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Temperature Preference as an Indicator of the Chronic Toxicity of Cupric Ions to Mozambique Tilapia

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Evaluation of the effects of environmental contaminants on aquatic communities has focused primarily on acute bioassays. These bioassays provide rapid and reproducible concentration response curves based on death as an endpoint. In recent years, however, emphasis has shifted towards monitoring sublethal effects of toxicants (e.g. modification of behavioral patterns, growth, reproduction), because a greater community biomass is generally exposed to chronically low levels of pollutants than to lethal levels (Kleerkopper 1976). Toxicological modification of specific behavioral patterns is often associated with the ability of a fish to survive environmental perturbations. Changes in fish behavior, however, may be complex and difficult to detect in the laboratory. An effective test, therefore, for evaluating the effects of aquatic contaminants on fish behavior must include a well-defined and quantifiable endpoint.

Temperature is an easily quantifiable parameter influencing both the behavior and survival of fishes. As poikilotherms, fish use behavioral responses to help regulate body temperature. When exposed to a thermal gradient, fish select a preferred temperature for optimum activity (depending on their acclimation temperature) (e.g. Meldrim and Gift 1971).

Fish thermoregulatory behavior may be altered by various toxic substances. Ogilvie and Anderson (1965) showed that Atlantic salmon (Salmo salar) exposed to sublethal DDT concentrations for 24 hr shifted their preferred temperature. Also, Opuszynski (1971) found that a 24 hr exposure of sublethal concentrations of copper (0.20 mg/l, 0.25 mg/l) caused a significant

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decrease in preferred temperature of fathead minnows (Pimephales promelas), although the results were confounded due to variations in copper concentrations.

Other toxic chemicals shown to have influenced fish thermoregulatory behavior include potassium cyanide (Anderson 1971), aldrin (Peterson 1973), and malathion (Peterson 1976). All of these studies involved short-term exposures to relatively high toxicant concentrations.

In this study, we examined the feasibility of using acute temperature preference tests to assess the chronic toxicity of low concentrations of free cupric ions to Mozambique tilapia, Oreochromis mossambicus (Peters).

MATERIALS AND METHODS

We exposed groups of 15 juvenile Mozambique tilapia to a cupric ion concentration of 0.06 mg/l and to control (no added copper) conditions for 8 d. Two control and two experimental groups per temperature (total 12 groups) were exposed at 15, 25, and 35°C. These temperatures were chosen to represent a range that Mozambique tilapia could survive for an extended time period. The exposure level was approximately one-half of the LC10 determined by a series of 48 hr rangefinder copper bioassays at 25 and 35°C (Welch 1985). After exposure to the toxicant, acute temperature preferences were determined, using a horizontal temperature gradient trough, for fish acclimated to each temperature. Fish were cultured in 37.8 L glass aquaria housed in refrigeration units. Using aquarium heaters, we achieved acclimation temperatures by increasing or decreasing water temperatures 1°C per day. Prior to the experiment, fish were held at the experimental acclimation temperature 24-48 hr. Fish were fed once per day. To ensure stomach constancy, we starved fish for 24 hr prior to the temperature preference test. During the experiment and acclimation period, photoperiod was maintained at 12:12 LD.

In order to maintain copper concentrations over an extended time period, we used a flow-through exposure system. This system consisted of a head box, diluter, and four exposure chambers formed by two 37.8 l glass aquaria divided in half by plastic mesh screens, all housed in a refrigeration unit. Laboratory water (alkalinity, 26.12 ± 0.52 mg/l; hardness, 64.10 ± 1.86 mg/l; pH, 6.57 ± 0.09) passed through an ion exchange filter and activated charcoal to remove impurities before entering the apparatus. A peristaltic pump introduced the toxicant, a 0.04 g/l solution of copper sulfate ($CuSO_4$), into the treatment aquaria via the diluter. Due to the varying degree of copper sulfate solubility at different temperatures, pump flow was adjusted at each temperature to produce a 0.06 mg/l cupric ion concentration in the experimental aquaria. Water flow adjustment provided approximately eight complete water exchanges per day in accordance with ASTM standards (American Society of Testing and Materials 1984a).

We measured cupric ion activity twice daily using a cupric ion-specific electrode (Orion Model 94-29). Cupric ion activity was expressed in mg/l because in dilute solutions ionic activity and concentration are practically identical (American Society of Testing and Materials 1984b). The electrode could detect cupric ion concentrations as low as 6.4×10^{-3} mg/l with reproducibility of 4%. Using the "known addition" procedure (American Society of Testing and Materials 1984b), we determined cupric ion concentrations. Temperature and dissolved oxygen, measured daily, remained within normal limits during all experiments.

In each experiment, two groups of tilapia were exposed to copper in one aquarium and two were exposed to control conditions in the other aquarium at a specific acclimation temperature. Because, at most 10 fish per day could be tested for acute temperature preference, each group of 15 fish was introduced into the exposure system over a period of four days to insure equal exposure times prior to temperature preference testing.

We determined acute temperature preference of control and copper-exposed fish in a horizontal temperature gradient trough (after Meldrim and Gift 1971) following the procedures of Stauffer et al. (1980). To begin a temperature preference test, one fish was randomly selected from an experimental group and introduced into the trough at its acclimation temperature for a 40 min orientation period. After this period, observations recorded at 15 sec intervals for 20 min (via overhead mirrors) included the location of the fish with respect to the nearest thermistor and the temperature at that location. Because of daylight time constraints, not all fish exposed to copper were tested for acute temperature preference.

Following completion of the temperature preference test, we calculated mean preferred temperature for each fish and a grand mean preferred temperature for each experimental group (copper-exposed vs. control). Because of isolative segregation (pseudoreplication) in the experimental design (Hurlbert 1984), inferential

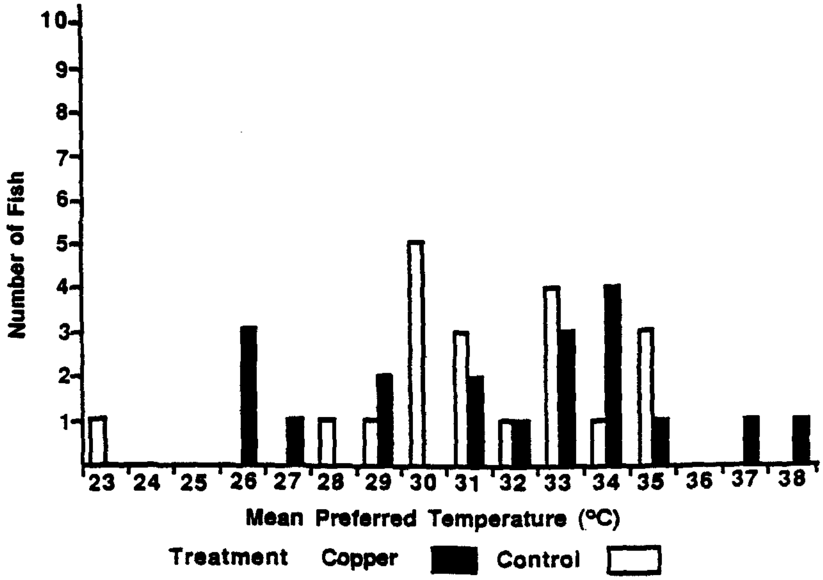

Figure 1. Mean preferred temperature distribution of Mozambique tilapia acclimated to 25°C and exposed to copper (dark bars) and control conditions (light bars).

statistics were inappropriate for data interpretation. Therefore, data were analyzed graphically.

RESULTS AND DISCUSSION

Mortality varied with acclimation temperature and toxicant exposure. There was 6.7% mortality among both copper-exposed groups at 25°C. At 35°C, one copper-exposed group suffered 13% mortality and the other 20%. All copper-exposed groups died, however, at 15°C after only 96 h of exposure. No mortalities occurred in the control groups.

Copper-exposed and control fish acclimated to 25°C preferred wide ranges of temperatures with considerable overlap between the two groups (Figure 1). The grand mean preferred temperatures were 31.7 ± 3.6°C for control fish (N=20) and 31.3 ± 2.8°C for copper-exposed fish (N=19). When fish were acclimated to 35°C, however, overlap in mean preferred temperature between copper-exposed and control groups decreased, with both groups preferring narrower temperature ranges (Figure 2). In addition, only three copper-exposed fish preferred temperatures of 34°C or greater, while control fish preferred temperatures greater than 34°C exclusively (Figure 2). Grand mean preferred temperatures of copper-exposed fish (N=19) and control fish (N=20) were 33.5 ± 1.6°C and 35.0

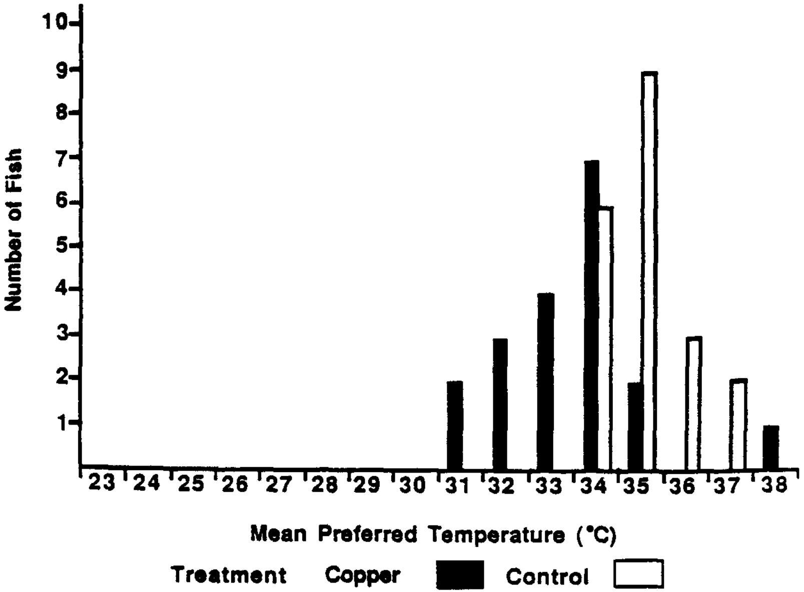

Figure 2. Mean preferred temperature distribution of Mozambique tilapia acclimated to 35°C and exposed to copper (dark bars) and control conditions (light bars).

± 0.9°C, respectively.

Temperature is a major factor influencing the effect of copper on fish. Chronic exposure of juvenile Mozambique tilapia to 0.06 mg/l cupric ion concentration at three temperatures resulted in three different responses: total mortality (15°C), low mortality (25°C), and low mortality plus behavior modification (35°C). Although it was not our intention to examine the lethal effects of copper during the acclimation period, differences in copper toxicity among acclimation groups are noteworthy because current EPA water quality criteria for copper does not take water temperature into consideration (Environmental Protection Agency 1980). The reasons for high cupric ion toxicity at 15°C are unclear. Cairns et al. (1978) found that goldfish (Carassius auratus) and channel catfish (Ictalurus punctatus) survived exposure to 3.5 mg/l total copper longer at 15°C than at 5°C. The lower lethal temperature for Mozambique tilapia has been reported to be 10°C (Lombard 1959). Perhaps, at temperatures approaching lower lethal levels, metallothionein (low molecular weight proteins capable of binding heavy metals) activity may be inhibited, although we are not aware of the existence of any data to support this.

Preference of copper-exposed tilapia acclimated to 35°C for lower temperatures than control fish may be related to a combination of copper and temperature-induced stress. An acclimation temperature of 35°C approaches the 38°C upper lethal temperature reported for this species (Allanson and Noble 1964). It is probable that, at stressful temperatures, gill efficiency may be lowered due to high gill ventilatory rates, copper-induced gill-tissue damage (Cardeilhac et al. 1979), and disruption of ionic balance across gill membranes (Stagg and Shuttleworth 1982). Opuszynski (1971) suggested, therefore, that fish exposed to copper may seek lower temperatures as refugia because lower gill efficiencies may be compensated by higher oxygen solubilities and decreased metabolic demands at lower temperatures.

We should note that although our experimental design lacked adequately replicated treatments, Stauffer (1986) conducted a similar experiment in our laboratory with tilapia from the same brood stock. He found that tilapia acclimated to 35°C preferred a mean temperature of 36°C, which is 1°C higher than the mean temperature preferred by our controls but 2.5°C higher than the mean temperature preferred by our copper-exposed tilapia acclimated to the same temperature.

Temperature is among the most important environmental parameters affecting the survival of fish. Temperature not only acts as a controlling factor (Fry 1971) governing the metabolic rates of fish, but also represents an axis of a fish's multidimensional niche (Magnuson et al. 1979). Changes in fish thermoregulatory behavior, therefore, after exposure to a contaminant may have ecological as well as physiological implications for fish populations (Beitinger and Fitzpatrick 1979).

In conclusion, acute temperature preference tests are sensitive indicators of chronic copper toxicity and may be used to detect changes in fish behavioral patterns caused by exposure to aquatic contaminants. Due to the complex relationship between temperature and copper toxicity, however, these tests should include several acclimation temperatures in order to thoroughly assess the toxicity of a contaminant. In addition, temperature preference tests meet the minimal criteria for behavioral toxicity tests suggested by Eisler (1979), in that the behavioral response pattern (preferred temperature) is important for species survival, the test is stable and reproducible, and it has a well-defined endpoint. Finally, the results are species specific (Reynolds and Casterlin 1976), economical, and require few subjective decisions on the part of the observer. Therefore, based on the results of this study, temperature preference tests can be used for establishing minimum acceptable toxicant levels.

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