

**THE EFFECT OF
TREATED ACID MINE DRAINAGE ON
STREAM MACROINVERTEBRATES
AND PERIPHYTIC ALGAE:
AN IN SITU MESOCOSM
EXPERIMENT**

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SUMMARY

A stream "mesocosm" was used to support an experiment designed to examine the effect of additions of treated acid mine drainage (AMD) on the composition and abundance of periphytic algae and aquatic invertebrates downstream of AMD discharge from the Equity mine site. An apparatus consisting of 10 flow-through troughs, suitable for invertebrate and periphytic algal colonization and growth was installed and tested at the site. After three weeks of running stream water without AMD through all troughs to allow colonization by stream invertebrates, additions of treated AMD at an operational dilution rate of 10% to 5 randomly allocated troughs was started and continued for three additional weeks. Water, algal, and insect (drift, adult emergence, and benthos) samples were collected from the 5 treated and 5 untreated troughs during and at the end of the 6-week experiment for examination of the effects of the AMD addition on indices of invertebrate abundance and algal growth. Results showed that the experimental approach using a mesocosm apparatus was highly sensitive and yielded analyses of variance with high power. Results showed conclusively that at the operational 10% AMD dilution rate, the addition of treated AMD to Foxy Creek does not impact on aquatic insect composition and abundance. The success of applying the mesocosm approach within a rigid and well structured experimental design in this study is discussed with respect to its application to many water quality problems. It can be a powerful tool to accurately determine ecosystem response curves for effluent discharges during mine operations or at closure. It would also be ideal for exploring alternative AMD treatment strategies at specific sites. An important advantage of the method over more common single-species bioassays, is the power of predicting changes in ecosystem functioning which is fundamental for establishing water quality criteria.

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1.0 INTRODUCTION

Acid mine drainage (AMD) is a widespread environmental problem. The literature on the biological effects of AMD is vast but it primarily includes laboratory bioassays of single taxa (Daniels *et al.* 1979). Most field studies of the effect of AMD on aquatic systems consist of monitoring studies which document temporal alterations in the chemical milieu and biological community in response to AMD additions (Dills and Rogers 1974, Hoehn and Sizemore 1977, Short *et al.* 1990). A few studies have used an experimental approach to determine the effects of AMD (eg. Peckarsky and Cook 1981). In most of these studies untreated AMD was released directly to the environment.

Despite the abundance of these data, water quality managers in British Columbia continue to deal with uncertainty in setting allowable dilution rates of AMD that is discharged into streams. A dilemma is that the abundance of monitoring information has little predictive power because of the lack of hypothesis testing that is inherent to those data. Hence, the assignment of allowable dilution rates and metals levels of AMD has been an arbitrary process. There are no site-specific experiments with which to determine what rates of AMD addition or concentrations of metals may be permitted. This problem is particularly acute in distinguishing the toxic effects of single elements in a chemical milieu that varies from site to site. Much of the present data also do not apply to variation in the handling of AMD. At the Equity Silver Mine, British Columbia, for example, AMD is treated with lime to decrease the acidity and the release flow rate is regulated. Experiments have not been done to examine change in ecosystem function due to stress (toxic or sublethal effects) associated with this specific processing of AMD.

Rather than consider the toxicity of individual elements and attempt to deal with constant changes in the degree of toxicity of those elements under changing chemical conditions, the chemical milieu in AMD may also be regarded as a single entity to

simplify assessments of toxic effects of AMD. This latter approach was used in the present study in an experiment designed to remove some uncertainty in the assignment of allowable dilution rates of the treated AMD that is discharged into Foxy Creek near the Equity Silver Mine operations. A flow-through mesocosm was designed and constructed to facilitate testing the null hypothesis that the present levels of treated AMD that are diluted in Foxy Creek (approximately 10 parts Foxy Creek water to one part treated AMD) do not affect the composition and abundance of periphytic algae or benthic macroinvertebrates. The emphasis here was to deal with the effects of the treated AMD (as a chemical milieu) on the stream community, an approach which closely examines "real" ecosystem responses, that are not confounded by artifacts associated with a laboratory. Hence, the experiment was run *in situ*. The insects were of primary interest because they are the direct food source for highly valued salmonids that inhabit downstream ecosystems. Any impact on the insect community may affect salmonid production downstream in the Bulkley River system (Wilkes 1987).

2.0 THE MESOCOSM APPROACH

Several approaches to the study of anthropogenic stresses on biological systems have been reported in the primary literature. There are laboratory bioassays of single species, mesocosm studies involving a few to many taxa, monitoring of whole systems, and mathematical modelling (Levin *et al.* 1989). Mesocosms are variously defined and, therefore, incorporate a range of designs and purposes (Gearing 1989). For our purposes, we will confine our meaning of the term, "mesocosm" to those replicated experimental units intended to mimic a natural ecosystem on the scale of relevant processes.

There are many advantages to the use of mesocosms in ecotoxicology. A mesocosm should allow the examination of the end result of many interacting organisms and processes in a situation similar to nature (Muirhead-Thomson 1987, Gearing 1989). This approach integrates a variety of system processes such as species interactions, nutrient flux, and energy flow, which cannot be imitated in a laboratory, single-species test. Thus, extrapolation of the results to the natural situation is more powerful than single species toxicity tests (Gearing 1989). For instance, Clements et al. (1989) found that exposure to dissolved copper made prey organisms more vulnerable to predation by a predatory stonefly. This finding suggested that toxic substances can create non-additive effects on members of the community. The ability to replicate mesocosms provides for a true experimental approach with appropriate controls and replication. Replication and appropriate controls allows inferential statistical tests of the null hypothesis of no treatment effect, which is not generally possible in monitoring studies (for an exception see Carpenter et al. [1989]). An experimental approach also permits the calculation of the power of the test (Cohen 1988, Peterman 1990) which estimates the ability of the test to detect real differences. Power estimates, in conjunction with the inferential statistics, removes uncertainty regarding the reality of the measured effect.

Stream mesocosms have seen increasing use in the past decade. Early use was mostly restricted to laboratory streams (reviewed by Warren and Davis [1971]). In recent years many investigators have used various designs of *in situ* stream mesocosms which allow for greater realism. One of the most important features of stream mesocosms in the opinion of Warren and Davis (1971) is the flow-through design which most closely embodies the natural functioning of stream systems. The important aspect of this characteristic is that streams function as open systems, and the flow-through design provides for exchange of nutrients, organic materials, and immigration/emigration processes of organisms. By the use of a flow-through design, the experimental unit can equilibrate and then the perturbation from this central state can be measured.

Stream mesocosms have been used to study a variety of processes. Recent work on the effects of acidification on benthic communities have made use of this approach (Servos and Mackie 1986, Allard and Moreau 1987, Hopkins et al. 1989). Studies of basic ecological processes such as nutrient and food limitation of stream organisms have used mesocosms for a variety of manipulations of nutrient input rates (Mundie *et al.* 1983, Perrin *et al.* 1987, Hill and Knight 1987, Richardson 1989). Studies of the effect of pesticides on benthic organisms have also used flow-through stream mesocosms (Muirhead-Thomson 1987).

Mesocosm studies are useful for deriving site-specific estimates of the effects of contaminants. In this sense, mesocosms are economical in defining effects before whole-system perturbation has occurred, or before attempting whole-stream manipulations as ameliorative measures. In the case of setting criteria for mine effluent they provide a powerful technique to test the actual effect of the effluent on the receiving community and thus they introduce a highly quantitative and definitive decision-making process for resource managers.

There are several disadvantages to mesocosm studies, primarily the reduced system realism which restricts the ability to extrapolate results (Levin *et al.* 1989). Some of the constraints on realism include the lack of structural heterogeneity typical of streams, lack of normal variation in rates of discharge, and the short-term nature of such studies. For many processes in streams, a mesocosm of a metre or more may suffice, e.g., periphyton growth, microbial respiration, and development of benthic invertebrate populations. However, the flow-through design which allows colonization of stream organisms also means that the fate of organisms emigrating from the mesocosm is unknown, i.e., it may have been normal movement, sublethal toxic effect, or death (Muirhead-Thomson 1987). Some taxa of benthic invertebrates typically have very high rates of movement, e.g., the mayfly *Baetis sp.*, and high rates of movement may dilute

the effects one could measure by high rates of turnover. The cost of constructing and moving mesocosms also may be restrictive under certain circumstances, although this may not be an important factor in resource management applications where the cost of not using a mesocosm and making an error in setting criteria levels (for AMD, for example) may far exceed the cost of a well run mesocosm experiment.

3.0 MATERIALS AND METHODS

The experiment was conducted at the confluence of Foxy Creek and the Lu Creek diversion canal (Figure 1). This location allowed a pipeline to be laid upstream in Foxy Creek to supply water for the mesocosm and it facilitated a second pipeline to be laid in the Lu Creek diversion canal to supply the treated AMD.

The AMD originated in a collection pond downslope of the Bessemer and Main waste dumps (Figure 1). From that pond, the raw AMD was pumped upslope to a treatment plant where the pH of the AMD was increased from about 2.3 up to 8.0-8.5 with the addition of lime. Sludge was allowed to settle in ponds adjacent to the treatment plant and the supernatant was decanted to the treated water storage pond prior to being pumped to the treated water discharge canal. The water then flowed into the Lu Creek diversion canal which discharged directly into Foxy Creek.

The mesocosm (Figure 2) consisted of ten flow-through troughs (each 1.52 m long x 0.2 m high x 0.2 m wide) that were assembled on a series of joists laid over top of the stream channel. Water and biota carried in suspension in Foxy Creek were supplied to the mesocosm via gravity through a 100m (6 inch 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 $0.5 \text{ L} \cdot \text{s}^{-1}$ without the use of valves. The use of valves was avoided as the turbulence that they create could cause trauma in the insects that were colonizing the troughs. The treated AMD was delivered via gravity through a second 260 m (2 inch diameter) pipeline that was laid in the treated water discharge canal. The intake for the treated AMD line was in the treated water discharge canal, upstream of the confluence with the Lu Creek diversion canal. A series of gate valves on the trough apparatus controlled the flow of AMD to the troughs to maintain a 10:1 flow dilution ratio, the same ratio which is maintained between Foxy Creek and discharge from the treated water discharge canal under normal operating conditions. Water temperature in the trough apparatus was monitored with a standard maximum-minimum thermometer that was read daily.

The troughs were fabricated from plexiglass. They included a mixing chamber at the inlet for Foxy Creek and treated AMD water. Downstream of the mixing chamber was a 1.2m section within which 3/4 inch-minus drain rock was laid to a depth of five cm. Downstream of the gravel was a 0.32 m section that was fitted with a sheet of open cell styrofoam DB (Snowfoam Products Ltd., El Monte, Cal.) that provided a surface for the sampling of periphyton biomass.

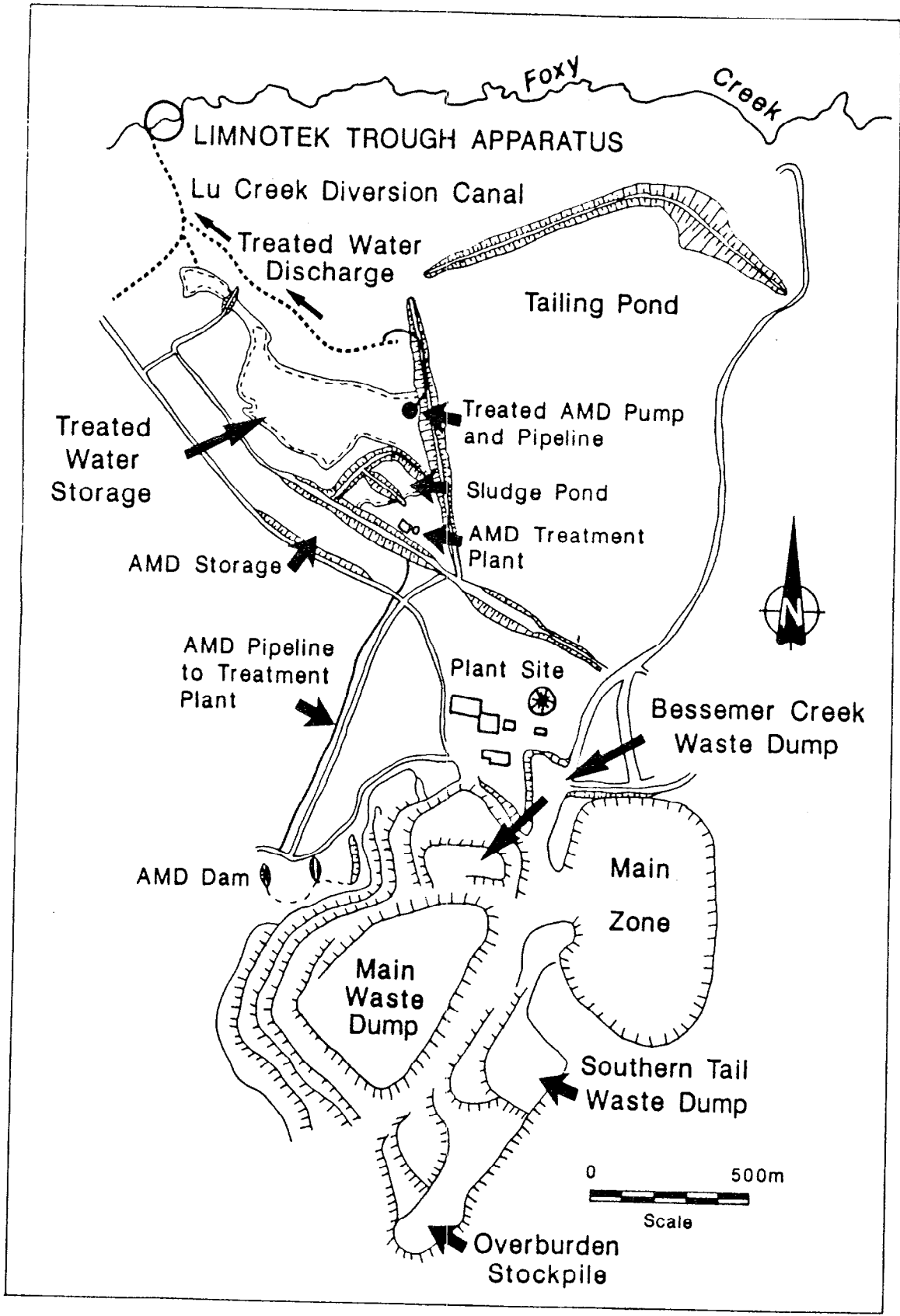


Figure 1. Location of the trough apparatus at the Equity Silver Mine.

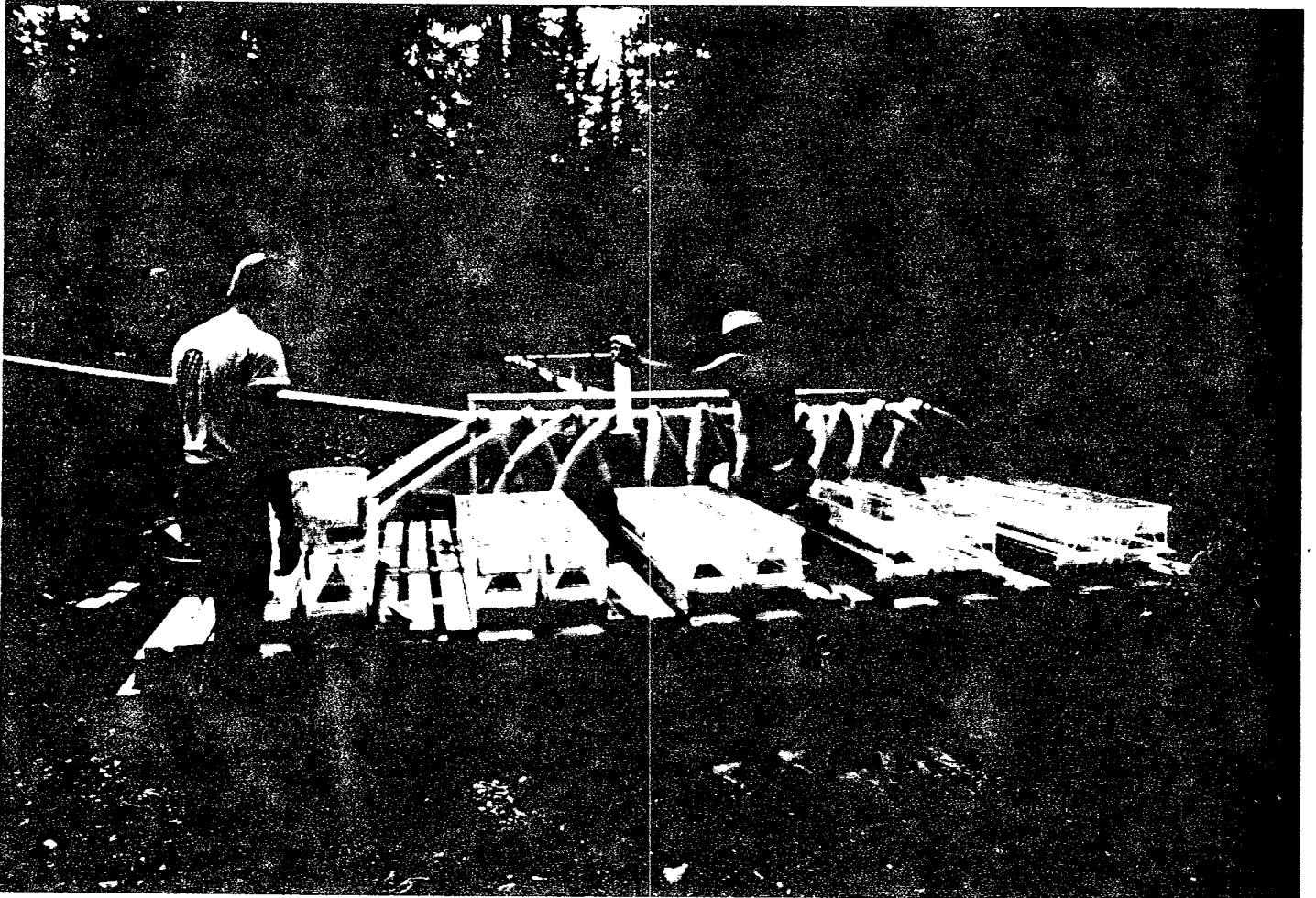


Figure 2. General view of the Limnotek Trough Apparatus in operation on Foxy Creek.

Water flow through all 10 troughs began on July 23, 1989. Three weeks were 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 (trough #2, 3, 5, 6, and 9) began on August 14, 1989. At that time, the collection of emerging insects began by placing a plexiglass emergence trap over top of each trough. The traps were designed to seal all openings on the top opening of the troughs, thus enabling the capture of all emerging insects. Openings on the side walls of the traps were fitted with 355 μm mesh Nitex netting to allow the free movement of air yet prevent the escape of captured insects. The experiment ended 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 shipped to Zenon Laboratories in Vancouver for analysis within 48 hours. Total alkalinity (acid neutralizing capacity) measured as the concentration of CaCO_3 ($\text{mg}\cdot\text{L}^{-1}$) was determined with an automated electrometer using pH 4.5 as the endpoint. Specific conductance was measured with a standard conductivity meter in units of $\mu\text{S}\cdot\text{cm}^{-1}$. pH was measured with an automated pH meter. Sulfate was determined using the automated methylthymol blue method. Nitrate plus nitrite was determined by automated cadmium reduction and soluble reactive phosphorus (SRP) was measured using the automated ascorbic acid method. Metals were determined by inductively coupled plasma techniques. Low level detection of copper and aluminum was run by graphite furnace methods. All procedures are described in APHA (1985).

Samples of periphyton to measure biomass accumulation were taken according to the accrual technique of Perrin *et al.* (1987). 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. The samples were retained in the vials and

stored frozen at -15°C for up to three weeks before shipment on dry ice to Zenon Laboratories in Vancouver. All samples were analyzed for chlorophyll *a* concentrations after extraction in 90% acetone using the fluorometric methods outlined in APHA (1985). Instrumentation was calibrated using fresh chlorophyll *a* standards.

Two time series of periphyton collections were run. The first (July 24 through August 10) was run to examine between-trough variation before the AMD additions started. The second (August 14 through September 7) completed the data to examine the effect of the treated AMD additions on periphyton biomass. 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. The percent composition of each algal taxa was described from counts taken at 500x magnification along several transects from subsamples that were allowed to settle in Utermohl chambers.

Aquatic insects were sampled in three ways. Drift of insects (emigration) from each trough was sampled over three hours and 24 hours one day prior, 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 plastic pails fitted with 253 μm mesh screen on an outlet opening. The pails continuously filtered all water to the 253 μm size fraction during the sampling period. The contents of each pail were preserved in 5% formalin. Emergence of adult insects 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. The water supply was shut off and the gravel substrata was transferred to a large stainless steel mixing bowl. The gravel was washed and removed from the bowl and the remaining water with the invertebrates and detritus was filtered through a 253 μm screen and preserved in 5% formalin.

Benthos samples were processed in the laboratory, first by separating size fractions by washing the samples through a series of nitex 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 6.4x magnification for fractions retained on the 1.0 mm sieve, and 16x magnification for the fraction retained on the 0.47 mm sieve. Sorted samples were preserved in 70% ethanol for counting and identification.

Identification of insects from drift, emergence and benthos samples were made using keys in Merritt and Cummins (1984), Cole (1969), Edmunds *et al.* (1976), and Wiggins (1977). All counts were made at 6.4x and 16x magnification. The counted benthos samples were retained for future reference.

4.0 RESULTS

4.1 Physical Data and Chemistry

Daily maximum and minimum water temperatures in the trough apparatus ranged from 14°C and 7.5°C respectively in late July to a peak of 16.0°C and 10.9°C respectively in early August. Thereafter, temperatures declined to a daily maximum of 6.3°C and minimum of 5.6°C in the first week of September.

Flows in each trough were easily maintained at 0.5 L·s⁻¹. At this rate, the average surface current velocity in each trough was 10 cm·s⁻¹ and the depth of water over the gravel substrata was about 2 cm. These physical conditions created a riffle-type environment which was maintained from the start of colonization until August 16. At that

time a 7 cm high wooden baffle was installed at the downstream end of the gravel section in each trough and flows were reduced to $0.4 \text{ L} \cdot \text{s}^{-1}$ to reduce surface turbulence, lower current velocities, and thus improve conditions for the emergence of adult insects. The baffles increased water depths over the gravel substrata to 7 cm and surface current velocities declined to $3 \text{ cm} \cdot \text{s}^{-1}$.

Concentrations of several chemical variables remained below detection limits throughout the study. In samples collected from the troughs, boron, barium, cadmium, chromium, molybdenum, and vanadium were always less than $0.01 \text{ mg} \cdot \text{L}^{-1}$. Cobalt and lead concentrations were always less than $0.1 \text{ mg} \cdot \text{L}^{-1}$ and nitrate plus nitrite-N concentrations were consistently less than $0.02 \text{ mg} \cdot \text{L}^{-1}$. Nickel was always less than $0.05 \text{ mg} \cdot \text{L}^{-1}$.

The remaining data are summarized in Table 1 for the period before and after the start of treated AMD additions. At the prescribed dilution rates used in the troughs, the addition of treated AMD increased the conductivity by 7.5 times over the control. This increase was mainly attributed to a 54-fold increase in sulphate concentrations, a 9.6-fold increase in calcium levels and a 5.1-fold increase in magnesium concentrations. Concentrations of manganese and zinc increased only slightly due to the AMD additions. The calculated concentrations of copper from analyses of samples collected from the treated water discharge canal indicated that it also increased marginally to reach $0.002 \text{ mg} \cdot \text{L}^{-1}$ in the treatment troughs. Aluminum concentrations did not change after the AMD addition. SRP concentrations did not change as a function of the AMD addition but they were remarkably high relative to what is found in most temperate streams. They are also high relative to the inorganic N concentrations (nitrate plus nitrite) and are in a range that would indicate that growth of the periphyton community was limited by nitrogen, not phosphorus which is more typically the case. Nitrogen deficiency has also been reported,

however, in the Nechako River, south of the Equity Mine area in the upper Fraser River system (Perrin 1988).

Table 1: Summary of chemical concentrations determined at the trough apparatus before and after the additions of treated AMD. Data are means, (standard error), sample size. Units are $\text{mg}\cdot\text{L}^{-1}$. With the exception of data for Al¹ and Cu¹, all concentrations were determined from samples collected at the outflow of the troughs.

Measure	Before AMD			After Start of AMD Control			Additions Treatment		
pH	7.36	(0.11)	30	7.44	(0.16)	25	7.42	(0.20)	25
Conductivity	47.2	(0.21)	20	51.8	(0.20)	5	386.0	(11.2)	5
Alkalinity	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	(1.30)	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	0.01	(<0.001)	30	0.009	(<0.001)	25	0.007	(<0.001)	24

¹The Al and Cu data are from samples collected as part of routine sampling by Equity Silver Mines Ltd. Values for dates before AMD additions were from samples collected in Foxy Creek upstream of the Lu Creek diversion canal. Values for dates after AMD additions were from samples collected at the same Foxy Creek site (control data) and in the Lu Creek diversion canal after correction for dilution in the troughs (treatment data).

4.2 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 less than 2.5% 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 from before AMD addition to 25 days later (Table 2).

Table 2. Comparison of the taxonomic composition of periphyton from control and treatment troughs, before and after the addition of treated AMD to the treatment troughs.

Taxon	Controls (%)		Treatment (%)	
	13 August	7 September	13 August	7 September
Diatoms				
<i>Hannaea</i>	61.1	64.6	67.0	60.9
<i>Diatoma</i>	11.4	9.8	11.8	13.8
<i>Synedra</i>	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

Before the treated AMD was added, periphyton biomass increased exponentially to reach a peak level of about $1.1 \mu\text{g} \cdot \text{cm}^{-2}$ over a time course of 18 days (Figure 3a). A repeated measures nested ANOVA indicated a significant trough effect within the control

or treatment ($p < 0.01$) but no significant treatment effect ($p > 0.5$). In this analysis the main factors were treatment and date, and the trough effect was examined by nesting trough within treatment. The nested F value (trough within treatment) was used as the error term to test for the effect of treatment. Despite the trough effect within treatment or control, this analysis indicated that the time course accumulation of chlorophyll *a* did not differ between the troughs allocated as controls and treatments before the treated AMD was added.

During the second time series (Figure 3b), the time course changes in periphyton accrual were very different from the previous data. The biomass increased in an erratic pattern reaching a peak level of 1.3 and 1.02 $\mu\text{g} \cdot \text{cm}^{-2}$ in the treatment and control troughs respectively. Volatile temporal changes in periphyton accrual during late summer has also been observed in other streams in British Columbia (Perrin 1988, Perrin *et al.* 1987). Based on evidence of algal-bacterial interactions reported by Kaplan and Bott (1989), Perrin (1989) has hypothesized that the phenomenon is related to an uncoupling of bacterial and algal growth at higher summer temperatures. Where bacteria directly utilize dissolved organic carbon (DOC) derived from algae earlier in the growing season, the bacteria may grow at a rate that is independent of the algae at higher temperatures later in the summer, potentially leading to very similar rates of energy production between autotrophy and heterotrophy. One may observe this phenomenon as an increase in variability of periphyton production, the declining phases being related to a net increase in heterotrophic activity. Another explanation for the trend, however, is that variability introduced by insect grazing may have increased in the latter period of the experiment. This effect is potentially important since invertebrates were not routinely picked from the styrofoam substrata. Although sloughing is often cited as a factor contributing to wide variation in periphyton accrual, it is not likely to be important in these data where the amount of biomass was very small.

By again using a repeated measures nested ANOVA, a trough effect within treatments and controls was not found ($p > 0.5$) but a treatment effect was significant ($p < 0.001$). The biomass levels in the troughs receiving the treated AMD were significantly greater than those in the controls. By only considering data from the day that peak biomass was measured, however, no significant treatment effect was found. Based on the procedure in Cohen (1988), the power of finding no difference when one might exist on the day that peak biomass was measured was 0.34 which is low enough to suggest that the statistical test for treatment effects on peak biomass was inconclusive.

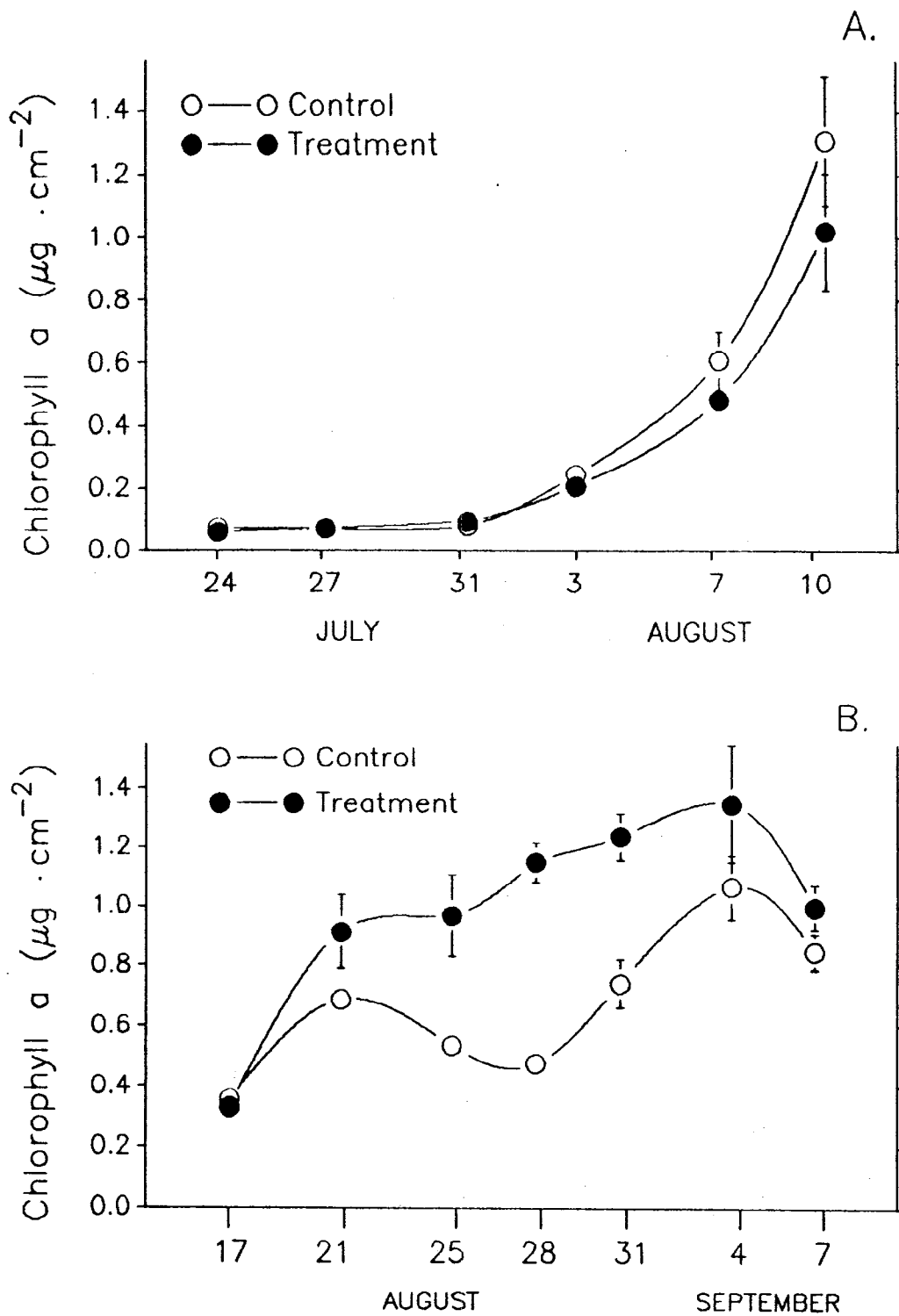


Figure 3. Time course changes in chlorophyll *a* concentrations on styrofoam substrata from A: July 23 through August 10 and B: from August 14 through September 7, in control and treatment troughs.

4.3 Macroinvertebrates

A complete listing of the invertebrate enumeration is given in Appendix 1 and a summary of the composition of the benthos and drift is shown in Table 3 and 4 respectively. We use "taxonomic richness" (Krebs 1978) as an index of diversity. It is essentially a measure of the community structure and is simply the number of taxonomic units per sample. Compared to more complex diversity indices based on information theory (ie. the Shannon-Wiener function; see Krebs 1978) that can be difficult to interpret and are often misused, the main advantage of using richness is its ease of interpretation. The taxonomic units in this study are genera, except for some identifications which proceeded only to the family level (Appendix 1).

At the end of the experiment an average of 2213 and 2468 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 were present in similar numbers.

Taxonomic richness of the drift was about 55% of that of the benthos (Table 4), a finding that is expected since not all taxa of the benthic community have equal tendencies to drift. Numbers of Baetids dominated the drift on all sampling dates. They were followed in importance by *Acari* sp., *Zapada* sp., and *Micrasema* sp. in the mid-August samples. In the August 30 samples, the Baetids numerically represented more than 85% of the community. *Zapada* sp. and chironomids of the Orthocladiinae followed in importance.

Generally, the emergence of aquatic insects was variable and the total numbers were low (Appendix 1). The most commonly collected taxa were midges of the subfamily Orthocladiinae. The only other taxa of significance were the Baetids which were captured occasionally and on the last sampling date, numbers of *Tanytarsini* were similar to those of the Orthocladiinae. Also included in the collections were non-aquatic species of the Hymenoptera, Collembola, Homoptera, and the fly families Muscidae and Scathopagidae. These specimens likely entered the mesocosm through the head tank when the top was opened for temperature readings and miscellaneous other tasks. These insects were omitted from statistical analyses.

4.3.1 Treatment effects on drift

The rates of drift before and after the initiation of AMD flow were tested using a three-way ANOVA. The factors were treatment, date (before or after AMD addition) and sampling period (3 or 24 h period). The interaction term for treatment x date is the measure of interest since a significant interaction would indicate that the rates of drift changed disproportionately from the treatment and control troughs across dates. Examples of this effect are shown for total abundance and taxonomic richness in Figure 4 where the slope of interaction lines do not differ significantly. Ten taxonomic groupings had sufficient numbers for analysis (listed in Table 4). Data were transformed to logarithms of $x+1$ prior to analysis. For both the 3 hour and 24 hour samples, none of the ten groupings had significant treatment x date interactions (all with $p > 0.18$) (Table 4; 24 hour data, Figure 4; 3 and 24 hour data). The critical level of alpha was determined to be 0.005 after the application of Bonferonni's correction for multiple comparisons (Rice 1989). This correction accounts for the possibility of significant random differences in multiple comparisons. The power of the comparison of drift rates between treatments on the day after the start of AMD flow was > 0.95 .

Table 3: Densities and taxonomic richness of benthos from experimental troughs at the Foxy Creek study site. Values include the mean and standard error; sample size = 5. The critical level of alpha 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 (i.e., 0.05/number of comparisons [=17]): see text for explanation.

Grouping	Control		Treatment		Probability of Effect
Total	2213.0	(120.5)	2468.0	(30.5)	>0.07
Taxonomic Richness	20.6	(0.68)	20.2	(0.74)	>0.68
Ephemeroptera					
<i>Baetis</i>	863.4	(83.8)	1090.8	(101.0)	>0.14
<i>Ameletus</i>	170.0	(12.7)	180.4	(27.4)	>0.8
<i>Cinygma</i>	48.4	(11.1)	73.8	(7.3)	>0.14
<i>Rhithrogena</i>	21.0	(3.3)	34.0	(3.9)	>0.03
<i>Epeorus</i>	3.0	(2.5)	1.6	(0.5)	>0.9
<i>Paraleptophlebia</i>	17.2	(2.1)	10.4	(1.7)	>0.03
EphemereIIDae	12.8	(1.8)	11.8	(1.5)	>0.7
Plecoptera					
<i>Zapada</i>	143.6	(14.7)	130.4	(9.5)	>0.5
Chloroperlidae	137.4	(9.9)	155.2	(21.9)	>0.5
<i>Doroneuria</i>	16.0	(1.5)	22.6	(4.5)	>0.15
<i>Isoperla</i>	12.4	(3.2)	16.2	(4.8)	>0.6
Trichoptera					
<i>Micrasema</i>	13.4	(5.1)	22.2	(11.3)	>0.5
others	3.4	(0.25)	3.0	(0.8)	>0.45
Diptera (Chironomidae)					
Tanypodinae	196.0	(21.6)	203.8	(18.6)	>0.75
OrthoclaIinae	405.0	(21.6)	394.8	(25.1)	>0.7
<i>Tanytarsini</i>	146.2	(17.7)	112.2	(12.7)	>0.15

Table 4: Summary of the composition of drift collected over 24 hour periods before (August 12-13 sample) and after (August 13-14 and August 30 samples) AMD additions. Data are mean numbers and standard errors.

Grouping	Control		Treatment		Probability of Effect
1: August 12-13 sample					
Total	92.6	(5.91)	97.0	(12.3)	
Richness	11.0	(0.84)	12.4	(0.40)	
<i>Baetis</i>	34.6	(3.71)	37.6	(14.4)	
<i>Epeorus</i>	0.0		0.4	(0.25)	
<i>Serratella</i>	1.0	(0.63)	0.6	(0.40)	
<i>Zapada</i>	12.0	(1.48)	13.6	(2.58)	
<i>Micrasema</i>	14.0	(3.19)	18.2	(3.69)	
<i>Parapsyche</i>	1.2	(0.97)	1.2	(0.37)	
Orthocladiinae	2.8	(0.37)	3.6	(0.81)	
Simuliidae	2.8	(0.86)	3.0	(1.09)	
<i>Acari</i>	15.6	(2.71)	10.6	(1.17)	
miscellaneous	8.6	(1.50)	8.2	(0.40)	
2: August 13-14 sample					
					P: (Treatment x Date)
Total	82.2	(10.28)	97.0	(6.67)	>0.40
Richness	10.8	(1.20)	10.2	(0.58)	>0.72
<i>Baetis</i>	30.2	(3.81)	41.2	(7.48)	>0.18
<i>Epeorus</i>	0.0		0.4	(0.25)	>0.35
<i>Serratella</i>	0.6	(0.40)	0.8	(0.20)	>0.50
<i>Zapada</i>	10.2	(1.85)	15.8	(4.60)	>0.98
<i>Micrasema</i>	9.4	(2.09)	10.0	(3.27)	>0.94
<i>Parapsyche</i>	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
<i>Acari</i>	17.4	(2.38)	20.6	(1.54)	>0.56
miscellaneous	8.4	(2.42)	4.8	(0.97)	>0.51

/Continued

Table 4: Continued

3: 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
<i>Baetis</i>	114.0	(18.2)	89.2	(4.87)	>0.22
<i>Epeorus</i>	0.0		0.2	(0.20)	
<i>Serratella</i>	0.2	(0.20)	0.2	(0.20)	
<i>Zapada</i>	5.8	(2.82)	2.6	(0.60)	>0.29
<i>Micrasema</i>	0.0		0.0		
<i>Parapsyche</i>	0.0		0.0		
Orthoclaadiinae	3.6	(0.40)	2.2	(0.58)	>0.08
Simuliidae	0.6	(0.25)	0.2	(0.20)	
<i>Acari</i>	0.0		0.0		
miscellaneous	8.6	(1.03)	5.0	(2.00)	>0.14

The 24 h drift samples from August 30 were analyzed with ANOVA. No taxonomic group revealed any treatment effect (all with $p > 0.08$; Table 4). Note that the critical level of alpha after the Bonferonni 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 (Figure 4). Both indices of samples from the treatment troughs appeared slightly higher for the 12-14 August period, but those from control troughs appeared greater on August 30. These differences were not significant (Table 4).

4.3.2 Treatment effects on benthos

Benthos samples were analyzed using ANOVA. Total densities were about 12% higher in treatment troughs relative to the control troughs (Table 3), but these differences were not significant ($p > 0.05$). The power of the test was 0.80. Each taxonomic group shown in Table 3 was analyzed separately but none were significantly different at a critical alpha of 0.003, the significance level applied after the Bonferroni correction. Two genera of mayflies (*Rhithrogenia* sp. and *Paraleptophlebia* sp.) had differences at the $0.05 > p > 0.03$ 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..

Species richness did not differ significantly between the benthic samples from the treatment and control troughs (ANOVA, $p > 0.68$).

4.3.3 Treatment effects on emergence

An ANOVA of logarithmically transformed emergence data with treatment and date as main effects was carried out. There was no significant effect of treatment ($p > 0.35$), while there were differences among dates ($p < 0.001$). Figure 5 indicates that emergence increased in a linear pattern that was independent of the addition of the treated AMD. This increase occurred despite declining temperatures. 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 to 5.9°C).

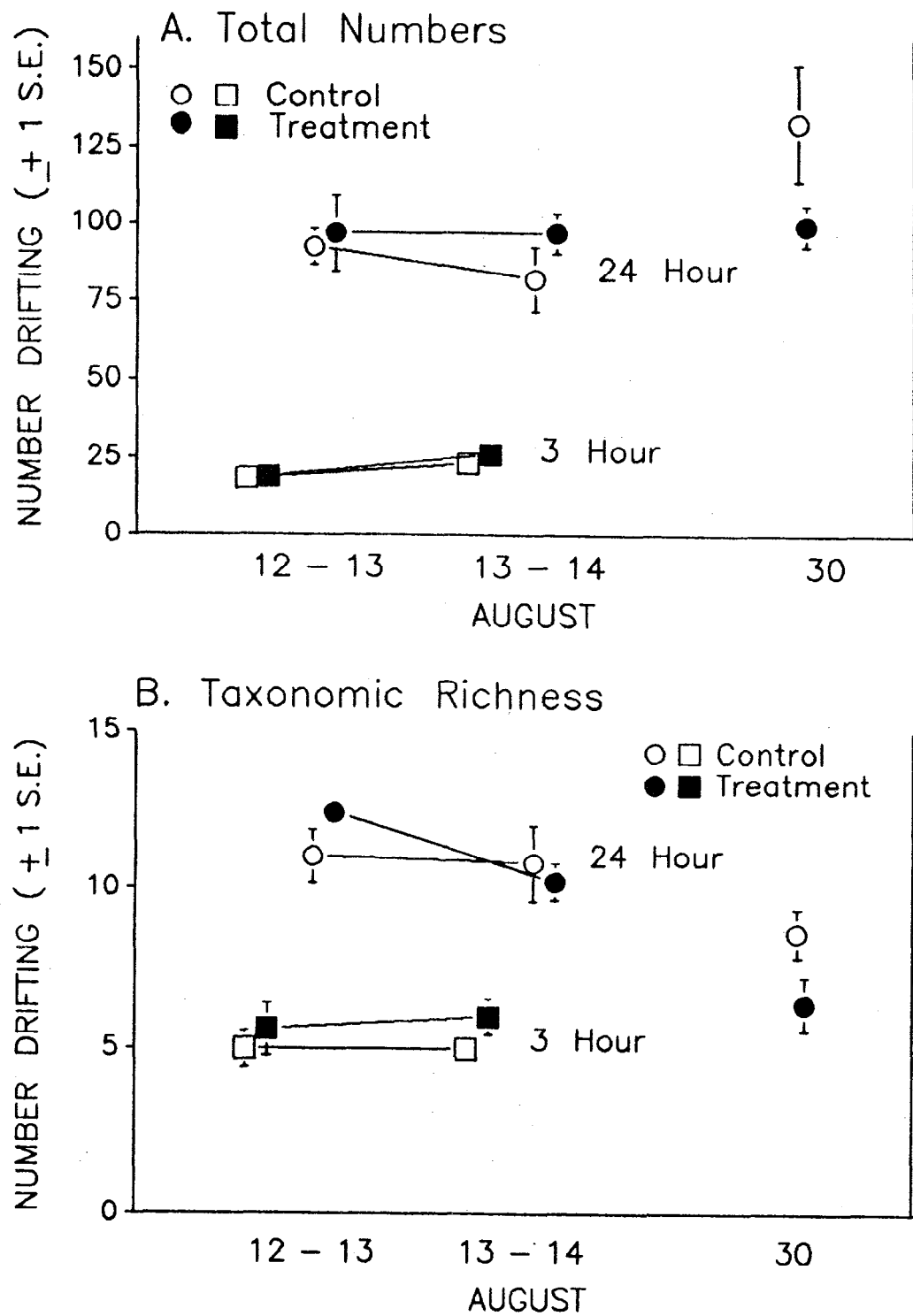


Figure 4. Total numbers (A) and taxonomic richness (B) of drifting invertebrates measured in 3 h and 24 h periods during August 12-14 and during a 24 h period on August 30. Lines drawn between data show the treatment x date interactions, none of which were significant. See text for significance values.

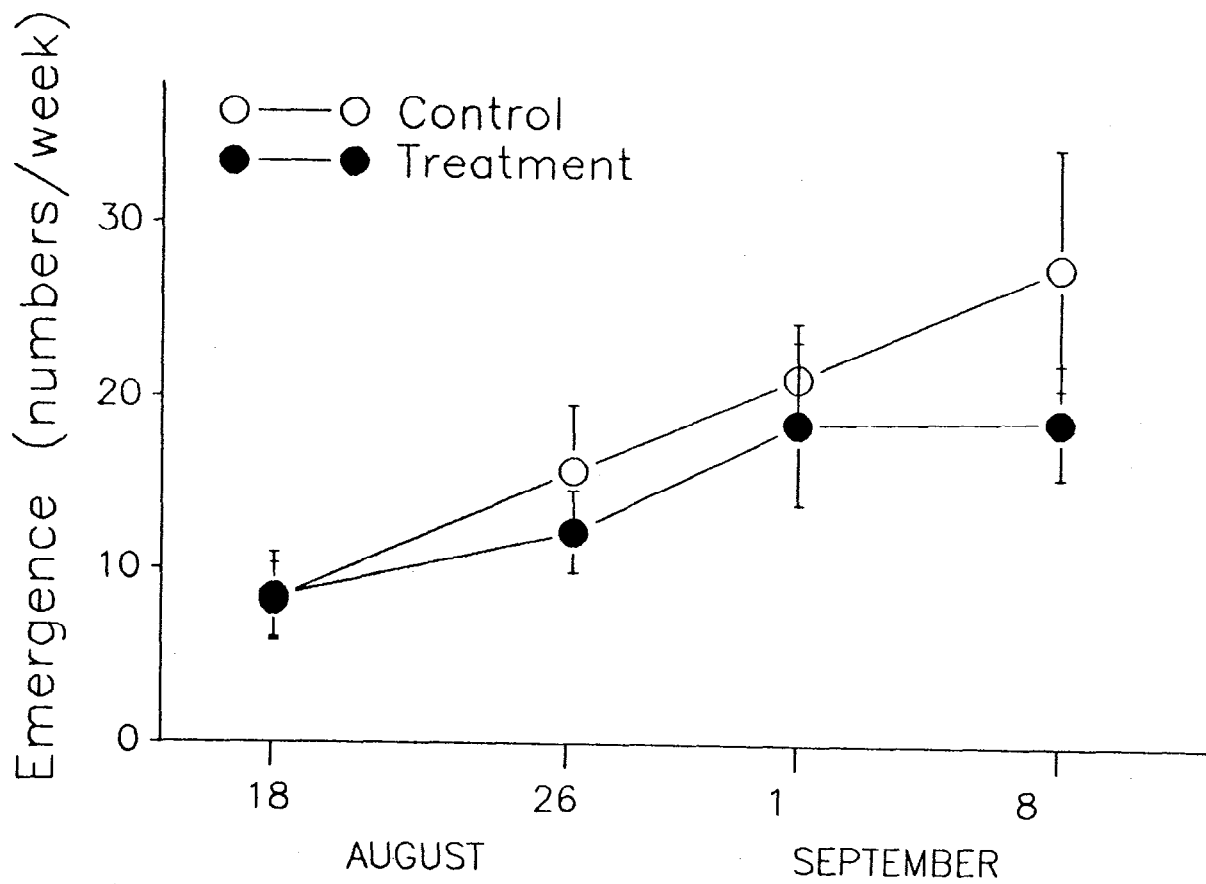


Figure 5. Time course changes in numbers of insects emerging from control and treatment troughs each week.

5.0 DISCUSSION

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. The realism of allowing natural assemblages of species to enter and leave the mesocosm in an open system allowed a direct comparison to the effects that would be expected in Foxy Creek. The troughs successfully supported the colonization and growth of periphyton and aquatic insect communities, thereby suggesting that the size of the troughs was appropriate for impact assessments at primary and secondary producer levels. The various ANOVA's which were used as the basic tool for examining the impact of AMD additions had power values in excess of 0.80 which is in the range that makes the results highly conclusive. Clearly, the use of five replicates was acceptable in testing the null hypothesis that the addition of treated AMD did not affect the abundance of periphytic algae and benthic insects.

With one exception, the addition of treated AMD from the Equity Mine drainage had no significant effect on the biological measures examined in the bioassay at the prescribed AMD dilution rate. This main finding suggests that at the dilution rate of 10 parts Foxy Creek water to one part treated AMD having chemical concentrations similar to those measured in this study, the discharge of treated AMD will not impact on the abundance of fish food organisms downstream of the Lu Creek diversion canal in Foxy Creek. It is interesting, however, that periphyton biomass increased significantly due to the AMD addition. Apparently, the treated AMD did not produce a toxic effect but rather an enhancement of algal biomass accrual. Since the treated AMD contained levels of several micronutrients, it is possible that any micronutrient deficiency was alleviated by the chemical additions. Although the procedures used in the experiment were sufficiently powerful to detect the treatment effect, it is important that the differences in

biomass levels (Figure 3) were very small and well within the range that can typically be found on substrata in a natural stream. The small enhancement effect, may have contributed to the 12% increase in total numbers of benthos in the treated troughs, although the increase in numbers of insects was not found to be significant.

The actual estimates of benthic densities were dependent on mesh size of the sampling gear and sorting sieves used. The number of taxa reported were undoubtedly only a small proportion of the total number of species that were actually present in the troughs. A large portion of the numbers of benthic invertebrates would have been missed by sorting through a sieve of 0.47 mm (e.g. Mundie et al. 1983). There is some evidence that the relatively small early stages of some insect species may be more sensitive to chemical manipulation than later stadia (Gauss et al. 1985). However, the mesh size used in this study retained the early instars of most taxa recorded. The main group of insects that might have been missed would be the chironomid midges. Generally, however, they are less sensitive to toxic substances than are other insect taxa. There is no reason *a priori* to imagine that the responses measured in the trough experiment might have been biased at different size scales. The use of a particular size sieve is a trade-off between including smaller taxa and individuals against the time required to process samples to such a small size fraction. In spite of missing many individuals, the larger size fraction (>0.47 mm) represents the largest portion of the benthic biomass. Since biomass is of greatest interest where impact to fish food organisms is concerned, the findings reported here are considered conclusive.

Acceptance of the null hypothesis that the discharge of treated AMD had no effect on benthic algae and insects suggests that the previous process of specifying loading rates of treated AMD to Foxy Creek has been successful in protecting the production of fish food organisms in Foxy Creek despite the absence of site-specific data. Before consideration of the use of the mesocosm approach for setting water quality objectives,

the specific chemistry of the diluted AMD and its potential effects on fish food-organisms was difficult to isolate without extensive testing of all species; a process that was largely impractical for both fiscal and scientific reasons. Limited budgets do not allow multispecies toxicity testing for a single site. In addition, the effects of high concentrations of calcium and magnesium that occur in treated AMD may ameliorate toxic effects of heavy metal contamination, since these cations have been shown to reduce biological binding sites for heavy metals (Campbell and Stokes 1985). In fact, it has proven difficult to identify speciation and complexation of metals in natural waters (Campbell and Stokes 1985). Hence, a greater use of site-specific tests of toxicity is required to establish quantitative guidelines. Since limited budgets have not allowed this testing, policy guidelines based on the use of present (albeit misleading) laboratory toxicity tests and personal experience has sufficed.

A great deal of the uncertainty associated with this process can be circumvented with the broad spectrum test provided by in situ mesocosms. The success of the mesocosm approach in this study in addition to the increasing use of flow-through stream mesocosms for basic and applied research into stream systems is indicative of the potential for such an approach to be more fully accepted as a technique for establishing water quality criteria for industrial users of water in British Columbia. Past uses of such systems in ecotoxicological studies has been limited by the failure to replicate, and thereby have no statistical power to draw inferences (e.g., Cooper and Stout 1985, Servos and Mackie 1986, Allard and Moreau 1987). Careful consideration of replication in a powerful experimental design that was used in this study is evidence that the technique can be further developed and applied where water quality criteria have yet to be established or where there is uncertainty in present water quality objectives. It may be particularly useful where the closure of existing mines is being considered and the management of drainage quality is crucial in the planning for closure.

Although the experimental design used in this study was simple, the mesocosm apparatus can be used for a variety of experimental tests. Future tests could elaborate on the basic experimental design by incorporating a gradation of treatment applications. Use of a series of graded treatments allows the definition of threshold effects as well as determining whether different functions or taxa are impaired at different levels. By calculating a graded response curve, a highly accurate measure of the minimum level of dilution of the treated AMD milieu that causes an impact on the colonization, abundance, drift, and emergence of fish food organisms can be identified.

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EQUITY SILVER: DRIFT
 August 12, 3hrs.

	TROUGH #									
	1	2	3	4	5	6	7	8	9	10
Coleoptera										
Elmidae	0	0	0	0	0	0	0	0	0	0
Hymenoptera										
Scelionidae	0	0	0	0	0	0	0	0	0	0
Eucoilidae	0	0	0	0	0	0	0	0	0	0
Braconidae	0	0	0	0	0	0	0	0	0	0
Diapriidae	0	0	0	0	0	0	0	0	0	0
Diptera										
Muscidae	0	0	0	0	0	0	0	0	0	0
Scathopagidae	0	0	0	0	0	0	0	0	0	0
Dolichopodidae	0	0	0	0	0	0	0	0	0	0
Ephydriidae	0	0	0	0	0	0	0	0	0	0
Chaoboridae	0	0	0	0	0	0	0	0	0	0
Ceratopogonidae	0	0	0	0	0	0	0	0	0	0
Chironomidae										
Tanypodini	0	0	0	0	0	0	0	0	0	0
Orthoclaadiinae	0	1	1	0	0	1	3	3	5	4
Tanytarsini	0	0	0	0	0	0	0	0	0	0
Simulidae										
Simulium sp.	0	1	1	1	0	0	0	0	0	2
ARACHNIDS	2	2	4	4	4	5	4	4	12	7

EQUITY SILVER: DRIFT
 August 12-13, 24hrs

	TROUGH #									
	1	2	3	4	5	6	7	8	9	10
Coleoptera										
Elmidae	0	0	0	0	0	0	0	0	0	0
Hymenoptera										
Scelionidae	0	0	0	0	0	0	0	0	0	0
Eucoilidae	0	0	0	0	0	0	0	0	0	0
Braconidae	0	0	0	0	0	0	0	0	0	0
Diapriidae	0	0	0	0	0	0	0	0	0	0
Diptera										
Muscidae	0	0	0	0	0	0	0	0	0	0
Scathopagidae	0	0	0	0	0	0	0	0	0	0
Dolichopodidae	0	0	0	0	0	0	0	0	0	0
Ephydriidae	0	0	0	0	0	0	0	0	0	0
Chaoboridae	0	0	0	0	0	0	0	0	0	0
Ceratopogonidae	0	0	0	0	0	0	0	0	0	0
Chironomidae										
Tanypodini	0	0	0	0	0	0	0	0	0	1
Orthocladiinae	3	6	2	3	3	5	4	2	2	2
Tanytarsini	0	0	0	0	0	0	0	0	0	0
Simulidae										
Simulium sp.	2	0	5	5	2	2	0	4	6	3
ARACHNIDS	25	14	12	9	10	7	17	12	10	15

EQUITY SILVER: DRIFT
 August 14

	TROUGH #									
	1	2	3	4	5	6	7	8	9	10
Coleoptera										
Elmidae	0	0	0	0	0	0	0	0	0	0
Hymenoptera										
Scelionidae	0	0	0	0	0	0	0	0	0	0
Eucoilidae	0	0	0	0	0	0	0	0	0	0
Braconidae	0	0	0	0	0	0	0	0	0	0
Diapriidae	0	0	0	0	0	0	0	0	0	0
Diptera										
Muscidae	0	0	0	0	0	0	0	0	0	0
Scathopagidae	0	0	0	0	0	0	0	0	0	0
Dolichopodidae	0	0	0	0	0	0	0	0	0	0
Ephydriidae	0	0	0	0	0	0	0	0	0	0
Chaoboridae	0	0	0	0	0	0	0	0	0	0
Ceratopogonidae	0	0	0	0	0	0	0	0	0	0
Chironomidae										
Tanypodini	0	0	0	0	0	0	0	0	0	0
Orthoclaadiinae	1	1	0	0	1	1	2	3	1	1
Tanytarsini	0	0	0	0	0	0	0	0	0	0
Simulidae										
Simulium sp.	0	0	1	0	0	1	0	0	1	0
ARACHNIDS	6	2	2	4	6	3	3	1	4	2

EQUITY SILVER: DRIFT
August 30

	TROUGH #									
	1	2	3	4	5	6	7	8	9	10
Coleoptera										
Elmidae	0	0	0	0	0	0	0	0	0	0
Hymenoptera										
Scelionidae	0	0	0	0	0	0	0	0	0	0
Eucoilidae	0	0	0	0	0	0	0	0	0	0
Braconidae	0	0	0	0	0	0	0	0	0	0
Diapriidae	0	0	0	0	0	0	0	0	0	0
Diptera										
Muscidae	0	0	0	0	0	0	0	0	0	0
Scathopagidae	0	0	0	0	0	0	0	0	0	0
Dolichopodidae	0	0	0	0	0	0	0	0	0	0
Ephydriidae	0	0	0	0	0	0	0	0	0	0
Chaoboridae	0	0	0	0	0	0	0	0	0	0
Ceratopogonidae	0	0	0	0	0	0	0	0	0	0
Chironomidae										
Tanypodini	0	0	0	1	0	0	0	0	0	0
Orthoclaadiinae	4	3	1	5	2	4	3	3	1	3
Tanytarsini	0	2	0	0	0	1	0	0	0	0
Simulidae										
Simulium sp.	0	0	0	1	0	0	1	0	1	1

ARACHNIDS

EQUITY SILVER: EMERGENCE
August 18

	TROUGH #									
	1	2	3	4	5	6	7	8	9	10
INSECTA										
Collembola	0	0	1	0	2	0	0	0	0	0
Ephemeroptera										
Siphonuridae										
Ameletus sp.	0	0	1	0	0	0	1	0	0	0
Baetidae										
Baetis sp.	0	1	1	0	0	0	0	0	1	0
Heptageniidae										
Cinygma sp.	0	0	0	0	0	0	0	0	0	0
Cinygmula sp.	0	0	0	0	0	0	0	0	0	0
Epeorus sp.	0	0	0	0	0	0	0	0	0	0
Rhithrogena sp.	0	0	0	0	0	0	0	0	0	0
Ephemerellidae										
Drunella sp.	0	0	0	0	0	0	0	0	0	0
Serratella sp.	0	0	0	0	0	0	0	0	0	0
Eurylophella sp.	0	0	0	0	0	0	0	0	0	0
Leptophlebiidae										
Paraleptophlebia sp.	0	0	0	0	0	0	0	0	0	0
Orthoptera										
Tridactylidae	0	0	0	0	0	0	0	0	0	0
Unknown	0	0	0	0	0	0	0	0	1	1
Plecoptera										
Nemouridae										
Zapada sp.	0	0	0	0	0	0	0	0	0	0
Perlidae										
Doroneuria sp.	0	0	0	0	0	0	0	0	0	0
Perlodidae										
Isoperla sp.	0	0	0	0	0	0	0	0	0	0
Chloroperlidae										
Sweltsa or Triznaka s	0	0	0	0	0	0	0	0	0	0
Unknown	0	0	0	0	0	0	0	0	0	0
Trichoptera										
Brachycentridae										
Micrasema sp.	0	0	0	0	0	0	0	0	0	0
Hydropsychidae										
Parapsyche sp.	0	0	0	0	0	0	0	0	0	0
Rhyacophilidae										
Rhyacophilia sp.	0	0	0	0	0	0	0	0	0	0
Limnophelidae	0	0	0	0	0	0	0	1	0	0

