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Aquatic Environments Limited

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TOXICITY OF SALINE GROUNDWATER FROM SYNCRUDE'S LEASE 17 TO FISH AND BENTHIC MACROINVERTEBRATES

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FOREWORD

The layer of clay which underlies the tar sand in the centre of Syncrude's mining area is dissected by several ancient river channels. These channels contain brackish water under enough pressure to threaten the stability of the mine walls. Pumping the water to the surface relieves the hydrostatic pressure but requires an environmentally safe disposal system. Aquatic Environments Ltd. (AEL) was hired by Syncrude to determine the toxicity of the water to fish and to smaller aquatic organisms. AEL's report is reproduced here in its entirety.

It is Syncrude's policy to publish its environmental consultants' final reports as they are received, withholding only proprietary technical information or that of a financial nature. Because we do not necessarily base our decisions on just one consultant's opinion, recommendations found in the text should not be construed as commitments to action by Syncrude.

Syncrude Canada Ltd. welcomes public and scientific interest in its environmental activities. Please address any questions or comments to Syncrude Environmental Affairs, 9915 - 108 Street, EDMONTON, Alberta, T5K 2G8.

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GLOSSARY OF TERMS

Incipient Lethal Level (ILL). "That level of an environmental entity beyond which 50% of a population of animals cannot live for an indefinite time" (Sprague, 1969)(This author considers Incipient LC50 to be a better term). The term ILL is not used in this report as it implies much longer test periods than were used here and also assumes a degree of acclimation to the environmental entity tested.

Median Lethal Concentration (LC50). The concentration which is toxic to 50% of animals tested over a stated exposure time; i.e., LC50 (96 hour) is the concentration causing 50% mortality in a 96 hour period. This parameter, calculated by the Reed-Muench method, was used exclusively in the present study. This is the term most commonly used in the North American literature and is equivalent to LD50 (Median Lethal Dose) used in pharmacology.

Median Tolerance Limit (TLM). The concentration of an environmental entity at which 50% of the animals tested are just able to survive in a stipulated exposure time. Similar to LC50 in meaning but usually obtained directly from plots of per cent mortality against Log_{10} concentration.

Osmolarity. An expression of concentration in osmotically active terms. For example, for sodium chloride (NaCl), an osmolar solution contains:

$$\frac{\text{Molecular Wt (g) per litre}}{\text{Dissociation Constant}} = \frac{58.45}{1.9} = 30.76 \text{ g/litre}$$

A milli-osmolar solution contains:

$$\frac{1 \text{ osmole}}{1000} = 0.03078 \text{ g/litre} = 30.8 \text{ mg/litre} = 30.8 \text{ ppm NaCl}$$

Thus, a 400 milli-osmolar solution = 400 x 30.8 NaCl equivalents or is equivalent to 12,320 ppm NaCl.

Osmolar units were used in this study as the ionic constituents of effluent are not known and osmolarity provides a convenient method of determining the sum of osmotic effects. Figure 1 indicates the relationship between osmolarity, the concentration of NaCl, and per cent NaCl.

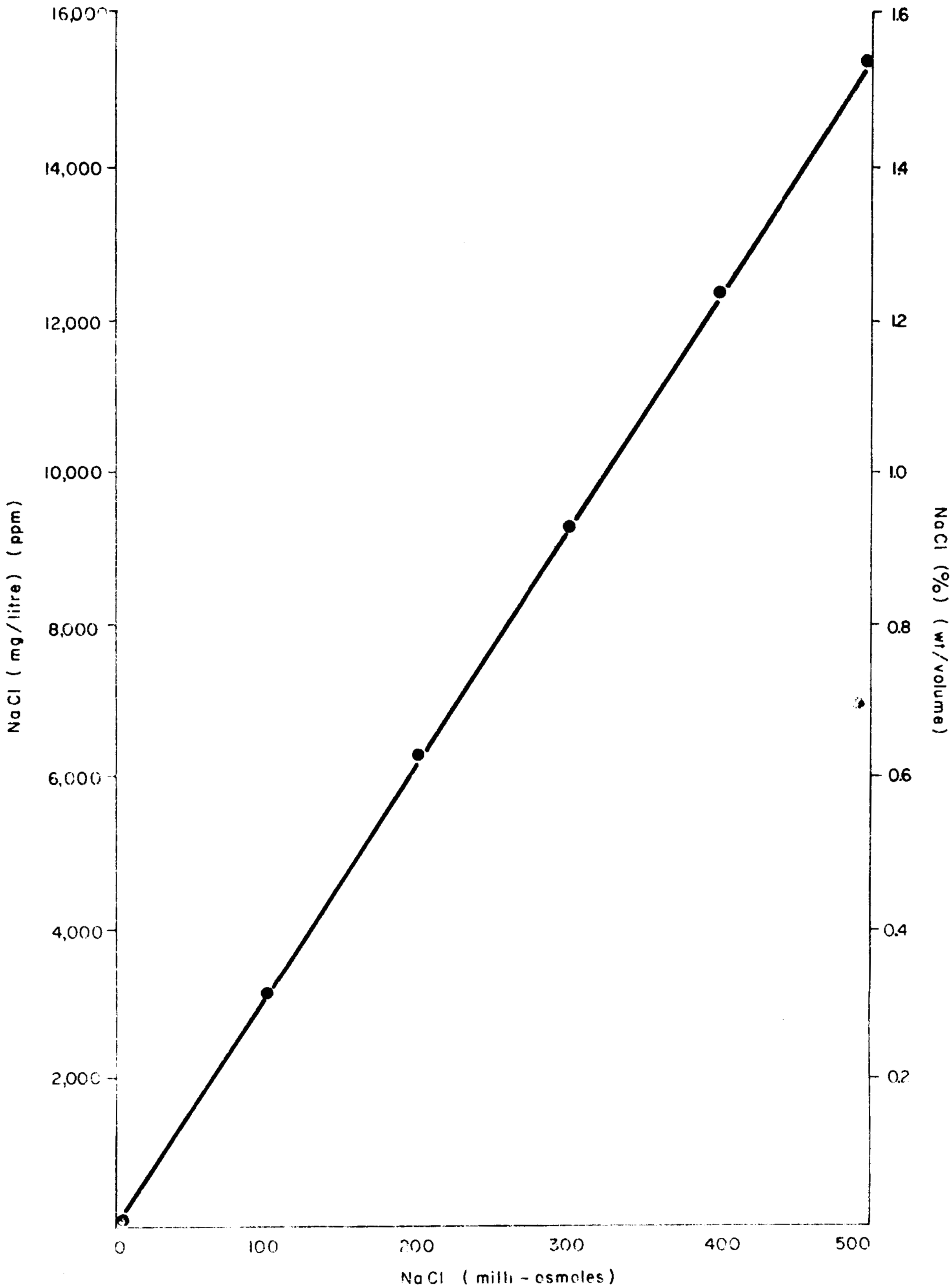

FIGURE 1. Relationship of osmolarity to concentration (ppm) and percent NaCl.

INTRODUCTION

The mining of the tar sands which are included in the area to be developed by Syncrude Canada Ltd. will require the dewatering of the mine pits. This will involve the pumping of large volumes of saline groundwater. Present plans call for its eventual disposal through Ruth Lake, the Poplar River and, finally, the Athabasca River. This study was designed to determine whether groundwater from the mine area is toxic to aquatic organisms and, if so, the concentrations at which this toxicity is expressed. A variety of species, including both fish and aquatic insects, was tested to determine the range of sensitivity among aquatic animals. The resultant data can, with some qualifications, be used to estimate the maximum safe concentrations of groundwater which can be added to natural waters with minimal risk of toxic effects.

METHODS

Acute Toxicity Tests

Fish

Juveniles and Adults

Juvenile rainbow trout 50 to 120 mm fork length and 6 to 13 g in weight were obtained from Townsend's Trout Lodge, Tacoma, Washington (Table 1). The trout were shipped by air in plastic bags surrounded by fibreglas insulation. The rainbow trout is the standard species for toxicological studies of fish and provides a basis for comparison with other studies.

Other fish were obtained from natural populations (Table 1). These included:

1) species collected from the Athabasca River in the vicinity of Syncrude's Lease 17 (lake chub, flathead chub, trout-perch, walleye),

2) species known to occur in the Athabasca River but collected from populations elsewhere (grayling, white sucker, lake chub),

3) the mountain whitefish which occurs in the middle and upper segments of the Athabasca River, though it has not yet been taken in the vicinity of Syncrude's Lease 17.

TABLE 1. Sources and size range of juvenile and adult fish used in toxicity studies. Unless otherwise indicated, all locations are in Alberta.

Species	Common Name	Location Captured or Obtained	Size Range Length (cm)	Size Range Weight (gm)
Salmo gairdneri	rainbow trout	Hatchery - Washington State	5.0-12.0	6.0-13.0
Prosopium williamsoni	mountain whitefish	Red Deer River	6.0-7.0	2.5-4.6
Thymallus arcticus	arctic grayling	Norman Wells area - N.W.T.	4.7-5.5	0.5-1.2
Couesius plumbeus	lake chub	Nose Creek	4.0-6.0	0.4-2.4
Couesius plumbeus	lake chub	Athabasca River	5.9-7.6	2.4-5.2
Platygobio gracilis	flathead chub	Athabasca River	8.7-11.3	6.1-11.8
Catastomus commersoni	white sucker	Nose Creek	3.0-7.0	2.5-8.0
Percopsis omiscomaycus	trout perch	Athabasca River	4.8-7.5	1.2-4.0
Stizostedion vitreum vitreum	walleye	Athabasca River	6.2-8.1	1.3-4.5

Fish from natural populations were captured either with a small-mesh, nylon marquissette beach seine or with a Smith Root Backpack Electroshocker. Captured fish were placed in plastic bags for transport. Those from remote locations were cooled with ice and shipped by air in insulated styrofoam chests.

On arrival in Calgary, all fish were taken to the University of Calgary freshwater aquarium where the plastic bags were placed in large aquaria so that temperature equilibration could occur. After several hours, the fish were released from the bags and entered the aquaria which were supplied with a continuous flow of dechlorinated city water which ranged in temperature from 6 to 9°C (seasonally dependent). Fish remained in these holding tanks for periods of 1 to 4 weeks during which they were fed a commercially prepared fish food.

As each test series began, the fish were removed from the holding facilities, placed in controlled environment chambers, and acclimated for one week in aerated 30 litre aquaria containing the diluent (control medium) at one of the two test temperatures, 5 or 15°C. Within the controlled environment chambers, temperatures varied ±0.5°C under conditions of constant light. Fish continued to be fed throughout the acclimation period.

After the acclimation period, the fish were distributed among the test aquaria. The latter contained either diluent alone (controls) or various concentrations of saline groundwater in diluent. For most of the tests conducted, Athabasca River water, obtained in the vicinity of Syncrude's Lease 17, was used as the diluent. The water was shipped to Calgary in fibreglas-lined, 45 gallon drums and stored at 5°C until used. To determine any possible toxicity of the Athabasca River water itself (e.g., as the result of deterioration in storage), a limited series of experiments was performed using dechlorinated Calgary tap water as a diluent. These experiments involved only rainbow trout and mountain whitefish.

The groundwater used in the experiments was obtained through the auspices of Syncrude Canada Ltd. from wells on Syncrude's Lease 17. Most of the groundwater used in the studies came from a well identified as 2600S, 4800E. However, a few early experiments, involving rainbow trout and mountain whitefish, were conducted using groundwater from two unidentified wells on Lease 17. Data describing the inorganic materials present in water from Well 2600S, 4800E are presented in Table 2 along with similar data for other wells in the vicinity. Values for various parameters for Well 2600S, 4800E fall within the range for other wells in the area. No comparable data are available for the two unidentified wells whose water was used in experiments. However, their waters did differ in osmolarity (one at 230 and one at 400 mOsm) from those of Well 2600S, 4800E (350 to 380 mOsm). In addition, water from the high osmolarity well was unusual in that it formed a heavy purple precipitate while in storage. A sample of this precipitate was subjected to semiquantitative analysis (Table 3) which indicated relatively high proportions of several metals.

TABLE 2. Results of water chemistry analysis for groundwater from wells on Syncrude Lease 17 including Well 2600S, 4800E, and other wells in the vicinity. Also, analyses for water from the Athabasca River in the vicinity of Lease 17. Data provided by Syncrude Canada Ltd. Values in mg/l unless otherwise indicated.

Element or Compound	2600S 4800E n=4			Wells in area n=72			Athabasca River n=4		
		Range			Range			Range	
	$\bar{x}$	high	low	$\bar{x}$	high	low	$\bar{x}$	high	low
Na	4,242.0	4,500	3,885	3,517.0	10,698	564	10.5	16	8
K	38.5	48	31	33.0	112	4	1.1	2	.6
Ca	48.5	96	14	56.0	316	3	35.3	44	33
Mg	51.3	59	35	69.6	411	3	9.3	14	6
Cl	4,488.0	4,807	3,890	4,560.0	19,854	19	66.0	99	28
HCO_3	3,188.0	3,523	2,626	2,016.7	3,431	532	111.0	132	78
SO_4	30.0	49	19	66.5	377	0	27.0	41	15
CO_3	166.0	446	nil	494.0	1,230	0	14.0	43	nil
SiO_2	-	-	-	22.4	27	18	-	-	-
PO_4	-	-	-	0.3	.41	.17	-	-	-
Nitrates	-	-	-	0.3	.75	.2	-	-	-

(Continued)

TABLE 2. Continued.

Element or Compound	2600S 4800E n= 4			Wells in area n=28			Athabasca River n=4		
		Range			Range			Range	
	$\bar{x}$	high	low	$\bar{x}$	high	low	$\bar{x}$	high	low
Aluminum	<1.00	-	-	1.60	5.20	<0.01	<1.00	-	-
Arsenic	<0.01	-	-	<0.01	-	-	<0.01	-	-
Barium	0.10	0.20	-	<1.00	-	-	<1.00	-	-
Boron	4.30	5.30	3.50	5.80	8.00	2.00	<1.00	-	-
Cadmium	<0.01	0.01	<0.01	<0.05	0.32	<0.01	<0.01	-	-
Chromium	<0.01	-	-	<0.01	-	-	<0.01	-	-
Cobalt	<0.03	0.07	<0.01	<0.01	-	-	<0.01	-	-
Copper	<0.01	0.01	<0.01	<0.01	-	-	<0.01	-	-
Iron	0.93	1.10	0.75	11.40	128.00	<0.01	0.65	0.91	0.43
Lead	<0.10	0.26	<0.01	0.05	0.22	<0.01	<0.01	-	-
Manganese	<0.01	0.01	<0.01	0.90	6.63	<0.01	<0.01	-	-
Mercury (ppb)	1.10	2.20	<0.20	<0.20	-	-	<0.20	-	-
Nickel	<0.02	-	-	<0.02	-	-	<0.02	-	-
Titanium	<1.00	-	-	<1.00	-	-	<1.00	-	-
Vanadium	<1.00	-	-	<1.00	-	-	<1.00	-	-
Zinc	0.02	0.02	<0.01	0.65	7.51	<0.01	0.03	0.06	<0.01
TDS (approx.)	12,262	13,537	10,504	10,864	36,613	1,146	280	672	169

TABLE 3. Chemical analysis of Syncrude groundwater precipitate from unidentified well. Further explanation in text.

	Lower Concentration Limit (ppm)	Number 144-03-03
Antimony	50	bcl
Arsenic	50	bcl
Barium	5	2,000
Beryllium	5	bcl
Bismuth	5	bcl
Boron	20	2,000
Cadmium	20	bcl
Calcium	0.05%	0.5%
Chromium	10	bcl
Cobalt	10	10
Copper	1	50
Gallium	2	5
Germanium	20	bcl
Iron	0.05%	1.0%
Lead	5	50
Magnesium	0.02%	0.2%
Manganese	5	200
Molybdenum	10	bcl
Nickel	5	50
Niobium	50	bcl
Silver	1	2
Strontium	20	1,000
Tantalum	200	bcl
Tellurium	200	bcl
Thorium	100	bcl
Tin	10	20
Titanium	5	500
Vanadium	10	20
Zinc	50	2,000
Zirconium	20	bcl

Concentration Range

>5,000 ppm =	>5,000 ppm	50 ppm =	25-100 ppm
5,000 ppm =	2,500-10,000 ppm	20 ppm =	10-50 ppm
2,000 ppm =	1,000 - 4,000 ppm	10 ppm =	5-20 ppm
1,000 ppm =	500 - 2,000 ppm	5 ppm =	2-10 ppm
500 ppm =	250 - 1,000 ppm	2 ppm =	1-4 ppm
200 ppm =	100 - 400 ppm	1 ppm =	0.5-2 ppm
100 ppm -	50 - 200 ppm	bcl =	below concentration limit.

Ranges for Iron, Calcium and Magnesium are report in %.

Water from wells on Lease 17 also contains a relatively high concentration of dissolved organic compounds. Some of these are dissolved gases which are rapidly volatized in the atmosphere (Table 4), others remain in solution. Indicators of the presence of organic compounds are the values for oil and grease and for phenols found in Beaver Creek during September, 1975, when large volumes of saline water were being discharged (Tables 5 and 6). Although, as the data indicate, organic compounds are present in the discharge water, these have not yet been subjected to detailed analysis and their exact identities are not known.

The methods used in the preparation of the test solutions and in the conduct of these tests are standard for acute toxicity tests of fish and other aquatic organisms and are those outlined by Duodoroff (1960) and Sprague (1969). The concentrations of groundwater used are indicated in Table 7. In some instances, tests at intermediate concentrations were eliminated because of a scarcity of fish.

Test solutions were prepared in 30 litre aquaria, placed in a constant environment chamber at the test temperature, and aerated under a glass cover for a period of at least 24 hours prior to the introduction of the test fish. At the beginning of the test, 5 to 20 fish (depending on availability and size) were placed in each aquarium. The glass cover was replaced and the aquarium gently aerated with a bubble-filter situated in the corner of the tank. Oxygen tensions were monitored daily using a Radiometer BMS3 PHM71 Blood Gas Analyser. The levels never dropped below 6.5 mg/l O_2 with most values being near saturation

TABLE 4. Analyses of gaseous components of groundwater from Syncrude Lease 17. Data supplied by Syncrude Canada Ltd.

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 5. Results of analyses of a mixture of saline groundwater and natural flow in Beaver Creek, Syncrude Lease 17. Data supplied by Syncrude Canada Ltd.

Date 1975	Chloride (mg/1)	pH	Suspended Solids (105°) (mg/1)	Oil and Grease (mg/1)	Phenols (mg/1)	C.O.D. (mg/1)
3/9	21.5	8.0	83.0	0.3	0.018	100.5
10/9	18.6	8.3	26.7	2.0	0.015	89.0
17/9	66.0	7.9	10.7	0.9	0.019	73.9
24/9	93.0	8.1	9.3	2.9	0.017	46.0

TABLE 6. Characteristics of saline water discharged into Beaver Creek from ponds by the test pit. Data supplied by Syncrude Canada Ltd.

Date 1975	Cl^- (mg/l)	Oil and Grease (mg/l)	pH
3/9	2,300	1.3	8.2
9/9	2,550	2.7	8.6
17/9	2,480	*	8.4
24/9	2,330	3.7	8.6

*Sample spoiled.

-All samples collected immediately downstream of discharge to Beaver Creek.

-Pumping from test pit ceased October 1, 1975.

-Estimated quantity for September - 600,000 cu. ft.

TABLE 7. Standard dilutions of saline groundwater and diluent (either Athabasca River water or dechlorinated Calgary tap water) used in acute toxicity tests of fish and benthic macroinvertebrates.

Concentration % groundwater	Volume groundwater (ℓ)	Volume diluent (ℓ)	Log_{10} concentration
0.00 (control)	0.00	30.00	-
0.12	0.04	29.96	0.92
1.25	0.38	26.63	0.10
5.01	1.50	29.50	0.70
12.60	3.78	26.22	1.10
31.60	9.48	20.52	1.50
50.10	15.10	14.90	1.70
79.40	23.82	6.18	1.90
100.00	30.00	0.00	2.00

(8 to 11 mg/1 in Calgary depending on solute concentrations). These are well above the 5 mg/1 which Duodoroff (1960) recommends as a minimum concentration for acute toxicity tests. pH values, recorded with the same instrument, ranged from 8.5 to 9.5 units during the test period. Osmolarity was also monitored (with an Advanced Instruments Osmometer) at the beginning and end of each test. In no case was there any significant change in osmolarity during the course of an experiment.

In most instances, fish remained in the tanks in constant light, without food, for a period of 168 hrs (7 days). Exceptions were a few tests (mountain whitefish at 15°C at 48 hrs; grayling at 15°C after 96 hrs) in which mortality in the control tank exceeded 10%, the maximum permissible level suggested by Duodoroff (1960). In these instances, the experiments were terminated and only those data recorded prior to the control mortality are presented in this report.

In one series of experiments with rainbow trout and mountain whitefish, the osmolarities of various test concentrations of groundwater were duplicated using artificial seawater salt (Utility Seven-Seas Marine Mix) diluted with Athabasca River water. This was an attempt to isolate the effects of osmotic stress from toxic effects of other constituents of saline groundwater from Syncrude's Lease 17. Experimental conditions were otherwise similar to those in the tests utilizing saline groundwater. Conditions throughout all tests are summarized in Table 8.

For most tests, mortalities were recorded each hour for the first

24 hour period and at the end of every 24 hour period thereafter to a total of 196 hours. Fish were judged to be dead when respiration had ceased and they did not respond when touched.

Statistical analysis included a calculation of LC50 (median lethal concentration at specific times) according to the Reed-Muench Method (Woolf, 1968).

Eggs and Alevins

Eyed rainbow trout eggs for acute toxicity tests were obtained from Townsend's Trout Lodge, Tacoma, Washington. The eggs were shipped by air to Calgary International Airport and then transported to AEL's laboratory. In the laboratory, the eggs, which had been cooled by crushed ice in transit, were transferred to pans of dechlorinated water, placed in controlled temperature units, and equilibrated to 5°C. The temperature within the unit was raised 5°C per day over the next three days to a final temperature of approximately 20°C. This high temperature was used to accelerate development and, during the ten day period of the experiment, the eggs progressed from the late eyed stage through hatching and the early alevin (yolk-sac) stage. The eggs remained at 20°C over the next two days and were then divided into 20 lots of 250 eggs apiece. Each lot was placed in a screen-bottomed plexiglas container which was then immersed in a 30 litre aquarium container holding either dechlorinated tap water (control) or various concentrations of saline groundwater from Well 2600S, 4800E diluted with dechlorinated tap water. The dilutions were those described above (Table 7) as standard for toxicity tests. The lots were replicated (i.e., two

TABLE 8. Summary of conditions during acute toxicity tests of saline groundwater from Syncrude Lease 17.

Species	Temperature (°C)	Diluent	Toxicant	Toxicant Osmolarity (mOsm)	Test Animals Average (No./tank)	Test Animals (total no.)
Salmo gairdneri (juveniles)	5	Athabasca	groundwater	230	4	26
		Athabasca	saline water	400	20	180
		Athabasca	groundwater	400	16	148
		Dechlorinated	saline water	400	20	180
		Dechlorinated	groundwater	400	17	139
	15	Athabasca	groundwater	230	5	27
(eggs/alevins)	20	Dechlorinated	groundwater	380	500	5,000
Prosopium williamsoni	5	Athabasca	groundwater	230	9	57
		Athabasca	groundwater	400	5	26
		Athabasca	saline water	400	7	63
		Dechlorinated	groundwater	230	10	60
	15	Athabasca	groundwater	230	5	25
		Athabasca	saline water	400	5	45
Thymallus arcticus	5	Athabasca	groundwater	375	12	112
	15	Athabasca	groundwater	375	12	108
Couesius plumbeus (Athabasca origin)	15	Athabasca	groundwater	350	10	90
Couesius plumbeus (Nose Creek origin)	5	Athabasca	groundwater	375	10	84
	15	Athabasca	groundwater	375	9	73

(Continued)

TABLE 8. Continued.

Species	Temperature (°C)	Diluent	Toxicant	Toxicant Osmolarity (mOsm)	Test Animals Average (No./tank)	Test Animals (total no.)
Platygobio gracilis	5	Athabasca	groundwater	350	5	42
Catastomus commersoni	5	Athabasca	groundwater	350	8	72
	15	Athabasca	groundwater	350	8	74
Percopsis omiscomaycus	5	Athabasca	groundwater	375	6	54
	15	Athabasca	groundwater	375	5	47
Stizostedion vitreum vitreum	5	Athabasca	groundwater	375	6	43
	15	Athabasca	groundwater	375	7	49
INSECT EXPERIMENTS						
Heptagenia marginalis	5	Athabasca	groundwater	350	20	180
	15	Athabasca	groundwater	350	10	90
Paraleptophlebia bicornuta	5	Athabasca	groundwater	375	10	90
Isogenus (Isogenoides) sp.	5	Athabasca	groundwater	375	9	81
	15	Athabasca	groundwater	350	5	45
Hydropsyche bifida	5	Athabasca	groundwater	375	15	135
	15	Athabasca	groundwater	350	15	135

containers were placed in each 30 litre aquarium) and two control tanks (four lots) were used.

During the course of the experiment, the aquaria were sheathed with aluminum foil and covered to exclude light, as the eggs and alevin stages are rather sensitive to this stimulus.

Each day, water temperatures and oxygen concentrations were monitored and mortalities were assessed. Water temperature was always close to ambient room temperature, approximately 20°C, and oxygen concentrations were near saturation throughout the experiment. The test was terminated at 200°C-days.

The methods of data analysis were similar to those described above for juvenile and adult fish.

Benthic Macroinvertebrates

Some preliminary acute toxicity tests were carried out with benthic macroinvertebrates collected in the Athabasca River near Syncrude's Lease 17 and in the Bow River near Calgary. The test species included the following:

Athabasca River

Order Ephemeroptera	*Heptagenia marginalis*

Bow River

Order Ephemeroptera	*Paraleptophlebia bicornuta*
Order Plecoptera	*Isogenus (Isogenoides)* sp.
Order Trichoptera	*Hydropsyche bifida*

The species taken in the Bow River are all representative of genera known to occur in the Athabasca River in the vicinity of Syncrude's Lease 17.

The specimens were collected by washing bottom materials through plastic screening. Captured invertebrates were transferred to filled plastic bags for shipment to the laboratory. Those from the Athabasca River were shipped by air in an ice-cooled insulated chest.

Groups of 5 to 20 individuals of each insect species were segregated in insect environment chambers consisting of a plastic (high impact polystrene) cup with its sides partially removed and replaced with fibreglas insect netting (W.L. Peters, Aquatic Entomology Laboratory, Florida A&M University, Tallahassee, personal communication). They were acclimated to Athabasca River water at test temperatures of 5° and 15°C for a period of five days then placed in 30 litre aquaria containing various concentrations of groundwater from Well 2600S, 4800E. The experimental procedures and the methods of data analysis were identical to those previously described for juvenile and adult fish.

Swimming Performance Tests

Swimming performance fatigue tests were carried out with rainbow trout obtained from the Sam Livingston Hatchery in Calgary. The fish were transferred to the aquarium facilities at the University of Calgary where they were held in dechlorinated Calgary tap water at 10°C. As each experiment began, the fish were removed from the holding facilities and placed in 20 litre acclimation aquaria, 10 fish per tank, where they were held for a period of 7 to 17 days. During the acclimation period, the tanks were aerated and filtered with glass wool and activated charcoal. Temperatures were maintained at the eventual test temperature, 5±0.1°C. In most experiments, the fish remained in the same medium throughout the acclimation period (Table 9). However, in two experiments (Nos. 6 and 7) the fish were acclimated to Athabasca River water for a period of four days then transferred to either 5%

(Experiment 6) or 10% (Experiment 7) groundwater for a further four days before fatigue tests began.

The fatigue tests were conducted in a tunnel respirometer (Figure 2) similar to that described by Brett (1964). All tests were performed at a velocity of 35 cm/sec. Dissolved oxygen was monitored throughout each experiment and maintained at saturation values. Temperatures were maintained at 5±0.1C.

Only fish with fork lengths between 9.0 cm and 10.9 cm were used in the experiments. Fish were transferred from the acclimation aquaria and tested individually in the respirometer. Each fish was tested only once.

After being placed in the test chamber of the respirometer, fish were allowed a 30 minute settling period during which the velocity was maintained at a slow 10 cm/sec. At the end of the settling period, the velocity was accelerated from 10 to 35 cm/sec in five equal steps each three minutes in duration. In most instances, timing started when the 35 cm/sec velocity was reached and ended when fish became fatigued. Fish were considered to be fatigued when they could no longer maintain a position upstream of the electrified screen at the downstream end of the chamber and were swept down onto the screen.

Some fish exhibited very erratic and excited behaviour in the test chamber, darting about in all directions. These fish either refused

TABLE 9. Summary of conditions during acclimation and test periods, swimming performance tests. In every case where groundwater is indicated, Athabasca River water was used as a diluent. All groundwater was from Well 2600S, 4800E.

Experiment	Acclimation Medium: Source	Acclimation Medium: Osmolarity (mOsm)	Acclimation Medium: Temperature (°C)	Test Water: Source	Test Water: Osmolarity (mOsm)	Test Water: Temperature (°C)	Number Test Fish
1	Calgary tap	6	5	Calgary tap	6	5	12
2	Calgary tap	6	5	Athabasca River	10	5	11
3	Athabasca River	10	5	Athabasca River	10	5	21
4	Athabasca River	10	5	10% groundwater	47	5	10
5	Athabasca River	10	5	20% groundwater	84	5	15
6	Athabasca River (4 days)	10	5	5% groundwater	28	5	18
	5% groundwater (4 days)	28	5				
7	Athabasca River (4 days)	10	5	10% groundwater	47	5	24
	10% groundwater (4 days)	47	5				

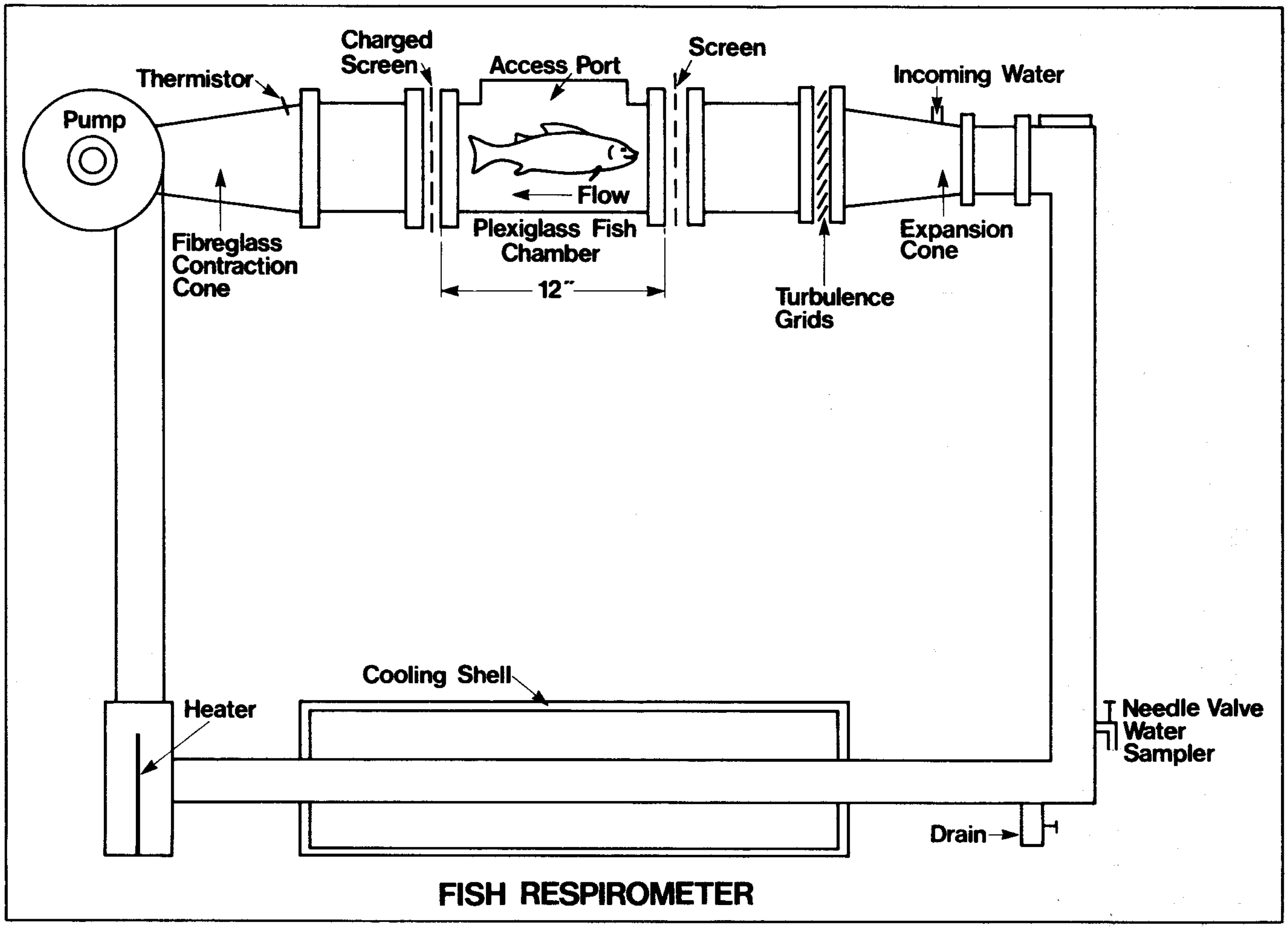

FIGURE 2. Fish respirometer. After Brett (1964).

to swim or became fatigued soon after the velocity was reached. All fish behaving in this manner, a total of nine, were recorded as "excited" and were omitted from data analysis.

RESULTS

Acute Toxicity Tests

Fish--Juveniles and Adults

Comparison of Athabasca River Water and Calgary Tap Water as Diluents

Figure 3 and Appendix Tables 3, 5, 8, 11 compare LC50's for rainbow trout and mountain whitefish exposed to groundwater concentrations in which either Athabasca River water or dechlorinated Calgary tap water were used as diluents. Groundwater of different osmolarities was used in testing the two species (400 mOsm for rainbow trout and 230 mOsm for mountain whitefish) so that the data are not strictly comparable. No significant difference in toxicity was observed between the two diluents but there is a trend towards an enhanced toxicity of groundwater when diluted with Athabasca River water. For rainbow trout, this trend is most apparent early in the experiment, up to 5 days. For mountain whitefish, it is most apparent from day 6 to the end of the experiment at day 11. This may be because the Athabasca River water already contains some of the toxic materials present in the groundwater.

Comparison Between Waters from Different Wells

For rainbow trout and mountain whitefish, data are available (Figure 4 and Appendix Tables 1, 3, 8, 9) to compare results for groundwater from two different sources, one with an osmolarity of 230 mOsm and the other of 400 mOsm.

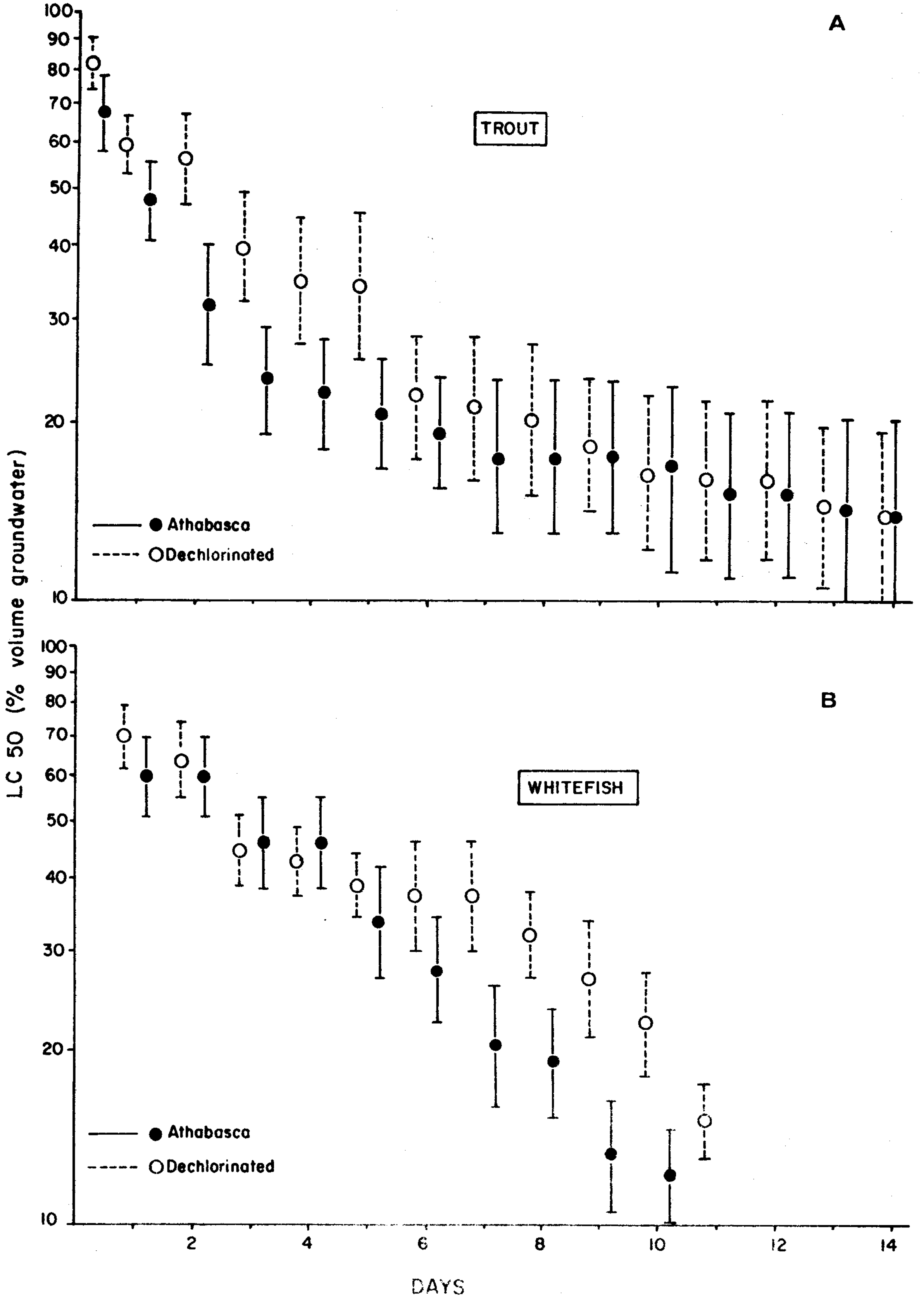

FIGURE 3. A. Comparison of LC50 values (and 70% confidence limits) for rainbow trout (*S. gairdneri*) tested at 5°C in saline groundwater diluted in either Athabasca River water or dechlorinated Calgary tapwater.
B. Comparison of LC50 values for mountain whitefish (*P. williamsoni*) tested at 5°C in saline groundwater diluted in either Athabasca River water or dechlorinated Calgary tapwater.

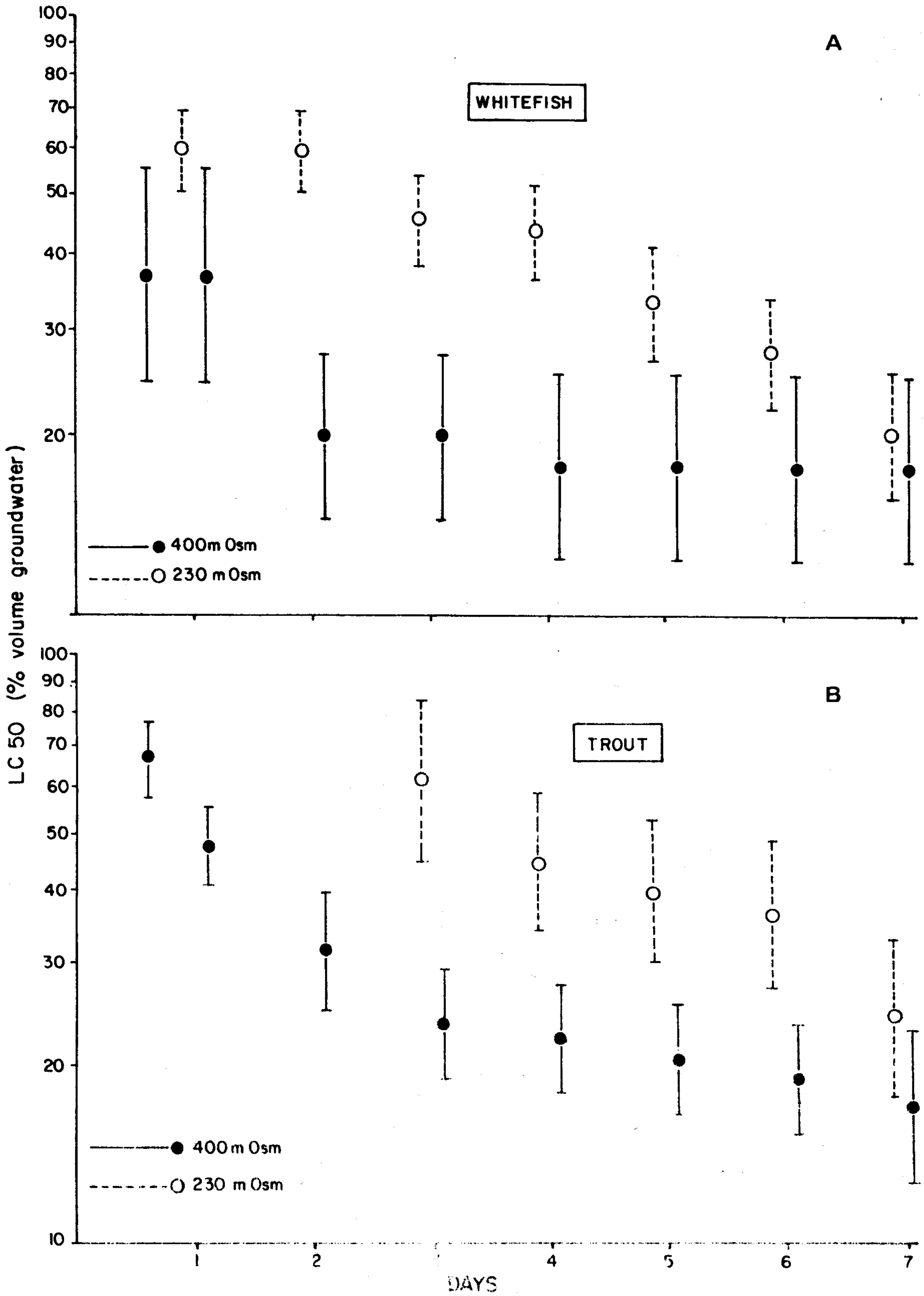

FIGURE 4. A. Comparison of LC50 values (and 70% confidence limits) for mountain whitefish (*P. williamsoni*) tested at 5°C in saline groundwater of 400 and 230 mOsmoles.

B. Comparison of LC50 values for rainbow trout (*S. gairdneri*) tested at 5°C in saline groundwater of 400 and 230 mOsmoles.

The tests were performed at 5°C. The data indicate that, for both species, the higher osmolarity water is considerably more toxic over most of the test period. This presumably reflects a greater concentration of toxicants in the higher osmolarity water. By the seventh day of the experiments, however, the LC50's for the low and high osmolarity waters are beginning to converge, suggesting that some factor, at least partly independent of concentration, is operative in the long term.

Comparisons of Mortalities at 5° and 15°C

For most species, data are available comparing LC50 values at 5° and 15°C (Figures 5 through 12, and Appendix Tables 1 through 25). For fish, there is a great deal of variation in response. For two species, the lake chub and grayling, mortalities were greater at the higher test temperature. Values for other species were variable from time period to time period. This is in contrast to the aquatic insects tested, for which mortalities were consistently and markedly higher at the more elevated test temperature.

Comparison of Effects of Groundwater and Artificial Sea-Salt

In order to separate the effects of osmotic stress from those of possible toxic materials included in groundwater from Syncrude's Lease 17, a series of tests was performed with rainbow trout and mountain whitefish to compare mortalities in solutions of groundwater and artificial sea-salt of similar osmolarity.

There are two distinct life history types within the species *Salmo* *gairdneri*, the freshwater-resident rainbow trout and the anadromous (sea-going) steelhead. Even the non-anadromous, freshwater resident rainbow populations are known to have a high tolerance to salinity (Conte and Wagner, 1965). Mountain whitefish, on the other hand, appear to be entirely freshwater resident and no anadromous populations are known. The species would be presumed to have a low tolerance to salinity.

Figures 13 and 14, Appendix Tables 2, 3, 9, 10 present data for rainbow trout and mountain whitefish tested in groundwater and artificial sea-salt, each with an osmolarity of 400 mOsm. Rainbow trout suffered no mortality in artificial sea-salt, but the solution was moderately toxic to mountain whitefish which began to die early in the experiment. Both species were susceptible to groundwater, though over most of the test period, rainbow trout were more resistant than mountain whitefish. By day 7, however, the LC50 values for the two species were very similar, suggesting that long exposure to groundwater would have a similar effect on the two species.

The results indicate that salinity, and a resultant osmotic stress, do not themselves cause mortality to rainbow trout at any length of exposure up to 168 hr or to mountain whitefish up to 72 hrs. Salinity may be a cumulative factor enhancing toxic effects due to other sources in the case of whitefish, but certainly cannot be regarded as the primary toxicant for even this more sensitive species. The results of this experiment suggest that we must look at other constituents of the groundwater to explain the toxicity levels observed.

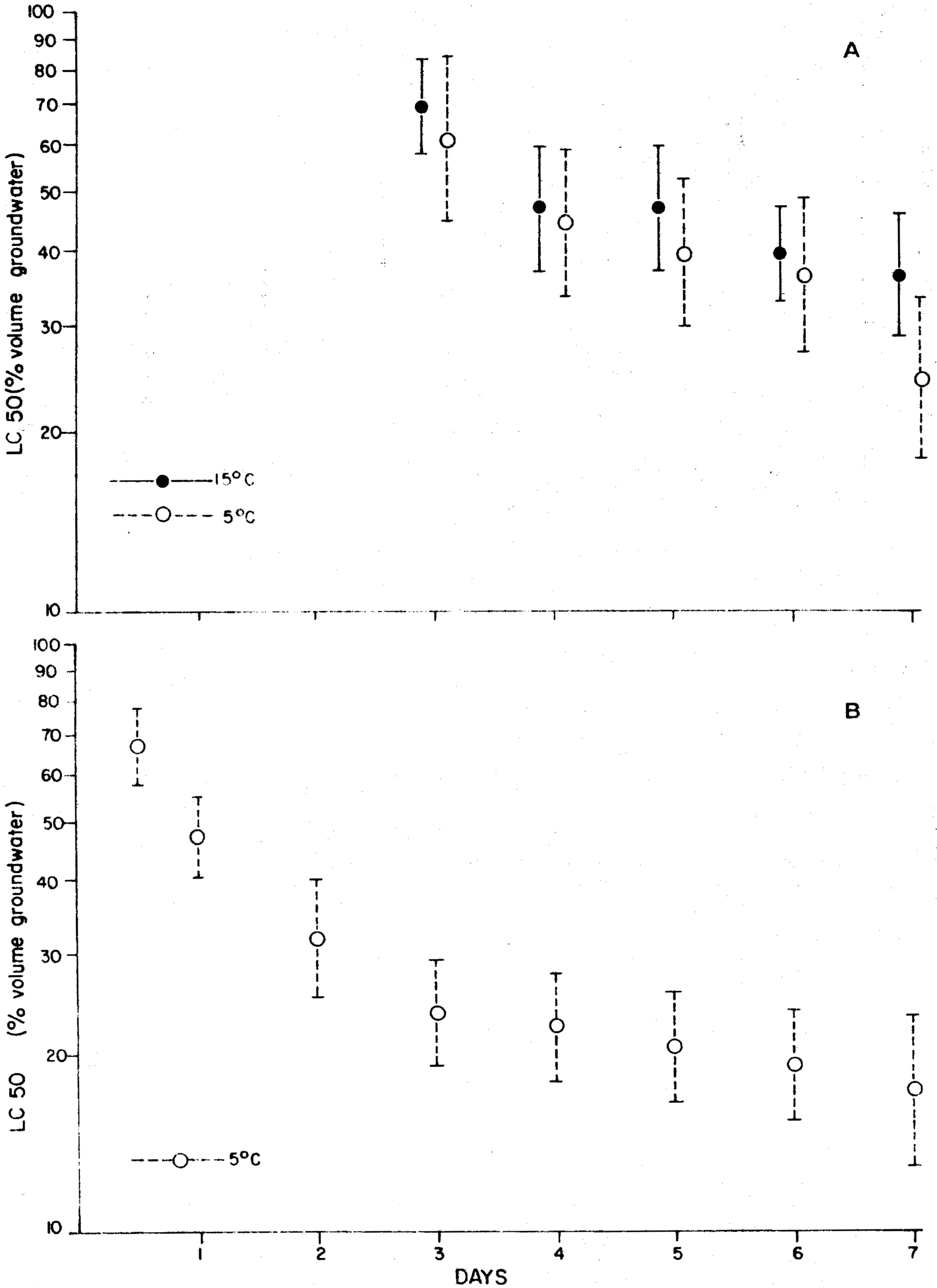

FIGURE 5. A. LC50 values and 70% confidence limits for rainbow trout *(S. gairdneri)* tested at 5° and 15°C in saline groundwater of 230 mOsmoles original concentration.

B. LC50 values for rainbow trout tested at 5°C in saline groundwater of 400 mOsmoles original concentration.

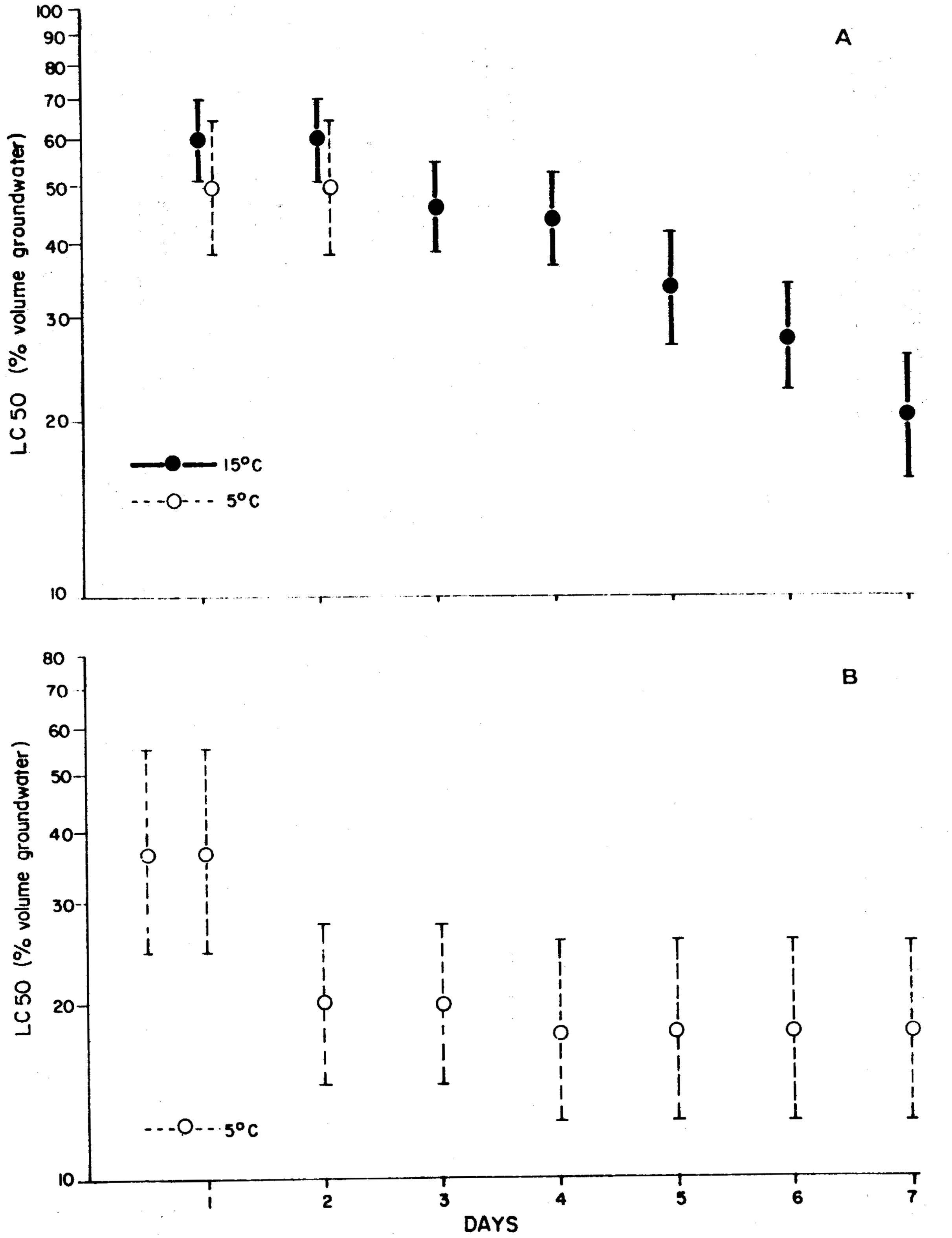

FIGURE 6. A. LC50 values and 70% confidence limits for mountain whitefish *(P. williamsoni)* tested at 5°C and 15°C in saline groundwater of 230 mOsmoles original concentration.
B. LC50 values for mountain whitefish tested at 5°C in saline groundwater of 400 mOsmoles original concentration.

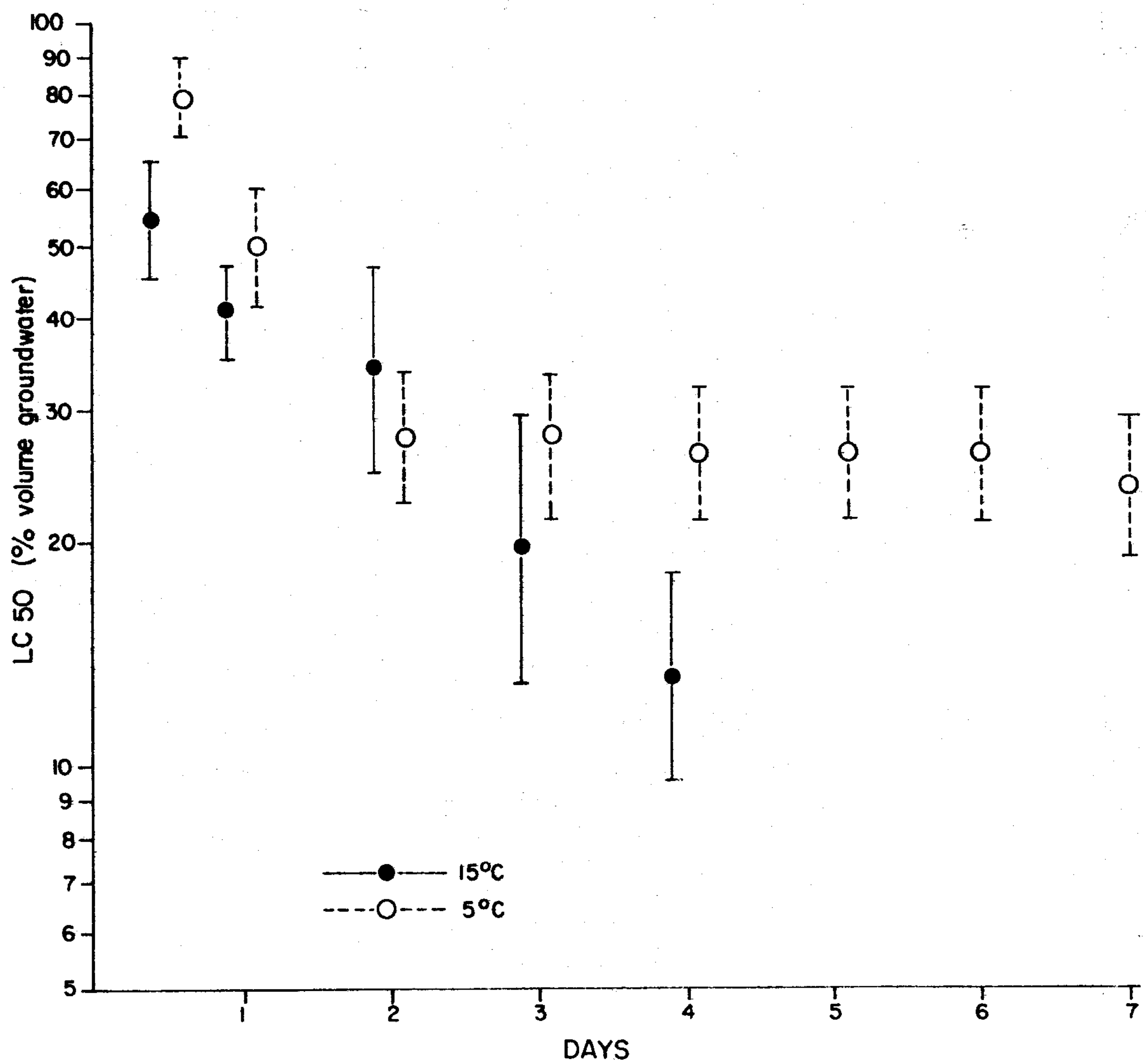

FIGURE 7. LC50 values and 70% confidence limits for grayling (*T. arcticus*) from the vicinity of Norman Wells, N.W.T., tested in saline groundwater at 5°C and 15°C.

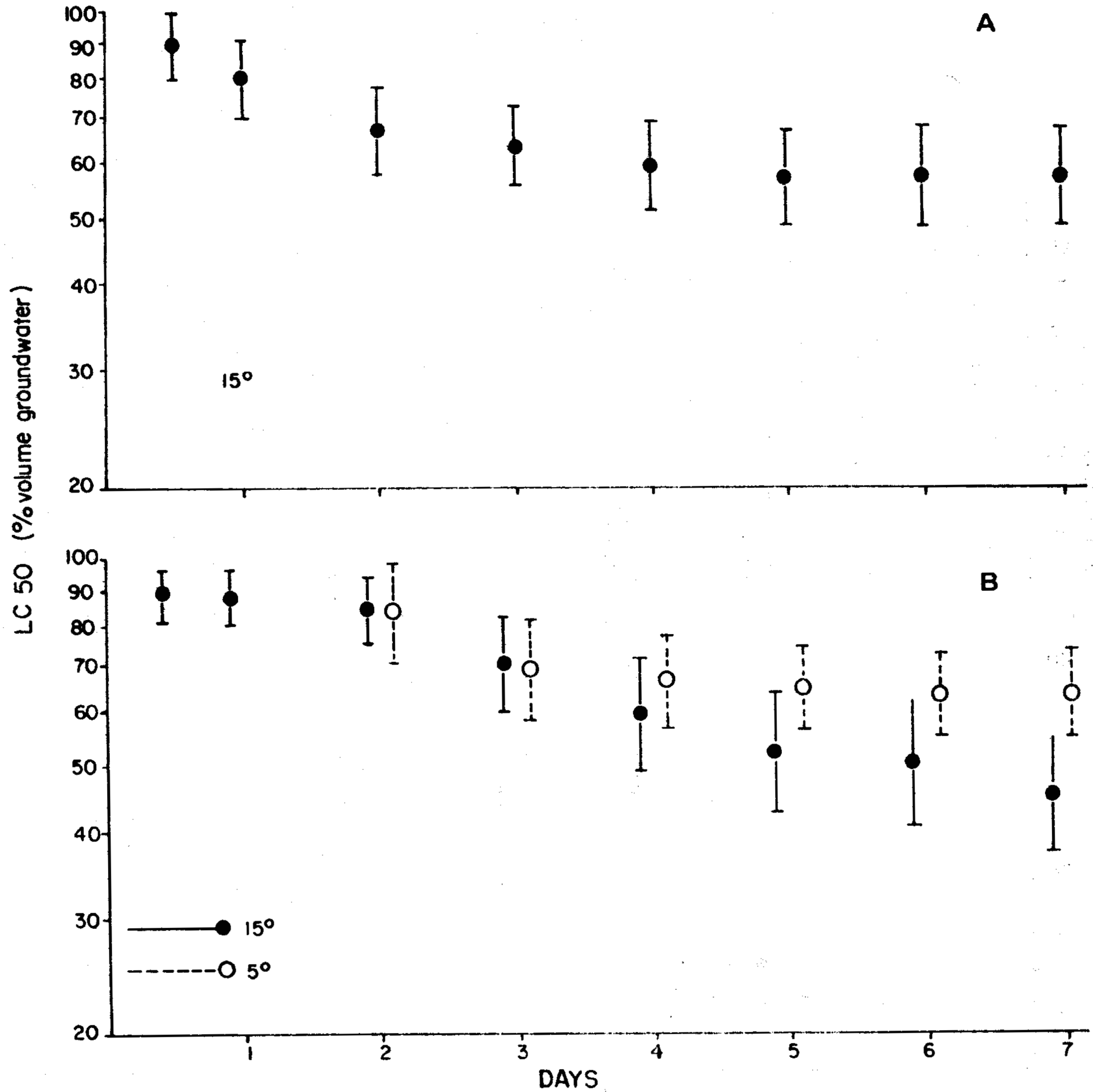

FIGURE 8. A. Comparison of LC50 values and 70% confidence limits of lake chub (*C. plumbeus*) from the Athabasca River tested at 15°C in saline groundwater.
B. Comparison of LC50 values and 70% confidence limits of lake chub from Nose Creek tested at 5° and 15°C in saline groundwater. (There was no mortality at 15°C to 24 hours).

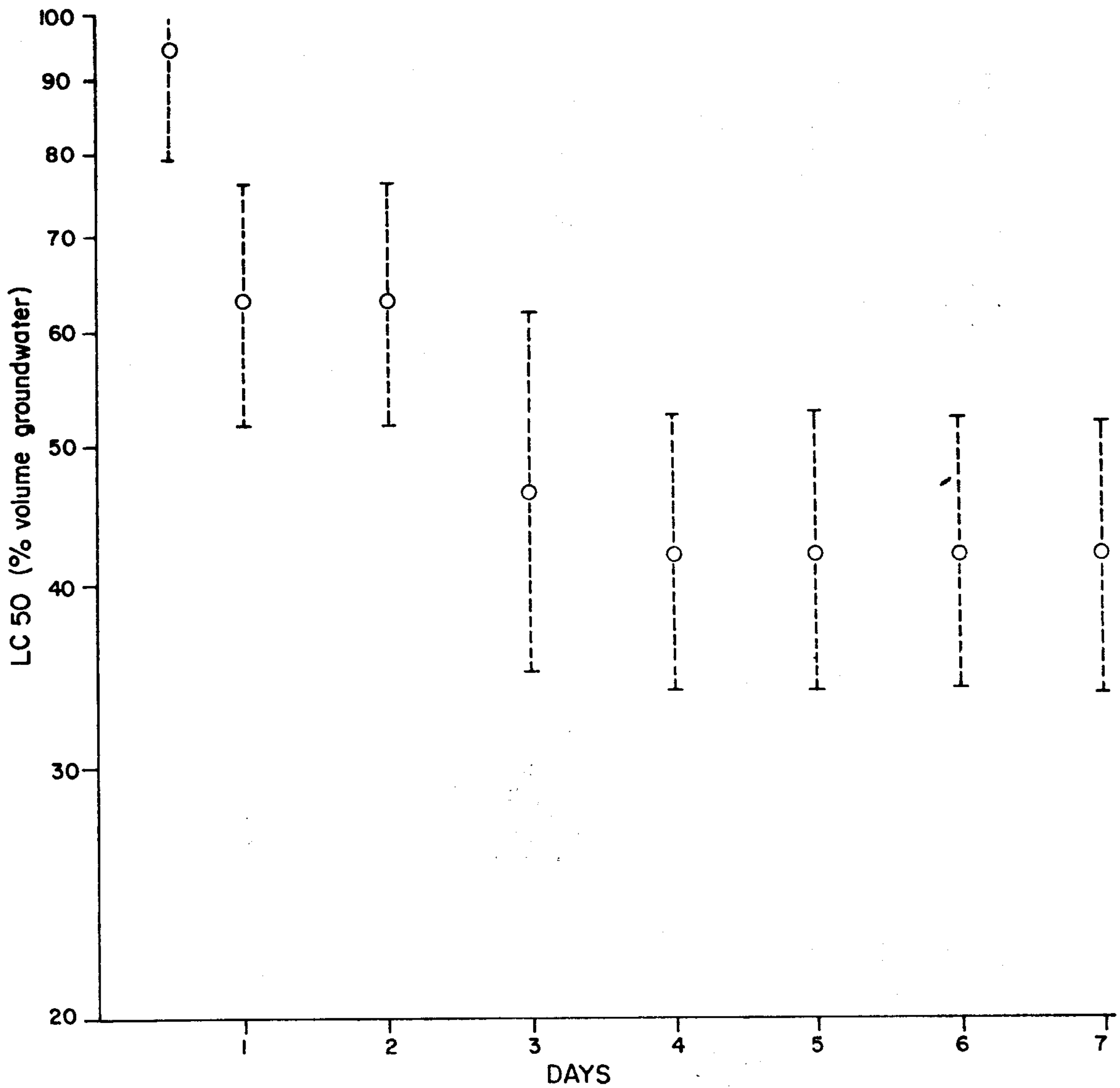

FIGURE 9. LC50 values and 70% confidence limits for flathead chub (*P. gracilis*) from the Athabasca River tested in saline groundwater at 5°C.

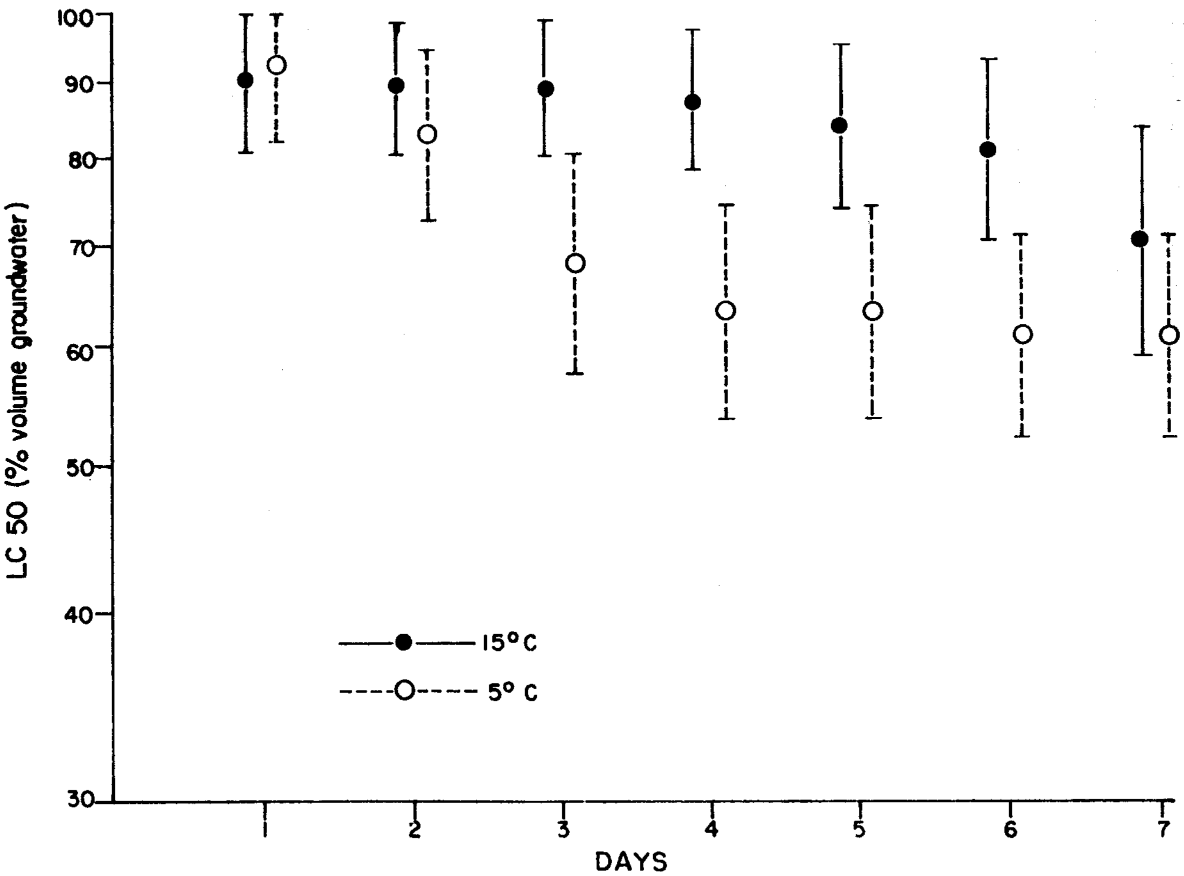

FIGURE 10. LC50 values and 70% confidence limits for white suckers (*C. commersoni*) from Nose Creek tested in saline groundwater at 5° and 15°C.

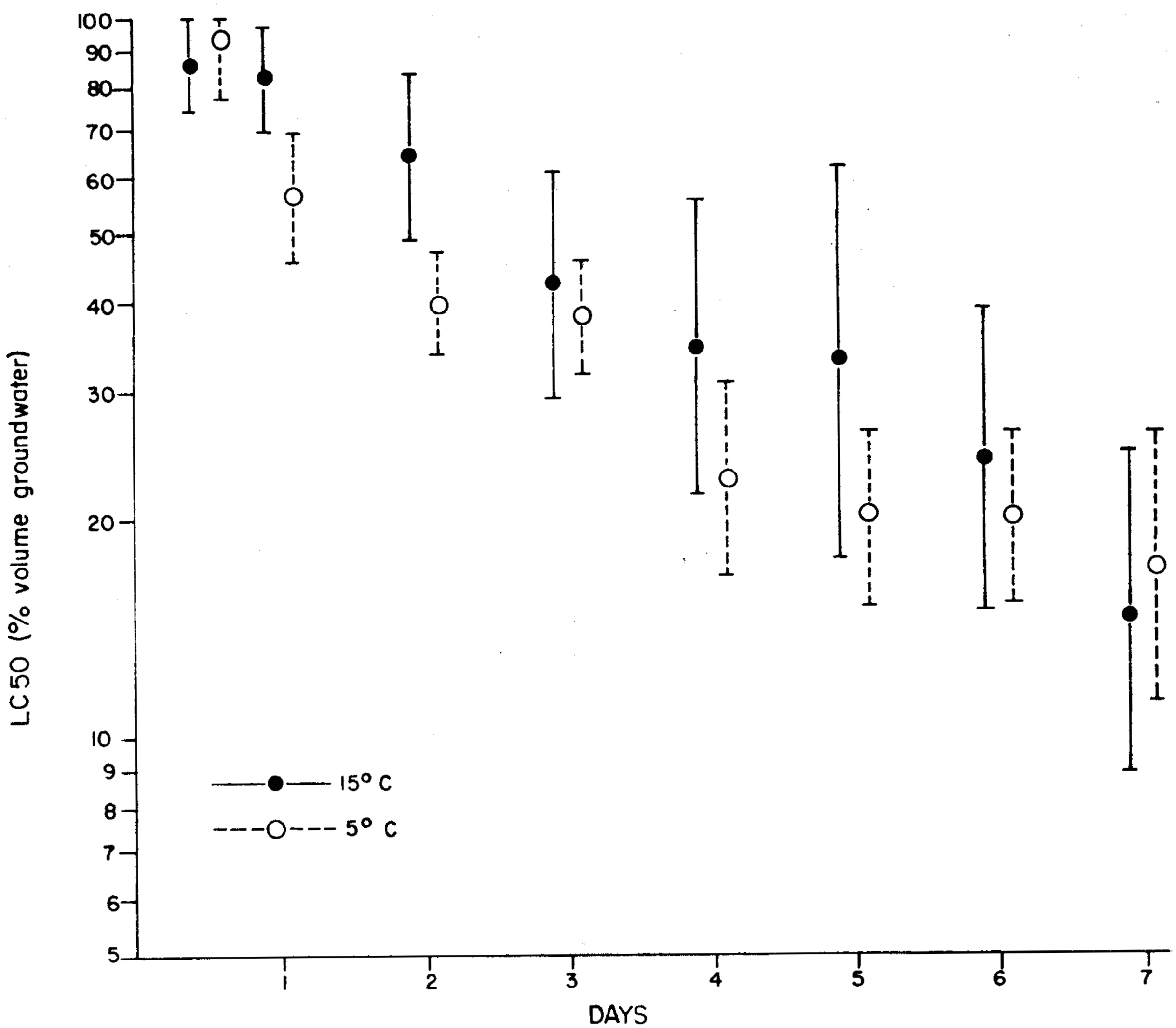

FIGURE 11. LC50 values and 70% confidence limits for trout-perch *(P. omiscomaycus)* from the Athabasca River tested in saline groundwater at 5° and 15°C.

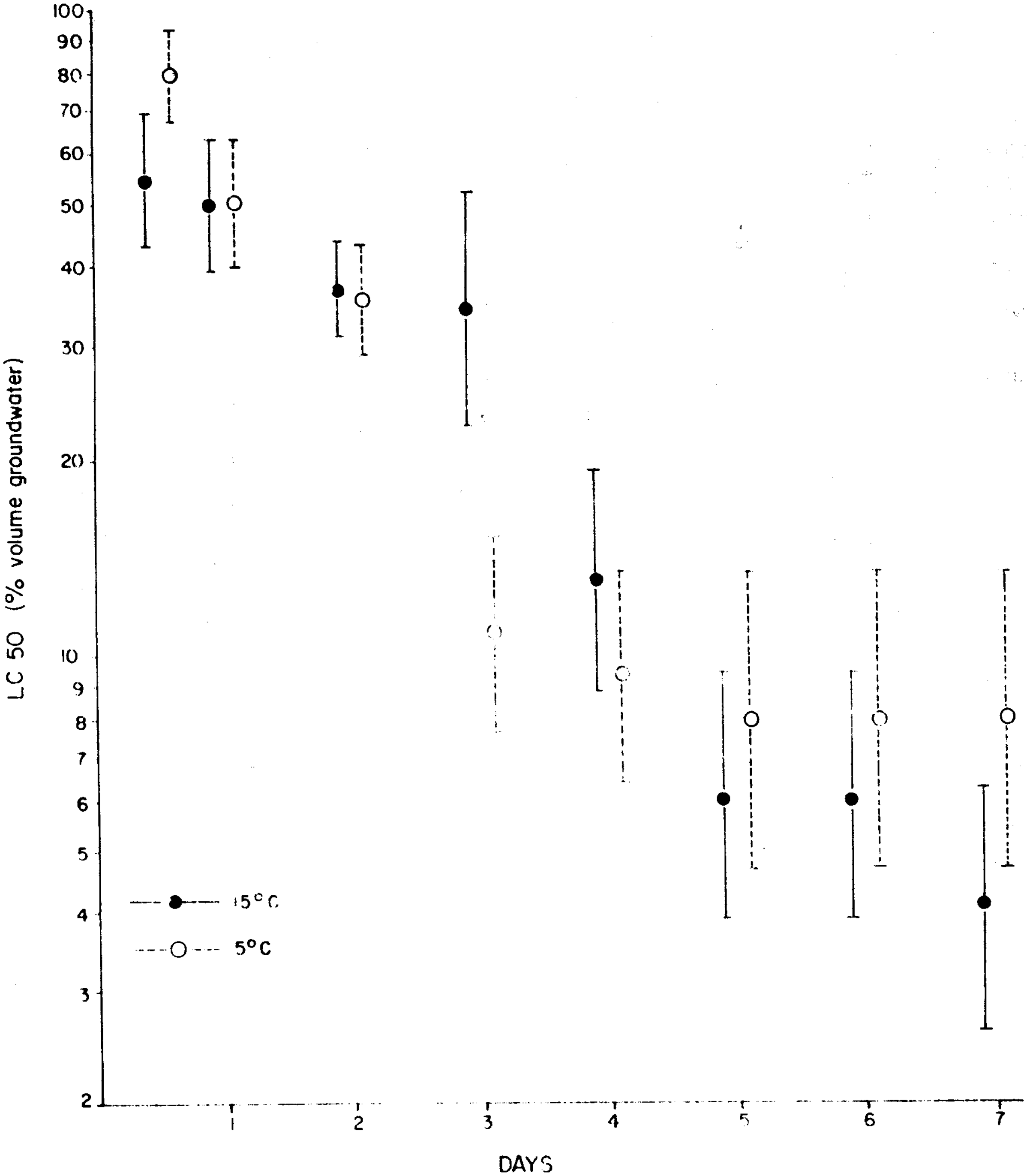

FIGURE 12. LC50 values and 70% confidence limits for walleye *(S. vitreum)* from the Athabasca River tested in saline groundwater at 5° and 15°C.

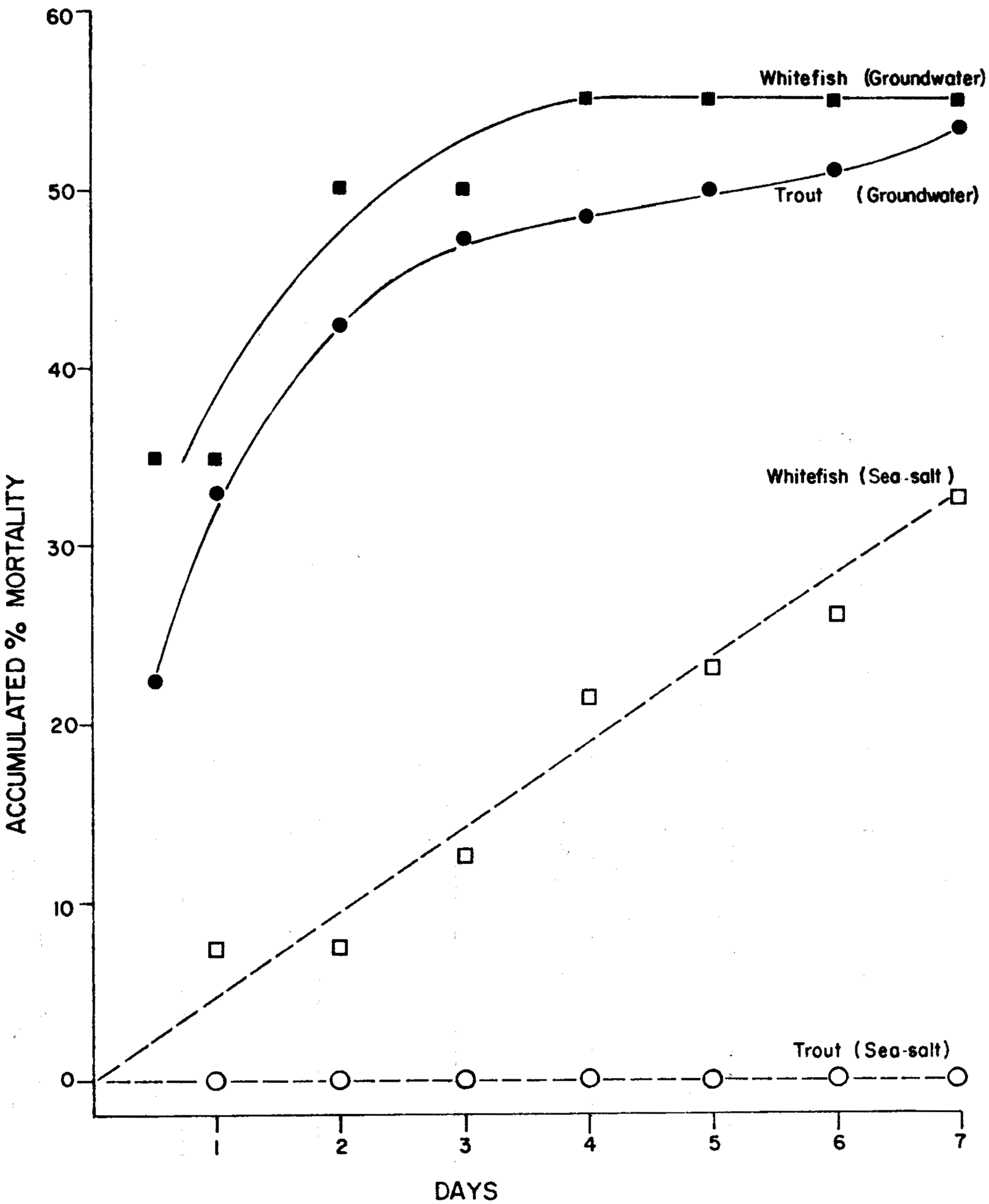

FIGURE 13. Comparison of accumulated percent mortality for mountain whitefish (*P. williamsoni*) and rainbow trout (*S. gairdneri*) tested at 5°C in solutions of saline groundwater diluted in Athabasca River water (solid lines) and artificial sea-salt diluted in Athabasca River water (dashed lines); all solutions at similar osmolarities.

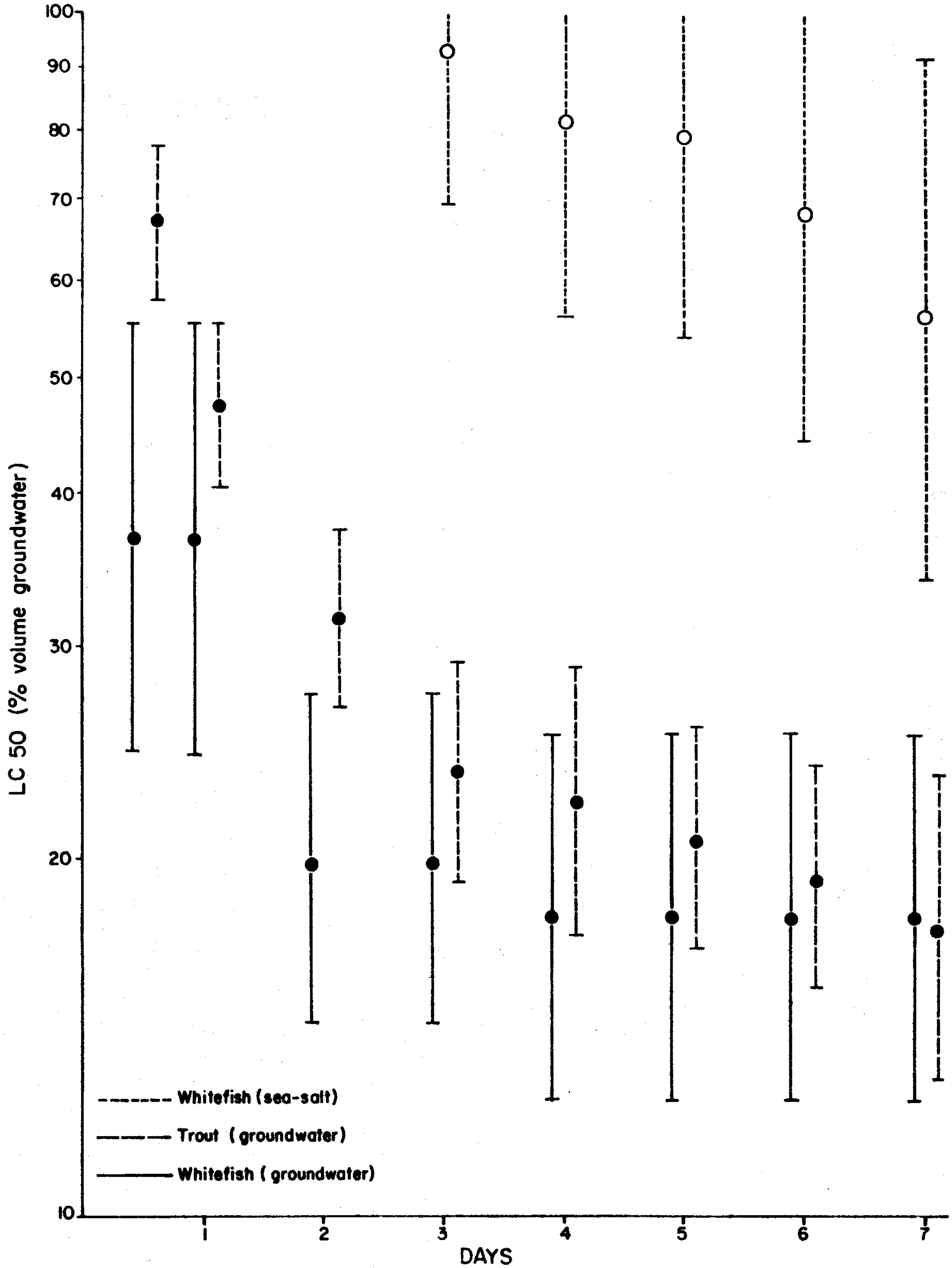

FIGURE 14. Comparison of LC50 values and 70% confidence limits for rainbow trout (*S. gairdneri*) and mountain whitefish (*P. williamsoni*) tested in both groundwater and artificial sea-salt of similar osmolarity at 5°C. Rainbow trout showed no mortality during exposure to artificial sea-salt.

Comparison of Sensitivity of Various Species

Figure 15 compares LC50 values at 5°C for various species of juvenile fish and for four species of benthic invertebrates. Among the fish, three groups can be distinguished:

1) two particularly resistant species, the lake chub and the white sucker,

2) a species, the flathead chub, with an intermediate response, and

3) a group of five species including the trout-perch, grayling, rainbow trout, walleye, and mountain whitefish, all of which are sensitive to the toxic effects of groundwater.

At 96 and 168 hours, the LC50's of the least sensitive species, the lake chub, are seven to eight times that of the most sensitive species, the walleye. An important aspect of the variation in sensitivity is that the resistant species are either suckers (Family Catostomidae) or minnows (Family Cyprinidae), species usually classified as coarse or trash fish, while four of the five most sensitive species, exclusive of the trout-perch, are important sports species.

Fish--Eggs and Alevins

During the course of the experiment, rainbow trout eggs appeared to suffer a relatively high mortality of approximately 25% (Table 10), possibly due to the effects of the elevated temperature (20°C) at which the experiment was conducted. The same high level of mortality was seen in both control groups, eggs not exposed to groundwater. Because of the high inherent mortality, the 10% maximum control mortality used in experiments with juvenile and adult fish could not be applied for eggs. It was, however, applied to the portion of the experiment involving alevins (the yolk-sac larval stage which occurs immediately after hatching).

At the lower range of concentrations used (0.12 to 50% groundwater), mortalities to eggs ranged from 25 to 35%, only slightly elevated from control mortalities (Table 10). At the two highest concentrations tested (79% and 100% groundwater), however, mortality levels were much higher, 50% and 65% mortality, respectively. Rainbow trout eggs were the most resistant to the effects of saline groundwater of any of the organisms and life history stages tested possibly because the covering of the eggs acts as a barrier to the passage of toxic substances.

Hatching success is illustrated in Figure 16. Hatching rate was approximately equivalent in the two "control" tanks as well as the five lowest concentrations (0.12% through 31% groundwater) where an average of 360 fry hatched per tank. At 50% groundwater, hatching success was slightly lower than this average. Substantially less success was seen in the last two tanks (79% and 100% respectively), where only 240 and 150 fry hatched respectively.

Alevins were much more sensitive to the toxic effects of groundwater than were eggs. Figure 17 represents the medial lethal concentration (LC50) for alevins. By 8 to 10 days (at 20°C) less than 7%

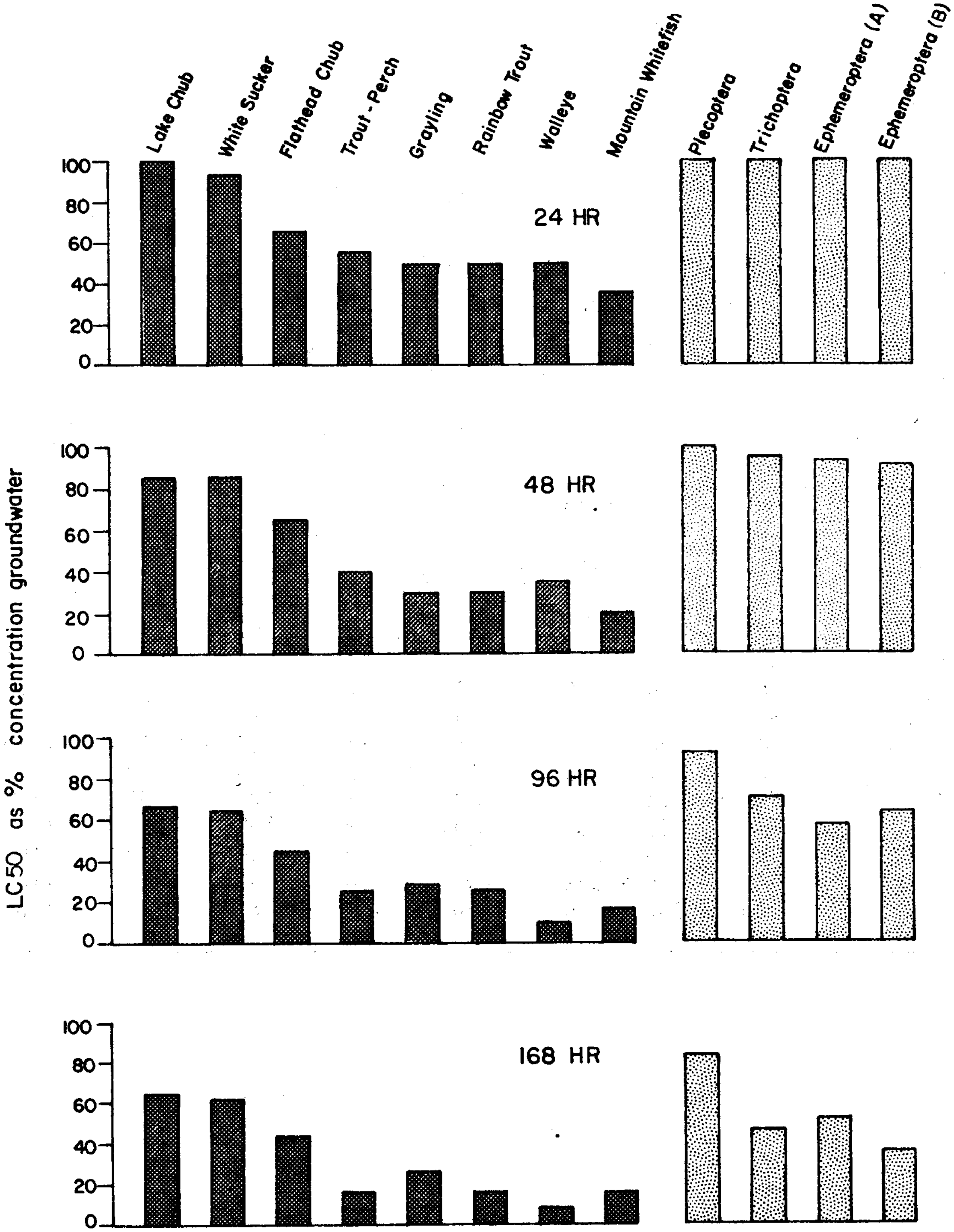

FIGURE 15. Comparison of LC50 values as percent concentration groundwater (osmolarity 350 to 400 mOsm) diluted in Athabasca River water. Values for 5°C. Species arranged in approximate order of increasing sensitivity.

TABLE 10. Cumulative mortality of rainbow trout eggs.

Concentration groundwater (% by volume)	n	(Total dead at end of time period) Hours 72	96	120	144	% Mortality (144 hrs.)
Control I	500	67	90	121	124	(25)
Control II	500	88	108	118	119	(24)
0.12 %	500	109	146	156	159	(32)
1.25 %	500	115	146	156	157	(31)
5 %	500	90	107	115	118	(24)
12 %	500	130	161	173	173	(35)
31 %	500	90	116	146	146	(29)
50 %	500	90	149	149	149	(30)
79 %	500	100	249	249	249	(50)
100%	500	325	325	325	325	(65)

Cumulative mortality of rainbow trout alevins.

Concentration groundwater (% by volume)	n	(Total dead at end of time period) Hours 72	96	120	144	168	240	% Mortality (240 hrs.)
Control I	374	1	2	3	4	7	27	(7.2)
Control II	384	0	0	2	4	4	7	(1.8)
0.12 %	342	3	9	10	12	15	24	(7.0)
1.25 %	343	4	9	11	13	17	31	(9.0)
5 %	383	1	10	13	23	26	62	(16.2)
12 %	331	14	53	69	101	301	331	(100)
31 %	359	20	189	359	359	359	359	(100)
50 %	320	130	320	320	320	320	320	(100)
79 %	240	180	240	240	240	240	240	(100)
100%	150	150	150	150	150	150	150	(100)

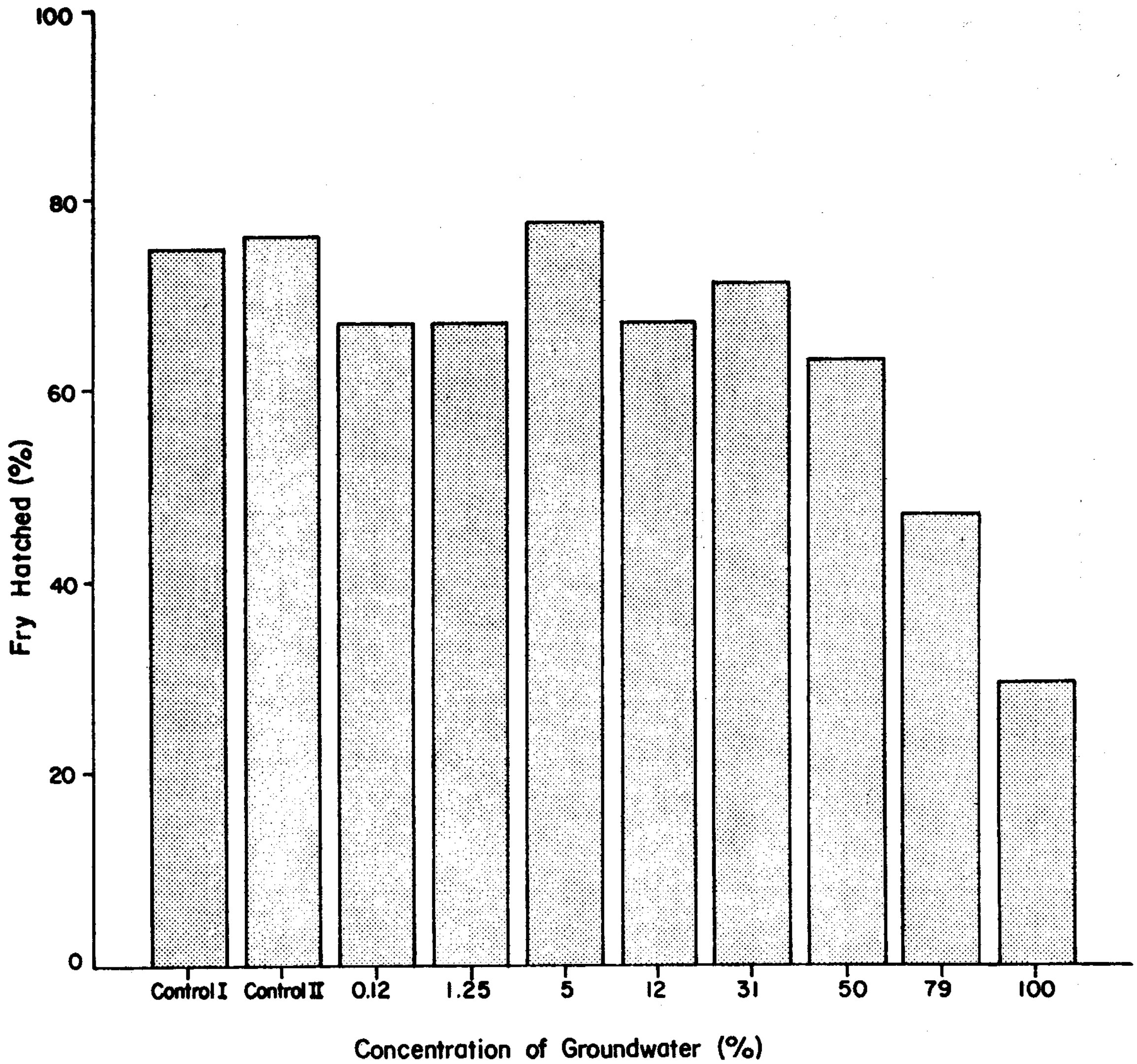

FIGURE 16. Percentage of rainbow trout (*S. gairdneri*) alevins hatched successfully from eggs at each test concentration level (20°C).

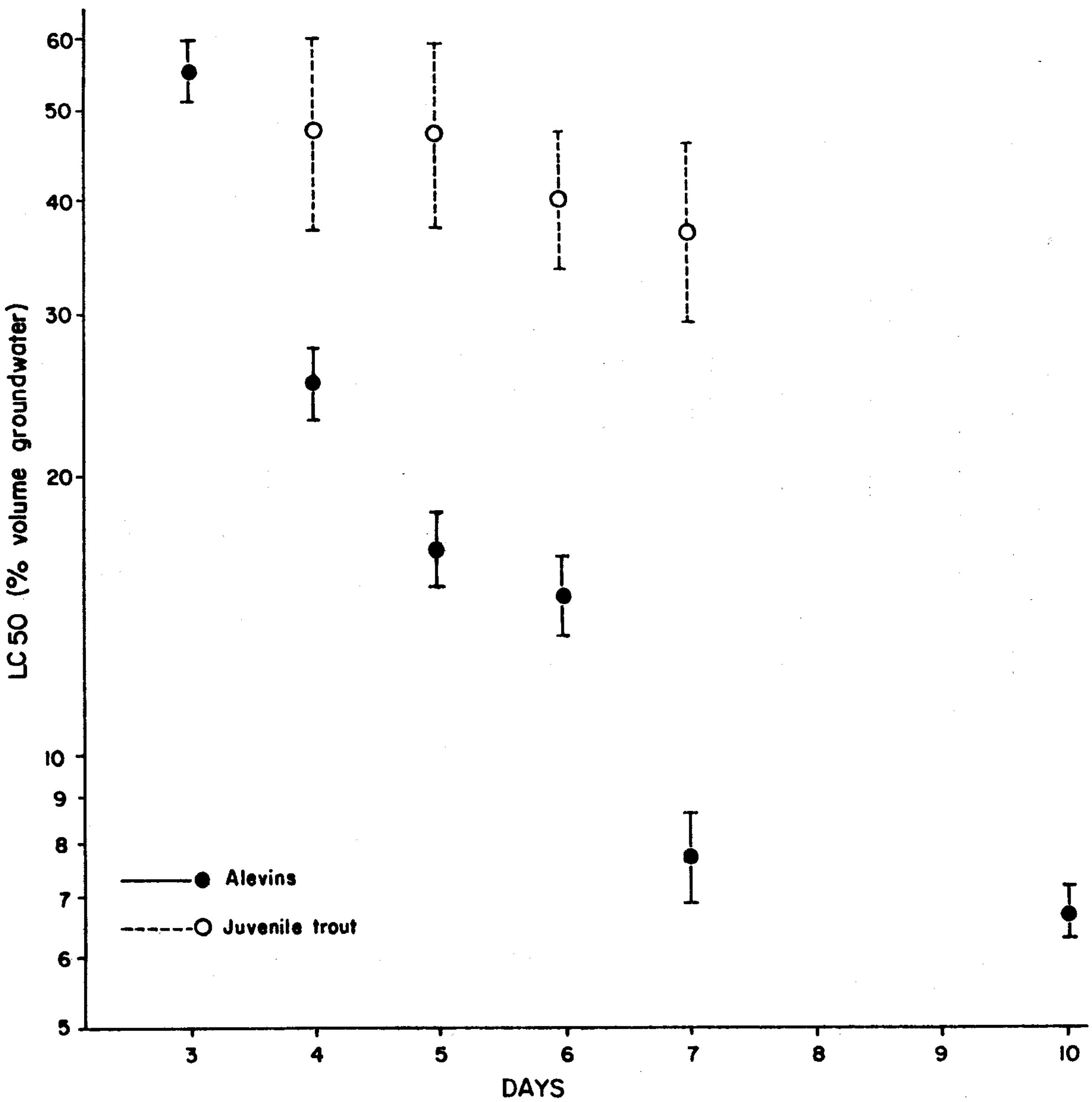

FIGURE 17. LC50 values for the alevins (95% confidence limits) and juveniles (70% confidence limits) of rainbow trout (*S. gairdneri*) exposed to saline groundwater, the former at 20°C and the latter at 15°C.

groundwater would be expected to kill one half of the trout fry present. In comparison, juvenile trout (at 15°C) showed an LC50 (7 days) of approximately 35%, indicating them to be significantly more resistant. Newly hatched fry are thus among the most sensitive types to be tested, while, except at exceptionally high groundwater concentrations, the eggs must be considered particularly resistant..

Benthic Macroinvertebrates

The experiments with benthic macroinvertebrates parallel those performed with juvenile fish. The resultant data are presented in Figures 18 through 21, and Appendix Tables 26 through 32, and summarized in Figure 15. An examination of the data indicates the following:

1) Of the species tested, the plecopteran *Isogenus* sp. is by far the most resistant to the toxic effects of Syncrude groundwater, and by 168 hrs is the only one whose LC50 exceeds that of the most tolerant fish.

2) Among the ephemeropterans, *Heptagenia marginalis* (obtained from the Athabasca River) is more resistant than that *(Paraleptophlebia bicornuta)* obtained from the Bow River (Figure 19).

3) In comparison with juvenile fish, the invertebrates tested have a short-term (to 48 hrs) tolerance which exceeds that of even the most tolerant fish (Figure 15), but in the longer term this difference

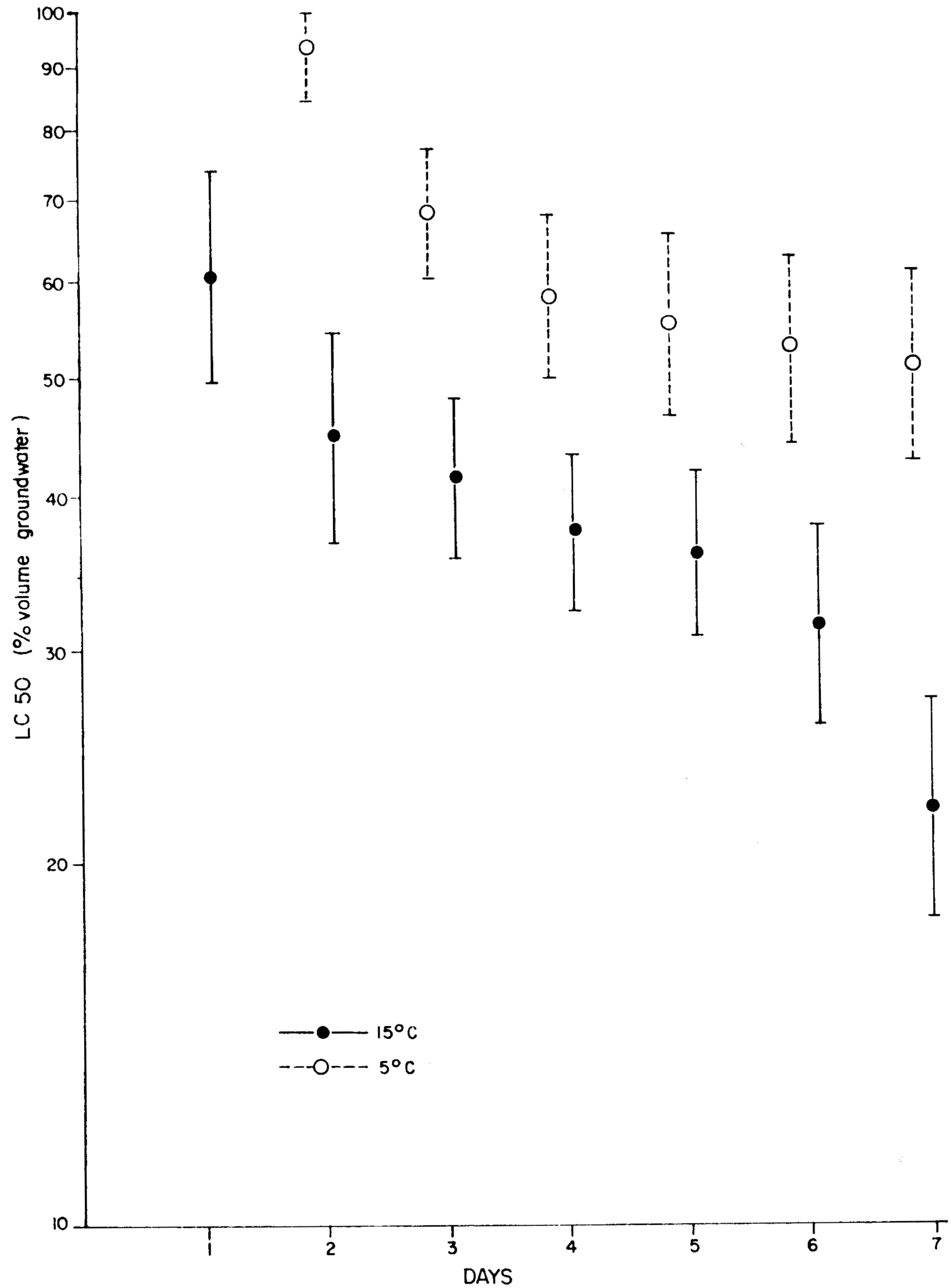

FIGURE 18. LC50 values and 70% confidence limits for a mayfly *(H. marginalis)* from the Athabasca River tested in saline groundwater at 5° and 15°C.

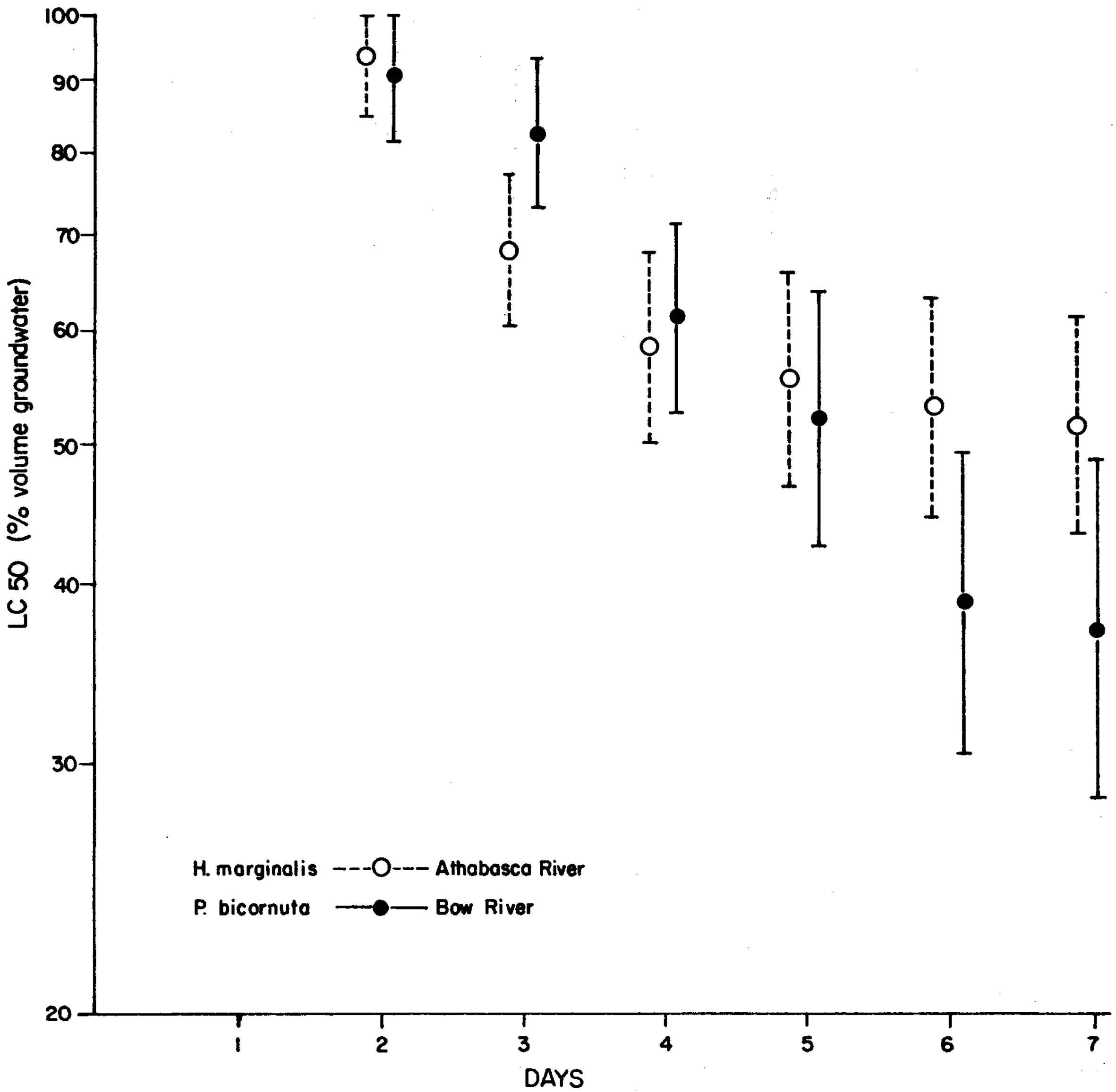

FIGURE 19. Comparison of LC50 values and 70% confidence limits for two species of mayfly, *Paraleptophlebia bicornuta* from the Bow River, and *Heptagenia marginalis* from the Athabasca River. Tests conducted in saline groundwater at 5°C.

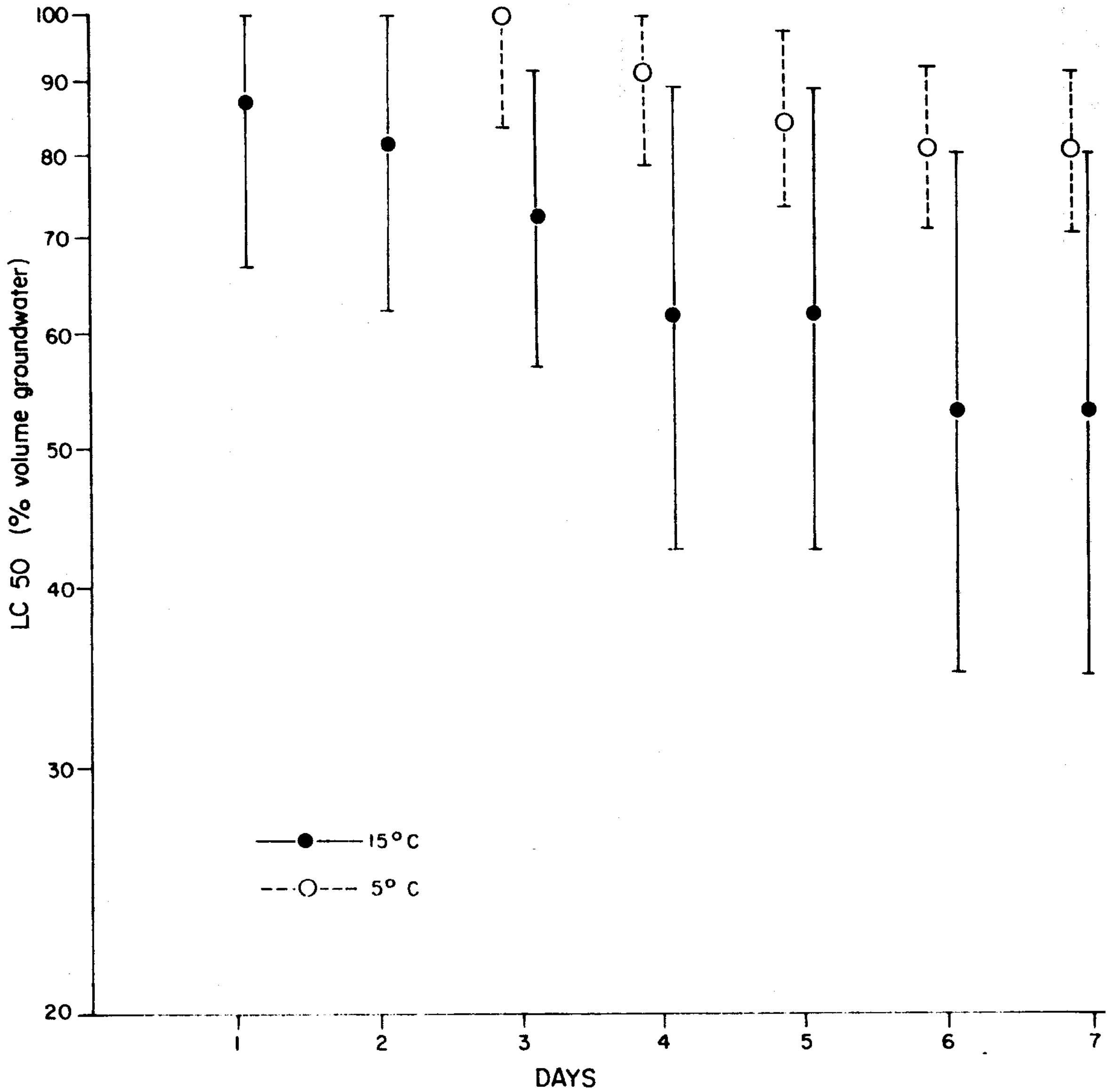

FIGURE 20. LC50 values and 70% confidence limits for a stonefly (*Isogenus* sp.) from the Bow River tested in saline groundwater at 5° and 15°C. There was insufficient mortality at 5°C to calculate LC50 at 24 and 48 hrs.

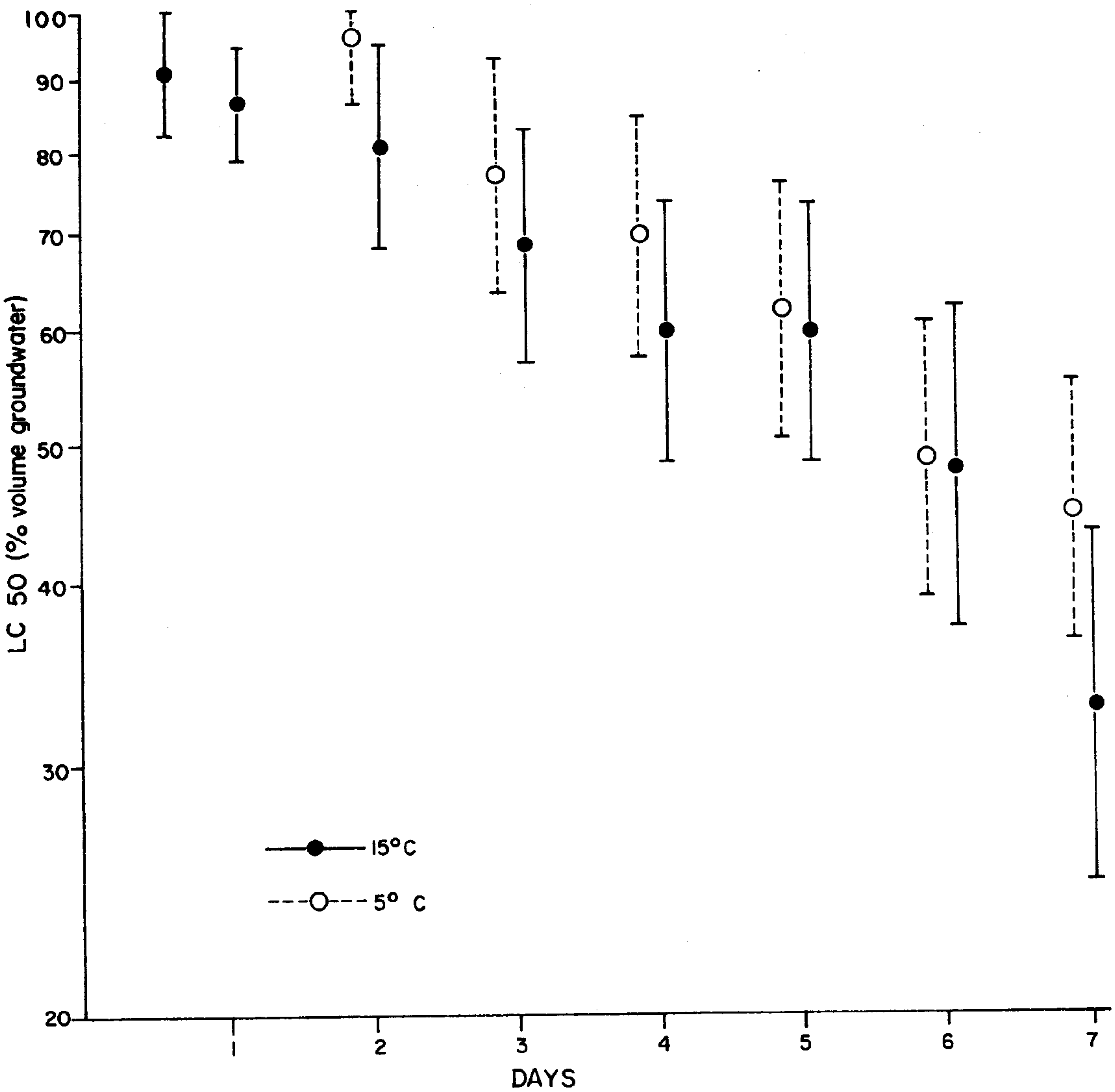

FIGURE 21. LC50 values and 70% confidence limits for a caddisfly *(H. bifida)* from the Bow River tested in saline groundwater at 5° and 15°C. There was insufficient mortality at 5°C to calculate LC50 at 12 and 24 hrs.

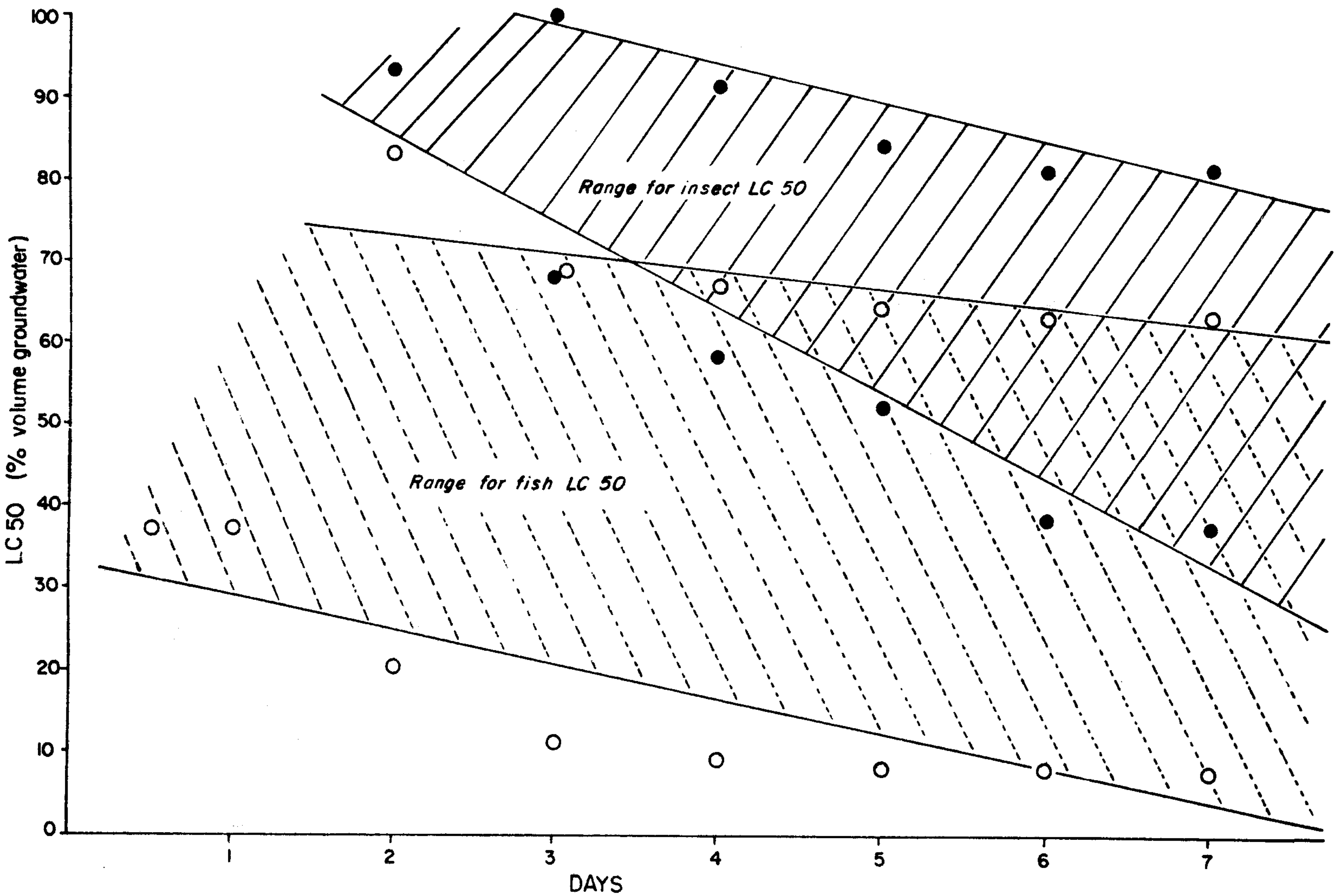

FIGURE 22. Approximate range of values for LC50 for fish and benthic invertebrates tested in saline groundwater at 5°C. Black circles indicate extreme values for insect species and open circles indicate extreme values for fish species (not including eggs).

is reduced. By 96 hrs, two, and by 168 hrs, three, of the four invertebrate species have values for LC50 which are lower than those of the two most tolerant species of fish. A comparison of ranges of LC50's for fish and benthic invertebrates (Figure 22) also indicates that the latter are more tolerant of saline groundwater than fish for the first few days of exposure but that with longer exposure, values for benthic invertebrates begin to overlap the upper range of values for fish.

4) Unlike juvenile fish, mortalities of benthic macroinvertebrates tested at 15°C were consistently greater (and thus LC50 values lower) than those tested at 5°C.

Swimming Performance Tests

Data for the swimming performance tests are summarized in Table 11. The data do indicate a tendency toward a reduction in swimming performance of those trout exposed to concentrations of groundwater (Figure 23), however, none of the differences in fatigue times was found to be statistically significant using either the t-test (Table 11) or the Mann-Whitney U-test.

Despite the fact that no significant differences could be demonstrated, some general comments can be made.

1) Fish tested in 10% groundwater, without prior exposure to groundwater, performed as well as controls.

2) Fish tested at 20% groundwater, without prior exposure to groundwater, tended to fatigue somewhat sooner than controls. (This difference might have been greater had not the tests at 20% been biased towards longer swimming times. This bias was the result of the fact that at 20% concentration, the electrified screen in the fish chamber was relatively ineffective because of the high conductivity of the water. Fish tended to rest with their tails on the screen for short periods between bursts of swimming activity.)

3) Fish tested at 5% or 10% groundwater concentration, which had been acclimated at the same concentration, tended to fatigue further than controls. Under these conditions, those tested at 10% tended to fatigue faster than those tested at 5% groundwater.

Overall, the results suggest that fish tested immediately after placement in moderate concentrations (to 10%) of groundwater, without prior experience, are unaffected in their swimming performance. However, fish exposed to high concentrations (20%) without prior exposure, or fish exposed to lower concentrations (5 and 10%) for a period of four days prior to testing were affected in their swimming performance. Apparently, at low to moderate concentrations, the physiological effects which reduce swimming performance only develop over a period longer than the duration of the performance test.

TABLE 11. Summary of data for swimming performance tests. All tests at 5°C. The t-tests results apply to both fatigue times and fish lengths to fatigue. ($\bar{x}$ mean, SD=standard deviation, SE=standard error, NS=not significant).

Experiment	Acclimation Medium	Test Medium		Fork Length	Fatigue Time (min.)	lengths to fatigue	t-test comparisons (significant if p<0.5)
1	Dechlorinated	Dechlorinated	$\bar{x}$=	9.90	18.20	3839	
	tap water	tap water	SD=	0.44	8.20	1701	
			SE=	0.13	2.40	491	
			n=	12	12	12	
2	Dechlorinated	Athabasca	$\bar{x}$=	10.10	34.40	7086	NS
	tap water	River water	SD=	0.38	29.70	5989	
			SE=	0.11	9.00	1806	NS
			n=	11	11	11	
3	Athabasca	Athabasca	$\bar{x}$=	9.80	18.50	3894	NS
	River water	River water	SD=	0.37	15.70	3168	
			SE=	0.08	3.40	691	
			n=	21	21	21	
4	Athabasca		$\bar{x}$	10.00	18.10	3774	NS
	River water	10% Groundwater	SD=	0.49	8.20	1647	
		90% River water	SE=	0.16	2.60	529	NS
			n=	10	10	10	NS
5	Athabasca		$\bar{x}$=	10.20	13.50	2774	NS
	River water	20% Groundwater	SD=	0.35	8.10	1653	
		80% River water	SE=	0.09	2.10	427	NS
			n=	15	15	15	
6	Athabasca River		$\bar{x}$=	10.00	15.30	3206	
	water(4 days)	5% Groundwater	SD=	0.97	11.00	2282	
	5% Groundwater	95% River water	SE=	0.23	2.60	538	
	(4 days)		n=	18	18	18	
7	Athabasca River		$\bar{x}$=	9.70	13.70	2888	NS
	water(4 days)	10% Groundwater	SD=	0.44	12.50	2541	
	10% groundwater	90% River water	SE=	0.09	2.50	519	
	(4 days)		n=	24	24	24	

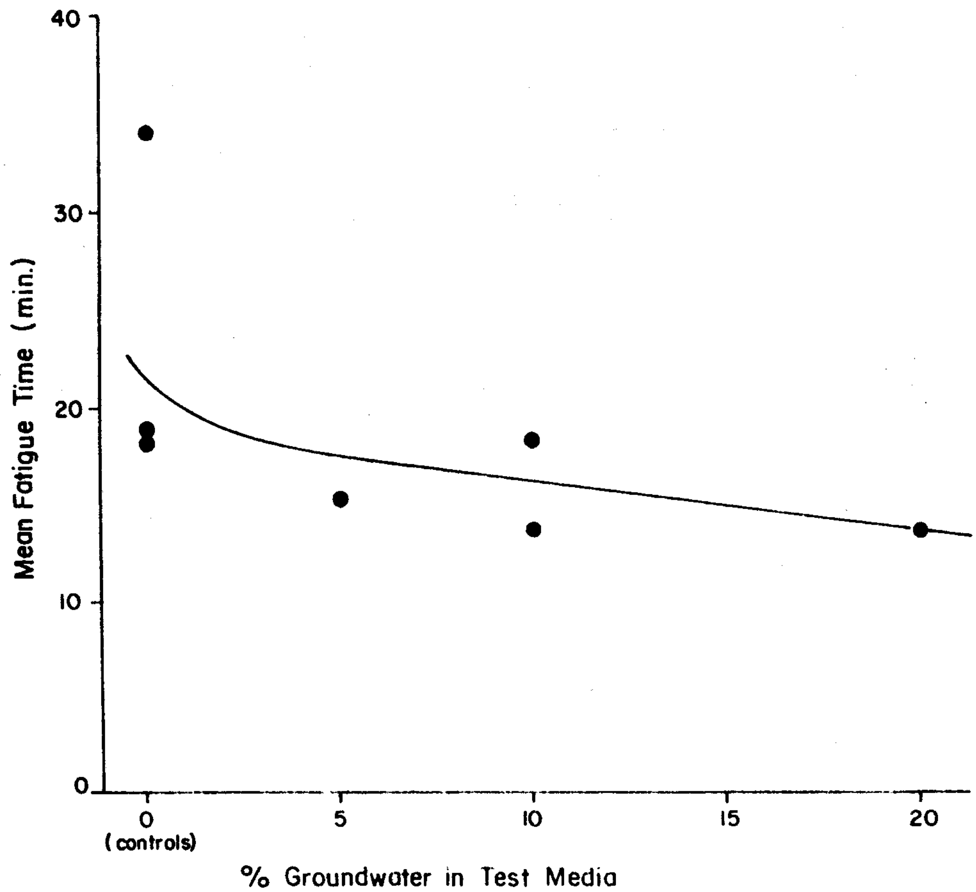

FIGURE 23. Relationship of mean fatigue time to concentration of groundwater in test media for juvenile rainbow trout (*S. gairdneri*) tested at 5°C.

DISCUSSION

The ultimate purpose of toxicity studies such as those reported here is to provide information concerning the probable effects of industrial effluents on the aquatic organisms inhabiting receiving waters. With sufficient information, guidelines can be established in advance to ensure that the degree of treatment of wastes and the dilution of effluents entering natural waters provide adequate protection to the aquatic ecosystem.

A first step in the process is to determine the level of protection which should be provided individual waterbodies. The Inland Waters Branch, Environment Canada, has devised a system of classification (Anonymous, 1972) which includes four different "levels of protection". Guidelines defining the four levels are summarized in Table 12. The following is a more detailed outline of the conditions to be expected at each of the four levels of protection:

> *"In waters to be maintained at Level I quality the degree of protection to the ecosystem and organisms is maximum: there is a maximum margin of safety. No degradation from the pristine or natural state (prior to settlement by man) occurs. Waters in wilderness areas would fall into this category. No additions to such waters should occur that would alter the ecosystem in any way. No increase in concentration of chemicals or of any harmful factor should occur. Waters which are naturally eutrophic or of naturally poor quality could fall in this category should the water protection agency decide that they be preserved in their natural state.*
>
> *"In waters to be maintained at Level II quality the degree of protection afforded to the ecosystem and aquatic organisms is high. There is considerable margin of safety before harmful levels of factors are reached. Some degradation from the natural or pristine state, as reflected in increased levels of certain chemicals or*

TABLE 12.
Guidelines defining various levels of protection from harmful factors for aquatic ecosystems (from Anonymous, 1972).

Level of Protection	Degree of Protection	Margin of Safety	Type of ecosystem of water body	Remarks
I	Maximum, i.e., pristine or natural state of ecosystem and water quality	Maximum margin	Any water body or portion thereof to be preserved in a pristine state -- e.g. those in wilderness areas or parks	No degradation
II	High, i.e., high level of ecosystem and water quality	High margin	Water bodies maintained at a high quality	Some degradation from Level I
III	Medium, i.e. medium level of ecosystem and water quality	Moderate margin	Waters receiving wastes to the extent that harmful effects do not occur and the indicated safety margin is obtained	Further degradation from Level II
IV	Minimum, i.e. at threshold of harmful effects	Nil	Waters used for discharge zones and other areas where only maintenance of minimum functioning of the ecosystem is possible	Degradation to threshold of harmful effects

other factors, might occur but should still permit a high quality to exist in the ecosystem and water body.

"In waters to be maintained at Level III quality the degree of protection afforded to the ecosystem and aquatic organisms is moderate. There is also a moderate margin of safety before harmful levels of factors are reached. Degradation (as measured by increases above natural values for certain chemicals or other harmful factors) would occur but would be maintained well below the threshold level for harmful effect.

"In waters to be maintained at Level IV quality the degree of protection to the ecosystem and organisms is minimal. There is no margin of safety and any slight increase over this limit would result in damage to aquatic life or the ecosystem. Such waters would be considered severely degraded and therefore this level would be used only for waters in discharge zones or areas where minimum functioning of the ecosystem is acceptable."

The Athabasca River has apparently not yet been classified with respect to level of protection. It is obviously not pristine and therefore does not quality for Level I. However, on the assumption that the river should receive the highest practical level of protection, we have assumed that water quality should be maintained at Level II.

To maintain various levels of protection, criteria can be defined for individual environmental factors (e.g., temperature, pH, suspended solids, dissolved oxygen, eutrophication). In this instance, the studies were designed to provide a baseline for assessing acute toxicity.

The results of the study clearly indicate that saline groundwater from Lease 17 is toxic to all the fish and the benthic invertebrates tested. No single toxic factor has been identified and it seems likely that the observed toxicity is the result of a complex of factors including the following:

1) salinity,

2) hydrocarbons,

3) heavy metals.

It seems an impossible task to separate the toxicity of the various constituents of a complex waste such as saline groundwater, with their individual, additive, and synergistic effects. A more practical approach, and the one pursued here, is to treat the effluent as it were a single, moderately toxic substance and to define appropriate dilution volumes on the basis of the effluent as a whole.

In determining the concentrations of non-cumulative toxicants which can be permitted in natural waters, the following information is required:

1) *An experimental determination, using local waters, of the concentration levels which are toxic to aquatic organisms.* In this instance, lethal levels have been expressed at LC50, the concentration lethal to 50% of the animals in a specified period of time. The 96 hr LC50 is most frequently used unless it is obvious that significant acute mortality continues past four days.

2) *An estimate of the threshold level of harmful effect.* This is the concentration above which sublethal effects could occur. Values for the threshold level can only be determined by experiments involving long-term exposure. Such experiments have not yet been carried out with saline groundwaters. Where definitive data are lacking, the Inland Waters Branch, Environment Canada, recommends the following (Anonymous, 1972):

> *"The estimate of the threshold level of harmful effect for a non-cumulative toxic pollutant should be 0.1 of the lethal threshold concentration for fish (i.e., 1/10 of the LC50). In no case should a value higher than this be used. If there is definite information that a lower fraction is necessary to avoid significant lethal effects, then the lower value should be used as the estimate of the threshold level of harmful effect."*

3) *An application factor.* This varies depending on the level of protection desired and the class of toxic materials involved. This value could be determined for individual situations but in the absence of precise information, the Inland Waters Branch recommends an application factor of 0.2 for streams afforded Level II protection.

As a basis for determining maximum permissible concentrations, it seems appropriate to use data for the most sensitive species tested. This is the walleye, a warm water sports species, common in the Athabasca River in the vicinity of the Syncrude Lease. The walleye had 96 hr LC50's of 9.3% and 13.0% at temperatures of 5 and 15°C, respectively. A simple calculation using the lower of the two values will provide one estimate of the maximum permissible percentage dilution of saline groundwater in Athabasca River water.

$$\text{LC50 (96 hr)} \times \text{Threshold Level} \times \text{Application Factor} = 9.3\% \times 0.1 \times 0.2 = 0.19\%$$

The results of the calculation suggest that, as long as saline effluent entering the Athabasca River does not constitute more than 0.19% of the total flow, juvenile walleye should not suffer any harmful effects. This would presumably also apply to the other species of fish and invertebrates which tests have shown to be less sensitive than the walleye. There are, however, some major problems with this approach which suggest that the calculated dilution should be treated as little more than a guideline.

First, the application factors on which the calculations are based assume that an accurate estimate of incipient lethal level is available. In fact, for most of the species tested, mortality continued to increase throughout the term of the experiments. For walleye (Tables 24 and 25) for example, there was significant mortality beyond 96 hr, especially at 15°C where the LC50 declined from 13.0% at 96 hr (4 days) to only 4.1% at 168 hr (7 days). A calculation based on the lower value indicates a maximum permissible dilution of only 0.08% (4.1 x 0.1 x 0.2), less than half that based on 96 hr values.

Second, both calculations are based on data for juvenile fish. Our studies suggest that, for rainbow trout at least, newly hatched alevins are even more sensitive than juveniles. If the same is true for the alevins of walleye and other species, a further reduction in maximum permissible dilution may be necessary to ensure that they do not suffer long-term effects.

Third, there may be other species, not tested, which are even more sensitive than the walleye.

Fourth, there is a presumption that the characteristics of both the saline groundwater and the receiving water or diluent remain approximately as they were during the tests. Undoubtedly, the characteristics of the saline effluent will be constantly changing as new pit areas are opened and as new groundwater sources are tapped. From chemical tests so far conducted, it would appear that water from Well 2600S, 4800E, with which most of the tests were conducted, is approximately average for groundwater sources on Lease 17 (Table 2). Some wells, however, have far higher concentrations of dissolved substances and their waters may be appreciably more toxic, requiring a reduction in the recommended dilution. Some wells, on the other hand, have lower concentrations of dissolved substances and discharge comprised largely of flow from these wells may allow an increase in the dilution ratio.

Obviously, a study such as this, which provides information concerning the acutely toxic effects of saline groundwater to a few life history stages of selected species of aquatic organisms cannot form the basis of a precise assessment of the impact of saline discharge on an ecosystem as complex as that of the Athabasca River. Aside from changes in the groundwater discharge, there is the possibility that the quality of the receiving water could change, either seasonally or as the result of the development of other facilities, either industrial or municipal, which cause a degradation of water quality in the area of stream influenced by the Syncrude discharge. Materials discharged into the river from other sources could have additive or synergistic effects causing an increase in toxicity even though the characteristics of the Syncrude effluent did not change.

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APPENDIX TABLES 1 - 32. Summary results of individual experiments with fish and benthic invertebrates.

TABLE 1. Results of acute toxicity studies with juvenile rainbow trout (*S. gairdneri*). Test conducted at 5°C with saline groundwater (230 mOsm) diluted in Athabasca River water. n=26.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100	4	0	1	1	3	4	4	4	4
56	4	0	0	0	1	2	3	4	4
32	4	0	0	0	0	0	0	0	2
18	4	0	0	1	1	1	1	1	1
10	4	0	0	0	1	1	1	1	1
Control	6	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	*	*	*	61.7	44.8	39.8	36.8	24.8
70% conf. limits	-	-	-	84.5	59.4	53.0	49.6	33.9
	-	-	-	45.0	33.7	30.0	27.3	18.3
95% conf. limits	-	-	-	100.0	77.9	69.7	66.0	45.5
	-	-	-	33.3	25.7	22.8	20.5	13.6

* Mortality not sufficient to calculate LC50.

TABLE 2. Results of acute toxicity studies with juvenile rainbow trout *(S. gairdneri)*. Test conducted at 5°C with artificial sea-salt (normal concentration range) diluted in Athabasca River water. n=180.

Cumulative mortality:

No mortality (0) in regular concentration range (0-400 mOsm), therefore no LC50 calculations possible.

TABLE 3. Results of acute toxicity studies with juvenile rainbow trout (*S. gairdneri*). Test conducted at 5°C with saline groundwater (400 mOsm) diluted in Athabasca River water. n=148.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100.0	16	13	16	16	16	16	16	16	16
79.4	16	12	16	16	16	16	16	16	16
50.1	16	2	7	13	13	13	14	16	16
31.6	16	1	3	8	10	11	11	11	11
12.6	16	1	1	1	1	1	1	1	1
5.0	16	0	0	1	2	2	2	2	3
1.25	16	0	0	1	1	1	1	1	1
0.12	16	0	0	0	2	2	3	3	5
Control	20	0	0	0	0	0	0	0	0

	n	192	216	240	264	288	312	336
100.0	16	16	16	16	16	16	16	16
79.4	16	16	16	16	16	16	16	16
50.1	16	16	16	16	16	16	16	16
31.6	16	11	11	11	14	14	15	15
12.6	16	1	1	1	1	1	1	1
5.0	16	3	3	3	3	3	3	3
1.25	16	1	1	1	1	1	2	2
0.12	16	5	5	6	6	6	6	7
Control	20	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	67.1	47.3	31.6	23.6	22.3	20.7	19.2	17.4
70% conf. limits	77.8	55.4	40.0	29.2	27.7	25.7	23.9	23.6
	57.9	40.4	25.0	19.1	18.0	16.7	15.5	12.9
95% conf. limits	89.6	64.4	49.4	35.8	34.0	31.5	29.5	31.5
	50.3	34.7	20.0	15.5	14.7	13.6	12.5	9.6

	192	216	240	264	288	312	336
LC50 (% volume groundwater)	17.4	17.4	16.8	15.2	15.2	14.3	13.8
70% conf. limits	23.6	23.6	23.2	21.0	21.0	20.4	20.2
	12.9	12.9	12.2	11.0	11.0	10.0	9.5
95% conf. limits	31.5	31.5	31.6	28.6	28.6	28.7	29.1
	9.6	9.6	9.0	8.0	8.0	7.1	6.6

TABLE 4. Results of acute toxicity studies with juvenile rainbow trout *(S. gairdneri)*. Test conducted at 5°C with artificial sea-salts (normal concentration range) diluted in Calgary tap water. n=180.

Cumulative mortality:

No mortality (0) in regular concentration range (0-400 mOsm), therefore no LC50 calculations possible.

TABLE 5. Results of acute toxicity studies with juvenile rainbow trout (*S. gairdneri*). Test conducted at 5°C with saline groundwater (400 mOsm) diluted in dechlorinated Calgary tap water. n=139.

Cumulative mortality:

Concentration groundwater (% by volume)	n	12	24	48	72	96	120	144	168
		Hours							
79.4	17	7	17	17	17	17	17	17	17
50.1	17	0	2	5	8	12	16	16	17
31.6	17	1	1	3	5	5	6	7	8
12.6	17	0	0	0	0	0	1	1	1
5.0	17	0	1	1	2	2	3	3	3
1.25	17	0	0	0	1	2	2	2	3
0.12	17	0	0	1	2	2	2	3	3
Control	20	0	0	1	1	1	1	1	1

	n	192	216	240	264	288	312	336
79.4	17	17	17	17	17	17	17	17
50.1	17	17	17	17	17	17	17	17
31.6	17	8	10	13	13	13	13	13
12.6	17	1	1	1	1	1	3	3
5.0	17	3	3	3	3	3	3	3
1.25	17	3	3	3	4	4	4	4
0.12	17	3	3	3	3	3	3	4
Control	20	1	1	1	1	1	1	1

LC50 and confidence limits:

	12	24	48	72	96	120	144	168
	Hours							
LC50 (% volume groundwater)	81.3	59.3	56.2	39.7	34.8	34.1	22.1	21.1
70% conf. limits	90.1	66.4	67.5	48.9	44.7	45.6	28.1	28.0
	73.3	53.0	46.8	32.3	27.1	25.9	17.3	15.9
95% conf. limits	99.4	74.0	80.5	59.7	56.9	60.4	35.6	36.7
	66.4	47.5	39.3	26.5	21.3	19.3	13.7	12.2

	192	216	240	264	288	312	336
LC50 (% volume groundwater)	20.3	18.4	16.4	16.0	16.0	14.4	13.9
70% conf. limits	27.3	23.9	22.2	21.9	21.9	19.7	19.0
	15.1	14.2	12.2	11.7	11.7	10.5	9.9
95% conf. limits	36.2	30.7	29.6	29.5	29.5	26.7	27.2
	11.4	11.1	9.1	8.6	8.6	7.7	7.1

TABLE 6. Results of acute toxicity studies on juvenile rainbow trout (*S. gairdneri*). Test conducted at 15°C with saline groundwater (230 mOsm) diluted in Athabasca River water. n=27.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100	5	0	0	0	5	5	5	5	5
56	5	0	0	0	1	3	3	5	5
32	5	0	0	0	0	0	0	0	1
18	5	0	0	0	0	1	1	1	1
Control	7	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	*	*	*	69.6	47.2	47.2	40.0	36.8
70% conf. limits	-	-	-	83.5	60.0	60.0	47.7	46.2
	-	-	-	58.1	37.1	37.1	33.6	29.3
95% conf. limits	-	-	-	99.3	75.5	75.5	56.4	57.4
	-	-	-	48.8	29.4	29.4	28.4	23.6

* Mortality not sufficient to calculate LC50.

TABLE 7. Results of acute toxicity studies with alevin rainbow trout (*S. gairdneri*). Test conducted at 20°C with saline groundwater (380 mOsm) diluted in dechlorinated Calgary tap water. n=3,400 approximately.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 72	96	120	144	168	240
100.0	150	150	150	150	150	150	150
79.4	240	180	240	240	240	240	240
50.1	320	130	320	320	320	320	320
31.6	359	20	189	359	359	359	359
12.6	331	14	53	69	101	301	331
5.0	383	1	10	13	23	26	62
1.25	343	4	9	11	13	17	31
0.12	342	3	9	10	12	15	24
Control II	384	0	0	2	4	4	7
Control I	374	1	2	3	4	7	27

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	Hatching			55.1	25.2	16.7	14.8	7.7
70% conf. limits				57.3	26.3	17.4	15.6	8.1
				53.0	24.1	15.9	14.0	7.3
95% conf. limits				59.5	27.4	18.2	16.4	8.6
				51.1	23.1	15.3	13.4	6.9

	240
LC50 (% volume groundwater)	6.7
70% conf. limits	7.0
	6.5
95% conf. limits	7.2
	6.3

TABLE 8. Results of acute toxicity studies with juvenile mountain whitefish (*P. williamsoni*). Test conducted at 5° with saline groundwater (230 mOsm) diluted with Athabasca River water. n=57.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100	10	4	10	10	10	10	10	10	10
56	9	0	4	4	5	5	7	9	9
32	9	0	0	0	1	1	3	3	4
18	9	0	0	0	1	1	2	3	4
10	10	0	0	0	1	2	2	2	4
Control	10	0	0	0	0	0	0	0	0

	n	192	216	240
100	10	10	10	10
56	9	9	9	9
32	9	5	6	8
18	9	4	7	7
10	10	4	5	5
Control	10	0	1	1

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	*	59.6	59.6	45.8	43.9	33.5	27.6	20.4
70% conf. limits	-	69.8	69.8	54.7	52.6	41.8	34.2	26.0
	-	50.9	50.9	38.4	36.7	26.8	22.3	15.9
95% conf. limits	-	81.3	81.3	64.8	68.5	51.7	41.9	33.0
	-	43.7	43.7	32.4	33.9	21.7	18.2	12.6

	192	216	240
LC50 (% volume groundwater)	19.1	13.0	12.1
70% conf. limits	23.8	16.3	14.5
	15.2	10.4	10.1
95% conf. limits	29.6	20.2	17.2
	12.3	8.4	8.5

* Mortality not sufficient to calculate LC50.

TABLE 9. Results of acute toxicity studies with juvenile mountain whitefish (*P. williamsoni*). Test conducted at 5°C with saline groundwater (400 mOsm) diluted in Athabasca River water. n=26.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
79.4	5	5	5	5	5	5	5	5	5
31.6	5	2	2	5	5	5	5	5	5
12.6	5	0	0	0	0	1	1	1	1
1.25	5	0	0	0	0	0	0	0	0
Control	6	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	36.8	36.8	20.0	20.0	17.8	17.8	17.8	17.8
70% conf. limits	55.5	55.5	27.4	27.4	25.4	25.4	25.4	25.4
	24.5	24.5	14.5	14.5	12.5	12.5	12.5	12.5
95% conf. limits	82.2	82.2	37.2	37.2	35.6	35.6	35.6	35.6
	16.5	16.5	10.7	10.7	8.9	8.9	8.9	8.9

TABLE 10. Results of acute toxicity studies with juvenile mountain whitefish (*P. williamsoni*). Test conducted at 5°C with artificial sea-salts (normal concentration range) diluted in Athabasca River water. n=63.

Cumulative mortality:

Concentration salt water (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100.0	7	0	0	0	2	3	3	3	4
79.4	7	0	0	0	0	1	1	1	1
50.1	7	0	0	0	0	1	1	1	1
31.6	7	1	2	2	2	2	2	3	3
12.6	7	0	0	0	0	0	0	1	2
5.0	7	0	1	1	2	3	4	4	4
1.25	7	0	0	0	0	1	1	1	1
0.12	7	1	1	1	1	1	1	1	2
Control	7	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume salt water)	*	*	*	93.6	81.2	79.4	68.4	56.2
70% conf. limits	-	-	-	125.7	115.5	116.9	105.6	92.5
	-	-	-	69.7	56.0	54.0	44.2	34.2
95% conf. limits	-	-	-	--- very large range -------				
	-	-	-					

* Mortality not sufficient to calculate LC50.

TABLE 11. Results of acute toxicity studies with juvenile mountain whitefish (*P. williamsoni*). Test conducted at 5°C with saline groundwater (230 mOsm) diluted in dechlorinated Calgary tap water. n=60.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 24	48	72	96	120	144	168
100	10	10	10	10	10	10	10	10
56	10	1	3	7	8	10	10	10
32	10	0	0	0	0	0	0	0
18	10	0	0	0	0	0	1	1
10	10	1	1	1	1	2	3	3
Control	10	0	0	1	1	1	1	1

		192	216	240	264
100	10	10	10	10	10
56	10	10	10	10	10
32	10	2	3	4	9
18	10	1	1	2	3
10	10	4	6	6	6
Control	10	1	1	1	1

LC50 and confidence limits:

	Hours 24	48	72	96	120	144	168
LC50 (% volume groundwater)	70.2	63.4	44.6	42.8	38.9	37.1	37.1
70% conf. limits	79.7	73.2	51.5	49.0	44.3	46.2	46.2
	61.8	54.9	38.7	37.3	34.2	29.8	29.8
95% conf. limits	90.0	84.1	59.4	53.8	50.1	57.1	57.1
	54.7	47.8	33.4	32.8	30.2	24.1	24.1

	192	216	240	264
LC50 (% volume groundwater)	32.0	26.8	22.3	15.1
70% conf. limits	38.6	33.9	27.5	17.6
	26.9	21.1	18.1	13.0
95% conf. limits	44.5	42.6	33.6	20.4
	22.8	16.8	14.8	11.2

TABLE 12. Results of acute toxicity studies with juvenile mountain whitefish (*P. williamsoni*). Test conducted at 15°C with saline groundwater (230 mOsm) diluted in Athabasca River water. n=18.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 24	48	72	96	120	144	168
100	4	4	4	---------terminated-----------				
56	4	2	2					
32	4	0	0					
Control	6	1	1					

LC50 and confidence limits:

	Hours 24	48	72	96	120	144	168
LC50 (% volume groundwater)	49.6	49.6	---------terminated-----------				
70% conf. limits	64.1 38.3	64.1 38.3					
95% conf. limits	82.2 29.9	82.2 29.9					

TABLE 13. Results of acute toxicity studies with juvenile mountain whitefish (*P. williamsoni*). Test conducted at 15°C with artificial sea-salts (normal concentration range) diluted in Athabasca River water. n=45.

Cumulative mortality:

Concentration salt water (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100.0	5	3	3	3	4	5	5	5	5
79.4	5	0	0	0	0	0	0	2	3
50.1	5	0	0	0	0	0	0	0	0
31.6	5	0	0	0	0	0	0	0	0
12.6	5	0	0	0	0	0	0	0	0
5.0	5	0	1	1	1	1	1	1	1
1.25	5	1	1	1	1	2	2	2	2
0.12	5	0	0	0	2	2	2	2	2
Control	5	0	0	0	0	1	1	1	1

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume salt water)	93.2	92.5	92.5	83.3	70.3	70.3	56.0	52.7
70% conf. limits	111.5	113.3	113.3	116.5	115.6	115.6	95.2	91.5
	77.8	75.4	75.4	59.8	42.8	42.8	32.9	30.3

TABLE 14. Results of acute toxicity studies with juvenile grayling (*T. arcticus*). Test conducted at 5°C with saline groundwater (375 mOsm) diluted in Athabasca River water. n=112.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100.0	12	12	12	12	12	12	12	12	12
79.4	12	6	12	12	12	12	12	12	12
50.1	12	0	5	12	12	12	12	12	12
31.6	12	0	2	7	7	7	7	7	8
12.6	12	0	0	0	0	0	0	0	0
5.0	12	0	0	0	0	1	1	1	1
1.25	12	0	0	0	0	0	0	0	0
0.12	12	0	0	0	0	0	0	0	0
Control	16	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	79.4	50.1	27.7	27.7	26.2	26.2	26.2	23.9
70% conf. limits	89.7	60.1	33.9	33.9	32.3	32.3	32.3	29.6
	70.3	41.8	22.7	22.7	21.2	21.2	21.2	19.3
95% conf. limits	100.8	71.5	41.0	41.0	39.6	39.6	39.6	36.4
	62.5	35.1	18.8	18.8	17.3	17.3	17.3	15.7

TABLE 15. Results of acute toxicity studies with juvenile grayling (*T. arcticus*). Test conducted at 15°C with saline groundwater (375 mOsm) diluted in Athabasca River water. n=108.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120
100.0	12	12	12	12	12	12	12
79.4	12	12	12	12	12	12	12
50.1	12	4	9	9	9	11	12
31.6	12	1	1	3	4	6	8
12.6	12	0	0	0	1	1	1
5.0	12	0	1	1	2	2	2
1.25	12	0	0	1	5	7	8
0.12	12	0	0	4	6	7	8
Control	12	0	0	0	1	1	3

LC50 and confidence limits:

	Hours 12	24	48	72	96
LC50 (% volume groundwater)	54.6	41.0	34.1	19.6	13.2
70% conf. limits	65.7	47.6	46.9	29.9	18.2
	45.4	35.4	24.8	12.8	9.6
95% conf. limits	78.6	55.0	63.5	44.9	24.8
	38.0	30.6	18.3	8.5	7.3

TABLE 16. Results of acute toxicity studies with juvenile lake chub (*C. plumbeus*), Athabasca origin. Test conducted at 15°C with saline groundwater (350 mOsm) diluted in Athabasca River water. n=90.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100.0	10	8	10	10	10	10	10	10	10
79.4	10	2	5	8	10	10	10	10	10
50.1	10	0	0	0	0	2	3	3	3
31.6	10	0	0	0	0	0	0	0	0
12.6	10	0	0	0	0	0	0	0	0
5.0	10	0	0	0	0	0	0	0	0
1.25	10	0	0	0	0	0	0	0	0
0.12	10	0	0	0	0	0	0	0	0
Control	10	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	89.1	79.4	66.8	63.1	59.5	57.1	57.1	57.1
70% conf. limits	100.0	90.6	77.5	72.0	69.0	66.9	66.9	66.9
	79.5	69.6	57.6	55.3	51.4	48.8	48.8	48.8
95% conf. limits	111.5	102.9	89.3	81.7	79.5	77.9	77.9	77.9
	71.2	61.3	50.0	48.7	44.6	41.9	41.9	41.9

TABLE 17. Results of acute toxicity studies with juvenile lake chub (*C. plumbeus*), Nose Creek origin. Test conducted at 5°C with saline groundwater (375 mOsm) diluted in Athabasca River water. n=84.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100.0	10	0	1	10	10	10	10	10	10
79.4	11	0	0	4	8	9	10	11	11
50.1	10	0	0	0	0	0	0	0	0
31.6	11	0	0	0	0	0	0	0	0
12.6	10	0	0	0	0	0	0	0	0
5.0	11	0	0	0	0	0	0	0	0
0.12	11	0	0	0	0	0	0	0	0
Control	10	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	*	*	83.4	68.8	66.4	64.5	63.1	63.1
70% conf. limits	-	-	99.9	81.2	77.4	74.7	72.5	72.5
	-	-	69.6	58.3	56.9	55.8	54.9	54.9
95% conf. limits	-	-	118.8	95.2	89.8	86.0	82.9	82.9
	-	-	58.6	49.7	48.1	48.5	48.0	48.0

* Mortality not sufficient to calculate LC50.

TABLE 18. Results of acute toxicity studies with juvenile lake chub (*C. plumbeus*), Nose Creek origin. Test conducted at 15°C with saline groundwater (375 mOsm) diluted in Athabasca River water. n=73.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100.0	9	8	9	9	9	9	9	9	9
79.4	9	1	1	3	5	7	9	9	9
50.1	9	0	0	0	1	2	3	3	4
31.6	9	0	0	0	1	1	1	1	1
12.6	9	0	0	0	0	1	1	1	1
5.0	9	0	0	0	0	0	0	0	0
1.25	9	0	0	0	0	0	0	1	1
Control	10	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	89.1	87.8	84.1	69.9	59.1	52.0	50.1	45.5
70% conf. limits	97.1	96.2	93.4	82.0	71.7	63.7	61.9	54.9
	81.0	80.2	75.8	59.6	48.7	42.5	40.5	37.7
95% conf. limits	107.4	104.9	103.3	95.5	86.3	77.5	76.0	65.9
	73.9	73.5	68.5	51.2	40.4	35.0	33.1	31.4

TABLE 19. Results of acute toxicity studies with juvenile flathead chub (*P. gracilis*). Test conducted at 5°C with saline groundwater (350 mOsm) diluted in Athabasca River water. n=42.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100.0	6	4	6	6	6	6	6	6	6
79.4	5	0	5	5	5	5	5	5	5
50.1	5	0	0	0	3	4	4	4	4
31.6	5	0	0	0	0	0	0	0	0
12.6	5	0	0	0	0	0	0	0	0
5.0	5	0	0	0	0	0	0	0	0
0.12	5	0	0	0	0	0	0	0	0
Control	6	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	94.4	63.1	63.1	46.4	42.2	42.2	42.2	42.2
70% conf. limits	112.0	76.8	76.8	62.0	52.5	52.5	52.5	52.5
	79.6	51.8	51.8	34.7	33.8	33.8	33.8	33.8
95% conf. limits	131.9	92.6	92.6	81.9	64.9	64.9	64.9	64.9
	67.5	42.9	42.9	26.3	27.4	27.4	27.4	27.4

TABLE 20. Results of acute toxicity studies with juvenile white suckers (*C. commersoni*). Test conducted at 5°C with saline groundwater (350 mOsm) diluted in Athabasca River water. n=72.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100.0	8	1	6	8	8	8	8	8	8
79.4	8	0	0	3	6	7	7	8	8
50.1	8	0	0	0	0	1	1	1	1
31.6	8	0	0	0	0	0	0	0	0
12.6	8	0	0	0	0	0	0	0	0
5.0	8	0	0	0	0	0	0	0	0
1.25	8	0	0	0	0	0	0	0	0
0.12	8	0	0	0	0	0	0	0	0
Control	8	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	*	92.6	83.2	68.1	63.1	63.1	61.1	61.1
70% conf. limits	-	104.5	94.9	80.8	74.6	74.6	71.5	71.5
	-	82.1	72.9	57.5	53.4	53.4	52.1	52.1
95% conf. limits	-	117.3	107.7	95.2	87.6	87.6	83.2	83.2
	-	73.1	64.2	48.7	45.4	45.4	44.8	44.8

* Mortality not sufficient to calculate LC50.

TABLE 21. Results of acute toxicity studies with juvenile white suckers (*C. commersoni*). Test conducted at 15°C with saline groundwater (350 mOsm) diluted in Athabasca River water. n=74.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100.0	9	1	7	9	9	9	9	9	9
79.4	9	0	0	0	0	1	3	4	6
50.1	8	0	0	0	0	0	0	0	0
31.6	8	0	0	0	0	0	0	0	0
12.6	8	0	0	0	0	0	0	0	0
5.0	8	0	0	0	0	0	0	0	0
1.25	8	0	0	0	0	0	0	0	0
0.12	8	0	0	0	0	0	0	0	0
Control	8	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	*	90.4	89.1	89.1	87.9	84.1	81.3	70.8
70% conf. limits	-	100.9	98.8	98.8	98.0	95.5	93.4	84.7
	-	81.0	80.4	80.4	78.8	74.2	70.8	59.2
95% conf. limits	-	112.0	109.1	109.1	108.8	107.7	106.6	100.6
	-	72.9	72.8	72.8	70.9	65.7	62.0	49.9

* Mortality not sufficient to calculate LC50.

TABLE 22. Results of acute toxicity studies with juvenile and adult trout-perch (*P. omiscomaycus*). Test conducted at 5°C with saline groundwater (375 mOsm) diluted in Athabasca River water. n=54.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100.0	6	3	6	6	6	6	6	6	6
79.4	6	2	6	6	6	6	6	6	6
50.1	6	0	2	6	6	6	6	6	6
31.6	6	0	0	0	1	4	5	5	5
12.6	6	0	0	0	0	0	0	0	0
5.0	6	0	0	0	0	0	0	0	0
1.25	6	0	0	0	0	0	0	0	1
0.12	6	0	0	0	0	1	1	1	2
Control	6	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	93.1	56.2	39.8	38.0	22.8	20.2	20.2	17.2
70% conf. limits	112.6	69.3	47.2	45.8	31.1	26.8	26.8	26.4
	77.0	45.6	33.6	31.5	16.7	15.2	15.2	11.2
95% conf. limits	135.1	84.6	55.6	54.8	42.0	35.1	35.1	39.8
	64.2	37.4	28.5	26.3	12.4	11.6	11.6	7.4

TABLE 23. Results of acute toxicity studies with juvenile and adult trout-perch (*P. omiscomaycus*). Test conducted at 15°C with saline groundwater (375 mOsm) diluted in Athabasca River water. n=47.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100.0	5	5	5	5	5	5	5	5	5
79.4	5	1	2	3	5	5	5	5	5
50.1	5	0	0	1	2	4	5	5	5
31.6	5	0	0	0	0	0	0	1	2
12.6	5	0	0	0	1	2	2	2	2
5.0	5	0	0	0	0	0	0	0	1
1.25	5	0	0	1	1	1	1	1	1
0.12	6	0	0	0	1	1	1	1	1
Control	6	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	86.6	82.5	64.2	42.5	34.7	33.1	24.3	14.9
70% conf. limits	100.0	97.6	83.9	61.2	55.8	62.0	39.3	25.1
	74.9	69.8	49.1	29.6	21.6	17.7	15.0	8.9
95% conf. limits	115.2	114.7	108.5	86.7	87.9	113.3	62.5	41.2
	65.1	59.4	38.6	20.9	13.7	9.7	9.3	5.4

TABLE 24. Results of acute toxicity studies with juvenile walleye (*S. vitreum*). Test conducted at 5°C with saline groundwater (375 mOsm) diluted in Athabasca River water. n=43.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100.0	7	7	7	7	7	7	7	7	7
79.4	6	3	6	6	6	6	6	6	6
50.1	6	0	3	6	6	6	6	6	6
31.6	6	0	0	2	6	6	6	6	6
5.0	6	0	0	0	0	0	0	0	0
1.25	6	0	0	0	1	2	3	3	3
Control	6	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	79.4	50.1	35.5	10.8	9.3	7.9	7.9	7.9
70% conf. limits	93.4	63.0	43.2	15.3	13.5	13.6	13.6	13.6
	67.5	39.9	29.1	7.6	6.4	4.6	4.6	4.6
95% conf. limits	109.0	78.4	52.3	21.4	19.2	22.8	22.8	22.8
	57.8	32.0	24.5	5.4	4.5	2.8	2.8	2.8

TABLE 25. Results of acute toxicity studies with juvenile walleye (*S. vitreum*). Test conducted at 15°C with saline groundwater (375 mOsm) diluted in Athabasca River water. n=49.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100.0	7	7	7	7	7	7	7	7	7
79.4	7	7	7	7	7	7	7	7	7
50.1	7	2	3	7	7	7	7	7	7
31.6	7	0	0	1	2	5	7	7	7
5.0	7	1	1	1	1	1	2	2	3
1.25	7	0	0	0	0	1	2	2	2
Control	7	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	54.9	50.1	36.8	34.7	13.0	6.0	6.0	4.1
70% conf. limits	69.6	63.3	43.9	52.6	19.4	9.4	9.4	6.3
	43.3	39.7	31.0	22.8	8.8	3.9	3.9	2.6
95% conf. limits	87.4	79.2	51.9	78.5	28.4	14.4	14.4	9.8
	34.5	31.7	26.2	15.3	6.0	2.5	2.5	1.7

TABLE 26. Results of acute toxicity studies with mayfly larvae (*H. marginalis*). Test conducted at 5° with saline groundwater (350 mOsm) diluted with Athabasca River water. n=180.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100.0	20	0	3	10	19	20	20	20	20
79.4	20	0	2	6	13	17	20	20	20
50.1	20	0	0	0	1	2	2	4	5
31.6	20	0	0	0	1	3	4	4	4
12.6	20	0	0	0	1	2	2	2	2
5.0	20	0	0	0	1	1	1	1	1
1.25	20	0	0	0	0	1	1	1	1
0.12	20	0	0	0	0	0	0	0	0
Control	20	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	*	*	93.8	68.3	58.5	55.5	53.1	51.7
70% conf. limits	-	-	104.0	77.4	68.2	66.0	63.4	61.7
	-	-	84.7	60.3	50.1	46.7	44.4	43.3
95% conf. limits	-	-	114.8	87.2	79.1	78.0	75.3	73.1
	-	-	76.7	53.5	43.2	39.5	37.5	36.5

* Mortality not sufficient to calculate LC50.

TABLE 27. Results of acute toxicity studies with mayfly larvae (*H. marginalis*). Test conducted at 15°C with saline groundwater (350 mOsm) diluted in Athabasca River water. n=90.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100.0	10	3	9	10	10	10	10	10	10
79.4	10	2	8	10	10	10	10	10	10
50.1	10	1	3	6	8	10	10	10	10
31.6	10	0	1	1	1	2	3	5	8
12.6	10	0	0	0	0	0	0	0	0
5.0	10	0	0	0	0	0	0	0	0
1.25	10	0	0	0	0	0	0	0	0
0.12	10	0	0	0	0	0	0	0	0
Control	10	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	*	60.7	44.8	41.5	37.6	36.0	31.6	22.4
70% conf. limits	-	74.1	54.6	48.2	43.5	42.2	38.1	27.6
	-	49.7	36.8	35.7	32.4	30.8	26.2	18.2
95% conf. limits	-	89.7	66.0	55.6	50.2	49.1	45.6	33.7
	-	41.7	30.5	30.9	28.1	26.4	21.9	14.9

* Mortality not sufficient to calculate LC50.

TABLE 28. Results of acute toxicity studies with mayfly larvae (*P. bicornuta*). Test conducted at 5°C with saline groundwater (375 mOsm) diluted in Athabasca River water. n=90.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100.0	10	0	3	8	10	10	10	10	10
79.4	10	0	1	1	4	9	10	10	10
50.1	10	0	0	0	0	2	4	6	7
31.6	10	0	0	0	0	0	1	3	3
12.6	10	0	0	0	0	0	0	2	2
5.0	10	0	0	0	0	0	0	0	0
1.25	10	0	0	0	0	0	0	0	0
0.12	10	0	0	0	0	0	0	0	0
Control	10	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	*	*	90.5	82.5	61.1	52.0	38.8	37.3
70% conf. limits	-	-	100.9	93.1	71.3	63.9	49.3	49.0
	-	-	81.1	73.1	52.4	42.4	30.5	28.4
95% conf. limits	-	-	112.1	104.6	82.7	78.5	62.0	63.7
	-	-	73.0	65.3	45.2	35.5	24.3	21.8

* Mortality not sufficient to calculate LC50.

TABLE 29. Results of acute toxicity studies with stonefly larvae (*Isogenus* sp.). Test conducted at 5°C with saline groundwater (375 mOsm) diluted in Athabasca River water. n=81.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100.0	9	1	1	1	3	5	7	9	9
79.4	9	0	0	1	3	3	4	4	4
50.1	9	0	0	0	0	0	0	0	0
31.6	9	0	0	0	0	0	0	0	0
12.6	9	0	0	0	0	0	0	0	0
5.0	9	0	0	0	0	0	0	0	0
1.25	9	0	0	0	0	0	0	0	0
0.12	9	0	0	0	0	0	0	0	0
Control	9	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	*	*	*	100.0	91.5	84.7	81.3	81.3
70% conf. limits	-	-	-	119.8	106.3	97.7	92.7	92.7
	-	-	-	83.5	78.8	73.5	71.2	71.2
95% conf. limits	-	-	-	-	122.7	111.9	105.3	105.3
	-	-	-	-	68.3	64.2	62.7	62.7

* Mortality not sufficient to calculate LC50.

TABLE 30. Results of acute toxicity studies with stonefly larvae (*Isogenus* sp.). Test conducted at 15°C with saline groundwater (375 mOsm) diluted in Athabasca River water. n=45.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100.0	5	2	3	5	5	5	5	5	5
79.4	5	0	1	1	2	2	2	3	3
50.1	5	0	1	1	1	1	1	1	1
31.6	5	0	1	1	1	2	2	3	3
12.6	5	0	0	0	0	1	1	1	1
5.0	5	0	0	0	0	0	0	0	0
1.25	5	0	0	0	0	0	0	0	0
0.12	5	0	0	0	0	0	0	0	0
Control	5	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	*	87.1	81.7	72.3	61.7	61.7	53.2	53.2
70% conf. limits	-	113.4	107.4	91.9	89.5	89.5	80.9	80.9
	-	66.9	62.2	56.9	42.5	42.5	35.0	35.0
95% conf. limits	-	146.3	139.4	115.7	127.9	127.9	120.9	120.9
	-	51.9	47.9	45.2	29.7	29.7	23.4	23.4

* Mortality not sufficient to calculate LC50.

TABLE 31. Results of acute toxicity studies with caddisfly larvae (*H. bifida*). Test conducted at 5°C with saline groundwater (375 mOsm) diluted in Athabasca River water. n=135.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100.0	15	2	4	7	10	12	12	14	14
79.4	15	1	2	3	6	6	6	8	11
50.1	15	0	0	0	6	6	8	8	8
31.6	15	0	0	0	3	3	4	5	5
12.6	15	0	0	0	0	0	0	2	2
5.0	15	0	0	0	0	0	0	0	0
1.25	15	0	0	0	0	1	1	1	1
0.12	15	0	0	0	0	0	0	0	0
Control	15	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	*	*	96.0	76.8	69.6	61.8	48.8	45.0
70% conf. limits	-	-	109.0	92.5	84.5	75.9	60.9	55.3
	-	-	86.3	63.8	57.3	50.2	39.0	36.6
95% conf. limits	-	-	122.0	110.6	101.7	92.5	75.4	67.5
	-	-	77.1	53.2	47.6	41.2	31.6	30.0

* Mortality not sufficient to calculate LC50.

TABLE 32. Results of acute toxicity studies with caddisfly larvae (*H. bifida*). Test conducted at 15°C with saline groundwater (350 mOsm) diluted in Athabasca River water. n=135.

Cumulative mortality:

Concentration groundwater (% by volume)	n	Hours 12	24	48	72	96	120	144	168
100.0	15	10	14	15	15	15	15	15	15
79.4	15	1	1	4	5	6	6	6	8
50.1	15	3	4	5	6	6	6	6	7
31.6	15	0	0	1	2	5	5	9	10
12.6	15	0	0	0	1	1	1	2	3
5.0	15	0	0	0	0	0	0	1	2
1.25	15	0	0	0	0	0	0	1	3
0.12	15	0	0	0	0	0	0	0	1
Control	15	0	0	0	0	0	0	0	0

LC50 and confidence limits:

	Hours 12	24	48	72	96	120	144	168
LC50 (% volume groundwater)	90.8	86.2	80.3	68.6	59.6	59.6	48.2	32.9
70% conf. limits	100.5	94.5	94.6	82.8	73.1	73.1	62.3	43.6
	82.0	78.7	68.2	56.9	48.5	48.5	37.3	24.9
95% conf. limits	110.8	103.1	110.7	99.1	88.9	88.9	79.7	57.1
	74.4	72.1	47.6	39.9	39.9	39.9	29.1	19.0