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The Effects of Heavy Metal Contamination on the Aquatic Biota of Buttle Lake and the Campbell River Drainage (Canada)

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Abstract. Concentrations of zinc and copper in a series of large oligotrophic lakes on Vancouver Island, Canada, have been increasing since mining operations began near the south end of Buttle lake in 1966. Declines in species diversity of both phytoplankton and periphyton have occurred since the beginning of mining activity with the disappearance of metal sensitive forms such as *Asterionella* and *Rhizosolenia*. Cladoceran and calanoid copepod numbers and species diversity increase as the metal concentrations decline downstream from the mine. Bioassays carried out with *Daphnia pulex* show increasing toxicity downstream. Metal concentrations in zooplankton and metallothionein concentrations in rainbow trout liver are correlated to metal concentrations in the water. The water was not acutely toxic to rainbow trout in laboratory simulations of Buttle Lake conditions or during *in situ* exposures (1 month). Specific sublethal deleterious effects have not yet been determined.

Western Mines (now Westmin Resources Ltd) began operation in 1966 of a copper, zinc, and lead mine at Myra Creek near the south end of Buttle Lake on Vancouver Island (Canada) (Figure 1). Since May 1967, tailings were discharged into Buttle Lake via a submerged outfall because mountainous terrain made land disposal difficult (Clark and Morrison 1982). Buttle Lake is the largest of a chain of lakes in the Campbell River drainage and consists of two basins. The south basin into which the tailings and Myra Creek flow is relatively small and shallow (Figure 1) and is separated by a sill from the main basin which comprises approximately 90% of the volume of the lake. Morphometry and turnover times of Buttle Lake and the other lakes in the system are shown in Table 1. The levels of Buttle and Upper Campbell Lakes are controlled by Strathcona Dam, Lower Campbell Lake is controlled by the Ladore Dam and John Hart Lake by John Hart Dam. Upper Quinsam Lake is located at the head of a series of lakes which flow into the Campbell River via the Quinsam River. Anadromous salmonids from the Campbell River are prevented from entering the lake system by Elk Falls. The native fish in these lakes consist of rainbow trout (*Salmo gairdneri*), cutthroat trout (*Salmo clarki*), Dolly varden char (*Salvelinus malma*), prickly sculpin (*Cottus asper*) and stickle back (*Gasterosteus aculeatus*). Various agencies have monitored metal concentrations and water chemistry in Buttle Lake and at a number of downstream locations. The majority of the data has been collected by the Waste Management Branch of the British Columbia Ministry of Environment; however, little information concerning biological effects was collected until 1980. A review of Waste Management Branch water quality data by Clark (1980) identified copper and zinc as the principal hazards to aquatic life in Buttle Lake. He described an increasing trend in zinc concentrations since the opening of the mine that was evident 90 km downstream in the Campbell River. This river supports large numbers of resident salmon and is the route

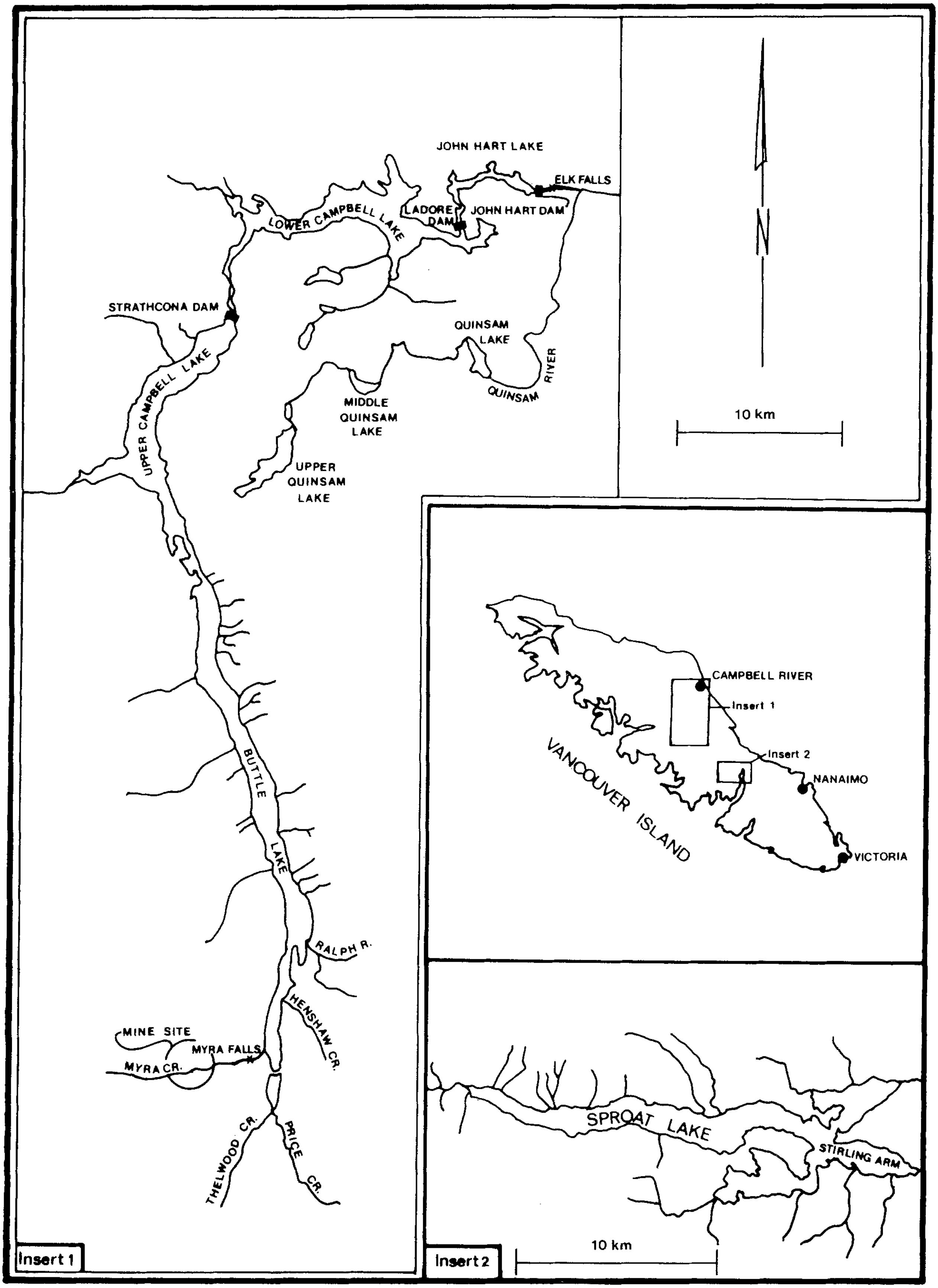

Fig. 1. Map of Vancouver Island and the Campbell River drainage showing the site of the mine

Table 1. Morphometry of the lakes in the Campbell River drainage (Nordin *et al.* 1984)

Lake	Surface area (S.A.) (km^2)	Volume ($dam^3 \times 10^3$)	Mean depth (m)	Maximum depth (m)	Elevation (m) as	Filling time (yr)	Littoral area (% S.A. <6m)
Buttle (south basin)	6.0	195	29	75	218	0.4	21
Buttle	35.3	1722	45	125	218	1.03	11
Upper Campbell	29.2	730	22	55	218	0.27	12
Lower Campbell	25.9	440	13	75	182	0.14	27
John Hart	3.6	41	13	25	132	0.01	21
Upper Quinsam	5.1	69	13	50	350	0.46	29

by which salmon raised at the Quinsam hatchery reach the sea. Alderdice and McLean (1982) concluded that steelhead trout (*Salmo gairdneri*) and chinook salmon (*Oncorhynchus tshawytscha*) in the Campbell River were subjected to metal concentrations sometimes approaching and occasionally exceeding the lethal threshold. The trend to increasing metal concentrations and concern over the deterioration of the water quality in Buttle Lake and the Campbell River drainage prompted a multi-disciplinary study involving the Ministry of Environment and the University of Victoria to determine the impact of the Westmin Mines operation on aquatic biota including fish. Studies were directed to provide systematic limnological information (data on benthos, periphyton, phytoplankton productivity, zooplankton standing crop, species composition, and diversity) and to determine metal accumulation in zooplankton and fish (sticklebacks, salmonid muscle, and liver).

In 1980, Barringer Magenta Ltd. (Rexdale, Ontario) collected stream sediment and water samples in 1980 in an exploratory survey of Buttle Lake tributaries. The Waste Management Branch (Ministry of Environment) reorganized the results of the report into a series of maps which showed that dissolved copper and zinc concentrations were far greater in the vicinity of the mine, in surface waters downstream, and in Myra Creek downstream of the mill than in upstream waters (Clark and Morrison 1982). Westmin Resources Ltd. was directed to investigate the contamination and hired British Columbia Research to conduct a more detailed survey. Intensive sampling revealed that acidification of waste rock in the area of the mine had occurred and that toxic concentrations of metals were entering Buttle Lake via Myra Creek upstream from the tailings disposal (British Columbia Research 1982). The mine developed a ground water collection and mine treatment system designed to reduce metal contamination in Myra Creek. Treatment began in November 1982.

This report is an overview of the effects of heavy metal contamination in Buttle Lake and the Campbell River drainage. It concentrates on detectable changes in the aquatic biota and evaluation of toxic hazard to salmonids in the area. Comparison of pre- and post-mine conditions has been difficult due to the lack of comprehensive biological data collected before the opening of the mine. However, this report provides useful comparative data for the development of monitoring programs for other sites, and documents the effects, in this case, of the discharge of heavy metals into a chain of oligotrophic lakes.

Methods

Water Chemistry

Routine sampling was carried out at six sites downstream of the mine as well as a control site—Upper Quinsam Lake. Sites were selected for maximum depth and included some sites that had previously been established. Water samples were taken at different depths using a Van Dorn type water sampler. The samples intended for analysis of dissolved metals (zinc, copper, cadmium, and lead) were filtered through a 0.45 μm filter and preserved with 4 ml/L of concentrated nitric acid and placed in coolers. Metal concentrations were determined by inductively coupled argon plasma and flame or graphite furnace atomic absorption spectrophotometry. Other analyses were performed according to McQuaker (1976) and included, phosphorus, nitrate, ammonia and total nitrogen; pH, alkalinity, calcium, magnesium, colour, turbidity, organic and inorganic carbon, as well as dissolved, suspended, inorganic, and organic residues.

Aquatic Biology

Chlorophyll a was sampled by collecting water samples at 1, 5, 10, 15, and 20 m. The samples were placed in a dark cooler and filtered the same day onto 0.45 μm membrane filters; magnesium carbonate buffer was added and the samples were frozen in sealed containers with silica gel dessicant.

Surface samples were taken for identification of phytoplankton, preserved in Lugol's solution and sent to the Environmental Laboratory for enumeration and identification. Phytoplankton growth rates were measured by carbon-14 uptake.

Samples were taken from 1, 5, 10, 15, and 20 m, placed in 350 mL glass stoppered BOD bottles and placed in dark coolers. On return to Victoria, each sample was inoculated with 1 μCi of C^{14} sodium bicarbonate and incubated for three or four hr under conditions of temperature and light which simulated conditions at 5 m depth. Samples were fixed with 1 ml of formalin and filtered onto 0.45 μm filters and placed in scintillation fluid (Aquasol II—New England Nuclear) (Nordin *et al.* 1984) for counting (LKB RacBeta).

Zooplankton samples taken for identification and biomass measurements were obtained by a vertical tow of 25 or 50 m with a 150 μm mesh conical net of 30 cm mouth diameter. Samples were preserved in isopropanol and sent to the Environmental Laboratory for enumeration, identification, and biomass calculations (Nordin *et al.* 1984). Zooplankton tissue samples for heavy metal analysis were obtained by towing two 64 μm conical nets of 1 m diameter behind the airplane or boat until sufficient biomass (10 g wet weight) had accumulated. These tows generally were of 10–20 min duration and filtered 1000 to 2000 m^3 of water. Samples were placed on ice until analysis by the Ministry of Environment Laboratory. On some trips, aliquots were frozen on dry ice and sent to the University of Victoria for analysis.

Salmonids were collected with gill nets during July and August 1981. Live fish were sacrificed, the livers were dissected and placed in plastic bags on dry ice. Muscle tissue was kept on ice and sent to the Environmental Laboratory for analysis. Sticklebacks (*Gasterosteus aculeatus*) were collected with minnow traps and seine nets. Whole fish were sent to the Environmental Laboratory for analysis. Analysis was performed by atomic absorption spectrophotometry after nitric acid digestion of tissues.

Hepatic metallothionein concentrations were determined by the Environmental Toxicology group at the University of Victoria (Roch *et al.* 1982). All livers were kept frozen from the time of sampling to analysis. Livers were homogenized in ice cold 0.9% NaCl and 10 mM Tris Buffer pH 8.6 with 8 passes of a stainless steel/teflon, Potter-Elvehjem homogenizer and made up to 5 mL with 0.9% NaCl. Livers larger than 0.5 g were homogenized in three volumes of buffer and a 0.2 mL aliquot was made up to 5 mL. The homogenate was heat denatured in a water bath at 80°C for five min. Denatured homogenates were kept in ice and filtered through a 0.45 μm filter. Metallothionein in the filtrate was determined by differential pulse polarography using a modification of the Brdicka (1933) procedure described by Olafson and Sim (1979) and standardized with metallothionein purified from the crab (*Scylla serrata*) (Olafson *et al.* 1979; Lerch *et al.* 1981). More recent samples were standardized with mouse metallothionein. Rainbow trout metallothionein has not been purified in sufficient quantities to use as a standard.

Details of the methods used in experiments to determine the toxicity of a metal mixture and of Buttle Lake water are reported in Roch and McCarter (1984a), Part I and Part II. The *in situ* toxicity experiments were carried out with net pens. Static replacement bioassays (96 hr) were conducted with a mixture of zinc, copper, and cadmium following the guidelines of Sprague (1973). A continuous flow bioassay using the metal mixture did not differ significantly ($P < 0.05$) from a static replacement bioassay carried out under the same conditions of temperature and hardness.

Qualitative phytoplankton samples were collected with standard plankton nets (64 μm) and preserved in a buffered solution of 5% formalin in 6 oz jars. (Austin and Munteanu 1984). Quantitative plankton samples were collected with a Kemmerer/Van Dorn bottle and preserved in buffered 5% formalin. Replicate subsamples were taken from homogeneously resuspended water samples and settled in 100 mL Utermohl sedimentation chambers with Lugol's solution. All organisms present in 48 microscope fields were identified whenever possible. The number of fields was determined with a minimum numbers graph.

Periphyton sampling by the Biology Department was carried out with a slotted steel frame supporting and exposing a number of horizontally and vertically oriented glass slides. Details of the construction and sampling are given by Austin (1983). Slides were transferred to jars containing 5% buffered formalin. A straight-edge scalpel was used to scrape the periphyton from the slides into the sample jar. Subsamples were withdrawn from the shaken sample and settled in Utermohl (Zeiss) sedimentation chambers with Lugol's solution. All organisms were enumerated in 20 random microscope fields and counts were converted to numbers per square millimeter.

Results and Discussion

Chemistry

Western Mines began disposing of concentrator tailings into Buttle Lake via a submerged outfall in May 1967. Dissolved copper, dissolved lead, total and dissolved zinc and color were measured between April 1966 and September 1973 and British Columbia Research (1974) concluded that no statistically apparent increase had occurred during this time for any of the characteristics measured at any of the lake or tributary stream sampling sites. Clark (1980) reported on metal concentrations that had been monitored since 1971 by the Waste Management Branch of the Ministry of Environment and identified a statistically significant increase in zinc concentrations (both total and dissolved) over the last decade at the surface and at 50 m. An increase in dissolved copper at surface locations was also evident. Figure 2 shows the increase in zinc concentrations since 1967 at the south end of the lake near the mine, at the north end of Buttle Lake and in the Campbell River 90 km downstream. Changes in dissolved zinc with depth and distance downstream are indicated in Figure 3.

Concern over the increasing metal concentrations at a time when the mine had proposed further expansion led to efforts to identify the sources of contamination. Previous monitoring of metal concentrations had been centered on the tailings disposal, but work carried out by British Columbia Research identified ground water contamination as the main source of metal loading in Buttle Lake via Myra Creek (Westmin Resources Ltd 1982).

Measurements of dissolved metals in the Lynx open pit and waste rock dump seepages by B.C. Research in November 1981, showed concentrations ranging from 13–78 mg Zn/L, 3.1–16.0 mg Cu/L and 0.07 to 0.31 mg Cd/L and pH ranging from 2.80 to 3.05. Total metal concentrations measured from June 1981 to May 1982 at the powerhouse above the mine operation were <0.005 mg Zn/L,

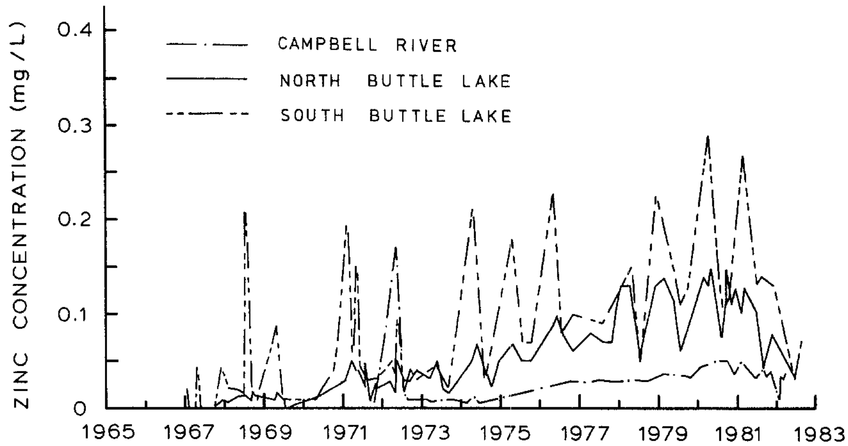

Fig. 2. Concentrations of zinc at 0–5 m depth in water of the Campbell River drainage from January 1, 1965 to August 31, 1982

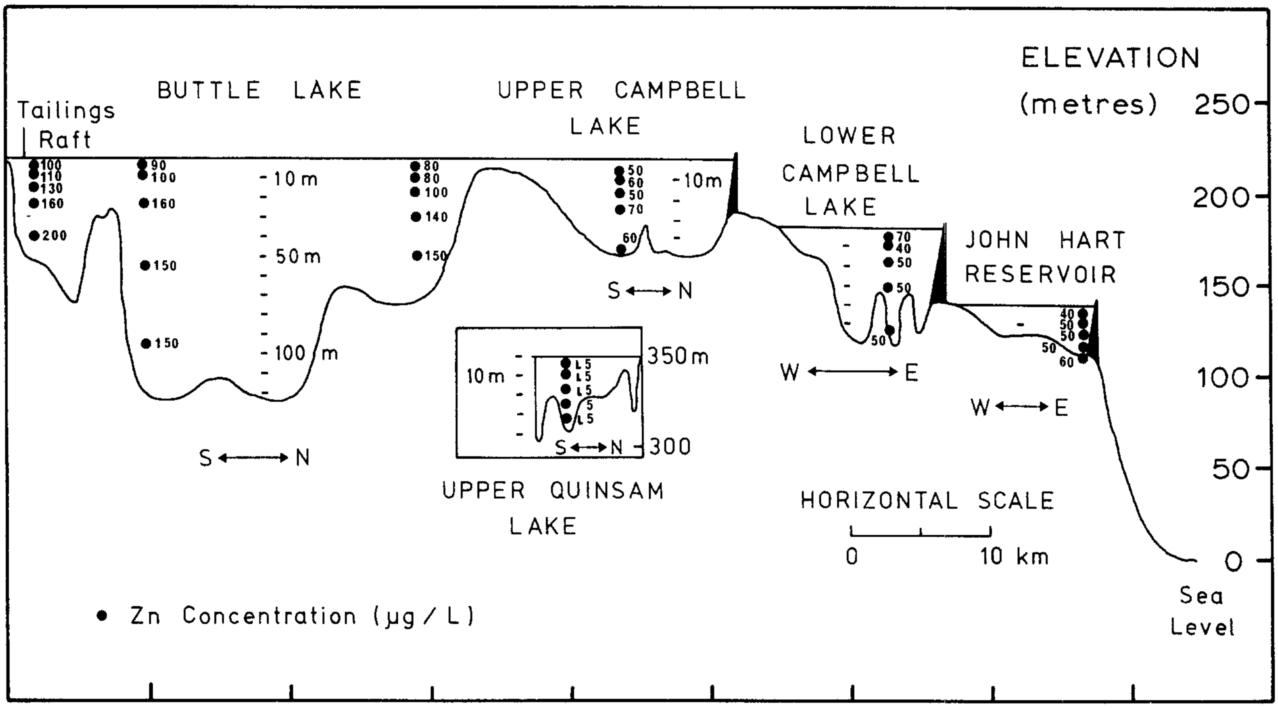

Fig. 3. Depth profile of the Campbell River drainage showing the variation of dissolved zinc concentration with depth

<0.001 mg Cu/L, and <0.000 5 mg Cd/L contrasted with 0.90 ± 0.47 mg Zn, 0.10 ± 0.06 mg Cu, and 0.0025 ± 0.0014 mg Cd/L (x ± S.D., $n = 33$) below the mine operations near the entry point of Myra Creek into Buttle Lake at Myra Falls. Dissolved metal concentrations averaged 98, 68, and 90% of total concentrations for zinc, copper, and cadmium. Mean daily estimates of dissolved zinc, copper, and cadmium entering Buttle Lake from Myra Creek during the period from June 1981 to May 1982 were 398 kg Zn/day, 29 kg Cu/day and 1.1 kg Cd/day. Figure 2 shows marked seasonal variation in the lakes with peaks occurring during periods of high rainfall Oct.–March and declines during the dry season with the lowest concentrations in August. These variations coincide with estimates of zinc, copper, and cadmium input from Myra Creek from June 1981 to May 1983. Bioassays (96 hr) of Myra Creek water conducted during this period resulted in at least 50% mortality of rainbow trout in all samples (Westmin Resources Ltd 1982).

British Columbia Research (1982) concluded that the Westmin Resources Ltd operation contributed 95% of the metal loadings in Myra Creek. Based on a simple computer model, these investigators estimated that the zinc loadings to Buttle Lake from Myra Creek represented 90% of the total zinc loading to the lake.

In 1980, British Columbia Research investigated the contribution of zinc tailings disposal to zinc

Table 2. Summary of major nutrients in the Campbell River drainage (Data from Clark and Morrison [1982] and Nordin *et al.* [1984])

	South Buttle	North Buttle	Upper Campbell	Upper Quinsam
Ammonia (mg/L-N)	<0.005 (7)	<0.005 (7)	<0.005 (8)	<0.005 (8)
Orthophosphate (mg/L-P)	<0.003 (7)	<0.003 (7)	<0.003 (8)	<0.003 (8)
Total phosphate (mg/L-P)	<0.003–.007 (7)	0.003–0.006 (7)	0.003–0.006 (8)	0.003–0.006 (8)
NO_2/NO_3 (mg/L-N)	<0.002–0.05 (7)	0.02–0.05 (7)	<0.02–0.04 (8)	<0.02–0.03 (8)
Silica (mg/L-SiO_2)	2.0–3.0 (4)	3.0–3.3 (5)	3.6–3.8 (4)	3.7–4.0 (4)
Mean extinction depth (M)	8.5 ± 3.4(5)[a]	9.9 ± 2.6(5)	8.1 ± 2.1(5)	10.3 ± 1.5 (n = 5)
Hardness (mg $CaCO_3$/L)	20–30 (2)	20–30 (2)	20–30 (2)	20–30 (2)
pH	6.54–7.3 (n = 10)	6.60–7.60 (n = 11)	6.85–7.30 (n = 10)	6.73–7.24 (n = 4)

[a] Mean ± standard deviation (N)

loading in the lake. Core samples in the tailings showed that they were unoxidized. An estimate of the contribution of dissolved zinc to Buttle Lake was 10% of the total loading. Pederson (1983) did a more detailed analysis of interstitial water in the tailings sediment and concluded that dissolved metals were lower in this water than in overlying lake waters, and concluded that the tailings deposit was not contributing a flux of dissolved zinc, copper, and cadmium to Buttle Lake and that oxidation of the deposit was insignificant.

Nutrients

Nutrient levels throughout Buttle Lake and the other lakes in the area (Table 2) are characteristic of the oligotrophic lakes on Vancouver Island. There are essentially no differences among the lakes with regard to pH, hardness, nitrogen, phosphorus, and silica concentrations. The low hardness (25 mg/L) as $CaCO_3$ and pH (7.1) are conditions which markedly increase the toxicity of heavy metals to salmonids and zooplankton in comparison to hard water (Spear 1981; Spear and Pierce 1979).

Phytoplankton Productivity

It is difficult to determine whether a decline in phytoplankton productivity has occurred since the mine began operation (since no data exist from the preoperational period). However, in terms of spatial patterns of phytoplankton distribution, heavy metals have depressed productivity from April to July 1981 as far downstream as the Central Buttle Lake site (Table 3). Phytoplankton incorporated significantly less ($P < 0.05$) C^{14} at the two locations closest to the mine during the period April to July than they did at downstream sites; however, primary productivity of S. Buttle Lake (the most contaminated site) over the longer six month period April to November 1981 (Nordin *et al* 1984) does not differ significantly ($P < 0.05$) with any of the other locations except Upper Quinsam lake.

Algal biomass as determined by chlorophyll a concentrations was below the detection limit (0.5 μg/L) in the majority of the samples because of the oligotrophic nature of the lakes. No trend related to metal contamination was evident.

Phytoplankton Species Composition

Species diversity as measured by the Shannon-Weaver diversity index declined from an average of 1.03 in the 1966, 1967, and 1968 samples to 0.77 in the 1980's samples at the most contaminated location (south Buttle Lake). In contrast, the index had changed very little from 0.85 to 0.77 at the north end of the lake during this time (Austin and Munteanu 1984). In 1966, the dominant phytoplankton association throughout Buttle Lake, as determined by qualitative and quantitative data, consisted of *Rhizosolenia eriensis, Asterionella formosa, Tabellaria fenestrata, Tabellaria flocculosa* and *Cyclotella bodanica*. McMynn and Larkin (1953) also reported the presence of *Asterionella formosa* in Buttle Lake. During 1967–68, changes in hierarchical dominance coincided with initial disturbances within the watershed. These changes may be a result of the commencement of the mining operation and road construction activities. In 1967, *C. bodanica* remained as a single dominant, while subdominants included *A. formosa, Peridinium* sp., *Melosira distans* v. *alpigena,* and *Dinobryon sertularia*. By 1968, *C. bodanica* was no longer dominant at the south end of the lake and only appeared as a subdominant at the north end. Dominants included *M. distans* v. *alpigena* and *Achnanthes microcephala*. In 1980, after 13 years of mining activity and significant increases in dissolved metals, previously abundant diatoms intolerant of metals, such as *Tabellaria fenestrata, Tabellaria flocculosa*

Table 3. Uptake of ^{14}C by phytoplankton (disintegration/min) (Nordin *et al.* 1984)

1981	South Buttle Lake	Central Buttle Lake	North Buttle Lake	Upper Campbell Lake	Lower Campbell Lake	John Hart Lake	Upper Quinsam Lake
April	52	61	121	124	113	128	162
May	110	143	151	133	138	147	200
July	78	78	245	138	134	113	92
August	53	47	59	52	45	44	96
September	41	34	36	36	34	33	185
November	44	35	42	42	52	54	74
Mean	63 ±	66 ±	109 ±	87 ±	86 ±	86 ±	135 ±
St. Dev.	26	41	81	49	48	49	54

Table 4. Zooplankton standing crop (1981) (organic content, dry wet- ash wet mg-M^2) (Nordin *et al.* 1984)

Lake	May	June	July	August	September	October	November
South Buttle	260.3	118.8	212.2	118.9	94.8	131.7	63.7
Central Buttle	213.6	69.3	185.3	191.0	101.8	100.5	79.2
North Buttle	165.5	73.5	50.9	169.0	186.7	83.5	46.7
Upper Campbell	292.8	232.0	1104.4	1007.3	265.9	203.7	207.9
Lower Campbell	359.4	333.9	325.4	356.1	274.4	472.5	295.7
John Hart	133.0	189.6	135.8	217.0	109.0	181.1	116.0
Upper Quinsam	496.6	585.6	408.9	362.2	639.4	632.4	853.1

and *A. formosa* had virtually disappeared (Austin and Munteanu 1984). The phytoplankton community in 1980–81 was dominated by *Navicula cryptocephala, Synedra acus,* and *Synedra filiformis* (Table 4). The distribution of species amongst major taxonomic groupings evidences a shift of relative representation to the extent of total disappearance by 1980. Species of Chlorophyta, Chrysophyta and Pyrrhophyta were better represented in 1960's samples while Cyanophyta do not appear to have changed significantly. Bacillariophyta, while of significance in 1966–68 samples have increased both in numbers and species in 1980–81 samples. Samples analyzed by the Ministry of Environment showed that *Rhizosolenia* was present in large numbers in early spring in Lower Campbell and John Hart lakes. *Asterionella* was common in Upper Quinsam Lake, but was absent in all lakes downstream of the mine. The presence of *Asterionella* and *Rhizosolenia* in large numbers before the operation of the mine in Buttle Lake suggests that heavy metal contamination has caused the decline in numbers of these species.

Periphyton Species Composition

The periphyton community in 1967 near the mine (S. Buttle Lake) consisted of a major dominant association of *Achnanthes microcephala, Achnanthes minutissima* and *Cyclotella stelligera.* Other common forms included *Melosira distans, Synedra filiformis, Synedra acus* and *Cymbella cymbiformis.* Although, the periphyton community was dominated by diatoms, the community also included several species of Desmids and other Chlorophyta, Cyanophyta, Chrysophyta and Pyrrhophyta (Austin 1983). The periphyton community at the north end of Buttle Lake was almost identical to that near the mine. The same three species of diatoms constituted the major dominant association and the minor dominant association included two additions, *Achnanthes flexella* and *Gomphonema olivaceum.*

In 1981, the community had changed substantially (Austin 1983). *A. minutissima,* which is believed to have a low tolerance to zinc (0.1–0.2 mg/L) and copper (Besch *et al.* 1972), had virtually disappeared. *C. stelligera,* previously abundant was found only in minute quantities in 1981. The three major dominants in 1967 had been replaced in 1981 by a single diatom *Navicula cryptocephala* which averages 66% of all cells counted at both the south and the north end of the lake. During the period of investigation from 1967 to 1981, the average species diversity decreased from 2.01 to 1.06 at the station nearest the zinc mine and from 2.32 to 1.05 at the north end of Buttle Lake. In 1981/82, species diversity was low throughout the system but tended to increase with increasing distance from the mine (Austin and Deniseger 1984). A number of species had decreased substantially in abundance, while only one species, *N. cryptocephala* had increased in abundance. Cell density dropped by an average of 56% at the south end of the lake during this time, however, a 43% increase in cell density occurred at the north end of the lake. The increase at the north end may have been due to a reduction of grazing pressure because of toxicity of metals to zooplankton. The most reasonable interpretations of the causes of alterations in the periphyton community is a combined effect of suspended and benthic fine particulates, toxicity of metal ions and reduced grazing pressure. Species number was at its lowest nearest the mine (S. Buttle Lake) as several diatoms intolerant of heavy metals were rare or absent. These species became more abundant at northerly stations and consequently species diversity tended to be higher at these stations.

Zooplankton

Zooplankton standing crop (Table 4) as measured by organic biomass (dry—ash weight) is highly variable at some sample locations from month to month and it is difficult to attribute any variation to the degree of metal contamination. Although an inverse correlation ($r = -0.759$) exists between mean biomass over the seven month period (May–Nov.) and metal concentration, the variation is also great.

Zooplankton Species Composition

Figures 4a and b show general trends relating the number of organisms present to the degree of metal contamination. The numbers of organisms in each group show great variation from month to month (Table 5), but a trend to decreasing numbers of cladocerans and particularly calanoid copepods is evident approaching the mine. Cladoceran species diversity decreases markedly toward the mine and only *Bosmina* is present at the south end of the lake. *Daphnia rosea* is common downstream of the mine site and in Upper Quinsam Lake. Whately (1969) reports that *Diaptomus* was the most common copepod at both the south and the north end of Buttle Lake and *Daphnia* was present in approximately the same numbers as *Bosmina* during 1967 and 1968. McMynn and Larkin (1953) report the presence of *Daphnia* and *Leptodora* in Buttle Lake before the operation of the mine. Sinclair (1965) found *Daphnia* sp. to be common at both the south and the north end of Buttle Lake and also reported the presence of *Leptodora, Holopedium* and *Polyphemus* sp. which were absent in 1981 samples. Historical data and the increase in the presence of *Daphnia* with distance downstream from the mine indicate that metal contamination has resulted in the decline of this genus due to increases in metal concentrations. Whately's data show that the mean numbers of *Diaptomus* (860) in his vertical tows were approximately 5-fold those of *Bosmina* (176) in eleven samples taken from August 1967 to December 1968 in the south basin of Buttle Lake. *Bosmina,* the only cladoceran in S. Buttle Lake (the most contaminated site), far outnumbered *Diaptomus* during 1981. Whately's data show a mean of 0.326 *Diaptomus* organisms per cm^2 in a 10 m tow, whereas Ministry of Environment data (Nordin *et al.* 1984) show 0.023 *Diaptomus* per cm^2 in a longer 50 m tow. If Whately (1969) had carried out 50 m tows, the difference would likely have been even greater. The comparison indicates that at least a 90% reduction in *Diaptomus* numbers has taken place since the early operation of the mine and 1981. Both *Diaptomus* and *Daphnia* would be important food sources for young rainbow trout and stickleback. McMynn and Larkin (1953) and Nilsson and Northcote (1981) found that zooplankton represented more than 50% of the stomach contents of rainbow trout from Upper and Lower Campbell Lakes.

Tests which have evaluated toxicity of metals to zooplankton in soft water are few in number and none have been previously reported for mixtures of metals comparable to those in Buttle Lake. Bioassays carried out by the Ministry of Environment with *Daphnia pulex* in 1982 show a gradient in toxicity downstream, with 60–70% dilutions of Buttle Lake water resulting in 50% mortality and 90% of John Hart Lake water resulting in 50% mortality in 96 hr (Nordin *et al.* 1984). Water from Upper Quinsam Lake resulted in less than 50% mortality in 96 hr. Although *Daphnia pulex* is not native to the Campbell River drainage, its susceptibility to metal is likely to be similar to the native species. Baudoin and Scoppa (1974) indicate a 48 hr LC50 of 40 μg Zn, and 5 μg Cu/L to *Dapnia hyalina* in water of 30 mg/L hardness. Marshall *et al.* (1983) report that zinc additions as low as 15 μg/L had a significant effect on zooplankton populations. Cadmium has also been shown to be toxic to zooplankton at 0.2–1.0 μg/L (Biesinger and Christensen 1972; Marshall 1978; Marshall and Mellinger 1980; Marshall *et al.* 1981). Concentrations in Buttle Lake have been far greater than those cited by these authors and are likely responsible for the virtual disappearance of *Dapnia* in the lake. Baudoin and Scoppa (1974) indicate 48 hr LC50's of 0.50 mg Cu or Zn per L to adult *Eudiaptomus padanus*. LC50's for *Cyclops abyssorum* were 5.5 and 2.5 mg/L for

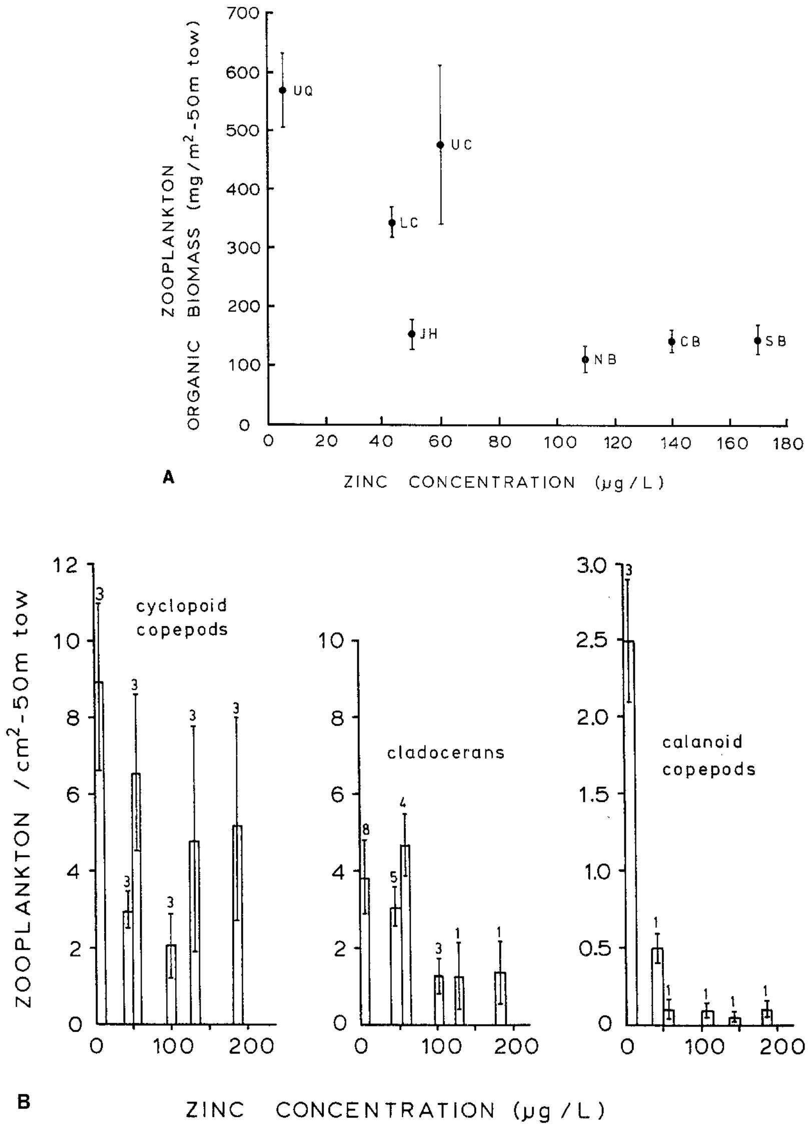

Fig. 4. A Mean and standard error (n = 7) of the organic biomass of zooplankton between May and November 1981 as a function of zinc concentration. The samples were taken from Upper Quinsam (UQ), John Hart (JH), Lower Campbell (LC), Upper Campbell (UC), North Buttle (NB), Central Buttle (CB) and South Buttle (SB) lakes B Mean and standard error (n = 5 or 6) of the number of cyclopoid copepods, cladocerans, and calanoid copepods from May to November 1981 as a function of zinc concentration. The number of species is indicated above the error bar

zinc and copper respectively. Although LC50's for *Eudiaptomus* were much greater than for *Daphnia hyalina,* the results indicate only that the adults were more resistant. Nauplii and copepodite stages of calanoid copepods may be far more sensitive than the adults. No correlation is evident between metal concentration and cyclopoid copepod numbers, suggesting that copepods may be relatively tolerant. However, *Daphnia* and *Diaptomus* show marked declines on the basis of historical comparisons and inverse correlation between numbers and metal contamination.

Benthos

Kathman (1982) described the littoral benthos of the lakes of the Campbell River watershed sampled during 1981. In considering the number of organisms, the highest total and average numbers are from Lower Campbell and John Hart (the most downstream sites). Upper Quinsam and John Hart have the most diverse fauna. The Buttle Lake sites generally had low total numbers of organisms and relatively low diversity. In considering the chironomids, *Procladius* was found most often at South Buttle and has been reported from contaminated sites (Simpson and Bode 1980). The genus *Thienemannimyia* is also known from sites subjected to toxic waste (Simpson and Bode 1980) and it was found only in South Buttle samples; however, some species of the genus are indicators of clean running water, so their presence is equivocal. It was also noted that stomach contents of chironomids varied among sites. Oligochaete setae and a few diatoms were observed at South Buttle, while at all other

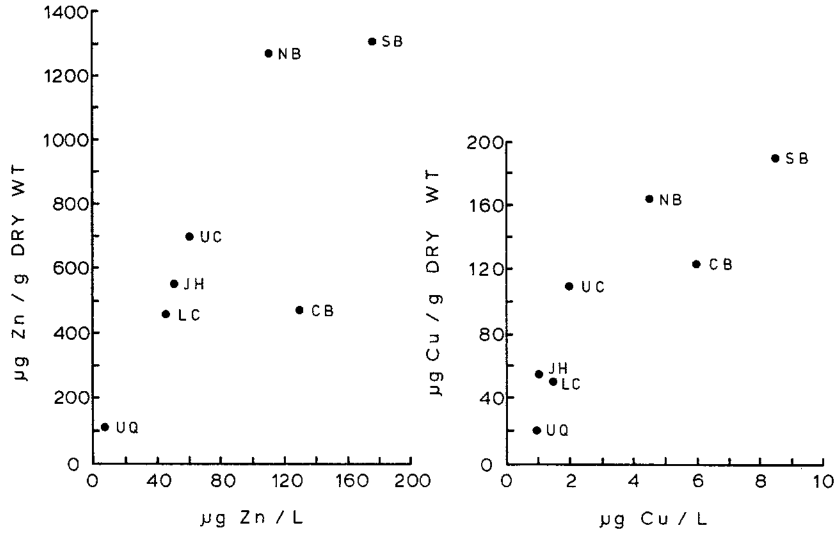

Fig. 5. Mean zinc and copper residues in zooplankton as a function of ambient concentrations. One sample was taken at the north (NB), central (CB) and south (SB) Buttle Lake stations. Six samples were taken at Upper Campbell (UC) Lake and seven at Lower Campbell (LC), John Hart (JH) and Upper Quinsam (UQ) lakes

sites contents included diatoms, zooplankton, tardigrades, oligochaete setae, and other chironomids, which indicates a more diversified and abundant food supply as distances from the discharge increase.

Oligochaetes were better indicators of heavy metal contamination. *Rhyacodrilus montana,* recently shown to be relatively tolerant to the metals cadmium and mercury (Chapman *et al.* 1982), was collected more often at South Buttle than any other species. Also noted in some specimens were setal aberrations, with enlarged and distorted dorsal pectinate setae, similar to those described by Milbrink (1980) in Swedish lakes with high metal concentrations. Milbrink (1983) also describes deformities in oligochaetes from contaminated areas. Reish *et al* (1974) also observed abnormalities in polychaetes subjected to sublethal concentrations of copper and zinc. No aberrations were found for *R. montana* or any other species at any other site. This indicates that levels of metals are sufficiently high at South Buttle to cause aberrations of those organisms able to withstand the contamination. Long-term effects, *i.e.,* viability, reproduction, etc. should be investigated to determine survival and growth of succeeding generations. Say and Giani (1981) found that accumulation of metals occurs in oligochaete cocoons as well as mature individuals, often in direct proportion to the amount in the water and sediment. In summary, the benthic fauna of South Buttle is severely depleted and contains several tolerant forms. North Buttle is quite similar.

Metal Residues in Tissue

Metal residues in phytoplankton were not determined because of the difficulty of obtaining the minimum 10 g required for tissue assays. Metal concentrations in zooplankton were determined from May to November 1981 and the analyses showed pronounced bioconcentration of metals (Nordin *et al.* 1984). Mean bioconcentration factors (metal concentration of tissue/metal concentration in water) at the six contaminated sites were approximately 1.1×10^4, 3.5×10^4, for zinc and copper. Bioconcentration factors of cadmium and lead were 1.7×10^4 and 2.8×10^4, respectively, at South Buttle Lake, the only location at which the metals were detectable. Zinc and copper concentrations in zooplankton (Figure 5) decline with decreasing metal concentrations in the water. Correlation coefficients between the 50th percentile metal concentrations during 1981 and the mean metal concentrations in zooplankton are 0.775 and 0.880 for zinc and copper, respectively. The correlations are significant for both zinc ($P < 0.05$) and copper ($P < 0.01$) but are not significant for cadmium and lead ($P < 0.05$), which are close to detection limits throughout the Campbell River drainage and Upper Quinsam Lake. Zinc and copper concentrations in whole stickleback (*Gasterosteus aculeatus*) declined with distance from the mine (Table 5a). Mean zinc concentrations in rainbow trout muscle are significantly correlated with the zinc concentration in the water ($P < 0.05$, $r = .798$), but copper, cadmium, and lead concentrations in rainbow trout caught at the south end of Buttle Lake do not differ from those at the control site-Upper Quinsam Lake.

Copper and cadmium concentrations in rainbow trout liver tissue (Table 5b) are significantly correlated to the degree of contamination, but zinc concentrations in the livers of rainbow trout, from South Buttle Lake, are not significantly different from those of fish caught in Upper Quinsam Lake, the control site. Roch *et al.* (1982) showed that he-

Table 5a. Metals in whole stickleback (*Gasterosteus aculeatus*) n = 1 Composite of 15–20 Animals (Nordin *et al.* 1984) (μg/g dry wt.)

Lake	Cd	Cu	Pb	Zn
South Buttle	2	28	<1	441
North Buttle	2	18	<1	367
Upper Campbell	1	9	<1	265
John Hart	1	7	<1	319
Sproat Lake (control)	<1	9	<1	147

Table 5b. Metals in rainbow trout (*Salmo gairdneri*) liver, mean ± S.D., n = 5, (Roch *et al.* 1982) (μg/g dry wt.)

Lake	Cu	Zn	Cd
South Buttle	539 ± 124	168 ± 50	19.4 ± 6.1
Upper Campbell	496 ± 238	161 ± 20	15.9 ± 7.0
John Hart	196 ± 39	123 ± 33	8.6 ± 2.9
Upper Quinsam	35 ± 14	123 ± 21	4.0 ± 0.6

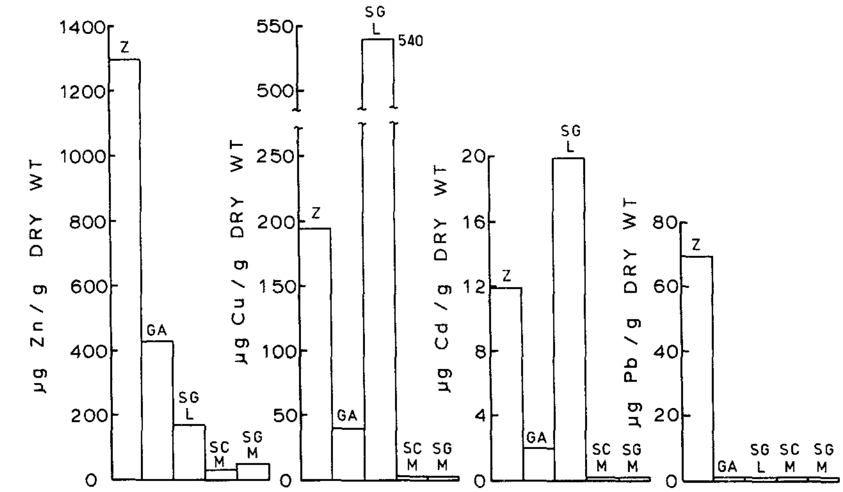

Fig. 6. Metal concentrations in aquatic biota from the south end of Buttle Lake in 1981. Key-zooplankton(Z), stickleback (*Gasterosteus aculeatus* GA), rainbow trout (*Salmo gairdneri* SG), liver (L) or muscle (M), and cutthroat trout (*Salmo clarki* SC) muscle (M)

patic metallothionein concentrations, copper concentrations, in both low and high molecular weight proteins in the liver and cadmium in high molecular weight proteins of rainbow trout are correlated with the degree of contamination. Zinc was not present in the low molecular weight proteins which include metallothionein. The absence of zinc in the metallothionein fraction may be explained by the binding constants of metals to metallothionein. Copper has a greater affinity than cadmium, which is greater than zinc (Rupp and Weser 1978). Although zinc is known to induce the synthesis of metallothionein, copper may displace it because of its greater affinity.

Figure 6 indicates the concentration of metals at various trophic levels. Zooplankton (Nordin *et al.* 1984) concentrate metals to the greatest degree, sticklebacks (Nordin *et al.* 1984) contain lesser quantities, and cutthroat trout (*Salmo clarki*) muscle contains less metal than sticklebacks, which are the main constitutent of the cutthroat trout's diet according to McMynn and Larkin (1953). Thus, biomagnification does not occur in the food chain with any of the metals. Comparison of metals in liver tissue shows that with the exception of lead, all metals are present in higher concentration in liver tissue than in muscle tissue. This difference is most pronounced with copper which is the primary constituent of metallothionein in these fish. The ratios of the metal concentrations in liver tissue as compared to muscle tissue reflect the binding affinity of these metals as metallothionein — copper > cadmium > zinc. Lead does not induce or bind metallothionein and the concentrations of this metal in liver and in muscle tissue are similar.

Toxicity of Heavy Metals to Salmonids in Buttle Lake

Background

In 1966, Wright Engineers Ltd. submitted a report to the Department of Fisheries which included bioassay results of tests with mill tailings. They concluded that a dilution of 3:1 resulted in 10% mortality over four days and that the mill effluent would have little or no affect on fish. Six bioassays conducted between 1967 and 1973 showed 10% mortality or less at 50% dilutions of a mill effluent and bioassays conducted twice annually by the mine since 1975 resulted in 96-hr LC50's with dilutions ranging from 28 to 100% (Clark and Morrison 1982). In a report commissioned by the B.C. Pollution Control Board, Langford (1969) concluded that laboratory acute bioassay measurements of simulated tailings, actual tailings and *in situ* bioassays indicated that toxic effects to rainbow trout could not occur. He recommended that a research station be established at Buttle Lake to study changes in the chemical, physical, and biological limnology. This recommendation was never implemented and it was largely assumed that metal concentrations in the lake would be non-toxic to fish because of sufficient dilution. British Columbia Research reported in 1974 that the tailings from the mine were not toxic to rainbow trout (96-hr exposure) if flocculants were added; they were unable to determine any detrimental effects of tailings disposal with the exception of increased copper concentrations in the liver tissue of native fish. It became evident in the late 1970's that metal concentrations in the lake were rising and Clark (1980) identified copper and zinc concentrations as a threat to salmonids. In a further report (Clark and Morrison 1982), the Waste Management Branch identified zinc, copper, and cadmium as exceeding US EPA (1980) criteria for protection of salmonids in soft water. Alderdice and McLean (1982) reviewed current data on toxicity of metals to salmonids and concluded that concentrations of zinc, copper, and cadmium in the Campbell River were close to and occasionally exceeded the threshold of lethality for steelhead trout (*Salmo gairdneri*) and chinook salmon (*Oncorhynchus tshawytscha*).

Field and Laboratory Assessment of Toxicity

During 1982, rainbow trout were placed in floating cylindrical net pens (0.5 m diameter, 1.5 m height, 1/8 inch mesh) at the south end of Buttle Lake to determine if the concentrations of metals were toxic over a four-week period. Mortality was less than 2% and could not be attributed to metal toxicity. Field bioassays were conducted by adding metals to uncontaminated water (Upper Quinsam Lake, Ralph River or Quinsam River) in the proportions characteristic of Buttle Lake, to determine concentrations which were acutely lethal. Toxicity increased with temperature (Q_{10} = 1.7). The 96-hr LC50 at 11°C in water of 23 mg/L hardness was 540 μg Zn, 27 μg Cu/L and 1.4 μg Cd/L as a mixture. At 21°C and 25 mg/L hardness as $CaCO_3$, the 96-hr LC50 was estimated to be 360 μg Zn, 18 μg Cu and 0.9 μg Cd/L. Thus, at 21°C, the concentrations of metals in the south basin of Buttle Lake were approximately 50% of the lethal concentrations (Roch and McCarter Part II 1984a).

Acute toxicity of the metal mixture to rainbow trout in laboratory experiments using a mixture of well and deionized water (25 mg/L hardness as $CaCO_3$) at 11°C is 500 μg Zn, 25 μg Cu and 1.3 μg Cd/L. A LC50 determined by a continuous flow bioassay was not significantly different ($P < 0.05$). Rainbow trout exposed to a mixture of 150 μg Zn, 7.5 μg Cu and <0.5 μg Cd/L showed less than one per cent mortality over a four-week period of continuous exposure (Roch and McCarter Part I 1984a). The results indicate that the toxicities of metals in the Laboratory and in the Campbell River drainage were similar.

From June 1981 to May 1982, bioassays (96 hr) that were carried out for Westmin Resources Ltd in water from Myra Creek showed that the water was toxic to rainbow trout in all samples (8) during this period (Westmin Stage II submission, Vol. 3 Part 1, Table 1.6.30). An LC50 calculated from the bioassay series is 480 μg Zn/L with estimated copper and cadmium concentrations of 36 and 1.2 μg/L, respectively. The LC10 is approximately 400 μg/L Zn, 30 μg/Cu, and 1 μg Cd/L as total metal. The bioassays were carried out at 15°C.

Toxicity of Buttle Lake Water from 96-hr Tests

Bioassays (96 hr) conducted with water from Buttle Lake and points downstream indicated no toxicity to swim up (0.15 g) rainbow trout (*Salmo gairdneri*) in dissolved metal concentrations as high as 200 μg Zn, 12 μg Cu, and 0.5 μg Cd/L on March 12, 1982 (Westmin Stage II Vol. 3 Part 1, Table 1.6–42).

One bioassay (96 hr) conducted at the University of Victoria with water from the south end of Buttle Lake resulted in mortality (4/10) of steelhead (*Salmo gairdneri*) parr. The total metal concentrations were 199 μg Zn, 14 μg Cu, and 0.7 μg Cd/L. Water from the same location was non-toxic (L5% mortality) in three tests with rainbow trout ranging from 0.3 to 1.2 g in weight. Metal concentrations ranged up to 173 μg Zn, 6 μg Cu, and 0.6 μg Cd/L.

Comparison with Data from the Literature

Metal tolerance by salmonids varies considerably with water quality, temperature, and developmental stage. Although it is accepted that hardness and alkalinity have a marked effect on toxicity, several researchers have reported widely varying toxicity of individual metals in waters of similar composition. A summary of acute toxicity data for copper in soft water (14–20 mg/L as $CaCO_3$) shows a range of LC50's for rainbow trout (*Salmo gairdneri*) between 10 and 100 μg Cu/L (Spear and Pierce 1979) and LC50's for zinc (Spear 1981) ranging between

90 and 1000 μg Zn/L in water ranging from 12–26 mg/L hardness as $CaCO_3$.

Predictions of toxicity are usually based on comparison of existing metal concentrations with laboratory LC50's determined under similar conditions of temperature and water quality. Chapman (1978) reported LC50's for steelhead trout (*Salmo gairdneri*) parr exposed to zinc, copper or cadmium in soft water (24 mg/L as $CaCO_3$) at a temperature of 12°C. The 200-hr LC50 concentrations reported by Chapman of 120 μg Zn, 15 μg Cu, and 0.9 μg Cd/L should be applicable to the prediction of the toxicity of heavy metals to rainbow trout (*Salmo gairdneri*) in Buttle toxicity of heavy metals to rainbow trout (*Salmo gairdneri*) in Buttle Lake. (25 mg/L hardness as $CaCO_3$) Combinations of copper and zinc show additive toxicity to rainbow trout (Lloyd 1961; Sprague 1964). These authors suggest that the action of metals as a mixture can be described by adding the fractions of the LC50s represented by the concentrations of individual metals. The concentrations of metals at the south end of Buttle Lake during May and June of 1982 were 140 μg Zn, 13 μg Cu, and 0.8 μg Cd/L at a temperature of 9–16°C and at 25 mg/L hardness. A prediction of toxicity based on Chapman's data for the same species, similar temperature, hardness of water and size of fish assuming additivity is $140/120 + 13/15 + 0.8/0.9 = 3$ toxic units indicating that severe mortality should have occurred. A large discrepancy exists between toxicity predicted in this manner from Chapman's data and that which occurred during *in situ* experiments, bioassays with Buttle Lake water and laboratory simulations of metal contamination in Buttle Lake.

Recently, Finlayson and Verrue (1982) reported a pronounced antagonistic (less than additive) response of chinook salmon (*Oncorhynchus tshawytscha*) when zinc, copper, and cadmium were present in a ratio of 150:12.5:1 (Zn:Cu:Cd) which is comparable to the ratio in Buttle Lake (400:20:1). The LC50 for the mixture was 4.8 toxic units based on the additive formula. These authors recommend developing water quality on a site specific and metal ratio specific basis.

Continuous flow bioassays with individual metals using juvenile (0.7–1.4 g) rainbow trout (*Salmo gairdneri*) at the University of Victoria toxicology laboratory indicated 7 day LC50 of 380 μg Zn, 50 μg Cu, and 1.3 μg Cd/L. The 7 day LC50 of the mixture (430 μg Zn, 2.2 μg Cu and 1.1 μg Cd/L) is 2.4 toxic units based on the additive formula.

Finlayson and Aschuckian (1979) tested the toxicity of an acid mine waste to steelhead trout (*Salmo gairdneri*) in the Spring Creek drainage, California and determined a 96-hr LC50 of 320 μg Zn, 16 μg Cu, and 0.8 μg Cd/L as total metals for swim up fry in soft (25–60 mg/L as $CaCO_3$) water at 11°C. These concentrations are comparable to values obtained for swim up rainbow trout (*Salmo gairdneri*) at the University of Victoria Toxicology Laboratory (330 μg Zn, 16 μg Cu, and 0.8 μg Cd/L). The former researchers determined an incipient lethal level (lLL–10% mortality) of 60 μg Zn, 10 μg Cu as compared to the 96-hr LC10 determined for swim up rainbow trout at the University of Victoria (130 μg Zn, 6.5 μg Cu, and 0.5 μg Cd/L). However, tests of Buttle Lake water showed no mortality of swim up rainbow trout at concentrations of 200 μg Zn, 12 μg Cu, and 0.5 μg Cd/L (Westmin Resources Ltd 1982). Considerable inconsistency can be found between lethal toxicity predicted from laboratory data and tests carried out *in situ* or with samples from Buttle Lake, but the tests conducted *in situ* are the most valid in assessing toxicity.

Incipient Lethal Level

In situ tests indicate that exposure of rainbow trout in the south basin of Buttle Lake did not cause mortality due to metal contamination. The incipient (threshold) lethal concentration was therefore greater than the ambient concentration of 140 μg Zn, 13 μg Cu, and 0.8 μg Cd/L for rainbow trout (*Salmo gairdneri*) parr. The most extreme conditions in Buttle Lake with respect to toxicity occur during the summer when the temperature near the surface reaches 20–22°C. The incipient lethal level (96–hr LC10) of a combination of metals comparable to the ratio of metals in Buttle Lake (400:20:1) added to uncontaminated water was 200 μg Zn, 10 μg Cu, and 0.5 μg Cd/L at 21°C and 16 mg/L hardness as $CaCO_3$.

Sublethal Effects

Short-term survival does not indicate that a population is safe; a number of sublethal deleterious effects of metals are described as a result of exposure to zinc (Spear 1981), copper (Spear and Pierce 1979) and cadmium exposure (NRCC 1979). These effects include depressed enzyme function, effects on behavior, temperature selection, swimming performance, chemosensory response, feeding, growth, osmoregulation, reproduction, and development. Very little work has been done in this study to assess sublethal effects on behavior, growth of wild fish or success of reproduction and development, because of the expertise required and the difficulties of assessing these effects in the natural envi-

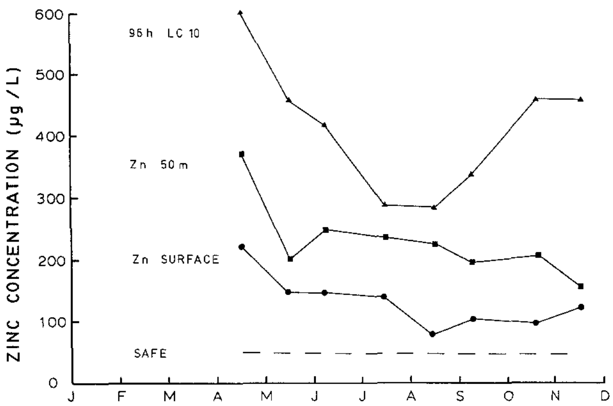

Fig. 7. Zinc concentrations at the surface and at depth in 1981 in S. Buttle Lake compared to the lethal toxicity of a metal mixture (Zn:Cu:Cd = 400:20:1) rainbow trout

ronment. A laboratory simulation of Buttle Lake conditions is being carried out at present to determine effects on development and growth of rainbow trout.

The distribution of metals in hepatic proteins including metallothionein was determined in wild rainbow trout from Buttle Lake (Roch *et al.* 1982) and showed elevated concentrations of copper in both low and high molecular weight protein and of metallothionein in proportion to the degree of zinc and copper contamination. Although inhibition of specific enzymes has not been shown, an excess of metals in the high molecular weight proteins which include the enzymes and functional proteins may interfere with their activities at sublethal concentrations.

Estimated Safe Concentrations

Metallothionein concentrations of 100 nanomoles/g/liver occur at metal concentrations of 50 µg Zn, 2.5 µg Cu, and <0.5 µg Cd/L in the water. This slight response to metals occurs at approximately 0.25 of the incipient lethal level for rainbow trout. As a predictor of sublethal toxicity, a value of 100 nanomoles/g provides a 75% margin of safety under conditions in Buttle Lake. In the absence of data on reproductive effects of metal mixtures on rainbow trout in soft water, the concentrations of 50 µg Zn, 2.5 µg Cu, and <0.5 µg Cd/L are recommended as the best available safe concentrations.

Figure 7 shows the monthly variation in metal concentrations at the south end of Buttle Lake during 1981 in comparison to the recommended safe level and to the 96-hr LC10 at the temperature that is characteristic of that month according to Clark and Morrison (1982). Although sublethal toxicity will vary considerably with temperature, it is difficult to quantify and is represented as a constant. Concerns raised by Alderdice and McLean (1982) with regard to toxic hazard of metals in the Campbell River were addressed in a further study (Roch and McCarter 1984b). Chinook salmon eggs exposed to John Hart Lake water and concentrations representative of the south end of Buttle Lake did not show greater mortality than the controls which were exposed to metal-free water. Reductions in growth (10%) were apparent over a 21 week exposure after hatch at a mean concentration of 51 µg Zn, 2.5 µg Cu, and <0.5 µg Cd/L and became more pronounced at higher concentrations. Safe concentrations designed to protect chinook salmon should not exceed these concentrations. Based on the ratio of Zn:Cu:Cd of 400:20:1 in the stock solution, the actual concentration of cadmium was 0.13 µg/L. Attempts should be made to lower the routine detection limits for cadmium to 0.2 µg/L.

Fish are not the most sensitive group of organisms in the lakes. The apparent major changes in species composition of phytoplankton and change in zooplankton species composition and standing crop provide evidence that much more severe effects have occurred in those groups of organisms than with the fish community. Although the toxicity of metals to the fish are obviously important, the toxicity of metals to the organisms on which the fish depend for their survival must also be considered. It is difficult to assess the degree to which a reduction in the food supply of rainbow trout may have affected population numbers or growth rate. Using the crude estimate of condition factor (Tesch 1968), there is essentially no difference between rainbow trout caught in 1981 (CF = 1.02, n = 15) and those caught by McMynn and Larkin in 1953 (CF = 1.02, n = 37) before the mine began operation. Measurement of annulus radii in systematic collections of scales from rainbow trout since the opening of the mine would have been useful in establishing whether increasing metal contamination had caused a decline in growth rate.

Recommendations for Further work

Periphyton

A more thorough understanding is required of the adaptive mechanisms of tolerant species and specific responses of sensitive species of periphyton in the presence of heavy metals. Periphyton assemblages as well as single species should be exposed to varying levels of heavy metals or other toxins in simulated field conditions in order to determine threshold concentrations of sensitivity.

Zooplankton

Toxicity data for *Daphnia* and other crustacean zooplankters using metal mixtures are limited and should be developed for mixtures of zinc, copper, and cadmium in varying ratios. More data are required relating toxicity to water hardness. Chronic toxicity data that can be used to estimate the risk to a population would be of great value in comparison with field data. Sensitivity of developmental stages of calanoid and cyclopoid copepods may vary and long-term exposures of nauplii and copepodite stages would be useful in assessing risk to the population.

Fish

More work is needed which describes the effect of the metal mixture characteristic of Buttle Lake on a long-term exposure of rainbown trout from hatch to the juvenile stage. This should include measurements of lethality, detrimental effects on growth, measurement of hepatic metallothionein, and histology of the gill, liver, and kidney. More knowledge is required to determine whether the metal combination has a detrimental effect on spermatogenesis and oogenesis. Mature rainbow trout should be exposed for periods of reproductive development to determine a threshold concentration of reproductive impairment. Little data are available concerning the toxicity of metals to cutthroat trout (*Salmo clarki*) and Dolly Varden Char (*Salvelinus malma*). Additional bioassays (96 hr) conducted with a mixture of metals characteristic of Buttle Lake with swim up fry of the three species present in the lake would establish relative tolerance. A thorough analysis for any histopathological effects of metals in wild rainbow trout, measurement of blood ion concentrations, and liver glycogen content would contribute to the assessment of the health of the fish present at the south end of Buttle Lake.

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