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Total Residual Chlorine: The Effect of Short-Term Exposure on the Emerald Shiner *Notropis atherinoides* (Rafinesque)

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Chlorine is used extensively for the antifouling of cooling systems and as a bacteriocide in waste-water treatment. Spent chlorine is usually discharged into nearby lakes and streams. Aquatic organisms near discharge areas are then subjected to intermittent chlorine exposure, normally toxic over prolonged periods. Civil engineers report that several mg/ℓ free chlorine must be maintained for 30 min for effective wastewater treatment (PIKE 1971). As a result, chlorine is often used in excess and, contrary to previous beliefs, chlorine is not readily converted to non-toxic anions by natural waters (BRUNGS 1976). Frequently total residual chlorine (TRC) concentrations in industrial discharges are both high and persistent. Although the use of chlorine as an antifouling agent is an intermittent procedure, aquatic organisms cannot anticipate this periodic treatment.

Chlorine is often used for wastewater treatment at concentrations of 0.5 to 1.0 mg/ℓ (BRUNGS 1973). These chlorine levels are toxic to aquatic organisms when discharged into natural systems (SPRAGUE & DRURY 1969, ZILLICH 1972, BRUNGS 1973, DICKSON et al. 1974).

This study investigates the toxic effects of short-term chlorine exposure or "chlorine shock" on the emerald shiner, Notropis atherinoides (Rafinesque).

Antifouling with chlorine is often associated with cooling systems and elevated water temperatures. This study focused on the effects of chlorine at two temperatures, 10 and 25°C. These temperatures were selected to determine if the effect of chlorine varied within the range of temperatures typically observed below effluent discharges. Specific aspects considered are the influence of size and age of the fish with respect to chlorine toxicity.

MATERIALS AND METHODS

The total residual chlorine (TRC) exposure study was conducted at the EPA National Environmental Research Laboratory, Duluth, Minnesota. A test apparatus was devised in which fish could be exposed to a slug dose of chlorine for a 30 min period.

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The apparatus, consisting of nine glass aquaria, was provided with a flow-through water system. Temperature was regulated to ±0.5°C by constant temperature water baths. A marriote bottle drip component was used to meter chlorine into the tanks.

Lake Superior water was used for the bioassays. Characteristics of the test water are provided by BIESINGER & CHRISTENSEN (1972). A sodium hypochlorite solution was used as the source of TRC.

The test organisms, Lake Superior emerald shiners, were obtained from a commercial bait dealer and included young-of-the-year, yearling and adult fish. Fish of each age were held separately in stock tanks where 10 and 25°C temperature acclimation was established for a minimum of 30 days prior to testing. All the fish were maintained on a diet of frozen brine shrimp and fed daily between 7:30 and 8:30 a.m. Food remaining after five min was considered excess and removed. All of the fish tanks were thoroughly cleaned as dictated by water conditions, and periodic prophylactic treatment with formalin and antibiotic was administered to the fish stock. TRC exposure tests were not run with fish subjected to chemical disease control until two weeks after treatment.

Young-of-the-year, yearling and adult emerald shiners were subjected to a 30 min TRC exposure at acclimation temperatures of 10 and 25°C The procedure for TRC exposure included four different phases:

Acclimation Phase: Fish of an age group were arbitrarily selected from stock tanks and placed in one of the test chambers. Tests were conducted with 10 adult or yearling fish, whereas 20 young-of-the-year fish were used per test. Fish of unusual size were not used and individuals that escaped once the acclimation phase began were not replaced. The fish were acclimated to the equipment for 24 h and not fed during this time.

Shock Phase: A predetermined volume of a sodium hypochlorite solution was added to the exposure test aquarium. Water flow and a continuous drip of the sodium hypochlorite solution were delivered at a known rate to maintain a relatively constant TRC concentration for a 30 min period. Preliminary checks were conducted to assure that the TRC concentration was constant throughout the test aquarium. Amperometric titration with a Sargent model XV polarograph was used to monitor TRC concentration during the shock phase and through the subsequent dilution procedure at 5 to 7 min intervals. A mean TRC concentration was calculated for the shock phase.

Dilution Phase: After the shock period sodium hypochlorite solution drip was discontinued and water flow increased. TRC was reduced to an undetectable level within 45 min of the beginning of this phase.

Observation Phase: Observations were made on responses of the fish throughout each test. After 24 h dead organisms were removed and measured for length and weight. The remaining fish were transferred to holding aquaria. Here they were fed daily and monitored for additional mortalities. At the end of the test, 96 h from the initial shock phase, surviving fish were sacrificed and measured for weight and length. Survivors of several tests were kept for 18 days to monitor delayed mortalities.

Control tests were performed utilizing ten fish per test. In these tests dilution water was added in place of a sodium hypochlorite solution. All other procedures were identical to those previously described.

RESULTS AND DISCUSSION

Probit analysis of the chlorine shock test results were performed using the method described by FINNEY (1971). Tests involving equipment failures, supersaturation and repeated 0% and 100% mortalities were omitted from the analysis. Figure 1 (probit analysis) presents for visual comparison the total residual chlorine based mortality for fish of the three age categories tested at 10 and 25°C.

The 95% confidence limits of the LC-50 values do not overlap between fish groups tested at 10 and 25°C (Table 1), nor are there any data points that overlap (Figure 1). Thus, TRC toxicity is significantly different at 10 and 25°C and the fish are approximately three times more sensitive at the higher temperature. CAIRNS et al. (1975 a & b) who believe mortality is due to asphyxia hypothesize that an increase in temperature would increase the respiratory demand for oxygen and would probably increase the toxicity of chlorine. Other work dealing with temperature and chlorine toxicity suggests a similar relationship. SCHNEIDER et al. (1974) working with brook trout found an increase in toxicity at 20°C. However, no increase was observed between 10 and 15°C. In contrast, DICKSON et al. (1977) working with goldfish at 14.5 and 20°C did not find temperature to influence toxicity significantly. It may be that tests at greater temperature extremes would have resulted in greater toxicity differences. Apparently, the range of temperatures selected for different species is critical (BROOKS & SEEGERT 1975).

Table 1 also represents the mean total and range of fish lengths for each group of fish studied. The fish length data were grouped into 5 mm size classes and presented as a percentage of the number of mortalities and the number of fish surviving. The T-test, method of paired comparisons (BAILEY 1959) indicates that the deviation of percent mortality from percent surviving within each size class is not significantly different from zero. In addition, the 95% confidence limits, calculated

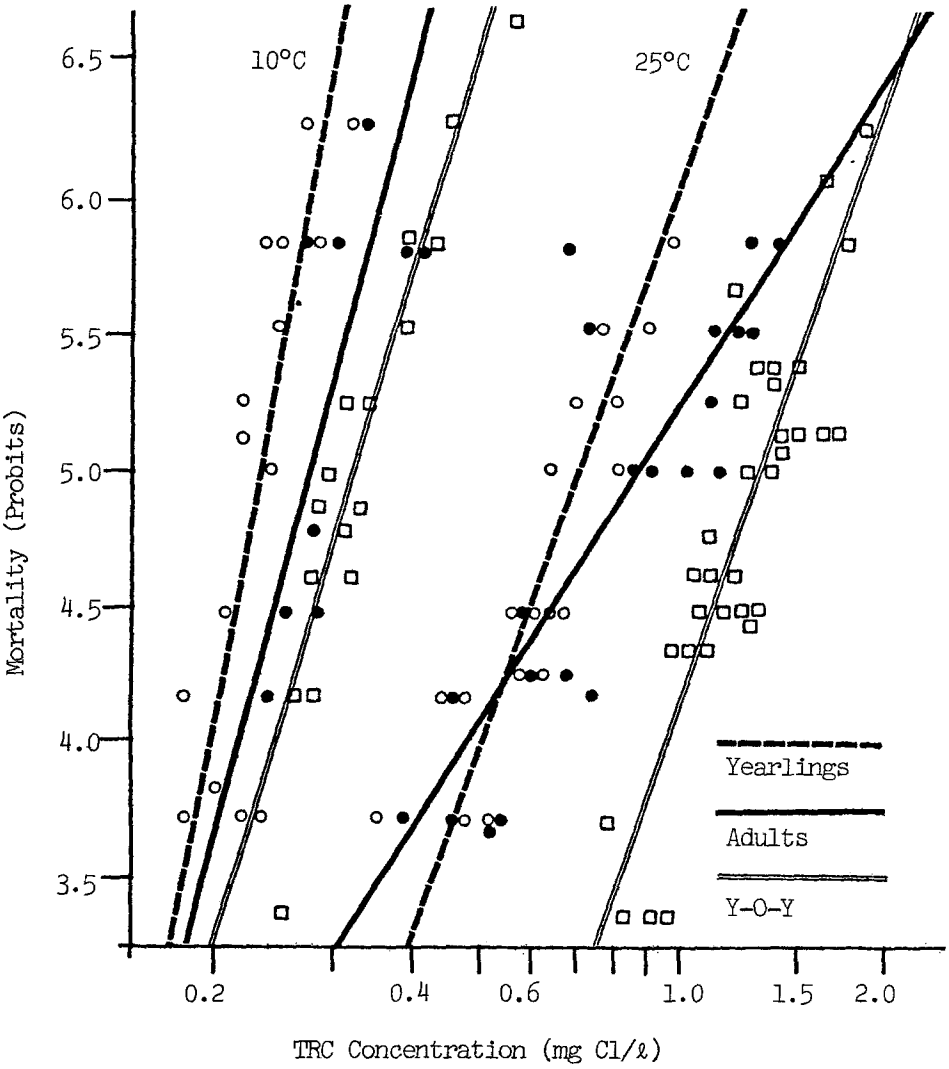

Figure 1. Mortality-probit analysis of emerald shiners (Notropis atherionoides) exposed to a 30 min total residual chlorine pulse.

by the method of MOSIMANN (1965), of percent mortality within each size class, overlap. Thus, TRC toxicity did not vary within the size classes tested, nor within the size ranges studied for young-of-the-year, yearling or adult emerald shiners. Similar results were reported by DICKSON et al. (1977) using Carrasius auratus as a test species.

A comparison of TRC toxicity between young-of-the-year, yearling and adult emerald shiners was made (Figure 1). The 95% confidence limits of the LC-50 values for yearling and adult fish overlap at 25°C. It was concluded that no significant difference in TRC toxicity could be detected. Young-of-the-year

TABLE 1

Characteristics of test organisms, Notropis atherinoides (Rafinesque), and the effects of a 30 minute total residual chlorine exposure at 10 and 25°C.

Fish Characteristics				Probit Analysis[1]			
		Fish Length			96 hr		
Age	N.	Ave. mm	Range mm	Temp.	LC-50 mg/ℓ	Confidence Limits	Chi-square
Y-O-Y	1217	43.2	39-52	10°C	1.32	1.27-1.36 mg/ℓ	0.31
				25°C	0.33	0.32-0.34	0.72
Yearlings	507	56.9	40-74	10°C	0.71	0.67-0.77	0.89
				25°C	0.23	0.22-0.24	0.15
Adults	500	85.6	70-115	10°C	0.87	0.77-1.01	0.05
				25°C	0.28	0.23-0.31	0.01

1. Tests involving equipment failures, supersaturation and repeated 0% and 100% mortalities were omitted from the analysis.

95% confidence limits do not overlap with either the yearling or adult LC-50 value limits at 25°C. It appears that young-of-the-year fish are slightly more resistant to TRC toxicity than yearling or adult fish. However, some of the yearling and adult data points overlap with the young-of-the-year data. The difference in observed TRC toxicity between young-of-the-year fish and yearling and adult fish is at best small.

The trends observed in TRC toxicity at 25°C were also evident at 10°C. The lower temperature, however, accentuated the differences. The 95% confidence limits of neither the young-of-the-year, yearling nor the adults overlap. As in the 25°C tests, the LC-50 values of the yearling and adults are similar but distinctly different from the LC-50 value of the young-of-the-year fish.

With the present testing procedure it cannot be determined whether the differences observed here are due to a size or age effect or a combination of both. Although the physiological effects of chlorine on fish is not well understood, the literature does indicate that the blood and gills are involved. Indeed BROOKS & SEEGERT (1975) suggest a multimode or site theory as possible mechanisms for the toxic action of chlorine. This makes the preceeding results even more difficult to interpret.

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