Bioassays on the Combined Effects of Chlorine, Heavy Metals and Temperature on Fishes and Fish Food Organisms Part I. Effects of Chlorine and Temperature on Juvenile Brook Trout (*Salvelinus fontinalis*)

by T. O. THATCHER, M. J. SCHNEIDER, and E. G. WOLF
Ecosystems Department
Battelle Pacific Northwest Labs.
Richland, Washington 99352

The effluents from power plants generally introduce heated water and at least one or several chemical pollutants into the receiving body of water. Chlorine is the biocide most commonly used in the cooling water system of power plants and thus, frequently occurs in these effluents. A recent report has estimated that up to 90 percent of the U.S. power plants in operation use chlorine for this antifoulant purpose (MCLEAN 1973). It is generally introduced as free chlorine from chlorine gas or hypochlorite. However, due to the presence of various substances with which it readily combines, chloramines and/or a wide variety of other combined forms commonly appear in power plant effluents or immediately downstream from their point of entry to the receiving waters.

Numerous studies appear in the literature concerning toxic properties of chlorine, both free available and combined available forms (the sum of which comprise total residual chlorine), to aquatic life. These have been reviewed thoroughly in two recent publications (BECKER and THATCHER 1973; BRUNGS 1973). Several of these studies indicated that brook trout were the most sensitive of the fishes studied. Brook trout data appearing in these publications differ notably, however. In one case 67 percent mortality occurred in 96 hours at 0.01 mg/l total residual chlorine (DANDY 1967) while in another, 0.083 mg/l was required to kill half the brook trout in 7 days (ARTHUR 1971-1972).

Several of the studies cited in these two literature reviews included fish food organisms, but only three contained information on crayfish. In one, the 7-day LC50 value was greater than 0.78 mg/l total residual chlorine (ARTHUR 1971-1972), while another investigator reported the "maximum lethal concentration" to be 1.2 mg/l as chloramines (COVENTRY et al. 1935). BRUNGS (1973) points out several problems among many of these studies, including different analysis techniques, and sometimes the failure to differentiate between free available and combined available chlorine forms. This latter point is important because free chlorine apparently is more toxic than chloramines and its effect occur more quickly. He considers amperometric titration analysis as the most favored method for determining total residual chlorine and the separate components of it.

Very few published reports have considered the combined stresses of chlorine and temperature alteration, and these only

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involved algae and zooplankters (BROOK and BAKER 1972; CAIRNS 1973; HIRAYAMA and HIRAMO 1970; MCLEAN 1973). In fact, however, only CAIRNS (1973) actually studied the combined effects of these two pollutants. The other three discussed combined effects but actually studied their effects separately. No data have been located pertaining to the combined effects of chlorine and temperature on freshwater fish or crayfish.

When an organism is simultaneously subjected to two toxic substances, combined effects are said to occur, and these may take one of three forms (WARREN 1971). If the total toxic effects of the combination are equal to the sum of the effects of the two substances when acting individually, their actions are said to be additive. If the combined effects of the two pollutants are greater than would be expected if they were merely additive, then they are said to be acting synergistically. If, however, the physiological effects of the two substances are less than expected if they were additive, they are then considered to be antagonistic.

The laboratory experiments reported in this study are an attempt to fill this void in the knowledge of the combined effects of temperature, chlorine, and heavy metals to aquatic organisms, especially fishes and fish food organisms. Part I of the study reports the results derived from exposing juvenile brook trout simultaneously to thermal alterations and a range of chlorine concentrations. Subsequent parts will focus on both acute and chronic effects of chlorine, heavy metals and temperature on other life states of brook trout, and various life stages of rainbow trout and crayfish, and perhaps one or two other species. The chronic experiments will include information on delayed mortality, growth, reproductive success and other physiological parameters important to these species. The data from these studies will be published at the end of each phase so that the information may be made immediately available to the scientific community. The information will be expecially pertinent for consideration in water quality criteria for the enhancement of aquatic life, and therefore, also in the preparation of environmental impact statements relative to the siting of thermal and chemical effluent emitting industries.

METHODS AND MATERIALS

The eastern brook trout, Salvelinus fontinalis (Mitchill), was selected as our experimental species. The choice was based on two primary considerations; first, it is a salmonid with wide distribution across the country, either as a native or introduced (TRAUTMAN 1957); secondly, the life cycle has been well documented and laboratory culture outlined (ENVIRONMENTAL PROTECTION AGENCY 1972).

The experimental stock was obtained from the Beitey Resort Hatchery at Valley, Washington in February when the fish were just

under 5 cm (2 in) in length. These had developed from eggs fertilized in early November (BEITEY 1974). They were transported to the lab in a refrigerated, aerated stainless steel tank without anesthetization. In the laboratory, they were maintained in four 65-gallon fiberglass aquaria. Each group was cold-branded and kept at a specific acclimation temperature (7, 10, 15 or 20°C) in the same quality water in which they would later be studied. The fish were fed daily with New Age dry pellets of appropriate size. Mortality during acclimation, in all cases, was less than 3 percent. At the beginning of each experiment, ten brook trout were introduced to each exposure chamber from a subsample of healthy acclimated fish. In any one experiment the length of the largest fish was not greater than 1.5 times the smallest. The average size of the experimental fish increased from about 7.5 cm (3 in) early in the study to about 18 cm (7 in) at the completion of the series of bioassays (TABLE I).

TABLE I

Information on Juvenile Brook Trout Used in Chlorine - Temperature Bioassays

Bioassay Number	Dates (1973)	Total Length (cm)	Acclimation Temperature	Bioassay Temperature
1	7/2-7/6	7.5-10	15 °C	15 °C
2	7/2-7/6	7.5-10	20	15
3	7/24-7/28	10-15	15	20
4	7/24-7/28	7.5-10	7	20
5	9/10-9/14	10-15	10	20
6	9/10-9/14	10-15	10	15
7	10/10-10/14	10-15	7	15
8	10/15-10/19	10-15	7	10
9	10/30-11/3	10-15	10	10
10	10/30-11/3	13-18	15	10
11	11/14-11/18	13-18	15	10
12	11/14-11/18	13-18	7	10
13	11/13-11/17	10-15	7	15

The water used in these bioassays was raw Columbia River water which supplies the wet labs of Battelle Pacific Northwest Laboratories, Richland, Washington. The water had been mechanically filtered, aerated, and temperature adjusted prior to delivery into the test chambers. Desired experimental temperatures were achieved by electrically controlled stainless steel valves which maintained the desired temperature within $\pm$ 0.4°C. The quality of this water was very good, being considered "Class A Excellent" (WATER POLLUTION CONTROL COMMISSION, STATE OF WASHINGTON 1967). A typical analysis showed the principal components and associated concentrations (in mg/l) as follows: total alkalinity 45-80, nitrate up to 2.0, fluoride 0.09-0.34, and copper up to 0.006. The filtration system kept the turbidity low. The effluent water from each exposure chamber went to a separate drain leading to a plant-wide special waste processing system.

The experimental glass aquaria were either 37.8 or 56.8 liter (10 or 15 gal) capacity, the former being used with a CHADWICK (1972) type serial diluter and the latter with a MOUNT-BRUNGS (1967) proportional diluter. Each diluter delivered about 1 l/min of solution to each of its six aquaria. Five received different predetermined concentrations of the toxicant and the sixth aquarium was used as a control chamber. All of the materials comprising the diluter and exposure containers were glass, tygon, aged neoprene stoppers, and silicone rubber cement, and are considered to add no toxicants to the system.

The criterion employed to detect differences in the combined effects of temperature change and exposure to chlorine was the common 96-hr LC50 value. These were determined by a computer program based on probit analysis which also gave the 24, 48, 72 and 96-hr LC values in increments of 5 percent from 5 to 95 percent mortality. In addition, the program yielded 95 percent fiducial limits for all these values.

Four acclimation temperatures were selected initially (7, 10, 15 and 20°C), however, due to disease problems the 20°C group of fish was discarded after bioassay Number 2. Individual groups of fish were acclimated at each temperature for at least 2 weeks prior to a bioassay. The experimental temperatures were maintained at 10, 15, or 20°C so that separate subsamples of each acclimation group were exposed to a range of chlorine values at three temperatures producing the desired conditions of combined stress of chlorine and altered temperature.

The chlorine levels were selected empirically such that the approximate 96-hr LC50 value would be near the middle of the five concentrations used during any one bioassay. Chlorine was measured daily by the amperiometric titration technique (APHA, AWWA and WPCF 1971), employing platinum electrodes and a polarograph for the end point detection. This method can measure total residual chlorine down to 0.5 ppb and can differentiate between free available and combined available forms. No free available chlorine

was ever detected in the exposure chambers.

In addition to chlorine, temperature was also monitored daily. The test temperatures were never more than 0.4°C different than that desired and in most cases were within 0.2°C. The total residual chlorine values in any one aquarium never varied more than 0.02 mg/l from the mean and no chlorine was ever detected in the control chambers. The pH and dissolved oxygen levels were checked during each experiment. The pH was always in the 7.8-8.2 range and dissolved oxygen was always greater than 8.0 mg/l.

These experiments spanned a 4-month period from July to November. During this time, the daily lighting scheme in the laboratory was 10 hrs of light and 14 hrs of darkness with increasing and decreasing light intensity at dawn and dusk.

On the first day of each experiment, chlorine was introduced through the dilution system into the exposure aquaria in the morning. Stable temperature and chlorine levels were reached within 1 to 2 hours in the aquaria. About 2 hours after the stable conditions were attained, a subsample of fish was removed from the acclimation aquaria, transferred to the exposure area in a plastic container, and randomly added (one at a time) to the test chambers. This transfer from acclimation to experimental chambers required about 15 min and had no observable deleterious effects on the fish. Mortality and general condition of the fish were recorded after 24, 48, 72 and 96 hrs. All dead fish were removed after each sample period, and all experimental fish were incinerated at the end of the experiment.

RESULTS

The 96-hr LC50 values from 13 continuous-flow bioassays with brook trout exposed to the combined effects of chlorine exposure and temperature alteration are presented in FIGURE 1. In general, there was no significant difference (at the 95% fiducial limit) between the 96-hr mortality values from experiments run at 10 or 15°C regardless of the previous acclimation temperature (7, 10, 15 and 20°C). However, in fish exposed to 20°C test temperature, all 96-hr LC50 values were significantly (at the 95 percent fiducial limits) less than at the lower test temperatures (10 and 15°C). It is also noteworthy that the lower 96-hr LC50 values at the 20°C test temperature are also independent of acclimation temperature.

A difference was also observed between early mortality and mortality after the 96 hrs. Four experiments were conducted during which fish (previously cold-branded) from two acclimation temperatures were exposed simultaneously in the same aquaria to the particular experimental conditions. The condition of the fish was noted both in the morning and the evening. During the first observation period that any mortality was observed in these tests (prior to 48 hours), the total number of deaths for the two acclimation groups was different (TABLE II). In each of the four cases,

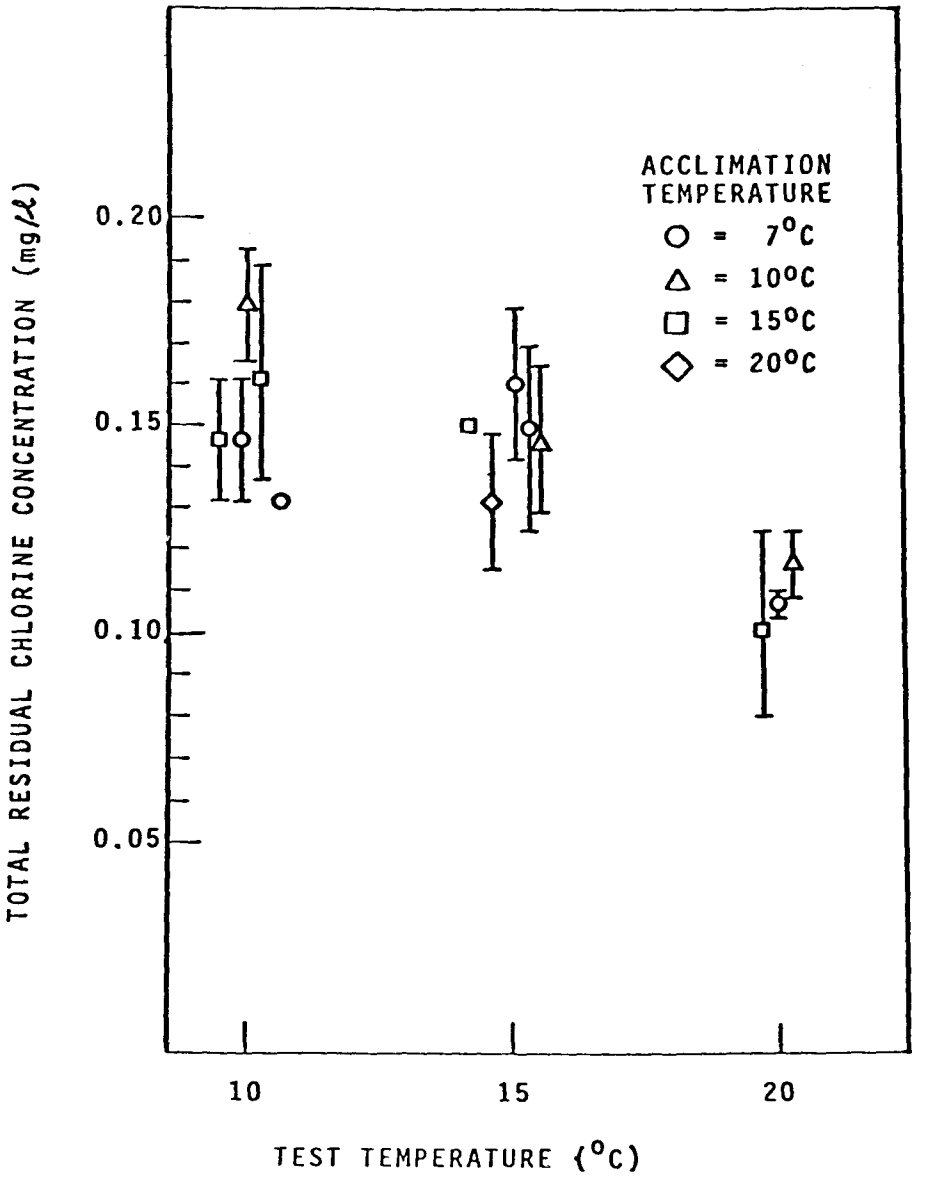

FIGURE 1. Thirteen 96-hr LC50 values for juvenile brook trout. The vertical bars indicate the 95% fiducial limits where these could be calculated.

TABLE II

Comparison of Early Mortality (Prior to 48 hrs) and 96-hr LC50 Values from Bioassays in Which Brook Trout From Two Different Acclimation Temperatures were Exposed to Chlorine Simultaneously in the Same Series of Chambers

Test Groups	96-hr LC50	95% Fiducial Limits	Early Mortality	Test Temp	ΔT
9	0.179	0.165-0.193	5.0%	10	0
10	0.163	0.136-0.188	11.7%	10	-5
12	0.146	0.131-0.161	6.7%	10	+3
11	0.146	0.131-0.161	13.3%	10	-5
1	0.153	-	0.0%	15	0
2	0.131	0.115-0.147	5.0%	15	-5
3	0.102	0.080-0.124	36.7%	20	+5
4	0.107	0.104-0.110	58.3%	20	+13

the group that had experienced the greater temperature change when transferred from the acclimation chamber to the bioassay aquaria experienced the higher mortality, regardless of whether the change was an increase or a decrease. In all of these experiments the LC50 values and the total deaths for the paired acclimation groups were not significantly different at the 95 percent fiducial limit after 96 hrs.

At the completion of four of the bioassays, the survivors were kept from 3 to 5 days in chlorine-free water. Of these chlorine-exposed fish (107) which were subsequently kept in clean water, only two died. Thus, delayed mortality was not typical, at least over the additional 3 to 5 day period.

DISCUSSION AND INTERPRETATION

These data indicate that at the experimental temperatures employed in this study, there is no additional effect of temperature upon the toxicity of chlorine juvenile brook trout when exposed for 96 hrs at 10 or 15°C. However, at the 20°C test temperature, 96-hr LC50 values were significantly lower at the 95 percent fiducial limit. Since there were no deaths in the control chambers the combined effects of thermal alteration and chlorine exposure indicated synergism under these conditions.

Absolute lethal concentrations can vary when going from laboratory to field situations due to the larger number of variables present in nature. However, the relationships developed in the laboratory bioassays would be expected to remain similar. Therefore, in a chlorine effluent area where the temperature approaches 20°C, the concentration of total residual chlorine (in particular the chloramines) necessary to be acutely lethal to brook trout would be about 30 percent lower than in other areas (or at other times) where the temperatures are in the 10 to 15°C range.

One possible explanation for the effect noted at these particular temperatures is that perhaps 10 to 15°C is the optimum temperature for the operation of the particular body functions in these fish which leads to their being able to withstand certain physiological stresses, in this case chlorine toxicity. Going further, perhaps this temperature range is the optimum for all the life promoting functions of this age of brook trout and therefore, this would obviously seem to be the condition under which they would be least susceptible to stresses. Supporting this latter idea are the findings of a previous study that the suitable range of temperatures for young brook trout growth and survival was from 9.8 to 15.4°C (MCCORMICK et al. 1972). Also the EPA recommended bioassay procedures for brook trout indicate that 15°C is the maximum desirable temperature for these studies. In similar studies, rainbow trout also seemed best able to withstand the toxic effects of mercury when the temperatures were near those optimum for their growth (THATCHER 1974).

The difference in mortality exhibited by these young brook trout prior to 48 hours of exposure compared to lethality after 96 hours is also noteworthy. Since this finding was complemented by (a) the absence of delayed mortality, and (b) the observations that the greater the temperature shock, the higher the mortality during these early stages; it appears that the effects of the previous temperature acclimation upon acute lethality of chlorine disappeared after about 2 days.

The absence of delayed mortality, if substantiated in further studies in this series, will become noteworthy for comparison with the toxic effects of chlorine upon crayfish. Preliminary data collected at our lab indicate that delayed mortality is common in crayfish tested in chlorine - temperature bioassays.

Additional studies in this series will determine the effects of temperature lower than 10°C upon the resistance to chlorine, and also will explore the combined effects of temperature alteration and chlorine, heavy metals, and other toxicant exposure on several sublethal parameters such as growth and reproduction in chronic bioassays. Rainbow and brook trout, and crayfish will be included in these studies, and the relative resistance of various life stages will also be investigated.

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