ORIGINAL ARTICLE

Abundance of an invasive bivalve, *Corbicula fluminea*, is negatively related to growth of freshwater mussels in the wild

Wendell R. Haag[1] | Jacob Culp[2] | Andrea N. Drayer[3] | Monte A. McGregor[4] | Drew E. J. White[3] | Steven J. Price[3]

[1]U.S. Forest Service, Southern Research Station, Center for Bottomland Hardwoods Research, Frankfort, KY, U.S.A.

[2]Kentucky Division of Water, Frankfort, KY, U.S.A.

[3]Department of Forestry and Natural Resources, University of Kentucky, Lexington, KY, U.S.A.

[4]Kentucky Department of Fish and Wildlife Resources, Center for Mollusk Conservation, Frankfort, KY, U.S.A.

Correspondence
Wendell R. Haag, U.S. Forest Service, Southern Research Station, Center for Bottomland Hardwoods Research, Frankfort, KY, U.S.A.
Email: wendell.haag@usda.gov

Funding information
U.S. Fish and Wildlife Service Ohio River Basin Fish Habitat Partnership; Kentucky Waterways Alliance; U.S. Forest Service, Daniel Boone National Forest and Southern Research Station; and McIntire-Stennis Cooperative Forestry Research Program (accession number 1001969).

Abstract

1. Causes of worldwide freshwater mussel declines are poorly understood, and the potential role of the invasive Asian Clam, *Corbicula fluminea*, has received little attention.

2. We measured survival and growth of captively-reared juveniles of four native mussel species during 84-day in situ exposures at 17 sites in the Rockcastle River system, Kentucky, U.S.A., where mussel declines are attributed to coal mining. We measured water temperature, a comprehensive array of water chemistry variables, and *Corbicula* abundance at each site during mussel exposures.

3. Mussel survival was high (mean = 85.4%), did not differ among species, and was not related to any measured factor. In contrast, growth varied among sites by an order of magnitude, but growth responses were nearly identical for all four species. We found little evidence of water pollution from coal mining or other sources, and pollution did not explain variation in mussel growth. Growth was best explained by a model including only temperature (positive effect) and *Corbicula* abundance (negative effect) without interaction.

4. Our model predicts 46% lower mass gain over 84 days for every 10-fold increase in *Corbicula* abundance regardless of temperature, but growth may be reduced to unsustainably low levels in cooler streams. Previous ideas about water pollution as a cause of low growth and mussel declines were not supported by our data. Instead, the predicted strong effects of *Corbicula*, combined with similar responses of four mussel species, suggest that *Corbicula* may be an important, but overlooked, factor in widespread mussel declines.

KEYWORDS
coal mining, competition, conservation, invasive species, water quality

1 | INTRODUCTION

Freshwater mussels (Order Unionoida) once dominated benthic biomass in many streams worldwide, but they are now among the most imperilled organisms on Earth (Lopes-Lima et al., 2017; Strayer et al., 2004). In the 1970s, entire mussel assemblages began to disappear rapidly in U.S.A. streams, and these declines continue across a large area (Haag, 2019). These declines are enigmatic because clear causes are not evident and other components of aquatic communities are relatively unaffected. Several anthropogenic sources of water pollution are proposed causes of mussel declines (e.g. ammonia, coal mining; Wang et al., 2007; Zipper et al., 2016). However,

these factors may not explain declines at large scales, and species responses can vary (Beggel et al., 2017; Denic et al., 2014; Haag, 2019).

Enigmatic mussel declines can be accompanied by sharply reduced juvenile growth, possibly indicating chronic effects of water pollution (Haag et al., 2019; Rogers et al., 2018). However, reduced growth is not consistently associated with any single anthropogenic water quality factor. Haag et al. (2019) found a negative correlation between mussel growth and agricultural contaminants, but agricultural contaminants were absent in some streams with low growth, suggesting that other factors were involved. Growth was also strongly influenced by catchment physiography. Physiographic variation among the streams studied by Haag et al. (2019) may have obscured subtle relationships between growth and other causal factors.

A potential factor in mussel declines that has received little attention is the invasive Asian Clam, *Corbicula fluminea* (hereafter *Corbicula*). *Corbicula* is one of the most widespread aquatic invasive species in the world and has major ecological and economic effects (Sousa et al., 2008). It colonised most of the U.S.A. by the 1970s, coincident with the advent of enigmatic mussel declines (Haag, 2019). Early studies failed to show unequivocal negative effects of *Corbicula* on native mussels, and, subsequently, it has been largely ignored in the U.S.A. (Haag, 2012; Strayer, 1999). However, *Corbicula* arrived more recently in Europe, where growing evidence supports negative effects on native bivalves (Ferreira-Rodríguez et al., 2018; Sousa et al., 2011). Another invasive bivalve, *Dreissena polymorpha*, is unequivocally linked to native bivalve extirpation in the northern U.S.A. and Canada due to food competition (Strayer, 1999). This suggests that *Corbicula* may have similar effects and could be an important, widespread factor in mussel declines.

We examined relationships between captively-reared juvenile mussel survival and growth, water temperature, water chemistry, and *Corbicula* abundance at 17 sites in the Rockcastle River system, Kentucky. This system has experienced severe mussel declines yet it has essentially no row-crop agriculture and basin physiography is relatively homogeneous. This allowed us to examine factors related to mussel survival and growth without potentially confounding effects of agricultural contaminants and variation in stream conditions. We exposed mussels to ambient conditions at each site for 84 days (average). We used four mussel species to evaluate differences in species responses. We evaluate the importance of water temperature, water chemistry, and *Corbicula* abundance in explaining variation in mussel survival and growth, and we discuss how these results inform our understanding of mussel declines.

2 | METHODS

2.1 | Study area

The Rockcastle River is a tributary of the Cumberland River in south-eastern Kentucky (Figure 1) that historically supported high mussel species richness (about 32 species), but mussels have declined dramatically throughout the catchment since the 1960s,

and some tributaries have lost essentially their entire mussel fauna (Haag, 2019). These declines have been attributed to coal mining; however, *Corbicula* colonised the system beginning in the late 1960s coincident with the beginning of mussel declines (Haag, 2019).

The catchment lies mostly on the Cumberland Plateau section of the Appalachian Plateaus physiographic province. Portions of some western tributaries lie on the Highland Rim section of the Interior Low Plateaus physiographic province. The Cumberland Plateau lies mostly on Pennsylvanian sandstones and shales, but some streams have downcut into Mississippian limestones; Highland Rim streams lie on Mississippian limestones. Streams are upland in character, moderately- to well-buffered, and flow over gravel, sand, and bedrock. Land use is 64% forest, 27% pasture/hay, 8% developed, and <1% row-crop (Haag et al., 2019). 4.6% of the catchment is within permitted coal mine boundaries, and the greatest extent of mining is in the South Fork Rockcastle River catchment (Figure 1; Table 1).

We chose 17 study sites to provide coverage throughout the catchment (Figure 1; Table 1), including four sites on the Rockcastle River and one to three sites on each major tributary.

2.2 | Study species and field deployment

Our study species were *Venustaconcha troostensis*, *Villosa taeniata*, *Lampsilis cardium*, and *Ptychobranchus subtentus*, all of which are native to the Rockcastle River system. *Venustaconcha troostensis* and *P. subtentus* are protected under the U.S. Endangered Species Act and have disappeared from much of their range (USFWS, 2020). *Villosa taeniata* and *L. cardium* are not of conservation concern. We produced juveniles of each species using broodstock obtained from the Rockcastle River system or elsewhere in the Cumberland River system (Appendix S1). We reared juveniles in a hatchery until they were about 4 months old. At that time, mean shell length and mass (blotted wet mass, including shell) were: *V. troostensis*, 4.6 mm ($\pm$0.6 *SD*), 0.019 g ($\pm$0.002); *V. taeniata*, 4.3 mm ($\pm$1.0), 0.009 g ($\pm$0.001); *L. cardium*, 5.9 mm ($\pm$1.3), 0.028 g ($\pm$0.007); *P. subtentus*, 4.3 mm ($\pm$0.6), 0.008 g ($\pm$0.001) (based on subsamples of 111–119 individuals of each species). We used these values as estimates of initial size for all sites.

We deployed mussels in streams between 5 and 7 June 2018. We placed mussels in concrete silos with a central chamber of 10.2-cm PVC pipe covered by a drain grate and containing a removable mussel holding chamber of 7.6-cm PVC covered on both ends by 1-mm screen (see Haag et al., 2019). Silos create an upwelling current through the central chamber, which delivers food and oxygen and carries away waste. We placed 15 mussels in each silo and each species in a separate silo. At all sites, we deployed six silos, including two each containing *V. troostensis*, *V. taeniata*, and *L. cardium*. We did not have enough *P. subtentus* to deploy them at all sites; we placed two silos of *P. subtentus* at five sites (HLC, MFR, RC2, RR3, SFR1). We acclimated mussels to stream temperatures by gradually replacing transport water with stream water over about 45 min. The mean difference of transport temperature − stream temperature was 1.2°C. We placed silos in runs 0.5–1 m deep to ensure that

FIGURE 1 Map of the Rockcastle River system, Kentucky, U.S.A., showing study sites. Yellow-shaded areas are permitted coal mine boundaries. See Table 1 for site codes. Inset map shows location of Kentucky (light shading) and the Rockcastle River system (dark shading) in the eastern U.S.A.
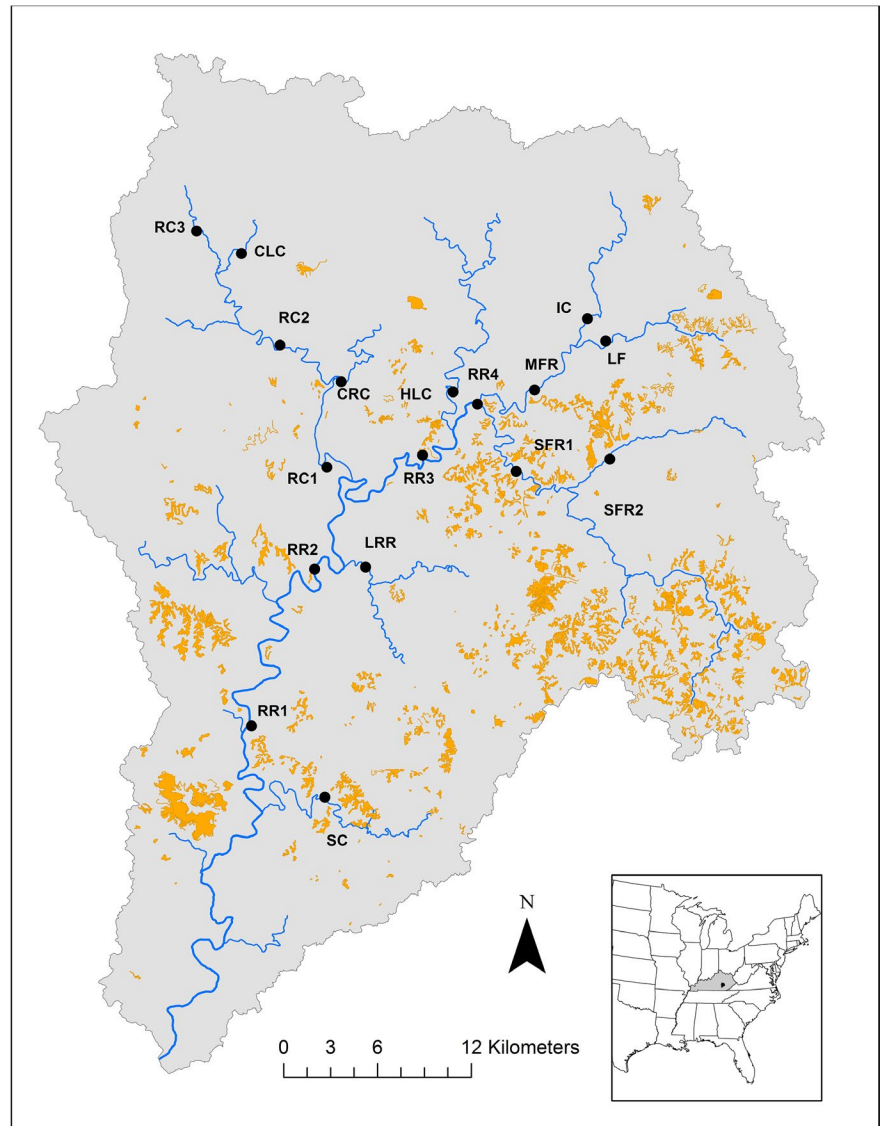
silos had sufficient water flow and remained immersed. We placed an IBWetland temperature logger (Alpha-Mach Inc., Sainte-Julie, Quebec, Canada; measurement interval = 90 min) in two silos at each site.

We retrieved silos on 28 and 29 August 2018. Exposure time averaged 84 days (82–85). Upon retrieval we recorded the number of live mussels in each silo, froze live mussels in liquid N, and returned them to the laboratory. In December 2018, we thawed samples and measured length and mass of all individuals.

2.3 | Water chemistry

We collected water samples monthly, while mussels were deployed, following Kentucky Division of Water and U.S. Environmental Protection Agency (USEPA) standard methods (Appendix S2). This resulted in four samples for all sites. Water samples were analysed at the Kentucky Division of Environmental Program Support (Frankfort, Kentucky) following USEPA standard methods. Kentucky

Division of Environmental Program Support measured 163 analytes in water samples (Table S1).

We assessed the extent of coal mining associated with each site using the U.S. Geological Survey's Streamstats v4.3.11 (https://streamstats.usgs.gov/ss/, accessed 18 March 2020) to create polygons encompassing the entire catchment upstream of each site. We imported these shapefiles into ArcGIS version 10.5 (ESRI) and overlaid a data layer of permitted mining boundaries (inactive and active, combined; Kentucky Division of Mine Permits, Frankfort, KY, https://kygeoportal.ky.gov/geoportal/catalog/main/home.page, accessed 18 March 2020). We then calculated the percentage of each site's catchment in permitted mine boundaries based on the total area of mine boundaries in each catchment and catchment area.

2.4 | *Corbicula* abundance

We estimated *Corbicula* abundance once at each site near the end of or shortly after the study (August to November 2018). At each site,

Stream	Site code	Site coordinates (UTM)		Catchment area (km^2)	Coal mining area (%)
		Latitude	Longitude		
Clear Creek	CLC	37.4255	−84.2893	60	0.00
Crooked Creek	CRC	37.3508	−84.2222	62	0.00
Horselick Creek	HLC	37.3231	−84.1422	161	0.74
Indian Creek	IC	37.3701	−84.0462	96	0.48
Laurel Fork	LF	37.3672	−84.0453	85	2.41
Little Rockcastle River	LRR	37.2444	−84.2076	145	1.61
Middle Fork Rockcastle River	MFR	37.3435	−84.0796	205	1.59
Roundstone Creek	RC1	37.3037	−84.2306	360	0.34
Roundstone Creek	RC2	37.3739	−84.2675	243	0.14
Roundstone Creek	RC3	37.4367	−84.3164	57	0.00
Rockcastle River	RR1	37.1504	−84.2906	1,590	3.58
Rockcastle River	RR2	37.2398	−84.2392	1,347	3.82
Rockcastle River	RR3	37.3097	−84.1525	754	5.20
Rockcastle River	RR4	37.3357	−84.1200	588	6.39
Sinking Creek	SC	37.0960	−84.2230	93	1.89
South Fork Rockcastle River	SFR1	37.2965	−84.0939	350	8.92
South Fork Rockcastle River	SFR2	37.2678	−84.0438	218	11.35

we established a 50-m reach including the silo location. We sampled cross-sectional transects at five randomly selected locations within the reach. Within each transect, we sampled a 0.018-m^2 circular quadrat (a 20-cm length of 15.2-cm diameter PVC pipe) every 1 m. We excavated all sediment to about 10-cm depth, washed sediment across a 2-mm mesh sieve, and counted all *Corbicula* in the sample. We estimated mean *Corbicula* abundance (number/m^2) across transects at a site based on the pooled number of *Corbicula* in each transect and the total area sampled. We measured the length of up to the first 50 *Corbicula* collected at each site, but we were unable to estimate biomass because too few individuals were collected at several sites. We encountered few wild, native mussels while sampling for *Corbicula*, but mussel abundance is low at most sites and our sampling was not designed to detect them or estimate their abundance.

2.5 | Data analysis

We calculated survival as the proportion of live individuals in each silo at the end of the experiment. We expressed growth as instantaneous growth (per day: ln[final mass in g/initial mass in g]/deployment period in days, Ricker, 1975; hereafter growth) based on the mean mass of all live individuals in each silo. Instantaneous growth is the exponential factor by which mass is predicted to increase each day; we used this measure instead of raw growth because it can be more easily compared with other studies (by accounting for differences in initial size and study duration) and it has better statistical properties. Growth responses among sites were nearly identical for all four species (see Results). Consequently, we computed mean mussel growth across all silos at each site ($n = 6$–8 silos/site). We used this generalised measure of growth as the response variable in all analyses involving water chemistry, water temperature, and *Corbicula* abundance.

A total of 43 water chemistry analytes were detected in at least one sample (Table S1). We reduced this dataset by omitting analytes detected at no more than three streams or present at uniformly low levels near the detection limit. Water hardness, calcium, and alkalinity were highly correlated ($r > 0.90$) and redundant; we retained only alkalinity. Reducing the dataset in this way left 18 water chemistry variables. We log-transformed water chemistry variables with skewness >1 (median skew after transformation = 0.65), and we used the mean value for each analyte across all monthly samples from a stream.

We calculated Spearman correlation coefficients between growth and survival and all retained water chemistry variables to examine general patterns in the data. We then used principal

components (PC) analysis based on correlation matrices to examine patterns of water chemistry among sites; we assessed interpretability of PC axes following Jackson (1993). We examined relationships between mussel growth and survival and water chemistry with principal components regression based on water chemistry PC scores for each site (Quinn & Keough, 2002).

We used Akaike information criteria (AIC; Burnham & Anderson, 2002) with a correction for small sample size (AIC_c) to evaluate a set of candidate models for predicting mussel growth based on the three variables that were significantly related to growth: water temperature, *Corbicula* abundance, and water chemistry PC1 (see Results). We then used coefficients from the best-supported model to evaluate the utility of this model for predicting growth by comparing those predictions with observed growth. All analyses were conducted in SAS version 9.4 (SAS Institute, Cary, NC, U.S.A.).

We used coefficients from the best-supported model (two-factor model with temperature and *Corbicula* and no interaction; see Results) to construct isoclines showing the predicted relationship between growth and temperature at different levels of *Corbicula* abundance. Because instantaneous growth depicts exponential changes in mass over time, values of instantaneous growth at a given temperature do not differ linearly among isoclines. Therefore, we also constructed isoclines based on predicted mass gain over the 84-d duration of our study [$Mass\ gain\ (g) = (M_i e^{gd}) - M_i$, where M_i is initial mass, e is the base of natural logarithms, g is instantaneous growth (d^{-1}, as g), and d is the number of days]; this allowed us to compute a single factor by which predicted mass gain decreased according to a specified increase in *Corbicula* abundance at any given temperature.

3 | RESULTS

We recovered all silos from all streams, but we omitted one silo at CLC that was displaced on the shore by flooding ($n = 111$ undisturbed silos).

3.1 | Mussel survival and growth

Mussel survival was generally high and averaged 85.4% across all silos (Figure 2). Survival was not significantly different among sites or species (two-factor generalised linear mixed model, sites: $F_{16,36} = 1.35$, $p = 0.223$; species: $F_{3,36} = 2.07$, $p = 0.121$). Mean survival was 77.3% ± 5.1 (SE) for *L. cardium*; 91.3% ± 10.1 for *P. subtentus*; 82.9% ± 5.1 for *V. taeniata*; and 94.5% ± 5.1 for *V. troostensis*.

Growth differed significantly among sites and species (two-factor generalised linear mixed model, sites: $F_{15,35} = 41.05$, $p < 0.0001$; species: $F_{3,35} = 22.12$, $p < 0.0001$; Figure 3). Growth was lowest for all species at CRC, IC, and HLC (growth = −0.002–0.010 day^{-1}, as g) and highest at Roundstone Creek and RR1 (0.019–0.034 day^{-1}, as g). Final individual length and mass ranged from a minimum of 4.2 mm (HLC, *P. subtentus*) and 0.0136 g (IC, *V. taeniata*) to a maximum of 14.9 mm and 0.4364 g (*L. cardium*, RC1). In general, growth was about one order of magnitude higher at the highest growth sites than at the lowest growth sites. Mean growth across species at each site was not related to mean survival (slope = 0.011, $p = 0.192$, $n = 17$).

Growth of *P. subtentus* and *V. taeniata* (least square mean growth = 0.0186 and 0.0185 day^{-1}, as g, respectively) did not differ but were significantly higher than *L. cardium* and *V. troostensis*, which did not differ from each other (least square means = 0.0129 and 0.0133, respectively; Tukey's HSD, α = 0.05). Despite the differences in the magnitude of growth among species, species responses among sites were nearly identical. All pairwise linear regressions of growth between species were highly significant ($r^2 = 0.803$–0.992, $p < 0.01$–.0001) and showed essentially 1:1 relationships. 95% confidence intervals around estimates of the slope and intercept included 1 and 0, respectively, for all relationships except *V. taeniata* versus *V. troostensis*, which showed a small, non-zero intercept (intercept = 0.006 ± 0.002 SE; slope not significantly different from 1).
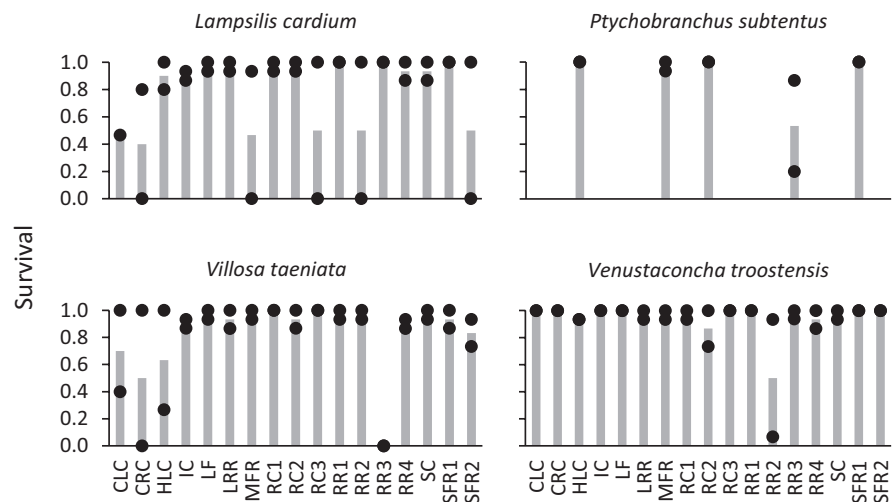

FIGURE 2 Juvenile mussel survival after 84-day exposures at 17 sites in the Rockcastle River system, Kentucky. Bars represent mean survival among silos at a site, and points represent individual silos. See Table 1 for site codes
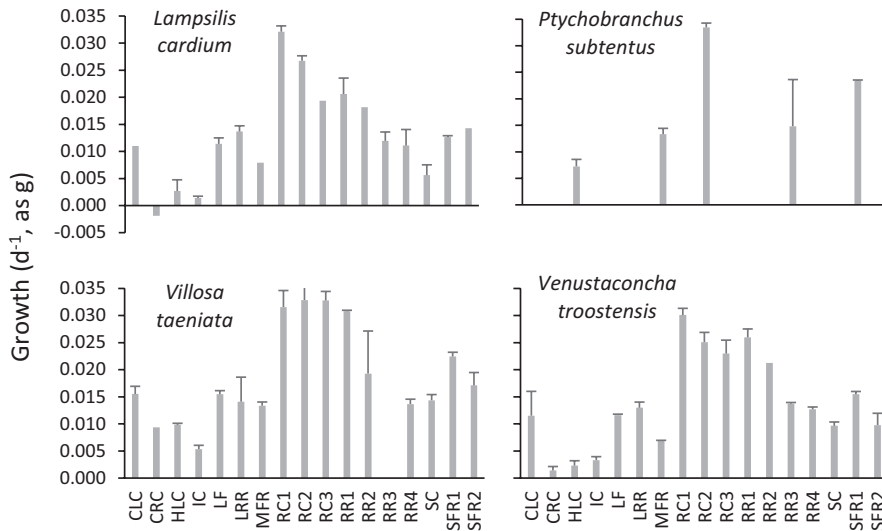
FIGURE 3 Mean (+*SE*) instantaneous growth of juvenile mussels after 84-day exposures at 17 sites in the Rockcastle River system, Kentucky. Data points without error bars are represented by only one observation (e.g., *Lampsilis cardium*, CLC); all individuals of *Villosa taeniata* died at RR3. See Table 1 for site codes

TABLE 2 Water chemistry values at 17 sites in the Rockcastle River system, Kentucky, from June to September 2018, and principal components loadings for each variable. The percentage of variation explained by each principal component is given below the component number. Correlations greater than or equal to 0.80 are bolded

Variable	Mean (SD)	Range	PC1 (53.5%)	PC2 (15.8%)
Alkalinity (as $CaCO_3$, mg/L)	69.8 (28.5)	34.9–129.0	0.18	**−0.93**
Al (µg/L)	134.4 (75.8)	52.7–285.4	**0.84**	−0.04
Ammonia (as *N*, mg/L)	0.014 (0.013)	0.000–0.045	0.76	0.07
Cl (mg/L)	5.2 (3.0)	1.0–11.6	**0.80**	−0.17
Specific conductivity (µmho/cm)	198.4 (48.9)	123.9–298.3	0.62	−0.56
Cu (µg/L)	0.65 (0.60)	0.14–2.69	**0.80**	0.18
Fe (mg/L)	0.34 (0.18)	0.12–0.79	0.79	0.45
Mg (mg/L)	5.3 (1.9)	3.2–11.1	0.77	0.23
Mn (µg/L)	93.0 (79.3)	27.4–357.5	0.77	0.54
Nitrate/nitrite (as *N*, mg/L)	0.27 (0.09)	0.17–0.53	0.40	0.12
Organic carbon, total (mg/L)	2.15 (0.66)	0.97–3.63	0.75	0.35
P (total, mg/L)	0.025 (0.012)	0.009–0.053	**0.84**	0.05
K (mg/L)	2.0 (0.6)	1.1–3.2	0.76	0.65
Na (mg/L)	4.5 (2.3)	1.6–9.5	**0.89**	0.12
Suspended solids, total (mg/L)	8.2 (9.0)	0.5–39.1	**0.82**	−0.14
SO_4 (mg/L)	23.1 (14.7)	7.2–67.9	0.44	**0.85**
Total Kjeldahl *N* (mg/L)	0.21 (0.20)	0.00–0.83	0.60	0.30
Turbidity (NTU)	9.3 (5.4)	3.7–19.5	**0.88**	0.08

3.2 | Water chemistry

There was little evidence of anthropogenic water quality degradation (Table 2). Nitrate and ammonia concentrations were uniformly low. Only three pesticides were detected at more than three sites, but their concentrations were near the detection limit (2,4-D, mean concentration = 0.032 µg/L; atrazine, 0.004 µg/L; hexachlor, 0.006 µg/L). There was little evidence of coal mining effects such as elevated conductivity, metals, or major ions. Aluminium was slightly elevated but was not correlated with the extent of mining in the catchment ($r_S = 0.09$, $p = 0.732$). Potassium ($r_S = 0.60$, $p = 0.010$) and SO_4 ($r_S = 0.70$, $p = 0.002$) were the only variables positively correlated with mining, but they were not conspicuously elevated (see Discussion). Alkalinity was the only variable negatively correlated with mining ($r_S = −0.71$, $p = 0.001$). Manganese was moderately elevated at several sites, but it was only weakly related to mining
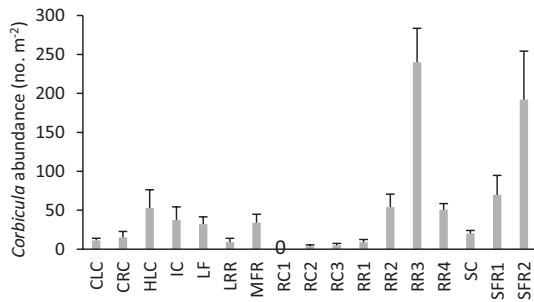
FIGURE 4 Mean (+*SE*) abundance of *Corbicula fluminea* at 17 sites in the Rockcastle River system, Kentucky. No *Corbicula* were encountered in samples at RC1. See Table 1 for site codes

($r_S = 0.45$, $p = 0.072$). The highest value of Mn (357.5 µg/L) occurred at the site with the greatest extent of mining (SFR2), but conductivity and other metals were not conspicuously elevated at that site, and elevated Mn occurred at sites with no mining (e.g. RC3, 182.0 µg/L).

Principal components analysis of 18 water chemistry variables revealed two interpretable axes (Table 2, Figure S1). Principal component axis 1 explained 53.5% of the variation in water chemistry among sites and contrasted sites with higher values of Na, turbidity, Al, P, total suspended solids (TSS), Cl, and Cu (Roundstone Creek, SFR2, LRR) with sites having lower values for those variables (HLC, IC). However, many other variables were highly correlated with PC1, and no variables were negatively correlated with PC1. Principal component axis 2 explained 15.8% of variation among sites, and it contrasted sites having higher alkalinity (Roundstone, CLC, CRC) with those having higher SO_4 (South Fork and LRR).

3.3 | *Corbicula* abundance

Corbicula abundance differed widely among streams (Figure 4). The highest abundances were at RR3 and SFR2 (240 and 192 m^{-2}, respectively). Abundance was <10 m^{-2} at RR1, LRR, and Roundstone Creek. We did not detect live *Corbicula* at RC1, but dead shells were present at the site. Mean *Corbicula* size among sites was 16.6 mm (±1.8 *SD*). *Corbicula* abundance was not related to water temperature or water chemistry PC1 ($p = 0.731$ and 0.244, respectively, $n = 17$, log *Corbicula* abundance + 1).

3.4 | Factors related to mussel survival and growth

Neither survival nor growth were correlated with catchment area or the extent of coal mining in the catchment ($r_S = -0.14$–0.19, $p = 0.486$–$.813$). Eleven out of eighteen water chemistry variables were positively correlated with mussel growth (Al, Cl, conductivity, Cu, Mg, total organic carbon, P, K, Na, TSS, turbidity; $r_S = 0.52$–0.79, $p = 0.032$–$<.001$). No variables were negatively correlated with growth, including variables potentially associated with coal mining (Mn, $r_S = 0.45$, $p = 0.073$; SO_4, $r_S = 0.10$, $p = 0.715$). Growth was positively related to PC1 (slope = 0.0018, $p = 0.005$, $r^2 = 0.423$, $n = 17$), indicating higher growth at sites with higher Na, turbidity, Al, P, TSS, Cl, Cu, and other variables. Growth was not related to PC2 ($p = 0.428$, $r^2 = 0.042$, $n = 17$). Survival was not related to PC1 or PC2 ($p = 0.643$ and 0.575, respectively, $n = 17$, arcsine survival).

Growth was positively related to water temperature and negatively related to *Corbicula* abundance, and both variables explained a moderate amount of variation in growth (Figure 5). However, both relationships had influential observations (studentised residuals >2.0), and the variance of residuals for growth increased with increasing values of water temperature. Survival was not related to water temperature or *Corbicula* abundance (temperature: slope = 0.040, $p = 0.462$; *Corbicula*: slope = −0.171, $p = 0.133$, $n = 17$; arcsine survival, log *Corbicula* abundance).

The best supported AIC model for explaining observed variation in growth among sites contained only water temperature and *Corbicula* abundance (Table 3). No other models were considered plausible based on ΔAIC_c values > 2 and low Akaike weights (w_i).

A model including water temperature, *Corbicula* abundance, and the interaction term was highly significant (ANOVA, $F_{3,13} = 14.07$, $p < 0.001$). Water temperature ($F_{1,13} = 25.95$, $p < 0.001$) and *Corbicula* abundance ($F_{1,13} = 14.57$, $p = 0.002$) were significant factors, but the interaction term was not significant ($F_{1,13} = 1.67$, $p = 0.218$). A reduced model omitting the interaction term also was highly significant ($F_{2,14} = 19.33$, $p < 0.001$; predicted growth = [0.0043 × temperature] − [0.0075 × log *Corbicula* abundance + 1] − 0.0704). Coefficients from the two-factor model without interaction predicted observed growth accurately: for the regression of observed growth on predicted growth, 95% confidence intervals for the slope and
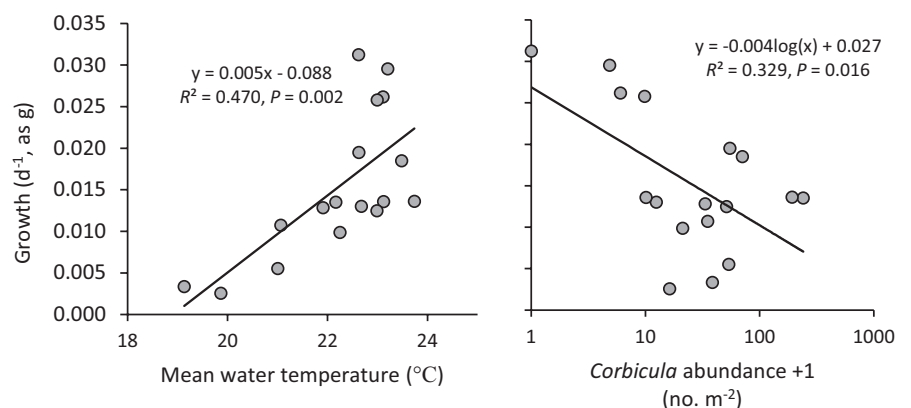

FIGURE 5 Mean instantaneous juvenile mussel growth in relation to water temperature and *Corbicula* abundance

Variables in model	r^2	AIC_c	ΔAIC_c	w_i	Evidence ratio
temperature, Corbicula	0.734	−178.532	0.000	0.726	–
temperature, Corbicula, PC1	0.741	−176.329	2.203	0.241	3.01
Corbicula, PC1	0.581	−170.802	7.730	0.015	47.67
temperature	0.470	−169.218	9.314	0.007	105.27
temperature, PC1	0.532	−168.899	9.632	0.006	123.45
PC1	0.423	−167.775	10.757	0.003	216.57
Corbicula	0.330	−165.202	13.330	0.001	784.21

TABLE 3 Akaike information criteria (AIC) for predicting mussel instantaneous growth based on water temperature, log *Corbicula* abundance, and water chemistry PC1. AIC_c is the AIC value corrected for small sample size; $\Delta AICc$ is the difference between the AIC_c value of a given model and the model with the lowest AIC_c value; w_i is the Akaike weight
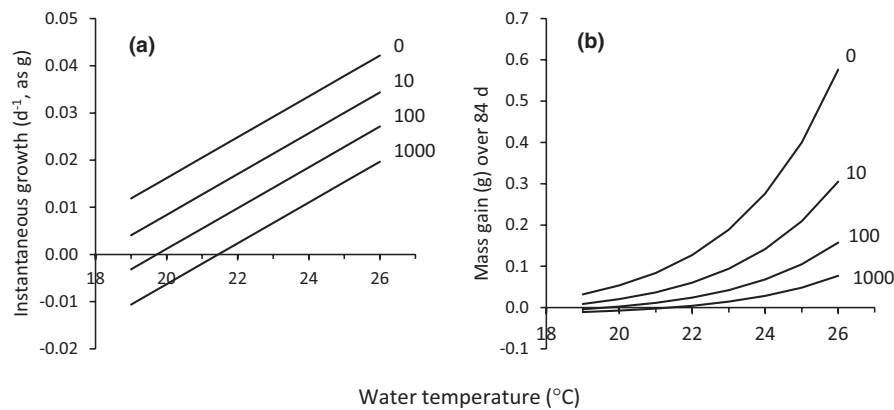

FIGURE 6 Isoclines showing predicted (a) instantaneous mussel growth and (b) mass gain in relation to water temperature at four levels of *Corbicula* abundance (0, 10, 100, 1,000 m^{-2}). Instantaneous growth represents the exponential factor by which predicted mass increases each day, and mass gain represents the predicted mass gained by mussels over 84 days (the duration of our study). Instantaneous growth isoclines were constructed using the equation: growth = (0.0043 × temperature) − (0.0075 × log *Corbicula* abundance + 1) − 0.0704; see text for calculation of mass gain

intercept contained 1 and 0, respectively (slope = 0.997 ± 0.155 *SE*; intercept = −0.00007 ± 0.00267), indicating a 1:1 relationship (Figure S2).

Assuming independent effects of temperature and *Corbicula* (i.e., no interaction), isoclines predicted sharply decreased growth with increasing *Corbicula* abundance at all temperatures (Figure 6). However, they predicted negative growth below about 21.5°C at 1,000 *Corbicula* m^{-2} and below about 19.8°C at 100 *Corbicula* m^{-2}. Isoclines predicted 46% lower mass gain over 84 days for every 10-fold increase in *Corbicula* abundance at any given temperature.

4 | DISCUSSION

The high survival we observed at all sites indicates a lack of acute toxicity to mussels in the Rockcastle River system, which is similar to previous findings at a larger scale (Haag et al., 2019). Low survival appeared to be largely a random occurrence, and survival was not related to any of the factors we examined. Low survival was probably caused by localised cage effects that reduced flow or otherwise created anoxic or other unfavourable conditions in some silos.

In contrast, growth varied widely and was extremely low at some sites, which could indicate chronic effects of water quality degradation (Haag et al., 2019; Rogers et al., 2018). However, we found little evidence of water pollution, and water chemistry was relatively uninformative for explaining variation in growth. Concentrations of all water chemistry variables with established ambient water quality criteria were far below those values (KDEP, 2016; USEPA, 2018), and we found no evidence for a negative relationship between growth and any anthropogenic water chemistry factor. The positive relationship of mussel growth with PC1 may reflect higher growth at sites with higher nutrients and overall productivity (e.g. P, K, total organic carbon; see Haag et al., 2019), but no variables inordinately contributed to this pattern. Several variables that were positively associated with PC1 and growth are injurious to mussels at high concentrations (e.g., Cu, Cl; Wang et al., 2007; Gillis, 2011), but concentrations of these elements were uniformly low and the correlations are probably spurious. The alkalinity gradient depicted by PC2 is easily explained because it contrasted streams with greater amounts of limestone in their catchments from those underlain primarily by sandstones and shales. The opposing gradient of SO$_4$ on PC2 may reflect coal mining pollution because coal deposits in Kentucky

are typically associated with sandstone and shale geology, and the sites with the highest scores on PC2 (South Fork) had the highest amount of coal mining in their catchments (see subsequent). However, PC2 explained little of the variation in water chemistry among sites and was not related to mussel growth.

Coal mining is widely invoked as a cause of mussel declines in the Appalachian region, including the Rockcastle River system (Houslet & Layzer, 1997; Zipper et al., 2016). We found no evidence of serious coal mining pollution. Elevated Mn and slightly elevated SO_4 in the South Fork may constitute a weak mining signal. However, our values for SO_4 and other mining indicators (e.g., conductivity, K, Mg) were within the ranges reported for streams without mining in their catchments; values in severely mine-impacted streams typically are one to two orders of magnitude higher than our values (Kunz et al., 2013; Lindberg et al., 2011; Pond et al., 2008). Manganese is naturally high throughout Kentucky, particularly in regions underlain by Pennsylvanian sandstones and shales (Fisher & Davidson, 2007), suggesting that elevated Mn is unrelated to mining. In the laboratory, Mn levels (500 μg/L) comparable to our highest observation (SFR2, 357.5 μg/L) resulted in metabolic suppression in a freshwater mussel, but this effect was not evident in mixtures containing other metals (Oliveira et al., 2018). Regardless, we did not find a negative relationship between mussel growth or survival and Mn or any other coal mining indicator.

Water temperature was strongly and positively related to growth, which is expected for ectotherms such as mussels (Carey et al., 2013). However, the heterogeneous variance of this relationship and the low AIC support for the model containing only temperature suggested that other factors also influenced growth. Akaike information criteria results supported a combined effect of temperature and *Corbicula* abundance on mussel growth, and the model containing both factors predicted observed growth remarkably well. The lack of interaction between temperature and *Corbicula* is important because it predicts that *Corbicula* negatively affects mussel growth in a similar way regardless of temperature. In this scenario (see Figure 6), mussels can sustain moderate growth (>0.010 day^{-1}) at any growing season temperature when *Corbicula* abundance is low (<10 m^{-2}). At moderate to high *Corbicula* abundance, growth will be substantially reduced at all temperatures, but growth in cooler streams will be reduced to unsustainably low levels. This suggests that, despite a constant effect, the ultimate outcome of growth reduction associated with *Corbicula* will differ according to stream conditions, and it provides explicit predictions for testing those effects.

Previously proposed negative effects of *Corbicula* on native mussels include competition for space or food; ingestion of mussel sperm, glochidia, or juveniles; displacement of juvenile mussels by *Corbicula* burrowing; ammonia toxicity produced during periodic *Corbicula* die-offs; and as a vector of disease (Haag, 2019; Strayer, 1999; Vaughn & Hakenkamp, 2001). In the context of our study, competition for food, ammonia toxicity, and disease are the only plausible explanations because our experimental mussels were not exposed to *Corbicula* directly. Ammonia toxicity and disease seem unlikely explanations because of the high survival we observed

at all sites, but these factors could have had sublethal effects (e.g. Newton & Bartsch, 2007). Ammonia and nitrate/nitrite concentrations were uniformly low during our study, but our monthly sampling could have missed brief spikes of ammonia; however, we saw no evidence of *Corbicula* die-offs at any site. Disease also seems unlikely because transmission to native mussels does not necessarily require high *Corbicula* abundance.

Reduction of food resources available to native mussels due to competition with *Corbicula* seems the most likely explanation for our results. Dense populations of *Corbicula* can remove a substantial percentage of organic matter from both the seston and the sediment (Hakenkamp & Palmer, 1999; Lauritsen, 1986; Leff et al., 1990). Our experimental animals were not exposed directly to the sediment, suggesting that reduction of seston alone is sufficient to negatively influence mussel growth.

Only two previous studies have shown strong evidence for reduction of mussel growth potentially related to food competition with *Corbicula*. Growth and carbohydrate content of adult mussels caged with different *Corbicula* densities (0, 100, 1,000, 2,000 m^{-2}) in a stream were negatively related to *Corbicula* density, but these responses differed only between the 0 and 2,000 m^{-2} treatments (Ferreira-Rodríguez et al., 2018). In contrast, growth of 1–3 day-old juvenile mussels exposed to different *Corbicula* densities (0–5000 m^{-2}) in the laboratory showed a sharp reduction in growth (as shell length added) with increasing *Corbicula* density, including a 78% reduction in growth at only 625 *Corbicula* per m^2 and a 93% reduction at 5,000 m^{-2} relative to the control treatment (Yeager et al., 2000). The discrepancy between these studies in the density of *Corbicula* necessary to produce a detectable effect probably relates to energetic differences between juvenile and adult mussels. Rapidly growing juveniles may have higher energetic requirements and lower energy stores (Bartsch et al., 2017)—and thus may be more vulnerable to food competition—than slow-growing adults, and their higher growth rate makes it easier to detect differences in growth.

Regardless of the mechanism, our study is the first we know of to show a negative relationship between mussel growth and *Corbicula* abundance in a wild, unmanipulated context. Our results from the field are similar to those of Yeager et al. (2000) from the laboratory. Our model predicts a 69% decrease in growth (as shell length added) at 625 *Corbicula* m^{-2}, and an 84% reduction at 5,000 m^{-2} relative to a density of 0 m^{-2} (calculated at 25°C, the temperature used by Yeager et al., 2000; Appendix S3; Table S2). However, our predictions cannot be compared directly to Yeager et al. (2000) because of large differences in initial mussel size and growing conditions. More importantly, our predictions are similar to Yeager et al. (2000) in that they support a measurable, negative effect of *Corbicula* on mussel growth even at relatively low *Corbicula* abundance.

We were surprised by the absence or low abundance of *Corbicula* at many of our sites. *Corbicula* frequently reaches abundances >1,000 m^{-2} (Hakenkamp & Palmer, 1999; Sousa et al., 2011). *Corbicula* abundance in our streams may have declined to a more stable equilibrium after a period of higher abundance during initial colonisation, similar to other invasive bivalves (Strayer & Malcom, 2006).

Unfortunately, historical trends in *Corbicula* abundance at our study sites are unknown. The apparent negative effects of *Corbicula* even at relatively low abundance may be because our Appalachian study streams are relatively unproductive in the broader context of eastern North America, resulting in a greater potential for food limitation.

Our results show that *Corbicula* may negatively affect native mussel growth in some situations, but the extent to which our results can be applied to other regions is unclear. By design, our study encompassed a relatively homogeneous area with regard to the physical characteristics of streams. A fuller understanding of the effects of *Corbicula* on native mussels requires evaluating those effects in a wider range of stream conditions. For example, if *Corbicula* is a food competitor, native mussels in more productive streams with greater food resources may be less vulnerable to competition. Evaluating our results in other contexts also requires incorporating the effect of variation in *Corbicula* size. *Corbicula* size was similar among our sites, which made *Corbicula* abundance a useful measure. Comparisons in different regions with potentially different *Corbicula* size structure will require estimating biomass, which better represents filtering capacity and, hence, the potential for competition.

Another important result of our study was the similarity in growth responses among mussel species, including imperilled and non-imperilled species. The differences in the magnitude of growth we observed among species were not unexpected because growth rates and maximum size differ widely among mussel species (Haag, 2012). More importantly, the similarity of growth for all four species among sites shows that they responded in nearly identical ways to conditions at the sites. All of our study species are in the same tribe (Lampsilini) and share similar life histories (Haag, 2012). Additional studies should examine growth of slower-growing or longer-lived mussel species in other phylogenetic groups. Nevertheless, our results do not support categorising these species as *sensitive* or *tolerant* to the effects of *Corbicula* or other, unmeasured stressors that may be present in the Rockcastle River system. The consistent responses we found across species suggest that *Corbicula* may be a major factor in widespread mussel declines.

ORCID

Wendell R. Haag https://orcid.org/0000-0001-8742-8381
Steven J. Price https://orcid.org/0000-0002-2388-0579

REFERENCES

Bartsch, M. R., Bartsch, L. A., Richardson, W. B., Vallazza, J. M., & Lafrancois, B. M. (2017). Effects of food resources on the fatty acid composition, growth and survival of freshwater mussels. *PLoS One*, *12*, e0173419. https://doi.org/10.1371/journal.pone.0173419

Beggel, S., Hinzmann, M., Machado, J., & Geist, J. (2017). Combined impact of acute exposure to ammonia and temperature stress on the freshwater mussel *Unio pictorum*. *Water*, *2017*(9), 455. https://doi.org/10.3390/w9070455

Burnham, K. P., & Anderson, D. R. (2002). *Model selection and inference: A practical information-theoretic approach*, 2nd ed. Springer-Verlag.

Carey, C. S., Jones, J. W., Hallerman, E. M., & Butler, R. S. (2013). Determining optimum temperature for growth and survival of laboratory-propagated juvenile freshwater mussels. *North American Journal of Aquaculture*, *75*, 532–542. https://doi.org/10.1080/15222055.2013.826763

Denic, M., Stoeckl, K., Gum, B., & Geist, J. (2014). Physiochemical assessment of *Unio crassus* habitat quality in a small upland stream and implications for conservation. *Hydrobiologia*, *735*, 111–122. https://doi.org/10.1007/s10750-013-1467-z

Ferreira-Rodríguez, N., Sousa, R., & Pardo, I. (2018). Negative effects of *Corbicula fluminea* over native freshwater mussels. *Hydrobiologia*, *810*, 85–95. https://doi.org/10.1007/s10750-016-3059-1

Fisher, R. S., & Davidson, B. (2007). *Groundwater quality in Kentucky: Manganese. Information Circular 14, Series 12.* : Kentucky Geological Survey. Available at: https://kgs.uky.edu/kgsweb/olops/pub/kgs/ic14_12.pdf, accessed January 31, 2020

Gillis, P. L. (2011). Assessing the toxicity of sodium chloride to the glochidia of freshwater mussels: Implications for salinization of surface waters. *Environmental Pollution*, *159*, 1702–1708. https://doi.org/10.1016/j.envpol.2011.02.032

Haag, W. R. (2012). *North American freshwater mussels: Natural history, ecology, and conservation*. Cambridge University Press.

Haag, W. R. (2019). Reassessing enigmatic mussel declines in the United States. *Freshwater Mollusk Biology and Conservation*, *22*, 43–60. https://doi.org/10.31931/fmbc.v22i2.2019.43-60

Haag, W. R., Culp, J. J., McGregor, M. A., Bringolf, R., & Stoeckel, J. A. (2019). Growth and survival of juvenile freshwater mussels in streams: Implications for understanding enigmatic mussel declines. *Freshwater Science*, *38*, 753–770. https://doi.org/10.1086/705919

Hakenkamp, C. C., & Palmer, M. (1999). Introduced bivalves in freshwater ecosystems: The impact of *Corbicula* on organic matter dynamics in a sandy stream. *Oecologia*, *119*, 445–451. https://doi.org/10.1007/s004420050806

Houslet, B. S., Layzer, J. B. (1997) Difference in growth between two populations of *Villosa taeniata* in Horse Lick Creek, Kentucky. In K. S. Cummings, A. C. Buchanan, C. A. Mayer, & T. J. Naimo (Eds.), *Conservation and management of freshwater mussels II: Initiatives for the future* (pp. 37–44). Proceedings of a UMRCC Symposium, 16–18, (October 1995), St Louis, Missouri. : Upper Mississippi River Conservation Committee.

Jackson, D. A. (1993). Principal components analysis: How many components are nontrivial and interpretable? *Ecology*, *74*, 2204–2214.

KDEP (Kentucky Department of Environmental Protection) (2016). *Surface water standards.* Available at https://www.epa.gov/sites/production/files/2014-12/documents/kywqs-standards.pdf accessed February 26, 2020

Kunz, J. L., Conley, J. M., Buchwalter, D. B., Norberg-King, T. J., Kemble, N. W., Wang, N., & Ingersoll, C. G. (2013). Use of reconstituted waters to evaluate effects of elevated major ions associated with mountaintop coal mining on freshwater invertebrates. *Environmental Toxicology and Chemistry*, *32*, 2826–2835. https://doi.org/10.1002/etc.2391

Lauritsen, D. D. (1986). Filter-feeding in *Corbicula fluminea* and its effect on seston removal. *Journal of the North American Benthological Society*, *5*, 165–172. https://doi.org/10.2307/1467704

Leff, L. G., Burch, J. L., & McArthur, J. V. (1990). Spatial distribution, seston removal, and potential competitive interactions of the bivalves *Corbicula fluminea* and *Elliptio complanata*, in a coastal plain stream. *Freshwater Biology*, *24*, 409–416. https://doi.org/10.1111/j.1365-2427.1990.tb00720.x

Lindberg, T. T., Bernhardt, E. S., Bier, R., Helton, A. M., Merola, B., Vengosh, A., & Guilio, R. T. (2011). Cumulative impacts of mountaintop mining on an Appalachian watershed. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(52), 20929–20934.

Lopes-Lima, M., Sousa, R., Geist, J., Aldridge, D. C., Araujo, R., Bergengren, J., … Zogaris, S. (2017). Conservation status of freshwater mussels in Europe: State of the art and future challenges. *Biological Reviews*, *92*, 572–607. https://doi.org/10.1111/brv.12244

Newton, T. J., & Bartsch, M. R. (2007). Lethal and sublethal effects of ammonia to juvenile *Lampsilis* mussels (Unionidae) in sediment and water-only exposures. *Environmental Toxicology and Chemistry*, *26*, 2057–2065. https://doi.org/10.1897/06-245R.1

Oliveira, L. F., Cabral, M. T., Risso, W. E., & Martinez, C. B. R. (2018). Single and combined effects of Zn, Mn and Fe on the neotropical freshwater bivalve *Anodontites trapesialis*: Bioaccumulation and biochemical biomarkers. *Ecotoxicology and Environmental Safety*, *161*, 735–745. https://doi.org/10.1016/j.ecoenv.2018.06.029

Pond, G. J., Passmore, M. E., Borsuk, F. A., Reynolds, L., & Rose, C. J. (2008). Downstream effects of mountaintop coal mining: Comparing biological conditions using family- and genus-level macroinvertebrate bioassessment tools. *Journal of the North American Benthological Society*, *27*, 717–737. https://doi.org/10.1899/08-015.1

Quinn, G. P., & Keough, M. J. (2002). *Experimental design and data analysis for biologists.* Cambridge University Press.

Ricker, W. E. (1975). *Computation and interpretation of biological statistics of fish populations.* : Department of the Environment, Fisheries and Marine Service. Available at: https://www.dfo-mpo.gc.ca/Library/1485.pdf, accessed July 24, 2020

Rogers, J. J., Henley, W. F., Weberg, A. G., Jones, J. W., & Cope, W. G. (2018). Assessment of growth, survival, and organ tissues of caged mussels (Bivalvia: Unionidae) in a river-scape influenced by coal mining in the southeastern USA. *Science of the Total Environment*, *645*, 1273–1286. https://doi.org/10.1016/j.scitotenv.2018.07.142

Sousa, R., Antunes, C., & Guilhermino, L. (2008). Ecology of the invasive Asian clam *Corbicula fluminea* (Müllere, 1774) in aquatic ecosystems: An overview. *Annales De Limnologie - International Journal of Limnology*, *44*, 85–94. https://doi.org/10.1051/limn:2008017

Sousa, R., Ilarri, M., Souza, A. T., Antunes, C., & Guilhermino, L. (2011). Rapid decline of the greater European peaclam at the periphery of its distribution. *Annales De Limnologie – International Journal of Limnology*, *47*, 211–219. https://doi.org/10.1051/limn/2011041

Strayer, D. L. (1999). Effects of alien species on freshwater mollusks in North America. *Journal of the North American Benthological Society*, *18*, 74–98. https://doi.org/10.2307/1468010

Strayer, D. L., Downing, J. A., Haag, W. R., King, T. L., Layzer, J. B., Newton, T. J., & Nichols, S. J. (2004). Changing perspectives on pearly mussels, North America's most imperiled animals. *BioScience*, *54*, 429–439. 10.1641/0006-3568(2004)054[0429:CPOPMN]2.0.CO;2

Strayer, D. L., & Malcom, H. M. (2006). Long-term demography of a zebra mussel (*Dreissena polymorpha*) population. *Freshwater Biology*, *51*, 117–130. https://doi.org/10.1111/j.1365-2427.2005.01482.x

USEPA (US Environmental Protection Agency) (2018). *National recommended water quality criteria—aquatic life criteria table.* : USEPA. Available at: https://www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table#table (accessed February 4, 2020).

USFWS (US Fish and Wildlife Service) (2020). *Endangered species.* https://www.fws.gov/endangered/ accessed July 24, 2020

Vaughn, C. C., & Hakenkamp, C. C. (2001). The functional role of burrowing bivalves in freshwater ecosystems. *Freshwater Biology*, *46*, 1431–1446. https://doi.org/10.1046/j.1365-2427.2001.00771.x

Wang, N., Ingersoll, C. G., Hardesty, D. K., Ivey, C. D., Kunz, J. L., May, T. W., … Barnhart, M. C. (2007). Acute toxicity of copper, ammonia, and chloride to glochidia and juveniles of freshwater mussels (Unionidae). *Environmental Toxicology and Chemistry*, *26*, 2036–2047. https://doi.org/10.1897/06-523R.1

Yeager, M. M., Neves, R. J., & Cherry, D. S. (2000). Competitive interactions between early life stages of *Villosa iris* (Bivalvia: Unionidae) and adult Asian clams (*Corbicula fluminea*). In R. A. Tankersley, D. I. Warmolts, G. T. Watters, B. J. Armitage, P. D. Johnson, & R. S. Butler (Eds.), *Freshwater Mollusk Symposia Proceedings. Part II. Proceedings of the First Freshwater Mollusk Conservation Society Symposium* (pp. 253–259) : Ohio Biological Survey Special Publication.

Zipper, C. E., Donovan, P. F., Jones, J. W., Li, J., Price, J. E., & Stewart, R. E. (2016). Spatial and temporal relationships among watershed mining, water quality, and freshwater mussel status in an eastern USA river. *Science of the Total Environment*, *541*, 603–615. https://doi.org/10.1016/j.scitotenv.2015.09.104

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.