BIOACCUMULATION OF ZINC IN PERIPHYTON AND INVERTEBRATES: LOTIC FIELD AND MICROCOSM STUDIES

by

Craig S. Harris

B.Sc., University of Alberta, 1989

PBD—Enviro. Tox., Simon Fraser University, 1996

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Abstract

Past studies directed at assessing the impact of mining effluent on the St. Mary River, BC focused on assessments of the diversity and biomass of invertebrates and benthic algae (periphyton). Results did not provide conclusive evidence of an impact that could be solely attributed to the mining effluent. Furthermore, these studies did not measure tissue metal levels. Therefore, this study evaluated whether zinc, the most conspicuous contaminant, was biologically available to the periphyton and if so, whether there was food chain transfer to invertebrates. Field evaluations of tissue zinc were complimented with laboratory stream microcosm studies. Flow-through microcosm experiments consisted of naturally cultured periphyton and a single grazing invertebrate. Treatments were designed to address two questions in the Fall 1997 and Spring 1998 respectively: 1) the role of iron oxides in zinc bioavailability and 2) zinc uptake, clearance, transfer and toxicity from an iron rich nutrient mixture. Zinc was quantified in abiotic and biotic compartments through flame atomic absorption. Laboratory periphyton community and population level effects were measured through pigment and taxonomic analysis. Periphyton as an indicator of bioavailable zinc has both strengths and weaknesses. In the controlled streams, periphyton accumulated zinc 10^3 times higher than ambient concentrations and was a sensitive indicator of the ability of iron oxides to reduce bioavailability. Population and community level effects were evident as dissolved zinc concentrations exceeded 50 $\mu g\ L^{-1}$. Although periphyton growth was reduced, the relative abundance of different genera of diatoms was also a sensitive indicator of zinc stress. In contrast to the laboratory, field studies were impeded by a lack of spatial consistency and associated inorganic debris, which made site specific comparisons of tissue zinc problematic. Both field and laboratory studies indicated significant food chain transfer. With the exception of predators, invertebrate tissue zinc correlated with periphyton zinc ($r^2 > 0.70$). Tolerant and sessile genera such as the filter feeding Hydropsychide proved to be the best indicator of zinc bioavailability in the field. In these dynamic freshwater resources, invertebrates spatially and temporally integrate environmental levels of metals. Therefore, they are much more reliable monitors as compared to standard chemical analysis, and it is recommended that they are used more frequently.

Acknowledgement

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The design and construction of the stream microcosm was an ambitious project, the completion of which was achieved with the support and technical expertise of the SFU machine shop personnel, Jamie Shoults and Dick Vanderwal, and electrician, Lloyd Cornwall. Many thanks to Bruce Leighton who's patience and helpful hints contributed to the setup, operation, biological design and dismantling of the microcosm. I would like to acknowledge my fellow students, Zainal Arifin, Lori Barjaktarovic and several laboratory assistants for all their suggestions, feedback and hard work. For tutorials and technical suggestions regarding the atomic absorption spectrophotometer I am grateful to Dr. Louis Peterson.

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Preface

This thesis was a logical blend of my geological background and more recent toxicological training. But more importantly, the research allowed me to explore the river valleys of British Columbia, a pastime that I have always loved. Through this project I have gained an appreciation of the wonderful biological world that exists in our freshwater ecosystems. As a result, I dedicate this thesis to the protection of what I consider the most valuable and beautiful freshwater habitats, our rivers and streams.

1 Chapter 1: Overall Introduction

The Federal Ministry of Fisheries and Oceans has been assigned the responsibility for sea coast, inland fisheries and administration of the Fisheries Act. This responsibility encompasses protection of marine and freshwater organisms and their habitat from the discharge of deleterious substances such as toxic metals. Protection of marine and inland waters can be achieved through the development of regulations such as Metal Mining Liquid Effluent Regulations (MMLER) passed in 1977 under the Fisheries Act. The regulations apply to new, expanded, and reopened metal mines. Guidelines were published at the same time for existing mines, such as Cominco's Sullivan Mine. Guidelines contain the same numerical limits for deleterious substances as the regulations, but are not legally binding unless a Federal or Provincial government agency imposes these limits in a permit or license issued under other legislation. The release of pollutants in effluents from metal mines is often related, among other factors, to 1) the natural characteristics of the ore, and 2) uncontrollable water flows into the mine, waste rock dumps, or tailings ponds. Tailings from abandoned or closed mines may continue to release substantial amounts of deleterious substances after mining and milling operations have ceased. Therefore, limits in the MMLER and Guidelines are based on the concentrations of deleterious substances in the effluent, rather than on the production rate of the mine. In addition to chemical and physical monitoring of the effluent, biological monitoring tools will enable the evaluation of the effectiveness of the regulations. Biomonitoring organisms can also be useful tools for assessment of industry's efforts in meeting environmental regulations.

History of Sullivan Mine Waste Disposal

Cominco's Sullivan Mine in Kimberley, B.C. is approaching its planned shutdown in the year 2001. The mine produces lead, zinc and silver with iron byproducts, and has been in operation since 1909. Since then, 140 million tonnes of ore and 15 million tonnes of waste rock have been removed from underground. Tailings disposal began in 1923 following development of a differential flotation process capable of separating the complex ore into lead, zinc and iron concentrate. Since then 90 million

tonnes of tailings containing iron sulfides have been discharged to ponds occupying 373 hectares of land. For over 70 years mine surface activity was centered 1 Km north of the town of Kimberley in the Mark Creek valley, with the main portal being 25 meters above the valley bottom. Waste rock from the underground was hauled out on a narrow gauge rail system and cast down the slopes along the north and south sides of the valley. The estimated 4.1 million tonnes of waste rock that has been deposited in the Mark Creek valley contains sulfide minerals, resulting in acid rock drainage (ARD) which has seeped into Mark Creek via surface flow and groundwater. This ARD into the creek has now caused elevated zinc concentrations in the creek, particularly during spring melts. In addition to mining, ammonium phosphate fertilizer was produced from excess iron sulfides and imported phosphate rock. The fertilizer process produced waste consisting of iron oxides and gypsum, the latter of which was deposited along the North shore of St. Mary River. Iron staining offshore from this tailings area developed as a result of Mark Creek discharges prior to 1979 (personal communication).

Responding to environmental concerns, Cominco commissioned a drainage water treatment plant at the Kimberley Operation in 1979 to treat mine drainage and tailings effluent. Lime added to a high density effluent sludge neutralizes the acid and precipitates metals into a non-leaching sludge. The sludge is discharged to a pond situated on the South side of St. Mary River. In preparation for shut down at the mine, ARD abatement has continued with projects to improve Mark Creek (Thomson et al., 1997) and reclaim the tailings ponds through continued operation of the collection and treatment systems in combination with a soil cover system (Gardiner et al., 1997).

Past Biological & Toxicological Assessments St. Mary River

Historical biological monitoring in the St. Mary River indicated reduced species richness in the benthic community downstream from Mark Creek, but provided no clear impact further downstream at Wycliffe (Figure 2). It is difficult to distinguish between those effects attributed to inorganic inputs from Sullivan Mine and organic inputs from the municipal sewage treatment plant. Sites downstream from Mark Creek confluence

had an absence of pollution sensitive invertebrate species (BC Environment, 1976). A later study by BC Environment (1981) found that invertebrate species richness directly downstream from Mark Creek was lower; but that biomass was higher. A comprehensive study conducted by BC Research in 1977 evaluated a number of measures of biological integrity between the reference Pumphouse location and the downstream Wycliffe location. It was determined that 1) algal productivity and species abundance were greater at Wycliffe, 2) benthic invertebrate abundance, biomass and species richness were greater at Wycliffe, and 3) benthic community composition shifted from predominantly scrapers at the Pumphouse to filter feeders and detritivoirs downstream. In agreement with BC Research, 1977, the Aquatic Effects Monitoring Field Evaluation commissioned by Natural Resources Canada (EVS, 1996), found invertebrate overall abundance was greater in the exposure zone, while species richness was unchanged. Benthic community structure may be reflecting increased primary production from nutrient loading, which would support a greater invertebrate abundance. The lack of apparent toxicity, despite increased zinc, iron and manganese, was hypothesized to be related to competition for binding sites from cations, primarily Ca^{2+} and Mg^{2+}, released from the ARD treatment plant (Appendix A: Table 12). However, these previous studies did not measure metal content of the sessile, benthic biological components of the river.

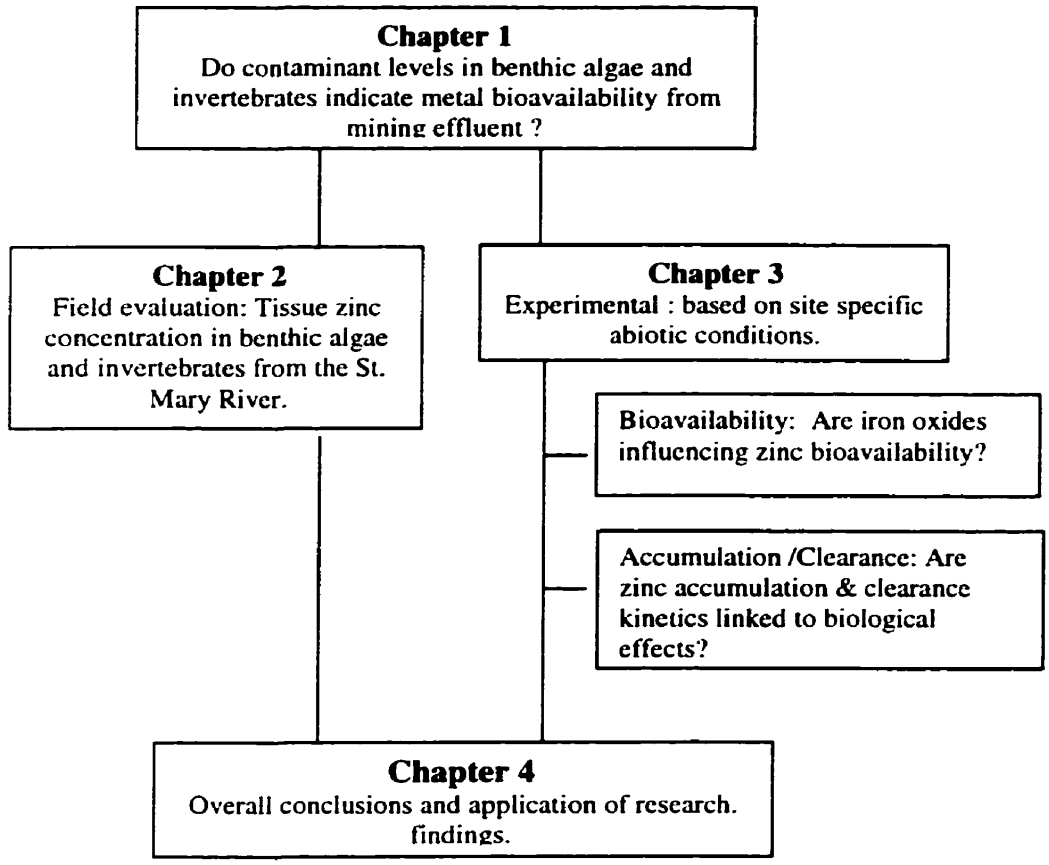
Benthic algae make excellent biomonitors because they are autotrophic and occupy a pivotal position in aquatic ecosystems at the interface of the chemical-physical and biotic components of the food web. Algae are primary energy sources for invertebrates in many mid-sized streams (Vannote et al., 1980), and they act as sinks for nutrients, including zinc (Stevenson, 1996). Because algae divide quickly they respond rapidly to shifts in environmental conditions and often are the first organisms to respond to environmental stress (Lowe and Pan, 1996).

Invertebrates make excellent metal indicators and should be sampled along with periphytic algae. Some are relatively tolerant to deleterious substances and abundant enough over reasonable distances to evaluate point source contamination. Benthic invertebrates represent secondary production in most rivers and streams (Allen, 1995).

This secondary productivity is important as the primary food supply for higher trophic predators such as several fish taxa (e.g. sculpins and salmonids) and certain birds such as American dippers (*Cinclus mexicanus*). As such, high levels of tissue contaminants accumulated by the invertebrates represent a significant route of exposure to the top predators living along and within any contaminated river or stream.

This study was undertaken to contribute to the existing studies with biological tissue contaminant concentrations. The primary purpose of the study was to address whether zinc released with complex mining effluent is bioavailable and, subsequently accumulated in periphytic benthic algae and macroinvertebrates within the St. Mary River. Laboratory microcosm stream studies complemented field results with site-specific questions related to: 1) the role of iron oxides in zinc bioavailablity; and 2) zinc accumulation, clearance, transfer and toxicity within a model stream ecosystem. An outline of the thesis is presented in Figure 1. Chapter 1: introduction and rationale; Chapter 2: field evaluations from Sullivan Mine; Chapter 3: experimental apparatus and designs for both bioavailability and accumulation/clearance bioassays; and finally Chapter 4: overall synthesis, conclusions and applications.

Figure 1: Schematic flow diagram of overall thesis project.



2 Chapter 2: Sullivan Field Evaluation

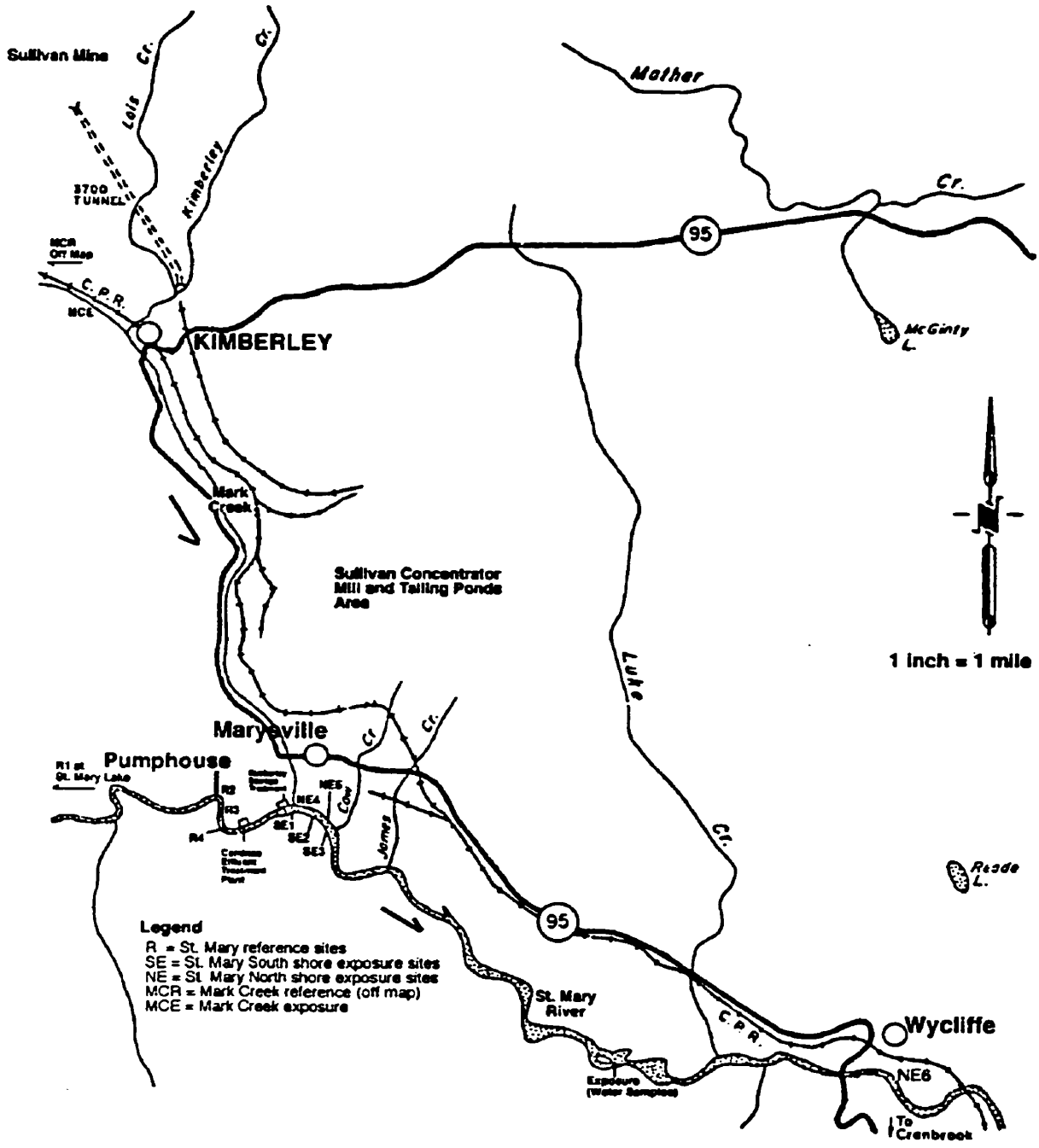
The St. Mary River, which receives treated effluent from Cominco's Sullivan Mine, is an erosional river with substrate consisting primarily of boulders and cobbles with few depositional areas. This important freshwater system drains into the Kootenay River, which in turn flows South across the United States border. Past biological studies (BC Environment, 1981; EVS, 1996) on the St. Mary River appeared to support the conclusion of a BC Research (1977) report that benthic community structure is reflecting changes in primary production. These changes have been attributed to the nutrient loading from both mining effluent and the sewage plant releases. Despite elevated concentrations of zinc, iron and manganese in the exposure area, no apparent adverse effects were observed on the benthic community. It was hypothesized (EVS, 1996) that the bioavailability of the metals may be reduced, in part, as a result of the cations released from the ARD treatment plant. If metals are bound with excess cations they should not accumulate in biological tissues. However, previous studies did not measure contaminant residue levels in the sessile, benthic biological components of the river. Therefore, one objective of this study was to determine if zinc, the primary contaminant from the mining, has accumulated in benthic periphyton and invertebrate tissue within the St. Mary River. Although tissue metal levels do not directly address the question of ecological impacts, they are correlate consistently to both ambient concentrations and decreases in a variety of ecological indices (Poulton et al., 1995, Hickey and Clements,1998). Additionally, by measuring zinc concentrations of sessile benthic organisms, the immediate spatial extent of the contamination can be delineated.

2.1 Methods

Study site: St. Mary River

The St. Mary River drains an area 2.360 km^2 before flowing into the Kootenay River, which flows South across the United States border. Historical mean annual flows at Wycliffe (1940's – 1990) were > 50 m^3 sec^{-1}, with peak flows in excess of 200 m^3 sec^{-1} occurring in June (Environment Canada, 1990). The St. Mary is an erosional system

Figure 2: Site map: Sullivan Mine and Concentrator in relation to Mark Creek and St. Mary River sample sites. Flow directions have been indicated. Wycliffe downstream exposure location (NE6).



Map adapted from BC Research , 1977

consisting primarily of riffle/run habitat with no significant depositional areas. Little riparian vegetation or in-stream woody debris was observed (personal observation, Aug. 97). Substrate is large, consisting primarily of boulder (>460 mm) and rock (180 – 460 mm). On average the depth in the reference zone is greater (>75 cm) than that in the exposure zone (>50 – 35 cm).

Sample locations

Field work took place during the last week in August 1997. Four reference and 6 exposure sites were selected for study along the St. Mary River (Figure 2). With the exception of South shore exposure, an attempt was made to sample as close to historical sites for comparison purposes (EVS, 1996 and BC Environment, 1976/81). One reference location was ~ 30 Km upstream at St. Mary Lake. The remaining 3 reference sites were along the North shore between the Pumphouse and the ARD treatment plant. The exposure zone was considered to begin at the confluence of St. Mary River with Mark Creek. It consisted of 3 sites along the North shore (NE4, NE5 and NE6) and, 3 along the South shore (SE1, SE2 and SE3). Habitat in the North shore reference sites consisted of deeper waters with a large cobble substrate. Sampling was undertaken one or two meters from shore. The North shore exposure had a similar habitat to that of the reference zone. On the South shore, shallower riffle habitats were encountered and it was possible to sample at least three to six meters out into the middle of the river. Mark Creek was not as intensely sampled as there were no invertebrates present in the exposure zone. As a result only two samples were taken, one each from a reference and an exposure zone (MCR, MCE).

Algae

Based on availability, chlorophyta (green algae), bryophyte (moss) and diatomaceous material (diatoms) were scraped off boulder and cobble substrate from within the $2m^2$ area. Material was rinsed with deionized water into a plastic beaker, transferred to acid washed 50 mL polypropylene sample bottles, and placed on ice. Samples were frozen until processed in the laboratory (Clements and Kiffney, 1994).

Collected material was thawed, filtered onto Whatman 42, (2.5 µm retention) ashless cellulose filters and dried at 60 ° C to a constant weight. After dried material had cooled to room temperature, weight was determined to the nearest 0.1 mg (Mettler AE 240). CEM microwave (MDS 2000) digestion (95% for 8 minutes followed by 60% for 6 minutes) took place in 13 mL of 8.0 M Bakers analysis lot ultra pure nitric acid (Bakers nitric acid). The cooled digests were transferred to acid washed plastic graduated centrifuge tubes and brought to exactly 13 mL in 8.0 M Bakers nitric acid. After centrifugation at 4000 rpm for 5 minutes, 10 mL of supernatant was extracted into 15 mL acid washed glass vials and diluted to 15 mL with double de-ionized water for a sample molarity of ~ 5.33. Samples were then aspirated directly into the atomic absorption spectrophotometer (AAS, Perkin Elmer 1100B with impact bead). Standards were prepared for both algae and invertebrate samples by diluting 16M Bakers nitric acid with double de-ionized water to match sample molarity. Quality control and assurance were ensured through the inclusion of process blanks, filter blanks and standard reference material. Reference materials consisted of National Institute Standards and Testing (NIST) oyster tissue (Appendix B: Tables 13 and 14).

The algae from the 1997 collection were preserved in Lugol's iodine solution, which inadvertently evaporated. The semi-permanent mounting technique presented in Lowe and LaLiberte (1996) using a clear glutaraldehyde fixative was employed for algae collected in August of 1998. Material was more plentiful and similar in appearance to that collected in 1997 (personal observation).

Invertebrates

Invertebrates were collected within riffle zones whenever possible. Boulders and cobbles in a 2 m^2 area were brushed off and disturbed invertebrates recovered by drift net (0.175 m^2 @ 250 µm mesh). The sample was placed into a large bin and the coarse debris removed. Both coarse and fine fractions were rinsed through sieves, 1.7 mm and 250 µm, respectively, and retained animals transferred with river water to zip lock bags. In the field, samples were stored on ice during which time gut contents would depurate

(Crawford and Luoma, 1994; Clements and Kiffney, 1994). Later, samples were frozen until processing back in the laboratory. Invertebrates were counted and keyed out to family and genus, where possible (Needham et al., 1969; Jewett, 1959; Frison, 1942; Clifford, 1991 and Merrit and Cummins, 1978). Length was measured with an occular micrometer calibrated to 6x power. Sorted, sized and enumerated invertebrates were dried for 24 hours @ 70° C and, after cooling to room temperature, dry weight was determined to the nearest 0.1 mg (Mettler AE 240). Biomass was transferred into acid washed plastic 15 mL graduated centrifuge tubes, unless the biomass was under 4 mg, then the material was transferred into a micro (1.5 mL) centrifuge tube. Digestion with either 3 mL (biomass > 10 mg), 2 mL (biomass between 10 - 4 mg) or 0.5 mL (biomass < 4 mg) 16M Bakers nitric acid took place within the centrifuge tubes in a conventional microwave oven. With their caps loosened, approximately 20 samples were subjected to medium power (75° C) for 1 minute, rotated and agitated, then heated at medium power for another minute. The digests were diluted up to 12, 6 and 1.5 mL respectively with double deionized water. After settling, the samples were aspirated directly from the centrifuge tubes into the Perkin Elmer AAS. Quality control and assurance were ensured through the inclusion of process blanks and standard reference material (Appendix B: Tables 13 and 14).

Data Analysis

All tissue zinc data were $\log_e$ transformed to stabilize the variance and create normal distributions for parametric analysis. Algae data were evaluated without taxonomic categories, with a two way ANOVA classified by site and geographical treatment zone. The geographical zones were upstream reference, North and South shore exposures, respectively. Invertebrate tissue zinc data were sorted into genus and pooled into geographic treatment zones, then analyzed with a two way ANOVA (covariance from algae zinc). $\log_e$ biomass and number of species were geographically pooled and analyzed with a two and one way ANOVA, respectively (covariance from depth). Due to unequal sample sizes, the Bonferroni multiple range comparison was employed to determine significance among the pooled means.

2.2 Results

St. Mary River Water Quality

Historical water quality data for St. Mary River and Mark Creek were obtained from the on site environmental engineer at Sullivan Mine. There are several sample locations along Mark Creek tracking the quality of the water as it passes through the upper and lower mine yards, and on through the towns of Kimberley and Marysville. St. Mary River has one reference location at the Pumphouse, one exposure location and one downstream site at Wycliffe (Figure 2). The Sullivan mine is primarily a zinc / lead mine with iron by products. The major metals showing increased concentrations in the exposure zone are iron, manganese and zinc. Calcium tends to increase downstream from the effluent release, which may be attributed to the lime used in the water treatment plant. Starting in 1995, seasonal sampling revealed that dissolved metal concentrations tended to be higher in the spring vs. summer or fall. Gradual reductions in the concentrations of all measured metals were evident over the history of record keeping in the St. Mary River from 1970 – 1998 (BC Research 1976, Cominco records).

Dissolved Zinc Gradient

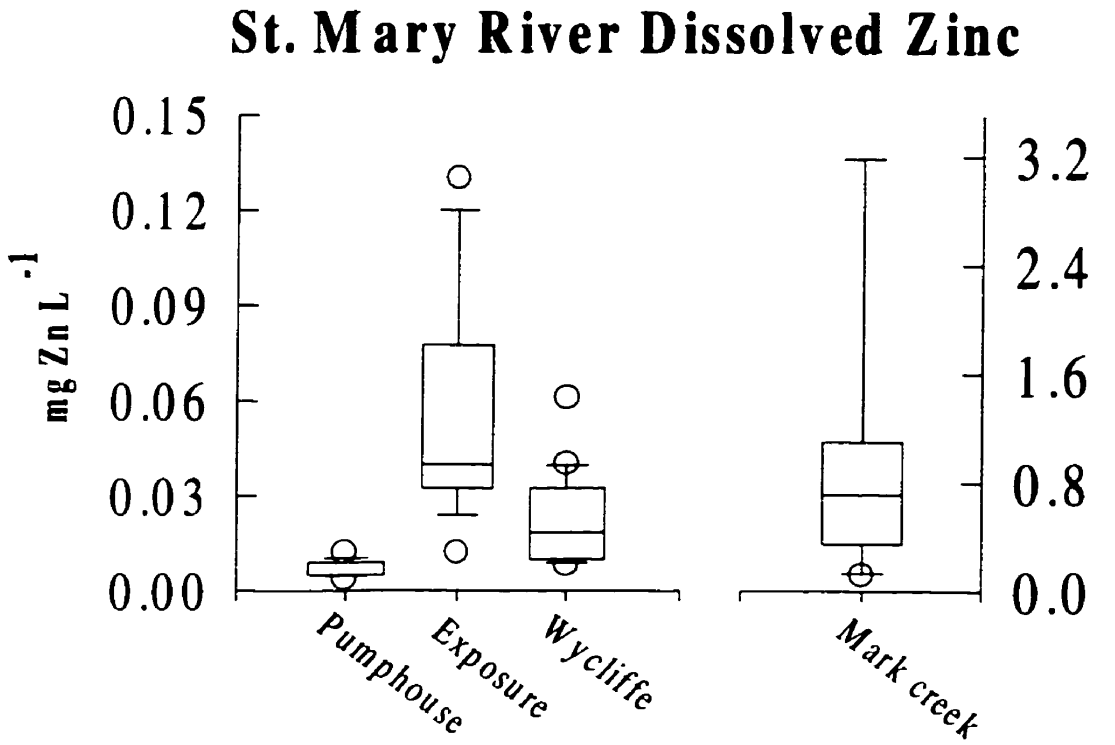
The distribution of dissolved zinc at all St. Mary sites and at one Mark Creek site directly upstream from the confluence were evaluated, using histograms, and were determined to be log normal distributions. Box plots illustrate the dispersion and skewness in the data (Figure 3). When probability estimates are required, such as with risk assessments, probability density curves can be generated as follows. Mean and standard deviation of $\log_e$ transformed data were calculated for each sample site and probability density curves (Figure 4) were created from the function given in Morgan and Henrion (1990) for log normal distributions:

$$F(x) = 1/\sigma(2\prod)^{0.5} \exp((- \ln x - \ln \mu)^2/2*\sigma^2$$

Ln μ = mean from the $\log_e$ data.
σ = standard deviation from the $\log_e$ data.

The width of the distribution represents the magnitude of the standard deviation, and the peak or mode of the distribution represents the most frequently measured values. Actual values recorded in October 1997 fall very close to the modal levels in each of the distributions. Mark Creek was 0.44 mg Zn L^{-1}, while St. Mary River sites were <0.005 mg Zn L^{-1}, 0.026 mg Zn $L^{-1}$and 0.021 mg Zn L^{-1} for reference, exposure and downstream locations, respectively.

Figure 3: Box plots for St. Mary River historical dissolved zinc between 1992 1997. Box divided at the median (50^{th}) encloses the upper and lower quartiles ($25 - 75^{th}$). Capped bars show the $10 - 90^{th}$ percentiles and all points outside these are shown as circles. Right scale is also mg L^{-1} and relates to the Mark Creek plot.



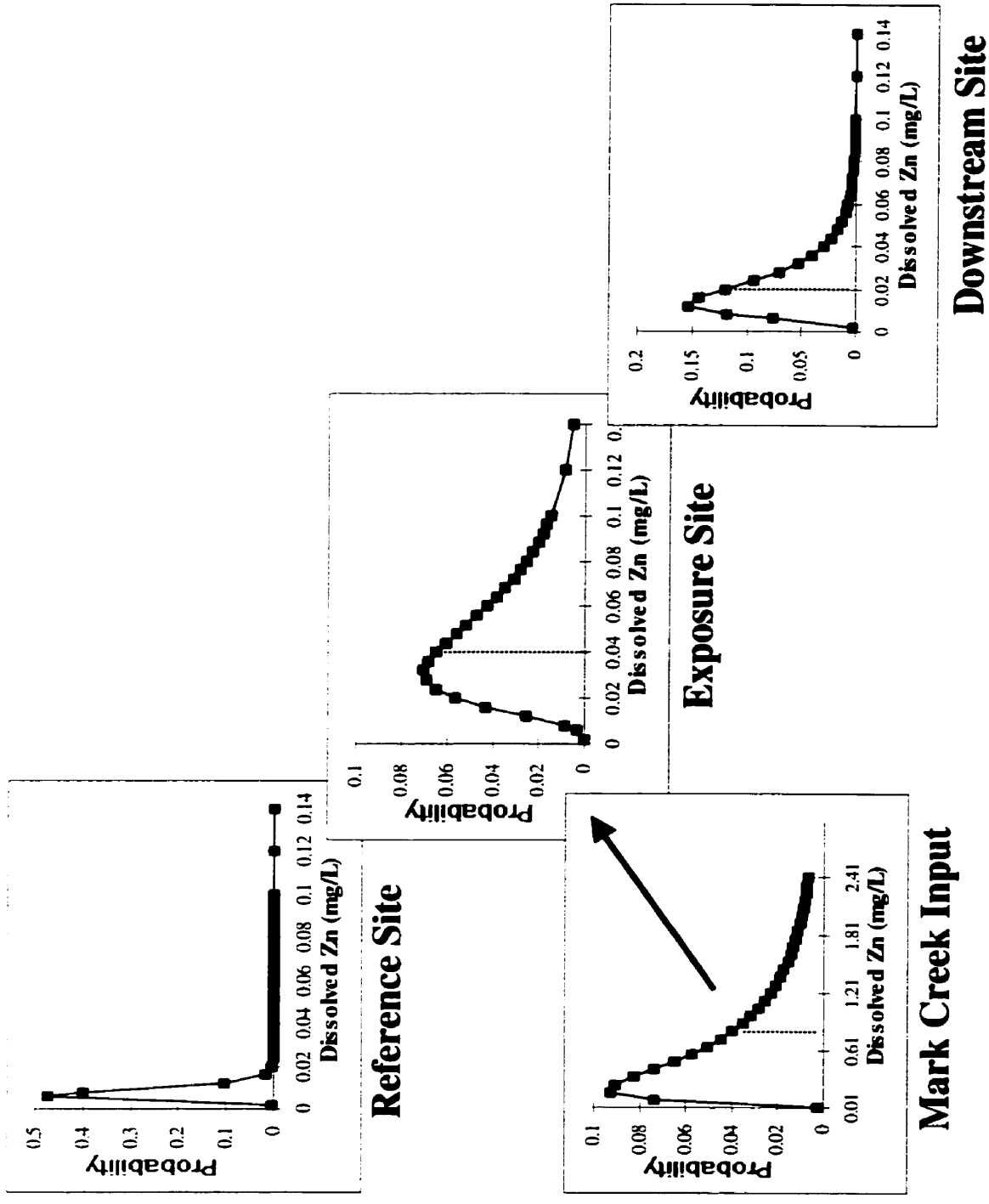
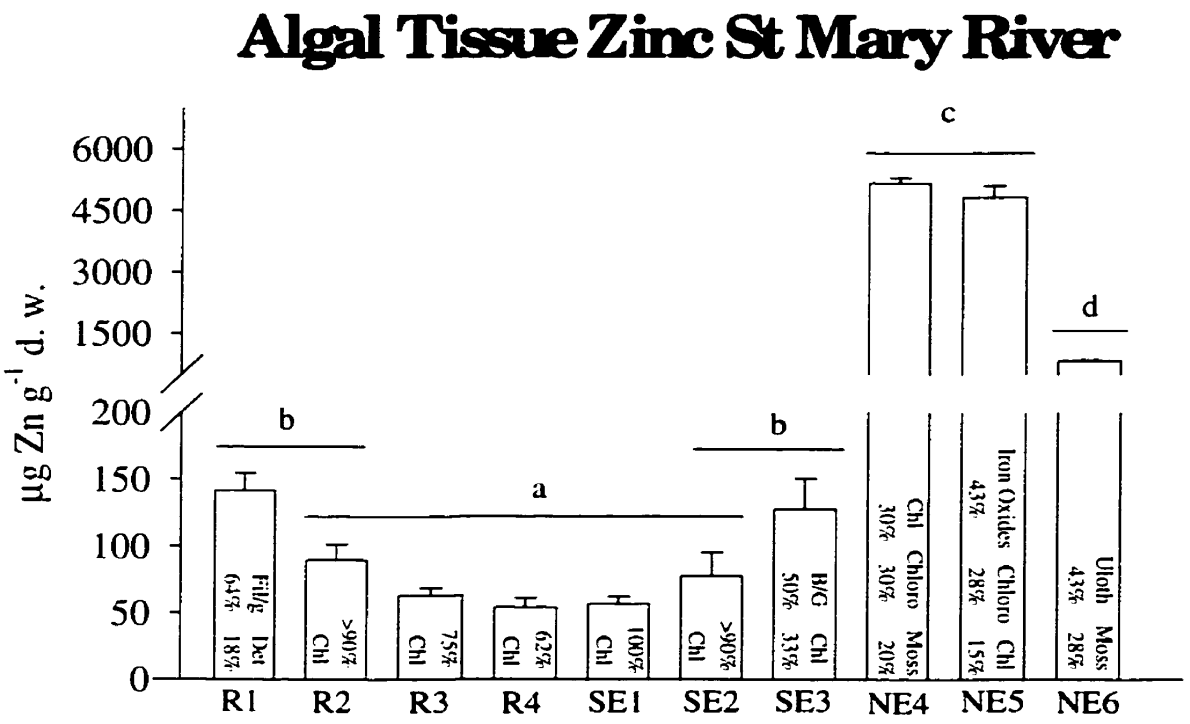


Figure 4: Log Normal Distributions representing historical dissolved zinc at St. Mary River and Mark Creek confluence. Y axis represents relative probability. Median (50^{th}) indicated by a dashed line and the mode, most frequently measured values at the peak of the curves. Skewness and width reflect the variance.

Periphyton collections and their zinc concentrations

The most dominant algal taxon collected and average tissue zinc concentrations (n = 3) are summarized in Figure 5 with details in Appendix D: Table 17. Mean periphyton zinc concentrations for each site helped pin-point the major source of the contamination on the North shore along Cominco's lands ($F = 250.85$, $\rho = 0.0001$, ANOVA). When these data are pooled into the geographical locations there are no significant differences between the reference and South shore exposure. Only the North shore exposure locations show significantly elevated concentrations of zinc. The Wycliffe location (NE6) is included in the North shore exposure treatment zone, although zinc levels are significantly reduced relative to those immediately downstream from Mark Creek (NE4 and NE5). The sampling technique under represents the Bacillariophyceae (diatoms), but for the purposes of using the periphyton as a bioindicator, diversity in the sample is not required. *Cladophora* has commonly been used as a bioindicator species in freshwater streams (Whitton, 1984 and Oertel, 1991) forming conspicuous green filamentous tufts on the surfaces of the cobble substrate. *Microspora*, another filamentous green, often occurs in tufts of *Cladophora* (Prescott, 1978). There was relatively consistent periphyton in the reference and South shore exposure. The North shore exposure locations were influenced by sewage treatment release, reduced light exposure and the iron staining along the old gypsum ponds. Periphyton collected included colonial green algae, increased bryophytes (moss) and abundant iron oxide material.

Figure 5: Periphyton composition and tissue zinc means with SE bars (n = 3). R denotes reference locations while SE and NE represent South and North shore exposure, respectively (See Figure 2). Statistical groupings (α = 0.05) are based upon Bonferroni multiple range test. Dominant periphyton composition within the bars as follows: Chl – *Cladophora* with *Microspora* , B/G – blue green filamentous, Fil/g - unknown green filamentous, Det – inorganic detritus, Chloro - chlorococcales, Uloth – *Ulothrix* (filamentous green). Diatoms, which are under represented in these samples, comprise the remaining percentage composition for all sites.



Invertebrate species numbers and biomass

Past studies of invertebrates in the St. Mary River (EVS, 1996 and BC Research, 1977) found both biomass and number of species increase in the exposure zone. Although the current study did not focus on detailed enumeration of all possible invertebrates, the drift netting of the macro invertebrates produced similar increased numbers of species downstream on both sides of the river (ANOVA: $F= 68.99$, $\rho = 0.0001$). The addition of the covariate of depth did not improve the overall significance of the model. Log_e transformed biomass was marginally significant, with least means indicating slightly reduced biomass from the South shore exposure zone, probably due to increased ephemeropterans (mayflies) (ANOVA: $F = 2.92$, $\rho = 0.0389$). Interestingly, both the sequential and partial sum of squares indicated that the covariate, depth, contributed more to the model variance than geographical treatment zone. A plot of depth vs. estimated biomass revealed an inverse relationship, which is consistent with preferential colonization of shallow riffle sections. The taxonomic data with the numbers of individuals has been summarized in Appendix D: Table 15.

Invertebrate tissue zinc

Routes of metal transfer can be explained by considering biological fundamentals such as physiology and feeding strategies. A portion of the fine filterable particulate organic debris within this medium order river is likely derived from autochthonous sources, algae (Lamberti, 1996 and Vannote et. al., 1980). As a consequence only the filter feeding invertebrates, Tricopterans, show significant variance associated with the addition of the co-factor, algal zinc to the general linear model (glm) (Table 1). Perlidae, being predators, would be expected to receive zinc from prey, such as simulide and chironomid larvae. Algal scrappers/collectors, Baedidae and Plecopterans (Chloroperlidae + Taeniopterygidae) showed zinc concentrations that follow algal trends; however, the addition of the co-factor, algal tissue zinc does not improve the significance of the glm. This suggests that other routes of exposure are likely, such as in dissolved form across respiratory gill structures. Smock (1983) found that feeding habit influenced

whole body metal concentrations in aquatic insects. Animals ingesting and living within the sediments had the highest concentrations, followed successively by the filter feeders, detritivoirs/scrapers, and lastly, surface feeding beetles and predators.

Table 1: St. Mary River Correlation: Algal tissue Zn with Invertebrate tissue Zn.

Taxon	PPCC	ρ value	Sample size
O: Plec. F: Chloro + Taenio	0.708	ρ =.986	9
O: Plec. F: Perlidae	0.529	ρ =.824	11
O: Tric. F: Hydropsychidae	0.808	ρ =.042	24
O: Tric. F: Brachycentriidae	0.816	ρ =.013	9
O: Ephe. F: Baetidae	0.813	ρ =.967	12

Note: ρ values indicate if the addition of the co-factor, algal zinc , increased the overall significance of the two way ANOVA (invertebrate tissue zinc = geographical zone * co-factor). PPCC – Pearson product correlation coefficient. Sample size is related the correlation (PPCC). O denotes Order with Plec = Plecopteran (Stoneflies); Tric = Tricopteran (Caddisflies); Ephe = Ephemeropteran (Mayflies). F denotes Family with Chloro = Chloroperlidae and Taenio = Taeniopterygidae.

Baetidae mayflies accumulated the highest tissue zinc (400 - 4000 $\mu g\ g^{-1}$). Tissue zinc concentrations in the Baedidae are increased, relative to reference, ~ 7.5 times directly downstream of Mark Creek. The filter feeding caddisflies, which represent the bulk of the secondary productivity within this river, accumulate much less; Hydropsychidae recorded levels of 250 - 980 $\mu g\ g^{-1}$ (smaller individuals achieve higher zinc concentrations) while the Brachycentriidae ranged between 460 - 1300 $\mu g\ g^{-1}$. Downstream tissue zinc in these Tricopterans is elevated ~ 2.5 times over background. The Perlidae stonefly represented the dominant invertebrate predator with zinc concentrations of 300 - 1000 $\mu g\ g^{-1}$ These zinc levels are high; but fall within the range found by others (Table 2). Appendix D: Table 16 lists the details.

Geographical trends show increased invertebrate whole body zinc concentrations along the South shore. Peak levels are attained on the North shore where Mark Creek meets the St. Mary River (Figure 6). Only the Hydropsychidae show significant differences among all three geographic areas, perhaps as a result of increased statistical power related to increased sample sizes. Baetidae and Perlidae show significant increases in whole body zinc in the North shore over those measured from both the South shore exposure and reference zones. In the case of Plecopterans and Brachycentriidae overlaps occurred among geographical zones; however, the whole body zinc measured in the North shore was still significantly elevated compared to those from the reference area.

Order: Ephemeropteran, Family: Heptagenniidae (illustrated), and two Trichopteran species, lacked sufficient sample sizes for statistical comparisons but still showed increased zinc downstream from Mark Creek confluence.

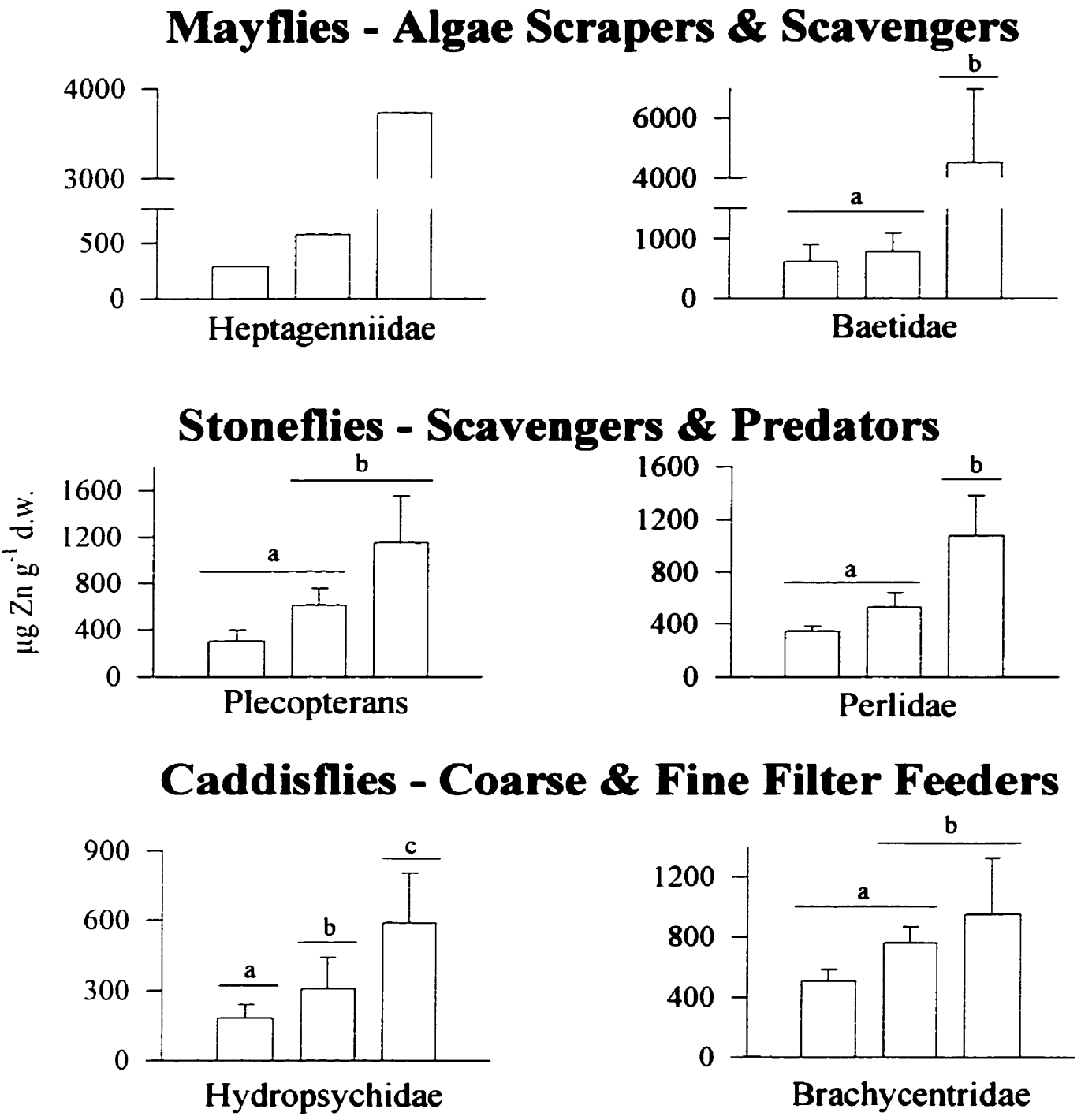


Figure 6: St. Mary River invertebrate (Family) tissue zinc. Pooled groups from left to right represent upstream reference, South and North shore exposure respectively (means with SE bars, *n* ranged from 3 to 10). Significantly different groups a, b and c indicated with bars, are based on Bonferroni multiple range test (α =0.05). Heptagenniidae lacked sufficient sample size for statistical comparison.

2.3 Discussion

Aquatic Plants as Ecological Indicators of Metal Contamination

Whitton (1984) outlines several potential advantages of using aquatic plants as indicators of metal contamination:

1) Plant monitors give an integrated picture of pollution within a system.

2) High levels of metals are accumulated, thus measurement of the levels in plants increases the sensitivity of detection.

3) Metals accumulated by plants gives a better indication of the fraction of metal in the environment which is likely to affect the aquatic ecosystem.

4) Dried plant material is easier to store over long periods and transport than water samples.

5) Information obtained may prove useful in the development of practical systems for the removal of heavy metals from mining and industrial effluents by encouraging the growth of plants.

Field sampling of periphyton indicated that point source zinc contamination was restricted to the North shore exposure, particularly downstream from Mark Creek confluence. Further downstream along the North shore at Wycliffe, the accumulated zinc in the benthic periphyton was reduced compared to sites immediately offshore from Cominco lands, but still elevated compared to reference sites. St. Mary periphyton sampling was problematic in certain locations. Sufficient growths were not always available, especially from riffle sections along the South shore exposure. When material was absent from the riffles, tufts from the slower flowing sides of the river were collected. These samples were likely outside the contamination plume from the effluent release and were certainly not affected by Mark Creek. Different aquatic plant material, such as bryophytes and chlorophytes would be expected to have very different background elemental concentrations (Emplain, 1976a and b; Whitton, 1984). Site to site comparisons would be more robust if a similar macroscopic form was encountered throughout the study region, for example *Cladophora*. Inorganic debris associated with

the collected periphyton can be a significant source of zinc. Sites carrying high suspended loads such as the outflow from St. Mary Lake (R1) and downstream at Wycliffe (NE6) had considerable inorganic debris. Iron oxides along the edge of the old gypsum ponds may be associated with bound zinc, which would confound measured zinc in the periphyton sample collected from NE5.

The use of periphyton as biomonitors of metal contamination has not become widely accepted. A number of difficulties arise when attempting to use either single freshwater alga species or whole periphyton mats. This study included, most studies must be opportunistic in their sampling; if aquatic plants are to be used routinely it will be necessary to include both algae and bryophytes that can be collected over at least three seasons. The other major problem is the lack of standardization in sample collection and washing techniques; macroscopic forms such as *Cladophora* and *Lemanea* are often associated with inorganic debris and epiphytes. One solution involves sampling the entire periphyton mat, which consists of all the microscopic algae, bacteria, and fungi on, or associated with the substrata. Newman (1989) cautions against using aufwuchs (periphyton with inorganic debris) as indicators of bioavailable metals because of abiotic contributions, particularly from hydroxides of iron and manganese which bind and co-precipitate metals. A better solution and recently favored technique involves the use of artificial substrates; however, these methods often encourage unnatural assemblages dominated by bacillariophyta, require more time in the field, and can be subject to vandalism. Aloi (1990) concluded a critical review of freshwater periphyton field methods stating that there will probably never be a narrow set of standard methods that are accepted by all researchers, and which are appropriate for all investigations. Experimental methods will continue to be constrained by time available, laboratory equipment and funding.

Insects as Alternative Indicators of Metal Contamination

Nehring (1976) noted that aquatic insects would make excellent monitors of heavy metal contamination and associated ecological impacts. Aquatic insects fulfilled his prerequisites for heavy metal biological monitors because;

1) insects are more tolerant than fish;

2) insects concentrate heavy metals in relative proportion to the metal content in the water and;

3) insects concentrate metal pollutants by a predictable factor over short periods of time.

Invertebrates (insect larvae) provided a more accurate picture of the bioavailability of zinc in the St. Mary River, relative to the periphyton. Both Hydropsychidae and Baetidae were abundant and appeared to be tolerant (Leland et al., 1989); consequently they are frequently used as indicators of ambient metal concentrations (Table 2). Fine particulate filter feeding caddisflies, Brachycentriidae are excellent candidates for indicator species as they are abundant and well correlated with algal zinc. Other invertebrates with increased mobility and diverse diets, such as predator stoneflies and collector mayflies and stoneflies, may not provide spatial consistency for monitoring metal contamination.

Both the United States Geological Survey and Environment Canada have endorsed the idea of using aquatic insects as indicators of heavy metal pollution, especially in cobble bottom rivers where sediment is rare (Crawford and Luoma, 1994; Cain et al., 1992; Carter and Fend, 1992). Recently the focus has shifted to correlating metal content in the insects with impacts on the benthic community structure as measured by the variety of matrices (Hickey and Clements, 1998; Poulton et al., 1995).

An overview of documented field studies in Table 2 suggests higher tissue metal concentrations are associated with historical mining activity in contrast to non-point source contamination. When compared to the other rivers world wide, the St. Mary River

would be considered as being at the high end of the scale for zinc contamination; however, contamination is not as severe as the superfund site in Arkansas River, Colorado. Composition of the benthic periphyton was rarely identified, and the evidence indicates zinc, a micro nutrient, may be regulated in algae and invertebrates. Elemental concentrations related to contamination will likely show some seasonal variation. Seasonal fluctuations can be attributed to a combination of dilution as the algal mats or invertebrates grow and reduced ambient concentrations after spring melt.

Table 2: Studies evaluating periphyton and invertebrate metal concentration in natural systems.

River or Stream	Location	Water Zinc µg/L filtered or * total	Sediment Algal or Periphyton Zinc µg/g d.w.	Invertebrate Zinc µg/g d.w.	Comments & Reference
Hayle & Gannel Rivers	Mount's Bay & Newlyn Downs (UK)	500–2,500 *	100 – 2,000 *Microspora, Spirogyra, Mougeotia, Zygogonium*		Foster (1982). Historic Cu, Sn & Pb mining regions. Cu, Pb & Fe concentration linear with ambient levels, but not Zn, suggesting regulation. Higher enrichment with lower ambient Zn.
Danube River	Hungarian reach north of Budapest	128- 4,000 Mean 507 ± 815	110 – 580 Strictly *cladophora*		Oertel (1991). Industrial pollution. Biomass inversely related to heavy metal content (dilution). Low standing crop between Jan – March.
Oker, Ecker, Radau, Grane, Laute & Tolle	Harz Mountains West Germany		840 – 8,000 Sediment	*Baetis rhodani* 1,300–9,400	Rehfeldt & Sochtig (1991). Industrial discharges. Species appear tolerant of heavy metals. Uptake across gills and via ingestion of contaminated diatoms.
Bayou d ' Inde	SW Louisiana USA		120 – 250 Periphyton		Ramelow et. al. (1992). Point source from industrial drainage ditch. Peaks in Pb , Cu & Zn accurately define the spatial extent.
Clark Fork River	Montana USA		54 – 1,073 Sediment	*Hydropsyche* 112 – 270 *Arctopsyche* 136 - 191	Cain & Luoma et. al. (1992). Historical mining in headwaters. Zinc in tissue less responsive than Cu & Cd. *Hydropsyche* best overall indicator.
Arkansas River	Colorado USA	138-1037 Seasonal	4,000 – 16,500 Periphyton	*Arctopsyche* – 1,000 *Baetis* 1,000 –7,500	Clements & Kiffney (1994). Leadville Mine and California Gulch (Superfund site). Spring samples higher.
Wah Diengling & Umkhrah streams	Shillong city, Meghalaya State India	4.3 - 37.6 *	38.3 - 109.2 Periphyton	*Hydropsyche* 114.1 - 204.0 *Baetis-* 201.8 - 639.4	Gupta (1996). Diffuse non-point source contamination. *Baetis* accumulates the highest, metals, especially Zn (distributes throughout all tissues).
Waiomu, Tararu & Tunakah-oia	Coromandel Peninsula New Zealand	<5 – 8,170	100 – 10,000 Periphyton		Hickey & Clements (1998). Historical gold exploration region. Concentration in biota and toxicity agree with community structure impacts.
St. Mary River	Kimberley BC Canada	<5 - 400	50 - 5,000 *Cladophora, microspora,* bryophytes	*Arctopsyche* 200 - 950 *Baetis-* 350 – 3,900.	Current study. Historical mining discharges. Peaks in both algae and invertebrate tissue Zn define spatial extent of contamination. Community impacts not found.

2.4 Conclusion

Despite the fact that invertebrates display individual variability in tissue zinc and are able to regulate this micro nutrient, they provided the best biological indicator of metal contamination in the St. Mary River. Hydropsychides, the larger and more abundant caddisflies, have been used by a variety of researchers and are considered the best indicators in this specific river system. These invertebrates reside in constructed dwellings primarily in high current riffle zones and feed by collecting organic debris in nets built between cobbles. They are also the primary invertebrates that benthic feeding fish, such as sculpins, prey upon (Allen, 1981). Brachycentriidae, the smaller caddisflies live in wooden cases and attach themselves to the backside of the cobbles in high current riffle/run sections. Both are relatively sessile organisms that integrate the overall contamination within the river.

Periphyton tissue metal levels are much more problematic when site to site comparisons are required. Consistent forms were not widely distributed enough to be comparable in this river system. Additionally, abiotic debris affects overall metal concentrations. As a result, natural periphyton metal concentrations may not always be reliable indicators of bioavailable metals. However, bacillariophyceae cultured on artificial substrates could provide both contaminant residue levels and insights into the effects of elevated metals on the population level (Lowe and Pan, 1996).

The pattern of contamination in St. Mary River can be interpreted by considering that samples collected from the South shore are likely within the mixing zone of natural waters and the effluent released from the ARD treatment plant, while those collected from the North shore are under the influence of effluent and sewage release, waters from Mark Creek confluence and the old gypsum ponds. Interpreting the results in this manner indicates that bioavailable metal levels from the effluent treatment plant are secondary in severity to those draining from Mark Creek. Alternatively, the contamination plume from the treatment plant may hug the North shore throughout this stretch of the river, resulting in no detectable increase in periphyton and invertebrate

tissue zinc along the South shore. In the past the perforated release pipe stretched across the entire river, diluting the effluent evenly; however, the pipe has been broken off and the majority of effluent is released into the middle of the river.

Future periphyton and invertebrate sampling should be conducted bi-annually. Artificial substrates could be used for culturing more uniform periphyton (diatoms) in the reference and exposures zones. Taxonomic analysis of such cultures would provide a rich data base for toxicological evaluations. The exposure zone should extend down to St. Eugene Mission, 27 Km downstream from Mark Creek confluence. Reference samples between St. Mary Lake and the Pumphouse would help characterize upstream ecology and background tissue contaminant levels. Additionally, the drifting particulate organic debris (seston) should be sampled for both composition and metal concentrations.

These findings suggested that zinc released from the effluent plant was significantly less bioavailable, as indicated by reduced periphyton and invertebrate tissue zinc concentration from South shore exposure sites. In contrast, the high tissue zinc concentration from the North shore exposure sites suggest that zinc was biologically available and, consequently accumulated to high levels. To further understand this geographical pattern of accumulation two separate hypothesis were developed and tested in laboratory microcosm studies conducted in the Fall of 1997 and Spring of 1998. The first experiment evaluated whether iron oxides, which are generated in the treatment plant, are able to reduce the biological availability of the associated zinc in the effluent. The second experiment was designed to test whether zinc accumulation, clearance and food chain transfer are linked to toxicological effects within a model stream ecosystem. Treatments from both experiments were derived from site specific abiotic characteristics.

3 Chapter 3: Experimental

Mining effluent is a complex mixture that can contain high levels of both dissolved and particulate iron (oxides). Sullivan's effluent treatment plant promotes the flocculation and precipitation of these oxides with the addition of calcium carbonate and air. Metal bioavailability from Sullivan mine treatment plant may be influenced by high iron and calcium released from the plant. Increases in alkalinity cations, Ca^{2+}, Mg^{2+}, will decrease metal uptake and toxicity in marine algae (Pellegrine et al., 1993; Braek et al., 1980). Another abiotic factor suggested to be affecting the metal bioavailabity at Sullivan is nutrient loading from the Kimberley sewage treatment plant (EVS, 1996). Increased nutrients have the opposite effects on uptake, by either stimulating growth or increasing polyphosphate bodies, which tend to absorb metals (Subramanian et al., 1994; Walsh and Hunter, 1992). Casini and Depledge, 1997 found that iron had no effect on the accumulation of zinc in the marine amphipod *Platorchestia platensis*. However, that study used artificially high concentrations of metals and did not allow for oxidation of the iron. Therefore, stream microcosm experiments were designed to address the role of iron and its influence on both the uptake and toxicity of zinc, the most conspicuous contaminant at Sullivan mine.

The first experiment conducted in the Fall of 1997, was designed to simulate the formation of iron oxides at the treatment plant by bubbling air directly into the carboys. Bioavailability of zinc was evaluated by concentrations accumulated in benthic periphytic algae. Furthermore, food chain transfer was tested by quantifying tissue zinc in grazing invertebrates.

The second experiment conducted in the Spring of 1998, was designed to test the hypothesis that iron may not have any ameliorating effect when aqueous dissolved zinc concentrations greatly exceed those of iron. Zinc/ iron concentrations were varied, by changing the relative concentration of zinc, to simulate levels found in the 3 geographical treatment zones of the St. Mary River. Accumulation and clearance of zinc in both algae and invertebrates were linked to population and community level toxicological effects. These effects were quantified through algal taxonomy and pigment analysis.

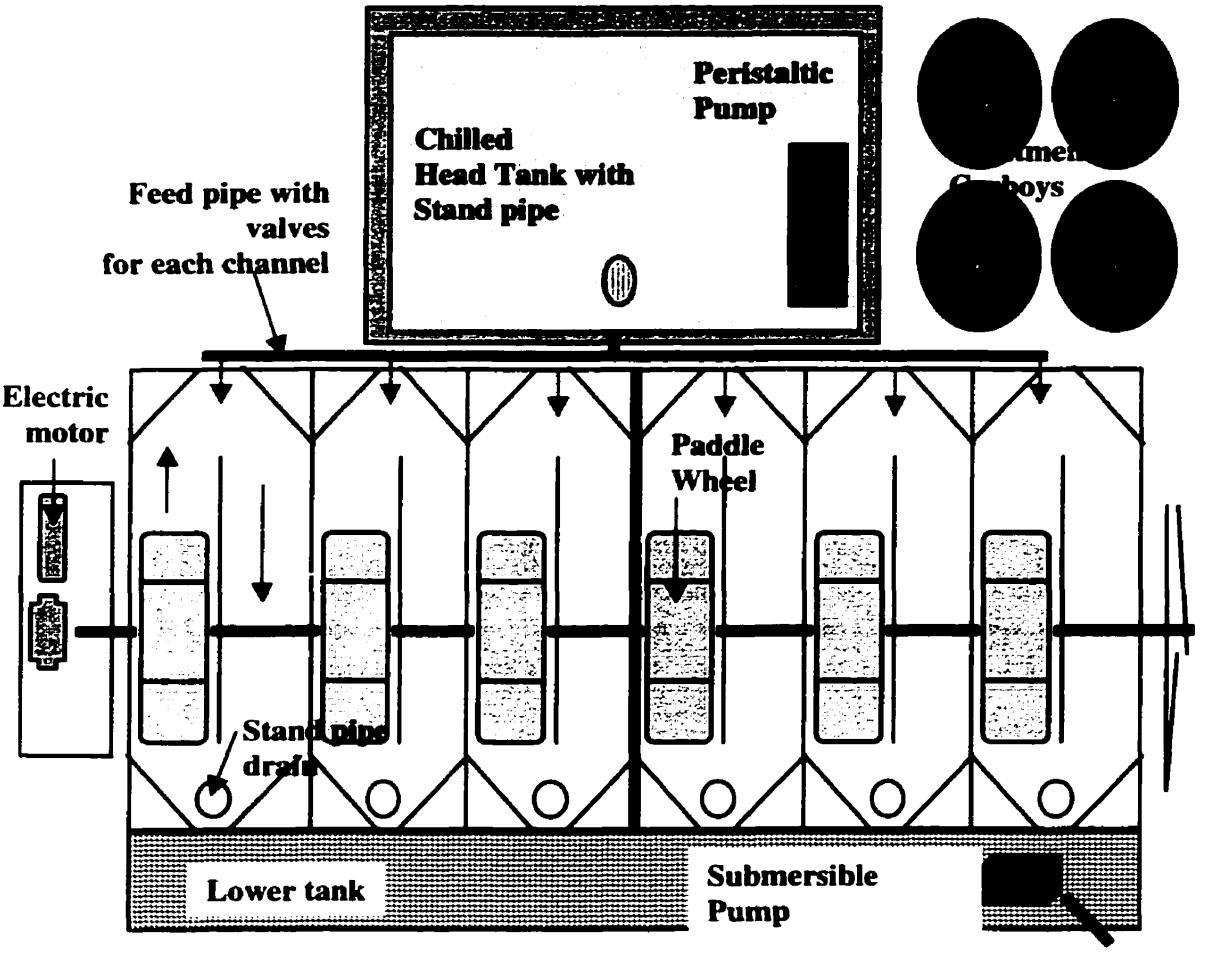
3.1 Apparatus Design

Replicate stream chambers offered the advantage of flow through or re-circulation with current, light and suitable substrate to support a representative biotic assemblage that provided realistic biogeochemical dynamics and food web interactions. The biological processes occurring in the stream chambers were integrated, as in natural streams, allowing for better extrapolation of findings to natural systems. A broad consideration of artificial stream research proceeded the final design and construction of the current system (McIntire et al., 1964; Matthews et al., 1990; Crossland et al., 1991; Shriner and Gregory, 1984; Lamberti and Steinman, 1993; Flum et al., 1993; Kiffney and Clements, 1994; Gruessner and Watzin, 1996; Palmer and Goetsch, 1997). A flow through oval design with a paddlewheel driven current similar to McIntire et al., 1964 and Kiffney and Clements, 1994 was chosen primarily because it offered superior current to support the greatest diversity of riffle insects (Pontasch and Cairns ,1989) and retention time could be controlled for toxicological applications. The scale for the chambers was selected on the basis of greater reality with an ability to support several trophic levels and the need for replication. The chambers were divided in the middle and measured 40 cm wide, 90 cm long and 12 cm deep for a capacity of 35 L (Figure 7). Each chamber drained through a stand pipe fitted with a mesh net at the bottom. De-chlorinated water, pH 5.7 – 6.2, entering the header tank was chilled by a series of PVC coils circulating coolant through a chiller unit mounted below the tank. Water from the Seymour watershed was used in all laboratory bioassays. Table 12 in Appendix A lists the average pH, hardness, dissolved organic carbon and various nutrients for the treated water from Seymour watershed in 1997. Temperature in the streams was maintained between 8 – 12° C in both the Fall 1997 and Spring 1998 experiments. The water that drained from each of the six streams collected into the lower tank. The water could be either returned to header supply via submersible pump (Little Giant, Model 5-MSP) for culturing homogeneous benthic algae or allowed to drain through a bulkhead fitting on the wall of the lower tank once toxicological treatments began. Current in each stream (5-20 cm sec^{-1} as measured by timing the distance traveled by a small float) was generated via a speed

controlled electric motor (Baldor 1/8 HP) attached to a 1 cm diameter stainless steel shaft which turned 6 PVC paddlewheels (Pontasch and Cairns, 1989). Fluorescent light banks, with true light (Industrial FT40T12) bulbs, provided full spectral light on a 14h :10h light/dark schedule. In 1997 light intensity was low with only 2 banks of lights, ~ 6 μE m^{-2} sec^{-1} in each stream as measured with an underwater cosine PAR sensor from LI-COR (set for air measurement). Two flow through 38 L fish tanks were piped into the drain valve on the header tank and provided acclimation chambers for invertebrates in 1997 (Scrimgeour et al., 1991).

In 1998 the microcosm apparatus was improved with the addition of six narrow gauge peristaltic cartridges with size 14 C-Flex (1.6mm I.D.) tubing. This allowed nutrients to be delivered from six separate 42 L carboys at a rate of ~ 10 mL min^{-1} into each stream channel (Flum et al., 1993). This in turn allowed for a slower overall dilution rate from the header tank (~ 800 mL min^{-1}) for a retention time of ~ 43 minutes. An extra bank of lights increased intensity to ~ 65 μE m^{-2} sec^{-1} which was sufficient to culture diatoms and chlorophytes from a local tributary (Cox , 1993). The third and final improvement was inline filtration (10 to 25 μm) of incoming water to eliminate colonization of chironomid larvae and algae growing upon the outside aeration/de-chlorination system at the Aquatic Alcan facility.

Figure 7: Schematic plan view of stream microcosm.



Note: De-chlorinated water from the Alcan Aquatic Facility was supplied to the header tank, which was elevated. Head pressure in the header tank was ensured by maintaining overflow through a bulk head fitting 3 inches below the top. A chiller unit mounted under the tank ensured water temperatures remained between 8 – 12 °C. Water drained through the stand pipe in the header tank to the 1 inch inside diameter feeder pipe that was fitted with valves to control the flow into each stream. Treatments were delivered from 42 L carboys through the peristaltic pump, which was situated below the head tank. Mixing was ensured through the current generated by the paddlewheels, which circulated the water clockwise within the channels. Water collected in the lower tank could be returned to the header tank or allowed to drain from the system. Fluorescent light fixtures arranged lengthwise across the channels provided full spectral light.

3.1.1 Bioavailability bioassay

The bioavailability bioassay was designed to simulate the formation of iron oxides at the Sullivan mine treatment plant. In addition to high concentrations of calcium and magnesium, iron and the associated hydroxides and oxides may have considerable influence on metal bioavailability. Past studies that evaluated metal bioaccumulation in mixtures that included iron had conflicting results. Cadmium accumulation in mayflies was significantly reduced (Gerhardt,1995); whereas the accumulation of zinc in an amphipod was unchanged when compared to singular treatments (Casini and Depledge, 1997). Both groups of researchers recognized that significant reductions in bioaccumulation would only occur with oxidation of Fe^{2+} to Fe^{3+}. The current study was designed to test whether zinc was less bioavailable to benthic periphyton in the presence of iron oxides. The oxidation of the iron was ensured by bubbling air into the treatment carboys. Food chain transfer of the zinc was tested by analyzing the accumulation of zinc by grazing snails. It was expected that stream chambers receiving the iron treatments would show significantly less zinc accumulation in periphyton, and consequently, less food chain transfer to the snails.

Experimental Design (Bioavailability Bioassay)

Six unglazed tiles (450 cm^2) were placed in Stoney Creek at the base of Burnaby Mountain for a 2 week inoculation period beginning in mid June 1997. Clay tiles were well suited to this application because they rapidly colonized and were easily transferred into the experimental streams (Lamberti and Resh, 1985). Starting July 07, 1997 through until October 05, 1997 a nutrient mixture was added to the header tank every 3 days to produce nominal levels of reactive phosphorus (10 $\mu g\ L^{-1}$), nitrate nitrogen (100 $\mu g\ L^{-1}$) and reactive silica (1000 $\mu g\ L^{-1}$) in each stream channel. The limitations of a four channel peristaltic pump made it impossible to deliver separate nutrient mixtures to all six streams, thus a simple nutrient mixture periodically delivered into the header tank served the purpose of stimulating algae growth. The choice of nominal levels of nutrients was a compromise between the high levels from both section 8 – 11 Standard Methods

(APHA, 1992) and Flum et. al., (1993) and the more realistic values from the Seymour watershed and the St. Mary River, Kimberley B.C. (Appendix A:Table 12).

During the initial culturing period the current was adjusted to ~ 6 cm sec^{-1} until benthic algae were established on the substrate, then current was increased to 15 cm sec^{-1} for the duration. After a two month culturing period, during which nutrient water was recycled, treatments were randomly assigned to the six streams and the system changed to flow-through for the next 30 days. Treatments consisted of two controls (no metal additions), two Zn^{2+} (50 µg L^{-1}) and two Zn^{2+} (50 µg L^{-1}) with Fe^{2+} (200 µg L^{-1}). Bi-weekly nutrient dosing continued throughout the experiment. Dilution water from the header tank to each stream was set at 1.3 L min^{-1} for a total retention time in the streams of 26 minutes.

Collection of approximately 180 gastropods (snails), *Physa gyrina* with some *Stagnicola catascopium* (National Museums, 1981), from the shallow shoals of Cultus Lake, B.C. occurred in early September, 1997. Snails were transferred to the laboratory in a cooler with lake water and placed into the acclimation tanks. To allow zinc levels in the periphyton to reach equilibrium, invertebrates were not stocked into the stream chambers until 10 days into treatment. The experimental chambers were repeatedly measured, 1 sample per chamber on each of 9 sample days over the 30 day bioassay for periphyton biomass and pigments. These data were used to evaluate whether the treatments were causing toxic effects on the periphyton. A similar repeated measures sample design was employed for aqueous zinc, algal and invertebrate tissue zinc. The results from aqueous zinc verified nominal concentrations in iron /zinc and zinc only channels were achieved. Algal tissue zinc concentration enabled the evaluation of bioavailability of the zinc in the presence and absence of iron and finally, invertebrate tissue zinc confirmed whether the zinc transferred up the food chain.

Methods

Biomass and Pigment Analysis

Two tiles, one for biomass and one for pigments, were randomly sampled from each stream on nine occasions throughout the thirty day experiment. Algae was scraped off the top, bottom and all sides of the 11 cm^2 tile and transferred onto a pre-weighed aluminum boat or 2 dram plastic vial for biomass and pigments, respectively. The biomass sample was dried at 60° C for > 48 hours prior to determination of dry weight to the nearest 0.1 mg (Mettler AE 240). Pigment samples were frozen in the dark until termination of the experiment.

Pigments were determined after methods of Wetzel and Likens (1991), slightly modified to reflect current sampling technique. The thawed algae was transferred into a glass grinding tube and ground with a teflon grinder in approximately 3 - 4 mL 90% acetone solution. This solution was transferred to a plastic 15 mL graduated centrifuge tube. The grinding tube was rinsed with 90 % acetone and recovered pigments were added to the centrifuge tube. Total volume was brought to either 5 or 6 mL depending on the intensity of the pigmentation. The samples were centrifuged in the IEC Centra MP4R at 3000 rpm for 5 minutes at 5° C. The supernatant was carefully transferred to a glass 4 mL, 1 cm pathlength cuvet and diluted to keep the recorded extinction values under 0.04. In order to calculate pigments for chlorophyll *a, b, c* and carotenoids, extinction coefficients were recorded at several wavelengths (750, 665, 664, 663, 647, 630, and 480ηm) on a Bausch and Lomb Spectronic 21. Post acidified phaeopigments were calculated by adding 0.1 mL 1N HCl per mL of extract directly to the cuvette cells and recording extinction coefficients at 750, 665, and 663 ηm. Samples were corrected for turbidity, and the machine was brought to 100% transmission with a 90% acetone blank every four samples.

Water (aqueous zinc)

Water samples were collected from each stream using acid washed 1 L glass jars. Immediately following collection, samples were brought to 1.0 M strength using Bakers nitric acid. Sub-samples from the 1 L samples were passed through 0.45 µm filter and aspirated directly into the flame atomic absorption spectrophotometer (Perkin Elmer 1100B with impact bead). The flame was adjusted for a hot lean burning blue flame. Standards were prepared to sample molarity in Bakers nitric acid and deionized water. Concentrations of zinc were calculated through standard curves. Results would be considered non-colloidal zinc, not dissolved as the filtration took place after acidification. Quality control was ensured through the inclusion of matrix blanks (Appendix B: Table 14).

Algal Samples

Algae (n =9/channel) were collected by scraping the sides of the stream channels with a plastic spatula and rinsing the material with header water, into a clean plastic beaker. Algae were immediately filtered and rinsed with de-ionized water, onto pre weighed GF filters (Whatman 934-AH, 1.5 µm retention) and dried at 60° C for > 48 hours. After cooling to room temperature, dry weights were recorded to the nearest 0.1 mg (Mettler AE 240). Algae with filters were ground in acid washed 12 mL glass vials before being transferred, with 10 mL 8.0 M Bakers nitric acid, into Teflon digestion vials. A CEM microwave MDS 2000 programmed at 85% power for 8 minutes followed by 60% power for 6 minutes was employed for digestion. After cooling, digests were transferred to acid washed plastic 15 mL graduated centrifuge tubes, brought to 10 mL volume with 8.0 M Bakers nitric acid and centrifuged at 3000 rpm for 5 minutes in the IEC Centra MP4R. Seven mL of supernatant was transferred into acid washed 12 mL vials and diluted with deionized water to a final volume of 11mL at ~ 5.0 molar. The samples were then aspirated directly into the AAS. Standards were prepared to sample molarity in Bakers nitric acid and double de-ionized water. Process blanks, filter blanks and standard reference material were passed through the procedures and aspirated along

with the samples. Reference materials consisted of NIST oyster tissue digested using CEM techniques (Appendix B: Tables 13 and 14).

Invertebrate Samples

After 48 hours of acclimation, 30 gastropods were stocked per stream for an initial density of 115 individuals m^{-2}. Lamberti et al. (1987) considered 350 snails m^{-2} a realistic density for their mesocosm grazing experiments. Snails were collected (2-6 per stream) each sample day, allowed to depurate over three hours and rinsed in de-ionized water to remove attached algae. The snails were frozen, thawed and the shells separated from the tissue. Tissues were dried at 60° C for at least 48 hours and dry weights recorded to the nearest 0.1 mg (Mettler AE240). Digestion took place in 7 mL 8.0 M Bakers nitric acid, as done for algae. Cooled digests were transferred to 15 mL graduated centrifuge tubes and brought to exactly 7 mL mark with 8.0 M Bakers nitric acid. The final 10 mL ~ 5.3 molar solution was aspirated into the AAS directly from the centrifuge tubes. A common standard curve was used for both algae and invertebrate samples. Process blanks and NIST bone ash reference material were processed with the samples, ensuring quality control, quality assurance (Appendix B: Tables 13 and 14).

Data Analysis

All analyses were preformed with $\log_e$ transformed data to stabilize variance and create a normal distribution for parametric analysis. Linear relationships between chlorophyll *a* and time represent algae growth rates and were treated by an analysis of covariance (Zar, 1984). To determine which slopes differed significantly a Student Newman Keuls (SNK) test was employed. Relationships between water and algae, algae and snail were tested for significant correlation (Minitab v 10.2). An algal enrichment factor was calculated by dividing the algal tissue zinc concentration (ppm) by the aqueous zinc (ppm). All time series measures of aqueous zinc, algal zinc, enrichment and snail zinc ($\mu g\ g^{-1}$ and $\mu g\ snail^{-1}$) were treated with a repeated measures ANOVA which takes into account the contributions from time and interactions between time and

treatments (SAS, 1998). Significance among treatments on each sampling day were further evaluated by Ryan - Einot - Gabriel - Welsch (REGWQ) multiple range tests.

Results

Algal Biomass and Pigments

Algal biomass and pigments were quantified in order to detect toxic effects from the treatments. Biomass varied over time, but the large variance within each treatment group precluded significant differences. Regression analysis produced r^2 values ranging from .314 from the control to .089 from zinc only streams. Analysis of covariance was not performed on these data because linear relationships did not exist.

All treatments showed increasing chlorophyll *a* per tile at similar rates up until day 19, thereafter both the zinc and iron/zinc treatments reached asymtotic values (Figure 8). The ratio of chlorophyll *a* : phaeopigment remained relatively constant throughout the experiment ($\rho = 0.370$, ANOVA). Although phaeopigments did not produce significant regressions, chlorophyll *a* was dependent on time (Table 3).

Table 3: Bioavailability bioassay: Chlorophyll *a* regression over time (growth curves).

Treatment Group	r^2	Slope ρ value
Control	0.786	<0.0001
Iron/Zn	0.827	<0.0001
Zinc alone	0.660	<0.0001

Note: Zinc alone = 50 μg Zn L^{-1}; Iron/ Zn = 50 μg Zn L^{-1} + 200 μg Fe L^{-1}; Control = no additions

Residuals were normally distributed and showed no trends. The slopes were significantly different ($F = 3.917$, $\rho < 0.010$, ANCOVA). The multiple range test evaluating differences among slopes produced the following results (Table 4).

Table 4: Bioavailability bioassay: Chlorophyll *a* SNK multiple range comparisons among treatment slopes.

Comparisons	Slope Q ratio	ρ value
Zinc & Iron/Zn	1.3607	Not Significant
Zinc & Control	2.1858	Not Significant
Iron/Zn & Control	3.5465	< 0.05

Note: Zinc alone = 50 μg Zn L^{-1}; Iron/ Zn = 50 μg Zn L^{-1} + 200 μg Fe L^{-1}; Control = no additions

These ANCOVA results indicate that the treatments of iron/zinc were adversely effecting the algal growth. The periphyton growths were mature (> 2 months) and were not homogeneously distributed throughout the streams. Within stream variance precluded any significant treatment effect when data were analyzed with a more rigorous ANOVA technique. The biomass data also suggested that variance is related to patchy distribution within the stream chambers. Toxicity cannot be conclusively determined from these analysis.

Aqueous Zinc

To verify that nominal concentrations of zinc did not differ between the iron/ zinc and zinc alone treatments water zinc was analyzed. Levels of aqueous zinc, without filtration at 0.45 μm, did not provide a reliable measure of zinc in water. Concentrations measured were regularly above 100 μg L^{-1} in all streams. High levels of zinc recorded were likely due to colloidal algae material collected with the water sample.

Sub-samples taken from the original 1 L sample jars which had been passed through a 0.45 μm syringe filter were reliable measures of the aqueous zinc concentrations. There were significant differences among treatments with variance related to lower concentrations measured in the control streams ($F = 22.39$, $\rho = 0.0157$, ANOVA; Figure 9). The peak on day nine was likely due to a serious fluctuation in the dilution water flowing from the header tank. Zinc concentrations in all chambers, with the exception of controls, were not significantly different.

Algal Tissue Zinc

In order to evaluate zinc bioavailability in the presence and absence of iron, tissue algal zinc was measured. Time averaged concentrations with standard errors were 130 ± 18, 460 ± 92 and 1530 ± 220 $\mu g\ g^{-1}$ in the control, iron/zinc and zinc treatments, respectively. The algae in the zinc only streams clearly accumulated the metal to a greater degree; however, significant differences occurred among all treatments ($F = 702.23$, $\rho = 0.001$, ANOVA; Figure 10). The algae integrated changes in the water zinc, especially in the absence of iron. Aqueous zinc correlated with algal tissue zinc ($r^2 = 0.317$, $\rho < 0.05$; Figure 12). This is a weak correlation primarily because of the poor sensitivity of the flame atomic absorption technique at these low aqueous zinc concentrations. The ameliorating effects of high iron on the bioaccumulation of zinc became very apparent when the data were presented as an algal enrichment factor ($F = 42.96$, $\rho = 0.0062$; Figure 10). Streams receiving zinc only had significantly higher enrichment factors (~35,000) than either control or iron/zinc treatments (~10,000). In fact, with the exception of the last day, neither control or iron/zinc treatment streams differed significantly in their enrichments. Zinc was clearly less bioavailable to the algae in the presence of iron.

Invertebrate Tissue Zinc

Food chain transfer of the zinc was evaluated through tissue zinc concentration in the grazing snails. Overall mean levels of zinc in the grazing snails were lower than the levels accumulated in algae. Means and standard errors for snail tissue zinc over the 6 sample days were: 99.36 ± 3.76, 249.45 ± 10.01, 377.51 ± 32.14 $\mu g\ g^{-1}$ for control, iron/zinc and zinc alone treatments, respectively. The time series analysis normalized to dry weight indicated significant differences among all treatments ($F = 557.1$, $\rho = 0.0001$, ANOVA). Body burden of zinc in the snails indicated that snails in the treatments receiving iron maintained their zinc burdens below 4 $\mu g\ animal^{-1}$; however, those in the zinc only streams had much higher variance with levels exceeding 12 $\mu g\ animal^{-1}$ after 25 days ($F = 125.29$, $\rho = 0.0013$; Figure 11). Zinc accumulation in snails did not show a

dramatic reduction due to high iron; however, the time series of body burden does suggest that the snails in iron treated streams were more able to regulate internal levels of zinc. Concentrations ($\mu g\ g^{-1}$ d.w.) in the snails related to the zinc concentrations in the algae ($r^2 = 0.850$; Figure 12). Although no biomagnification of the zinc occurred in the snails, there was evidence that contaminated benthic algae may be a significant route of food chain transfer to higher trophic levels.

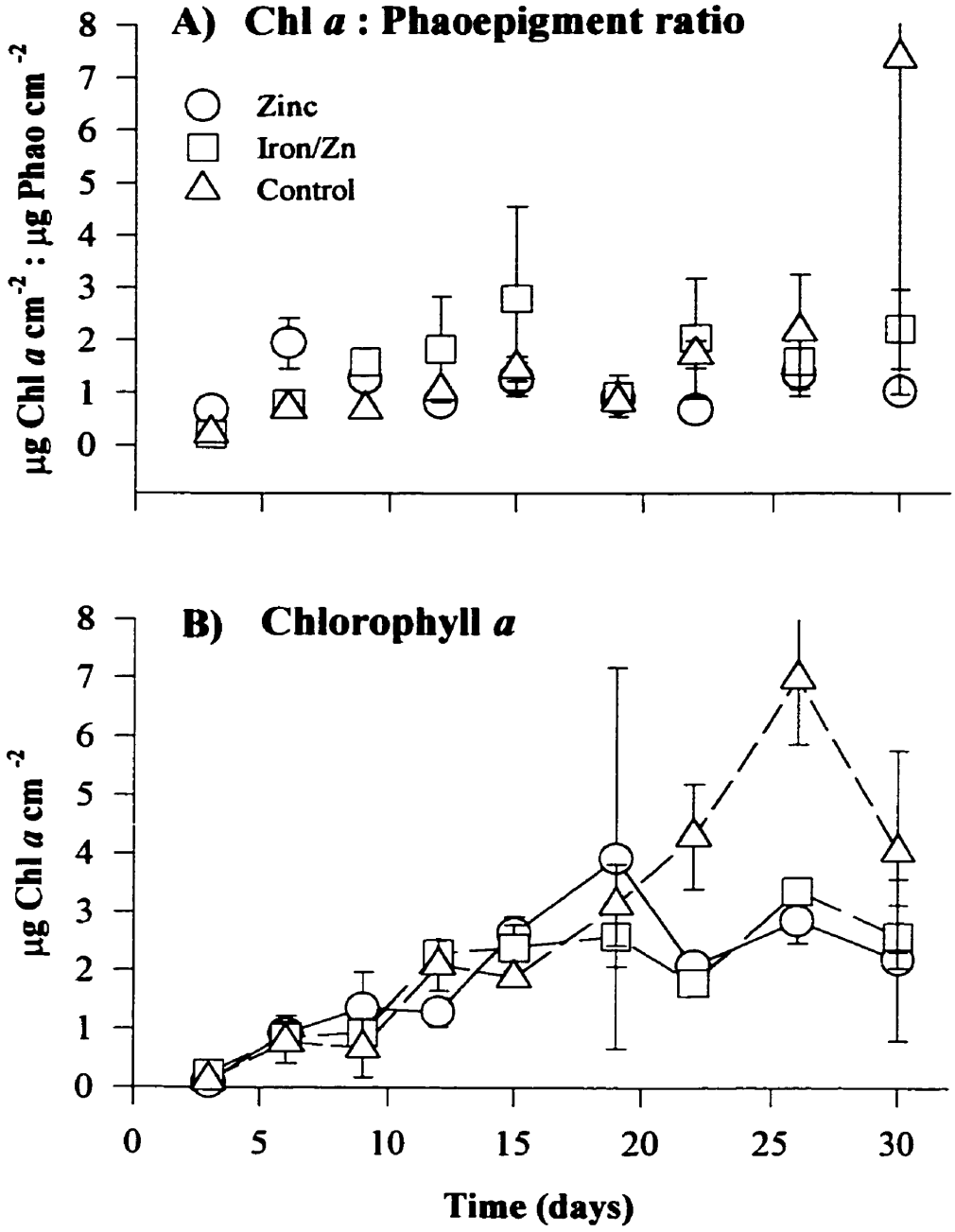


Figure 8: Bioavailability bioassay: Time series algal pigments (means with SE bars $n = 2$ per treatment).

A): Ratio Chlorophyll *a* (Chl *a*) : Phaeopigments.

B) Chlorophyll *a* growth curves. Growth curves from Iron/Zn treatment were significantly reduced ($\rho < 0.05$, ANCOVA) after 20 days of growth.

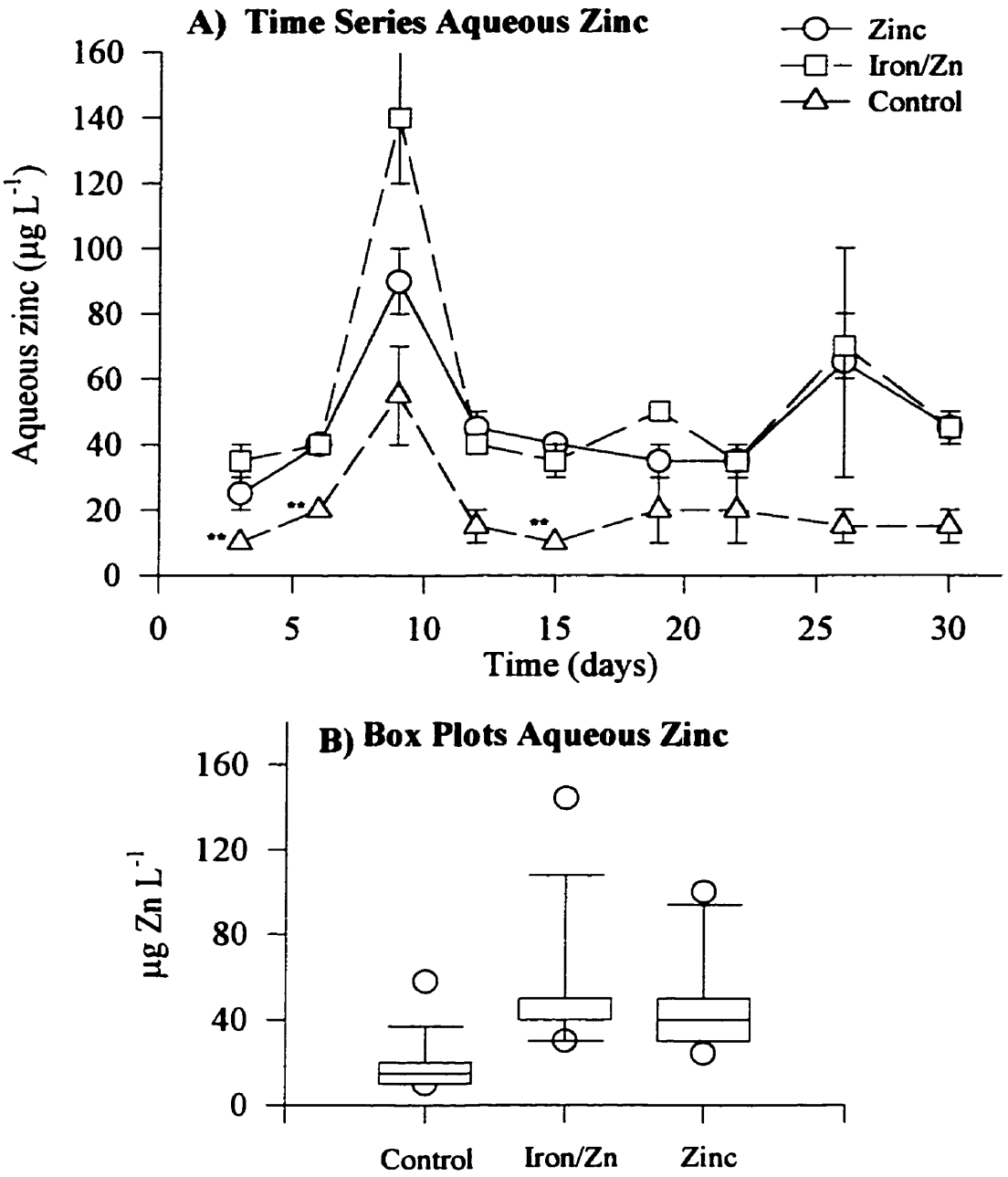


Figure 9: Bioavailability bioassay: Aqueous Zinc

A) Time series aqueous zinc (means with SE bars, $n = 2$ per treatment). REGWQ multiple range comparisons among treatment on each sample day ($\rho < 0.05$ **).

B) Box plots of aqueous zinc pooled over the 30 days of the bioassay for each treatment ($n = 18$). Box divided at the median (50th) encloses the upper and lower quartiles (25 – 75th). Capped bars show the 10 – 90th percentiles and all data points outside the 90th as circles.

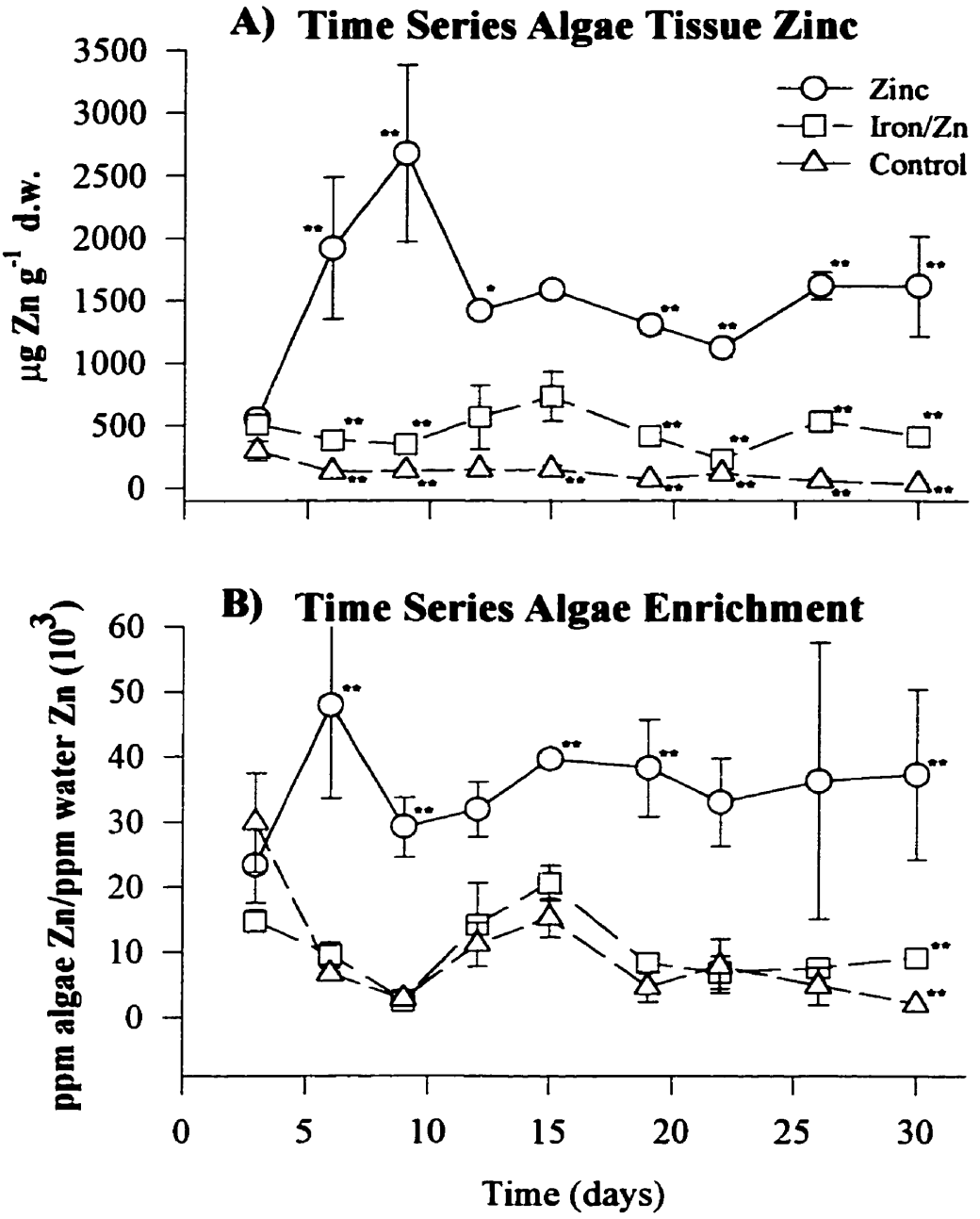


Figure 10: Bioavailability bioassay: Algal Zinc

A) Time series algal tissue zinc normalized to dry weight (means with SE bars, $n = 2$ per treatment). REGWQ multiple range comparison among treatments on each sample day ($\rho < 0.05$ **) or * significantly higher than control only.

B) Time series algal tissue zinc enrichment (means with SE bars, $n = 2$ per treatment). REGWQ multiple range comparison among treatments on each sample day ($\rho < 0.05$ **)

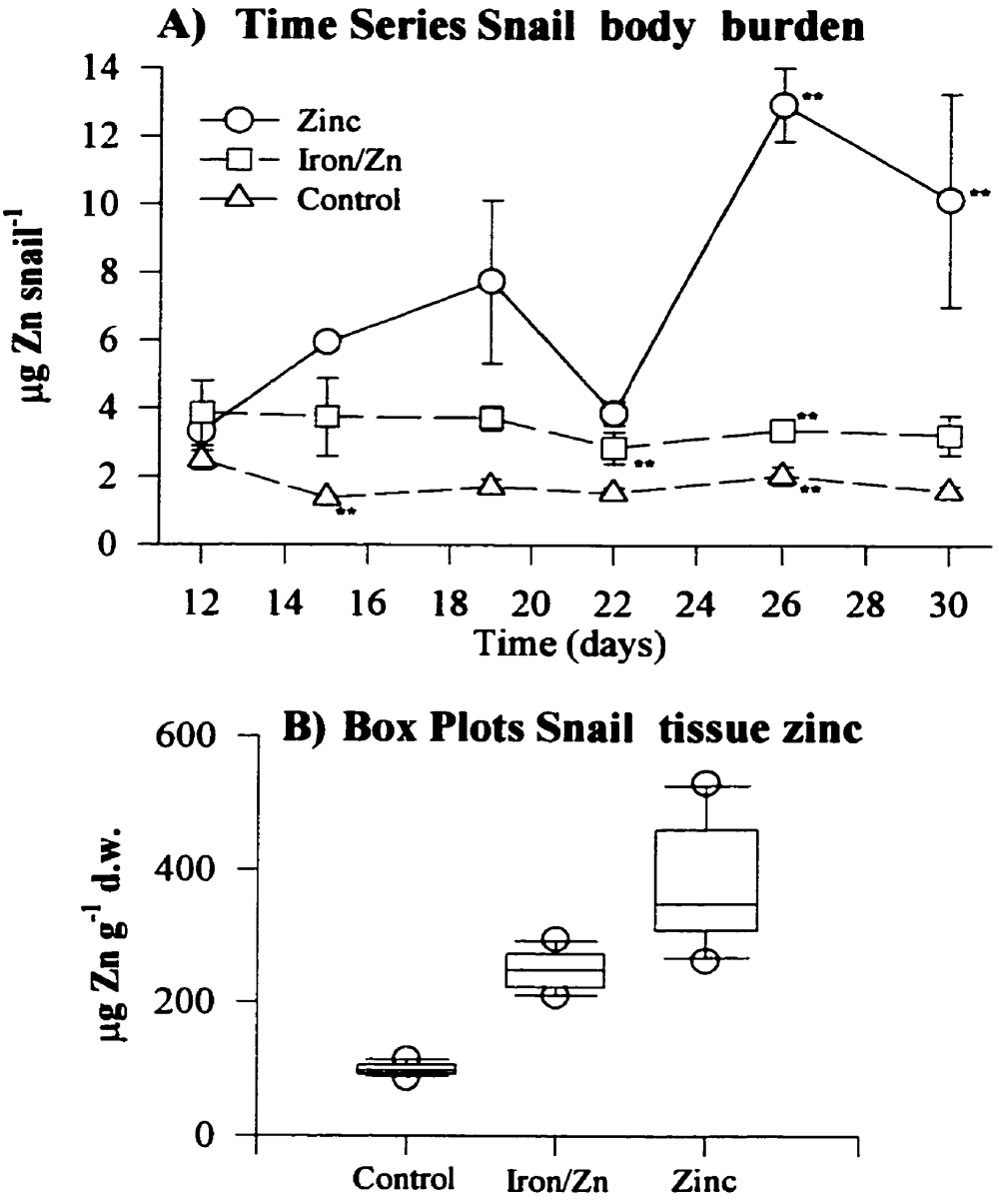


Figure 11: Bioavailability bioassay: Snail Zinc

A) Time series snail body burden zinc (means with SE bars, $n = 2$ per treatment). REGWQ multiple range comparison among treatments on each sample day ($\rho < 0.05$ **).

B) Box plots for snail tissue zinc normalized to dry weight, pooled over the 30 day bioassay ($n = 12$ per treatment). Box divided at the median (50^{th}) encloses the upper and lower quartiles ($25 - 75^{th}$). Capped bars show the $10 - 90^{th}$ percentiles and all data points outside the 90^{th} as circles.

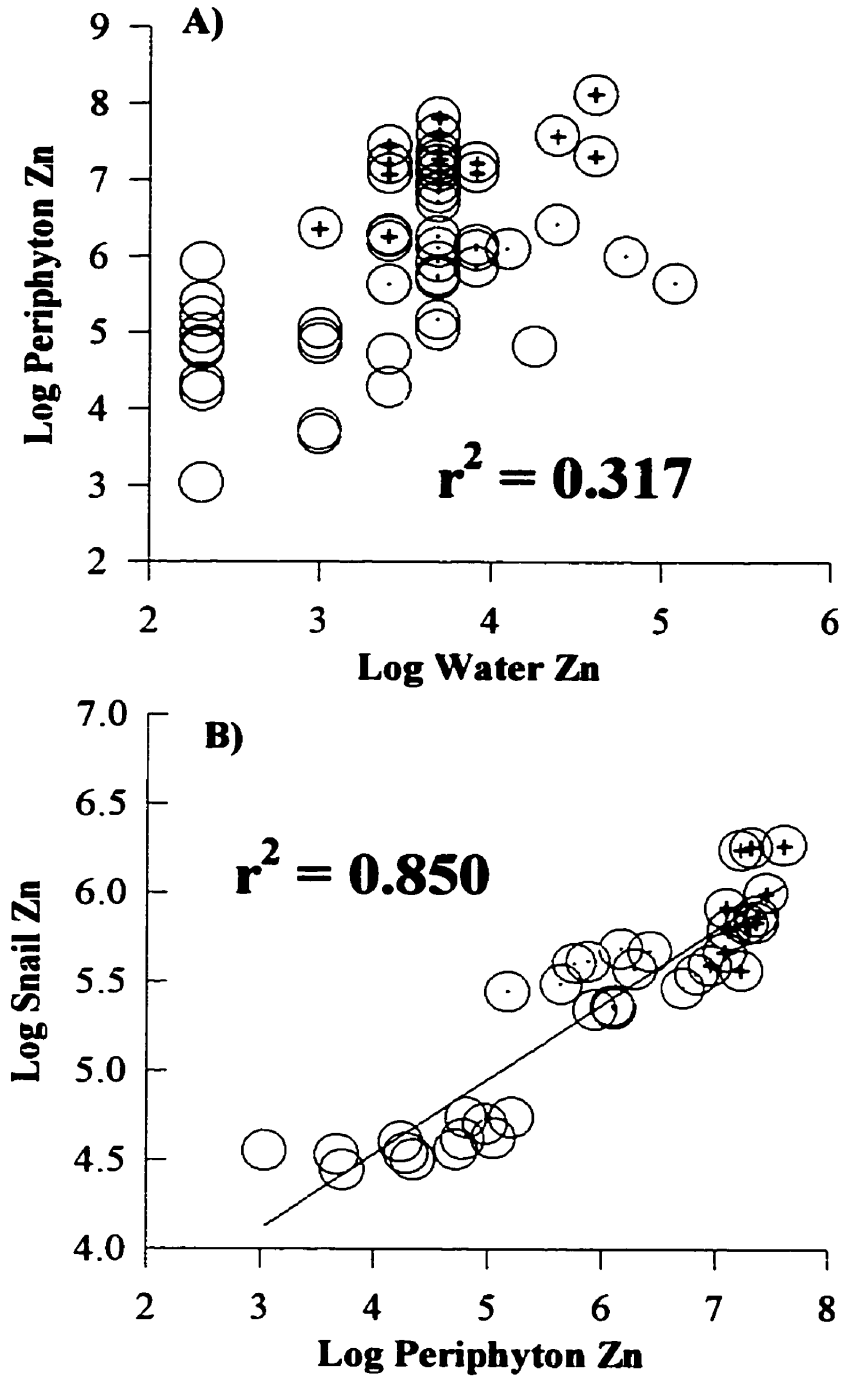


Figure 12: Bioavailability bioassay: Correlation of Log_e transformed data pooled over the 30 day bioassay. Plain circles - control treatment, Circles with dots – Iron/Zn treatment and Circles with crosshairs – Zinc alone treatment

A) Water zinc – Periphyton zinc. **B)** Periphyton zinc – Snail zinc.

Discussion: Bioavailability bioassay

Iron in the treatment effectively reduced the bioaccumulation of the zinc in the algae and this subsequently reduced the food chain transfer to the grazing snails. Creating conditions that favoured the formation of iron oxides within the carboys likely precipitated out iron oxides within the carboys and along the tubing before entering the streams (personal observation). The proportion of aqueous zinc within the streams with added iron was significantly less bioavailable, as indicated by the algal enrichment. With the exception of the analysis on chlorophyll *a* slopes, no toxicological effects were detected with these levels of zinc and iron.

Toxicological tests with combinations of zinc and iron conducted by Wang (1985) had inconclusive results. At zinc levels below 1.0 mg L^{-1} iron seemed to be antagonistic or had no effect, but as zinc levels increased to 1.0 mg L^{-1} and greater, iron seemed to be synergistic on algal respiration. Studies by Stauber and Florence (1985a and b) on marine *Nitzschia closterium*, suggest that iron deficient medium actually increased copper toxicity. Whether or not impairment of chlorophyll *a* occurred in the streams that received additions of iron, it is unlikely that such impairment would have contributed to the dramatic reduction in amounts of accumulated zinc.

Iron and manganese oxides and hydroxides are very important components of sediment that affect metal bioavailablity (Campbell and Tessier, 1989). These oxides and hydroxides ameliorated both accumulation and toxicity of copper in a marine diatom by adsorbing the metal on the membrane surfaces and preventing penetration into the cells (Stauber and Florence, 1985a and b). Gerhardt (1995) found that in the presence of iron, the mayfly *Leptophlebia marginata* accumulated significantly less cadmium. Fe^{2+} and Fe^{3+} were localized in the gut and adsorbed to the body and gills. Casini and Depledge (1997) found that in the presence of iron the marine amphipod *Platorchestia platensis* accumulated no more zinc than in singular treatment, and cadmium–iron combinations showed significantly higher accumulation of the cadmium. However, the authors used

very high nominal concentration of all metals (100 $\mu g\ L^{-1}$) and prevented any changes in speciation, which are critical to metal bioavailabilty. Other chelators that can bind to metals and reduce their toxicity and bioaccumulation are amino acids, organic matter, humic acids, fulvic acid and EDTA (Rai et al., 1981).

The algal enrichment factor illustrates that the proportion of the aqueous zinc that was bioavailable (as measured by periphyton uptake) was reduced in those streams treated with iron. Brooks and Rumsby (1965) used what was considered biologically available dissolved species in the denominator when they described enrichment of trace metals in bivalves off the coast of New Zealand. Enrichment ratios in this study agree with those found by Foster (1984) and Whitton (1982) for zinc in filamentous green algae ($10^3 - 10^4$) and *Lemanea fluviatilis* ($10 * 10^3 - 50 * 10^3$), respectively. Both researchers observed an increasing enrichment with decreasing ambient zinc, suggesting internal regulation of this micro nutrient. This negative correlation was reported by Whitton (1984) to occur with cadmium and lead; although Foster (1984) found increased enrichment with increasing ambient copper.

Snails are important herbivores in many freshwater systems. These animals feed by rasping organic matter from stream substrate with a toothed, tongue-like radula (Stewart and Hill, 1993). When high densities are achieved, grazing can greatly reduce the thickness and biomass of the periphyton and indirectly affect other invertebrates (Steinman, 1996). Grazing snails can exert a top down control over periphyton growth and prolong the turnover rate of both nutrients and toxicants in and out of the periphyton. Stewart and Hill (1993) proposed that by excluding protected grazers (snails) from highly contaminated reaches allowed for easily predated invertebrates like chironomid and baetidae to prosper, thus increasing the food chain transfer of the contaminant from periphyton to fish.

This study supports Stewart and Hill's (1993) hypothesis that snails could serve as long term sinks of nutrients, or toxicants, through the action of grazing contaminated periphyton. Snail tissue zinc was less sensitive to the immediate abiotic characteristics, but was proportionally related to concentrations in the food (algae). Although this

experiment did not address the question of zinc regulation, there is a considerable body of evidence that indicates invertebrates can regulate this micro-nutrient.

Dallinger and Wieser (1984) found that in feeding experiments with the terrestrial snail *Helix pomatia* zinc accumulated in most organs during the loading period and was then redistributed to the midgut gland, where metallothioneins bound up to 70% of the metal by the end of the experiment. Lead and cadmium also accumulated to a high degree in the midgut gland; however, copper was more evenly distributed throughout the organism. Zinc regulation has been reported in freshwater amphipods (Bryan, 1967) shore crab *Carcinus maenas (L.)* (Chan and Rainbow, 1993); estuarine prawn *Pandalus montagui* (Nugegoda and Rainbow, 1987) and a freshwater prawn *Macrobrachium malcolmsonii* popular as an aqua culture species in India (Vijayram and Geraldin, 1996).

Conclusion

Iron oxides reduced the adsorption and internal accumulation of zinc in freshwater algae. Snail zinc tissue concentrations were maintained significantly lower than those in the periphyton and did not respond to the iron in the treatment, but they were highly correlated to zinc concentrations in the algae ($r^2 > 0.80$). Provided total iron greatly exceeds total zinc and that significant oxides are formed, one could hypothesize that natural streams receiving similarly characterized effluents would show reduced bioavailability as measured by algal and invertebrate tissue zinc. Field evaluations from St. Mary River agree with these experimental findings. The results from the St. Mary River indicate that the zinc released from the treatment plant is significantly less biologically available than the zinc released from Mark Creek, which lacks the associated iron oxides.

3.1.2 Accumulation/Clearance Bioassay

High levels of accumulated zinc in periphyton and invertebrates sampled along the St. Mary North shore exposure suggested that the zinc was bioavailable. It was hypothesized that iron may not have any ameliorating effect when the dissolved zinc concentrations greatly exceeded those of iron. Therefore, this second experiment was designed as a toxicological bioassay with three levels of zinc delivered from an iron rich nutrient mixture. These treatments reflected realistic zinc/ iron ratios from reference, South and North shore exposures in St. Mary River. This experiment tested whether there were any ameliorating effects on zinc accumulation and toxicity from the iron enriched nutrient mixtures. By varying the concentration of zinc it was possible to link accumulated levels of zinc in the algae and invertebrates to adverse effects. Better control over periphyton was achieved which enabled toxicological endpoints at the community and population level to be evaluated. It was expected that uptake and toxicity would be reduced in the medium zinc treatment, when compared to the high treatment.

Experimental Design (Accumulation/ Clearance Bioassay)

Diatoms from the Seymour River tributary were cultured on six (840cm^2) nytex 250 µm nets left unattended between April and May, 1998. They were held in place on the water surface by string tied up into the riparian growth. Ambient light intensity on this partly overcast morning was ~ 150 µE m^{-2} sec^{-1}. Netting was retrieved and transferred back to the laboratory streams in poly bags with river water. Nets were randomly assigned to the six streams and the attached periphyton was washed off into the streams (leaving the net in for 48 hours) with no current. The benthic periphyton rapidly colonized the tile substrate.

An iron rich (100 µg L^{-1}) nutrient mixture was delivered continuously into each stream and immigration of chironomid larvae was minimized through in line filtration. Measures of dissolved nitrate and silica throughout the experiment provided evidence of the flow control and dilution rates. A portion of the among stream nutrient variance is likely due to differing periphyton uptake. The two streams with the highest light

intensity recorded lower median nitrate. Silica, which is incorporated into the diatom cell walls, should be related to the abundance of diatoms in the periphyton. Nitrate means ranged from a high of 120 $\mu g\ L^{-1}$ in stream 3 to a low of 97.25 $\mu g\ L^{-1}$ in stream 5, with low variance ($F=1.18$, $\rho > 0.1$, ANOVA; Figure 13). Silica ranged from 1240 $\mu g\ L^{-1}$ in stream 1 to 1211 $\mu g\ L^{-1}$ in stream 2 ($F=0.02$, $\rho > 0.1$, ANOVA; Figure 13). Variance over time for silica was significant; but all streams experienced similar fluctuations and the impact on treatment effects analyzed as repeated measures is insignificant (Figure 13).

Collection of approximately 180 caddisfly larva: Limnephilide (*Dicosmoecus)* from a pool downstream from the periphtyton cultures occurred mid May, 1998. This holarctic genus is confined to western North America. Larvae are usually found on rocks in running water and require two years for completion of their life cycle (Wiggins, 1996). While most species are considered generalized shredders/detritivors, Gotceitus and Clifford (1983) found 1st & 2nd instars feed primarily on diatoms and fine organic debris, which was the case during early May in the Seymour demonstration forest. Immediately following collection, the caddisflies were stocked into the stream channels 30 each, for an initial density of 115 m^{-2}. This is below the density that Lamberti et al. (1987) considered realistic for mesocosm grazing experiments with *Dicosmoecus* caddisflies (200 m^{-2}).

Treatments began 4 days after stocking and involved adding 0.1 L, 0.6 L and 1.2 L of a hydrous zinc sulfate stock to each 44 L nutrient carboy. Targeted final concentration were ~ 10 $\mu g\ L^{-1}$, ~ 60 $\mu g\ L^{-1}$ and ~ 120 $\mu g\ L^{-1}$ for duplicate randomly assigned low, medium and high streams. No air was bubbled into the carboys; however, oxygen saturation was ensured with 4 air hoses tied around the standpipe draining the header tank. Clearance phase involved reducing the nominal zinc concentration in all of the 6 stream channels to ~ 10 $\mu g\ L^{-1}$. This clearance period began after 22 days and the nutrient delivery was terminated on day 35.

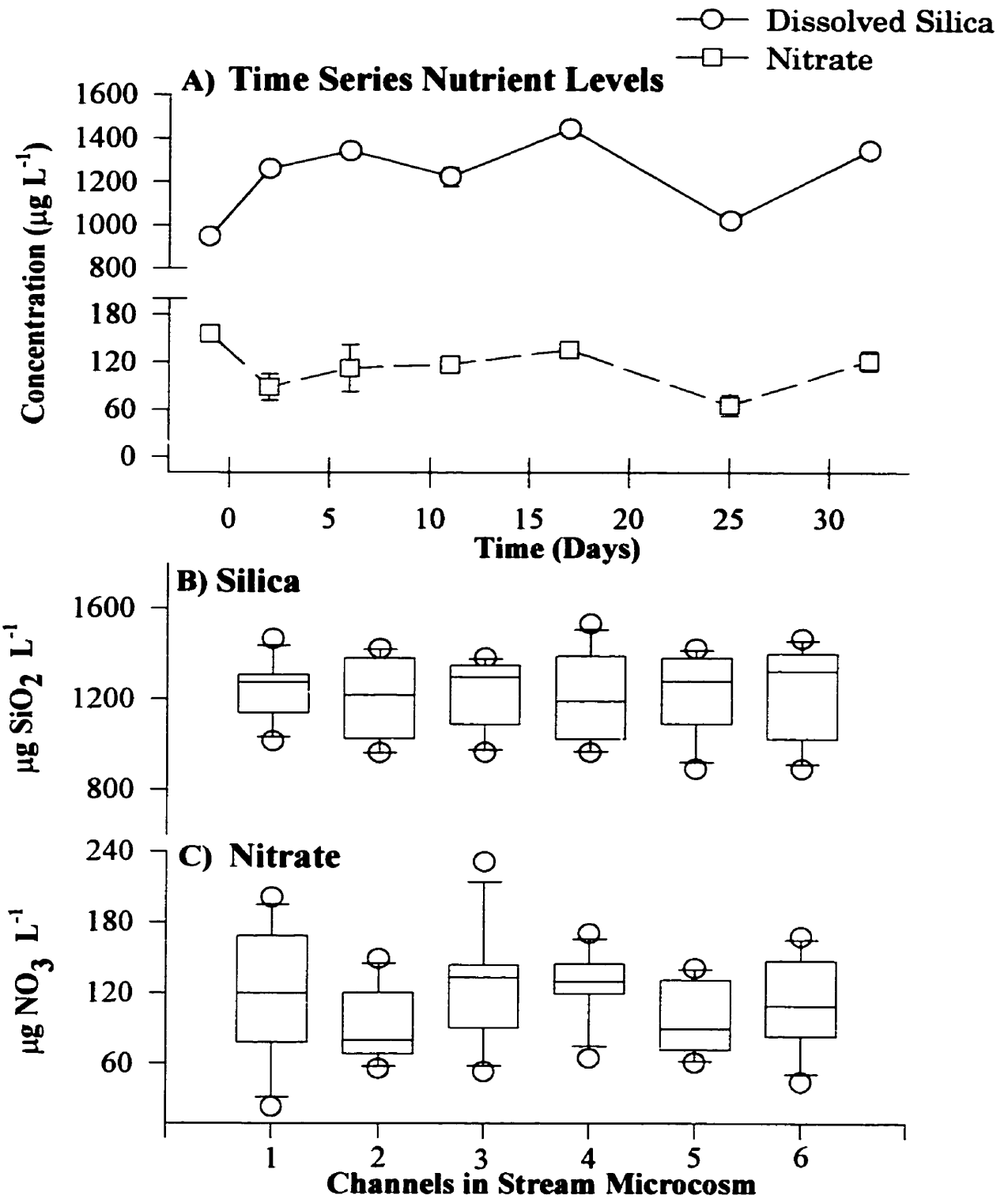


Figure 13: Accumulation/Clearance Bioassay: **A)** Time series depicting pooled nutrient results from all six channels for each sample day (means with SE bars, $n = 6$). **B)** Silica and **C)** Nitrate box plots pooled over 35 day duration for each channel ($n = 7$ per channel). Light: 74, 80, 65, 75, 77, and 61 µE m^{-2} sec^{-1} channels 1 through 6 respectively.

Methods

Dissolved Nitrate & Silica

In order to confirm nutrient levels and flow rates in the microcosm, nutrients were measured on 7 days throughout the course of the experiment commencing before the zinc treatment began. Single samples were taken from each stream and the header tank on each sample day. Process blanks of double de-ionized water were processed along with the samples. All samples and blanks were passed through an acid washed syringe 0.45 μm Millex – HV, Millipore filter into a 100 mL glass jar and 50 mL plastic centrifuge tube for silica and nitrate, respectively. The samples were analyzed immediately by colorimetric methods outlined in Wetzel and Linkens (1991).

Nitrate was measured by passing the alkaline-buffered solution through a cadmium column reducing the nitrate to nitrite with an efficiency greater than 95%. The nitrite is reacted with a colour agent that forms a stable pink azo dye. Nitrite concentration can be determined very accurately using this technique with an applicable range between 10 – 1000 μg NO_3-N L^{-1} (APHA, 1992). Extinction coefficients for all samples and standards were recorded at a wavelength of 543 ηm (1 cm path length plastic cuvet in Pharmacia LKB-Ultrospec 3). Distilled water was employed as the reference blank. All standards and samples were corrected by subtracting for the process blank and linear regression procedures were employed to obtain sample concentrations.

Dissolved silicate (SiO_2^{2-}) was measured by reaction with ammonium molybdate to form a yellow silicomolybdate, which was subsequently reduced by sodium sulfite to produce the stable blue molybdate colour. This method is accurate to at least ± 20 μg SiO_2^{2-} L^{-1} (Wetzel and Linkens, 1991). Extinction coefficients for the samples, standards and blanks were measured at 700 ηm in a plastic 1cm pathlength cuvet in the Pharmacia LKB-Ultrospec 3. All standards and samples were subtracted from the process blank and regression procedures were used to determine concentrations of dissolved silica. On 4 occasions throughout the experiment reactive phosphorus and total

nitrite were measured, but neither of these nutrients were detected with the procedures employed. The limit of detection for phosphorus was 10 $\mu g\ L^{-1}$.

Algal Pigments

For the evaluation of periphyton community health algae pigments were analyzed. Three tiles were sampled per stream on 7 occasions commencing 4 days prior to the addition of the caddisflies (Day = 0) and continuing until day 38. A randomization scheme was used to determine which tile was to be sampled from three distinct quadrates in the stream channels. Algae scrapped from the $11 cm^2$ area were processed immediately according to previously described procedures. The samples were only held in the round, glass 4 mL cuvets for transfer to a quartz 1 cm pathlength cuvet. The extinction coefficients were measured at 750, 665 and 480 ηm on Pharmacia LKB-Ultrospec 3 using 90% acetone as the blank. Monochromatic chlorophyll *a*, plant carotenoids and post acidified phaeopigments were calculated.

Aqueous Dissolved Zinc

To quantify the dissolved zinc three replicate samples per stream, each sampling day, were passed through an acid washed syringe fitted with a 0.45 µm filter (Millex – HV, Millipore) into a pre acidified (Bakers nitric acid) 100 mL polypro containers. The final volume was 50 mL (1.0 M) which was aspirated directly into flame atomic absorption within two hours of collection. Fresh standards were prepared, as previously described, to sample molarity each sample day. Concentrations of zinc were calculated from standard curves and quality control was ensured through the inclusion of process blanks of acidified double deionized water (Appendix B: Tables 13 and 14).

Algal Tissue Zinc

Algae samples were collected to monitor the accumulation (n=6, 3 replicate day^{-1}) and clearance (n =3, 3 replicate day^{-1}) of the zinc. The procedure involved scraping material off the walls, using a fine mesh fish net, in 3 locations between the input and

stand pipe. The diatomaceaous material was then rinsed from the net with head tank water into a clean, labeled 250 mL plastic beaker. Once in the laboratory the contents of each beaker were filtered onto labeled, pre weighed, ashless cellulose filters (Whatman 42, 2.5 µm retention). Dried samples were digested in 10 mL, 10.0 M Bakers nitric acid with a two stage CEM microwave digestion recipe set at 95% power for 8 minutes followed by 85% power for 6 minutes. Final sample volume was 11 mL (5.45 M). Standards were prepared to sample molarity in Bakers nitric acid and double deionized water. Quality control and assurance were ensured through the inclusion of process blanks, filter blanks and NIST tomato leaf reference material (Appendix B: Tables 13 and 14).

Invertebrate Samples

Caddisflies were collected to track the accumulation (n=6, 3 replicates day $^{-1}$) and clearance (n=2, 3 replicate day $^{-1}$) of zinc. The collected invertebrates were allowed to depurate gut contents in header tank water for over four hours, then euthanesticized with the addition of 95% ethanol. The caddisflies were removed from their casings, rinsed in double deionized water, then dried at 60° C for at least 48 hours. After cooling to room temperature, dry weights were determined to the nearest 0.1 mg (Mettler AE 240) and the biomass transferred to acid washed, plastic 15 mL graduated centrifuge tubes. Digestion took place within the centrifuge tubes with 2.0 mL 16.0 M Bakers nitric acid using a conventional microwave. With the caps loosened, 48 samples at a time were processed through 3 stages at full power (95° C) for two minutes each. To promote homogeneous digestion samples were agitated and rotated between stages. Digests were diluted up to a final volume of 6 mL at ~ 5.3 molar and allowed to settle in the fridge prior to being aspirated into the AAS directly from the centrifuge tubes. Fresh standards were prepared in Bakers nitric acid 5.33 molar matrix. Quality control and assurance were ensured through the inclusion of process blanks and NIST oyster tissue reference material digested in the conventional microwave (Appendix B: Tables 13 and 14).

Algal Taxon Samples

In order to track population changes one 11 cm^2 tile was sampled from each stream on 4 occasions starting 10 days prior to stocking the caddisflies. Material was scraped into a 2 dram glass vial and preserved and mounted according to procedures outlined in Lowe and LaLiberte (1996) for semipermanent mounts of soft algae. The procedure is a modification by Stevenson (1984) of Taft's (1978) glucose mounts. Briefly, the material is preserved in a clear 2% glutaraldehyde fixative, then mounted into a mixture of Taft's glucose, which consists of formaldehyde and light Karo corn syrup. There was not enough material to perform diatom cleaning, which involves oxidizing the organic material such that only silica cell walls of the diatoms remain. Through cell morphology it was still possible to identify the diatoms to family, genus level within the soft mounts.

Data Analysis

All data were $\log_e$ transformed prior to analysis to stabilize variance and create a normal distribution for parametric analysis. The nutrient data were treated with one way ANOVA to test the among stream variance. The caddisfly dry weights were regressed over time (up until day 22) for each treatment group and tested for homogeneity of variance in the slopes by the analysis of covariance outlined in Zar (1984). Chlorophyll *a* data were sub-divided into accumulation phase (day 0 – day 24) and clearance phase (day 24 – day 38). These growth curves were evaluated with the analysis of covariance. Significant differences among slopes were determined with a student Newman Keuls (SNK) multiple range test. To determine relationships between zinc in water and algae, algae and caddisfly ($\mu g\ g^{-1}$) Pearson product correlations were employed (Minitab v 10.2). Time series of algal and caddisfly tissue zinc were treated with a repeated measures ANOVA (SAS v 6.12) which takes into account the contributions from both the time and interactions between time and treatments. Significant differences among treatments on each sampling day were determined with REGWQ multiple range test. Dissolved zinc had numerous non detection points, therefore, these data were analyzed using a univariate general linear model substituting the mean square from the streams

nested in treatments as the denominator in the F ratio (SAS, 1985). Algal tissue zinc accumulation (day 3 – 22) and clearance (day 22 – 35) were treated as regressions over time. Among treatment slope coefficients were tested for significant differences with the analysis of covariance and SNK multiple range test (Zar, 1984).

Algal taxon data were ranked as follows: 3 (dominant), 2 (abundant), 1 (present), 0 (not present). Unfortunately the data base was not robust enough to be subjected to a multivariate analysis, and the general linear model produced trends in the errors even after square root transformations. The samples were pooled over the 4 days and nonparametric measures of heterogeneity were calculated for each stream using all taxon. These nonparametric procedures were repeated for the diatom taxa only. The Simpson's Index and Shannon Wiener Functions discussed in Krebs (1999) were employed and differences among treatments tested with a one way ANOVA. Additionally, changes in diatom abundance over time revealed important effects, thus histograms of the proportion diatom abundance were plotted, against time, by treatment group.

Results

Aqueous Dissolved Zinc

Dissolved zinc was measured in order to verify nominal concentration. Medians, the points (50^{th} percentile) in which an equal number of data points lie above and below and means, the average of all the data points, for the dissolved zinc measures were influenced by non detection in the lowest treatment streams (<10 $\mu g\ L^{-1}$). These data points have been omitted from the analysis. The means and sample sizes from Figure 14 are presented below in Table 5.

Table 5: Accumulation/ Clearance bioassay: Dissolved zinc pooled over both accumulation and clearance periods. SE denotes standard error. Treatments from Low to High are 10, 60 and 120 μg Zn L^{-1} nominal concentrations respectively.

Treatment Group	Mean ($\mu g\ L^{-1}$)	SE	Sample size
Low	22	5.08	16
Medium	29	2.07	32
High	64.5	5.15	29

The time series of dissolved zinc indicates treatment effects are only marginally significant ($F=5.84$, $\rho < 0.10$, ANOVA; Figure 15). The data set was dominated by the variance within treatments and time. Concentrations were approximately half of projected nominal levels, due to significant uptake in the algae.

Algal Tissue Zinc

Algal tissue zinc was analyzed to test accumulation and clearance characteristics and to link accumulated levels to ambient dissolved zinc. The means and standard error for the treatment groups are tabulated below (Table 6), with full distributions shown in box plots (Figure 14).

Table 6: Accumulation/ Clearance bioassay: Algal tissue zinc pooled over accumulation and clearance periods. SE denotes standard error. Treatments from Low to High are 10, 60 and 120 μg Zn L^{-1} nominal concentrations respectively.

Treatment Group	Mean (μg g^{-1} d.w.)	SE	Sample size
Low	579.53	24.05	54
Medium	1177.66	82.43	54
High	2318.15	202.84	54

Algal zinc was related to dissolved zinc ($r^2 = 0.410$; Figure 17); however, the correlation suffered from poor instrument sensitivity and numerous non-detection points.

Time series of the complete data set confirms that accumulation was proportional to the ambient dissolved zinc ($F = 331.41$, $\rho = 0.0003$, ANOVA; Figure 15). All treatments were significantly different after 10 days and remained so until 4 days into the clearance stage ($t = 26$), when they converged. Not enough data points were available to characterize the initial adsorption phase(day 0 – 3); however the gradual linear uptake that followed was characterized (Table 7). The results suggest that this second phase of uptake was independent of the concentration of zinc in the channels, with both medium and high treatments showing no difference in slope coefficients.

Table 7 Accumulation/ Clearance bioassay: Algal tissue zinc accumulation and clearance rates.

Treatment Group	Accumulation rate Slope coefficient	r^2	Clearance rate Slope coefficient	r^2
Low	0.0147*	0.162	-0.0225*	0.192
Medium	0.0348	0.683	-0.1020*	0.854
High	0.0388	0.553	-0.1394*	0.847

Note: Accumulation: day 3 – 22, 5 sample days with 3 replicates per day. Clearance: day 22 – 35, 4 sample days with 3 replicates per day. R squared values to the right of the coefficients correspond to the $\log_e$ transformed linear relationships. * indicates significant difference among slope coefficients (ANCOVA, $p < 0.05$).

The rate of clearance in the algae was proportional to the total accumulated levels (including both adsorbed and absorbed portions). All slope coefficients describing the clearance rates were significantly different (Table 7). Half times calculated from the high and medium treatments were approximately 3 and 4 days, respectively. After four days, clearance was 61, 48 & 20 % of the total zinc accumulated in high, medium and low treatments, respectively. These values increase to 83, 75 & 27 % after thirteen days of clearance. Less than ten days into the clearance period, algae collected from all streams showed no significant difference in elemental zinc content. However, the overall mean remained elevated in comparison to that calculated for day = 0, indicating that algae may make good longer term indicators of periodic metal input to freshwater systems. Algae was a better measure of biological available zinc, when compared to direct measures of dissolved species. This is because algae continuously integrated the abiotic zinc concentrations and detection was not a problem.

Invertebrate Tissue Zinc

In order to test whether significant food chain transfer of zinc occurred the concentration in the tissue of grazing caddisflies were analyzed. The means and standard errors for the treatment groups are presented in Table 8 and illustrated in Figure 14. Algal zinc correlated with caddisfly zinc ($r^2 = 0.743$; Figure 17). The strength in this correlation suggests that the grazing caddisflies are accumulating metal from the food (algae).

Table 8: Accumulation/ Clearance bioassay: Caddisfly tissue zinc pooled from both accumulation and clearance periods. SE denotes standard error. Treatments from Low to High are 10, 60 and 120 μg Zn L^{-1} nominal concentrations respectively.

Treatment Group	Mean (μg g^{-1} d.w.)	SE	Sample size
Low	414.62	12.47	48
Medium	623.41	43.38	46
High	949.67	60.74	46

Time series of μg Zn g^{-1} d.w. illustrated large variance among medium and high treatments up until day twenty two when the high treatment increased to 1400 μg g^{-1} d.w., whereas those in medium streams dropped to below 800 μg g^{-1} d.w. ($F = 81.02$, $\rho = 0.0025$, ANOVA; Figure 16). Body burden time series illustrated that caddisflies from both high and medium treatments showed very similar zinc burdens up until day 22; after which they dramatically diverged to create the only significant point ($F = 19.51$, $\rho = 0.0191$, ANOVA; Figure 16). The clearance pattern for body burden (μg $individual^{-1}$) is similar to that observed in the data normalized to dry weight; caddisflies from high treatments persisted with high zinc burdens even though those in the algae had been reduced. After nine days of clearance caddisflies had reduced total accumulated zinc by 48, 43 & 15 % in high, medium and low treatments respectively. Thirteen days into the clearance period, caddisflies from the high streams were unchanged, while those in the medium and low streams managed to clear 46 & 31 % of their peak levels respectively. Caddisflies from medium streams seem to be reducing zinc burdens even before treatments were reduced to ~10 μg L^{-1}. The data suggests that even though grazing invertebrates maintain lower levels of zinc, they retain it longer than algae. Invertebrates that tend to have less biomass, such as those from the high treatment stream channels, will show a higher tissue zinc concentration when normalized to dry weight.

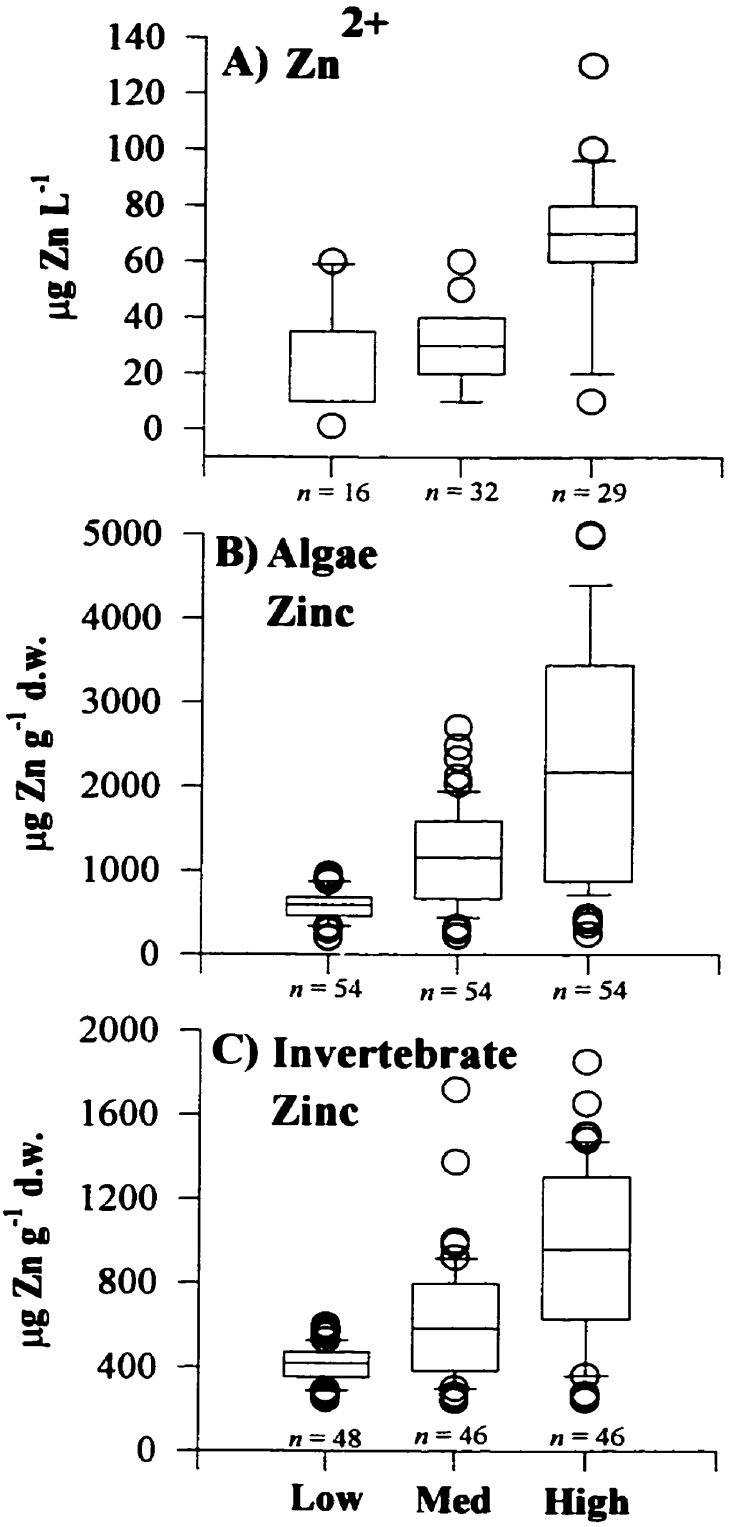


Figure 14: Accumulation/Clearance bioassay: Box plots pooled over the 35 day bioassay **A)** dissolved zinc, **B)** algal tissue zinc and **C)** caddisfly tissue zinc. Treatment groups defined by 10 , 60 and 120 µg Zn L^{-1} nominal concentrations for Low, Medium and High respectively. Box divided at the median (50^{th}) encloses the upper and lower quartiles ($25 - 75^{th}$). Capped bars show the $10 - 90^{th}$ percentiles and all data points outside the 90^{th} as circles.

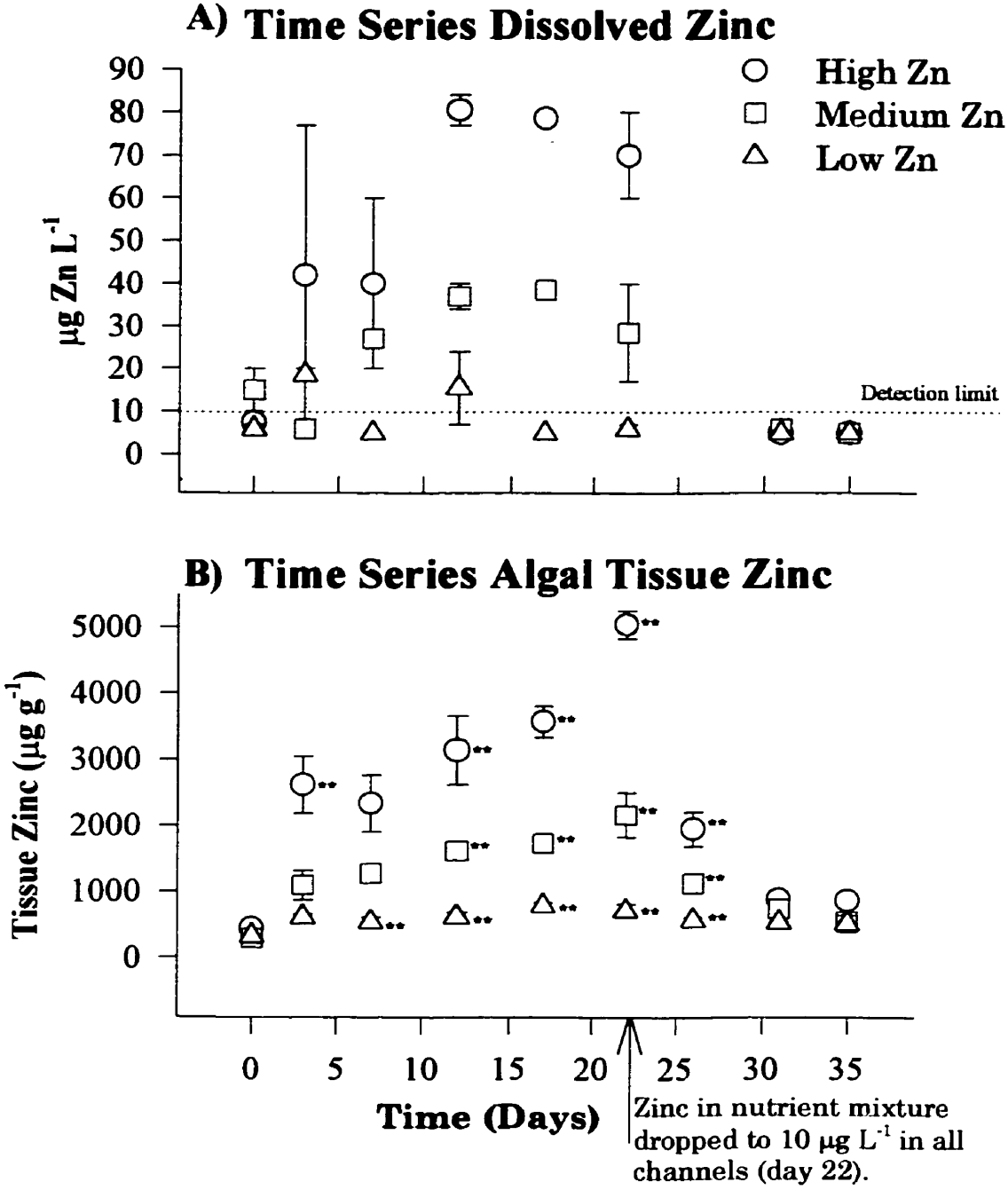


Figure 15: Accumulation/Clearance Bioassay: Low, Medium and High correspond to 10, 60 and 120 µg Zn L^{-1} nominal concentrations delivered in nutrient mixtures. 2 Channels per treatment.

A) Time series dissolved zinc (means with SE bars, $n = 2$ per treatment). Significance among treatment precluded by large variance over time and numerous non - detection points which were dropped from the analysis.

B) Time series algal tissue zinc (means with SE bars, $n = 2$ per treatment). REGWQ multiple range comparison among treatments on each sample day ($\rho < 0.05$ **).

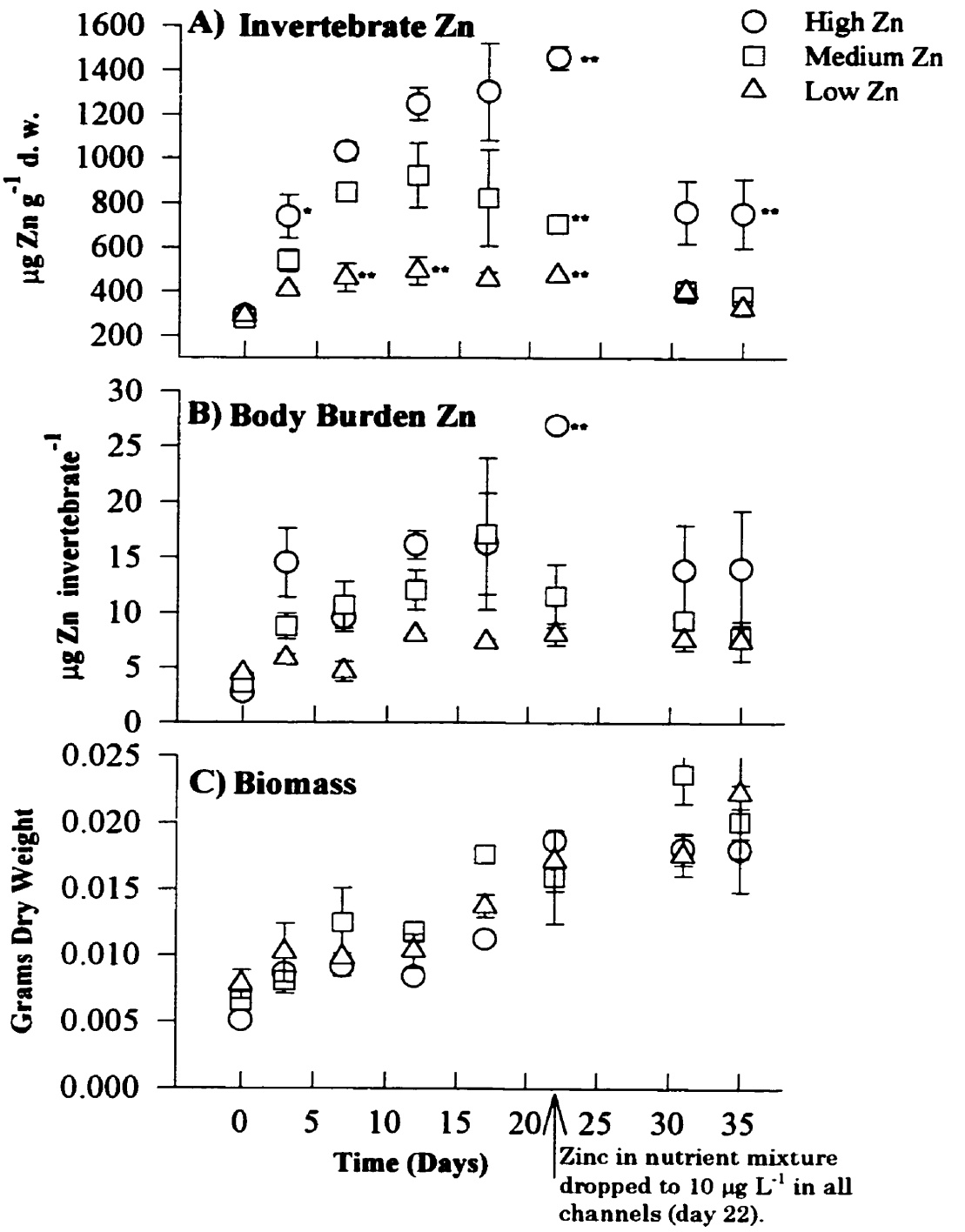


Figure 16: Accumulation/Clearance Bioassay: Invertebrate Zinc and Biomass. All symbols are means with SE bars, n= 2 per treatment. REGWQ multiple range comparison among treatments on each sample day ($\rho < 0.05$ **), significantly higher than low only *. Treatments: 10, 60 and 120 µg Zn L^{-1} nominal concentration for Low, Medium and High respectively. **A)** Time series caddisfly tissue zinc (µg g^{-1}). **B)** Time series body burden zinc (µg $individual^{-1}$). **C)** Time series caddisfly growth curves. No significant difference in slopes ($\rho > 0.05$, ANCOVA).

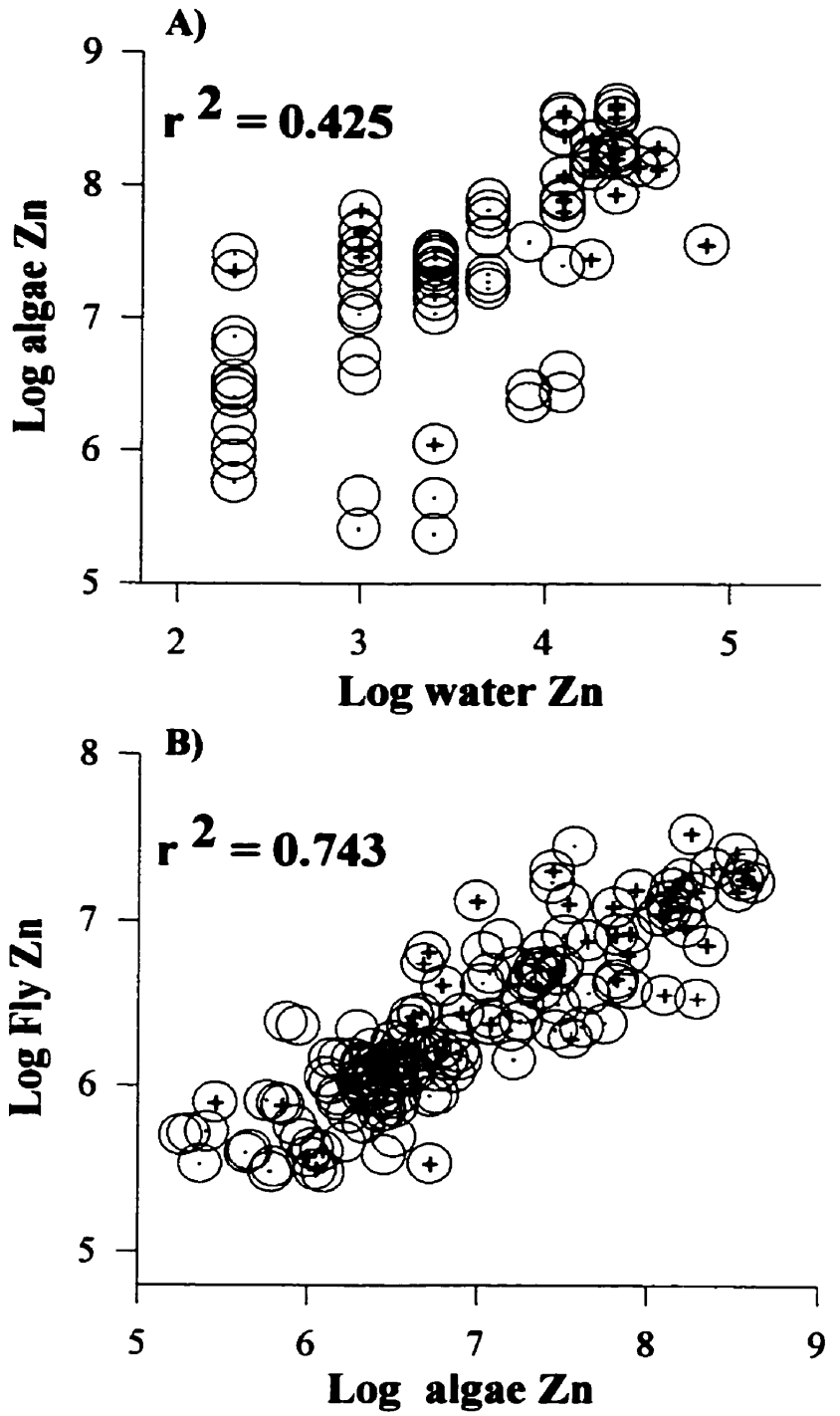


Figure 17: Accumulation/Clearance Bioassay: Correlation of Log_e transformed data pooled from both accumulation (0 - 22 days) and clearance (22 - 35 days) periods. Plain circles - Low (10 µg Zn L^{-1}), circles with dots - Medium (60 µg Zn L^{-1}) and circles with crosshairs High (120 µg Zn L^{-1}).

A) Water zinc – Periphyton zinc. B) Periphyton zinc – Caddisfly zinc.

Algal Pigments

Algal pigments were analyzed in order to detect toxic effects from the zinc treatments. The time series plot of the algal pigments are presented in Figure 18. There is a dramatic reduction in all pigments measured after the introduction of the caddisflies to the streams, day = 0. This effect was not heavy grazing pressure, but a physical disturbance on the loosely established benthic periphyton. The carotenoid pigments paralleled the trend of the chlorophyll *a* and will not be discussed further. Phaeopigment remained low and constant among all treatments until very late in the experiment when levels increased substantially in the medium and low treatments. The ratio of Chlorophyll *a* : Phaeopigment is maintained above 1 until the clearance phase when values increase dramatically. The accumulation phase showed dose related reductions in the algal growth curves. The high treatment shows very little growth, in fact, without the data point from day 24, the slope coefficient would not be significantly different from zero. Chlorophyll *a* regression data for the accumulation phase are tabulated below (Table 9).

Table 9: Accumulation/ Clearance bioassay: Chlorophyll *a* accumulation phase (day 0 –24) growth regression over time (n = 12 per treatment).

Treatment Group	r^2	Slope ρ value
Low	0.483	<0.0001
Medium	0.514	<0.0001
High	0.150	= 0.061

Note: Treatments from Low to High are 10, 60 and 120 µg Zn L^{-1} respectively.

Residuals were normally distributed and did not violate any regression assumptions. Growth curves were significantly different ($F = 5.572$, $\rho < 0.001$, ANCOVA) and the multiple range test produced the following results (Table 10).

Table 10: Accumulation/ Clearance bioassay: Chlorophyll *a* accumulation phase SNK multiple range test among treatment slopes. Treatments from Low to High are 10, 60 and 120 μg Zn L^{-1} respectively.

Comparisons	Slope Q ratio	ρ value
Low & Medium	0.49534	N/S
Low & High	4.26604	~ 0.01
Medium & High	4.76140	< 0.005

Clearly the highest treatment streams have reduced chlorophyll *a* to such an extent that no growth was observed within the three samples taken inside the accumulation period. All regression relationships for the clearance phase (day 24 - 38) produced significant slope coefficients with the lowest r^2 of 0.421 from the low treatment ($F = 3.66$, $\rho < 0.02$, ANCOVA). However, this significance was related to differences in the intercepts, not the slopes. Therefore, benthic periphyton in all stream channels showed healthy growth after the zinc stress was removed, indicating this biotic component of the benthos has significant resilience.

Algal Composition

Algal compositional changes were monitored to detect toxic effects from the zinc treatments. Overall algal composition was balanced between bacillariophyta (diatoms) and chlorophyta (green algae) with lesser amounts of cyanophyta (blue green algae) Bacillariophyta were dominated by genera from families Fragilariaceae and Naviculaceae. Chlorophyta was represented by many families, Gloeocystaceae, Ulotrichaceae and Zygnemataceae the dominant ones. The cyanophyta were dominated by members of the family Chroococcales (Appendix C: Figure 20). Table 11 shows the diversity indices of Simpson's and Shannon-Wiener expressed in terms of genus #'s with standard errors along with the relative proportion of green algae (greens) to diatoms. Although the diversity indices show decreases with increasing zinc, significance was precluded by the large variance, particularly in the high treatment. The ratio of greens : diatoms increases with increasing zinc; however, the significance level is only $\rho = 0.10$.

Table 11: Accumulation/ Clearance bioassay: Heterogeneity indices and greens : diatom ratio for complete algal populations (± SE, $n = 2$). Treatments from Low to High are 10, 60 and 120 µg Zn L^{-1} respectively.

Index	Low Zn	Medium Zn	High Zn
Simpson's	14.93 ± 1.22	12.81 ± 0.984	11.27 ± 3.170
Shannon –Wiener	18.05 ± 0.258	15.72 ± 0.583	14.09 ± 3.351
Greens : Diatom ratio	0.5335 ± 0.0022	0.6479 ± 0.1121	0.7848 ± 0.0848

Histograms of the proportion of diatoms are presented in Figure 19. The early collections from Seymour tributary were dominated by chain like *Fragilaria* and this was the case throughout all the streams on May 05, 1998, 10 days before treatments started. By day 17 the distribution of diatoms in the lowest & medium treatment stream chambers were evenly spread between several genera in families Fragilariaceae and Naviculaceae, while high treatment streams remained heavily weighted toward one genus, *Synedra*. Day 31, 9 days into the clearance phase of the experiment a new genus, *Gomphonema* joined the already diverse communities in the low and medium stream chambers, whereas increases in family Naviculaceae marked the high streams. By day 38 the high streams remained dominated by *Synedra* (> 50%); however, *Gomphonema* and *Meridion*, which had previously not been detected in either high streams, occurred in both. Simpson's index was significantly reduced in high streams ($\rho < 0.10$) and that from Shannon – Wiener was significantly reduced at $\rho < 0.05$.

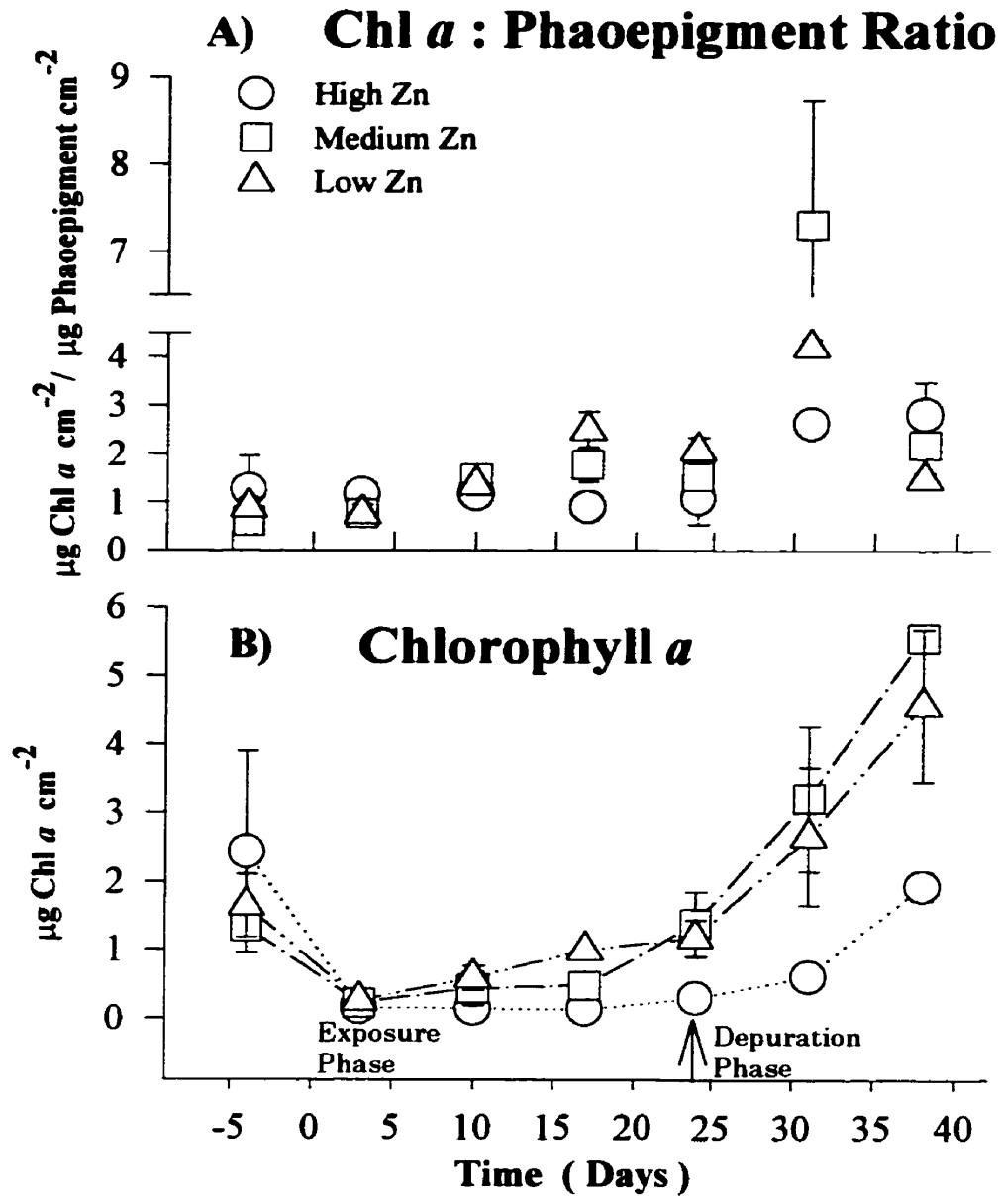


Figure 18: Accumulation/ Clearance Bioassay: Algal Pigment Analysis. Means with SE bars, $n = 2$ per treatment. Treatments: 10, 60 and 120 µg Zn L^{-1} nominal concentrations representing Low, Medium and High respectively.

A) Chlorophyll *a* (Chl *a*) : Phaeopigment ratio.

B) Chlorophyll *a* growth curves from 4 day prior to introducing the caddisflies through until 16 days into the clearance phase. Through the accumulation phase high treatments had reduced algal growth ($\rho < 0.05$, ANCOVA). Clearance phase slopes were not significantly different.

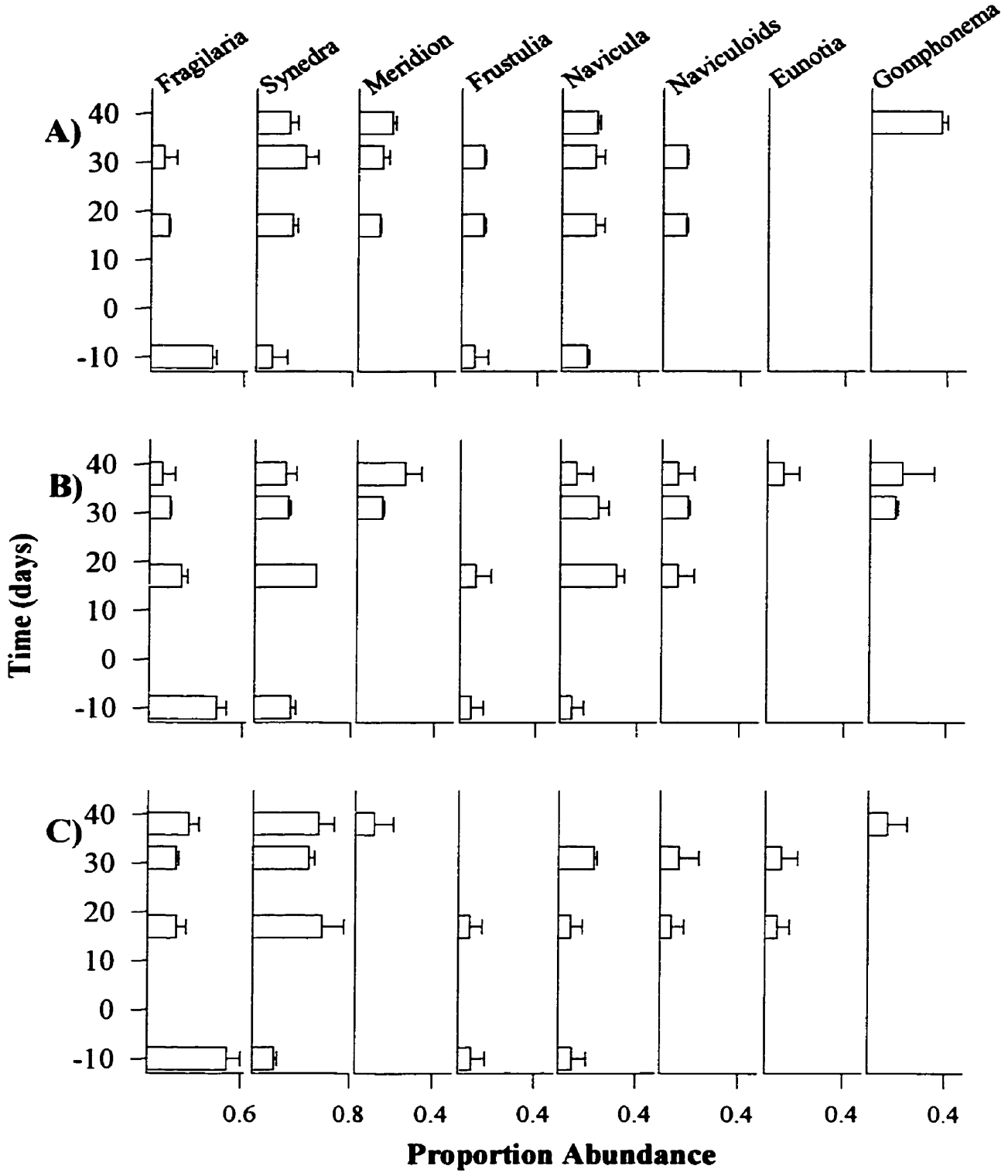


Figure 19: Accumulation/ Clearance Bioassay: Histograms of proportion abundance through time for dominant diatoms with SE bars, $n = 2$ per treatment. **A)** Low zinc (10 µg L^{-1}); **B)** Medium zinc (60 µg L^{-1}) **C)** High zinc (120 µg L^{-1}) all nominal concentrations. Treatments begin day 0 and stop day 22. Shannon - Wierner index of heterogeneity reduced in the high treatments ($\rho < 0.05$, ANOVA).

Discussion: Accumulation/ Clearance bioassay

Uptake and Clearance of Zinc

No ameliorating effects of the high (~100 $\mu g\ L^{-1}$) iron in the nutrient mixture were apparent in the bioaccumulation data. Several factors combined will affect metal bioavailability, but as noted by Casini and Depledge (1997), changes in metal speciation appear to be required. It is unlikely that significant iron oxides formed in the 43 minutes the oxygenated mixture circulated. The rate of oxidation, $Fe^{2+} \rightarrow Fe^{3+}$ is highly sensitive to pH. At pH 7.0 the $t_{1/2}$ is 36 minutes; however at pH 6.5 the $t_{1/2}$ becomes 360 minutes (Davison and Seed, 1983). The pH range in the bioassay water, which was supplied from the Seymour watershed falls between 5.7 – 6.2.

The uptake of zinc in the periphyton was proportional to the ambient dissolved concentration, consistent with previous studies (Cushing and Rose, 1970; Saygideger, 1998). The accumulation, especially with $\log_e$ transformed data (Appendix C: Figure 21), suggest a two phase uptake, which has been described by Genter (1996): 1st rapid metabolism independent phase with binding to cell walls and external surfaces; 2nd slower metabolism dependent phase with transport across cell membranes. There is still uncertainty as to whether periphyton zinc adsorption/absorption reaches saturation or not. A continuous linear increase in algal tissue zinc through the 22 day accumulation phase was observed in this study. In contrast, saturation kinetics have been reported by several authors who have employed radio tracers Bachmann (1963), Gutknecht (1965) and Knauer (1996). Cushing and Rose (1970) attributed the asymptotic relationship they observed to decreasing ambient levels of ^{65}Zn. When they corrected this by maintaining constant flow-through chambers, no saturation occurred. Growth rate can influence accumulation and has likely contributed to the linear uptake of zinc in the current study. Bates et al., (1985) noted zinc transport into *Chlamydomonas variabilis* increased linearly during exponential growth phase, then seemed to plateau.

There are two separate mechanism driving the apparent two phase uptake observed. Adsorptive accumulation, which Genter (1996) called biosorption is the mechanism driving the 1st phase of uptake. This rapid and reversible process is not influenced by light, temperature or metabolic inhibitors (Garnham et al., 1992) and occurs with dead periphyton (Cushing and Rose, 1970). Biosorption can account for substantial total amounts of metals accumulated. Garnham et al., (1992) reported 50% of measured zinc in *Chlorella salina* was attributed to biosorption. Adsorbed metal can be easily rinsed away with distilled water, or a chelator such as EDTA. Although algal material in this study was filtered with de-ionized water, a considerable proportion of the measured zinc could still be attributed to biosorption. The rapid reduction in algal tissue zinc through the clearance phase supports the conclusion that significant amounts of zinc were adsorbed, rather than internally absorbed. EDTA should be employed in rinsing if it is necessary to distinguish between adsorbed and absorbed metal.

Absorptive accumulation, which is the mechanism driving the 2nd phase of uptake, involves either active transport or passive diffusion. This mechanism is characteristically slower (hours to days) and can be influenced by temperature, light, metabolic inhibitors and the growth medium (Garnham et al., 1992). Cushing and Rose (1970) noted a cyclical uptake of ^{65}Zn during 12 hour photoperiod experiments, increasing during daytime and dropping during dark respiration. Photosynthesis consumes CO_2 (dropping pH) and produces O_2 in the microhabitat surrounding the cells, with the opposite occurring during respiration (Genter, 1996). Cushing and Rose (1970) suggested that this pH shift was the driving mechanism that lead to the cyclical uptake observed, rather than direct metabolic mechanisms.

Accumulation of zinc by the grazing caddisflies confirmed food chain transfer, which agrees with the Fall, 1997 Bioavailability bioassay results. Regulation of zinc, an essential metal, may have occurred with these invertebrates. The strength of the correlation between caddisfly zinc and algal zinc suggest that most zinc is being taken up through the food supply. These larval caddisflies also have abdominal gills, which may be a significant route of exposure.

Toxicological Effects

Reduced algal growth was observed in the highest treatments streams (> 50 µg Zn L^{-1}). Total biomass measures like chlorophyll *a* may not respond to stress on a community scale if the abundance of sensitive species are replaced by tolerant populations. In this study, the suppression of the pigments was simply due to decreased density and was not related to interference in the pigment metabolism within each individual cell (Saint – Louis et al., 1994). Zinc may be interfering with the uptake of PO_4 , or precipitating out PO_4, creating possible PO_4 limitation and inhibiting growth in the high streams (Kuwabara, 1985 and Verma, 1993). Phaeopigments are generally considered to be a result of chlorophyll degradation (Round, 1981). The dramatic increase of these pigments toward the end of the clearance phase was likely due to increased senescence within the thickening periphyton.

On a population level toxicological effects were apparent in the highest treatment streams. The diatom genera *Frustulia*, *Meridion* and *Gomphonema* appeared to be the most sensitive to zinc, while *Synedra*, *Fragilaria* and various naviculoids were less sensitive. *Eunotia*, which was not observed in low treatments, seemed to prefer high levels of zinc. Experimental studies by Williams and Mount (1965) concluded that *Synedra ulna* was highly tolerant of zinc. Field surveys in the New Lead Belt of Missouri (Wixson and Bolter, 1971) determined three diatom genera were useful indicators of mine discharge. *Synedra* and *Navicula* were found to be tolerant, while *Cymbella* was intolerant. Genter et al. (1987), in a series of outdoor stream mesocosm studies spanning three seasons, concluded zinc treatments as low as 50 µg L^{-1} had communities dominated by greens and blue greens, especially in the summer. Population effects in diatoms proved to be a sensitive indicator of the zinc gradient. Genera dominating controls and lacking from zinc treatments were *Melosira /Cocconeis*, *Gomphonema* and *Navicula* in spring, summer and fall respectively. *Fragilaria*, *Achnanthes* and certain *Nitzschia* species were all less affected by the zinc. With green

algae, Coccoid and Oocystis families dominated in high treatment, while filamentous *Ulothrix* did well in controls.

Conclusion

Zinc uptake in periphyton was proportional to ambient dissolved zinc. Full saturation was not observed, probably due to the fact that the algal biomass was increasing throughout the experiment. Levels of tissue zinc in the grazing caddisflies were related to those in the periphyton and body burden was regulated in these invertebrates. The rapid clearance of the tissue zinc from the periphyton is further evidence of the highly sensitive nature of this biological material to the ambient metal concentrations. In a natural setting invertebrates may provide a better indicator of periodic metal exposure, as they seem to retain metal burdens over longer periods. Periphyton community level effects are evident with decreased chlorophyll *a*, at zinc concentrations exceeding 50 $\mu g\ L^{-1}$. Consistent with Genter et al., (1987), population changes occurred when ambient zinc levels exceeded 50 $\mu g\ L^{-1}$, with the exclusion of sensitive diatoms and the promotion of tolerant chlorophyta and cyanophyta. More attention to sampling and enumeration of benthic algae would increase the ability to detect slight changes, thus increasing the statistical power of the system with only 2 channels per treatment.

4 Chapter 4 : Overall Conclusions

Provincial and Federal Environment Ministries in cooperation with the Department of Fisheries and Oceans administers and monitors compliance with the regulations and guidelines under the Metal Mining Liquid Effluent Regulations (MMLER). This environmental code limits the concentrations of arsenic, copper, lead, nickel, zinc, total suspended matter and radium ^{226} that may be discharged in effluent from new, expanded and reopened metal mines. Existing mines, such as Sullivan Mine, are not bound by the specific law; however they are expected to meet the same numerical values set forth in the MMLER. The only biological aspect of the regulations is Environment Canada's guidelines for an acute lethality bioassay in which rainbow trout are exposed to undiluted effluent for 96 hours. These regulations do not fully address possible impacts on biological integrity within the receiving environment. More sensitive biological monitors within the receiving environment should be considered for inclusion in the regulations.

A variety of organisms have been examined as biomonitors for metals including fish, benthic invertebrates, macrophytes and algae. The effectiveness of any one organism as a biomonitor depends on our understanding of the organism and their interactions with metals and, most importantly, their limitations as a biomonitor. When the receiving water is a stream or river with a cobbled bottom, benthic periphytic algae and invertebrates offer an advantage over fish. With the exception of drifting and mobile invertebrates, these organisms are sessile and able to spatially and temporally integrate the inorganic contamination. Algae have been shown to respond rapidly to the changing abiotic conditions in controlled microcosm studies where a similar composition existed; however, in the natural setting it was much more problematic. Natural periphytic algae are indicative of metal bioavailability, but finding similar forms of algae over a given reach in the river and associated inorganic debris make spatial comparisons difficult. Benthic invertebrates, and particularly the filter feeding caddisflies were abundant and found to be the best overall biomonitors in the St. Mary River.

Both experimental and field evaluations support the conclusion that algae serve as a sink for bioavailable zinc, proportionally accumulating concentrations tens of thousands of times greater than ambient levels. Zinc levels in St. Mary periphyton, immediately downstream from Mark Creek, were elevated in relation to reference concentrations by a factor of 65; which was reduced to a factor of 10, 27 Km downstream at Wycliffe. In the microcosms algae responded quickly to decreases in the dissolved zinc with rapid clearance of accumulated amounts. High concentrations (> 50 $\mu g\ Zn\ L^{-1}$) were linked to impaired algae growth and limited biodiversity in diatom populations in controlled microcosm studies; however, this has yet to be verified in the St. Mary River.

Zinc accumulated in the autotrophic base of the food web within rivers and streams can be transferred up the food chain through algae grazing and filter feeding on drifting organic debris. Conclusions from both these experimental and field studies support the hypothesis that invertebrate feeding habit influences the whole body zinc concentrations. Hydropsychidea caddisflies and Baetidea mayflies have been proven to be tolerant of heavy metal contamination and represent valuable world wide biomonitors for inorganic contamination in freshwater rivers and streams. In conjunction with periphyton, invertebrates integrate the overall contamination in the system, thus provide a more consistent monitor when compared to water sample analysis.

Geographical comparisons in the St. Mary River support the conclusion that zinc released from the ARD treatment plant is less bioavailable than discharges from Mark Creek. Although there are a number of abiotic factors that may contribute to this apparent reduced bioavailability, experimental results from 1997 suggest that iron oxide formation in the treatment process can be a significant factor.

Application of Research

The results of this research can be applied toward remedial management action at the Sullivan Mine and various other metal mines. For example, in cooperation with the town of Kimberley, sewage could be released upstream in Mark Creek promoting

periphyton growth. This growth would act as a biological sink for the dissolved zinc resulting in reduced discharge to St. Mary River. A metal analysis on the sewage treatment plant release may indicate whether or not metal loading is occurring from this source. It is likely that Mark Creek discharges hugging the North shore contribute to iron staining along the old gypsum ponds. General health of the river in this region is impaired, which may be related to elevated metal concentrations from Mark Creek, or a disruption in the habitat due to iron oxide deposits. Reclaimation of this habitat, by either reducing Mark Creek discharges, or cleaning the iron deposits could promote healthy periphyton growth.

Tissue analysis data should be included in every baseline study for proposed new mine sites. In addition to quantifying natural background contaminant levels, the technique could prove valuable as a prospecting tool, such as occurs with some terrestrial plants. With further field data and controlled microcosm studies, predictive quantitative models can be developed. These models can be used in risk assessments and decision analysis to help quantify transfer into the primary and secondary production in a given freshwater ecosystem. The water quality data would be treated as stochastic (having a random natural variation) and could be expressed in terms of probabilities (Figure 3). A random selection from the distribution would be fed into a model, the output of which would be whole body metal concentrations for a given biological component ($\mu g\ g^{-1}$). Model parameters would have their own probabilities reflecting uncertainty in the model form and fit. This process would be repeated several thousand times with a Monte Carlo simulation software such as Crystal Ball v. 4.0 producing forecasted tissue contaminant concentrations which could then be utilized to predict impacts or predator transfer (Figure 22). Future research toward this goal would be focused on the uncertainty surrounding models parameters.

5 References

Allen, J.D., 1981. Predator – prey relationships in streams. In: J.R., Barnes and G.W., Minshall [Eds], Stream Ecology, Application and Testing of General Ecological Theory. Plenum Press, New York.

Allen, J.D., 1995. Stream Ecology: Structure and Function of Running Waters. London. Chapman Hall.

Aloi, J., 1990. A critical review of recent freshwater periphyton field methods. *Can. J. Fish. Aquatic Sci.* **47**, 656-670.

APHA American Public Health Association. 1992. Standard Methods for Examination of Water and Wastewater. 18th edition. A.E., Greenberg, L.S., Clesceri, and A.D., Eaton [Eds].

Bachmann, J.D., 1963. Zinc ^{65} in studies of the freshwater zinc cycle. In: V. Schultz and A. Klement [Eds.], Radioecology. Reinhold.

BC Environment, 1976. Kootenay Air and Water Quality Study, Phase 1. Water quality in region 4, the lower Kootenay River Basin. Department of Environment, Water Resource Service (Excerpt).

BC Environment, 1981. APD Bulletin 20. Kootenay Air and Water Quality Study, Phase 2. Water quality in the Kootenay River basin. Ministry of the Environment, Assessment and Planning Division (Excerpt).

BC Environment, 1990. BC Historical Streamflow Summary. Ministry of the Environment.

BC Research, 1977. Biological Monitoring of the St. Mary River March 1976 – April 1977.

Braek, G.S., Malnes, D., and Jensen, A., 1980. Heavy metal tolerance of marine phytoplankton: Combined effects of zinc and cadmium on growth and uptake in some marine diatoms. *J. Exp. Mar. Biol. Ecol.* **42**, 39–54.

Brooks, R.R. and Rumsby, M.G., 1965. The biogeochemistry of trace element uptake by some New Zealand bivalves. *Limnol. Oceanogr.* **10**, 521-527.

Bryan, G.W., 1967. Zinc regulation in the freshwater crayfish (including some comparative copper analysis). *J. Exp. Biol.* **46**, 281-292.

Cain, D.J., Luoma, S.N., Carter, J.L. and Fend, S.V., 1992. Aquatic insects as bioindicators of trace element contamination in cobble-bottom rivers and streams. *Can. J. Fish. Aquat. Sci.* **49**, 2141-2154.

Campbell, P.G.C. and Tessier, A., 1989. Geochemistry and bioavailability of trace metals in sediments. In: A. Boudou. and F. Ribeyre [Eds.], Aquatic Ecotoxicology: Fundamental Concepts and Methodology. Vol. 2., CRC Press.

Casini, S. and Depledge, M.H., 1997. Influence of copper, zinc and iron on cadmium accumulation in the Talitrid Amphipod, *Platorchestia platensis. Bull. Environ. Contam. Toxicol.* **59**, 500-506.

Chan, H.M. and Rainbow, P.S., 1993. The accumulation of dissolved zinc by the shore crab *Carcinus maenas* (L.). *Ophelia.* **38**, 13-30.

Clements, W.H. and Kiffney, P.M., 1994. Integrated laboratory and field approach for assessing impacts of heavy metals at the Arkansas River, Colorado. *Enviro. Tox. Chem.* **13** (3), 397-404.

Clifford, H.F., 1991. Aquatic Invertebrates of Alberta: An Illustrated Guide. University of Alberta press.

Cox, E., 1993. Freshwater diatom ecology: developing an experimental approach as an aid to interpreting field data. *Hydrobiologia.* **269/270**, 447–452.

Crawford, K. and Luoma, S.N., 1994. Guidelines for Studies of Contaminants in Biological Tissues for the National Water-Quality Assessment Program. U.S. Geological Survey. Open File Report 92-494.

Crossland, N.O., Mitchell, G.C., Bennett, D. &and Maxted, J., 1991. An outdoor artificial stream system designed for ecotoxicological studies. *Ecotoxicology and Environmental Safety.* **22**, 175–183.

Cushing C.E. and Rose, F.L., 1970. Cycling of zinc ^{65} by Columbia River periphyton in a closed lotic microcosm. *Limnol. Oceanogr.* **15**, 762-767.

Dallinger, R.; and Wieser, W., 1984. Patterns of accumulation, distribution and liberation of Zn, Cu, Cd and Pb in different organs of the land snail *Helix pomatia* L. *Comp. Biochem. Physiol.* **79C**(1), 117-124.

Davison, W. and Seed, G., 1983. The kinetics of the oxidation of ferrous iron in synthetic and natural wastes. *Geochim. Cosmochim. Acta.* **47**, 67–79.

Emplain, A., 1976a. Estimation de la pollution par Metaux Lourds dans la Somme par l'Analyse des bryophytes aquatiques. *Bull. Francais Piscicult.* **48**, 138–142.

Emplain, A., 1976b. Les bryophytes aquatique utilises comme traceurs de la contamination en Metaux Lourds des Eaux Douces. *Mem. Soc. Royal Bot. Belgium.* **7**(11), 141–156.

EVS Environmental Consultants, 1996. Field Evaluation. Aquatic Effects Monitoring: Field Survey Report Sullivan Mine Site. Prepared for Natural Resources Canada CANMET. Ottawa, Ontario.

Foster, P., 1982. Species associations and metal contents of algae from rivers polluted by heavy metals. *Freshwater Biology.* **12**, 17-39.

Flum, T., Huxel, G.L., LaRue, C.S., Hardison, B., Duncan, J.R. and Drake, J.A., 1993: A closed artificial stream for conducting experiments requiring a controlled species pool. *Hydrobiologia.* **271**, 75-85.

Frison, T.H., 1942. Studies of North American Plecoptera. Natural History Survey Division. Urbana, Illinois.

Gardiner, R.T., Dawson, B.B. and Gibson, G.G., 1997. Application of ARD abatement technology in reclamation of tailing ponds at Cominco Ltd. Sullivan Mine. Fourth International Conference on Acid Rock Drainage Proceedings, Vol 1, Vancouver, BC.

Garnham, G.W., Codd, G.A. and Gadd, G.M., 1992. Kinetics of uptake and intracellular location of cobalt, manganese and zinc in the estuarine green alga *Chlorella salina. App. Microbiol. Biotechnol.* **37**, 270-276.

Genter, R.B., Cherry, D.S., Smith, E.P. and Cairns (Jr.) J., 1987. Algal – periphyton population and community changes from zinc stress in stream mesocosms. *Hydrobiologia.* **153**, 261-275.

Genter, R.B., 1996. Ecotoxicology of inorganic chemical stress. In: R. J. Stevenson, M.L. Bothwell and R.L. Lowe [Eds], Algal Ecology Freshwater Benthic Ecosystems. Academic Press.

Gerhardt, A., 1993. Review of impact of heavy metals on stream invertebrates with special emphasis on acid conditions. *Water, Air, and Soil Pollution.* **66**, 289-314.

Gerhardt, A., 1995. Joint and single toxicity of Cd and Fe related to metal uptake in the mayfly *Leptophlebia marginata* (L.) (Insecta). *Hydrobiologia.* **306**, 229-240.

Gotceitus, V. and Clifford, H.F., 1983. Life history of *Discosmoecus atripes* (Hagen) in a rocky mountain stream of Alberta. *Can. J. of Zoology.* **61**, 586–596.

Gruessner, B. and Watzin, M.C., 1996. Response of aquatic communities from a Vermont stream to environmentally realistic atrazine exposure in laboratory microcosms. *Environ. Tox. Chem.* **15**(4), 410–419.

Gutknecht, J., 1965. Uptake and retention of cesium ^{137} and zinc ^{65} by seaweeds. *Limnol. Oceanogr.* **10**, 58-66.

Gupta, S.L., 1989. Interactive effects of nitrogen and copper on growth of cyanobacterium *Microcystis*. *Bull. Environ. Contam. Toxicol.* **42**, 270-275.

Gupta, A., 1996. Heavy metals in water, periphytonic algae, detritus, and insects from two streams in Shillong, Northeastern India. *Environmental Monitoring and Assessment.* **40**, 215-223.

Hickey, C.W. and Clements, W.H., 1998. Effects of heavy metals on benthic macroinvertebrate communities in New Zealand streams. *Environ. Tox. Chem.* **17**(11), 2338–2346.

Jewett, S.G., 1959. The Stoneflies (Plecoptera) of the Pacific Northwest. Oregon State College Press.

Karr, J.R., 1991. Biological integrity: a long neglected aspect of water resource management. *Ecological Applications.* **1**, 66–84.

Kiffney, P.M. and Clements, W.H., 1994. Effects of heavy metals on a macroinvertebrate assemblage from a rocky mountain stream in experimental microcosms. *J. North American Benthological Soc.* **13**(4), 511–523.

Knauer, K., 1996. Interactions of trace metals (Cu, Zn, and Mn) with freshwater phytoplankton. Swiss Federal Institute of Technology, Zurich (Switzerland), abstract only.

Krebs, C.J., 1999. Ecological Methodology, 2nd Edition. Addison – Wesley Longman, Inc.

Kuwabara, J.S., 1985. Phosphorus – zinc interactive effects on growth by *Selenastrum capricornutum* (Chlorophyta). *Environ. Sci. Technol.* **19**, 417-421.

Lamberti, G.A., 1996. Role of benthic algae in lotic food webs. In: R. J. Stevenson, M.L. Bothwell and R.L. Lowe [Eds], Algal Ecology Freshwater Benthic Ecosystems. Academic Press.

Lamberti, G.A., Feminella, J.W., and Resh, V.H., 1987. Herbivory and interaspecific competition in a stream caddisfly population. *Oecologia.* **73**, 75–81.

Lamberti, G.A. and Steinman, A.D.[Eds], 1993. Research in artificial streams: applications, uses, and abuses. *J. North American Benthological Soc.* **12**(4), 313–384.

Lamberti, G.A. and Resh, V.H., 1985. Comparability of introduced tiles and natural substrates for sampling lotic bacteria, algae and macroinvertebrates. *Freshwater Biology* **15**, 21–30.

Leland, H.V., Fend, S.V. and Dudley, T.L., 1989. Effects of copper on species composition of benthic insects in a Sierra Nevada, California, stream. *Freshwater Biology.* **21**, 163–179.

Lowe, R.L. and LaLiberte, G.D., 1996. Benthic stream algae: distribution and structure. In: F.R. Hauer, and G.A. Lamberti [Eds], Methods in Stream Ecology. Academic Press Inc. San Diego.

Lowe, R.L. and Pan, Y., 1996. Benthic algal communities as biological monitors. In: R. J. Stevenson, M.L. Bothwell and R.L. Lowe [Eds], Algal Ecology Freshwater Benthic Ecosystems. Academic Press.

Matthews, W.J., Gelwick, F.P. and Gardner, T.J., 1990. A simple system of replicated recirculating experimental streams. *J of Freshwater Ecology.* **5**, 437–443.

McIntire, C.D., Garrison, R.L., Phinney, H.K. and Warren, C.E., 1964. Primary production in laboratory streams. *Limnology and Oceanography.* **9**, 92–102.

Morgan, G. and Henrion, M., 1990. Uncertainty: A guide to dealing with uncertainty in quantitative risk and policy analysis. Cambridge University Press. pp 89.

Merritt, R.W. and Cummins, K.W. [Eds]. 1978. An Introduction to the Aquatic Insects of North America. Kendall/Hunt Publishing Company.

National Museum of Canada, 1981. The freshwater molluscs of Canada. National Museum of Natural History, Ottawa, On. pp. 446.

Needham, J.G., Traver, J.R., and Yin-Chi, Hsu, 1969. The Biology of Mayflies – Systematic account of North American Species. Classey Publication, Hampton, Middlesex. England.

Nehring, B.R., 1976. Aquatic insects as biological monitors of heavy metal pollution. *Bull. Enviro. Contamination Tox.* **15** (2), 147-153.

Newman, M.C. and McIntosh, A.W., 1989. Appropriateness of *aufwuchs* as a monitor of bioaccumulation. *Environmental Pollution.* **60**, 83-100.

Nugegoda, D. and Rainbow, P.S., 1987. Zinc uptake and regulation by the sublittoral prawn, *Pandalus montagui* (Crustacea: Decapoda). *Estuarine Coastal Shelf Sci.* **26**, 619-632.

Oertel, N., 1991. Heavy metal accumulation in *Cladophora glomerata* (L.) Kutz in the River Danube. *Ambio.* **20** (6), 264-268.

Palmer, C.G. and Goetsch, P.A., 1997. The calibration of an artificial stream system used to investigate the tolerances of South African riverine invertebrates to selected water quality variables. *Arch. Environ. Contam. Toxicol.* **32**, 22–31.

Patrick, R. and Reimer, C., 1966. The diatoms of the United States exclusive of Alaska and Hawaii. Vol 1. Academy of Natural Sciences. Philadelphia.

Pentecost, A., 1984. Introduction to Freshwater Algae. Richmond Publishing Company, Surrey, Great Britain.

Pellegrini, M., Laugier, A., Sergent, M., Phantanlu, R., Valls, R., and Pellegrini, L., 1993. Interactions between the toxicity of the heavy metals cadmium, copper, zinc in combinations and the detoxifying role of calcium in the brown alga *Cystoseira barbata*. *J. Applied Phycology.* **5**, 351-364.

Pontasch, K.W. and Cairns, J.Jr., 1989. Establishing and maintaining laboratory – based microcosms of riffle insect communities: their potential for multispecies toxicity tests. *Hydrobiologia.* **175**, 49–60.

Poulton, B.C., Monda, D.P., Woodward, D.F., Wildhaber, M.L., and Brumbaugh, W.G., 1995. Relations between benthic community structure and metals concentrations in aquatic macroinvertebrates: Clark Fork River, Montana. *Journal of Freshwater Ecology.* **10** (3), 277-293.

Prescott, G.W., 1978. How to Know the Freshwater Algae, 3rd Edition.. WM. C. Brown Company Publishers. Dubuque, Iowa.

Rai, L.C., Gaur, J.P. and Kumar, H.D., 1981. Phycology and heavy metal pollution. *Biological Review.* **56**, 99-151.

Ramelow, G.J., Biven, S.L., Zhang, Y. and Beck, J.N. with Young, J.C., Callahan, J.D., and Marcon, M.F., 1992. The identification of point sources of heavy metals in an industrially impacted waterway by periphyton and surface sediment monitoring. *Water, Air and Soil Pollution.* **65**, 175-190.

Rehfeldt, G. and Sochtig, W., 1991. Heavy metal accumulation by *Baetis rhodani* and macrobenthic community structure in running waters of the N'Harz Mountains (Lower Saxony, France). *Entomol. Gener.* **16** (1), 31-37.

Round, F.E., 1981. The Ecology of Algae. Cambridge University Press.

Saint–Louis, R., Pelletier, E., Marsot, P., and Fournier, R., 1994. Distribution et effets du chlorure de tributyletain et de ses produits de degradation sur la croissance de l'algue marine *Pavlova lutheri* en culture continue. *Water Res.* **28**, 2533–2544.

SAS Institute Inc., 1985. SAS User's Guide: Basics. SAS Institute Inc. Cary, NC, USA.

Saygiderger, S., 1998. Bioaccumulation and toxicity of zinc in *Spirogyra fluviatilis hilse* (Chlorophyta). *Water, Air, and Soil Pollution.* **101**, 323–331.

Scrimgeour, G.J., Culp, J.M., Bothwell, M.L., Wrona, F.J. and McKee, M.H., 1991. Mechanisms of algal patch depletion: importance of consumptive and non-consumptive losses in mayfly - diatom systems. *Oecologia.* **85**, 343–348.

Shriner, C. and Gregory, T., 1984. Use of artificial streams for toxicological research. *CRC Critical Reviews in Toxicology.* **13**(3), 253–281.

Smock, L.A., 1983. The influence of feeding habits on whole-body metal concentrations in aquatic insects. *Freshwater Biology.* **13**, 301-311.

Stauber, J.L. and Florence, T.M., 1985a. The influence of iron on copper toxicity to the marine diatom , *Nitzschia closterium* (Ehrenberg). *Aquatic Toxicology.* **6**, 297–305.

Stauber, J.L. and Florence, T.M., 1985b. Interactions of copper and manganese: A mechanism by which manganese alleviates copper toxicity to the marine diatom, *Nitzschia closterium* (Ehrenberg). *Aquatic Toxicology.* **7**, 241–254.

Stewart, A.J. and Hill, W.R., 1993. Grazer, periphyton and toxicant movement in streams. *Environ. Tox. Chem.* **12**, 955–957.

Steinman, A.D., 1996. Effects of grazers on freshwater benthic algae. In: R. J. Stevenson, M.L. Bothwell and R.L. Lowe [Eds], Algal Ecology Freshwater Benthic Ecosystems. Academic Press.

Stevenson, R.J., 1984. Procedures for mounting algae in syrup medium. *Transactions of the American Microscopical Society.* **103**, 320–321.

Stevenson, R.J., 1996. An introduction to algal ecology in freshwater benthic habitats. In: R. J. Stevenson, M.L. Bothwell and R.L. Lowe [Eds], Algal Ecology Freshwater Benthic Ecosystems. Academic Press Inc. San Diego.

Subramanian, V.V., Sivasubramanian, V., and Gowrinathan, K.P., 1994. Uptake and recovery of heavy metals by immobilized cells of *Aphanocapsa pulchra* (Kutz) *J. Environ. Sci. Health* Part A. **A29**, 1723–1733.

Taft, C.E., 1978. A mounting medium for freshwater plankton. *Transactions of the American Microscopical Society.* **97**, 263–264.

Thomson, D.G., Manly, J.K., Kitchin, R.F., Ecnkooren, N.E., Davies, M.P., Gibson, G.G. and Dawson, B.B., 1997. Mark Creek improvement project control of ARD in the Lower Mine yard Kimberley, BC. Fourth International Conference on Acid Rock Drainage Proceedings, Vol 1, Vancouver, BC.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R. and Cushing, C.E., 1980. The river continuum concept. *Can. J. Fish. Aquatic Sci.* **37**, 130–137.

Verma, S.K., Singh, R.K., and Singh, S.P., 1993. Copper toxicity and phosphate utilization in the cyanobacterium *Nostoc calcicola*. *Bull. Environ. Contam. Toxicol.* **50**, 192-198.

Vijayram, K. and Geraldine, P., 1996. Are the heavy metals cadmium and zinc regulated in freshwater prawns? *Ecotoxicology and Environmental Safety.* **34**, 180–183.

Walsh, R.S. and Hunter, K.A., 1992. Influence of phosphorus storage on the uptake of cadmium by the marine alga *Macrocystis pyrifera*. *Limnol. Oceanogr.* **37**, 1361–1369.

Wang, W., 1985. Effects of iron and zinc interactions on algal communities. In: R.D. Cardwell, R. Purdy and R.C. Bahner [Eds.], Aquatic Toxicology and Hazard Assessment: Seventh Symposium. ASTM. pp. 187–201.

Whitton, B.A., 1984. Algae as monitors of heavy metals in freshwaters. In: L. E. Shubert [Ed], Algae as Ecological Indicators. Academic Press, London.

Wetzel, R.G. and Linkens, G.E., 1991. Limnological Analysis, 2nd Edition. Springer – Verlag, New York.

Wiggins, G.B., 1996. Larvae of the North American Caddisfly Genera (Trichoptera), 2nd Edition. University of Toronto press.

Williams, L.G. and Mount, D.I., 1965. Influence of zinc on periphyton communities. *American J. Bot.* **52**, 26–34.

Wixson, B.A. and Bolter, E., 1971. Evaluations of stream pollution and trace substances in New Lead Belt of Missouri. In: D.D. Hemphill [Ed], Trace Substances in Environmental Health. Proceedings. University of Missouri, Columbia.

Zar, J.H., 1984. Biostatistical Analysis. 2nd Edition. Prentice Hall Inc., Englewood Cliffs, New Jersey.

6 Appendix

Appendix A

Table 12: Average nutrient concentrations from a variety of artificial and natural studies. All concentration in $\mu g\ L^{-1}$.

Chemical	Nutrient	Target	APHA, 1992	Flum et al. 1993.	St. Mary (Ref)	St Mary (Exp)	Seymour water
$NaNO_3$	N	100	4,200	200	70	190	70
$Na\ H_2\ PO_4 * H_2O$	P	10	186	14	nd	nd	nd
$CaSO_4 *2H_2O$	Ca		1,200	20,000	9,080	18,720	1,700
$MgSO_4 * 7H_2O$	Mg		2,900	7,500	2,070	5,080	160
$ZnSO_4 * H_2O$	Zn	**	1.57	26	9	128	nd
$Na_2MoO_4 * H_2O$	Mo	0.05	2.88	2	nd	nd	-
H_3BO_4	B	30	32.5	54	42	50	nd
$FeCl_3 * 6H_2O$	Fe	**	33	800	60	130	140
$Na_2SiO_3 * 9H_2O$	Si	1,000	-	10000	490	620	3,200
Hardness $CaCO_3$			-	-	29,500	61,240	1,700
pH			-	-	7.6	7.6	6.0
DOC	C		-	-	800	1,100	2,100

Note: ** Zinc & Iron treatment nutrients and no additional Ca or Mg added. St. Mary values taken from EVS, 1996 and Seymour watershed from GVRD values reported from 1997. Nutrients below detection denoted with "nd" and a dash indicates not reported. **Target**: nominal concentration in laboratory bioassays. De chlorinated Seymour watershed water was used in both bioassay experiments.

Appendix B

Table 13: QA/QC reference material recovery. All concentration $\mu g\ g^{-1}$.

Year	Material	Digest tech.	Certified ± 95% CI	Measured ± SE	Recovery	Sample size
1997	Oyster	CEM	830 ± 57	1301.4 ± 126.6	156.8 %	19
1997	Bone ash	Conventional	181 ± 3	188.0 ± 7.96	103.9 %	18
1998	Oyster	CEM	830 ± 57	1015.8 ± 373.3	122.4 %	4
1998	Oyster	Conventional	830 ± 57	841.6 ± 59.1	101.4 %	10
1998	Tomato leaf	CEM	30.9 ± 0.7	41.7 ± 5.32	134.9%	12

Note: Oyster tissue (NIST 1566a), Bone ash (NIST 1400), Tomato leaves (NIST 1573a). St. Mary samples included in the 1997 reference runs.

Table 14: QA/QC filter blanks and process blanks. Filters $\mu g\ g^{-1}$, all other were $\mu g\ L^{-1}$.

Year	Filters (SE)	Algae blanks	Invert blanks	Water blanks
1997	§ 57.6 ± 15.3 $\mu g\ g^{-1}$	nd	nd	nd
1998	¥ 11.23 ± 0.905 $\mu g\ g^{-1}$	nd	nd	nd

Note: Level of zinc detection with Flame Atomic Absorption is 10 $\mu g\ L^{-1}$. Filter blanks (§ glass fiber & ¥ ashless cellulose) were subtracted from algae and any applicable reference analysis.

Appendix C

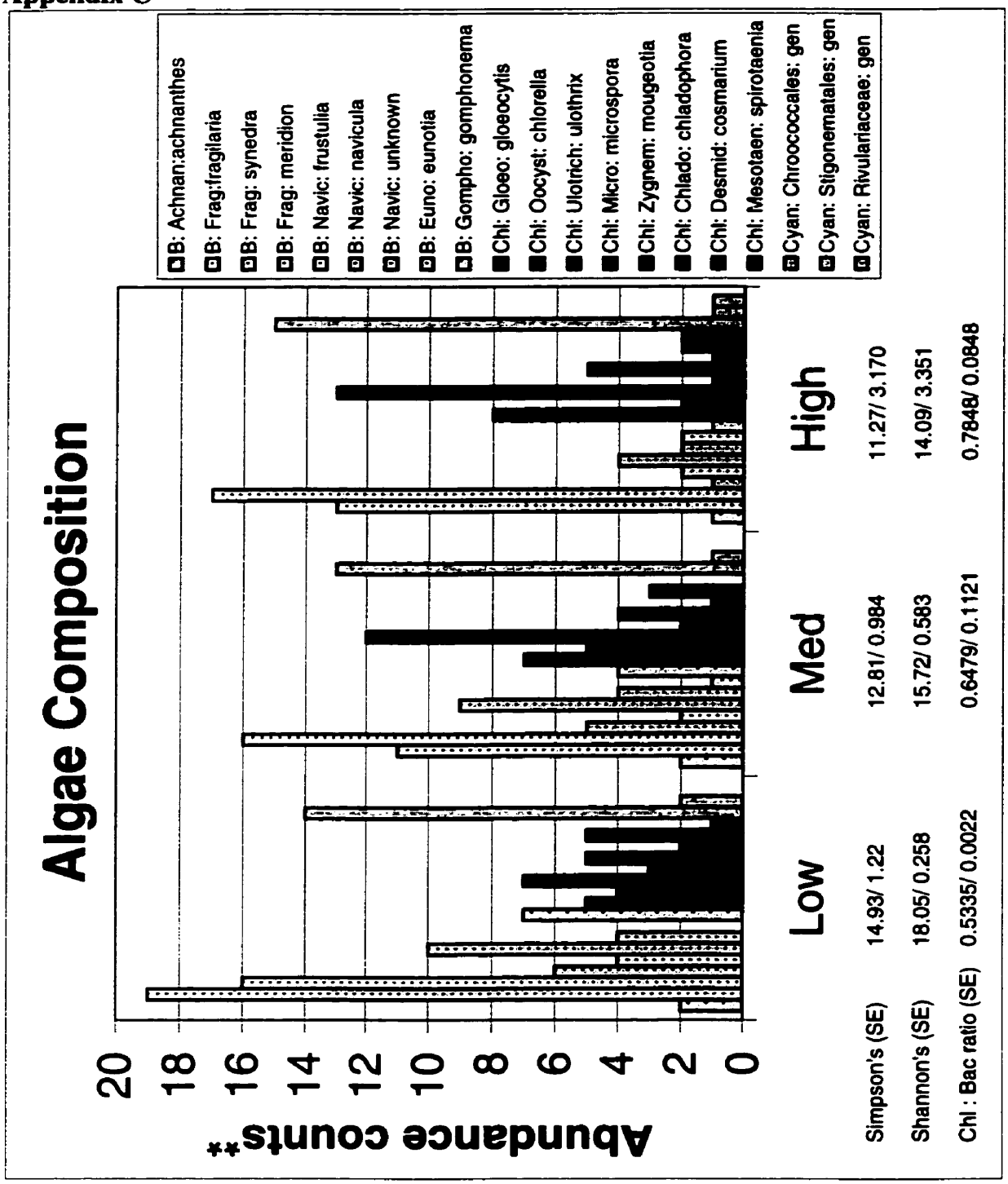


Figure 20: Accumulation/Clearance Bioassay: Overall algal composition. **Abundance ranking: (3) Dominant, (2) Abundant, (1) Present, (0) Not observed. Legend from top to bottom follows left to right on the bar graph. Low , Medium and High denote 10, 60 and 120 µg Zn L^{-1} nominal concentration. Indices of heterogeneity with standard errors across the bottom (n = 2 per treatment). Chl, Bac denote Chlorophyta and Baccilariophyceae respectively.

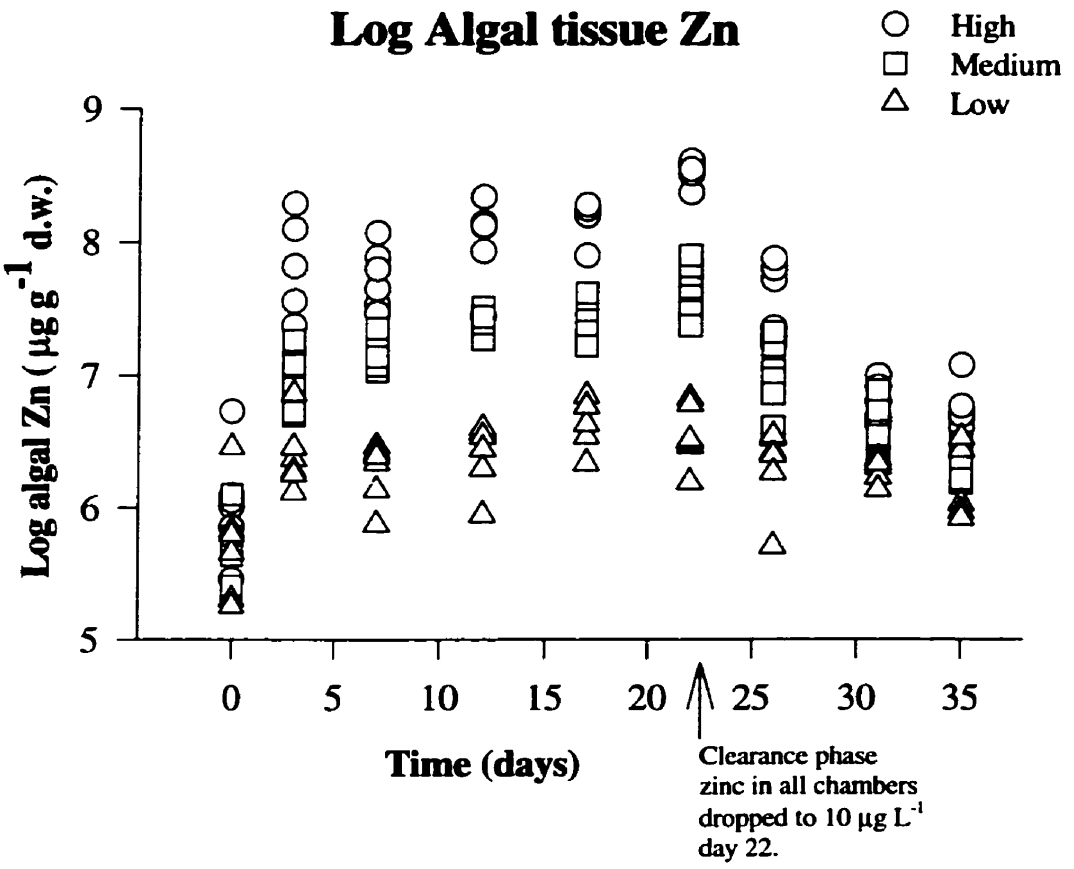


Figure 21: Accumulation/ Clearance Bioassay: Time Series Log_e transformed algal tissue zinc. Treatments: 10, 60 and 120 µg Zn L^{-1} nominal concentrations for Low, Medium and High respectively. Symbols are individual replicates (n = 6 per treatment per day). Pattern shows initial rapid "adsorptive" phase uptake, followed by the gradual linear increase in algal zinc: "absorptive" phase. Clearance is linear in this log_e transformed data.

Appendix D

Table 15: St. Mary River :Table of species numbers , August 1997. MC - Mark Creek reference.

Taxa		Mark Creek & St. Mary Reference					St. Mary Exposure South Shore			St. Mary Exposure North Shore		
Order: Family	**Genus**	MC	R1	R2	R3	R4	SE1	SE2	SE3	NE4	NE5	NE6
O: Ephemeroptera												
F: Baetidae	indeterminant		20	24	50	45	~70	45	>100	72	>130	65
F: Ephemerellidae	indeterminant											
	drunella						1	1	2		1	
	ephemeralla						9	7	6		2	
F: Heptageniidae	indeterminant	27		15								
	cinygma							11	~100	28	50	15
	rhithrogena						23	11	~80		20	
O: Plecoptera												
F: Chloroperlidae	indeterminant		>100							7	5	
F: Perlidae	acroneuria		18	5			8	8	10	15		8
F: Taeniopterygidae	brachyptera			4		10	32	34	45	20	10	6
O: Trichoptera												
F: Brachycentridae	brachycentrus			60	43	45	80	24	80	140	~80	55
F: Hydropsychidae	arcotopsyche	3	>130	27	29	146	123	103	110	122	68	74
F: Limnephilidae	indeterminant	1	1									1
F: Rhyacophilae	acroneuria		6							3		4
O: Diptera												
F: Simuliidae	indeterminant		>1000									

Table 16: St. Mary River invertebrate tissue zinc concentrations $n = 1$ ($\mu g\ g^{-1}$).

		Mark Creek & St. Mary Reference					St. Mary Exposure South Shore			St. Mary Exposure North Shore		
Order: Family	**Genus**	MC	R1	R2	R3	R4	SE1	SE2	SE3	NE4	NE5	NE6
O: Ephemeroptera												
F: Baetidae	indeterminant		421	724	976	350	1126	1126	708	4698	2243	2685
F: Ephemerellidae	indeterminant											
	drunella						199	221	301		-	
	ephemeralla						-	485	320		-	
F: Heptageniidae	indeterminant	195		290								
	cinygma							-	767	-	-	-
	rhithrogena						328	-	882			
O: Plecoptera												
F: Chloroperlidae	indeterminant		-									
F: Perlidae	acroneuria		338	355			345	601	574	1028		964
F: Taeniopterygidae	brachyptera		255	-		411	449	745	633	1567	765	1127
O: Trichoptera												
F: Brachycentridae	brachycentrus			475	594	443	638	809	832	1330	944	581
F: Hydropsychidae	arcotopsyche(L)	278	160	168	228	173	214	229		458	463	402
F: Hydropsychidae	arcotopsyche(S)		291	229		102	248	359	560	965	646	416
F: Limnephilidae	indeterminant	685	486									1911
F: Rhyacophilae	acroneuria		201							756		671
O: Diptera												
F: Simuliidae	indeterminant		231									

Note: L & S denote large and small Hydropsychide. Dashes signify insufficient biomass for metal analysis.

Appendix D continued

Table 17: St. Mary River Periphyton composition and associated mean ($n = 3$) tissue zinc ($\mu g\ g^{-1}$) .

Taxa	Mark Creek		St. Mary Reference				St. Mary Exposure South Shore			St. Mary Exposure North Shore		
	MCE	MCR	R1	R2	R3	R4	SE1	SE2	SE3	NE4	NE5	NE6
Bacillariophyceae												
tabularia			**	*	*	*			*	*	*	
fragilaria			**			*		*				
others			*		*	*				*		*
Bryophytes												
moss?		***								**		**
Chlorophyta												
unknown filament			***									
cladophora			*?	***	***	***	***	**	**	**	*	
microspora				**	**	**	**	***		*		
ulothrix												***
chlorella										**	*	
pseudosphaerocytis										*	*	
Cyanophyta												
unknown filament									***			
Inorganic debris												
iron oxides	***		**								***	*
mean tissue zinc (n=3)	5984	90.52	142	89.6	62.9	54.1	56.5	77.6	128	5178	4839	847

Note: *** - dominant taxa, ** - abundant taxa, * - present taxa.

Appendix E

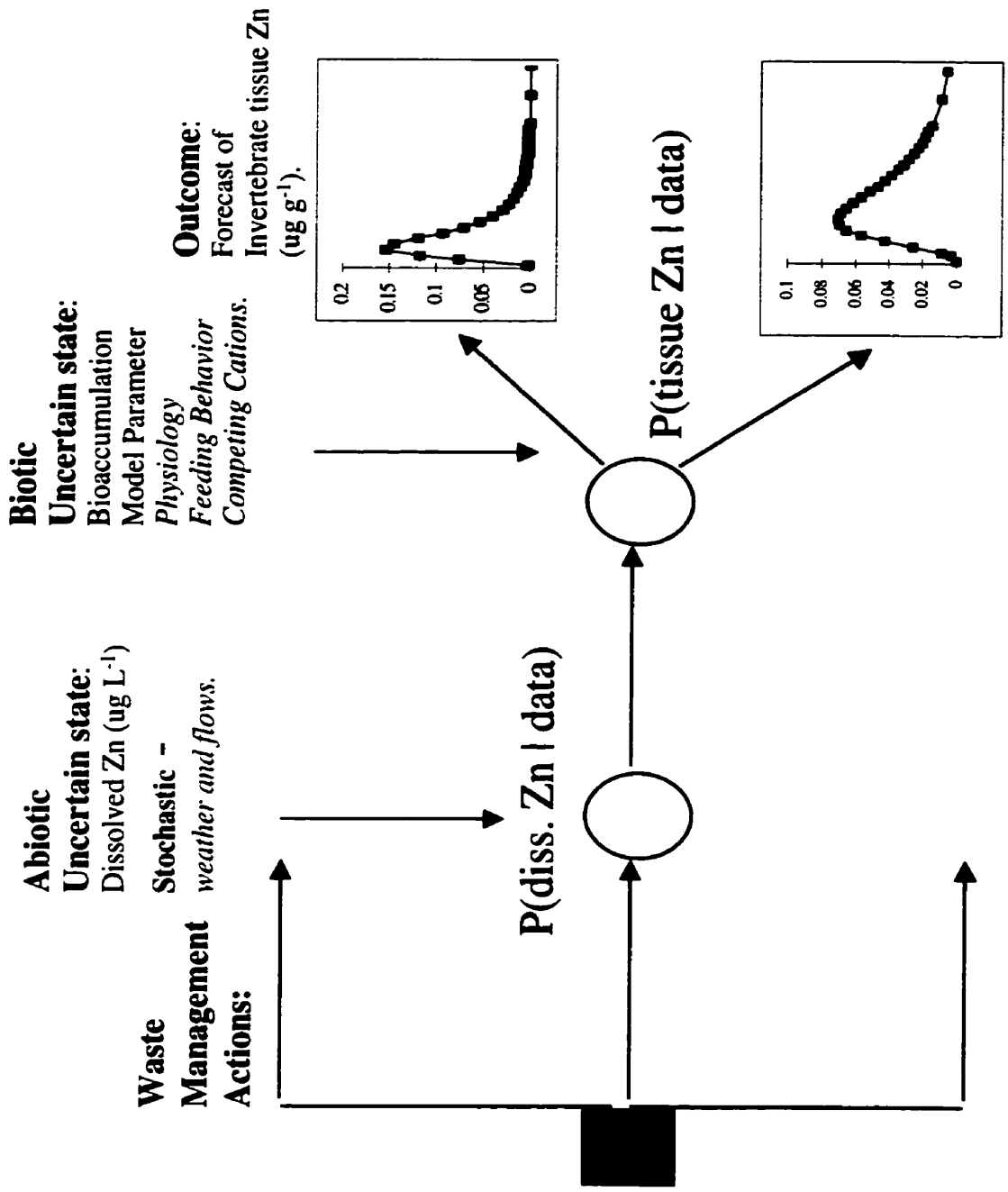


Figure 22: Structure of decision analysis tree. Management actions originate from a problem statement. Uncertainty nodes as circles and ρ (Zn | data) represents the probability of a zinc concentration given the data. These techniques are founded in the Bayesian statistical paradigm. Forecasted outcomes (y-axis as relative probability) can be used to predict further food chain transfer or quantify ecological impacts.