FINAL REPORT

State: West Virginia

Project Number: F-18-R

Project Type: Research

Project Title: To develop laboratory facilities to conduct long-term chronic bioassays on the effects of acid water on aquatic organisms.

Period Covered: July 1, 1974 to June 30, 1976

Job Objectives: To determine chronic effects of iron and aluminum on brook trout.

ABSTRACT

During an 11 month period, all developmental stages of brook trout (*Salvelinus fontinalis*) were continually exposed to different concentrations of iron and aluminum. Data collected indicate that concentrations of iron should not exceed 1.37 mg/l and concentrations of aluminum should not exceed 0.58 mg/l.

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INTRODUCTION

Waters draining from coal mines typically contain a complex mixture of minerals in concentrations that may be toxic to animals (Parsons, 1957). Amoung these mineral compounds are sulfuric acid and the acid salts of iron, aluminum, zinc, lead, and copper. The toxicity of mine wastes to aquatic animals is doubtless a function of more than one factor, including the concentration of dissociated hydrogen and sulfate ions, increased osmotic pressure caused by high concentrations of mineral salts, increased carbon dioxide tensions resulting from low pH values, oxygen reduction by the oxidation of metals, and possible synergism of metallic cations. In addition, smothering blankets of iron salts deposited on stream beds reduce the numbers and possibly the variety of benthic inverebrates and algae.

The effects of acid water and of several metals which enter the aquatic ecosystem as a result of mining activities have been intensively studied. The chronic effects of low pH has been described by Mount (1973) for fathead minnows and by Menendez (1976) for brook trout. The effects of various metals are described in a review by McKim et al (1973).

Two metals of concern for which little chronic data is available are iron and aluminum. Both metals are found in abundance and are associated with acid mine drainage and land disturbances associated with mining activities.

The reaction of iron and the formation of iron salts in natural waters has been described by many authors. Among these are Sykora, Smith and Synak (1972) and Smith and Sykora (1976). They describe the effects of lime-neutralized iron hydroxide suspensions on brook trout and coho salmon.

Aluminum is the third most abundant element in the earth's crust and the most abundant metal. However its natural occurrence is limited to highly insoluble complex minerals. The behavior of aluminum in solution is extremely complex and it forms a variety of complexes with water. The behavior of aluminum in natural waters has been described in detail by Hem and Roberson (1967), Hem (1968) and Roberson and Hem (1969).

Everhart and Freeman (1973) describe the toxicity to rainbow trout of aluminum complexes in basic and neutral media.

The study described here is an attempt to determine the chronic effects of iron and aluminum on the various life history stages of the brook trout (*Salvelinus fontinalis*).

METHODS

The methodology employed in conducting the long-term exposure was generally similar to that of McKim and Benoit (1971) and is described in detail by Menendez (1976).

Toxicant solution used in the iron exposure was prepared by dissolving reagent grade ferrous sulfate ($FeSO_4 \cdot 7H_2O$) into 20 l of dilution water acidified with 10 ml

of concentrated HCl to prevent precipitation. The aluminum stock solution was prepared by dissolving the powder form of the metal in 15 ml HCl per l of stock solution. The stock solutions were introduced from a Mariotte bottle to the diluter. The delivery rates were adjusted to produce nominal concentrations of 10.00, 5.00, 2.50, 1.25, and 0.63 mg/l for both metals. Since both stock solutions were highly acidic, it was necessary to introduce a solution of 1.N. sodium hydroxide into the system to maintain pH values at the same level as the controls. This was accomplished using a delivery system identical to that used for the toxicants.

Daily water samples (100 ml) from each test tank were composited over a 5-day period for the analysis of total iron and aluminum. The Phenanthrolene method was used for iron and the Eriochrome Cyanine R method for aluminum (APHA, 1971). Metal concentrations were determined on a Beckman spectrophotometer. Both methods were checked periodically for accuracy using atomic absorption analysis.

The chemical and physical characteristics of the dilution water has been previously described by Menendez (1976).

RESULTS

Iron Exposure

Physical and Chemical Conditions

The results of the weekly composite samples for each of the iron concentrations tested are presented in Table 1. All mean iron concentrations were significantly different from each other (P=0.05).

The pH values during the test ranged from 6.5 to 7.4 with a mean of 7.2±0.19. Mean dissolved oxygen was 8.3±0.82, and values were never below 70% saturation in any test tank.

Adult Exposure

After five months exposure the effects on survival, growth, and reproduction of adult brook trout varied with the concentration of toxicant. Total mortality of brook trout occurred at the highest iron concentration (10.76 mg/l). Survival was reduced significantly (P=0.05) at 5.88 mg/l and none of the females surviving at this concentration spawned although all had mature gonads.

Survival, growth and reproduction in 2.79, 1.37, 0.70 mg/l iron and in the controls (0.07 m/l iron) were similar.

Tests with Eggs and Alevins from Exposed Parents

Hatchability of eggs from exposed parents was reduced significantly at an iron concentration of 2.79 mg/l. Failure of eggs to hatch is believed due to ferric hydroxide precipitates which coated the eggs. Hatchability at 1.37, 0.70 and control (0.07) was similar (Table 4).

The highest concentration at which eggs were spawned (2.79 mg/l) caused a 45% mortality of alevins 12 weeks after hatching (Table 4). An alevin mortality of 38% was observed at 1.37 mg/l, and 28% at 0.70 mg/l. These mortalities did not differ significantly from the controls.

Tests with Alevins from Unexposed Parents

Significant mortality (P=0.05) of alevins was observed at iron concentrations below 1.37 mg/l compared to the control. Alevins exposed to iron concentrations of 10.76 and 5.88 mg/l experienced mortalities of 98% and 76% respectively. Most of this mortality occurred during the first week of exposure.

During the 12 week exposure, the growth rate of alevins was reduced at all iron concentrations, with significant differences (P=0.05) observed at iron concentrations greater than 1.37 mg/l (Fig. 1).

Growth measurements were also made on alevins from exposed parents and approximately the same results were observed.

Discussion

The results reported here indicate that iron does adversily affect the various life history stages depending on the concentration. Adult brook trout appear able to tolerate concentrations as high as 2.79 mg/l with little effect on survival, growth or reproduction. However hatchability, survival and growth of alevins was significantly reduced at concentrations greater than 1.37 mg/l. Thus it appears that the early stages of the brook trout are more sensitive than other stages in the life cycle.

The results presented here differ considerably from those reported by Sykora, Smith and Synak (1972) and Smith and Sykora (1976). They report no effects of iron concentrations as high as 10.0 mg/l on any life history stage of the brook trout. It should be pointed out however that their research was aimed at determining the effects of particulate suspensions, and fish were exposed to concentrations of lime-neutralized iron hydroxide at pH values above 8.0.

The pH values during this exposure were maintained near 7.0. At this level more iron would remain in solution. Comparison of results obtained in this experiment with those mentioned above would indicate that iron is toxic only in solution and not in the suspended form.

Aluminum Exposure

Physical and Chemical Conditions

The results of the weekly composite samples for each of the aluminum concentrations tested are presented in Table 4. All mean aluminum concentrations were significantly different from each other (P=0.05).

The pH values during the test ranged from 6.7 to 8.0 with a mean of 7.13±0.24. Extreme values (pH 8.0) were recorded on only two occasions. Mean dissolved oxygen was 7.8±0.62, and values were never below 70% saturation in any test tank.

Adult Exposure

After 5 months exposure, the effects of different concentrations on adult brook trout varied. Total mortality was observed at a concentration of 10.75 mg/l (Table 5) while concentrations of 5.90 and 2.71 mg/l decreased survival significantly. Growth was reduced slightly at concentrations higher than 0.58 mg/l. There was no statistical difference in egg production at any of the concentrations in which brook trout survived and spawned.

Tests with Eggs and Alevins from Exposed Parents

Hatchability of eggs from exposed parents was reduced at aluminum concentrations higher than 0.58 mg/l (Table 6). Most egg mortality occurred prior to the eyed-stage and may have resulted from coatings of precipitates which occurred in the incubation chambers. Alevins that did successfully hatch at the higher concentrations were less active than those hatched at lower concentrations, and did not begin feeding as readily.

Alevin mortality in aluminum concentrations from 0.58 to 5.90 mg/l ranged from 39% to 42% (Table 6), while the mortality rate among control alevins was 26%.

Tests with Alevins from Unexposed Parents

Mortality rates of alevins from unexposed parents was significant only at 10.75 mg/l (Table 6). There were no significant differences in mortality rates at the other concentrations tested, with exception of 2.71 mg/l where a 60% mortality was observed. The reason for this is unknown. During the 12 week exposure, the growth rates of alevins were reduced at all aluminum concentrations above 0.58 mg/l (Fig. 2).

Growth measurements were also made on alevins from exposed parents and, although their growth was slower, no significant differences could be found ($P=0.05$).

Discussion

The results obtained in this experiment indicate that aluminum concentrations above 1.24 mg/l reduce survival of adult trout and that growth is reduced above concentrations of 0.58 mg/l. Spawning and egg viability was not affected by concentrations up to 5.90 mg/l.

Aluminum concentrations greater than 0.58 mg/l caused reduced hatchability and reduced growth of alevins. Survival of alevins from exposed parents was reduced at all concentrations tested, however survival of alevins from unexposed parents was similar for all concentrations except 10.75 mg/l where significant mortality was observed. These alevins were hatched and held in control water before being exposed to the different aluminum concentrations and thus may have been in better condition. This difference in mortality of alevins from exposed and unexposed parents was not observed in the iron exposure tests.

Growth rates of alevins were reduced at concentrations greater than 0.58 mg/l.

The data obtained in this experiment indicate that for the species of fish tested in the dilution water used, the concentrations of aluminum should not exceed

0.58 mg/l. In a study on the effects of aqueous aluminum complexes in neutral and basic media to rainbow trout fingerlings Freeman and Everhart (1971) found the toxicities of various concentrations were highly dependent on pH. Dissolved concentrations over 1.5 mg/l aluminum caused drastic physiological and behavioral aberrations as well as acute mortality. Toxic effects of suspended aluminum, while they are more noticeable at low concentrations, are not as concentration dependent as for dissolved forms. They conclude the safe concentrations of either dissolved or suspended aluminum is well below 0.5 mg/l. Everhart and Freeman (1973) found that fertilization of rainbow trout eggs was not affected by the concentration of aluminum tested, and most mortalities occurred during hatching and in the post swim-up state.

SUMMARY

Adult brook trout were able to tolerate iron concentrations as high as 2.79 mg/l.

Hatchability, survival and growth of alevins were reduced at concentrations greater than 1.37 mg/l iron.

The data obtained indicate that for the species tested and the dilution water used at pH 7.2 iron concentrations should not exceed 1.37 mg/l.

Aluminum concentrations above 1.24 mg/l reduced survival of adult brook trout.

Growth of adults was reduced at aluminum concentrations above 0.58 mg/l.

Spawning and egg viability were not affected by aluminum concentrations up to 5.90 mg/l.

Aluminum concentrations greater than 0.58 mg/l caused reduced hatchability and growth of alevins.

Survival of alevins from exposed parents was reduced at all concentrations, while survival of alevins from unexposed parents was reduced only at the highest aluminum concentrations.

The data obtained in this experiment indicate that for the species tested and the dilution water used, the concentration of aluminum should not exceed 0.58 mg/l.

RECOMMENDATIONS

It is recommended the exposure tests be conducted at different pH levels and that tests to determine the combined effects of iron and aluminum be initiated.

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Prepared by: Raymond Menendez
Raymond Menendez
Project Leader

Approved by: Peter E. Zurbuch
Peter E. Zurbuch
Assistant Chief, Research

Reviewed by: William Santonas
William Santonas, Supervisor
Planning & Biometrics

Submitted by: Robert D. Ross
Robert D. Ross
Federal Aid Coordinator

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Table 1. Iron content of water from test tanks (35 5-day composite samples for each concentration)

Nominal Iron Mg/l	Measured Iron Concentrations Mean	Range
10.00	10.76 (2.02)[a]	7.50-15.00
5.00	5.88 (1.47)	4.70-9.75
2.50	2.79 (0.45)	2.00-3.50
1.25	1.37 (0.15)	1.00-1.70
0.63	0.70 (0.71)	0.51-0.90
Control	0.07 (0.05)	0.04-0.15

[a]Standard deviation

Table 2. Survival, growth, and spawning of yearling brook trout (*Salvelinus fontinalis*) after 5 mo. exposure to various iron concentrations (14 fish per concentration).

	Mean Iron Concentrations					
	10.76	5.88	2.79	1.37	0.70	Control 0.05
Survival (%)	0	36	78	64	100	100
Mean initial wt.(g)	191(41.36)[a]	193(32.66)	203(31.29)	204(26.74)	207(31.23)	202(43.72)
Mean terminal wt.(g)	-	259(53.36)	276(23.36)	286(66.10)	302(27.46)	294(47.00)
% increase	-	34	35	41	46	45
No. Surviving Males						
gonads mature	-	2	5	4	5	4
gonads immature	-	-	-	-	-	1
No. Surviving Females						
gonads mature	-	3	6	6	9	9
gonads immature	-	-	-	-	-	-
No. Females Spawning	-	0	4	4	6	7
Total Viable Eggs Spawned	-	0	2528	1824	3072	3142
Mean Viable Eggs Spawned per female	-	-	632	456	512	449

[a]Standard deviation

Table 3. Effects of various iron concentrations on trout hatchability, and observed mortality of alevins from parents exposed 5 months. The effects of iron on observed mortality of alevins from unexposed parents are also included.

Iron Concentrations Mg/l	Eggs from exposed parents Hatchability %	Observed Alevin Mortality (%)	
		From exposed parents	From unexposed parents
10.76	-	-	98(100)
5.88	-	-	76(100)
2.79	69(8)[a]	45(100)[b]	44(100)
1.37	89(8)	38(100)	32(100)
0.70	87(8)	28(100)	25(100)
Control	91(8)	24(100)	24(100)

[a]Number of Spawnings Checked
[b]Number of Alevins Exposed

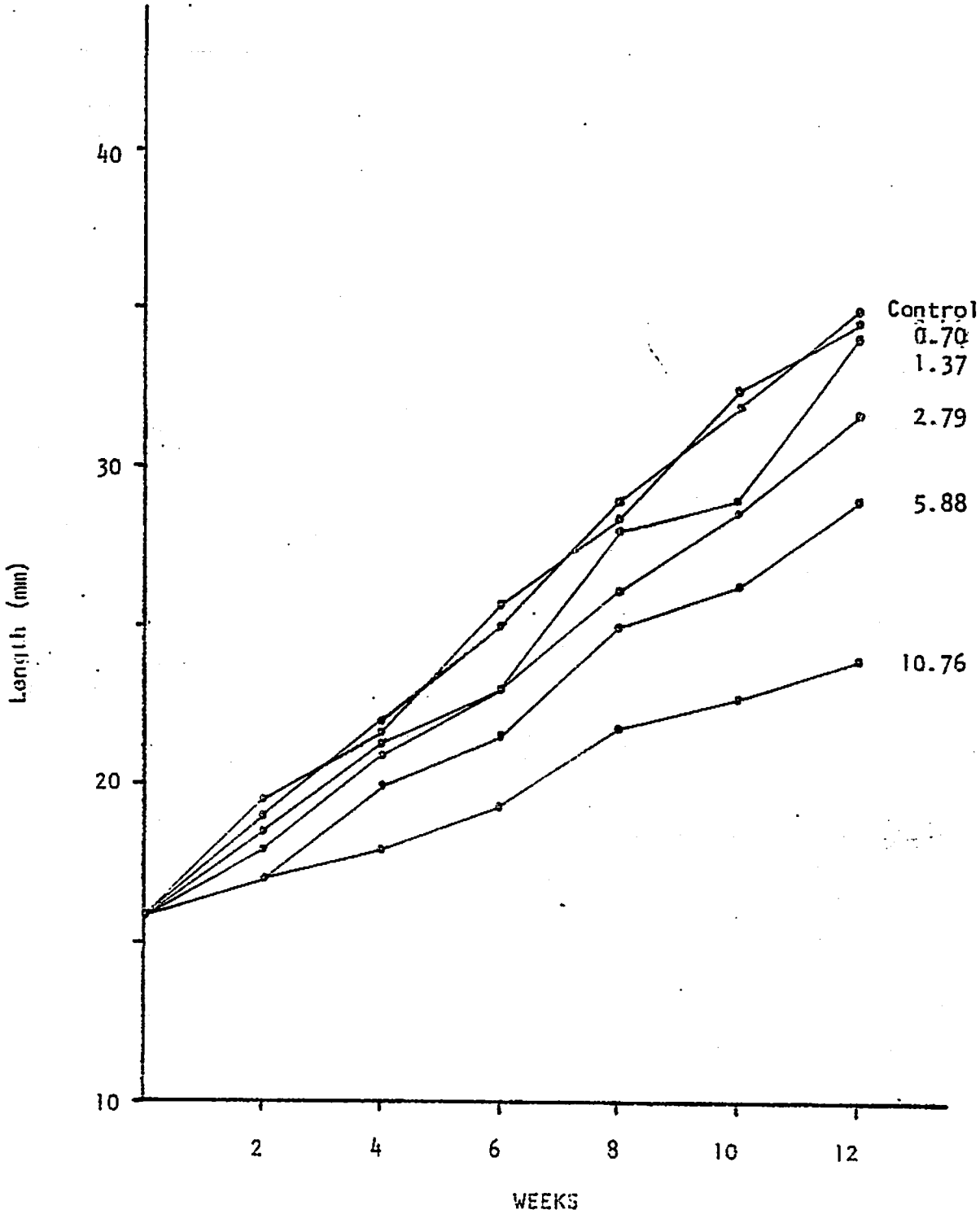

Figure 1. Growth rates of alevin brook trout from unexposed parents placed in five concentrations of iron. Iron concentrations are expressed in mg/l.

Table 4. Aluminum content of water from test tanks (35 5-day composite samples for each concentration)

Nominal Al. Mg/l	Measured Aluminum Concentrations	
	Mean	Range
10.00	10.75 (.96)[a]	8.60-12.50
5.00	5.90 (.16)	4.50-6.70
2.50	2.71 (.70)	1.50-4.10
1.25	1.24 (.21)	0.90-1.42
0.63	0.58 (.15)	0.40-0.81
Control	0.10 (.40)	0.04-0.20

[a]Standard deviation

Table 5. Survival, growth, and spawning of yearling brook trout (*Salvelinus fontinalis*) after 5 mo. exposure to various AL_2 concentrations.

	Mean AL_2 Concentrations					
	10.75	5.90	2.71	1.24	0.58	(Control) 0.10
Survival (%)	0	66	66	83	92	100
Mean initial wt.(g)	264.1(53.73)[a]	284.9(51.52)	248.42(47.85)	257.83(58.46)	237.0(10.922)	251.5(49.99)
Mean terminal wt.(g)	-	300.0(74.13)	284.87(72.21)	289.80(63.00)	292.27(40.22)	318.9(68.49)
% increase	-	5.3	14.7	12.39	23	26.8
No. Surviving Males						
gonads mature	-	3	3	3	3	4
gonads immature	-	0	0	0	1	0
No. Surviving Females						
gonads mature	-	5	5	7	7	8
gonads immature	-	0	0	0	0	0
No. Females Spawning	-	4	4	6	6	6
Total Viable Eggs Spawned	-	1913	2207	2938	3075	2860
Mean Viable Eggs Spawned per female	-	478	552	490	512	477

[a]Standard deviation

Table 6. Effects of various aluminum concentrations on trout hatchability, and observed mortality of alevins from parents exposed 5 months. The effects of aluminum on observed mortality of alevins from unexposed parents are also included.

Aluminum Concentration Mg/l	Eggs from exposed parents Hatchability %	Observed Alevin Mortality (%)	
		From exposed parents	From unexposed parents
10.75	-	-	74(100)
5.90	55(8)[a]	40(100)[b]	38(100)
2.71	67(8)	42(100)	60(100)
1.24	64(8)	41(100)	27(100)
0.58	80(8)	39(100)	25(100)
0.10 (Control)	87(8)	26(100)	26(100)

[a]Number of Spawnings Checked
[b]Number of Alevins Exposed

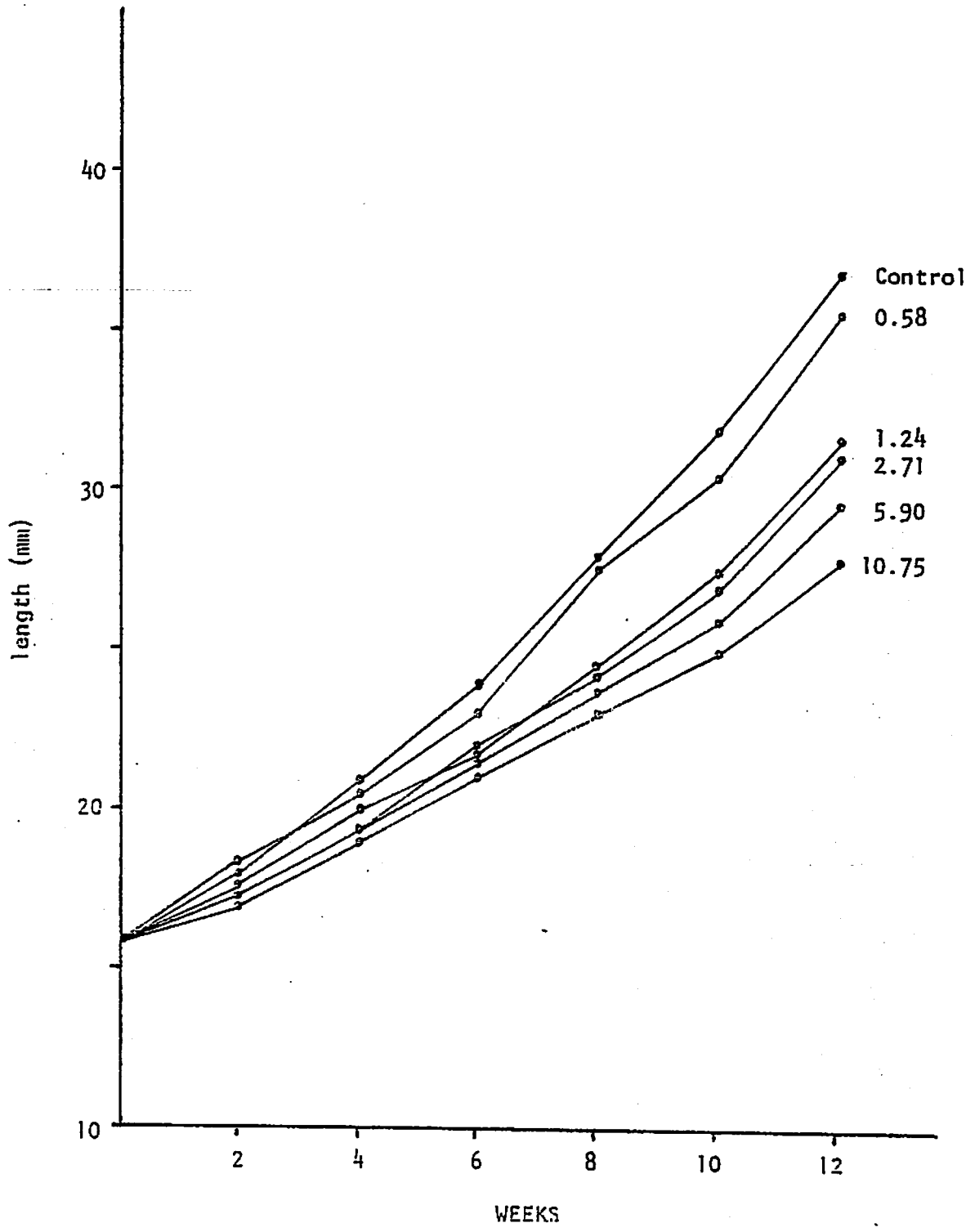

Figure 2. Growth rates of alevin brook trout from unexposed parents placed in five concentrations of aluminum. Aluminum concentrations are expressed in mg/l.