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STREAM PERIPHYTON AND BENTHIC INSECT RESPONSES TO ADDITIONS OF TREATED ACID MINE DRAINAGE IN A CONTINUOUS-FLOW ON-SITE MESOCOSM

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Abstract—An on-site continuous-flow trough mesocosm was used to examine changes in the composition and abundance of periphytic algae and benthic invertebrates from additions of a solution of treated acid mine drainage (AMD). Five control and five treatment troughs supported an assemblage of periphyton and invertebrates that colonized from water withdrawn from Foxy Creek, a stream that receives limed AMD from the Equity Silver Mine, central British Columbia, Canada. A water intake for the mesocosm was located upstream of the AMD discharge. The treated AMD was delivered to the apparatus through a pipeline laid in a canal that carried the AMD to Foxy Creek. After three weeks of colonization in the troughs, additions of one part AMD to 10 parts Foxy Creek water was delivered to the treatment troughs and continued for three weeks. Analyses of variance of measurements of abundance and biomass indexes contained high power values and indicated that the AMD addition did not significantly change the algal and insect composition and abundance. Advantages and disadvantages of the mesocosm with regard to the relative sensitivity of the measured parameters for use in examining effects of the AMD additions are discussed. The conclusion was that quantitative on-site experimentation using the mesocosm apparatus is a powerful approach in setting guidelines for AMD discharge mainly due to its capability of integrating ecosystem processes in experiments where hypothesis testing is possible.

Keywords—Mesocosm   Periphyton   Benthos   Acid mine drainage (AMD)

INTRODUCTION

Acid mine drainage (AMD) is a widespread environmental problem that has been examined extensively in the literature. Most reports of effects of AMD on aquatic biota are laboratory bioassays of single taxa [1]. Field studies are less common and deal mainly with monitoring temporal changes in the chemical milieu and biological community in response to AMD additions [2–4]. Only a few studies have used experimental approaches to examine in situ effects of AMD [5]. Largely due to logistic constraints (i.e., examining in situ effects after an AMD discharge has occurred), most of these field studies are upstream-to-downstream comparisons of water-quality indexes, lacking the necessary controls to infer causality.

Despite the abundance of these data, predictions of stream ecosystem responses to AMD additions have been crude at best, given that complex ecosystem processes that may be affected by a variety of chemicals [6] cannot be distinguished in most single-organism bioassays or monitoring studies. The general inability to extrapolate findings from the laboratory to the field leaves water-quality managers with considerable uncertainty in setting allowable dilution rates of AMD that is discharged into streams. The general lack of information to indicate sublethal effects and to predict food-web or geochemical interactions [7] demands site-specific, in situ experiments that allow testing of explicit hypotheses at the community or ecosystem level.

A continuous-flow, on-site, mesocosm apparatus was used to test the null hypothesis that the di-

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lution of 10 parts water to one part treated AMD does not affect the composition and abundance of periphytic algae or benthic macroinvertebrates in Foxy Creek, a stream that receives discharge of limed AMD from the Equity Silver Mine, located in north-central British Columbia, Canada. The 10:1 dilution ratio was established by regulatory agencies but is presently not supported with technical rationale to show that the dilution is adequate to protect downstream ecosystems.

The use of a stream mesocosm has many advantages over other techniques used for testing toxic effects of AMD. Streams function as open systems, and the flow-through design of a trough, having suitable substrata to support a representative biotic assemblage, provides for exchange of components necessary in geochemical dynamics, behavioral interactions of organisms, food-web interactions, and so forth. Biological processes are integrated in a trough mesocosm, as they are in a natural stream, thus allowing for extrapolation of findings to natural ecosystems [8]. As stream water continuously flows through the troughs, system processes can be allowed to equilibrate, then a perturbation from this central state can be measured. The ability to replicate experimental units in a mesocosm also provides for a true experimental approach with appropriate controls for the testing of hypotheses, which is generally not possible in other field monitoring studies. The power of significance tests [9,10] can be determined to provide estimates of the ability of the tests to detect real differences. Uncertainty is then removed in the process of setting regulations for AMD. Considering these advantages, it is not surprising that experiments using mesocosms have been increasingly used to examine a variety of toxicants, including acidification [11] and pesticides [12], as well as for examining basic ecosystem processes [13].

Disadvantages to mesocosm studies relate to constraints on realism, including the lack of structural heterogeneity in streams, lack of normal variation in rates of discharge, and the short-term nature of such studies. Many of these variables, however, can be manipulated in mesocosm designs to make the system as simple or as complex as required. Mesocosms also have short water residence times that may not allow chemical equilibria to become established after additions of mixtures of metals. Processes in the mesocosm generally represent worst-case conditions for biota because many metals remain bioavailable within the troughs yet may be complexed or precipitated downstream and become less bioavailable. An important factor is that the fate of organisms emigrating from the mesocosm is unknown. Although the fate of animals may be important in toxicity tests of individual taxa (in which case a mesocosm would likely not be used), the actual fate of animals was not important in this study. Whether animals emigrated from the troughs as a behavioral response to additions of AMD or whether they died from the AMD was irrelevant. Any selective disappearance of taxa from a mesocosm receiving AMD was considered evidence of an impact of the AMD additions.

Throughout this study, the treated AMD (raw AMD that is limed to increase the pH from 2.2 to about 8) is considered a single chemical entity despite its well-known complexity. There was little point in examining the toxicity of individual constituents of the AMD when interactions and speciation of chemicals are difficult or impossible to predict [14]. Most important is that the experiment was designed as an on-site test of system responses to routine AMD discharge.

MATERIALS AND METHODS

The experiment was conducted at the confluence of Foxy Creek and a drainage canal that carried treated AMD from the Equity Mine site (Fig. 1). This location allowed a pipeline to be laid upstream in Foxy Creek to supply water to the mesocosm, and it facilitated a second pipeline to be laid in the canal to supply the treated AMD.

The mesocosm consisted of 10 flow-through troughs (each 1.52 m long × 0.2 m high × 0.2 m wide) that were fabricated with 0.64 cm clear Plexiglas® and assembled on a series of joists laid over the stream channel. The design has been described in a previous article [15]. Water and biota carried in suspension in Foxy Creek were supplied to the mesocosm by gravity through a 100-m (15.24 cm diameter) pipeline installed in Foxy Creek. The pipeline was fitted to a head tank, and water was delivered to each trough through a standpipe assembly. The standpipe for each trough could be slightly rotated to maintain water flow at 0.4 $L \cdot s^{-1}$ without the use of valves. The treated AMD was delivered by gravity through the second 260-m (5 cm diameter) pipeline laid in the Lu Creek diversion canal (Fig. 1), which carried the treated AMD from the water treatment facilities at the mine. A series of gate valves on the trough apparatus controlled the flow of AMD to the troughs to maintain a 10 part water to 1 part AMD flow dilution ratio, the

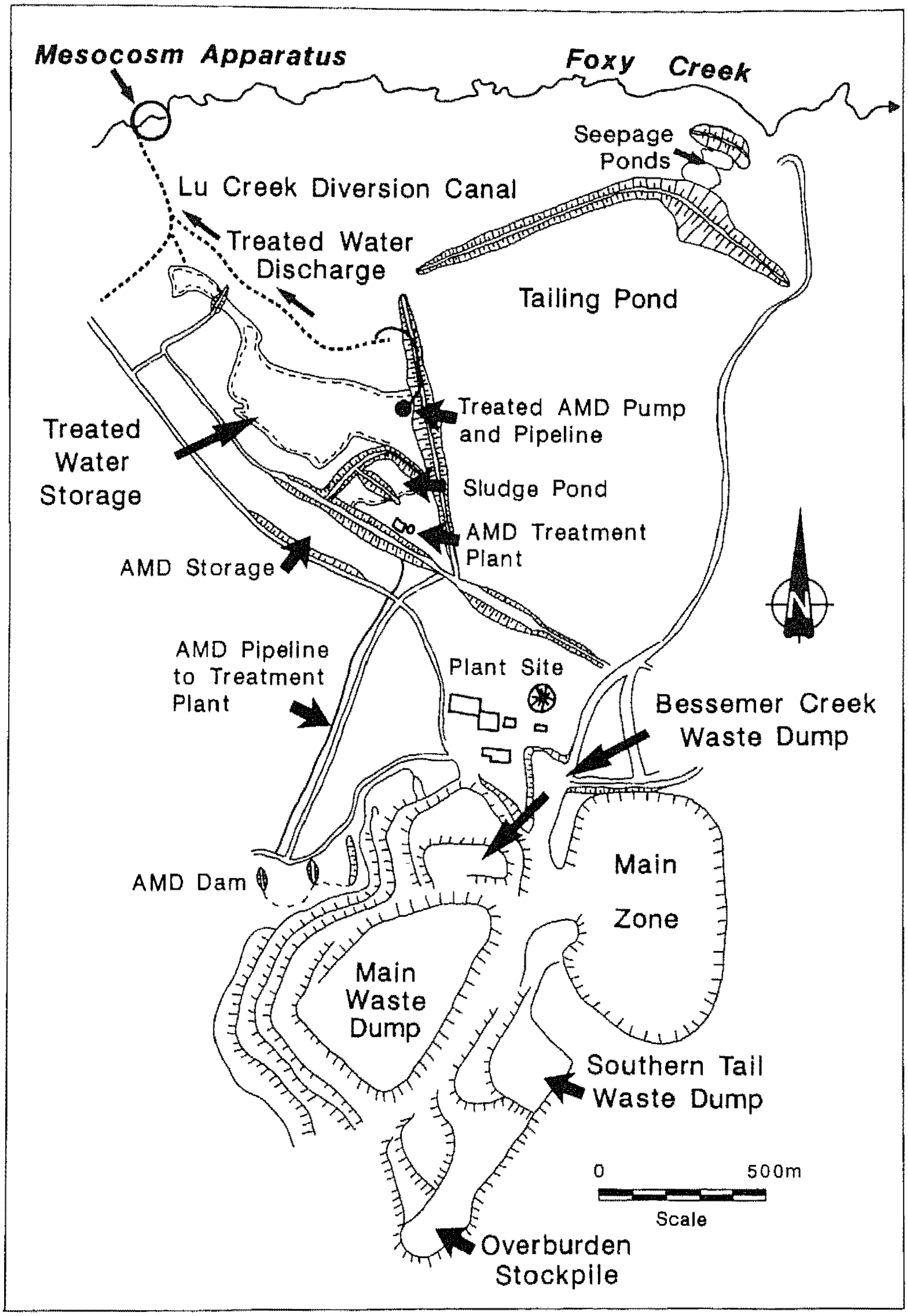

Fig. 1. Layout of the Equity Silver Mine and location of the experimental trough apparatus.

same ratio that had been established by regulatory authorities for AMD discharge from the mine.

A mixing chamber consisting of an angled baffle that created turbulence in the water and AMD supply was located at the head of each trough. Downstream of the mixing chamber was a 1.2-m section within which 2-cm-diameter drain rock was laid to a depth of 5 cm. Downstream of the gravel was a 0.32-m section fitted with a sheet of open cell Styrofoam® DB (Snowfoam Products Ltd., El Monte, CA) that provided a surface for the sampling of periphyton biomass. The gravel and Styrofoam sections were separated with a 7-cm-high baffle that produced a water depth of 7 cm over the gravel. At the flow rate of 0.4 $L \cdot s^{-1}$, the surface current velocity was 6 $cm \cdot s^{-1}$. Water leaving the gravel section flowed over the baffle and dropped onto the Styrofoam surface. The water then flowed in a laminar pattern over the Styrofoam at a depth of 1 cm.

Water flow through all 10 troughs began on July 23, 1989. Three weeks was allowed for colonization of the troughs by aquatic insects. The troughs were randomly assigned to serve as controls or treatments (AMD addition). The flow of AMD to the five treatment troughs began on August 13, 1989. At that time, the collection of emerging insects began by placing a Plexiglas emergence trap over each trough. The traps were identical to those designed and reported in a previous article [15]. Trap sizing was adjusted to seal the top opening of the troughs, thus preventing the airborne escape of emerging insects. The mesocosm was shut down with the termination of water and AMD flow on September 8, 1989.

Water samples were collected from the outflow of each trough on eight occasions for analysis of dissolved metals and macronutrients. Samples were filtered in the field and analyzed for total alkalinity (acid-neutralizing capacity), specific conductance, pH, sulfate, nitrate plus nitrite-N, soluble reactive phosphorus (SRP), and a suite of metals. Laboratory procedures followed those described in the American Public Health Association manual [16]. Metals were determined by inductively coupled plasma techniques. Low-level detection of copper and aluminum was measured by graphite furnace methods.

Samples of periphyton used to measure biomass accumulation were taken according to the accrual technique reported in a previous article [17]. Briefly, the biomass attached to, or settled on, the Styrofoam substrata in each trough was collected weekly by removing a core with the open end of a light-tight 7-dram plastic vial. All samples were frozen in the field at −15°C and shipped on dry ice to laboratory facilities within three weeks. All samples were analyzed for chlorophyll-*a* concentrations using fluorometric methods [16] after extraction in 90% acetone. Change in biomass was followed through two time series (July 24–August 10 and August 14–September 7), each of which began with the installation of a clean substratum. At the end of each time series, an additional core was extracted from each trough and preserved in Lugol's solution for later taxonomic examination according to methods outlined in [18].

Drift of aquatic insects from each trough was sampled over 3 h as well as over 24 h one day before and one day following AMD additions. An additional 24-h collection was made on August 30 to examine drift rates after an extended period of AMD addition. Drift samples were collected in 11 L plastic pails fitted with 253-μm mesh screen on an outlet opening. The pails were hung at the downstream ends of the troughs and continuously filtered all water to the 253-μm size fraction during the sampling period. Adult insects emerging from the troughs were collected continuously in the emergence traps. The traps were emptied weekly, and the insects were preserved in 5% formalin. Benthos samples were collected at the end of the experiment (September 8) by removing the entire contents of each trough.

In the laboratory, the insect samples were washed through a series of sieves (1.0-, 0.47-, 0.1-mm mesh). Insects retained on the 1.0- and 0.47-mm sieves were preserved in 70% ethanol for further sorting. The remaining size fraction was preserved for future reference. Benthic insects were separated from the detritus and algae using a dissecting microscope at 6.4× magnification for fractions retained on the 1.0-mm sieve and 16× magnification for the fraction retained on the 0.47-mm sieve. Sorted samples were preserved in 70% ethanol for counting and identification. Identification of the insects from drift, emergence, and benthos samples was made using comprehensive keys [19–22]. All counts were made at 6.4× and 16× magnification for the 1.0- and 0.47-mm size fractions, respectively.

RESULTS

Physical and chemical

Daily maximum and minimum water temperatures in the trough apparatus ranged from 14 and 7.5°C, respectively, in late July to a peak of 16.0 and 10.9°C, respectively, in early August. Thereafter, temperatures declined to a daily maximum of

6.3°C and a minimum of 5.6°C in the first week of September.

Concentrations of boron, barium, cadmium, chromium, molybdenum, and vanadium were always less than the detection limit of 0.01 mg/L. Cobalt and lead concentrations were always <0.1 mg/L and nitrate- plus nitrite-N concentrations were consistently <0.02 mg/L. Nickel was always <0.05 mg/L.

Data for the remaining chemical variables are summarized in Table 1 for the period before and after the start of treated AMD additions. At the 10% dilution rate, the addition of treated AMD increased the conductivity by 7.5 times that of the control. This increase was attributed mainly to a 54-fold increase in sulfate concentrations, a 9.6-fold increase in calcium levels, and a 5.1-fold increase in magnesium concentrations. Manganese concentrations increased by 0.05 mg/L, but zinc levels increased by only 0.001 mg/L to reach 0.011 mg/L due to the AMD additions. This zinc concentration is well below the 0.03-mg/L level for protection of aquatic life that is identified in Canadian water-quality guidelines [23]. Concentrations of copper increased marginally in the treatment troughs to reach 0.002 mg/L, the same concentration that is identified in Canadian water-quality guidelines [23] as the guideline for protection of aquatic life. Aluminum concentrations did not change after the start of the AMD addition, but were marginally greater than the Canadian water-quality guideline of 0.1 mg/L. SRP concentrations also did not change from ambient concentrations that were between 0.007 and 0.010 mg/L.

Periphyton

Samples of periphyton collected from the Styrofoam were dominated by diatoms (Table 2). *Hannaea* sp., *Diatoma* sp., and *Synedra* sp. were the most common genera. Chlorophytes, including *Ulothrix* sp., *Closterium* sp., *Mougeotia* sp., and *Cosmarium* sp. represented <2.5% (by numbers) of the periphyton community. Trace numbers of the blue-green alga *Anabaena* sp. were also found. There were no major changes in the composition of algae collected from control and treatment troughs over the 25 d of AMD addition.

A repeated-measures, nested analysis of variance (ANOVA) (trough nested in treatment) of the chlorophyll-*a* concentrations before the AMD addition indicated no difference in biomass levels between control and treatment troughs ($p > 0.2$) through time, thus confirming that the randomized allocation of controls and treatments did not produce differences in biomass as an artifact of the trough layout. Areal biomass increased exponentially, reaching a mean peak biomass (PB) of 1.1 $\mu g/cm^2$ over the 18 d.

After clean substrata were installed and the AMD addition was started, periphyton biomass was consistently greater in the treatment troughs than the controls over the 25-d time series (Fig. 2).

Table 1. Chemical concentrations at full mixing of treated acid mine drainage (AMD) and Foxy Creek water before and after additions of treated AMD

	Before AMD additions			After the start of AMD additions: Control			Treatment		
Measure	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*
pH	7.36	0.11	30	7.44	0.16	25	7.42	0.20	25
Conductivity (μmhos/cm)	47.2	0.21	20	51.8	0.2	5	386.0	11.2	5
Alkalinity (mg/L $CaCO_3$)	25.0	0.35	30	24.1	0.54	25	26.7	0.67	25
SO_4	1.37	0.05	30	2.53	0.27	25	137.9	7.08	25
Ca	5.19	0.06	30	5.06	0.02	25	48.5	1.3	25
Fe	0.05	0.002	30	0.087	0.007	25	0.078	0.005	25
Mg	2.04	0.01	30	2.12	0.01	25	10.83	0.28	25
Mn	0.01	0.00	30	0.01	0.00	25	0.06	0.002	24
Zn	0.01	0.00	30	0.01	0.00	25	0.011	0.0006	24
Al	0.122	0.068	2	0.117	0.02	6	0.121	0.026	5
Cu	<0.001	<0.001	2	<0.001	<0.001	6	0.002	<0.001	5
SRP[a]	0.01	<0.001	30	0.009	<0.001	25	0.007	<0.001	24

Data, left to right for each column, are means, standard error, and numbers of samples collected throughout the experiment at the outflow of the troughs. All units are milligrams per liter unless otherwise indicated.
[a]Soluble reactive phosphorus.

Table 2. Comparison of the taxonomic composition of periphyton from control and treatment troughs, before (August 13) and after (September 7) the addition of treated AMD to the treatment troughs. Data for diatom genera indicate relative abundance of all diatoms.

	Controls (%)		Treatment (%)	
Taxon	August 13	September 7	August 13	September 7
Diatoms	97.4	97.5	98.6	98.7
Hannaea	61.1	64.6	67.0	60.9
Diatoma	11.4	9.8	11.8	13.8
Synedra	15.0	14.3	9.0	6.7
Others	7.5	8.8	10.7	17.3
Chlorophyta	2.3	2.4	1.2	1.3
Cyanophyta	0.3	0.1	0.2	0.0

Another repeated-measures, nested ANOVA showed that the differences were highly significant ($p < 0.0005$). In the troughs receiving the AMD additions, PB reached 1.3 $\mu g/cm^2$ after 22 d compared to a peak of 1.02 $\mu g/cm^2$ over the same time period in the controls.

Macroinvertebrates

At the end of the experiment, an average of 2,213 and 2,468 animals were found in the benthos of each of the control and treatment troughs, respectively. This total number was represented by about 20 taxa (Table 3). Baetid mayflies dominated the community. Chironomids of the Orthocladiinae were next most common, followed by *Ameletus* sp., *Zapada* sp., Chloroperlidae, Tanypodinae, and Tanytarsini, each of which was present in similar numbers.

"Taxonomic richness" [24] is used as an index of diversity in this paper. It is essentially a measure of community structure and is simply the number of taxonomic units per sample. Compared to more complex diversity indexes based on information theory (i.e., the Shannon–Wiener function [24]) that can be difficult to interpret and are often misused, richness is easy to interpret. Taxonomic units used in this study are genera except for some identifications that proceeded only to the family level (Tables 3 and 4).

Taxonomic richness of the drift was about 55% (by numbers) of that of the benthos (compare Tables 3 and 4), a finding that was expected because numbers of animals in the drift were <5% (by numbers) of the benthic population. Most benthic animals will eventually drift, but the proportions of common taxa in benthos and in drift are

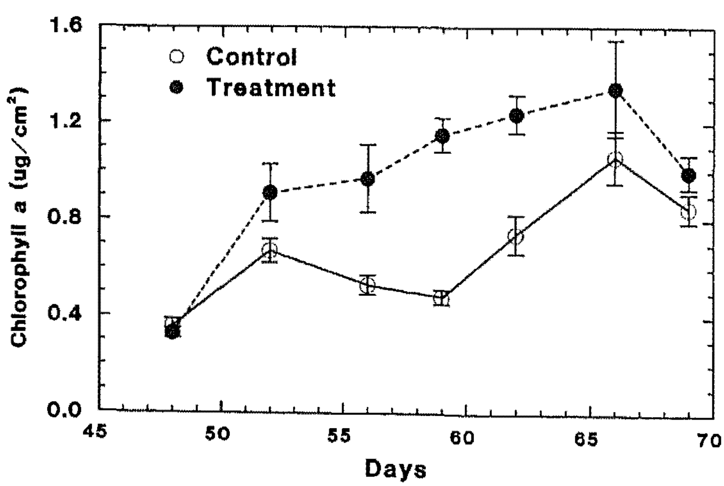

Fig. 2. Time course changes in mean chlorophyll-*a* concentrations (±1 SE) on Styrofoam® substrata in control and treatment troughs after the start of acid mine drainage (AMD) additions (August 14–September 7).

Table 3. Mean numbers and taxonomic richness (±1 SE) of benthos collected from five replicate control and treatment troughs on September 8

Grouping	Control Mean	Control ±1 SE	Treatment Mean	Treatment ±1 SE	Probability of effect
Total numbers	2,213.0	120.5	2,468.0	30.5	>0.07
Taxonomic richness	20.6	0.68	20.2	0.74	>0.68
Ephemeroptera					
Baetis	863.4	83.8	1,090.8	101.0	>0.14
Ameletus	170.0	12.7	180.4	27.4	>0.80
Cinygma	48.4	11.1	73.8	7.3	>0.14
Rhithrogena	21.0	3.3	34.0	3.9	>0.03
Epeorus	3.0	2.5	1.6	0.5	>0.90
Paraleptophlebia	17.2	2.1	10.4	1.7	>0.03
Ephemerellidae	12.8	1.8	11.8	1.5	>0.70
Plecoptera					
Zapada	143.6	14.7	130.4	9.5	>0.50
Chloroperlidae	137.4	9.9	155.2	21.9	>0.50
Doroneuria	16.0	1.5	22.6	4.5	>0.15
Isoperla	12.4	3.2	16.2	4.8	>0.60
Trichoptera					
Micrasema	13.4	5.1	22.2	11.3	>0.50
Others	3.4	0.25	3.0	0.8	>0.45
Diptera (Chironomidae)					
Tanypodinae	196.0	21.6	203.8	18.6	>0.75
Orthocladiinae	405.0	21.6	394.8	25.1	>0.70
Tanytarsini	146.2	17.7	112.2	12.7	<0.15

The critical alpha level for comparisons of the numbers of animals of each taxa between the control and treatment troughs was 0.003 after applying the Bonferroni correction for multiple comparisons.

usually very different. Numbers of Baetids dominated the drift on all sampling dates. They were followed in importance by Acari, *Zapada* sp., and *Micrasema* sp. in the mid-August samples. In the August 30 samples, the Baetids numerically represented more than 85% (by numbers) of the community. *Zapada* sp. and chironomids of the Orthocladiinae followed in importance.

The emergence of aquatic insects was generally variable, and the total numbers were low (Table 5). The most commonly collected taxa were midges of the subfamily Orthocladiinae. The only other taxa of significance were the Baetids and the Ceratopogonids, which were captured occasionally. On the last sampling date, numbers of Tanytarsini increased and exceeded those of the Orthocladiinae. *Simulium* sp. also became more important on the last two sampling dates.

In more than 70% of the samples, nonaquatic species of the Hymenoptera, Collembola, and Homoptera and of the fly families Muscidae and Scatophagidae were found. These collections were not included in calculations of total numbers or taxonomic richness in Table 5. Although the Dolichopodid flies may be considered terrestrial, they are common in lotic margins. Because they have a semiaquatic life history, they were included in Table 5.

Several taxa found in the emergence traps were never encountered in either the benthos or the drift, likely because they were too small to be retained on mesh sizes used to sieve the samples and also due to specific habitat preferences. The very small sizes of the Ceratopogonidae and *Simulium* sp. likely passed the 0.4-mm sieve and hence might have been missed. Because the counts of *Cinygmula* sp., Limnophelidae, Empididae, and the Coleoptera were only occasional and irregular, it is unlikely that they had become established in the troughs and might simply have entered the trough apparatus as they were emerging or drifting near the water intake. The Dolichopodidae have terrestrial larval stages and would not be expected to become established in the benthic community. The Ephydridae are lentic brine or shore flies that might have originated from the many ponds that are close to Foxy

Table 4. Composition of drift (mean numbers ± 1 SE) collected over 24-h periods before (August 12–13 sample) and after (August 13–14 and August 30 samples) acid mine drainage (AMD) additions

Grouping	Control Mean	Control ±1 SE	Treatment Mean	Treatment ±1 SE	Probability of effect
August 12–13 sample					
Total	92.6	5.91	97.0	12.3	
Richness	11.0	0.84	12.4	0.40	
Baetis	34.6	3.71	37.6	14.4	
Epeorus	0.0		0.4	0.25	
Serratella	1.0	0.63	0.6	0.40	
Zapada	12.0	1.48	13.6	2.58	
Micrasema	14.0	3.19	18.2	3.69	
Parapsyche	1.2	0.97	1.2	0.37	
Orthoocladiinae	2.8	0.37	3.6	0.81	
Simuliidae	2.8	0.86	3.0	1.09	
Acari	15.6	2.71	10.6	1.17	
Miscellaneous	8.6	1.50	8.2	0.40	
August 13–14 sample					*P*: (treatment × date)
Total	82.2	10.28	97.0	6.67	>0.40
Richness	10.8	1.20	10.2	0.58	>0.72
Baetis	30.2	3.81	41.2	7.48	>0.18
Epeorus	0.		0.4	0.25	>0.35
Serratella	0.6	0.40	0.8	0.20	>0.50
Zapada	10.2	1.85	15.8	4.60	>0.98
Micrasema	9.4	2.09	10.0	3.27	>0.94
Parapsyche	0.4	0.25	1.0	0.63	>0.71
Orthocladiinae	4.0	0.84	2.0	0.45	>0.23
Simuliidae	1.6	0.68	0.4	0.25	>0.78
Acari	17.4	2.38	20.6	1.54	>0.56
Miscellaneous	8.4	2.42	4.8	0.97	>0.51
August 30 sample					*P*: (control <> treatment)
Total	132.8	18.69	99.6	6.69	>0.13
Richness	8.6	0.75	6.4	0.81	>0.08
Baetis	114.0	18.2	89.2	4.87	>0.22
Epeorus	0.0		0.2	0.20	
Serratella	0.2	0.20	0.2	0.20	
Zapada	5.8	2.82	2.6	0.60	>0.29
Micrasema	0.0		0.0		
Parapsyche	0.0		0.0		
Orthocladiinae	3.6	0.40	2.2	0.58	>0.08
Simuliidae	0.6	0.25	0.2	0.20	
Acari	0.0		0.0		
Miscellaneous	8.6	1.03	5.0	2.00	>0.14

The probability of a treatment by date interaction, indicating a disproportionate change in drift rate in the August 13 to 14 samples or an absolute difference between treatments and controls in the August 30 samples, is indicated in the last column.

Creek and particularly the holding ponds that are near the mine tailings area. Similarly, the Chaoboridae are lake dwellers and would not be expected to become established in the benthic community of the troughs.

Treatment effects on benthos

Benthos samples were analyzed by ANOVA with treatment as the main factor. The results were summarized by total abundance, taxonomic richness, and abundance of individual taxa (Table 3).

Total densities were about 12% (by numbers) higher in treatment troughs than the control troughs, but these differences were not significant ($p > 0.07$). The power of the test was 0.80. A total of 18 taxonomic groups were examined separately for treatment effects, but none was significantly different at a critical alpha of 0.003, the signifi-

Table 5. Mean numbers and taxonomic richness (±1 SE) of emergence collected from five replicate control (C) and treatment (T) traps on four dates after the initiation of acid mine drainage (AMD) additions

	August 18		August 26		Sept. 1		Sept. 8	
Grouping	C	T	C	T	C	T	C	T
Total	6.8	8.2	15.8	12.0	21.0	18.6	28.2	12.8
	(3.1)	(5.2)	(8.5)	(5.3)	(7.5)	(7.3)	(14.7)	(8.0)
Richness	3.2	2.8	4.4	3.0	4.8	5.8	4.0	3.4
	(1.3)	(2.0)	(0.9)	(1.0)	(1.6)	(1.1)	(0.7)	(0.9)
Ephemeroptera								
Ameletus sp.	0.2	0.2	0.2	0			0.2	0
Baetis sp.	0	0.6	0.2	1	0.2	0.4	0.2	0.2
Cinygmula sp.					0.2	1.4		
Tricoptera								
Limnephilidae	0.2	0						
Coleoptera								
Elmidae	0.2	0						
Unknown			0.2	0	0	0.6		
Diptera								
Dolichopodidae	0.8	0.8	1.6	1.6	0.6	1	0	0.6
Ephydridae	0.2	0.2	1	0.2	0.4	0.4	0.2	0
Chaoboridae			0	0.2	0.2	0		
Ceratopogonidae	0.2	0.2	2	0.8	1	0.2	0.4	0.4
Orthocladiinae	4.4	6.0	9	8.2	14.2	11.4	7.8	5.2
Tanytarsini	0.4	0	1	0	3.4	2	16.8	12.2
Simulium sp.			0.6	0	0.6	1	1.8	0.2
Empididae					0.2	0.2		

cance level applied after the Bonferroni correction for multiple comparisons [25]. This correction accounts for the possibility of significant random differences in multiple comparisons. Two genera of mayflies (*Rhithrogena* sp. and *Paraleptophlebia* sp.) had differences at $0.05 > p > 0.03$, but this was not significant when the Bonferroni correction was applied. The relative differences in numbers of these genera between the control and treatment were also not consistent: Numbers of *Rhithrogena* sp. were greater in the treatment troughs compared to the controls, but the opposite was the case for numbers of *Paraleptophlebia* sp.

Taxonomic richness did not differ significantly between the treatment and control troughs ($p > 0.68$).

Treatment effects on drift

The rates of drift before and after the initiation of AMD flow were tested using a three-way ANOVA, with treatment, date (before or after AMD addition), and sampling period (3- or 24-h period) the main factors. The interaction term for treatment times date was the measure of interest because a significant interaction would indicate that the rates of drift changed disproportionately from the treatment and control troughs across dates. Ten taxonomic groupings had sufficient numbers for analysis (listed in Table 4). Data were transformed to logarithms of $x + 1$ before analysis. For both the 3- and the 24-h samples, none of the 10 groupings had significant treatment by date interactions (all with $p > 0.18$) (Table 4, 24-h data; Fig. 3, 3- and 24-h data). The critical level of alpha was 0.005, determined after the application of Bonferroni's correction. The power of the comparison of drift rates between treatments on the day after the start of AMD flow was >0.95.

The 24-h drift samples from August 30 were also analyzed with ANOVA, and no taxonomic group revealed any treatment effect (all with $p > 0.08$; Table 4). The critical level of alpha after the Bonferroni correction was 0.008. A similar finding applied to the total number of insects drifting ($p > 0.13$) and for taxonomic richness ($p > 0.08$).

Neither taxonomic richness nor total numbers in the drift differed significantly across treatments for any drift sampling period (Fig. 3). Both indexes of samples from the treatment troughs appeared slightly higher for the August 12 to 14 period, but

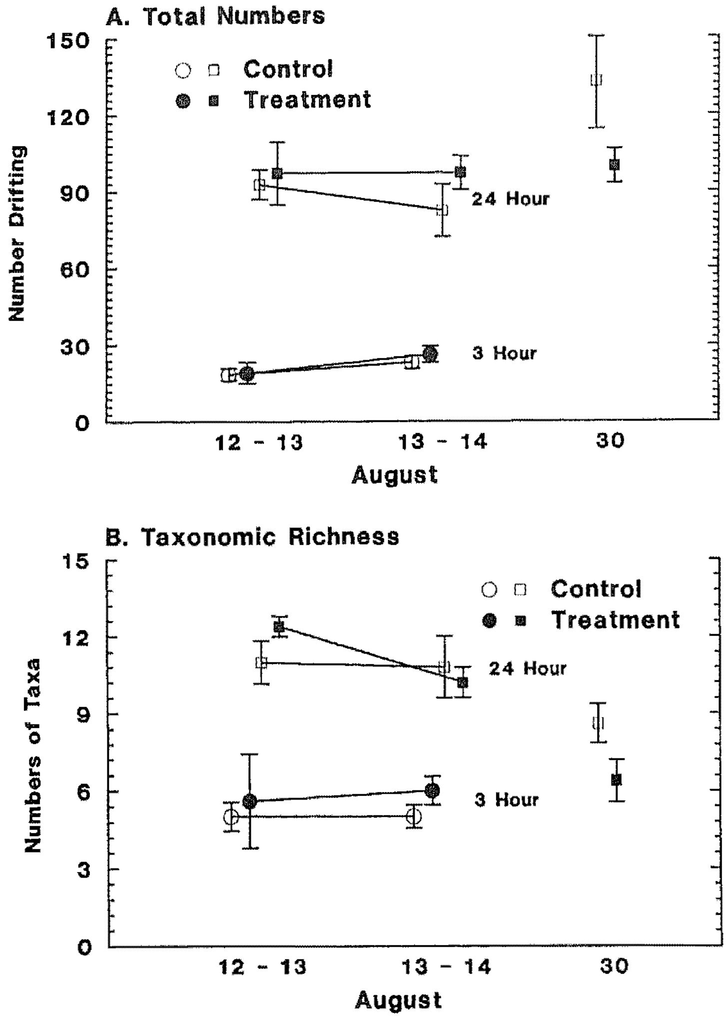

Fig. 3. Total numbers (A) and taxonomic richness (B) of drifting invertebrates measured in 3- and 24-h periods during August 12 to 14 and during a 24-h period on August 30. Lines drawn between data show the treatment × date interactions, none of which was significant. See text for significance values. Acid mine drainage (AMD) additions were initiated on August 13.

those from control troughs appeared greater on August 30. These differences were not significant (Table 4).

Treatment effects on emergence

An ANOVA of logarithmically transformed emergence data with treatment and date as main effects indicated no significant effect of treatment ($p > 0.35$), whereas there were differences among dates ($p < 0.001$). Emergence increased in a linear pattern that was independent of the addition of the treated AMD. Total abundance was <10 animals per week when the experiment started but increased to 20 to 30 animals per week at the end. The greatest emergence occurred during the week of lowest temperatures (maximum daily temperature ranged from 6.3 to 10.8°C, and the minima ranged from 5.6–5.9°C).

DISCUSSION

The addition of treated AMD from the Equity Mine had no significant negative effect on any of the biological measures examined in the experiment at the prescribed 10:1 dilution rate. The only change that the treated AMD addition had on the stream mesocosm was a significant increase in areal biomass of periphyton. Apparently, the treated AMD did not produce a toxic effect but rather an enhancement of algal biomass accrual. Because the treated AMD contained levels of several micronutrients, it is possible that any micronutrient deficiency was alleviated by the chemical additions. Although a treatment effect was apparent, it is important that the differences in biomass were very small and well within the range that can typically be found in a natural stream. This small difference could have been caused by reduced grazing effects, but as no treatment effect on invertebrate abundance was detected, this possibility is less likely than changes in nutrient deficiency. The analyses of invertebrate abundance suggest that at the dilution rate of 10 parts Foxy Creek water to one part treated AMD that has chemical concentrations similar to those measured in this study, the discharge of treated AMD will not impact on the abundance of macroinvertebrates downstream of the Lu Creek diversion canal in Foxy Creek. Because macroinvertebrates are the primary size fraction of animals that are consumed by salmonids [26], this finding indicates no impact on the abundance of fish food organisms by the addition of treated AMD over a three-week period.

The use of troughs as stream mesocosms in this study provided a powerful experimental approach for assessing the effect of additions of treated AMD on ecosystem processes at the Equity Mine site. This finding suggests that replicated, in situ stream mesocosms offer the experimental testing of explicit hypotheses while providing a high degree of realism by incorporating most of the natural ecosystem processes [15]. The realism provided by such an approach made possible the strong inference of the effects of treated AMD additions on the Foxy Creek system. The troughs successfully supported periphyton and aquatic insect communities, and the various ANOVAs used as the basic tool for examining the impact of AMD additions had power values in excess of 0.80. It has been suggested that, in general, power values ≥ 0.8 are necessary for results of significance tests to be conclusive [10]. By obtaining high power values in the drift and benthos analyses, we have confidence that the experimental and equipment design used in this study was suitable for producing conclusive evidence that the null hypothesis of no effect of the added AMD could not be rejected.

Although several measures of invertebrate response to the AMD additions were examined, it is worth noting that with the present mesocosm design, the most convincing data on responses came from changes in numbers in the benthos. Very subtle treatment effects in the drift may be difficult to detect in small troughs because drift leaving all troughs is a combination of what is coming in from the stream and passing through the troughs and what is being produced by processes in the troughs themselves. The trough drift may be far exceeded by stream drift, thus masking treatment effects. Hence, drift measurements are useful mainly when treatments produce large changes in numbers of animals that leave the troughs relative to numbers drifting through the troughs. In our study, drift measurements were used to examine an immediate behavioral response to the onset of AMD additions. Our null hypothesis was that any change in the chemical milieu would not lead to a large exodus from the benthic community, and the results were highly supportive of this hypothesis. The emergence data were considered relatively imprecise largely due to the small numbers of animals collected. Total numbers were about 10 times lower than those found in identical emergence traps used in a mesocosm established in Carnation Creek, a small coastal stream in British Columbia [15]. The main difference between experiments was that in the present study, surface current velocities were double those at Carnation Creek. More animals would be expected to be lost in water outflow from our apparatus during the process of emergence. Benthos measurements, by comparison, were not confounded by flow-through dynamics in the troughs and thus were more sensitive to treatment effects than the other measurements.

An advantage of mesocosm experiments is that they can be run for any period of time and in so doing can be used to examine time-related changes in ecosystem dynamics. In comparison to conventional toxicity bioassays that last no more than a few days in a laboratory, the present experiment may be considered a chronic test. Lab bioassays, however, deal only with one or a few organisms, and interactions within and between species assemblages are ignored. The present trough experiment, by contrast, involved a minimum of three weeks for an assemblage of periphytic algae and benthic insects to become established, and the toxicity test dealt with a combination of acute effects on individual organisms plus changes in the integrated structure and function of the trough assemblage. At sublethal levels of an added toxicant, changes in these latter processes may not be detectable for days or weeks after the toxicant is added. In the present study, a three-week period of AMD addition was arbitrarily selected as a reasonable time to detect changes in the benthic community. It is well accepted, however, that sublethal effects may require longer periods to be noticeable in the mesocosm and thus in an actual stream ecosystem. It is known, for example, that very small, early stages of insects are more sensitive to additions of toxicants than are larger animals [11,27], and where this is important, numbers of larger animals may not change for an extended period of time in relation to reduced recruitment by younger larval stages. Although early instars of many taxa were enumerated in our benthos samples, it is unknown what proportion of the smallest animals were lost by sorting with a 0.47-mm sieve. We did recover large animals that undoubtedly represented the majority of biomass and the fraction that is most likely to be consumed by salmonids. But the potential importance of smallest size classes in determining populations in the longer term suggests that the experiment be considered an acute test of effects of the AMD additions. A longer term experiment or complete enumeration of smallest animals would be required to confirm longer term changes in the structure and function of Foxy Creek.

An important assumption of this study is that results from the mesocosm can be extrapolated to Foxy Creek. It is understood that the lack of structural heterogeneity in the mesocosm may produce an assemblage of organisms that is not identical to that in the stream. Taxonomic richness approximated 20 taxa, which is probably less than that in Foxy Creek. But the mesocosm did support an interacting assemblage that was derived from Foxy Creek and thus could be considered representative for examining reductions in numbers of animals in an interactive system from the treated AMD additions.

The ability to extrapolate findings from a mesocosm to a stream is important from a regulatory perspective. Agencies that are involved in permitting waste discharges use published criteria [23] as an overall guideline. These data give receiving-water criteria for individual metals, but information on effects of levels of metal mixtures is notably lacking. Consequently, effluent permits that must identify critical levels of metal mixtures do so without support of technical rationale. The value of the present experiment is the ability to extrapolate findings of effects of treated AMD on a representative assemblage of organisms to determine potential changes that may occur in Foxy Creek. The present findings indicate that the discharge of treated AMD in a dilution ratio of 10 parts water to 1 part treated AMD will not produce short-term changes in the abundance of benthic insects that are available for consumption by salmonids. The data suggest that present permit regulations in effect at the Equity Mine are adequate for short-term protection of the benthic community in Foxy Creek.

Although the design of this experiment was simple, more elaborate experiments incorporating a graded series of chemical additions or factorial designs are possible. There is potential for defining threshold effects and considering taxon-specific or process-specific impairment at different rates of treatment. An incorporation of a gradation of treatments to define threshold effects is likely to be most useful. By calculating a graded response curve, a measure of the minimum level of dilution of the treated AMD milieu that causes an impact on ecosystem processes could be identified.

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