Recovery Status of Freshwater Mussels (Bivalvia: Unionidae) in the North Fork Holston River, Virginia

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Abstract: To determine the degree of recovery of mussels from mercury (Hg) contamination in the North Fork Holston River (NFHR) downstream of the Superfund site at Saltville, Virginia (NFHRM 80.3), 19 sites were sampled using catch-per-unit-effort (no./h) sampling method and 3 sites were surveyed with quadrats (0.25 m^2). Nine species of live freshwater mussels were observed in the river, and juveniles were noted at 5 sites (30 juveniles of 4 species). The first mussel assemblage, as defined by numerous animals of multiple species, was located at NFHRM 59.9, approximately 20.4 river miles downstream of Saltville. The greatest number of species was observed at NFHRM 11.0 (5 species), while the greatest mussel density (2.6 mussels/m^2), the greatest number of juveniles (11), and the greatest species richness of juveniles (3 species) were observed at NFHRM 13.5. Random catch-per-unit-effort at surveyed sites, as well as the number of juvenile species observed, were correlated to total Hg, but not methylmercury content, as measured in *Corbicula fluminea* (Müller, 1774) from proximate sites. Based on the appearance of multiple species and age classes, as well as the presence of juvenile mussels, recovery of freshwater mussels begins to occur roughly 20 river miles downstream of the Hg contaminated Superfund site at Saltville.

Key Words: freshwater mussels, Unionidae, North Fork Holston River, mercury, survey

The North Fork Holston River (NFHR) in southwestern Virginia flows in a generally southwestern direction through Bland, Smyth, Washington, and Scott counties to its confluence with the mainstem Holston River in Tennessee (Fig. 1). This Tennessee River tributary is a moderately hard-water stream with $CaCO_3$ concentrations from 35 mg/l to 239 mg/l. Hill *et al.* (1974) described the NFHR as having a high riffle-pool ratio, and a substratum consisting mainly of sand, gravel, and cobble. Average discharge for the period from October 1931 to December 1981 was estimated at 25.7 m^3/s near Gate City, Scott County, Virginia. The ${}_7Q_{10}$ for this period was estimated at 1.7 m^3/s, and the average monthly summer flow (July through September) is roughly 9.7 m^3/s.

The river is surrounded by moderate agricultural and low urban development, and has historically supported a high faunal diversity. Environmental degradation above Saltville, Virginia has been minimal, but large quantities of elemental mercury (Hg) and chloride salts were discharged into the river at Saltville during this century, contaminating more than 128 km of this river to its confluence with the Holston River in Tennessee (Sheehan *et al.*, 1989; Seivard *et al.*, 1993). Utilizing local salt deposits, the Mathieson Alkali Works began production of soda ash in 1894 at Saltville, Virginia (Seivard *et al.*, 1993). In 1950, the Mathieson Chemical Company (merging with the Olin Corporation in 1954 to become the Olin-Mathieson Chemical Company) began operation and continued until 1972, using Hg in an electrolytic process to convert sodium chloride to chlorine and caustic soda. It is estimated that, in addition to Hg, the plant produced approximately 862 metric tons of $CaCl_2$ and 522 metric tons of NaCl per day for extended periods (Hill *et al.*, 1974). Two settling ponds were created adjacent to the river to store this waste, covering approximately 122 ha. In 1977, the ponds contained an estimated 100 metric tons of Hg (Carter 1977). Seivard *et al.* (1993) estimated that in the final years of plant operation, as much as 1,814 metric tons of salt and 34 kg of Hg were deposited daily into these settling ponds, the surrounding soils, and to the river. An estimated 100 g of Hg per day continued to seep from these ponds into the river (Carter, 1977). The area was designated as a Superfund site by the U. S. Environmental Protection Agency in 1982.

Detrimental effects to the stream fauna were of cat-

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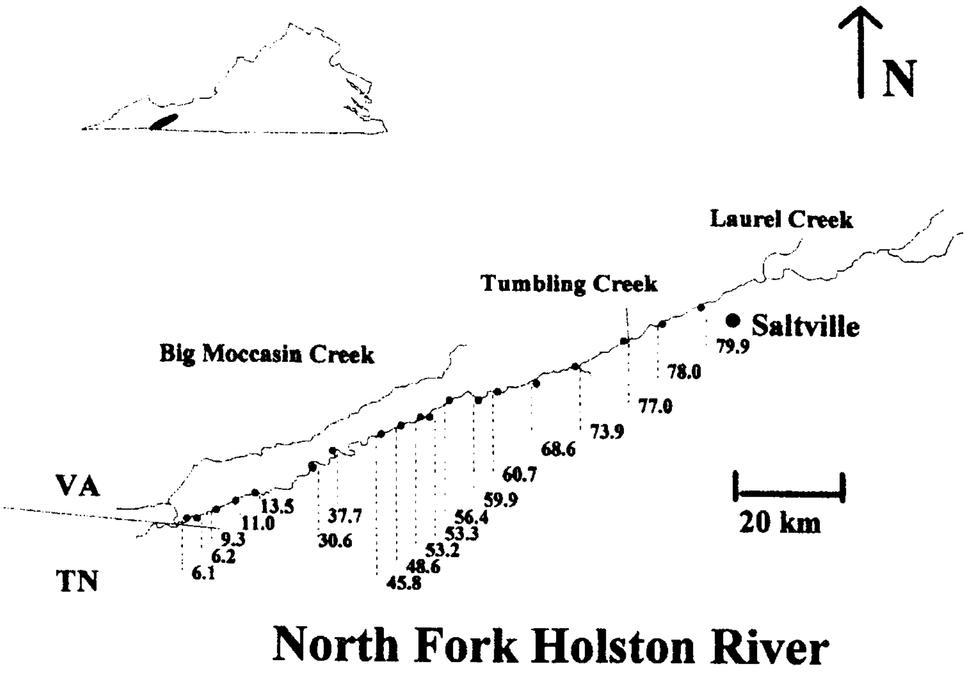

Fig. 1. Locations of survey sites in the North Fork Holston River, Virginia. River miles are measured from the confluence of the NFHR with the mainstem Holston River, Tennessee.

astrophic proportions, essentially extirpating the freshwater mussel fauna downstream of Saltville. The loss of mussels apparently began early in this century. Stansbery (1972) quotes Adams (1915) as stating, "I was told by a resident that occasionally the 'alkali' refuse came down (the NFHR at Saltville) in such quantity as to give the river a milky color." In 1918, Ortmann reported more than 42 species of freshwater mussels above and downstream of Saltville. By 1973, only one species was reported below Saltville, and 11 species upstream (Hill *et al.*, 1974). In 1976, fish tissue obtained at 6 sampling stations along 113 km of river below Saltville contained at least twice the level of Hg allowed by the FDA; therefore, a ban on fish consumption was initiated (Carter, 1977). Mercury contamination continues to be a problem, with concentrations in the tissue of Asian clams (*Corbicula fluminea* Müller, 1774) obtained 121 km downstream of Saltville being eight times higher than levels at an upstream control site (Seivard *et al.*, 1993).

In 1993, a faunal survey noted recovery of benthic invertebrates at North Fork Holston River Mile (NFHRM) 76.7, below the Saltville plant (which is located at NFHRM 80.3). The survey recorded diversity and abundance measures at NFHRM 61.0 that were comparable to an upstream reference site (Woodward-Clyde Engineering 1993). The survey recorded 4 species of mussels at 3 different sites downstream of Saltville: wavyrayed lampmussel, *Lampsilis fasciola* Rafinesque, 1820; pocketbook, *L. ovata* (Say, 1817); mountain creekshell, *Villosa vanuxemensis* (I. Lea, 1838); and rainbow mussel, *V. iris* (I. Lea, 1829). We note that *V. iris* in the NFHR seems to be a complex of 2 sibling species; therefore, every designation of *V. iris* within this document represents this complex.

Other unpublished surveys, as well as translocation records, indicate additional river locations where live mussels occur. A fish survey of the NFHR in 1993 recorded 5 sites containing live mussels (unidentified), from below Saltville to the Tennessee state line at NFHRM 8.0. During this survey, shells of *Lampsilis fasciola*, *Villosa iris* and flutedshell, *Lasmigona costata* (Rafinesque, 1820) also were collected at 13 sites between river miles 79.6 and 6.2 (Leftwich 1994). Ahlstedt (1979) and Sheehan *et al.* (1989) conducted two mussel translocation programs in the NFHR below Saltville between 1975 and 1985. Seventeen species were translocated to 8 sites. These translocations were conducted with mussels of the Clinch River (CR) at Kyles Ford, Copper Creek in the CR basin, and Big Moccasin Creek, a tributary of the NFHR. Based on these and previous records, there was circumstantial evidence that live mussels were present at 19 sites within the NFHR below Saltville, Virginia.

Our study was conducted to summarize the results of previous unionid surveys of this river, to assess the degree of recovery of freshwater mussels downstream of Saltville, Virginia, and to examine the possible relationship between the distributional pattern of mussels and Hg at selected sites in the river.

METHODS

The degree of recovery of unionid mussels, including location, species richness, abundance, and reproduction in the NFHR downstream of Saltville, Virginia, was determined for 19 sites. The level of survey effort expended at a site was defined by catch-per-unit-effort (CPUE) values recorded, and a predetermined level of CPUE triggered further survey effort. The area of each site was surveyed using a CPUE snorkeling technique (termed random CPUE within this study). Because of differences in ability and experience of snorkelers in locating mussels, the CPUE of the principle investigator (Henley) was used to trigger subsequent sampling. If the CPUE value exceeded 5 mussels/h at a site, transect CPUE surveys were conducted. If the CPUE value exceeded 10 mussels/h, 0.25 m^2 quadrat sampling along existing transect lines also was conducted. All survey work occurred from June to August 1995 during low flow conditions (Table 1 and Fig. 1).

At each site, a random CPUE survey was conducted by a crew of 2 to 5 people to confirm the presence of mussels, their relative abundance, and the position of mussel aggregations. During a random CPUE survey, only visible mussels were counted; few rocks were overturned. Observed mussels were left in position, and their locations were marked with fluorescent flags. As with all survey techniques used, mussels were examined to record species, sex and gravidity, measured for length and width (mm), and returned to the exact location of collection. Random CPUE values were calculated by dividing the number of mussels observed by total effort in hours.

Table 1. Survey sites and associated survey methods conducted on the North Fork Holston River below Saltville, Virginia from June to August 1995.

NFHR Mile	Random CPUE (no./h)	Transect CPUE (no./h)	Transect Quadrats (no./m2)
79.9	X		
78.0	X		
77.0	X		
73.9	X		
68.6	X		
60.7	X		
59.9	X	X	
56.4	X	X	
53.3	X		
53.2	X	X	X
48.6	X		
45.8	X		
37.7	X		
30.6	X	X	X
13.5	X	X	X
11.0	X	X	
9.3	X		
6.2	X		
6.1	X		

We emphasize that transects were not randomly selected, but were positioned to include mussel aggregations discovered during the random CPUE survey. Ten transects per study site were positioned 5 m apart. Catch-per-unit-effort surveys were conducted to include 1 m on either side of transect lines. A 2 m length of rebar with a painted center-line was used during surveys to aid surveyors in remaining within transect width limits. Thus, transect CPUE provided an estimate of relative abundance, and an estimate of observable mussels/m^2. During these surveys, most cobbles larger than 25 cm were overturned (and replaced) to determine the presence of mussels. Survey crews consisted of 3 to 6 individuals of varied experience, but a core crew of the same 3 individuals was always present. Catch-per-unit-effort was calculated as previously described.

For further quantification, 0.25 m^2 quadrats were randomly positioned on existing transect lines using a random numbers table. Each transect was surveyed with 10 quadrats, totaling 100 per study site. Quadrats were excavated to hardpan, or to approximately 25 cm, and substratum was later replaced. Survey crews consisted of 3 to 6 individuals of varied experience, but the core crew of 3 individuals was always present.

The following sample size formula was used to estimate the precision of density estimates with a sample size of 100 quadrats per site (Downing and Downing 1992):

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

where: $A = cm^2$ covered by each replicate sample (in this case 2500 cm^2),

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

Using this formula, a precision of 21% was calculated. All statistical analyses and graphics were conducted and generated using Minitab 10.5[2] (Minitab, Inc., College Station, Pennsylvania). Site quadrat data were analyzed for distributional type using the Chi Squared Goodness-of-Fit Test (Elliott 1977). Because these data fit the negative binomial distribution, a measure of aggregation ($\hat{k}$) was estimated using a maximum likelihood technique (Krebs 1989).

In addition to random and transect CPUE (no./h) and density estimations (no./m^2), data obtained from the combination of these survey techniques facilitated estimations of the number of species present and reproduction within the mussel aggregations at the sites. The presence of

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juveniles (< 20mm) at a site indicated recent reproduction. Since CPUE (no./h), density (no./m^2), and species presence were recorded for sites surveyed, these values were regressed on NFHR mile location. To verify ages of mussels collected, shell material collected from the river was measured for length (mm), and aged by counting external annual rings. These measurements were recorded by species and sex for dimorphic species (Neves and Moyer 1988). From these shell measurements, length and age were regressed for species and sex. Regression equations allowed length measurements of live mussels to be converted to ages.

Relationships between the distributional patterns of bivalves and the presence of Hg in the river were examined. Measures of relative abundance of mussels, number of species present, and methylmercury and total Hg content (mg/l, dry weight) in *Corbicula fluminea* also were regressed on NFHRM (Seivard *et al.*, 1993). Since there was not an exact correspondence between the sites surveyed for Hg content in the Seivard study and the sites surveyed in this study, Hg content values from the nearest sites were used. We collected tissue from *Villosa iris* for determination of total Hg content (mg/l, dry weight) which was analyzed by Hazelton Environmental Services, Inc., Madison, WI using the cold vapor atomic absorption method. For this analysis, 3 replicates of 3 *V. iris* per replicate were taken from NFHRM 84.5, 53.2, and 30.6, totaling 9 mussels sampled per site. For comparison, total Hg content in *C. fluminea* (mg/l, dry weight) collected from the NFHR is also included, as reported by Woodward-Clyde Engineering (1993) and the U. S. Geological Survey (G. Johnson, unpub. data).

RESULTS AND DISCUSSION

A mixture of sand, cobble, boulder, and bedrock substrata characterized survey sites. Ranges of physical measurements recorded at NFHR survey sites were: mean depths of 34.1 cm to 69.7 cm; mean widths of 30.6 m to 59.5 m; and mean velocities of 0.13 m/s to 0.31 m/s. Water temperatures varied from 20 to 32°C.

Nine species of live freshwater mussels were observed in surveys of the NFHR, including the purple wartyback, *Cyclonaias tuberculata* (Rafinesque, 1820); fineraryed pigtoe, *Fusconaia cuneolus* (I. Lea, 1840); wavyrayed lampmussel, *Lampsilis fasciola*; pocketbook, *L. ovata*; flutedshell, *L. costata*; Tennessee clubshell, *Pleurobema oviforme* (Conrad, 1834); kidneyshell, *Ptychobranchus fasciolaris* (Rafinesque, 1820); rainbow mussel, *V. iris*; and mountain creekshell, *V. vanuxemensis* (Table 2). *L. fasciola* and *V. iris* were observed at all sites surveyed with transect CPUE; *L. ovata* was observed at and downstream of NFHRM 53.2; and, *C. tuberculata, F. cuneolus, L. costata, P. fasciolaris*, and *P. oviforme* were collected only at or downstream of NFHRM 13.5. Measures of relative abundance of mussels varied widely from site to site, including the primary investigator's CPUE and mean transect CPUE. The first mussel assemblage, as defined by numerous animals of multiple species, was located at NFHRM 59.9, approximately 20.4 river miles downstream of Saltville (NFHRM 80.3). Evidence of reproduction (juvenile mussels) was documented at NFHRM 56.4, 53.2, 30.6, 13.5, and 11.0, and included the species *L. fasciola, L. ovata, V. iris*, and *V. vanuxemensis* (Table 3). Relative abundance and evidence of reproduction (juveniles and multiple age classes) was highest at NFHRM 53.2 and 13.5, with decreased levels at other sites.

Table 2. Species observed at 19 sampling sites on the North Fork Holston River, Virginia from June through August 1995. (X, live mussels; *, juveniles.

	North Fork Holston River Survey Site (NFHRM)																		
	79.9	78.0	77.0	73.9	68.6	60.7	59.9	56.4	53.3	53.2	48.6	45.8	37.7	30.6	13.5	11.0	9.3	6.2	6.1
Cyclonaias tuberculata (Rafinesque, 1820)																X			
Fusconaia cuneolus (I. Lea, 1840)																			X
Lampsilis fasciola Rafinesque, 1820	X					X	X	X	X	X	X	X	X	X	X*	X*	X	X	X
Lampsilis ovata (Say, 1817)					X	X			X	X*		X		X	X*	X	X	X	X
Lasmigona costata (Rafinesque, 1820)																		X	
Pleurobema oviforme (Conrad, 1834)															X				
Ptychobranchus fasciolaris (Rafinesque, 1820)																X			
Villosa iris (I. Lea, 1829)				X			X	X*	X	X*	X	X	X	X	X	X	X	X	X
Villosa vanuxemensis (I. Lea, 1838)					X	X	X	X*	X	X	X			X					

Table 3. Survey results from 19 sampling sites on the NFHR from June through August 1995. Transect CPUE, transect CPUE density, and quadrat density values are site means. (CPUE, no./h; density, no./m^2).

	North Fork Holston River Survey Site (NFHRM)																		
	79.9	78.0	77.0	73.9	68.6	60.7	59.9	56.4	53.3	53.2	48.6	45.8	37.7	30.6	13.5	11.0	9.3	6.2	6.1
Investigator CPUE	1	0	0	0	0	0	5	5	5	11	4	3	2	8	22	13	4	3	6
Random CPUE	.50	0.0	0.0	.50	2.0	3.0	11.0	8.0	8.6	34.3	16.5	2.9	4.9	13.6	30.8	17.0	2.3	2.6	5.3
Transect CPUE							2.96	3.75		8.28				5.90	18.6	11.2			*
Transect CPUE Density Estimate							0.04	0.13		0.19				0.15	0.45	0.40			*
Quadrat Density Estimate (± 21%)										2.20					2.60	1.76			*
Juveniles Observed								2		7				5	11	5			
Species with Juveniles Observed								2		2				1	3	2			

*Survey disrupted by the activity of another investigator.

It should be noted that between 1991 and 1995, 852 adult mussels of 9 species were translocated from sites in the Clinch River and upstream of Saltville to NFHRM 13.5 (R. Neves, unpub. data). This was the site with the highest recruitment, as evidenced by the collection of 11 juvenile mussels (Table 3). Collection of juveniles of 3 species indicates that this location is being repopulated by reproduction from translocated animals, because of the prior absence of mussels at this site. The site will presumably become a source of recruitment of juvenile mussels to proximate river reaches in future years.

In general, the number of age classes for all species was highest at NFHRM 53.2, and the number of age classes at downstream sites where random CPUE equaled or exceeded 5 mussels/h remained roughly constant. At most sites, *Lampsilis fasciola*, *Villosa vanuxemensis*, *V. iris*, and *L. ovata* were represented by multiple age classes. The number of age classes and relative abundance of *L. fasciola*, *V. iris*, and *L. ovata* generally increased with distance from Saltville, while age classes and relative abundance of *V. vanuxemensis* decreased. Generally, the number of *V. iris* increased, while the number of *V. vanuxemensis* decreased proceeding downstream. The number of *L. ovata* and *L. fasciola* generally remained similar with downstream river mile, except that the number of *L. fasciola* peaked at NFHRM 53.2.

A comparison of our survey results with those of Ortmann (1918) and Hill *et al.* (1974) revealed that the number of species in the river downstream of Saltville, Virginia has decreased over time (Tables 3 and 4). Between the early 1900s and 1972, the number of reported mussel species decreased from 24 to 1 within the river reach of this study (Ortmann 1918; Hill *et al.*, 1974). From the results of our survey we see that the number of species recorded in the reach has increased from 1 to 9 since 1972 (Table 3). Using maximum ages of live mussels per site, we conclude that recolonization or reproduction within aggregations began at least by the early to mid-1980s. In addition to an increase in the number of species observed downstream of Saltville since the Hill *et al.* (1974) survey, there also appears to have been a shift in the pattern of species richness within the river this century (Ortmann, 1918). Whereas we found species richness to be highest at NFHRM 11.0, the highest species richness in the early 1900s occurred at NFHRM 59.3 (26 species) and 39.2 (24 species) (Table 4). Thus, not only has the number of mussel species reported from the river been drastically reduced, but the spatial distribution of aggregations within the river has shifted.

Descriptive characteristics of mussel assemblages at sites surveyed, such as the total number of mussels observed ($r^2 = 0.40$, $p = 0.18$), the number of juveniles detected ($r^2 = 0.06$, $p = 0.35$), number of age classes for all species ($r^2 = 0.24$, $p = 0.33$), and number of age classes for *L. fasciola* and *V. iris* ($r^2 = 0.23$, $p = 0.33$ and $r^2 = 0.34$, $p = 0.24$, respectively) did not increase significantly with distance downstream of Saltville. Also, random CPUE (no./h) ($r^2 = 0.10$, $p = 0.18$) and mean site quadrat density (no./m^2) ($r^2 = 0.06$, $p = 0.95$) were not correlated to river mile location. Mean transect CPUE was marginally related to NFHRM ($r^2 = 0.64$, $p = 0.06$). The Anderson-Darling test for normality (Sokal and Rohlf 1995) showed that all mussel assemblage characteristics were normally distributed ($p > 0.05$), except random CPUE ($p < 0.001$) and number of juvenile species observed ($p < 0.01$). Transformations indicated by the Box-Cox transformation procedure (Sokal and Rohlf, 1995) did not substantially increase r^2 and associated p values, when these variables were regressed on river mile. Thus, mussel assemblage characteristics did not seem to exhibit a curvilinear relationship to river mile location.

Site quadrat data from surveyed mussel aggregations followed a negative binomial distribution, using the Chi Square Goodness-of-Fit Test (Elliott 1977). Chi square values (x^2), associated p value ranges, and $\hat{k}$ estimates were: NFHRM 53.3, $x^2 = 0.53$ ($0.90 < p < 0.95$) with $\hat{k} = 10.000$; NFHRM 13.5, $x^2 = 6.18$ ($0.25 < p < 0.50$) with $\hat{k} = 0.495$; and NFHRM 11.0, $x^2 = 2.95$ ($0.50 < p < 0.75$) with $\hat{k} =$

Table 4. Species presence at survey sites with comparisons to survey findings of Ortmann (1918) (O) and Hill *et al.* (1974) (H). Holding ponds at Saltville, Virginia are at NFHRM = 80.3. Ortmann site locations are approximate. Ortmann (1918) binomials were revised to conform to current taxonomic names according to Parmalee and Bogan (1998).

	North Fork Holston River Survey Site (NFHRM)																	
	82.8 (O)	79.9	79.0 (H)	59.9	59.3 (O)	56.4	53.2	45.8	45.0 (H)	39.2 (O)	30.6	20.9 (O)	13.5	11.0	8.8 (O)	6.3 (H)	6.2	6.1
Actinonaias ligamentina (Lamark, 1819)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-
A. pectorosa (Conrad, 1834)	X	-	-	-	X	-	-	-	-	X	-	X	-	-	X	-	-	-
Alasmidonta marginata Say, 1818	X	-	-	-	X	-	-	-	-	X	-	-	-	-	-	-	-	-
A. viridis (Rafinesque, 1820)	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Amblema plicata (Say, 1817)	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-
Cyclonaias tuberculata (Rafinesque, 1820)	-	-	-	-	X	-	-	-	-	X	-	-	-	X	-	-	-	-
Elliptio dilatata (Rafinesque, 1820)	-	-	-	-	X	-	-	-	-	X	-	X	-	-	X	-	-	-
Epioblasma brevidens (I. Lea, 1831)	-	-	-	-	X	-	-	-	-	-	-	-	-	-	X	-	-	-
E. capsaeformis (I. Lea, 1834)	-	-	-	-	X	-	-	-	-	X	-	-	-	-	X	-	-	-
E. haysiana (I. Lea, 1834)*	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-
E. torulosa gubernaculum (Reeve, 1865)*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-
E. triquetra (Rafinesque, 1820)	-	-	-	-	X	-	-	-	-	X	-	-	-	-	X	-	-	-
Fusconaia barnesiana (I. Lea, 1838)	X	-	-	-	-	-	-	-	-	X	-	X	-	-	X	-	-	-
F. cor (Conrad, 1834)	-	-	-	-	X	-	-	-	-	X	-	X	-	-	X	-	-	-
F. cuneolus (I. Lea, 1840)	-	-	-	-	X	-	-	-	-	X	-	-	-	-	-	-	-	X
F. subrotunda (I. Lea, 1831)	-	-	-	-	X	-	-	-	-	X	-	-	-	-	X	-	-	-
Lampsilis fasciola Rafinesque, 1820	X	X	-	X	X	X	X	X	-	X	X	X	X	X	X	X	X	X
L. ovata (Say, 1817)	X	-	-	-	X	-	X	X	-	X	X	-	X	X	-	-	X	X
Lasmigona costata (Rafinesque, 1820)	X	-	-	-	X	-	-	-	-	X	-	X	-	-	X	-	X	-
Lemiox rimosus (Rafinesque, 1831)	-	-	-	-	X	-	-	-	-	-	-	X	-	-	-	-	-	-
Lexingtonia dolabelloides (I. Lea, 1840)	X	-	-	-	X	-	-	-	-	X	-	X	-	-	-	-	-	-
Medionidus conradicus (I. Lea, 1834)	X	-	-	-	X	-	-	-	-	X	-	X	-	-	-	-	-	-
Pegias fabula (I. Lea, 1838)	X	-	-	-	-	-	-	-	-	X	-	X	-	-	-	-	-	-
Pleurobema oviforme (Conrad, 1834)	X	-	-	-	X	-	-	-	-	X	-	X	X	-	-	-	-	-
Ptychobranchus fasciolaris (Rafinesque, 1820)	-	-	-	-	X	-	-	-	-	X	-	-	-	X	-	-	-	-
P. subtentum (Say, 1835)	X	-	-	-	-	-	-	-	-	X	-	X	-	-	X	-	-	-
Quadrula cylindrica (Say, 1817)	-	-	-	-	X	-	-	-	-	X	-	-	-	-	X	-	-	-
Q. intermedia (Conrad, 1836)	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-
Strophitus undulatus (Say, 1817)	X	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-
Toxolasma lividus Rafinesque, 1831	X	-	-	-	-	-	-	-	-	-	-	X	-	-	X	-	-	-
Villosa fabalis (I. Lea, 1831)	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-
V. iris (I. Lea, 1829)	X	-	-	X	X	X	X	X	-	X	X	X	X	X	-	-	X	X
V. perpurpurea (I. Lea, 1861)	-	-	-	-	X	-	-	-	-	X	-	-	-	-	-	-	-	-
V. vanuxemensis (I. Lea, 1838)	X	-	-	X	X	X	X	-	-	X	X	-	-	-	X	-	-	-
Total Species Observed	16	1	0	3	26	3	4	3	0	24	4	14	4	5	16	1	4	4

*extinct

0.369. Because these data fit the negative binomial distribution, it was appropriate to use $\hat{k}$ as an estimate of site aggregation. It is important to note that higher $\hat{k}$ values reflect lower levels of mussel aggregation or clumping (Elliott 1977). Values of $\hat{k}$ can range from 0 to $+\infty$, with values from 0 to about 8 indicating distributional aggregation (Poole 1974). Thus $\hat{k}$ values > 8 may indicate a distribution of mussels that approaches the Poisson or random distribution. On this basis, mussel distributions at NFHRM 13.5 and 11.0 may be viewed as highly aggregated. Since the $\hat{k}$ value associated with NFHRM 53.2 is > 8, mussels at this site were considered to be approaching a random distribution. Elliott (1977) noted that if the same number and size of quadrats are obtained at survey locations, then $\hat{k}$ may be used to compare aggregations. These requirements were fulfilled in our survey.

Results of our study show no evidence of mussel assemblages immediately below Saltville, between NFHRM 79.9 and 59.9. Between Saltville and river mile 59.9, only isolated individuals of mussel species were observed. Possible explanations for this are the lingering effects of Hg, or insufficient numbers of mussels and suitable fish hosts to support reproduction. A translocation of sufficient specimens of species such as *Villosa iris* and

Lampsilis fasciola into this river reach would allow an assessment of its suitability for recolonization. Comparison of the body burdens of Hg in *Corbicula fluminea* and *V. iris* from the U. S. Geological Survey (G. Johnson, unpub. data), Woodward-Clyde (1993), Seivard *et al.* (1993), and our values shows the need for further Hg monitoring (Table 5 and Fig. 2). Although most studies show Hg levels > 1 mg/l dry weight, content values between studies are inconsistent and likely vary between body burdens in *C. fluminea* versus *V. iris*. Further study is needed to show whether levels of Hg in *C. fluminea* and *V. iris* are comparable for specimens of similar age.

Within this study, random CPUE and total number of species observed were the only mussel assemblage characteristics that were related to total Hg content in *Corbicula fluminea*, as measured by Seivard *et al.* (1993) at proximate survey sites ($r^2 = 0.69, p = 0.02$ and $r^2 = 0.77$, $p = 0.13$, respectively). There was a noticeable absence of younger age classes and a decrease in total mussels observed at sites surveyed at locations upstream of NFHRM 60.0. Total Hg content in *C. fluminea* was inversely correlated to river mile locations ($r^2 = 0.79$, $p = 0.003$), whereas methylmercury content in C. fluminea was not related to river mile ($r^2 = 0.52$, $p = 0.04$), as reported by Seivard *et al.* (1993). Two conclusions seem evident from this information. First, the probability of the effect of Hg on mussel recruitment (recent and historic) in the river increases with proximity to Saltville; and secondly, that effect is seemingly related to total Hg and not methylmercury. It is interesting that measures of relative abundance of mussels estimated by our study were inversely correlated to total Hg content rather than methylmercury content, since methylmercury is known to produce toxic effects in aquatic organisms (Chang *et al.*, 1974; Khan and Weis, 1987; Stinson and Mallatt, 1989; Chen and McNaught, 1992). Perhaps some form of Hg in the total Hg component that was not tested has had a contributing effect.

When examining the relationships between site assemblage characteristics, such as the number of mussel species and random and transect CPUE versus methylmer-

Table 5. Mean total mercury content (mg/l, dry weight) in *Corbicula fluminea* and *Villosa iris* from selected North Fork Holston River sites (G. Johnson, unpub. data; Woodward-Clyde, 1993; Seivard *et al.*, 1993; this study). Settling ponds at Saltville are at NFHRM 80.3.

	Seivard *et al.* (1993) *C. fluminea*		Woodward-Clyde (1993) *C. fluminea*		G. Johnson, unpub. data *C. fluminea*		This Survey *V. iris*	
Site	Mean	s	Mean	s*	Mean	s**	Mean	s
91.5			NP					
88.5			< 0.20					
85.6			< 0.20					
84.5	0.18	0.02					1.54***	1.61
81.3			0.13*					
79.9	3.34	0.42						
76.7			NP					
74.0			0.49*					
71.0			0.52	0.12				
69.9	3.46	0.21			1.50			
66.3			NP					
63.8			< 0.26					
60.7			0.48*					
60.2	2.65	0.04						
58.2			< 0.20					
56.4			< 0.28					
53.2			0.51	0.08			2.67	0.53
51.3	1.79	0.15						
39.7	2.38	0.23						
30.6							5.27***	3.94
30.1	1.94	0.20						
22.8	1.89	0.14						
7.6	1.47	0.13						
4.8					1.40			

*Most standard deviations for Woodward-Clyde data were not included because some data were presented as < 0.20 or of only one datum; **standard deviations of G. Johnson (unpub. data) not provided; ***one datum was comparatively high for the site (3.39 mg/l, dry weight); ****one datum was comparatively high for the site (9.73 mg/l, dry weight).

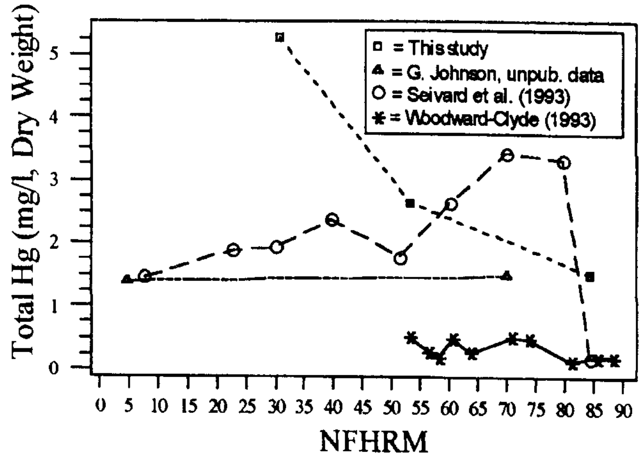

Fig. 2. Mean total mercury content (mg/l, dry weight) in *Corbicula fluminea* and *Villosa iris* from selected sites on the North Fork Holston River (G. Johnson, unpub. data; Woodward-Clyde, 1993; Seivard *et al.*, 1993; this study). Accuracy of data points of Woodward-Clyde (1993) are suspect (represented at < 0.20 or by one datum). Settling ponds at Saltville are at NFHRM 80.3.

cury content in *Corbicula fluminea* (Seivard *et al.*, 1993), an interesting phenomenon is seen. At NFHRM 45.8 through 30.6, decreases in site assemblage characteristics occur, and associated with these decreases are increases in total Hg in *C. fluminea* at NFHRM 39.7(Tables 3 and 5 and Fig. 2). Although total Hg is seemingly related to reduced mussel assemblages in this reach of the river, further survey would be required to confirm this apparent effect. Seivard *et al.* (1993) found a general decreasing trend in total Hg content in *C. fluminea* downstream of Saltville, Virginia. The exception to this decreasing trend was observed at NFHRM site 39.7, where total Hg content increased (Seivard *et al.*, 1993). Mercury content in *C. fluminea* measured by the U. S. Geological Survey (G. Johnson, unpub. data) also decreased with distance from Saltville, whereas similar data measured by Woodward-Clyde (1993) showed no trend (Table 5 and Fig. 2). Additional testing is needed to determine whether the decrease in total Hg with downstream distance from Saltville is real or spurious. If the elevated Hg levels in *C. fluminea* near NFHRM 30.0 through 40.0 are representative of elevated levels of Hg in river sediment, then mussel aggregations downstream, such as at NFHRM 13.5 and 11.0, could be at future risk if such a contaminated sediment load is being transported downstream.

When considering the re-establishment of mussel species reported by Ortmann (1918) in the river downstream of Saltville, Virginia, and the current absence of most of those species in the upper and lower river, the need for a mussel restoration program becomes apparent. Of the 33 species reported by Ortmann (1918) downstream of Saltville, Hill *et al.* (1974) and Barr *et al.* (1993) observed 11 and 10 of these species upstream, respectively. Consequently, translocation of mussels from another river within the same basin will be essential for the re-establishment of mussel species that were present historically.

In addition to previous translocation, habitat requirements of translocated species, and possible contaminant effects, the selection of sites should be guided by evidence of current reproduction, historical pattern of mussel aggregations, and the availability of suitable fish hosts. Our results indicate that NFHRM 11.0, 13.5, 30.6, and 53.2 are suitable sites for reintroduction. The river reach between NFHRM 40 through 60 should be evaluated in further assessments of site suitability, as this stretch of the river historically supported a high diversity of species (Ortmann, 1918).

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