WATER POLLUTION STUDIES

Federal Aid Project #F-243R-4

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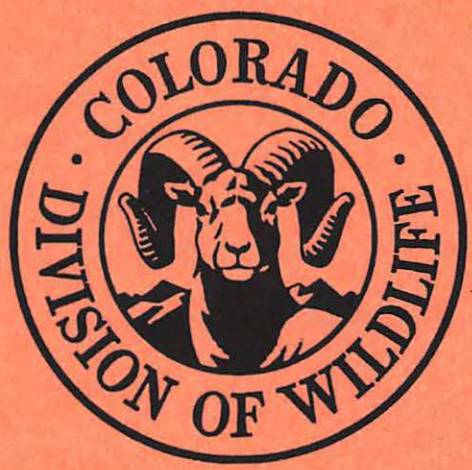

John Mumma, Director

Federal Aid in Fish and Wildlife Restoration

Job Final Report

Colorado Division of Wildlife

Fish Research Section

Fort Collins, Colorado

August 1997

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The results of the research investigations contained in this report represent work of the authors and may or may not have been implemented as Division of Wildlife policy by the Director or Wildlife Commission.

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JOB FINAL REPORT

State: Colorado

Project No. F243R-4

Title: Water Pollution Studies

Period Covered: July 1, 1996 to June 30, 1997

Principal Investigator: Patrick H. Davies
Co-investigators: Stephen F. Brinkman, Matthew McIntyre

Objective: To develop quantitative chemical and toxicological data on the toxicity of pollutants to aquatic life, investigate water pollution problems in the field, and provide expertise in aquatic chemistry and aquatic toxicology.

STUDY PLAN 1: REGULATORY AND LEGAL ACTIVITIES

Objective: To provide technical assistance to regulatory and legal entities toward the development, implementation, and enforcement of water quality standards needed to protect or enhance the aquatic resources of Colorado.

Job 1. Water quality standards for the protection of aquatic life in Colorado

Objectives:

1. To apply research results and toxicological information from literature toward the development, enactment, and implementation of water quality standards and appropriate aquatic life use classifications.

2. To provide technical information and/or expert testimony in aquatic toxicology and aquatic chemistry in agency meetings, regulatory hearings, and/or court litigations as needed to protect aquatic resources of Colorado.

3. To develop or compile toxicological and chemical data on toxicants for which state or federal governments have not developed a standard.

ACCOMPLISHMENT:

Studies on the chronic toxicity of silver to rainbow and brown trout in waters of different hardness have been completed. We have also completed experiments to evaluate effects of mountain and plains sediments on the acute toxicity of silver in soft and hard waters. These data are currently being analyzed. Currently, we are initiating additions experiments with sediments to assess there impact on the toxicity of different silver compounds. We are also initiating tests on the potential of "so called" insoluble silver compounds to be chronically toxic to fish. These experiments will be written up shortly and submitted as part of the state's exhibits at a November, 1997, hearing to consider evidence to again promulgate chronic silver standards in Colorado.

In June, we completed early life stage (ELS) toxicity tests with manganese on larval brook trout. We also conducted similar chronic toxicity tests on unacclimated brook trout fry to assess the chronic toxicity of manganese to brook trout and to assess the ability of brook trout to acclimate, i.e., tolerate high concentration of Mn from having been embryonically exposed. We agreed to perform these experiments in response to concerns of EPA regarding the protection of brook trout in the West Fork of Clear Creek with standards we helped to develop for brown trout. These data are also currently be analyzed and will be presented at the water quality standards hearing in November.

STUDY PLAN 2: LABORATORY STUDIES

Objective: To research and develop information on, or analytical tools in, aquatic chemistry and aquatic toxicology to better assess toxic responses of pollutants to aquatic life in laboratory and natural waters, such as the Arkansas River.

Job 1. Chemical Equilibria and Kinetic Effects on the Bioavailability and Toxicity of Metals to Aquatic Life

Objective: To develop analytical methods using Ion Chromatography, ion separation and/or ultrafiltration to measure toxic fractions and effects of chemical kinetics on toxicity of zinc, copper, lead, cadmium and/or silver to *Ceriodaphnia dubia*, rainbow trout, brown trout and/or fathead minnows in waters of different complexing capacity. Concurrently, investigate effects of chemical kinetics on results obtained from toxicity tests.

Job 2. Use of Biochemical Methods to Measure Disruption of Ion Regulation and Stress in Aquatic Organisms Exposed to Metals

Objective: To develop biochemical methods to measure effects on enzyme systems using electrophoresis or other methods to assess stress in rainbow and brown trout exposed to zinc, copper, lead and/or cadmium.

Job 3. Investigations on the Toxicity of Silver to Aquatic Organisms in Waters Different Complexing Capacity

Objective: To develop acceptable toxicant concentrations of silver for rainbow trout, brown trout, and/or fathead minnows in hard, high alkaline, and soft, low alkaline waters.

Job 4. Effects of Calcium Hardness, Inorganic and Organic Ligands and Sediments on Toxicity of Metals to Aquatic Organisms

Objective: To determine antagonistic effects of calcium hardness in low alkaline waters and the effects of specific inorganic and organic ligands and sediments on acute and long-term toxicity of zinc, copper, lead, cadmium, and/or silver to rainbow trout, brown trout and/or fathead minnows.

Job 5. Investigations on Enhanced Toxicity of Unionized Ammonia to Fish at Cold Water Temperatures

Objective: To determine effects of temperature on toxicity of unionized ammonia to rainbow trout and fathead minnows or other warmwater species at optimal and less than 5 °C water temperatures.

Job 6. Effects of Episodic Exposure on Toxicity and Sensitivity of Aquatic Life to Intermittent Exposure to Metals

Objective: To determine toxic effects and organism sensitivity to intermittent exposure of zinc, copper, lead, and/or cadmium to rainbow trout, brown trout and/or fathead minnows, and their ability to acquire and/or lose tolerance.

Job 7. Investigations on Enhanced Toxicity of Water-Borne Metals to Aquatic Life Exposed to Dietary Sources of Metals

Objective: To determine effects of water-borne zinc, copper, cadmium, lead and/or manganese on their toxicity to rainbow and brown trout following and/or concurrent with exposure to dietary metals.

Job 8. Investigations on Effects and Interactions of Multiple Metal Exposure on Toxicity to Aquatic Life

Objective: To determine effects of exposure of rainbow trout and/or brown trout to zinc, copper, cadmium, lead, and manganese at different combinations found in Colorado's mining areas. Will require an ability to measure bioavailable forms on metals as outlined in Job 1.

Job 9. Investigations of Analytical Methods to Measure Rotenone Concentrations in the Field and Antimycin Concentrations in the Laboratory

Objective: To develop a field method to measure rotenone and a laboratory method for measuring antimycin at concentrations less than 5 ppb using cation exchange chromatography.

ACCOMPLISHMENTS

Effects of Chronic Exposure of Boreal Toad Tadpoles (Bufo boreas) to Cadmium

INTRODUCTION

Boreal toads (*Bufo boreas*) have declined in Colorado over the last quarter century and have been state-listed as endangered since November 1993 and federally listed as "warranted but precluded" since March 1995 (Goettl [eds.] and the Boreal Toad Recovery Team 1997). The Colorado Division of Wildlife Aquatic Toxicology Laboratory is assisting with investigations into possible causes of this decline by evaluating water quality characteristics that may limit their survival and distribution. These efforts include analysis of water samples collected from current and historic breeding ponds, developing techniques to measure effects of toxicants (heavy metals, pesticides, deicing compounds) on tadpoles, and conducting

experiments to determine toxicity of selected metals to boreal toad tadpoles. During this segment we report the results of two chronic toxicity tests assessing the effects of cadmium and copper exposure to boreal toad tadpoles.

METHODS AND MATERIALS

A serial diluter (Benoit et al. 1982) delivered seven concentrations of cadmium (as cadmium sulfate) and control to completely randomized 2.2 liter glass exposure chambers at a rate of twenty mls/minute. The experiment was replicated four times with nominal cadmium exposure concentrations of 216, 72, 24, 12, 6, 3, 1.5 and 0 μg Cd/l. Source water consisted of dechlorinated Fort Collins tap water. Two fluorescent blacklights and two florescent full spectrum (Repta Sun) bulbs suspended approximately 12 inches above the exposure chambers provided artificial lighting with 16 hour light and 8 hour dark photoperiod.

Twenty boreal toad tadpoles (*Bufo boreas*) about 10 days post hatch were randomly placed in each exposure chamber. Tadpoles were fed a mixture of Mazuri, Silver Cup trout food and frozen organic romaine lettuce *ad libitum*. Exposure chambers were checked daily for mortality. Dead tadpoles were removed, blotted dry with a paper towel and weighed.

Water quality parameters were measured weekly in alternating replicates. Hardness and alkalinity were determined according to Standard Methods (APHA). pH was determined using an Orion Research pH meter 811 calibrated daily using pH 7.00 and 4.00 pH buffers. Conductivity was determined using a YSI Model 35 conductance meter. Anions were determined using a Dionex 4005i ion chromatograph.

Water samples for cadmium analysis were collected weekly in each exposure level from alternating replicates. Acid leachable as well as dissolved cadmium (passing through a 0.45 micron filter) were analyzed. Water samples for cadmium analysis were preserved by acidification to pH<2 using Ultrex nitric acid. Cadmium concentrations in water samples were measured using an Instrumentation Laboratory Video 22 atomic absorption spectrometer with air-acetylene flame or Thermo Jarrell Ash SH4000 with CTF 188 graphite furnace. Both instruments used Smith-Hieftje background correction.

Five tadpoles were randomly removed from each exposure chamber after 13, 28 and 56 days of exposure and terminally anesthetized with MS-222. Total length, snout-vent length, and Gosner stage of amphibian development were recorded (Gosner 1960). Total length and snout-vent length were not measured on tadpoles collected after 56 days because of metamorphosis. The tadpoles were rinsed with deionized water, blotted dry with a paper towel, pooled and weighed in preweighed polypropylene centrifuge tubes. Tubes containing the tadpoles were placed in a drying oven at 80°C and dried to constant weight. Trace metal grade nitric acid was added to the tubes which were then heated for eight hours in a water bath at 60°C. Trace metal grade hydrogen peroxide was added and the tubes heated an additional eight hours. The digests were then diluted to volume with deionized water and analyzed for cadmium content using an Instrumentation Laboratory Video 22 atomic absorption spectrometer with air-acetylene flame and Smith-Hieftje background correction.

Statistics

Analysis of variance (ANOVA) was performed on mortality and wet weight using SAS computer software (SAS 1989). Mortality data were transformed by arcsine squareroot prior to analysis (Snedecor and Cochran 1980). Assumptions of normality and homogeneity of variance were evaluated using Shapiro Wilk's test and Bartlett's test respectively ($p<0.10$). Treatment means were compared using one-tailed Dunnett's multiple comparison test ($p<0.05$) to evaluate differences from control.

RESULTS

Water quality characteristics of exposure water are shown in Table 1. Water quality characteristics were stable throughout the test with the exception of dissolved oxygen. Dissolved oxygen in the source water was relatively low (3.25-5.34 mg/L) during the first two weeks of exposure but this did not seem to adversely affect the tadpoles. Aeration of the source water after the first two weeks increased dissolved oxygen to near saturation for the remainder of the test. Total and dissolved cadmium concentrations and associated mortality are shown in Table 2. A large majority of total cadmium present in exposure water is dissolved which is characteristic of the soft water used in this experiment. Survival of boreal toad tadpoles was not affected by the cadmium exposure concentrations ($p>0.05$). However cadmium exposure had profound effects on development and growth. Gosnerian development was not different after 13 days of exposure but was significantly reduced after 28 days exposure to 241 and 71 μg Cd/L and after 56 days was reduced by concentrations greater than 7.2 μg Cd/L (Figure 1.). Total length was significantly reduced at cadmium concentrations of 7.2 μg/L and greater after 13 days exposure and reduced at 4.9 μg/L and greater after 28 days exposure (Figure 2). Snout-vent length was significantly reduced above exposure concentrations of 16.2 and 4.9 μg/L after 13 and 28 days exposure respectively (Figure 3). Wet weight was reduced in tadpoles exposed to a cadmium concentration of 4.9 μg/L or greater after thirteen and 28 days. After 56 days of exposure, wet weight was reduced at cadmium exposures greater than of 37.5 μg/L (Figure 4). Figure 5 shows that cadmium is rapidly accumulated by boreal toad tadpoles. Whole body cadmium content increased in all exposure levels compared to the control (Figure 5). The influence of duration of exposure on whole-body cadmium content does not follow a clear trend. Cadmium content increased between thirteen days and 28 days of exposure but decreased considerably between 28 and 56 days of exposure.

DISCUSSION

Boreal toad tadpoles are much more resistant to the lethal effects of cadmium than other aquatic vertebrates. For example, 96 hour LC50s for salmonids range between 1.4 and 3.8 μg/L (Chapman 1978, Spehar and Carlson 1984, Buhl and Hamilton 1991, Davies et al. 1993). Furthermore, the tadpoles in this experiment consumed food that was present in the exposure water for extended periods of time. It is likely that cadmium sorbed onto the food resulting in dietary as well as waterborne exposure to cadmium. The results of this experiment indicate that boreal toad tadpoles tolerate very high concentrations of cadmium. The presence of elevated whole body cadmium content does not necessarily indicate the potential for reduced survival. The concentrations of cadmium used here were relatively high and would not be present except in the most metal-impacted areas. However, cadmium exposure dramatically impacted growth and development of *Bufo boreas* tadpoles. Reduced growth may result in decreased overwinter survival. A delay in development could lead to death if the tadpole is unable to metamorphose before onset of winter; a likely possibility given the short summer at the higher elevations where boreal toads breed.

Whole body content of cadmium was dose-dependent and overall increased between 13 and 28 days. However cadmium content decreased between 28 and 56 days. The most likely explanation is that metamorphosis results in significant excretion of cadmium.

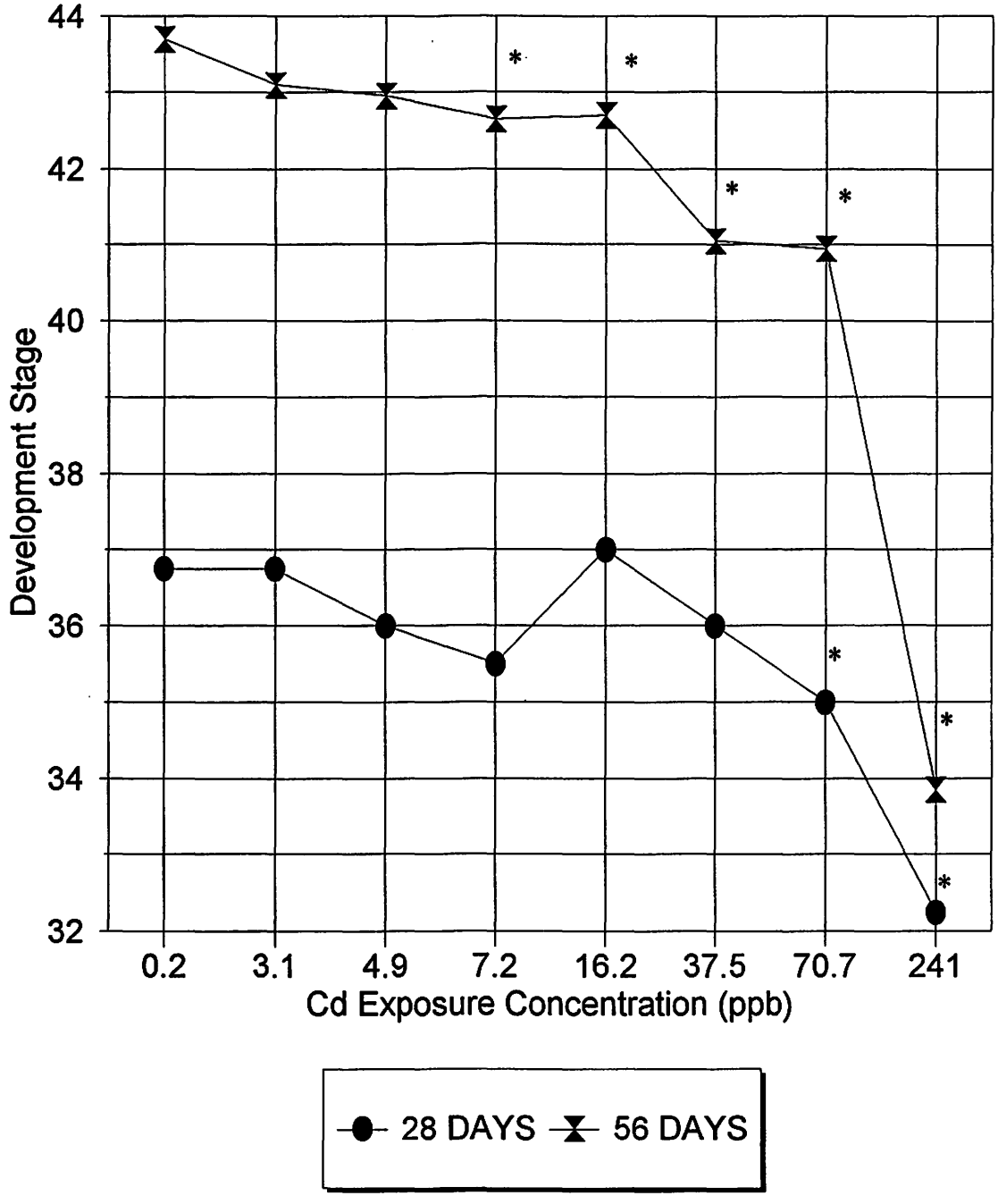

Figure 1. Gosner development of boreal toads tadpoles exposed to cadmium. Asterisk indicates treatment significantly less than control ($p<0.05$).

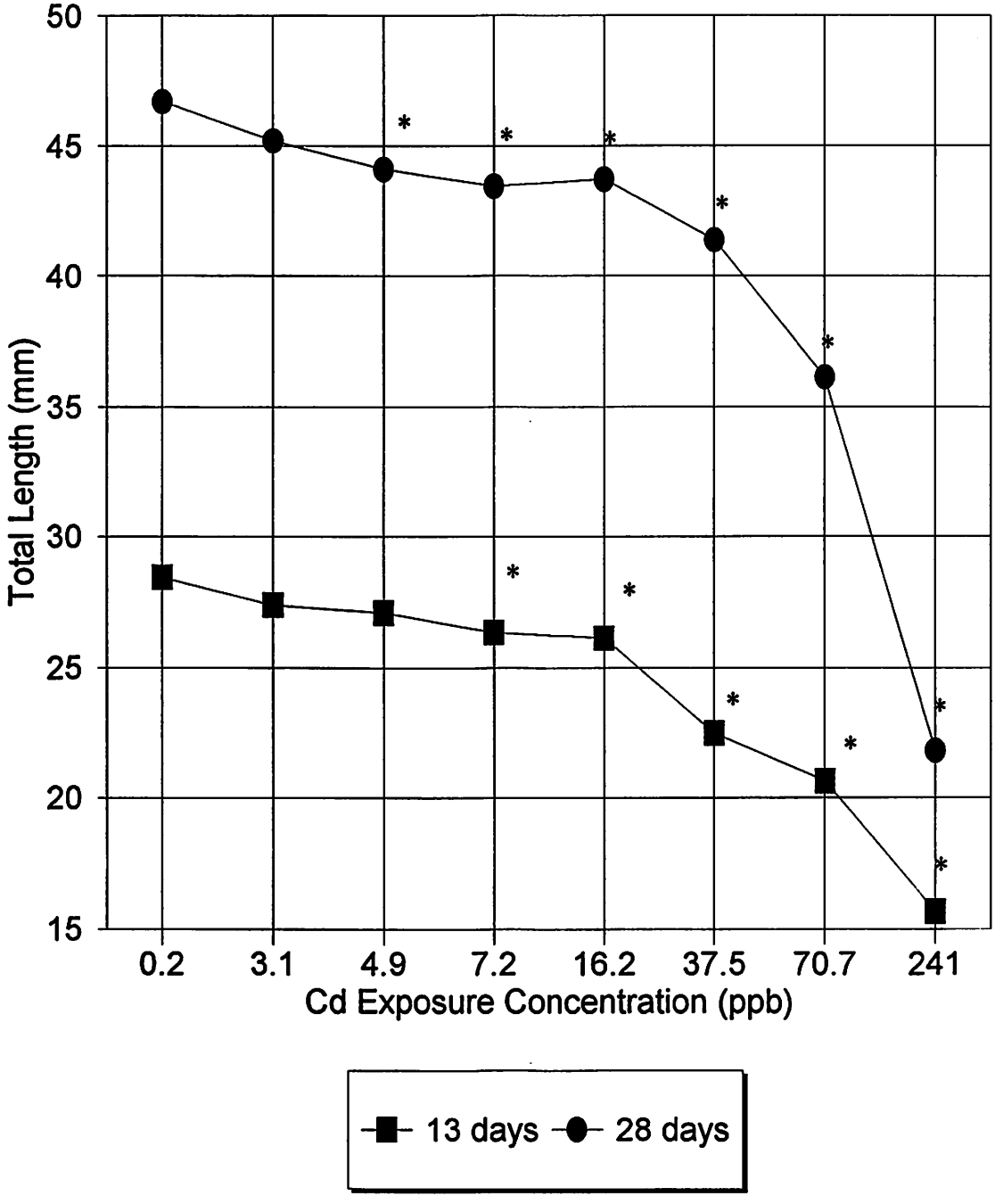

Figure 2. Total length (mm) of boreal toads tadpoles exposed to cadmium for 13 and 28 days. Asterisk indicates significantly lower than control ($p<0.05$).

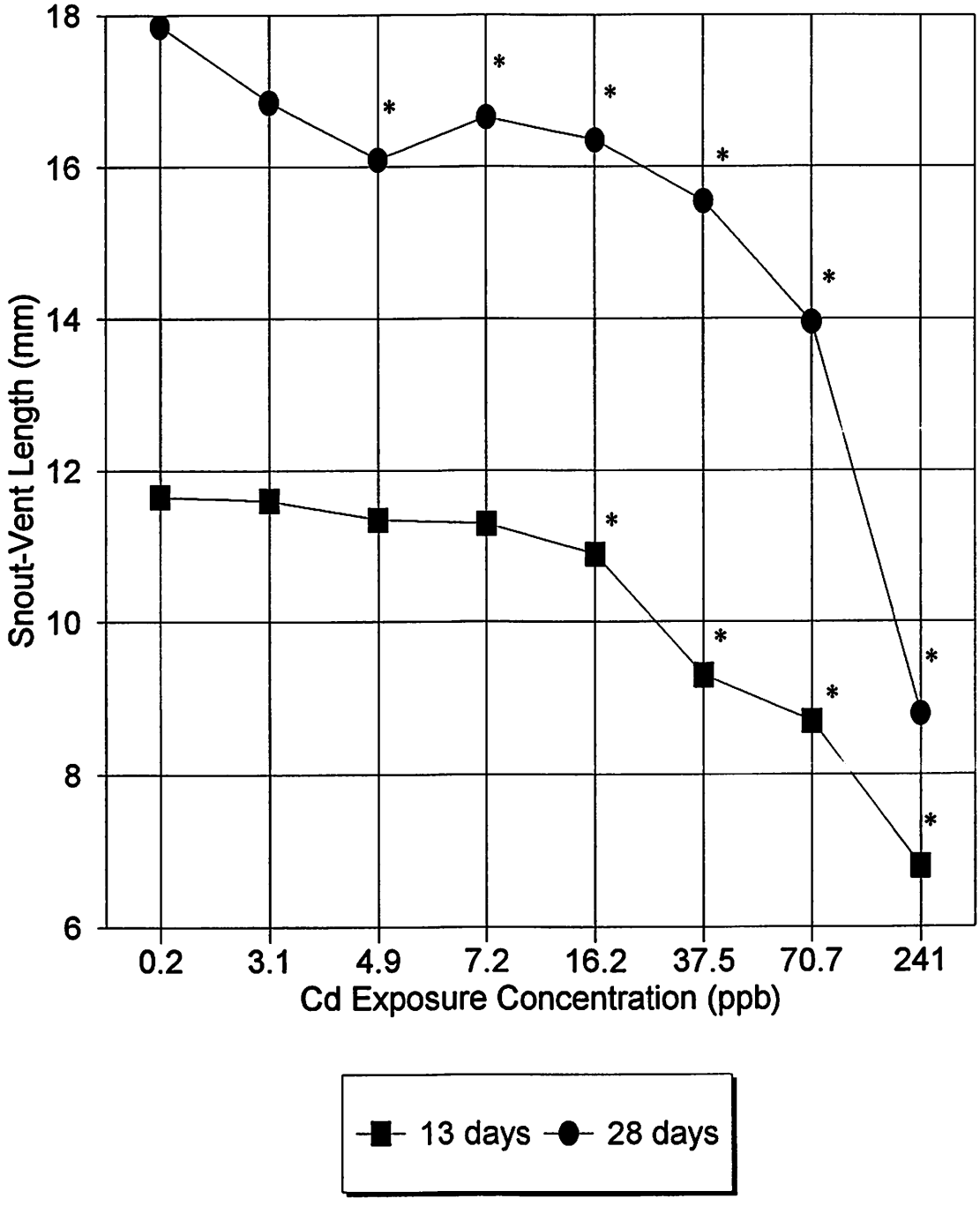

Figure 3. Snout-vent length (mm) of boreal toad tadpoles exposed to cadmium for 13 and 28 days. Asterisk indicates significantly lower than control ($p<0.05$).

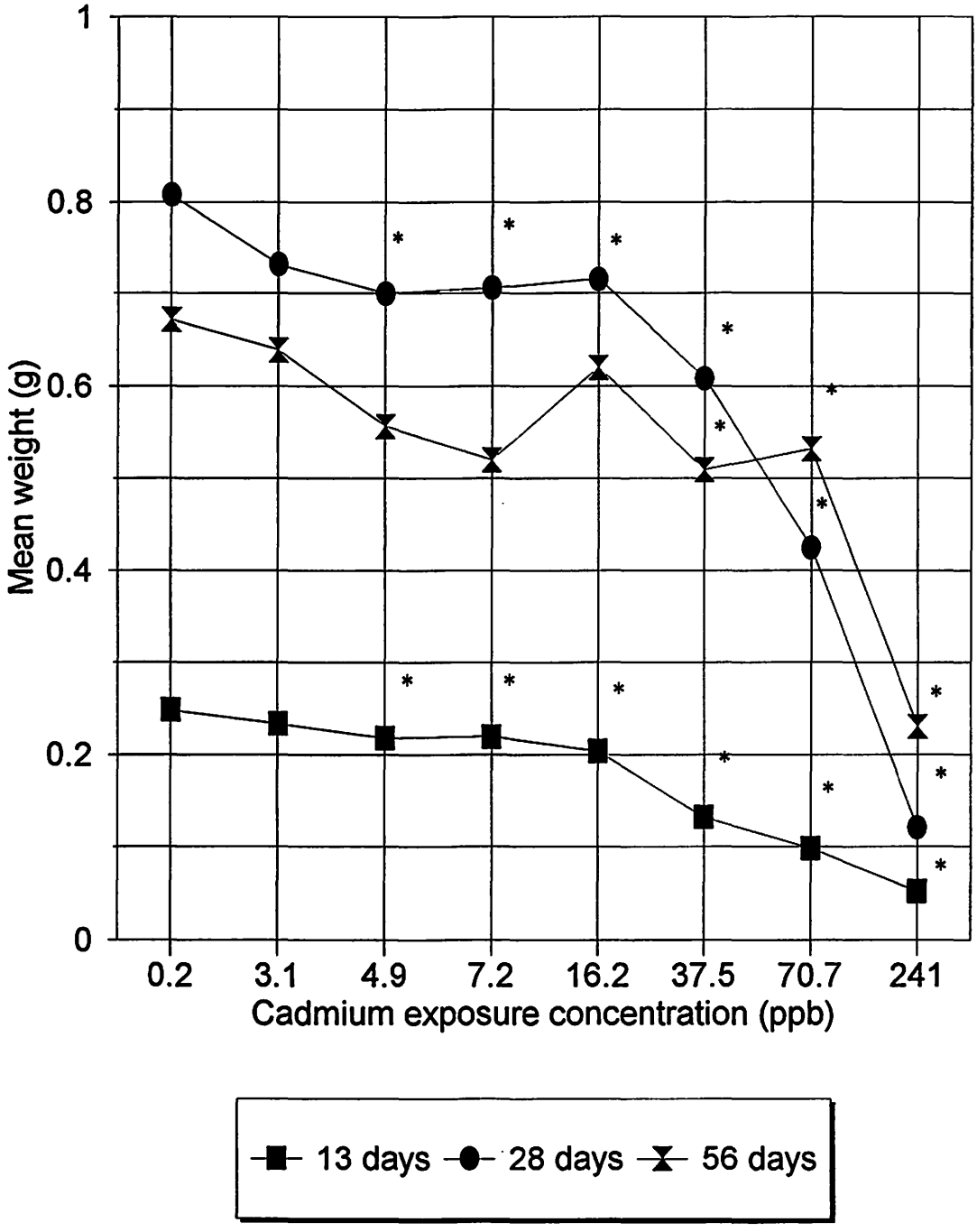

Figure 4. Mean wet weight (g) of boreal toads tadpoles after 13, 28, and 56 days exposure to cadmium. Asterisk indicates significantly less than control ($p<0.05$).

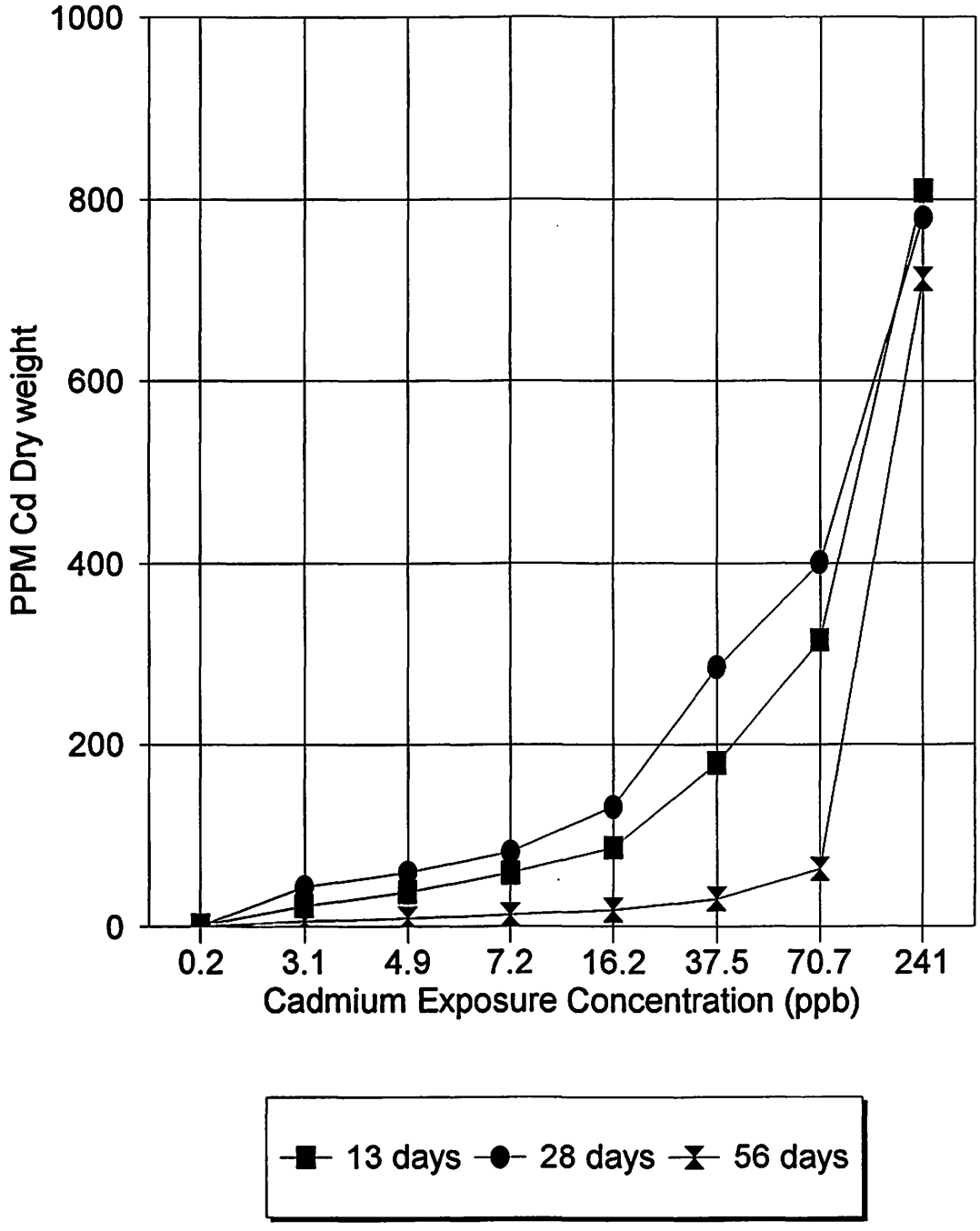

Figure 5. Whole body cadmium content (mg/Kg dry weight) of boreal toad tadpoles exposed to cadmium for 13, 28, and 56 days. All treatments greater than control ($p<0.05$)

Table 1. Water quality characteristics of exposure water used in cadmium toxicity test conducted on boreal toad tadpoles.

	MEAN	STANDARD DEVIATION	RANGE
HARDNESS (mg $CaCO_3$/L)	50.4	2.2	44.0-55.0
ALKALINITY (mg $CaCO_3$/L)	37.1	4.7	32.8-65.8
pH (S.U.)	7.25	0.13	6.97-7.46
DISSOLVED OXYGEN (mg O_2/L)	5.65	1.36	3.21-7.70
TEMPERATURE (°C)	19.1	0.6	18.1-20.0
CONDUCTIVITY (μS/cm)	102.8	3.6	95.0-114.0
FLUORIDE (mg/L)	0.88	0.21	0.37-1.14
CHLORIDE (mg/L)	2.08	0.35	1.54-2.76
NITRATE (mg/L)	0.31	0.36	0.00-1.2
PHOSPHATE (mg/L)	0.06	0.17	0.00-0.62
SULFATE (mg/L)	11.82	0.25	11.5-12.3

Table 2. Total and dissolved cadmium exposure concentrations (standard deviations) and associated survival of boreal toad tadpoles exposed for 47 days.

Nominal Cd Conc (μg/L)	Measured Total (μg/L)	Measured Dissolved (μg/L)	Percent Survival
216	241.0 (38.3)	202.3 (16.3)	85.0 (0.6)
72	70.7 (11.1)	63.5 (10.6)	87.5 (0.5)
36	37.5 (7.0)	32.8 (6.7)	90.0 (0.8)
12	16.2 (2.6)	14.2 (2.5)	87.5 (0.5)
6.0	7.2 (2.3)	6.6 (1.9)	92.5 (0.5)
3.0	4.9 (1.9)	3.7 (1.2)	87.5 (1.3)
1.5	3.1 (1.2)	2.7 (1.1)	87.5 (1.3)
0	0.2 (0.1)	<0.1 (0.0)	92.5 (0.5)

Effects of Chronic Exposure of Boreal Toad Tadpoles (Bufo boreas) to Copper

MATERIALS AND METHODS

A serial diluter (Benoit et al. 1982) delivered seven concentrations of copper (as copper sulfate pentahydrate) and control to completely randomized 2.2 liter glass exposure chambers at a rate of twenty mls/minute. The experiment was replicated four times with nominal copper exposure concentrations of 100, 50, 25, 12.5, 6.25, 3.12 1.56 and 0 μg Cu/L. Source water consisted of dechlorinated Fort Collins tap water. Two fluorescent blacklights and two florescent full spectrum (Repta Sun) bulbs suspended approximately 12 inches above the exposure chambers provided artificial lighting with 16 hour light and 8 hour dark photoperiod.

Twenty-five boreal toad tadpoles about 10 days post hatch were randomly placed in each exposure chamber. Tadpoles were fed a mixture of Mazuri, Silver Cup trout food and frozen organic romaine lettuce *ad libitum*. Exposure chambers were checked daily for mortality. Dead tadpoles were removed, blotted dry with a paper towel and weighed.

Water quality parameters were measured weekly in alternating replicates. Hardness and alkalinity were determined according to Standard Methods (APHA). pH was determined using an Orion Research pH meter 811 calibrated daily using pH 7.00 and 4.00 pH buffers. Conductivity was determined using a YSI Model 35 conductance meter. Anions were determined using a Dionex 4005i ion chromatograph.

Water samples for copper analysis were collected weekly in each exposure level from alternating replicates. Acid leachable as well as dissolved copper (passing through a 0.45 micron filter) were analyzed. Water samples for copper analysis were preserved by acidification to pH<2 using Ultrex nitric acid. Copper concentrations in water samples were measured using an Instrumentation Laboratory Video 22 atomic absorption spectrometer with air-acetylene flame or Thermo Jarrell Ash SH4000 with CTF 188 graphite furnace. Both instruments used Smith-Hieftje background correction.

Five tadpoles were randomly removed from each exposure chamber after 14 and 42 days of exposure and terminally anesthetized with MS-222. The tadpoles were rinsed with deionized water, blotted dry with a paper towel, pooled and weighed in preweighed polypropylene centrifuge tubes. Tubes containing the tadpoles were placed in a drying oven at 80°C and dried to constant weight. Trace metal grade nitric acid was added to the tubes which were then heated for eight hours in a water bath at 60°C. Trace metal grade hydrogen peroxide was added and the tubes heated an additional eight hours. The digests were then diluted to volume with deionized water and analyzed for copper content using an Instrumentation Laboratory Video 22 atomic absorption spectrometer with air-acetylene flame and Smith-Hieftje background correction.

Statistics

The 96-hour median lethal concentration (LC50) was determined using Spearman-Karber (Hamilton et al. 1977, Hamilton et al. 1978). Analysis of variance (ANOVA) was performed on mortality and wet weight using SAS computer software (SAS 1989). Mortality data were transformed by arcsine squareroot prior to analysis (Snedecor and Cochran 1980). Assumptions of normality and homogeneity of variance were evaluated using Shapiro Wilk's test and Bartlett's test respectively ($p<0.10$). Treatment means were compared using one-tailed Dunnett's multiple comparison test ($p<0.05$) to evaluate differences from control.

RESULTS

Water quality characteristics of exposure water are shown in Table 3. Water quality characteristics were stable throughout the test. Total and dissolved copper concentrations and associated mortality during the acute phase of the toxicity test are shown in Table 4. A majority of total copper present in exposure water is dissolved which is characteristic of the soft water used in this experiment. The 96-hour median lethal concentration (LC50) is 69.4 μg Cu/L based on total copper concentrations. Copper concentrations and associated mortality following 43 days of exposure are shown in Table 5. Copper concentrations were fairly consistent throughout the duration of the test. The lowest observed effect level (LOEC) based on mortality occurred at a copper concentration of 33.8 μg Cu/L with the no observed effect level (NOEC) at 15.2 μg Cu/L. The maximum allowable toxicant concentration (MATC) is 22.7 μg Cu/L based on the geometric mean of the LOEC and NOEC. Weight of tadpoles was unaffected by copper exposure after 14 days but was reduced at a concentration of 15.2 μg Cu/L after 42 days (Figure 6). Development of tadpoles were unaffected by copper exposure. Whole body copper content was higher at all copper exposures when compared to control.. Whole body copper content did not change between 14 and 42 days when based on wet weight (Figure 7) but decreased over time when based on dry weight (Figure 8).

DISCUSSION

The results of this experiment indicate that boreal toad tadpoles are very sensitive to copper. Care should be taken to minimize contamination of water by copper and brass fittings if boreal toad tadpoles are reared in captivity. The LC50 for boreal toad tadpoles found here (69.4 μg Cu/L) agrees reasonably well with the LC50 of 57.1 μg Cu/L found using a static-renewal test (Davies and Brinkman 1996). These values are similar to those derived using rainbow trout (*Oncorhynchus mykiss*) in similar water quality (46.6-55.7 μg Cu/L this report). The MATC for boreal toad tadpoles chronically exposed to copper is about one-third the LC50 at 22.7 μg Cu/L. The MATC derived from the previously mentioned static-renewal test was somewhat higher at 37.4 μg Cu/L. The lower value reported here probably results from increased mortality resulting from much longer exposures (42 verses 10 days for the static-renewal test). The similarity of the results of the ten-day static-renewal and 42-day flow-through test indicate that short-term toxicity tests may provide accurate estimates of chronic mortality. However short-term exposure would not provide much information on sublethal effects such as reduction of growth and development found with chronic exposure to

cadmium. Whole-body copper content followed a dose-dependent relationship and was a sensitive indicator of exposure to copper. However elevated levels do not necessarily indicate ill-effects. Interpretation of whole-body copper content was affected by whether results are based on wet weight or dry weight. Whole-body copper content was unchanged over time when based on wet weight but decreases if based on dry weight; probably because the water content of tadpoles decrease as they develop and metamorphose. Copper exposure was lethal to tadpoles at low concentrations but did not drastically affect growth or development. This contrasts sharply with cadmium which was not directly fatal but significantly reduced growth and development.

Table 3. Water quality characteristics of exposure water used in copper toxicity test conducted on boreal toad tadpoles.

	MEAN	STANDARD DEVIATION	RANGE
HARDNESS (mg $CaCO_3$/L)	49.5	1.5	47-52
ALKALINITY (mg $CaCO_3$/L)	37.1	5.1	33.8-64.0
pH (S.U.)	7.36	0.14	7.08-7.62
DISSOLVED OXYGEN (mg O_2/L)	6.70	0.90	5.0-8.1
TEMPERATURE (°C)	18.4	0.4	17.6-19.1
CONDUCTIVITY (μS/cm)	102.2	2.0	97-106
FLUORIDE (mg/L)	0.9	0.2	0.5-1.2
CHLORIDE (mg/L)	2.0	0.2	1.5-2.3
NITRATE (mg/L)	<0.5	0.3	<0.5-0.6
PHOSPHATE (mg/L)	<0.5	0.0	<0.5
SULFATE (mg/L)	11.9	0.2	11.6-12.2

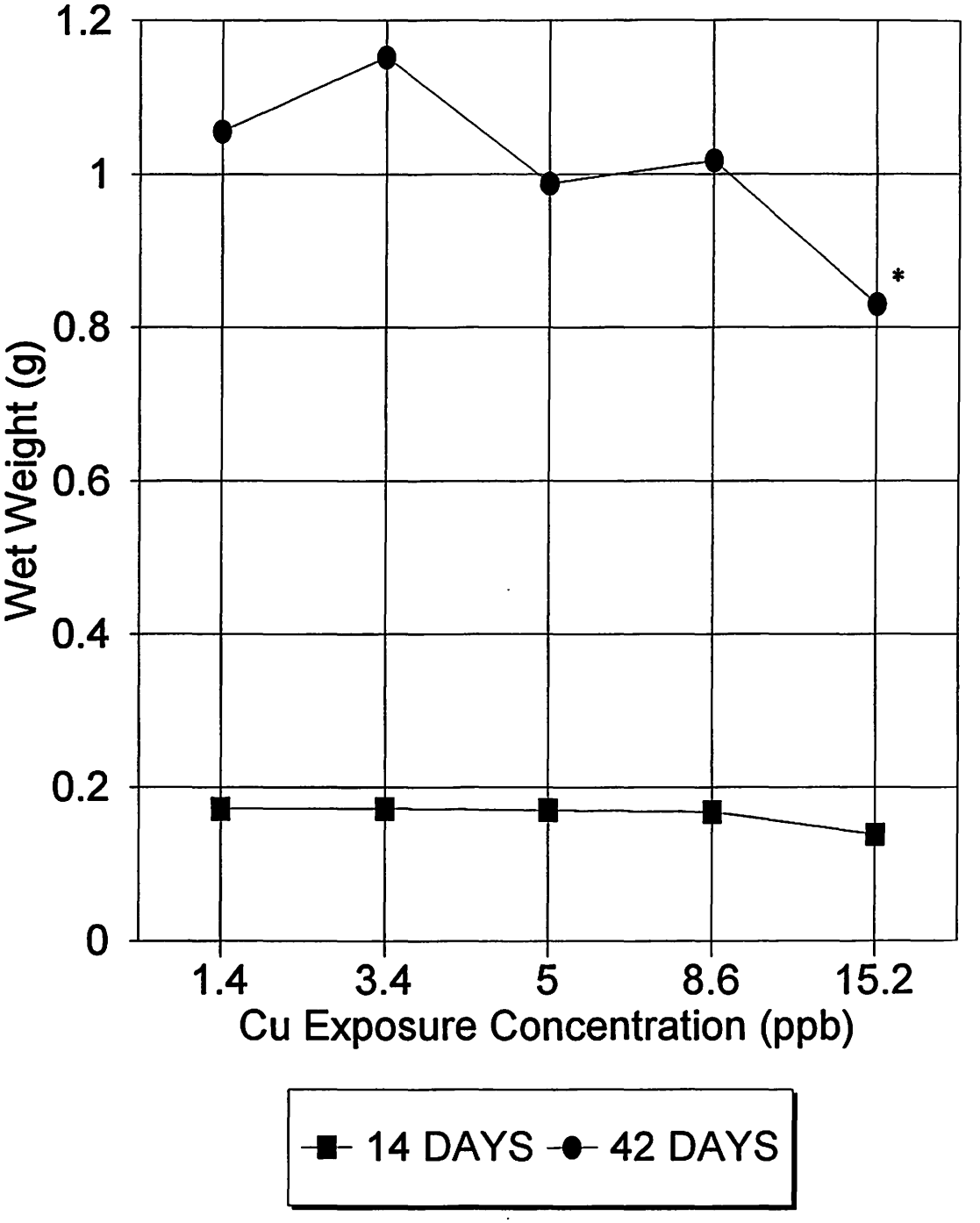

Figure 6. Mean wet weight (g) of boreal toad tadpoles after 14 and 42 days of exposure to copper. Asterisk indicates significantly less than control.

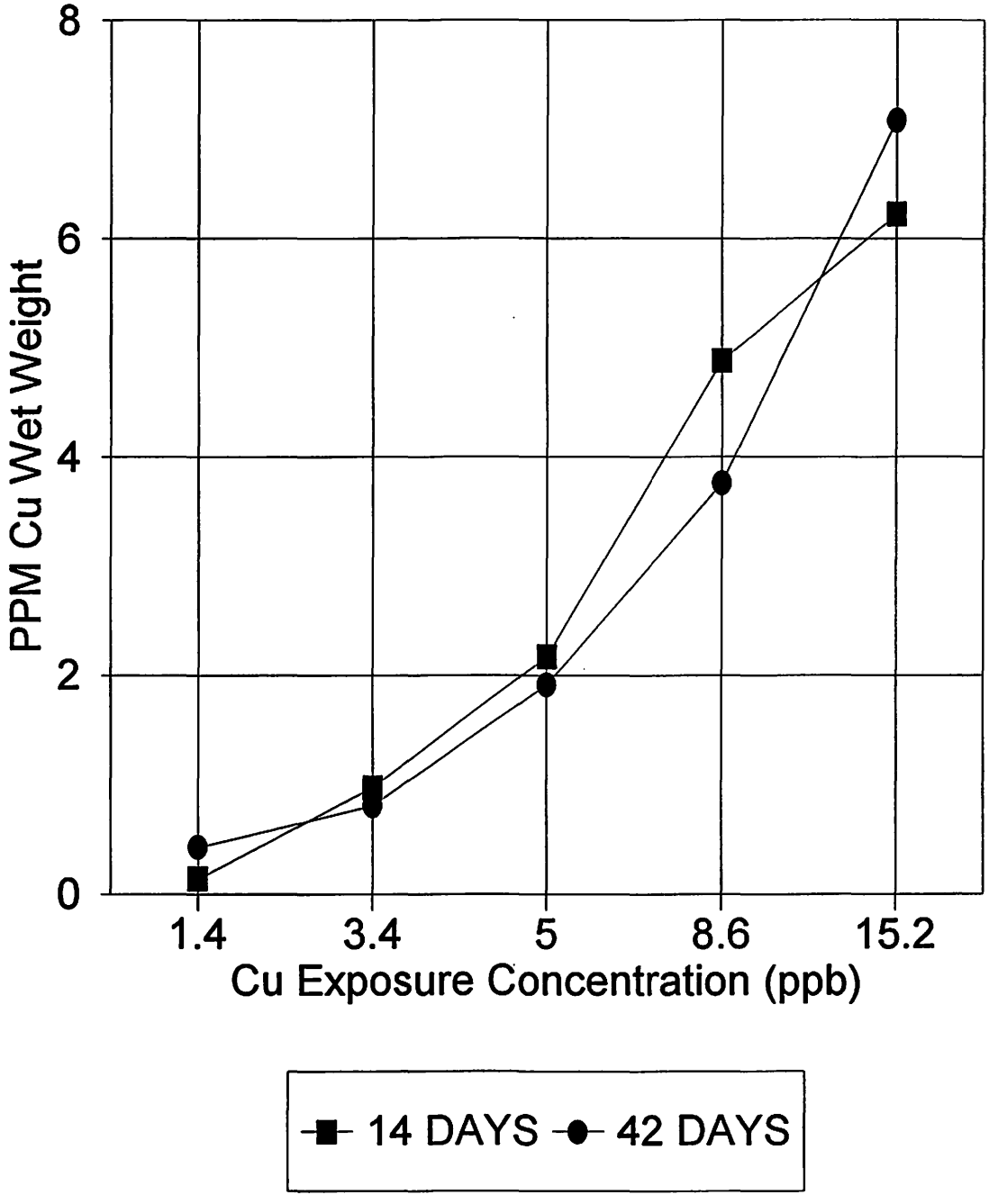

Figure 7. Whole body copper content (mg/Kg wet weight) of boreal toad tadpoles exposed to copper for 14 and 42 days. All treatments greater than control.

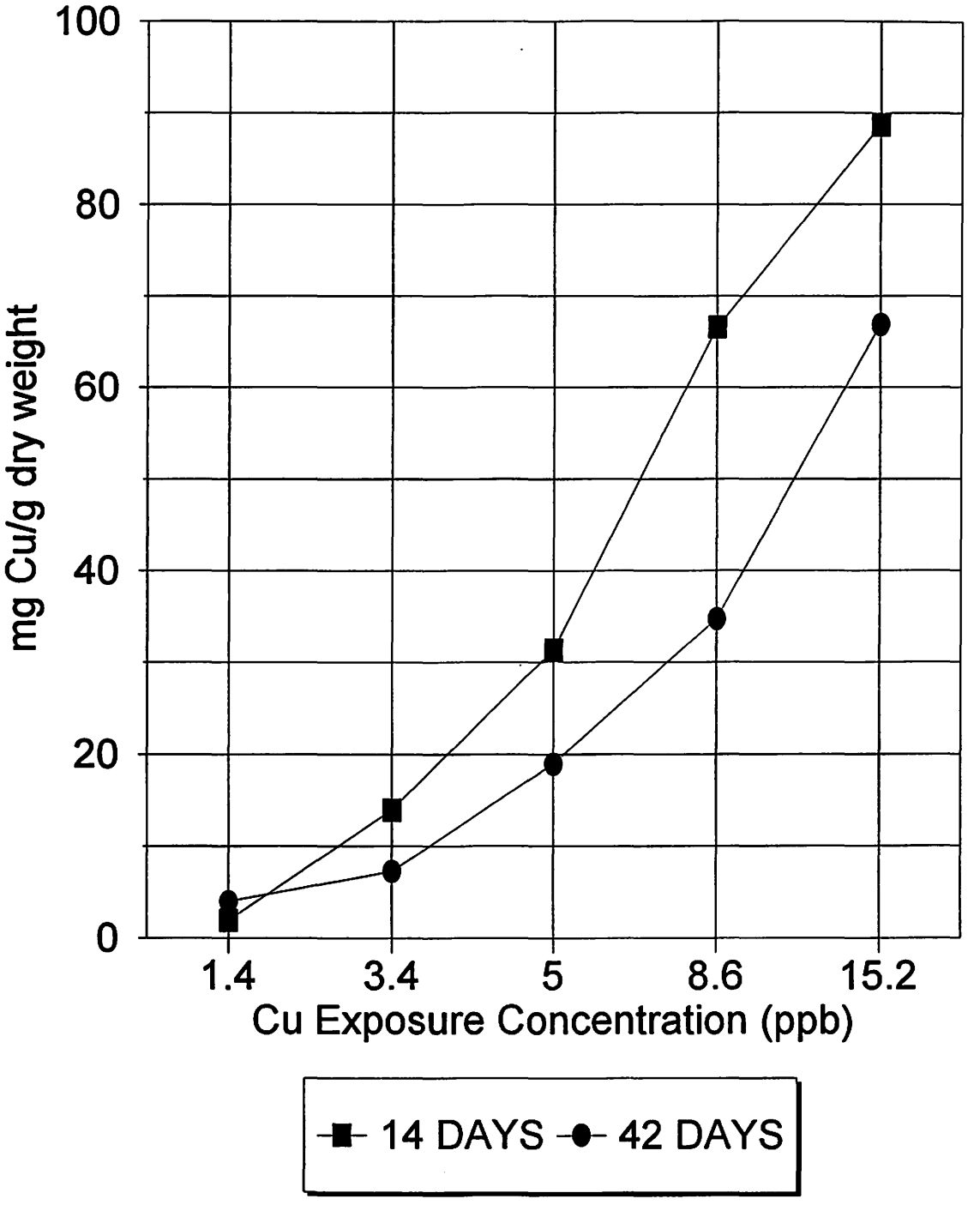

Figure 8. Whole body copper content (mg/Kg dry weight) of boreal toad tadpoles exposed to copper for 14 and 42 days. All treatments greater than control.

Table 4. Total and dissolved copper exposure concentrations and associated acute (96 hour) mortality of boreal toad tadpoles.

Nominal Conc (μg/L)	Measured Total (μg/L)	Measured Dissolved (μg/L)	Percent Mortality
100	100	85	94.0 (0.05)
50	60	55	26.0 (0.08)
25	34	32	1.0 (0.02)
12.5	19.4	12.4	0.0 (0.0)
6.25	5.9	5.4	0.0 (0.0)
3.12	3.4	2.0	1.0 (0.02)
1.56	2.2	1.1	1.0 (0.02)
0	<0.5	<0.5	0.0 (0.0)

LC50 (95% C.I.) = 69.4 (65.6-73.5) μg Cu/L

Table 5. Total and dissolved copper exposure concentrations (standard deviations) and associated survival of boreal toad tadpoles chronically exposed for 43 days.

Nominal Conc (μg/L)	Measured Total (μg/L)	Measured Dissolved (μg/L)	Percent Mortality
100	92.0 (11.3)	82.5 (3.5)	100 (0)*
50	63.5 (4.9)	58.5 (4.9)	100 (0)*
25	33.8 (1.0)	32.8 (2.2)	97 (4)*
12.5	15.2 (2.1)	13.0 (0.8)	24 (17)
6.25	8.6 (1.7)	7.1 (1.1)	18 (20)
3.12	5.0 (1.3)	3.9 (1.3)	10 (10)
1.56	3.4 (1.6)	2.8 (1.4)	16 (11)
0	1.4 (1.7)	<0.5 (0.9)	11 (6)

* Significantly higher than control ($p<0.05$)

Maximum allowable toxicant concentration (MATC) = 22.7 μg Cu/L

**Effects of pH on Survival of Rainbow Trout (*Oncorhynchus mykiss*)
and Brown Trout *(Silvelinus fontinalis)*
Exposed to a 3:1 Ratio of Copper to Zinc in Soft Water**

INTRODUCTION

The Colorado Division of Wildlife (CDOW), Aquatic Toxicology Laboratory was asked to conduct a toxicological evaluation on catchable rainbow trout using water quality conditions similar to those found in Terrace Reservoir on the Alamosa River below the Summitville Super Fund Site. The study involved two issues: (I) EPA wanted information on concentrations of copper (Cu) and zinc (Zn) at which catchable rainbow trout could survive water quality conditions currently found in Terrace reservoir. (II) The Division of Wildlife in consultation with other state agencies desired information on the survival of catchable rainbow trout exposed to Cu and Zn at water quality conditions existing in Terrace Reservoir prior to 1986 when the Summitville Consolidated Mining Corporation, Inc (SCMCI) began mining the site. Water quality conditions under these two scenarios are substantially different, especially pH which will largely control the bioavailability and toxicity of Cu and Zn. An examination of current water quality data and data on conditions prior to 1986 defined water quality conditions needed for the two studies. (Table 1).

Experiments were conducted to allow concentrations of Cu and Zn to attain equilibrium with water quality conditions and ligands characteristic of the reservoir prior to fish exposure. This was to assure that long-term toxicity effects will reflect equilibrium conditions of metals in the reservoir. CDOW's research laboratory is unique in its capability of investigating effects of chemical kinetics on the toxicity of metals under continuous flow conditions essential to studies on trout at different water quality conditions.

Table 6. Test conditions (bold) and summer water quality characteristics for pH, hardness, and the ratio of Cu to Zn in Terrace Reservoir currently and prior to 1986.

Water Quality	Currently	Prior to 1986
pH (Range) 95% C.I.	**5.0** (4.7 to 6.6)	**7.0** (6.7 to 8.3) 7.1 to 7.4
Hardness, mg/L (Range) 95% C.I.	**40** (12 to 140)	**40** (10 to 80) 26 to 49
Cu:Zn Ratio	**3:1**	**3.1**

METHODS AND MATERIALS

Scenario (I)

Toxicant Delivery System

Water used in the toxicity experiments was adjusted to a pH of 5 using reagent grade sulfuric acid. Sodium sulfate was also added to the source water to provide a nominal sulfate concentration of 75 mg/liter, a level typically found in acid mine drainage. The acidic and sulfate modified water was pumped to modified

Mount and Brungs (1967) proportional diluters where a 3:1 ratio of Cu to Zn was added. Nominal concentrations of 90, 68, 50, 29, 19, 9, and 0 μg Cu/liter and 30, 22, 17, 10, 5.4, 3.0, and 0 μg Zn/liter were used at a water hardness of about 50 mg/liter. A second control tank, identified as the complex control, was used to allow an evaluation of potential toxicity of modified experimental water. This control consisted of source water at ambient pH and sulfate (i.e., no addition of sulfuric acid or sodium sulfate) and a water hardness of 50 mg/liter. Water was delivered from the diluter, at a rate of 500 mls per minute, to 435 liter polyethylene equilibrium tanks which overflowed to 91 liter aquaria containing catchable rainbow trout with a length of 25 cm. These flow and volume conditions will provide a minimum 95% replacement time of about 48 hrs. This time interval will allow Cu and Zn to attain equilibrium with ligands in the water and stabilize pH prior to fish being exposed. The experiment will be replicated with an identical toxicant delivery and equilibration system.

Similar, replicate, flow-through toxicity test conducted at a pH of 7. Ten, 20 cm rainbow trout were added to each of the test aquaria. Nominal concentrations of 120, 90, 67.5, 43.2, 21.6, 12.0, and 0 μg Cu/liter and 40, 30, 22.4, 14.4, 7.2, 4.0, and 0 μg Zn/liter were used at a water hardness of 50 mg/liter, as $CaCO_3$. In one test, water from the diluter was delivered directly to 90 liter aquaria containing rainbow trout. Water in another test was first delivered to aging or chemical equilibria tanks described above at ambient pH. Toxicological differences from these two experiments will allow us an ability to assess possible kinetic effects on chemical equilibria and toxicity.

Acute and Long-term Toxicity Tests

Where possible acute data was collected during the acute phase of long-term toxicity tests. Twenty-five centimeter rainbow trout were acclimated to a water hardness of approximately 50 mg/liter, as $CaCO_3$, for two weeks before initiating the experiments. Diluter flows were initiated to fill the 435 liter equilibrium tanks with test solutions of Cu and Zn at a pH of 5. Just prior to overflow of test solutions from equilibrium tanks into test aquaria, eight rainbow trout were randomly placed into 45 liters of ambient control water (pH 7.3 and a temperature of 8°C) in each of the 90 liter test aquaria. This conditioning minimized possible shock and death of fish from being transferred from source water directly to pH 5 experimental water at 12° C. Eight rainbow trout used in each of the 90 liter aquaria to remain within ASTM loading requirements for flow-through toxicity testing.

During the acute phase of the experiments, fish mortality was monitored and recorded every two hours during the day. Total length and weight was taken from dead fish. Ninety-six hour LC50's were derived using appropriate methodologies, Trimmed Spearman-Karber or probit analysis, depending on the mortality pattern observed. Rainbow trout were not fed during the acute phase of the experiments. During the chronic phase, aquaria were inspected daily for dead fish. Feeding rates were based on weight of fish that died during the acute phase (Piper et al., 1982). Experiments were run for a period of 30 days. Length and weight data were collected from dead fish and surviving fish upon terminating the experiments. Chronic values were calculated from the geometric mean of no-effect/effect concentrations.

We also investigated effects of pH 5 water on rainbow trout slowly acclimated to changes in temperature and pH. Eight, catchable rainbow trout were placed into an aquarium filled with ambient source water of a hardness of 50 mg/liter, pH 7.5, and a temperature of 8° C. Over a two hour period, the temperature was increased to 11° C. We then started adding water at a pH 5.24 to the aquarium. The pH slowly decreased from a pH of 7.49 to 5.3 over a three day acclimation period.

Water Quality Characteristics and Metal Analysis

Hardness, alkalinity, conductivity, temperature and DO were measured weekly in all aquaria according to Standard Methods (APHA, 1985). pH was monitored daily and adjusted as needed to maintain pH levels at 5. Sulfate, chloride, fluoride, nitrate, and phosphate anions were analyzed weekly by ion chromatography (IC). Water samples were collected daily during the acute phase of the experiments and analyzed for Cu and Zn using flame atomic absorption spectrophotometer (AAS) with Smith-Hieftje background correction. During the chronic phase of experiments, we collected water samples daily in 15 ml polystyrene tubes with seven day composites stored in 125 ml high-density polyethylene (HDPE) bottles acidified with Ultrex triple-distilled nitric acid to pH <2. Cu concentrations less than flame detection limits were analyzed by graphite furnace AAS with Smith-Hieftje background correction.

Quality Assurance - Quality Control Protocols

Analysis of Cu and Zn by flame AAS:

Analysis was performed on a Instrumentation Laboratory Video 22 atomic absorption spectrometer using air-acetylene flame with Smith-Hieftje background correction. The instrument is calibrated each day that analysis are performed. Standard curves consist of five standards and a blank. The standard curve is modified every twenty five samples using the "autozero" and "autocalibration" feature. The autozero must measure within one detection limit or the instrument is recalibrated. The autocalibration must analyze between 90 and 110% of nominal or the instrument is recalibrated. An external reference standard is analyzed every twenty five samples. Recovery of the reference standard must be between 90 and 110 percent or problem is corrected and the previously twenty five samples are then reanalyzed. Five percent of samples are reanalyzed to ensure reproducibility of analytical results. Five percent of samples are spiked to ensure good recovery.

Analysis of Cu by graphite furnace AAS:

Instrumentation included a Thermo Jarrell Ash SH4000 atomic absorption spectrometer (AAS) with a CTF 188 graphite furnace using Smith-Hieftje background correction. Each day that analyses are performed, the monochrometer is calibrated, graphite electrodes and optics are cleaned, and the instrument is calibrated. Standard curves consist of five standards and a blank. The standard curve is modified every fifteen samples using the "autozero" and "autocalibration" feature. An external reference standard is analyzed every fifteen samples. Recovery of the reference standard must be between 90-110 percent or the problem is corrected and the previous fifteen samples are reanalyzed. Five percent of the samples are reanalyzed as duplicates to ensure reproducibility. Five percent of the samples are spiked to ensure good recovery. Ammonium phosphate (0.1%) is used as a matrix modifier for standards and samples.

Analysis of anions:

Anions were analyzed using a Dionex 4000i chromatograph and AS4A ion-exchange column with conductivity detection. The instrument is calibrated each day that analyses are performed. Standard curves consist of five standards and a blank. An external reference standard is analyzed every fifteen samples. Recovery of the reference standard must be between 90-110 percent or the problem is corrected and the previous fifteen samples are reanalyzed. Five percent of the samples are reanalyzed as duplicates to ensure reproducibility of results. Five percent of the samples are spiked to ensure good recovery. The separator column and micro-membrane suppressor are cleaned as needed.

Scenario (II)

We conducted similar experiments with rainbow trout under Scenario (II), except acute and long-term toxicity were run at pH 7 and ambient sulfate levels in the water, as opposed to pH 5 and added sulfate described under Scenario (I). Acute and Chronic test procedures were the same as explained in Scenario (I), except complex control aquaria were not needed since ambient water conditions were not modified. Duplicate experiments were run under unaged and aged conditions to assess possible differences in results from a condition where equilibrium has not been attained (A)- Unaged experiment, and where equilibrium has been attained (B) - Aged experiment. Equilibrium conditions and tanks were the same as explained in the pH 5 experiments, except pH and sulfates levels of the source water was not modified.

Brown Trout Exposure

With juvenile brown trout available, we took the opportunity to also assess the toxicity of a 3:1 Cu to Zn ratio on brown trout in 50 mg/liter hardness water at a pH of 5. Acute and chronic methods were the same described for the rainbow trout tests at pH 5, except fifteen, 60 mm brown trout were placed into each of the experimental aquaria following the same conditioning procedures used in the pH 5 rainbow trout studies. We also evaluated effects of pH on brown trout placed directly into pH 5 water at 12° C without a tempering or acclimation period.

RESULTS

Acute and Chronic Toxicity of 3:1 Cu:Zn Ratio to Rainbow Trout at pH 7

In water of pH 7 or less, Cu and Zn exist primarily in soluble, bioavailable forms. The lower the pH, the greater the toxicity because of increases in the ionic, bioavailable forms. For every unit decrease in pH, for example going from pH 7 to 6, there is a 100 fold increase in toxic forms of divalent metals like Cu and Zn. At pH 7, the 96-hr LC50 of a 3:1 Cu to Zn ratio to catchable rainbow trout was 54.7 μg Cu/liter and 17.3 μg Zn/liter in unaged water, where equilibrium conditions have not been attained (Table 7). Under equilibrium conditions, afforded by water with Cu and Zn being retained in large equilibrium tanks (aged) prior to flowing to aquaria containing fish, the 96-hr LC50 was significantly less than that obtained in the unaged experiment (Table 7). This indicates a kinetic effect in which some complexation of Cu and Zinc occurred in the equilibrium tanks that did not occur in the unaged experiment. This occurred even at pH 7 where Cu and Zn exist primarily in an ionic form. Under chronic or long-term exposure, chronic values (those concentrations presumed to be safe) were virtually identical (Table 8). This indicates, that equilibrium conditions were achieved in unaged aquaria under long-term exposure which did not occur during the acute, short-term exposure period.

Acute and Chronic Toxicity of 3:1 Cu:Zn Ratio to Rainbow Trout at pH 5

Not only do we see greatly increased toxicity of Cu and Zn but highly toxic conditions of pH, alone, to acutely exposed, catchable rainbow trout (Table 9). A 96-hr acute toxicity of 6.07 and 3.78 μg/liter for Cu and Zn, respectively, from combined, replicate data at pH 5 (Table 9), was considerably lower than the 46.6 and 15.0 μg/liter for Cu and Zn obtained in the similarly aged experiment at pH 7. A significant portion of the toxicity observed is attributed to pH alone. In control tanks of pH 5, containing no added Cu or Zn, an average mortality for acutely exposed rainbow trout was over 56 percent over a four day period (Table 9). Unlike the pH 7 experiment, a chronic value could not be calculated for the chronically exposed rainbow trout. At water pH of 5, there was no Cu:Zn concentration at which no observed effect (NOEC) occurred (Table 10). Under long-term exposure to Cu and Zn, pH (in the absence of added Cu and Zn) remained the

principal toxic agent resulting in 75 percent mortality to catchable rainbow trout in less than a five day period. Nearly 100 percent mortality occurred in the lowest Cu and Zn concentrations tested (Table 10). This is in contrast to results obtained for rainbow trout slowly acclimated to pH 5 water over a period of 3 days. No mortality occurred with these trout maintained at a pH of 5 for 30 days. However, these fish were not exposed to Cu and Zn.

Acute and Chronic Toxicity of 3:1 Cu:Zn Ratio to Brown Trout at pH 5

Brown trout proved to be considerably more resistant to reduced pH,.and Cu:Zn toxicity than were catchable rainbow trout. This occurred with much smaller, 60 mm, brown trout versus 250 mm rainbow trout. Generally smaller fish are more sensitive to the toxic effects of metals than larger fish. Acute and chronic toxicity values, for brown trout exposed to Cu and Zn at pH 5, were very similar to those found for rainbow trout at pH 7 (Table 11 versus Tables 7 and 8, respectively). Brown trout, without conditioning or tempering, were transferred directly from ambient water characteristics of pH 7.3 and a temperature of 8° C to pH 5 water at a temperature of 12° C. These fish showed no mortality after a period of 30 days.

Water quality characteristics and pH conditions are given for each of the experiments (Table 12 and Table 13, respectively). Similarly, Table 14 provides information on anion concentrations measured during the studies. Length and weight data are provided on rainbow and brown trout used in the different experiments (Table 15).

Table 7. Acute exposure concentrations, mortality, and 96-Hr LC50 (μg/L) and (95 % C.I.) for rainbow trout exposed to a 3:1 Cu to Zn ratio in (A) unaged and (B) aged, soft (50 mg/L hardness, as $CaC0_3$) water at pH of 7. Metals data in parentheses give standard deviation.

Nominal Cu	120	90.0	67.5	43.2	21.6	12.0	0
Nominal Zn	40.0	30.0	22.4	14.4	7.2	4.0	0
	(A) UNAGED						
Cu (μg/L)	116 (14.9)	100 (0)	72.5 (4.5)	43.0 (4.0)	21.0 (1.0)	9.5 (3.5)	<5 (0)
Zn (μg/L)	44.7 (7.0)	40.0 (0)	25.0 (3.0)	12.5 (1.5)	5.00 (1.0)	<5 (0)	<5 (0)
% Mortality	100	100	90	10	0	0	0
96-Hr LC50 = 54.7 μg Cu/L (47.5 - 63.1) and 17.3 μg Zn/L (14.3 - 20.9)							
	(B) AGED						
Cu (μg/L)	111 (12.7)	82.0 (0)	66.0 (9.0)	37.5 (8.5)	23.5 (3.5)	11.0 (5.0)	<5 (0)
Zn (μg/L)	41.8 (3.3)	32.0 (0)	22.5 (1.5)	12.0 (1.0)	5.5 (1.5)	<5 (0)	<5 (0)
% Mortality	100	100	90	20	0	0	0
96-Hr LC50 = 46.6 μg Cu/L (40.2 - 54.2) & 15.0 μg Zn/L (12.3 - 18.3)							

Table 8. Thirty day exposure concentrations, mortality, and Chronic Value (μg/L) for rainbow trout exposed to a 3:1 Cu to Zn ratio in (A) unaged and (B) aged, soft (50 mg/L hardness, as $CaC0_3$) water at pH of 7. Metals data in parentheses give standard deviation.

Nominal Cu	120	90.0	67.5	43.2	21.6	12.0	0
Nominal Zn	40.0	30.0	22.4	14.4	7.2	4.0	0
	(A) UNAGED						
Cu (μg/L)	116 (14.9)	100 (0)	72.5 (4.5)	35.0 (4.7)	19.0 (1.2)	9.88 (0.2)	<5 (0)
Zn (μg/L)	44.7 (7.0)	40.0 (0)	25.0 (3.0)	12.9 (0.2)	6.50 (1.1)	<5 (0)	<5 (0)
% Mortality	100	100	90	10	0	0	0
Chronic Value = 25.8 μg Cu/L and 9.2 μg Zn/L							
	(B) AGED						
Cu (μg/L)	111 (12.7)	82.0 (0)	66.0 (9.0)	34.1 (2.1)	18.6 (2.9)	9.2 (1.1)	<5 (0)
Zn (μg/L)	41.8 (3.3)	32.0 (0)	22.5 (1.5)	12.0 (1.6)	7.6 (1.3)	<5 (0)	<5 (0)
% Mortality	100	100	90	20	0	0	0
Chronic Value = 25.2μg Cu/L & 9.5 μg Zn/L							

Table 9. Acute exposure concentrations, mortality, and 96-Hr LC50 (μg/L) and (95 % C.I.) for rainbow trout exposed to a 3:1 Cu to Zn ratio in soft (50 mg/L hardness, as $CaC0_3$) water at pH of 5. Complex control (CC) had pH of 7.3. Metals data in parentheses give standard deviation.

Nominal Cu	90	67.5	50.4	32.4	16.2	9.0	0.0	0 (CC)
Nominal Zn	30.0	22.5	16.8	10.8	5.4	3.0	0.0	0 (CC)
	REPLICATE A							
Cu (μg/L)	--- [a]	68.0 (8.5)	53.0 (2.8)	30.3 (6.9)	19.7 (2.3)	14.0 (1.2)	5.5 (1.3)	<1.0 ---
Zn (μg/L)	---	22.3 (4.0)	18.0 (5.7)	12.0 (0)	5.0 (0)	<5 (0)	<5 (3.5)	<5 ---
% Mortality	---	100	100	100	75	62.5	62.5	0
96-Hr LC50 = 5.65 μg Cu/L (0.42 - 10.4) and 2.53 μg Zn/L (0.19-3.64)								
	REPLICATE B							
Cu (μg/L)	88.0 (5.7)	74.0 (2.8)	55.5 (2.1)	32.4 (0.8)	20.0 (2.3)	12.6 (0.9)	6.2 (0.8)	7.0 ---
Zn (μg/L)	31.3 (4.0)	24.7 (3.1)	16.5 (0.7)	8.5 (0.7)	7.0 (0)	<5 (0)	<5 (0)	<5 ---
% Mortality	100	100	100	100	100	75	50	0
96-Hr LC50 =6.69μg Cu/L (1.79 - 9.73) & 4.08 μg Zn/L (1.51 - 4.76)								
(COMBINED) 96-Hr LC50 = 6.07 μg Cu/L (2.65 - 8.90) & 3.78 μg Zn/L (1.94 - 4.40)								

[a] No fish added to high concentration tank

Table 10. Thirty day exposure concentrations, mortality, and Chronic Value (μg/L) for rainbow trout exposed to a 3:1 Cu to Zn ratio in soft (50 mg/L hardness, as $CaC0_3$) water at pH of 5. Complex control (CC) had pH of 7.3. Metals data in parentheses give standard deviation.

Nominal Cu	90	67.5	50.4	32.4	16.2	9.0	0.0	0 (CC)
Nominal Zn	30.0	22.5	16.8	10.8	5.4	3.0	0.0	0 (CC)
	REPLICATE A							
Cu (μg/L)	---	67.7 (6.0)	53.0 (2.0)	30.0 (5.8)	19.4 (2.3)	13.5 (1.6)	5.9 (1.2)	4.7 (3.6)
Zn (μg/L)	---	24.5 (2.1)	22.5 (0.7)	11.5 (0.7)	7.40 (1.4)	<5 (0)	<5 (0)	<5 (0)
% Mortality	---	100	100	100	87.5	87.5	75.0	0
Chronic Value = No C.V. at pH 5, do not have a NOEC [a]								
	REPLICATE B							
Cu (μg/L)	88.0 (5.7)	74.0 (2.8)	55.5 (2.1)	32.4 (0.8)	20.0 (2.3)	12.6 (0.9)	6.2 (0.8)	7.0 ---
Zn (μg/L)	31.3 (4.0)	24.7 (3.1)	16.5 (0.7)	8.5 (0.7)	7.0 (0)	<5 (0)	<5 (0)	<5 ---
% Mortality	100	100	100	100	100	100	75	0
Chronic Value = No C.V. at pH 5, do not have a NOEC [a]								

[a] No Observed Effect Concentration

Table 11. Acute and chronic exposure concentrations, mortality, and toxicity for brown trout exposed to a 3:1 Cu to Zn ratio in soft (50 mg/L hardness, as $CaC0_3$) water at pH of 5. Complex control (CC) had pH of 7.3. Metals data in parentheses give standard deviation.

Nominal Cu	67.5	50.4	32.4	16.2	9.0	0.0	0(CC)
Nominal Zn	22.5	16.8	10.8	5.4	3.0	0.0	0(CC)
	ACUTE						
Cu (μg/L)	85.5 (2.5)	56.5 (0.5)	34.5 (0.5)	21.5 (0.5)	14.0 (0)	6.30 ---	5.40 ---
Zn (μg/L)	34.5 (1.5)	21.5 (0.5)	13.5 (0.5)	7.0 (0)	4.5 (0.5)	4.0 (2.0)	11.0 ---
% Mortality	100	21.4	7.14	0	0	0	0
96-Hr LC50 = 60.9 μg Cu/L (54.0 - 68.7) and 23.7 μg Zn/L (20.2 - 26.9)							
	CHRONIC						
Cu (μg/L)		51.4 (3.3)	27.7 (2.4)	18.2 (2.4)	13.3 (1.4)	7.27 (1.9)	5.3 (0.1)
Zn (μg/L)		28.2 (6.1)	17.2 (6.2)	12.0 (6.7)	8.20 (4.0)	5.33 (0.5)	5.7 (4.5)
% Mortality	100	71.4	35.7	0	13.3	6.7	0
Chronic Value = 22.4 μg Cu/L & 14.4 μg Zn/L							

Table 12. Water quality characteristic (mg/L) for pH, hydrogen ion concentration, hardness, alkallinity, dissolved oxygen, temperature (° C), and conductivity (mS/m) were measured during the 3:1 Cu to Zu toxicity test conducted at pH of 7 and 5. Standard deviation in parentheses.

	EXPOSURE AQUARIA			COMPLEX CONTROL		
	Rainbow Trout - Cu:Zn Toxicity Tests at pH 7					
	Unaged	**Aged**				
pH	7.47(0.13)	7.42 (0.13)				
Hardness	47.1 (2.0)	47.4 (2.6)				
Alkalinity	35.3 (1.1)	35.5 (1.5)				
D. O.	9.36 (0.26)	9.09 (0.35)				
Temperature	11.5 (0.2)	11.9 (0.4)				
Conductivity	83.0 (2.2)	83.4 (2.4)				
	Rainbow Trout - Cu:Zn Toxicity Tests at pH 5					
	Rep. A	Rep. B	Mean	Rep. A	Rep. B	Mean
pH	5.00 (0.30)	5.12 (0.48)	5.07 (0.42)	7.29 (0.09)	7.25 (0.07)	7.27 (0.08)
H ion conc	$1.27x10^{-5}$	$1.06x10^{-5}$	$1.14x10^{-5}$	$5.18x10^{-8}$	$5.66x10^{-8}$	$5.44x10^{-8}$
Hardness	49.8 (0.4)	49.3 (5.6)	49.5 (4.5)	52.4 (5.2)	49.2 (0.6)	50.8 (3.8)
Alkalinity	3.8 (0.5)	2.3 (0.8)	2.9 (3.0)	41.7 (8.7)	37.0 (1.4)	39.4 (6.3)
D. O.	9.01 (0.56)	8.85 (0.56)	8.91 (0.56)	8.22 (0.51)	8.88 (0.48)	8.55 (0.58)
Temperature	12.3 (0.4)	12.5 (0.5)	12.4 (0.5)	11.4 (0.5)	11.3 (0.5)	11.4 (0.5)
Conductivity	140.2 (2.6)	141.5 (2.3)	141.0 (2.5)	92.0 (3.8)	92.0 (2.1)	92.0 (2.9)
	Brown Trout - Cu:Zn Toxicity Tests at pH 5					
pH	5.08 (0.51)			7.22 (0.02)		
H ion conc	$1.25x10^{-5}$			$6.08x10^{-8}$		
Hardness	49.3 (5.0)			49.5 (0.1)		
Alkalinity	2.3 (0.8)			36.6 (2.3)		
D. O.	8.89 (0.44)			8.95 (0.08)		
Temperature	12.8 (0.7)			11.0 (0.07)		
Conductivity	143.0 (3.2)			90.4 (1.7)		

Table 13. Water quality characteristic for pH, hydrogen ion concentration, hardness, alkallinity, dissolved oxygen, temperature, and conductivity collected during the Rainbow Trout Acclimation Test and the Non-Acclimated Brown Trout Toxicity Test in water of pH 5. Standard deviation in parentheses.

Water Quality Char.	Rainbow T. 3 Day Acclimation	Non-Acclimated Brown Trout
pH (units)	5.36 (0.51)	4.79 (0.52)
H ion concentration	$1.27x10^{-5}$	$2.7x10^{-5}$
Hardness (mg/L)	51.7 (2.7)	48.8
Alkalinity (mg/L)	5.9 (6.1)	0
D. O. (mg/L)	8.35 (0.15)	9.07
Temperature (°C)	12.5 (0.6)	12.7
Conductivity (mS/m)	138.0 (5.2)	153.0

Table 14. Anion concentrations (mg/liter) for fluoride, chloride, nitrate, phosphate, and sulfate were measured during the 3:1 Cu to Zu toxicity tests on rainbow and brown trout exposed to pH 5 water. Standard deviation in parentheses.

	EXPOSURE AQUARIA			COMPLEX CONTROL		
	Rainbow Trout - Cu:Zn Toxicity Tests at pH 5					
	Rep. A	Rep. B	Mean	Rep. A	Rep. B.	Mean
Fluoride	1.0 (0.2)	1.0 (0.1)	1.0 (0.2)	1.1 (0.1)	0.8 (0.2)	0.9 (0.2)
Chloride	2.6 (0.2)	2.7 (0.2)	2.6 (0.2)	2.5 (0.2)	2.4 (0.4)	2.5 (0.3)
Nitrate	<0.5 (0.3)	<0.5 (0.2)	<0.5 (0.3)	<0.5 (0.2)	<0.5 (0.3)	<0.5 (0.2)
Phosphate	<0.5 (0)	<0.5 (0)	<0.5 (0)	<0.5 (0)	<0.5 (0)	<0.5 (0)
Sulfate	69.7 (0.8)	70.0 (1.3)	69.8 (1.0)	12.7 (0.6)	12.7 (0.5)	12.7 (0.5)
	Brown Trout - Cu:Zn Toxicity Tests at pH 5					
Fluoride	0.6 (0.2)	NA		NA	NA	
Chloride	2.3 (0.4)					
Nitrate	0.5 (0.2)					
Phosphate	<0.5 (0)					
Sulfate	67.7 (5.7)					

Table 15. Mean length (mm) and weight (g) data from fish that died during acute, chronic stages and survivors at termination of the 3:1 Cu to Zu toxicity tests on rainbow and brown trout conducted at pH of 7 and 5. Standard deviation in parentheses.

	Rainbow Trout - Cu:Zn Toxicity Tests at pH 7					
	UNAGED			AGED		
	Acute	Chronic	Survivors	Acute	Chronic	Survivors
Length	200 (35)	215 (17)	219 (24)	216 (22)	220 (14)	222 (30)
Weight	110.7 (78.4)	84.7 (28.8)	108.5 (36.9)	110.0 (31.2)	111.6 (26.2)	126.0 (41.4)
	Rainbow Trout - Cu:Zn Toxicity Tests at pH 5					
	REPLICATE A			REPLICATE B		
	Acute	Chronic	Survivors	Acute	Chronic	Survivors
Length	230 (28)	214 (56)	248 (21)	239 (19)	248 (27)	249 (21)
Weight	159.1 (119.6)	146.2 (20.2)	162.6 (43.4)	154.1 (36.7)	182.3 (67.2)	175.7 (47.8)
	Brown Trout - Cu:Zn Toxicity Tests at pH 5					
	Acute	Chronic	Survivors	Acute	Chronic	Survivors
Length	60.7 (5.8)	53.8 (6.1)	66.3 (9.8)	NA	NA	NA
Weight	2.09 (0.72)	1.39 (0.44)	2.91 (1.61)	NA	NA	NA

CONCLUSIONS AND DISCUSSION

These studies clearly define conditions necessary for sustaining a put and take rainbow trout fishery in the Alamosa River and Terrace Reservoir located below the Summitville Super Fund Site. EPA's desire for information on concentrations of Cu and Zn needed for a catchable rainbow trout fishery in Terrace Reservoir can not be achieved. Catchable rainbow trout are highly sensitive to pH conditions in Terrace Reservoir even in the absence of lethal levels of Cu and Zn. At pH of 5, characteristic of the reservoir, 75 percent of 25 cm rainbow trout died within five days. This mortality occurred even with a conditioning period of 11 hours where pH was slowly decreased from a pH 7.3 to 5. With a slowly induced acclimation period of 3 days, these data suggest that catchable rainbow trout could survive pH 5 levels for a period of at least 30 days, but not in the presence of elevated concentrations of Cu and Zn. Of the trout species tested, brown trout were the most tolerant of reduce pH levels. They were also more tolerant of Cu and Zn concentrations than rainbow trout in water of similar quality. However, Cu and Zn concentrations in the Alamosa River and Terrace Reservoir would need to be reduced significantly to maintain a brown trout fishery at reduced levels of pH.

STUDY PLAN 3: TECHNICAL ASSISTANCE

Objective:

To provide expertise, consultation, evaluation and training in aquatic toxicology and aquatic chemistry to Division of Wildlife personnel, and other state and federal agencies.

Job 1. Water Quality Assistance to Other Personnel

Objectives:

1. To oversee the training and evaluation of metal analysis by laboratory technicians.
2. To assist Division and other state and federal personnel in the analysis and toxicological assessment of water quality data.
3. To develop and maintain a quality assurance program to evaluate the quality of analytical results for metals.
4. To collect and analyze metals concentrations in samples from the Arkansas River.

ACCOMPLISHMENTS

Water quality characteristics and or metal analyses were performed for the following persons and agencies:

Will Clements, Colorado State University (CSU)
Barb Horn, Colorado Division of Wildlife (CDOW)
John Woodling, CDOW
Mark Jones, CDOW
Greg Policky, CDOW
George Schisler, CSU
Del Nimmo, National Biological Survey (NBS)

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