

**INFLUENCE OF MULTIPLE DISTURBANCES ON STREAM  
STRUCTURE AND FUNCTION**

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## ABSTRACT

We investigated the influence of multiple disturbances on ecosystem structure and function in a headwater stream adjacent to an abandoned arsenic mine using an upstream (reference) and downstream (mine-influenced) comparative reach approach. In this study, floods were addressed as a pulse disturbance, and the abandoned arsenic mine was characterized as a press disturbance. Chronically elevated levels of arsenic were specifically addressed as a ramp disturbance. Stream ecosystem structure and biogeochemical functioning were characterized monthly over a period from July to December 2004 by determining benthic organic matter standing stocks, ecosystem metabolism, and by using solute additions to examine differences in phosphorus uptake and hydrology over the monitoring period. Influences of the press disturbance were evident in the mine-influenced reach where arsenic concentrations ( $254 \pm 39 \mu\text{g/L}$ ) were >30 higher than in the reference reach ( $8 \pm 1 \mu\text{g/L}$ ). However, in almost all cases the presence of the abandoned arsenic mine appeared to exert little influence on reach-scale measures of ecosystem structure and function (e.g., organic matter standing crops, phosphorus uptake). Conversely, floods (i.e., pulse disturbances) influenced organic matter standing stocks and hydrologic interactions between the stream and transient storage zones in both study reaches. Interactions between press and pulse disturbances were evident in several cases and illustrated by phosphorus uptake responses. Phosphorus uptake was best predicted by coarse particulate organic matter standing stocks in the reference reach. However, in the reach exposed to the press

disturbance (i.e., mine-influenced reach), both coarse particulate organic matter standing stocks and characteristics of the pulse disturbance regime (i.e., number of days post-flood) were significant predictors of phosphorus uptake. Within the mine-influenced reach, arsenic concentrations increased from 16–600  $\mu\text{g/L}$  and were addressed as a ramp disturbance. Analysis of phosphorus uptake in the mine-influenced reach across a gradient of arsenic concentrations correlated with Michaelis-Menton models of enzyme kinetics in the presence of a competitive inhibitor. These results suggest that arsenic appears to competitively inhibit phosphorus uptake by microbial assemblages in the mine-influenced reach. Results from this study highlight the fact that ecotoxicological studies at the ecosystem scale should consider not only contaminant influences, but rather place its implications within the extant disturbance regime generated from both natural and anthropogenic sources.

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## **DEDICATION**

To Rev. Presbyter Philemon Sevastiades

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## INTRODUCTION

Ecological disturbances occur when potentially damaging forces such as forest fires, floods, and anthropogenic activities are imposed upon habitat space occupied by a population, community, or ecosystem (Lake 2000). Disturbances may result in the death of organisms, depletion of consumable resources, or degradation of habitat structure (Lake 2000). Lake (2000) went on to argue that disturbances differ in their temporal pattern of intensity and duration and can be broadly separated into three primary categories: pulse, press, and ramp. Historically, disturbance ecology in streams has focused on pulse disturbances, such as floods, that have discrete, short-term influence on lotic ecosystems (Resh et al. 1988, Lake 2000). Press disturbances are characterized by a long-term, sustained influence on an ecosystem. Most press disturbances are the result of anthropogenic activities such as changes in land use, logging, and mining (Lake 2000). The concept of a ramp disturbance was first introduced by Lake (2000) whereby the disturbance intensity increases steadily with time, as may occur with droughts. While the term may not have been explicitly used, previous research has described ramp-type influences of disturbance. For example, Boulton and Stanley (1995) described the increasing influence of a drought on interstitial invertebrates of streams as a gradual press disturbance.

Discrete disturbance regimes may characterize some ecosystems (i.e., a specific disturbance frequency and intensity may dictate ecosystem stability and successional recovery). However, many ecosystems experience a mix of disturbance types simultaneously; thus, responses to disturbance may require knowledge of disturbance interaction and history. Ross et al. (2004) emphasized the need to approach a disturbance within the framework of the ecosystem disturbance regime. For example, Collier and Quinn (2003) surmised that the magnitude and duration of responses to major pulse disturbances (i.e., floods) can depend on the presence or absence of an underlying press disturbance (i.e.,

agricultural land use). Parkyn and Collier (2004) further suggested that, in some cases, the influence of a press disturbance may only be evident after a pulse disturbance.

Stressors on aquatic ecosystems resulting from mining activity can persist for extended periods of time (Courtney and Clements 2002). Mining activity potentially imposes a template of multiple disturbance regimes on aquatic ecosystems, including increased acidity and heavy metal concentrations, precipitation of metal oxides on sediments, and sedimentation (Kelly 1988, Courtney and Clements 2002). These stressors often represent a press disturbance, although they may also be pulsatile in nature (Johnston and Keough 2002). Thus, studies examining the effects of mining on aquatic ecosystems should not be limited to assessing the influence of a single disturbance when a regime of multiple disturbances may exist.

In addition to the multiple interacting disturbance regimes that may result from mining practices, the effects of contaminants may be manifested at all levels of biological organization (Clements 2000). Because preservation of biological integrity includes protecting higher levels of biological organization (e.g., ecosystem structure and function), some researchers (e.g., Gessner and Chauvet 2002) have suggested that field responses of ecosystem structure and function are more ecologically relevant than effects at lower levels. Clements (2000) further suggested that one of the major goals of ecotoxicology should be to develop an improved mechanistic understanding of ecologically significant responses to contaminants.

As a result of Clements (2000) and other research, Chaffin et al. (*in press*) addressed the effects of arsenic (As) on organic matter (OM) processing in an Appalachian Mountain headwater stream. Arsenic is recognized as a toxic metalloid linked to skin, bladder, and other cancers (National Research Council 1999). Arsenic is also toxic to many prokaryotes and eukaryotes (Oremland and Stolz 2003). Chaffin et al. (*in press*) demonstrated that

elevated levels of arsenic decreased leaf decomposition rates in a headwater stream, representing altered ecosystem function. The decrease in leaf breakdown rates was attributed to the toxic effects of arsenic on the macroinvertebrate community (e.g., shredders *sensu* Merritt and Cummins (1984)). While the study by Chaffin et al. (*in press*) suggests that arsenic altered a fundamental ecosystem function primarily mediated by metazoans, less is known about how chronically elevated levels of arsenic influence ecosystem functions such as nutrient uptake or ecosystem metabolism that are principally controlled by microbial assemblages.

In the current study, we address how multiple disturbances alter ecosystem function in a headwater stream adjacent to an abandoned arsenic mine. Ecosystem metabolism and the nutrient spiraling concept are used to address how floods (i.e., pulse disturbance), abandoned arsenic mine (i.e., press disturbance), and longitudinal gradients in arsenic concentration (i.e., ramp disturbance) alter fundamental ecosystem functions and how these disturbances may interact to organize ecosystem behavior. To address our question, stream ecosystem structure and biogeochemical functioning were characterized monthly over a period from July to December 2004 by determining benthic organic matter standing stocks and ecosystem metabolism and by using solute additions to determine changes in nutrient uptake and hydrology over the monitoring period.

## **METHODS**

### *Study site*

Research was conducted in a headwater stream located at the site of the former Brinton Arsenic Mine in southwestern Virginia, USA. From 1903-1919, arsenopyrite was removed from mine shafts within a few hundred meters of the stream, and several tailing piles were deposited directly adjacent to the stream channel. Tailings consist of primarily

small, unconsolidated sediments replete with iron oxides from roasting and weathering. Tailing piles and adjacent areas exhibit extensive signs of erosion and mass wasting into the stream channel from runoff.

The study site (Digital Appendix A) included an upstream reference reach and a downstream mine-influenced reach. The reference reach extended 140 m upstream of the tailing piles and is heavily forested with deciduous trees (Chaffin et al. *in press*). No evidence of mining activity exists in the reference reach. The mine-influenced reach extends 90 m downstream of the upstream extent of the tailing piles. Approximately 60 m of the mine-influenced reach is virtually devoid of vegetation along the side of the stream adjacent to the tailing piles (Digital Appendix A). The remaining 30 m of the reach are covered with vegetation similar to that in the reference reach. Although arsenic concentrations increase significantly along the 90-m reach, concentrations of other toxic elements (i.e., Copper, Zinc, and Cadmium) were not comparably elevated (Chaffin et al. *in press*).

#### *Stream hydrogeochemistry*

Chemical properties of water within the reference and mine-influenced reaches were measured at 10 m intervals (9–12 transects) within each reach on a monthly basis. After collection, all water samples were filtered using glass fiber filters (Whatman GF/F, 0.70  $\mu\text{m}$  pore size), and stored in new polypropylene tubes. Water samples were analyzed for anions (chloride (Cl), nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ) and phosphorus ( $\text{PO}_4\text{-P}$ )) using a Dionex DX-500 with an AS14A column following USEPA standard methods (1993). Triplicate samples were collected for determination of arsenic at 20 m intervals within each reach. Samples were preserved with nitric acid and analyzed for total arsenic using a Graphite Furnace Atomic Absorption Spectrophotometer with Zeeman background correction (USEPA 1992).

*Stream reach structure: light, geomorphology, and biological characteristics*

Channel insolation (as % incident light) was determined by comparing a 1-min integration of light intensity at 5-m intervals within each reach using HOBO-Light Intensity Logger (Onset Computer Corp., Pocasset, MA, USA) to a separate logger exposed to direct sunlight on a single date during mid-day prior to leaf fall.

Benthic particle size classes were compared at the beginning (July 2004) and end (December 2004) of the study within each reach using granulometry techniques (Bunte and Abt 2001). Volume of large woody debris (LWD) was measured using the line-transect method (Wallace and Benke 1984). Wood mass as ash-free dry mass (AFDM; kg/m<sup>2</sup>) was calculated using a specific gravity of 0.356 g AFDM/cm<sup>3</sup> (Wallace et al. 2000). Debris dams were defined as collections of woody debris with a single piece at least 5 cm in diameter that extended the width of the stream (Valett et al. 2002) and were enumerated once in each reach.

Epilithic OM, chlorophyll *a*, fine benthic organic matter (FBOM, particles <1 mm), and coarse particulate organic matter (CPOM, wood and leaves >1 mm) were quantified as the mean of five samples taken from random locations along each reach. Samples were collected from August–December 2004. Rock scrapings of a known area were used to determine epilithic OM as AFDM (mg/m<sup>2</sup>) following combustion at 550 °C for 45 min and chlorophyll *a* (mg/m<sup>2</sup>) using hot ethanol extraction (Sartory and Grobbelaar 1984). Coarse particulate organic matter was collected from each location using a cylindrical sampling device (0.05 m<sup>2</sup>) and standing crops determined as AFDM (g/m<sup>2</sup>). Fine benthic organic matter (g/m<sup>2</sup>) was sampled by sealing the same cylindrical sampling device to the stream bottom, determining average depth (m) of stream water in sampling device, agitating sediments to 5-cm depth, determining AFDM concentration (g/m<sup>3</sup>) in a 250-mL subsample, and calculating standing stock as the product of concentration and depth.

### *Solute injections*

A conservative tracer (NaCl) and a biologically active solute (KH<sub>2</sub>PO<sub>4</sub>) were released under base-flow conditions at approximately monthly intervals throughout the study (Stream Solute Workshop 1990) to determine reach scale phosphorus uptake and hydrogeomorphic characteristics. Target enrichment concentrations for solutes were 3 mg/L chloride (Cl) and 50 µg/L PO<sub>4</sub>-P. Duplicate (n = 2) samples were collected from each transect at plateau and analyzed for PO<sub>4</sub>-P. Breakthrough specific conductance curves were recorded at 2-min intervals by monitoring changes in temperature-corrected conductivity using an automated sonde (Hydrolab model 4A, Austin, Texas, USA).

### *Solute injections: solute transport modeling*

Dilution gauging techniques (Stream Solute Workshop 1990) and one-dimensional modeling of solute transport (Bencala and Walters 1983), including inflow and transient storage was used to characterize hydrologic variables in each study reach. Wetted channel width ( $w$ , m) was measured at 5-m intervals along each reach prior to solute injections. Average discharge ( $Q$ , L/s) was calculated using dilution gauging techniques at each transect (Stream Solute Workshop 1990). Lateral inflow ( $Q_L$ , L/s) was calculated as the change in discharge from the head to the base of the reach, normalized to a 100-m reach. Background corrected conductivity data (i.e., Cl breakthrough curve) were used to analyze solute transport using the model of Runkel (1998). Variables obtained from the model characterize features of both the surface channel and storage zone. Damkohler coefficients (Wagner and Harvey 1997) calculated for injection experiments ranged from 0.65–4.23 with a mean of 1.82 and are within the range suggested by Harvey and Wagner (2000) for adequate estimation of model parameters. Quantified surface parameters included cross-section area ( $A$ , m<sup>2</sup>), stream velocity ( $u$ , m/s), and dispersion ( $D$ , m<sup>2</sup>/s). Depth ( $z$ , m) was calculated from average

discharge, stream width, and velocity (obtained from model output). Output variables for the storage zone include cross-sectional size ( $A_s$ , m<sup>2</sup>) and exchange coefficient ( $\alpha$ , s<sup>-1</sup>), a measure of the percent of water entering the storage zone per unit time. The normalized storage zone area ( $A_s/A$ ) was used to represent the storage zone size relative to the channel cross-sectional area. The proportion of median travel time due to storage ( $F_{med}$ , %) was calculated using an empirical formula provided by Runkel (2002), utilizing a 100-m reach length.

*Solute injections: calculation of phosphorus uptake*

Dilution- and background-corrected plateau PO<sub>4</sub>-P samples from each transect were natural-log transformed and regressed with distance downstream. The regression coefficient ( $K_L$ , m<sup>-1</sup>) was used to determine the phosphorus uptake velocity ( $V_f$ , mm/s; Stream Solute Workshop 1990) as:

$$V_f = K_L \cdot u \cdot z \cdot 1000 \quad (1)$$

Areal phosphorus uptake ( $U$ , µg/m<sup>2</sup>/hr) was calculated as the product of  $V_f$ , mean background PO<sub>4</sub>-P concentration, and a unit conversion factor of 3600 (Stream Solute Workshop 1990).

*Ecosystem Metabolism*

Gross primary production (GPP) and ecosystem respiration (R) were determined using the diel dissolved oxygen mass balance technique concurrent with solute injections (Bott 1996, Young and Huryn 1998). Oxygen concentrations (O<sub>2</sub>, mg/L) and temperature (°C) were recorded at 10-min intervals for a 24-hr period with a single automated sonde located at the base of each reach. Exchange of dissolved oxygen with the atmosphere was calculated from the average oxygen saturation deficit within the study reach, temperature, barometric pressure (each monitored at 10-min intervals), and reaeration rates determined



from the dilution-corrected decline of sulfur-hexafluoride (SF<sub>6</sub>) gas during steady state conditions. Gross primary production and R were calculated using the single station technique (Bott 1996).

#### *Sediment phosphorus sorption characteristics*

In order to assess the potential for abiotic processes (i.e., sorption to sediments) of reach scale phosphorus uptake, benthic sediment cores (1.5-cm diameter, 5-cm depth) were collected for laboratory sorption assays along both reaches from areas consisting of fine sediments (<4 mm). Filtered, stream water was obtained from each location and used as the matrix for sorption assays. A second water samples was also collected, filtered, and preserved with nitric acid at each location for determination of dissolved arsenic. Phosphorus uptake/sorption (mg/m<sup>2</sup>/hr) was determined on intact (live) and mercuric chloride (HgCl<sub>2</sub>) amended (killed) sediments to assess biotic and abiotic influence on phosphorus sorption capacity (Meyer 1979). Filtered stream water from each sample location (100 mL) was added to 20–30 g of wet sediments and enriched to 50 µg/L PO<sub>4</sub>-P (i.e., similar to field enrichment concentrations, *sensu* Bache and Williams 1971). Samples were shaken for 10 s every 15 min. After 1 hour, a 15-mL aliquot was removed, filtered, and analyzed for PO<sub>4</sub>-P. Phosphorus sorption/uptake (mg/m<sup>2</sup>/hr) was determined by dividing the change in PO<sub>4</sub>-P by the sediment core area (i.e., 3.08x10<sup>-4</sup> m<sup>2</sup>) and incubation time (i.e., 1 hr).

#### *Analysis of natural and anthropogenic disturbance regimes*

For the purposes of this study, floods were characterized as natural pulse disturbances (Lake 2000). Base flow discharge in the study stream was approximately 0.5 L/s during summer and fall (Chaffin et al. *in press*). Increased flow was classified as a ‘flood’ when discharge exceeded 8 L/s or flow required to mobilize a particle of 5.6-mm diameter (i.e.,

approximately 50% of the stream bed composition) by determining the shear velocity necessary to move a given sediment particle (Gordon et al. 2004) and relationships between discharge and velocity (Wollheim et al. 2001). Flood regime was characterized by several derived variables, including days-post-flood (number of days since a flood), maximum discharge within 2 months prior to sampling, and the cumulative number of floods (beginning May 2004) prior to a sampling event.

The presence of the abandoned arsenic mine was characterized as a press disturbance. The influence of press disturbances was analyzed by comparing reach-scale measures of structure and function. Increasing arsenic concentrations within the mine-influenced reach were used to assess how a ramp disturbance influences phosphorus uptake. Accordingly, the mine-influenced reach was separated into three, 30-m sub-reaches with increasing arsenic concentration. Average arsenic concentration and phosphorus uptake velocity were determined from replicate samples collected from three transects established within each sub-reach of the mine-influenced reach across all sampling periods.

### *Statistical analysis*

All statistical analysis was done using SAS (version 9.1 SAS Institute Inc., Cary, North Carolina, USA). Hydrologic stability was assessed by comparing the coefficients of variation for all hydrologic parameters. The coefficient of variation for each hydrologic parameter (e.g.,  $Q$ ,  $A_s/A$ ) was determined ( $n = 6$  for each variable) in each reach, and a paired t-test by hydrologic variable was completed in order to address directional trends in temporal variability. Sediment sorption characteristics in the reference and mine-influenced reaches were compared using two-way analysis of variance using reach (reference and mine-influenced) and treatment (live vs. killed) as main effects. Within the mine-influenced reach, the influence of arsenic concentrations on sediment phosphorus sorption capacity was

determined using one-way analysis of variance. Reach-scale comparisons of physical and chemical properties (e.g., stream geomorphology, solute transport parameters), biotic structure (e.g., organic matter standing stocks) and ecosystem function (e.g., uptake velocity and ecosystem metabolism), were done using paired t-tests. Relationships between structure, function, and responses to disturbance (e.g., flooding, arsenic gradients) were explored using linear regression models. Comparisons of regression parameter estimates between reaches were completed by assessing the interaction term between variables (Ott and Longnecker 2001).

## RESULTS

### *Physical, chemical, and hydrogeomorphic characteristics*

Across all sampling dates, average arsenic concentration within the reference reach was 8 µg/L (Table 1) and varied little spatially along the reach (3–26 µg/L, Figure 1). Average arsenic concentrations in the mine-influenced reach were more than 30 times the reference values (Table 1) and increased dramatically along the reach to approximately 600 µg/L (Figure 1). Average NO<sub>3</sub>-N concentrations were generally >500 µg/L in both reaches and significantly higher in the reference reach ( $P = 0.001$ , Table 1). In contrast to the relatively high NO<sub>3</sub>-N concentrations, PO<sub>4</sub>-P concentrations were at or below detection limits (<5 µg/L) throughout the study (Table 1).

Channel insolation was relatively constant in the reference reach, averaging <5% incident light, but did reach 20% in areas where the canopy thinned (Digital Appendix B). Insolation values at the head and base of the mine-influenced reach were comparable to the reference reach (~5%) where a normal canopy cover existed. However, adjacent to the tailings, percent insolation increased to over 80% due to lack of vegetation in areas near the mine tailings (Digital Appendix B).

No major differences in stream sediment particle distribution were observed between reaches at the beginning of the study (Digital Appendix C) when median particle size was approximately 3–4 mm. Over the period of the study, the median particle size did not significantly change in the reference reach, and proportions of particles within size classes remained relatively constant. In the mine-influenced reach, the proportion of fine sediments (<4 mm) declined by 20% across the study period, resulting in an increase in the median particle size from 4 to 12 mm (Digital Appendix C).

Stream discharge at the bottom of the mine-influenced reach determined from a flume varied from 1.5 L/s to >44 L/s (Figure 2). Highest discharge corresponded to hurricanes Frances (September 8, 2004), Ivan (September 17, 2004), Jeanne (September 28, 2004), and two flashfloods (October 13 and November 24, 2004). Average stream depth and velocity were numerically similar but differed significantly between reach types (Table 1). Lateral-inflow in the mine-influenced reach was more than double the reference reach ( $P = 0.032$ ). Channel cross-section area was similar in both reaches (Table 1) but discharge was higher in the mine-influenced reach throughout the study as a result of higher stream velocity (Table 1). Average absolute size of the transient storage zone ( $A_s$ ) was approximately 1.7 times greater in the reference reach, but mean values were not significantly different ( $P = 0.090$ , Table 1). When corrected for channel cross-section area ( $A$ ), normalized storage zone size ( $A_s/A$ ) was only 1.1 times greater in the reference reach (Table 1). The percent of median residence time spent in storage (i.e.,  $F_{med}$ ) varied from 9–58% and was significantly greater in the reference reach ( $P = 0.018$ , Table 1).

Hydrogeomorphic characteristics (Table 1) in the mine-influenced reach were significantly more temporally variable than in the reference reach ( $P = 0.016$ ). Coefficients of variation for all hydrogeomorphic variables varied from 14%–80 % and were, on average, 24% higher in the mine-influenced reach (51.7%) than the reference reach (41.7%).

The number of floods ( $Q > 8$  L/s) and magnitude of floods observed in each reach differed over the study. Interpolation of discharge in the reference reach from the flume at the base of the mine-influenced reach indicate that flood magnitude in the reference reach was approximately 46% lower than that in the mine-influenced reach ( $r^2 = 0.974$ ,  $P < 0.001$ ,  $n = 6$ ). Six floods occurred in the reference reach, two with a maximum discharge of 24 L/s. A total of nine floods occurred in the mine-influenced reach, two with a maximum discharge  $>44$  L/s. In general, flooding regime influenced transient storage zone characteristics similarly in both the reference and mine-influenced reaches (Digital Appendix D). Across both reaches  $A_s/A$  ( $r^2 = 0.698$ ,  $P < 0.001$ ) and  $F_{med}$  ( $r^2 = 0.726$ ,  $P < 0.001$ ) decreased over the course of the study with increasing cumulative number of floods (Digital Appendix D). In the mine-influenced reach, cumulative floods caused a rapid non-linear decline, whereas a linear decline was observed in the reference reach. Maximum discharge was not significantly related to transient storage zone characteristics in the reference reach. However, both  $A_s/A$  ( $r^2 = 0.667$ ,  $P = 0.44$ ) and  $F_{med}$  ( $r^2 = 0.745$ ,  $P = 0.027$ ) declined linearly with increasing flood intensity in the mine-influenced reach (Digital Appendix D). Relationships in both cases were also significant when data from the reference and mine-influenced reaches were combined. Transient storage zone characteristics were not significantly related to days-post-flood in either reach. However, a combined model indicates that  $A_s/A$  increased linearly with days-post-flood ( $r^2 = 0.470$ ,  $P = 0.014$ ).

#### *Large woody debris and benthic standing stocks*

Standing stocks of large woody debris were similar in the reference and mine-influenced portions of the stream (Table 1). While reach estimates of LWD biomass differed by  $<5\%$ , distribution of wood within the two reaches was very different. Debris dam frequency within the reference reach (8.33/100 m) was nearly three times that in the mine-influenced reach (3.33/100 m, Table 1). Average chlorophyll *a* standing crops were  $<1.52$

mg/m<sup>2</sup> in both reaches (Table 1) and did not differ significantly between reaches ( $P = 0.326$ ). Maximum epilithic chlorophyll *a* on bare rock was 62 mg/m<sup>2</sup> in the mine-influenced reach and 9 mg/m<sup>2</sup> in the reference reach just prior to the first major flood (i.e., hurricane Frances). Average FBOM standing stocks varied from 17–113 g/m<sup>2</sup> and were approximately two times lower in the mine-influenced reach than in the reference reach. Mean values did not differ significantly ( $P = 0.057$ , Table 1). Reach estimates of CPOM standing stocks reflected changes in leaf litter abundance. While CPOM standing stocks were always higher in the reference reach, mean values were not significantly different ( $P = 0.079$ , Table 1).

Following floods, temporal change was evident for some aspects of organic matter characteristics. No floods were observed in the 44 days prior to hurricane Frances, and chlorophyll *a* standing crops were 0.53 mg/m<sup>2</sup> and 6.79 mg/m<sup>2</sup> in the reference and mine-influenced reach respectively (Digital Appendix E). After hurricane Frances (September 8, 2004), chlorophyll *a* standing crops were extremely low (<0.01 mg/m<sup>2</sup>). Chlorophyll *a* then increased linearly with DPF in the reference ( $r^2 = 0.387$ ,  $P = 0.378$ ,  $n = 4$ ) and mine-influenced ( $r^2 = 0.960$ ,  $P = 0.020$ ,  $n = 4$ ) reaches, although they never reached pre-hurricane Frances levels in either reach. Maximum standing crops observed 17 to 25 days after hurricane Frances were on the order of a magnitude smaller than those observed prior to the hurricane. The FBOM in the reference reach increased significantly with DPF ( $r = 0.974$ ,  $P = 0.005$ ,  $n = 5$ ; Digital Appendix E). A similar trend observed in the mine-influenced reach was not statistically significant ( $r^2 = 0.063$ ,  $P = 0.684$ ,  $n = 5$ ) due primarily to a single outlier ( $r^2 = 0.709$ ,  $P = 0.158$  with the point removed).

#### *Sediment phosphorus sorption characteristics*

Phosphorus uptake on ‘live’ and ‘killed’ sediment cores from both reaches averaged 11 µg/m<sup>2</sup>/hr and were not significantly different ( $P = 0.581$ ,  $n = 31$ ) at concentrations similar

to those created by phosphorus additions to the stream. Sorption capacity estimates were therefore combined to compare differences between reaches. Sediment from the mine-influenced reach had 1.2 times greater potential to sorb phosphorus ( $P = 0.011$ , Table 2), and sediment from both reaches were able to account for  $\text{PO}_4\text{-P}$  uptake in the range of 9–11  $\text{mg/m}^2/\text{hr}$ .

Within the mine-influence reach, sediment sorption characteristics varied across a range of arsenic concentrations (Figure 3). No significant differences (Fishers LSD,  $P > 0.05$ ) were observed between the sorption capacity of sediments collected from near the head (263  $\mu\text{g/L As}$ ) and at the base (2822  $\mu\text{g/L As}$ ) of the mine-influenced reach. Sediment collected from middle region (823  $\mu\text{g/L}$ ) of the mine-influenced reach had significantly lower (Fishers LSD,  $P < 0.05$ ) phosphorus sorption potential.

#### *Ecosystem metabolism and phosphorus retention*

No detectable GPP was observed throughout the study in either reach. Across reaches, R varied from 1.3–5.6  $\text{g O}_2/\text{m}^2/\text{d}$  during the study. Average respiration was not significantly different ( $P = 0.116$ , Table 2) between reaches but was higher in the reference ( $4.4 \pm 0.4 \text{ g O}_2/\text{m}^2/\text{d}$ ) than the mine-influenced reach ( $3.3 \pm 0.5 \text{ g O}_2/\text{m}^2/\text{d}$ ). Ecosystem respiration was not tied to benthic standing stocks, and correlations with CPOM, FBOM, and chlorophyll *a* were not significant ( $P > 0.05$ ).

Over the 6-month study period, mean  $V_f$  for each reach was identical (0.027  $\text{mm/s}$ , Table 2) and not significantly different ( $P = 0.796$ ). At the same time,  $U$  ranged from 270–756  $\mu\text{g/m}^2/\text{hr}$ , and did not significantly differ between reaches ( $P = 0.796$ , Table 2). Because  $\text{PO}_4\text{-P}$  concentrations were always below detection limit,  $U$  was calculated as  $V_f$  multiplied by a constant background phosphorus concentration (i.e., 5  $\mu\text{g/L}$ ) and is thus statistically identical to  $V_f$ . Uptake velocities increased with decreasing transient storage ( $A_s/A$ ,  $r = -$

0.704;  $P = 0.011$ ,  $n = 12$ ) and  $F_{med}$  ( $r = -0.680$ ;  $P = 0.015$ ,  $n = 12$ ). Uptake velocities were also positively related to CPOM standing crops (Digital Appendix F) in the reference ( $r^2 = 0.906$ ,  $P = 0.013$ ,  $n = 5$ ) and mine-influenced ( $r^2 = 0.784$ ,  $P = 0.046$ ,  $n = 5$ ) reaches. Relationships between CPOM and  $V_f$  within reaches were not significantly different from each other ( $P > 0.05$ ). No relationships were observed between  $V_f$  and other standing stock estimates. Regression models, including both CPOM standing stock and DPF, explained 99% of the variance in  $V_f$  in the mine-influenced reach ( $r^2 = 0.989$ ,  $P = 0.023$ ) while DPF did not significantly add to the regression model in the reference reach.

Phosphorus uptake also varied across a gradient of arsenic concentrations. Average arsenic concentrations within sub-reaches in the mine-influenced reach ranged from 25–625  $\mu\text{g/L}$  (Figure 1), and arsenic concentrations always increased across the sub-reaches (i.e., from ‘low’ to ‘high’). Phosphorus uptake velocities calculated for the 30-m sub-reaches ranged from 0.009–0.091 mm/s. At high arsenic concentrations,  $V_f$  was always low; similarly, at low arsenic concentrations,  $V_f$  was generally high. Overall, a non-linear, hyperbolic model related  $V_f$  and arsenic in the mine-influenced reach ( $r^2 = 0.858$ ,  $P < 0.001$ ,  $n = 15$ , Figure 4).

## DISCUSSION

### *Ecosystem metabolism and phosphorus uptake*

Similar to other studies of forested headwater streams, no detectable GPP was observable, most likely due to light limitation (Mulholland et al. 2001). Although, recent studies (e.g., Hall and Tank 2005) have suggested that minor groundwater inputs may bias measures of ecosystem metabolism and potentially are a source of error in estimates in our study, measured respiration rates (1.3–5.4  $\text{gO}_2/\text{m}^2/\text{d}$ ) are similar to other values reported for headwater streams. In the eastern United States, respiration in headwater streams generally



varies from 1.5–6.9 gO<sub>2</sub>/m<sup>2</sup>/d (Mulholland et al. 1997, 2001, Webster et al. 2003). Unlike other studies that observed links between ecosystem metabolism and CPOM standing stocks (Hedin 1990), channel hydraulic conditions (Mulholland et al. 1997), or nutrient spiraling metrics (Hall and Tank 2003), significant relationships were not found among these variables in the present study.

Streams in the southern Appalachian Mountains are generally phosphorus limited (Webster et al. 1995) and the demand for PO<sub>4</sub>-P is higher than for NO<sub>3</sub>-N (Webster et al. 1991). Many studies have suggested (e.g., Davis and Minshall 1999, Valett et al. 2002) that  $V_f$  is an appropriate metric for biological comparisons of nutrient demand among systems because it accounts for hydrologic variation (e.g., stream depth and velocity). Uptake velocities observed in both the reference and mine-influenced reaches in this study are relatively close to many other reported values of  $V_f$  (e.g., 0.01–0.08 mm/s) for phosphorus limited streams (Mulholland et al. 1997, Davis and Minshall 1999, Sabater et al. 2000), but they were substantially lower than those reported for southern Appalachian streams of old-growth forest (Valett et al. 2002).

Although we are able to estimate phosphorus uptake using solute injections, we cannot isolate the mechanisms responsible because phosphorus may be sequestered by both biotic and abiotic processes. The role of sediments in abiotically retaining/sorbing phosphorus has been well established. Some studies have demonstrated that PO<sub>4</sub>-P uptake in streams is driven by abiotic factors (Meyer 1979) while other researchers have attributed it to biotic processes (Munn and Meyer 1990). Higher sorption potentials observed in the mine-influenced reach are most likely due to sorption characteristics of weathering products of arsenopyrite, including iron hydroxides (Dove and Rimstidt 1985) to which phosphorus is strongly adsorbed (Tadanier et al. 2002). Within a sediment depth of 5 cm, microcosm results suggest that sediment <4 mm could account for phosphorus uptake of approximately

4.6 mg/m<sup>2</sup>/hr in the reference and 4.4 mg/m<sup>2</sup>/hr in the mine-influenced reaches. Calculated  $U$  for phosphorus uptake based on solute releases (using geometric mean of corrected plateau concentrations) varied from 2.4–4.4 mg/m<sup>2</sup>/hr in the reference and 2.7–5.3 mg/m<sup>2</sup>/hr in the mine-influenced reaches, both within the range of uptake potentials derived from sediment microcosm experiments.

Abiotic removal of phosphorus should also be enhanced by increased water residence time as described by measures of transient storage. Previous studies have shown that nutrient uptake increased with storage size (i.e.,  $A_s/A$ ; Mulholland et al. 1997). Uptake velocities in this study, however, decreased significantly with increasing transient storage extent (e.g.,  $A_s/A$ ), suggesting that biotic or abiotic uptake occurring within the storage zone is of relatively little influence. Lack of evident pools, eddies, or backwaters make it unlikely that transient storage is associated with surface features and suggests that phosphorus uptake occurs in association with the benthic surface rather than the hyporheic zone (i.e., transient storage zone). Although abiotic sorption may be able to account for all phosphorus uptake, Mulholland et al. (1997) suggested that sorption comes quickly to isotopic equilibrium and cannot represent a long-term sink for added solutes.

Results from this study suggest that phosphorus retention occurs in association with the benthic surface, a notion supported by significant correlations between benthic CPOM standing stocks and phosphorus uptake. Mulholland et al. (1985) observed strong relationships between leaf litter standing stock and phosphorus uptake where the highest uptake was observed when CPOM standing stocks were greatest. Similarly, in this study 78–91% of the variation in  $V_f$  was explained by CPOM standing stocks, and  $V_f$  in both reaches was greatest when CPOM standing stocks was maximal. Nutrient uptake is therefore probably influenced by both biotic and abiotic processes not in the storage zone but rather

associated with benthic compartments in the stream channel, including microbial biofilms on leaf material and/or sediments of the streambed.

#### *Press disturbance influence on reach structure and function*

Several studies have shown that functional measures (e.g., ecosystem metabolism, leaf decomposition) are sensitive to disturbance (e.g., Gessner and Chauvet 2002, Chaffin et al. *in press*), although functional responses to toxic stressors may differ. Chaffin et al. (*in press*) explored how elevated arsenic concentrations influence leaf decomposition at the Brinton Arsenic Mine and found that elevated arsenic concentrations significantly decreased leaf breakdown rates. However, results from this study suggest that not all reach-scale processes are equally altered by elevated arsenic concentrations.

Macroinvertebrates, especially shredders (i.e., organisms that process CPOM; *sensu* Merritt and Cummins 1984), play an important role as detrital processors (Wallace et al. 1982) and appear to be adversely affected by elevated levels of arsenic, resulting in decreased leaf breakdown rates in the mine-influenced reach (Chaffin et al. *in press*). However, other functional characteristics, such as ecosystem metabolism and nutrient uptake, are primarily controlled by microbial communities (Hall and Meyer 1998). Results from studies at the Brinton Arsenic Mine demonstrate that processes controlled by metazoans are drastically altered (Chaffin et al. *in press*), while our study suggests that those controlled by microbial communities are less affected, even at arsenic concentrations nearly 70 times greater than those set as chronic toxic levels for metazoans (USEPA 2002). This conclusion is supported by prior studies of arsenic influence on microbial assemblages and functioning. Speir et al. (1999) demonstrated that the presence of elevated levels of arsenic did not inhibit microbial respiration or decrease microbial biomass in soils. Further, Turpeinen et al. (2004) found

that, while exposure to high levels of arsenic resulted in reduced microbial diversity, soil metabolic activity was not altered.

#### *Pulse disturbance influence on ecosystem structure and function*

Pulse disturbances (e.g., floods) have long been recognized as a dominant factor controlling stream ecosystem structure and function (e.g., Resh et al. 1988). Floods appear to play a significant role in structuring the physical and hydrologic characteristics of the reference and mine-influenced reach at the Brinton Arsenic Mine. Although floods appear to change sediment particle size distribution, organize the extent of the transient storage zone, and OM standing stocks, they did not have a similar effect on most ecosystem functions (e.g., phosphorus uptake and ecosystem metabolism) quantified in this study. The influence of floods on phosphorus uptake were not apparent when floods were considered as discrete disturbance regime.

#### *Interactions between press and pulse disturbances*

Similar to other studies (e.g., Parkyn and Collier 2004, Ross et al. 2004), interaction between pulse and press disturbances was evident for both structural and functional characteristics. Results from comparison of sediment particle size distribution (Digital Appendix C) suggest that the mine-influenced reach was less resistant to pulse disturbances (i.e., flooding) than the reference reach. The decrease in resistance to pulse disturbances in the mine-influenced reach is most likely due to the lack of debris dams (Bilby and Likens 1980).

The decreased resistance to floods was also apparent in the increased temporal variation in hydrogeomorphic characteristics in the mine-influenced reach. Consequently, significant relationships between flood regime and hydrogeomorphic characteristics (Digital

Appendix D) were observed in the mine-influenced reach but not in the reference reach. These patterns suggest that the mine-influenced reach, under the influence of an active press disturbance, was more susceptible to flood (i.e., pulse) disturbances.

Multiple linear regression models indicate that floods did influence phosphorus uptake in the mine-influenced reach but had no influence on phosphorus uptake in the reference reach. This interaction suggests that in order to understand phosphorus uptake dynamics in an ecosystem exposed to a press disturbance, relationships with additional disturbance regimes such as pulse disturbances (i.e., floods) need to be considered. Phosphorus uptake was highest directly after a flood and may be due to an influx of new sediment from mine tailings and higher abiotic uptake on open sorption sites of imported sediments (Meyer 1979).

#### *Ramp disturbance influence on phosphorus uptake*

Due to the wide range of arsenic concentrations observed in this study, a reach-scale approach may not capture the spatial variability of the disturbance regime within the mine-influenced area. We, therefore, employed a sub-reach analysis within the mine-influenced reach and examined phosphorus uptake across a gradient of arsenic concentrations to determine if a ramp disturbance regime (i.e., gradient of arsenic concentrations) was a more appropriate descriptor of arsenic influence on phosphorus uptake.

A recent study by Speir et al. (1999) demonstrated that the relationship between phosphatase activity and arsenic in soils was best described by a hyperbolic decline. At the same time, they observed no negative effects of arsenic on either microbial respiration or biomass. As a result, Speir et al. (1999) summarized that the decline in phosphatase activity conformed to Michaelis-Menton (M-M) kinetics and was a response to competitive inhibition of phosphatase activity by arsenic due to the chemical similarities between arsenate and

phosphate. Arsenate, the dominant form of arsenic in aquatic ecosystems, is a molecular analog to phosphate (Oremland and Stolz 2003, Mkandawire et al. 2004) and other research with plants has suggested that arsenate competes with phosphorus for the same uptake carriers. Competition between phosphate and arsenate may explain reduction of phosphorus uptake when arsenic is present (Mkandawire et al. 2004).

Nutrient uptake in streams has also been shown to conform to M-M kinetics (Dodds et al. 2002, Earl 2004) where  $U$  increases asymptotically to a maximum with increasing nutrient concentration. Because spiraling metrics ( $V_f$  and  $U$ ) are mathematically related, applying the M-M model to  $U$  also dictates how  $V_f$  will respond to increasing nutrient concentration (Earl 2004). Uptake velocity is thus described by a non-linear, hyperbolic decline with increasing nutrient concentration:

$$V_f = \frac{U_{\max}}{K_m + C} \quad (2)$$

where  $U_{\max}$  = maximum uptake,  $K_m$  = half-saturation constant, and  $C$  = nutrient concentration (Earl 2004). In this study,  $\text{PO}_4\text{-P}$  concentrations were always at or below detection limits and represented as a constant ( $5 \mu\text{g/L}$ ). For a given stream condition,  $U_{\max}$  and  $V_f$  are constants. Since background  $\text{PO}_4\text{-P}$  concentration was expressed as a constant for calculation purposes,  $V_f$  should be relatively constant throughout the study. However, because previous research has suggested that arsenic may competitively inhibit microbial activity, chronically elevated arsenic concentrations may exert a stronger influence on phosphorus uptake characteristics within the mine-influence reach than variation in ecosystem attributes such as organic matter standing stocks.

Within the mine-influenced reach, the sub-reach analysis of phosphorus uptake provides a range of  $V_f$  across a gradient of arsenic concentrations. A M-M model for  $V_f$  that takes into the account the presence of a competitive inhibitor (i.e., arsenate) could be used to assess if arsenic is behaving as a competitive inhibitor of microbial phosphorus uptake. If

arsenic is serving as a competitive inhibitor of phosphorus uptake,  $K_m$  for  $V_f$  increases by a factor of:

$$1 + \frac{I}{K_I} \quad (3)$$

where  $I$  = concentration of the inhibitor and  $K_I$  = the dissociation constant for the enzyme-inhibitor complex (Campbell 1999). The M-M model for  $V_f$  can then be adjusted to account for the presence of a competitive inhibitor:

$$V_f = \frac{U_{\max}}{K_m \left( 1 + \frac{I}{K_I} \right) + C} \quad (4)$$

In order to fit this model to our experimental data, we assumed that the half saturation constants were similar (i.e.,  $K_m = K_I$ ) and that the availability of phosphorus was constant (i.e., 5  $\mu\text{g/L}$ ). Based on these assumptions,  $V_f$  should decline hyperbolically with increasing arsenic concentration and our data are well represented by a mod of this type (Figure 4).

Model output estimates derived from a non-linear fit of equation 4 using Sigma Plot 9.0 of M-M parameters were  $U_{\max} = 31 \text{ mg/m}^2/\text{hr}$  and  $K_m = 113 \text{ }\mu\text{g/L}$ . Wen et al. (1997) observed that  $K_m$  values for phosphorus uptake in 37 species of freshwater algae ranged from 1–867  $\mu\text{g/L}$ .

Although we were able to estimate M-M parameters that were similar to other studies, the primary result of this analysis was that the data was best described by our derived model of competitive inhibition of phosphorus uptake typical of enzyme kinetics and observed in other studies (e.g., Speir et al. 1999). However, the decline in phosphorus uptake could also be due to a downstream decrease in abiotic sorption potential as a result of increased quantities of arsenic sorbed to sediment surfaces. Due to the similarities between arsenate and phosphate, arsenate competes for the same binding sites on sediment although not as strongly as phosphate (Rubinos et al. 2003). However, no significant decline in sediment

sorption potential was observed across a wide gradient of arsenic concentrations, suggesting that chronically elevated levels of arsenic are not altering abiotic sorption characteristics in this stream. Further, we observed that low  $V_f$  values occurred in conjunction with elevated arsenic concentrations, whether these concentrations occurred in sub-reaches characterized by low, medium, or high arsenic concentrations. Finally, we observed strong correlations between phosphorus uptake and CPOM standing stocks but not with increased interaction with the transient storage zone. Together, these results suggest that arsenic may not alter microbial phosphorus uptake as a toxin, but rather as a chemical inhibitor.

### *Ecosystem implications*

At the ecosystem scale, several studies have suggested that measures of ecosystem function are useful parameters in assessing the functional integrity of streams (e.g., Gessner and Chauvet 2002). Results presented here provide an example of how multiple disturbance regimes alter ecosystem structure and function across both temporal and spatial scales. Results of this study, in conjunction with Chaffin et al. (*in press*), suggest that in order to assess the influences of a toxin at an ecosystem scale, a range of functional characteristics need to be addressed. Chaffin et al. (*in press*) emphasized a lack of resistance in the macroinvertebrate community and its implications for organic matter processing. Here we emphasize that processes associated with microbial assemblages (i.e., ecosystem metabolism) appear to be highly resistant to elevated concentrations of arsenic. Additionally, toxins such as arsenic may not alter biotic activity due to their toxic characteristics as was demonstrated in Chaffin et al. (*in press*) but rather as a result of its chemical characteristics as highlighted in this study at the ecosystem scale and in other studies as well (e.g., Speir et al. 1999).

Results from this study along with others (Parkyn and Collier 2004, Ross et al. 2004) continue to highlight the fact that disturbance regimes interact to alter ecosystem structure



and function. Therefore, in light of Clements' (2000) call for ecotoxicological studies to take place at the ecosystem scale, such studies should consider not only contaminant influences but rather place its implications within the extant disturbance regime generated from both natural and anthropogenic sources.

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Table 1. Characterization of chemical, hydrologic, and organic matter standing stock features of the reference and mine-influenced reaches at the Brinton Arsenic Mine. P values derived from paired t-tests are given in the final column (bold values <0.05). Dashes indicate no reach-scale replication

Stream reach characteristics	Reference reach	Mine-influenced reach	P
<b>Chemical characteristics<sup>†</sup></b>			
As ( $\mu\text{g/L}$ )	$8 \pm 1$	$254 \pm 39$	<b>0.004</b>
PO <sub>4</sub> -P ( $\mu\text{g/L}$ )	<5	<5	--
NO <sub>3</sub> -N ( $\mu\text{g/L}$ )	$781 \pm 63$	$497 \pm 59$	<b>0.001</b>
<b>Hydrologic characteristics<sup>†</sup></b>			
Discharge ( $Q$ , L/s)	$1.7 \pm 0.4$	$2.4 \pm 0.8$	0.101
Lateral inflow ( $Q_L$ , L/s/100 m)	$0.4 \pm 0.1$	$0.9 \pm 0.2$	<b>0.032</b>
Wetted width ( $w$ , m)	$0.66 \pm 0.04$	$0.74 \pm 0.06$	<b>0.013</b>
Depth ( $z$ , m)	$0.05 \pm 0.01$	$0.04 \pm 0.01$	<b>0.040</b>
Stream x.s. area ( $A$ , m <sup>2</sup> )	$0.03 \pm 0.01$	$0.03 \pm 0.01$	0.083
Velocity ( $u$ , m/s)	$0.06 \pm 0.01$	$0.09 \pm 0.01$	<b>0.006</b>
Storage zone x.s. area ( $A_s$ , m <sup>2</sup> )	$0.05 \pm 0.01$	$0.03 \pm 0.01$	0.090
Exchange coefficient ( $\alpha$ , s <sup>-1</sup> )	$0.0005 \pm 0.0001$	$0.0008 \pm 0.0003$	0.302
Normalized storage zone area ( $A_s/A$ , m <sup>2</sup> /m <sup>2</sup> ) <sup>‡</sup>	$1.72 \pm 0.03$	$1.56 \pm 0.08$	0.704
$F_{med}$ (%) <sup>‡</sup>	$40.6 \pm 0.2$	$24.6 \pm 0.4$	<b>0.018</b>
<b>Organic matter standing stocks</b>			
Wood mass (kg/m <sup>2</sup> )	0.85	0.82	--
Debris dam frequency (no./100 m)	8.33	3.33	--
Chlorophyll $a$ (mg/m <sup>2</sup> ) <sup>††</sup>	$0.15 \pm 0.96$	$1.52 \pm 1.32$	0.326
Epilithic OM (g/m <sup>2</sup> ) <sup>††</sup>	$0.17 \pm 0.03$	$1.22 \pm 0.80$	0.250
FBOM (g/m <sup>2</sup> ) <sup>††</sup>	$68 \pm 13$	$37 \pm 8$	0.057
CPOM (g/m <sup>2</sup> ) <sup>††</sup>	$116.6 \pm 35.5$	$54.0 \pm 13.8$	0.079

Notes: <sup>†</sup>Data are means  $\pm$  SE for six reach averages from July–December 2004.

<sup>‡</sup>Means and SE were calculated on square-root transformed data; tabular values were back-transformed.

<sup>††</sup>Data are means  $\pm$  SE for five reach average values from August–December 2004.



Table 2. Ecosystem respiration, phosphorus uptake, and sediment assays in the reference and mine-influenced reaches at Brinton Arsenic Mine. Bold P values indicate significant differences between reaches (i.e.,  $P < 0.05$ ).

Steam reach characteristics	Reference reach	Mine-influenced reach	P
Ecosystem metabolism <sup>†</sup>			
Gross primary production ( <i>GPP</i> , g O <sub>2</sub> /m <sup>2</sup> /d)	BDL	BDL	--
Ecosystem respiration ( <i>R</i> , g O <sub>2</sub> /m <sup>2</sup> /d)	4.4 ± 0.4	3.3 ± 0.5	0.116
Phosphorus uptake metrics <sup>‡</sup>			
Uptake velocity ( <i>V<sub>f</sub></i> , mm/s)	0.027 ± 0.004	0.027 ± 0.003	0.796
Areal uptake ( <i>U</i> , µg/m <sup>2</sup> /hr)	477 ± 68	492 ± 52	0.796
Sediment sorption characteristics <sup>††</sup>			
50 µg/L enrichment ( mg/m <sup>2</sup> /hr)	9 ± 0	11 ± 0	<b>0.011</b>

Notes: <sup>†</sup>Data are means ± SE for five reach averages from August–December 2004. P values derived from paired t-tests given in the final column.

<sup>‡</sup>Data are means ± SE for six reach averages from July–December 2004. P values derived from paired t-tests given in the final column.

<sup>††</sup>Data are means ± SE of both live and killed sediment assays from the reference and mine-influenced reaches. P values derived from t-tests given in the final column.

BDL = below detection limit.

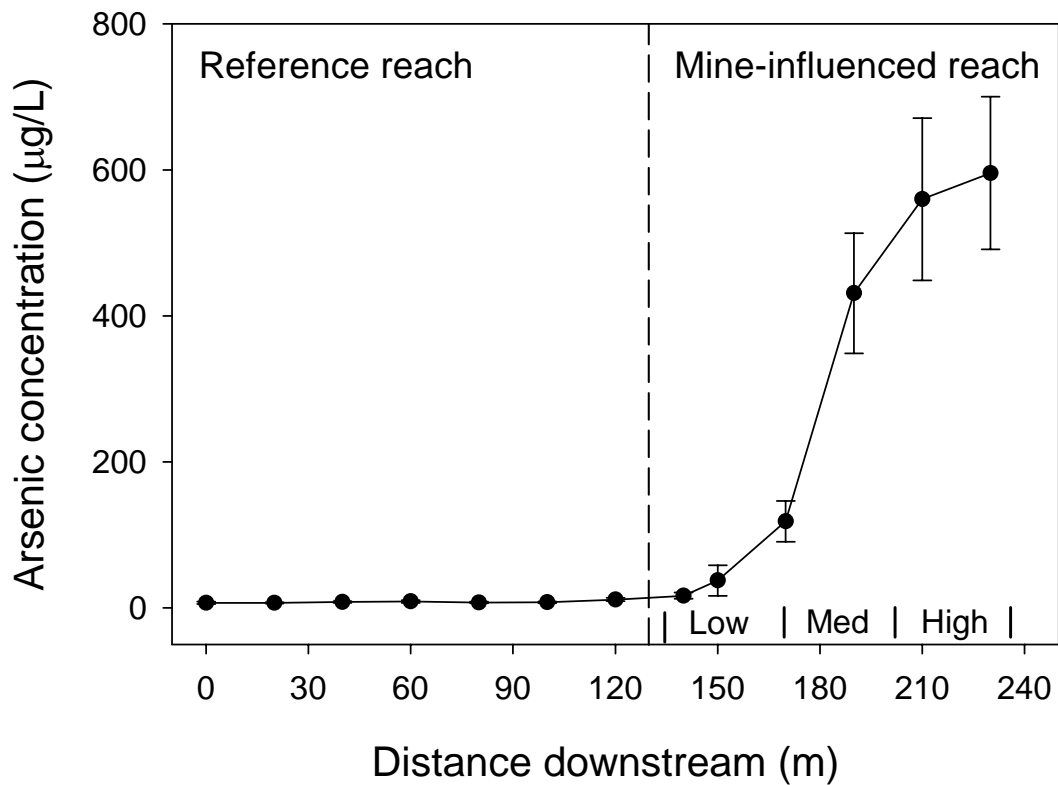


Figure 1. Arsenic concentrations (mean  $\pm$  SE) from July–December 2004 for the reference and mine-influenced reaches at the Brinton Arsenic Mine. Data are means  $\pm$  SE for six monthly values at each site (error bars may be within point symbols). Monthly values were determined from triplicate samples at each location. Sub-reaches within the mine-influenced reach are identified as ‘low’, ‘medium’, and ‘high’ in reference to average ambient arsenic concentration.

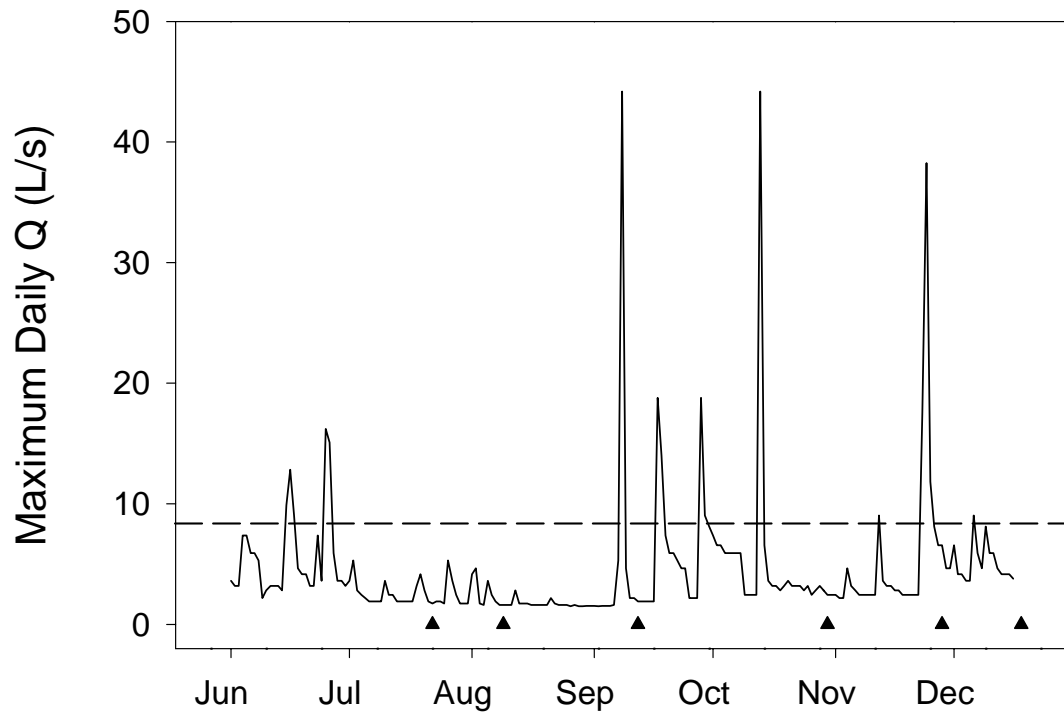


Figure 2. Maximum daily discharge determined from a Tracon fiberglass H-flume at the bottom of the mine-influenced reach at Brinton Arsenic Mine. Triangles indicate when sampling of stream structure and function occurred. Floods occurred when maximum daily discharge exceeded 8 L/s (i.e., above the dashed line).

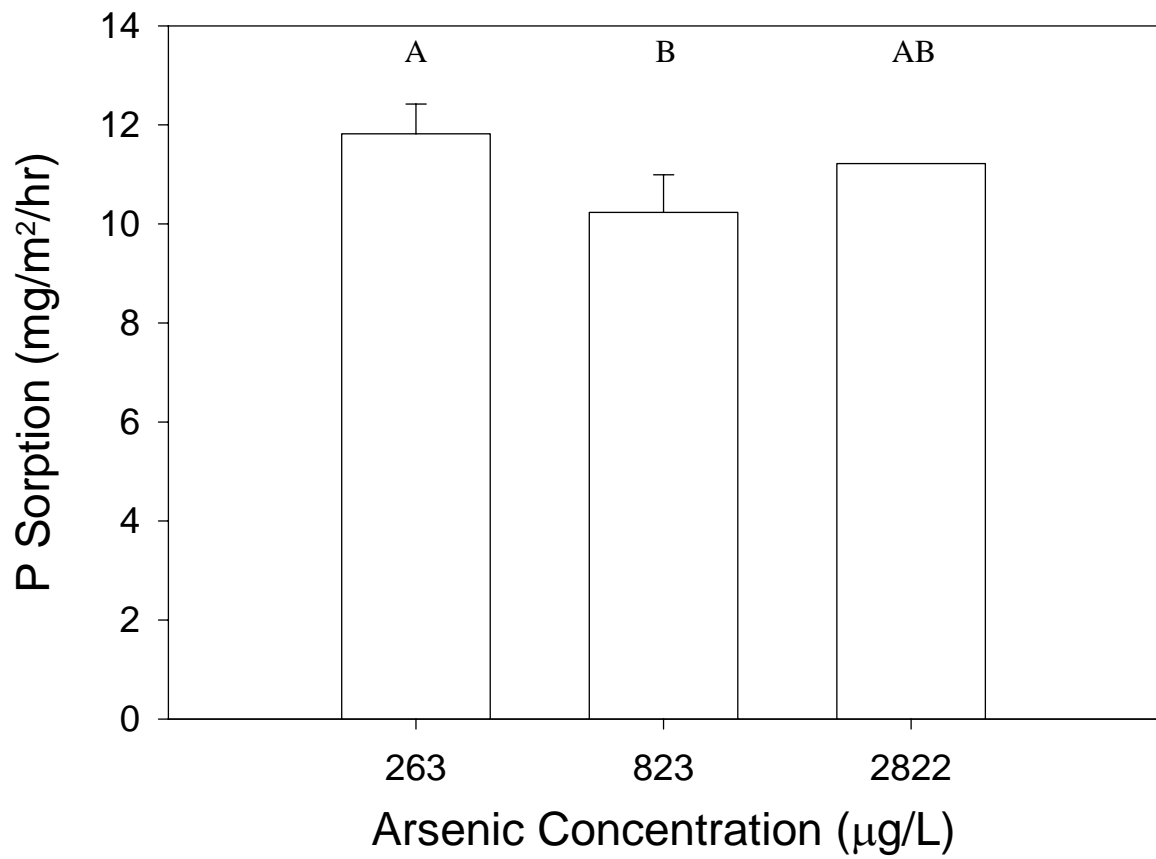


Figure 3. Comparison of abiotic sediment sorption potential across a gradient of arsenic concentrations in the mine-influenced reach. Letters indicate significant groupings ( $P < 0.05$ ).

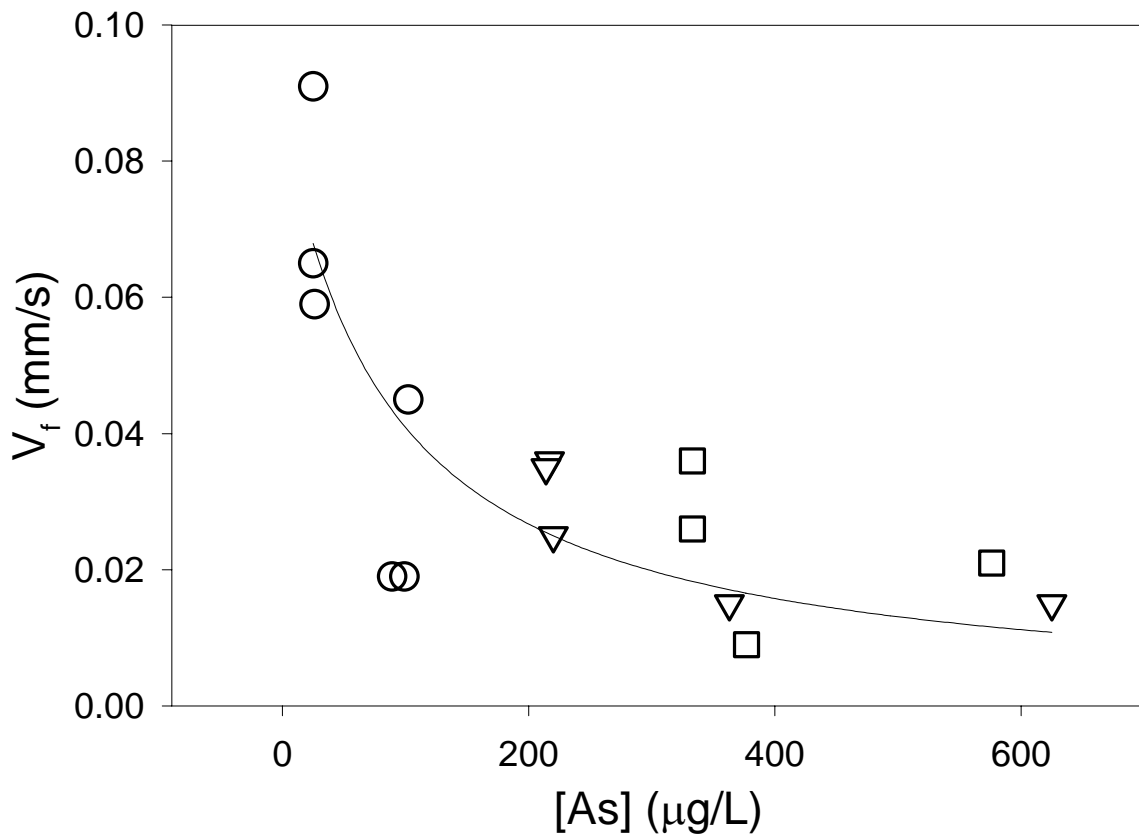
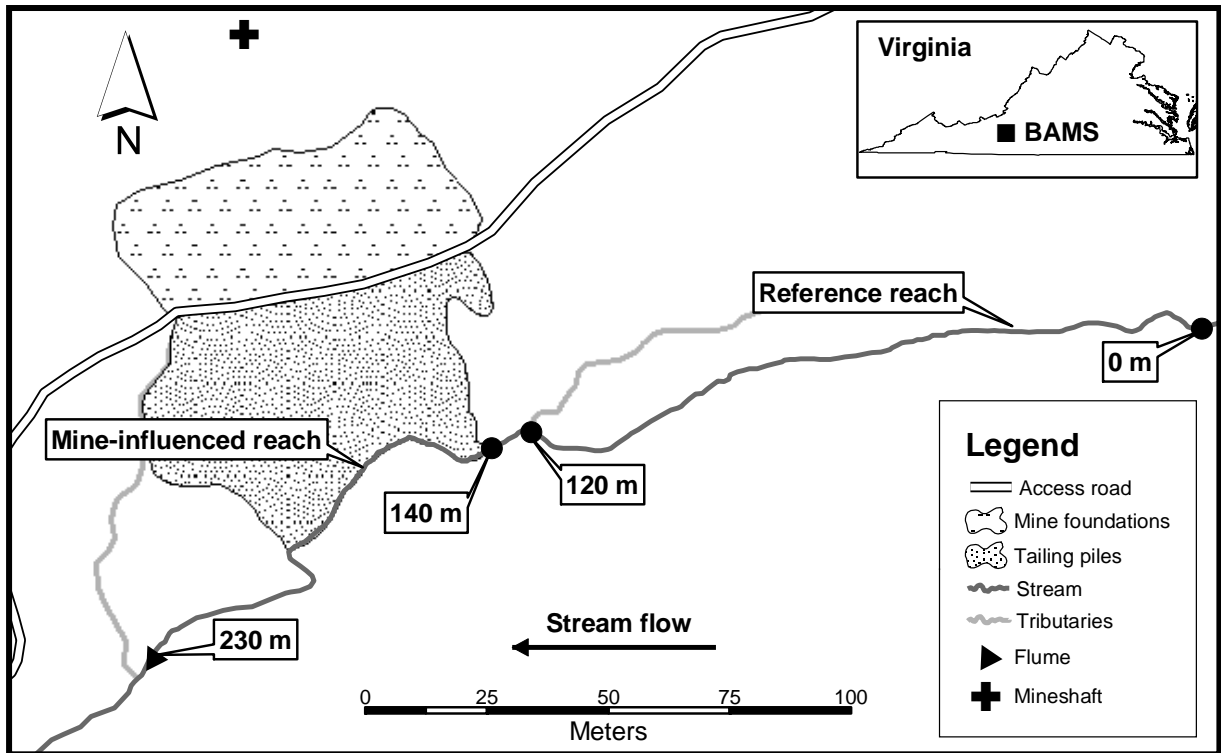
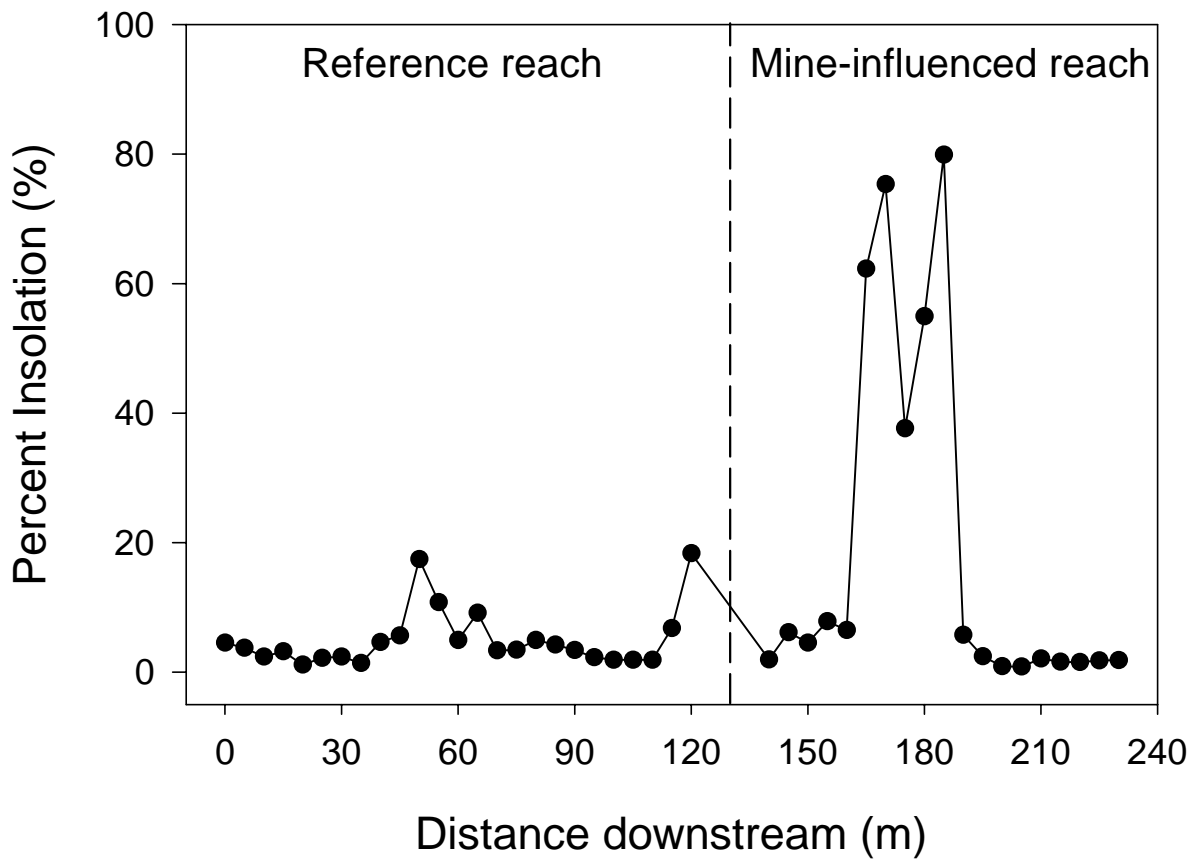


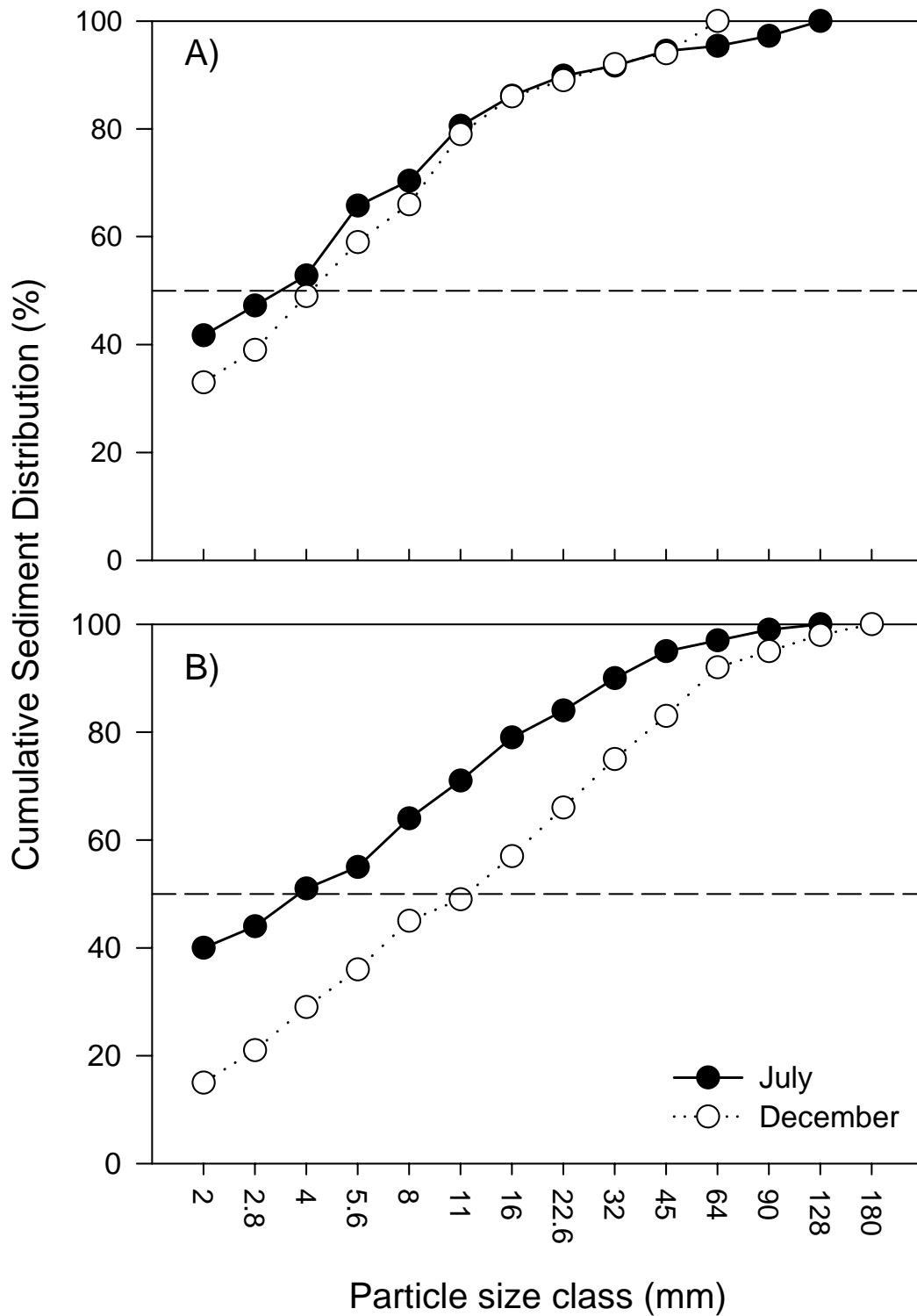
Figure 4. Relationship between phosphorus uptake efficiency ( $V_f$ , mm/s) and mean arsenic ( $\mu\text{g/L}$ ) in the mine-influenced reach at the Brinton Arsenic Mine ( $r^2 = 0.858$ ,  $P < 0.001$ ,  $n = 15$ ).  $V_f$  and mean arsenic concentrations were calculated from 30-m sub-reaches representing low (open circles), medium (open triangles), and high arsenic (open squares) concentrations (Figure 2).



Digital Appendix A. Site location map for Brinton Arsenic Mine. The reference reach extends from 0–120 m downstream and ends directly above the tributary. The mine-influenced reach begins at the top of the tailing piles and extends 90 m downstream.

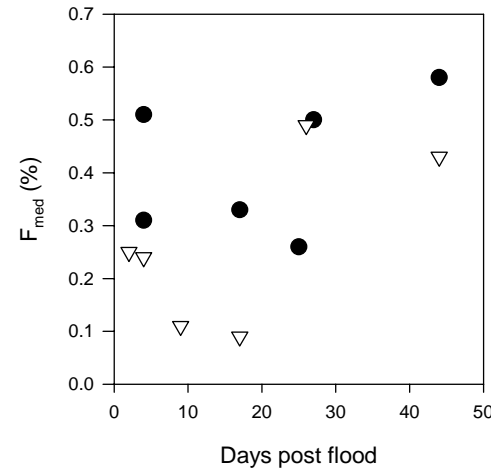
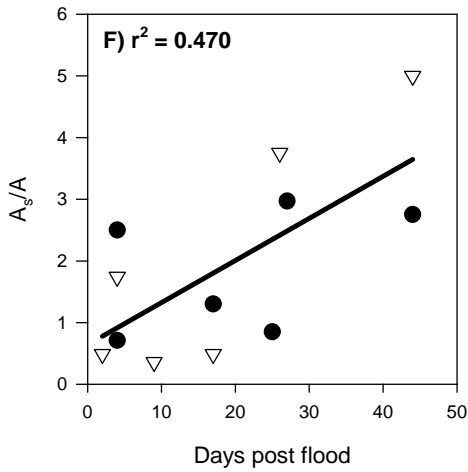
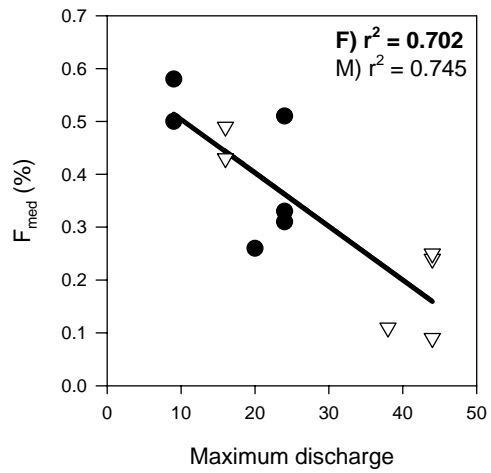
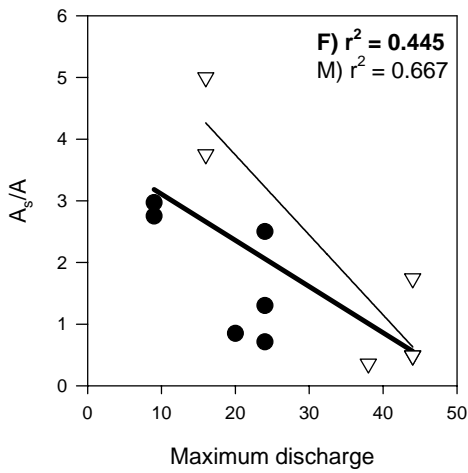
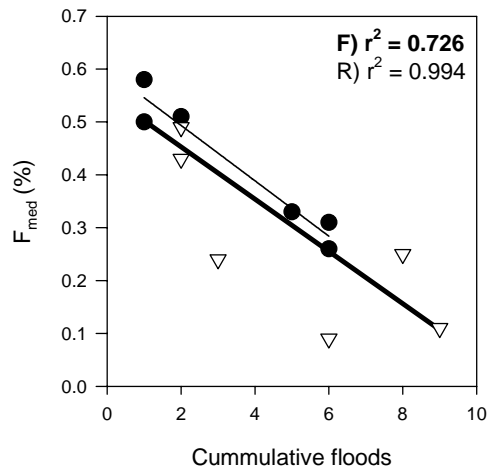
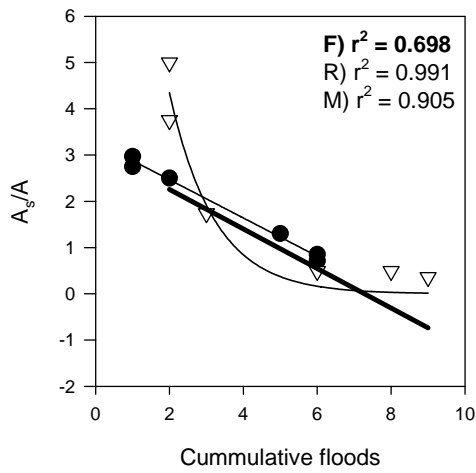


Digital Appendix B. Percent insolation for the reference and mine-influenced reaches at the Brinton Arsenic Mine. Data represent the mean of a 1-min integration at noon prior to leaf fall.

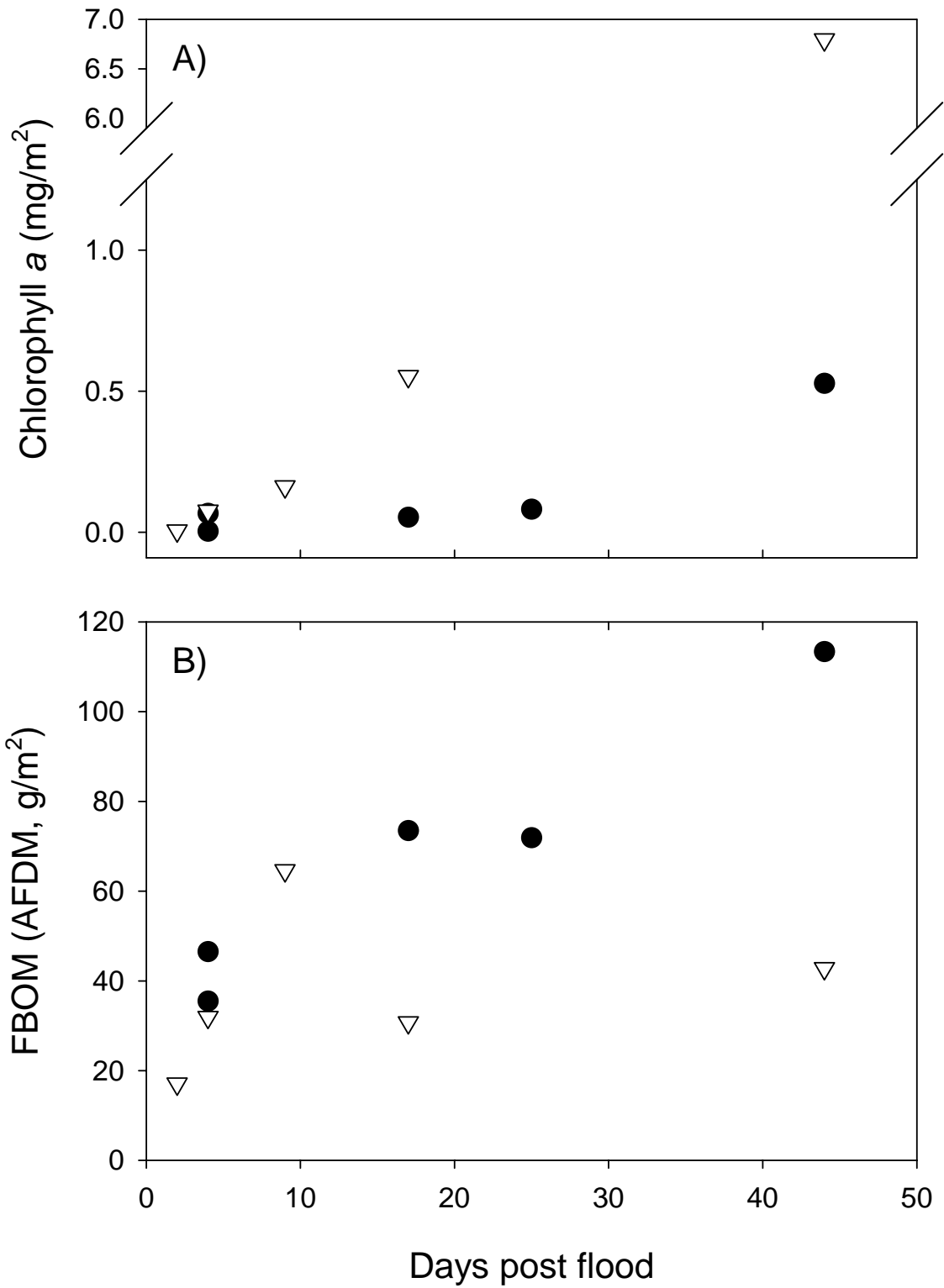


Digital Appendix C. Cumulative distribution for sediment particle sizes in the reference (A) and mine-influenced (B) reaches at the Brinton Arsenic Mine at the start (July) and end (December) of the study. Distributions are derived from 100 random samples taken from each reach.

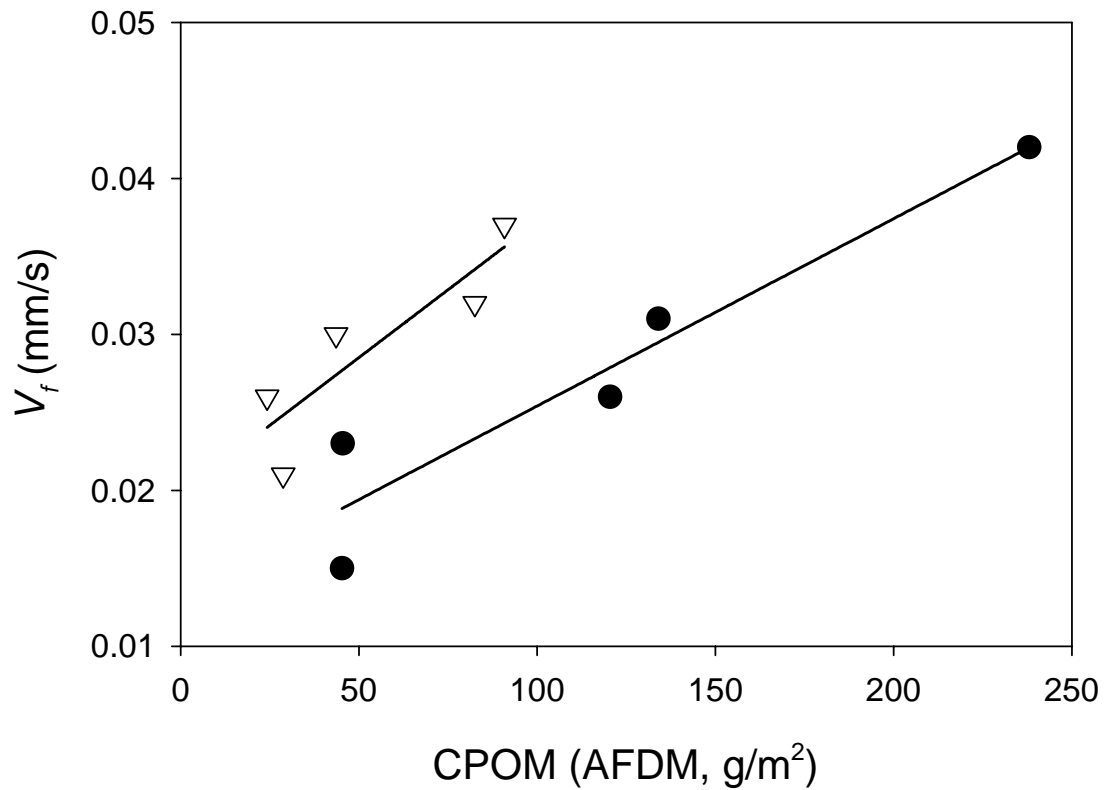




Digital Appendix D. Influence of flooding on transient storage in the Brinton Arsenic Mine in the reference (closed circles) and mine-influenced (open triangles) reaches. Regressions indicate significant correlations (i.e.,  $P < 0.05$ ). Bold regression coefficients and heavy lines represent models (F) that include data from both reference and mine-influenced reaches. Non-bold regression coefficients represent a reduced model including data from either the reference (R) or mine-influenced (M) reaches.



Digital Appendix E. Relationship between (A) chlorophyll *a* standing crop (mg/m<sup>2</sup>) and (B) FBOM (g/m<sup>2</sup>) and days post flood (DPF) in the reference (closed circles) and mine-influenced (open triangles) reaches at the Brinton Arsenic Mine study site.



Digital Appendix F. Relationship between phosphorus uptake velocity ( $V_f$ , mm/s) and CPOM ( $\text{g/m}^2$ ) in the reference (closed circles) and mine-influenced reach (open triangles) at Brinton Arsenic Mine. Regression models are:  $y = mx + b$  for the reference reach ( $r^2 = 0.906$ ,  $P = 0.013$ ,  $n = 5$ ),  $y_1 = m_1x + b_1$  for the mine-influenced reach ( $r^2 = 0.784$ ,  $P = 0.046$ ,  $n = 5$ ), and  $y_{\text{tot}} = m_{\text{tot}}x + b_{\text{tot}}$  for the combined model ( $r^2 = 0.538$ ,  $P = 0.016$ ,  $n = 10$ ).

# CURRICULUM VITA

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## EDUCATION

**M.S., Virginia Polytechnic Institute and State University, Blacksburg, VA** 2005

- Biological Science: Aquatic Ecology

**B.S., University of Wisconsin, Superior—Superior, WI** 1998-2003

- Double Major: Biology and Political Science
- Double Minor: Chemistry and Geology

## RESEARCH EXPERIENCE

**Virginia Tech Stream Team—Blacksburg, VA** 2003-Present

*Graduate Research Assistant*

- Part of a team of researchers including eleven graduate students and three professors conducting research on headwater streams throughout Virginia and North Carolina

**Center for Limnology, University of Wisconsin, Madison—Madison, WI** 2002-2003

*Undergraduate Research*

- Studied spatial heterogeneity of soluble reactive phosphorus in streams
- Researched nutrient retention based on stream sediment characteristics
- Performed sample analysis and methods development on an array of analytical equipment
- Conducted extensive field sampling and data collection

**Environmental Protection Agency, ORD—Duluth, MN** Summer, 2001

*Physical Environmental Science Technician*

- Participated in Lake Michigan coastal wetland REMAP research
- Researched nutrient dynamics of wetlands and tributaries
- Conducted extensive field sampling and data collection
- Performed sample analysis and methods development on an array of analytical equipment

## TEACHING EXPERIENCE

- Graduate Teaching Assistant: Freshwater Ecology, Virginia Tech 2004
- Graduate Teaching Assistant: General Biology, Virginia Tech 2003-2004
- Teaching Assistant: General Chemistry, University Wisconsin Superior 2001

## WORK EXPERIENCE

- Natural Resources Engineering—Duluth, MN** 2002-2003  
*Geographic Information Systems Technician*
- Collected and analyzed environmental data
  - Interfaced with government and private agencies
  - Extensively analyzed environmental data using GIS
- Enbridge Energy Company, Inc.—Duluth, MN** 2001-2002  
*Environmental Analyst Student Intern*
- Assisted in the development of a pipeline risk assessment model
  - Liaised with government and private agencies
  - Collected, interpreted, and analyzed environmental data

## PROFESSIONAL SOCIETY MEMBERSHIPS

- North American Benthological Society
- Society of Environmental Toxicology and Chemistry

## PUBLICATIONS

- Lottig, N.R., H.M. Valett, J.R. Webster and M.E. Schreiber. Influence of multiple disturbances on stream structure and function. (*In prep*).
- Lottig, N.R. and E.H. Stanley. Benthic sediment influence on phosphorus concentrations in a headwater stream. (*In prep*).

## PRESENTATIONS

- Lottig, N.R., H.M. Valett, J.R. Webster and M.E. Schreiber. 2005. Influence of multiple disturbance regimes on ecosystem structure and function. North American Benthological Society.
- Lottig, N.R. and H.M. Valett. 2004. Influence of elevated arsenic concentrations on nutrient retention in a headwater stream. North American Benthological Society.
- Orr, C.H., K.L. Rogers, N.R. Lottig and E.H. Stanley. 2004. Phosphorus uptake, channel geometry, and sediment size before and after small dam removal in Boulder Creek, Wisconsin. North American Benthological Society.
- Schreiber, M.E., H.M. Valett, M. Gentry, B. Brown, J. Brookshire and N.R. Lottig. 2004. Geologic Controls on arsenic discharge to a mine-impacted headwater stream. Geological Society of America.
- Lottig, N.R., E.H. Stanley. 2003. Benthic sediment influence on spatial heterogeneity of soluble reactive phosphorus concentrations in Boulder Creek. North American Benthological Society.
- Lottig, N.R. and E.H. Stanley. 2003. Soluble reactive phosphorus dynamics of boulder creek. University of Wisconsin—Superior Research Symposium.
- Lottig, N.R., K.D. Benton and M.J. Miller. 2003. A comparison of the incidence of disease on two coral reefs in Belize, C.A. University of Wisconsin—Superior Research Symposium.
- Lottig, N.R. 2002. Nutrient dynamics of Boulder Creek. (Invited Presentation). Department of Limnology and Oceanography. University Wisconsin Madison.

## POSTERS

- Reinhardt, L.P., N.R. Lottig, H.M. Valett and M.E. Schreiber. 2005. Influence of elevated arsenic concentrations on microbial respiration and phosphorus uptake in a headwater stream. North American Benthological Society.
- Lottig, N.R., H.M. Valett, J.R. Webster and M.E. Schreiber. 2005. Influence of multiple disturbance regimes on ecosystem structure and function. 2<sup>nd</sup> Annual Virginia Tech Biology Department Research Symposium.
- Reinhardt, L.P., N.R. Lottig, H.M. Valett and M.E. Schreiber. 2005. Influence of elevated levels of arsenic on microbial activity in a headwater stream. 2<sup>nd</sup> Annual Virginia Tech Biology Department Research Symposium.
- Lottig, N.R. and E.H. Stanley. 2003. Benthic sediment influence on phosphorus concentrations in a headwater stream. 1<sup>st</sup> Annual Virginia Tech Biology Department Research Symposium.