transportation features that were classified as primary, secondary, or local roads. Railroads were included in this category because of the high levels of herbicides used in their maintenance.

Unpaved roads were not thought to have the potential for large-scale, catastrophic threats that paved roads have. The most significant potential threat from unpaved roads was believed to be heavy sediment inputs, which were believed to affect aquatic life in certain portions of the BSF (O’Bara et al. 1982). Unpaved roads included graveled and dirt vehicular roads and walking, biking, ATV, and horse trails, which occur throughout the BSF watershed.

Distance variables from site to agriculture and urban development were not included because of the added complexity of measuring distance between threat data in a raster layer and site locations in a point layer. If large amounts of agriculture and urban development would have been present near sites, these would have been important variables to include in assessing threat. However, few of these threats existed in the BSF land cover data, so agriculture and urban distance variables were not included.

An index was constructed to rank the sites on a relative scale of potential impact. The index used categories of not impacted (1), somewhat impacted (2), and impacted (3) for each of the ten variables, depending on proximity or frequency of that variable (Table 3.3). Sites were assigned an index value of 1, 2 or 3 that corresponded to the impact category into which the measured variable fell. Because coal mining is believed to be a very severe threat to mussels, index values of 1, 3 or 5 were assigned in order to weight sites with coal mining threats more heavily. Index values for each threat were summed to estimate overall threat level at a site. Higher summed index values indicated sites that were in close proximity to threats and/or had higher frequencies of threats in a 2 km area, whereas lower summed index values indicated sites that were far from threats and/or had lower frequency of threats in a 2 km area. The summed index values across sites were grouped into categories of low, moderate, or high threats based on natural breaks in the frequencies of summed index values.

Specific threshold values of threats that correspond to not impacted, somewhat impacted, and impacted mussel or other aquatic fauna ranged widely in the literature.

Usually available threat thresholds came from studies evaluating aquatic systems in other geographic areas, which may not be consistent with actual threshold values in the Cumberland Plateau region. Threat values also may vary by what researchers deem 'impacted'. In this study, a range of threshold values was used in three separate indices. The first index used 'expert opinion' values, which were median threat values from published literature. The threshold values used as category bounds in this index seem to best represent levels at which biological impacts would appear (Table 3.3). The second index used 'liberal' values that assigned 'not impacted' status to sites with threats at closer distances or with threats at higher intensities than those of expert opinion values (Table 3.3). The last index used 'conservative' values that assigned 'not impacted' status to sites with threats further away or with threats in lower intensities than the expert opinion (Table 3.3). Low- and high-ranking sites were compared by each index; however, the expert opinion index was generally used to assign threat levels to sites.

Measured values of threat variables at each site were analyzed with principal components analysis (PCA) to seek relationships among sites in terms of measured threat variables. Threat variables also were analyzed with a Spearman rank correlation to identify which variables, if any, were correlated strongly with each other or with the final summed index value of a site. An additional Spearman rank correlation was performed on the summed index values of the three indices. SAS statistical package software (SAS Institute 2002) was used to complete all procedures.

Conservation value

Data on mussel species at each of the 39 sites evaluated in the threat assessment were obtained from Ahlstedt et al. (2005). Nineteen of the sites were on the mainstem

BSF; the remaining 20 sites were on tributaries of the BSF. Surveys were conducted by snorkeling and occurred throughout the year (Ahlstedt et al. 2005). The number of individuals of each species and the time spent searching for mussels were recorded for each site. Twenty-six species were reported, including a live individual believed to be *Pleurobema cava*, but its identification was not confirmed. That individual was included in these analyses as well as recently dead, identifiable shell material, or relic shells, found at some sites (Appendix 3-B).

Four measures of conservation value were calculated for each site: 1) species richness, 2) number of endangered species, 3) index of centers of density (ICD) (Winston and Angermeier 1995), and 4) value of an area (VA) (Filipe et al. 2004). Species richness and number of endangered species were simple metrics to assess conservation value among sites. Species richness indicated sites with many mussel species and was calculated by summing the number of species per site. The number of endangered species indicated which sites have rare and highly imperiled species, as well as legal requirements for their conservation. The number of endangered species equals those federally listed as endangered (no threatened species were reported in the BSF surveys).

ICD is a more complex metric that designates specific sites as 'population centers'. The ICD metric calculates the proportion of a species' density at one particular site relative to all other sites in the study area. It then averages species density proportions at that site for all species found there. A site with large proportions of abundance of several species is a high quality site. Focusing on sites with relatively high ICD values should allow managers to best conserve a diversity of species into the future. ICD was calculated by using the following equations from Winston and Angermeier

(1995):

$$x_{i,k} = D_{i,k} \Big/ \sum_{j=1}^{T} D_{i,j} \qquad (1)$$

$$ICD_k = \frac{1}{R_k} \sum_{i=1}^{S} x_{i,k} \qquad (2)$$

where *D* is density of species *i*, *R* is number of species observed at site *k*, *S* is total number of species collected in the region, *T* is total number of sites, and *j* and *k* vary from 1 to *T*. In my calculations, catch per unit effort (CPUE) was used an index of density.

VA (value of an area) was the most complex metric included in analyses. It combined information on species distribution and abundance, rankings of species' endangerment, and proportional abundances of species at a site to designate sites of highest conservation value. There were two steps in assigning VA to a site. First, each species was assigned an individual conservation value (VS_k) that depended on its abundance (*O*), distribution (*T*), and endangerment (*E*) (equation 3). For example, species that were found in low numbers, in only a few sites within the study area, or were federally endangered received higher individual species values, while species that were common in the study area, were widely distributed in the study area, or were listed as stable received lower individual species values (Appendix 3-C). Filipe et al. (2004) included a ranking of species endemism in VA, but in this analysis, endemism was replaced with endangerment. Next, at each site, a species' value was multiplied by its proportional presence at a site relative to all other sampled sites, and these values were summed for all species at a site (equation 4). This provided a value for the area (or site).

Equations from Filipe et al. (2004) are as follows:

$$VS_k = \frac{\left(a \frac{1/O_k}{\sum_{i=1}^{S} 1/O_i} + b \frac{1/\ln T_k}{\sum_{i=1}^{S} 1/\ln T_i} + c \frac{1/E_k}{\sum_{i=1}^{S} 1/E_i} \right)}{3} * 100 \qquad (3)$$

$$VA_j = \sum_{k=1}^{S} \left(P_{k,j} * VS_k \right) \qquad (4)$$

In equation 3, S is the number of species considered, O_k is the total number of sampling sites where species k occurred in all samples, T_k is the total number of captured individuals of species k in all samples, and E_k is the endangered value of species k according to its global status range. Variables a, b, and c are weighting factors that can fluctuate according to the importance placed on conserving distribution (a), abundance (b), or endangerment (c), as long as their sum is 3. Since T_k ranged from 1 to 1907, the natural log of (T_k + 1) was used. Endangered values were assigned as follows: federally endangered species were assigned a value of 1, species listed as having ‘special concern’ in Williams et al. (1993) were assigned a value of 2, and all other species were assigned a value of 3. A value of 1 was used for variables a, b, and c (Appendix 3-C). In equation 4, $P_{k,j}$ is the proportion of CPUE for species k at site j, and VS_k is the conservation values of species k.

The top 5 sites in each measure were compared. Site rankings of conservation value among measures were analyzed with a Spearman rank correlation analysis, using SAS statistical package software (SAS Institute 2002).

Results

Threat assessment

Using data from the Office of Surface Mining, expertise of Ron Cornelius (NPS), and the NPS on-line database, a total of 3,147 coal mines were located in the BSF watershed, which include operational, abandoned and/or reclaimed shaft and surface mines and coal washing facilities. Number of mines within 2 km of mussel sites ranged from 0 to 33 (Table 3.4). Mean distance from site to nearest coal mine was 11.5 ± 1.49 (se) km. Expert opinion index scores for both coal mining variables ranged from 1 to 3, with a mode of 1 (Table 3.5). Mine locations were mainly in the southern and eastern portions of the BSF watershed.

There are 1,436 oil and gas wells in the BSF watershed, using data from Ron Cornelius and the NPS on-line database. Number of wells within 2 km of sites ranged from 0 to 54 (Table 3.4). Mean distance to nearest well was 1.75 ± 0.2 km. Expert opinion index scores for well variables ranged from 1 to 3, with a mode of 1 for distance to nearest well, and a mode of 3 for number of wells (Table 3.5). Most wells were located in the Clear Fork and North White Oak Creek watersheds.

Two major highways cross the mainstem BSF, including State Route 297 at Leatherwood Ford (BSF02 and BSF03) and State Highway 92 at Yamacraw (BSF19). Two major highways cross tributary streams, including U.S. Highway 27 at New River

(NR1), and State Highway 52 at Clear Fork River (CF2) and White Oak Creek (WOC1). Railroads also crossed the New River near NR1 and about 5 km upstream of NR2. Numerous other small roads and trails crossed streams throughout the watershed. Two major equestrian trails cross the BSF at Station Camp Ford (BSF08) and Big Island Ford (BSF11). Mean distance from site to nearest paved crossing was 3.7 (±0.60) km; mean distance from site to nearest unpaved crossing was 2.8 (±0.75) km. Mean paved road density was 0.66 (±0.15) km/km^2; mean unpaved road density was 1.20 (±0.18) km/km^2. Expert opinion index scores ranged from 1 to 3 for distance to nearest crossing and road density for both in paved and unpaved variables. The mode for all road variables was 2, except for paved road density when the mode was 1 (Table 3.5).

Land cover in the BSF watershed included few urban and agricultural areas. Percent urban area within 2 km of sites ranged from 0% to 2.01%, with a mean of 0.35% (±0.08); percent agriculture ranged from 0.04% to 8.07%, with a mean of 0.97% (±0.29) (Table 3.4). Expert opinion index scores for % urban ranged from 1 to 2, with one site receiving a score of 2; all other sites received a score of 1. All 39 sites received a score of 1 in the % agriculture variable (Table 3.5).

Summed site index scores ranged from 10 to 25, 10 to 22, and 11 to 27 in the expert opinion, liberal, and conservative indices, respectively (Table 3.6, Figure 3.2). However, rankings of sites among indices were strongly correlated. All correlation coefficients were above 0.60 (Table 3.7). The highest score possible in all indices was 34; the lowest score possible was 10. Threat variable index scores by site are listed in Table 3.5, Appendices 3-D & 3-E.

Two of the least threatened sites, Peter's Bridge on Clear Fork River (CF1) and the shoal upstream of the mouth of Bear Creek (BSF15), received a score of 10 using the expert opinion and liberal indices, indicating a relatively low threat level. CF1 and BSF15 also scored lowest in the conservative index (11 and 14, respectively). Other sites that scored relatively low were Clear Fork upstream of Burnt Mill Bridge (CF4) and 500 yards from mouth (CF5), the mainstem BSF at (BSF01), Parch Corn Creek (BSF09), mouth of William's Creek (BSF12) shoal downstream of Oil Well Branch (BSF13) and mouth of Heuling Branch (BSF14), New River at Highway 27 bridge (NR1) and near mouth (NR3), North White Oak Creek upstream of Coyle Branch (NWO4), No Business Creek near mouth (NBC1) and upstream of ATV crossing on Laurel Fork of North White Oak Creek (LF1) (Figure 3.3).

The site near Blue Heron (BSF18) received the highest score in all indices, scoring 25, 22, 27 in the expert opinion, liberal, and conservative index, respectively, indicating a relatively high threat level. Other sites that scored relatively high were the mainstem BSF upstream of Leatherwood Ford (BSF02), Leatherwood Ford (BSF03), upstream of Salt Branch (BSF16), Big Shoal (BSF17) and Yamacraw (BSF19), Clear Fork at Brewster Bridge (CF2) and Sheep Ranch (CF3), William's Creek (WC1), and North White Oak at mouth of Mill Creek (NWO1), Zenith (NWO2), upstream of the confluence of Laurel Fork (NWO3), and upstream of mouth (NWO6) (Figure 3.4).

In PCA, the first two principal components accounted for almost 50% of the variation. The first component was influenced most strongly by distance to nearest well and % urban variables (Table 3.8). The second component was influenced most strongly by distance to nearest unpaved crossing and number of wells (Table 3.8) No single variable explained a large amount of variation among sites. Some trends between site variation based on measured threats and the expert opinion summed index value were apparent (Figure 3.5). Low-scoring, low-threat sites were associated with low numbers of wells and low distances to unpaved crossings.

Correlation analysis also showed that threat variables were somewhat correlated. The strongest correlations occurred between well distance and number of wells (-0.73), paved road crossing distance and paved road density (-0.65), unpaved road crossing distance and unpaved road density (-0.64), mine distance and number of mines (-0.58), and mine distance and percent urban (-0.5). Two variables correlated with the final summed index value of sites, which were distance to nearest unpaved crossing and number of mines with coefficients of -0.53 and 0.51, respectively (Table 3.9).

Conservation value

Among sites, species richness ranged from 0 to 23, number of endangered species ranged from 0 to 6, ICD values ranged from 0 to 0.37, and VA values ranged from 0 to 24.0 (Table 3.10). The shoal downstream of Station Camp Creek (BSF08) and Big Island (BSF11) on the mainstem BSF ranked highest in species richness (20 and 23, respectively), number of endangered species (5 and 6, respectively), and VA (23.2 and 24.0, respectively) (Table 3.10). The highest ICD value (0.37) was for the sight on North White Oak Creek upstream of its confluence with Laurel Fork (NWO4). At this site, only one species was present, and 37% of its entire sampled CPUE was located at that particular site.

Eight sites contained no mussels and were ranked at the bottom of all measures. These sites included the mainstem BSF at the confluence of Clear Fork and New rivers

(BSF01), Laurel Fork upstream of ATV crossing (LF1), No Business Creek 500 yards from mouth (NBC1), New River at the Highway 27 bridge (NR1) and 500 yards from the mouth (NR3), North White Oak Creek at the mouth of Mill Creek (NWO1), Station Camp Creek upstream of mouth (SCC1), and William's Creek upstream of mouth (WC1).

BSF sites typically had higher species richness than tributary sites, but a few tributary sites ranked above some BSF sites. Mainstem sites also tended to have a higher number of endangered species, although, some tributary sites negated that generalization. In the ICD measure, a tributary site (NWO3) ranked highest, and more tributary sites ranked above mainstem sites in this measure than in others. However, there are some mainstem sites that also rank high in this measure. In the VA measure, mainstem sites tend to rank above tributary sites.

When evaluating conservation value measures, the top 5 sites from each were compared, except when reporting number of endangered species. Only the top four sites having five or more endangered species were compared; four sites contained three endangered species, so considering only the top five sites with this measure was not feasible (Table 3.11). The shoal downstream of Station Camp Creek on the mainstem BSF ranked among the top 5 sites for all conservation value measures. Big Island ranked among the top 5 sites for 3 measures. Sites in the top 5 for at least 2 of the measures included the site upstream of Rough Shoals Branch (BSF04), downstream of Rough Shoals Branch (BSF06), Parch Corn Creek (BSF09), the shoal downstream of Oil Well Branch (BSF13), and the shoal upstream of Bear Creek (BSF15) on the mainstem BSF. Sites that were in the top for only one measure included sites on North White Oak Creek upstream from confluences of Laurel Fork (NWO3) and Coyle Branch (NWO4), which ranked high in ICD.

Correlation analysis showed that all measures were somewhat correlated (Table 3.12). VA and ICD ranks were the most correlated of all conservation value ranks (0.92), while ranks using number of endangered species and ICD were least correlated (0.62). Ranks using number of endangered species typically were the least correlated of any of the species composition measures, with values ranging from 0.62 to 0.69. Although measures showed tendencies to rank sites similarly, rankings were not completely identical.

To look for trends between measured threat variables and conservation value, conservation value according to each measure and results of threat variable PCA were overlaid (Figures 3.6, 3.7, 3.8, and 3.9). The highest conservation value sites were typically associated with low distance to unpaved road crossings, low number of wells, moderate distance to wells, and moderate percent of land in urban use.

Discussion

Threat assessment

In general, sites in the BSF watershed had scores at the low end of all possible values in the threat assessment, indicating that some measured threats in the BSF are not at maximum levels of potential impact for mussels. The highest scoring site received a score of 25 out of 34 in the expert opinion index. Percent of the watershed in urban and agriculture was relatively small at all sites, which kept the summed index scores from reaching the highest possible values. A study by Gaydos et al. (1986) analyzed aerial photos taken between 1974 and 1976, and estimated that the Cumberland Plateau in Tennessee was approximately 75% forest, 20% agriculture and open land, 3% mining, and 2% urban or rural residential. Using 1992 NLCD land cover percentages, the entire BSF watershed was 94.1% forested, 4.5% agriculture, 0.6% urban or developed 0.4% open water, 0.3% barren, and 0.1% wetlands. If the BSF was similar to the rest of the Cumberland Plateau in the 1970s, amount of forest cover has increased and agricultural areas have decreased in past decades, and urban areas continue to make up little of the land cover. Large amounts of forest in a watershed (i.e., greater than 90%) are typically protective of water quality, and have been shown to be associated with high fish and macroinvertebrate IBI scores in Wisconsin (Wang et al. 1997).

The most widespread threat throughout the BSF seems to be transportation corridors and, in specific areas, coal mines and oil and gas wells. No sites appeared to be threatened by urban or agricultural land use since all sites received scores of 'not impacted' in these categories except one site that scored 'somewhat impacted' for % urban. Sites that had high summed index values typically had high densities of roads, were close to a crossing, and were threatened by either coal mines or oil and gas wells. Many of the sites with low summed index values also had high road densities and were near crossings, but were typically unimpacted by coal mines or oil and gas wells.

Sites that were nearest coal mines or had high numbers of mines nearby were located on the lower portions of the main stream BSF. Blue Heron, an abandoned mining camp that is now an interpretive center, is located on the bank of BSF and has relatively high urban land use and road densities, as well as several abandoned mines. Sites nearest oil and gas wells or with high numbers of wells nearby were mostly located in North

While Oak Creek or Clear Fork River. Sites on the mainstem BSF that also scored high in well variables were usually in upstream areas near the confluence of Clear Fork or North White Oak Creek. Even though mine and well threats tended to be somewhat clustered by sub-watershed, mines and wells were distributed haphazardly throughout the sub-watershed. Because of this, sites within a sub-watershed did not always have similar overall threat levels. For example, Clear Fork River contained sites with some of the lowest and highest summed index values.

Sites with lowest land use threats were generally located in the middle sections of the mainstem BSF and on tributaries in the west-central portion of the watershed, while sites on the lower BSF and on southwestern tributaries usually had higher threats. Results of the current land use threat assessment are similar to results from previous and ongoing assessments of water quality in the BSF, with a few exceptions (O'Bara et al. 1982, Rikard et al. 1986, G. Johnson, U.S. Geological Survey, personal communication). Lower portions of the BSF and its associated tributaries tend to have somewhat poorer water quality than other portions of the BSF (O'Bara et al. 1982, G. Johnson, personal communication), which may be due to the high land use threats in that region. The New River also tends to have somewhat poorer water quality than other areas in the BSF watershed (Rikard et al. 1986, G. Johnson, personal communication), but has only low to moderate levels of threats from nearby land uses. This indicates that sites may be affected by land uses further upstream than were considered here. Other tributaries with variable levels of land use threats, such as North White Oak Creek and Clear Fork, tend to have water quality parameters that reflect slight degradation, while not being severely degraded throughout their length (O'Bara et al. 1982, Rikard et al. 1986, G. Johnson, unpublished data).

In general, the threat indices seemed to work well in evaluation of BSF sites. No variables were strongly correlated or redundant, and the index provided a good overall assessment of threat level at a site, relative to other sites. A multi-variate approach seems to be appropriate to evaluate threat levels among sites since no single variable could indicate threat level at a site. Using different bounds for 'not impacted', 'somewhat impacted', and 'impacted' categories did not change site rankings to a great extent, although rankings were not completely identical. Given the lack of region-specific category bounds, the expert opinion index seems most appropriate for evaluation of threats at BSF sites.

Efforts were made to use the most accurate, available data to assess threats. However, several potential sources for error were evident. In using GIS datasets, there is always some level of uncertainty associated with measuring distance between specific points and with using outdated data. This could be reduced by obtaining the latest available data in fine-scale formats. Another possible problem was introduced when deciding what threat variables to measure. For example, mine locations included both active and inactive sites, which likely do not have the same effects on mussels, but were lumped together for ease of analysis. If more was known about impacts from different mine types, that information could be used to refine the index matrix. Another difficulty in the assessment comes from using a specific distance to determine whether sites are likely impacted. Usually researchers do not know specific effective distances of threats or effects of combined threats to mussel populations. The values used here were 'expert opinions' at whether a site would be impacted, and should not be taken to represent findings of actual threshold intensities or effective distances. This analysis should reinforce the fact that those threshold threat values remain largely unknown, and may vary from region to region. Further investigations into biological impacts from measured threats in the BSF would help to reduce these uncertainties.

Conservation value

The shoal downstream of Station Camp Creek (BSF08) on the mainstem BSF was of high conservation value in all analyzed measures; Big Island (BSF11) on the mainstem BSF was of high conservation value in 3 of the 4 measures. These sites contain high numbers of species, several multiple endangered species, fairly large centers of density for numerous species, and high abundances of species with high individual values. These sites represent areas unique in the BSF and should be of high importance to managers for conservation.

Other sites that ranked high in all measures of conservation value include the mainstem BSF near Rough Shoals Branch (BSF04, BSF05, BSF06), the mouth of Steven's Branch (BSF07), Parch Corn Creek (BSF09 & BSF10), the mouth of William's Creek (WC1), shoal downstream of Oil Well Branch (BSF13), shoal upstream of Bear Creek (BSF15), and Big Shoal (BSF17), which are all mainstem sites. Previous studies confirmed that endangered species typically occur at sites with high mussel diversity and that mussel diversity typically increases with larger stream size (Vaughn and Pyron 1995). An exception to this was the occurrence of the endangered Cumberland elktoe in tributary sites that usually had low species richness. The Cumberland elktoe seems to be a headwater species and was often the dominant or only species at a site. Sites containing the Cumberland elktoe were often not as species-rich as the previously listed sites, but typically supported a sizeable population of this species. Sites such as this include all sites on Clear Fork (CF1-5), five sites on North White Oak Creek (NWO2-6), and White Oak Creek (WOC1). If managers were to maintain only sites with high species richness, sites with the endangered Cumberland elktoe would largely be ignored.

ICD site rankings had interesting comparisons with site rankings of species richness and number of endangered species. Sites that were centers of high density for several species were not always the same ones that had high species richness. For example, two North White Oak sites (NWO3 & NWO4) and two mainstem BSF sites (BSF06 & BSF04) had highest indices of population density. However, these sites had species richness of 1, 2, 8 and 16, respectively, and number of endangered species of 1, 1, 0, and 2, respectively. This ICD metric identified some sites of conservation value that were not as highly ranked using the simpler metrics.

VA was the most complex metric of the four used, but had similar rankings to ICD and species richness. Sites that were highly ranked in this measure had several rare or endangered species and contained high densities of several species. A major part of this metric was the value assigned to individual species. The species value equation gave high values to endangered species, which was desirable. However, one flaw was that the value of a species was greatly influenced by distribution within the NRRA; some species were found at only one site which resulted in a large value for a species, even though it may be common throughout its range outside the NRRA (Appendix 3-C). Even though some common, non-endangered species were given high individual species values, ranks of sites according to this value should be applicable to addressing which sites are of highest conservation value.

Management recommendations and future land use

By combining results of the threat and conservation value assessments, appropriate management actions can be identified on a site-by-site basis. This threat assessment was meant to relate what threats may potentially impact sites in the BSF based on published impacts to mussels and other aquatic biota at sites in other watersheds. It was not meant to categorize specific land uses that affect distribution of mussels. Sites varied with respect to level of threat and conservation value. Theoretically, sites with high conservation value also should have low threats, while sites with low conservation value should have high threats. However, this was not always the case with this data set. Some sites of high conservation value were relatively unthreatened, while others were moderately to highly threatened. The reverse was also true; no mussels were found at sites that were relatively unthreatened and some mussels were found at sites that were relatively threatened (Appendix 3-F). With little data on past mussel populations, it is difficult to link land use threats with actual biological impacts. Long-term monitoring of mussel populations can help managers discover which threats actually impact mussel fauna, and can further refine management recommendations in the BSF NRRA.

BSF NRRA managers should be aware of sites with high conservation value and moderate to high threats. These sites include the mainstem BSF upstream and at Rough Shoals Branch (BSF04 & BSF05), downstream of Rough Shoals Branch (BSF06), mouth of Steven's Branch (BSF07), the shoal downstream of Station Camp Creek (BSF08), the island downstream of Parch Corn Creek (BSF10), Big Island (BSF11), and Big Shoal

(BSF17), and North White Oak Creek upstream of confluence with Laurel Fork (NWO3) (Appendix 3-G). Mussels are not known to be currently impacted from the identified threats, although no assessment of recruitment has been conducted. These sites should be monitored for potential declines over time. When possible, land use threats should be reduced or removed from areas surrounding these sites, or if land uses intensify, relocation of mussels may need to be considered.

Several sites had high conservation value and low threat levels. At these sites, threats that may affect mussels are minimal, and those levels should be maintained to prevent potential negative impacts to mussels. All such sites were on the mainstem BSF, and specifically were the mouth Parch Corn Creek (BSF09), mouth of William's Creek (BSF12), shoal downstream of Oil Well Branch (BSF13), and shoal upstream of Bear Creek (BSF15) (Appendix 3-H).

There were several sites with low to moderate threat levels that currently have low to moderate conservation values. These sites included the BSF at the confluence of New River and Clear Fork (BSF01) and mouth of Heuling Branch (BSF14), North White Oak Creek at upstream of confluence with Coyle Branch (NWO4), at the O and W ATV crossing (NWO5), Laurel Fork upstream of ATV crossing (LF1), New River at Highway 27 bridge (NR1), Silcox Ford (NR2) and upstream of mouth (NR3), No Business Creek near mouth (NBC1), Crooked Creek near mouth (CC1), Station Camp Creek near mouth (SCC1), White Oak Creek below Highway 52 bridge (WOC1), and Clear Fork upstream of Peter's Bridge (CF1), upstream of Burnt Mill Bridge (CF4) and upstream of mouth (CF5) (Appendix 3-I). Some of these sites contained few to no mussels and may be suitable for mussel restoration, although a more thorough habitat and water quality analysis is needed before a mussel relocation is considered.

Some sites had moderate to high levels of threats, and were not of high conservation value. Sites such as this included the BSF at Leatherwood Ford (BSF02 & BSF03), below gauge above Salt Branch (BSF16), Blue Heron (BSF18) and Yamacraw (BSF19), William's Creek near mouth (WC1), North White Oak Creek at mouth of Mill Creek (NWO1), Zenith (NWO2) and upstream of mouth (NWO6), and Clear Fork at Brewster Bridge (CF2) and Sheep Ranch (CF3) (Appendix 3-J). These sites are not recommended for restoration unless land uses are mitigated or more thorough research shows that identified threats will not impact mussels. If mussels are restored before mitigation, there is a high probability that restoration will fail because of negative effects of land uses at these sites.

Future changes in land use may alter the recommended action for a site. Oil and gas wells may be capped or mines closed and reclaimed, but potential impacts from past threats can remain. For example, a reclaimed surface mine area in the New River watershed suddenly became unstable in summer 2005 and resulted in a large landslide that filled downstream areas with sediment for several kilometers (S. Bakaletz, personal communication). As land uses change over time, probabilities of sites being impacted by threats also will change.

Few predictions can be made on how land uses will change in the BSF watershed in the next several decades. It is unknown whether more oil and gas wells will be drilled, but no new permits have been issued in Tennessee since the early 1990s (E. Foust, Tennessee Dept. of Environment and Conservation, personal communication).

Fluctuating fuel costs dictate the frequency and duration of currently active wells, which makes it difficult to predict the effects that these operations may have on mussel populations. However, no significant changes are projected to take place in the near future, and the NRRA will not allow new drilling on its property.

Future coal mining is of concern for mussel populations in the BSF (Layzer and Anderson 1992). Since the passage of the Surface Mine Control and Reclamation Act in 1977, the amount of coal mining has decreased in the BSF watershed because of the cost of producing electricity while adhering to EPA guidelines. However, with improved technology, the high sulfur coal in the BSF watershed is now more affordable to mine and use, and there is a possibility that strip mining may start soon in the New River drainage on a large scale (V. Morel, National Parks Conservation Association, personal communication). If mining proceeds and significant steps are taken to alleviate potential downstream impacts, this operation may have limited effect on mussels. However, it seems likely that surface mining on a large scale such as this will have some negative repercussions on mussel fauna in the BSF (Layzer and Anderson 1992), and non-government organizations are filing petitions to stop additional mining (V. Morel, personal communication).

With the exception of mining in the New River drainage, no other activities are scheduled or documented that may alter land use significantly in the BSF watershed. Within the NRRA, a few currently forested areas are scheduled to be returned to native glade and prairie vegetation (NPS 2003b), but these areas are small and should not affect mussels. Land use on private land may change, but mostly likely it will remain mostly forested in the near future. The number of roads, railways and trails in the NRRA should not change dramatically. According to the NRRA's master plan, one new campground, some picnic areas, and a few trails are scheduled to be constructed in the next 15 years. The impacts from these activities should have no adverse effect on mussel areas, but proximity of proposed actions to high conservation value sites should be considered and possibly relocated away from these sites. Current land uses seem to be suitable for sustaining freshwater mussels, and, with well-planned restoration efforts, populations of most species should continue to thrive in the BSF NRRA.

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Table 3.1: Summary of threshold threat values and citations used to define index category bounds for selected land uses.

Land use threat	Associated stressor(s)	Levels associated with impacts	Source
Coal mines	Acid mine drainage (AMD), heavy metals, sedimentation (soil or coal fines)	AMD sites affected up to 40 km of stream	Nichols & Bulow 1973
		Up to 10 km of stream impacted from coal processing effluent	Kennedy et al. 2003
		Sediment from surface mines moved 15 km downstream within 4 yrs	Houp 1993
Oil and gas wells	Brine wastes, extraction spills	Steams influenced by close proximity wells (within 100 m); high numbers of wells in area associated with mussel decline	Warren & Haag 2005
		Oil from spill accumulated 40 km downstream	Poulton et al. 1997
Agricultural land	Sedimentation, excess nutrient runoff, channel instability, altered hydrology	Stream habitat and fish IBI scores decreased when agriculture comprised 50% of land use	Wang et al. 1997
		Land use within 2-3 km affected water quality	Houlahan & Findlay 2004
		Some impacts to streams occur when land >30% 'cleared', severe impacts occur when land is >60% 'cleared'; no impacts occur when area is 'completely' forested	Bryce et al. 1999
		Strong relationships between land use and fish and base flow using 1.6 or 3.2 km distances	Wang et al. 2001

Table 3.1: Summary of threshold threat values and citations used to define index category bounds for selected land uses.

Land use threat	Associated stressor	Levels associated with impacts	Source
Roads, rails, trails	Sedimentation, heavy metals, hydrocarbons, de-icing salts, bridge-related impacts (i.e. spills, altered habitat, etc.)	Impacts can extend >1 km downstream	Forman & Alexander 1998
		Varied impacts from major spills, e.g. Certus spill imapcted 11 km of stream, diesel fuel spill impacted macroinvertebrates within 3 km	Jones et al. 2001; Keller et al. 1998
		Road density between 0.5 and 1.5 km/km^2 starts to affect streams	Bryce et al. 1999
		Stream within 100 m of road drainage site is influenced by road	Maltby et al. 1995
		Road contaminants detected 300 m from road	Gjessing et al. 1984
Urban land	Altered hydrology, pollutants in runoff	Streams begin to show habitat damage when urban areas reach 2% of watershed	Booth & Jackson 1997
		Urban impacts become severe between 10% and 20% of watershed	Wang et al. 1997; Booth & Jackson 1997; Wheeler et al. 2005
		Fish and macroinvertebrate IBI scores showed strongest relationship with land use (especially urban and mining areas) within 2 km	Diamond & Serveiss 2001; Diamond et al. 2002
		Major changes in fish and base flow when impervious areas reached 8-12%	Wang et al. 2001; Wheeler et al. 2005

Table 3.2: Sources of geographical information used in BSF threat assessment.

Geographical feature	Description	Source
Paved road crossing	Includes in-stream and above-stream crossings of paved roads and railways	National Park Service database Ron Cornelius, NPS GIS specialist
Unpaved road crossing	Includes in-stream and above-stream crossings of unpaved roads, hiking, biking and horse trails	National Park Service database Ron Cornelius, NPS GIS specialist
Paved road density	Length of all paved roads and railways in a site's drainage basin within 2 km	National Park Service database Ron Cornelius, NPS GIS specialist
Unpaved road density	Length of all unpaved roads, hiking, biking and horse trails in a site's drainage basin within 2 km	National Park Service database Ron Cornelius, NPS GIS specialist
Coal mine areas	Includes active, abandoned, surface and shaft mines	National Park Service database Ron Cornelius, NPS GIS specialist Office of Surface Mining
Oil or gas well locations	Includes active and capped oil and gas wells	National Park Service database Ron Cornelius, NPS GIS specialist
% Agriculture	Includes pasture, hay field, row crops	National Land Cover Dataset (NLCD) 1992
% Urban	Includes low and high intensity residential areas and commercial/ industrial/ transportation areas (little to no overlap with roads and trails)	National Land Cover Dataset (NLCD) 1992
Elevation raster	Digital elevation map of land in BSF watershed (30 m resolution)	National Elevation Dataset

Table 3.3: Indices used in BSF threat assessment. An index value of 1 was assigned if no impacts were associated with that level of threat; a value of 2 when there is somewhat of an impact; and a value of 3 when that level of threat would severely impact a site. For coal mining threats, a value of 3 was assigned for the somewhat impacted category, and a value of 5 for the impacted category. Impact categories are based on values from published literature. Paved and unpaved road density, number of mines and wells, and percent agriculture and urban land were calculated for a drainage area within 2 km of a site.

Expert Opinion Index

	Index value		
Land use threat	**1**	**2 (*3)**	**3 (*5)**
Paved road crossing proximity (km)	>5	1-5	<1
Unpaved road crossing proximity (km)	>2	0.5-2	<0.5
Paved road density (km/km^2)	<0.5	0.5-1.5	>1.5
Unpaved road density (km/km^2)	<0.5	0.5-1.5	>1.5
Coal mine proximity (km)*	>2	1-2	<1
# coal mines*	0	1	>1
Well proximity (km) (oil or gas)	>2	1-2	<1
# wells (oil or gas)	0	1	>1
% Ag	<30%	30-50%	>50%
% Urban or industrial	<2%	2-10%	>10%

Liberal Index

	Index Value		
Land use threat	**1**	**2 (*3)**	**3 (*5)**
Paved road crossing proximity (km)	>2	0.5-2	<0.5
Unpaved crossing proximity (km)	>1	0.1-1	<0.1
Paved road density (km/km^2)	<0.5	0.5-1.5	>1.5
Unpaved road density (km/km^2)	<0.5	0.5-1.5	>1.5
Coal mine proximity (km)*	>1	0.1-1	<0.1
# coal mines*	0	1	>1
Well proximity (km) (oil or gas)	>1	0.1-1	<0.1
# wells (oil or gas)	0	1	>1
% Ag	<30%	30-60%	>60%
% Urban or industrial	<10 %	10-20%	>20%

Table 3.3: Indices used in BSF threat assessment. An index value of 1 was assigned if no impacts were associated with that level of threat; a value of 2 when there is somewhat of an impact; and a value of 3 when that level of threat would severely impact a site. For coal mining threats, a value of 3 was assigned for the somewhat impacted category, and a value of 5 for the impacted category. Impact categories are based on values from published literature. Paved and unpaved road density, number of mines and wells, and percent agriculture and urban land were calculated for a drainage area within 2 km of a site.

Conservative Index

	Index Value		
Land use threat	**1**	**2 (*3)**	**3 (*5)**
Paved road crossing proximity (km)	>10	1-10	<1
Unpaved crossing proximity (km)	>5	1-5	<1
Paved road density (km/km^2)	<0.5	0.5-1.5	>1.5
Unpaved road density (km/km^2)	<0.5	0.5-1.5	>1.5
Coal mine proximity (km)*	>10	2-10	<2
# coal mines*	0	1	>1
Well proximity (km) (oil or gas)	>10	2-10	<2
# wells (oil or gas)	0	1	>1
% Ag	<10%	10-30%	>30%
% Urban or industrial	<2%	2-10%	>10%

Table 3.4: Measured values of threat variables by site in the BSF watershed.

Site	Paved crossing distance (km)	Unpaved crossing distance (km)	Paved road density (km/km^2)	Unpaved road density (km/km^2)	Coal mine proximity (km)	# Coal mines w/in 2 km	Well proximity (km) (oil or gas)	# Wells w/in 2 km (oil or gas)	% Ag	% Urban
BSF01	6.48	4.05	0.00	0.32	4.65	0	1.37	10	0.08	0.33
BSF02	2.52	1.33	0.00	2.45	16.04	0	0.15	3	0.38	0.48
BSF03	0.11	0.11	0.94	1.54	16.28	0	0.38	3	1.63	0.32
BSF04	1.56	0.22	0.19	1.09	21.02	0	0.32	1	0.40	0.12
BSF05	2.55	0.10	0.02	1.19	22.00	0	1.31	4	0.58	0.05
BSF06	2.66	0.22	0.00	1.30	22.12	0	1.42	4	0.59	0.10
BSF07	3.22	0.13	0.00	1.12	22.68	0	1.47	2	0.12	0.18
BSF08	0.10	0.03	0.32	2.20	28.91	0	3.17	0	0.72	0.00
BSF09	1.73	0.60	0.78	2.42	30.54	0	3.11	0	0.12	0.06
BSF10	1.82	0.25	0.65	2.03	30.62	0	3.19	0	0.10	0.05
BSF11	5.97	0.49	0.50	2.59	7.98	0	1.10	1	0.11	0.14
BSF12	1.73	1.09	0.55	1.43	10.74	0	3.86	0	0.04	0.90
BSF13	9.36	1.44	0.00	0.97	3.25	0	2.76	0	0.09	0.47
BSF14	9.94	0.68	0.00	0.65	3.82	0	0.70	1	0.11	0.34
BSF15	12.69	2.68	0.00	0.00	2.34	0	2.46	0	0.20	0.64
BSF16	5.00	1.03	0.15	1.45	0.22	1	2.74	0	0.11	0.43
BSF17	2.93	1.55	0.00	1.08	1.59	2	4.20	0	0.05	0.90
BSF18	6.86	0.03	3.15	5.53	0.58	2	4.41	0	2.39	2.01
BSF19	0.37	0.79	3.46	0.34	0.48	33	3.75	0	0.16	0.10

Table 3.4: Measured values of threat variables by site in the BSF watershed.

Site	Paved crossing distance (km)	Unpaved crossing distance (km)	Paved road density (km/km^2)	Unpaved road density (km/km^2)	Coal mine proximity (km)	# Coal mines w/in 2 km	Well proximity (km) (oil or gas)	# Wells w/in 2 km (oil or gas)	% Ag	% Urban
CC1	0.34	23.19	0.85	0.00	11.04	0	0.85	3	0.08	0.00
CF1	5.48	12.38	0.39	0.00	22.81	0	-	0	0.70	0.00
CF2	0.06	0.42	2.07	0.70	7.83	0	0.90	13	5.01	0.21
CF3	0.43	5.37	2.15	0.00	19.57	0	0.44	25	0.04	0.04
CF4	1.70	1.69	1.98	0.92	2.07	0	2.31	0	8.07	1.83
CF5	6.26	3.83	0.00	0.19	8.42	0	1.43	3	0.04	0.09
LF1	11.75	0.91	0.25	1.78	-	0	2.79	0	0.25	0.06
NBC1	-	1.54	0.00	1.74	7.12	0	1.60	1	0.18	0.00
NR1	0.07	6.36	3.18	0.06	4.81	0	-	0	4.06	1.32
NR2	0.00	13.53	0.25	0.04	2.72	0	0.13	54	1.19	0.40
NR3	7.76	7.23	0.00	0.32	4.34	0	1.38	12	0.31	0.92
NWO1	4.40	0.06	0.60	0.83	1.25	1	3.52	0	5.61	0.03
NWO2	0.14	0.54	0.67	0.69	7.09	0	0.91	12	0.30	0.02
NWO3	2.38	0.46	0.32	1.77	9.32	0	1.45	4	0.23	0.00
NWO4	3.09	2.98	0.00	1.15	13.97	0	1.19	2	0.12	0.00
NWO5	2.64	0.90	0.25	0.79	16.57	0	0.49	2	0.23	0.00
NWO6	0.29	0.23	0.33	0.74	17.26	0	1.18	4	0.13	0.03
SCC1	12.28	0.66	0.00	3.48	-	0	0.83	1	0.35	0.00
WC1	4.45	0.12	0.00	1.58	9.65	0	0.89	7	0.12	0.00
WOC1	0.03	8.50	1.71	0.14	14.75	0	0.35	1	2.73	1.14

Table 3.5: Index values assigned using the expert opinion index by site.

SITE	Paved crossing distance (km)	Unpaved crossing distance (km)	Paved road density (km/km^2)	Unpaved road density (km/km^2)	Coal mine proximity (km)	# Coal mines w/in 2 km	Well proximity (km) (oil or gas)	# Wells w/in 2 km (oil or gas)	% Ag	% Urban	Total
BSF01	1	1	1	1	1	1	2	3	1	1	**13**
BSF02	2	2	1	3	1	1	3	3	1	1	**18**
BSF03	3	3	2	3	1	1	3	3	1	1	**21**
BSF04	2	3	1	2	1	1	3	2	1	1	**17**
BSF05	2	3	1	2	1	1	2	3	1	1	**17**
BSF06	2	3	1	2	1	1	2	3	1	1	**17**
BSF07	2	3	1	2	1	1	2	3	1	1	**17**
BSF08	3	3	1	3	1	1	1	1	1	1	**16**
BSF09	2	2	2	3	1	1	1	1	1	1	**15**
BSF10	2	3	2	3	1	1	1	1	1	1	**16**
BSF11	1	3	2	3	1	1	2	2	1	1	**17**
BSF12	2	2	2	2	1	1	1	1	1	1	**14**
BSF13	1	2	1	2	1	1	1	1	1	1	**12**
BSF14	1	2	1	2	1	1	3	2	1	1	**15**
BSF15	1	1	1	1	1	1	1	1	1	1	**10**
BSF16	1	2	1	2	5	3	1	1	1	1	**18**
BSF17	2	2	1	2	3	5	1	1	1	1	**19**
BSF18	1	3	3	3	5	5	1	1	1	2	**25**
BSF19	3	2	3	1	5	5	1	1	1	1	**23**

Table 3.5: Index values assigned using the expert opinion index by site.

SITE	Paved crossing distance (km)	Unpaved crossing distance (km)	Paved road density (km/km^2)	Unpaved road density (km/km^2)	Coal mine proximity (km)	# Coal mines w/in 2 km	Well proximity (km) (oil or gas)	# Wells w/in 2 km (oil or gas)	% Ag	% Urban	Total
CC1	3	1	2	1	1	1	3	3	1	1	**17**
CF1	1	1	1	1	1	1	1	1	1	1	**10**
CF2	3	3	3	2	1	1	3	3	1	1	**21**
CF3	3	1	3	1	1	1	3	3	1	1	**18**
CF4	2	2	3	2	1	1	1	1	1	1	**15**
CF5	1	1	1	1	1	1	2	3	1	1	**13**
LF1	1	2	1	3	1	1	1	1	1	1	**13**
NBC1	1	2	1	3	1	1	2	2	1	1	**15**
NR1	3	1	3	1	1	1	1	1	1	1	**14**
NR2	3	1	1	1	1	1	3	3	1	1	**16**
NR3	1	1	1	1	1	1	2	3	1	1	**13**
NWO1	2	3	2	2	3	3	1	1	1	1	**19**
NWO2	3	2	2	2	1	1	3	3	1	1	**19**
NWO3	2	3	1	3	1	1	2	3	1	1	**18**
NWO4	2	1	1	2	1	1	2	3	1	1	**15**
NWO5	2	2	1	2	1	1	3	3	1	1	**17**
NWO6	3	3	1	2	1	1	2	3	1	1	**18**
SCC1	1	2	1	3	1	1	3	2	1	1	**16**
WC1	2	3	1	3	1	1	3	3	1	1	**19**
WOC1	3	1	3	1	1	1	3	2	1	1	**17**

Table 3.6: Summed index scores and rankings from three indices used in threat assessment.

Expert Opinion		Liberal		Conservative	
Site	Score	Site	Score	Site	Score
BSF15	10	BSF15	10	CF1	11
CF1	10	CF1	10	BSF15	14
BSF13	12	BSF13	11	BSF12	15
BSF01	13	BSF01	12	LF1	15
CF5	13	CF5	12	BSF13	16
LF1	13	NR3	12	NR1	16
NR3	13	BSF12	13	NWO4	17
NR1	14	LF1	13	NR3	17
BSF12	14	NWO4	13	BSF08	17
NWO4	15	NBC1	13	BSF09	17
BSF09	15	NR1	14	BSF10	17
BSF14	15	CF4	14	BSF04	17
CF4	15	BSF14	14	CC1	17
NBC1	15	BSF05	14	SCC1	17
BSF08	16	BSF06	14	WOC1	17
BSF10	16	BSF07	14	BSF01	18
NR2	16	BSF09	15	CF5	18
SCC1	16	BSF10	15	BSF02	18
BSF05	17	BSF17	15	BSF05	18
BSF06	17	BSF04	15	BSF06	18
BSF07	17	BSF11	15	BSF07	18
NWO5	17	BSF16	15	NWO5	18
BSF04	17	NR2	15	CF4	18
BSF11	17	NWO5	15	NBC1	18
CC1	17	SCC1	15	NR2	18
WOC1	17	BSF02	15	CF3	18
BSF02	18	NWO3	15	BSF14	19
NWO3	18	BSF08	16	NWO6	19
BSF16	18	NWO1	16	BSF16	20
CF3	18	NWO6	16	BSF11	21
NWO6	18	CC1	16	NWO3	21
BSF17	19	WOC1	16	WC1	21
NWO1	19	WC1	16	BSF03	21
WC1	19	CF3	17	BSF17	22
NWO2	19	NWO2	18	NWO1	22
BSF03	21	BSF03	19	NWO2	22
CF2	21	CF2	19	CF2	23
BSF19	23	BSF19	21	BSF19	25
BSF18	25	BSF18	22	BSF18	27

Table 3.7: Spearman rank correlation coefficients for summed index values among expert opinion, liberal, and conservative indices.

	Expert opinion	Liberal	Conservative
Expert opinion	1		
Liberal	0.88	1	
Conservative	0.82	0.63	1

Table 3.8: PCA summary of measured values of threat variables among sites and loadings of ten habitat variables on the first two principal components, and percent of total variance explained by each component.

	Axis 1	**Axis 2**
Eigenvalues	2.64	0.26
Cumulative % of Eigenvalues	1.99	0.46
Component loadings		
Distance to paved crossing	0.135	-0.227
Distance to unpaved crossing	-0.253	0.435
Paved road density	0.364	0.383
Unpaved road density	0.336	-0.329
Distance to mine	-0.244	-0.336
No. of mines	0.231	0.203
Distance to well	0.462	-0.152
No. of wells	-0.295	0.421
% Agriculture	0.281	0.326
% Urban	0.421	0.203

Table 3.9: Spearman rank correlation coefficients for measured values of threat variables and summed index value among sites.

	Paved crossing distance	Unpaved crossing distance	Paved road density	Unpaved road density	Mine distance	Well distance	% Ag	% Urban	No. mines	No. wells	Index value
Paved crossing distance	1										
Unpaved crossing distance	0.03	1									
Paved road density	-0.65	-0.06	1								
Unpaved road density	0.22	-0.64	-0.13	1							
Mine distance	-0.13	-0.23	-0.06	0.30	1						
Well distance	0.10	-0.18	0.20	0.09	-0.58	1					
% Ag	0.24	-0.07	0.16	0.12	-0.20	0.46	1				
% Urban	-0.23	0.05	-0.17	-0.25	0.11	-0.42	-0.73	1			
No. Mines	-0.31	-0.24	0.33	0.03	0.00	0.01	-0.04	-0.03	1		
No. Wells	-0.03	0.22	0.09	-0.13	-0.50	0.22	0.13	-0.22	0.14	1	
Index value	-0.41	-0.53	0.38	0.23	-0.16	0.51	-0.23	0.25	0.18	-0.09	1

Table 3.10: Comparison of site ranking among four conservation value measures: number of species, number of endangered species (# E), index of centers of density (ICD), and value of an area (VA). Eight sites contained no mussels. *BSF 11 numbers include one live individual tentatively identified as the endangered *Pleurobema clava*.

Site	# Species	Site	# E	Site	ICD	Site	VA
BSF11	23*	BSF11	6*	NWO3	0.3652	BSF11	23.984
BSF08	20	BSF08	5	BSF06	0.2437	BSF08	23.220
BSF15	20	BSF09	5	BSF08	0.1686	BSF06	8.216
BSF09	19	BSF15	5	NWO4	0.1539	BSF04	7.305
BSF13	17	BSF05	3	BSF04	0.1418	BSF13	6.412
BSF04	16	BSF10	3	BSF11	0.1263	BSF09	5.629
BSF05	16	BSF13	3	BSF13	0.1260	BSF05	4.727
BSF10	16	BSF17	3	BSF12	0.1069	BSF10	3.852
BSF17	15	BSF04	2	BSF09	0.1058	BSF12	2.663
BSF12	13	BSF12	1	BSF05	0.0986	BSF17	1.626
BSF14	13	BSF14	1	BSF10	0.0881	BSF07	1.544
BSF07	11	CC1	1	CF4	0.0809	BSF15	1.446
BSF16	10	CF1	1	BSF07	0.0752	NWO3	1.159
BSF06	9	CF2	1	CF3	0.0690	BSF16	1.049
CF3	8	CF3	1	CF2	0.0659	NWO4	0.943
NR2	8	CF4	1	CF1	0.0637	CF3	0.919
BSF03	7	CF5	1	WOC1	0.0603	CF4	0.824
BSF18	7	NR2	1	BSF16	0.0525	BSF14	0.782
BSF02	6	NWO2	1	BSF17	0.0454	CF1	0.744
CF1	6	NWO3	1	CC1	0.0409	WOC1	0.725
CF2	6	NWO4	1	NWO2	0.0406	CF2	0.657
CF4	6	NWO5	1	BSF15	0.0367	BSF02	0.338
WOC1	6	NWO6	1	BSF02	0.0344	CC1	0.337
CF5	5	WOC1	1	NWO5	0.0329	BSF03	0.203
CC1	4	BSF01	0	BSF14	0.0296	BSF18	0.193
NWO4	2	BSF02	0	CF5	0.0200	CF5	0.165
BSF19	1	BSF03	0	BSF03	0.0163	NWO2	0.129
NWO2	1	BSF06	0	BSF18	0.0146	NWO5	0.104
NWO3	1	BSF07	0	NWO6	0.0065	NR2	0.068
NWO5	1	BSF16	0	NR2	0.0052	NWO6	0.021
NWO6	1	BSF18	0	BSF19	0.0028	BSF19	0.004
BSF01	0	BSF19	0	BSF01	0.0000	BSF01	0.000
LF1	0	LF1	0	LF1	0.0000	LF1	0.000
NBC1	0	NBC1	0	NBC1	0.0000	NBC1	0.000
NR1	0	NR1	0	NR1	0.0000	NR1	0.000
NR3	0	NR3	0	NR3	0.0000	NR3	0.000
NWO1	0	NWO1	0	NWO1	0.0000	NWO1	0.000
SCC1	0	SCC1	0	SCC1	0.0000	SCC1	0.000
WC1	0	WC1	0	WC1	0.0000	WC1	0.000

Table 3.11: Comparison of top sites from conservation value measures and their threat assessment ranking using the expert opinion index. Species composition measures are species richness (# species), number of threatened and endangered species (# T&E), index of centers of density (ICD), and value of an area (VA).

Site	**# Species**	**#T&E**	**ICD**	**VA**	**Threat ranking**
BSF04			X	X	Moderate
BSF06			X	X	Moderate
BSF08	X	X	X	X	Moderate
BSF09	X	X			Low
BSF11	X	X		X	Moderate
BSF13	X			X	Low
BSF15	X	X			Low
NWO3			X		High
NWO4			X		Low

Table 3.12: Spearman correlation coefficients for conservation value measures among sites. All coefficients are significant at α=0.05.

	ICD	Species richness	No. endangered	VA
ICD	1			
Species richness	0.69	1		
No. endangered	0.62	0.69	1	
VA	0.92	0.89	0.67	1

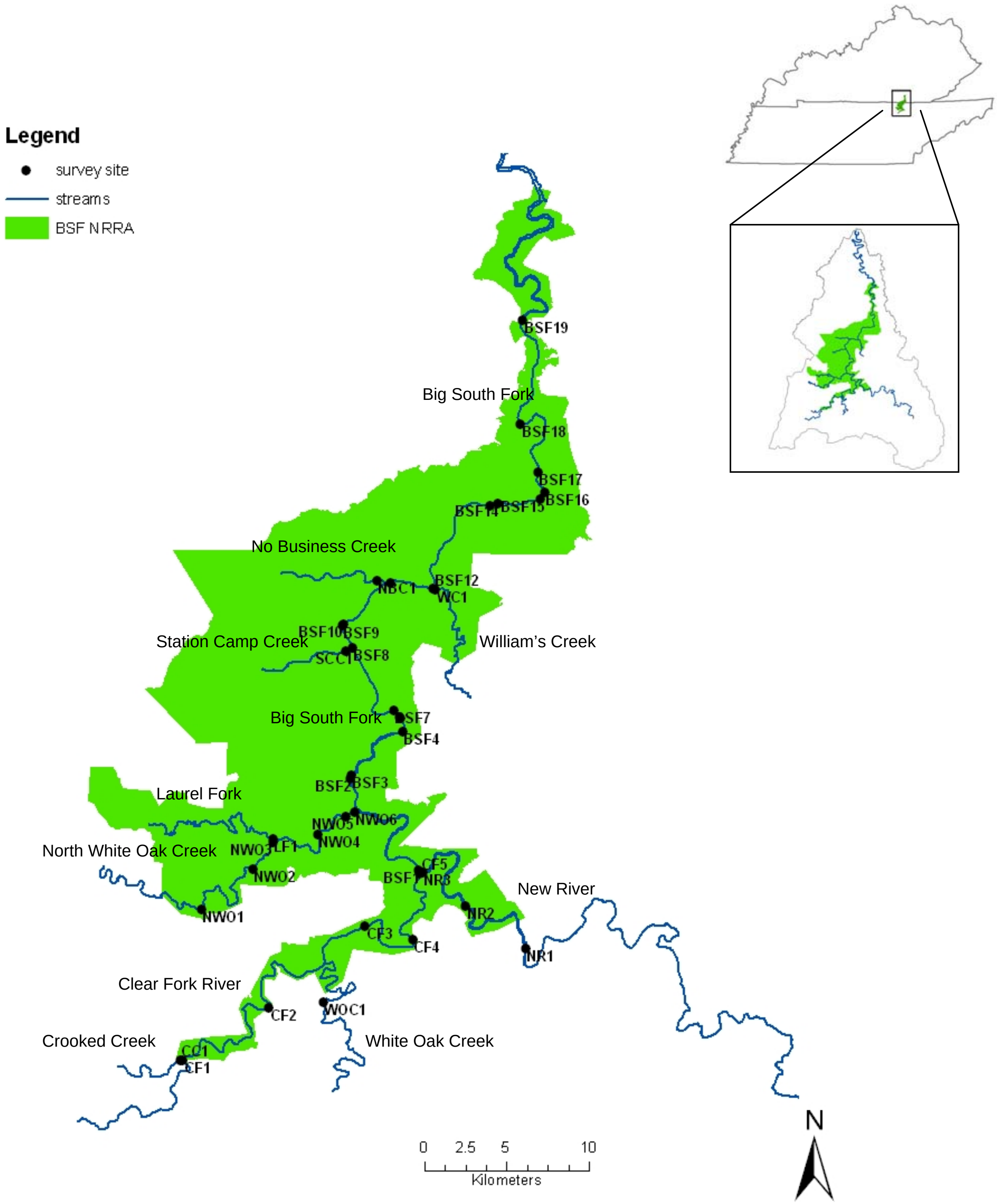


Figure 3.1: Map of Big South Fork National River and Recreation Area and survey site locations. The Big South Fork River watershed is highlighted in inset.

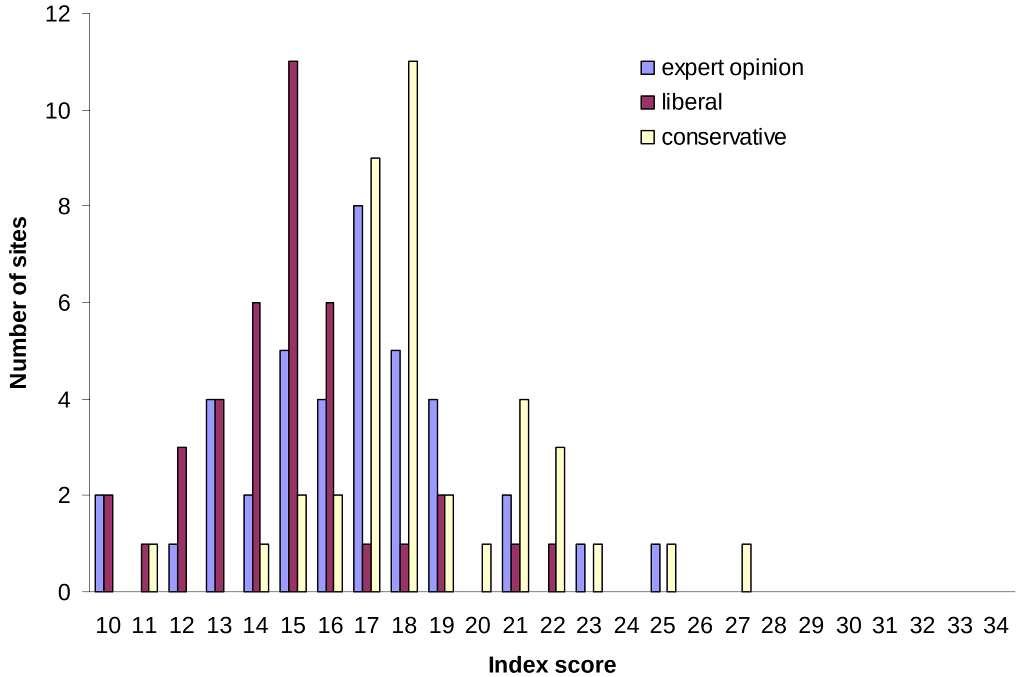


Figure 3.2: Number of sites per index score for the three threat indices used. Possible index scores range from 10 to 34.

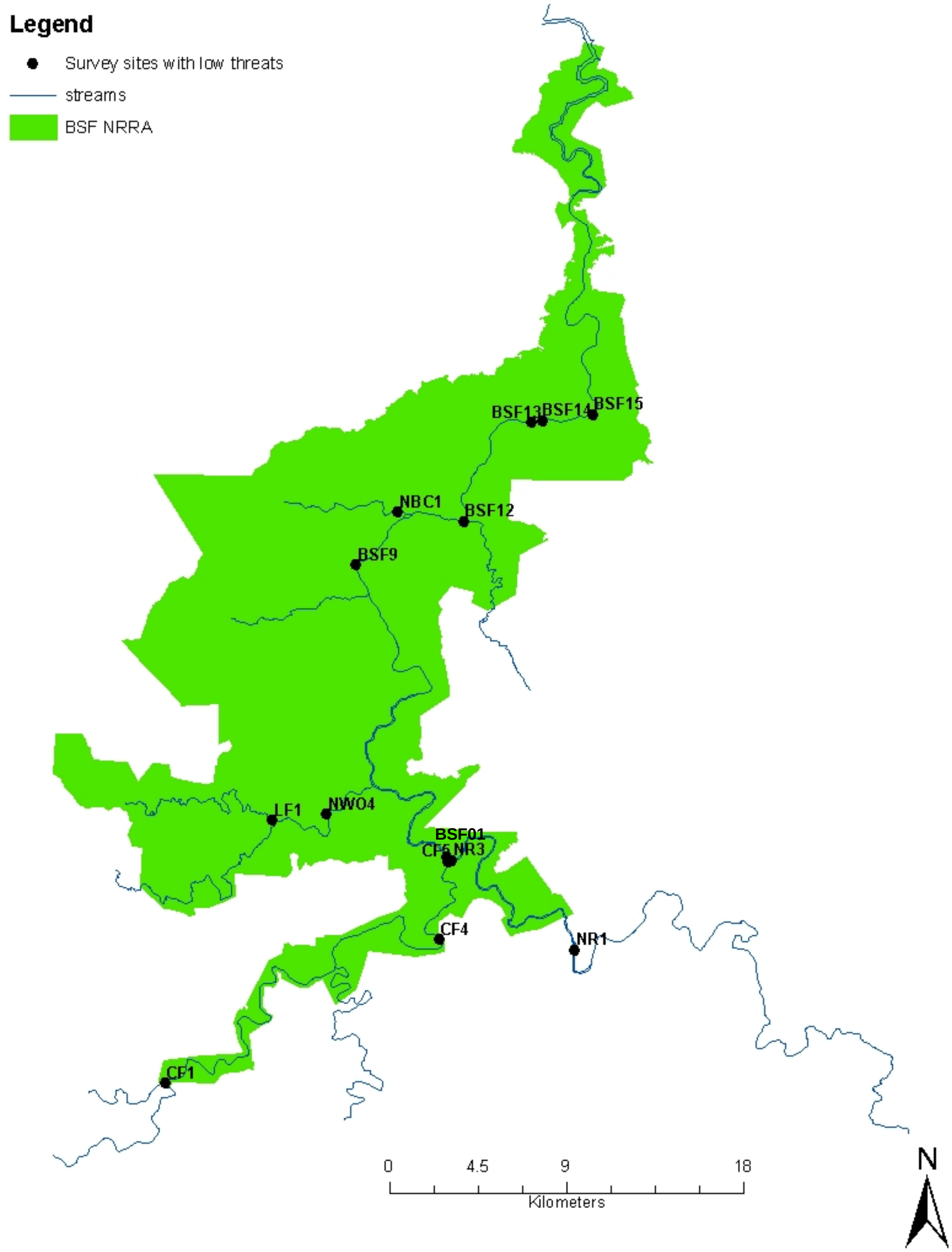


Figure 3.3: Distribution of sites with low threats. Threat level designation based on expert opinion summed index value.

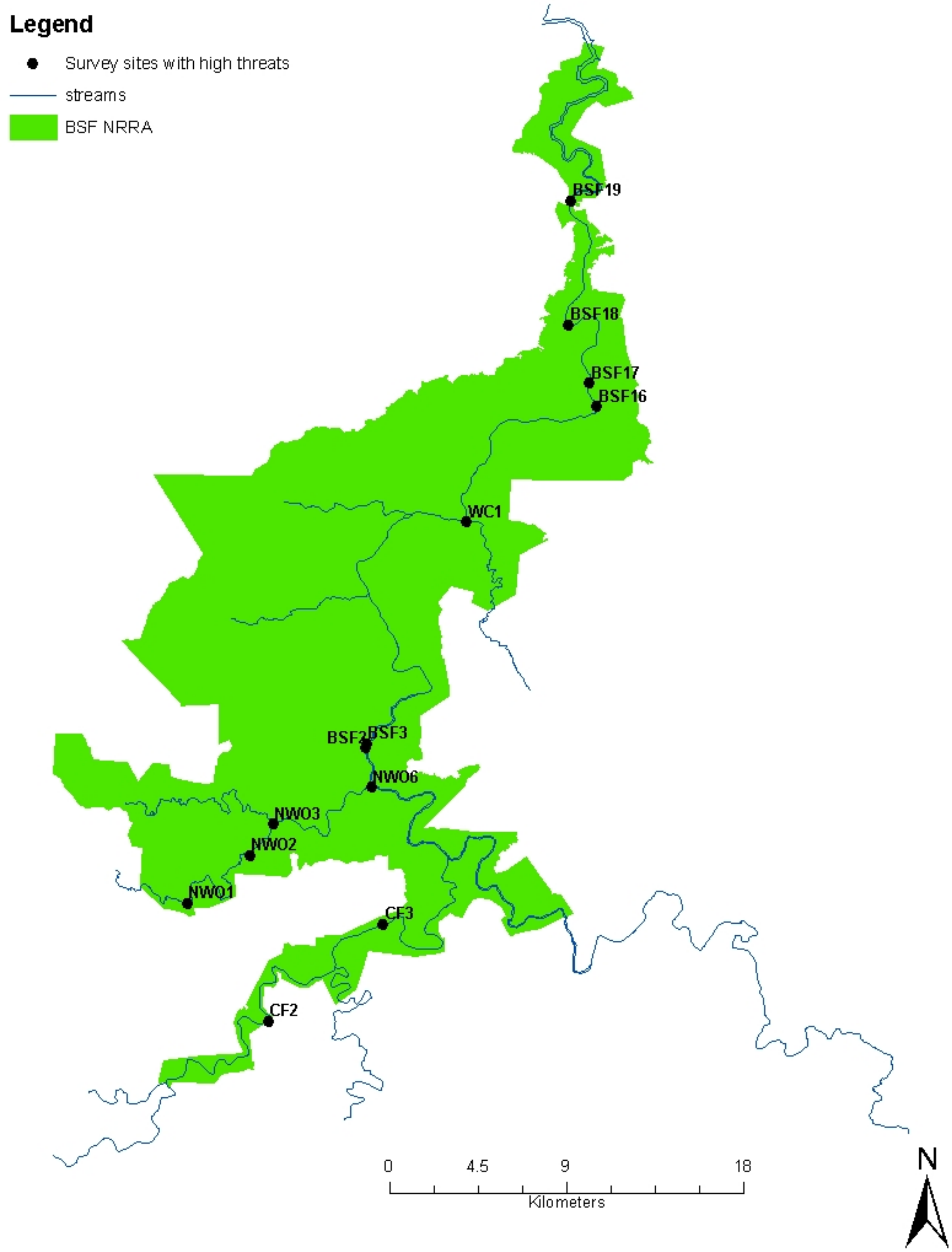


Figure 3.4: Distribution of sites with high threats. Threat level designation based on expert opinion summed index value.

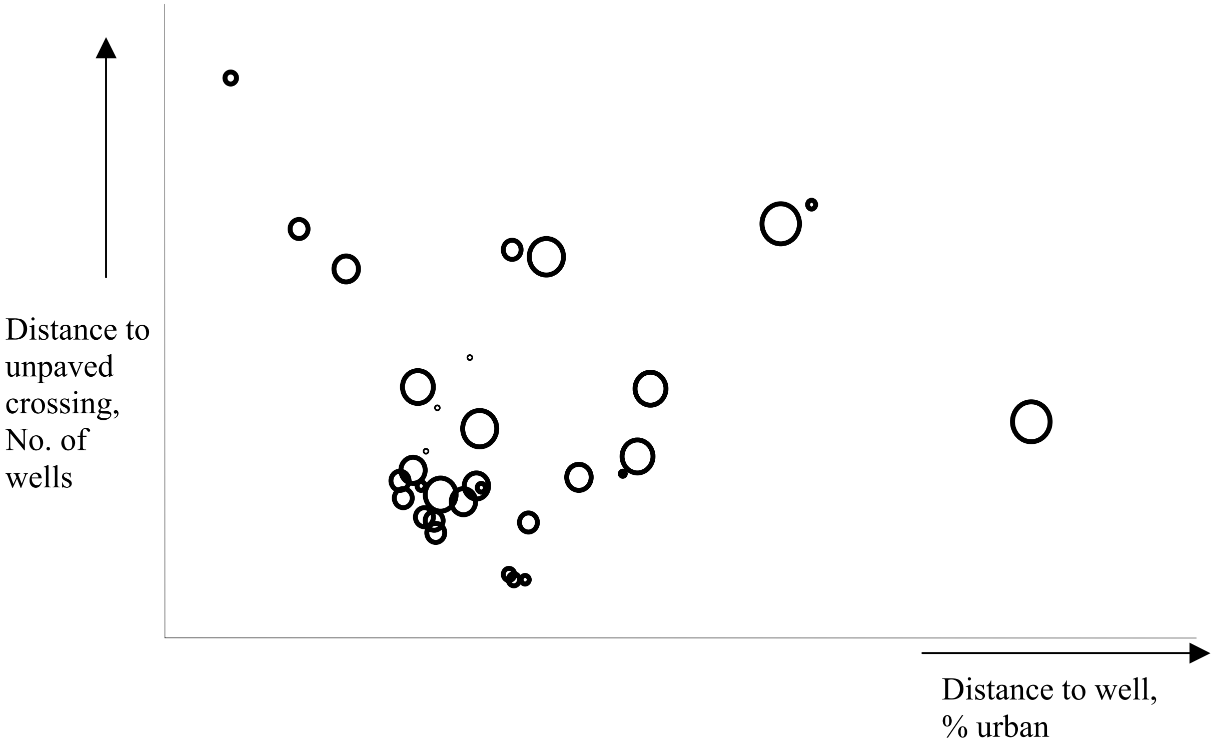


Figure 3.5: Graphical presentation of principal component scores for measured threat variable values across sites. Bubble size represents score assigned to a site using the expert opinion index, with smaller bubbles representing low scores and lower threats.

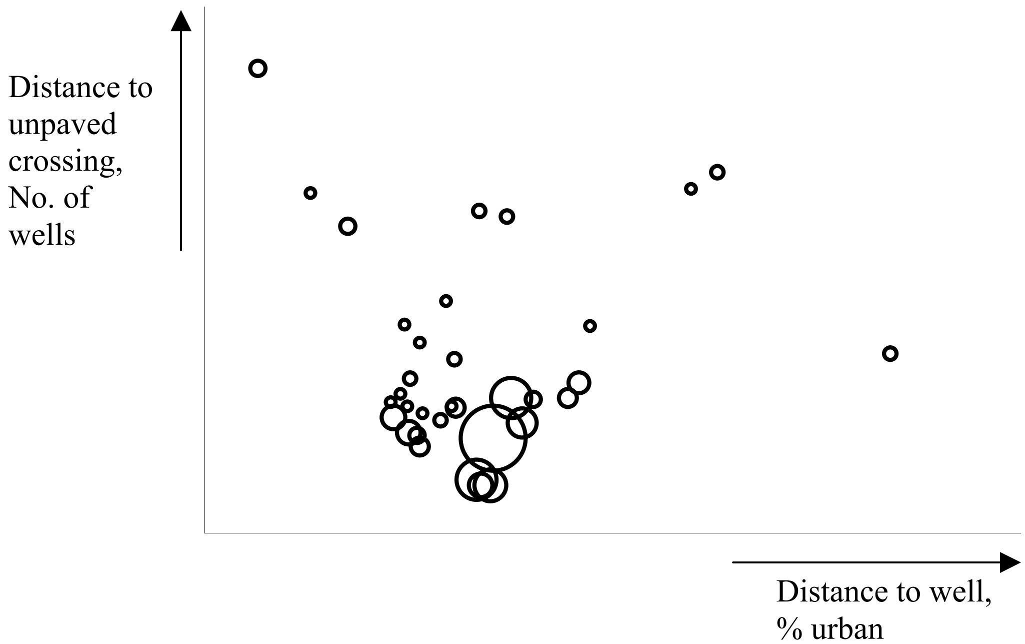


Figure 3.6: Graphical presentation of principal component scores for measured threat variable values across sites. Bubble size represents conservation value score assigned to a site using species richness measure, with larger bubbles representing higher conservation values.

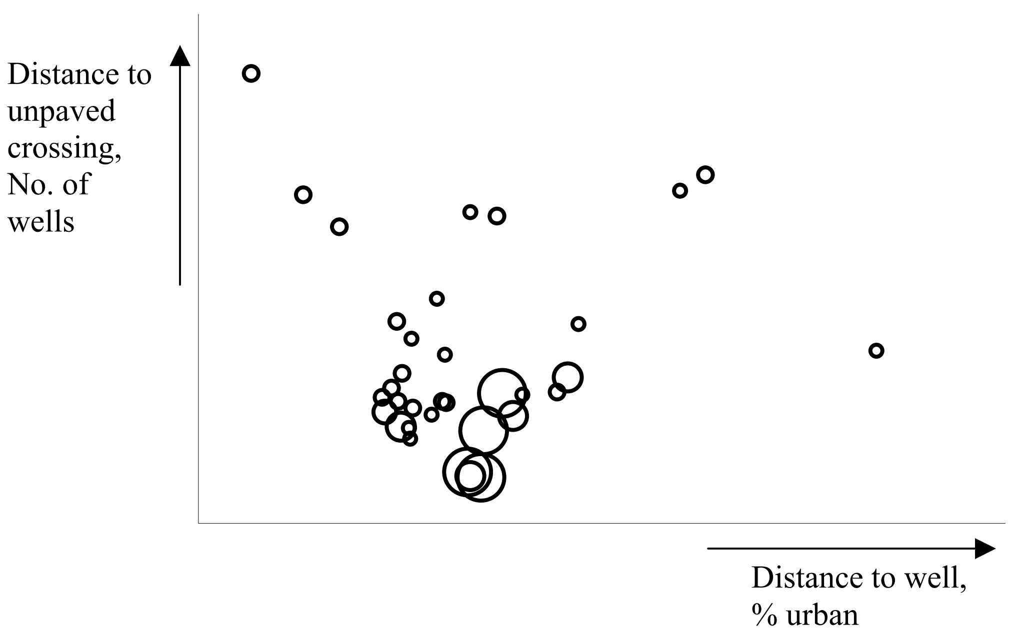


Figure 3.7: Graphical presentation of principal component scores for measured threat variable values across sites. Bubble size represents conservation value score assigned to a site using endangered species richness measure, with larger bubbles representing higher conservation values.

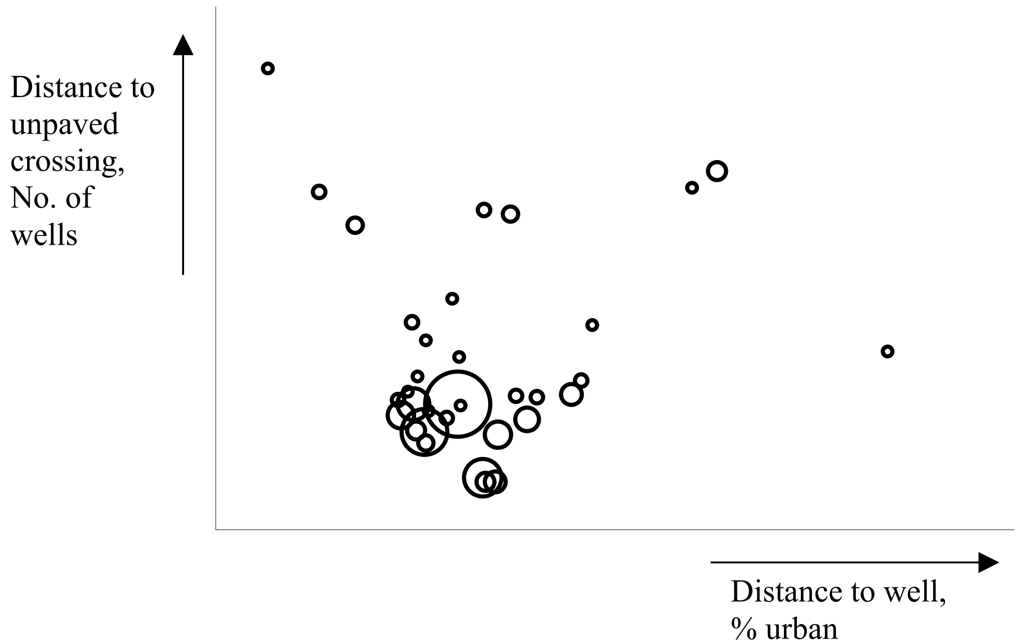


Figure 3.8: Graphical presentation of principal component scores for measured threat variable values across sites. Bubble size represents conservation value score assigned to a site using the ICD measure, with larger bubbles representing higher conservation values.

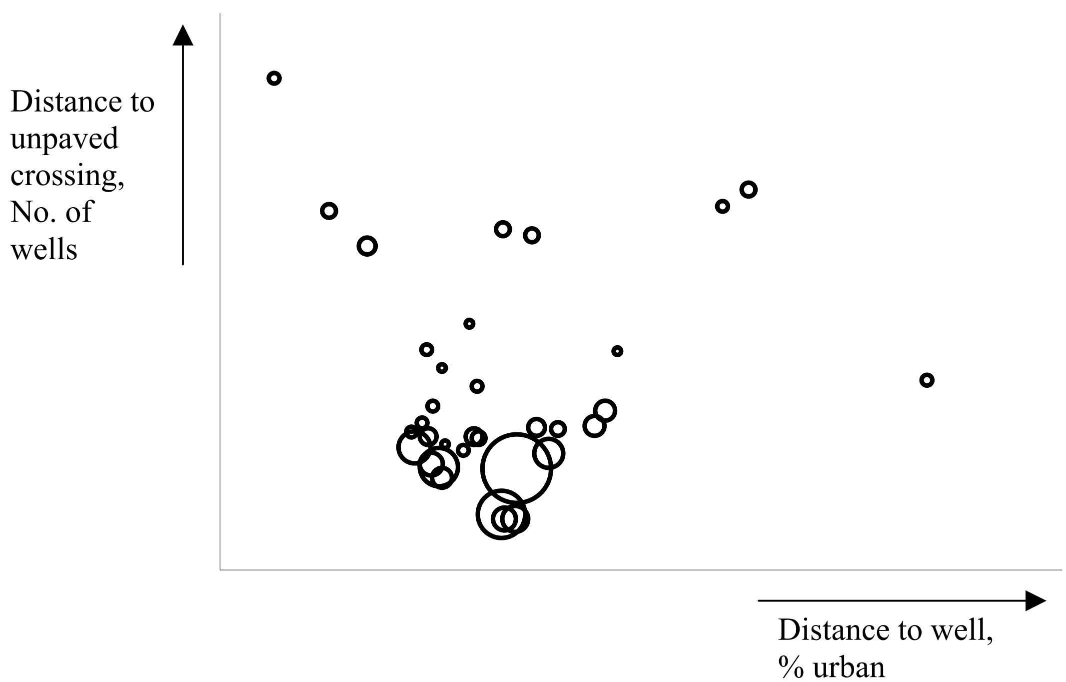


Figure 3.9: Graphical presentation of principal component scores for measured threat variable values across sites. Bubble size represents conservation value score assigned to a site using the VA measure, with larger bubbles representing higher conservation values.

Appendix 3-A: Descriptions of mussel sampling sites in Ahlstedt et al. (2005) with permission.

Site	Stream name	Description	Latitude	Longitude	River mi/km
BSF01	Big South Fork Cumberland River	confluence of Clear Fork and New R	362528	843725	77/124
BSF02	Big South Fork Cumberland River	upstream of Leatherwood Fd	362832	844007	70.5/113
BSF03	Big South Fork Cumberland River	Leatherwood Fd	362841	844005	70/113
BSF04	Big South Fork Cumberland River	upstream of Rough Shoals Br	363004	843756	67.5/109
BSF05	Big South Fork Cumberland River	mouth of Rough Shoals Br	363027	843801	67/108
BSF06	Big South Fork Cumberland River	downstream of Rough Shoals Br	363033	843805	66.5/107
BSF07	Big South Fork Cumberland River	mouth of Stevens Br	363045	843816	66/106
BSF08	Big South Fork Cumberland River	shoal downstream of Station Camp Cr	363251	843953	62.5/101
BSF09	Big South Fork Cumberland River	Parch Corn Cr	363332	844018	61.5/99
BSF10	Big South Fork Cumberland River	island downstream of Parch Corn Cr	363339	844017	61.3/99
BSF11	Big South Fork Cumberland River	Big Island	363457	843840	59/95
BSF12	Big South Fork Cumberland River	mouth of Williams Cr	363446	843633	57/92
BSF13	Big South Fork Cumberland River	shoal downstream of Oil Well Br	363726	843413	52.3/84
BSF14	Big South Fork Cumberland River	mouth of Hueling Br	363728	843356	52/84
BSF15	Big South Fork Cumberland River	shoal upstream of Bear Cr	363737	843160	50.3/81
BSF16	Big South Fork Cumberland River	below gage above Salt Br	363749	843154	50/80
BSF17	Big South Fork Cumberland River	Big Shoal	363827	843211	49.2/79
BSF18	Big South Fork Cumberland River	Blue Heron	364006	843249	45.5/73
BSF19	Big South Fork Cumberland River	Yamacraw	364331	843238	40.5/65
CC1	Crooked Creek	300 yards upstream from mouth	361928	844717	.2/.3
CF1	Clear Fork	upstream of Peters Bridge	361928	844712	20/32
CF2	Clear Fork	Brewster Bridge	362106	844341	14/23
CF3	Clear Fork	Sheep Ranch (gas drilling site)	362342	843939	7/11
CF4	Clear Fork	upstream of Burnt Mill Bridge	362312	843744	3.7/6
CF5	Clear Fork	500 yards upstream from mouth	362523	843724	.3/.5
LF1	Laurel Fork	upstream from ATV crossing	362638	844319	.3/.5
NBC1	No Business Creek	500 yards upstream from mouth	363501	843849	.3/.5
NR1	New River	Highway 27 bridge	362250	843310	9/14
NR2	New River	Silcox Ford	362416	843533	4.7/7.6
NR3	New River	500 yards upstream from mouth	362526	843717	.3/.5
NWO1	North White Oak Creek	mouth of Mill Creek	362425	844619	.3/.5
NWO2	North White Oak Creek	Zenith	362542	844409	--
NWO3	North White Oak Creek	upstream from confluence with Laurel Fk	362630	844319	--
NWO4	North White Oak Creek	upstream from confluence with Coyle Br	362647	844130	--
NWO5	North White Oak Creek	ATV crossing -- O and W ATV crossing	362718	844020	1/1.6
NWO6	North White Oak Creek	500 yards upstream of mouth	362729	843958	.3/.5
SCC1	Station Camp Creek	500 yards upstream from mouth	363251	843953	.3/.5
WC1	Williams Creek	100 yards upstream from mouth	363442	843627	.1/.16
WOC1	White Oak Creek	below Hwy. 52 Bridge near Rugby	362114	844124	5.5/8.8

Appendix 3-B: Data from mussel survey by Ahlstedt et al. (2005) with permission. Totals on this page include only mainstem sites. Relic shells were included in total species counts and are highlighted in grey.

	Site																		
Mussel species	BSF 01	BSF 02	BSF 03	BSF 04	BSF 05	BSF 06	BSF 07	BSF 08	BSF 09	BSF 10	BSF 11	BSF 12	BSF 13	BSF 14	BSF 15	BSF 16	BSF 17	BSF 18	BSF 19
Actinonaias pectorosa	0	0	0	3	4	1	2	58	50	5	14	6	20	5	44	7	10	0	0
Alasmidonta atropurpurea	0	0	0	2	3	0	0	2	2	0	5	0	0	0	2	0	0	0	0
Alasmidonta marginata	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Alasmidonta viridis	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elliptio crassidens	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Elliptio dilatata	0	2	1	6	9	0	1	101	403	63	235	37	78	24	136	4	20	3	0
Epioblasma brevidens	0	0	0	0	7	0	0	161	54	1	16	6	5	4	6	1	1	1	0
Epioblasma f. walkeri	0	0	0	0	0	0	0	2	87	1	7	0	15	0	1	0	0	0	0
Lampsilis cardium	0	7	4	7	45	14	20	150	59	1	60	4	26	11	27	2	1	0	0
Lampsilis fasciola	0	2	2	6	17	11	4	91	56	3	41	6	8	5	8	0	2	0	0
Lasmigona costata	0	2	1	13	34	0	7	62	58	5	31	1	3	1	18	1	5	1	0
Leptodea fragilis	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Ligumia recta	0	0	0	1	2	0	2	17	12	0	6	1	1	0	4	0	2	0	0
Medionidus conradicus	0	0	0	0	0	0	0	0	0	2	1	0	11	1	1	0	0	0	0
Pegias fabula	0	0	0	0	0	0	0	1	55	15	10	0	23	0	2	0	1	0	0
Pleurobema clava	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Pleurobema oviforme	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0
Pleurobema sintoxia	0	0	0	4	24	12	8	661	263	2	452	102	55	50	266	4	4	0	0
Potamilus alatus	0	1	2	23	117	0	39	140	187	5	94	59	14	15	26	3	28	19	0
Ptychobranchus fasciolaris	0	0	0	1	6	1	1	11	33	1	21	5	7	10	27	2	3	2	0
Quadrula p. pustulosa	0	12	6	9	114	38	25	338	127	0	88	9	8	18	79	5	2	0	1
Strophitus undulatus	0	0	0	1	11	1	0	67	23	1	15	2	0	0	5	0	0	3	0
Tritogonia verrucosa	0	0	12	0	2	18	18	95	4	1	6	0	1	0	5	0	1	0	0
Villosa iris	0	0	0	7	0	0	0	0	12	4	8	0	4	3	11	0	1	0	0
Villosa taeniata	0	0	0	2	0	0	0	2	37	4	15	1	1	2	9	1	2	1	0
Villosa trabalis	0	0	0	3	4	0	0	8	23	0	6	0	0	0	1	0	1	0	0
Total specimens	0	26	28	89	402	97	127	1969	1545	114	1134	239	280	149	678	30	84	30	1
Total species	0	6	7	16	16	8	11	20	19	16	23	13	17	13	20	10	16	7	1
Total sampling hrs	1.5	3	12	3	13	3	6	69	52	5.5	58.5	5	11	16	35.5	3	6.5	17.5	7

Appendix 3-B: Data from mussel survey by Ahlstedt et al. (2005) with permission. Totals on this page include only tributary sites. Relic shells were included in total species counts and are highlighted in grey.

Mussel species	CF 1	CF 2	CF 3	CF 4	CF 5	CC 1	WOC 1	NR 1	NR 2	NR 3	Site NWO 1	NWO 2	NWO 3	NWO 4	NWO 5	NWO 6	LF 1	SC C1	NB C1	WC 1
Actinonaias pectorosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Alasmidonta atropurpurea	74	14	8	9	1	32	61	0	2	0	0	14	42	66	17	3	0	0	0	0
Alasmidonta marginata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Alasmidonta viridis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elliptio crassidens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elliptio dilatata	34	2	2	13	1	22	2	0	1	0	0	0	0	0	0	0	0	0	0	0
Epioblasma brevidens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Epioblasma f. walkeri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lampsilis cardium	10	6	3	12	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0
Lampsilis fasciola	6	5	15	6	3	4	5	0	1	0	0	0	0	1	0	0	0	0	0	0
Lasmigona costata	24	96	68	64	0	0	40	0	12	0	0	0	0	0	0	0	0	0	0	0
Leptodea fragilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ligumia recta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Medionidus conradicus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pegias fabula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pleurobema clava	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pleurobema oviforme	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pleurobema sintoxia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potamilus alatus	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Ptychobranchus fasciolaris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Quadrula p. pustulosa	0	0	1	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Strophitus undulatus	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tritogonia verrucosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Villosa iris	18	4	5	12	0	0	11	0	1	0	0	0	0	0	0	0	0	0	0	0
Villosa taeniata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Villosa trabalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total specimens	166	127	104	116	7	59	120	0	21	0	0	14	42	67	17	3	0	0	0	0
Total species	6	6	8	6	5	4	6	0	8	0	0	1	1	2	1	1	0	0	0	0
Total sampling hrs	9.5	6	5	6	2	5	6.5	3.5	10.5	1.5	3	3	1	2	4.5	4	1	3	2	1

Appendix 3-C: Species values (VS_k) used in calculating species composition measure, VA. Number of specimens is total number of individuals collected by Ahlstedt et al. (2005), number of sites is the number of sites out of 39 where an individual of that species was found. The status of 'endangered' refers to federal designation, 'special concern' refers to species listed as such in Williams et al. (1993), 'stable' refers to species that are thought to be stable throughout their range (Parmalee and Bogan 1998).

Species	No. specimens	No. sites	Status	VS_k
Pleurobema clava	1	1	endangered	11.646
Alasmidonta marginata	1	1	special concern	10.441
Elliptio crassidens	1	1	stable	10.039
Leptodea fragilis	1	1	stable	10.039
Pleurobema oviforme	2	2	special concern	6.383
Alasmidonta viridis	4	2	special concern	5.527
Villosa trabalis	46	7	endangered	3.889
Epioblasma f. walkeri	113	6	endangered	3.863
Pegias fabula	107	7	endangered	3.752
Epioblasma brevidens	263	12	endangered	3.355
Medionidus conradicus	16	5	special concern	3.243
Alasmidonta atropurpurea	359	19	endangered	3.175
Ligumia recta	48	10	special concern	2.463
Actinonaias pectorosa	229	14	special concern	2.104
Villosa taeniata	77	12	stable	1.897
Lampsilis cardium	473	24	special concern	1.893
Strophitus undulatus	131	11	stable	1.861
Tritogonia verrucosa	163	11	stable	1.835
Villosa iris	101	14	stable	1.798
Ptychobranchus fasciolaris	131	15	stable	1.741
Pleurobema sintoxia	1907	14	stable	1.550
Potamilus alatus	773	17	stable	1.541
Lampsilis fasciola	308	24	stable	1.527
Quadrula p. pustulosa	883	19	stable	1.501
Lasmigona costata	547	22	stable	1.499
Elliptio dilatata	1200	24	stable	1.428

Appendix 3-D: Index values assigned to mainstem sites on the Big South Fork using liberal index categories by site.

SITE	Paved crossing distance (km)	Unpaved crossing distance (km)	Paved road density (km/km^2)	Unpaved road density (km/km^2)	Coal mine proximity (km)	# Coal mines w/in 2 km	Well proximity (km) (oil or gas)	# Wells w/in 2 km (oil or gas)	% Ag	% Urban	Total
BSF01	1	1	1	1	1	1	1	3	1	1	**12**
BSF02	1	1	1	3	1	1	2	3	1	1	**15**
BSF03	3	2	2	3	1	1	2	3	1	1	**19**
BSF04	2	2	1	2	1	1	2	2	1	1	**15**
BSF05	1	2	1	2	1	1	1	3	1	1	**14**
BSF06	1	2	1	2	1	1	1	3	1	1	**14**
BSF07	1	2	1	2	1	1	1	3	1	1	**14**
BSF08	3	3	1	3	1	1	1	1	1	1	**16**
BSF09	2	2	2	3	1	1	1	1	1	1	**15**
BSF10	2	2	2	3	1	1	1	1	1	1	**15**
BSF11	1	2	2	3	1	1	1	2	1	1	**15**
BSF12	2	1	2	2	1	1	1	1	1	1	**13**
BSF13	1	1	1	2	1	1	1	1	1	1	**11**
BSF14	1	2	1	2	1	1	2	2	1	1	**14**
BSF15	1	1	1	1	1	1	1	1	1	1	**10**
BSF16	1	1	1	2	3	3	1	1	1	1	**15**
BSF17	1	1	1	2	1	5	1	1	1	1	**15**
BSF18	1	3	3	3	3	5	1	1	1	1	**22**
BSF19	3	2	3	1	3	5	1	1	1	1	**21**

Appendix 3-D: Index values assigned to tributary sites of the Big South Fork using liberal index categories by site.

SITE	Paved crossing distance (km)	Unpaved crossing distance (km)	Paved road density (km/km^2)	Unpaved road density (km/km^2)	Coal mine proximity (km)	# Coal mines w/in 2 km	Well proximity (km) (oil or gas)	# Wells w/in 2 km (oil or gas)	% Ag	% Urban	Total
CC1	3	1	2	1	1	1	2	3	1	1	**16**
CF1	1	1	1	1	1	1	1	1	1	1	**10**
CF2	3	2	3	2	1	1	2	3	1	1	**19**
CF3	3	1	3	1	1	1	2	3	1	1	**17**
CF4	2	1	3	2	1	1	1	1	1	1	**14**
CF5	1	1	1	1	1	1	1	3	1	1	**12**
LF1	1	2	1	3	1	1	1	1	1	1	**13**
NBC1	1	1	1	3	1	1	1	2	1	1	**13**
NR1	3	1	3	1	1	1	1	1	1	1	**14**
NR2	3	1	1	1	1	1	2	3	1	1	**15**
NR3	1	1	1	1	1	1	1	3	1	1	**12**
NWO1	1	3	2	2	1	3	1	1	1	1	**16**
NWO2	3	2	2	2	1	1	2	3	1	1	**18**
NWO3	1	2	1	3	1	1	1	3	1	1	**15**
NWO4	1	1	1	2	1	1	1	3	1	1	**13**
NWO5	1	2	1	2	1	1	2	3	1	1	**15**
NWO6	3	2	1	2	1	1	1	3	1	1	**16**
SCC1	1	2	1	3	1	1	2	2	1	1	**15**
WC1	1	2	1	3	1	1	2	3	1	1	**16**
WOC1	3	1	3	1	1	1	2	2	1	1	**16**

Appendix 3-E: Index values assigned to mainstem sites on the Big South Fork using conservative index categories by site.

SITE	Paved crossing distance (km)	Unpaved crossing distance (km)	Paved road density (km/km^2)	Unpaved road density (km/km^2)	Coal mine proximity (km)	# Coal mines w/in 2 km	Well proximity (km) (oil or gas)	# Wells w/in 2 km (oil or gas)	% Ag	% Urban	Total
BSF01	2	2	1	1	3	1	3	3	1	1	**18**
BSF02	2	2	1	3	1	1	3	3	1	1	**18**
BSF03	3	3	2	3	1	1	3	3	1	1	**21**
BSF04	2	3	1	2	1	1	3	2	1	1	**17**
BSF05	2	3	1	2	1	1	3	3	1	1	**18**
BSF06	2	3	1	2	1	1	3	3	1	1	**18**
BSF07	2	3	1	2	1	1	3	3	1	1	**18**
BSF08	3	3	1	3	1	1	2	1	1	1	**17**
BSF09	2	3	2	3	1	1	2	1	1	1	**17**
BSF10	2	3	2	3	1	1	2	1	1	1	**17**
BSF11	2	3	2	3	3	1	3	2	1	1	**21**
BSF12	2	2	2	2	1	1	2	1	1	1	**15**
BSF13	2	2	1	2	3	1	2	1	1	1	**16**
BSF14	2	3	1	2	3	1	3	2	1	1	**19**
BSF15	1	2	1	1	3	1	2	1	1	1	**14**
BSF16	2	2	1	2	5	3	2	1	1	1	**20**
BSF17	2	2	1	2	5	5	2	1	1	1	**22**
BSF18	2	3	3	3	5	5	2	1	1	2	**27**
BSF19	3	3	3	1	5	5	2	1	1	1	**25**

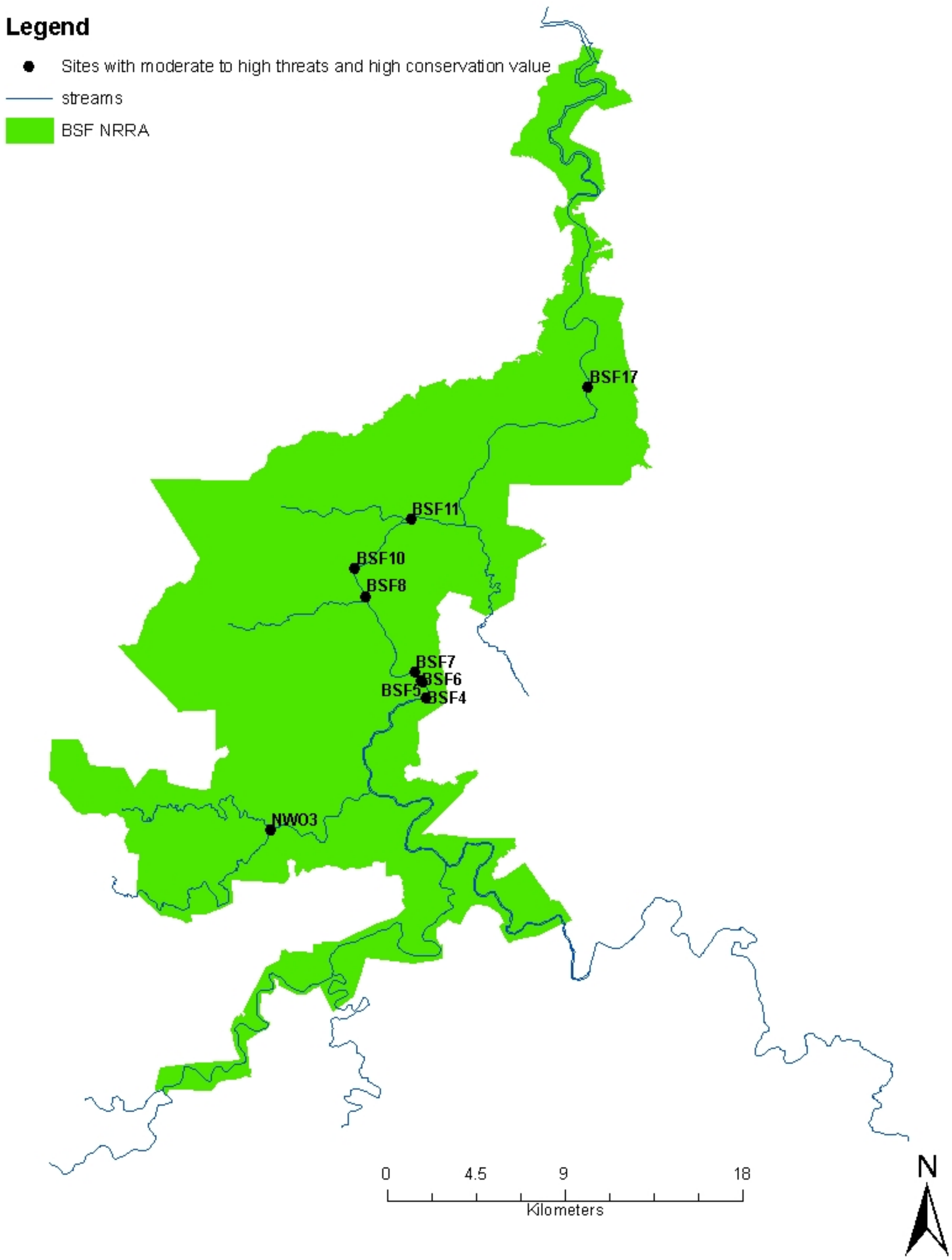
Appendix 3-E: Index values assigned to tributary sites of the Big South Fork using conservative index categories by site.

SITE	Paved crossing distance (km)	Unpaved crossing distance (km)	Paved road density (km/km^2)	Unpaved road density (km/km^2)	Coal mine proximity (km)	# Coal mines w/in 2 km	Well proximity (km) (oil or gas)	# Wells w/in 2 km (oil or gas)	% Ag	% Urban	Total
CC1	3	1	2	1	1	1	3	3	1	1	**17**
CF1	2	1	1	1	1	1	1	1	1	1	**11**
CF2	3	3	3	2	3	1	3	3	1	1	**23**
CF3	3	1	3	1	1	1	3	3	1	1	**18**
CF4	2	2	3	2	3	1	2	1	1	1	**18**
CF5	2	2	1	1	3	1	3	3	1	1	**18**
LF1	1	3	1	3	1	1	2	1	1	1	**15**
NBC1	1	2	1	3	3	1	3	2	1	1	**18**
NR1	3	1	3	1	3	1	1	1	1	1	**16**
NR2	3	1	1	1	3	1	3	3	1	1	**18**
NR3	2	1	1	1	3	1	3	3	1	1	**17**
NWO1	2	3	2	2	5	3	2	1	1	1	**22**
NWO2	3	3	2	2	3	1	3	3	1	1	**22**
NWO3	2	3	1	3	3	1	3	3	1	1	**21**
NWO4	2	2	1	2	1	1	3	3	1	1	**17**
NWO5	2	3	1	2	1	1	3	3	1	1	**18**
NWO6	3	3	1	2	1	1	3	3	1	1	**19**
SCC1	1	3	1	3	1	1	3	2	1	1	**17**
WC1	2	3	1	3	3	1	3	3	1	1	**21**
WOC1	3	1	3	1	1	1	3	2	1	1	**17**

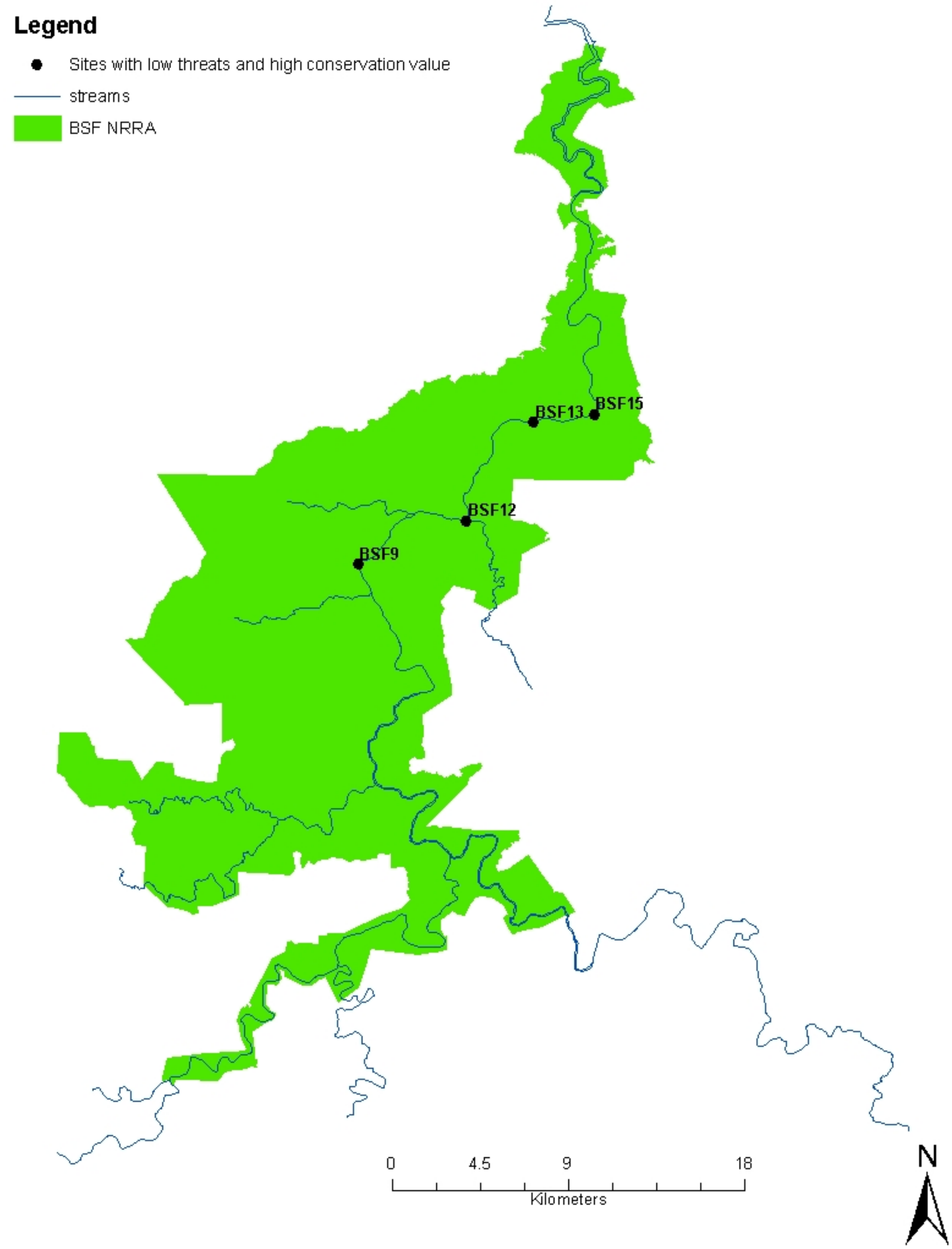
Appendix 3-F: List of recommended management actions based on relative conservation value and relative threat ranking for sites in the BSF watershed. The conservation value designation is based on rankings of sites among all measures used. The threat ranking category is based on rankings of sites from the expert opinion index.

Threat ranking	Conservation Value	Management action	Sites
Low	High	Preventative maintenance (4 sites)	BSF09, BSF12, BSF13, BSF15
Moderate to high	High	Monitoring, land use mitigation (9 sites)	BSF04, BSF05, BSF06, BSF07, BSF08, BSF10, BSF11, BSF17, NWO3
Low to moderate	Low to moderate	Restoration possible (15 sites)	BSF01, BSF14, CC1, CF1, CF4, CF5, LF1, NWO4, NWO5, NR1, NR2, NR3, NBC1, SCC1, WOC1
High	Low to moderate	Land use mitigation, then restoration (11 sites)	BSF02, BSF03, BSF16, BSF18, BSF19, CF2, CF3, NWO1, NWO2, NWO6, WC1

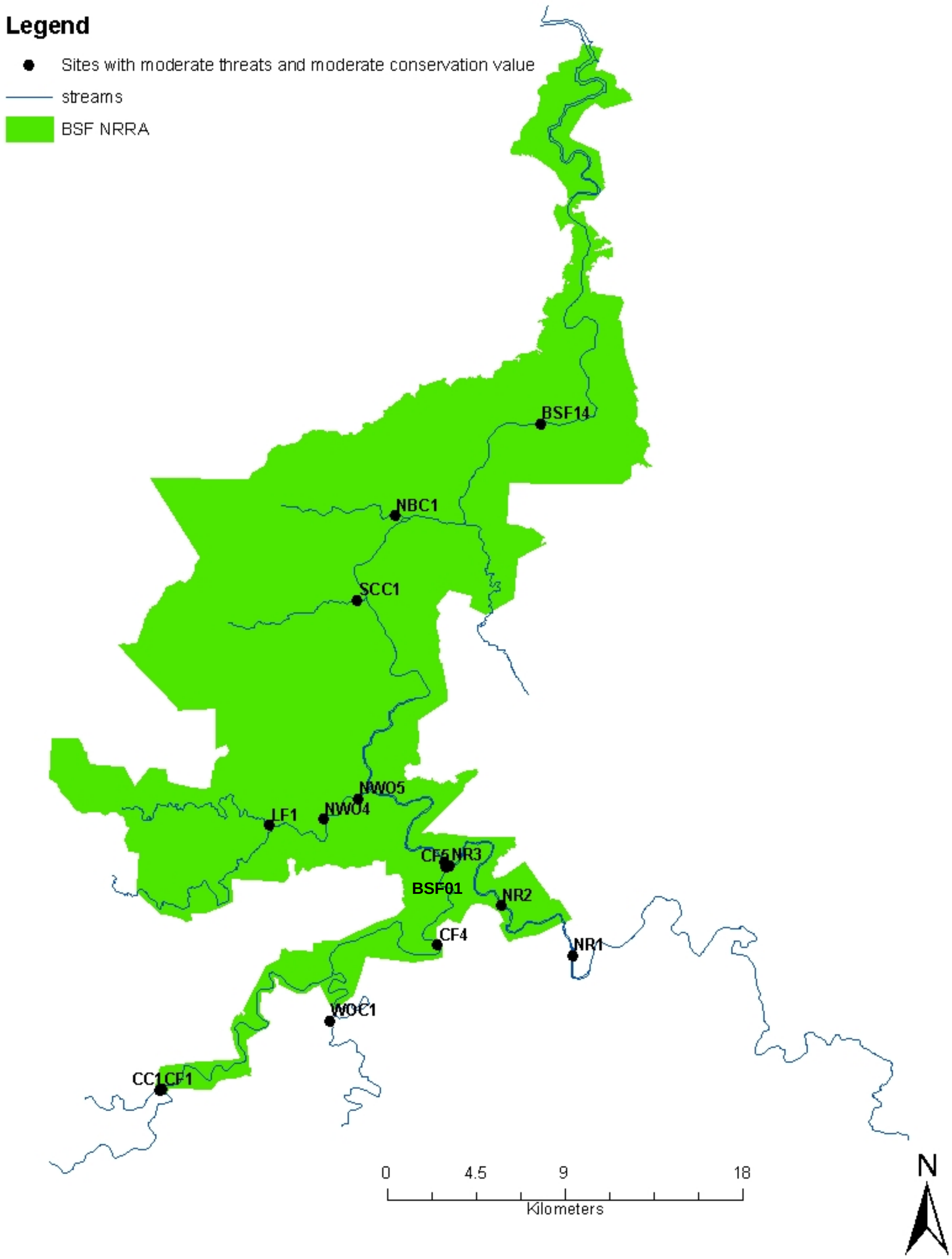
Appendix 3-G: Distribution of mussel sites with moderate to high threats and high conservation value. BSF 17 and NWO 3 have relatively high threats, and all other sites have moderate threats.



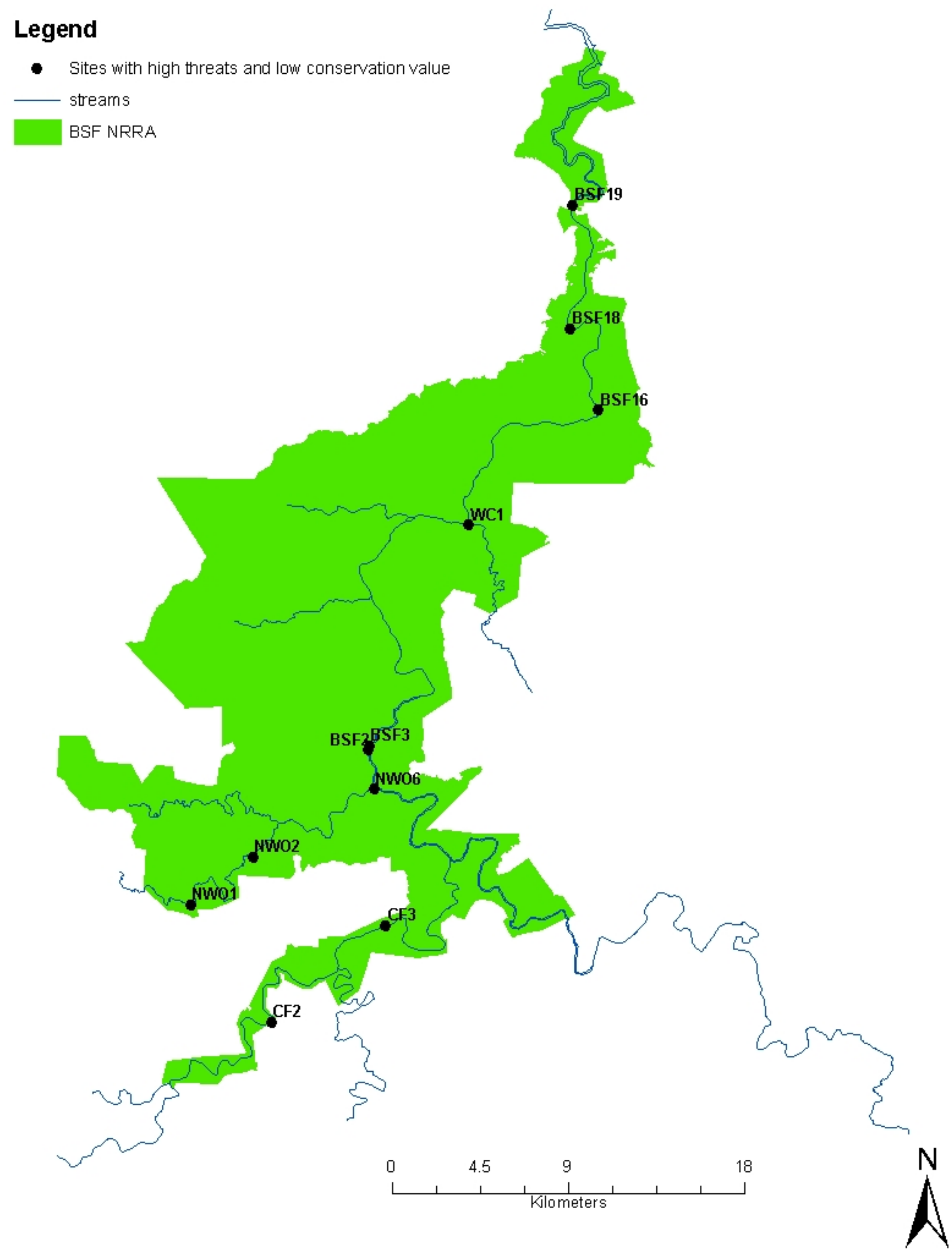
Appendix 3-H: Distribution of mussel sites with low threats and high conservation value.



Appendix 3-I: Distribution of mussel sites with moderate to low threats and moderate to low conservation value. Sites BSF01, BSF14, CF 1, CF4, CF5, LF1, NR1, NR3, NBC1, and NWO4 have relatively low threats and may be good candidates for mussel restoration.



Appendix 3-J: Distribution of mussel sites with high threats and low to moderate conservation value.



VITA

Jennifer Guyot was born in Perryville, Missouri in 1980, and lived there with her parents, Jim and Bonnie Guyot, and siblings, Susan, Luke, and Kate, until graduating from St. Vincent High School in 1998. She then attended the University of Missouri in Columbia where she received a Bachelor of Science degree in Fisheries and Wildlife in May 2003. In August of 2003, she enrolled at Virginia Polytechnic Institute and State University in Blacksburg, Virginia, and began her thesis research studying freshwater mussel restoration efforts in the Big South Fork National River and Recreation Area in Tennessee and Kentucky. In the spring of 2006, she completed her Master of Science degree in Fisheries and Wildlife Sciences at Virginia Tech. In January 2006 she began her professional career as a Fisheries Management Biologist with the Missouri Department of Conservation.