

Supplemental Materials and Methods

S2.2 Mussel Propagation, Deployment, and Collection

All mussels used in this study were propagated by standard host fish infection techniques at either the Freshwater Mollusk Conservation Center at Virginia Tech in Blacksburg, VA, USA (all *V. iris* and a portion of *L. fasciola*) or the Virginia Department of Wildlife Resources Aquatic Wildlife Conservation Center in Marion, VA, USA (also supplied *L. fasciola*). *Villosa iris* were ~17 months old and ranged in size 15-40 mm at the time of deployment. Approximately half of the *L. fasciola* ranged in size 40-45 mm, while the other half were 20-22 mm, owing to the two source facilities. We used a random number generator to randomly select individuals of *V. iris* (n = 18) and *L. fasciola* (n = 11) for deployment in each cage and silo. Fewer *L. fasciola* were deployed per cage/silo to account for their larger size compared to *V. iris* and achieve similar biomass per cage/silo in both field seasons. We also reserved a random subset of 161 *V. iris* and 179 *L. fasciola* and immediately preserved them in a freezer to provide baseline samples for tissue contaminant analyses.

We deployed juvenile mussels in sediment cages at the 8 mainstem sites and in water column silos at all 12 sites (8 mainstem and 4 tributary sites). The cages were constructed from polyethylene industrial containers with mesh sides and base (406.4 mm x 304.8 mm x 114.3 mm; Schaefer Systems, 52356, US Plastic Corporation, Lima, Ohio, USA) with plastic mesh (6.34 mm) affixed to the inside of the cages on the top, sides, and bottom to contain mussels and allow water to flow through the cages (Supplemental Figure 1A). At each Clinch River mainstem site, 6 silos and 6 cages were placed in habitats with a substrate mixture of gravel and sand and in locations of historic mussel populations, based on

published literature, expert consensus, and the presence of dead shells. Cages were partially buried to ensure the tops of the cages were flush with the sediment-water interface. At all sites (mainstem and tributaries), we placed 6 concrete mussel silos to house mussels in the water column [~ 6 cm above the sediment-water interface. Mussels were placed in cylindrical chambers (14 cm) with plastic mesh (6 mm mesh size) on the top and bottom to allow water to circulate through the chamber (Supplemental Figure 1B).

We deployed *V. iris* on 5-6 June 2012, and *L. fasciola* on 31 July-1 August 2013. Mussels were deployed later in the second summer due to a series of storms and subsequent high-water levels in the river. Once mussels were deployed, cages and silos were monitored monthly throughout the deployment period and cleaned by fanning the water directly above the cage or silo to remove any fine sediment deposition. We retrieved *V. iris* from all sites on 23-25 October 2012, and retrieved *L. fasciola* on 5-7 November 2013. Upon collection, mussels were transported on ice to the laboratory where we transferred them to a -20°C freezer for storage. Prior to analyses, mussels were thawed, measured, weighed and dissected; mussel tissue was equally divided (from 3 cages or silos per sample) to create two composite tissue samples per site. The composite tissue samples were stored frozen (-80°C) until chemical analyses were performed.

Resident mussels (*Actinonaias pecterosa* and *V. iris*) were collected at all sites where they occurred. Methods for resident mussel collection are found in Cope et al. [11]. For the present study, resident mussels were analyzed for stable isotopes in the same manner as the mussels deployed in cages and silos (Section 2.3). Resident mussels could only be sampled at mainstem sites due to availability.

S2.3 Environmental Media Collection, Laboratory Analyses, and Quality Control

Shell cross-sections were pre-digested in warm hydrogen peroxide (30% v/v, Fisher Scientific) to remove all organic matter. Remaining mineral shell material was digested with a MARS 5 HP 500 Plus™ system microwave digestion system (CEM Corporation, Matthews, NC, USA) in 5 mL of trace metal grade nitric acid (Fisher Scientific). Digestions made use of either an entire valve, or representative fragments not exceeding 0.50 g dry weight. We digested two reagent blank samples and two standard reference material samples (DOLT-03, dogfish liver, National Research Council of Canada) in each set of 40 samples. Digests were analyzed using an inductively coupled plasma mass spectrometer (Agilent, 7500cx, Santa Clara, CA, USA).

Samples had detectable concentrations of several elements, with the percentage of samples with concentrations below the limits of detection ranging from 0.3% (Zn) to 98.61% (Cr). Specifically, the elements had the following percentages of samples below detection: Al = 0.7%, As = 27.5%, Cd = 93.0%, Co = 2.8%, Cr = 98.6%, Cu = 10.1%, Fe = 0.7%, Hg = 90.6%, Mn = 0.7%, Ni = 3.1%, Pb = 93.4%, Se = 58.2%, V = 69.3%, U = 98.3% and Zn = 0.0%.

S2.4.2 Growth

The size distribution of *L. fasciola* deployed in 2013 was bimodal (Supplemental Figure 2), owing to the different hatchery sources of the two size groups. To account for this, we pooled data within each site and performed modal analyses using the NORMSEP procedure in FiSAT II (version 1.2.2, Food and Agriculture Organization of the United Nations, Rome, Italy) to assign the mussels into small and large size groups by length. Separation index (SI) values from NORMSEP ranged from 4.2 to 7.9 (mean = 6.2), well above the threshold considered as 'reliable separation' (SI > 2). To calculate our growth rate metric (mm d⁻¹),

we subtracted the mean initial length from the mean final length at each site and divided this value by the respective deployment period (143 days in 2012 and 99 days in 2013). Site-specific initial lengths were only available in for *L. fasciola* in the 2013 season. For the 2012 season, we used the subset of initial lengths from *V. iris* collected as baseline mussels ($n = 161$) to randomly generate values for initial lengths. Although 2013 growth rates were higher overall in small mussels (mean = 0.03 mm d^{-1}) compared to large ones (mean = 0.01 mm d^{-1}) mussels, there was a strong positive and significant correlation between site-specific growth rates of small and large mussels in both silos ($r_s = 0.91$, $p < 0.0001$) and cages ($r_s = 0.87$, $p = 0.005$). Therefore, we considered it most parsimonious to use the average growth rate between the two size groups to facilitate comparisons between study years.

S2.4.4 Tissue Concentrations of Potential Toxicants

The Scheirer-Ray-Hare (SRH) extension of the Kruskal-Wallis test, a two-way non-parametric analog of ANOVA, was used to determine if tissue concentrations of individual contaminants (metals or PAHs) differed significantly between deployment methods (cages vs. silos), controlling for site by using an interaction factor. A relatively small number of elements (1 of 21 in 2012; 8 of 21 in 2013) exhibited significantly different concentrations between cages and silos, therefore, we pooled cage and silo tissue concentration data by site.

We found a significant and strong linear relationship between tissue Al and Si concentrations in each year ($p < 0.001$; $r^2 > 0.90$), supporting this conclusion. Standardization to aluminum (Al) resulted in the spatial patterns of most elements mirroring patterns in Al, due to the large range of Al concentrations between sites in 2012

and the large differences between 2012 and 2013 averages. Therefore, tissue values for each site were natural log transformed to improve normality, pooled across years to improve replication, and compared using ANOVA with a Tukey-Kramer *post hoc* test. This approach ensured a conservative analysis of significant differences between sites by incorporating all variability within the test. Alternatively, individual year/species natural log transformed data sets were pooled by spatial category similar to Rogers et al., [13]: mainstem upstream of ZOD, within ZOD, downstream of ZOD, and tributaries. These spatial groupings were compared using ANOVA with a Tukey-Kramer *post hoc* test. Pearson's correlation compared the spatial patterns from these categories between years and found the majority (11 of 19, or 63.6%) had negative relationships, of which most (10 of 11) were strong or very strong correlations ($|r| \geq 0.60$). Only 3 elements (Ba, Hg, Mn) had strong or very strong positive correlations. This suggests that spatial patterns in elements were typically reversed between years and rarely consistent. This inconsistency further supported pooling data across years/species to remain conservative in describing spatial patterns.

Many of our statistical approaches were previously published in Cope et al. [11]. In brief, we performed cluster analyses on deployed mussel tissue concentrations (separately for metals and PAHs) to determine covariance of analytes between sites (clustering by site) and within sites (clustering by analyte). Specifically, we conducted hierarchical agglomerative cluster analyses using the average group linkage method and Euclidean distances in Statistical Analysis Software (SAS, version 9.3, SAS Institute, Cary, NC, USA). Data were standardized to account for the orders of magnitudes difference between analyte means. To determine the optimal number of clusters (groups) in each analysis, we

assessed multiple diagnostics, including the pseudo- F and pseudo- t^2 statistics (generated by SAS), and the relationship between the percent of total variation explained versus the number of clusters (e.g., this would range between 1 and 12 potential clusters because there were 12 sites). We then used PC-ORD (version 6.16, MjM Software Design, Gleneden Beach, OR, USA) to generate two-way dendrograms for each cluster analysis.

Additionally, we conducted Spearman (r_s) correlation to determine whether tissue analyte concentrations in deployed mussels were related to those of environmental media: total surface water, dissolved fraction, pore water and bed sediment. Correlations with surface water were conducted with the closest sampling times, due to the variable nature of analyte concentrations in that medium. In a similar manner, we also compared site-specific concentrations in the tissues of deployed mussels with resident mussel population densities [12].