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FRESHWATER MUSSEL (UNIONIDAE) ABUNDANCE AND DIVERSITY UPSTREAM AND DOWNSTREAM OF A SUPERFUND SITE ON THE NORTH FORK HOLSTON RIVER, SALTVILLE, VIRGINIA

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ABSTRACT The North Fork Holston River (NFHR) historically supported 33 unionid mussel species downstream of Saltville, VA. Because of industrial contamination over decades from a chlor-alkali plant, a U.S. Environmental Protection Agency Superfund Site (SITE) was created with Hg and MeHg designated as contaminants of concern. Mussel surveys were conducted at 18 NFHR locations to determine abundance, species richness, and recruitment upstream and downstream of the SITE. Seventeen unionid species were collected, and mean species richness of upstream sites (8.8 species, $n = 6$ sites) was greater than the mean of downstream locations (3.8 species, $n = 12$). The catch-per-unit-effort mean from upstream sites (10.4 mussels/h, $n = 3$ sites) was greater than the mean of downstream sites (3.5 mussels/h, $n = 12$). Mean density of upstream (1.8 mussels/m^2, $n = 6$ sites) sites was higher than observed at downstream (1.0 mussels/m^2, $n = 8$) locations. Results show that species richness in the entire lower NFHR is less than observed upstream, and measures of mussel abundance and recruitment also are severely depressed in the ~35 km reach downstream of the SITE, where no juvenile and very few adult mussels were collected. The influences of a wide array of contaminants, including Hg, MeHg, Cl^-, major ions, and trace elements, from the SITE on downstream recovery of unionid mussels are discussed.

KEY WORDS: freshwater mussels, North Fork Holston River, Superfund Site, mercury, chlor-alkali plant

INTRODUCTION

The North Fork Holston River (NFHR) in southwest Virginia and northeast Tennessee historically supported a highly diverse mussel fauna (Unionidae). Diversity was greatly diminished by pollution due to industrial activities from a chemical production facility adjacent to the NFHR at Saltville, Smyth County, VA. Utilizing local salt deposits, the Olin Corporation and its predecessors (Olin Mathieson Chemical Corporation, Mathieson Chemical Company, and Mathieson Alkali Works) used the facility to produce soda ash, caustic soda, and/or chlorine from 1895 to 1972 (USEPA 2012). Waste disposal practices resulted in the daily release of toxic effluents (Hg, various salts, and other contaminants) into the NFHR and leaching into adjacent soils from two chemical–waste disposal ponds (labeled Ponds 5 and 6) adjacent to the river at ~ NFHR km (NFHRKM) 131.5 (Carter 1977, Seivard et al. 1993, Henley and Neves 1999, USEPA 2012). In the early 1970s, the states of Virginia and Tennessee initiated fish consumption bans and catch-and-release fishing regulations for the NFHR due to unacceptable concentrations of methylmercury (MeHg) in fish tissues, and these regulations remain in effect (USEPA 2012). The area containing the chemical-waste ponds and the former chlor-alkali plant was designated by the U.S. Environmental Protection Agency (USEPA) as the Saltville Waste Disposal Ponds Superfund Site (SITE) on the U.S. National Priorities List in 1983. The USEPA completed a risk assessment in 1986, and produced a Record of Decision in 1987, which identified Hg and MeHg as contaminants of concern (USEPA 2012).

Compared with historical records, the mussel fauna downstream of the SITE was nearly extirpated. In 1912 and 1913, 33 species of mussels were observed downstream of Saltville in Virginia and 37 species in Tennessee (Ortmann 1918). By 1972, however, only one species was observed downstream of the SITE in Virginia (Hill et al. 1974). About 20 y later, Woodward Clyde Engineering (1993) reported four species of mussels downstream of the SITE. In 1995, Henley and Neves (1999) collected nine mussel species downstream, and observed the first multispecies assemblage, as defined by numerous mussels of multiple species, downstream ~35 km downstream of the SITE at NFHRKM 96.4. At survey locations in this 35 km reach, Henley and Neves (1999) observed only isolated individuals of three mussel species and no juvenile mussels. Thus, a reach of special concern regarding mussel occurrence and reproduction exists from the SITE to ~35 km downstream.

The objective of this study was to compare abundance, species richness, and recruitment of mussels at survey locations upstream and downstream of the SITE. Downstream survey sites were grouped into two zones: the first zone included locations from the SITE to ~35 km downstream, and the second zone included locations from ~35 km downstream to just past the Virginia–Tennessee state line (~94 km in length). Mussel abundance, species richness, and recruitment data from survey sites were analyzed to determine overall upstream and

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downstream differences, as well as differences among the downstream zones. Potential effects of a wide array of contaminants from the SITE on downstream unionid mussels were discussed, and critical research needs were introduced. Further, how emphasis on Hg and MeHg as sole designated contaminants of concern may lead to underestimation of complexity and severity of downstream pollution and effects on resident mussels has also been discussed.

MATERIALS AND METHODS

A total of 18 NFHR locations were surveyed for unionid mussels from September 2004 to October 2005, including 6 upstream and 12 downstream of the SITE (Fig. 1). For convenience, the date of all sampling is presented as 2005. Three river zones (RZ) for subsequent data analyses were designated. The zones included survey locations upstream of the SITE ($n = 6$; NFHRKM 147–158) and two zones downstream. The first downstream RZ was termed D1, and encompassed the reach from immediately downstream of the SITE to the Highway 19/58 Bridge in Washington County, VA (~35 km downstream of the SITE, where Henley and Neves (1999) found no mussel assemblages in 1995; $n = 5$; NFHRKN 89–131). The second downstream RZ was termed D2, and extended from the downstream boundary of D1 to just past the Virginia–Tennessee state line, with one survey site in Tennessee ($n = 7$; NFHRKM 7–131) (Table 1). Site abbreviations associated with these NFHRKM survey locations are subsequently used. The abbreviations were based on whether survey sites were located upstream (U) or downstream (D) of the SITE; as stated, the two downstream RZ are designated as D1 and D2; and the last numeral in the site abbreviations designates upstream to downstream sequence of sites within RZ (Tables 1 and 2, Fig. 1). For example, abbreviation D2–2 denotes that the survey location at NFHRKM 49.3 is the second downstream site in RZ D2. Upstream sites were located at NFHRKM (site abbreviation) 157.3 (U-1), 155.7 (U-2), 154.4 (U-3), 153.4 (U-4), 151.7 (U-5), 147.3 (U-6), and downstream sites were at 128.6 (D1–1), 117.9 (D1–2), 110.4 (D1–3), 96.4 (D1–4), 89.8 (D1–5), 65.7 (D2–1), 49.3 (D2–2), 35.1 (D2–3), 21.7 (D2–4), 10.0 (D2–5), 9.8 (D2–6) in Virginia and 7.2 (D2–7) in Tennessee. For latitude and longitude coordinates of the sites, see Henley and Neves (2006).

At 15 NFHR locations, timed mask-and-snorkel surveys [catch-per-unit-effort (CPUE), mussels/h] were conducted by 8–22 biologists to determine locations of mussel aggregations for subsequent quadrat (0.25 m^2) surveys (Table 1). Effort expended at survey sites during CPUE searches ranged from 4.0 to 22.0 person-hours (Table 1). During CPUE surveys, mussels were located, their positions flagged, measured (mm)

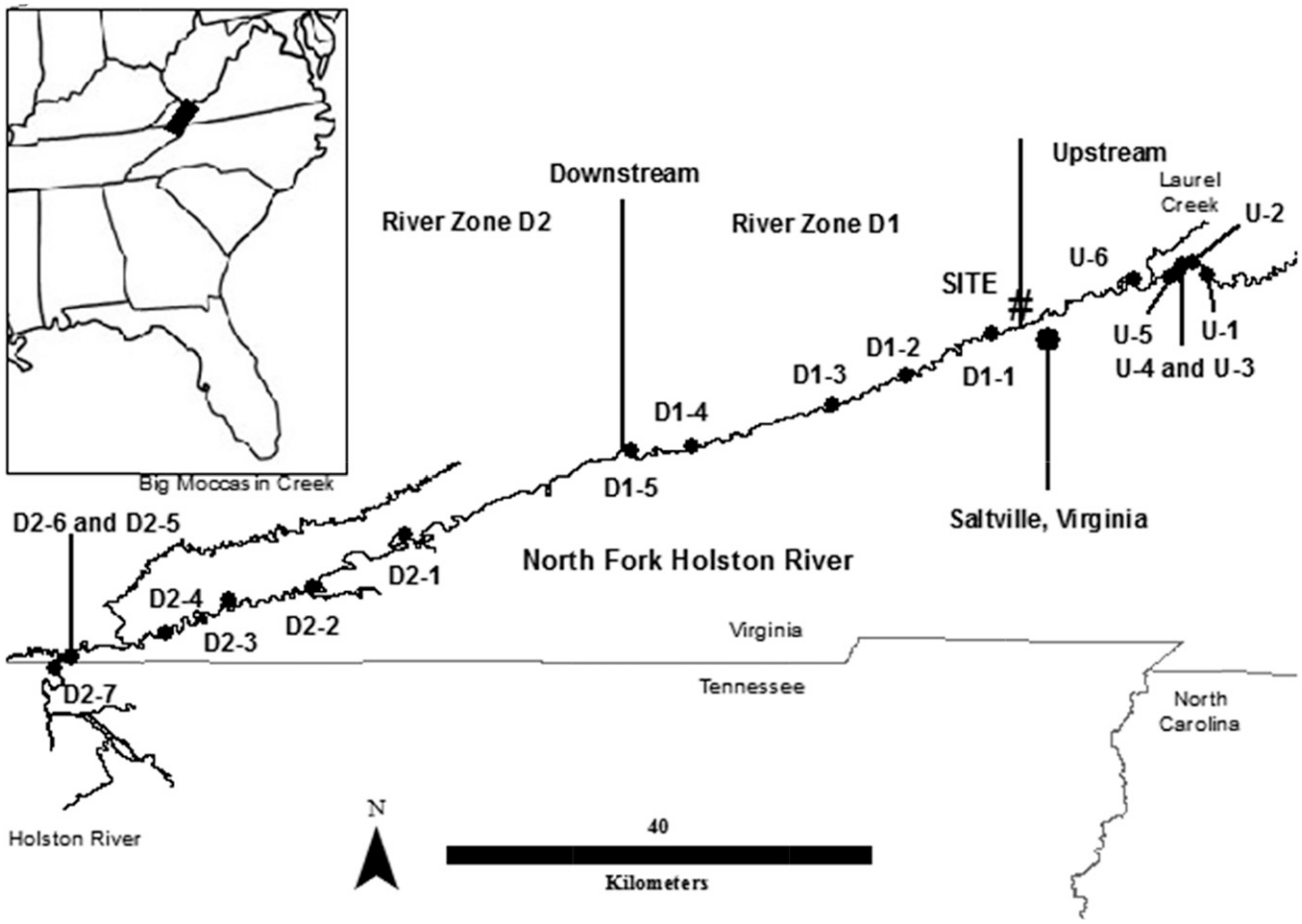

Figure 1. NFHR survey locations upstream and downstream of the SITE at Saltville, VA. Downstream RZ D1 and D2 are delineated in the map. See text for interpretation of site codes.

TABLE 1.

Results of CPUE (mussels/h) and 0.25 m^2 quadrat (mussels/m^2) surveys at NFHRKM locations in RZ upstream and downstream of the USEPA SITE (NFHRKM ~131.5) at Saltville, VA.

	NFHR sites																			
RZ	Upstream						Downstream													
							Zone D1						Zone D2							
Site (NFHRKM)	157.3	155.7	154.4	153.4	151.7	147.3	128.6	117.9	110.4	96.4	89.8	65.7	49.3	35.1	21.7	10.0	9.8	7.2		
Site abbreviation*	U-1	U-2	U-3	U-4	U-5	U-6	D1–1	D1–2	D1–3	D1–4	D1–5	D2–1	D2–2	D2–3	D2–4	D2–5	D2–6	D2–7		
Quadrat density†	0.1	3.0	2.8	2.8	1.6	0.8	–	0.1	0.04	0.6	0.8	–	1.1	2.5	1.3	–	1.2	–		
CPUE‡	1.6	21.0	–	–	8.7	–	0.6§ (0.3)	0.9§ (0.2)	1.8	7.0	3.3	0.4	6.9	5.0	7.7	0.8	5.9	1.5		
CPUE effort (person-hours)	13.0	7.0	–	–	11.0	–	11.0§ (1.0)	13.0§ (1.0)	15.4	9.0	22.0	16.0	14.0	15.0	14.8	4.0	8.0	10.8		
Number of species collected	2	10	11	11	9	10	4	3	3	3	3	2	6	2	3	5	9	3		
Quadrat density for juveniles	0.00	0.18	0.11	0.04	0.11	0.04	–	0.00	0.00	0.00	0.00	–	0.04	0.07	0.07	–	0.00	–		

D1 and D2 are designated RZ downstream of the SITE.

* First letter in site abbreviations denote survey locations upstream (U) or downstream (D) of the SITE; the two downstream RZ are designated as D1 and D2; and the last numeral represents upstream to downstream sequence of sites within RZ.

† Because of low mussel abundances observed during CPUE surveys and subsequent consensus of surveying biologists, quadrat surveys were not conducted at sites D1–1, D2–1, D2–5, and D2–7 (see text).

‡ Results of Jones and Neves (2007) CPUE surveys were used to determine mussel aggregation locations at sites U-3, U-4, and U-6, therefore, CPUE surveys at these sites were not conducted during this study (see text).

§ Mean (±SE) of two separate CPUE surveys (see text).

for length, identified to species, and returned to their flagged position in the substrate. By consensus of the surveying biologists, juveniles were defined as lengths less than or equal to 20 mm (Henley and Neves 1999). Jones and Neves (2007) conducted CPUE surveys in 2000–2004 at sites U1–3, U1–4, and U1–6; but because of funding limitations of the present study, CPUE surveys were not conducted at the earlier-mentioned sites. River positions of mussel aggregations at these sites, as determined by Jones and Neves (2007), were subsequently surveyed with quadrats during this study.

At 14 locations, 0.25 m^2 quadrat surveys were conducted (Table 1). Data from these surveys were in mussel counts per quadrat. Each of the 14 sites was surveyed with 110 quadrats, along 11 bank-to-bank transects, with 10 quadrats per transect. The first downstream random-start transect location was determined using a random number table, whereas subsequent transects were 10 m apart. Quadrats were randomly positioned along transect lines using a random number table. River substrata bounded by quadrat margins were collected and sieved (12.7 mm^2 mesh size) to collect mussels for species and gender identification and length measurement (mm). The substrate and mussels were returned to quadrat locations of excavation.

Data analyses were conducted using SAS 9.3 (SAS Institute, Incorporated, Cary, NC) and Minitab 16.2 (Minitab, Incorporated, College Station, PA). Site density (mussels/m^2) and recruitment estimates (number of juveniles per site and mussel lengths) were generated from counts of mussels in quadrats. Species richness at sites was determined using data from the quadrat and CPUE surveys. Species richness and CPUE values were grouped by sites upstream versus downstream of the SITE and in RZ, and were analyzed using a general linear model and Bonferroni *post hoc* multiple comparisons tests in Minitab. Species richness and CPUE data residuals were tested for normality using the Ryan–Joiner test in Minitab; residuals of these data were normally distributed ($n = 18$ for species richness and $n = 15$ for CPUE; Ryan–Joiner statistic ranged from 0.949 to 0.986; $P > 0.06$).

Counts of mussels in quadrats (mussels/0.25 m^2 quadrat) were analyzed using generalized linear mixed models (GLIMMIX) in SAS to determine differences among upstream and downstream zones. Within the mixed models, RZ was a fixed factor, quadrat and transect were designated as random factors, and the data distribution was set with DIST = NEGBIN. Quadrat data (mussel counts in quadrats) provided adequate fit to the negative binomial distribution (SAS, COUNTREG, and FREQ: df = 6, $X^2 = 5.10$, $P = 0.531$). Site was designated as the sampling unit within RZ, with the GLIMMIX subject and group subcommands. Least square means were compared between upstream and downstream and among RZ using Bonferroni *post hoc* multiple comparisons tests (significant at $P < 0.05$). Because counts of mussels in quadrats were statistically analyzed, mussel densities were not; however, because of usual reporting practice, mussel densities also are presented in the results. Because of the rarity of juvenile mussels, and subsequent dominance of zeros in the juvenile-quadrat dataset, GLIMMIX could not fit an appropriate model to the data (X^2/df < 0.17, where the proportion should be ~1). Therefore, densities of juvenile mussels from sites are presented without statistical results. Mean (±SE) numbers of species, numbers of mussels collected, CPUE, and densities are presented for survey locations upstream and downstream of the SITE and in RZ.

RESULTS

Species

Seventeen species of mussels were collected during CPUE and quadrat surveys; 13 mussel species were observed at 6 survey locations upstream of the SITE and 12 species were found at 12 downstream locations (Table 2). Mean species richness was higher at upstream survey sites (8.8 ± 1.4) than the mean from those downstream (3.8 ± 0.6) (df = 1, $F = 15.61$, $P = 0.001$). Mean number of species also was significantly higher at upstream sites than site means in the separate downstream D1 and D2 RZ (3.2 ± 0.2 and 4.3 ± 1.0, respectively), whereas mean species at sites in D1 and D2 were not different (df = 2, $F = 7.83$, $P = 0.005$; Table 1). During all upstream and downstream surveys combined, only one mussel was collected each of *Actinonaias ligamentina* (Lamarck, 1819), *Amblema plicata* (Say, 1817), and *Fusconaia cuneolus* (Lea, 1840) (U.S. federally endangered); less than or equal to five specimens were collected each of *Cyclonaias tuberculata* (Rafinesque, 1820), *Fusconaia cor* (Conrad, 1834) (federally endangered), *Lasmigona costata* (Rafinesque, 1820), and *Pleuronaia barnesiana* (Lea, 1838). Although *Ptychobranchus subtentum* (Say, 1825) (federally endangered) was relatively abundant at upstream sites, only one specimen was collected downstream of the SITE (Table 2). Individuals of *Pleuronaia dolabelloides* (Lea, 1840) (federally endangered) were observed only at upstream survey sites.

Recruitment

Eighteen juvenile mussels of four species were collected in quadrats at 8 of the 18 survey locations. The four species were *Lampsilis fasciola* (Rafinesque, 1820) (2 juveniles), *Medionidus conradicus* (I. Lea, 1834) (1), *Villosa iris* (Lea, 1829) (11), and *Villosa vanuxemensis* (Lea, 1838) (4) (Table 2). No juvenile mussels were observed during CPUE sampling efforts. Thirteen juveniles were collected upstream of the SITE, no juveniles were collected from RZ D1, and five juveniles were collected from D2. Densities of juveniles from sites ranged from 0.0 to 0.18 juveniles/m^2 (Table 1); mean juvenile densities from upstream and downstream sites were 0.08 (±0.03) and 0.02 (±0.01), and means from sites in RZ D1 and D2 were 0.00 (±0.00) and 0.04 (±0.02), respectively (Table 1). Therefore, evidence of recent mussel recruitment was documented only upstream and in RZ D2.

Abundance

The CPUE mean (mussels/h) from upstream sites (10.4 ± 5.7) was significantly greater than the downstream mean (3.5 ± 0.8) (df = 1, $F = 5.37$, $P = 0.037$). The CPUE means for RZ D1 and D2 were 2.7 (±1.2) and 4.0 (±1.2), respectively, with each zone including two sites with CPUE values less than 1.0 mussel/h (Table 1).

TABLE 2.

Species occurrence at NFHRKM locations in RZ upstream and downstream of the USEPA SITE (NFHRKM ~131.5) at Saltville, VA.

	NFHR sites																	
							Downstream											
RZ	Upstream						Zone D1					Zone D2						
Site (NFHRKM)	157.3	155.7	154.4	153.4	151.7	147.3	128.6	117.9	110.4	96.4	89.8	65.7	49.3	35.1	21.7	10.0	9.8	7.2
Site abbreviation*	U-1	U-2	U-3	U-4	U-5	U-6	D1–1	D1–2	D1–3	D1–4	D1–5	D2–1	D2–2	D2–3	D2–4	D2–5	D2–6	D2–7
Actinonaias ligamentina	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	X[1]	–
Actinonaias pectorosa	–	X[1]	X	X	X	X	–	–	–	–	–	–	–	–	–	–	X[1]	X[1]
Amblema plicata	–	–	–	–	–	–	–	–	–	–	–	–	X[1]	–	–	–	–	–
Cyclonaias tuberculata	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	X[1]	X[1]
Fusconaia cor FE	–	–	X[1]	X	–	X[1]	–	–	–	–	–	–	–	–	–	–	–	–
Fusconaia cuneolus FE	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	X[1]	–
Lasmigona costata	–	X[1]	–	X[1]	–	X[1]	–	–	–	–	–	–	–	–	–	X[1]	–	–
Lampsilis fasciola	–	X[1]	X	X†	X	X	X[1]	X[1]	X[1]	X	X[1]	X[1]	X†	X[1]	X	X	X[1]	–
Lampsilis ovata	–	–	X	–	–	–	X[1]	–	–	–	–	–	X[1]	–	X[1]	X	X[1]	–
Medionidus conradicus	X	X	X†	X	X	X	–	–	–	–	–	–	–	–	–	–	–	–
Pleurobema oviforme	–	–	X	X	X	X[1]	–	–	–	–	–	–	–	–	–	–	–	–
Pleuronaia barnesiana	–	X	–	–	–	–	–	–	–	–	–	–	X[1]	–	–	–	–	–
Pleuronaia dolabelloides FE	–	X[1]	X	X	X	X[1]	–	–	–	–	–	–	–	–	–	–	–	–
Ptychobranchus fasciolaris	–	X[1]	X	X	X	X	–	–	–	–	–	–	–	–	–	–	–	–
Ptychobranchus subtentum FE	–	X	X	X	X	–	–	–	–	–	–	–	–	–	–	–	X[1]	–
Villosa iris	X	X†	X†	X	X†	X†	X	X	X[1]	X	X	X[1]	X†*	X†	X†	X	X[1]	X[1]
Villosa vanuxemensis	–	X†	X	X	X†	X	X[1]	X	X	X	X	–	X[1]	–	–	X[1]	X[1]	–

D1 and D2 are designated RZ downstream of the SITE.
The superscript number 1 indicates only one specimen of the species was collected.
The superscript FE indicates U.S. federally endangered.
The symbol † indicates Juvenile(s) collected at site.
* See Table 1 for interpretation of site codes.

Mussel counts in quadrats from upstream sites were significantly greater than counts from downstream sites (df = 1, $F = 7.87$, $P = 0.0058$). Mussel counts in quadrats from sites upstream and in zone D2 were significantly higher than counts from D1 sites, whereas those upstream and in D2 were not different (df = 2, $F = 17.9$, $P < 0.0001$). Mean site densities (mussels/m^2) for upstream and downstream zones were 1.8 (±0.5) and 1.0 (±0.3) mussels/m^2, respectively; mean site densities for RZ D1 and D2 were 0.4 (±0.2) and 1.5 (±0.3), respectively (Table 1).

DISCUSSION

The results indicated that the SITE near Saltville serves as a point of delineation in the NFHR for mussel occurrence, abundance, and recruitment. Species richness, CPUE, and counts of mussels in quadrats were significantly lower at downstream survey locations compared with those upstream of the SITE. Analyses showed that species richness and counts of mussels in quadrats at survey locations in RZ D1 were significantly lower than at upstream locations. Few mussels from D1 during quadrat and visual surveys were collected, and no juvenile mussels were observed. These findings are consistent with those of Henley and Neves (1999), who reported a dearth of mussels and no recruitment in approximately the first 35 km downstream of the SITE. Thus, zone D1 continues to be a reach of special concern regarding mussel occurrence and reproduction. Although counts of mussels in quadrats at sites upstream and in RZ D2 were not significantly different, species richness in D2 was significantly less than upstream. Therefore, it appears that habitat conditions downstream of the SITE continue to limit mussel abundance and recruitment in RZ D1 and species richness in D1 and D2.

A comparison of results of this study with those of Henley and Neves (1999) shows that meaningful recovery of mussels did not occur downstream of the SITE during the period between 1995 and 2005. Eight identical locations downstream of the SITE were surveyed for CPUE during both studies, including four sites each in zones D1 and D2 (Table 3). The only river location common to both studies that was sampled with quadrats was site D2–4. From 1995 to 2005, CPUE remained relatively constant and low in the upper portion of zone D1 (sites D1–1, D1–2, and D1–3), but CPUE decreases were observed at the farthest downstream site in zone D1 (D1–4) and at most survey locations in zone D2 (Table 3). At site D2–4, where quadrat surveys were conducted during both studies, mussel density in 2005 was half of the density observed in 1999 (Table 3). Examination of the numbers of species observed at survey locations common to both projects shows apparent gains in species richness in 2005 at most sites; however, several of the species observed downstream of the SITE in 2005 were represented by only one mussel (Tables 2 and 3).

The paucity of mussel abundance and recruitment for ~35 km (downstream zone D1) and species richness for ~120 km (zones D1 and D2) downstream of the SITE is biologically significant and emphasizes the impact of historic pollution in the lower NFHR. Although contaminant concentration data were not collected during this study, a review of relevant data demonstrates the spatial relationship between contaminant concentrations and impaired mussel populations, and highlights the need for further research on the effects of contaminant mixtures and their probable presence downstream of the SITE.

Initial research by Hill et al. (1974), Carter (1977), Turner and Lindberg (1978), and Hildebrand et al. (1980) documented pervasive, unmitigated delivery of Hg from the chemical–waste holding ponds, occurrences of Hg and MeHg in downstream biota, and subsequent biotic effects. These studies helped to establish a rationale for regulatory action by USEPA, with designation of the SITE on the U.S. National Priorities List in 1983. Despite mitigation actions that have reduced Hg input to the river from the SITE, recent studies also have documented high levels of legacy total Hg in sediment and pore water that may partially explain the low mussel abundances and species richness downstream (Echols et al. 2009, USEPA 2012). Survival and growth of caged Asian clams *Corbicula fluminea* (Müller, 1774) at river locations downstream of the SITE were inversely correlated with sediment Hg concentrations (Echols et al. 2009). Downstream concentrations ($\bar{X} = 0.99 \pm 0.35$ mg Hg/kg,

TABLE 3.

Comparison of results from surveys conducted in 1995 (Henley and Neves 1999) and in 2005 (this study).

	NFHR sites															
	Downstream															
RZ	Zone D1								Zone D2							
Site (NFHRKM)	128.6		117.9		110.4		96.4		49.3		21.7		10.0		9.8	
Site abbreviation*	D1–1		D1–2		D1–3		D1–4		D2–2		D2–4		D2–5		D2–6	
Survey year	1995	2005	1995	2005	1995	2005	1995	2005	1995	2005	1995	2005	1995	2005	1995	2005
Density (mussels/m^2)†	–	–	–	–	–	–	–	–	–	–	2.6	1.3	–	–	–	–
CPUE (mussels/h)	0.5	0.6	0.5	0.9	2.0	1.8	11.0	7.0	13.6	6.9	30.8	7.7	2.6	0.8	5.3	5.9
Number of species‡	1^1	4^3	1^1	3^1	2^2	3^2	3	3	4^1	6^3	4^1	3^1	4	5^2	4^1	9^9
Number of juveniles§	0	0	0	0	0	0	0	0	5^1	1^1	11^3	1^1	0	0	0	0
Density of juveniles	–	–	–	–	–	–	–	–	–	–	0.25	0.07	–	–	–	–

Sites in downstream RZ D1 and D2 are presented in the table. Results are presented only for sites where similar surveys were conducted during both studies.

* See Table 1 for interpretation of site codes.

† Only site D2–4 was surveyed with quadrats in 1999 and this project.

‡ Superscript indicates number of mussel species observed at survey location.

§ Superscript indicates number of species with juveniles observed at survey location.

$n = 7$ sampling sites) of Hg in river sediment samples were higher than sample concentrations from upstream reference locations ($\bar{X} = 0.02 \pm 0.01$ mg Hg/kg, $n = 2$ sites), with a maximum total Hg concentration of 2.82 mg/kg measured ~2.6 km below the SITE (Echols 2007). At NFHRKM 96.0 (~35 km downstream of the SITE), the mean concentration of Hg in river sediment measured from 1990 to 1999 was 2.76 mg/kg (±0.79, $n = 6$), with all data above the consensus-based threshold probable effects concentration of 0.18 mg/kg (MacDonald et al. 2000). For reference, the USEPA severe effects level for Hg in sediment is 2.0 mg/kg (Buchman 2008). The highest concentration of Hg in interstitial water (2.1 µg/L) was observed ~80 km downstream of the SITE, which is approximately three times the USEPA chronic screening benchmark of 0.77 µg/L (Buchman 2008, Echols et al. 2009).

Although Hg and MeHg have been designated as contaminants of concern at the SITE, it is unlikely that Hg and MeHg are solely responsible for suppression of mussel recovery; rather, it is probable that restraint on recovery occurs from effects of a wide array of contaminants from the SITE that includes Hg and MeHg. Chloride concentrations from various sources at and near the SITE also may inhibit downstream mussel recovery. Over the course of operation, the chemical plant historically used massive amounts of calcium and sodium chloride salts during industrial processes (Hill et al. 1974). In the final years of the plant's operation, an estimated ~1,630,000 kg/day of these salts were deposited into the chemical waste–holding ponds, adjacent soils, and river at the SITE (Sheehan et al. 1989, Seivard et al. 1993). Also, major salt deposits exist at and near Saltville, and extraction occurred beginning in the late 1700s, with significant industrial salt production occurring during the U.S. Civil War and continuing to chemical plant closure in 1972 (Whisonant 1996, USEPA 2012). Brine ponds in the town of Saltville are associated with the abandoned salt mines, and water levels of these ponds are maintained by a piped-discharge that empties into the river ~1.7 km upstream of the SITE at NFHRKM 134.1. Although the SITE and this brine discharge are major contributors of Cl^- to the river, it is noteworthy that several Cl^- National Pollutant Discharge Elimination System permits have been issued for private, municipal, and corporate discharges at and near the SITE, and contributions of Cl^- from these permittees to the river are unknown (VADEQ 2006, Echols et al. 2009). Because of elevated levels of Cl^- in the vicinity of the SITE, the Virginia Department of Environmental Quality designated a 7.2 km reach of the NFHR, beginning just upstream of the brine discharge and including the SITE, as impaired (VADEQ 2006, 2012).

Chloride concentrations measured in water samples from the NFHR beside the SITE boundary varied widely and ranged from ~210 to 26,000 mg/L, whereas Cl^- levels at the brine discharge ranged from ~2,970 to 4,600 mg/L (Echols et al. 2009, Wang et al. 2010, Henley et al. 2013). For reference, the USEPA's recommended acute and chronic Water Quality Criteria for Cl^- are 860 mg/L and 230 mg/L, respectively (NOAA 2016). Exposures of juvenile mussels to 100% concentration of the brine discharge water caused 100% and 40% mortality in *Lampsilis fasciola* and *Lampsilis ovata* (Say, 1817), respectively, and mortality was attributed to concentrations of Cl and Na; the dilution EC50 during brine water exposures for *Villosa iris* was 68% (Wang et al. 2010). However, recent results showed that mean Cl^- concentrations dissipated from 2,747 mg/L at the brine pipe to 158 mg/L at a measurement location approximately 100 m downstream of the pipe, but increased again downstream at seepage points beside the SITE (measured range from 255 to 17,983 mg/L) (Henley et al. 2013). Downstream Cl^- data are limited, with only one dataset obtained downstream of the SITE at NFHRKM 96.0 (~35 km downstream), where mean Cl^- concentration from 1990 to 2001 was 93.7 mg/L (range 39–205 mg/L, $n = 120$) (VADEQ 2006).

Mussel species and life stages within species exhibit varied sensitivity to Cl^-, and there are indications that the range of Cl^- concentrations observed at and downstream of the SITE could produce acute or chronic effects in downstream mussels. Thus, Cl^- may be a significant pollutant that inhibits mussel occurrence and also recruitment in zone D1. The 24-h Cl^- LC50 estimates for glochidia of multiple species vary widely, ranging from 113 to 3,257 mg/L (Bringolf et al. 2007, Valenti et al. 2007, Cope et al. 2008, Gillis 2011). Reduced encystment of glochidia on host fish and juvenile transformation of *Elliptio complanata* (Lightfoot, 1786) occurred at 3 mg NaCl/L (~1,820 mg/L Cl^-) (Blakeslee et al. 2013). The 96-h LC50 for juvenile *Villosa iris* exposed to NaCl was 1.66 g/l (~1,010 mg/L Cl^-), and reduced oxygen consumption in adult *E. complanata* exposed to 2 mg NaCl/L (1.21 mg Cl/L) occurred within 24 h, and consumption rate did not recover after 28 days (Pandolfo et al. 2012). High Cl^- concentrations in the 35 km reach downstream of the SITE probably inhibit mussel recruitment, but this needs to be determined.

The formation of mercury-chloride complexes, including $HgCl^+$, $HgCl_2$, $HgCl_3^-$, and $HgCl_4^{2-}$ can occur in aquatic environments like the NFHR where elevated Cl^- exists in the presence of Hg (Hahne and Kroontje 1973, USEPA 1987, Ravichandran 2004). We can, however, find no documentation of testing for mercury-chloride complexes from any NFHR locations. Several authors have observed toxic effects of $HgCl_2$ on freshwater and marine bivalves, including immunotoxicity, genotoxicity to gametes, reduced fertilization, embryotoxicity, reduced larval metamorphosis, and reduced growth and survival of larvae and juveniles (Calabrese et al. 1973, Brunelli et al. 1986, Beiras and His 1994, Boening 2000, Fournier et al. 2001, Sauvé et al. 2002, Valenti et al. 2005, 2007, Duchemin et al. 2008; Fathallah et al. 2010).

Pollution in the lower NFHR is likely more complex than just presence of Hg, Cl^-, and possibly their complexes. Fairly recent results showed that a wide array of contaminants enters the river from SITE run-off and groundwater seepages. High concentrations of Ca, Na, K, SO_4, ammonia, trace elements, conductivity, as well as Cl^- were observed in water samples from collection locations in the river adjacent to the chemical-holding ponds and a water diversion ditch between the two waste ponds (Wang et al. 2010, Henley et al. 2013). During these studies, conductivity in the river adjacent to the SITE ranged from ~1,000 to 64,000 µS/cm, indicating that active seeps from the waste ponds exist and elevate conductivity (Wang et al. 2010, Henley et al. 2013). Serial dilution tests using water samples from Pond 6 (most downstream of the two waste ponds) and diversion ditch seeps showed conductivity EC50s for juveniles of *Villosa iris* ranging from ~7,100 to 10,300 µS/cm (Wang et al. 2010). Water samples from seepages adjacent to Pond 6 and the diversion ditch also were acutely toxic to juveniles of *Lampsilis fasciola* and *Lampsilis ovata*, and exposures to serial dilutions of these two samples estimated LC50s for *V. iris* juveniles at 16%

concentration of Pond 6 and 43% concentration of the diversion ditch seepages (Wang et al. 2010). Toxicity tests on two occasions using reconstituted water from the Pond 6 seepage site indicated that concentrations of Ca (8,550 and 2,200 mg/L), Na (6,840 and 1,590 mg/L), Cl^- (22,200 and 7,320 mg/L), and K (47 and 17 mg/L) were sufficiently elevated to cause toxicity to juveniles of *V. iris* (Wang et al. 2010). Juveniles of *V. iris* also showed 100% mortality during 28 days *in situ* exposures to sediment from a Pond 6 seepage point (Wang et al. 2010). At the same Pond 6 seepage location where Wang et al. (2010) collected their water samples, Henley et al. (2008) observed 93.8% and 100% mortality of juveniles and adults of *V. iris* deployed *in situ* after just 41 h of exposure, and severe necrosis in histologically prepared gill, kidney, and digestive gland tissues was observed after only 22-h exposure. Because concentrations of conductivity and ions at the SITE are elevated enough to cause effects to mussels, they have likely inhibited mussel recovery in at least RZ D1, but this needs to be substantiated by further research.

Effects of cyclical mobilization of legacy contaminants during flood events have not been addressed in literature concerning the SITE. Activities at the SITE have contributed to high levels of total Hg in sediment and floodplain soils downstream. Maximum observed concentrations of total Hg in floodplain soils were above the USEPA ecological soil screening level of 0.1 mg/kg, dry weight at the majority of 28 sampling sites downstream of the SITE, with one datum greater than 22 mg/kg observed ~23 km downstream of the SITE (MACTEC Engineering and Consulting 2006, Buchman 2008). To date, research on occurrence of Hg in the water column of the lower NFHR has been conducted during normal- or low-flow conditions. Results from research conducted in other river systems, however, indicate that transportation of Hg in lotic systems occurs during high-flow events due to mobilization of particulate-bound Hg and MeHg (Balogh et al. 1997, Mason and Sullivan 1998, Whyte and Kirchner 2000, Riscassi et al. 2016). As a result of their findings, Whyte and Kirchner (2000) suggested that Hg stream monitoring programs that do not include water quality measurements obtained during high-flow episodes might greatly underestimate actual Hg transport. Since 1907, flood stage was exceeded at the U.S. Geological SurveyGaging Station near Saltville 47 times (USGS 2013). It is important to note that no results were found of testing for other contaminants except Hg in sediment or floodplain soils from downstream locations, but recent results of Henley et al. (2013) showed a wide array of pollutants including various metals in water samples along the Site. Results from Henley et al. (2013) showed that concentrations of 12 metals and metalloids were strongly associated with mortality and vital organ damage in *in situ* exposures to *Villosa iris* deployed to the river beside the chemical-holding ponds. Thus, other contaminants probably exist in sediment and soils downstream of the SITE that have never been detected. Our concern is that when metal-laded particulates are cyclically mobilized from large reaches in the lower NFHR, recurring broad-scale transformations of Hg and possibly other metals probably occur (Stein et al. 1996, Ullrich et al. 2001, Eggleton and Thomas 2004). Such mobilization may be yet another factor that contributes to inhibition of downstream mussel recovery, but this needs to be determined.

Comparison of results from Henley and Neves (1999) and this study show that mussel recovery downstream of the SITE did not occur during the period from 1995 to 2005. Downstream conditions continue to limit mussel abundance and recruitment in D1 and species richness in D1 and D2, and zone D1 remains a reach of special concern regarding mussel occurrence and reproduction. Past research emphasis on only two contaminants of concern (Hg and MeHg) has probably led to underestimation of the degree and complexity of pollution in the lower NFHR. Future research needs to investigate the wide array of other stressors from the SITE, including concentrations of Cl^-, metal-Cl^- complexes, major ions, and trace elements in the NFHR during normal flow and flood conditions to determine their mobility and estimate effects on recovery of downstream mussel populations.

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