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THE EFFECT OF SUBLETHAL CONCENTRATIONS OF ZINC ON GROWTH AND PLASMA GLUCOSE LEVELS IN RAINBOW TROUT, *Salmo gairdneri* (RICHARDSON)

T. A. WATSON[1] and B. A. McKEOWN[2]

Abstract: The long term effects of three sublethal concentrations of zinc (0.214, 0.52 and 1.12 ppm) on growth and plasma glucose concentration in yearling rainbow trout *Salmo gairdneri* (Richardson) were investigated. Analysis of covariance of percent weight increase revealed that a significant inhibition of growth ($P<0.05$) in the 1.12 ppm zinc-exposed fish had occurred. Plasma glucose showed a significant hyperglycemia ($P<0.05$) in all three zinc-exposed groups of fish after 7 days exposure and in the 1.12 ppm zinc-exposed group after 63 days. The hyperglycemia observed has been explained as possibly resulting from activation of the pituitary-interrenal axis by the stress of zinc causing mobilization of tissue glycogen.

INTRODUCTION

Zinc is a naturally occurring heavy metal in many Canadian streams, rivers and lakes. However, this element has been found in much higher concentration than background levels in the effluents from mining and milling processes. These effluents containing zinc have caused pollution of adjacent bodies of water, damaging aquatic ecosystems and causing fish mortalities.[13,14,16]

To develop water quality criteria for protection of aquatic ecosystems from excessive levels of zinc, a number of laboratory performed acute-toxicity bioassays have been conducted from which "safe levels" are derived.[1,14] Although these studies provide valuable information they do not evaluate the effects of low concentrations of zinc on fish for prolonged periods of time.

A number of studies reported that sublethal concentrations of zinc over a long-term exposure had an inhibiting effect on the growth of guppies, *Poecilia reticulatus*,[6,7] fathead minnows, *Pimephales promelas*[2] and the minnow, *Phoxinus phoxinus*.[1] The results of these studies indicate that growth provides a sensitive measure for the effects of sublethal concentrations of zinc.

Blood glucose has been shown to be a sensitive indicator of environmental stress and the stress induced by handling, forced activity, thermal shock and contact with certain chemical pollutants.[4,5,8,9,12,15,19] Blood glucose could, therefore, be a useful indicator for the relative sublethal toxicity of zinc.

To assess the sublethal toxicity of zinc to rainbow trout (*Salmo gairdneri* R.), an 85-day exposure was conducted. During the test period, weight measurements and plasma glucose concentrations were determined.

MATERIALS AND METHODS

The rainbow trout used in this experiment were obtained as eggs from the Normandale Hatchery (Ontario) in December, 1973. They were hatched in running well-water at 10 ± 1 C. Lighting was supplied by fluorescent light fixtures controlled by a timer adjusted to simulate a natural light period. Fish were fed daily

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ad libitum on a commercial pellet diet.[3] All fish were acclimated to one 500 l capacity circular fibre-glass tank at 10 C for 3 months. The experiment was initiated when the fish were approximately 45 g in size.

Two weeks before initiating the experiment, 160 fish were removed from the 500 l tank, anaesthetized in 50 ppm MS222,[4] weighed and randomly distributed equally among five 50 l capacity enamelled tanks. Three of these five tanks were used for zinc exposure and the other two served as controls. The fish now in the 50 l capacity tanks were placed on a more controlled feeding regime (once daily at 1% of the total weight of fish in each tank) which continued until the end of the experiment. Three diluting apparatuses[15] delivered 1 l/min of zinc sulphate solution to each of three tanks. Approximately 50 l of aerated water was present in each tank and with the flow rate described above, 99% replacement was achieved in 3.8 hrs. Stock solutions of zinc were prepared by adding reagent grade zinc sulphate ($ZnSO_4 \cdot 7H_2O$)[5] to distilled water. Analysis of variance was performed on the weight observations to ensure homogeneity among the five test tanks.

Following the two week acclimation the fish were again anaesthetized and weighed; also, approximately 200 μl of blood was withdrawn by heart puncture with syringes from 5 to 10 fish chosen at random from four of the five tanks (the fifth tank served as an unbled control group to test the effects of heart puncture on growth and plasma glucose concentration). After the weight measurements were recorded and the blood samples collected on a given sampling day, the fish were returned to their appropriate tanks. The blood collected in the syringes was placed in ammonium heparinized capillary tubes, centrifuged and the plasma stored at —30 C until analyzed for plasma glucose concentration. On the 85th day blood samples were collected from the unbled control group as well as the other four treatments by the caudal severing technique described by Leatherland and Ensor.[10] Storage of the blood until glucose analysis was the same as mentioned above.

Blood sampling and weight measurements were conducted at 0, 7, 21, 42, 63, and 85 days. Weights were recorded for fish in all five experimental groups.

Plasma glucose and weight data were collected at the same time of day (1000-1200 hrs) to minimize circadian fluctuations. Plasma glucose concentration was determined using the hexokinase and glucose-6-phosphate dehydrogenase enzyme assay kit.[6] Analysis of variance was performed on plasma glucose and weight. In addition to analysis of variance on weight, the percent weight increase was calculated (as a percentage from the day 0 mean weight) and data were then analyzed by regression analysis and analysis of covariance.

Measurements of hardness (EDTA tritimetric method) and dissolved oxygen content (unmodified Winkler method) were carried out by the methods of Taras *et al.*[17] Temperature and pH were also recorded. The concentration of zinc (ppm) was measured weekly by atomic flame absorption.

RESULTS

Table 1 shows the physical-chemical properties and the observed zinc concentrations of water used in this experiment.

Mean weights ($\pm$ S.E.) and analysis of variance of the weight observations are presented in Table 2. No significant differences ($P>0.05$) were observed among the weights of the three zinc-exposed or two control groups. The plotted values of percent weight increase vs. time and results of analysis of covariance and regression analysis of these data are given

[3] Martin Feed Mill Company, Elmira, Ontario, Canada.
[4] Kent Laboratories, Vancouver, British Columbia, Canada.
[5] Fisher Scientific Company, Don Mills, Ontario, Canada.
[6] Sigma, St. Louis, Missouri, U.S.A. 63118.

TABLE 1. Physical-chemical properties and observed zinc concentrations of the water used in this experiment.

Tank	Mean observed zinc concentration (mg/l)	95% concentration limits (mg/l)	Oxygen confidence range (mg/l)	N	Temperature ° C (± S.E.)	N	pH* (± S.E.)	N *	Hardness* EDTA ± 95% confidence limits (mg/l)	N
1 (control)	<0.1	——	7.4-8.5	8	10.0 ± 0.1	15	——	—	——	—
2 (control)	<0.1	——	7.4-8.5	8	10.0 ± 0.1	15	——	—	——	—
3	0.214	± 0.035	7.9-8.6	8	10.0 ± 0.1	15	7.3 ± 0	8	374.0 ± 5.89	15
4	0.52	± 0.077	7.8-8.3	8	10.0 ± 0.1	15	——	—	——	—
5	1.12	± 0.153	7.5-8.7	8	10.0 ± 0.1	15	——	—	——	—

* Determinations made from one tank.

TABLE 2. Mean weights ± Standard Error of rainbow trout exposed to various zinc concentrations.

Tank	Zinc-Treatment	14-Days Prior to Experiment	Day 0	Day 7	Day 21	Day 42	Day 63	Day 85
1	control not bled	47.1 ± 2.68	46.1 ± 2.90	48.3 ± 2.81	53.4 ± 3.99	63.4 ± 5.34	75.8 ± 6.44	89.2 ± 7.37
2	control not bled	43.7 ± 2.91	45.2 ± 3.79	47.1 ± 3.95	51.5 ± 5.47	58.5 ± 6.64	71.0 ± 7.85	87.6 ± 8.55
3	0.214	43.1 ± 2.14	41.9 ± 2.53	43.6 ± 2.48	47.2 ± 3.41	54.6 ± 4.21	64.2 ± 5.13	76.8 ± 6.35
4	0.52	43.9 ± 2.53	42.9 ± 2.50	45.8 ± 2.90	49.1 ± 3.08	56.8 ± 3.65	67.7 ± 4.34	80.6 ± 5.07
5	1.12	43.7 ± 2.29	42.1 ± 2.52	42.7 ± 2.35	46.9 ± 3.03	53.1 ± 3.57	61.5 ± 4.43	71.0 ± 5.31
		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

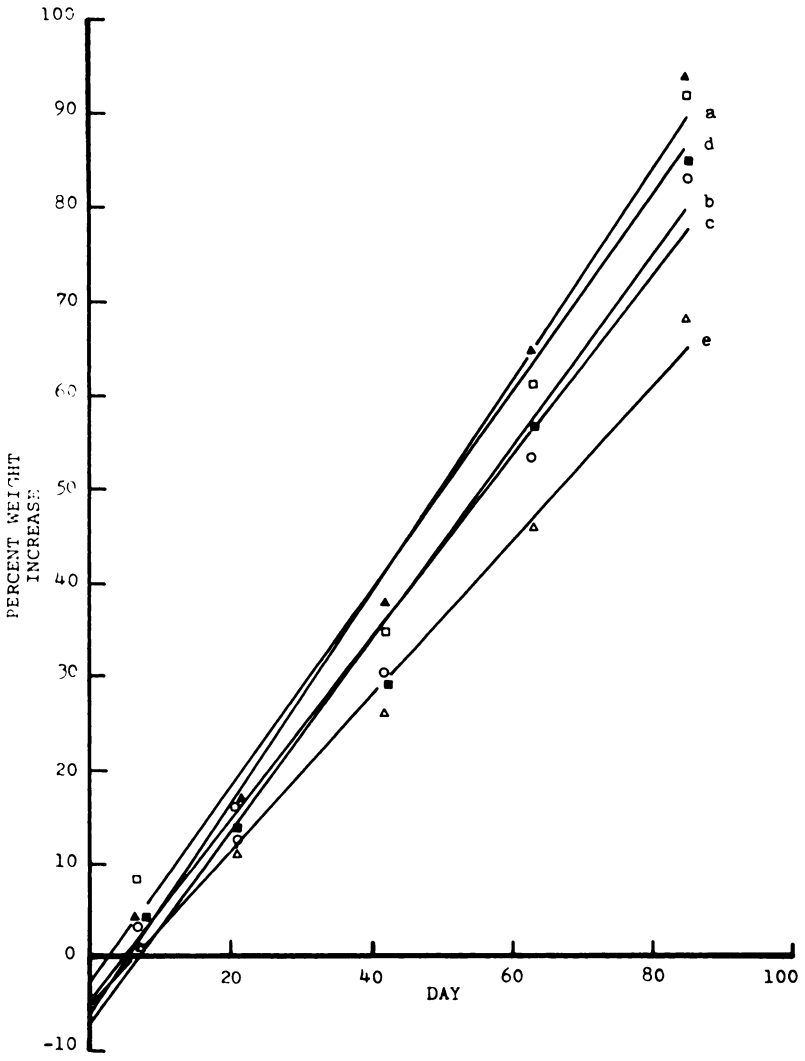

FIGURE 1. The relationship between percent increase in weight and time for yearling rainbow trout exposed for 85 days to zinc. Results of ANOCOVA show a significant difference in slopes and elevations at $P < 0.05$ but no difference in slopes or elevations when the 1.12 ppm zinc growth line was absent.

(a) Percent weight increase vs. time—control not bled
Growth curve = —4.7058 + 1.11X
$R^2 = 99.26$ $r = 0.99$. ▲———▲

(b) Percent weight increase vs. time—control bled
Growth curve = —4.88 + 0.999X
$R^2 = 98.04$ $r = 0.99$. ■———■

(c) Percent weight increase vs. time —0.214 ppm zinc
Growth curve = —5.274 + 0.975X
$R^2 = 98.15$ $r = 0.99$. ○———○

(d) Percent weight increase vs. time —0.52 ppm zinc
Growth curve = —3.185 + 1.055X
$R^2 = 98.43$ $r = 0.99$ □———□

(e) Percent weight increase vs. time —1.12 ppm zinc
Growth curve = —4.87 + 0.823X
$R^2 = 98.76$ $r = 0.99$. △———△

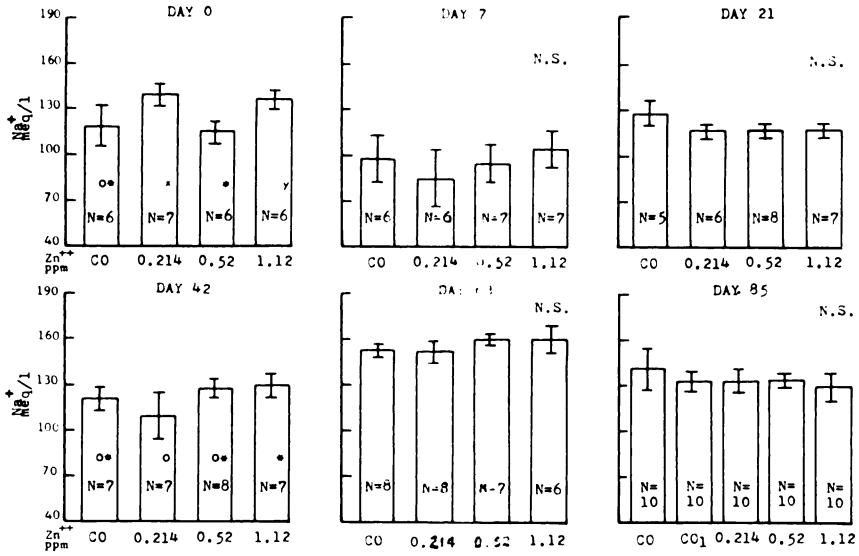

FIGURE 2. Mean plasma glucose concentrations (mg %) of yearling rainbow trout at the various sampling days with 95% confidence limits and results of ANOVA and Scheffe's comparison of means test. CO—bled control group. CO_1—unbled control group.
N.S.—not significant at $P > 0.05$.
Means significantly different ($P < 0.05$) from one another are distinguished by symbols; °*.

in Fig. 1. All five groups of fish showed a highly significant linearity in growth rate ($P<0.05$) over the exposure period. Analysis of covariance revealed that a significant inhibition of growth rate occurred in the 1.12 ppm zinc-exposed group of fish and that the rates of growth in the two controls and the 0.214 and 0.52 ppm zinc-exposed groups were not significantly different ($P>0.05$).

Analysis of variance on glucose indicated that significant hyperglycemia occurred on day 7 in the three zinc-exposed groups of fish (Fig. 2) On this day the mean glucose concentration of the control group (103.05 mg %) was significantly lower ($P<0.05$, Scheffe's test) than the means obtained from the zinc-exposed fish, *viz.*, 115.5 mg % (0.214 ppm zinc), 116.8 mg % (0.52 ppm zinc) and 115.3

mg % (1.12 ppm zinc). On day 63 a significant hyperglycemia ($P<0.05$) was observed in the 1.12 ppm zinc-exposed group's value of 71.92 mg %. No significant differences ($P>0.05$) in plasma glucose concentrations were observed on days 0, 21, 42, and 85 among treatments (Fig. 2).

A few mortalities occurred during the experiment; two fish in the bled control group, one in the 0.214 ppm zinc-exposed group, and three in the 1.12 ppm zinc-exposed group. No mortalities occurred in the unbled control and the 0.52 ppm zinc-exposed groups. The mortalities observed occurred before the experiment began in three of the six cases; of the other three one was attributed to heart puncture, two to starvation. None of the dead fish were included in any statistical calculations.

DISCUSSION

The results of regression analysis and analysis of covariance indicate that a significant ($P<0.05$) inhibition of growth occurred during an 85-day exposure of yearling rainbow trout to 1.12 ppm zinc. From the statistical tests employed it was not possible to determine the point at which the 1.12 ppm zinc-exposed groups differentiated in growth rate from the other four treatments. However, visual observation of the 1.12 ppm zinc-exposed group's growth curve shows that it was different from the others as early as 20 days exposure to 1.12 ppm zinc. This would indicate that growth experiments as short as 20 days should be sufficient to observe a suppressed growth rate.

A number of authors have reported compensatory growth of fish after an initial inhibition from exposure to a toxicant: fathead minnows in solutions of copper,[11] cichlids in solutions of potassium pentachlorophenate,[3] and minnows in solutions of zinc.[1] These studies, however, were conducted for at least 150 days and do not imply that a similar compensating growth should occur in rainbow trout. Nevertheless, it is a possibility that should be considered during longer-term exposures of rainbow trout to zinc. Since the fish in all five tanks received the same amount of food (based on 1% total body weight/tank), the decreased growth rate observed in the 1.12 ppm zinc-exposed group cannot be due to the consumption of less food since the fish consumed all the food presented. This suggests that assimilation of the food[18] and/or energy transformation[1] were in some way impaired.

The results of the plasma glucose determinations (Fig. 2) show that hyperglycemia occurred on day 7 in all three zinc-exposed groups of fish ($P<0.05$). However, on days 21 and 42, there were no significant differences ($P>0.05$) in plasma glucose among treatments, suggesting the fish were able to accommodate or adapt to the stress of these concentrations of dissolved zinc. On day 63 the 1.12 ppm zinc-exposed group's plasma glucose concentration was significantly greater ($P<0.05$) than the 0.214 ppm zinc-exposed group's but not greater than the other treatments. This observation is difficult to interpret but it may represent a difficulty in sampling or analysis, that was unnoticed at the time.

The hyperglycemia possibly may be attributed in part to the stress of dissolved zinc stimulating the pituitary-interrenal axis. Evidence for this is given by a number of other experimenters who observed hyperglycemia from numerous and varied stress conditions and correlated the hyperglycemia with the mobilization of tissue glycogen *via* the increased production of ACTH, corticosteroids and catecholamines.[4,12,9,5,8,19,15] Also Watson[18] found increased interrenal steroidogenic activity in rainbow trout subjected to sublethal concentrations of zinc in a long-term exposure.

The results of this research show that growth in part is a useful parameter in assessing the toxicity of sublethal concentrations of zinc and when included with plasma glucose measurement provides a further insight into the mechanisms of zinc-induced growth inhibition in rainbow trout.

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