

**STREAM COMMUNITY RESPONSES
TO A GRADIENT OF
ACID MINE DRAINAGE ADDITIONS**

MEND Report 4.7.3b

This project was prepared for
British Columbia Acid Mine Drainage Task Force through funding from Energy, Mines
and Resources Canada and the British Columbia Ministry of Energy, Mines and Petroleum
Resources under the Canada/British Columbia Mineral Development Agreement, by
Environment Canada, the British Columbia Ministry of Environment, and Noranda
Minerals Inc.

August 1992

**STREAM COMMUNITY RESPONSES
TO A GRADIENT OF
ACID MINE DRAINAGE ADDITIONS**

**LIMNOTEK RESEARCH AND DEVELOPMENT INC.
Vancouver, British Columbia**

August 1992

This project was prepared for British Columbia Acid Mine Drainage Task Force through funding from Energy, Mines and Resources Canada and the British Columbia Ministry of Energy, Mines and Petroleum Resources under the Canada/British Columbia Mineral Development Agreement, by Environment Canada, the British Columbia Ministry of Environment, and Noranda Minerals Inc.

SUMMARY

Relationships were examined between chemical variation associated with additions of acid mine drainage (AMD) to a stream, and changes in lotic community structure. The experiment was run in Foxy Creek, close to an AMD seepage source from the Equity Silver Mine, north-central B.C. The AMD was metered into a 16 channel, flow-through mesocosm in an experimental design that included seven replicated AMD dilutions: Control, 1:25000, 1:10000, 1:2500, 1:1000, 1:250, 1:25. Alkalinity and pH varied hyperbolically with AMD dilutions. At dilutions less than 1:1000, there was clear evidence that acid neutralizing capacity of the stream was exceeded and pH dropped abruptly from neutrality. Metals levels increased by several orders of magnitude with declining dilution of the AMD. Metals which changed most from the AMD additions were those known to be most toxic to aquatic organisms. Analysis of copper complexing capacity indicated that at the lowest dilution of AMD (1:25), dissolved organic ligands were completely hydrolyzed. At higher dilutions, complexation capacity increased, thus suggesting reduced potential for toxicity at those dilutions. At highest dilutions, other ligands may have been important in complexing metal cations. Evidence of protonation of organic ligands at the 1:25 dilution indicated "competition" between H^+ and cations including Cd, Cu, and Zn and the possibility that despite the high concentrations of aquo ions, hydrolysis of exchange sites may have reduced direct toxicity by metal binding. A separate group of processes that potentially contributed to toxicity included the ingestion of metals bound to a series of low and high energy binding sites on food particles. Relatively loose sediment bound metals that included in ranked order; $Cd > Zn > Cu > Mn$ could have contributed to toxicity via ingestion. The abundance of the diatom dominated periphyton community and mayfly/chironomid dominated benthos declined with increasing doses of AMD according to logarithmic decay functions. Of the taxon that dominated the benthic community, only the abundance of the Orthocladinae did not change as a function of the AMD additions. Insect drift analyses indicated an immediate avoidance response to the onset of AMD additions, the response by mayflies occurring within three hours and that by the Tanytarsini within 24 hours. Drift also increased with higher doses of the AMD. Crude estimates of insect turnover rates indicated that the mayfly population was transient and the chironomids settled for longer periods in the troughs. Models of indices of benthic response to the gradient of AMD additions suggested that the structure of the benthic community in Foxy Creek would be changed with an AMD dilution of less than or equal to 1:25,000. Comparison of these models to CCREM guidelines indicated that levels of Cd, Mn, Zn, Al, and Fe that were found to cause change in community structure as part of a complex metals mixture were less than the critical levels indicated in the CCREM guidelines. Only copper levels found to cause change as part of the mixture were above the CCREM guideline. This comparison did not indicate that the CCREM guidelines are wrong. Rather, this study suggests that models based on community level responses to experimental dilutions of chemical mixtures are appropriate predictors to be used after an initial screening using CCREM guidelines, when questions related to systems no less complex than communities are of interest. The concentration of a metal in solution that causes change in community structure can be lower when that metal is part of a complex acidic mixture with other metals compared to when the same metal acts individually on single organisms or simple populations which is the level of complexity in most tests forming the basis of CCREM guidelines.

ACKNOWLEDGEMENTS

This project was prepared for the British Columbia Acid Mine Drainage Task Force through funding from Energy, Mines and Resources Canada and the British Columbia Ministry of Energy, Mines and Petroleum Resources under the Canada/British Columbia Mineral Development Agreement, by Environment Canada, the British Columbia Ministry of Environment, and Noranda Minerals Inc. Liaison with Brian Wilkes (MOE, Smithers), Benoit Godin (EC, North Vancouver), Bob Patterson (Equity Silver, Houston), Dr. John Errington (MEMPR, Victoria), and Paul Kluckner (EC, West Vancouver) is greatly appreciated and was essential in making the project a success. Ray Carrier and Duane Hogberg assisted with the field operations. Ray Carrier is also thanked for his assistance with data preparations and selected aspects of the literature review. Karen Needham and Gareth Williams conducted the insect enumeration and Mary Bolin performed the algal enumeration. Dr. John Richardson assisted with the statistical analyses and discussion with him on several aspects of the findings was greatly appreciated.

TABLE OF CONTENTS

	Page
SUMMARY	ii
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	iv
LIST OF FIGURES	v
LIST OF TABLES	vii
1.0 INTRODUCTION	1
1.1 Background	1
1.2 Study Objectives	4
2.0 MATERIALS AND METHODS	4
2.1 Study Site	4
2.2 Mesocosm Operation	6
2.3 Physical Data	8
2.4 Water Chemistry	9
2.5 Sediment Chemistry	10
2.6 Periphyton	12
2.7 Aquatic Insects	13
3.0 RESULTS AND DISCUSSION	14
3.1 Physical Variables	14
3.2 Chemical Relationships	15
3.3 Periphyton	34
3.4 Aquatic Insects	42
3.4.1 Insect Drift	42
3.4.2 Adult Insects	48
3.4.3 Benthos	50
3.5 AMD Toxicity in Foxy Creek	53
4.0 REFERENCES CITED	57

LIST OF FIGURES

	Page
Figure 1. Location of the mesocosm apparatus at the Equity Silver Mine	5
Figure 2. Schematic layout of the mesocosm apparatus	7
Figure 3. Mean daily temperature in Foxy Creek during the study period	14
Figure 4. Mean concentrations of total Cu (a), Fe (b), and Al (c) extracted from the trough sediments for each dilution of AMD additions	22
Figure 5. Change in the capacity of organic ligands to complex copper as a function of the rate of AMD addition	23
Figure 6. Partitioning of Cu, Fe, and Al determined by sequential extraction of one sample collected from each treatment	25
Figure 7. Mean concentrations of total Cd (a), Mn (b), and Zn (c) extracted from the trough sediments for each dilution of AMD additions	28
Figure 8. Partitioning of Cd, Mn, and Zn determined by sequential extraction of one sample collected from each treatment	29
Figure 9. Loss on ignition of sediments collected across the AMD gradient	31
Figure 10. Log-log regressions showing the effect of AMD additions on total periphytic algae cell density and diatoms only	37
Figure 11. Log-log regressions showing the effect of AMD additions on the cell density of the Cyanophyta and Chlorophyta	38
Figure 12. Log-log regression showing the effect of AMD additions on the periphytic algae richness	39
Figure 13. Total number of aquatic insects drifting over paired time durations (3h and 24h) before and after the onset of AMD additions and on three subsequent 24h periods during AMD additions	44

Figure 14.	Numbers of <i>Baetis sp.</i> drifting over paired time durations (3h and 24h) before and after the onset of AMD additions and on three subsequent 24h periods during the AMD additions	46
Figure 15.	Numbers of Tanytarsini drifting over paired time durations (3h and 24h) before and after the onset of AMD additions and on three subsequent 24h periods during the AMD additions	47
Figure 16.	Numbers of chironomid adults drifting over paired time durations (3h and 24h) before and after the onset of AMD additions and on three subsequent 24h periods during the AMD additions	49
Figure 17.	Log-log regressions showing the effect of AMD additions on total benthos and Tanytarsini	52

LIST OF TABLES

	Page
Table 1. Layout of treatments and replication within the seven levels of AMD addition in the proposed Equity 1990 experiment	8
Table 2. Mean concentrations (\pm 1 SD) of metals and other descriptive indices in raw AMD from the upper seepage pond and Foxy Creek water in the mesocosm control troughs in August and September, 1990	15
Table 3. Mean concentrations (\pm 1 SD) of dissolved metals and other chemical indices measured in the seven dilutions of AMD in Foxy Creek water	19
Table 4. Relative abundance of algal taxa found in the mesocosm troughs on September 8	35
Table 5. Relationships between algal density and AMD additions as determined from log-log regressions	40
Table 6. Peak biomass (PB) concentrations of chlorophyll <i>a</i> by treatment in the mesocosm troughs	41
Table 7. Estimates of turnover rates of dominant insect groups found in the troughs on September 6	48
Table 8. Summary statistics for the final densities of benthos	51
Table 9. Comparison of metals concentrations that caused a change in the structure of the mesocosm community with levels established in water quality guidelines (CCREM)	56

1.0 INTRODUCTION

1.1 Background

An objective of managing freshwater ecosystems is to establish water quality guidelines that will prevent or minimize change in ecosystem structure and function downstream of chemical discharges. The scientific nature of this statement implies that a fundamental, quantitative relationship exists to form the basis of a guideline. It infers that an hypothesis can be tested to examine effects of contaminants on ecosystem processes or that data exists with sufficient precision and accuracy that effects can be predicted. The objective suggests that through experimental science or a data management process, resource managers can recommend guidelines with the knowledge that a particular system will not change at a specific level or within a certain range of contaminant additions.

There are two divergent approaches to testing effects of toxicants on aquatic ecosystems. Each is fundamentally based on the view that effects of chemical additions to lentic or lotic systems must be known before those additions begin and thus water quality guidelines can be established to protect downstream ecosystems and water users. Each is based on doing experiments but that is where the similarities end.

The first approach is the short term bioassay. It is a standardized procedure, run in a laboratory, and is based on determining if a single organism or a homogeneous or mixed population dies within a predefined period of time of exposure to a toxicant. This has been the conventional approach to toxicity testing, originally conceived by Hart et al. (1945) and later endorsed by Doudoroff et al. (1951). The emphasis on testing stress or death on single organisms or simple populations dominates the toxicological literature (reviewed by EVS, 1990) and to date forms the basis of water quality guidelines (CCREM 1989). The techniques are quick (results are available within a week), they can be performed as a battery of tests which makes them relatively inexpensive yet precise, and they have legal acceptance which makes them useful as evidence in the permitting process. The acute lethality tests for *Daphnia sp.* (EPS

1/RM/11, 1990), rainbow trout (EPS 1/RM/9, 1990), and three spine stickleback (EPS 1/RM/10, 1990) are examples of the legally accepted bioassays in Canada but lethal tests have been performed on wide numbers of species (eg. Mackie 1989) as long as they can be successfully collected from the field and be kept in a laboratory long enough to perform the tests.

The second approach is actually a group of alternatives that have an ecological basis. They are increasingly being examined because of the view that there is no scientific basis for extrapolation of bioassay results to predict ecosystem responses (Cairns and Pratt 1989). Regulators will obviously assign allowable levels of toxicants that are less than those known to kill organisms in bioassay tests but sublethal effects remain unresolved. The bioassay gives no information on changes in relative dominance of species, species interactions, biogeochemical cycles, and the temporal sequence of changes in trophic structure, all of which are necessary for determining effects on water quality, fisheries, and other water users downstream. Schindler (1987) suggests that bioassays are basically inadequate for establishing cause and effect relationships for ecosystems because they ignore the importance of top predators which can be important in systems where "trophic cascading" (Carpenter et al. 1985) is known to affect trophic structure and system biogeochemistry (eg. Hessen and Nilssen, 1986). To include the dynamics of trophic cascading, testing effects of chemical additions requires experimental manipulations of whole ecosystems such as those conducted at the Experimental Lakes Area (ELA) in northwestern Ontario (eg. Schindler et al. 1985).

Obviously, most management problems do not allow whole ecosystem perturbations such as the those used at ELA mainly because they are expensive, and do not provide information within a time frame for short term decision making that is usually required for industrial developments. Many would argue that whole ecosystem studies are unavoidable to address the management of pollutants that affect large areas (ie. the acid rain problem over north eastern North America) and certainly, work at the ELA has been influential in establishing data with which management decisions can be made at the international level. On smaller spatial scales, alternate approaches are required to establish regulations. Effects of acidification in the

Andirondack Lakes have been examined with complicated models (Gherini et al. 1985) but Schindler (1987) points out that many models do not include relevant processes and are prone to giving misleading results of chemical perturbations. Monitoring studies have also been considered for "impact assessments" but for many of those related to stream ecosystems, the indices selected for measurement have been insensitive or inappropriate for examining ecosystem responses to chemical doses or their duration has been insufficient to detect real effects (Larkin 1984). More importantly, impact assessments generally lack statistical rigor and controls from which treatment effects can be measured. The fact that these assessments continue to be used (usually at great cost to a proponent) indicates that there is some difficulty in establishing alternate approaches to monitoring. Even recent proposals outlining "environmental effects monitoring" indicate that this "new" approach is fundamentally based on comparisons of spatially separated sites. This approach is in fact no different from conventional monitoring and it has a continuing problem of not being able to isolate causal effects from changes in water chemistry.

For assessments of chemical manipulations in streams, the use of mesocosms is gaining wide acceptance for obtaining quantitative evidence of responses by representative communities to chemical manipulation (Allard and Moreau 1987, Hart and Robinson 1990, Mundie et al. 1983, Mundie et al. 1991, Perrin 1992). Stream mesocosms located in the field (recirculating lab systems are not considered here) function as open systems and their flow-through design provides for the exchange of components necessary to represent the stream biogeochemistry, behavioral interactions of organisms, food web interactions, etc. Biological processes are integrated in a trough mesocosm, as they are in the natural stream, thus allowing for considerable extrapolation of findings to larger stream reaches. Work with continuous-flow trough mesocosms at the Equity Silver Mine in 1989 (Perrin et al 1992) showed that replicated experimental units in a mesocosm were suitable to test hypotheses of effects of treated acid mine drainage (AMD) additions to Foxy Creek, a second order stream that receives treated AMD from the mine. Results had power values well in excess of 0.8, the minimum level recommended by Peterman (1990) to be confident that the outcome of statistical tests is conclusive.

1.2 Study Objectives

This study continues from the 1989 work at Equity to test the effects of a gradient of raw AMD additions to a Foxy Creek mesocosm. Six troughs were added to the 10-trough layout used in 1989 to quantitatively describe the relationship between raw AMD dilution and indices of abundance and composition of the macrobenthos and periphyton communities. Functional response curves that are representative of in-stream responses intuitively can provide powerful tools for setting water quality guidelines by describing the range of AMD dilutions that causes shifts in community structure and increases in mortalities of organisms. Together with descriptions of changes in chemical dynamics at each of the AMD treatments, this approach is comprehensive and provides far more relevant information on community processes than can be accomplished with even the most intensive lab bioassay or monitoring study.

2.0 MATERIALS AND METHODS

2.1 Study Site

The experiment was conducted in Foxy Creek, immediately downslope of AMD seepage ponds located at the base of tailings dam #1 at the Equity Silver Mine (Figure 1). This location allowed a water supply pipeline to be laid upstream in Foxy Creek and it provided easy access to the raw AMD contained in the seepage pond. Power was available from a pumphouse located adjacent to the seepage ponds.

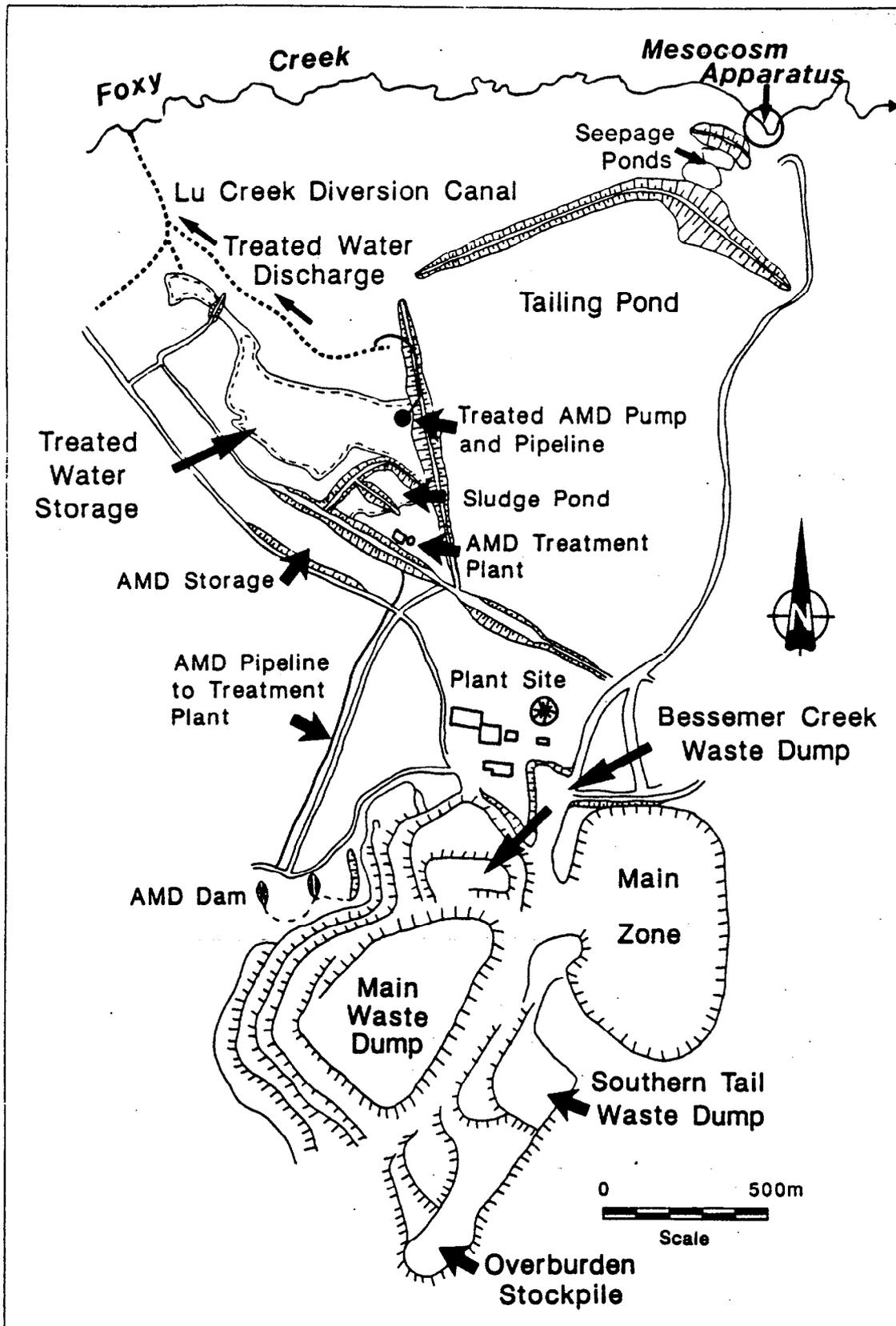


Figure 1. Location of the mesocosm apparatus at the Equity Silver Mine.

2.2 Mesocosm Operation

The mesocosm consisted of 16 flow-through troughs (each 1.52m long x 0.2m wide) that were fabricated with 0.64 cm clear acrylic and assembled on a series of joists laid over the stream channel. The design followed that originally conceived by Mundie et al (1991) and subsequently modified for use at the Equity mine (Figure 2). Water and biota carried in suspension in Foxy Creek were supplied to the mesocosm via 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 that was used to adjust water flow in each trough to $0.3 \text{ L} \cdot \text{s}^{-1}$. Raw AMD from the upper seepage pond was delivered to the troughs by gravity through a second 170 m (5 cm diameter) pipeline and metered into each trough with Teflon needle valves (Cole Parmer Instrument Co., Chicago) for the two highest treatments and a peristaltic pump (Technicon Autoanalyzer pump head) for the other treatments (Table 1). Because waste from the mesocosm would increase metals levels in Foxy Creek to concentrations close to those cited in current waste guidelines, a collection basin was constructed and all waste was pumped upslope and discharged into the lower AMD seepage pond. The control of waste at the site was authorized under Ministry of Environment Approval No. NE-265 issued from the Assistant Regional Waste Manager for the Skeena Region.

A mixing chamber consisting of an angled baffle that created turbulence was located at the head of each trough. Downstream of the mixing chamber was a 1.2m 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 that was fitted with a sheet of open cell styrofoam DB (Snowfoam Products Ltd. El Monte, California) that provided a surface for the sampling of periphyton biomass. The gravel and styrofoam sections were separated with a 7 cm high baffle which produced a water depth of 7 cm over the gravel. The surface current velocity was $5 \text{ cm} \cdot \text{s}^{-1}$. 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.

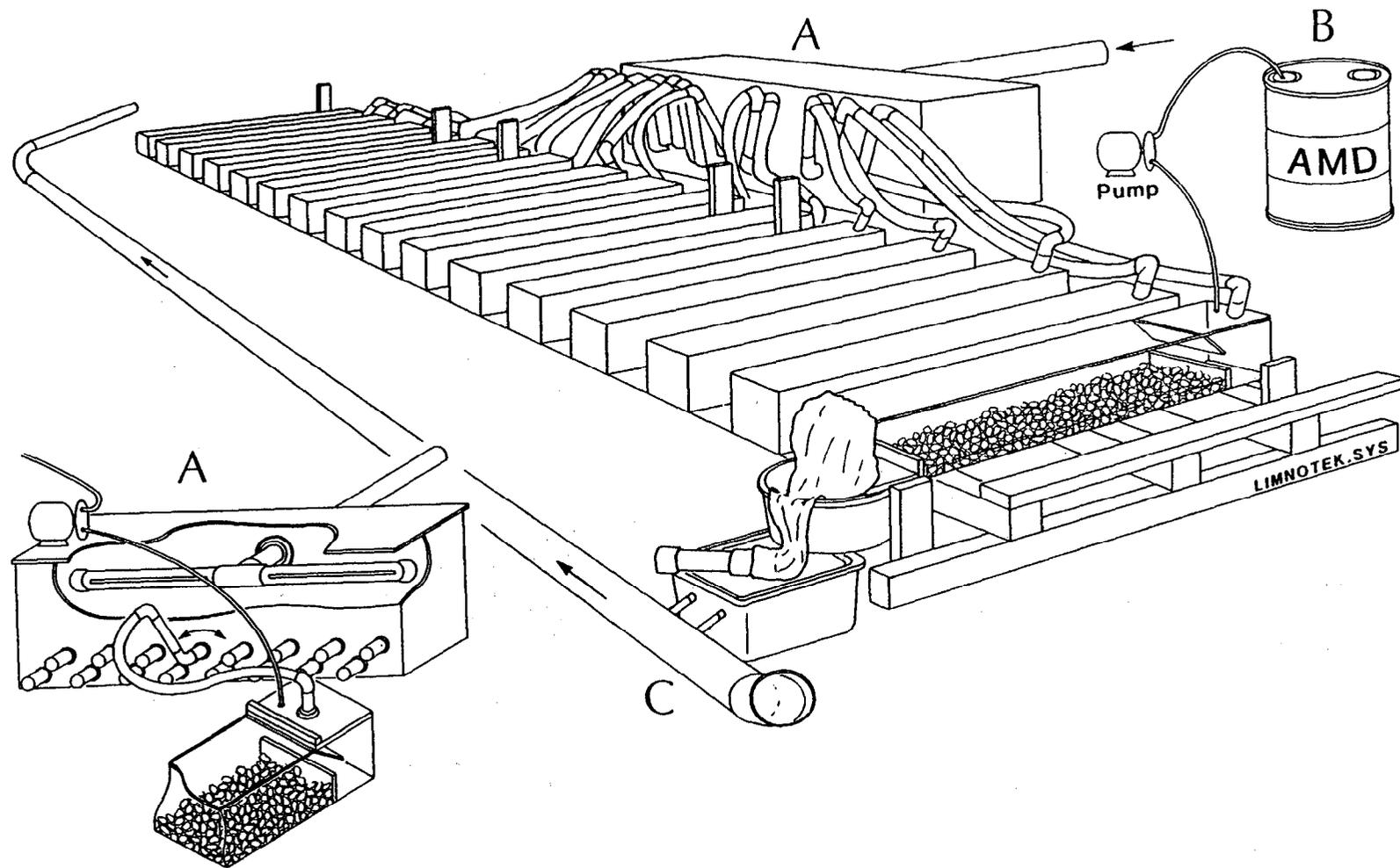


Figure 2. Schematic layout of the mesocosm apparatus: **A**, head tank assembly showing inflow pipe, water distribution inside the tank, outflow regulated by standpipes, and mixing chamber of each trough showing water inflow and chemical injection line; **B**, reservoir of AMD that was used as the supply for troughs receiving the AMD delivery by pump; **C**, trough outflow fitted with drift net, collection tank, and waste line that discharged to a collection basin which was continuously emptied into the upslope seepage pond using a submersible pump.

Water flow through the troughs began on July 23, 1990. Three weeks were allowed for colonization of the troughs by aquatic insects. The AMD treatments (Table 1) were then randomly assigned among troughs and AMD flow was started on August 15. When the AMD additions started, a sheet of acrylic (0.3 cm thickness) was placed on top of each trough to prevent the airborne escape of insects. Frequent sampling with drift samplers that were placed on the outlet of all troughs was used to enumerate adult and larval drift rates of all insects. The mesocosm was shut down with the termination of water and AMD flow on September 8.

Table 1. Layout of treatments and replication within the seven levels of AMD addition in the proposed Equity 1990 experiment. AMD flow rates are based on a total flow rate of $300 \text{ ml} \cdot \text{s}^{-1}$ in each trough.

Treatment #	AMD Dilution (AMD/Water)	AMD Flow Rate ($\text{ml} \cdot \text{min}^{-1}$)	# Replicates
1	no AMD added	0	3
2	1:25,000	0.72	2
3	1:10,000	1.8	2
4	1:2,500	7.2	2
5	1:1,000	18.0	2
6	1:250	72.0	2
7	1:25	720.0	3

2.3 Physical Data

Water flow through the troughs and AMD flows were checked and adjusted as necessary at least four times per week. A calibrated V-notch weir was used to check trough flow rates. AMD input was checked with the graduated cylinder and stopwatch method. Foxy Creek temperatures were continuously measured with a Ryan J-90 (Redmond, Washington)

thermograph that was installed in the trough head tank beginning when the AMD additions started. Before that date, mid-day instantaneous temperatures were measured on three separate occasions.

2.4 Water Chemistry

Water samples were collected from each of the 16 troughs on Aug. 16, Aug 23, Aug. 30, and Sept. 6.

Aliquots for dissolved metals analyses were filtered in the field, preserved with ultra-pure nitric acid to reduce the pH to < 1 and were shipped to the Environmental Protection Service (EPS) laboratory in West Vancouver for analysis within 48 hours using methods outlined in EPS (1988). Metals scans were run using inductively coupled argon plasma emission spectroscopy (ICP). Graphite furnace atomic absorption spectroscopy was used for copper and cadmium which were expected to be in trace concentrations in the more dilute treatments.

Samples for alkalinity and conductivity analyses were collected in 300 ml plastic bottles that were slowly filled to maximum capacity to minimize gas exchange within the bottle. Samples were shipped in cool storage to Vancouver for analyses using standard methods (APHA 1985).

pH measurements were made in the field using a hand held meter equipped with a combination electrode. The meter was calibrated in the field using three buffer solutions.

As part of routine operations at the mine, raw AMD from the seepage pond was sampled twice weekly for pH, total acidity, and conductivity measurements, and for analyses of sulphate, copper, zinc, and iron concentrations (total and dissolved). Atomic absorption spectroscopy (APHA 1985) was used for the metals analyses. An ICP scan was run on a sample collected in January, 1991 for additional description of metals levels. An ICP scan was also run on AMD

from the seepage pond by the Waste Management Branch as part of monitoring activities. These data were used to describe the levels of total and dissolved metals in the raw AMD.

Sulfate concentrations in Foxy Creek were described from results of sampling at a site approximately 300 m downstream from where the mesocosm was located (Figure 1). Monthly collections are made here as part of the mine operating permit.

On August 30, an additional water sample was collected from one trough of each treatment and immediately shipped on ice to CB Research International Corp (CBR), Sidney, B.C. for determination of the capacity of copper to bind to organic ligands. The lab work was completed under a separate contract to CBR (Imber 1991) in which the following methods are described:

The concentration of organic ligands able to bind copper were determined indirectly by voltametry. In this method the free Cu^{2+} ion is plated onto a mercury electrode. The voltage is then scanned and Cu^{2+} is stripped off the electrode. The removal of Cu^{2+} causes a drainage in current that is measured by a polarograph. The organic ligands are then titrated with increased quantities of Cu^{2+} . The resulting titration curves show a break in the gradient of the curve that corresponds to the saturation of the organic ligands with Cu^{2+} . Before the break point is reached, Cu^{2+} is in equilibrium with the organic ligand and some ionic Cu^{2+} becomes bound to the organic ligand. The bound Cu^{2+} cannot be detected and causes a reduction in the Cu^{2+} plated onto the mercury drop. After the break point, the ligand is saturated with Cu^{2+} and all additional Cu^{2+} added is detected. From these titrations the ligand concentrations and the stability constant may be determined using procedures described by Imber (1991).

2.5 Sediment Chemistry

On the final day of the experiment, benthos and sediment were collected simultaneously. As described in section 2.6, zoobenthos was collected in a net secured at the outlet of each

trough while the entire contents of each trough was agitated by hand for 5 min. Bottom sediment that had accumulated in each trough during the course of the experiment was resuspended during this process. Material that passed the 250 μm net that captured the insects was retained in a plastic collection basin. After a 24 hr settlement period, water was decanted from each basin and the remaining sediment was placed in sample bottles and shipped cool to Vancouver for analysis. One subsample from each container was air dried, sieved to $< 63 \mu\text{m}$, digested with aqua regia, and analyzed for metals using inductively coupled plasma (ICP) procedures. A homogenized portion of the dried material was also ignited at 550 $^{\circ}\text{C}$ in a muffle furnace. The loss on ignition was reported as volatile residue.

Another subsample of the original material from one trough of each treatment (#1,2,4,5,6,7,12) was air dried and sieved to $< 63 \mu\text{m}$ for sequential extraction of metals using the methods described by Tessier et al (1979). In sequential order, the following extractions were performed:

1. **Exchangeable metals** were those bound to sediments by weak adsorption. These metals are thought to be released from the sediment by changes in the ionic strength in ambient media. The subsample was extracted with 1M MgCl_2 initially at pH 7 and room temperature for one hour on a wrist action shaker.
2. **Carbonate metals** are those bound to carbonates from which release is associated with a lowering of pH. Residue from extraction 1 was leached with 1M sodium acetate adjusted to pH 5 with acetic acid at room temperature for 5 hrs. on a wrist action shaker.
3. **Iron and manganese oxides** can have metals bound to them but under reducing conditions, the complex is unstable, thus releasing bound metals. Residue from extraction #2 is further extracted at 96 $^{\circ}\text{C}$ for 6 hrs. with 0.04M $\text{NH}_4\text{OH.HCL}$ in 25% (v/v) acetic acid.
4. The **organic bound fraction** includes metals that are bound to organic matter and can be released under oxidative degradation. Residue from extraction #3 is further extracted at 85 $^{\circ}\text{C}$ for 5 hrs. with 0.02 M HNO_3 and 30% H_2O_2 adjusted to pH 2 with HNO_3 and then at room temperature with 3.2M NH_4OAc in 20% (v/v) HNO_3 on a wrist action shaker for 30 min.

5. **Residual metals** include the strong-acid extractable metals remaining in the sediment after removal of all other fractions. Original dried subsamples were digested with HNO₃ and HCl in a microwave oven yielding the total metals (TM) fraction. Residual metals concentrations were determined as the difference between TM and the sum of concentrations determined from the other extractions.

Concentrations of metals from each fraction were measured using ICP methods.

In reporting results from the extractions, it was recognized that there may have been limitations with the method, as there are with many other methods that have been reported. Campbell et al. (1988) provide a summary of these methods and points out that the various metal sinks are often closely associated and it cannot be assumed that any one particular extraction will remove all of one metal from a specific sink. Based on quantitative comparisons of methods by Campbell et al (1988), it is clear that there is no one method that can be recommended for particular types of samples. Hence, the data are used here only to provide a qualitative description of the general partitioning of metals among the metal sinks that may have changed in relative importance through the gradient of AMD additions.

2.6 Periphyton

Samples of periphyton were collected from the styrofoam DB (El Monte, California) substratum that were installed in the downstream area of each trough (Figure 2). New substratum were installed in each trough on August 17. In three to four day intervals up to September 7 (a duration of 21 days) a 2 cm diameter core of the styrofoam and adhering biomass was removed from each trough, frozen at -15°C, and shipped on dry ice to the EPS lab in West Vancouver for analysis of chlorophyll *a* concentrations using the spectrophotometric procedure reported in APHA (1985). On the final sampling day (September 7), an additional core was extracted from each substrata and preserved in Lugol's solution for later taxonomic analysis of the attached community. Quantitative cell counts were made at 500x magnification in Utermohl chambers. Only cells containing cytoplasm were enumerated. A minimum of 100 individuals of the dominant species and a minimum of 300 cells in total were counted in

subsamples removed from the original material adhered to the styrofoam. Cells were enumerated from any filamentous forms that were found.

2.7 Aquatic Insects

Insect drift from the troughs (emigration) was sampled over three hours and 24 hours before (August 13 through 14) and at the onset (August 15) of AMD additions. Additional 24 hour samples were collected on August 17, 23, 26, and September 6. The samples were collected in drift nets that were fitted to the end of the troughs (Figure 2). The nets continuously filtered all water to the 253 μm size fraction. Contents were preserved in 5% formalin.

At the end of the experiment, zoobenthos was collected by washing the entire contents of each trough into a 250 μm mesh nitex net that was secured to the outlet. The gravel in each trough was agitated by hand for a standard period of 5 min. Benthos that was retained in the net was preserved in 5% formalin. In the laboratory, the drift and benthos were split into four subsamples of equal volume and particulate distribution and one subsample was enumerated at 40X magnification using a stereomicroscope. All insects were sorted from the detrital matter at 10X magnification and enumerated at 40X magnification under a stereomicroscope. Results were extrapolated to total sample volume.

Adults and larval stages of aquatic insects were identified to the generic level using keys and descriptions in Merritt and Cummins (1984), Cole (1969), Edmunds et al. (1976), Wiggins (1977), McAlpine et al (1981), and Baumann et al. (1977).

3.0 RESULTS AND DISCUSSION

3.1 Physical Variables

Once initially set to $0.3 \text{ L} \cdot \text{s}^{-1}$, water flow in the troughs required only minor adjustments. On any one date, measured flows were different from intended flows by $< 0.03 \text{ L} \cdot \text{s}^{-1}$.

AMD flows also varied little. The metering pump was very accurate and no adjustments to its operation were necessary. Minibore tubing at the pump head was changed once during the four weeks of AMD addition. Flow from the needle valves was more variable and the micrometer required adjustment at least once per week. Flows at the 1:25 and 1:250 dilutions varied by no more than 15% between settings.

When the AMD additions started, mean daily temperatures were about 15°C , the maximum for the experimental period (Figure 3). Thereafter, temperatures declined, reaching about 8°C by the end of August and 6.5°C by September 8. Before the AMD was added, instantaneous mid-day temperatures ranged between 14 and 16°C .

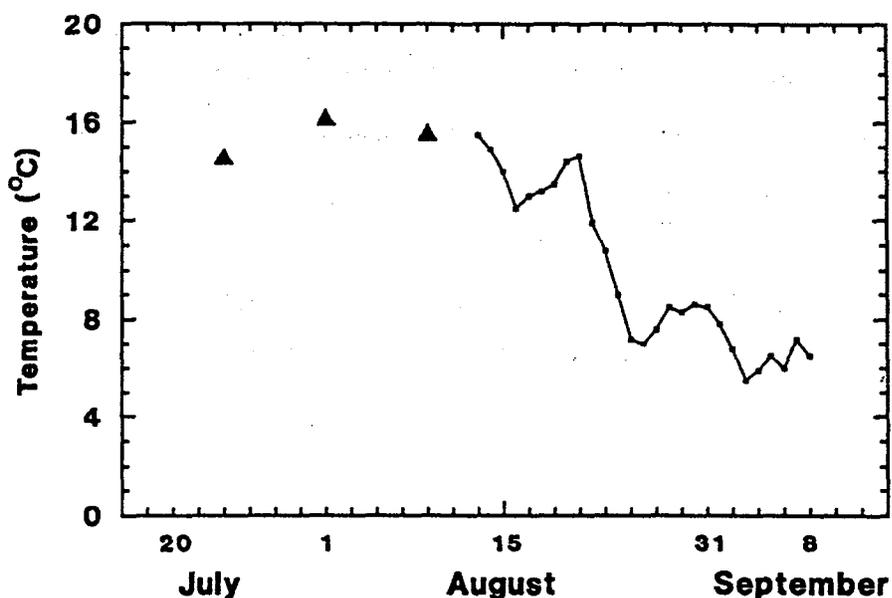


Figure 3. Mean daily temperature in Foxy Creek during the study period. Individual data points indicate instantaneous mid-day measurements taken before AMD additions started.

3.2 Chemical Relationships

The Equity AMD was an acidic mixture having high conductivity, no alkalinity, and sulfate and metals concentrations that were several times greater than those in Foxy Creek water (Table 2). At the low pH of about 2.5, there were no differences between mean concentrations of dissolved and total Cu ($P > 0.2$), Fe ($P > 0.8$), or Zn ($P > 0.3$) in the AMD (Table 2), thus confirming that high acidity maintained metals in the dissolved form. There was no temporal trend in metals levels in the AMD during the experiment and the CV in metals concentrations was $< 4.5\%$ except for Fe which had a CV of 11.5%. These findings are consistent with longer term observations which indicate low variation in levels of metals and SO_4 in the AMD seepage pond within and between late summer periods over several years (B. Patterson, Equity Silver Mines, pers comm).

Table 2. Mean concentrations (± 1 SD) of total metals and other descriptive indices in raw AMD from the upper seepage pond and dissolved metals in Foxy Creek water in the mesocosm control troughs in August and September, 1990.

Test	M.O.E. Raw AMD	Equity Silver Raw AMD (n=9) Total metals	Equity Silver Raw AMD (n=9) Dissolved metals	Control Troughs (n=12)
pH (relative units)	2.47	2.52 \pm 0.05		7.08 \pm 0.31
Conductivity ($\mu\text{S} \cdot \text{cm}^{-1}$)	7100	6946 \pm 168		166 \pm 9
Total hardness (mg $\text{CaCO}_3 \cdot \text{L}^{-1}$)	1670			73 \pm 8
Ca + Mg hardness (mg $\text{CaCO}_3 \cdot \text{L}^{-1}$)				73 \pm 8
Alkalinity (mg $\text{CaCO}_3 \cdot \text{L}^{-1}$)				44 \pm 4
SO_4^{2-} (mg $\cdot \text{L}^{-1}$)	6050	7187 \pm 325		53.5 \pm 20.2
Ag		<15		<10
Al (mg $\cdot \text{L}^{-1}$)	346	360		<0.05
As		6980		<50
B	2200	<100		<10

Test	M.O.E. Raw AMD	Equity Silver Raw AMD (n=9) Total metals	Equity Silver Raw AMD (n=9) Dissolved metals	Control Troughs (n=12)
Ba	7010	<10		19 ± 1
Be		37		<1
Ca (mg · L ⁻¹)	407	419		20 ± 3
Cd	170	520		<0.1
Co	1500	1600		<5
Cr	260	<15		<5
Cu (mg · L ⁻¹)	45.8	40.4 ± 1.6	39.5 ± 1.3	0.003 ± 0.001
Fe (mg · L ⁻¹)	1120	987.4 ± 178.3	970.9 ± 167	0.055 ± 0.025
K (mg · L ⁻¹)				3 ± 1
Mg (mg · L ⁻¹)	222	271		6 ± 0.23
Mn	46000	38000		3 ± 2
Mo	<10	<30		<10
Na (mg · L ⁻¹)				3.43 ± 0.198
Ni	5800	4080		<20
P		12900		<100
Pb	3	<50		<50
Sb		<200		<50
Se		<200		<50
Si (mg · L ⁻¹)		38.5		8.1 ± 0.56
Sn				<50
Sr		3170		199 ± 10
V	320	152		<10
Zn (mg · L ⁻¹)	22.6	28.6 ± 1.6	27.9 ± 1.1	0.009 ± 0.011

There were exceptions to the typically high concentrations of metals in the AMD. Ba levels were always less than $10 \mu\text{g} \cdot \text{L}^{-1}$ which was less than the Ba concentrations in Foxy Creek; Ag and Cr levels were $< 15 \mu\text{g} \cdot \text{L}^{-1}$; Pb and Mo concentrations were < 50 and $30 \mu\text{g} \cdot \text{L}^{-1}$ respectively; B was $< 100 \mu\text{g} \cdot \text{L}^{-1}$; and Sb and Se levels were each $< 200 \mu\text{g} \cdot \text{L}^{-1}$. These values were the detectable limits of the ICP scan. Without detection, any difference in concentrations between control and raw AMD solutions could not be determined. Pb analyses using graphite furnace methods were run for the MOE data and additional analyses run by Environment Canada (B. Godin, Environment Canada, North Vancouver, pers comm.) confirmed that Pb was consistently $< 5 \mu\text{g} \cdot \text{L}^{-1}$ in the raw AMD.

Concentrations of dissolved metals and other chemical indices that were measured at the outflow of each treatment are summarized in Table 3. Changes in concentrations of the other metals and nonmetallic elements were variable but could be separated into four groups according to change in concentration by treatment. Barium, potassium, selenium, and boron concentrations (Group 1) did not change over the AMD dilution gradient. These elements were always measured in the Foxy Creek supply water and no change through the treatment gradient indicated that additions from the AMD were small and could not be distinguished from the background levels. Group 2 (Si, Na, and Sr) was also detectable throughout the gradient, but unlike in group 1, element concentrations increased slightly at the lowest dilution indicating a noticeable addition to Foxy Creek levels from the AMD only at that dilution. Group 3 included elements that remained at their detection limits for most treatments and increased only at the lowest AMD dilutions. The concentrations of elements in this group were relatively low in Foxy Creek but their presence in the AMD resulted in measurable levels beginning at the 1:1000 dilution. The group included arsenic, beryllium, cobalt, chromium, nickel, and phosphorus. Concentrations of aluminum, cadmium, copper, iron, manganese, and zinc (Group 4) generally increased with

decreased dilution throughout the gradient. Their high concentrations in the AMD clearly exceeded background levels in Foxy Creek at all AMD dilutions.

Acid neutralizing capacity (ANC), measured as alkalinity was remarkably constant from the highest to the 1:1000 dilutions given that the values were in a range which is sensitive to acid additions (Galloway 1983). Hence, there was no large drop in pH down to the 1:1000 dilution (Table 3). At the 1:250 dilution, the additional acid had clearly consumed ANC and at the lowest dilution, the capacity of the system to combine H^+ ions was completely exceeded, as indicated by 0 alkalinity. The pH dropped accordingly. Conductivity followed the changes in ANC by increasing from about $165 \mu S \cdot cm^{-1}$ in dilutions down to 1:1000 and then rising sharply at the lower dilutions, coincidentally with higher levels of Ca, Mg, Na, and metals of groups 3 and 4. Hardness also increased over the dilution gradient but total hardness increased proportionately more than did hardness determined only from the Ca and Mg levels. This divergence indicated increased contribution by metals to hardness at the lower dilutions.

Table 3. Mean concentrations (± 1 SD) of dissolved metals and other chemical indices measured in the seven dilutions of AMD in Foxy Creek water (n=4). Units are $\mu\text{g} \cdot \text{L}^{-1}$ unless otherwise noted.

Analysis	Control	1:25000	1:10000	1:2500	1:1000	1:250	1:25
pH (relative units)	7.08 \pm 0.31	6.95 \pm 0.35	6.95 \pm 0.22	6.88 \pm 0.21	6.34 \pm 0.48	5.72 \pm 0.25	3.48 \pm 0.28
Alkalinity (mg CaCO ₃ · L ⁻¹)	44 \pm 4	48 \pm 12	36 \pm 14	43 \pm 6	38 \pm 3	28 \pm 9	0
Conductivity ($\mu\text{S} \cdot \text{cm}^{-1}$)	166 \pm 9	166 \pm 8	169 \pm 8	163 \pm 15	172 \pm 6	190 \pm 10	724 \pm 216
Total Hardness (mg CaCO ₃ · L ⁻¹)	73.4 \pm 8	72.9 \pm 10	75.3 \pm 9	76.3 \pm 10	76.6 \pm 10	82.3 \pm 12	234 \pm 29
Mg + Ca Hardness (mg CaCO ₃ · L ⁻¹)	73.0 \pm 8	72.2 \pm 10	74.0 \pm 9	74.8 \pm 10	73.2 \pm 10	80.4 \pm 11	156 \pm 19
Ca (mg · L ⁻¹)	19.6 \pm 3.0	19.4 \pm 3.7	19.9 \pm 3.0	20.2 \pm 3.6	19.5 \pm 3.5	21.1 \pm 3.6	35.6 \pm 5.6
Mg (mg · L ⁻¹)	5.8 \pm 0.2	5.8 \pm 0.3	5.9 \pm 0.4	5.9 \pm 0.3	6.0 \pm 0.3	6.7 \pm 0.6	16.3 \pm 1.6
GROUP 1							
Ba	19.3 \pm 1.4	19.1 \pm 1.9	19.4 \pm 0.7	19.5 \pm 1.4	19.0 \pm 1.1	19.4 \pm 1.5	19.2 \pm 1.2
K (mg · L ⁻¹)	2.8 \pm 1.2	3.3 \pm 2.4	2.8 \pm 1.5	2.7 \pm 1.8	3.0 \pm 1.6	2.9 \pm 1.8	2.8 \pm 1.6
Se	52.5 \pm 9.2	51.6 \pm 7.4	<50	50.4 \pm 3.9	52.9 \pm 11	<50	<50
B	11 \pm 6	14.3 \pm 14	10.5 \pm 3.9	10.4 \pm 3.9	16.9 \pm 17.9	<10	9.9 \pm 3.2
GROUP 2							
Si (mg · L ⁻¹)	8.1 \pm 0.6	7.9 \pm 0.5	8.1 \pm 0.3	8.2 \pm 0.5	8.0 \pm 0.3	8.1 \pm 0.4	9.7 \pm 0.6
Na (mg · L ⁻¹)	3.4 \pm 0.2	3.3 \pm 0.1	3.5 \pm 0.1	3.4 \pm 0.1	3.6 \pm 0.1	4.1 \pm 0.4	11.7 \pm 1.7
Sr	199 \pm 10	197 \pm 12	200 \pm 8	202 \pm 10	198 \pm 7	210 \pm 12	303 \pm 21.4
GROUP 3							
As	54 \pm 13	<50	<50	50 \pm 4	<50	50.5 \pm 3.9	198 \pm 78
Be	<1	<1	<1	<1	<1	<1	2.3 \pm 0.5
Co	<5	<5	<5	<5	4.6 \pm 1.2	7.4 \pm 3.3	118.7 \pm 68.2
Cr	<5	<5	<5	<5	<5	<5	5.0 \pm 1.7
Ni	<20	<20	<20	<20	<20	18.0 \pm 3.7	189 \pm 31.2
P	<100	<100	<100	<100	112 \pm 36	<100	392 \pm 117
GROUP 4							
Al	<50	68 \pm 32	86 \pm 52	136 \pm 43	346 \pm 112	103 \pm 65	14675 \pm 2412
Cd	<0.1	<0.1	0.17 \pm 0.1	0.12 \pm 0.05	0.24 \pm 0.08	0.64 \pm 0.29	16.9 \pm 3.8
Cu	2.5 \pm 1.3	6.7 \pm 4.3	13.0 \pm 11	13.5 \pm 5.1	36.5 \pm 12	51.4 \pm 27.1	1530 \pm 247
Fe	55 \pm 25	134 \pm 60	308 \pm 277	322 \pm 161	788 \pm 422	722 \pm 423	26530 \pm 13730
Mn	3.3 \pm 2.0	8.5 \pm 6.0	27 \pm 44	18.1 \pm 5.6	50.1 \pm 16	159 \pm 70	1657 \pm 595
Zn	8.5 \pm 11	9.1 \pm 8.2	23.6 \pm 27	11.8 \pm 12.8	31.3 \pm 32	94.5 \pm 47.6	1147 \pm 222

As mentioned earlier, the form of a metal and pH is thought to determine the "degree" of toxicity that will affect a stream community. It is well established that metal complexes with organic and inorganic ligands tend to dissociate as pH decreases, thus releasing metal aquo ions into solution (Leckie and James 1975). There is a wide body of evidence suggesting that biological diversity, productivity, and biomass is coincidentally reduced with declining pH and increasing concentrations of free metal ions (Boudou and Ribeyre 1989). So strong has this relationship been that high acidity is often considered to infer high concentrations of dissolved metals and high levels of toxicity. Campbell and Stokes (1985) clearly point out, however, that changes in pH and metals concentrations may produce antagonistic effects in organisms. Low pH does not infer that metals in solution are toxic. It follows that declining species richness or productivity at low pH does not imply that relatively high concentrations of free metal ions actually cause those changes or shifts in ecosystem structure. Rather, there appears to be groups of metals that respond to pH shifts in different ways and organisms may be sensitive either to the metals speciation or other processes associated with changes in acidity (eg. membrane depolarization which will affect metal uptake, conformational changes at the metal-binding sites, and altered affinities of membranes for metals, (Campbell and Stokes 1985, Campbell et al. 1988)).

Visual observations of metal precipitation and evidence of metal partitioning in the trough sediments, and measurements of the complexing capacity of copper with organic matter provided some clues to the potential toxicity of metals present in the AMD. The focus here was on those metals which existed in the cationic form and had the potential to be highly available and toxic. This restriction limited the list to Cu, Cd, Mn, and Zn. Al was included because of its known leaching response to acidification (Cronan and Schofield 1979). Fe was included because of massive ferric oxide precipitates that are associated with the mixing of AMD with surface water and the role of iron in the coprecipitation of other metals (Jenne 1968).

The most obvious effect of AMD addition was the appearance of a hydrous ferric oxide precipitate that covered the surface of the gravel substratum in the troughs in amounts corresponding to the gradient of AMD added. At the 1:25 dilution, the solids accumulated to

a depth of more than 3 cm, an orange "froth" developed on the water surface, and metal lattice particulates were always collected in the nets used to capture outmigrating insects. No precipitate was observed at dilutions greater than 1:2500. This visual change was confirmed with the measurement of a large increase in total Fe in the sediments collected from troughs receiving the highest dose of AMD (Figure 4b). As dissolved Fe increased from 55 to 722 $\mu\text{g}\cdot\text{L}^{-1}$ in the surface water over the dilution range from the control to 1:250 (Table 3), Fe bound in the sediments increased by about 3,000 $\mu\text{g}\cdot\text{g}^{-1}$. At the 1:25 dilution, dissolved Fe concentrations increased a further 26,000 $\mu\text{g}\cdot\text{L}^{-1}$ and sediment bound Fe increased by 160,000 $\mu\text{g}\cdot\text{g}^{-1}$. The process for this phenomenon is well known: with increasing pH on the addition of AMD to stream water, the Fe^{3+} oxidation state is favoured (Pagenkopf 1978), which can be readily hydrolysed to form the precipitate, the amount being in direct proportion to the gradient of dissolved Fe concentrations, as was observed in the troughs.

The sequential extraction suggested that about 15% of the precipitated iron was associated with iron oxide fraction down to the 1:250 dilution (Figure 6). At the lowest dilution, however, from which the precipitate was most obvious, the relative importance of the iron oxide fraction increased to about 25% of the total iron extracted. In addition to the obvious association with iron oxides, up to 80% of Fe in the control and dilutions down to 1:250 was also found in residual materials that generally includes silicate based minerals. At the 1:25 dilution, about 60% remained in this residual fraction. More than 80% of the Al extracted from the sediments (Figure 4c) was also bound in this fraction (Figure 6) which indicates the presence of clay minerals (eg. aluminum silicates). Although up to 80% of the total iron was not recovered until the final extraction, iron oxides are known to be associated with these clays (Jenne 1977). Hence, it is not surprising that complexed iron was recovered in both the iron/manganese oxide and residual extractions.

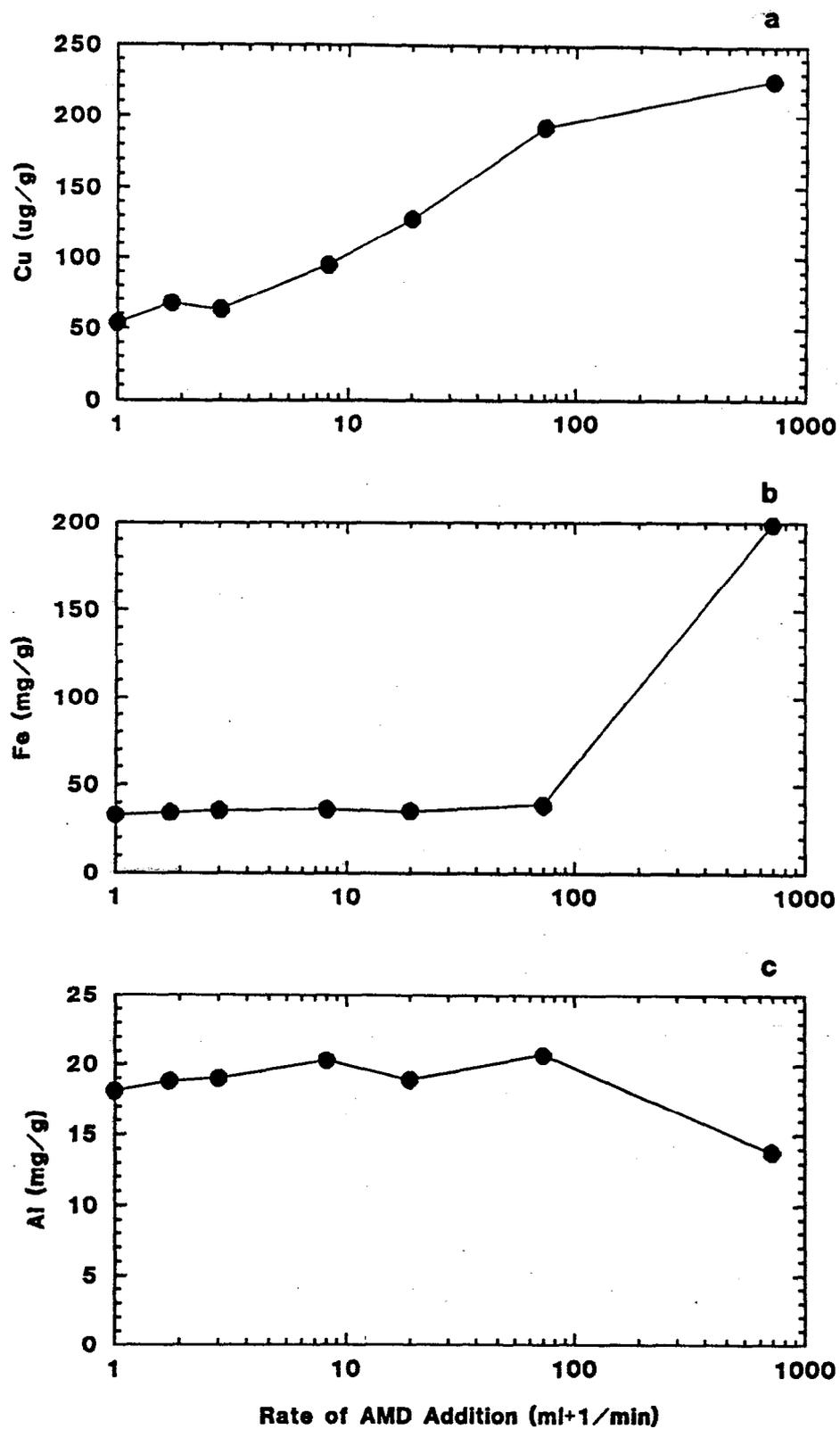


Figure 4. Mean concentrations of total Cu (a), Fe (b), and Al (c) extracted from the trough sediments for each dilution of AMD additions. $n=3$ for lowest and highest dilutions; $n=2$ for all other dilutions.

Sediment bound copper also increased with lower dilutions of the AMD (Figure 4a). A minimum of $50 \mu\text{g} \cdot \text{g}^{-1}$ was measured in the control troughs and up to $225 \mu\text{g} \cdot \text{g}^{-1}$ was found at the 1:25 AMD dilution. This increase occurred with rising dissolved Cu concentrations in solution across the gradient but with increasing and then decreasing complexing capacity (Figure 5).

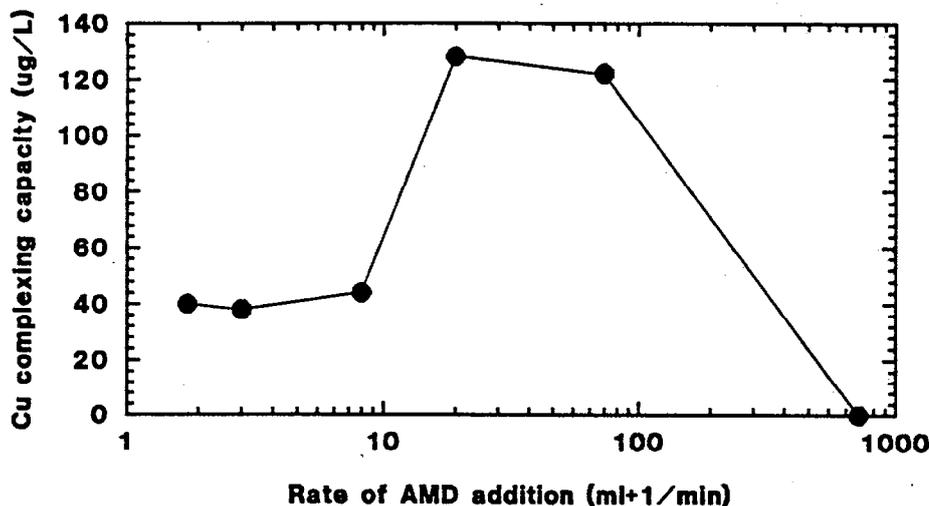


Figure 5. Change in the capacity of organic ligands to complex copper as a function of the rate of AMD addition. Data were reprinted from Imber (1991).

The decrease in complexing capacity at the AMD dilution of 1:25 supports other findings that copper speciation is highly sensitive to variation in pH (Anderson and Rubin 1981). The similar concentrations of total and dissolved copper concentrations in the AMD (section 3.2) indicated that Cu^{2+} in an aquo ionic form was dominant in the AMD, indicating that ligands were completely protonated. Using the terminology from Campbell and Stokes (1985), the hydrolysis equilibrium was shifted far to the left as indicated in equation 1.



where M^{z+} is a generalized metal cation of charge $+z$.

Upon mixing with the stream water, the equilibrium would have shifted to the right thus favouring increased metals complexation. This shift would have been strongly favoured at the

high dilution end of the gradient (1:25,000) but relatively weakly favoured at the low dilution end (1:25). The measurements of complexing capacity supported this theory by indicating no capacity to complex copper to the organic ligands at the pH in the 1:25 dilution (mean of 3.5). That pH is below the boundaryline (on Eh/pH diagrams) separating complete protonation from some metal complexation. Hence, protonation was favoured in the water sample from the 1:25 dilution that was used to measure the complexing capacity and in that respect was similar to the raw AMD. Maximum complexing capacity was found between pH 5.7 and 6.3 but it was about 3 fold lower at the higher dilutions covering a pH range from 6.8 to 7.1. This lower capacity to complex with organics at the highest pH suggests that other ligands were outcompeting the organics for the titrated copper.

Potential ligands and complexation substrates that were involved in the copper partitioning were indicated by the sequential extraction (Figure 6). Copper was complexed with ligands and adsorbed on exchange sites across the entire AMD gradient, including the lowest dilution (pH 3.5). The relative increase in dissolved copper was greater than that of sedimentary copper with declining AMD dilutions (compare Table 3 with Figure 4), and this generally supports the theory of increasing free aquo ions with reduced pH in the surface water. Clearly, however, there was not a general dissociation of copper/ligand complexes and desorption processes with rising concentrations of H^+ associated with the lower pH in the sediments (Figure 4 and 6), but rather an increase with the higher AMD loadings. With declining pH and AMD dilutions there was a shift from associations having high energy binding sites (on residual materials) to a greater association with low energy binding sites (adsorption and association with carbonates and iron oxides). High energy residual copper complexes declined from more than 60% to <30% of sedimentary copper over the AMD gradient. Weakly adsorbed copper was found only at the lowest dilution of AMD and there was an increase in the carbonate fraction up to the 1:250 dilution. The relative copper/organic ligand association was the greatest of all the metals examined and it remained at about 30% of the total sedimentary copper. There was also a strong association with iron or manganese oxides which was apparent as an increase from <5% to more than 25% of the copper being associated with those oxides over the AMD gradient (Figure 6).

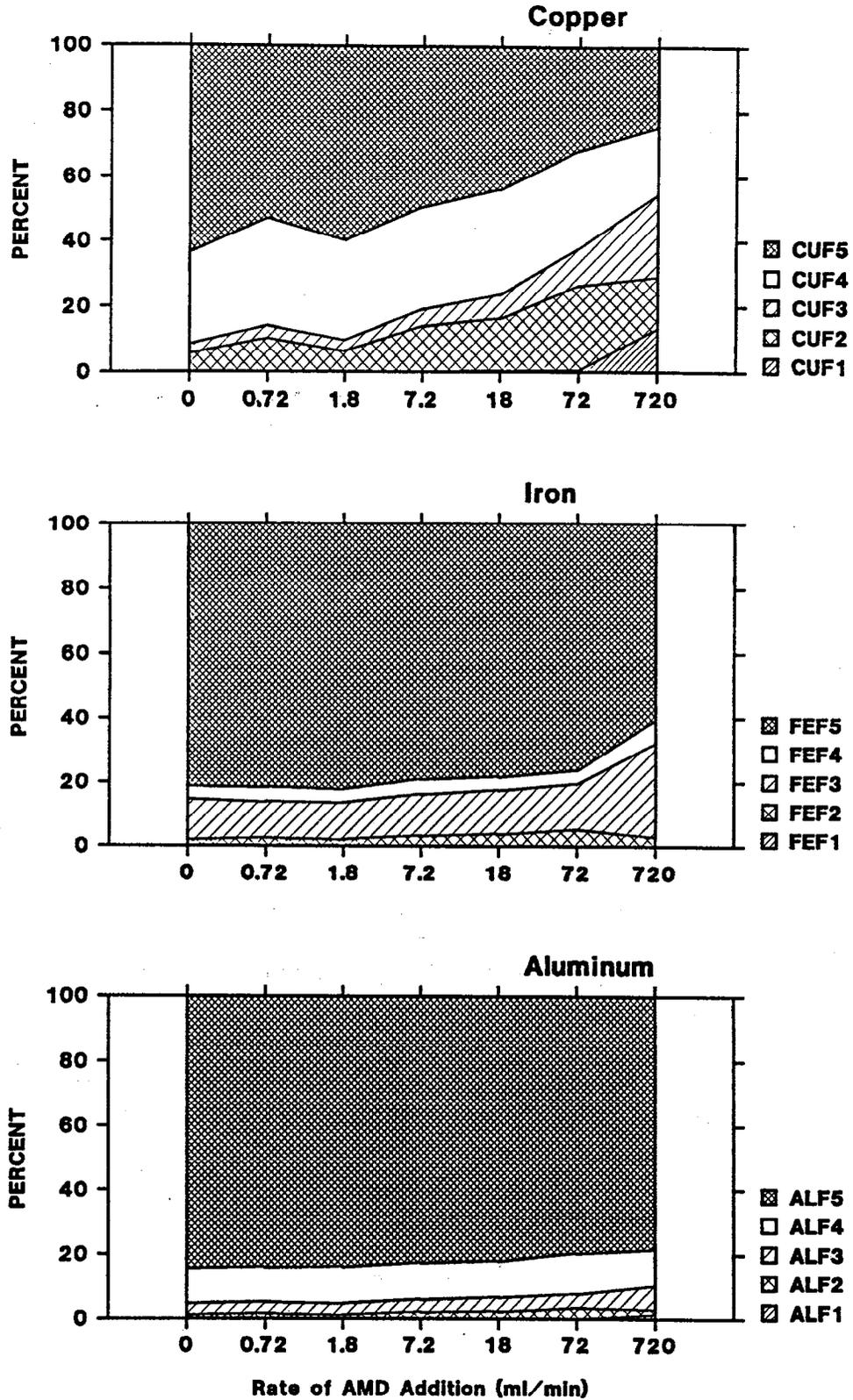


Figure 6. Partitioning of Cu, Fe, and Al determined by sequential extraction of one sample collected from each treatment. The suffix F1....5 included with each label in the graphics keys corresponds to exchangeable, carbonate, Fe + Mn oxide, organic bound, and residual partitions respectively.

This copper partitioning in the sediments was particularly interesting since it indicated a different relationship with pH compared to that found for the surface water (Figure 5). There had to be separate processes determining the speciation of copper between the dissolved (surface water) and particulate (sediment) phases. The difference was most apparent at the 1:25 AMD dilution that was characterized by a pH of 3.5 and an extensive $\text{Fe}(\text{OH})_3$ precipitate. Copper complexation in the sediments increased with the higher AMD additions and the organic/copper association, which did not occur in solution, was more than 30% of all copper complexes in the sediment at the 1:25 dilution. By strictly using pH as a determinant of complexation, there should have been lower complexation and adsorption of copper in favour of ligand hydrolysis at the pH of 3.5. This relationship is taken from that in the dissolved phase in which the dissolved organic ligands were hydrated at the lowest pH and other multi-ligand systems complexed copper at higher pH. But, in the sediments, it was clear that factors influencing relationships between pH and adsorption and precipitation, in particular, were also important in determining the copper/sediment associations. Campbell et al (1988) cite factors including total metal concentration, the nature and concentration of competing metals, the nature and concentration of ligands, and reaction time for equilibrium to become established as being important in this regard. The interaction of these factors apparently caused an increase in complexation in the sediments even down to the 1:25 dilution and a pH of 3.5.

Another important factor may have been the dense $\text{Fe}(\text{OH})_3$ precipitate which could have restricted or prevented active transfer of water between the surface water and pore water. Acting as a boundary layer, the precipitate may have isolated surface and bottom processes. A pH dependant shift from desorption to adsorption can occur within a very narrow pH range (Campbell et al 1988) which may have occurred across the precipitate boundary layer.

Other conflicting evidence related to the relationship between surface pH and metal precipitation was the formation of the $\text{Fe}(\text{OH})_3$ precipitate at the lowest AMD dilution. If we assume that the redox potential (Eh) of the supply water was between 500 and 600 mV which is typical of well oxidized surface water of lakes and streams (Wetzel 1975), then the pH had to be near 4 for any precipitate to form. A pH of 4 is near the boundaryline between the

formation of $\text{Fe}(\text{OH})_3$ and ionic divalent Fe^{++} given this ambient redox potential (Eh-pH diagram for iron speciation in Stumm and Morgan 1970). If the pH was any lower, the precipitate would not have formed (ie. as is typical of raw AMD). However, it is also clear that dissolved iron concentrations were increasing with declining dilutions and the order of magnitude increase in AMD additions between the two lowest dilutions resulted in a disproportionate increase of the dissolved concentrations by 36 times. This observation suggests there was no clear cut separation of iron species in the troughs. The ionic species may have been favoured at the 1:25 dilution but other factors (eg. high concentration of the dissolved species) allowed formation of the precipitate.

Like iron, the other metal included in Group 4 (Table 3) that was important in redox reactions was manganese (Figure 7b, 8). It was strongly associated with the sediments in AMD dilutions down to 1:1000 but at lower dilutions, that association declined by several times (Figure 7b). The drop in total Mn concentrations in the sediment corresponded to a pH of not more than 6.3. That level is close to the boundaryline on the pH-Eh diagram for Mn that separates the insoluble MnO_2 precipitate and the ionic divalent species (Mn^{2+}). Hence at dilutions $< 1:1000$, in which pH was < 6.3 , the ionic species was favoured thus reducing the potential formation of the manganese oxide precipitate. At dilutions $> 1:1000$, the MnO_2 species was favoured, thus yielding high total Mn levels in the sediment of 1.8 - 2.0 $\text{mg} \cdot \text{g}^{-1}$. The oxidation of manganese is also slower than that of iron which suggests that the oxidation of Mn^{2+} may not have been complete within the troughs.

Like iron, sediment bound manganese was associated mainly with the metal oxides and residual minerals at all AMD dilutions (Figure 8). Unlike iron, however, about 20% of the sedimentary Mn was also associated with the carbonate ligands across all treatments except the 1:25. At the higher concentrations of other metals and the low pH in that treatment (Table 3), the carbonate-Mn association dropped to about 7% in favour of weak adsorption to substrates that may have included clays, other metal oxides, and organic matter.

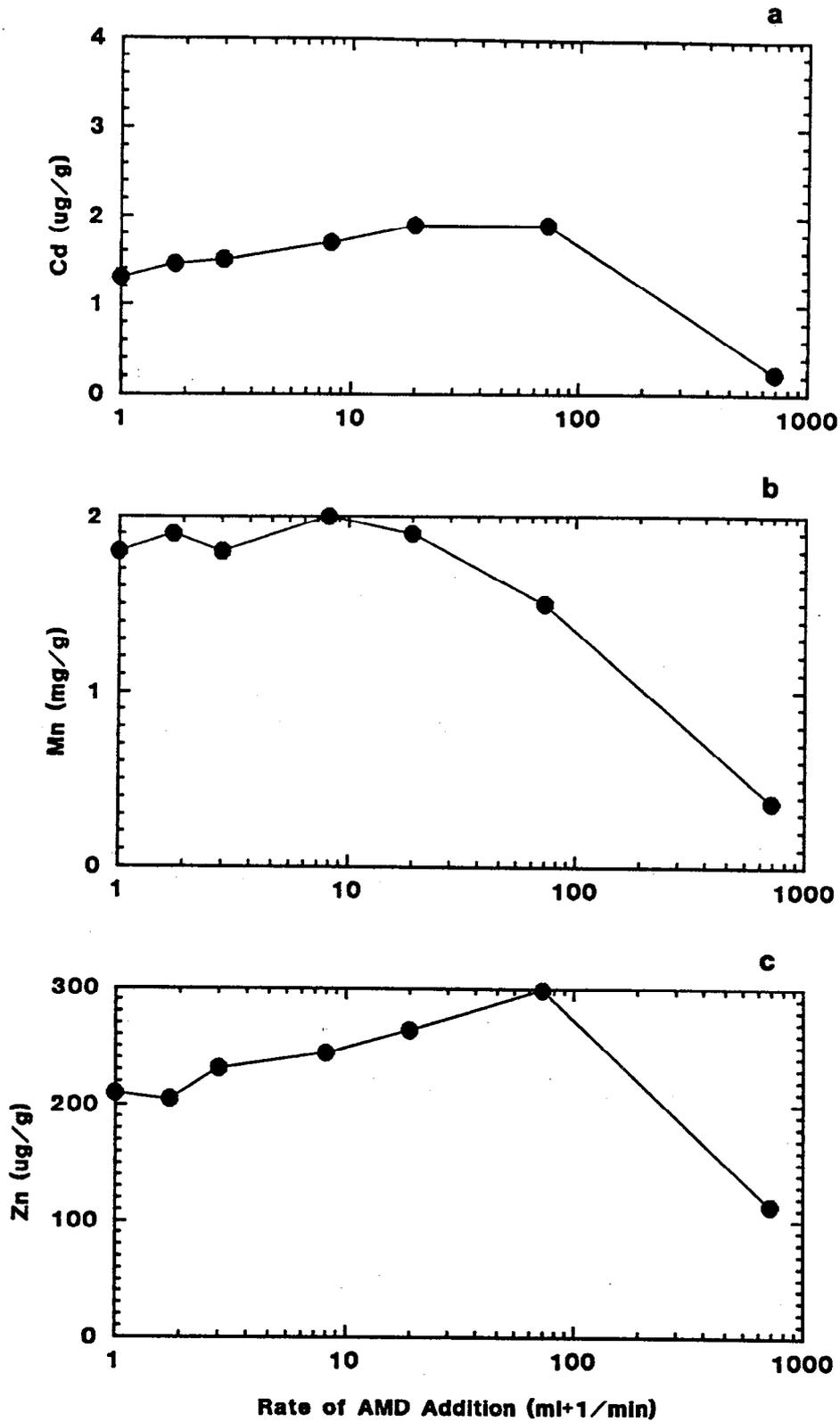


Figure 7. Mean concentrations of total Cd (a), Mn (b), and Zn (c) extracted from the trough sediments for each dilution of AMD additions. $n=3$ for lowest and highest dilutions; $n=2$ for all other dilutions.

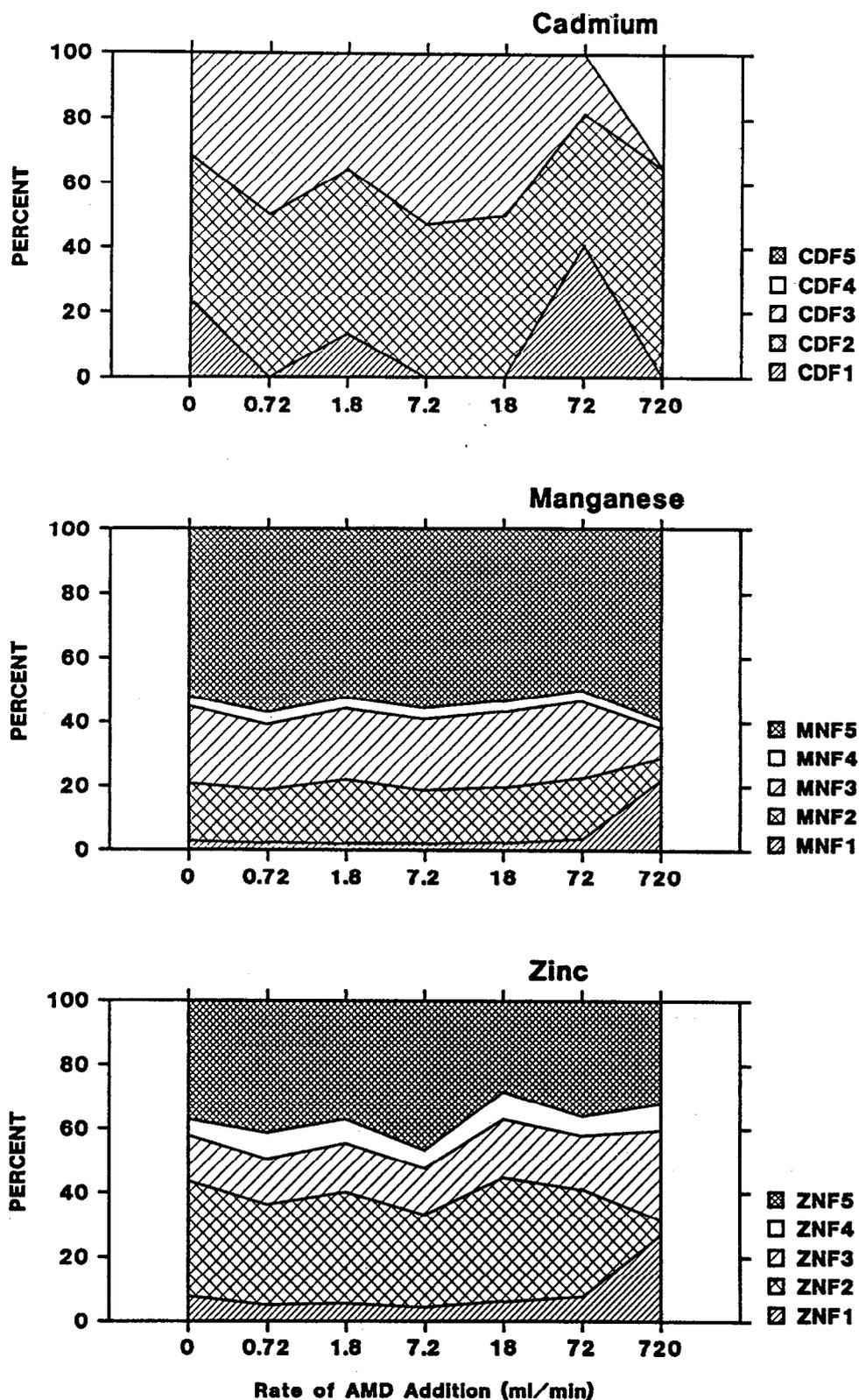


Figure 8 Partitioning of Cd, Mn, and Zn determined by sequential extraction of one sample collected from each treatment. The suffix F1....5 included with each label in the graphics keys corresponds to exchangeable, carbonate, Fe + Mn oxide, organic bound, and residual partitions respectively.

The two remaining metals, Cd and Zn each showed similar trends in total concentration in the sediments across the treatments. Total concentrations increased with rising dissolved concentrations and declining AMD dilutions to 1:250. With the further drop in pH at the 1:25 dilution and the very large increase in dissolved metal concentrations, both the Cd and Zn levels in the sediment declined markedly (Figure 7). Unlike the similar trend for manganese that was probably related to redox processes, Cd and Zn are more involved in adsorption/desorption reactions that include electrostatic attraction to negatively charged particles or adsorption to oxide surfaces. Since these processes are strongly pH dependant, it is possible that adsorption was favoured at $\text{pH} > 5.7$ (mean pH at 1:250 dilution) but not when $\text{pH} < 5.7$. Different metals also have varying affinities to adsorption depending on metal(s) concentrations and the nature and concentration of any ligands that were present. Generally the series of binding strengths, $\text{Zn} < \text{Cd} < \text{Cu}$ is observed on hydrous oxides and on humates (Campbell et al 1988). Hence, adsorption of Cu may have outcompeted both the Zn and Cd for binding sites at the lowest pH at which the binding capacity for all metals would have been weakened. Those metals having the weakest substrate affinity (Cd and Zn) were least likely to be associated with the available substrates.

The distribution of Cd and Zn was, however, very different between the various sediment metal partitions (Figure 8). Less than 10% of Zn was extracted from weakly exchangeable substrates except at the 1:25 dilution in which that partition increased to almost 30% likely due to "flooding" of adsorption sites by the increase in dissolved concentrations to more than $1 \text{ mg} \cdot \text{L}^{-1}$ (Table 3). The distribution of Cd at those sites was inconsistent and variable, probably reflecting variation in amounts extracted between the carbonate and exchangeable fraction rather than actual variation in binding affinity on substrates across the treatments. The carbonates are well known to be a sink for Cd largely via Cd substitution for Ca and Mg on mineral surfaces or in crystal lattices and not surprisingly, this fraction represented about 50% of the sedimentary Cd across all treatments. Carbonates were also a sink for Zn (30% of total Zn) except at the 1:25 dilution which favoured the lower energy binding in place of the carbonates. Fe and Mn oxides also provided a substrate for Cd and Zn. Oxide bound Cd was about 40% of total Cd except at dilutions $< 1:1000$ in which organic ligand complexes increased in importance. At the

lowest dilution, the organic matter content of the sediments increased by almost 10% (Figure 9), perhaps in relation to the mortalities of benthic algae and insects (section 3.3 and 3.4). This finding is consistent with evidence that the partitioning of Cd can be related to the relative dominance of available substrates. About 15% of Zn was consistently associated with metal oxides and only 5% was in the organic phase and this difference did not change across the AMD gradient. However, unlike Cd, Zn was extracted from the residual fraction in amounts up to 40% of the total concentration. The distinction here was that Zn is a relatively common metal occurring in all treatments at concentrations several times the levels of Cd. Zn is abundant in common minerals whereas Cd is not.

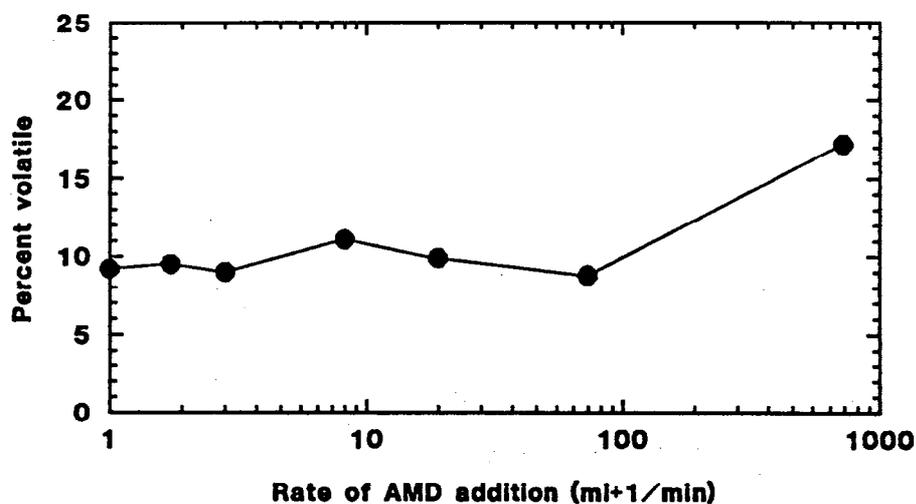


Figure 9. Loss on ignition of sediments collected across the AMD gradient.

To elicit toxic effects at the cellular level, a given metal must interact with cell membranes on which there are functional groups having a negative charge (alcohol, carboxylic acid, amine, etc.) that will bind metals (Beveridge and Murray 1980). Usually, metals having high free energy (ie. free aquo ions) are those that are most readily bound at cell surfaces (Sundra and Guillard 1976) although metal transport can also occur with dissolved organic nutrients (eg. amino acids and proteins). Based on the test for complexation capacity of copper, it is clear that aquo forms of Cu^{2+} were common at the lowest dilution of the AMD which

means that copper in that treatment had the potential to be highly toxic. Since a pH near 4.0 appears to distinguish protonation from potential complexation of copper and given that the same can be true for other metals (Imber, CBR International, Pers Comm.), it is possible that the other important metals (Group 4 in Table 3) were also in ionic form, had the highest affinity for uptake, and thus had the potential to contribute to metabolic change and perhaps mortalities in the biological community at that lowest AMD dilution. For the other treatments, there would obviously have been a declining probability of toxic effects developing with the increasing AMD dilutions. The complexing capacity for copper was found to be relatively high at the 1:1000 and 1:250 dilutions and was probably complexed with other ligands at the higher dilutions (eg. manganese or iron oxides). If this was true for the other metals, then species having the highest free energy would have been relatively unimportant and the potential for toxicity would have been diminished sequentially at those treatments.

Of the several factors that can affect the cellular uptake of metal ions (reviewed by Campbell et al. 1988), effects of H^+ concentration was particularly important in this study. H^+ can effectively outcompete metal cations at cellular exchange sites at low pH when the concentration of free H^+ increases (Campbell and Stokes 1985). This hydrolysis of exchange sites is particularly effective in reducing the binding of copper, cadmium, and zinc (Campbell and Stokes 1985) and thereby reduce the toxicity of these metals. Hence, the lowest AMD dilution had the potential to be most toxic, strictly from a chemical speciation point of view, but actual toxicity associated at least from copper, cadmium, and zinc may have been countered by competitive interactions with protonation of cellular binding sites. Given this scenario, any high energy metal species that may have occurred in solution at the higher dilutions (albeit the copper speciation data suggest this would be minimal) could have the potential to be more toxic than the much higher concentrations of ionic metals at the lowest AMD dilution.

Because of these confounding effects of acidity and metal speciation, it is apparent that metal speciation data from a mesocosm, such as the one used in this study, cannot be used to predict toxicity within a benthic community. The only possibility of this working may be if factors determining metal uptake could be controlled. Given the large range of interacting

factors, this task would be near impossible within a field based mesocosm facility.

Further complicating the question of potential toxicity of the Group 4 metals is the direct ingestion of metals by the aquatic insects. The mesocosm supported a community of insects dominated by mayflies, stoneflies, true beetles, caddisflies and chironomids (section 3.4). These taxa included animals having wide ranging food collection strategies including shredding (wood, leaves, periphyton mats), the collection of fine particulate organic matter (FPOM), scraping periphyton, and some predation on other animals. Hence, animal feeding in the troughs would have ingested sedimentary material that included the low and high energy bound metals shown in Figures 4,6,7,and 8. The affinities for uptake via ingestion may be rated according to binding strength associated with complexation and adsorption reactions: dissolved > exchangeable > carbonates > iron/manganese oxides > organics > residual minerals. Although placed in this series, the residual fraction is probably unavailable since it consists mainly of mineral bound metals. However, metals that are weakly adsorbed on the surfaces of clays may be important since relatively little energy is required for desorption and the metals could be ingested by various collector insects. Metals occurring with particulate and dissolved organic matter are fundamentally important since this is the primary food source of the aquatic insects. Copper dominated this fraction in all treatments and would be expected to be important in contributing to any toxicity caused by direct ingestion. The digestive process can be active in releasing weakly bound metals for uptake across cell membranes. Weakly bound metals associated with inorganic materials (eg. sand, oxides, lattices of carbonates) may also be ingested and metals released particularly by animals that filter or collect undissociated fine particulate material that was primarily organic in origin but could also have contained small inorganic particles entrained with the collected particles. Of the toxic metals examined in the sequential extraction (Figure 6 and 8), a series contributing to potential toxicity can be established based on a qualitative rating of binding energies. Using this approach, Cd > Zn > Cu > Mn. In other words, Cd had the greatest potential to cause toxic effects (mainly associated with low energy binding sites) and Mn had the lowest potential. Al was not included in the series since it was mostly associated with strongly bound residual silicates which are unavailable for uptake. Fe was not included because it is generally considered non-toxic.

In summary, the gradient of AMD dilutions produced important shifts in the acid neutralizing capacity of Foxy Creek and in concentrations of metals known to be toxic. The capacity of Foxy Creek to consume H^+ ions was exceeded near the 1:250 dilution but was severely stressed at the 1:1000 dilution. In this range, significant drops in pH are clear. If the raw AMD is diluted less, ANC is exceeded and pH drops in response to increased levels of H^+ ions. Metals that dominated the metal mixture from the increase in AMD levels included those known to be most toxic to aquatic organisms (Group 4; Table 3). At the lowest dilution of AMD, protonation was evident based on measurements of copper complexation capacity. At higher AMD dilutions, copper complexation and perhaps complexation of other metals on organic ligands was relatively high, thus indicating reduced potential for toxicity at those levels. At the highest dilutions, other ligands (eg. manganese or iron oxides) may have been important in complexing metals. Evidence of protonation of ligands at the 1:25 dilution indicates competition between H^+ and cations including Cd, Cu, and Zn and the possibility that despite the high concentrations of the aquo ions, protonation may have reduced toxicity. Hence, from low to high AMD additions confounding processes "competed" to cause net effects on toxicity in the biological community. A separate group of processes that potentially contributed to toxicity included the ingestion of metals bound to a series of low and high energy binding sites on food particles. Relatively loosely sediment bound metals that included in ranked order Cd, Zn, Cu, and Mn, could have contributed to toxicity via this direct ingestion.

3.3 Periphyton

Sixteen genera of periphytic algae were found in the troughs: one Cyanophyte (*Anabaena* sp.), 3 Chlorophytes (*Spirogyra* sp., *Ankistrodesmus* sp., and *Closterium* sp.), and 12 diatoms (Table 4). Rare diatom genera grouped as "others" in Table 4 included *Hannaea*, *Gomphonema*, *Diatoma*, *Navicula*, *Amphipleura*, *Epithemia*, and *Cymbella*.

At AMD dilutions greater than 1:250, diatoms consistently dominated the periphytic community (Table 4) with relative abundance of 69 - 94%. Chlorophytes were least abundant and the Cyanophytes abundance ranged between 4 and 27% of the community.

At dilutions less than 1:250, the relative importance of the diatoms declined by about three fold and the blue green abundance increased to more than 60% of the community. These data support other observations of a monoculture of *Anabaena sp.* surviving on gravel substrata in a riffle that separated the two AMD seepage ponds (Perrin, Limnotek Research and Development Inc., Unpub. data). The relative abundance of the chlorophytes also increased at the lower dilutions but the cells were found in a patchy distribution, occurring in some troughs but not others, thus producing highly variable cell counts (Figure 11).

Table 4. Relative abundance of algal taxa found in the mesocosm troughs on September 8

Group	Rate of AMD Addition (ml/min)						
	0	0.72	1.8	7.2	18	72	720
Cyanophytes	13.4	5.0	27.0	14.4	3.6	32.4	61.4
Chlorophytes	0.4	1.5	4.1	0.003	2.1	39.3	0
Diatoms	86.3	93.5	69.0	85.3	94.3	28.4	38.7
<i>Synedra</i>	1.1	1.0	8.0	5.4	3.7	13.2	10.0
<i>Cocconeis</i>	49.2	48.6	49.6	89.0	44.7	48.4	9.9
<i>Nitzschia</i>	7.9	6.0	0	1.5	25.8	26.5	0
<i>Achnanthes</i>	27.2	32.7	29.5	0	16.4	0	50.0
<i>Fragilaria</i>	13.8	11.1	9.5	3.0	6.4	0	30.0
Others	0.8	0.6	3.4	1.1	3.0	11.9	0

Of the five common diatoms listed in Table 4, *Cocconeis sp.* was clearly the dominant genera, accounting for about half of the diatom community at most treatment levels. Its relative abundance increased at the 1:2500 dilution but it almost disappeared at the lowest dilution. *Achnanthes sp.* and *Fragilaria sp.* followed in importance at the higher dilutions but declined relative to *Nitzschia sp.* at the lower dilutions.

Although major shifts in relative dominance of all taxa appeared at the two lower dilutions, small numbers of cells at those dilutions (Figure 10) caused large shifts in relative abundance. Because of the low numbers, these large changes in relative abundance are not considered important.

Double log transform regressions showed that in all taxa, the addition of AMD resulted in a logarithmic decline in numbers of cells (Figure 10). At lowest dilutions, the periphyton community was essentially decimated by the AMD. Highly significant relationships were found for total algal cells and for diatoms only (Figure 10). Relatively low numbers of Cyanophytes and Chlorophytes in patchy distributions resulted wide confidence limits and poor fits of the regression models (Figure 11).

Diatoms, which numerically dominated the community, also dominated the fit and error associated with the regression models. Diatoms alone produced a best fit ($R^2=0.73$) and a highly significant model ($P<0.001$). The introduction of scatter from the addition of chlorophytes and cyanophytes still produced a significant model, but the added variation slightly reduced the R^2 value for total cells to 0.60. This comparison suggests that numerically dominant taxa will be the best predictors of periphyton response to AMD additions. Indeed this analysis can be carried further to consider the possibility of an "index" taxa for periphyton in Foxy Creek. Since *Cocconeis sp.* clearly dominated the diatom community, it should also predict the general diatom response with even less error and better fit than the other diatoms or all diatoms combined. Minor species tend to occur in a patchy distribution and combinations of taxa introduces variation and a poorer fit to a predictive model. This was in fact the case: of all the diatoms and groups of taxa tested, *Cocconeis sp.* produced the best fit ($R^2=0.78$) in the most significant model ($P<0.0001$, Table 5). From this analysis, *Cocconeis sp.* can be considered a sensitive indicator of periphyton community response to AMD additions in 1990. Since taxa that are known only to recur as a dominant of the community intuitively should be considered as indicators and *Cocconeis sp.* was only a minor taxa in 1989 (Richardson and Perrin 1990), it follows that the diatoms taken as a group should be considered for indexing purposes.

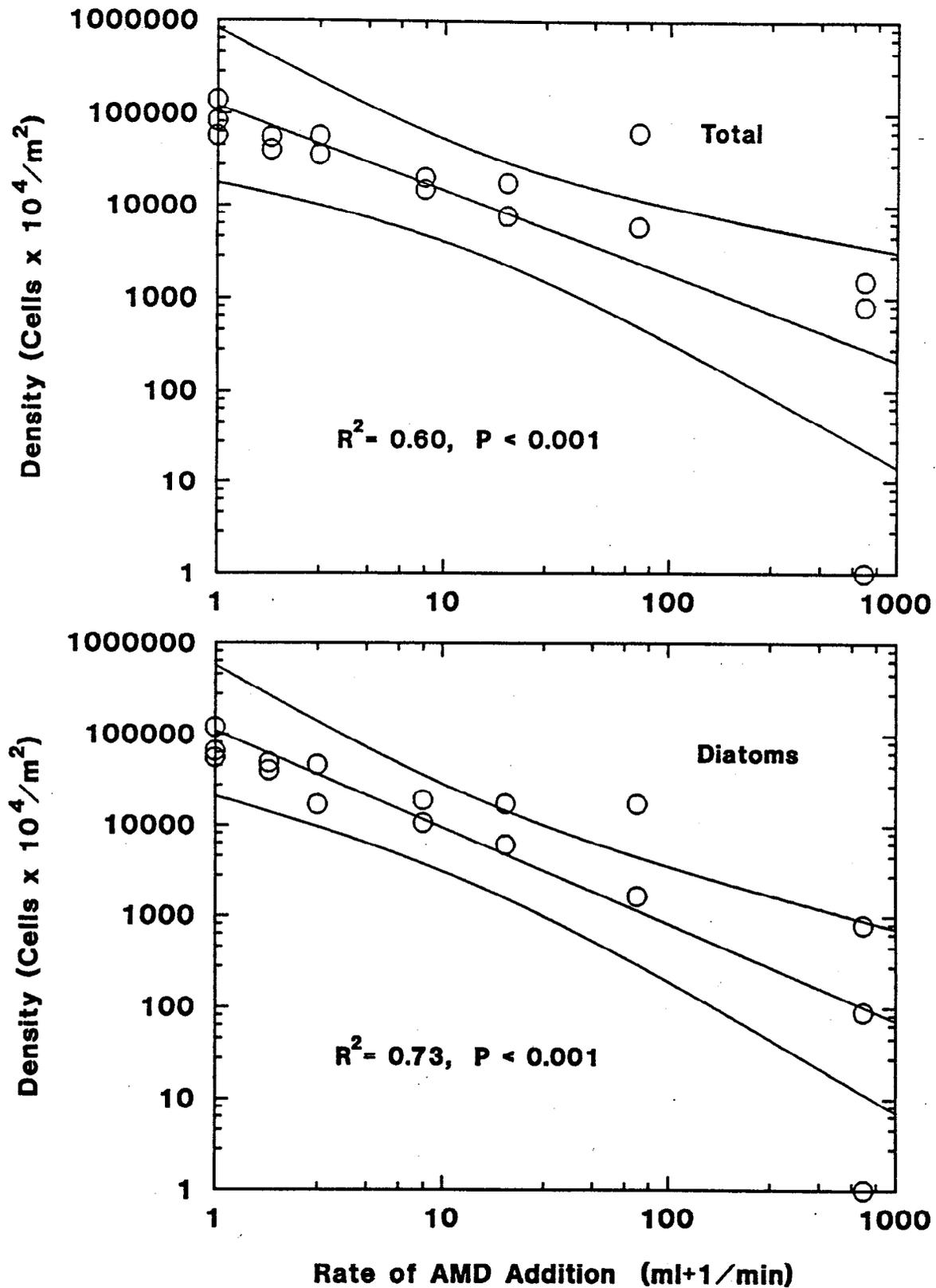


Figure 10. Log-log regressions showing the effect of AMD additions on total periphytic algae cell density (top) and diatoms only (bottom). All transforms were $\log(x + 1)$. The calculated regression model with 95% confidence limits is plotted with the $x + 1$ data.

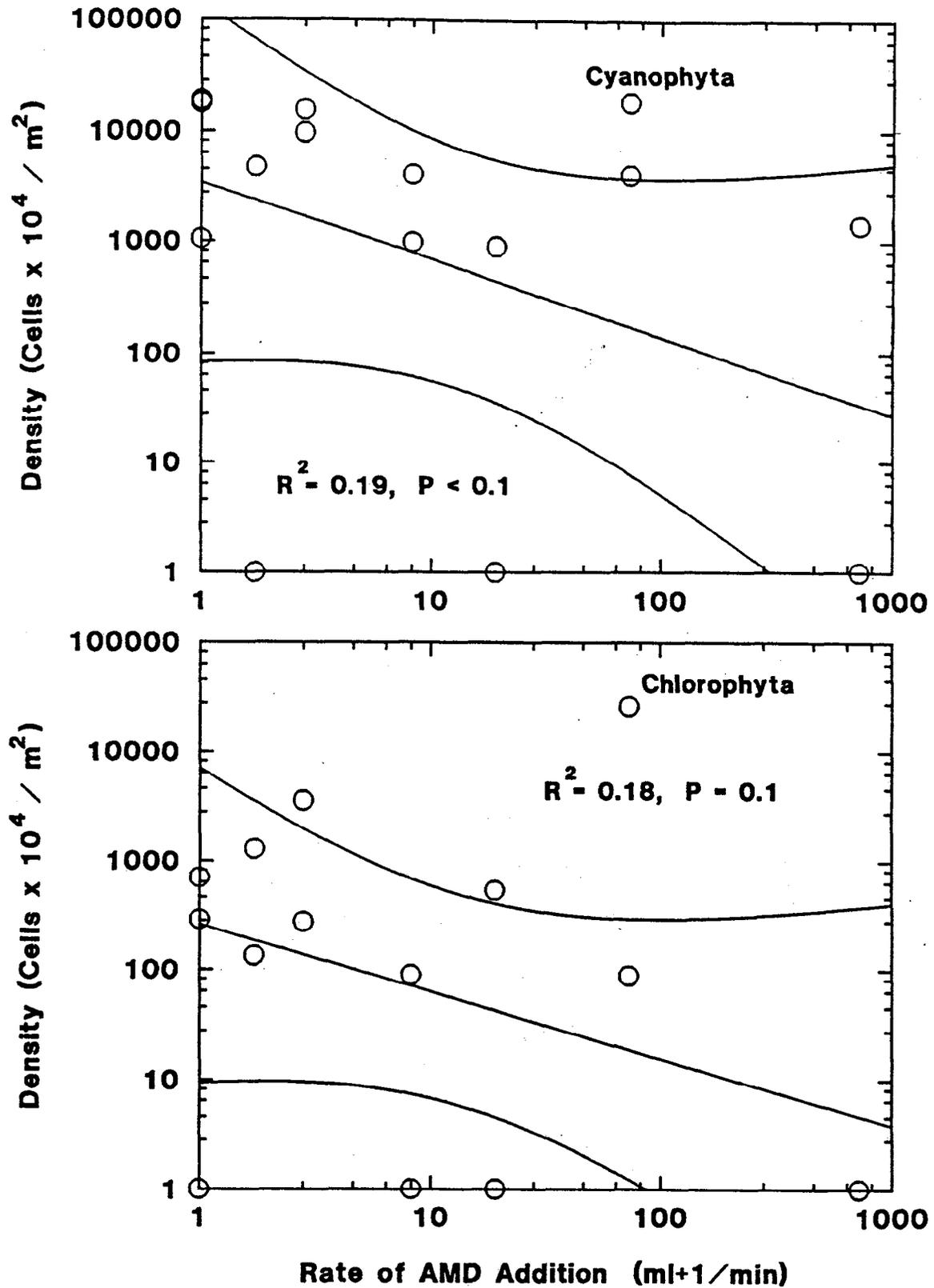


Figure 11. Log-log regressions showing the effect of AMD additions on the cell density of the Cyanophyta (top) and Chlorophyta (bottom). All transforms were $\log(x + 1)$. The calculated regression model with 95% confidence limits is plotted with the $x + 1$ data.

Generic richness was also found to decline with additions of the AMD (Figure 12). Of the 16 algal genera that were found in the mesocosm, ten of those were generally found together in the periphyton community that was not affected by the AMD. This number declined to 6 - 8 genera at AMD additions up to $72 \text{ ml} \cdot \text{min}^{-1}$ but coincidentally with the collapse of algal numbers at the lowest dilution, the numbers of genera also dropped. The data fit a significant logarithmic regression ($P < 0.001$, Table 5, Figure 12).

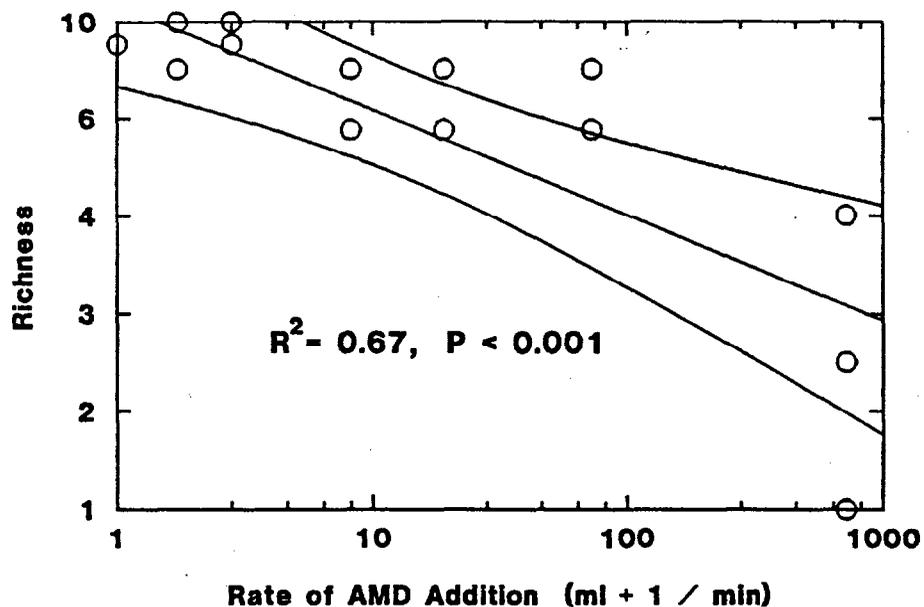


Figure 12. Log-log regression showing the effect of AMD additions on the periphytic algae richness. All transforms were $\log(x + 1)$. The calculated regression model with 95% confidence limits is plotted with the $x + 1$ data.

Table 5. Relationships between algal density and AMD additions as determined from log-log regressions.

Group	Probability	R ²	Slope
Cyanophytes (<i>Anabaena</i> sp)	<0.1	0.19	-0.70
Chlorophytes	0.101	0.18	-0.61
Diatoms	<0.001	0.73	-0.85
<i>Synedra</i> sp.	<0.01	0.41	-0.65
<i>Cocconeis</i> sp.	<0.0001	0.78	-1.26
<i>Nitzschia</i> sp.	<0.03	0.30	-0.97
<i>Achnanthes</i> sp.	<0.004	0.47	-1.34
<i>Fragilaria</i> sp.	<0.0001	0.68	-1.21
Other diatoms	<0.05	0.25	-0.64
Richness	<0.001	0.67	-0.82

Chlorophyll *a* concentrations were measured from samples collected through time with the objective of examining changes in indices of accrual as a function of treatment, but the data were inconsistent with the cell count - AMD relationships. The cell count information was considered more reliable in that it is virtually free of lab error. Peak biomass can be used as an index of growth in periphyton (Bothwell 1989) and these data (Table 6) do not follow the AMD gradient. Rather than declining to low levels at the highest addition of AMD, as the cell count data suggest (Figure 10), the concentrations increased by several times over most of the other values. There was also no consistent number of days within which PB was attained across the treatment gradient.

One explanation for the discrepancy is spectral interference in the spectrophotometer from products associated with the iron oxide precipitates. The samples collected particularly from the troughs receiving the two lowest dilutions of AMD were severely contaminated with the

precipitates. Precipitates and coloration of the substrata were also visually apparent in many other samples collected from troughs receiving the higher dilutions. The possibility of spectral interference by this material has not been examined in the literature and its importance remains unresolved. In cooperation with the EPS lab, however, tests are presently underway to specifically examine the effect of coloration associated with iron oxides on the spectrophotometric analysis of chlorophyll *a*.

The low mean values, inconsistency, and variation in the chlorophyll data made it highly insensitive to effects of AMD additions. An analysis of covariance was run to test if slopes of the accrual time course curves differed significantly. In this analysis, treatment was assigned as the covariate and day was the main effect. The result showed an insignificant effect of the AMD additions ($P > 0.2$). Considering the very clear effects of AMD additions on cell counts and the potential errors in the chlorophyll analyses, the chlorophyll data are not considered a reliable indicator of changes in the periphyton community of Foxy Creek in relation to additions of AMD.

The only consistent evidence from the data is that the concentrations were all very low. This finding is similar to measurements of periphyton accrual in 1989 in Foxy Creek and do suggest that the stream is extremely unproductive. Measurements using similar procedures in nutrient-deficient streams at lower altitudes where the productive growing season is longer routinely show PB levels of 10 - 40 $\mu\text{g}/\text{cm}^2$ attained in a 20-day time course (Perrin 1990). Levels $< 1 \mu\text{g}/\text{cm}^2$ in Foxy Creek indicate extreme limitation to periphyton growth.

Table 6. Peak biomass (PB) concentrations of chlorophyll *a* by treatment in the mesocosm troughs. The day that PB was measured after installation of clean substrata accompany the data.

Index	Rate of AMD addition (ml/min)						
	0	0.72	1.8	7.2	18	72	720
PB ($\mu\text{g}/\text{cm}^2$)	0.95	0.66	0.79	0.65	0.47	0.32	1.55
Day of PB	20	18	4	11	18	18	15

3.4 Aquatic Insects

Most insects found in the troughs were from the Ephemeroptera (mayflies), Plecoptera (stoneflies), Coleoptera (beetles), Tricoptera (caddisflies), and Chironomidae (chironomids). Mayflies were dominated by the baetids with fewer numbers from the heptagenids, leptophlebrids, and ephemerebrids. The stoneflies were dominated by *Zapada sp.*, *Isoperla sp.*, and the chloroperbrids (*Sweltza* or *Triznaka sp.*). A few Hemiptera were found but these were of terrestrial origin and were not included in the subsequent analyses. The beetles were also rare, many of which were terrestrial: only the elmids (aquatic beetles) were included in the analyses. There were relatively few caddisflies present and they were dominated by *Arctopsyche sp.*, *Ryacophila sp.*, and *Micrasema sp.* Occasionally simuliids were found: they tended to occur in some troughs but not others which is consistent with their known clumped distributions in natural substrata. Other flies of the Ceratopogonidae were consistently found but they were considered rare since their numbers were only about 1% of the chironomid numbers. The chironomids were dominated by the Orthoclaadiinae and Tanytarsini. *Tanypodini sp.* was also consistently found but in relatively low numbers. Water mites of the order Acarina were also consistently found and in some drift samples occurred in similar numbers to the chironomids. Unlike the findings from the 1989 project at Equity, up to 200 annelid worms were found in the benthos of each trough. These were not keyed out below the phylum level.

3.4.1 Insect Drift

The effect of the AMD gradient on emigration of animals from the troughs was examined in three hour and 24 hour sampling periods immediately before and after the onset of AMD additions as well as on three separate 24 periods thereafter. The objective was first to quantify any immediate avoidance of the AMD mixture (3 hour sampling) as well as longer term changes in emigration as a function of the AMD added.

Emigration was examined with two way ANCOVA using treatment as the covariate. Each pair (3 hr and 24 hr) of samples collected before and after the start of AMD additions were considered main effects. The main test was to determine whether the slopes of the drift-treatment relations changed significantly from before to after the onset of AMD additions. It was tested by examining the interaction term of slope due to treatment in the presence and absence of the AMD additions. The three sets of 24-hour samples that were collected on other dates after the initiation of AMD dosing were analyzed with ANCOVA again using treatment as the covariate.

Drift rates of all animals combined was significantly affected by the startup of AMD additions. There was a clear treatment effect (AMD addition) ($P < 0.001$) and the interaction term was also significant ($P < 0.001$) thus indicating a difference in slopes of the model before and after the AMD addition was started (Figure 13). In the three hour set before the AMD was added, drift rates were similar across all troughs. Within three hours after the start of AMD additions, drift from the troughs receiving highest doses increased about 8 fold due to the AMD addition. The effect was not noticeable at dilutions $\geq 1:1000$ (AMD additions ≤ 18 ml/min). In the 24-h set, drift after the AMD additions started was less than or equal to that before the additions in all treatments except at the highest dose from which drift increased about 1.5 times that measured before treatment. The response was less evident in the 24-h set but it did continue through the longer period after the pronounced response within the 3 hour period. Observations at the site indicated that the outmigration was immediate. Within seconds after the AMD flow was started, a mass migration of mayflies was visually apparent from the troughs receiving the two highest doses of AMD.

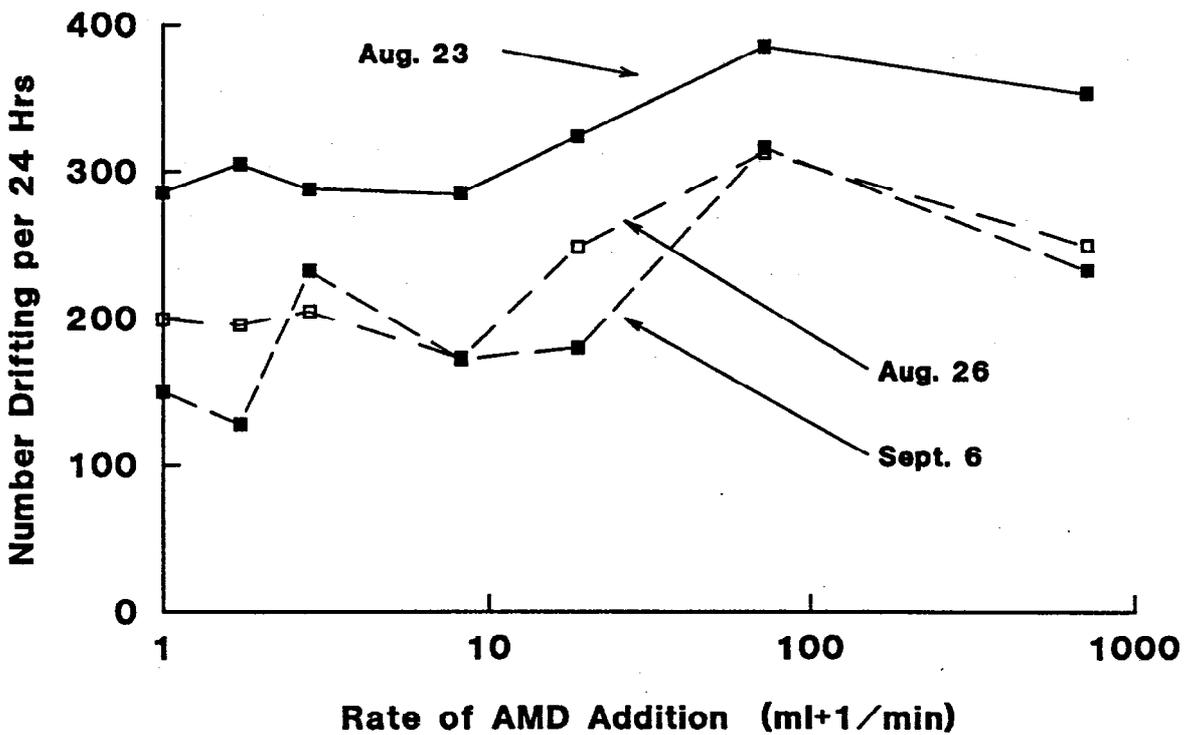
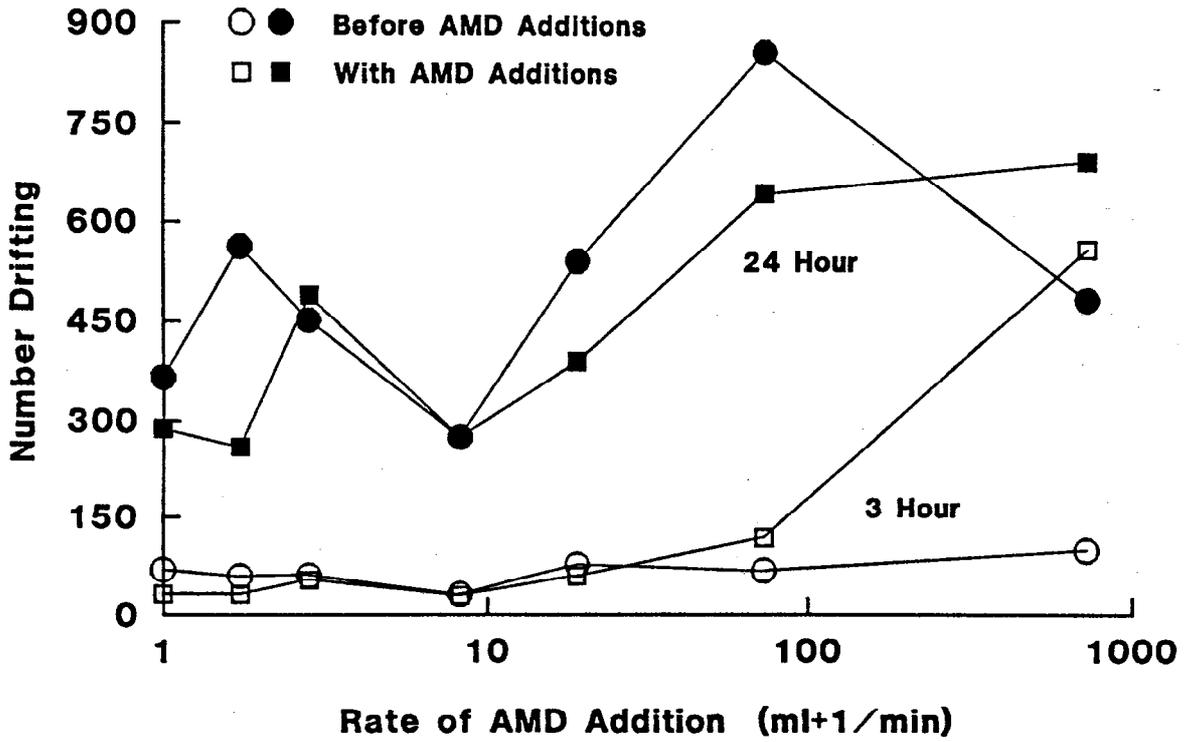


Figure 13. Total number of aquatic insects drifting over paired time durations (3h and 24h) before and after the onset of AMD additions (top) and on three subsequent 24h periods during AMD additions (bottom).

Baetis sp. and Tanytarsini were two of the numerically dominant taxa in all drift and they were mainly responsible for responses by total numbers. These two genera alone accounted for about 70% of total numbers drifting (compare Figures 14,15 with Figure 13). The drift rates of both also increased with the onset of AMD additions (Figures 8 and 9). The response by *Baetis sp.* in the 3 hr set was in particular very similar to that of the combination of all animals, thus indicating that *Baetis sp.* dominated the immediate outmigration after AMD additions started. Unlike the immediate response by *Baetis sp.*, a change in the drift of Tanytarsini was not apparent in the first three hours after AMD additions started but was striking within 24 hours, the effect occurring at higher AMD dilutions than were apparent from the *Baetis sp.* drift. Unlike *Baetis sp.* and Tanytarsini the orthoclad chironomids and aquatic mites did not exhibit significant changes in drift rates with respect to the AMD additions.

The effects of treatment on drift rates remained obvious during the two weeks following the onset of AMD additions. The ANCOVA for these three sampling periods showed significant effects of treatment on emigration rates of total numbers ($P < 0.04$, Figure 13), *Baetis sp.* ($P < 0.02$, Figure 14), Tanytarsini ($P < 0.04$, Figure 15), and Acarina ($P < 0.004$). Again, however, the orthocladiinae exhibited no trend in emigration rates with respect to treatment.

A time effect was also apparent in numbers drifting from August 23 to the end of the experiment. Total numbers of animals declined significantly over the two weeks ($P < 0.004$, Figure 13), the effect being particularly noticeable between August 23 and August 26. Contributing to this effect were significant declines in Tanytarsini ($P < 0.001$, Figure 15), Orthocladiinae ($P < 0.0001$), and Acarina ($P < 0.003$). A likely explanation for this trend is reduced metabolic activity associated with declining temperature (Figure 3). Temperatures in Foxy Creek declined from about 12°C to 5°C through this two week period, a decline which may have passed an activity threshold in the animals. Any threshold, however, did not hold for all aquatic insects. Drift of *Baetis sp.*, did not exhibit a time effect ($P = 0.415$, Figure 14) and if anything, actually increased between late August and September 6.

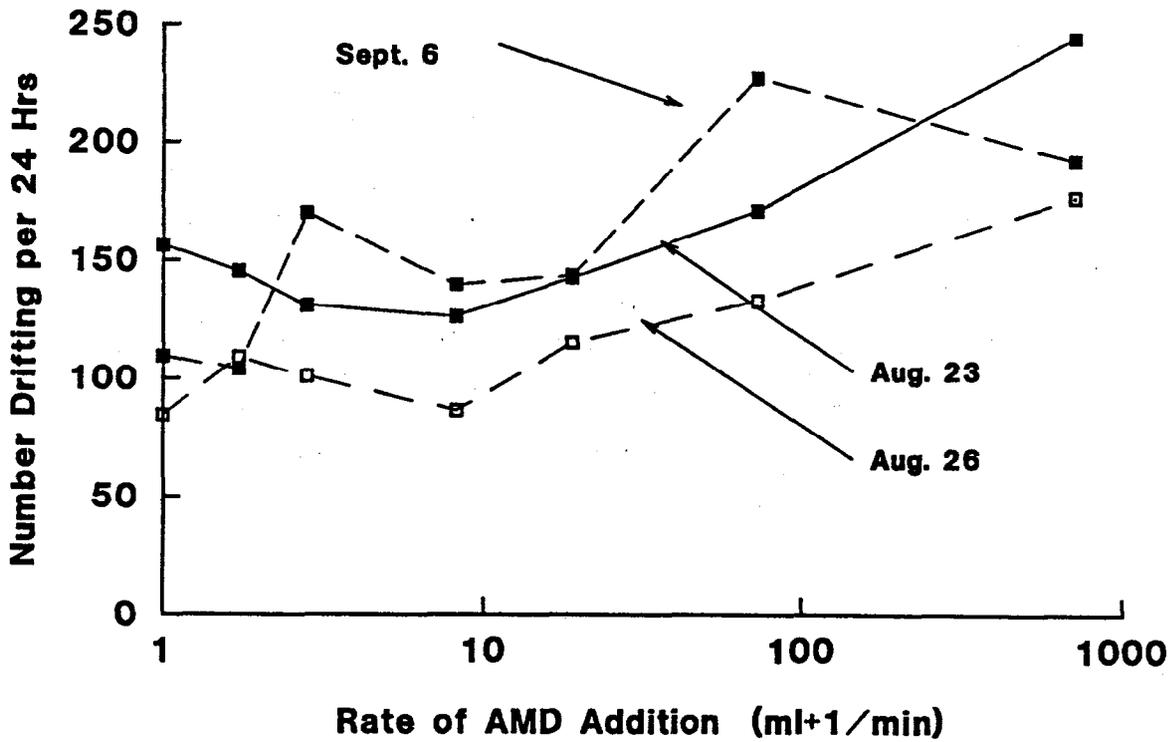
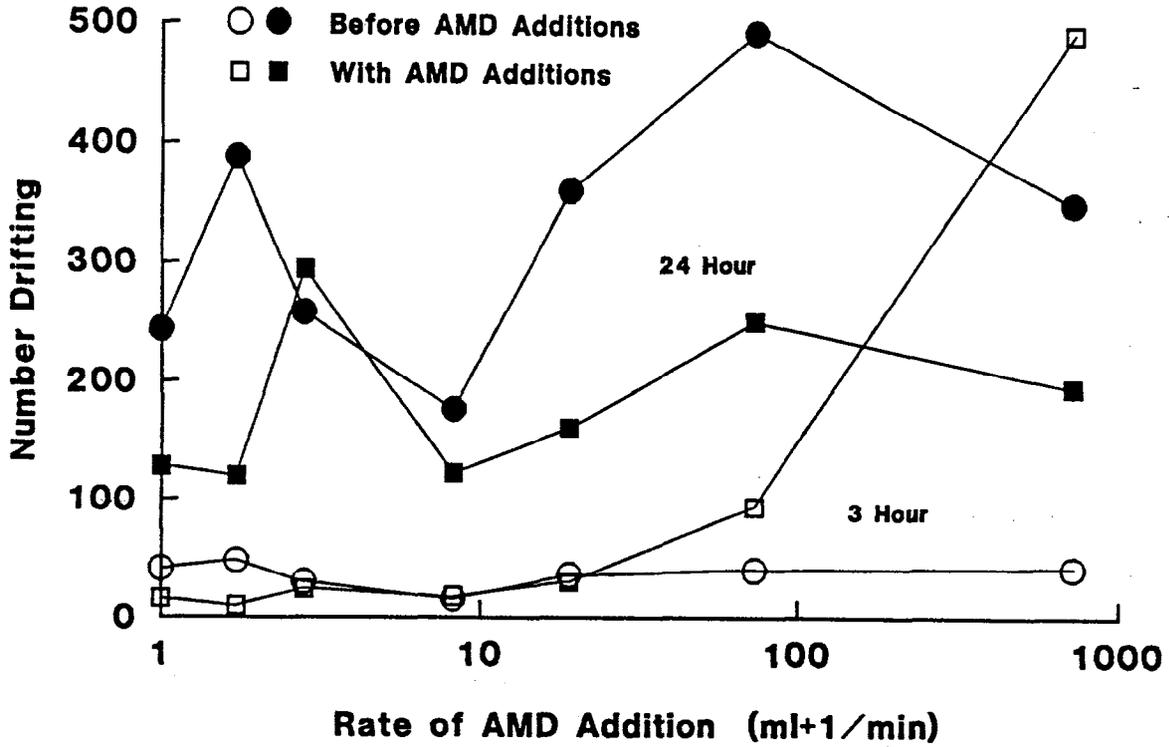


Figure 14. Numbers of *Baetis sp.* drifting over paired time durations (3h and 24h) before and after the onset of AMD additions (top) and on three subsequent 24h periods during the AMD additions (bottom).

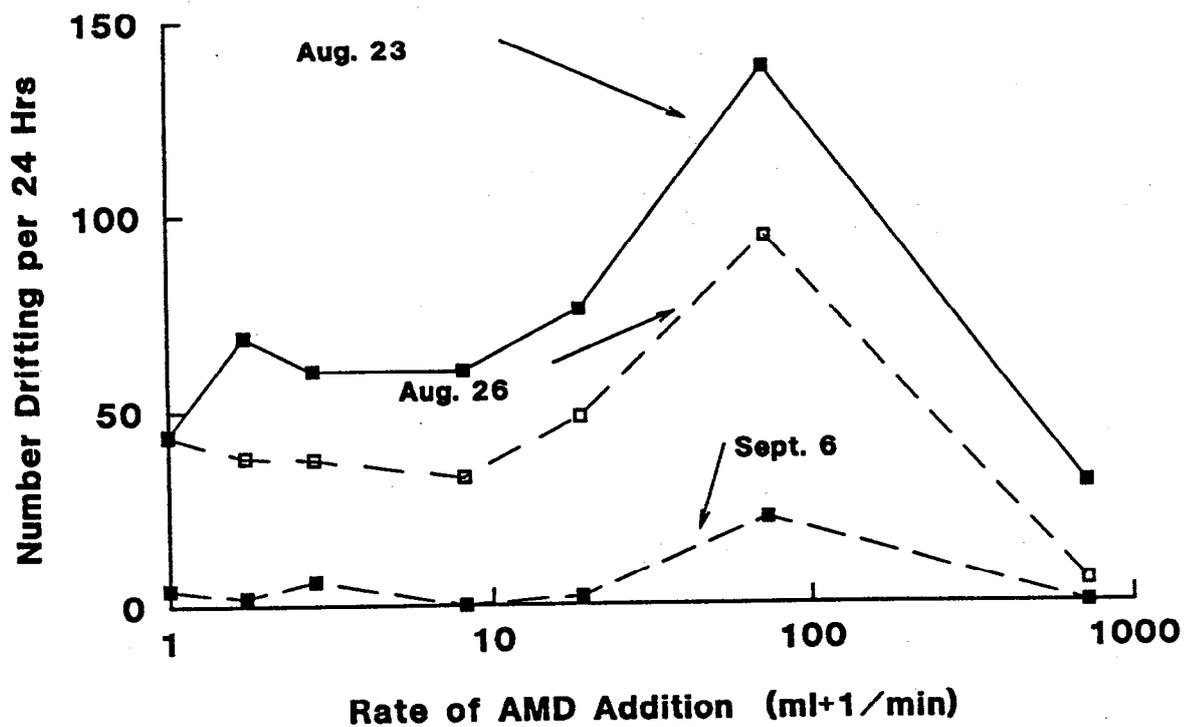
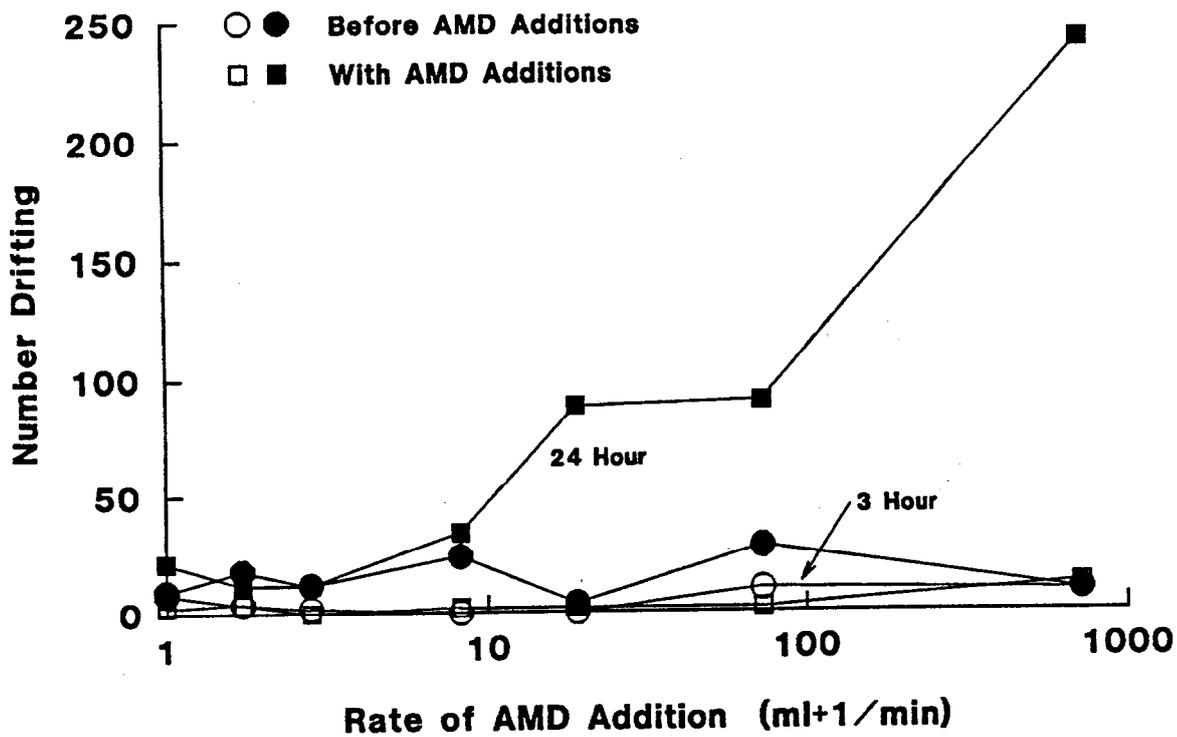


Figure 15. Numbers of Tanytarsini drifting over paired time durations (3h and 24h) before and after the onset of AMD additions (top) and on three subsequent 24h periods during the AMD additions (bottom).

A crude assessment of turnover rates of organisms in the troughs was estimated from the ratio of drift to benthos. Since benthos was not collected until September 6, the estimates were only determined for that date (Table 7). From these estimates it appeared that *Baetis sp.* had a high turnover rate even in the control troughs. At a 47% turnover rate, the community was turning over in less than three days. With increasing additions of AMD, turnover rate was affected by declining benthos (Figure 17) and relative to benthos present, the numbers of outmigrating baetids increased. The turnover rates at higher doses of AMD are extremely high and may indicate that immigrating animals didn't even settle in the troughs after immigration at those treatments. Turnover rates of all animals combined were less obvious at the higher AMD dilutions but abruptly increased at AMD additions of 72 ml/min and higher. Again, however, the orthoclads were unique: their turnover rate did not show a clear increase with the AMD additions.

Table 7. Estimates of turnover rates of dominant insect groups found in the troughs on September 6.

Group	Rate of AMD addition (ml/min)						
	0	0.72	1.8	7.2	18	72	720
Total	0.063	0.053	0.088	0.056	0.084	0.405	0.283
<i>Baetis sp.</i>	0.465	0.552	0.847	1.201	7.197	14.722	4.88
Orthoclads	0.065	0.026	0.123	0.037	0.041	0.062	0.06

3.4.2 Adult Insects

While sorting and enumerating the drift samples, adult stages of aquatic insects were routinely found and counted separately from the immature stages. These counts were not expected to reveal total numbers of adults since any emergence would have been lost from the mesocosm through the openings at either end of each trough. Hence, the counts included adults that (1) were in the process of emerging but were entrained with the surface current and were swept into the drift nets; (2) those that were captured in the mesocosm intake and were simply moving through the mesocosm; and (3) those that were killed in the mesocosm as a function of the AMD additions. Only the chironomid adults were found in sufficient numbers in all treatments to allow any interpretations (Figure 16). All other captures were incidental.

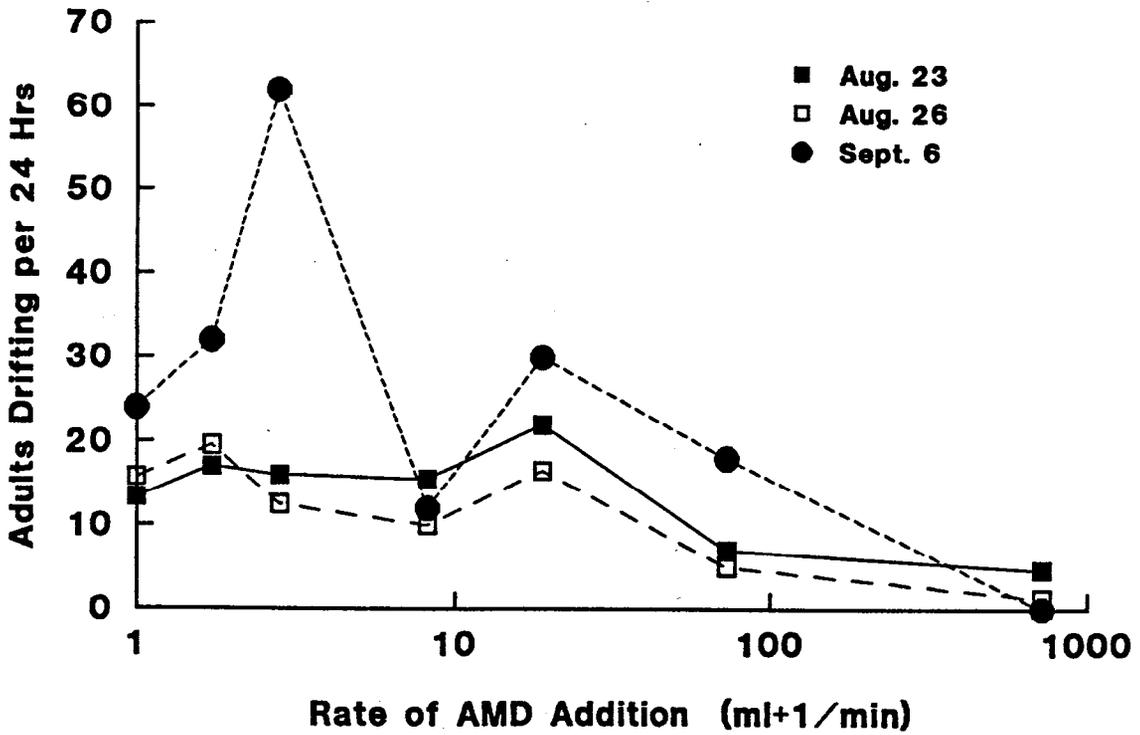
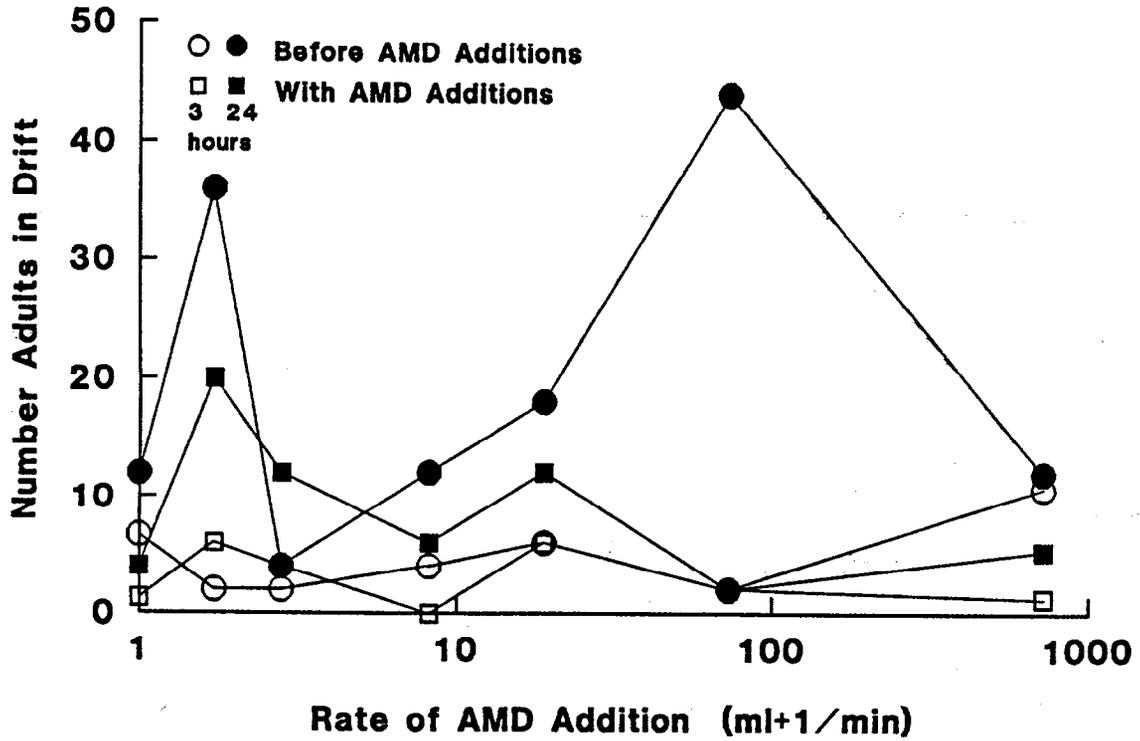


Figure 16. Numbers of chironomid adults drifting over paired time durations (3h and 24h) before and after the onset of AMD additions (top) and on three subsequent 24h periods during the AMD additions (bottom).

Adult drift in relation to sampling duration and treatment was highly variable and inconclusive (Figure 16). Numbers were generally higher in the 24h set than in the 3h set but no effect of the onset of AMD additions was apparent. However, in the 24h sample sets collected between August 23 and September 6, numbers declined with increasing additions of AMD, a finding that supports trends in the other drift parameters. There was no clear temporal trend, however, over the three sampling dates.

3.4.3 Benthos

The densities of benthos at the end of the experiment were tested using log-log regressions. Total density declined with increasing AMD addition as did *Tanytarsini* (Table 8, Figure 17), the single most common taxon in the benthos. Table 8 shows that most taxa declined significantly with increasing dose of AMD. The exception was the orthoclad chironomids which did not decline. The simuliids showed a slight increase with the AMD addition but their numbers were very low and were not considered important in indicating a general response.

Unlike richness of the periphyton, insect richness did not change with treatment, thus indicating that most taxon remained in the troughs despite large decreases in numbers of animals with increasing dose of AMD.

Table 8. Summary statistics for the final densities of benthos. The critical level of alpha was 0.0038 after correction for multiple comparisons using the Bonferroni procedure (Rice 1988).

Group	P (*=sig)	R ²	Slope	Grand Mean
Total numbers	<0.0001*	0.68	-0.186	1777
Richness	0.47	0.04	-0.0071	
<i>Baetis sp.</i>	0.0025*	0.49	-0.29	84
Heptageniidae	<0.0001*	0.78	-0.48	9
Paraleptophlebia	0.0002*	0.64	-0.64	7.7
Zapada	<0.0001*	0.85	-0.85	58
Isoperla	0.0021*	0.5	-0.47	29
Chloroperlidae	<0.0001*	0.7	-0.47	177
Elmidae	0.9854	0	-0.001	10
Tricoptera	0.0479	0.25	-0.15	23
Simuliidae	0.0269	0.3	+0.25	1.3
Orthoclaadiinae	0.5245	0.03	+0.028	223
Tanypodinae	0.0017*	0.52	-0.3	12
Tanytarsini	<0.0001*	0.69	-0.23	793
Oligochaeta	0.0467	0.25	-0.20	45

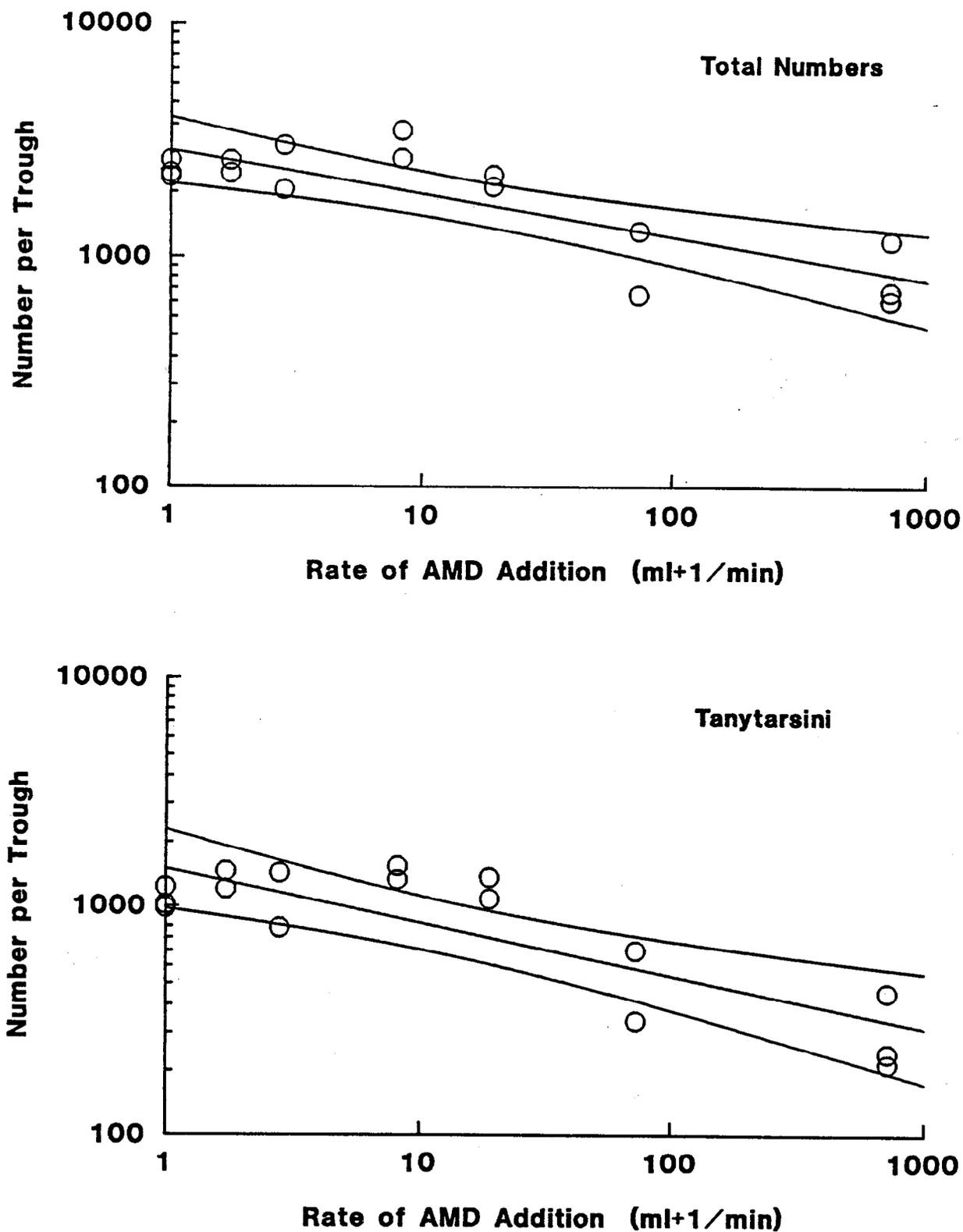


Figure 17. Log-log regressions showing the effect of AMD additions on total benthos (top) and Tanytarsini (bottom). All transforms were $\log(x + 1)$. The calculated regression model with 95% confidence limits is plotted with the $x + 1$ data.

3.5 AMD Toxicity in Foxy Creek

Results from this study have confirmed that additions of untreated AMD to Foxy Creek would cause a significant decline in the abundance of important components of the lotic community. The reduction in the abundance of the flora and fauna infers that the AMD was toxic to the community that was supported in the mesocosm. Obviously, evidence of negative effects of high acidity and metals concentrations in solution is not new. Several relevant studies are cited in section 3.2 of this paper. Recently, Moore et al. (1991) found that AMD was persistent over long reaches of a large order stream in Montana and that declines in the abundance of components of benthic communities occurred coincidentally with AMD transport. Campbell and Stokes (1985) is a key paper in citing many observations of relationships between metals, acidity, and toxicity.

The present findings are unique, however, in demonstrating a direct causal link between AMD and reduced abundance of components of a stream community, they indicate sensitive and insensitive flora and fauna to AMD additions, they provide quantitative models that can be used to predict functional responses of organisms and complex groups of organisms, and from these models, threshold levels of AMD additions can be identified that affect the various components of the community. There is a great deal of emphasis in the toxicity literature on establishing effects of individual metals on individual organisms (eg. EVS 1990) and on separating effects of changes in acidity from changes in metals concentrations and these studies are important in understanding physiological adaptations to contaminant stress. But, from a ecological point of view where the interest is on change in ecosystem structure and function these literature are inadequate because they give no evidence of changes at the ecosystem level. The advantage of using an experimental design that tested a gradient of AMD additions in a mesocosm environment is that quantitative causal relationships were established at several levels of community organization. Only this level of data analysis is adequate with which to make quantitative decisions on allowable levels of effluent dilution in receiving environments.

A key finding from the study was that decreased dilution of AMD caused an increase in acidity and concentrations of metals known to be toxic to aquatic biota and that

a corresponding logarithmic decrease in the abundance of periphytic algae and benthos that was highly significant could be quantified in predictive models. Of all periphytic taxon known to be important in trophic production, the diatoms, particularly *Cocconeis sp.*, dominated the responses and showed decreases in abundance beginning at the highest dilution of AMD tested (addition of 0.72 ml/min; AMD dilution of 1:25,000). Numbers of cells were reduced by almost two orders of magnitude with dilutions reduced to 1:250 and were virtually eliminated at a dilution of 1:25. The benthos also exhibited a logarithmic decline with AMD additions but the individual data that are plotted with the model in Figure 17 suggest that a threshold existed between AMD additions of 18 and 72 ml · min⁻¹ (AMD dilutions of 1:1000 and 1:250) at which point there was a downward step in numbers of benthic insects. This response may not only be related to direct toxic effects on the benthos but the response corresponded with a marked increase in the accumulation of the iron oxide precipitate and higher rates of emigration. Hence, there may be additive physical and behavioral effects combining with any direct mortalities at the two lowest AMD dilutions. It is important, however, that a highly significant logarithmic decay function was found for the combined benthos data. This finding suggests that effects of AMD additions on benthos at dilutions greater than 1:1000 cannot be ruled out.

Although it was clear that the AMD additions caused declines in the abundance of the flora and fauna, the experiment did not distinguish between mortality and emigration. The drift data clearly showed an immediate and pronounced outmigration caused by the AMD addition, particularly of the baetid mayflies and the chironomid tribe, Tanytarsini. The immediacy of this response suggests that the animals were avoiding the AMD additions although acute mortality was possible. The very high turnover rates of *Baetis sp.* also suggests that avoidance was part of the mayfly response. Since *Baetis* was found in the benthos but had turnover rates that exceeded 100% at AMD dilutions less than 1:10000, many animals must have drifted through the troughs without actually staying for more than a day. The increase in turnover rates with increasing additions of the AMD suggests the throughput effect increased at reduced AMD dilutions. It remains uncertain, however, what proportion of the throughput or other drift included animals that were actually killed in the troughs compared to that which was acutely avoiding the AMD mixture.

Responses to the AMD additions were taxon specific or observations were controlled by dominant taxon. Of the periphyton, the diatoms, and particularly *Cocconeis sp.* numerically dominated the community. Hence, the response by the diatoms determined the overall change in periphyton abundance. In the benthos and drift, the abundance of the numerically dominant taxon which included *Baetis sp.*, the stoneflies, *Zapada sp.* and Chloroperlidae, and the chironomid tribe, Tanytarsini all declined with increasing additions of AMD and thus determined the overall insect response. The Orthoclaadiinae, however, showed no response to the AMD, yet it also was a numerically important taxa. It may be that orthoclads are insensitive to the AMD at the dilutions tested. If this were true, they would have experienced reduced competition or predation from the concomitant reduction in density of other taxon. The turnover rates of the orthoclads were lower than most (Table 8), so there is no indication that the densities were maintained by high rates of colonization. They indeed did survive even the highest additions of AMD, quite unlike any other taxon.

Data from this study can be used as a test for existing regulations in Foxy Creek or other subalpine stream that supports a similar community. To avoid change in the structure of the benthic community due to AMD additions, the present data suggest that raw AMD dilutions would have to be greater than 1:25,000. This conclusion is based on the relationships between abundance of community components and AMD additions which indicated some change in structure even at the highest AMD dilution. Each of the metals in Group 4 in Table 3, with the possible exception of Al (complexed at high energy residual binding sites in sediment), potentially caused some of the observed changes in ecosystem structure. The concentrations of these metals at the dilution of observed changes (1:25,000 dilution) can be identified from Table 3. These data are compared to CCREM guidelines in Table 9.

Table 9. Comparison of metals concentrations that as a mixture caused a change in the structure of the mesocosm community with levels established in water quality guidelines (CCREM) for protection of freshwater aquatic life.

Metal	Concentration in the metals mixture that caused change in community structure ($\mu\text{g} \cdot \text{L}^{-1}$)	CCREM guideline ($\mu\text{g} \cdot \text{L}^{-1}$)
Copper	6.7 ± 4.3	2
Cadmium	<0.1	0.2
Manganese	8.5 ± 6.0	no guideline
Zinc	9.1 ± 8.2	30
Aluminum	68 ± 32	100 at pH ≥ 6.5
Iron	134 ± 60	300

Since the CCREM guidelines are largely based on evidence from single species or simple population bioassays, these data suggest that under exposure to complex metal mixtures, the concentrations of individual metals in those mixtures are potentially toxic at lower concentrations than may be apparent when tested alone. With the exception of copper, the CCREM guideline for each metal in Table 9 was higher than the level found to cause a change in community structure by the metals mixture tested in this study. Hence, this comparison further indicates that the CCREM guideline for metals must only be considered a screening tool for the end user, and that experimentation is required to define more accurate levels of metals to be established in the permitting process. If experimentation is not possible, the CCREM guidelines must be regarded as maximum levels and that at those guidelines, some change in community structure may be expected when the discharge of acidic metal mixtures is in question.

4.0 REFERENCES CITED

- Allard, M. and G. Moreau. 1987. Effects of experimental acidification on a lotic macroinvertebrate community. *Hydrobiologia* 144:37-49.
- Anderson, M.A. and A.J. Rubin. 1981. Adsorption of Inorganics at Solid-Liquid Interfaces. Ann Arbor Science Publ. Ann Arbor, MI. 357 pp.
- APHA. 1985. Standard Methods for the Examination of Water and Wastewater. (16th Edition).
- Baumann, R.W., A.R. Gaufin, and R.F. Surdick. 1977. The stoneflies (Plecoptera) of the Rocky Mountains. *Mem. Am. Ent. Soc.* 31:1-208.
- Beveridge, T.J. and R.G.E. Murray. 1980. Sites of metal deposition in the cell wall of *Bacillus subtilis*. *J. Bacteriol.* 141: 876-887.
- Bothwell, M.L. 1989. Phosphorus-limited growth dynamics of lotic periphyton diatom communities: areal biomass and cellular growth rate responses. *Can. J. Fish. Aquat. Sci.* 46:1293-1301.
- Boudou, A. and F. Ribeyre. 1989. Aquatic Ecotoxicology: Fundamental Concepts and Methodologies. CRC Press. Boca Raton. Fl.
- Cairns, J. and J.R. Pratt. 1989. The scientific basis of bioassays. *Hydrobiologia.* 188/189:5-20.
- Campbell, P.G.C. and P.M. Stokes. 1985. Acidification and toxicity of metals to aquatic biota. *Can. J. Fish. Aquat. Sci.* 42: 2034-2049.
- Campbell, P.G.C., A.G. Lewis, P.M. Chapman, A.A. Crowder, W.K. Fletcher, B.Imber, S.N. Luoma, P.M. Stokes, and M. Winfrey. 1988. Biologically available metals in sediments. National Research Council of Canada Publication No. NRCC 27694. Ottawa, Canada. 298p.
- Carpenter, S.R., J.F. Kitchell, and J.R. Hodgson. 1985. Cascading trophic interactions and lake productivity. *Bioscience* 35:634-639.
- CCREM. 1989. Canadian Water Quality Guidelines. Canadian Council of Resource and Environment Ministers. Ottawa.
- Cole, F. R. 1969. The Flies of Western North America. University of California Press, Los Angeles, California.
- Cronan, C.S. and C.L. Schofield. 1979. Aluminum leaching response to acid precipitation: effects on high elevation watersheds in the Northeast. *Science (Wash. DC)* 204: 304-306.

Doudoroff, P., B.G. Anderson, G.E. Burdick, P.S. Galtsoff, R. Patrick, E.R. Strong, E.W. Surber, and W.M. van Horn. 1951. Bio-assay for the evaluation of acute toxicity of industrial wastes to fish. *Sewage Ind. Wastes* 23: 1380-1397.

Edmunds, G.F., S.L. Jensen, and L. Berner. 1976. *The Mayflies of North and Central America*. University of Minnesota Press, Minneapolis, Minnesota.

EPS. 1988. *Metals in Water. Methods manual*. 8 pp.

EPS. 1990. Biological test method: Acute lethality test using rainbow trout. Environment Canada Environmental Protection Series Report EPS 1/RM/9. 51p.

EPS. 1990. Biological test method: Acute lethality test using threespine stickleback. Environment Canada Environmental Protection Series Report EPS 1/RM/10. 45p.

EPS. 1990. Biological test method: Acute lethality test using *Daphnia spp.* Environment Canada Environmental Protection Series Report EPS 1/RM/11. 57p.

EVS Consultants Ltd. 1990. Literature Review for biological monitoring of heavy metals in aquatic environments. B.C. Ministry of Energy, Mines, and Petroleum Resources. Acid Mine Drainage Task Force. Victoria, B.C. 127p.

Galloway, J.N. 1983. Effects on aquatic chemistry. In: A.P. Altshaller and R.A. Linhurst (Ed). *The Acid Deposition Phenomenon and its Effects: Critical Assessment Review Papers*. pp 4-1 - 4-162. USEPA Washington. D.C. EPA-600-8-83-016B.

Gherini, S.A., L. Mok, R.J.M. Hudson, and G.F. Davis. 1985. The ILWAS model: formulation and application. *Water, Air, Soil Pollution* 26:425-459.

Hart, D.D. and C.T. Robinson. 1990. Resource limitation in a stream community: phosphorus enrichment effects on periphyton and grazers. *Ecology* 71:1494-1502.

Hart, W.B., P. Doudoroff and J. Greenbank. 1945. The evaluation of the toxicity of industrial wastes, chemicals, and other substances to freshwater fishes. Waste Control Laboratory, Atlantic Refining Co. Philadelphia, Pa. 376 p.

Hessen, D.O. and J.P. Nilssen. 1986. From phytoplankton to detritus and bacteria: effects of short-term nutrient and fish perturbations in a eutrophic lake. *Arch. Hydrobiol.* 105:273-284.

Imber, B. 1991. Development of an ion speciation model for environmental assessment in aquatic systems. Contract report from CB Research Corp. for B.C. Ministry of Energy, Mines and Petroleum Resources. 17p.

Jenne, E.A. 1968. Controls on Mn, Fe, Co, Ni, Cu, and Zn concentrations in soils and water: the significant role of hydrous Mn and Fe oxides. *Adv. Chem. Ser.* 73: 337-387.

- Jenne, E.A. 1977.** Trace element sorption by sediments and soils - sites and processes. In *Molybdenum in the Environment*. Vol. 2. Edited by W.R. Chappell and K.K. Petersen. Marcel Dekker, New York, NY. pp.425-553.
- Larkin, P.A. 1984.** A commentary on environmental impact assessment for large projects affecting lakes and streams. *Can. J. Fish. Aquat. Sci.* 41:1121-1127.
- Leckie, J.O. and R.O. James. 1975.** Control mechanisms for trace metals in natural waters. In: A.J. Rubin (Ed.), *Aqueous-Environmental Chemistry of Metals*. Ann Arbor Science Publishers, Ann Arbor, Mich., pp. 1-76.
- McAlpine, J.F., B.V. Peterson, G.E. Shewell, H.J. Teskey, J.R. Vockeroth, and D.M. Wood (coords.). 1981.** Manual of Neararctic Diptera, Vol.1. Res. Branch Agric. Can. Mongr. 27. 674p.
- Mackie, G.L. 1989.** Tolerances of five benthic invertebrates to hydrogen ions and metals (Cd, Pb, Al). *Arch. Environ. Contam. Toxicol.* 18:215-223.
- Merritt, R.W. and K.W. Cummins (eds). 1984.** An Introduction to the Aquatic Insects of North America. 2nd. edition. Kendal-Hunt Publ. Dubuque, Iowa. 721p.
- Moore, J. N., S. N. Luoma, and D. Peters. 1991.** Downstream effects of mine effluent on an intermontane riparian system. *Can. J. Fish. Aquat. Sci.* 48: 222-232.
- Mundie, J.H., S.M. McKinnell, and R.E. Traber. 1983.** Responses of stream zoobenthos to enrichment of gravel substrates with cereal grain and soybean. *Can. J. Fish. Aquat. Sci.* 40:1702-1712.
- Mundie, J.H., K. Simpson, and C.J. Perrin. 1991.** Responses of stream periphyton and benthic insects to increases in dissolved inorganic phosphorus in a mesocosm. *Can. J. Fish. Aquat. Sci.* 48(11):2061-2072
- Pagenkopf, G.K. 1978.** Introduction to natural water chemistry. Marcel Dekker, Inc. New York, New York. 271 pp.
- Perrin, C.J. 1990.** Pilot fertilization of the Nechako River II: Nitrogen-limited periphyton production and water quality studies during treatment of the upper river. Nechako Fisheries Conservation Program, Project RM89-4.
- Perrin, C. J., B. Wilkes, and J.S. Richardson. 1992.** Stream periphyton and benthic insect responses to additions of treated acid mine drainage in a continuous-flow on-site mesocosm. *Environ. Tox. and Chem.* 11(11). In Press.
- Peterman, R.M. 1990.** Statistical power analysis can improve fisheries research and management. *Can. J. Fish. Aquat. Sci.* 47:2-15.
- Rice, W.R. 1988.** Analyzing tables of statistical tests. *Evolution.* 43:223-225.

- Richardson, J.S. and C.J. Perrin. 1990.** The effect of treated acid mine drainage on stream macroinvertebrates and periphytic algae: an in situ mesocosm experiment. B.C. Ministry of Energy, Mines, and Petroleum Resources. Acid Mine Drainage Task Force. Victoria. 33p.
- Schindler, D.W., K.H. Mills, D.F. Malley, D.L. Findlay, J.A. Shearer, I.J. Davies, M.A. Turner, G.A. Linsey, and D.R. Cruikshank. 1985.** Long-term ecosystem stress: the effects of eight years of acidification on a small lake. *Science* 228: 1395-1401.
- Schindler, D.W. 1987.** Detecting ecosystem response to anthropogenic stress. *Can. J. Fish. Aquat. Sci.* 44(Suppl.):6-25.
- Sunda, W.G. and R.R.L. Guillard. 1976.** The relationship between cupric ion activity and the toxicity of copper to phytoplankton. *J. Mar. Res.* 34: 511-529.
- Tessier, A., P.G.C. Campbell, and M. Bisson. 1979.** Sequential extraction procedures for the speciation of particulate trace metals. *Anal. Chem.* 51(7): 844-851.
- Wetzel, R.G. 1975.** *Limnology*. W.B. Saunders. Philadelphia, PA. 743pp.
- Wiggins, G.B. 1977.** *Larvae of the North American Caddisfly Genera (Tricoptera)*. University of Toronto Press, Toronto, Ont.