Table 1
General chemical and biological parameters at the two stream sites used for experiments in the study, Slippery Rock Creek (at AMD impacted Station 65) and Wolf Creek (unimpacted reference stream)[a]

Parameter	Slippery Rock Creek	Wolf Creek
Discharge ($m^3\ s^{-1}$)	0.1–3.5	0.4–6.7
Width at low flow (m)	4.3	6.7
pH	6.3 (5.5–6.6)	7.7 (6.5–8.1)
Alkalinity (mg $CaCO_3\ l^{-1}$)	11.7 (4.1–40.0)	76.2 (24.9–149.9)
Acidity (mg $CaCO_3\ l^{-1}$)	8.3 (3.4–13.6)	2.5 (0–8.3)
Sulfate (mg l^{-1})	255 (245-260)	70 (60-79)
Soluble Fe (mg l^{-1})	0.40 (0.20–1.88)[b]	0.04 (bd–0.19)[c]
Soluble Mn (mg l^{-1})	4.10 (bd–9.50)[b,c]	0.08 (bd–0.1)[c]
Soluble Al (mg l^{-1})	0.10 (bd–0.55)[b,c]	0.14 (bd–0.40)[b,c]
Soluble Zn (mg l^{-1})	0.07 (bd–0.12)[b,c]	0.04 (bd–0.10)[c]
Sediment Fe (g kg^{-1})	51.0 (20.6–99.0)	44.9 (13.7–114.9)
Sediment Mn (g kg^{-1})	4.1 (0.4–16.4)	0.8 (0.3–6.7)
Sediment Al (g kg^{-1})	27.9 (23.8–30.5)	37.0 (27.3–63.2)
Sediment Zn (g kg^{-1})	0.3 (0.1–1.2)	0.2 (0.2–0.7)
Macroinvertebrate density (number m^{-1})	4 (0–54)	897 (369–1764)
Marcoinvertebrate species richness	2 (0–6)	27 (15–34)
Epilithic periphyton density (number cm^{-1})	1.7×10^5 (2.2×10^5–6.9×10^5)	1.2×10^6 (1.1×10^5–1.2×10^7)
Epilithic periphyton species richness	21 (14–31)	23 (18–37)

[a] Values are medians and ranges based on quarterly sampling from 1995 to 2000. Sediment concentrations are for the clay fraction of sediment.
[b] Concentration of maximum dissolved metal exceeds either USEPA's or Pennsylvania's continuous water quality standard for freshwater.
[c] bd, below detection limits.

order tributary of Slippery Rock Creek and of good water quality (Table 1). It is approximately 30 km downstream of the headwater area and drains a mix of woodland and agricultural areas.

3. Materials and methods

3.1. Substratum experiment

To examine the effects of AMD precipitate on epilithic invertebrates and periphyton, 30.5×30.5×4.0 cm wooden frames with 1.0 cm^2 open mesh, plastic bottoms were filled with cobble-sized substrata obtained from a quarry. To obtain substratum treatments with an AMD coating, frames filled with either sandstone or limestone cobble were placed in a highly impacted, untreated 2nd order AMD stream in the headwaters of Slippery Rock Creek for 3 weeks to accumulate a precipitate coating approximately 0.5 mm thick (estimated visually by scraping the substratum). This stream contains few to no macroinvertebrates and a visual inspection of the cobbles in the trays indicated no invertebrates were present after the 3-week exposure. These trays were then transferred in water-filled containers to a circumneutral stream with good water quality, Wolf Creek, on 13 October 1998. On the same date, substratum trays containing either clean, washed limestone or clean, washed sandstone treatments were also placed in Wolf Creek. Five replicates of the four substratum treatments, clean (control) sandstone, clean (control) limestone, AMD coated sandstone and AMD coated limestone, were located randomly in a long riffle of the stream for a 4-week exposure. Metals were sampled from the substratum in the trays before and after being placed in Wolf Creek. Substratum in the trays were sampled for macroinvertebrates and periphyton at the end of the 4-week exposure.

To examine changes in metal concentrations on the substrata during the 4-week exposure, randomly selected cobbles from trays of each treatment ($n = 5$) were analyzed for metals before and after being placed into Wolf Creek. The entire surface of each rock was scrubbed with a hard bristle, plastic-brush using a 2% HNO_3 solution. Material scrubbed and rinsed from the cobble was digested for metal analyses using nitric acid (digestion procedure 303E, APHA, 1998). Concentrations of Al, Fe, Mn, and Zn were determined using a Perkin Elmer Plasma 400, inductively coupled plasma spectrophotometer (ICP; preliminary studies have shown these to be the most abundant metals). Accuracy and precision of metal analyses were determined by running duplicates, blanks, standards and spiked samples at a frequency of approximately 1 per 10 normal samples. Samples outside of limits of acceptability (10%) were rerun (APHA, 1998).

Invertebrates from each tray were sampled by placing a 500-μm mesh net directly downstream of the tray and rubbing invertebrates off each cobble by hand into the net. Invertebrate samples were preserved in 70% ethanol and individuals identified to genus using primarily Peckarsky et al. (1990). Prior to invertebrate sampling, one randomly selected cobble was taken from three of the five trays for each treatment to sample periphyton (i.e. three replicates per treatment for periphyton samples). Periphyton was scraped from a 5.3 cm^2 area on the rocks using a SG-92 sampler following the protocols of the USGS National Water Quality Assessment monitoring program (Porter et al., 1993). Algal samples were preserved with Lugol's Solution. Each algal sample was homogenized with a Ten Broeck tissue grinder. To estimate periphyton density, 250–900 algal units were counted from random fields in Palmer Counting Cells. Soft algae were identified in this count with diatoms lumped into one taxonomic category. The sample was then digested in concentrated nitric acid, rinsed, dried onto coverslips, mounted in NAPHRAX, and 300 cleared diatom valves identified to species at 1000× magnification. The proportion of diatom species in this count was used to estimate their abundance in the count for periphyton density. Primary references for identification of algae were, Krammer and Lange-Bertalot (1986, 1988, 1991a, b), and Whitford and Schumacher (1984).

Concentrations for each metal on substrata before and after the experiment were compared using a split-plot analysis of variance (ANOVA) with substratum treatment as the whole plot and sample date (before or after) as the subplot. When factor effects were significant, means were separated using Fisher's protected least significant difference (FPLSD) values using the appropriate standard errors for whole or subplots (Peterson, 1985). Differences among substratum treatments in mean density, species diversity, and relative abundance of individual taxa for macroinvertebrates and periphyton were determined by a completely randomized, single factor, ANOVA. For all tests, the level of significance used was $P<0.05$. When needed, logarithmic or square-root transformations were used to normalize the data prior to ANOVA. The effect of treatments on the entire assemblage of macroinvertebrates or periphyton, respectively, was tested by canonical correspondence analysis (CCA) using nominal variables in the environmental data set to define treatments. A significant affect of treatment on the whole assemblage was determined by the *F*-ratio for a Monte Carlo permutation test (ter Baak and Šmilauer, 1998). The level of significance used was $P<0.05$ with 199 permutations.

3.2. Aqueous exposure of caddisflies

Uptake of metals from stream water by hydropsychid caddisflies was measured in a 4th order stream affected by AMD (Slippery Rock Creek at Station 65) and the unimpacted, reference stream (Wolf Creek) on 16–22 November 2000. Live hydropsychid caddisflies (mostly *Hydropsyche* sp.) were collected from Wolf Creek. Half of the collected individuals were brought back to the laboratory in a cooler where they were killed by bubbling nitrogen gas into the water at 25 °C overnight. To create respective live and dead treatments, either 10 live or 10 dead caddisflies were placed into cages. Cages were constructed of 15 cm long sections of 6×6 cm square plastic pipe glued to bricks. Open ends of the cages were covered by 500 μm mesh netting and a few small rocks from Wolf Creek were placed into the cages as substrata for the caddisflies. Cages were placed 30–40 cm deep in riffles of Wolf Creek (current velocity 9–27 cm s^{-1}) and in a section of Slippery Rock Creek affected by mine drainage (Station 65, current speed 12–24 cm s^{-1}), with mesh ends perpendicular to the flow. There were seven replicate cages of live and dead caddisflies, respectively, in each of the two stream sites. Cages were retrieved after 5 days and the number of live and dead individuals counted in situ for the live cages. Caddisflies were rinsed with distilled water, placed into plastic petri dishes and frozen at −80 °C. In the laboratory, caddisflies were freeze dried using a Labconco Freezone 4.5 freeze drier, weighed, and digested for metals analyses using nitric acid (APHA, 1998). Concentrations of Fe, Al, Mn, Zn, Cd and Pb were determined with an ICP as previously described. The same laboratory procedure was used to determine whole-body concentrations of metals for 10 randomly selected live caddisflies collected in Wolf Creek at the start of the experiment. Whole-body concentrations for each metal in the cage treatments were compared using a split-plot ANOVA with stream site (reference or AMD) as the whole plot and caddisfly condition (live or dead) as the subplot. For all tests, the level of significance used was $P<0.05$. When needed, logarithmic or square-root transformations were used to normalize the data prior to ANOVA.

At the start and end of the caddisfly exposure at the two sites, alkalinity, acidity, conductivity, pH, temperature, dissolved oxygen, and current velocity were measured in the field (APHA, 1998). In addition, 200-ml water samples were filtered through 0.45 μm filters to determine concentrations of dissolved Fe, Al, Mn, Pb, Cd, and Zn. Filters were digested using nitric acid to determine concentrations of the above metals in seston at the two sites. Metals for samples were analyzed by ICP with quality assurance protocols as described above.

4. Results

4.1. Substratum experiment

Iron was the most abundant metal on the rocks for all substrata treatments with values on AMD substrata being at least an order of magnitude higher than for Al, Mn or Zn (Fig.1). Iron concentrations on both sandstone and limestone were significantly higher on AMD substrata than control substrata, and there was a significant interaction between substrata treatment and change in concentrations during the 4-week exposure in Wolf Creek (Fig. 1A). Iron concentration did not change significantly on either the sandstone or limestone control substrata but significantly decreased on both AMD substrata. Aluminum concentrations were significantly different among substrata treatments and were higher on AMD sandstone than control sandstone substrata. There was a significant interaction between substrata treatment and the changes in Al concentrations, with all treatments losing aluminum during the exposure except sandstone control (Fig. 1B). Concentrations of manganese and zinc on the rocks were quite a bit lower than for Fe and Al, with all values less than 0.01 mg cm^{-2}. Changes in Mn concentrations on the rocks were also significantly affected by the substrata treatment. Unlike the other metals, control substrata treatments significantly gained Mn during the experiment, but there was not a significant increase on the AMD treatments which had higher initial Mn concentrations (Fig. 1C). Zinc concentrations on the substrata were relatively low, and there were no significant main affects of treatments or change during exposure. Although there was a significant interaction between substrata type and concentrations of Zn before and after exposure, there were no consistent trends in the changes (Fig. 1D).

After the 4-week exposure in Wolf Creek, mean macroinvertebrate densities were not significantly different among substrata treatments, although they were slightly higher on control treatments (Fig. 2). Shannon diversity values for macroinvertebrates varied from 1.0 to 1.4 and were not significantly different among treatments (Table 2). Taxonomic composition of the macroinvertebrates on the four substrata treatments was similar, with the stonefly, *Taeniopteryx* sp., and the mayfly, *Isonychia* sp., dominating the assemblages. Each of the five most abundant macroinvertebrate taxa did not differ significantly in their relative abundance

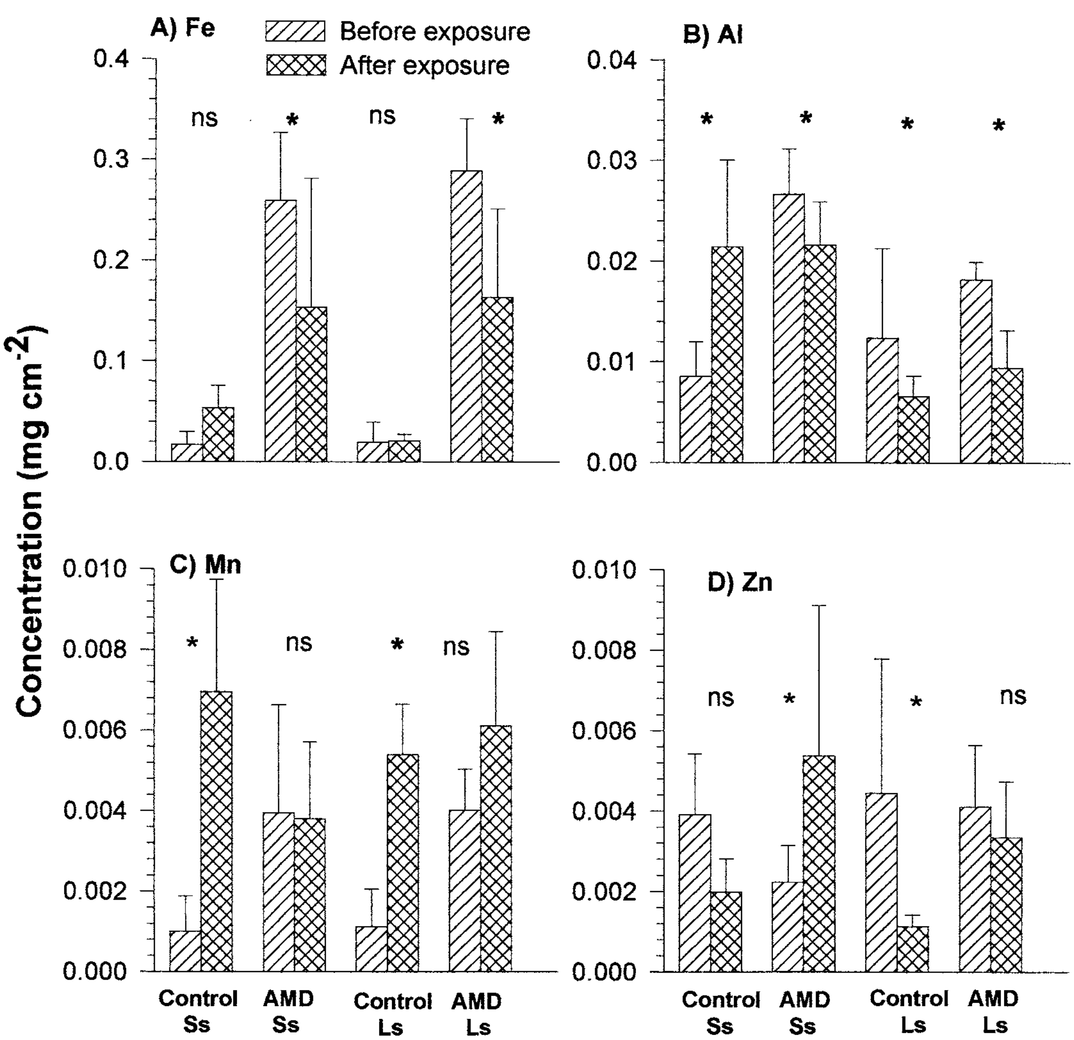

Fig. 1. Mean concentrations of Fe, Mn, Al and Zn on four substrata treatments before and after exposure in an unimpacted reference stream, Wolf Creek. Asterisks indicate a significant change ($P<0.05$) in concentration during the exposure within each treatment. Error bars are 1 SE, $n=5$. Ss, sandstone, Ls, limestone.

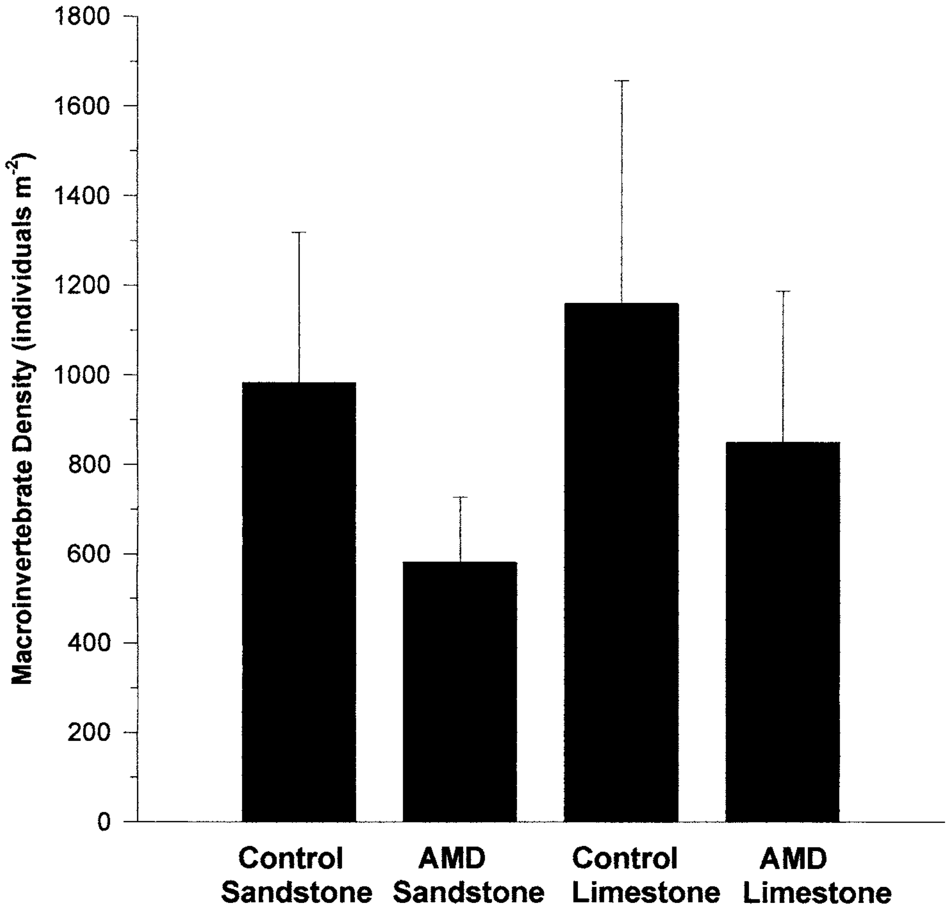

Fig. 2. Mean density of macroinvertebrates on four substrata treatments after a 4-week exposure in an unimpacted reference stream, Wolf Creek. Error bars are 1 SE, $n=5$.

Table 2
Taxonomic composition, as percent, for the most abundant macroinvertebrate taxa and Shannon Diversity values on four substrata treatments[a]

	Control sandstone	AMD sandstone	Control limestone	AMD limestone
Genus				
Taeniopteryx	45.3 (11.7)	31.8 (9.3)	39.2 (12.3)	31.7 (8.5)
Isonychia	14.2 (4.9)	36.7 (10.1)	24.9 (5.3)	40.9 (6.6)
Stenonema	7.9 (3.7)	15.3 (4.3)	16.6 (4.8)	9.4 (3.7)
Cheumatopsyche	5.1 (2.4)	3.4 (1.8)	3.2 (1.2)	3.5 (1.5)
Caenis	0.6 (0.4)	4.7 (3.6)	6.6 (4.7)	9.4 (7.7)
Diversity				
Shannon Diversity	1.0 (0.3)	1.3 (0.1)	1.4 (0.2)	1.3 (0.1)

[a] Values are means (1 SE), $n=5$.

among the treatments, respectively (Table 2). Multivariate analysis (CCA) of entire macroinvertebrate assemblages on substrata in each tray indicated no significant difference among treatments based on the permutation test.

Mean periphyton densities were about 40% higher on the substrata coated with AMD, however there was considerable variability among replicates within treatments, and mean densities on four substrata treatments were not significantly different (Fig. 3). Mean diversity values of periphyton assemblages varied from 3.1 to 3.4 for the four substrata and also were not significantly different (Table 3). Relative abundance of taxa in the periphyton was very similar among all the treatments. The flora was dominated by diatoms typically found on natural substrata in Wolf Creek such as *Nitzschia dissipata*, *Navicula gregaria* and *Navicula cryptocephala*. None of the six most abundant periphyton taxa were significantly different in their relative abundance among the four substrata treatments, respectively (Table 3).

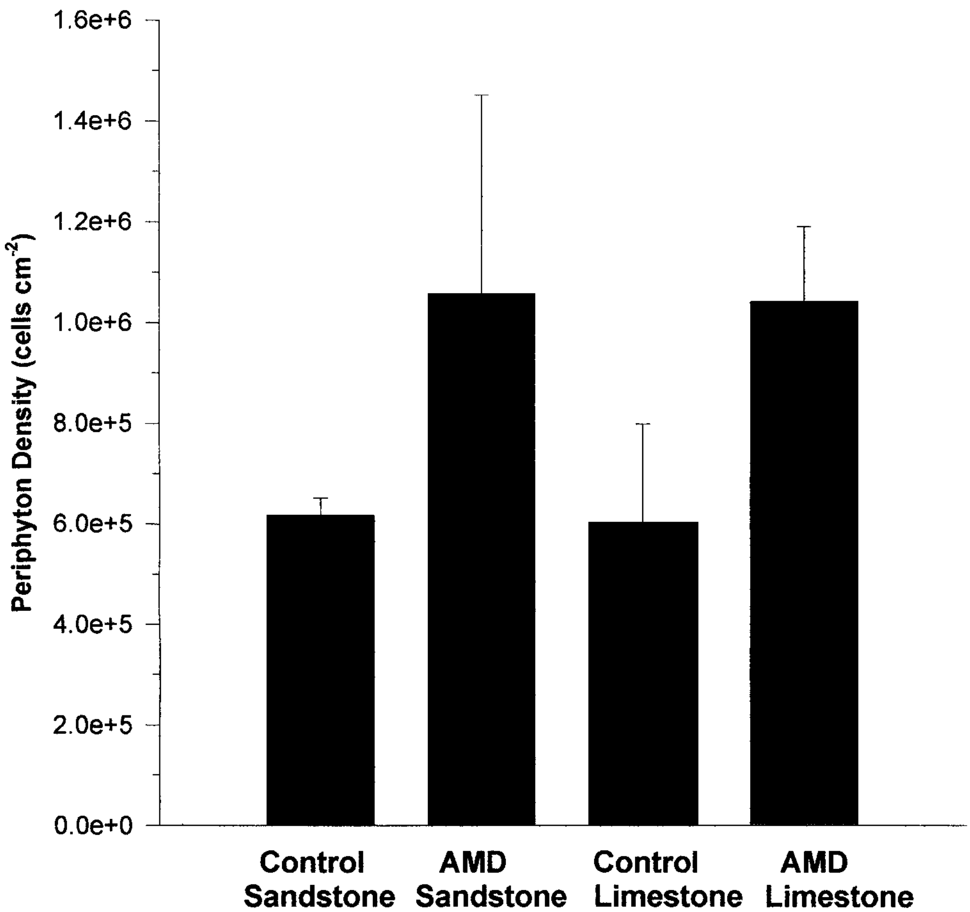

Fig. 3. Mean density of periphyton on four substrata treatments after a 4-week exposure in an unimpacted reference stream, Wolf Creek. Error bars are 1 SE, $n=3$.

Table 3
Taxonomic composition, as percent, for the most abundant periphyton taxa and Shannon Diversity on four substrata treatments[a]

	Control sandstone	AMD sandstone	Control limestone	AMD limestone
Genus				
Nitzschia dissipata	25.9 (1.2)	28.9 (2.6)	30.2 (1.4)	34.8 (2.3)
Navicula gregaria	19.6 (1.2)	22.0 (2.9)	19.6 (2.9)	21.1 (3.4)
Navicula cryptocephala	9.6 (1.2)	9.4 (1.2)	9.9 (1.1)	5.9 (1.2)
Navicula minuscula	8.7 (1.9)	7.9 (2.6)	6.1 (1.1)	9.5 (1.4)
Navicula tripunctata	5.8 (1.3)	4.6 (0.8)	6.8 (1.3)	4.6 (0.7)
Navicula cryptotenella	2.1 (0.2)	2.9 (0.7)	2.5 (0.4)	2.5 (0.5)
Diversity				
Shannon Diversity	3.2 (0.2)	3.4 (0.2)	3.2 (0.1)	3.1 (0.3)

[a] Values are means (1 SE), $n=3$.

Periphyton assemblages as a whole were not significantly different among treatments based on the CCA permutation test.

4.2. Aqueous exposure of caddisflies

During the 5-day caddisfly exposure, Wolf Creek had a higher pH and alkalinity, and was lower in the conductivity and acidity than the AMD affected site in Slippery Rock Creek. Temperature at the two sites was about the same and dissolved oxygen was slightly higher in Wolf Creek (Table 4). Concentrations of Fe and Mn dissolved in the water and in the seston were one to two orders of magnitude higher at the Slippery Rock Creek site than in Wolf Creek, but concentrations were only slightly higher for Zn, Pb, and Cd (Table 5).

In Wolf Creek, 91.4% (SE = 3.4) of the caddisflies in the live treatment survived during the 5-day exposure,

Table 4
Ranges of chemical and physical characteristics in Slippery Rock Creek (at AMD impacted Station 65) and Wolf Creek (unimpacted reference stream) during 5-day exposure of caddisflies

Parameter	Wolf Creek	Slippery Rock Creek
pH	8.3–8.7	6.7–6.8
Alkalinity (mg l^{-1})	98.0–110.0	15–25
Acidity (mg l^{-1})	0.0	8–12
Temperature (C)	4.2	4.7
Dissolved oxygen (mg l^{-1})	15.0	11.0
Conductivity (μS cm^{-1})	430	690

Table 5
Ranges of concentrations for dissolved metals and metals in seston (>0.45 micrometers) in Slippery Rock Creek (at AMD impacted Station 65) and Wolf Creek (unimpacted reference stream) during 5-day exposure of caddisflies[a]

Metal Species	Wolf Creek	Slippery Rock Creek
Dissolved Fe	0.05–0.06	0.87–0.93
Dissolved Mn	0.02–0.03	3.00–2.90
Dissolved Al	0.15–0.13	0.17–0.17
Dissolved Zn	0.01–0.01	0.01–0.01
Dissolved Pb	0.17–0.18	0.25–0.26
Dissolved Cd	bd[a]	0.02–0.02
Seston Fe	0.54–0.54	2.76–3.04
Seston Mn	0.04–0.04	5.80–5.90
Seston Al	3.48–4.70	3.40–3.52
Seston Zn	bd[a]	0.04–0.12
Seston Pb	0.34–0.34	0.50–0.54
Seston Cd	0.02–0.02	0.04–0.04

[a] bd, below detection limits. All values are mg l^{-1}.

which was significantly higher than the 79.0% (SE = 5.2) survival in Slippery Rock Creek (t-test, $P<0.05$). Concentrations of Pb were below detection limits for all caddisfly samples. The combined concentration of the metals measured (Fe, Al, Mn, Zn, Cd) in the caddisflies was significantly different between sites and between live and dead treatments, but there was not a significant interaction between the two factors (Fig. 4A). Whole-body concentrations of most individual metals were higher in caddisflies placed in the AMD site than in the reference stream, with concentrations of Fe and Al being one to two orders of magnitude greater than for the other measured metals (Fig. 4). Iron was significantly greater in the AMD site and in dead caddisflies than for the reference stream and live caddisflies, respectively. Both Al and Mn concentrations were significantly greater in dead caddisfly bodies, but there was not a significant affect of stream site. There were no significant effects of stream site or live/dead treatment for concentrations of Cd or Zn in the caddisfly bodies (Fig. 4). Concentrations of all the above metals in the live caddisflies exposed in Wolf Creek were very similar to concentrations found in the ambient caddisflies selected at random from the stream (Table 6; Fig. 4).

5. Discussion

The results suggest that AMD precipitate on hard substrata in coal mining impacted streams has the potential to be lost if the chemical influence of the aqueous AMD discharge is completely treated. Iron and Al are the most abundant heavy metals in mine drainage discharges into Slippery Rock Creek and therefore it was not surprising that they were the most abundant metals precipitated onto the substratum surface in the trays. The entire surface of each limestone and sandstone cobble was covered with approximately a 0.5 mm, adhering layer of yellow/orange precipitate, which was reduced but still visible after 4 weeks in the reference stream. There was very little, to no, loose, unconsolidated floc of AMD precipitate associated with the substrata in the trays, probably because it would be removed by the relatively high current velocities. Concentrations for all metals were similar on the two substratum types (sandstone and limestone) within each treatment, although significant interaction terms indicate that the sandstone substrata often accumulated more metals when exposed to AMD. It was thought the dissolution of carbonate from the limestone would increase the pH at the surface and potentially increase precipitation of metals. However, the surface of our sandstone cobbles (glacial deposit origin) was rougher than the limestone (Vanport formation) and possibly may have provided more surface area for precipitates to form. The low pH and high sulfate concentrations at the AMD site used to create the precipitate treatment, and the yellow/orange color of the AMD coating on the cobbles indicate that the precipitate was mostly composed of iron oxyhydroxides with adsorbed sulfates that bind heavy metals (Hedin et al., 1994; Rose and Ghazi, 1997; Webster et al., 1998). Rose and Ghazi (1997) found that when these types of precipitates are placed in more alkaline conditions, sulfate and its associated heavy metals desorb, but that the iron oxyhyroxide minerals of the precipitate remain insoluble. Conditions were different in our study in that we were examining release of the precipitate from the substrata and not its complete dissolution, and exposures were for 5 weeks in a fast current rather than 24 h on a laboratory shaker. Nevertheless, the significant reductions in Fe and Al concentration on substrata coated with AMD in circumneutral Wolf Creek in our study could be related to the desorption of sulfate, which may destabilize the oxyhydroxides enough to release them from the rock surface. The mostly uniform loss of precipitate from the entire surface (top and bottom) of the cobbles indicated that loss was primarily through chemical dissolution

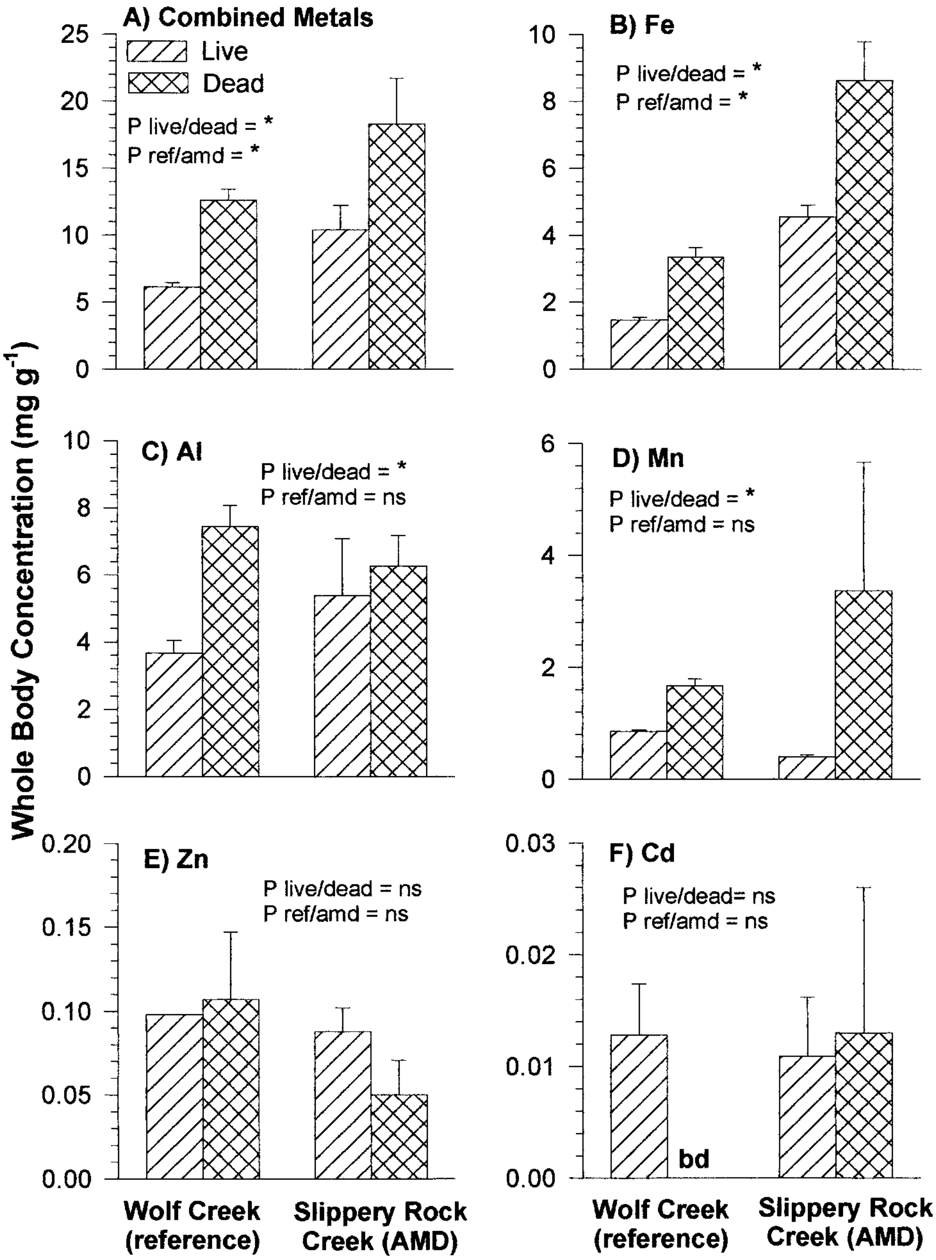

Fig. 4. Mean whole-body concentrations of Fe, Al, Mn, Zn, Cd and combined metals for live and dead hydropsychid caddisflies after a 5-day exposure in Slippery Rock Creek (at AMD impacted Station 65) and Wolf Creek (unimpacted reference stream). Error bars are 1 SE, $n=7$. bd, below detection limits. Asterisks indicate a significant difference ($P<0.05$) in concentration between live and dead caddisflies within a stream site, or between stream sites for all caddisflies.

rather than physical abrasion from the current. Concentrations of Zn and Mn on the substrata were 1–2 orders of magnitude lower than Fe and Al. Under oxidizing conditions, precipitation of Mn is much greater at $pH>6$ (Hedin et al., 1994) and thus more likely in Wolf Creek than the AMD site, however aqueous concentrations in Wolf Creek were very low. It is likely changes in the concentration of Zn and Mn after exposure in Wolf Creek may have been relatively more affected by formation of a biofilm containing these elements than by changes in mineral solubility or adsorption.

After the 4-week exposure in Wolf Creek, there were no significant differences between AMD coated and control substrata in abundance or taxonomic composition of invertebrates or periphyton. Several aquatic invertebrates have been shown to be tolerant of high to moderate levels of dissolved Fe (Rasmusssen and Lindegaard, 1988; Gerhardt, 1992; Rousch et al., 1997). However, studies of mine drainage in which stream pH values are circumneutral and dissolved metals low, have suggested that loose, unattached precipitates of ferric iron hydroxides can have devastating affects on invertebrate

Table 6
Whole-body concentrations of metals in 10 live hydropsychid caddisflies selected at random from the reference stream, Wolf Creek

Metal	Whole-body concentration (mg g^{-1})
Fe	1.36
Mn	0.96
Al	3.86
Zn	0.06
Pb	bd[a]
Cd	bd[a]
Total	6.24

[a] bd, below detection limits.

fauna through burial of substrata, clogging of gill surfaces, reductions in vision, disruptions in feeding, and general abrasiveness (Carrithers and Bulow, 1973; Hoehn and Sizemore, 1977; Letterman and Mitsch, 1978; Scullion and Edwards, 1980; Gray, 1996). In our study, similar precipitate affects were probably greatly reduced because the AMD substrata had a relatively thin, adhered coating of precipitate with no loose floc covering the rocks. The latter probably would have washed away in the current of Wolf Creek. Macroinvertebrates found on the AMD substrata were similar to the control substrata and typical of the fauna found in Wolf Creek (DeNicola and Stapleton, 2000), which suggests that the adhering precipitate of AMD was relatively nontoxic, and that physical affects associated with fine flocculated precipitate probably have a greater negative impact on macroinvertebrate fauna. Similar results were reported by McKight and Feder (1984) who found that benthic macroinvertebrate assemblages at a site with low pH, high dissolved metals, and an adhering crust of metal precipitate on the substratum were similar in abundance and diversity to an unimpacted site. However, at lower sites in the watershed, where flocculent metal precipitate covered the stream bed, invertebrates were extremely impacted.

We do not know how long the sampled macroinvertebrates were resident on the substrata in our study, and it could be argued that they represented drifting organisms that were only there for a period too short to be affected by the AMD precipitate. Of the mayflies found on the tray substrata, several common taxa were heptageniid mayflies that are scrapers and clingers, which are generally more rare in the drift (Giller and Malmqvist, 1998). The stonefly, *Taeniopteryx,* is also a sprawler/clinger and usually not as common in the drift. While most caddisflies generally tend to be rare in drift, the family Hydropsychidae is more common (Giller and Malmqvist, 1998). The mayfly, *Isonychia*, is a good swimmer and probably the taxon most likely to be common in the drift (Cloud and Stewart, 1974).

Periphyton densities on substrata coated with AMD precipitate were slightly higher but not significantly different than control substrata. Perrin et al. (1992) found periphyton exposed to diluted AMD from a silver mine had greater biomass than control and hypothesized that micronutrients in the diluted AMD may have stimulated growth. In general, studies of overall AMD affects on periphyton have shown a greater impact on species composition than biomass (Warner, 1971; Verb and Vis, 2000), especially in relation to reductions in pH (DeNicola, 2000). In our results, the composition of the periphyton assemblages was not affected by the AMD coating and the flora was typical of the epilithic flora of Wolf Creek (DeNicola and Stapleton, 1999). The most severe effect of mine drainage on epilithic periphyton appears to be related to burial by loose, iron precipitate floc, whose affects are independent of pH changes. Descriptions of periphyton sample site characteristics in the studies of Warner (1971), McKight and Feder (1984), and Verb and Vis (2000), indicate that substrata covered with iron hydroxide floc had periphyton that was both lower in biomass and diversity than substrata that had only an adhering precipitate coat. In areas with high precipitation of metals, floc reduces light availability, greatly lowering periphyton biomass. In addition, there can be a shift from attached, epilithic species toward motile, epipelic species on hard substrata buried with a layer of iron hydroxide floc (Warner, 1971; Nichols and Bulow, 1973; McKnight and Feder, 1984; Verb and Vis, 2000). The lack of impact of AMD precipitate on periphyton assemblages in our study corroborates the hypothesis that an adhesive metal precipitate coat has less of an affect on periphyton than burial by the substrata in iron hydroxide floc.

Given the lack of impact of AMD precipitate in the substratum experiment, we examined whether the aqueous chemical environment of Slippery Rock Creek still affected invertebrates despite improved water quality from passive treatment of AMD discharges. Hydropsychid caddisflies are useful sentinel organisms for examining metal uptake because they are ubiquitous and accumulate metals from filter feeding in higher concentration than for all other feeding groups except sediment-deposit feeders (Smock, 1983). Lower survival of caged caddisflies in the AMD impacted Slippery Rock Creek most likely resulted from the higher concentrations of metals in the water and/or in the seston that they are filtering as a food source. While most aquatic insects accumulate metals in proportion to their availability in the environment, the nature of the relationship depends on the metal, its form, its bioavailability, and other interacting chemical factors such as pH and hardness (Gerhardt, 1994; Hare, 1992; Brown, 1977). Toxicity is most related to uptake of metals in the free ion form in the water or from food, and occurs when uptake exceeds the organisms ability to regulate the metal or bind it in a nonlethal form (Hare, 1992; Gerhardt, 1993). While the pH of Slippery Rock Creek during the exposure was over 6.0, it was lower than the pH of Wolf Creek and it was likely to have a greater concentrations of more toxic, free-ion forms of the metals (Table 1). Caged caddisflies in the AMD impacted stream had significantly higher concentrations of most of the measured metals, with concentrations of Fe and Al being the highest. Most aquatic animals regulate Fe well and LC50 values for dissolved Fe are relatively high (3–300 mg l^{-1}; Gerhardt, 1993, 1994). Precipitation of Fe hydroxides can decrease survival because of disruption of intestine membranes, clogging of the digestive tract, and coating of gill surfaces (Gerhardt, 1992, 1993). Although no visible orange precipitate was seen on the caddisflies placed in the AMD site, precipitate formed on the cages in that stream indicating that some precipitation of Fe on the organisms was more likely than in Wolf Creek. Toxicity of Al on aquatic insects appears to result mainly from affects on gills and respiration (Rosenberg and Resh, 1993). While dissolved ionic Al can disrupt ion transport across gill membranes, respiration can be also affected in part by precipitation of Al hydroxide on gill surfaces. Krantzberg and Stokes (1988) found maximum Al body burdens of chironomids were greatest between pH 5.1 and 5.6, a level close to the pH at our AMD site.

Comparison of whole-body concentrations in live vs. dead caddisflies implies that although active ingestion of metals can lead to toxic internal affects, passive uptake of metals on external body surfaces also can be an important uptake mechanism in hydropsychid caddisflies, which may lead to chronic lethal affects associated with gills or sensitive surfaces. It was surprising that dead caddisflies had higher total body concentrations than live for all metals, as most studies find little difference in metal uptake between live and dead aquatic insects (Timmermans et al., 1992). Iron in the gut contents of hydropsychid caddisflies make up 34–60% of the Fe in the whole body (Smock, 1983; Cain et al., 1995), and we assumed active uptake of filtered seston would increase metal concentrations in live organisms in the AMD stream. Dead caddisflies were depurate and their mass was about half that of live, thus it appears that metals associated with passive surface sorption increased the whole-body concentrations in dead organisms. Other factors that could increase the concentration of metals in dead vs. live caddisflies are the release metals sequestered in granules near the body surface after death (Krantzberg and Stokes, 1988), the release of proteins in killed organisms that then adsorb metals from solution (Timmermans et al., 1992), the lack of metal regulation/excretion in dead organisms, and the possibility that metals precipitated on the retreats and nets of live hydropsychid caddisflies, rather than on the body surface (Letterman and Mitsch, 1978; Brown, 1977).

6. Conclusions

Understanding the relative roles of substratum and aqueous chemical affects of AMD on benthic organisms is critical to successful remediation of impacted streams. The building of passive systems to treat coal mine discharges entering streams has increased substantially in the past 5 years (Milavec, 2000; Rossman et al., 1997), but there has been little examination of the relative roles of the benthic vs. aqueous environment on the recovery of stream benthos. Results from this study indicate that aqueous AMD chemical environment in Slippery Rock Creek had a greater affect on organisms than the chemical precipitate on substrata. Hard substrata coated with AMD precipitate had a significant decrease in the most abundant metals, Fe and Al, and a benthic flora and fauna similar to control substrata when placed into an unimpacted aqueous environment, whereas caddisflies exposed to a moderately impacted aqueous AMD environment had significantly higher mortality and metal concentrations in their bodies. While treatment of several large AMD inputs into Slippery Rock Creek have improved the overall aqueous environment, water chemistry appears to continue to limit the recovery of benthic organisms. Increased AMD discharge during storm events can overwhelm or bypass treatment systems, and the temporary deterioration in stream water quality can increase the negative impact on benthic organisms (Verb and Vis, 2000; DeNicola and Stapleton, 1999). Moreover, decades of accumulated AMD precipitate deposited on the substratum may now be out of equilibrium with the treated aqueous environment, and thus be a source of aqueous metals. Our study suggests improvement in water quality resulting from passive treatment systems should aid in the recovery of the chemical and biological environment of the substrata in AMD affected streams. While AMD affected substrata recovered quickly in our transplant experiment, the cobble was coated with a thin, recently deposited precipitate, which is most representative of riffle areas where flocculent precipitates do not accumulate. Sections of other streams and Slippery Rock Creek that have accumulated layers of encrusted and flocculent AMD precipitate may take substantially longer to recover given improved water quality.

Acknowledgements

We are grateful to Scott Daly, Heather Doyle, Rebecca Henry, Jeremy Kazio, Shymana Satiapillaii, and Taylor Zenter for their assistance in the field and metals analysis in the laboratory. Robert Hedin, Robert Bode, John Constable, and an anonymous reviewer provided comments and suggestions for the manuscript. C. David McIntire offered advice on canonical correspondence analysis. This project was funded in part by a Pennsylvania State Growing Greener Grant, administered by the Slippery Rock Watershed Coalition and Stream Restoration Inc.

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Chemical and Biological Monitoring of Slippery Rock Creek, PA

Associated with Installation of Passive Treatment Systems to Treat Acid Mine Drainage

For the Period Fall 1999- Fall 2001

Final Report to PA DEP

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Keywords: Acid mine drainage, passive treatment, anoxic limestone drain, vertical flow wetland, macroinvertebrates, periphtyon, stream restoration

Abstract

A 70 km^2 area in the headwaters of Slippery Rock Creek in Western Pennsylvania is impacted by acid mine drainage (AMD). Twelve stations, two of which are in unimpacted control streams, were sampled in fall 1999, spring 2000, fall 2000, spring 2001 and fall 2001 to monitor changes in water chemistry resulting from continued passive treatment in the watershed. Seven of the 12 sites were monitored for changes in epilithic and epipelic algae, and in macroinvertebrates in riffle areas. Values for pH and alkalinity in some tributaries and sites in the lower watershed increased slightly during the 1999-2001 period. Since monitoring began in 1995, there have been long term increases in alkalinity in the Seaton Creek tributary and in the two sites lowest in the watershed (65 and 67), which reflect the substantial restoration efforts in the Seaton Creek drainage and upstream sites in the main stem during this period. Overall, pH and alkalinity values in the streams are fairly good, however they can temporarily decrease during periods of high flow, presumably through flushing out of mine pools. Although concentrations of dissolved zinc and nickel have decreased in Seaton Creek, values of dissolved iron and manganese remain above EPA standards for organisms and could be limiting biological recovery.

The percentages of acidic indicator taxa of benthic algae are higher at AMD impacted sites than the reference sites, however the reduction in their relative abundance since 1995 at several sites is a sign of improving conditions. Macroinvertebrates remain severely impacted at AMD sites. Macroinvertebrate density and richness at the impacted sites remain well below those for the reference streams. One of the most important factors limiting macroinvertebrate recovery is probably the high amount of fine sediments in the watershed that make an extremely poor substrate for the invertebrates in the most of main stem. Despite these problems, AMD sensitive taxa (i.e., mayflies, stoneflies and caddisflies) are beginning to be found in low numbers on hard substrata, downstream in the watershed (Site 65).

The continued high concentrations of sulphate in the streams indicates that mine drainage still enters the watershed, but the concurrent improvement in alkalinity demonstrates that treatment systems are successfully reducing AMD impacts in the watershed. This coupled with the slightly improving trends in the benthic algal flora and invertebrate fauna suggests further recovery in the watershed as future restoration efforts occur. Recommendations are for a better understanding of the hydrology of the watershed, improving the substrate quality in the streams, and the need for experimental work to determine specifically which chemical factors are limiting the recovery of the biota.

Introduction

Slippery Rock Creek in Western Pennsylvania, USA is a tributary in the upper Ohio River Watershed that drains a 1,100 km^2 area. The Slippery Rock Creek Watershed has been severely impacted by acid mine drainage (AMD) for more than a century, predominantly from coal mining activities in a 70 km^2 area at the headwaters of the stream. Contact of AMD water with stream water of higher pH can precipitate large amounts of iron hydroxides on the substrata, as well as lower the pH of the stream (Boult et al. 1994, Robb and Robinson 1995). These effects usually result in drastic reductions in benthic macroinvertebrate abundance and diversity (e.g., Dills and Rogers 1974, Letterman and Mitsch 1978, Scullion and Edwards 1980, DeNicola and Stapleton 2000), and significant changes in benthic algal communities (Verb and Vis 2000).

Measures to abate AMD discharges have been implemented in the watershed since the 1970's and have improved water quality in the lower Slippery Rock Creek watershed, however, the main stem and tributaries in the headwaters area remain impacted by AMD. Three types of passive treatment systems have been installed since 1995 to treat discharges in the headwaters of the watershed; aerobic pond/wetlands, anoxic limestone drains (ALD), and vertical flow wetlands. When correctly designed for the flow rate and chemistry of the discharge, passive treatment systems have been shown to be extremely effective in improving water quality (Hedin et al. 1994, Robb and Robinson 1995). In addition, there has been extensive reclamation of land area polluted by coal refuse in the watershed.

Monitoring of chemical and biological parameters began in 1995 (full scale monitoring started in 1996) to evaluate the effectiveness of passive treatment technology in restoring the stream ecosystems of Slippery Rock Creek in the headwaters area. Our objective has been to compare chemical and biological parameters in impacted stream sites in the watershed to unimpacted reference streams prior to and during the restoration process. A total of 9 abatement projects were completed in the headwaters area by 1999 to reduce AMD impacts. Results of quarterly monitoring from 1995-1999 are summarized in the final report submitted to the PA DEP in 1999 (DeNicola and Stapleton 1999).

In brief, the results of the 1995-1999 monitoring indicated overall, there had been perhaps a slight improvement in pH and alkalinity at some of the headwater sites downstream of treatment systems, but little improvement in aqueous or sediment metal concentrations. Most chemical parameters in the watershed were quite variable over time within each site. At AMD impacted sites, taxonomic composition for both epilithic and epipelic algae was more affected than algal densities. Impacted sites were dominated by taxa characteristic of low pH waters. Invertebrate density and diversity were greatly reduced at AMD affected sites in the headwaters compared to references streams, and did not appear to improve from 1995-1999. Macroinvertebrate taxa at AMD impacted sites were mostly diptera and hydropsychid caddisflies. At the end of that period there appeared to be little, if any, significant recovery in macroinvertebrates and algae in the 5 years following the initial building of treatment systems in the headwaters area. We concluded that toxic concentrations of iron, manganese, aluminum and zinc were probably impacting the macroinvertebrates more than low pH or alkalinities, and that complex interactions in the hydrology of the mine pools may have caused temporarily harmful concentrations of many chemical parameters in the streams. In addition, much of the substrate at the impacted sites was dominated by clay, which is a very poor substrate for macroinvertebrates (DeNicola and Stapleton 1999).

There has been more treatment of AMD and restoration of the watershed since 1999. On the main stem of Slippery Rock Creek, 1 more passive treatment system was completed in 2000, with another nearing completion. There has also been extensive recent restoration in Seaton Creek, a major tributary of Slippery Rock Creek in the headwaters, with 4 sites of land reclamation and several large vertical flow systems installed. As a result, there are now currently approximately 12 passive treatment systems in the headwaters of Slippery Rock Creek treating about 500 million gallons of water a year (Fig 1). This removes about 190 tons/yr of acidity, 8 tons/yr of Al and 150 tons/yr of Fe (see the Watershed Coalition web site, www.srwc.org). The purpose of the present study was to continue monitoring of the stream sites twice a year from the fall of 1999 through the fall of 2001 to determine if conditions in the watershed were improving as a result of the increase in restoration efforts. Note, there were two funding sources for monitoring during this period, a Bureau of Land and Water Conservation Nonpoint Source 319 Project that funded the restoration efforts at discharge SR89, and a Growing Greener Grant that funded restoration for SR 96. Since the monitoring study was continuous, data for the entire 1999-2001 period are provided in this one report. Also, we report some long term trends in the streams for 1995-2001.

In addition to monitoring, a set of experiments that examined the relative roles of substrata and aqueous affects of AMD on invertebrates and algae in Slippery Rock Creek was completed in 2000. Information on this study is provided in a separate, self-contained, attached document, which has been accepted for publication in the journal *Environmental Pollution.*

Methods

Detailed methods were provided in the QA/QC work plan originally written for this study in 1996 and are available from the authors or the PA DEP. Below is a brief summary of the methods. Sample dates for analyses for each parameter are given in Table 1.

Study Design

Twelve sites in the watershed (Fig. 1) were sampled for selected chemical parameters in fall 1999, spring 2000, fall 2000, spring 2001 and fall 20001 (Table 1). Seven of the sites are in the main stem of Slippery Rock Creek (2-4th order streams) within the headwaters area and impacted by AMD (sites 44, 46, 60, 63, 64, 65 and 67), 3 are AMD impacted tributaries (49, 62, and 68), and 2 sites are in "control" or reference streams that are unimpacted by AMD (Fig. 1). One of the control streams is a 1st order unimpacted tributary within the headwaters (Site 61), the other is Wolf Creek, a 4th order stream that is in the Slippery Rock Creek watershed but not in the headwaters area. Biological samples were taken at a subset of the sites (44, 46, 60, 61, 65, 67 and Wolf Creek) on the dates specified in Table 1.

Water Chemistry

A large beaver dam appeared just downstream of the confluence of the tributary at Sites 46 and 49 in the fall of 2000, flooding the entire area. As a result, the two sites were merged into a pond and no longer represented the two different streams. Water samples from the area were take below the confluence and referred to as Site 46. Thus there are no samples for Site 49 after this date. Temperature, dissolved oxygen, pH, alkalinity, acidity, conductivity and sulphate were measured in the field following Standard Methods (APHA 1989). Water samples for dissolved element analysis were filtered in the field through a 0.45 micrometer Millipore Filter and fixed with concentrated HNO_3 to a pH < 2.0. Unfiltered water samples for total elemental analysis were fixed with concentrated HNO_3 in the field. In the laboratory, unfiltered samples were digested with HNO_3 following Standard Methods (APHA 1989). Dissolved and total water samples were analyzed for Fe, Mn, Ni, Pb, Al, Zn, Cd, Si, Mg and Ca using a Perkin Elmer Plasma 400 inductively coupled plasma spectrophotometer as outlined in APHA 1989. Discharged was measured at each site on each sample date using a Marsh McBirney flow meter.

Macroinvertebrates and Algae

Macroinvertebrates in riffles were sampled at Sites 44, 46, 60, 61, 65 and Wolf Creek using a Surber sampler. Samples in riffles areas were not taken at Site 67 because shallow riffles were not present in this area of the stream. The deep pond produced by the large beaver dam built just downstream of Site 46 in the fall of 2000 prevented invertebrate sampling at that site on that and all subsequent dates. Three replicate samples were taken at the sites on the sample dates and the organisms preserved in 70% ethanol. In the laboratory, individuals were sorted and identified to the lowest taxonomic level possible (usually genus). Chironomid larvae were cleared in KOH and mounted in CMCP10 on microscope slides for identification.

Epilithic periphyton algae (algae that grows on rocks) was scraped from 3 rocks in riffle areas at Sites 44, 46, 60, 61, 65, 67, and Wolf Creek, respectively, using an SG-92 sampler following the protocols of the USGS National Water Quality Assessment (NAWQA) monitoring program (Porter et al. 1993). Samples were not taken at Site 67 in the spring 2001 because reconstruction of the bridge at the site prevented access to the rock substrata. Because of the time consuming nature of counting algal samples, the 3 rock scrapings were combined into a single composite sample for each site on the sample dates. Epipelic periphyton (algae that grow on the sediment surface) was sampled at the same sites following the coring method of NAWQA (Porter et al. 1993). Two cores were combined into 1 composite sample per site per date. Algal samples were preserved with Lugol's Solution. Algal counts for density were done in Palmer Cells with diatoms lumped into one taxonomic category. The sample was then digested in HNO_3, rinsed, dried onto coverslips and mounted in NAPHRAX for diatom identification (DeNicola et al. 1990). All algae were identified to species.

Results and Discussion

The complete set of values for all water chemistry parameters and discharge on each sample date are given in Tables 2 and 3

pH, alkalinity and acidity

Pennsylvania water quality standards cite pH values below 6 has being detrimental to aquatic life, although severe effects usually are found below 5.5. Values for pH in the headwaters ranged from 4.5 to 7.8, with values of 5.5 or lower occurring on some dates from Sites 44, 62, 64 and 68 (Fig. 2). State water quality standards list impacts occurring below an alkalinity of 20 mg/l for most streams, and sites in the headwaters area almost always below this (Fig. 3). However, the low alkalinity at the small reference stream in the headwaters is also occasionally below 20 mg/l, suggesting a naturally low buffering capacity of the geology of the area in general. The most downstream sites in the headwaters, 65 and 67, were generally greater than this threshold during the study period (Fig. 2) Alkalinity and pH values were substantially higher in the large reference stream, Wolf Creek, primarily because it is in a different geologic group than the headwaters (Potsville vs. Allegheny) with more tributaries intersecting the Vanport Limestone outcrop geology. Acidity values were quite variable, reflecting to some extent the difficulties involved in measuring acidity accurately (APHA 1989). Overall acidities were highest at Sites 46/49, 63 and 64, but often values were similar to those found in the small reference site, 61 (Table 2).

Temporal trends in pH, acidity and alkalinity at each site during the 2 year period covered by this report are difficult to discern. However, from 1999-2001 there do appear to be some increases in pH and alkalinity at Sites 62, 64, 65 and 67 (Figs. 2 and 3). The recent improvement in pH and alkalinity at Site 62 may be related to the construction an anoxic limestone drain in the area in 1998. While this treatment system is a short distance downstream from Site 62, it increased the flow from the AMD discharge, which perhaps lowered the mine pool in the deep mine in the hillside. This may have dried up small discharges above Site 62 that were coming out of the same mine pool. Longer term trends based on monitoring at the sites since 1995 also show improvements. Site 68 is on Seaton Creek, and drains an area that has received substantial restoration since 1998. For the time period 1995-2001 there are positive increases in alkalinity and pH at this site (Figs. 4 and 5). Sites 65 and 67 are at the bottom of the headwaters and therefore changes in their water chemistry should reflect the overall impact of restoration efforts in the entire watershed. Site 67 is upstream of the Seaton Creek inlet and Site 65 below. Both these sites have shown increases in pH and alkalinity since 1995, which presumably reflects the positive affects of restoration on water chemistry in the headwaters (Figs. 6-9). While the significance of these long term trends were not tested statistically for this report, they do indicate a trend of improving water quality.

Sulphate, conductivity and dissolved oxygen

Sulphate and conductivity are good general chemical indicators of AMD and were highest overall for Sites 65 and 68 (Table 2). This reflects the influence of the Seaton Creek drainage in the headwaters. Station 68 is on lower Seaton Creek and Site 65 is on the main stem of Slippery Rock Creek below the junction with Seaton Creek (Fig. 1). The Seaton Creek drainage has had some of the most severe mine drainage problems in the watershed but has recently be the focus of substantial restoration efforts. As seen above, there has been an improvement in pH and alkalinity at these stations since 1995. We began measuring sulphate and conductivity starting in 1999 and do not have long term trends. For the period 1999-2001 there are no consistent decreases in these measures at Sites 68, 65 or 67, but treatment systems generally do not decrease sulphate concentrations. Sulphate reduction is very slow in the anaerobic environments of vertical flow systems, and aerobic processes below the treatment keep sulphate high. That fact that sulphate (which drives conductivity values) remains high in the streams is an indicator that AMD inputs from the watershed continue. However, the concurrent increases in alkalinity and pH of the streams indicates that treatment systems are effectively improving water quality by treating these discharges (Robert Hedin, pers. comm.).

Dissolved oxygen values at all sites were usually near saturating most of the study period (Table 2), and should not have a negative effect on the biota.

Soluble Metals

Soluble iron concentrations were highest at Site 44, which is located above all treatment, and at Site 63 (Fig. 10). Site 63 is just downstream from substantial input of iron from AMD discharges SR96 and SR89. Treatment systems for these discharges are currently under construction and should greatly reduce iron and input of other metals to this site in the near future. Pennsylvania water quality standards list maximum iron levels for aquatic life at 1.5 mg/l for total and 0.3 mg/l for dissolved. These standards were greatly exceeded a certain times of the year at all impacted sites in the watershed except Site 67 and probably have a negative impact on biological recovery in the streams (Table 3 and Fig 10).

Overall, dissolved iron concentrations at most impacted sites in the watershed have not changed much since 1995-1999 monitoring report (DeNicola and Stapleton 1999). However, soluble iron showed a general decrease in concentrations at Site 67, the lowest point in the watershed above the Seaton Creek input (Fig 11). This may reflect the removal of iron by treatment systems further upstream. There no changes in iron concentrations at Site 65 and perhaps an increasing trend at Site 68 since1995 (Figs. 12 and 13). This may reflect the fact that significant AMD input remains in the parts of the Seaton Creek drainage, although several of these areas are targeted for future restoration.

The New York State standard for dissolved Manganese is 0.3 mg/l (PA does not have a dissolved standard). Mean manganese concentrations do appear to be a bit lower than they were during the 1995-1999 monitoring (DeNicola and Stapleton 1999) but impacted sites in headwaters area still exceed the standards at some point during the 1999-2001 study (Table 3). Concentrations were highest at Sites 44, 46, 62 and 68 (Table 3). Manganese is more difficult than iron to remove using passive treatment (Hedin 1990) and may play a significant role in limiting biological recovery at some sites in the watershed.

Mean values of dissolved aluminum at impacted sites were similar to values for reference sites, generally less than 0.3 mg/l (Table 3) and haven't change much since the previous monitoring period. Pennsylvania aluminum standards depend on the species of organism, the standard for New York is 1.0 mg/l total Al, and is exceeding at several sites in the watershed (Table 3). Nontoxic silicon is associated with aluminum as aluminum silicates in minerals, and had similar trends in concentrations as aluminum in the aqueous samples

Dissolved lead was lowest at the small reference site, 61, but other sites, except 68, had similar concentrations to Wolf Creek (Table 3). There are no biota standards for lead in surface water for PA, and EPA standards depend on the organism.

Concentrations of soluble zinc were generally higher at the impacted sites than the reference sites from 1999-2001 (Table 3), however most values are below the toxic threshold minimum, 0.1 mg/l. During the 1995-1999 monitoring period mean concentrations of dissolved zinc were quite high (near or above 0.1 mg/1) for Sites 63 and 68 and seem to have decreased since then.

In general, concentrations of soluble nickel and cadmium at the impacted sites were similar to values at the reference sites, indicating little impact (Table 3). Average nickel concentrations were very high at Site 68 (> 0.1 mg/l) during the 1995-1999 monitoring period and have drop significantly since then. Cadmium concentrations are similar to what they were in 1995-1999 (Table 3 and DeNicola and Stapleton 1999).

Aqueous concentrations for both calcium and magnesium were lowest at the small reference stream (Site 61). Concentrations in Wolf Creek were similar to impacted sites except for Sites 68 and 65, which were considerably higher (Table 3). One would expect higher levels of calcium and magnesium at lower pH sites from the dissolution of their alkaline minerals. Higher calcium levels in the watershed could also result from dissolution of calcium from limestone in ALD's and vertical flow systems. Given the high sulfate levels in the impacted streams (200-700 mg/l), high calcium may result in precipitation of gypsum ($CaSO_4$) in the streams. These trend are similar to what they were during the 1995-1999 monitoring period (DeNicola and Stapleton 1999).

Effect of discharge on water chemistry:

Although there appears to be a trend in improvement in some water quality parameters, patterns are difficult to establish because flow conditions can influence concentrations. For example, alkalinity at Sites 60 and 64 in the middle of the watershed, tended to decrease at high discharges during the period 1995-2002 (Figs.14 and 15). A similar relationship exists for other sites as well. Changes in pH and iron concentrations also tend to be related to flow at some sites, but the trends were not as clear as for alkalinity. Dills & Rogers (1974) found high surface runoff during wet periods often increased dilution of AMD inputs, however we found the opposite.

Rather than diluting AMD, increased precipitation probably flushes out mine pools and increases surface runoff from coal refuse in the Slippery Rock Creek watershed, which degrades water quality. As stated in our 1999 monitoring report, a better understanding of the hydrology of the area is needed in order to explain some of the natural variability in water chemistry.

Benthic algal density

Epilithic algal densities generally increased as stream size becomes larger downstream (Fig. 16). Densities in the 4th order Wolf Creek are approximately 100 times higher than at most of the upstream sites. Density values at all sites were very similar to those found in the 1995-1999 monitoring (DeNicola and Stapleton 1999). Benthic algal densities in streams are usually limited by light and thus often increase as stream size increases and the canopy becomes more open (Vannote et al. 1979). Others have found that differences in periphyton densities are generally controlled more by light or nutrients than from chemical affects of AMD (Verb and Vis 2000). In the previous monitoring prior to 1999, densities at the 1st order reference site (61) were generally higher than that at the AMD impacted sites such as 44, 46 and 60, indicating some impact from AMD. For the period 1999-2001, the control site densities are more similar to the AMD sites, indicating perhaps a reduction in the AMD affects (Fig. 16). Impacts of AMD on epilithic algae is usually due to the unconsolidated fine sediment (clays and iron oxide precipitates) burying the rocks, rather than a purely chemical affect from AMD (DeNicola and Stapleton, In Press). However, rock substrata at Sites 44, 46 and 60 did not seem visually to have less fine sediment deposits than in previous years.

Epipelic algal densities follow a similar trend as the epilithon, except for Site 60, which had very high densities on several sample dates (Fig. 17). Densities were lower for Sites 44, 46, 65 and 67, probably reflecting the more unstable substrate in these areas. Temporal changes in both epilithon and epipelon densities do not appear to be related to installation of treatment systems for AMD, which is not surprising given that previous studies have shown that algal species composition is generally affected by AMD, while algal densities are not (Verb and Vis 2000).

Benthic algal species composition

Relative abundance values for each taxon in each benthic algae samples are given in Appendix I.

Mean species diversity of the sites for the whole study (i.e., dates pooled for each site) indicates that Wolf Creek had the highest diversity for epilithon and epipelon (Tables 4 and 5). Acidic diatom taxa were higher at the AMD affected sites than Site 61, indicating an AMD impact remains. Species richness trends at the sites were quite variable for both epilithon and epipelon, and occasionally were higher at Site 44 than Wolf Creek (Figs. 18 and 19). This discrepancy is in part an artifact of the way the samples are counted. The cyanobacteria, *Phormidium*, usually dominated the algal assemblages at all sites except Wolf Creek. As a result, sites such as 44, have very few diatoms, and in the separate count of 300 diatoms many rare species are encountered that increase the species richness of the sample. Comparison of the upstream impacted sites to the small reference stream, 61, indicates that richness values were variable but similar among the sites for most dates. In fact, richness was higher at Sites 44 and 46 than Site 61 on many dates (Figs. 18 and 19). Serious impact on benthic algal richness and diversity does not begin to appear until below pH 4.5 (DeNicola 1999), which rarely occurred at any of the sites. However, many species of diatoms have narrow pH tolerances and are good indicators of pH, thus while there is little change in diversity there was a shift in species composition at the AMD impacted sites.

Comparing the similarities in average species composition for the whole study (dates pooled for each site) among sites indicates that Wolf Creek is most different from all other sites in both epilithic an epipelic species composition (Tables 4 and 5). Dominant taxa in both the epilithon and epipelon in Wolf Creek were the diatoms *Navicula gregaria* and *Navicula lanceolata* (Tables 4 and 5). There were also many species in this diverse flora that are typical for a clean water, moderately productive, high pH stream. The cyanobacteria, *Phormidium* is a dominant taxon at the other sites, indicating it was tolerant of their AMD impacts. Several types of cyanobacteria have been found to be tolerant to heavy metals (Genter 1996), but generally not grow well in very acidic conditions. Diatom taxa typical of low pH are species of *Eunotia* and *Anomoeonies*, which also can be abundant in severely impacted AMD streams (Verb and Vis 2000, Warner 1971). These taxa are generally most abundant at Site 60. Site 61 does have several acid tolerant diatoms, which is not surprising given its pH and low alkalinity. Generally these taxa are less abundant and *Phormidium* more abundant at Site 60 compared to the AMD impacted sites.

Overall, the species composition of epilithic and epipelic algae at the AMD affected sites has fewer acidic diatoms than in 1995-1999, indicating some improvement in the past few years. For example, Sites 46 and 60 have less than half the percentage of acidic epilithic diatom taxa than they contained in 1995-1999. A similar decrease occurred for Sites 65 and 67, although smaller. At the same time, the percentage of acidic taxa at Sites 44 and 61 has not changed from 1995-2001, which is to be expected since 44 is upstream of all installed treatment and 61 is a reference stream.

To examine whether benthic algae indicates substantial improvement during the 1999-2001 period, changes in the abundance of acid indicator species as a percentage of only diatoms were examined. For epilithic diatoms, sites 44, 46 and 60 had overall the highest percentage of acid indicator species, indicating they are the most AMD impacted sites (Table 6). There is much variability over time at each site, however, there appears to be a bit of a decrease in the percentage of acidic diatom species at Site 60 and perhaps Site 46. Although this is not a significant statistical relationship it might denote a future trend of biological improvement at some of the AMD impacted sites. The percentage of acidic diatoms is higher for all sites in the epipelon, and there is no indication of a decrease in their abundance at any of the sites for the past 2.5 years (Table 7).

Macroinvertebrates

Relative abundance values for each taxon in macroinvertebrates samples are given in Appendix II.

Density and species composition in riffles in pools

Mean macroinvertebrates densities in riffles were 1-2 orders of magnitude higher at the 2 control sites (61 and Wolf Creek) than at the most AMD impacted sites on most dates (Fig. 20). The densities of macroinvertebrates at most of the sites have not changed dramatically from the 1995-1999 monitoring period. Densities at Site 60 were above 1000 individuals/m2 in 1996 and 1998, but for most of 1999-2000 no macroinvertebrates were found in samples. There has been a substantial change in the hydrology at this site in the past 4 years resulting from the building of a very large beaver dam just downstream. This has increase water depth and decreased flow, which can have a negative impact on the macroinvertebrate community. As a result it is difficult to determine how the installation of treatment systems has affect the invertebrates at this site. Densities at Sites 44 and 46 remain extremely low. However, Site 44 is upstream of all treatment and Site 46 has also been dramatically affected by beaver dams preventing sampling the past few years. Macroinvertebrate densities at Site 65 are generally greater than for the period 1995-1999, but still remain quite low compared to the reference sites.

Mean taxa richness (number of different genera present) of macroinvertebrates in riffle areas was less than 12 for all AMD impacted sites on all sample dates, but ranged from 8-30 at the two reference sites (Fig. 21). Of the impacted sites, Site 44 and 65 had the highest overall richness, but was still quite a bit less than for the reference sites. Richness was consistently higher at Site 65 in 1999-2001 than in 1995-1999, but still quite low. Species richness at Sites 44, 46 and 60 were similar or lower to what they were in the pervious monitoring period.

Composite samples of the sites (i.e., averaged over dates) indicate there were no extremely dominant taxa at the two reference sites (Table 8). The caddisfly *Hydropsyche* and the riffle beetle *Optioservus* were the most abundant taxa in Wolf Creek, whereas craneflies, stoenflies, riffle beetles and chironomids were abundant at the diverse Site 61. Site 44 had an abundance of the dipterans *Tipula* and *Hexotoma*. In general, dipterans are one of the more tolerant groups to AMD (Letterman and Mitsch, 1978, Scullion and Edwards 1980). The filter feeding caddisfly, *Cheumatopsyche*, was abundant in riffles at Sites 60 and 65. This taxon has been found to be tolerant of AMD in several other studies (Warnick and Bell, 1969, Letterman and Mitsch, 1978) and to effects of heavy metals in western US streams (Clements et al. 1988). Recent studies indicate that the chironomid midge genus, *Parametriocnemus* may be a good indicator of mine drainage in western PA (Steve Harris, pers. comm.). This taxon was present at Sites 44, 61 and 65. The species composition of Site 46 is deceptive. So few organisms were found there, that relative abundances become inflated by the presence of one individual (Table 8).

Mayflies are considered one of the most sensitive orders of aquatic insects to AMD (Warnick and Bell 1969), as well as to isolated effects of low pH and heavy metals (Clements et al. 1988). While stoneflies and caddisflies are more tolerant of low pH, they are in general considered indicators of good water quality. As a result the EPT index (the number of mayfly + stonefly + caddisfly taxa) is widely used to assess water quality. The value of the EPT index has increased at Site 65 over time during the period of 1999-2001 (Table 9). Moreover, it has increased at this site from the 1995-1999 monitoring period. While the number of EPT taxa and densities of invertebrates were low at the site relative to the reference, there has been an improvement in the number of clean water taxa found at site. Although represented by only 1-2 individuals, the presence of the mayflies *Serratella* and *Caenis*, the caddisflies *Pycnopsyche* and *Chimarra*, and the stonefly *Paracapnia* are indications that conditions have improved at Site 65 in the past 7 years (Appendix II and DeNicola and Stapleton 1999).

The low density and taxa richness of the benthic macroinvertebrates in riffles at AMD impacted sites relative to the control sites corresponds to many other studies of AMD effects (Dills and Rodgers 1974, Letterman and Mitsch 1978). Based only on the average pH and alkalinites, the Slippery Rock headwaters should be able to support a larger and more diverse invertebrate fauna (Hoehn and Sizemore 1977). However, some of the chemical data indicated that concentrations of heavy metals are near or at the toxic threshold for stream fauna, and probably are having the greatest affect on invertebrates. There is also great variability in water quality over time, and the overall low invertebrate densities probably result from impacts during periods of high AMD input. In addition, mining disturbance in a watershed greatly increases soil erosion, which alone can have as large or larger a detrimental affect on invertebrates as water chemistry (Hoehn and Sizemore 1977). The watershed in headwaters area of Slippery Rock Creek has been highly disturbed from mining and has a lot of naturally occurring clay. The increased sediment load in the steams together with iron precipitates from AMD bury substrate and reduce invertebrate density and diversity. Sites such as 46 have deep clay and silt deposits that are extremely unstable and poor habitats for invertebrates. It is probably the combination of burial by fine sediments and toxic levels of metals at certain times during the year that are still affecting the macroinvertebrates fauna in the treatment area. The relatively hard substrata of parts of Site 65 might be one reason why there has been some improvement in the types of macroinvertebrates found at this site.

Overall Assessment of Water Quality at the Sample Sites in Slippery Rock Creek

The chemical conditions in the Slippery Rock Creek watershed have generally improved in in the short and long term. From 1999-2001 there has been improvement in the lower watershed and some tributaries in pH and alkalinities. In addition, the restoration efforts in the Seaton Creek tributary have resulted in increased pH and alkalinity in that stream since 1995. The improvement in these parameters in the lower watershed (Sites 65 and 67) since 1995, indicate an overall neutralization of acid impacts by the treatment systems. The continued high concentrations of sulphate indicates that mine drainage still enters the watershed, but the concurrent improvement in alkalinity demonstrates that treatment systems are successfully reducing AMD impacts in the watershed.

Dissolved metal concentrations have not changed as much as alkalinity in the watershed since 1995, except for substantial decreases in zinc and nickel in Seaton Creek (Site 68). Dissolved iron remains fairly high at most sites and above the EPA standard, but it is relatively nontoxic to organisms. The major affect of iron on the biology of the stream is from precipitation of iron oxides onto the substrata burying organisms (DeNicola and Stapleton, In Press). Relatively high concentrations of manganese remain at many AMD impacted sites, and maybe a potential limitation to biological recovery. This could be a continued problem in the future because passive treatment is not as effective at removing manganese relative to iron and aluminum. We recommend examining the effects of manganese on the invertebrates experimentally.

The hydrologic connection between mine pools, groundwater discharges, overland flow and the stream appears to be complex. Subsurface flow into the streams that are not discrete discharges may represent a substantial input of AMD. In general, it appears that periods of increased rainfall and flow tend to flush out mine pools and temporarily increase AMD impacts. These events may limit the biological recovery of the streams. We reiterate our recommendation from the 1999 report, that soliciting a hydrologist to examine these connections would a be great help in planning further restoration.

The percentages of acidic indicator taxa of benthic algae are higher at AMD impacted sites than the reference sites, however the reduction in their relative abundance since 1995 at several sites is a sign of improving conditions. Macroinvertebrate density and richness at the impacted sites remain well below those for the reference streams. The average pH values in the watershed should be able to support a more healthy invertebrate community, although temporary poor water quality during periods of high flow could be limiting overall recovery. Several metals exceed water quality standards for biota at many of the sites, especially manganese and iron. These metals are probably having some impact on the invertebrate fauna either through direct toxicity, or by precipitates burying substrata. One of the most important factors limiting macroinvertebrate recovery is probably the high amount of fine sediments in the watershed that make an extremely poor substrate for the invertebrates in the most of main stem. Despite these problems, AMD sensitive taxa are beginning to found lower in the watershed on hard substrata (Site 65). Although few in number, they indicate that conditions have improved to the point where a few individuals can survive.

Although funding for monitoring of these sites in the Slippery Rock Creek watershed has run out, we will attempt to continue monitoring on our own at a reduced level. As restoration efforts continue in the watershed we expect to see a continued improvement in chemical conditions in the streams. However, unstable sediments with high levels of metals may persist in the streams and limit the rate of biological recovery. Continued restoration of land should reduce sediment loading, and eventually allow natural scouring to improve the substrata in the stream beds.

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Acknowledgements

This project is funded by a 319 nonpoint source pollution grant from the Pennsylvania Department of Environmental Protection and by a Growing Greener Grant. The authors thank the Knox Office of the Pennsylvania DEP for assistance in the field, Stream Restoration Inc. and Bob Hedin for information on treatment systems.

None of this research would have been possible without the valuable contributions of the following students from Slippery Rock University: Taylor Zenter, Heather Doyle, Shymana Satiapillai, Joana Conrad, Robin Synder and Holly Martinchek..

Table 1. Sample dates and codes for biological and chemical parameters.

Date	Code	pH, Alk & Dis. Metals	Total Aq. Metals	Algae	Surber
21Oct99	Fall99	x	x	x	x
23Mar00	Spr00	x	x	x	x
19Sept00	Fall00	x	x	x	x
8Mar01	Spr01	x	x	x	x
25Sept01	Fall01	x	x	x	x

Table 2

Field Data for Slippery Rock Creek Fall 99- Fall 02

Site	Date	Temp C	pH	Alk (mg/l)	Acd (mg/l)	SO4 (mg/l)	DO (mg/l)	Cond (mS/cm)	Flow (m3/s)
44	10/21/1999	9	5.10	19.90	27.60	200	8	0.38	0.012
46	10/21/1999	8	6.90	40.10	18.70	250	10	0.50	0.027
49	10/21/1999	8	6.80	27.60	27.50	250	9	0.48	0.003
60	10/21/1999	13	6.20	17.80	17.10	260	9	0.59	0.010
61	10/21/1999	7	7.50	17.20	15.00	46	9	0.16	0.001
62	10/21/1999	8	4.60	2.10	33.40	295	8	0.52	0.016
63	10/21/1999	10	6.60	14.50	57.20	370	7	0.61	nd
64	10/21/1999	7	5.00	4.50	37.90	270	8	0.51	0.046
65	10/21/1999	9	5.50	48.00	28.00	390	6	0.88	0.130
67	10/21/1999	10	5.70	29.20	20.00	250	7	0.48	nd
68	10/21/1999	8	5.50	30.50	19.50	750	8	1.20	0.067
WC	10/21/1999	10	7.10	150.40	4.70	80	11	0.52	0.454
44	3/23/2000	8	5.90	9.60	8.80	3	nd	0.23	0.122
46	3/23/2000	9	6.20	11.40	5.70	80	nd	0.30	0.156
49	3/23/2000	10	5.70	8.20	6.40	70	nd	0.24	0.061
60	3/23/2000	11	5.90	6.30	4.20	60	nd	0.25	0.256
61	3/23/2000	9	6.70	6.00	3.20	50	nd	0.11	0.030
62	3/23/2000	9	6.00	7.90	2.70	90	nd	0.17	0.054
63	3/23/2000	10	6.50	7.90	5.50	70	nd	0.26	nd
64	3/23/2000	9	5.70	4.00	5.90	140	nd	0.25	1.750
65	3/23/2000	10	6.10	10.20	4.30	260	nd	0.46	0.954
67	3/23/2000	10	5.90	5.70	5.10	180	nd	0.27	nd
68	3/23/2000	10	6.00	9.80	7.20	450	nd	0.71	0.303
WC	3/23/2000	10	9.00	59.20	0.00	70	nd	0.30	3.340
44	9/19/2000	14	5.40	22.60	33.50	92	9	0.33	0.017
46&49	9/19/2000	15	5.60	29.70	35.00	108	nd	0.42	0.070
60	9/19/2000	18	6.60	26.00	29.00	80	9	0.42	0.067
61	9/19/2000	14	5.90	21.30	24.50	44	8	0.18	0.011
62	9/19/2000	nd	5.20	18.40	26.50	100	nd	0.38	0.012
63	9/19/2000	15	7.00	35.50	75.20	180	3	0.44	nd
64	9/19/2000	nd	5.50	20.70	64.20	110	6	0.41	0.138
65	9/19/2000	17	5.80	34.80	18.10	245	8	0.68	0.250
67	9/19/2000	17	5.90	36.90	25.40	136	7	0.40	0.273
68	9/19/2000	15	5.60	27.10	26.80	630	8	1.07	0.084
WC	9/19/2000	nd	7.60	130.00	46.00	79	11	0.48	0.548
44	3/8/01	6.2	6.3	10	6.2	108	7	0.32	0.049
46&49	3/8/01	4.4	6.32	9.8	4.1	140	11	0.32	0.122
60	4/27/01	20.3	5.92	5.4	8.8	98	nd	0.28	0.321
61	3/8/01	5.3	6.39	4.6	5.2	12	10	0.11	0.015
62	3/8/01	5	6.15	3.7	3.1	76	nd	0.40	0.008
63	4/27/01	19.3	6	4.2	7.9	110	9	0.26	nd
64	3/8/01	4.1	5.9	3.4	4.2	130	8	0.31	0.243
65	3/8/01	4.5	6.76	15.7	1.9	255	3	0.53	0.634
67	3/8/01	4.5	6.35	7.6	3.4	144	8	0.31	0.585
68	3/8/01	4.7	6.77	20.7	3.8	560	10	0.85	0.247
WC	3/8/01	4.7	9.31	76	0	60	11	0.43	1.465

Table 2 Cont.

Site	Date	Temp C	pH	Alk (mg/l)	Acd (mg/l)	SO4 (mg/l)	DO (mg/l)	Cond (mS/cm)	Flow (m3/s)
44	9/25/01	14	6.64	25.6	12.9	70	9	0.39	0.023
46&49	9/25/01	13	6.73	31.5	10.2	170	8	0.50	0.075
60	9/25/01	12	6.4	22	12.8	140	8	0.50	0.117
61	9/25/01	13	6.78	18	11	32	10	0.14	0.001
62	9/25/01	13	6.27	15.4	16.4	100	11	0.38	0.073
63	nd	nd	nd	nd	nd	nd	nd	nd	nd
64	9/25/01	14	5.65	10.3	25.3	175	5	0.52	nd
65	9/25/01	14	6.63	31	8.9	380	10	0.82	0.351
67	9/25/01	15	6.76	31	12	165	9	0.56	nd
68	9/25/01	13	6.71	32.5	13.7	490	8	1.13	0.073
WC	9/25/01	14	8.3	183	0	77	11	0.50	0.479

Table 3

Station #	Quarter	Aluminum Soluble (mg/l)	Iron Soluble (mg/l)	Manganese Soluble (mg/l)	Calcium Soluble (mg/l)	Magnesium Soluble (mg/l)	Silicon Soluble (mg/l)	Cadmium Soluble (mg/l)	Lead Soluble (mg/l)	Nickel Soluble (mg/l)	Zinc Soluble (mg/l)	Aluminum Total (mg/l)	Iron Total (mg/l)	Manganese Total (mg/l)	Calcium Total (mg/l)	Magnesium Total (mg/l)	Silicon Total (mg/l)	Cadmium Total (mg/l)	Lead Total (mg/l)	Nickel Total (mg/l)	Zinc Total (mg/l)
44	Fall 99	0.30	3.60	1.41	42.10	11.97	5.72	0.03	0.23	0.02	0.03										
46	Fall 99	0.27	1.59	1.28	64.80	14.19	5.64	0.04	0.35	0.02	0.02										
49	Fall 99	0.29	3.93	1.96	53.61	20.36	4.29	0.04	0.28	0.01	0.02										
60	Fall 99	0.25	0.23	0.25	66.42	16.71	4.14	0.04	0.35	0.01	0.06										
61	Fall 99	0.24	0.23	0.27	14.90	6.58	3.19	0.01	0.15	0.01	0.05										
62	Fall 99	0.31	2.57	2.16	62.67	21.08	5.21	0.04	0.34	0.04	0.05										
63	Fall 99	0.44	14.35	1.35	80.36	21.07	5.86	0.05	0.43	0.03	0.08										
64	Fall 99	0.38	3.88	2.26	55.27	15.87	5.21	0.03	0.28	0.02	0.03										
65	Fall 99	0.25	2.645	6.825	108.18	33.935	3.965	0.05	0.46	0.025	0.025										
67	Fall 99	0.22	0.23	0.28	51.95	15.40	2.91	0.03	0.26	0.01	0.06										
68	Fall 99	0.26	0.4	11.615	166.685	57.915	5.145	0.07	0.65	0.05	0.065										
WC	Fall 99	0.22	0.06	0.04	61.48	12.88	1.90	0.03	0.27	0.00	0.02										
44	Spring 00	0.25	0.75	1.62	20.66	7.89	4.18	0.01	0.14	0.03	0.03										
46	Spring 00	0.25	0.80	0.72	27.44	8.34	4.27	0.02	0.23	0.02	0.01										
49	Spring 00	0.24	0.28	0.91	20.30	8.43	3.53	0.02	0.16	0.02	0.03										
60	Spring 00	0.24	0.22	0.80	24.69	8.02	3.61	0.02	0.20	0.02	0.10										
61	Spring 00	0.24	0.04	0.21	7.99	3.65	2.96	0.01	0.14	0.02	0.00										
62	Spring 00	0.24	0.21	0.53	14.44	6.28	3.51	0.01	0.12	0.02	0.04										
63	Spring 00	0.24	0.63	0.82	26.17	8.55	3.75	0.02	0.19	0.02	0.03										
64	Spring 00	0.26	0.18	0.65	24.64	7.64	3.68	0.02	0.18	0.01	0.05										
65	Spring 00	0.245	0.13	2.12	48.75	16.175	3.55	0.03	0.29	0.035	0.03										
67	Spring 00	0.26	0.13	0.62	26.77	8.41	3.32	0.02	0.17	0.02	0.02										
68	Spring 00	0.25	0.57	6.98	87.49	32.11	4.69	0.05	0.42	0.08	0.11										
WC	Spring 00	0.24	0.10	0.04	33.59	6.55	1.04	0.02	0.21	0.01	0.02										
44	Fall 00	0.25	2.49	1.47	33.81	10.77	5.47	0.03	0.26	0.03	0.01	1.26	2.60	1.34	30.03	9.32	5.07	0.03	0.21	0.02	0.02
46	Fall 00	0.26	0.43	1.16	54.11	12.40	6.02	0.04	0.35	0.02	0.00	1.99	1.58	1.03	49.07	11.22	5.53	0.04	0.36	0.02	0.01
49	Fall 00	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
60	Fall 00	0.245	0.155	0.535	48.93	13.435	3.98	0.04	0.36	0.015	0	1.095	1.835	0.575	50.15	13.635	4.595	0.04	0.35	0.025	0.01
61	Fall 00	0.25	0.19	0.43	16.09	7.01	3.63	0.02	0.23	0.03	0.01	1.48	1.78	0.56	14.46	6.23	4.42	0.02	0.17	0.03	0.03
62	Fall 00	0.25	1.26	1.62	42.37	15.24	4.88	0.04	0.34	0.04	0.01	2.93	3.14	1.60	39.17	13.89	5.23	0.04	0.33	0.04	0.03
63	Fall 00	0.26	9.48	1.00	57.39	16.21	5.02	0.05	0.40	0.03	0.01	1.19	9.97	0.88	52.96	14.74	5.10	0.05	0.37	0.02	0.02
64	Fall 00	0.245	1.235	0.925	50.795	13.97	4.825	0.04	0.34	0.035	0.005	1.23	3.16	0.77	40.98	12.17	4.41	0.04	0.25	0.02	0.01
65	Fall 00	0.19	0.39	3.325	83.025	25.74	4	0.04	0.375	0.02	0.01	2.515	1.51	2.865	71.825	22.915	4.16	0.05	0.415	0.025	0.025
67	Fall 00	0.17	0.23	0.20	45.42	13.41	3.47	0.03	0.25	0.01	0.00	1.32	0.74	0.18	39.84	12.29	3.61	0.03	0.25	0.02	0.00
68	Fall 00	0.20	0.46	10.65	153.86	51.61	5.09	0.07	0.63	0.06	0.04	0.92	0.81	9.22	134.86	47.89	4.85	0.08	0.64	0.05	0.05
WC	Fall 00	0.18	0.07	0.04	60.17	12.14	2.78	0.03	0.28	0.01	0.00	1.52	0.23	0.05	51.56	11.12	2.88	0.04	0.37	0.01	0.01

Total Metals for Fall99 andSpring 00 Did not pass QC and will be rerun

Table 3 Cont.

Station #	Quarter	Aluminum Soluble (mg/l)	Iron Soluble (mg/l)	Manganese Soluble (mg/l)	Calcium Soluble (mg/l)	Magnesium Soluble (mg/l)	Silicon Soluble (mg/l)	Cadmium Soluble (mg/l)	Lead Soluble (mg/l)	Nickel Soluble (mg/l)	Zinc Soluble (mg/l)	Aluminum Total (mg/l)	Iron Total (mg/l)	Manganese Total (mg/l)	Calcium Total (mg/l)	Magnesium Total (mg/l)	Silicon Total (mg/l)	Cadmium Total (mg/l)	Lead Total (mg/l)	Nickel Total (mg/l)	Zinc Total (mg/l)
44	Spring 01	0.28	1.41	2.38	27.30	10.78	4.51	0.02	0.21	0.04	0.06	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
46	Spring 01	0.26	1.52	1.30	37.70	11.48	4.73	0.03	0.28	0.03	0.04	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
49	Spring 01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
60	Spring 01	0.31	1.91	0.68	29.42	9.79	3.89	0.02	0.21	0.02	0.02	2.45	2.20	0.59	26.42	8.52	4.27	0.02	0.22	0.03	0.01
61	Spring 01	0.28	0.08	0.21	7.87	3.77	3.01	0.01	0.11	0.01	0.02	1.58	0.28	0.19	7.61	3.40	3.53	0.01	0.12	0.01	0.00
62	Spring 01	0.29	0.44	1.01	21.87	9.33	3.88	0.02	0.18	0.03	0.10	8.30	1.00	0.90	21.91	8.28	4.84	0.02	0.19	0.03	0.02
63	Spring 01	0.31	1.235	0.67	28.045	9.37	3.865	0.02	0.235	0.025	0.025	2.035	1.41	0.58	24.885	8.51	3.78	0.025	0.195	0.015	0.035
64	Spring 01	0.30	0.37	0.93	32.82	9.75	4.24	0.03	0.27	0.03	0.02	2.13	0.56	0.83	29.24	9.17	4.17	0.03	0.22	0.02	0.02
65	Spring 01	0.27	1.16	3.14	63.11	19.35	4.00	0.05	0.37	0.04	0.04	3.46	1.60	2.79	57.58	18.08	4.18	0.05	0.35	0.03	0.04
67	Spring 01	0.27	0.18	0.85	31.77	9.98	3.85	0.03	0.23	0.02	0.01	2.37	0.44	0.76	28.35	9.28	3.82	0.03	0.21	0.02	0.02
68	Spring 01	0.26	2.57	7.30	119.91	38.93	4.65	0.07	0.57	0.07	0.08	2.44	3.07	6.61	107.91	35.73	4.58	0.07	0.56	0.06	0.08
WC	Spring 01	0.26	0.10	0.09	44.31	9.01	1.52	0.03	0.28	0.02	0.01	1.48	0.29	0.07	40.31	8.53	1.58	0.04	0.29	0.01	0.00
44	Fall 01	0.28	5.43	3.26	71.47	20.29	9.08	0.05	0.34	0.04	0.07	0.70	1.68	0.98	19.84	5.55	3.14	0.02	0.16	0.01	0.01
46	Fall 01	0.26	2.42	6.685	121.1	34.88	9.29	0.07	0.5	0.05	0.09	1.39	1.225	1.745	29.24	8.355	2.97	0.025	0.21	0.025	0.01
49	Fall 01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
60	Fall 01	0.25	0.39	0.39	73.38	18.05	3.87	0.05	0.34	0.01	0.06	6.00	1.22	0.22	30.68	7.32	2.73	0.03	0.22	0.01	0.00
61	Fall 01	0.24	0.28	0.29	17.10	7.05	4.35	0.02	0.11	0.01	0.01	1.08	0.44	0.10	5.88	2.24	2.26	0.02	0.13	0.02	-0.01
62	Fall 01	0.26	2.63	3.31	78.08	25.30	7.21	0.05	0.34	0.04	0.08	1.67	1.35	0.83	19.64	5.91	2.43	0.02	0.21	0.02	0.00
63	Fall 01	0.255	1.515	0.645	103.115	25.53	4.985	0.06	0.435	0.015	0.09	1.55	1.645	0.22	31.595	7.805	2.21	0.035	0.305	0.03	0.005
64	Fall 01	0.30	1.43	3.69	74.50	20.07	5.07	0.05	0.32	0.03	0.09	1.96	1.06	1.53	29.76	8.01	2.73	0.03	0.32	0.05	0.02
65	Fall 01	0.25	0.25	3.99	130.78	38.40	4.17	0.07	0.50	0.03	0.03	3.00	0.53	1.67	55.52	16.26	2.42	0.05	0.39	0.05	0.02
67	Fall 01	0.24	0.48	1.83	108.50	30.10	5.75	0.06	0.44	0.02	0.04	3.53	0.69	0.57	34.34	9.20	2.88	0.04	0.29	0.04	0.01
68	Fall 01	0.27	1.32	13.14	270.99	92.87	6.13	0.12	0.84	0.07	0.09	3.61	0.60	3.11	66.32	22.48	2.14	0.05	0.47	0.04	0.02
WC	Fall 01	0.24	0.04	0.09	67.06	13.92	2.23	0.04	0.27	0.01	0.02	1.38	0.11	0.05	24.28	5.12	1.23	0.03	0.26	0.04	0.00

Table 4

Epilithic algae 1999-2001, sites pooled for date. Relative abundance for taxa over 4%.
Taxa in bold are indicative of acid water.

Label	Site 44	Site 46	Site 60	Site 61	Site 65	Site 67	Wolf Crk
Class size	5	5	5	5	5	5	5
Species ID							
Ach. minutissima	0.0583	0.4210	0.2811	0.3486	0.4396	0.5488	0.0704
Amp. pusilla	0.0021	0.0000	0.0000	0.0000	0.0000	0.0000	0.0805
Eun. minor	0.0139	0.0340	0.0779	0.0297	0.0765	0.0132	0.0000
Gomp. parvulum	0.0249	0.0093	0.0155	0.0250	0.0722	0.0136	0.0065
Nitz. frust.NV	0.0017	0.0027	0.0087	0.0045	0.0070	0.0017	0.0578
Phormidium	0.6109	0.3415	0.1855	0.3708	0.0858	0.1900	0.0064
Stigeoclonium	0.0040	0.0000	0.0016	0.0479	0.0000	0.0014	0.0000
Nav. gregarica	0.0212	0.0018	0.0032	0.0022	0.0006	0.0032	0.1859
Nav. tripunctat	0.0067	0.0000	0.0000	0.0000	0.0000	0.0000	0.0513
Amon. vitrea	0.0140	0.0542	0.2155	0.0091	0.0410	0.0277	0.0007
Eun. exigua	0.0492	0.0135	0.0302	0.0163	0.0096	0.0022	0.0000
Nav. cryptocephala	0.0021	0.0006	0.0035	0.0022	0.0000	0.0011	0.0451
Dent. kuetzingii	0.0001	0.0023	0.0042	0.0011	0.0000	0.0440	0.0013
Diversity HE	1.9600	1.8203	2.3670	1.9505	2.3835	1.8272	3.1026
Richness N(0)	88.0000	59.0000	59,0000	47.0000	61.0000	58.0000	61.0000

Run: Relative abundance of taxa in epipelon 1999-20001, pooled by date. Relative abundnace
Taxa in bold are indicative of acidic conditions.

Label	Site 44	Site 46	Site 60	Site 61	Site 65	Site 67	Wolf Creek
Species ID							
Ach. lanceolata	0.0598	0.1099	0.1543	0.1271	0.1539	0.2797	0.0193
Amp. perpusilla	0.0000	0.0000	0.0000	0.0000	0.0013	0.0000	0.0602
Eun. minor	0.0023	0.0574	0.1152	0.0291	0.0768	0.0846	0.0013
Frag. const. venter	0.0000	0.0006	0.0121	0.0000	0.0210	0.1143	0.0233
Gomp. parvulum	0.0395	0.0308	0.0117	0.0328	0.0356	0.0275	0.0123
Nitz. palea	0.0082	0.0693	0.0237	0.0056	0.0626	0.0546	0.0559
Phormidium sp.	0.7163	0.2423	0.1257	0.5183	0.1534	0.0566	0.0066
Nav. gregarica	0.0036	0.0217	0.0011	0.0028	0.0243	0.0104	0.1720
Nav. lanceolata	0.0000	0.0000	0.0000	0.0010	0.0040	0.0012	0.0443
Anom. vitrea	0.0010	0.0574	0.2728	0.0033	0.1419	0.0442	0.0000
Eun. exigua	0.0320	0.0722	0.0522	0.0430	0.0797	0.0100	0.0006
Euglena sp.	0.0000	0.0417	0.0000	0.0030	0.0000	0.0000	0.0024
Diversity HE	1.4803	2.8956	2.6351	2.1550	2.9905	2.8813	3.5156
Richness N(0)	69.0000	55.0000	62.0000	66.0000	75.0000	56.0000	77.0000

Table 6 Change in percent of epilithic diatoms that are acid indicators.

Site	Fall99	Spr00	Fall00	Spr01	Fall01
Site 44	9.67	23.79	31.23	14.93	22.19
Site 46	14.00	22.98	3.89	15.95	0.89
Site 60	32.54	17.83	14.00	18.94	8.60
Site 61	4.30	17.33	7.00	15.84	2.65
Site 65	9.33	9.00	14.00	15.46	6.89
Site 67	0.97	5.08	1.99	1.97	7.67
Wolf Creek	0	0	0	0	0

Table 7

Change in percent of epipelic diatoms that are acid indicators.

Site	Fall99	Spr00	Fall00	Spr01	Fall01
Site 44	24.54	20.27	20.79	22.00	20.79
Site 46	10.63	28.00	42.57	38.24	13.67
Site 60	20.33	39.40	17.33	39.33	16.07
Site 61	11.21	9.30	22.33	25.00	22.50
Site 65	13.77	27.12	23.38	28.67	14.67
Site 67	14.10	20.20	7.32	12.00	–
Wolf Creek	0.00	0.00	0.00	0.00	0.98

Data: SLIPPERY ROCK CREEK SURBER FALL 1999-FALL 2001

Date: 22 Feb 2002

Table 8 Relative abundance for dominant taxa at each site (dates pooled).

Taxon	Site 44	Site 46	Site 61	Site 65	Wolf Crk	Site 60
Antocha	0.0090	0.0000	0.0000	0.0000	0.0414	0.0000
Hexotoma	0.0757	0.0000	0.0864	0.0000	0.0014	0.0000
Tipula	0.1368	0.0000	0.0721	0.0108	0.0000	0.0000
Tabanus	0.0829	0.0000	0.0200	0.0000	0.0000	0.0000
Hydropsuyche	0.0222	0.0000	0.0082	0.0000	0.0871	0.1567
Cheumatopsyche	0.0273	0.0000	0.0375	0.2046	0.1584	0.7236
Oulimnius	0.0000	0.0000	0.0635	0.0270	0.0084	0.0000
Optioservus	0.0000	0.0000	0.0586	0.2500	0.2209	0.0000
Nigronia	0.0287	0.0000	0.0556	0.0108	0.0058	0.0000
Neohermes	0.0000	0.0000	0.0000	0.0556	0.0000	0.0000
Orthocladius	0.0000	0.0000	0.0000	0.0000	0.0722	0.0000
Parametriocnemus	0.0222	0.0000	0.0726	0.0402	0.0000	0.0000
Eukiefferiella	0.1001	0.0000	0.0112	0.0000	0.0412	0.0000
Telopelopia	0.0476	0.0000	0.0010	0.0000	0.0012	0.0000
Baetidae	0.0000	0.1667	0.0089	0.0000	0.0000	0.0000
Isonychia	0.0000	0.0000	0.0000	0.0000	0.0875	0.0000
Perloidae	0.0667	0.0000	0.0060	0.0000	0.0000	0.0000
Paracapnia	0.0142	0.0000	0.0959	0.0108	0.0022	0.0000
Lanthus	0.0000	0.0000	0.0615	0.0000	0.0006	0.0000
Gomphus	0.0000	0.1667	0.0000	0.0000	0.0000	0.0000
Gammarus	0.0000	0.0000	0.0000	0.1280	0.0278	0.0000
Oligochaeta	0.0666	0.5667	0.0191	0.0000	0.0000	0.0000
Diversity	2.9844	1.1494	3.3098	2.4905	2.7700	0.9933

Table 9 EPT values for invertebrates.

Site	Fall 1999	Spring 2000	Fall 2000	Spring 2001	Fall 2001
44	0	1	0	3	1
46	0	1	-	-	-
60	0	0	0	0	5
61	8	7	6	5	6
65	2	0	4	1	4
Wolf Creek	8	9	9	7	8

Figure Captions

Fig. 1. Map of the hadwaters of Slippery Rock Creek indicating the location of sample sites, passive treatment systems for acid mine drainage discharges, and the area of land reclamation. Wolf Creek is approximately 30 km west of the headwaters and not on the map..

Fig. 2. Change in pH at the sample stations.

Fig. 3. Change in alkalinity at the sample stations.

Fig. 4. Temporal change in pH at Site 68 (Seaton Creek) for the period 1995-2001. Best fit line, not tested for significance.

Fig. 5. Temporal change in alkalinity at Site 68 (Seaton Creek) for the period 1995-2001. Best fit line, not tested for significance.

Fig. 6. Temporal change in pH at Site 65 for the period 1995-2001. Best fit line, not tested for significance.

Fig. 7. Temporal change in alkalinity at Site 65 for the period 1995-2001.Best fit line, not tested for significance.

Fig. 8. Temporal change in pH at Site 67 for the period 1995-2001.Best fit line, not tested for significance.

Fig. 9. Temporal change in alkalinity at Site 67 for the period 1995-2001. Best fit line, not tested for significance.

Fig. 10. Change in dissolved iron concentration at the sample stations.

Fig. 11. Temporal change in dissolved iron at Site 67 for the period 1995-2001. Best fit line, not tested for significance.

Fig. 12. Temporal change in dissolved iron at Site 65 for the period 1995-2001. Best fit line, not tested for significance.

Fig. 13. Temporal change in dissolved iron at Site 68 for the period 1995-2001. Best fit line, not tested for significance.

Fig. 14. The relationship between discharge and alkalinity at Site 60. Best fit line, not tested for significance.

Fig. 15. The relationship between discharge and alkalinity at Site 64. Best fit line, not tested for