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Effects of Phenol Exposure on the Thermal Tolerance Ability of the Central Stoneroller Minnow

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Recent studies have shown that environmental pollutants that exhibit lethal effects in fish may also induce physiological changes at sublethal concentrations. These changes, in turn, can affect a fish's ability to tolerate environmental stresses. For example, sublethal exposure to trace metals has been shown to reduce thermal tolerance in some fishes (Watenpaugh and Beitenger 1985). Exposure to a variety of pollutants has been reported to affect respiration and metabolism in numerous aquatic organisms (Katz 1979).

A common pollutant often released into aquatic systems as a component of complex industrial effluents is phenol. This organic compound is utilized directly or is a by-product in a number of industrial applications, including oil refineries, coke ovens, resin manufacturers, and various chemical production facilities (McKee and Wolf 1963). Phenols are also a major organic constituent in coal conversion wastewater effluents, ranging in concentration in these materials from 5,000 to 12,000 mg/L (USDOE 1981). Often, industrial effluents (especially those associated with cooling systems or coal conversion) may affect receiving water quality both chemically and thermally. The purpose of the present study was to assess the effects of sublethal phenol exposure to the thermal tolerance measured as critical thermal maxima (CTMax) of the central stoneroller minnow (Campostoma anomalum). This species is widespread and abundant throughout the central and mideastern United States and is often a major component of stream fish communities (Trautman 1981; Pflieger 1975).

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MATERIALS AND METHODS

Adult central stoneroller minnows (47.5+8.9 mm mean standard length) were collected from Harker's Run, a second-order stream in southwestern Ohio (84°42'30" W, 39°31'00" N), between October 1985 and July 1986. Fish were acclimated and tested in deionized water that was reconstituted with salts (pH=7.8, total hardness=45 mg $CaCO_3$/L, alkalinity=30 mg $CaCO_3$/L, Marking 1970) and were fed commercial trout chow every 2-3 days. Prior to phenol exposure and thermal tolerance determination, fish were acclimated at either 7.5 C (+1.0 C) or 23.0 C (+1.0 C) for a minimum of 2 wks but no more than 3 wks. Fish were maintained in glass aquaria provided with constant aeration throughout the acclimation and phenol exposure procedures, and a 12L:12D photoperiod was used throughout the study.

The acute toxicity of phenol was determined using a static renewal 48-h toxicity test (ASTM 1981). Phenol was dissolved in 20 L of aerated reconstituted water at concentrations determined from preliminary toxicity tests conducted on stoneroller minnows. Twelve fish were tested at each of five concentrations, and the test groups were matched with a single control group. During acute toxicity determination, test waters were completely replaced every 24 h. Phenol concentrations were measured at the beginning and end of each 24-h period using a 4-aminoantipyrine colorometric test (APHA 1971). Absorbance was measured with a Beckman DU-2 UV-Vis spectrophotometer (Beckman Instruments, Inc., Fullerton, CA 92634) equipped with a Gilford (Gilford Instrument Laboratories, Inc., Oberlin, OH 44074) modernization system (Model 252-2). Final toxicant concentrations were calculated by averaging the four values obtained for each test concentration. Mortality data were collected every 24 h and subjected to probit analysis (Finney 1971) to provide a 48-h LC50 estimate.

Based on the results of the 48-h LC50, four sublethal phenol concentrations were selected as exposure concentrations: 6.0, 8.0, 10.0, and 12.0 mg/L. Following acclimation to either 7.5 C or 23 C, stoneroller minnows were exposed to the phenol concentrations under conditions identical to those utilized in the 48-h toxicity test. Phenol exposed and control group fishes were tested for thermal tolerance immediately following the 48-h dosing period.

The critical thermal maximum (CTMax) as defined by Cox (1974) was used as a measure of thermal tolerance. Three to five fish from a single test group were placed into a 1-L, flat-bottomed glass bowl containing 900 mL of clean reconstituted water. The bowl was wrapped with a rheostatically controlled heating tape. The water was then heated at a rate of 1.0 C/min until the endpoint was reached. The endpoint (CTMax) was defined as that temperature at which a fish lost equilibrium and could no longer maintain an upright position. During testing, constant aeration was provided to reduce the potential of low oxygen stress, and all CTMax determinations were conducted at the same time each day to

minimize possible diel variations in thermal tolerance. Upon attaining the endpoint, the fish was returned to water at the acclimation temperature and allowed to recover. Data for fishes that did not recover was not used in the statistical analyses. Differences in mean CTMax among control and exposure groups were analyzed with ANOVA and Duncan's new multiple range test (Barr et al. 1976).

RESULTS AND DISCUSSION

The phenol 48-h LC50 for central stoneroller minnows acclimated at 23 C was 17.9 mg/L with a 95% confidence interval extending from 15.8 to 20.2 mg/L. This value is within the range of acute phenol toxicity values reported for other fish species. A 48-h LC50 of 7.5 mg/L was reported (USEPA 1973) for rainbow trout (Salmo gairdneri), and a 48-h LC50 of 47.4 mg/L was determined (Angus 1983) for mosquitofish (Gambusia affinis). Gupta et al. (1983) reported 48-h LC50 values ranging from 7.4 to 18.95 mg/L for Notopterus notopterus when tested under different dissolved oxygen levels. In the present study, no mortality was observed at or below a phenol concentration of 12.0 mg/L.

During sublethal exposure, the measured concentrations of phenol varied by no more than 6% of the nominal concentrations of 6.0, 8.0, 10.0, and 12.0 mg/L. The acute sublethal exposures resulted in a significant lowering of thermal tolerance in fish at each acclimation temperature. For fish acclimated at 7.5 C, the control group had a mean CTMax of 28.8 C. The control value did not differ significantly ($p>0.05$) from the mean values determined for the 6.0 (X=29.2 C), 8.0 (X=29.0 C), or 10.0 mg/L (X=27.9 C) exposure groups. The mean CTMax value (X=27.1 C) for the 12 mg/L exposure group did not differ significantly from the 10 mg/L group, but was significantly reduced from the values for the control and other exposure groups (Figure 1).

The mean CTMax value for control fish acclimated at 23 C was 35.8 C. No significant differences ($p>0.5$) were found among mean CTMax values for control, 6.0 (35.5 C), and 8.0 mg/L (35.3 C) test groups (Figure 1). Central stoneroller minnows exposed to a phenol concentration of 10 mg/L had a mean CTMax value (34.7 C) that did not differ from the CTMax of fish exposed to 8.0 mg/L, but was significantly lower than the values for the control and 6.0 mg/L groups ($p<0.05$). The mean CTMax value determined for the 12 mg/L test group (32.9 C) was significantly reduced from the values determined for the control and other exposure groups.

The effects of sublethal exposures of phenol on fishes have been reported by numerous investigators. These effects include lethargy, loss of equilibrium, and body deformations (Holcombe et al. 1984), desquamation of gill tissues, with epithelium lifting from basement membranes (Hawkes 1977), damage to blood vessels and extravasation of blood in gill lamellae (Waluga 1966), and increased mucus production on gills and skin (Holcombe et al. 1984).

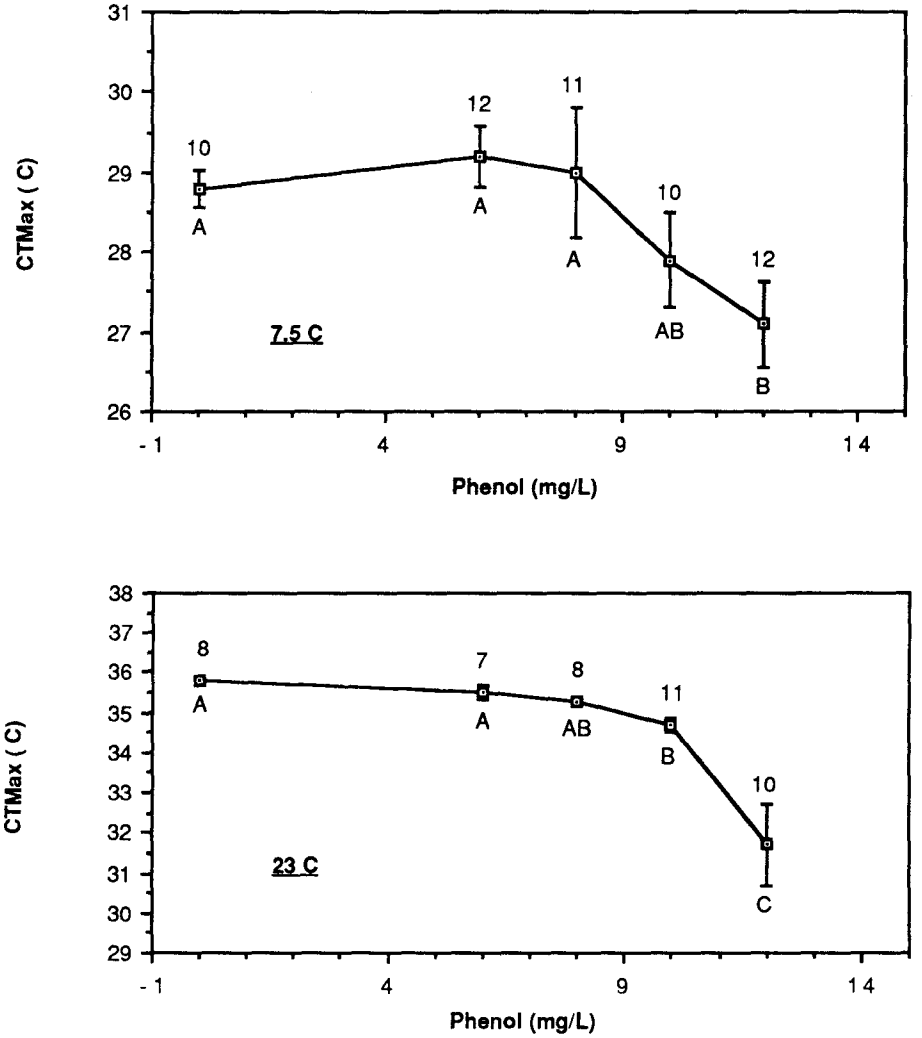

Figure 1. Critical thermal maxima (CTMax) of central stoneroller minnows (Campostoma anomalum) acutely exposed at 7.5 C and 23 C to sublethal concentrations of phenol. Points within the boxes represent mean CTMax values, and vertical lines represent the standard error of the mean. Sample sizes are given above the standard error bars for each phenol concentration tested. Within each graph, mean values that share a common letter are not significantly different (Duncan's new multiple range test, $p<0.05$, Barr et al. 1976).

The results of the present study indicate that acute exposure to sublethal concentrations of phenol acts to decrease thermal tolerance in stoneroller minnows. Temperature is important in a variety of physiological processes in fishes, such as in the structure and metabolism of cell membranes (Hazel 1984) and in

oxygen uptake associated with metabolic requirements (Ultsch et al. 1978). Because many of these temperature-related processes are dependent upon normal gill functioning, the observed decrease in thermal tolerance in the central stoneroller minnow may be a function of the impacts of sublethal phenol exposure on gill tissues.

Although the range of CTMax values exhibited by central stoneroller minnows acclimated to 7.5 C may never be encountered in winter, water temperatures at or exceeding the reduced CTMax values exhibited by the fish acclimated to 23 C and exposed to 12 mg/L of phenol may often be encountered during summer months, and also in waters receiving thermally enriched industrial effluents. Stream concentrations of phenol have been reported to exceed 30 mg/L (Angus 1983), and temperatures exceeding 30 C may regularly occur in small, unshaded streams throughout the range of this species (Tramer 1977; Matthews 1986). Thus, central stoneroller minnow habitats that normally experience high temperatures may become unsuitable when phenol concentrations are at or above 12 mg/L.

The loss of the central stoneroller minnow from stream habitats habitats may affect other, sympatric species. For example, Power and Matthews (1983) reported that the presence or absence of central stoneroller minnows strongly affected the character and standing crops of attached algal communities in stream pools. In addition, this species represents an important prey item for many piscivorous fish species (Scalet 1977). Thus, the loss of stoneroller minnows from a habitat could directly or indirectly result in faunal changes in these areas.

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