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STATUS OF FRESHWATER MUSSELS IN THE MIDDLE FORK HOLSTON RIVER, VIRGINIA

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ABSTRACT

Six sites in the Middle Fork Holston River (MFHR), Virginia, were surveyed in 2010 and 2011 using catch-per-unit-effort (CPUE, no./h) and 0.25 m^2 quadrats to assess changes in the mussel fauna since a previous survey in 1998. Since 1998, species richness declined from 15 to 11, compared to a historical richness of at least 20 species. To date, extirpated species are dominated by short-lived species, but all remaining species are declining rapidly. Mussel abundance, both as density (number/m^2) and CPUE, declined by ≥50% since 1998 at most sites, and several species collected during this study were represented by only a few individuals. There was no evidence of recent reproduction at the survey sites. Although the federally endangered *Epioblasma florentina aureola* appears to be extirpated, two species proposed for federal listing, *Pleuronaia dolabelloides* and *Ptychobranchus subtentum*, persist in the river. The MFHR appears to be another example of an enigmatic mussel decline characterized by curtailed recruitment and subsequent erosion of the fauna over time, despite a lack of obvious impacts to the stream. Twenty-six reaches in the MFHR watershed are listed as impaired, primarily by sediment and *E. coli*, suggesting that nutrient enrichment coupled with increases in streambed embeddedness could produce elevated substrate ammonia concentrations, which are toxic to juvenile mussels. In addition, limited sediment quality data indicate that metals, PCBs, and DDE are present in the stream and also may inhibit recruitment or have sublethal effects on adult mussels. The MFHR is an important refuge for the diverse Tennessee River basin mussel fauna, and identification and remediation of specific factors responsible for mussel declines are urgently needed.

KEY WORDS Freshwater mussels, Unionidae, Middle Fork Holston River

INTRODUCTION

The Middle Fork Holston River (MFHR) is a tributary of the Tennessee River system, and it historically supported a freshwater mussel fauna of at least 20 species (Henley et al., 1999). The MFHR potentially is an important conservation refuge for the unique mussel fauna of this region, and it previously supported one of only two remaining populations of *Epioblasma florentina aureola*; however, the fauna of the river has declined substantially in recent decades. By 1998, only 15 species were collected from the river, but abundances were low for most species and evidence of recent recruitment was absent at nearly all sites (Henley et al., 1999). These observations suggest a steady decline in mussel diversity and abundance throughout the river. Mussel abundance was exceptionally low downstream of the towns of Atkins, Marion, and Chilhowie, indicating possible effects of point source discharges. We resurveyed the MFHR in 2010 and 2011 to assess the current status of the mussel fauna with particular emphasis on documenting changes over the 12 years since the survey of Henley et al. (1999).

METHODS AND MATERIALS

The MFHR flows southwest through Wythe, Smyth, and Washington counties, southwestern Virginia, to its confluence with the South Fork Holston River near Abingdon (Fig. 1). The watershed lies within the Ridge and Valley physiographic province and is underlain primarily by limestone bedrock (Henley et al., 1999). Average discharge for the period between 1932 and 2010 at the USGS gaging station near Meadowview, Virginia, was 6.9 (±1.9 SD) cms, with a mean monthly summer flow of 3.8 (±2.6) cms and mean annual peak flow of 131.2 (±72.8) cms (USGS, 2012). The watershed covers approximately 625 km^2, and current land uses are roughly 49% forest, 41% pasture, 9% residential, and 1% cropland (USEPA, 2010). About 8 km of the stream are impounded by Edmonson Dam, located at MFHRKM 22.5, and approximately 2.4 km of the lower river are influenced by South Holston Lake. There is a small (approximately one m high), damaged dam at MFHRKM 32.7 in Chilhowie, Smyth County, that probably does not inhibit fish passage. Also, there is an approximately 2.4 m high milldam at MFHRKM 31.6 at DeBusk Mill, Washington County. This milldam does inhibit fish passage.

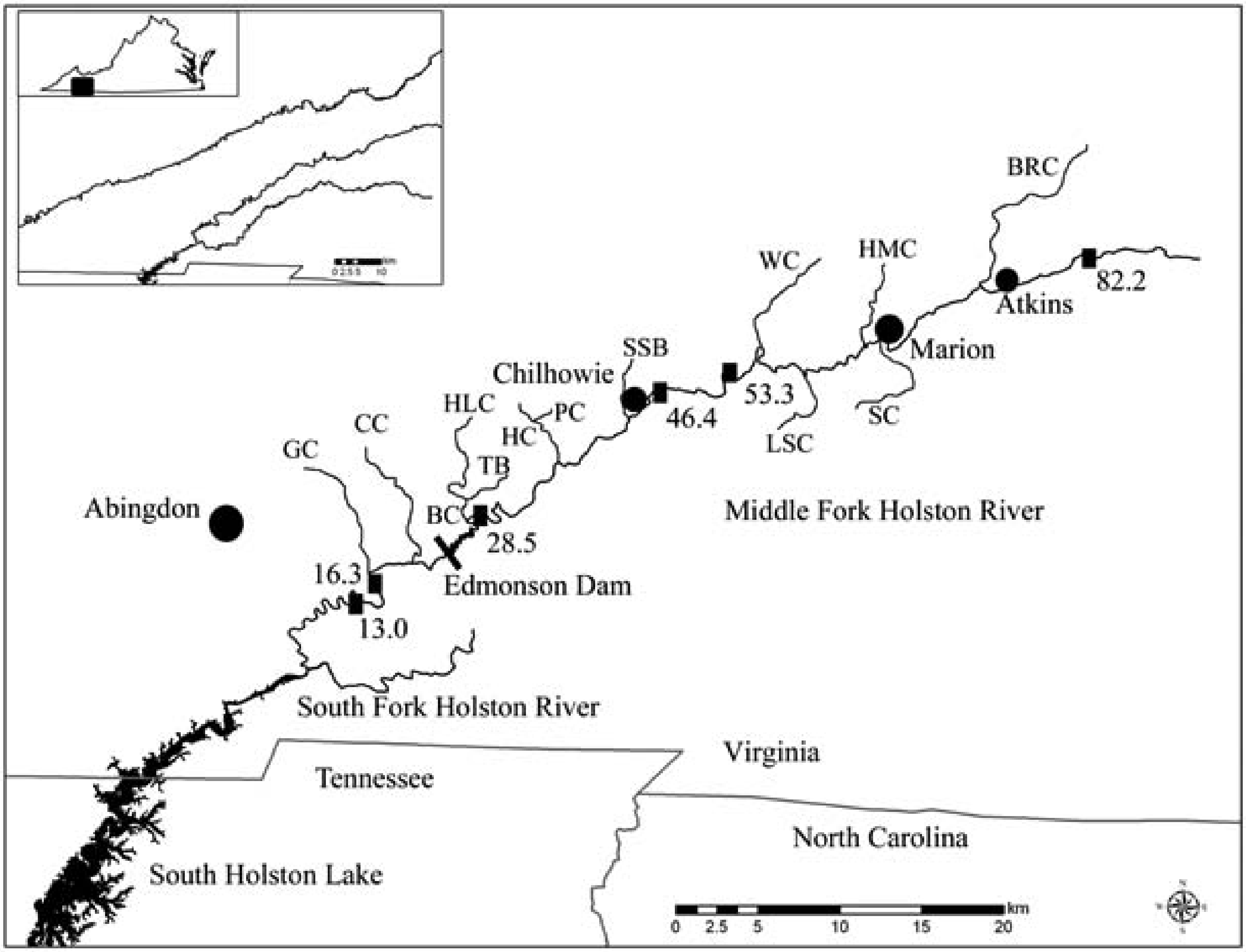

FIGURE 1

Mussel survey locations (indicated by squares and river kilometer) in the Middle Fork Holston River, Virginia, in 2010 and 2011. Circles indicate location of major towns. Tributaries are Greenway Creek (GW), Cedar Creek (CC), Hall Creek (HLC), Tattle Branch (TB), Byers Creek (BC), Hutton Creek (HC), Plum Creek (PC), Sulphur Springs Branch (SSB), Walker Creek (WC), Laurel Springs Branch (LSB), Hungry Mother Creek (HMC), Staley Creek (SC), and Bear Creek (BRC) (see impaired reach and tributary listings in Appendix 2.

We sampled freshwater mussels at six sites in the MFHR in 2010 and 2011 (Table 1; Fig. 1). Sample methods were similar to Henley et al. (1999). Mussel abundance at each site was measured in two ways: visual survey and quadrat sampling. These two methods were used because visual surveys cover more area and thus provide better estimates of site richness, but quadrat sampling provides better estimates of mussel density and size structure (Vaughn et al., 1997; Strayer & Smith, 2003). Survey crews consisted of at least two trained biologists for both methods.

TABLE 1

Study sites and results of mussel sampling in the Middle Fork Holston River, Virginia, during 2010. CPUE = catch per unit effort. Asterisks indicate that the absence of mussels in this study was due to bridge construction and prior mussel relocation (see text).

					Visual sampling		Quadrat sampling		
Site	County	Latitude (N)	Longitude (W)	Reach Length (m)	Total search time (person h)	Mussel CPUE(no./h)	No. of Transects	No. of Quadrats	Mussel density (no./m^2)
82.8	Smyth	36°53'19.08"	81°20'48.79"	60	1.67	0.00*	14	65	0.00*
53.3	Smyth	36°49'12.04"	81°37'08.08"	40	4.00	0.50	9	36	0.44
46.4	Smyth	36°50'14.36"	81°30'30.16"	50	3.40	2.34	15	60	0.07
28.5	Washington	36°50'06.38"	81°35'43.79"	120	17.10	6.20	24	96	0.33
16.3	Washington	36°44'23.98"	81°46'53.46"	40	2.00	88.00	9	36	1.67
13.0	Washington	36°41'32.28"	81°51'53.85"	45	2.00	0.00	10	40	0.10

Visual surveys were conducted using mask and snorkel to search the river bottom for mussels. During the searches, moderate-sized cobbles were overturned to locate mussels. When a live mussel was observed, its position in the substrate was marked by a flag. After visual survey at a site was complete, mussels marked by flags were removed, identified to species, measured for length, and returned to their original substrate position. The average duration of visual surveys across sites was 5.0 person-h (range = 1.7 – 17.1), and mussel abundance was expressed as catch per unit effort (CPUE, number/h).

Quadrat sampling was conducted with 0.25 m^2 quadrats positioned along transect lines perpendicular to the river channel. We sampled 36-96 quadrats on 9-24 transects at each site depending on site length (Table 1). The position of the first transect at each site was determined by selecting an arbitrary starting position at the downstream end of the sample reach, and then using a random number table to determine the number of paces upstream from the starting position for placement of the first transect. All subsequent transects were placed at 5 m intervals in an upstream direction. Four quadrats were randomly placed along each transect using a random number table, and quadrats were excavated to hardpan or to approximately 25 cm. Substrate from quadrats was not sieved, but we attempted to examine excavated substrate carefully for the presence of juvenile or other small mussels. Mussel densities were expressed as number/m^2. Mussels in each quadrat were identified to species, measured for length, and replaced at the point of collection.

We used a generalized linear mixed model (GLIMMIX, SAS Institute Incorporated, Cary, North Carolina) to test for statistical differences in mean density between 1998 amd 2010 at each site. The response variable (site mussel density) was designated as having a Poisson distribution with transects and quadrats set as random variables (quadrats within transects). The quadrat data provided adequate fit to the Poisson distribution (SAS generalized linear model, GENMOD: df=532, deviance X^2=561.12, p=0.185). We compared mean river-wide CPUE between 1998 and 2010 using natural log-transformed CPUE data and a paired t-test (Minitab 16, Minitab Incorporated, College Station, Pennsylvania). In previous surveys, including 1998, *Lasmigona holstonia* was only found in the headwaters of the river near MFHRKM 82.8 (Table 2). After conducting our survey in 2010, we learned that bridge reconstruction had occurred at this site in 2002, and an effort was made at that time to translocate as many individuals of this species as possible to another nearby site (Mair & Neves, 2002). This may explain the absence of *L. holstonia* in our survey at MFHRKM 82.8. Therefore, data from MFHRKM 82.8 were not included in the statistical analyses.

TABLE 2
Historical changes in mussel species richness in the Middle Fork Holston River.

Species	Study				
	Ortmann (1918)	Stansbery and Clench (1974)	Neves et al. (1980)	Henley et al. (1999)	This study
Actinonaias ligamentina			X		
Actinonaias pectorosa		X	X	X	X
Alasmidonta marginata	X	X	X		
Alasmidonta viridis	X				
Cyclonaias tuberculata		X		X	X
Elliptio dilatata	X	X	X	X	X
Epioblasma f. aureola	X	X	X	X	
Lampsilis fasciola	X	X	X	X	X
Lampsilis ovata		X	X		
Lasmigona costata	X	X	X	X	
Lasmigona holstonia		X		X	
Medionidus conradicus	X	X	X	X	
Pegias fabula		X			
Pleurobema oviforme	X	X	X	X	X
Pleuronaia barnesiana	X	X	X	X	X
Pleuronaia dolabelloides		X	X	X	X
Ptychobranchus fasciolaris		X	X	X	X
Ptychobranchus subtentum	X	X	X	X	X
Villosa iris	X	X	X	X	X
Villosa vanuxemensis	X	X	X	X	X
Total Species	12	18	16	15	11

Two types of data from the Virginia Department of Environmental Quality (VDEQ) were obtained to assess potential causes of mussel declines in the MFHR: a list of reaches in the MFHR and tributaries impaired for recreational and/or aquatic life uses under the criteria of sections 303(d) and 305(b) of the US Clean Water Act (VDEQ, 2010; 2012), and results of sediment contaminant analyses from two sites (T. Frasier, VDEQ, Abingdon, Virginia, unpublished data). Sites with sediment analysis were MFHRKM 16.3 (only metals analyzed from one sediment sample from 2008) and 42.0 (metals and organics contaminants analyzed from 20 collection dates from 1981 to 1998). Although MFHRKM 42.0 was not one of our survey sites, sediment results from this site probably represent past activities from upstream locations in the watershed, including the towns of Atkins, Marion, and Chilhowie, Virginia. Sediment samples were analyzed for metals and organic compounds by the Division of Consolidated Laboratory Services, Department of General Services, Richmond, Virginia, using USEPA methods for sample preparation (3005A) and analysis (200.8) (USEPA, 1992; 1994). We reported only contaminants that were above detection limits from the VDEQ sediment database. We compared sediment contaminant concentrations measured at MFHRKM 16.3 and 42.0 with the consensus-based freshwater sediment quality guidelines of MacDonald et al. (2000), and when guidelines were not provided for a certain contaminant, freshwater sediment-screening benchmarks of USEPA (2006) were used.

RESULTS

Mussel species richness in the MFHR has declined substantially in recent decades (Table 2). Ortmann (1918) surveyed only 2 sites in the MFHR in 1912 and 1913 but found 12 species. Stansbery and Clench (1974) surveyed 21 sites in the late 1960s and early 70s and found 18 species, and Neves et al. (1980) collected 17 species at nine sites in the late 1970s; together, these surveys reported 20 species present in the river prior to 1980. In 1998, Henley et al. (1999) found 15 species, but we found only 11 species in 2010, representing roughly half of historical richness. Only one site yielded no mussels (MFHR 82.8; see Methods). At each of our five study sites where mussels were observed, richness was approximately half that observed in 1998 (Fig. 2; Appendix 1), and several species were extremely rare.

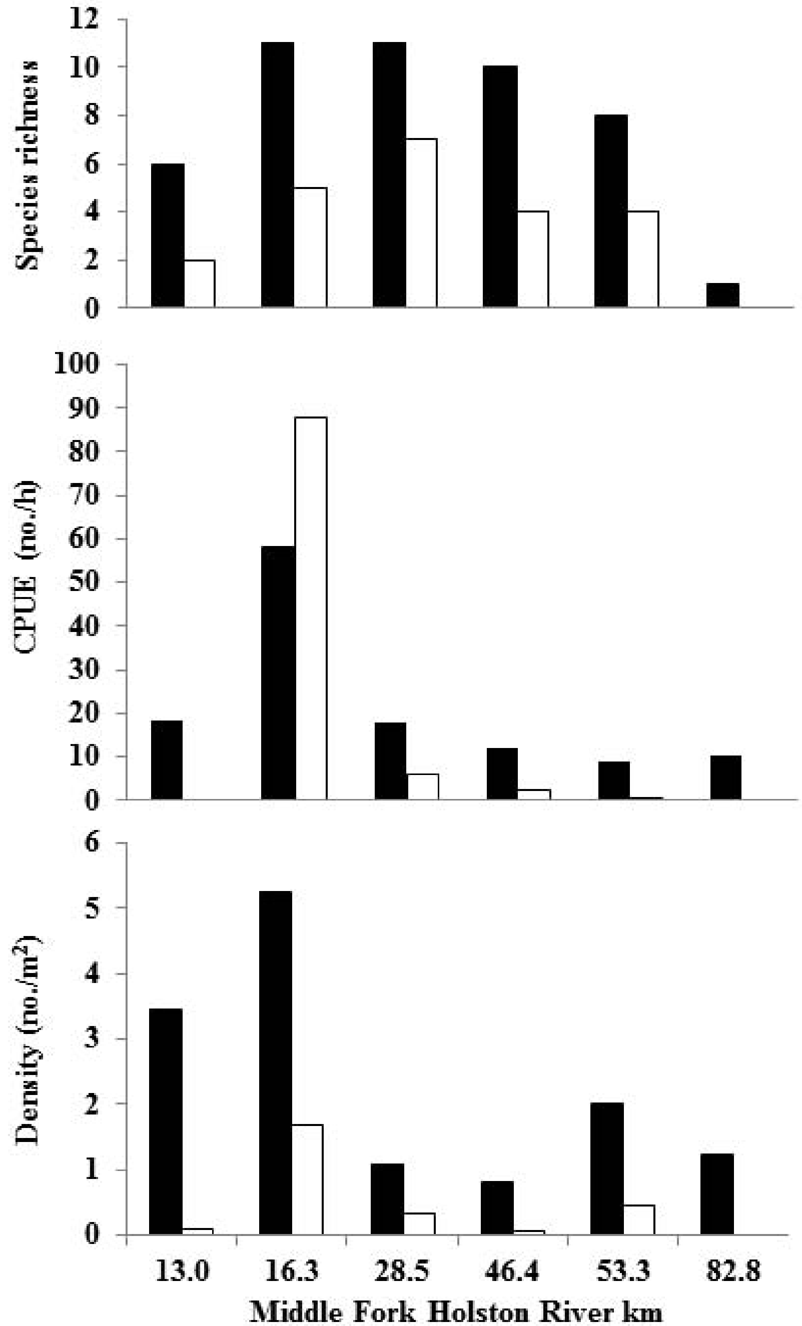

FIGURE 2

Changes in mussel assemblages at six sites in the Middle Fork Holston River (MFHR) from 1998 to 2010. Black bars are results of 1998 surveys and white bars are 2010. No mussels were found at MFHR kilometer location 82.8 in 2010 due to mussel relocation prior to our survey (see text).

Lampsilis fasciola and *Pleurobema oviforme* each were represented by single individuals, and *Elliptio dilatata* and *Pleuronaia barnesiana* were represented by only 4 and 5 individuals, respectively. Two species proposed for federal listing as endangered were collected during this survey: *Ptychobranchus subtentum* at MFHRKM 46.4 and *Pleuronaia dolabelloides* at MFHRKM 16.3 and 13.0. *Epioblasma f. aureola* was collected only at MFHRKM 28.5 in 1998, but the species was not observed in 2010. In subsequent sampling in 2013, one live *P. subtentum* was collected at MFHRKM 16.3 (one of our sample sites), and numerous *P. dolabelloides* were collected at MFHRKM 15.1 (a previously unsurveyed site; D. Schilling, personal communication).

Mussel abundance declined dramatically from 1998 to 2010 (Fig. 2; Appendix 1). Mussel abundance, as measured by CPUE, declined by ≥50% at all sites except for MFHR 16.3, where CPUE was higher in 2010 (88.0 mussels/h in 2010 versus 58.3/h in 1998). Mean (±SD) river-wide CPUE declined only slightly but significantly from 23.1±20.1/h in1998 to 19.4±38.4/h in 2010 (*ln*CPUE, *n*=5, *t*=2.45, p=0.035), but the small magnitude of this difference is a result of the high CPUE at MFHR 16.3 in 2010, which was nearly 20X higher than all other sites in 2010. River-wide mussel density declined more dramatically from 2.5±1.8/m^2 in 1998 to 0.5±0.7/m^2 in 2010 (*df*=1, *F*=36.55, p<0.0001). A few species appeared to show slight increases in abundance at some sites (Appendix 1), but the precision of these estimates is low, and these apparent increases probably are not statistically significant.

There was no evidence of recent recruitment at any of our survey sites. The mean size of each species indicated that all individuals were adults, and many individuals appeared to be of advanced age (Table 3). The smallest individual observed was a 28.5 mm *Villosa iris*. Subsequent sampling at MFHRKM 16.3 in 2013 found a few *Pleuronaia dolabelloides* that were estimated to be 7-10 years old (D. Schilling, personal communication).

TABLE 3

Mean and minimum lengths (in parentheses, both mm) of mussels collected in the Middle Fork Holston River in 2010. Values are from combined results of CPUE and quadrat sampling; integers below size measurements are the number of collected and measured individuals; if only one mussel was measured at a site, then no minimum size is provided. No mussels were found at MFHRKM 82.8 due to mussel relocation prior to our survey (see text).

	Middle Fork Holston River Sites (km)				
Species	**53.3**	**46.4**	**28.5**	**16.3**	**13.0**
Actinonaias pectorosa	-	-	110.3 (107.4) 3	118.8 (95.0) 124	-
Cyclonaias tuberculata	-	-	-	83.7 (75.0) 9	-
Elliptio dilatata	72.0 1	88.5 1	88.1 (86.1) 2	-	-
Lampsilis fasciola	46.1 1	-	-	-	-
Pleuronaia barnesiana	-	64.4 1	57.0 (52.3) 4	-	-
Pleuronaia dolabelloides	-	-	-	78.3 (57.0) 44	86.0 1
Pleurobema oviforme	-	-	57.2 1	-	-
Ptychobranchus fasciolaris	-	-	87.3 (70.6) 40	108.7 (98.0) 14	-
Ptychobranchus subtentum	-	100.4 (89.7) 6	-	-	-
Villosa iris	45.1 1	-	49.7 (28.5) 31	-	-
Villosa vanuxemensis	38.4 (32.4) 3	49.2 1	46.2 (34.0) 33	-	-

Twenty-six reaches of the MFHR and tributaries are classified as impaired for recreational and/or aquatic life uses by the VDEQ (VDEQ, 2010; 2012; Appendix 2). All of the sites we surveyed are in impaired reaches except MFHRKM 13.0. Causes of impairment in the watershed listed by VDEQ include sediment, *Escherichia coli*, fecal coliform, and alterations to benthic macroinvertebrate communities, all generally attributed to unrestricted livestock access to water bodies, animal feeding operations, crop production, grazing in riparian zones, and the category "rural (residential areas)" that presumably describes inadequate and failing residential septic systems (VDEQ 2010, 2012). Of particular importance to remaining mussel populations, including the federal candidate species *Pleuronaia dolabelloides* and *Ptychobranchus subtentum*, is the impaired reach that contains site MFHRKM 16.3 (VAS-O05R-MFHR3A00, recreational impairment), and two impaired tributaries that enter MFHR in this area (Greenway Creek, VAS-O05R-GRW01A02, aquatic life and recreational impairments; and Cedar Creek, VAS-O05-CED01A94 and VAS-O05-ECE01A02, aquatic life and recreational impairments) (Appendix 2). The reach of the MFHR that contains MFHRKM 46.4 where *P. subtentum* also was found is impaired (VAS-O04R-MFH01A00, recreational impairment). In impairment notes related to this reach, VDEQ states that DDT was detected in sediment samples.

Many contaminant concentrations measured in sediment at MFHRKM 42.0 were above suggested screening levels, but no metal concentrations were above these levels at MFHRKM 16.3 (Appendix 3). At MFHRKM 42.0, mean concentrations of antimony, iron, lead, manganese, and selenium were above sediment quality guidelines (SQG); however, concentrations of arsenic, cadmium, mercury, nickel, zinc, total polychlorinated biphenyls (PCBs), and dichlorodiphenyldichloroethylene (DDE, breakdown byproduct of DDT) also periodically rose above SQGs (Appendix 3). The contaminant levels measured from sediment from MFHRKM 42.0 result from upstream activities, including those in and around the towns of Atkins, Marion, and Chilhowie.

DISCUSSION

The mussel fauna of the MFHR has declined dramatically since 1998 in both species richness and abundance, and the lack of recruitment portends a further diminishment of the fauna. However, this decline appears to have begun prior to 1998 judging by the meager evidence of recruitment and the disappearance of several species at the time of the Henley et al. (1999) survey. The decline of the MFHR fauna is yet another example of an unexplained, enigmatic mussel decline characterized by a gradual erosion of mussel diversity apparently due to curtailment of recruitment (Haag, 2012). It is noteworthy that most species that have disappeared from the MFHR are short-lived (life span < about 20 y; e.g., *Alasmidonta* spp., *Epioblasma florentina aureola*, *Lasmigona* spp., *Medionidus conradicus*, *Pegias fabula*) (Haag & Rypel, 2010). The remaining fauna is composed mainly of long-lived species (lifespan > 30 y) such as *Actinonaias pectorosa*, *Cyclonaias tuberculata*, *Elliptio dilatata*, *Pleuronaia* spp., and *Ptychobranchus fasciolaris*, but the uniformly large size of these individuals suggests that they recruited prior to the appearance of factors that now limit recruitment. As in other streams that have experienced enigmatic mussel declines, factors responsible for the lack of recruitment in the MFHR are unknown. Sediment and fecal bacteria inputs are sources of use impairment in the MFHR. The river is prone to extended periods of high turbidity after rain events, and in our experience, the water clears much more slowly after these events than in other streams in southwestern Virginia. No studies have determined primary sources of these contaminants, but our observations suggest that unrestricted cattle access and erosion in riparian zones are major causes. This also is concordant with the fact that 41% of the watershed is pastureland (USEPA, 2010). The effect of fecal bacteria on mussel survival is unknown, and there is little evidence for a direct negative effect of sediment (Haag, 2012). However, extended periods of suspended solids, as seen in the MFHR, can cause sharply reduced fertilization of mussel eggs (Gascho Landis et al., 2013).

Both of these factors may be indirectly involved in mussel declines via their role in increasing ammonia concentrations in stream sediments. Juvenile mussels are highly sensitive to ammonia (Augspurger et al., 2003; Geist & Auerswald, 2007; Wang et al., 2007a, 2007b). Animal manure is a major source of nutrient enrichment and eutrophication in streams, which also can lead to elevated ammonia, and unrestricted cattle access to streams is linked to increased total nitrogen, total phosphorus, total suspended solids, ammonium, turbidity, and *E. coli* (Vidon et al., 2008). Sedimentation can further exacerbate ammonia levels in the streambed by reducing interstitial sediment oxygen concentrations, which in turn reduces the ability of nitrifying bacteria to convert ammonia to less toxic nitrates. Because juvenile mussels reside primarily in and feed on sediments, they may be inordinately exposed to elevated ammonia in the streambed (Cope et al., 2008; Strayer & Malcom, 2012). Testing of sediment ammonia and oxygen concentrations in the MFHR is urgently needed to evaluate this potential cause of mussel declines. In addition, a wide variety of landowner incentive programs designed to restrict cattle access to streams are available through agencies such as the National Resource Conservation

Service, and promotion of these programs could help reduce this source of contamination in the watershed.

The available sediment data are not contemporary with our study period, but the persistence of metals, PCBs, DDE, and other compounds in sediment suggests that they still may limit juvenile survival. Resuspension and reoxygenation of these sediment contaminants during sustained turbidity events also may contribute to their continued bioavailability (Eggleton & Thomas, 2004). In addition to juvenile mortality, chronic exposure may result in sublethal effects to remaining adult mussels in the MFHR. Many of these compounds can negatively affect gamete production and quality and larval survival in other organisms including marine bivalves (Bayne et al., 1981; McDowell et al., 1999; Pocar et al., 2003; Tay et al., 2003; Lewis & Ford, 2012), but their effects on freshwater mussels are largely unknown.

Sediment data show considerable variation in contaminant levels among sites. Metals were not above screening guidelines at MFHRKM 16.3, but many exceeded screening levels at MFHRKM 42.0. Edmonson Dam (MFHRKM 22.5) and DeBusk milldam (MFHRKM 31.6) may act as settling basins that intercept many contaminants before they reach the lower river. This phenomenon may partially explain the higher mussel abundance that we observed at MFHRKM 16.3 in the lower river, but the lack of recruitment even at this site indicates the presence of significant stressors throughout the river.

The MFHR is an important refuge for the diverse Tennessee River basin mussel fauna, and identification and remediation of specific factors responsible for mussel declines are urgently needed. Mitigation efforts throughout the river should to be guided by results of sediment and pore-water contaminant analyses. Therefore, we recommend that Virginia state agencies coordinate the collection of sediment and interstitial water samples at sites along the length of the river for determinations of organic and inorganic contaminants. Possible links among these data and current industrial discharges in the watershed need to be determined. We also suggest immediate implementation of best management practices (BMPs, e.g., riparian restoration, fencing, and alternative water sources) that would reduce nutrient and sediment inputs. An overarching motivation for these efforts is that the drastic decline in mussel species richness and abundance is an indicator of highly degraded conditions in the river and its watershed, which affects all stakeholders in the region. Without determination of specific stressors and appropriate mitigation, the mussel fauna of the MFHR likely will disappear completely as remaining individuals become senescent and die.

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APPENDIX 1

Species relative abundance and density at six sites in the MFHR in 1998 (Henley et al., 1999) and 2010 (this study). Top values for each species are CPUE (number/h) and bottom values are density (number/m^2). Asterisks indicate that the absence of mussels in this study was due to bridge construction and prior mussel relocation (see text). Dashes indicate species not observed.

Species	Middle Fork Holston River Sites (km)											
	82.8		53.3		46.4		28.5		16.3		13.0	
	1998	2010	1998	2010	1998	2010	1998	2010	1998	2010	1998	2010
Actinonaias pectorosa	-	-	-	-	-	-	-	0.18 0.00	48.4 2.88	59.00 0.67	3.53 0.20	-
Cyclonaias tuberculata	-	-	-	-	-	-	-	-	0.49 0.00	3.50 0.22	0.94 0.12	-
Elliptio dilatata	-	-	2.86 0.88	0.25 0.00	1.00 0.08	0.29 0.00	0.17 0.00	0.12 0.00	-	-	-	-
Epioblasma f. aureola	-	-	-	-	-	-	0.17 0.00	-	-	-	-	-
Lampsilis fasciola	-	-	0.14 0.00	0.00 0.11	0.50 0.00	-	1.17 0.00	-	0.25 0.00	-	-	-
Lasmigona costata	-	-	-	-	-	-	-	-	0.50 0.00	-	-	-
Lasmigona holstonia	10.3 1.24	* *	-	-	-	-	-	-	-	-	-	-
Medionidus conradicus	-	-	-	-	1.00 0.00	-	2.00 0.08	-	-	-	-	-
Pleurobema oviforme	-	-	3.29 0.44	-	6.00 0.28	-	8.50 0.40	0.06 0.00	1.47 0.24	-	2.11 0.00	-
Pleuronaia barnesiana	-	-	0.14 0.12	-	0.50 0.12	0.29 0.00	1.50 0.12	0.23 0.00	0.98 0.12	-	-	-
Pleuronaia dolabelloides	-	-	0.29 0.00	-	0.50 0.00	-	1.00 0.00	-	5.66 1.76	19.50 0.56	11.5 3.12	0.00 0.10
Ptychobranchus fasciolaris	-	-	-	-	-	-	0.83 0.12	2.16 0.13	0.00 0.24	6.00 0.22	0.24 0.00	-
Ptychobranchus subtentum	-	-	-	-	0.50 0.12	1.47 0.07	-	-	0.25 0.00	-	-	-
Villosa iris	-	-	0.14 0.00	0.00 0.11	0.50 0.08	-	1.17 0.20	1.58 0.17	0.25 0.00	-	-	-
Villosa vanuxemensis	-	-	2.14 0.56	0.25 0.22	1.50 0.12	0.29 0.00	1.00 0.16	1.87 0.04	-	-	-	-
Site Total	10.3 1.24	* *	9.00 2.00	0.50 0.44	12.00 0.80	2.34 0.07	18.01 1.08	6.20 0.33	58.25 5.24	88.00 1.67	18.32 3.44	0.00 0.10

APPENDIX 2

Stream reaches in the Middle Fork Holston River (MFHR) watershed listed as impaired by Virginia Department of Environmental Quality under sections 303 (d) and 305 (b) of the Clean Water Act (VDEQ, 2010; 2012). In the VDEQ database, all assessment unit labels are preceded by the prefix 'VAS-'; note that Cedar and Staley creeks each have two impaired assessment units. In columns under "Impairment Cause", the first value is the length (km) of the impaired reach, and the second value (in parentheses) is the actual or target date for Total Maximum Daily Load (TMDL) development. Cause category 4A indicates that TMDL is not necessary or previously developed, and 5A indicates that TMDL is required. Impairment causes are determined by exceedance of impairment thresholds. Benthic impairment cause was determined using the Virginia Stream Condition Index (VDEQ, 2010). Tributary locations are shown on Figure 1.

				Impairment Cause				Use Impairment	
Assessment Unit (MFHR reach)	Stream	County	Cause Category	*Escherichia coli*	Fecal coliform	Benthic	Sediment	Recreation	Aquatic Life
O05R_BYS01A94	Byers Creek	Washington	4A	0.8 (2002)	-	0.8 (2004)	-	√	√
O05R_CED01A94 O05R_ECE01A02	Cedar Creek	Washington	4A	10.5 (2002)	3.0 (2002)	13.5 (2004)	13.5 (2004)	√	√
O05R_HAL01A94	Hall Creek	Washington	4A	-	10.9 (2002)	10.9 (2004)	10.9 (2004)	√	√
O05R_HTO01A94	Hutton Creek	Washington	4A	7.7 (2002)	-	7.7 (2004)	7.7 (2004)	√	√
O05R_MFHR04A00	MFHR	Smyth & Washington	4A	-	14.7 (2014)	14.7 (2020)	-	√	√
O05R_MFHR03A00	MFHR	Washington	4A	4.2 (2014)	-	-	-	√	-
O05R_MFHR05A00	MFHR	Washington	4A	-	5.9 (2014)	5.9 (2014)	-	√	√
O03R_MFHR01A00	MFHR	Smyth, Washington, & Wythe	4A	28.9 (2014)	8.8 (2014)	-	-	√	-
O03R_MFHR02A00	MFHR	Smyth, Washington, & Wythe	4A	8.3 (2014)	8.3 (2014)	-	-	√	-
O03R_MFHR05A04	MFHR	Smyth, Washington, & Wythe	4A	5.2 (2022)	5.9 (2014)	5.9 (2014)	-	√	√
O03R_MFHR04A98	MFHR	Smyth, Washington, & Wythe	4A	-	6.8 (2014)	-	-	√	-
O05R_PLU01A02	Plum Creek	Washington	4A	-	3.5 (2002)	3.5 (2004)	-	√	√
O05R_TAT01A02	Tattle Branch	Washington	4A	-	4.4 (2002)	4.4 (2004)	4.4 (2004)	√	√
O05R_XCG01A02	Unnamed tributary to Hall Creek	Washington	4A	-	2.7 (2002)	2.7 (2004)	2.7 (2004)	√	√
O05R_XCD01A02	Unnamed tributary to Hutton Creek	Washington	4A	-	6.5 (2002)	6.5 (2004)	6.5 (2004)	√	√
O05R_XDY01A08	Unnamed tributary to MFHR	Washington	4A	1.4 (2020)	-	-	-	√	-
O05R_CWF01A02	West Fork Cedar Creek	Washington	4A	-	2.5 (2002)	2.5 (2004)	2.5 (2004)	√	√
O03R_BER01A02	Bear Creek	Smyth	5A	9.0 (2022)	-	-	-	√	-
O03R_BER02A04	Bear Creek	Smyth	5A	-	-	6.5 (2020)	-	-	√
O05R_GRW01A02	Greenway Creek	Washington	5A	7.8 (2020)	-	7.8 (2022)	-	√	√
O04R_HUN02A02	Hungry Mother Creek	Smyth	5A	7.8 (2018)	-	-	-	√	-
O04R_LRL1A04	Laurel Springs Creek	Smyth	5A	3.3 (2018)	-	-	-	√	-
O03R_STA01A02 O03R_STA01B10	Staley Creek	Smyth	5A	11.0 (2022)	-	-	-	√	-
O04R_SUL01A12	Sulphur Springs Branch	Smyth	5A	11.0 (2024)	-	-	-	√	-
O04R_WAL01A02	Walker Creek	Smyth	5A	20.6 (2018)	-	-	-	√	-

APPENDIX 3

Mean (±SE) contaminant concentrations in sediment collected by Virginia Department of Environmental Quality (VDEQ) at Middle Fork Holston River kilometer locations 42.0 (Washington-Smyth county line approximately 2.4 km downstream of Chilhowie, Virginia; 20 collection dates during 1981 to 1998) and 16.3 (see Table 1 for location; one collection date in 2008). *n* is the number of observations that were above detection limits. Sediment screening levels are criteria used to evaluate the risk of an observed contaminant concentration to aquatic organisms as follows. TEC is the consensus-based threshold effects concentration below which harmful effects on sediment-dwelling organisms are not expected (MacDonald et al., 2000; clarification of TEC for selenium provided by A. D. Lemly, USDA Forest Service and Department of Biology, Wake Forest University, Winston-Salem, North Carolina, personal communication). PEC is the consensus-based probable effects concentration above which harmful effects on sediment-dwelling organisms are expected to occur frequently (MacDonald et al., 2000). FSSB are freshwater sediment screening benchmarks used to evaluate sediment data from Superfund sites and to classify ecological risk (USEPA, 2006). Effect range – low (ERL) is the concentration below which adverse effects would be rarely observed (MacDonald et al., 2000). Lowest effect level (LEL) is the concentration below which no effects on the majority of sediment-dwelling organisms are expected (MacDonald et al., 2000). FSSB criteria were used only when TEC or PEC were not available.

	Site									
	42.0				16.3			Sediment Screening Levels		
Analyte	**Mean**	**SE**	**Range**	***n***	**Mean**	**SE**	***n***	**TEC**	**PEC**	**FSSB**
Metals (mg/kg DW)										
Aluminum	13,584	1,270	9,390 – 17,400	5	8,620	-	1	-	-	-
Antimony	20.0	13.0	7.0 – 33.0	2	-	-	-	-	-	2.0 ERL
Arsenic	7.3	0.8	2.0 -13.2	11	-	-	-	9.79	33.0	-
Cadmium	0.8	0.3	0.2 – 2.5	7	-	-	-	0.99	4.98	-
Chromium	21.8	1.3	13.9 – 31.0	14	17.8	-	1	43.4	111	-
Copper	21.8	1.6	10.1 – 31.0	14	15.0	-	1	31.6	149	-
Iron	21,170	978	18,700 – 23,700	5	15,200	-	1	-	-	20,000 LEL
Lead	36.0	5.2	13.0 – 87.5	14	22.4	-	1	35.8	128	-
Manganese	650.2	63.1	495 – 819	5	358.0	-	1	-	-	460 LEL
Mercury	0.10	0.03	0.10 – 0.30	6	-	-	-	0.18	1.06	-
Nickel	20.0	1.1	9.6 – 25.0	14	13.7	-	1	22.7	48.6	-
Selenium	4.0	2.0	2.1 – 6.0	2	-	-	-	-	-	2.0 TEC
Thallium	1.6	0.6	1.0 – 2.1	2	-	-	-	-	-	-
Zinc	102.9	6.9	76.7 – 146.0	13	89.2	-	1	121	459	-
Total PCBs (μg/kg DW)	25.8	24.8	1.0 – 100.0	4	-	-	-	59.8	676	-
DDE (μg/kg DW)	6.8	6.7	0.1 – 40.0	6	-	-	-	3.16	31.3	-