Propagation of Juvenile Freshwater Mussels (Bivalvia: Unionidae) and Assessment of Habitat Suitability for Restoration of Mussels in the Clinch River, Virginia

by

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(ABSTRACT)

Freshwater mussel propagation techniques were tested at the Virginia Department of Game and Inland Fisheries Aquatic Wildlife Conservation Center through a series of three experiments. Experiment 1 tested the suitability of a pond and raceway for rearing juvenile oystermussels (*Epioblasma capsaeformis*) and wavyrayed lampmussel (*Lampsilis fasciola*). This experiment was prematurely terminated due to predation on mussels by fathead minnows (*Pimephales promelas)*. Experiment 2 evaluated growth and survival of juvenile rainbow mussels in outdoor troughs and indoor aquaria. There was no significant difference in survival or growth between the two systems. Experiment 3 used troughs similar to those in Experiment 2 to rear *E. capsaeformis* and *L. fasciola* under two silt regimes. Survival for Experiment 3 was significantly greater for *L. fasciola*. The comparison between silt regimes indicated that individuals in the high-silt treatment had better survival than those in the low-silt treatment. The difference between these 2 treatments may be a reflection of increased escapement in the low-silt treatment, which may have resulted from more frequent disturbance during sampling. Growth of *L. fasciola* was significantly greater than *E. capsaeformis*, and was greater in the low-silt treatment.

A habitat survey of the Clinch River, Virginia was conducted from Blackford, Clinch River Kilometer (CRK) 478 to the Tennessee border, CRK 325. Physical characteristics identified in the survey were combined with water quality and impact source data to develop a habitat suitability index for freshwater mussels within this study reach. Model parameters were indexed and weighted to give a final suitability ranking. Habitat units having the highest overall ranking included: Nash Ford (CRK 449), Artrip (CRK 442), several riffles and runs below Carterton (CRK 417), upstream of Mill Island (CRK 389.5), and Pendleton Island (CRK 365), and Speers Ferry (CRK 333-325). Potential locations for habitat restoration projects and additional monitoring were also identified.

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Table of Contents

Chapter 1. Survival and Growth of Juvenile Freshwater Mussels Reared at the Aquatic Wildlife Conservation Center

List of Figures

Chapter 1. Survival and Growth of Juvenile Freshwater Mussels Reared at the Aquatic Wildlife Conservation Center

List of Tables

Chapter 2: GIS-based habitat model to identify Clinch River sites suitable for the release of captively propagated freshwater mussels

List of Figures

Chapter 2: GIS-based habitat model to identify Clinch River sites suitable for the release of captively propagated freshwater mussels

List of Tables

Chapter 2: GIS-based habitat model to identify Clinch River sites suitable for the release of captively propagated freshwater mussels

List of Appendices

GENERAL INTRODUCTION

Status and life history

Of the nearly 300 species of freshwater mussels in North America, 70 species are listed on the federal register as threatened or endangered (USFWS 2002), and at least 143 others are afforded a lesser level of government protection (Williams et al. 1992, Richter et al. 1997). Due to the decline in numbers and the recent extinction of 30 mussel species (Parmalee and Bogan 1998), nationwide restoration and preservation activities have been initiated to conserve the remaining fauna. Freshwater mussel populations face several challenges to survival and reproduction, including impoundments, declines in water quality, sedimentation, and introduction of exotic species (Williams et al. 1992). Some of these issues are being addressed through legislation to prevent further habitat loss and decline in numbers; e.g., addition of zebra mussels (*Dreissena polymorpha*) to the Lacey Act's "dirty list", passage of the 1990 Non-Indigenous Aquatic Nuisance Prevention and Control Act, and adoption of best management practices by the U. S. Forest Service and other forest management agencies (Prud'homme and Greis 2002). However, for many endangered mussel species, populations are seemingly already below the numbers necessary for successful natural reproduction.

While freshwater mussels can inhabit lakes and reservoirs, the fauna is most diverse in rivers and streams having substantial flow (Johnson and Brown 2000). Juvenile and adult mussels live either partially or completely buried in the substrate, filtering algae, detritus and microorganisms from the water column (Fuller 1974). Most species prefer clean, silt-free, shoals of cobble and gravel interspersed with sand (Fuller 1974, Williams et al. 1992).

Freshwater mussels have a complex reproductive cycle with a larval stage that parasitizes fish hosts before transforming into a free-living juvenile mussel. The cycle begins when male mussels release sperm into the water column, which are subsequently filtered out by conspecific females. Mature eggs are fertilized when water, transporting sperm passes over the gills. Fertile eggs are then brooded in the gill marsupium until they reach a mature larval stage, called a glochidium. Duration of brooding larvae is categorized as either long-term (bradytictic) or short-term (tachytictic); (Ortman 1911). Long-term brooders typically spawn during late summer, brood young over winter, and release glochidia the following spring and early summer. Short-term brooders spawn in the spring and release glochidia that summer. Fully developed glochidia must be released in close proximity to a suitable host fish, to become encysted on its gills or fins. Glochidia remain encysted for 2-6 wk, then transform into juvenile mussels and drop off into the substrate, causing little or no harm to the host fish. Though some mussel species are generalists, capable of successfully parasitizing many species of fish, most are specialists able to transform on only a few host fish species (Fuller 1974, Williams et al. 1992, Parmalee and Bogan 1998).

With endangered species restoration in mind, techniques have been developed to successfully propagate mussels in captivity. Using these techniques, glochidia from gravid females can be collected, infested onto appropriate host fish, and then transformed juveniles can be cultured for release into the wild. While considerable success has been realized, methods for propagation are still being refined in order to increase juvenile survival during culture.

Propagation efforts

Researchers have developed a variety of techniques for culturing juvenile mussels over the last century, and have achieved varying degrees of success. In one of the earliest documented efforts, Lefevre and Curtis (1910) attempted to rear juvenile mussels in aquaria, but observed 100 % mortality within the first month of culture. There is a gap in mussel propagation literature from roughly 1920 to 1980. However, after passage of the Endangered Species Act in 1973, 23 species of freshwater mussels were added to the list of federally protected species. Interest in mussel propagation was renewed as populations continued to decline, and recovery efforts for critically endangered species looked to captive culture and relocation. A study by Hudson and Isom (1984) was perhaps the first successful attempt to culture juvenile freshwater mussels in the laboratory. Since then, efforts by Buddensiek (1995), Gatenby et al. (1996), O'Beirn et al. (1998), Starkey, et al. (1998), Hanlon (2000), Jones (pers. comm.) and other unpublished studies have diversified and improved mussel culture techniques.

Goals of propagation include producing large numbers of viable juvenile mussels and then rearing them in a protected environment through the vulnerable first stages of life. Captive propagation can yield a far greater number of juveniles per gravid mussel than natural infestations, by increasing the proportion of glochidia that successfully infest host fish (Neves and Widlak 1988). Furthermore, a temperature-controlled and predator-free environment may support faster growth and better survival of newly metamorphosed mussels when compared to the wild condition (Beaty 1997).

Researchers have tested a variety of culture systems. Gatenby et al. (1996) used aerated culture dishes to rear juveniles of *Villosa iris* (Lea, 1829), whereas O'Beirn et al. (1998) utilized recirculating culture systems for studies with *Lampsilis fasciola* (Rafinesque, 1820). J. Jones (pers. comm.) designed a 'mesocosm' recirculating system to rear a variety of mussel species. The mesocosm is a multi-stage recirculating system incorporating several natural biological processes, including bacteria growth and detritus decomposition. This mesocosm approach attempts to mimic a natural stream system and provides a more diverse diet for newly metamorphosed juveniles. All of these systems, however, require frequent maintenance and supplemental feeding with cultured algae. In contrast, Hanlon (2000) reared juveniles of *L. fasciola* in hatchery raceways at the Aquatic Wildlife Conservation Center of the Virginia Department of Game and Inland Fisheries, in Marion, Virginia. Juveniles in this hatchery system required minimal maintenance and no supplemental feeding, and, although a less controlled environment, summer survival rates exceeded those of most published studies. It is, as yet, unknown whether mussel culture in these raceways can achieve similar success in rearing endangered juvenile mussels.

Reintroduction as a tool for recovery

The capability to produce juvenile mussels in the laboratory will be of little conservation value if these individuals do not survive after release. Key to this issue is the identification of suitable locations for release. Currently, there are many locations where populations are critically low or extirpated, but that may once again support viable populations through augmentation or reintroduction (Henley and Neves 1999). Concern over outbreeding depression and loss of rare alleles has fueled opposition to introduction of captive-reared juveniles (Riusech and Barnhart 1998, Hoeh et al. 1999, Rogers 1999). Additionally, since habitat degradation and poor water quality are two of the primary reasons for the initial declines, it is not certain that propagated individuals could survive and reproduce.

Issues of conservation genetics and habitat quality, however, must be weighted against the urgency of recovery and the threat of extinction. Reintroduction of individuals may help endangered species in a number of ways. Reintroduction of species into historic sites can increase the number of healthy populations, and thus reduce the possibility of species extinction from a stochastic event. Augmentation of existing small populations can improve reproductive success throughout the population (e.g., avoidance of the Allee effect; Meffe and Carroll 1997). Augmentation of small or isolated populations also can increase genetic variability, enhancing resilience to environmental changes. Evidence of the need for population enhancement for these reasons is provided both in the literature and in the news media. Griffith et al. (1989) suggest that habitat loss and fragmentation may disrupt dispersal mechanisms, and for species with limited dispersal abilities, translocation or reintroductions may be required to maintain genetic variability. Supporting this is a study by Berg et al. (1998), who found *Elliptio dilatata* (Rafinesque, 1820) to have very low population variation (mean heterozygosity = 0.12) within 10 different sites in Big Darby Creek, Ohio.

The risk of stochastic events to isolated populations of endangered mussels was evidenced in 1997, when a tanker-truck spilled 1400 gallons of a zinc compound into the Clinch River. This spill resulted in the extirpation of mollusks and most other aquatic fauna for > 10 river km, including more than 200 individuals of three federally endangered mussel species (Jones et al. 2001). The spill location narrowly avoided contaminating Indian Creek, a tributary of the Clinch River, which harbors the only known reproducing population of the tan riffleshell, *Epioblasma florentina walkeri* (Wilson and Clark, 1914) in Virginia.

In September 2000, the U. S. Fish and Wildlife service published their final policy regarding controlled propagation of threatened and endangered species (USFWS 2000). The policy supports controlled propagation for purposes of recovery related research, maintaining refugia populations, providing plant or animals for reintroduction, or conserving species at risk of imminent extinction or extirpation. Controlled propagation, according to this policy can only be used if it is a recommended recovery method in the approved species recovery plan. To this end, all prepared recovery plans for listed mussels include juvenile propagation and release into historic habitats as a means of stabilizing and eventually delisting endangered species. The research presented in this thesis attempts to expand knowledge and improve techniques used for mussel propagation and reintroduction.

Literature cited

Beaty, B. B. 1997. Development of juvenile culture techniques and testing of potential biomarkers of environmental stress in freshwater mussels (Bivalvia: Unionidae). Doctoral dissertation. Virginia Polytechnic Institute and State University, Blacksburg, VA.

Berg, D.J., E.G. Cantonwine, W.R. Hoeh, and S.I. Guttman. 1998. Genetic structure of *Quadrula quadrula*: little variation across large distances. Journal of Shellfish Research 17:1365-1373.

Buddensiek, V. 1995. The culture of juvenile freshwater pearl mussels *Margaritifera margaritifera* in cages: a contribution to conservation programmes and the knowledge of habitat requirements. Biological Conservation 74:33-40.

Fuller, S. 1974. Clams and mussels (Mollusca: Bivalvia). *In* Pollution Ecology of Freshwater Invertebrates. C. W. Hart Jr. and S. L. H. Fuller, editors. New York: Academic Press.

Gatenby, C. M., R. J. Neves, and B. C. Parker. 1996. Influence of sediment and algal food on cultured juvenile freshwater mussels. Journal of the North American Benthological Society 15:597-609.

Griffith, B., J. M. Scott, J. W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool: status and strategy. Science 245:477-480.

Hanlon, S. D. 2000. Release of juvenile mussels into a fish hatchery raceway: a comparison of techniques. Master's Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Henley W. F. and R. J. Neves. 1999. Recovery status of freshwater mussels (Bivalvia: Unionidae) in the North Fork Holston River, Virginia. American Malacological Bulletin 15(1):65-73.

Hoeh, W. R., K. S. Frazer, E. Naranho-Garcia, M. B. Black, D. J. Berg, and S. I. Guttman. 1999. Correlation between mating system and distribution of genetic variation in *Utterbackia* (Bivalvia: Unionidae). Journal of Shellfish Research 17(5): 1-11.

Hudson, R. G. and B. G. Isom. 1984. Rearing juveniles of freshwater mussels (Unionidae) in a laboratory setting. The Nautilus 98(4):129-138.

Johnson, P. D., and K. M. Brown. 2000. The importance of microhabitat factors and habitat stability to the threatened Louisiana pearlshell, *Margaritifera hembeli*. Canadian Journal of Zoology 78:271-277.

Jones, J. W., R. J. Neves, M. A. Patterson, C. R. Good, A. DiVittorio. 2001. A status survey of freshwater mussel populations in the upper Clinch River, Tazewell County, Virginia. Banisteria 17:20-30.

Lefevre, G. and W. C. Curtis. 1910. Experiments in the artificial propagation of freshwater mussels. Bulletin of the U. S. Bureau of Fisheries 28:615-626.

Meffe, G. K. and C. R. Carroll. 1997. Principles of Conservation Biology. Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts.

Neves, R. J. and J. C. Widlak. 1988. Occurrence of glochidia in stream drift and on fishes of the Upper North Fork Holston River, Virginia. American Midland Naturalist 119(1): 111-120.

O'Beirn, F. X., R. J. Neves, and M. B. Steg. 1998. Survival and growth of juvenile freshwater mussels (Unionidae) in a recirculating aquaculture system. American Malacological Bulletin 14:165-171.

Ortman, A. E. 1911. Monograph of the naiades of Pennsylvania. Memoirs of the Carnegie Museum 4:279-347.

Parmalee, P. W. and A. E. Bogen. 1998. The Freshwater Mussels of Tennessee. The University of Tennessee Press, Knoxville, TN.

Prud'homme, B. A. and J. G. Greis. 2002. Best management practices in the South. Pages 519-535 *in* D. N. Wear and J. G. Greis, editors. Southern Forest Resource Assessment. General Technical Report SRS-53. Asheville, NC. U. S. Department of Agriculture, Forest Service Southern Research Station.

Richter, B. D., D. P. Braun, M. A. Mendelson, and L. L. Master. 1997. Threats to imperiled freshwater fauna. Conservation Biology 11:1081-1093.

Riusech, F. A. and M. C. Barnhart. 1998. Host suitability and utilization in *Venustaconcha ellipsiformis* and *Venustaconcha pleasii* (Bivalvia: Unionidae) from the Ozark plateaus. Pages 83-91 *in* R. A. Tankersley, A., D. I. Warmolts, G. T. Watters, B. J. Armitage, P. D. Johnson, and R. S. Butler, editors. Proceedings of the Freshwater Mollusk Symposia. Ohio Biological Survey, Columbus, Ohio.

Rogers, S. O. 1999. Population biology of the tan riffleshell (*Epioblasma florentina walkeri*) and the effects of substratum and light on juvenile mussel propagation. Master's Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Starkey, R. W., A. G. Eversole, and D. E. Brune. 1998. Growth and survival of juvenile and adult freshwater mussels in the partitioned aquaculture system . Pages 109-114 *in* R. A. Tankersley, A., D. I. Warmolts, G. T. Watters, B. J. Armitage, P. D. Johnson, and R. S. Butler, editors. Proceedings of the Freshwater Mollusk Symposia. Ohio Biological Survey, Columbus, Ohio.

U. S. Fish and Wildlife Service. 2000. Policy regarding controlled propagation of species listed under the Endangered Species Act. Federal Register 65(183): 56916-56922.

U. S. Fish and Wildlife Service. 2002. Endangered and threatened wildlife and plants: box score. Endangered Species Bulletin 19(2):32

Williams, J. D., M. L. Warren, K. S. Cummings, J. L. Harris, R. J. Neves. 1992. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18:6-22.

Chapter 1: Survival and Growth of Juvenile Freshwater Mussels Reared at the Aquatic Wildlife Conservation Center, Marion, Virginia

INTRODUCTION

In 1996, facilities at the Buller Fish Cultural Station of the Virginia Department of Game and Inland Fisheries were allocated to the conservation of non-game aquatic fauna. The resulting Aquatic Wildlife Conservation Center (AWCC) renovated several hatchery raceways and a pond to accommodate goals of long-term holding and culture of freshwater mussels. Since the creation of AWCC, numerous experiments have been conducted to determine the feasibility of rearing juvenile mussels at this facility. Hanlon (2000) was successful in rearing wavyrayed lampmussels, *Lampsilis fasciola* (Rafinesque, 1820) in raceways, but his experiments experienced problems with fish predation as well as reduced growth and survival attributed to excessive silt deposition. My research commenced in summer 2001, with the goal to design and test several low maintenance culture systems for culturing mussels at the AWCC. These systems were to be evaluated using both common and endangered mussels to identify species-specific culture preferences. Growth and survival of newly metamorphosed mussels were used to evaluate a pond, a raceway, and flow-through troughs as rearing environments.

A series of three experiments were conducted at the AWCC during the summers of 2001 and 2002. The first experiment compared rearing conditions in a holding pond and in the hatchery raceway. This study was abandoned after 2 wk (sampling event 1) due to nearly 100 % mortality of juvenile mussels. The second experiment, also conducted in the summer of 2001, compared growth and survival of the rainbow mussel, *Villosa iris* (Lea, 1829) reared in two different systems, indoor flow-through aquaria and outdoor PVC troughs. Experiment 3 was conducted in the summer of 2002, and compared growth and survival of endangered oystermussel, *Epioblasma capsaeformis* (Lea 1834) and a common mussel species, *L. fasciola*, under two silt treatments.

Study area

Culture experiments were conducted at the AWCC near Marion, Virginia (Figure 1.1). The hatchery consists of a series of holding ponds and raceways fed from a small impoundment on the South Fork Holston River (SFHR), at river mile 105. Water for all treatments is fed through two ponds. The first is a 12,140 m^2 pond used for muskellunge (*Esox masquinongy*) rearing. Water is piped from this pond to a lower holding pond (60 x 18 x 1 m), supplemented by water pumped directly from the SFHR. In 1999, the lower pond was lined with sand and a polyethylene liner to prevent leakage. Pipes from the holding pond supply the raceway with water warmer (due to solar exposure) than occurs in the river. Temperature data from summers of 1999 and 2001 indicate that the retaining pond increased mean daily water temperatures in the raceway to 20.2° C and 19.0°C, an average of 2.7 and 1.8 °C, respectively, warmer than water in the SFHR. Discharge volume from the pond can be adjusted with a valve, and water directly from the river can be simultaneously fed to the culture building and raceways to maintain consistent flow. Summer flow through the raceway is maintained at 720 L/min (velocity = 0.13 cm/sec). With all flow through the raceways and the culture buildings coming from the pond (no supplemental flow from the river), turnover time for the pond is approximately 25 hr.

All three of these experiments were designed to assist hatchery personnel in establishing techniques for successfully rearing endangered juvenile mussels. The basic designs of these experiments were developed to accommodate limited personnel and space availability. Other hatchery characteristics also were accommodated; these obstacles are discussed individually.

Silt- Silt in water drawn from the river naturally accumulates in the hatchery ponds and raceways. The first holding pond also serves as a settling pond; however, due to the short turnover period in the pond (approximately 25 hr), much of the silt is carried to the raceways. Silt deposits throughout the raceways, but accumulation is greatest at the head of the raceway. Silt is a concern for the survival of freshwater mussels, especially during the juvenile stages (Henley et al. 2000). Silt must be physically removed from containers holding juvenile mussels to prevent lethal levels of accumulation.

Filamentous algae- Filamentous algae growth (primarily *Cladophora* sp.) is abundant in the outdoor raceways during mid to late summer (June-September). Algae growth does not appear to inhibit juvenile mussel survival; in fact, observations at the hatchery may indicate that algae growth in some quantity is beneficial to juveniles by providing a refuge from predators. Filamentous algae do, however, create difficulty when sampling juveniles in experimental treatments. Juveniles become entangled in the long hair-like strands and are difficult to detect. In addition to the increased effort required to sample juveniles in algae, the loss of juveniles in algae may cause overestimation of mortality.

Predation- Several mussel predators have been identified at the AWCC. The two primary predators are flatworms (*Macrostomum* sp.) and fathead minnows (*Pimephales promelas)*. Flatworms in the genus *Macrostomum* have been identified in several studies as predators of juvenile mussels (Sickel 1998, Zimmerman et al. 2003). Predation by flatworms seems to be size-constrained and does not noticeably affect juvenile survival after age 2-4 wk of age (400-500μm long). Hanlon (2000) identified fathead minnows as predators of juvenile freshwater mussels at this facility.

Escapement- Previous experiments indicate that juvenile mussels are highly mobile. Laboratory observations by Hanlon (2000) and Hanlon and Levine (2001) indicate that mussels can climb vertical surfaces of containers and escape from treatment units. Designing treatment units at the hatchery to eliminate, or more realistically, reduce escapement is a challenge. Screens are not practical at AWCC, as silt and algae accumulation require at least daily cleaning to prevent clogging and overflow.

Temperature- Juvenile growth is directly influenced by water temperature. Studies indicate that approximately 15° C marks the lower critical temperature for mussel growth (Beaty 1997). Although AWCC is a warmwater hatchery, water temperatures consistently dip below 15° C during the months of October through April. Juvenile mussel trials are therefore restricted to summer months at the hatchery, with experiments commencing in May or June and culminating in September.

Species selection

The rainbow mussel, *Villosa iris* (Lea, 1829), wavyrayed lampmussel, *Lampsilis fasciola* (Rafinesque, 1820), and oyster mussel, *Epioblasma capsaeformis* (Lea, 1834), were the preferred species to use in these experiments for several reasons. First, information from several studies provides background data on survival rates for rearing these species in captivity. Recirculating systems at the Freshwater Mussel Conservation Center (FMCC) at Virginia Tech have yielded survival rates for *V. iris* ranging between 8 and 30 % (Gatenby et al.1996, Beaty 1997, Gatenby et al. 1997, O'Beirn et al. 1998). Growth and survival of *L. fasciola* in both laboratory and hatchery conditions are available from studies by Steg (1997) and Hanlon (2000). J. Jones (pers. comm., Virginia Tech, Blacksburg, Virginia) has cultured *E. capsaeformis* at FMCC since 1998. Secondly, host fishes for these three mussel species are known (Zale and Neves 1982, Yeager 1995), and we have had good success in producing large numbers of juveniles in the laboratory. Lastly, sufficient numbers of gravid females can be collected to produce the necessary number of juveniles for these experiments. The latter is particularly important for *E. capsaeformis*, as it is federally endangered and of limited availability.

MATERIALS AND METHODS

Production of Juveniles

All juvenile mussels used in the culture experiments were produced by induced infestations on host fish (Zale and Neves 1982) at the FMCC, Blacksburg, Virginia. Gravid mussels were collected by snorkeling. Females of *V. iris* were collected from Indian Creek, Tazewell County, VA, and *L. fasciola* and *E. capsaeformis* from the Clinch River, Hancock County, TN. After collection, all mussels were transported to FMCC in coolers filled with river water. Infestations were conducted by flushing glochidia from the gills of the gravid female into a Petri dish using a large bore needle and syringe. Suitable host fish were placed in a container with sufficient water to cover them. Glochidia collected from the gravid females were mixed in the water containing host fish, and an airstone was used to aerate the water and maintain glochidia in suspension. Infestation of the glochidia onto the gills of fish then occurred passively via fish respiration. The gills of the fish were periodically examined until they appeared sufficiently infested, which occurred after approximately 45 min. The fish were then placed in aquaria and held until transformation and juvenile excystment occurred. Juveniles were collected by siphoning the bottoms of the tanks and passing the siphonate through a 120-µm-mesh sieve. Once collected, juveniles were enumerated, measured, transported to the hatchery, and randomly assigned to treatments.

Experiment 1: Utilization of holding ponds and a raceway for rearing mussels

Gravid females of *L. fasciola* and *E. capsaeformis* were collected from the Clinch River, Hancock County in May 2001. Largemouth bass (*Micropterus salmoides*) from the New River served as host fish for *L. fasciola*, while banded sculpin (*Cottus carolinae*) from the South Fork Holston River were used to transform glochidia of *E. capsaeformis*. Both fish species were collected using a backpack electroshocker. Newly metamorphosed juveniles were placed at the AWCC on May 29, 2001. For this experiment, 10 shoebox-size containers (32 x 19 x 11 cm) holding 100 newly metamorphosed *L. fasciola* and 2 mm of coarse limestone sand (1000-2000 µm) were placed on the bottom of the retaining pond (PB). Ten similar containers were suspended in the pond 0.3 m below the surface of the water (PF), and another 10 containers were placed in the raceway (RW), for a total of 3000 juveniles of *L. fasciola* (Figure 1.2). The same configuration also was arranged using 3000 newly metamorphosed *E. capsaeformis*. Including the PB, PF, and RW treatments for both species of mussels, there were a total of 60 containers with 100 mussels in each.

To estimate silt accumulation in treatment locations, 3 empty shoebox-sized containers were placed at each location: PB, PF, RW. Concurrent with each sampling event, contents of the shoeboxes were individually collected in water-tight containers, transported to Virginia Tech, and dried in an oven at 96°C for approximately 4 d, or until all water had evaporated. Dry weights were calculated by subtracting container weight from the combined weight of the container and silt. Dry weights of silt for the 3 replicates were averaged for each location to produce a mean dry weight, which was later used for comparisons among locations.

Containers assigned to the PF treatment were suspended by one of 5 cages constructed to hold four containers (two of each mussel species being tested). Floating cages were constructed with 10.1 cm PVC to create a 1-m square floating collar. A 1 x 0.75-m aluminum grate was attached to the PVC collar by the corners using 1.25-cm nylon rope. All 4 containers were secured to the grate using elastic cords. An 11-kg sandbag was used to anchor each cage at a given location within the pond. Containers (floating and bottom) in the pond were randomly assigned to locations near the outflow end to utilize water having the highest algal density and water temperature. To eliminate possible raceway effects, treatments in the raceway were all placed in a single raceway unit.

Twenty days post-metamorphosis, half of all containers (5 from each treatment and species) were sampled. Substrate in the container was separated from the juveniles by first washing it through a series of sieves. A 600-μm sieve retained most of the coarse sand substrate while allowing the juveniles to pass through unharmed. A 150-μm sieve below the 600-μm sieve was small enough to collect the juveniles, but large enough to allow most of the accumulated silt to pass through. Some of the pre-sieved sand particles and invertebrates passed through the coarse sieve, so material in the 150-µm sieve was then washed into an elutriator and tumbled for 3 min. The elutriator was constructed of a 90 cm long clear PVC pipe, 5 cm in diameter with a PVC couple joined to a 5-cm x 2.5-cm reducer. A 2.5-cm ball valve was attached to the reducer, and a 1.4-cm garden hose fed out of the other end of the ball valve. A piece of 200-µm Nitex mesh was placed between the couple and reducer to prevent juveniles from falling into the ball valve. This elutriator separated particles by density; lighter particles, including juvenile mussels, are lifted out the terminal end of the PVC pipe and more dense particles (sand) remain in the pipe. Water and lifted particles were caught in a 150-µm sieve. Water was drawn through the elutriator using a 0.5-hp magnetic drive pump.

<u>Experiment 2: Feasibility of using troughs and aquaria for rearing mussels</u>

Rainbow mussels (*V. iris)* were collected from Indian Creek, a tributary to the Clinch River in Tazewell County, Virginia. Infestations were conducted on rock bass (*Ambloplites rupestris*) from the New River, which were collected using a backpack electroshocker. For this experiment, 4 PVC troughs were used to rear 800, 10 to 14 day-old *V. iris*, or 200 per trough (Figure 1.3). Troughs were constructed from 15.2-cm diameter schedule-40 PVC pipe, cut longitudinally with caps on each end. Approximately 8 cm from the downstream end of the pipe, a 1-cm hole was drilled through the pipe, and a 1.3-cm male adapter was pushed through and tightened by a female adapter on the outside of the pipe (Figure 1.4). The male adapter served as a standpipe for water to flow out of the trough while maintaining water level at approximately 85% capacity. Total volume of the troughs was approximately 7 L. All troughs were located outdoors, and were covered by a 50 % shade cloth to reduce sun exposure, lessen effects of precipitation, and reduce algae growth.

Bottoms of the troughs were covered with coarse sand to a depth of 2 mm (particle size = 1000-2000 μm). Juveniles were distributed throughout the entire trough and were not confined to dishes. The flow rate in the troughs was held constant at 7 L/min. An additional 400 *V. iris* were held in four 13 x 7 x 6-cm glass dishes (100 mussels per dish) in 4 indoor flow-through aquaria. Substrate in the dishes consisted of coarse sand 2 mm in depth. Flow rates in the aquaria were approximately 3 L/min.

Juvenile mussels in all troughs and glass dishes were sampled at 2-wk intervals for 8 wk. At each sampling event, all juveniles in the container were enumerated, and 10 individuals from each container were randomly selected for length and width measurements to estimate growth rate (Chalkey 1943, Hanlon 2000). Methods for separating juveniles from the substrate and accumulated silt were similar to those described for Experiment 1.

Due to considerable juvenile predation by fathead minnows (*Pimephales promelas*) in Experiment 1, all water flowing into the troughs first passed through a 500-μm filter to prevent entry of minnows. Minnows were not observed in the aquaria, and therefore no filtration precautions were used for the aquaria systems.

Experiment 3: Comparison of survival and growth of mussels under two silt regimes

This experiment is similar in design to trials conducted for Experiment 2 in Summer 2001, but differed in species tested, trough diameter, and sampling design.

Troughs for this trial were similar to those described in Experiment 2, except that the diameter of the pipe used for construction was 7.6 cm rather than 15.3 cm. Total volume of each trough was approximately 2 L. Successful mussel culture in similarly designed containers in Summer 2001 (see Experiment 2), and by Hanlon and Levine (2001), initiated this system design. Practical considerations, including space and budget constraints, were met with this design. The individual troughs provided statistically independent replicates, were efficient to sample, and since recovery rates in Experiment 2 were high, this design was thought to limit emigration of juvenile mussels from the treatment units.

Water entered the system from the 1000 m^3 holding pond, passed through a 500-μm Nitex mesh, to remove potential predators such as fathead minnows, and was held in a 380-L reservoir. Water left the reservoir through a 3.2-cm bulkhead, approximately 8 cm from the bottom. The pipe leading out of the reservoir was expanded to 5 cm and was split every 2 m by a 5-cm T-joint. A manifold serving 5 troughs was attached to each T-joint. The manifold was constructed of 5-cm pipe, cut every 25-cm and joined with a 5 x 5 x 1.9-cm reducing-T. Each 1.9-cm reducing-T fed an individual trough via an adjustable ball valve that regulated flow (Figure 1.5). Silt naturally accumulated in the troughs as it was carried by the water from the holding pond. The low-silt treatment had silt physically removed every 2 wk, whereas silt in the high-silt treatment was allowed to accumulate for 6wk.

For this experiment, 10 PVC troughs were used to rear 1000 newly metamorphosed *E. capsaeformis* (100/trough), and an additional 10 troughs contained 2000 *L. fasciola* (200 juvenile mussels/replicate trough). The bottoms of the troughs were covered with coarse sand to a depth of 2 mm (particle size = 1000-2000 µm). Juveniles were distributed throughout the entire trough and not confined to dishes. The flow rate in the troughs was held constant at 1 L/min.

Ten of the troughs (5 with *L. fasciola* and 5 with *E. capsaeformis*) were sampled every 2 wk for 10 wk. At each sampling, juveniles in the trough were enumerated, and 10 individuals were randomly selected for length and width measurements to estimate growth rate. Juveniles were separated from substrate in the treatment containers by first washing the water and substrate through a 250-µm sieve. After sieving, substrate was washed into an elutriator and tumbled for 3 min to separate juveniles from coarse sand. Juveniles in the 10 troughs in the high-silt treatment were only sampled at weeks 6 and 10.

Sediment

Sediment accumulation was quantified by collecting the sieved contents of each sampled trough. Water, substrate, silt, and juveniles in the trough were poured through a 200-µm sieve. Juveniles and substrate were retained in the sieve, and the water and silt that passed through the sieve was collected in a 19-L bucket. The sieve contents were washed thoroughly with silt-free water to ensure that all silt was collected in the bucket. The volume of water and silt in the bucket was diluted to 14 L, and a 1-L grab sample was collected from the homogeneously mixed slurry. The slurry sample was transported to Virginia Tech for determination of silt dry weight. Samples were shaken to ensure uniform mixing and 200 ml of the slurry was poured into a pre-weighed Pyrex dish. Samples were dried at 97°C for 5 d, until all water had evaporated and silt was completely dry. Dried samples were weighed, and the weight of the glass dish tared.

Weights of dried samples were used to calculate the quantity of silt in the entire trough. Silt samples were not ashed for determination of organic content. The presence of flocculant material mixed in the deposited silt indicated that some of the material referred to as 'silt' was actually fine particulate organic matter (FPOM).

Water Chemistry

Water chemistry parameters were tested during biweekly sampling events. A Hach™ DR/2000 spectrophotometer was used to measure total ammonia (NH_3), hardness, alkalinity, nitrite (NO_2) and nitrate (NO_3) from water samples collected at AWCC and transported to the FMCC. Dissolved oxygen and pH were measured using a YSI meter on site, and temperature was recorded hourly with an Onset Optic Stowaway data logger (Onset Computer Corporation, Pocasset, MA).

Algal biomass

Algal density was quantified by analyzing chlorophyll α content. A water sample from the distribution reservoir was collected in an amber-colored bottle and transported on ice to Virginia Tech for chlorophyll analysis. Procedures for chlorophyll extraction and estimation follow those outlined by Standard Methods for the Examination of Water and Wastewater (APHA et al. 1989) for spectrophotometric determination. The only variation from this method was the use of ethanol for chlorophyll extraction rather than acetone (Webb et al. 1992). Algal biomass was reported as mg/m^3 and was calculated as:

$$\frac{26.7\,(664_b - 665_a) * V1}{V2 * L}$$

V1 = volume of alcohol (L)
V2 = volume of sample filtered (M^3)
L = light path length of cuvette
664_b = absorbance at 664 nm
665_a = absorbance at 665 nm with acid addition

Data Analysis

For the three juvenile culture experiments, I tested for significant differences in growth and survival among treatments and species. Since individual mussels were not uniquely marked and sample sizes were small, it was not possible to use the repeated measures approach to statistical testing. Data on both growth and survival are presented graphically, and where appropriate, comparisons among treatments and species were made using data collected at the final observation. Because long-term survival was the desired outcome, comparison of data at test culmination was considered most important. Differences were tested at a significance level of $\alpha = 0.05$. All results are reported as the mean ± standard error.

Fisher's exact test

Given marginal frequencies in a table and assuming that two factors in the table are not related, Fisher's test computes the likelihood of obtaining cell frequencies as or more extreme than observed ones. For small samples, this probability can be computed exactly by counting all possible tables that can be constructed based on marginal frequencies.

Mantel-Haensel Test

The Mantel-Haensel test (Q_{MH}) is a non-parametric test for binomial data in 2x2 contingency tables and was used for survival comparisons in Experiment 3. The Q_{MH} was used to test the null hypothesis that survival did not differ between species. Individuals of the same species and treatment were pooled for analysis. The test requires a total sample size of at least 30, and that both treatments begin with the same number of individuals. Since the *L. fasciola* treatments started with 200 individuals per trough and

E. capsaeformis began with 100, values for *E. capsaeformis* were doubled to match those of *L. fasciola*. After doubling, a contingency table for Experiment 3 was constructed as follows:

TREATMENT=LOW-SILT (final sampling event)

species Survival ID

Frequency Row Percent	Yes	No	Total
E. capsaeformis	6 0.06	994 99.4	1000
L. fasciola	58 5.80	942 94.20	1000
Total	64	1936	2000

Survival ID = Y for survival to that day or N for dead on that day

RESULTS

<u>Experiment 1: Utilization of holding ponds and raceway for rearing mussels</u>

Survival

Five replicates were sampled from each of the 3 treatments and each of the 2 species (30 total observations) at 14 days post-metamorphosis. Juveniles of *L. fasciola* and *E. capsaeformis* placed in all 3 treatments (PF, PB, and RW) experienced high mortality prior to this first sampling event (Figure 1.6). Survival of *L. fasciola* in the raceway treatment ranged between 0 and 10 % (mean 7.2 %) at 14 days post-metamorphosis, and survival of *E. capsaeformis* in the same treatment ranged between 7 and 19 % (mean = 10.5 %). Survival in the PF treatment was 0-8 % (mean = 3.6 %) and

1-10 % (mean = 4.2 %) for *L. fasciola* and *E. capsaeformis*, respectively. In the PB treatment, survival ranged between 0 and 8 % (mean = 2.8 %), and was 0-7 % (mean = 1.7 %) for *L. fasciola* and *E. capsaeformis*, respectively.

Statistical analyses were conducted on survival data collected at week 2. Survival did not differ between *L. fasciola* and *E. capsaeformis* within any of the 3 rearing locations (Fisher's exact test; $p>0.05$). Survival of *L. fasciola* did not differ between treatment locations; however, survival of *E. capsaeformis* was greater in RW than in PB ($p = 0.002$) or in PF ($p = 0.02$; Fig 1.6). As predation threats were similar for both species in all 3 treatments, this may indicate that *E. capsaeformis* is more suited for rearing in a raceway than a pond.

Despite using a 150-µm sieve to collect the juveniles when separating from the substrate, little if any shell material was observed in the containers to account for the missing or dead individuals. A 150-µm sieve is fine enough to collect newly metamorphosed juveniles; therefore, it is unlikely that shell material present in the containers would have been missed. During the experiment, fathead minnows were observed in both the experimental pond and raceway. Minnows were introduced as forage for muskellunge in the pond that fed the experimental holding pond. Due to high mortality and the likelihood that this mortality was caused by minnow predation, Experiment 1 was terminated after the first sampling event, 2 wk post-metamorphosis.

Growth

Lengths and widths of all surviving mussels were measured (Figure 1.7). Pooled across treatments, the 68 *L. fasciola* alive at sampling event 2 averaged 385 µm in length. Separated by treatment, mean lengths were 374, 376, and 387 µm for PF, PB, and RW, respectively. For the 78 surviving *E. capsaeformis*, average length was 354 µm. For PF, PB, and RW locations, lengths were 358, 375, and 352 µm, respectively. There was no significant difference in length for either species among treatment locations (PB, PF, RW). Juveniles of *L. fasciola* showed a significantly greater increase in length (i.e. growth) than *E. capsaeformis* ($p = 0.03$) in all 3 treatments.

Silt

Silt accumulation in the treatment containers varied substantially among locations. Mean dry weights of silt were 10.31 mg, 6.19 mg, and 3.57 mg for PB, PF, and RW treatments, respectively. There were significant differences among all pairs of treatment locations ($p<0.05$).

Water quality

Chlorophyll-α Water samples were collected on 29 May, at the start of the experiment, and at first sampling on 11 June. Samples were analyzed for chlorophyll-α content, but both samples contained undetectable levels of chlorophyll.

Temperature Data loggers recorded water temperature in the three treatment locations and at the intake pipe in the South Fork Holston River. Temperatures were plotted to show the degree of solar warming as water flowed from the river through the holding pond and into the raceway (Figure 1.8). Temperatures increased in the following order: river < pond bottom < raceway < pond floating. Daily average temperatures differed significantly among all locations.

<u>Experiment 2: Feasibility of using troughs and aquaria for rearing mussels</u>

Survival

Juveniles of *V. iris* in Experiment 2 were placed in treatments 14 days post-metamorphosis. Survival of juveniles in Experiment 2 was better than either of the other two experiments and did not show the high early mortality observed in Experiments 1 and 3. Mean survival in aquaria was 77.0 ± 9.9 % after 14 days, and 60.8 ± 6.4 % and 54.8 ± 9.2 % after 28 and 42 days, respectively. In the outdoor troughs, mean survival was 72.8 ± 7.9 %, 51.3 ± 9.4 %, and 42.6 ± 6.4 % after 14, 28, and 42 days, respectively (Figure 1.9). At the end of the 8-wk trial, mean survival of *V. iris* was 51.3 ± 8.7 % in the aquaria and 39.9 ± 7.5 % in the PVC troughs. Due to the high variability of survival between replicates, these differences were not significant when compared using Fisher's test ($p>0.05$).

Growth

Growth rates tended to be higher in the outdoor troughs, but due to high variability length differences in valves between treatments were not significant ($p>0.05$), with lengths at 90 days post-metamorphosis averaging 794 μm in the aquaria and 857μm in the troughs (Figure 1.10). Any difference between growth rates can likely be attributed to slightly higher average daily temperature (0.75°C) in the PVC troughs than in the aquaria (Figure 1.11). Growth appeared linear, lacking any obvious growth "spurts" within the first 90 days post-metamorphosis (Figure 1.9).

Experiment 3: Comparison of survival and growth of mussels under two silt regimes

Survival

Silt comparison trials for *E. capsaeformis* and *L. fasciola* ran for 70 days. Survival trends are demonstrated graphically (Figure 1.12), but statistical comparisons were made only on days 40 and day 70. On these two dates, survival estimates were computed for both the high-silt and low-silt treatments to compare silt level as well as species. As replicates were randomly assigned to troughs, surviving individuals from the 5 replicates of each species and treatment were grouped together for statistical analysis.

Observations from the low-silt treatment indicate that the greatest mortality occurred within the first 2 wk post-metamorphosis, after which mortality rates slowed. Results indicate that individuals in the high-silt treatment had significantly greater survival than those in the low-silt treatment, when compared with a 2-tailed Fisher's Exact Test (298 vs. 64). *L. fasciola* had significantly greater survival than *E. capsaeformis* at both high-silt (170 vs. 128) and low-silt (58 vs. 6) treatments.

Growth

A growth model was fit to the mean length of juveniles in each trough. The slopes of these curves were compared using a t-test to evaluate differences in growth rates between species. Growth rates of *L. fasciola* were greater than that of *E. capsaeformis* ($p < 0.001$). Using length measurements of juveniles from the low-silt treatment only, plots illustrate growth trends for both species tested (Figure 1.13). Individuals from all replicates were combined to provide an overall average for each species. Average lengths of each species at both the starting point (age 0) and experiment terminus (age 70) are different (Figure 1.13). Additionally, significant differences between high and low-silt treatments of the same species were detected (Figure 1.14). Length measurements of 70-day-old juveniles of *L. fasciola* for high-silt and low-silt treatments were compared using a t-test, which indicated that juveniles in the low-silt treatment were larger at 70 days of age ($p < 0.0001$). A similar comparison was done for *E. capsaeformis* using length measurements at 40 days, as an insufficient number of individuals survived to day 70 for statistical comparisons. The t-test performed on length measurements of mussels 40 days old indicated that individuals in the low-silt treatment were significantly larger in size than individuals in the high-silt treatment ($p < 0.0001$).

Silt

Silt accumulation in the troughs was highly variable through time (Figure 1.15). Variability among sampling events can be attributed to rain or other events that affected turbidity in the SFHR. Variation among replicates at the same sampling event is more difficult to explain. Although no apparent distribution trend was observed among trough locations, some troughs contained greater numbers of macroinvertebrates than others. Isopods, which are known to consume detritus, were the dominant invertebrates in the troughs. Anecdotal observations indicate that troughs containing high densities of isopods had less silt accumulation than those having fewer isopods. It is also possible that some silt settled within the pipes feeding water to the troughs.

Mean silt accumulations for the 2 wk periods between low-silt sampling events were 4.27 ± 0.53, 3.35 ± 0.47, 2.73 ± 0.46, 4.01 ± 0.67, and 4.25 ± 1.10 mg of silt for sample events 1 through 5, respectively. Accumulations for the high-silt treatments were 5.47 ± 1.4 and 6.99 ± 1.7 mg after 6 and 10 wk, respectively. The first sampling event of the high-silt treatment co-occurred with low-silt sampling event 3 and the final high-silt sampling event with low-silt event 5. Silt accumulation in the high-silt treatment was significantly greater than the low-silt treatment after 6 and 10 wk ($p<0.05$). Silt accumulation for both high-silt sampling events was lower than the cumulative means of the low-silt accumulation at events 3 and 5.

Water chemistry

Chemical analyses Water quality parameters measured at each sampling event ranged as follows: total ammonia, 0.02-0.06 mg/L, total hardness 90-136 mg/L, alkalinity 66-140 mg/L, nitrate 0.1-0.7 mg/L, nitrite 0.001-0.015 mg/L, pH 6.78-7.38, and DO 7.08-7.7 mg/L (Table 1.1). There were no obvious temporal trends for the water chemistry parameters measured. The summer of 2002 was characterized by drought conditions. Sediment and nutrient imputs due to runoff were minimal throughout the summer experiment due to low amounts of precipitation.

Chlorophyll α Algal biomass in the distribution reservoir was low throughout the experiment. Algal densities did not reach detectable levels of chlorophyll until sample event 3 (6 wk: 20 July). Chlorophyll levels increased from 2.00 mg/m^3 on 20 July to 8.01 mg/m^3 on 3 August and maintained the same density at sampling event 5 (10 wk: 19 August) (Table 1.1). In spite of a holding pond to enhance temperature and algal production, primary production of phytoplankton was low.

DISCUSSION

Experiment 1: Utilization of holding ponds and a raceway for rearing mussels

Survival of juveniles in this experiment was low, but these findings are still useful for assessing hatchery facilities for grow-out of juveniles. Rearing juvenile mussels in ponds would be the least labor-intensive method for using traditional fish hatcheries for mussel propagation. However, the large volume of water passing through the pond limits options available for predator exclusion. Since both sampling efficiency and predator exclusion are required for successful juvenile mussel culture, the ponds at AWCC are not suitable for rearing newly metamorphosed mussels of these species at this time.

Fathead minnows (*Pimephales promelas*) entered the experimental pond in water flowing from the upper muskellunge-rearing pond, where they were intentionally released as a forage fish. It is unlikely that minnows would have survived the withdrawal pump and been able to enter the experimental pond, had the river been the sole source of water feeding the pond. Mussel predation by fathead minnows may have been reduced had a predatory fish species, such as largemouth bass, been introduced into the rearing environments (pond and raceway) to reduce minnow numbers. This strategy was not used during these experiments for several reasons. First, predation rate would have been higher in the raceway than in the pond due to the more confined space in the raceway, and less cover options for prey, leading to a bias in comparisons between the 2 treatments. Secondly, larger predator fish likely would have disturbed substrate and mussels and possibly could have overturned the containers. Lastly, predation potential by fathead minnows was grossly underestimated, and the need for their control or elimination was unanticipated.

Fathead minnows are not noted to be molluscivores; they are generalist bottom feeders consuming benthic invertebrates living in the substrate (Jenkins and Burkhead 1993). It seems unlikely that minnows would be major predators of juvenile mussels in the wild. However, a lack of substrate and cover in the pond and raceway, lack of predators and competitors, and the unnaturally high density of minnows likely exacerbated predation rates. A study by Bergström and Englund (2002) suggests that predation rates in medium-scaled experiments overestimate natural predation rates due to the concentration of predator and prey species. A lack of alternative cover and predators likely encouraged minnows to forage in the open experimental containers, which resulted in the extreme loss of juvenile mussels.

Although the results of this study may discourage the use of ponds for grow-out of mussels, some studies indicate that ponds can be successfully used for holding adult and juvenile mussels of some species. W. Henley (pers. comm., Virginia Tech, Blacksburg, Virginia) successfully held adults of *L. fasciola*, *V. vanuxemensis*, and *V. iris* in an earthen pond. All three of these species naturally inhabit streams rather than lentic environments. Although these mussels were not assayed for physiological condition, females were able to sustain energy stores required to become gravid during the 14-mo holding period. Dunn and Layzer (1997) held adult mussels of 20 different species in ponds for 12-14 mo. All of the mussels held were riverine; however, survival was variable among species. Similarly, Mummert (2002) reared juvenile *L. fasciola* and *V. iris* in a flow-through pond and observed 49.8 % survival of *V. iris* but only 6.3 % survival of *L. fasciola*. Differences in survival among species may be attributed to species-specific microhabitat tolerances.

Silt tolerance of juvenile mussels has not been tested in laboratory studies; however, some researchers speculate that newly metamorphosed mussels are less tolerant of high-silt loads due to a reliance on interstitial spaces. Studies by Ryan (1991) indicate a general decline in many invertebrate species with an increase in interstitial sediments. Silt loads for both pond treatments were significantly higher than in the raceway. If newly metamorphosed mussels are as sensitive to silt loading as other lotic invertebrates, then many ponds may not be appropriate rearing environments for juvenile mussels even if predation and escapement can be controlled or eliminated. Mussel survival may differ, in ponds with longer water turnover time or those fed by streams with lower silt loads.

Experiment 2: Feasibility of using troughs and aquaria for rearing mussels

Compared to the aquaria, the outdoor troughs had 0.75 C° higher temperatures and potentially greater food availability for juvenile mussels. Immediately preceding the water distribution head for the troughs is a 10-m stretch of raceway that acts as a second (smaller) warming pond (Figure 1.3). Higher temperatures and greater food availability should promote increased growth rates when compared to juveniles reared in aquaria (Buddensiek 1995, Beaty 1997). Additionally, Rogers (1999) found that light exposure had no significant effect on juvenile survival, but positively influenced growth rates for juveniles of *L. fasciola*. Although outdoor systems in this experiment were covered with 50% shade cloth, they still received more light than their indoor counterparts. The drawback of these outdoor systems is increased vulnerability to predators, especially flatworms (*Macrostomum* sp.). Sediments collected from the end of this raceway contained flatworms large enough to consume juvenile mussels over 400 µm in length

(Jess Jones, pers. comm.). Additionally, solar exposure increased the amount of filamentous algae growth in the outdoor troughs, making recovery of juvenile mussels tedious and difficult.

Although there is no way to quantify the extent of predation or sampling error, it seems that most of the apparent difference in survival rates between the two treatments can be explained by sampling difficulty in the outdoor troughs resulting from filamentous algae growth and predation by flatworms. During my sampling events, 6 mussels were observed in the guts of flatworms. Since flatworms consume mussels by digesting soft parts and regurgitating empty valves, it is not possible to visually distinguish between predated juveniles and those that died from other causes (Zimmerman et al. 2003).

Filamentous algae (*Cladophora* sp.) inhibited adequate sampling, but there was no evidence that these algae negatively affected juvenile growth or survival in the outdoor troughs. Juveniles became entangled in clumps of algae. Algae clumps were light enough to be separated by the elutriator, however searching algae for juveniles was tedious and introduced a source of error. The amount of effort required to sample troughs was 4 times greater than that required to sample indoor aquaria lacking filamentous algae. The primary concern with algal blooms in aquatic systems is a reduction in dissolved oxygen (DO) upon decomposition. Dissolved oxygen was measured during sampling events in the water distribution reservoir and in the troughs containing mussels. Values of DO were similar between the trough and the feeding reservoir and averaged 6.3 mg/L, which were safely above the 5 mg/L recommended for unionids (Havlik and Marking 1987).

Experiment 3: Comparison of survival and growth of mussels under two silt regimes

The effect of sampling has been shown to negatively affect survival of juvenile mussels in the laboratory (Beaty 1997). However, in a more natural hatchery environment, the beneficial effects of sampling for juveniles may outweigh the potential harm. While sampling disturbs mussel positioning and introduces the possibility of mortality from rough handling or accidental loss (passing through the sieves, getting stuck in a piece of apparatus, etc.), sampling also removes accumulated sediments that may be detrimental to juvenile mussel survival and growth. Observations by Hanlon (2000) suggest that at the AWCC, where substantial sediments are transported into the raceway in river water, periodic sampling and silt removal may positively affect growth and survival. However, sampling of juvenile mussels is a tedious and labor-intensive activity. A long-term goal for the AWCC is to rear large numbers of juvenile mussels and maintain them in captivity for several months to years. Results from this experiment were designed to develop sampling periodicity guidelines that will allow the hatchery to successfully rear juveniles with minimal sampling effort. Although it does not appear from the results of this experiment that a lethal level of silt was determined, I suggest that containers holding juvenile mussels be sampled approximately every 2 months to remove accumulated silt. This periodicity may need to be shortened during summers of high precipitation and higher silt loads.

In this experiment, survival in the high-silt treatment was significantly higher than that in the low-silt treatment. This is in contrast with findings of other researchers, which indicate that excessive amounts of silt reduce survival of at least some species of freshwater mussels (Imlay 1972, Marking and Bills 1980, Hanlon 2000). Three factors may have influenced this experiment; emigration of juveniles, overestimation of actual silt present versus FPOM, and a relatively low silt accumulation in the high-silt treatment. Hanlon and Levine (2001) observed emigration from treatment troughs similar to those used in this experiment. Their troughs were indoor recirculating units, using dechlorinated city water supplemented with an algal feed. The relatively clean and closed nature of the recirculating systems allowed them to design traps to catch and thereafter quantify juvenile escapement from the troughs. Such traps were not possible on the stream-fed systems that I used, as silt, filamentous algae, and invertebrates in the water would rapidly clog screen traps and cause overflow. According to Hanlon and Levine (2001), counts of juveniles found in traps indicate a 7.1 % cumulative emigration after 8 d and a significant decline in emigration thereafter. Emigration also appeared to be more prevalent in higher flow treatments. Flow rates in my experiments were similar to the high flow rates of Hanlon and Levine (2001). Based on their results and the survival trend observed in my experiments, I suspect that emigration accounted for some portion of specimen losses attributed to mortality. Unfortunately, there is no way to quantify the amount of emigration from the troughs. However, since construction and flow rates were similar in all troughs, transport of juveniles should not have differed between treatments. I have no information on similarity or differences in mobility between species.

Although no direct mortality was observed as a result of sampling activities, i.e., no individuals were obviously crushed, sampling could have negatively affected juveniles by displacement. After sampling, juveniles were replaced in troughs filled with water, but with flow turned off and a capped standpipe. Flow remained off for approximately

10 min to allow juveniles to settle in the substrate. It is possible that this disturbance may have prompted emigration.

It is also probable that the amount of silt accumulation in the troughs was overestimated because the amount of organic matter was not subtracted from the total amount of settled material. Fine particulate organic matter is lighter than inorganic silt and generally flocculates on the surface of the substrate rather than settling into interstitial spaces. I recommend for future experiments to determine ash-free dry weight in addition to dry weight to calculate the ratio of FPOM to inorganic silt.

It seems that 5 wk of silt accumulation during the high-silt treatment did not exceed lethal levels. More specifically, it seems that any negative effects of the additional silt in the high-silt treatment did not exceed the potentially negative effects of sampling. For reasons unknown, silt accumulation in the high-silt treatment was less than the cumulative volume of silt deposited in the low-silt treatment. While silt accumulation at days 40 and 70 were significantly higher in the high-silt treatment than in the low-silt treatment, the difference in silt accumulation between the two treatments was not as distinct as expected.

Based on the results of this trial, it seems that the trough design used in Experiments 2 and 3 is not appropriate for rearing newly metamorphosed mussels because of potentially high emigration. However, high survival and recovery rates for *V. iris* in Experiment 2 and the tapering trend of emigration by older juvenile mussels observed by Hanlon and Levine (2001) and Uryu et al. (1996) indicate that older juveniles are less mobile. Based on this information, I recommend that the troughs as designed in this experiment are more appropriate for juveniles greater than 1 month old.

Experiment 3 was intended to assist hatchery personnel in establishing a sampling periodicity that would maximize survival and growth of captively cultured mussels. Results of this experiment indicate that the act of sampling and the disturbance it induces may have a negative effect on mussel survival or retention. High volumes of silt accumulation did retard growth of the two species tested, but in this case, the severity of sampling induced mortality or loss likely outweighed any potential negative effects that reduced growth might have had on survival. Researchers speculate that high silt levels negatively affect young mussels (Henley et al. 2000). My results suggest that silt accumulation levels of up to 10 mg (approximately 5 mg/L) are not lethal to juvenile mussels, but that high silt loads do reduce growth rates.

Overall water quality at the AWCC, however appears to be within suitable ranges for holding and rearing freshwater mussels. Henley et al. (2001) summarized known water quality tolerances for freshwater mussels and other sensitive aquatic organisms. Conservative criteria levels from the literature include: pH minimum of 6.0 (Peterson 1987), 0.09 mg/L NH_3, 0.19 mg/L NO_2 (Myers-Kinzie 1998), and 180 mg/L NO_3 (Rubin and Elmaraghy 1977). Levels of nitrate (NO_3), nitrite (NO_2), ammonia (NH_3), pH, and dissolved oxygen (DO) recorded at AWCC for the summer of 2002 were within these tolerances ranges for unionids according to these sources.

CONCLUSIONS AND RECOMMENDATIONS

Based on the experiments conducted at AWCC in 2001 and 2002, several recommendations and conclusions can be made. Although previous studies indicate that ponds can be used to maintain adults of some species, newly metamorphosed mussels held in ponds are too vulnerable to predation to be held in this culturing environment. Additionally, the high volume of water and heavy silt loads transported into a pond limit the options available for container design that would prevent juvenile emigration. If mesh were to be used to retain mussels in a treatment container, it would quickly become clogged with silt and inhibit water flow into the container. Recovery of juveniles escaping from holding containers would be laborious and impractical.

Also apparent from the experiments is that water needs to be filtered before entering juvenile culture systems. A mesh size of 500 μm was sufficiently small to eliminate predacious fathead minnows from the troughs in these experiments; however, this size is not small enough to remove flatworms and other potentially problematic invertebrates, such as ostracods, chironomids, and isopods.

Future culture systems must be designed with the utmost attention to juvenile emigration, especially during the first 2-4 wk post-metamorphosis. No simple design to meet this challenge is apparent. Filters require frequent maintenance and cleaning, and they also increase the risk of system failure and increase costs. If a pre-system filter becomes clogged with silt or debris, water flow to the system may be completely stopped, and a juvenile trap on the outflow (the standpipe in these experimental systems) could clog and overflow the system. Given these scenarios, it is mandatory that an attendant be available for daily maintenance of culture systems.

An option for successful mussel rearing is to culture mussels in a laboratory setting for the first 2-4 wk post-metamorphosis. Clean, predator-free water allows for juvenile traps to be fitted to outflow structures during this highly mobile period without risk of clogging, and limits the possibility of predator-induced mortality. When juveniles become larger and less likely to emigrate from containers, they can be introduced then to a less controlled hatchery environment for long-term grow-out.

Aquatic Wildlife Conservation Center

Marion, Virginia

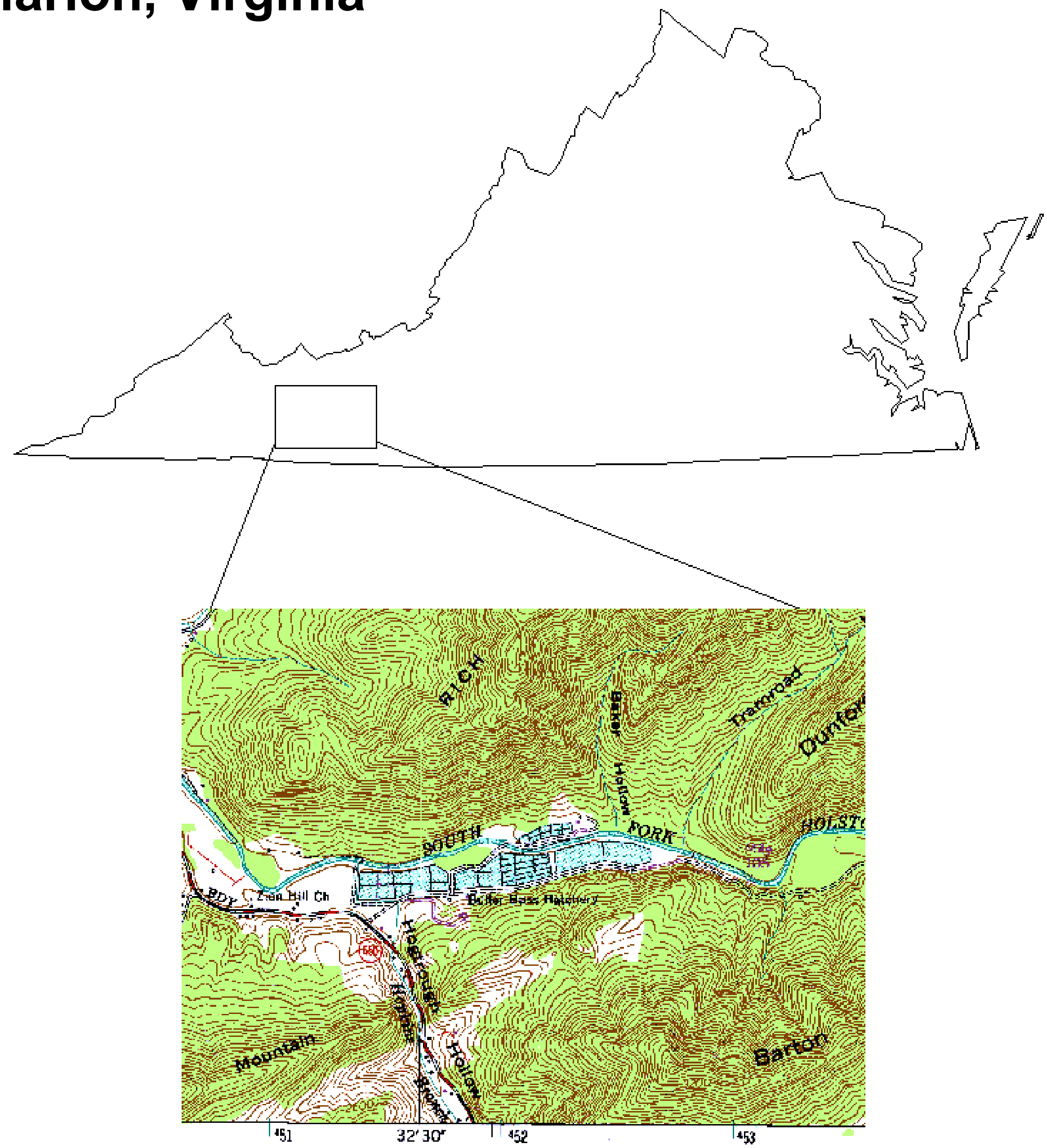


Figure 1.1. Aquatic Wildlife Conservation Center at the Buller Bass Hatchery, Marion, Virginia.

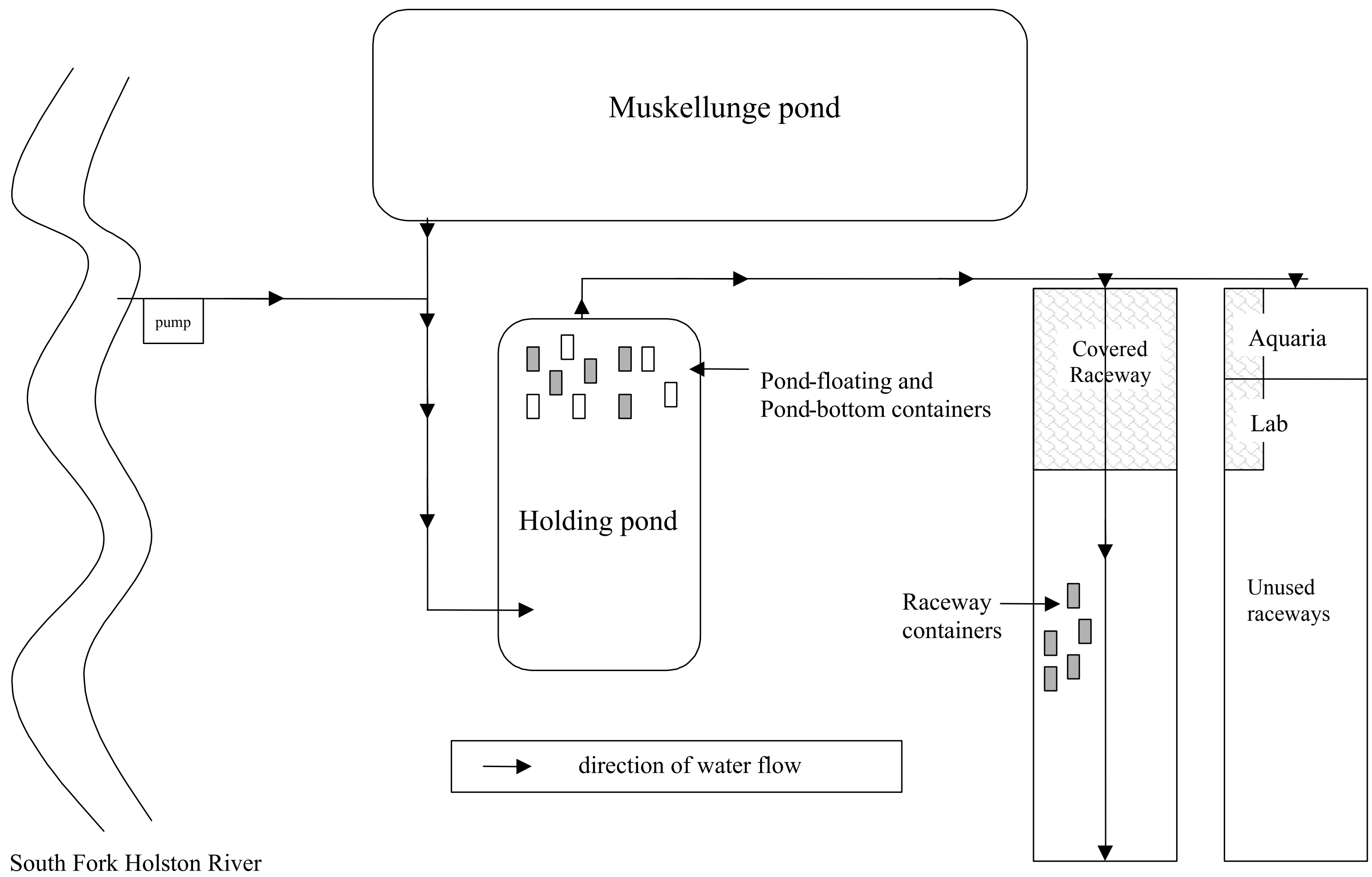


Figure 1.2. Layout for Experiment 1 showing rearing environments for *E. capsaeformis* and *L. fasciola*. Pond-floating and pond-bottom treatments were both positioned towards the outflow end of the pond, and the raceway treatments were all held in the far left sub-raceway unit.

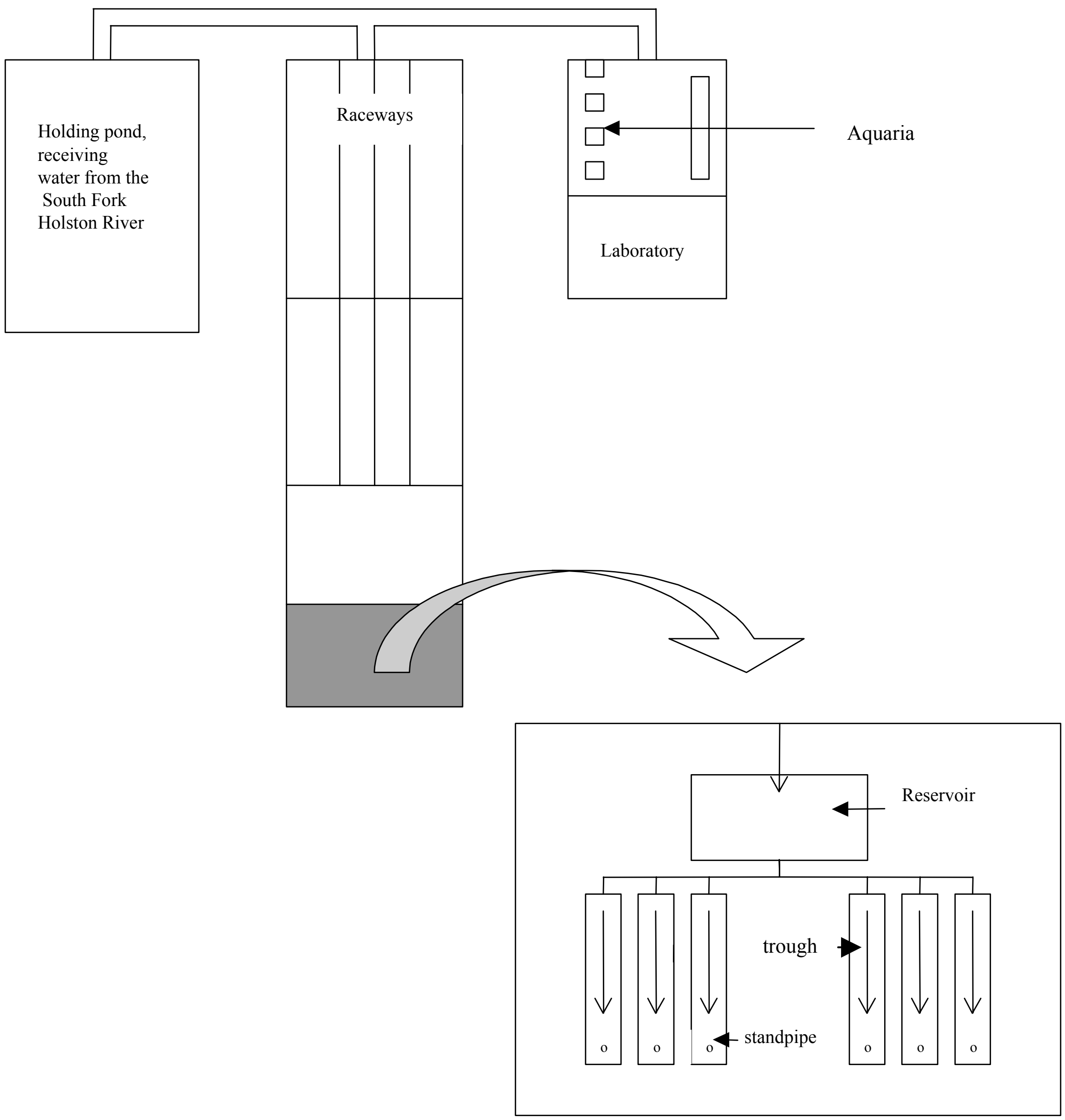


Figure 1.3. Schematic design for Experiment 2; a survival and growth comparison between rearing *V. iris* in outdoor troughs and indoor aquaria.

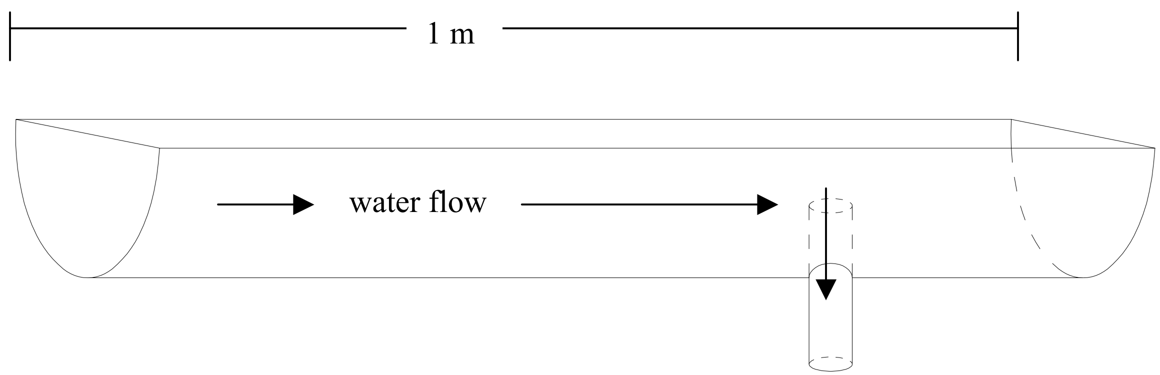


Figure 1.4. Trough design used in Experiments 2 and 3. Troughs used in Experiment 2 were 15.3-cm in diameter, where troughs used in Experiment 3 were 7.6-cm in diameter.

lf = low silt; *L. fasciola*
lc = low silt; *E. capsaeformis*
hf = high silt; *L. fasciola*
hc = high silt; *E. capsaeformis*

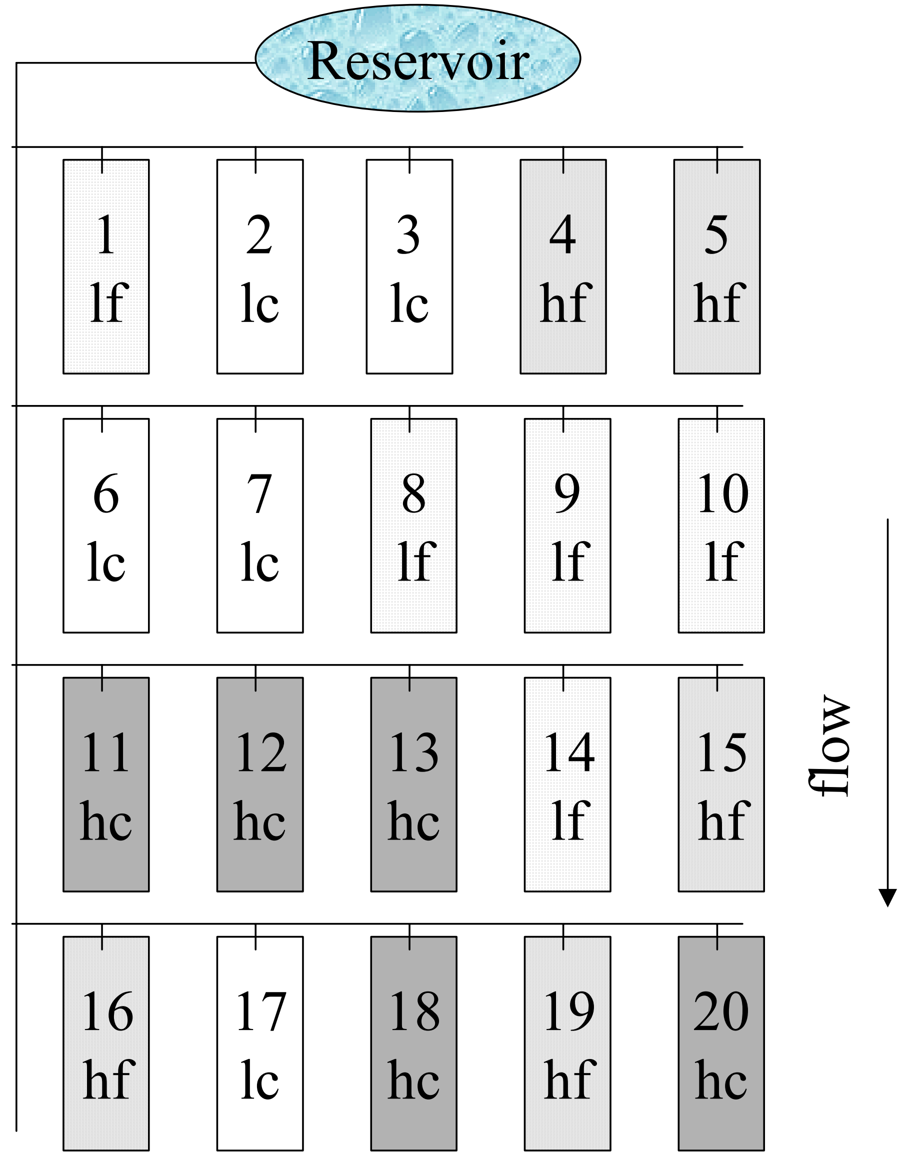


Figure 1.5. Schematic design of 20 troughs used in Experiment 3. Treatments and species were randomly assigned to troughs. Each trough contained either 100 *E. capsaeformis* juveniles or 200 *L. fasciola* juveniles. Trough colors in the illustration indicate silt and species combinations.

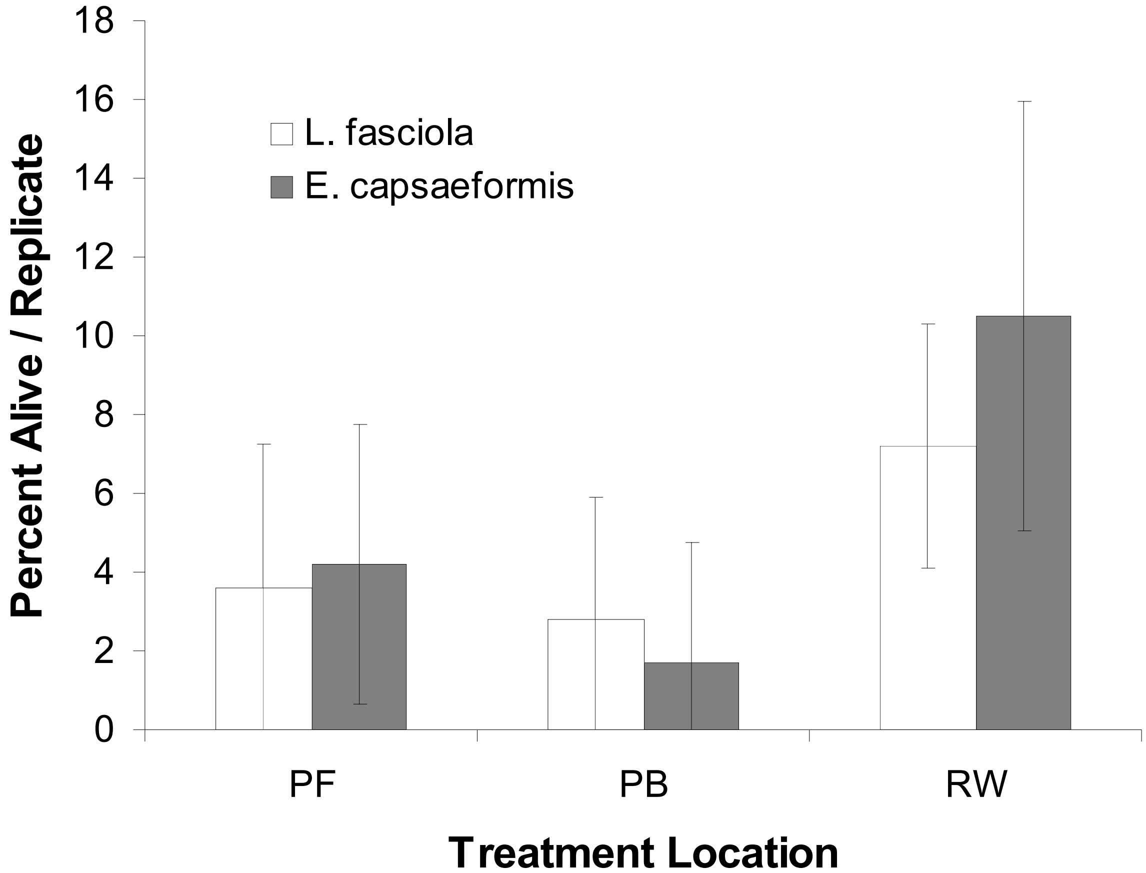


Figure 1.6. Survival of juvenile mussels in pond-floating (PF), pond-bottom (PB) and raceway (RW) locations (Experiment 1). Plotted are the means of 5 replicates (100 mussels/replicate) for each treatment. Error bars indicate standard error of the means.

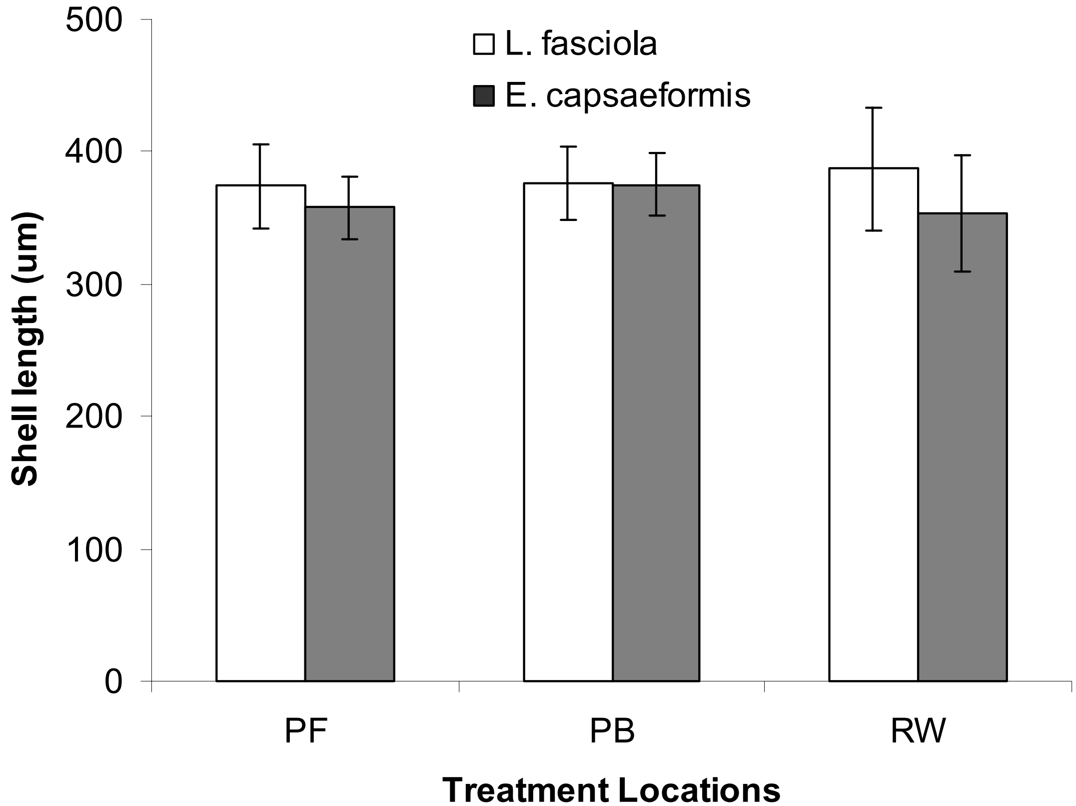


Figure 1.7. Mean length of juvenile mussels in pond-floating (PF), pond-bottom (PB) and raceway (RW) locations (Experiment 1). Error bars indicate standard error of the means.

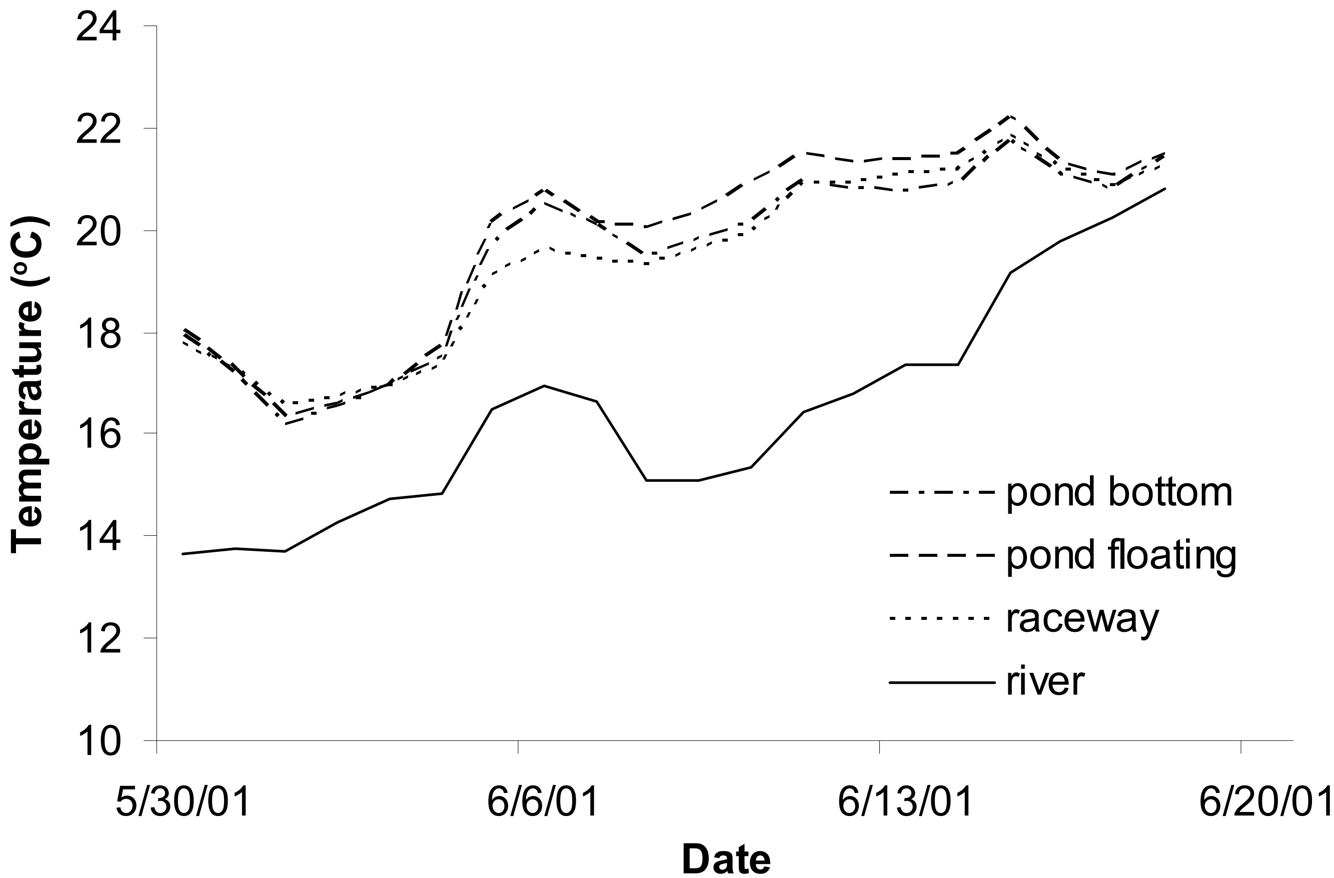


Figure 1.8. Mean daily water temperatures (ºC) during Experiment 1. Data loggers were placed at all three treatment locations and in the South Fork Holston River.

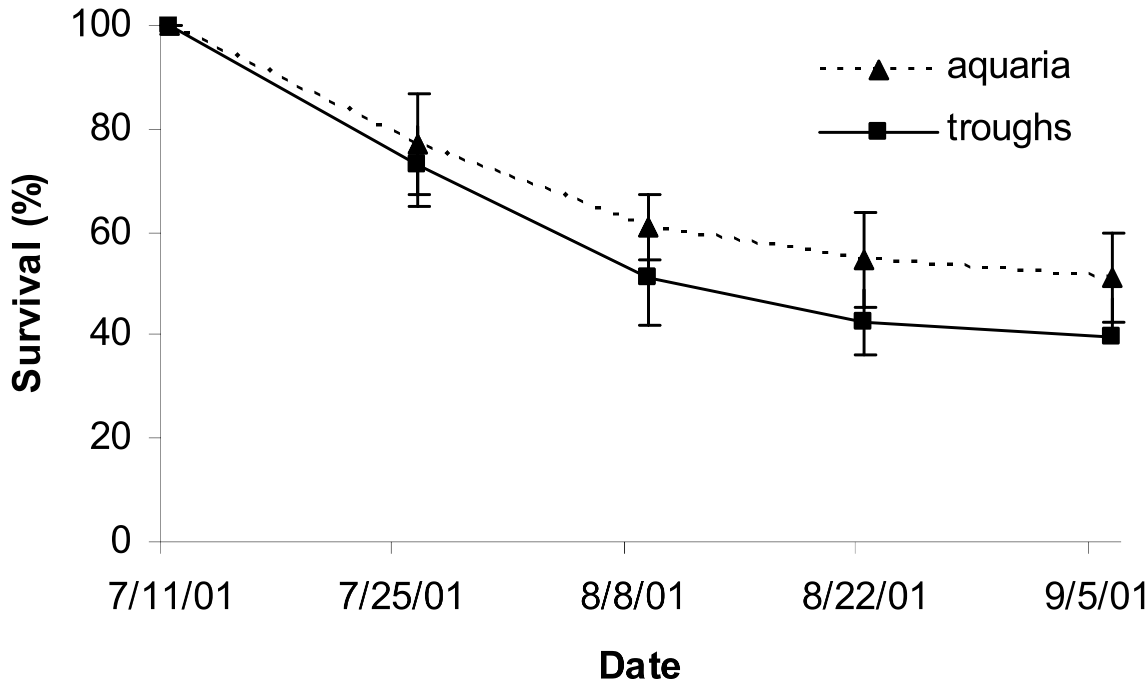


Figure 1.9. Survival (mean ± SE) of *V. iris* reared in outdoor PVC troughs and indoor aquaria. Differences in survival between treatments are not significant ($p = 0.095$).

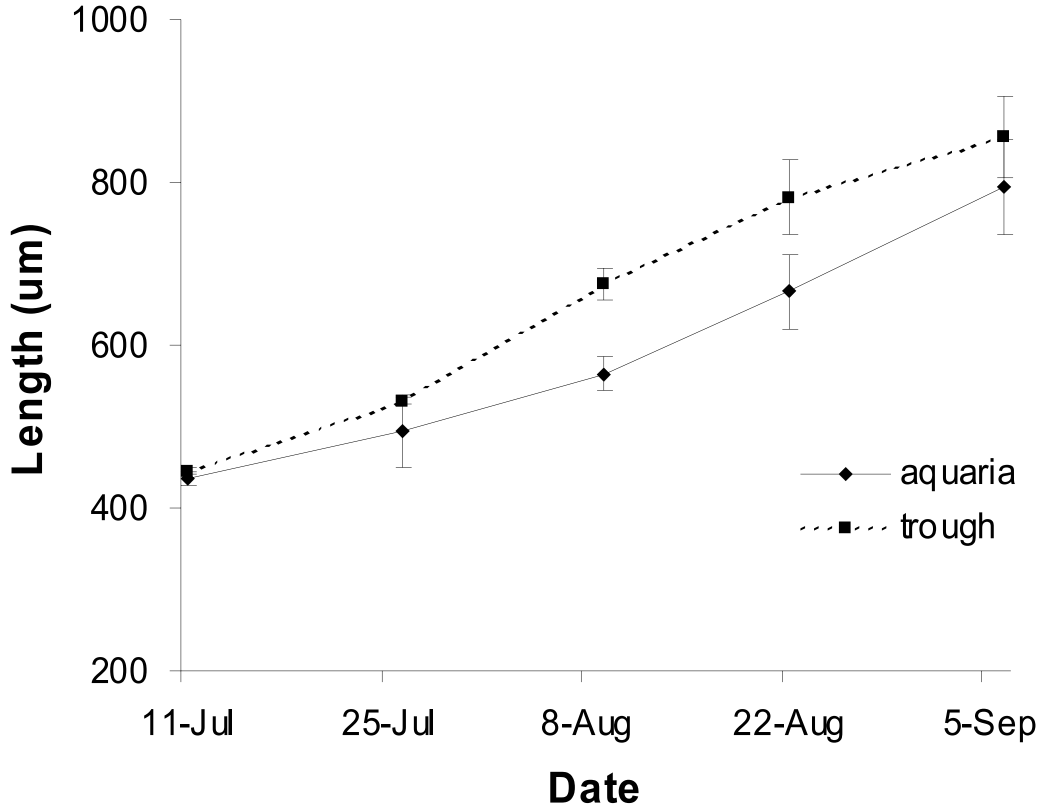


Figure 1.10. Mean shell lengths of *V. iris* in Experiment 2. Lengths of mussels reared in outdoor troughs and indoor aquaria did not differ significantly after 8 wk ($p>0.05$).

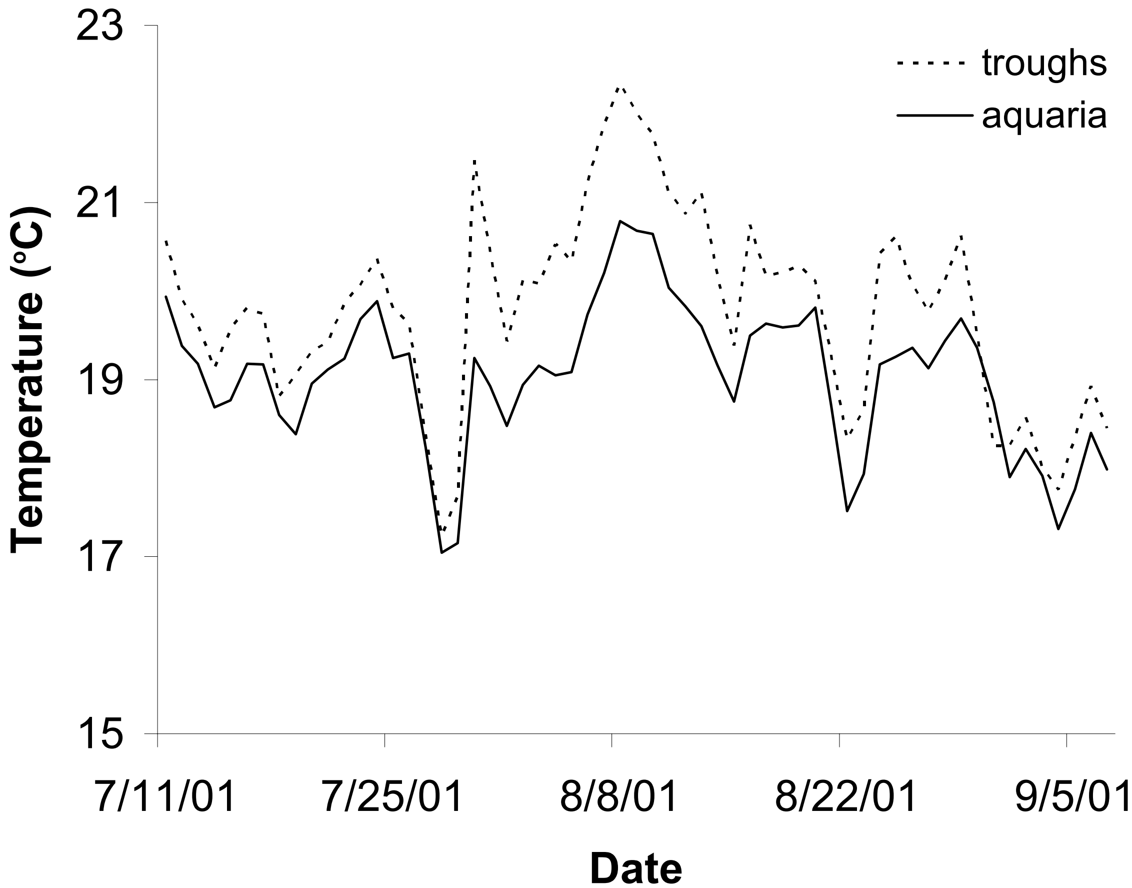


Figure 1.11. Average daily temperatures in outdoor troughs and indoor aquaria. Daily average temperatures in troughs were 0.75 C° higher than in indoor aquaria.

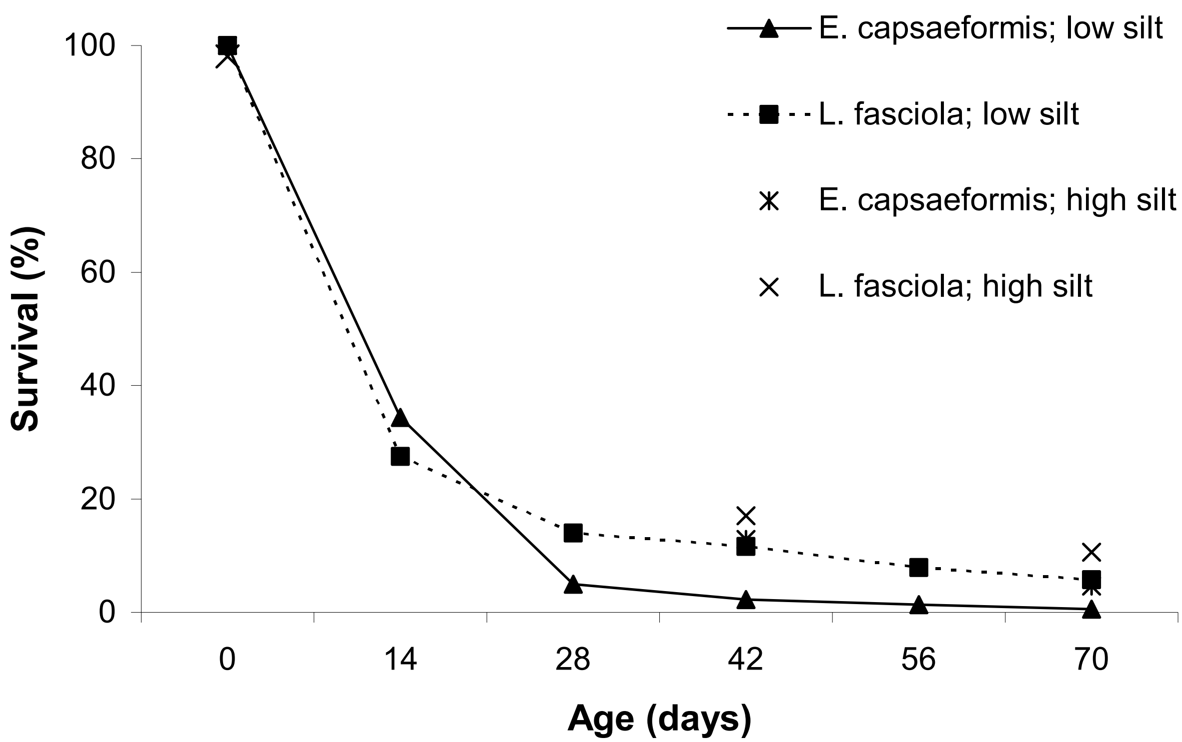


Figure 1.12. Mean % survival per trough of *E. capsaeformis* and *L. fasciola* under two silt regimes. High-silt treatments are marked only on days 0, 42 and 70, when juveniles were enumerated.

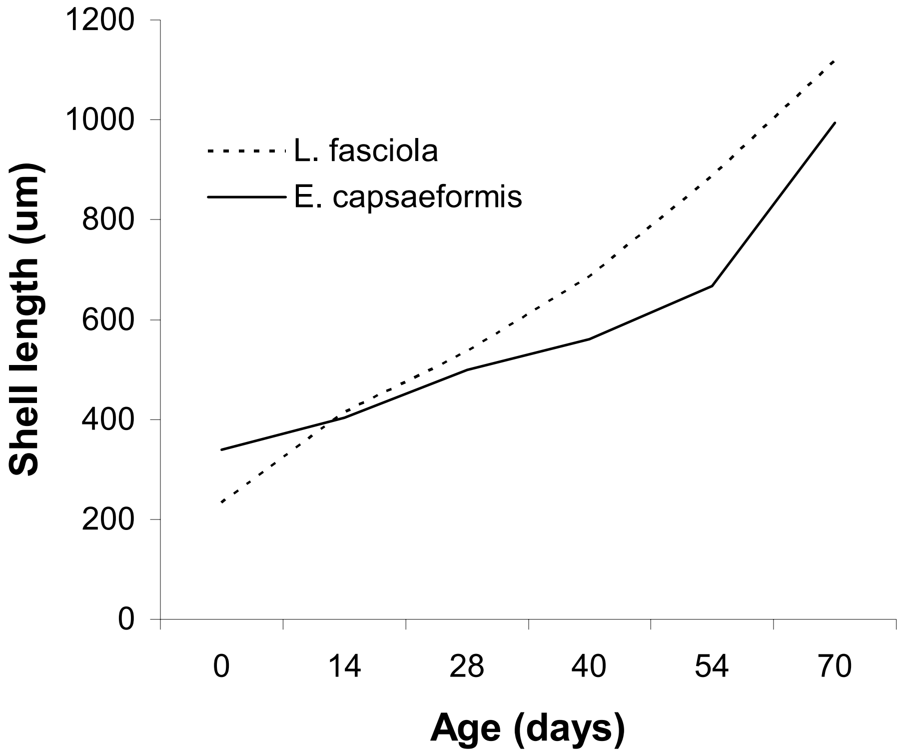


Figure 1.13. Mean lengths of *L. fasciola* and *E. capsaeformis* in the low-silt treatments. Growth rates differed between species ($p<0.0001$).

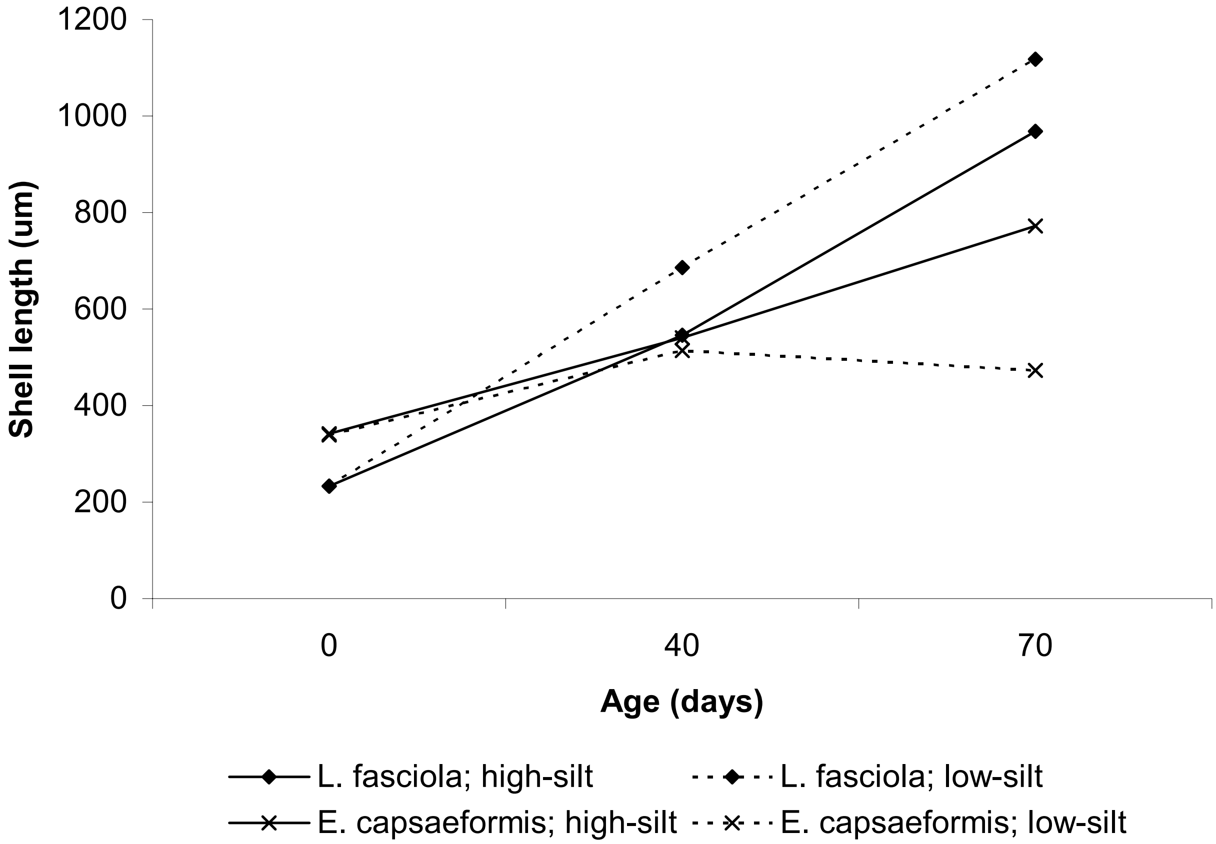


Figure 1.14. Length comparisons between *L. fasciola* and *E. capsaeformis* in high-silt and low-silt treatments. Lengths of *L. fasciola* at day 40 are significantly greater in low-silt vs. high-silt treatments ($p < 0.0001$). Statistical comparisons for *E. capsaeformis* at day 70 were not possible due to insufficient sample size.

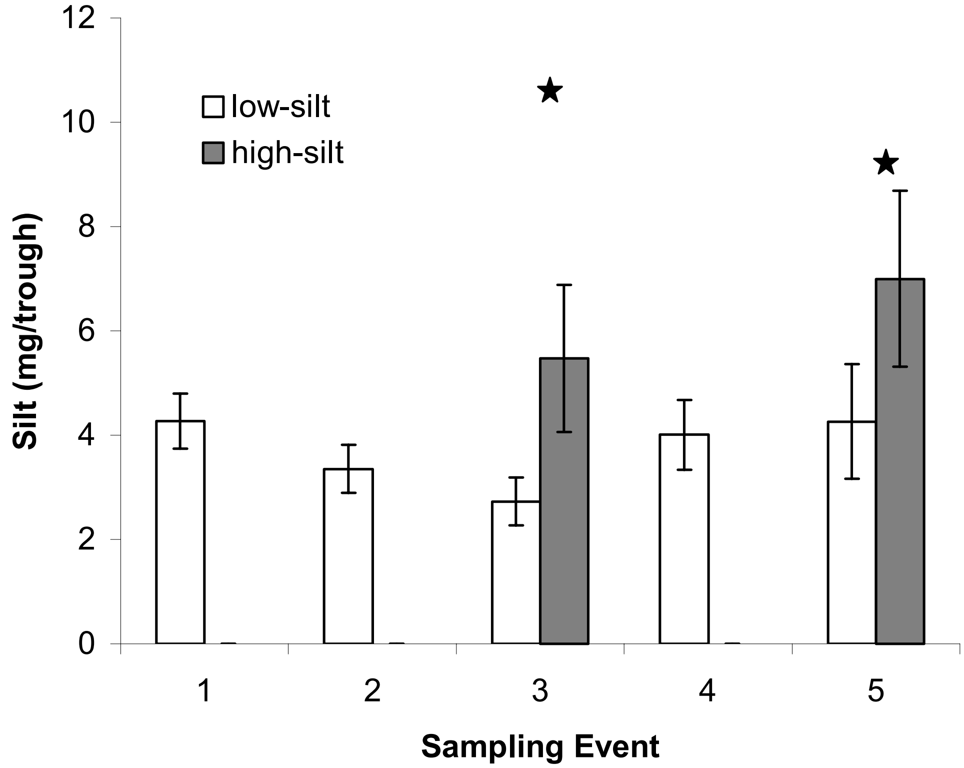


Figure 1.15. Silt (± SE) accumulation in troughs. Stars indicate the cumulative means from low-silt estimates that were expected in the high-silt treatment.

Table 1.1. Water chemistry measurements for Experiment 3. Water samples were collected from the system reservoir.

SAMPLING DATE	AMMONIA (mg/L)	HARDNESS (mg/L)	ALKALINITY (mg/L)	NITRATE (mg/L)	NITRITE (mg/L)	pH	DO (mg/L)	Chlorophyll α (mg/m^3)
10-Jun-02	0.03	114	66	0.7	0.015	7.2	7.7	Undetected level
24-Jun-02	0.02	90	89	0.1	0.011	7.2	7.6	Undetected level
08-Jul-02	0.03	97	80	0.6	0.006	7.1	7.3	Undetected level
20-Jul-02	0.06	96	86	0.6	0.005	7.4	7.6	2.00
03-Aug-02	0.05	96	140	0.4	0.008	7.1	7.6	8.01
19-Aug-02	0.03	136	84	0.1	0.001	6.8	7.1	8.01
MEAN	**0.04**	**105**	**91**	**0.4**	**0.008**	**7.1**	**7.5**	**--**

Literature cited

APHA (American Public Health Association), American Water Works Association, and Water Environment Federation. 1989. Standard methods for the examination of water and wastewater, 17th edition. APHA, Washington, D. C.

Beaty, B. B. 1997. Development of juvenile culture techniques and testing of potential biomarkers of environmental stress in freshwater mussels (Bivalvia: Unionidae). Doctoral dissertation. Virginia Polytechnic Institute and State University, Blacksburg, VA.

Bergström, U. and G. Englund. 2002. Estimating predation rates in experimental systems: scale-dependent effects of aggregative behaviour. Oikos 97:251-259.

Buddensiek, V. 1995. The culture of juvenile freshwater pearl mussels *Margaritifera margaritifera* in cages: a contribution to conservation programmes and the knowledge of habitat requirements. Biological Conservation 74:33-40.

Chalkley, H. W. 1943. Method for the quantitative morphologic analysis of tissues. Journal of the National Cancer Institute 4(7):47-53.

Dunn, C. S. and J. B. Layzer. 1997. Evaluation of various holding facilities for maintaining freshwater mussels in captivity. Pages 205-213 *in* K. S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo, editors. Conservation and Management of Freshwater Mussels II: initiatives for the future. Proceedings of a UMRCC Symposium. Upper Mississippi River Conservation Committee, Rock Island, Illinois.

Gatenby, C. M., R. J. Neves, and B. C. Parker. 1996. Influence of sediment and algal food on cultured juvenile freshwater mussels. Journal of the North American Benthological Society 15:597-609.

Gatenby, C. M., B. C. Parker, and R. J. Neves. 1997. Growth and survival of juvenile rainbow mussels, *Villosa iris* (Lea, 1829) (Bivalvia: Unionidae), reared on algal diets and sediment. American Malacological Bulletin 14(1):57-66.

Hanlon, S. D. 2000. Release of juvenile mussels into a fish hatchery raceway: a comparison of techniques. Master's Thesis. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Hanlon, S. D. and J. Levine. 2001. Preliminary studies on the life history and propagation of several North Carolina freshwater mussels. Final Report to the U. S. Fish and Wildlife Service, Raleigh, NC.

Havlik, M. E. and L. L. Marking. 1987. Effects of contaminants on naiad mollusks (Unionidae). U. S. Department of Interior, Fish and Wildlife Service: Resource Publication 164.

Henley, W. F., M. A. Patterson, R. J. Neves, and A. D. Lemly. 2000. Effects of sedimentation and turbidity on lotic food webs: a concise review for natural resource managers. Reviews in Fisheries Science 8(2):125-139.

Henley, W. F., L. L. Zimmerman, and R. J. Neves. 2001. Design and evaluation of recirculating water systems for maintenance and propagation of freshwater mussels. North American Journal of Aquaculture 63:144-155.

Imlay, M. J. 1972. Greater adaptability of freshwater mussels to natural rather than to artificial displacement. Nautilus 86:76-79.

Jenkins, R. E. and N. M. Burkhead. 1993. Freshwater Fishes of Virginia. American Fisheries Society, Bethesda, Maryland.

Marking, R. H. and T. D. Bills. 1980. Acute effects of silt and sand sedimentation on fresheater mussels. Pages 204-211*in* J. L. Rasmussen, editor. Proceedings of the Symposium on Upper Mississippi River Bivalve Mollusks. Upper Mississippi River Conservation Committee, Rock Island, Illinois.

Mummert, A. K. 2002. Evaluating the feasibility of rearing juvenile freshwater mussels in a flow-through pond system at White Sulphur Springs National Fish Hatchery. Master's Thesis. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Myers-Kinzie, M. L. 1998. Factors affecting survival and recruitment of unionid mussels in small mid-western streams. Doctoral Dissertation. Purdue University, West Lafayette, Indiana.

O'Beirn, F. X., R. J. Neves, and M. B. Steg. 1998. Survival and growth of juvenile freshwater mussels (Unionidae) in a recirculating aquaculture system. American Malacological Bulletin 14:165-171.

Peterson, R. H. 1987. Influence of water pH on frequency of collection of certain invertebrates during lake and stream surveys in New Brunswick and Nova Scotia. Department of Fisheries and Oceans, Canadian Technical Report, Number 1523, St. Andrews, New Brunswick.

Rogers, S. O. 1999. Population biology of the tan riffleshell (*Epioblasma florentina walkeri*) and the effects of substratum and light on juvenile mussel propagation. Master's Thesis. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Rubin, A. J., and G. A. Elmaraghy. 1977. Studies on the toxicity of ammonia, nitrate and their mixtures to guppy fry. Water Research 11:927-935.

Ryan, P. A. 1991. Environmental effects of sediment on New Zealand streams: a review. New Zealand Journal of Marine and Freshwater Research 25:207-221.

Sickel, J. B. 1998. Gluttonous feeding behavior in the Rhabdocoel, *Macrostomum* sp, induced by larvae of the Asiatic clam, *Corbicula fluminea* Journal of Freshwater Ecology 13(1):135-137

Steg, M. B. 1997. Identification of host fishes and experimental culture of juveniles for selected freshwater mussel species in Virginia. Master's Thesis. Virginia Polytechnic Institute and State University, Blacksburg, VA.

Uryu, Y., K. Iwasaki, and M. Hinoue. 1996. Laboratory experiments on behaviour and movement of a freshwater mussel, *Limnoperna fortunei* (Dunker). Journal of Molluscan Studies 62:327-341.

Webb, D. J., B. K. Burnison, A. M. Trimbee, and E. E. Prepas. 1992. Comparison of chlorophyll α extractions with ethanol and dimethyl sulfoxide/acetone, and a concern about spectrophotometric phaeopigmentation correction. Canadian Journal of Fisheries and Aquatic Sciences 49:2331-2336.

Yeager, B. L. and C. F. Saylor. 1995. Fish hosts for four species of freshwater mussels (Pelecypoda: Unionidae) in the upper Tennessee River drainage. American Midland Naturalist 133:1-6.

Zale, A. V., and R. J. Neves. 1982. Fish hosts of four species of lampsiline mussels (Mollusca: Unionidae) in Big Moccasin Creek, Virginia. Canadian Journal of Zoology 60(11):2535-2542.

Zimmerman, L. L., D. G. Smith, and R. J. Neves. 2003. Control of Predacious Flatworms (*Macrostomum* sp.) in Culturing Juvenile Freshwater Mussels (Bivalvia: Unionidae). North American Journal of Aquaculture. In Press.

Chapter 2: Ecological risk assessment to identify Clinch River sites suitable for the release of captively propagated freshwater mussels

INTRODUCTION

Recovery plans for endangered freshwater mussels include goals of propagation and release of juvenile mussels to augment or expand the range of extant populations. Identifying suitable locations to release these propagated individuals is critical to their survival. The absence of mussels from a site known to previously support them may be due to many factors. These factors can be divided into two categories: 1) stochastic or pulse stresses, such as a chemical spill, severe drought, or other isolated habitat disturbance resulting in a one-time mussel kill, from which the fauna has not been able to naturally recover, or 2) chronic or press stresses that may inhibit survival or recruitment, such as loss of fish hosts, stream channelization, or continuous exposure to toxicants or high sediment loads (Bender et al. 1984). The first step in identifying suitable habitat for release of propagated mussels is to characterize which of the two types of impacts is responsible for the initial decline or extirpation. Releasing captively propagated mussels into an area with high levels of press stresses is unlikely to result in successful colonization.

Many studies have attempted to characterize habitat requirements and potential stressors for freshwater mussels. Altered hydrology due to river impoundment and the introduction of exotic species, especially zebra mussels (*Dreissena polymorpha*) are documented as some of the most influential press stresses (Richter et al. 1997, Vaughn and Taylor 1999). Many chemical pollutants have also been identified as stressors to freshwater mussels. Toxicity tests with juvenile and adult freshwater mussels indicate altered metabolic activity or mortality as common responses to various types of effluent and landscape runoff. McKinney and Wade (1996) found concentrations of 2.2 % of paper mill effluent to impair survival of juvenile paper pondshell, *Anodonta imbecillis* in a 9-day LC50 trial. This effluent was approximately 4 times more toxic to mussels than daphnids. The eastern elliptio, *Elliptio complanata* showed significantly inhibited cholinesterase activity during 96-hour exposure tests with aldicarb and aciphate pesticides (Moulton et al. 1996). McCann (1992) demonstrated negative affects of coal-related products on juvenile freshwater mussels in the Powell River, Virginia. Wastewater discharge also has been noted as the cause of mussel declines in the Blanco River, Texas (Horne and McIntosh 1979). Likewise, in a survey of the Middle Fork Holston River, Virginia, Henley et al. (1999) showed association between reduced numbers of mussels and number of mussel species downstream of permitted wastewater discharges. Point source discharges similar to those mentioned, as well as other possible sources such as acid mine drainage and urban runoff, could be negatively affecting mussel populations in the Clinch River.

One method for identifying environmental stressors is to characterize potential impacts through an ecological risk assessment (Suter 1993). Risk assessments are a method for rating a site based on the probability it will be adversely affected by human activity. Risk assessments are generally used to assist decisionmakers understand potential effects of a proposed stress source. Although most risk assessments have been calculated for individual stressors at a site-specific scale, potential risk of multiple anthropogenic stressors can be assessed at a regional scale (Wiegers et al. 1998, Bryce et al. 1999). The regional scale, multiple-stressor ecological risk assessment framework developed by Wiegers et al. (1998) was used to develop my study on the Clinch River.

One goal of mussel propagation and reintroduction is to reduce the possibility of a catastrophic event, e.g. chemical spill or drought, eliminating remaining populations of a species. An additional goal is to assist natural recolonization by filling in distributional gaps between populations. The current method for selecting captively cultured mussel release sites is to either return propagated mussels to the same river reach where the brood stock originated or to release them in an area known to harbor healthy mussel populations (Jones et al. 2003). Current methods for release site selection are appropriate for augmenting existing populations, but do not support these other two objectives.

Several methods have been used to identify risk factors and potential mussel release sites in the Clinch River watershed. In the 1980s, the Tennessee Valley Authority's (TVA) Cumberland Mollusk Conservation Program embarked on a study to synthesize information to determine suitable release sites for several endangered Cumberlandian mussel species. This study resulted in the publication of 9 reports examining a wide array of physical, limnological, botanical, and zoological components of 19 stream reaches in the upper Tennessee and Cumberland River systems (TVA 1986). This project required almost 10 years of planning and monitoring, and a large crew of experienced biologists and water quality specialists. Ultimately, a great deal of biological data was collected; however, only 19 650-m stream reaches had been monitored as potential reintroduction sites (TVA 1986). Data collected in these studies helped identify stream characters common to highly productive mussel sites, but did not identify specific habitat preferences or water quality tolerances needed to predict locations of future mussel beds. The result was a ranking of the 19 reaches in terms of their suitability for mussel relocation (TVA 1986).

Rather than long-term chemical and hydrological monitoring, another technique frequently used to measure stream health is an Index of Biota Integrity (IBI); (Karr and Chu 1999). The basis of IBI is that many biological, chemical, and hydrologic factors can be gauged by examining the biological communities living in a stream reach. Fish species presence and guild composition can relate specific stream impacts without years of monitoring. Biological monitoring is a useful technique for gauging multiple stream habitat parameters with a single sampling. It does, however, require substantial sampling effort for large survey areas and in many cases is difficult to compare results with those of other studies due to non-standardized methodology. IBI rankings of stream integrity are time and cost effective and often yield results similar to ecological risk assessments (Diamond and Serveiss 2001). For the scope of this study, even this abbreviated monitoring technique was not economically or temporally feasible.

This paper describes the process and application of an ecological risk assessment (EcoRA) for the Clinch River in Virginia. The purpose of this risk assessment was to identify locations along the Clinch River having the highest quality mussel habitat and the lowest risk of anthropogenic impacts. Presumably, these locations that could successfully support captively cultured endangered mussels. The analysis of this risk assessment was conducted in two parts. The first analysis looked at physical habitat availability and distribution, and a second analysis ranked habitat based on potential risk factors. The two analyses were then combined to identify habitats with the highest available physical habitat and the lowest risk to anthropogenic threats. Due to the large study area (150 river-km) and the desire to evaluate all available stream habitats at a fine resolution, an EcoRA was determined to be the most appropriate approach.

Study Area

The focus of this study is the Clinch River in southwest Virginia. The Clinch River originates in Tazewell County, Virginia, and flows 312 km to the southwest through Russell, Scott, and Wise counties in Virginia. The Clinch's largest tributary, the Powell River, begins in Wise County and flows southwest towards Norris Lake in eastern Tennessee. The Powell River continues to be impacted by coal mining, therefore, the analysis of this study focuses only on the Clinch River because of the greater potential for restoration.

The Clinch River has been selected for this study for three primary reasons; first, the system contains a diverse mussel fauna (Stansbery 1973, Ahlstedt 1999). Secondly, because the entire watershed is located in a relatively undeveloped area of southwestern Virginia with low human population, physical habitat remains in good condition. Lastly, the Clinch River harbors 18 endangered mussel species, and it is expected to receive captively propagated mussels as part of recovery attempts for many of those species.

Land cover in the Clinch River watershed is dominated by forest (69 %) and agriculture (28 %); (Diamond et al. 2002). Primary agricultural products from this region are tobacco and beef cattle. Coal mining is an important industry and land-based activity within the watershed. Most mining activity is restricted to coal seams in the western portion of the Clinch watershed (especially around the Guest River) and even more so in the Powell River watershed. Aside from coal mining, coal processing, and agriculture, there is nominal industry in the watershed.

METHODS

Habitat Survey

The method used for habitat mapping of the Clinch River is an adaptation of the Basinwide Visual Estimation Technique (BVET); (Hankin and Reeves 1988, Dolloff et al. 1993). Unlike most BVET surveys that examine habitat by walking upstream, we surveyed the Clinch River by boat, floating downstream, and stopping at selected habitat sections. Where possible, we floated downstream in a zigzag fashion to better gauge mean characteristics for each habitat unit. Deviations from standard BVET were necessary due to the large size and length of river being surveyed. Perhaps the largest deviation from standard BVET protocol was the use of a laser rangefinder (Bushnell Yardage Pro 400, Bushnell Co. Overland Park, Kansas) to measure unit lengths and widths rather than using hip chains or measuring tapes. Due to the ease of measuring distance with a laser rangefinder, actual width measurements were taken at each habitat unit rather than estimations. Therefore, there was no need for post-survey area calculations.

The survey appraised stream and riparian characters from Clinch River Kilometer (CRK) 478 at Blackford (Russell County, VA) to the Tennessee-Virginia state line (CRK 325) at Scott County, VA and Hancock County, TN (Figure 2.1). Habitat units characterized by low water velocity, streambed gradient near zero, and a smooth water surface were classified as pools, while habitats with relatively steep gradient, shallow water, high surface turbulence, and high velocity were considered riffles; runs were characterized by shallow water, high velocity, and low surface turbulence (Platts et al. 1983). Unit lengths were measured from the upstream extent to a point, e.g. tree branch or rock outcrop, on the downstream end using a rangefinder. The maximum range of the rangefinder model used was approximately 350 m in a straight line. Units exceeding 350 m in length or that contained sharp bends had multiple lengths recorded and subsequently combined. In addition to length, a GPS unit was used to collect latitude and longitude coordinates at the start of each new habitat unit. The start of a new habitat unit designated the end of the previous unit.

At each habitat unit, data were collected on several habitat features including unit length, stream width, substrate composition, substrate embeddedness, riparian land use, bank erosion potential, and mean unit depth (see Appendix A). Measured parameters and methods were selected based on the protocol described in Dolloff et al. (1993), logistical constraints due to the size and length of river sampled, and biological requirements of freshwater mussels. Habitat data for the main channel and permanently wetted side channels were recorded. Unit length was measured from obvious breaks between habitats; e.g. logs, small waterfalls, ledges, etc. If a single unit contained a mix of habitats, the dominant habitat was recorded as the unit type. Stream width measured the average wetted width of the habitat unit. Width was measured with a laser rangefinder in several (2 to 5 depending on unit length) locations to estimate average width. Average depth was measured using a graduated wading rod marked in 5 cm intervals. Multiple depth measurements from various sections of the stream channel were recorded for each habitat unit and values were averaged.

Some habitat features were described as categories. Substrate composition was divided into 4 particle-size categories; fine (<8mm), medium (8mm – 30cm), large (>30cm), and bedrock. The percentage of substrate within the unit representing each category was estimated visually. Riparian use was divided into 6 categories; forested, pasture, row crop, urban, residential, and industrial. Riparian land use was considered for a 30-m adjacent to the stream for both the right and left bank. If multiple uses occurred in the habitat unit on the same bank, only the dominant land use for that bank was recorded. In the case that left and right banks differed in land use, both uses were noted and averaged for the analysis. For example, if the left bank was predominantly forest (category 4) and the right bank mostly row crop (category 2), the habitat unit category for land use was 3. Bank condition categories were based on the mean bank erosion potential of a habitat unit. A low percentage of vegetated bank area was considered at high risk for erosion. The percent coverage was averaged for the entire habitat unit, including both banks. Class I represents banks > 85% covered by vegetation, Class II represents 50-84% vegetated banks with minor to moderate erosion, Class III represents 25-49% vegetated, and Class IV represents highly eroded banks less than 25% vegetated. Embeddedness was defined as a percent of total surface area in which interstitial spaces between substrate particles were filled with sand, silt, or clay. Embeddedness was estimated by randomly selecting 5 stones at each habitat unit and observing the mean depth of silt coverage on the rock. An embeddedness rank of 4 corresponds to a mean of less than 25 percent of the substrate covered with silt. Other categories include 25-50, 50-75, and 75-100 percent embedded for classes 3, 2, and 1, respectively.

Cattle access points were also identified during the habitat survey. Concentrated cattle access was determined by an absence of bank vegetation, cow manure on the stream bank, compacted bank soil from cattle traffic, and the presence of cattle in or next to the river. Additional miscellaneous features such as bridges, discharge pipes and tributaries were noted, and latitude and longitude coordinates were recorded.

GIS Development

Parameters entered into the GIS and the related proximities of influence for potential impacts were selected based on data availability and previous studies on sources of stress to aquatic fauna. Goudreau et al. (1993) identified monochloramine, a byproduct from wastewater treatments plants, to negatively affect mussel populations in the Clinch River up to 3.7 km downstream of outfalls. Mines and mine-related activities, including AMD, abandoned mine lands, and coal processing plants, have been repeatedly identified as being detrimental to aquatic fauna (Nichols and Bulow 1973, Keller and Zam 1991). Proximity to bridges (both vehicular and rail) was included in the analysis due to hydrocarbon and silt inputs to the river from road runoff and the possibility of unintentional spills of toxic substances during traffic accidents. Accidents, such as one that occurred in Cedar Bluff, Virginia, impacted the Clinch River for approximately 11 km downstream (Jones et al. 2001).

Data for GIS layers were obtained from a variety of sources (Table 2.1). Hydrography and road (improved and unimproved) coverages were obtained from the USGS as digital line graphs (DLG) at the 1:100,000 scale. The Virginia Department of Mines, Minerals, and Energy, Division of Mined Land Reclamation provided data for all existing and acid mine drainage (AMD) sites and areas permitted for mining. The Virginia Department of Environmental Quality supplied data for permitted municipal and industrial wastewater discharges. Data were entered into the GIS as geo-referenced layers of "impacts". All images were projected to Zone 17 of the Universal Transverse

Mercator (UTM) system, North American Datum 1927 (NAD 1927). Locations measured in latitude and longitude coordinates in the field were converted to UTM, NAD 1927.

Model Development

Each habitat unit was ranked by physical habitat characteristics measured during the stream survey, including substrate embeddedness, bank stability, riparian land use, and habitat type (Table 2.2). Habitat units were considered unsuitable for mussel habitation and eliminated from analysis if >80 % of the substrate comprised bedrock or if mean depth exceeded 150 cm. The elimination of habitats dominated by bedrock was based on the results of Church (1997), who found highly productive mussel sites to be negatively correlated with high percent bedrock. I judged that a high proportion of bedrock would provide insufficient substrate for burrowing and impair mussels' ability to maintain position. Mean depth exceeding 150 cm was chosen as a cutoff because deep habitat units could not be thoroughly surveyed. Water turbidity inhibited visual estimation of substrate composition and embeddedness. There is also some evidence that most mussel species common to the Clinch River are associated with shallow microhabitats (Church 1997, Johnson and Brown 2000). Church (1997) identified braidedness as a character common to most high quality mussel habitats. All braided units were ranked as 4, whereas non-braided units were given a rank of 2. Because riffles and runs are typically high quality mussel habitats (Church 1997) I ranked riffles and runs as 4 and pools as 2.

After the BVET survey of habitat availability and distribution, each habitat unit was evaluated for its likelihood to be affected by a list of anthropogenic impacts. Habitat units were then ranked by risk potential from listed impacts. Habitat units were ranked based on their distance downstream from potential impacts, including bridges, point-source discharges (PSDs), towns, cattle feedlots, and confluences with tributaries affected by AMD. Each impact was evaluated separately. Distances from potential impacts were calculated in ArcView GIS software using the Network Analyst extension (ESRI, Redland, CA). Impact coverages were entered as 'facilities', with each habitat unit loaded as an 'event'. The distance between facility and event was calculated following the stream network, in an upstream direction only. All permitted discharges located on the Clinch River and its tributaries were included as potential impacts. Due to a lack of data, all other proximity measurements were taken from habitat unit to nearest potential impact on the mainstem Clinch River, ignoring potential impacts located on tributaries.

Ranking of Parameters

Parameters of each habitat unit were entered into a data matrix. Of the 461 habitat units identified in the river survey, 316 were identified as potentially suitable after the elimination of units exceeding 150 cm average depth or 80 % bedrock substrate. For each of the 316 potentially suitable habitat units, 13 equally-weighted parameters were entered into the matrix, 5 parameters reflecting physical habitat and 8 parameters related to potential risk (Table 2.2). Rankings of each parameter ranged from 1 (unsuitable) to 4 (most favorable). The highest possible combined score for each habitat unit in the analysis was 52.

Risk potentials for proximity analyses were divided into distance-to-impact categories. For all parameters used in the analysis, higher index values indicated habitats less likely to be influenced by a potential impact. Categories were divided as follows:

habitat units <1000 m from a potential impact received a ranking of 1, 1000-5000 m ranked as 2, 5001-10,000 were 3, and > 10,000 were assigned a ranking of 4 (Table 2.2). Studies by Goudreau (1993) indicate that point-source discharge from wastewater treatment plants can negatively impact freshwater mussels for up to 15 km thus, categories representing potential impact from PSD were increased to include distances up to 15 km (Table 2.2). All risk category designations were based on available sensitivity data and my best professional judgment.

An additional value was entered in the matrix to provide a relative weighting for the distance to tributary confluence affected by AMD. All tributaries of the Clinch River were ranked between 1 and 4 based on the number of AMD sites located on the tributary. The Guest River watershed contained the highest number of AMD sites and was ranked as 1. Lick, Dumps, and Swords creeks had at least 13 AMD sites within their watersheds and ranked as 2. Russell, Weaver, Stock, Cove, and Lewis creeks, as well as Big Spring Branch and Bull Run all had AMD sites identified within their watersheds and were ranked as 3. Any habitat units located > 10,000 m from the confluence of one of the aforementioned tributaries were ranked as 4 (Table 2.2).

A weighting value was also added to the matrix to accommodate multiple PSD within 15, 000 m of the habitat unit. All habitat units with 5-6 identified PSD within 15,000 m upstream were given a rank of 1, 3-4 PSD within 15,000 m were ranked as 2, and 1-2 PSD ranked as 3. If no PSD were identified within 15,000 upstream, the habitat unit was given a rank of 4 (Table 2.2).

Index Structure

Indices were calculated separately for physical habitat parameters and potential threats. There were 5 parameters describing physical habitat, including, habitat index, embeddedness, riparian land use, bank condition, and braidedness. The 8 risk factors included bridge, rail, cattle, town, AMD, and PSD proximities. The risk index also included the relative ranking for each AMD and a rating corresponding to the number of PSD within 15 km upstream. All 13 of the index parameters had a maximum rank of 4 and a minimum rank of 1 (Table 2.2). The maximum possible score for the habitat analysis was 20, and the maximum score for the risk factors was 32.

Host Fish Distribution

Because mussel distribution may be tied to the distribution of host fishes (Watters 1992, Haag and Warren 1998). I attempted to include fish distribution in the model. A database of fish occurrence in the Clinch River was compiled from a variety of stream surveys contained in the Biota of Virginia (BOVA) database maintained by the Virginia Department of Game and Inland Fisheries. This database included more than 300 collections for the mainstem Clinch River, conducted by a variety of researchers between 1964 and 1988. The fish occurrence data were entered into a GIS, and after plotting these surveys, it became obvious that many of the survey coordinates recorded in the database were not precise enough to allow association with specific habitat units. Consequently, efforts to associate fish occurrence with habitat units were abandoned.

Next, I investigated the possibility of a trend in fish distribution across the study area, which might influence the suitability of habitat units for mussel habitation (Angermeier and Schlosser 1989, Watters 1992). Species richness of surveys included in

BOVA was plotted against UTM Northing coordinates in an attempt to identify a potential distribution gradient within the study reach. Northing was used as an approximation of river km. Due to an incomplete knowledge of host fish suitability for many mussels, specific fish species were not identified as being more ecologically important; species richness was the only parameter used. The resulting plot of species richness against longitude indicated no trend ($r^2 = -0.008$; $p = 0.42$) (Figure 2.2). Some of the variability in species richness among samples is likely due to inconsistency in sampling effort and technique. To standardize for this inconsistency, a subset of fish surveys having standardized methodology were selected and plotted against northing coordinates (Figure 2.3). No significant trend on the mainstem Clinch River within the bounds of my study area was detected ($R^2 = -0.0878$; $p = 0.063$; $n = 8$). These fish surveys indicate, however, that there is high species diversity throughout the study area, ranging from 24 to 39 species per site. Based on this information, I concluded that fishes were abundant throughout the study area, and there was no heterogeneity in habitat quality based on fish species diversity.

Water Quality

Water quality data for most of the Clinch River are lacking. The Virginia Department of Environmental Quality has monitored 24 stations on the Clinch River, Virginia, but currently maintains only 2 stations, one at Carbo (6BCLN206.70) and the other near the TN border (6BCLN271.50). Of the parameters measured at these stations, fecal coliform is the only parameter that periodically exceeded drinking water standards. This concurs with findings reported for water quality in the entire upper Tennessee River basin (Hampson et al. 2000). Mussel sensitivity levels for fecal coliform have not been identified.

RESULTS

Habitat Quality

Forest dominated riparian land cover (71 %) throughout the study reach. Agricultural categories were mostly located in the upper and middle portion of the survey reach, upstream of Burtons Ford (CRK 402). The study reach contained 125 braided habitat units. Of the 316 habitat units identified in the stream survey, 274 were classified as riffles or runs, and 42 were pools. Although pools were found throughout the study reach, long pools, some exceeding 1 km in length, were located in the lower portion of the river. Substrate embeddedness was low to moderate for most of the study reach. None of the habitat units exceeded 75 % embeddedness, and only 6 % of the units were 50-75 % embedded. Although mostly localized to the confluence areas, degrees of embeddedness increased below the confluences of Thompson (CRK 444), Weaver (CRK 441), Stony (CRK 367) and Cove (CRK 360.5) creeks. An extended reach of high embeddedness (25-75%) occurred from the mouth of Lick Creek (CRK 363) to approximately 2 km downstream of the Guest River (CRK 391).

Stream banks were mostly vegetated. Of the 316 habitat units, 124 had a mean bank coverage >85 %, and only 28 units had mean bank coverage < 50 %. Of these 28 units with high erosion potential, only 5 were located upstream of Castlewood (CRK 417); the remaining 23 units were all located downstream of Dungannon (CRK 380). Habitat units with high erosion potential were not necessarily co-located with concentrated cattle access. The majority of cattle access locations were in the upper portion of the study area, whereas non-vegetated banks were more common in the lower portion.

Habitat units were ranked based on physical characteristics including, habitat index (riffle/run or pool), embeddedness, riparian land use, bank condition, and braidedness (Table 2.3; Table 2.4). Out of a possible 20 (5 parameters with a maximum score of 4), scores ranged from 12 to 20, with 183 habitat units receiving a score < 18 (< 90 %) and 130 receiving a score ≥ 18 (≥ 90 %); (Table 2.4). Based on this analysis, the Clinch River from Nash Ford (CRK 449) down to Pendleton Island (CRK 365) has largest area of high quality habitat (Figure 2.4).

Risk Analysis

Coal mining is a pervasive threat to mussels in the Clinch River watershed. Permits granted by the Virginia Division of Mined Lands and Reclamation (DMLR) indicate that 69 km^2 (2.3 %) of the Clinch River watershed within Virginia is permitted for mining. A higher percentage of the Powell River watershed is held in permits — 125 km^2 (10.5 %) of the Powell watershed within Virginia. Also provided by DMLR was a database of 314 acid mine drainage (AMD) sites within the Clinch River watershed. AMD sites were identified by DMLR by collecting water samples from randomly selected sites. If a sample contained high levels of iron or manganese, or had pH below 6.0, additional water samples were collected upstream of the original sample site to locate the source of perturbation. As was expected, many of the AMD sites are located within mine permit areas. At least 33 of the 86 Clinch River tributaries in my survey reach are affected by AMD. Clinch River tributaries containing the most AMD sites included

Guest River (118), Indian Creek (24), Lick Creek (18), Swords Creek (13), and Dumps Creek (13) (Table 2.5). No data concerning the quantity of AMD from each site were available.

Thirteen roadway bridges crossed the Clinch River within the study area. High traffic routes include the Route 58 bridge crossing at Speers Ferry, Route 72 crossing upstream of Pendleton Island, Route 65 in Dungannon, Route 58 in Saint Paul, and Route 82 in Cleveland. Seven railroad bridges cross the Clinch River in the study area.

Richlands (CRK 515), Cleveland (CRK 438), Carbo (CRK 429), Saint Paul (CRK 410), and Dungannon (CRK 381) were the 5 towns identified on the Clinch River. Richlands, in Tazewell County, is located approximately 30 km upstream of the study area, and is the largest of the 5 towns with a population of 4456 (U. S. Census Bureau 1990). Saint Paul is the second largest town with 1000 people. Cleveland, Carbo, and Dungannon are home to fewer than 250 people per town.

The Virginia Department of Environmental Quality identified 49 permitted municipal and industrial discharges to the Clinch River and its tributaries; 12 of these occur on the mainstem. Although non-permitted discharges have been observed along the Clinch River, primarily untreated septic waste (Diamond and Serveiss 2001), no non-permitted discharges were specifically identified or added to the point source discharge database. Identified cattle access points were located in the upper portion of the study reach, and no concentrated access points were identified downstream of CRK 385.

Analysis of the 8 parameters associated with potential threats resulted in scores ranging from 14 to 32 (44-100 % of total possible). A total of 60 habitat units scored as ≥ 90%. Most of the low risk habitats were located around Nash Ford, below Nash Ford, Artrip, Pendleton Island, above Clinchport, and the section near the Tennessee border (Figure 2.5). Additionally, risk parameters were analyzed individually within the matrix of physically suitable habitat units (those ranking ≥ 90 % of physical habitat score). Any habitat unit whose suitability percentage was reduced by more than 1 % was considered to be at risk of degradation by the stressor. For example, if a habitat unit had a score of 90 % (18 out of a possible score of 20) based on physical parameters and scored 83 % (20 out of a possible 24) after the potential risk of nearby town activities was added to the matrix, the habitat unit was considered potentially degraded. Distribution maps of habitat suitability including each of the potential stressors were created (Appendices B-G).

Combined Analysis

Risk rankings were merged with those of physical habitat. The physical habitat ranking had 130 units with ≥ 90 % of the possible score. There were 110 units with high quality physical habitat that did were considered to be at high risk for anthropogenic impacts (Figure 2.6; Table 2.6). Additionally, out of the 60 sites in the lowest risk category (≥ 90 %), 40 sites did not fall in the high physical habitat category (Figure 2.7; Table 2.7). There were only 20 habitat units with high physical habitat and low risk potential, e.g. scoring ≥ 90 % of the highest score for physical habitat and ≥ 90 % for threats. These 20 habitats clustered around Nash Ford (CRK 449), below Nash Ford and at the head of Pendleton Island (CRK 365). There were 62 habitat units scoring ≥ 75 % for threats and ≥ 90 % for physical habitat (Figure 2.8). In addition to the aforementioned clusters, low risk and high quality habitat units were distributed near Artrip (CRK 442), below Carterton, Mill Island (389.5), Grays Island, and a few isolated spots around Pinnacles (CRK 457), Clinchport (CRK 343), and Speers Ferry (CRK 333).

For comparison purposes, after looking at the physical and risk parameters separately, the 5 parameters describing physical habitat and 8 describing potential threats, were summed to derive an overall combined score (0-52) for each unit. These overall index totals were plotted against the number of habitat units receiving each ranking (Figure 2.9). Habitat quality categories were separated based on the closest quartile divisions for reference purposes. Out of a maximum of 52, the highest-ranking habitat unit received a score of 51; the lowest rank for a habitat unit was 28. Habitat quality categories were derived from scores as follows: Excellent (51-44), Good (43-41), Fair (40-38), Poor (37-28). A total of 100 habitat units fell within the excellent category, with 76 considered good, 82 fair and 55 poor.

Habitat units with an overall rank of Excellent were distributed throughout the stream reach (Table 2.8). Clusters of excellent units were located (starting from upstream) at Pinnacle (CRK 457), Nash Ford (CRK 449), Artrip (CRK 442), upstream of Mill Island (CRK 389.5), Pendleton Island (CRK 365), Clinchport and upstream (CRK 356-343), and several riffles below Speers Ferry (CRK 333-325) (Figure 2.10). Sites ranking as poor primarily clustered around the towns of St. Paul and Carbo (Table 2.9). Both of these areas have multiple permitted discharges, including a water treatment plant in St. Paul and a coal-fired power plant in Carbo. The distribution of excellent and poor sites in the combined score analysis were similar to those resulting from the separated risk and physical habitat analysis. All habitat units scoring $\geq 75\%$ for threats and $\geq 90\%$ for physical habitat were also included in the Excellent category (Table 2.8).

DISCUSSION

Comparisons of the EcoRA developed in this project with the study by TVA's Cumberland Mollusk Conservation Program from the 1980s have limited overlap. Of the 30 sites monitored as part of the TVA project, only Pendleton Island (CRK 364) was located in the Clinch River, VA. An overall ranking of the 30 TVA sites placed Pendleton Island as the fifth most suitable for transplant of the birdwing pearlymussel (*Lemiox rimosus*) and Cumberland monkeyface (*Quadrula intermedia*) (TVA 1986). Pendleton Island also ranked in the highest habitat quality category of this study. Other sites observed in recent surveys to support healthy mussel populations, such as Clinchport (CRK 343), also ranked in the highest category for overall quality as calculated in this study. Cleveland Island (CRK 436), recently surveyed and containing diverse and reproducing mussel populations (M. Pinder, Virginia Department of Game and Inland Fisheries, Blacksburg, Virginia, pers. comm.) scored lower due to its close proximity to the town of Cleveland, the Route 82 bridge, and a wastewater treatment plant.

Given a large survey area, ranking risks based on extensive surveys is an ideal method for developing site assessments. Monitoring techniques such as IBI or other site intensive surveys are excellent for identifying stream health in discrete locations; however, time and budget constraints generally allow only a few sites to be surveyed. Additionally, site selection for monitoring is frequently biased towards areas that are easily accessible, e.g. near bridge crossings, boat launches, or other public access points. For endangered species introductions, these access points are the least favorable due to increased stream and riparian disturbance from stream users or litter and petroleum runoff from highway traffic. The complete habitat survey conducted as part of this project permitted all available stream habitats to be included in the analysis.

Habitat suitability indices (HSI) are another approach frequently used to describe the capacity of a given habitat to support a selected fish or wildlife species (USFWS 1981). HSI models can be developed for a single species or group of related species over a large survey area. The variables incorporated into an HSI model include species-specific life requisite parameters, including physical, chemical, and biological characteristics. Because the model parameters are based on species requirements rather than site information, the models can frequently be applied to many locations (USFWS 1980). In the absence of species life history information, however, parameters are based on assumptions, generalizations, or extrapolations from other species (Scott et al. 2002). Inaccurate data inputs may lead to inaccurate indices. Long-term chemical sensitivity limits for freshwater mussels are mostly unidentified (Environmental Contaminants Specialists 2001). Likewise, many specific life history parameters are also unknown and would have to be estimated for calculating an HSI. Given this lack of data, as well as the lack of stream-wide water quality monitoring, a risk assessment model based on proximity to potential impacts seemed the most appropriate approach for assessing relative water quality and habitat suitability.

Estimations and weighting may, with future monitoring or additional sensitivity data, need to be refined or adjusted to achieve more accurate suitability rankings. For watersheds with more concentrated impacts, it may be necessary to categorize threats differently than presented in this analysis. Bridge crossings, towns, and other potential risk factors were sufficiently dispersed that it was rare for a habitat unit to have multiple stressors from one category within 10 km upstream. The exception to this was permitted discharges. To weight units with multiple PSDs, an additional parameter was added to the matrix representing the number of PSD within 15 km. For the Clinch River PSD was the only risk factor requiring this additional weighting; however, if a similar model were used in other watersheds, this is type of weighting may be necessary for other parameters.

Additionally, with source-specific information regarding potential impacts, e.g. volume and content of permitted discharge or amount of AMD, it would be possible to weight sources relative to their impact potential. The categorical ranking of habitat units, however, provides a straightforward method for ranking relative habitat quality in the absence of complete stressor sensitivity and biological knowledge.

My study is a progression of other risk assessment studies completed for the Clinch River watershed. Studies by Diamond and Serveiss (2001) and Diamond et al. (2002) identified negative correlations between stressors, including mining, agriculture, and episodic spills and aquatic diversity in the Clinch River. My analysis examines the potential for these threats to affect available habitat. With information generated from the BVET survey, habitat availability for the entire Clinch River was incorporated into risk assessment models. Documenting habitat types and the distribution of threats provides a continuous view of habitat availability and risk potential that have previously been absent. Resource managers can use this complete habitat and risk analysis to best identify locations for restoration activities.

CONCLUSIONS

The initial objective of this project was to identify release sites in the Clinch River for endangered mussels. My recommendations for release sites include the 62 habitat units listed in Table 2.10 that have high quality physical habitat and low risk of anthropogenic impact. Most of the sites identified in this table are sites that were identified during the stream survey as either harboring live mussels or have been previously surveyed and are known to have a diverse mussel fauna.

In addition to the stated objective of selecting mussel release sites, this analysis has also identified stream reaches that would be excellent candidates for habitat restoration or to receive additional monitoring. There were 40 low risk sites but were not considered to have optimal physical habitat. Physical habitat parameters for these sites should be examined to identify the source of the reduced physical habitat rating. Sites receiving a reduced rating based on low bank vegetation cover or high risk riparian land use should be considered for restoration projects, including the creation of riparian buffer strips or bank stabilization. Additional water quality or biological monitoring should be conducted at the 110 stream reaches identified as having excellent physical habitat but being at high risk for anthropogenic impacts. If monitoring indicates that these high-risk sites are impacted, they may be areas where partnership projects can be initiated to reduce anthropogenic stressors. Identifying restoration sites using this analysis could assist in selecting priority stream reaches critical for faunal conservation.

The Clinch River was, in some ways, an ideal study area for this type of analysis. The entire 150 km survey reach was deep enough to be canoed, access points were sufficient to accommodate reasonable paddling distances per survey-day, and the stream gradient is moderate with no impoundments or dangerous (greater than Class III) rapids to portage. Rivers that do not fit these criteria may require substantial effort to obtain physical habitat data. Remote sensing from satellite images may enable some habitat data to be obtained without conducting the visual habitat survey (see Appendix H).

My approach should be broadly applicable. Anthropogenic risk parameters that were incorporated into this model should be available for any watershed. Alternatively, they could likely be calculated from maps or other nationally available digital data sources. Risk parameters for another river can be adjusted to accommodate activities of that watershed.

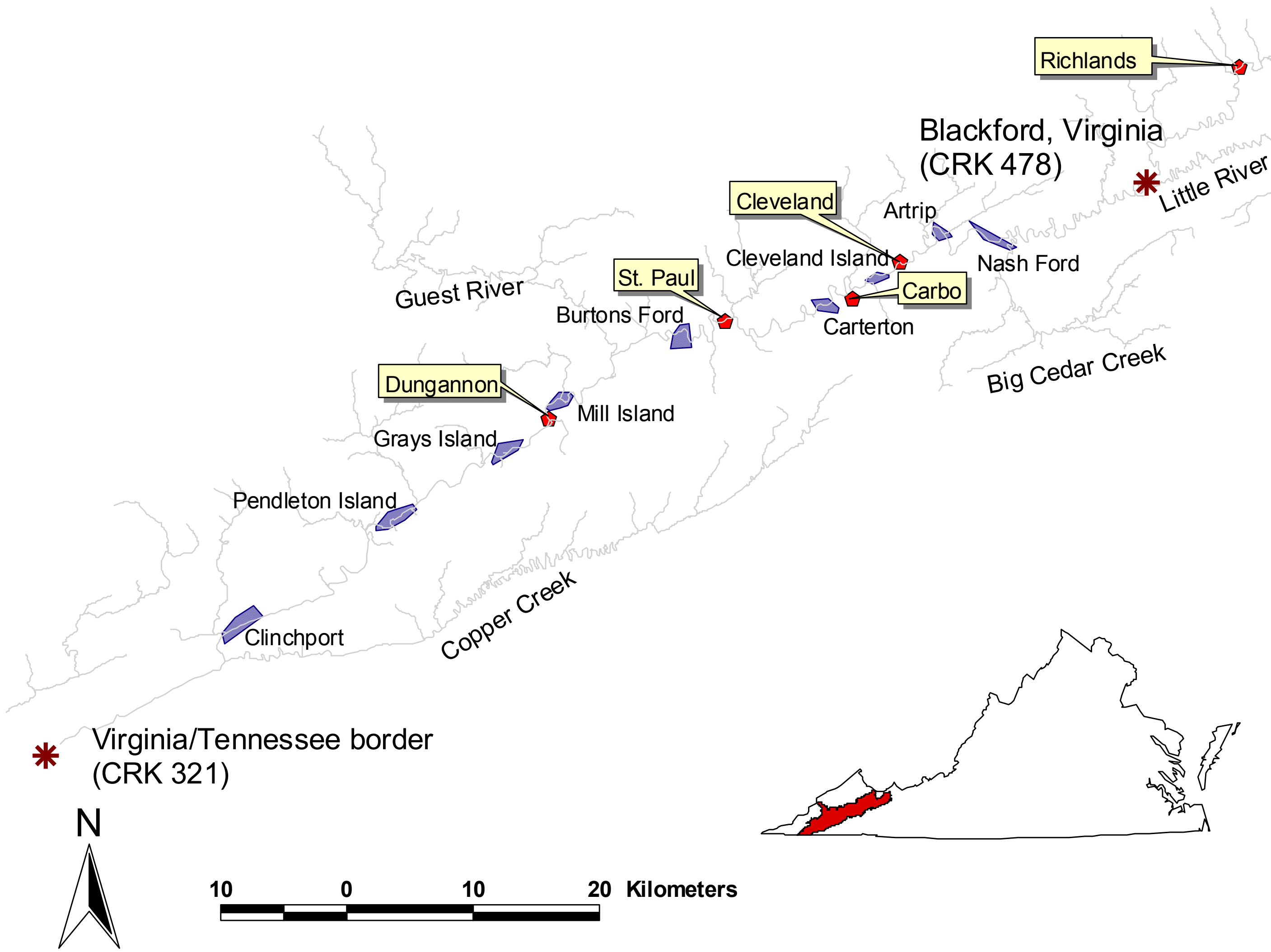


Figure 2.1. Extent of habitat survey of the Clinch River, which began at Clinch River kilometer (CRK) 478 in Blackford, VA and continued to the Virginia/Tennessee border at CRK 321.

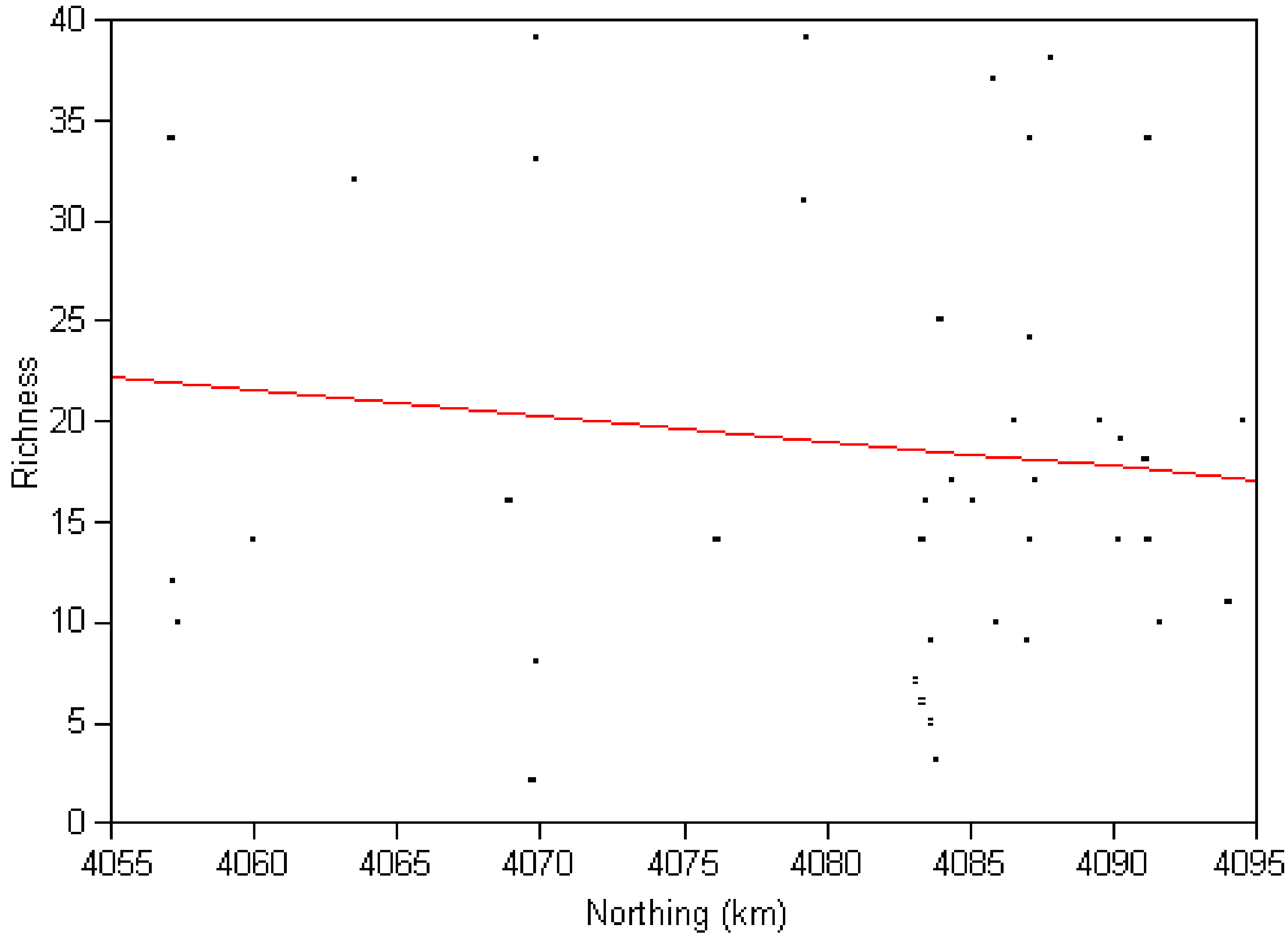


Figure 2.2. Regression of fish species richness against UTM Northing (km). Lesser UTM values indicate the downstream extent of the survey area. There was no trend in species richness over a longitudinal gradient (r^2adj = -0.008; p = 0.42). Fish surveys were obtained from the Biota of Virginia database maintained by the Virginia Department of Game and Inland Fisheries.

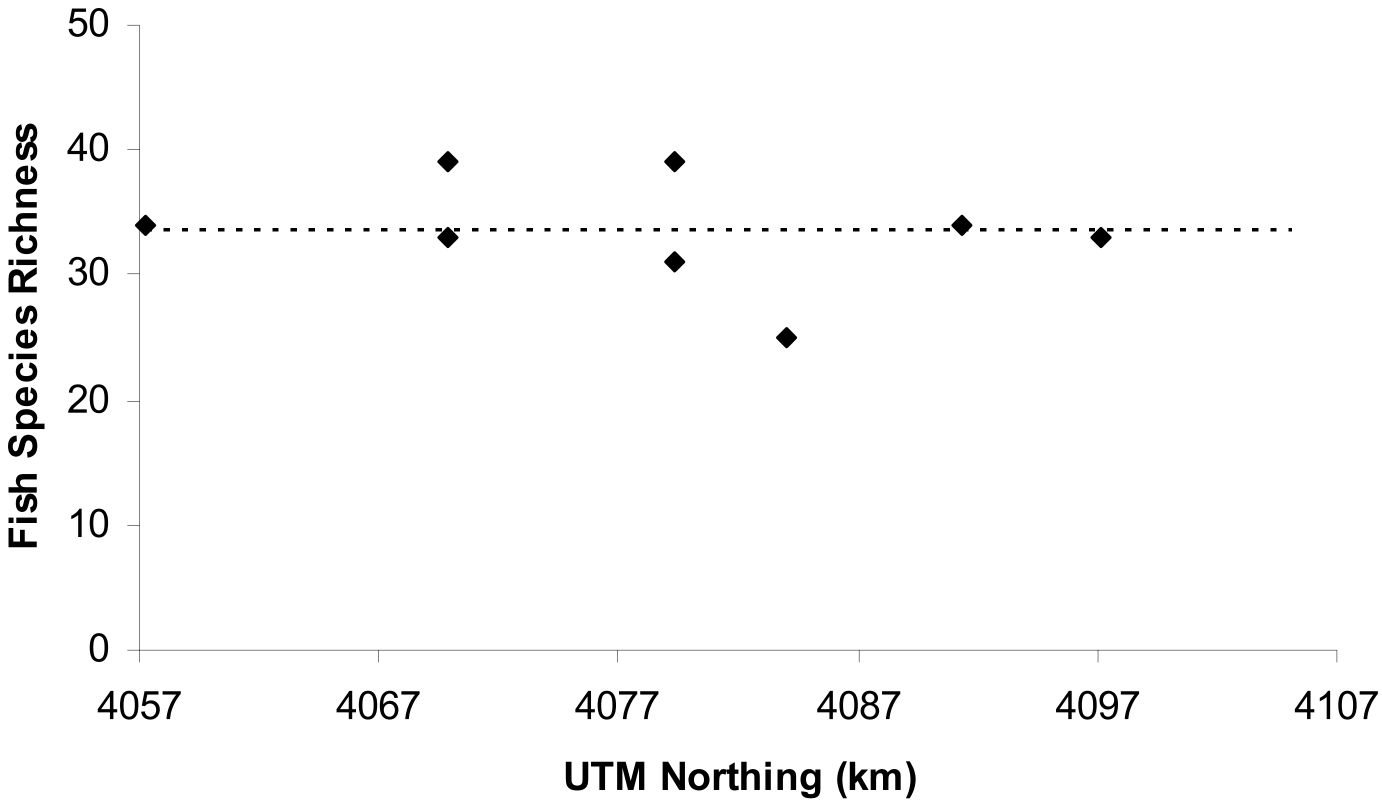


Figure 2.3. Species richness of selected surveys on the Clinch River plotted against UTM Northing. Lesser UTM values indicate the downstream extent of the survey area. To standardize for methodology, all surveys plotted here were conducted by P. L. Angermeier. Fish species richness did not exhibit a significant trend in the extent of my study area (R^2 = -0.0878; p = 0.06; n = 8). Data are included in the Biota of Virginia database maintained by the Virginia Department of Game and Inland Fisheries.

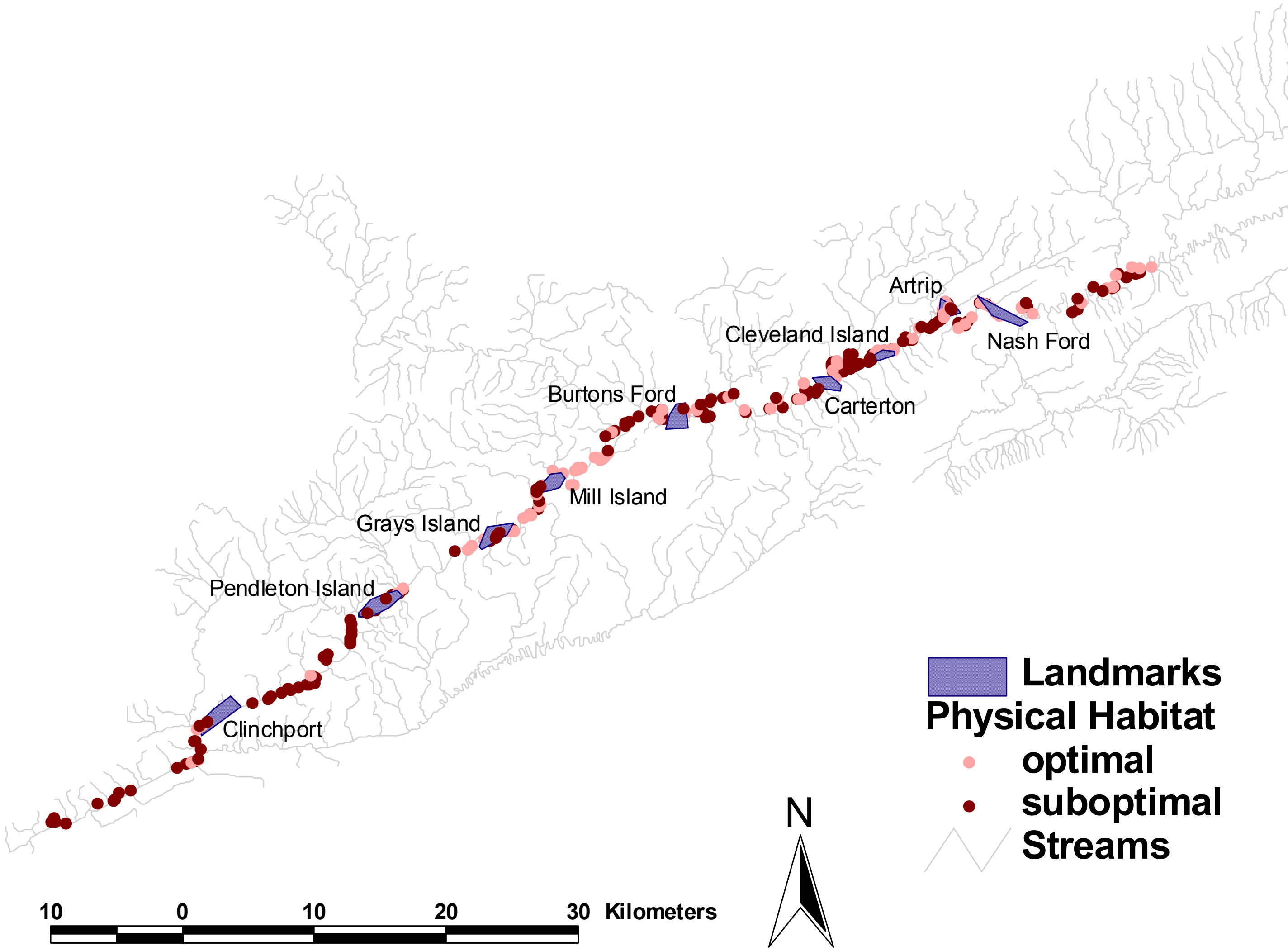


Figure 2.4. Distribution of habitat unit ranks based on physical habitat parameters. Optimal habitat received a ≥ 90% score based on habitat index (riffle/run or pool), embeddedness, riparian land use, bank condition, and braidedness. Suboptimal habitat received < 90% score based on the same parameters.

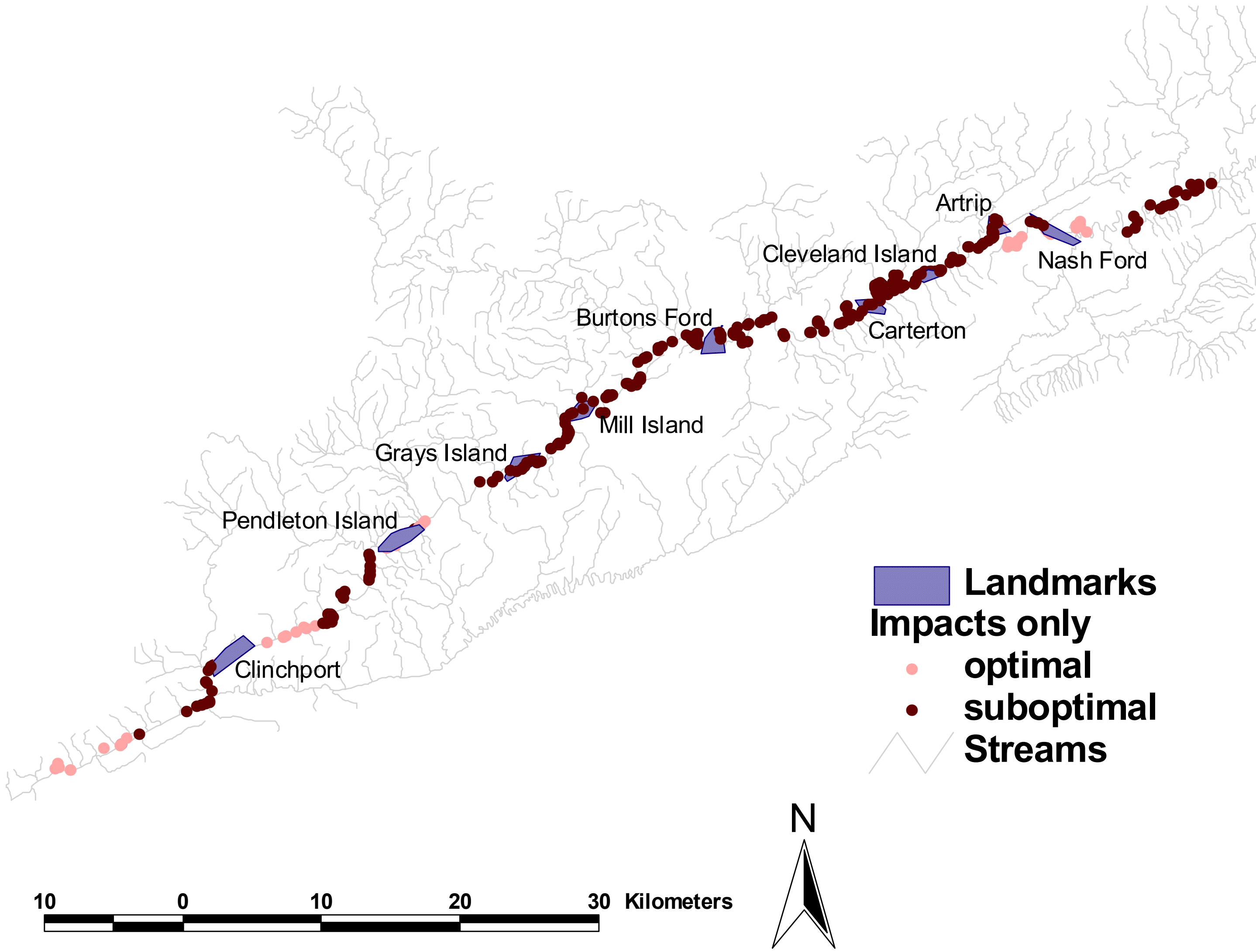


Figure 2.5. Distribution of habitat unit ranks based on risk parameters. Optimal habitat received a ≥ 90% score based on bridge, rail, acid mine drainage, point-source discharge, cattle and town proximity parameters. Suboptimal habitat received < 90% score based on the same parameters.

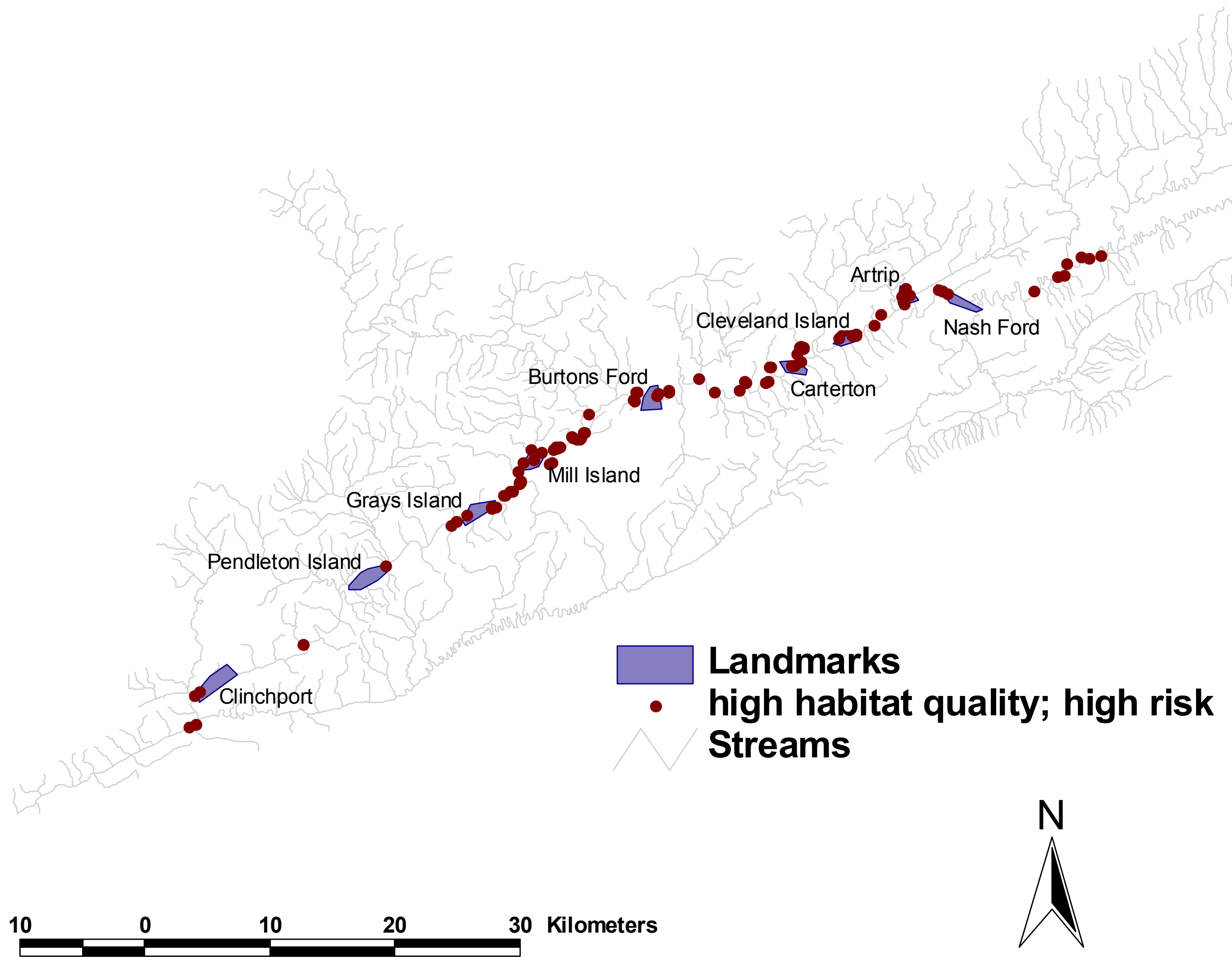


Figure 2.6. Distribution of habitat units having physical habitat scores ≥ 90 %, but at high risk for anthropogenic impacts (< 90 % of possible score).

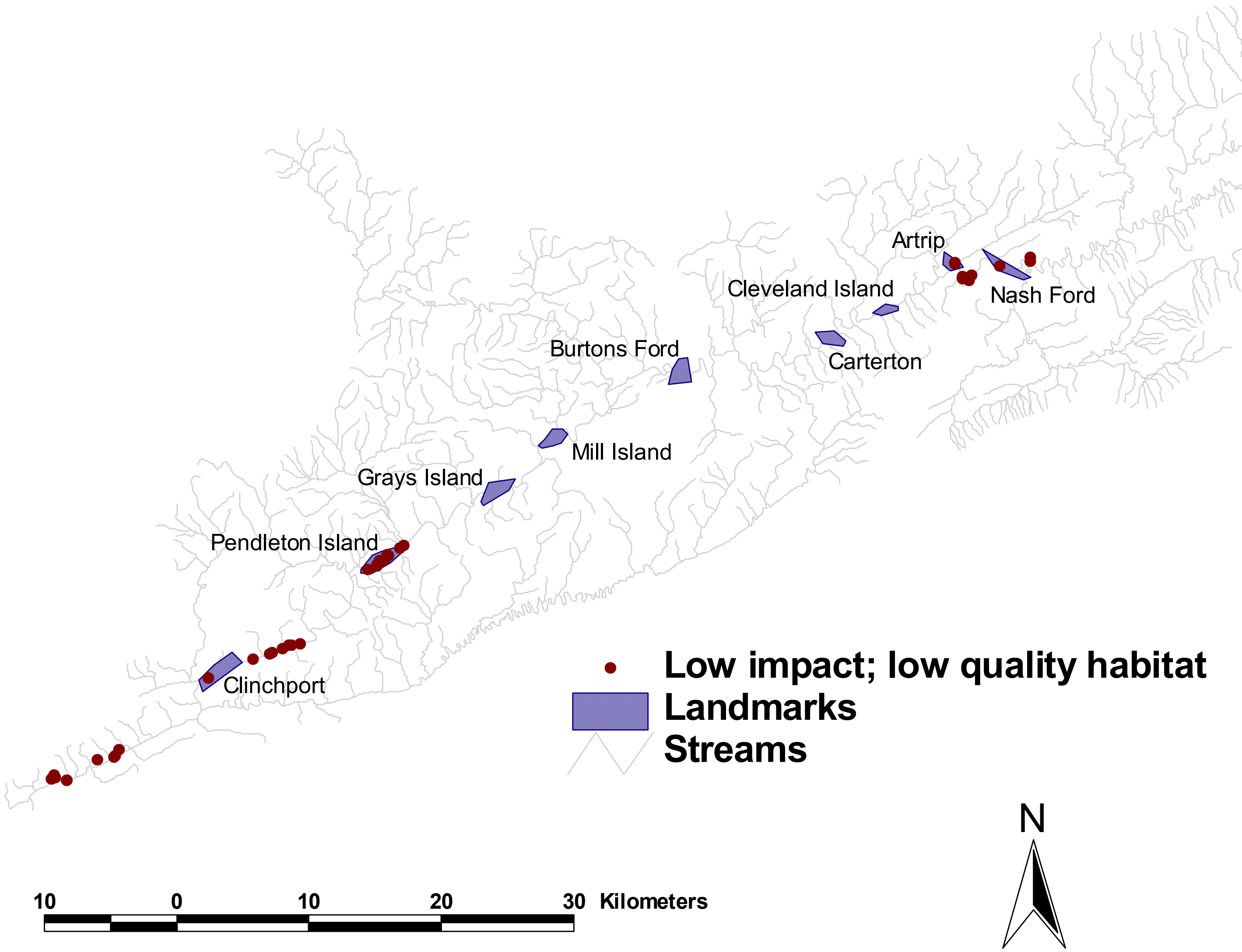


Figure 2.7. Habitat units at low risk, but having lower quality physical habitat (< 90 %).

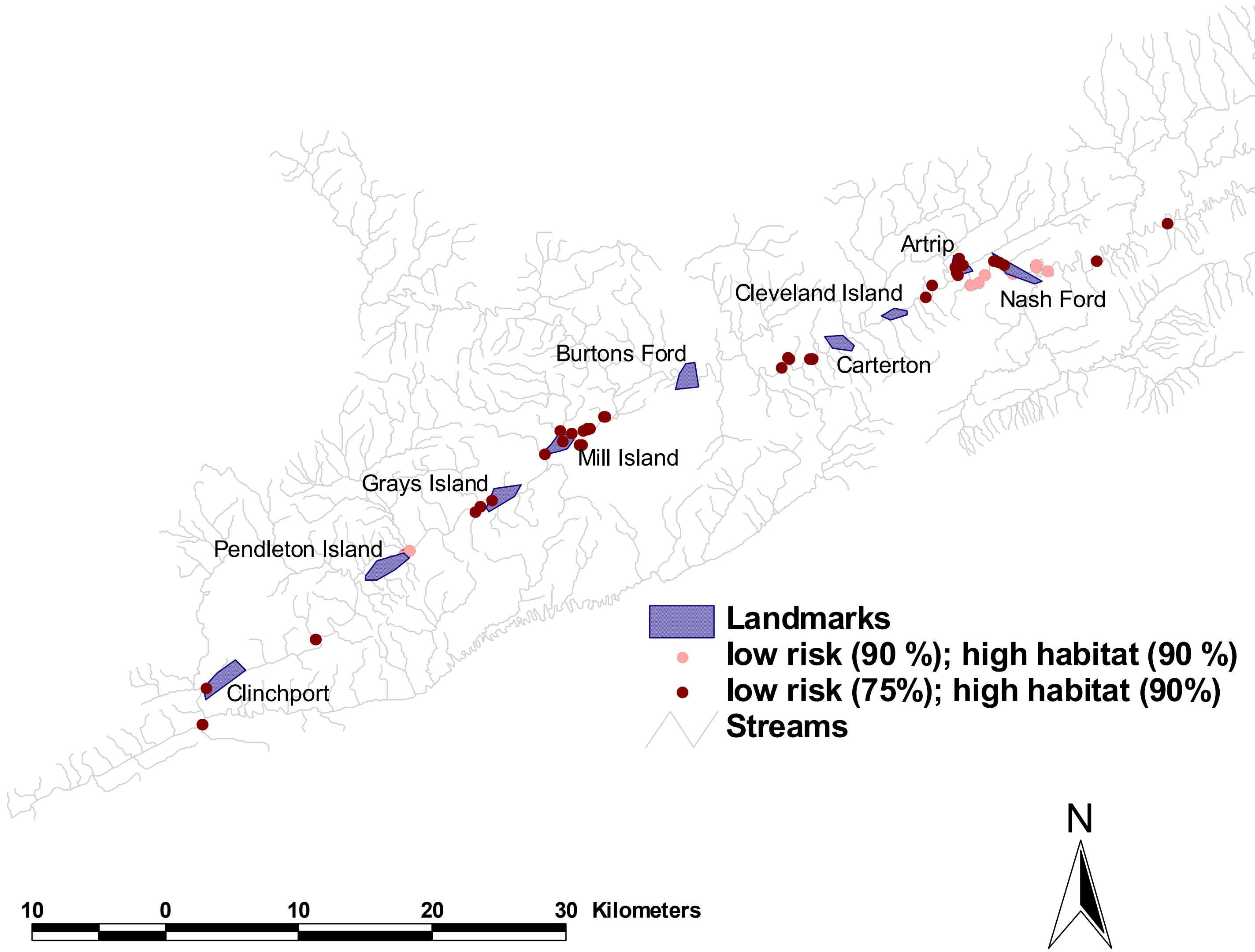


Figure 2.8. Distribution of habitat units with low risk to anthropogenic impacts and having high physical habitat scores ≥ 90 %. There are 20 habitat units in the lowest risk category (≥ 90 %) and 62 habitat units if the criteria is lowered to ≥75 %.

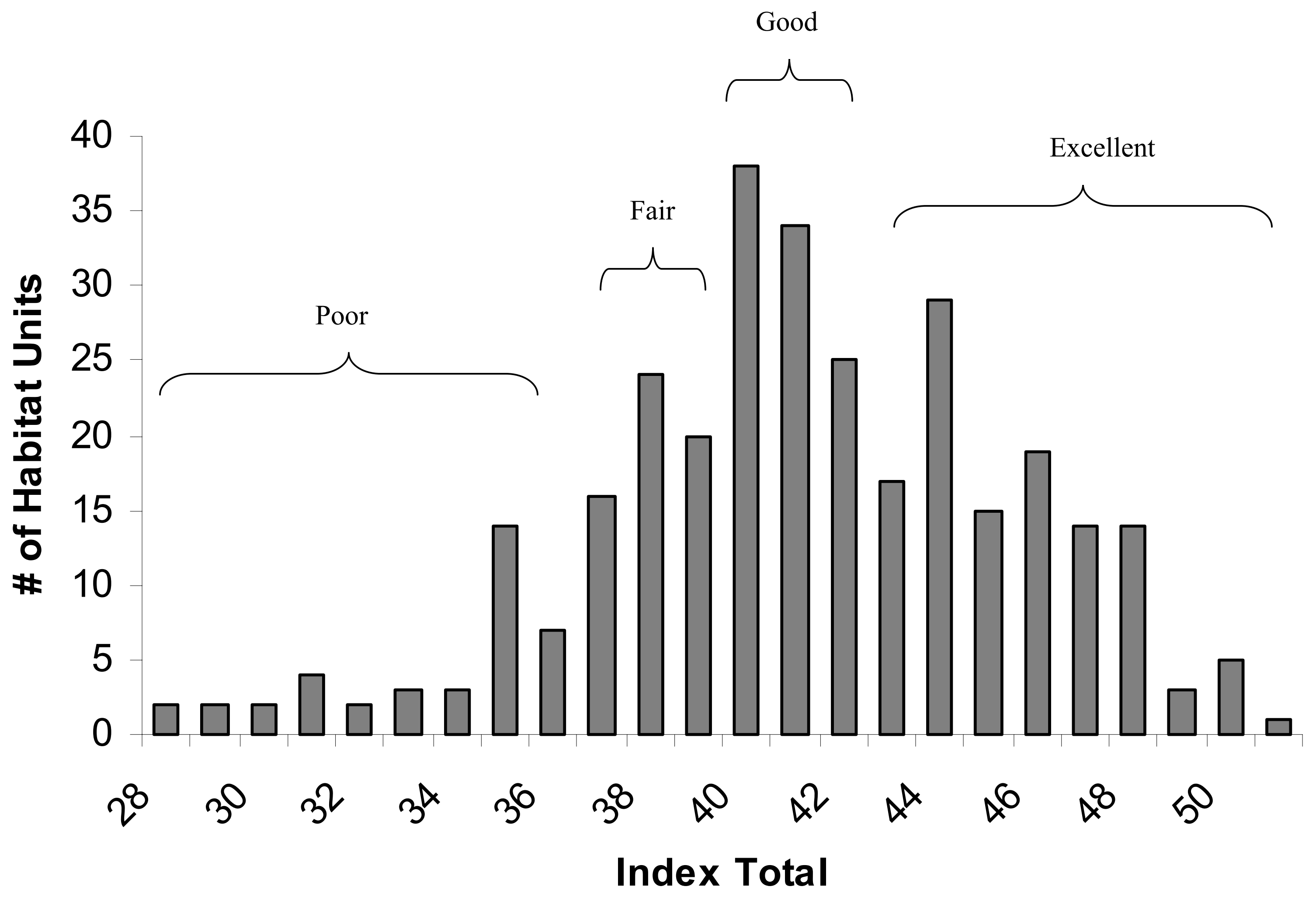


Figure 2.9. Distribution of index totals for all habitat units; 100 units were considered excellent, 76 good, 82 moderate, 36 fair, and 55 poor. Category divisions were based on nearest quartile divisions. Index total incorporates physical habitat and risk potential.

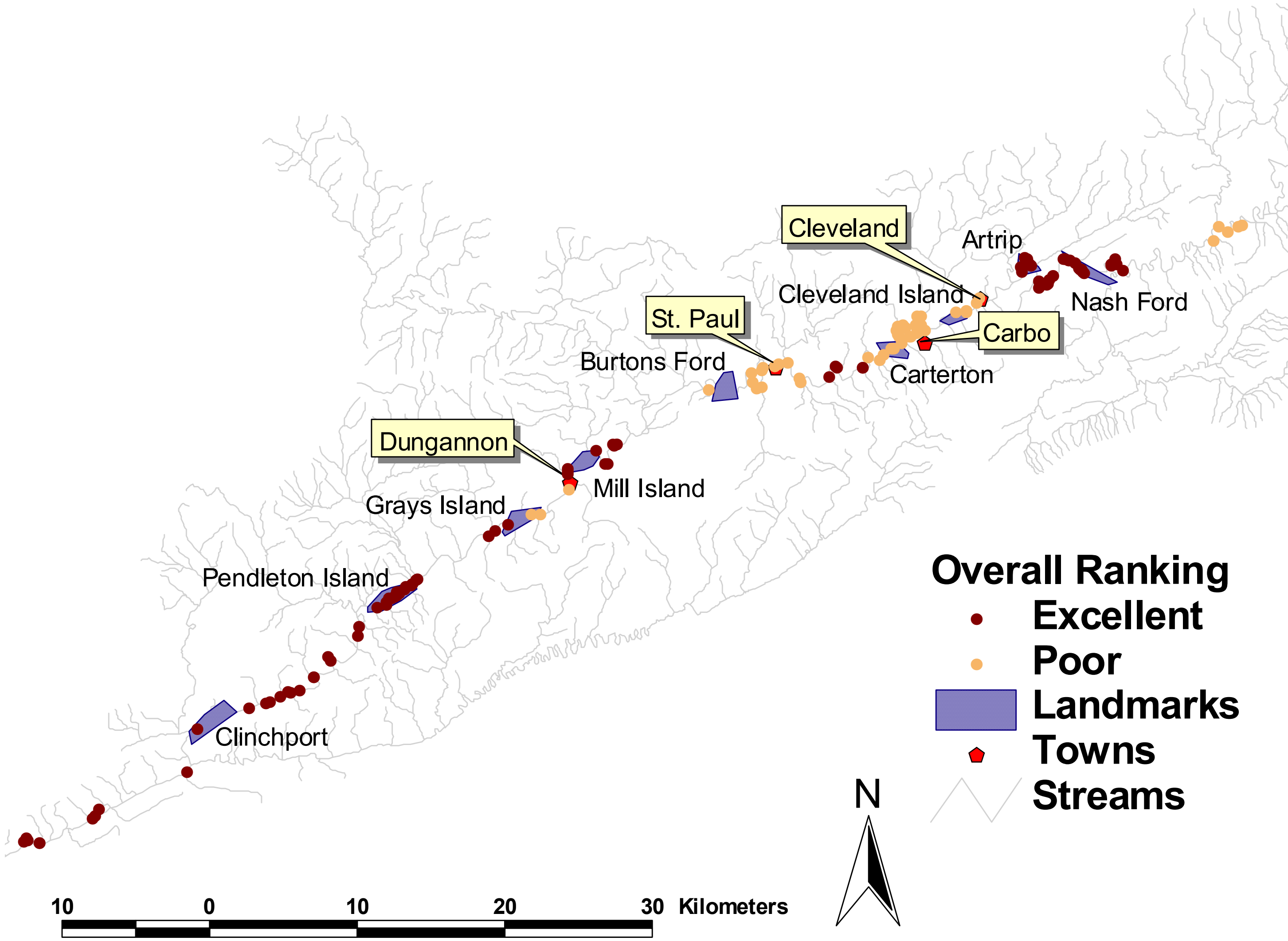


Figure 2.10. Distribution of Excellent and Poor habitat ranking categories throughout the Clinch River survey area. Rankings are based on combined scores for physical habitat quality and risk potential.

Table 2.1 Geographical information used in the relative risk model.

Name	Description	Source
PSD	Permitted discharges	Virginia Department of Environmental Quality
AMD	Identified acid mine drainage sites located on the mainstem Clinch River and its tributaries.	Virginia Department of Mined Land Reclamation
Streams	River locations	U.S. Geological Survey
Bridges	Automobile bridge locations	Virginia Department of Transportation
Fish	Fish surveys conducted on the mainstem Clinch River	Biota of Virginia; Virginia Department of Game and Inland Fisheries
Rail	Railroad bridge locations	TIGER (U. S. Census Bureau)
Watersheds	Watershed boundaries	National Elevation Data (USGS)

Table 2.2. Index values for all parameters entered in the habitat suitability matrix.

Parameter	Index Value			
	1	2	3	4
HABITAT QUALITY				
Bank stability (% vegetated or rock)	<25 %	49-25 %	50-84%	>85 %
Riparian land use	urban / industrial	row crop / residential	pasture	forest
Embeddedness	>75 %	51-75 %	26-50 %	0-25 %
Braided	--	no	--	yes
Habitat type	--	pool	--	riffle/run
THREATS				
Bridge proximity (km)	<1	1-5	5-10,	>10
Rail bridge proximity (km)	<1	1-5	5-10,	>10
Acid mine drainage (AMD) tributary proximity (km)	<1	1-5	5-10,	>10
AMD tributary ranking	Guest	Lick, Dumps, Swords	all other AMD tributaries	No AMD tributaries within 10,000m
Permitted discharge (PSD) proximity (km)	<5	5-10	10-15	>15
# PSD within 15km	5-6	3-4	1-2	0
Cattle access proximity (km)	<1	1-5	5-10,	>10
Distance to town (km)	<1	1-5	5-10,	>10

Table 2.3. Legend for abbreviations used in tables.

Abbreviation	Description
Hab unit	Habitat unit identifying the type of habitat (riffle, run, or pool) and a unique identifying number
LAT	Latitude measured in decimal degrees
LONG	Longitude measured in decimal degrees
Hab index	Habitat index distinguishing riffles and runs versus pools
Embed	Embeddedness of substrate
Riparian	Riparian land use adjacent to the stream
Bank	Bank stability (% vegetated or rock)
Braided	Braided channel versus single channel
Bridge	Automobile bridge
Rail	Railroad bridge
AMD	Tributary affected by identified Acid Mine Drainage
trib weight	Tributary ranking based on the number of identified AMD sites on the tributary
PSD	Permitted point-source discharge on the mainstem Clinch River and its tributaries
# psd	Number of PSD within 15 km upstream of habitat unit
Cattle	Concentrated cattle access
City	Town located near the Clinch River

Table 2.4. Habitat units ranked as ≥ 90 % of optimum based on physical parameters only.

Hab Unit	LAT	LONG	Hab index	Embed	Riparian	Bank	Braided	Habitat total	% of total
ri102	36.7665	-82.5806	4	3	4.0	3	4	18	90
ri106	36.7618	-82.5922	4	3	4.0	3	4	18	90
ri11	36.9641	-82.0756	4	4	3.0	3	4	18	90
ri112	36.7087	-82.6540	4	3	3.5	3	4	18	90
ri121	36.6497	-82.7486	4	3	4.0	3	4	18	90
ri13	36.9681	-82.0802	4	4	4.0	4	2	18	90
ri22	36.9559	-82.1015	4	4	3.5	4	2	18	90
ri26	36.9715	-82.1187	4	4	3.5	4	2	18	90
ri32	36.9452	-82.1463	4	4	2.0	4	4	18	90
ri34	36.9381	-82.1626	4	4	4.0	4	2	18	90
ri37	36.9372	-82.1702	4	4	4.0	4	2	18	90
ri50	36.9141	-82.2382	4	4	3.5	4	2	18	90
ri52	36.9027	-82.2433	4	4	3.0	3	4	18	90
ri58	36.8951	-82.3294	4	4	3.0	3	4	18	90
ri6	36.9811	-81.9820	4	4	3.5	4	2	18	90
ri66	36.8884	-82.3605	4	4	4.0	4	2	18	90
ri7	36.9820	-81.9765	4	4	4.0	4	2	18	90
ri73	36.8612	-82.4077	4	4	4.0	4	2	18	90
ri74	36.8598	-82.4116	4	4	4.0	4	2	18	90
ri75	36.8616	-82.4157	4	4	4.0	4	2	18	90
ri78	36.8520	-82.4520	4	4	4.0	4	2	18	90
ri79	36.8425	-82.4333	4	4	4.0	4	2	18	90
ri80	36.8421	-82.4354	4	4	4.0	4	2	18	90
ri83	36.8500	-82.4426	4	4	4.0	4	2	18	90
ri84	36.8447	-82.4500	4	4	4.0	4	2	18	90
ri85	36.8420	-82.4589	4	4	4.0	4	2	18	90
ri87	36.8356	-82.4642	4	4	4.0	4	2	18	90
ri9	36.9633	-82.0749	4	4	3.0	3	4	18	90
ri90	36.8274	-82.4627	4	4	3.5	4	2	18	90
ri93	36.8189	-82.4753	4	4	3.5	2	4	18	90
ri94	36.8102	-82.4834	4	3	4.0	3	4	18	90
ri99	36.7996	-82.5185	4	3	3.5	3	4	18	90
ru1	36.9966	-81.9438	4	4	4.0	4	2	18	90
ru10	36.9711	-82.0028	4	4	3.5	4	2	18	90
ru104	36.8916	-82.3398	4	3	3.0	4	4	18	90
ru119	36.8605	-82.4092	4	4	4.0	4	2	18	90
ru120	36.8614	-82.4146	4	4	4.0	4	2	18	90

Table 2.4. Habitat units ranked as ≥ 90 % of optimum based on physical parameters only.

Hab Unit	LAT	LONG	Hab index	Embed	Riparian	Bank	Braided	Habitat total	% of total
ru123	36.8521	-82.4321	4	4	4.0	4	2	18	90
ru126	36.8293	-82.4618	4	3	3.0	4	4	18	90
ru130	36.8101	-82.4833	4	3	4.0	3	4	18	90
ru135	36.7996	-82.5185	4	3	4.0	3	4	18	90
ru141	36.7597	-82.5927	4	3	4.0	3	4	18	90
ru142	36.7588	-82.5942	4	3	4.0	3	4	18	90
ru155	36.7087	-82.6540	4	3	4.0	3	4	18	90
ru164	36.6738	-82.7457	4	3	3.5	3	4	18	90
ru171	36.6480	-82.7541	4	3	4.0	3	4	18	90
ru18	36.9641	-82.0441	4	4	4.0	4	2	18	90
ru21	36.9668	-82.0533	4	4	4.0	4	2	18	90
ru29	36.9653	-82.0776	4	4	4.0	4	2	18	90
ru34	36.9534	-82.1081	4	4	3.5	4	2	18	90
ru44	36.9381	-82.1626	4	4	3.0	3	4	18	90
ru47	36.9603	-82.1198	4	4	3.5	4	2	18	90
ru48	36.9381	-82.1626	4	4	4.0	4	2	18	90
ru53	36.9530	-82.1408	4	4	3.5	4	2	18	90
ru56	36.9372	-82.1671	4	4	3.0	3	4	18	90
ru59	36.9353	-82.1781	4	4	3.5	4	2	18	90
ru6	36.9905	-81.9738	4	4	4.0	4	2	18	90
ru74	36.9280	-82.2123	4	3	4.0	3	4	18	90
ru78	36.9149	-82.2176	4	4	3.0	3	4	18	90
ru82	36.9136	-82.2391	4	4	3.5	4	2	18	90
ru91	36.8968	-82.2668	4	4	3.0	3	4	18	90
ru93	36.8956	-82.2892	4	4	3.0	3	4	18	90
ri10	36.9633	-82.0749	4	3	4.0	4	4	19	95
ri100	36.7965	-82.5228	4	4	3.5	3	4	19	95
ri103	36.7658	-82.5818	4	3	4.0	4	4	19	95
ri117	36.6707	-82.7498	4	4	3.5	3	4	19	95
ri12	36.9641	-82.0756	4	3	4.0	4	4	19	95
ri122	36.6496	-82.7485	4	4	4.0	3	4	19	95
ri15	36.9699	-82.0854	4	4	3.0	4	4	19	95
ri16	36.9699	-82.0854	4	4	4.0	3	4	19	95
ri17	36.9707	-82.0889	4	3	4.0	4	4	19	95
ri20	36.9604	-82.0967	4	3	4.0	4	4	19	95
ri27	36.9658	-82.1215	4	4	4.0	3	4	19	95
ri3	36.9951	-81.9540	4	4	2.5	4	4	19	95

Table 2.4. Habitat units ranked as ≥ 90 % of optimum based on physical parameters only.

Hab Unit	LAT	LONG	Hab index	Embed	Riparian	Bank	Braided	Habitat total	% of total
ri35	36.9371	-82.1643	4	4	4.0	3	4	19	95
ri36	36.9370	-82.1658	4	4	4.0	3	4	19	95
ri38	36.9369	-82.1756	4	4	4.0	3	4	19	95
ri44	36.9283	-82.2105	4	4	4.0	3	4	19	95
ri45	36.9236	-82.2145	4	4	4.0	3	4	19	95
ri46	36.9180	-82.2116	4	4	3.5	3	4	19	95
ri47	36.9150	-82.2200	4	4	4.0	3	4	19	95
ri54	36.9032	-82.2614	4	4	3.5	3	4	19	95
ri59	36.8951	-82.3294	4	4	3.0	4	4	19	95
ri63	36.8947	-82.3584	4	4	4.0	3	4	19	95
ri64	36.8948	-82.3587	4	4	4.0	3	4	19	95
ri70	36.8784	-82.4011	4	3	4.0	4	4	19	95
ri72	36.8645	-82.4051	4	3	4.0	4	4	19	95
ri89	36.8284	-82.4616	4	4	3.0	4	4	19	95
ri91	36.8216	-82.4698	4	3	4.0	4	4	19	95
ri95	36.8101	-82.4873	4	4	4.0	3	4	19	95
ri98	36.8043	-82.5085	4	4	4.0	3	4	19	95
ru103	36.8957	-82.3293	4	4	3.0	4	4	19	95
ru105	36.8937	-82.3394	4	3	4.0	4	4	19	95
ru118	36.8644	-82.4048	4	3	4.0	4	4	19	95
ru125	36.8293	-82.4618	4	3	4.0	4	4	19	95
ru128	36.8188	-82.4758	4	4	3.5	3	4	19	95
ru129	36.8096	-82.4872	4	4	4.0	3	4	19	95
ru140	36.7612	-82.5916	4	4	4.0	3	4	19	95
ru22	36.9681	-82.0532	4	3	4.0	4	4	19	95
ru26	36.9618	-82.0732	4	3	4.0	4	4	19	95
ru4	36.9956	-81.9606	4	3	4.0	4	4	19	95
ru46	36.9381	-82.1626	4	3	4.0	4	4	19	95
ru54	36.9370	-82.1649	4	4	4.0	3	4	19	95
ru71	36.9295	-82.2111	4	4	4.0	3	4	19	95
ru72	36.9278	-82.2098	4	3	4.0	4	4	19	95
ru79	36.9148	-82.2188	4	4	4.0	3	4	19	95
ru86	36.9031	-82.2407	4	4	4.0	3	4	19	95
ru87	36.9031	-82.2407	4	4	4.0	3	4	19	95
ru89	36.9024	-82.2607	4	4	3.5	3	4	19	95
ru90	36.9024	-82.2607	4	4	3.5	3	4	19	95
ri21	36.9604	-82.0967	4	4	4.0	4	4	20	100

Table 2.4. Habitat units ranked as ≥ 90 % of optimum based on physical parameters only.

Hab Unit	LAT	LONG	Hab index	Embed	Riparian	Bank	Braided	Habitat total	% of total
ri25	36.9672	-82.1152	4	4	4.0	4	4	20	100
ri28	36.9626	-82.1204	4	4	4.0	4	4	20	100
ri48	36.9151	-82.2200	4	4	4.0	4	4	20	100
ri55	36.9046	-82.3026	4	4	4.0	4	4	20	100
ri57	36.8951	-82.3294	4	4	4.0	4	4	20	100
ri76	36.8533	-82.4291	4	4	4.0	4	4	20	100
ri77	36.8539	-82.4296	4	4	4.0	4	4	20	100
ri88	36.8285	-82.4620	4	4	4.0	4	4	20	100
ri92	36.8211	-82.4703	4	4	4.0	4	4	20	100
ri96	36.8095	-82.4872	4	4	4.0	4	4	20	100
ru110	36.8892	-82.3619	4	4	4.0	4	4	20	100
ru121	36.8539	-82.4267	4	4	4.0	4	4	20	100
ru122	36.8539	-82.4267	4	4	4.0	4	4	20	100
ru127	36.8212	-82.4687	4	4	4.0	4	4	20	100
ru137	36.7697	-82.5765	4	4	4.0	4	4	20	100
ru23	36.9681	-82.0532	4	4	4.0	4	4	20	100
ru50	36.9375	-82.1626	4	4	4.0	4	4	20	100
ru52	36.9378	-82.1622	4	4	4.0	4	4	20	100
ru73	36.9279	-82.2095	4	4	4.0	4	4	20	100

Table 2.5. Summary of Clinch River tributaries affected by identified acid mine drainage (AMD) sites. A total of 314 AMD sites were identified in the Virginia Division of Mined Lands database for the Clinch River drainage. Habitat units are coded as run (ru), riffle (ri), and pool (p). Those streams listed with multiple habitat units at the confluence represent braided channels.

Receiving Stream	**Number of AMD sites in watershed**	**Clinch River habitat unit at tributary confluence**
Stock Creek	3	ru164
Cove Creek	3	ru148
Stony Creek	1	ru137
Staunton Creek	1	ri99, ru135
Corder Branch	1	ri84
Guest River	119	ri71
Bull Run	4	ru113
Russell Creek	8	ru106, ri63, ri64
Robinette Branch	2	p70, ru74, ri56
Lick Creek	18	ru94
Big Spring Branch	3	ru92, p67
Russian Branch	1	p67
Sexton Branch	1	ru86, ru87
Unnamed tributary	1	ru91
Unnamed tributary	1	ru90
Unnamed tributary	1	ru88
Dry Run	2	ri50, ru82
Unnamed tributary	4	ri49
Eagle Nest Branch	1	ru75
Unnamed tributary	1	ri46
Dumps Creek	14	ri41
Weaver Creek	5	ri30, ru49
Thompson Creek	2	ru37, ru38
Lewis Creek	5	ri5, ru6
Little River	1	ru1
Swords Creek	13	above survey area

Table 2.6. Habitat units with high physical habitat quality (≥ 90% of optimal), but at high risk for anthropogenic threats (<90 %).

LABEL	LAT	LONG	Low impact (≥ 90 % of best possible score)	Physical habitat (< 90 % of best possible score)
ri44	36.9283	-82.2105	53	95
ru44	36.9381	-82.1626	53	90
ru48	36.9381	-82.1626	53	90
ru71	36.9295	-82.2111	53	95
ru72	36.9278	-82.2098	53	95
ru73	36.9279	-82.2095	53	100
ru74	36.9280	-82.2123	53	90
ri45	36.9236	-82.2145	56	95
ri47	36.9150	-82.2200	56	95
ri48	36.9151	-82.2200	56	100
ru79	36.9148	-82.2188	56	95
ri46	36.9180	-82.2116	59	95
ri55	36.9046	-82.3026	59	100
ru46	36.9381	-82.1626	59	95
ru93	36.8956	-82.2892	59	90
ri34	36.9381	-82.1626	63	90
ru50	36.9375	-82.1626	63	100
ru6	36.9905	-81.9738	63	90
ru78	36.9149	-82.2176	63	90
ri3	36.9951	-81.9540	66	95
ri35	36.9371	-82.1643	66	95
ri36	36.9370	-82.1658	66	95
ri37	36.9372	-82.1702	66	90
ri50	36.9141	-82.2382	66	90
ri57	36.8951	-82.3294	66	100
ri59	36.8951	-82.3294	66	95
ri66	36.8884	-82.3605	66	90
ri88	36.8285	-82.4620	66	100
ri89	36.8284	-82.4616	66	95
ri90	36.8274	-82.4627	66	90
ri91	36.8216	-82.4698	66	95
ru110	36.8892	-82.3619	66	100
ru125	36.8293	-82.4618	66	95
ru126	36.8293	-82.4618	66	90
ru127	36.8212	-82.4687	66	100
ru54	36.9370	-82.1649	66	95
ru56	36.9372	-82.1671	66	90
ru82	36.9136	-82.2391	66	90
ri117	36.6707	-82.7498	69	95

Table 2.6. Habitat units with high physical habitat quality (≥ 90% of optimal), but at high risk for anthropogenic threats (<90 %).

LABEL	LAT	LONG	Low impact (≥ 90 % of best possible score)	Physical habitat (< 90 % of best possible score)
ri38	36.9369	-82.1756	69	95
ri6	36.9811	-81.9820	69	90
ri7	36.9820	-81.9765	69	90
ri72	36.8645	-82.4051	69	95
ri92	36.8211	-82.4703	69	100
ri93	36.8189	-82.4753	69	90
ru103	36.8957	-82.3293	69	95
ru104	36.8916	-82.3398	69	90
ru105	36.8937	-82.3394	69	95
ru118	36.8644	-82.4048	69	95
ru128	36.8188	-82.4758	69	95
ru171	36.6480	-82.7541	69	90
ru4	36.9956	-81.9606	69	95
ru52	36.9378	-82.1622	69	100
ru59	36.9353	-82.1781	69	90
ru90	36.9024	-82.2607	69	95
ri58	36.8951	-82.3294	72	90
ri63	36.8947	-82.3584	72	95
ri64	36.8948	-82.3587	72	95
ri70	36.8784	-82.4011	72	95
ri73	36.8612	-82.4077	72	90
ri74	36.8598	-82.4116	72	90
ri85	36.8420	-82.4589	72	90
ri94	36.8102	-82.4834	72	90
ri95	36.8101	-82.4873	72	95
ri96	36.8095	-82.4872	72	100
ru119	36.8605	-82.4092	72	90
ru129	36.8096	-82.4872	72	95
ru130	36.8101	-82.4833	72	90
ri75	36.8616	-82.4157	75	90
ri76	36.8533	-82.4291	75	100
ri77	36.8539	-82.4296	75	100
ru120	36.8614	-82.4146	75	90
ru121	36.8539	-82.4267	75	100
ru122	36.8539	-82.4267	75	100
ru164	36.6738	-82.7457	75	90
ru53	36.9530	-82.1408	75	90
ri121	36.6497	-82.7486	78	90
ri122	36.6496	-82.7485	78	95
ri28	36.9626	-82.1204	78	100

Table 2.6. Habitat units with high physical habitat quality (≥ 90% of optimal), but at high risk for anthropogenic threats (<90 %).

LABEL	LAT	LONG	Low impact (≥ 90 % of best possible score)	Physical habitat (< 90 % of best possible score)
ri32	36.9452	-82.1463	78	90
ri52	36.9027	-82.2433	78	90
ri54	36.9032	-82.2614	78	95
ri78	36.8520	-82.4520	78	90
ri84	36.8447	-82.4500	78	90
ri98	36.8043	-82.5085	78	95
ri99	36.7996	-82.5185	78	90
ru1	36.9966	-81.9438	78	90
ru10	36.9711	-82.0028	78	90
ru123	36.8521	-82.4321	78	90
ru47	36.9603	-82.1198	78	90
ru86	36.9031	-82.2407	78	95
ru87	36.9031	-82.2407	78	95
ru89	36.9024	-82.2607	78	95
ri27	36.9658	-82.1215	81	95
ri79	36.8425	-82.4333	81	90
ri80	36.8421	-82.4354	81	90
ru91	36.8968	-82.2668	81	90
ri83	36.8500	-82.4426	84	90
ri87	36.8356	-82.4642	84	90
ri100	36.7965	-82.5228	88	95
ri102	36.7665	-82.5806	88	90
ri112	36.7087	-82.6540	88	90
ri13	36.9681	-82.0802	88	90
ri15	36.9699	-82.0854	88	95
ri16	36.9699	-82.0854	88	95
ri17	36.9707	-82.0889	88	95
ri25	36.9672	-82.1152	88	100
ri26	36.9715	-82.1187	88	90
ru135	36.7996	-82.5185	88	90
ru155	36.7087	-82.6540	88	90

Table 2.7. Habitat units with low physical habitat quality (< 90% of optimal), but at low risk for anthropogenic threats (≥90 %). These sites are recommended for potential habitat restoration.

LABEL	LAT	LONG	Low impact (≥ 90 % of best possible score)	Physical habitat (< 90 % of best possible score)
p133	36.6996	-82.6731	91	75
ri105	36.7618	-82.5905	91	85
ri107	36.7618	-82.5905	91	85
ri108	36.7612	-82.5916	91	85
ri109	36.7614	-82.5904	91	85
ri115	36.6991	-82.6711	91	80
ri116	36.6927	-82.6898	91	85
ru139	36.7620	-82.5915	91	85
ru158	36.7005	-82.6643	91	75
ru159	36.6967	-82.6791	91	85
ru160	36.6937	-82.6871	91	80
p119	36.7581	-82.5963	94	70
ri124	36.6216	-82.8181	94	75
ri23	36.9556	-82.1074	94	80
ri24	36.9570	-82.1076	94	85
ru143	36.7589	-82.5942	94	85
ru144	36.7578	-82.5980	94	85
ru145	36.7556	-82.6005	94	75
ru146	36.7540	-82.6003	94	80
ru147	36.7520	-82.6071	94	75
ru174	36.6260	-82.8150	94	85
ru19	36.9710	-82.0499	94	85
ru32	36.9580	-82.1000	94	80
ru33	36.9546	-82.1016	94	80
ru35	36.9562	-82.1074	94	75
p115	36.7665	-82.5806	97	85
ri101	36.7689	-82.5773	97	85
ru161	36.6895	-82.7030	97	80
ru162	36.6760	-82.7413	97	80
ru175	36.6207	-82.8194	97	65
ru177	36.6190	-82.8333	97	60
ru20	36.9682	-82.0494	97	80
ru28	36.9645	-82.0762	97	75
ru37	36.9662	-82.1137	97	80
ru38	36.9664	-82.1141	97	85
ri126	36.6058	-82.8683	100	60
ri127	36.6052	-82.8715	100	60
ru179	36.6044	-82.8592	100	70
ru180	36.6044	-82.8592	100	60
ru181	36.6080	-82.8700	100	65

Table 2.8. Data matrix of habitat units ranking as Excellent for overall relative ranking, including physical habitat parameters and risk potential. Habitat units marked with an * were also scored ≥ 90 % for physical habitat and ≥ 75 % for risk factors.

Hab Unit	LAT	LONG	Hab index	Embed	Riparian	Bank	Braided	Bridge	Rail	AMD	trib weight	PSD	# psd	Cattle	City	Total
p70	36.9018	-82.3169	2	3	2.0	4	2	1	1	2	3	2	2	3	1	28
ru68	36.9346	-82.2003	4	4	1.3	3	2	1	2	1	2	1	1	4	2	28
p48	36.9246	-82.1996	2	4	1.0	3	2	2	2	2	2	1	1	4	3	29
p47	36.9266	-82.2003	2	4	1.3	3	2	2	2	2	2	1	1	4	3	29
p49	36.9225	-82.2063	2	4	4.0	3	2	2	2	2	2	1	1	4	1	30
ru96	36.9030	-82.3076	4	3	1.0	3	2	2	2	2	3	2	2	3	1	30
p51	36.9286	-82.2137	2	4	3.0	3	2	2	2	2	2	2	1	4	2	31
p52	36.9277	-82.2150	2	4	3.0	3	2	2	2	2	2	2	1	4	2	31
ri42	36.9289	-82.2010	4	4	1.3	4	2	1	2	2	2	1	1	4	3	31
ru69	36.9296	-82.2011	4	4	1.3	4	2	1	2	2	2	1	1	4	3	31
p72	36.8903	-82.3178	2	4	4.0	3	2	2	2	2	3	2	1	3	2	32
ru70	36.9220	-82.2048	4	4	4.0	3	2	2	2	2	2	1	1	4	1	32
ri41	36.9345	-82.1971	4	4	1.0	3	2	3	2	1	2	2	3	4	2	33
ru67	36.9292	-82.1968	4	3	3.0	3	2	2	1	1	3	2	3	4	2	33
ru75	36.9280	-82.2146	4	4	3.0	3	2	2	2	2	2	2	1	4	2	33
p50	36.9278	-82.2093	2	4	4.0	3	4	2	2	2	2	2	1	4	2	34
p73	36.8894	-82.3219	2	4	3.0	4	2	2	2	3	3	2	1	4	2	34
ru95	36.9046	-82.3052	4	4	2.0	3	2	2	2	1	3	2	2	3	4	34
ru92	36.8934	-82.2883	4	4	3.5	3	2	2	1	1	3	2	3	2	4	35
p3	36.9927	-81.9535	2	3	3.0	3	2	2	4	3	2	3	3	1	4	35
p33	36.9435	-82.1550	2	4	2.0	3	2	2	3	2	3	4	4	3	1	35
p7	36.9826	-81.9749	2	3	3.0	3	2	3	4	2	3	2	3	1	4	35
p76	36.8989	-82.3260	2	4	3.0	4	2	3	2	3	3	2	1	4	2	35
p98	36.8256	-82.4633	2	3	3.0	3	2	1	4	4	4	3	3	2	1	35
ri56	36.9005	-82.3179	4	3	4.0	4	2	2	2	2	3	3	2	3	1	35
ru101	36.8931	-82.3240	4	3	3.0	4	2	2	2	3	3	2	1	4	2	35
ru44	36.9381	-82.1626	4	4	3.0	3	4	1	3	3	3	1	3	1	2	35
ru48	36.9381	-82.1626	4	4	4.0	4	2	1	3	3	3	1	3	1	2	35
ru74	36.9280	-82.2123	4	3	4.0	3	4	2	2	2	2	2	1	4	2	35
ru76	36.9256	-82.2156	4	4	4.0	3	2	2	3	2	2	2	1	4	2	35
ru77	36.9221	-82.2136	4	4	4.0	3	2	2	3	2	2	2	1	4	2	35
ru94	36.9057	-82.2983	4	3	2.0	3	2	2	2	1	3	3	3	3	4	35
p61	36.9092	-82.2372	2	4	3.0	3	2	2	4	3	2	3	1	4	3	36
ri44	36.9283	-82.2105	4	4	4.0	3	4	2	2	2	2	2	1	4	2	36
ru42	36.9462	-82.1529	4	3	2.0	3	2	2	3	2	3	4	4	3	1	36

Table 2.8. Data matrix of habitat units ranking as Excellent for overall relative ranking, including physical habitat parameters and risk potential. Habitat units marked with an * were also scored ≥ 90 % for physical habitat and ≥ 75 % for risk factors.

Hab Unit	LAT	LONG	Hab index	Embed	Riparian	Bank	Braided	Bridge	Rail	AMD	trib weight	PSD	# psd	Cattle	City	Total
ru66	36.9265	-82.1965	4	4	3.0	3	2	2	1	3	3	2	3	4	2	36
ru71	36.9295	-82.2111	4	4	4.0	3	4	2	2	2	2	2	1	4	2	36
ru72	36.9278	-82.2098	4	3	4.0	4	4	2	2	2	2	2	1	4	2	36
ru80	36.9107	-82.2258	4	3	3.0	3	2	2	3	3	2	3	1	4	3	36
ri5	36.9914	-81.9714	4	4	3.5	3	2	2	4	1	3	2	3	1	4	37
ru5	36.9887	-81.9647	4	3	3.0	3	2	2	4	3	2	3	3	1	4	37
p102	36.8103	-82.4843	2	2	4.0	2	4	2	4	4	4	2	2	3	2	37
p103	36.8105	-82.4907	2	3	4.0	3	2	2	4	4	4	2	2	3	2	37
p34	36.9378	-82.1632	2	2	4.0	4	4	2	3	3	3	1	3	4	2	37
p55	36.9149	-82.2176	2	4	4.0	3	4	3	3	3	2	2	1	4	2	37
ri45	36.9236	-82.2145	4	4	4.0	3	4	2	3	2	2	2	1	4	2	37
ri47	36.9150	-82.2200	4	4	4.0	3	4	1	3	3	2	2	1	4	2	37
ru109	36.8883	-82.3579	4	3	4.0	3	2	4	1	2	2	3	3	2	4	37
ru3	36.9916	-81.9570	4	3	3.0	3	2	2	4	3	2	3	3	1	4	37
ru58	36.9373	-82.1709	4	2	4.0	4	2	2	3	3	3	1	3	4	2	37
ru65	36.9262	-82.1946	4	4	3.0	4	2	2	1	3	3	2	3	4	2	37
ru73	36.9279	-82.2095	4	4	4.0	4	4	2	2	2	2	2	1	4	2	37
ru79	36.9148	-82.2188	4	4	4.0	3	4	1	3	3	2	2	1	4	2	37
ru81	36.9077	-82.2284	4	4	3.0	3	2	2	3	3	2	3	1	4	3	37
ru93	36.8956	-82.2892	4	4	3.0	3	4	2	1	1	3	2	3	3	4	37
ri46	36.9180	-82.2116	4	4	3.5	3	4	2	3	3	2	2	1	4	2	38
ri49	36.9082	-82.2342	4	4	3.5	3	2	2	3	3	2	3	1	4	3	38
ru51	36.9526	-82.1320	4	2	3.5	3	2	2	2	1	3	4	4	3	4	38
ru7	36.9818	-81.9773	4	3	3.5	3	2	3	4	2	3	2	3	1	4	38
p0	36.9671	-82.0058	2	3	3.0	2	2	4	4	3	3	4	3	1	4	38
p106	36.8053	-82.4991	2	3	4.0	2	4	2	4	4	4	2	2	3	2	38
p84	36.8934	-82.3679	2	4	4.0	3	2	4	2	2	2	4	3	2	4	38
p87	36.8795	-82.3992	2	3	4.0	4	2	4	2	2	3	2	3	3	4	38
p96	36.8413	-82.4609	2	4	4.0	3	2	4	4	3	1	3	2	2	4	38
p99	36.8218	-82.4698	2	3	4.0	4	4	2	4	4	4	1	2	2	2	38
ri123	36.6471	-82.7580	4	4	3.0	3	2	1	2	2	3	4	2	4	4	38
ri34	36.9381	-82.1626	4	4	4.0	4	2	1	3	3	3	1	3	4	2	38
ri48	36.9151	-82.2200	4	4	4.0	4	4	1	3	3	2	2	1	4	2	38
ri51	36.9070	-82.2363	4	4	3.0	3	2	2	4	3	2	3	1	4	3	38
ri71	36.8761	-82.4067	4	3	4.0	4	2	4	3	2	1	2	2	3	4	38

Table 2.8. Data matrix of habitat units ranking as Excellent for overall relative ranking, including physical habitat parameters and risk potential. Habitat units marked with an * were also scored ≥ 90 % for physical habitat and ≥ 75 % for risk factors.

Hab Unit	LAT	LONG	Hab index	Embed	Riparian	Bank	Braided	Bridge	Rail	AMD	trib weight	PSD	# psd	Cattle	City	Total
ru111	36.8915	-82.3635	4	3	4.0	4	2	4	1	2	2	3	3	2	4	38
ru112	36.8925	-82.3647	4	2	4.0	4	2	4	2	2	2	3	3	2	4	38
ru113	36.8894	-82.3788	4	3	4.0	4	2	4	2	1	3	1	3	3	4	38
ru117	36.8661	-82.4046	4	3	4.0	4	2	4	3	2	1	2	2	3	4	38
ru169	36.6486	-82.7510	4	3	4.0	3	2	1	2	2	3	4	2	4	4	38
ru46	36.9381	-82.1626	4	3	4.0	4	4	1	3	3	3	1	3	3	2	38
ru6	36.9905	-81.9738	4	4	4.0	4	2	2	4	1	3	2	3	1	4	38
ru78	36.9149	-82.2176	4	4	3.0	3	4	3	3	3	2	2	1	4	2	38
ru85	36.9054	-82.2359	4	4	3.0	3	2	2	4	3	2	3	1	4	3	38
ri50	36.9141	-82.2382	4	4	3.5	4	2	2	3	3	2	3	1	4	3	39
ri90	36.8274	-82.4627	4	4	3.5	4	2	1	4	4	4	3	2	2	1	39
ru82	36.9136	-82.2391	4	4	3.5	4	2	2	3	3	2	3	1	4	3	39
p31	36.9448	-82.1477	2	3	2.0	3	4	2	3	2	3	4	4	3	4	39
p64	36.9026	-82.2439	2	4	3.0	3	2	2	4	4	4	3	1	4	3	39
ri30	36.9556	-82.1283	4	3	3.0	3	4	2	2	1	3	4	4	2	4	39
ri37	36.9372	-82.1702	4	4	4.0	4	2	2	3	3	3	1	3	4	2	39
ri4	36.9928	-81.9535	4	4	3.0	4	2	2	4	3	2	3	3	1	4	39
ri40	36.9306	-82.1823	4	4	3.0	3	2	2	4	3	3	2	3	4	2	39
ri55	36.9046	-82.3026	4	4	4.0	4	4	2	2	1	3	2	2	3	4	39
ri61	36.8937	-82.3394	4	3	3.0	3	4	3	3	3	3	3	3	1	3	39
ri66	36.8884	-82.3605	4	4	4.0	4	2	4	1	2	2	3	3	2	4	39
ru106	36.8946	-82.3585	4	3	4.0	3	2	4	4	1	2	3	3	2	4	39
ru108	36.8915	-82.3589	4	2	4.0	3	2	4	4	2	2	3	3	2	4	39
ru126	36.8293	-82.4618	4	3	3.0	4	4	1	4	4	4	3	2	2	1	39
ru132	36.8092	-82.4954	4	3	4.0	3	2	2	4	4	4	2	2	3	2	39
ru172	36.6438	-82.7664	4	3	3.0	3	2	2	2	3	3	4	2	4	4	39
ru49	36.9549	-82.1289	4	4	3.0	3	2	2	2	1	3	4	4	3	4	39
ru56	36.9372	-82.1671	4	4	3.0	3	4	2	3	3	3	1	3	4	2	39
ru60	36.9343	-82.1806	4	4	4.0	3	2	2	3	3	3	2	3	4	2	39
ri3	36.9951	-81.9540	4	4	2.5	4	4	2	4	3	2	2	3	1	4	40
ri39	36.9314	-82.1823	4	4	3.5	3	2	2	4	3	3	2	3	4	2	40
ri6	36.9811	-81.9820	4	4	3.5	4	2	3	4	2	3	2	3	1	4	40
ri93	36.8189	-82.4753	4	4	3.5	2	4	2	4	4	4	1	2	3	2	40
ru59	36.9353	-82.1781	4	4	3.5	4	2	2	3	3	3	2	3	4	2	40
ru61	36.9310	-82.1820	4	4	3.5	3	2	2	4	3	3	1	2	4	4	40

Table 2.8. Data matrix of habitat units ranking as Excellent for overall relative ranking, including physical habitat parameters and risk potential. Habitat units marked with an * were also scored ≥ 90 % for physical habitat and ≥ 75 % for risk factors.

Hab Unit	LAT	LONG	Hab index	Embed	Riparian	Bank	Braided	Bridge	Rail	AMD	trib weight	PSD	# psd	Cattle	City	Total
ru62	36.9312	-82.1820	4	4	3.5	3	2	2	4	3	3	2	3	4	2	40
ru63	36.9291	-82.1833	4	4	3.5	3	2	2	4	3	3	2	3	4	2	40
ru8	36.9793	-81.9853	4	4	3.5	3	2	3	4	2	3	3	3	1	4	40
ri114	36.7038	-82.6505	4	3	4.0	2	2	1	2	3	3	4	4	4	4	40
ri118	36.6627	-82.7515	4	3	4.0	3	2	4	2	2	3	3	2	4	4	40
ri29	36.9572	-82.1244	4	3	3.0	3	2	1	2	4	4	4	4	2	4	40
ri33	36.9444	-82.1484	4	3	3.0	3	2	2	3	2	3	4	4	3	4	40
ri35	36.9371	-82.1643	4	4	4.0	3	4	2	3	3	3	1	3	4	2	40
ri36	36.9370	-82.1658	4	4	4.0	3	4	2	3	3	3	1	3	4	2	40
ri59	36.8951	-82.3294	4	4	3.0	4	4	3	3	3	3	2	2	2	3	40
ri60	36.8938	-82.3288	4	4	3.0	4	2	3	3	3	3	2	2	4	3	40
ri68	36.8859	-82.3867	4	3	4.0	4	2	4	2	2	3	2	3	3	4	40
ri69	36.8835	-82.3905	4	3	4.0	4	2	4	2	2	3	2	3	3	4	40
ri7	36.9820	-81.9765	4	4	4.0	4	2	3	4	2	3	2	3	1	4	40
ri8	36.9823	-81.9932	4	3	4.0	4	2	3	4	2	3	3	3	1	4	40
ri89	36.8284	-82.4616	4	4	3.0	4	4	1	4	4	4	3	2	2	1	40
ri91	36.8216	-82.4698	4	3	4.0	4	4	2	4	4	4	1	2	2	2	40
ru104	36.8916	-82.3398	4	3	3.0	4	4	3	3	3	3	3	3	1	3	40
ru107	36.8944	-82.3595	4	3	4.0	4	2	4	4	1	2	3	3	2	4	40
ru114	36.8851	-82.3904	4	3	4.0	4	2	4	2	2	3	2	3	3	4	40
ru116	36.8781	-82.4020	4	2	4.0	4	2	4	3	2	3	2	3	3	4	40
ru12	36.9645	-82.0112	4	3	4.0	4	2	1	4	3	3	4	3	1	4	40
ru125	36.8293	-82.4618	4	3	4.0	4	4	1	4	4	4	3	2	2	1	40
ru131	36.8101	-82.4883	4	4	4.0	3	2	2	4	4	4	2	2	3	2	40
ru133	36.8070	-82.4976	4	3	4.0	2	4	2	4	4	4	2	2	3	2	40
ru134	36.8067	-82.4976	4	2	4.0	3	4	2	4	4	4	2	2	3	2	40
ru148	36.7477	-82.6219	4	3	3.0	2	2	2	4	1	3	4	4	4	4	40
ru170	36.6480	-82.7541	4	2	4.0	3	4	1	2	2	3	4	3	4	4	40
ru171	36.6480	-82.7541	4	3	4.0	3	4	1	2	2	3	4	2	4	4	40
ru50	36.9375	-82.1626	4	4	4.0	4	4	1	3	3	3	1	3	4	2	40
ru54	36.9370	-82.1649	4	4	4.0	3	4	2	3	3	3	1	3	4	2	40
ru64	36.9277	-82.1916	4	3	4.0	4	2	2	4	3	3	2	3	4	2	40
p66	36.9037	-82.2619	2	4	3.5	2	2	3	4	4	4	4	3	1	4	41
ri117	36.6707	-82.7498	4	4	3.5	3	4	4	1	1	3	3	2	4	4	41
ri31	36.9534	-82.1394	4	4	3.5	3	2	2	2	2	3	4	4	3	4	41

Table 2.8. Data matrix of habitat units ranking as Excellent for overall relative ranking, including physical habitat parameters and risk potential. Habitat units marked with an * were also scored ≥ 90 % for physical habitat and ≥ 75 % for risk factors.

Hab Unit	LAT	LONG	Hab index	Embed	Riparian	Bank	Braided	Bridge	Rail	AMD	trib weight	PSD	# psd	Cattle	City	Total
ri94	36.8102	-82.4834	4	3	4.0	3	4	2	4	4	4	2	2	3	2	41
ru128	36.8188	-82.4758	4	4	3.5	3	4	2	4	4	4	1	2	3	2	41
ru90	36.9024	-82.2607	4	4	3.5	3	4	3	4	4	4	4	1	1	1	41
p126	36.7310	-82.6212	2	4	3.0	2	2	3	4	2	3	4	4	4	4	41
p131	36.7027	-82.6574	2	4	4.0	2	2	2	3	3	3	4	4	4	4	41
p67	36.8960	-82.2673	2	4	3.0	3	2	3	4	4	4	4	2	2	4	41
ri113	36.7076	-82.6508	4	3	4.0	3	2	1	2	3	3	4	4	4	4	41
ri125	36.6216	-82.8181	4	3	4.0	2	4	3	3	4	4	4	4	4	1	41
ri38	36.9369	-82.1756	4	4	4.0	3	4	2	3	3	3	2	3	4	2	41
ri57	36.8951	-82.3294	4	4	4.0	4	4	3	3	3	3	2	2	2	3	41
ri58	36.8951	-82.3294	4	4	3.0	3	4	3	3	3	3	2	2	4	3	41
ri62	36.8956	-82.3404	4	4	4.0	3	2	3	3	4	4	3	3	1	3	41
ri65	36.8925	-82.3600	4	3	4.0	4	2	4	4	2	2	3	3	2	4	41
ri72	36.8645	-82.4051	4	3	4.0	4	4	4	3	2	1	2	2	4	4	41
ri73	36.8612	-82.4077	4	4	4.0	4	2	4	3	2	1	3	2	4	4	41
ri74	36.8598	-82.4116	4	4	4.0	4	2	4	3	2	1	3	2	4	4	41
ri85	36.8420	-82.4589	4	4	4.0	4	2	4	4	3	1	3	2	2	4	41
ri88	36.8285	-82.4620	4	4	4.0	4	4	1	4	4	4	3	2	2	1	41
ri97	36.8035	-82.5037	4	4	4.0	3	2	3	4	4	4	2	2	3	2	41
ru103	36.8957	-82.3293	4	4	3.0	4	4	3	3	3	3	2	2	4	2	41
ru105	36.8937	-82.3394	4	3	4.0	4	4	3	3	3	3	3	3	1	3	41
ru110	36.8892	-82.3619	4	4	4.0	4	4	4	1	2	2	3	3	2	4	41
ru118	36.8644	-82.4048	4	3	4.0	4	4	4	3	2	1	2	2	4	4	41
ru119	36.8605	-82.4092	4	4	4.0	4	2	4	3	2	1	3	2	4	4	41
ru127	36.8212	-82.4687	4	4	4.0	4	4	2	4	4	4	1	2	2	2	41
ru130	36.8101	-82.4833	4	3	4.0	3	4	2	4	4	4	2	2	3	2	41
ru156	36.7069	-82.6496	4	3	4.0	3	2	1	2	3	3	4	4	4	4	41
ru163	36.6737	-82.7456	4	2	4.0	3	4	3	4	1	3	3	2	4	4	41
ru167	36.6621	-82.7508	4	3	4.0	3	2	4	2	2	3	4	2	4	4	41
ru39	36.9448	-82.1492	4	4	3.0	3	2	2	3	2	3	4	4	3	4	41
ru4	36.9956	-81.9606	4	3	4.0	4	4	2	4	3	2	3	3	1	4	41
p125	36.7373	-82.6199	2	3	3.5	3	2	3	4	2	3	4	4	4	4	42
p97	36.8311	-82.4622	2	4	3.5	4	2	4	4	4	4	3	3	2	2	42
ru153	36.7234	-82.6404	4	3	3.5	3	2	3	1	3	3	4	4	4	4	42
ru164*	36.6738	-82.7457	4	3	3.5	3	4	3	4	1	3	3	2	4	4	42

Table 2.8. Data matrix of habitat units ranking as Excellent for overall relative ranking, including physical habitat parameters and risk potential. Habitat units marked with an * were also scored ≥ 90 % for physical habitat and ≥ 75 % for risk factors.

Hab Unit	LAT	LONG	Hab index	Embed	Riparian	Bank	Braided	Bridge	Rail	AMD	trib weight	PSD	# psd	Cattle	City	Total
ru165	36.6731	-82.7475	4	4	3.5	3	2	4	4	1	3	3	2	4	4	42
ru53*	36.9530	-82.1408	4	4	3.5	4	2	2	2	2	3	4	4	3	4	42
ru88	36.8973	-82.2568	4	4	3.5	3	2	3	4	4	4	4	1	1	4	42
p129	36.7082	-82.6518	2	3	4.0	3	2	4	2	3	3	4	4	4	4	42
ri119	36.6572	-82.7463	4	3	4.0	3	2	4	2	2	3	4	3	4	4	42
ri120	36.6506	-82.7485	4	3	4.0	2	4	4	2	2	3	4	2	4	4	42
ri63	36.8947	-82.3584	4	4	4.0	3	4	4	4	1	2	3	3	2	4	42
ri64	36.8948	-82.3587	4	4	4.0	3	4	4	4	1	2	3	3	2	4	42
ri70	36.8784	-82.4011	4	3	4.0	4	4	4	2	2	3	2	3	3	4	42
ri75*	36.8616	-82.4157	4	4	4.0	4	2	4	3	2	1	3	3	4	4	42
ri92	36.8211	-82.4703	4	4	4.0	4	4	2	4	4	4	1	2	3	2	42
ri95	36.8101	-82.4873	4	4	4.0	3	4	2	4	4	4	2	2	3	2	42
ru120*	36.8614	-82.4146	4	4	4.0	4	2	4	3	2	1	3	3	4	4	42
ru129	36.8096	-82.4872	4	4	4.0	3	4	2	4	4	4	2	2	3	2	42
ru149	36.7450	-82.6206	4	3	3.0	3	2	3	4	1	3	4	4	4	4	42
ru152	36.7331	-82.6209	4	3	3.0	2	2	3	4	2	3	4	4	4	4	42
ru173	36.6282	-82.8046	4	3	2.0	3	2	3	3	3	3	4	4	4	4	42
ru45	36.9628	-82.1209	4	3	2.0	2	4	3	2	4	4	4	4	2	4	42
ru52	36.9378	-82.1622	4	4	4.0	4	4	1	3	2	3	4	4	3	2	42
ru55	36.9452	-82.1463	4	4	3.0	3	2	2	3	2	3	4	4	4	4	42
ru57	36.9446	-82.1474	4	3	3.0	2	4	2	3	2	3	4	4	4	4	42
p26	36.9591	-82.1211	2	4	3.5	4	2	1	2	4	4	4	4	4	4	43
ri99*	36.7996	-82.5185	4	3	3.5	3	4	3	4	4	4	3	3	3	1	43
ru10*	36.9711	-82.0028	4	4	3.5	4	2	4	4	2	3	3	3	2	4	43
ru47*	36.9603	-82.1198	4	4	3.5	4	2	1	2	4	4	4	4	2	4	43
ri121*	36.6497	-82.7486	4	3	4.0	3	4	4	2	2	3	4	2	4	4	43
ri32*	36.9452	-82.1463	4	4	2.0	4	4	2	3	2	3	4	4	3	4	43
ri52*	36.9027	-82.2433	4	4	3.0	3	4	2	4	4	4	3	1	4	3	43
ri78*	36.8520	-82.4520	4	4	4.0	4	2	4	4	2	1	3	3	4	4	43
ri84*	36.8447	-82.4500	4	4	4.0	4	2	4	4	3	1	4	3	2	4	43
ri96	36.8095	-82.4872	4	4	4.0	4	4	2	4	4	4	2	2	3	2	43
ru1*	36.9966	-81.9438	4	4	4.0	4	2	4	4	2	2	2	3	4	4	43
ru11	36.9740	-82.0063	4	3	4.0	4	2	4	4	3	3	3	3	2	4	43
ru123*	36.8521	-82.4321	4	4	4.0	4	2	4	4	2	1	3	3	4	4	43
ru136	36.7960	-82.5331	4	3	4.0	2	2	3	4	4	4	3	3	4	3	43

Table 2.8. Data matrix of habitat units ranking as Excellent for overall relative ranking, including physical habitat parameters and risk potential. Habitat units marked with an * were also scored ≥ 90 % for physical habitat and ≥ 75 % for risk factors.

Hab Unit	LAT	LONG	Hab index	Embed	Riparian	Bank	Braided	Bridge	Rail	AMD	trib weight	PSD	# psd	Cattle	City	Total
ru157	36.7029	-82.6551	4	3	4.0	3	2	2	3	3	3	4	4	4	4	43
ru177	36.6190	-82.8333	4	3	4.0	2	2	3	4	4	4	4	4	4	4	43
ru43	36.9634	-82.1207	4	3	3.0	4	2	3	2	4	4	4	4	2	4	43
p119	36.7581	-82.5963	2	3	3.5	1	4	2	4	4	4	4	4	4	4	44
p133	36.6996	-82.6731	2	4	4.0	3	2	2	3	4	4	4	4	4	4	44
p24	36.9707	-82.1174	2	4	3.5	4	2	3	1	4	4	4	4	4	4	44
ri110	36.7347	-82.6208	4	3	3.5	3	2	3	4	2	3	4	4	4	4	44
ri127	36.6052	-82.8715	4	3	2.5	3	2	4	4	4	4	4	4	4	4	44
ri54*	36.9032	-82.2614	4	4	3.5	3	4	3	4	4	4	4	1	1	4	44
ru151	36.7400	-82.6200	4	3	3.5	3	2	3	4	2	3	4	4	4	4	44
ru175	36.6207	-82.8194	4	3	3.5	3	2	3	4	4	4	4	4	4	4	44
ru40	36.9695	-82.1165	4	2	3.5	4	2	3	1	4	4	4	4	4	4	44
ru89*	36.9024	-82.2607	4	4	3.5	3	4	3	4	4	4	4	1	1	4	44
ri122*	36.6496	-82.7485	4	4	4.0	3	4	4	2	2	3	4	2	4	4	44
ri126	36.6058	-82.8683	4	3	4.0	2	2	4	4	4	4	4	4	4	4	44
ri14	36.9697	-82.0841	4	4	3.0	3	2	1	4	4	4	4	3	4	4	44
ri18	36.9707	-82.0889	4	3	3.0	3	4	1	4	4	4	4	3	3	4	44
ri76*	36.8533	-82.4291	4	4	4.0	4	4	4	3	2	1	3	3	4	4	44
ri77*	36.8539	-82.4296	4	4	4.0	4	4	4	3	2	1	3	3	4	4	44
ri79*	36.8425	-82.4333	4	4	4.0	4	2	4	4	3	1	3	3	4	4	44
ri80*	36.8421	-82.4354	4	4	4.0	4	2	4	4	3	1	3	3	4	4	44
ri86	36.8387	-82.4644	4	4	3.0	4	2	4	4	4	4	3	2	2	4	44
ri98*	36.8043	-82.5085	4	4	4.0	3	4	3	4	4	4	2	2	4	2	44
ru121*	36.8539	-82.4267	4	4	4.0	4	4	4	3	2	1	3	3	4	4	44
ru122*	36.8539	-82.4267	4	4	4.0	4	4	4	3	2	1	3	3	4	4	44
ru124	36.8374	-82.4643	4	4	3.0	4	2	4	4	4	4	3	2	2	4	44
ru158	36.7005	-82.6643	4	3	4.0	2	2	2	3	4	4	4	4	4	4	44
ru180	36.6044	-82.8592	4	2	2.0	2	4	4	4	4	4	4	4	4	4	44
ru31	36.9704	-82.0899	4	4	3.0	3	2	1	4	4	4	4	3	4	4	44
ru86*	36.9031	-82.2407	4	4	4.0	3	4	2	4	4	4	3	1	4	3	44
ru87*	36.9031	-82.2407	4	4	4.0	3	4	2	4	4	4	3	1	4	3	44
ru91*	36.8968	-82.2668	4	4	3.0	3	4	3	4	4	4	4	1	2	4	44
ri111	36.7217	-82.6434	4	4	3.5	3	2	4	2	3	3	4	4	4	4	45
ru138	36.7649	-82.5854	4	4	3.5	3	2	1	4	4	4	4	3	4	4	45
ru145	36.7556	-82.6005	4	2	3.5	3	2	2	4	4	4	4	4	4	4	45

Table 2.8. Data matrix of habitat units ranking as Excellent for overall relative ranking, including physical habitat parameters and risk potential. Habitat units marked with an * were also scored ≥ 90 % for physical habitat and ≥ 75 % for risk factors.

Hab Unit	LAT	LONG	Hab index	Embed	Riparian	Bank	Braided	Bridge	Rail	AMD	trib weight	PSD	# psd	Cattle	City	Total
ru147	36.7520	-82.6071	4	3	3.5	2	2	2	4	4	4	4	4	4	4	45
ru154	36.7197	-82.6413	4	4	3.5	3	2	4	2	3	3	4	4	4	4	45
ru160	36.6937	-82.6871	4	4	3.5	2	2	2	3	4	4	4	4	4	4	45
ri104	36.7639	-82.5864	4	3	4.0	4	2	1	4	4	4	4	3	4	4	45
ri115	36.6991	-82.6711	4	4	4.0	2	2	2	3	4	4	4	4	4	4	45
ri124	36.6216	-82.8181	4	3	4.0	3	4	3	3	4	4	4	4	4	4	45
ri27*	36.9658	-82.1215	4	4	4.0	3	4	3	2	4	4	4	4	1	4	45
ri28*	36.9626	-82.1204	4	4	4.0	4	4	1	2	4	4	4	4	2	4	45
ri83*	36.8500	-82.4426	4	4	4.0	4	2	4	4	3	1	4	3	4	4	45
ri87*	36.8356	-82.4642	4	4	4.0	4	2	4	4	4	4	3	2	2	4	45
ru181	36.6080	-82.8700	4	3	4.0	3	2	4	4	4	4	4	4	4	4	45
ru35	36.9562	-82.1074	4	4	2.0	3	2	2	4	4	4	4	4	4	4	45
ri105	36.7618	-82.5905	4	2	3.5	3	4	1	4	4	4	4	4	4	4	46
ri112*	36.7087	-82.6540	4	3	3.5	3	4	4	2	3	3	4	4	4	4	46
ri116	36.6927	-82.6898	4	4	3.5	3	2	2	3	4	4	4	4	4	4	46
ri26*	36.9715	-82.1187	4	4	3.5	4	2	3	1	4	4	4	4	4	4	46
ru139	36.7620	-82.5915	4	2	3.5	3	4	1	4	4	4	4	4	4	4	46
ru146	36.7540	-82.6003	4	3	3.5	3	2	2	4	4	4	4	4	4	4	46
ru28	36.9645	-82.0762	4	3	3.5	2	2	4	4	4	4	4	3	4	4	46
ru33	36.9546	-82.1016	4	3	3.5	3	2	2	4	4	4	4	4	4	4	46
ri102*	36.7665	-82.5806	4	3	4.0	3	4	4	4	4	4	4	3	3	2	46
ri107	36.7618	-82.5905	4	2	4.0	3	4	1	4	4	4	4	4	4	4	46
ri108	36.7612	-82.5916	4	3	3.0	3	4	1	4	4	4	4	4	4	4	46
ri109	36.7614	-82.5904	4	2	4.0	3	4	1	4	4	4	4	4	4	4	46
ri13*	36.9681	-82.0802	4	4	4.0	4	2	1	4	4	4	4	3	4	4	46
ri23	36.9556	-82.1074	4	4	3.0	3	2	2	4	4	4	4	4	4	4	46
ru135*	36.7996	-82.5185	4	3	4.0	3	4	3	4	4	4	3	3	4	3	46
ru155*	36.7087	-82.6540	4	3	4.0	3	4	4	2	3	3	4	4	4	4	46
ru159	36.6967	-82.6791	4	4	4.0	3	2	2	3	4	4	4	4	4	4	46
ru179	36.6044	-82.8592	4	2	3.0	3	4	4	4	4	4	4	4	4	4	46
ru32	36.9580	-82.1000	4	3	4.0	3	2	2	4	4	4	4	4	4	4	46
ri100*	36.7965	-82.5228	4	4	3.5	3	4	3	4	4	4	3	3	4	3	47
ru144	36.7578	-82.5980	4	3	3.5	2	4	2	4	4	4	4	4	4	4	47
ru161	36.6895	-82.7030	4	4	3.5	2	2	3	4	4	4	4	4	4	4	47
ri106*	36.7618	-82.5922	4	3	4.0	3	4	1	4	4	4	4	4	4	4	47

Table 2.8. Data matrix of habitat units ranking as Excellent for overall relative ranking, including physical habitat parameters and risk potential. Habitat units marked with an * were also scored ≥ 90 % for physical habitat and ≥ 75 % for risk factors.

Hab Unit	LAT	LONG	Hab index	Embed	Riparian	Bank	Braided	Bridge	Rail	AMD	trib weight	PSD	# psd	Cattle	City	Total
ri15*	36.9699	-82.0854	4	4	3.0	4	4	1	4	4	4	4	3	4	4	47
ri16*	36.9699	-82.0854	4	4	4.0	3	4	1	4	4	4	4	3	4	4	47
ri17*	36.9707	-82.0889	4	3	4.0	4	4	1	4	4	4	4	3	4	4	47
ri24	36.9570	-82.1076	4	4	3.0	4	2	2	4	4	4	4	4	4	4	47
ru143	36.7589	-82.5942	4	3	4.0	2	4	2	4	4	4	4	4	4	4	47
ru162	36.6760	-82.7413	4	3	4.0	3	2	3	4	4	4	4	4	4	4	47
ru174	36.6260	-82.8150	4	3	4.0	4	2	3	3	4	4	4	4	4	4	47
ru19	36.9710	-82.0499	4	3	4.0	4	2	3	4	4	4	4	4	3	4	47
ru20	36.9682	-82.0494	4	2	4.0	4	2	3	4	4	4	4	4	4	4	47
ru37	36.9662	-82.1137	4	4	2.0	4	2	3	4	4	4	4	4	4	4	47
ri22*	36.9559	-82.1015	4	4	3.5	4	2	2	4	4	4	4	4	4	4	48
ru34*	36.9534	-82.1081	4	4	3.5	4	2	2	4	4	4	4	4	4	4	48
p115	36.7665	-82.5806	2	3	4.0	4	4	4	4	4	4	4	3	4	4	48
ri101	36.7689	-82.5773	4	4	4.0	3	2	4	4	4	4	4	3	4	4	48
ri11*	36.9641	-82.0756	4	4	3.0	3	4	4	4	4	4	4	3	3	4	48
ri20*	36.9604	-82.0967	4	3	4.0	4	4	2	4	4	4	4	3	4	4	48
ri25*	36.9672	-82.1152	4	4	4.0	4	4	3	1	4	4	4	4	4	4	48
ri9*	36.9633	-82.0749	4	4	3.0	3	4	4	4	4	4	4	3	3	4	48
ru140*	36.7612	-82.5916	4	4	4.0	3	4	1	4	4	4	4	4	4	4	48
ru141*	36.7597	-82.5927	4	3	4.0	3	4	2	4	4	4	4	4	4	4	48
ru142*	36.7588	-82.5942	4	3	4.0	3	4	2	4	4	4	4	4	4	4	48
ru18*	36.9641	-82.0441	4	4	4.0	4	2	3	4	4	4	4	4	3	4	48
ru21*	36.9668	-82.0533	4	4	4.0	4	2	4	4	4	4	3	3	4	4	48
ru38	36.9664	-82.1141	4	2	3.0	4	4	3	4	4	4	4	4	4	4	48
ri21*	36.9604	-82.0967	4	4	4.0	4	4	2	4	4	4	4	3	4	4	49
ru22*	36.9681	-82.0532	4	3	4.0	4	4	4	4	4	4	3	3	4	4	49
ru29*	36.9653	-82.0776	4	4	4.0	4	2	4	4	4	4	4	3	4	4	49
ri10*	36.9633	-82.0749	4	3	4.0	4	4	4	4	4	4	4	3	4	4	50
ri103*	36.7658	-82.5818	4	3	4.0	4	4	4	4	4	4	4	3	4	4	50
ri12*	36.9641	-82.0756	4	3	4.0	4	4	4	4	4	4	4	3	4	4	50
ru23*	36.9681	-82.0532	4	4	4.0	4	4	4	4	4	4	3	3	4	4	50
ru26*	36.9618	-82.0732	4	3	4.0	4	4	4	4	4	4	4	3	4	4	50
ru137*	36.7697	-82.5765	4	4	4.0	4	4	4	4	4	4	4	3	4	4	51

Table 2.9. Data matrix of habitat units ranking as Poor for overall relative ranking, including physical habitat parameters and risk potential.

Hab Unit	LAT	LONG	Hab index	Embed	Riparian	Bank	Braided	Bridge	Rail	AMD	trib weight	PSD	#psd	Cattle	City	Total
p70	36.9018	-82.3169	2	3	2.0	4	2	1	1	2	3	2	2	3	1	28
ru68	36.9346	-82.2003	4	4	1.3	3	2	1	2	1	2	1	1	4	2	28
p48	36.9246	-82.1996	2	4	1.0	3	2	2	2	2	2	1	1	4	3	29
p47	36.9266	-82.2003	2	4	1.3	3	2	2	2	2	2	1	1	4	3	29
p49	36.9225	-82.2063	2	4	4.0	3	2	2	2	2	2	1	1	4	1	30
ru96	36.9030	-82.3076	4	3	1.0	3	2	2	2	2	3	2	2	3	1	30
p51	36.9286	-82.2137	2	4	3.0	3	2	2	2	2	2	2	1	4	2	31
p52	36.9277	-82.2150	2	4	3.0	3	2	2	2	2	2	2	1	4	2	31
ri42	36.9289	-82.2010	4	4	1.3	4	2	1	2	2	2	1	1	4	3	31
ru69	36.9296	-82.2011	4	4	1.3	4	2	1	2	2	2	1	1	4	3	31
p72	36.8903	-82.3178	2	4	4.0	3	2	2	2	2	3	2	1	3	2	32
ru70	36.9220	-82.2048	4	4	4.0	3	2	2	2	2	2	1	1	4	1	32
ri41	36.9345	-82.1971	4	4	1.0	3	2	3	2	1	2	2	3	4	2	33
ru67	36.9292	-82.1968	4	3	3.0	3	2	2	1	1	3	2	3	4	2	33
ru75	36.9280	-82.2146	4	4	3.0	3	2	2	2	2	2	2	1	4	2	33
p50	36.9278	-82.2093	2	4	4.0	3	4	2	2	2	2	2	1	4	2	34
p73	36.8894	-82.3219	2	4	3.0	4	2	2	2	3	3	2	1	4	2	34
ru95	36.9046	-82.3052	4	4	2.0	3	2	2	2	1	3	2	2	3	4	34
ru92	36.8934	-82.2883	4	4	3.5	3	2	2	1	1	3	2	3	2	4	35
p3	36.9927	-81.9535	2	3	3.0	3	2	2	4	3	2	3	3	1	4	35
p33	36.9435	-82.1550	2	4	2.0	3	2	2	3	2	3	4	4	3	1	35
p7	36.9826	-81.9749	2	3	3.0	3	2	3	4	2	3	2	3	1	4	35
p76	36.8989	-82.3260	2	4	3.0	4	2	3	2	3	3	2	1	4	2	35
p98	36.8256	-82.4633	2	3	3.0	3	2	1	4	4	4	3	3	2	1	35
ri56	36.9005	-82.3179	4	3	4.0	4	2	2	2	2	3	3	2	3	1	35
ru101	36.8931	-82.3240	4	3	3.0	4	2	2	2	3	3	2	1	4	2	35
ru44	36.9381	-82.1626	4	4	3.0	3	4	1	3	3	3	1	3	1	2	35
ru48	36.9381	-82.1626	4	4	4.0	4	2	1	3	3	3	1	3	1	2	35
ru74	36.9280	-82.2123	4	3	4.0	3	4	2	2	2	2	2	1	4	2	35
ru76	36.9256	-82.2156	4	4	4.0	3	2	2	3	2	2	2	1	4	2	35
ru77	36.9221	-82.2136	4	4	4.0	3	2	2	3	2	2	2	1	4	2	35
ru94	36.9057	-82.2983	4	3	2.0	3	2	2	2	1	3	3	3	3	4	35
p61	36.9092	-82.2372	2	4	3.0	3	2	2	4	3	2	3	1	4	3	36
ri44	36.9283	-82.2105	4	4	4.0	3	4	2	2	2	2	2	1	4	2	36
ru42	36.9462	-82.1529	4	3	2.0	3	2	2	3	2	3	4	4	3	1	36

Table 2.9. Data matrix of habitat units ranking as Poor for overall relative ranking, including physical habitat parameters and risk potential.

Hab Unit	LAT	LONG	Hab index	Embed	Riparian	Bank	Braided	Bridge	Rail	AMD	trib weight	PSD	#psd	Cattle	City	Total
ru66	36.9265	-82.1965	4	4	3.0	3	2	2	1	3	3	2	3	4	2	36
ru71	36.9295	-82.2111	4	4	4.0	3	4	2	2	2	2	2	1	4	2	36
ru72	36.9278	-82.2098	4	3	4.0	4	4	2	2	2	2	2	1	4	2	36
ru80	36.9107	-82.2258	4	3	3.0	3	2	2	3	3	2	3	1	4	3	36
ri5	36.9914	-81.9714	4	4	3.5	3	2	2	4	1	3	2	3	1	4	37
ru5	36.9887	-81.9647	4	3	3.0	3	2	2	4	3	2	3	3	1	4	37
p102	36.8103	-82.4843	2	2	4.0	2	4	2	4	4	4	2	2	3	2	37
p103	36.8105	-82.4907	2	3	4.0	3	2	2	4	4	4	2	2	3	2	37
p34	36.9378	-82.1632	2	2	4.0	4	4	2	3	3	3	1	3	4	2	37
p55	36.9149	-82.2176	2	4	4.0	3	4	3	3	3	2	2	1	4	2	37
ri45	36.9236	-82.2145	4	4	4.0	3	4	2	3	2	2	2	1	4	2	37
ri47	36.9150	-82.2200	4	4	4.0	3	4	1	3	3	2	2	1	4	2	37
ru109	36.8883	-82.3579	4	3	4.0	3	2	4	1	2	2	3	3	2	4	37
ru3	36.9916	-81.9570	4	3	3.0	3	2	2	4	3	2	3	3	1	4	37
ru58	36.9373	-82.1709	4	2	4.0	4	2	2	3	3	3	1	3	4	2	37
ru65	36.9262	-82.1946	4	4	3.0	4	2	2	1	3	3	2	3	4	2	37
ru73	36.9279	-82.2095	4	4	4.0	4	4	2	2	2	2	2	1	4	2	37
ru79	36.9148	-82.2188	4	4	4.0	3	4	1	3	3	2	2	1	4	2	37
ru81	36.9077	-82.2284	4	4	3.0	3	2	2	3	3	2	3	1	4	3	37
ru93	36.8956	-82.2892	4	4	3.0	3	4	2	1	1	3	2	3	3	4	37
ri46	36.9180	-82.2116	4	4	3.5	3	4	2	3	3	2	2	1	4	2	38

Table 2.10. Habitat units with high physical habitat quality (< 90% of optimal), but at low risk for anthropogenic threats (≥75 %). These sites are recommended for potential mussel release sites.

LABEL	LAT	LONG	Low impact (≥ 75 % of best possible score)	Physical habitat (≥ 90 % of best possible score)	Approximate Location
ru1	36.9966	-81.9438	78	90	Blackford
ru10	36.9711	-82.0028	78	90	Puckett Hole
ru18	36.9641	-82.0441	94	90	Pinnacle
ru22	36.9681	-82.0532	94	95	Pinnacle
ru23	36.9681	-82.0532	94	100	Pinnacle
ru21	36.9668	-82.0533	94	90	Pinnacle
ru26	36.9618	-82.0732	97	95	Nash Ford
ri9	36.9633	-82.0749	94	90	Nash Ford
ri10	36.9633	-82.0749	97	95	Nash Ford
ri11	36.9641	-82.0756	94	90	Nash Ford
ri12	36.9641	-82.0756	97	95	Nash Ford
ru29	36.9653	-82.0776	97	90	Nash Ford
ri13	36.9681	-82.0802	88	90	Nash Ford
ri15	36.9699	-82.0854	88	95	Nash Ford
ri16	36.9699	-82.0854	88	95	Nash Ford
ri17	36.9707	-82.0889	88	95	Nash Ford
ri20	36.9604	-82.0967	91	95	Below Nash Ford
ri21	36.9604	-82.0967	91	100	Below Nash Ford
ri22	36.9559	-82.1015	94	90	Below Nash Ford
ru34	36.9534	-82.1081	94	90	Below Nash Ford
ri25	36.9672	-82.1152	88	100	Artrip
ri26	36.9715	-82.1187	88	90	Artrip
ru47	36.9603	-82.1198	78	90	Artrip
ri28	36.9626	-82.1204	78	100	Artrip
ri27	36.9658	-82.1215	81	95	Artrip
ru53	36.9530	-82.1408	75	90	Above Cleveland
ri32	36.9452	-82.1463	78	90	Above Cleveland
ru86	36.9031	-82.2407	78	95	Below Carterton
ru87	36.9031	-82.2407	78	95	Below Carterton
ri52	36.9027	-82.2433	78	90	Below Carterton
ru89	36.9024	-82.2607	78	95	Above Castlewood
ri54	36.9032	-82.2614	78	95	Above Castlewood
ru91	36.8968	-82.2668	81	90	Above Castlewood
ru120	36.8614	-82.4146	75	90	Above Mill Island
ri75	36.8616	-82.4157	75	90	Above Mill Island
ru121	36.8539	-82.4267	75	100	Above Mill Island
ru122	36.8539	-82.4267	75	100	Above Mill Island
ri76	36.8533	-82.4291	75	100	Above Mill Island
ri77	36.8539	-82.4296	75	100	Above Mill Island
ru123	36.8521	-82.4321	78	90	Above Mill Island

Table 2.10. Habitat units with high physical habitat quality (< 90% of optimal), but at low risk for anthropogenic threats (≥75 %). These sites are recommended for potential mussel release sites.

LABEL	LAT	LONG	Low impact (≥ 75 % of best possible score)	Physical habitat (≥ 90 % of best possible score)	Approximate Location
ri79	36.8425	-82.4333	81	90	Above Mill Island
ri80	36.8421	-82.4354	81	90	Above Mill Island
ri83	36.8500	-82.4426	84	90	Above Mill Island
ri84	36.8447	-82.4500	78	90	Above Mill Island
ri78	36.8520	-82.4520	78	90	Above Mill Island
ri87	36.8356	-82.4642	84	90	Above Mill Island
ri98	36.8043	-82.5085	78	95	Grays Island
ri99	36.7996	-82.5185	78	90	Grays Island
ru135	36.7996	-82.5185	88	90	Grays Island
ri100	36.7965	-82.5228	88	95	Grays Island
ru137	36.7697	-82.5765	97	100	Pendleton Island
ri102	36.7665	-82.5806	88	90	Pendleton Island
ri103	36.7658	-82.5818	97	95	Pendleton Island
ru140	36.7612	-82.5916	91	95	Pendleton Island
ri106	36.7618	-82.5922	91	90	Pendleton Island
ru141	36.7597	-82.5927	94	90	Pendleton Island
ru142	36.7588	-82.5942	94	90	Pendleton Island
ri112	36.7087	-82.6540	88	90	Craft Mill
ru155	36.7087	-82.6540	88	90	Craft Mill
ru164	36.6738	-82.7457	75	90	Clinchport
ri122	36.6496	-82.7485	78	95	Speers Ferry
ri121	36.6497	-82.7486	78	90	Speers Ferry

Literature cited

Ahlstedt, S. A., and J. D. Tuberville. 1999. Quantitative reassessment of the freshwater mussel fauna in the Clinch and Powell Rivers, Tennessee and Virginia. Pages 72-97 in K. S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo, editors. Conservation and Management of Freshwater Mussels II: initiatives for the future. Proceedings of a UMRCC Symposium. Upper Mississippi River Conservation Committee, Rock Island, Illinois.

Angermeier, P. L. and I. J. Schlosser. 1989. Species-area relationship for stream fishes. Ecology 70:1450-1462.

Bender, E. A., T. J. Case, and M. E. Gilpin. 1984. Pertubation experiments in community ecology: theory and practice. Ecology 65(1):1-13.

Bryce, S. A., D. P. Larsen, R. M. Hughes, and P. R. Kaufmann. 1999. Assessing relative risks to aquatic ecosystems: a mid-Appalachian case study. Journal of the American Water Resources Association. 35(1):23-36.

Church, G. W. 1997. Macrohabitat factors affecting distribution patterns of freshwater mussels in the Clinch River (Virginia, Tennessee). Doctoral Dissertation. Virginia Polytechnic Institute and State University, Blacksburg, VA.

Diamond, J. M. and V. B. Serveiss. 2001. Identifying sources of stress to native aquatic fauna using a watershed ecological risk assessment framework. Environmental Science and Technology 35:4711-4718.

Diamond, J. M., D. W. Bressler, and V. B. Serveiss. 2002. Assessing relationships between human landuses and the decline of native mussels, fish, and macroinvertebrates in the Clinch and Powell river watershed, USA. Environmental Toxicology and Chemistry 21(6):1147-1155.

Dolloff, C. A., D. G. Hankin, and G. H. Reeves. 1993. Basinwide estimation of habitat and fish populations in streams. General Technical Report SE-83. Asheville, North Carolina: U.S. Department of Agriculture, Forest Service, Southeastern Forest Experimental Station.

Environmental Contaminants Specialists in MO, NC, PS and VA. 2001. Developing Water Quality Standards for Recovery of Imperiled Freshwater Mussels (Family Unionidae). Proposal for Environmental Contaminants Programs of Regions 3, 4, and 5. U. S. Fish and Wildlife Service.

Goudreau, S. E., R. J. Neves, and R. J. Sheenan. 1993. Effects of wastewater treatment plant effluents on freshwater mollusks in the upper Clinch River, Virginia, USA. Hydrobiologia 252:211-230.

Hagg, W. R. and M. L Warren. 1998. Role of ecological factors and reproductive strategies in structuring freshwater mussel communities. Canadian Journal of Fisheries and Aquatic Sciences 55:297-306.

Hampson, P. S., M. W. Treece, G. C. Johnson, S. A. Ahlstedt, and J. F. Connell. 2000. Water Quality in the Upper Tennessee River Basin, Tennessee, North Carolina, Virginia, and Georgia 1994-1998. U. S. Geological Survey Circular 1205.

Hankin D. G. and G. H. Reeves. 1988. Estimating total fish abundance and total habitat area in small streams based on visual estimation methods. Canadian Journal of Fisheries and Aquatic Sciences 45:834-844.

Henley, W. F., R. J. Neves, L. L. Zimmerman, R. Winterringer. 1999. A survey of freshwater mussels in the Middle Fork Holston River, Virginia. Banisteria 14:15-24.

Horne, F. and S. McIntosh. 1979. Factors influencing distribution of mussels in the Blanco River of central Texas. The Nautilus 94:119-133.

Johnson, P. D., and K. M. Brown. 2000. The importance of microhabitat factors and habitat stability to the threatened Louisiana pearlshell, *Margaritifera hembeli*. Canadian Journal of Zoology 78:271-277.

Jones, J. W., R. J. Neves, M. A. Patterson, C. R. Good, A. DiVittorio. 2001. A status survey of freshwater mussel populations in the upper Clinch River, Tazewell County, Virginia. Banisteria 17:20-30.

Jones, J. W., R. Mair, and R. J. Neves. 2003. Life history and artificial culture of endangered mussels. Annual Report submitted to Tennessee Wildlife Resources Agency, Nashville, Tennessee.

Karr, J. R. and E. W. Chu 1999. Restoring life in running waters: better biological monitoring. Island Press, Washington D. C.

Keller, A. E., and S. G. Zam. 1991. The acute toxicity of selected metals to the fresh-water mussel, *Anodonta-imbecilis*. Environmental Toxicology and Chemistry 10(4):539-546.

McCann, M. T. 1992. Toxicity of zinc, copper, and sediments to early life stages of freshwater mussels in the Powell River, Virginia. M. S. Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. 143 pp.

McKinney, A. D. and D. C. Wade. 1996. Comparative response of *Ceriodaphnia dubia* and juvenile *Anodonta imbecillis* to pulp and paper mill effluents discharged to the Tennessee River and its tributaries. Environmental Toxicology and Chemistry 15(4):514-517.

Moulton, C. A., W. J. Flemming, and C. E Purnell. 1996. Effects of two cholinesterase-inhibiting pesticides on freshwater mussels. Environmental Toxicology and Chemistry 15(2):131-137.

Nichols, L. E. and F. J. Bulow. 1973. Effects of acid mine drainage on the stream ecosystem of the east fork of the Obey River, Tennessee. Journal of the Tennessee Academy of Science 48:30-39.

Platts, W. D., W. F. Megahan, and G. W. Minshall. 1983. Method for evaluating stream, riparian, and biotic conditions. General Technical Report INT-138. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Research Station.

Richter, B. D., D. P. Braun, M. A. Mendelson, and L. L. Master. 1997. Threats to imperiled freshwater fauna. Conservation Biology 11:1081-1093.

Scott J. M., P. J. Heglund, M. L. Morrison, J. B. Haufler, M. G. Raphael, W. A. Wall, and F. B. Samson. 2002. Predicting Species Occurrences: Issues of Accuracy and Scale. Island Press, Washington D. C.

Stansbery, D. S. 1973. A preliminary report on the naiad fauna of the Clinch River in the southern Appalachian Mountains of Virginia and Tennessee (Mollusca: Bivalvia: Unionoida). Bulletin of the American Malacological Union for 1972:20-22.

Suter, G. W., editor. 1993. Ecological Risk Assessment. Lewis Publishers, Chelsea, Michigan.

Tennessee Valley Authority. 1986. Cumberlandian mollusk conservation program. Activity 9: Selection of Transplant Sites and Habitat Characterization. Office of Natural Resources and Economic Development, Tennessee Valley Authority Knoxville, Tennessee.

U. S. Fish and Wildlife Service. 1980. Habitat Evaluation Procedures (HEP) Ecological Services Manual 102. U. S. Department of Interior Fish and Wildlife Service, Division of Ecological Services. Government Printing Office, Washington D. C.

U. S. Fish and Wildlife Service. 1981. Standards for the Development of Suitability Index Models Ecological Services Manual 102. U. S. Department of Interior Fish and Wildlife Service, Division of Ecological Services. Government Printing Office, Washington D. C.

Vaughn, C. C. and C. M. Taylor. 1999. Impoundments and the decline of freshwater mussels: a case study of an extinction gradient. Conservation Biology 13(4):912-920.

Watters, G. T. 1992. Unionids, fishes, and the species-area curve. Journal of Biogeography 19:481-490.

Wiegers, J. K., H. M. Feder, L. S. Mortensen, D. G. Shaw, V. J. Wilson, and W. G. Landis. 1998. A regional multiple-stressor rank-based ecological risk assessment for the fjord of Port Valdez, Alaska. Human and Ecological Risk Assessment 4(5):1125-1173.

Appendix 2-A. Record sheet for Clinch River BVET survey.

Record Sheet for Clinch River BVET Survey

Substrate	
Fine	< 8mm
Medium	8mm - 30cm
Large	>30cm
bedrock	

Riparian Use
forested (F)
urban (U)
residential (R)
industrial (I)
pasture (P)
row crop (C)

Mussel presence
live (L)
subfossil (S)
recent dead (R)
none (N)

Bank Condition	
Class I	>85 % vegetated
Class II	84-50 % vegetated
Class III	49-25% vegetated
Class IV	24-0% vegetated

Embeddedness	
1	>75%
2	51-75%
3	26-50%
4	5-25%
5	<5%

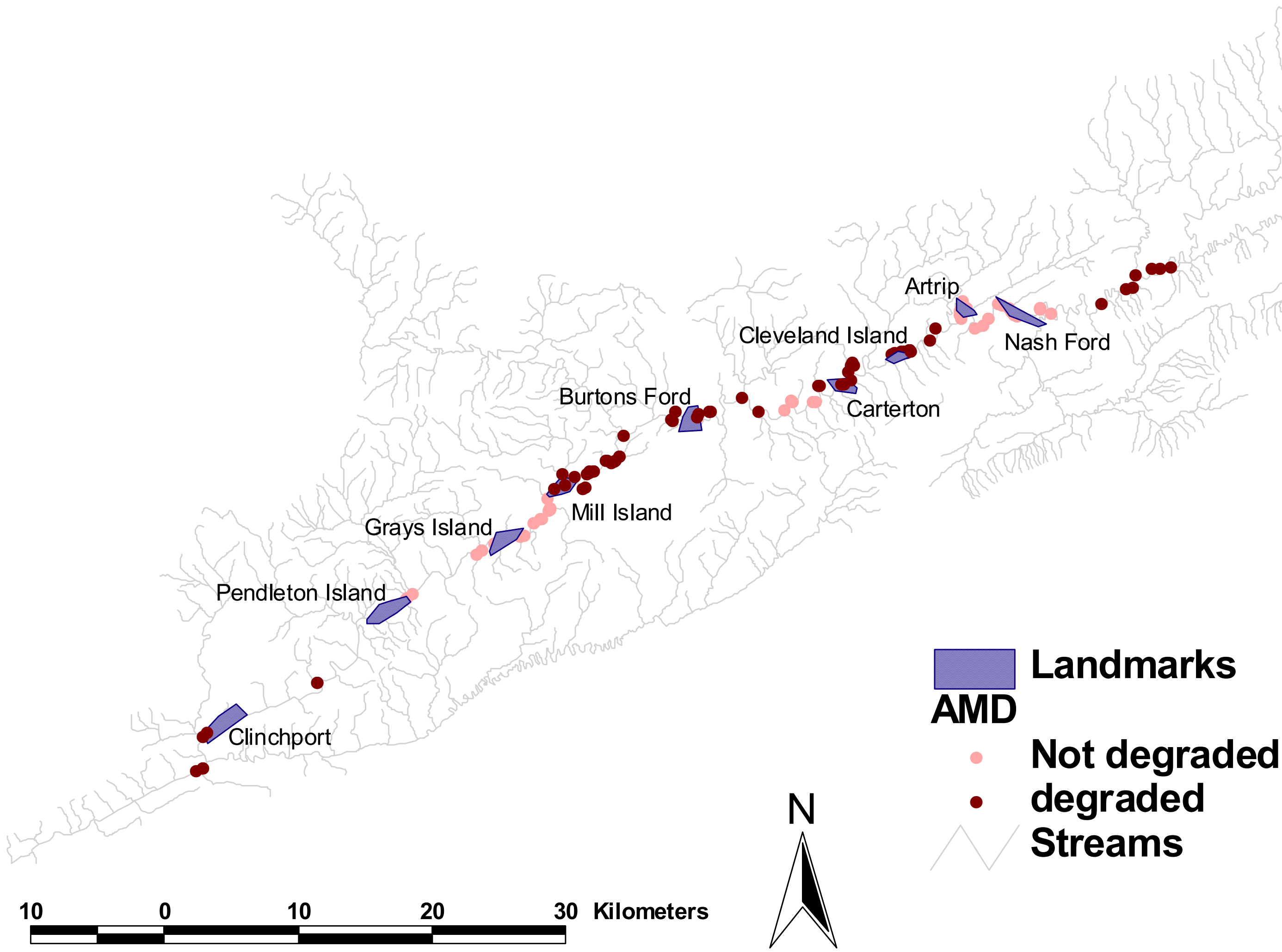
Record for every habitat unit:

Section Number: record new reach where there is a major change in channel type
Unit Type: pool/glide, riffle, or run
Unit Number: consecutive
Distance: (m) at upstream end of unit (measured with rangefinder)
Lat/Long: at upstream end of unit
Estimated Width: (m) average wetted width (measured with rangefinder)
Average Depth: (cm) measured with graduated wading rod
Substrate percentages: fine, med, large, bedrock
Embeddedness: % of stone surface covered with silt (rank on quartiles)
Riparian Use: dominate riparian activity within 30 m of stream
Bank Erosion Potential: quartile ranking of bank area vegetated
Mussels or shells present: live, subfossil, recently dead, none

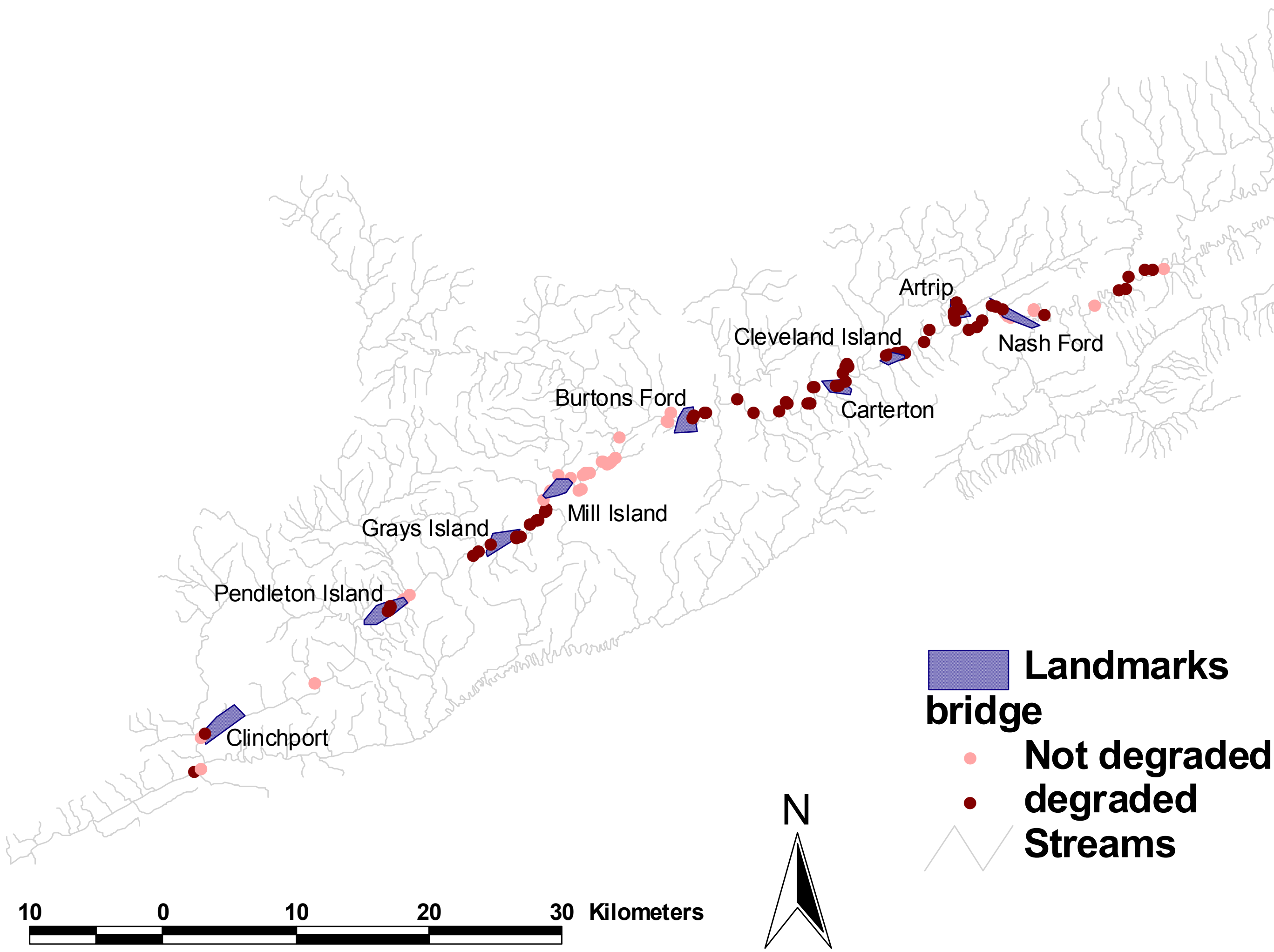
Miscellaneous Features:

Tributary (TRIB) distance, in on L or R
Bridge (BRIDGE) distance, height, width, riparian influence
End of pipe discharge (PIPE) distance, in on L or R
Rock Ledge (LEDGE)
Trash Dump (DUMP) L or R
Presence of coal fines (COAL)

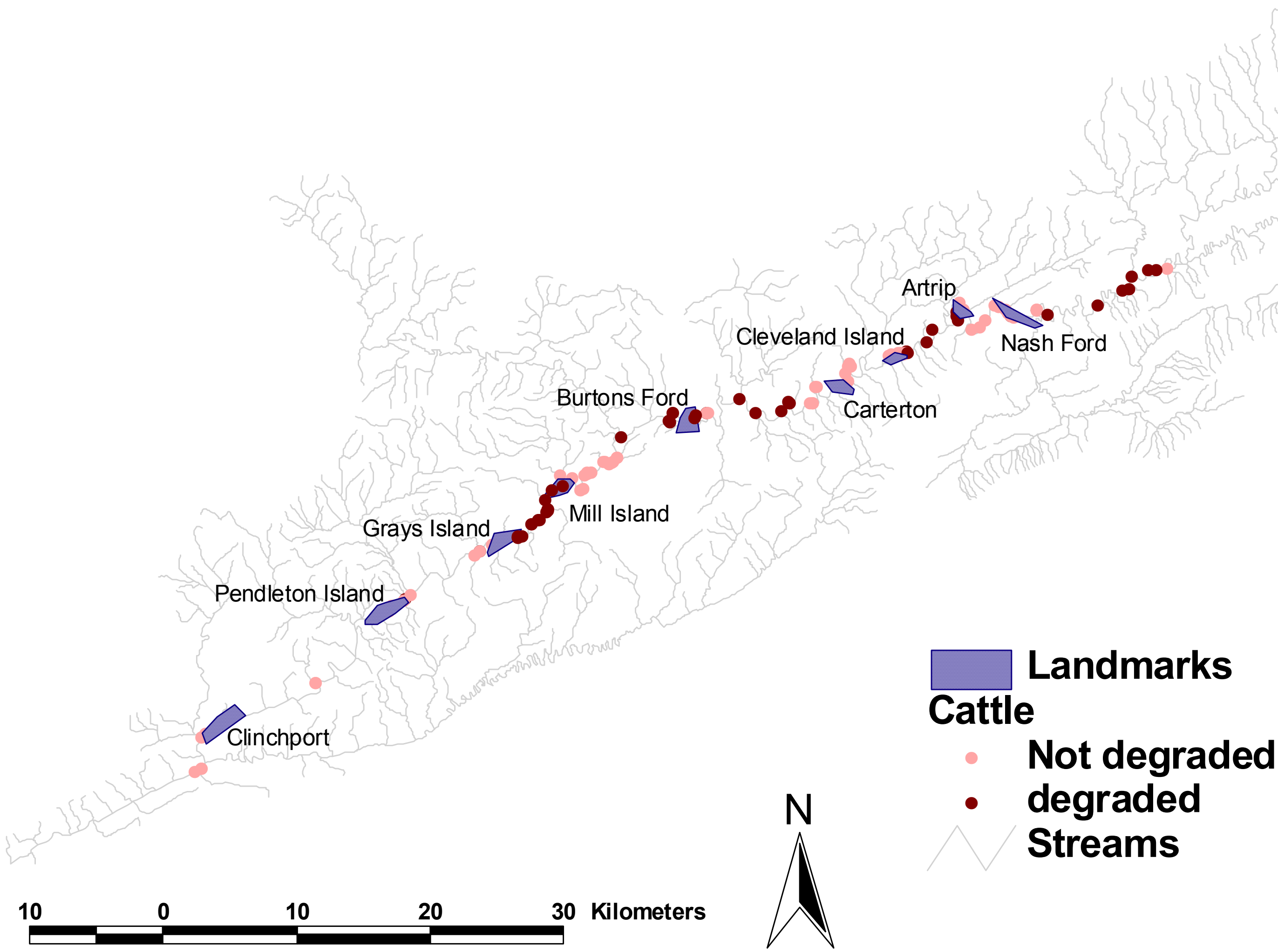
Appendix 2-B. Distribution of habitat units whose suitability percentage was reduced by more than 1 % due to proximity to potential acid mine drainage impacts.



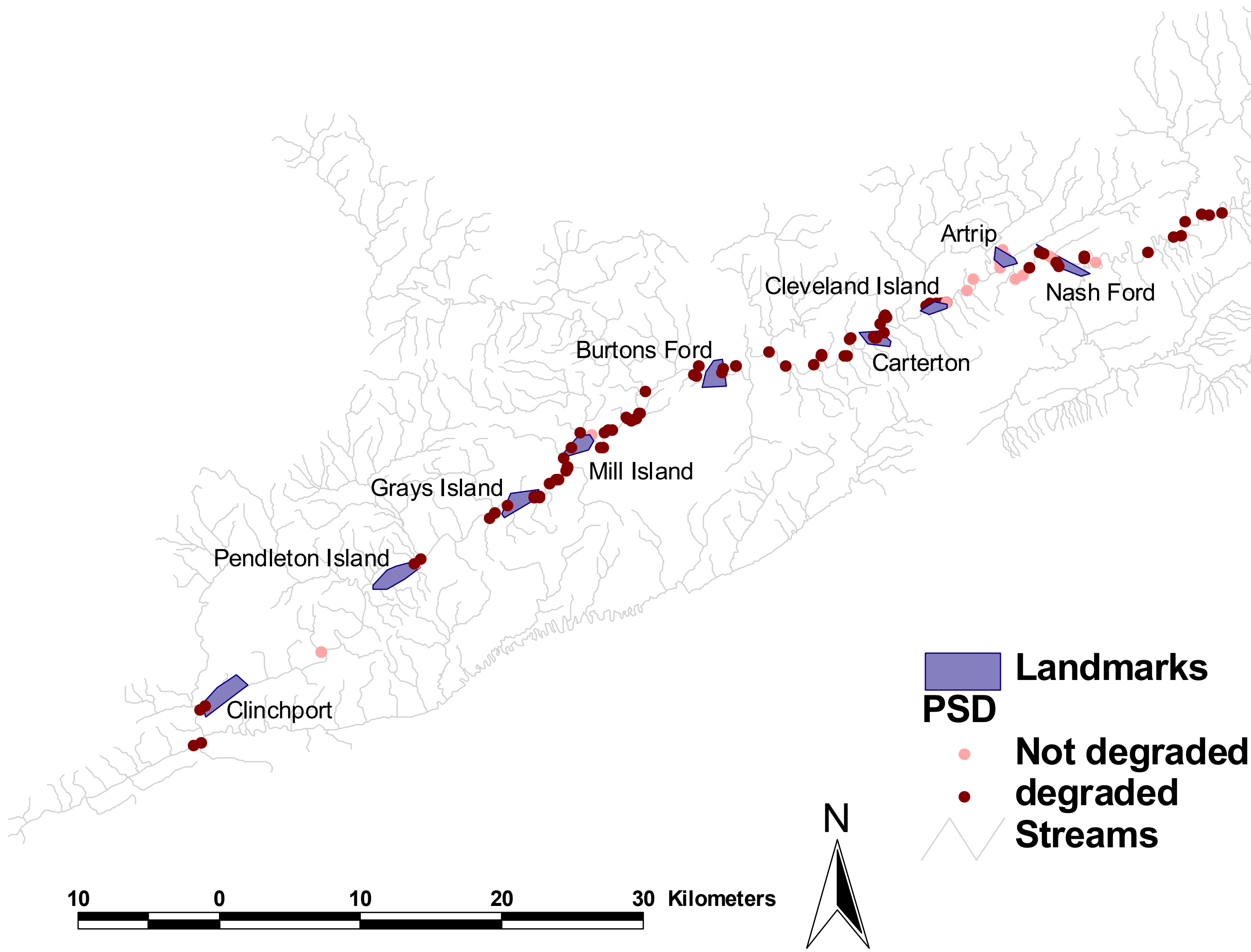
Appendix 2-C. Distribution of habitat units whose suitability percentage was reduced by more than 1 % due to proximity to potential vehicular bridge impacts.



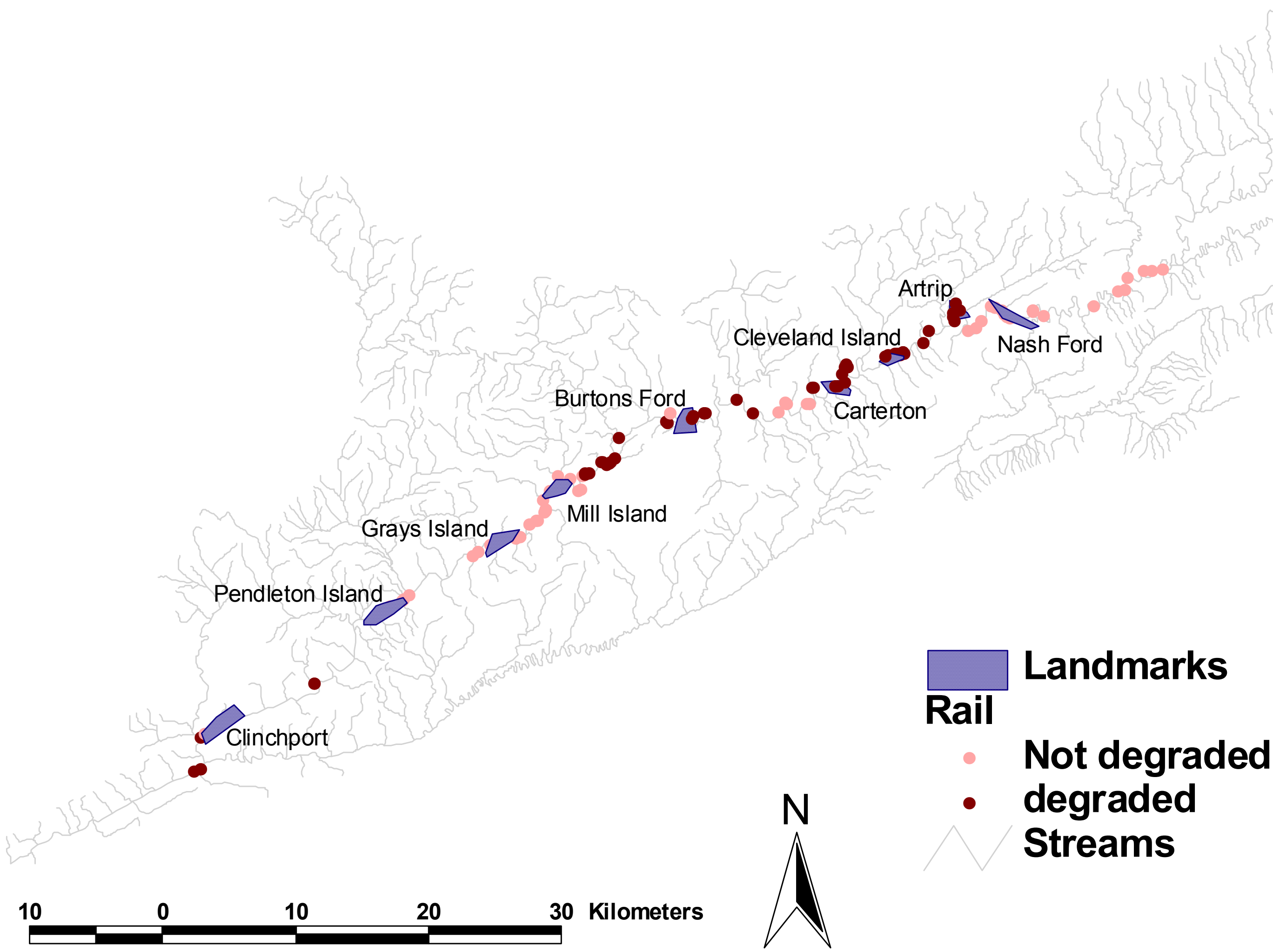
Appendix 2-D. Distribution of habitat units whose suitability percentage was reduced by more than 1 % due to proximity to potential cattle impacts.



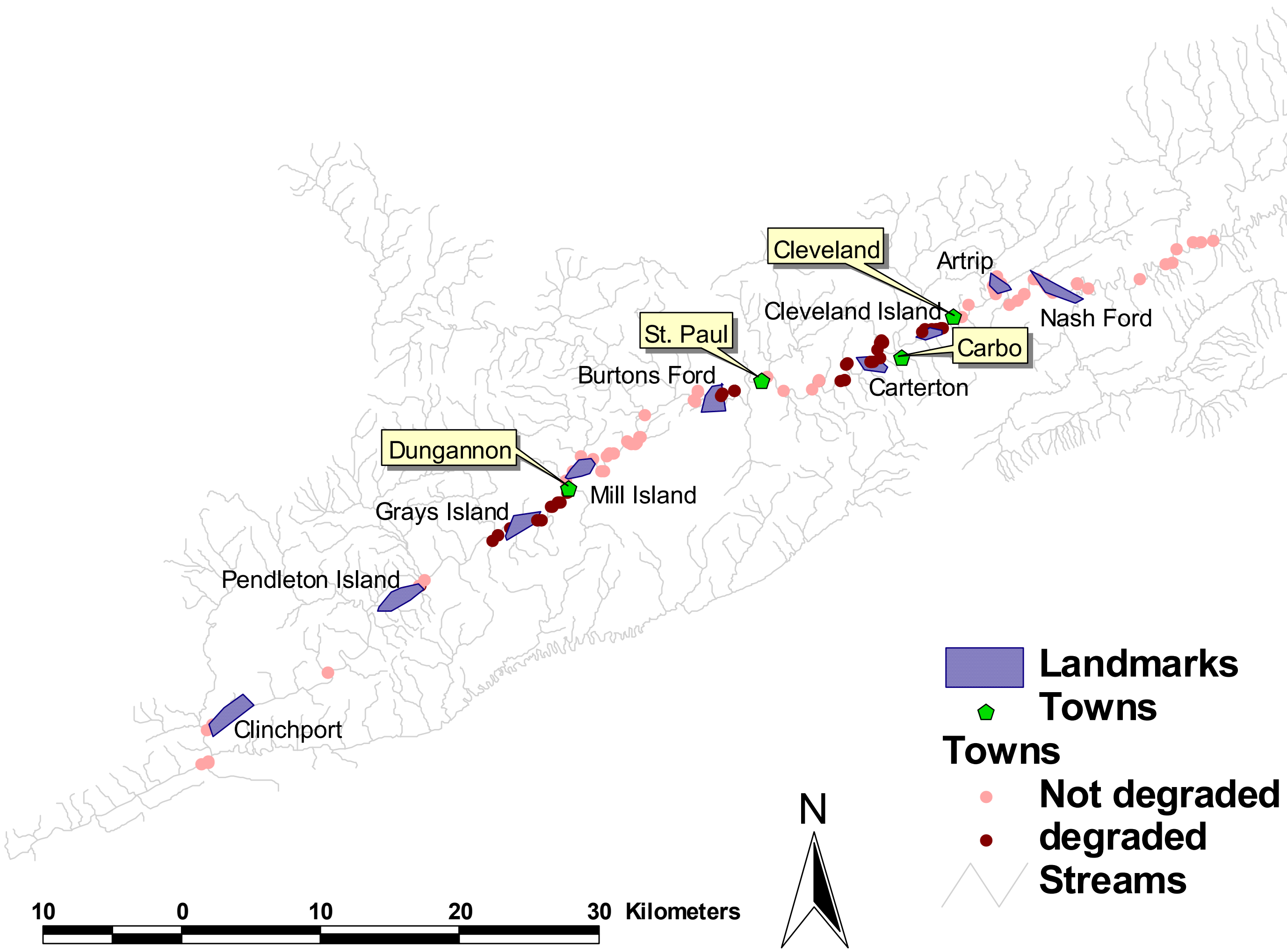
Appendix 2-E. Distribution of habitat units whose suitability percentage was reduced by more than 1 % due to proximity to potential permitted discharge impacts.



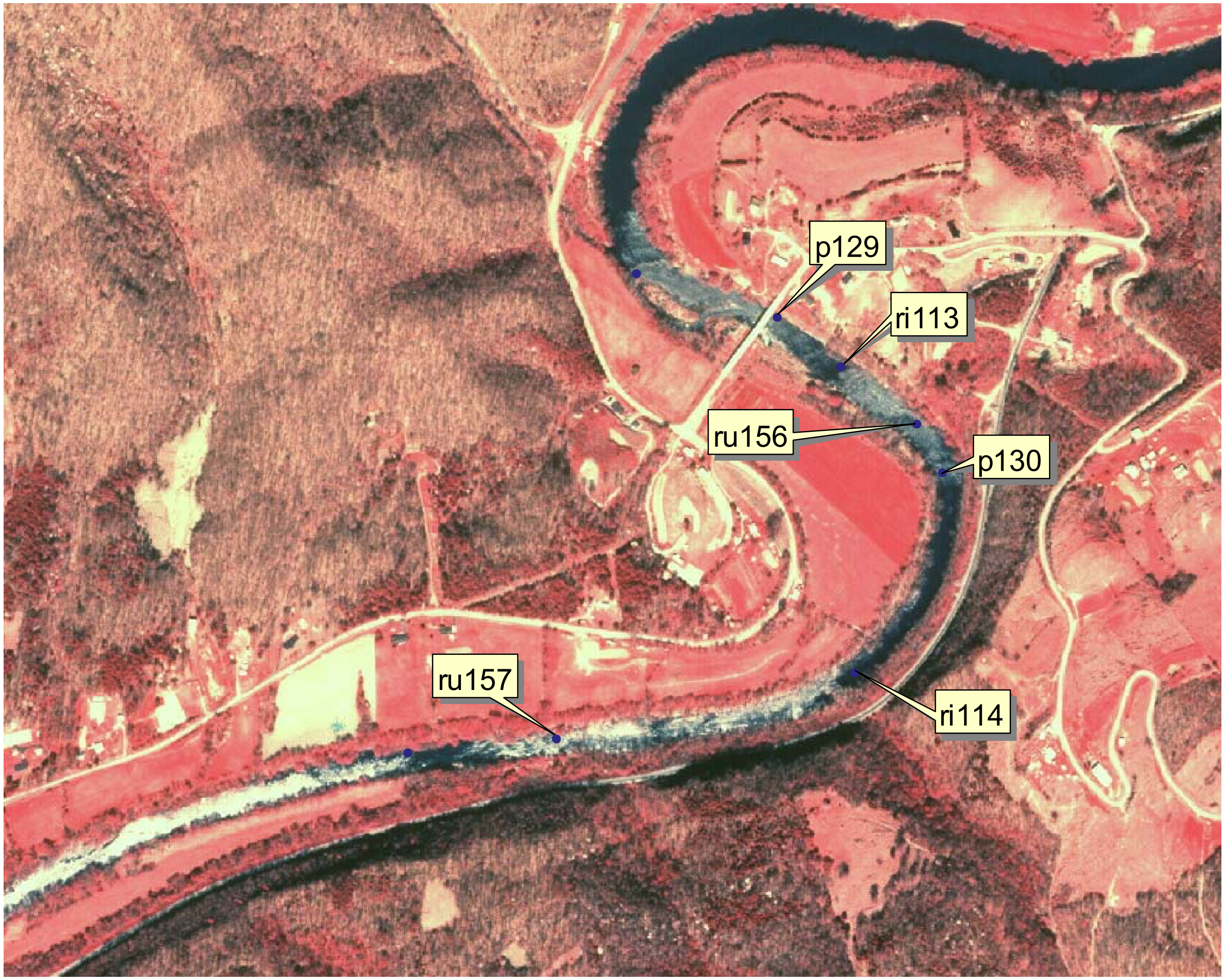
Appendix 2-F. Distribution of habitat units whose suitability percentage was reduced by more than 1 % due to proximity to potential railroad bridge impacts.



Appendix 2-G. Distribution of habitat units whose suitability percentage was reduced by more than 1 % due to proximity to potential town impacts.



Appendix 2-H. Digital ortho quarter quad (DOQQ) of the Clinch River overlaid with habitat unit locations. Each point represents the start of a new habitat unit and continues to the start of the next downstream habitat unit. River flow conditions may have differed between the date of the ground survey and those of the aerial photograph, but demonstrate the possibility of using remote sensing to obtain some habitat availability information.



VITA

Lora Leigh Zimmerman was born in Harrisonburg, Virginia on March 12, 1975 to Fred and Kathy Zimmerman. After graduating from Spotswood High School in May 1993, she enrolled at Virginia Tech in the department of Environmental Science. In May of 1997, she completed her bachelors degree in Envronmental Science and began working as a technician in the Virginia Tech Department of Fisheries and Wildlife. For 3.5 years, she worked with Dr. Richard Neves researching and surveying freshwater mussels. In August 2000, she enrolled at Virginia Tech as a graduate research assistant, pursuing a Master's of Science in Fisheries Science. In May 2001, she entered the Student Career Experience Program with the U. S. Fish and Wildlife Service, working at the Southwestern Virginia Field Office in Abingdon, Virginia. She received her Master's degree in January 2003 and looks forward to starting her career with the U. S. Fish and Wildlife Service in Charleston, South Carolina.