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TOXICITY OF CADMIUM TO EARLY LIFE STAGES OF BROWN TROUT (*SALMO TRUTTA*) AT MULTIPLE WATER HARDNESSES

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Abstract—Toxicity of cadmium to early life stages of brown trout (*Salmo trutta*) was determined at multiple water hardnesses. Increasing water hardness decreased cadmium toxicity. Postswimup fry were much more sensitive than embryos and larvae. Chronic values from early life stage tests initiated with eyed embryos were 3.52, 6.36, and 13.6 μg Cd/L at water hardnesses of 30.6, 71.3, and 149 mg/L, respectively. In tests initiated with 30-d postswimup fry, chronic values were 1.02, 1.83, and 6.54 μg Cd/L at water hardnesses of 29.2, 67.6, and 151 mg/L, respectively. Higher chronic values from the early life stage tests compared to tests initiated with swimup fry likely are caused by acclimation during cadmium-tolerant embryo and larval stages. Growth was not affected by cadmium in the early life stage tests but was negatively affected in tests initiated with fry at water hardnesses of 29.2 and 67.6 mg/L. Concentrations of cadmium that reduced growth were higher than those that increased mortality. Median lethal concentrations for swimup fry after 96 h were 1.23, 3.90, and 10.1 μg Cd/L at water hardnesses of 29.2, 67.6, and 151 mg/L, respectively. Test results enable prediction of acute mortality of brown trout swimup fry based on cadmium concentration and water hardness.

Keywords—Cadmium Brown trout Hardness Acclimation

INTRODUCTION

An estimated 2,080 km of streams in Colorado, USA are affected by metals [1]. Cadmium is commonly found as a contaminant in the Colorado mineral belt and often is associated with waters affected by historic mining activities. Brown trout (*Salmo trutta*) are an important component of Colorado ecosystems in many headwater streams, but their densities often are reduced because of metal contamination [2]. Limited cadmium toxicity data indicate that brown trout is perhaps the most acutely sensitive aquatic species tested [3]. Median lethal concentrations (LC50s) after 96 h were 1.4, 2.39, and 1.87 μg Cd/L in water hardnesses of 43.5, 37.6, and 36.9 mg/L, respectively, as $CaCO_3$ [4,5]. The chronic value was 16.49 μg Cd/L at a water hardness of 250 mg/L from a life-cycle test with brown trout [6]. A brown trout early life stage (ELS) test resulted in a chronic value of 6.67 μg Cd/L at a water hardness of 44 mg/L [7]. Curiously, hardness-adjusted, 96-h LC50s are much lower than chronic values derived from life-cycle and ELS tests. Life-cycle and ELS tests typically start with a tolerant life stage. Acclimation that occurs during a tolerant life stage results in reduced toxicity during a subsequent, sensitive life stage [4,8,9,10]. In contrast, acute toxicity tests usually are conducted using unacclimated organisms during a sensitive life stage.

The first objective of the present study was to develop a relationship that could predict mortality of brown trout fry based on cadmium concentration and water hardness. The second objective was to compare toxicity of cadmium in tests initiated with embryo and larval life stages and postswimup fry. To achieve these test objectives, toxicity tests were conducted using both life stages at water hardnesses of 30, 75, and 150 mg/L as $CaCO_3$.

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MATERIAL AND METHODS

Organisms

Brown trout embryos were obtained as newly eyed eggs from the Colorado Division of Wildlife Research Hatchery (Bellevue, CO, USA). The source of the eggs was a Colorado Division of Wildlife spawning operation using feral brown trout in the North Delaney Butte Reservoir (Colorado, USA). Ten eggs were placed into each exposure chamber for the ELS tests. Additional eggs were placed in 90-L glass aquaria, hatched, and later used in the fry toxicity tests. Eggs began hatching approximately 14 d after initiation of exposure. Brown trout embryos remained as sac fry for approximately 27 d before reaching the swimup stage. Fry were fed appropriately sized trout food (Silver Cup; Nelson and Sons, Murray, UT, USA) four times daily (twice daily on weekends and holidays) at an estimated rate of 3% body weight/d on absorption of the yolk sac. Trout food was supplemented with a concentrated suspension of brine shrimp nauplii (age, <24 h; San Francisco Bay Brand, Newark, CA, USA). The ELS test exposure continued for an additional 14 d postswimup. Total exposure duration for the ELS tests was 55 d.

The fry toxicity tests used 34-d postswimup fry from the same lot of eggs as the ELS tests. Fry were not fed during the initial 96 h of exposure but were subsequently fed twice daily (once on weekends and holidays) at an estimated rate of 3% body weight/d. Exposure for the fry toxicity tests was 30 d.

Exposure apparatus

Water from an on-site well was diluted with either dechlorinated Fort Collins municipal tap water or reverse-osmosis water to obtain nominal water hardnesses of 30, 75, and 150 mg/L as $CaCO_3$ (designated 30H, 75H, and 150H, respectively). Each water mixture was maintained at a constant hardness through the use of conductivity controllers. Identical modified continuous-flow diluters [11] were constructed of Teflon®,

Table 1. Water-quality characteristics (mean [SD]) used for early life stage (ELS) and fry tests[a]

	30 Hardness		75 Hardness		150 Hardness	
	ELS	Fry	ELS	Fry	ELS	Fry
Hardness (mg/L)	30.6 (2.1)	29.2 (0.9)	71.3 (2.7)	67.6 (1.5)	149 (7)	151 (2)
Alkalinity (mg/L)	22.9 (1.3)	21.7 (0.8)	51.5 (1.6)	47.9 (1.1)	107 (5)	107 (2)
pH (S.U.)	7.72 (0.12)	7.54 (0.13)	7.75 (0.14)	7.60 (0.10)	7.83 (0.14)	7.51 (0.12)
Temperature (°C)	11.6 (0.4)	11.7 (0.1)	12.0 (0.3)	11.4 (0.2)	11.8 (0.4)	11.8 (0.4)
Conductivity (μS/L)	52.9 (2.0)	51.5 (0.5)	123 (5)	115 (2)	255 (8)	260 (2)
Dissolved oxygen (mg/L)	8.49 (0.58)	8.61 (0.22)	8.61 (0.67)	8.88 (0.17)	8.32 (0.64)	8.58 (0.14)

[a] 30 Hardness, 75 Hardness, and 150 Hardness refer to 30, 75, and 150 mg/L, respectively, as $CaCO_3$.

polyethylene, and polypropylene components. The diluters delivered five exposures with a 50% dilution ratio and an exposure control. A flow splitter allocated each concentration equally among four replicate exposure chambers at a rate of 40 ml/min each. Exposure chambers consisted of polyethylene containers with a capacity of 2.8 L. Test solutions overflowed from exposure chambers into water baths, which were maintained at 12°C using temperature-controlled recirculators. Chemical stock solutions were prepared by dissolving a calculated amount of reagent-grade cadmium sulfate ($CdSO_4$) in deionized water. The chemical stock solutions were delivered to the diluters via peristaltic pumps at a rate of approximately 2.0 ml/min. New stock solutions were prepared as needed during the toxicity tests. Dim fluorescent lighting provided a 12:12-h light:dark photoperiod. Diluters and toxicant flow rates were monitored daily to ensure proper operation. Fish loading during the ELS test was less than 0.63 g/L of tank volume and less than 0.04 g/L of flow per 24 h. During the fry tests, loading never exceeded 2.2 g/L of tank volume and was less than 0.11 g/L of flow per 24 h. Fish loading was much less than the suggested maximum levels [12].

ELS test methods

The number of hatched eggs and the mortality of eggs and fry were monitored and recorded daily. Dead fry were blotted dry with a paper towel, and total length (to the nearest mm) and weight (to the nearest 0.001 g) were measured and recorded. At the end of the tests, surviving fish from each exposure chamber were terminally anesthetized and blotted dry with a paper towel, and total lengths and weights were measured and recorded.

Water-quality characteristics of exposure water were measured weekly in all treatment levels within a replicate. Different replicates were selected each week for sampling. Hardness and alkalinity were determined according to standard methods [13]. A Thermo Orion 635 meter (ThermoFisher, Waltham, MA, USA) was used to measure pH and conductivity. Dissolved oxygen was measured using an Orion 1230 dissolved oxygen meter (ThermoFisher). The conductivity, pH, and dissolved oxygen meters were calibrated before each use.

Water samples for cadmium analyses were collected weekly from each exposure level with surviving fry. Exposure water was passed through a 0.45-μm filter (Acrodisc; Pall Life Sciences, Ann Arbor, MI, USA), collected in disposable polystyrene tubes (Falcon, Franklin Lakes, NJ, USA), and immediately preserved with high-purity nitric acid to pH less than 2. Water samples were analyzed using a SH4000 atomic absorption spectrometer with CTF 188 graphite furnace (Thermo Jarrell Ash, Waltham, MA, USA) and Smith-Hieftje background correction. Dibasic ammonium phosphate (0.1%) was used as a matrix modifier. The spectrometer was calibrated before each use and the calibration verified using a certified standard (High Purity Standards, Charleston SC, USA). Sample splits and spikes were collected at each sampling event to verify analytical reproducibility and recovery.

Fry test methods

Brown trout fry experiments utilized the same exposure apparatus as the ELS tests. Test methods were identical to the ELS test methods with the following exceptions: Water-quality characteristics were determined daily, and cadmium concentrations were measured three times during the initial 96 h and weekly thereafter. Fry were not fed during the initial 96 h of exposure but were fed twice daily thereafter (once daily on weekends). Cadmium exposure lasted for a total of 30 d.

Water-quality characteristics of both ELS and fry tests were near target levels and consistent over the duration of the experiments, as evidenced by relatively low standard deviations (Table 1). Mean recovery was 99% for quality assurance standard and 101% for spiked samples. Mean percentage difference between sample splits was 7%. The detection limit was less than 0.08 μg Cd/L.

Statistical analysis

Statistical analyses of data were conducted using Toxstat Version 3.5 software [14]. Analysis of variance (ANOVA) was used to test toxicity end points that included hatching success, sac fry survival, swimup survival, mean time to hatch, and lengths, weights, and biomass of surviving fish at test termination. Hatching success and survival data were arcsine square root transformed before ANOVA [15]. Normality and homogeneity of variances were tested using chi-square and Levene's test, respectively. Treatment means were compared to the control using Williams' one-tailed test [16,17] at $p < 0.05$. Mortality data from the 30H fry test did not meet the assumption of homogeneity of variance and were analyzed using Steel's many-one rank test. The highest cadmium concentration not associated with a treatment effect (e.g., decreased survival or decreased body weight) was designated as the no-observed-effect concentration (NOEC). The lowest concentration of cadmium 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 concentration estimated to cause a 20% reduction in organism performance compared with the control (IC20) [18] was calculated using the combined weight of surviving organisms from each treatment (biomass or standing crop). The 96-h LC50s were estimated by the trimmed Spearman-Karber technique [19] using log-transformed cadmium concentrations and 10% trim. Regressions of end points and hard-

Table 2. End points and associated cadmium chronic values (μg/L) of early life stage (ELS) and fry tests[a]

	30 Hardness		75 Hardness		150 Hardness	
	ELS	Fry	ELS	Fry	ELS	Fry
Time to hatch	>4.87 (4.87, ND)	—	>8.64 (8.64, ND)	—	>19.1 (19.1, ND)	—
Hatch success	>4.87 (4.87, ND)	—	>8.64 (8.64, ND)	—	>19.1 (19.1, ND)	—
Sac fry survival	>4.87 (4.87, ND)	—	>8.64 (8.64, ND)	—	>19.1 (19.1, ND)	—
Swimup fry survival	3.52 (2.54, 4.87)	1.02 (0.74, 1.40)	6.36 (4.68, 8.64)	1.83 (1.30, 2.58)	13.6 (9.62, 19.1)	6.54 (4.81, 8.88)
Weight	>4.87 (4.87, ND)	1.95 (1.40, 2.72)	>8.64 (8.64, ND)	3.4 (2.58, 4.49)	>19.1 (19.1, ND)	>16.4 (16.4, ND)
IC20	2.22	0.87	4.01	2.18	13.6	6.62
96-h LC50	—	1.23	—	3.9	—	10.1

[a] 30 Hardness, 75 Hardness, and 150 Hardness refer to 30, 75, and 150 mg/L, respectively, as $CaCO_3$. LOEC = lowest-observed-effect concentration; ND = not detected; NOEC = no-observed-effect concentrations; NOEC and LOEC in parentheses.

ness were performed using the Statistical Analysis System Proc GLM and assuming a log–log relationship [20]. Proc Genmod was used for the logistic regression of mortality with hazard quotient (HQ).

RESULTS

ELS tests

Mean time to hatch, hatching success, and sac fry survival were not significantly affected at any cadmium concentrations in any of the tests. Hatching success was 70 to 95% and exceeded 80% in the controls. Mortality during the sac fry stage was very low. Metal-related mortality occurred shortly after swimup, after absorption of the yolk sac, and when fry began exogenous feeding. In the 30H test, mortality was significant at 4.87 μg Cd/L (LOEC) but not at 2.54 μg Cd/L (NOEC). The LOEC and NOEC for the 75H test was 8.64 and 4.68 μg Cd/L, respectively. Mortality in the 150H test was significant at 19.1 μg Cd/L (LOEC) but not at 9.62 μg Cd/L (NOEC). The chronic values based on survival were 3.52, 6.36, and 13.6 μg Cd/L in water hardnesses of 30.6, 71.3, and 149 mg/L, respectively, as $CaCO_3$ (Table 2). No significant effects on growth, as measured by lengths and weights of fry at test termination, were detected. The IC20s based on biomass at test termination were 2.22, 4.71, and 13.6 μg Cd/L in water hardnesses of 30.6, 71.3, and 149 mg/L, respectively (Table 2).

Acute fry tests

No mortality occurred in the control or lowest exposure concentration during the 96-h acute exposures. Mortality increased with increasing cadmium concentration, resulting in complete mortality at 5.64 μg Cd/L in 30H. In 75H, 97.5% mortality was observed at 8.86 μg Cd/L, and in 150H, 90% mortality was observed at 16.4 μg Cd/L. The slopes of the concentration–response curves appeared to decrease as the water hardness increased. The 96-h LC50s were 1.23, 3.90, and 10.1 μg Cd/L at water hardnesses of 29.2, 67.6, and 151 mg/L, respectively (Table 2).

Chronic fry tests

Little additional metal-related mortality occurred after the initial 96 h of exposure. In the 30H exposures, low levels of mortality were observed at 0.42 and 0.74 μg Cd/L, but this was not significantly greater than control at $p = 0.05$. In 30H, mortality was significant at 1.40 μg Cd/L (LOEC) but not at 0.74 μg Cd/L (NOEC). The LOEC and NOEC for the 75H exposures were 2.58 and 1.30 μg Cd/L, respectively. Mortality in the 150H test was significant at 8.88 μg Cd/L (LOEC) but not at 4.81 μg Cd/L (NOEC). The chronic values based on survival were 1.02, 1.83, and 6.54 μg Cd/L at water hardnesses of 29.2, 67.6, and 151 mg/L, respectively (Table 2). Reduced growth, as measured by weight at test termination, was detected in the 30H and 75H tests. The weight of the single surviving fish at 2.72 μg Cd/L in the 30H test was significantly less than control. In the 75H test, mean weights of surviving fish in 4.49 and 8.86 μg Cd/L were significantly less than controls. No effect on growth was detected in the 150H test. The IC20s based on biomass at test termination were 0.87, 2.18, and 6.62 μg Cd/L in water hardnesses of 30.6, 71.3, and 149 mg/L, respectively (Table 2).

DISCUSSION

A break in a water line leading to the toxicology laboratory led to premature termination of the ELS tests after 41 d posthatch (14 d postswimup). The recommended duration of salmonid ELS tests is 60 d posthatch [20] or at least 30 d postswimup [12]. A majority of metal-related mortality occurred shortly after swimup and then quickly tapered off. It is unlikely that significant additional mortality would have taken place had the test continued for an additional 20 d. Negative effects on growth in the ELS tests may have occurred if the exposure continued for a longer duration.

Cadmium exposure to brown trout eggs did not affect mean time to hatch. Time to hatch of brown trout eggs has been altered by exposure to zinc [9,10] and to manganese [21]. Exposure to silver accelerated hatching of rainbow trout (*Oncorhynchus mykiss*) eggs [22]. Hatching success and sac fry mortality were unaffected by the cadmium concentrations used in the ELS tests. Egg and sac fry life stages of salmonids generally are more tolerant to metal exposure than the subsequent swimup fry stage [23,24]. Metal-related mortality in the ELS tests occurred shortly after brown trout embryos reached the swimup stage and began exogenous feeding. No effect of cadmium exposure on growth was detected in any of the ELS tests. In contrast, reduced growth in the fry tests was detected at 30H and 75H but not at 150H. Growth impacts occurred at cadmium concentrations greater than those that led to increased mortality.

The most sensitive end point tested was the IC20. The inhibitory concentration is interpolated from a concentration–response relationship and provides an estimate for a reduction of biological performance—in this case, a reduction of 20% biomass. Biomass at test termination reflects the effects of exposure on both survival and growth. Chronic values based on NOEC and LOEC are determined using hypothesis testing and can be influenced by selection of exposure concentrations

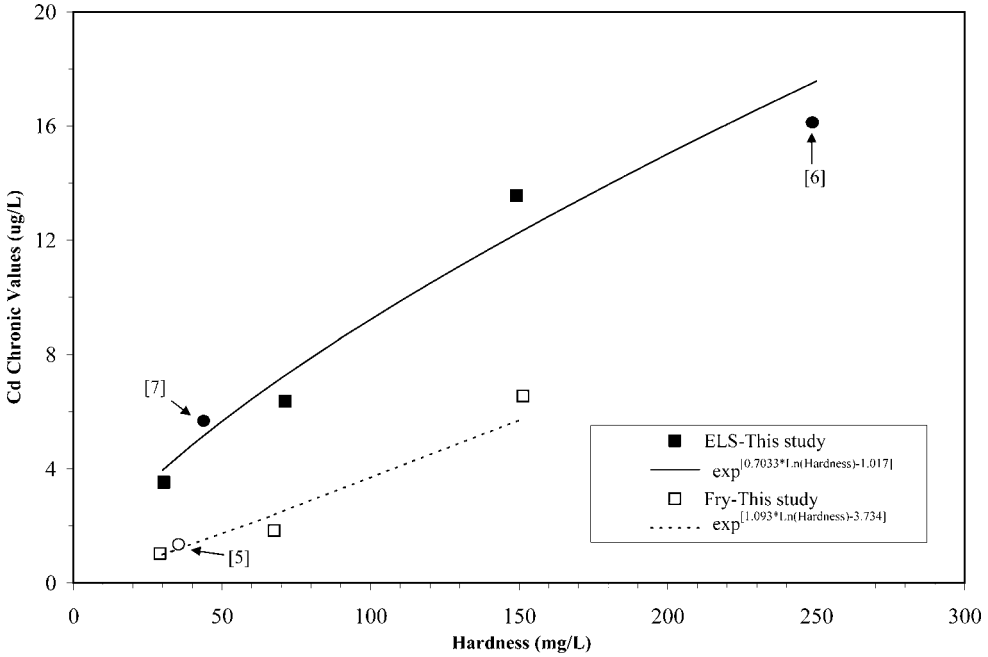

Fig. 1. Brown trout cadmium chronic values from brown trout life cycle–early life stage tests and fry at different water hardnesses.

and variability of the data set. Furthermore, chronic values provide little information regarding the magnitude of the effect at the LOEC. For fry but not ELS tests, the IC20 and the chronic value based on mortality were in close agreement. In contrast, chronic values from the 30H and 75H ELS tests were considerably greater than the corresponding IC20s. High variability inherent to ELS tests may decrease statistical power to detect reduced survival or biomass.

The ELS chronic values from the present study, along with previously reported values from ELS and life-cycle tests, show decreasing chronic toxicity with increasing hardness (Fig. 1). Results from the present study are in good agreement with those in the existing literature. The regression of the ELS chronic values, including an ELS test at a water hardness of 44 mg/L [6] and a life-cycle test at a water hardness of 250 mg/L [7], provides a good fit, with a correlation coefficient of 0.97. The regression equation for the ELS/life-cycle tests is

$$\text{brown trout ELS/life cycle chronic Cd} = \exp\{0.7033[\ln(\text{hardness})] - 1.017\}$$

Chronic values from tests initiated with fry are substantially lower than chronic values derived from ELS and life-cycle tests (Fig. 1). The equation describing the regression line for the fry tests (correlation coefficient, 0.97) is

$$\text{brown trout fry chronic Cd} = \exp\{1.093[\ln(\text{hardness})] - 3.734\}$$

Chronic end points of the ELS tests were consistently greater than those in the tests initiated with fry and even exceeded 96-h LC50s (Table 2). Exposure during cadmium-tolerant egg and larval stages probably resulted in acclimation. Consequently, exposed organisms were more tolerant to lethal effects during the subsequent, sensitive swimup fry stage [8,10,25,26]. Similarly, the U.S. Environmental Protection Agency (EPA) cadmium criteria document derived a brown trout species mean chronic value of 5.004 μg Cd/L, which is much greater that the reported species mean chronic value of 1.613 μg Cd/L [3]. The U.S. EPA water-quality criteria are intended to protect all life stages of an organism; however, chronic criteria derived from tests in which acclimation occurred may not protect sensitive life stages. The U.S. EPA criteria guidance document acknowledges that acclimation during chronic tests could lead to an acute to chronic ratio of less than two. In such cases, an acute to chronic ratio of two is assumed, because acclimation and continuous exposure in field situations cannot be assured.

Unacclimated brown trout fry from clean tributaries or upstream of a cadmium source could migrate into, or be washed into, cadmium-contaminated reaches. Those fry likely will experience cadmium toxicity in a manner similar to the tests initiated with fry rather than to that of the ELS tests. Also, brown trout fry can lose acclimation to metals once exposure to metals is discontinued [9,27] (Lara L. Gasser, 1998, Master's thesis, Colorado State University, Fort Collins, CO, USA). Migration into a clean tributary could lead to a loss of acclimation, followed by toxicity on return to a contaminated stream reach. Loss of acclimation also could occur during spring runoff, when dilution from spring snowmelt substantially reduces metal concentrations in streams.

Acute toxicity of cadmium decreased as hardness increased (Fig. 2). In addition to data from the present study, the regression of LC50s with hardness included three previous studies (1.4, 2.39, and 1.87 μg Cd/L at water hardnesses of 43.5, 37.6, and 36.9 mg/L, respectively) [4,5]. The regression equation estimating the brown trout cadmium LC50 based on water hardness is (correlation coefficient, 0.95)

$$\text{brown trout Cd LC50} = \exp\{1.258[\ln(\text{hardness})] - 3.999\}$$

The equation above, relating the cadmium LC50 and water hardness, can be used to normalize cadmium exposure concentrations. Assuming that half the LC50 is a safe concentration [20], a HQ for brown trout can be calculated by dividing a cadmium exposure concentration at a given hardness ($[Cd]_h$) by half the estimated LC50 at that hardness ($LC50_h$):

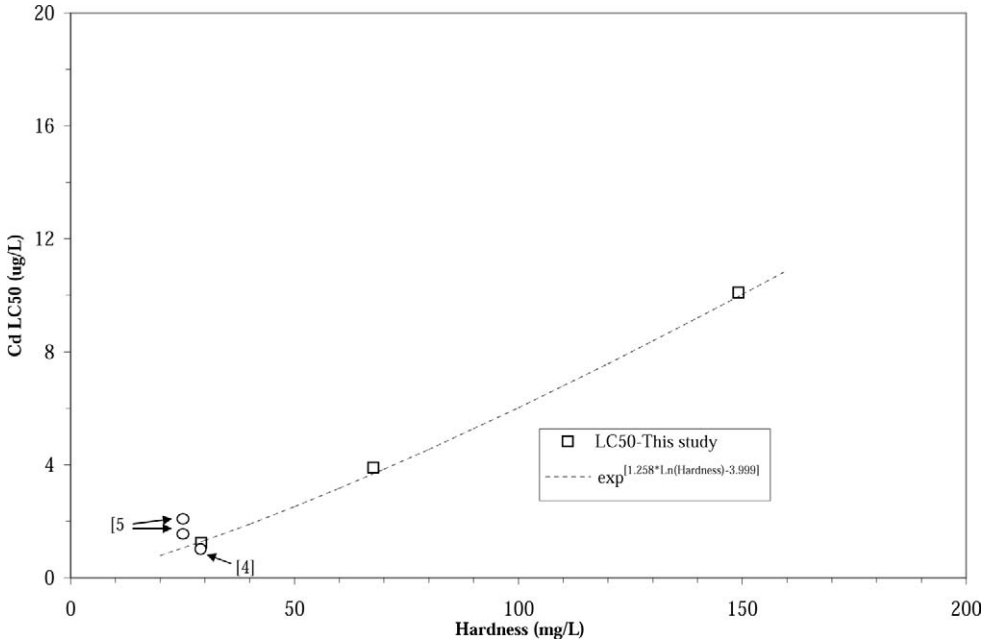

Fig. 2. Brown trout cadmium median lethal concentrations (LC50) at different water hardnesses.

$$\text{brown trout HQ} = [\text{Cd}]_h/(0.5 \cdot \text{LC50}_h)$$

Percentage mortality plotted against the HQ exhibits a characteristic sigmoid-shaped curve (Fig. 3). Acute mortality data from the three tests reported here as well as from two previous tests [5] are included. The fit of the curve is reasonable considering the range of hardness (30–150 mg/L) and size of organisms (0.48–7.00 g). Exposure concentrations and associated mortality were not reported by Spehar and Carlson [4] and, consequently, were not used in the regression. That particular study is represented by a single point with 50% mortality at the reported LC50 divided by the hardness-predicted LC50. The equation for the line relating cadmium HQ and brown trout mortality is

$$\text{96-h brown trout mortality (\%)} = 100/[1 + \exp(-2.4011\text{HQ} + 5.067)]$$

Figure 3 and the associated regression equation can be used to predict brown trout swimup fry mortality given cadmium concentration and hardness. Alternatively, hardness-based concentrations of cadmium can be calculated for the protection of brown trout based on an acceptable level of mortality.

CONCLUSIONS

Cadmium is highly toxic to brown trout swimup fry. Embryos and larvae are less sensitive. Chronic tests initiated with tolerant life stages may lead to acclimation, resulting in lower

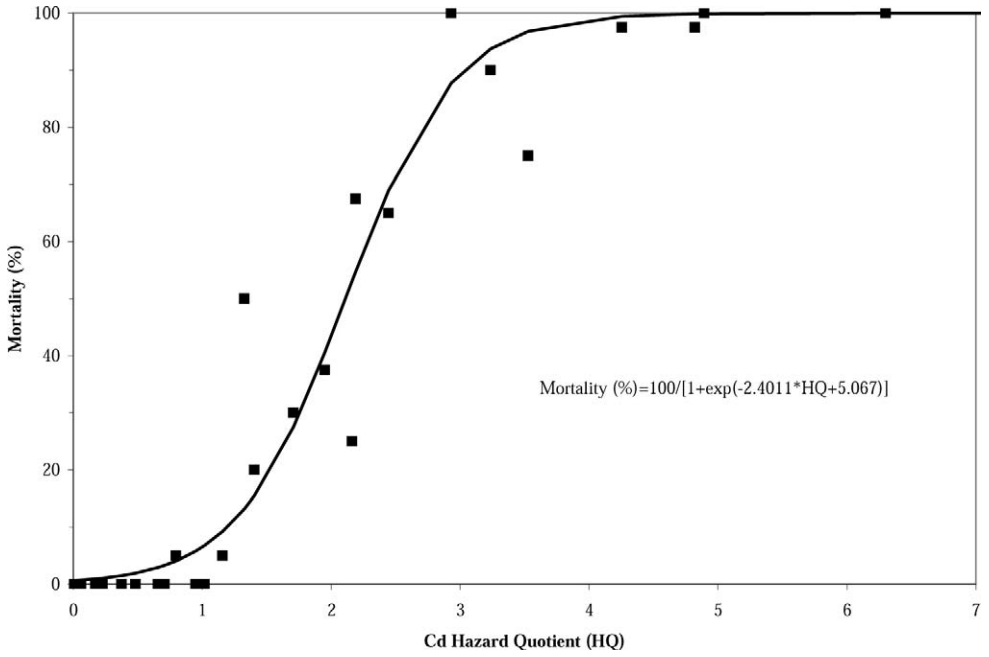

Fig. 3. The 96-h brown trout mortality (%) as a function of cadmium hazard quotient (HQ).

mortality during more sensitive life stages. In such instances, ELS tests may underestimate chronic toxicity. Cadmium toxicity is decreased by water hardness in a predictable manner. The LC50s of cadmium to brown trout fry can be estimated from water hardness. The ratio of measured cadmium concentration to the estimated LC50 can be used to estimate acute brown trout mortality.

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