Freshwater Mussels (Bivalvia: Margaritiferidae and Unionidae) of the Buffalo River Drainage, Tennessee

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Abstract - The Buffalo River in Tennessee historically contained a rich diversity of freshwater mussels. Sampling efforts in the 1980s documented declines in most of the main channel. Recent collection data indicated recovery in the upper and lower reaches of the river. The objective of this study was to update the current status, distribution, and species composition of the mussel fauna in the main channel and major tributaries through qualitative sampling, and document community structure using quantitative sampling at the most diverse location in the main channel for use in future monitoring efforts. In the qualitative sampling portion of this study, timed searches established catch-per-unit effort (CPUE) at intervals of ~5 river miles in the main channel and tributaries. We recorded a total of 36 species at 62 sites, including 3 federally protected species: *Margaritifera monodonta* (Spectaclecase), *Pleuronaia dolabelloides* (Slabside Pearlymussel), and *Theliderma cylindrica* (Rabbitsfoot). An additional 3 species being considered for the federal endangered species list were also found extant in the main channel: *Obovaria subrotunda* (Round Hickorynut), *Pleuronaia barnesiana* (Tennessee Pigtoe), and *Toxolasma lividum* (Purple Lilliput). Multiple sites in the upper and lower mainstem were suitable for reintroduction of species. These findings should be considered in future management and conservation efforts.

Introduction and Historical Review

Tennessee is one of the most biologically rich inland states and has the highest number of freshwater fish species (Boschung and Mayden 2004, Etnier and Starnes 1993) and the second highest number of freshwater mussels among US states (Parmalee and Bogan 1998, Williams et al. 2008). Parmalee and Bogan (1998) reported 129 mussel taxa historically known to occur in Tennessee, and subsequent taxonomic changes and recent surveys have raised this number to 139 (G.R. Dinkins, unpubl. data). To date, Tennessee has lost roughly 26 taxa to extinction or statewide extirpation, lowering the total number of extant mussel species to 113, of which 3 have uncertain status (G.R. Dinkins, unpubl. data).

The Buffalo River joins the Duck River ~15.5 river miles upstream of the confluence with the Tennessee River, and is the largest tributary to the Duck River. The Duck River has long been recognized as exceptionally diverse, and is home to roughly 151 species of fish, 66 species of mussels, and 22 species of aquatic snails (Ahlstedt et al. 2017, Etnier and Starnes 1993, Hubbs et al. 2011). In contrast, the Buffalo River has received little attention except for occasional surveys at a few isolated locations on the main channel.

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Only a few published surveys have documented the historical mussel fauna of the Buffalo River. Arnold Ortmann visited the Buffalo River in 1922 during a mussel study of the Duck River drainage (Ortmann 1924) and sampled near Riverside (Buffalo River Mile [BRM] 80.5). He noted a substantial mussel fauna, and reported 20 species. In 1931, Calvin Goodrich and Henry van der Schalie sampled BRM 80.5, BRM 41.0, BRM 31.6, and BRM 19.1. Their results, published over 40 y after the survey, added 13 species to Ortmann's list (van der Schalie 1973). Goodrich and van der Schalie's most speciose site was BRM 19.1 (near Lobelville), where they reported 29 species. Van der Schalie (1973:49) noted that "the Duck and Buffalo rivers had a surprisingly rich mussel fauna, both in the numbers of species and in individuals". Based on the number of mussels found at BRM 41.0, BRM 31.6, and BRM 19.1, Van der Schalie (1973:49) concluded the Duck and Buffalo Rivers "have some of the finest shoals in the world but, as previously indicated, the mussels have now been depleted drastically".

The decline of the mussel fauna in the Buffalo River was also noted by Isom and Yokley (1968). In their field notes accompanying speciments deposited in the Museum of Biological Diversity at Ohio State Universtiy (OSUM), they wrote, "The Buffalo is a clear, cool, rapidly flowing small river with no evident pollution. In spite of limestone bedrock, cobbles, and gravel, the mollusks were not abundant." Isom and Yokley found no live mussels or dead shells when they visited BRM 80.5, but they found 16 species at BRM 74.8 and added several new species to the historical record. David Stansbery visited the Buffalo River in 1972 and made this entry in his fieldnotes accompanying speciments deposited in OSUM: "Water low, clear and cool … Naiades [freshwater mussels] all but absent, a single *Toxolasma lividus lividus* [= *Toxolasma lividum* Rafinesque, 1831] was taken alive along with a few dead shells of other species." In 1980, biologists from the Tennessee Valley Authority (TVA) sampled 24 sites on the Buffalo River as part of the Cumberlandian Mollusk Conservation Program and Columbia Dam project on the Duck River (Ahlstedt 1991). They found only 3 or fewer live species at any site, and most sites yielded none. Initially, more sites were to be surveyed, but the study was terminated because of the paucity of mussels encountered. These surveys did not investigate the reason(s) for the decline of the mussel fauna in the Buffalo River, but Fitz (1973) and Mast and Turk (1999) suggested the decline was associated with degraded water quality caused by anthropogenic activities.

In 2002, a survey of the Duck River drainage included 5 sites in the Buffalo River previously surveyed by earlier investigators (Ahlstedt et al. 2017). Of the 9 species found across the 5 sites, none represented a new species for the river. Those authors observed severe substrate destabilization and silt accumulation, and mussels were extremely rare.

Despite the lack of a drainage-wide survey, based on these reports, the Buffalo River was known to have contained 43 species, including 7 that are now federally endangered: *Epioblasma ahlstedti* (Duck River Dartersnapper), *E. aureola* (Golden Riffleshell), *Hemistena lata* (Cracking Pearlymussel), *Pleuronaia dolabelloides* (Slabside Pearlymussel), *Ptychobranchus subtentus* (Fluted Kidneyshell),

Theliderma cylindrica (Rabbitsfoot), and *Toxolasma cylindrellus* (Pale Lilliput) (Appendix 1). In 2012, we recognized the need for a thorough survey of the Buffalo River when biologists from the TVA brought several fresh dead mussels collected during a fish survey in the lower reach to the McClung Museum of Natural History and Culture at the University of Tennessee. In this sample of shells, we identified 2 species not previously reported from the Buffalo River: *Margaritifera monodonta* (Spectaclecase [endangered]) and *Ellipsaria lineolata* (Butterfly). The sample also included *Eurynia dilatata* (Spike), which had not been seen in the Buffalo River since 1922 (Ortmann 1924), *Cyclonaias pustulosa* (Pimpleback), and *Pleuronaia dolabelloides*, not seen since 1931 (van der Schalie 1973).

Study Area

The Buffalo River is located in western Middle Tennessee in the Western Highland Rim of the Interior Low Plateau physiographic province (Mast and Turk 1999) and is one of the state's longest free-flowing rivers (201 km) (Fig. 1). Rising in Lawrence County, the river flows west through Lewis and Wayne counties, then turns north to flow through Perry and Humphreys counties, draining ~1227 km^2. The upper Buffalo River, in Lawrence County, is designated a "State Scenic River" under the Tennessee State Scenic Rivers Act. The Western Highland Rim is highly dissected by perennial streams that create irregular topography consisting of peneplain ridgelines and flat-bottomed drainages, often separated by steep-sided ridges. The Western subdivision of the Highland Rim province encompasses the entire Buffalo River, and varies in elevation from 157 m to 305 m. Mississippian limestone, chert, sandstone, and shale compose primary deposits, with sinkholes, caves, and karst topography occurring throughout the region (Smalley 1981). Mean temperatures vary from 8.8 °C in January to 31.6 °C in July. Mean annual precipitation is 142 cm, with peak precipitation occurring in May and December. May is generally the wettest month and averages 15.5 cm of precipitation. The driest months are August through October. Mast and Turk (1999) and the Tennessee Department of Environment and Conservation (TDEC 2005) described land use in the Buffalo River watershed as primarily forest (77%) and agriculture (18%). Over 90% of the watershed is private land, and urban development constitutes less than 1%. Despite its rural setting, the Buffalo River drainage experienced considerable development from the mid-1960s to mid-1980s.

Methods

Museum records

We collected records from several museums to supplement the list of species in past survey reports: Carnegie Museum of Natural History (CM), McClung Museum of Natural History and Culture at the University of Tennessee (UTMM), North Carolina State Museum of Natural Sciences (NCMNS), Museum of Biological Diversity at The Ohio State University (OSUM), and University of Michigan Museum of Zoology (UMMZ).

Qualitative sampling

We sampled 47 sites in the main channel Buffalo River and 15 sites in tributary streams between October 2012 and December 2015 (Table 1, Fig. 1). We selected sampling locations surveyed in previous investigations and new sites that we considered to possess suitable habitat. Each locality was recorded in decimal degrees

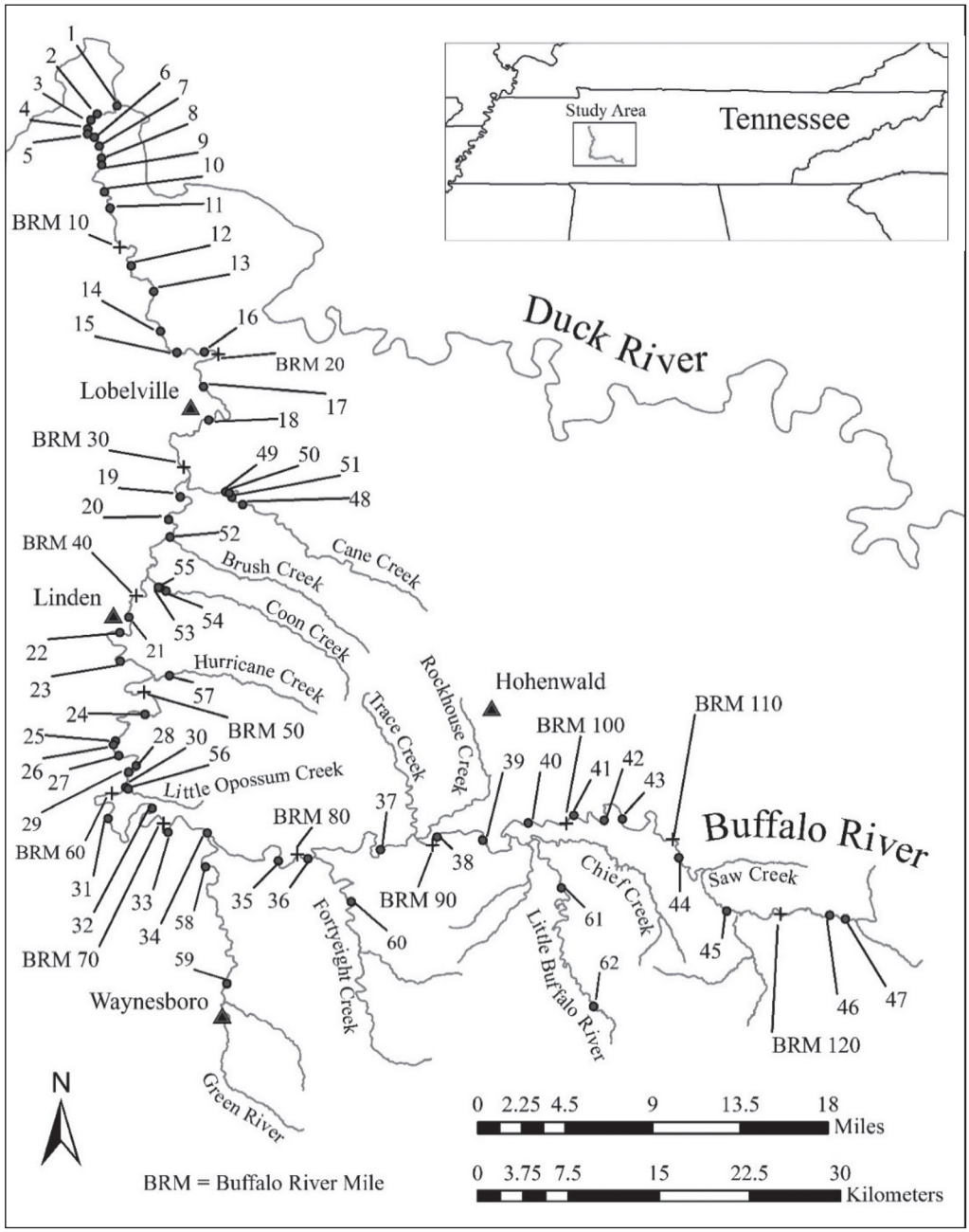

Figure 1. Sampling localities for mussels in the Buffalo River drainage in Lawrence, Lewis, Wayne, Perry, and Humphreys counties, TN.

Table 1. Location of mussel survey sites in the Buffalo River system, TN. [Table continued on following page.]

Site/location	Latitude (°N)	Longitude (°W)	River-mile	Date
1 Buffalo River	35.9957330	87.8407500	0.2	11 October 2012
2 Buffalo River	35.9894400	87.8555600	1.9	16 November 2012
3 Buffalo River	35.9850500	87.8603100	2.2	16 November 2012
4 Buffalo River	35.9784300	87.8626100	2.8	16 November 2012
5 Buffalo River	35.9746800	87.8627500	3.2	16 November 2012
6 Buffalo River	35.9722400	87.8577100	3.5	17 November 2012
7 Buffalo River	35.9657000	87.8541400	4.0	17 November 2012
8 Buffalo River	35.9567100	87.8524000	4.6	17 November 2012
9 Buffalo River	35.9517200	87.8522000	4.9	17 November 2012
10 Buffalo River	35.9316590	87.8501960	6.8	11 October 2013
11 Buffalo River	35.9195000	87.8461000	8.0	11 October 2013
12 Buffalo River	35.8766670	87.8303500	12.0	11 October 2013
13 Buffalo River	35.8576170	87.8135670	13.8	30 August 2013
14 Buffalo River	35.8279670	87.8086830	16.3	30 August 2013
15 Buffalo River	35.8121830	87.7964500	17.7	30 August 2013
16 Buffalo River	35.8126170	87.7759000	19.4	30 August 2013
17 Buffalo River	35.7867330	87.7766670	22.4	29 August 2013
18 Buffalo River	35.7620830	87.7727500	25.8	29 August 2013
19 Buffalo River	35.7049100	87.7937700	32.0	10 August 2013
20 Buffalo River	35.6880300	87.8026200	34.3	10 Aug 2013
21 Buffalo River	35.6154300	87.8320300	41.4	9 August 2013
22 Buffalo River	35.6040700	87.8387900	42.8	9 August 2013
23 Buffalo River	35.5827400	87.8385200	45.6	9 August 2013
24 Buffalo River	35.5432900	87.8201000	52.1	9 August 2013
25 Buffalo River	35.5235500	87.8419670	55.1	18 December 2012
26 Buffalo River	35.5206330	87.8435670	55.6	18 December 2012
27 Buffalo River	35.5125267	87.8395990	56.0	18 December 2012
28 Buffalo River	35.5050500	87.8266330	57.4	18 December 2012
29 Buffalo River	35.5004000	87.8322330	58.0	18 December 2012
30 Buffalo River	35.4890835	87.8342990	59.0	18 December 2012
31 Buffalo River	35.4657500	87.8476000	62.7	21 June 2013
32 Buffalo River	35.4734830	87.8148670	67.4	21 June 2013
33 Buffalo River	35.4556670	87.8029500	70.6	21 June 2013
34 Buffalo River	35.4551000	87.7737670	73.5	25 May 2013
35 Buffalo River	35.4343500	87.7210000	78.7	25 May 2013
36 Buffalo River	35.4360170	87.6990500	80.5	25 May 2013
37 Buffalo River	35.4427170	87.6451170	87.0	24 May 2013
38 Buffalo River	35.4521500	87.6030170	90.6	24 May 2013
39 Buffalo River	35.4497700	87.5690900	93.6	17 July 2013
40 Buffalo River	35.4625800	87.5352000	98.2	17 July 2013
41 Buffalo River	35.4681200	87.5012900	100.6	17 July 2013
42 Buffalo River	35.4645100	87.4786700	102.9	16 July 2013
43 Buffalo River	35.4654800	87.4652900	105.3	17 July 2013
44 Buffalo River	35.4367000	87.4232300	111.3	17 July 2013
45 Buffalo River	35.3971400	87.3877100	116.7	18 July 2013
46 Buffalo River	35.3939800	87.3111500	121.0	18 July 2013
47 Buffalo River	35.3911100	87.2994600	122.0	18 July 2013
48 Cane Creek	35.7048170	87.7554670	3.4	11 December 2012
49 Cane Creek	35.7086670	87.7601500	2.4	11 December 2012

using a hand-held global positioning system unit (Garmin GPSMAP 76Cx unit, WAAS enabled) and is reported in river miles because this is the increment used in US Geological Survey 7.5 minute topographic maps.

Mussels encountered at each sampling location were removed from the substrate and held in a submerged mesh bag until they were sorted and identified. Fresh-dead (shiny nacre, hinge ligament intact) and relic shells (weathered periostracum, faded or chalky nacre) were identified, counted, recorded on a field data sheet, and taken to UTMM where they were cleaned, verified, and catalogued. Live mussels were measured (anterior to posterior in millimeters) using dial calipers, photographed, and returned to the substrate. Each site was surveyed by at least 2 experienced biologists for 1 h or more. Shorelines and adjacent submerged habitats were searched for shells deposited by Ondatra zibethicus (L.) (Muskrat) or high water.

Quantitative sampling

Based on the results of the qualitative survey, we chose the most diverse and abundant mussel community for quantitative sampling in November 2013 (shoal at BRM 3.2). We established parallel transects in an area measuring 67 m long and 35 m wide (2345 m^2). Transects were oriented perpendicular to the shoreline, spaced at 6-m intervals, and spanned the wetted channel. A total of 100 quadrats (0.25 m^2) evenly spaced 4 m apart along the transects was sampled using skin diving gear and SCUBA, and each quadrat was excavated to an attempted minimum depth of 15 cm. Water depth varied from a few centimeters along the right descending bank to 2 m in the thalweg, located near the left descending bank.

Analyses

We calculated species richness, relative abundance, and CPUE (determined as number of observed live and fresh dead mussels/snorkel time per observer x number of observers) for each site. Numerous studies involving freshwater mussels have documented the potential bias of selecting for larger individuals in qualitative versus quantitative sampling methods (Hornbach and Deneka 1996, Miller and

Table 1, continued.

Site/location	Latitude (°N)	Longitude (°W)	River mile	Date
50 Cane Creek	35.7077819	87.7575284	2.6	11 December 2012
51 Cane Creek	35.6992830	87.7475330	4.1	11 December 2012
52 Brush Creek	35.6751170	87.8015000	0.1	12 December 2012
53 Coon Creek	35.6349000	87.8045330	1.3	12 December 2012
54 Coon Creek	35.6375500	87.8088670	0.9	12 December 2012
55 Coon Creek	35.6377000	87.8101801	0.7	12 December 2012
56 Lower Opossum Creek	35.4878336	87.8325710	0.1	18 December 2012
57 Hurricane Creek	35.5721100	87.8019500	0.9	10 August 2013
58 Green River	35.4299440	87.7750700	2.3	19 December 2015
59 Green River	35.3431100	87.7592800	10.6	19 December 2015
60 Fortyeight Creek	35.4040640	87.6667760	4.4	19 December 2015
61 Little Buffalo River	35.4143080	87.5105710	4.8	19 December 2015
62 Little Buffalo River	35.3261060	87.4866630	15.3	19 December 2015

Payne 1993, Miller et al. 1993, Obermeyer 1998). To test for this potential bias, we conducted a 2-tailed Kolmogorov–Smirnov (K–S) nonparametric test (Smirnov 1948) to examine size-class variance in qualitative versus quantitative sampling data for the most abundant species, *Pleuronaia dolabelloides*. We hypothesized qualitative size-class data would vary significantly from quantitative size-class data. We conducted our analyses in XLSTAT statistical software. Taxonomy follows Williams et al. (2017).

Results

Museum records

The search for Buffalo River specimens at 5 museums yielded a total of 32 species, including 4 new records from 16 collecting localities (Appendix 2): *Epioblasma turgidula* (Turgid Blossom), *Megalonaias nervosa* (Giant Washboard), *Ligumia subrostrata* (Pondmussel), and *Utterbackiana suborbiculata* (Flat Floater). *Epioblasma turgidula* (2 males and 1 female) and *Megalonaias nervosa* were collected by H. Athearn in 1966 at the Gilmore Bridge near Lobelville (BRM 22.6) (J. Smith, NCMNS, Raleigh, NC, pers. comm.). Athearn also collected *M. nervosa* at the US Route 412 bridge near Linden (BRM 41.0). In July 1994, S. Ahlstedt found *Ligumia subrostrata* (Pondmussel) in an overflow channel of the Buffalo River at Mill Bridge (BRM 24.0). There are only a few records of this species from Tennessee, but it is found throughout the Mississippi River basin (Parmalee and Bogan 1998). *Ligumia subrostrata* typically occurs in areas with little to no current (Williams et al. 2008), and it seems to be particularly adaptable to newly created ponds or channels, where it can be abundant. Its presence in an overflow channel of the Buffalo River, while well upstream of its previously known range in the Tennessee River drainage, is consistent with its habitat preference. *Utterbackiana suborbiculata* (Flat Floater) was found at Beardstown (BRM 32.6) in 1973. A large, single valve of this species was found in the Buffalo River and is cataloged at UTMM. *Utterbackiana suborbiculata* appears to be expanding its range upstream in both the Tennessee and Cumberland river drainages (Bates 1962, Parmalee and Bogan 1998) and it is now found in the Tennessee River as far upstream as Knoxville and in the Cumberland River as far upstream as Hartsville (G. Dinkins, pers. observ.). Williams et al. (2008) speculated *U. suborbiculata* has spread into the Tennessee River due to conditions associated with impoundment of the formerly free-flowing river. While the Buffalo River is completely unimpounded, Kentucky Lake impounds the lower Duck River upstream to its confluence with the Buffalo River.

Qualitative results

We found a total of 33 species in the qualitative survey; 2 additional species were found after the qualitative survey was completed and were added to the qualitative survey results (Appendix 3). We observed live or fresh dead mussels at 30 of the 47 main channel sites. By far, the most diverse sites were BRM 3.2 and BRM 3.5. We found no live mussels at the 5 most upstream sites (BRM 105.3 to

BRM 122.0). Mussel diversity varied in the main channel, and although mussels were sparse in the middle reaches compared to historical sampling efforts, we detected significant increases in the upper and lower Buffalo River. We documented *Lampsilis fasciola* (Wavyrayed Lampmussel) and *Villosa taeniata* (Painted Creekshell) at 18 and 21 sites, respectively; these were the most abundant and widespread species. Species richness and CPUE were correlated throughout the river. Sites in the middle portion of the river (BRM 30.0 to BRM 98.0) supported few live mussels. Species richness was similarly low in this stretch of the river. Tributaries to the Buffalo River (Sites 48 to 60, 62) were devoid of mussels except for a single relic *Strophitus undulatus* (Creeper) found in the Little Buffalo River at Site 61. We did not observe *Strophitus undulatus* in the main channel of the Buffalo River. The apparent lack of mussels in Buffalo River tributaries mirrors recent findings from sampling in tributaries of the Duck River (K. Irwin, Tennessee Tech University, Cookeville, TN, unpubl. data). For this reason, tributary sampling results are not included in Appendix 3.

Length–frequency data indicated recent recruitment for *V. taeniata* (20–89 mm), *L. fasciola* (56–86 mm), *Pleuronaia barnesiana* (Tennessee Pigtoe) (27–59 mm), and *P. dolabelloides* (36–70 mm). We observed multiple size-classes of *Potamilus alatus* (Pink Heelsplitter) and *T. cylindrica*, but there were insufficient numbers to infer recent recruitment. During quantitative sampling at BRM 3.2, a large, submerged muskrat midden was located just outside of the study area. After a brief assessment, it became evident this midden contained a large number of fresh dead shells; these numbers are omitted from the study results.

Quantitative results

Quadrat excavations yielded 178 live individuals representing 20 species and an average of 1.8 mussels per 0.25-m^2 quadrat (Table 2). We collected 1 live *Actinonaias ligamentina* (Mucket) in the quadrat excavations, which was the only individual of the species found in our study. Two federally protected species, *P. dolabelloides* and *T. cylindrica*, comprised nearly 30% of the live mussels in the samples; of these, *P. dolabelloides* was the most abundant (26.4%). There were multiple size-classes (11–65 mm) of this species, which indicated recent recruitment. Other species with multiple size-classes included *Cyclonaias pustulosa*, *Cyclonaias tuberculata* (Purple Wartyback), and *Eurynia dilatata*. Evidence of recent recruitment was inconclusive for *Lampsilis fasciola*, *P. barnesiana*, *T. cylindrica*, *Tritogonia verrucosa* (Pistolgrip), and *Truncilla truncata* (Deertoe) due to insufficient number of individuals.

There was no significant difference in size-classes of *P. dolabelloides* in qualitative versus quantitative data ($P = 0.093$), suggesting qualitative sampling methods were not biased toward detecting larger individuals.

Species of conservation concern

Margaritifera monodonta (Spectaclecase).

Margaritifera monodonta was once widespread in the Mississippi, Ohio, and Missouri River basins from Minnesota and western Pennsylvania south to the Gulf

of Mexico (Parmalee and Bogan 1998). It was listed as federally endangered in 2012 (USFWS 2012). Prior to this study, it was not known to occur in the Buffalo River. Two live individuals were found in 1998 in the Tennessee River at RM 170, approximately 60 river-miles downstream of the mouth of the Duck River (Hubbs and Jones 2000), and in the early 2000s, a single individual was observed in the Duck River (USFWS 2012). To our knowledge, no additional individuals of this species have been found in the Duck River, and it appears to be equally rare in the Buffalo River. The periostracum of the specimen brought to us by the TVA fish-sampling crew was fairly weathered but not overly so, and the nacre was lustrous. We cannot precisely estimate how much time had elapsed since its death, but we have found shells of *M. monodonta* from other locations in the Tennessee River drainage that appeared weathered soon after death; consequently, it is likely the specimen found by the TVA was alive within 1 y of its shell being found, and from this we infer that there is an extant but extremely small population remaining in the Buffalo River.

Epioblasma ahlstedti (Duck River Dartersnapper).

Epioblasma ahlstedti historically occurred in the Duck and Buffalo rivers, as well as the Tennessee River at Muscle Shoals and Shoal Creek, a large direct tributary to the Tennessee River (Jones 2004). Currently, it is restricted to ~48.3 river km in the Duck River, from the Old Columbia Dam upstream to Lillard Mill

Table 2. Summary of quantitative sampling at BRM 3.2. Number = number observed among 100 quadrats. % Occurrence = percent of occurrence across all quadrat samples. Number of quadrats = number of quadrats occupied by species. Size (min–max if more than 1 observed) is presented for live individuals only.

Species	Number	% occurrence	# of quadrats	Size (mm)
Actinonaias ligamentina	1	0.6	1	49
Actinonaias pectorosa	1	0.6	1	14
Cyclonaias pustulosa	15	8.4	10	20–61
Cyclonaias tuberculata	46	26.0	25	18–99
Ellipsaria lineolata	3	1.7	2	22–54
Elliptio crassidens	3	1.7	3	111–141
Eurynia dilatata	20	11.2	15	15–85
Lampsilis fasciola	6	3.3	5	47–82
Lampsilis ovata	2	1.1	2	16–74
Lasmigona costata	2	1.1	2	82–149
Megalonaias nervosa	1	0.6	1	88
Obliquaria reflexa	1	0.6	1	29
Pleuronaia barnesiana	5	2.8	5	18–59
Pleuronaia dolabelloides	47	26.4	28	11–65
Potamilus alatus	2	1.1	2	109–113
Theliderma cylindrica	6	3.3	5	64–94
Toxolasma lividum	1	0.6	1	16
Tritogonia verrucosa	6	3.3	4	64–89
Truncilla truncata	9	5.0	8	18–37
Villosa taeniata	1	0.6	1	84
Total	178			

(Jones and Neves 2010, Parmalee and Bogan 1998). Its historical occurrence in the Buffalo River is based on 1 specimen found in the Buffalo River in 1931 (van der Schalie 1973) near the Humphreys/Perry County border (approximate BRM 19.0). We did not find this species in this survey. *Epioblasma ahlstedti* was listed as federally endangered in 1997 (as *Epioblasma capsaeformis* [Oyster Mussel]; USFWS 1997).

Epioblasma aureola (Golden Riffleshell).

Epioblasma aureola presumably occurred in numerous tributary streams of the middle and upper Tennessee River system downstream to the Duck and Buffalo rivers, but is now thought to occur only in Indian Creek, a tributary to the upper Clinch River in southwestern Virginia (Jones and Neves 2010). Its historical occurrence in the Buffalo River is based on 3 specimens found in the Buffalo River in 1931 (van der Schalie 1973) near the Humphreys/Perry County border (approximate BRM 19.0). We did not find this species in this survey. *Epioblasma aureola* was listed as federally endangered in 1977 (as *Epioblasma florentina walkeri* [Wilson and Clark] [Tan Riffleshell]; USFWS 1977).

Epioblasma turgidula (Turgid Blossom).

Epioblasma turgidula appears to have been endemic to the Tennessee and Cumberland River drainages in Alabama and Tennessee, where it occurred in numerous large tributary rivers and streams (Parmalee and Bogan 1998, Williams et al. 2008). H. Athearn collected 3 individuals of this species (presumably alive) in September 1966 in the Buffalo River at Gilmore Bridge (BRM 22.6) (J. Smith, pers. comm.). *Epioblasma turgidula* is presumed extinct and was last seen alive in 1972 in the upper Duck River (Stansbery 1976). Johnson (1978) included records for the species from the St. Francis River in Arkansas, but its taxonomic status outside of the Tennessee and Cumberland systems is uncertain and yet unresolved (J. Harris, Arkansas State University, Jonesboro, AR, pers. comm.). It was listed as federally endangered in 1976 (USFWS 1976).

Hemistena lata (Cracking Pearlymussel).

Hemistena lata is restricted to the Ohio River drainage, including the Tennessee and Cumberland rivers (Watters et al. 2009). There are 2 records from the Buffalo River: 1 individual was collected at BRM 19.1 in 1931 (van der Schalie 1973) and an unknown number were collected at BRM 74.8 in 1965 (Isom and Yokley 1968). We did not find *H. lata* in this survey. *Hemistena lata* was listed as federally endangered in 1989 (USFWS 1989).

Obovaria subrotunda (Round Hickorynut).

Obovaria subrotunda is known from parts of the Great Lakes Basin and is widespread in the Tennessee, Cumberland, and Ohio River drainages (Haag and Cicerello 2016, Parmalee and Bogan 1998, Watters et al. 2009, Williams et al. 2008). *Obovaria subrotunda* was first reported from the Buffalo River in 1922 (Ortmann 1922), and it was subsequently collected in 1931 and 1965 (Isom and Yokley 1968, van der Schalie 1973). We found 1 live individual at BRM 3.2 during a timed search but did not find it in the quantitative sampling at this same location. The

USFWS is currently conducting a status assessment to determine if *O. subrotunda* warrants federal protection under the Endangered Species Act (ESA).

Pleuronaia barnesiana (Tennessee Pigtoe).

Pleuronaia barnesiana occurs in medium-sized creeks to large rivers in the Tennessee River drainage (Parmalee and Bogan 1998, Williams et al. 2008). *Pleuronaia barnesiana* was relatively widespread in the Buffalo River. We found live or dead shells at 13 locations from just upstream of the Duck River confluence to BRM 102.9. In the quantitative survey at BRM 3.2, *P. barnesiana* comprised ~3% of the live mussels found in the quadrats and several age classes were evident. The USFWS is currently conducting a status assessment to determine if *P. barnesiana* warrants federal protection under the ESA.

Pleuronaia dolabelloides (Slabside Pearlymussel).

Pleuronaia dolabelloides is endemic to the Tennessee and Cumberland River drainages and was listed as federally endangered in 2013 (USFWS 2013a). This species was one of the most abundant and wide-ranging species we found in the Buffalo River. Individuals collected from quadrats at BRM 3.2 exhibited a healthy size-class distribution (11–65 mm), with strong evidence of recent recruitment. Currently, healthy communities of *P. dolabelloides* persist in the upper and lower reaches of the Buffalo River (e.g., BRM 102.9 and BRM 3.2). Clinal variation in shell morphology for this species is consistent with observations made by Ortmann (1924) and Van der Schalie (1973); individuals collected upstream (e.g., BRM 102.9) were notably compressed compared to individuals at downstream sites (e.g., BRM 3.2).

Pleurobema oviforme (Tennessee Clubshell).

Pleurobema oviforme historically occurred in small to large rivers in the Tennessee and Cumberland River drainages, but it has disappeared from much of its historical range and is in danger of disappearing from Kentucky (Haag and Cicerello 2016). We observed relic shells of *P. oviforme* at 2 sites during this study—BRM 3.2 and BRM 3.5. The USFWS is currently conducting a status assessment to determine if *P. oviforme* warrants federal protection under the ESA.

Ptychobranchus subtentus (Fluted Kidneyshell).

Ptychobranchus subtentus is endemic to the Tennessee and Cumberland River drainages (Parmalee and Bogan 1998). It appears to be extirpated from Alabama (Garner et al. 2004), and populations have declined dramatically in Kentucky (Haag and Cicerello 2016). We did not find *P. subtentus* in this survey, and it has not been seen in the Buffalo River since 1922 at BRM 90.4 (Ortmann 1924). *Ptychobranchus subtentus* was listed as federally endangered in 2013 (USFWS 2013a).

Theliderma cylindrica (Rabbitsfoot).

Theliderma cylindrica is endemic to the Tennessee River drainage and was listed as threatened in 2013 (USFWS 2013b). The Buffalo River was not included as critical habitat for *T. cylindrica*, and a recent status update assumed it was extirpated from the river (USFWS 2015). During our qualitative search, *T. cylindrica*

was found to be mostly restricted to the lower reach of the Buffalo River. We documented live individuals at BRM 2.2, BRM 3.2, and BRM 17.7. Quantitative sampling at BRM 3.2 produced 6 *T. cylindrica* from 5 quadrat samples.

Toxolasma cylindrellus (Pale Lilliput).

Toxolasma cylindrellus is endemic to the middle and lower Tennessee River drainage in Tennessee and Alabama (Parmalee and Bogan 1998, Williams et al. 2008). It was thought to have been extirpated from the Duck River, but was rediscovered in 2015 in Lick Creek, a tributary to the Duck River in Maury County (D. Hubbs, Tennessee Wildlife Resource Agency,Camden, TN, pers. comm.). Prior to this discovery, the only known population occurred in a tributary of the Paint Rock River in Alabama and Tennessee. *Toxolasma cylindrellus* was last seen in the Buffalo River in 1922 at BRM 80.5 (Ortmann 1924), and we did not find the species in this survey. *Toxolasma cylindrellus* was listed as federally endangered in 1976 (USFWS 1976).

Toxolasma lividum (Purple Lilliput).

Toxolasma lividum occurs in the Ohio River drainage, including the Cumberland and Tennessee rivers, the Arkansas River drainage, and in the Lake Erie drainage (Parmalee and Bogan 1998, Watters et al. 2009). In the Buffalo River, it was rare and restricted to lower reaches where it occurred along the margins of backwater areas and shallow pools in soft substrate. We found a single individual during quantitative sampling at BRM 3.2. The taxonomic status of populations in the Cumberland River drainage are under review (D. Campbell, Gardner-Webb University, Boiling Springs, NC, pers. comm.), and the USFWS is currently conducting a status assessment to determine if *T. lividum* warrants federal protection under the ESA.

The following species were not detected in our survey and may be extirpated from the Buffalo River. The date of last occurrence is given in brackets following the common name: *Alasmidonta marginata* (Say) (Elktoe) [1965], *Alasmidonta viridis* (Rafinesque) (Slippershell Mussel) [1922], *Lasmigona complanata* (Barnes) (White Heelsplitter) [1931], *Ligumia subrostrata* (Say) (Pondmussel) [1994], *Medionidus conradicus* (Lea) (Cumberland Moccasinshell) [1968], *Pleurobema sintoxia* (Rafinesque) (Round Pigtoe) [1931], *Ptychobranchus fasciolaris* (Rafinesque) (Kidneyshell) [1965], *Utterbackia imbecillis* (Say) (Paper Pondshell) [1931], and *Utterbackia suborbiculata* [1973].

Discussion and Recommendations

The Buffalo River is a rare example of a large unimpounded stream. Our review of published literature, unpublished reports, and museum records along with our survey results indicate that the river once contained 51 freshwater mussel species (Appendix 4). Most of the river's mussel fauna was nearly extirpated in the years following the early surveys by Ortmann, which was documented in subsequent surveys by Goodrich and van der Schalie, Isom and Yokley, and Ahlstedt. As a result, management decisions and conservation efforts aimed at recovering

imperiled species in the Tennessee River system have largely overlooked the Buffalo River's potential. The present survey recorded 36 species in the main channel of the river and an additional species found a few months before fieldwork began (*Margaritifera monodonta*). Of these, 29 species were represented by live or fresh-dead material, and 8 species were represented by relic shells only. There were 14 species present in the historical record that we did not detect.

We rarely encountered the degree of species richness or abundance noted in previous studies. In our qualitative survey, 22 species were represented by 10 or fewer live or fresh dead individuals and 7 species were represented by relic shells only. A substantial reach of the Buffalo River had few or no live mussels (BRM 32.0 to 98.2; BRM 105.3 to BRM 122.0). The middle reach from BRM 32.0 to BRM 58.0 contained only *L. fasciola* and *V. taeniata*. The paucity of mussels in the middle reach is comparable to the survey data from 1980 (Ahlstedt 1991). Of the 30 species still present in the river, at least 8 species that historically occurred in the upper reaches of the river are now restricted to the lower reaches: *Actinonaias ligamentina*, *Actinonaias pectorosa* (Pheasantshell), *Lampsilis cardium* (Plain Pocketbook), *Lampsilis ovata* (Pocketbook), *Lasmigona costata* (Flutedshell), *Leptodea fragilis* (Fragile Papershell), *Obovaria subrotunda*, and *T. verrucosa*.

We observed obvious signs of stream-bank instability and significant erosion and siltation, likely due to clearing of riparian vegetation adjacent to agricultural operations, in various stretches of the river. In general, most of these reaches produced low CPUE values. Another factor that may be inhibiting recovery of the mussel communities is elevated levels of toxic metals. Denton (2007) reported high levels of mercury in the tissue of *Micropterus dolomieu* (Lacepède) (Smallmouth Bass) at BRM 17.7, which prompted TDEC to issue a fish-consumption advisory for the lower Buffalo River extending from BRM 31.6 to the confluence with the Duck River. Naimo (1995) reported that elevated levels of mercury can alter growth, filtration efficiency, enzyme activity, and behavior in freshwater mussels. Gravel dredging and unrestricted livestock access have also destabilized many of the tributaries. We found no mussels in the tributaries except for a single, relic shell of *Strophitus undulatus* in the Little Buffalo River at mile 4.8. Despite reaches with unstable habitat and heavy sedimentation, the Buffalo River and its tributaries have excellent fish diversity, (C. Saylor, TVA, Knoxville, TN, pers. comm.), and support multiple federally listed fishes including *Etheostoma boschungi* (Wall and Williams) (Slackwater Darter) and *Erimonax monachus* (Cope) (Spotfin Chub) (Etnier and Starnes 1993).

Analysis of spatial distributions demonstrated several findings of immediate conservation importance. Between BRM 3.2 to BRM 4.9, we found 18 species that occurred nowhere else in the Buffalo River, and a number of species that are apparently restricted to its lower reaches (e.g., *Lampsilis cardium*, *Lampsilis teres* [Yellow Sandshell], *Obliquaria reflexa* [Threehorn Wartyback], *Pleurobema cordatum* [Ohio Pigtoe], *Theliderma cylindrica*, *Cyclonaias pustulosa*, and *Quadrula quadrula* [Mapleleaf]) have not been reported in the Buffalo River since 1931 (van der Schalie 1973). We documented 5 new records for the drainage in this reach (*Margaritifera monodonta*, *Arcidens confragosus* [Rock Pocketbook], *Fusconaia*

subrotunda, [Longsolid], *Ligumia recta*, [Black Sandshell], and *Theliderma metanevra* [Monkeyface]). With the exception of *F. subrotunda*, all were reported in the lower Duck River by Schilling and Williams (2002).

In addition, the area extending from BRM 100.6 to BRM 102.9 should be re-examined and monitored in the future; BRM 102.9 had populations of both *P. barnesiana* and *P. dolabelloides*. We observed evidence of recent recruitment for both species. Habitat at sites in this upper reach appeared excellent, with minimal evidence of disturbance. We found no mussels above BRM 102.9, further increasing the importance of preserving this isolated reach.

The continued monitoring and conservation of mussel assemblages identified in this study is vital to future management of mussel resources in the Buffalo River. Fish hosts of threatened and endangered mussels should also be monitored. Quadrat excavations conducted at BRM 3.2 provide a baseline for future quantitative mussel monitoring. Because it is a tributary to the Duck River, the Buffalo River has the potential, with the proper attention and management, to regain much of its lost mussel biodiversity. Sedimentation and channel instability appear to be the primary factors impacting mussel habitat in the river. We strongly recommend that landowners in the Buffalo River drainage be encouraged to implement best management practices. Better management of riparian zones and limiting cattle movements near main-channel mussel habitats could play a pivotal role in mitigating sedimentation and habitat degradation in the Buffalo River.

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Literature Cited

Ahlstedt, S.A. 1991. Cumberlandian mollusk conservation program. Activity 1: Mussel surveys in six Tennessee Valley streams. Walkerana 5(13):123–160.

Ahlstedt, S.A., J.R. Powell, R.S. Butler, M.T. Fagg, S.F. Novak, S.R. Palmer, and P.D. Johnson. 2017. Historical and current examination of freshwater mussels (Bivalvia: Margaritiferidae, Unionidae) in the Duck River Basin, Tennessee. Malacological Review 45/46:163

Bates, J.M. 1962. The impact of impoundment on the mussel fauna of Kentucky Reservoir, Tennessee River. American Midland Naturalist 68(1):232–236.

Boschung, J.T., Jr., and R.L. Mayden. 2004. Fishes of Alabama. Smithsonian Books, Washington, DC. 736 pp.

Denton, G.M. 2007. Mercury levels in Tennessee fish. Tennessee Department of Environment and Conservation, Division of Water Pollution Control, Nashville, TN.

Etnier, D.A., and W.C. Starnes. 1993. The Fishes of Tennessee. University of Tennessee Press, Knoxville, TN. 696 pp.

Fitz, R. 1973. Tennessee Valley streams: Their fish, bottom fauna, and aquatic habitat. Buffalo River Drainage Basin. Division of Forestry, Fisheries and Wildlife Development, Fisheries and Waterfowl Resources Branch. Tennessee Valley Authority, Knoxville, TN. 1973.

Garner, J.T., H. Blalock–Herod, A.E. Bogan, R.S. Butler, W.R. Haag, P.D. Hartfield, J.J. Herod, P.D. Johnson, W.W. McGregor, and J.D. Williams. 2004. Freshwater mussels and snails. Pp. 1358, *In* R.A. Mirarchi (Ed.). Alabama Wildlife. Volume 1. A Checklist of Vertebrates and Selected Invertebrates: Aquatic Mollusks, Fishes, Amphibians, Reptiles, Birds, and Mammals. The University of Alabama Press, Tuscaloosa, AL. 212 pp.

Haag, W.R., and R.R. Cicerello. 2016. A distributional atlas of the freshwater mussels of Kentucky. Scientific and Technical Series 8. Kentucky State Nature Preserves Commission, Frankfort, KY. 299 pp.

Hornbach, D.J., and T. Deneka. 1996. A comparison of a qualitative and a quantitative collection method for examining freshwater mussel assemblages. Journal of the North American Benthological Society 15(4):587–596.

Hubbs, D.W., and A. Jones. 2000. 1998 statewide commercial mussel report. Tennessee Wildlife Resources Agency Fisheries Report 00–08. Nashville, TN. 36 pp.

Hubbs, D., S. Chance, L. Colley, and R.S. Butler. 2011. 2010 Duck River quantitative mussel survey. Tennessee Wildlife Resources Agency, Fisheries Division Report 11–04. Nashville, TN.

Isom, B.G., and P. Yokley Jr. 1968. The mussel fauna of the Duck River in Tennessee, 1965. American Midland Naturalist 80(1):34–42.

Jenkinson, J.J. 1988. Resurvey of freshwater mussel stocks in the Duck River, Tennessee. Tennessee Valley Authority, Knoxville, TN.

Johnson, R.I. 1978. Systematics and zoogeography of *Plagiola* (=*Dysnomia* = *Epioblasma*), an almost extinct genus of freshwater mussels (Bivalvia: Unionidae) from Middle North America. Bulletin of the Museum of Comparative Zoology 148(6):239–320.

Jones, J.W. 2004. A holistic approach to taxonomic evaluation of two closely related endangered freshwater mussel species, the Oyster Mussel (*Epioblasma capsaeformis*) and Tan Riffleshell (*Epioblasma florentina walkeri*). M.Sc. Thesis. Virginia Polytechnic Institute and State University, Blacksburg, VA. Available online at http://scholar.lib.vt.edu/theses/ available/etd–03302004–153127/. Accessed June 2015.

Jones, J.W., and R. J. Neves. 2010. Descriptions of a new species and a new subspecies of freshwater mussels, *Epioblasma ahlstedti* and *Epioblasma florentina aureola* (Bivalvia: Unionidae), in the Tennessee River drainage, USA. The Nautilus 124:77–92.

Mast, M.A., and J.T. Turk. 1999. Environmental characteristics and water quality of hydrologic benchmark network stations in the eastern United States, 1963–95. US Geological Survey Circular 1173(A):158.

Miller, A.C., and B.S. Payne. 1993. Qualitative versus quantitative sampling to evaluate population and community characteristics at a large-river mussel bed. American Midland Naturalist 130(1):133–145.

Miller, A.C., B.S. Payne, D.J. Shafer, and L.T. Neil. 1993. Techniques for monitoring freshwater bivalve communities in large rivers. Pp. 145–158, *In* K.S. Cummings, B.C. Buchanan, and L.M. Koch (Ed.). Conservation and Management of Freshwater Mussels. Proceedings of a UMRCC Symposium, 12–14 October 1992, St. Louis, MO. Upper Mississippi River Conservation Committee, Rock Island, IL.

Naimo, T.J. 1995. A review of the effects of heavy metals on freshwater mussels. Ecotoxicology 4(6):341–362.

Obermeyer, B.K. 1998. A comparison of quadrats versus timed snorkel searches for assessing freshwater mussels. American Midland Naturalist 139(2):331–339.

Ortmann, A.E. 1924. The naiad-fauna of the Duck River in Tennessee. American Midland Naturalist 9(2):28.

Parmalee, P.W., and A.E. Bogan. 1998. The Freshwater Mussels of Tennessee. The University of Tennessee Press, Knoxville, TN. 147 pp.

Schilling, E.M., and J.D. Williams. 2002. Freshwater mussels (Bivalvia: Margaritiferidae and Unionidae) of the Lower Duck River in Middle Tennessee: A historic and recent review. Southeastern Naturalist 1(4):403–414.

Smalley, G.W. 1981. Classification and evaluation of forest sites on the western Highland Rim and Pennyroyal. General technical Report SO–30, US Department of Agriculture Forest Service, Southern Forest Experiment Station, Asheville, NC. 120 pp.

Smirnov, N. 1948. Table for estimating the goodness-of-fit for empirical distributions. Annals of Mathematical Statistics 19:279–281.

Stansbery, D.H. 1976. Naiad mollusks. Pp. 42–52, *In* H.T. Boschung (Ed.). Endangered and Threatened Plants and Animals of Alabama. Alabama Museum of Natural History Bulletin 2. 93 pp.

Tennessee Department of Environment and Conservation (TDEC). 2005. Total maximum daily load (TDML) for pathogens in the Buffalo River watershed (HUC 06040004). State of Tennessee, Department of Environment and Conservation, Division of Water Pollution Control, January 2005. Available online at http://www.state.tn.us/environment/wpc/tmdl/approvedtmdl/BuffaloF2.pdf. Accessed December 2013.

US Fish and Wildlife Service (USFWS). 1976. Endangered and threatened wildlife and plants; endangered status for 159 taxa of animals. Federal Register 41:24062–24067.

USFWS. 1977. Endangered and threatened wildlife and plants; determination of endangered status for Tan Riffleshell; final rule. Federal Register 42(163):42351–42353.

USFWS. 1989. Endangered and threatened wildlife and plants; determination of Cracking Pearlymussel (*Hemistena* (=*Lastena*) *lata*) to be an endangered species. Federal Register 54(187):39850–39863.

USFWS. 1997. Endangered and threatened wildlife and plants; determination of endangered status for the Culberland Elktoe, Oyster Mussel, Cumberlandian Combshell, Purple Bean, and Rough Rabbitsfoot; final rule. Federal Register 62(7):1647–1658.

USFWS. 2012. Endangered and threatened wildlife and plants; determination of endangered status for the Sheepnose and Spectaclecase mussels throughout their range; final rule. Federal Register 80(83):24691–24774.

USFWS. 2013a. Endangered and threatened wildlife and plants; endangered species status for the Fluted Kidneyshell and Slabside Pearlymussel; final rule. Federal Register 78(187): 59269–59287.

USFWS. 2013b. Endangered and threatened wildlife and plants; endangered status for the Neosho Mucket and threatened status for the Rabbitsfoot; final rule. Federal Register 78(180):57076–57097.

USFWS. 2015. Endangered and threatened wildlife and plants; designation of critical habitat for Neosho Mucket and Rabbitsfoot; final rule. Federal Register 80(83):24691–24774.

van der Schalie, H. 1973. The mollusks of the Duck River drainage in central Tennessee. Sterkiana 52:45–56.

Watters, G.T., M.A. Hoggarth, and D.H. Stansbery. 2009. The Freshwater Mussels of Ohio. The Ohio State University Press, Columbus, OH. 421 pp.

Williams, J.D., A.E. Bogan, and J.T. Garner. 2008. Freshwater mussels of Alabama and the Mobile Basin. University of Alabama Press, Tuscaloosa, AL. 908 pp.

Williams, J.D., A.E. Bogan, R.S. Butler, K.S. Cummings, J.T. Garner, J.L. Harris, N.A. Johnson, and G.T. Watters. 2017. A revised list of the freshwater mussels (Mollusca: Bivalvia: Unionida) of the United States and Canada. Freshwater Mollusk Biology and Conservation 20:33–58.

Appendix 1. List of mussel species documented in the Buffalo River, by survey year. 1922 = Ortmann (1924), 1931 = Van der Schalie (1973), 1965 = Isom and Yokley (1968), 1980 = Ahlstedt (1991), 2002 = Ahlstedt et al. (2017), and 2011 = TVA field crew (unpubl. data).

Species	1922	1931	1965	1980	2002	2011	Present study
Margaritiferidae							
Margaritifera monodonta						X	
Unionidae							
Actinonaias ligamentina		X	X	X			X
Actinonaias pectorosa	X	X	X		X		X
Alasmidonta marginata	X		X				
Alasmidonta viridis	X						
Amblema plicata		X		X		X	X
Arcidens confragosus							X
Cyclonaias pustulosa		X				X	X
Cyclonaias tuberculata		X	X	X		X	X
Ellipsaria lineolata						X	X
Elliptio crassidens	X	X	X		X		X
Eurynia dilatata	X					X	X
Epioblasma ahlstedti		X					
Epioblasma aureola		X					
Fusconaia subrotunda							X
Hemistena lata		X	X				
Lampsilis cardium	X	X					X
Lampsilis fasciola	X		X			X	X
Lampsilis ovata	X	X	X			X	X
Lampsilis teres		X				X	X
Lasmigona complanata		X					
Lasmigona costata	X	X	X			X	X
Leptodea fragilis		X		X			X
Ligumia recta							X
Megalonaias nervosa							X
Obliquaria reflexa		X					X
Obovaria subrotunda	X	X	X				X
Pleurobema cordatum	X	X					X
Pleurobema oviforme	X	X	X				X
Pleurobema sintoxia		X					
Pleuronaia barnesiana	X			X	X		X
Pleuronaia dolabelloides	X	X				X	X
Potamilus alatus		X	X			X	X
Ptychobranchus fasciolaris			X				
Ptychobranchus subtentus	X						
Pyganodon grandis		X	X		X		X
Quadrula quadrula		X					X
Strophitus undulatus	X	X					X
Theliderma cylindrica		X					X
Theliderma metanevra							X

Species	1922	1931	1965	1980	2002	2011	Present study
Toxolasma cylindrellus	X						
Toxolasma lividum					X	X	X
Tritogonia verrucosa		X	X			X	X
Truncilla truncata		X				X	X
Utterbackia imbecillis		X					
Villosa iris	X	X		X	X	X	X
Villosa taeniata	X		X		X		X
Villosa vanuxemensis	X	X		X	X	X	X
Number of Sites Sampled	1	4	2	24	5	1	62
Total (48 species)	20	31	16	7	8	17	36

Appendix 2. Mussel species from the Buffalo River and cataloged at Carnegie Museum of Natural History (CM), University of Tennessee, McClung Museum of Natural History and Culture (UTMM), North Carolina State Museum of Natural Sciences (NCMNS), Museum of Biological Diversity at The Ohio State University (OSUM), and University of Michigan Museum of Zoology (UMMZ).

Species	CM	UTMM	NCMNS	OSUM	UMMZ
Actinonaias pectorosa	X		X	X	X
Alasmidonta marginata	X		X	X	
Alasmidonta viridis	X				
Amblema plicata		X	X		X
Cyclonaias tuberculata		X	X	X	X
Elliptio crassidens			X		X
Epioblasma ahlstedti					X
Epioblasma aureola					X
Epioblasma turgidula			X		
Hemistena lata				X	
Lampsilis cardium	X				
Lampsilis fasciola	X	X	X		
Lampsilis ovata			X	X	
Lasmigona costata	X		X		
Ligumia subrostrata		X			
Megalonaias nervosa			X		
Obovaria subrotunda	X		X	X	
Pleurobema oviforme	X			X	
Pleuronaia barnesiana	X				
Pleuronaia dolabelloides	X	X	X		
Potamilus alatus			X		
Ptychobranchus subtentus	X				
Pyganodon grandis		X	X		
Theliderma cylindrica			X		
Strophitus undulatus	X				
Toxolasma cylindrellus	X				
Toxolasma lividum			X	X	
Utterbackia imbecillis		X			
Utterbackiana suborbiculata		X			
Villosa iris	X		X		
Villosa taeniata	X	X	X		
Villosa vanuxemensis	X		X	X	
Number of species	15	9	19	9	6
Number of locations from which specimens originated	1	6	2	5	4

Appendix 3. Summary of mussels found in the Buffalo River during qualitative collections in 2012–2013, and an opportunistic collection made in 2016 at Buffalo River Mile (BRM) 3.2. Number of live and fresh dead mussels combined; number of relic shells in parentheses and shells collected in 2016 in brackets. Catch-per-unit effort (CPUE) is based on combined number of live and fresh dead found in qualitative sampling only and does not include opportunistic collection. Percent collected (% coll.) is calculated using the combined number of live mussels and dead shells. Species richness is the combined number of live mussels and dead shells. [A]Federally protected species. [B]New drainage record

Site	1	2	3	4	5	6	7	8	9	10	11
BRM	0.2	1.9	2.2	2.8	3.2	3.5	4.0	4.6	4.9	6.8	8.0
Species											
Actinonaias pectorosa					(1)						
Amblema plicata	2							2	(1)		(1)
Arcidens confragosus[B]					[1]						
Cyclonaias pustulosa					4	(41)			1		
Cyclonaias tuberculata	1				3	(17)	2(2)	2(1)	1	(1)	
Ellipsaria lineolata[B]	3					(1)					
Elliptio crassidens					2						
Eurynia dilatata	2		(1)		2(2)	(14)	(5)	1(1)	1(1)	1(2)	
Fusconaia subrotunda[B]								(2)			
Lampsilis cardium	1(1)										
Lampsilis fasciola	2		1(1)	(1)	2(1)	(3)	1(2)	1(2)	2(1)	1	
Lampsilis ovata					1	(2)	1				
Lampsilis teres					[1]						
Lasmigona costata		(1)			(1)	1		(1)	1		
Leptodea fragilis					1	1			(1)		
Ligumia recta[B]								(1)			
Obliquaria reflexa					(1)	2					
Obovaria subrotunda					1						
Pleurobema cordatum						(1)					
Pleurobema oviforme					(1)	(13)					
Pleuronaia barnesiana	6			2	1(2)	(20)	(4)	(1)		1	
Pleuronaia dolabelloides[A]		6			1	2(3)	(58)	1(3)	(6)		1
Potamilus alatus		1			2(3)	1(1)			(1)		
Pyganodon grandis					(1)						
Quadrula quadrula						1(1)					
Theliderma cylindrica[A]			2		6	(1)					
Theliderma metanevra[B]					[1]						
Toxolasma lividum					2	1(1)					
Tritogonia verrucosa	4									1(1)	
Truncilla truncata					1(4)	(10)					
Villosa iris								(1)		(1)	
Villosa taeniata									1(1)		
Villosa vanuxemensis					(1)		(1)				
Effort (person hours)	1.5	1	1	1	1.5	1.5	1	1	1	1	1
Total live and fresh dead	27	1	3	3	34	7	5	6	7	5	0
Total relic	1	1	2	1	21	284	17	16	6	5	1

Site	1	2	3	4	5	6	7	8	9	10	11
BRM	0.2	1.9	2.2	2.8	3.2	3.5	4.0	4.6	4.9	6.8	8.0
CPUE	18	1	3	3	20	4	5	6	7	5	0
Species richness	9	2	3	3	23	18	7	10	9	7	1

Site	12	13	14	15	16	17	18	19	20	21	22
BRM	12.0	13.8	16.3	17.7	19.4	22.4	25.8	32.0	34.3	41.4	42.8
Species											
Actinonaias pectorosa											
Amblema plicata						(1)					
Arcidens confragosus[B]											
Cyclonaias pustulosa											
Cyclonaias tuberculata				(1)		(1)					
Ellipsaria lineolata[B]											
Elliptio crassidens											
Eurynia dilatata					(1)	1					
Fusconaia subrotunda[B]											
Lampsilis cardium											
Lampsilis fasciola		2(2)	4(1)	2	2		1				
Lampsilis ovata				1							
Lampsilis teres											
Lasmigona costata					1(1)	1	1				
Leptodea fragilis			1(1)	1	1						
Ligumia recta[B]											
Obliquaria reflexa											
Obovaria subrotunda											
Pleurobema cordatum											
Pleurobema oviforme											
Pleuronaia barnesiana		(2)		1							
Pleuronaia dolabelloides[A]		(1)	(2)	(3)	1		1				
Potamilus alatus				1	2						
Pyganodon grandis											
Quadrula quadrula											
Theliderma cylindrica				1							
Theliderma metanevra[B]											
Toxolasma lividum											
Tritogonia verrucosa	1					1					
Truncilla truncata											
Villosa iris											
Villosa taeniata				1	1	2	1				2(2)
Villosa vanuxemensis											
Effort (person hours)	1	1	1	1	1	1	1	1	1	1	1
Total live and fresh dead	1	2	5	9	7	6	3	0	0	0	2
Total relic	1	6	5	1	2	2	0	0	0	0	2
CPUE	1	2	5	9	7	6	3	0	0	0	2
Species richness	2	3	3	9	6	7	3	0	0	0	1

Site	23	24	25	26	27	28	29	30	31	32	33
BRM	45.6	52.1	55.1	55.6	56.0	57.4	58.0	59.0	62.7	67.4	70.6
Species											
Actinonaias pectorosa											
Amblema plicata											
Arcidens confragosus[B]											
Cyclonaias pustulosa											
Cyclonaias tuberculata				(1)		(1)					
Ellipsaria lineolata[B]											
Elliptio crassidens				(1)							
Eurynia dilatata											
Fusconaia subrotunda[B]											
Lampsilis cardium											
Lampsilis fasciola			1	(1)		1(2)					
Lampsilis ovata											
Lampsilis teres											
Lasmigona costata											
Leptodea fragilis											
Ligumia recta[B]											
Obliquaria reflexa											
Obovaria subrotunda											
Pleurobema cordatum											
Pleurobema oviforme											
Pleuronaia barnesiana					(1)				2		
Pleuronaia dolabelloides[A]											
Potamilus alatus								1			
Pyganodon grandis											
Quadrula quadrula											
Theliderma cylindrica[A]											
Theliderma metanevra[B]											
Toxolasma lividum											
Tritogonia verrucosa											
Truncilla truncata											
Villosa iris									(1)		
Villosa taeniata		(1)	1	1	3(7)	(4)	(2)		1	(1)	2
Villosa vanuxemensis								1			
Effort (person hours)	1	1	1	1	1	1	1	1	1	1	1
Total live and fresh dead	0	0	2	1	3	1	0	2	3	0	2
Total relic	0	1	0	3	8	7	2	0	1	1	0
CPUE	0	0	2	1	3	1	0	2	3	0	2
Species richness	0	1	2	4	2	3	1	2	3	1	1

Site	34	35	36	37	38	39	40	41	42	43
BRM	73.5	78.7	80.5	87.0	90.6	93.6	98.2	100.6	102.9	105.3
Species										
Actinonaias pectorosa										
Amblema plicata										
Arcidens confragosus[B]										
Cyclonaias pustulosa										
Cyclonaias tuberculata										
Ellipsaria lineolata[B]										
Elliptio crassidens		(1)								
Eurynia dilatata										
Fusconaia subrotunda[B]										
Lampsilis cardium										
Lampsilis fasciola						1			1	
Lampsilis ovata										
Lampsilis teres										
Lasmigona costata										
Leptodea fragilis										
Ligumia recta[B]										
Obliquaria reflexa										
Obovaria subrotunda										
Pleurobema cordatum										
Pleurobema oviforme										
Pleuronaia barnesiana						(9)		3(5)	5(3)	
Pleuronaia dolabelloides[1]								2	5	
Potamilus alatus										
Pyganodon grandis										
Quadrula quadrula										
Theliderma cylindrica[A]										
Theliderma metanevra[B]										
Toxolasma lividum										
Tritogonia verrucosa										
Truncilla truncata										
Villosa iris										
Villosa taeniata	(3)	3		2		(8)	(4)	7(42)	12(13)	(5)
Villosa vanuxemensis								2	1	
Effort (person hours)	1	1	1	1	1	1	1	1	1	1
Total live and fresh dead	0	3	0	2	0	1	0	14	24	0
Total relic	3	1	0	0	0	17	4	47	16	5
CPUE	0	3	0	2	0	1	0	14	24	0
Species richness	1	2	0	1	0	3	1	4	5	1

	Site	44	45	46	47		Site	Distrib.	%
	BRM	111.3	116.7	121.0	122.0	Totals	freq.	(BRM)	coll.
Species									
Actinonaias pectorosa						(1)	1	3.2	0.2
Amblema plicata						4(3)	5	0.2–22.4	1.1
Arcidens confragosus[B]						1	1	3.2	0.2
Cyclonaias pustulosa						5(41)	3	3.2–4.9	7.9
Cyclonaias tuberculata						9(25)	10	0.2–57.4	5.9
Ellipsaria lineolata[B]						3(1)	2	0.2–3.2	0.7
Elliptio crassidens						2(2)	3	0.2–78.7	0.7
Eurynia dilatata						8(27)	10	0.2–22.4	6.0
Fusconaia subrotunda[B]						(2)	1	4.6	0.2
Lampsilis cardium						1(1)	1	0.2	0.4
Lampsilis fasciola						25(17)	19	0.2–102.9	7.3
Lampsilis ovata						3(2)	4	3.2–17.7	0.9
Lampsilis teres						1	1	3.2	0.2
Lasmigona costata						4(4)	8	1.9–25.8	1.6
Leptodea fragilis						5(2)	6	3.5–19.4	1.1
Ligumia recta[B]						(1)	1	4.6	0.2
Obliquaria reflexa						2(1)	2	3.2–3.5	0.5
Obovaria subrotunda						1	1	3.2	0.2
Pleurobema cordatum						(1)	1	3.5	0.2
Pleurobema oviforme						(14)	2	3.2–3.5	2.4
Pleuronaia barnesiana						21(47)	14	0.2–102.9	11.2
Pleuronaia dolabelloides[A]						20(76)	14	0.3–102.9	16.6
Potamilus alatus						8(5)	7	1.9–59.0	2.2
Pyganodon grandis						(1)	1	3.2	0.2
Quadrula quadrula						1(1)	1	3.5	0.4
Theliderma cylindrica[A]						9(1)	4	2.2–17.7	1.7
Theliderma metanevra[B]						1	1	3.2	0.2
Toxolasma lividum						3(1)	2	3.2–3.5	0.7
Tritogonia verrucosa						7(1)	4	0.2–22.4	1.4
Truncilla truncata						1(14)	2	3.2–3.5	2.6
Villosa iris						(3)	3	4.6–62.7	0.7
Villosa taeniata						40(93)	23	4.9–102.9	22.9
Villosa vanuxemensis						4(2)	5	3.2–102.9	1.0
Effort (person hours)		1	1	1	1				
Total live and fresh dead		0	0	0	0	189			
Total relic		0	0	0	0	390			
CPUE		0	0	0	0				
Species richness		0	0	0	0	33			

Appendix 4. Conservation status of mussels of the Buffalo River, TN. Federal status according to USFWS (2016). Species with Unknown status were either undetected or represented by relic shells only. E = Endangered, T = Threatened, X = Extinct.

		Status	
Scientific name	Common name	USFWS	Buffalo River
Margaritiferidae			
Margaritifera monodonta (Say)	Spectaclecase	E	Extant
Unionidae			
Actinonaias ligamentina (Lamarck)	Mucket		Extant
Actinonaias pectorosa (Conrad)	Pheasantshell		Extant
Alasmidonta marginata Say	Elktoe		Unknown
Alasmidonta viridis (Rafinesque)	Slippershell		Unknown
Amblema plicata (Say)	Threeridge		Extant
Arcidens confragosus (Say)	Rock Pocketbook		Extant
Cyclonaias pustulosa (Lea)	Pimpleback		Extant
Cyclonaias tuberculata (Rafinesque)	Purple Wartyback		Extant
Ellipsaria lineolata (Rafinesque)	Butterfly		Extant
Elliptio crassidens (Lamarck)	Elephantear		Extant
Epioblasma ahlstedti Jones and Neves	Duck River Dartersnapper	E	Unknown
Epioblasma aureola Jones and Neves	Golden Riffleshell	E	Unknown
Epioblasma turgidula (Lea)	Turgid Blossom	E,X	Unknown
Eurynia dilatata Rafinesque	Spike		Extant
Fusconaia subrotunda (Lea)	Longsolid		Unknown
Hemistena lata (Rafinesque)	Cracking Pearlymussel	E	Unknown
Lampsilis cardium Rafinesque	Plain Pocketbook		Extant
Lampsilis fasciola Rafinesque	Wavyrayed Lampmussel		Extant
Lampsilis ovata (Say)	Pocketbook		Extant
Lampsilis teres (Rafinesque)	Yellow Sandshell		Extant
Lasmigona complanata (Barnes)	White Heelsplitter		Unknown
Lasmigona costata (Rafinesque)	Flutedshell		Extant
Leptodea fragilis (Rafinesque)	Fragile Papershell		Extant
Ligumia recta (Lamarck)	Black Sandshell		Unknown
Ligumia subrostrata (Say)	Pondmussel		Unknown
Megalonaias nervosa (Rafinesque)	Washboard		Extant
Obliquaria reflexa Rafinesque	Threehorn Wartyback		Extant
Obovaria subrotunda (Rafinesque)	Round Hickorynut		Extant
Pleurobema cordatum (Rafinesque)	Ohio Pigtoe		Unknown
Pleurobema oviforme (Conrad)	Tennessee Clubshell		Unknown
Pleurobema sintoxia (Rafinesque)	Round Pigtoe		Unknown
Pleuronaia barnesiana (Lea)	Tennessee Pigtoe		Extant
Pleuronaia dolabelloides (Lea)	Slabside Pearlymussel	E	Extant
Potamilus alatus (Say)	Pink Heelsplitter		Extant
Ptychobranchus fasciolaris (Rafinesque)	Kidneyshell		Unknown
Ptychobranchus subtentus (Say)	Fluted Kidneyshell	E	Unknown
Pyganodon grandis (Say)	Giant Floater		Unknown

		Status	
Scientific name	Common name	USFWS	Buffalo River
Quadrula quadrula (Rafinesque)	Mapleleaf		Extant
Strophitus undulatus (Say)	Creeper		Unknown
Theliderma cylindrica (Say)	Rabbitsfoot	T	Extant
Theliderma metanevra (Rafinesque)	Monkeyface		Extant
Toxolasma cylindrellus (Lea)	Pale Lilliput	E	Unknown
Toxolasma lividum Rafinesque	Purple Lilliput		Extant
Tritogonia verrucosa (Rafinesque)	Pistolgrip		Extant
Truncilla truncata Rafinesque	Deertoe		Extant
Utterbackia imbecillis (Say)	Paper Pondshell		Unknown
Utterbackiana suborbiculata (Say)	Flat Floater		Unknown
Villosa iris (Lea)	Rainbow		Unknown
Villosa taeniata (Conrad)	Painted Creekshell		Extant
Villosa vanuxemensis (Lea)	Mountain Creekshell		Extant
Total (51 species)			