ALBERTA OIL SANDS
ENVIRONMENTAL
RESEARCH PROGRAM

A Laboratory Study of Long-term Effects of Mine Depressurization Groundwater on Fish and Invertebrates

Project WS 2.6.1

December 1980

Alberta
ENVIRONMENT
15th Floor, Oxbridge Place
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Edmonton, Alberta, Canada
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ALBERTA OIL SANDS ENVIRONMENTAL RESEARCH PROGRAM

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AOSERP Report 113

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The Hon. J.W. (Jack) Cookson
Minister of the Environment
222 Legislative Building
Edmonton, Alberta

Sir:

Enclosed is the report "A Laboratory Study of Long-term Effects of Mine Depressurization Groundwater on Fish and Invertebrates".

This report was prepared for the Alberta Oil Sands Environmental Research Program, through its Water System, under the Canada-Alberta Agreement of February 1975 (amended September 1977).

Respectfully,

W. Solodzuk, P.Eng.
Chairman, Steering Committee, AOSERP
Deputy Minister, Alberta Environment

A LABORATORY STUDY OF LONG-TERM EFFECTS OF MINE DEPRESSURIZATION GROUNDWATER ON FISH AND INVERTEBRATES

by

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for

ALBERTA OIL SANDS ENVIRONMENTAL RESEARCH PROGRAM

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ABSTRACT

This study was conducted to determine long-term toxic effects, on selected species of fish and invertebrates, of mine depressurization at concentrations non-lethal in acute toxicity tests. The study also includes chronic toxicity experiments, studies of sub-lethal effects of groundwater, and a literature review.

The results of groundwater monitoring indicate that during 6 mo of storage, there was a decline in concentration of almost all chemical parameters tested. However, in contrast to previous studies, some heavy metals (iron, lead, nickel, and zinc) showed an increase in concentration with storage time.

Mine depressurization groundwater was acutely toxic to the three species of invertebrates tested. The 96 h LC_{50} for the two mayfly species, *Caenis simulans* and *Paraleptophlebia cornuta*, was 68.75 and 64.28%, respectively. The 96 h LC_{50} for the amphipod, *Hyalella azteca*, was 50%.

Results of chronic toxicity tests for the three invertebrates species indicated that percent survivorship was highest in control and 3% groundwater concentrations. The mine depressurization groundwater had an inhibitor effect on the larval growth and emergence of the two mayfly species tested. The result was less obvious in the experiment with *H. azteca*. Cannibalism appeared to be an important factor contributing to higher mortalities in amphipods.

The salinity of mine depressurization groundwater affected the osmoregulatory function of the two mayfly species. Chloride cell density was of some predictive value in determining osmoregulatory stress in the test animals. Accumulation of both Cu and Zn occurred in tissues of *Caenis* and *Hyalella*, particularly after chronic exposure to the higher concentrations of groundwater.

The 90 d LC_{50} was 8.5 to 9% for rainbow trout, 13.2% for lake chub, and 5.8% for white suckers. Initial exposure to sublethal levels of groundwater caused significant elevation of operculate pumping frequency and coughing rate. Significant depression of opercular pumping frequency occurred during chronic exposure. Measurement of both operculate pumping and coughing rates might constitute a practical biomonitoring method because of the speed of response and ease of measurement.

A 1 h exposure to mine depressurization water was toxic to fertilized lake whitefish eggs at concentrations as low as 10%. Mortality increased with time.

The review of the literature available on the components and effects of mine depressurization groundwater is presented. The review includes sections on chemical properties of groundwater, the effects of storage on groundwater, acute toxicity studies done using groundwater, studies of sublethal effects of groundwater, and a summary of work done on individual components (heavy metals, cations, and anions) commonly found in groundwater.

Because more data are available on the acute toxicity of mine depressurization groundwater, the determination of tolerance limits should be made with greater reliance on this test. Information from other sources should be used to verify decisions derived from acute toxicity data.

ACKNOWLEDGEMENTS

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This research project was funded by the Alberta Oil Sands Environmental Research Program, a joint Alberta-Canada research program established to fund, direct, and co-ordinate environmental research in the Athabasca Oil Sands area of northeastern Alberta.

1. INTRODUCTION

1.1 BACKGROUND

Underlying the oils sands in the centre of the mining area of Syncrude Canada Ltd. are several ancient river channels. The water contained in these channels is brackish with high concentrations of sodium, chloride, and bicarbonate ions, together with a variety of other organic and inorganic components. Because a hydrostatic head is maintained by the overlying strata, this groundwater is under artesian pressure. During the open-pit mining process, the hydrostatic balance is disturbed as the overburden is removed. This unbalanced uplift threatens the stability of the mine floor and walls. In order to maintain the water table below the level of the mine, water must be depressurized by pumping it to the surface. This mine depressurization water is collected in impoundments and eventually discharged into the Athabasca River.

1.2 SCOPE

The objective of the study described in this report was to determine the long-term toxic effects, on selected species of fish and invertebrates, of mine depressurization water at concentrations non-lethal in acute (96 h) toxicity tests. Previous studies commissioned by the Alberta Oil Sands Environmental Research Program (AOSERP) and Syncrude Canada Ltd. (McMahon et al. 1976; Lake and Rogers 1979; Giles et al. 1979) have established the acute toxicity of the mine depressurization groundwater to both fish and invertebrates (Figure 1). There is, however, virtually no information on the chronic toxicity and sublethal effects of the saline groundwater. The present work includes chronic toxicity experiments, studies of sub-lethal effects of the groundwater, and a literature review.

1.3 TERMS OF REFERENCE

The Terms of Reference for this study defined the methods of collecting and handling groundwater, the fish species selected for the experiments, and the scope of the literature review.

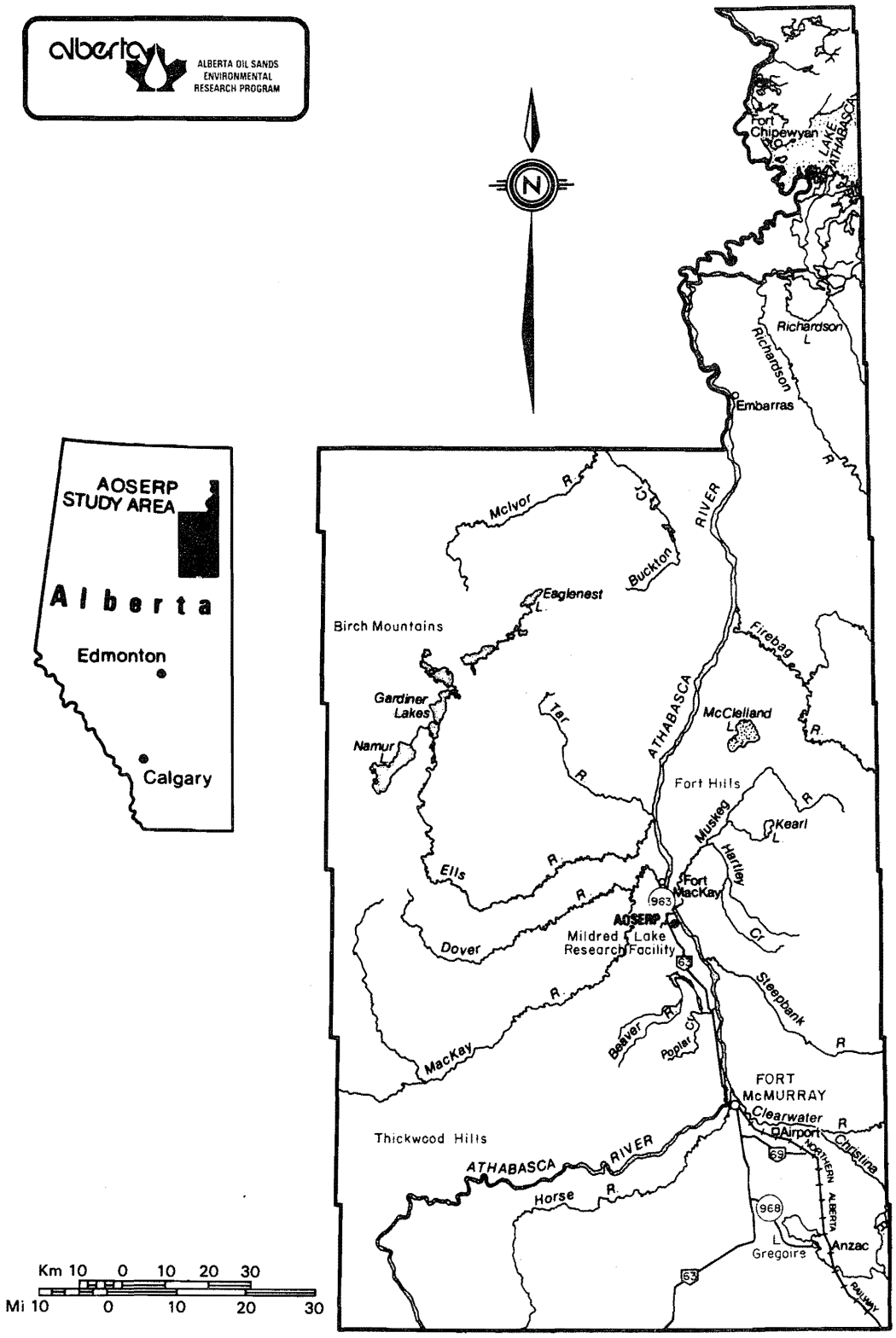

Figure 1. Alberta Oil Sands Environmental Research Program study area.

1.4 PLAN OF PRESENTATION

Summaries of the material in this report are placed at the beginning. The first part of the volume is devoted to the reports on the experimental work; the second part is devoted to a brief literature review.

2. SUMMARY AND CONCLUSIONS

2.1 EXPERIMENTAL STUDIES

2.1.1 Groundwater

During storage, a major decrease in concentrations occurred in almost all the chemical parameters tested. Contrary to the observations in some previous studies, certain heavy metals (iron, lead, nickel, and zinc) showed an increase in concentration with storage time. This increase may have been due to the resuspension of the heavy metals into the solution during storage or it may have represented variations in the metal content between the drums.

2.1.2 Aquatic Invertebrates

The mine depressurization groundwater was acutely toxic to invertebrates tested. The 96 h LC_{50} (concentration at which 50% of the animals die) for *Paraleptophlebia bicornuta* was 64.28%, for *Caenis simulans* 68.75%, and for *Hyalella azteca* 50%.

Since toxicity of the mine depressurization groundwater was lower at both ends of the test concentration range, the invertebrate chronic lethality experiments indicated the dose-mortality relationship was non-linear. The non-lethal or "safe" concentration for the invertebrates tested appeared to be less than 3% groundwater.

The final larval stage nymphs of the mayfly *Paraleptophlebia bicornuta* were more sensitive to the toxicant than the younger nymphs. The mine depressurization groundwater had an inhibiting effect on the larval growth and emergence of the two mayfly species tested. The result was less obvious in the experiment on the amphipod *Hyalella azteca*.

The salinity of the mine depressurization groundwater affected the osmoregulatory function of the two mayfly species tested. Chloride cell density appeared to have certain predictive value in quantifying osmoregulatory stress in the test animals.

Accumulation of heavy metal ions occurred in the tissues of both *Caenis* and *Hyalella* after chronic exposure to the various concentrations of groundwater tested. This was particularly so at the higher concentrations.

2.1.3 Fish

The 90 d LC_{50} for rainbow trout, lake chub, and white sucker was 8.5 to 9% for trout, 13.2% for chub, and 5.8% for suckers. As was true of the invertebrate experiments, the dose-mortality relationship was non-linear. No significant difference in the LC_{50} was observed in the two rainbow trout experiments using groundwater of different ages.

Exposure, both acute and chronic, to sublethal levels of groundwater caused significant elevation of opercular pumping frequency and coughing rate on initial exposure to groundwater, and significant depression of opercular pumping frequency during chronic exposure. Because of the ease of measurement and speed of response, measurement of opercular and coughing rates together would seem to constitute a practical biomonitoring method.

Sublethal exposure also resulted in an increase in blood haematocrit in fish, most likely an osmo-induced increase in red blood cell volume.

Sublethal exposure caused a marked buildup of major ionic constituents of the groundwater Na^{+} and K^{+} ions, and an accumulation of heavy metal ions (Cu^{++} and Zn^{++}) in the fish tissues.

Mucus production and buildup in the gills of fishes occurred on exposure to groundwater. The mucus production may have induced some respiratory stress.

A 1 h exposure to mine depressurization water during the fertilization process was toxic to lake whitefish eggs at concentrations as low as 10%. Mortality increased with time and, after 100 d incubation, ranged from 21.7%, after a 1 h exposure to a 10% groundwater solution, to 23.2% at 25% concentrations, 38.9% at 50% concentrations, and 85.5% at 100% concentrations.

2.2 LITERATURE REVIEW

A review of the data (including that generated in the present study) on the toxicity of mine depressurization groundwater indicated that they fall into several general categories. A brief summary of the work done in each category follows.

2.2.1 Acute Toxicity Tests

Seventy-three acute toxicity tests (96 to 240 h) have been performed on fish and invertebrates.

2.2.2 Chronic Toxicity Tests

Six tests (54 to 90 d) have been performed, three on fish, and three on invertebrate species.

2.2.3 Critical Life-Stage Tests

Toxicity tests (both acute and chronic) have been performed on critical life stages of rainbow trout (eggs, alevins), mountain whitefish (sperm, fertilized eggs, unfertilized eggs); mayflies--*Hexagenia rigida* (fertilized eggs, parthogenetic eggs), *Paraleptophlebia bicornuta* (penultimate larval stage), and *Caenis simulans* (penultimate larval stage).

2.2.4 Behavioural Response Tests

Swimming performance tests for rainbow trout and avoidance tests for lake whitefish and the amphipod *Gammarus lacustris* have been done.

2.2.5 Physiological Reponses

Physiological parameters tested for fish (rainbow trout, lake chub, white sucker) during acute and chronic exposure to mine depressurization water included histopathological studies on gill, kidney, and liver tissue; cardiovascular/respiratory responses; opercular pumping frequencies; coughing frequencies; haematocrit; ion accumulation; and growth. For the invertebrates *Caenis simulans* and

Hyalella azteca, physiological parameters observed under chronic conditions included moulting frequencies, emergence success, osmoregulation, growth, and ion accumulation.

2.2.6 Field Toxicity Studies

Only one field investigation has been conducted. This study was done on Beaver Creek and controlled microcosm experiments were notably absent.

2.3 SUGGESTIONS FOR FUTURE STUDIES

In order to determine tolerance limits for the aquatic communities in the AOSERP area, a subjective judgement would have to be made, based on the toxicological data so far obtained. These data indicate that, in short-term laboratory exposures, mine depressurization groundwater is moderately toxic. During chronic laboratory exposures, negative responses have been elicited even at low groundwater concentrations (2 to 3%).

Because there is more information available on the acute toxicity of mine depressurization groundwater, the determination of tolerance limits should be made with greater reliance on this category of data. Data derived from other tests should be used to crosscheck decisions derived from the acute toxicity data. A final assessment of the hazard of mine depressurization groundwater should also be based on a thorough consideration of the following questions:

1. Are the physical and chemical properties of mine depressurization groundwater sufficiently understood to prevent "ecological surprises"?
2. Could concentrations of the mine water discharged be predicted with confidence?
3. What is the relationship of LC_{50}'s to anticipated concentrations of mine depressurization groundwater? $LC_{50} \geq$ x 100 anticipated acute exposure level or $LC_{50} \leq$ x 10 anticipated acute exposure level?

4. What is the relationship between anticipated groundwater concentrations and the maximum allowable concentration derived from critical life-stage testing?
5. Will exposures from possible accidents (eg., spills) be localized and temporary? Can they be minimized by proper handling and planning?
6. Will biomagnification of heavy metals from the groundwater be great enough to harm aquatic organisms? Can it harm species at a higher trophic level in the food chain?
7. What methods could be used to monitor the effects of mine depressurization groundwater on the aquatic ecosystem?

3. GROUNDWATER MONITORING

3.1 METHODS

Mine depressurization groundwater used throughout this study was collected from Wells M_1, 78, and 79 on Syncrude Lease 17. In August 1977, a total of approximately 20 500 L groundwater was collected from the three wells and stored in 204 L polyethylene-lined steel drums. The drums were immediately trucked to Calgary and stored in a commercial cold storage warehouse at $5^{\circ}C$.

As required by the Terms of Reference for this study, the toxicant used was made by mixing the groundwater from the three wells in the following proportions:

Well	Volume
M_1	2/3
78	1/6
79	1/6

During the course of the study, chemical analyses of the composite groundwater were performed periodically to determine the effect of storage on its chemical composition. Chemical analyses were carried out by Chemex Labs (Alberta) Ltd. in Calgary.

3.2 RESULTS

The results of groundwater monitoring are summarized in Table 1. The results clearly indicate that, during the 6 mo of storage, there was a decline in concentration of almost all the chemical parameters for which analyses were performed. The groundwater became less saline and there was a reduction in both alkalinity and hardness. The pH of the groundwater fluctuated but tended to become more acidic with time. The oxygen demand of the composite groundwater increased during the first month of storage and then decreased. Concentrations of both organic and inorganic carbon decreased. Contrary to previous observations by McMahon et al. (1976) and Giles et al. (1979), some heavy metals (iron, lead, nickel, and zinc) showed an increase in concentration with storage time.

Table 1. Changes in the chemical composition of composite mine depressurization groundwater during the study period, and chemical composition of the Calgary city water (diluent).

Date of Analysis	Composite Mine Depressurization Groundwater						Calgary City Water
Parameter[a]	29 Aug. 1978	5 Sept. 1978	21 Sept. 1978	24 Oct. 1978	18 Dec. 1978	26 Feb. 1979	26 Feb. 1979
Na	6 000.0	6 000.0	6 750.0	5 300.0	2 250.0	2 100.0	212.0
K	50.0	51.0	60.0	43.0	22.5	10.0	0.40
Ca	63.0	60.0	98.0	55.0	45.0	34.0	47.5
Mg	115.0	114.0	12.0	113.0	62.0	20.5	14.20
Cl	8 710.0	8 414.0	7 683.0	8 002.0	2 710.0	2 800.0	2.0
HCO_3	3 090.0	2 821.0	3 293.0	3 243.0	2 860.0	1 297.4	160.3
CO_3	75.0	<1.0	1.0	1.0	<1.0	<1.0	<1.0
NH_3•N	10.7	11.0	11.0	10.5	10.5	2.75	0.05
CN	<0.001	<0.001	<0.001	<0.001	0.009	0.01	<0.001
Al	0.09	0.10	0.08	<0.04	<0.04	NA[b]	NA
Ar	0.0005	<0.0002	<0.0002	<0.0002	0.0018	<0.0012	<0.0002
B	8.0	8.3	8.1	4.6	0.8	2.5	<0.02
Cd	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Co	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cu	0.022	<0.001	0.05	0.006	0.014	0.014	0.019

continued ...

Table 1. Continued.

Parameter	Composite Mine Depressurization Groundwater						Calgary City Water
Date of Analysis	29 Aug. 1978	5 Sept. 1978	21 Sept. 1978	24 Oct. 1978	18 Dec. 1978	26 Feb. 1979	26 Feb. 1979
Fe	0.43	0.12	0.06	0.70	0.15	1.04	0.02
Pb	<0.002	<0.002	<0.002	0.014	0.004	0.026	0.002
Hg	0.0003	0.0003	0.002	0.0036	<0.0001	<0.0001	<0.0001
Ni	<0.001	<0.001	<0.001	<0.001	<0.001	0.005	0.001
P (total)	0.04	0.006	0.01	0.007	0.011	0.003	<0.003
Zn	NA	NA	NA	0.002	0.006	0.10	0.003
pH	8.5	8.35	7.6	7.55	8.3	7.9	8.1
Alkalinity ($mg/L\ CaCO_3$)	5 241.0	2 312.0	2 700.0	2 658.0	2 340.0	1 063.4	131.4
Hardness ($mg/L\ CaCO_3$)	617.5	606.0	293.0	590.0	368.0	169.4	177.1
COD	630.0	752.0	812.0	534.0	574.0	47.0	5.0
TOC	40.0	15.0	22.0	15.5	2.6	34.0	4.0
TIC	710.0	555.0	707.0	662.5	562.0	255.0	31.5
Total Suspended Solids	NA	104.0	3.3	4.7	24.0	32.8	<0.4

continued ...

Table 1. Concluded.

Date of Analysis	Composite Mine Depressurization Groundwater						Calgary City Water
Parameters	29 Aug. 1978	5 Sept. 1978	21 Sept. 1978	24 Oct. 1978	18 Dec. 1978	26 Feb. 1979	26 Feb. 1979
Total Hydrocarbons	7.0	7.4	3.9	5.3	3.2	0.10	<0.10
Turbidity (JTU)	9.0	25.0	5.3	6.0	5.8	20.5	0.8
Oil and Grease	6.2	6.2	3.4	4.2	2.0	0.60	0.10

a All concentrations are expressed in mg/L unless otherwise stated.

b NA - not available.

4. AQUATIC INVERTEBRATES

4.1 INTRODUCTION

The objective of the invertebrate studies was to determine the long-term effects of saline mine depressurization groundwater on selected invertebrate species. The species studied were nymphs of the mayflies *Caenis simulans* and *Paraleptophlebia bicornuta*, and the amphipod *Hyalella azteca*. Preliminary acute toxicity tests, chronic toxicity tests, and studies of sublethal effects were done on all three species. The latter studies included examination of test animals for changes in moulting frequency, emergence, growth, osmoregulation, and ion accumulation.

4.2 CHRONIC TOXICITY

4.2.1 Methods

Groundwater was collected from Wells M_1, 78, and 79 on Syncrude Lease 17. The water from the three wells was shipped to Calgary in polyethylene-lined steel drums with a capacity of 204 L, and stored at $5^{o}C$ until used. The water from the three wells was mixed in the following proportions: two-thirds from Well M_1, one-sixth from Well 78, and one-sixth from Well 79. The composite well water was used as the test toxicant.

Chemical characteristics of the composite stock solution were monitored throughout the study period. Monitoring results are presented in Section 3. Test solutions in each tank were monitored for dissolved O_2 concentration, pH, temperature, and conductivity. The results are tabulated in Table 2.

Calgary city water was used as the diluent in this study. Before use, the tapwater was filtered through a carbon filter and dechlorinated with sodium thiosulphate. It was then aerated and warmed to $15^{o}C$.

Table 2. Summary of routine physico-chemical analyses from chronic bioassays with rainbow trout (Experiments 1 and 2), lake chub, and white sucker.

Concentration (%)	Temperature N	Mean	Range	Conductivity N	Mean	Range	Dissolved Oxygen N	Mean	Range	pH N	Mean	Range
Rainbow Trout (Experiment 1)												
Week 1 (24-30 October):												
12.5	14	15.2	15.0-16.0	6	3230	3120-3360	10	7.7	6.0-8.7	6	8.3	8.2-8.5
10.0	14	15.2	15.0-16.0	6	2580	2520-2640	10	7.4	6.4-8.8	6	8.2	8.1-8.3
8.0	14	15.2	15.0-16.0	6	2216	2100-2280	10	7.5	5.6-9.1	6	8.2	8.1-8.3
4.0	14	15.2	15.0-16.0	6	1320	1260-1380	10	8.0	6.8-9.2	6	8.2	8.1-8.3
2.0	14	15.2	15.0-16.0	6	868	840- 888	10	8.2	7.4-9.0	6	8.1	8.1-8.2
Control	14	15.2	15.0-16.0	6	366	336- 398	10	7.9	6.8-9.5	6	7.9	7.8-8.1
Week 2 (31 October to 6 November)												
12.5	8	15.4	15.0-16.5	2	2940	2880-3000	8	8.1	7.4-8.5	4	8.4	8.4-8.4
10.0	8	15.4	15.0-16.5	2	2640	2640-2640	8	8.0	7.6-9.2	4	8.3	8.3-8.3
8.0	8	15.4	15.0-16.5	2	2220	2160-2280	8	8.0	7.4-9.0	4	8.2	8.1-8.3
4.0	8	15.3	15.0-16.0	2	1380	1380-1380	8	8.1	7.4-9.5	4	8.1	7.9-8.2
2.0	8	15.3	15.0-16.0	2	912	912- 912	8	7.9	7.0-8.4	4	7.9	7.8-8.0
Control	8	15.3	15.0-16.0	2	348	348- 348	8	8.0	7.4-8.4	4	8.0	7.9-8.1
Week 3 (7-8 November):												
12.5	6	16.0	16.0	4	3270	2760-4440	8	8.0	7.5-9.0	6	8.5	8.4-8.5
10.0	6	16.0	16.0	4	2475	2340-2640	8	7.8	7.1-9.2	6	8.4	8.3-8.4

continued ...

Table 2. Continued.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
8.0	6	16.0	16.0	4	2220	2160-2280	8	7.9	7.3-9.4	6	8.3	8.1-8.4
4.0	6	15.8	15.5-16.0	4	1335	1260-1380	8	7.9	7.5-9.0	6	8.2	8.1-8.3
2.0	6	15.8	15.5-16.0	4	891	888- 900	8	7.9	7.0-9.2	6	8.1	8.0-8.3
Control	6	15.8	15.5-16.0	4	330	312- 348	8	8.1	7.4-9.5	6	8.0	7.9-8.1
Week 4 (14-20 November):												
12.5	4	15.3	15.0-15.5	4	1350	1200-1560	10	8.6	8.0-9.6	2	8.2	8.2-8.3
10.0	4	15.3	15.0-15.5	4	1110	1000-1260	10	8.2	7.5-9.2	2	8.1	8.1-8.2
8.0	4	15.3	15.0-15.5	4	1059	920-1200	10	8.4	7.6-9.6	2	8.1	8.0-8.2
4.0	4	15.3	15.0-15.5	2	774	768- 780	5	8.7	7.6-9.9	1	8.0	8.0
2.0	4	15.3	15.0-15.5	4	588	552- 684	10	8.7	7.6-9.8	2	8.1	8.0-8.1
Control	4	15.3	15.0-15.5	4	291	276- 300	10	8.7	6.8-10.0	2	8.0	7.9-8.0
Week 5 (21-27 November):												
12.5	2	16	16	4	3210	3000-3360	10	8.2	7.2-9.0	4	8.5	8.4-8.6
10.0	2	16	16	4	2580	2400-2760	10	8.1	7.1-9.0	4	8.3	8.3
8.0	2	16	16	4	1980	1560-2220	10	8.5	7.8-10.0	4	8.3	8.3-8.4
4.0	1	16	16	2	1350	1320-1380	5	8.6	8.0-9.5	2	8.2	8.2-8.3
2.0	2	16	16	4	1107	840-1440	10	8.5	7.8-9.4	4	8.2	8.1-8.2
Control	2	16	16	4	327	300- 360	10	8.4	7.8-9.6	4	8.1	8.0-8.1
Week 6 (28 November to 4 December):												
12.5	4	15.2	15.0-15.5	4	1980	1440-2520	10	8.3	7.6-9.0	4	8.2	8.0-8.3
10.0	4	15.3	15.0-15.5	4	1575	1320-1980	10	8.4	7.8-9.1	4	8.2	8.0-8.3

continued ...

Table 2. Continued.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
8.0	4	15.3	15.0-15.5	4	1239	1104-1440	10	8.4	7.5-9.0	4	8.1	7.9-8.3
4.0	2	15.3	15.0-15.5	2	918	780-1056	5	8.5	8.1-8.7	2	8.1	7.9-8.3
2.0	4	15.3	15.0-15.5	4	585	504- 696	10	8.4	6.8-9.0	4	7.9	7.6-8.2
Control	4	15.3	15.0-15.5	4	303	288- 324	10	8.5	7.7-9.6	4	7.9	7.7-8.2
Week 7 (5-11 December):												
12.5	4	15.5	15.0-16.0	4	1548	1464-1584	10	8.0	6.5-9.4	4	8.2	8.1-8.3
10.0	4	15.5	15.0-16.0	4	1317	1248-1356	10	7.9	6.8-9.6	4	8.2	8.1-8.2
8.0	4	15.5	15.0-16.0	4	1092	1032-1152	10	8.1	7.0-9.4	4	8.1	8.1-8.2
4.0	2	15.5	15.0-16.0	2	768	744- 792	5	8.3	7.7-9.2	2	8.1	8.1
2.0	4	15.5	15.0-16.0	4	534	504- 588	10	8.1	6.0-9.3	4	8.0	7.9-8.0
Control	4	15.5	15.0-16.0	4	369	312- 468	10	8.1	6.0-9.1	4	7.9	7.9-8.0
Week 8 (12-18 December):												
12.5	4	15.0	15.0	4	1509	1440-1560	10	7.7	6.4-8.3	4	8.1	8.0-8.2
10.0	4	15.0	15.0	4	1335	1260-1440	10	7.7	7.1-8.8	4	8.1	8.0-8.3
8.0	4	15.0	15.0	4	1122	1104-1140	10	7.9	7.4-8.4	4	8.1	8.0-8.2
4.0	2	15.0	15.0	2	780	768- 792	5	8.3	7.8-8.6	2	8.0	8.0
2.0	4	15.0	15.0	4	561	516- 600	10	7.9	6.6-8.8	4	7.9	7.9-8.0
Control	4	15.0	15.0	4	330	324- 348	10	7.9	7.2-8.6	4	7.8	7.8-7.9
Week 9 (19-15 December):												
12.5	2	15.3	15.0-15.5	2	1395	1350-1440	6	8.0	7.5-8.6	2	8.2	8.2

continued ...

Table 2. Continued.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
10.0	2	15.8	15.5-16.0	2	1179	1164-1194	6	7.9	7.4-8.5	2	8.2	8.1-8.2
8.0	2	15.3	15.0-15.5	2	1147	1100-1194	6	8.0	7.4-8.4	2	8.2	8.2
4.0	1	15.0	15.0	1	720	720	3	8.3	8.0-8.6	1	8.2	8.2
2.0	2	15.0	15.0	2	492	492	6	7.9	6.4-8.7	2	8.1	8.0-8.2
Control	2	15.0	15.0	2	294	294	6	8.0	7.6-8.6	2	8.0	7.9-8.0
Week 10 26 December to 2 January):												
12.5	4	14.5	14.0-15.0	6	1454	1368-1656	10	8.2	6.7-9.0	6	8.1	7.7-8.3
10.0	4	14.5	14.0-15.0	6	1304	1170-1512	10	8.2	7.6-8.9	6	8.1	7.7-8.4
8.0	4	14.5	14.0-15.0	6	1150	1068-1320	10	8.3	7.0-8.9	6	8.0	7.9-8.3
4.0	2	14.5	14.0-15.0	3	752	696- 792	5	8.6	8.4-8.9	3	8.1	7.9-8.2
2.0	4	14.5	14.0-15.0	6	538	504- 588	10	8.5	7.7-9.2	6	8.0	7.8-8.1
Control	4	14.5	14.0-15.0	6	316	300-336	10	8.5	8.0-9.2	6	7.9	7.8-8.1
Week 11 (3-9 January):												
12.5	4	15.8	15.5-16.0	4	1452	1380-1500	6	7.4	6.0-8.0	4	8.2	8.1-8.3
10.0	4	15.8	15.5-16.0	4	1218	1152-1284	6	7.5	6.0-8.4	4	8.1	8.0-8.2
8.0	4	15.8	15.5-16.0	4	1116	1092-1128	6	7.9	7.0-8.4	4	8.1	8.0-8.2
4.0	2	15.0	15.0	2	726	708- 744	3	8.1	7.8-8.4	2	8.0	7.9-8.0
2.0	4	15.0	15.0	4	557	522- 588	6	7.7	6.0-8.4	4	8.0	7.9-8.1
Control	4	15.0	15.0	4	311	282- 348	6	7.9	7.1-8.2	4	7.9	7.8-8.0

continued ...

Table 2. Continued.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
Week 12 (10-16 January):												
12.5	5	15.7	15.0-16.0	4	1566	1500-1620	10	7.8	6.8-9.0	4	8.1	8.0-8.2
10.0	6	15.7	15.0-16.0	4	1224	1008-1308	10	7.9	7.0-9.3	4	8.0	8.0-8.1
8.0	6	15.3	15.0-16.0	4	1143	1056-1224	10	8.2	7.4-9.2	4	8.0	7.9-8.1
4.0	6	15.3	15.0-16.0	2	786	756- 816	5	8.4	8.0-9.4	2	8.0	7.9-8.1
2.0	6	15.3	15.0-16.0	4	546	492- 576	10	8.3	7.0-9.6	4	7.9	7.8-8.0
Control	6	15.3	15.0-16.0	4	330	312- 348	10	8.2	7.4-9.6	4	7.8	7.8-8.0
Week 13 (17-19 January):												
12.5	2	15.0	15.0	2	1560	1500-1620	6	7.4	7.0-7.9	2	8.2	8.1-8.3
10.0	2	15.0	15.0	2	1278	1236-1320	6	7.7	6.0-8.5	2	8.2	8.2
8.0	2	15.0	15.0	2	1164	1152-1176	6	7.9	7.6-8.3	2	8.1	8.1-8.2
4.0	2	15.0	15.0	1	744	744	3	8.4	8.2-8.7	1	8.1	8.1
2.0	2	15.0	15.0	2	546	528- 564	6	8.4	8.0-8.6	2	8.0	7.9-8.0
Control	2	15.0	15.0	2	318	312- 324	6	8.2	7.9-8.7	2	7.8	7.8-7.9
Rainbow Trout (Experiment 2)												
Week 1 (8-14 November):												
12.5	6	15.5	15.0-16.5	4	2275	2340-3000	8	8.1	7.6-8.8	4	8.4	8.2-8.6
10.0	6	15.5	15.0-16.5	4	2586	2520-2664	8	8.3	7.6-9.5	4	8.4	8.2-8.5
8.0	6	15.5	15.0-16.5	4	1416	960-2280	8	8.5	7.8-9.6	4	8.3	8.2-8.6
4.0	6	15.5	15.0-16.5	4	1332	1260-1380	8	8.0	6.1-9.6	4	8.2	8.1-8.4

continued ...

Table 2. Continued.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
2.0	6	15.5	15.0-16.5	4	831	768- 900	8	8.4	7.9-9.2	4	8.2	8.1-8.2
Control	6	15.5	15.0-16.5	4	294	252- 324	8	8.3	7.4-9.5	4	8.3	8.1-8.7
Week 2 (15-21 November):												
12.5	4	16.0	16.0	4	1418	1350-1500	10	8.5	7.2-9.6	2	8.3	8.3-8.4
10.0	4	16.0	16.0	4	1320	1200-1380	10	8.2	6.0-9.5	2	8.1	8.1
8.0	4	16.0	16.0	4	1104	1008-1152	10	8.8	7.6-9.8	2	8.2	8.1-8.2
4.0	4	16.0	16.0	4	744	720- 768	10	8.2	6.0-10.0	2	8.1	8.1
2.0	4	16.0	16.0	4	546	528- 576	10	8.6	7.2-9.8	2	8.0	8.0
Control	4	16.0	16.0	4	296	259- 324	10	8.7	7.2-9.8	2	7.9	7.8-8.0
Week 3 (22-28 November):												
12.5	2	15.0	15.0	2	3240	3120-3360	10	8.3	7.6-9.2	4	8.5	8.4-8.5
10.0	2	15.0	15.0	2	2610	2520-2700	10	8.3	7.8-9.2	4	8.5	8.4-8.5
8.0	2	15.0	15.0	2	2070	1920-2220	10	8.3	8.0-9.0	4	8.4	8.3-8.4
4.0	2	15.0	15.0	2	1380	1320-1440	10	8.5	8.2-9.0	4	8.3	8.2-8.4
2.0	2	15.0	15.0	2	900	888- 912	10	8.2	7.6-8.9	4	8.2	8.1-8.3
Control	2	15.0	15.0	2	342	336- 348	10	8.1	6.7-9.0	4	8.0	7.8-8.0
Week 4 (29 November to 5 December):												
12.5	6	16.0	16.0	4	2439	1596-3360	10	7.2	6.6-7.7	4	8.2	7.9-8.5
10.0	6	16.0	16.0	4	2049	1476-2700	10	7.4	6.6-8.0	4	8.2	7.9-8.5
8.0	6	16.0	6.0	4	1659	1236-2220	10	7.8	7.6-8.0	4	8.3	8.0-8.5

continued ...

Table 2. Continued.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
4.0	6	16.0	16.0	4	1104	816-1440	10	7.5	6.0-8.1	4	8.1	7.8-8.4
2.0	6	16.0	16.0	4	744	576- 912	10	7.8	6.4-8.3	4	8.0	7.9-8.3
Control	6	16.0	16.0	4	342	324- 360	10	7.4	6.6-8.2	4	7.9	7.7-8.1
Week 5 (6-12 December):												
12.5	6	15.2	15.0-15.5	4	1524	1416-1620	10	7.5	5.6-8.2	4	8.1	8.0-8.1
10.0	6	15.2	15.0-15.5	4	1299	1200-1380	10	7.8	7.4-8.4	4	8.1	8.0-8.2
8.0	6	15.2	15.0-15.5	4	1107	1032-1176	10	7.9	7.4-8.3	4	8.1	8.0-8.2
4.0	6	15.2	15.0-15.5	4	696	624- 756	10	7.9	7.2-8.4	4	8.0	7.9-8.1
2.0	6	15.2	15.0-15.5	4	519	456- 600	10	8.1	7.6-8.9	4	8.0	7.9-8.0
Control	6	15.2	15.0-15.5	4	294	246- 348	10	7.8	6.0-9.2	4	7.9	7.8-8.0
Week 6 (13-19 December):												
12.5	4	16.0	16.0	4	1551	1500-1584	8	8.3	7.9-8.6	4	8.3	8.2-8.3
10.0	4	16.0	16.0	4	1263	1200-1320	8	8.3	7.8-8.6	4	8.2	8.2
8.0	4	16.0	16.0	4	1107	1080-1140	8	8.1	7.9-8.2	4	8.1	8.0-8.1
4.0	4	16.0	16.0	4	750	744- 768	8	8.2	8.0-8.4	4	8.1	8.1
2.0	4	16.0	16.0	4	564	540- 588	8	8.0	7.7-8.5	4	8.0	7.9-8.1
Control	4	16.0	16.0	4	321	312- 324	8	8.3	8.2-8.4	4	7.9	7.9-8.0
Week 7 (20-26 December):												
12.5	4	15.5	15.0-16.0	2	1482	1464-1500	6	8.5	8.0-9.0	2	7.9	7.9
10.0	4	15.5	15.0-16.0	2	1212	1188-1236	6	8.7	8.3-9.0	2	7.9	7.9-8.0

continued ...

Table 2. Continued.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
8.0	4	15.5	15.0-16.0	2	1074	1032-1116	6	8.6	8.1-9.0	2	8.0	7.9-8.0
4.0	4	15.5	15.0-16.0	2	696	684- 708	6	8.6	8.0-9.2	2	8.0	8.0
2.0	4	15.5	15.0-16.0	2	516	504- 528	6	8.5	7.8-8.8	2	8.0	8.0
Control	4	15.5	15.0-16.0	2	294	288- 300	6	8.3	8.0-8.6	2	8.0	7.9-8.0
Week 8 (27 December to 3 January):												
12.5	4	15.5	15.0-16.0	6	1590	1500-1680	12	8.4	7.8-9.2	5	8.2	8.1-8.2
10.0	4	15.5	15.0-16.0	6	1346	1236-1440	12	8.5	7.8-9.2	6	8.2	8.1-8.3
8.0	4	15.5	15.0-16.0	6	1177	1080-1260	12	8.4	7.8-9.0	6	8.0	8.0-8.1
4.0	4	15.0	14.5-15.5	6	776	660- 864	12	8.4	7.6-9.3	6	7.9	7.8-8.0
2.0	4	15.0	14.5-15.5	6	556	456- 624	12	8.5	7.7-9.4	6	8.0	7.8-8.1
Control	4	15.0	14.5-15.5	6	310	288- 348	12	8.5	7.9-9.3	6	7.8	7.6-8.1
Week 9 (4-10 January):												
12.5	6	15.3	14.0-16.0	4	1479	1368-1560	6	8.4	8.0-8.9	4	8.3	8.3-8.5
10.0	6	15.3	14.0-16.0	4	1260	1200-1356	6	8.4	8.1-8.8	4	8.3	8.2-8.3
8.0	6	15.3	14.0-16.0	4	1086	1020-1152	6	8.2	7.8-8.7	4	8.2	8.1-8.3
4.0	6	15.3	14.0-16.0	4	720	648- 768	6	8.3	7.8-9.2	4	8.0	7.9-8.2
2.0	6	15.3	14.0-16.0	4	519	444- 552	6	8.3	7.8-9.0	4	8.1	8.0-8.1
Control	6	15.3	14.0-16.0	4	317	282- 348	6	8.1	7.9-8.4	4	7.7	7.1-8.1
Week 10 (11-17 January):												
12.5	6	15.3	15.0-16.0	6	1514	1428-1620	10	8.2	7.4-8.8	6	8.3	8.3-8.4

continued ...

Table 2. Continued.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
10.0	6	15.3	15.0-16.0	6	1396	1380-1416	10	8.4	8.0-8.8	6	8.3	8.1-8.4
8.0	6	15.3	15.0-16.0	6	1160	1080-1200	10	8.2	7.8-8.6	6	8.0	8.0-8.1
4.0	6	15.3	15.0-16.0	6	804	768- 828	10	8.3	7.6-8.8	6	8.0	7.9-8.2
2.0	6	15.3	15.0-16.0	6	580	540- 624	10	8.7	8.1-9.2	6	8.0	7.9-8.1
Control	6	15.3	15.0-16.0	6	336	276- 408	10	8.6	8.0-9.0	6	7.9	7.8-8.0
Week 11 (18-24 January):												
12.5	4	15.5	15.0-16.0	4	1568	1530-1620	8	8.3	8.0-8.8	4	8.4	8.2-8.6
10.0	4	15.5	15.0-16.0	4	1374	1320-1440	8	8.5	8.1-8.9	4	8.3	8.2-8.6
8.0	4	15.5	15.0-16.0	4	1167	1116-1200	8	8.3	8.0-8.5	4	8.3	8.1-8.5
4.0	4	15.5	15.0-16.0	4	831	804- 840	8	7.9	5.8-8.9	4	8.3	8.1-8.4
2.0	4	15.3	14.5-16.0	4	618	576- 672	8	8.3	7.2-9.0	4	8.2	8.0-8.3
Control	4	15.3	14.5-16.0	4	405	384- 432	8	0.3	7.9-8.6	4	8.2	8.0-8.4
Week 12 (25-31 January):												
12.5	4	15.0	15.0	4	2319	1620-3000	4	8.2	7.5-8.7	4	8.2	7.6-8.8
10.0	4	15.0	15.0	4	1950	1380-2520	4	8.2	7.4-9.0	4	8.3	8.2-8.4
8.0	4	15.0	15.0	4	1686	1212-2160	4	8.2	7.2=9.1	4	8.1	7.9-8.2
4.0	4	15.0	15.0	4	1074	792-1320	4	8.2	6.9-8.9	4	8.3	8.1-8.4
2.0	4	15.0	15.0	4	729	612- 864	4	8.3	7.4-9.0	4	8.1	8.1-8.2
Control	4	15.0	15.0	4	381	360- 408	4	8.2	7.6-8.8	4	8.1	8.0-8.1

continued ...

Table 2. Continued.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
Lake Chub												
Week 1 (21-27 September):												
25.0	6	15.3	15.0-16.0	6	4490	3900-5040	14	8.5	7.2-10.2	6	8.4	8.1-8.7
12.5	6	15.3	15.0-16.0	6	2980	2760-3120	14	8.5	7.4-10.3	6	8.3	7.9-8.5
8.0	6	15.3	15.0-16.0	6	2175	2040-2280	14	8.2	7.4-10.1	6	8.22	8.1-8.4
6.0	6	15.7	15.0-16.0	6	1920	1800-2010	14	8.3	7.0-10.2	6	8.14	7.8-8.3
3.0	6	15.6	15.0-16.0	6	1496	1164-1812	14	8.3	7.4-10.1	6	8.13	8.0-8.2
Control	6	15.4	15.0-16.0	6	289	258- 336	14	8.4	7.6- 9.8	6	8.12	7.6-8.5
Week 2 (28 September to 4 October):												
25.0	4	15.5	15.0-16.0	6	6153	5720-6720	10	7.8	7.0- 8.4	6	8.5	8.4-8.7
12.5	4	15.5	15.0-16.0	6	3134	2580-3744	10	7.9	7.0- 8.6	6	8.4	8.3-8.5
8.0	4	15.5	15.0-16.0	6	2186	2040-2400	10	8.1	7.2- 8.8	6	8.3	8.2-8.4
6.0	4	15.5	15.0-16.0	6	1906	1830-1980	10	8.0	6.8- 9.0	6	8.2	8.1-8.3
3.0	4	15.5	15.0-16.0	6	1235	1152-1320	10	8.1	7.0- 9.0	6	8.1	8.0-8.2
Control	4	15.5	15.0-16.0	6	283	240-312	10	8.1	6.9- 9.0	6	7.8	7.8-7.9
Week 3 (5-11 October):												
25.0	4	15.8	15.5-16.0	6	5650	4620-6360	10	8.0	7.2-8.6	6	8.5	8.4-8.5
12.5	4	15.8	15.5-16.0	6	3120	2580-3360	10	7.7	6.4-8.8	6	8.3	8.2-8.4
8.0	4	15.8	15.5-16.0	6	2190	2040-2340	10	8.1	7.0-9.0	6	8.2	8.2-8.3
6.0	4	15.8	15.5-16.0	6	1765	1680-1860	10	8.4	7.8-9.4	6	8.2	8.0-8.3

continued ...

Table 2. Continued.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
3.0	4	15.8	15.5-16.0	6	1082	1056-1128	10	8.3	7.8-9.3	6	8.0	7.9-8.1
Control	4	15.8	15.5-16.0	6	308	276- 348	10	8.5	8.1-9.2	6	7.9	7.8-8.0
Week 4 (12-18 October):												
25.0	6	15.4	15.0-15.5	4	6330	5760-6960	10	7.8	6.8-9.4	6	8.5	8.5-8.6
12.5	6	15.3	15.0-15.5	6	3290	3000-3480	10	7.6	7.0-8.2	6	8.3	8.3-8.5
8.0	6	5.3	15.0-15.5	6	2270	2160-2460	10	7.8	7.2-8.9	6	8.3	8.3-8.4
6.0	6	15.3	14.5-15.5	6	1745	1680-1830	10	8.0	6.8-9.0	6	8.2	8.1-8.3
3.0	6	15.3	14.5-15.5	6	1116	1008-1200	10	8.3	7.0-9.4	6	8.2	8.1-8.3
Control	6	15.3	15.0-15.5	6	333	312- 360	10	8.2	6.9-9.2	6	7.9	7.9-8.1
Week 5 (19-25 October):												
25.0	4	15.0	15.0	6	6050	5640-6360	8	8.0	7.3-8.6	6	8.5	8.4-8.6
12.5	4	15.0	15.0	6	3360	3240-3480	8	7.9	7.1-8.4	6	8.4	8.3-8.4
8.0	4	15.0	15.0	6	2370	2280-2520	8	8.2	7.6-8.8	6	8.3	8.3-8.4
6.0	4	15.0	15.0	6	1890	1860-1980	8	7.6	6.5-8.4	6	8.2	8.1-8.2
3.0	4	15.0	15.0	6	1140	1080-1176	8	8.3	7.6-8.8	6	8.2	8.1-8.3
Control	4	15.0	15.0	6	320	300- 360	8	8.4	8.1-9.0	6	7.9	7.8-8.0
Week 6 (26 October to 1 November):												
25.0	4	14.8	14.5-15.0	6	5500	5040-5880	10	7.8	7.3-8.4	6	8.5	8.3-8.7
12.5	4	14.8	14.5-15.0	6	1990	2880-3120	10	7.6	6.8-8.4	6	8.3	8.3-8.4
8.0	4	14.8	14.5-15.0	6	2220	2160-2280	10	7.6	6.2-8.4	6	8.3	8.2-8.4

continued ...

Table 2. Continued.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
6.0	4	14.8	14.5-15.0	6	1700	1560-1800	10	7.5	6.0-9.5	6	8.2	8.1-8.3
3.0	4	14.8	14.5-15.0	6	1122	1080-1154	10	7.9	7.2-9.0	6	8.2	8.1-8.2
Control	4	14.8	14.5-15.0	6	358	348- 360	10	7.8	7.0-9.6	6	8.0	7.9-8.0
Week 7 (2-8 November):												
25.0	6	15.5	14.5-16.0	4	5690	5000-6000	6	8.2	7.8-8.6	6	8.3	7.9-8.6
12.5	6	15.5	14.5-16.0	4	3090	2000-3120	6	8.0	7.6-8.6	6	8.1	7.9-8.4
8.0	6	15.5	14.5-16.0	4	2310	2160-2400	6	8.1	7.6-8.7	7	8.1	7.9-8.3
6.0	6	15.5	14.5-16.0	4	1749	1620-1860	6	8.4	7.9-9.0	6	8.1	7.9-8.2
3.0	6	15.5	14.5-16.0	4	1056	1032-1104	6	8.0	7.6-8.3	6	8.1	8.0-8.1
Control	6	15.5	14.5-16.0	4	336	312- 372	6	8.6	8.2-9.5	6	8.0	7.8-8.1
Week 8 (9-15 November):												
25.0	4	15.0	15.0	4	5260	5000-5640	10	7.9	7.4-8.2	4	8.4	8.2-8.7
12.5	4	15.0	15.0	4	2910	2880-3000	10	7.9	7.4-9.4	4	8.4	8.2-8.5
8.0	4	15.0	15.0	4	2250	2220-2280	10	7.9	7.2-8.2	4	8.3	8.2-8.4
6.0	4	15.0	15.0	4	1770	1680-1860	10	7.8	7.3-8.2	4	8.3	8.3-8.4
3.0	4	15.0	15.0	4	1104	1080-1152	10	8.0	7.4-8.4	4	8.3	8.2-8.3
Control	4	15.0	15.0	4	288	228- 324	10	8.0	7.4-8.4	4	8.2	8.1-8.2
Week 9 (16-22 November):												
25.0	6	14.8	14.0-15.5	6	2490	2340-2640	10	8.4	7.2-9.8	2	8.4	8.4-8.5
12.5	6	14.8	14.0-15.5	6	1449	1260-1560	10	8.5	7.4-10.0	2	8.2	8.2-8.2

continued ...

Table 2. Continued.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
8.0	6	14.8	14.0-15.5	6	1104	1020-1152	10	8.5	7.6-9.7	2	8.2	8.2-8.2
6.0	6	14.8	14.0-15.5	6	894	816- 936	10	8.7	7.8-10.1	2	8.1	8.1-8.1
3.0	6	14.8	14.0-15.5	6	624	680- 636	10	8.8	7.8-10.0	2	8.1	8.0-8.1
Control	6	14.8	14.0-15.5	6	286	270- 312	10	8.8	7.6-9.8	2	8.0	8.0-8.0
Week 10 (23-29 November):												
25.0	4	15.5	15.0	6	5600	5040-6120	10	8.4	7.6-9.4	6	8.5	8.2-8.7
12.5	4	15.5	15.0	6	3200	3000-3360	10	8.4	7.9-9.5	6	8.4	8.2-8.6
8.0	4	15.5	15.0	6	2220	2160-2280	10	8.4	7.8-9.6	6	8.3	8.1-8.5
6.0	4	15.5	15.0	6	1760	1620-1920	10	8.1	6.7-9.2	6	8.3	8.2-8.4
3.0	4	15.5	15.0	6	1100	996-1176	10	8.2	6.8-9.6	6	8.2	8.0-8.3
Control	4	15.5	15.0	6	323	276- 360	10	8.4	7.7-9.8	6	8.0	7.9-8.3
Week 11 (30 November to 6 December):												
25.0	4	15.3	15.0-15.5	4	2637	2436-2880	10	8.2	7.3-8.6	4	8.3	8.2-8.4
12.5	4	15.3	15.0-15.5	4	1596	1464-1680	10	8.2	7.2-8.6	4	8.1	8.0-8.3
8.0	4	15.3	15.0-15.5	4	1191	1164-1200	10	8.3	7.8-8.6	4	8.1	8.0-8.2
6.0	4	15.3	15.0-15.5	4	1008	960-1032	10	8.0	7.3-8.5	4	7.9	7.8-8.1
3.0	4	15.3	15.0-15.5	4	651	624- 672	10	8.2	7.6-8.6	4	8.0	7.9-8.1
Control	4	15.3	15.0-15.5	4	327	312- 336	10	8.3	7.7-8.7	4	7.7	7.5-8.0
Week 12 (7-13 December):												
25.0	4	15.0	15.0	6	2740	2520-3000	8	8.0	7.3-8.4	6	8.3	8.2-8.4

continued ...

Table 2. Continued.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
12.5	4	15.0	15.0	6	1590	1500-1680	8	7.8	6.8-8.6	6	8.0	8.0-8.1
8.0	4	15.0	15.0	6	1084	1032-1140	8	8.0	7.3-8.6	6	8.0	7.9-8.1
6.0	4	15.0	15.0	6	906	840- 936	8	8.2	7.9-8.6	6	7.9	7.9-8.0
3.0	4	15.0	15.0	6	624	588- 672	8	8.1	7.8-8.6	6	7.9	7.8-8.0
Control	4	15.0	15.0	6	318	288- 336	8	8.2	7.9-8.6	6	7.8	7.7-7.9
Week 13 (14-19 December):												
25.0	4	14.8	14.5-15.0	4	2790	2640-2880	6	8.2	7.6-9.0	4	8.4	8.3-8.4
12.5	4	14.8	14.5-15.0	4	1509	1440-1560	6	8.3	7.8-9.0	4	8.2	8.1-8.4
8.0	4	14.8	14.5-15.0	4	1128	1092-1164	6	8.2	7.9-8.5	4	8.2	8.0-8.3
6.0	4	14.8	14.5-15.0	4	972	888-1044	6	8.2	7.8-8.6	4	8.2	8.0-8.3
3.0	4	14.8	14.5-15.0	4	618	600- 648	6	8.2	8.0-8.6	4	8.0	7.9-8.1
Control	4	14.8	14.5-15.0	4	291	276- 312	6	8.3	7.9-8.6	4	7.9	7.7-8.1
White Sucker												
Week 1 (14-20 September):												
25.0	14	15.3	15.0-16.0	8	5754	4514-6960	14	7.8	7.0-8.8	12	8.5	8.2-8.6
12.5	14	15.5	15.0-16.0	8	3480	3120-3600	14	7.7	6.5-8.2	12	8.4	8.3-8.5
8.0	14	15.4	15.0-16.0	8	2504	2980-3600	14	7.7	6.8-8.5	12	8.3	8.0-8.5
3.0	14	15.3	15.0-16.0	8	1025	936-1080	14	7.8	6.5-8.4	12	8.0	7.9-8.2
2.0	14	15.3	15.0-16.0	8	827	744- 984	14	7.8	6.5-8.3	12	8.0	7.8-8.3
Control	14	15.3	15.0-16.0	8	275	246- 300	14	7.9	6.5-8.5	12	7.9	7.6-8.1

continued ...

Table 2. Continued.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
Week 2 (21-27 September):												
25.0	10	15.1	15.0-15.5	4	5678	5280-6000	14	8.0	7.0-9.4	6	8.2	7.6-8.5
12.5	10	15.1	15.0-15.5	4	2775	2280-3240	14	8.1	6.4-9.4	6	8.2	7.8-8.4
8.0	10	15.1	15.0-15.5	4	2315	2190-2520	14	7.7	6.2-8.6	6	8.0	7.8-8.2
3.0	10	15.1	15.0-15.5	4	1173	972-1530	14	8.4	7.6-9.4	6	8.0	7.9-8.2
2.0	10	15.1	15.0-15.5	4	963	924- 996	14	7.9	6.2-9.4	6	7.9	7.8-8.0
Control	10	15.1	15.0-15.5	4	276	264- 288	14	8.4	7.8-9.3	6	7.9	7.6-8.2
Week 3 (28 September to 4 October):												
25.0	14	15.0	15.0	4	6270	5400-7200	10	7.5	5.0-8.4	6	8.4	8.3-8.5
12.5	14	15.0	15.0	4	2985	2700-3240	10	7.9	7.0-8.6	6	8.3	8.2-8.4
8.0	14	15.0	15.0	4	2218	2040-2340	10	7.7	6.6-8.2	6	8.2	8.1-8.3
3.0	14	15.0	15.0	4	1182	960-1530	10	7.9	7.6-8.6	6	8.1	8.0-8.2
2.0	14	15.0	15.0	4	882	768- 984	10	7.8	7.0-8.4	6	8.0	7.9-8.1
Control	14	15.0	15.0	4	300	264- 324	10	7.9	7.4-8.4	6	7.9	7.8-8.0
Week 4 (5-11 October):												
25.0	8	15.3	15.0-16.0	6	6440	5640-7200	10	8.5	7.8-9.0	6	8.3	8.0-8.6
12.5	8	15.3	15.0-16.0	5	2880	2580-3600	8	7.9	6.8-9.2	5	8.2	8.0-8.4
8.0	8	15.3	15.0-16.0	4	2160	2040-2400	7	7.9	6.8-9.4	5	8.2	8.1-8.2
3.0	8	15.3	15.0-16.0	6	1097	864-1290	10	8.5	7.8-9.8	6	8.0	7.9-8.1
2.0	8	15.3	15.0-16.0	5	854	792- 936	8	8.4	8.0-9.4	5	8.0	7.9-8.1
Control	8	15.3	15.0-16.0	6	283	240- 312	10	8.7	8.2-9.6	6	7.7	7.4-7.9

continued ...

Table 2. Concluded.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
Week 5 (12-18 October):												
25.0	4	15.5	15.0-16.0	6	6330	6180-6600	10	8.9	8.3-9.4	6	8.5	8.2-8.8
12.5	4	15.5	15.0-16.0	2	3780	3600-3960	4	9.0	8.3-9.6	2	8.5	8.5-8.7
8.0	4	15.5	15.0-16.0	2	2415	2370-2460	4	8.8	8.0-9.6	2	8.4	8.2-8.6
3.0	4	15.5	15.0-16.0	6	1227	404-1380	10	8.3	7.6-9.2	6	8.1	8.0-8.3
2.0	4	15.5	15.0-16.0	3	928	888- 960	5	8.3	7.4-9.5	3	8.1	8.0-8.2
Control	4	15.5	15.0-16.0	6	325	312- 336	10	8.7	7.9-9.8	6	7.5	7.8-8.1
Week 6 (19-25 October):												
25.0	2	15.0	15.0	6	6440	6240-6600	8	8.0	7.6-8.4	6	8.7	8.6-8.8
12.5	-	-	-	-	-	-	-	-	-	-	-	-
8.0	-	-	-	-	-	-	-	-	-	-	-	-
3.0	2	15.0	15.0	6	1202	1104-1284	8	8.2	7.6-8.7	6	8.3	8.2-8.3
2.0	1	15.0	15.0	3	936	912- 960	4	8.3	7.8-8.9	3	8.3	8.2-8.4
Control	2	15.0	15.0	6	374	348- 408	8	8.3	7.5-9.8	6	8.0	8.0-8.1
Week 7 (26 October to 1 November):												
25.0	6	14.7	14.0-15.0	5	5376	5040-5880	9	7.9	7.4-8.4	5	8.6	8.1-8.8
12.5	-	-	-	-	-	-	-	-	-	-	-	-
8.0	-	-	-	-	-	-	-	-	-	-	-	-
3.0	5	14.7	14.0-15.0	5	111	1020-1200	9	7.7	7.4-8.2	5	8.3	8.2-8.4
2.0	2	14.7	14.0-15.0	3	848	792- 912	5	7.7	7.2-8.0	2	8.3	8.2-8.3
Control	6	14.7	14.0-15.0	6	340	300- 396	10	7.8	7.2-8.2	6	8.1	8.0-8.2

Three invertebrate species were collected for the chronic toxicity tests: *Caenis simulans*, *Paraleptophlebia bicornuta*, and *Hyalella azteca*. *Caenis* nymphs, 3 to 5 mm in length, and *Hyalella*, 6 to 8 mm in length, were collected from the West Interceptor Ditch on Syncrude Lease 8 July 1978. *Paraleptophlebia* were collected from the Bow River, approximately 0.5 km west of the Calgary Zoo, on 2 August 1978.

The invertebrates were captured in dipnets or kick nets and then rinsed through fine mesh sieves to separate them from other organisms and debris. Animals from the Syncrude site were placed in plastic bags filled with water. The sample bags were packed in insulated styrofoam coolers containing ice. Before shipment, the water in the bags was saturated with oxygen.

In Calgary, the samples were delivered to the laboratory at Aquatic Environments Ltd. To allow the water temperature to equilibrate, the bags containing the invertebrates were placed in large aquaria in a controlled environment chamber. Several hours later, the bags were opened and the animals released into the aquarium water (dechlorinated City of Calgary tapwater maintained at $15^{o}C$). The aquaria were provided with 3 to 4 cm of an inert, sterilized, silica sand substrate.

Caenis and *Paraleptophlebia* were supplied with soaked leaves and their attached periphyton (including *Spirogyra* sp.) to serve as both food and cover. *Hyalella* were fed commercial Tetramin fish food (recommended by B. G. deMarch, Biologist, Canada Freshwater Institute, Winnipeg, personal communication) and a mixture of filamentous green algae (again including *Spirogyra* sp.), cultured in 1 L Erlenmeyer flasks at a constant $20^{o}C$, and spinach. The spinach was commercial frozen spinach. The mixture was prepared by mixing less than 2 g of a spinach/algae inoculum, homogenized in a blender, with 50 mL of dechlorinated city water and 5 mL of pond water. The mixture was stirred at least twice daily and allowed to decay for several days before being fed to the *Hyalella*. The animals were fed daily to a level in excess of what was eaten the day before.

To establish the range of toxic concentrations, preliminary 96 h acute toxicity tests were performed on all species using methods outlined in Standard Methods (1975). Five organisms were randomly selected and separated into insect environment chambers consisting of plastic cups with the sides partially replaced with fibreglass insect netting. The chambers were placed in aquaria containing 100, 50, 25, 12.5, 5, and 0% groundwater by volume. The groundwater/diluent mixture had been aerated to bring the dissolved O_2 to levels above 7.0 ppm. The experiment was run in duplicate so that a total of 10 organisms was exposed to each concentration.

The highest concentration used in chronic toxicity testing was non-lethal at 96 h. For all three invertebrates, this concentration was 25% by volume groundwater. The intermediate concentrations were the 96 h LC_{50} times an application factor of 0.1. The remaining concentrations were chosen as near approximations of decilog intervals given in Standard Methods (1975). All chronic toxicity tests were performed at 15°C.

During the chronic toxicity tests, 90% of the water was changed weekly by siphoning it into trays and replacing it with the same volume and concentration of groundwater. Unless dissolved O_2 levels were already in excess of 7 ppm, the replacement groundwater was aerated to raise the levels beyond 7 ppm before being added to the aquaria. The groundwater was also continuously aerated during the course of the experiment. The aquaria were fitted with glass covers to minimize evaporation of water and volatile substances.

Dissolved O_2 and temperature were monitored and recorded daily. Groundwater pH and conductivities were initially recorded every second day, but later in the tests these two parameters were recorded only twice a week, immediately before and after the introduction of replacement groundwater. Tables 3 to 5 summarize test concentrations for each invertebrate species.

Caenis nymphs, weighing an average of 0.0022 g per individual, were introduced into 1.5 L aquaria provided with a substrate of sterilized insect netting. Silica sand was not used during acclimation because it was found that the small nymphs could not be

Table 3. Summary of routine chemical and physical parameters recorded during the chronic exposure of *Paraleptophlebia bicornuta* nymphs to saline groundwater.

Concentration (%)	Temperature			Conductivity			Dissolved Oxygen			pH		
	N	Mean	Range	N	Mean	Range	N	Mean	Range	N	Mean	Range
Week 1:												
25.0	1	15	-	1	5040	-	1	5.9	-	1	8.7	-
18.0	1	15	-	1	4560	-	1	6.0	-	1	8.6	-
11.0	1	15	-	1	3000	-	1	6.2	-	1	8.45	-
6.4	1	15	-	1	1740	-	1	6.0	-	1	8.3	-
3.0	1	15	-	1	840	-	1	6.4	-	1	8.1	-
Control	1	15	-	1	240	-	1	5.9	-	1	8.0	-
Week 2:												
25.0	1	15	-	1	5160	-	1	6.3	-	1	8.8	-
18.0	1	15	-	1	4380	-	1	6.1	-	1	8.7	-
11.0	1	15	-	1	2880	-	1	6.3	-	1	8.4	-
6.4	1	15	-	1	1674	-	1	6.2	-	1	8.3	-
3.0	1	15	-	1	864	-	1	6.4	-	1	8.2	-
Control	1	15	-	1	252	-	1	6.0	-	1	8.1	-
Week 3:												
25.0	1	15	-	1	5172	-	1	6.8	-	1	8.75	-
18.0	1	15	-	-	Terminated	-	-	Terminated	-	-	Terminated	-
11.0	1	15	-	1	3024	-	1	6.44	-	1	8.4	-
6.4	1	15	-	1	1704	-	1	6.3	-	1	8.35	-
3.0	1	15	-	1	816	-	1	6.1	-	1	8.15	-
Control	1	15	-	1	264	-	1	6.3	-	1	8.1	-

Table 4. Summary of routine chemical and physical parameters recorded during the chronic exposure of *Caenis simulans* nymphs to saline groundwater.

Concentration (%)	Temperature			Conductivity			Dissolved Oxygen			pH		
	N	Mean	Range	N	Mean	Range	N	Mean	Range	N	Mean	Range
Week 1 (12 to 18 September):												
25.0	14	15.2	15.0-16.0	6	6090	4800-6720	14	7.9	7.2-8.4	12	8.55	8.4-8.7
18.0	14	15.4	15.0-16.0	6	4640	3840-4980	14	8.0	7.0-8.6	12	8.48	8.3-8.6
10.0	14	15.4	15.0-16.0	6	2760	2520-2940	14	7.9	6.9-8.4	12	8.3	8.1-8.5
6.8	14	15.5	15.0-16.0	6	2090	1500-2340	14	7.9	7.2-8.6	12	8.2	8.1-8.3
3.0	14	15.2	15.0-16.0	6	1186	1080-1320	14	7.7	7.0-8.3	12	7.9	7.8-8.1
Control	14	15.2	15.0-16.0	6	372	306- 456	14	7.9	7.2-8.4	12	7.75	7.4-8.0
Week 2 (19 to 25 September):												
25.0	14	15.1	15.0-15.5	4	5370	3840-6240	14	8.2	7.8-8.8	8	8.6	8.5-8.7
18.0	14	15.2	15.0-16.0	4	4620	4200-4920	14	8.3	7.8-9.2	8	8.45	8.3-8.6
10.0	14	15.3	15.0-16.0	4	2640	2400-2880	14	8.3	7.7-9.0	8	8.3	8.1-8.4
6.8	14	15.2	15.0-15.5	4	2153	1920-2370	14	8.1	7.2-8.6	8	8.2	8.0-8.3
3.0	14	15.2	15.0-16.0	4	1107	1008-1200	14	8.3	7.9-9.0	8	8.0	7.9-8.2
Control	14	15.1	15.0-15.5	4	377	336- 456	14	8.3	7.6-9.0	8	7.9	7.7-8.0
Week 3 (26 September to 2 October):												
25.0	14	14.8	14.0-15.5	4	6450	6000-6960	12	8.0	7.0-8.6	6	8.65	8.6-8.7
18.0	14	14.8	14.0-16.0	4	4578	4200-5040	12	8.0	7.4-8.4	6	8.5	8.4-8.6
10.0	14	14.8	14.0-15.5	4	2618	2160-2880	12	8.1	7.4-8.7	6	8.45	8.4-8.5
6.8	14	14.8	14.0-15.0	4	2057	1536-2370	12	7.9	7.0-8.4	6	8.3	8.2-8.4
3.0	14	14.7	14.0-15.0	4	1050	864-1200	12	8.0	7.0-8.5	6	8.2	8.1-8.3
Control	14	14.6	14.0-15.0	4	352	288- 435	12	8.0	7.2-8.6	6	8.0	7.9-8.1

continued ...

Table 4. Continued.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
Week 4 (3 to 9 October):												
25.0	14	15.0	15.0-15.5		5620	3840-6480	10	8.0	7.2-8.4	3	8.54	8.6-8.7
18.0	14	15.0	15.0-15.0	6	4740	4500-5040	10	8.0	7.2-8.4	3	8.56	8.5-8.6
10.0	14	15.0	15.0-15.5	6	2820	2760-2880	10	8.0	7.3-8.4	3	8.4	8.3-8.4
6.8	14	15.0	15.0-15.0	6	2080	2040-2160	10	8.0	7.4-8.6	3	8.3	8.2-8.3
3.0	14	15.0	15.0-15.5	6	1113	1002-1200	10	8.2	7.4-8.6	3	8.1	8.0-8.2
Control	14	15.0	15.0-15.5	6	368	312- 420	10	8.0	7.3-8.4	3	8.0	7.9-8.0
Week 5 (10 to 16 October):												
25.0	14	15.0	15.0-15.5	6	5880	4680-6720	10	8.5	7.8-9.2	6	8.7	8.6-8.7
18.0	14	15.1	15.0-15.5	6	4480	3780-5040	10	8.5	7.2-9.4	6	8.5	8.5-8.6
10.0	14.	15.1	15.0-15.5	6	2720	2520-2880	10	8.4	7.8-9.0	6	8.4	8.3-8.4
6.8	14	15.2	15.0-16.0	6	2040	1740-2160	10	8.8	8.2-9.5	6	8.3	8.2-8.3
3.0	14	15.2	15.0-16.0	6	1122	1020-1176	10	8.6	7.5-9.4	6	8.1	8.1-8.2
Control	14	15.2	15.0-16.0	6	341	288- 372	10	8.8	8.2-9.5	6	7.9	7.9-8.0
Week 6 (17 to 23 October):												
25.0	14	15.1	15.0-15.5	6	6130	6000-6300	10	7.8	7.0-8.7	6	8.5	8.4-8.6
18.0	14	15.1	15.0-15.5	6	4796	4680-4920	10	7.8	7.1-8.2	6	8.5	8.4-8.6
10.0	14	15.2	15.0-16.0	6	2790	2640-3120	10	7.8	7.2-8.5	6	8.3	8.3-8.4
6.8	14	15.2	15.0-16.0	6	2120	1980-2280	10	7.8	7.0-9.0	6	8.3	8.1-8.3
3.0	14	15.2	15.0-16.0	6	1137	1056-1228	10	7.9	7.9-9.0	6	8.1	8.0-8.2
Control	14	15.2	15.0-16.0	6	449	324- 576	10	7.9	7.5-8.3	5	8.0	7.8-8.1

continued ...

Table 4. Continued.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
Week 7 (24-30 October):												
25.0	14	15.0	15.0	6	6000	5760-6120	8	8.0	7.4-8.6	6	8.6	8.5-8.6
18.0	14	15.0	15.0	6	4490	4080-4800	8	8.0	7.5-8.7	6	8.5	8.4-8.6
10.0	14	15.0	15.0	6	2730	2520-2880	8	7.7	7.0-8.4	6	8.4	8.3-8.5
6.8	14	15.0	15.0	6	2070	1920-2220	8	7.8	7.3-8.7	6	8.4	8.2-8.5
3.0	14	15.0	15.0	6	1132	1050-1260	8	7.8	7.3-8.5	6	8.2	8.0-8.3
Control	14	15.0	15.0	6	422	396- 456	8	8.0	7.5-8.9	6	8.0	7.8-8.1
Week 8 (31 October to 6 November):												
25.0	14	15.0	14.0-16.0	4	5895	5800-6000	8	7.9	7.1-8.4	4	8.6	8.5-8.7
18.0	14	15.0	14.0-16.0	4	4603	4560-4680	8	7.8	7.0-8.2	4	8.5	8.5-8.5
10.0	14	15.1	15.0-16.0	4	2689	2580-2800	8	7.8	7.1-8.4	4	8.3	8.3-8.3
6.8	14	15.1	15.0-16.0	4	1894	1860-1970	8	7.6	6.9-8.4	4	8.2	8.2-8.3
3.0	14	15.1	15.0-16.0	4	1233	1152-1260	8	7.8	7.0-8.4	4	8.15	8.1-8.2
Control	14	15.1	15.0-16.0	4	411	408- 420	8	7.6	7.1-8.4	4	7.9	7.9-8.0
Week 9 (7-13 November):												
25.0	14	15.5	15.0-16.5	4	5880	4800-6480	8	7.6	6.8-8.2	4	8.6	8.5-8.7
18.0	14	15.5	15.0-16.5	4	4470	3500-5040	8	7.7	7.5-8.0	4	8.5	8.4-8.6
10.0	14	15.5	15.0-16.5	4	2592	2400-2880	8	7.7	7.3-8.2	4	8.3	8.3-8.4
6.8	14	15.5	15.0-16.5	4	2040	1980-2100	8	7.6	7.2-8.2	4	8.2	8.2-8.3
3.0	14	15.5	15.0-16.5	4	1131	1068-1200	8	7.7	7.5-8.1	4	8.2	8.1-8.4
Control	14	15.5	15.0-16.5	4	510	444- 576	8	7.7	7.3-8.0	4	8.0	7.8-8.2

continued ...

Table 4. Continued.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
Week 10 (14-20 November):												
25.0	14	15.4	15.0-16.0	4	3330	3000-3600	10	8.2	7.5-9.2	2	8.5	8.5-8.5
18.0	14	15.4	15.0-16.0	4	2790	2520-3120	10	8.3	7.6-9.4	2	8.5	8.4-8.5
10.0	14	15.4	15.0-16.0	4	1733	1680-1830	10	8.1	7.5-9.2	2	8.3	8.3-8.3
6.8	14	15.4	15.0-16.0	4	1319	1284-1350	10	8.1	7.4-9.1	2	8.3	8.3-8.3
3.0	14	15.4	15.0-16.0	4	837	696- 912	10	8.2	7.5-9.6	2	8.3	8.3-8.3
Control	14	15.4	15.0-16.0	4	396	360- 432	10	8.3	7.6-9.6	2	8.1	8.1-8.2
Week 11 (21-27 November):												
25.0	14	15.1	15.0-15.5	4	5550	5160-5760	8	7.7	7.2-8.8	4	8.7	8.7-8.7
18.0	14	15.1	15.0-15.5	4	3960	3360-4440	8	8.1	7.8-8.7	4	8.6	8.6-8.6
10.0	14	15.1	15.0-15.5	4	2415	1980-2880	8	7.6	6.5-7.9	4	8.4	8.3-8.5
6.8	14	15.1	15.0-15.5	4	1560	1440-1860	8	7.8	7.6-8.1	4	8.4	8.3-8.4
3.0	14	15.1	15.0-15.5	4	1185	1128-1260	8	7.8	7.5-8.2	4	8.2	8.2-8.2
Control	14	15.1	15.0-15.5	4	386	336- 444	8	7.7	7.3-8.2	4	8.0	8.0-8.0
Week 12 (28 November to 4 December):												
25.0	14	15.1	15.0-16.0	4	3690	3540-3840	10	8.1	7.2-8.4	4	8.5	8.3-8.6
18.0	14	15.1	15.0-16.0	4	2640	2400-2784	10	8.1	7.6-8.4	4	8.5	8.4-8.5
10.0	14	15.1	15.0-16.0	4	1607	1380-1836	10	8.1	7.3-8.4	4	8.3	8.2-8.5
6.8	14	16.1	16.0-16.5	4	1146	960-1356	10	7.9	7.2-8.4	4	8.3	8.1-8.4
3.0	14	16.1	16.0-16.5	4	729	648- 816	10	8.1	7.5-8.4	4	8.1	8.0-8.3
Control	14	16.0	15.5-16.5	4	399	348- 444	10	8.0	7.7-8.2	4	8.0	7.8-8.2

continued ...

Table 4. Concluded.

Concentration (%)	Temperature			Conductivity			Dissolved Oxygen			pH		
	N	Mean	Range	N	Mean	Range	N	Mean	Range	N	Mean	Range
Week 13 (5-11 December):												
25.0	14	15.4	15.0-15.5	4	2775	2640-2880	12	8.1	7.2-8.4	4	8.4	8.3-8.5
18.0	14	15.4	15.0-15.5	4	2198	2160-2220	12	8.1	7.8-8.3	4	8.2	8.0-8.4
10.0	14	15.4	15.0-15.5	4	1293	1150-1440	12	8.2	8.0-8.4	4	8.2	8.2-8.2
6.8	14	15.5	15.0-16.0	4	1029	960-1116	12	8.0	7.6-8.4	4	8.1	8.0-8.2
3.0	14	15.5	15.0-16.0	4	648	520- 732	12	8.1	8.0-8.3	4	8.0	7.9-8.1
Control	14	15.5	15.0-16.0	4	339	320- 360	12	8.1	7.8-8.2	4	8.0	7.9-8.0
Week 14 (12-18 December):												
25.0	14	14.9	14.5-15.0	4	2568	2436-2760	8	7.9	7.2-8.6	4	8.3	8.0-8.4
18.0	14	14.9	14.5-15.0	4	2124	2100-2160	8	8.1	7.6-8.4	4	8.2	8.2-8.3
10.0	14	14.9	14.5-15.0	4	1338	1320-1356	8	7.9	6.9-8.6	4	8.2	8.1-8.2
6.8	14	15.0	15.0-15.0	4	1044	996-1092	8	7.9	7.1-8.4	4	8.1	8.0-8.2
3.0	14	15.0	15.0-15.0	4	711	684- 744	8	8.2	7.6-8.6	4	8.0	7.9-8.1
Control	14	16.0	15.0-15.0	4	384	360- 420	8	7.7	6.2-8.4	4	7.9	7.6-8.0
Week 15 (19 December):												
25.0	2	15.0	-	-	-	-	2	8.3	8.2-8.4	-	-	-
18.0	2	15.0	-	-	-	-	2	8.2	8.2-8.2	-	-	-
10.0	2	15.0	-	-	-	-	2	8.1	8.0-8.3	-	-	-
6.8	2	15.0	-	-	-	-	2	8.0	8.0-8.1	-	-	-
3.0	2	15.0	-	-	-	-	2	8.1	7.9-8.4	-	-	-
Control	2	15.0	-	-	-	-	2	8.0	8.0-8.1	-	-	-

Table 5. Summary of routine chemical and physical parameters recorded during the chronic exposure of *Hyalella azteca* to saline groundwater.

Concentration (%)	Temperature			Conductivity			Dissolved Oxygen			pH		
	N	Mean	Range	N	Mean	Range	N	Mean	Range	N	Mean	Range
Week 1 (13-19 September):												
25.0	14	15.0	15.0-15.0	6	6577	6300-7080	14	8.0	7.0-9.2	14	8.4	7.9-8.7
18.0	14	15.2	15.0-16.0	6	4920	4440-5520	14	8.0	6.4-9.4	14	8.4	8.0-8.5
10.0	14	15.1	15.0-16.0	6	2880	2640-3120	14	8.0	6.4-9.4	14	8.2	7.8-8.4
5.0	14	15.1	15.0-16.0	6	1620	1560-1680	14	8.1	7.4-9.2	14	8.1	8.0-8.2
3.0	14	15.1	15.0-16.0	6	1236	1080-1320	14	8.1	7.0-9.2	14	7.9	7.6-8.1
Control	14	15.0	15.0-15.0	6	399	360- 432	14	8.2	7.4-9.4	14	7.7	7.5-7.8
Week 2 (20-26 September):												
25.0	14	15.2	15.0-15.5	4	6600	6120-6840	14	8.2	7.0-9.4	6	8.4	7.7-8.8
18.0	14	15.2	15.0-15.5	4	4875	4800-5040	14	8.3	7.4-10.1	6	8.2	7.6-8.6
10.0	14	15.2	15.0-15.5	4	2925	2760-3000	14	8.4	7.5-9.8	6	8.3	7.8-8.5
5.0	14	15.2	15.0-15.5	4	1695	1560-1800	14	8.1	7.4-8.6	6	8.2	7.7-8.4
3.0	14	15.2	15.0-15.5	4	1068	1032-1104	14	8.1	7.0-8.6	6	8.1	8.0-8.3
Control	14	15.2	15.0-15.5	4	303	252- 360	14	7.5	4.0-8.4	6	7.9	7.7-8.2
Week 3 (27 September to 3 October):												
25.0	14	15.1	15.0-15.5	6	6210	6120-6300	12	7.6	6.9-8.2	6	8.5	8.2-8.8
18.0	14	15.1	15.0-15.5	6	4330	3720-4680	12	7.6	6.0-8.8	6	8.5	8.2-8.7
10.0	14	15.1	15.0-15.5	6	2730	2640-2880	12	7.5	6.1-8.4	6	8.2	8.0-8.5
5.0	14	15.1	15.0-15.5	6	1525	1440-1620	12	8.0	6.6-8.6	6	8.3	8.1-8.4
3.0	14	15.1	15.0-15.5	6	1044	960-1080	12	8.2	7.4-8.8	6	8.2	8.1-8.3
Control	14	15.1	15.0-15.5	6	294	264- 324	12	8.0	7.2-8.6	6	8.1	8.0-8.2

continued ...

Table 5. Continued.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
Week 4 (4-10 October):												
25.0	14	15.2	15.0-16.0	4	6060	5760-6360	8	8.2	7.7-8.8	4	8.7	8.6-8.7
18.0	14	15.2	15.0-16.0	4	4440	4080-4560	8	8.3	7.6-9.0	4	8.6	8.5-8.6
10.0	14	15.2	15.0-16.0	4	2640	2520-2760	8	8.5	8.2-9.0	4	8.4	8.3-8.5
5.0	14	15.1	15.0-15.5	4	1590	1500-1680	8	8.6	8.4-8.9	4	8.2	8.1-8.3
3.0	14	15.1	15.0-15.5	4	1049	996-1104	8	8.5	8.2-9.0	4	8.0	8.9-8.1
Control	14	15.2	15.0-16.0	4	315	282- 348	8	8.4	8.2-8.9	4	7.8	7.7-7.8
Week 5 (11-17 October):												
25.0	14	15.3	15.0-16.0	6	6340	6240-6480	10	8.3	7.2-9.4	6	8.6	8.3-8.9
18.0	14	15.3	15.0-16.0	6	4760	4440-5040	10	8.3	7.2-9.4	6	8.5	8.4-8.7
10.0	14	15.3	15.0-16.0	6	2740	2640-2880	10	8.8	8.0-9.2	6	8.4	8.2-8.5
5.0	14	15.3	15.0-16.0	6	1605	1470-1680	10	8.8	8.0-10.0	6	8.3	8.1-8.4
3.0	14	15.3	15.0-16.0	6	1230	1104-1500	10	8.4	7.0-9.7	6	8.1	7.9-8.4
Control	14	15.3	15.0-16.0	6	369	312-408	10	8.6	7.7-9.6	6	7.9	7.8-8.0
Week 6 (18-24 October):												
25.0	14	15.4	15.0-16.0	6	6380	6120-6720	8	7.5	7.4-7.8	6	8.6	8.2-8.8
18.0	14	15.4	15.0-16.0	6	4520	4200-4920	8	7.4	6.2-8.2	6	8.6	8.5-8.7
10.0	14	15.4	15.0-16.0	6	2800	2760-2880	8	7.5	7.0-8.0	6	8.3	8.1-8.5
5.0	14	15.4	15.0-16.0	6	1660	1560-1740	8	7.7	7.3-8.3	6	8.3	8.2-8.5
3.0	14	15.4	15.0-16.0	6	1180	1092-1260	8	7.7	7.2-8.2	6	8.2	8.0-8.3
Control	14	15.4	15.0-16.0	6	428	396- 504	8	7.6	7.0-8.2	6	8.0	7.9-8.2

continued ...

Table 5. Continued.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
Week 7 (25-31 October):												
25.0	14	15.0	15.0	6	6160	6000-6480	10	7.4	6.8-8.2	6	8.7	8.6-8.8
18.0	14	15.0	15.0	6	4520	4200-4800	10	7.2	6.2-8.0	6	8.6	8.4-8.6
10.0	14	15.0	15.0	6	2750	2520-3000	10	7.2	6.5-8.0	6	8.4	8.4-8.5
5.0	14	15.0	15.0	6	1630	1560-1740	10	7.7	7.0-8.4	6	8.3	8.2-8.4
3.0	14	15.0	15.0	6	1164	1080-1260	10	7.2	6.2-8.0	6	8.2	8.1-8.3
Control	14	15.0	15.0	6	481	450- 540	10	7.5	7.2-7.9	6	8.0	8.0-8.1
Week 8 (1-7 November):												
25.0	14	15.3	15.0-16.0	2	6180	6120-6240	8	7.6	7.4-8.0	4	8.4	8.0-8.7
18.0	14	15.3	15.0-16.0	2	4380	4320-4440	8	7.6	7.0-8.1	4	8.4	8.0-8.8
10.0	14	15.3	15.0-16.0	2	3473	2510-2610	8	7.8	7.4-8.0	4	8.4	8.2-8.6
5.0	14	15.3	15.0-16.0	2	1545	1500-1590	8	7.6	7.2-8.0	4	8.3	8.1-8.4
3.0	14	15.3	15.0-16.0	2	1050	996-1104	8	7.4	6.3-8.1	4	8.2	8.0-8.4
Control	14	15.3	15.0-16.0	2	372	360- 384	8	7.6	6.9-8.2	4	8.1	7.9-8.2
Week 9 (8-14 November):												
25.0	14	15.1	15.0-16.0	4	6210	6120-6360	8	8.6	8.0-9.6	4	8.7	8.6-8.9
18.0	14	15.1	15.0-16.0	4	4410	4320-4560	8	8.6	8.2-9.6	4	8.6	8.5-8.7
10.0	14	15.1	15.0-16.0	4	2775	2700-2880	8	8.5	8.0-9.6	4	8.4	8.4-8.5
5.0	14	15.1	15.0-16.0	4	1466	1410-1512	8	8.5	8.1-9.6	4	8.4	8.4-8.5
3.0	14	15.1	15.0-16.0	4	0168	972-1140	8	8.5	7.4-9.5	4	8.3	8.2-8.4
Control	14	15.1	15.0-16.0	4	360	300- 468	8	8.5	8.0-9.2	4	8.1	8.1-8.2

continued ...

Table 5. Continued.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
Week 10 (15-21 November):												
25.0	14	15.2	15.0-16.0	4	2865	2700-3000	10	8.5	7.4-9.8	2	8.5	8.5-8.6
18.0	14	15.2	15.0-16.0	4	2070	1980-2160	10	8.2	6.2-9.6	2	8.5	8.5-8.5
10.0	14	15.2	15.0-16.0	4	1343	1260-1410	10	8.4	6.8-9.9	2	8.2	8.2-8.2
5.0	14	15.2	15.0-16.0	4	888	804- 936	10	8.5	7.6-9.9	2	8.2	8.1-8.2
3.0	14	15.2	15.0-16.0	4	705	648- 756	10	8.6	7.5-10.0	2	8.1	8.0-8.1
Control	14	15.2	15.0-16.0	4	375	336- 420	10	8.6	7.8-9.8	2	7.9	7.9-8.0
Week 11 (22-28 November):												
25.0	14	15.0	15.0	4	6150	6000-6240	10	8.3	7.8-8.6	4	8.8	8.7-8.9
18.0	14	15.0	15.0	4	4320	4200-4440	10	8.3	7.8-8.6	4	8.6	8.5-8.7
10.0	14	15.0	15.0	4	2730	2640-2760	10	8.3	7.9-8.7	4	8.5	8.4-8.6
5.0	14	15.0	15.0	4	1575	1560-1620	10	8.3	7.9-8.7	4	8.3	8.2-8.5
3.0	14	15.0	15.0	4	1077	1044-1128	10	8.3	7.8-8.8	4	8.2	8.1-8.4
Control	14	15.0	15.0	4	357	324- 396	10	8.2	7.9-8.4	4	8.0	7.9-8.2
Week 12 (29 November to 5 December):												
25.0	14	15.0	15.0	4	2940	2880-3000	10	8.1	7.8-8.3	4	8.4	8.3-8.4
18.0	14	15.0	15.0	4	2040	1800-2280	10	8.1	8.0-8.2	4	8.3	8.3-8.3
10.0	14	15.0	15.0	4	1425	1380-1500	10	8.1	7.8-8.4	4	8.2	8.2-8.2
5.0	14	15.0	15.0	4	921	876-1008	10	8.3	7.1-8.4	4	8.1	8.0-8.2
3.0	14	15.0	15.0	4	708	672- 756	10	8.2	7.8-8.5	4	8.0	7.9-8.0
Control	14	15.0	15.0	4	356	324- 384	10	8.2	7.9-8.5	4	7.9	7.8-7.9

continued ...

Table 5. Concluded.

Concentration (%)	Temperature N	Temperature Mean	Temperature Range	Conductivity N	Conductivity Mean	Conductivity Range	Dissolved Oxygen N	Dissolved Oxygen Mean	Dissolved Oxygen Range	pH N	pH Mean	pH Range
Week 13 (6-11 December):												
25.0	14	15.2	15.0-16.0	4	2547	2424-2700	8	7.8	7.3-8.2	4	8.5	8.4-8.5
18.0	14	15.2	15.0-16.0	4	2022	1980-2100	8	7.5	6.1-8.2	4	8.3	8.2-8.4
10.0	14	15.2	15.0-16.0	4	1338	1272-1380	8	8.1	7.8-8.2	4	8.3	8.2-8.4
5.0	14	15.2	15.0-16.0	4	879	840- 924	8	8.0	7.8-8.2	4	8.2	8.1-8.3
3.0	14	15.2	15.0-16.0	4	642	612- 660	8	8.0	7.8-8.2	4	8.0	7.9-8.1
Control	14	15.2	15.0-16.0	4	341	300- 408	8	7.9	7.7-8.1	4	7.9	7.8-8.0

readily seen against a background of sand. The loading density was based on initial weights and was a maximum of 80 individuals per container, although most concentrations had fewer than this number. The loading density was 0.176 g/1.5 L or 1.17 g/10 L.

Paraleptophlebia experiments were run in 3 L aquaria with an inert silica sand substrate. At the beginning of the chronic toxicity tests, the study population contained black wing pad as well as non-black wing pad individuals, affording an opportunity to examine the effects of groundwater on the survivorship of both life history stages. Both mayfly species were held in aquaria covered with insect netting to ensure there was no loss of emergent adults.

Mortality checks were done at least once a day. All moults and dead and emerged mayflies were removed. The dead animals and the emergent moults of both species were fixed for a minimum of 8 h in a solution of 0.1 mol $AgNO_3$ dissolved in 1 N HNO_3 to stain the chloride cells for osmoregulation studies. The gills were dissected off and mounted on slides in balsam/monoethyl ether. Emergent mayflies were fixed in a 70% ethyl alcohol and stored for later examination. *Caenis* moults (except the emergent ones) were frozen for ion accumulation studies. At the end of the chronic toxicity tests, a minimum of five live nymphs were fixed for chloride cell counts, and the remainder were frozen for determining accumulation of heavy metal ions.

Hyalella azteca tests were run in 1 L beakers. The initial loading density, based on an average weight of 0.0046 g per individual and a maximum of 35 individuals in each container, was 0.161 g/1 L or 0.6 g/10 L. Mortality checks were done at least once a day, when all dead animals were removed and frozen for ion accumulation studies. Cannibalism became evident in *Hyalella* in the third week of the tests. Insect netting was used to divide the beakers into two sections in an attempt to minimize the effects. On 4 October 1978, five amphipods were obtained at each concentration for determining accumulation of heavy metal ions. All animals remaining at the end of the chronic toxicity tests were used for ion accumulation analysis.

4.2.2 Results

Preliminary acute toxicity tests were performed on the three invertebrate species to determine maximum short-term (96 h) non-lethal groundwater concentrations. The results are summarized in Table 6 and presented in more detail in Table 7. The results indicate that the *Caenis* mayfly nymphs were most tolerant of the saline groundwater and *Hyalella* were least tolerant. All animals in 25% and lower test concentrations survived the 96 h test period.

In the chronic toxicity tests, all organisms that responded to tactile stimulation were considered survivors. In addition, emerged mayflies were considered survivors because they had successfully completed their life cycles. The number of surviving mayflies was counted by subtracting the number of mortalities from the number of test animals originally introduced (N). "Calculated survivors" for *Hyalella* were also computed in this manner. The term "actual survivors" designated the number of *Hyalella* remaining in each concentration during the weekly checks. This distinction was necessary because of the problem of predation among these animals.

Results of the chronic toxicity tests for *Paraleptophlebia*, *Caenis*, and *Hyalella* are summarized in Figures 2 to 5 and in Tables 8 to 11. For all three test species, the data indicate that percent survivorship was highest in control and 3% groundwater concentrations. The highest mortalities, however, occurred at different concentrations for different test species. *Paraleptophlebia* black wing pad nymphs had higher mortalities than the younger, non-black wing pad nymphs (Figure 3).

In general, the survivorship of *Hyalella* in saline groundwater was lower than that of *Caenis*. Cannibalism appeared to be an important factor contributing to the higher mortalities. Percent loss due to cannibalism in the amphipod experiment was calculated by subtracting the actual percent survivorship from the calculated percent survivorship. The percent loss due to cannibalism was lowest in the control and highest in the 25% concentration, suggesting an increase in aggressive behaviour with an increase in groundwater concentration. Populations in 3, 5, 10, and 18% concentrations showed intermediate

Table 6. Results of 96 h acute toxicity studies with *Paraleptophlebia bicornuta* (mayfly), *Caenis simulans* (mayfly), and *Hyalella azteca* (amphipod).

Test Animal	LC_{50} (% Volume Groundwater)	Standard Error	Confidence Limits
Paraleptophlebia bicornuta	64.28	7.58	± 14.86
Caenis simulans	68.75	7.02	± 13.76
Hyalella azteca	50.00	7.90	± 15.48

Table 7. Cumulative mortality of the nymphs of *Caenis mcdunnough*, *Paraleptophlebia bicornuta*, and the amphipod *Hyalella azteca* in a static 96 h bioassay.

Test Animal	Groundwater Concentration (% by volume)	N	Hours of Exposure 0.25	0.5	2	4	8	24	48	72	96
Paraleptophlebia bicornuta	100	10	0	0	0	0	0	10	-	-	-
	50	10	0	0	0	0	0	3	3	4	6
	25	10	0	0	0	0	0	0	0	0	0
	12.5	10	0	0	0	0	0	0	0	0	0
	5	10	0	0	0	0	0	0	0	0	0
	Control	10	0	0	0	0	0	0	0	0	0
LC_{50} (% groundwater) =	64.28										
Standard error =	7.58										
95% confidence limits =	±14.86										
Caenis mcdunnough	100	10	0	0	0	0	1	4	10	-	-
	50	10	0	0	0	0	0	0	0	0	2
	25	10	0	0	0	0	0	0	0	0	0
	12.5	10	0	0	0	0	0	0	0	0	0
	5	10	0	0	0	0	0	0	0	0	0
	Control	10	0	0	0	0	0	0	0	0	0
LC_{50} (% groundwater) =	68.75										
Standard error =	7.02										
95% confidence limits =	±13.76										

continued ...

Table 7. Concluded.

Test Animal	Groundwater Concentration (% by volume)	N	Hours of Exposure 0.25	0.5	2	4	8	24	48	72	96
Hyalella azteca	100	10	0	0	0	0	0	3	10	-	-
	50	10	0	0	0	0	0	0	1	3	5
	25	10	0	0	0	0	0	0	0	0	0
	12.5	10	0	0	0	0	0	0	0	0	0
	5	10	0	0	0	0	0	0	0	0	0
	Control	10	0	0	0	0	0	0	0	0	0
LC_{50} (% groundwater) =	50										
Standard error =	7.9										
95% confidence limits =	±15.48										

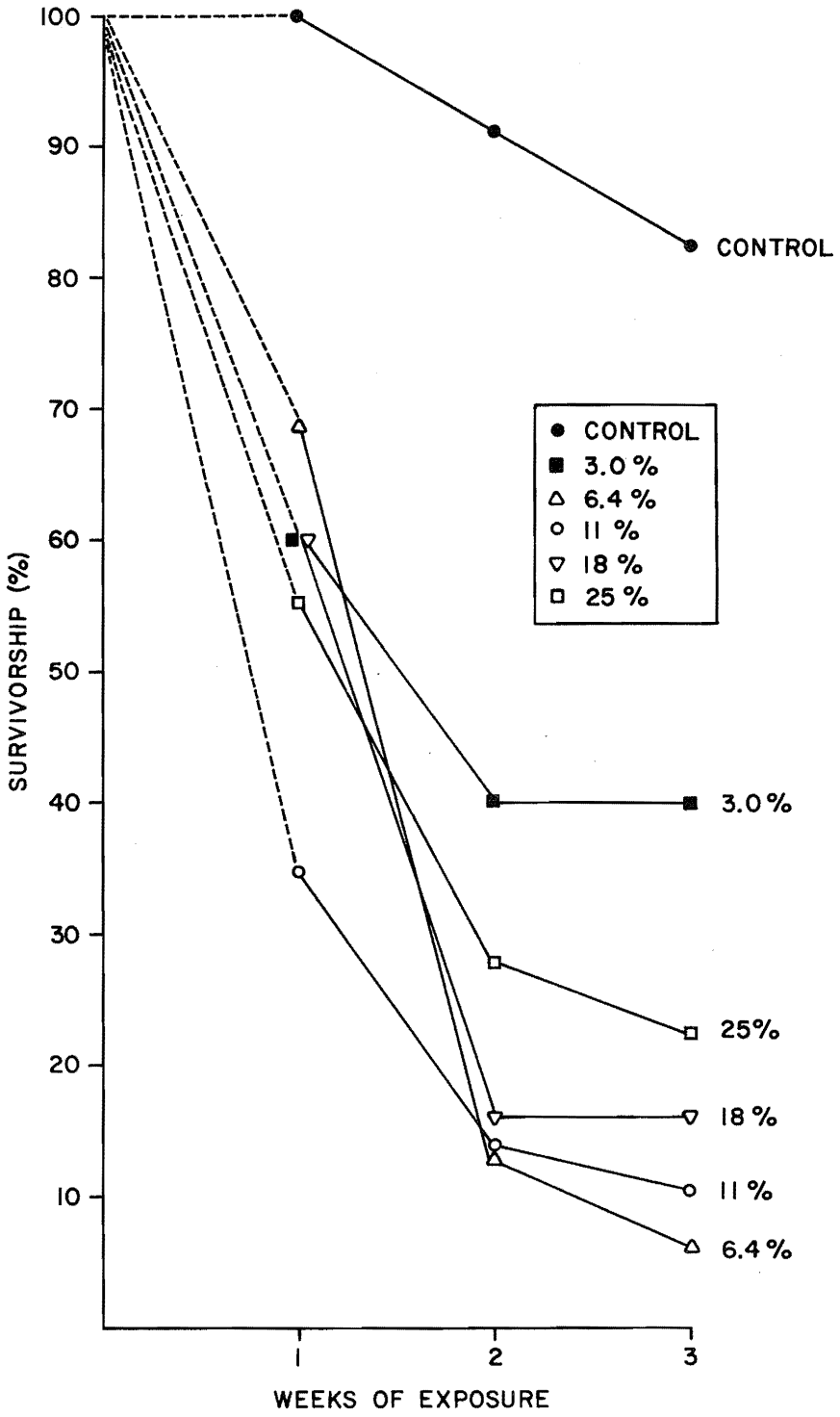

Figure 2. Percent survivorship in *Paraleptophlebia bicornuta* exposed to various saline groundwater concentrations.

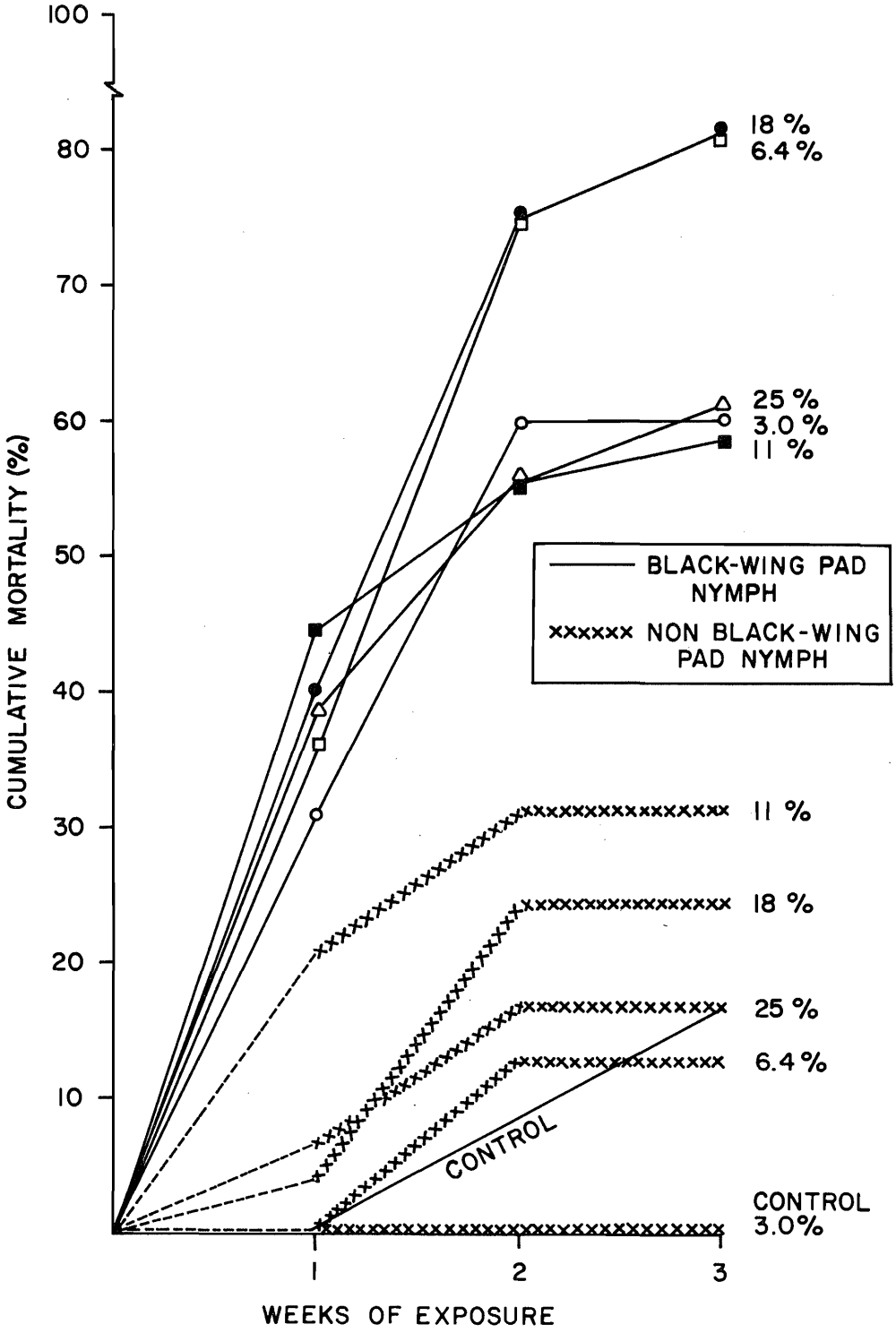

Figure 3. Cumulative percent mortality in black wing-pad vs. non-black wing-pad nymphs of *Paraleptophlebia bicornuta*.

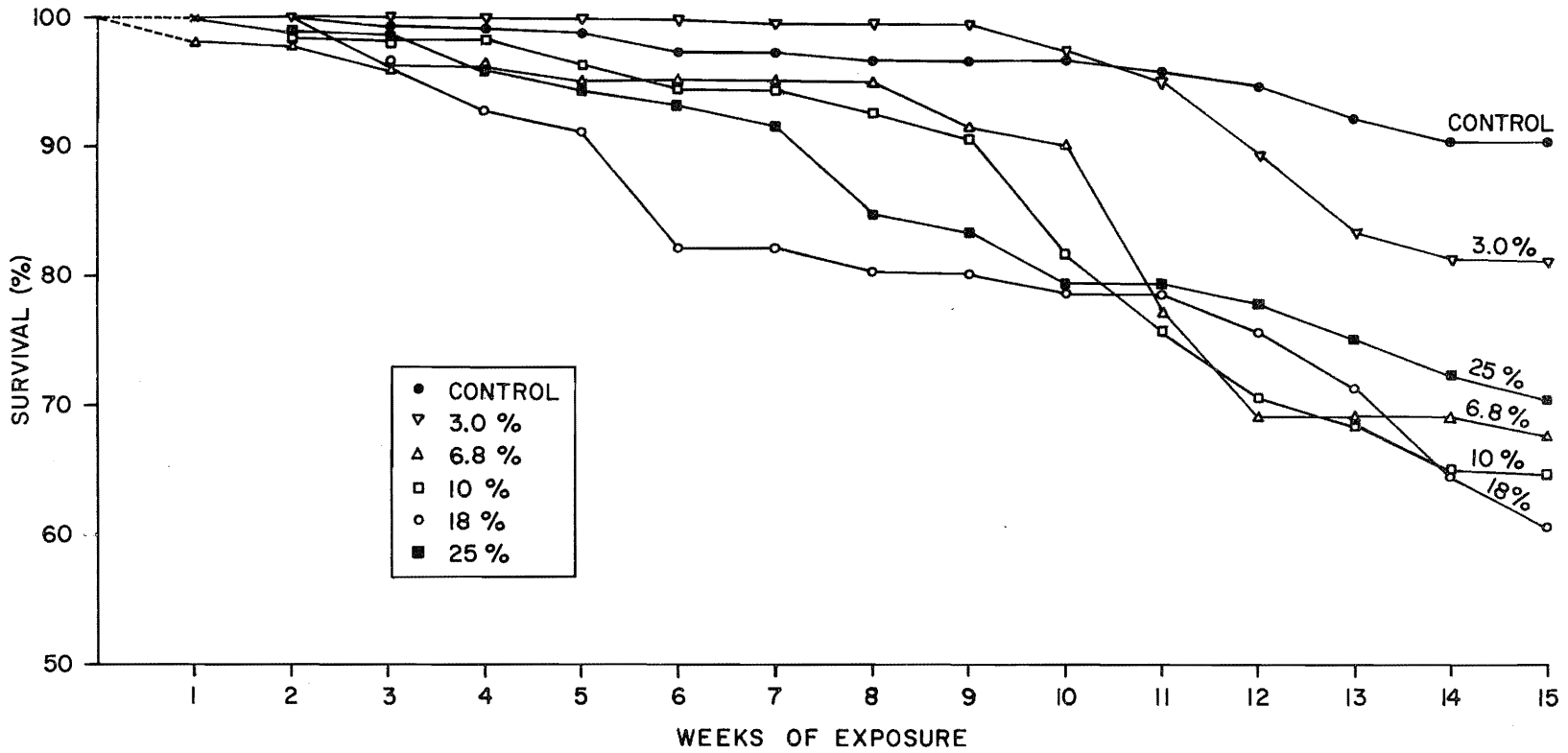

Figure 4. Percent survival of *Caenis simulans* exposed to various groundwater concentrations.

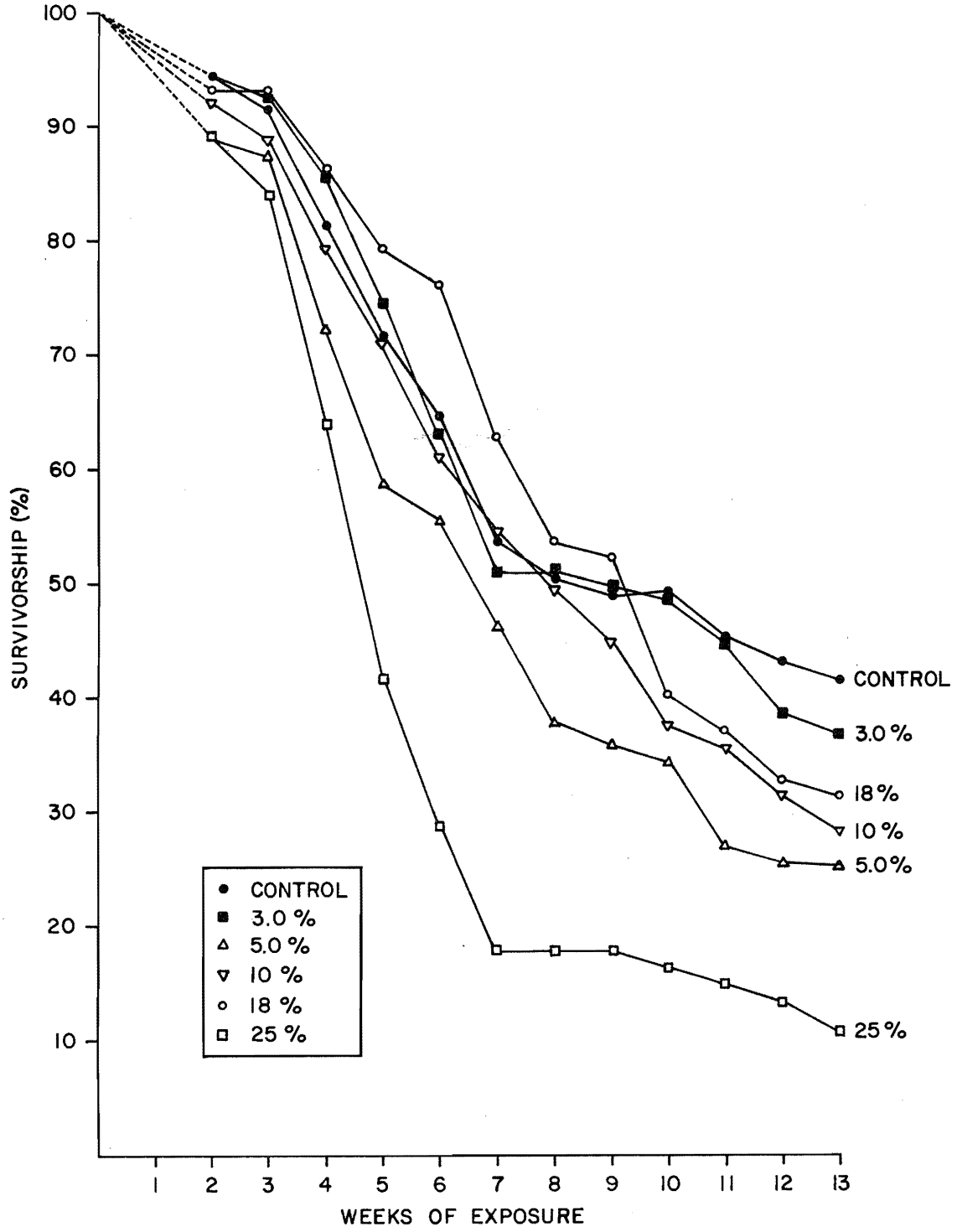

Figure 5. Percent actual survivorship in *Hyalella azteca* exposed to saline groundwater.

Table 8. Survivorship in *Paraleptophlebia bicornuta* exposed to various concentrations of saline groundwater.

Concentration (% Volume Groundwater)	N	Weeks	No. of Mortalities	No. of Survivors	% Survivorship
Control	12	1	0	12	100
		2	1	11	91.7
		3	2	10	83.3
3.0	10	1	4	6	60
		2	6	4	40
		3	6	4	40
6.8	16	1	5	11	68.75
		2	14	2	12.5
		3	15	1	6.25
11.0	29	1	19	10	34.5
		2	25	4	13.8
		3	26	3	10.3
18.0	25	1	10	15	60
		2	21	4	16
		3	21	4	16
25.0	18	1	8	10	55.6
		2	13	5	27.8
		3	14	4	22.2

Table 9. Cumulative percent mortality in black wing pad and non-black wing pad *Paraleptophlebia bicornuta*.

Concentration (% Volume Groundwater)	Weeks of Exposure	N	Black Wing Pad		Non-Black Wing Pad	
			No. of Mortalities	Cumulative % Mortality	No. of Mortalities	Cumulative % Mortality
Control	1	12	0	0	0	0
	2		1	8.3	0	0
	3		2	16.7	0	0
3.0	1	10	4	40	0	0
	2		6	60	0	0
	3		6	60	0	0
6.4	1	16	5	31.25	0	
	2		12	75	2	12.5
	3		13	81.25	2	12.5
11.0	1	29	13	44.8	6	20.7
	2		16	55.2	9	31.0
	3		17	58.6	9	31.0
15.0	1	25	9	36	1	4.0
	2		15	60	6	24.0
	3		15	60	6	24.0
25.0	1	18	7	38.9	1	5.6
	2		10	55.6	3	16.7
	3		11	61.1	3	16.7

Table 10. Survivorship in *Caenis mcdunnough* exposed to various concentrations of saline groundwater.

	Concentrations (% Volume Groundwater)																	
	Control			3.0			6.8			10.0			18.0			25.0		
Weeks	N	No. of Survivors	% Survivorship	N	No. of Survivors	% Survivorship	N	No. of Survivors	% Survivorship	N	No. of Survivors	% Survivorship	N	No. of Survivors	% Survivorship	N	No. of Survivors	% Survivorship
1	156	156	100	102	102	100	62	61	98.4	54	54	100	56	56	100	72	72	100
2		156	100		102	100		61	98.4		53	98.2		56	100		71	98.6
3		155	99.4		102	100		60	96.8		53	98.2		54	96.4		71	98.6
4		155	99.4		102	100		60	96.8		53	98.2		52	92.9		69	95.8
5		153	98.1		102	100		59	95.2		52	96.3		51	91.1		68	94.4
6		152	97.4		101	99.0		59	95.2		51	94.4		46	8.21		67	93.1
7		152	97.4		101	99.0		59	95.2		51	94.4		46	82.1		66	91.7
8		151	96.8		101	99.0		59	95.2		50	92.6		45	80.4		61	84.7
9		151	96.8		101	99.0		57	91.9		49	90.7		45	80.4		60	83.3
10		151	96.8		99	97.0		56	90.3		44	81.5		44	78.6		57	79.2
11		150	96.2		97	95.1		48	77.4		41	75.9		44	78.6		57	79.2
12		148	94.9		91	89.2		43	69.4		38	70.4		41	73.2		56	77.8
13		144	92.3		85	83.3		43	69.4		37	68.5		40	71.4		54	75.0
14		141	90.4		83	81.4		43	69.4		35	64.8		36	64.3		52	72.2
15		141	90.4		83	81.4		42	67.8		35	64.8		34	60.7		51	70.8

Table 11. Survivorship of *Hyalella azteca* exposed to various concentrations of saline groundwater.

Concentration (% Volume Groundwater)	Weeks	N	1 No. of Recoverable Mortalities	2 Cumulative No. of Mortalities	3 No. of Survivors Calculated	4 No. of Survivors Actual	5 % Survivorship Calculated	6 % Survivorship Actual	% Loss Due To Cannibalism (Column 5 and Column 6)
Control	1	70	3	3	67	-	95.7	ND	-
	2	70	1	4	66	66	94.3	94.3	0
	3	70	0	4	66	64	94.3	91.4	2.9
	4	70	3	7	63	57	90.0	81.4	8.6
	5	70	0	7	63	50	90.0	71.4	18.6
	6	65	2	9	56	42	86.2	64.6	21.6
	7	65	2	11	54	35	83.1	53.8	29.3
	8	65	2	13	52	33	80.0	50.8	29.2
	9	65	0	13	52	32	80.0	49.2	30.8
	10	65	0	13	52	32	80.0	49.2	30.8
	11	65	2	15	50	29	76.9	44.6	32.3
	12	65	1	16	49	28	75.4	43.1	32.3
	13	65	0	16	49	27	75.4	41.5	33.9
3.0	1	70	0	0	70	-	100	ND	-
	2	70	0	0	70	66	100	94.3	5.7
	3	70	0	0	70	65	100	92.9	7.1
	4	70	0	0	70	60	100	85.7	14.3
	5	70	2	2	68	52	97.1	74.3	22.8
	6	65	1	3	62	41	95.4	63.1	32.3
	7	65	1	4	61	33	93.8	50.7	43.1
	8	65	0	4	61	33	93.8	50.7	43.1
	9	65	1	5	60	32	92.3	49.2	43.1
	10	65	2	7	58	30	89.2	46.2	43.0
	11	65	0	7	58	29	89.2	44.6	44.6
	12	65	3	10	55	25	84.6	38.5	46.1
	13	65	1	11	54	24	83.1	36.9	46.2
5.0	1	72	0	0	72	ND	100	ND	-
	2	72	1	1	71	64	98.6	88.9	9.7
	3	72	0	1	71	63	98.6	87.5	11.1
	4	72	0	1	71	52	98.6	72.2	26.4
	5	72	2	3	69	42	95.8	58.4	37.4

continued ...

Table 11. Continued.

Concentration (% Volume Groundwater)	Weeks	N	1 No. of Recoverable Mortalities	2 Cumulative No. of Mortalities	3 No. of Survivors Calculated	4 No. of Survivors Actual	5 % Survivorship Calculated	6 % Survivorship Actual	% Loss Due To Cannibalism (Column 5 and Column 6)
	6	67	0	3	64	37	95.6	55.2	40.4
	7	67	1	4	63	31	94.0	46.3	47.7
	8	67	4	8	59	25	88.1	37.3	50.8
	9	67	0	8	59	24	88.1	35.8	52.3
	10	67	1	9	58	23	86.6	34.3	52.3
	11	67	0	9	58	18	86.6	26.9	59.7
	12	67	1	10	57	17	85.1	25.4	59.7
	13	67	0	10	57	17	85.1	25.4	59.7
10.0	1	72	2	2	70	ND	97.2	ND	-
	2	72	1	3	69	66	95.8	91.7	4.1
	3	72	0	3	69	64	95.8	88.9	6.9
	4	72	1	4	68	57	94.4	79.2	15.2
	5	72	1	5	67	51	93.1	70.8	22.3
	6	67	2	7	60	44	89.5	65.7	23.8
	7	67	0	7	60	36	89.5	53.7	35.8
	8	67	0	7	60	33	89.5	49.3	40.2
	9	67	1	8	59	30	88.1	44.8	43.3
	10	67	1	9	58	25	86.6	37.3	49.3
	11	67	0	9	58	24	86.6	35.8	50.8
	12	67	3	12	55	21	82.1	31.3	50.8
	13	67	1	13	54	19	80.6	28.4	52.2
18.0	1	72	0	0	72	ND	100	ND	-
	2	72	2	2	70	67	97.2	93.1	4.1
	3	72	0	2	70	67	97.2	93.1	4.1
	4	72	1	3	69	62	95.8	86.1	9.7
	5	72	1	4	68	57	94.4	79.2	15.2
	6	67	1	5	62	51	92.5	76.1	16.4
	7	67	1	6	61	42	91.0	62.7	28.3
	8	67	1	7	60	36	89.5	53.7	35.8
	9	67	1	8	59	35	88.0	52.2	35.8
	10	67	3	11	56	27	83.6	40.3	43.3
	11	67	2	13	54	25	80.6	37.3	43.3
	12	67	3	16	51	22	76.1	32.8	43.3
	13	67	0	16	51	21	76.1	31.3	44.8

continued ...

Table 11. Concluded.

Concentration (% Volume Groundwater)	Weeks	N	1 No. of Recoverable Mortalities	2 Cumulative No. of Mortalities	3 No. of Survivors Calculated	4 No. of Survivors Actual	5 % Survivorship Calculated	6 % Survivorship Actual	% Loss Due To Cannibalism (Column 5 Column 6)
25.0	1	72	0	0	72	ND	100	ND	-
	2	72	3	3	69	64	95.8	88.9	6.9
	3	72	1	4	68	60	94.4	83.3	11.1
	4	72	1	5	67	46	93.1	63.9	29.2
	5	72	0	5	67	30	93.1	41.7	51.4
	6	67	1	6	61	19	91.0	28.4	62.6
	7	67	0	6	61	12	91.0	17.9	73.1
	8	67	0	6	61	12	91.0	17.9	73.1
	9	67	0	6	61	12	91.0	17.9	73.1
	10	67	0	6	61	11	91.0	16.4	74.6
	11	67	0	6	61	10	91.0	14.9	76.1
	12	67	1	7	60	9	89.6	13.4	76.2
	13	67	1	8	59	7	88.1	10.5	77.6

values for percent loss due to cannibalism, but cannibalism did not increase proportionately with the increase in saline groundwater concentration (Table 11).

4.3 SUBLETHAL EFFECTS

In addition to chronic toxicity tests for survivorship, studies were undertaken to examine the test species for changes in moulting frequency, emergence, growth, osmoregulation, and ion accumulation. Each experiment is presented in the following sections.

4.3.1 Moulting Frequency

4.3.1.1 Rationale. Moulting is a complex growth mechanism governed internally by the insect's neuro-endocrine system. The number of moults is not constant and depends on such factors as heredity, external environmental conditions, or nutrition (Wigglesworth 1965). This experiment attempted to determine the effect of saline groundwater on the growth process of invertebrates, using moulting frequency as a growth index.

4.3.1.2 Methods. Moulting frequency was computed daily or weekly as a ratio of the total number of cast skins recovered to the total number of test animals in a particular test concentration. In addition, a mean moulting frequency was calculated for each test species at each test concentration.

4.3.1.3 Results. Moulting frequency results are summarized in Figures 6 to 8. The results indicate that *Paraleptophlebia* moulting frequencies were higher in control and 3% groundwater concentrations. At 6.4% and higher concentrations of groundwater, moulting frequencies were reduced. Analyses, using Duncan's New Multiple Range Test, showed there were statistically significant differences between the control and the 6.4 and 11% test groups, and between the 3% and the 6.4% test

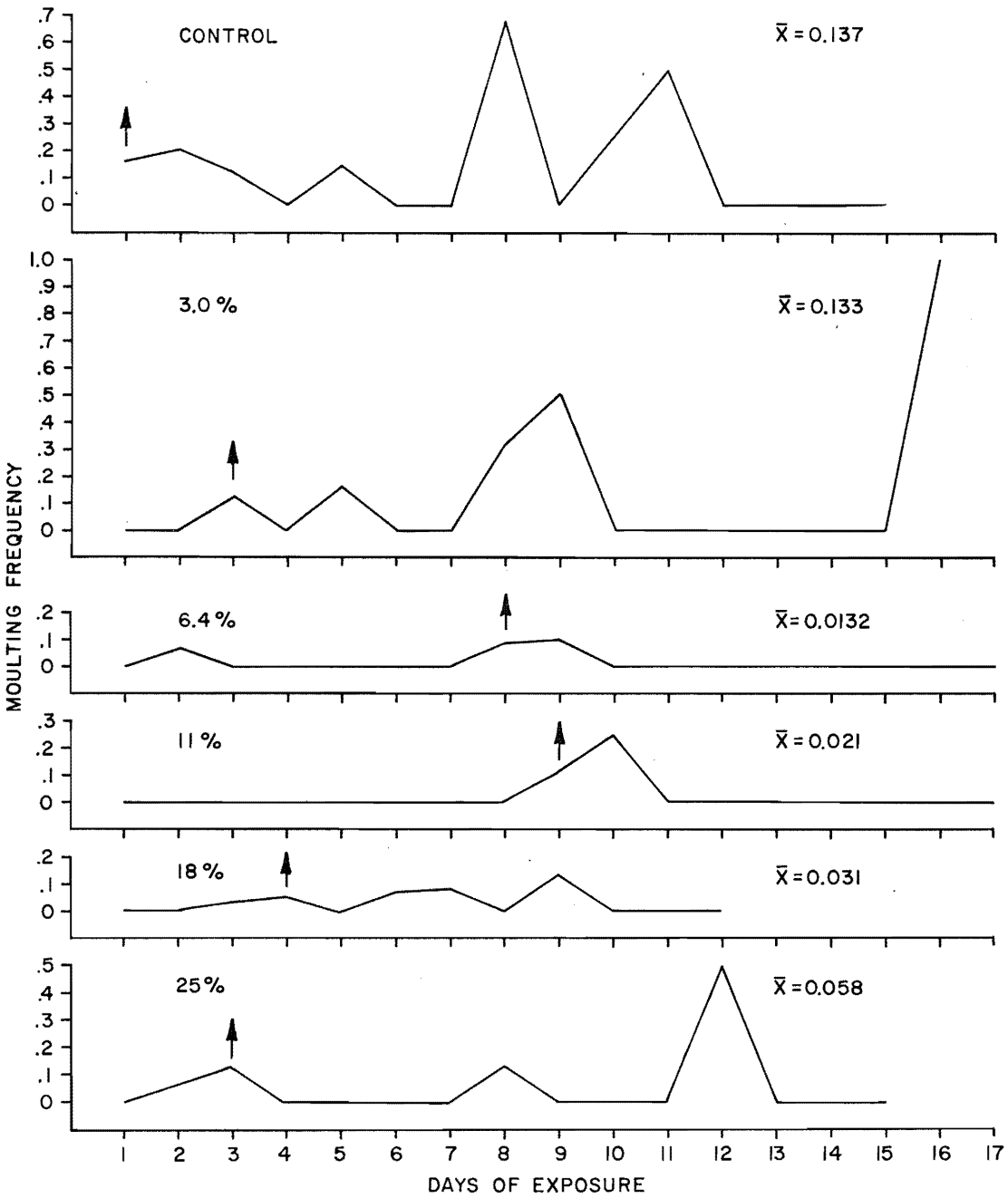

Figure 6. Moulting frequency of *Paraleptophlebia bicornuta* nymphs exposed to saline groundwater (x = mean moulting frequency; arrow indicates the first day of adult emergence).

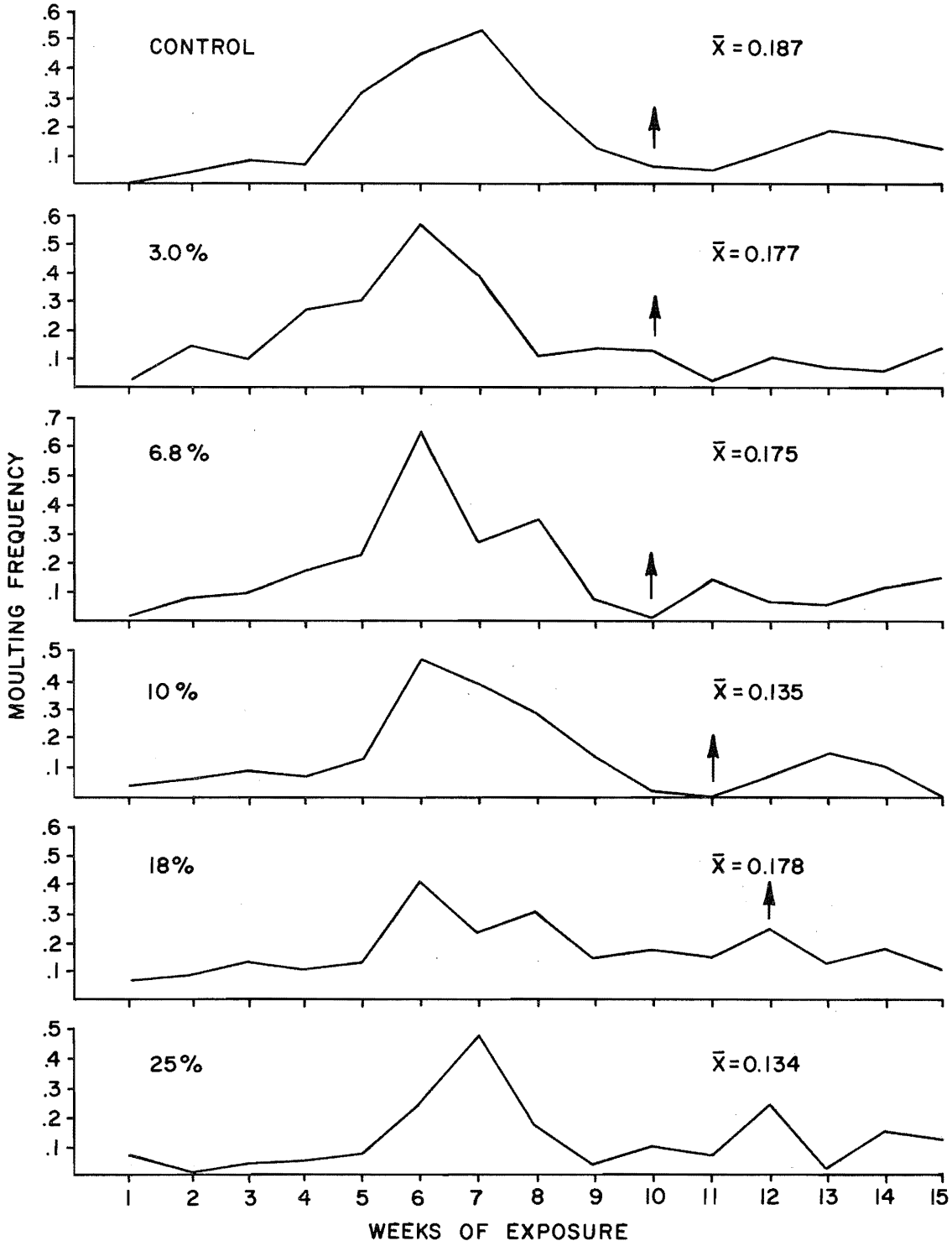

Figure 7. Moulting frequencies of *Caenis simulans* nymphs exposed to saline groundwater. ($\bar{x}$ = mean moulting frequency; arrow indicates the first week of adult emergence).

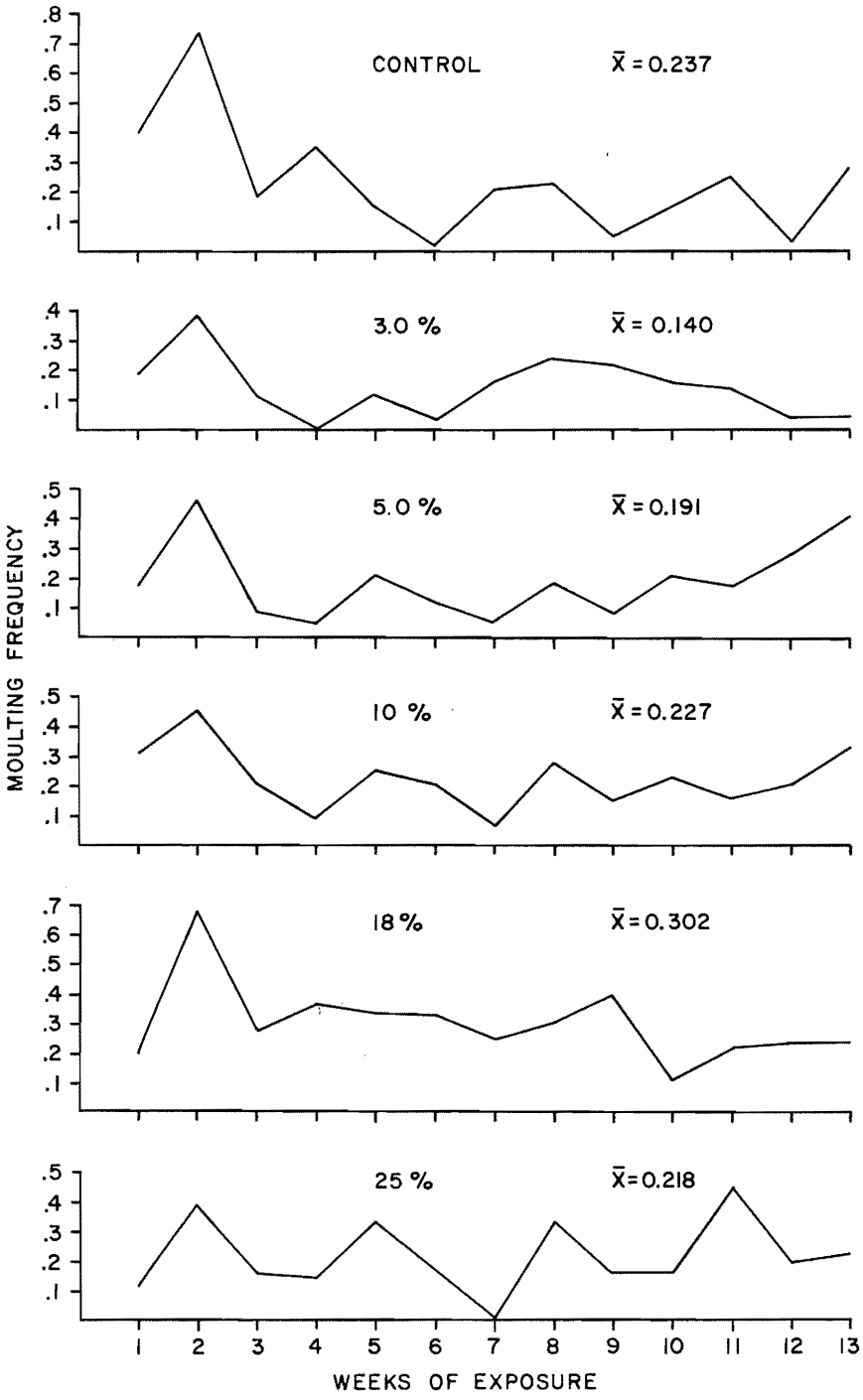

Figure 8. Moulting frequencies of *Hyalella azteca* exposed to saline groundwater ($\bar{x}$ = mean moulting frequency).

groups. A distinct delay in the laboratory emergence of final instar nymphs into the winged stage was observed at control, 3, 6.4, and 11% groundwater concentrations (Figure 6).

In the *Caenis* mayflies, there was no statistically significant difference between the moulting frequencies of the various test groups, although the control had the highest mean value and the 25% test group had the lowest. All the test groups exhibited a peak in moulting activity during the sixth to seventh week of the experiment. Nymphs reared in test solutions, however, demonstrated a distinct delay in emergence of final instar nymphs into the winged stage. This delay ranged from 1 wk at 10% to 2 wk at 18% to a complete inhibition of emergence at the 25% concentration (Figure 7).

Interpretation of the *Hyalella* moulting data was complicated by the increasing cannibalism among the test animals as the experiment progressed. After an initial period of 1 to 2 wk, during which there appeared to be little intraspecific feeding, the test animals were observed eating cast skins (Figure 8). Consequently, only the data for the first 2 wk of the experiment appear to be reliable. During this initial period, moulting frequency was highest in the control group and lowest at the 25% groundwater concentration.

4.3.2 Emergence in Mayflies

4.3.2.1 Rationale. In mayflies, the transformation of the aquatic nymph into the aerial subimago is termed "emergence". Emergence is controlled by a host of environmental conditions such as temperature of air and water, water currents, moon phase, humidity, wind, rainfall, and photoperiod, as well as physiological factors such as neuroendocrine secretions and the physiological rhythms of the animal (Corbet 1964; Humpesch 1971; Thibault 1971; Fremling 1973a; Fremling 1973b; Langford 1975; Peters and Peters 1977).

This experiment attempted to determine the effect of saline groundwater concentrations on the emergence success of both *Paraleptophlebia* and *Caenis* mayflies.

4.3.2.2 Methods. Daily records were kept of emergence in both mayfly species. Cumulative percent emergences at various test concentrations were calculated.

4.3.2.3 Results. Emergence results are summarized in Figures 9 and 10. The results indicate that, in *Paraleptophlebia*, the highest cumulative percent emergence (83%) recorded was for the control group, and the lowest (6.25%) was for the 6.4% concentration group (Figure 9). A delay in emergence was observed for all groundwater concentrations; however, the delay was not proportional to the strength of groundwater concentration.

In the *Caenis* group, the highest cumulative percent emergence (21%) occurred in the 6.8% concentration test group, and the lowest occurred in the 25% concentration test group. No emergence at all occurred at this concentration (Figure 10). A 1 to 2 wk delay in emergence was observed in the 10% concentration test groups, and a 2 wk delay was observed for the animals in the 18% concentrations.

No external morphological abnormalities were observed for the aerial subimagos of either species.

4.3.3 Growth

4.3.3.1 Methods. At the end of the chronic toxicity experiments, *Caenis* nymphs from different concentrations of groundwater were measured to determine total body length, head width, and thorax width. The results were computed using Duncan's New Multiple Range Test.

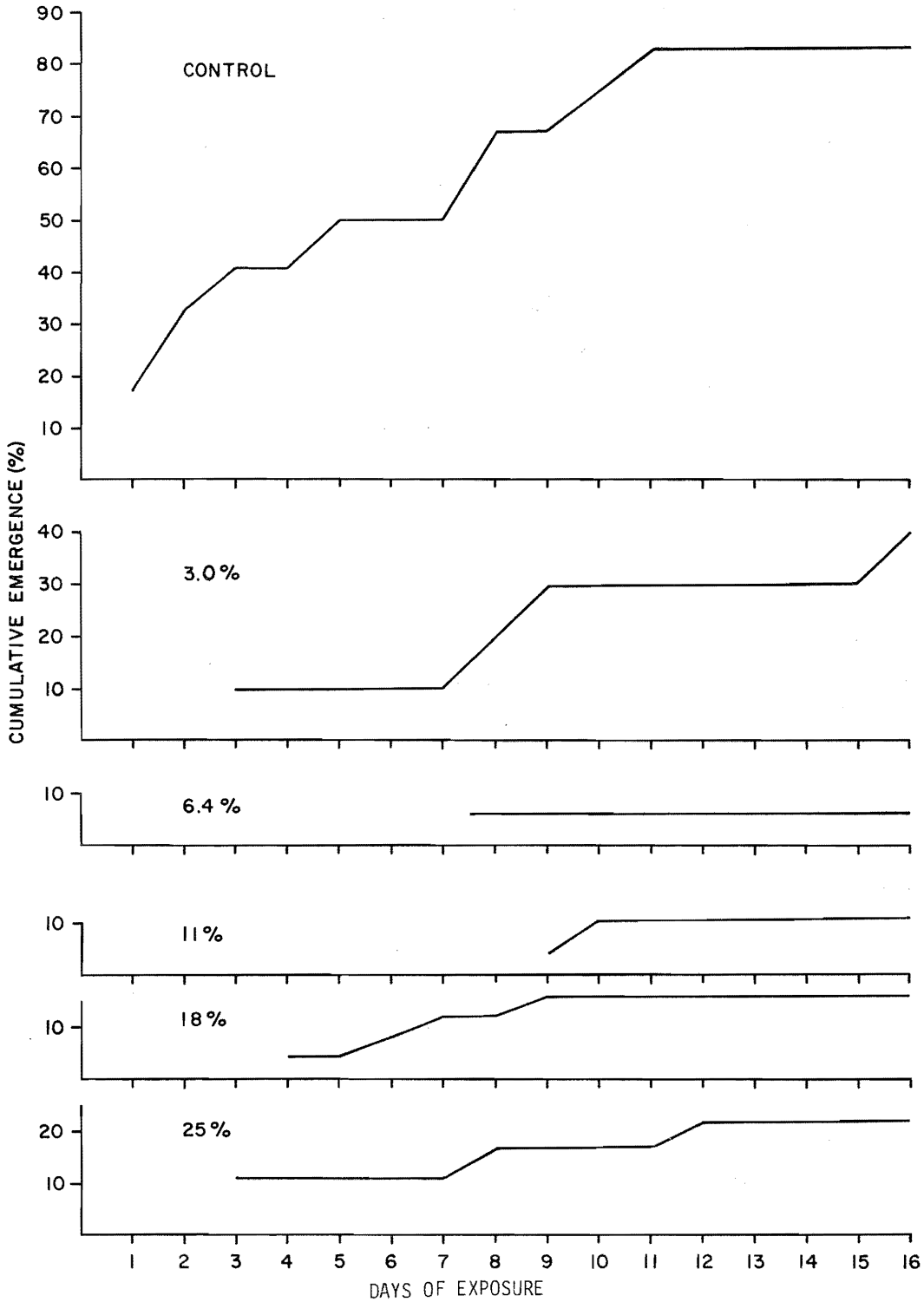

Figure 9. Cumulative percent emergence of *Paraleptophlebia bicornuta* nymphs exposed to saline groundwater.

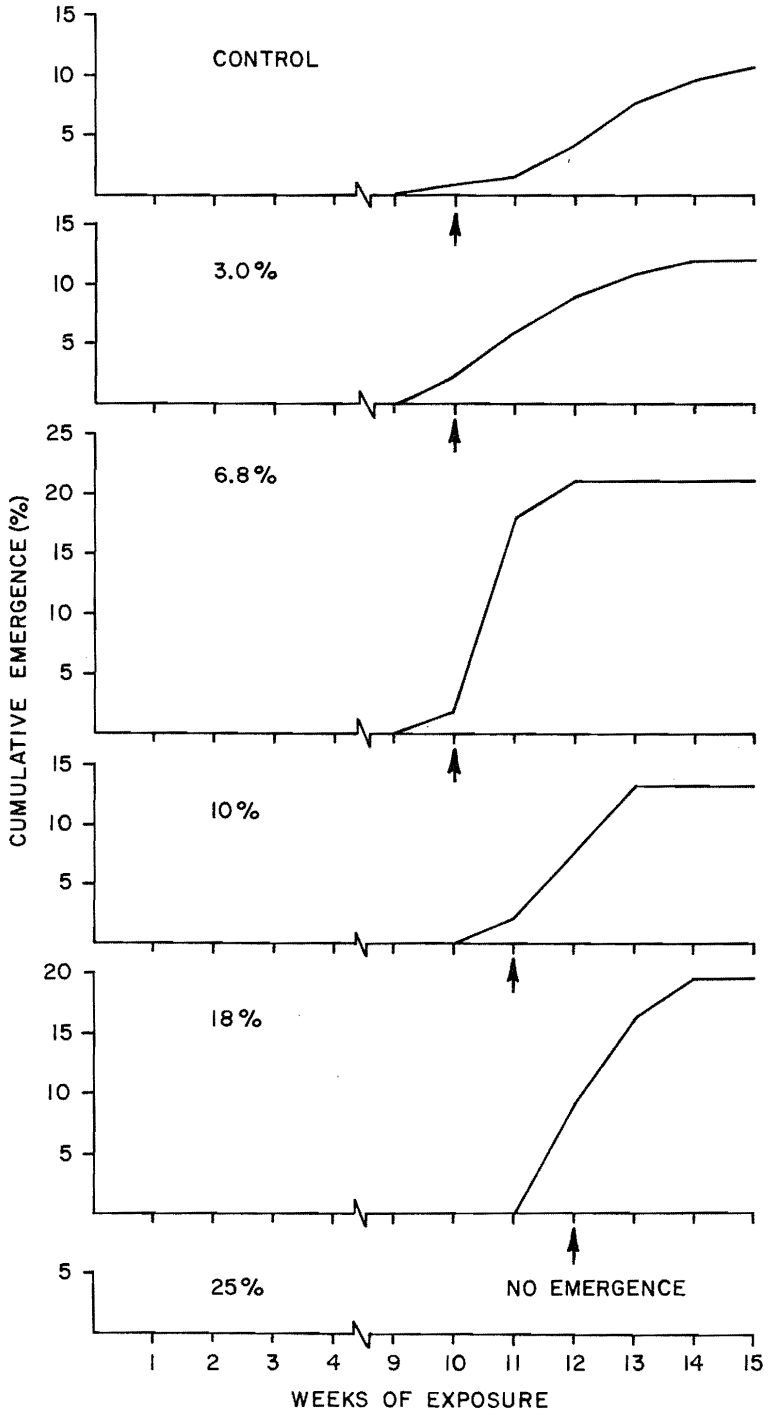

Figure 10. Cumulative percent emergence of *Caenis simulans* nymphs exposed to saline groundwater (arrow indicates first week of emergence).

4.3.3.2 Results. The results indicated no significant differences ($p > 0.01$) in measurements of any of the three morphological characters. Observations of the nymphs under a dissecting microscope indicated, however, that there were differences in the shapes and proportions between the bodies of control nymphs and those in 10, 18, and 25% concentrations of groundwater.

Nymphs in the control group could be separated, using scatter diagrams (Figures 11 to 13), from those in the 10, 18, and 25% concentrations when two dimensions (head width and total body length) were compared. A triangular graph method also succeeded in separating the control and 10% concentration test groups. In this instance, head widths, thorax widths, and total body lengths were compared (Figure 14). It would appear that the saline groundwater has a subtle effect on the allometric growth of *Caenis* nymphs.

4.3.4 Osmoregulation

4.3.4.1 Rationale. Since saline groundwater is hypotonic in relation to the haemolymph of mayflies, an experiment was designed to determine whether saline groundwater might cause osmoregulatory stress in freshwater mayfly species. The mayfly species were selected for this study because both *Paraleptophlebia* and *Caenis* possess ion-absorbing cuticular structures known as chloride cells.

The number of chloride cells is related to external salinity; i.e., cell density increases as salt concentration decreases and *vice versa* (Wichard et al. 1973; Komnick and Wichard 1975; Wichard 1975; Wichard and Heuss 1975; Wichard et al. 1975). In addition to the numerical variation, chloride cells also respond to different salinities with changes in fine structure. Biological, morphological, and histochemical evidence supports the assumption that the cells are osmoregulatory and are involved in the absorption of chloride ions.

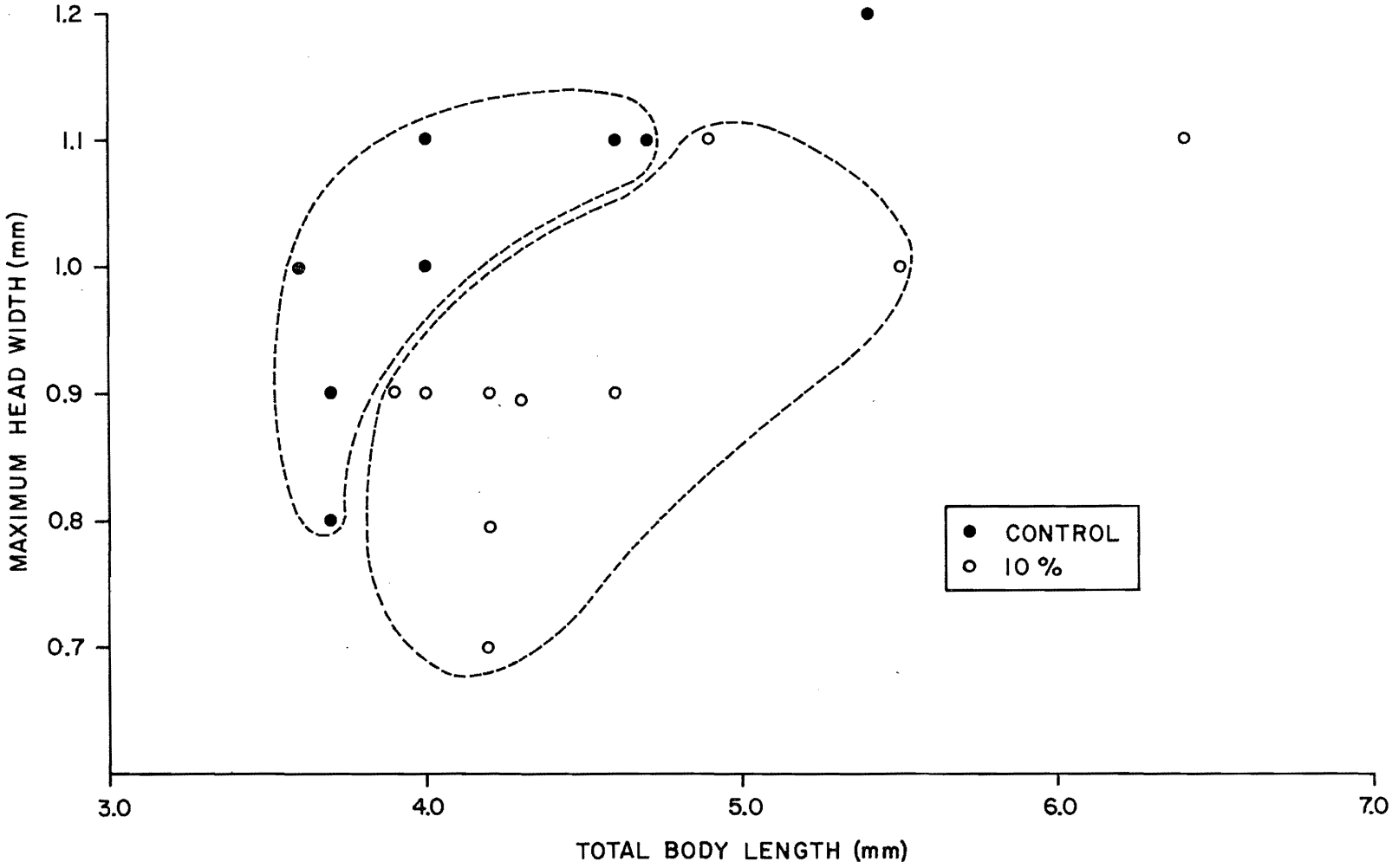

Figure 11. A scatter diagram separating *Caenis simulans* nymphs of the control group from the 10% concentration test group.

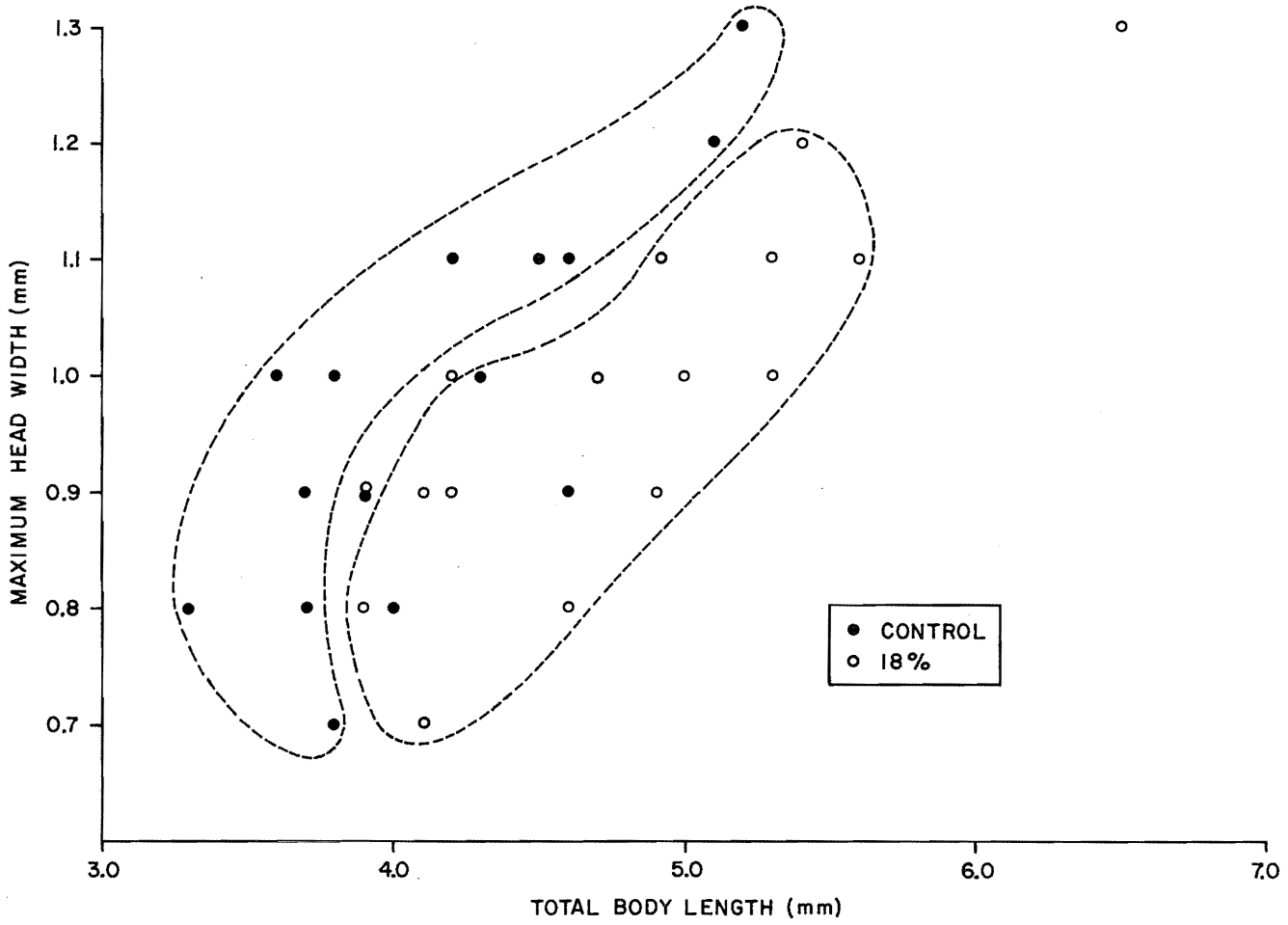

Figure 12. A scatter diagram separating *Caenis simulans* nymphs of the control group from the 18% concentration test group.

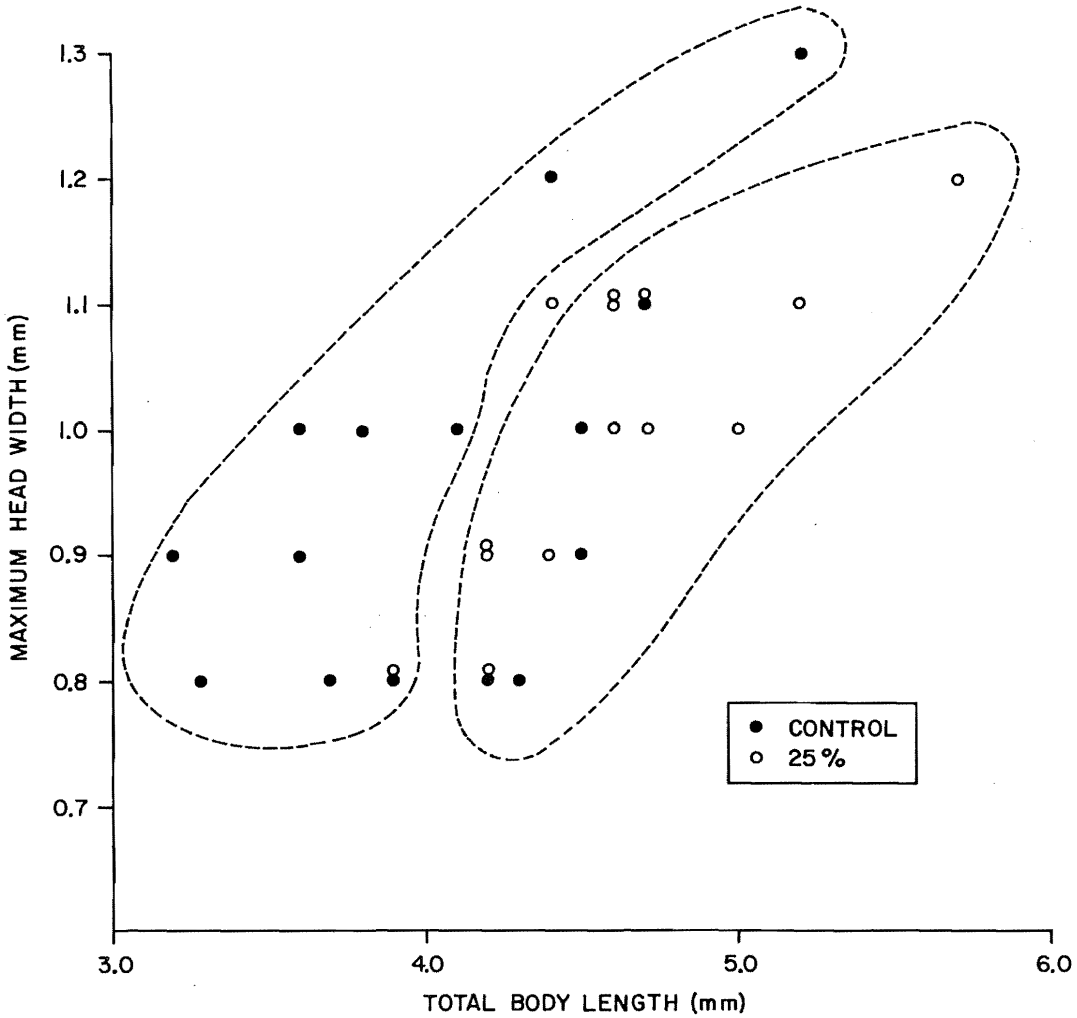

Figure 13. A scatter diagram separating *Caenis simulans* nymphs of the control group from the 25% concentration test group.

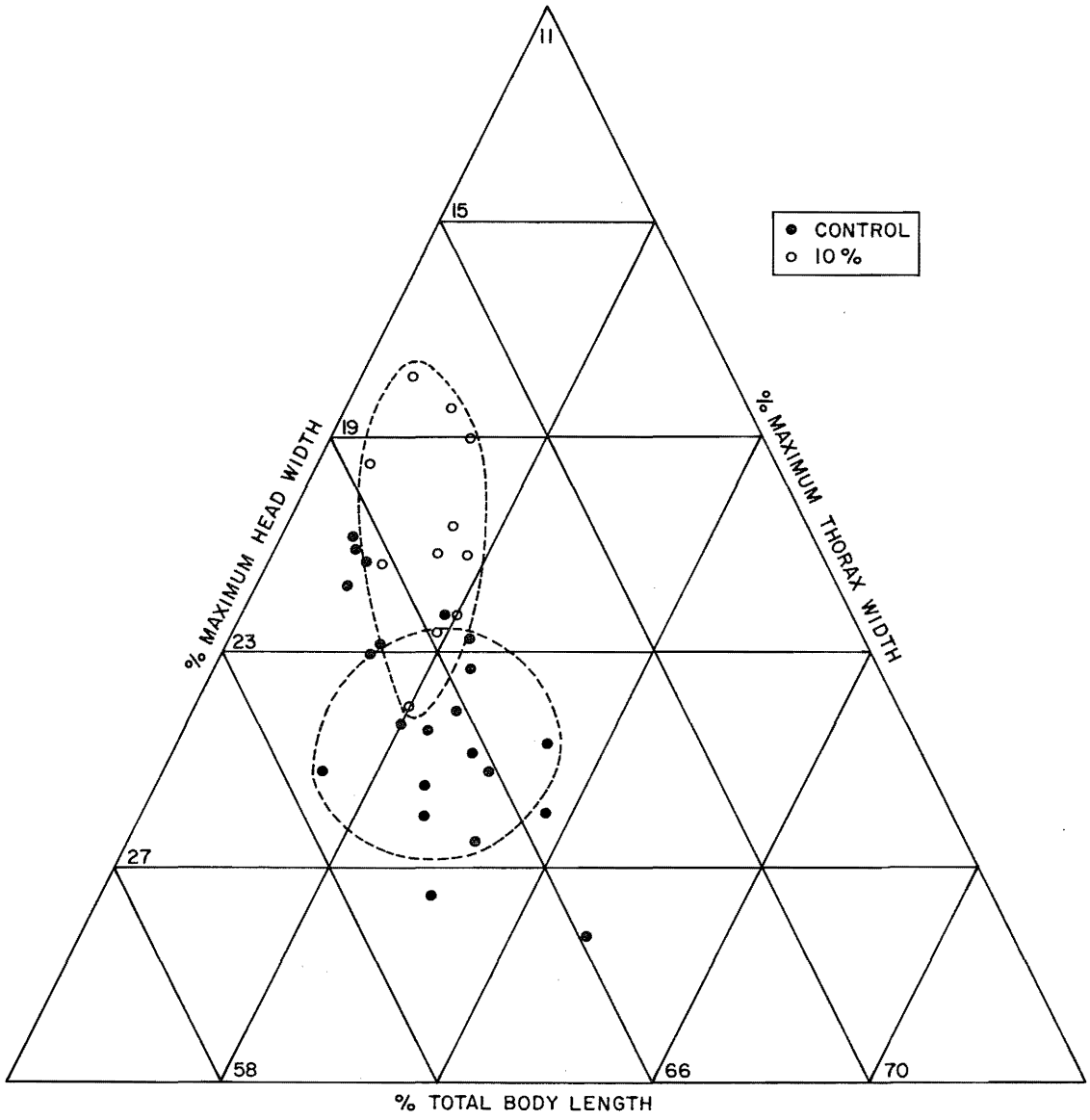

Figure 14. Triangular graph of the head width, thorax width, and total body length of *Caenis simulans* nymphs from the control and 10% saline groundwater test groups.

Because of the simple numerical relationship between chloride cell density and salinity, enumeration of chloride cells might be an ideal biomonitoring technique to detect salinity changes in the aquatic environment.

4.3.4.2 Methods. At the end of the chronic toxicity experiments, the abdominal gills of *Paraleptophlebia* and *Caenis* were treated with $AgNO_3$ and the number of chloride cells per gill lamella counted.

4.3.4.3 Results. The results are presented in Tables 12 to 14 and Figures 15 and 16. The results indicate that there is a curvilinear relationship between chloride cell density and salinity. Saline groundwater lowered the chloride cell density in the gills of all the test animals, reducing the area available for ion absorption. Since the patterns of changes in chloride cell density appear to parallel that of survivorship (Figures 15 and 16), chloride cell density may have predictive value in determining and quantifying osmoregulatory stress in some aquatic animals.

4.3.5 Ion Accumulation

4.3.5.1 Rationale. Although bioaccumulation of heavy metals has been studied in fish, little work has been done on invertebrates. In this experiment, the accumulation of Cu and Zn in the tissues of the mayfly *Caenis* and the amphipod *Hyalella* was examined after chronic exposure of the animals to a range of concentrations of groundwater.

4.3.5.2 Methods. Whole *Caenis* and *Hyalella* were towel-dried and weighed. Pooled samples were used because at least 10 animals were required to produce a sufficiently large sample to determine all ion concentrations. The weighed animals were placed in a glass-teflon homogenizer and hand-homogenized in 50 volumes of glass-distilled water. The homogenate was centrifuged three times for 15 min at 3000 G.

Table 12. Mean chloride cell density per gill lamella in *Paraleptophlebia bicornuta* after 2 wk of adaptation in saline groundwater.

% Groundwater Concentration	N	Mean Chloride Cell Density	S.E.
Control	2	1549.0	± 30.0
3.0	4	1041.5	± 35.9
6.4	1	404.0	
11.0	4	894.5	± 39.0
18.0	4	880.0	± 38.9
25.0	4	997.5	± 68.7

Table 13. Mean chloride cell density per opercular gill lamella in *Caenis simulans* after 15 wk of adaptation in saline groundwater.

% Groundwater Concentration	N	Mean Chloride Cell Density	S.E.
Control	12	846.75	± 40.39
3.0	11	497.90	± 54.60
6.8	8	340.0	± 22.70
10.0	8	543.13	± 35.40
18.0	6	462.16	± 20.16
25.0	6	421.67	± 21.20

Table 14. Mean chloride cell density per third gill lamella in *Caenis simulans* after 15 wk of adaptation in saline groundwater.

% Groundwater Concentration	N	Mean Chloride Cell Density	S.E.
Control	20	192.1	± 10.3
3.0	18	158.5	± 12.7
6.8	6	123.6	± 20.2
10.0	10	121.7	± 14.8
18.0	8	94.5	± 6.7
25.0	8	129.75	± 11.49

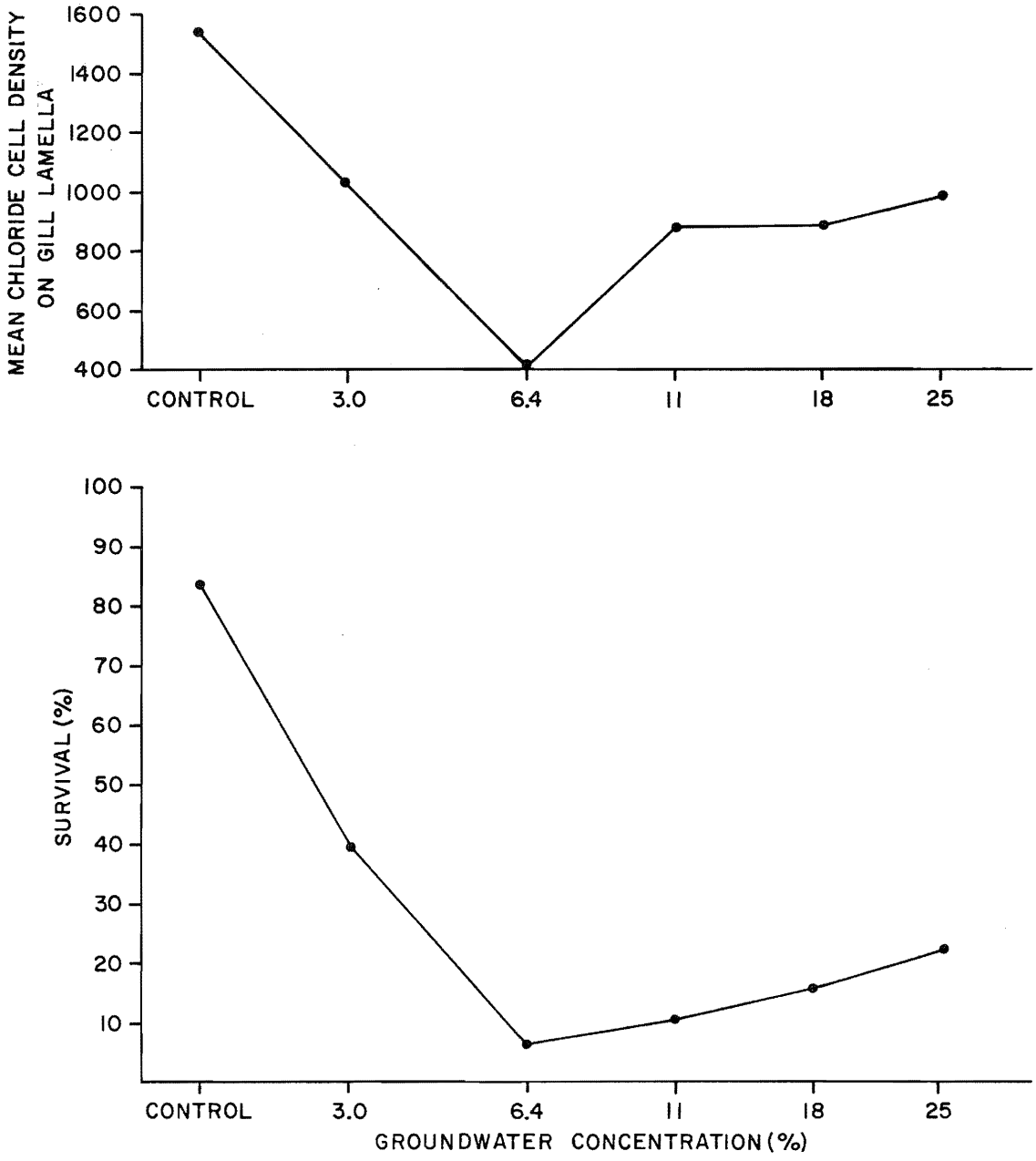

Figure 15. Mean chloride cell density per gill lamella compared to percent survival in *Paraleptophlebia bicornuta* nymphs after two weeks of adaptation in saline groundwater.

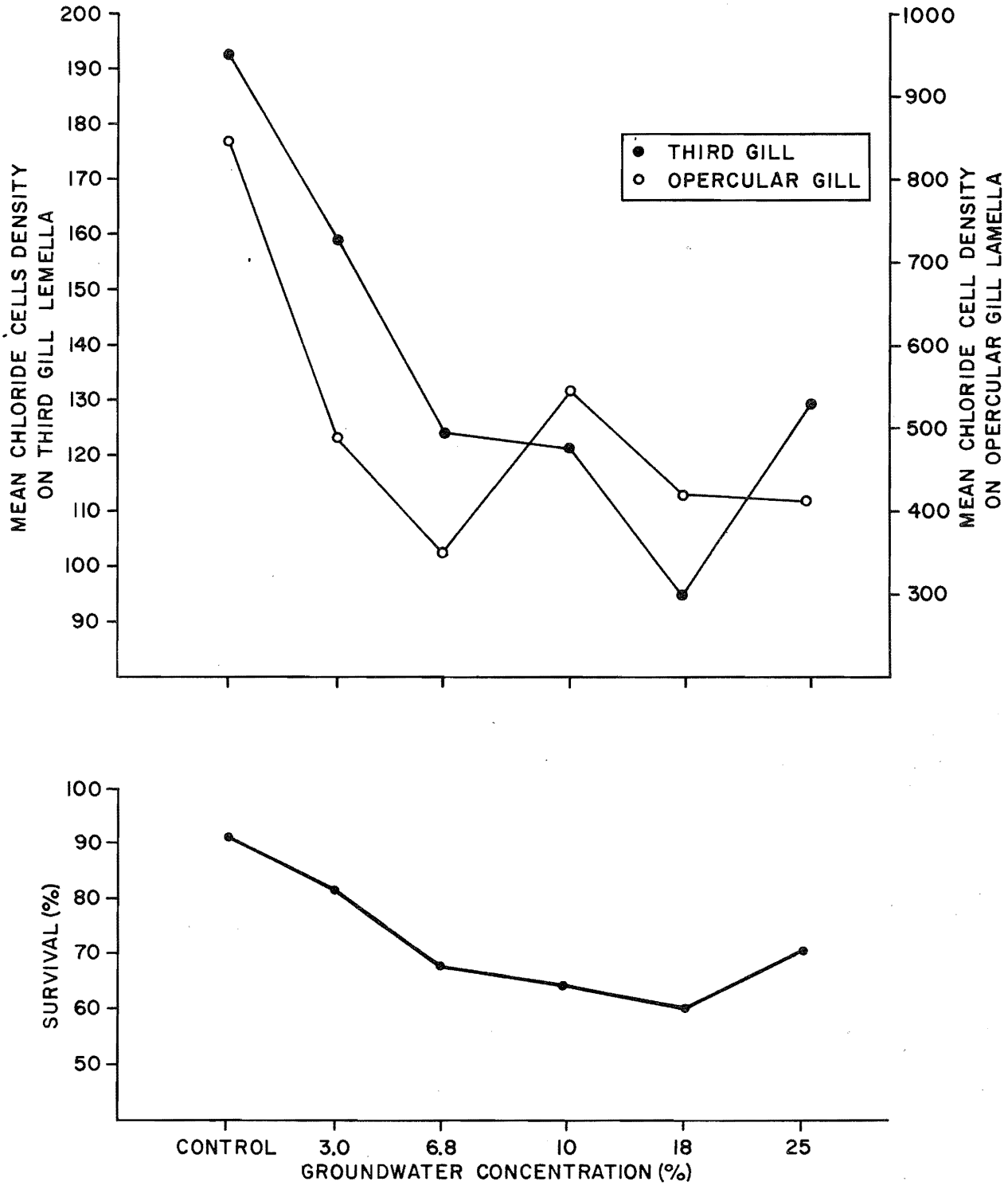

Figure 16. Mean chloride cell density per third gill lamella and opercular gill lamella compared to percent survival in *Caenis simulans* after 15 weeks of adaptation in saline groundwater.

After the last spin, the supernatant was stored frozen in Nalgene containers. The sloughed-off cuticles of *Caenis* and *Hyalella* were prepared for ion analysis using the same method. Again, pooled samples were used.

Ionic concentrations of Cu and Zn in the supernatants were determined using a Jarrel Ash Model 850 Atomic Absorption Spectrophotometer and an air/acetylene flame. Further details are given in Section 5.3.4.2, which describes a similar analysis for fish samples.

4.3.5.3 Results. The ion accumulation results are presented in Table 15. The results show levels of both Cu and Zn in the tissues of both species were similar to those recorded for gill tissue from fish (Section 5.3.4.3). Accumulation of both ions occurred at higher levels in the animals from higher groundwater concentrations.

Levels of both metals were also measured in the sloughed-off cuticle remaining after moulting. This was done in an attempt to determine whether excess heavy metal ions might be excreted by this route in insects. Since the levels of both ions were lower in the cuticle than in the tissues and did not increase with increasing groundwater concentrations, this did not seem to be the case. The only possible exception was Cu in *Hyalella* cuticle at the highest concentrations tested.

Table 15. Accumulation of copper and zinc in tissues and in the exuviae of invertebrates exposed to mine depressurization groundwater.

% Groundwater Concentration	Copper		Zinc	
	Tissues	Exuviae	Tissues	Exuviae
Caenis simulans				
Control	13	5	7.0	4.4
3.0	13	4	7.2	5.2
6.8	17	3	16.4	5.0
10.0	12	7	15.5	3.4
18.0	10	4	16.5	5.2
25.0	14	4	19.0	5.9
Hyalella azteca				
Control	15.4	6.5	6.5	<2.0
3.0	14.6	7.5	8.6	2.5
5.0	16.5	6.5	7.1	1.5
10.0	21.0	6.5	6.3	2.5
18.0	24.0	10.0	15.1	2.0
25.0	23.0	10.5	19.5	<2.0

5. FISH

5.1 INTRODUCTION

The objective of this portion of the study was to determine the long-term effects of mine depressurization groundwater on selected species of fish, using groundwater concentrations non-lethal at 96 h. Adverse impact of mine depressurization groundwater on aquatic ecosystems may result, not only from acute toxicity which causes the immediate death of an organism, but also from chronic toxicity and sublethal effects which may exert a long-term influence on a population's abundance, distribution, and diversity.

In the following sections, both chronic toxicity and sublethal effects are examined. Parameters for the latter include opercular frequency, coughing frequency, changes in hematocrit, ion accumulation, gill damage, growth, and fertilization success.

5.2 CHRONIC TOXICITY

5.2.1 Methods

Preliminary acute toxicity (96 h) experiments were conducted for lake chub, white sucker, and rainbow trout to determine the non-lethal range of groundwater concentrations. These initial experiments followed the methods outlined in Standard Methods (1975). The results are summarized in Table 16.

Chronic toxicity tests at non-lethal concentrations were conducted for each species. These tests also followed standard bioassay procedures (Standard Methods 1975). Tests were conducted under semi-static conditions which required 90% of the test solution to be replaced every 7 d. The test solution was mixed in the proportions described in Section 4.2, using Calgary City tapwater as a diluent. Before being used, the tapwater was treated to eliminate chlorine by filtering it through charcoal and adding sodium thiosulphate. It was then aerated for at least 24 h in a 228 L fibreglass storage tank.

Table 16. Ninety-six hour LC_{50} values and maximum non-lethal concentrations for rainbow trout, lake chub, and white sucker exposed to mine depressurization groundwater at 15°C.

Species	96 h LC_{50}	Maximum Non-Lethal Concentrations
Rainbow Trout	36.73% ± 6.35	12.5%
Lake chub[a]	59.5%	31.6%
White Sucker	26.9% ± 5.0	25.0%

[a] Data from McMahon et al. 1976.

The experiments were conducted in two walk-in, constant (15°C) temperature rooms at the Aquatic Environments laboratory in Calgary. Photoperiods were controlled to simulate summer daylight and darkness periods. During the test, fish were held in glass tanks measuring 27 x 25 x 41 cm, and were fed twice weekly (the day before water changes and 2 d after water changes) on a diet of Trout Starter Ration (Unifeed).

Chemical characteristics of the composite stock solution were monitored throughout the study period. Monitoring results are presented in Section 3. Test solutions in each tank were monitored for dissolved O_2 concentration, pH, temperature, and conductivity. The results are tabulated in Table 2. The experiments were run in duplicate at five different concentrations (all non-lethal at 96 h) plus the control (diluent water only). Table 17 summarizes the test concentrations, durations, and the number and condition of test species. For each experiment LC_{50} values were determine by the Muench and Reed method (Woolf 1968).

5.2.2 Results

Results of the 90 d chronic bioassay are expressed as LC_{50}'s and are summarized in Table 18 and Figures 17 to 20. The results indicate that the 90 d median lethal concentration values for the three test species were all approximately 25% of their 96 h LC_{50} values. In all four chronic toxicity experiments, the dose-mortality relationship was non-linear.

In the lake chub experiment, cumulative mortality was highest at 3% concentration. No mortality occurred at the highest test concentration (25%). For white sucker, mortality was initially highest in the median concentration range (8 to 12.5%); however, cumulative mortalities were similar for all the concentrations after 8 wk exposure. In the first rainbow trout test (Experiment No. 1), cumulative mortality was highest at the 4% concentration and lowest at 2%. Under similar experimental conditions, rainbow trout in Experiment No. 2 also had their highest mortality at 4%, but their lowest was at 12.5% concentration.

Table 17. A summary of the concentration of mine depressurization groundwater, number and species of fish used in replicates A and B, test durations, size and source of fish tested in the chronic toxicity bioassays.

	Rainbow Trout (Experiment #1)		Rainbow Trout (Experiment #2)		Lake Chub		White Sucker	
Duration:	90 d (24 Oct 1978 to 21 Jan 1979)		90 d (8 Nov 1978 to 5 Feb 1979)		90 d (21 Sept 1978 to 19 Dec 1979)		54 d (13 Sept 1978 to 6 Nov 1978)	
Source:	Spring Creek Hatchery, Montana		Spring Creek Hatchery, Montana		Nose Creek, Calgary		Nose Creek, Calgary	
Mean Length: (Before Bioassay)	6.33 cm (n = 36)		--		--		5.1 cm (n = 91)	
Mean Weight: (Before Bioassay)	2.27 g (n = 36)		--		--		1.2 g (n = 91)	
Groundwater:	Conc. (%)	No. of Fish	Conc. (%)	No. of Fish	Conc. (%)	No. of Fish	Conc. (%)	No. of Fish
	12.5 A	10	12.5 A	9	25.0 A	15	25.0 A	17
	12.5 B	11	12.5 B	9	25.0 B	16	25.0 B	10
	10.0 A	10	10.0 A	8	12.5 A	19	12.5 A	15
	10.0 B	9	10.0 B	8	12.5 B	19	12.5 B	13

continued ...

Table 17. Concluded.

Groundwater:	Rainbow Trout (Experiment #1)		Rainbow Trout (Experiment #2)		Lake Chub		White Sucker	
	Conc. (%)	No. of Fish	Conc. (%)	No. of Fish	Conc. (%)	No. of Fish	Conc. (%)	No. of Fish
	8.0 A	10	8.0 A	8	8.0 A	18	8.0 A	15
	8.0 B	10	8.0 B	8	8.0 B	25	8.0 B	17
	4.0 A	8	4.0 A	9	6.0 A	17	3.0 A	22
	4.0 B	11	4.0 B	10	6.0 B	19	3.0 B	15
	2.0 A	11	2.0 A	8	3.0 A	15	2.0 A	16
	2.0 B	9	2.0 B	9	3.0 B	19	2.0 B	15
	Control A	8	Control A	9	Control A	14	Control A	20
	Control B	10	Control B	8	Control B	16	Control B	17

Table 18. Median lethal concentrations (%) of mine depressurization groundwater for rainbow trout, lake chub, and white sucker in 90 d semi-static chronic toxicity bioassays.

Species	LC_{50} S.E.
Rainbow trout (Experiment No. 1)	8.5 ± 1.0
Rainbow trout (Experiment No. 2)	9.0 ± 1.3
Lake chub	13.2 ± 1.2
White sucker	5.8 ± 1.3

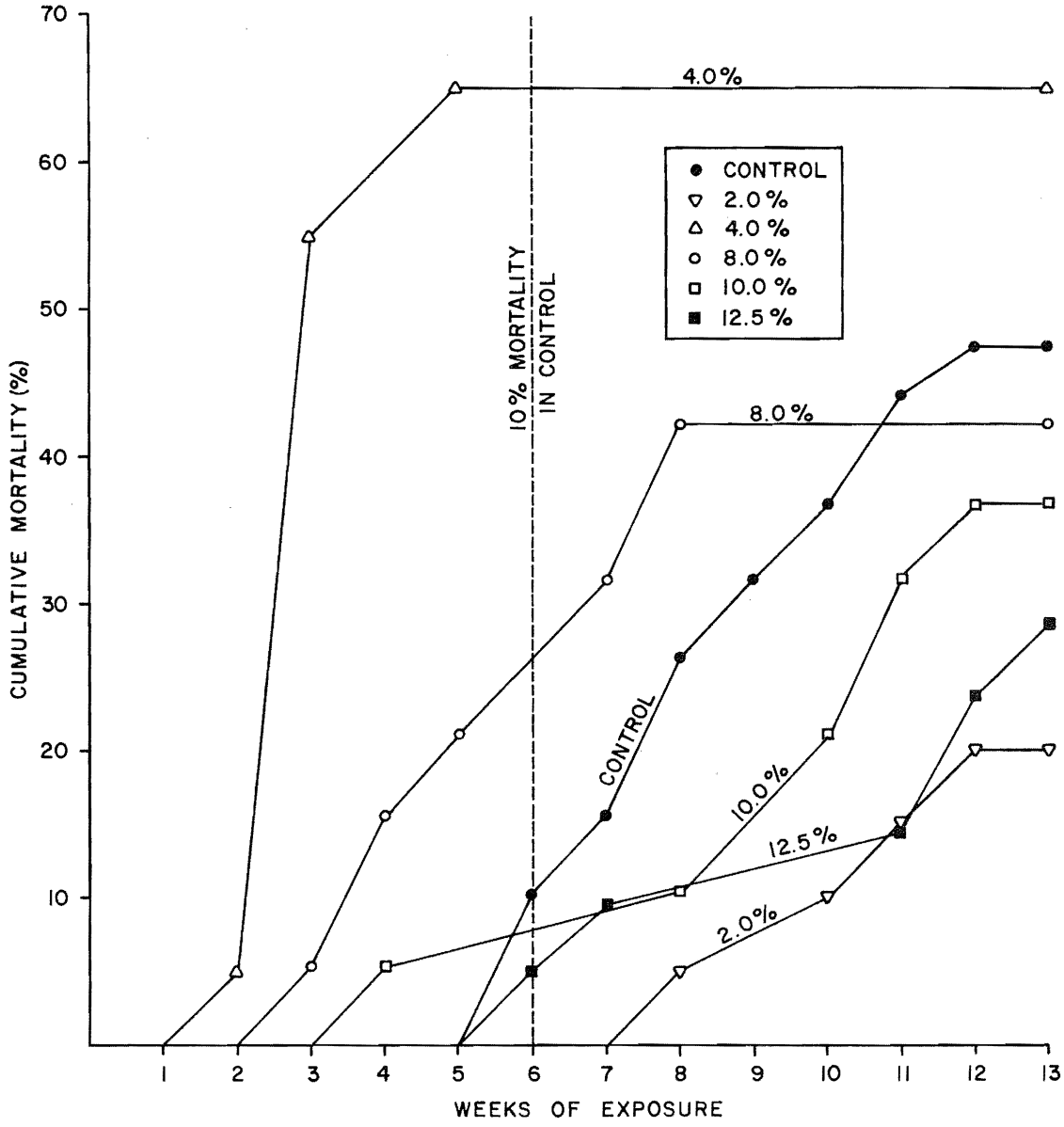

Figure 17. Cumulative percent mortality of rainbow trout (Experiment No. 1) after 13 wk of exposure to various concentrations of mine depressurization groundwater.

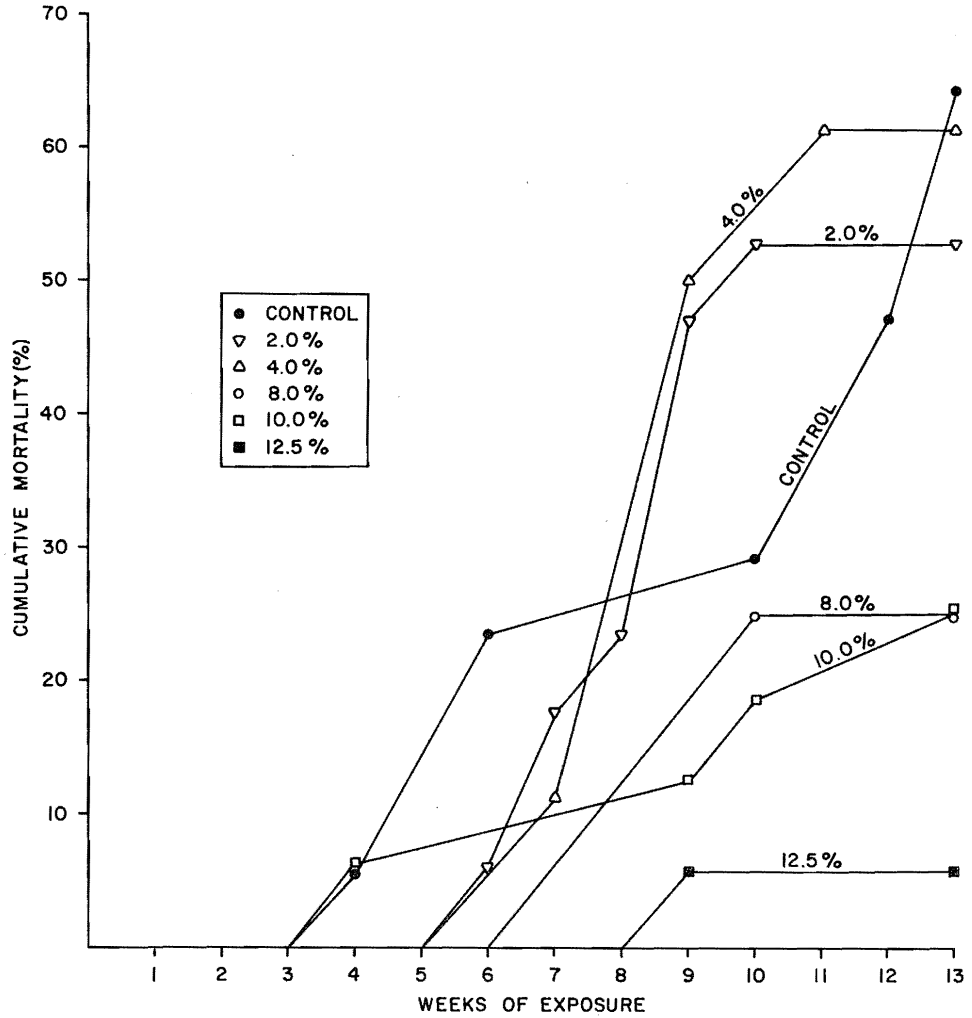

Figure 18. Cumulative percent mortality of rainbow trout (Experiment No. 2) after 13 wk of exposure to various concentrations of mine depressurization groundwater.

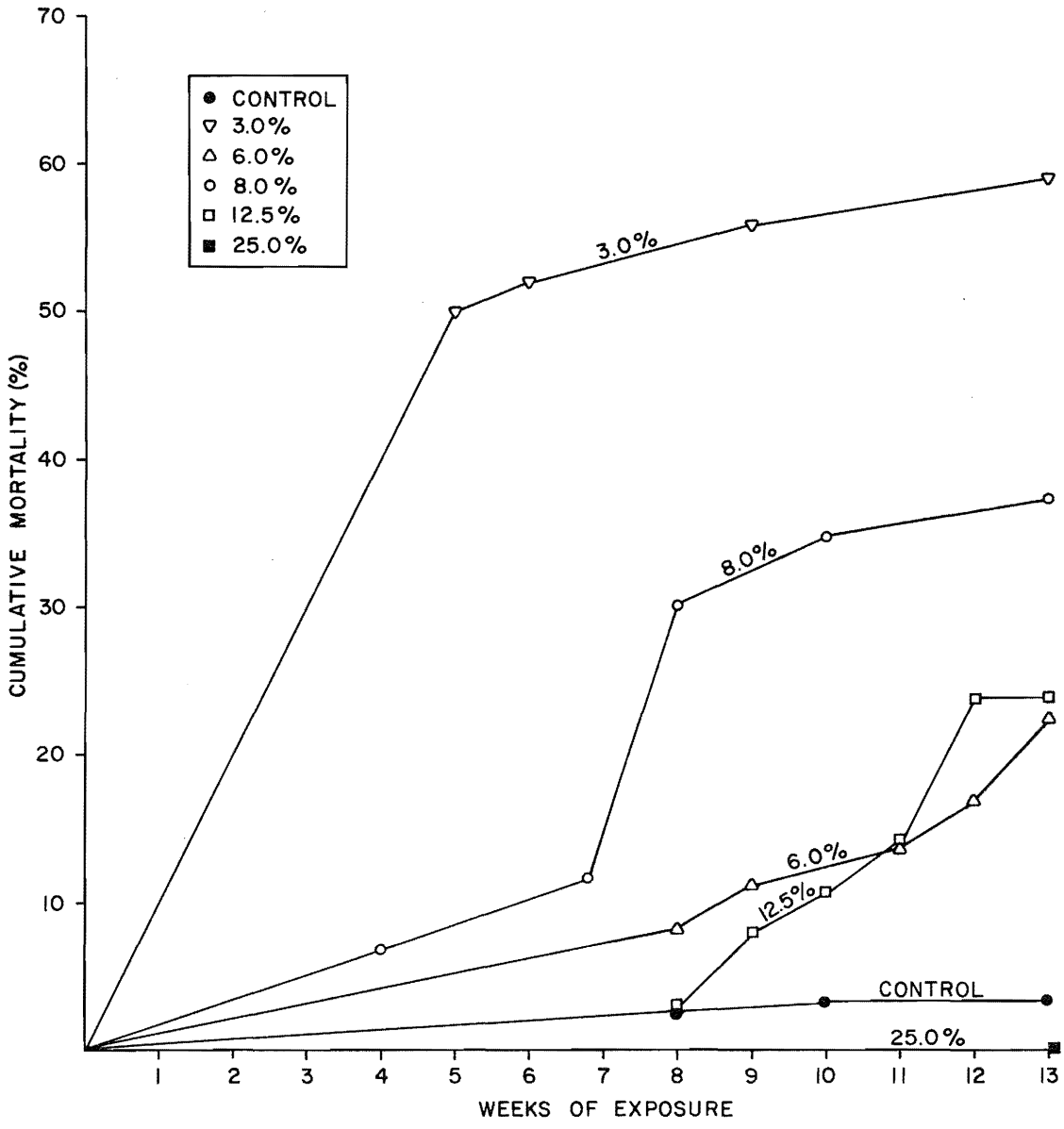

Figure 19. Cumulative percent mortality of lake chub after 13 wk of exposure to various concentrations of mine depressurization groundwater.

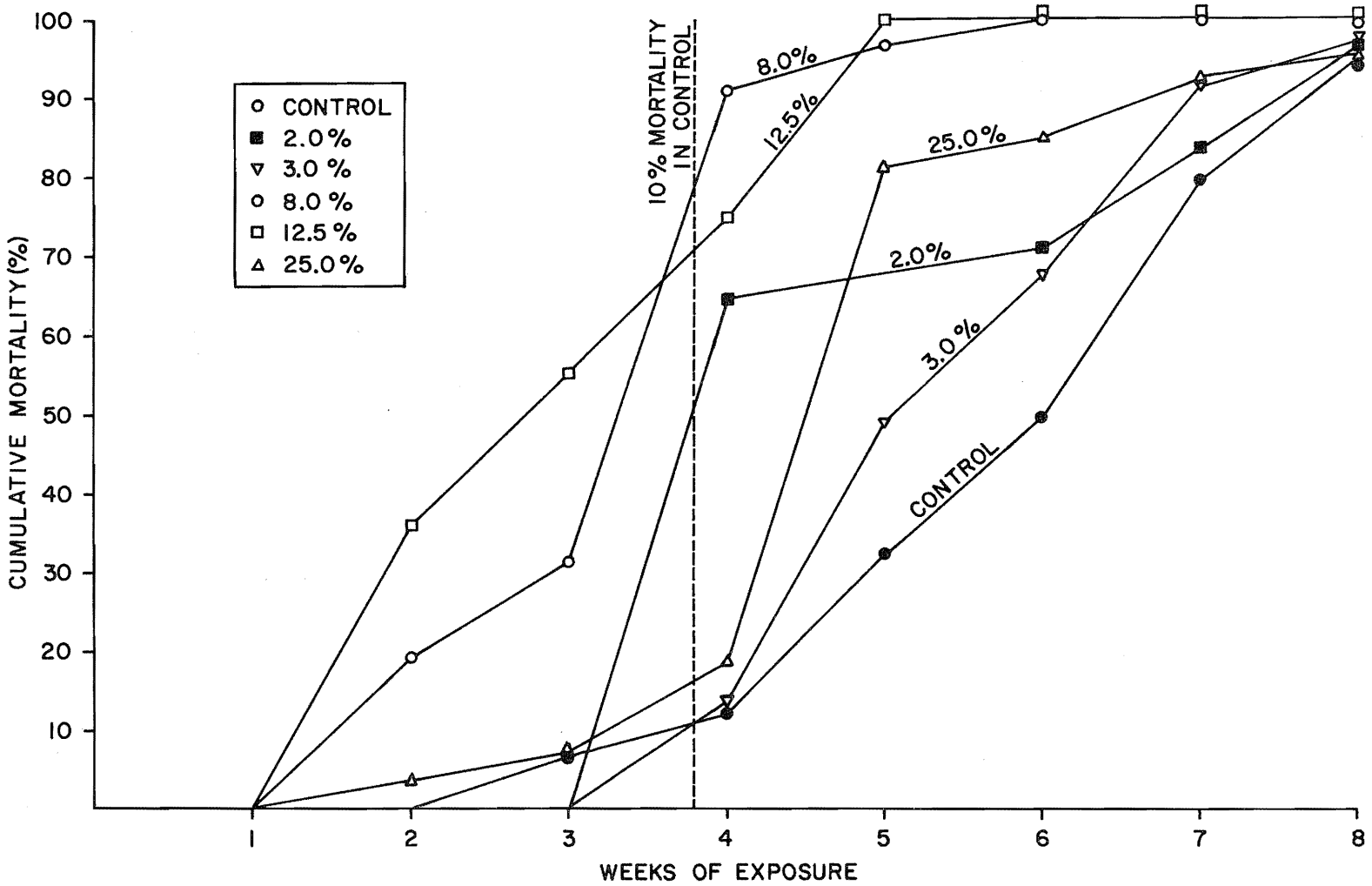

Figure 20. Cumulative percent mortality of white sucker after 8 wk of exposure to various concentrations of mine depressurization groundwater.

5.2.3 Discussion

In both the rainbow trout experiments and the lake chub experiment, toxicity of the mine depressurization groundwater appeared greatest at the 3 to 4% range. Toxicity also appeared to be suppressed at either end of the concentration scale. Similarly, in the initial stages of the white sucker experiment (2 to 3 wk exposure), toxicity of the groundwater was greatest in the 8 to 12.5% range. These results seem to indicate that, at a certain range of concentration, components of groundwater may interact to increase its toxicity. Below or beyond this particular concentration range, the components of groundwater either do not interact or may have an infra-additive or antagonistic interaction (Warren 1971) which decreases toxicity.

5.3 SUBLETHAL EFFECTS

5.3.1 Opercular Frequency

5.3.1.1 Rationale. The number of opercular movements per minute (f_{op}) may be quickly and easily monitored on even small fishes by simply counting the movements of the operculum over a known time period. Since the ventilatory pump of fishes does not operate at fixed volume, the opercular frequency does not allow accurate quantification of either ventilation volume (the amount of water pumped per unit time) or O_2 uptake (the amount of O_2 removed per unit time). Nonetheless, the measurements do allow the following qualitative assessments:

1. Metabolic O_2 demand; i.e., opercular frequency increases with an increase in the animal's requirement for O_2;
2. Efficiency of O_2 uptake; i.e., opercular frequency increases with decrease of blood O_2 or increase of blood CO_2 levels; thus, factors which cause disruption of either gill gas exchange of blood O_2 transport will create an increase in opercular frequency; and

3. Qualitative assessment of the initial levels of "stress", "disturbance" or "irritation" caused by the testing; i.e., these influences increase activity which results in elevation of opercular frequency.

Although residual O_2 bioassays have been used with some degree of success, opercular frequency is particularly relevant to toxicological studies because it is the only indicator of "respiration" that can be used economically on the very small fishes preferred for toxicological testing. The direct observation method requires no apparatus, can be used on fishes within an existing group and in the same container, and, most important, requires no disturbance, restraint, or handling of the fishes under test.

5.3.1.2 Methods. Opercular frequencies were determined by visual counting on five fish, in control water and at each concentration of groundwater. Operators were not screened from the fish, but operator movements were minimized to avoid disturbing the fish. At each count, the number of opercular beats was determined for at least two 30 s periods for each fish. Readings were taken only from fish showing minimal locomotor activity. Results from fish that became active during the counting period were discarded. For trout, considered the most sensitive species of the three, readings were taken immediately following introduction to test conditions, after 4, 24, 48, and 96 h, and each week thereafter up to 13 wk. All other species were assessed weekly during the course of the chronic toxicity experiment.

5.3.1.3 Results. During the acute toxicity test (96 h), measurements of opercular beats were made on rainbow trout exposed to 5 to 100% concentrations of stored groundwater. Precipitation during storage (see Section 3) had caused a reduction in salinity of the groundwater to a concentration, expressed in Na ion equivalents, only 30% of the original level. Because no deaths were recorded during the 96 h period, this result can be recorded as an acute sublethal exposure.

Changes in the frequency of opercular pumping are summarized in Figures 21 and 22. Disturbance and handling caused elevated frequencies immediately after the animals had been introduced into the water. This was evident even in the control water (Figure 21). Significantly greater ($p < 0.05$, Duncan's New Multiple Range Test) increases, however, were noted at all test concentrations, indicating that all concentrations of groundwater were sensed by the fish and may have induced increased water flow across the gills. This response was probably due to the increased O_2 consumptions resulting from mild avoidance. The increase was clearly dose-related (Figure 21) with higher concentrations causing a greater increase in frequency.

Four hours following transfer to the test solution, opercular frequencies had fallen significantly in all fish tested, presumably as they acclimated to the experimental conditions. Nonetheless, frequencies remained significantly elevated over control values in all concentrations of groundwater.

A further reduction in opercular frequency of all fish had occurred by 24 h exposure; but, with the exception of those fish held in 12.5% concentrations, all rates were still significantly over control values. At 48 h, differences between control and test groups were smaller, but all test levels were still higher than control and those in 50% concentrations were not significantly so. At 72 to 96 h, all values had decreased and no test concentration was significantly elevated over control values.

Three fish species, lake chub, white sucker, and rainbow trout, were selected for a study of opercular frequency during chronic (13 wk) exposure to sublethal concentrations of groundwater. Results for each species are presented both as weekly means of frequency for each concentration (Figure 23) and as grand means for all measurements at each concentration over the length of the exposure (Figure 24).

In general, chronic exposure to groundwater caused depression of opercular frequency, particularly at the higher levels tested. Experimental results for lake chub, white sucker, and rainbow trout are examined in turn in the following paragraphs.

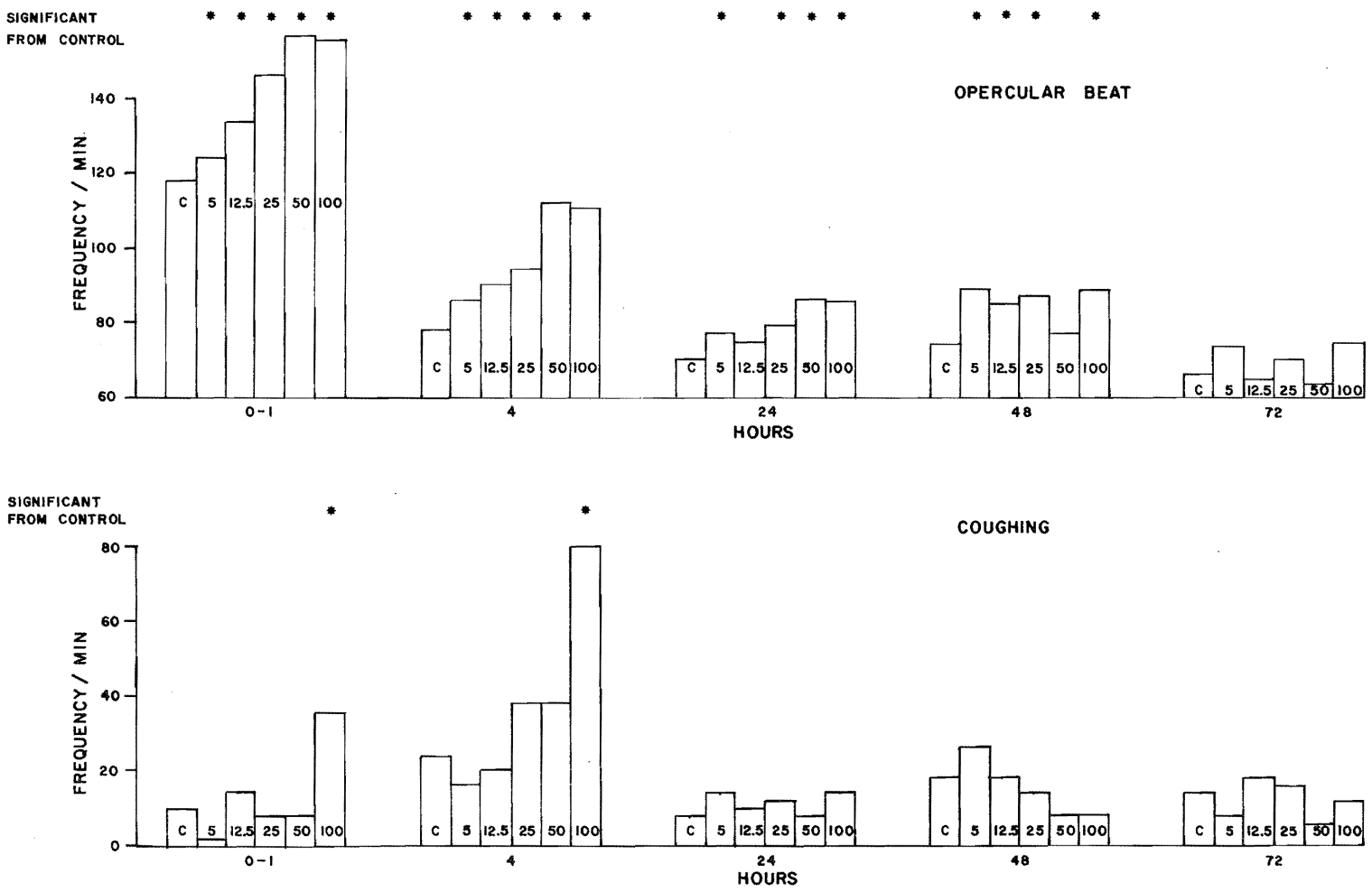

Figure 21. Opercular and coughing frequencies of rainbow trout during acute sublethal exposure to various concentrations of mine depressurization groundwater.

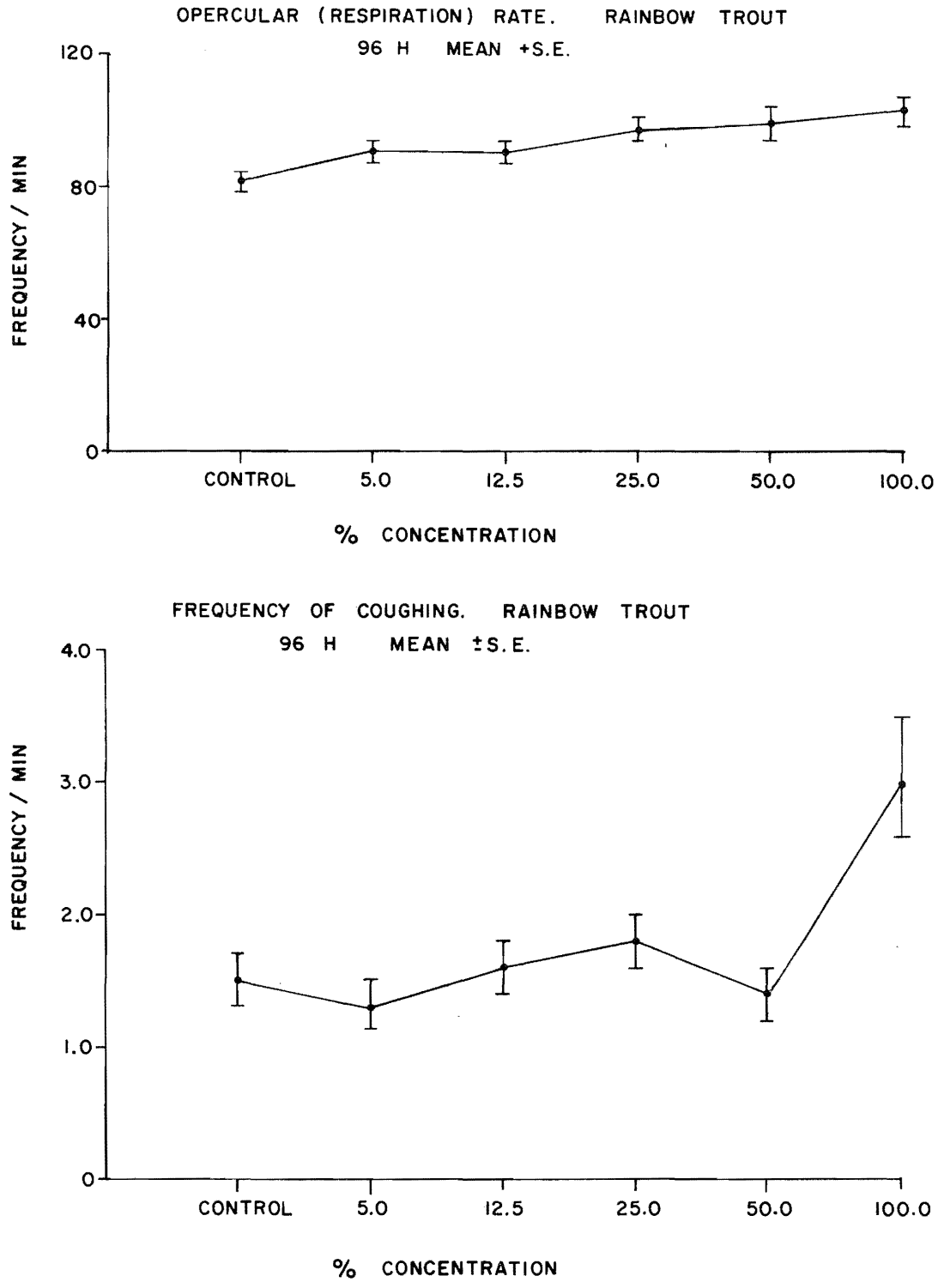

Figure 22. Opercular and coughing frequencies of rainbow trout at 96 h exposure to various concentrations of mine depressurization groundwater.

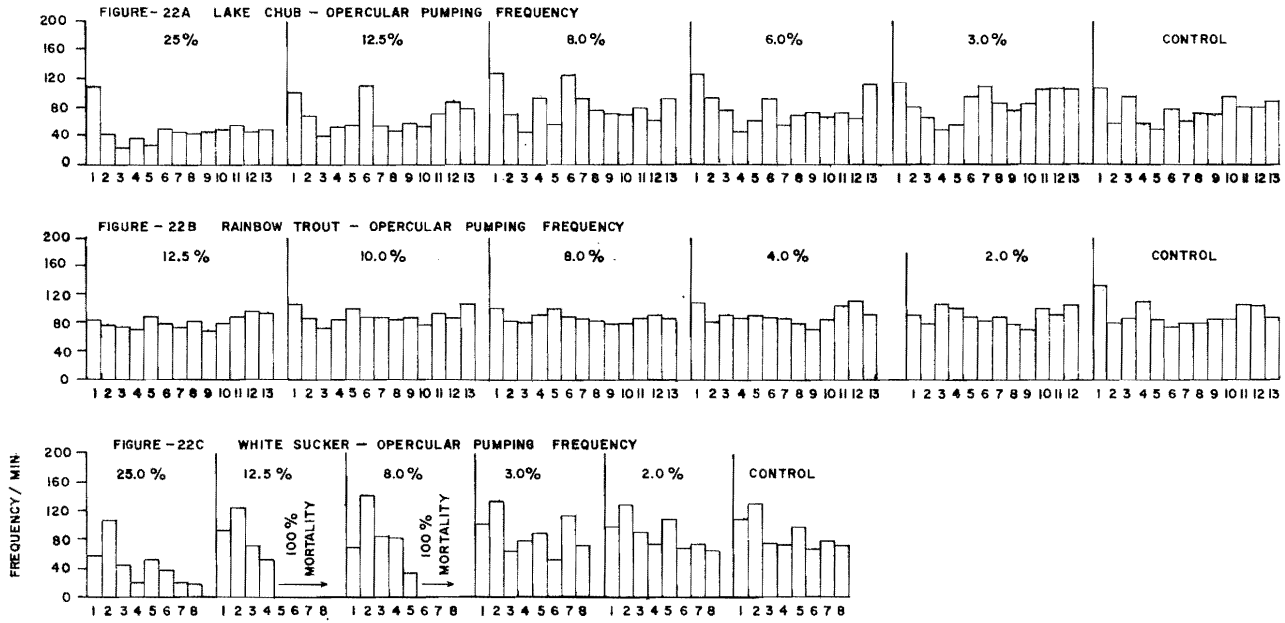

Figure 23. Weekly means of opercular and coughing frequencies of rainbow trout, lake chub, and white sucker during chronic (13 wk) exposure to sublethal concentrations of mine depressurization groundwater. (Continued)

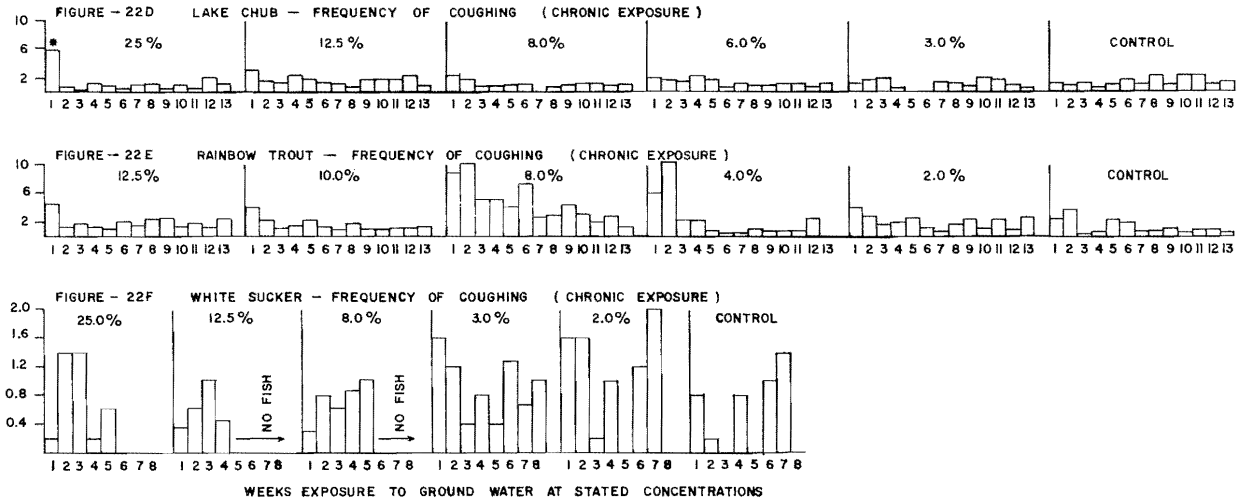

Figure 23. Concluded.

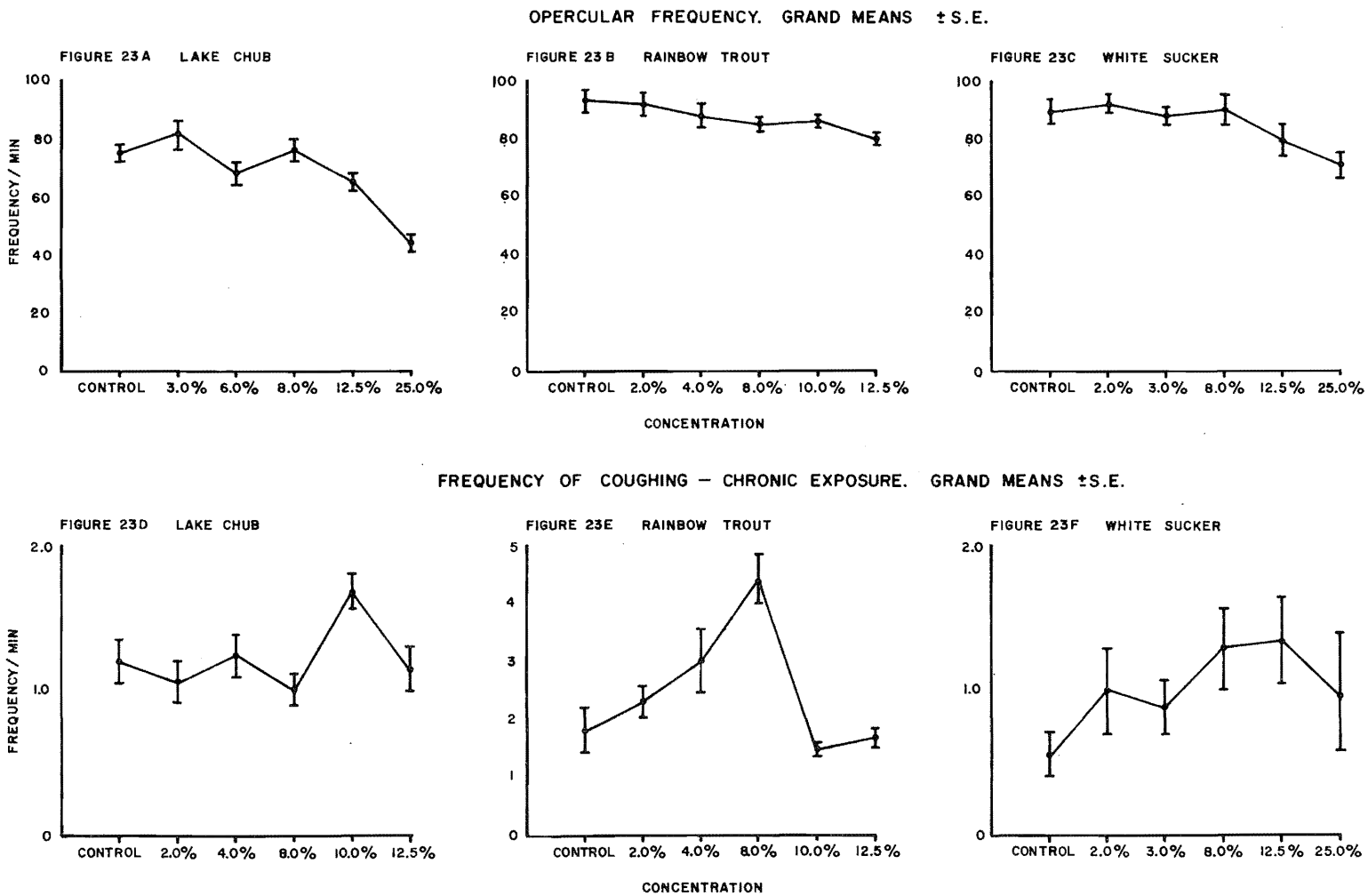

Figure 24. Grand means of opercular and coughing frequencies of rainbow trout, lake chub, and white sucker during chronic (13 week) exposure to sublethal concentrations of mine depressurization groundwater.

Lake chub were tested at concentrations ranging from 2 to 25% groundwater. During the course of the experiment, precipitation of ions occurred in the stored groundwater, reducing ionic levels up to 66% (measured as Na ion). In the first week of exposure, opercular frequencies were elevated in control and in all test concentrations. This effect may have been due to the residual effects of the disturbance noted for the short-term exposure of rainbow trout. However, following the initial increases, marked and progressive decreases in opercular frequency occurred at all test concentrations (Figure 23). Some reduction also occurred in control fish and was attributed to acclimation to experimental conditions. However, a significantly greater decrease, compared to control values, was observed for 11 of 13 wk in the chub exposed to 25% groundwater, and for 4 of 13 wk in chub exposed to 12.5% (Figure 23). Over the first 4 wk, this decrease could be ascertained in all test groups, but in the lower concentrations frequencies were rarely significantly below control levels. No significant difference was seen at these lower levels for the remainder of the 13 wk exposure. Expressed as a mean frequency over the entire 13 wk period (Figure 24), marked decreases in rates (35%) were seen only for animals exposed to 25% groundwater.

White suckers were tested over the full sublethal concentration range of 2 to 25%. In the first week, significant decreases in opercular frequency below control values were observed in the three highest concentrations, 8, 12.5, and 25% (Figure 23). In the second week, pronounced hyperventilation of unknown cause was observed. Following the second week, animals at 25% showed pronounced decreases in opercular frequency, which were significantly below control values for the remainder of the test. Because fish deaths occurring in these groups caused early termination of the experiments, no significant suppression can be calculated for fish at 8 or 12.5% concentrations. No significant difference was observed between control and 2 to 3% groups (Figure 24).

Rainbow trout were tested at groundwater concentrations ranging from 2 to 12.5%. In measurements taken during the first week of exposure, opercular frequency rates were elevated on in the control group and at the 2% level. At higher concentrations, significant

decreases below control levels were observed at all test concentrations above 4% (Figure 23). Following the first week, frequencies generally decreased in fish exposed to the higher concentrations. The decrease was rarely significantly different from control values for the balance of the 13 wk test. Examination of the grand means (Figure 24) demonstrated a slight but consistent dose-related decrease in opercular frequency. The decrease was not significant.

5.3.1.4 Discussion. Counts of opercular frequency have been used in the past to assess the respiratory effects of exposure to inorganic chemicals (Skidmore 1970), organic chemicals (Lunn et al. 1976), mixed chemical and particulate material in pulp mill effluent (Schaumberg et al. 1967), and, most recently, liquid effluent from a petroleum refinery (Sprague et al. 1978b). Increases in opercular frequency may be associated with:

1. Increases in O_2 demand caused by the disturbance or activity that may be involved in avoidance responses;
2. Decreases in O_2 supply that might result from the salination of water (i.e., displacement of dissolved O_2);
3. A decrease in O_2 transport across the gills caused by a thickening of the respiratory surface (see Section 5.3.5); or
4. A reduction in O_2 transport efficiency caused by poisoning of the animal's hemoglobin.

Although decreases in opercular frequency occur in all animals as they acclimate to their experimental regimes, a decrease to below control values is observed less often (Marchetti 1962) and presumably involves depression of respiration either by depression of the central nervous system (particularly the respiratory generating and control centres) or a general depression of metabolism.

In the present study, opercular frequency rates recorded for control rainbow trout at 15°C (91 ± 2 SE) were similar to those recorded for the same species and size of fish (94 ± 11) by Sprague et al. (1978b) using an electrical recording technique. Control opercular frequencies for lake chub and white sucker were slightly lower, and may have been associated with routine activity levels

lower than those of trout. No comparative figures could be found in the literature for these species.

Both increases and decreases in opercular frequency were observed in fish exposed to sublethal concentrations of groundwater. Initial increases (Figure 21) in control fish no doubt reflected a general rise in activity levels, resulting from disturbance. Where significant increases over control levels were observed, however, as they were in the first day of exposure for rainbow trout (Figure 21), avoidance activity or reduction of respiratory efficacy must have been involved.

The increase in opercular frequencies in trout exposed to saline groundwater was slightly larger than the increase exhibited by the same species exposed to 100% effluent from a petroleum refinery (Sprague et al. 1978b) and following exposure of trout exposed to lethal concentrations of Zn (Skidmore 1970). As suggested before, because many substances present in groundwater can cause increases in opercular frequency (eg., Na^+, Cu^{++}, Zn^{++}, NH_3, NH_4^+, phenols, etc.), it is virtually impossible to single out one or more likely constituents. That this response was observed within 1 h in concentrations as low as 5% groundwater indicates the sensitivity of this measurement for detection of sublethal effects. However, as pointed out by Sprague et al. (1978b) and others, these measurements are influenced slightly by low level pollution but grossly by activity. As the activity may not be related to the test substance in use, this effect often seriously reduces the ability of this method to discriminate between low-level concentrations. Utilization of animals only at rest, or at controlled activity levels, may improve the discrimination ability of this test.

The increased opercular frequencies observed in the first hours of exposure were not maintained, but declined rapidly (Figure 21) and, by the end of a 96 h exposure, were not significantly different from control values. As a result, this response is primarily important in the first 1 to 2 d exposure. Consquently, although some increase in opercular frequency was observed within the first week of the chronic toxicity tests, no further increases were apparent

after the second week. Consequently, increases in opercular frequency provide a sensitive basis for testing sublethal groundwater effects only within the first 24 to 48 h exposure.

The above cannot be construed as implying opercular rate changes do not result from chronic exposure to groundwater: they do. But the response is not elevation, but suppression of opercular rates. As discussed above, decreases may involve depression of either the respiratory centres or of metabolism. Decreases in opercular frequency in response to chemicals has been previously reported in the literature, though infrequently (Marchetti 1962). Adelman et al. (1976) reported respiratory rate depression in goldfish and flathead minnows prior to death caused by lethal levels of Na salts, and exposure to 19 µL/L benzene caused respiratory depression in fish (Meyerhoff 1975). Both authors postulated central nervous depression, and both substances were found in groundwater samples. Since the composition of groundwater is extremely complex, variable, and poorly quantified, much more testing would be needed to isolate the active substance or substances.

5.3.2 Coughing Frequency

5.3.2.1 Rationale. Coughing in fishes is a rapid, complex series of high-amplitude movements of the buccal floor and operculae, which apparently causes a momentary rapid reversal of water flow across the gills (Hughes and Shelton 1958). Since coughing increases with the presence of particulate matter in the ambient water, this action may be used to clear the gills. Other substances are also active in elevated coughing rates, including heavy metals such as Zn (Skidmore 1970), DDT (Schaumburg et al. 1967), and other pesticides (Lunn et al. 1976). Because coughing rates also increase in many species of fish in response to O_2 depletion, either in blood or in ambient water, they may provide a rough index of gill gas exchange efficiency, as well as gill irritation.

Coughs can be easily counted, even in small fishes. The "coughing pattern" is distinctive. With practice, it can be distinguished by its rapid spasmodic nature from the slower maximal distension of the buccal apparatus termed "yawning". The response develops rapidly at very low concentrations, providing a potential measure for assessment of sublethal effects of water-borne pollutants.

5.3.2.2 Methods. Coughing frequencies were counted throughout the chronic toxicity tests under the same conditions and using the same techniques as those already described for opercular frequency measurements (Section 5.3.1.2).

5.3.2.3 Results. During the preliminary acute toxicity test (96 h), counts of coughing frequency were made on rainbow trout exposed to 5 to 100% concentrations of stored groundwater. An account of the effects of storage on groundwater concentrations is given in Section 5.3.1.3. No deaths were recorded during the 96 h test.

The frequency of coughing was elevated significantly over control values (Figure 21) for the 4 h following introduction into the highest concentrations used (100%). After the initial 4 h period, coughing rates varied between fish and between readings on the same fish, but no significant differences were observed over the 96 h test period.

During the chronic (13 wk) exposure to sublethal concentrations of groundwater, coughing frequencies were determined on the same samples of fish described for opercular frequency testing (Section 5.3.1.3). Data were recorded for lake chub, white sucker, and rainbow trout. The results are presented as histograms showing weekly mean frequency at each concentration (Figure 23) plus grand means of all results at each concentration for the entire 8 to 13 wk test period (Figure 24).

In general, coughing frequencies were increased by the higher sublethal concentrations of groundwater, but the fish apparently adapted quickly and the response was rarely significant after 2 wk exposure. Detailed results for lake chub, white sucker, and rainbow trout are discussed in turn in the following paragraphs.

Lake chub had generally low coughing frequencies. Usually only 1 to 2 min^{-1} coughing movements were observed in this species. No change in coughing frequency was observed in control fish for the duration of the 13 wk chronic toxicity test. Increased frequency of coughing, probably indicating irritation of the gills, could be seen in the first week of testing at the two highest concentrations used, 12.5 and 25% (Figure 23), but the increase was significant only at the 25% concentration. The amalgamated grand means (Figure 24) confirm that coughing frequency did not differ significantly in any group after the first week, but may suggest a slight overall increase in frequency of coughing at 12.5%.

White sucker coughing frequencies were lower (Figures 23 and 24) than the other two species. Frequencies greater than 3 min^{-1} were rarely observed. A complex response was seen in the first week of exposure. Animals exposed to 2 and 3% groundwater showed an increased coughing frequency over control fish; however, the higher test groups, 8, 12, and 25% showed suppression of the response. In the second week, elevated coughing frequencies were observed at all groundwater concentrations. In subsequent weeks, considerable fluctuation was observed in all groups, perhaps due to disease apparent in this group at this time.

Rainbow trout had slightly higher frequencies (1 to 10 min^{-1}) as may be expected from this more sensitive species (McMahon et al. 1976). Highest coughing rates were observed in all groups at the start of the test but, unlike those recorded for lake chub, increases in trout persisted for 2 wk. All test groups of fish had greater frequency of coughing than control fish in the first week of testing, but these differences were only significant for trout at 4 and 8% concentrations. Greater frequencies of coughing persisted into the second week for trout exposed to 8% concentrations and for 6 wk for trout at 4% concentrations, but the response was apparently suppressed at the higher concentrations (Figure 23). Examination of the amalgamated grand means (Figure 24), showed a marked trend to increased coughing rates at the lower concentrations of groundwater, but a marked suppression at higher levels (10 and 25%). Note that this suppression

of coughing frequency was associated with some slight suppression of opercular frequency at these higher concentrations (Section 5.3.1.3).

5.3.2.4 Discussion. Increase in coughing frequency is associated with the presence of particulate matter in the ambient water (Schaumberg et al. 1967, Hughes 1975) and also with the presence of DDT (Schaumberg et al. 1967), Zn (Skidmore 1970), Cu (Drummond et al. 1973), Hg (Drummond et al. 1974), dieldren (Lunn et al. 1976), and soluble oil fractions (Thomas and Rice 1975, Sprague et al. 1978b). These effects may be associated with irritation or clogging of the gill surface, causing fish to reflux water violently across the gills (Hughes and Shelton 1958), but are also seen in some fishes in response to asphyxia (low O_2 associated with high CO_2)(McMahon unpublished data). Consequently, increased coughing frequencies may result from gill irritation or damage, or from O_2 depletion as a result of gill irritation or damage.

Coughing frequencies observed in control trout (1 to 2 min^{-1}) were similar to those recorded by others for this species (Sprague et al. 1978b). Rates in lake chub and white sucker were lower, but no comparable results were found in the literature.

Increases in coughing rates in trout, even on first exposure, were slightly lower than those in response to effluent from a pulp mill (Schaumburg et al. 1967) or from an oil refinery (Sprague et al. 1978b). These latter reports, however, concern exposure to higher concentrations of effluent. In the present study, elevated coughing levels were most readily apparent in the first few hours of exposure. It is possible that a general respiratory depressant effect of groundwater may have depressed the coughing response, causing its early attenuation. Because the fish apparently adapt quickly, this response, like elevation of opercular rate, may be best used early in the exposure period.

The value of the coughing response as a tool for rapid assessment of pollutant activity, both at lethal and sublethal concentrations, has been recently discussed by Sprague et al. (1978b). Coughing seems to be an indicator of gill irritation or fouling, or

possibly of more general stress (Sprague et al. 1978b), and might provide a rapid method of assessing the effects of a wide range of chemicals. Since coughing frequencies can be readily monitored by observation, using inexpensive apparatus, they could be useful in providing a test method for predicting lethal groundwater levels. Also possible is their use in assessing sublethal effects. The rapid attenuation of the coughing response, however, may argue against its use as a tool for assessing "no effect" or "safe" levels of groundwater concentration.

5.3.3 Changes in Hematocrit

5.3.3.1 Rationale. Changes in hematological properties have not often been used in toxicological studies, even though they may be of considerable importance (Hughes 1976). One such property is the Hematocrit (Hct), or volume of red blood cells (RBC) expressed as a percentage of plasma volume. Increase in the Hct of fish blood was associated with capture and other stresses by Fletcher (1975). The effect is probably due, not so much to destruction of the RBC's, but to swelling of the RBC's caused by changes in O_2 or CO_2 levels, blood osmolarity, or ionic concentration. Change in Hct values could also be associated with exposure to hypersaline waters, which causes imbalance of blood ionic levels, and to pollutants such as heavy metals, which may cause destruction of the RBC's. As the groundwater under test is highly saline and contains moderate levels of heavy metal ions, Hct levels were measured in control and test fish at the end of each experiment.

5.3.3.2 Methods. A drop of blood, produced by incision of the exposed heart ventricle, was taken from individual fish and introduced by capillary action into clean heparinized (ammonium heparin) hematocrit tubes. With practice, a sufficient amount (50 to 100 µL) of blood could be obtained from these small fish. Occasionally, when hemolysis occurred in the tubes, samples were discarded. Blood samples were stored on ice (above $0^{\circ}C$) for several hours before being

centrifuged in an International HNG equipped with a hematocrit head. Samples were spun for 5 min at approximately 3000 G to separate and pack the RBC's. Readings were expressed as percent of total volume occupied by RBC's. No correction was made for the minute volume of plasma trapped between the packed RBC's.

5.3.3.3 Results. Hematocrit was measured in the blood of trout exposed for 96 h to concentrations of groundwater ranging from 5 to 100%. Results of this short-term, sublethal exposure are shown in Table 19. The highest mean value was observed in fish exposed to 12.5% and the lowest from fish exposed to 100% groundwater. The differences were not significant.

Mean percent Hct values were also calculated for lake chub, white sucker, and rainbow trout exposed to sublethal concentrations of groundwater for 13 wk. Values for both control species and species exposed to concentrations of groundwater ranging from 2 to 25% are presented in Table 20. In all chronic exposures, Hct was increased over control levels by exposure to groundwater with concentrations greater than 2%. No significant difference ($p > 0.05$; Student's Test) was found between the control values for the species studied. Hct values for control trout and other fish were higher than those reported for trout (or other fish) in the literature (Wood et al. 1979), but values for juvenile trout have not previously been determined and may be typically higher.

5.3.3.4 Discussion. Hematocrit values for all fishes exposed to groundwater concentrations above 2% showed values elevated over control levels. The degree of elevation was least in trout, but it must be remembered that these animals were exposed to groundwater which had lost up to 70% of its ionic content due to precipitation in storage. Though the lack of information on the effects of pollutants on hematological parameters, combined with the complex chemical composition of the groundwater used in this study, rules out attributing this effect to any specific constituent, two general effects may have been involved:

Table 19. Hematocrit values form juvenile rainbow trout exposed for 4 d (96 h) to stated concentrations of saline groundwater ($\bar{x} \pm SD$).

Concentration %	0 (Control)	5	12.5	25	50	100
$\bar{x}$ Hct SD	38.7 ± 4	36.0 ± 4	40.5 ± 3	35.9 ± 3	36.3 ± 3	32.6 ± 5
(n)	(7)	(5)	(4)	(9)	(4)	(5)

Table 20. Hematocrit values of fish after 13 wk exposure to various concentrations of saline groundwater.

% Concentration	Rainbow Trout (RT_2)	Rainbow Trout (Combined)	Lake Chub	White Sucker
2	37 ± 3[a] (12)	36.1 ± 3.6[a] (22)	-	30 ± 6 (7)
3	-	-	41.9 ± 1.7[a] (8)	42 ± 7[a] (5)
4	41.5 ± 4[a] (6)	37.4 ± 5.4[a] (13)	-	-
6	-	-	39.6 ± 4[a]	-
8	40.2 ± 3[a] (10)	38.16 ± 3.2[a] (18)	38.1 ± 6 (13)	41.8 ± 2[a] (8)
10	39.7 ± 6[a] (11)	37.8 ± [a] (21)	- -	- -
12.5	39.1 ± 4[a] (16)	38.11 ± 3.8[a] (26)	37.3 ± 5 (9)	-
25	-	-	40.3 ± 5[a] (16)	41.5 ± 14[a] (6)
Control	34.3 ± 2 (8)	34.6 ± 3.4 (16)	36.6 ± 2.7 (9)	33.2 ± 5 (9)

[a] $p < 0.05$.

1. Treatment with saline groundwater causes imbalance in blood ionic levels. It is not known whether these increases occur largely in the RBC or plasma fraction, but owing to the complex interactions which occur between RBC and plasma ionic pools, ionic imbalance across the RBC membrane might easily result. This imbalance would cause osmotic imbalance across the RBC membrane with consequent swelling of the RBC and a corresponding increase in Hct.

2. Changes in O_2 and CO_2 levels in the blood, possibly resulting from reduced opercular activity, can also cause ionic and osmotic imbalance across the RBC membrane, causing swelling of the RBC and an apparent rise in Hct. Long-term reductions in blood O_2 levels can stimulate erythropoiesis in fish (Wood and Johansen 1972). Increase in gill mucus production did occur in fish at all groundwater concentrations (see Section 5.3.5.3) and could have caused some reduction in O_2 transfer across the gills. This reduction, combined with the depressed ventilation, may have produced some hypoxia in the blood of the fish and caused a slight stimulation of RBC production in compensation.

Because Hct levels vary even in control fish, differences as small as those observed in the present study can rarely be demonstrated to be significant. This problem, combined with the difficulty of obtaining blood from these small fishes, may limit the use of this parameter for sublethal assessment.

5.3.4 Ion Accumulation

5.3.4.1 Rationale. High salinity is an important consideration in toxicological testing as it has both direct and indirect effects on assays. Direct effects of salinity are illustrated by the work of Bryan (1976) who clearly demonstrated increased survival time at higher salinities in the worm *Nereis diversicolor* exposed to 1 ppm $CuNO_3$. Other authors (Mount 1968; Pagenkopf et al. 1974) have demonstrated decrease in the toxicity of heavy metals to fish with increasing hardness, alkalinity, or $HCO_3^-/CO_3^=$ ion concentrations in fresh water. As well, salinity itself may be toxic, and combine with other toxicants to create additive effects. Direct effects will vary with the osmoregularity ability of the fish. McMahon et al.(1976), for example, showed saline-enhanced toxicity in whitefish but not in trout exposed to groundwater.

Even though exposure to high salinity may not be directly toxic, it may nonetheless influence toxicity of other constituents indirectly. Increased salinity may alter the physiological condition of the fish, increasing the energy cost of regulating its internal environment, and thereby increasing the potential toxicity of other constituents that may be present. For these reasons, levels of Na^+, K^+, Ca^{++}, and Mg^{++} were measured in the blood, gill, and liver tissues of fishes at the end of each chronic toxicity test.

Moderate levels of heavy metals are found in the groundwater used in this study. Though these heavy metals rarely occur in amounts sufficient to cause acute toxicity, even small amounts may be biologically important. During chronic exposure, for example, low levels of heavy metals and other toxicants may create important behavioural and reproductive effects. Further, many heavy metals interact complexly with other metals or organic constituents to compound toxic effects (Eaton 1973; Calamari and Marchetti 1973). Finally, many fishes concentrate toxicants within their body tissues where heavy metal accumulation could eventually reach lethal levels.

The present study attempted to assess the buildup of two representative heavy metals, Cu and Zn, in the blood, gill, and liver tissues of fishes after chronic exposure to different concentrations of groundwater. An attempt was also made to assess the buildup of two other metal ions, Pb and V, but the small size of the tissue samples prevented precise analyses of these metals.

5.3.4.2 Methods. Blood, gill, and liver samples were taken from fishes following chronic exposure to different concentrations of groundwater. The groundwater was moderately to highly saline. Major contributing ions were Na^+ and Cl^-. Substantial contributions were also made by HCO_3^- and $CO_3^=$ ions, giving the water a high hardness and alkalinity. Small, but environmentally important, levels of a range of heavy metal ions were also involved (Table 1), as were many organic compounds.

Whole blood concentrations of Na, K, Pb, Cu, Zn, and V were determined. Blood samples were diluted in 50 volumes of double glass-distilled water. Haemolysis of RBC's occurred immediately after water was added to the blood. The diluted samples were centrifuged twice at 3000 G for 15 min to remove RBC membranes. The supernatant from each sample was placed in a separate nalgene container and frozen until required for ion analysis. Calcium and Mg concentrations were measured only in the white sucker.

Concentrations of Pb, Zn, V, and Cu were determined in gill filaments and liver tissue. Gill filament and liver sample wet weights were recorded and the tissues placed in a glass-teflon tissue homogenizer. Each tissue was homogenized by hand in 50 volumes of double glass-distilled water. The homogenate was spun three times for 15 min at 3000 G. The final supernatant was used in the ion assay. All metal associated with tissue protein may not have been removed in the extraction because acid extraction was not used. Consequently, values obtained for tissue ion concentrations were relative and not absolute. The supernatant was stored frozen.

Ion concentrations were determined using a Jarrel Ash Model 850 Atomic Absorption Spectrophotometer. Sodium, K, Mg, Ca, Cu, Zn, and Pb concentrations were measured using an air/acetylene flame. Vanadium concentrations were determined using a nitrous oxide/ acetylene flame. Potassium was not added to suppress ionization when measuring V, Ca, and Na because the K concentration in the samples was already greater than 0.1%. A concentration of 0.1% $LaCl_3$ was added to samples during the measurement of Ca and Mg to suppress ionization interference caused by the air/acetylene flame.

Concentrations of Mg, Ca, Cu, Zn, Pb, and V were determined from the supernatant obtained during sample preparation. (The dilution of the original tissue was 1:50.) Before Na and K concentrations were measured, the supernatant was further diluted in 40 volumes of double glass-distilled water. Total dilution was 1:2000.

In order to obtain sufficient sample volume for the measurement of ions, it was necessary to pool tissue and blood samples from each group. To measure all ions, a minimum sample size of 10 mL was required, 1.5 mL for each ion. The sample was aspirated through the flame at a rate of 5 mL/min. A 10 s aspiration was required for flame equilibration. Three absorbance values were recorded for each sample, each value being the absorbance integrated over a 4 s period.

5.3.4.3 Results. Levels of the major components of groundwater decreased two to threefold (to 30% original concentration) during the course of the experiment. (See Table 1 for detailed analysis.) In the early stages of the experiment, when lake chub and white sucker were under study, appreciable changes occurred in the groundwater ionic content during the actual assay period. For this reason, Na and K ionic content for each experimental dilution have been reported for both the beginning and the end of the test period (Table 21). All experiments on rainbow trout were conducted at the end of the storage period, when concentrations were lower but more stable. By the end of the experiment, levels of all ions, even in the highest groundwater concentrations, were markedly lower (Tables 21 and 22) than those normally found in the tissues of animals.

Table 21. Changes of sodium (Na), potassium (K), copper (Cu), and zinc (Zn) in tissues of lake chub and white sucker following chronic exposure to a series of mine depressurization groundwater dilutions.

	Groundwater				Blood					Gill		Liver	
	Na^+	K^+	Cu^{++}	Zn^{++}	Na^+	K^+	Cu^{++}	Zn^{++}		Cu^{++}	Zn^{++}	Cu^{++}	Zn^{++}
Lake Chub													
Control	212	0.4			1720	1550	28	9		10	13	18	22
3.0%	373(408)	1.06 (2.19)			1920	1450	20	11		-	-	-	-
6.0%	334(604)	1.73 (3.98)			1850	2000	26	9		12	15	21	20
8.0%	375(735)	2.17 (5.17)			1995	1375	36.5	9		9	17	23	25
12.5%	467(1029)	3.16 (7.85)			1820	2100	29	10		13	14	22	23
25.0%	722(1840)	5.93 (15.3)			2030	2350	33	10.3		14	19	26	29
White Sucker					Na^+	K^+	Ca^+	Mg^{++}	Zn^{++}				
Control	212	0.4			2143	1450	290	70	7.5	11	10		
2.0%	253(343)	0.84 (1.59)			2269	1110	290	53	9.5	16	11	7.5	20
3.0%	273(408)	0.06 (2.19)			2394	1150	280	80	12.5	15	15	12.5	28
8.0%	375(735)	2.17 (5.17)			2350	1410	325	50	10.0	15	10	7.5	20
12.5%	467(1029)	3.16 (7.85)			2430	1200	290	60	9.5	13	14	7.5	28
25.0%	722(1846)	(5.93)			2130	1600	290	80	9.5	18	14	7.5	28

Table 22. Changes of sodium (Na), potassium (K), copper (Cu), and zinc (Zn) in rainbow trout following acute and chronic exposure to sublethal concentrations of mine depressurization groundwater. All results are expressed in mg/L (ppm).

	In Test Water				Blood				Gill		Liver		
	Na^+	K^+	Cu^{++}	Zn^{++}	Na^+	K^+	Cu^{++}	Zn^{++}	Cu^{++}	Zn^{++}	Cu^{++}	Zn^{++}	
Rainbow trout 96 h:													
Control	212	0.4	na	0.003	2528	1091	6.4	14.1	14.0	7.0	25	21.0	
5%	306	0.88	na	0.008	2310	1153	8.9	16.9	18.0	7.8	26	26.5	n =
12.5%	448	1.6			2871	1285	6.9	12.5	16.0	7.5	32	26.8	10 P.U.
25%	684	2.8			2769	1291	7.3	14.1	19.0	10.0	29	30.7	per
50%	1206	5.2			2651	1237	8.2	14.6	22.0	10.0	31	30.9	sample
100%	2100	10	na	0.10	2819	1487	9.1	15.9	20.0	9.5	32	28.9	
Rainbow trout 90 d:													
Control	212	0.4	na	0.003	2542	1144	6.7	13.5	11.0	7.8	23	10.8	
2%	253	0.84			2758	1282	6.8	10.1	12.5	7.8	22	10.4	n =
4%	294	1.28			2639	1108	5.5	9.7	11.5	7.9	24	11.9	12 to 20
8%	375	2.17			2629	1110	5.4	14.5	15.0	8.5	27.5	16.7	ie., RT
10%	416	2.61			2689	1276	5.7	10.3	12.5	9.2	25	15.3	+ RT_2
12.5%	722	3.16	na	0.10	3015	1274	7.9	12.2	15.5	10.1	28.5	15.0	

In control trout, ionic concentrations of whole blood ranged from 2528 to 2542 mg/L^{-1} for Na; 1091 to 1144 mg/L^{-1} for K; 6.4 to 6.7 mg/L^{-1} for Cu; and 13.5 to 14.1 mg/L^{-1} for Zn. These levels were similar to those reported in the literature for this species (Skidmore 1970). Any small differences may have been due to differences in the environmental history, holding conditions, or larger size of Skidmore's fish. Values for Cu in gill tissue were similar to those of brook trout (McKim and Benoit 1974). Control levels of blood Na in lake chub and white sucker were lower than those in trout; levels of K and Cu were higher in chub than in trout (Tables 21 and 22). No comparable data for these species could be found in the literature. Since samples were usually pooled to obtain a sufficiently large sample for analysis, the data cannot be statistically compared among species.

By the end of the preliminary acute toxicity test (96 h), appreciable accumulation of all ions tested had occurred. Despite the reduced ionic concentration of the groundwater at the start of the last acute toxicity test on rainbow trout, noticeable accumulation of all ions tested (often in the lower concentrations of groundwater) was noted by the end of the experiment (Table 22).

During the chronic toxicity tests, accumulation of each of the ions tested occurred in each fish species during the 8 to 13 wk exposure. Accumulation occurred in blood, gill, and liver tissues. The accumulations were loosely dose-dependent: accumulation of some ions occurred at the lowest concentrations; the greatest accumulations occurred at the highest concentrations. In the following paragraphs, results for lake chub, white sucker, and rainbow trout are presented in turn.

In lake chub, blood Na levels increased slightly over the control values after chronic exposure to 3% groundwater. In the highest groundwater concentration (25%), blood Na increased by 18%. Increases in blood K, Cu, and Zn were variable in fish exposed to lower concentrations, but all increased at exposures above 12.5%. The largest increases (Na equal to 18%, K equal to 51%, Cu equal to 18%, Zn equal to 15%) were observed at 25% groundwater concentrations. Following exposure to groundwater concentrations above 8%, marked

increases in Cu and Zn levels in both gill and liver tissues were noted. Highest accumulation again occurred at 25% groundwater. Percent accumulation was higher in gill and liver tissue than in blood. In gill tissue, increases in Cu equal to 40% and in Zn equal to 46% were found; in liver tissue, the increases in Cu were equal to 44% and in Zn equal to 38%. Calcium and Mg levels remained essentially unchanged.

In rainbow trout, the highest concentration of groundwater used for chronic toxicity tests was 12.5% ion-depleted groundwater. At this concentration, the groundwater contained only 722 mg/L Na^+, only threefold above control levels. Despite the low Na levels in the groundwater, blood Na levels were elevated in trout at all concentrations, reaching levels 20% above control levels after exposure to the 12.5% concentration (Table 22). Blood levels of K (+10%) and Cu (+12.5%) were elevated slightly. Only Zn levels showed no appreciable increase. As they were in lake chub, much larger increases were observed in both gill and liver tissues (Cu equal to +41% and Zn equal to +30% in gill, and Cu equal to +21% and Zn equal to +39% in liver). As might be expected from the lower maximal concentration and greater ionic depletion of groundwater, tissue increases were slightly lower than those for lake chub. Calcium and Mg levels remained essentially unchanged.

Because of fish deaths, few white suckers were available for examination. The maximum exposure time (8 wk) was less than that of rainbow trout and lake chub (13 wk). In general, the results showed trends similar to those described for trout and lake chub. Sodium and Zn levels were increased in animals exposed to groundwater. Potassium levels were increased only at the highest concentrations, and Ca and Mg levels remained essentially unchanged.

5.3.4.4 Discussion. Ion concentrations were measured in three tissues: blood, liver, and gill. Blood was tested because it represents the transportation system within the body. Liver tissue was tested as the most likely site in which excess ions, especially heavy metals, might be sequestered within the body. Gill tissue

was tested because it represents the most probable point of entry for the ions and the area of greatest contact with the external environment.

Initially, the groundwater tested was moderately to heavily saline (6700 mg/L Na^+) and contained appreciable amounts of other ions as well. Owing to precipitation during storage, however, the ionic content was reduced to 30% of the original ionic content. By the end of chronic testing, levels of all ions in the test solution had decreased to below acute lethal levels. Despite this decrease, accumulation of all ions tested occurred within the tissues of both fish and invertebrates (see Section 4.3.6), often in the most dilute groundwater concentrations. These accumulations could have important biological effects.

Before precipitation occurred in the groundwater, Na levels (6700 mg/L) approached lethal levels (6 d LC_{50}) for goldfish (7332 mg/L) and fathead minnows (7650 mg/L)(Adelman et al. 1976). Levels similar to these probably played a direct role in mortality during the initial acute toxicity experiments. McMahon et al. (1976) showed saline-induced mortality in mountain whitefish at similar concentrations. Even following precipitation and dilution to sublethal levels, the concentration of both Na and K in the test solutions clearly exceeded the animals' regulatory abilities, causing the concentrations of these ions in the blood to increase.

Both Na and K ions are important regulators of metabolism. They are involved in maintenance of electrical potential in nerve cells. Changes in their concentrations can create physiological changes in the body. For example, an increase in ionic concentration causes increased O_2 affinity of fish blood (Johansen et al. 1978), reducing the efficiency of O_2 delivery to the tissues. Increased Na levels also cause interference with the body's H ion excretion process, causing acidosis which has serious metabolic effects. Change in Hct, probably arising from a change in RBC volume caused by ionic imbalance between RBC and plasma, has been discussed in Section 5.3.3.4 of this report.

Buildup of the heavy metals Cu and Zn usually occurs in both fish blood and tissues. As a rule, the levels attained are probably below acute lethal levels for single substances, but could reach lethal levels during chronic exposures by contributing to:

1. A cumulative toxic syndrome in combination with other heavy metals (Eaton 1973);
2. A cumulative toxic syndrome in combination with other inorganic compounds (Broderius and Smith 1979);
3. A cumulative toxic syndrome in combination with organic compounds (Calamari and Marchetti 1973); or
4. A long-term buildup to lethal levels in the tissues.

McKim and Benoit (1974) found no accumulation of Cu in a chronic (3 mo) exposure to sublethal concentrations (<10 $\mu g/L^{-1}$) in brook trout, but in the present study marked increases were observed over a similar exposure period. It cannot be stated that these increased levels pose a biological danger to these fishes (dose-dependent mortality was not observed); nevertheless, reproductive and behavioural effects have been noted at lower ionic levels. Sprague (1964) reported marked avoidance by Atlantic salmon in concentrations as low as 4 µg/L Cu and Brungs (1969) calculated that a similar dose (5 µg/L) Zn could reduce fecundity by 20%.

In summary, accumulation of ions could have serious metabolic effects. Without long-term studies, however, it is impossible to determine just how severe the effects of a long-term chronic exposure to groundwater would be.

5.3.5 Gill Damage

5.3.5.1 Rationale. Many substances toxic to fishes cause damage to the gills. The effects can range from:

1. Slight irritation of the sensitive gill surface causing a secretion of mucus into the interlamellar grooves; to
2. Clogging and distortion of the lamellar shape; to
3. Disturbance and bending of the lamellae epithelia; to

4. Loosening and separation of the lamellae epithelia. These effects are separate but form a series. They are very difficult to quantify (Hughes 1976), especially when damage is slight. The functional effect of all these changes is to cause thickening of the gill epithelia accompanied by edema, increasing the diffusion path and decreasing gaseous exchange at the gill surface. This reduction in gas exchange is particularly marked for O_2 because this gas diffuses 30 times more slowly through aqueous media and tissues than does CO_2. As a consequence, the net result of many kinds of pollution is relative hypoxia (inability of the gill surface to take up sufficient O_2).

5.3.5.2 Methods. Gills dissected from control and experimental animals were wrapped in two layers of aluminum foil and placed in airtight polythene bags to prevent dessication during freezing. Prior to sectioning, the frozen tissues were mounted on metal chucks. The chucks were precooled to -70°C by placing them in CO_2 ice pellets. Cryoform mountant (International Equipment Co., Needham Heights, Massachusetts) was placed on the head of the coated chuck. The tissue was then placed in the mountant and held in position until frozen. Freezing was complete within 15 s. The exposed tissue was covered with mountant medium which, upon freezing, provided support for the tissue during sectioning.

Transverse cryostat sections, 20 µm thick, were cut from each tissue sample and air-dried at room temperature for approximately 10 min. The cryostat consisted of a microtome in a cryostat cabinet (Slee Ltd., London, UK). The cryostat cabinet temperature was maintained between -15 and -20°C while the tissues were sectioned with a knife cooled to -70°C with CO_2 ice. Sections were stained using the modified haematoxylin and eosin method described in Chayen et al. (1969) and examined using a light microscope (Reichert, Austria).

5.3.5.3 Results. Histological gill damage was not observed in response to chronic, sublethal exposure to even the highest concentrations of groundwater. Mucus buildup, however, indicative of a protective response to protect the gills from irritation, was observed in the gills of fishes exposed to even the lowest concentrations of groundwater (2 to 3%). Mucus production was most marked in rainbow trout exposed to concentrations above 10%. No mucus buildup was observed in the gills of control fishes (Table 23).

5.3.5.4 Discussion. Mucus buildup seems to be a sensitive but not quantitative detector of low levels of gill irritation. At higher concentrations, Cu in the groundwater has been shown to cause serious damage to gill tissues (Skidmore 1970; Hughes and Perry 1976). The concentrations used during the present study caused no gill damage, but the increased mucus production may well have reduced the O_2 transport capabilities of the gill surface by increasing the diffusing distance. Despite the apparent sensitivity of the mucus buildup response, this phenomenon is difficult to quantify and difficult to assess outside the laboratory. As a result, it may not be a useful indicator of sublethal pollution levels.

Because no histological effects could be demonstrated, no histochemistry studies were performed on the gills of these fish.

5.3.6 Growth

5.3.6.1 Rationale. Growth of an organism is dependent on internal (or metabolic) conditions and on external (or environmental) factors. Warren (1971) indicated that pollutants can affect the efficiency of food conversion in animals and thereby impose stress on the animal's growth. Growth rate has also been suggested as a good indicator of stress by Sprague (1971). Measurements of growth rate and conversion efficiency are also considered a more sensitive indicator of sublethal toxic effects than swimming performance (Webb and Brett 1973).

Table 23. Gill morphology following chronic exposure (90 d) to various concentrations of mine depressurization groundwater. Secondary lamellae and filaments on four gill arches from each of four fish were examined for each concentration.

Concentration (%)	Mucus
Rainbow trout (Experiment #1):	
Control	-
2.0	++
4.0	+
8.0	++
10.0	+++
12.5	++
Rainbow trout (Experiment #2):	
Control	-
2.0	++
4.0	++
8.0	++
10.0	++
12.5	+++
White sucker [a]:	
Control	-
2.0	-
3.0	+
8.0	++
12.5	++
25.0	++
Lake chub:	
Control	-
3.0	++
6.0	++
8.0	++
12.5	++
25.0	++

[a] Only 56 d of exposure.

5.3.6.2 Methods. In this study, the lengths and wet weights of rainbow trout and lake chub were taken after 90 d exposure to several concentrations of mine depressurization groundwater. The Duncan New Multiple Range Test was used to determine the statistical significance of the test results.

5.3.6.3 Results. Results are summarized in Tables 24 and 25. The results of the statistical analysis indicate no significant differences (at 1% probability level) in wet weights and lengths of fish exposed chronically to any concentration of mine depressurization groundwater.

5.3.6.4 Discussion. The results are not unusual for a toxicant as complex as mine depressurization groundwater. Lett et al. (1976) found pollutants containing heavy metals initially reduced the appetite of test fish but, after chronic exposure, fish exhibited an increased appetite. The groundwater, which also contains a substantial amount of heavy metals, probably did not materially affect growth in the test fish.

5.3.7 Fertilization Success

5.3.7.1 Rationale. The purpose of this study was to examine the toxic effects of saline groundwater on fertilization success of whitefish during spawning.

During the spawning period, there are two critical periods. The first is a brief period prior to fertilization when sperm and unfertilized ova are in direct contact with external contaminants. The second is during the next half-hour or so when eggs imbibe water and become water hardened. During the latter period, materials such as Cd may move rapidly across the vitillitive membrane (Rosenthal and Sperling 1974). The rate of movement declines as eggs become water hardened. An exposure time of 1 h was chosen in this experiment to examine the effect of saline groundwater on gametes, combined with the effect of saline groundwater uptake during the period of water imbibation after fertilization.

Table 24. Wet weights (g) of rainbow trout and lake chub following 90 d exposure to various concentrations (%) of mine depressurization.

Species	Concentration (%)					
	Control	2.0	4.0	8.0	10.0	12.5
Rainbow trout	4.32	3.74	4.36	3.28	4.88	3.91
(Experiment No. 1)	(n = 10)	(n = 16)	(n = 6)	(n = 12)	(n = 12)	(n = 15)
Rainbow trout	5.92	4.89	4.07	5.21	5.28	4.05
(Experiment No. 2)	(n = 6)	(n = 8)	(n = 7)	(n = 12)	(n = 12)	(n = 17)
	Control	3.0	6.0	8.0	12.5	25.0
Lake chub	1.19	1.30	1.27	1.33	1.22	1.14
	(n = 29)	(n = 14)	(n = 28)	(n = 27)	(n = 27)	(n = 31)

Table 25. Total lengths (cm) of rainbow trout and lake chub following 90 d of exposure to various concentrations (%) of mine depressurization groundwater.

Species	Concentration (%)					
	Control	2.0	4.0	8.0	10.0	12.5
Rainbow trout	7.3	6.8	6.85	6.7	7.5	7.0
(Experiment No. 1)	(n = 10)	(n = 16)	(n = 6)	(n = 12)	(n = 12)	(n = 15)
Rainbow trout	8.3	7.83	7.47	8.05	8.03	7.19
(Experiment No. 2)	(n = 6)	(n = 8)	(n = 7)	(n = 12)	(n = 12)	(n = 17)
	Control	3.0	6.0	8.0	12.5	25.0
Lake chub	5.1	5.2	5.3	5.3	5.1	4.9
	(n = 29)	(n = 14)	(n = 28)	(n = 27)	(n = 27)	(n = 31)

The lake whitefish was chosen for this study for three reasons:

1. Lake whitefish is the only major fall spawning species in the AOSERP study area (the timing of the study was inappropriate for spring spawning species);
2. A large whitefish spawning run is known to occur in the mainstem of the Athabasca River (Jones et al. 1978); and
3. Lake whitefish is a major species in the Peace-Athabasca Delta and Lake Athabasca commercial and domestic fisheries.

5.3.7.2 Methods. Eighteen lake whitefish (four females and 14 males) were collected alive by beach seine from spawning grounds on the Athabasca River on 16 October 1978 (Jones et al. 1978). Their sex products were immediately stripped and pooled in two dry containers, one for ova and one for milt. Prior to testing, the sex products were stored in a portable cooler packed with ice.

Within 1 h of stripping, 25 mL ova and 1 mL milt were mixed together in plastic buckets containing 500 mL of either plain water from the Athabasca River (control) or one of seven saline groundwater solutions: 100, 50, 25, 10, 1, 0.1, and 0.01% by volume. Each solution was tested twice. The first and last tests in each series were control solutions (Athabasca River water) so that a systematic variable such as a drop in gamete viability over time could be identified. The groundwater solutions were tested in a random sequence. The same sequence was used for both replicates. The sequence, in order, was: first control, 0.1, 10, 0.01, 100, 50, 1, 25%, and, finally, the second control. A single batch of composite mine depressurization groundwater was used in all tests. The chemical composition of the groundwater was similar to that of the 21 September 1978 sample described in Table 1. To allow water temperatures to equilibrate, all solutions were stored in the Athabasca River (water temperature 5.5^{o}C) for at least 1 h before testing.

After incubating in the test solutions for 1 h, the fertilized eggs were gently transferred to plastic sieve buckets and thoroughly rinsed in the Athabasca River. After rinsing, each sample was placed in a plastic bag containing 25 mL water. The bags were aerated with O_2, sealed, and packed with ice in portable coolers. The eggs were shipped to The University of Calgary where they were placed randomly in the compartments of a vertical stack incubator. The eggs were incubated at 2.0 ± 5°C for the duration of the experiment.

From 16 October 1978 to 14 February 1979, the eggs were examined at regular intervals for the presence of dead (opaque or fungused) eggs which were removed and enumerated. The experiment was ended 23 February when a sudden increase in water temperature resulted in greatly increased mortality rates for both control and saline groundwater test treatments. The remaining eggs were counted in order to determine the total number of eggs in each treatment.

5.3.7.3 Results. During the 1 h exposure period, whitefish eggs adhered to the bottom of the plastic buckets in both the control and saline groundwater solutions up to, and including, 25%. Eggs in the 50 and 100% solutions of saline groundwater failed, however, to develop the normal stickiness.

Data on the cumulative mortality of lake whitefish eggs exposed to various concentrations of saline groundwater during fertilization are summarized in Table 26. In each treatment, the difference between the first and second replicate at any one time was very small, with little overlap between treatments. Values for the first and second replicates were therefore averaged.

Egg mortality in the second set of controls was considerably higher than the first set (Table 26, Figure 25). Evidently, there was a reduction in gamete viability over the time it took to mix the eggs and milt with the various concentrations of saline groundwater, a procedure which took about 1 h.

Table 26. The cumulative mortality of lake whitefish eggs exposed to various concentrations of saline groundwater for 1 h during fertilization on 16 October 1978.

Treatment	Initial No.	Cumulative Egg Mortality (%) Over Time								
		5 Nov 78	13 Nov 78	23 Nov 78	28 Nov 78	13 Dec 78	5 Jan 79	17 Jan 79	5 Feb 79	14 Feb 79
First Control:										
First Replicate	2698	0.5	1.4	1.6	2.2	2.5	2.8	2.9	4.3	7.3
Second Replicate	2498	2.4	3.2	3.5	4.0	4.8	4.9	5.0	5.4	6.3
Mean	2598	1.5	2.3	2.5	3.1	3.7	3.9	3.9	4.9	6.8
100% Saline Groundwater:										
First Replicate	2385	14.5	19.0	41.9	76.5	84.5	84.8	85.0	85.4	85.8
Second Replicate	2338	7.0	11.3	53.7	77.4	84.4	84.7	84.7	85.0	85.1
Mean	2361	10.7	15.1	47.8	76.9	84.5	84.7	84.9	85.2	85.5
50% Saline Groundwater:										
First Replicate	1822	24.5	26.0	ND[a]	29.2	29.6	30.0	30.3	33.1	36.7
Second Replicate	2700	25.4	28.6	ND	31.7	32.3	32.9	34.1	37.4	41.1
Mean	2261	24.9	27.7	ND	30.5	30.7	31.5	32.2	35.3	38.9

continued ...

Table 26. Continued.

Treatment	Initial No.	Cumulative Egg Mortality (%) Over Time								
		5 Nov 78	13 Nov 78	23 Nov 78	28 Nov 78	13 Dec 78	5 Jan 79	17 Jan 79	5 Feb 79	14 Feb 79
25% Saline Groundwater:										
First Replicate	2528	14.1	17.5	ND	21.1	21.9	23.3	25.5	ND	ND
Second Replicate	2573	9.6	12.9	ND	17.2	18.3	18.6	19.3	20.9	23.2
Mean	2551	11.9	15.2	ND	19.1	20.1	20.9	22.4	ND	ND
10% Saline Groundwater:										
First Replicate	2277	6.7	10.8	ND	12.1	12.7	13.4	14.1	17.4	19.9
Second Replicate	2334	6.7	13.4	15.1	15.9	16.4	17.0	17.5	20.2	23.5
Mean	2305	6.7	12.1	ND	14.0	14.5	15.2	15.8	18.3	21.7
1% Saline Groundwater:										
First Replicate	2898	4.7	6.4	ND	9.5	9.9	10.5	12.6	16.4	20.3
Second Replicate	2660	3.0	4.1	ND	7.5	7.9	8.6	10.0	14.1	18.5
Mean	2779	3.9	5.3	ND	8.5	8.9	9.5	11.3	15.3	19.4
0.1% Saline Groundwater:										
First Replicate	2621	0.9	2.3	2.9	3.2	3.7	3.9	4.0	4.7	6.6
Second Replicate	2855	0.6	1.4	2.1	2.8	3.5	3.8	3.9	4.4	5.1
Mean	2738	0.7	1.9	2.5	3.0	3.6	3.9	3.9	4.5	5.9

continued ...

Table 26. Concluded.

Treatment	Initial No.	Cumulative Egg Mortality (%) Over Time								
		5 Nov 78	13 Nov 78	23 Nov 78	28 Nov 78	13 Dec 78	5 Jan 79	17 Jan 79	5 Feb 79	14 Feb 79
0.01% Saline Groundwater:										
First Replicate	2540	4.7	6.5	ND	8.7	9.1	9.6	10.3	12.2	14.1
Second Replicate	2939	3.7	4.5	4.8	5.7	6.1	6.8	7.2	9.8	12.6
Mean	2739	4.2	5.5	ND	7.2	7.6	8.2	8.7	11.0	13.3
Second Control:										
First Replicate	2215	3.5	5.1	ND	11.5	12.2	13.5	14.7	18.1	20.6
Second Replicate	2848	3.3	5.0	9.9	11.9	12.3	13.3	13.9	16.0	17.8
Mean	2531	3.4	4.1	ND	11.7	12.3	13.4	14.3	17.1	19.2

[a] ND = no data.

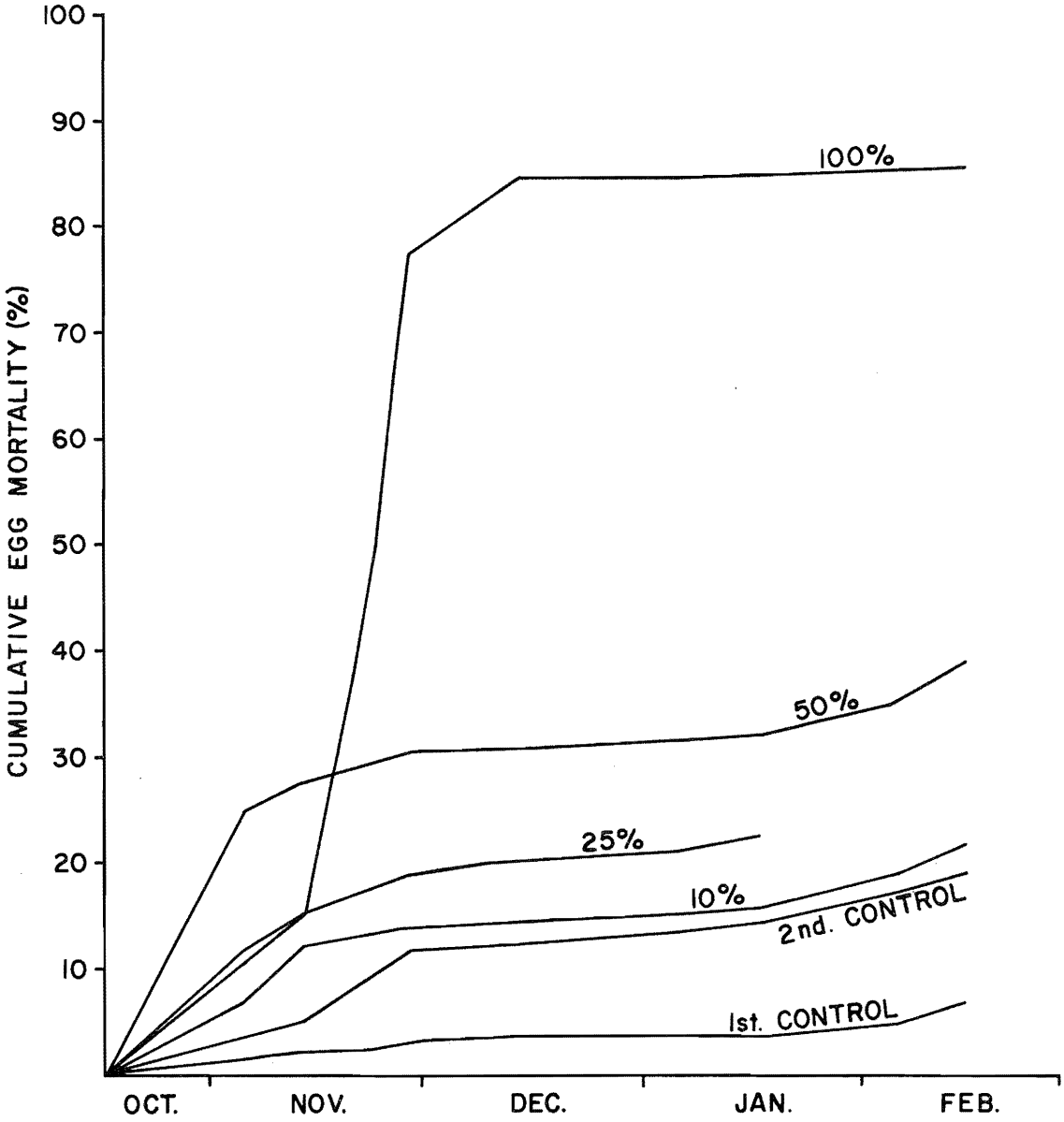

Figure 25. Cumulative mortality of lake whitefish eggs exposed to various concentrations (10.0 to 100.0%) of saline groundwater for 1 h during fertilization.

5.3.7.4 Discussion. There is no evidence that exposure to saline groundwater concentrations up to and including 1.0% affects the fertilization and incubation success of whitefish eggs. By the end of the experiment, the cumulative egg mortality for 0.01 to 1.0% saline groundwater concentrations ranged from 5.9 to 19.4%, compared to 6.8 and 19.2% for the first and second set of controls (Table 29). Egg mortality resulting from a 1 h exposure to 10% saline groundwater was, however, consistently higher than both controls throughout the course of the experiment (Figure 25). Since the 10% solution of saline groundwater was also the first groundwater solution tested (see order of testing in Section 5.3.7.2), probably very little of the observed mortality can be attributed to a decline in gamete viability. Progressively higher egg mortalities were observed for higher concentrations of saline groundwater, ranging from 23.2% for exposure to 25% solution, to 85.8% for exposure to 100% solutions at the end of the study period (Figure 25).

6. LITERATURE REVIEW

This portion of the report is a brief review of the literature available on the components and effects of mine depressurization groundwater. It includes sections on the chemical properties of groundwater, the effects of storage on groundwater, acute toxicity studies done using groundwater, studies of the sublethal effects of groundwater, and a summary of work done on individual components found in groundwater.

6.1 CHEMICAL PROPERTIES OF GROUNDWATER

Data on the chemical characteristics of mine depressurization groundwater were found in McMahon et al. (1976), Lake and Rogers (1979), and Giles et al. (1979). The chemical composition of groundwater varied from well to well and from day to day. Table 27 summarizes the available data on the chemical composition of groundwater, and Table 28 gives a list of the gaseous components of groundwater.

6.2 EFFECTS OF STORAGE ON GROUNDWATER

McMahon et al. (1976) and Giles et al. (1979) detected major changes in the chemical composition of mine depressurization groundwater during storage. The latter noted that storage of a composite groundwater sample for 27 d at $4^{o}C$ resulted in large changes in chemical composition (Table 29).

McMahon et al. (1976) found that water from a high osmolarity well formed an unusual heavy purple precipitate during storage. An analysis of the precipitate indicated high concentrations of several metals, including Ba, B, Cd, Co, Cu, Fe, Pb, Mn, Ni, V, and Zn. Giles et al. (1979) found that As and Se did not change in concentration during a 30 d storage period, but concentrations of Mn decreased by 92.0%, Cu by 91.4%, Hg by 92.0%, and Zn by 75.0%. These observations indicate that some heavy metal components of groundwater precipitate during storage.

Table 27. Chemical composition of mine depressurization water from Syncrude Canada Ltd. Lease 17 (Lake and Rogers 1979). Also, analysis for water from the Athabasca River in the vicinity of Lease 17 (McMahon et al. 1976).

Parameter[a]	Mine Depressurization Groundwater		Athabasca River	
	Mean	Range Low to High	Mean	Range Low to High
Calcium	71.46	1.0 to 318.0	35.3	33.0 to 44.0
Magnesium	133.89	47.0 to 253.0	9.3	6.0 to 14.0
Sodium	5 622.63	2 150.0 to 7 900.0	10.5	8.0 to 16.0
Potassium	45.44	19.0 to 65.0	1.1	0.6 to 2.0
Chloride	7 615.47	2 250.0 to 10 500.0	66.0	28.0 to 99.0
Sulphate	10.22	0.5 to 78.0	27.0	15.0 to 41.0
Total Alkalinity	2 628.78	1 524.0 to 4 452.0		
pH	7.55	6.9 to 9.1		
Carbonate	27.18	0.0 to 396.0	14.0	0.0 to 43.0
Bicarbonate	3 150.29	1 828.5 to 5 427.0	111.0	78.0 to 132.0
Total Hardness	741.58	295.0 to 1 377.0		
Fluoride	0.71	0.48 to 1.25		
Silica	4.39	2.0 to 18.0		
Conductivity	25 658.50	9 400.0 to 48 000.0		
Colour T_1	25.31	5.0 to 98.0		

continued ...

Table 27. Continued.

Parameter[a]	Mine Depressurization Groundwater				Athabasca River	
	Mean	Range Low to High			Mean	Range Low to High
Colour T_2	98.62	98.0	to	99.0		
Colour T_3	96.62	96.0	to	97.0		
Tannin and Lignin	0.62	0.1	to	2.0		
Total Residue	15 479.32	8 810.0	to	19 330.0		
Total Filtered Residue	14 688.68	5 768.0	to	19 240.0		
Total Filtered Residue Fixed	16 268.39	5 376.0	to	19 140.0		
Total Nonfiltered Residue	55.84	0.4	to	436.0		
Total Nonfiltered Residue Fixed	47.33	0.4	to	394.0		
Turbidity	32.16	0.01	to	298.0		
Surfactants	0.32	1.02	to	188.0		
Humic Acid	1.17	1.08	to	1.36		
Total Organic Carbon	189.42	1.0	to	9 319.0		
Total Inorganic Carbon	579.19	250.0	to	820.0		
Nitrate	0.10	0.1	to	ND		
NO_2 + NO_3(Nitrogen)	0.037	0.01	to	0.061		
NH_3	7.69	2.2	to	13.65		

continued ...

Table 27. Continued.

Parameter	Mine Depressurization Groundwater				Athabasca River	
	Mean	Range Low to High			Mean	Range Low to High
Total Kjeldahl Nitrogen	11.37	4.8	to	22.9		
Total Phosphorus	0.17	0.0005	to	1.74		
Total Phosphate	0.52	0.2	to	0.81		
Ortho Phosphorus	0.06	0.005	to	0.242		
Phenol	0.0055	0.001	to	0.029		
Oil and Grease	2.43	0.1	to	36.3		
Sulphide	0.051	0.02	to	0.11		
Cyanide	0.014	0.01	to	0.035		
Total Hydrocarbon	15.01	0.001	to	324.0		
Biological Oxygen Demand	3.67	2.0	to	8.0		
Chemical Oxygen Demand	321.4	10.0	to	1 282.0		
Cadmium	0.013	0.001	to	0.053	<0.01	
Chromium +6	0.008	0.002	to	0.036	<0.01	
Copper	0.015	0.001	to	0.032	<0.01	
Iron	1.51	0.04	to	7.45	0.65	0.43 to 0.91
Lead	0.028	0.002	to	0.142	<0.01	
Manganese	0.194	0.65	to	1.2	<0.01	

continued ...

Table 27. Concluded.

Parameter	Mine Depressurization Groundwater			Athabasca River	
	Mean	Range Low to High		Mean	Range Low to High
Silver	0.011	0.005	to 0.03		
Zinc	0.024	0.001	to 0.2	0.03	0.01 to 0.06
Vanadium	0.004	0.001	to 0.05	<1.00	
Selenium	0.0014	0.0005[b]	to 0.0037		
Mercury	0.0034	0.0001[b]	to 0.07	<0.0002	
Arsenic	0.004	0.0002[b]	to 0.02	<0.01	
Nickel	0.059	0.002[b]	to 0.32	<0.02	
Aluminum	0.16	0.005[b]	to 2.3	<1.00	
Cobalt	0.05	0.002[b]	to 0.165	<0.01	
Boron	2.60	0.48	to 7.08	<1.0	
Total Dissolved Solids	15 561.16	9 319.00	to 19 245.00	280.0	169.0 to 672.0
PCB's	0.00015	< 0.0001[b]	to 0.0006		
Total Carbon	757.31	21.0	to 1 130.0		

[a] Values as mg/L, except:

Conductivity in µmhos/cm

Turbidity in JTU

Metals as totals mg/L

Alkalinity and Hardness expressed as Calcium Carbonate

Nitrate, NO_2 + NO_3 expressed as N

Phosphorus T expressed as PO_4

Phosphorus O expressed as P

pH in pH units

Table 28. Analysis of gaseous components of groundwater from Syncrude Lease 17 (McMahon et al. 1976).

Element or Compound	Number of Samples	$\bar{x}$ (mol %)	Range	
			High	Low
H_2	5	0.01	0.04	0.00
He	3	0.00		
N_2	5	4.30	11.61	0.00
CO_2	6	11.70	28.70	5.70
H_2S	6	0.00		
C_1	6	83.90	91.70	70.80
C_2	6	0.34	1.70	0.05
C_3	6	0.12	0.57	0.00
iC_4	6	0.01	0.06	0.00
C_4	6	0.08	0.34	0.00
iC_5	5	0.05	0.22	0.00
C_5	5	0.01	0.05	0.00
C_6	5	0.07	0.33	0.00
C_7+	5	0.20	0.52	0.00

Table 29. Percentage variation in concentration of certain components of mine depressurization groundwater during a storage time of 27 d. Data from Giles et al. (1979).

Variation in Concentration During Storage (%)	Parameter	
	Increase	Decrease
>75	Sulphate, carbonate, oil and grease, chemical oxygen demand	Calcium, tannin and lignin, phenol, total organic carbon
50 to 75	Total inorganic carbon	Total phosphorus
25 to 50		Bicarbonate, total hardness
20 to 25	Ammonia nitrogen	Potassium, fluoride, total alkalinity

6.3 ACUTE TOXICITY OF GROUNDWATER

Acute toxicity bioassays have been performed on nine species of fish and nine species of invertebrates. The results of these tests are summarized in Table 30. In general, the results indicate mine depressurization groundwater was acutely toxic to all fish and invertebrates tested. Because of the chemical complexity of groundwater, no single toxic factor was isolated; however, toxicity apparently resulted from a combination of factors such as salinity and the presence of ammonia, hydrocarbons, and heavy metals.

The major conclusions of McMahon et al. (1976), Lake and Rogers (1979), and Giles et al. (1979) are summarized in the following three sections.

6.3.1 Variations in Groundwater Toxicity

McMahon et al. (1976) reported no significant differences in the toxicity of mine depressurization groundwater when dechlorinated City of Calgary water and Athabasca River water were used as diluents. Giles et al. (1979), using City of Winnipeg water, supported this observation. Lake and Rogers (1979), using dechlorinated City of Edmonton water, reported results comparable to field toxicity tests using Athabasca River water as a diluent.

Toxicity of groundwater varied with storage time. Lake and Rogers (1979) found the toxicity of groundwater decreased for the first 10 days of storage and increased thereafter. Giles et al. (1979) found the toxicity increased with storage time. In concentrations of groundwater ranging from 30 to 100% by volume, median survival times ranged from 1.6 to 115.0 h in groundwater stored for 8 d, in contrast to a range of 0.75 to 2.2 h in groundwater stored for 18 d.

Toxicity varied from well to well. For groundwater from different wells in Syncrude Lease 17, Lake and Rogers (1979) reported LC_{50} values for trout-perch ranging from 21 to 40%, and for rainbow trout from 59.2 to 80%. This groundwater had been stored for 1 d.

Table 30. Summary of acute toxicity data for mine depressurization groundwater.

No. of Individuals	Temp (^{0}C)	D.O. (ppm)	pH	Time (hr)	Toxicity (% Vol. Groundwater)	Comments	Ref.
Salmo gairdneri - Rainbow trout (juvenile)							
26	5	>6.5	8.5 to 9.5	96	LC_{50} 44.8	Static bioassay; diluent -	McMahon
				168	LC_{50} 24.8	Athabasca River; toxicant	et al.
						osmolarity = 230 mOsm	1976
148	5	>6.5	8.5 to 9.5	96	LC_{50} 22.3	Static bioassay; diluent -	
				168	LC_{50} 17.4	Athabasca River; toxicant	
				240	LC_{50} 16.8	osmolarity = 400 mOsm	
139	5	>6.5	8.5 to 9.5	96	LC_{50} 34.8	Static bioassay; diluent -	
				168	LC_{50} 21.1	Calgary dechlorinated water;	
				240	LC_{50} 16.4	toxicant osmolarity = 400 mOsm	
27	15	>6.5	8.5 to 9.5	96	LC_{50} 47.2	Static bioassay; diluent -	
				168	LC_{50} 36.8	Athabasca River; toxicant	
						osmolarity = 230 mOsm	
Salmo gairdneri (alevin)							
3 400	20	>6.5	8.5 to 9.5	96	LC_{50} 25.2	Static bioassay; total darkness	
				168	LC_{50} 7.7	diluent - Calgary dechlorinated	
				240	LC_{50} 6.7	water; toxicant osmolarity = 380	
						mOsm	

continued...

Table 30. Continued.

No. of Individuals	Temp (°C)	D.O. (ppm)	pH	Time (hr)	Toxicity (% Vol. Groundwater)	Comments	Ref.
Salmo gairdneri (juvenile)							
50	15	>90% air-sat	8.6 to 9.0	240	LC_{50} 19-35	Static; SSR[a]; diluents - Athabasca R. & Winnipeg dechlorinated water	Giles et al. 1979
Prosopium williamsoni - Mountain whitefish (juvenile)							
57	5	>6.5	8.5 to 9.5	96	LC_{50} 43.9	Static; diluent - Athabasca R.; toxicant osmolarity = 230 mOsm	McMahon et al. (1976)
				168	LC_{50} 20.4		
				240	LC_{50} 12.1		
26	5	>6.5	8.5 to 9.5	96	LC_{50} 17.8	Static; diluent - Athabasca R.; toxicant osmolarity = 400 mOsm	
				168	LC_{50} 17.8		
60	5	>6.5	8.5 to 9.5	96	LC_{50} 42.8	Static; diluent - Calgary dechlorinated; toxicant osmolarity - 230 mOsm	
				168	LC_{50} 37.1		
				240	LC_{50} 22.3		
18	15	>6.5	8.5	48	LC_{50} 49.6	Static; diluent - Calgary dechlorinated water; toxicant osmolarity - 230 mOsm	

continued ...

Table 30. Continued.

No. of Individuals	Temp (°C)	D.O. (ppm)	pH	Time (hr)	Toxicity (% Vol. Groundwater)	Comments	Ref.
Thymallus arcticus - Arctic grayling (juvenile)							
112	5	>6.5	8.5 to 9.5	96	LC_{50} 26.6	Static; diluent - Athabasca	
				168	LC_{50} 23.9	R.; toxicant osmolarity =	
						375 mOsm	
108	15	>6.5	8.5	96	LC_{50} 13.2		
Couesius plumbeus - Lake chub (juvenile) Athabasca River origin							
90	15	>6.5	8.5 to 9.5	96	LC_{50} 59.5	Static; diluent - Athabasca	McMahon
				168	LC_{50} 57.1	R.; toxicant osmolarity =	et al.
						350 mOsm	1976
Couesius plumbeus - Lake chub (juvenile) Nose Creek, Calgary, origin							
84	5	>6.5	8.5 to 9.5	96	LC_{50} 66.4	Static; diluent - Athabasca	
				168	LC_{50} 63.1	R.; toxicant osmolarity =	
						375 mOsm	
73	15		8.5 to 9.5	96	LC_{50} 59.1		
				168	LC_{50} 45.5		
Platygobio gracilis - Flathead chub (juvenile)							
42	5	>6.5	8.5 to 9.5	96	LC_{50} 42.2	Static; diluent - Athabasca	

Continued ...

Table 30. Continued.

No. of Individuals	Temp (°C)	D.O. (ppm)	pH	Time (hr)	Toxicity (% Vol. Groundwater)	Comments	Ref.
Platygobio gracilis - Flathead chub (juvenile) con't							
				168	LC_{50} 42.2	River; toxicant osmolarity = 350 mOsm	
Catastomus commersoni - White sucker (juvenile)							
72	5	>6.5	8.5 to 9.5	96	LC_{50} 63.1	Static; diluent - Athabasca	
				168	LC_{50} 61.1	R.; toxicant osmolarity = 350 mOsm	
74	15	>6.5	8.5 to 9.5	96	LC_{50} 87.9		
				168	LC_{50} 70.8		
Stizostedion vitreum - Walleye (juvenile)							
43	5	>6.5	8.5 to 9.5	96	LC_{50} 9.3	Static; diluent - Athabasca	McMahon
				168	LC_{50} 7.9	R.; toxicant osmolarity = 375 mOsm	et al. 1976
49	15	>6.5	8.5 to 9.5	96	LC_{50} 13.0		
				168	LC_{50} 4.1		
Coregonus clupeaformis - Lake whitefish (juvenile)							
60	15	9.5	8.0 to 8.7	96	LC_{50} 62.0	Static; diluent - Winnipeg	Giles et
		10.1		168	LC_{50} 37.5	dechlorinated water	al. 1979

continued ...

Table 30. Continued.

No. of Individuals	Temp (°C)	D.O. (ppm)	pH	Time (hr)	Toxicity (% Vol. Groundwater)	Comments	Ref.
Percopsis omiscomaycus - Trout perch (juvenile, adult)							
54	5	>6.5	8.5 to 9.5	96	LC_{50} 22.8	Static; diluent - Athabasca	McMahon
				168	LC_{50} 17.2	R.; toxicant osmolarity =	et al.
						375 mOsm	1976
47	15	>6.5	8.5 to 9.5	96	LC_{50} 34.7		
				168	LC_{50} 14.9		
60	15	9.7	8.2	240	LC_{50} 45.0	Static; diluent - Athabasca	Lake and
						R.; effluent storage time =	Rogers
						12 days; composite effluent	1979
60	15	9.7	8.2	240	LC_{50} 40.0	Static; diluent - Athabasca	
						R.; effluent storage time =	
						18 days; composite effluent	
60	15	9.7	8.2	96	LC_{50} 28.3	SSR; diluent - Athabasca R.;	Lake and
						effluent storage time =	Rogers
						1 day; Well #2 effluent	1979
60	15	9.7	8.2	96	LC_{50} 31.3	SSR; diluent - Athabasca R.;	
						effluent storage time =	
						1 day; Well #3 effluent	

continued ...

Table 30. Continued.

No. of Individuals	Temp (°C)	D.O. (ppm)	pH	Time (hr)	Toxicity (% Vol. Groundwater)	Comments	Ref.
Percopsis omiscomaycus - Trout perch (juvenile, adult) con't							
60	15	9.7	8.2	96	LC_{50} 32.0	SSR; diluent - Athabasca R.; effluent storage time = 5 days; composite effluent	Lake and Rogers 1979
60	15	9.7	8.2	96	LC_{50} 50.1	SSR; diluent - Athabasca R.; effluent storage time = 2 days; Well #1 effluent	
60	15	9.7	8.2	96	LC_{50} 37.4	SSR; diluent - Athabasca R.; effluent storage time = 1 day; Well #4 effluent	
60	15	9.7	8.2	96	LC_{50} 24.4	SSR; diluent - Athabasca R.; effluent storage time = 1 day; Well #5 effluent	
Daphnia magna - Water flea							
30	19	7.8	8.6 to 9.6	24	LC_{50} >6.25	Static; diluent - Winnipeg dechlorinated water; composite effluent	Giles et al. 1979
		9.4		96	LC_{50} 20-50		

continued ...

Table 30. Continued.

No. of Individuals	Temp (°C)	D.O. (ppm)	pH	Time (hr)	Toxicity (% Vol. Groundwater)	Comments	Ref.
Gammarus lacustris - Scuds							
50	15	9.6 10.8	8.1 to 8.6	96	LC_{50} 90-100	Static; diluent - Winnipeg dechlorinated water	
Artemia salina - Brine shrimp							
20	19	8.7				No effects; at all concentrations, on hatching efficiency	
Orconectes virilis - Crayfish							
60	15			264	LC_{50} >100	Static; diluent - Winnipeg dechlorinated water	
Chironomus tentans - Blood worm							
180	25			48 96	LC_{50} 69.0 LC_{50} 62.0	Static; diluent - Athabasca River	
180	20			48 96	LC_{50} 69.0 LC_{50} 63.0	Static; diluent - Winnipeg dechlorinated water	Giles et al. 1979

continued ...

Table 30. Continued.

No. of Individuals	Temp (°C)	D.O. (ppm)	pH	Time (hr)	Toxicity (% Vol. Groundwater)	Comments	Ref.
Hydropsyche bifida - Caddisfly larvae							
135	5	>6.5	8.5 to 9.5	96	LC_{50} 69.6	Static; diluent - Athabasca	McMahon
				168	LC_{50} 45.0	R.; toxicant osmolarity =	et al.
						375 mOsm	1976
135	15	>6.5	8.5 to 9.5	96	LC_{50} 59.6	Static; diluent - Athabasca	
				168	LC_{50} 32.9	R.; toxicant osmolarity =	
						350 mOsm	
Salmo gairdneri (juvenile)							
60	15	9.7	7.6	96	LC_{50} 68.6	SSR; effluent storage time =	Lake and
						1 day; diluent - Edmonton	Rogers
						dechlorinated water;	1979
						composite effluent	
60	15	9.7	7.6	96	LC_{50} 55.2	Continuous flow; effluent	
						storage time = 1 day;	
						diluent - Edmonton dechlor-	
						inated water; composite	
						effluent	

continued ...

Table 30. Continued.

No. of Individuals	Temp (°C)	D.O. (ppm)	pH	Time (hr)	Toxicity (% Vol. Groundwater)	Comments	Ref.
Heptagenia marginalis - Mayfly larvae							
180	5	>6.5	8.5 to 9.5	96	LC_{50} 58.5	Static; diluent - Athabasca	McMahon
				168	LC_{50} 51.7	R.; toxicant osmolarity =	et al.
						350 mOsm	1976
90	15	>6.5	8.5 to 9.5	96	LC_{50} 37.6		
				168	LC_{50} 22.4		
Paraleptophlebia bicornuta - Mayfly larvae							
90	5	>6.5	8.5 to 9.5	96	LC_{50} 61.1	Static; diluent - Athabasca	
				168	LC_{50} 37.3	R.; toxicant osmolarity =	
						375 mOsm	
Isogenus (Isogenoides) - Stonefly nymphs							
81	5	>6.5	8.5 to 9.5	96	LC_{50} 91.5	Static; diluent - Athabasca	
				168	LC_{50} 81.3	R.; toxicant osmolarity =	
						375 mOsm	
45	15	>6.5	8.5 to 9.5	96	LC_{50} 61.7		
				168	LC_{50} 53.2		

continued ...

Table 30. Continued.

No. of Individuals	Temp (°C)	D.O. (ppm)	pH	Time (hr)	Toxicity (% Vol. Groundwater)	Comments	Ref.
60	15	9.7	7.6	96	LC_{50} 80.0	Continuous flow; effluent storage time = 10 days; diluent - Edmonton dechlorinated water; composite effluent	
60	15	9.7	7.6	96	LC_{50} 11.1	Continuous flow; effluent storage time = 29 days; diluent - Edmonton dechlorinated water; composite effluent	Lake and Rogers 1979
60	15	9.7	7.6	96	LC_{50} 64.3	SSR; effluent storage time = 1 day; diluent - Edmonton dechlorinated water; Well #4 effluent	
60	15	9.7	7.6	96	LC_{50} 38.4	SSR; effluent storage time = 10 days; diluent - Edmonton dechlorinated water; Well #2 effluent	

continued ...

Table 30. Concluded.

No. of Individuals	Temp (°C)	D.O. (ppm)	pH	Time (hr)	Toxicity (% Vol. Groundwater)	Comments	Ref.
60	15	9.7	7.6	96	LC_{50} 64.8	SSR; effluent storage time = 10 days; diluent - Edmonton dechlorinated water; Well #1 effluent	
60	15	9.7	7.6	96	LC_{50} 65.6	SSR; effluent storage time = 17 days; diluent - Edmonton dechlorinated water; Well #5 effluent	

[a] SSR: semi-static 90% molecular replacement every 24 h.

6.3.2 Toxicity to Aquatic Invertebrates

Invertebrate 96 h LC_{50} values ranged from 20 to >100%. The arithmetic mean LC_{50} was 63.0%; the geometric mean was 63.5%. The invertebrates tested had a short-term tolerance exceeding that of even the most tolerant fish but, over longer exposure periods, this difference was reduced. Invertebrate mortalities were consistently and markedly higher at 15°C than they were at 5°C.

6.3.3 Toxicity to Fish

Fish 96 h LC_{50} values ranged from 9 to 88%. The arithmetic mean LC_{50}, based on data summarized in Table 30 was 32.1%; the geometric mean was 29.3%.

At high concentrations of groundwater, fish exhibited signs of stress, including rapid opercular movements, loss of equilibrium, darkening of colour, gasping at the water surface, and haemorrhaging of the gills. Alevins (newly hatched fry) were among the most sensitive types to be tested. Rainbow trout eggs were more resistant to the effects of groundwater than any of the organisms and life history stages tested.

Some species of fish (lake chub, Arctic grayling) had mortalities consistently greater at 15°C than at 5°C; however, other species showed no variability.

6.4 SUBLETHAL EFFECTS OF GROUNDWATER

6.4.1 Histopathological Effects

Exposure of rainbow trout to sublethal concentrations of mine depressurization groundwater induced definite histopathological changes in both gills and kidneys and, to a lesser extent, in liver (Giles et al. 1979). Histopathological lesions were observed in the secondary gill lamellae and kidneys of juvenile trout exposed to groundwater concentrations ranging from 6.25 to 50%. The degenerative histological changes observed could explain the apparent increase in the mortality rate observed in the latter stages of the 10 d acute toxicity bioassays. The accumulation of tissue damage in gills and

and kidneys may have reduced the animals' ability to maintain physiological processes such as ionic-osmotic regulation, waste excretion, and blood-gas transport.

6.4.2 Cardiovascular/Respiratory Effects

At 10, 20, and 25% concentrations of mine depressurization groundwater, there were no consistent sublethal responses in heart rate, metabolic rate, buccal pressure, or ventilation rate in adult rainbow trout observed over a 24 h exposure period (Giles et al. 1979).

6.4.3 Avoidance/Preference Responses

The amphipod *Gammarus lacustris* exhibited an extreme avoidance reaction to groundwater concentrations ranging from 0.1 to 35%. The whitefish, *Coregonus clupeaformis*, neither avoided nor preferred these concentrations (Giles et al. 1979).

6.4.4 Swimming Performance

Swimming performance tests indicated a tendency toward reduction in swimming performance of trout exposed to groundwater. None of the differences in fatigue time was found to be statistically significant (McMahon et al. 1976).

6.5 INDIVIDUAL COMPONENTS OF GROUNDWATER

Because substantial information on the chemical composition of mine depressurization groundwater is now available, a review of the existing data on the toxicity of individual groundwater components is presented. This review is limited to a discussion of selected heavy metals, cations, and anions commonly found in groundwater.

6.5.1 Dissolved Heavy Metals

Gregory (1974) discussed the more recent literature on the toxicity of heavy metals to fish. The subject has been reviewed by Doudoroff and Katz (1953), Doudoroff (1957), and McKee and Wolf (1963). Thompson (1974) also summarized much of this work and the Environmental Protection Agency (EPA) reviewed many of the current studies on heavy

metal toxicity (EPA 1973). For ease of reference, the 15 heavy metals discussed here are presented in alphabetical order.

Aluminum — A lethal concentration of Al 0.07 mg/L has been reported for sticklebacks (Jones 1939); however, McKee and Wolf (1963) reported that levels as high as 0.25 mg/L in acid water were not harmful to fish. In mine depressurization groundwater, the range of Al concentration values reached 2.3 mg/L, well above the reported toxic concentrations.

Boron — High levels of B are tolerated by fish. Turnbull et al. (1954) found that, for bluegill sunfish, the 24 h LC_{50} of boron trifluoride at 20°C is 15 000 mg/L. LeClerc and Delaminck (1955) reported that the minimum lethal dose for minnows exposed to boric acid (H_3BO_3) for 6 h in hard water was 19 000 mg/L. The average concentrations of B in groundwater was 2.6 mg/L, well below the lethal levels.

Cadmium — Data summarized in EPA (1976) set the following criteria for the protection of freshwater aquatic life from the adverse effects of Cd:

1. For cladocerans and salmonid fishes, 0.0004 mg/L in soft water and 0.0012 mg/L in hard water were considered within tolerance limits;
2. For less sensitive aquatic life, 0.004 mg/L in soft water and 0.012 mg/L in hard water were considered within tolerance limits.

McKee and Wolf (1963) reported that for fish, lethal concentrations of Cd varied from 0.01 to 10 mg/L depending on conditions. In a number of the groundwater samples, the lower range of concentrations was exceeded (0.001 to 0.053 mg/L). Schweiger (1961) found that 0.03 mg/L Cd was not harmful to 1 and 2 y old tench, carp, rainbow trout, and char; however, McKim and Eaton (unpublished data 1971) found the safe level for coho salmon fry was between 0.004 and 0.001 mg/L.

Cobalt

Cobalt concentrations of 1.0 mg/L were not harmful to 1 y old tench, carp, rainbow trout, and char (Schweiger 1961); however, higher concentrations of 10 mg/L were lethal to sticklebacks (Jones 1939). Cobalt concentrations of groundwater were well below these concentrations (0.002 to 0.165 mg/L).

Copper

The average concentration of Cu in the groundwater (0.015 mg/L) was less than any of the LC_{50}'s summarized by Gregory (1974) (Table 31) or reported by Howarth and Sprague(1978) for rainbow trout (0.02 to 0.5 mg/L). The EPA (1976) summarized data which showed that:

1. Copper concentrations below 0.025 mg/L were not acutely toxic to most fish common in fresh waters in the United States;
2. Copper sulphate was less toxic to trout in hard water than in soft water; and
3. Salmonids were, in general, more sensitive to Cu toxicity than centrarchids.

None of the fish species for which data are presented in Table 8 had LC_{50}'s less than the average concentrations (0.015 mg/L) of groundwater, but several had LC_{50}'s within the upper limit of concentrations recorded for groundwater (0.032 mg/L). These included *Pimephales promelas* (LC_{50} 0.022 mg/L), *Salmo salar* (0.032 mg/L), and *Salvelinus fontinalis* (0.032 mg/L).

Among the invertebrates for which data are presented in Table 31, only *Daphnia magna*, tested without food, (LC_{50} = 0.0098 mg/L) had an LC_{50} for Cu less than the average concentration of groundwater (0.015 mg/L). However, several other results showed effects at concentrations less than the upper limit (0.032 mg/L) so far measured for mine water. These effects included reproductive impairment in *Daphnia magna* at 0.022 mg/L and a 96 h LC_{50} of 0.020 mg/L for *Sammarus pseudolimnalus*.

Table 31. Summary of copper toxicity data.

Species	n	Size or or Age	Source of Copper	Temp (^{0}C)	D.O. (mg/L)	pH
Pimephales promelas	10	1→2 g	$CuSO_4 \cdot 5H_2O$	25	7.8	7.5 8.4→7.4
Lepomis macrochirus	10	1→2 g	$CuSO_4 \cdot 5H_2O$	25	7.8	7.5 8.4→7.4
Carassius auratus	10	1→2 g	$CuSO_4 \cdot 5H_2O$	25	7.8	7.5
Lebistes reticulatus	10	0.1→0.2 g	$CuSO_4 \cdot 5H_2O$	25	7.8	7.5
Salmo salar	5	14.3 cm (10.2→19.5)	$CuSO_4 \cdot 5H_2O$	15	-	7.3→7.6
Salmo salar	8	9.2 cm (7.2→10.9)	-	17.0→0.5	-	7.0→7.4
Pimephales promelas	5	adults	-	25±1	7.2→7.9	6.9→7.2
(no hatching or fry survival at 0.0184; OK at 0.0106 mg/L)						
Pimephales promelas	5	adults	$CuSO_4$	23	7.0	∿ 7.5
(no spawning or hatching at ≥0.032 mg/L; OK at 0.014 to 0.015 mg/L)						

continued ...

Table 31. Continued.

Species	n	Size or Age	Source of Copper	Temp (°C)	D.O. (mg/L)	pH
Salvelinus fontinalis	10	14 mo	$CuSO_4 \cdot 5H_2O$	12±1	∿10	∿7.5
Ictalurus nebulosus	5	7 mo	$CuSO_4 \cdot 5H_2O$	23	∿7	∿7.5
Acroneuria lycorias	10	-	$CuSO_4 \cdot 5H_2O$	18.5	8.0	6.8
Ephemerella subvaria	10	-	$CuSO_4 \cdot 5H_2O$	18.5	8.0	6.9
Hydropsyche betteni	10	-	$CuSO_4 \cdot 5H_2O$	18.5	8.0	6.8
Daphnia magna	10	12±12 h	$CuCl_2$	18±1	∿9.0	7.7
						7.4→8.2
Daphnia magna	10	12±12 h	$CuCl_2$	18±1	∿9.0	7.7
						7.4→8.2
Daphnia magna	10	12±12 h	$CuCl_2$	18±1	∿9.0	7.7
						7.4→8.2
Daphnia magna	5	12±12 h	$CuCl_2$	18±1	∿9.0	7.7
						7.4→8.2
Daphnia magna	5	12±12 h	$CuCl_2$	18±1	∿9.0	7.7
						7.4→8.2

continued ...

Table 31. Continued.

Species	n	Size or Age	Source of Copper	Temp (°C)	D.O. (mg/L)	pH
Oronectes rusticus	50	30 to 35 mm	$CuSO_4$	20	-	7.8
	100	10 to 11 mm	$CuSO_4$	20	-	7.8
	(0.06 to 0.125 mg/L was threshold for newly hatched)					
Campeloma decisum	10	-	$CuSO_4$	15±1	∿9.5	∿7.7
	10	-	$CuSO_4$	15±1	∿9.5	∿7.7
	(survival <50% in 0.0147 and 0.028 mg/L)					
Physa integra	10	-	$CuSO_4$	15±1	∿9.5	∿7.7
	10	-	$CuSO_4$	15±1	∿9.5	∿7.7
	(survival <40% in 0.014 and 0.028 mg/L)					
Gammarus pseudolimnaeus	"	-	$CuSO_4$	15±1	∿9.5	∿7.7
	"	-	$CuSO_4$	15±1	∿9.5	∿7.7
	(survival< 40% in 0.0148 and 0.028 mg/L)					
Gammarus pseudolimnaeus	10	-	$CuSO_4$	15±1	∿9.5	∿7.7
	(<50% survived to adult stage in ≤0.008 mg/L)					
Salmo salar	5(or 3)	-	-	11.0→12.0	8.0→9.5	6.5→7.3
	(Tetagouche H_2O and fish)					

continued ...

Table 31. Continued.

Species	$CaCO_3$ (mg/L)	Time	LC_{50} for metal (mg/L)	Comments	Reference
Pimephales promelas	20	96 h	0.022 0.025		Pickering and
Pimephales promelas	360	96 h	1.14 1.76		Henderson 1966
Lepomis macrochirus	20	96 h	0.66		
Lepomis macrochirus	360	96 h	10.2	←Interpolation	
Carassius auratus	20	96 h	0.036		
Lebistes reticulatus	20	96 h	0.036		
Salmo salar	20		0.048	ILL	Sprague 1964
Salmo salar			0.032	ILL	Sprague and Ramsay 1965
Pimephales promelas	∿32	96 h	0.075	Meas. conc.	Mount and Stephen
Pimephales promelas	∿32	96 h	0.084	Static bioassay nominal conc.	1969
Pimephales promelas	198	96 h	0.47	Cont. flow meas. conc.	Mount 1968
Pimephales promelas	198	96 h	0.43	Static bioassay meas. conc.	
Salvelinus fontinalis	∿42	96 h	0.09	0.11 cont. flow	McKim and Benoit 1971

continued ...

Table 31. Continued.

Species	$CaCO_3$ (mg/L)	Time	LC_{50} for metal (mg/L)	Comments	Reference
Salvelinus fontinalis	∿42	8 mo	0.0325	(a) 43% survival of adults (b) slightly reduced growth (c) less viable eggs than at 0.0019 to 0.0174 mg/L	McKim and Benoit 1971
Ictalurus nebulosus	202	96 h	0.17	0.19	Brungs et al. 1973
Acroneuria lycorias	40	96 h	8.3		Warnick and Bell 1969
Ephemerella subvaria	40	48 h	0.32		
Hydropsyche betteni	46	14 d	32.0		
Daphnia magna	45.3 (44-53)	48 h	0.0098	Without food	Biesinger and Christensen 1972
Daphnia magna	(44-53)	48 h	0.06	With food	
Daphnia magna	(44-53)	3 wk	0.044	0.035 to 0.055	

continued ...

Table 31. Concluded.

Species	$CaCO_3$ (mg/L)	Time	LC_{50} for metal (mg/L)	Comments	Reference
Daphnia magna	45.3 (44-53)	3 wk	0.022	16% repro. impair.	Biesinger and Christensen 1972
Daphnia magna	(44-53)	3 wk	0.035	50% repro. impair.	
Oronectes rusticus	100-125	96 h	3.0	cont. flow	Hubschman 1967
Oronectes rusticus	100-125	<25 h	1.0	cont. flow	
Campeloma decisum	∿45	96 h	1.7	2.0 and 1.4	Arthur and
Campeloma decisum	∿45	6 wk			Leonard 1970
Physa integra	∿45	96 h	0.039	0.041 and 0.037	
Physa integra	∿45	6 wk			
Gammarus	∿45	96 h	0.020	0.022 and 0.019	
pseudolimnaeus	∿45	6 wk			
"	∿45	9 wk			
Salmo salar	77		0.065		Côté 1971 (2)
Salmo salar	77		0.065		
Salmo salar	33		0.062		

It appears that Cu concentrations in groundwater were insufficient to cause acute toxicity in most species of fish and aquatic invertebrates. NAS/NAE (1974:12), however, recommended that:

> ...once a 96 h LC_{50} has been determined using the receiving water in question and the most sensitive important species in the locality as the test organisms, a concentration of copper safe to aquatic life in that water can be estimated by multiplying the 96 h LC_{50} by an application factor of 0.1.

If this application factor was used, the concentration of Cu in groundwater did exceed tolerance limits. As a consequence, Cu may have contributed to the overall toxicity of the groundwater.

Anderson and Weber (1975) found that mixtures of Cu and Zn have 2.6 times the toxicity of the predicted additive effect. Sprague (1964) observed a twofold increase in toxicity for mixtures of Cu and Zn over that predicted by simple addition of effects.

Chromium

Concentrations of total Cr less than 0.1 mg/L appeared to have no adverse effect on freshwater aquatic life (EPA 1976). This concentration was not exceeded in the groundwater (Table 27). Toxicity data from Gregory (1974) (Table 32) confirmed that the Cr concentrations in groundwater were lower than the LC_{50} level for fish.

Iron

Data describing the toxicity of Fe to various aquatic invertebrates were variable. The mayfly *Ephemerella subvaria* seemed to be particularly sensitive with a 96 h LC_{50} of only 0.32 mg/L, 50 times lower than that of two other invertebrates tested under similar conditions (Table 33). McKee and Wolf (1963) found that, at a pH of 6.7, concentrations of Fe less than 2.0 mg/L were acutely toxic to a variety of fish including carp, pike, tench, and trout. In contrast, Ellis (1937) described a concentration of 50 mg/L as the upper limit for fish life. The average concentration of Fe (1.51 mg/L) in groundwater exceeded this value. Consequently, Fe may be implicated in some of the toxic effects of the groundwater.

Table 32. Summary of some toxicity data for chromic salts, chiromates, and dichromates (Gregory 1974).

	Species	n	Size or Age	Source of Chromium	Temp (°C)	D.O. (ppm)	pH
1.	*Daphnia magna*	-	4 ± 4 h	$CrCl_3$	Lake Erie Water		
2.	*Acroneuria lycorias*	10	-	$CrCl_3 \cdot 6H_2O$	18.5	8.0	6.8
3.	*Ephemerella subvaria*	10	-	$CrCl_3 \cdot 6H_2O$	18.5	8.0	6.9
4.	*Hydropsyche betteni*	10	-	$CrCl_3 \cdot 6H_2O$	18.5	8.0	7.0
5.	*Daphnia magna*	5	12 ± 12 h	$CrCl_3 \cdot 6H_2O$	18±1	~9	7.74
6.	*Daphnia magna*	5	12 ± 12 h	$CrCl_3 \cdot 6H_2O$	18±1	~9	(7.4-8.2)
7.	*Daphnia magna*	5	12 ± 12 h	$CrCl_3 \cdot 6H_2O$	18±1	~9	7.74
8.	*Daphnia magna*	10	$\leq$ 12 h	Sodium Chromate	23±1	double distilled ref. H_2O	
9.	*Pimephales promelas* fathead minnow	10	1 to 2 g	Potassium Chromate	25	7.8	7.5
10.	*Pimephales promelas* fathead minnow	10	1 to 2 g	Potassium Dichromate	25	7.8	7.5
11.	*Pimephales promelas* fathead minnow	10	1 to 2 g	Potassium Dichromate	25	7.8	8.4→7.4

continued ...

Table 32. Continued.

	Species	n	Size or Age	Source of Chromium	Temp (°C)	D.O. (ppm)	pH
12.	*Lepomis macrochirus* bluegill	10	1 to 2 g	Potassium Dichromate	25	7.8	7.5
13.	*Lepomis macrochirus* bluegill	10	1 to 2 g	Potassium Dichromate	25	7.8	8.4→7.4
14.	*Carassius auratus* goldfish	10	1 to 2 g	Potassium Dichromate	25	7.8	7.5
15.	*Lebistes reticulatus* guppy	10	0.1 to 0.2 g	Potassium Dichromate	25	7.8	7.5
16.	*Lepomis macrochirus* bluegill	10	5 to 9 g	Potassium Dichromate	20 ± 1	aerated	∿6.5
17.	*Lepomis macrochirus* bluegill	10	5 to 9 g	Potassium Dichromate	20 ± 1	aerated	∿8.0

continued ...

Table 32. Continued.

	Species	$CaCO_3$ (ppm)	Time	LC_{50} for metal (ppm)	Comments	Ref.
1.	*Daphnia magna*		64 h	<1.2	thresh. conc. for immobilization	Anderson 1948
2.	*Acroneuria lycorias*	50	7 d	32.0		Warnick and Bell 1969
3.	*Ephemerella subvaria*	50	96 h	16.0		"
4.	*Hydropsyche betteni*	50	7 d	32.0		"
5.	*Daphnia magna*	45.3 (44 to 53)	3 wk	2.0	(0.65 to 2.6)	Biesinger and Christensen 1972
6.	*Daphnia magna*	"	3 wk	0.33	16% repro.impair.	"
7.	*Daphnia magna*	"	3 wk	0.60	50% repro.impair.	"
8.	*Daphnia magna*	"	100 h	0.42	for salt	Freeman and Fowler 1953
9.	*Pimephales promelas* fathead minnow	20	96 h	45.6		Pickering and Henderson 1966

continued ...

Table 32. Concluded.

	Species	$CaCO_3$ (ppm)	Time	LC_{50} for metal (ppm)	Comments	Ref.
10.	*Pimephales promelas* fathead minnow	20	96 h	17.6		Pickering and Henderson
11.	*Pimephales promelas* fathead minnow	360	96 h	27.3	Static bioassays	1966
12.	*Lepomis macrochirus* bluegill	20	96 h	118.0		"
13.	*Lepomis macrochirus* bluegill	360	94 h	133.0		"
14.	*Carassius auratus* goldfish	20	96 h	37.5		"
15.	*Lebistes reticulatus* guppy	20	96 h	30.0		"
16.	*Lepomis macrochirus* bluegill	45	96 h	110.0		Trama and Benoit 1960
17.	*Lepomis macrochirus* bluegill	45	96 h	120.0		"

Table 33. Summary of data describing the toxicity of iron on aquatic invertebrates.[a]

Species	n	Source of Iron	Temp (°C)	pH	D.O. (mg/L)	Time	$CaCO_3$ (mg/L)	LC_{50} for metal (mg/L)
Acroneuria lycorias	10	$FeSO_4$	18.5	7.7	8.0	9 d	48	16.0
Ephemerella subvaria	10	$FeSO_4$	18.5	8.2	8.0	96 h	48	0.32
Hydropsyche betteni	10	$FeSO_4$	18.5	8.1	8.0	7 d	50	16.0

[a] Data from Warnick and Bell (1966).

Lead — Lead concentrations less than 0.03 mg/L were considered safe for aquatic life by NAS/NAE (1974). The average concentration of Pb in groundwater was slightly lower than this level (0.028 mg/L) but was exceeded in some cases (i.e., by at least one sample at 0.142 mg/L). A comparison of Pb concentrations found in groundwater with those found to result toxicity to aquatic organisms (Table 34) indicated that:

1. Lead concentrations in groundwater were far below those which cause acute toxicity to fish; and
2. Lead concentrations were below those which cause acute toxicity to a variety of invertebrates. However, there was some indication of chronic effects in the form of reproductive impairment in *Daphnia* at concentrations within the range described for groundwater.

Manganese — McKee and Wolf (1963) stated that concentrations of 1.0 mg/L Mn were not deleterious to fish and aquatic life. Only the upper ranges of Mn concentrations in groundwater exceeded this concentration.

Mercury — Concentrations of 0.004 to 0.02 mg/L Hg have been reported harmful to freshwater fish (McKee and Wolf 1963). Schweiger (1961), however, reported that 0.2 mg/L Hg was not harmful to 1 and 2 y old tench, carp, rainbow trout, and char. The average concentration of Hg in the groundwater (0.0034 mg/L) was less than the reported harmful level; however concentrations as high as 0.07 have been recorded.

EPA (1976) summarized data concerning bioaccumulation and concentration of methyl mercury in fish tissues, as well as some data concerning toxic and chronic effects of various mercuric compounds on freshwater fish. Based on these data, a maximum concentration of 0.000 05 mg/L total Hg was recommended for the protection of human consumers of freshwater fish. This concentration is much lower than the concentrations in groundwater.

Table 34. Summary of lead toxicity data.

	Species	n	Size or Age	Source of Lead	Temp (°C)	D.O. (mg/L)	pH
1.	*Pimephales promelas*	10	1 to 2 g	$PbCl_2$	25	7.8	7.5
2.	*Pimephales promelas*	10	1 to 2 g	$PbCl_2$	25	7.8	8.4→7.4
3.	*Pimephales promelas*	10	1 to 2 g	Lead acetate	25	7.8	7.5
4.	*Lepomis macrochirus*	10	1 to 2 g	$PbCl_2$	25	7.8	7.5
5.	*Lepomis macrochirus*	10	1 to 2 g	$PbCl_2$	25	7.8	8.4→7.4
6.	*Carassius auratus*	10	1 to 2 g	$PbCl_2$	25	7.8	7.5
7.	*Lebistes reticulatus*	10	0.1 to 0.2 g	$PbCl_2$	25	7.8	7.5
8.	*Acroneuria lycorias*	10	-	$PbSO_4$	18.5	8.0	7.3
9.	*Ephermerella subvaria*	10	-	$PbSO_4$	18.5	8.0	7.0
10.	*Hydropsyche betteni*	10	-	$PbSO_4$	18.5	8.0	7.1
11.	*Daphnia magna*	10	12 ± 12 h	$PbCl_2$	18±1	~9.0	7.74
12.	*Daphnia magna*	5	12 ± 12 h	$PbCl_2$	18±1	~9.0	(7.74-7.78)
13.	*Daphnia magna*	5	12 ± 12 h	$PbCl_2$	18±1	~9.0	"
14.	*Daphnia magna*	5	12 ± 12 h	$PbCl_2$	18±1	~9.0	"

continued ...

Table 34. Concluded.

	Species	$CaCO_3$ (mg/L)	Time	LC_{50} for metal (mg/L)	Comments	Ref.
1.	*Pimephales promelas*	20	96 h	5.58 to 7.33		Pickering
2.	*Pimephales promelas*	360	96 h	482.0		and Henderson
3.	*Pimephales promelas*	20	96 h	7.48	Static bioassays	1966
4.	*Lepomis macrochirus*	20	96 h	23.8	Some pptn	"
5.	*Lepomis macrochirus*	360	96 h	442.0		"
6.	*Carassius auratus*	20	96 h	31.4		"
7.	*Lebistes reticulatus*	20	96 h	20.6		"
8.	*Acroneuria lycorias*	54	>14 d	64.0		Warnick and
9.	*Ephemerella subvaria*	52	7 d	16.0		Bell 1969
10.	*Hydropsyche betteni*	54	7 d	32.0		"
11.	*Daphnia magna*	45.3	48 h	0.45	With food	Besinger and
12.	*Daphnia magna*	(44 to 53)	3 wk	0.30		Christensen
13.	*Daphnia magna*	"	3 wk	0.03	16% repro. impair.	1972
14.	*Daphnia magna*	"	3 wk	0.10	50% repro. impair.	"

Nickel — Data from Gregory (1974) (Table 35) indicated sublethal effects on *Daphnia magna* reproduction at Ni concentrations found in the groundwater (0.002 to 0.32 mg/L). These concentrations were, however, within the tolerance range for most aquatic organisms even when a suggested application factor of 0.01 was used (EPA 1976).

Anderson et al. (1979) found that mixing V and Ni resulted in decreased lethal potency of the mixture, and that both Ni and V, singly or in combination, interacted with phenol to cause a synergistic form of multiple toxicity.

Selenium — A concentration of 2.0 mg/L Se (as sodium selenite) has been reported toxic to goldfish in 8 d and lethal in 18 to 46 d (McKee and Wolf 1963). The concentration of Se in groundwater average 0.0014 mg/L, far below this level. EPA (1976) recommended an application factor 0.01 times the 96 h LC_{50}, determined using a sensitive resident species, as the maximum tolerance level for freshwater aquatic life. If resident species were similar in sensitivity to goldfish, it seems unlikely Se makes a major contribution to the overall toxicity of groundwater.

Silver — EPA (1976) summarized data which showed a wide variation in the toxicity of Ag compounds to aquatic life, which was affected by the dissociation characteristic of these compounds. Based on the little information available, a factor of 0.01 times the LC_{50}, determined by bioassays of a sensitive resident species, was recommended as the maximum tolerance level.

Toxic levels of silver nitrate (0.003 mg/L) have been reported for sticklebacks. The LC_{50} for guppies was reported at 0.0043 mg/L (McKee and Wolf 1963). The concentrations of Ag in groundwater (0.005 to 0.03 mg/L) might have exceeded these concentrations, making it likely that Ag contributed to the overall toxicity of the groundwater.

Table 35. Summary of some nickel toxicity data.[a]

Species	n	Size or Age	Source of Nickel	Temp (C)	D.O. (ppm)	pH	$CaCO_3$ (ppm)	Time
1. *Pimephales promelas*	10	1 to 2 kg	$NiCl_2 \cdot 6H_2O$	25	7.8	7.5	20	96 h
2. *Pimephales promelas*	10	1 to 2 kg	$NiCl_2 \cdot 6H_2O$	25	7.8	8.4 to 7.4	360	96 h
3. *Lepomis macrochirus*	10	1 to 2 kg	$NiCl_2 \cdot 6H_2O$	25	7.8	7.5	20	96 h
4. *Lepomis macrochirus*	10	1 to 2 kg	$NiCl_2 \cdot 6H_2O$	25	7.8	8.4 to 7.4	360	96 h
5. *Carassius auratus*	10	1 to 2 kg	$NiCl_2 \cdot 6H_2O$	25	7.8	7.5	20	96 h
6. *Lebistes reticulatus*	10	0.1-0.2 g	$NiCl_2 \cdot 6H_2O$	25	7.8	7.5	20	96 h
7. *Acroneuria lycoria*	10	-	$NiSO_4 \cdot 6H_2O$	18.5	8.0	7.0	40	96 h
8. *Ephemerella subvaria*	10	-	$NiSO_4 \cdot 6H_2O$	18.5	8.0	7.0	40	96 h
9. *Hydropsyche betteni*	10	-	$NiSO_4 \cdot 6H_2O$	18.5	8.0	7.0	40	14 d
10. *Daphnia magna*	10	12 12 h	$NiCl_2$	18 1	9.0	7.74 (7.4-8.2)	45.3 (44-53)	48 h
11. *Daphnia magna*	10	12 12 h	$NiCl_2$	18 1	9.0	"	"	48 h
12. *Daphnia magna*	5	12 12 h	$NiCl_2$	18 1	9.0	"	"	3 wk
13. *Daphnia magna*	5	12 12 h	$NiCl_2$	18 1	9.0	"	"	3 wk
14. *Daphnia magna*	5	12 12 h	$NiCl_2$	18 1	9.0	"	"	3 wk
15. *Pimephales promelas*	-	-	$NiSO_4$	-	-	-	200	

continued ...

Table 35. Concluded.

Species	LC_{50} for metal (ppm)	Comments	Reference
1. *Pimephales promelas*	4.58 5.18	Static bioassay	Pickering and Henderson
2. *Pimephales promelas*	42.40 44.50		1966
3. *Lepomis macrochirus*	5.18 5.36		
4. *Lepomis macrochirus*	39.60		
5. *Carassius auratus*	9.82		
6. *Lebistes reticulatus*	4.45		
7. *Acroneuria lycoria*	33.50		Warnick and Bell
8. *Ephemerella subvaria*	4.0		1969
9. *Hydropsyche betteni*	64.0		
10. *Daphnia magna*	0.51	Without food	Biesinger and
11. *Daphnia magna*	1.12	With food	Christensen
12. *Daphnia magna*	0.13	(0.098 to 0.178) 50% repro. impair.	1972
13. *Daphnia magna*	0.03	16% repro. impair.	
14. *Daphnia magna*	0.095		
15. *Pimephales promelas*	0.40	"Safe conc."	

[a] Data from Gregory (1974).

Vanadium

Giles et al. (1979) found that V as vanadium peroxide was moderately toxic to rainbow trout and whitefish (96 h LC_{50} for trout 6.4 mg/L; for whitefish 17.4 mg/L) and that toxicity increased slightly with decreasing pH. Sprague et al. (1978a) found that pentavalent V concentrations of 2.4 to 5.6 mg/L were lethal to rainbow trout in 7 d. Over a range of pH from 5.5 to 8.8, V was slightly more toxic at intermediate pH values (6.6 to 7.7) and slightly less toxic in acid water than in alkaline water. Acute toxicity to rainbow trout was slightly greater in soft water (30 mg/L $CaCO_3$ out of the range 30 to 350 mg/L $CaCO_3$). There were no apparent effects of size upon resistance to V at lethal concentrations. For American flagfish, 28 d LC_{50}'s were reported at 0.9 to 1.9 mg/L.

Anderson et al. (1979) found that pentavalent V at a concentration of 4.8 mg/L was lethal to rainbow trout in 7.2 d. The 96 h LC_{50} was 11.5 mg/L and the 14 d LC_{50} was 2.8 mg/L. These authors also reported gill irritation, high frequency of coughing, loss of equilibrium, and loss of appetite in rainbow trout during exposure to sublethal concentrations of V. Histopathological lesions were observed in lamellar epithelium and boundary membranes of the gut.

Giles et al. (1979) observed pronounced histopathological lesions in the gills and kidneys of trout exposed to sublethal concentrations of V. The extent of damage increased with length of exposure. Eyed eggs of trout were 250 to 300 times more resistant to V than fingerlings. The toxicant did not appear to induce histopathological lesions in the developing embryos. Acute exposure to V did not produce significant changes in nine cardiovascular/respiratory parameters or liver catalase and xanthine oxidase of adult trout. Juvenile whitefish actively avoided V at concentrations $\geq$ 0.5 mg/L.

Sprague et al. (1978a) observed an increase in early mortality of American flagfish exposed to sublethal concentrations >0.17 mg/L V. The egg-fry stage appeared to be the most sensitive to V on chronic exposure. After 28 d, or for larger fish throughout exposure, these concentrations appeared to produce no obvious effects. Exposure to 0.17 mg/L caused no mortality but produced a marginal decrease in the growth rate of second generation fry. Concentrations of 0.41 mg/L had positive effects. Females increased in length and weight in comparison with controls, and spawning was more rapid and prolific. Based on these observations, the lower threshold for chronic toxicity of V to American flagfish was determined to be 0.08 mg/L. There was no evidence that V had any long-term cumulative toxicity, nor were there deleterious effects upon the hatchability of eggs.

Mean concentration of V in the groundwater was 0.004 (0.001 to 0.05) mg/L, hardness was usually greater than 35 mg/L $CaCO_3$, and pH was usually 8.0 to 8.3. Together these data suggested that V in the groundwater was, in itself, unlikely to be toxic to aquatic organisms.

Anderson et al. (1979) found that mixing V and Ni resulted in decreased lethal potency of the mixture and that both Ni and V, singly or in combination, interacted with phenol to cause a synergistic form of multiple toxicity.

Zinc

Thompson (1974) discussed the variable and contradictory evidence regarding lethal concentrations of Zn. Data from Gregory (1974) (Table 36) indicated that most of the 96 h LC_{50}'s reported were lower than the mean concentration of Zn in groundwater (0.024 mg/L). However, if the recommended factor of 0.01 (EPA 1976) were applied to some of the reported LC_{50} values, the upper range of Zn concentration would exceed the tolerance levels for aquatic life. Lake and Rogers (1979) noted in one of their bioassay experiments that two fish in the highest concentrations of groundwater

Table 36. Summary of zinc toxicity data.

Species	n	Size or Age	Source of Zinc	Temp (°C)	D.O. (mg/L)	pH	$CaCO_3$ (mg/L)
1. *Salmo gairdneri*	10	Fingerlings	$ZnSO_4 \cdot 7H_2O$	17.5	-	6.6→6.7	12
2. *Salmo gairdneri*	10	"	$ZnSO_4 \cdot 7H_2O$	17.5	-	7.0→7.2	50
3. *Salmo gairdneri*	10	"	$ZnSO_4 \cdot 7H_2O$	17.5	-	7.6→7.8	320
4. *Lepomis macrochirus*	10	1→2 g	$ZnSO_4 \cdot 7H_2O$	25.0	7.8	7.5	20
5. *Lepomis macrochirus*	10	1→2 g	$ZnSO_4 \cdot 7H_2O$	15.0	7.8	7.5	20
6. *Lepomis macrochirus*	10	1→2 g	$ZnCl_2$	25.0	7.8	7.5	20
7. *Lepomis macrochirus*	10	1→2 g	$ZnSO_4 \cdot 7H_2O$	25.0	7.8	8.4→7.4	360
8. *Carassius auratus*	10	1→2 g	$ZnSO_4 \cdot 7H_2O$	25.0	7.8	7.5	20
9. *Lebistes reticularus*	10	0.1→0.2 g	$ZnSO_4 \cdot 7H_2O$	25.0	7.8	7.5	20
10. *Pimephales promelas*	10	1→2 g	$ZnSO_4 \cdot 7H_2O$	25.0	7.8	7.5	20
11. *Pimephales promelas*	10	1→2 g	$ZnSO_4 \cdot 7H_2O$	15.0	7.8	7.5	20
12. *Pimephales promelas*	10	1→2 g	$ZnSO_4 \cdot 7H_2O$	25.0	7.8	8.4→7.4	360
13. *Pimephales promelas*	10	1→2 g	$ZnSO_4 \cdot 7H_2O$	25±1	7.3	6.0	50
14. *Pimephales promelas*	10	1→2 g	$ZnSO_4 \cdot 7H_2O$	25±1	7.9	6.0	100
15. *Pimephales promelas*	10	1→2 g	$ZnSO_4 \cdot 7H_2O$	25±1	7.2	6.0	200
16. *Pimephales promelas*	10	1→2 g	$ZnSO_4 \cdot 7H_2O$	25±1	6.8	7.0	50

continued ...

Table 36. Continued.

Species	n	Size or Age	Source of Zinc	Temp (°C)	D.O. (mg/L)	pH	$CaCO_3$ (mg/L)
17. *Pimephales promelas*	10	1→2 g	$ZnSO_4 \cdot 7H_2O$	25± 1	-	7.0	100
18. *Pimephales promelas*	10	1→2 g	$ZnSO_4 \cdot 7H_2O$	25±1	6.8	7.0	200
19. *Pimephales promelas*	10	1→2 g	$ZnSO_4 \cdot 7H_2O$	25±1	7.4	8.0	50
20. *Pimephales promelas*	10	1→2 g	$ZnSO_4 \cdot 7H_2O$	25±1	6.6	8.0	100
21. *Pimephales promelas*	10	1→2 g	$ZnSO_4 \cdot 7H_2O$	25±1	7.1	8.0	200
22. *Pimephales promelas*	10	2→3 g	$ZnSO_4 \cdot 7H_2O$	23.0	6.7	7.7	203
				(1.7→10.8)		(7.4→8.3)	(192→221)
23. *Pimephales promelas*	10	2→3 g	$ZnSO_4 \cdot 7H_2O$	23.0	"	"	"
24. *Salmo salar*	5	14.3 cm	$ZnSO_4 \cdot 7H_2O$	15.0	-	7.3→7.6	20
25. *Salmo salar*		(10.2→ 9.2)					
	5	"	$ZnSO_4 \cdot 7H_2O$	15.0	-	7.3→7.6	20
26. *Salmo salar*	5	"	$ZnSO_4 \cdot 7H_2O$	5.0	-	7.3→7.6	20
27. *Salmo salar*	5	"	$ZnSO_4 \cdot 7H_2O$	5.0	-	7.3→7.6	20
28. *Salmo salar*	8	9.2 cm	-	17±0.5	-	7.0→7.4	14
29. *Salmo salar*		(7.2→10.2)	-	17±0.5	-	7.0→7.4	14
30 *Daphnia magna*	10	12±12 h	$ZnCl_2$	18±1	∿9.0	7.7	45
						(7.4→8.2)	(44→53)

continued ...

Table 36. Continued.

Species	n	Size or Age	Source of Zinc	Temp (°C)	D.O. (mg/L)	pH	$CaCO_3$ (mg/L)
31. *Daphnia magna*	10	12±12 h	$ZnCl_2$	18±1	∿9.0	7.7 (7.4→8.2)	45.3 (44.0→53)
32. *Daphnia magna*	10	12±12 h	$ZnCl_2$	18±1	∿9.0	"	"
33. *Daphnia magna*	10	12±12 h	$ZnCl_2$	18±1	∿9.0	"	"
34. *Daphnia magna*	10	12±12 h	$ZnCl_2$	18±1	∿9.0	"	"
35. *Acroneuria lycurias*	10	-	$ZnSO_4 \cdot 7H_2O$	18.5	8.0	7.6	50
36. *Ephemerella subvaria*	10	-	$ZnSO_4 \cdot 7H_2O$	18.5	8.0	7.6	54
37. *Hydropsyche betteni*	10	-	$ZnSO_4 \cdot 7H_2O$	18.5	8.0	7.6	52

continued ...

Table 36. Continued.

Species	Time	LC_{50} for metal (mg/L)	Comments	Ref.
1. *Salmo gairdneri*	48 h	4	Zinc ppt at high pH values	Lloyd 1960
2. *Salmo gairdneri*	48 h	0.5	Static bioassays	
3. *Salmo gairdneri*	48 h	5.46 4.85 4.61	Based on zinc added some ppt	Pickering and Henderson 1966
4. *Lepomis macrochirus*	96 h	6.44		
5. *Lepomis macrochirus*	96 h	5.37		
6. *Lepomis macrochirus*	96 h			
7. *Lepomis macrochirus*	96 h	40.90	(Interpolation) pH decreased	
8. *Carassius auratus*	96 h	6.44	Static bioassays	
9. *Lebistes retucularus*	96 h	1.27		
10. *Pimephales promelas*	96 h	0.96 0.78		
11. *Pimephales promelas*	96 h	6.44		
12. *Pimephales promelas*	96 h	33.40		
13. *Pimephales promelas*	96 h	12.5 13.8	Cont. flow	Mount 1966
14. *Pimephales promelas*	96 h	18.5 28.0		
15. *Pimephales promelas*	96 h	29.0 35.5	ZnSO $7H_2O$ reduced	
16. *Pimephales promelas*	96 h	13.7 6.2	the pH at higher levels	

continued ...

Table 36. Continued.

Species	Time	LC_{50} for metal (mg/L)		Comments	Ref.
17. *Pimephales promelas*	96 h	12.5	12.5		
18. *Pimephales promelas*	96 h	19.0	13.6		
19. *Pimephales promelas*	96 h	4.7	5.1		
20. *Pimephales promelas*	96 h	8.1	9.9	pH 8- zinc ppt	
21. *Pimephales promelas*	96 h	8.2	15.5		
22. *Pimephales promelas*	96 h	12.0	13.0	Static bioassays	Brungs 1969
23. *Pimephales promelas*	96 h	8.4	10.0	Cont. flow	
24. *Salmo salar*	96 h	0.6	ILL	Cont. flow	Sprague 1964
25. *Salmo salar*	7 h	2.89		Cont. flow	
26. *Salmo salar*		0.9			
27. *Salmo salar*	28 h	$\geq$ 2.89	ILL[a]		
28. *Salmo salar*	-	0.42			Sprague and
29. *Salmo salar*	-	-			Ramsay 1965
30. *Daphnia magna*	48 h	0.10		Cont. flow (without	Biesinger and
31. *Daphnia magna*	48 h	0.28		food)	Christensen
Daphnia magna	48 h	0.28		With food	1972
32. *Daphnia magna*	3 wk	0.14	0.15 0.17		
33. *Daphnia magna*	3 wk	0.07		16% repro. impair.	
34. *Daphnia magna*	3 wk	0.102		50% repro. impair.	

continued ...

Table 36. Concluded.

Species	Time	LC_{50} for metal (mg/L)	Comments	Ref.
35. *Acroneuria lycurias*	14 d	32.0		Warnick and Bell
36. *Ephemerella subvaria*	10 d	16.0		1969
37. *Hydropsyche betteni*	11 d	32.0		

[a] ILL - Initial Lethal Level.

(100%) exhibited some signs of haemorrhaging from the gills as well as some clogging of the gills with mucus. Chronically toxic concentrations caused general enfeeblement, widespread histological changes to organs, and retardation of growth and maturation.

Copper and Zn are known to react synergistically. Anderson and Weber (1975) found that mixtures of Cu and Zn have 2.6 times the toxicity of the predicted additive effect. Sprague (1964) observed a twofold increase in toxicity for mixtures of Cu and Zn over that predicted by simple addition of the effects of each. Skidmore (1964) reported that salts of alkaline-earth metals are antagonistic to the action of Zn salts, whereas salts of some heavy metals have synergistic effects in soft water.

6.5.2 Cations

A comparison of mine depressurization groundwater with Athabasca River water (Table 30) shows that Na ion concentrations are highly elevated, and that the Ca ion concentrations are only twice the river concentrations. Magnesium and K ion concentrations are intermediate to these extremes. Cations Ca, Mg, K, and Na are reviewed in the following paragraphs.

Calcium

Water hardness in fresh water is caused principally by dissolved Ca and Mg ions. Doudoroff and Katz (1953) have presented data showing that increasing Ca concentrations reduced the toxicity of other heavy metals. McKee and Wolf (1963) also found that Ca in water reduces the toxicity of many heavy metals to fish and other aquatic forms. Experimental data summarized by Gregory (1974) (Table 37) indicated that increased Ca in water, due to increased hardness, decreased the sensitivity of certain freswhater species to Zn. McKee and Wolf (1963) reported that the lethal threshold concentration for sticklebacks is 800 mg/L and levels toxic to fish have been reported between 300 and

Table 37 . Summary of toxicity data for zinc in various concentrations of calcium.

Zinc Salt	n	Temp (C)	Time	$CaCO_3$ (mg/L)	LC_{50} for metal (mg/L)	Reference
Salmo gairdneri						
$ZnSO_4 \cdot 7H_2O$	10	17.5	48 h	12	∿4.0	Lloyd 1960
$ZnSO_4 \cdot 7H_2O$	10	17.5	48 h	50	0.5	
$ZnSO_4 \cdot 7H_2O$	10	17.5	48 h	320	5.46 4.85 4.61	Pickering and Henderson 1966
Lepomis macrochirus						
$ZnSO_4 \cdot 7H_2O$	10	25.0	96 h	20	6.4	
$ZnSO_4 \cdot 7H_2O$	10	15.0	96 h	20	5.3	
$ZnSO_4 \cdot 7H_2O$	10	25.0	96 h	360	40.9	
Pimephales promelas						
$ZnSO_4 \cdot 7H_2O$	10	25.0	96 h	20	0.9 0.78	
$ZnSO_4 \cdot 7H_2O$	10	15.0	96 h	20	6.4	
$ZnSO_4 \cdot 7H_2O$	10	25.0	96 h	360	33.4	
$ZnSO_4 \cdot 7H_2O$	10	25.0 ±1	96 h	50	12.5 13.8	Mount 1966
$ZnSO_4 \cdot 7H_2O$	10	25.0 ±1	96 h	100	18.5 28.0	
$ZnSO_4 \cdot 7H_2O$	10	25.0 ±1	96 h	200	29.0 35.5	
$ZnSO_4 \cdot 7H_2O$	10	25.0 ±1	96 h	50	13.7 6.2	
$ZnSO_4 \cdot 7H_2O$	10	25.0 ±1	96 h	100	12.5 12.5	
$ZnSO_4 \cdot 7H_2O$	10	25.0 ±1	96 h	200	19.0 13.6	

continued ...

Table 37 . Concluded.

Zinc Salt	n	Temp (C)	Time	$CaCO_3$ (mg/L)	LC_{50} for metal (mg/L)	Reference
$ZnSO_4 \cdot 7H_2O$	10	25.0 ±1	96 h	50	4.7 5.1	Mount 1966
$ZnSO_4 \cdot 7H_2O$	10	25.0 ±1	96 h	100	8.1 9.9	
$ZnSO_4 \cdot 7H_2O$	10	25.0 ±1	96 h	200	8.2 15.5	

1000 mg/L Ca. Calcium concentrations reported for groundwater were 1.0 to 318 mg/L.

Magnesium McKee and Wolf (1963) stated that magnesium chloride and magnesium nitrate can be toxic to fish at concentrations between 100 and 400 mg/L Mg; however, some freshwater fish have been found in saline lake water containing over 1000 mg/L Mg. The concentration of Mg in groundwater exceeded the toxic level indicated by some of the experimental data summarized by Gregory (1974) Table 38.

Potassium Potassium has been found to be toxic to fish at concentrations of 50 to 200 mg/L; however, the toxicity is reduced by the presence of Ca and Na (McKee and Wolf 1963). The concentrations of K in groundwater (19 to 65 mg/L) were generally below the toxic concentrations.

Sodium Concentrations of Na 500 to 1000 mg/L have been found to be toxic to fish (McKee and Wolf 1963). Other reports, however, indicated that concentrations as high as 7870 mg/L were harmless to sticklebacks (Doudoroff and Katz 1953). The mean concentration of Na in groundwater (5622/6 mg/L) exceeded the toxic level indicated in all data given by Gregory (1974) (Table 39).

6.5.3 Anions

In comparison with water from the Athabasca River, chloride and bicarbonate concentrations were very high in mine depressurization groundwater (Table 30). Generalizations about specific ion toxicity were difficult because each mixture with other salts had to be evaluated separately. Gregory (1974) discussed the toxicity of selected anions. Carbonate, bicarbonate, and chloride anions are reviewed in the following paragraphs.

Carbonate Carbonates and bicarbonates were seldom considered to be detrimental to aquatic organisms, but their buffering action and effect on pH may have contributed to toxicity at high pH values (McKee and Wolf 1963).

Table 38. Summary of toxicity data for magnesium salts.[a]

Magnesium Salt	n	Temp (C)	Time	Salt	LC_{50} Magnesium (ppm)	Comments	Reference
Daphnia magna							
$MgCl_2 \cdot 6H_2O$	10	18 ± 1	48 h		140	Without food	Biesinger and
$MgCl_2 \cdot 6H_2O$	10	18 ± 1	48 h		322	With food	Christensen
$MgCl_2 \cdot 6H_2O$	5	18 ± 1	3 wk		190	167 to 217	1972
$MgCl_2 \cdot 6H_2O$	5	18 ± 1	3 wk		82	16% repro. impair.	
$MgCl_2 \cdot 6H_2O$	5	18 ± 1	3 wk		125	50% repro. impair.	
$MgSO_4$	ND[b]	ND	96 h	788	159		Dowden and Bennett 1965
Lepomis macrochirus							
$MgSO_4$	ND	ND	24 h	1 900	383		
Lymaea sp.							
$MgSO_4$	ND	ND	96 h	6 250	1 262		
					↑	If anhydrous salts, these values will be slightly less.	

[a] Data from Gregory (1974).

[b] ND = No Data.

Table 39. Summary of toxicity data for sodium salts.[a]

Sodium Salt	n	Temp (C)	Time	Salt	LC_{50} Sodium (ppm)	Comments	Reference
Daphnia magna							
Na_2CO_3	10	23 ± 1	100 h	102	44.3		Freeman and
Na_2SO_4	10	23 ± 1	100 h	4 547	1 405.0		Fowler 1953
$NaHSO_3$	10	23 ± 1	100 h	102	23.0		
Na_2SiO_3	10	23 ± 1	100 h	247	93.0		
NaCl	10	23 ± 1	38 h	3 318	1 303		Dowden 1960
Na_2CO_3	10	23 ± 1	38 h	265	115		
Na_2SO_4	10	23 ± 1	38 h	2 564	830		
$NaNO_3$	10	23 ± 1	38 h	3 581	968		
Na_2SiO_3	10	23 ± 1	38 h	494	80		
Culex pipiens - larvae							
Na_2CO_3	10	23 ± 1	48 h	600	260		Dowden and
$NaHCO_3$	10	23 ± 1	48 h	2 000	489		Bennett 1965
$NaHSO_3$	10	23 ± 1	48 h	300	66		
Hyallela							
Na_2CO_3	ND[b]	ND	96 h	67	29		Dowden and
Na_2CO_3	ND	ND	24 h	67	29		Bennett 1965

continued ...

Table 39. Concluded.

Sodium Salt	n	Temp (C)	Time	Salt	LC_{50} Sodium (ppm)	Comments	Reference
Lepomis macrochirus							
Na_2CO_3	ND	ND	24 h	385	167		Dowden and Bennett 1965
Daphnia magna							
NaCl	10	18 ± 1	48 h		1 640	Without food	Biesinger and
NaCl	10	18 ± 1	48 h		1 820	With food	Christensen
NaCl	5	18 ± 1	3 wk		1 480	1 180 to 1 840	1972
NaCl	5	18 ± 1	3 wk		680	16% repro. impair.	
NaCl	5	18 ± 1	3 wk		1 020	50% repro. impair.	

[a] Data from Gregory (1974).

[b] ND - No Data.

Chloride Concentrations of 400 mg/L Cl have been found to be lethal to trout. Concentrations as high as 2000 mg/L have been reported as harmless to some fish (McKee and Wolf 1963). These concentrations were exceeded by those of groundwater (2250 to 10500 mg/L).

6.6 FIELD TOXICITY OF GROUNDWATER

The only field investigations performed using mine depressurization groundwater have been those on Beaver Creek (RRCS 1974). Beaver Creek flows across the leasehold of Syncrude Canada Ltd. Its lower reaches are utilized as a receiving body for saline water discharged from dragline operations on the Syncrude mining site. The major conclusions of the RRCS (1974) study were:

1. Chlorides and turbidity have increased in Beaver Creek as a result of groundwater discarge;
2. Mayfly and stonefly nymphs have largely disappeared from the sampling sites; and
3. No adverse effects of saline discharges were observed on either fish or aquatic macrophytes.

Field studies to assess the survival of fish and invertebrates held under experimental conditions in Beaver Reservoir, a body of water which receives mine depressurization groundwater, were sponsored by Syncrude Canada Ltd., but no results are yet available (J. Retallack, Limnologist, Syncrude Canada Limited, personal communication).

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8. LIST OF AOSERP RESEARCH REPORTS

Report	Project	Reference
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2	AF 4.1.1	Kristensen, J., B.S. Ott, and A.D. Sekerak. 1976. Walleye and goldeye fisheries investigations in the Peace-Athabasca Delta--1975. Prep. for the Alberta Oil Sands Environmental Research Program by LGL Ltd., Environmental Research Associates. AOSERP Report 2. 103 pp.
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