The Freshwater Mussels (Bivalvia: Unionidae) of the Harpeth River Drainage and the Upper and Middle Duck River Tributaries, Tennessee

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Kristin Leigh Irwin

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ABSTRACT

The Cumberland and Tennessee River drainages harbor high diversity and endemism of freshwater mussels. The faunas of the Harpeth River drainage and Duck River tributaries have been disproportionally understudied relative to other Cumberlandian streams. Forty-two sites on 23 tributaries in the Harpeth River drainage and a 21-kilometer reach of the main channel were assessed qualitatively for freshwater mussels. Relic shells of four species were observed in eight sites on four of the tributaries. Twenty species were observed in the main channel including the discovery of a new Harpeth River drainage record: *Simsponaias ambigua*. Catch per unit effort (CPUE) ranged from 0.0 to 32.0 mussels/h. *Lampsilis fasciola* and *Potamilus alatus* were the most abundant species. Mussel populations were fragmented and all species exhibited primarily large size-classes. Eighty-three sites on 37 tributaries in the upper and middle Duck River drainage were sampled qualitatively. Nineteen species were observed and 12 were collected live or fresh dead, and CPUE ranged from 0.0 to 58.0 mussels/h. *Villosa vanuxemensis* and *Villosa taeniata* were the most abundant and widespread species observed. Live mussels were found in only five tributaries, although mussels historically occurred in 17 of the sampled streams. Length frequency analysis indicated recent recruitment for four species in Big Rock Creek. Results of canonical correspondence analysis for both drainages revealed no association between environmental variables and mussel community structure (live and fresh dead individuals combined), likely a result of low densities. Two sites on Big Rock Creek in the Duck River drainage were sampled quantitatively using 0.25-m^2 quadrats. Densities were 0.33 and 1.27 mussels/0.25m^2 and species richness ranged from four to five. Quantitative sampling indicated that qualitative timed searches may be sufficient for detecting recruitment in small streams. Anthropogenic alteration has resulted in extensive loss of freshwater mussel habitat, leading to local extirpations and a reduction of diversity and abundance in both watersheds.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

North America is a global hotspot for freshwater mussel (Bivalvia: Margaritiferidae and Unionidae) diversity with over 300 species of historical occurrence (Carter et al. 2011). Recent estimates indicate that approximately 72% of freshwater mussel species in North America are imperiled, being designated as special concern, threatened, or endangered (Williams et al. 1993). The causes of such declines include habitat loss, sedimentation, reduction of water quality, and loss of compatible host fishes (Haag 2012). Due to this diversity and imperilment, the study of freshwater mussels has become an increasingly important focus of aquatic conservation.

Native freshwater mussels serve important roles in aquatic environments, providing the ecosystem services of water filtration, nutrient cycling, and biodeposition (Howard and Cuffey 2006, Vaughn 2017). They can make up a large component of the benthic community in streams and are a food source for other organisms, such as muskrats (*Ondatra zibethicus*) and river otters (*Lontra canadensis*). Freshwater mussels are ecosystem engineers and are useful bioindicators of water quality and habitat stability (Watters 2000, Chowdhury et al. 2016, Archambault et al. 2016).

The collective knowledge on the current distributions of freshwater mussel species is still being assembled. Several large published works have documented the distributions of freshwater mussels across particular states, regions, and drainages (Parmalee and Bogan 1998, Watters et al. 2002, Williams et al. 2008). Smaller tributary systems have been notoriously overlooked in field sampling, although a few studies of North American streams exist (Chastain et al. 2005, Shasteen et al. 2013). Increased mussel diversity and abundance has been positively related to an increase in stream size (Daniel and Brown 2013), deterring monitoring and management efforts in small tributaries. However, species continue to be rediscovered in small streams, such as the rediscovery of *Lampsilis virescens* in the Little Emory River (Gerry Dinkins, McClung Museum of Natural History and Culture, pers. comm.) and *Toxolasma cylindrellus* in Lick Creek (Don Hubbs, Tennessee Wildlife Resources Agency, pers. comm.) in Tennessee.

The term “Cumberlandian” was coined by malacologist Arnold E. Ortmann to describe the combined freshwater mussel fauna of the Tennessee and Cumberland River drainages. The Cumberlandian faunal region historically harbored 110 species, with 32 species being endemic to the drainage (Haag 2012). The fauna of the two rivers share 83 species, making them a suitable study combination. The Harpeth River, a tributary to the Cumberland River, and the Duck River, a tributary to the Tennessee River, were chosen for extensive tributary surveys. A selected section of the Harpeth River was also assessed. The objective of these surveys were to: 1) Evaluate the current status and distribution of freshwater mussels in the Harpeth River drainage and the upper and middle Duck River tributaries, 2) Establish a quantitative, long-term monitoring site on one tributary in each drainage, and 3) Search for patterns between reach-scale environmental factors and freshwater mussel community structure in each river drainage.

PART 1

THE FRESHWATER MUSSELS OF THE HARPETH RIVER DRAINAGE

Abstract

The Harpeth River is a tributary to the Cumberland River, which is one of the most biologically diverse river drainages in North America (Haag 2012). Despite this, freshwater mussel populations in the Harpeth River have not been thoroughly studied. Due to limited research and the possibility of the drainage hosting a wide range of species, the Harpeth River was selected for a descriptive study. Historical review revealed records of only five species for the tributaries: *Alasmidonta viridis, Fusconaia flava, Lampsilis fasciola, Ptychobranchus subtentus,* and *Villosa vanuxemensis*. Forty-two sites on 23 tributaries were qualitatively sampled for freshwater mussels. No live or fresh dead mussels were found in Harpeth River tributaries. Relic shells of four species were collected from eight sites on four streams: Jones Creek, Turnbull Creek, South Harpeth River, and Little Harpeth River. Three new species records for the Harpeth River tributaries were collected: *Potamilus alatus*, *Villosa iris*, and *Villosa taeniata*. Thirty-four sites on 19 tributaries were devoid of mussels. This study suggests at least eight species occurred historically in the tributaries of the Harpeth River, although all species appear to be extirpated.

A 21-km reach of the main channel Harpeth River was sampled following the tributary surveys. A review of museum collections and other available data revealed that at least 45 species occurred historically in the Harpeth River drainage. Twenty species were collected from the survey reach, although only 10 were collected live. A total of 46 live individuals were observed. *Lampsilis fasciola*, *Potamilus alatus*, and *Villosa taeniata* were the most abundant species collected. Shells collected were primarily of large size-classes, indicating a lack of recent recruitment. Two fresh dead individuals of *Simpsonias ambigua* were collected during this survey and represented a new species record for the Harpeth River drainage. This represents the first time this species has been collected in the Cumberland River drainage since the 1960s. Mussel populations were fragmented throughout the sample reach, and catch per unit effort ranged from 0.0 to 32.0 mussels/h. Increased urban development and agricultural activity have resulted in a broad-scale loss of suitable aquatic habitat for freshwater mussels. While this study indicates that mussels have been extirpated from the tributaries, more extensive surveying is required to evaluate the population viability of mussels remaining in the main channel Harpeth River.

Chapter 1: The Harpeth River Watershed and Historical Review of Freshwater Mussels

Study Area

The Harpeth River rises in western Rutherford County, Tennessee (TN) and flows primarily westward for 201 km to its mouth at the Cumberland River near Ashland City, TN. It is one of the longest free-flowing river in Tennessee and drains an area of approximately 2,253 km^2. This system has five major tributaries: Jones Creek, Turnbull Creek, South Harpeth River, Little Harpeth River, and West Harpeth River. The river is known historically to harbor approximately 85 species of fish and 25 species of freshwater mussels (Etnier and Starnes 1993, Parmalee and Bogan 1998).

The river occurs in both the Western Highland Rim and Central Basin physiographic provinces, resulting in a variety of topographic and geologic features throughout the drainage (Safford 1869, Smalley 1981). The Western Highland Rim is characterized by hilly terrain incised by streams with elevations of 122 to 305 m above sea level. Its rivers are characterized by chert gravel and sand substrates and water with moderate to low primary productivity. Geology is primarily Mississippian limestone, shale, and chert, and soils are acidic and high in phosphate. The Central Basin is dominated by Ordovician limestones and characterized by relatively level topography. Streams are highly productive and covered with large expanses of limestone bedrock (Etnier and Starnes 1993).

The Tennessee Department of Environment and Conservation (TDEC) describes land cover in the Harpeth River watershed as being composed of deciduous forest (45.8%) and pasture (43.5%), with urban development comprising only 2.2% (TDEC 2002). However, urban development is projected to increase in the watershed. The Harpeth Conservancy was established in 1999 to monitor and restore the water quality, aquatic biota, and habitat of the Harpeth River drainage.

Historical Review

Published Studies

Only two published studies exist documenting the mussel fauna of the Harpeth River drainage. Samuel N. Rhoads surveyed the Harpeth River near Bellevue in Davidson County and the South Harpeth River six miles south of Bellevue in 1895. He observed ten species in the main channel and three species in the South Harpeth River (Pilsbry and Rhoads 1896) (Table 1).

Table 1. Species reported from the Harpeth River and South Harpeth River in 1895. All species were observed live (Pilsbry and Rhoads 1896).

Species	**Harpeth River**	**South Harpeth River**
Alasmidonta viridis		X
Amblema plicata	X	X
Epioblasma capsaeformis	X	
Fusconaia flava	X	
Lampsilis cardium	X	
Lampsilis teres	X	
Lasmigona costata	X	
Obovaria subrotunda	X	
Ptychobranchus subtentus		X
Pyganodon grandis	X	
Strophitus undulatus	X	
Tritogonia verrucosa	X	
Villosa taeniata		X
Total: 13		

Following Rhoad's collections in 1895, the Harpeth River was not surveyed evidently for almost two decades. Charles B. Wilson and Howard W. Clark surveyed the Harpeth River approximately five miles above its mouth in 1914, and reported the presence of five species (Table 2). They noted that freshwater mussel populations had declined prior to their study, and suggested the lock and dam below the mouth of the Harpeth River was detrimental to the freshwater mussel populations in the lower portion of the river (Wilson and Clark 1914).

Table 2. Species collected in the Harpeth River by Wilson and Clark in the early 1900s. All species were observed live (Wilson and Clark 1914).

Species
Fusconaia flava
Lampsilis cardium
Lampsilis complanata
Lampsilis teres
Potamilus ohiensis
Total: 5

Unpublished Studies

Unpulished freshwater mussel data from the Harpeth River drainage were also compiled. The Tennessee Wildlife Resources Agency (TWRA) surveyed the Harpeth River for freshwater mussels in 2002 (Hubbs et al. 2002). They sampled seven sites and observed 27 species (Appendix A), adding 21 records to the species list from published studies (Pilsbry and Rhoads 1896, Wilson and Clark 1914). Field notes from these surveys mentioned the issues of siltation, erosion, and unconsolidated substrate. Recruitment was noted for several species at Collier Bend in Dickson County, and this site harbored the most diverse and abundant freshwater mussel community.

Museum Records

Museum records were compiled to improve accuracy of the historical species list, and proved vital to the evaluation of the historical fauna in the Harpeth River drainage. Collections that housed specimens from the Harpeth River were Academy of Natural Sciences of Drexel University (ANS), Museum of Biological Diversity at The Ohio State University (OSUM), North Carolina State Museum of Natural Sciences (NCM), McClung Museum of Natural History and Culture (MCM), National Museum of Natural History (NMNH), University of Michigan Museum of Zoology (UMMZ), Carnegie Museum of Natural History (CMNH), and the Illinois Natural History Survey (INHS).

Table 3. Species records from the Harpeth River drainage housed in museum collections. "M" denotes that a specimen was collected in the main channel. "T" denotes that a specimen was collected in a tributary.

Species	**ANS**	**OSUM**	**NCM**	**MCM**	**NMNH**	**UMMZ**	**CMNH**	**INHS**
Actinonaias pectorosa		M		M	M			
Alasmidonta viridis	M, T			M				
Amblema plicata	M	M		M			M	
Cyclonaias pustulosa		M			M			
Cyclonaias tuberculata		M		M				
Cyprogenia stegaria					M			
Dromus dromas	M	M						
Elliptio crassidens				M				
Epioblasma capsaeformis		M	M					
Epioblasma obliquata		M				M		
Epioblasma turdigula					M			
Epioblasma walkeri				M				
Eurynia dilatata		M		M				
Fusconaia flava	M, T	M		M		M	M	
Lampsilis cardium	M	M		M				
Lampsilis fasciola	M, T	M, T		M				
Lampsilis teres	M						M	
Lasmigona complanata	M	M		M		M	M	
Lasmigona costata	M	M		M	M			M
Leptodea fragilis				M		M	M	
Ligumia subrostrata				M				
Medionidus conradicus	M							
Megalonaias nervosa		M		M				
Obliquaria reflexa				M				
Obovaria subrotunda	M	M		M		M		M
Pleurobema sintoxia				M				
Potamilus alatus				M		M	M	
Ptychobranchus fasciolaris				M				
Ptychobranchus subtentus	T							
Pyganodon grandis	M	M		M				
Quadrula fragosa				M				
Quadrula quadrula				M			M	
Strophitus undulatus	M			M	M		M	
Theliderma cylindrica		M			M			
Toxolasma lividum			M	M				
Toxolasma parvum							M	
Tritogonia verrucosa	M		M	M				
Truncilla truncata				M			M	
Utterbackia imbecillis		M		M				
Villosa iris		M						
Villosa taeniata	M, T	T		M				
Villosa vanuxemensis	T							
Total: 42								

Museum records alone indicated at least 42 species occurred historically in the Harpeth River, and five of those occurred in tributaries (Table 4). Exact locality information and collection date was not always provided, such as for *Epioblasma turgidula* and *Cyprogenia stegaria* from the National Museum of Natural History. Several of the shells collected by Samuel N. Rhoads in 1895 were deposited at the Academy of Natural Sciences, and thus are represented twice. Information from museum collections documented additional surveys in the Harpeth River drainage. A record of *Epioblasma obliquata* from the University of Michigan Museum of Zoology suggested Isaac Lea acquired specimens from the Harpeth River in the 1800s. Arnold E. Ortmann surveyed the Harpeth River at several location in 1921, as evidenced by records from the Carnegie Museum of Natural History. Records from The Ohio State University indicated that Don Manning sampled several Harpeth River sites in 1977.

Museum records accounted for most of the species known from the drainage. All species except *Actinonaias ligamentina*, *Potamilus ohiensis*, and *Truncilla donaciformis* were housed in at least one museum collection. In summary, the historical review from all datasets showed that 45 species occurred historically in the Harpeth River drainage. This diversity along with the sporadic nature in which the Harpeth River has been sampled revealed the necessity of an extensive freshwater mussel inventory.

Chapter 2: Qualitative Assessment of Freshwater Mussels in the Harpeth River Drainage

Methods

The following section provides information on the methods used to evaluate the status of freshwater mussel populations in the Harpeth River drainage. Mussel taxonomy follows the most recent revision of North American freshwater mussel taxonomy made by Williams et al. (2017).

Tributary and Site Selection

Tributaries were selected based on historical occurrence of mussels, access, land cover, and information provided by personal communication with experts in the area. If a specific site locality was known for a species in the historical record, those sites were resampled. The number of sites on each tributary depended on stream size, stream placement in the drainage, and the presence, absence, and composition of mussel populations.

Forty-two sites on 23 tributaries were sampled (Table 4, Figure 1). Following tributary sampling, a 20-km section of the Harpeth River in Cheatham County was surveyed (Figure 2).

Tributary Sampling

A qualitative survey method was selected because this type of sampling is most efficient for surveying species richness over a large area (Obermeyer 1998). Surveys were completed by a minimum of two individuals. These surveys consisted of visual searches along with digging in substrate to find specimens that were partially emerged or buried in the substrate. Survey methods and the time spent at each location were tailored to be site-specific and were influenced by water depth and clarity, substrate type, and accessibility. In some streams, the search for mussels included snorkeling. Mesohabitats searched included riffles, pools, silt along banks, water willow (*Justicia americana*) beds, and under large, flat rocks.

Table 4. Sites sampled in selected Harpeth River tributaries and sampling dates.

Site	Locality	Latitude	Longitude	Date
HT1	Jones Creek at State Route 47	36.14323	-87.28739	6/20/2016
HT2	Jones Creek at Old County House Road	36.15599	-87.28137	6/23/2016
HT3	Jones Creek above Timber Ridge Road	36.21925	-87.20377	6/20/2016
HT4	Jones Creek below Timber Ridge Road	36.22472	-87.19891	6/20/2016
HT5	Sulphur Fork Creek at Old Stage Road	36.16379	-87.37231	10/7/2017
HT6	Sulphur Fork Creek along State Route 47	36.16161	-87.31901	6/23/2016
HT7	Little Jones Creek at Westfield Road	36.13228	-87.37813	6/23/2016
HT8	Little Jones Creek at Hicks Road	36.12891	-87.36270	6/23/2016
HT9	Little Jones Creek at Columbia Road	36.13095	-87.33645	6/23/2016
HT10	Leatherwood Creek at Percy Harris Road	36.14873	-87.14605	10/7/2017
HT11	Turnbull Creek at Old Cox Pike	35.96616	-87.19802	6/24/2016
HT12	Turnbull Creek at White Road	36.00426	-87.22067	6/24/2016
HT13	Turnbull Creek at State Route 96	36.03222	-87.21341	6/21/2016
HT14	Turnbull Creek at Turnbull Road	36.07480	-87.19810	6/21/2016
HT15	Turnbull Creek at Ullrich Landing Park	36.09598	-87.14123	6/21/2016
HT16	Nails Creek at Nails Creek Road	36.00627	-87.30009	6/21/2016
HT17	Nails Creek at Hogan Road	36.01026	-87.27678	6/21/2016
HT18	Nails Creek at Deal Road	36.02948	-87.21364	6/21/2016
HT19	Little Turnbull Creek at Dice Lampley Road	35.97839	-87.15879	6/22/2016
HT20	Parker Creek at South Tidwell Road	35.97515	-87.25681	6/24/2016
HT21	Barren Fork at New Hope Road	35.94427	-87.25225	6/22/2016
HT22	Brush Creek at South Harpeth Road	36.07727	-87.08069	10/8/2017
HT23	West Fork Brush Creek at Brush Creek Road	36.05914	-87.09395	10/8/2017
HT24	South Harpeth River at Old Harding Pike	36.01897	-87.02672	3/9/2017
HT25	South Harpeth River at Anderson Road	36.06785	-87.05734	3/9/2017
HT26	East Fork Creek along Big East Fork Road	35.99939	-87.02697	3/9/2017
HT27	Caney Fork Creek at Caney Fork Road	35.93818	-87.09721	3/9/2017
HT28	West Harpeth River at Thompson Station Road	35.80610	-86.85130	5/10/2016
HT29	West Harpeth River at Highway 31	35.82986	-86.88242	6/22/2016
HT30	West Harpeth River along West Harpeth Road	35.84402	-86.90687	6/22/2016
HT31	West Harpeth River at Boyd Mill Pike	35.92536	-86.96913	10/8/2017
HT32	Leipers Fork at Bailey Road	35.88597	-87.00546	10/6/2017
HT33	Leipers Fork at Floyd Road	35.89496	-86.99538	10/6/2017
HT34	Murfrees Fork at Carl Road	35.88111	-86.96263	10/8/2017
HT35	Nelson Creek at Cox Road	35.84555	-86.69901	5/9/2017
HT36	Arrington Creek off Nolensville Pike	35.88960	-86.66480	5/10/2017
HT37	Arrington Creek at Highway 96	35.86680	-86.69530	5/10/2017
HT38	Mayes Creek at Wilson Pike	35.90905	-86.74943	5/10/2017
HT39	South Steward Hills Branch at Cecil Lewis Drive	35.90886	-86.77358	5/9/2017
HT40	Beech Creek at Curray Ingram Academy	36.03037	-86.86032	5/9/2017
HT41	Little Harpeth River at Vaughn Road	36.04960	-86.90480	5/9/2017
HT42	Little Harpeth River at Edwin Warner Park	36.05080	-86.91540	5/9/2017

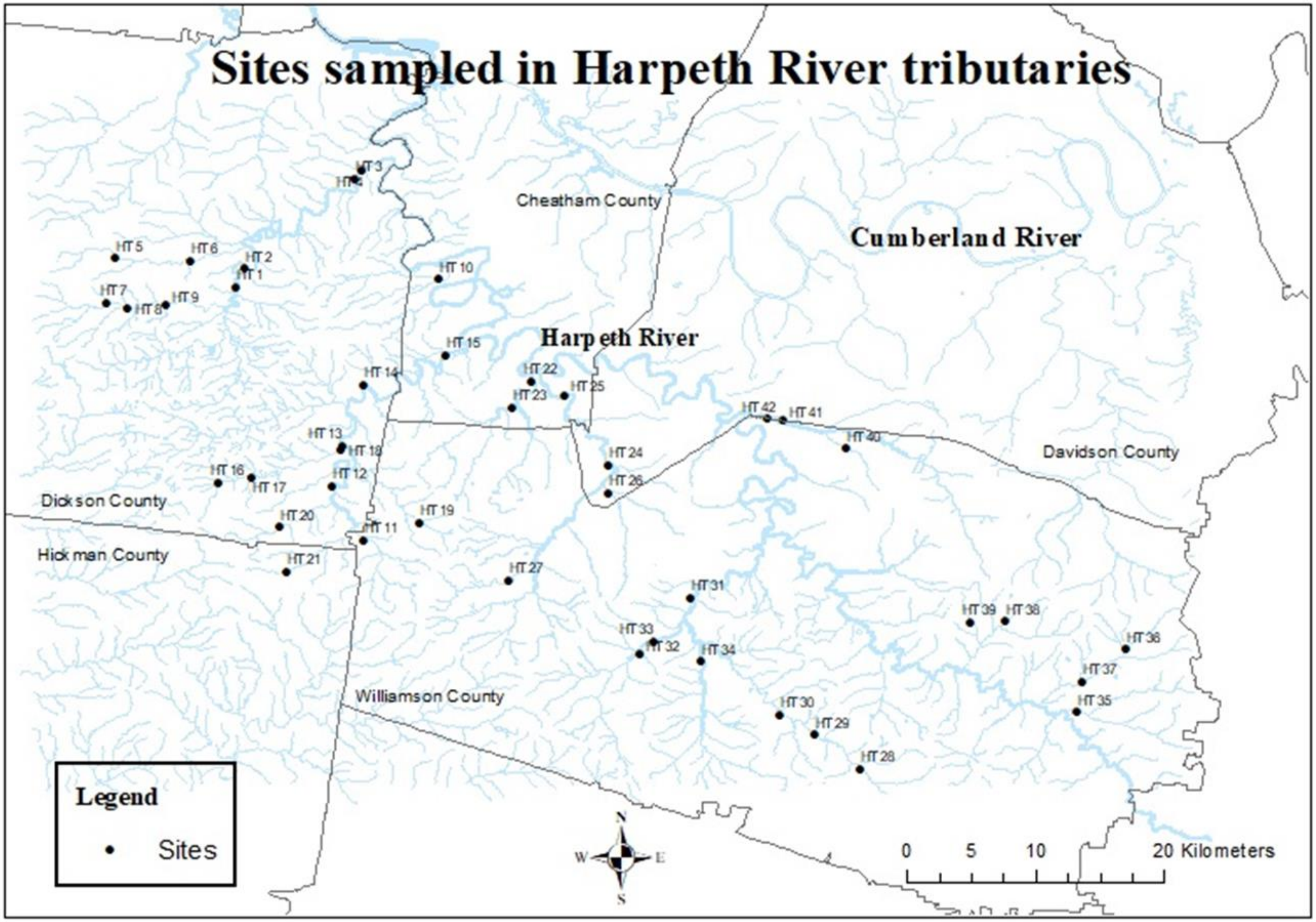

Figure 1. Map of sites sampled in Harpeth River tributaries.

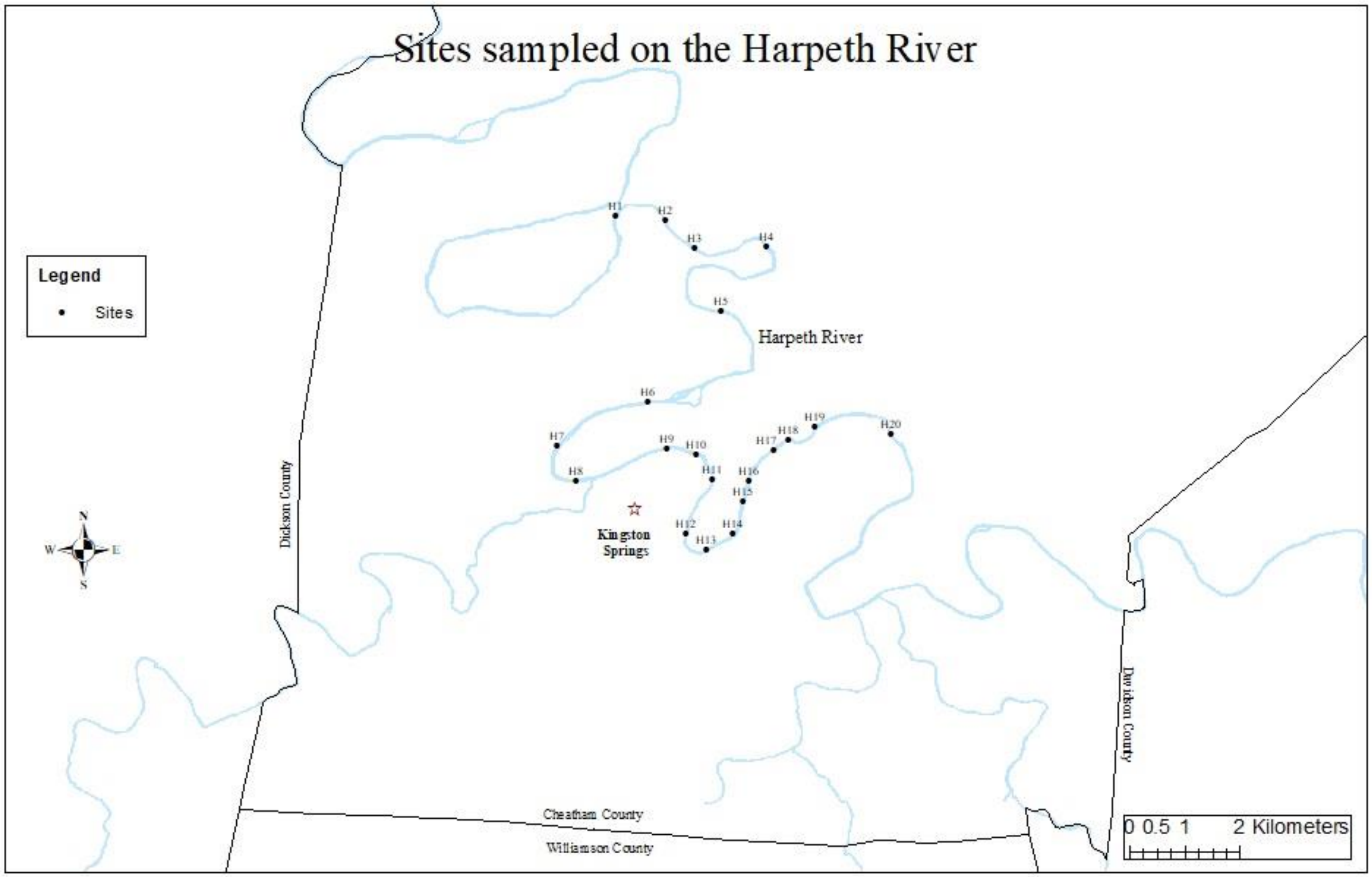

Figure 2. Map of sites sampled on the main channel Harpeth River.

Care was taken to ensure that major habitats were searched to avoid biasing results toward species of particular habitat preferences. Exposed gravel bars and stream banks were searched for the presence of deposited shell material and muskrat middens. The amount of time spent at each location depended on the presence or absence of mussels and mussel habitat and was recorded at each site. The survey team sampled for a distance sufficiently far enough away from various impacts at the site, such as the scouring effect of bridges. Exact sampling localities were recorded in decimal degrees with a handheld Global Positioning System (GPS) device. Live individuals were identified, sexed where applicable, and returned to the substrate where found. Live individuals were measured in millimeters for total shell length (anterior to posterior end) using calipers. Fresh dead shells (tissue still intact, shiny nacre) and relic shells (weathered and worn) were taken to the McClung Museum of Natural History and Culture. Specimens returned to the museum were identified and catalogued.

Main Channel Sampling

Twenty sites were sampled on the main channel Harpeth River in September 2017. Four or five surveyors floated the river in kayaks and stopped to sample each time suitable mussel habitat was encountered. Sampling methods employed during tributary sampling were used, however each site was sampled for a minimum of one person hour.

Reach-Scale Environmental Factors

For the purpose of this study, a reach is defined as the length of a site that was sampled. Several environmental characteristics were qualitatively examined at each site (Appendix B, Appendix C). Factors measured were substrate composition, erosion, and riparian buffer quality. The percentage of substrate composed of organic material (detritus, vegetation), silt, sand, gravel, cobble, boulder, and bedrock throughout the entire site was qualitatively estimated. Sites were given scores of erosion extent and riparian buffer quality using a scale derived from the Evironmental Protection Agency bioassessment protocols (Barbour et al. 1999). A score of "low" was given to sites that had less than 5% of their bank eroded, and a score of "moderate" was assigned to sites that had only intermittent erosion. Sites that exhibited bank sloughing and erosion throughout more than 40% of the reach were given a score of "severe". Riparian buffer quality at each site was given a score of excellent, moderate, or poor. Sites in which riparian cover was extensively removed were given a score of “poor”. Sites with a riparian buffer that was extensive but extended eight meters or less from the bank were assigned a score of “moderate”. A score of “excellent” was given to sites where the riparian cover was extensive and extended at least 18 meters from the bank. The presence or absence of fish, gastropods, and the non-native Asian Clam *Corbicula fluminea* were also recorded.

Data Analysis

Metrics analyzed at each site were species richness and catch per unit effort (CPUE). Relative abundance was estimated with CPUE using the following equation: CPUE = *(number of observed live or fresh dead mussels / survey time per observer * number of observers)*. Live and fresh dead individuals were included in the calculation of CPUE, but relic shells were excluded. Total shell length was used as a vector for age, and length-frequency data were plotted as histograms by species. In order to discern recruitment, external growth rings were used to determine the average length of a two year old individual for particular species. Lengths of ten relic shells for each species were averaged, and these lengths are reported accordingly throughout the remainder of this document.

Canonical Correspondence Analysis (CCA) was used to detect patterns between reach-scale environmental factors and mussel community composition. This is a multivariate, constrained ordination technique for biotic community data that was conducted using the software package PC-ORD (6.0/2002, MjM Software Design, Gleneden Beach, OR). The main matrix was species presence and absence (live or fresh dead) from the 20 sites sampled on the main channel Harpeth River. A constant of +0.01 was added to account for sparsity in the dataset (i.e, many absences). The secondary matrix was composed of the environmental variables of erosion scores, riparian buffer scores, and substrate composition for each site. The null hypothesis of no relationships between matrices was tested. Statistical significance of non-random community structure was evaluated at $\alpha = 0.05$.

Results

Tributary Surveys

Relic shells of four species were collected from eight sites on four tributaries to the Harpeth River: Jones Creek, Turnbull Creek, South Harpeth River, and Little Harpeth River (Table 5). The remaining 34 sites on 19 tributaries revealed no evidence of freshwater mussel populations. Catch per unit effort was 0.0 for all sites sampled, and species richness ranged from 0-2. Records found in the historical review and the current study indicate at least eight species historically occurred in the Harpeth River tributaries. This study documented the extirpation of all 8 species, although it introduced four species to the historical record. Substrate composition, erosion, riparian buffer quality and aquatic life varied throughout the tributaries sampled (Appendix B, Appendix C). Due to the low density and lack of live or fresh dead mussels observed, a quantitative survey was not conducted in any of the Harpeth River tributaries.

Table 5. Species collected from Harpeth River tributaries. "R" denotes relic individuals. Only sites from which mussels were collected are included.

	Site							
Species	**HT1**	**HT2**	**HT4**	**HT15**	**HT24**	**HT25**	**HT41**	**HT42**
Potamilus alatus		R	R					
Villosa iris	R			R		R		
Villosa taeniata					R		R	R
Villosa vanuxemensis							R	
Species Richness	1	1	1	1	1	1	2	1
CPUE (mussels/hour)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Jones Creek

No historical records exist for Jones Creek, however two species were collected during this survey (Table 5). The lower sites on Jones Creek (Sites HT3 and HT4) suffered from severe gravel scouring, although fish were notably abundant (Appendix B, Appendix C). Two tributaries to Jones Creek were sampled. There are no records of mussels from Sulphur Fork Creek or Little Jones Creek, and no mussels were observed during this study.

Leatherwood Creek

No records were found for Leatherwood Creek during the historical review, and no mussels were observed during the current study.

Turnbull Creek

No records for Turnbull Creek or its tributaries were found during the historical review. This study introduced two species records for the stream (Table 5), although these species have been extirpated. Four tributaries to Turnbull Creek were sampled. No mussels were observed in Nails Creek, Little Jones Creek, Parker Creek, or Barren Fork. Turnbull Creek's tributaries were composed of primarily bedrock substrate (Appendix B). Nails Creek was surrounded by mature deciduous forest and suffered from little to no erosion. Contrastingly, Little Jones Creek and Parker Creek had degraded riparian buffers and moderate to severe erosion.

Brush Creek

No historical records were found for Brush Creek or West Fork Brush Creek, and no mussels were observed during this study.

South Harpeth River

Five species occurred historically in the South Harpeth River (Table 3). Relic individuals of *Villosa taeniata* and *Villosa iris* were observed during this study (Table 5). This was the first reported occurrence of *V. iris* in the stream. This study documents the loss of six species of freshwater mussel from the South Harpeth River. Both sites sampled (Sites HT24 and HT25) were eroded and had unstable gravel substrates (Appendix B). Two tributaries to the South Harpeth River were sampled. No historical records were found for East Fork Creek or Caney Fork Creek. No evidence of mussels was found in those streams during this study, although sites sampled on those streams (Sites HT26 and HT27) had suitable substrate composition and riparian buffers.

West Harpeth River

Although *Alasmidonta viridis* was collected from the West Harpeth River in 2002 (Steven Ahlstedt, U.S. Geological Survey, pers. comm.), no evidence of freshwater mussels was found in this study. The West Harpeth River suffered from severe erosion and poor riparian cover, and two of the sites sampled were almost eroded to bedrock (Sites HT28 and HT29). Cattle were observed in the creek at Site H30, and agricultural activity was adjacent to all sites sampled. Freshwater mussels were not observed in Leipers Fork or Murfrees Fork. The substrate of both tributaries was primarily unstable gravel. *Lampsilis fasciola* and *Villosa taeniata* historically occurred in Leipers Fork, but populations appear to have been extirpated.

Little Harpeth River

No records were found in the historical review for the Little Harpeth River, although relic *Villosa iris* and *Villosa vanuxemensis* were collected during this study (Table 5). Gastropods and fish were abundant in the stream (Appendix C). Although the substrate at Site H42 was composed mostly of bedrock, Site H41 consisted of suitable, heterogeneous substrate (Appendix B). Beech Creek and South Steward Hills Branch were void of mussels, and both streams were eroded to bedrock.

Main Channel Survey

Twenty species were collected during the qualitative surveys of the main channel Harpeth River (Table 6). Forty-six individuals of ten species were collected live. Fresh dead shells were collected for all species except *Ptychobranchus fasciolaris*. Species richness ranged from five to fifteen per site, and CPUE ranged from 0.8 to 32.0 mussels/h (Appendix D). Catch per unit effort was highest in the middle section of the survey reach, but species richness varied throughout the study area. Site H13 contained a large amount of fresh dead shells deposited along the left descending bank, and this accounted for the disproportionately high CPUE at that site. Live mussels persisted only in patchy assemblages throughout the survey reach. *Lampsilis fasciola*, *Potamilus alatus*, and *Villosa taeniata* were the species most commonly encountered. The majority of shells collected were of large length, indicating a lack of recruitment. However, *Lampsilis fasicola* was observed spawning at Site H14. Two fresh dead individuals of

Simpsonaias ambigua were observed (Sites H5 and H11), and this was the first reported occurrence of this species from the Harpeth River drainage.

Sections of the Harpeth River suffered from severe erosion and a cleared riparian buffer (Appendix E). Substrate was primarily gravel at most of the sites sampled, although some were dominated by bedrock. Live gastropods and *Corbicula fluminea* were observed at all 20 sites sampled (Appendix F).

Table 6. Species collected and shell length ranges from sites sampled in the Harpeth River. Live individuals were counted. “FD” denotes fresh dead and “R” denotes relic individuals. Length range includes only live individuals.

Species	Condition	Length Range (mm)
Amblema plicata	FD, R	-
Cyclonaias tuberculata	4, FD, R	94-106
Eurynia dilatata	2, FD, R	65-93
Lampsilis cardium	1, FD, R	68
Lampsilis fasciola	12, FD, R	40-88
Lampsilis teres	FD, R	-
Lasmigona costata	FD, R	-
Leptodea fragilis	2, FD, R	60-114
Megalonaias nervosa	1, FD, R	189
Obliquaria reflexa	FD, R	-
Potamilus alatus	11, FD, R	97-138
Ptychobranchus fasciolaris	R	-
Quadrula quadrula	FD, R	-
Tritogonia verrucosa	2, FD, R	90-104
Simpsonaias ambigua	FD, R	-
Truncilla donaciformis	FD, R	-
Truncilla truncata	FD, R	-
Villosa iris	1, FD, R	55
Villosa taeniata	10, FD, R	65-90
Villosa vanuxemensis	FD, R	-
Total: 20	**Total Live: 46**	

Canonical Correspondence Analysis

The CCA results suggested there was no significant relationship between the species and environmental matrices (Table 7), thus there was no community structure (i.e., spatial distribution of species was random). Axis 1 explained most of the variation at 14% (Table 8). but it was not statistically significant (Eigenvalue= 0.266, Spp-Envt. Corr.= 0.912, p= 0.1191).

Table 7. Harpeth River CCA results. Monte Carlo test results for eigenvalues and species-environmental correlations based on randomized data from 998 runs.

		Randomized Data			
Axis	**Real Data**	**Mean**	**Minimum**	**Maximum**	**p**
	Eigenvalue				
1	0.266	0.221	0.119	0.341	0.1191
2	0.158	0.158	0.070	0.238	
3	0.096	0.111	0.032	0.194	
	Spp-Envt Corr.				
1	0.912	0.874	0.671	0.981	0.1852
2	0.898	0.829	0.521	0.963	
3	0.717	0.773	0.451	0.947	

Table 8. Axis summary statistics from Harpeth River CCA.

	Axis 1	**Axis 2**	**Axis 3**
Eigenvalue	0.266	0.158	0.096
Variance in species data			
% variance explained	14.0	8.3	5.1
Cumulative % explained	14.0	22.3	27.4
Pearson Correlation, Spp-Envt	0.912	0.898	0.717
Kendall (Rank) Corr., Spp-Envt	0.633	0.410	0.441

Species Accounts

Information from museum records, unpublished and published surveys, and records from the present study reveals that 46 species of native freshwater mussels historically occurred in the Harpeth River drainage. General distribution information and habitat preferences are provided by Parmalee and Bogan (1998). Federal conservation status is provided, where applicable, from the United States Fish and Wildlife Service (USFWS) listing of endangered and threatened species (USFWS 2017). Information on species petitioned for listing under the Endangered Species Act is from the Center for Biological Diversity's (CBD) Southeastern aquatic species petition list (CBD 2010). Additional non-regulatory conservation status is given according to personal communication with Dr. James Williams (Florida Museum of Natural History).

Actinonaias ligamentina (Lamarck, 1819) – Mucket

Actinonaias ligamentina has a widespread distribution in the Mississippi River drainage, including the Cumberland River and several of its tributaries. It is found at shallow depths in a variety of water currents and substrates. Relic individuals were collected from the Harpeth River in 2002 (Hubbs et al. 2002), although this is the only known occurrence of the species in the drainage. No individuals were observed during the present study. *Actinonaias ligamentina* is considered stable throughout its range, and more sampling is required to determine its status in the Harpeth River.

Actinonaias pectorosa (Conrad, 1834) – Pheasantshell

Actinonaias pectorosa is endemic to the Tennessee and Cumberland River drainages. It is typically found in shallow depths in riffles with sand and gravel substrates. Relic individuals were collected in the lower Harpeth River in 2002 (Hubbs et al. 2002). This species was not collected during the current study. *Actinonaias pectorosa* is petitioned to be listed under the Endangered Species Act by the USFWS, and more sampling is required to evaluate its status in the Harpeth River.

Alasmidonta viridis (Rafinesque, 1820) - Slippershell

Alasmidonta viridis has a widespread distribution throughout the Mississippi River drainage, including the Cumberland River and several of its tributaries. It was observed in the South Harpeth River in 1895 (Pilsbry and Rhoads 1896). One live individual was collected from the West Harpeth River in 2002 (Steven Ahlstedt, United States Geological Survey, pers. comm.), and live individuals were collected from the Harpeth River in 2002 (Hubbs et al. 2002). This species was not observed during the current study. *Alasmidonta viridis* is considered vulnerable throughout its range, and additional surveying is necessary to determine its status in the Harpeth River drainage.

Amblema plicata (Say, 1817) – Threeridge

Amblema plicata is widespread throughout the Mississippi River drainage and the Interior Basin, and occurs in the Cumberland and Tennessee River drainages in a variety of habitats. This species was collected from the Harpeth River near Bellevue in 1895 (Pilsbry and Rhoads 1896). Live individuals were collected from the Harpeth River in 2002 (Hubbs et al. 2002). Fresh dead individuals were collected from one location on the Harpeth River (Site H13), and relic individuals were collected at several other sites during the present study (Appendix D). *Amblema plicata* is considered stable throughout its range, and more surveying is needed to evaluate its status in the Harpeth River.

Cyclonaias pustulosa (Lea, 1831) – Pimpleback

Cyclonaias pustulosa occurs throughout the entire Mississippi River drainage and has a widespread distribution in the state of Tennessee. It is found in a variety of habitats, including reservoirs. Live individuals were collected from the lower Harpeth River in 2002 (Hubbs et al. 2002). This species was not observed during the present study. *Cyclonaias pustulosa* is considered stable throughout its range, and additional sampling is required to assess its status in the Harpeth River.

Cyclonaias tuberculata (Rafinesque, 1820) – Purple Wartyback

Cyclonaias tuberculata has a widespread distribution throughout the Mississippi River drainage, and is extant in the Tennessee and Cumberland River drainages. It occurs in a wide variety of habitats. Live individuals were collected from the Harpeth River in 2002, along with relic individuals from several other locations (Hubbs et al. 2002). Four live individuals were collected during the present study (Sites H13 and H14) and sizes ranged from 94-106 mm in length (Appendix D). Relic shells were collected from all sites sampled on the main channel. *Cyclonaias tuberculata* is stable throughout its range, and is extant in the Harpeth River drainage.

Cyprogenia stegaria (Rafinsque, 1820) – Fanshell

Cyprogenia stegaria occurs throughout the Ohio, Cumberland, and Tennessee River drainages. It is typically found in shallow water in sand and gravel substrates. This species was collected in the Harpeth River (NMNH), although the collection date is unknown. It has not been collected in subsequent surveys, and no individuals were observed during the present study.

Cyprogenia stegaria is federally endangered, and it is presumed extirpated from the Harpeth River.

Dromus dromas (Lea, 1834) – Dromedary Pearlymussel

Dromus dromas is endemic to the Tennessee and Cumberland River drainages. It is typically observed in shoal and riffle habitats in sand and gravel. Museum records (OSUM) indicate Isaac Lea reported this species from the Harpeth River in the 1800s. This species has not been recorded in subsequent surveys, and was not observed during the present study. *Dromus dromas* is federally endangered, and is presumed extirpated from the Harpeth River.

Elliptio crassidens (Lamarck, 1819) – Elephantear

Elliptio crassidens has a widespread distribution throughout the Mississippi River drainage and occurs in the Tennessee and Cumberland River drainages. It typically occurs in large rivers, although it has been observed in smaller streams. Museum records (MCM) indicate that one relic individual was collected from the lower Harpeth River in 1982. No individuals have been observed in subsequent surveys, and this species was not observed during the current study. *Elliptio crassidens* is considered vulnerable throughout its range, and is likely extirpated from the Harpeth River.

Epioblasma capsaeformis (Lea, 1834) – Oystermussel

Epioblasma capsaeformis is endemic to the Tennessee and Cumberland River drainages. It is primarily found in shallow riffles in sand and gravel. It was observed in the Harpeth River in 1895 (Pilsbry and Rhoads 1896). Museum records (OSUM) reveal that Herbert D. Athearn reported this species from the Harpeth River in 1964. No individuals were collected during the present survey. *Epioblasma capsaeformis* is federally endangered, and is likely extirpated from the Harpeth River.

Epioblasma obliquata (Rafinesque, 1820) – Catspaw

Epioblasma obliquata historically occurred in the St. Lawrence, Ohio, and Cumberland River systems as well as the Tennessee River near Muscle Shoals, Alabama. It has been extirpated throughout much of its former range, and typically occurs in riffle habitats of medium to large-sized rivers. Museum records (UMMZ) indicate Isaac Lea reported this species from the Harpeth River in the 1800s. It has not been reported from the Harpeth River since that date, and no evidence of the species was found during the current survey. *Epioblasma obliquata* is federally endangered, and is presumed extirpated from the Harpeth River.

Epioblasma turgidula (Lea, 1858) – Turgid Blossom

Epioblasma turgidula occurred historically in the Tennessee and Cumberland River drainages as well as several rivers in the Ozark region. This species occurred in small to medium-sized rivers in shoal habitat. Museum records (NMNH) indicate that seven individuals of this species were collected from the Harpeth River, although no collection date was reported. It has not been collected in subsequent surveys, and no individuals were observed during the current study. *Epioblasma turgidula* is federally endangered, and it is considered extinct throughout its range.

Epioblasma walkeri (Wilson and Clark, 1914) – Tan Riffleshell

Epioblasma walkeri is endemic to the Tennessee and Cumberland River drainages. It typically occurs in coarse sand and gravel in moderate current. Museum records (NMNH, MCM) indicate that this species was collected in the Harpeth River sometime in the 1800s. It has not been recorded in subsequent surveys, and no individuals were collected during the present study. *Epioblasma walkeri* is federally endangered, and it is presumed extirpated from the Harpeth River.

Eurynia dilatata (Rafinesque, 1820) – Spike

Eurynia dilatata has a widespread distribution throughout the Mississippi River drainage and the Interior Basin. It is extant in the Cumberland River drainage and occurs in a variety of habitats. Live individuals were collected from the lower Harpeth River in 2002 (Hubbs et al. 2002). Two live individuals were collected during this study from Site H16 (65 and 93 mm) and it was collected in fresh dead and relic condition at several other sites (Appendix D). This species is considered stable throughout its range, and remains extant in the Harpeth River.

Fusconaia flava (Rafinesque, 1820) – Ohio Pigtoe

Fusconaia flava is widespread throughout the Mississippi River drainage, and is currently extant in the Cumberland River drainage. It typically occurs in a variety of water depths in sand and gravel. It was reported from the Harpeth River and the South Harpeth River in early surveys of the drainage (Pilsbry and Rhoads 1896, Wilson and Clark 1914). Live individuals were collected in the lower Harpeth River along with relic individuals from several other sites in 2002 (Hubbs et al. 2002). No individuals were collected during the current study. *Fusconaia flava* is considered stable throughout its range, and additional surveys are needed to determine its status in the Harpeth River drainage.

Lampsilis cardium Rafinesque, 1820 – Plain Pocketbook

Lampsilis cardium is widely distributed throughout the Mississippi River drainage and the Great Lakes Basin and remains extant in the Cumberland River drainage. It occurs in a variety of substrates and water depths. This species was observed in early surveys of the Harpeth River (Pilsbry and Rhoads 1896, Wilson and Clark 1914). Live individuals were collected from the lower Harpeth River along with relic individuals at several other sites in 2002 (Hubbs et al. 2002). One live individual (68 mm) was collected in the Harpeth River during this study (Site H16), along with relic and fresh dead individuals at several additional sites (Appendix D). *Lampsilis cardium* is considered stable throughout its range and remains extant in the Harpeth River.

Lampsilis fasciola Rafinesque, 1920 -Wavy-rayed Lampmussel

Lampsilis fasciola has a widespread distribution throughout the Great Lakes basin and upper Mississippi River drainage, and it is extant in the Cumberland River drainage. While typically found in small to medium-sized rivers in less than three feet of water, it is tolerant of a variety of habitats. Museum records (ANS) indicate that it was collected from the South Harpeth River in 1895. Live individuals were collected from several locations on the Harpeth River in 2002 (Hubbs et al. 2002). Twelve live individuals ranging from 40-88 mm were collected at several locations (Sites H4, H7, H10, H13, H14, and H16) during the present survey (Appendix

D). *Lampsilis fasciola* is considered stable throughout its range. It remains extant in the Harpeth River, although it is presumed extirpated from its tributaries.

Lampsilis teres (Rafinesque, 1820) – Yellow Sandshell

Lampsilis teres is widely distributed throughout the Mississippi River drainage, and is extant in the Cumberland River drainage. It typically occurs in mud and sand substrates and is tolerant of impounded conditions. *Lampsilis teres* was collected from the main channel Harpeth River during early surveys of the drainage (Pilsbry and Rhoads 1896, Wilson and Clark 1914). It has not been collected during subsequent surveys, including the current study. *Lampsilis teres* is considered stable throughout its range, and may be extirpated from the Harpeth River.

Lasmigona complanata (Barnes, 1823) – White Heelsplitter

Lasmigona complanata is widespread throughout the Mississippi River drainage and the Great Lakes basin. It remains extant in the Cumberland River drainage and is observed in a variety of habitats. *Lasmigona complanata* was collected in the Harpeth River in the early 1900s (Wilson and Clark 1914). Live individuals were collected from two locations on the Harpeth River in 2002 (Hubbs et al. 2002). No individuals were observed during the current study. *Lasmigona complanata* is considered stable throughout its range, and additional sampling is needed to evaluate its status in the Harpeth River.

Lasmigona costata (Rafinesque, 1820) – Flutedshell

Lasmigona costata is widely distributed throughout the Mississippi River drainage and the Great Lakes basin. It remains extant in the Cumberland River drainage, and is typically collected in medium-sized rivers with moderate current in sand and gravel. It was observed in the Harpeth River in 1895 (Pilsbry and Rhoads 1886). Live individuals were collected from several sites on the Harpeth River in 2002 (Hubbs et al. 2002). One fresh dead individual was observed in the Harpeth River (Site H6) along with relic specimens from several sites during the current study (Appendix D). *Lasmigona costata* remains stable throughout its range, and additional sampling is required to evaluate its status in the Harpeth River.

Leptodea fragilis (Rafinesque, 1820) – Fragile Papershell

Leptodea fragilis has a widespread distribution throughout the Mississippi River drainage and the Great Lakes Basin. It is extant in the Cumberland River and several of its tributaries. It is suited to a variety of habitats, including impounded conditions. Live individuals were collected from two locations on the Harpeth River in 2002 (Hubbs et al. 2002). One live individual (114 mm) was collected from Site H13, and another (60 mm) was collected at Site H16 (Appendix D). Fresh dead and relic shells were collected at several other sites. *Leptodea fragilis* is considered stable throughout its range and remains extant in the Harpeth River.

Ligumia subrostrata (Say, 1831) – Pondmussel

Ligumia subrostrata has a widespread distribution throughout the Mississippi River drainage and occurs in the lower Cumberland River drainage. It is observed in a variety of habitats including lakes, small streams, and larger rivers at shallow depths. Museum records (MCM) reveal this species was collected from the Harpeth River in 1994, however it has not been collected in subsequent surveys. No individuals were observed during the current study. *Ligumia subrostrata* is considered stable throughout its range, and more extensive sampling is required to assess its status in the Harpeth River.

Medionidus conradicus (Lea, 1834) – Cumberland Moccasinshell

Medionidus conradicus is endemic to the Tennessee and Cumberland River drainages. It is often observed in sand and gravel in moderate to strong current as well as in cracks of bedrock. This species was reported from the Harpeth River in the early 1900s, as evidenced by one museum record (ANS). No individuals were observed during the present study. *Medionidus conradicus* is a candidate species to be listed under the Endangered Species Act by the USFWS, and it may be extirpated from the Harpeth River.

Megalonaias nervosa (Rafinesque, 1820) – Washboard

Megalonaias nervosa is widespread throughout the Mississippi River drainage and is extant in the Cumberland River drainage. It is typically observed in large rivers in slow current, although it also occurs in smaller streams. Relic individuals were collected in the lower Harpeth

River in 2002 (Hubbs et al. 2002). One live individual (189 mm) was collected from the Harpeth River during this survey (Site H13), and fresh dead and relic shells were collected at several additional sites (Appendix D). *Megalonaias nervosa* is considered stable throughout its range and remains extant in the Harpeth River.

Obliquaria reflexa Rafinesque, 1820 – Threehorn Wartyback

Obliquaria reflexa has a widespread distribution throughout the Mississippi River drainage and occurs in the Alabama and Mobile River drainages. It is extant in the Cumberland River and several of its tributaries. This species is tolerant of a variety of habitats, including impounded conditions and depths of up to 20 feet. Fresh dead individuals were collected from the lower Harpeth River in 2002 (Hubbs et al. 2002). Fresh dead and relic shells were collected from several sites on the Harpeth River during the present survey (Appendix D). *Obliquaria reflexa* is considered stable throughout its range, and additional surveying is needed to discern its status in the Harpeth River.

Obovaria subrotunda (Rafinesque, 1820) – Round Hickorynut

Obovaria subrotunda has a historically widespread distribution in the Ohio, Tennessee, and Cumberland River drainages, but has been extirpated from parts of its range. It is typically observed in medium to large-sized rivers with moderate flow in sand and gravel substrate. It was reported from the Harpeth River near Bellevue in 1895 (Pilsbry and Rhoads 1896). Relic individuals were collected from the lower Harpeth River in 2002 (Hubbs et al. 2002), and it was not observed during this study. *Obovaria subrotunda* is petitioned for listing under the Endangered Species Act and may be extirpated from the Harpeth River.

Pleurobema sintoxia (Rafinesque, 1820) – Round Pigtoe

Pleurobema sintoxia has a widespread distribution throughout the upper Mississippi River drainage, although its range has been reduced in the Cumberland River drainage. It is typically found in medium-sized rivers in sand and gravel substrate. Museum records (MCM) reveal that this species was collected from the Harpeth River near Franklin in 1988. No individuals have been reported in subsequent surveys, and none were observed during this study.

Pleurobema sintoxia is considered vulnerable throughout its range, and it may be extirpated from the Harpeth River.

Potamilus alatus (Say, 1817) - Pink Heelsplitter

Potamilus alatus is widespread throughout the Interior Basin and the Mississippi River drainage. It is extant in the Cumberland River drainage and is found in a variety of habitats. Live individuals were collected from two locations on the Harpeth River in 2002 (Hubbs et al. 2002). No historical records were found of this species for any of the Harpeth River tributaries. However, this species was one of the most abundant species observed during the main channel survey of this study. Relic individuals were observed at two sites on Jones Creek (Sites HT2 and HT4). Eleven live individuals were observed at three sites on the main channel (Sites H9, H13, and H20) and ranged from 97-138 mm in length. Fresh dead and relic shells were observed at several other sites on the Harpeth River (Appendix D). *Potamilus alatus* is considered stable throughout its range. It is extant in the Harpeth River but is presumed to be extirpated from its tributaries.

Potamilus ohiensis (Rafinesque, 1820) – Pink Papershell

Potamilus ohiensis is widespread throughout the Mississippi River drainage, including the Cumberland River drainage. It is found in a variety of substrates in slow current, and is tolerant of impounded conditions. This species was collected in the Harpeth River in the 1900s (Wilson and Clark 1914). No individuals were collected during the present study. *Potamilus ohiensis* is considered stable throughout its range, but it may be extirpated from the Harpeth River.

Pytchobranchus fasciolaris (Rafinesque, 1820) - Kidneyshell

Ptychobranchus fasciolaris has a widespread distribution throughout the Ohio, Tennessee and Cumberland River drainages and is found in a variety of habitats. It was collected live in the Harpeth River near Franklin in 2002 (Hubbs et al. 2002). One relic individual was collected during the current survey (Site H9). *Ptychobranchus fasciolaris* is considered vulnerable throughout its range, and more extensive sampling is required to determine its status in the Harpeth River.

Ptychobranchus subtentus (Say, 1825) – Fluted Kidneyshell

Ptychobranchus subtentus is endemic to the Tennessee and Cumberland River drainages and has been extirpated from much of its former range. It is typically found in the riffles of small streams in sand and gravel substrate. It was reported from the South Harpeth River in 1895 (Pilsbry and Rhoads 1896). No individuals were observed during the current study. *Ptychobranchus subtentus* is federally endangered, and is likely extirpated from the Harpeth River drainage.

Pyganodon grandis (Say, 1829) – Giant Floater

Pyganodon grandis has a widespread distribution throughout the Interior Basin and the Mississippi River drainage. It is extant in the Cumberland River drainage and is found in a variety of habitats. This species was observed in the Harpeth River in 1895 (Pilsbry and Rhoads 1896). Live individuals were collected from two locations on the Harpeth River in 2002 (Hubbs et al. 2002), although it was not observed during this study. *Pyganodon grandis* is considered stable throughout its range, and additional sampling is required to evaluate its status in the Harpeth River.

Quadrula fragosa (Conrad, 1835) – Winged Mapleleaf

Quadrula fragosa had a historically widespread distribution in the Ohio, Tennessee, and Cumberland River drainages, but it has been extirpated throughout much of its former range. It is typically observed in medium to large-sized rivers. Museum records (MCM) indicate that this species was collected from the Harpeth River near Pegram in 1988. It has not been collected in subsequent surveys, and no individuals were observed during the current study. *Quadrula fragosa* is federally endangered, and is likely extirpated from the Harpeth River.

Quadrula quadrula (Rafinesque, 1820) – Mapleleaf

Quadrula quadrula is widespread throughout the Mississippi River drainage and occurs in the Cumberland River and several of its tributaries. It is found in a variety of habitats and has adapted to impounded conditions. It was collected live from two locations on the Harpeth River in 2002 (Hubbs et al. 2002). One fresh dead individual was observed during this survey (Site H6), and relic individuals were observed throughout the survey reach (Appendix D). *Quadrula quadrula* is considered stable throughout its range, although more sampling is required to assess its status in the Harpeth River.

Simpsonaias ambigua (Say, 1825) – Salamander Mussel

Simpsonaias ambigua has been reported from the Lake St. Clair drainage in Canada and from the Ohio River system. It has been collected from the East Fork Stones River, a Cumberland River tributary, but a small population in the Duck River may represent the only remaining population in Tennessee (Gangloff and Folkerts 2006). This species is often observed under large flat rocks in slow current and in mud adjacent to *Justicia americana* patches. Two fresh dead individuals were collected during this survey (Sites H5 and H11). This is a new species record for the Harpeth River drainage and is the first collection of the species from the Cumberland River drainage in Tennessee since the 1960s. *Simpsonaias ambigua* is petitioned for listing under the Endangered Species Act, and additional surveying is required to evaluate its status in the Harpeth River.

Strophitus undulatus (Say, 1817) – Creeper

Strophitus undulatus has a widespread distribution throughout the Interior Basin, the Great Lakes Basin, and the Mississippi River drainage. It occurs in the Cumberland River and several of its tributaries in Tennessee. This species is tolerant of a wide variety of habitats. It was reported from the Harpeth River in 1895 (Pilsbry and Rhoads 1896). Museum records (CMNH) indicateArnold E. Ortmann collected this species in the Harpeth River in 1921, and additional records (MCM) reveal the last known date of collection was 1976. No individuals were observed during this survey. *Strophitus undulatus* is considered stable throughout its range but may be extirpated from the Harpeth River.

Theliderma cylindrica (Say, 1817) – Rabbitsfoot

Theliderma cylindrica has a widespread distribution throughout the Ohio, Tennessee, and Cumberland River drainages as well as the lower Mississippi River drainage. It is typically collected in medium to large-sized rivers in shoal habitat. Museum records (OSUM) indicate that Isaac Lea reported this species from the Harpeth River in the 1800s. No individuals were observed during the current study. *Quadrula cylindrica* is federally endangered and is presumed extirpated from the Harpeth River.

Toxolasma lividum Rafinesque, 1831 – Purple Lilliput

Toxolasma lividum has a historical distribution throughout the Ohio, Cumberland, and Tennessee River drainages, but has been extirpated throughout much of its former range. It is typically observed in small to medium-sized rivers and large creeks in sand and gravel substrate. Relic individuals were collected from the Harpeth River in 2002 (Hubbs et al. 2002), but none were observed during this study. *Toxolasma lividum* is petitioned to be listed under the Endangered Species Act, and more sampling is necessary to evaluate its status in the Harpeth River.

Toxolasma parvum (Barnes, 1823) – Lilliput

Toxolasma parvum has a widespread distribution throughout the Mississippi River drainage, including the Cumberland River drainage in Tennessee. This species is tolerant of reservoirs, but also occurs in small to large rivers. Museum records (CMNH) indicate that Arnold E. Ortmann reported this species from the Harpeth River in 1921. No individuals have been observed in subsequent surveys, including the present study. *Toxolasma parvum* is considered stable throughout its range but may be extirpated from the Harpeth River.

Tritogonia verrucosa (Rafinesque, 1820) – Pistolgrip

Tritogonia verrucosa has a widespread distribution throughout the Mississippi River and Gulf slope drainages, and occurs throughout the state of Tennessee in a variety of habitats. This species was observed in the Harpeth River as early as 1895 (Pilsbry and Rhoads, 1896). It was collected live from four locations on the Harpeth River in 2002 (Hubbs et al. 2002). One live individual (90 mm) was collected from Site HM8, and one live individual (104mm) was collected from Site H14 during the current study. Fresh dead individuals were also collected at several sites (Appendix D). *Tritogonia verrucosa* is considered stable throughout its range, and it is extant in the Harpeth River.

Truncilla donaciformis (Lea, 1828) – Fawnsfoot

Truncilla donaciformis has a widespread distribution throughout the Mississippi River drainage and occurs in the Cumberland River and several of its tributaries in Tennessee. It is tolerant of a variety of habitats, including impounded conditions. Fresh dead individuals were collected from the lower Harpeth River in 2002 (Hubbs et al. 2002). One fresh dead individual was collected during the current study (Site H10). *Truncilla donaciformis* is considered stable throughout its range, although more sampling is needed to assess its status in the Harpeth River.

Truncilla truncata Rafinesque, 1820 – Deertoe

Truncilla truncata is widespread throughout the Mississippi River drainage, and is present in the Cumberland River drainage in a wide variety of habitats. Live individuals were collected from the lower Harpeth River in 2002 (Hubbs et al. 2002). Fresh dead individuals were observed at three sites on Harpeth River during the present study (Sites H11, H13, and H15), along with relic shells from several additional sites (Appendix D). *Truncilla truncata* is considered stable throughout its range, and additional surveys are required to evaluate its status in the Harpeth River.

Utterbackia imbecillis (Say, 1829) – Paper Pondshell

Uttebackia imbecillis is widespread throughout the Interior and Great Lakes Basins and the Mississippi River drainage, including the Cumberland River and several of its tributaries. This species thrives in impounded conditions. Museum records (OSUM, MCM) indicate that it was collected from the Harpeth River in 1965 and 1976. Live individuals were collected from the Harpeth River in 2002 (Hubbs et al. 2002). No individuals were observed during the present survey. *Utterbackia imbecillis* is considered stable throughout its range, but more sampling is required to determine its status in the Harpeth River.

Villosa taeniata (Conrad, 1834) - Painted Creekshell

Villosa taeniata is endemic to the Tennessee and Cumberland River drainages. It is typically found in gravel and sand at shallow depths in moderate current. This species was reported from the South Harpeth River in 1895 (Pilsbry and Rhoads 1896). Live individuals were collected from two locations on the Harpeth River along with fresh dead shells at additional sites in 2002 (Hubbs et al. 2002). Relic individuals were collected from one site on the South Harpeth River (Site HT24) and two locations on the Little Harpeth River (Sites HT41 and HT42). Ten live individuals ranging from 65-90mm in length were collected from six sites during the current survey (Sites H6, H8, H9, H13, H15, and H20). Fresh dead and relic shells were collected at several other sites (Appendix D). *Villosa taeniata* is considered stable throughout its range. It is extant in the main channel Harpeth River, but appears to be extirpated from its tributaries.

Villosa iris (I. Lea, 1829) - Rainbow

Villosa iris has a widespread distribution in the Ohio, Cumberland, and Tennessee River drainages. It is often found in shallow riffles in relatively strong current. Fresh dead individuals were collected from the lower Harpeth River in 2002 (Hubbs et al. 2002). One live individual (55 mm) was collected from the Harpeth River during this survey (Site H13). Relic individuals were collected from Jones Creek (Site HT1), Turnbull Creek (Site HT15), and the South Harpeth River (Site HT25) during this study. This study is the first report of *Villosa iris* from tributaries to the Harpeth River. *Villosa iris* is considered stable throughout its range. Additional sampling is required to evaluate its status in the Harpeth River, although it is likely extirpated from its tributaries.

Villosa vanuxemensis (I. Lea, 1838) - Mountain Creekshell

Villosa vanuxemensis is endemic to the Tennessee and Cumberland River drainages. It is typically found in small to medium-sized streams in shallow riffles in gravel and cobble substrate. Museum records (ANS) indicate that it was collected from the South Harpeth River in 1895. No historical records were found of this species for the main channel Harpeth River. One relic individual was collected from the Little Harpeth River and one from the main channel during this study (Sites HT41 and H10). These represent two new distributional records for the species. *Villosa vanuxemensis* is considered vulnerable throughout its range, and it appears to be extirpated from the Harpeth River and its tributaries.

Discussion and Recommendations

This study demonstrates that the Harpeth River historically harbored a rich freshwater mussel fauna of at least 46 species, almost doubling previous estimates of diversity (Starnes and Bogan 1988, Parmalee and Bogan 1993). Unfortunately, this fauna has not been surveyed extensively or monitored regularly over time. Mussel extirpations from the lower river were reported as early as 1914 (Wilson and Clark), although species collected by Arnold E. Ortmann in 1921 indicate the fauna remained more intact upstream. *Epioblasma capsaeformis* was collected from the Harpeth River in 1964 by Herb Athearn. The occurrence of five species from the sensitive genus *Epioblasma* in the Harpeth River implies the river likely had dense mussel assemblages at one point in time. The intermittency of freshwater mussel sampling in the Harpeth River inhibits a clearer comprehension of the fauna's history.

This study also illustrates that mussels have been extirpated from the tributaries to the Harpeth River. Only five species were found to exist in the tributaries based on the historical review. Whether this species richness is historically accurate or a result of limited sampling is difficult to discern. *Ptychobranchus subtentus* has been reported from the South Harpeth River but not from the main channel (Pilsbry and Rhoads 1896), indicating the necessity of tributary sampling during drainage distribution studies. The possibility that additional species occurred in the tributaries is likely, but the opportunity to report them has likely been missed, because the environmental degradation of tributary sites is so pervasive. Freshwater mussel populations still remain in the main channel Harpeth River, although they do not appear to be thriving. While twenty species were found in the main channel, most species only remain in isolated and fragmented populations throughout the area sampled (Appendix D).

One feasible explanation for the low density of freshwater mussels observed during this study is lack of suitable habitat. Substrate was unstable and bank erosion was severe throughout the study area, likely a result of riparian vegetation being removed for agriculture and urban development. These conditions, in combination with severe flood events in the drainage has created unstable bottom habitat for mussel populations, especially in the tributaries. Water quality is a concern for the watershed as well, and 58% of the main channel does not meet state water quality standards (TDEC 2016). The issues of low dissolved oxygen, pollution from phosphorous and wastewater effluent, and sedimentation are also prevalent throughout the drainage. Mussel abundance may be low due to factors not measured here, such as poor water quality, toxins, and insufficient fish host abundance needed for larval glochidia. Although parts of the watershed remain relatively remote, the predicted growth of the city of Franklin poses an additional threat to the Harpeth River. Mussel populations downstream of urban areas tend to be less abundant and diverse, and are often dominated by larger size classes (Gillis et al 2017). The freshwater mussels observed reflected these attributes, and will likely worsen with continued anthropogenic alteration, unless restorative actions occur.

Freshwater mussel species exhibit a wide variety of habitat preferences, and this can lead to naturally patchy assemblages (Thorp and Covich 2010). Despite this, studies has documented that species composition can be driven by various local and landscape-scale environmental variables. One would expect that mussel asemblages would be non-random, since stream fishes, which are the primary host for larval unionid mussels, readily exhibit non-random patterns in species assembly (Yeager et al. 2011, Alford 2014). Particle size of substrate, conductivity, and increased agricultural land conversions have proven significant in driving freshwater mussel assemblage composition in South Dakota (Faltys 2016). The failure to detect patterns between reach-scale environmental characteristics and mussel assemblage variation in the Harpeth River may be attributed to several factors. Freshwater mussels may be impacted by large-scale watershed characteristics like hydrological changes (i.e., flow regime) and increased bed load brought on by greater land use towards agriculture and urban development throughout a site's upstream drainage. Landscape-scale factors such as the riparian buffer of the entire upstream watershed of a reach have been found to drive mussel community composition more than the riparian buffer at the sample site (Atkinson et al. 2012). The riparian buffer scores collected during this study were likely not sufficient enough to account for changes in mussel community structure. Patterns can be particularly difficult to detect in highly fragmented and degraded systems (Hornbach et al. 2017). Suitable habitat was fragmented and mussel populations were isolated during this study. This in combination with the low density of mussels collected may have inhibited the detection of a pattern in mussel species assembly.

This survey highlights that future freshwater mussel sampling in the Harpeth River tributaries should not be a priority. If restoration of freshwater mussels in the tributaries becomes a goal for management, it will depend on propagation and reintroduction due to the lack of present populations. Current restorations should be continued and new projects should be considered working directly to improve the habitat of the Harpeth River tributaries, especially the major five streams in the drainage. Habitat restoration will be vital to the success of any potential mussel reintroductions in the tributaries.

The section of the Harpeth River surveyed during this study was strictly a preliminary assessment of freshwater mussels in the drainage. More extensive sampling of the main channel is necessary to accurately evaluate the status and distribution of freshwater mussels in the Harpeth River. The discovery of a previously unknown population of *Simpsonaias ambigua* highlights the degree in which the Harpeth River has been neglected in freshwater mussel surveys. This species is notoriously difficult to detect and is likely overlooked in sampling (Haag and Cicerello 2016). Future surveys will better inform the USFWS's listing decision of this species. An extensive freshwater mussel survey of the Harpeth River is highly recommended based on the results of this study.

PART 2:

THE FRESHWATER MUSSELS OF THE UPPER AND MIDDLE DUCK RIVER TRIBUTARIES

Abstract

The Duck River is one of the most biologically diverse rivers in North America, and has been extensively surveyed for freshwater mussels. The tributaries, especially those in the upper and middle Duck River drainage, have not been the focus of research for several decades. Due to these knowledge gaps and the potential for unknown populations of cryptic, listed, and petitioned species, a survey of the upper and middle tributaries of the Duck River drainage was conducted. This study revealed that 29 species occurred historically in the tributaries to the upper and middle Duck River tributaries.

Eighty-three sites on 37 tributaries from Lick Creek upstream to Crumpton Creek were sampled qualitatively for freshwater mussels. Nineteen species were observed, but only twelve of those species were collected in live or fresh dead condition. Live mussels were collected from 11 sites in five tributaries. Seven sites on six other tributaries had only fresh dead shells. Twelve sites contained only relic shells, and 53 sites on 23 tributaries had no evidence freshwater mussels. Evidence of recruitment of *Villosa vanuxemensis*, *Villosa taeniata*, and *Medionidus conradicus* was observed in Big Rock Creek. *Villosa vanuxemensis* was the most common and abundant species collected during tributary sampling. This study suggests that 59% of the species known to have occurred historically in the study area are extirpated.

Quantitative sampling was conducted at two sites on Big Rock Creek in Marshall County. The first site was below Berea Church, and the second site was upstream below a mill dam. Species richness was four at both sites, although *Pleuronaia barnesiana* was limited to the first site and *Villosa iris* to the second site. Density was 0.33 mussels/0.25m^2 below Berea Church, but was much higher below the mill dam at 1.27 mussels/0.25m^2. *Villosa vanuxemensis* composed 58.6% of species composition at Berea Church, and 76.8% below the mill dam. A two-tailed Kolmogorov-Smirnov test indicated length frequency distributions of *Villosa vanuxemensis* differed between qualitative and quantitative sampling below the mill dam ($d=0.254$ $a=0.05$, $p=0.012$). However, qualitative sampling was successful at documenting recruitment, indicating that timed searches may be sufficient for sampling small tributaries.

Broad habitat degradation was observed throughout the drainage, and mussel populations were isolated and restricted to a few tributaries. Continued habitat restoration will be vital to the management of the freshwater mussels of the upper and middle Duck River tributaries.

Chapter 3: The Duck River Watershed and Historical Review of Freshwater Mussels

Study Area

The Duck River is one of the most biologically diverse rivers in North America with approximately 155 species of fish and 66 species of freshwater mussels (Etnier and Starnes 1993, Hubbs et al. 2011). Rising in Coffee County, the river flows for approximately 435 km until it empties into the Tennessee River in Humphreys County. It is the largest river contained entirely within the state of Tennessee and drains an area of approximately 12,949 km^2. It flows through three different physiographic provinces: the Eastern Highland Rim, the Central Basin, and the Western Highland Rim (Theis 1936). Streams of the Eastern and Western Highland Rim are characterized by chert gravel and sand substrates, while those in the Central Basin include large stretches of bedrock intermixed with finer substrates (Etnier and Starnes 1993).

The Duck River is impounded at Normandy Dam in Manchester and downstream at Shelbyville Dam but is primarily free-flowing throughout most of its length. Due to the river's large size and variable topography, researchers have often separated the river into the upper, middle, and lower Duck River drainages. For the purposes of this study, the upper and middle Duck River tributaries are designated as the streams sampled from Lick Creek in Maury County upstream to Crumpton Creek in Coffee County.

The Duck River watershed is composed of rural landscapes of pastures, agricultural fields, and woodlands intermixed with urban areas. The Duck River is the sole water supply for more than 250,000 people, and conversion of agricultural land for urban development is increasing at a high rate in the watershed (Sally Palmer, The Nature Conservancy, pers. comm.). The Duck River is the focus of several freshwater mussel propagation and reintroduction efforts by state and federal agencies and serves as the location for several experimental populations (Don Hubbs, Tennessee Wildlife Resources Agency, pers. comm.). Therefore, numerous agencies such as the Tennessee Department of Agriculture (TDA), the Duck River Watershed Association (DRWA), the Tennessee Wildlife Resources Agency (TWRA), and The Nature Conservancy (TNC) are actively working to monitor and protect the Duck River drainage.

Historical Review

The following section describes species occurrences from published studies, unpublished data, and museum records from selected tributaries of the upper and middle Duck River drainage. Streams that were not sampled during the current study are not reported in the following historical review.

Published Studies

The freshwater mussel fauna of the Duck River has been extensively surveyed (Ortmann 1924, Isom and Yokley 1968, Van der Schalie 1973, Schilling and Williams 2002, Ahlstedt et al. 2017). Published studies that include the tributaries to the Duck River are less numerous, although the Buffalo River was recently the focus of a drainage-wide survey (Reed 2014).

Arnold E. Ortmann sampled Garrison Fork in Wartrace during a survey of the Duck River basin in the early 1920s (Ortmann 1924). He described the stream as "fordable, with a great variety of conditions of current, bottom, etc." He collected seven species from one shallow riffle, and all species except for *Lampsilis ovata* were observed live (Table 9).

Table 9. Species collected by Arnold E. Ortmann from Garrison Fork in 1924.

Species	**Condition**
Lampsilis fasciola	Live
Lampsilis ovata	Fresh Dead
Lasmigona costata	Live
Medionidus conradicus	Live
Pleuronaia barnesiana	Live
Strophitus undulatus	Live
Villosa vanuxemensis	Live
Total: 7	

Billy G. Isom and Paul Yokley resampled Garrison Fork near Fairfield in the 1960s. The substrate was reported to be mostly bedrock, and only *Villosa vanuxemensis* was observed (Isom and Yokley 1968).

One specimen of *Pegias fabula* was reported in 1986 from an archaeological excavation at the McCollum Rockshelter (40MU390) along Fountain Creek in Maury County (Parmalee and Klippel 1986). This was the only record found of this species from a tributary to the Duck River.

A large-scale survey of the Duck River watershed was conducted in 2001 and 2002 (Ahlstedt et al. 2017). Twenty-one species were collected from 14 of the upper and middle Duck River tributaries, including live individuals of eleven of these species (Appendix G). Of note is the collection of one live individual of *Lasmigona c.f. holstonia* from North Fork Creek at Kennedy Road in Bedford County.

Unpublished Studies

The Tennessee Valley Authority (TVA) sampled Big Rock Creek, East Rock Creek, and Fountain Creek in 1990 in response to the collection of *Toxolasma cylindrellus* from Big Rock Creek in 1989 (Ahlstedt and Saylor 1990). They sampled four sites on Big Rock Creek in Marshall County, collecting a total of 14 species of which only six were collected live. Recruitment was noted to be occurring for several species at approximately creek mile 5.0 (Berea Church). They sampled the stream at creek mile 3.3 (The Sheep Neck), and noted severe erosion and cattle manure-covered substrate. East Rock Creek was reported to be turbid and difficult to access. Three sites were sampled and a total of seven species were collected, although five were only collected in relic condition. One site on Fountain Creek was sampled from which unstable substrate and algal growth was reported. *Lampsilis cardium* was collected fresh dead and *Potamilus alatus* was collected in relic condition. Species collected during this survey have been tabulated with the records from 2001-2002 (Appendix G). This was done due to the similarity of sites sampled and the proximity of the two surveys in time.

Museum Records

In order to supplement the historical species list, museum databases were queried for records from the Duck River tributaries (Table 10). The collections that housed species from the selected Duck River tributaries were the Museum of Biological Diversity at The Ohio State University (OSUM), North Carolina State Museum of Natural Sciences (NCM), University of Michigan Museum of Zoology (UMMZ), Illinois Natural History Survey at Prairie Research

Institute (INHS), McClung Museum of Natural History and Culture (MCM), National Museum of Natural History (NMNH), Carnegie Museum of Natural History (CMNH), and the Field Museum of Natural History (FMNH). Occasionally, only one record for a species was present in a collection, such as *Toxolasma cylindrellus* (MCM). Some collection material from the published and unpublished data was donated to museum collections. Therefore, some records found in the museum records overlap with aforementioned studies.

Table 10. Species records from upper and middle Duck River tributaries mussels housed in museum collections.

Species	**OSUM**	**NCM**	**UMMZ**	**INHS**	**MCM**	**NMNM**	**CMNH**	**FMNH**
Actinonaias pectorosa					X			
Alasmidonta viridis	X							
Amblema plicata	X							
Lampsilis cardium	X							
Lampsilis facsiola	X		X		X		X	
Lasmigona costata	X							
Medionidus conradicus	X						X	
Obovaria subrotunda	X							
Pleurobema oviforme	X							X
Pleuronaia barnesiana	X		X	X	X	X	X	X
Pleuronaia dolabelloides	X		X				X	
Potamilus alatus					X			
Pyganodon grandis	X			X				
Strophitus undulatus							X	
Theliderma cylindrica	X							
Toxolasma cylindrellus					X			
Toxolasma lividum	X			X				
Utterbackia imbecillis	X							
Villosa iris	X			X	X			
Villosa taeniata	X	X		X	X			
Villosa vanuxemensis	X	X		X	X		X	
Total (21 species)								

The complete historical review indicates a total of 27 species occurred historically in the selected upper and middle Duck River tributaries. *Lampsilis ovata* and *Lampsilis cardium* are combined into *Lampsilis cardium* for the purposes of this study due to taxonomic complications (Williams et al. 2008). Several of the species found in the historical review are petitioned to be listed by the USFWS under the Endangered Species Act. These include *Actinonaias pectorosa*, *Medionidus conradicus*, *Obovaria subrotunda*, *Pleuronaia barnesiana*, and *Toxolasma lividum*

(CBD 2010). The lack of recent data and the potential for the presence of petitioned species prompted the current study's evaluation of freshwater mussels in the upper and middle Duck River tributaries.

Chapter 4: Qualitative Assessment of Freshwater Mussels in the Upper and Middle Duck River Tributaries

Methods

Tributary and Site Selection

Tributaries and sites were selected using the same methodology described in Chapter 2. Reference the section “Tributary and Site Selection” in Part 1 of this thesis (Chapter 2; Methods). A total of 83 sites on 37 tributaries were sampled in the Duck River drainage from Lick Creek upstream to Crumpton Creek (Table 11, Figure 3).

Qualitative Sampling

Due to objectives being identical, the methods employed in qualitative sampling of the upper and middle Duck River tributaries were identical to those reported in Chapter 2. Reference the sections “Tributary Sampling” and “Reach-scale Factors” in Part 1 of this thesis (Chapter 2; Methods).

Data Analysis

Analyses for data collected in the Duck River tributaries were similar to those used in Chapter 2. Reference the section “Data Analysis” in the Part 1 of this thesis (Chapter 2; Methods). Performance of CCA was the same except for differences in the composition of the matrices. The main matrix was presence and absence data of species collected live or fresh dead from the sampled Duck River tributaries. A constant of 0.01 was added to account for sparsity in the dataset. The secondary matrix was composed of erosion and riparian buffer scores and substrate composition at those sites. A null hypothesis of no relationship between the two matrices was tested at $\alpha = 0.05$.

Table 11. Sites sampled in the Duck River tributaries and sampling dates.

Site	Locality	Latitude	Longitude	Date
D1	Cathey's Creek at Stephenson Schoolhouse Road	35.63319	-87.30540	7/12/2016
D2	Cathey's Creek along Booker Farm Road	35.63643	-87.26895	7/12/2016
D3	Big Bigby Creek at Lawrenceburg Highway	35.48558	-87.23890	7/13/2016
D4	Big Bigby Creek at State Route 166	35.54292	-87.23551	7/12/2016
D5	Big Bigby Creek at State Route 412	35.61747	-87.20948	7/12/2016
D6	Big Bigby Creek at Southall Road	35.65100	-87.24646	7/12/2016
D7	Greenlick Creek at Highway 50	35.63475	-87.12930	8/9/2017
D8	Greenlick Creek at Hicks Lane	35.64333	-87.12153	8/27/2017
D9	Little Bigby Creek at Indian Hills Road	35.53810	-87.08986	6/13/2017
D10	Little Bigby Creek at Highway 245	35.57341	-87.07119	8/9/2017
D11	Fountain Creek at Pullens Mill Road	35.46252	-87.00214	10/1/2016
D12	Fountain Creek at Graham Road	35.47700	-86.98619	7/13/2016
D13	Fountain Creek at Seavy Height Road	35.51816	-86.94195	7/13/2016
D14	Fountain Creek at former Blue Springs Road	35.55997	-86.96142	10/1/2016
D15	Silver Creek at Bryant Road	35.54780	-86.94887	8/9/2017
D16	Big Rock Creek off McBride Road	35.49490	-86.76420	6/7/2018
D17	Big Rock Creek at Wallace Thompson Road	35.50444	-86.76708	6/7/2018
D18	Big Rock Creek downstream from mill dam	35.53217	-86.76853	10/2/2017
D19	Big Rock Creek at Berea Church	35.53780	-86.76910	10/2/2017
D20	Big Rock Creek off Verona Caney Road	35.55190	-86.76871	10/2/2017
D21	Big Rock Creek above The Sheepneck	35.56047	-86.76371	5/24/2018
D22	East Rock Creek at John Shaw Road	35.46422	-86.64691	8/28/2017
D23	East Rock Creek at Phillips Road	35.48087	-86.68019	10/3/2017
D24	East Rock Creek at Highway 11	35.50292	-86.71260	10/3/2017
D25	East Rock Creek at Wade Brown Road	35.52290	-86.71700	10/3/2017
D26	Sinking Creek at Warner Bridge Road	35.50843	-86.58424	8/9/2017
D27	Sinking Creek at Wheel Road	35.53545	-86.59013	7/26/2017
D28	Sugar Creek at Sandusky Road	35.39706	-86.51775	9/18/2017
D29	Sugar Creek at Highway 130	35.43558	-86.51774	9/19/2017
D30	Sugar Creek at Highway 64	35.45629	-86.50872	7/25/2017
D31	Blue Stocking Branch at Sandusky Road	35.39561	-86.52475	9/18/2017
D32	Flat Creek off New Harmon Road	35.37090	-86.42380	6/14/2017
D33	Flat Creek at Flat Creek Farms	35.37334	-86.42329	7/25/2017
D34	Flat Creek along New Harmon Road	35.38620	-86.41480	7/25/2017
D35	Flat Creek at Mill Road	35.40664	-86.42608	6/14/2017
D36	Thompson Creek at Highway 16	35.44741	-86.32890	9/19/2017
D37	Thompson Creek at Three Forks Bridges Road	35.45488	-86.33238	9/19/2017
D38	Crumpton Creek at Rutledge Falls Road	35.42215	-86.13653	9/19/2017
D39	Wiley Creek at Rutledge Falls Road	35.42264	-86.13559	9/19/2017
D40	Perry Creek at Duncan Road	35.59597	-86.09104	9/19/2017
D41	Garrison Fork at Fairfield Pike	35.56642	-86.28332	10/7/2017

Table 11. Continued

Site	Locality	Latitude	Longitude	Date
D42	Garrison Fork at Kellertown Road	35.54115	-86.29313	10/7/2017
D43	Garrison Fork at Knob Creek Road	35.52059	-86.31846	9/18/2017
D44	Garrison Fork at Haley Road	35.48622	-86.33582	9/18/2017
D45	Wartrace Creek at Highway 82	35.58817	-86.33935	6/14/2017
D46	Wartrace Creek at Winnette Ayers Park	35.52777	-86.34034	6/14/2017
D47	Little Hurricane Creek at Old Nashville Dirt Road	35.52518	-86.47286	7/25/2017
D48	Little Hurricane Creek at Highway 64	35.53257	-86.51650	7/25/2017
D49	Fall Creek down from Pinkston Road	35.58330	-86.48860	7/26/2017
D50	Fall Creek at Gregory Mill Road	35.56440	-86.51657	9/19/2017
D51	Fall Creek at Ben Williams Road	35.55064	-86.54247	9/19/2017
D52	Hurricane Creek at Burns Road	35.55021	-86.49013	9/19/2017
D53	North Fork Creek at North Fork Baptist Church	35.59930	-86.53618	6/15/2017
D54	North Fork Creek at Highway 41 and Kennedy Road	35.58357	-86.55045	6/15/2017
D55	North Fork Creek at Halls Mill Road	35.59268	-86.58672	9/19/2017
D56	Alexander Creek at Barber Road	35.61667	-86.53314	6/15/2017
D57	Weakly Creek from mouth to Halls Mill Road	35.59579	-86.58681	9/20/2017
D58	Clem Creek at Old Pencil Mill Road	35.59568	-86.59830	9/19/2017
D59	Wilson Creek at Wright Road	35.61557	-86.64137	8/28/2017
D60	Wilson Creek at Highway 270	35.60007	-86.65963	9/20/2017
D61	Spring Creek at Mount Vernon Road	35.66906	-86.68134	6/13/2017
D62	Spring Creek at Highway 270	35.60316	-86.69662	9/20/2017
D63	Caney Creek at Lunns Store Road	35.61444	-86.76430	8/28/2017
D64	Caney Creek at Highway 99	35.60923	-86.76924	9/20/2017
D65	Flat Creek at Hazelwood Road	35.68509	-86.80144	10/7/2017
D66	Flat Creek at Highway 431	35.66535	-86.83037	10/7/2017
D67	Rutherford Creek at Jim Warren Road	35.72543	-86.86646	7/24/2017
D68	Rutherford Creek at Double Branch Road	35.67576	-86.98094	9/30/2016
D69	Rutherford Creek at mouth of Carter's Creek	35.67667	-87.00921	6/22/2016
D70	Rutherford Creek at Northpoint Road	35.66970	-87.01436	10/7/2017
D71	Rutherford Creek at confluence with Duck River	35.64677	-87.04258	9/30/2016
D72	Aenon Creek at Duplex Road	35.75270	-86.87452	7/24/2017
D73	Aenon Creek at McCory Lane	35.74274	-86.87993	7/24/2017
D74	McCutcheon Creek at Harvey Park	35.75931	-86.92297	7/24/2017
D75	Carter's Creek along Carter's Creek Road	35.69686	-87.00642	10/1/2016
D76	Carter's Creek at lower RR crossing	35.67714	-87.01113	6/22/2016
D77	Knob Creek along Knob Creek Road	35.69111	-87.08164	6/30/2017
D78	Knob Creek at Chickasaw Trace State Park	35.66273	-87.09156	6/30/2017
D79	Snow Creek at Craig Bridge Road	35.69480	-87.18833	6/29/2017
D80	Leipers Creek at Water Valley Road	35.74122	-87.18915	6/29/2017
D81	Leipers Creek along Leipers Creek Road	35.72345	-87.19288	6/29/2017
D82	Leipers Creek at Leipers Creek Road	35.70698	-87.19446	6/29/2017
D83	Lick Creek at Lick Creek Road	35.84127	-87.19289	6/14/2017

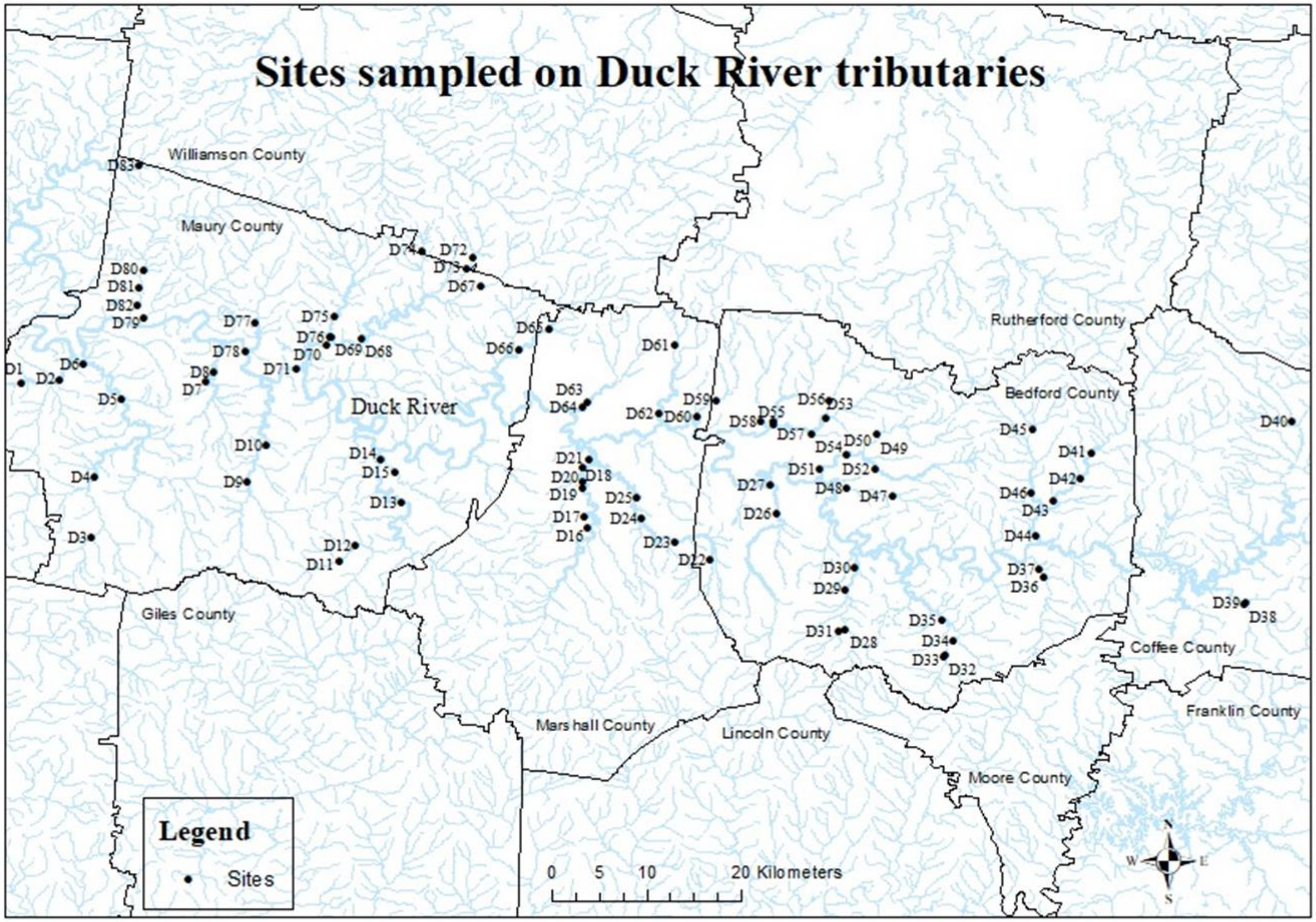

Figure 3. Map of sites sampled in Duck River tributaries.

Results

Tributary Surveys

Prior to this survey, 27 species were known from the upper and middle Duck River tributaries. This survey provided records of two new species: *Epioblasma triquetra* and *Tritogonia verrucosa*. Nineteen species of freshwater mussels were observed during this study (Table 12). Eight of these species were collected live, and an additional four were collected in fresh dead condition. Seven species were observed only by relic shells.

Table 12. Species observed in upper and middle Duck River tributaries.

Species	Condition
Alasmidonta viridis	FD,R
Amblema plicata	R
Cyclonaias tuberculata	R
Epioblasma triquetra	R
Lampsilis cardium	FD, R
Lampsilis fasciola	L, FD, R
Lasmigona costata	R
Medionidus conradicus	L, FD, R
Pleuronaia barnesiana	L, FD, R
Pleuronaia dolabelloides	L, FD, R
Pyganodon grandis	L, FD, R
Theliderma cylindrica	R
Toxolasma cylindrellus	FD, R
Toxolasma lividum	FD, R
Tritogonia verrucosa	R
Utterbackia imbecillis	R
Villosa iris	L, FD, R
Villosa taeniata	L, FD, R
Villosa vanuxemensis	L, FD, R
Total: 19	

Live individuals were collected from 11 sites in five tributaries: Big Rock Creek, Sugar Creek, Flat Creek, Rutherford Creek, and Aenon Creek. Fresh dead individuals were collected at seven sites from an additional six streams: Big Bigby Creek, Fountain Creek, Garrison Fork, Wartrace Creek, Carter’s Creek, and Lick Creek. An additional 12 sites only had relic individuals. Twenty tributaries and 53 sites revealed no evidence of freshwater mussel populations. Species richness ranged from 0-10, and CPUE ranged from 0.0 to 58.0 mussels/h (Appendix H).

Due to the overall low abundance of mussels observed, recruitment success was indiscernible for most species across the sites sampled. Length-frequency histograms were only useful for four species from two sites on Big Rock Creek (D18 and D19). *Villosa vanuxemensis* exhibited the healthiest length-frequency distribution at both sites sampled (Figure 4, Figure 5). The petitioned *Medionidus conradicus* also displayed recruitment (<20mm) at both sites (Figure 6, Figure 7). Recruitment within the last two years was observed for *Villosa iris* at site D19 but not at site D18 (Figure 8, Figure 9). Juvenile individuals (<25mm) of *Villosa taeniata* were observed at site D18, but the individuals collected at site D19 were all of larger size classes (Figure 10, Figure 11).The environmental and biological reach-scale factors recorded were variable throughout the sites sampled (Appendix I, Appendix J). This study suggests that 17 of the 29 species known to have occurred historically in the upper and middle Duck River tributaries are extirpated (Appendix L).

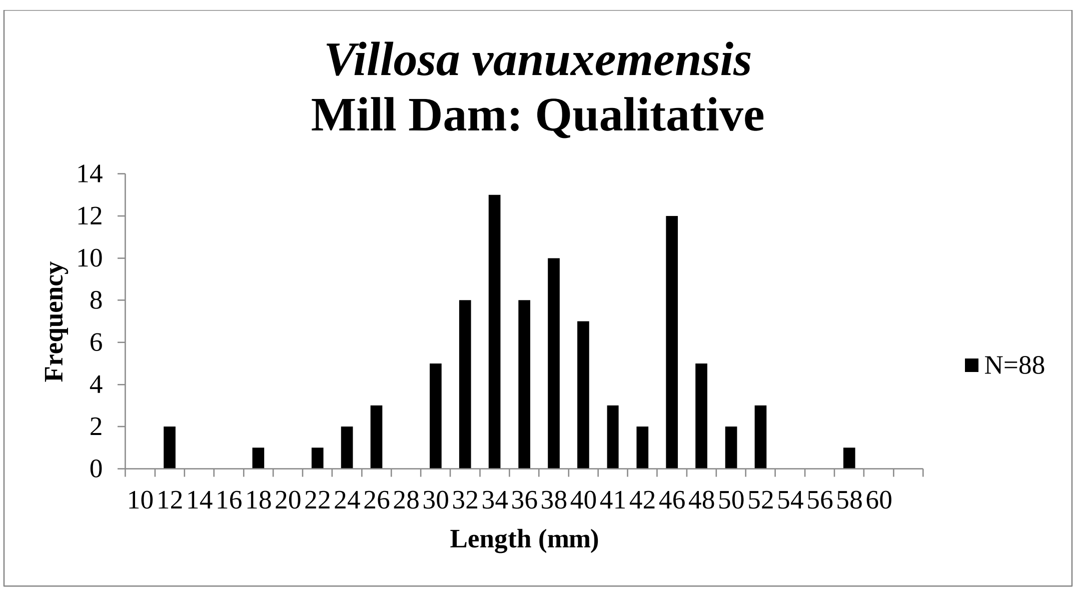

Figure 4. Length frequency histogram of *Villosa vanuxemensis* collected from Big Rock Creek (Site D18).

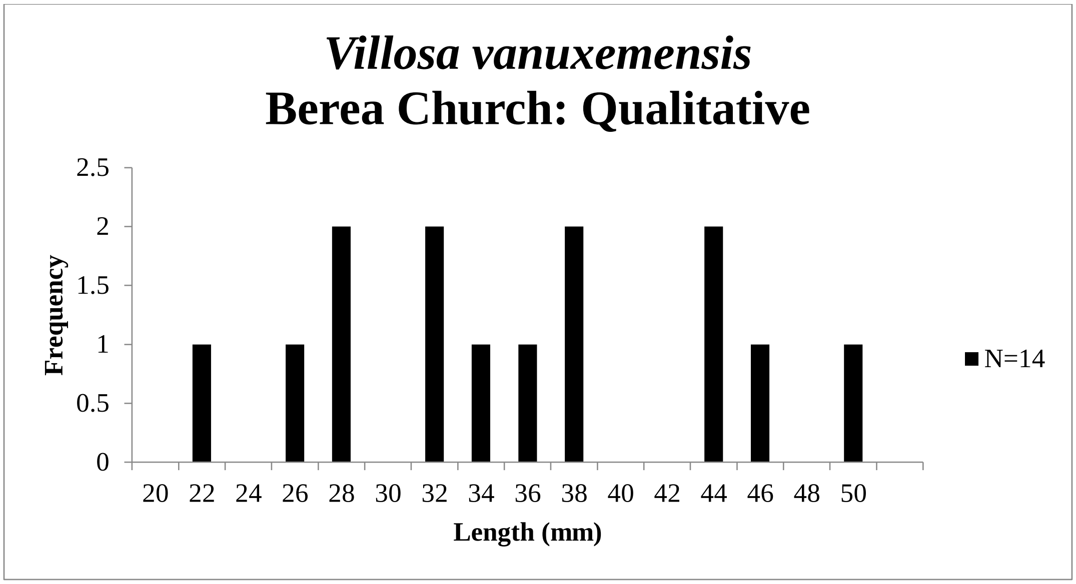

Figure 5. Length frequency histogram of *Villosa vanuxemensis* collected from Big Rock Creek (Site D19).

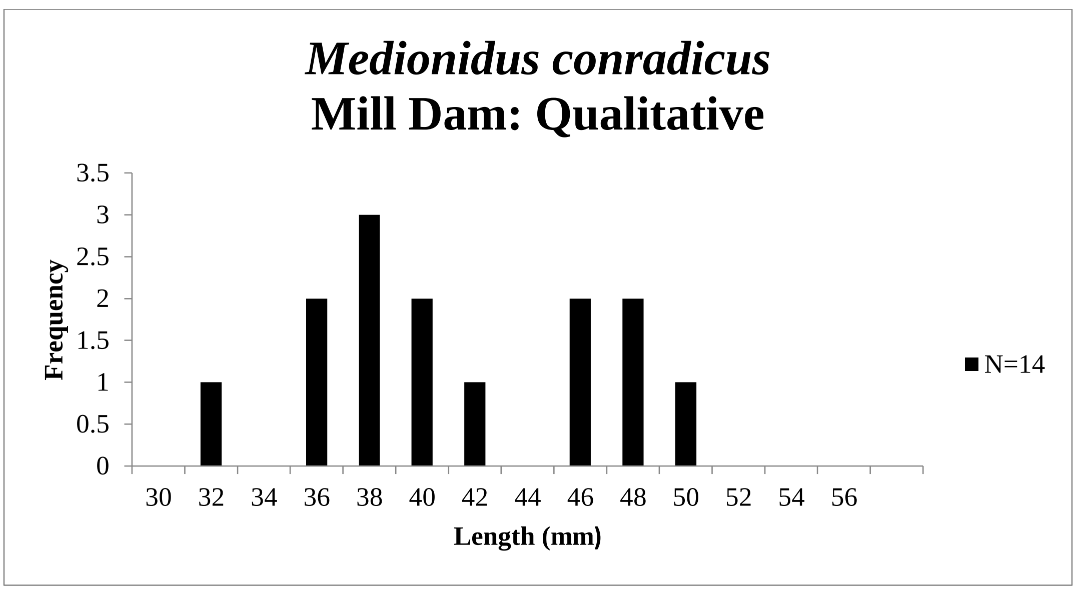

Figure 6. Length frequency histogram of *Medionidus conradicus* collected from Big Rock Creek (Site D18).

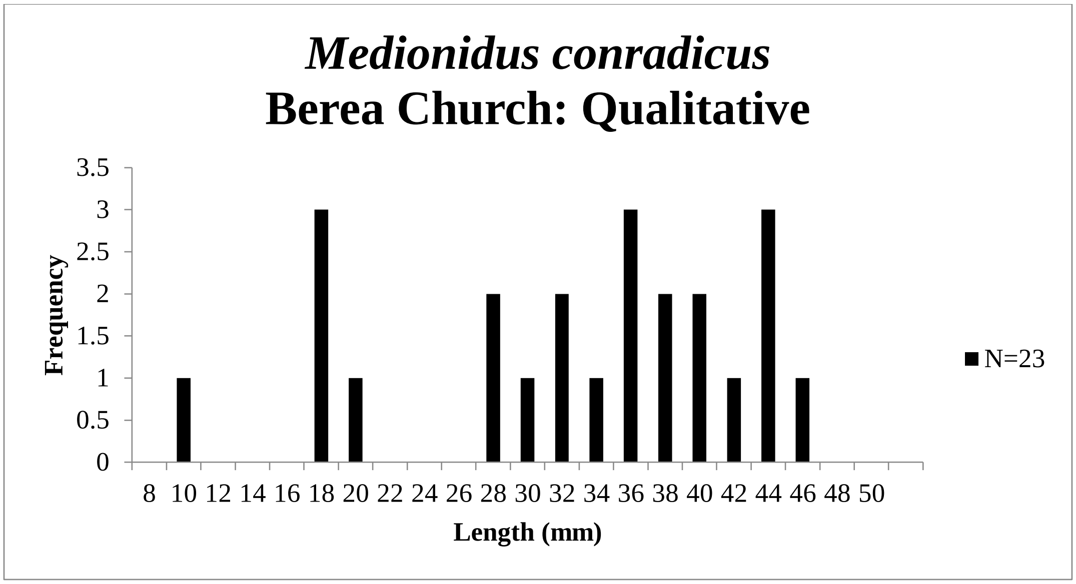

Figure 7. Length frequency histogram of *Medionidus conradicus* collected from Big Rock Creek (Site D19).

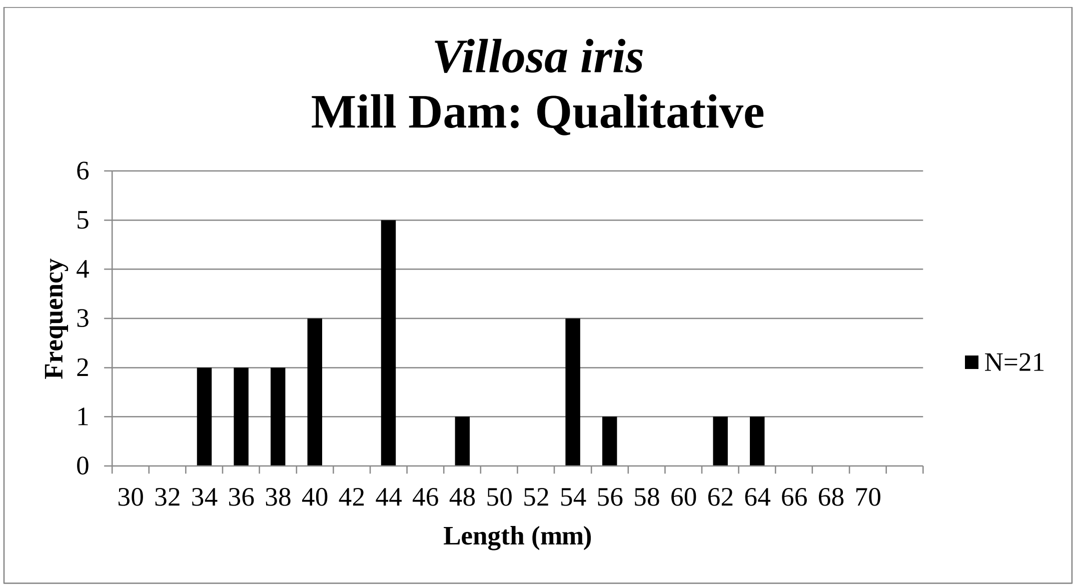

Figure 8. Length frequency histogram of *Villosa iris* collected from Big Rock Creek (Site D18).

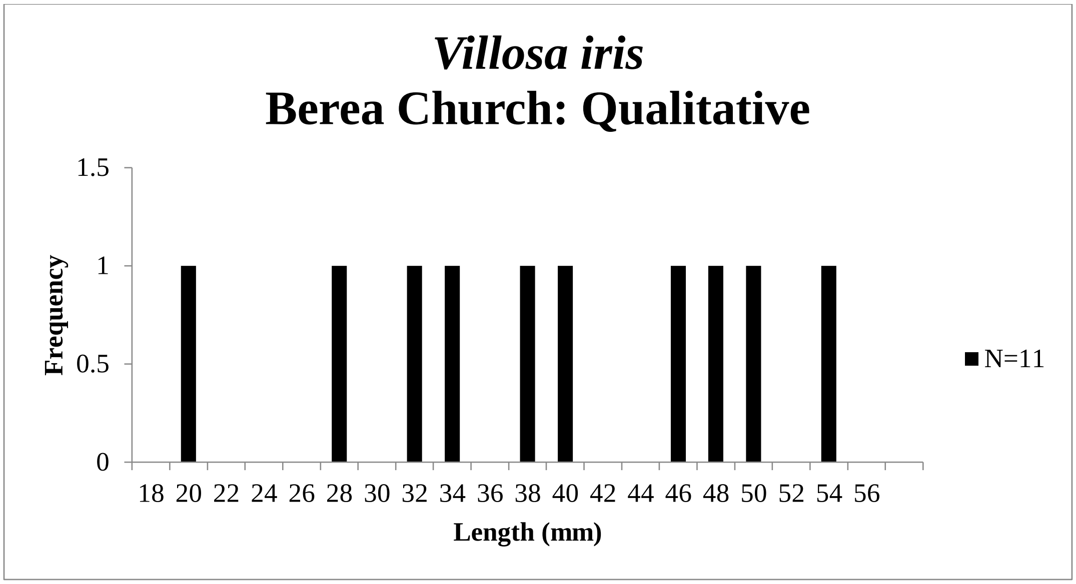

Figure 9. Length frequency histogram of *Villosa iris* collected from Big Rock Creek (Site D19).

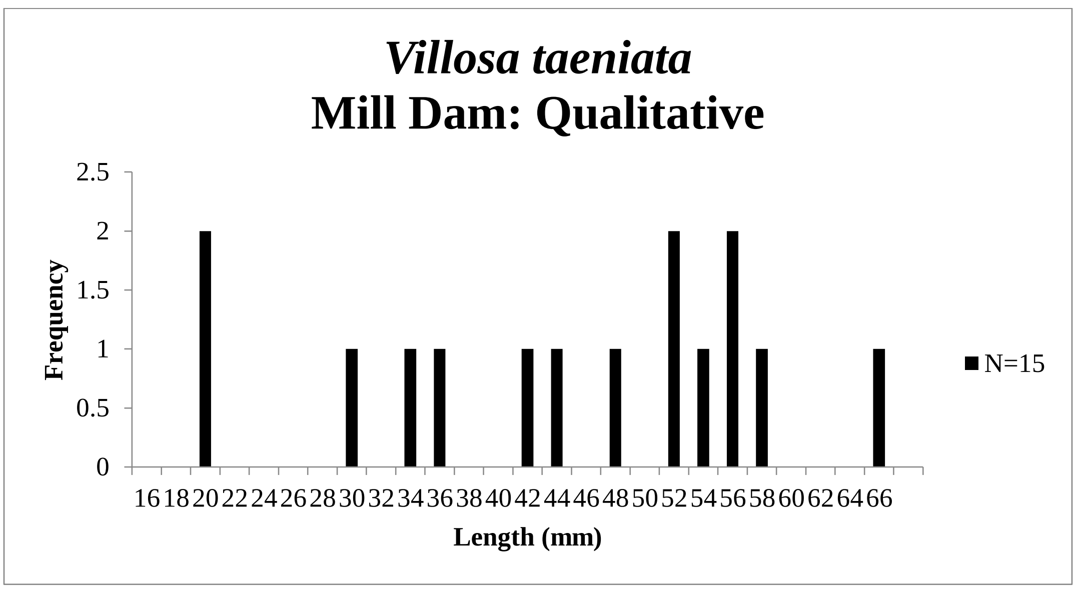

Figure 10. Length frequency histogram of *Villosa taeniata* collected from Big Rock Creek (Site D18).

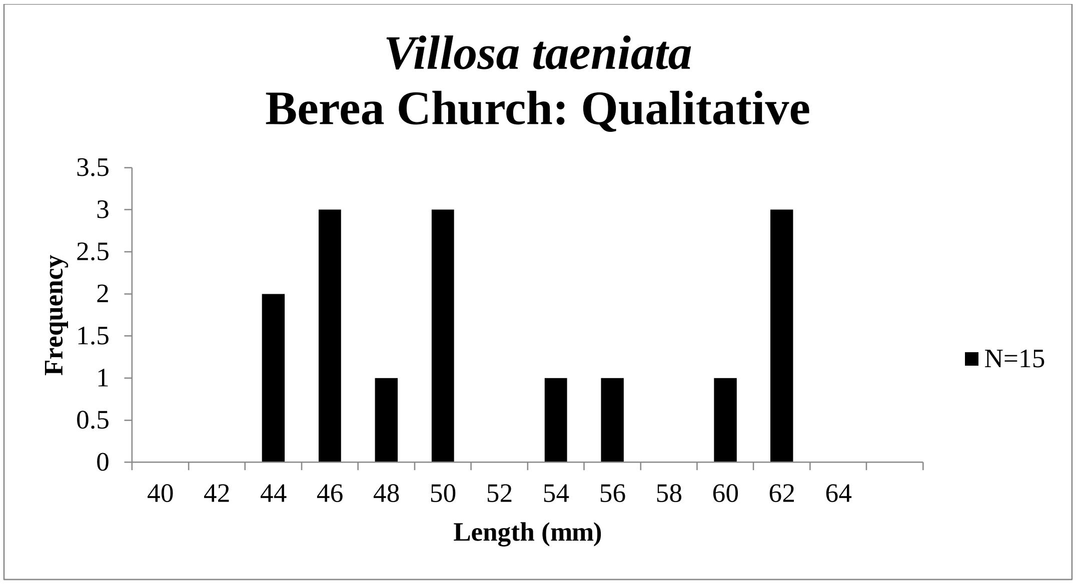

Figure 11. Length frequency histogram of *Villosa taeniata* collected from Big Rock Creek (Site D19).

Cathey's Creek

No historical records were found for Cathey's Creek, and both sites sampled during this study (Sites D1 and D2) were void of mussels.

Big Bigby Creek

Pleuronaia barnesiana and *Lasmigona costata* occurred historically in Big Bigby Creek, and the former was collected in relic condition from site D4 during this study. *Lampsilis fasciola* was collected fresh dead from Site D6 and represents a new species record for the stream. The sites sampled on Big Bigby Creek had low erosion, suitable riparian buffers, and variable substrate composition (Appendix I)

Greenlick Creek

No historical records were found for Greenlick Creek, and no mussels were observed during this study. Both sties sampled (Sites D7 and D8) were impacted by scoured gravel substrate.

Little Bigby Creek

No records were found for Little Bigby Creek in the historical review, and both sites sampled during this study lacked mussels. Site D11 was eroded to bedrock and the riparian buffer had been removed from several areas.

Fountain Creek

Seven species occurred historically in Fountain Creek (Appendix L). *Lampsilis fasciola* and *Villosa iris* were collected fresh dead from Fountain Creek during this study (Site D14). Relic individuals of *Pleuronaia barnesiana* and *Amblema plicata* were collected during this survey (Site D13), representing two new species records for the stream. This study documents the extirpation of seven of the nine species known to have occurred in Fountain Creek. Site D12 was eroded to bedrock, and agricultural activity was present adjacent to all sites sampled (Appendix I). Three species occurred historically in Silver Creek, a tributary to Fountain Creek: *Actinonaias ligamentina*, *Lampsilis cardium*, and *Medionidus conradicus*. No mussels were observed in Silver Creek during this study.

Big Rock Creek

Seventeen species were found in the historical record for Big Rock Creek (Appendix L). Fifteen species were collected during the current study, and species composition varied throughout the sites sampled. Three new species were collected in relic condition during this survey: *Epioblasma triquetra*, *Lasmigona costata*, and *Tritogonia verrucosa*. Only nine species were collected live or fresh dead, documenting the extirpation of 11 species from Big Rock Creek. Habitat capable of supporting abundant mussel beds was abundant in three of the six sites sampled (Sites D18, D19, and D20). The most downstream site sampled was primarily composed of unstable silt and gravel substrate (Site D21), and bedrock was the dominate substrate at the upper two locations (Sites D17 and D18). Historical records of nine species were found for East Rock Creek (Appendix L). Only one relic individual of *Villosa taeniata* was collected during this survey (Site D25). East Rock Creek, a tributary to Big Rock Creek, suffered from siltation, and private landowners of Site D25 had built an artificial boulder dam that interrupted flow. This study documents the loss of all nine freshwater mussel species from East Rock Creek.

Sinking Creek

Records of *Pyganodon grandis*, *Villosa iris* and *Villosa vanuxemensis* were found for Sinking Creek during the historical review. No freshwater mussels were observed in Sinking Creek during this study. Both sites sampled were predominately bedrock, and Site D27 was dry.

Sugar Creek

One record of *Toxolasma lividum* was found for Sugar Creek during the historical review. *Villosa taeniata* and *Pyganodon grandis* were collected from Site D30, representing two new species records for the stream. All three sites sampled were predominately bedrock (Appendix I). Active construction of a bridge was present on Site D29, and Site D28 was completely scoured to bedrock and dry. No records were found for Blue Stocking Branch during the historical review, and no mussels were observed during the current study. The location sampled (Site D31) was scoured to bedrock and dry at the time of sampling.

Flat Creek – South

Ten species were found in the historical record for Flat Creek in Bedford County (Appendix L). Live individuals of *Medionidus conradicus*, *Pleuronaia barnesiana*, *Villosa taeniata*, and *Villosa vanuxemensis* were collected from Flat Creek at two sites sampled during this study (Sites D32 and D34). This study documents that four of the ten species known to have occurred in Flat Creek remain extant in the stream. Sites D32 and D34 had heterogeneous substrate, low erosion, good riparian cover, and abundant aquatic life (Appendix I, Appendix J).

Thompson Creek

Records of *Medionidus conradicus* and *Pleuronaia barnesiana* were found for Thompson Creek in the historical records. No mussels were observed during this study, despite the fact that the same site from which the two species were collected in 2001 was resampled (Site D37).

Crumpton Creek

No records were found for Crumpton Creek or its tributaries during the historical review, and no mussels were found during this study. The site sampled was above Rutledge Falls and the substrate consisted of primarily bedrock (Site D38), however erosion was low and the riparian buffer was extensive (Appendix I). Wiley Creek was also sampled and no mussels were observed. Substrate composition was similar in Wiley Creek (Site D39).

Perry Creek

No historical freshwater mussel records were found for Perry Creek, and the site sampled during this study was void of mussels. The site suffered from severe gravel scouring, and the landowner adjacent to the site reported that the creek was occasionally dry throughout the summer and fall (Site D40).

Garrison Fork

Eight species were found to have historically occurred in Garrison Fork (Appendix L), however only relic *Pleuronaia barnesiana* and two fresh dead *Villosa taeniata* were collected from one site during this study (Site D43). Garrison Fork has suffered a loss of freshwater mussel habitat through a lack of suitable substrate composition, and three of the four sites sampled were eroded to bedrock (Sites D41, D42, and D44). An old mill dam was present at the upstream boundary of Site D41. This study documents the extirpation of seven species from Garrison Fork. Wartrace Creek, a tributary to Garrison Fork, was sampled at two locations (Sites D45 and D46). Records of *Pyganodon grandis and Medionidus conradicus* were found during historical review for Wartrace Creek. Relic *Pleuronaia barnesiana* and *Villosa iris* along with fresh dead *Villosa vanuxemensis* were observed at Site D46, introducing three new species records for the creek. This study documents the extirpation of four of the five species known to have occurred in Wartrace Creek.

Little Hurricane Creek

No historical records were found for Little Hurricane Creek, and no mussels were observed at the two sites sampled during this study. The stream suffered from a limited riparian buffer and agricultural activity was observed at both sample locations (Sites D47 and D48).

Fall Creek

Records of seven freshwater mussel species were found for Fall Creek during the historical review (Appendix L). Relic shells of three species were collected during this survey: *Toxolasma lividum*, *Villosa taeniata*, and *Villosa vanuxemensis. Toxolasma lividum* and *Villosa taeniata* represent new species records for the stream. Substrate composition appeared suitable for mussels at Site D49, but was composed of primarily bedrock at Sites D50 and D51 (Appendix I). This study documents the potential loss of all nine species known to have historically occurred in Fall Creek. Records of *Pyganodon grandis* and *Villosa vanuxemensis* were found for Hurricane Creek, a tributary to Fall Creek, during the historical review. No mussels were observed in Hurricane Creek during this study.

North Fork Creek

Records of eleven species were found for North Fork Creek during the historical review (Appendix L). Relic shells of six species were observed during this study: *Alasmidonta viridis*, *Pleuronaia barnesiana*, *Pyganodon grandis*, *Toxolasma lividum*, *Villosa iris*, and *Villosa taeniata*. *Alasmidonta viridis* represented a new species record for North Fork Creek. This study documented the extirpation of all eleven species known to have historically occurred in North Fork Creek. The stream suffered from severe gravel scouring and limited riparian cover (Appendix I). Three tributaries to North Fork Creek were sampled during this study: Alexander Creek, Clem Creek, and Weakly Creek. Historical records of *Pyganodon grandis* and *Villosa iris* were found for Alexander Creek. Relic specimens of four new species were observed during this survey: *Pleuronaia barnesiana*, *Toxolasma lividum*, *Utterbackia imbecillis*, and *Villosa vanuxemensis*. This study indicates that all six species known to have historically occurred in Alexander Creek have been extirpated. No species records were found for Clem Creek or Weakly Creek during the historical review, and no mussels were observed in either stream during this survey. Both streams were scoured to bedrock, and the site sampled on Clem Creek was dry (Site D58).

Wilson Creek

One record of *Toxolasma lividum* was found for Wilson Creek during the historical review. No mussels were observed at either two sites sampled during this study. Both sites sampled (Sites D59 and D60) were composed primarily of bedrock substrate (Appendix 1). Bedrock was the dominate substrate at both sites (Appendix I).

Spring Creek

No records were found for Spring Creek during the historical review. No mussels were observed during this survey. Substrate was primarily bedrock at both sites sampled (Sites D61 and D62), although erosion was low and riparian cover was moderately sufficient (Appendix I). Fish and gastropods were notably abundant at both sites (Appendix J).

Caney Creek

Historical records for *Utterbackia imbecillis* were found for Caney Creek, although no mussels were observed during this study. Substrate at both sites (D63, D64) was composed primarily of bedrock (Appendix I).

Flat Creek – North

No records were found from Flat Creek in Maury County, and both sites sampled during this study were void of mussels. Bedrock was the dominate substrate at both sample locations (Sites D65 and D66), and aquatic life was sparse throughout the stream (Appendix I, Appendix J).

Rutherford Creek

Historical records of *Lampsilis cardium*, *Pleuronaia barnesiana*, *Pyganodon grandis*, and *Villosa vanuxemensis* were found for Rutherford Creek. Live individuals of *Villosa vanuxemensis* and relic *Pleuronaia barnesiana* were collected during this survey. Relic shells of *Amblema plicata* and *Villosa iris* were collected during this study, representing two new species records for Rutherford Creek. This study documents the extirpation of five of the six species known to have occurred in the stream. Carters Creek, a tributary to Rutherford Creek, was sampled during this study. Records of *Actinonaias ligamentina*, *Pyganodon grandis* and *Villosa vanuxemensis* were found in the historical review. Fresh dead individuals of *Villosa vanuxemensis* were collected during this survey. *Villosa iris* was also collected in relic condition during this survey, and is a new species record for Carters Creek. This study indicates that three of the four species known to have occurred in Carters Creek have been extirpated. Aenon Creek and McCutcheon Creek, two tributaries to Rutherford Creek, were sampled. No species records were found for either stream during the historical record. While McCutcheon Creek was void of mussels, one individual of *Villosa vanuxemensis* and a relic shell of *Pleuronaia barnesiana* were observed in Aenon Creek. This study introduced two new species records for Aenon Creek, although *Pleuronaia barnesiana* has been extirpated from the stream.

Knob Creek

No historical records were found for Knob Creek, and no mussels were observed during this study.

Snow Creek

No records were found for Snow Creek during the historical review, and no mussels were collected at the site sampled during this survey.

Leipers Creek

No species records were found for Leipers Creek during the historical review. Relic shells of *Villosa iris* and *Villosa vanuxemensis* were collected during this study. These two species are new records for Leipers Creek, although this study indicates that they have been extirpated from the stream.

Lick Creek

Historical records of *Toxolasma cylindrellus* and *Villosa taeniata* were found for Lick Creek. Fresh dead shells of these two species along with *Villosa vanuxemensis* and *Alasmidonta viridis* were collected during this survey. This study indicates that the four species known to occur in Lick Creek remain extant in the stream. Although substrate was primarily bedrock at the site sampled (D83), erosion was minimal and riparian cover was optimal (Appendix I). *Fundulus catenatus*, the fish host for the federally endangered *Toxolasma cylindrellus*, was abundant throughout the site.

Canonical Correspondence Analysis

Results of the CCA suggested no significant association between mussel community structure and environmental variables. Axis 1 explained most of the variance at 19% (Table 14). However, it was not significant, thus mussel species distribution was random (Eigenvalue= 0.470, Spp-Env Corr.= 0.768, p= 0.09).

Table 13. Duck River tributary CCA results. Monte Carlo test results for eigenvalues and species-environmental correlations based on randomized data from 998 runs.

		Randomized Data			
Axis	**Real Data**	**Mean**	**Minimum**	**Maximum**	**p**
	Eigenvalue				
1	0.470	0.316	0.106	0.775	0.0911
2	0.181	0.167	0.056	0.626	
3	0.106	0.081	0.009	0.237	
	Spp-Envt Corr.				
1	0.786	0.768	0.523	0.996	0.4174
2	0.676	0.639	0.306	0.955	
3	0.442	0.540	0.187	0.841	

Table 14. Axis summary statistics from Duck River tributary CCA.

	Axis 1	Axis 2	Axis 3
Eigenvalue	0.470	0.181	0.106
Variance in species data			
% variance explained	19.0	7.3	4.3
Cumulative % explained	19.0	26.3	30.5
Pearson Correlation, Spp-Envt	0.786	0.676	0.442
Kendall (Rank) Corr., Spp-Envt	0.391	0.232	0.151

Species Accounts

This study reports that 29 species historically occurred in the upper and middle Duck River tributaries. The following species accounts are compiled from museum records, unpublished and published surveys, and data from the current study. Accounts include general distribution information, habitat preferences, and known occurrences in the Duck River tributaries. Distribution and habitat information is provided by Parmalee and Bogan (1998). Federal status is provided by the USFWS listings (USFWS 2017). Information on species petitioned for listing is provided from the CBD petition list of Southeastern aquatic species (CBD 2010). Non-regulatory conservation status is given according to personal communication with Dr. James Williams (Florida Museum of Natural History).

Actinonaias ligamentina (Lamarck, 1819) – Mucket

Actinonaias ligamentina has a widespread distribution in the Mississippi River drainage, including the Tennessee River and several of its tributaries. It is typically collected in moderate current at shallow depths. Relic specimens were collected from Silver Creek and Carters Creek in 2001 (Ahlstedt et al. 2017). No individuals were observed during the current study. *Actinonaias ligamentina* is considered stable throughout its range, and it is likely extirpated from the upper and middle Duck River tributaries.

Actinonaias pectorosa (Conrad, 1834) – Pheasantshell

Actinonaias pectorosa is endemic to the Tennessee and Cumberland River drainages. This species is typically found in larger rivers to large creeks in relatively shallow water with swift current. Museum records (MCM) indicate that this species was collected from Fountain Creek in 1980. It has not been collected in subsequent surveys, and no individuals were observed during the current study. *Actinonaias pectorosa* is petitioned for listing under the Endangered Species Act, and it appears to be extirpated from the middle and upper Duck River tributaries.

Alasmidonta viridis (Rafinesque, 1820) – Slippershell

Alasmidonta viridis has a widespread distribution throughout the upper Mississippi River drainage, including the smaller tributary streams of the Tennessee River drainage. It is found in sand and gravel substrate as well as in mud along patches of American water willow (*Justicia americana*). Museum records (OSUM) indicate that this species was collected from East Rock Creek in 1976. One relic specimen was collected from North Fork Creek in 2001 (Ahlstedt et al. 2017). Two fresh dead shells were collected in Lick Creek (Site D83), and one relic individual was collected from North Fork Creek (Site D54). *Alasmidonta viridis* is considered stable throughout its range and remains extant in Lick Creek.

Amblema plicata (Say, 1817) – Threeridge

Amblema plicata has a wide distribution throughout the Mississippi River drainage and is common in the Tennessee River drainage. It is found in a variety of habitats. Museum records (OSUM) indicate that it was collected from East Rock Creek in 1976. A large amount of relic shells were observed in the creek in 1990 and 2002 in from Big Rock Creek (Ahlstedt and Saylor 1990, Ahlstedt et al. 2017). Relic individuals were collected from one site on Big Rock Creek (Site D19), one site on Rutherford Creek (Site D68), and two sites on North Fork Creek (Sites D53 and D55) during the current study. *Amblema plicata* is considered stable throughout its range, but is presumed to be extirpated from the upper and middle Duck River tributaries.

Cyclonaias tuberculata (Rafinesque, 1820) – Purple Wartyback

Cyclonaias tuberculata has a widespread distribution throughout the Mississippi River drainage and the Interior Basin. It remains extant throughout the Tennessee River drainage and is a habitat generalist. Relic individuals were collected from Big Rock Creek in 1990 (Ahlstedt and

Saylor). One relic individual was collected from lower Big Rock Creek (Site D21). *Cyclonaias tuberculata* is considered stable throughout its range, and is likely extirpated from the upper and middle Duck River tributaries.

Epioblasma triquetra (Rafinesque, 1820) – Snuffbox

Epioblasma triquetra has a widespread distribution in the Mississippi River drainage and the Interior and Great Lakes Basins, including the Tennessee River drainage. It typically occurs in shallow riffles in gravel and sand. No historical records were found for the upper and middle Duck River tributaries. One relic individual was collected from lower Big Rock Creek (Site D21) during this study, representing a new distributional record for the species. *Epioblasma triquetra* is federally endangered, and is likely extirpated from the upper and middle Duck River tributaries.

Eurynia dilatata Rafinesque, 1820 – Spike

Eurynia dilatata has a widespread distribution in the Mississippi River drainage and the Interior Basin. It is extant in the Tennessee River drainage and occurs in a variety of habitats. One live individual was collected from Flat Creek in Bedford County in 2001 (Ahlstedt et al. 2017). No individuals were observed during the current study. *Eurynia dilatata* is considered vulnerable throughout its range, and appears to be extirpated from the upper and middle Duck River tributaries.

Lampsilis cardium Rafinesque, 1820 – Plain Pocketbook

Lampsilis cardium is widely distributed throughout the Mississippi drainage, and is extant in the Tennessee River drainage. It occurs in a wide variety of habitats. Arnold E. Ortmann reported *Lampsilis ovata* from Garrison Fork in 1924, and this species has been grouped with *Lampsilis cardium* based on taxonomic issues (Williams et al. 2008). Museum records (OSUM) indicate that this species was collected from Flat Creek in 1972 and from Rutherford Creek in 1976. One fresh dead individual was collected from Fountain Creek and relic individuals were collected from Big Rock Creek in 1990 (Ahlstedt and Saylor 1990). Relic individuals were collected from North Fork Creek and Silver Creek in 2001 (Ahlstedt et al. 2017). One fresh dead individual was collected from Big Rock Creek during the current study (Site D18). *Lampsilis cardium* is considered stable throughout its range, but appears to be extirpated from all except one of the upper and middle Duck River tributaries.

Lampsilis fasciola Rafinesque, 1820 – Wavy rayed Lampmussel

Lampsilis fasciola has a wide distribution in the Mississippi drainage and the Great Lakes Basin, and is common throughout the Tennessee River drainage in a variety of habitats. It was collected from Garrison Fork in 1922 (Ortmann 1924). Museum records (MCM) indicate that it was collected from Fountain Creek in 1980. One live specimen was collected from Big Rock Creek in 2001 (Ahlstedt et al. 2017). One live individual (20 mm) was collected from Big Rock Creek during this survey (Site D18). Fresh dead individuals were collected from Big Bigby Creek (Site D6) and Fountain Creek (Site D14). *Lampsilis fasciola* is considered stable throughout its range. It appears to be extirpated from Garrison Fork, but remains extant in Big Rock Creek, Big Bigby Creek, and Fountain Creek.

Lasmigona costata (Rafinesque, 1820) – Flutedshell

Lasmigona costata has a widespread distribution in the Great Lakes Basin and Mississippi River drainages, including the Tennessee River and several of its tributaries. It typically occurs in large creeks to medium-sized rivers with moderate to strong current in coarse sand and gravel. Arnold E. Ortmann reported this species from Garrison Fork in 1924. Museum records (OSUM) indicate that it was collected from Flat Creek in Bedford County in 1972. One live individual was observed in Big Bigby Creek along with relic individuals in North Fork Creek in 2001 (Ahlstedt et al. 2017). One relic specimen was collected from Big Rock Creek during the current study (Site D19). *Lasmigona costata* is considered stable throughout its range, but appears to be extirpated from the upper and middle Duck River tributaries.

Lasmigona sp. *c.f. holstonia*

This species, described as *Lasmigona diversa*, was not recognized in the most recent North American mussel taxonomy revision (Williams et al. 2017). This species is reported to be restricted to the upper Duck River drainage and upper Caney Fork systems (Layzer et al. 1993). One live individual was collected in 2001 from North Fork Creek (Ahlstedt et al. 2017). This species was not observed during the current study. *Lasmigona* sp. *c.f. holstonia* may be extirpated from the upper and middle Duck River tributaries.

Medionidus conradicus (I. Lea, 1834) – Cumberland Moccasinshell

Medionidus conradicus is endemic to the Tennessee and Cumberland drainages, where it occurs in sand and gravel as well as under large, flat rocks. Arnold E. Ortmann reported this species from Garrison Fork in 1924. Live individuals were reported from Big Rock Creek and Flat Creek (Bedford County) along with relic individuals from Garrison Fork, East Rock Creek, and Silver Creek in 2001 (Ahlstedt et al. 2017). Fifty live individuals were collected from four sites throughout Big Rock Creek during the current survey (Sites D17, D18, D19, and D20). Recruitment was observed at Sites D18 and D19. Seven live individuals (all > 30 mm) were collected from two sites on Flat Creek (Sites D32 and D34). *Medionidus conradicus* is petitioned for listing under the Endangered Species Act. While it was once relatively widespread in the upper and middle Duck River tributaries, it remains extant only in Big Rock Creek and Flat Creek.

Obovaria subrotunda (Rafinesque, 1820) – Round Hickorynut

Obovaria subrotunda had a historically widespread distribution in the Ohio, Tennessee, and Cumberland River drainages but has become increasingly rare. It is typically collected in medium to large-sized rivers with moderate current in sand and gravel substrate. Relic individuals of this species were collected from Big Rock Creek in 1990 (Ahlstedt and Saylor 1990). It has not been observed in subsequent surveys, and no individuals were observed during the current study. *Obovaria subrotunda* is petitioned for listing under the Endangered Species Act, and may be extirpated from the upper and middle Duck River tributaries.

Pegias fabula (I. Lea, 1838) – Littlewing Pearlymussel

Pegias fabula is endemic to the Tennessee and Cumberland River drainages. This species is restricted to creeks and small to medium-sized rivers, and it is often buried under large rocks. Archaeological specimens are known from Fountain Creek (Parmalee and Klippel, 1986), but no other collection records exist from the Duck River tributaries. *Pegias fabula* is federally endangered, and is extirpated from the tributaries to the upper and middle Duck River.

Pleurobema oviforme (Conrad, 1834) – Tennessee clubshell

Pleurobema oviforme is a Cumberlandian endemic that is observed in large rivers to small, shallow streams with moderate current in gravel and sand. Museum records (FMNH) indicate that Arnold E. Ortmann collected this species from Garrison Fork in 1922, although he only reported the presence of *Pleuronaia barnesiana* (Ortmann 1924). Similar shell characteristics of the two species often make it difficult to discern between them. Museum records (OSUM) reveal that this species was collected from Flat Creek (Bedford County) in 1972. It has not been collected in subsequent surveys, and no individuals were observed during the current study. *Pleurobema oviforme* is a petitioned for listing under the Endangered Species Act, and is presumed to be extirpated from the upper and middle Duck River tributaries.

Pleuronaia barnesiana (I. Lea, 1838) – Tennessee Pigtoe

Pleuronaia barnesiana is a Cumberlandian endemic and is extant in the Tennessee River drainage. It occurs small to medium-sized river at shallow depths in sand, silt, and gravel. This species was collected from Garrison Fork in 1922 (Ortmann 1924). Museum records indicate that this species historically occurred in Big Bigby Creek (NMNH) and Rutherford Creek (OSUM). Live individuals were collected from Big Rock Creek along with relic shells from North Fork Creek, Garrison Fork, Flat Creek (Bedford County), Fall Creek, Thompson Creek, and East Rock Creek in 2001 (Ahlstedt et al. 2017). One live individual (53 mm) was collected from Big Rock Creek (Site D18), and two live individuals (81 mm, 78 mm) were observed in Flat Creek (Sites D32 and D34) during the current study. Relic shells were collected from Big Bigby Creek (Site D4), Fountain Creek (Site D13), Garrison Fork (Site D43), Wartrace Creek (Site D46), North Fork Creek (Sites D53 and D55), Alexander Creek (Site D56), Rutherford Creek (Site D69), and

Aenon Creek (Site D72). *Pleuronaia barnesiana* is petitioned for listing under the Endangered Species Act. While it was once widespread throughout the upper and middle Duck River tributaries, it remains only in Flat Creek and Big Rock Creek.

Pleuronaia dolabelloides (I. Lea, 1840) – Slabside Pearlymussel

Pleuronaia dolabelloides is a Cumberlandian endemic and occurs in isolated populations in the state of Tennessee. It is typically found in sand, gravel, and cobble in moderate to strong current. Museum records (OSUM, UMMZ) indicate that this species was collected from Fall Creek in 1924. Live individuals were collected from Big Rock Creek in 1990 (Ahlstedt and Saylor 1990), and relic individuals were collected from North Fork Creek in 2001 (Ahlstedt et al. 2017). Three live individuals (30, 40, 42 mm) were collected from Big Rock Creek during the current study (Site D19). *Pleuronaia dolabelloides* is federally endangered. It remains extant in Big Rock Creek but is likely extirpated from the other upper and middle Duck River tributaries.

Potamilus alatus (Say, 1817) – Pink Heelsplitter

Potamilus alatus has a widespread distribution in the Mississippi River drainage, and is extant in the Tennessee River system. It is found in a wide variety of habitats. Museum records (MCM) indicate that it was collected from Fountain Creek in 1980. Relic individuals were collected from Fountain Creek in 1990 (Ahlstedt and Saylor 1990). *Potamilus alatus* is considered stable throughout its range, but is likely extirpated from the upper and middle Duck River tributaries.

Ptychobranchus fasciolaris (Rafinesque, 1820) – Kidneyshell

Ptychobranchus fasciolaris has a widespread distribution throughout the Ohio, Tennessee, and Cumberland River drainages. It occurs in a wide variety of habitats. Relic individuals of this species were collected from Big Rock Creek in 1990 and from Fall Creek in 2001 (Ahlstedt and Saylor 1990, Ahlstedt et al. 2017). No individuals were observed during the current study. *Ptychobranchus fasciolaris* is considered vulnerable throughout its range, and this study suggests it is extirpated from the upper and middle Duck River tributaries.

Pyganodon grandis (Say, 1829) – Giant Floater

Pyganodon grandis has a widespread distribution throughout the Mississippi River drainage, and is extant in the Tennessee River drainage. Museum records (OSUM) indicate it has been observed in Alexander Creek, Wartrace Creek, Rutherford Creek, Big Rock Creek, Sinking Creek, and Thompson Creek. Relic individuals were collected from North Fork Creek, Wartrace Creek, Hurricane Creek, Fall Creek, and Fountain Creek in 2001 (Ahlstedt et al. 2017). Fresh dead individuals were collected from Sugar Creek (Site D30) and relic shells were found in North Fork Creek (Site D53) during the current survey. *Pyganodon grandis* is considered stable throughout its range. This species was once widespread throughout the upper and middle Duck River tributaries, but is may only remain extant in Sugar Creek.

Strophitus undulatus (Say, 1817) – Creeper

Strophitus undulatus has a widespread distribution throughout the Mississippi River drainage and the Interior Basin, including the Tennessee River and several of its tributaries. This species is tolerant of a variety of habitats. It has been reported from Garrison Fork (Ortmann 1924), and relic individuals were collected from East Rock Creek in 2001 (Ahlstedt et al. 2017). No individuals were observed during the current study. *Strophitus undulatus* is considered stable throughout its range, but may be extirpated from the upper and middle Duck River tributaries.

Theliderma cylindrica (I. Lea, 1860) – Rabbitsfoot

Theliderma cylindrica has a widespread distribution in the Mississippi River drainage but has become increasingly rare throughout its former range. It typically occurs in large creeks to large rivers in shoal habitats. Relic individuals were collected from Big Rock Creek in 2001 (Ahlstedt et al. 2017). One relic shell was collected from lower Big Rock Creek (Site D21) during the current study. *Theliderma cylindrica* is federally endangered, and is likely extirpated from the upper and middle Duck River tributaries

Toxolasma cylindrellus (Lea, 1868) – Pale Lilliput

Toxolasma cylindrellus is endemic to the Cumberlandian region and is currently restricted to tributaries in the Tennessee River system. It typically thrives in small, shallow tributaries with moderate current in sand and gravel. This species was reported from Big Rock Creek in the 1990s (Ahlstedt and Saylor 1990). Museum records (MMC) indicate that this species was collected from Lick Creek in 2015. One fresh dead and several relic shells were observed in Lick Creek during the current study (Site D83). *Toxolasma cylindrellus* is federally endangered. It remains in extant in Lick Creek, but is presumed extirpated from the other upper and middle Duck River tributaries.

Toxolasma lividum (Rafinesque, 1831) – Purple Lilliput

Toxolasma lividum has a widespread historical distribution in the Ohio, Tennessee, and Cumberland River drainages, but has been extirpated throughout much of its former range. Museum records (OSUM) reveal it historically occurred in Sugar Creek, Wilson Creek, North Fork Creek, and Big Rock Creek. Live individuals were collected from Big Rock Creek and relic individuals were collected from North Fork Creek in 2001 (Ahlstedt et al. 2017). Two fresh dead shells were observed in Big Rock Creek (Site D19) during this study. Relic shells were collected from Fall Creek (Site D49), North Fork Creek (Sites D54 and D55), and Alexander Creek (Site D56). *Toxolasma lividum* is petitioned for listing under the Endangered Species Act. This species has been extirpated from much of its historical range in the upper and middle Duck River tributaries.

Tritogonia verrucosa (Rafinesque, 1820) – Pistolgrip

Tritogonia verrucosa has a widespread distribution in the Mississippi River drainage, including the Tennessee River and several of its tributaries. It occurs in a wide variety of habitats. No historical records were found of this species for the upper and middle Duck River tributaries. One relic individual was collected from Big Rock Creek during this survey (D19). *Tritogonia verrucosa* is considered stable throughout its range, and this study suggests that it is extirpated from the upper and middle Duck River tributaries.

Utterbackia imbecillis (Say, 1829) – Paper pondshell

Utterbackia imbecillis is widespread throughout the Mississippi River drainage and the Interior Basin, and is a common species throughout the Tennessee River drainage. It is particularly common in impounded conditions. Museum records (OSUM) indicate that it was collected in Caney Creek in 1989. One relic individual was collected from Alexander Creek (Site D56) during this study. *Utterbackia imbecillis* is considered stable throughout its range, but may be extirpated from the upper and middle Duck River tributaries.

Villosa iris (I. Lea, 1829) – Rainbow

Villosa iris has a widespread distribution throughout the Ohio, Cumberland, and Tennessee River drainages. It typically occurs in shallow riffles in moderate to strong current and along the margins of vegetation beds. Museum records indicate that it was collected from Big Rock Creek in 1990 (INHS). Live individuals from North Fork Creek and relic shells from Flat Creek, Sinking Creek, East Rock Creek, and Fall Creek were collected in 2001 (Ahlstedt et al. 2017). Forty-six live individuals were collected from Big Rock Creek across three sites during this study (Sites D18, D19, and D20). Relic shells were also collected from Fountain Creek (Site D14), Wartrace Creek (Site D46), North Fork Creek (Site D54), Rutherford Creek (Site D71), and Carters Creek (Site D75). *Villosa iris* is considered stable throughout its range. This study suggests that this species was once widespread throughout the upper and middle Duck River tributaries, but it only remains extant in Big Rock Creek.

Villosa taeniata (Conrad, 1834) - Painted Creekshell

Villosa taeniata is endemic to the Tennessee and Cumberland River drainages. It is typically observed in shallow water with moderate current in sand and gravel substrate. Live individuals were collected from Big Rock Creek, North Fork Creek and Flat Creek in 2001 (Ahlstedt et al. 2017). Ninety-one live individuals were collected from four sites across Big Rock Creek during this survey (Sites D17, D18, D19, and D20). Eleven live individuals were collected from Flat Creek in Bedford County (D32, 34). One live individual (35 mm) was collected from Sugar Creek (Site D30). Fresh dead shells were collected from Garrison Fork (Site D43) and Lick Creek (Site D83). Relic shells were collected from East Rock Creek (Site

D25) and Fall Creek (Sites D49 and D50). *Villosa taeniata* is considered vulnerable throughout its range, and remains extant in several upper and middle Duck River tributaries.

Villosa vanuxemensis (I. Lea, 1838) - Mountain Creekshell

Villosa vanuxemensis is endemic to the Cumberlandian region and is extant in several tributaries of both the Tennessee and Cumberland Rivers. It typically occurs in riffles of small to medium-sized rivers in sand and gravel substrate. This species was reported from Garrison Fork in the 1900s (Ortmann 1924, Isom and Yokley 1968). Museum records (MCM) indicate that it was last collected in Hurricane Creek in 1979 and from Rutherford Creek in 2003. Live individuals were collected from Big Rock Creek, North Fork Creek, Sinking Creek, and Carters Creek along with relic shells from Wartrace Creek, East Rock Creek, Fall Creek, Flat Creek (Bedford County), and Thompson Creek in 2001 (Ahlstedt et al. 2017). This was the most widespread and abundant species collected during the current study. A total of 114 live individuals were collected from four sites across Big Rock Creek (Sites D17, D18, D19, and D20). Twenty-three live individuals (all > 40mm) were collected from two sites in Flat Creek (Sites D32 and D34), and two individuals (43 mm, 46 mm) were observed in Rutherford Creek (Site D69). One live individual (50 mm) was collected from Aenon Creek (Site D73). *Villosa vanuxemensis* is considered vulnerable throughout its range.The range of this species has been reduced throughout the upper and middle Duck River tributaries, although it remains extant in four streams.

Discussion and Recommendations

This is the first extensive assessment of freshwater mussel populations in the upper and middle Duck River tributaries, and this studydocuments the extirpation of 17 of the 29 species known to have historically occurred in the study area. Due to the extensive amount of sampling conducted during this study, it is presumed that species collected only in relic condition have been extirpated from the area. Due to the diversity in the main channel, it is possible diversity was even higher in the tributaries before anthropogenic alteration to the watershed occurred. Several factors, such as habitat loss, poor water quality, unstable substrate, and loss of suitable fish hosts have likely contributed to the loss of mussel diversity and abundance observed during this study.

Suitable habitat for freshwater mussels was limited in most of the tributaries sampled. Urban development is increasing rapidly throughout the Duck River drainage, resulting in the large-scale removal of riparian vegetation. As a result, the river is suffering an increase in siltation (Don Hubbs, TWRA, pers. comm.). Bedrock was the dominate substrate observed in several tributaries (Appendix I), and this limits the ability of mussels to thrive in those streams. Substrate permeability and stability are crucial to support the burrowing activity and long-term survival of freshwater mussels (Allen and Vaughn 2009, Pandolfo et al. 2016). North Fork Creek and Garrison Fork suffer from unstable substrates and severe gravel scouring. These tributaries once harbored diverse mussel populations (Appendix L). The instability of their substrates in combination with high flow events in the drainage will likely prohibit freshwater mussel populations from reestablishing themselves unless degradation is addressed with restorative efforts.

No pattern was detected between the reach-scale factors examined (erosion, riparian buffer and substrate composition) and the structure of freshwater mussel assemblages. Multivariate analysis is commonly used in the field of aquatic ecology to examine co-occurrence patterns among species in a community, however its applicability to streams with low densities can be limited (Faltys 2016). Live or fresh dead mussels were absent at 78% of sites, and these low densities may have prevented a pattern from being detected. The parameters selected and the methodology of their collection may not have been ideal for discerning patterns through multivariate analysis. Substrate composition has been significantly linked to patterns of mussel species richness and abundance (Bird 2017). Bird analyzed substrate composition by collecting samples and sieving them to quantitatively estimate the percent composition of each particle size. The qualitative assessment of surface substrate conducted during this study may not have been sufficient in categorizing the substrate composition at sites, and this may have impacted the results of the analysis. Landscape-scale factors such as forest coverage within a watershed have been found to significantly drive freshwater mussel assemblage structure (Burlakova et al 2011). The riparian cover throughout entire tributaries, rather than at each site, would have been a more useful parameter for the analysis performed during this study.

Populations of several species remain relatively intact in Big Rock Creek (Appendix H). The Nature Conservancy (TNC) is currently conducting a long-term restoration project in the Big Rock Creek drainage. In the last decade, restorative actions have included bank stabilization, riparian buffer improvement, and riffle construction (Sally Palmer, The Nature Conservancy, pers. comm.). Species abundance has improved in Big Rock Creek since it was last surveyed in 2001 (Ahlstedt et al. 2017), indicating that the restoration methods used by TNC may have been beneficial for freshwater mussel species. Although cause-effect relationships cannot be determined by this study due to lack of independent replication, continued, regular monitoring of the mussels in Big Rock Creek will not only help document the success of this restoration, but will inform future management decisions in the Duck River watershed. The populations observed below the mill dam (Site D18) were particularly abundant. Small, intact dams have been found to enhance freshwater mussel populations by providing stable geomorphology (Gangloff et al. 2011). The mill dam on Big Rock Creek may be providing a refuge for freshwater mussels, and any future plans to remove this dam should consider the success of these populations. The TWRA is currently performing reintroductions of the federally endangered *Toxolasma cylindrellus* (Don Hubbs, TWRA, pers. comm.), a species that historically occurred in Big Rock Creek. It is recommended Big Rock Creek be considered as an additional relocation site for this species.

Freshwater mussels collected in Flat Creek (Bedford County) were all of large size classes, although species composition was similar to that of Big Rock Creek (Appendix H). Flat Creek should be considered as a candidate stream for restoration, especially due to the presence of the petitioned *Medionidus conradicus* and *Pleuronaia barnesiana*. Implementation of the practices used by the TNC in the Big Rock Creek drainage are recommended for the improvement of mussel habitat.

These extensive tributary surveys not only documented broad extirpations, but documented the presence of previously unknown populations of important species. Several species collected during this study are candidates for listing under the Endangered Species Act (CBD 2010). Both *Medionidus conradicus* and *Pleuronaia barnesiana* were collected live during this study. *Toxolasma lividum* was only collected in fresh dead condition from one tributary, but this species was found in relic condition throughout this study (Appendix L). Information from this extensive study gives a more accurate representation of these species' current distributions and will aid the USFWS in their listing decisions.

Chapter 5: Quantitative Assessment of Freshwater Mussels in Big Rock Creek

Introduction

Freshwater mussel survey methods range from qualitative, semi-quantitative, and quantitative depending on survey objectives and logistics. Qualitative surveys often consist of timed searches in which a large area is sampled for a particular amount of time. This type of sampling is more efficient for estimating species richness, but may underestimate the presence of smaller individuals (Obermeyer 1998). Quantitative surveys are more rigorous and are often conducted using 0.25-m² quadrats to randomly sample a particular percentage of area. Quadrat sampling allows surveyors to more accurately estimate mussel densities and recruitment, but may be less suitable for detection of rare species compared to timed searches (Kovalak 1986, Miller and Payne 1993). The combination of qualitative and quantitative survey methods is ideal for evaluating the status of freshwater mussel populations. Due to the diversity and abundance observed in Big Rock Creek during the qualitative surveys during this study, two sites were chosen for quadrat sampling. This is the first quantitative survey to be conducted in Big Rock Creek and will serve as a baseline for future assessments of freshwater mussel populations.

Methods

Sites that exhibited the highest freshwater mussel diversity and abundance during qualitative surveys were considered for additional quadrat sampling. Two locations were selected for quantitative sampling on Big Rock Creek in Marshall County.

Berea Church

The area selected for quantitative sampling was chosen based upon habitat conditions, substrate composition, and accessibility. A 20-m transect was set delineating the upstream and downstream boundaries of the selected study area. The area was separated into ten, 2-m lanes (Figure 12). Each lane ran perpendicular across the width of the channel or until habitat was no longer able to be sampled. Lanes were separated into 1 m x 2 m cells, enough to allow for a total of eight 0.25 m^2 settings. A random number generator was used to select a number from one to eight, which determined the placement of the quadrat sampler. Before each quadrat was sampled a surface substrate description was qualitatively recorded as percentage cover of organic matter, silt, sand, gravel, cobble, boulder, and bedrock within the quadrat. If a quadrat was placed on an area composed exclusively of bedrock, the quadrat was moved one quadrat over and sampled in the new location. If the second quadrat was also bedrock, that section was sampled. Each quadrat was excavated until the channel bed was reached or to a depth of approximately 15 cm. All live mussels were collected in mesh bags and identified, sexed where applicable, measured for length in millimeters, and placed back into the substrate where found. A total area of 22.25 m^2 was sampled and a total of 89 quadrats were excavated. Mussel density was calculated as total number of mussels/0.25m^2. Confidence intervals (95%) for average mussel density were calculated by bootstrapping using XLSTAT statistical software. Following quantitative sampling, a qualitative time search of 2 person hours was performed outside the perimeter of the sample grid to capture any additional species outside the area that may have been missed.

Mill Dam

A 24-m transect was placed from upstream to downstream boundary of the selected study reach. The area was separated into eleven, two meter lanes (Figure 13). The same methodology used at the Berea Church site was repeated, although the shape and dimensions of the survey area differed. A total area of 18.5 meters^2 was surveyed and 74 quadrats were sampled. Due to the presence of extensive bedrock, certain sections were excluded from the study area. The perimeter surrounding the quantitative grid was sampled qualitatively for 2 person hours after quadrat sampling was completed. A two-tailed Kolmogorov-Smirnov test was used at $\alpha = 0.05$ to check for significant differences in size distribution between the two sampling methods below the mill dam for *Villosa vanuxemensis*. Qualitative data collected on October 2, 2017 from Site D18 was compared to data collected during quadrat sampling. Data from the previous years' time search was selected over the 2017 timed search of the perimeter around the quantitative grid due to a closer similarity of number of live individuals. The null hypothesis that the two length frequency distributions followed the same distribution was tested using XLSTAT software.

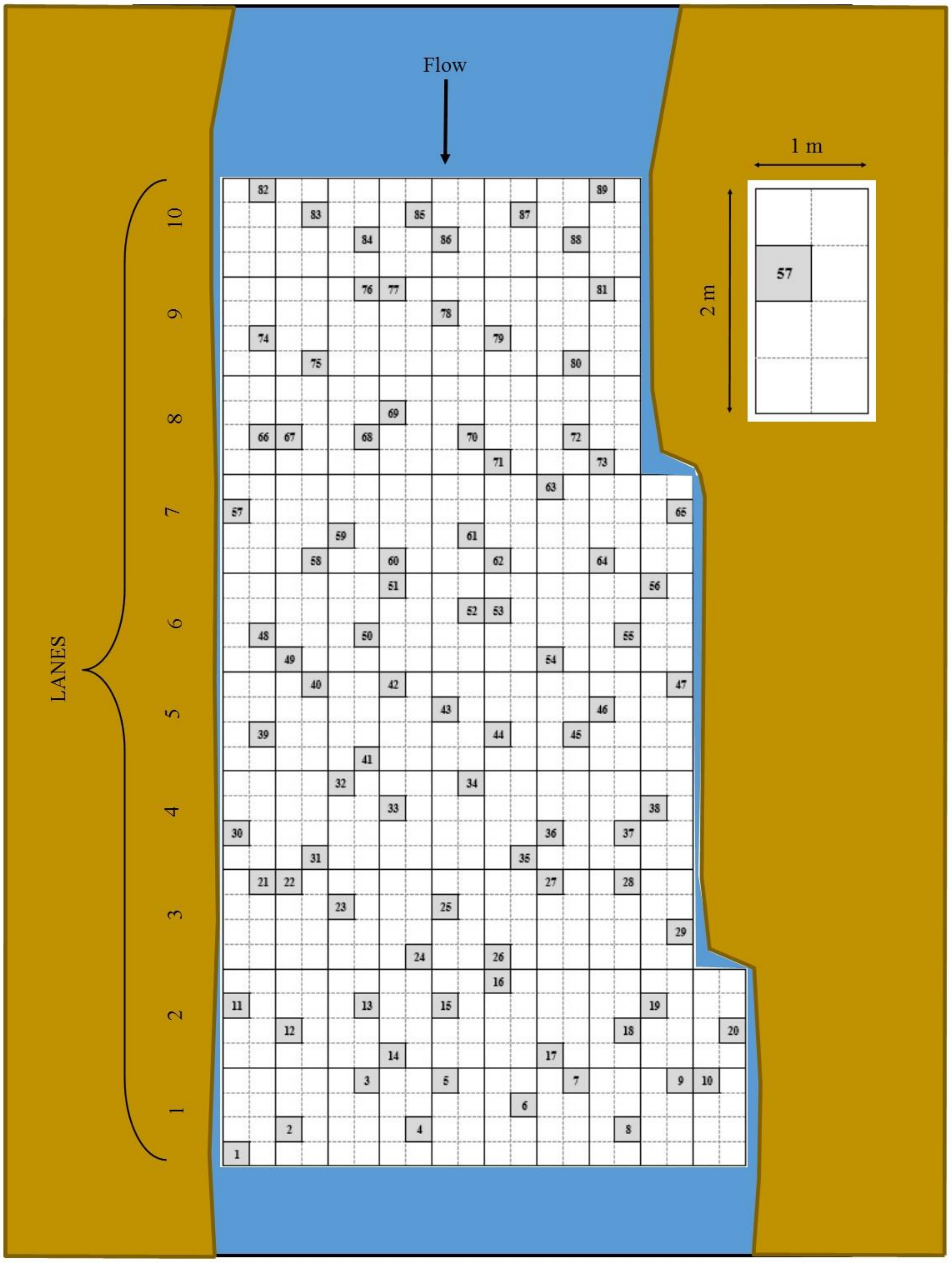

Figure 12. Quantitative grid sampled in Big Rock Creek at Berea Church site.

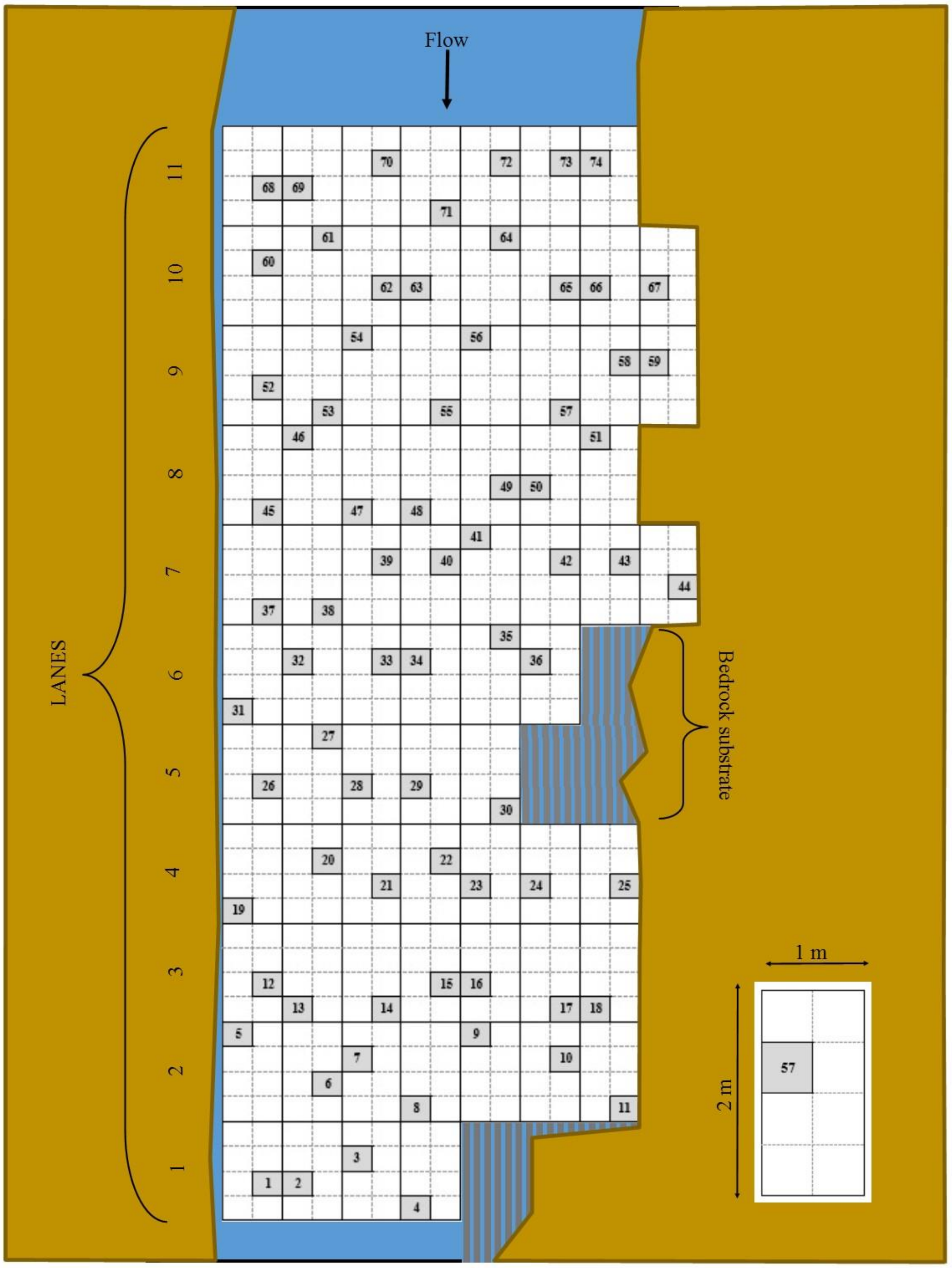

Figure 13. Quantitative grid sampled in Big Rock Creek at mill dam site.

Results

Berea Church

Freshwater mussel density was estimated to be 0.33 mussels/0.25m² (Table 15). Species richness was four and a total of 29 mussels were collected from 89 quadrats. *Villosa vanuxemensis* and *Villosa taeniata* were the most common species observed, comprising 58.6% and 31% of all individuals (Table 16). Juveniles of all species except for *Medionidus conradicus* were collected, indicating recent recruitment. Fifty-six live mussels of the same four species were collected during the time search conducted after quadrat sampling with a CPUE of 28.0 mussels/h (Table 17, Appendix M).

Mill Dam

Density was higher below the mill dam at 1.27 mussels/0.25m² (Table 15). A total of 95 mussels were collected from 74 quadrats. Twenty-seven mussels were collected during the timed search conducted outside the perimeter of the quadrat grid with a CPUE of 14.5 mussels/hour (Table 18, Appendix M). Two individuals of *Pleuronaia barnesiana* were collected during the qualitative search, and this increased species richness at the site to five. Multiple age classes were observed in the length frequency distribution of *Villosa vanuxemensis* collected during quantitative sampling (Figure 14).

Results from the two-tailed Kolmogorov-Smirnov test indicate a significant difference between length frequency distributions of *Villosa vanuxemensis* collected qualitatively and quantitatively from below the mill dam (D=0.254, alpha=0.05, p=0.012)(Table 19).

Table 15. Summary results of quantitative sampling on two sites in Big Rock Creek.

	Berea Church	**Mill Dam**
# of quadrats sampled	89	74
Area sampled (m²)	22.25	18.5
Species richness	4	4
# of mussels	29	95
% of quadrats occupied	20.22%	56.76%
Density (mussels/0.25m²)	0.33	1.27
95% Confidence Intervals	0.173 - 0.486	0.926 - 1.568

Table 16. Number of individuals observed, percent composition, and length range of species collected from quadrats from Big Rock Creek at Berea Church.

Species	# Observed	%	Length range (mm)
Villosa vanuxemensis	17	58.6	19-49
Villosa taeniata	9	31.0	10-72
Pleuronaia barnesiana	2	6.9	33-72
Medionidus conradicus	1	3.5	48
Total	**29**	**100**	

Table 17. Species and number of mussels collected during timed search (2 person hours) in area surrounding quantitative sampling grid at two sites on Big Rock Creek.

	Berea Church	Mill Dam
Villosa vanuxemensis	19	17
Villosa taeniata	24	6
Villosa iris	0	0
Medionidus conradicus	12	2
Pleuronaia barnesiana	1	2
Total	**56**	**27**

Table 18. Number of individuals observed, percent composition, and length range of species collected from quadrats from Big Rock Creek at the mill dam.

Species	# Observed	%	Length range (mm)
Villosa vanuxemensis	73	76.8	15-61
Villosa taeniata	12	12.6	23-58
Villosa iris	5	5.3	38-50
Medionidus conradicus	5	5.3	25-39
Total	**95**	**100**	

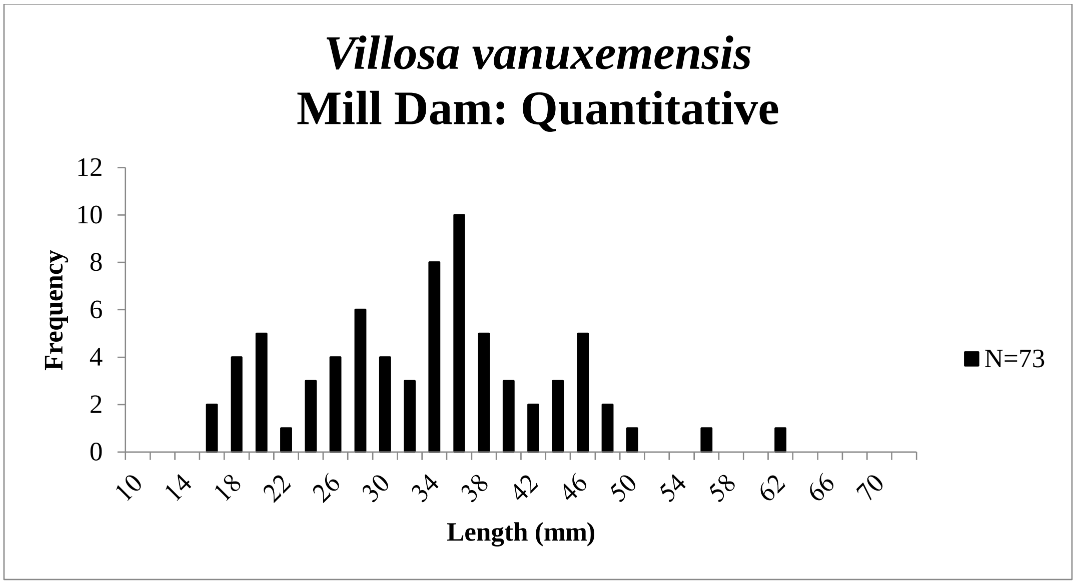

Figure 14. Length frequency histogram of *Villosa vanuxemensis* collected from Big Rock Creek during quantitative sampling at mill dam site.

Table 19. Results from a two-tailed Kolmogorov-smirnov test for length frequency distributions of *Villosa vanuxemensis*. The test compared qualitative data collected on October 10, 2017 (Site D18) and quantitative data collected June 6, 2018.

	Method	
Parameter	Qualitative	Quantitative
Minimum size	12.000	15.000
Maximum size	58.000	61.000
Mean	36.545	32.932
Standard Deviation	8.122	9.740
Total # of observations	88	73
p-value	0.012	
alpha	0.05	

Discussion

Substrate permeability is important for the viability of freshwater mussel populations. Smaller particle sizes, such as sand, gravel and cobble, provide flow refuges for mussels, which have been found to drive abundance and species assemblages (Di Maio and Corkum 1997; Cope et al. 2003). Bedrock substrate lacks these refugia from high flow events that mussels require for survival. The inability of freshwater mussels to establish dense assemblages in bedrock has been reported (Layzer and Madison 1995), and this study suggests mussel populations in Big Rock

Creek were excluded from ares in which substrate was predominately bedrock. Mussels did not occur in quadrats in which bedrock composed more than 50% of the substrate (Appendix O). Substrate compositions observed from quadrats at the mill dam indicated that the species utilized a mixture of sand, gravel, and cobble. The restoration project that began a decade ago on Big Rock Creek has possibly prevented the loss of heterogeneous substrate made of silt, sand, gravel, and cobble, and boulder, likely enabling mussel populations to expand.

More live individuals (N=56) were collected during the time search following quantitative sampling than from the excavated quadrats (N=29) at Berea Church. Mussels were found to be abundant in the riffle mesohabitat above the quantitative grid, which was entirely within a run mesohabitat. Placement of the quadrat grid may have excluded patches of suitable mussel habitat, leading to an underestimation of mussel density. This highlights the benefit of combining qualitative and quantitative sampling. The qualitative time search below the mill dam increased species richness at the site by one with the addition of *Pleuronaia barnesiana*. Quantitative sampling underestimated species richness at the mill dam sites, and this indicates the importance of qualitative sampling for detection of rare species.

Results from the Kolmogorov-Smirnov test revealed the length frequency distribution of *Villosa vanuxemensis* significantly differed between the two survey methods. The fact that the minimum size collected during qualitative (12 mm) was smaller than that of quantitative sampling (15 mm) suggests the timed search successfully documened the presence of recruitment. Studies suggest that qualitative sampling can underestimate recruitment and be biased to individuals of larger size (Hornbach and Deneka 1996). Although a larger number of juveniles (<25 mm) were collected during quantitative sampling, recruitment was still documented in qualitative sampling (Figure 4, Figure 14). This discrepancy between sampling methods may not be as prominent in small stream such as Big Rock Creek, where the channel width was as narrow as four meters in parts of the sample reach. Comparison of length frequency histograms of *Villosa vanuxemensis* observed during both sampling methods indicated a healthy size distribution and successful recruitment of small juveniles (Figure 4, Figure 14). The overall number of live individuals collected from combining qualitative and quantitative sampling in 2017 (N=90) was comparable to the number collected during qualitative sampling in 2016 (N=88). This study indicates that timed searches may be sufficient enough for streams of small size if the objective is to document recruitment and estimate abundance. However, quantitative sampling is required for an accurate estimation of density and should still be considered a necessary tool in the long-term monitoring of mussel populations in small tributary streams.

CONCLUSION

Freshwater mussels have become a focal point for aquatic conservation in recent decades, but knowledge on the distribution of species is still developing. The Harpeth River once harbored a rich fauna of 46 species, and 29 species historically occurred in the selected Duck River tributaries. This diversity has been greatly underestimated in the past, which highights the importance of continued status and distributional studies. The tributary streams of the Harpeth and Duck River drainages have suffered a loss of freshwater mussel diversity, and this is attributed to habitat loss resulting from large-scale anthropogenic alterations to the landscapes of both watersheds. These evaluations of the current status and distribution of freshwater mussels in the Harpeth River drainage and upper and middle Duck River tributaries will be an important tool for future work in the Cumberlandian region. Plans to expand the scope of both projects are currently being organized by non-profit organizations and government agencies.

Harpeth River

Based on the present study, subsequent freshwater mussel surveys in the Harpeth River tributaries are not recommended. Mussels were absent from 81% of sites and 83% of the tributaries sampled during the duration of this study. Habitat loss has reduced native mussel densities and this issue should be addressed prior to considering the reintroduction of freshwater mussels. Current habitat restorations should be continued along with new projects to improve habitat availability for freshwater mussels. Contrastingly, an extensive survey of the main channel Harpeth River is required to discern which freshwater mussel species remain extant in the system. The discovery of the petitioned *Simsponaias amibigua* reveals the need for surveying tailored to that species' habitat preferences: large, flat rocks in slow moving water and along *Justicia americana* patches. This survey will aid in informing the USFWS decision regarding the federal listing of this species.

Duck River

Freshwater mussels were once widespread throughout the upper and middle Duck River tributaries, but habitat loss has diminished these populations to isolated assemblages in a select few tributaries. Freshwater mussels occurred in seventeen of the sampled tributaries, but communities have been extirpated from ten of those streams. Approximately 59% of the species that historically occurred in upper and middle Duck River tributaries have been extirpated.

Previously unknown populations of endangered (*Pleuronaia dolabelloides*) and petitioned (*Pleuronaia barnseiana*, *Medionidus conradicus*) species were reported during this survey, highlighting the necessity of surveying tributaries when assessing freshwater mussel populations. An extensive survey of the lower Duck River tributaries is being planned in collaboration with Tennessee Technological University. In addition to freshwater mussel sampling, a habitat suitability model will be developed using mussel presence and absence data collected throughout all of the Duck River tributaries. This comprehensive study will inform management on important decisions regarding the freshwater mussel populations in the Duck River drainages.

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APPENDICES

Appendix A: Species collected from seven sites on the Harpeth River by Hubbs et al. in 2002. "L" denotes live, "FD" denotes fresh dead, and "R" denotes relic individuals.

Species	Collier Bend	HWY 100	Sneed Road	Below Franklin Sewage Outfall
Actinonaias ligamentina		R		
Actionaias pectorosa	R			
Alasmidonta viridis				
Amblema plicata	R		L, R	R
Cyclonaias tuberculata	L, R	R	FD, R	R
Cyclonaias pustulosa	L, R			
Eurynia dilatata	L			
Fusconaia flava	L, RD			R
Lampsilis cardium	L	R	FD, R	R
Lampsilis fasciola	R	L, R	L, FD, R	R
Lasmigona complanata	L, FD		L	R
Lasmigona costata	L	L, FD, R	L, R	
Leptodea fragilis	L	L, R	R	R
Megalonaias nervosa	R			
Obliquaria reflexa	FD, R		R	
Obovaria subrotunda	R			
Potamilus alatus	L	L, R	FD, R	FD, R
Ptychobranchus fasciolaris	R			L, R
Pyganodon grandis	L		R	R
Quadrula quadrula	L, R		L, R	
Toxolasma lividum				
Tritogonia verrucosa	L	L, FD, R	L, FD, R	L, R
Truncilla donaciformis	FD, R			
Truncilla truncata	L	R	FD, R	
Utterbackia imbecillis				
Villosa iris	FD			
Villosa taeniata		FD, R	FD, R	R

Species	Above Arno Road	Cox Road	HWY 41
Actinonaias ligamentina			
Actionaias pectorosa			
Alasmidonta viridis		L, FD	
Amblema plicata	R	R	R
Cyclonaias tuberculata			
Cyclonaias pustulosa			
Eurynia dilatata			
Fusconaia flava	R	FD, R	R
Lampsilis cardium	R		
Lampsilis fasciola	R	L, FD	
Lasmigona complanata			
Lasmigona costata	R	L	
Leptodea fragilis			
Megalonaias nervosa			
Obliquaria reflexa			
Obovaria subrotunda			
Potamilus alatus			
Ptychobranchus fasciolaris	R		
Pyganodon grandis		R	L, FD, R
Quadrula quadrula			
Toxolasma lividum		R	R
Tritogonia verrucosa			
Truncilla donaciformis			
Truncilla truncata			
Utterbackia imbecillis			L
Villosa iris			
Villosa taeniata	L, R	L, R	

Appendix B: Reach-scale factors recorded at Harpeth River tributary sites.

						Substrate %			
Site	**Erosion**	**Riparian**	**Organic**	**Silt**	**Sand**	**Gravel**	**Cobble**	**Boulder**	**Bedrock**
HT1	Moderate	Excellent				60	30		10
HT2	Low	Excellent				60	30		10
HT3	Severe	Moderate				80	10		10
HT4	Severe	Moderate				80	10		10
HT5	Moderate	Moderate				60			40
HT6	Low	Moderate	10			80			10
HT7	Low	Moderate				70	20		10
HT8	Low	Excellent				25	25		50
HT9	Low	Moderate				30	10		60
HT10	Moderate	Poor				30			70
HT11	Low	Moderate			10	40	40		10
HT12	Low	Moderate			10	40	40		10
HT13	Moderate	Excellent			20	80			
HT14	Moderate	Moderate				90			10
HT15	Moderate	Moderate			20	80			
HT16	Low	Excellent				10			90
HT17	Low	Excellent				60	30		10
HT18	Low	Excellent			10	70			20
HT19	Severe	Poor				10			90
HT20	Moderate	Moderate				10			90
HT21	Low	Moderate			10	90			
HT22	Moderate	Moderate				50			50
HT23	Severe	Poor				10			90
HT24	Moderate	Poor	10			50	40		
HT25	Moderate	Moderate			10	50	40		
HT26	Low	Excellent				60	40		
HT27	Low	Excellent			10	80	10		
HT28	Moderate	Poor				20			80
HT29	Severe	Moderate				30			70
HT30	Severe	Poor		50		50			
HT31	Severe	Poor				80			20
HT32	Moderate	Moderate				80	20		
HT33	Moderate	Moderate				100			
HT34	Moderate	Moderate				70			30
HT35	Moderate	Poor							100
HT36	Severe	Moderate				10	20	10	60
HT37	Moderate	Moderate				20	20		60
HT38	Severe	Moderate				20	10		70
HT39	Severe	Poor				10			90
HT40	Moderate	Moderate				10			90
HT41	Moderate	Excellent			10	40	30	10	10
HT42	Moderate	Excellent				10	10		80

Appendix C: Aquatic life recorded at Harpeth River tributary sites.

Site	**Fish**	***Corbicula***	**Gastropods**
HT1	Present	Relic	Relic
HT2	Abundant	Live	Live
HT3	Abundant	Live	Live
HT4	Abundant	Live	Live
HT5	Absent	Absent	Relic
HT6	Rare	Absent	Relic
HT7	Present	Absent	Relic
HT8	Present	Live	Live
HT9	Abundant	Live	Live
HT10	Present	Relic	Absent
HT11	Present	Relic	Relic
HT12	Present	Relic	Live
HT13	Present	Live	Live
HT14	Absent	Relic	Absent
HT15	Present	Relic	Live
HT16	Abundant	Absent	Live
HT17	Abundant	Absent	Live
HT18	Rare	Relic	Relic
HT19	Rare	Relic	Live
HT20	Rare	Absent	Live
HT21	Rare	Absent	Relic
HT22	Rare	Relic	Absent
HT23	Absent	Relic	Absent
HT24	Abundant	Live	Live
HT25	Present	Relic	Relic
HT26	Present	Live	Live
HT27	Rare	Relic	Live
HT28	Present	Live	Relic
HT29	Present	Relic	Relic
HT30	Rare	Live	Relic
HT31	Rare	Relic	Relic
HT32	Rare	Relic	Relic
HT33	Present	Relic	Relic
HT34	Rare	Relic	Absent
HT35	Absent	Absent	Absent
HT36	Rare	Relic	Absent
HT37	Present	Live	Live
HT38	Present	Absent	Live
HT39	Rare	Absent	Relic
HT40	Present	Absent	Absent
HT41	Abundant	Live	Live
HT42	Present	Live	Relic

Appendix D: Species richness and catch per unit effort reported at 20 Harpeth River sites. Live and fresh dead individuals were combined. "R" denotes relic individuals. Species richness includes live, fresh dead, and relic mussels. Catch per unit effort includes live and fresh dead mussels.

	Site Number													
Species	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**	**H9**	**H10**	**H11**	**H12**	**H13**	**H14**
Amblema plicata									R	R	R	R	2	
Cyclonaias tuberculata	R	R	1	R	1	R	R	R	2	R	R	R	6	2
Eurynia dilatata				R	R	R	R	1	5	2	7	R	2	1
Lampsilis cardium						R				R		R		R
Lampsilis fasciola	R		R	1	R	R	1	1	1	2	5	1	5	8
Lampsilis teres		R								R	1			R
Lasmigona costata					R	1	R	R	R	R	R	R		
Leptodea fragilis		1		R	2	R		R	R	R		1	2	R
Megalonaias nervosa										1			2	R
Obliquaria reflexa		R				R	1		1	R	1		3	R
Potamilus alatus	1	R	1	1	1	R	R	R	5	2	1		4	8
Ptychobranchus fasciolaris									R					
Quadrula quadrula		R	R			1				R				
Simpsonaias ambigua					1						1			
Tritogonia verrucosa		1	1		R			1		R		R	3	1
Truncilla donaciformis										1				
Truncilla truncata	R	R		R	R	R	R	R	R		4		4	R
Villosa iris		R	1	R			R							R
Villosa taeniata	R		2	R	4	4	R	3	4	R	3	2	6	R
Villosa vanuxemensis										R			1	
Species Richness	5	9	7	8	10	11	9	9	11	15	11	9	12	13
CPUE (mussels/hour)	1.0	2.0	5.0	2.0	9.0	4.5	2.0	6.0	10.8	7.0	22.0	3.2	32.0	17.6

	Site Number					
Species	**H15**	**H16**	**H17**	**H18**	**H19**	**H20**
Amblema plicata	R	R	R	R	R	
Cyclonaias tuberculata	1	1	R	1		R
Eurynia dilatata	1	5		R	R	R
Lampsilis cardium		1	R	R		R
Lampsilis fasciola	R	1	R	1	1	R
Lampsilis teres						R
Lasmigona costata	R	R	R	R	R	R
Leptodea fragilis	R	1		R	R	1
Megalonaias nervosa	R	R	R			R
Obliquaria reflexa	1			R	1	R
Potamilus alatus	2	R	1	3	2	8
Ptychobranchus fasciolaris						
Quadrula quadrula				R		R
Simpsonaias ambigua						
Tritogonia verrucosa	2					R
Truncilla donaciformis						
Truncilla truncata	2	R	R	R	R	R
Villosa iris		R		R		R
Villosa taeniata	3	3	R	2	R	3
Villosa vanuxemensis						
Species Richness	11	12	8	13	9	15
CPUE (mussels/hour)	7.2	9.6	0.8	5.6	3.2	9.6

Appendix E: Reach-scale factors recorded at Harpeth River sites.

			Substrate %						
Site	**Erosion**	**Riparian**	**Organic**	**Silt**	**Sand**	**Gravel**	**Cobble**	**Boulder**	**Bedrock**
H1	Moderate	Excellent				70	20		10
H2	Moderate	Moderate				80	20		
H3	Severe	Moderate				90	10		
H4	Severe	Moderate				80	10		10
H5	Moderate	Moderate			10	60	20		10
H6	Moderate	Moderate	10			80	10		
H7	Moderate	Moderate				40	10		50
H8	Moderate	Excellent		20		40			40
H9	Moderate	Moderate				80	20		
H10	Severe	Poor		20		80			
H11	Severe	Moderate				70			30
H12	Moderate	Moderate				90	10		
H13	Moderate	Excellent			10	40	30	10	10
H14	Moderate	Moderate				50	40		10
H15	Moderate	Moderate			10	50	40		
H16	Severe	Moderate				50	40		10
H17	Moderate	Excellent			10	80			10
H18	Severe	Moderate			10	80			10
H19	Moderate	Moderate		20		80			
H20	Severe	Poor		20	10	70			

Appendix F: Aquatic life recorded at main channel Harpeth river sites.

Site	**Fish**	***Corbicula***	**Gastropods**
H1	Present	Live	Live
H2	Abundant	Live	Live
H3	Abundant	Live	Live
H4	Abundant	Live	Live
H5	Absent	Live	Live
H6	Present	Live	Live
H7	Present	Live	Live
H8	Present	Live	Live
H9	Abundant	Live	Live
H10	Present	Live	Live
H11	Present	Live	Live
H12	Present	Live	Live
H13	Abundant	Live	Live
H14	Absent	Live	Live
H15	Present	Live	Live
H16	Abundant	Live	Live
H17	Abundant	Live	Live
H18	Present	Live	Live
H19	Present	Live	Live
H20	Present	Live	Live

Appendix G. Species collected from selected Duck River tributaries in 1990 and 2001 (Ahlstedt and Saylor 1990, Ahlstedt et al. 2017). "L" denotes live, "FD" denotes fresh dead, and "R" denotes relic individuals.

	Tributary				
Species	**Carters Creek**	**Thompson Creek**	**Big Bigby Creek**	**Fall Creek**	**Big Rock Creek**
Actinonaias ligamentina	R				
Amblema plicata				R	R
Cyclonaias tuberculata					R
Elliptio dilatata					L
Lampsilis cardium					R
Lampsilis fasciola					L
Lasmigona costata			L		
Lasmigona c.f. holstonia					
Medionidus conradicus					L
Obovaria subrotunda					R
Pleuronaia barnesiana		R		R	L
Pleuronaia dolabelloides					L
Potamilus alatus					
Ptychobranchus fasciolaris				R	R
Pyganodon grandis				R	
Strophitus undulatus					
Theliderma cylindrica					R
Toxolasma lividum					L
Villosa iris				R	
Villosa taeniata					L
Villosa vanuxemensis	L	R		R	L

Species	East Rock Creek	North Fork Creek	Tributary Garrison Fork	Wartrace Creek	Hurricane Creek
Actinonaias ligamentina					
Amblema plicata		R			
Cyclonaias tuberculata					
Elliptio dilatata					
Lampsilis cardium		R	R		
Lampsilis fasciola					
Lasmigona costata	R	R			
Lasmigona c.f. holstonia		L			
Medionidus conradicus	R		R		
Obovaria subrotunda					
Pleuronaia barnesiana	R	R	R		
Pleuronaia dolabelloides		R			
Potamilus alatus					
Ptychobranchus fasciolaris					
Pyganodon grandis	R	R		R	R
Strophitus undulatus	R				
Theliderma cylindrica					
Toxolasma lividum	R	R			
Villosa iris	L, FD	L			
Villosa taeniata		L			
Villosa vanuxemensis	FD	L		R	

	Tributary			
Species	**Flat Creek**	**Sinking Creek**	**Fountain Creek**	**Silver Creek**
Actinonaias ligamentina				R
Amblema plicata				
Cyclonaias tuberculata				
Elliptio dilatata				
Lampsilis cardium			FD	R
Lampsilis fasciola				
Lasmigona costata				
Lasmigona c.f. holstonia				
Medionidus conradicus	L			R
Obovaria subrotunda				
Pleuronaia barnesiana	R			
Pleuronaia dolabelloides				
Potamilus alatus			R	
Ptychobranchus fasciolaris				
Pyganodon grandis			R	
Strophitus undulatus				
Theliderma cylindrica				
Toxolasma lividum				
Villosa iris	R	R		
Villosa taeniata	L			
Villosa vanuxemensis	R	L		

Appendix H: Species richness and catch per unit effort reported at 83 Duck River tributary sites. Live and fresh dead individuals were combined. "R" denotes relic individuals. Species richness includes live, fresh dead, and relic mussels. Catch per unit effort includes live and fresh dead mussels.

	Site Number													
Species	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**	**D11**	**D12**	**D13**	**D14**
Alasmidonta viridis														
Amblema plicata													R	
Cyclonaias tuberculata														
Epioblasma triquetra														
Lampsilis cardium														
Lampsilis fasciola						1							R	1
Lasmigona costata														
Medionidus conradicus														
Pleuronaia barnesiana				R									R	
Pleuronaia dolabelloides														
Pyganodon grandis														
Theliderma cylindrica														
Toxolasma cylindrellus														
Toxolasma lividum														
Tritogonia verrucosa														
Utterbackia imbecillis														
Villosa iris														1
Villosa taeniata														
Villosa vanuxemensis														
Species Richness	0	0	0	1	0	1	0	0	0	0	0	0	3	2
CPUE (mussels/hour)	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0

							Site Number							
Species	**D15**	**D16**	**D17**	**D18**	**D19**	**D20**	**D21**	**D22**	**D23**	**D24**	**D25**	**D26**	**D27**	**D28**
Alasmidonta viridis														
Amblema plicata					R		R							
Cyclonaias tuberculata							R							
Epioblasma triquetra							R							
Lampsilis cardium				1										
Lampsilis fasciola						1								
Lasmigona costata					R									
Medionidus conradicus			3	16	29	10	R							
Pleuronaia barnesiana				1	R		R							
Pleuronaia dolabelloides					3		R							
Pyganodon grandis														
Theliderma cylindrica							R							
Toxolasma cylindrellus														
Toxolasma lividum				R	2		R							
Tritogonia verrucosa					R		R							
Utterbackia imbecillis														
Villosa iris				21	12	16	R							
Villosa taeniata			5	21	40	58	R				R			
Villosa vanuxemensis			3	103	29	12	R							
Species Richness	0	0	3	7	10	5	10	0	0	0	1	0	0	0
CPUE (mussels/hour)	0.0	0.0	5.5	54.3	58.0	32.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

	Site Number													
Species	**D29**	**D30**	**D31**	**D32**	**D33**	**D34**	**D35**	**D36**	**D37**	**D38**	**D39**	**D40**	**D41**	**D42**
Alasmidonta viridis														
Amblema plicata														
Cyclonaias tuberculata														
Epioblasma triquetra														
Lampsilis cardium														
Lampsilis fasciola														
Lasmigona costata														
Medionidus conradicus				3		4								
Pleuronaia barnesiana				1		1								
Pleuronaia dolabelloides														
Pyganodon grandis		7												
Theliderma cylindrica														
Toxolasma cylindrellus														
Toxolasma lividum														
Tritogonia verrucosa														
Utterbackia imbecillis														
Villosa iris														
Villosa taeniata		1		6	R	5								
Villosa vanuxemensis				11	1	12								
Species Richness	0	2	0	4	2	4	0	0	0	0	0	0	0	0
CPUE (mussels/hour)	0.0	9.3	0.0	16.8	0.5	22.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

	Site Number													
Species	**D43**	**D44**	**D45**	**D46**	**D47**	**D48**	**D49**	**D50**	**D51**	**D52**	**D53**	**D54**	**D55**	**D56**
Alasmidonta viridis												R	R	
Amblema plicata											R			
Cyclonaias tuberculata														
Epioblasma triquetra														
Lampsilis cardium														
Lampsilis fasciola														
Lasmigona costata														
Medionidus conradicus														
Pleuronaia barnesiana	R			R							R		R	R
Pleuronaia dolabelloides														
Pyganodon grandis											R			
Theliderma cylindrica														
Toxolasma cylindrellus														
Toxolasma lividum							R					R	R	R
Tritogonia verrucosa														
Utterbackia imbecillis														R
Villosa iris				R								R		
Villosa taeniata	2						R	R						
Villosa vanuxemensis				6			R				R	R	R	R
Species Richness	2	0	0	3	0	0	3	1	0	0	4	4	4	4
CPUE (mussels/hour)	3.9	0.0	0.0	8.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

	Site Number													
Species	**D57**	**D58**	**D59**	**D60**	**D61**	**D62**	**D63**	**D64**	**D65**	**D66**	**D67**	**D68**	**D69**	**D70**
Alasmidonta viridis														
Amblema plicata												R		
Cyclonaias tuberculata														
Epioblasma triquetra														
Lampsilis cardium														
Lampsilis fasciola														
Lasmigona costata														
Medionidus conradicus														
Pleuronaia barnesiana													R	
Pleuronaia dolabelloides														
Pyganodon grandis														
Theliderma cylindrica														
Toxolasma cylindrellus														
Toxolasma lividum														
Tritogonia verrucosa														
Utterbackia imbecillis														
Villosa iris														
Villosa taeniata														
Villosa vanuxemensis												R	7	
Species Richness	0	0	0	0	0	0	0	0	0	0	0	2	2	0
CPUE (mussels/hour)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.9	0.0

	Site Number												
Species	**D71**	**D72**	**D73**	**D74**	**D75**	**D76**	**D77**	**D78**	**D79**	**D80**	**D81**	**D82**	**D83**
Alasmidonta viridis													2
Amblema plicata													
Cyclonaias tuberculata													
Epioblasma triquetra													
Lampsilis cardium													
Lampsilis fasciola													
Lasmigona costata													
Medionidus conradicus													
Pleuronaia barnesiana		R											
Pleuronaia dolabelloides													
Pyganodon grandis													
Theliderma cylindrica													
Toxolasma cylindrellus													1
Toxolasma lividum													
Tritogonia verrucosa													
Utterbackia imbecillis													
Villosa iris	R				R							R	
Villosa taeniata													4
Villosa vanuxemensis		3	6		2	3						R	
Species Richness	1	2	1	0	2	1	0	0	0	0	0	2	3
CPUE (mussels/hour)	0.0	6.0	2.0	0.0	4.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0	7.0

Appendix I. Reach-scale factors recorded at Duck River tributary sites.

						Substrate %			
Site	**Erosion**	**Riparian**	**Organic**	**Silt**	**Sand**	**Gravel**	**Cobble**	**Boulder**	**Bedrock**
D1	Low	Excellent				50	50		
D2	Low	Excellent				70	20		10
D3	Low	Excellent				40	10	50	
D4	Low	Moderate				90	10		
D5	Low	Moderate			10	20	10		60
D6	Low	Excellent				50	50		
D7	Low	Moderate				50			50
D8	Low	Excellent		10		80			10
D9	Severe	Moderate				40	20		40
D10	Severe	Moderate				20			80
D11	Moderate	Moderate				10			90
D12	Moderate	Poor				10			90
D13	Low	Excellent				40	40		20
D14	Low	Excellent				70	10		20
D15	Low	Moderate					10		90
D16	Low	Excellent				20			80
D17	Low	Excellent				20	20		60
D18	Low	Excellent			10	40	40		10
D19	Low	Excellent			10	40	40		10
D20	Low	Excellent			10	40	30		20
D21	Moderate	Excellent			50	50			
D22	Low	Moderate		20		20			60
D23	Moderate	Moderate		10					90
D24	Moderate	Moderate				20	10		70
D25	Moderate	Moderate		20		10			70
D26	Moderate	Excellent							100
D27	Moderate	Excellent				Dry			
D28	Moderate	Moderate				Dry			
D29	Low	Poor		10					90
D30	Moderate	Excellent				10	10		80
D31	Moderate	Moderate				Dry			
D32	Low	Excellent				30	50		20
D33	Low	Excellent				40	20		40
D34	Low	Excellent				50	50		
D35	Severe	Moderate			20	80			
D36	Low	Excellent				50	50		
D37	Low	Excellent				20	70		10
D38	Low	Excellent				5			95
D39	Low	Excellent							100
D40	Severe	Moderate				50	40		10
D41	Moderate	Excellent				20			80
D42	Moderate	Moderate				20			80

.

						Substrate %			
Site	**Erosion**	**Riparian**	**Organic**	**Silt**	**Sand**	**Gravel**	**Cobble**	**Boulder**	**Bedrock**
D43	Moderate	Excellent				90			10
D44	Moderate	Moderate							100
D45	Moderate	Excellent				70			30
D46	Moderate	Excellent				40			60
D47	Moderate	Poor			50	50			
D48	Moderate	Poor		20					80
D49	Moderate	Excellent		20		40	40		
D50	Low	Excellent				10	10		80
D51	Low	Excellent				5			95
D52	Moderate	Moderate				50	40		10
D53	Low	Moderate				20			80
D54	Moderate	Moderate		20		80			
D55	Moderate	Excellent				40			60
D56	Moderate	Poor		20		80			
D57	Moderate	Moderate			10				90
D58	Moderate	Poor				Dry			
D59	Moderate	Moderate				15	15		70
D60	Moderate	Moderate							100
D61	Low	Moderate			10	20			70
D62	Low	Excellent				20			80
D63	Moderate	Moderate				20			80
D64	Moderate	Moderate							100
D65	Moderate	Moderate							100
D66	Moderate	Moderate				10			90
D67	Moderate	Excellent				10			90
D68	Low	Excellent				70	30		10
D69	Low	Excellent			10	70			20
D70	Moderate	Excellent				30			70
D71	Low	Excellent				40	20		40
D72	Severe	Excellent		10		70	20		
D73	Severe	Excellent		10		70	20		
D74	Severe	Excellent				50	50		
D75	Low	Excellent				40			60
D76	Low	Excellent			10	40	40		10
D77	Low	Excellent				30			70
D78	Severe	Moderate				40	10		50
D79	Moderate	Moderate				30	30		40
D80	Moderate	Excellent				20			80
D81	Low	Moderate				10	10		80
D82	Moderate	Moderate				50	40		10
D83	Low	Excellent		10		30	20		40

Appendix J: Aquatic life recorded at Duck River tributary sites.

Site	Fish	*Corbicula*	Gastropods
D1	Abundant	Present	Live
D2	Present	Present	Live
D3	Present	Relic	Live
D4	Present	Present	Absent
D5	Present	Present	Absent
D6	Abundant	Present	Live
D7	Present	Relic	Relic
D8	Rare	Relic	Live
D9	Rare	Relic	Relic
D10	Rare	Relic	Absent
D11	Rare	Relic	Relic
D12	Rare	Relic	Live
D13	Rare	Relic	Live
D14	Present	Present	Live
D15	Present	Absent	Absent
D16	Absent	Relic	Relic
D17	Abundant	Live	Live
D18	Abundant	Live	Live
D19	Abundant	Live	Live
D20	Abundant	Live	Live
D21	Present	Live	Relic
D22	Abundant	Live	Relic
D23	Rare	Relic	Absent
D24	Rare	Relic	Live
D25	Rare	Relic	Live
D26	Absent	Relic	Absent
D27		Dry	
D28		Dry	
D29	Absent	Relic	Absent
D30	Rare	Live	Absent
D31		Dry	
D32	Abundant	Live	Live
D33	Abundant	Relic	Relic
D34	Abundant	Live	Live
D35	Rare	Live	Live
D36	Abundant	Relic	Live
D37	Rare	Live	Live
D38	Rare	Relic	Live
D39	Absent	Relic	Live
D40	Rare	Relic	Absent
D41	Rare	Relic	Relic
D42	Rare	Relic	Relic

Site	Fish	*Corbicula*	Gastropods
D43	Abundant	Relic	Live
D44	Rare	Rare	Absent
D45	Rare	Relic	Relic
D46	Present	Live	Live
D47	Absent	Absent	Relic
D48	Rare	Absent	Absent
D49	Present	Live	Live
D50	Abundant	Relic	Relic
D51	Abundant	Relic	Live
D52	Present	Relic	Live
D53	Present	Live	Relic
D54	Absent	Relic	Relic
D55	Abundant	Relic	Live
D56	Present	Relic	Live
D57	Rare	Relic	Live
D58		Dry	
D59	Rare	Absent	Absent
D60	Rare	Absent	Absent
D61	Present	Live	Live
D62	Abundant	Relic	Live
D63	Rare	Absent	Absent
D64	Present	Absent	Rare
D65	Absent	Relic	Relic
D66	Absent	Absent	Absent
D67	Present	Relic	Live
D68	Present	Relic	Relic
D69	Abundant	Live	Live
D70	Present	Relic	Relic
D71	Present	Relic	Relic
D72	Abundant	Live	Relic
D73	Abundant	Live	Relic
D74	Rare	Relic	Relic
D75	Abundant	Live	Relic
D76	Abundant	Live	Live
D77	Present	Relic	Live
D78	Present	Relic	Live
D79	Present	Relic	Live
D80	Present	Relic	Live
D81	Present	Relic	Live
D82	Rare	Relic	Live
D83	Abundant	Live	Live

Appendix K: Letters assigned to Duck River tributaries for Appendix L.

Letter	**Stream Name**
A	Cathey's Creek
B	Big Bigby Creek
C	Greenlick Creek
D	Little Bigby Creek
E	Fountain Creek
F	Silver Creek
G	Big Rock Creek
H	East Rock Creek
I	Sinking Creek
J	Sugar Creek
K	Blue Stocking Branch
L	Flat Creek - South
M	Thompson Creek
N	Crumpton Creek
O	Wiley Creek
P	Perry Creek
Q	Garrison Fork
R	Wartrace Creek
S	Little Hurricane Creek
T	Fall Creek
U	Hurricane Creek
V	North Fork Creek
W	Alexander Creek
X	Weakly Creek
Y	Clem Creek
Z	Wilson Creek
AA	Spring Creek
BB	Caney Creek
CC	Flat Creek - North
DD	Rutherford Creek
EE	Aenon Creek
FF	McCutcheon Creek
GG	Carter's Creek
HH	Knob Creek
II	Snow Creek
JJ	Leipers Creek
KK	Lick Creek
Total	**37**

Appendix L: Comparison of historical and current (this study) occurrences of species in Duck River tributaries. Stream names correspond to the letters assigned in Appendix K. "L" denotes live, "FD" denotes fresh dead, and "R" denotes relic shells.

	A		**B**		**C**		**D**		**E**		**F**		**G**	
Historical(H) | Current (C) Species	H	C	H	C	H	C	H	C	H	C	H	C	H	C
Actinonaias ligamentina											X			
Actinonaias pectorosa									X					
Alasmidonta viridis														
Amblema plicata										R			X	R
Cyclonaias tuberculata													X	R
Epioblasma triquetra														R
Eurynia dilatata													X	
Lampsilis cardium									X		X		X	FD
Lampsilis facsiola				FD					X	FD			X	L
Lasmigona costata			X											R
Lasmigona c.f. holstonia														
Medionidus conradicus											X		X	L
Obovaria subrotunda													X	
Pegias fabula									X					
Pleurobema oviforme														
Pleuronaia barnesiana			X	R						R			X	L
Pleuronaia dolabelloides													X	L
Potamilus alatus									X					
Ptychobranchus fasciolaris													X	
Pyganodon grandis									X				X	
Strophitus undulatus														
Theliderma cylindrica													X	R
Toxolasma cylindrellus													X	
Toxolasma lividum													X	FD
Tritogonia verrucosa														R
Utterbackia imbecillis														
Villosa iris									X	FD			X	L
Villosa taeniata													X	L
Villosa vanuxemensis													X	L

	H		I		J		K		L		M		N	
Historical(H) | Current(C) Species	H	C	H	C	H	C	H	C	H	C	H	C	H	C
Actinonaias ligamentina														
Actinonaias pectorosa														
Alasmidonta viridis	X								X					
Amblema plicata	X													
Cyclonaias tuberculata														
Epioblasma triquetra									X					
Eurynia dilatata														
Lampsilis cardium									X					
Lampsilis fasciola														
Lasmigona costata									X					
Lasmigona c.f. holstonia														
Medionidus conradicus	X								X	L	X			
Obovaria subrotunda														
Pegias fabula														
Pleurobema oviforme									X					
Pleuronaia barnesiana	X								X	L	X			
Pleuronaia dolabelloides														
Potamilus alatus														
Ptychobranchus fasciolaris														
Pyganodon grandis			X			L								
Strophitus undulatus	X													
Theliderma cylindrica														
Toxolasma cylindrellus														
Toxolasma lividum	X				X									
Tritogonia verrucosa														
Utterbackia imbecillis														
Villosa iris	X		X						X					
Villosa taeniata	X	R				L			X	L				
Villosa vanuxemensis	X		X						X	L				

	O		P		Q		R		S		T		U	
Historical(H) | Current(C) Species	H	C	H	C	H	C	H	C	H	C	H	C	H	C
Actinonaias ligamentina														
Actinonaias pectorosa														
Alasmidonta viridis														
Amblema plicata											X			
Cyclonaias tuberculata														
Epioblasma triquetra														
Eurynia dilatata														
Lampsilis cardium					X									
Lampsilis fasciola					X									
Lasmigona costata					X									
Lasmigona c.f. holstonia														
Medionidus conradicus					X		X							
Obovaria subrotunda														
Pleurobema oviforme					X									
Pleuronaia barnesiana					X	R		R			X			
Pleuronaia dolabelloides											X			
Potamilus alatus														
Ptychobranchus fasciolaris											X			
Pyganodon grandis							X				X		X	
Strophitus undulatus					X									
Theliderma cylindrica														
Toxolasma cylindrellus														
Toxolasma lividum												R		
Tritogonia verrucosa														
Utterbackia imbecillis														
Villosa iris								R			X			
Villosa taeniata						FD						R		
Villosa vanuxemensis					X			FD			X	R	X	

	V		W		X		Y		Z		AA		BB	
Historical(H) | Current(C)														
Species	H	C	H	C	H	C	H	C	H	C	H	C	H	C
Actinonaias ligamentina														
Actinonaias pectorosa														
Alasmidonta viridis		R												
Amblema plicata	X													
Cyclonaias tuberculata														
Epioblasma triquetra														
Eurynia dilatata														
Lampsilis cardium	X													
Lampsilis fasciola														
Lasmigona costata	X													
Lasmigona c.f. holstonia	X													
Medionidus conradicus														
Obovaria subrotunda														
Pegias fabula														
Pleurobema oviforme														
Pleuronaia barnesiana	X	R		R										
Pleuronaia dolabelloides	X													
Potamilus alatus														
Ptychobranchus fasciolaris														
Pyganodon grandis	X		X											
Strophitus undulatus														
Theliderma cylindrica														
Toxolasma cylindrellus														
Toxolasma lividum	X	R		R					X					
Tritogonia verrucosa														
Utterbackia imbecillis				R									X	
Villosa iris	X	R	X											
Villosa taeniata	X													
Villosa vanuxemensis	X	R		R										

	CC		DD		EE		FF		GG		HH		II	
Historical(H) | Current(C) Species	H	C	H	C	H	C	H	C	H	C	H	C	H	C
Actinonaias ligamentina									X					
Actinonaias pectorosa														
Alasmidonta viridis														
Amblema plicata				R										
Cyclonaias tuberculata														
Epioblasma triquetra														
Eurynia dilatata														
Lampsilis cardium			X											
Lampsilis fasciola														
Lasmigona costata														
Lasmigona c.f. holstonia														
Medionidus conradicus														
Obovaria subrotunda														
Pegias fabula														
Pleurobema oviforme														
Pleuronaia barnesiana			X	R		R								
Pleuronaia dolabelloides														
Potamilus alatus														
Ptychobranchus fasciolaris														
Pyganodon grandis			X						X					
Strophitus undulatus														
Theliderma cylindrica														
Toxolasma cylindrellus														
Toxolasma lividum														
Tritogonia verrucosa														
Utterbackia imbecillis														
Villosa iris				R						R				
Villosa taeniata														
Villosa vanuxemensis			X	L		L			X	FD				

	JJ		KK	
Historical (H) | Current(C) Species	H	C	H	C
Actinonaias ligamentina				
Actinonaias pectorosa				
Alasmidonta viridis				FD
Amblema plicata				
Cyclonaias tuberculata				
Epioblasma triquetra				
Eurynia dilatata				
Lampsilis cardium				
Lampsilis fasciola				
Lasmigona costata				
Lasmigona c.f. holstonia				
Medionidus conradicus				
Obovaria subrotunda				
Pegias fabula				
Pleurobema oviforme				
Pleuronaia barnesiana				
Pleuronaia dolabelloides				
Potamilus alatus				
Ptychobranchus fasciolaris				
Pyganodon grandis				
Strophitus undulatus				
Theliderma cylindrica				
Toxolasma cylindrellus			X	FD
Toxolasma lividum				
Tritogonia verrucosa				
Utterbackia imbecillis				
Villosa iris		R		
Villosa taeniata			X	FD
Villosa vanuxemensis		R		FD

Appendix M: Species collected from 89 quadrats from Big Rock Creek, Berea Church Site.

Quadrat	**Berea Church** Species observed			
	Medionidus conradicus	*Pleuronaia barnesiana*	*Villosa taeniata*	*Villosa vanuxemensis*
1				
2				
3				
4				
5				
6				
6				
7				
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29				
30				
31				
32				
33				
34				
35			1	1
36				2
37				
38				
39				1
40				
41				

	Berea Church			
Quadrat	Species observed			
	Medionidus conradicus	*Pleuronaia barnesiana*	*Villosa taeniata*	*Villosa vanuxemensis*
42				
43				
44				
45			1	1
46			1	
47				
48				
49				
50				
51				
52				
53			1	1
54				
55				
56				
57				
58				
59				
60				2
61				
62				
63				
64				
65				
66				1
67				
68				
69		1	1	
70				1
71			2	
72				
73		1		
73				
74				2
75				
76				
77			1	2
78	1		1	
79				

Quadrat	Berea Church Species observed *Medionidus conradicus*	*Pleuronaia barnesiana*	*Villosa taeniata*	*Villosa vanuxemensis*
80				
81				
82				1
83				
84				
85				1
86				1
87				
88				
89				

Appendix N: Species collected from 74 quadrats from Big Rock Creek, Mill Dam Site.

	Mill Dam			
Quadrat	Species Observed			
	Medionidus conradicus	*Villosa iris*	*Villosa taeniata*	*Villosa vanuxemsensis*
1				2
2	1			1
3				
4				
5				
6				3
7				2
8			1	2
9				
10				
11				
12	1			
13				1
14				2
15		1		
16				1
17			1	
18				
19				
20				
21				2
22				3
23				
24				3
25	1			2
26				
27				
28				2
29				
30			2	2
31				
32				5
33				2
34				2
35		2		4
36				1
37				1
38			1	
39			1	4
40				
41				
42				3

	Mill Dam			
Quadrat			Species Observed	
	Medionidus conradicus	*Villosa iris*	*Villosa taeniata*	*Villosa vanuxemsensis*
43				2
44	1			1
45				3
46				
47				1
48			1	4
49				
50				
51				
52				
53			1	
54				1
55				2
56				
57				
58			1	3
59				
60				2
61			1	
62				
63				
64				
65				
66			1	
67				
68			1	
69				2
70				
71				
72				
73	1			1
74		2		1

Appendix O: Substrate data from 74 quadrats sampled on Big Rock Creek, Mill Dam site.

			Substrate composition				
Quadrat	**Organic**	**Silt**	**Sand**	**Gravel**	**Cobble**	**Boulder**	**Bedrock**
1	0	0	0.5	0.5	0	0	0
2	0	0	0	0.5	0	0	0.5
3	0	0	0	0.5	0	0	0.5
4	0	0	0.25	0	0	0	0.75
5	0	0	0.5	0.5	0	0	0
6	0	0	0.3	0.7	0	0	0
7	0	0	0.4	0.6	0	0	0
8	0	0	0.1	0.9	0	0	0
9	0	0	0.1	0.8	0.1	0	0
10	0	0	0	0	0	0	1
11	0	0	0	0.1	0.2	0	0.7
12	0	0	0.5	0.5	0	0	0
13	0	0	0.75	0.25	0	0	0
14	0	0	0.4	0.6	0	0	0
15	0	0	0.4	0.6	0	0	0
16	0	0	0.4	0.5	0	0.1	0
17	0	0	0	0	0.5	0	0.5
18	0	0	0	0	0.8	0	0.2
19	0.5	0	0.5	0	0	0	0
20	0.1	0	0.6	0.3	0	0	0
21	0	0	0.5	0.5	0	0	0
22	0.1	0	0.4	0.5	0	0	0
23	0.1	0.1	0	0.6	0	0.2	0
24	0	0	0	0.6	0.4	0	0
25	0	0	0	0.6	0.4	0	0
26	0	0	0.1	0.7	0.2	0	0
27	0	0	0	0.7	0.3	0	0
28	0	0	0.25	0.4	0	0	0.35
29	0	0	0	0.6	0.4	0	0
30	0	0	0	0.6	0.4	0	0
31	0.15	0	0.35	0.5	0	0	0
32	0	0	0	0.6	0.4	0	0
33	0	0	0	0.6	0.4	0	0
34	0	0	0.1	0.6	0	0.3	0
35	0	0	0	0.1	0.1	0.8	0
36	0	0	0	0.1	0.2	0.7	0

			Substrate composition				
Quadrat	**Organic**	**Silt**	**Sand**	**Gravel**	**Cobble**	**Boulder**	**Bedrock**
40	0	0	0.2	0.8	0	0	0
41	0	0	0.1	0.9	0	0	0
42	0	0	0.2	0.3	0	0.5	0
43	0	0	0	0.3	0	0.7	0
44	0	0.1	0.25	0.35	0.1	0.2	0
45	0	0	0.7	0.3	0	0	0
46	0	0	0.5	0.5	0	0	0
47	0	0	0.2	0.8	0	0	0
48	0	0	0.4	0.5	0	0.1	0
49	0	0	0	0	0	0	1
50	0	0	0	0	0	0	1
51	0	0.2	0	0.3	0	0.5	0
52	0	0	0.7	0.3	0	0	0
53	0.4	0	0.6	0	0	0	0
54	0	0	0.6	0.4	0	0	0
55	0	0	0.2	0.8	0	0	0
56	0	0	0	0	0	0	1
57	0	0	0	0	0	0	1
58	0	0	0	0.7	0	0	0.3
59	0	0	0.5	0.5	0	0	0
60	0.1	0	0.3	0.6	0	0	0
61	0	0	0.4	0.6	0	0	0
62	0	0	0	0.3	0	0	0.7
63	0	0	0	0	0	0	1
64	0	0	0	0.1	0	0	0.9
65	0	0	0	0	0	0	1
66	0	0	0	0.9	0.1	0	0
67	0	0.4	0.4	0.1	0.1	0	0
68	0	0	0	0.6	0.4	0	0
69	0	0	0.1	0.9	0	0	0
70	0	0	0	0	0	0	1
71	0	0	0	0	0	0	1
72	0	0.2	0	0	0	0	0.8
73	0	0	0.1	0.5	0.4	0	0
74	0	0	0	0	0.1	0.9	0

VITA

Kristin Irwin grew up in Spring Hill, Tennessee and earned a Bachelor of Science degree in Environmental Studies from the University of Tennessee in 2016. She worked as an undergraduate research assistant for Dr. Michael McKinney studying invasive mollusks, and has worked as the Collections Manager of Malacology at the McClung Museum of Natural History and Culture since 2015. Kristin enrolled in the department of Wildlife and Fisheries Science at the University of Tennessee in 2016, where she has worked as a graduate research assistant for Dr. Brian Alford while pursuing her Master's degree. Her research focuses on the status and distribution of freshwater mussels in Central Tennessee.