Bull. Environ. Contam. Toxicol. (1995) 55:230–236

Environmental Contamination and Toxicology

Reciprocal Influences of Temperature and Copper on Survival of Fathead Minnows, *Pimephales promelas*

V. L. Richards, T. L. Beitinger

Department of Biological Sciences, University of North Texas, Post Office Box 5218, Denton, Texas 76203, USA

Received: 28 November 1994/Accepted: 7 March 1995

Contemporary ecological concerns of accelerated global warming, increase in toxic chemicals and loss of biodiversity make relevant studies of tolerance of various organisms to abiotic variables. In this study, the reciprocal effects of temperature and copper on survival of fathead minnows, *Pimephales promelas*, were determined. Temperature tolerance of fishes is limited by a cornucopia of biotic and abiotic factors (Hutchison 1976), including various toxicants (see review of Beitinger and McCauley 1990). Not only do chemicals affect temperature tolerance of fishes, temperature influences the sensitivity of fish to toxic chemicals; however, the relationship between temperature and lethality is complex, difficult to predict, and has not been the focus of many studies.

Copper, a necessary trace element in animal metabolism and ubiquitous in aquatic environments, was selected as our test toxicant. Hodson et al. (1979) reported copper concentrations of one to 20 µg/L in unpolluted surface waters in the United States. Copper sulfate ($CuSO_4$), is an algicide, bactericide and herbicide for ponds, lakes and fish hatcheries (Klussmann and Davis 1988). Also, copper is recommended as a fungicide for a variety of ornamental plants and crops, and in various chemical forms enters the environment through mining, smelting, and refining activities (e.g., Hodson et al. 1979). Copper is toxic in parts per billion concentrations (µg/L) and is an EPA priority pollutant. In this research two null hypotheses were tested: (1) temperature has no effect on the lethality of copper sulfate, and (2) sublethal concentrations of copper do not affect the upper temperature tolerance of fathead minnows.

MATERIALS AND METHODS

Fathead minnows, obtained from a local culture, were held under a LD 14:10 photoperiod at 22°C and fed daily. Four constant acclimation temperatures (5, 12, 22 and 32°C) were chosen. Minnows acclimated to 5 and 12°C were cooled at 1°C per day until their acclimation temperature was reached. The water temperature of minnows acclimated to 32°C was raised 2 to 3° C per day until 32°C was reached. Minnows acclimated to 22°C were maintained at the culture temperature. Once at the appropriate acclimation temperature, minnows were

Correspondence to: T. L. Beitinger

held for a minimum of seven days before trials. Fish were fed flake food daily during acclimation; however, feed was withheld one day prior to and during the 96-hr LC50 trials. Standard concentrations from 0.1563 to 5 mg/L of cupric sulfate ($CuSO_4 \cdot 5H_2O$) were used as the copper source. Absorbance levels of prepared samples were measured (± 0.001) by direct-flame atomic absorption on a Perkin-Elmer 2380 Atomic Absorption Spectrophotometer and compared to absorbance levels for samples via linear regression. The resulting regression model was used to estimate actual copper concentrations (± 10 µg/L) in the samples. Reconstituted hard water (APHA 1992) with hardness of 160 to 180 mg/L $CaCO_3$ and alkalinity of 110 to 120 mg/L $CaCO_3$ was used in all experiments.

Two replicate trials to estimate 96-hr LC_{50}s were conducted with a control and five copper concentrations (100, 200, 300, 400, and 500 µg/L) at each of the four acclimation temperatures in aerated glass aquaria. Water pH (meter, ± 0.1 units), hardness (color-metric titration, ± 2 mg $CaCO_3$/L), alkalinity (titrametric method, ± 5 mg $CaCO_3$/L), and dissolved oxygen (meter, ± 0.1 mg O_2/L) were measured. Either five or six fathead minnows were tested per replicate for each copper concentration. After 1.5, 3, 6, 12, 24, 48, 72, and 96 hr, dead minnows were removed, weighed (± 0.1 mg), and measured (± 0.1 cm). Copper concentrations were measured before fish were added and again at the completion of each trial; these concentrations were averaged to estimate exposure concentrations in subsequent temperature tolerance tests.

Mortality data were analyzed using the Trimmed Spearman-Karber method (Hamilton et. al. 1977; APHA 1992). The LC_{50}s obtained at the four acclimation temperatures were compared by examining 95% confidence intervals for overlap using the formula:

$$f_{1,2} = \frac{1.96\ SE_{diff}}{LC50_{1,2}} = \text{antilog}\sqrt{(\log f_1)^2 + (\log f_2)^2}$$

where f is the factor for 95% confidence limits of the LC_{50}, i.e., the confidence limits are LC_{50} x f and LC_{50} ÷ f (f=antilog of two standard deviations of the log LC_{50}). If the ratio of the greater LC_{50} to the smaller LC_{50} exceeds the value of $f_{1,2}$, the LC_{50}s were considered significantly different at a α = 0.05 (APHA 1992). Percent mortality was converted to probit units and regressed against acclimation temperature for each copper concentration.

After a 96-hr median LC_{50} was determined for each acclimation temperature, reciprocal experiments were performed to test the effect of sublethal copper exposure on the upper temperature tolerance via the critical thermal maxima (CTMax). This methodology has been reviewed for fishes by Beitinger and McCauley (1990). Nominal copper concentrations used represent 25, 50 and 75% of the 96-hr LC_{50} that we determined for each acclimation temperature.

Water quality parameters were taken, cupric sulfate was added and copper concentrations were measured before minnows were introduced. After minnows had been exposed to copper for 24 hr, 10 minnows were transferred to a 38-l aquarium with 10 partitioned chambers and a circulating thermoregulator. Water temperature was measured to ± 0.1°C by a calibrated Cole-Parmer Thermistor Thermometer probe. Aeration and mixing was provided by a 10-cm airstone placed along the length of the aquarium. Water temperature was increased at a constant 0.3°C/min. An endpoint of loss of equilibrium combined with loss of righting ability was used. When a minnow reached this endpoint, the temperature was recorded (= CTMax) and the minnow was immediately returned to its acclimation temperature and observed for recovery. The weight (± 0.1 mg) and length (± 0.1 cm) of each minnow were recorded. All trials were performed between 0800 and 1300 to minimize possible diel fluctuations in CTMax (Hutchison 1976).

CTMax data for each acclimation temperature / copper group were tested for normality (Shapiro Wilks W test with α=0.05) and Hartley's F_{max} (α=0.01) test assessed homogeneity of variances. Parametric ANOVA with multiple range tests were used to detect statistical differences (α=0.05) within and between acclimation temperature groups.

RESULTS AND DISCUSSION

The ranges of pH, hardness, and alkalinity across the eight, 96-hr LC_{50} trials were 7.8 to 8.5 pH units, 148 to 180 mg/L as $CaCO_3$, and 125 to 145 mg/L as $CaCO_3$, respectively. Fish weights and lengths ranged from 0.13 to 3.23 g and 1.9 to 5.5 cm, respectively. Coefficients of determination relating estimated copper concentrations from standard concentrations exceeded 0.99. Partial mortality occurred at all acclimation temperatures; however, only at 12°C was a 100% "kill" observed. Percent mortality expressed as probit units was linearly regressed on $\log_{10}$ copper concentration to determine if these transformed variables were related. Only at the 32°C acclimation temperature was a statistically significant model produced. Lack of statistical significance at the other three test temperatures may be explained by the relatively small range of copper concentrations, 400 mg/L, used in these trials.

Our 96-hr LC_{50}s ranged from 297 µg/L at 12°C to 513 µg/L at 32°C, a 1.72-fold difference (Fig. 1). These values compare favorably with those predicted by Hodson's (1979) model, 404 and 506 µg/L, which relates copper lethality to water hardness for fathead minnows. Other studies of copper lethality in fathead minnows include Mount (1968), Pickering et al. (1977) and Schubauer-Berigan et al. (1993).

Surprisingly, there have been few studies concerning the effects of acclimation temperature on chemical toxicity. Our results support Welch et al. (1989) who cited temperature as a major factor influencing the toxicity of copper to fish. Copper lethality appears to be quadratically related to temperature (Fig. 2). The regression model [LC_{50} (µg/L) = 608.93 - 38.32(AC) + 1.11(AC^2)] where AC = acclimation temperature (°C)) describing the quadratic relationship was not statistically significant (p=0.0871), since only four observations were available. Nonetheless it mathematically describes the relationship observed in these toxicity experiments. The median 96-hr LC_{50}s estimated by a trimmed Spearman-Karber method were separated into two statistically distinct groups: <u>32°C 5°C</u> >

22°C 12°C. Copper was most toxic to fathead minnows at 12 and 22°C and less toxic at 5 and 32°C. Cairns et al. (1978) reported a similar "depression" in the 24-hr median lethal concentrations of copper at a mid-range temperature for the golden shiner (*Notemigonius crysoleucus*), bluegill (*Lepomis macrochirus*) and channel catfish (*Ictalurus punctatus*) when tested at 5, 15, and 30°C. Copper was more toxic to Mozambique tilapia (*Oreochromis mossambicus*) at 15°C than at 25 and 35°C (Welch et al. 1989).

Few studies have examined the effect of acclimation temperature on CTMax. Each of the control mean CTMaxs for the four acclimation temperatures (28.6, 30.7, 36.4 and 40.4°C, respectively) were significantly distinct (SNK, α=0.05). These values define the upper limits of a CTM temperature tolerance polygon for fathead minnows acclimated between 5 and 32°C (Fig. 3). Little variation was observed as coefficients of variation were all less than 3.1%. Linear regression analysis generated a highly significant (p = 0.0001) model (CTMax (°C) = 25.91 + 0.4567 (acclimation temperature, °C) which explains almost 97% of the observed variation in CTMax. This model indicated that for each 1°C increase in acclimation temperature, CTMax increased by 0.46°C.

Temperature tolerance of fish is often compromised by exposure to heavy metals and other toxic chemicals (see review by Beitinger and McCauley 1990). In our experiments, acute sublethal exposure to copper significantly decreased the CTMax for fathead minnows at three of the four acclimation temperatures (see Fig. 2). Of the four acclimation temperatures, copper exerted the greatest effect

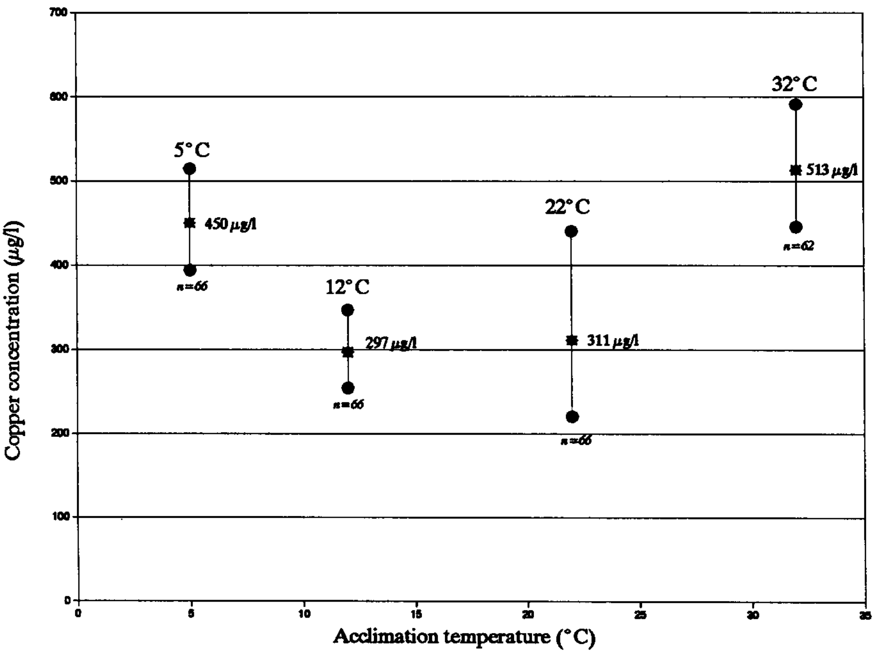

Figure 1. Median lethal concentrations (LC_{50}) after 96-hr exposure ± 95% confidence intervals for each acclimation temperature. The LC_{50} concentration is shown at the median and sample size is given below each bar.

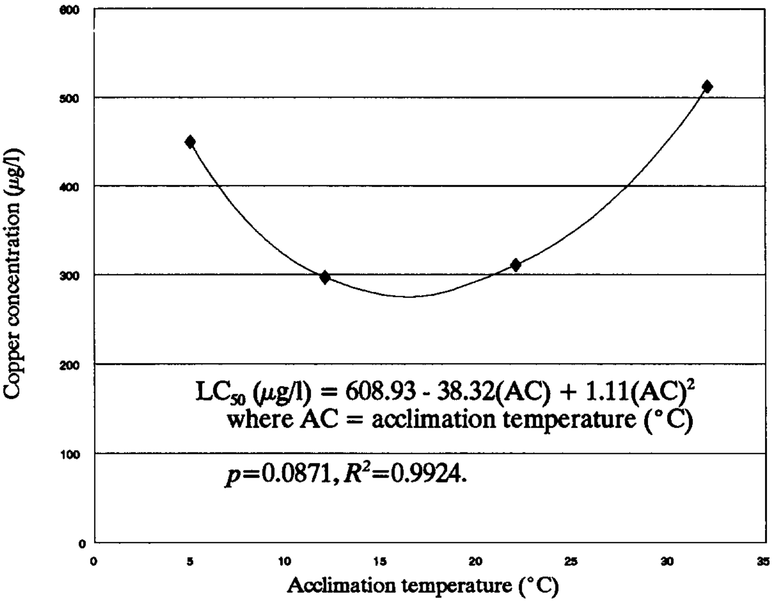

Figure 2. Polynomial regression of 96-hr LC_{50} and acclimation temperature.

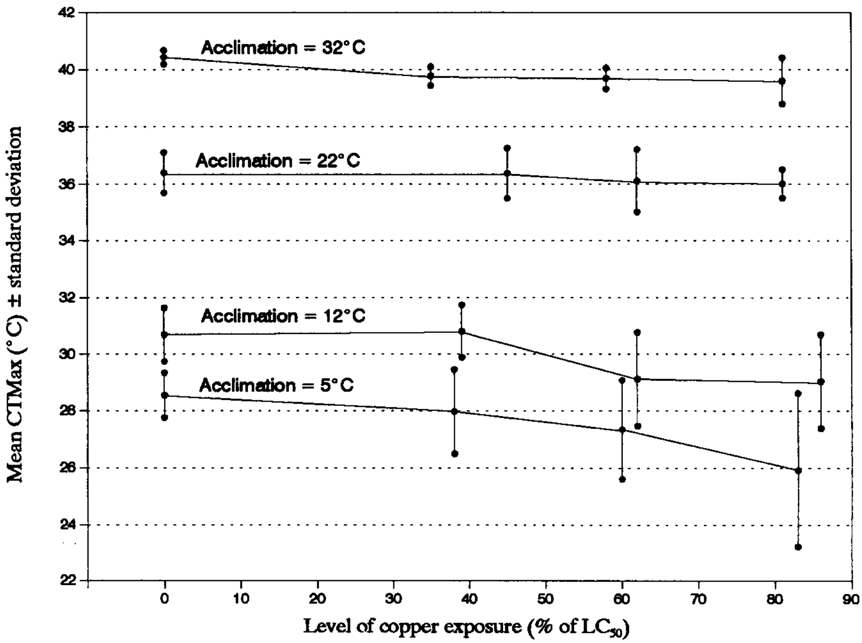

Figure 3. Mean CTMax ± one standard deviation for four acclimation temperatures after a 24-hr sublethal exposure to copper at 25, 50, and 75% of the 96-hr LC_{50}. Bars are positioned at the level of copper measured during the exposure. Sample size is nine or ten for each group.

at 5°C, where CTMax decreased by 2.6°C relative to controls. Unexpectedly, at 22°C a drop of only 0.4°C was seen between the control group and the group exposed to 81% of the 96-hr LC_{50}. The greatest change between exposure levels was seen in 12°C-acclimated fish between 37 and 58% of the LC_{50}, where the CTMax dropped 1.7°C. Statistically significant differences were found in mean CTMax among exposure groups at 5°C ($p = 0.017$), 12°C ($p = 0.005$), and 32°C ($p = 0.0016$) (one-way ANOVA and multiple range test, both with $\alpha=0.05$). Not only was the mean response affected, variances tended to increase with increasing copper exposure. Higher exposure levels (greater than 40% of the LC_{50}) caused a more dramatic drop in CTMax at the lower test temperatures (5 and 12°C), suggesting that copper inhibited the ability to tolerate increasing temperature to a greater degree at lower metabolic rates.

These results agree with tests of Lydy and Wissing (1988) who found that sublethal exposures to copper (ranging from approximately 38% to 59% of the 96-hr LC_{50}) reduced the thermal tolerances of the fantail darter (*Etheostoma flabellare*) and the johnny darter (*E. nigrum*) 7.6 and 5.2°C, respectively. No other studies of the effects of copper on CTMax were found, but similar results have been reported for other heavy metals including cadmium (Middaugh et al. 1975) and nickel (Becker and Wolford 1980).

This research clearly demonstrates that acclimation temperature significantly affects the 96-hr median lethal concentration in fathead minnows. Copper is lethal in the µg/L range and a quadratic relationship is hypothesized between acclimation temperature and copper lethality. In addition, exposure to copper adversely affects the ability of fathead minnows to withstand high temperatures. Although the responses seen at the four different acclimation temperatures were not identical, sublethal copper exposure and CTMax were inversely related at three of the four acclimation temperatures.

REFERENCES

American Public Health Association (1992) Standard methods for the examination of water and wastewater, 18th edition. APHA, Washington, D. C.

Becker CD, Wolford MG (1980) Thermal resistance of juvenile salmonids sublethally exposed to nickel, determined by the critical thermal maximum method. Environ Poll Ser A Ecol Biol 21:181-189

Beitinger TL, McCauley RW (1990) Whole-animal physiological processes for the assessment of stress in fishes. J of Great Lakes Res 16:542-575

Cairns J Jr, Buikema AL Jr, Heath AG, Parker BC (1978) Effects of temperature on aquatic organism sensitivity to selected chemicals. Bull 106 from Virginia Water Resources Research Center, Blacksburg, Virginia, pp. 1-88

Hamilton MA, Russo RC, Thurston RV (1977) Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ Sci Tech 11:714-719

Hodson PV, Borgmann U, Shear H (1979) Toxicity of copper to aquatic biota. In: Nriagu JO (ed) Copper in the Environment, Part 2: Health Effects. John Wiley & Sons Inc., New York, pp. 307-372

Hutchison VH (1976) Factors influencing thermal tolerances of individual organisms. In: Esch GW, McFarlane RW (eds) Thermal Ecology II. United States National Technical Information Service, US Department of Commerce, Springfield, Virginia, CONF-750425, pp. 10-26

Klussman WG, Davis JT (1988) Common aquatic plants of Texas. Texas Agricultural Extension Service B-1018, Texas A&M University System, College Station, TX

Lydy MJ, Wissing TE (1988) Effect of sublethal concentrations of copper on the critical thermal maxima (CTMax) of the fantail (*Etheostoma flabellare*) and johnny (*E. nigrum*) darters. Aquat Toxicol 12:311-322

Middaugh DP, Davis WR, Yoakum RL (1975) The response of larval fish, *Leiostomus xanthurus*, to environmental stress following sublethal cadmium exposure. Contri Mar Sci 19:13-19

Mount DI (1968) Chronic toxicity of copper to fathead minnows (*Pimephales promelas* Rafinesque). Wat Res 2:215-223

Pickering QH, Brungs WA, Gast MH (1977) Effect of exposure time and copper concentration on reproduction of the fathead minnow *(Pimephales promelas).* Wat Res 11:1079-1083

Schubauer-Berigan MK, Dierkes JR, Monson PD, Ankley GT (1993) pH-dependent toxicity of Cd, Cu, Ni, Pb and Zn to *Ceriodaphnia dubia, Pimephales promelas, Hyalella azteca* and *Lumbriculus variegatus*. Environ Toxicol Chem 12:1261-1266

Welch TJ, Stauffer JR Jr, Morgan RP II (1989) Temperature preference as an indicator of the chronic toxicity of cupric ions to Mozambique tilapia. Bull Environ Contam Toxicol 43:761-768