Toxicity of Intermittent Chlorination to Bluegill, *Lepomis macrochirus*: Interaction with Temperature

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Introduction

Treatment of water by chlorination is undertaken for two reasons: protection of the public health and antifouling in industry (BRUNGS, 1973). The primary impetus for the work reported here came from this second use of chlorine.

Steam-electric generating plants use chlorine to reduce slime in the condensers, permitting more efficient cooling behind the turbine thereby yielding a greater generating potential. Chlorine is added to the cooling water to maintain a residual chlorine concentration of about 0.5-1.0 mg/l at the condenser. In many plants water goes from the condenser to the cooling towers where further cooling takes place before the water is recirculated (DRALEY, 1972).

There is considerable evaporation of water from the cooling towers which tends to concentrate suspended material in the water. Periodically (2 to 4 times daily), cooling-tower blowdown is performed to wash out the accumulated solids and this chlorinated water is released into a stream or lake. Thus, the biota in the receiving water is exposed to "pulses" of residual chlorine rather than a constant concentration.

Because most of the bioassay data in the literature was determined from continuous exposure to chlorine, they are not readily applicable to the normal intermittent release of chlorine by some industries. Furthermore, CAIRNS, *et al*. (1975) reviewed the literature on the effects of temperature to toxicity of many substances and found information on chlorine lacking.

The objectives of this study were to determine the following: (1) the concentrations of chlorine that are lethal to bluegill sunfish (*Lepomis macrochirus*) exposed intermittently in the laboratory, and (2) the effect that water temperature has on the lethality of chlorine to this species.

Methods and Materials

Juvenile bluegill of 4.6 $\pm$ 0.02 cm (mean $\pm$ S. E.) average length and 3.91 $\pm$ 0.11 g average weight were obtained from the King and Queen Virginia State Hatchery and were treated with oxytetracycline as a prophylatic. They were fed ground Purina Trout Developer daily.

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For convenience the bluegill were maintained in laboratory holding tanks that were not temperature controlled (range 12-20 C). It is important to note that the fish were tested at the colder temperatures when the holding temperature was near the lower end of the range and tested at the warmer temperatures when the holding tank temperature was near the upper end of the range.

Ten bluegill, which had been acclimated in 190-liter aquaria to each test temperature (6, 15, 25, and 32 C) for a minimum of 2 weeks, were placed in each bioassay container and further acclimated for 48 h before chlorine exposure. The fish were not fed for 24 h prior to or during the test.

A laboratory system (Figure 1) was built to simulate the pattern of change in chlorine concentration of water below a steam-electric generating plant as described by DICKSON et al. (1974). Chlorine, as calcium hypochlorite, was introduced to the bioassay system in an intermittent pulsing manner. Blacksburg tap water was dechlorinated by passage through activated charcoal filters to produce dilution water. Dechlorinated dilution water flowed into ten 25-liter glass aquaria on a continuous flow basis throughout the experiment. The water flow was monitored using size 5 Gilson flowmeters, and was controlled with tension tubing clamps. Flow rates of 560 ml/min, which allowed 99% turnover of the water in each aquarium in approximately 180 minutes, were maintained.

Chlorine was introduced into the dilution water using timer-controlled model 50 Harvard metering pumps for 45 minutes three times daily, at 0700 h, 1500 h and 2300 h for 7 days (Figure 2). Chlorine was added to achieve peaks of total residual chlorine concentration of 0.21, 0.31, 0.41 and 0.52 mg/l, respectively, in each of 4 aquarium at the temperatures used in the test. Both free and total chlorine peaks were monitored by amperometric titration (Standard Methods, 1973) using a Wallace and Tiernan model A790 titrator. Chlorine was monitored three times daily during the first 3 days of each 7 day experimental run, and once or twice daily during the remaining 4 days of the experiment. Four replicate bioassays were run at each temperature. A summary of the water quality data monitored during the experiments is given in Table I. Two statistical parameters were obtained in the time-until-death analysis. The first was the median lethal time (LT_{50}) which is the time required for the death of 50% of the test organisms at a specific concentration of chlorine. The second was the median lethal concentration (LC_{50}) which is the concentration at which the death of 50% of the test organisms occurs. The LC_{50} is associated with a specific time interval.

The LC_{50} and the LT_{50} were calculated by the probit analysis methods first proposed by BLISS (1935, 1937). In the LT_{50} analyses, resistance time in minutes was converted to $\log_{10}$ and a regression was determined on percent mortality converted to probit. In the LC_{50} analyses, each concentration was converted to $\log_{10}$ and regressed on percent mortality as probit. The LT50 and LC50 data were computed with a program written by Dr. Ford Calhoun (personal communication) from FINNEY'S (1971) equations.

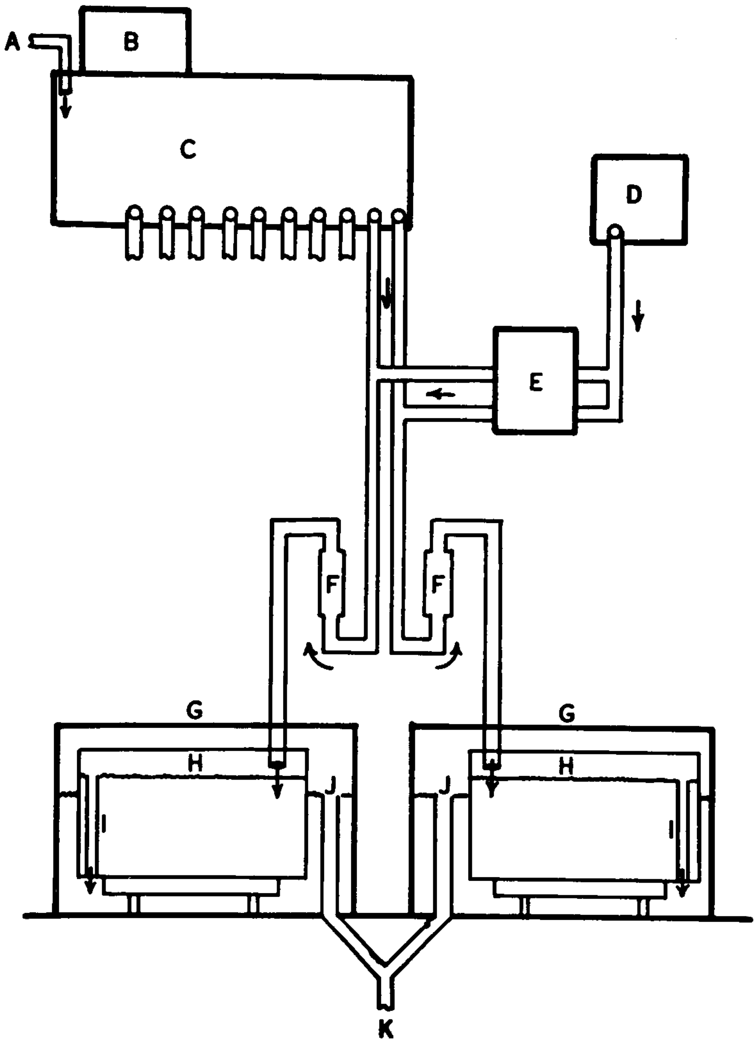

Figure 1. Diagram of bioassay system used for intermittent exposure to chlorine: A. dechlorinated tapwater source for dilution water; B. temperature control; C. dilution water reservoir; D. chlorine stock reservoir; E. metering pump; F. flowmeters; G. water baths for temperature control; H. bioassay aquaria; I. standpipes for bioassay aquaria; J. standpipe for water bath; K. drain for bioassay system.

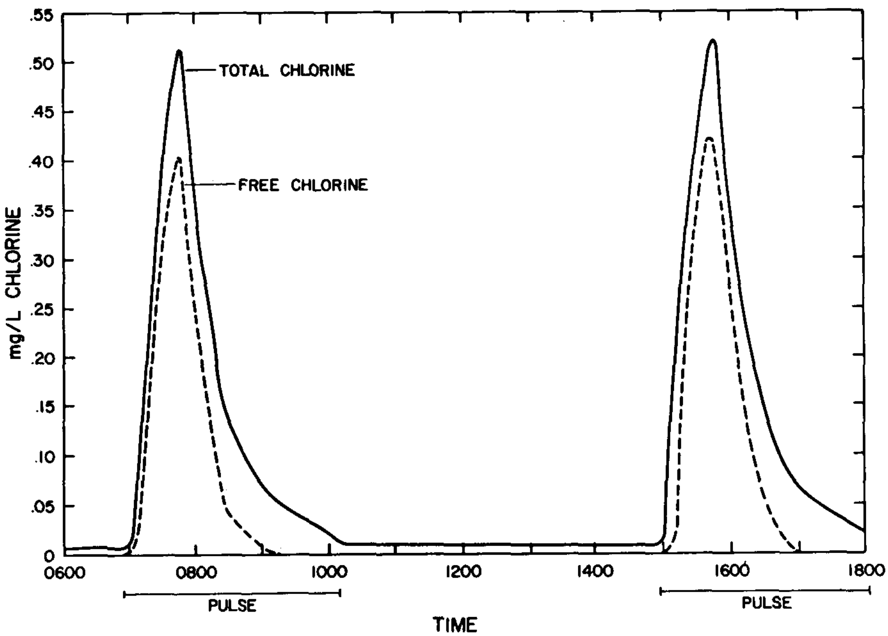

Figure 2. Example of two successive chlorine pulses at the highest concentration (0.52 mg/l total chlorine and 0.41 mg/l free residual chlorine).

Table I. Water Quality Parameters for Dilution Water

Total Hardness:	41.5-46.3 mg/l
Alkalinity:	42-45 mg/l $CaCO_3$
pH:	7.25-7.55 units
Zinc:	0-0.007 mg/l
Copper:	undetectable
Conductivity:	99-110 μMHO

Moribund and surviving fish were taken from the bioassay containers, sized for weight and length and prepared for histological studies reported on separately (BASS, BERRY, and HEATH, in preparation).

Results

No deaths occurred in control tanks at any of the four temperatures used. Likewise, no deaths occurred at any of the four experimental temperatures at 0.21 mg/l peak total chlorine, although these fish did appear to be in distress during the chlorination pulse at the two highest temperatures and especially at 32 C. These fish were near the surface and were showing a definite coughing reflex during the pulse. They also were less responsive to the observer's presence than those fish exposed to chlorine at lower temperatures. Less than 15% mortality occurred at 0.31 mg/l peak total chlorine with the highest mortality observed at 6 C. Some bluegills exposed to 0.42 or 0.52 mg/l peak total chlorine became lighter in color during and after exposure, and they died shortly thereafter. This was infrequently observed.

The mean total chlorine in Table II refers to the mean concentration for the entire experimental period. The peak free and total chlorine concentrations were measured at the maximum in the aquaria during the intermittent pulse (refer to Figure 2). A notable temperature effect was found at a peak total chlorine concentration of 0.52 mg/l where the LT_{50} was 74 h at 6 C and respectively decreased to about 20 h at 32 C. These LT_{50} values correspond to the free chlorine concentrations of 0.44 and 0.39 mg/l, respectively (Table II). This shows that the free chlorine constituted approximately 85% of the peak total chlorine at 6 C while it was only 75% of the peak total chlorine at 32 C.

There appears to be little temperature effect on the lethal concentration (Table III). The 96 h LC_{50} was approximately 0.40-0.45 mg/l peak total chlorine at all four temperatures. Extending the exposure time to 168 h (7 days) does not significantly reduce the LC_{50} except at 6 C where it is lowered to 0.33 mg/l.

Discussion

Although there were no deaths at 0.2 mg/l peak total chlorine, the behavior of the fish exposed at the two highest temperatures definitely indicated that they were under stress. The threshold of 0.3 mg/l peak total chlorine observed in this study, at which chlorine becomes toxic to bluegills, is rather distinct. This is above the 0.2 mg/l limit proposed by BRUNGS (1973) for situations where warm-water fish may be exposed intermittently to residual chlorine. The color changes observed in some of the test bluegill, and subsequent mortality was not observed frequently enough to be stated as a general pattern.

TABLE II.

Average Median Lethal Time (LT_{50}) of Four Replications for Juvenile Bluegill at Different Concentrations of Residual Chlorine and Four Temperatures.

Temp. (C)	Mean Total Chlorine (mg/l)	Peak Free Chlorine (mg/l)	Peak Total Chlorine (mg/l)	LT_{50} (h)	95% Confidence Interval (h)
6	0.073	0.440	0.516	74.0	75.3-72.7
6	0.060	0.375	0.420	105.6	107.6-103.9
6	0.045	0.265	0.307	181.1	201.0-171.8
6	0.037	0.165	0.210	0	0
15	0.074	0.425	0.524	62.4	63.3-61.5
15	0.060	0.310	0.416	162.9	174.9-154.1
15	0.045	0.185	0.310	*	*
15	0.037	0.140	0.280	0	0
25	0.074	0.415	0.520	44.7	46.3-43.1
25	0.060	0.300	0.415	72.2	77.7-67.5
25	0.045	0.205	0.312	134.4	212.5-34.5
25	0.038	0.130	0.212	0	0
32	0.074	0.390	0.523	19.9	21.1-18.7
32	0.060	0.290	0.417	*	*
32	0.046	0.195	0.311	*	*
32	0.037	0.110	0.211	0	0

*Insufficient data to calculate
0 No mortality

TABLE III.
Average Median Lethal Concentration (LC_{50}) of Total Residual Chlorine for Juvenile Bluegill at Four Temperatures.

Temp. (C)	Time (h)	Mean Total LC_{50} (mg/l)	95% Confidence Interval	Peak Total LC_{50} (mg/l)	95% Confidence Interval
6	72	0.075	0.086-0.071	0.532	0.611-0.500
6	96	0.064	0.067-0.062	0.452	0.472-0.429
6	168	0.048	0.050-0.045	0.331	0.348-0.312
15	96	0.063	0.066-0.061	0.443	0.461-0.426
15	168	0.060	0.062-0.057	0.415	0.432-0.348
25	48	0.076	0.097-0.069	0.539	0.693-0.483
25	72	0.059	0.063-0.056	0.410	0.436-0.385
25	96	0.057	0.060-0.054	0.392	0.415-0.369
25	168	0.054	0.057-0.052	0.373	0.391-0.354
32	24	0.071	0.075-0.068	0.501	0.533-0.478
32	48	0.067	0.069-0.065	0.470	0.487-0.453
32	72	0.067	0.069-0.065	0.470	0.487-0.453
32	96	0.065	0.067-0.063	0.455	0.473-0.411

Referring to Table II, the LT_{50} at 0.5 mg/l peak total chlorine showed a definite temperature effect. However, a temperature effect on the LC50 was not apparent. Although the temperature appears to have little effect on the LC_{50}, it may make the sublethal levels more stressful. The 96 h LC_{50} of approximately 0.4 mg/l for bluegill was higher than most of the values for related species that were found in the literature and were based on continuous exposure experiments (ARTHUR, 1971; ARTHUR and EATON, 1971; ESVELT, 1971).

In this study most of the total residual chlorine was in the free form which would be the "worst case" because of the greater toxicity of free chlorine as compared with a combined chloramine form (ZILLICH, 1972; BRUNGS, 1973). Other work in progress in this laboratory on different species indicates the combined form has a higher LC_{50} (i.e. is less toxic).

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