WATER POLLUTION STUDIES

Federal Aid Project #F-243R-7

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Federal Aid in Fish and Wildlife Restoration

Job Final Report

Colorado Division of Wildlife

Fish Research Section

Fort Collins, Colorado

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JOB PROGRESS REPORT

State: Colorado

Study No. F243R-7

Title: Water Pollution Studies

Period Covered: July 1, 2000 to June 30, 2001

Principal Investigator: Patrick H. Davies
Co-investigator: Stephen F. Brinkman

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 A: 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 A1. Water quality standards for the protection of aquatic life in Colorado

Job Objectives:

A1.1 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.

A1.2 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.

A1.3 To develop or compile toxicological and chemical data on toxicants for which state or federal governments have not developed a standard.

Work Schedule: Personnel will provide information and expertise in aquatic toxicology and chemistry in hearings before the Water Quality Control Commission, court litigations and as needed by other regulatory agencies. Consequently, a specific work schedule cannot be provided except as provided in Job A1.3a. Approach: A1.1 through A1.3.

ACCOMPLISHMENTS**:**

Approximately ??? percent of Pat Davies time was spent assisting Vicky Peters, of the Colorado Attorney Generals Office, and State and Federal Trustee's on issues related to Natural Resources Damage (NRD) claims, restoration needs and goals, and settlemments on the Arkansas River at Leadville, CO. These negotiations will continue.

STUDY PLAN B: 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 B1. Chemical Equilibria and Kinetic Effects on the Bioavailability and Toxicity of Metals to Aquatic Life

Job 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 B2. Use of Biochemical Methods to Measure Disruption of Ion Regulation and Stress in Aquatic Organisms Exposed to Metals

Job 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 B3. Investigations on the Toxicity of Silver to Aquatic Organisms in Waters of Different Complexing Capacity

Job Objective: To develop acceptable toxicant concentrations of silver for cold- and warmwater fishes in hard high alkaline, and soft low alkaline waters and assess the toxicity of different silver compounds.

Job B4. Effects of Calcium Hardness, Inorganic and Organic Ligands and Sediments on Toxicity of Metals to Aquatic Organisms

Job 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 B5. Investigations on Enhanced Toxicity of Unionized Ammonia to Fish at Cold Water Temperatures

Job 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 B6. Effects of Episodic Exposure on Toxicity and Sensitivity of Aquatic Life to Intermittent Exposure to Metals

Job 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 B7. Investigations on Enhanced Toxicity of Water-Borne Metals to Aquatic Life Exposed to Dietary Sources of Metals

Job 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 B8. Investigations on Effects and Interactions of Multiple Metal Exposure on Toxicity to Aquatic Life

Job 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 B1.

ACCOMPLISHMENTS:

Toxicity test have been performed to evaluate possible synergistic toxicity relationships between copper and zinc using both brown and brook trout. Data from these experiments are being analyzed and evaluated and will be reported next segment.

STUDY PLAN C: 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 C1. 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.

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

Phil Schler, Colorado Division of Wildlife (CDOW)
John Woodling, CDOW
Barb Horn, CDOW

Rotenone analyses were performed for :

Sherman Hebein, CDOW

Analytical Assistance and Laboratory facilities were provided for:

Barry Nehring, CDOW
George Schisler, CDOW
Blair Prusha, CSU

Interpretation of toxicity information was provided for:

Andrew Archuleta, U.S. Fish and Wildlife Service
Lee Provanka, Dept of Health and Environment
Vicky Peters, Attorney Generals Office
Angus Campbell, Department of Health and Environment
Joe Gorsuch, Kodak
Will Tully, Bureau of Reclamation

Two toxicity experiments were conducted to provide toxicity data for personnel in the habitat section. The first test was conducted to determine to toxicity of zinc to mottled sculpin, a species which has failed to recover in metal impacted areas of the Eagle River despite improved water quality and the return of brown and rainbow trout to these areas. The second test was conducted to evaluate the effect of a copper and zinc mixture to brook trout in a water hardness of 88 mg $CaCO_3$/l.

ACUTE AND CHRONIC TOXICITY OF ZINC TO THE MOTTLED SCULPIN *COTTUS BAIRDI*

Abstract - The acute and chronic toxicity of zinc to feral mottled sculpin (*Cottus bairdi*) was measured using a 13 day and a 30 day, flow-through toxicity test, respectively. Hardness of exposure water was 48.6 mg/l $CaCO_3$ and 46.3 $CaCO_3$ in the acute and chronic tests, respectively, pH slightly above neutral and temperature near 12°C. The median lethal concentration (LC50) after 96 hours was 156 µg Zn/l, but decreased with duration of exposure to an incipient LC50 of 38 µg Zn/l after 9 days. This was the lowest zinc LC50 reported for any fish species. The incipient LC50 from the 30 d chronic test was was 32 µg Zn/l. The 30 day chronic no-effect and lowest effect concentrations were 16 µg Zn/l (no mortality) and 27 µg Zn/l (32% mortality), respectively. No effects on growth were observed. The results of these tests suggest that mottled sculpin may experience acute and chronic toxicity at zinc concentrations lower than any other fish species tested to date. Protection of aquatic communities in stream reaches contaminated by metals appear to require determination of zinc toxicity to lotic species other than trout.

INTRODUCTION

Although the toxicity of zinc to fish has been well documented and reviewed (Hogstrand and Wood 1996), the toxicity of this metal is not well known for cold water, littoral species such as sculpin (*Cottus* spp). Sculpin species inhabit many streams in the western U. S (Lee et al. 1980), including two species in Colorado (Woodling 1985), the mottled sculpin (*Cottus bairdi*) and the Paiute sculpin (*Cottus beldingi*). Sculpin were absent and trout numbers were depressed in a 19.3 km segment of the Eagle River downstream of an inactive mining operation dating to the 1800s near Minturn, Colorado, U.S.A. (unpublished Colorado Division of Wildlife fish monitoring data). At the same time, sculpin were present in the mainstem Eagle River immediately upstream of the mine operation, downstream of the stream reach impacted by mine operation, and in the mouths of three tributaries that enter the mainstem in the 19.3 km metal contaminated reach. Sculpin failed to appear, while brown trout numbers increased, in the 19.3 km reach during the course of a ten year federally mandated restoration program that began in 1988. Zinc was the principal metal of concern through the last half of a ten year period. Based on these observations, Sculpin appeared to be more sensitive to zinc contamination of the Eagle River than trout species.

The toxicity of zinc to various trout species has been determined through multiple toxicity tests and incorporated into a US EPA criteria document (USEPA 1987). If sculpin are more sensitive to zinc than trout species, existing criteria and restoration objectives may not be adequate to protect a diverse aquatic community. Determining the relative metal toxicity of variety of native lotic species is required to assure that appropriate water quality criteria and restoration objectives are chosen for stream reaches contaminated by metals.

The current study had three objectives. The first objective was to develop a 96h zinc LC50 value for recently hatched wild *C. bairdi* to assess acute toxicity. The second objective was to conduct a 30 day chronic toxicity test to determine both mortality rates and decreased growth rates of mottled sculpin surviving zinc exposure. Thirdly, results were compared to data for salmonids to determine relative zinc sensitivity.

METHODS AND MATERIALS

Organisms

A total of 134 recently emerged *C. bairdi* were collected from the White River approximately 5 km east of Meeker, Colorado, U.S.A. on August 8, 2000 for the acute toxicity test using a Smith Root backpack electrofisher unit. Hardness and conductivity at the site of collection was 240 mg/L $CaCO_3$

and 454 μS/cm, respectively. The fish were immediately transported in an aerated, iced cooler to the Colorado Division of Wildlife Aquatic Toxicology Laboratory in Fort Collins, Colorado. Upon arrival, fish were immersed in a 3% sodium chloride solution for 3 minutes to remove potential ectoparasites, then placed in a glass aquarium supplied with a mixture of dechlorinated Ft Collins municipal tap water and onsite well water. The mixture approximated that of the White River (hardness 225 mg/L $CaCO_3$, conductivity 450 μS/cm, temperature 18°C). Over the next 36 hours, the ratio of well water was slowly decreased until only dechlorinated Ft Collins municipal tap water with a hardness of 50 mg/L $CaCO_3$ was supplied. The fish were acclimated to dechlorinated Ft Collins municipal tap water for ten days prior to initiation of the acute toxicity test. Five of the original 134 sculpin died during transport to the laboratory in Fort Collins. No additional mortality occurred during the acclimation period. Fish were fed a concentrated suspension of brine shrimp nauplii (San Francisco Bay Brand) supplemented with starter trout chow (Silver Cup). Fish were observed feeding on both types of food.

A second group of 170 mottled sculpin were collected on August 30, 2000 at the same White River location for the 30 day chronic exposure. These organisms were handled in the same manner as the first group. There were no mortalities during the transportation to the Fort Collins laboratory or during the 21 day acclimation period prior to the start of the chronic test.

Toxicant

Chemical stock solutions were prepared by dissolving a calculated amount of reagent grade zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) (Mallincrodt) in deionized water. New stock solutions were prepared as needed during the two toxicity tests.

Acute test Methods

A continuous-flow diluter (Benoit et al. 1982) was used to deliver the exposure solutions. The source water consisted of dechlorinated Fort Collins municipal tap water. The diluter was constructed of polyethylene and polypropylene components with silicone stoppers and Nalgene FDA food grade vinyl tubing. A zinc stock solution was delivered to the diluter via a peristaltic pump (Cole-Palmer model C/L) at a rate of 2.2 mls/minute. The diluter delivered five concentrations of zinc and control. Nominal zinc concentrations for the acute test were 1000, 500, 250, 125, 62, and 0 μg Zn/L. A flow splitter allocated each concentration equally among four replicate exposure chambers at a rate of 40 mls/minute. Exposure chambers consisted of polyethylene containers with a capacity of 2.8 liters. Test solutions overflowed from the exposure chambers into a water bath maintained at 12 °C using a temperature-controlled recirculator (VWR Scientific Products Recirculator). A photocell regulated the fluorescent lighting to provide a natural August photoperiod for Ft Collins.

Five mottled sculpin were randomly placed in each of the exposure chambers for the acute toxicity test. Mortality was monitored and recorded several times daily. Dead sculpin were measured for TL (mm), blotted dry with a paper towel and weighed (g). Sculpin did not receive food during the first 96 hours but thereafter were fed a concentrated suspension of brine shrimp nauplii supplemented with starter trout chow twice daily on weekdays and once daily on weekends. After eight days, an additional treatment was initiated to provide mortality data at a nominal zinc concentration of 31 μg Zn/L. Five sculpin were randomly added to each of the four replicates at 31 μg Zn/L and treated as described previously. Zinc exposure continued for 13 days for all nominal concentrations. Control fish were monitored for 21 days. Aquaria were siphoned to remove uneaten food and feces as needed.

Randomly chosen surviving fish and mortalities from the acute toxicity test were preserved in Bouin's solution and taken to the Colorado Division of Wildlife Aquatic Animal Health Laboratory for necropsy and examination. Preserved specimens were examined with a dissecting scope for parasitic infestations and then dissected. Various tissues were embedded in paraffin, sectioned, stained and examined with a compound microscope for both disease and internal parasites.

Chronic test Methods

Seven mottled sculpin were randomly placed in each exposure chamber for the 30 day chronic toxicity test exposure. Mortality was monitored daily. Nominal zinc concentrations for the chronic test were 200, 100, 50, 25, 15.2, and 0 μg Zn/L. Flow rate of the zinc stock solution was reduced to 1.6 ml/min. A natural September-October photoperiod for Ft Collins was created as defined above. All other aspects of fish care and handling and test methods were the identical to the acute test. Lengths and weights of all fish that survived the 30 day exposure were determined following terminal anesthesia with metomidate hydrochloride (Wildlife Pharmaceuticals Inc.).

Water quality analysis

Exposure water characteristics were measured daily during the acute test on weekdays from two randomly selected aquaria. Exposure water characteristics during the chronic test were measured weekly in each nominal concentration from a randomly selected replicate. Hardness and alkalinity were determined according to Standard Methods (APHA 1985). pH was measured using an Orion Research pH meter 811 calibrated prior to each use with pH 7.00 and pH 4.00 buffers. Conductivity was determined using a YSI Model 35 conductance meter. Dissolved oxygen was measured using a YSI Model 58 dissolved oxygen meter.

Water samples for zinc analysis were collected daily during the acute test from all exposure levels within a replicate. Replicates were alternated. Water samples for zinc analysis were collected daily during the chronic test for the first seven days and weekly thereafter from one randomly selected replicate chamber for each nominal concentration. Total (acid soluble) samples were collected in disposable polystyrene tubes (Falcon) and immediately preserved with Ultrex, triple distilled, nitric acid to pH <2. Dissolved samples were passed through a 0.45 μm filter (Acrodisc) prior to acidification. Water samples were analyzed for zinc using an Instrumentation Laboratory Video 22 (Allied Analytical Systems, Franklin, MA) atomic absorption spectrometer with air-acetylene flame and Smith-Hieftje background correction. The spectrometer was calibrated prior to each use and the calibration verified using a NIST traceable QAQC standard from an outside source.

Statistics

Median lethal concentrations (LC50) were based on dissolved zinc concentrations and estimated by the Trimmed Spearman-Karber technique (Hamilton et al. 1977, 1978) using Toxstat version 3.5 software (West Inc.). The lengths, weights and survival of sculpins used in the chronic test were analyzed using analysis of variance (ANOVA). Survival proportions were arcsine transformed prior to ANOVA (Snedecor and Cochran 1980). Treatment means were compared to the control using William's one-tailed test ($p<0.05$). Sculpin length and weight data were normal with homogeneity of variance according to Shapiro-Wilk's test and Bartlett's test, respectively (Weber et al. 1989). However, transformed survival proportions failed both tests.

RESULTS

Acute test

Exposure water hardness averaged 48.6 mg $CaCO_3$ /l, temperature 12.2 C and pH was slightly basic at 7.4 (Table 1). Measured zinc concentrations were consistent for the duration of the test in each of the treatments and close to the desired nominal concentrations (Table 2). Dissolved and total zinc concentrations were virtually identical. The 96h LC50 was 156 μg Zn/L. All sculpin exposed to dissolved zinc concentrations greater than or equal to 487 μg/L died within 96 hours (Table 2). Mortality in lower zinc concentrations increased with duration of exposure. Complete mortality occurred at all exposure concentrations greater than or equal to 69 μg/L by the ninth day of the test, and 40% mortality was observed at a concentration of 34 μg/L (Table 2). No additional mortality occurred after 9 days through the end of test at 13 days. All fish in the control treatments survived. Median lethal concentrations (LC50s) declined with time with an incipient LC50 of 38 μg/L at nine days (Table 3). The average length of mottled sculpin used in the acute test was 31.4 mm with a range of 24 mm to 40 mm. These fish were considered to be young-of-the-year because 21 mm mottled sculpin collected on July 30, 2000 at the same location still had a yolk sac.

Gross and microscopic examination of organisms used in the tests failed to find external or internal parasites or disease.

Chronic test

Exposure water characteristics of exposure were also constant during the chronic test (Table 1) and were similar to the acute test. As with the acute test, measured zinc concentrations were consistent for the duration of the test in each of the treatments and close to the desired nominal concentrations (Table 4). Dissolved and total zinc concentrations were virtually identical. All sculpin exposed to dissolved zinc concentrations greater than or equal to 104 μg/L died by the 14th day of the 30 day test (Table 4). At the culmination of the 30 day chronic test 32% mortality had occurred at a concentration of 27 μg Zn/L with no mortality at a concentration of 16 μg Zn/L. No fish died after the 19th day of the chronic test. The incipient LC50 was 32 μg Zn/l.

Median lethal concentrations for 5 days through 13 days were similar to the comparable values determined at the same times during the 13 day acute test. The 30 day test provided a replicate study for portions of the 13 day acute test.

The average length and weight of all sculpin that died within the first 96 h of the 30 day chronic test were 36.8 mm and 0.545 g, respectively (Table 5). Mean length and weight of fish surviving the chronic exposure were 41.3 mm and 0.707 g, respectively. The increase in length of 4.5 mm was significant ($p<0.0001$, t-test using a pooled variance with an F test to verify variance equality) as was the increase in weight ($p<0.0016$) of 0.162 g. No differences in length and weight were determined among control fish, sculpin in the 16 μg Zn/L exposure level or sculpin that survived exposure at 27 μg Zn/L during the 30 day chronic test (Table 4).

DISCUSSION

Mottled sculpin were more sensitive to zinc than freshwater trout and char during acute exposures. The 96 h LC50 for the mottled sculpin was 156 μg/L at a hardness of 48.6 mg/L $CaCO_3$. In comparison, the lowest recorded 96 h LC50 for juvenile rainbow trout (*Oncorhynchus mykiss*) in water at a hardness of 46.8 mg/L $CaCO_3$ was 370 μg/L (Holcombe and Andrew 1978). Juvenile brook trout (*Salvelinus fontinalis*) were less sensitive than sculpin with a lowest recorded 96 h LC50 in soft water (46.8 mg/L $CaCO_3$) of 1,550 μg /L (Holcombe and Andrew 1978). The 96 h LC50 for juvenile brown trout (*Salmo trutta*) was 760 μg/L during an in-situ exposure in Georgetown Reservoir in Colorado (Sinley et al. 1974). Only one fish species, the striped bass (*Morone saxatillis*), appeared to be more sensitive to acute zinc exposure than the mottled sculpin. The striped bass species mean zinc 96 h LC50 (normalized to a hardness of 50 mg/l $CaCO_3$) was 119 μg/L (USEPA 1987). The mottled sculpin appeared to be the second most sensitive fish species to acute zinc exposure for which data were available.

The mottled sculpin was also more sensitive to zinc than freshwater trout and char during 30 d chronic exposures. The 30 day LC50 for mottled sculpin was 32 μg/L. In comparison, the lowest zinc chronic value for *O. mykiss* was 276 μg/L (USEPA 1987). The chronic zinc value for *S. fontinalis* was 854 μg/L (USEPA 1987). The chronic value was 36 μg Zn/L (Spehar 1976 a,b)for the flagfish (*Jordaneila floridae*), the only fish species with chronic zinc sensitivity similar to the mottled sculpin. Although little chronic zinc data exist for freshwater fish the mottled sculpin appeared to be the most sensitive fish species to chronic zinc exposure for which data were available.

Ninety six hours is used as the basis for defining acute toxicity for fish. However, 96 h appears to be an insufficient duration to accurately measure acute toxicity of zinc to mottled sculpin. The zinc concentration resulting in death to mottled sculpin decreased five fold as length of exposure increased from 4 days to 7 or 8 days. Significant sculpin mortality continued to occur until about 14 days of exposure. In comparison, studies conducted with brown trout found that a large majority of mortality over the course of 30 days occurred during the initial 96 hours of exposure (Davies and Brinkman 1994, 1999). In instances where mortality continues past the initial 96 h of exposure, the use of incipient or timeless LC50 may be preferred to describe acute toxicity.

Use of feral fish could influence the outcome of toxicity tests. Disease and/or parasitic infections and the stress of capture, transportation or captivity could induce mortality at lower concentrations than in cultured fish. Neither of these two groups of factors appeared to influence the results of the current toxicity tests. External parasites were either not present in the mottled sculpin collected from the White River or were removed by the salt treatment when the test organisms were moved into the Fort Collins toxicity test center. Disease and/or internal parasitic infections were not evident and did not appear to influence the results of the acute test. Capture, transportation or captivity did not appear to have any significant effects since there was no mortality during either the 10 day acclimation prior to the acute test or during the 21 day acclimation period prior to the chronic toxicity test. In addition, no control fish died during either of the toxicity tests. Observations during fish care activities demonstrated that the sculpin readily began taking food and were active in the aquaria. All sculpin surviving zinc exposure during the 30 day chronic toxicity test increased in both length and weight at the same magnitude and rate as the control fish. If fish had been stressed during the exposure to 16 μg Zn/L then differences in length and weight compared to control fish probably may have been observed. Lack of control fish mortality, similarity of results in median LC50 values at comparable time periods and similar growth rates among control and exposed fish would provide indications that use of feral fish did not greatly influence results. Use of wild fish in the toxicity tests may be preferable. Wild populations encountering any zinc exposure in natural conditions where the presence or absence of diseases and parasites would only be another set of factors impacting survival.

Results of the current study indicated that the mottled sculpin may not survive and reproduce at zinc concentrations which do not affect various salmonids. This observation is consistent with field observations on the Eagle River where sculpin are remain absent from a stream reach where trout numbers increased following a reduction in zinc concentrations. Lower instream concentrations are required for mottled sculpin to persist as viable populations. Much of the available zinc toxicity test data were developed using fish species that are spawned and reared in aquaculture facilities. Little data exist for most lotic fish species that are not amenable to fish culture techniques. A need exists to study more riverine fish species such as longnose dace, and flannel mouth sucker that would be expected to inhabit stream reaches contaminated by zinc.

Death was the measurable end-point during the chronic test, no growth differences were observed at any zinc concentration where test organisms survived. Sublethal responses to zinc using mottled sculpin need to be determined. Additional toxicity tests of longer duration would be required to determine possible growth effects, hormone stress response at zinc levels less than the incipient lethal concentration.

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Table 1. Mean, standard deviation and range of water quality characteristics of exposure water used for zinc toxicity tests (acute and chronic) conducted with mottled sculpin.

	pH (S.U.)	Temp. (°C)	Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)	Conductivity (μS/cm)	Oxygen (mg /L O_2)
Acute test						
Mean	7.38	12.2	48.6	36.0	85.2	9.1
Std.Dev.	0.13	0.3	1.1	1.4	2.1	0.19
Range	7.2-7.6	11.6-12.8	46.2-50.4	34.0-39.0	81.4-90.5	8.8-9.5
Chronic test						
Mean	7.56	11.8	46.3	35.9	81.3	8.8
Std.Dev.	0.08	0.3	2.7	1.0	2.5	0.2
Range	7.4-7.7	11.2-12.5	42.8-49.8	34.6-37.8	78.3-87.8	8.4-9.3

Table 2. Acute toxicity test. Mean zinc concentrations (µg/L) and associated mortality (%) of mottled sculpin at different durations of exposure. Standard deviations are in parentheses.

Nominal Zn (µg/L)	0	31.2	62.5	125	250	500	1000
Total Zn (µg/L)	<10 (3)	35 (3)	70 (4)	138 (10)	251 (7)	489 (12)	1005 (25)
Dissolved Zn (µg/L)	<10 (2)	34 (3)	69 (4)	128 (4)	249 (7)	487 (12)	1001 (28)
Mortality							
96 Hr %	0 (0)	10 (12)	10 (20)	35 (25)	80 (16)	100 (0)	100 (0)
5 Day %	0 (0)	15 (19)	25 (38)	75 (25)	90 (12)	100 (0)	100(0)
6 Day %	0 (0)	20 (23)	55 (30)	90 (12)	100 (0)	100 (0)	100 (0)
7 Day %	0 (0)	30 (26)	80 (16)	95 (10)	100 (0)	100 (0)	100 (0)
8 Day %	0 (0)	35 (30)	90 (20)	100 (0)	100 (0)	100 (0)	100 (0)
9 Day %	0 (0)	40 (28)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)
13 Day %	0 (0)	40 (28)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)

Table 3. Median lethal concentrations of zinc and 95% confidence intervals (µg/L) to mottled sculpin at different durations of exposure.

Duration of exposure	LC50 Estimate (µg/L) Acute	LC50 Estimate (µg/L) Chronic
96 Hours	156 (125-193)	
5 Days	92 (71-120)	94 (72-122)
6 Days	62 (47-80)	57 (40-80)
7 Days	45 (37-55)	48 (40-55)
8 Days	41 (34-51)	42 (37-46)
9 Days	38 (31-48)	38 (34-43)
13 Days	38 (31-48)	33 (29-38)
21 Days	--	32 (28-37)
30 Days	--	32 (28-37)

Table 4. Chronic toxicity test. Mean zinc concentrations (μg/L) and associated mortality (%) of mottled sculpin at different durations of exposure. Standard deviations are in parentheses.

Nominal Zn (μg /L)	0	12.5	25	50	100	200
Total Zn (μg /L)	<5 (4)	15 (2)	28 (2)	53 (2)	104 (3)	216 (10)
Dissolved Zn (μg /L)	<5 (2)	16 (3)	27 (2)	53 (2)	102 (2)	210 (5)
Mortality						
96 Hr (%)	0 (0)	0 (0)	0 (0)	4 (7)	54 (14)	32 (18)
7 Day (%)	0 (0)	0 (0)	0 (0)	68 (24)	93 (8)	96 (7)
14 Day (%)	0 (0)	0 (0)	29 (30)	96 (7)	100 (0)	100 (0)
21 Day (%)	0 (0)	0 (0)	32 (29)	100(0)	100 (0)	100 (0)
30 Day (%)	0 (0)	0 (0)	32 (29)	100 (0)	100 (0)	100 (0)

Table 5. Mean lengths and weights of mottled sculpin used in acute toxicity test and those surviving three concentrations in chronic toxicity test. Standard deviations are in parentheses.

	Acute test	chronic test		
Nominal Zn µg/L		0	12.5	25
Mean length (mm)	36.8	40.6 (1.1)	39.6 (1.1)	40.2 (2.6)
mean weight (g)	0.545	0.672 (0.053)	0.623 (0.055)	0.634 (0.110)

Effects of a copper and zinc mixture on survival and growth of brook trout (*Salvelinus fontinalis*) at a water hardness of 86 mg/l.

Introduction

This test was initiated to investigate possible effects of exposure to a mixture of copper and zinc to brook trout. A water hardness of 86 mg/l was selected because it was the mean hardness of segment 4a during the months of May, June and July (personal communication, Barb Horn, Colorado Division of Wildlife).

Material and Methods

Organisms

Test organisms were received as eyed eggs from Ten Sleep Hatchery in Wyoming. Eggs were hatched in a glass aquarium that received a constant flow of dechlorinated Fort Collins municipal tap water. Water quality characteristics were approximately 50 mg/l as calcium carbonate, 34 ppm alkalinity and pH 7.5. Upon swimup, fry were fed a mixture of brine shrimp napali and Silver Cup trout starter. The brine shrimp-trout chow mixture was slowly adjusted until fry were fed entirely trout chow. Twenty four hour prior to initiation of the experiment, the water quality characteristics were adjusted to deliver a water hardness of 86 mg/l as calcium carbonate, achieved by mixing dechlorinated Fort Collins municipal tap water and onsite well water.

Toxicant

Zinc was added as reagent grade zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) (Mallincrodt). Copper was added as reagent grade copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) (Mallincrodt). Chemical stocks for toxicity test were prepared by dissolving a calculated amounts of $ZnSO_4 \cdot 7H_2O$ and $CuSO_4 \cdot 7H_2O$ in deionized water to achieve the nominal stock solution concentration. The resulting stock solution was delivered to the test diluters as described below. New stock solutions were prepared as needed during the tests.

Test Methods

A modified continuous-flow diluter (Benoit et al. 1982) was used to deliver the exposure solutions. The source water consisted of a mixture of dechlorinated Fort Collins municipal tap water and an onsite well water mixed to provide a water hardness of 86 mg/l. The diluter was constructed of polyethylene and polypropylene components with silicone stoppers and Nalgene FDA food grade vinyl tubing. The chemical stock solution was delivered to the diluter via a peristaltic pump (Cole-Palmer model C/L) at a rate of 1.5 mls/minute. The diluter delivered five concentrations of metal mixture with a 50% dilution ratio and control. Operation of the diluter and toxicant flow were monitored daily to ensure proper performance. The conductivity of dilution water was monitored daily to ensure a proper mixture of well and tap water. Target metal concentrations for the test were 80 ppb Cu+ 5200 Zn, 40 Cu+2600 Zn, 20 Cu+1300 Zn, 10 Cu+650 Zn, and 5 Cu+325 Zn. A flow splitter allocated each concentration equally among four replicate exposure chambers at a rate of 40 mls/minute each. Exposure chambers consisted of polyethylene containers with a capacity of 2.8 liters. Test solutions overflowed from the exposure chambers into a water bath which was maintained at 12°C using a temperature-controlled recirculator (VWR Scientific Products

Recirculator). A photocell regulated the fluorescent lighting to provide a natural photoperiod for Ft Collins in April.

Ten brook trout fry were randomly placed in each exposure chamber. Mortality was monitored and recorded daily. Dead organisms were measured for total length (mm), blotted dry with a paper towel and weighed (g). Tests organisms were fed #1 trout chow twice daily (once daily on weekends) at a rate of approximately 5% body weight per day. Aquaria were siphoned to remove uneaten food and feces as needed. After 30 days of exposure, surviving fish were terminally anaesthetized, and length and weight of surviving organisms recorded.

Water quality characteristics of exposure water were measured weekly from two randomly selected aquaria. Hardness and alkalinity were determined according to Standard Methods (APHA 1985). pH was measured using an Orion Research pH meter 811 calibrated prior to each use with pH 7.00 and pH 4.00 buffers. Conductivity was determined using a YSI Model 35 conductance meter. Dissolved oxygen was measured using a YSI Model 58 dissolved oxygen meter.

Metal Analysis

Water samples from each exposure concentration within a replicate were collected weekly from aquaria with surviving fry. Replicates were alternated each week. Total (acid soluble) samples were collected in disposable polystyrene tubes (Falcon) and immediately preserved with Ultrex® triple distilled nitric acid to pH <2. Dissolved samples were passed through a 0.45 m filter (Acrodisc) prior to acidification. Water samples were analyzed for zinc using an Instrumentation Laboratory Video 22 (Allied Analytical Systems, Franklin, MA) atomic absorption spectrometer with air-acetylene flame and Smith-Hieftje background correction. Copper concentrations greater than 15 µg/l were analyzed using an air-acetylene flame AA. Lower levels of copper were analyzed using a Thermo Jarrell Ash SH4000 spectrometer with a CTF controlled temperature graphite furnace. The spectrometers were calibrated prior to each use and the calibration verified using a NIST traceable QAQC standard from an outside source.

Statistical analyses

Statistical analyses were conducted using Toxstat version 3.5 software (West Inc. 1996). Ninety six hr median lethal concentrations (LC50) were based on dissolved metal concentrations and estimated by the Trimmed Spearman-Karber technique (Hamilton et al. 1977, 1978). Lengths and weights of brook trout surviving the 30 day test were analyzed using analysis of variance (ANOVA). Survival data were transformed by arcsine square root prior to ANOVA (Snedecor and Cochran 1980). Normality and homogeneity of variances were tested using Shipiro-Wilk's test and Barlett's test, respectively (Weber et al., 1989). Treatment means were compared to the control using William's one-tailed test ($p<0.05$) (Williams 1971, Williams 1972). The highest copper and zinc concentration not associated with a treatment effect (e.g. increased mortality, decreased body weight) was designated as the no-observed-effect concentration (NOEC). The lowest concentration of copper and zinc that was associated with a treatment effect was designated as the lowest-observed-effect concentration (LOEC). Chronic values were calculated as the geometric mean of the LOEC and NOEC.

The inhibition concentration (IC_{20} value), the concentration estimated to cause a 20% reduction in organism performance compared with the control, was also calculated (USEPA 1993) using the combined weight of surviving organisms from each treatment.

Results

Water quality characteristics of exposure water were constant during the test (Table 1). Mean water hardness of the exposure water was 84.2 mg/l, slightly less than the target value of 86 mg/l. Temperature was near 12°C and pH above neutral at 7.4. Dissolved oxygen was near saturation.

Table 2 shows the exposure concentrations and associated mortality and lengths and weights of surviving brook trout. Copper and zinc exposure concentrations were constant throughout the 30 day duration of the test. Nearly all copper and zinc in total samples were present as dissolved. Dissolved copper represented 92% of the total while dissolved zinc represented 99% of the total (total concentrations not shown). All mortality occurred during the initial 96 hours of exposure. Survival in the lower exposure treatments was good, exceeding 95%. The highest two exposures experienced complete mortality. Lengths and weights of surviving brook trout show a decreasing trend with increasing exposures concentrations.

The endpoints estimated for the test are summarized in Table 3. The 96 hr median lethal concentration (LC50) based on dissolved metals concentrations was 16.0 μg/l for copper with a 95% confidence interval of 14.2-18.0. For zinc, the LC50 was 1085 with a confidence interval of 961-1224. The mortality data set did not have homogeneity of variances and was nonnormal. However, there is a clear effect on survival at 20.8 μg/l and 1329 μg/l for copper and zinc, respectively. The no observed effect concentration (NOEC) based on mortality was 8.6 μg/l and 612 μg/l for the copper zinc mixture respectively. Based on surviving length, the NOEC and LOEC for copper was 4.6 and 8.6 μg/l, respectively. For zinc, The NOEC and LOEC was 284 and 612, respectively. The NOEC and LOEC for surviving weight were identical to those based on surviving length. The 20% inhibition concentration (IC20) based on biomass at the end of the 30 day test was estimated at 8.1 and 571 μg/l for copper and zinc, respectively.

Discussion

The results of this test clearly show impacts to brook trout exposed to a mixture of 8.6 μg/l copper and 612 μg/l zinc in waters with a hardness around 85 mg/l. While mortality did not occur at these levels, there was a strong effect on growth. Growth tends to be a more sensitive endpoint for toxicity than mortality. It should be emphasized that the endpoints reported here are for a mixture of copper and zinc and should not be used separately.

Table 1. Mean, standard deviation and range of water quality characteristics of exposure water used for the 30 day copper zinc mixture toxicity test conducted on brook trout.

	pH (S.U.)	Temperature (°C)	Hardness (ppm $CaCO_3$)	Alkalinity (ppm $CaCO_3$)	Conductivity (S/cm)	Dissolved Oxygen (mg O_2/L)
Mean	7.45	11.9	84.2	61.4	1151.3	8.9
Std. Dev.	0.11	0.2	1.2	0.8	2.8	0.3
Range	7.25-7.65	11.5-12.2	82.4-85.8	60.0-63.0	146.2-156.8	8.4-9.4

Table 2. Copper and zinc exposure concentrations and associated mortality after 96 hrs, 30 days and surviving lengths and weights. Standard deviations in parentheses.

Dissolved Copper (µg/l)	<1.0 (0.3)	4.6 (0.9)	8.6 (0.4)	20.8 (2.2)	38 -	82 -
Dissolved Zinc (µg/l)	<10 (3)	284 (25)	612 (30)	1329 (45)	2840 -	5820 -
96 Hr Mortality (%)	2.5 (5.0)	0 (0)	2.5 (5.0)	75.0 (23.8)	100 (0)	100 (0)
30 Day Mortality (%)	2.5 (5.0)	0 (0)	2.5 (5.0)	75.0† (23.8)	100† (0)	100† (0)
Surviving Length (mm)	44.6 (0.9	43.8 (0.9)	40.9* (1.4)	33.9* (5.1)	--	--
Surviving Weight (g)	0.791 (0.065)	0.735 (0.033)	0.587* (0.058)	0.296* (0.171)	–	--

† Significantly greater than control
* Significantly less than control ($p<0.05$)

Table 3. Summary of endpoints of copper and zinc (µg/l) to brook trout in water hardness of 85 mg/l. 95% confidence intervals in parenthesis where available.

Endpoint	Copper	Zinc
96 Hr Median lethal concentration	16.0 (14.2-18.0)	1085 (961-1224)
Chronic value based on survival	13.4	902
Chronic value based on surviving length	6.3	417
Chronic value based on surviving weight	6.3	417
IC_{20}	8.1	571

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