A Quantitative Survey of the Freshwater Mussel Fauna in the Powell River of Virginia and Tennessee, and Life History Study of Two Endangered Species, *Quadrula sparsa* and *Quadrula intermedia*.

Matthew S. Johnson

Thesis submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Master of Science

in

Fisheries and Wildlife Sciences

William F. Henley, Co-Chairperson

Richard J. Neves, Co-Chairperson

Andrew Dolloff

January 28, 2011

Blacksburg, Virginia

Keywords: Freshwater Mussels, Powell River, Survey, Unionidae, *Quadrula sparsa*, *Quadrula intermedia*, Life-History.

A Quantitative Survey of the Freshwater Mussel Fauna in the Powell River of Virginia and Tennessee, and Life History Study of Two Endangered Species, *Quadrula sparsa* and *Quadrula intermedia*.

by

Matthew S. Johnson

(Abstract)

Qualitative and quantitative sampling was conducted along a 165 km reach of the river from PRKM 269.4 near Dryden, VA to PRKM 104.8 near Harrogate, TN. Twenty-nine species were observed throughout the entire river, and the highest diversity of 23 species was collected at PRKM 152.6. Mussel abundances (mussels/person-h and mussels/m^2) ranged from 0.33 to 21.98 mussels/person-h and 0.00 to 2.24 mussels/m^2. Recent recruitment (individuals < 40 mm, depending on the species) was observed for 15 species, including the endangered *Epioblasma brevidens*, *Lemiox rimosus*, and *Quadrula intermedia*. The greatest number of species (6) with evidence of recent recruitment also was found at PRKM 152.6.

Data from the quantitative survey were used to simulate several sampling protocols that could be used to develop a long-term monitoring program for the Powell River. Five sites, PRKMs 197.9, 171.4, 159.6, 152.6, and 129.4, were selected for long-term monitoring because of high mussel densities and species richness. Six sampling protocols were simulated using the statistical program MONITOR to determine which protocols, if any, could monitor statistically significant changes in mussel abundance at rates $\pm$ 10%. Each of the simulated sampling protocols lasted between 15 and 30 y, and employed quantitative sampling at 3 to 5 y intervals. None of the sampling protocols simulated during this study were able to detect declines in mussel abundance $\leq$ 10%. Two sampling programs were able to detect increases in mussel abundance $\geq$ 6% when the level of significance was $\geq$ 0.10, and four sampling programs were able to detect a density increase of $\geq$ 8% when the level of significance was 0.05.

Despite the inability to monitor declines in mussel abundances, a long-term monitoring program is needed for the Powell River. Because qualitative sampling has been repeatedly shown to document species presence more effectively than quantitative sampling, it should be used to monitor changes in species presence and distribution. Quantitative sampling should be employed to monitor juvenile recruitment and changes in size-class structure of populations. Quantitative sampling also should be conducted to monitor overall mussel abundance at sites. Despite the inability to statistically detect changes in mussel density in the Powell River, quantitative sampling can provide valuable information, and the data collected can be used to qualitatively monitor changes in total density at sites.

Both species share a similar distribution in the Powell River. Eighteen specimens of *Quadrula sparsa* were collected between PRKM 230.9 and 152.6, and 68 individuals of *Q. intermedia* were collected between PRKM 230.9 and 129.4. The highest density of each species was collected at PRKM 152.6, and recent recruitment was observed at PRKMs 152.6 and 153.4. Fresh-dead and relic shells of both species were thin-sectioned to determine individual growth rate and life span. These species complete the majority of their growth during the first 10 y of life, and likely live for a total of 40 to 50 y. One gravid female of *Q. intermedia* was collected during this study, but no gravid females of *Q. sparsa* were observed.

Acknowledgements

The U. S. Fish and Wildlife Service field offices in Gloucester, Virginia, Abingdon, Virginia, and Asheville, North Carolina provided funding for this project. Dr. William Henley provided technical assistance, guidance, and support that were invaluable during the completion of this thesis. I would like to thank Dr. Richard J. Neves for introducing me to freshwater mussels as a summer field technician, and then providing me the opportunity to continue researching these under-appreciated animals as a graduate student. I also would like to thank my committee member, Dr. Andrew Dolloff for his guidance and support.

This project would not have been possible without the help and support of those who have worked at the Freshwater Mollusk Conservation Center during my time as research staff and a graduate student. I would like to thank Jess Jones and Brett Ostby for providing a wealth of advice and experience. Additionally, I would like to thank Dan Hua, Tim Lane, Joyce Barkley, Caitlin Carey, Nick King, Meghann Vincie, Jake Carter, and Adam Wilson for their countless hours assisting me in the field and providing friendship during my time at the FMCC. I would also like to thank my fellow graduate students in the Department of Fisheries and Wildlife Sciences.

Most importantly, I would like to thank my family and Mary for supporting me through all of my ups and downs. Without their love and encouragement, I would not be where I am today.

Table of Contents

List of Tables

Chapter 1: A Survey of the Freshwater Mussel Fauna in the Powell River, Virginia and Tennessee

List of Figures

Chapter 1: A Survey of the Freshwater Mussel Fauna in the Powell River, Virginia and Tennessee

Appendix C

Chapter 2. Evaluation of Long-term Quantitative Sampling Programs for Selected Sites in the Powell River, Virginia and Tennessee.

Chapter 3. Population Status of the Appalachian monkeyface, *Quadrula sparsa* (Lea, 1841), and Cumberland monkeyface, *Quadrula intermedia* (Conrad, 1836), in the Powell River, Virginia and Tennessee.

Chapter 1

A Survey of the Freshwater Mussel Fauna in the Powell River, Virginia and Tennessee.

(Abstract)

The Powell River is inhabited by a historically rich mussel fauna, which includes nine federally endangered species. However, recent quantitative sampling in the Powell River has recorded a decline in mussel densities at multiple sites over the past 30 y. In 2008 and 2009, a qualitative and quantitative survey of 22 sites was completed to document richness, relative abundance, density (mussels/m^2 and mussels/person-h), and size-class structure of resident populations. Sites were divided into three river-zones (Upper, Middle, and Lower), and differences in species occurrence and abundance were compared among zones.

The qualitative portion of the survey (n = 1403 person-h) encountered 29 species and 15,088 individuals at 22 sites; catch per unit effort ranged from 0.33 to 21.98 mussels/person-h. Four species documented in previous surveys; spectaclecase (*Cumberlandia monodonta)*, oystermussel *(Epioblasma capsaeformis)*, finerayed pigtoe *(Fusconaia cuneolus)*, and cracking pearlymussel (*Hemistena lata)*, were not observed during this survey, and it is likely that *E. capsaeformis* and *H. lata* have been extirpated from the river. Live individuals (n = 265) were collected of seven of the nine federally endangered species previously reported in the Powell River. Statistical analysis determined that sites in the Upper Zone had significantly fewer species than the other zones, and significantly lower abundances than those found in the Lower Zone.

The quantitative portion of the survey, consisting of 50 to 200 quadrats of 0.25m^2 at 21 sites, recorded 19 species of mussels; mean mussel densities ranged from 0.00 to 2.24 mussels/m^2. As with the qualitative sampling methods, comparisons among river zones determined that sites in the Upper Zone had significantly lower species richness and mean mussel densities than downstream. The failure to reach a stable mean density estimates during requisite sample size evaluations showed that the number of quadrats sampled during this study were generally inadequate to produce accurate density estimates at sites within the three survey zones.

Introduction

The Powell River originates near Norton in Wise County, Virginia, and flows in a southwesterly direction to converge with the Clinch River in Norris Reservoir (Figure 1). The Powell River watershed drains an area of approximately 2,470.9 km^2, with 1,429.7 km^2 in Virginia, and 1,041.2 km^2 in Tennessee (Tennessee Department of Environment and Conservation [TDEC] 2007). Numerous parallel ridges and subterranean drainages characterize the Powell watershed. Steep riverbanks provide poor riparian cover that makes the river potentially vulnerable to human activities (TDEC 2007). The river's substrate types consist predominately of sand, gravel, and cobble. Major land use categories in Virginia are forest (61.3%), agriculture (29.5%), and coal mining, industrial, and urban development (9.2%) (Eckert et al. 2004). The Tennessee portion of the watershed is comprised of forest (75.0%), agriculture (21.0%), ponds (3.0%), and a mixture of commercial and residential areas (1.0%) (TDEC 2007).

The fish and mussel faunas in the Powell River, as with the Clinch River, are some of the most diverse in the United States due to lack of both glaciation and sea-level rises, and isolation from other river systems. Historically, the Powell River was inhabited by 41 species of freshwater mussels (Ortmann 1918). Ahlstedt et al. (2005) lists 36 species as recently remaining in the Powell River drainage. Seven species in the Powell River are federally listed, including the dromedary pearlymussel (*Dromus dromas*, Lea 1834), shiny pigtoe (*Fusconaia cor*, Conrad 1834), fine-rayed pigtoe (*F. cuneolus*, Lea 1840), cracking pearlymussel (*Hemistena lata*, Rafinesque 1820), birdwing pearlymussel (*Lemiox rimosus*, Rafinesque 1831), Cumberland monkeyface (*Quadrula intermedia,* Conrad 1836), and Appalachian monkeyface (*Q. sparsa,* Lea 1841). The Powell River is one of the last two remaining refuges for *Q. intermedia* and *Q. sparsa*.

The river's mussel fauna was already experiencing a noticeable decline due to anthropogenic effects during Ortmann's survey. He noted that a large portion of the mussel fauna in the river had already been decimated downstream of a wood extraction plant in Big Stone Gap, Virginia (Ortmann 1918). Between the 1960's and 1990's, mussels in other portions of the river became increasingly rare (Ahlstedt et al 2005). Stansbery (1973) used sampling data collected between 1963 and 1971 to confirm his initial observations that the mussel fauna in the Powell River was declining.

Numerous other surveys over the past 40 y have documented a decline in the abundance of freshwater mussels in the Powell River (Ahlstedt and Brown 1979, Neves et al 1980, Dennis 1981, Ahlstedt 1986, Eckert et al. 2005, Ahlstedt et al. 2005). Ahlstedt and Brown (1979) were unable to find 9 of the species observed by Ortmann (1918), and presumed that 6 of the headwater species had been extirpated from the main-stem river due to heavy siltation and mining activities. Three additional species were believed to have been extirpated due to the construction of Norris Dam. More recently, Ahlstedt et al. (2005) documented a decline in mussel densities at 5 long-term monitoring sites in the Powell River between 1979 and 2004, including PRKMs 230.9, 188.8, 179.9, 171.4, and 159.6. The shoals at PRKMs 188.8, 179.9, 171.4, and 159.6 were sampled 6 times in the 25 y period, and each site exhibited overall declines in total mussel density and number of species collected. The shoal at PRKM 230.9 was sampled 3 times during this period, and also showed declines in total mussel density and number of species collected. The shoal at PRKM 193.4 was only sampled during 2004. Of the sites surveyed by Ahlstedt et al. (2005), PRKM 188.8 experienced the greatest declines in both total mussel density, from 11.14 to 1.24 mussels/m^2, and number of species collected, from 16 to 7 species, respectively.

Mussel declines in this river have been largely attributed to habitat degradation caused by agricultural practices, urban land use, and coal mining (Dennis 1981, Ahlstedt and Tuberville 1997, Diamond et al. 2002, Ahlstedt et al. 2005). Ahlstedt et al. (2005) stated that mussel distributions and abundances are influenced by the co-occurrence with mine lands in the watershed. Additional studies have shown that sediments contaminated with by-products from coal mining activities are a potential factor leading to mussel decline (McCann and Neves 1992). Black-water events (coal fines released into the river) have occurred several/many times over the last 100 y in the Powell River watershed (Ahlstedt et al. 2005). Declines in mussel densities have been observed following some black-water events. Following a period in the early 1980's, when the entire river was known to occasionally run black with coal fines (Ahlstedt 1986), a mussel die-off was observed in 1983 between PRKMs 230.9 and 104.8 (Jenkinson and Ahlstedt 1988). In order to understand what possible effects these anthropogenic events are having on the river's diverse mussel fauna, it is imperative that researchers have current data regarding species presence and distribution, mussel abundances, and size-class structures.

Statement of Objectives

The goal of this study is to document current mussel occurrences and abundances at selected sites in the Powell River, Virginia and Tennessee, and provide base-line data for a future long-term monitoring program of the mussel fauna in the river. Specific objectives are as follows:

1) document mussel abundances (mussels/h and mussels/m^2) at selected sites in the Powell River, Virginia and Tennessee;
2) document individual species occurrences and percentages of species collected using qualitative and quantitative sampling methods at these selected sites;
3) document current size-class distributions of all mussels collected at these selected sites; and
4) make statistical comparisons between 3 pre-defined river zones to determine whether differences exist in species richness or abundance among different portions of the river.

Materials and Methods

Site Selection

In 2008 and 2009, 22 sites were sampled in an approximately 165 km stretch of the Powell River from Powell River Kilometer (PRKM) 269.4 to PRKM 104.8, including shoals at PRKMs 269.4, 266.3, 263.0, 246.9, 236.3, 230.9, 206.6, 198.8, 197.9, 193.4,188.8, 180.7, 179.9, 171.4, 159.6, 153.4, 153.0, 152.6, 136.2, 135.8, 129.4, and 104.8 (Fig. 1and Table 1). Sites were selected for survey based on the locations of previously documented living mussel assemblages (Dennis 1981, Ahlstedt 1991a, Wolcott and Neves 1994, Ahlstedt et al. 2005, Eckert et al. 2006) (Table 2). Previously un-surveyed sites also were selected following a preliminary qualitative snorkel survey of other areas. This survey consisted of snorkel sampling throughout the selected shoal. These sites were selected for additional qualitative and quantitative survey if a mussel aggregation was observed during these preliminary snorkel surveys.

The dimensions of each site were determined by locating the upstream and downstream points of suitable riffle habitat. These points formed the upstream and downstream boundaries of the sites, and all habitats between these points were surveyed. The boundaries of all sites were documented by collecting GPS coordinates for each site's four corners (Appendix 1). Based on spatial relationship to other sites, and for the sake of statistical comparison, sites were placed into one of three zones. Zones were defined as distinct reaches of the river, and contained all sites sampled within that defined reach. The zones were defined as Upper (including PRKMs 269.4, 266.3, 263.0, 246.9, 236.3, 230.9, 206.6), Middle (including PRKMs 198.8, 197.9, 193.4, 188.8, 180.7, 179.9, 171.4, 159.6), and Lower (including PRKMs 153.4, 153.0, 152.6, 136.2, 135.8, 129.4, 104.8). Sites downstream of PRKM 104.8 were not sampled during this survey because below this point the river begins to experience effects from Norris Reservoir.

Site Surveys

Sites were qualitatively and quantitatively sampled. During qualitative sampling, the entire river bottom of each site was sampled using a catch-per-unit-effort (CPUE, mussels/h and species/h) sampling technique. The level of quantitative sampling varied for each site based on the likelihood of mussel occurrence; therefore, sites upstream of PRKM 193.4 were sampled using $\leq$ 102 quadrats [PRKM 269.4 = 100 (0.25m^2 quadrats); PRKM 266.3 = 100; PRKM 263.0 = 100; PRKM 246.9 = 50; PRKM 236.3 = 100; PRKM 230.9 = 100; PRKM 206.6 = 100; PRKM 198.8 = 102; PRKM 197.9 = 102], and sites downstream of PRKM 193.4 were sampled using >

100 quadrats [PRKM 193.4 = 192 (0.25m^2 quadrats); PRKM 188.8 = 153; PRKM 180.7 = 150; PRKM 179.9 = 130; PRKM 171.4 = 200; PRKM 159.6 = 150; PRKM 153.4 = 150; PRKM 153.0 = 151; PRKM 152.6 = 150; PRKM 136.2 = 150; PRKM 135.8 = 150; PRKM 129.4 = 150; PRKM 104.8 = 150]. A greater number of quadrats was used at sites downstream of PRKM 193.4, because these sites are of greater importance when monitoring mussel diversity, as the sites are inhabited by several federally threatened or endangered species. Due to poor river conditions (high turbidity), only 50 quadrats were sampled at PRKM 246.9.

Qualitative Sampling

Substrate-surface sampling, including snorkeling or visually searching using view buckets, was conducted at each site to determine species presence and CPUE (mussels/h and species/h) values. Survey crews consisted of 3 to 20+ people with varying levels of experience. A core crew of 3 individuals was present at every sampling event. Few rocks were moved during qualitative sampling, and only mussels visible at the surface were counted. The entire wetted width of the river was sampled at each site. As mussels were found, surveyors left mussels undisturbed in the substrate, and marked the location of the mussel by inserting a florescent flag in the substrate adjacent to the mussel. A separate data-collecting crew followed the snorkelers to removed flagged mussels from the substrate and identify the individual to species. Each mussel's gender was recorded, if the species was sexually dimorphic, and the shell length of each individual was measured using calipers. All mussels then were immediately returned to the location in the river substrate where collected. All specimens of *Q. sparsa* and *Q. intermedia* were checked for gravidity before being returned to the river.

Two methods of qualitative sampling were used. The expected presence of federally endangered mussels determined which of the following qualitative methods was used. Expected presence of federally endangered mussels was based on previous qualitative and quantitative data (Ahlstedt 1994, Ahlstedt 2005, Wolcott and Neves 1995, Jess Jones, USFWS, Virginia Tech, personal unpublished data).

Brief Qualitative Survey. When federally endangered mussels were deemed unlikely to be present at a site, based on previous qualitative and quantitative data collected during other surveys, qualitative snorkel sampling was used to collect mussels. During this sampling, snorkelers initiated sampling at the downstream boundary of the site, and continued to the upstream boundary of the site. Samplers then utilized a random search method with lateral sweeps to cover as much habitat as possible. Sampling continued until the entire area had been searched. Sites that were declared as having a low likelihood of being inhabited by federally endangered mussels included PRKMs 269.4, 266.3, 263.0, 246.9, 236.3, 230.9, 206.6, 198.8, 197.9.

Extensive Qualitative Survey. When federally endangered mussels were likely to be present at a site, based on qualitative and quantitative data collected during historical surveys, the site was partitioned into 1.5 m-wide lanes, extending from the downstream to the upstream boundaries. Lanes were created by inserting 1.2 m x 2 cm rebar into the substrate at the upstream and downstream boundaries, and tying string between the two endpoints. Flagging tape was placed every 10 m starting at the downstream post, and continued until the upstream post. Lanes were oriented parallel to flow across the wetted width of the site. Surveyors initiated sampling at the downstream boundary of each lane, and continued upstream towards the upstream site boundary. All mussel positions in relationship to the 10 m markers in a lane were recorded. Sites that were designated as having a high likelihood of habitation by federally endangered mussels included PRKMs 180.7, 179.9, 171.4, 159.6, 153.4, 153.0, 152.6, 136.2, 135.8, 129.4, and 104.8. Because of ongoing sampling by the Virginia Department of Game and Inland Fisheries (VDGIF), there was a deviation in sampling methods at PRKMs 193.4 and 188.8. In order to maintain sampling protocol continuity within an existing monitoring program (Eckert et al. 2005), random CPUE sampling was used at these sites.

Quantitative Sampling

As previously explained, the number of quadrats sampled at a site depended on the expected presence of endangered mussel species. At sites where endangered species were unlikely to be present, $\leq$ 100 quadrats were sampled. The sites where 100 or fewer quadrats were sampled include PRKMs 269.4, 266.3, 263.0, 246.9, 236.3, 230.9, 206.6, 198.8, and 197.9. Greater than 100 quadrats were sampled at sites endangered species were likely to be present. The sites where > 100 quadrats were used included PRKMs 180.7, 171.4, 159.6, 153.4, 153.0, 152.6, 136.2, 135.8, 129.4, and 104.8.

The following formula (Strayer and Smith 2003) was used to determine the precision of the density estimates:

$$n = m^{-0.5} CV^{-2} \quad ,$$

where, n = number of quadrats sampled,

and, m = mean number of mussels per quadrat,

and, CV = coefficient of variation (standard error divided by the mean).

The number of quadrats sampled at the shoals at PRKMs 193.4 and 188.8 differed from previous methodology, because sampling at these sites was conducted in collaboration with long-term sampling by VDGIF. At these sites, quadrat sampling was already conducted by VDGIF in a portion of the site that was qualitatively considered to contain the highest densities of mussels. In order to obtain a mussel density estimate for the entire site, quadrat data from VDGIF's quantitative sampling were combined with quadrat data collected from locations that were outside of VDGIF's sampling area.

Quantitative surveys employed a systematic sampling design that incorporated a single random start (Strayer and Smith 2003). Transects were perpendicular to flow, and were evenly spaced across the full length of the site, from downstream to upstream boundary. The position of the first downstream transect was determined by random number table. A random number was used to determine the distance (m) upstream from an arbitrary point on the right ascending bank at the site's downstream boundary. Once the position of the initial transect was determined, a random number table was used again to determine the distance (m) from the right-ascending bank; the first quadrat would be placed on the initial transect. Following the selection of a starting point from the random number table, quadrats were placed at evenly spaced intervals along each transect. Quadrats were spaced so that approximately 10 quadrats would be sampled along each transect. Quadrats were designated consecutively in alternating directions on each transect; i.e., right ascending bank to left ascending bank on first transect, followed by left ascending bank to right ascending bank on second transect, etc. If insufficient space existed between the final quadrat on a transect and the river bank, the difference between the remaining spacing distance, and distance to the river bank would be continued on the following transect, and quadrat sampling would resume. For example, if quadrats were evenly spaced at 5 m apart, and only 3 m remained between the final quadrat and the river bank, the first quadrat on the following transect would be place 2 m from the river bank.

Each quadrat was excavated to a depth of 25 cm or until bedrock. In each quadrat, all mussels were collected, identified, sexed (if possible), measured for maximum shell length (mm, anterior to posterior), and denoted as visible on the surface of the substrate or undetectable at the surface. Mussels were then returned to the substrate directly adjacent to the quadrat, and substrate that was excavated from the quadrat was returned. During the quadrat survey, mussels with any portion of their shell above the substrate were noted as "surface" on the data sheets, and mussels only visible during excavation were noted as "buried".

Abundance

Using data collected during qualitative and quantitative sampling, mussel abundances (mussels/h and mussels/m^2) were calculated for each site. Mean abundance values were created for each pre-defined zone by averaging abundance values of all surveyed sites within that zone. One-way ANOVA was used to determine whether significant differences in abundances existed between the three river zones (Upper, Middle, and Lower). Multiple comparison analysis (Fisher's Least Significant Difference, LSD) was used to compare differences in zone means. Correlations (r) among mussels/person-h, mussels/m^2, and PRKM also were calculated.

Post-hoc power analyses were used to determine the level of power achieved by the test. Achieving an appropriate level of power shows that there is an acceptable likelihood that the statistical test will reject H_0 when H_a is true. Power analyses were performed on the ANOVA tests used for zone comparisons of abundances (mussels/m^2 and mussels/person-h), and species presence. Achieved levels of power were calculated using α-values of 0.05, 0.10, and 0.15.

Species Occurrence and Percentages

The number of species present at each site was determined using the combined data collected during qualitative and quantitative sampling. As with the abundance data, the number of species collected at sites using both qualitative and quantitative sampling methods was compared using one-way ANOVA with multiple comparisons by Fisher's LSD. Post-hoc power analysis also was conducted on both qualitative and quantitative data. Correlation (r) between the number of species collected at a site and PRKM was calculated. A percentage of total mussels collected during semi-quantitative and quantitative sampling was calculated for each species.

Evidence of Reproduction

Based on the work of Ahlstedt et al (2005), mussels < 40 mm were considered to be recent recruits. Because of their smaller maximum lengths, specimens of *Epioblasma brevidens, E. triquetra*, *Lemiox rimosus, Medionidus conradicus, Villosa iris* and *Villosa vanuxemensis* that were < 30 mm were considered as evidence of recent recruitment.

Requisite Sample Size Evaluation

To determine whether an adequate number of quadrats were sampled to obtain a stable estimate of density (mussels/0.25m^2), a program was created in Minitab 15 to calculate partial sum densities from quadrat data collected at each site during this survey. Partial sums were created by summing the number of mussels that had been collected from sequential quadrats. This process was completed from first to final quadrat used during a site's quantitative sampling. Partial sums were divided by the number of quadrats sampled for that particular partial sum to create a partial sum density. A cumulative mean density was then calculated by averaging all the partial sum densities. These partial sum densities then were plotted in consecutive order, and compared to the cumulative mean density. If the partial sum means eventually remained within ± 10% of the cumulative mean density, sampling at the site was considered to have reached a stable density estimate.

Data Analysis

All statistical analyses were performed using Minitab 15 (Minitab Inc., State College, Pennsylvania), SAS 9.2 (SAS Inst., Cary, North Carolina), JMP 8 (SAS Inst., Cary, North Carolina), and G*Power 3.1 (Institute for Experimental Psychology, Heinrich Heine University, Düsseldorf, Germany). Graphs and tables were produced using Minitab 15, SAS 9.2, and Excel 2007 (Microsoft Inc., Redmond, Washington). P-values of < 0.05 were considered significant.

Results

Abundance

Qualitative Sampling

Total mussel CPUE ranged from 0.33 mussels/h to 20.97 mussels/h at sampled sites, and generally increased from upstream to downstream ($r = 0.66$, $p < 0.05$) (Table 3 and Fig. 2). Analysis of variance showed that significant differences existed among total mussel CPUE data within the 3 pre-defined zones ($F = 9.77$, $p < 0.05$) (Table 4). Multiple comparisons determined that means of mussel CPUE from sites in the Upper Zone were significantly lower than those from sites in the Lower zone ($p < 0.05$). No significant differences exist between the Upper and Middle Zones ($p > 0.05$) or between the Middle and Lower zones ($p > 0.05$). *Post hoc* power analysis of the ANOVA used to compare zone data determined that powers of 0.94, 0.97, and

0.98 were achieved when conducting analysis with α-values of 0.05, 0.10, and 0.15, respectively (Table 5).

Quantitative Sampling

The estimated mussel densities based on quadrat data from the sampling sites ranged from no mussels/m^2 to 2.25 mussels/m^2 (0.56 mussels/quadrat) (Table 6). Because no mussels were collected during quadrat sampling at PRKMs 269.4, 266.3, 263.0, and 246.9, mussel densities could not be estimated at those sites. The shoal at PRKM 135.8 had the highest estimated mussel density at 2.25 mussels/m^2. Precisions associated with mussel density estimates at the sites ranged from 0.22 to 0.09 (Table 7), and coefficients of variation ranged from 0.42 to 1.31 (Table 7). There was a significant correlation between mussel density and PRKM ($r = 0 .57$, $p < 0.05$) (Fig. 3). Analysis of variance found significant differences among mussel densities of the river zones ($F_{2,19} = 1.19$, $p < 0.05$) (Table 4), and Fisher's LSD *post hoc* analysis determined that the density of the Upper Zone was significantly less than the densities of the Middle and Lower zones ($p < 0.05$). No significant differences occurred between the Middle and Lower zones ($p > 0.05$) (Table 4). *Post hoc* power analysis determined that powers of 0.88, 0.94, and 0.96 were achieved when conducting analyses with α-values of 0.05, 0.10, and 0.15, respectively (Table 9).

Species Occurrence and Percentages

Qualitative Sampling

Mussels were found at every site sampled during qualitative surveys. Twenty-nine species, represented by 15,088 individuals, were found during qualitative sampling (Table 8 and 9). The highest number of species (23) was collected at PRKM 152.6. Only one species was collected at PRKM 263.0 and PRKM 146.9 (Table 5). Federally endangered species were collected at 14 of the 22 surveyed sites. No federally endangered species were found upstream of PRKM 230.9 (Table 9).

When examining data obtained from qualitative sampling techniques, the mussel fauna appeared to be most diverse in the middle and downstream portions of the Powell River, from PRKM 197.9 to PRKM 180.7 and from PRKM 153.4 to PRKM 135.8. Correlation analysis showed a general decline in species diversity from downstream to upstream ($r = 0.79$, $p < 0.05$) (Fig. 4). Statistical analysis (one-way ANOVA) determined that significant differences occurred between the number of species collected in each of the three pre-defined zones ($F = 19.94$, $p < 0.05$) (Table 4). *Post hoc* Fisher's LSD test showed that the number of species collected in the Upper Zone was significantly lower than the number of species collected in the Middle or Lower zones. *Post hoc* power analysis determined that powers of 0.99 were achieved when using α-values of 0.05, 0.10, and 0.15 (Table 5).

Non-Listed Species. The pheasantshell, *Actinonaias pectorosa,* was the most common species collected during qualitative sampling, represented 47.83% of all mussels collected, and occurred at 19 of 22 sampled sites between PRKM 266.3 and PRKM 104.8. The mucket, *Actinonaias ligamentina* (28.17%) was the second most abundant species collected, and occurred at 17 sites between PRKM 236.3 to PRKM 104.8. The Cumberlandian moccasinshell, *Medionidus conradicus* (5.77%) occurred at 15 sites between PRKM 206.6 and PRKM 104.8. The next most commonly occurring species were the spike, *Elliptio dilatata* (4.43%), threeridge, *Amblema plicata* (3.6%), and purple wartyback, *Cyclonaias tuberculata* (3.1%). Each of these species was collected at 16 sites during qualitative sampling. *Elliptio dilatata* had the widest distribution of these species, and was collected between PRKM 266.3 and PRKM 104.8 *Amblema plicata* and

Cyclonaias tuberculata were collected between PRKM 230.9 and PRKM 104.8. The kidneyshell, *Ptychobranchus fasciolaris* (1.78%), was collected at 17 sites between PRKM 269.4 and PRKM 104.8, and wavyrayed lampmussel, *Lampsilis fasciola* (0.61%) was collected at 16 sites between PRKM 169.4 and PRKM 104.8. The pocketbook, *Lampsilis ovata* (0.63%) and rainbow mussel, *Villosa iris* (0.32%) were each collected at 12 sites between PRKM 230.9 and PRKM 104.8. The remaining species collected during qualitative sampling were the elephantear, *Elliptio crassidens* (0.03%), Tennessee pigtoe, *Fusconaia barnesiana* (0.04%), longsolid, *Fusconaia subrotunda* (0.21%), flutedshell, *Lasmigona costata* (0.19%), black sandshell, *Ligumia recta* (0.14%), pink heelsplitter, *Potamilus alatus* (0.01%), pimpleback, *Quadrula pustulosa* (0.03%), and mountain creekshell, *Villosa vanuxemensis* (0.11%). These species were all found at fewer than 10 sites between PRKM 236.3 and PRKM 104.8 (Table 10).

Federally Listed and Candidate Species. Seven federally listed species also were collected during qualitative surveys. The dromedary pearlymussel, *Dromus dromas* (0.78%) and Cumberland monkeyface, *Quadrula intermedia* (0.45%) were each collected at 12 sites between PRKM 230.9 and PRKM 104.8. The Cumberland combshell, *Epioblasma brevidens* (0.40%) was collected at 11 sites between PRKM 193.4 and PRKM 104.8. The shiny pigtoe, *Fusconaia cor* (0.13%), birdwing pearlymussel, *Lemiox rimosus* (0.1%), rough rabbitsfoot, *Quadrula c. strigillata* (0.05%), and Appalachian monkeyface, *Quadrula sparsa* (0.11%) were each collected at fewer than 10 sites between PRKM 236.3 and PRKM 104.8 (Table 10). Four of the five federal candidate species in the Powell River also were collected during qualitative surveys. The sheepnose, *Plethobasus cyphyus* (0.68%) was collected at 13 sites between PRKM 198.8 and PRKM 104.8. The snuffbox, *Epioblasma triquetra* (0.05%), slabside pearlymussel, *Lexingtonia* *dolabelloides* (0.03%), and fluted kidneyshell, *Ptychobranchus subtentum* (0.23%) were each collected at fewer than 10 sites between PRKM 236.3 and 104.8 (Table 10).

Quantitative Sampling

Quantitative sampling yielded 580 mussels of 19 species, including 4 federally endangered and 1 candidate species (Tables 6 and 11). The number of species collected by quantitative sampling increased from upstream to downstream ($r = 0.825$, $p < 0.05$) (Fig. 4). Statistical analysis (one-way ANOVA) determined that significant differences occurred among the number of species collected in each of the three pre-defined zones (F = 20..01, $p < 0.05$) (Table 4), and *post hoc* Fisher's LSD test showed that the number of species collected in the Upper Zone was significantly lower than the number of species collected in the Middle or Lower zones. *Post-hoc* power analysis determined that powers of 0.99 were achieved when using α-values of 0.05, 0.10, and 0.15 (Table 5).

Non-Listed Species. As with qualitative sampling, *A. pectorosa* (41.72%) and *A. ligamentina* (17.93%) were the most commonly collected species, and were collected at 17 and 16 sites, respectively. *M. conradicus* (14.14%) and *E. dilatata* (8.1%) were the next most commonly collected species during quantitative sampling, and were each collected at 12 sites. The species *A. plicata* (2.41%), *C. tuberculata* (2.76%), *E. crassidens* (0.17%), *F. subrotunda* (0.86%), *L. fasciola* (2.76%), *L. ovata* (0.34%), *L. costata* (0.17%), *P. fasciolaris* (2.59%), *V. iris (*3.1%), and *V. vanuxemensis* (0.69%) were all collected at fewer than 10 sites. The species *F. barnesiana*, *L. recta*, and *P. alatus* were not collected during quantitative sampling (Table 11).

Federally Listed and Federal Candidate Species. Specimens of *D. dromas* (0.52%), *E. brevidens* (1.03%), *Q. c. strigillata* (0.34%), and *L. rimosus* (0.17%) were each collected at fewer than 10 sites. *P. cyphyus* (0.34%) was the only federal candidate species collected during quantitative sampling, and was found at fewer than 10 sampled sites. The species *E. triquetra*, *F. cor*, *L. dolabelloides*, *P. subtentum*, *Q. intermedia*, *Q. pustulosa*, and *Q. sparsa* were not collected during quantitative sampling (Table 11).

Size-class Structure

All species except *P. alatus* and *Q. pustulosa*, which were only represented by single individuals, were represented by multiple size-classes**.** Fifteen species, including the three endangered species, *E. brevidens*, *L. rimosus*, and *Q. intermedia*, were represented by mussels below the pre-defined threshold (< 30 mm or < 40 mm depending upon species) to indicate recent recruitment (Appendix 2). Species with no size classes < 40 mm were uncommon during the survey. The greatest number of species showing recent recruitment was found at PRKM 152.6, *A. ligamentina*, *A. pectorosa*, *A. plicata*, *C. tuberculata*, *E. brevidens*, and *L. rimosus*.

Requisite Sample Size Evaluation

Examination of mean stability plots showed that the numbers of quadrats used at sites during this survey were generally insufficient to achieve stable mean density estimates. For the majority of the sampled sites (PRKM 236.3, 230.9, 206.6, 197.9, 193.4, 188.8, 180.7, 153.4, 153.0, 136.2, 135.8, 129.4, and 104.8), the estimated mean density varied by greater than ± 10% of the cumulative mean density. The numbers of quadrats used were sufficient to achieve stable mean density estimates at only 5 of the 22 sites sampled during this study; PRKM 198.8, 179.9, 171.4, 159.6, 152.6 (Table 7). The sample size required to achieve a stable mean could not be assessed at 4 sites, because no mussels were found during quantitative sampling: PRKMs 269.6, 266.3, 263.0 and 246.9.

Comparison of Sampling Techniques

Initially, correlation analysis was used to determine if CPUE values calculated during this study maintained any form of correlation with the abundance values calculated from quantitative sampling. Analysis showed that a slight positive correlation between the two sampling methods did exist during this study ($r = 0.464$, $p < 0.05$, Fig. 5). Simple linear regression was then used to determine if CPUE could serve as a predictive indicator of mussel abundances measured through quantitative methods. Regression analysis showed that the CPUE data collected at individual sites during this study was not predictive of quantitative abundances estimated for the CPUE data's corresponding sites ($y = 0.0543x + 0.2647$, $r^2 = 0.215$, $p > 0.05$).

Discussion

Species Richness and Abundance

Of the 41 species historically reported as inhabiting the main-stem Powell River (Ortmann 1918), only 29 (70%) were collected during this study. The following species have likely been extirpated from the river since Ortmann's (1918) survey: elktoe, *Alasmidonta marginata* (Say 1818), oystermussel, *Epioblasma capsaeformis* (1834), acornshell, *Epioblasma haysiana* (Lea 1834), forkshell, *Epioblasma lewisii* (Walker 1910), green blossom *Epioblasma torulosa gubernaculum* (Reeve 1865), Tennessee heelsplitter, *Lasmigona holstonia* (Lea 1838), littlewing pearlymussel, *Pegias fabula* (Lea 1838), creeper, *Strophitus undulatus* (Say 1817), purple lilliput, *Toxolasma lividus* (Rafinesque 1831), rayed bean, *Villosa fabalis* (Lea 1831), and purple bean, *Villosa perpurpurea* (Lea 1861). Several of these species were headwater species, and were likely impacted by upstream pollution (Ahlstedt and Brown 1979, Dennis 1981). It is likely that the cracking pearlymussel, *Hemistena lata* (Rafinesque 1820), also has been extirpated from the lower river. However, there is a remote chance that *H. lata* still inhabits portions of the river since the species is difficult to detect because they remain deeply buried in the substrate for significant portions of its life-time (Ahlstedt 1991b).

The spectaclecase, *Cumberlandia monodonta* (Say 1829), finerayed pigtoe, *Fusconaia cuneolus* (Lea 1840), and the Tennessee clubshell, *Pleurobema oviforme* (Conrad 1834), could also possibly inhabit the river. Although live specimens of *C. monodonta* were not collected, relic to long-dead shell material was collected, indicating that a population of the species may still persist in the Powell River. The species *F. cuneolus* and *P. oviforme* also could inhabit the river in low densities. The shell morphologies of these species are similar to those of *F. cor* and *L. dolabelloides*, and could have potentially been misidentified during this study. In this survey, *P. alatus* and *Q. pustulosa* were represented by single older specimens indicating a low likelihood that these two species are recruiting and may soon become extirpated from the river.

Differences are apparent when species composition is compared among the three river zones of this study. The species richness in the Upper Zone is significantly less than the Middle and Lower zones (Table 4). These results show that significant declines in mussel diversity have occurred in the river upstream of PRKM 236.3. Data collected during this study suggest that the mussels in the Upper Zone have been subjected to factors that have caused declines in species richness since 1918. Although declines have occurred, it appears that mussels in the Middle and Lower zones have not been as seriously impacted by these factors as has the Upper Zone, and the declines in species richness have not been as severe.

Considering the significant differences between the Upper Zone and Middle and Lower Zones in mussel abundances and species richness, it may be more appropriate to view the Powell

River as consisting of two zones (Upper and Middle/Lower), rather than the three zones examined during this study. The two zones would consist of sites upstream of PRKM 230.6 (Upper Zone) and those downstream of PRKM 230.6 (Middle/Lower Zone). Admittedly the study only surveyed sites to PRKM 104.8, and sites downstream of this may prove to have significantly different abundances or total species present. Additional sampling of sites downstream of this site would be needed to determine if sites downstream of PRKM 104.8 would be included in the Middle/Lower Zone or if an additional zone exists.

As with species richness, significant differences in abundances exist among the survey's three river zones. The Upper Zone has significantly lower mussel abundances than the Middle and Lower zones. Fewer than five mussels were collected at each of the four sites upstream of PRKM 246.9, and these sites will soon be devoid of mussels. Only 38 mussels were collected at PRKM 236.3, and all mussels may soon be extirpated from this site if the apparent trend continues.

The exact causes of these declines are unknown. Other authors have attributed the declines in mussel abundance and diversity in the Virginia portion of the river to coal mining, agriculture and development, but such conclusions cannot be made using data from this survey. It also is unknown whether factors that have caused these declines are still currently impacting the mussel fauna, or if the current abundance and richness of the mussel fauna is a result of previous effects.

Comparison of Sampling Techniques

Estimated abundances for each site varied greatly between estimated derived from qualitative methods and estimates derived from quantitative methods. Generally, abundance estimates varied greatly from site to site (Tables 6 and 9). This is because the qualitative sampling methods used during this study did not just account for time physically surveying each site. The time estimates included total time at each site. This time included what was needed to organize and initiate sampling, as well as identify and measure individuals once sampling had begun. This inflated time measurement for each site can cause CPUE estimates for sites to be lower than if only the time physically searching for mussels was counted. Consequently, it would be inappropriate to make statistical comparisons between CPUE data collected during this study and CPUE data collected during other studies.

Despite showing a slight correlation between CPUE and an abundance estimate determined during quantitative sampling, CPUE values calculated during this study should not be considered an appropriate predictor of mussel abundance at a site. Unfortunately, this is likely the result of the aforementioned shortcomings associated with the sampling techniques utilized during this study. If the time measured during qualitative sampling had more accurately reflected time-searched, rather than time-on-site, a stronger correlation (r) may not have only existed between the abundances calculated using qualitative and quantitative sampling techniques, but the coefficient of determination (r^2) may have proven to be large enough that CPUE could become predictive of quantitative density estimates. Additional CPUE and quantitative abundance data would be required to perform this analysis.

Size-class Structure

Some historical length-frequency data exist for mussels in the Powell River, so it is possible to put current length frequencies into an historical context. Neves et al. (1980) and Wolcott and Neves (1994) recorded mussel lengths during their quantitative surveys, and observed a decline in the number of individuals in smaller age classes of *A. pectorosa* at PRKM 188.8. When visually compared to these previous studies, results of this survey showed a shift to smaller size-classes at this site (Fig. 6). Despite this shift towards smaller size-classes, an overall decrease in abundance appears to have continued, and fewer large individuals (> 100 mm) were collected during the current study. Mussels > 100 mm accounted for greater than 50% of *A. pectorosa* collected by Neves (1980) and Wolcott and Neves (1994), but account for only 5% of those collected during this study. Similar trends were observed with *A. ligamentina* at PRKM 188.8 (Fig. 7). The loss of smaller individuals (< 70 mm) since the late 1970's and loss of larger individuals (> 100 mm) shows that dramatic changes in the size-class structure of these two species at PRKM 188.8 have occurred in recent decades (Fig. 7).

The loss of larger individuals during this study is likely the result of a lack of juvenile recruitment in recent decades, which was evident by the lack of smaller individuals observed by Wolcott and Neves (1994). The shift towards smaller size-classes during this study shows that recruitment has increased in both *A. pectorosa* and *A. ligamentina*, when compared to Wolcott and Neves (1994), and that abundances of both *A. pectorosa* and *A. ligamentina* should increase in the future. These changes in size-class structure and abundances are important because *A. pectorosa* and *A. ligamentina* accounted for a large portion of all mussels collected using qualitative sampling methods during this study, at 46.7% and 24.8% respectively. Consequently, changing abundances of both species would be major driving factors when monitoring changes in overall mussel abundance estimates at PRKM 188.8. Changes in total abundance at PRKM 188.8 are likely to close reflect changes in abundances of those two species, and management decisions based on changing mussel abundances are going to be highly influenced by size-class fluctuations of *A. pectorosa* and *A. ligamentina*.

Evaluation of Sample Sizes

Requisite sample size evaluation showed that the number of quadrats used to sample each site during this study were often insufficient to obtain a stable mean density estimate within ± 10% of the cumulative mean density. Not obtaining stable density estimates, in addition to having large CV's for many sites, questions the accuracy of the density estimates (mussels/m^2) calculated during this study. These concerns could be addressed by re-sampling sites with greater numbers of quadrats to determine the appropriate number of quadrats needed to obtain a stable mean density estimate.

However, because mussels are clumped in distribution and generally have low densities throughout the river, the number of quadrats needed to obtain stable density estimates and smaller CV's could become very large. As the number of quadrats needed to obtain a stable mean increases, the time and funding required to complete the survey will increase. The increase in required funding and time can make a thorough survey less feasible. Therefore, although the numbers of quadrats sampled during this survey were generally inadequate to obtain a stable density estimate, they do provide an approximate density of mussels at a site within a precision of at least 22%. It is important to remember that although the densities estimated during this study are fairly precise, there is no way of knowing their accuracy relative to the true mussel densities at each site without conducting a total mussel census. Conducting a census at each site would cause significant disturbance to mussel habitat and require exorbitant levels of man-power to conduct. Therefore, although it is not possible to verify the accuracy of the resulting estimated densities, quantitative sampling should include at least 150 quadrats at each site because this sample size obtains a precision of < 0.09. The data collected and analyses performed in this study will be used in Chapter 2 of this thesis to evaluate potential long-term sampling protocols, and make recommendations for a monitoring program.

Significance of the Powell River

Despite experiencing significant declines in abundance and diversity during recent decades, the Powell River still contains one of the most diverse mussel fauna in the southeastern United States. Additionally, it contains rare, viable populations of two federally endangered species, *Quadrula sparsa* and *Quadrula intermedia*. The eventual loss of these fauna is not a foregone conclusion, as evidenced by collecting recently recruited individuals of not only commonly found species, but also rare and endangered species. Observing these recently recruited individuals demonstrates that the Powell River still has the potential to rebound from previous and current anthropogenic impacts. For this reasons, it is imperative that research, restoration, and monitoring continue in the Powell River. When the continued existence of unionids is threatened throughout many areas in the country, it would be tragic to allow a mussel assemblage as diverse as that found in the Powell River to be lost.

Conclusions and Recommendations

1) The majority of sites in the Upper Zone were characterized by poor species diversity and low abundances, including PRKMs 269.4, 266.3, 263.0, and 246.9. At these sites, < 5 individuals were found during qualitative sampling, and no mussels were collected using quantitative sampling. Despite having low abundances, these sites should be sampled to document levels of recovery in the future. Initially qualitative sampling would be sufficient to monitor the presence of mussel species. If substantial populations reappear at these sites, quantitative sampling to determine abundances would be appropriate.

Conducting quantitative sampling at these sites prior to the documentation of a mussel assemblage is not warranted.

2) The highest levels of species richness and abundance are found at sites in the Middle and Lower zones of the river, and large stretches of these river zones contain sites that remain un-sampled. Sites within this reach should be selected and sampled using qualitative and quantitative methods to acquire a greater and more comprehensive documentation of the status of the Powell River mussel fauna.

3) Based on finding smaller individuals during qualitative sampling, several common species, including *A. ligamentina*, *A. pectorosa*, *E. dilatata*, and *L. fasciola*, may be recruiting. Also, three federally endangered species, including *E. brevidens*, *Q. intermedia*, and *Q. sparsa*, may have recruited recently based on finding individuals in smaller size-classes. During this study, a wider range of size classes was collected during qualitative sampling; however, the individuals collected during qualitative sampling may not be an accurate representation of the true size-class structure of the river's mussel fauna. Therefore, despite the fact that qualitative sampling found a wider range of size-classes than quantitative sampling, quantitative sampling should be used to monitor changes in size class structure in the future.

4) Quadrat sample sizes were generally inadequate to estimate a stable mean density. Future surveys should use larger sample sizes if possible, in order to obtain abundance estimates that more accurately represent true abundances.

5) A long-term sampling program needs to be developed to adequately monitor changes in mussel species diversity and total mussel densities at selected sites in the Powell River. When developing this monitoring program, biologists and resource managers should

consider statistical validity prior to initiating a survey, and determine whether sampling designs dictated by budget constraints will generate statistically significant results.

6) In order to slow or halt the decline of mussels in the Powell River, suitable release sites for artificially produced juveniles should be identified. Some sites previously used for juvenile release, PRKM 179.9, have exhibited little to no juvenile survival. This study has identified several sites that are currently supporting natural reproduction, including PRKMs 152.6, 153.4, 188.8, and 197.9, and could potentially serve as release sites for artificially propagated juveniles.

Literature Cited

Ahlstedt, S. A. 1983. The molluscan fauna of the Elk River in Tennessee and Alabama. American Malacological Bulletin 1:43-50.

Ahlstedt, S. A. 1991a. A Cumberlandian Mollusk Conservation Program: Mussel surveys in six Tennessee Valley streams. Walkerana 5:123-160.

Ahlstedt, S. A. 1991b. Twentieth century changes in the freshwater mussel fauna of the Clinch River (Tennessee and Virginia). Walkerana 5(13):73-122.

Ahlstedt, S.A., M.T. Fagg, R.S. Butler, and J.F. Connell. 2005. Long-term trend information for freshwater mussel populations at twelve fixed-station monitoring sites in the Clinch and Powell rivers of eastern Tennessee and southwestern Virginia, 1979-2004. Unpublished Report**,** U.S. Fish and Wildlife Service, Cookeville, Tennessee.

Ahlstedt, S. A. and J. D. Tuberville. 1997. 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. Upper Mississippi river conservation Committee, Rock Island, Illinois.

Coker, R.E., A.F. Shira, H.W. Clark, and A.D. Howard. 1921. Natural history and propagation of fresh-water mussels. Bulletin of the Bureau of Fisheries. 37:77-181

Dennis, S. D. 1981. Mussel fauna of the Powell River, Tennessee and Virginia. Sterkiana 71:1-7.

Diamond, J. M., D. W. Bressler, and V. B. Serveiss. 2001. Assessing relationships between human land uses and the decline of native mussels, fish, and macro-invertebrates in the Clinch and Powell River watershed, USA. Environmental Toxicology and Chemistry 21:1147-1155.

Eckert, N. L., J. J. Ferraro, M. J. Pinder, and B. T. Watson. 2004. Freshwater mussel and spiny riversnail survey of SR 833 Bridge and Fletcher Ford, Powell River, Virginia: augmentation monitoring sites - 2004, Unpublished Report, Virginia Department of Game and Inland Fisheries Wildlife Diversity Division.

Jenkinson, J. J. and S. A. Ahlstedt. 1988. Semi-quantitative reassessment of the freshwater mussel fauna in the Powell River, Tennessee and Virginia. Tennessee Valley Authority (TVA), River Basin Operations/Water Resources. Knoxville, Tennessee.

Layzer, J. B. and Khym, J. R. 2005. Fish hosts for glochidia of the pheasantshell, *Actinonaias pectorosa*. Walkerana 14:79-85.

Lefevre, G. and W.C. Curtis. 1910. Reproduction and parasitism in the Unionidae. Journal of Experimental Zoology 9:79-115

Miller, A. C. and B. S. Payne. 1988. The need for quantitative sampling to characterize size demography and density of freshwater mussel communities. American Malacological Bulletin 6:49-54.

Neves, R. J. 1991. Mollusks. Virginia's endangered species. Department of Game and Inland Fisheries. K. Terwilliger. Blacksburg, McDonald and Woodward Publishing Company**:**251-320.

Neves, R.J., A.E. Bogan, J. D. Williams, S. A. Alstedt, and P.W. Hartfield. 1997. Status of aquatic mollusks in the southeastern United States: A downward spiral of diversity. In: G.W. Bense and D. E. Collins, editors. Aquatic fauna in peril: The southeaster perspective. Special publication 1, Southeastern Aquatic Research Institute, Lenz Design and Communications, Decatur, Georgia: 43-85.

Neves, R. J., G. B. Pardue, E. F. Benfield, and S. D. Dennis. 1980. An evaluation of endangered mollusks in Virginia. Final Report, Virginia Commission of Game and Inland Fisheries, Project No. E-F-1, Richmond, Virginia.

Ortmann, A. E. 1918. The nayades (freshwater mussels) of the upper Tennessee drainage. Proceedings of the American Philosophical Society 57:521-626.

Parmalee and Bogan. 1998. Freshwater mussels of Tennessee. Knoxville, The University of Tennessee Press.

Reuling, F.H. 1919. Acquired immunity to an animal parasite. Journal of Infectuous Diseases 24:337-346

Strayer, D. L. and D. R. Smith 2003. A guide to sampling freshwater mussel populations. Bethesda, Maryland, American Fisheries Society.

Tennessee Department of Environment and Conservation, Division of Water Pollution Control. 2007. Powell River Watershed (06010206) of the Tennessee River Basin Watershed water quality management plan. Knoxville, Tennessee.

Williams, J. D., M. L. Warren Jr., K. S. Cummings, J. L. Harris, and R. J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries. 18: 6-22.

Wolcott, L. T. and R. J. Neves. 1994. Survey of the freshwater mussel fauna of the Powell River, Virginia. Banisteria 3:1-14.

Table 1. Survey sites, zone classification, and survey methods used in the Powell River, Virginia and Tennessee, during 2008 and 2009. PRM = Powell River Mile, PRKM = Powell River Kilometer.

Zone	**Site Number**	**PRM**	**PRKM**	**Site**	**Qualitative**	**Quantitative**
Upper	1	167.4	269.4	Dryden	X	X
Upper	2	165.5	266.3	Rte. 619 Bridge	X	X
Upper	3	163.4	263.0	Swimming Hole	X	X
Upper	4	153.4	246.9	Shafer Ford	X	X
Upper	5	146.8	236.3	Cheekspring Ford	X	X
Upper	6	143.5	230.9	Sewell Bridge	X	X
Upper	7	128.4	206.6	Hall Ford	X	X
Middle	8	123.5	198.8	Snodgrass Ford	X	X
Middle	9	123.0	197.9	Island Below Snodgrass	X	X
Middle	10	120.2	193.4	Rte. 833 Bridge	X	X
Middle	11	117.3	188.8	Fletcher Ford	X	X
Middle	12	112.3	180.7	Bales Ford	X	X
Middle	13	111.8	179.9	Fugate Ford	X	X
Middle	14	106.5	171.4	McDowell Shoal	X	X
Lower	15	99.2	159.6	Buchanan Ford	X	X
Lower	16	95.3	153.4	Bar above Brooks Bridge	X	X
Lower	17	95.1	153.0	Brooks Bridge	X	X
Lower	18	94.8	152.6	Bar below Brooks Bridge	X	X
Lower	19	84.6	136.2	Yellow Shoals		X
Lower	20	84.4	135.8	Below Yellow Shoals	X	X
Lower	21	80.4	129.4	Double S Bend	X	X
Lower	22	65.1	104.8	Above Rte. 25E Bridge	X	X

Table 2. Sites selected for survey during previous freshwater mussel surveys. PRM = Powell River Mile, PRKM = Powell River Kilometer

Site Number	PRM	PRKM	Neves et al. (1980)	Dennis (1981)	Ahlstedt (1991a)	Wolcott and Neves (1994)	Eckert et al. (2004)	Ahlstedt (2005)
1	**167.4**	**269.4**	-	X	X	X	-	-
2	**165.5**	**266.3**	-	X	-	X	-	-
3	**163.4**	**263.0**	-	-	X	X	-	-
4	**153.4**	**246.9**	-	-	X	X	-	-
5	**146.8**	**236.3**	-	-	-	X	-	-
6	**143.5**	**230.9**	-	-	X	X	-	X
7	**128.4**	**206.6**	X	-	X	X	-	-
8	**123.5**	**198.8**	-	-	-	-	-	-
9	**123.0**	**197.9**	-	-	X	X	-	-
10	**120.2**	**193.4**	X	X	-	X	X	X
11	**117.3**	**188.8**	X	X	X	X	X	X
12	**112.3**	**180.7**	-	-	X	-	-	-
13	**111.8**	**179.9**	-	X	X	-	-	X
14	**106.5**	**171.4**	X	X	X	-	-	X
15	**99.2**	**159.6**	X	X	X	-	-	X
16	**95.3**	**153.4**	-	-	-	-	-	-
17	**95.1**	**153.0**	-	-	-	-	-	-
18	**94.8**	**152.6**	-	-	X	-	-	-
19	**84.6**	**136.2**	-	-	X	-	-	-
20	**84.4**	**135.8**	-	-	-	-	-	-
21	**80.4**	**129.4**	-	-	X	-	-	-
22	**65.1**	**104.8**	-	-	X	-	-	-

Table 3. Total mussels observed and catch per unit effort (mussels/h and species/h) at 21 sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. PH = person-hours on site PRKM = Powell River Kilometer

Zone	**Site Number**	**PRKM**	**Total Mussels**	**Total Species**	**PH**	**mussels/h**	**species/h**
Upper	1	**269.4**	4	3	3.0	1.33	1.00
Upper	2	**266.3**	2	2	3.0	0.67	0.67
Upper	3	**263.0**	1	1	3.0	0.33	0.33
Upper	4	**246.9**	4	1	4.5	0.89	0.22
Upper	5	**236.3**	38	4	7.5	5.07	0.53
Upper	6	**230.9**	85	12	18.8	4.53	0.64
Upper	7	**206.6**	79	10	8.8	9.03	1.14
Middle	8	**198.8**	25	7	9.0	2.78	0.78
Middle	9	**197.9**	2579	22	123.0	20.97	0.18
Middle	10	**193.4**	1738	20	223.0	7.79	0.09
Middle	11	**188.8**	948	18	189.0	5.02	0.10
Middle	12	**180.7**	540	21	62.5	8.64	0.34
Middle	13	**179.9**	178	14	78.0	2.28	0.18
Middle	14	**171.4**	286	17	101.0	2.83	0.17
Lower	15	**159.6**	513	15	118.0	4.35	0.13
Lower	16	**153.4**	1084	20	58.0	18.69	0.34
Lower	17	**153.0**	267	13	66.5	4.02	0.20
Lower	18	**152.6**	4297	23	194.3	22.12	0.12
Lower	20	**135.8**	553	20	32.0	17.28	0.63
Lower	21	**129.4**	1286	16	61.5	20.91	0.26
Lower	22	**104.8**	581	18	39.0	14.90	0.46
		Overall:	**15088**	**29**	**1403.25**	**10.75**	**0.40**

Table 4. Differences in mussel CPUE, species richness using quantitative and qualitative sampling, and mussel density (mussel/m^2) within the pre-defined Upper, Middle, and Lower zones of the Powell River, Virginia and Tennessee, during 2008 and 2009. Sites were divided into zones as follows (Upper: PRKM's 269.4, 266.3, 263.0, 246.9, 236.3, 230.9, and 206.6; Middle: 198.8, 197.9, 193.4, 188.8, 180.7, 179.9, and 171.4; Lower: 159.6, 153.4, 153.0, 152.6, 136.2, 135.8, 129.4, and 104.8) [1] Like letters in columns are not significantly different ($p > 0.05$) per Fisher's LSD *post hoc* analysis.

Mussel CPUE		**Quantitative Species Richness**		**Qualitative Species Richness**		**Mussel Density**	
$F = 9.77$ $p < 0.05$		$F = 20.01$ $p < 0.05$		$F = 19.95$ $p < 0.05$		$F = 7.14$ $p < 0.05$	
Zone	**Mean**	**Zone**	**Mean**	**Zone**	**Mean**	**Zone**	**Mean**
Upper	3.12a[1]	**Upper**	1.42a	**Upper**	4.71a	**Upper**	0.12a
Middle	6.83 ab	**Middle**	6.87b	**Middle**	16.75b	**Middle**	0.95b
Lower	16.32b	**Lower**	8.14b	**Lower**	18.33b	**Lower**	1.07b

Table 5. Levels of power achieved during this studies as determined through *post hoc* power analysis of ANOVA of mussel density, species richness during qualitative and quantitative sampling, and mussel CPUE within the Upper, Middle, and Lower zones using α-values 0.05, 0.10, and 0.15. Higher levels of power show a statistical test's ability to detect significant differences among zones. Mussel density = mussels/m^2, Mussel CPUE = mussels/h

α	**Mussel Density**	**Quantitative Species Richness**	**Qualitative Species Richness**	**Mussel CPUE**
0.05	0.88	0.99	0.99	0.94
0.10	0.94	0.99	0.99	0.97
0.15	0.96	0.99	0.99	0.98

Table 6. Densities (mussels/m^2) of mussel species collected during quantitative sampling at 22 sites in the Powell River, Virginia and Tennessee, 2008 and 2009.

	Powell River Site (Site Number and PRKM)										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Species	**269.4**	**266.3**	**263**	**246.9**	**236.3**	**230.9**	**206.6**	**198.8**	**197.9**	**193.4**	**188.8**
Actinonaias ligamentina	-	-	-	-	0.04	-	0.12	0.12	0.27	0.06	0.31
Actinonaias pectorosa	-	-	-	-	0.04	0.16	0.2	-	1.14	0.94	0.5
Amblema plicata	-	-	-	-	-	-	0.04	-	-	-	-
Cyclonaias tuberculata	-	-	-	-	-	-	-	-	0.04	-	0.03
Dromus dromas	-	-	-	-	-	-	-	-	-	0.02	-
Elliptio dilatata	-	-	-	-	0.04	-	0.08	-	0.4	0.19	0.13
Elliptio crassidens	-	-	-	-	-	-	-	-	-	-	-
Epioblasma brevidens	-	-	-	-	-	-	-	-	-	-	0.03
Fusconaia subrotunda	-	-	-	-	-	-	-	-	0.08	0.02	-
Lampsilis fasciola	-	-	-	-	-	-	-	0.08	-	-	0.08
Lampsilis ovata	-	-	-	-	-	-	-	-	-	0.02	-
Lasmigona costata	-	-	-	-	-	-	0.04	-	-	-	-
Lemiox rimosus	-	-	-	-	-	-	-	-	-	0.02	-
Lexingtonia dolabelloides	-	-	-	-	-	-	-	-	-	0.17	-
Medionidus conradicus	-	-	-	-	-	-	-	-	-	-	0.16
Plethobasus cyphyus	-	-	-	-	-	-	-	-	-	-	-
Ptychobranchus fasciolaris	-	-	-	-	0.08	-	-	-	0.04	-	0.03
Quadrula c. strigillata	-	-	-	-	-	-	-	-	-	-	-
Villosa iris	-	-	-	-	-	-	-	-	-	0.08	0.08
Villosa vanuxemensis	-	-	-	-	-	-	-	-	-	-	-
Mean Density (mussels/m^2):	**0.00**	**0.00**	**0.00**	**0.00**	**0.20**	**0.16**	**0.48**	**0.20**	**1.97**	**1.52**	**1.35**
Total Quadrats:	**100**	**100**	**100**	**50**	**100**	**100**	**100**	**102**	**102**	**192**	**153**
Total Species:	**0**	**0**	**0**	**0**	**4**	**1**	**5**	**2**	**6**	**9**	**9**

Table 6. Continued.

Species	Powell River Site (Site Number and PRKM)										
	12	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
Species	**180.7**	**179.9**	**171.4**	**159.6**	**153.4**	**153**	**152.6**	**136.2**	**135.8**	**129.4**	**104.8**
Actinonaias ligamentina	0.16	0.12	0.14	0.19	0.16	0.24	0.48	0.27	0.05	0.11	0.05
Actinonaias pectorosa	0.03	0.37	0.2	0.27	0.4	0.05	0.53	0.37	0.11	1.12	0.24
Amblema plicata	0.03	-	-	-	0.03	0.13	0.11	-	0.03	-	0.03
Cyclonaias tuberculata	-	0.07	-	-	-	0.11	0.05	0.05	0.03	0.05	0.03
Dromus dromas	-	0.03	-	-	-	-	0.03	-	-	-	-
Elliptio dilatata	0.03	0.12	0.08	0.03	-	-	-	-	0.05	0.11	-
Elliptio crassidens	-	-	-	-	-	-	-	-	0.03	-	-
Epioblasma brevidens	-	-	-	0.03	-	-	0.03	0.03	-	0.03	0.03
Fusconaia subrotunda	0.03	-	-	-	0.03	-	-	-	-	-	-
Lampsilis fasciola	0.03	0.03	-	-	0.03	-	0.03	-	0.05	0.08	0.05
Lampsilis ovata	-	-	-	0.03	-	-	-	-	-	-	-
Lasmigona costata	-	-	-	-	-	-	-	-	-	-	-
Lemiox rimosus	-	-	-	-	-	-	-	-	-	-	-
Lexingtonia dolabelloides	-	-	-	-	-	-	-	-	-	-	-
Medionidus conradicus	-	0.12	0.14	0.05	0.05	0.03	0.05	0.45	0.08	0.67	0.13
Plethobasus cyphyus	0.03	-	-	-	-	0.03	-	-	-	-	-
Ptychobranchus fasciolaris	0.03	-	-	-	0.05	0.03	0.03	-	0.05	0.08	0.03
Quadrula c. strigillata	0.03	-	-	-	-	-	-	-	-	-	-
Villosa iris	0.08	0.06	0.02	-	-	0.05	-	0.08	-	-	-
Villosa vanuxemensis	0.03	-	-	-	-	0.03	-	-	-	-	0.03
Mean Density (mussels/m^2):	**0.51**	**0.92**	**0.58**	**0.60**	**0.75**	**0.70**	**1.34**	**1.25**	**0.48**	**2.25**	**0.62**
Total Quadrats:	**150**	**130**	**200**	**150**	**150**	**151**	**150**	**150**	**150**	**150**	**150**
Total Species:	**10**	**8**	**5**	**6**	**6**	**9**	**9**	**9**	**7**	**8**	**9**

Table 7. Number of quadrats, mussel density, standard deviation, precision, and evaluation of mean density stability from samples taken in the Powell River, Virginia and Tennessee, 2008 and 2009. Mussel Density = mussels/m^2, SD = standard deviation, CV = coefficient of variation, (*) = no mussels collected during quantitative sampling, (+) = mussels were collected, but sample size was insufficient to achieve a stable mean, (number) = approximate number of quadrats needed to achieve a stable mean density within ± 10% of the cumulative mean density. PRKM = Powell River Kilometer

Site	# of Quadrats	Mean	SD	CV	Precision	Stable Mean
269.4	100	0.00	0.00	-	-	**
266.3	100	0.00	0.00	-	-	**
263.0	100	0.00	0.00	-	-	**
246.9	50	0.00	0.00	-	-	**
236.3	100	0.20	0.26	1.31	0.21	*
230.9	100	0.16	0.20	1.23	0.22	*
206.6	100	0.48	0.43	0.90	0.17	*
198.8	102	0.20	0.26	1.31	0.21	*
197.9	102	1.97	1.06	0.54	0.12	*
193.4	192	1.52	0.71	0.47	0.09	*
188.8	153	1.35	0.62	0.46	0.11	*
180.7	150	0.51	0.39	0.76	0.14	*
179.9	130	0.92	0.52	0.57	0.13	100
171.4	200	0.58	0.41	0.70	0.11	150
159.6	150	0.60	0.37	0.62	0.13	130
153.4	150	0.75	0.52	0.70	0.12	*
153.0	151	0.70	0.47	0.68	0.13	*
152.6	150	1.34	0.56	0.42	0.11	80
136.2	150	1.25	0.74	0.59	0.11	*
135.8	150	0.48	0.38	0.80	0.14	*
129.4	150	2.25	1.06	0.47	0.09	*
104.8	150	0.62	0.44	0.72	0.13	130

Table 8. Mussel species collected during qualitative and quantitative sampling in the Powell River, Virginia and Tennessee, during 2008 and 2009.

Scientific Name[1]	**Common Name**
Actinonaias ligamentina	mucket
Actinonaias pectorosa	pheasantshell
Amblema plicata	three-ridge
Cyclonaias tuberculata	purple wartyback
Dromus dromas[2]	dromedary pearlymussel
Elliptio dilatata	spike
Elliptio crassidens	elephant-ear
Epioblasma brevidens[2]	Cumberlandian combshell
Epioblasma triquetra[3]	snuffbox
Fusconaia barnesiana	Tennessee pigtoe
Fusconaia cor[2]	shiny pigtoe
Fusconaia subrotunda	long-solid
Lampsilis fasciola	wavy-rayed lampmussel
Lampsilis ovata	pocketbook
Lasmigona costata	fluted-shell
Ligumia recta	black sandshell
Lemiox rimosus[2]	birdwing pearlymussel
Lexingtonia dolabelloides	slabside pearlymussel
Medionidus conradicus	Cumberland moccasinshell
Potamilus alatus	pink heelsplitter
Plethobasus cyphyus[3]	sheepnose
Ptychobranchus fasciolaris	kidneyshell
Ptychobranchus subtentum[3]	fluted kidneyshell
Quadrula cylindrica strigillata[2]	rough rabbitsfoot
Quadrula intermedia[2]	Cumberland monkeyface
Quadrula pustulosa	pimpleback
Quadrula sparsa[2]	Appalachian monkeyface
Villosa iris	rainbow mussel
Villosa vanuxemensis	mountain creekshell

[1] Nomenclature from Parmalee and Bogan (1998)
[2] Federally endangered species
[3] Federal candidate species

Table 9. Mussels collected during qualitative sampling of 21 sites in the Powell River, Virginia and Tennessee, 2008 and 2009.

	Powell River Site (Site Number and PRKM)										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Species	**269.4**	**266.3**	**263.0**	**246.9**	**236.3**	**230.9**	**206.6**	**198.8**	**197.9**	**193.4**	**188.8**
Actinonaias ligamentina	-	-	-	-	7	11	23	9	609	192	235
Actinonaias pectorosa	1	-	-	4	28	53	34	9	1573	1287	443
Amblema plicata	-	-	-	-	-	2	3	1	8	11	34
Cyclonaias tuberculata	-	-	-	-	-	2	4	1	9	9	24
Dromus dromas	-	-	-	-	-	-	-	-	2	7	6
Elliptio dilatata	-	1	-	-	2	3	7	-	246	137	56
Elliptio crassidens	-	-	-	-	-	-	-	-	-	-	-
Epioblasma brevidens	-	-	-	-	-	-	-	-	-	1	6
Epioblasma triquetra	-	-	-	-	-	-	-	-	-	-	-
Fusconaia barnesiana	-	-	-	-	-	-	-	-	3	-	-
Fusconaia cor	-	-	-	-	-	-	-	-	12	4	-
Fusconaia subrotunda	-	-	-	-	1	6	2	-	7	9	3
Lampsilis fasciola	1	-	-	-	-	1	2	-	1	11	5
Lampsilis ovata	-	-	-	-	-	1	-	-	1	2	12
Lasmigona costata	-	-	-	-	-	-	1	-	1	-	-
Ligumia recta	-	-	-	-	-	-	-	-	-	-	-
Lemiox rimosus	-	-	-	-	-	-	-	-	3	2	4
Lexingtonia dolabelloides	-	-	-	-	-	-	-	-	3	1	-
Medionidus conradicus	-	-	-	-	-	-	1	1	49	33	63
Potamilus alatus	-	-	-	-	-	-	-	-	-	-	-
Plethobasus cyphyus	-	-	-	-	-	-	-	1	5	1	6
Ptychobranchus fasciolaris	2	1	1	-	-	-	2	-	8	14	23
Ptychobranchus subtentum	-	-	-	-	-	-	-	-	26	1	-
Quadrula c. strigillata	-	-	-	-	-	3	-	-	-	-	-
Quadrula intermedia	-	-	-	-	-	1	-	-	7	6	6
Quadrula pustulosa	-	-	-	-	-	-	-	-	2	-	-
Quadrula sparsa	-	-	-	-	-	1	-	-	-	1	2
Villosa iris	-	-	-	-	-	1	-	3	3	9	14
Villosa vanuxemensis	-	-	-	-	-	-	-	-	1	-	6
Total Mussels:	**4**	**2**	**1**	**4**	**38**	**85**	**79**	**25**	**2579**	**1738**	**948**
Total Species:	**3**	**2**	**1**	**1**	**4**	**12**	**10**	**7**	**22**	**20**	**18**

Table 9. Continued.

	Powell River Site (Site Number and PRKM)									
	12	**13**	**14**	**15**	**16**	**17**	**18**	**20**	**21**	**22**
Species	**180.7**	**179.9**	**171.4**	**159.6**	**153.4**	**153.0**	**152.6**	**135.8**	**129.4**	**104.8**
Actinonaias ligamentina	291	57	88	145	388	132	1717	153	80	113
Actinonaias pectorosa	64	59	74	270	485	17	1776	159	646	235
Amblema plicata	42	11	35	15	26	35	194	66	12	48
Cyclonaias tuberculata	17	7	14	7	48	53	146	53	29	45
Dromus dromas	6	7	5	8	10	2	61	2	-	1
Elliptio dilatata	22	17	12	10	4	-	52	17	59	24
Elliptio crassidens	-	-	2	-	-	-	2	1	-	-
Epioblasma brevidens	1	-	1	1	4	2	26	5	12	1
Epioblasma triquetra	-	-	-	-	2	-	4	-	1	-
Fusconaia barnesiana	-	-	-	-	-	-	-	1	-	2
Fusconaia cor	-	1	-	-	-	-	1	1	-	-
Fusconaia subrotunda	-	-	-	-	2	-	1	1	-	-
Lampsilis fasciola	5	2	1	1	6	3	9	5	34	5
Lampsilis ovata	9	-	4	4	15	-	31	5	10	1
Lasmigona costata	3	-	-	4	-	1	12	2	-	5
Ligumia recta	1	-	2	-	1	3	10	2	1	1
Lemiox rimosus	2	-	-	-	-	-	4	-	-	-
Lexingtonia dolabelloides	-	-	-	-	-	-	-	-	-	-
Medionidus conradicus	16	8	24	38	58	9	83	60	358	69
Potamilus alatus	1	-	-	-	-	-	-	-	-	-
Plethobasus cyphyus	32	3	7	-	3	4	33	3	2	2
Ptychobranchus fasciolaris	12	1	5	4	23	2	96	12	38	25
Ptychobranchus subtentum	-	-	-	-	3	-	4	-	1	-
Quadrula c. strigillata	1	-	-	-	1	-	-	1	-	2
Quadrula intermedia	3	3	8	3	3	4	23	-	1	-
Quadrula pustulosa	-	-	-	-	-	-	3	-	-	-
Quadrula sparsa	1	-	-	1	1	-	9	-	-	-
Villosa iris	7	1	3	2	-	-	-	4	-	1
Villosa vanuxemensis	4	1	1	-	1	-	-	-	2	1
Total Mussels:	**540**	**178**	**286**	**513**	**1084**	**267**	**4297**	**553**	**1286**	**581**
Total Species:	**21**	**14**	**17**	**15**	**20**	**13**	**23**	**20**	**16**	**18**

Table 10. Percentage composition all mussels collected during qualitative sampling of 21 sites in the Powell River, Virginia and Tennessee, during 2008 and 2009.

Species	**Number Collected**	**Percent Composition**
Actinonaias ligamentina	4250	28.17
Actinonaias pectorosa	7217	47.83
Amblema plicata	543	3.60
Cyclonaias tuberculata	468	3.10
Dromus dromas	117	0.78
Elliptio dilatata	669	4.43
Elliptio crassidens	5	0.03
Epioblasma brevidens	60	0.40
Epioblasma triquetra	7	0.05
Fusconaia barnesiana	6	0.04
Fusconaia cor	19	0.13
Fusconaia subrotunda	32	0.21
Lampsilis fasciola	92	0.61
Lampsilis ovata	95	0.63
Lasmigona costata	29	0.19
Ligumia recta	21	0.14
Lemiox rimosus	15	0.10
Lexingtonia dolabelloides	4	0.03
Medionidus conradicus	870	5.77
Potamilus alatus	1	0.01
Plethobasus cyphyus	102	0.68
Ptychobranchus fasciolaris	269	1.78
Ptychobranchus subtentum	35	0.23
Quadrula c. strigillata	8	0.05
Quadrula intermedia	68	0.45
Quadrula pustulosa	5	0.03
Quadrula sparsa	16	0.11
Villosa iris	48	0.32
Villosa vanuxemensis	17	0.11
Total:	**15,088**	**100.00**

Table 11. Percentage composition of mussels collected during quantitative sampling in the Powell River, Virginia and Tennessee, during 2008 and 2009.

Species	**Number Collected**	**Percent Composition**
Actinonaias ligamentina	104	17.93
Actinonaias pectorosa	242	41.72
Amblema plicata	14	2.41
Cyclonaias tuberculata	16	2.76
Dromus dromas	3	0.52
Elliptio dilatata	47	8.10
Elliptio crassidens	1	0.17
Epioblasma brevidens	6	1.03
Epioblasma triquetra	0	0.00
Fusconaia barnesiana	0	0.00
Fusconaia cor	0	0.00
Fusconaia subrotunda	5	0.86
Lampsilis fasciola	16	2.76
Lampsilis ovata	2	0.34
Lasmigona costata	1	0.17
Ligumia recta	0	0.00
Lemiox rimosus	1	0.17
Lexingtonia dolabelloides	0	0.00
Medionidus conradicus	82	14.14
Potamilus alatus	0	0.00
Plethobasus cyphyus	2	0.34
Ptychobranchus fasciolaris	15	2.59
Ptychobranchus. subtentum	0	0.00
Quadrula c. strigillata	1	0.17
Quadrula intermedia	0	0.00
Quadrula pustulosa	0	0.00
Quadrula sparsa	0	0.00
Villosa iris	18	3.10
Villosa vanuxemensis	4	0.69
Total:	**580**	**100.00**

Figure 1. Sites surveyed in the Powell River, Virginia and Tennessee, during the summers of 2008 and 2009.

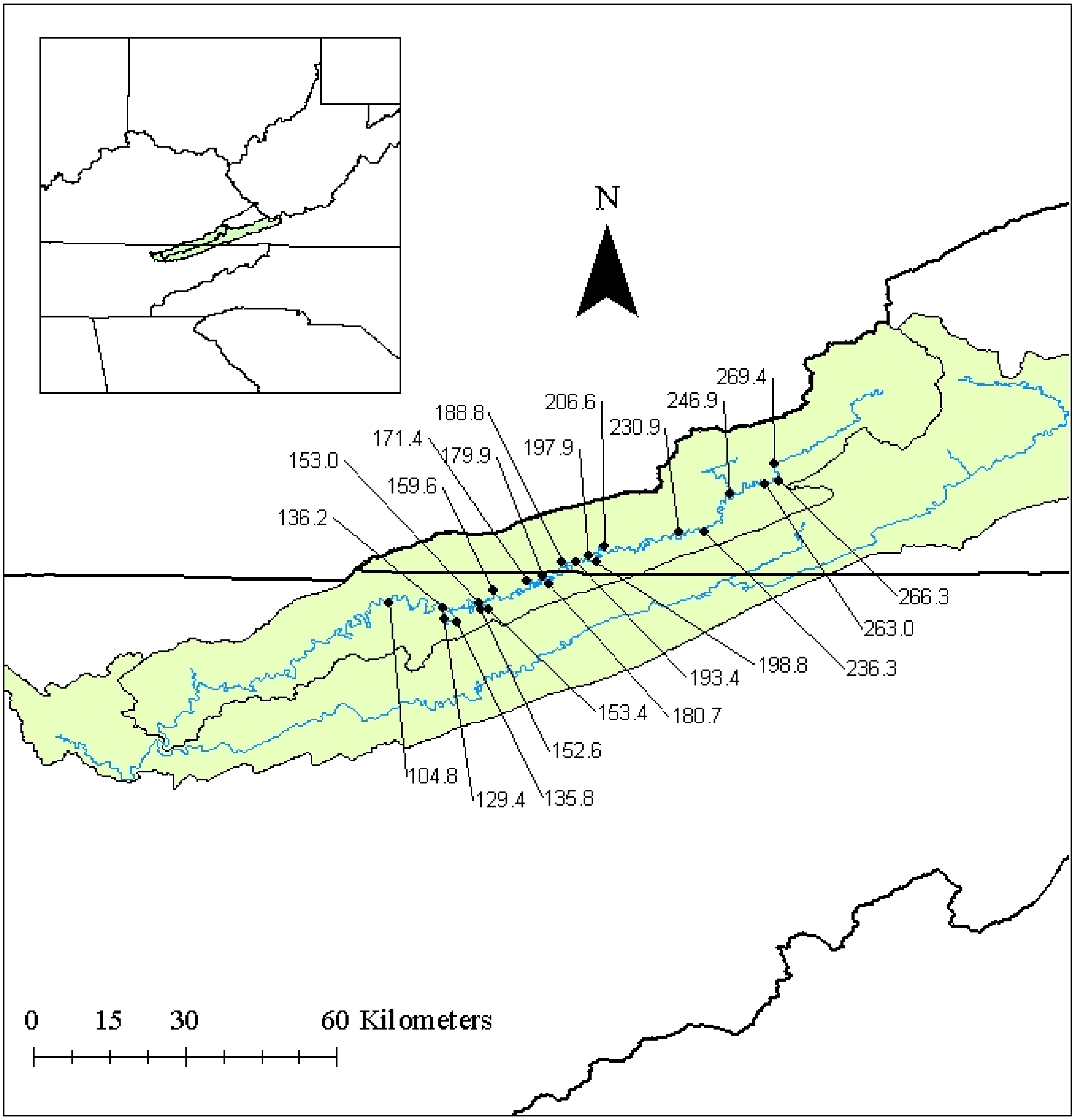


Figure 2. Plot of total mussel CPUE (mussels/h) at sites (PRKM) in the Powell River, Virginia and Tennessee, during qualitative sampling in 2008 and 2009. Qualitative sampling was not conducted at PRKM 136.2 due to resource constraints. Correlation between mussel CPUE and PRKM: $r = 0.66$, $p < 0.05$.

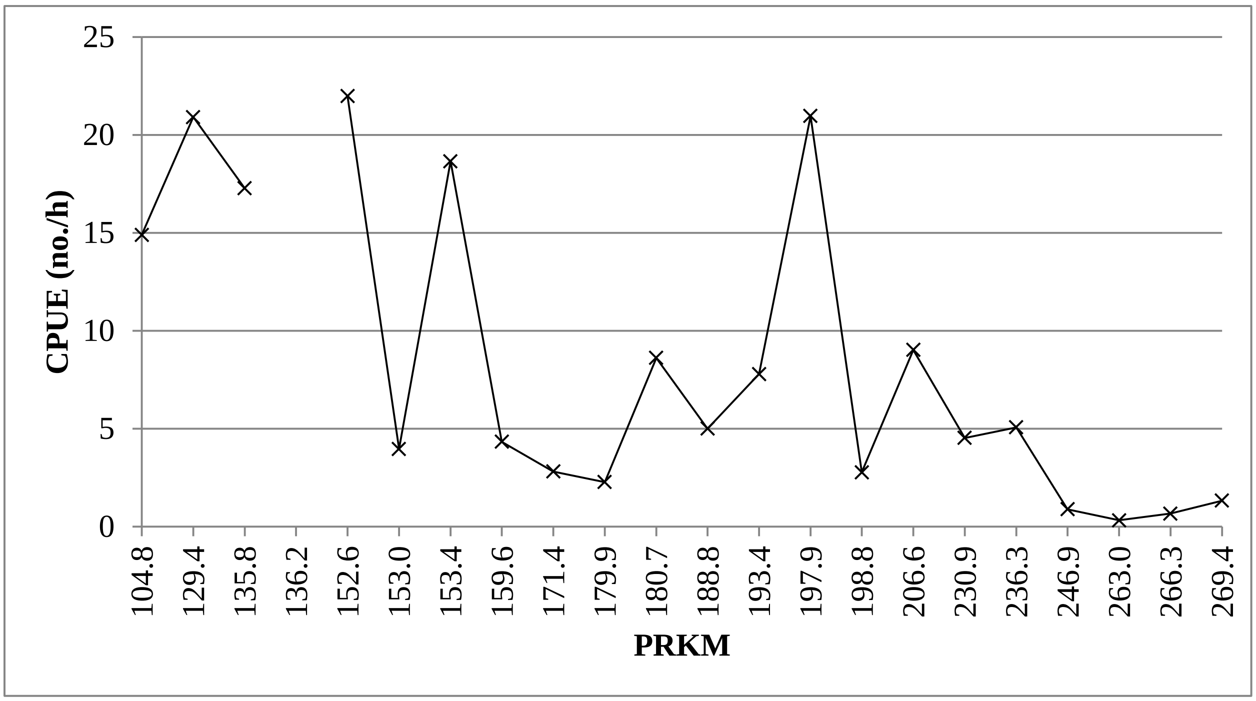


Figure 3. Plot of estimated mussel densities at sites (PRKM) in the Powell River, Virginia and Tennessee, during quantitative sampling in 2008 and 2009. Correlation between mussel density and PRKM: $r = 0.57$, $p < 0.05$.

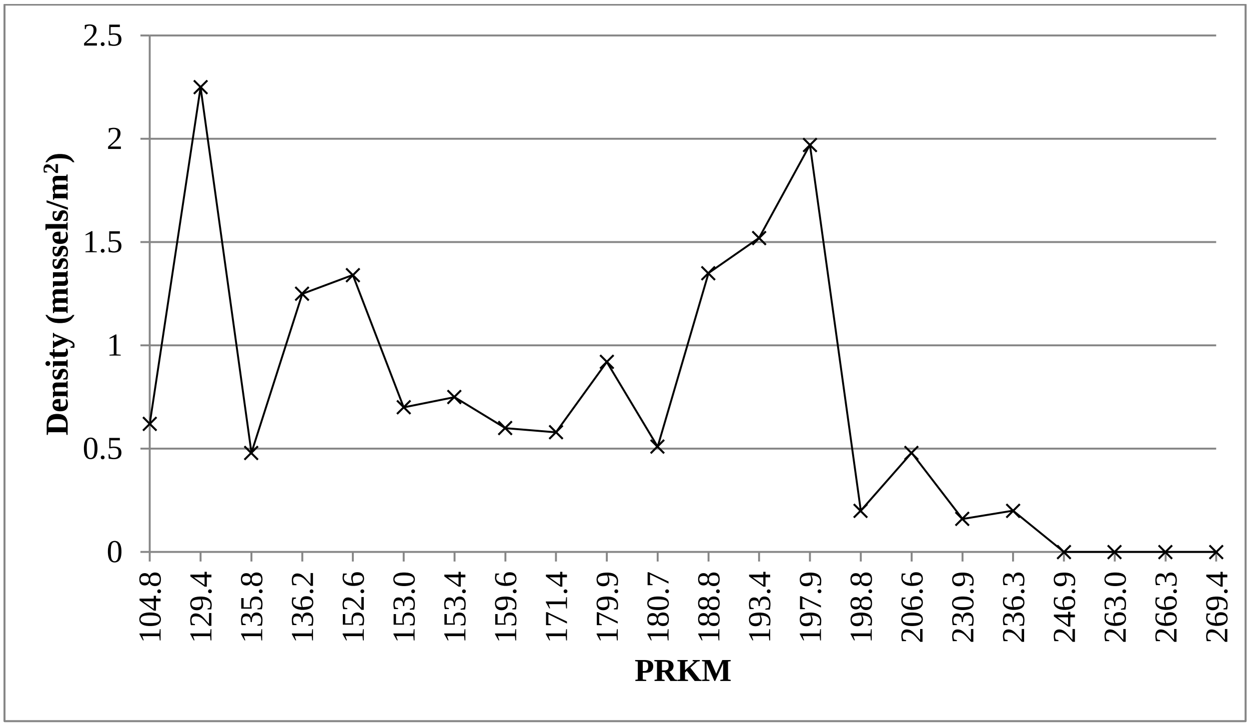


Figure 4. Plots of number of species observed at selected sites (PRKM) in the Powell River, Virginia and Tennessee, during qualitative and quantitative surveys in 2008 and 2009. Qualitative sampling was not conducted at PRKM 136.2 due to resource constraints. Dashed line = species observed during qualitative sampling; solid line = species observed during quantitative sampling. Correlation between number of species and PRKM: qualitative sampling: $r = 0.79$, $p < 0.05$. quantitative sampling: $r = 0.83$, $p < 0.05$.

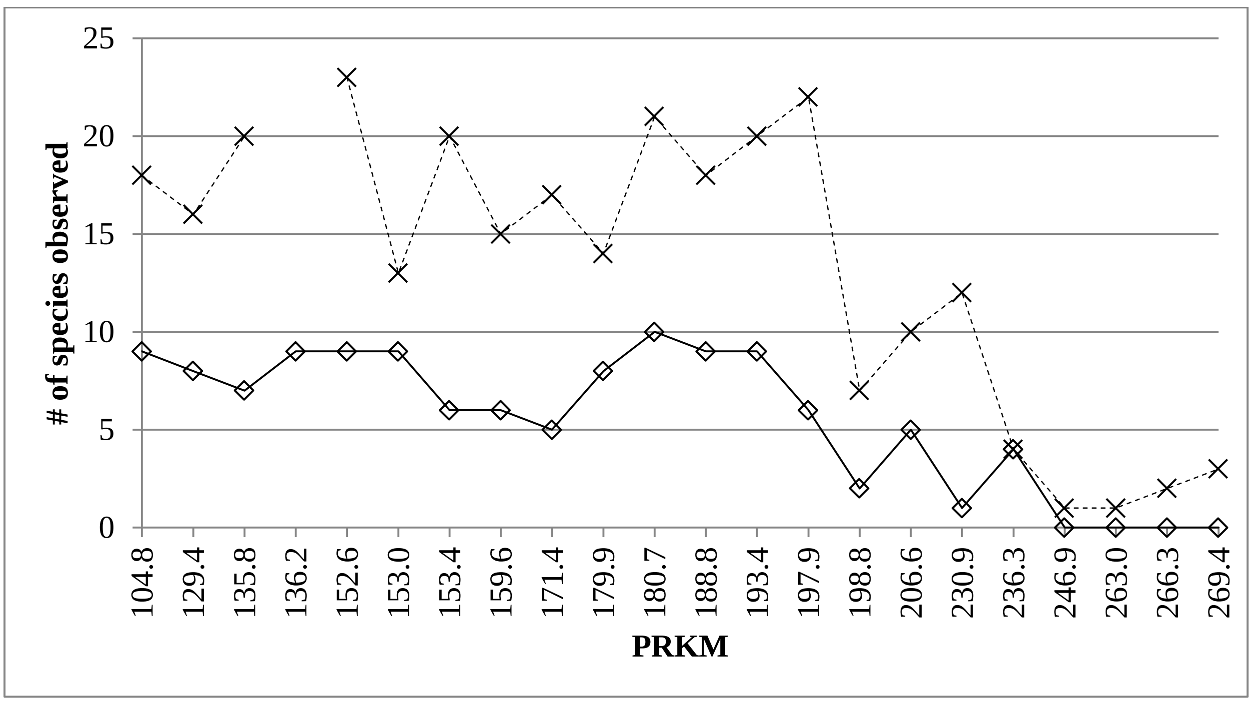


Figure 5. Correlation and simple linear regression of mussel abundances as estimated through qualitative sampling methods against mussel abundances estimated through quantitative sampling methods. Correlation analysis: $r = 0.46$, $p < 0.05$; simple Linear Regression: $r^2 = 0.215$, $p > 0.05$; regression line: $y = 0.0543x + 0.2647$.

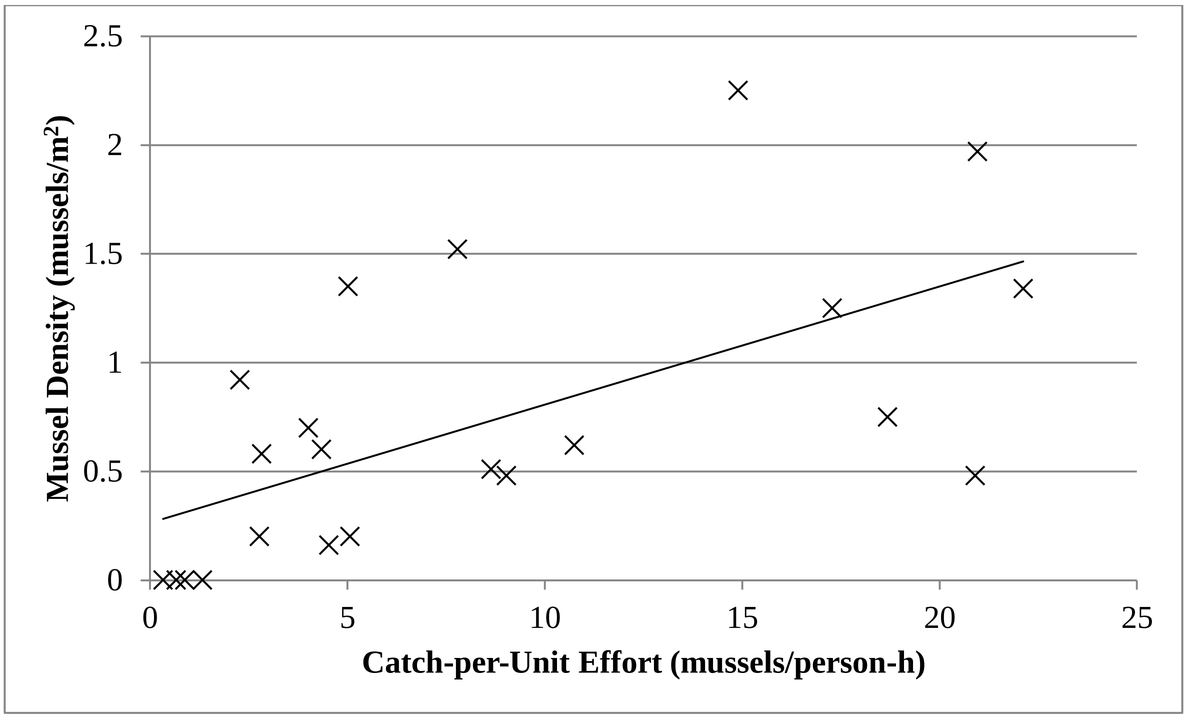


Figure 6. Comparisons of length frequency histograms of *Actinonaias pectorosa* collected through quantitative methods from: a) 2008 and 2009; b)1988 and 1989 (Wolcott and Neves 1994); and c) 1978 and 1979 (Neves 1980).

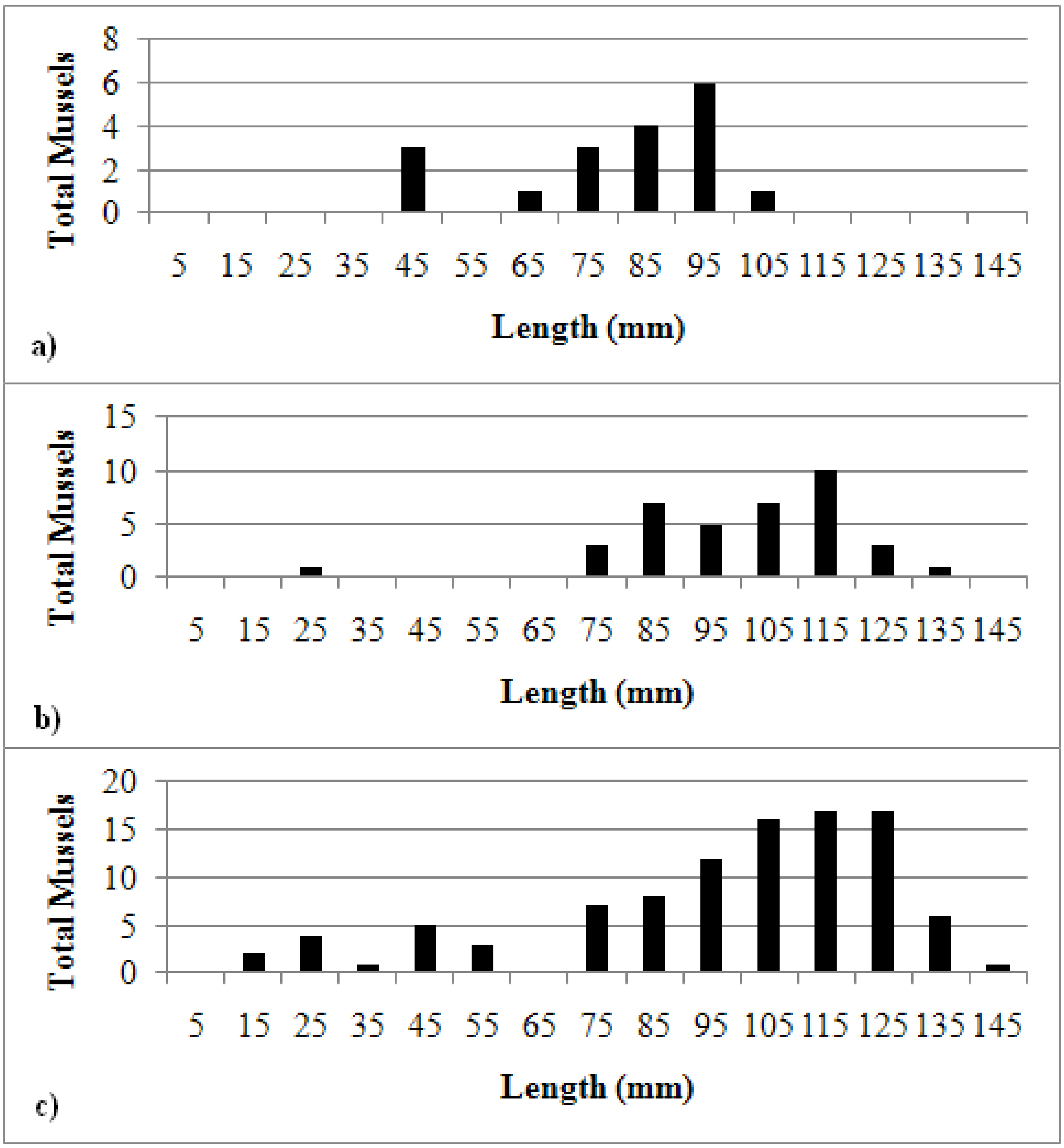
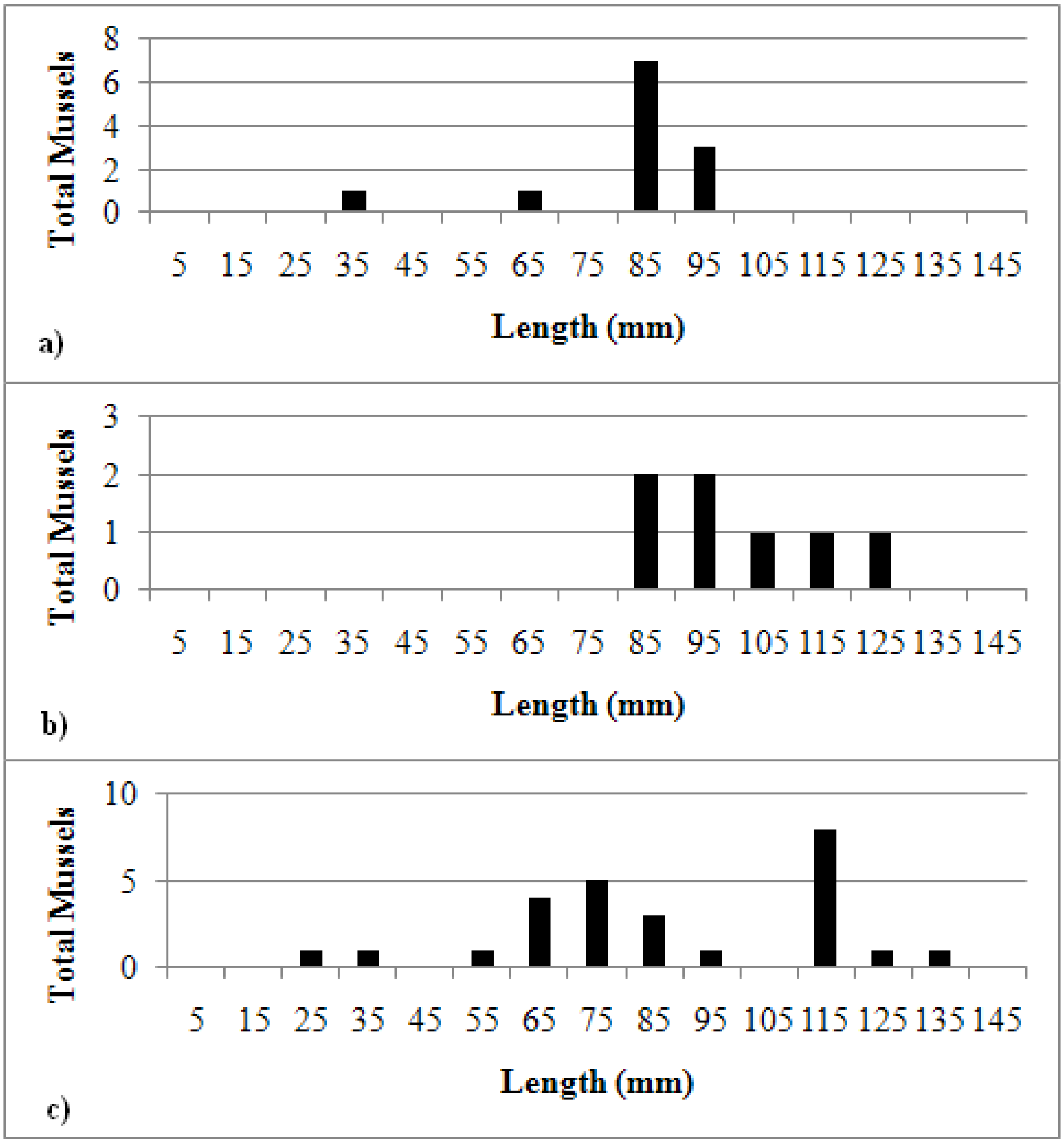


Figure 7. Comparisons of length frequency histograms of *Actinonaias ligamentina* collected through quantitative methods from: a) 2008 and 2009; b)1988 and 1989 (Wolcott and Neves 1994); and c) 1978 and 1979 (Neves 1980).



APPENDIX A

GPS Markers for Surveyed Sites in the Powell River, Virginia and Tennessee.

Table 1. Site locations described by Powell River kilometer (PRKM), commonly-used site name, and GPS coordinates for the four corners of the surveyed area.

PRKM	Site Name	GPS Coordinates	
269.4	Dryden	N 36.78298, W -82.92490	N 36.78293, W -82.92452
		N 36.78159, W -82.92495	N 36.78237, W -82.92431
266.3	Rte. 619 Bridge	N 36.76568, W -82.92158	N 36.76552, W -82.92140
		N 36.76556, W -82.92189	N 36.76531, W -82.92178
263	Swimming Hole	N 36.76104, W -82.94687	N 36.76076, W -82.94672
		N 36.76093, W -82.94767	N 36.76075, W -82.94761
246.9	Shafer Ford	N 36.72342, W -83.00205	N 36.72341, W -83.02203
		N 36.72300, W -83.02220	N 36.77295, W -83.02208
236.3	Cheekspring Ford	N 36.67591, W -83.05487	N 36.67579, W -83.05490
		N 36.67636, W -83.05540	N 36.67620, W -83.05554
230.9	Sewell Bridge	N 36.66301, W -83.09427	N 36.66302, W -83.09388
		N 36.66183, W -83.09450	N 36.66209, W -83.09404
206.6	Hall Ford	N 36.63610, W -83.22828	N 36.63586, W -83.22841
		N 36.63620, W -83.22860	N 36.63601, W -83.22876
198.8	Snodgrass Ford	N 36.61965, W -83.24989	N 36.61940, W -83.24996
		N 36. 61992, W -83.25065	N 36.61966, W -83.25076
197.9	Island Below Snodgrass	N 36.61855, W -83.25779	N 36.61832, W -83.25779
		N 36.61875, W -83.25888	N 36.61839, W -83.25868
193.4	Rte. 833 Bridge	N 36.371694, W -83.17513	N 36.371594, W -83.17404
		N 36.371242, W -83.17989	N 36.371191, W -83.17908

Table 1. Continued.

PRKM	Site Name	GPS Coordinates	
188.8	Fletcher Ford	N 36.36136, W -83.17384	N 36.36130, W -83.17402
		N 36.36181, W -83.17434	N 36.36179, W -83.17446
180.7	Bales Ford	N 36.58296, W -83.33298	N 36.58293, W -83.33288
		N 36.58209, W -83.33216	N 36.58199, W -83.33175
179.9	Fugate Ford	N 36.58382, W -83.33606	N 36.58384, W -83.33643
		N 36.58575, W -83.33654	N 36.58571, W -83. 33694
171.4	McDowell Shoal	N 36.57497, W -83.36272	N 36.57507, W -83.36245
		N 36.57376, W -83.36155	N 36.57390, W -83.36134
159.6	Buchanan Ford	N 36.55819, W -83.42336	N 36.55806, W -83.42250
		N 36.55861, W -83.42425	N 36.55855, W -83.42441
153.4	Bar above Brooks Bridge	N 36.53524, W -83.44164	N 36.53438, W -83.44154
		N 36.53524, W -83.44259	N 36.53477, W -83.44289
150	Brooks Bridge	N 36.53497, W -83.44586	N 36.53477, W -83.44594
		N 36.53523, W -83.44732	N 36.53495, W -83.44729
152.6	Bar below Brooks Bridge	N 36.53742, W -83.45206	N 36.53732, W -83.45230
		N 36.53905, W -83.45348	N 36.53888, W -83.45365
136.2	Yellow Shoals	N 36.52887, W -83.50992	N 36. 52894, W -83.50959
		N 36.52792, W -83.50875	N 36.52813, W -83.50842
135.8	Below Yellow Shoals	N 36.51836, W -83.49685	N 36.51854, W -83.49655
		N 36.51670, W -83.49577	N 36.51675, W -83.49548
129.4	Double S Bend	N 36.52263, W -83.51653	N 36.52284, W -83.51670
		N 36.52295, W -83.51579	N 36.52303, W -83.51605
104.8	Above Rte. 25E Bridge	N 36.54878, W -83.61327	N 36.54849, W -83.61293
		N 36.54804, W -83.61394	N 36.54790, W -83.61367

Appendix B

Size-Class Distributions of Mussels Collected during Qualitative Sampling of Sites in the Powell River, Virginia and Tennessee, during 2008 and 2009.

Table 1. Size-class distribution of *Actinonaias ligamentina* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	***Actinonaias ligamentina* Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Cheekspring Ford	-	-	-	-	-	-	-	-	-	3	-	4	-	-
Sewell Bridge	-	-	-	-	-	-	-	-	3	6	1	-	1	-
Hall Ford	-	-	-	-	-	-	1	1	7	6	6	2	-	-
Snodgrass Ford	-	-	-	-	-	-	-	-	1	2	6	-	-	-
Island below Snodgrass Ford	-	-	1	-	-	1	15	43	266	223	48	10	2	-
Rte. 833 Bridge	-	-	-	-	-	-	3	13	93	65	13	5	-	-
Fletcher Ford	-	-	-	-	-	-	3	22	128	65	13	3	1	-
Bales Ford	-	-	-	-	-	2	-	8	73	159	39	7	2	-
Fugate Ford	-	-	-	-	-	1	1	2	13	26	10	4	-	-
McDowell Shoal	-	-	-	-	-	-	2	10	49	26	-	1	-	-
Buchanan Ford	-	1	-	-	-	1	3	6	19	46	41	21	7	-
Bar above Brooks Bridge	-	-	-	-	-	-	4	17	22	111	128	76	27	3
Brooks Bridge	-	-	-	-	-	-	2	3	9	22	38	43	14	1
Below Brooks Bridge	-	2	-	-	2	6	47	151	218	652	479	116	40	4
Below Yellow Shoals	-	-	-	-	-	-	6	14	19	32	59	19	3	1
Double S Bend	-	-	-	-	-	-	6	13	6	29	18	8	-	-
Above Rte. 25E Bridge	-	-	-	-	-	2	2	13	13	22	37	19	5	-
Total	0	3	1	0	2	13	95	316	939	1495	936	338	102	9

Table 2. Size-class distribution of *Actinonaias pectorosa* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	Actinonaias pectorosa **Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Dryden	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Shafer Ford	-	-	-	-	-	-	-	-	-	-	2	2	-	-
Cheekspring Ford	-	-	-	-	-	-	-	-	-	1	8	15	3	-
Sewell Bridge	-	-	-	-	-	-	-	-	-	9	25	11	8	-
Hall Ford	-	-	-	-	-	-	-	-	2	10	13	8	1	-
Snodgrass Ford	-	-	-	-	-	-	-	-	-	5	4	-	-	-
Island below Snodgrass Ford	-	-	1	2	2	2	9	35	147	583	652	128	12	-
Rte. 833 Bridge	-	-	1	-	-	3	12	71	223	582	360	34	-	1
Fletcher Ford	-	-	-	1	5	1	19	63	133	148	65	8	-	-
Bales Ford	-	-	-	-	-	-	1	3	12	29	18	1	-	-
Fugate Ford	-	-	-	-	-	-	-	6	8	23	17	3	1	-
McDowell Shoal	-	-	-	-	-	-	1	8	27	35	3	-	-	-
Buchanan Ford	-	-	-	-	1	1	4	27	63	84	76	12	2	-
Bar above Brooks Bridge	-	-	-	-	1	2	7	29	57	112	194	73	10	-
Brooks Bridge	-	-	-	-	-	-	-	1	2	4	5	3	1	-
Below Brooks Bridge	-	1	-	-	4	4	33	111	298	468	611	227	17	2
Below Yellow Shoals	-	-	-	-	-	-	2	8	26	50	59	11	2	1
Double S Bend	-	1	2	1	-	14	43	95	187	173	111	16	3	-
Above Rte. 25E Bridge	-	-	-	-	-	2	14	32	65	64	36	19	2	1
Total	0	2	4	4	13	29	145	489	1250	2380	2260	571	62	5

Table 3. Size-class distribution of *Amblema plicata* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	***Amblema plicata* Median size-class (mm)**														
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**	**145**
Sewell Bridge	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-
Hall Ford	-	-	-	-	-	-	-	1	1	-	1	-	-	-	-
Snodgrass Ford	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Island below Snodgrass Ford	-	-	-	-	-	-	1	-	1	3	2	-	-	-	-
Rte. 833 Bridge	-	-	-	-	-	-	-	1	1	6	2	1	-	-	-
Fletcher Ford	-	-	-	-	-	1	1	3	12	10	3	2	2	-	-
Bales Ford	-	-	-	-	-	-	-	1	4	17	14	5	1	-	-
Fugate Ford	-	-	-	-	-	-	-	-	-	4	5	1	-	-	-
McDowell Shoal	-	-	-	-	-	1	1	4	11	11	2	2	3	-	-
Buchanan Ford	-	-	1	-	-	1	1	-	1	6	3	1	-	1	-
Bar above Brooks Bridge	-	-	-	-	-	-	2	-	2	8	4	5	4	1	-
Brooks Bridge	-	-	-	-	-	1	-	-	3	9	5	6	6	5	-
Below Brooks Bridge	-	-	-	1	-	3	6	8	36	77	43	15	3	2	-
Below Yellow Shoals	-	-	-	-	1	-	-	1	1	32	24	3	2	1	1
Double S Bend	-	-	-	-	-	2	-	-	2	4	4	-	-	-	-
Above Rte. 25E Bridge	-	-	-	-	-	3	8	3	10	9	10	5	-	-	-
Total	0	0	1	1	1	12	20	22	85	198	122	47	21	10	1

Table 4. Size-class distribution of *Cyclonaias tuberculata* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	***Cyclonaias tuberculata* Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Sewell Bridge	-	-	-	-	-	-	-	1	1	-	-	-	-	-
Hall Ford	-	-	-	-	-	1	-	2	1	-	-	-	-	-
Snodgrass Ford	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Island below Snodgrass Ford	-	-	-	-	-	-	3	2	1	3	-	-	-	-
Rte. 833 Bridge	-	-	-	-	-	2	1	4	2	-	-	-	-	-
Fletcher Ford	-	-	-	-	-	1	11	8	3	1	-	-	-	-
Bales Ford	-	-	-	-	-	-	3	6	6	1	1	-	-	-
Fugate Ford	-	-	-	-	-	1	2	-	3	-	-	1	-	-
McDowell Shoal	-	-	-	-	-	-	11	3	-	-	-	-	-	-
Buchanan Ford	-	-	-	-	-	-	-	3	1	1	1	-	-	-
Bar above Brooks Bridge	-	-	-	-	-	1	2	8	14	7	12	4	-	-
Brooks Bridge	-	-	-	-	-	-	-	3	3	19	12	15	1	-
Below Brooks Bridge	-	-	-	1	-	4	12	27	36	35	26	3	1	1
Below Yellow Shoals	-	-	-	-	-	2	6	12	13	8	11	1	-	-
Double S Bend	-	-	-	-	-	-	3	13	10	3	-	-	-	-
Above Rte. 25E Bridge	-	-	-	1	-	-	1	11	20	8	2	-	1	-
Total	0	0	0	2	0	12	55	103	114	87	65	24	3	1

Table 5. Size-class distribution of *Dromus dromas* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	Dromus dromas **Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Island below Snodgrass Ford	-	-	-	-	-	1	-	1	-	-	-	-	-	-
Rte. 833 Bridge	-	-	-	-	-	1	3	3	-	-	-	-	-	-
Fletcher Ford	-	-	-	-	-	2	3	1	-	-	-	-	-	-
Bales Ford	-	-	-	-	-	4	1	1	-	-	-	-	-	-
Fugate Ford	-	-	-	-	-	4	1	2	-	-	-	-	-	-
McDowell Shoal	-	-	-	-	-	4	1	-	-	-	-	-	-	-
Buchanan Ford	-	-	-	-	-	2	5	1	-	-	-	-	-	-
Bar above Brooks Bridge	-	-	-	-	-	1	6	-	1	1	-	-	-	-
Brooks Bridge	-	-	-	-	1	-	-	-	1	-	-	-	-	-
Below Brooks Bridge	-	-	-	-	-	4	28	22	4	2	1	-	-	-
Below Yellow Shoals	-	-	-	-	-	-	1	1	-	-	-	-	-	-
Above Rte. 25E Bridge	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Total	0	0	0	0	1	23	49	33	6	3	1	0	0	0

Table 6. Size-class distribution of *Epioblasma brevidens* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	Epioblasma brevidens **Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Rte. 833 Bridge	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Fletcher Ford	-	-	-	-	5	1	-	-	-	-	-	-	-	-
Bales Ford	-	-	-	-	1	-	-	-	-	-	-	-	-	-
McDowell Shoal	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Buchanan Ford	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Bar above Brooks Bridge	-	-	-	-	3	1	-	-	-	-	-	-	-	-
Brooks Bridge	-	-	-	-	2	-	-	-	-	-	-	-	-	-
Below Brooks Bridge	-	-	-	3	7	7	4	4	1	-	-	-	-	-
Below Yellow Shoals	-	-	-	-	5	-	-	-	-	-	-	-	-	-
Double S Bend	-	-	-	-	6	6	-	-	-	-	-	-	-	-
Above Rte. 25E Bridge	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Total	0	0	0	3	33	15	4	4	1	0	0	0	0	0

Table 7. Size-class distribution of *Epioblasma triquetra* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	Epioblasma triquetra **Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Bar above Brooks Bridge	-	-	-	-	1	1	-	-	-	-	-	-	-	-
Below Brooks Bridge	-	-	-	-	3	1	-	-	-	-	-	-	-	-
Double S Bend	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Total	0	0	0	0	5	2	0	0	0	0	0	0	0	0

Table 8. Size-class distribution of *Elliptio dilatata* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	***Elliptio dilatata* Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
619 Bridge	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Cheekspring Ford	-	-	-	-	-	-	-	2	-	-	-	-	-	-
Sewell Bridge	-	-	-	-	-	-	-	1	2	-	-	-	-	-
Hall Ford	-	-	-	-	-	1	4	2	-	-	-	-	-	-
Island below Snodgrass Ford	-	-	-	1	2	8	90	94	33	14	3	1	-	-
Rte. 833 Bridge	-	-	-	-	1	5	61	55	12	3	-	-	-	-
Fletcher Ford	-	-	-	-	2	8	25	19	2	-	-	-	-	-
Bales Ford	-	-	-	-	1	9	11	1	-	-	-	-	-	-
Fugate Ford	-	-	-	-	2	2	6	3	3	-	1	-	-	-
McDowell Shoal	-	-	-	-	1	4	4	2	1	-	-	-	-	-
Buchanan Ford	-	-	-	-	-	-	2	5	3	-	-	-	-	-
Bar above Brooks Bridge	-	-	-	-	-	-	2	2	-	-	-	-	-	-
Below Brooks Bridge	-	-	-	-	-	4	8	27	10	2	1	-	-	-
Below Yellow Shoals	-	-	-	-	3	2	7	2	1	2	-	-	-	-
Double S Bend	-	-	-	-	5	21	20	12	2	-	-	-	-	-
Above Rte. 25E Bridge	-	-	-	-	1	10	6	4	3	-	-	-	-	-
Total	0	0	0	1	18	74	246	232	72	21	5	1	0	0

Table 9. Size-class distribution of *Elliptio crassidens* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	Elliption crassidens **Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
McDowell Shoal	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Below Brooks Bridge	-	-	-	-	-	-	-	-	-	-	1	1	-	-
Below Yellow Shoals	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Total	0	0	0	0	0	0	0	0	0	0	2	1	1	1

Table 10. Size-class distribution of *Fusconaia barnesiana* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	Fusconaia barnesiana **Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Island below Snodgrass Ford	-	-	-	-	-	-	1	2	-	-	-	-	-	-
Below Yellow Shoals	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Double S Bend	-	-	-	-	1	1	-	-	-	-	-	-	-	-
Total	0	0	0	0	1	2	3	2	0	0	0	0	0	0

Table 11. Size-class distribution of *Fusconaia cor* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	Fusconaia cor **Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Island below Snodgrass Ford	-	-	-	-	-	2	3	4	2	1	-	-	-	-
Rte. 833 Bridge	-	-	-	-	-	-	2	2	-	-	-	-	-	-
Fugate Ford	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Below Brooks Bridge	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Below Yellow Shoals	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Total	0	0	0	0	0	3	7	6	2	1	0	0	0	0

Table 12. Size-class distribution of *Fusconaia subrotunda* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	Fusconaia subrotunda **Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Cheekspring Ford	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Sewell Bridge	-	-	-	-	-	-	-	-	5	-	1	-	-	-
Hall Ford	-	-	-	-	-	1	1	-	-	-	-	-	-	-
Island below Snodgrass Ford	-	-	-	-	-	1	3	3	-	-	-	-	-	-
Rte. 833 Bridge	-	-	-	-	-	-	2	3	4	-	-	-	-	-
Fletcher Ford	-	-	-	-	-	-	1	2	-	-	-	-	-	-
Bar above Brooks Bridge	-	-	-	-	-	-	1	-	1	-	-	-	-	-
Below Brooks Bridge	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Below Yellow Shoals	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Total	0	0	0	0	0	2	8	10	10	1	1	0	0	0

Table 13. Size-class distribution of *Lampsilis fasciola* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	Lampsilis fasciola **Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Dryden	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Sewell Bridge	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Hall Ford	-	-	-	-	-	1	1	-	-	-	-	-	-	-
Island below Snodgrass Ford	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Rte. 833 Bridge	-	-	-	-	2	2	5	-	2	-	-	-	-	-
Fletcher Ford	-	-	1	-	1	2	1	-	-	-	-	-	-	-
Bales Ford	-	-	-	-	2	3	-	-	-	-	-	-	-	-
Fugate Ford	-	-	-	-	-	1	1	-	-	-	-	-	-	-
McDowell Shoal	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Buchanan Ford	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Bar above Brooks Bridge	-	-	-	-	1	4	1	-	-	-	-	-	-	-
Brooks Bridge	-	-	-	-	1	2	-	-	-	-	-	-	-	-
Below Brooks Bridge	-	-	-	-	-	3	6	-	-	-	-	-	-	-
Below Yellow Shoals	-	-	-	-	-	2	3	-	-	-	-	-	-	-
Double S Bend	-	-	1	-	7	16	8	2	-	-	-	-	-	-
Above Rte. 25E Bridge	-	-	-	-	1	3	1	-	-	-	-	-	-	-
Total	0	0	2	0	16	41	28	3	2	0	0	0	0	0

Table 14. Size-class distribution of *Lampsilis ovata* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	Lampsilis ovate **Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Sewell Bridge	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Island below Snodgrass Ford	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Rte. 833 Bridge	-	-	-	-	-	-	-	-	1	1	-	-	-	-
Fletcher Ford	-	1	1	5	-	1	2	1	1	-	-	-	-	-
Bales Ford	-	-	-	-	-	-	-	4	1	1	1	2	-	-
McDowell Shoal	-	-	-	-	-	-	-	2	1	-	1	-	-	-
Buchanan Ford	-	-	-	-	-	-	-	-	2	-	2	-	-	-
Bar above Brooks Bridge	-	-	-	-	-	3	-	3	3	2	3	1	-	-
Brooks Bridge	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Below Brooks Bridge	-	-	-	-	-	-	5	9	10	5	2	-	-	-
Below Yellow Shoals	-	-	-	-	-	-	-	1	3	-	-	-	1	-
Double S Bend	-	-	-	-	-	-	-	2	3	4	1	-	-	-
Above Rte. 25E Bridge	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Total	0	1	1	5	0	4	7	23	26	14	10	3	1	0

Table 15. Size-class distribution of *Lasmigona costata* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	Lasmigona costata **Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Island below Snodgrass Ford	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Bales Ford	-	-	-	-	-	-	1	-	2	-	-	-	-	-
Buchanan Ford	-	-	-	-	-	-	-	-	4	-	-	-	-	-
Brooks Bridge	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Below Brooks Bridge	-	-	-	-	-	1	3	5	2	-	1	-	-	-
Below Yellow Shoals	-	-	-	-	-	-	-	1	1	-	-	-	-	-
Above Rte. 25E Bridge	-	-	-	-	-	-	-	2	-	2	1	-	-	-
Total	0	0	0	0	0	2	5	8	9	2	2	0	0	0

Table 16. Size-class distribution of *Ligumia recta* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	***Ligumia recta* Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Bales Ford	-	-	-	-	-	-	-	-	-	-	-	-	1	-
McDowell Shoal	-	-	-	-	-	-	-	-	-	1	-	1	-	-
Bar above Brooks Bridge	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Brooks Bridge	-	-	-	-	-	-	-	-	-	-	-	1	1	1
Below Brooks Bridge	-	-	-	-	-	-	-	-	-	-	1	4	2	-
Below Yellow Shoals	-	-	-	-	-	-	-	-	1	-	1	-	-	-
Double S Bend	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Above Rte. 25E Bridge	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Total	0	0	0	0	0	0	0	0	1	1	2	7	5	2

Table 17. Size-class distribution of *Lemiox rimosus* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	Lemiox rimosus **Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Island below Snodgrass Ford	-	-	-	2	1	-	-	-	-	-	-	-	-	-
Rte. 833 Bridge	-	-	-	-	2	-	-	-	-	-	-	-	-	-
Fletcher Ford	-	-	-	1	3	-	-	-	-	-	-	-	-	-
Bales Ford	-	-	-	2	-	-	-	-	-	-	-	-	-	-
Below Brooks Bridge	-	-	-	-	2	-	-	-	-	-	-	1	1	-
Total	0	0	0	5	8	0	0	0	0	0	0	1	1	0

Table 18. Size-class distribution of *Lexingtonia dolabelloides* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	Lexingtonia dolabelloides **Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Island below Snodgrass Ford	-	-	-	-	-	-	1	1	-	-	-	-	-	-
Rte. 833 Bridge	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Total	0	0	0	0	0	0	1	2	0	0	0	0	0	0

Table 19. Size-class distribution of *Medionidus conradicus* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	***Medoniadas conradicus* Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Hall Ford	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Snodgrass Ford	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Island below Snodgrass Ford	-	-	-	5	15	27	2	-	-	-	-	-	-	-
Rte. 833 Bridge	-	-	-	2	22	8	1	-	-	-	-	-	-	-
Fletcher Ford	-	-	-	9	42	12	-	-	-	-	-	-	-	-
Bales Ford	-	-	-	5	11	-	-	-	-	-	-	-	-	-
Fugate Ford	-	-	-	1	6	1	-	-	-	-	-	-	-	-
McDowell Shoal	-	-	1	10	13	-	-	-	-	-	-	-	-	-
Buchanan Ford	-	-	-	5	22	11	-	-	-	-	-	-	-	-
Bar above Brooks Bridge	-	-	1	7	32	18	-	-	-	-	-	-	-	-
Brooks Bridge	-	-	-	6	1	2	-	-	-	-	-	-	-	-
Below Brooks Bridge	-	-	2	18	47	16	-	-	-	-	-	-	-	-
Below Yellow Shoals	-	-	3	11	40	5	1	-	-	-	-	-	-	-
Double S Bend	-	-	14	105	202	37	-	-	-	-	-	-	-	-
Above Rte. 25E Bridge	-	-	1	13	44	11	-	-	-	-	-	-	-	-
Total	0	0	22	198	497	149	4	0	0	0	0	0	0	0

Table 20. Size-class distribution of *Plethobasus cyphyus* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	Plethobasus cyphyus **Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Snodgrass Ford	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Island below Snodgrass Ford	-	-	-	-	-	-	1	1	2	-	1	-	-	-
Rte. 833 Bridge	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Fletcher Ford	-	-	-	-	-	1	1	2	2	-	-	-	-	-
Bales Ford	-	-	-	-	-	-	1	4	17	8	2	1	-	-
Fugate Ford	-	-	-	-	-	-	-	1	1	1	-	-	-	-
McDowell Shoal	-	-	-	-	-	-	3	4	-	-	-	-	-	-
Bar above Brooks Bridge	-	-	-	-	-	-	-	-	2	1	-	-	-	-
Brooks Bridge	-	-	-	-	-	-	-	-	2	2	-	-	-	-
Below Brooks Bridge	-	-	-	-	-	1	1	15	12	4	-	-	-	-
Below Yellow Shoals	-	-	-	-	-	-	-	2	1	-	-	-	-	-
Double S Bend	-	-	-	-	-	-	-	-	2	-	-	-	-	-
Above Rte. 25E Bridge	-	-	-	-	-	-	-	-	2	-	-	-	-	-
Total	0	0	0	0	0	2	8	29	43	17	3	1	0	0

Table 21. Size-class distribution of *Potamilus alatus* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	Potamalus alatus **Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Bales Ford	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Total	0	0	0	0	0	0	0	0	0	0	0	1	0	0

Table 22. Size-class distribution of *Ptychobranchus fasciolaris* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	Ptychobranchus fasciolaris **Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Dryden	-	-	-	-	-	-	-	-	-	-	2	-	-	-
Rte. 619 Bridge	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Swimming Hole	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Hall Ford	-	-	-	-	-	-	-	-	2	-	-	-	-	-
Island below Snodgrass Ford	-	-	-	-	-	1	-	-	4	1	2	-	-	-
Rte. 833 Bridge	-	-	-	-	-	-	2	3	5	4	1	-	-	-
Fletcher Ford	-	-	-	-	-	-	2	3	8	6	3	1	-	-
Fugate Ford	-	-	-	-	-	-	-	-	-	-	-	1	-	-
McDowell Shoal	-	-	-	-	-	-	1	-	2	2	-	-	-	-
Buchanan Ford	-	-	-	-	-	-	-	2	1	1	-	-	-	-
Bar above Brooks Bridge	-	-	-	-	-	-	1	6	8	6	2	-	-	-
Brooks Bridge	-	-	-	-	-	1	-	-	-	-	-	1	-	-
Below Brooks Bridge	-	-	-	-	-	-	3	23	28	30	11	-	-	1
Below Yellow Shoals	-	-	-	1	-	-	-	2	1	2	5	1	-	-
Double S Bend	-	-	-	-	-	2	7	12	13	4	-	-	-	-
Above Rte. 25E Bridge	-	-	-	-	1	-	3	8	8	2	2	1	-	-
Total	0	0	0	1	1	4	19	59	81	58	29	5	0	1

Table 23. Size-class distribution of *Ptychobranchus subtentum* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	Ptychobranchus subtentum **Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Island below Snodgrass Ford	-	-	-	-	-	1	12	11	2	-	-	-	-	-
Rte. 833 Bridge	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Bar above Brooks Bridge	-	-	-	-	-	-	-	2	-	-	1	-	-	-
Double S Bend	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Total	0	0	0	1	0	1	12	13	3	0	1	0	0	0

Table 24. Size-class distribution of *Quadrula c. strigillata* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	Quadrula. c. strigillata **Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Sewell Bridge	-	-	-	-	-	-	-	1	2	-	-	-	-	-
Bales Ford	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Bar above Brooks Bridge	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Below Yellow Shoals	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Above Rte. 25E Bridge	-	-	-	-	-	1	-	1	-	-	-	-	-	-
Total	0	0	0	0	0	2	1	3	2	0	0	0	0	0

Table 25. Size-class distribution of *Quadrula intermedia* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	***Quadrula intermedia* Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Sewell Bridge	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Island below Snodgrass Ford	-	-	-	-	-	2	-	-	-	-	-	-	-	-
Rte. 833 Bridge	-	-	-	1	-	2	3	-	-	-	-	-	-	-
Fletcher Ford	-	-	-	-	1	3	2	-	-	-	-	-	-	-
Bales Ford	-	-	-	-	1	-	1	-	-	-	-	-	-	-
Fugate Ford	-	-	-	-	1	1	1	-	-	-	-	-	-	-
McDowell Shoal	-	-	-	-	-	5	2	-	-	-	-	-	-	-
Buchanan Ford	-	-	-	-	-	1	1	-	-	-	-	-	-	-
Bar above Brooks Bridge	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Brooks Bridge	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Below Brooks Bridge	-	-	-	-	-	2	2	-	-	-	-	-	-	-
Double S Bend	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Total	0	0	0	2	3	17	14	0	0	0	0	0	0	0

Table 26. Size-class distribution of *Quadrula pustulosa* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	***Quadrula pustulosa* Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Island below Snodgrass Ford	-	-	-	-	-	1	1	-	-	-	-	-	-	-
Total	0	0	0	0	0	1	1	0	0	0	0	0	0	0

Table 27. Size-class distribution of *Quadrula sparsa* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	Quadrula sparsa **Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Sewell Bridge	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Rte. 833 Bridge	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Fletcher Ford	-	-	-	-	1	1	-	-	-	-	-	-	-	-
Bales Ford	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Buchanan Ford	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Bar above Brooks Bridge	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Below Brooks Bridge	-	-	-	-	-	-	3	-	-	-	-	-	-	-
Total	0	0	0	0	2	2	5	1	0	0	0	0	0	0

Table 28. Size-class distribution of *Villosa iris* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	Villosa iris **Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Sewell Bridge	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Snodgrass Ford	-	-	-	-	2	1	-	-	-	-	-	-	-	-
Island below Snodgrass Ford	-	-	-	-	1	-	1	1	-	-	-	-	-	-
Rte. 833 Bridge	-	-	-	-	4	5	-	-	-	-	-	-	-	-
Fletcher Ford	-	-	-	5	2	4	2	-	-	-	-	-	-	-
Bales Ford	-	-	-	-	4	2	1	-	-	-	-	-	-	-
Fugate Ford	-	-	-	-	-	-	1	-	-	-	-	-	-	-
McDowell Shoal	-	-	1	-	1	1	-	-	-	-	-	-	-	-
Buchanan Ford	-	-	-	-	-	2	-	-	-	-	-	-	-	-
Brooks Bridge	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Below Yellow Shoals	-	-	-	-	2	2	-	-	-	-	-	-	-	-
Above Rte. 25E Bridge	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Total	0	0	1	7	16	18	5	1	0	0	0	0	0	0

Table 29. Size-class distribution of *Villosa vanuxemensis* collected during qualitative sampling at selected sites in the Powell River, Virginia and Tennessee, during 2008 and 2009. Size-classes are divided into 10 mm increments; median values presented.

	***Villosa vanuxemensis* Median size-class (mm)**													
Site	**5**	**15**	**25**	**35**	**45**	**55**	**65**	**75**	**85**	**95**	**105**	**115**	**125**	**135**
Island below Snodgrass Ford	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Fletcher Ford	-	-	-	1	4	-	1	-	-	-	-	-	-	-
Bales Ford	-	-	-	-	3	1	-	-	-	-	-	-	-	-
Fugate Ford	-	-	-	-	1	-	-	-	-	-	-	-	-	-
McDowell Shoal	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Bar above Brooks Bridge	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Double S Bend	-	-	-	-	-	-	2	-	-	-	-	-	-	-
Above Rte. 25E Bridge	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Total	0	0	0	2	11	1	3	0	0	0	0	0	0	0

Appendix C

Mean Stability Plots of Partial Sum Densities from Quantitative Sampling at Sites in the Powell River, Virginia and Tennessee, during 2008 and 2009.

Figure 1. Mean stability plot of sequential sample sizes used during quantitative sampling of PRKM 236.3 on the Powell River, Virginia and Tennessee, 2008 and 2009. $\bar{X}$ and Solid line = cumulative mean density; dashed lines = ± 10% of cumulative mean density.

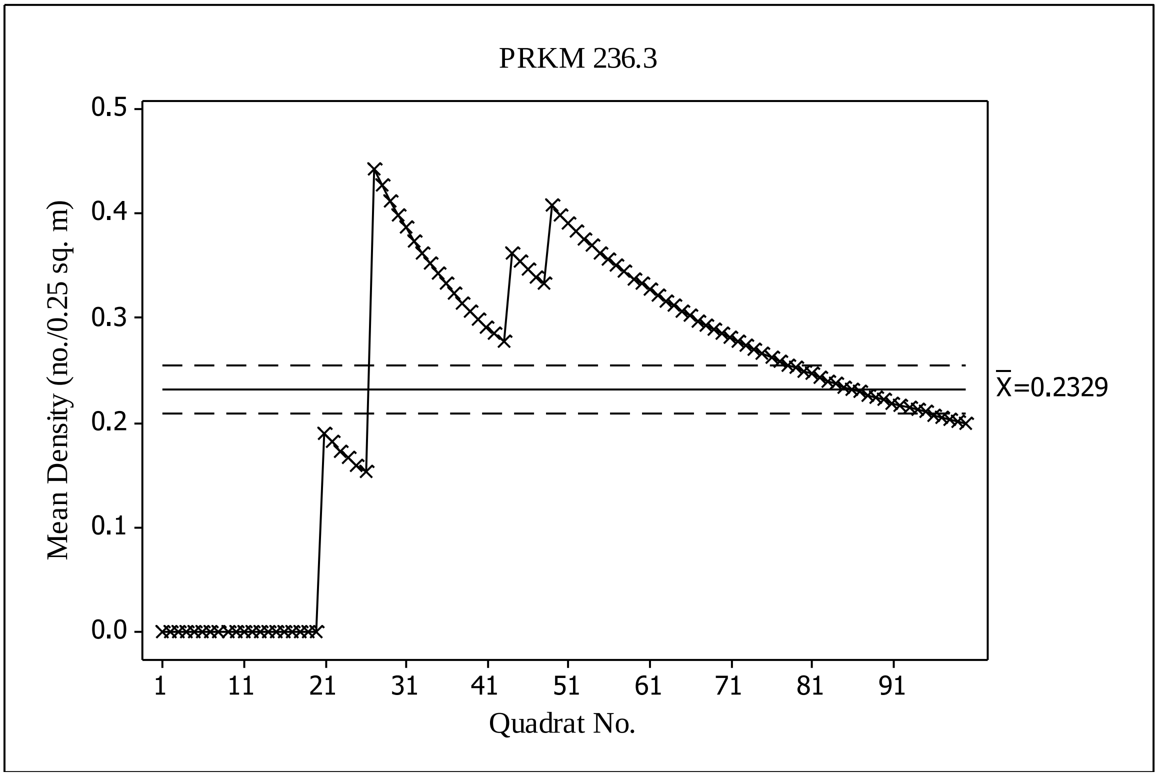


Figure 2. Mean stability plot of sequential sample sizes used during quantitative sampling of PRKM 230.9 on the Powell River, Virginia and Tennessee, 2008 and 2009. $\bar{X}$ and Solid line = cumulative mean density; dashed lines = ± 10% of cumulative mean density.

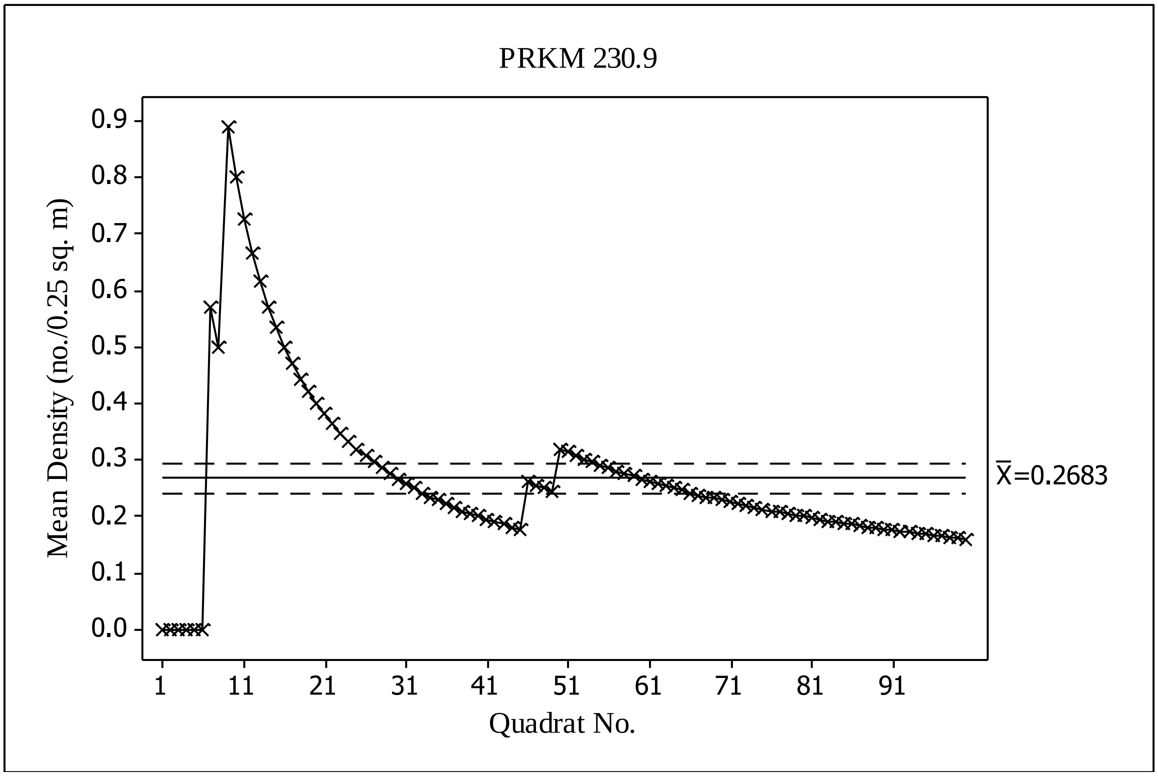


Figure 3. Mean stability plot of sequential sample sizes used during quantitative sampling of PRKM 206.6 on the Powell River, Virginia and Tennessee, 2008 and 2009. $\bar{X}$ and Solid line = cumulative mean density; dashed lines = ± 10% of cumulative mean density.

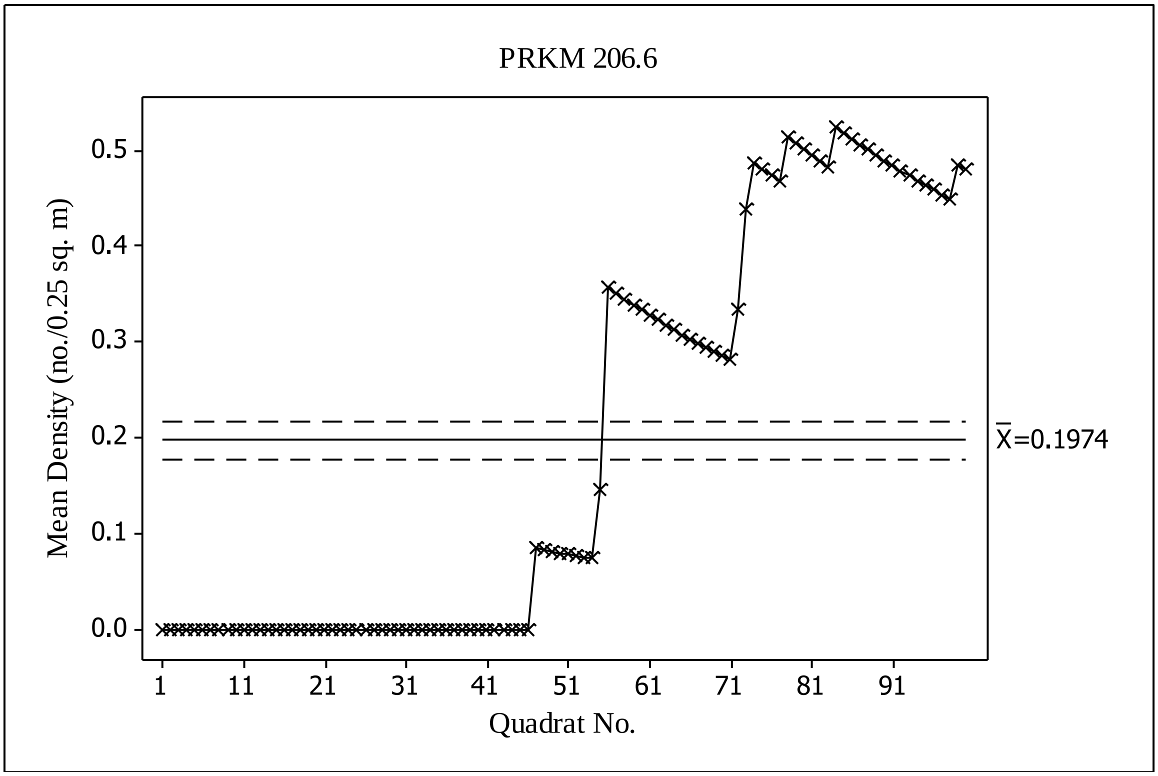


Figure 4. Mean stability plot of sequential sample sizes used during quantitative sampling of PRKM 198.8 on the Powell River, Virginia and Tennessee, 2008 and 2009. $\bar{X}$ and Solid line = cumulative mean density; dashed lines = ± 10% of cumulative mean density.

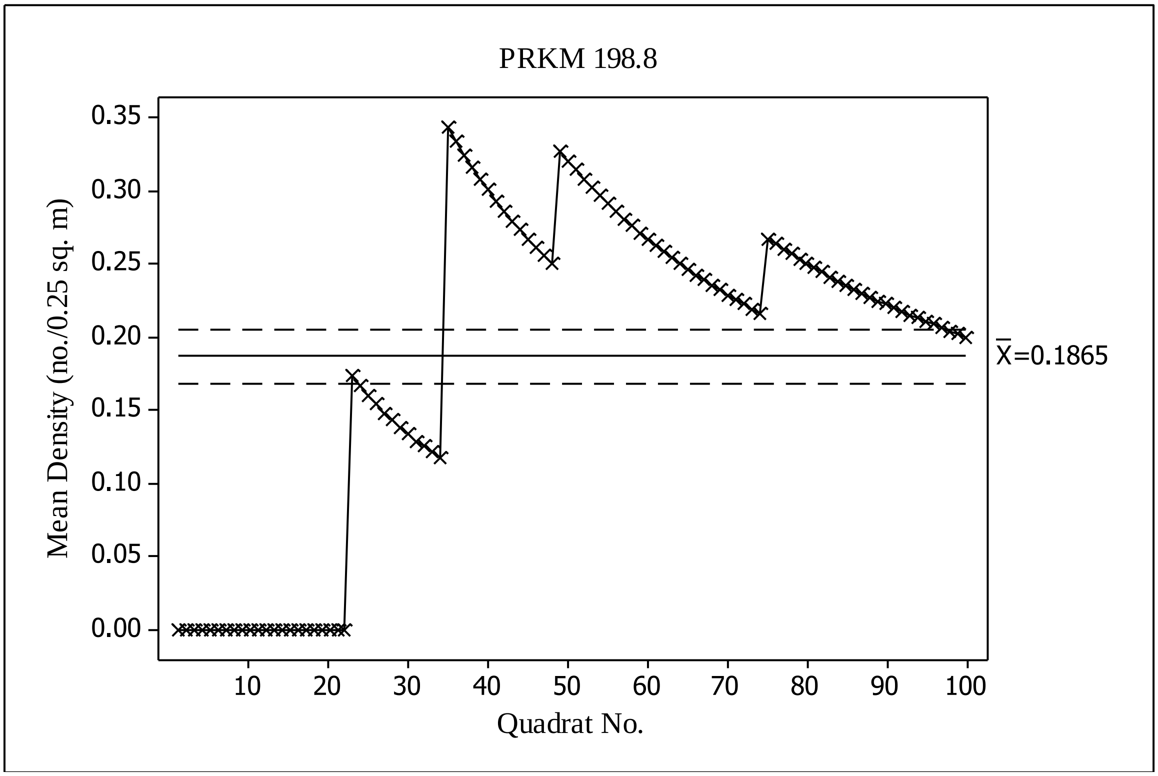


Figure 5. Mean stability plot of sequential sample sizes used during quantitative sampling of PRKM 197.9 on the Powell River, Virginia and Tennessee, 2008 and 2009. $\bar{X}$ and Solid line = cumulative mean density; dashed lines = ± 10% of cumulative mean density.

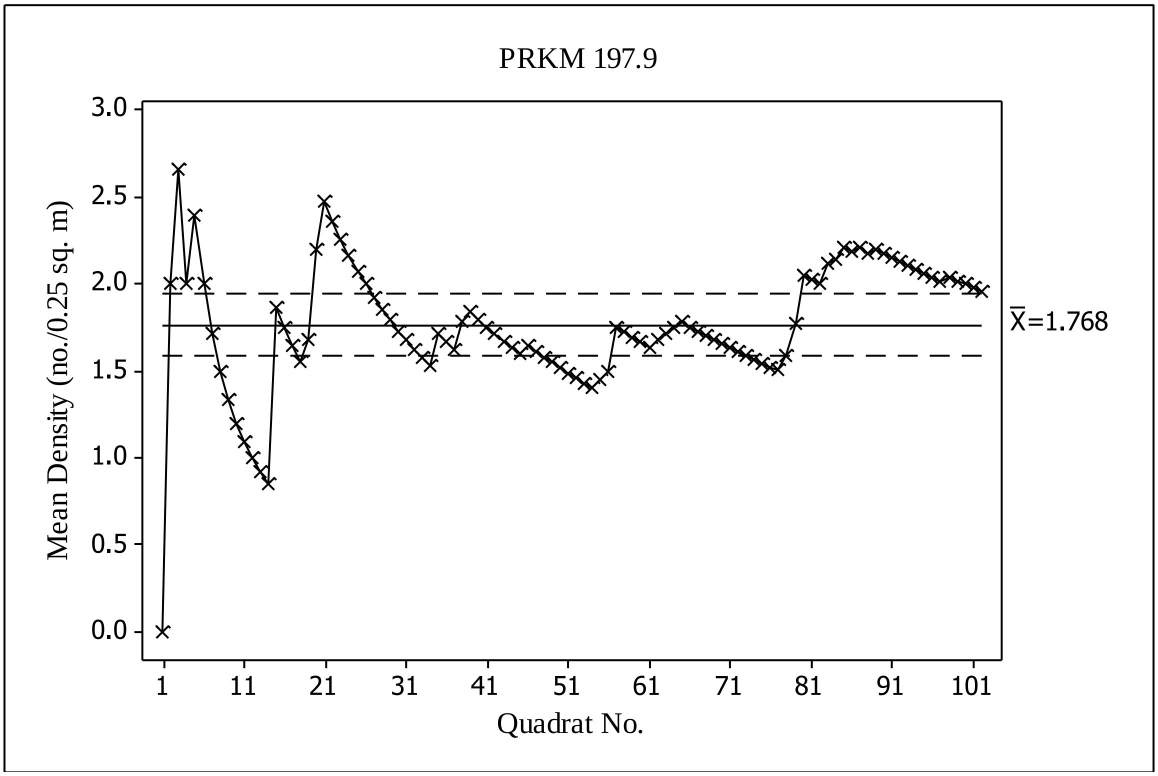


Figure 6. Mean stability plot of sequential sample sizes used during quantitative sampling of PRKM 193.4 on the Powell River, Virginia and Tennessee, 2008 and 2009. $\bar{X}$ and Solid line = cumulative mean density; dashed lines = ± 10% of cumulative mean density.

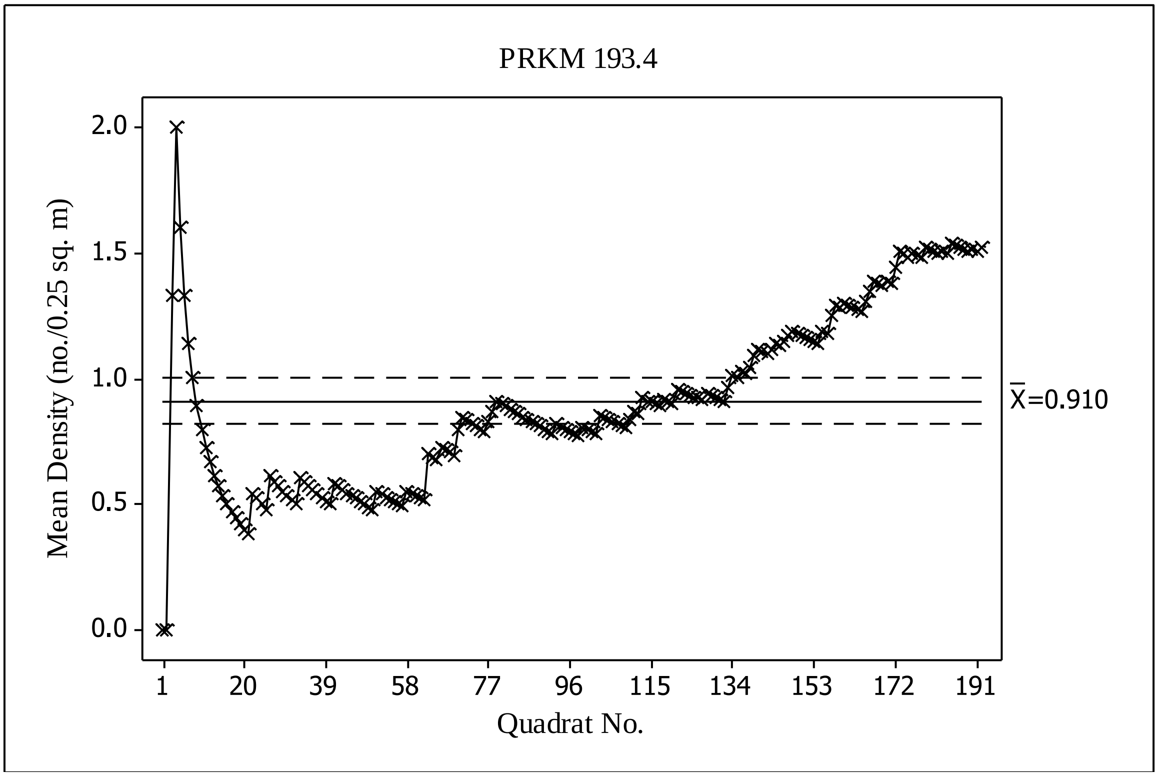


Figure 7. Mean stability plot of sequential sample sizes used during quantitative sampling of PRKM 188.8 on the Powell River, Virginia and Tennessee, 2008 and 2009. $\bar{X}$ and Solid line = cumulative mean density; dashed lines = ± 10% of cumulative mean density.

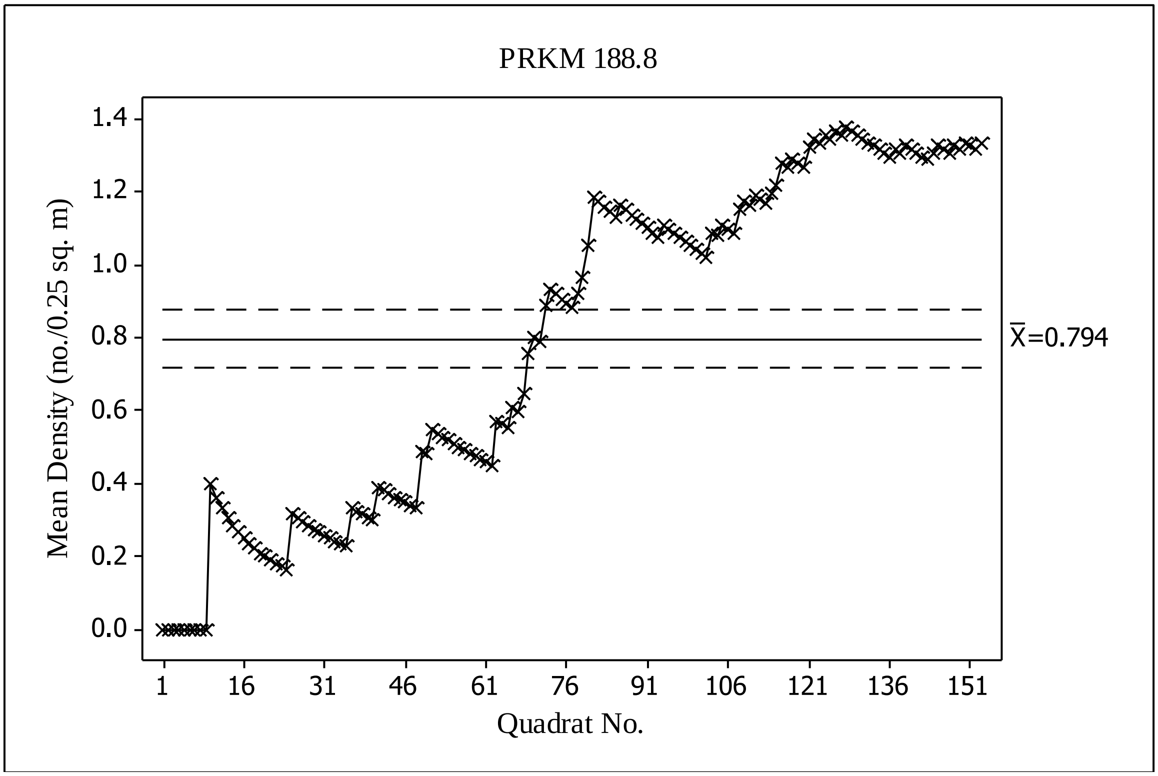


Figure 8. Mean stability plot of sequential sample sizes used during quantitative sampling of PRKM 180.7 on the Powell River, Virginia and Tennessee, 2008 and 2009. $\bar{X}$ and Solid line = cumulative mean density; dashed lines = ± 10% of cumulative mean density.

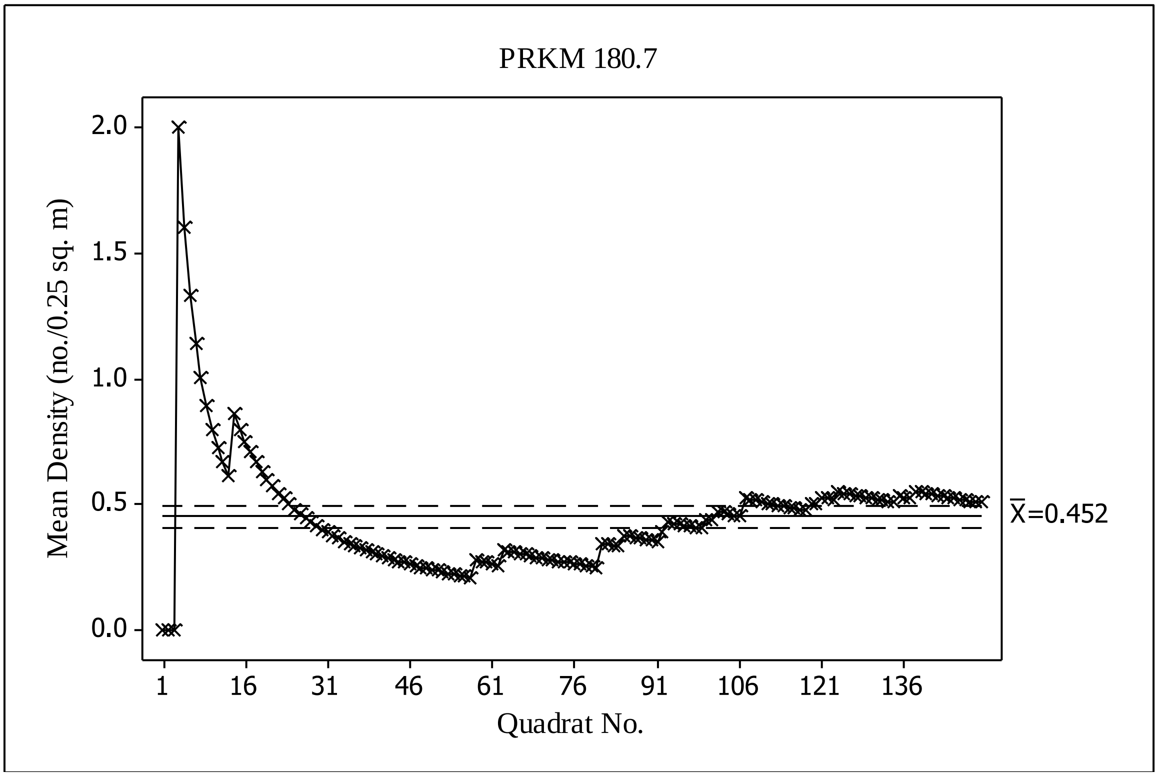


Figure 9. Mean stability plot of sequential sample sizes used during quantitative sampling of PRKM 179.9 on the Powell River, Virginia and Tennessee, 2008 and 2009. $\bar{X}$ and Solid line = cumulative mean density; dashed lines = ± 10% of cumulative mean density.

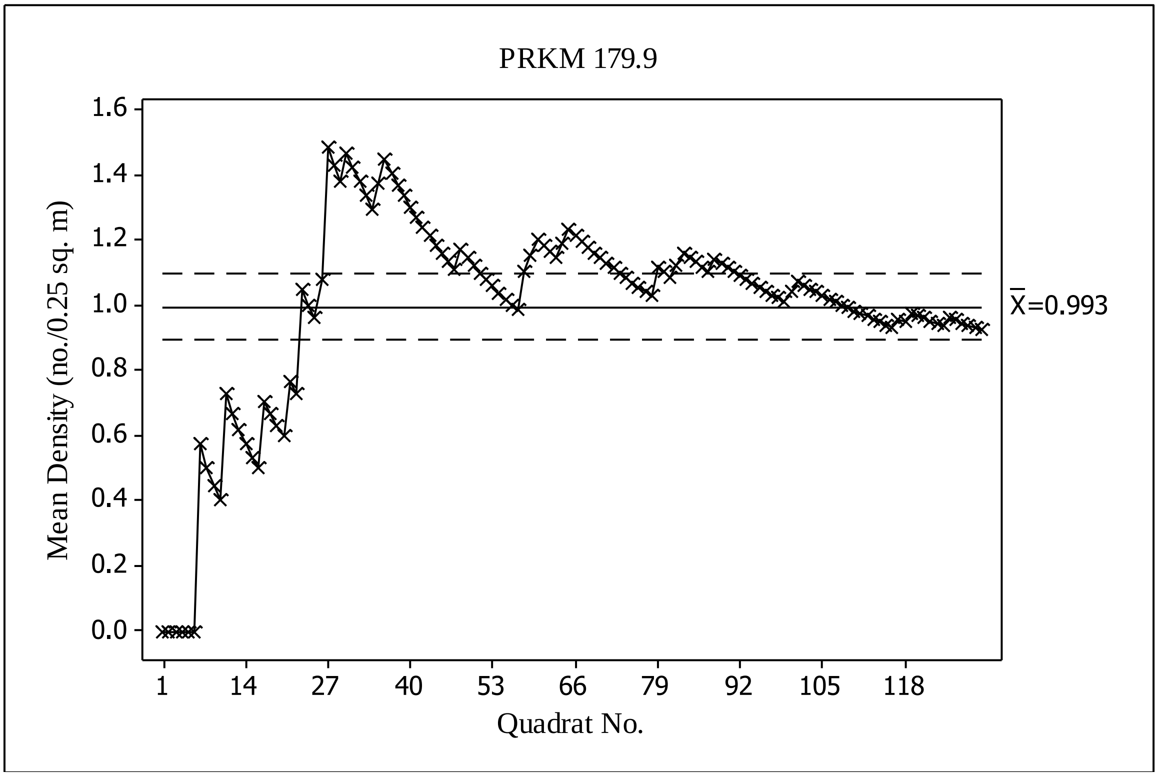


Figure 10. Mean stability plot of sequential sample sizes used during quantitative sampling of PRKM 171.4 on the Powell River, Virginia and Tennessee, 2008 and 2009. $\bar{X}$ and Solid line = cumulative mean density; dashed lines = ± 10% of cumulative mean density.

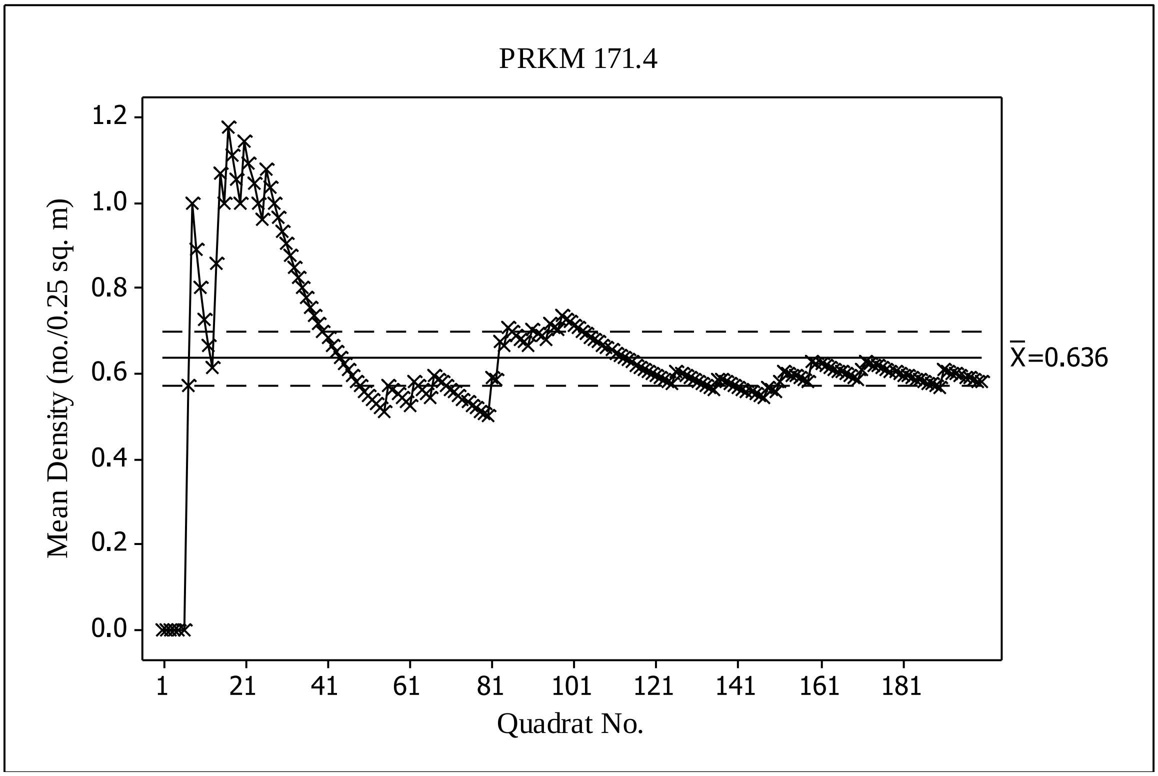


Figure 11. Mean stability plot of sequential sample sizes used during quantitative sampling of PRKM 159.6 on the Powell River, Virginia and Tennessee, 2008 and 2009. $\bar{X}$ and Solid line = cumulative mean density; dashed lines = ± 10% of cumulative mean density.

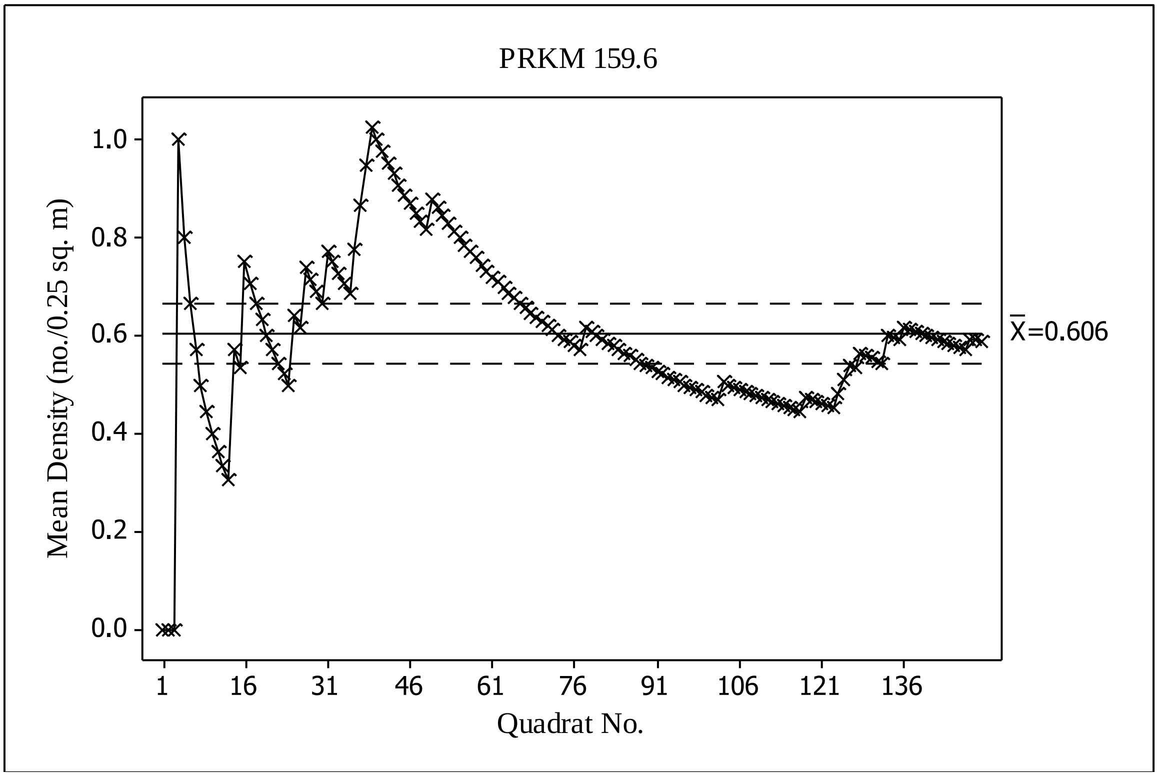


Figure 12. Mean stability plot of sequential sample sizes used during quantitative sampling of PRKM 153.4 on the Powell River, Virginia and Tennessee, 2008 and 2009. $\bar{X}$ and Solid line = cumulative mean density; dashed lines = ± 10% of cumulative mean density.

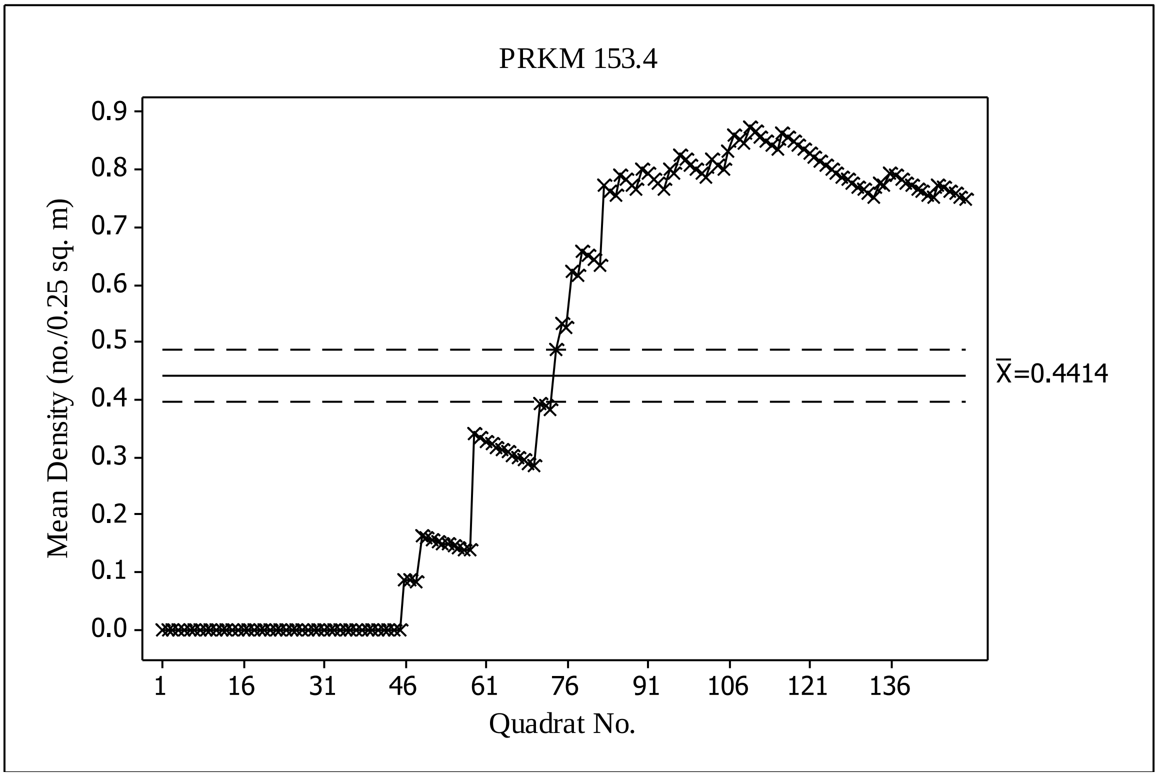


Figure 13. Mean stability plot of sequential sample sizes used during quantitative sampling of PRKM 153.0 on the Powell River, Virginia and Tennessee, 2008 and 2009. $\bar{X}$ and Solid line = cumulative mean density; dashed lines = ± 10% of cumulative mean density.

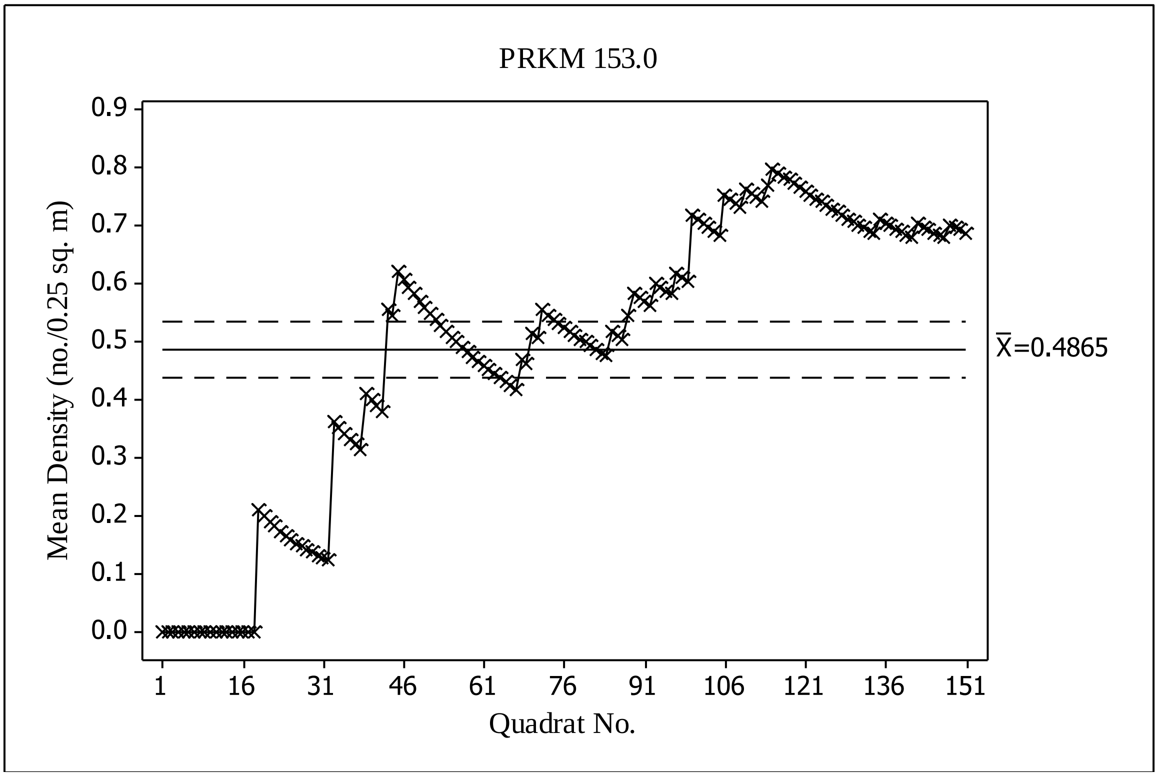


Figure 14. Mean stability plot of sequential sample sizes used during quantitative sampling of PRKM 152.6 on the Powell River, Virginia and Tennessee, 2008 and 2009. $\bar{X}$ and Solid line = cumulative mean density; dashed lines = ± 10% of cumulative mean density.

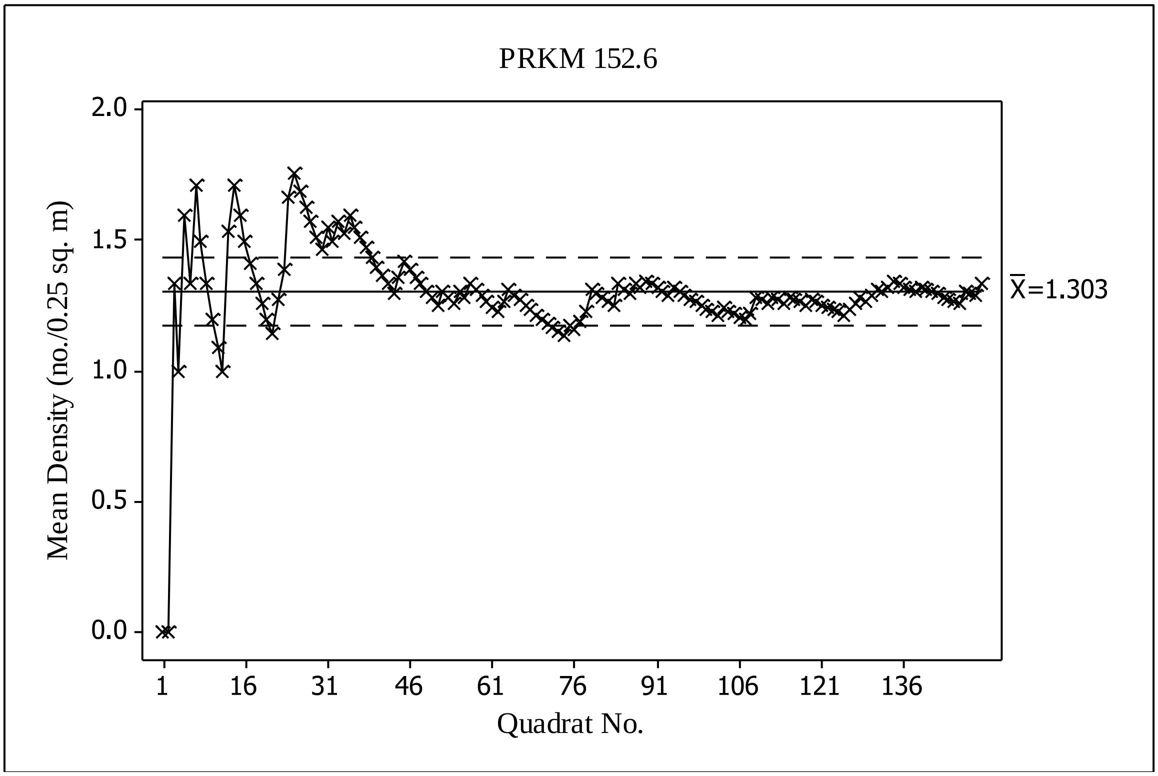


Figure 15. Mean stability plot of sequential sample sizes used during quantitative sampling of PRKM 136.2 on the Powell River, Virginia and Tennessee, 2008 and 2009. $\bar{X}$ and Solid line = cumulative mean density; dashed lines = ± 10% of cumulative mean density.

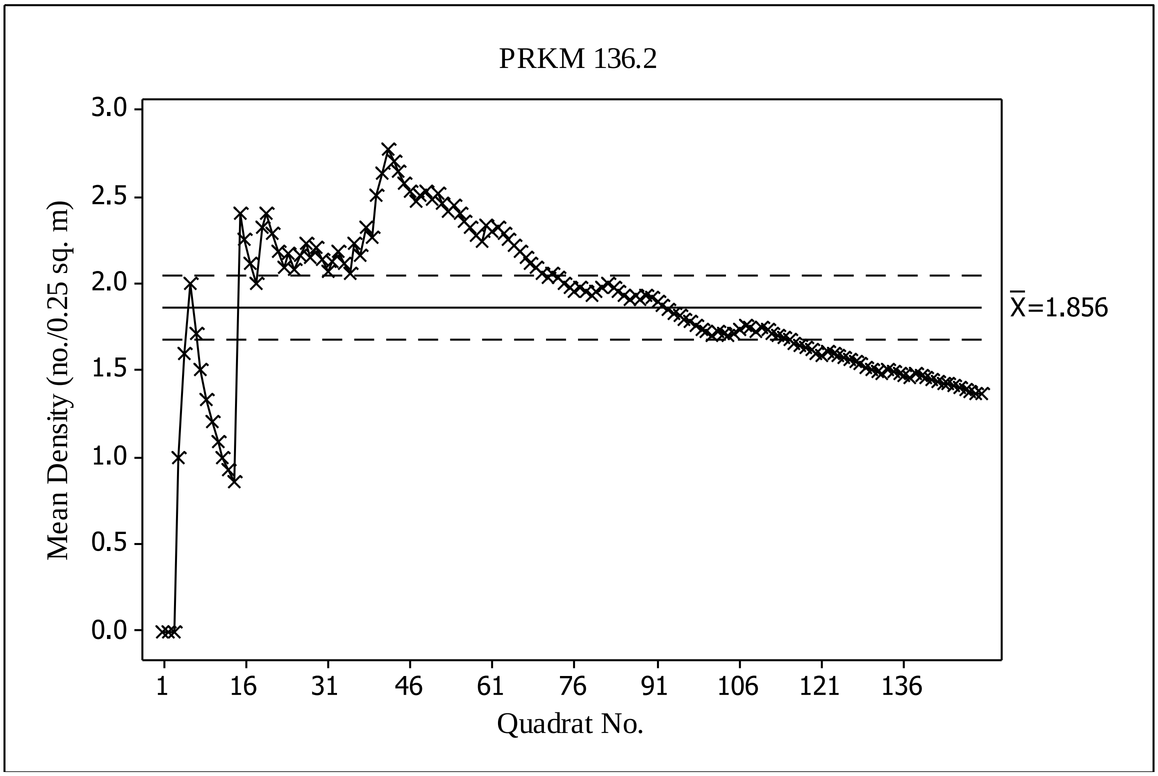


Figure 16. Mean stability plot of sequential sample sizes used during quantitative sampling of PRKM 135.8 on the Powell River, Virginia and Tennessee, 2008 and 2009. $\bar{X}$ and Solid line = cumulative mean density; dashed lines = ± 10% of cumulative mean density.

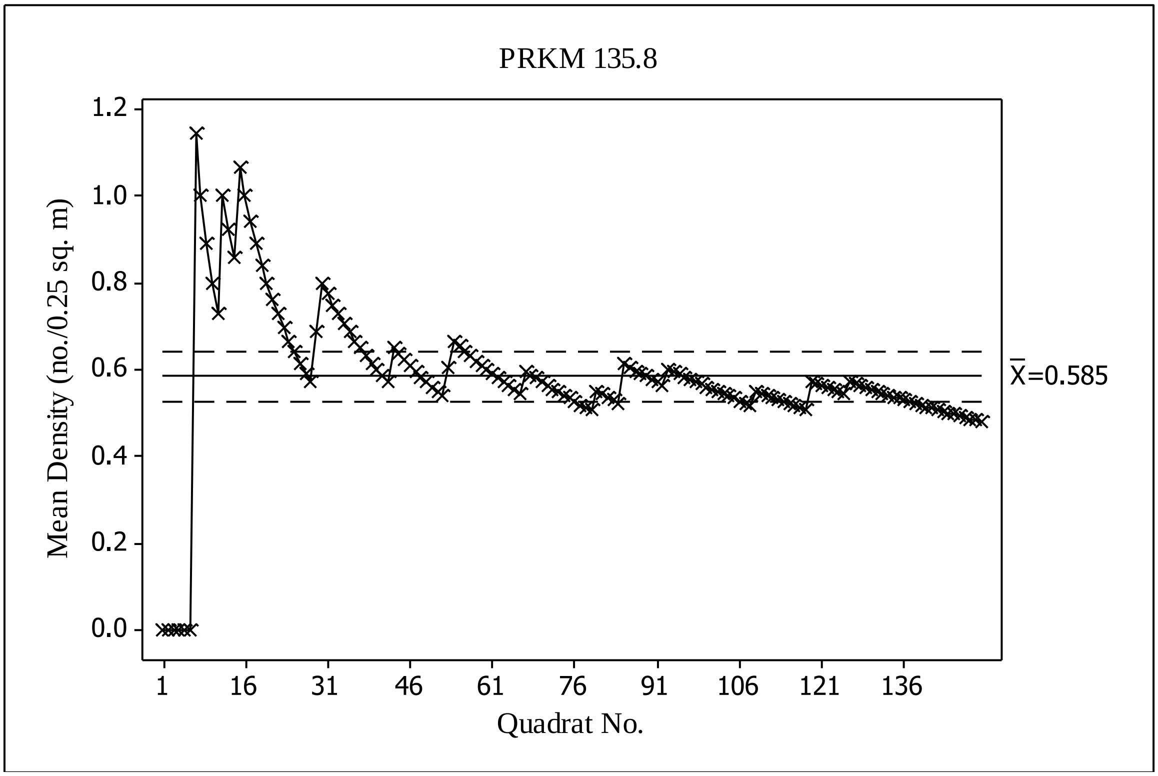


Figure 17. Mean stability plot of sequential sample sizes used during quantitative sampling of PRKM 129.4 on the Powell River, Virginia and Tennessee, 2008 and 2009. $\bar{X}$ and Solid line = cumulative mean density; dashed lines = ± 10% of cumulative mean density.

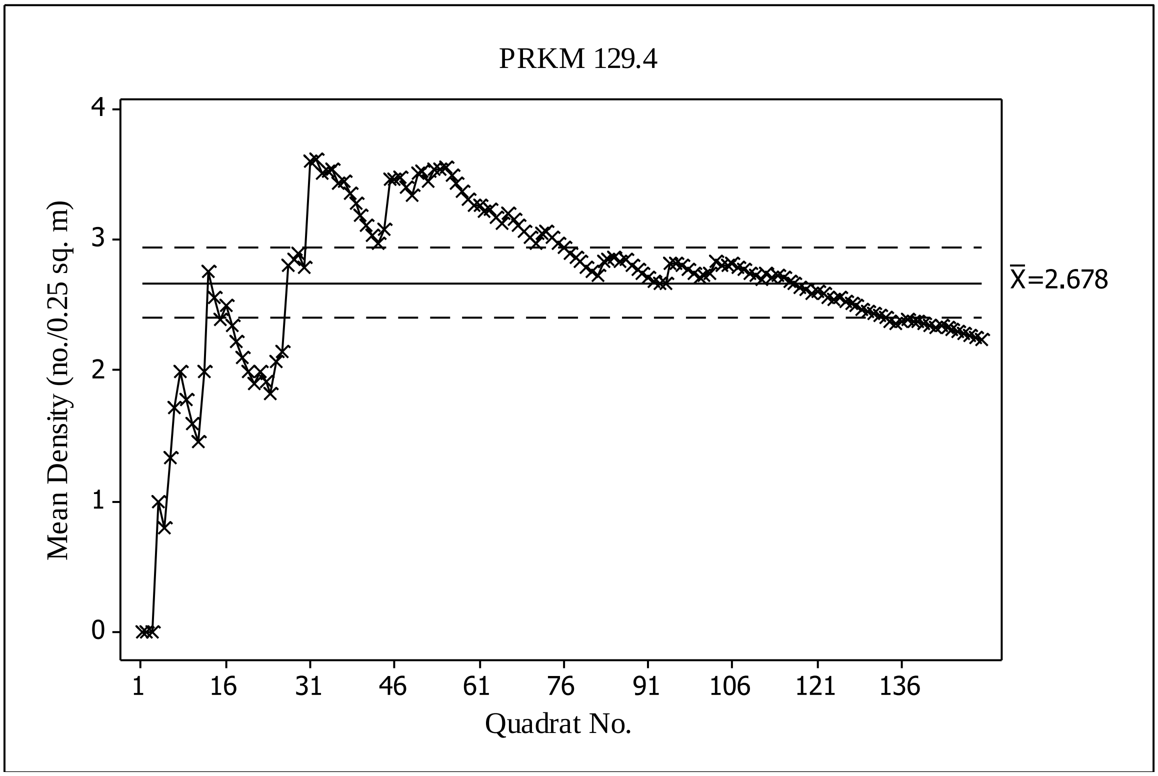
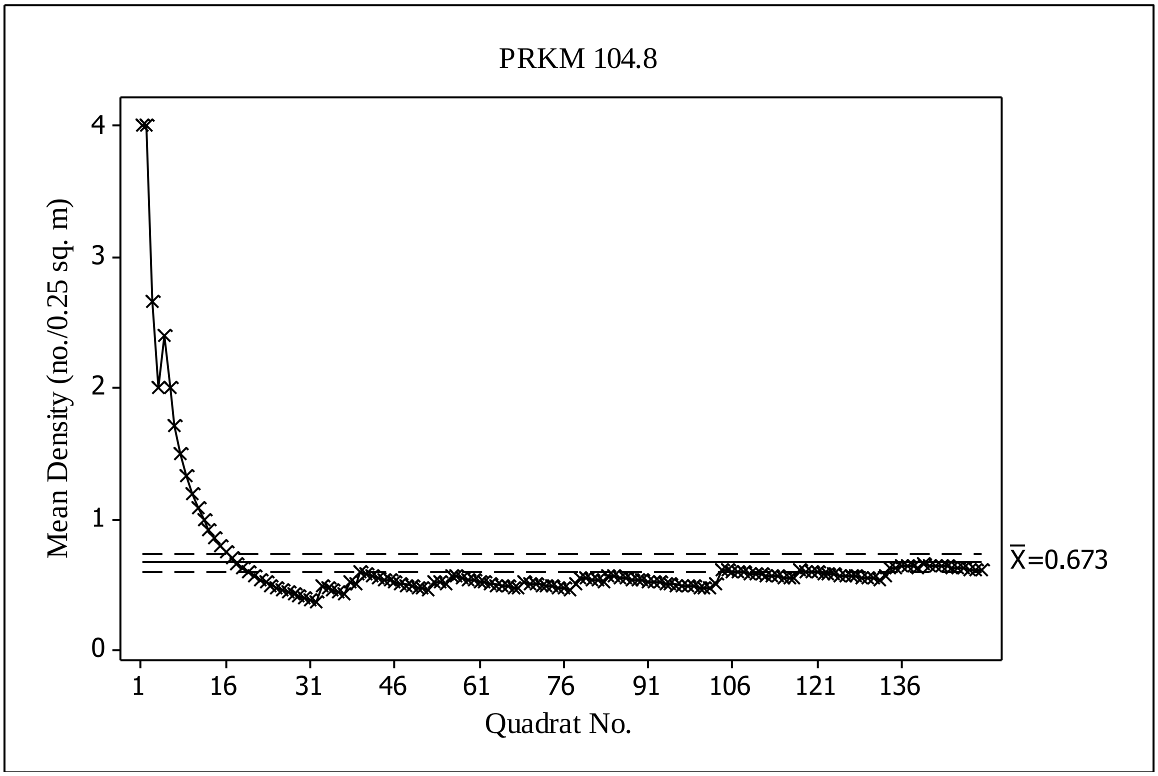


Figure 18. Mean stability plot of sequential sample sizes used during quantitative sampling of PRKM 104.8 on the Powell River, Virginia and Tennessee, 2008 and 2009. $\bar{X}$ and Solid line = cumulative mean density; dashed lines = ± 10% of cumulative mean density.



Chapter 2

Evaluation of Long-term Quantitative Sampling Programs for Mussels at Selected Sites in the Powell River, Virginia and Tennessee.

(Abstract)

The freshwater mussel fauna of the Powell River has declined in recent decades, from 41 species to approximately 32 species. To adequately detect declines in mussel density, a long-term monitoring program that has appropriate sample sizes and sufficient duration (with appropriate sampling intervals) is needed, while maintaining an acceptable level of power. This study used several statistical programs to test potential sampling protocols for capability to compare differences in mussel density among three river zones, and to detect changes in mussel density ± 10% at individual sites.

A priori power analyses determined that ≥ 9 sites would have to be sampled to compare mussel CPUE, mussel density, or total species presence among three river zones, and achieve a statistical power of ≥ 0.80. It is unlikely that sufficient funds would be available to finance such a monitoring program in the future, and it was determined that making comparisons among river zones would not be possible. Six potential sampling programs, with varying durations and sampling intervals, were simulated using 3 levels of significance (0.05, 0.10, and 0.15) to determine whether the program obtained sufficient power when monitoring changes in mussel density at individual sites. None of the sampling programs simulated in this study were able to detect declines in mussel density ≤ 10%. Several of the sampling programs were able to detect increases in mussel density of ≥ 6%. Although none of the simulated sampling protocols were able to detect declines of mussel density ≤ 10% through quantitative sampling, recommendations for the use of quantitative and qualitative sampling are made based on their capability to

qualitatively assess population status, using species presence, size-class structure, and juvenile presence. A recommendation to perform simulations for the sampling protocols ability to detect declines in mussel density > 10% also is made.

Introduction

The goal of ecological monitoring is to 1) inform the researcher when the study system deviates from a desired state, 2) measure success of conservation or management actions, or 3) detect the effects of disturbances in the study system (Legg and Nagy 2006). Achievement of the program goals depends on the program's sampling design and its ability to detect statistically significant changes in target population. Developing an adequate monitoring program would prevent resource managers from making uninformed policy and management decisions.

There are no standard requirements for developing a valid, long-term monitoring program, but Legg and Nagy (2006) made several recommendations for good monitoring design, including an experimental approach to sampling design by developing null and alternative hypotheses, selecting methods that are appropriate to the objectives and study system, and ensuring adequate spatial and temporal replication. Strayer (1986) believes that a necessity of any sampling program is that it has a straight-forward design and utilizes sampling techniques easily repeated by individuals who have little or no specialized training. While this may be true, the ultimate test of an effective monitoring program is its ability to collect data that will reject a null hypothesis if it is false (Krebs 1989, Legg and Nagy 2006). For example, if the mussel fauna were declining in the Powell River over a 30 y period, a sufficient sampling program would be able to use data collected during that period, and show that a particular rate of decline is occurring using statistical analyses. An inadequate sampling program would not be able to document that rate of decline using statistical analyses. In order to correct this, either the methods used during the sampling program would need to be amended or the goals of the sampling program, detecting a certain rate of change in density, would need to be amended to meet the limitations of the sampling protocol.

Despite being well understood in statistical literature, the importance of estimating the power of a statistical test (1 – the probability of Type II error, or the probability of a statistical test rejecting the null hypothesis when it is false) often is not considered during the design stage of an ecological experiment (Toft and Shea 1983, Peterman 1990). If the statistical test used to analyze an ecological experiment has insufficient power, the researcher can potentially make erroneous interpretations of the data collected during the experiment (Fowler et al 1998). Therefore, it is imperative that an *a priori* power analysis be conducted during the planning stages of a monitoring program, because it will not be possible to correct the issue of insufficient power later in the monitoring process. For example, if quantitative sampling is conducted for multiple years with insufficient sample sizes, it is not possible for researchers to correct this problem by adding additional quadrats after sampling has concluded. Researchers would be forced to use the data available, which may not be adequate to produce statistically valid conclusions. If the sampling produces no statistically valid conclusions, the data collected are unable to answer the questions originally asked.

Due to their significant declines in recent decades (Neves et al. 1997, Ricciardi and Rasmussen 1999), freshwater mussels are becoming a focus of ecological monitoring as bio-indicators for pollution (Foster and Bates 1978, Angelo et al. 2007, Webb et al. 2008). It is imperative that researchers follow the recommendations of Legg and Nagy (2006) when developing mussel monitoring programs in the future. Otherwise, it is possible that those conducting the monitoring programs will be unable to properly analyze the data collected, and will lead to a waste of financial and personnel resources.

The goal of this study is to evaluate several potential monitoring programs, evaluate their ability to adequately measure changes in mussel density at selected sites in the Powell River, and make recommendations for a statistically valid, long-term monitoring program. Relying on the software MONITOR to simulate multiple sampling programs, this study will determine which programs, if any, would obtain sufficient power to make statistically valid conclusions on changes in mussel density at selected sites in the Powell River. MONITOR evaluates the adequacy of sampling programs based on initial mean densities and standard deviations of the sampling data, program duration, sampling frequency and intervals between sampling events, and population growth rates, all of which must be considered when developing a successful monitoring program. Most importantly, MONITOR forces the researcher to consider what statistical analyses will be performed on the data at the conclusion of the program, and what levels of power and significance are desired.

Statement of Objectives

Estimating temporal trends in animal populations is often a focus of resource managers. Unfortunately, sampling protocols that can determine statistically valid conclusions regarding these temporal changes are not often utilized. The goal of this study is to use data and results presented in Chapter 1 to develop a statistically valid sampling protocol that can monitor changes in mussel abundance at selected sites in the Powell River. Specific objectives are as follows:

1) determine sample sizes (number of sites and number of 0.25 m^2 quadrats) needed to detect changes in mussel densities (mussels/0.25 m^2), while maintaining appropriate levels of statistical power and precision, at selected sites in the Powell River;
2) simulate sampling protocols over 15 y to 30 y periods to evaluate the ability of potential sampling programs to detect changes in total mussel density, < ±10% at selected sites; and
3) make recommendations for long-term sampling protocols for the Powell River.

Materials and Methods

Site Selection

Long-term monitoring sites were selected from those sampled in Chapter 1. Several factors were considered when selecting these sites, including species richness, presence of threatened or endangered species, total mussel density, accessibility, and proximity to human impacts. Ideal sites would 1) contain a rich mussel fauna that includes threatened and endangered species, 2) have a high total mussel density, 3) be easily accessible, but not directly adjacent to detrimental anthropogenic influences (industrial areas, excessive agriculture runoff, or extensive development), and 4) have been previously surveyed.

Five sites were selected for potential long-term monitoring, including shoals at PRKM's 197.9, 171.4, 159.6, 152.6, and 129.4 (Table 1). Each site met at least 2 of the 4 pre-determined criteria. Site PRKM 152.6 was selected because it is inhabited by a rich mussel fauna that includes several threatened and endangered species, is easily accessible, and is not close to any known major anthropogenic impacts. The shoals at PRKM's 197.9 and 129.4 were selected because they contained the two highest densities of mussels of all sites sampled during the

Chapter 1 survey (1.97 mussels/m^2 and 2.25 mussels/m^2, respectively), and are protected from human influence. The shoal at PRKM 197.9 also is the uppermost site in the Powell River with a dense mussel fauna, and would be a good point to measure potential impacts in Virginia. The shoal at PRKM 129.4 is one of the most downstream sites from the known anthropogenic impacts of southwest Virginia. The sites at PRKMs 171.4 and 159.6 should continue to be surveyed because of known mussel density and species richness at the sites. The five sites selected for long-term monitoring span approximately 70 km of river, and provide adequate spatial coverage of the river upstream of Norris Reservoir (Figure 1).

Determining Adequate Sample Size

Sampling Sites

Using mussel density data from Chapter 1, the program G*Power 3.1 (Institute for Experimental Psychology, Heinrich Heine University, Düsseldorf, Germany) determined the number of sampling sites required to achieve appropriate levels of statistical power when making comparisons among the three river zones created in Chapter 1 (Upper, Middle, and Lower). *A prior* power analyses were conducted for mussel density, total species, and mussel CPUE using significance levels of 0.05, 0.10, and 0.15.

Quadrats

Quantitative data from the Chapter 1 survey were used to determine the appropriate number of quadrats to accurately estimate mussel density. Mean stability plot evaluation of quantitative data from Chapter 1 determined that the numbers of quadrats used during this study were generally inadequate to provide accurate mussel density estimations. Therefore, the following formula was employed to determine the required number of quadrats needed to obtain a desired level of precision using previously collected data for mean mussel density or mussels/m^2 (Downing and Downing 1992)

$$n = 1 \times \left(\frac{mussels / m^2}{10{,}000 / A} \right)^{-0.5} \times D^{-2}$$

where, n = sample size,

and, A = cm^2 covered by each replicate sample (2,500 cm^2 in this study),

and, D = SE/m = the desired accuracy of density estimates.

This process was performed in two ways. First, the number of quadrats needed to obtain the same level of precision for each site was calculated for several levels of precision, 0.20, 0.15, 0.10, 0.09, and 0.08. Second, several standard numbers of quadrats were simulated to determine the obtained level of precision using that pre-set number of quadrats. The standard numbers of quadrats calculated for this study were 75, 100, 125, and 150 quadrats. It is important to note that the number of quadrats recommended by this formula only provides an estimate of precision and not accuracy of density estimates.

Evaluation of Long-Term Sampling Programs

The program MONITOR (Gibbs and Ene 2010) was used to test the statistical power of a potential survey protocol's ability to detect trends in overall mussel density at the river sites surveyed during this study. MONITOR uses Monte Carlo procedures to create numerous simulated sets of survey data and determines how often the pre-defined survey program detects trends that occur in the sampling data. The user can input multiple sampling parameters into the theoretical monitoring program, including the number of sites surveyed, frequency at which the sites are surveyed, total duration of the survey program, significance level used to detect population trends, and whether the user wants to detect a positive and/or negative trend in population growth. Site densities and standard deviations from Chapter 1 were used as input parameters for MONITOR.

MONITOR was used to evaluate the ability of six potential monitoring programs to detect change in mussel density at the selected sites in the river. All sampling programs assumed exponential growth, used 2-tailed significant tests (to detect positive and/or negative trends), and were simulated using 1000 replications. The program uses exponential growth as a default during simulations unless a differing growth rate is supplied. Not enough is known about mussel population growth to insert a different population growth parameter; therefore, the default exponential population growth rates were used during this study. Three levels of significance were evaluated, 0.05, 0.10, and 0.15. Truncated, normal distributions were used for each simulation. By using a truncated, normal distribution, the program would only simulate density measurements $\geq$ 0.00 mussels/m^2. Non-truncated, normal distributions would produce negative density measurements, and log-normal distribution measurements would not simulate densities equaling zero.

Nineteen growth trends were evaluated, including -50%, -40%, -30%, -20%, -10%, -8%, -6%, -4%, -2%, 0%, 2%, 4%, 6%, 8%, 10%, 20%, 30%, 40%, and 50%. These trends were tested for sampling scenarios involving 15 y, 24 y, 25 y, and 30 y programs, sampled at 3 y or 5 y intervals. As labeled in this thesis, a 15/3 sampling program would last 15 y with sampling events every 3 y during years 0, 3, 6, 9, 12, and 15.

Data Analysis

All statistical analyses were performed using G*Power 3.1 (Institute for Experimental Psychology, Heinrich Heine University, Düsseldorf, Germany) and MONITOR 11.0.0 (Gibbs and Ene 2010). Tables were produced using Excel 2007 (Microsoft, Inc., Redmond, Washington), and the figure was produced using ArcMap 9.3.1 (Environmental Systems Research Institute, Redlands, California).

Results

Appropriate Sample Sizes

Sampling Sites. The number of sample sites needed to obtain pre-determined levels of power, 0.80, 0.90, or 0.95, with corresponding levels of significance of 0.15, 0.10, or 0.05 varied greatly for zone comparisons of total mussel density, mussel CPUE, or total mussel species collected. Using a level of significance of 0.05, the number of sites required to obtain a statistical power of 0.80 when comparing total mussel density among zones was 15 sites. In order to obtain a statistical power of 0.95, using the same level of significance, a sampling program would need to sample 30 sites. When attempting to detect significant differences in mussel CPUE or total mussel species, when $\alpha = 0.05$, 12 and 9 sites are needed, respectively, to achieve a statistical power of 0.80. In order to achieve a statistical power of 0.95, 30 and 27 sites would need to be sampled to compare mussel CPUE and total mussel species, respectively (Table 2).

Quadrats. The number of quadrats required to achieve a desired level of precision of 0.20 ranged from 17 to 33 quadrats, and between 104 to 205 quadrats for a precision of 0.08 (Table 3). The calculated precisions for sampling 75 quadrats at all sites ranged from 0.094 to 0.132.

The protocol sampling of 150 quadrats at all sites achieved the highest levels of precision, 0.067 to 0.094 (Table 4).

Long-Term Sampling Simulations

A total of 6 potential long-term sampling protocols were simulated using the program MONITOR (Table 5) to determine which protocols could detect statistically significant increases or decreases in mussel density at selected sites in the Powell River. Generally, all of the sampling protocols simulated were able to detect increases in mussel abundance, but were unable to detect decreases in abundance.

Each potential sampling program utilized 150 quadrats. The degree of power obtained by each sampling protocol varied widely with differing sampling durations, sampling intervals, significance level, or type of trend (positive or negative). If a 30/3 sampling program assumes a significance level of 0.05, then it will be able to detect an increase in mussel density at 8% per sampling interval (Table 5). The same program also would be able to detect 8% increases in density during a 24/3 program, although with slightly lower power (1.00 versus 0.80), and would be unable to detect increases in density smaller than 10% during a 15/3 program. A sampling protocol with sampling intervals of 5 y achieved comparable levels of power for 15/5, 25/5, and 30/5 programs (Table 5). All simulated programs with assumed significance levels of 0.10 and 0.15 achieved higher levels of power than those with assumed significance levels of 0.05. Two 30/3 programs, with set significance levels of 0.10 and 0.15, were able to detect mussel density increases of 6% (Table 5). No sampling protocols, regardless of significance levels, sampling durations, or sampling intervals, were able to detect population declines at any of the rates tested during this study ($\leq$ 50%).

Discussion

Sample Sizes

Sampling Sites

Power analyses determined the number of sampling sites required to make comparisons in species presence and abundance (mussels/m^2 and mussels/person-h) among river zones (Upper, Middle, and Lower). In order to maintain an appropriate level of statistical power, 0.80, the number of sampling sites ranged from 9 to 15 sites (species presence: 9, mussel CPUE : 12, mussel density (mussels/m^2): 15, when assuming $\alpha = 0.15$). It is unlikely that any long-term sampling program would have the required resources to routinely sample this many sites. So, the statistical comparison of river zones during a long-term monitoring program does not appear to be a reasonable sampling design for monitoring the Powell River mussel fauna.

Quadrats

Systematic quadrat sampling, with one or multiple random starts, would be suitable for documenting the presence of juvenile mussels. One major deviation from the methodology used during this study would be the number of quadrats used during each survey. Using the Strayer and Smith's (2003) precision formula, it was determined that sampling 150 quadrats (0.25 m^2) at each of the selected sites (PRKMs 197.9, 171.4, 159.6, 152.6, and 129.4) would achieve a precision of at least < 0.10. This is not an unreasonable number of quadrats to be sampled per site in the river. Generally, a disproportionate number of quadrats sampled in the river lacks mussels, and quantitative sampling can be completed quickly. Sampling the proposed 750 quadrats could be easily completed in 75-80 person-hours. Excavating this number of quadrats will provide a precise estimate of total mussel density at the site, and exert a reasonable effort to collect juveniles. The level of statistical precision obtained by this number of quadrats more than justifies the level of effort required. Sampling more than 150 quadrats would be preferred, as increased sample size will provide greater precision in density estimates (Strayer and Smith 2003), but the level of effort required may extend beyond reasonable costs.

Evaluation of Monitoring Programs

The monitoring programs simulated during this study were able to detect increases in density at individual sites, but were not able to detect decreases in density. Monitoring changes in total mussel density is often an objective of mussel programs, and any proposed program for the Powell River would have this objective. Unfortunately, simulations using data from Chapter 1 suggest that the 15 to 30 y monitoring programs simulated, using 3 or 5 y intervals, would not be able to detect declines in mussel density ≤ 50% or increases in mussel density of ≤ 6% with a suitable level of power. This shortcoming in the potential monitoring programs is likely caused by sparse mussel populations and high deviations in quadrat data caused by non-random patterns of mussel distribution.

Three changes that could be made to the monitoring programs to potentially remedy this issue are as follows: 1) increase quadrat sample size to lower within-sample deviation, or 2) decrease interval lengths between sample periods to increase the number of sampling events during the program, or 3) some combination of the two. Neither of these proposed solutions would be realistic options. As previously mentioned, increasing quadrat sample sizes would likely require more resources than could reasonably be allocated. The same could be said for decreasing the intervals between sampling events.

For example, simulations determined that a sampling program could detect an 8% increase in mussel density with a statistical power of 0.90 if 150 quadrat sampling events occurred every 3 y during a 24 y period, 9 sampling events, and a significance level of 0.05 was used during calculations. In order to determine whether these changes are observed on a river-wide basis, this sampling program would need to use 24 sites, 8 sites/river zone, in order to obtain a 0.90 power at a 0.05 level of significance for detecting significant differences in mussel density among zones. Using 150 quadrats per site across the 24 sites, a total of 3,600 quadrats would need to be excavated during each sampling event. Through the duration of the 24 y monitoring program, a total of 32,400 quadrats would need to be excavated. It is unlikely that a monitoring program requiring this number of quadrats to only detect an 8% increase in mussel density would be funded. It is even less likely that a monitoring program with the ability to detect a comparable decrease in mussel density, requiring much larger sample size and sampling events, would justify its cost.

Potential Sampling Designs

Detecting Rare or Endangered Species

Future surveys in the Powell River will likely focus on documenting the continued presence of threatened and endangered species. A successful monitoring program would need to collect presence/absence data in both a cost-efficient and time-efficient manner. Currently, a simple, timed, random CPUE sampling design remains the most cost-effective method for monitoring species richness, and in turn, rare species presence (Vaughn et al. 1997, Obermeyer 1998, Metcalfe-Smith et al. 2000). This method allows surveyors to cover large areas and many habitat types while exerting relatively little effort. Unfortunately, CPUE measurements do not always adequately represent mussel abundances, and researchers are attempting to develop a sampling method that simultaneously detects rare mussel species and estimates mussel densities (Obermeyer 1998, Smith et al. 2003, Smith 2006).

One methodology that has been proposed as a potential solution to the quantitative sampling of rare or clustered mussel populations is adaptive cluster sampling. Unfortunately, the method can be marred with inefficiency if populations are common or insufficiently clustered, and it is difficult to determine when the technique is appropriate without having *a priori* data on the focus population's density and distribution (Smith et al. 2003). In order to obtain these data, a form of random CPUE sampling would be required to determine the degree of clustering at a site. This makes the process time-consuming as researchers need to conduct one form of sampling to determine whether another form of sampling is applicable.

Smith (2006) proposed a method for determining the amount of sampling necessary to detect a rare mussel species based upon search area, an estimated number of mussels found at the survey site, and search efficiency (detectability). Estimating search efficiency relies on a suite of factors that are directly related to the target animal's behavior, color, size, shape, and reproductive activities. Search efficiency also would be impacted by dominant substrate types, vegetative cover, and water temperature and turbidity. It is difficult to accurately estimate search efficiency for mussel aggregations, due to site differences, unique behaviors, and life-history characteristics of each mussel species. The difficulty to verify search efficiency makes any estimation of required sampling area using Smith's formula difficult to apply to a real-world setting.

Unfortunately, both of these suggested alternate methodologies make mussel sampling more time-intensive, and rely on estimations of search efficiency or degree of clustering, neither of which may be easily available for the focal population. Therefore, it is reasonable to use the random CPUE sampling method to detect species presence at a site.

Juvenile Recruitment

As mussel populations decline and more species are extirpated from their native ranges, biologists have an increasing desire to monitor juvenile recruitment. Qualitative sampling methods have generally proven ineffective in collecting juveniles when compared to quantitative sampling (Obermeyer 1998). This is because some form of sediment excavation is often required to locate smaller individuals and increase search efficiency (Hornbach and Deneka 1996). For these reasons, the excavation of quadrats is recommended for juveniles mussels in the Powell River.

Recommended Sampling Program Design

Qualitative and quantitative sampling should be conducted at PRKMs 197.9, 171.7, 159.6, 152.6, and 129.4 on a 5 y rotating schedule to monitor the mussel fauna into the future (Tsble 6). Using this scheduling format, one of the five sites will be sampled each year, and the other four sites will be sampled in a consistent order over the following 4 y. This would allow each site to be sampled in 5 y intervals without using the level of resources needed to sample all five sites in a single year.

Regardless of the number of sites and sampling order selected for future monitoring, random CPUE surface sampling should be conducted to monitor changes in species presence and distribution. One hundred-fifty 0.25 m^2 quadrats should be sampled utilizing a one- or three-random start, systematic sampling design to monitor changes in recruitment and size-class structure. Utilizing this number of quadrats would obtain ≤ 0.094 precision at each of the selected sites. All quadrats should be excavated to increase the likelihood of collecting smaller individuals and document recent recruitment. Although this sampling program would not allow managers to detect statistically significant declines in mussel density, it would allow them to make qualitative assessments of the mussel fauna and enact appropriate management policies.

Conclusions and Recommendations

1) Future mussel monitoring programs should consider statistical validity prior to implementation. Properly developing a program *a priori* will lead to more efficient uses of funding, personnel time, and statistically testable hypotheses and defensible conclusions.

2) Financial and personnel restraints would prevent any future monitoring program from sampling the number of sites needed to make statistical comparisons among the river zones described in Chapter 1.

3) Potential long-term monitoring programs should consider life-history characteristics of the study species. Due to several species of mussels being short-lived, a program with 3 to 5 y intervals between sampling events should be considered.

4) Although, the quadrat surveys simulated during this study cannot adequately detect decreases in mussel densities ≤ 10%, they could still be used to detect juvenile recruitment in the river and detect projected increases in total mussel density.

5) Additional simulations of quadrat surveys should be conducted to determine whether monitoring programs could statistically detect declines in mussel density > 10%.

6) In order to achieve levels of precision that are < 0.10, future sampling protocols should sample 150 quadrats.

7) Further analysis of mussel population growth patterns are needed to develop more accurate monitoring simulations. The simulations conducted in this study assume exponential population growth, which does not accurately represent the actual growth pattern of mussel populations.

8) None of the simulated sampling protocols would adequately detect declines in mussel density (mussels/m^2). However, qualitative and quantitative sampling should be conducted in the future to monitor species presence, juvenile recruitment, and size-class structure. Documenting these community characteristics will allow researchers to qualitatively assess the status of mussel populations, despite being unable to make statistical comparisons of sampling events over time.

9) Long-term sampling should be conducted at PRKMs 197.9, 171.4, 159.6, 152.6, and 129.4. Sampling should be conducted for 30 y in 5 y intervals using random CPUE to collect species presence/absence data, and $\geq$ 150 - 0.25 m^2 quadrats should be collected to estimate mussel densities (mussels/m^2). Quadrat sampling should be conducted using a one- or 3-random start, systematic sampling design to obtain adequate spatial coverage of the entire site.

Literature Cited

Ahlstedt, S.A., M.T. Fagg, R.S. Butler, and J.F. Connell. 2005. Long-term trend information for freshwater mussel populations at twelve fixed-station monitoring sites in the Clinch and Powell rivers of eastern Tennessee and southwestern Virginia, 1979-2004. Unpublished Report**,** U.S. Fish and Wildlife Service, Cookeville, Tennessee.

Angelo, R. T., M. S. Cringan, D. L. Chamberlain, A. J. Stahl, S. G. Haslouer, and C. A. Goodrich. 2007. Residual effects of lead and zinc mining on freshwater mussels in the Spring River Basin (Kansas, Missouri, and Oklahoma, USA). Science of the Total Environment 384: 467-496.

Dennis, S. D. 1981. Mussel fauna of the Powell River, Tennessee and Virginia. Sterkiana 71:1-7.

Foster, R. B., and J. M. Bates. 1978. Use of freshwater mussels to monitor point source industrial discharges. Environmental Science and Technology. 12: 958-962.

Fowler, J., L. Cohen, and P. Jarvis. 1998. Practical Statistics for Field Biology. 2nd Edition. Wiley, New York.

Gibbs, J. P. and Eduard Ene. 2010. Program Monitor: estimating the statistical power of ecological monitoring programs. Version 11.0.0.

Hornbach, D. J., and T. Deneka. 1996. A comparison of a qualitative and a quantitative collection method for examining freshwater mussel assemblages. Journal of the North American Benthological Society 15: 587-596.

Jenkinson, J. J. and S. A. Ahlstedt. 1988. Semi-quantitative reassessment of the freshwater mussel fauna in the Powell River, Tennessee and Virginia. Tennessee Valley Authority (TVA), River Basin Operations/Water Resources. Knoxville, Tennessee.

Krebs, C. J. 1989. Ecological Methodology. Harper Row, New York.

Lagg, C. J. and L. Nagy. 2006. Why most conservation monitoring is, but need not be, a waste of time. Journal of Environmental Management. 78: 194-199.

Metcalfe-Smith, J. L., J. Di Maio, S. K. Staton, and G. L. Mackie. 2003. Effect of sampling effort on the efficiency of the timed search method for sampling freshwater mussel communities. Journal of the North American Benthological Society 19: 725-732.

Neves, R.J., A.E. Bogan, J. D. Williams, S. A. Ahlstedt, and P.W. Hartfield. 1997. Status of aquatic mollusks in the southeastern United States: A downward spiral of diversity. In: G.W. Bense and D. E. Collins, editors. Aquatic Fauna in Peril: The Southeastern Perspective. Special publication 1, Southeastern Aquatic Research Institute, Lenz Design and Communications, Decatur, Georgia: 43-85.

Obermeyer, B. K. 1998. A comparison of quadrats versus timed snorkel searches for assessing freshwater mussels. American Midland Naturalist 139:331-339.

Peterman, R. M. 1990. Statistical power analysis can improve fisheries research and management. Canadian Journal of Fisheries and Aquatic Science 47:2-15.

Ricciardi, A., and J. B. Rasmussen. 1999. Extinction rates of North American freshwater fauna. Conservation Biology 13: 1220-1222.

Smith, D. R. 2006. Survey design for detecting rare freshwater mussels. Journal of the North American Benthological Society 25: 701-711.

Smith, D. R., R. F. Villella, and D. P. Lemarie. 2003. Application of adaptive cluster sampling to low-density populations of freshwater mussels. Environmental and Ecological Statistics 10: 7-15.

Strayer, D. 1986. Long-term ecological studies: An illustrated account of their design, operation, and importance to ecology. Occasional Publication of the Institute of Ecosystem Studies, New York Botanical Garden, No. 2.

Strayer, D. L. and D. R. Smith 2003. A Guide to Sampling Freshwater Mussel Populations. Bethesda, Maryland, American Fisheries Society.

Toft, C. A. and P. J. Shea. 1983. Detecting community-wide patterns: estimating power strengthens statistical inference. American Naturalist 122:618-625.

Vaughn, C. C., C. M. Taylor, and K. J. Eberhard. 1997. A comparison of effectiveness of timed searches vs. quadrat sampling in mussel surveys, p. 157 – 162 in K. S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo (eds.). Conservation and Management of Freshwater Mussels II: Initiatives for the uture. Proceedings of an Upper Mississippi River Conservation Committee (UMRCC) symposium, 16-18 October 1995, St. Louis, Missouri. Upper Mississippi River Conservation Committee, Rock Island, Illinois.

Webb, K., C. Craft, and E. Elswick. 2008. The evaluation of the freshwater western pearl mussel, *Margaritifera falcata* (Gould, 1850), as a bioindicator through the analysis of metal partitioning and bioaccumulation. Northwest Science 82: 163:173.

Table 1. Site locations described by Powell River kilometer (PRKM), commonly-used site name, and GPS coordinates for the four corners of the surveyed area.

PRKM	Site Name	GPS Boundaries	
197.9	Island Below Snodgrass	N 36.61855, W -83.25779	N 36.61832, W -83.25779
		N 36.61875, W -83.25888	N 36.61839, W -83.25868
171.4	McDowell Shoal	N 36.57497, W -83.36272	N 36.57507, W -83.36245
		N 36.57376, W -83.36155	N 36.57390, W -83.36134
159.6	Buchanan Ford	N 36.55819, W -83.42336	N 36.55806, W -83.42250
		N 36.55861, W -83.42425	N 36.55855, W -83.42441
152.6	Bar below Brooks	N 36.53742, W -83.45206	N 36.53732, W -83.45230
		N 36.53905, W -83.45348	N 36.53888, W -83.45365
129.4	Double S Bend	N 36.52263, W -83.51653	N 36.52284, W -83.51670
		N 36.52295, W -83.51579	N 36.52303, W -83.51605

Table 2. *A priori* power analysis to determine sample sizes (0.25 m^2 quadrats) to achieve desired powers of, 0.80, 0.90, and 0.95, for total mussel density, mussel CPUE, and total species present, using pre-defined significance levels, 0.05, 0.10, and 0.15, and mean and SD data from individual sites qualitatively and quantitatively sampled for freshwater mussels in the Powell River, Virginia and Tennessee, 2008 and 2009. The numbers within the table represent the total number of sites needed to achieve the desired level of power with pre-determined level of significance. For example, if comparing mussel CPUE among three zones, 18 total sites (6 sites per zone) would be needed to achieve a power of 0.80, with a pre-defined significance level of 0.05, when attempting to detect significant differences in mussel CPUE.

	Mussel Density			**Mussel CPUE**			**Total Species Present**		
	Significance level (α)			**Significance level (α)**			**Significance level (α)**		
Power	**0.05**	**0.1**	**0.15**	**0.05**	**0.1**	**0.15**	**0.05**	**0.1**	**0.15**
0.95	30	24	21	27	21	18	15	12	9
0.90	24	21	18	21	18	15	12	9	9
0.80	21	15	15	18	15	12	12	9	9

Table 3. Required quadrat sample sizes to obtain predetermined levels of precision at selected sites in the Powell River, Virginia and Tennessee. Total mussel density = mussels/0.25 m^2 obtained during the Chapter 1 mussel survey.

			Precision				
PRKM	**Site Name**	**Total Mussel Density**	**0.2**	**0.15**	**0.10**	**0.09**	**0.08**
197.9	Island Below Snodgrass	1.96	18	32	71	89	112
171.4	McDowell Shoal	0.58	33	58	131	162	205
159.6	Buchanan Ford	0.59	33	58	130	161	203
152.6	Bar Below Brooks	1.33	22	39	87	107	135
129.4	Double S Bend	2.24	17	30	67	83	104

Table 4. Predicted precision obtained using predetermined numbers of quadrats at selected sites in the Powell River, Virginia and Tennessee. Total mussel density = density estimation obtained during the Chapter 1 mussel survey.

			Number of Quadrats			
PRKM	**Site Name**	**Total Mussel Density**	**75**	**100**	**125**	**150**
197.9	Island Below Snodgrass Ford	1.96	0.98	0.84	0.076	0.069
171.4	McDowell Shoal	0.58	0.132	0.115	0.102	0.094
159.6	Buchanan Ford	0.59	0.132	0.114	0.102	0.093
152.6	Bar Below Brooks Bridge	1.33	0.108	0.093	0.083	0.076
129.4	Double S Bend	2.24	0.094	0.82	0.073	0.067

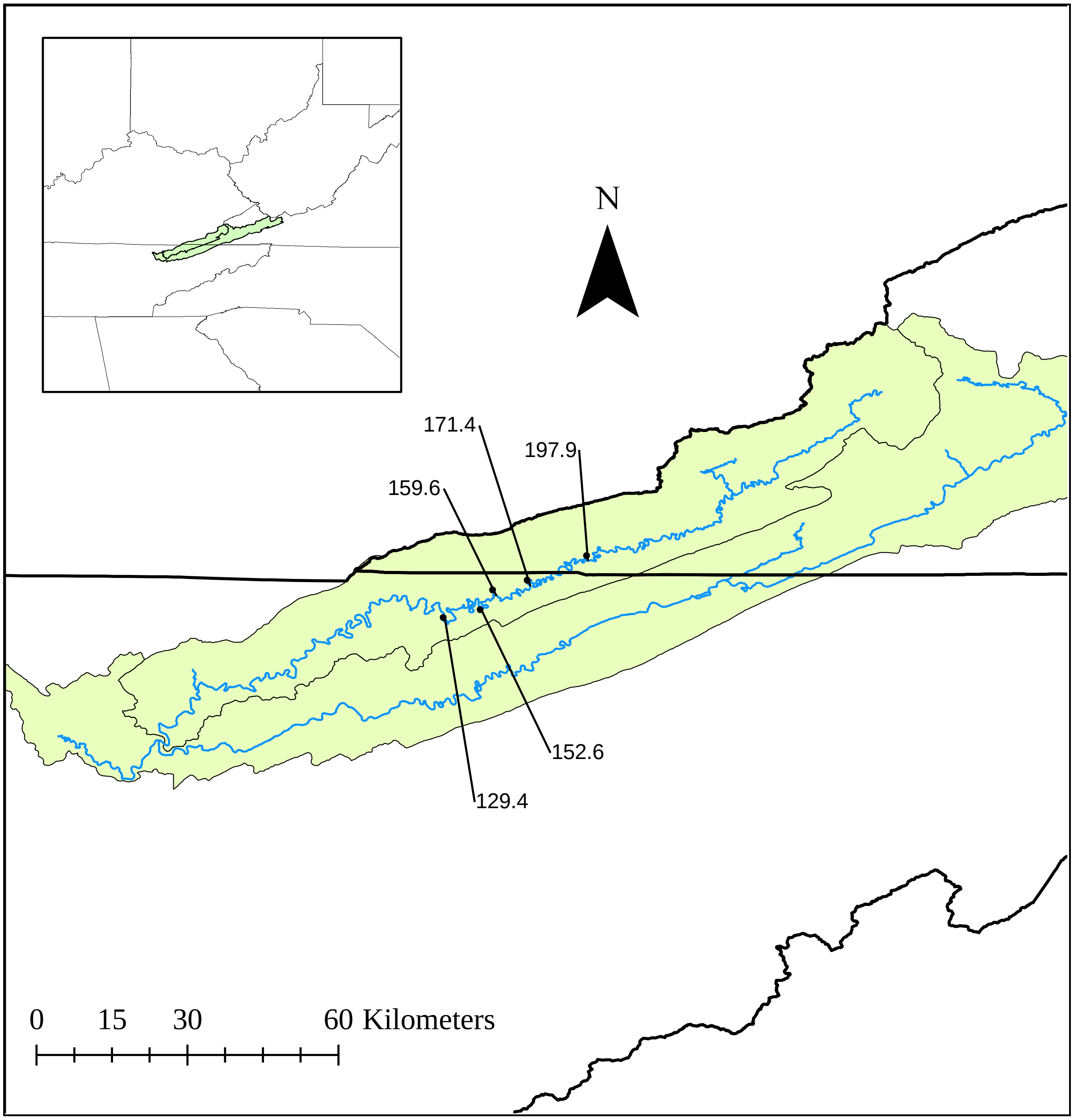
Table 5. Statistical power associated with potential sampling protocols at PRKMs 197.9, 171.4, 159.6, 152.6, and 129.4. Potential program descriptions are total duration of the program and the sampling interval during the program; i.e. 15/3 = a program with a duration of 15 y and sampling occurring every 3 y. A total of 150 quadrats were used for each sampling event, and a 2-tailed test was used to monitor potential positive and negative trends.

	Potential Sampling Programs																	
	Significance Level (α) = 0.05						Significance Level (α) = 0.10						Significance Level (α) = 0.15					
Trend	**15/3**	**15/5**	**24/3**	**25/5**	**30/3**	**30/5**	**15/3**	**15/5**	**24/3**	**25/5**	**30/3**	**30/5**	**15/3**	**15/5**	**24/3**	**25/5**	**30/3**	**30/5**
50%	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
40	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
30	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20	0.99	0.99	1	1	1	1	0.99	0.99	1	1	1	1	1	0.99	1	1	1	1
10	0.47	0.42	0.99	0.99	1	1	0.6	0.55	0.99	0.98	1	1	0.68	0.68	0.99	0.99	1	0.99
8	0.1	0.1	0.9	0.87	1	1	0.18	0.18	0.95	0.95	1	1	0.26	0.23	0.96	0.97	1	1
6	0.08	0.04	0.25	0.27	0.79	0.66	0.11	0.09	0.39	0.41	0.82	0.82	0.14	0.14	0.49	0.48	0.93	0.87
4	0.03	0.03	0.07	0.05	0.11	0.11	0.07	0.08	0.11	0.12	0.2	0.2	0.12	0.11	0.15	0.12	0.27	0.12
2	0.04	0.03	0.05	0.04	0.04	0.03	0.07	0.08	0.07	0.08	0.08	0.08	0.13	0.13	0.11	0.17	0.15	0.21
0	0.04	0.02	0.03	0.03	0.04	0.04	0.09	0.07	0.07	0.07	0.07	0.09	0.11	0.11	0.1	0.13	0.1	0.11
-2	0.04	0.04	0.02	0.03	0.04	0.02	0.06	0.07	0.07	0.08	0.05	0.07	0.11	0.11	0.12	0.12	0.14	0.1
-4	0.03	0.03	0.03	0.03	0.03	0.04	0.06	0.06	0.09	0.07	0.08	0.08	0.13	0.09	0.1	0.13	0.12	0.1
-6	0.03	0.03	0.04	0.03	0.03	0.04	0.07	0.07	0.07	0.06	0.07	0.07	0.12	0.12	0.12	0.11	0.11	0.12
-8	0.03	0.03	0.04	0.04	0.03	0.03	0.06	0.08	0.09	0.08	0.06	0.08	0.11	0.11	0.1	0.11	0.12	0.14
-10	0.05	0.06	0.12	0.10	0.16	0.15	0.10	0.09	0.17	0.17	0.21	0.22	0.15	0.14	0.23	0.22	0.26	0.27
-20	0.15	0.14	0.25	0.23	0.30	0.25	0.20	0.20	0.31	0.32	0.41	0.40	0.27	0.25	0.37	0.39	0.45	0.42
-30	0.22	0.20	0.31	0.30	0.38	0.35	0.26	0.25	0.40	0.35	0.45	0.48	0.36	0.33	0.48	0.46	0.52	0.55
-40	0.28	0.22	0.41	0.31	0.46	0.36	0.30	0.31	0.50	0.38	0.48	0.52	0.43	0.35	0.55	0.53	0.55	0.60
-50%	0.28	0.24	0.41	0.37	0.47	0.41	0.36	0.34	0.52	0.42	0.56	0.54	0.49	0.38	0.60	0.56	0.62	0.63

Table 6. A sample, proposed rotating sampling schedule based on sampling five sites over a 5 y period.

PRKM	Site Name	Sampling Year
197.9	Island Below Snodgrass Ford	2012
171.4	McDowell Shoal	2013
159.6	Buchanan Ford	2014
152.6	Bar Below Brooks Bridge	2015
129.4	Double S Bend	2016
197.9	Island Below Snodgrass Ford	2017
171.4	McDowell Shoal	2018
159.6	Buchanan Ford	2019
152.6	Bar Below Brooks Bridge	2020
129.4	Double S Bend	2021
197.9	Island Below Snodgrass Ford	2022
171.4	McDowell Shoal	2023
159.6	Buchanan Ford	2024
152.6	Bar Below Brooks Bridge	2025
129.4	Double S Bend	2026

Figure 1. Proposed sampling sites for long-term monitoring in the Powell River, Virginia and Tennessee.



Chapter 3

Population Status of the Appalachian monkeyface, *Quadrula sparsa* (Lea, 1841), and Cumberland monkeyface, *Quadrula intermedia* (Conrad, 1836), in the Powell River, Virginia and Tennessee

(Abstract)

This study determined the current distribution, and selective life history characteristics of the two species. Sixteen and 68 individuals of *Q. sparsa* and *Q. intermedia*, respectively, were collected using qualitative snorkel survey methods at 22 sites within an approximately 100 km reach of the Powell River, between PRKMs 232.7 and 136.1. Due to their low densities and subsurface habitat, it is possible that both species occur in very low densities at additional sites outside of this range. Several young specimens < 10 y in age were collected at two sites in the river, PRKMs 153.3 and 136.1.

An age-growth curve was developed for each species by counting internal and external growth annuli on collected valves. Fifteen valves (50 - 80 mm) and 27 valves (37 - 74mm) of *Q. sparsa* and *Q. intermedia*, respectively, were thin-sectioned for age determination. Ages of these shells ranged from 14-44 y for Q. sparsa and 7-37 y for *Q. intermedia.* Based on the sizes and ages from thin-sectioned valves, neither species appears to live beyond approximately 50 y. Fourteen fish species were collected to test as potential hosts, but no viable glochidia were collected for either species.

Introduction

The genus *Quadrula* is comprised of 20 recognized species throughout North America. Populations of only 7 of the 20 species within the genus are considered currently stable. Five species have been declared federally endangered, an additional five species have been declared imperiled, and three species are presumed extinct (Table 1) (Williams et al. 1993, Turgeon et al. 1998, Serb et al. 2003). Geographic ranges of species within the genus vary greatly. Some species have an extremely wide distribution, across entire river systems like the Mississippi, while other species are highly endemic and only inhabit a few rivers (Serb et al. 2003).

Quadrula sparsa (Lea 1841), Appalachian monkeyface, is a Cumberlandian species that has been reported historically in the upper Tennessee River drainage (United States Fish and Wildlife Service [USFWS] 1983a), including the Powell (Stansbery 1976), Clinch (Stansbery 1973 & 1976, Ahlstedt 1991a), and Holston rivers (Lea, 1841, Simpson 1914). Historical records of *Q. sparsa* distributions are confusing at best due to identification discrepancies. Ortmann (1918) combined *Q. sparsa* with *Q. tuberosa* (Lea 1840), rough rockshell, and *Q. intermedia* (Conrad 1836), Cumberland monkeyface, under *Q. intermedia*. Because of this confusion, it is unknown whether *Q. sparsa* and *Q. tuberosa* were distinct species. If the two species were in fact synonymous, *Q. sparsa* also inhabited the headwaters of the Cumberland River (Parmalee and Bogan 1998).

Quadrula sparsa is currently only known to inhabit the Powell and Clinch rivers upstream of Norris Reservoir (USFWS 1983a). The remnant population of *Q. sparsa* in the Powell River resides in a 72.4 km reach (PRKM 136.5 to PRKM 210.2), and is considered extremely small and at a high risk of extirpation (Ahlstedt 2005). In the recent past, live individuals have been found in the Clinch River at CRKM 435.8, but they are not believed to be members of a viable population (Jess Jones, USFWS, Virginia Tech, personal communication).

Adults of *Q. sparsa* are not clearly sexually dimorphic. Both sexes have a yellowish-brown periostracum, and often have small green chevrons (triangles). Parmalee and Bogan (1998) describe the shell as, "…triangular to irregularly rhomboidal in outline, solid, and rather sparsely tuberculate" (Fig. 1). Females are believed to be tachytictic brooders (short-term) and become gravid in late spring. When spawning, individuals have been observed lying on their side on river substratum (Ahlstedt 1991). No host fish information is available for this species.

Quadrula intermedia also is a Cumberlandian species that has been historically reported in the upper Tennessee River system, including the Powell (Stansbery 1976), Clinch (Ortmann 1918, Stansbery 1976), Holston (Ortmann 1918), Duck (Ortmann 1924, Stansbery 1976), and Elk rivers (Ortmann 1924, Stansbery 1976). Additional archaeological specimens have been collected from the lower Clinch and Tennessee rivers. The only viable populations are found currently in the Powell River (PRKM 136.2 to PRKM 210.2) and Duck River (DRKM 287.2 to DRKM 262.0) (USFWS 1982).

Like *Q. sparsa*, *Q. intermedia* is not clearly sexually dimorphic. Both sexes have a greenish-yellow periostracum that darkens as the individual matures, and exhibits green chevrons and sometimes broken green rays. The shell is described as, "…solid, only slightly inflated, and sub-elliptical, sub-orbicular, or sub-quadrate in outline." (Parmalee and Bogan 1998) (Fig. 2). Like other species in the genus *Quadrula*, *Q. intermedia* is a tachytictic brooder, and females have been observed lying on their side on substratum during the spawning period (Parmalee and Bogan 1998).

The USFWS developed species recovery plans for both *Q. sparsa* and *Q. intermedia* in 1982 and 1983, respectively. These plans outlined several goals that would help the USFWS meet the overall objectives of maintaining and restoring viable populations of these species to significant portions of their former ranges (USFWS 1982, USFWS 1983a). The general goals of these plans are to (1) protect and enhance habitats containing remaining populations, and (2) establish populations in additional rivers where these species occurred historically.

In spite of having an existing recovery framework for more than 20 y, little has been accomplished to meet the goals set for the recovery of these species. Information regarding the current distribution and status of both Powell River populations are generally lacking, and what does exist was collected by surveys with differing goals and sampling techniques. These differences can make data difficult to analyze statistically. There is also a significant lack of basic life history information, such as growth rate, life span, and fish host species, for both *Q. sparsa* and *Q. intermedia*. Because these basic data have not been collected and analyzed, it is difficult to assess the condition of both species in the Powell River.

Knowledge of fish-host suitability is crucial to successful population augmentation and reintroduction. Discerning which fish species transform the maximum number of glochidia to juvenile mussels is vital to efficiently propagate freshwater mussels. Currently, fish host testing has not been conducted for *Q. sparsa*; therefore, no hosts have been identified for propagation purposes. *Erimystax dissimilis*, streamline chub, and *Erimystax insignis*, blotched chub, have been identified tentatively as hosts for *Q. intermedia* (Table 2) (Neves 1981, Yeager and Saylor 1995), but additional testing needs to confirm their suitability. Collecting additional information on fish host suitability for these two species is integral to successful population augmentation and creation of additional populations in other historically significant rivers.

Statement of Objectives

The goal of this study is to assess the current distributions and abundances of *Q. sparsa* and *Q. intermedia* in the Powell River, Virginia and Tennessee, and to provide life history metrics that are useful to the recovery of these populations. Specific objectives are as follows:

1) collect life history information for *Q. sparsa* and *Q. intermedia*, including growth rate and life span;
2) assess current population distribution and status of both species in the Powell River, Virginia and Tennessee; and
3) locate suitable release sites in the Powell River, Virginia and Tennessee, determine fish hosts for and propagate *Q. sparsa* and *Q. intermedia* juveniles for release at these sites.

Methods

Age and Growth

Fresh-dead shells and relic shells of individuals were thin-sectioned using the procedures described in Clark (1980) and Neves and Moyer (1988), along a continuous portion of shell between the umbo and ventral margin. Shells were cut using a Buehler Isomet low-speed saw with a diamond-impregnated blade (Buehler, Evantston, Illinois). The line of cut extended from the center of the umbo to the ventral edge, and remained perpendicular to the external growth rings when possible. Cut valves were adhered (2-Ton Clear Epoxy, Illinois Tool Works, Devcon, Massachusetts) to one or multiple petrographic microslides (27 x 46 mm or 25 x 75 mm). Slides were vacuum-sealed to the saw's petrographic chuck and sectioned to a thickness of approximately 0.8 mm. Shell-sections were then polished with 150-, 220- and 320-grit sandpaper to remove scratches that occurred during cutting. Sections were read using a microscope, and only lines that began at the umbo and continued completely to the ventral margin were counted. Lines that were not continuous were considered false annuli, and not counted. Because no individuals representing early age classes were collected, lengths for individuals <5 y were obtained by measuring the early external annuli (1 to 10 y) of older individuals. The accuracy of external versus internal aging was assessed by comparing the slope of the linear regression of internal ages on external ages and a theoretical 1:1 relationship between internal and external ages, using the following formula (Fischer 1921):

$$t = \frac{b_1 - b_2}{s_{b_1 - b_2}}$$

where, b = slope,

and, S = standard error of the slope.

Distribution and Length

During the summers of 2008 and 2009, qualitative substrate sampling, consisting of snorkeling or wading with view buckets, was used to collect live specimens, fresh-dead individuals, and relic shells at 22 sites between PRKM 104.8 and PRKM 230.9. The methods used for qualitative sampling are described in Chapter One. Live individuals were used to delineate current distributions of the two species in the Powell River. Recently dead and relic shell material was collected to age and create von Bertalanffy age-to-growth curves for both species. All live individuals were collected, measured, and aged using external growth annuli during 2008 and 2009. All live individuals and shells were measured at the widest point from posterior to anterior margins. Non-gravid individuals were immediately returned to their points of collection, but females that were thought to be potentially gravid were held for further observation at the Freshwater Mollusk Conservation Center at Virginia Tech (FMCC).

Fish Host Testing

Potential brood stock was collected by snorkeling, and examined for gravidity by carefully opening the valves to view the gills. If the individual was possibly gravid, it was placed in a closed container, and transported in an aerated cooler to either the FMCC or the Aquatic Wildlife Conservation Center (AWCC) at Buller Hatchery, Virginia Department of Game and Inland Fisheries in Marion, Virginia. Mussels were held in flow-through systems, and gravidity was checked periodically to monitor any progress towards producing viable glochidia. When a mussel was determined to not be gravid, it was returned to the site of collection.

Previous testing by Yeager and Saylor (1995) identified two species of *Erimystax* as suitable host species for *Q. intermedia*, whereas other fish groups such as suckers (catostomids) and darters (percids) were unsuitable for glochidia transformation (Table 2). Fish host testing of other species in the genus *Quadrula* has identified several ictalurids as suitable fish hosts (Fuller 1974, 1978; Yeager and Neves 1986; Steingraeber et al. 2007). In this study, fourteen fish species were collected to test as potential fish hosts (Table 2). Fish were purchased from a commercial aquaculture facility or collected through electro-shocking, kick seining, or herd seining. *Erimystax dissimilis*, streamline chub, and *Percina aurantiaca*, tangerine darter, were held in a Min-O-Cool system (Frigid Units Inc., Toledo, Ohio) with approximately 5 cm of small pebble substrate. All other fish were held in recirculating aquaculture systems consisting primarily of 75.7 L aquaria with no substrate. Pieces of PVC pipe were placed in each tank to provide cover for the fish. All systems were maintained between 19 and 21 C. Because no testing was conducted, all wild-caught fish were released, and purchased fish were euthanized.

Data Analysis

A one-way ANOVA was used to determine whether the lengths of shell-only specimens and live mussels were significantly different from one another. The Kolmogorov-Smirnov goodness-of-fit test was used to determine distribution of shell length data. Simple linear regression was used to determine accuracy of external aging by comparing ages estimated by counting internal growth lines as well as external growth rings. Fisher's test to compare regression line slopes (Fisher 1921) was used to compare the slopes of the regression of internal ages on external ages and the slope of a 1:1 relationship between internal and external ages. Data collected during sectioning also was used to develop a non-linear growth model, also known as a von Bertlanaffy growth curve. All statistical analyses were conducted using GLM, NLIN, REG, SLR, and UNIVARIATE procedures in SAS 9.2 (SAS Inst., Cary, North Carolina). Parameters of the von Bertalanffy growth equation were calculated and fit using nonlinear procedures in SAS. Additional graphs and figures were produced using Excel 2007 (Microsoft Corp., Redmond, Washington) and Minitab 15 (Minitab Inc., State College, Pennsylvania).

Results

Quadrula sparsa

Age and Growth. Fresh-dead and relic shell material of 15 specimens of *Q. sparsa* were successfully sectioned and aged. The mean length of shells of dead individuals was 65.8 mm (SD = 9.7) and ranged from 50 to 80 mm. Two additional shells were not sectioned due to poor shell quality, and excluded in the analysis. Ages of the sectioned shells ranged from 13 to 44 y.

The mean age of these shells was approximately 28 y (SD = 9.1). Using age and length data collected from the shell material, mean lengths (l) for each age (t) were used to develop a von Bertalanffy growth equation, $L_t = 67.8 \text{ mm} \times (1\text{-}e^{-0.2016(t- -0.0585)})$, which was used to predict length values at age. Annual growth averaged 4.8 mm/y through the first 13 y of life, and declined to 0.16 mm/y thereafter (Table 4 and Fig. 4).

Poor periostracum condition prevented external aging of growth rings on all 15 *Q. sparsa* shells used for sectioning. Because shells were not externally aged, the concurrence of external ages with internal ages could not be calculated.

Distribution and Length. A total of 18 live specimens of *Q. sparsa* were collected from 9 of the 23 sites surveyed during the summers of 2008 and 2009 (Table 3). These mussels were collected between PRKM 193.4 in Virginia and PRKM 152.6 in Tennessee. The greatest number of *Q. sparsa* was collected at PRKM 152.6 (Table 3).

The mean length of live individuals was 61.65 mm (SD = 11.31), and sizes ranged from 44.5 to 80.3 mm (Fig. 3). The largest live individuall (80.3 mm) was collected at PRKM 152.6, and the smallest (44.5 mm) was collected at PRKM 153.4. The Kolmogorov-Smirnov goodness-of-fit test found shell lengths of both shell-only specimens and live individuals to be normally distributed ($p > 0.05$). ANOVA found that lengths of shell-only specimens were not significantly different from those of live individuals ($F_{1, 30} = 0.82$, $p > 0.05$)

Fish Host Testing. Six female mussels were collected for potential fish host testing, but no individuals produced viable glochidia for fish host testing. The six individuals were returned to their site of collection.

Quadrula intermedia

Age and Growth. Twenty-seven freshly dead and relic shells of *Q. intermedia* were sectioned for internal age analysis. Ages of the sectioned shells ranged from 7 to 35 y. The mean age of shells of dead individuals was approximately 23 y (SD = 9.2). Mean lengths (l) for each age (t) were used to develop a the von Bertalanffy growth equation, $L_t = 65.3 \text{ mm} \times (1-e^{-0.1744(t - 0.3867)})$, which was used to predict length values. Annual growth averaged 4.6 mm/y through the first 14 y of life, and declined to 0.3 mm/y thereafter (Table 5 and Fig. 7).

The internal and external age estimates of 9 mussels were compared using a simple linear regression. Mussels were selected for this analysis because of high quality periostracum and the author's ability to be confident when externally aging these individuals. The linear regression of internal ages on external ages was significant ($r^2 = 0.55$, $p < 0.05$), and the slope of the regression line was 0.71. Fisher's test to compare regression slopes showed that the regression between internally and externally assigned ages was significantly higher than a theoretical 1:1 relationship between internal and external ages ($t = 3.79$, $p < 0.05$). This shows that counting external growth lines of *Q. intermedia* shells over-estimated predicted ages when compared to ages obtained through reading internal growth rings during this study.

Distribution and Length. A total of 68 live specimens of *Q. intermedia* were collected from 12 of the 23 surveyed sites (Table 3). These individuals were collected between PRKM 229.8 in Virginia and PRKM 129.4 in Tennessee. The highest numbers of *Q. intermedia* were collected between PRKM 193.4 and PRKM 152.6 (Table 3).

The mean length of live individuals was 57.0 mm (SD = 6.8), and lengths ranged from 38.7 to 68.3 mm (Fig. 6). Both the largest and smallest individuals were collected at PRKM

153.0. The mean length of shell specimens was 59.4 mm (SD = 10.0), and lengths ranged from 37 to 74 mm. The Kolmogorov-Smirnov goodness-of-fit test was used to screen shell-only and live individual shell length data for normality and determined that both data sets were normally distributed ($p > 0.05$). ANOVA showed that the lengths of shell-only specimens were not significantly different from those of live individuals ($F_{1, 93} = 1.85$, $p > 0.05$). Although the shells collected during this study were not significantly different from the live individuals collected in this particular study, shell material collected from sampling sites should not be assumed to be representative of the live populations at the those sites.

Fish Host Testing. Four individuals were collected for potential fish host testing, but no individuals produced viable glochidia. One gravid individual was collected from PRKM 171.4 on June 9, 2008, and held at the AWCC. The glochidia of the female were not mature when collected, and were aborted within 3 days of collection. Because no other individuals containing glochidia were collected, no fish host testing was conducted.

Discussion

Life History Characteristics

Growth, Age, and Lifespan

Both *Q. sparsa* and *Q. intermedia* achieve most of their growth in the first 10 to 15 y of life, and growth for both species declines dramatically thereafter. Based on the relic shell material collected in this study, neither species appears to live beyond 50 y. Dark coloration of the periostracum, erosion of periostracum near the umbo, tubercules, and closely laid growth rings of older individuals make it extremely difficult to externally age individuals older than 15 – 20 y. Comparisons of internally- and externally-derived ages showed that external aging did not concur with the mussel's internal age. Based on the difficulties associated with externally aging individuals, including worn periostricum and closely laid growth lines, internally aging individuals appears to be the more reliable aging method. More shell material is required to develop a functional von Bertlanaffy age-to-growth curves for both species. Age at sexual maturity is still unknown for both species, so individuals < 10 y were considered to be sub-adults for the purposes of this study.

Comparing the slope of the regression line of the theoretical 1:1 internal to external age estimation (b = 1.00) to that of the external versus internal age relationship computed in this study (b = 0.701), showed that externally aged individuals over-predicted internal ages by almost 30%. The disparity between these two slopes confirmed that externally aged specimens of *Q. intermedia* do not agree with the age of live individuals when aged by counting internal growth lines. Similarities in shell morphology also allow *Q. intermedia* to act as a surrogate species for ages of *Q. sparsa*, and it is reasonable to believe that the same trend in underestimating ages would apply to *Q. sparsa*.

Collecting size-at-age data from older individuals in this manner is a common practice when all age classes are not represented in a shell collection (Bruenderman and Neves 1993, Jones et al. 2004). Unfortunately, this method can have the undesired effect of biasing the curve to more closely reflect the growth rates of those individuals used to perform the calculations. Because of the small sample sizes used, bias possibly exists in the growth curves developed for both *Q. sparsa* and *Q. intermedia*. Data from additional sectioned shells should be added to the growth curve to correct for this possible bias.

Distribution and Status

Distribution

The upstream boundaries for the distributions of *Q. sparsa* and *Q. intermedia* have decreased since the 1988-1989 survey. Since the 1979 survey, the downstream boundary of *Q. sparsa* also has decreased, but that of *Q. intermedia* has increased. Previous surveys have observed both species inhabiting a 96.6 km reach of the Powell River between PRKM 232.7 and PRKM 136.1 (Wolcott and Neves 1994, Steven Ahlstedt, TVA retired., personal communication). The 2008-2009 survey collected *Q. sparsa* between PRKM 229.8 and PRKM 152.6, and collected *Q. intermedia* between PRKM 229.8 to PRKM 129.4. This study suggests that the distribution of *Q. sparsa* has declined by approximately 19.4 km in this reach of the Powell River since 1988 – 1989, but the distribution of *Q. intermedia* may have expanded by approximately 3.8 km.

Smith (2003) states that the probability of detecting a rare species is determined by abundance of the species, spatial distribution within the site, sampling effort, search efficiency, and the distribution of sampling effort within the site. The cryptic nature of *Q. sparsa* and *Q. intermedia*, which can create a low search efficiency, combined with the species' low abundances, make it difficult to determine the true probability of having detected *Q. sparsa* or *Q. intermedia* at each sampled site. It is possible that they still inhabit areas reported in previous surveys, but went undetected during the most recent survey, or inhabited areas where they went undetected in previous surveys. Therefore, it is possible that the changes in distribution noted in this survey are the result of insufficient sampling effort at low-density sites, or greater sampling effort than previous surveys at other locations.

Increased sampling effort will be needed to reduce the risk of Type II error, failing to detect a species when it is in fact present, and determine whether *Q. sparsa* and *Q. intermedia* continue to inhabit portions of the river where they were found in previous surveys. It is also possible that both species currently inhabit un-sampled shoals outside of the current distributions described above. Additional sampling at un-sampled sites outside of the known distribution is needed to confirm these current distributions in the Powell River.

Status

In their respective recovery plans, *Q. sparsa* and *Q. intermedia* have been documented as inhabiting the upper Powell River, but becoming increasingly rare within their range (USFWS 1982, USFWS 1983a). Results of this study show that neither the abundances nor distributions of either species have improved since the development of their respective recovery plans, and that both species are still at high risk of extirpation from the Powell River. Data collected during the summers of 2008 and 2009 suggest that low abundances of *Q. sparsa* and *Q. intermedia* occur in portions of an approximately 100 km reach of river, between PRKM 229.8 and PRKM 129.4. Outside of a 0.8 km reach between PRKM 153.3 and PRKM 152.5, no specimens of *Q. sparsa* or *Q. intermedia* were externally aged to < 10 y. The lack of mussels in this age range confirms that little recruitment has occurred outside of this 0.8 km reach of the river in recent years. Within the 0.8 km reach, mussels aged to be < 10 y accounted for 23% of the total *Q. intermedia* found, and 20% of the total *Q. sparsa* found (Appendix 2). Unless recruitment occurs in additional portions of the river, and older individuals are replaced by new recruits, overall abundances will decline, and the center of both populations may become restricted to this 0.8 km reach of the river.

Because qualitative searches of the substrate surface were used to collect individuals during this study, it is likely that smaller or buried individuals were undetected. It is common in many mussel species for smaller individuals to remain buried in the substrate until later in life (Strayer and Smith 2003). As a result, a majority of these smaller individuals are not collected unless substrate is excavated. Because this study used qualitative sampling methods to collect *Q. sparsa* and *Q. intermedia*, it is likely that the smaller size classes of both species went undetected. For these reasons, additional excavation should be conducted at sites where multiple size classes were collected, to determine whether recruitment is occurring at these shoals. Because the densities of *Q. intermedia* are higher at these sites, in relation to other sampled sites in the river, this additional excavation should be conducted at shoals located at PRKM 197.9 and PRKM 171.4. Because the shoals at PRKM 193.4 and PRKM 188.8 are already intensely sampled by VDGIF, they do not require additional quantitative sampling.

Because both populations appear to be sustainably recruiting in only a small portion of their ranges, the potential threats to both populations are large. Coal mining and poor agriculture practices could have additional impacts on the mussel fauna in the Powell River (Dennis 1981, Ahlstedt and Tuberville 1997, Diamond et al. 2002, Ahlstedt et al. 2005). The low densities and long-lived nature of both species could cause even small reductions in survival or reproductive output to have large detrimental impacts on the population as a whole. The continued survival and reproduction of adults between PRKM 153.3 and PRKM 152.5 is of utmost importance for the survival of both populations. The abundances of the current populations in the Powell River must be enhanced, and the re-establishment of populations in additional rivers is important for species survival in lieu of these potential threats.

Determination of Fish Hosts, Propagation, and Release

Fish Hosts

Because no viable glochidia were obtained, no fish host testing was possible during this study. There is no information on fish hosts for *Q. sparsa*, and tentative hosts for *Q. intermedia* must be verified. Determination of fish hosts for *Q. sparsa* and *Q. intermedia* is of utmost importance for the recovery of populations in the Powell River. The precarious nature of these species requires determination of fish hosts for propagation purposes. If either species is to have a realistic chance of recovery, collection of gravid females and fish host testing must be attempted on a yearly basis until suitable hosts are determined.

In future studies, the following fish species should be tested for host-fish suitability for both *Q. sparsa* and *Q. intermedia*: *Erimytax dissimilis*, *E. insignis*, *E. cahni*, slender chub, *Ictalurus punctatus*, channel catfish, *Noturus eleutherus*, mountain madtom, *N. flavipinnis*, yellowfin madtom, *Phenacobius uranops*, stargazing minnow. All species listed exist in the Powell River, and have documented distributions that overlap those of *Q. sparsa* and *Q. intermedia* (Jenkins and Burkhead 1993). *Erimystax chani* and *Phenacobius uranops* have been selected because both species are closely related to *E. dissimilis* and *E. insignis* (Jenkins and Burkhead 1993), which have been tentatively identified as host fish for *Q .intermedia* (Yeager and Saylor 1995). Despite the conservation states of *E. cahni* and *N. flavipinnis*, each designated as a federally threatened species (USFWS 1983b, USFWS 1983c), they should be tested as host fish due to their similar distributions, in addition to their similarities with identified host fish for congener mussels.

Propagation

During this study, the highest densities of reproducing specimens of *Q. sparsa* and *Q. intermedia* were recorded between PRKM 153.4 and PRKM 152.5. I placed an aggregation consisting of 9 *Q. sparsa* and 23 *Q. intermedia* at PRKM 152.5 to facilitate reproduction of both species. This aggregation could serve potentially as a source of brood stock for future propagation efforts for *Q. sparsa* and *Q. intermedia*.

The Duck River sustains a second viable population of *Q. intermedia*, which provides a second source of brood stock for fish host confirmation and propagation of juveniles. Before mussels from the Duck River population are used for fish host confirmation or juvenile propagation, a genetics study should be conducted to determine whether the two populations are genetically divergent enough to prevent releasing Duck River produced juveniles into the Powell River. If the populations are found to be sufficiently similar, gravid females can be collected from the Duck River, and their glochidia can be used to confirm *Erimystax dissmilis* and *Erimystax insignis* as fish hosts. Juveniles produced from Duck River mussels could also be used to augment the Powell River population, and vice versa.

Using artificial fish blood media is another method that can be used to propagate juvenile freshwater mussels (Isom and Hudson, 1982). Recent improvements in methodologies have allowed the use of artificial media to become a viable method of producing juvenile mussels without the use of a natural host fish (Leroy Koch, USFWS, personal communication). While releasing juveniles through this method may be a future way of augmenting the Powell River populations, determining the natural host fish must be given priority to promote natural recovery in the Powell River.

Juvenile Grow-Out and Release

The presence of mussels < 10 y at the shoals between PRKM 153.3 and PRKM 152.5 confirm that appropriate environmental conditions occur there to foster juvenile survival and reproduction for both species. Additionally, the favorable conditions of these sites have already been documented in high levels of growth and survival of propagated juveniles of other mussel species held at PRKM 153.3 (Dan Hua, Virginia Tech, unpublished data

The presence of relatively young *Q. sparsa* and *Q. intermedia*, along with data from these other juveniles held at these sites, show that the shoals between PRKM 153.3 and PRKM 152.5 are suitable release sites for any propagated juveniles.

Additional Powell River sites that exhibited recruitment of other mussel species, and could be tested as potential release sites are PRKM 197.9, PRKM 193.4, PRKM 188.8, PRKM 171.4, and PRKM 129.4 (Appendix 2). Each of these shoals has the potential to support juvenile mussels, and they should be studied further for suitable release sites, such as PRKM 153.3.

Conclusions and Recommendations

1) Low abundances of *Q. sparsa* and *Q. intermedia* inhabit an approximately 100 km reach of the Powell River between PRKM 232.7 and PRKM 136.1. Un-sampled sites on the margins of this distribution should be sampled to verify presence or absence.
2) Young individuals were collected at shoals between PRKM 153.3 and PRKM 152.5. Because only qualitative surface sampling was used, the tendency of young mussels to remain buried in the substrate, sites with multiple size-classes should be subjected to excavation sampling to assess recent recruitment.

3) Fish host testing was not possible during this study due to lack of viable glochidia. As a result, no fish hosts are known for *Q. sparsa*, and fish hosts need to be confirmed for *Q. intermedia*.
4) Small individuals were found at multiple shoals between PRKM 153.3 and PRKM 152.5. These sites have exhibited the ability to support young *Q. sparsa* and *Q. intermedia*, and should be the primary release site of juveniles of *Q. sparsa* or *Q. intermedia* propagated in the future.
5) The shoals at PRKM 197.9, PRKM 193.4, PRKM 188.8, PRKM 171.4, and PRKM 129.4 exhibited reproduction of other mussel species, and bay be suitable release sites for propagated juveniles of *Q. sparsa* or *Q. intermedia.*

Literature Cited

Ahlstedt, S. A. 1991. Cumberlandian Mollusk Conservation Program: Mussel surveys in six Tennessee Valley streams. *Walkerana* 5:123-160.

Ahlstedt, S. A. and J. D. Tuberville. 1997. 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. Upper Mississippi river conservation Committee, Rock Island, Illinois.

Ahlstedt, S.A., M.T. Fagg, R.S. Butler, and J.F. Connell. 2005. Long-term trend information for freshwater mussel populations at twelve fixed-station monitoring sites in the Clinch and Powell rivers of eastern Tennessee and southwestern Virginia, 1979-2004. Unpublished Report**,** U.S. Fish and Wildlife Service, Cookeville, Tennessee.

Bruenderman, S. A. and R. J. Neves. 1993. Life history of the endangered fine-rayed pigtoe (*Fusconaia cuneolus*) (Bivalvia:Unionidae) in the Clinch River, Virginia. *American Malacological Bulletin* 10:83-91.

Clark, G. R. 1980. Study of molluscan shell structure and growth lines using thin sections. Pages 603–606 in D. C. Rhoads and R. A. Lutz (editors). Skeletal growth in aquatic organisms. Plenum Press, New York, New York.

Crowley, T. E. 1957. Age determination in *Anodonta*. *Journal of Conchology* 24: 201-207.

Dennis, S. D. 1981. Mussel fauna of the Powell River, Tennessee and Virginia. *Sterkiana* 71:1-7.

Diamond, J. M., D. W. Bressler, and V. B. Serveiss. 2001. Assessing relationships between human land uses and the decline of native mussels, fish, and macro-invertebrates in the

Clinch and Powell River watershed, USA. Environmental Toxicology and Chemistry 21:1147-1155.

Fisher, R. A. 1921. On the probable error of a coefficient of correlation deduced from a small sample. *Metron* 1:3-32.

Fuller, S. L. H. 1974. Clams and mussels (Mollusca: Bivalvia). Pages 215-273 in C. W. Hart, Jr. and S. L. H. Fuller (editors). Pollution Ecology of Freshwater Invertebrates. Academic Press, New York, New York.

Fuller, S. L. H. 1978. Fresh-water mussels (Mollusca: Bivalvia: Unionidae) of the upper Mississippi River: Observations at selected sites within the 9-foot channel navigation project on behalf of the U. S. Army Corps of Engineers, Academy of Natural Sciences of Philadelphia.

Haskin, H. H. 1954. Age determination in molluscs. *Transactions of the New York Academy of Sciences* 16:300-304.

Isom, B. G. and R. G. Hudson, 1982. In vitro culture of parasitic freshwater mussel glochidia. *The Nautilus* 96:147–151.

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

Jones, J. W., R. J. Neves, S. A. Ahlstedt, and R. A. Mair. 2004. Life history and propagation of the endangered dromedary pearlymussel (*Dromus dromas*) (Bivalvia:Unionidae). *Journal of the North American Benthological Society* 23:515-525.

Lea, I. 1841. Continuation of Mr. Lea's paper on freshwater and land shells. *Proceedings of the American Philosophical Society* 2:81-83.

Neves, R. J. and S. N. Moyer. 1988. Evaluation of techniques for age determination of freshwater mussels (Unionidae). *American Malacological Bulletin* 6:179-188.

Neves, R. J., L. R. Weaver, and A. V. Zale. 1985. An evaluation of host fish suitability for glochidia of *Villosa vanuxemi* and *V. nebulosa* (Pelecypoda: Unionidae). *American Midland Naturalist* 113:13-19.

Neves, R. J. 1991. Mollusks. Pages 251-320 in K. Terwilliger (editor). Virginia's Endangered Species. Department of Game and Inland Fisheries. Blacksburg, McDonald and Woodward Publishing Company.

Ortmann, A. E. 1918. The nayades (freshwater mussels) of the upper Tennessee drainage. *Proceedings of the American Philosophical Society* 57:521-626.

Ortmann, A. E. 1924. The naiad fauna of Duck River in Tennessee. *American Midland Naturalist* 9:18-62.

Parmalee and Bogan. 1998. Freshwater Mussels of Tennessee. The University of Tennessee Press. Knoxville, Tennessee.

Serb, J. M., J. E. Buhay, and C. Lydeard. 2003. Molecular systematics of the North American freshwater bivalve genus *Quadrula* (Unionidae: Ambleminae) based on mitochondrial ND1 sequences. *Molecular Phylogenetics and Evolution* 28:1-11.

Simpson, C. T. 1914. A Descriptive Catalogue of the Naiads, or Pearly Freshwater Mussels. Bryant Walker, Detroit, Michigan.

Stansbery D. H. 1964. The Mussel (Muscle) Shoals of the Tennessee River revisited. American Malacological Union Annual Report.

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

Stansbery, D. H. 1976. Status of endangered fluviatile mollusks in central North America: *Q. sparsa* (Lea, 1841). Ohio State University Resource Foundation Report to Department of the Interior, USFWS, Bureau of Sport Fish and Wildlife.

Steingraeber, M. T., M. R. Bartsch, and J. E. Kalas. 2007. Thermal criteria for early life stage development of the winged mapleleaf mussel (*Quadrula fragosa*). *American Midland Naturalist* 157:297-311.

Strayer, D. L. and D. R. Smith. 2003. A Guide to Sampling Freshwater Mussel Populations.

American Fisheries Society. Bethesda , Maryland.

Turgeon, D. D., J. F. Quinn, A. E. Bogan, E. V. Coan, F. G. Hochberg, W. G. Lyons, P. M. Mikkelsen, R. J. Neves, C. F E. Roper, G. Rosenberg, B. Roth, A. Scheltema, F. G. Thompson, M. Vecchione, and J. Williams. 1998. Common and Scientific Names of Aquatic Invertebrates from the United States and Canada: Mollusks. 2nd edition. Special Publication 26. American Fisheries Society, Bethesda, Maryland.

U.S. Fish and Wildlife Service. 1982. Cumberland monkeyface pearly mussel recovery plan. U.S. Fish and Wildlife Service, Atlanta, Georgia.

U.S. Fish and Wildlife Service. 1983a. Appalachian monkeyface pearly mussel recovery plan. U.S. Fish and Wildlife Service, Atlanta, Georgia.

U.S. Fish and Wildlife Service. 1983b. Recovery plan for the slender chub (*Hybopsis cahni*). Atlanta, Georgia.

U.S. Fish and Wildlife Service. 1983c. Recovery plan for the yellowfin madtom (*Noturus flavipinnis*). Atlanta, Georgia.

Williams, J. D., M. L. Warren Jr., K. S. Cummings, J. L. Harris, and R. J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. *Fisheries*. 18: 6-22.

Wolcott, L. T. and R. J. Neves. 1994. Survey of the freshwater mussel fauna of the Powell River, Virginia. *Banisteria* 3:1-14.

Yeager, B. L. and R. J. Neves. 1986. Reproductive cycle and fish hosts of the rabbit's foot mussel, *Quadrula cylincrica strigillata* (Mollusca: Unionidae) in the upper Tennessee River drainage. *American Midland Naturalist* 16:329-340.

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.

Table 1. *Quadrula* species organized by their respective species groups and associated conservation states: CS, currently stable; SC, special concern; I, imperiled (endangered or threatened, but not federally listed); E, federally endangered with listing date; *, presumed extinct (Serb et al. 2003)

Species	Conservation status
Quadrula species group	
Quadrula apiculata (Say, 1829)	CS
Quadrula fragosa (Conrad 1835)	E, 1991
Quadrula nobilis (Conrad, 1854)	Not currently recognized
Quadrula quadrula (Rafinesque, 1820)	CS
Quadrula rumphiana (Lea, 1852)	SC
Pustulosa species group	
Quadrula asperata (Lea, 1861)	CS
Quadrula aura (Lea 1859)	SC
Quadrula couchiana (Lea, 1860)	I*
Quadrula houstonensis (Lea, 1859)	I
Quadrula keineriana (Lea, 1852)	Not currently recognized
Quadrula nodulata (Rafinesque, 1820)	CS
Quadrula petrina (Gould, 1855)	I
Quadrula refulgens (Lea, 1868)	CS
Metanevra species group	
Quadrula c. cylindrica (Say, 1817)	I
Quadrula c. strigillata (Wright, 1898)	E, 1997
Quadrula intermedia (Conrad, 1836)	E, 1976
Quadrula metanevra (Rafinesque, 1820)	CS
Quadrula sparsa (Lea, 1841)	E, 1976
Quadrula stapes (Lea, 1831)	E, 1987*
Quadrula tuberosa (Lea, 1840)	I*

Table 2. Fish species collected for host testing of *Quadrula intermedia*. (+) = species positively identified as host fish; * fish host testing did not occur due to lack of viable glochidia.

Fish species	Yeager and Saylor (1995)
Ambloplites rupestris	
Aplodinotus gruniens	x
Campostoma anomalum	x
Caripiodes sp. (juvenile)	
Cottus carolinae	
Cyprinella galactura	x
C. spiloptera	x
Dorosoma cepedianum	x
Erimystax dissimilis	+
E. insignis	+
Etheostoma blennioides	x
E. camurum	x
E. rufilineatum	
E. simoterum	x
E. zonale	x
Fundulus catenatus	x
Ictalurus punctatus	x
Lepomis cyanellus	
L. macrochirus	
Luxilus chrysocephalus	x
L. coccogenis	x
Lythurus ardens	x
L. lirus	x
Macrhybopsis aestivalis	x
Micropterus salmoides	
Morone mississippiensis	x
Moxostoma duguesnei	x
Nocomis micropogon	x
Notropis amblops	x
N. leuciodus	x
N. rebellus	x
N. spectrunculus	x
Noturus insignis	
N. eleutherus	
Percina caprodes	x
P. evides	x
P. aurantiaca	
Pimephales notatus	x
P. vigilax	x
Pomoxis annularis	x
Rhinichthys atratulus	x

Table 3. Sites where *Quadrula sparsa* and *Quadrula intermedia* were collected during the 2008-2009 Powell River survey; (-) = no individuals collected.

PRKM	**Site**	***Q. sparsa***	***Q. intermedia***
269.4	Dryden	-	-
266.3	619 Bridge	-	-
263.0	Swimming Hole	-	-
246.9	Shafer Ford	-	-
236.3	Cheekspring Ford	-	-
230.9	Sewell Bridge	1	1
206.6	Hall Ford	-	-
198.8	Snodgrass Ford	-	-
197.9	Island Below Snodgrass Ford	2	7
193.4	833 Bridge	1	6
188.8	Fletcher Ford	2	6
180.7	Bales Ford	1	3
179.9	Fugate Ford	*	3
171.4	McDowell Shoal	*	8
159.6	Buchanan Ford	1	3
153.4	Bar above Brooks Bridge	1	3
153.0	Brooks Bridge	-	4
152.6	Bar below Brooks Bridge	9	23
136.2	Yellow Shoals	-	-
135.8	Below Yellow Shoals	-	-
129.4	Double S Bend	-	1
104.8	Above 25E Bridge	-	-
	Total:	18	68

* *Q. sparsa* are known to be present at this site, but specimens were not collected during this sampling effort

Table 4. Observed and predicted shell lengths-at-age of *Quadrula sparsa* collected from the Powell River, Virginia and Tennessee, 2008 to 2009. (-) = no data

Internal Annulus	No. of Individuals	Observed Length (mm)		Predicted Length (mm)	Growth Increment (mm)
		Mean	Range		
0	-	-	-	0.79	0
1	2	13.1	12.3-13.8	13.02	12.23
2	2	22.5	20.8-24.2	23.02	10.00
3	2	30.3	27.7-32.8	31.19	8.17
4	2	36.2	34.7-37.6	37.87	6.68
5	2	43.8	43.8	43.33	5.46
6	2	49.9	49.2-50.5	47.80	4.46
7	2	54.2	53.9-54.5	51.45	3.65
8	2	57.6	57-58.2	54.43	2.98
9	2	59.5	58.7-60.3	56.87	2.44
10	-	-	-	58.86	1.99
11	-	-	-	60.49	1.63
12	-	-	-	61.82	1.33
13	1	50	50	62.91	1.09
14	1	59	59	63.80	0.89
15	-	-	-	64.52	0.73
16	1	54	54	65.12	0.59
17	-	-	-	65.60	0.49
18	-	-	-	66.00	0.40
19	-	-	-	66.33	0.32
20	-	-	-	66.59	0.27
21	-	-	-	66.81	0.22
22	1	66	66	66.99	0.18
23	1	55	55	67.13	0.14
24	-	-	-	67.25	0.12
25	-	-	-	67.35	0.10
26	-	-	-	67.43	0.08
27	-	-	-	67.49	0.06
28	-	-	-	67.54	0.05
29	2	69.5	59-80	67.59	0.04
30	1	56	56	67.62	0.04
31	2	75	74-76	67.65	0.03
32	-	-	-	67.67	0.02
33	1	67	67	67.69	0.02
34	-	-	-	67.71	0.02
35	2	71	67-75	67.72	0.01
36	-	-	-	67.73	0.01
37	-	-	-	67.74	0.01
38	-	-	-	67.75	0.01
39	1	73	73	67.75	0.01
40	-	-	-	67.76	0.00
41	-	-	-	67.76	0.00
42	-	-	-	67.77	0.00
43	-	-	-	67.77	0.00
44	1	76	76	67.77	0.00

Table 5. Observed and predicted shell lengths-at-age of *Quadrula intermedia* collected from the Powell River, Virginia and Tennessee, 2006 to 2009. (–) = no data.

Internal Annulus	No. of Individuals	Observed Length (mm) Mean	Observed Length (mm) Range	Predicted Length (mm)	Growth Increment (mm)
0	-	-	-	-4.55	**0**
1	7	9.6	7-12.1	6.62	11.17
2	7	14.5	10.8-20.5	16.00	9.38
3	7	21.5	13.8-38.2	23.89	7.88
4	8	28	18.5-47	30.51	6.62
5	8	34.8	25.3-54.1	36.07	5.56
6	8	41.6	33.9-58.6	40.74	4.67
7	9	46.2	37-60.4	44.67	3.92
8	7	51.1	41.2-62.2	47.96	3.30
9	3	59.5	50.2-65.6	50.73	2.77
10	2	53.55	48-59.1	53.06	2.33
11	1	51	51	55.01	1.95
12	1	51	51	56.65	1.64
13	1	54	54	58.03	1.38
14	1	62	62	59.18	1.16
15	1	55	55	60.16	0.97
16	-	-	-	60.97	0.82
17	-	-	-	61.66	0.69
18	3	51.7	47-59	62.24	0.58
19	-	-	-	62.72	0.48
20	-	-	-	63.47	0.75
21	-	-	-	63.75	0.29
22	2	56.5	52-61	63.99	0.24
23	-	-	-	64.20	0.20
24	1	65	65	64.37	0.17
25	2	65	57-73	64.51	0.14
26	-	-	-	64.63	0.12
27	3	70.3	68-73	64.73	0.10
28	1	74	74	64.82	0.08
29	-	-	-	64.89	0.07
30	-	-	-	64.95	0.06
31	1	61	61	65.00	0.05
32	-	-	-	65.04	0.04
33	1	71	71	65.07	0.04
34	2	62.5	58-67	65.10	0.03
35	2	67.5	67-68	65.13	0.02
36	-	-	-	65.15	0.02
37	1	65	65	65.16	0.01

Figure 1. Photo of *Quadrula sparsa* showing external morphology. Length = 62 mm.

Figure 2. Photo of *Quadrula intermedia* showing external morphology. Length = 65 mm.

Figure 3. Histogram of median lengths (mm) of live *Quadrula sparsa* collected in qualitative sampling of the Powell River, Virginia and Tennessee, from 2008 and 2009 (N = 18).

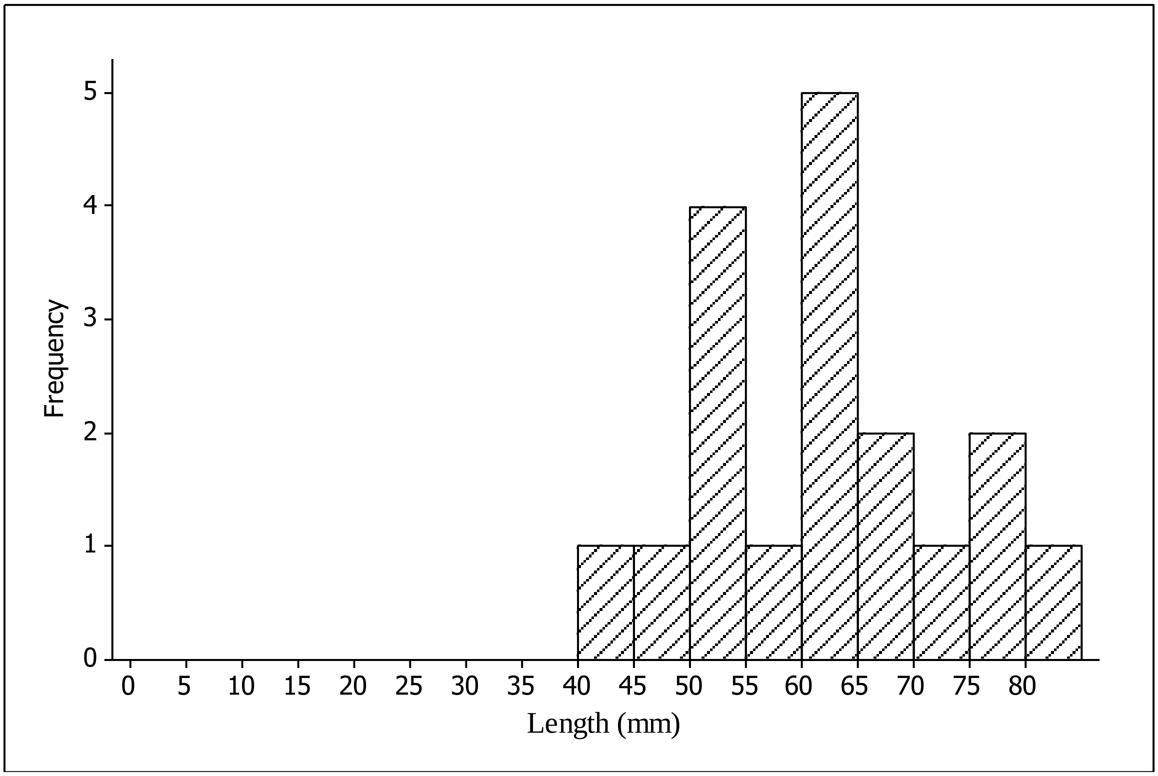


Figure 4. A von Bertalanffy growth curve developed using shells of *Quadrula sparsa* collected from the Powell River, Virginia and Tennessee, in 2008 and 2009.

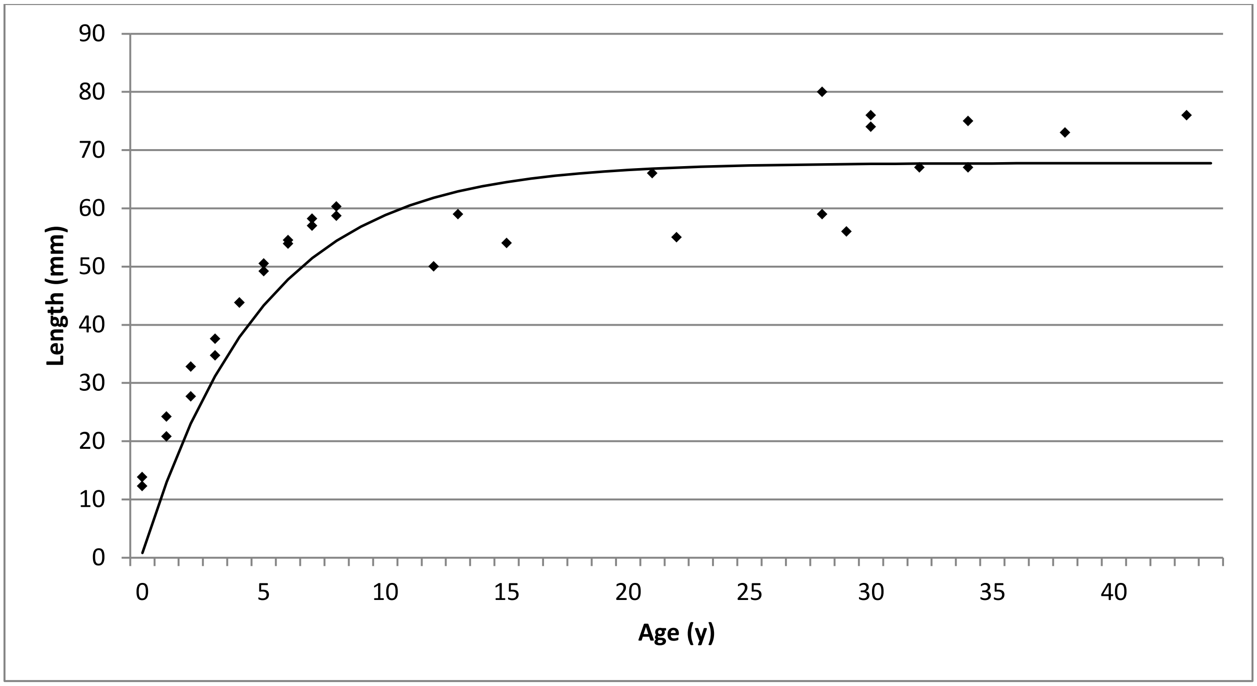


Figure 5. Comparison of age estimates obtained by thin-sectioning valves and by counts of external growth rings of *Quadrula intermedia* collected from the Powell River, Virginia and Tennessee, in 2008 and 2009 ($r^2 = 0.55$, $p < 0.05$). Data points below the 1:1 line represent under-estimates of mussel ages by counting external growth rings.

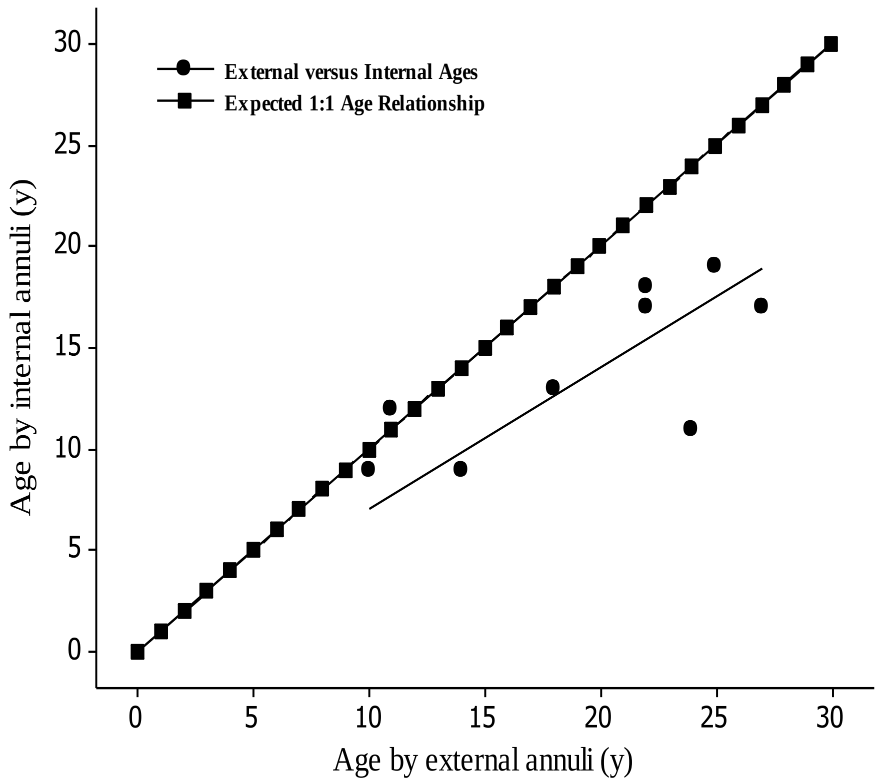


Figure 6. Histogram of median lengths (mm) of live specimens of *Quadrula intermedia* collected by qualitative sampling from the Powell River, Virginia and Tennessee, from 2008 and 2009 (N = 68).

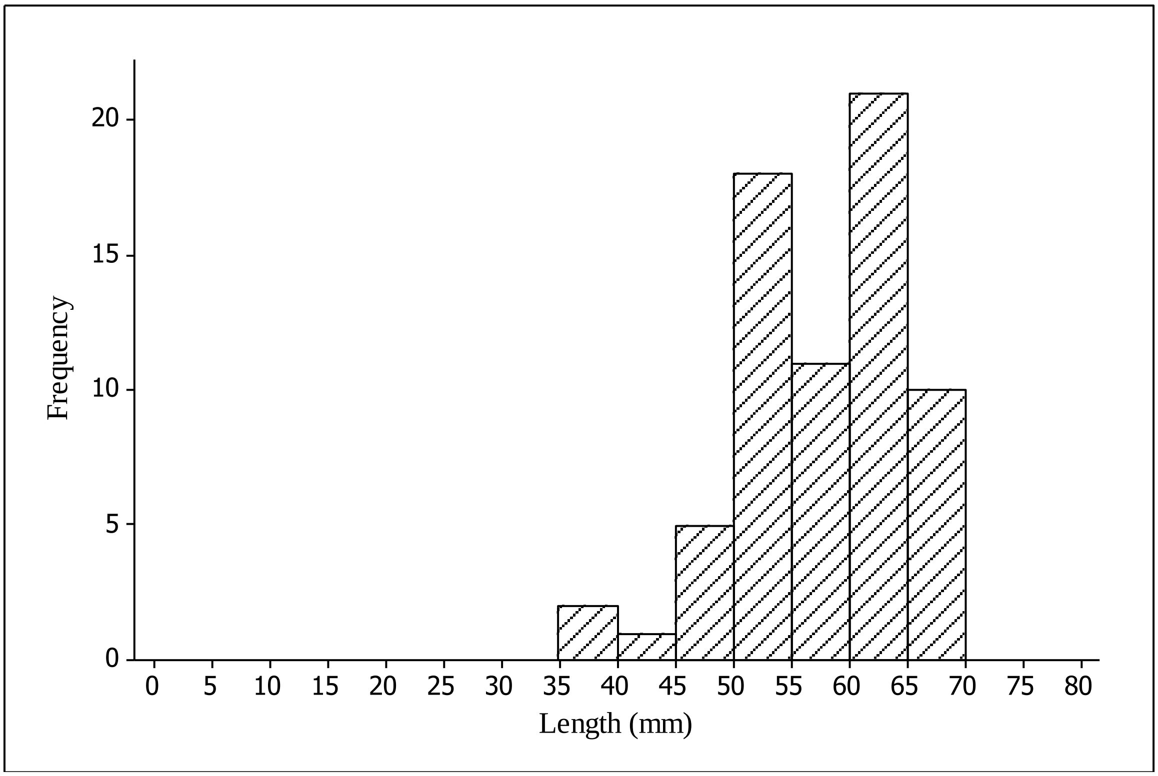
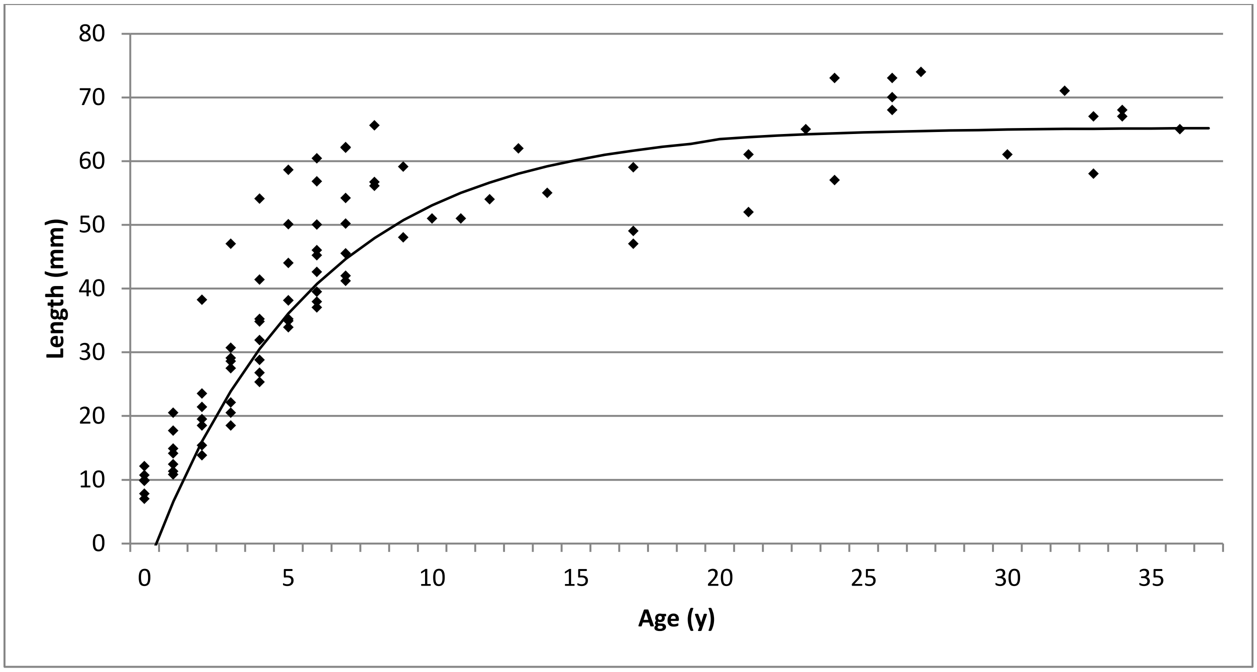


Figure 7. A von Bertalanffy growth curve developed from shells of *Quadrula intermedia* collected in the Powell River, Virginia and Tennessee, in 2008 and 2009.



Appendix A

Death of a specimen of *Quadrula intermedia*: Red Series T995/T996

Red Series T995/T996 was originally collected at PRKM 153.4 on April 17, 2009, and re-located to a stockpile at PRKM 179.9. The individual was collected from the stockpile on May 26, 2009, and held at the FMCC until it was returned to the stockpile on June 1, 2009. The mussel was transported in a sealed container to collect any aborted glochidia. The container contained no substrate. The individual was checked for gravidity on May 29, 2009 with the assistance of Jess Jones. The fresh-dead shell of the individual was discovered on June 29, 2009, while monitoring other individuals in the stockpile for gravidity. There were no obvious signs of muskrat involvement in the individual's death, and no soft-tissue remained attached to the shell. The shell was collected and used for thin-sectioning. The individual was 60.6 mm long and externally aged to be approximately 20 y old (Appendix 2).

Appendix B

Records of *Quadrula sparsa* and *Quadrula intermedia* collected and tagged in quantitative surveys of the Powell River, Virginia and Tennessee, 2008 and 2009.

Table 1. Records of individuals of *Quadrula sparsa* collected from the Powell River, Virginia and Tennessee, 2008 to 2009.

PRKM	Site Name	Length (mm)	Tag Numbers	Collection Date
152.6	Below Brooks Bridge	50	A801, A802	3-Jun-09
152.6	Below Brooks Bridge	52	A850	17-Jun-09
152.6	Below Brooks Bridge	62	A841	17-Jun-09
152.6	Below Brooks Bridge	62.4	A783, A784	26-May-09
152.6	Below Brooks Bridge	64	A844	17-Jun-09
152.6	Below Brooks Bridge	69	A785, A786	26-May-09
152.6	Below Brooks Bridge	72	A847	17-Jun-09
152.6	Below Brooks Bridge	79.5	A787, A788	26-May-09
152.6	Below Brooks Bridge	80.3	A789, A790	26-May-09
153.4	Bar Above Brooks Bridge	44.5	X274, X275	10-Jul-08
159.6	Buchanan Ford	62	X265, X266	24-Jul-08
171.4	McDowell Shoal	*	*	25-Mar-09
180.7	Bales Ford	55	X295, X296	7-Aug-08
188.8	Fletcher Ford	46	S709	1-Sep-09
188.8	Fletcher Ford	51	S708	1-Sep-09
193.4	833 Bridge	69	S706, S707	4-Aug-09
197.9	Island Below Snodgrass	52	A821, A822	28-Jul-09
197.9	Island Below Snodgrass	61	A819, A820	28-Jul-09
230.9	Sewell Bridge	78	A770, A769	27-May-09

* data not recorded due to shell quality or lack of proper instruments

Table 2. Records of individuals of *Quadrula intermedia* collected from the Powell River, Virginia and Tennessee, 2008 to 2009.

PRKM	Site Name	Length (mm)	Tag Numbers	Collection Date
129.4	Double S Bend	67	A817, A818	11-Jun-09
152.6	Bar below Brooks	41	A799, A800	3-Jun-09
152.6	Bar below Brooks	46	A815, A816	3-Jun-09
152.6	Bar below Brooks	47	A797, A798	3-Jun-09
152.6	Bar below Brooks	48.5	A777, A778	26-May-09
152.6	Bar below Brooks	50.6	A781, A782	26-May-09
152.6	Bar below Brooks	51.5	A775, A776	26-May-09
152.6	Bar below Brooks	52	A803, A804	3-Jun-09
152.6	Bar below Brooks	53	S688, S689	26-May-09
152.6	Bar below Brooks	53	A846	17-Jun-09
152.6	Bar below Brooks	55	A848	17-Jun-09
152.6	Bar below Brooks	56	A813, A814	3-Jun-09
152.6	Bar below Brooks	59	A845	17-Jun-09
152.6	Bar below Brooks	59.1	A773, A774	26-May-09
152.6	Bar below Brooks	60	A847	17-Jun-09
152.6	Bar below Brooks	62	A779, A780	26-May-09
152.6	Bar below Brooks	62	S690, S691	26-May-09
152.6	Bar below Brooks	62	A810, A809	3-Jun-09
152.6	Bar below Brooks	62	A812, A811	3-Jun-09
152.6	Bar below Brooks	63	A807, A808	3-Jun-09
152.6	Bar below Brooks	64	A805, A806	3-Jun-09
152.6	Bar below Brooks	65	S692, S693	26-May-09
152.6	Bar below Brooks	66	A849	17-Jun-09
152.6	Bar below Brooks	67	A842	17-Jun-09
153.0	Brooks Bridge	38.7	A763, A764	1-May-09
153.0	Brooks Bridge	53.2	A765, A766	1-May-09
153.0	Brooks Bridge	58	Not Tagged	26-Jun-08
153.0	Brooks Bridge	68.3	A767, A768	1-May-09
153.4	Bar above Brooks	39.75	X276, X 277	10-Jul-08
153.4	Bar above Brooks	60.6	T995, T996	17-Apr-09
153.4	Bar above Brooks	66.7	T993, T994	17-Apr-09
159.6	Buchanan Ford	59	X263, X 264	24-Jul-08
159.6	Buchanan Ford	60.8	T991, T992	17-Apr-09
159.6	Buchanan Ford	65	X267, X 268	24-Jul-08

PRKM	Site	Length (mm)	Tag Numbers	Collection Date
171.4	McDowell Shoal	50	X163, L253	9-Jun-08
171.4	McDowell Shoal	51	X160, L256	9-Jun-08
171.4	McDowell Shoal	52	X176	9-Jun-08
171.4	McDowell Shoal	54	X174, X175	9-Jun-08
171.4	McDowell Shoal	55	X161, L255	9-Jun-08
171.4	McDowell Shoal	55	X166, X167	9-Jun-08
171.4	McDowell Shoal	61	X172, X173	9-Jun-08
171.4	McDowell Shoal	62	X168, X169	9-Jun-08
179.9	Fugate Ford	48	X178	25-May-08
179.9	Fugate Ford	50	X177	25-May-08
179.9	Fugate Ford	60	X179	25-May-08
180.7	Bales Ford	54	X293, X294	7-Aug-08
180.7	Bales Ford	55	A791, A792	2-Jun-09
180.7	Bales Ford	61	X297, X298	7-Aug-08
188.8	Fletcher Ford	49	S710	1-Sep-09
188.8	Fletcher Ford	50	S713	1-Sep-09
188.8	Fletcher Ford	51	S714	1-Sep-09
188.8	Fletcher Ford	53	S715	1-Sep-09
188.8	Fletcher Ford	61	S711	1-Sep-09
188.8	Fletcher Ford	63	S712	1-Sep-09
193.4	833 Bridge	54	S700, S701	4-Aug-09
193.4	833 Bridge	59	S698, S699	4-Aug-09
193.4	833 Bridge	60	S696, S697	4-Aug-09
193.4	833 Bridge	61	S704, S705	4-Aug-09
193.4	833 Bridge	62	S694, S695	4-Aug-09
193.4	833 Bridge	65	S702, S703	4-Aug-09
197.9	Island Below Snodgrass	52	A835, A836	28-Jul-09
197.9	Island Below Snodgrass	53	A833, A834	28-Jul-09
197.9	Island Below Snodgrass	59	A825, A826	28-Jul-09
197.9	Island Below Snodgrass	60	A832, A831	28-Jul-09
197.9	Island Below Snodgrass	62	A823, A824	28-Jul-09
197.9	Island Below Snodgrass	62	A830, A829	28-Jul-09
197.9	Island Below Snodgrass	66	A827, A828	28-Jul-09
230.9	Sewell Bridge	65	A771, A772	27-May-09