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Resistance of Temperature Tolerance Ability of Green Sunfish to Cadmium Exposure

Roxie Carrier and T. L. Beitinger

Division of Environmental Sciences, Department of Biological Sciences, North Texas State University, Denton, Texas 76203

Fish are in intimate contact with their aqueous environment, and hence, are quickly susceptible to stressful changes in their chemical milieu. It is expected that stressful chemicals present in the external aquatic environment would induce observable physiological changes, even at sublethal concentrations. Carrier and Beitinger (in press) reported that cadmium at LC5, LC10 and LC20 concentrations (of 96-h LC50) highly significantly decreased the temperature tolerance of red shiners _Notropis lutrensis_ and fathead minnows, _Pimephales promelas_. After 10 days, temperature tolerance measured as critical thermal maximum (CTM) of cadmium-exposed fish decreased by 2.3 to 4.4 C in red shiners and 4.2 to 5.7 C in fathead minnows.

Since sunfishes, family Centrarchidae appear to be more resistent to certain types of chemicals (e.g., nitrite, see Huey et al. 1982; Palachek and Tomasso 1984), we decided to employ similar methodology to test the effects of cadmium on temperature tolerance of green sunfish, _Lepomis cyanellus_. The literature (e.g., Palachek and Tomasso 1984) and research in our laboratory led us to hypothesize that cadmium exposure would not reduce temperature tolerance as much in a centrarchid such as green sunfish as in red shiners and fathead minnows.

MATERIALS AND METHODS

Juvenile green sunfish (0.5 to 2.5 g) were obtained from a local hatchery and maintained at 20 C in aerated, charcoal-filtered reconstituted hardwater (USEPA 1975) for at least 1 week before trials. Fish were fed flake food daily.

Since published cadmium LC50 values for fish are variable and dependent on water quality, we determined 96-h LC50 values for green sunfish in reconstituted hardwater. Lethal static toxicity tests were conducted over 96 h in separate 19-L aerated aquaria at 20 C. The following variables were monitored: oxygen (Winkler calibrated meter, $\pm$0.1 mg/L), pH (meter, $\pm$0.01 units), alkalinity

Send reprint requests to T.L.Beitinger at the above address

(bromcresol green, methyl red titration method, American Public Health Association 1975; sensitivity ±0.1 mg $CaCO_3$/L), hardness (EDTA titrimetic method, American Public Health Association 1975; sensitivity ±0.1 mg $CaCO_3$/L) and metal concentrations were monitored at the beginning, conclusion and at three other times during the 96-h LC50 determinations. Five cadmium concentrations were duplicated with 10 fish exposed to each concentration during the lethality trials. Food was withheld from fish during the trials. Dead (no response to touch) fish in each aquarium were counted and removed at 1, 2, 4, 8, and 24 h, and daily thereafter (Peltier and Weber 1983). The LC50 was determined by a program of Stephan (1977). Results from these lethality trials allowed calculation of sublethal cadmium concentrations for exposures prior to CTM trials. Cadmium exposures equivalent to 24, 37 and 45 % of lethal concentrations for 96-h exposures were tested.

Thermal tolerance was evaluated by the critical thermal maximum, CTM, introduced by Cowles and Bogert (1944) and defined by Cox (1974). The selected test endpoint was loss of equilibrium coupled with loss of righting response. These criteria were definitive and repeatable. Consistent with the initial definition of CTM methodology, a 100 % recovery of test fish occurred when fish at this endpoint were quickly returned to their acclimation temperature.

Cadmium (as cadmium sulfate) was dissolved in 189 L of hardwater at 20 C. Water was aerated and charcoal filtered during cadmium exposures. Preliminary trials indicated that cadmium concentrations were not influenced by charcoal filtration. During the 10-day cadmium exposures, pH, alkalinity and hardness remained consistently at 8.0, 85.5 mg/L and 136.8 mg/L, respectively.

Approximately 100 fish were placed in each of four aquaria (control and three cadmium concentrations). Fish were removed from each aquaria for CTM measurements after 1, 5 and 10 days of cadmium exposure. The CTM of 12 fish per cadmium exposure were determined simultaneously in a 60 L "long" aquarium containing aerated hardwater. The test chambers consisted of two adjacent rows of six 10 cm x 13 cm x 25 cm (high) plastic mesh enclosures. Temperature was increased at a constant rate of 0.30 C/ min following the recommendations of Becker and Genoway (1977) by two circulating thermoregulators and monitored with a digital thermometer (±0.01 C) until the endpoint criterion (loss of equilibrium combined with loss of righting ability) of each fish was met.

Water samples collected during tests were acidified with nitric acid (Baker trace metal analysis grade) and refrigerated in nalgene bottles for later analysis of cadmium concentrations by flame atomic absorption (±0.01 mg/L, Perkin-Elmer 1982). Measured cadmium concentrations for LC50 determination and sublethal exposures remained stable throughout the testing periods. The coefficients of variation did not exceed 13.2 %.

RESULTS AND DISCUSSION

Nominal cadmium concentrations for lethal testing were 0, 3, 5, 7, 9 and 11 mg Cd/L. Actual measured concentrations differed by no more than 5.4 % from nominal during lethality trials.

Probit analysis determined a 96-h LC50 of 11.52 mg Cd/L with a 95 % confidence interval extending from 10.44 to 16.86 mg/L. During these trials, mortalities ranged from none to 41.2 %.

Our 96-h LC50 of 11.52 mg Cd/L for green sunfish is encompassed by LC50 values for this species, 2.84 to 66.0 mg/L, previously reported by Pickering and Henderson (1966). The disparity of these values appear to be a function of the water hardness used in the determination of these three LC50s. In our trials hardness averaged 85.5 mg $CaCO_3$/L, whereas in the trials of Pickering and Henderson (1966), the lower LC50 estimate corresponded to 20 mg $CaCO_3$/L and the highest occurred at 360 mg $CaCO_3$/L. These three LC50 values are significantly linearly related to water hardness ($r = 0.998$, $p = 0.035$). In hard water, $CaCO_3$ apparently "binds" with cadmium, reducing its availability and, hence, more cadmium is needed to produce the same percentage of lethality, i.e., the LC50 is higher. These results further support the idea that water quality variables can greatly influence the toxicity of chemicals; consequently, care should be taken when comparing LC50 values derived under different water qualities.

Prior to CTM trials, experimental fish were exposed to the following cadmium concentrations: 2.76, 4.22 and 5.17 mg/L. All of these exposures are less than an LC5 assuming linearity of response over the range of our LC50 determination.

At all exposures, green sunfish were fairly inactive, with an apparent uniform distribution of fish throughout the aquaria. Individuals did not exhibit any obvious symptoms of stress.

We found little variation in the CTMs of controls (Table 1). Coefficients of variation for days 1, 5, and 10 were all less than 2 %. Means of control green sunfish measured on these 3 days ranged from 35.79 to 35.92 C and were not significantly different. The consistency and stability of the control data provide an excellent baseline to assess the effects of water-borne cadmium on thermal tolerance.

Given the tightness of the control data, any cadmium-induced change in CTM would be detected as statistically significant. Nevertheless, neither cadmium concentration ($p > 0.80$) nor exposure time ($p > 0.80$) had a significant affect of CTM in this species (two-way analysis of variance). The mean CTMs for all 12 groups ranged from 35.40 to 36.17 C, a difference of only 0.77 C or 2.2 %. In this table, the effect of cadmium concentration is read vertically while time of exposure appears horizontally.

Table 1. Critical Thermal Maxima (mean and standard deviation with n in parenthesis) of *Lepomis cyanellus* exposed to sublethal concentrations of cadmium.

Treatment	Day 1	Day 5	Day 10
Control	35.79 ± 0.69 (9)	35.76 ± 0.40 (11)	35.92 ± 0.56 (9)
2.76 mg/L	35.84 ± 0.66 (9)	35.79 ± 0.66 (11)	35.35 ± 0.53 (8)
4.22 mg/L	35.40 ± 1.00 (10)	35.93 ± 1.04 (8)	36.17 ± 0.65 (8)
5.17 mg/L	35.80 ± 0.94 (10)	35.80 ± 0.71 (8)	35.62 ± 0.13 (8)

The observed lack of effect of cadmium on the ability of green sunfish to tolerate temperature is noteworthy, since the other species examined exhibited not only a statistically significant but ecologically important decrease in CTM subsequent to cadmium exposure. The highest cadmium concentration that green sunfish were exposed to for 10 days in this research (5.17 mg/L) approaches the 96 h LC50 for red shiners (6.62 mg/L) and exceeds the 3.56 mg/L 96 h LC50 for fathead minnows that we measured under the same temperature, hardness and water quality (Carrier and Beitinger in press).

Several as yet unresolved possibilities could explain our findings. First it is possible that cadmium uptake rates may be lower in green sunfish. Palachek and Tomasso (1984) reported that the unusually high nitrite LC50 measured in another centrachid, the largemouth bass, appears to be the result of differential selectivity of gill epithelium to particular ions. Also it is possible that detoxification processes are well developed in this species. Detoxification of cadmium appears to be through a combination of copper and zinc binding isoforms of metallothionein and a low molecular weight, metal-inducible protein capable of binding cadmium (Price-Haughey et al. 1986; Petering and Fowler 1986; Kay et al. 1986). Sequestering of cadmium is complicated by past metal exposure history, which effects activation of detoxification proteins and may also play a role in the variation in response to cadmium. Finally cadmium depuration rates may be high.

Regardless of the mechanism(s) green sunfish and centrarchids in general appear to be hardy to a variety of environmental conditions. This characteristic undoubtedly has assisted their wide zoogeographical distribution. In addition, this same characteristic makes green sunfish a much less sensitive indicator of environmental contamination than other possible test species such as fathead minnows.

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