# In situ studies with Asian clams (*Corbicula fluminea*) detect acid mine drainage and nutrient inputs in low-order streams

**David J. Soucek, Travis S. Schmidt, and Donald S. Cherry**

**Abstract**: In situ Asian clam (*Corbicula fluminea* [Müller]) studies may effectively mirror resident community responses to both acute toxicants and nutrient inputs in low-order streams. Clam survival and growth after 30 days in situ were compared with benthic macroinvertebrate community structural changes caused by acid mine drainage (AMD) and nutrient loading (measured as nitrate) in a small subwatershed of the North Fork Powell River, Virginia, U.S.A. Clam survival distinguished between two different levels of impact due to acidic, neutralized, and intermittent AMD inputs and was positively correlated with water column pH and negatively correlated with conductivity and metal concentrations. Survival was also positively correlated with relative abundance of the order Ephemeroptera, the most sensitive macroinvertebrate taxonomic group to AMD in this system. Clam growth was not related to AMD inputs but was positively correlated with nitrate concentrations and the relative abundance of the collector–filterer functional feeding group. These results suggest that transplanted clam studies accurately reflect benthic macroinvertebrate community responses to multiple stressors from point and nonpoint sources.

**Résumé** : Des études in situ sur la Mye asiatique (*Corbicula fluminea* [Müller]) peuvent représenter de façon réaliste les réactions de la communauté qui habite des ruisseaux d'ordre inférieur tant à des contaminations aiguës qu'à des apports de nutriments. La survie des myes et leur croissance après 30 jours in situ ont été comparées aux changements dans la structure de la communauté de macroinvertébrés benthiques causés par des rejets miniers acides (AMD) et des charges de nutriments (mesurées en nitrates) dans un petit sous-bassin hydrographique de la rivière North Fork Powell, en Virginie, aux U.S.A. Les données de survie des myes permettent de distinguer deux niveaux d'impacts causés par des apports d'AMD acides, neutralisés et intermittents; la survie est en corrélation positive avec le pH de la colonne d'eau et en corrélation négative avec la conductivité et les concentrations de métaux. La survie est aussi corrélée positivement avec l'abondance relative des Éphéméroptères, le groupe taxonomique de macroinvertébrés le plus sensible à l'AMD dans ce réseau. La croissance des myes n'est pas en corrélation avec les apports d'AMD, mais elle est directement reliée aux concentrations de nitrates et à l'abondance relative de la guilde alimentaire des collecteurs-filtreurs. Ces résultats laissent croire que des études sur des myes transplantées dans des cours d'eau reflètent de façon précise les réactions de la communauté de macroinvertébrés benthiques à des sources de stress multiples d'origine définie et diffuse.

[Traduit par la Rédaction]

## Introduction

Complex combinations of environmental factors regulate aquatic community structure and population distributions (Merritt and Cummins 1996). Lotic benthic macroinvertebrate communities in particular are structured by biotic factors such as competition and predation and a number of abiotic factors, including climate, water quality, nutrient availability, stream order, and substrate composition. Studies of pollution impacts on aquatic communities are often inconclusive because a pollutant, such as an industrial effluent, may contain several interacting components. Some components may enhance an aspect of the community by providing a limited nutrient, while others have an inhibitory effect by causing toxicity (Wiederholm 1984). Ecosystems often receive inputs from multiple stressors, including both point and nonpoint sources, further confounding our understanding of community responses to pollutants.

In situ toxicity tests have gained popularity as replacements for, or supplements to, standard bioassessment tools such as laboratory toxicity testing and benthic macroinvertebrate sampling. They are thought to provide more environmental realism than laboratory tests by incorporating continuous exposure over an extended period of time to the multiple stressors that regulate indigenous communities and are less time and labor intensive than macroinvertebrate community sampling (Cherry 1996). The Asian clam *Corbicula fluminea* [Müller] is particularly useful as a biomonitoring tool because of its availability, 1- to 3-year life span, sedentary nature, sensitivity to different types of pollutants, and ability to accumulate organic pollutants and heavy metals (Doherty and Cherry 1988; Doherty 1990). Transplanted

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clams have been used widely to detect anthropogenic impacts in aquatic ecosystems, with endpoints ranging from survival and growth (Belanger 1991) to cellulolytic activity (Farris et al. 1988), valve movement (Allen et al. 1996), and DNA strand breakage (Black 1997).

While numerous studies have employed *Corbicula* as a biomonitoring tool, very few researchers (Farris et al. 1988) have compared transplanted Asian clam responses with those of benthic macroinvertebrate communities. The purpose of this study was to evaluate the correlation between transplanted Asian clam and indigenous community responses to acid mine drainage (AMD) and nutrient loading in first- to third-order streams. These objectives were accomplished by comparing the toxicological endpoints of clam survival and growth with benthic macroinvertebrate community indices, as community responses to both AMD and nutrient loading are well characterized (Wiederholm 1984; Hilsenhoff 1988; Kelly 1988). We hypothesized that (*i*) clam survival may be limited by acutely toxic AMD inputs in the upper portions of the watershed (first- to third-order streams) and (*ii*) clam growth is likely to be related to trophic status in low-order streams lacking AMD because they are filter feeders. Changes in those endpoints should be related to shifts in relative abundance of ecologically different groups of benthic macroinvertebrates. Therefore, clam growth and survival endpoints in low-order streams may provide different types of information to ecologists or risk assessors by responding in different ways to different types of pollutants.

## Materials and methods

### Sampling stations and groups

Puckett's Creek flows via Straight Creek and Stone Creek into the North Fork of the Powell River, Virginia, U.S.A., which contributes to the Tennessee River drainage (Fig. 1). Asian clam populations were well established in the Powell River before this study was conducted, so potential introduction of an exotic species was not a concern. Fifteen sampling stations were selected in Puckett's Creek and its tributaries, including Lick Branch, which received input from several AMD discharges. In addition, four stations were selected in higher-order (fourth to sixth) AMD-influenced streams of the same subwatershed of the North Fork of the Powell River. Stations were categorized based on level of AMD input as determined by location, stream order, and mean pH. Categorization of stations facilitated determination of clam sensitivity to the different types of AMD impacts relative to the benthic macroinvertebrate communities by providing treatments to compare using analysis of variance (ANOVA).

Five groups of stations were constructed as follows. The first group ($n$ = 5) consisted of stations that were upstream of all known AMD inputs in Puckett's Creek and Lick Branch. Stream order for these five stations ranged from first to second. The second group ($n$ = 3) consisted of stations ranging from first to third order and were subjected to intermittent AMD input. The intermittent designation was based on a wide pH range over time (e.g., 3.17–6.30 for station LB-5) or being downstream of such a station but upstream of any continuous AMD input (Soucek et al. 2000). The third group ($n$ = 4) consisted of those stations in Lick Branch (first to second order) continuously subjected to AMD input and having acidic mean pH values (≤4.50). The fourth group ($n$ = 3) consisted of stations in the lower, third-order section of Puckett's Creek below the confluence of Lick Branch; these stations were continuously subjected to AMD input but had circumneutral mean pH values (6.11–7.42) because of dilution. Therefore, group 4 stations will be referred to as neutralized AMD stations. The fifth group ($n$ = 4) consisted of stations in Straight Creek, Stone Creek, and the North Fork of the Powell River. While all group five stations were downstream of various AMD inputs, they were differentiated from group 4 as having a higher level of dilution, being located in fourth- to sixth-order streams.

One additional station (SC-2) was selected in a third-order reach of the adjacent Straight Creek but was not used in the primary analyses because it was located in a different creek upstream of AMD input. This station was suspected of receiving diluted domestic input (possibly sewage) based on observations and nutrient measurements and therefore was used as a case study of clam growth in low-order streams where nutrient concentrations were elevated. Sampling at this station was conducted concurrently with, and was identical to, that for the other stations in terms of types of samples collected and analyzed.

### Water column chemistry

To characterize stations according to the degree of AMD input and nutrient levels, water samples were collected at each station for analysis of selected water quality parameters on four occasions from fall 1997 to summer 1998. Either samples were brought to the laboratory, stored for 24 h at 4°C, and measurements taken under laboratory conditions or measurements were taken in the field. Sample pH was measured using an Accumet® (Fisher Scientific, Pittsburgh, Pa.) pH meter equipped with an Accumet® gel-filled combination electrode (accuracy <±0.05 pH unit at 25°C). Conductivity measurements were made using a Hach® (Hach, Loveland, Co.) conductivity/TDS meter. Dissolved oxygen, alkalinity, and hardness data for the watershed are provided in Soucek et al. (2000). In addition, water column samples were prepared for metals analysis according to standard methods (U.S. Environmental Protection Agency 1991). Samples were analyzed by inductively coupled plasma spectrometry for total aluminum (Al) and iron (Fe), which were previously determined to be the dominant metals in the system (Soucek et al. 2000). Lower detection limits were 0.001 and 0.002 $mg \cdot L^{-1}$ for Al and Fe, respectively. Water samples also were analyzed for nitrate ($NO_3$) according to standard methods (U.S. Environmental Protection Agency 1979).

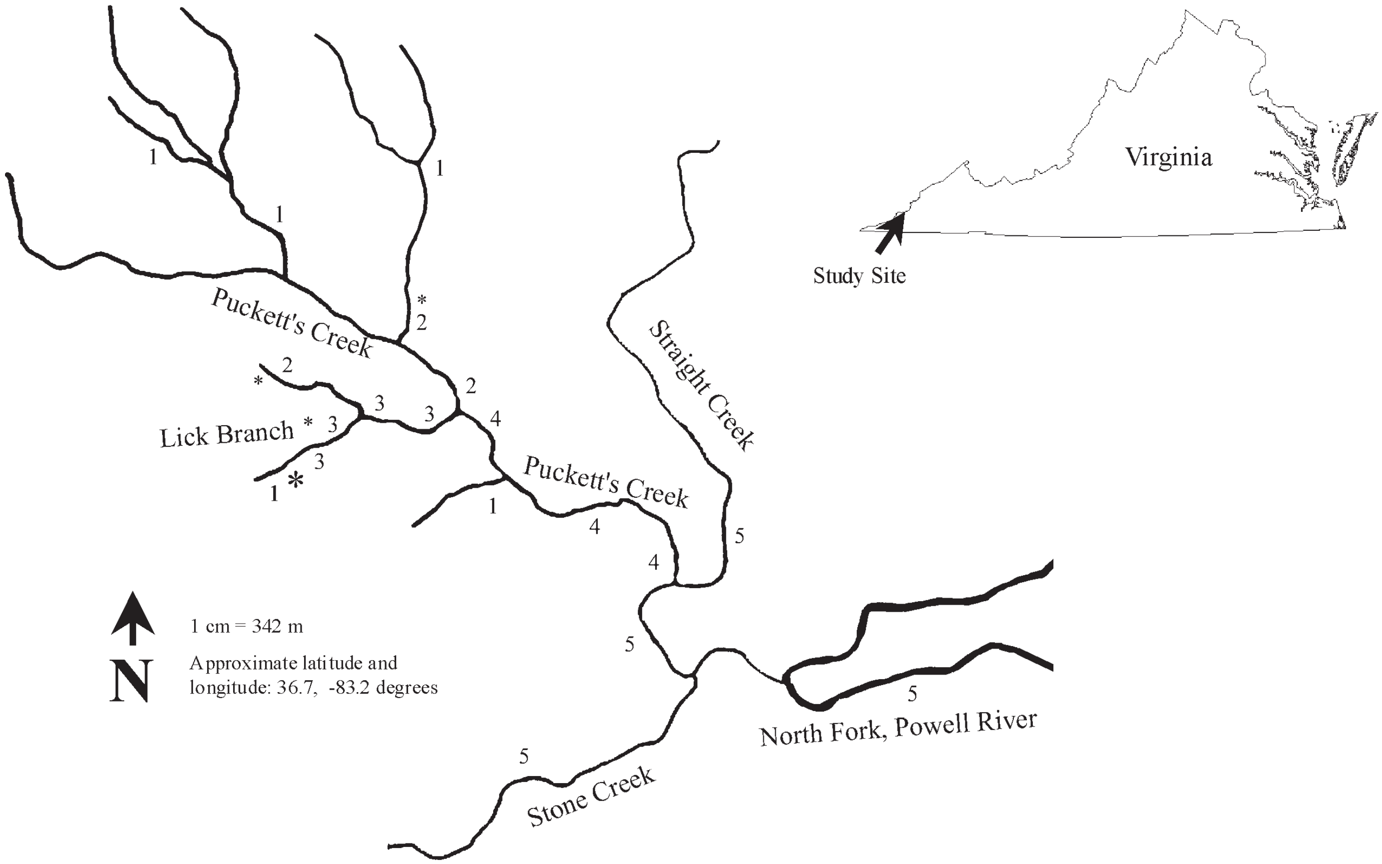
Mean values for individual sampling stations were calculated for parameters of which more than one measurement was taken (i.e., pH and conductivity). Then, mean values for station groups 1–5 were compared by ANOVA. Student's $t$ test was used for post hoc pairwise analysis at the $\alpha$ = 0.05 significance level.

### Benthic macroinvertebrate sampling

Benthic macroinvertebrate surveys were conducted according to the U.S. Environmental Protection Agency Rapid Bioassessment Protocols (Plafkin et al. 1989). Riffle, run, pool, and shoreline rooted areas were thoroughly sampled for 20 min per site using dip nets with 800-μm mesh. Two replicate samples were collected per site and mean values for all indices were calculated for each site. All organisms in each sample were identified to the lowest practical taxonomic level (usually genus) using standard keys (Pennak 1989; Merritt and Cummins 1996). Chironomids were identified as either subfamily Tanypodinae or non-Tanypodinae.

For community analyses, taxa were placed into six categories: Ephemeroptera, Plecoptera, Trichoptera, Hydropsychidae, Chironomidae, and "other." The "other" taxa category included the Megaloptera, Odonata, Coleoptera, nonchironomid Diptera, and noninsects. The Trichoptera group included hydropsychid caddisflies, but hydropsychids were placed into an additional category because they may reach high densities in response to dilute organic inputs or mild eutrophication (Wiederholm 1984). The relative abundance of each taxonomic group was calculated for each station by dividing, for example, the number of mayflies by the total num-

**Fig. 1.** Diagram of the Puckett's Creek watershed showing locations of sampling stations by group and AMD discharges. 1, upstream stations; 2, intermittent AMD stations; 3, acidic AMD stations; 4, neutralized AMD stations; 5, higher-order stations. The large asterisk indicates a continuously flowing AMD discharge, while the small asterisks indicate intermittent discharges.




ber of organisms. These values were compared within each station group by ANOVA and post hoc tests as described previously to determine which type of organisms were dominant within each station group.

Each taxon identified also was assigned to a functional feeding group as described in Merritt and Cummins (1996). Feeding groups included collector–gatherers, shredders, collector–filterers, scrapers, and predators. Tanypod chironomids were considered predators, and non-tanypod chironomids were collector–gatherers. Similar analyses were conducted as with the taxonomic groups, i.e., mean relative abundances of feeding groups were compared within station groups.

To further test for the potential presence of dilute organic input at station SC-2, family-level biotic index (FBI) values were calculated for the five upstream (group 1) stations and for station SC-2 (Hilsenhoff 1988). The FBI value gives an indication of the level of organic pollution from sources such as agricultural runoff or sewage based on tolerance values of arthropod families found at the site, with higher scores indicating more organic pollution.

### In situ clam toxicity testing

For in situ toxicity tests, Asian clams were collected from the New River near Ripplemead, Va., using clam rakes. Clams were held in Living Streams® (Toledo, Ohio) at the Ecosystem Simulation Laboratory, Virginia Tech, Blacksburg, Va., until use. Individual clams were given one of five distinctive marks and measured for width to the nearest 0.01 mm using Vernier calipers prior to placement into bags for testing. Testing procedures consisted of tying five mesh bags, each bag containing five clams, to stakes at each sampling station. Bags were 18 cm wide by 36 cm long with a mesh size of about 0.5 cm$^2$. At the end of 30 days, clam bags were collected from each testing station and transported on ice to the laboratory. Clams were counted as dead or alive; clams found with valves separated, or that were easily opened, were considered dead. Surviving clams were measured again for width, and growth was calculated by subtracting beginning from ending width. Mean clam survival and growth values for station groups 1–5 were compared using ANOVA and pairwise analyses, as described previously, at the $\alpha = 0.05$ level.

### Correlation analysis

To compare benthic macroinvertebrate sampling data with Asian clam toxicity test data for the watershed, bivariate correlation analyses were conducted. Both clam survival and growth were compared with taxonomic and feeding group relative abundances. Values from all 19 stations were included in the analysis for clam survival, but stations where all clams died were excluded from the correlation analyses for clam growth. Additional correlation analyses were conducted between clam survival/growth and water chemistry parameters to determine if the clam endpoints were related to AMD inputs or to nutrient levels at a given station. Significance of correlation was determined at the $\alpha = 0.05$ level.

## Results

### Water chemistry

The most extreme values for the four AMD-related parameters analyzed were observed at group 3 stations, where mean pH (3.71) was lowest and conductivity (1216 μmho·$cm^{-1}$), Al (30.7 mg·$L^{-1}$), and Fe (17.09 mg·$L^{-1}$) were highest (Table 1). The intermittent nature of AMD input at the group 2 stations was demonstrated by relatively high standard deviations about the means for pH, conductivity, Al, and Fe. The group 4 stations had a circumneutral average pH (6.84) but elevated values for mean conductivity (559 μmho·$cm^{-1}$) and Al (2.2 mg·$L^{-1}$). Mean water chemistry values for the group 5 stations were similar to those for the group 1 stations except for nominally elevated mean conductivity (469 μmho·$cm^{-1}$) and a significantly higher mean $NO_3$ concentration (0.40 mg·$L^{-1}$) than for any of the lower-order station groups (1–4).

### Benthic macroinvertebrate communities

Benthic macroinvertebrate communities were sensitive to the various types of AMD input. At the group 1 stations, upstream of AMD input, Ephemeroptera and Plecoptera were the dominant taxa, making up 41.3 and 31.6%, respectively, of the mean total abundances (Table 2). The intermittent AMD stations (group 2) had similar assemblages in terms of which taxa were dominant, but mayflies (Ephemeroptera) were slightly less abundant than stoneflies (Plecoptera), chironomid numbers decreased, and relative abundance of the "other" group was not significantly different from that of the mayflies and stoneflies. At the acidic AMD stations, chironomids accounted for more than half of the organisms, while mayflies, stoneflies, and caddisflies made up less than 15% of the assemblages. The group 4 stations were dominated by the "other" taxa group, and at the group 5 or higher-order stations, no single group was dominant, as no significant differences were observed.

Changes also were observed in relative abundances of functional feeding groups in response to AMD. The collector–gatherer functional feeding group was dominant at all stations except for the neutralized AMD group (Table 2). At the group 1 stations, shredders were codominant, having a significantly higher relative abundance than the other three feeding groups. While shredders and collector–gatherers had high relative abundance values at the group 2 intermittent AMD stations, their values were not significantly higher than for the other groups. Collector–gatherers dominated the acidic AMD stations with a relative abundance of 67.5%. Predators were the dominant group at the neutralized AMD stations, making up almost half of the assemblages. At the higher-order group 5 stations, collector–gatherers and collector–filterers made up the greatest proportion of the macroinvertebrate assemblages, followed by predators, shredders, and scrapers.

### In situ clam toxicity

The clam survival endpoint was sensitive to AMD inputs, differentiating between two levels of environmental impact. Average survival of clams at the end of the 30-day test was highest in groups 1 and 5 (92.8 and 92.0%, respectively), while none of the clams placed in the group 3 acidic AMD impacted stations survived (Table 3). As illustrated by standard deviations about means, survival at the stations with intermittent (group 2) and neutralized AMD input (group 4) was variable, with intermediate mean values of 54.6 and 36.0%, respectively. Groups 1 and 5 had significantly higher survival than group 2, which in turn had significantly higher survival than group 3 stations.

Clam growth was variable and less sensitive to AMD inputs when excluding all sites where 100% mortality of clams was observed (Table 3). While station groups consisting of first- to third-order reaches (groups 1, 2, and 4) had substantially lower growth (–0.08 to 0.06 mm) than the higher-order group 5 stations (0.37 mm), differences were not significant due to high variability.

### Correlation analysis

Clam survival data compared with benthic macroinvertebrate indices produced three significant correlations (Table 4). Clam survival was negatively correlated with the relative abundance of chironomids and positively correlated with the relative abundance of mayflies (Ephemeroptera). The only functional feeding group that was correlated with clam survival was the scraper group, with a positive significant relationship.

Clam growth was negatively correlated with percent abundance of stoneflies (Plecoptera) but positively correlated with hydropsychid caddisflies and chironomids (Table 4). Growth also had a significant negative relationship with percent abundance of shredders and a positive relationship with collector–filterers.

Comparison of in situ toxicity test parameters with water chemistry data indicated that clam survival was significantly positively correlated with pH and negatively correlated with conductivity and Al and Fe concentrations in the water column (Table 5). $NO_3$ was not significantly correlated with clam survival; however, $NO_3$ had a significantly positive relationship with clam growth.

### Clam responses to nutrient loading

Comparing nutrient levels and their effects in the absence of AMD, the upstream group 1 stations in Puckett's Creek had a mean $NO_3$ concentration of 0.16 ± 0.09 mg·$L^{-1}$, while station SC-2, a similar type of station in the adjacent Straight Creek watershed, had 0.56 mg $NO_3$·$L^{-1}$. Mean clam growth was substantially higher at station SC-2 (1.1 mm) compared with the mean value for the group 1 stations (0.062 ± 0.25 mm). Likewise, percent abundance of collector–filterers and FBI score were substantially higher in SC-2 (79.2% and 3.86, respectively) compared with the means for the Puckett's Creek group 1 stations (6.7 ± 6.4% and 2.54 ± 0.48, respectively).

## Discussion

These results suggest that Asian clams may be used to detect two different types of pollution in headwater streams: acute toxicants and dilute nutrient inputs. In addition, in situ clam tests appear to accurately reflect benthic macroinvertebrate responses to these types of pollutants. Clam survival was sensitive to various levels of AMD inputs and was correlated with dominance of the most AMD-sensitive taxonomic groups (i.e., mayflies). Conversely, clam growth was correlated with dominance of benthic macroinvertebrate groups (i.e., hydropsychids and chironomids) that have been observed to thrive downstream of mild organic or nutrient inputs.

**Table 1.** Mean (±SD) water chemistry values for station groups in the Puckett's Creek watershed.

| Station group | pH | Conductivity (μmho·cm$^{-1}$) | Al (mg·L$^{-1}$) | Fe (mg·L$^{-1}$) | $NO_3$ (mg·L$^{-1}$) |
|---|---|---|---|---|---|
| 1 | 7.27 ± 0.48 A | 187 ± 99 A | 0.2 ± 0.1 A | 0.04 ± 0.02 A | 0.16 ± 0.09 B |
| 2 | 6.63 ± 1.35 A | 525 ± 180 B | 9.2 ± 10.8 A | 4.32 ± 4.60 A | 0.13 ± 0.04 B |
| 3 | 3.71 ± 0.69 B | 1216 ± 365 C | 30.7 ± 13.4 B | 17.09 ± 19.12 A | 0.19 ± 0.07 B |
| 4 | 6.84 ± 0.66 A | 559 ± 6 B | 2.2 ± 1.9 A | 0.09 ± 0.07 A | 0.14 ± 0.04 B |
| 5 | 7.76 ± 0.30 A | 469 ± 187 AB | 0.19 ± 0.2 A | 0.08 ± 0.11 A | 0.40 ± 0.18 A |

**Note:** Means in a vertical series followed by the same letter are not significantly different (α = 0.05, ANOVA and Student's *t* test). Group: 1, no AMD impact (*n* = 5); 2, intermittent AMD (*n* = 3); 3, acidic AMD (*n* = 4); 4, neutral AMD (*n* = 3); 5, higher order (*n* = 4).

**Table 2.** Mean (±SD) percent composition by taxonomic and functional feeding group for the five station types in the Puckett's Creek watershed.

| | Upstream group 1 (*n* = 5) | Intermittent group 2 (*n* = 3) | Acidic AMD group 3 (*n* = 4) | Neutralized AMD group 4 (*n* = 3) | Higher-order group 5 (*n* = 4) |
|---|---|---|---|---|---|
| **Taxonomic group** | | | | | |
| Plecoptera | 31.6 ± 22.5 A | 41.5 ± 19.1 A | 10.9 ± 15.7 BC | 25.8 ± 19.9 AB | 9.9 ± 3.3 A |
| Ephemeroptera | 41.3 ± 18.2 A | 25.4 ± 14.4 AB | 2.2 ± 3.6 C | 1.4 ± 2.4 C | 19.6 ± 14.3 A |
| Trichoptera | 9.6 ± 6.3 B | 9.9 ± 9.8 BC | 0.4 ± 0.7 C | 20.9 ± 6.0 B | 18.7 ± 8.4 A |
| Hydropsychidae | 6.4 ± 6.2 B | 6.2 ± 8.8 BC | 0.4 ± 0.7 C | 13.2 ± 6.4 BC | 16.6 ± 9.9 A |
| Chironomidae | 6.5 ± 9.6 B | 2.1 ± 2.5 C | 59.4 ± 28.7 A | 6.7 ± 5.9 BC | 17.3 ± 9.2 A |
| Other | 11.0 ± 12.6 B | 20.9 ± 13.6 ABC | 27.0 ± 15.7 B | 44.8 ± 13.7 A | 34.9 ± 18.2 A |
| **Feeding group** | | | | | |
| Collector–gatherers | 42.9 ± 18.0 A | 28.3 ± 12.9 A | 67.5 ± 27.4 A | 7.4 ± 2.5 BC | 37.0 ± 12.7 A |
| Scrapers | 8.6 ± 3.9 B | 6.7 ± 3.5 A | 1.5 ± 1.8 B | 4.0 ± 5.3 C | 8.6 ± 6.5 C |
| Collector–filterers | 6.7 ± 6.4 B | 7.0 ± 9.2 A | 0.4 ± 0.8 B | 15.4 ± 8.6 BC | 23.2 ± 10.3 AB |
| Shredders | 28.3 ± 22.5 A | 40.0 ± 23.8 A | 11.8 ± 17.0 B | 26.8 ± 14.9 AB | 11.7 ± 6.6 BC |
| Predators | 8.5 ± 1.9 B | 17.8 ± 10.6 A | 18.6 ± 12.9 B | 46.2 ± 16.4 A | 19.3 ± 8.9 BC |

**Note:** Means in a vertical series followed by the same letter are not significantly different (α = 0.05, ANOVA and Student's *t* test).

**Table 3.** Mean (±SD) survival and growth in Asian clam in situ toxicity tests for station groups in the Puckett's Creek watershed.

| Station group | Clam survival (%) | Clam growth (mm) |
|---|---|---|
| 1. Upstream | 92.8 ± 5.2 A | 0.06 ± 0.25 A |
| 2. Intermittent AMD | 54.6 ± 47.7 B | –0.05 ± 0.04 A |
| 3. Acidic AMD | 0.0 ± 0 C | na |
| 4. Neutralized AMD | 36.0 ± 34.2 BC | –0.08 ± 0.03 A |
| 5. Higher order | 92.0 ± 5.6 A | 0.37 ± 0.18 A |

**Note:** Means in a vertical series followed by the same letter are not significantly different (α = 0.05, ANOVA and Student's *t* test); na, not available.

**Table 4.** Correlation coefficients (*r*) between Asian clam in situ toxicity test endpoints and taxonomic and functional feeding groups of benthic macroinvertebrates in the Puckett's Creek watershed.

| Comparison | *r* | *p* |
|---|---|---|
| Clam survival vs. Chironomidae | –0.526 | 0.0208 |
| Clam survival vs. Ephemeroptera | +0.496 | 0.0304 |
| Clam survival vs. scrapers | +0.469 | 0.0428 |
| Clam growth vs. Plecoptera | –0.675 | 0.0113 |
| Clam growth vs. Hydropsychidae | +0.650 | 0.0161 |
| Clam growth vs. Chironomidae | +0.819 | 0.0006 |
| Clam growth vs. shredders | –0.663 | 0.0135 |
| Clam growth vs. collector–filterers | +0.745 | 0.0035 |

**Note:** All significant relationships ($p \leq 0.05$) are shown.

Clam survival effectively distinguished between different levels of environmental impact due to acidic, neutralized, and intermittent AMD inputs in this small watershed. While significant mortality of transplanted clams has been observed in response to organochlorine contamination (Hayward et al. 1996) and sewage treatment plant effluents (Belanger 1991), most transplant studies with Asian clams have investigated sublethal responses to pollutants such as DNA strand breakage (Black 1997), enzyme activity (Farris et al. 1988), bioaccumulation (e.g., Andrès et al. 1999; Gunther et al. 1999), and valve movement (Allen et al. 1996). These types of responses have been sensitive to low levels of pollution, but they may be too time intensive for a preliminary bioassessment of a whole watershed. In this study, the clam survival endpoint was sensitive to two different levels of environmental impact, and these survival responses were correlated with AMD-related water chemistry parameters such as pH, conductivity, and metal concentrations.

The benthic macroinvertebrate community responses to AMD in Puckett's Creek were similar to those observed in other AMD-impacted watersheds. For example, mayfly numbers often are depressed by mine drainages (e.g., Roback and Richardson 1969; Merrett et al. 1991; Nelson and Roline 1996). In Puckett's Creek, they were the most sensitive taxonomic group to AMD inputs, dominating the assemblages upstream of AMD inputs but comprising less than 2.5% of the assemblages at the acidic and neutralized AMD stations. Their relative abundance also was low at the stations with intermittent AMD inputs. Chironomid relative abundance

**Table 5.** Correlation coefficients (*r*) between Asian clam in situ toxicity test endpoints and water quality data for the Puckett's Creek watershed.

| Comparison | *r* | *p* |
|---|---|---|
| Clam survival vs. pH | +0.732 | 0.0008* |
| Clam survival vs. conductivity | –0.763 | 0.0004* |
| Clam survival vs. Al | –0.653 | 0.0045* |
| Clam survival vs. Fe | –0.535 | 0.0270* |
| Clam survival vs. $NO_3$ | +0.337 | 0.1865 |
| Clam growth vs. pH | +0.472 | 0.1423 |
| Clam growth vs. conductivity | +0.205 | 0.5453 |
| Clam growth vs. Al | –0.197 | 0.5617 |
| Clam growth vs. Fe | +0.173 | 0.6106 |
| Clam growth vs. $NO_3$ | +0.647 | 0.0313* |

**Note:** An asterisk indicates a significant relationship ($p \leq 0.05$).

also responded to AMD impacts, as has been observed in other studies (e.g., Roback and Richardson 1969; Armitage 1980; Rutherford and Mellow 1994), increasing in relative abundance at the acidic AMD stations compared with unimpacted sites. These findings suggest that this watershed was a good system to use in a comparison of transplanted Asian clam responses to AMD with those of the resident community.

To our knowledge, one other study has compared transplanted Asian clam responses with indigenous community responses (Farris et al. 1988). In that study, clam cellulolytic activity was related to the presence of suspended particles with bound metals, while invertebrate communities were most severely impacted where dissolved metals were highest. Clam and macroinvertebrate community endpoints were not compared statistically. In the present study, clam survival was positively correlated with relative abundance of Ephemeroptera and negatively correlated with Chironomidae. These were the most and least sensitive taxonomic groups, respectively, to various levels of AMD input. Thus, clam survival in transplant tests appears to mirror benthic macroinvertebrate community responses to AMD in this watershed.

Clam growth could not be determined at the most severely impacted sites (group 3) because all clams died, and at stations where clams survived, growth was minimal except in the fourth- to sixth-order stations (group 5). Furthermore, clams decreased in size at the group 2 and 4 stations, as has been observed in response to low levels of copper, zinc, and chrysotile asbestos (Belanger et al. 1986*a*, 1986*b*, 1990), but those means were not significantly different from the mean for the upstream stations due to variability within groups.

Although clam growth was not statistically sensitive to AMD inputs and was not significantly correlated with AMD-related chemistry parameters, it was positively correlated with $NO_3$ concentrations, suggesting that the nutrient loads explained variations in clam growth in this headwater system. Growth was greatest at the larger group 5 stations, which had the highest $NO_3$ concentrations and the greatest flow, being fourth- to sixth-order streams. Clam growth was negatively correlated with relative abundance of the Plecoptera taxonomic group and the shredder functional feeding group and positively correlated with relative abundance of hydropsychids, chironomids, and collector–filterers, providing further evidence of this nutrient-level connection with clam growth.

As filter feeders, the success of Asian clams in terms of building biomass is dependent on suspended and (or) dissolved carbon as a food source. Coarse particulate organic matter is generally the major carbon source in a small system of first- to third-order streams. Thus, shredders, which feed upon coarse particulate organic matter, should be one of the dominant functional feeding groups in low-order streams (Vannote et al. 1980), and they had the second highest relative abundance at the group 1 stations of this study. Collector–filterers generally are not as abundant as shredders in headwater streams because of the relative lack of fine particulate or dissolved organic matter. Therefore, the poor clam growth at the group 1 stations is not unusual; however, these results have other bioassessment implications.

Based on these data, one should expect poor growth of Asian clams at stations upstream of acutely toxic inputs in headwater systems, as was observed for the group 1 stations, unless some artificial nutrient source is available. Both hydropsychids (collector–filterers) and chironomids (collector–gatherers) have been observed to reach high densities in response to dilute eutrophication or organic input (Wiederholm 1984). Clam growth in this watershed was significantly positively correlated with the relative abundance of both hydropsychids and chironomids. Therefore, robust growth of clams in a first- to third-order stream may also be an indicator of dilute anthropogenic organic or nutrient inputs (i.e., dilute sewage or agricultural runoff).

To test this hypothesis, we conducted the comparison of clam growth and macroinvertebrate community indices for the Puckett's Creek group 1 stations versus the values for station SC-2, which was suspected of receiving elevated nutrient inputs. The $NO_3$ concentration at station SC-2 was 3.5-fold higher than the mean for the Puckett's Creek stations, and FBI scores and percent collector–filterer values responded accordingly with 1.5- and 11.8-fold increases, respectively. An FBI score of 2.54 (the average for Puckett's Creek group 1 stations) corresponds to an "excellent" rating with "organic pollution unlikely," while a score of 3.86 (station SC-2) indicates "possible slight organic pollution" (Hilsenhoff 1988). Likewise, clam growth was about 18-fold greater at station SC-2 than the mean for the Puckett's Creek upstream stations. Thus, clam growth in transplant toxicity tests appears to be related to nutrient levels and accurately reflects benthic macroinvertebrate responses to nutrient loading.

While clam survival and growth may provide much information about a small watershed, the tests are simple to conduct and cost little in terms of time and materials. Disadvantages of transplant studies with Asian clams include potential vandalism of test containers, predation upon test organisms, and potential release of propagules into previously uncolonized areas (Cherry 1996). However, these data suggest that transplanted clams may be useful tools for preliminary reconnaissance of small headwater systems that Asian clams have already colonized.

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