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Impaired *Acroneuria* sp. (Plecoptera, Perlidae) Populations Associated with Aluminum Contamination in Neutral pH Surface Waters

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Abstract. Our aim was to quantify impairment to invertebrate predator populations, particularly to *Acroneuria* sp. (Plecoptera, Perlidae), downstream of an acid mine drainage-impacted tributary to the North Fork of the Powell River, southwestern Virginia. Predatory insects comprised 9.0 ± 1.3% of the total abundance at the three stations upstream of the impacted tributary, but were significantly reduced ($p = 0.0039$) downstream (3.9 ± 0.6%). *Acroneuria* sp. populations followed the same trend, with the upstream average (2.3 and 2.8%) being significantly higher ($p < 0.05$) than the downstream averages (0.2 and 0%) during 1999 and 2000, respectively. Using correlation analysis, we evaluated the relationship between the percent abundance of *Acroneuria* sp. throughout this reach and metal concentrations in water, sediment, and biological tissues (invertebrate predators and primary consumers). Water column aluminum (Al) concentration was the only parameter that was significantly correlated with percent *Acroneuria* sp. abundance, with correlation coefficients of −0.845 and −0.873 during 1999 and 2000, respectively. While this correlation exists, it may not indicate a causal relationship, and experiments should be conducted to determine the long-term toxicity of various Al species to perlid stoneflies.

Numerous studies have documented the impacts of acid mine drainage (AMD) upon aquatic communities within acidified stream reaches (Armitage 1980; Kelly 1988). These impacts include reduced taxonomic richness and abundance, and/or a shift from pollution-sensitive to pollution-tolerant species. Beyond the zone of pH depression, a variety of community impacts have been documented. For example, sediment toxicity and bioaccumulation of metals such as copper (Cu), cadmium (Cd), and zinc (Zn) have been associated with benthic macroinvertebrate impairment in hard-rock mine impacted areas (Kemble *et al.* 1994; Besser *et al.* 2001; Kiffney and Clements 1993). Solid iron (Fe) flocculent (a product of pyrite oxidation) is thought to cause ecosystem impairment by smothering habitat and reducing primary productivity (McKnight and Feder 1984; Scullion and Edwards 1980); it also can cause direct acute toxicity to *Daphnia magna* in the absence of dissolved Fe (Soucek *et al.* 2000a). In addition, aluminum (Al) has been observed to cause acute and chronic toxicity to fish and invertebrates in circumneutral mixing zones below acidic tributaries (Campbell *et al.* 2000; Rosseland *et al.* 1992; Soucek *et al.* 2001). These findings indicate that AMD is a complex pollutant that can impair aquatic ecosystems in a variety of ways.

The Clinch-Powell River system of northeastern Tennessee and southwestern Virginia, identified by The Nature Conservancy as a conservation priority of national importance, has experienced a decline in biodiversity (Chaplin *et al.* 2000). The decline in Powell River unionid mussel communities in particular has largely been attributed to coal mining activity (McCann 1993; Neves 1991). There are at least 10 AMD impacted tributaries in the upper Powell watershed, but the effects of these tributary level inputs upon the biota of the mainstem of the Powell and its North Fork (NFP) are not well characterized.

The most severely impacted sub-watershed within the NFP is Stone/Straight Creek, because of input from two highly impacted tributaries, Ely and Puckett's Creeks (Cherry *et al.* 2001, Soucek *et al.* 2000b). Community diversity and taxonomic richness are not significantly impaired and pH is not depressed in the NFP downstream of Stone/Straight Creek relative to upstream levels (Soucek *et al.* 2001b); however, during the course of a two-year study, we observed a reduction in the abundance of *Acroneuria* sp., a predatory stonefly (Plecoptera) of the family Perlidae, below this tributary. The purpose of this study was to quantify the impairment to invertebrate predators in general and particularly to *Acroneuria* sp. populations. We further attempted to determine if the observed impairment was associated with water column or sediment metal concentrations, or with bioaccumulation of metals in tissues via food-borne uptake.

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Materials and Methods

Sampling Stations

This study was conducted during the Summers (June through August) of 1999 and 2000. Six sampling stations were selected in the North Fork of the Powell River (NFP), Lee County, VA, USA (Figure 1). Three stations were located upstream of Stone/Straight Creek, at NFP km-10 (Cherry *et al.* 2001, Soucek *et al.* 2000b), while the remaining three were downstream of the tributary. The last one was located just upstream of the NFP confluence with the Powell River mainstem (NFP km-0). To determine if the size of the river was a factor in reduced predator numbers, a seventh site was sampled for benthic macroinvertebrates in the second year of the study. This site was at Fletcher Ford, a freshwater mussel preserve ~57 km downstream of the NFP/Powell River confluence.

Benthic Macroinvertebrate Sampling

Benthic macroinvertebrate surveys were conducted in June of both years according to the U.S. Environmental Protection Agency Rapid Bioassessment Protocols (RBP) with slight modifications (Barbour *et al.* 1999). Modifications of RBP protocols included the fact that riffle, run, pool, and shoreline rooted areas were sampled for 5 min per site using dip-nets with 800-μm mesh dipnet, rather than for 20 "jabs" with a 500-μm mesh dipnet. Two replicate samples were collected per site and mean values for all indices were calculated for each site during each year. All organisms in each sample were identified to the lowest practical taxonomic level (usually genus) using standard keys (Merritt and Cummins 1996; Pennak 1989). Chironomids were identified as either subfamily Tanypodinae or non-Tanypodinae. Each taxon identified was assigned to a functional feeding group as described in Merritt and Cummins (1996). Feeding groups included collectors, shredders, scrapers, and predators. Tanypod chironomids were considered predators, and non-tanypod chironomids were collectors. Percent abundance of each functional feeding group was calculated (e.g., number of predators divided by the number of organisms in a sample = % predators), as was percent *Acroneuria* sp. abundance. Mean values for percent predator abundance and percent *Acroneuria* sp. abundance for the three stations upstream of Stone/Straight Creek were compared to those at the three downstream stations using Student's T-test in the JMP-IN® software package (Sall and Lehman 1996) with significance determined at the $\alpha = 0.05$ level.

Water Column and Sediment Chemistry/Metals

Water samples were collected at each station for analysis of selected water quality parameters during June and August of each year. Samples either were analyzed in the field or were brought to the laboratory and stored for 24 h at 4°C before measurements were taken under laboratory conditions. Sample pH was measured using an Accumet® (Fisher Scientific, Pittsburgh, PA, USA) pH meter equipped with an Accumet® gel-filled combination electrode (accuracy $< \pm 0.05$ pH at 25°C). Conductivity measurements were made using a Hach® (Hach, Loveland, CO, USA) conductivity/TDS meter. Dissolved oxygen (measured in the field), alkalinity, and hardness were analyzed according to standard methods (APHA *et al.* 1995).

Sediment samples were collected using a polyurethane scoop, placed in freezer bags and stored at 4°C until analysis for metals. Both sediment and water column samples were prepared for metals analysis according to standard methods (US EPA 1991). Samples were analyzed at the Virginia Tech Soil Testing Laboratory via inductively coupled plasma (ICP) spectrometry for total recoverable (i.e., unfiltered) aluminum (Al), iron (Fe), copper (Cu), and zinc (Zn), which were previously determined to be the dominant four metals in the system (Soucek *et al.* 2000b). Lower detection limits for digested samples (μg/L) were 1.0, 2.0, 1.7, and 0.5 for Al, Fe, Cu, and Zn, respectively, and sediment concentrations (mg/kg) were calculated from aqueous digestion concentrations according to dry weight (USEPA 1991).

Bioaccumulation Study

To determine if bioaccumulation and/or biomagnification of metals from insects at the primary consumer level to predatory insects was associated with *Acroneuria* sp. population variations, an additional set of benthic macroinvertebrate samples was collected in August 2000 for measurement of metal concentrations. Available habitat was sampled qualitatively using a D-frame net, and organisms were sorted and identified in the field according to functional feeding groups (Merrit and Cummins 1996). Predators and primary consumers (including shredders, scrapers, and collectors) were placed in separate vials and chilled but not frozen for 24 h to allow organisms to clear their gut contents. Samples then were frozen and prepared for metals analysis according to standard methods (U.S. EPA 1991). Tissues were analyzed for Al, Fe, Cu, and Zn at the Virginia Tech Soil Testing Laboratory. Lower detection limits for digested samples (μg/L) were again 1.0, 2.0, 1.7, and 0.5 for Al, Fe, Cu, and Zn, respectively, and tissue concentrations (mg/kg) were calculated from aqueous digestion concentrations according to dry weight (US EPA 1991).

Using average metal concentrations for predators and primary consumers at the six sites, bioaccumulation factors (BF) were calculated for the four analyzed metals. For example, the average concentration of Cu in predatory insects for the six sampling stations was divided by the average concentration in primary consumers at the six sites. The same process was used for the other three metals, and the mean concentrations ($n = 6$) for predators and primary consumers were compared statistically with Student's t-test using JMP-IN® software (Sall and Lehman 1996). Thus, if for a given metal, $BF > 1$ and the mean values were significantly different, biomagnification occurred. If $BF < 1$ and means were significantly different, trophic dilution occurred. Simple transfer ($BF = 1$) occurred if mean concentrations in predators and primary consumers were not significantly different (Newman 1995).

Bivariate Correlation Analysis

To statistically evaluate factors associated with *Acroneuria* sp. numbers downstream of the Stone/Straight Creek tributary, bivariate correlation analysis was conducted using JMP-IN® software (Sall and Lehman 1996). Percent abundance of *Acroneuria* sp. was used as the dependent variable, and metal concentrations in sediment, water column, predatory insects, and primary consumers were the independent variables. Water column metals data were available for both 1999 and 2000, so separate analyses were conducted for both years. Significance of correlation was determined at the $\alpha = 0.05$ level.

Results

Benthic Macroinvertebrate Sampling

Generally, two-year average values indicated that shredders decreased in relative abundance from ~25% at the furthest

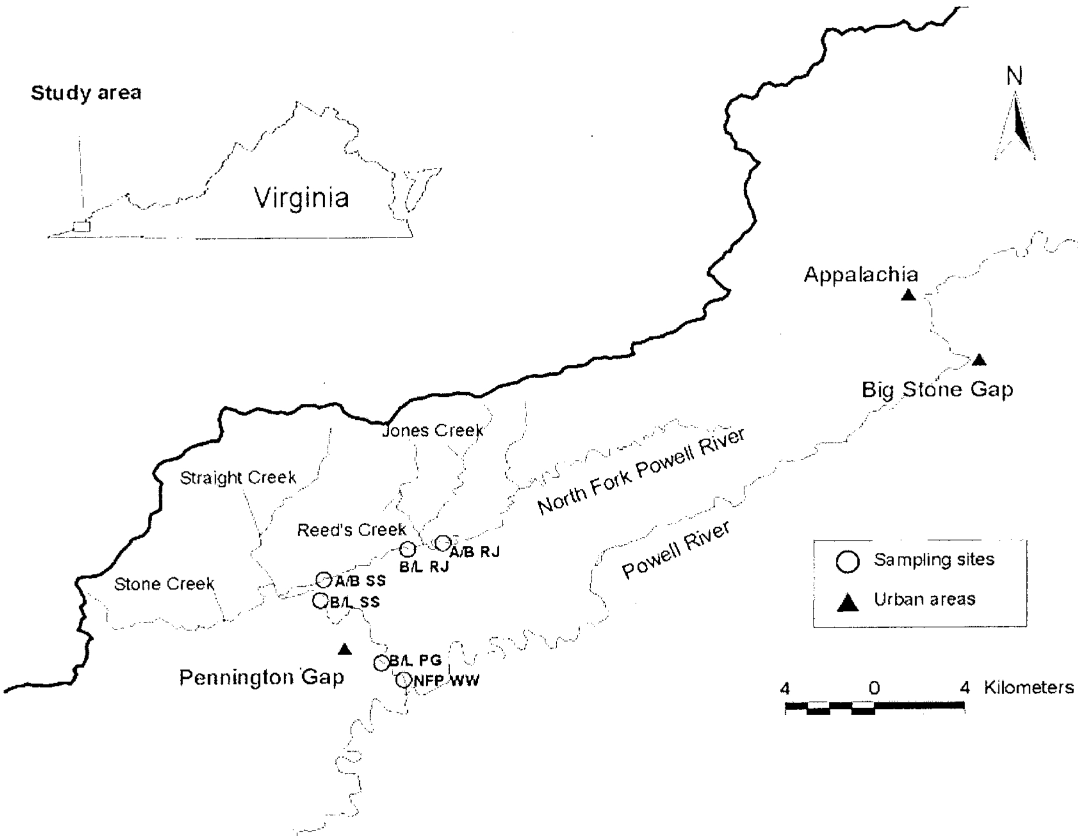

Fig. 1. Map of study area indicating sampling stations. A/B = above; B/L = below; RJ = Reed/Jones Creek; SS = Stone/Straight Creek; PG = Pennington Gap; NFP WW = North Fork Powell at Woodway

upstream station to ~5% just upstream of the confluence with the Powell mainstem (Figure 2). Conversely, there was a general trend of increasing relative abundance of scrapers from the furthest upstream site (~22%) to the bottom one (~47%). Percent abundance of collectors was variable from site to site, ranging from ~43% to 71%, but decreasing again to ~43% at the furthest downstream site. Two-year average values for percent abundance of predatory invertebrates dropped substantially beginning at the station downstream of Stone/Straight Creek (Figure 2). Comparing averages for the three stations upstream of Stone/Straight to those for the three downstream stations, predatory invertebrates comprised an average of 9.0 ± 1.3% of the total abundance at the upstream stations, but were significantly reduced ($p = 0.0039$) at the three downstream stations (3.9 ± 0.6%).

Acroneuria sp. populations followed the same trend as that of predatory invertebrates in general (Figure 3). During 1999, the average (n = 3) percent *Acroneuria* sp. abundance was significantly higher upstream of Stone/Straight Creek (2.3 ± 0.5%) than the downstream average (0.2 ± 0.3%), and averages were similarly significantly different in 2000 (2.8 ± 0.1% upstream and 0 ± 0% downstream). Actual average abundances for the upstream (n = 3) and downstream (n = 3) sites were 7.2 ± 2.4 and 0.1 ± 0.2, respectively, for 1999, and 5.4 ± 0.5 and 0, respectively, for 2000. In both years, upstream mean abundances were significantly higher than the downstream averages ($p < 0.05$). While no perlid stoneflies were found in

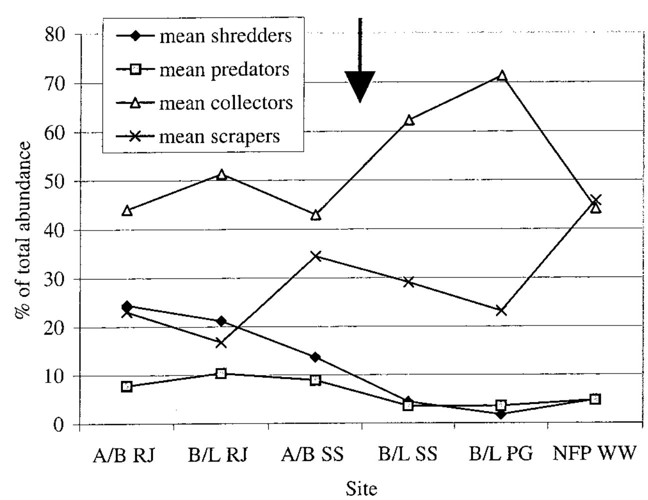

Fig. 2. Two-year mean values for benthic macroinvertebrate functional feeding groups at six sites moving from upstream to downstream in the North Fork of the Powell River. Arrow indicates the point at which Stone/Straight Creek enters the North Fork. A/B = above; B/L = below; RJ = Reed/Jones Creek; SS = Stone/Straight Creek; PG = Pennington Gap; NFP WW = North Fork Powell at Woodway

the three downstream stations during the 2000 sampling season, percent predator abundance was 10.1% and *Acroneuria* sp.

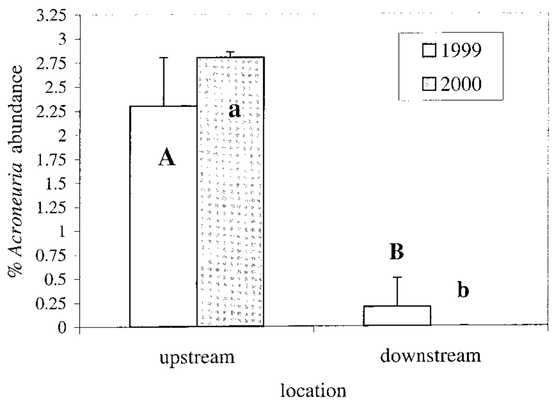

Fig. 3. Percent *Acroneuria* abundance at stations upstream (n = 3) and downstream (n = 3) of the Stone/Straight Creek tributary to the North Fork Powell River for 1999 and 2000. Different upper or lower case letters indicate significantly different means ($p < 0.05$)

comprised 1.4% of the total abundance at Fletcher Ford, ~57 km downstream of the NFP/Powell mainstem confluence.

Water Column and Sediment Chemistry/Metals

In general, water column Al concentrations were higher throughout the system in 1999 than in the 2000 sampling season, with a general trend of increased concentrations of Al at the station below Stone/Straight Creek in both years (Table 1). The two-year average values for the three stations upstream of Stone/Straight ranged from 15 to 24 μg Al/L, while the average value for the station below Stone/Straight was 51 μg Al/L, decreasing to 48 and 33 μg Al/L moving further downstream. The trend for Fe concentrations was not as distinct (Table 1). Concentrations of Cu and Zn in the water column were always below detection limits (1.7 and 0.5 μg/L, respectively). Average values for pH, conductivity, alkalinity, and hardness were consistent throughout the system with average values of 7.9 ± 0.1, 486 ± 56 μS/cm, 75 ± 22 mg/L as $CaCO_3$, and 165 ± 5 mg/L as $CaCO_3$, respectively. Dissolved oxygen was always at saturation.

Sediment metals concentrations were variable and did not follow a distinct trend; Al and Cu concentrations were lower at the first station downstream of Stone/Straight Creek (B/L SS) than those at the station just upstream of the tributary (A/B SS, Table 1). Concentrations of Fe in sediments were virtually the same at those two sites while Zn increased nominally at B/L SS.

Bioaccumulation Study

No general trend of increased tissue metal concentrations was observed downstream of Stone/Straight Creek; in fact, concentrations of all four metals were lower in predators downstream of the tributary than at the station just upstream of Stone/Straight Creek (Table 2). Comparing average tissue metal concentrations at the six stations, the general trend was that predators had lower concentrations than primary consumers (Figure 4). Concentrations in primary consumers were significantly higher ($p < 0.05$) than in predators for Al (24.6 and 11.6 g/kg, respectively), Fe (39.7 and 20.3 g/kg, respectively), and Zn (329.3 and 182.2 mg/kg, respectively). The average BF values of 0.48, 0.52, and 0.55 for Al, Fe, and Zn, respectively, indicated "trophic dilution." Concentrations of Cu were not

Table 1. Water column and sediment metal concentrations for two years at sites within the North Fork of the Powell River

	Water column metals					
	Al (μg/L)			Fe (μg/L)		
	1999	2000	mean	1999	2000	mean
A/B RJ	30	1	15	41	93	67
B/L RJ	47	1	24	79	93	86
A/B SS	41	5	23	71	103	87
B/L SS	56	45	51	53	137	95
B/L PG	62	35	48	65	124	95
NFP WW	57	9	33	44	90	67

	Sediment metals			
	Al (g/kg)	Fe (g/kg)	Cu (mg/kg)	Zn (mg/kg)
A/B RJ	19	105	6	330
B/L RJ	49	261	6	343
A/B SS	28	135	17	360
B/L SS	25	136	11	391
B/L PG	30	235	5	349
NFP WW	31	281	10	365

Yearly values are averages for two sampling occasions, and mean values are averages for both years.
Water column Cu and Zn levels were below detection limits.
A/B = above; B/L = below; RJ = Reed/Jones Creek; SS = Stone/Straight Creek; PG = Pennington Gap; NFP WW = North Fork Powell at Woodway.

Table 2. Metal concentrations in predatory insect and potential prey insect tissues at sites within the North Fork of the Powell River

	Predatory insects			
	Al (g/kg)	Fe (g/kg)	Cu (mg/kg)	Zn (mg/kg)
A/B RJ	9.0	14.4	23.3	239.7
B/L RJ	7.2	14.4	20.9	189.2
A/B SS	18.2	34.7	22.1	229.1
B/L SS	9.2	16.4	18.1	196.7
B/L PG	14.2	27.8	25.1	158.0
NFP WW	14.9	20.1	47.8	141.4

	Primary consumers (prey)			
	Al (g/kg)	Fe (g/kg)	Cu (mg/kg)	Zn (mg/kg)
A/B RJ	26.9	42.9	10.8	305.4
B/L RJ	25.7	41.4	24.1	459.1
A/B SS	24.1	41.5	18.3	282.3
B/L SS	22.4	36.1	19.4	389.7
B/L PG	24.2	39.4	15.9	255.1
NFP WW	25.4	42.3	21.1	369.4

A/B = above; B/L = below; RJ = Reed/Jones Creek; SS = Stone/Straight Creek; PG = Pennington Gap; NFP WW = North Fork Powell at Woodway.

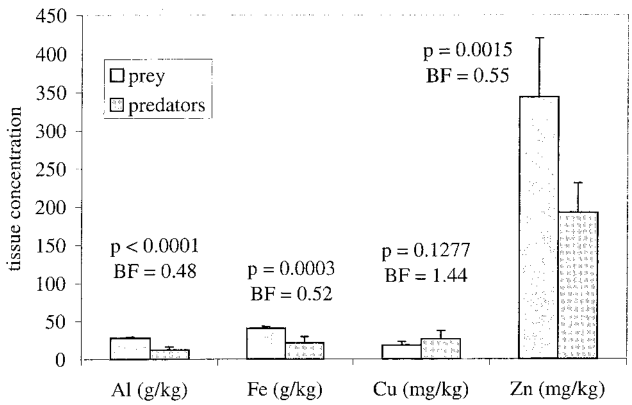

Fig. 4. Tissue concentrations of four metals in benthic macroinvertebrates of two trophic levels. Values shown are means (± SD) for the six sampling sites in the North Fork of the Powell River. BF = bioaccumulation factor, prey = primary consumers

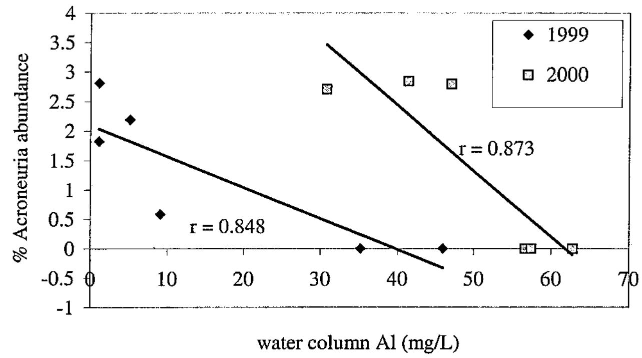

Fig. 5. Correlation analysis of water column Al concentrations and percent abundance of *Acroneuria* at the six sampling stations in 1999 and 2000. Both correlations are significant ($p < 0.05$)

significantly different ($p = 0.1277$) between predators (26.6 mg/kg) and primary consumers (18.8 mg/kg) indicating that the BF value of 1.44 was not significantly different from 1.0, and that "simple transfer" occurred.

Bivariate Correlation Analysis

Water column Al concentration was significantly ($p < 0.05$) correlated with percent *Acroneuria* abundance in the NFP. During both 1999 and 2000, strong negative correlation coefficients (−0.848 and −0.873, respectively) were obtained between the two parameters (Figure 5). Bivariate correlation analysis failed to produce significant correlations between percent *Acroneuria* abundance and any of the other parameters measured in this study.

Discussion

Percent abundances of non-predatory functional-feeding groups changed moving from the furthest upstream to the furthest downstream station, but the changes could largely be explained by the River Continuum Concept (Vannote *et al.* 1980). For example, percent abundance of shredders decreased moving downstream; this was expected because the amount of coarse particulate organic matter (CPOM), upon which shredders feed, decreases with increasing stream size. Conversely, scrapers increased in percent abundance, a phenomenon associated with increased amounts of primary production (periphyton) as stream size increases. Relative abundance of collectors was variable, with the largest value occurring at the site below the city of Pennington Gap, potentially due to dilute sewage inputs.

Although relative abundance of predators should remain fairly consistent with increasing stream size (Vannote *et al.* 1980), their numbers dropped significantly below Stone/Straight Creek. In fact, *Acroneuria* sp. was only found at one site below Stone/Straight Creek on one occasion during the two-year study; however, it was found much further downstream in a year when it was not collected in samples at the three stations downstream of Stone/Straight Creek, suggesting that the phenomenon was not a function of stream size. Stoneflies are known to be sensitive to chemical degradation in Appalachian streams, especially metal inputs, as part of the EPT (Ephemeroptera-Plecoptera-Trichoptera) index (Soucek *et al.* 2000b; Wallace *et al.* 1996). Density of *Acroneuria* sp. has been observed to be reduced by overflow from fly-ash settling ponds (Specht *et al.* 1984), and experimental studies have demonstrated the sensitivity of other stoneflies to extreme acidic and alkaline pH (Lechleitner *et al.* 1985) and various pesticides (Breneman and Pontasch 1993; Harrahy *et al.* 1994).

According to our analyses, the factor most strongly correlated with variation in *Acroneuria* sp. numbers from site to site during both sampling seasons was total Al in the water column. While Al generally is not thought to be a toxic influence in neutral pH surface waters because of its low solubility, studies suggesting otherwise are accumulating. These include laboratory studies with *Daphnia magna* (Havas 1985), and mixing zone studies with fish (Rosseland *et al.* 1992). In addition, recent work (Campbell *et al.* 2000) indicates that snail behavior is significantly altered by grazing upon extracellular mucopolysaccharides that have bound polyhydroxy-Al at neutral pH. Furthermore, we have observed acute toxicity to *Ceriodaphnia dubia* at pH > 7.0 when organisms were exposed to $\sim$1.3 to 2.8 mg Al/L shortly after acidic solutions were diluted and neutralized (Soucek *et al.* 2001). The Criterion Continuous Concentration (CCC) for Al (the estimate of the highest concentration to which aquatic communities can be exposed indefinitely without unacceptable effects) is 87 μg/L at pH 6.5 to 9.0 (U.S. EPA 1999). While the average Al concentration downstream of Stone/Straight Creek was only 50 μg/L, individual measurements at this site were as high as 89.9 μg/L. Chronic continuous exposure to these concentrations of Al may be toxic to perlid stoneflies.

Aquatic invertebrates are not considered to be as sensitive to Al as fish are, and the toxic effects are generally considered to be of an ionoregulatory nature under slightly acidic conditions (Gensemer and Playle 1999). Several authors have suggested that the mode of Al toxicity to fish is transformation of Al^{3+} to polymers/colloids by hydrolysis with increased pH. Fish gills then act as a nucleating surface onto which the polymers precipitate, leading to suffocation (Exley *et al.* 1996; Neville and Campbell 1988). Because perlid stoneflies have large external gills, they may be more susceptible to Al toxicity than many other benthic macroinvertebrates, and Guerold *et al.* (1995) have shown that, under acidic conditions, Al has a strong affinity for chloride cells located on the gills and thoracic segments of *Perla*, another perlid stonefly. Laboratory experiments and field transplant studies should be conducted to verify the hypothesis that the gill structure or size of perlids makes them sensitive to Al precipitation under neutral pH conditions.

The results of the resident invertebrate whole body metals analysis did not provide support to the hypothesis that bioaccumulation of metals through water, sediment, or prey items was the cause of decreased predator percent abundance downstream of Stone/Straight Creek. In fact metal concentrations in predators (no perlid stoneflies were collected) were lower downstream of the impacted tributary than they were upstream. Further, mean tissue concentrations in predators were lower than those found in primary consumers at the six stations, as has been observed in other studies (Smock 1983). Some have suggested that Zn increases in concentration at higher invertebrate trophic levels (Timmermans *et al.* 1989); however, most studies indicate that Cu and Zn are not biomagnified in predators (Goodyear and McNeil 1999). Our results support this contention, and our BF values for Cu (1.44) and Zn (0.55) are similar to mean values of 1.2 (range of 1.0 to 1.9) for Cu and 0.31 (range of 0.15 to 0.63) for Zn found by Besser *et al.* (2001). Thus, while accumulation of metals in tissues has been documented to impair benthic macroinvertebrate communities and food webs (Beltman *et al.* 1999; Besser *et al.* 2001), the reduction in predators in general and particularly *Acroneuria* populations downstream of Stone/Straight Creek does not appear to be associated with food-borne metal uptake.

In conclusion, we have observed the virtual elimination of the perlid stonefly, *Acroneuria*, downstream of an AMD impacted tributary in the North Fork of the Powell River. Despite the fact that stream order can play a role in structuring benthic macroinvertebrate communities (Vannote *et al.* 1980), its occurrence further downstream at Fletcher Ford indicates that this disappearance is not associated with the size or discharge of the river. We evaluated the relationship between *Acroneuria* numbers throughout this reach and metal concentrations in water, sediment, and biological tissues (invertebrate predators and primary consumers), and found that the strongest association was with water column Al concentrations. We hypothesize that the large gill structure of these organisms may play a role in their apparent sensitivity to Al. While this correlation exists, it does not indicate a causal relationship, and studies of Al impacts on perlids should be conducted to provide support to the hypothesis that Al is the cause of impaired *Acroneuria* populations downstream of Stone/Straight Creek.

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