SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 24, No. 6, pp. 1515–1517, 2005

Printed in the USA
0730-7268/05 $12.00 + .00

Short Communication

ZINC TOXICITY TO THE MOTTLED SCULPIN (*COTTUS BAIRDI*) IN HIGH-HARDNESS WATER

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(*Received* 5 *May* 2004; *Accepted* 14 *December* 2004)

Abstract—The median 96-h lethal zinc concentration (LC50) was 439 μg Zn/L (hardness of 154 mg/L as $CaCO_3$) for feral mottled sculpin (*Cottus bairdi*), decreasing to a median incipient lethal level of 266 μg Zn/L after 13 d. The 30-d chronic value was 255 μg Zn/L. The acute toxicity–hardness (ln-ln) slope of 1.022 exceeded that of the current U.S. Environmental Protection Agency zinc criteria. The mottled sculpin is the second most sensitive fish species for which toxicity data are available.

Keywords—Zinc Mottled sculpin Toxicity test Acute toxicity Chronic toxicity

INTRODUCTION

Cottidae were absent from waters in the western United States with elevated zinc concentrations, although trout populations were present [1–4]. Existing U.S. Environmental Protection Agency (U.S. EPA) criteria and various stream restoration objectives may not be adequate to protect diverse aquatic communities if sculpin are more sensitive to zinc than trout species. Laboratory tests demonstrated the mottled sculpin, *Cottus bairdi*, was extremely sensitive to zinc in soft water [3]. Sensitive species must be protected to ensure that appropriate water quality criteria and restoration objectives are chosen for zinc-contaminated stream reaches. The current study had two objectives: first, to develop acute and chronic toxicity data for recently hatched wild *C. bairdi* in high-hardness water, and, second, to determine if the relationship between water hardness and zinc toxicity for sculpin was similar to other species for which zinc toxicity data are available.

MATERIALS AND METHODS

Recently emerged *C. bairdi* were collected from the White River approximately 5 km east of Meeker, Colorado, USA, on August 8, 2002, using a backpack electrofishing unit with pulsed DC current. Hardness, conductivity, and dissolved zinc levels at the collection site were 240 mg/L as $CaCO_3$, 454 μs/cm, and <10 μg Zn/L, respectively. The fish were collected, transported, received, and maintained at the Colorado Division of Wildlife Toxicology Laboratory in Fort Collins, Colorado, USA, as previously described [3]. After a 26-d holding period, well water was added to dechlorinated Fort Collins municipal tap water to increase the water hardness to 150 mg/L as $CaCO_3$. The organisms were acclimated to this hardness for 18 d before starting the 30-d toxicity test. Toxicant diluter, test methods, water quality analyses, and zinc analyses were the same as in a previous *C. bairdi* toxicity test [3] except that dilution water consisted of dechlorinated Fort Collins municipal tap water and on-site well water mixed to create a nominal hardness of 150 mg/L as $CaCO_3$. The photoperiod was 12:12-h light:dark. Four replicates were used with nominal zinc exposure concentrations of 800, 400, 200, 100, 50, and 0 μg Zn/L. A continuous-flow diluter [5] was used along with a flow splitter to deliver exposure concentrations. Seven fish were randomly chosen and placed in each exposure and each control chamber. Fish were fed a concentrated suspension of brine shrimp nauplii (San Francisco Bay Brand, Newark, CA, USA) mixed with starter trout chow (Silver Cup, Hanford CA, USA). Fish were not fed during the 96-h acute toxicity test.

All median lethal concentrations to 50% (LC50) of the test organisms were estimated by the trimmed Spearman–Karber technique [6,7] with Toxstat® software (Ver 3.5; Western EcoSystems Technology, Cheyenne, WY, USA). The median incipient lethal level concentrations (ILL50) were the LC50 values derived at the time mortality ceased. Lengths, weights, and survival of sculpin among treatments used in the chronic test were analyzed by analysis of variance [8]. Treatment means were compared to the control by Williams'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 Bartletts's test, respectively. Lengths and weights of fish at the beginning and end of the 30-d test were compared using a t test ($p < 0.05$) to determine if the organisms grew during the test period.

RESULTS

Exposure water hardness averaged 154 mg/L as $CaCO_3$ (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). The estimated 96-h LC50 was 439 μg Zn/L. All sculpin exposed to dissolved 778 μg Zn/L (the highest concentration) died by the ninth day. Mortality at 379 μg Zn/L increased from 46% at 96 h to 85% at 13 d. No mortality was observed at an exposure of 50 μg Zn/L or in the controls. No additional mortality occurred after 13 d through the end of the 30-d test. The LC50 values declined with time to an ILL50 of 266 μg Zn/L at 13 d (Table 3). Mortality at the no-observed-effect concentration of 172 μg Zn/L was statistically similar to the controls. The statistically significant no-observed-effect concentration and lowest-observed-effect concentration were 172 and 379 μg Zn/L, respectively. A 30-d chronic value (the geometric mean of the

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Table 1. Mean, standard deviation, and range of water quality characteristics of exposure water used for zinc toxicity tests conducted with mottled sculpin. $n = 18$

	pH (SU)[a]	Temp. (°C)	Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)	Conductivity (μs/ cm)	Oxygen (mg/L)
Mean	7.5	12.4	154	110	254	8.2
Standard deviation	0.09	0.4	8.9	7.1	20	0.32
Range	7.4–7.7	11.7–13.1	138–167	95–118	229–2284	7.6–8.8

[a] SU = standard unit.

no-observed-effect concentration and lowest-observed-effect concentration) was estimated to be 255 μg Zn/L for mottled sculpin in water of hardness 154 mg/L as $CaCO_3$.

The average length of the mottled sculpin was 27 mm at time of capture as determined from the fish that died in transit to the laboratory. The average length of mottled sculpin used in the acute test was 35 mm with a range of 30 to 42 mm. These fish were considered to be young-of-the-year [3]. The average length of fish surviving the 30-d exposure was 38 mm. Significant growth was observed in the test organisms during both acclimation and the 30-d test. No differences in length or weight were seen in fish surviving the 30-d exposure period among the different exposure levels (Table 2).

DISCUSSION

Use of feral fish did not appear to influence results of the current study. Mortality occurred (37 died) during the 24-h transportation period from point of collection to the laboratory. However, no additional mortality was observed during acclimation of the more than 300 surviving test organisms or to control fish during the toxicity test. Nonlethal differences in growth were not observed at any zinc concentration where test organisms survived the toxicity test.

The sculpin averaged 27 mm total length and average weight of 0.289 g when first collected. Fish grew significantly during the acclimation period to an average total length of 35 mm total length and average weight of 0.442 g, indicating that feed, health, and holding conditions were adequate for the test organisms. The mottled sculpin continued to grow through the 30-d test.

The relative sensitivity of mottled sculpin to zinc to other aquatic species was assessed by comparing zinc toxicity data determined in this study to current U.S. EPA zinc criteria [9]. All mottled sculpin toxicity data from the current study and two prior studies [3,10] were normalized to a hardness of 50 mg/L as $CaCO_3$, and a genus mean acute value was calculated. The genus mean acute value for mottled sculpin is 182 μg Zn/L. The mottled sculpin appears to be the third most sensitive aquatic species to zinc for which data are available [9,11] based on a comparison of genus mean acute values. The striped bass (*Morone saxatillis*) was the only fish species that appeared more sensitive to acute zinc exposure than the mottled sculpin. The mottled sculpin was more sensitive to zinc than any salmonid species.

The mottled sculpin was sensitive to the acute toxic effect of zinc in relatively hard water (154 mg/L as $CaCO_3$), the same result previously found in soft water (49 mg/L as $CaCO_3$) [3]. A third toxicity test on mottled sculpin found a 96-h LC50 of 590 μg Zn/L at a hardness of 156 mg/L as $CaCO_3$ [10] in an exposure test limited to 4 d. An acute toxicity–hardness (ln-ln) slope of 1.022 ($r^2 = 0.95$) resulted from a combination of all three 96-h LC50 values available for the mottled sculpin. The slope of 1.022 lies within the range of 0.5603 reported for bluegill (*Lepomis macrochirus*) and 1.644 for the guppy (*Poecilia reticulata*) but exceeds the pooled mean slope of 0.8473 in the current U.S. EPA, acute zinc criteria [9].

Comparing the relative chronic toxicity of mottled sculpin to zinc with other fishes was not possible because of a paucity of data in the literature. Neither the original U.S. EPA zinc criteria document [11] nor the 1995 zinc update [9] contained references to chronic zinc toxicity tests with fish in water in hardness greater than 45 mg/L as $CaCO_3$.

Calculation of a chronic value from this study (255 μg Zn/L) suggested that the U.S. EPA–recommended chronic exposure of 165 μg Zn/L at a hardness of 150 mg/L as $CaCO_3$ would be protective of mottled sculpin. However, zinc criteria would not be protective of mottled sculpin in lower-hardness water [3]. Apparently, the toxicity of zinc to sculpin is attenuated by hardness to a greater degree than other species used to determine the existing U.S. EPA criteria. Mottled sculpin may not be protected at hardness levels less than 109 mg/L as $CaCO_3$ based on a comparison of the hardness response of this species with the current zinc criteria [9]. We expect acute mortality to wild mottled sculpin exposed to zinc concentrations deemed safe using the current U.S. EPA criteria at hardness levels less than 109 mg/L as $CaCO_3$. Additional toxicity tests to mottled sculpin or other *Cottus* species are needed to validate the hardness response of the genus to zinc. Also, zinc toxicity tests are needed to determine how protective the U.S. EPA zinc criteria are to a wide variety of fish species for which data are not currently available at hardness concentrations in excess of 50 mg/L as $CaCO_3$.

The sensitivity of mottled sculpin, relative to trout species, was evident in field observations of the Eagle River in Colorado, USA. Brook trout and brown trout but not sculpin inhabited three Eagle River sampling sites where dissolved zinc concentrations ranged from 315 to 711 μg Zn/L at hardness concentrations reaching 130 mg/L as $CaCO_3$ (John Woodling,

Table 2. Mean zinc concentrations (μg/L), mortality (%), total length (mm), and weight (g) of mottled sculpin after 30 d of exposure. Standard deviations are in parentheses. * = significantly different than control at $p < 0.05$. $n = 9$, five in first 4 d and weekly thereafter

Nominal Zn	0	50	100	200	400	800
Dissolved Zn	<5 (3)	50 (6)	94 (9)	172 (17)	379 (16)	778 (21)
Mortality	0	0	7 (8)	8 (9)	86 (20)*	100 (0)*
Length	938.2 (1)	38.1 (1.5)	37.1 (1.9)	38.6 (2.1)	40.4 (0.5)	—
Weight	0.47 (0.042)	0.50 (0.060)	0.49 (0.04)	0.51 (0.08)	0.65 (0.06)	—

Table 3. Median lethal concentrations to 50% of test organisms (LC50) of zinc and 95% confidence intervals (μg/L) to mottled sculpin at different durations of exposure

Duration of exposure	LC50 estimate (μg/L)	95% confidence interval
4 d (96 h)	439	290–664
5 d	302	245–372
6 d	283	243–328
7 d	278	239–324
8 d	279	243–321
9 d	273	242–309
13 d	266	240–295
15 d	266	240–295
30 d	266	240–295

personal communication). As such, lower in-stream zinc concentrations are required for mottled sculpin to recolonize the portion of the Eagle River where zinc concentrations have not eliminated trout species. In this instance, sculpin serve as an indicator species for water quality in waters where the both groups are expected to be present.

The 96-h LC50 concentration (439 μg Zn/L) decreased to 302 μg Zn/L (35%) by day 5. The LC50 decreased to 266 μg Zn/L by day 13. No mortality occurred following day 12. The use of ILL50 data may be preferable to describe acute toxicity in instances where high levels of mortality are measured for a few days past the initial 96-h exposure. Additional toxicity tests are required with mottled sculpin, including early life stage testing, to determine sublethal effects of zinc at incipiently lethal levels.

Acknowledgement—Funding for this study was provided in part by the U.S. Fish and Wildlife Service Federal Aid Grant F-243-R10. We wish to acknowledge the assistance of Shannon Albeke and Daria Hansen and the editing efforts of Brighid Kelly and two anonymous reviewers.

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