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

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Abstract—The acute and chronic toxicity of zinc to wild mottled sculpin (*Cottus bairdi*) was measured with 13-d and 30-d flow-through toxicity tests, respectively. Exposure water hardness was 48.6 mg/L as $CaCO_3$ and 46.3 mg/L as $CaCO_3$ in the acute and chronic tests, respectively; pH was slightly above neutral; and temperature near 12°C. The median lethal concentration (LC50) after 96 h was 156 μg Zn/L, but decreased with exposure duration to a median incipient lethal level (ILL50) of 38 μg Zn/L after 9 d, the lowest zinc LC50 reported for any fish species. The 30-d chronic no-effect and lowest-effect concentrations were 16 μg Zn/L (no mortality) and 27 μg Zn/L (32% mortality), respectively. The ILL50 was 32 μg Zn/L. No sublethal growth differences were observed during the chronic test. Analysis of the results from these tests suggested 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 seem to require determination of zinc toxicity to lotic species other than trout and other species amenable to aquaculture.

Keywords—Zinc Mottled sculpin Toxicity test Acute toxicity Chronic toxicity

INTRODUCTION

Although the toxicity of zinc to several fish species has been well documented and reviewed [1], the toxicity of this metal is not well known for other cold water, littoral fishes such as sculpin (*Cottus* spp). Sculpin species inhabit many streams in the western United States [2], including two species in Colorado [3], the mottled sculpin (*Cottus bairdi*) and the Paiute sculpin (*Cottus beldingi*). Sculpin were absent and trout numbers were depressed in a 19.3-km-long segment of the Eagle River downstream of an inactive mining operation dating to the 1800s near Minturn, Colorado, USA (Colorado Division of Wildlife, Denver, CO, USA, unpublished data).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-long metal-contaminated reach. Sculpin failed to appear, whereas brown trout (*Salmo trutta*) numbers increased, in the 19.3-km-long reach during the course of a 10-year federally mandated restoration program that began in 1988. Zinc was the principal metal of concern through the last one half of a 12-year period. Sculpin seemed to be more sensitive to zinc contamination of the Eagle River than were trout species.

The toxicity of zinc to various trout species has been determined through multiple toxicity tests and is used in deriving the U.S. Environmental Protection Agency ambient water quality criteria for zinc [4]. If sculpin are more sensitive to zinc than are trout species, existing criteria and restoration objectives may not be adequate to protect a diverse aquatic community. Determining the relative metal toxicity of a 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.

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The current study had three objectives. The first objective was to develop a 96-h zinc median lethal concentration (LC50) value for recently hatched wild *C. bairdi* to assess acute toxicity. The second objective was to conduct a 30-d chronic toxicity test to determine both mortality rates and decreased growth rates of mottled sculpin that survived zinc exposure. The third objective was to compare results to data for salmonids to determine relative zinc sensitivity.

METHODS AND MATERIALS

Organisms

A total of 134 recently emerged *C. bairdi* was collected from the White River approximately 5 km east of Meeker, Colorado, USA, on August 8, 2000, for the acute toxicity test by using pulsed direct current from a backpack electrofisher unit (Smith Root, Vancouver, WA, USA). Hardness and conductivity at the site of collection were 240 mg/L as $CaCO_3$ and 454 μS/cm, respectively. The fish were transported in an aerated, iced cooler to the Colorado Division of Wildlife Aquatic Toxicology Laboratory in Fort Collins, Colorado, USA. Upon arrival, fish were immersed in a 3% sodium chloride solution for 3 min to remove potential ectoparasites, then placed in a glass aquarium supplied with a mixture of dechlorinated Fort Collins municipal tap water and on-site well water. The mixture approximated that of the White River (hardness 225 mg/L as $CaCO_3$, conductivity 450 μS/cm, temperature 18°C). Over the next 36 h, the amount of well water used was slowly decreased until only dechlorinated municipal tap water was used (hardness 50 mg/L as $CaCO_3$). The fish were acclimated to dechlorinated municipal tap water for 10 d before initiation of the acute toxicity test. Five of this group died during transport to the laboratory. No additional mortality occurred during the acclimation period. Fish were fed a concentrated suspension of brine shrimp nauplii (San Francisco Bay Brand, Newark, CA, USA) supplemented with starter trout chow (Silver Cup, Hanford, CA, USA). Fish were observed feeding on both types of food.

A second group of 170 mottled sculpin was collected on August 30, 2000, at the same White River location for the 30-d chronic exposure. These organisms were collected, transported, and handled in the same manner as the first group. No mortality occurred during the transportation to the laboratory or during the 21-d acclimation period before initiation 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, Paris, KY, USA) in deionized water. New stock solutions were prepared as needed during the two toxicity tests.

Acute test methods

A continuous-flow diluter [5] was used to deliver the exposure solutions. The source water consisted of dechlorinated municipal tap water. The diluter was constructed of polyethylene and polypropylene components and Nalgene food-grade vinyl tubing. A zinc stock solution was delivered to the diluter via a peristaltic pump (model C/L, Cole-Palmer, Barrington, IL, USA) at a rate of 2.2 ml/min. The diluter delivered five concentrations of zinc and control. Nominal zinc concentrations for the acute test were 1,000, 500, 250, 125, 62.5, 31.2, and 0 μg Zn/L. A flow splitter allocated each concentration equally among four replicate exposure chambers at a rate of 40 ml/min. Exposure chambers consisted of polyethylene containers with a capacity of 2.8 L. Test solutions overflowed from the exposure chambers into a water bath that was maintained at 12°C with a temperature-controlled recirculator (Polyscience, Nile, IL, USA). An outdoor photocell regulated the fluorescent lighting to provide a natural August photoperiod of 13 h of daylight and 11 h of darkness each day.

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 total length (mm), blotted dry with a paper towel, and weighed (g). Sculpin did not receive food during the first 96 h 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 8 d, 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 in the preceding paragraph. Zinc exposure continued for 13 d for all nominal concentrations. Control fish were monitored for 21 d. Aquaria were siphoned to remove uneaten food and feces as needed.

Randomly chosen surviving fish and dead fish 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 microscope 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-d chronic toxicity test exposure. Mortality was monitored daily. Nominal zinc concentrations for the chronic test were 200, 100, 50, 25, 12.5, and 0 μg Zn/L. Flow rate of the zinc stock solution was reduced to 1.6 ml/min. A natural September–October photoperiod of 11.5 h of daylight and 12.5 h of darkness was created as defined above. All other aspects of fish care and handling and test methods were identical to those of the acute test. Lengths and weights of all fish that survived the 30-d exposure were determined after the fish were killed with metomidate hydrochloride (Wildlife Laboratories, Fort Collins, CO, USA).

Water quality analysis

Exposure water characteristics were measured daily during the acute test on weekdays in two randomly selected aquaria. Exposure water characteristics during the chronic test were measured weekly in one randomly selected replicate chamber for each nominal concentration. Hardness and alkalinity were determined according to standard methods [6]. Measurements for pH were conducted with a pH meter (model 811, Orion, Cambridge, MA, USA) calibrated before each use with pH 7.00 and pH 4.00 buffers. Conductivity was determined with a conductance meter (model 35, Yellow Springs Instruments, Yellow Springs, OH, USA). Dissolved oxygen was measured with a dissolved oxygen meter (model 58, Yellow Springs Instruments).

Water samples for zinc analysis were collected daily during the acute test from all exposure concentrations. A different replicate tank was sampled daily from each exposure concentration. Water samples for zinc were collected daily during the chronic test for the first 7 d and weekly thereafter from one randomly selected replicate chamber for each exposure concentration. Total (acid soluble) samples were collected in disposable Falcon polystyrene tubes (Becton Dickinson, Franklin Lakes, NJ, USA) and immediately preserved with triple distilled nitric acid (Ultrex®, Phillipsburg, NJ, USA) to pH < 2. Dissolved samples were passed through a 0.45-μm filter (Acrodisc, Ann Arbor, MI, USA) before acidification. Water samples were analyzed for zinc with an Instrumentation Laboratory Video 22 (Allied Analytical Systems, Franklin, MA, USA) atomic absorption spectrometer with air–acetylene flame and Smith–Hieftje background correction. The spectrometer was calibrated before each use and the calibration was verified with a National Institute of Standards and Technology (Gaithersburg, MD, USA) traceable standard from an outside source.

Statistics

All LC50 values were based on dissolved zinc concentrations and estimated by the trimmed Spearman–Karber technique [7,8] with Toxstat® software (Ver. 3.5, Western EcoSystems Technology, Cheyenne, WY, USA). The median incipient lethal level (ILL50) concentrations were the LC50 values derived at the time mortality ceased. The lengths, weights, and survival of sculpin used in the chronic test were analyzed by analysis of variance (ANOVA). Survival proportions were arcsine transformed before ANOVA [9]. Treatment means were compared to the control by 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.

RESULTS

Acute test

Exposure water hardness averaged 48.6 mg/L as $CaCO_3$, temperature 12.2°C, and pH was slightly basic at 7.4 (Table

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[a]

	pH (SU)	Temperature (°C)	Hardness (mg/L as $CaCO_3$)	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
SD	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
SD	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

[a] SU = standard units; SD = standard deviation.

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 96-h LC50 was 156 μg Zn/L. All sculpin exposed to dissolved zinc concentrations greater than or equal to 487 μg/L died within 96 h. 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 d through the end of test, at 13 d. All fish in the control treatments survived. The LC50 values declined with time, with an ILL50 of 38 μg/L at 9 d (Table 3). The average length of mottled sculpin used in the acute test was 31.4 mm, with a range of 24 to 40 mm. These fish were considered to be young-of-the-year because 21-mm-long mottled sculpin collected 8 d before collection of test organisms 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 also were constant during the chronic test (Table 1) and were similar to those of 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 2). Dissolved and total zinc concentrations were virtually identical. All sculpin exposed to dissolved zinc concentrations greater than or equal to 53 μg/L died by the 19th day of the 30-d test. At the culmination of the 30-d chronic test, a 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 ILL50 was 32 μg Zn/L.

Even though the nominal exposure concentrations were different during the two toxicity tests, the 30-d test provided a replicate study for portions of the 13-d acute test. The LC50s determined for day 5 through day 13 during the chronic test were similar to LC50s calculated at the same times during the acute test (Table 3).

The average length and weight of all sculpin that died within the first 96 h of the 30-d chronic test were 36.8 mm and 0.545 g, respectively. 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 with a pooled variance with an F test to check variance equality), as was the increase in weight ($p < 0.0016$) of 0.162 g. Mean length and weight of control fish at the end of the chronic test were 40.6 mm and 0.67 g, respectively. Mean lengths of fish in the chronic exposure at 16 μg Zn/L or fish surviving the exposure at 27 μg Zn/L were 39.6 mm and 40.2 mm, respectively, whereas mean weights were 0.62 g and 0.63 g. No differences in length and weight were determined among control fish, sculpin in the exposure at 16 μg Zn/L, or sculpin that survived exposure at 27 μg Zn/L during the 30-d chronic test.

DISCUSSION

Mottled sculpin inhabit trout streams throughout much of western Colorado. The water quality of many of these streams continues to be degraded by metal loadings attributable to past and present mining activities, some of which date back to the 1860s. A wealth of data have been developed regarding the effects of metals on trout because of the sensitivities of the various species to metals, ease of aquaculture, and economic importance. However, effects of zinc on sculpin were un-

Table 2. Mean zinc concentrations (μg/L) (13-d acute tests and 30-d chronic tests). Standard deviations are given in parentheses

	Concentration						
Acute test							
Nominal Zn (μg/L)	0	31.2	62.5	125	250	500	1,000
Total Zn (μg/L)	<10 (3)	35 (3)	70 (4)	138 (10)	251 (7)	489 (12)	1,005 (25)
Dissolved Zn (μg/L)	<10 (2)	34 (3)	69 (4)	128 (4)	249 (7)	487 (12)	1,001 (28)
Chronic test							
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)	

Table 3. Median lethal concentrations (LC50s) of zinc (95% confidence intervals) to mottled sculpin at different durations of exposure

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

known. The results of these toxicity tests indicated that mottled sculpin were more sensitive to acute and chronic zinc exposure than were other freshwater trout and char.

The 96-h LC50 for the mottled sculpin was 156 μg Zn/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 as $CaCO_3$ was 370 μg Zn/L [10]. Juvenile brook trout (*Salvelinus fontinalis*) were less sensitive than sculpin, with a lowest recorded 96-h LC50 in soft water (46.8 mg/L as $CaCO_3$) of 1,550 μg Zn/L [10]. The 96-h zinc LC50s for brown trout for fish of different ages were 392, 871, and 1,033 μg Zn/L in toxicity tests that used the same water source as the present study [11]. Only one fish species, the striped bass (*Morone saxatillis*), seemed to be more sensitive to acute zinc exposure than the mottled sculpin. The striped bass mean zinc 96-h LC50 (normalized to a hardness of 50 mg/L as $CaCO_3$) was 119 μg/L [3]. The mottled sculpin seemed to be the second most sensitive fish species to acute 96-h zinc exposure for which data were available.

In addition, the mottled sculpin was more sensitive to chronic zinc exposure than freshwater trout and char. The 30-d LC50 for mottled sculpin was 32 μg Zn/L. In comparison, the lowest zinc chronic value for *O. mykiss* was 276 μg/L [4]. The chronic value was 36 μg Zn/L [12,13] for the flagfish (*Jordaneila floridae*), the only fish species with chronic zinc sensitivity similar to that of the mottled sculpin. The mottled sculpin seemed to be the most sensitive fish species to chronic zinc exposure, although relatively few chronic toxicity tests have been performed on other fish species.

An exposure of 96 h is the defined basis for assessing acute toxicity for fish [4]. However, 96 h seemed to be an insufficient exposure to accurately assess acute zinc toxicity in the mottled sculpin. The zinc concentration that resulted in mottled sculpin death decreased fourfold during the acute test as exposure time period increased from 4 d to 7 and 8 d. In addition, the onset of mottled sculpin acute mortality was delayed in comparison to mortality observations made during 96-h acute toxicity tests to various trout and char species. In our past toxicity work, most trout mortalities occurred during the second 24-h period of 96-h toxicity tests conducted at similar hardness levels and temperatures. Few if any trout died during the last 24-h period of a given 96-h toxicity test. In contrast, sculpin mortality did not begin until the third 24-h period of either the acute or chronic exposure test of the current study. In instances where high levels of mortality are measured for a few days past the initial 96-h exposure, the use of ILL50 data may be preferable to describe acute toxicity.

The ILL50 determinations for both the acute and chronic test were similar. Mottled sculpin exposed to ILL50 zinc concentrations from 38 μg Zn/L to 32 μg Zn/L experienced significant mortality in a time period of 9 to 19 d, respectively, in the acute and chronic toxicity tests. However, current U.S. Environmental Protection Agency zinc criteria [14] suggest that a 30-d exposure at an average zinc level of 64 μg Zn/L or 61.5 μg Zn/L would be protective at the hardness levels of the test water in this study. The ILL50 levels for *C. bairdi* determined in our study are approximately 50% less than the zinc concentrations assumed to be safe based on current U.S. Environmental Protection Agency zinc criteria. We expect short-term mortality for wild mottled sculpin populations exposed to zinc concentrations currently expected to be safe, based on current U.S. Environmental Protection Agency chronic zinc criteria.

Use of wild fish could influence the outcome of toxicity tests. Disease or parasitic infections and the stress of capture, transportation, or captivity could induce mortality at lower concentrations than in cultured fish. Disease and external or internal parasitic infections were not evident and did not seem to influence the results of either the acute or chronic test. Capture, transportation, or captivity did not seem to have any influence on test results because no mortality occurred during either the 10- or 21-d acclimation periods before the acute or chronic tests. In addition, no control fish died during either of the toxicity tests. Lack of control fish mortality coupled with a significant increase in length and weight of all fish surviving the chronic test compared to fish that died in the initial 96 h also were indications that use of wild fish did not greatly influence results.

Examination of results of the current study indicated that the mottled sculpin will not survive exposure to zinc concentrations that support brown trout and brook trout. This observation was consistent with field observations on a metal-contaminated segment of the Eagle River in Colorado. Brook trout and brown trout, but not sculpin, inhabited three Eagle River sampling sites where dissolved zinc concentrations ranged from 315 μg Zn/L to 711 μg Zn/L after a metal contamination reduction program (Colorado Division of Wildlife, unpublished data). Low numbers of sculpin were collected at a fourth downstream Eagle River site in the years after dissolved zinc concentrations dropped from 330 μg Zn/L to less than 166 μg Zn/L. Sculpin were always collected through the multiyear sampling program at an upstream reference site where dissolved zinc exceeded a 10 μg Zn/L detection limit in only 3 of 12 annual low-flow, high-metal-concentration sampling events (range 15–71 μg Zn/L). No obvious habitat differences existed to preclude sculpin colonization at the middle three Eagle River sampling sites. However, Eagle River hardness concentrations were approximately twice those in the present study. Still lower instream zinc concentrations are required for mottled sculpin to colonize the three middle Eagle River sampling sites.

Much of the available zinc toxicity test data were developed with fish species that are routinely spawned and reared in aquaculture facilities, such as trout. Few 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 (*Rhinichthys cataractae*) and flannel mouth sucker (*Catostomus latipinnis*), that would be expected to inhabit stream reaches where zinc toxicity usually has been defined based on trout.

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 with mottled sculpin need to be determined. Additional toxicity tests would be required to determine possible growth effects and changes in production of stress hormones such as cortisol [15] at zinc levels less than the ILLs determined in this study.

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