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Thermal Tolerance of Red Shiner *(Cyprinella lutrensis)* After Exposure to Atrazine, Terbufos, and Their Mixtures

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Sublethal effects of pollutants on fish species occur in response to alterations in their physiological performance (Chagnon and Hlohowskyj 1989; Watenpaugh and Beitinger 1985; Watenpaugh et al. 1985). Toxicity of most chemicals to fish has been reported to increase as temperature increases (Cairns et al. 1975; Johnson 1968). Temperature can aggravate toxicity by increasing the uptake of contaminants delivered to hypoxic tissues which ultimately affect fish thermal tolerance (Rombough and Garside 1977; Jacobs et al. 1981). Exposure to sublethal chemical concentrations can decrease thermal tolerance of fish measured by their Critical Thermal Maximum (CTM) response using the CTM method. Exposure of stoneroller minnows (Camuostoma anomalum) to phenols (Chagnon and Hlohowskyj 1989) fathead minnows (Pimephales promelas) to selenate (Watenpaugh et al. 1985) and to cadmium (Carrier and Beitinger 1988) and red shiner (Cyprinella lutrensis) to cadmium (Carrier and Beitinger 1988) and phenols (Chagnon and Hlohowskyj 1989) resulted in highly significantly reduced thermal tolerance. Thermal tolerance was reported to decrease in response to chemical impacts on gill tissues, liver tissues, and lipid depletion (Becker and Wolford 1980) resulting from increased metabolic activities (Becker and Wolford 1980; Chagnon and Hlohowskyj 1989; Watenpaugh and Beitinger 1985). CTM is recognized widely as a powerful tool to reflect the general physiological conditions of organisms in response to sublethal concentrations of contaminants and lowering resistance to hypoxia (Becker and Wolford 1980; Carrier and Beitinger 1988; Watenpaugh and Beitinger 1985; Watenpaugh et al. 1985). The CTM is defined as "the arithmetic mean of the collective thermal point at which locomotor-y activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death" (Cowles and Bogert 1944).

Matthews and Hill (1977) found that red shiner adapts well to environmental variations. However, they reported greater tolerance of this species to lower temperatures than to higher temperatures. Red shiner CTM means were reported in the range of 35.9 to 36.4°C within the American Great Plains streams (Matthews 1986) 27.8 to 33.2°C in the Platte River, and 35.7 to 38.7°C under laboratory conditions after acclimation at 22°C (Fessell 1996). Hubbs and Miller (1948) found red shiners from a warm spring in New Mexico to withstand

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temperatures as high as 39.5°C. Ambient water temperature is considered the primary factor affecting the metabolic rate and performance of fish (Jacobs et al. 1981). Since water temperature in Great Plains rivers often reach the thermal limits of red shiner (36°C) it is important to evaluate the influence of pollutants on their thermal tolerance. The objective of this study was to evaluate thermal tolerance of red shiners after exposure to sublethal concentrations of atrazine, terbufos, and their mixtures using the CTM method.

MATERIALS AND METHODS

Red shiners (C. lutrensis), 40-68 mm standard length were collected by seining from the Platte River in central Nebraska, treated with NaCl (0.00025 mg/L), brought to the laboratory, and treated with malachite green (≅ 0.1 mL/L) to reduce stress and infections. The experimental design was a split-plot. Main unit treatments of 23°C and 30°C were arranged in a randomized complete block with three blocks each. The subunit treatments were the chemical treatments plus a control (ten treatments in all) and were completely randomized within the main units. Ten fish were randomly allocated to each tank within a temperature-chemical combination. Thirty-liter glass aquaria, continuously aerated with biological sponge filters, were used to hold the fish. Fish were fed once daily with commercial food flakes and allowed to acclimate for two weeks on a 12: 12 photoperiod before the 14-d bioassays.

Fourteen day triplicate bioassays were conducted at 23°C and at 30°C after the 14-d acclimation period using 0, 10, 100, and 1000 µg atrazinen, 0, 1, 10, and 100 µg terbufos/L, and their mixtures, according to a randomized complete block design (RCBD) and a factorial treatment with temperature as the main factor. To apply the required concentrations of pesticides, stock solutions were prepared by dissolving chemicals in acetone as a solvent carrier. The controls received acetone only (1 mL/L water).

To determine the CTM, 3 fish of 10 were taken randomly from each aquarium with minimal disturbance at the end of the bioassays and placed in glass beakers tilled with water from the same tank, which was used to hold fish. Stirrers were placed in the beakers to insure uniformity of heat transfer and reduce oxygen stress throughout the water column. Hot plates with stirring were used to heat individual fish from ambient temperature at a rate of 1°C per minute, until fish became motionless and lost equilibrium. Temperature and time to the loss of equilibrium were recorded. Statistical analysis of CTMs was conducted following Dunnett's procedure to compare all treatments to the control and reflected a split-plot design by temperature in a randomized block design (three blocks per temperature). All tests were conducted at 0.05 level of significance.

RESULTS AND DISCUSSION

CTM values of red shiners unexposed to the pesticides were 36.5 (± 0.5) at 23°C

(n = 9) and 38.3 (± 1.5) at 30°C (n = 9). There was a significant interaction (P= 0.01) between temperature and pesticide exposure on CTM. Decrease of CTMs was significant when all pesticide treatments (nine treatments/block) were compared to the controls or without the controls at 23°C (n = 81) and 30°C (n = 81) (Table 1). Also, when all treatments of atrazine, terbufos, or their mixtures were compared separately to the controls at 23°C and 30°C (n = 27), fish CTMs decreased and tested significant (Table 2). There was a strong correlation between pesticide concentration and CTM response (Table 2).

Table 1. Test of significance of the critical thermal maximum (CTM) Of Cyprinella lutrensis after 14-d exposure to atrazine, terbufos, and their mixtures at 23°C and 30°C

Temperature	Treatment means excluding controls (±SE)	Null Hypothesis: Means of all pesticide treatments excluding controls are not different (n = 81 / temperature)		Treatment means including controls (±SE)	Null Hypothesis: Means of all pesticide treatments including controls are not different (n = 90 / temperature)	
		F-values	P-values		F-values	P-values
23°C	34.5 (± 0.8)	19.88	0.0001*	34.8 (± 0.6)	15.02	0.0001*
30°C	35.0 (± 1.3)	12.63	0.0005*	35.2 (± 1.3)	5.16	0.0015*

* indicates significance of all pesticide treatments combined at $\alpha = 0.05$ using Dunnett's procedure.

Table 2. Test of significance of the critical thermal maximum (CTM) of Cyprinella lutrensis of all treatments per pesticide vs. the control after 14-d exposure to atrazine, terbufos, and their mixtures at 23°C and 30°C

Pesticide treatments including control at 23°C	Mean square	F-Value	P-Value	Correlation between pesticide concentration and CTM
Atrazine vs. control	19.00083	52.01	0.0001*	0.78
Terbufos vs. control	28.83000	78.91	0.0001*	0.75
Mixtures vs. control	28.83000	78.91	0.0001*	0.58
Pesticide treatments including control at 30°C	Mean square	F-Value	P-Value	Correlation between pesticide concentration and CTM
Atrazine vs. control	84.149	9.30	0.0069*	0.80
Terbufos vs. control	51.391	5.68	0.0284*	0.83
Mixtures vs. control	91.668	10.13	0.0052*	0.98

* indicates significance of all pesticide treatments as compared to the control at $\alpha = 0.05$ using Dunnett's procedure, n = 27 per pesticide.

Generally, thermal tolerance of red shiner after exposure to atrazine, terbufos, and their mixtures decreased significantly at both temperatures, especially at the high concentrations as indicated from their CTMs. Reduction of red shiner thermal tolerance has been reported as a result of exposure to phenol at LC_5, LC_{10}, and LC_{20} (Chagron and Hlohowskyj 1989) and to cadmium at the same concentrations (Carrier and Beitinger 1988). King et al. (1985) reported red shiners in the Brazos River from north-central Texas when found closest to Moris Shippard Dam, to exhibit lower thermal tolerance which might indicate

environmental perturbation.

When each level (n = 9) of the treatments per pesticide was compared to the control, differences in CTMs were observed. The values of fish CTM were significantly reduced after exposure to all concentrations of atrazine (10, 100, and 1000 µg/L), terbufos (1, 10, and 100 µg /L), and their mixtures (1+10, 10 +100, and 100 + 1000 µg/L) at 23°C (Figure 1, 2, and 3) despite the small variability between effects, and only significant after exposure to 1000 µg atrazine/L and 100 µg terbufos/L + 1000 µg atrazine/L at 30°C (Figure 1 and 3).

Even though, CTM mean values at 30°C decreased significantly only at 1000 µg atrazine/L and at 100 µg terbufos/L + 1000 µg atrazine/L, thermal tolerance reduction was marked for all levels as compared to controls (Figure 1 and 3). Also, significance was established for the overall chemical average at both temperatures. The reason for the lack of significance at lower and medium concentrations of atrazine and the pesticide mixtures, and at all terbufos concentrations at 30°C may be attributed to either the larger variability in fish CTM responses or due to the small sample size, (n=9) per level, (n= 27) per chemical, (n= 81) for all chemicals excluding the controls, and (n=90) including the controls. Fish thermal tolerance, measured in terms of their CTMs, decreased significantly at all concentrationa at 23°C and highly significantly at the highest mixtures concentration at 30°C as opposed to each chemical separately, might indicate additive effects.

A temperature of 30°C was responsible for further lowering of C. lutrensis thermal tolerance which may indicate increased toxicity and/or lowered dissolved oxygen. The toxicity of most chemicals to fishes has been reported to increase as temperature increases (Cairns et al. 1975; Felts and Heath 1984; Johnson 1968). Possible mechanisms for this temperature effects might be increasing the absorption of contaminants delivered to hypoxic tissues or by reducing dissolved oxygen or rapid respiration, which allows further entry of toxicants (Felts and Heath 1984). Thus, red shiners and possibly other fish species are likely to be severely harmed upon exposure to environmental contaminants at higher temperatures, mostly when a habitat is characterized by low nutrition and low dissolved oxygen. The impacts of these pesticides on red shiners can be confirmed from their behavioral and histomorphological alteration, responsible for physiological dysfunction. Hence, red shiner may not be able to adapt metabolically to the stress imposed by toxicants and high temperature simultaneously. This may explain the decrease in fish thermal tolerance due to pesticide use in this study and can be attributed to: (1) impacts on red shiner gill tissues caused by multiple changes in respiratory histomorphology (Becker and Wolford 1980; Chagnon and Hlohowskyj 1989; Felts and Heath 1984; Watenpaugh et al. 1985) (2) possible hypoxia (Jacobs et al. 981) and (3) lipid consumption to adjust for the increased metabolic activities to mitigate stress caused by exposure to pollutants (Felts and Heath 1984). These mechanisms might be sufficient to explain the reduction of red shiners thermal tolerance after

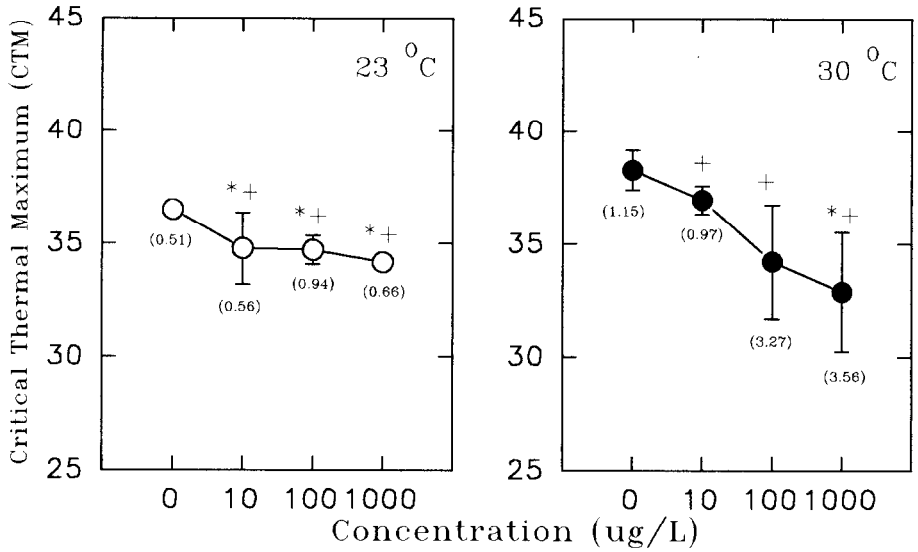

Figure 1. Thermal tolerance of Cyprinella lutrensis exposed to various atrazine concentrations at 23°C and 30°C. *significant at $\alpha = 0.05$ using Dunnett's procedure. +significant based on overall average chemical as compared to the controls, ( ) = ± SD.

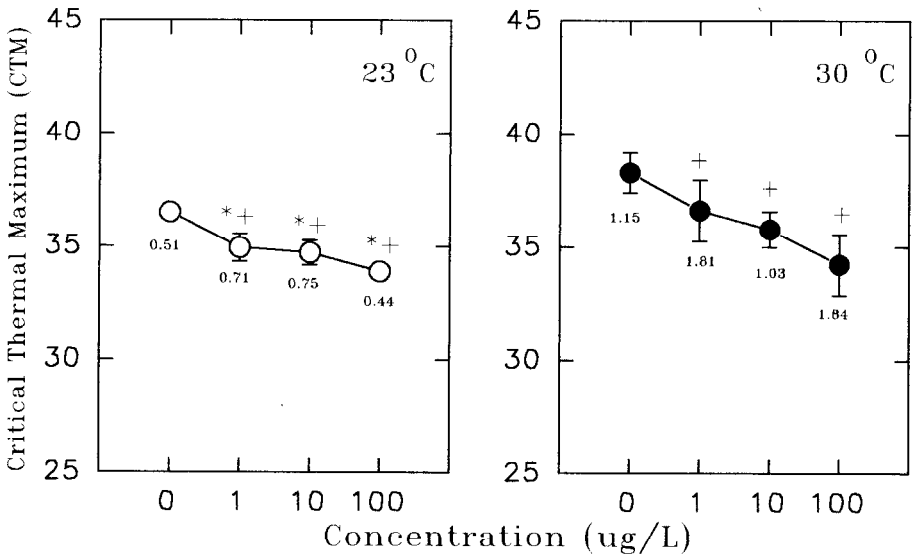

Figure 2. Thermal tolerance of Cyprinella lutrensis exposed to various terbufos levels at 23°C and 30°C. *significant at $\alpha = 0.05$ using Dunnett's procedure. +significant based on overall average chemical as compared to the controls, ( ) = ± SD.

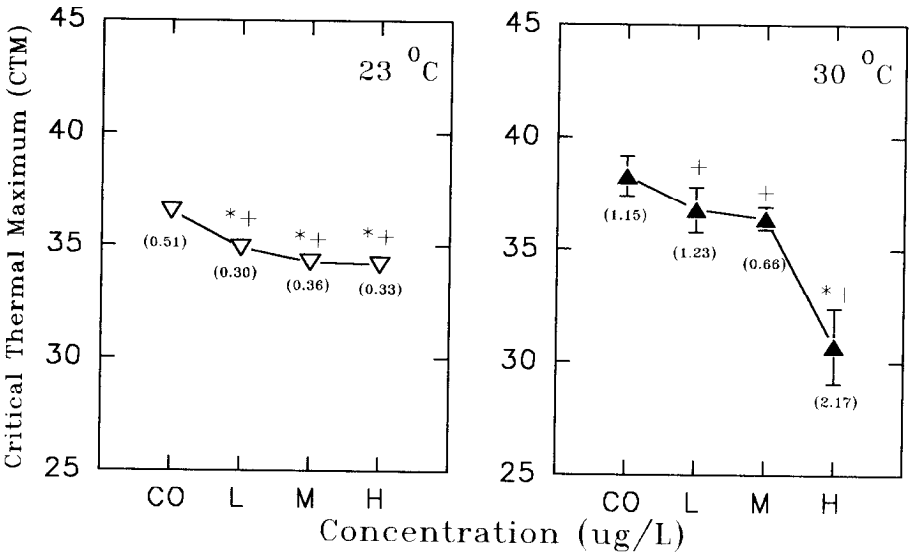

Figure 3. Thermal tolerance of Cyprinella lutrensis exposed to various atrazine plus terbufos mixture concentrations at 23°C and 30°C. CO = control, L = low mixture level, M = medium mixture level, H = high mixture level. *significant at α = 0.05 using Dunnett's procedure. +significant based on overall average chemical as compared to controls, ( ) = ± SD.

pesticides exposures in this study. Thus, if these agricultural chemicals run into aquatic habitats, fish species may perish, especially under the warm summer temperatures and long-term exposures.

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