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Freshwater Mussel Shells as Environmental Chronicles: Geochemical and Taphonomic Signatures of Mercury-Related Extirpations in the North Fork Holston River, Virginia

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This study utilized freshwater mussel shells to assess mercury (Hg) contamination in the North Fork Holston River that extirpated (caused local extinctions of) a diverse mussel fauna. Shells (n = 366) were collected from five sites situated upstream (two sites), just below (one site), and downstream (two sites) of the town of Saltville, Virginia, where Hg was used to produce chlorine and caustic soda from 1950 to 1972. Shell samples were used to test the (*1*) utility of geochemical signatures of shells for assessing the spatial variation in Hg levels in the river relative to the contamination source and (*2*) value of taphonomy (post-mortem shell alteration) for distinguishing sites that differ in extirpation histories. Geochemical signatures of 40 shells, analyzed using atomic absorption spectroscopy, indicated a strong longitudinal pattern. All shells from the two upstream sites had low Hg concentrations (<5–31 μg/kg), shells directly below Saltville had variable, but dramatically higher concentrations (23–4637 μg/kg), and shells from the two downstream sites displayed intermediate Hg levels (<5–115 μg/kg) that declined with distance from Saltville. Two pre-industrial shells, collected at Saltville in 1917, yielded very low Hg estimates (5–6 μg/kg). Hg signatures were consistent among mussel species, suggesting that Hg concentrations were invariant to species type; most likely, highly variable Hg levels, both across sites and through time, overwhelmed any interspecific differences in Hg acquisition. Also, a notable post-mortem incorporation of Hg in mussel shells seemed unlikely, as the Hg content was not correlated with shell taphonomy (r = 0.18; p = 0.28). The taphonomic analysis (n = 366) showed that the degree of shell alteration reliably distinguished sites with different extirpation histories. At Saltville, where live mussels have been absent for at least 30 years, shells were most heavily altered and fragmented. Conversely, fresh-looking shells abounded upstream, where reproducing mussel populations are still present. In summary, relic shells offered valuable spatio-temporal data on Hg concentrations in a polluted ecosystem, and shell taphonomic signatures discriminated sites with different extirpation histories. The shell-based strategies exemplified here do not require sampling live specimens and may augment more standard strategies applied to environmental monitoring. The approach should prove especially useful in areas with unknown extirpation and pollution histories.

Introduction

There is a growing realization that shells of benthic organisms may provide important and otherwise inaccessible historical insights into the anthropogenic changes of aquatic ecosystems, thus augmenting ongoing efforts of conservation biologists and environmental scientists (*1*–*12*). These new strategies have been recently applied to marine habitats, demonstrating the value of long dead shellfish as archives of the recent history of now degraded ecosystems (*3*, *9*, *10*, *12*–*15*). Shells of freshwater mussels may also offer a suitable target because they litter many streams, rivers, and lakes of the world and should provide data comparably valuable to those provided by marine shellfish. Yet, except for a handful of recent studies (*8*, *11*), shell-based strategies, which require minimal (if any) live collecting, have remained underutilized in environmental research of freshwater habitats.

The main goal of this study was to explore the utility of shell-based techniques for studying freshwater ecosystems severely affected by Hg contamination. The study focused on the North Fork Holston River (Virginia), a freshwater ecosystem that historically supported very diverse mussel populations (*16*), but was subsequently affected by catastrophic-scale Hg contamination by the chlor-alkali industry (*17*, *18*). This river provides a highly suitable testing ground for demonstrating the utility of shell-based approaches for studying the history of local ecosystems affected by metal pollution.

Such approaches should be broadly applicable to diverse environmental issues. First, Hg pollution affects many aquatic ecosystems worldwide (*7*, *19*, *20*). In particular, the chlor-alkali industry, which utilized a Hg cell in the electrolysis process (*21*, *22*) for over 100 years, has led to the degradation of numerous rivers and extirpation of many species (*23*). Second, bivalve mollusks are among the key bio-monitoring tools used globally to assess the state of aquatic ecosystems (*4*, *19*, *20*, *24*, *25*), including anthropogenic increases in sediment load, changes in dissolved oxygen content, impoundments, channelization, and pollutants (*26*). Third, due to anthropogenic stresses, freshwater mussels are disappearing from historically abundant areas at alarming rates: 70% of North American mussels are extinct, endangered, or in need of special protection (*26*). Not surprisingly, freshwater mollusks have been studied extensively by conservation biologists and environmental researchers (*1*, *4*, *7*, *11*, *27*, *28*). However, most of those investigations have focused on live-collected mussels, and shells have remained largely unexplored as a potential source of environmental information. Fourth, mussel shells may record the history of severe metal pollution. Divalent metals, in particular, may be incorporated into the calcareous shell as metabolic analogues to calcium (*4*, *12*), so shells can act as recorders

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of the metal contamination during the organism's lifetime (*11*). Analyzing shell geochemistry is also attractive because soft tissues of mollusks are highly sensitive to short-term variations in water chemistry, recording primarily chemical conditions of the water at the time of sampling (*10*). In contrast, shells are the long-term recorders of the pollution history of an area (*3, 4, 7, 9–12*). Finally, the post-mortem alteration of mussel shells (i.e., their taphonomic signatures) may provide insights into local extirpation history. Areas lacking reproducing populations are likely to be dominated by severely altered specimens. Conversely, in areas supporting extant populations, death assemblages should include a higher proportion of fresh-looking shells derived from recently dead specimens. Yet, despite intense taphonomic research of marine shellfish, the applicability of using taphonomic patterns to evaluate freshwater ecosystems is still in its infancy (*29*).

This study evaluates whether (1) the geochemical signatures of Hg extracted from shells can offer a separate documentation of the contamination history of a polluted river system and (2) the taphonomic signature of freshwater shells can provide independent assessments of the local extirpations events. If successful, the shell-based strategies combining geochemical and taphonomic strategies could become a functional research tool applicable to other freshwater ecosystems affected by metal pollutants. To test the geochemical approach, Hg concentrations were analyzed in shells collected at five locations along the North Fork Holston River, including sites upstream, just below, and downstream of the contamination source. Concurrently, the extent of shell alteration at each site was quantified using a variety of taphonomic features. The resulting taphonomic patterns were then used to assess whether such signatures can be used reliably to distinguish sites with different Hg contamination history.

History of the Study Area

The Olin-Mathieson Chemical Co. utilized natural, underground salt deposits located in Saltville, Virginia, to produce chlorine and caustic soda. The plant used a Hg cell in the production process from 1950 until 1972, when the factory was permanently closed because discharge water standards could not be met cost-effectively (*30*). Two unlined settling ponds that covered 44 ha (*23*) and ran 4.16 km along the river were used to settle particulates from the Hg-contaminated waste slurry (*30*). The ponds drained directly into the river via pipes through a dike (*23*). By 1957, the elemental Hg and chloride salts led to extirpations of mussel populations as far as 112 km downstream of Saltville (*31*). The Hg contamination also was evident in fish samples collected in 1977, more than 160 km downstream of Saltville (*17*). An estimated 1814 metric tons of salt and 34 kg of Hg were deposited per day into the plant's settling ponds during the final years of operation (*18*). After plant closure, an estimated ~100 g of Hg seeped from these ponds into the river daily. In addition, 99 773 kg of Hg was reported on the grounds where the "cell building" once stood (*17*).

The North Fork Holston River is a moderate-hardwater, riffle-pool stream, with substrate composed primarily of sand, gravel, and rubble (*30*). Historically, the river had an extremely diverse freshwater mussel fauna. Ortmann (*16*) reported 42 species of freshwater mussels in the river, including 33 species downstream of Saltville. This originally high diversity relates to a favorable geological setting. In many places, the river flows directly over limestone bedrock, enriching the water with calcium, while numerous sandy pool areas provide habitats rich in nutrients (*32*).

The mussel diversity decreased dramatically in the North Fork Holston River following the Hg contamination. In 1998, only nine species of mussels were found living downstream

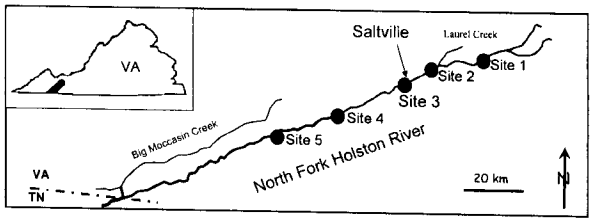

FIGURE 1. A schematic map of the study area showing the five sampling sites. The inset map shows the regional location of the study area (modified after Henley and Neves, 1999). The black arrow indicates the approximate position of the Hg contamination source at Saltville.

of Saltville (*33*). Five of these nine species were only found below river mile 13.5, nearly 107 km downstream of Saltville (*33*). Beginning in 1975, transplanting restoration efforts at multiple sites downstream of Saltville have reintroduced mussels from sites upstream of Saltville and from the nearby Clinch River (*33, 34*). However, in the late 1990s, reproducing individuals were found at only 4 of 19 downstream sites at which live mussels were reintroduced (*33*).

Material and Methods

Sampling Sites. Five collection sites used in previous research on mussels of the region were chosen on the North Fork Holston River (Figure 1) (*33*). Two sites are located upstream of Saltville at river miles 96 (site 1) and 85 (site 2). Site 3 is located at river mile 79.9, directly below the Olin-Mathieson settling ponds in Saltville. The two downstream sites are located at river miles 68.6 (site 4) and 56 (site 5).

Stream Gradient. The stream gradient was calculated as a proxy for the energy of each site. The distance between topographic contour lines that encompassed each site was measured on U.S.G.S. topographic maps. The difference between the elevations of the two encompassing contour lines was divided by the distance between them. These calculations produced an estimate of average stream gradient for each stretch of the river.

Sampling of Mussel Shells. Due to scarcity of shells in the lower North Fork Holston River, collection along transects (*33*) or in grids was not feasible. Consequently, collection efforts consisted of systematic, exhaustive surveys of sampling sites with all shells and shell fragments collected by hand-picking. The surveys were focused along the banks because most of shells were found in the soft sediment near the shoreline. To ensure comparable sampling intensity across sites, ~45 min was spent collecting at each site during each of the three trips to each site. The surficial sampling strategy may bias collecting efforts against larger, better preserved specimens, which often die in situ and are not likely to be altered as heavily as when exposed on the surface. However, because the same procedure was followed at all sites, data yielded estimates that were meaningful for comparison.

Taphonomic Analysis of Shells. Taphonomic analysis refers to the study of post-mortem physical, chemical, and biological alterations of shells, bones, and other skeletal remains. Freshwater mussels are expected to acquire diverse post-mortem alteration because their shells consist of high-organic, nacreous, aragonite, and are thus vulnerable to processes that operate in high-energy fluvial systems. Also, the weak hinge ligament in most species makes shells prone to post-mortem disarticulation (*29*).

All collected mussel shells and shell fragments were categorized as right or left valve, and whole specimen or shell fragment. The maximum anterior–posterior length of each shell was measured with electronic calipers to the nearest 0.1 mm. The genus and species were identified for the reasonably complete valves and then classified as a "thick" or "thin" species.

A taphonomic scoring system was adopted by modification of standard rank systems developed for marine mollusks (*35–39*). However, the taphonomic scores may not be comparable across different studies because there is a significant operator bias (*40*), and the scores may vary also due to environmental and taxonomic differences across studies. All shells and shell fragments were ranked for the following four taphonomic variables: (1) degree of fragmentation, (2) valve edge rounding, (3) shell exterior surface alteration, and (4) presence of articulation. All four grades pertain exclusively to extrinsic (typically post-mortem) shell modifications and should not be confused with biologically mediated deformations that may alter shell morphology during the growth of the mussel (*41*). For each taphonomic variable, a score of 0 was assigned for unaltered specimens, and increasingly higher ranks (up to 4 ranks in the case of some types of alterations that allowed for distinguishing multiple intermediate alteration stages) were assigned to remains displaying an increasing degree of alteration. For each specimen, the total taphonomic grade (TTG) was calculated as the arithmetic sum of scores assigned for the four individual grades. Museum specimens, live-collected in 1917 (see below), were not analyzed for taphonomic signatures because any alteration displayed by these shells would reflect museum storage of live-collected specimens, not relevant for studying post-mortem taphonomic history.

Preparation of Shells for Hg Analysis. Digital photographs of each specimen were taken prior to the chemical analyses. The shells were then soaked and scrubbed in bleach to remove any extraneous organic material or sediment that may have been attached. Shells were scrubbed with a nylon brush to remove the periostracum. Each specimen was consistently sampled from the same shell area to minimize the amount of variability in chemical signatures due to the heterogeneity of the shell (i.e., the prismatic and nacreous layers, cleaned of the periostracum, along the ventral margin). The sampled pieces were powdered using a ceramic ball-mill that was cleaned with 12 N hydrochloric acid and rinsed with distilled water between uses.

Hg Analysis of Shells. Five shells from various species at each site were selected initially to investigate whether Hg could be detected in the shells. The powdered samples were analyzed for Hg at the Activation Laboratories Ltd. (Canada) by cold vapor FIMS (flow injection Hg system) using a Perkin-Elmer atomic absorption spectrometer with detection limits of 5 μg/kg. Prior to statistical analysis, shells with Hg concentrations below the detection limits were assigned the value of 5 μg/kg (i.e., the highest possible value). Duplicate samples, analyzed to assess the laboratory error, displayed variation below the detection limit.

Following the initial analysis, all subsequent Hg samples were constrained to one species, *Pleurobema oviforme*, the Tennessee clubshell. This species was selected because it (1) can be found at all five sampling sites; (2) is native to the river; (3) has a robust shell that may survive for many years after death; (4) showed high levels of Hg in the initial analysis; and (5) occurs in museum collections that include specimens live-collected in pre-industrial times.

Two shells of *P. oviforme*, collected from Saltville in 1917 by C. C. Adams, were also analyzed. These shells were obtained from the Carnegie Museum of Natural History to establish background Hg levels prior to the Hg pollution event.

Statistical Analyses. Given the scarcity of the shells at some of the sampling sites, the sample sizes for this study were small. Consequently, data were pooled by groups of sites (e.g., "upstream" from "downstream") to maintain statistically reasonable sample sizes. The pooling of data across sites makes statistical test results more conservative.

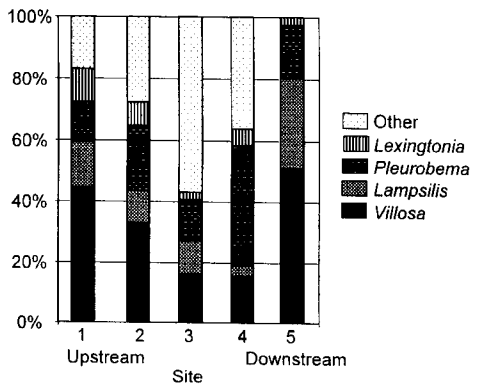

FIGURE 2. The taxonomic (genus-level) composition of shell assemblages of freshwater mussels from the North Fork Holston River based on bulk sampling at the five sampling sites (n = 266).

For example, the addition of specimens from the increasingly distant downstream sites (especially site 5) is expected to lower the estimates of the average Hg concentration of shells, making it potentially more difficult to statistically distinguish the contaminated from uncontaminated sites.

Due to small sample sizes and the inclusion of many rank and nominal variables, the statistical analyses focused primarily on nonparametric rank techniques (e.g., Wilcoxon two-sample median test, Spearman rank correlation test) and standard contingency tests for enumeration data (log-likelihood G ratio) (*42*). All statistical decisions were based on the significance level of $\alpha = 0.05$. Tests were performed using Statistical Analysis Software (*43*).

Results

Fourteen species were found in the death assemblage at the five sites (see Appendix 2, Supporting Information). Four genera occurred in notable numbers at all five sites (Figure 2). The sites varied significantly in terms of relative abundance of dominant genera (G = 67.3; $p < 0.0001$; df = 12; log-likelihood ratio χ^2 G-test).

Shell Hg Concentrations Relative to the Pollution Source. The initial Hg analysis (see Supporting Information for data summary), based on specimens from eight species of seven distinct genera, indicated that shells collected upstream from Saltville had Hg concentrations below 10 μg/kg (Figure 3A). In contrast, shells collected directly below the pollution source at Saltville contained substantially elevated Hg concentrations, with multiple specimens exceeding 100 μg/kg; the highest value was 176 μg/kg, and mean value was 31.7 μg/kg. Shells collected further downstream had Hg concentrations that decreased with increasing distance from Saltville (Figure 3A). The maximum Hg value at site 4 was 115 μg/kg (mean = 42 μg/kg), and declined to 14 μg/kg at site 5 (mean = 8 μg/kg), making it similar to estimates for the most upstream site 1 (approximately 25.6 km upstream from Saltville).

The Hg analysis of only *P. oviforme* shells (Figure 3B) was consistent with the mixed-genera analysis, but exhibited an even more dramatic spike in Hg concentration at, and downstream of, Saltville (note the y-axis is log-transformed). One specimen had Hg levels of 4637 μg/kg, several hundred times higher than recorded for any shell collected upstream. Results of Hg analyses suggested slightly elevated Hg levels at site 2, the closest site above Saltville (Figure 3). As in the case of the mixed genera analysis, the Hg content in *P. oviforme* shells decreased downstream, with increasing distance from the contamination point at Saltville.

The mixed-genera shells and *P. oviforme* shells did not differ significantly in their median Hg concentrations (Table 1). The lack of significant statistical differences between the

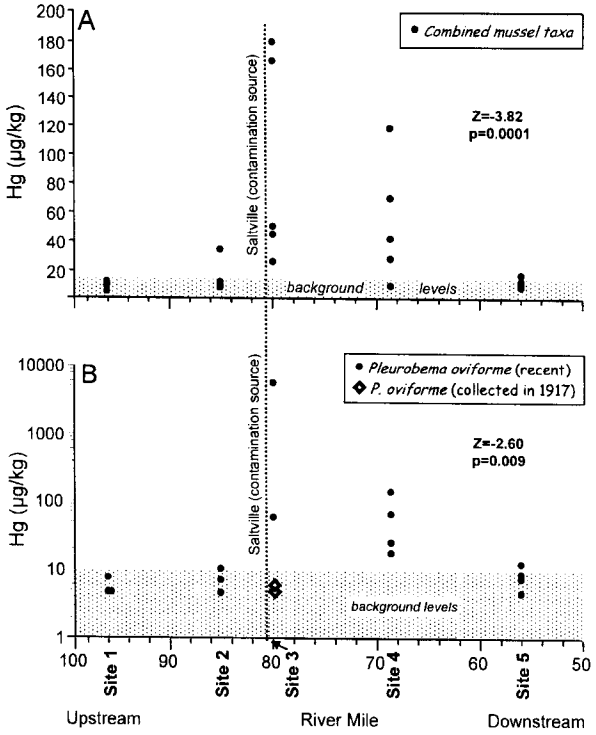

FIGURE 3. Results of the Hg analysis; each data point represents an estimate of the Hg content derived from different specimens of dead-collected shells. The hatched area represents expected background levels of Hg in uncontaminated habitats. The background levels are based on the highest concentrations (of 9 µg/kg) measured in shells from site 1, which was assumed unaffected by the Hg pollution (located >20 km upstream of the contamination point). (A) Results from reconnaissance study of specimens from several different genera. (B) The larger study (note log-scale *y*-axis) restricted to the species *Pleurobema oviforme*. Note that the highest Hg values downstream of Saltville were 2–3 orders of magnitude higher than those observed upstream. Specimens collected in 1917, prior to the contamination event, had levels of Hg comparable to background levels observed upstream.

two analyses, and the striking similarity of Hg patterns among sites (Figure 3A vs B), warrants pooling the data across taxa (such pooling was needed to obtain sufficient sample sizes for statistical analyses across sites).

The pooled data were first grouped into "upstream" (sites 1 and 2) and "downstream" (sites 3–5) categories. Comparison of these two groups showed that the upstream shells had significantly lower Hg concentrations than the downstream shells (Table 1). The resulting difference in Hg concentration was even more dramatic when site 5 was excluded from the analysis (Table 1). When the data were restricted to *P. oviforme* shells only, the significant pattern observed for pooled data persisted (Table 1).

The two shells collected from Saltville in 1917 had very low levels of Hg (6 µg/kg or less), comparable to the low levels observed at site 1 (Figure 3B). Because only two specimens were available for analysis, statistical tests are not applicable. Nevertheless, it is remarkable that all post-industrial shells from Saltville displayed Hg concentrations that were higher (by 2–3 orders of magnitude, in most cases) than those recorded by the pre-industrial shells.

Taphonomic Signatures and Extirpation Patterns. The shells with the highest total taphonomic grade, that is, those that were most heavily altered and fragmented when all taphonomic variables were combined, dominated in areas at site 3 directly downstream from the contamination point (Figure 4A). In contrast, upstream sites, unaffected directly by the Hg contamination, contained many less altered shells with lowest total taphonomic grades. Site 4 had specimens that varied in the total taphonomic grade, many still exhibiting a high degree of alteration. The total taphonomic grade decreased from site 4 to site 5, the farthest site downstream (Figure 4A). Site 5, with similar numbers of shells with high and low taphonomic grades, was comparable to sites 1 and 2 (upstream). The downstream shells were significantly more taphonomically altered than the shells collected from upstream sites ($G = 27.8$; $p = 0.0005$; df = 8; G-test). The median taphonomic grade was also significantly higher at sites downstream from Saltville when compared to upstream sites ($Z = -3.66$; $p = 0.0003$; $n = 366$; Wilcoxon two-sample test with normal approximation). The same pattern also was seen when each taphonomic variable was examined separately (Table 2; Figure 5B; see also Supporting Information). For each of those four taphonomic variables, the observed differences between the "upstream" and "downstream" sites were statistically significant (Table 2).

Thick-shelled and thin-shelled species varied significantly in relative abundance among sites (Figure 5A; $G = 23.3$; $p = 0.0001$; df = 4; G-test). Although thin-shelled taxa exhibited significantly lower median taphonomic grades ($Z = 5.15$, $p < 0.0001$, thick, $n = 201$, median = 5, thin, $n = 114$, median = 3; Wilcoxon test), both thick-shelled and thin-shelled species revealed the same taphonomic trend: the highest taphonomic grade at site 3 and lower taphonomic grades upstream and downstream of the contamination point (Figure 5B). For the thick shells, this trend was statistically significant ($G = 19.6$; $p = 0.007$; df = 7; G-test). The thin shells were too few to be assessed statistically. However, with data grouped into the upstream and downstream sites, the difference in the total taphonomic grade was significant (Table 2).

The taphonomic pattern was unrelated to stream gradient (Figure 5B). Sites 1 and 2 represented the highest-energy (steepest-gradient) hydrodynamic regimes, but contained shells with a low degree of taphonomic alteration. Sites 3 and 4 had much lower stream gradients, yet contained shells with higher taphonomic grades than sites 1 and 2. The variation in stream gradient among the five studied sites was minor (1.84–1.23 m/km), such that hydrologic regimes were comparable among sites.

Discussion

Shell Hg Concentrations Relative to the Pollution Source. Geochemical analysis of shells of dead mussels showed that specimens collected upstream (sites 1 and 2) of the pollution source had low Hg concentrations (<10 µg/kg). The slightly elevated Hg levels at site 2 provide tentative evidence supporting anecdotal reports of undocumented landfill sites upstream of Saltville. Shells collected directly downstream of the pollution source contained significantly higher Hg concentrations, reflecting their close downstream proximity to the plant. Shells collected further downstream have Hg concentrations that decreased with increasing distance from Saltville, which may be a signature of downstream dilution of the Hg discharging from the plant at Saltville.

Results of this study corroborated recent research showing that freshwater mussel shells can provide a record of metal pollution. Markich et al. (*11*) used the freshwater bivalve shell of *Velesunio angasi* as an indicator of trace metal levels in a stream affected by acid mine drainage. In the Markich study, the whole shell was analyzed by bulk sampling, and the annual growth increments were analyzed using secondary ion mass spectrometry (SIMS). Similar to this study, Markich et al. (*11*) found that metal concentrations (copper, manganese, zinc, uranium, nickel, cobalt, and lead) in the whole shell decreased with increasing distance from the pollution source. They also observed that there were no significant

TABLE 1. Summary of Geochemical Results for Hg Analysis in Mussel Shells Collected from the North Fork Holston River

sample groups	sample size	Hg concentrations in shells (μg/kg)					Wilcoxon two-sample test	
		min	max	mean	median	standard deviation	Z	p
all data pooled	39[a]	5	4637	144	8	739.4		
mixed genera	21	5	176	32	9	48.3	−1.34	0.18
Pleurobema oviforme	18	5	4637	276	6.5	1089		
upstream sites (1−2)	17	5	31	8	6	6.1	−3.83	0.0001
downstream sites (3−5)	22	5	4637	250	21.5	981.2		
upstream sites (1−2)	17	5	31	8	6	6.1	4.33	<0.0001
downstream sites (3−4)	14	7	4637	387	44	1224		
site 1	9	5	9	6	6	1.4	24.06[b]	<0.0001
site 2	8	5	31	10	6.5	8.8		
site 3	6	23	4637	848	104.5	1858		
site 4	8	7	115	42	32	35.5		
site 5	8	5	14	8	7.5	2.9		
upstream sites (1−2)	8[c]	5	8	6	5.5	1.0	−2.70	0.007
downstream sites (3−4)	10[c]	5	4637	491	17	1457		

[a] Excludes two specimens collected in 1917. [b] χ^2 parameter value for nonparametric Anova (Kruskal−Wallis test). [c] Includes only *P. oviforme* data.

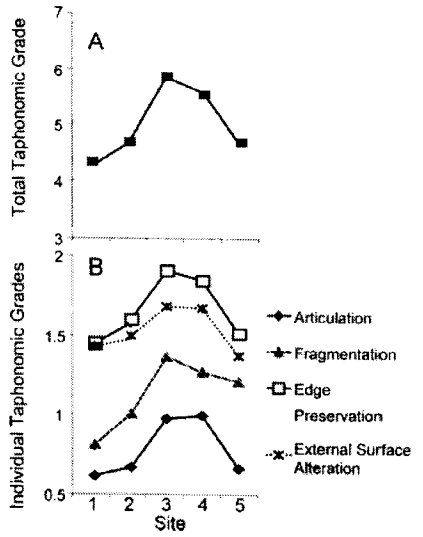

FIGURE 4. Changes in the overall taphonomic grade of shell assemblages (i.e., the extent of physicochemical shell alteration) along the river as observed in dead-collected shells from the five targeted sites. (A) The change in the grade of taphonomic variables combined (the total taphonomic grade) as observed in dead-collected shells from five targeted sites. (B) The change in average grade of individual taphonomic variables, as observed in dead-collected shells from the five targeted sites.

differences in the metal concentrations over the lifetime of the animals, represented by analysis of growth intervals. Both studies illustrated that bivalve shells can be successfully used to document metal pollution.

The use of multiple species of mussels for ecotoxicological comparisons across sites may obscure resulting patterns due to differences in rates of accumulation and excretion of heavy metals (*44*). It is therefore encouraging that not only *P. oviforme* shells, but all analyzed mussel species showed a significant increase in Hg levels below Saltville. Apparently, in the case of extensive Hg contamination, the obscuring effects of interspecific differences in vital effects are too minute to affect the primary geochemical signal exerted by pollutants.

The low levels of Hg in pre-industrial shells collected from Saltville confirm that elevated levels of Hg were not present in the Saltville area in 1917. Thus, the high levels of Hg in shells collected recently at site 3 are unlikely to record a natural (geological) source of Hg eroded by the river over multiple centuries, but rather, reflect recent (post-1917) anthropogenic release of Hg to the river at Saltville.

Given that intermittent shell transport may be expected for high-energy rivers, it is remarkable that the Hg signal is so clearly manifested spatially along the longitudinal cross-section of the North Fork Holston River. This strong spatial fidelity suggests that downstream shell transport must be very limited. This is perhaps not surprising given that reworking and transport of partly or entirely buried shells of large mussels are unlikely to be common even during seasonally high floods. This observation is also consistent with previous taphonomic studies, which suggested high fidelity of signals preserved in death assemblages of freshwater mussels (*29*).

Finally, Thomas and Bendell-Young (*6*) indicated that there may be potential metal contamination on the surface of the *Macoma balthica* shell that was exposed to the contaminated water. This problem does not appear to affect the shells analyzed in this study, as there is no correlation between the total taphonomic grade (a proxy for the duration of post-mortem exposure time) and Hg concentration (see Supporting Information).

Taphonomic Signatures and Extirpation Patterns. In the studied freshwater system, taphonomic signatures of dead shells provide a proxy for distinguishing sites with active (reproducing) mussel populations from sites affected by local extirpation events. Upstream areas that have had a continuous input of recently dead shell material exhibited younger taphonomic signatures. Site 3, directly below the contamination source, exhibited the oldest taphonomic signature. This site had been devoid of viable populations for at least 30 years, and thus was likely to be dominated by old specimens that have been altered by the prolonged action of various taphonomic factors operating in fluvial systems. The areas downstream exhibited an intermediate taphonomic signature, likely reflecting the fresh-dead shell input from mussels reintroduced in post-industrial restoration efforts. In sum, the taphonomic index of shells proved to be a reliable predictor for distinguishing affected and unaffected sites along the river. This approach may be a useful tool for identifying freshwater communities with unknown extirpation histories, especially for regions similar to the study area in faunal composition, climate, and local geomorphology (e.g., other comparably small rivers of the southeastern USA). In addition, the spatial trends in taphonomic signatures among the five sampled sites (Figures 4 and 5) were consistent with patterns revealed by Hg analysis (Figure 3) and provided additional, albeit more indirect, evidence suggesting that

TABLE 2. Summary of Statistical Tests for Differences in Taphonomic Grades Across Sites[a]

compared data and variables	upstream sites (1 and 2) n	upstream sites (1 and 2) median	downstream sites (3 and 4) n	downstream sites (3 and 4) median	Wilcoxon (two-sample) median test |Z|	Wilcoxon (two-sample) median test P
articulation						
all[c]	172	1	134	1	6.84	<0.0001
thick[c]	90	1	91	1	5.36	<0.0001
thin[c]	65	1	28	1	3.72	0.0002
fragmentation						
all[c]	172	0	134	1	2.96	0.003
thick[b]	90	0	91	1	2.03	0.04
thin	65	0	28	0.5	1.46	0.14
edge preservation						
all[c]	172	2	134	2	5.08	<0.0001
thick[c]	90	2	91	2	3.80	0.0001
thin	65	1	28	2	1.85	0.06
external surface alteration						
all[c]	172	1	134	2	3.34	0.0008
thick[c]	90	2	91	2	2.88	0.004
thin	65	1	28	1	0.56	0.58
total taphonomic grade						
all[c]	172	4.5	134	6	4.58	<0.0001
thick[c]	90	5	91	6	3.40	0.0007
thin[c]	65	3	28	5	2.30	0.02

[a] Site 5 was excluded from the analyses reported here (the analyses with site 5 included are similar in most cases). The mussel species were grouped into thickness categories as follows: "thick" (*Actinonaias pectorosa, Alasmidonta marginata, Fusconaia barnesiana, Fusconaia cor, Lampsilis fasciola, Lampsilis ovata, Lexingtonia dolabelloides, Pleurobema oviforme, Ptychobrancus subtentum, Ptychobrancus fasciolaris*) and "thin" (*Elliptio dilatata, Medionidus conradicus, Villosa iris, Villosa vanuxemensis*). [b] Tests significant at $\alpha = 0.05$ level. [c] Tests significant at $\alpha = 0.005$ level.

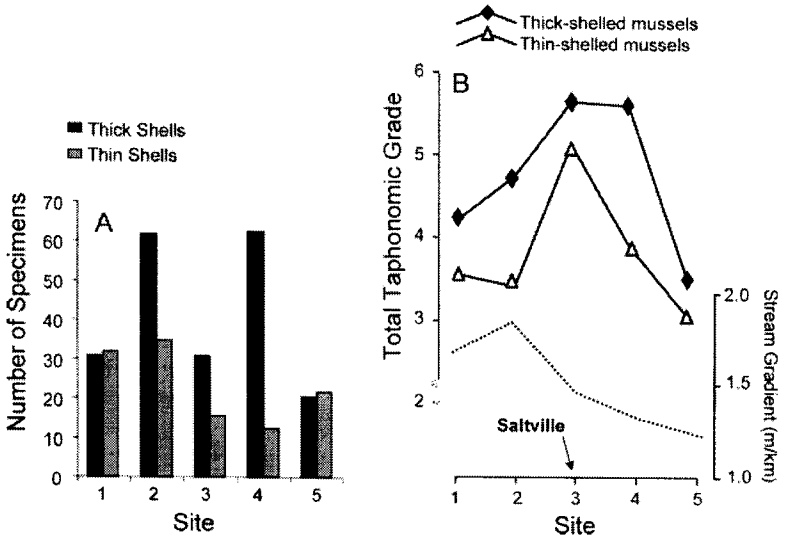

FIGURE 5. Changes in the overall taphonomic grade of shell assemblages (i.e., the extent of physicochemical shell alteration) along the river as observed in dead-collected shells from the five targeted sites. (A) The composition of dead shell assemblages across the five targeted sites with shells grouped into thick (more robust valves)- or thin-shelled (less durable valves) species of freshwater mussels. (B) Results for thick- and thin-shelled species of freshwater mussels. The taphonomic grade is compared to the stream gradient at each site (a proxy for hydrodynamic regime of each site) estimated from topographic maps.

the extirpation patterns related to the Hg pollution source in Saltville.

The taphonomic patterns documented in this study are consistent with high fidelity between the composition of shell death assemblages and live mussel populations postulated previously by Cummins (*29*), who concluded that fresh-dead shells are continuously contributed to the death assemblage in habitats with active, reproducing populations. However, following a local extirpation event, shell input is either arrested or, at best, limited to few transport-battered shells (if more pristine sites still exist upstream). As in the case of Hg patterns (see above), the strong spatial taphonomic signal indicates that extensive downstream transport of shells is limited in the studied river system. Also, the lack of correlation between stream gradient and taphonomic signatures suggested that extirpation history played a primary role in controlling the taphonomic signatures of mussel shells in the river (this observation may not apply necessarily to rivers with different substrates or more variable hydrologi or chemical gradients).

The differences in taphonomic signatures of thick-shelled and thin-shelled species most likely reflect the fact that robust shells can withstand more battering before being completely destroyed and thus can obtain a higher taphonomic grade than thin-shelled taxa. Yet, both thick-shelled and thin-shelled species revealed the same taphonomic trend related

to the pollution source, again suggesting that taphonomic patterns were controlled primarily by the presence or absence of reproducing populations.

Closing Remarks. The shell-based techniques presented here provided useful, otherwise inaccessible insights into the extirpation of freshwater mussels and Hg pollution history of the North Fork Holston River. Shell chemistry provided an independent record of the presence and longitudinal variation of Hg contamination in the river, and taphonomic signatures of shell assemblages offered a reliable tool for differentiating sites with different extirpation histories. The shell-based strategies demonstrated here could be of particular importance in areas with unknown extirpation and pollution histories.

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Supporting Information Available

Site location information (Appendix 1), list of species recovered at each site (Appendix 2), Hg content in shells collected in this study (Appendix 3), Hg content in specimens collected in 1917 (Appendix 4), summary of taphonomic data (Appendix 5), and the comparison of the overall taphonomic grade and the Hg content of shells (Appendix 6). This material is available free of charge via the Internet at http://pubs.acs.org.

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