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Effect of the Aquatic Herbicide Endothal on the Critical Thermal Maximum of Red Shiner, *Notropis lutrensis*

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The influence of temperature upon the toxicity of chemicals to species of fish has received extensive research attention. This topic has been reviewed by Cairns et al. (1975 a, b). A reciprocal interactive experimental approach is the determination of the effects of a chemical on the temperature tolerance and/or resistance of fish.

Endothal [7-oxabicyclo(2,2,1) heptane-2,3-dicarboxylic acid] is an organic contact herbicide that is used on both pre- and post-emergent stages, as well as a defoliant of cotton and other crops (Armstrong, 1974; Martin, 1964; Penwalt, 1982) . The dipotassium salt of endothal (Aquathol-K) has been successfully used to control Elodea, Ceratophyllum, and other nuisance aquatic plants (Armstrong, 1974; Pennwalt, 1982). Studies by Simsiman et al. (1976) and Pennwalt (1982) indicate that Aquathol-K, at weed control levels, 0.05 - 5.0 mg/L, has little adverse effect on the aquatic environment. Aquathol-K does not affect survival or reproduction of bluegill sunfish (Lepomis macrochirus) when present at 5 mg/L (Serns, 1977).

Aquatic plant control is an important problem in the southern United States. The U. S. Army Corps of Engineers currently plan to use Aquathol-K to control Eurasian watermilfoil (Myriophyllum spicatum) in Pat Mayse lake (north of Paris, Texas). Since southern lakes and reservoirs receive large amounts of solar radiation and achieve high surface water temperatures, the effect of endothal on thermal tolerances of aquatic organisms is an environmentally relevant question. The objective of this study was to determine if the ability of the red shiner (Notropis lutrensis) to tolerate high temperatures is affected by prior exposure to Aquathol-K. The critical thermal maximum (CTM) method was used to quantify temperature tolerance. The CTM test which is both a parameter and a method (Becker and Genoway, 1979) was developed by Cowles and Bogert (1944) with more recent revisions provided by Lowe and Vance (1955), Hutchison (1961), Cox (1974) and Becker and Genoway (1979). The CTM reflects an animal's ability to adjust to a progressive increase in ambient temperature. In their recent review Paladino et al. (1980) conclude that the CTM approach is a powerful tool for studying the physiology of stress and adaptation in fish.

MATERIALS AND METHODS

Red shiners (Notropis lutrensis) captured by seining in Denton County, Texas, were brought to the laboratory and separated into three groups. Each group was placed into a 19-L all-glass aquarium with continuous aeration and charcoal filtration. The three holding aquaria were placed in a 75-L water bath maintained at 22 C $\pm$ 0.1 C by two circulating 1000 w thermoregulators, and a photoperiod cycle of LD 12:12. During a two-week acclimation period, water temperatures were measured daily while dissolved oxygen, ammonia and pH were measured on alternate days. These parameters were determined using a calibrated mercury thermometer, Winkler-calibrated Yellow Springs Instruments oxygen meter (0.1 mg O_2/L sensitivity), Corning specific ion meter (0.001 mg NH_3 - N/L sensitivity) and a Beckman Expandomatic pH meter (0.1 pH unit sensitivity), respectively. Fish were fed daily with pulverized trout chow in amounts that could be consumed within five minutes. Uneaten food was removed.

After the 2-week acclimation period, dilute solutions of Aquathol-K were added to two holding aquaria to obtain initial nominal concentrations of 1 and 30 mg/L acid equivalents (a.e.) of endothal. The third aquarium which contained no endothal, served as a control. The fish were exposed to the endothal for 48-h. Water samples were taken at the beginning and end of the exposure period for bacterial (1% PCA - Standard Methods, 1980) and endothal analyses. The initial and final endothal concentrations in the treatment aquaria were determined by the extraction procedure of Sikka and Rice (1973) with subsequent analyses using a Hewlett-Packard 5710A Gas Chromatograph (N-P detector) and 3390-A Integrator with 0.001 mg/L endothal-imide (a.e.) sensitivity.

Twelve fish were randomly selected from each holding aquarium for CTM determinations. Six fish were tested per trial, one in each of six separate chambers within the CTM test apparatus. The water was gently aerated and thermally controlled with two circulating thermoregulators (Haake Model E52). Starting at the acclimation temperature (22 C), temperature was increased at a constant rate of 0.3 C/min until a CTM response was observed in all six fish. The selected CTM endpoint criterion was the temperature at which a fish lost its righting ability. Temperature in the test chamber was measured with a Digitec 5810 thermometer (0.01 C sensitivity). A 2-factor ANOVA was employed to determine if observed CTM values were significantly affected (α = 0.05) by endothal exposure, fish length or their interaction.

RESULTS

Dissolved oxygen, pH and ammonia, given as arithmetic ranges in Table 1, did not vary significantly among the three groups (one-way ANOVA for each parameter, all $p > 0.05$).

TABLE 1. Arithmetic ranges of three water quality parameters measured at 1, 5, 7, 10, 12 and 14 days of the acclimation period in the control and two exposure groups. Oxygen and ammonia are in mg/L.

		Endothal Exposures	
Parameter	Control	1 mg/L	30 mg/L
Oxygen	7.1 to 9.0	6.5 to 9.2	7.9 to 8.9
pH	7.6 to 8.3	7.0 to 8.2	7.8 to 8.3
Ammonia	0.006 to 0.241	0.006 to 0.322	0.007 to 0.230

Similarly we found no major differences in bacterial counts among the three groups. Endothal concentrations were close to nominal values at the beginning of the exposure period (Table 2); however, at the conclusion of the 48-hr exposures, endothal concentrations decreased by approximately 34% in both aquaria.

TABLE 2. Endothal concentrations at the beginning and end of the 48-hr exposure period

	Endothal Concentration (mg/L)		
Group	Initial	Final	% lost
Control	0.000	0.000	--
1 mg/L	0.974	0.642	34.2
30 mg/L	30.980	10.656	34.4

Mean total lengths of the 12 fish from each group selected for CTM trials varied from 33.3 to 34.5 mm and were not significantly different (ANOVA, $p > 0.05$). More importantly, our 2-factor ANOVA clearly demonstrated that the CTM was not affected by endothal exposure, fish total length or interaction between endothal concentration and fish length (all $p > 0.05$). Not only are the group means and standard deviations for our CTM determinations remarkably similar (Table 3), these data are exceptionally consistent. The standard deviation expressed as a percentage of its respective mean (i.e. coefficient of variation or C.V.) ranged from only 1.25 to 1.35% (Table 3).

TABLE 3. Critical thermal maximum of N. lutrensis exposed to endothal

		CTM (C)		
Group	N	$\bar{X}$	S.D.	C.V.*
Control	12	36.18	0.49	1.35
1 mg/L	12	35.81	0.46	1.33
30 mg/L	12	35.94	0.45	1.25

* = coefficient of variation

DISCUSSION

Few studies have examined the effects of toxic chemicals on the CTM of fish. Possibly the first research of this type was conducted by Silbergeld in 1973. Etheostoma nigrum pretreated with 2.3 ug/L of dieldrin had significantly higher mortality rates when heated at 1 C/h than untreated fish. Paladino and Spotila (1978) found that exposure to sublethal concentrations of arsenic produced a pronounced decline in the CTM of swim-up muskellunge fry, Esox masquinongy. Also, mean CTM for rainbow trout, Salmo gairdneri, was significantly decreased by sublethal amounts of water-borne nickel, as low as 1.5 mg/L (Becker and Wolford, 1980). The results of these studies support a hypothesis that the stress of exposure to toxicants decreases the ability of a fish to withstand the additional stress of increasing ambient temperature.

In contrast, our research clearly demonstrated that endothal, as Aquathol-K, does not significantly alter the CTM of Notropis lutrensis at concentrations of 1 mg/L and 30 mg/L acid equivalence. The 1 mg/L test concentration is well within the range of use for aquatic weed control, and the 30 mg/L test concentration was six times the highest recommended concentration for aquatic use (Martin, 1964; Pennwalt, 1982). We conclude that Aquathol-K does not significantly affect the CTM of red shiners within the range of concentrations recommended for control of aquatic weeds. Results for endothal are similar to those for tetracycline hydrochloride. Schubauer et al. (1979) reported that exposure to this antibiotic did not change the CTM of treated fish, Notropis cornutus, relative to controls.

The mean CTM of 36.0 C for red shiner from our combined data (N = 36) is slightly lower than reported values of 38.99 C (Matthews and Maness, 1979) and 37.8 to 38.0 C (King et al. in review). In both of these studies, red shiners were acclimated to 25 C instead of 22 C. It has been well documented that increases in CTM accompany increases in acclimation temperature for many vertebrate species including fish (e.g. Hutchison 1976, Hassan and Spotila 1976, Kaya 1978; Lee and Rinne 1980).

Also, a difference in the selected endpoint criteria helps account for the greater disparity between our CTM value and that of Matthews and Maness (1970). The loss of righting response (our endpoint) occurs prior to the onset of muscular spasms (endpoint of Matthews and Maness, 1979); consequently our approach would yield lower CTM values.

Since endothal is usually degraded within one to five days by microbial activity (Pennwalt 1982), it has low persistence in aquatic environments. Also, endothal is lethal to nontarget species such as fish at relatively high concentrations. Surber and Pickering (1962) and Davis and Hughes (1963) have reported 24-h LC-50 values of 320 to 450 mg/L for bluegill (Lepomis macrochirus). In addition to these desirable characteristics, endothal even at a concentration six-fold greater than maximum recommended for aquatic use, does not reduce the ability of red shiners to resist high temperatures.

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REFERENCES

ARMSTRONG, J. G.: U. S. Dept. Comm. PB-235 447 (1974).
BECKER, C. D. and R. G. GENOWAY: Env. Biol. Fish. 4:245 (1979).
BECKER, C. D. and M. G. WOLFORD: Environ. Pollut. Ser. A.21: 181 (1980).
CAIRNS, J. Jr., A. G. HEATH and B. C. PARKER: Hydrobiologia 47:267 (1975).
CAIRNS, J. JR., A. G. HEATH and B. C. PARKER: J. Water Poll. Control Fed. 47: 1464 (1975).
COWLES, R. B. and C. M. BOGERT: Bull. Amer. Mus. Nat. Hist. 83:265 (1944).
COX, D. K.: In: J. W. Gibbons and R. R. Sharitz (eds.) Thermal Ecology (1974).
DAVIS, J. T. and J. S. HUGHES: S. Weed Conf. Proc. 16:337 (1963).
HASSAN, K. C. and J. R. SPOTILA: In: G. W. Esch and R. W. McFarlane (eds.) Thermal Ecology II (1976).
HUTCHISON, V.E.: Physiol. Zool. 34:97 (1961).
HUTCHISON, V.E.: In: G. W. Esch and R. W. McFarlane (eds.) Thermal Ecology II (1976).
KAYA, C. M.: Prog. Fish-Cult. 40:138 (1978).
KING, T. L., E. G. ZIMMERMAN and T. L. BEITINGER: in review.
LEE, R. M. and J. N. RINNE: Trans. Amer. Fish. Soc. 109:632 (1980).
LOWE, C. H. Jr., and V. J. VANCE: Science 122:73 (1955).
MARTIN, H.: Pesticide Manual. 2nd Ed. British Crop Protection Council. (1964).

MATTHEWS, W. J. and J. D. MANESS: Amer. Midl. Nat. 102:374 (1979).
PALADINO, F. V. and J. R. SPOTILA: J. Thermal Biol. 3:223 (1978).
PALADINO, F. V., J. R. SPOTILA, J. P. SCHUBAUER and K. T. KOWALSKI: Rev. Can. Biol. 39:115 (1980).
PENNWALT Corporation: Sales literature, Tacoma, Wash. (1982).
SCHUBAUER, J. P., C. L. SCOTT, K. T. KOWALSKI and J. R. SPOTILA: Prog. Fish-Cult. 42:48 (1980).
SERNS, S. L.: Water Res. Bull. 13:71 (1977).
SIKKA, H. C. and C. P. RICE: J. Agr. Food Chem. 21:842 (1973).
SILBERGELD, E.L.: Environ. Sci. Tech. 7:846 (1973).
SIMSIMAN, G. V., T. C. DANIEL and G. CHESTERS: Res. Rev. 62: 116 (1976).
Standard Methods for the Examination of Water and Wastewater. 15th ed. 1134 p. (1980).
SURBER, E. W. and Q. H. PICKERING: Prog. Fish-Cult. 24:164 (1962).

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