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TOXICITY OF RESIDUAL CHLORINE COMPOUNDS TO AQUATIC ORGANISMS

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TOXICITY OF RESIDUAL CHLORINE COMPOUNDS TO AQUATIC ORGANISMS

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FOREWORD

The advantages and disadvantages of chlorination for disinfection and slime control have been the subject of intensive debate over the past several years. Included in that debate are such considerations as the need for disinfection in all cases, and the possibility of mitigating the adverse effects of chlorination (while retaining the beneficial effects) by variations in the dose regime.

This report presents results and interpretations of research on the effects of chlorine and inorganic chloramines on several species of fish, a crayfish, and laboratory stream communities. Among other things, it was found that the relationship between the concentration-time integral of exposure under a particular exposure regime and the toxic effects of that exposure could be generalized to predict effects on fish under different exposure regimes. These results should aid in establishing a basis for the possible controlled use of chlorine to achieve its purpose with minimal damage to aquatic life.

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ABSTRACT

Laboratory studies on the acute and chronic toxicity of chlorine and inorganic chloramines to trout, salmon, minnows, bullhead, largemouth bass, and bluegill were conducted. Acute toxicity under continuous and intermittent patterns of exposure as well as behavioral, reproduction, development, and growth responses to low level exposures to residual chlorine compounds were determined. But not all patterns of toxicant exposure or all responses of all fish species were studied. Acute and chronic toxicities of chloramines to crayfish were investigated. Algae, invertebrates, including insects, and juvenile salmon were exposed continuously to relatively low levels of residual chloramine compounds in laboratory stream communities. The acute toxicities of inorganic chloramines, as measured by 96-hour LC50 values, were less than 100 µg/l for salmonids and were a function of life history stage, body size, and some water quality conditions. Whereas adult trout may live indefinitely at concentrations near 50 µg/l, the LC50 values for late developmental stages--fry and very small juveniles--were not much above this concentration. Effects on growth of alevins and juveniles had threshold concentration values between about 10 and 22 µg/l, effects being quite marked at 22 µg/l. In intermittent exposure to relatively high concentrations of free residual chlorine, mortality was found to be a rather consistent function of the area under the time-concentration curves of exposure, for different forms, durations, and frequencies of such patterns of exposure. Behavioral responses of fish, such as avoidance of chlorinated water which could be advantageous in nature and lethargic swimming, surfacing, and sinking to the bottom which would probably be harmful were studied. Such behaviors occur not only at acutely toxic concentrations but also at lower ones. It was necessary to introduce concentrations ranging from 100 to 800 µg/l of chloramine into laboratory stream communities to maintain mean residual concentrations near 20 µg/l. No marked effects on algal or insect abundances or on survival and production of juvenile salmon were observed at this and lower concentrations in the laboratory streams. It is doubtful that the amperometrically determined residual concentrations of chloramines in the streams consisted predominantly of inorganic chloramines, organic chloramines perhaps being an important constituent under the stream conditions. Little is known of the toxicity of organic chloramines. The amount of inorganic chloramines introduced to maintain desired residual concentrations appears to have been a function of the amount of organic material in the stream communities.

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INTRODUCTION

Present and probable future use of chlorine for disinfection and for slime control presents a hazard to aquatic life that makes the establishment and enforcement of adequate standards important. Effluent and receiving water standards intended to provide protection for aquatic organisms ought to ensure that concentrations of toxic materials permitted in receiving waters are no higher than is consistent with the persistence of desired aquatic communities and high levels of production of species man uses. For lack of empirical and theoretical knowledge, few if any toxic substance standards are known to provide such needed protection of aquatic resources. Use of chlorine for disinfection and slime control and what is known of its toxicity have led some to consider chlorine to be a general and serious water pollution problem. And yet only recently have even modest levels of research on this problem been forthcoming. The research here reported is intended to extend the basis for setting standards to protect some species of fish and invertebrates from direct and indirect effects of chlorine and inorganic chloramines.

Chlorine in water may exist as free residual in the forms of chlorine gas, hypochlorous acid, and hypochlorite ion, and as combined residual--the chloro-derivatives. One important group of chloro-derivatives includes the inorganic chloramines monochloramine (NH_2Cl), dichloramine ($NHCl_2$), and trichloramine (NCl_3). One or more of these compounds are formed when water containing ammonia is chlorinated. Monochloramine and dichloramine are the predominant forms of inorganic chloramines found in receiving waters. Total residual chlorine (TRC) is the sum of the concentrations of free and combined residuals as measured by amperometric titration analysis.

Residual chlorine compounds are known to be present in many receiving waters in concentrations toxic to fish (Brungs, 1973). Through studies conducted in natural streams receiving chlorinated effluents, the Michigan Department of Natural Resources (Anonymous, 1971) found the 96-hour LC50 for rainbow trout (*Salmo gairdneri*) to be 22.8 µg/l total residual chlorine; concentrations high enough to cause mortality persisted for 0.8 mile down stream from the point of discharge. Nonchlorinated effluent was not toxic to the trout. In chlorinated sewage effluent, the 96-hour LC50 for fathead minnows (*Pimephales promelas*) was found to be between 80 and 190 µg/l total residual chlorine (3.2 and 7 percent effluent); no mortality occurred in 100 percent effluent that was dechlorinated (Zillich, 1972). Arthur et al. (1971) found that growth and survival of fathead minnow larvae were reduced at 108 µg/l total residual chlorine in sewage effluent. Tsai (1973) reported that a species diversity index for fish in rivers in Maryland, Virginia, and Pennsylvania dropped to zero below chlorinated effluent outfalls when mean total residual chlorine concentrations were about 250 μg/l. Sprague and Drury (1969) conducted laboratory experiments in which concentrations as low as 1 μg/l of TRC were avoided by rainbow trout. Others have shown avoidance responses of various freshwater and estuarine fishes at relatively low TRC concentrations under laboratory and field conditions (Meldrim et al., 1974; Tsai and Fava, 1975; Bogardus, et al., MS ; and Basch and Truchan, 1976). Not much is known about the effects of intermittent exposure of fish to chlorine, but deaths and abnormal behavior have been observed where chlorinated effluents were being discharged intermittently (Basch and Truchan, 1976). Laboratory studies have been conducted on the acute toxicity of chlorine to bluegill, yellow perch, and several species of salmonids, as affected by temperature, exposure duration, and exposure frequency (e.g. Stober and Hanson, 1974; Brooks and Seegert, 1977, and Bass and Heath, in press). Tolerance of fish to chlorine has been shown to decrease with increasing duration and frequency of exposure.

There are sound reasons, we believe, for man not to alter aquatic and other natural communities any more than absolutely necessary to ensure his own well being. But activities of man that impinge upon aquatic systems are likely to alter in some degree their communities. Even if we take the perhaps too narrow a view that, at a minimum, man must protect fish and other species of particular interest, it becomes necessary to maintain the physical conditions and biological communities making possible the persistence, the production, and acceptable yields of these species. For such species of interest, receiving water standards for toxicants ought to insure not only that reproduction, development, growth, and behavior of individuals and the persistence and production of their populations are not directly affected by toxicants, but also that biological communities providing species of interest with food and other resources are maintained. Different life history stages and biological responses of species of interest are known to vary widely in sensitivity to toxicants, and such sensitivity is also known to be a function of water quality conditions. All these considerations should be borne in mind as the necessary context for interpretation of any results obtained through research on particular species.

The results and interpretations reported here, as well as any conclusions and recommendation derived from them, are based on research on the effects of chlorine and inorganic chloramines on several species of fish, a crayfish, and laboratory stream communities. This research included studies on the reproduction, development, growth, and survival of salmon and trout exposed to chronically as well as acutely toxic concentrations of chlorine and inorganic chloramines. The effects of water temperature, pH, and total alkalinity on acute toxicity of inorganic chloramines were also determined. Acute toxicity of chlorine to largemouth bass as well as effects of chlorine and chloramines on behavior were determined, when the fish were intermittently exposed. Both acute and chronic toxicity of inorganic chloramines to crayfish were determined. Preliminary studies of acute toxicity and behavioral effects of chlorine were conducted with other species of freshwater fish. Finally, we conducted laboratory stream studies, in which communities of algae, insects, other invertebrates, and juvenile salmon were exposed for relatively long periods of time to low concentrations of residual chlorine compounds.

The above summary of the research conducted suggests that a rather wide variety of methodological approaches was employed in determining acute and chronic toxicity of chlorine and inorganic chloramines to aquatic organisms. But the relatively limited support and time available for this research prevented really adequate replication of experiments of particular kinds, and thus many of the results should be viewed as being of a preliminary nature. In planning and conducting this research, we and the Environmental Protection Agency scientists advising us decided that, in view of the breadth, complexity, and seriousness of chlorine toxicity problems and the scarcity of pertinent information, it was most immediately important to obtain information on a variety of responses of several species to chlorine and inorganic chloramines under different sets of conditions. Although we are not satisfied with the precision obtained in most of the experiments, taken together the results of these experiments rather clearly indicate exposures of aquatic organisms to these toxicants that would be harmful. The conclusions and recommendations reflect this.

CONCLUSIONS AND RECOMMENDATIONS

1. The acute toxicity of free chlorine and inorganic chloramines, as determined by 96-hour continuous exposure to constant concentrations, differs considerably among species, among individuals of different sizes, and among different life history stages. Crayfish, for example, may have 96-hour LC50 values near 900 μg/l inorganic chloramines, whereas such values for salmon and trout are likely to be less than 100 μg/l. But adult salmonids may live indefinitely at concentrations near 50 μg/l, which are close to the LC50 values for fry and very small juveniles. And adult crayfish may succumb to concentrations near 50 μg/l when molting. Chronic effects on growth and other responses of salmonids may occur at concentrations near 20 μg/l or even lower. Thus the difference between acutely toxic and chronically toxic concentrations of inorganic chloramines is very great for some species and really quite small for others. When considered along with differences in sensitivity of different life history stages, this makes it apparent that information on acute toxicity--as measured by 96-hour LC50 values--alone is quite inadequate for determining levels of residual chlorine compounds that will not be deleterious to aquatic life in receiving waters when exposure is more or less continuous.

2. Chlorination of some industrial effluents will present difficult and perhaps unexpected problems in the toxicology of aquatic organisms. We found that chlorination of a kraft pulp and paper effluent--which was biologically stabilized and exhibited no toxicity in 96 hours at 100 percent concentrations--rendered this effluent acutely toxic at concentrations near 20 percent, even though only 1 mg/l of inorganic chloramines was added and no residuals were detectable in the test solutions. Certainly chlorination of effluents should be undertaken only when there is a demonstrable need and then only minimal effective treatments should be employed, after possible toxic effects on aquatic organisms have been evaluated.

3. Intermittent introduction of free chlorine, such as for one hour in every 24 hours, has important power plant and other industrial applications in slime organism control. There must be an infinite number of possible concentration-time patterns and frequencies of introduction of chlorine for such applications; some of these present serious toxic problems to fish and other aquatic organisms in receiving waters. But so many different patterns and frequencies of chlorine introduction are employed in slime control, the difficulty of evaluation of toxic hazard is very great. We have found that the area, expressed as mg/l x minutes, under concentration-time curves of exposure makes it possible to generalize to different exposure regimes results obtained under particular regimes. Time, concentration, and frequency of exposure boundary conditions of application

of this method of expressing bioassay results should be further explored, because it could be very useful in simplifying analysis, interpretation, and application of studies on intermittent exposure to chlorine. We gain the impression that unnecessarily high concentrations, durations, and frequencies of chlorine treatment in slime control are often employed. This is not only needlessly harmful to aquatic life but economically wasteful. Properly controlled use of chlorine can, we believe, generally achieve its purpose with little or no damage to fish populations.

4. Under some circumstances, relations between temperature and the toxicity of free and other residual forms of chlorine are likely to be important. Temperature is generally a factor in determining the toxicity of substances to aquatic organisms. It is likely to be especially important when organisms are exposed to toxicants at water temperatures higher than the ones to which they have been acclimated. This may occur when chlorine residuals are present in thermal discharges from power plants.

5. Especially under conditions of more or less continuous exposure of aquatic populations to residual chlorine compounds, as occurs where chlorinated sewage effluents are discharged, effects of quite low residual concentrations on development, reproduction, growth, and production of fish and other organisms are of concern. Our studies have shown that threshold concentrations of inorganic chloramines having effects on the growth of salmonid alevins and juveniles probably lie somewhere between 10 and 20 μg/l of total residual chlorine as determined by amperometric titration. Effects on growth are quite marked at concentrations near 20 μg/l. Field studies by others indicate that salmonid populations may be eliminated from areas where they are subjected to such levels of exposure. Much more emphasis needs to be given to studies of reproduction, development, and growth of fish and other organisms likely to be exposed more or less continuously to such relatively low levels of residual chlorine compounds. There is no way that 96-hour acute toxicity studies can provide adequate information on which to base the protection of aquatic organisms from such conditions.

6. Studies of the possible effects of various levels and durations of exposure of fish to residual chlorine compounds are also important. Fish have the capacity to avoid chlorinated waters under some conditions, and this could enhance the possibility of their persistence. Laboratory studies have demonstrated such capacity, but the extent to which fish are actually able to avoid chlorinated discharges in nature cannot be determined by extrapolation from laboratory studies. Careful field studies, perhaps employing underwater observations, would be very worthwhile. We have shown behavioral modification of fish by acutely toxic and lower concentrations of chlorine and inorganic chloramines, under conditions of intermittent exposure. Such behavioral modifications include lethargic swimming, surfacing, and sinking to the bottom. Similar observations have been made in the field, and there is every reason to believe that these and other behavioral modifications are deleterious to fish populations exposed intermittently to relatively high concentrations of residual chlorine compounds.

7. We introduced continuously into laboratory stream communities--including algae, invertebrates, and juvenile salmon--concentrations of inorganic chloramines from about 100 to 800 μg/l. This was necessary to maintain experimental mean residual concentrations up to about 20 μg/l, as determined by amperometric titration. The introduced concentrations needed to maintain desired experimental concentrations were seasonally very dependent on the amount of organic material, especially algal materials, present in the laboratory streams. Mean concentrations of 20 μg/l TRC and less had little if any apparent effect upon the algae, stream invertebrates, and juvenile salmon, under the conditions in these streams. But we are uncertain as to the forms of residual chlorine compounds that were present. It is unlikely that inorganic chloramines were the main persistent form, probably more of the TRC being present as organic chloramines, the toxicity of which we know little. We do not wish to suggest that in nature reproduction and other life processes of insects and other invertebrates would not be harmed by concentrations near those we maintained. Insect reproduction, for example, cannot be adequately evaluated by means of our laboratory streams as presently designed and housed. Much more needs to be done to determine the occurrence of different forms of residual chlorine under different conditions and the effects of these on aquatic organisms, both in the laboratory and in the field.

METHODS AND MATERIALS

PREPARATION AND INTRODUCTION OF TOXICANTS

In most of our experiments with inorganic chloramines, test solutions were formed by mixing solutions of sodium hypochlorite (NaOCl) and ammonium chloride (NH_4Cl) in a molar ratio of 0.7:1. The solution of each chemical was prepared with well water in a 20- or 45-liter Mariotte bottle. By connecting the atmospheres of the bottles containing the two chemicals, the flows from the two bottles mixed at equal volumes on entering a mixing chamber. Retention in the mixing chamber was about 3 hours, sufficient time for no free residual chlorine to be detectable in any test solution of inorganic chloramines entering the dilution systems. Test solutions of free residual chlorine were prepared and introduced in the same way, except that hypochlorite stock solutions were not mixed with other chemicals.

In the laboratory stream studies which required the introduction of high concentration solutions, stock solutions of sodium hypochlorite were kept in a collapsible bottle submersed in a cool water bath. This procedure reduced exposure to air and minimized loss of chlorine gas. Stock solutions of ammonium chloride were kept in a Mariotte bottle. The two solutions were transferred with a tubing pump to a closed mixing bottle having a retention time of several hours for formation of inorganic chloramines. The tubing pump was controlled by a flowing-mercury switch located on the "dipping bird" mechanism of a dilution system similar to that described by Mount and Brungs (1967). To reduce gas formation in the closed mixing bottle, NaOCl and NH_4Cl concentrations were adjusted to achieve a pH of about 8. This adjustment was necessary because a high NaOCl concentration has a pH high enough for release of ammonia upon mixing with NH_4Cl. Conversely, the presence of too much NH_4Cl produced acid conditions and the release of chlorine gas.

Concentrations of free residual chlorine and inorganic chloramines were determined using a Wallace and Tiernan amperometric titrator, the titrant (0.0564N phenylarsene oxide) being added to the test solutions with a micropipette. Concentrations were determined in micrograms per liter (μg/l) or milligrams per liter (mg/l) of total residual chlorine (TRC). Concentrations as low as 1 μg/l of TRC could, with adequate precision, be determined in test solutions.

Flowing water dilution systems were used for all experiments. In the partial chronic and the embryo-alevin experiments, 2-liter proportional diluters, similar in design to the one described by Mount and Brungs (1967), were used. An 8-liter proportional diluter was used in combination with a modified Chadwick et al. (1972) type dilution system in the laboratory stream studies. The dilution systems used in the acute toxicity, growth, and behavior experiments were similar to the one described by Chadwick et al. (1972).

WATER QUALITY AND PHYSICAL CONDITIONS

Well water was used for dilution in all experiments, except for the sand-filtered Oak Creek water used in the laboratory stream studies and the sand-filtered Willamette River water used in the acute toxicity studies with pulp and paper mill effluent. The creek and river waters were pumped through ultraviolet light to destroy pathogens. In order to keep the temperature of the sand-filtered dilution waters well within the maximum temperature range of creeks and rivers in western Oregon, water chillers were used for cooling during summer. Temperature control of well water was maintained with submersible heating elements located in the dilution water reservoir boxes.

Temperatures of control water and test solutions were determined with hand thermometers; pH was determined by means of an Orion Model 801 Analyzer. Total alkalinity was determined by the bromcresol green-methyl red indicator method. Dissolved oxygen concentrations were analyzed by the modified Winkler method.

Except for the laboratory stream studies, all experiments were conducted in laboratories having constant temperature control. Photoperiod was kept at the regime for the Corvallis, Oregon, latitude (45°N) by adjustments made every two weeks. The laboratory streams were kept under natural photoperiod, but the quality and quantity of light were altered somewhat by the translucent plastic roof of the building housing the streams.

EXPERIMENTAL ANIMALS

The species of animals used in these studies included: coho salmon (Oncorhynchus kisutch); brook trout (Salvelinus fontinalis); cutthroat trout (Salmo clarki); crayfish (Pacifasticus trowbridgi); brown bullhead (Ictalurus nebulosus); redside shiner (Richardsonius balteatus); blackside dace (Rhinichthys osculus); bluegill (Lepomis macrochirus); and largemouth bass (Micropterus salmoides). The locations from which these species were obtained are listed in Table 1, and the experiments in which the species were used are listed in Table 2.

METHODS FOR DETERMINING ACUTE TOXICITY: CONTINUOUS EXPOSURE

For 96-hour acute toxicity experiments, 10 fish, or 8 crayfish, having nearly the same individual body weights were tested in each of twelve 45-liter glass aquaria. Small fish (alevins and fry) and the crayfish were tested in 15 liters of control (well) water or test solutions to insure adequate circulation of the water around the animals, since they remained on or near the aquaria bottoms. Larger fish were tested in 30 liters of solution. Two groups of animals were tested at each toxicant concentration

TABLE 1. SOURCES OF SPECIES OF FISH AND THE CRAYFISH USED ON EXPERIMENTS

Species	Source and location of collection
Coho salmon	Fall Creek Hatchery, Oregon Department of Fish and Wildlife, Western Oregon
Brook trout alevins and juveniles	Raised from embryos obtained from Beities Resort, Valley, WA.
Cutthroat trout juveniles	Fall City Hatchery, Washington State Depart-Department of Fisheries, Fall City, WA.
adults	Black Creek, Western Oregon
Crayfish	Alsea River and Fall Creek, Western Oregon
Redside shiner	Esmond Creek, Western Oregon
Blackside dace	Alsea River, Western Oregon
Bluegill, brown bullhead and largemouth bass	Farm ponds near Corvallis, OR.

TABLE 2. THE EXPERIMENTS IN WHICH EACH SPECIES WAS USED.

Experiment	Species
Acute Toxicity:	
Continuous exposure -	Coho salmon, brook trout, cutthroat trout, and crayfish
Intermittent exposure -	Largemouth bass, bluegill, brown bullhead, redside shiner, blackside dace, and cutthroat trout
Chronic Toxicity:	
Partial chronic -	Cutthroat trout, brook trout, and crayfish
Development, survival and growth -	Coho salmon
Behavior -	Largemouth bass, bluegill, brown bullhead, redside shiner, blackside dace, and cutthroat trout
Laboratory Streams:	Coho salmon

and for controls. Coho salmon alevins and brook trout alevins were tested in darkness to simulate streambed conditions, and at 10 C, but other tests were conducted in light and at 15 C unless stated otherwise. The flow rate of control water or test solution into each aquarium was maintained at 500 ml/min.

The effects of temperature (10.8 and 15.0 C), total alkalinity (135 and 320 mg/l as $CaCO_3$), and pH (7.0, 7.5, and 8.1) on the acute toxicity of chloramines to juvenile coho salmon were determined in separate tests. The alkalinity of the well water was increased by the addition of a saturated solution of sodium bicarbonate, changes of pH being made and controlled by the addition of carbon dioxide or 0.2 N sodium hydroxide.

For tests with coho salmon, the 96-hour median lethal concentrations (LC50) were determined by calculating the logit linear regression line (Ashton, 1972). Percentage of mortality was plotted on a logit scale and concentration of toxicant was plotted as TRC on a base 10 log scale. A computer program designed by D. A. Pierce (Department of Statistics, Oregon State University) was used to test for significant differences ($P \leq 0.05$) between the slopes and intercepts of logit regression lines. For tests with other species, the 96-hour LC50's were determined by calculating probit regression lines (Finney, 1971). Probits of mortality were plotted against concentration of toxicant on a base 10 log scale. Regression analyses were performed with a Monroe Model 1766 calculator.

In 96-hour acute toxicity experiments with chlorinated and nonchlorinated secondary-treated pulp and paper mill effluent (SKME), 10 juvenile coho salmon of nearly equal body weight were tested in each of twenty-two 45-liter glass aquaria. Each aquarium contained 30 liters of control water or test solution. Separate dilution systems were used for the chlorinated and the nonchlorinated effluent in each test. The effluent was chlorinated with a solution of inorganic chloramines (71 percent) and free residual chlorine (29 percent) immediately before it entered the dilution system. Concentrations of 18, 32, 56, and 100 percent chlorinated and nonchlorinated SKME were used in the first three tests. The 18 percent concentration was replaced by a 75 percent concentration in a fourth test. Two replicates were tested for each set of control and treatment conditions. The flow rate to each aquarium was maintained at 330 ml/min. The concentrations of chlorinated SKME between which the 96-hour LC50 values occurred were estimated by plotting percent mortality against percent chlorinated SKME on a base 10 log scale.

The effluent was from a kraft liner board mill and had 24 hours of primary treatment, which was followed by 7 days of secondary treatment in biological stabilization ponds. An additional 10 days of biological treatment in a small stabilization pond--operated by the National Council for Air and Stream Improvement (NCASI)--was provided to produce an effluent of high and and relatively consistent quality, one that might be characteristic of stabilized kraft process effluents produced by this mill in the future. The BOD of the SKME was determined by personnel of the NCASI.

The animals used in the acute toxicity tests with chlorinated well water and SKME were acclimated to the laboratory conditions for at least 3 days prior to being tested. During the acclimation period, the fish were fed either Oregon moist pellet or Tubifex. The crayfish were fed a mixture of alfalfa and Oregon Test Diet (Lee et al., 1967). The fish and crayfish were not fed for 24 hours prior to testing and during the tests.

METHODS FOR DETERMINING ACUTE TOXICITY: INTERMITTENT EXPOSURE

Fish were subjected to short-term exposure to residual chlorine, of which 97.04 percent was free residual chlorine. Two types of time-toxicant concentration exposures were tested. These will be referred to as square and spike exposure patterns. They represent the extremes of time-concentration relationships found in the field at the points of discharge of intermittently chlorinated waters (G. Nelson, EPA, personal communication). The square exposure patterns were produced by adding the toxicant to the aquaria at constant rates for predetermined periods of time. Chlorine concentrations reached a plateau level 20 minutes after the toxicant flow as initiated. With but one exception, the toxicant flow was terminated at 60 minutes, and the toxicant was completely flushed from the aquaria after an additional 30 minutes. Thus, total exposure time was 90 minutes. For bioassays in which spike exposure patterns were employed, both toxicant and dilution water flows were manipulated to achieve the desired time-conncentration curves. Each spike exposure pattern was characterized by a rapid rise to a peak toxicant concentration, followed immediately by a rapid decline in concentration. Spike patterns having different peak concentrations and different durations were used in the tests. One pattern peaked in concentration 5 minutes after initiation of toxicant flow, and others peaked at 29 and 22 minutes. Total exposure times for these three spike exposures were 51, 60, and 63 minutes, respectively. The concentrations of total residual chlorine for each exposure pattern were determined at 2 to 10 minute intervals. The fish, which had been fed a daily ration of Oregon moist pellet, were starved for 24 hours prior to being tested and during the tests.

Most of the intermittent exposure tests were conducted with largemouth bass in aquaria. But channels in which the fish were forced to swim against a current were used in some tests. And other species were also studied in aquarium experiments. In each of the aquarium tests, six bass of nearly the same weight were acclimated to the test conditions (except for toxicant) for 30 minutes in a 45-liter glass aquarium containing 30 liters of water. These fish were then exposed to the toxicant for a predetermined period of time, after which they were maintained in water without toxicant for observation for the duration of the 96-hour test. The quality of the well water used in these studies was as follows: dissolved oxygen, 7.4 mg/l; hardness, 128 mg/l; total alkalinity, 148 mg/l; pH 7.94; and temperature, 24.3 C, with the exception of one experiment in which temperature was varied.

Previous studies at our laboratory had shown that the tolerance of coho salmon for residual chlorine in acute toxicity tests is a function of body size (Larson et al., in press). Preliminary tests were conducted to determine if body weight affected the tolerance of the bass for short-term exposures of chlorine. The tested fish were divided into two weight classes, one being 3.87 ± 0.15, the other 5.93 ± 0.28 g/fish, dry weight. Groups of each weight class were subjected to one 90-minute square pattern of exposure. The LC50, based on observation of mortality but not exposure for 96 hours, for the two classes differed by approximately 1.2 mg/l TRC (mean plateau concentration from 20 to 60 minutes), the smaller fish being the more sensitive. On the basis of these preliminary results, fish weight was standardized in each experiment, although the weights varied from test to test (Table 3).

TABLE 3. MEAN DRY WEIGHT AND STANDARD DEVIATION OF LARGEMOUTH BASS TESTED IN THE INTERMITTENT EXPOSURE EXPERIMENTS WITH FREE RESIDUAL CHLORINE.

Experiment	Mean dry weight per fish (g)	Standard deviation
Weight effects (90-minute square exposure)		
Class 1	3.87	0.15
Class 2	5.93	0.28
Exposure regime		
90-minute square exposure	8.60	0.20
53-minute low spike exposure	8.50	0.20
63-minute high spike exposure	8.51	0.24
Exposure duration		
90-minute square exposure	8.92	0.36
150-minute square exposure	8.92	0.53
Exposure frequency		
one 90-minute square exposure	6.18	0.19
two 90-minute square exposures	6.15	0.32
Temperature effects (90-minute square exposure)		
13.1 C (acclimation and test)	4.53	0.36
24.3 C	4.64	0.28
13.1 24.3 C	4.62	0.13

In one experiment, groups of bass were exposed to 51- or 63-minute spike patterns of exposure, or to the 90-minute square pattern of exposure. The mean plateau concentrations of TRC for square exposure patterns ranged from 2.35 to 3.32 mg/l. The peak TRC concentrations were from 8.21 to 11.93 mg/l and from 5.73 to 9.06 mg/l for the 51- and 63-minute spike patterns of exposure, respectively. The effects of the three types of exposures were compared on the basis of areas under the time-concentration curves. These areas were measured using a compensating planimeter and expressed as mg/1 x minutes of total residual chlorine. The curves for all tests were graphed using the following scale: a 10 minute exposure to 1 mg/1 TRC equalled 5.9 cm.

The effect of exposure frequency on survival was examined by subjecting some groups of bass to single 90-minute square patterns of exposure, while other groups were subjected to two such exposures, a two-hour recovery period separating the exposures. The effect of exposure duration on survival was investigated by subjecting groups of bass to either a 90-minute or a 150-minute square pattern of exposure. For the latter, toxicant flow into the aquaria was terminated at 120 minutes.

In other experiments, cutthroat trout, brown bullhead, bluegill, blackside dace and redside shiner were tested separately. Groups of each species of fish were subjected to one 90-minute square pattern of exposure to free residual chlorine and then kept in freshwater for the remainder of the 96-hour tests. The number of tests, range of fish weights, number of fish used in each aquarium, and the water quality in each test are shown in Table 4.

METHODS FOR DETERMINING CHRONIC TOXICITY: PARTIAL CHRONIC TESTS

The main objective of partial chronic experiments is to determine, for the species of interest, the life history stages most sensitive to a toxicant when constant exposure has occurred over all life stages. The method requires a period of exposure of separate groups of sexually maturing adult organisms to different concentrations of a toxicant and subsequent exposure of embryo, alevin, and juvenile life stages to the same toxicant concentrations. The experimental animals used in our partial chronic experiments with chloramines were brook trout, cutthroat trout, and crayfish (Table 5). Because it was not possible to collect brook trout and crayfish from the field until midsummer, the gonadal products of these species were near maturity at the beginning of each experiment.

Ten stainless steel aquaria, two at each toxicant concentration and two well water controls, were used in each partial chronic experiment with fish. Each aquarium was 90 cm long, 30 cm wide, and 38 cm deep. In experiments with trout, each aquarium contained 81 liters of water 30 cm deep. Other aquaria used in the crayfish tests contained 27 liters of water 10 cm deep.

In tests with trout, the aquaria were covered with screens for shading. Spawning trays, either pans of gravel or pans covered with plastic screening upon which rocks had been glued, were placed in the aquaria. Fertilized eggs from each group of fish were placed in a cup positioned at the water surface in the same aquarium. The cups were so designed that water flow was upward and around developing embryos and alevins (Larson et al., in press).

TABLE 4. NUMBER AND WEIGHT OF FISH AND WATER QUALITY CONDITIONS IN THE INTERMITTENT EXPOSURE EXPERIMENTS WITH FREE RESIDUAL CHLORINE

Species	No. of tests	Mean dry weight per fish (g)	Standard deviation	No. of fish tested in each aquarium	Acclimation and test temperature (C)	pH	Total alkalinity (mg/1)
Cutthroat trout	6	2.21	0.25	5	12.5	8.1	150
Brown bullhead	2	0.43	0.04	4	12.5 → 22.0	8.1	150
	4	0.36	0.04	4	24.0 → 22.0	8.0	150
Redside shiner	10	0.99	0.05	6	20.6	8.0	158
Blackside dace	8	0.02	0.003	10	19.6	8.0	155
	5	0.33	0.05	6	19.6	8.0	155
Bluegill	8	0.85	0.08	6	25.2	8.1	150

TABLE 5. SPECIES, DURATION, AND SIZE OF ANIMALS IN PARTIAL CHRONIC TESTS.

Species	Test no.	Duration of experiment	Mean wet weight or mean carapace length per animal
Brook trout	1	December 4, 1973 to January 11, 1974	65.4 g
	2	August 9, 1974 to March 12, 1975	57.7 g
Cutthroat trout	1	January 14 to August 5, 1974	79.4 g
	2	August 23, 1974 to May 26, 1975	79.9 g
Crayfish	1	August 20, 1974 to May 1, 1975	41.1 mm
	2	August 14, 1975 to August 14, 1976	40.5 mm

In tests with crayfish, each animal was isolated by partitioning each aquarium into 10 compartments with plastic screens. Only during the mating period were male and female crayfish placed together, each male being placed with a particular female. After mating, the animals were again isolated. The number of females extruding clusters of embryos was recorded. The survival of adults and embryos was checked daily.

In each partial chronic experiment, four concentrations of total residual chlorine plus a well water control were tested, and each was replicated (Table 6). Monochloramine was the predominant species of chlorine in each experiment, this averaging about 90.3, 87.8, and 90.1 percent of the total residual chlorine in experiments with brook trout, cutthroat trout, and crayfish, respectively. Concentrations of the toxicant in the test aquaria were determined 3 to 7 times each week during each experiment.

In tests with trout, water temperature was approximately 12 C in July and August, and near 10 C during other months. In crayfish tests, the water was heated in summer to approximately 16 C, gradually decreased to ambient (10 C) level by January, and then gradually increased to 16 C by the following summer. The pH ranged from 7.0 to 8.1 in tests with trout and ranged from 7.5 to 8.1 in crayfish tests. Total alkalinity ranged from 130 to 150 mg/l (as $CaCO_3$) in all tests. Dissolved oxygen concentrations were always slightly less than 100 percent of the air saturation level. Illumination at the water surface of the test aquaria was about 175 lux.

Adult trout were fed Oregon Test Diet and adult crayfish were fed a mixture of Oregon Test Diet and alfalfa. The diet of the crayfish was supplemented with pieces of frozen herring.

METHODS FOR DETERMINING CHRONIC TOXICITY: SURVIVAL, DEVELOPMENT, AND GROWTH

It is not always possible, or even appropriate, to subject a species to a toxicant under chronic or partial chronic test conditions. It may be more practical or appropriate to expose particular life stages of the species to a toxicant at concentrations and for periods of time similar to exposures these stages would have in such tests. For anadromous salmonid species, for example, it would seem appropriate to conduct tests on the freshwater life stages, because it is during this period that the species are most likely to be exposed to particular toxicants. We determined for coho salmon--whose progeny spend about one year in fresh water before migrating to sea--the effects of exposures to inorganic chloramines on the survival, development, and growth of embryos and alevins, and on the growth of juveniles, which were not at the migratory stage of their development.

Coho salmon embryos and alevins were exposed to concentrations of total residual chlorine of 47 μg/l and lower (Table 7). Survival and development of the embryos, size of alevins at hatching, and time of hatching, as well as subsequent survival and growth to yolk sac absorption were determined. Immediately after eggs were fertilized in well water on November 22, 1973, about 300 embryos (estimated by volume) were placed in each of 12 chambers designed so that water flowed continuously upward from the chamber bottoms to

TABLE 6. MEAN CONCENTRATIONS (AND STANDARD DEVIATIONS) OF INORGANIC CHLORAMINES, IN MICROGRAMS PER LITER, TO WHICH GROUPS OF TEST ORGANISMS WERE SUBJECTED DURING THE PARTIAL CHRONIC EXPERIMENTS.

Group	Brook trout		Cutthroat Trout		Crayfish	
	1973-74	1974-75	1974	1974-75	1974-75	1975-76
A	0 (control)	0	0	0	0	0
B	5.3 (1.0)	5.4 (5.0)	4.3 (1.3)	5.9 (2.4)	38.7 (15.9)	25.2 (5.4)
C	11.0 (1.6)	10.7 (3.7)	9.2 (3.0)	10.5 (3.3)	80.1 (33.7)	48.6 (9.5)
D	21.7 (2.9)	21.8 (7.2)	23.1 (7.0)	18.8 (6.4)	170.3 (73.5)	99.4 (20.4)
E	44.7 (5.3)	50.1 (15.7)	54.9 (19.9)	48.4 (16.2)	373.4(124.0)	194.0 (46.2)

pass around the developing embryos (Larson et al., in press). Each of four test concentrations and for the two controls were replicated (Table 7). The chambers were kept in light proof boxes to approximate natural streambed light conditions. Temperature was maintained between 9.8 and 10.5 C; total alkalinity and pH were about 135 mg/l and 7.5.

TABLE 7. MEAN CONCENTRATIONS (AND STANDARD DEVIATION) OF RESIDUAL INORGANIC CHLORAMINES, IN MICROGRAMS PER LITER, TO WHICH GROUPS OF COHO SALMON WERE SUBJECTED DURING THE EMBRYO-ALEVIN EXPERIMENT.

Group	TRC (μg/l)
A	0 (well water control)
B	0
C	5.2 (1.0)
D	11.3 (1.9)
E	22.7 (3.3)
F	47.0 (6.1)

The instantaneous growth rates of the alevins in each chamber were determined by randomly removing 25 alevins each week, from January through February 11, and then dividing them into five groups of five fish each. The yolk sacs were removed from the alevins and then each group of animals was dried at 70 C for 7 days before dry weight was determined. Instantaneous rates (k) were calculated for each group using the following formula:

$$k = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1}$$

where W_2 was the final dry weight of each group of five fish at time T_2 and W_1 was the initial dry weight at time T_1.

The caloric content of alevins (without yolk) was determined on January 28 and February 4 and 11, and that of the yolk was determined on the two February sampling dates, by means of a Parr Oxygen Calorimeter and standard methods. To determine the content in the yolk, one group of five alevins was removed from each chamber on each sampling date, dried, weighed, and then oxidized in the calorimeter. The caloric content in the yolk was determined by the difference in the content of alevins without yolk and those with yolk.

At the conclusion of above experiment, each of the remaining groups of fish (now fry) was transferred to a 10-liter plastic aquarium and exposed to the concentration of toxicant to which it had been previously exposed. These fish were fed a daily ration of Oregon Test Diet and were later used in the first growth experiment with juveniles, which began on May 1, 1974.

One purpose of the first growth experiment was to compare effects of the toxicant on the growth of juvenile fish exposed continuously to the toxicant since fertilization to effects on fish exposed only during the growth test. A second purpose of this experiment was to determine if there were any residual effects of the toxicant on the growth of fish in water containing no toxicant after these fish had been exposed to the toxicant since fertilization. To do this, control fish from the embryo-alevin experiment were divided into eight lots of 15 fish each (referred to as Group A) and exposed to one of three concentrations of toxicant or well water or control conditions (Table 8). Furthermore those fish which had been exposed to 5 or 11 μg/l total residual chlorine in the embryo-alevin experiment were divided into two lots of 15 fish each (referred to as Group B) and exposed to nearly the same concentrations of the toxicant in the growth test. In both groups (A and B), one lot of fish at each toxicant concentration and control was fed Oregon Test Diet at a rate of 4 percent of their initial dry weight each day, while the other lot was fed at a rate of 8 percent. The initial weights were estimated from the weight of a lot of 15 fish that had been dried at 70 C for seven days at the beginning of the experiment. Finally, a third group (C) of fish was used to estimate the residual effects of the toxicant on the growth of fish that had been exposed to 5, 11, or 23 μg/l total residual chlorine in the embryo-alevin experiment. This was done by placing one lot of 15 fish from each concentration and control into water containing no toxicant and feeding them at the 8 percent ration level (Table 8). The temperature of the well water in these tests was maintained at 10 to 11 C, and total alkalinity and pH were about 135 mg/l and 7.5.

TABLE 8. MEAN CONCENTRATIONS OF INORGANIC CHLORAMINES, IN MICROGRAMS PER LITER, TO WHICH GROUPS OF COHO SALMON WERE EXPOSED DURING THE EMBRYO-ALEVIN AND THE CONCENTRATION (AND STANDARD DEVIATION) TO WHICH THEY WERE SUBJECTED AS JUVENILES DURING THE FIRST GROWTH EXPERIMENT.

Group	Embryo-alevin test	First growth test
A	0 (control)	0 (control
		4.9 (2.8)
		10.9 (4.2)
		22.3 (8.9)
B	5.2	4.9 (2.8)
	11.3	10.9 (4.2)
C	0	0
	5.0	0
	11.3	0
	22.7	0

The mean relative growth rate of each lot of fish in the 21-day experiment was determined with the following formula:

$$GR = \frac{W_2 - W_1}{0.5\ (W_1 + W_2)\ \ (T_2 - T_1)}$$

where W_2 was the final dry weight at time T_2 and W_1 was the estimated initial dry weight at time T_1. At the end of the experiment, the fish were not fed for 24 hours before being sacrificed, dried at 70 C for seven days, and then weighed. Gross efficiency of food conversion for each lot of fish was calculated by dividing the dry weight of food eaten into the change in dry weight of the fish during the test. Food consumption was determined by subtracting the dry weight of uneaten food from the dry weight of the food offered to each lot of fish. Each day the uneaten food was removed from each aquarium before that day's ration was fed to the fish.

The purpose of the second growth test, which began on January 10, 1975 and lasted for 14 days, was to determine the effects of residual chlorine on the growth of juvenile coho salmon not previously exposed to the toxicant. These fish were older and larger than those used in the previous growth test. The fish were divided into 12 lots of eight fish each and exposed to either well water (control) or to one of three concentrations of inorganic chloramines (Table 9). At each treatment, one lot was fed a ration (Oregon Test Diet) of 3 percent of their estimated initial dry body weight, another was fed a 6 percent ration, and a third lot was fed to repletion each day. The methods for feeding, removing the uneaten food, and for calculating the growth of each lot of fish were the same as those described above, except the fish were starved for 48 hours before each lot was sacrificed, dried, and weighed. Quality of the well water used in this study was as follows: temperature, 15 C; total alkalinity, 135 mg/liter; and pH, 7.5.

TABLE 9. MEAN CONCENTRATIONS (AND STANDARD DEVIATIONS) OF INORGANIC CHLORAMINES, IN MICROGRAMS PER LITER, TO WHICH GROUPS OF COHO SALMON JUVENILES WERE EXPOSED DURING THE SECOND GROWTH EXPERIMENT.

Group	TRC (μg/l)
A	0 (control)
B	5.2 (1.2)
C	9.9 (3.6)
D	23.2 (8.0)

METHODS FOR DETERMINING EFFECTS ON BEHAVIOR

Observations on changes in behavior of bass were made during each acute toxicity test under conditions of intermittent exposure to free residual chlorine. Most of the observations were made during the exposure period, other observations being made at 24-hour intervals while the fish were held in toxicant-free water for the remainder of the 96-hour tests. The time of first occurrence of particular behavioral responses was usually recorded. These responses are described in the appropriate section under Results and Interpretation. In tests with cutthroat trout, brown bullhead, bluegill, blackside dace, and redside shiner, qualitative observations of behavior were made according to the procedures outlined for bass.

During the intermittent exposure experiments, the behavior of largemouth bass was altered even when exposures to chlorine were not acutely toxic. Thus we attempted to determine the threshold concentrations at which the bass exhibited particular behaviors, when subjected to intermittent exposure to chlorine (97 percent free residual) in glass aquaria. The test conditions were those described in the section entitled Methods for Determining Acute Toxicity : Intermittent Exposure. The behavior of individual bass was continuously observed during each exposure, either a 90-minute square pattern or a 63-minute spike pattern, and then for one to two minutes at 24-hour intervals for the remainder of the 96-hour tests. In most cases, two fish were tested together, one having a dry weight of about 14 grams and the other about 7 grams. Paired fish were kept together in a test aquarium for five days prior to being tested. The fish were fed a daily ration of Oregon moist pellets supplemented with pieces of frozen herring, but they were not fed for 24 hours before being tested and during the tests.

Effort expended by fish to maintain their position in flowing water could affect their tolerance to toxicants. We subjected bass, having nearly the same body weight as those used in the intermittent exposure regime experiment to similar exposures to chlorine under conditions in which the fish were forced to swim against a current. In each test, individual bass were acclimated to the test conditions (except for toxicant) for 60 minutes, and then the fish were subjected to either a 90-minute square pattern of exposure or a 60-minute spike pattern of exposure. For the latter, peak concentrations of total residual chlorine occurred at 29 minutes after the tests were started. After being subjected to either type of exposure, the fish were kept in the experimental apparatus for 24 hours before being transferred to 45-liter glass aquaria in which the toxicant was not present for the remainder of the 96-hour tests. The behavior of the bass was continuously observed during the exposures and for one to two minutes at 24-hour intervals thereafter.

The experimental apparatus used in these studies is shown in Figure 1. It consisted of a half-round aluminum trough, painted light blue, with two straight sections connected at the ends with semi-circular sections. The fish were tested in a straight section of the trough. Two stainless steel screens, one at each end of the test section, restricted the movements of the fish to within the section. A piece of styrofoam was placed over a portion of the test section to provide cover for the fish. Water current in the trough was maintained at 13.4 centimeters per second with a submersible pump. Toxicant was added upstream from the test section for these experiments on the effects of swimming activity on tolerance of the fish for chlorine. The animals were continuously subjected to the toxicant during each exposure and were unable to avoid exposure.

Although aquatic organisms are often unable to avoid exposure to toxicants in nature, there is evidence that fish may be able to avoid or move out of areas receiving intermittent discharges of chlorine (Basch and Truchan, 1976). In view of this, the following experiment was conducted with largemouth bass given the opportunity of either remaining in intermittent discharges of chlorine or moving into water containing no toxicant. Our purposes were to determine if bass would avoid such exposure conditions and, if so, whether or not such avoidance was correlated with the concentrations of the toxicant. Exposures to either inorganic chloramines or free residual chlorine were tested in order to determine if fish responded differently to these forms at similar concentrations of total residual chlorine.

In this experiment, bass having a mean dry weight per fish of 9.18 grams were tested individually on six successive days. The experimental apparatus was designed so that the fish had freedom of movement between a section into which the toxicant was introduced and a section without toxicant (Fig. 1). During each of the first three days each bass was acclimated to the apparatus for 30 minutes and then observed for 60 minutes in order to establish the average expected time the fish spent in the two sections of the apparatus. During the next three days, the same procedure was followed each day, except that toxicant was introduced during the 60-minute observation (exposure) period in each test. Then, after toxicant was no longer present, the section of the apparatus in which each fish was located 15 minutes after each 60-minute exposure was recorded. All observations were made through a small opening in a black plastic sheet which surrounded the apparatus. Each fish was kept in a separate 45-liter glass aquarium for one week prior to being tested and during periods between tests. The fish were fed a daily ration of Oregon moist pellets, supplemented with pieces of frozen herring, about 24 hours before being tested. At the conclusion of each series of tests on a fish, it was starved for an additional 24 hours and then sacrificed, dried, and weighed.

Changes in the amount of time each fish spent in the two section of the apparatus, between tests without toxicant and those with toxicant, were determined using the following formula (after Tsai and Fava, 1975):

$$\text{Percent change} = 100\left(1 - \frac{T_t}{T_c}\right)$$

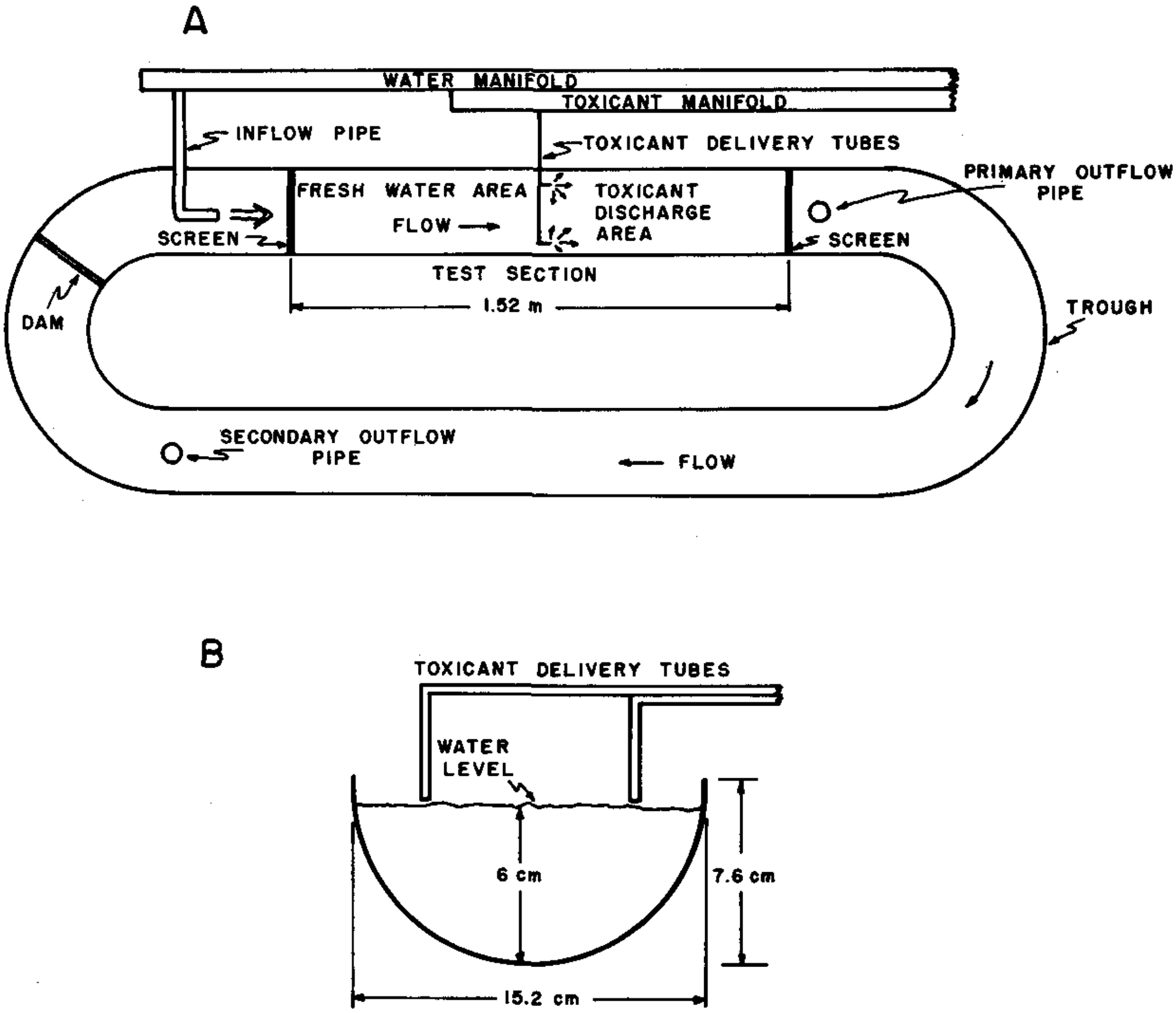

Figure 1. Schematic diagrams of the experimental apparatus used in the avoidance experiment with largemouth bass: (A) top view of the entire apparatus; (B) cross sectional view of the middle of the test section.

where T_t was the total time spent in the discharge section divided by the observation (exposure) time (60 minutes) in each test with toxicant, and T_c was the mean time spent in the toxicant section divided by the observation time in each test without toxicant. In addition to determining changes in the time fish spent in the two sections, a comparison was also made between the actual amount of exposure each fish received and the total possible exposure in each test with toxicant. This was represented by means of the following formula:

$$\text{Exposure} = \frac{T_s \times T_e}{T_c}$$

where T_s was the time spent in the toxicant section, T_e was the total possible exposure (expressed as mg/l x minutes) under the time-concentration curve, and T_c was the total possible time of exposure (60 minutes).

In each toxicant test, the maximum concentration of inorganic chloramines or free residual chlorine in the toxicant section was reached within a minute after the test began, and the concentration dropped to zero within a minute after toxicant introduction was terminated. The water current in the control and toxicant sections was about 4 cm per second, a velocity found on the basis of dye studies to be sufficient to keep toxicant out of the control section. The concentrations of toxicant in the toxicant section of the apparatus were determined by analysis of samples taken from the primary outflow pipe (Fig. 1) at 1 to 10-minute intervals during each 60-minute test with toxicant. Samples of test solutions used in free residual chlorine experiments contained a small percentage of inorganic chloramine, but solutions of inorganic chloramines did not contain detectable amounts of free residual chlorine (Table 10).

TABLE 10. FORMS OF RESIDUAL CHLORINE COMPOUNDS PRESENT IN THE INTERMITTENT EXPOSURE EXPERIMENTS AND THEIR MEAN PERCENTAGE (AND STANDARD DEVIATION).*

Form of residual chlorine	Percent of total residual chlorine: Tests using free residual chlorine	Percent of total residual chlorine: Tests using inorganic chloramines
Free chlorine	94.2 (1.1)	0
Monochloramine	5.1 (1.1)	97.2 (1.2)
Dichloramine	0.7 (0.7)	2.8 (1.1)

* Average water quality of the well water in each test was as follows: Temperature, 25C; pH, 7.9; dissolved oxygen, 7.5 mg/l; total alkalinity (as $CaCO_3$), 145 mg/l, and hardness, 126 mg/l.

LABORATORY STREAM COMMUNITY METHODS

Communities of aquatic plants and animals in laboratory streams have provided a useful way to study possible influences of toxicants on communities in receiving waters. In this portion of our program, 12 separate stream communities were established to determined the influence of inorganic chloramines on the biomasses of algae and invertebrate animals and on the production of juvenile coho salmon at TRC concentrations of about 20 μg/l or less.

The laboratory streams used in these studies were similar in design to those described in detail by McIntire et al. (1964). Each stream was contained in a plywood trough 3.3 m long, 66 cm wide, and 25 cm deep, divided into two channels by a median plywood partition open at each end to permit the water to circulate. The floors of the troughs were covered with gravel and stones in such a way as to form a pool at each end alternating with riffles along the straight channels. Electrically driven paddle wheels provided current velocities up to about 24 cm/sec over the riffles. The water in each stream was exchanged at about 2 liters per minute with sand-filtered creek water.

Benthic organisms were initially established in the streams by repeated stocking with organisms collected from nearby creeks, by egg deposition of adult insects, and possibly by some immigration of algal cells and invertebrate animals through the water supply. Colonization began in September 1974, and by April of the following spring the stream communities were well established. The salmon were not introduced into the streams until after the colonization period.

Benthic organisms, including macro-invertebrates and periphyton, were sampled at approximately three-week intervals. A 0.093 m (1 ft^2) section of stream bottom was sealed off with two partitions and the enclosed substrate was scrubbed to remove attached organisms. After cleaning, the gravel was removed and the water containing organisms was siphoned into a 100-micron mesh plankton net. The sample was then divided into two parts with a soil-type sample splitter. Half of the sample was returned to the stream, the other half being retained and frozen until the macro-invertebrates could be removed with the aid of a binocular microscope. The remaining sample materials were then analyzed for chlorophyll A, B, and C and for the amount of organic matter, determined by loss-on-ignition, by means of methods described by Strickland and Parsons (1968).

Juvenile coho salmon were each marked by a "cold brand" (Everest and Edmundson, 1967) to identify individual fish. From a group of fish acclimated to the dilution water for at least two weeks, fish were selected for uniformity in body size. These were starved for 48 hours to eliminate differences in the weights owing to stomach contents, anesthetized in MS-222, blotted dry, and weighed in a tared water bath on a top-loading balance accurate to 0.05 grams. Weighed fish were then distributed to each of the 12 streams at the beginning of the experiments. At the end of each experiment, and at about 21 day intervals, the fish in each stream were removed and reweighed. Mean relative growth rates and production rates (growth rate x mean biomass) were calculated.

RESULTS AND INTERPRETATION

ACUTE TOXICITY OF CHLORAMINES WHEN EXPOSURE CONTINUOUS

Over the past two decades, the 96-hour acute toxicity bioassay has become the most generally employed means of evaluating and setting standards for toxic materials introduced into aquatic systems. It is generally recognized that concentrations of toxic substances near the 96-hour median lethal concentration (LC50) cannot be permitted over appreciable areas or for more than a few hours, if aquatic life is to be protected. In consequence, there has developed a tendency to adopt some fractional proportion of the 96-hour LC50 of particular toxic substances in standards for the protection of particular aquatic species. There is no sound empirical or theoretical basis for this practice, because the relationships between concentrations leading to death in a short period of time and those affecting important biological responses such as reproduction and growth have not been and cannot be expected to be generally representable by simple coefficients or "application factors" (Warren, 1971). It is only in the absence of adequate empirical and theoretical knowledge that use of application factors can be justified as a necessary expedient in the first stages of dealing with pressing problems of water pollution control. Nevertheless, acute toxicity bioassays have had and will continue to have an important role in the solution of these problems. They do provide one possible means for biologically assaying the relative toxicities of substances and effluents and for determining the relative sensitivity of different species and life history stages to acutely toxic conditions. And they also permit some evaluation of the effects of other water quality conditions on the toxicity of substances and effluents. The acute toxicity of chloramines to different life stages of coho salmon, brook trout, and cutthroat trout under conditions of continuous exposure are considered in this section. Influences of other water quality conditions on acute toxicity of chloramines are also considered. In the next section, the acute toxicity of free residual chlorine to largemouth bass and other species of fish when exposure was intermittent will be discussed.

The acute toxicity of chloramines to coho salmon, brook trout, and cutthroat trout, as measured by 96-hour LC50's, is a function of the developmental stage and body size of the fish. Alevins (yolk sac extending outside the body cavity) are more tolerant than are late fry (yolk sac inside the body cavity) and early juvenile stages (Fig. 2A; Table 11, 12). Median lethal concentrations of chloramines for very small juvenile coho salmon ranged from 57 to 66 μg/l (Table 11). With increasing body size, the 96-hour LC50's for juveniles of this species increased to about 80 μg/l, near the tolerance level of the alevin stage (Fig. 2A; Table 11). In developing from alevin through fry and then into juvenile stages, brook

TABLE 11. LIFE HISTORY STAGE, MEAN DRY WEIGHT PER FISH, WATER QUALITY CONDITIONS, AND THE 96-HR LC50 OF INORGANIC CHLORAMINES IN EACH ACUTE TOXICITY TEST WITH COHO SALMON. DRY WEIGHTS OF ALEVINS AND FRY DO NOT INCLUDE YOLK.

Life stage	Mean dry weight per fish (g)	Temp. (C)	Alkalinity (mg/l)	pH	96-hr LC50 TRC (μg/l)
Alevin	0.0238	9.9	135	7.4	83
Alevin	0.0549	10.2	135	7.6	80
Fry	0.0727	10.3	135	7.4	79
Juvenile	0.123	10.5	135	7.6	66
		15.0			62
Juvenile	0.114	10.8	135	7.6	57
		15.0			57
Juvenile	0.227	15.0	135	7.5	64
				7.0	72
Juvenile	0.210	15.0	135	7.0	72
				7.5	72
				8.1	53
Juvenile	0.234	15.0	135	7.0	72
				7.5	72
				8.2	60
Juvenile	0.240	15.0	135	7.5	71
				8.1	65
Juvenile	1.150	15.0	135	7.5	82
			316		82
Juvenile	1.550	15.0	135	7.5	82
			322		82
Juvenile*	1.530	15.0	135	7.4	81

* 1973 year-class; all other groups from 1974 year-class

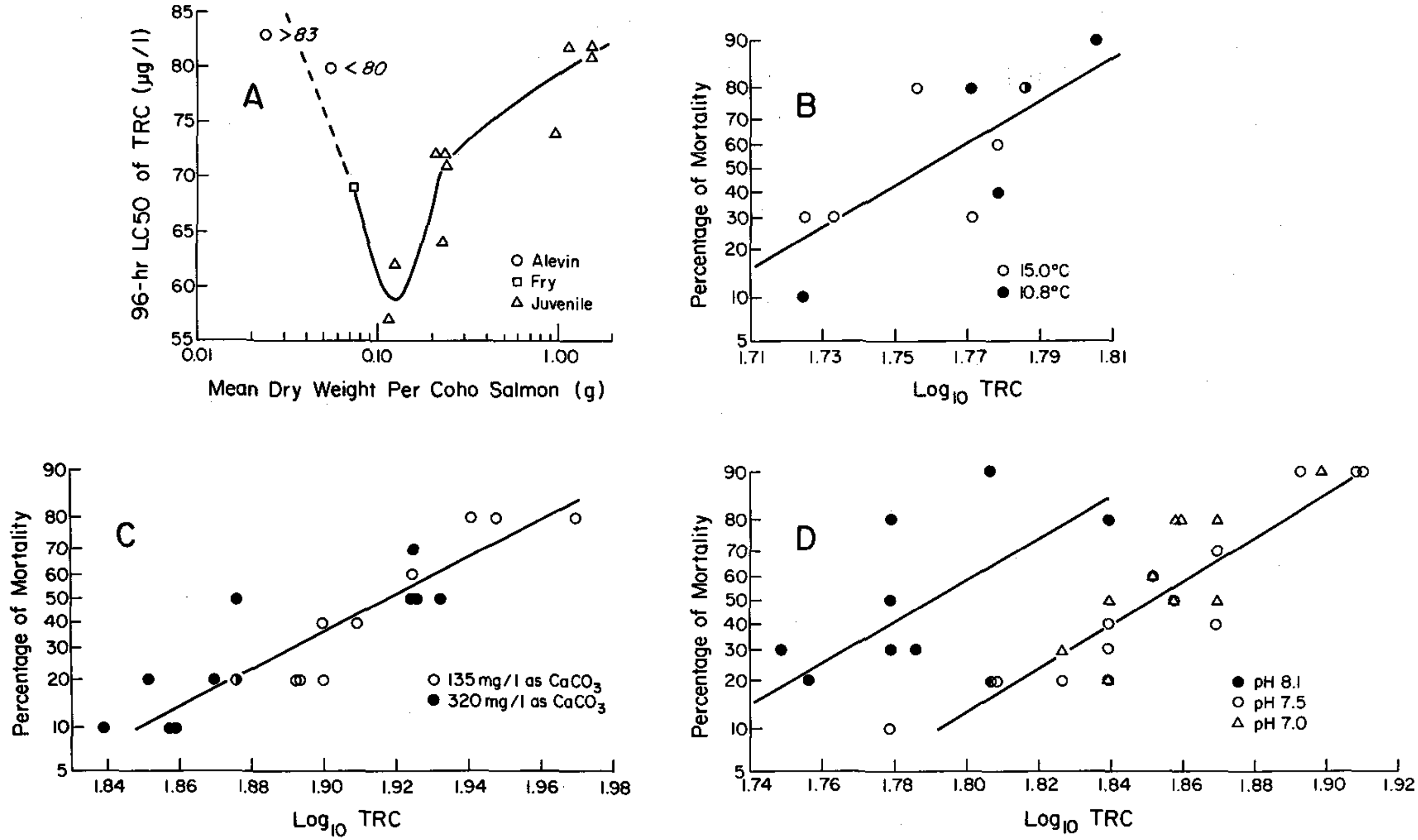

Figure 2. Acute toxicity of inorganic chloramines (TRC) to coho salmon in 96-hour tests as affected by: (A) fish weight; (B) temperature; (C) total alkalinity; and (D) pH. Toxicant concentrations (µg/l) on the abscissal axes in B, C, and D are expressed as base 10 logarithms.

trout exhibited the same pattern of initial decrease and then increase in tolerance to chloramines (Table 12). Figure 3 clearly illustrates the increase in tolerance of juvenile cutthroat trout with increase in body weight.

TABLE 12. LIFE HISTORY STAGES, MEAN DRY WEIGHT PER FISH, WATER QUALITY CONDITIONS, AND THE 96-HR LC50 OF INORGANIC CHLORAMINES IN EACH ACUTE TOXICITY TEST WITH BROOK TROUT AND CUTTHROAT TROUT. DRY WEIGHTS OF ALEVINS AND FRY DO NOT INCLUDE YOLK.

Species	Life Stage	Mean dry wt per fish (g)	Temp (C)	Total alkalinity	pH	96-hr LC50 TRC (μg/l)
Brook trout	Alevin	.006	10.1	148	7.7	insufficient deaths
	Alevin	.006	10.6	142	7.8	105.5
	Alevin	.009	10.8	180	7.8	90.6
	Fry	.012	10.8	180	7.8	81.8
	Juvenile	.041	11.3	150	7.8	90.6
	Juvenile	.983	11.1	130	7.7	88.4
Cutthroat trout	Juvenile	.546	15.1	145	7.7	74.5
	Juvenile	.761	12.3	155	7.7	81.7
	Juvenile	.803	12.7	143	7.7	83.1
	Juvenile	1.255	12.3	155	7.7	94.7
	Juvenile	1.297	10.1	150	7.7	94.0

Temperature was not found to affect the acute toxicity of chloramines to juvenile coho salmon significantly ($P > 0.05$), in tests conducted at 10.8 C and 15.0 C (Fig. 2B). Total alkalinity, in tests conducted at 135 mg/l and 320 mg/l, did not affect significantly the toxicity of chloramines to the juvenile salmon (Fig. 2C). Although there was no significant difference in the acute toxicity of chloramines tested at pH 7 and pH 7.5, toxicity was significantly higher at pH 8.1 (Fig. 2D).

Extensive monitoring of stabilized kraft pulp and paper mill effluents (SKME) at our laboratory has demonstrated that, when biologically stabilized to levels of BOD near 15 mg/l, these effluents extremely rarely result in any mortality in 96 hours, even when tested at 100 percent concentration (Robinson-Wilson and Seim, MS). But the addition of 1 mg/l of chloramines to SKME in one test resulted in acute toxicity of the effluent at a dilution of 18 percent by volume (Table 13). In this and the other three acute toxicity tests conducted, the results were inadequate to estimate 96-hour LC50's, but the effluent was rendered toxic. In three of the tests the concentration range within which the LC50's occurred could be estimated (Table 13). After mixing with the effluent, no residual chloramines could be detected by amperometric titration, when chloramines were added at concentrations of 1.0, 1.7, and 2.3 mg/l.

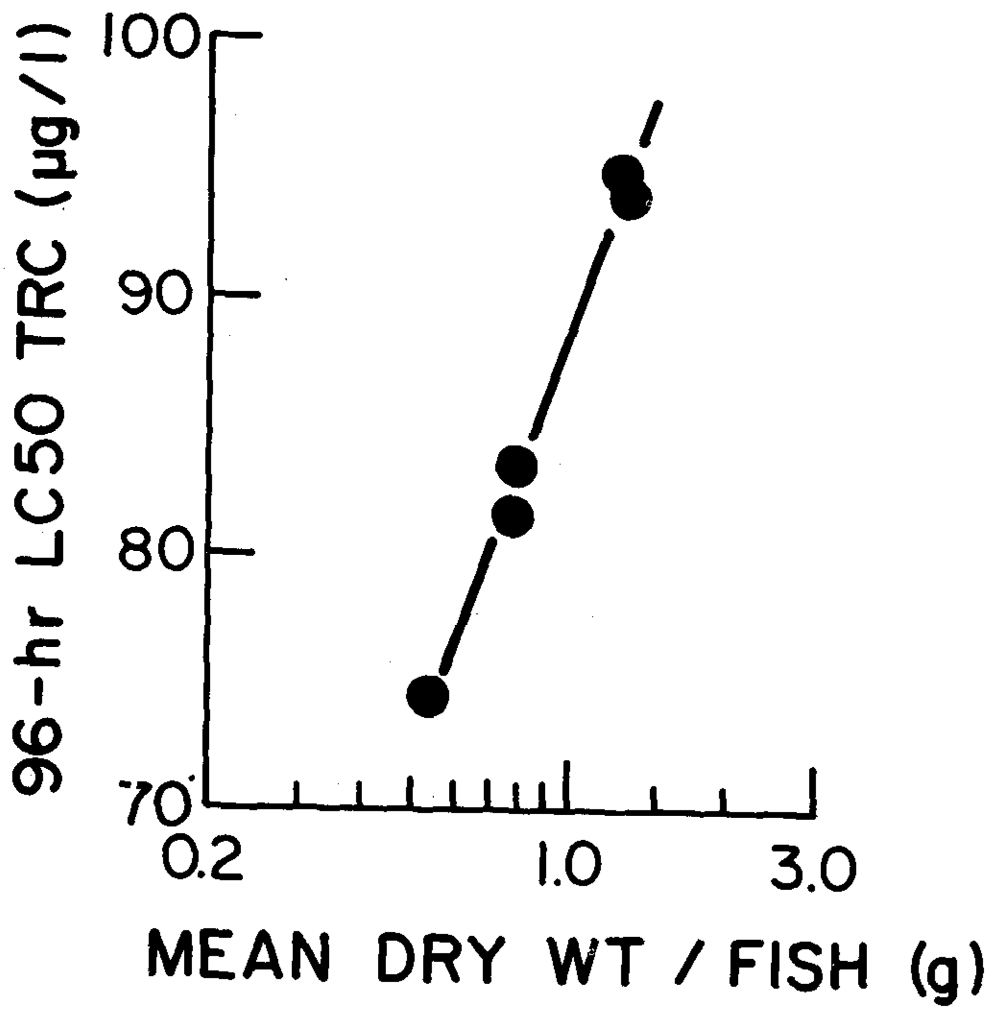

Figure 3. Relationship between the mean dry weight(g) per fish and the 96-hour LC50 of inorganic chloramines (TRC) for juvenile cutthroat trout.

TABLE 13. CONCENTRATIONS OF SECONDARILY TREATED KRAFT PAPER MILL EFFLUENT (SKME) BETWEEN WHICH THE 96-HR LC50 OCCURRED IN CHLORINATED SKME ACUTE TOXICITY TESTS WITH COHO SALMON.*

Starting date of test	Mean dry wt per fish (g)	Milligrams of chlorine added per liter of 100% SKME	Mean temperature (C)			Concentrations of chlorinated SKME between which the 96-hr LC50 occurred (%)
			River water	Non-chlorinated 100% SKME	Chlorinated 100% SKME	
7-17-75	0.14	1.0	19.1	22.1	22.0	18 - 32
7-20-75	0.72	1.7	19.1	23.6	22.0	56 -100
7-30-75	0.60	1.7	16.9	20.6	19.8	insufficient deaths
8-6-74	1.10	2.3	17.7	17.9	17.8	56 -100

*pH ranged from 7.8 to 8.3, dissolved oxygen from 6.1 to 8.9 mg/l, and BOD from 9 to 17.6 mg/l over all tests.

Adult crayfish are much more tolerant of high concentrations of chloramines than are fish in 96-hour tests. In two tests, we were unable to estimate the 96-hour LC50's even though the highest concentration tested was 749 µg/l (Table 14).

ACUTE TOXICITY OF FREE CHLORINE WHEN EXPOSURE INTERMITTENT

When chlorine is employed for slime control in power generation facilities or industrial processes, its introduction is generally intermittent, with some regular intervening period. This is unlike the continuous introduction of this toxicant generally practiced in sewage disinfecting applications. The concentration of chlorine introduced, the duration of introduction, and the frequency of introduction are parameters that can be varied so as to achieve effective slime control. Because concentration, duration, and frequency of chlorine introduction will determine the time pattern of concentrations of chlorine in natural waters and thus exposure of aquatic organisms to this toxicant, it is important to determine what, if any, pattern of utilization of chlorine for slime control could minimize or eliminate harmful effects on fish and yet achieve its objectives. In slime control applications, the total amounts of chlorine employed, when introduction is intermittent, may not be great and, when mixed in most receiving waters, may not result in concentrations high enough to be either acutely or chronically toxic to fish and organisms in their food chains. But in the immediate area of effluent introduction, there may occur concentrations of chlorine and derivative compounds high enough to be acutely toxic on short exposures, to have deleterious effects on the behavior of fish, or to have chronic effects . For these reasons, we have evaluated the effects on largemouth bass of free chlorine at different concentrations and exposure durations and frequencies. Acute toxicity--as measured by survival--and some behavioral effects were investigated with largemouth bass. Preliminary acute toxicity and behavioral studies with bluegill, blackside dace, redside shiner, brown bullhead, and cutthroat trout were also conducted. In this section, the acute toxicity of chlorine under conditions of intermittent exposure will be considered; results and interpretation of behavioral studies will be presented in a later section.

One of the major problems of general application of the results of bioassays of the acute toxicity of chlorine to fish under conditions of intermittent exposure is that the concentration, duration, and frequency of exposure of fish to chlorine are highly variable at different locations, because very different sets of values of these parameters are employed for slime control at different power plant installations. Conceivably, bioassays would need to be employed to determine the effects of slime control practices at each power facility. This would require a rather extensive bioassay undertaking, and the results might not prove to be very useful for setting general standards for the protection of fish. Figure 4 illustrates three general time-concentration curves representing patterns of chlorine concentration that may occur in receiving waters, with a single application of chlorine treatment for slime control. The curves in Figure 4 also illustrate the main time-concentration patterns of chlorine to which we exposed test fish, with single or multiple exposures. We have named these patterns high-spike pattern of exposure (peak about 10 mg/l TRC, mainly free chlorine),

TABLE 14. MEAN DRY WEIGHT (WITHOUT CHELIPEDS) AND CARPACE LENGTH PER ANIMAL, WITH QUALITY CONDITIONS, AND RANGES OF CONCENTRATIONS OF INORGANIC CHLORAMINES TESTED IN EXPERIMENTS IN WHICH INSUFFICIENT DEATHS OCCURRED TO ESTIMATE THE 96-HR LC50 VALUE FOR CRAYFISH.

Test	Date	Mean dry weight per animal (g)	Mean carapace length per animal (mm)	Temp. (C)	pH	Alkalinity (mg/l)	Dissolved oxygen (μg/l)	TRC (μg/l)
1	9-19-74	0.928	23.4	15.1	7.7	140	9.5	306 - 530
2	9-26-74	2.540	33.6	15.3	7.8	143	8.0	449 - 749

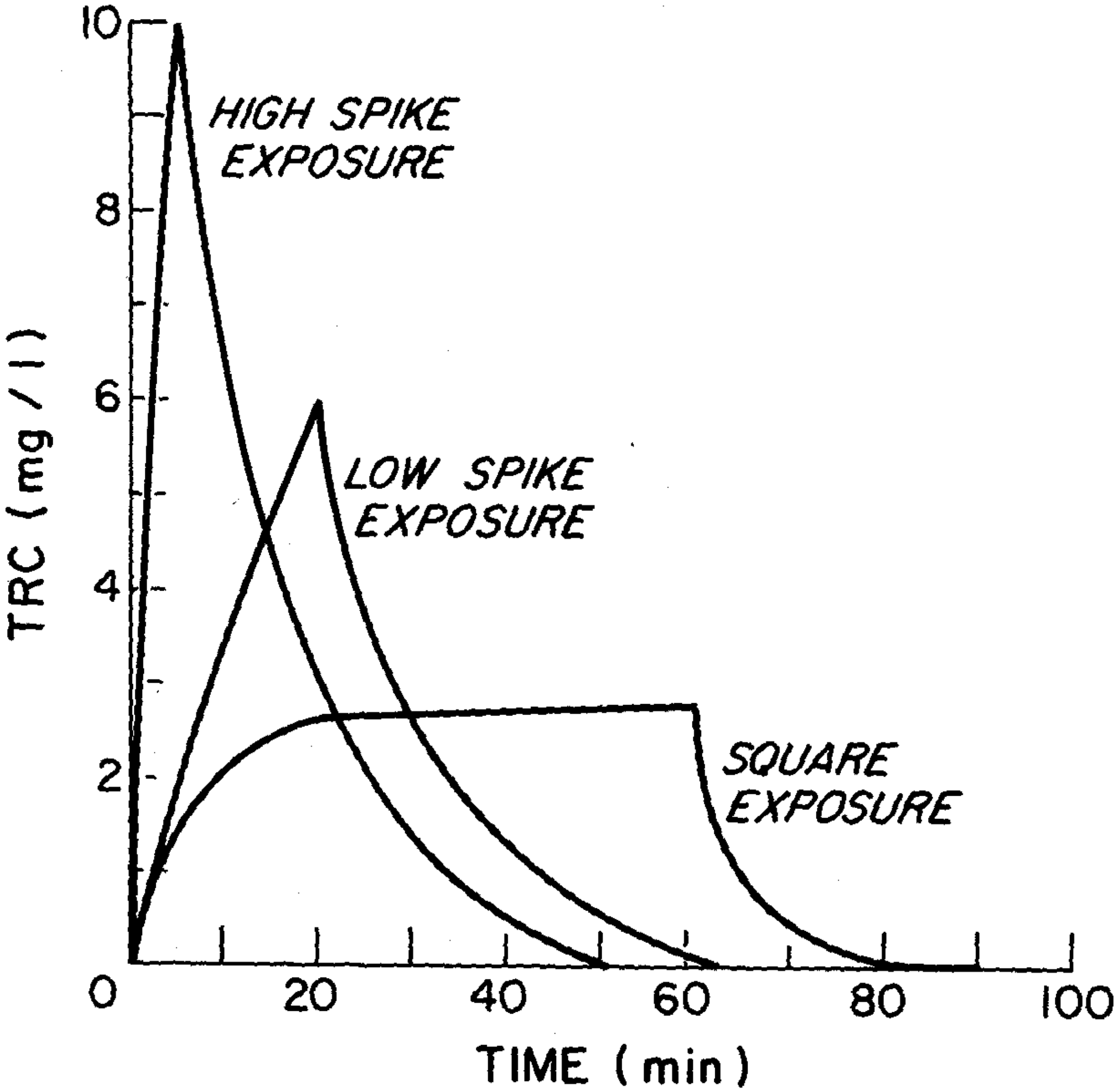

Figure 4. Examples of the time-concentration relationships of the square pattern of exposure and of the high and low spike patterns of exposure to free residual (TRC).

low-spike pattern of exposure (peak about 6 mg/l TRC), and square pattern of exposure (plateau about 3 mg/l TRC). Mortality of fish can be related to peak concentrations, plateau concentrations, mean concentrations, or the time-concentration area of exposure (mg/l x minutes). The first three alternatives are quite acceptable for reporting the results of particular experiments or exposure patterns, but results so reported cannot be generally applied to other patterns. We will present evidence that the last procedure--relating mortalities to the time-concentration area of exposure--yields very similar results for different exposure patterns, at least for those we tested. This result could be very important for generalization of the results of tests conducted on particular patterns and setting general standards based on such tests. More extensive work should be conducted to confirm this and to clearly establish its parametric boundaries, if reliable application of this procedure is to be made.

Figure 5 presents the results of an experiment in which different groups of largemouth bass were exposed to what we have called high-spike pattern of exposure, low-spike pattern of exposure, and square pattern of exposure. The peaks of the high-spike patterns ranged from 8.21 to 11.93 mg/l of chlorine; those for the low-spike patterns from 5.73 to 9.06 mg/l; and the mean plateau concentrations for the square patterns ranged from 2.35 to 3.32 mg/l. Even so, the relationships between mortality of the largemouth bass and the area under the time-concentration exposure curve were not significantly different for the three quite different exposure patterns (Fig. 5).

Similarly, mortality of largemouth bass was closely related to total area beneath time-concentration exposure curves, when the fish were exposed to two 90-minute square patterns or to one 90-minute and one 150-minute square pattern, with two-hour intervals between exposures (Fig. 6 C, F). This was so even though clearly distinct mortality-concentration relationships were found when the mortalities were graphed in relation to mean plateau concentration (Fig. 6 A, D) and to mean concentration during the entire exposure (Fig. 6 B, E). Again, area under the time-concentration exposure curve appears to be a way of generalizing the results of different patterns of exposure to chlorine. It should, however, again be noted that the interval between these exposures was only two hours, which was clearly not enough time for recovery from chlorine intoxication of the exposed fish. Were much longer recovery periods to have occurred, it is quite likely that relationships between mortalities and total areas under successive exposure curves would not be so well defined.

Both test temperature and body size were shown to influence the tolerance of largemouth bass to chlorine when exposed to a single square pattern. The fish were much more tolerant at 13.1 C than at 24.3 C, and were least tolerant when acclimated at 13.1 C and exposed to chlorine at 24.3 C (Fig. 7). And bass having a mean weight of 5.93 grams were more tolerant to chlorine than bass weighing 3.75 grams (Fig. 8).

Mortality of cutthroat trout occurred, under conditions of the square pattern of exposure, when the fish were exposed just once for 90 minutes to a pattern having a mean plateau concentration of about 985 µg/l (Table 15).

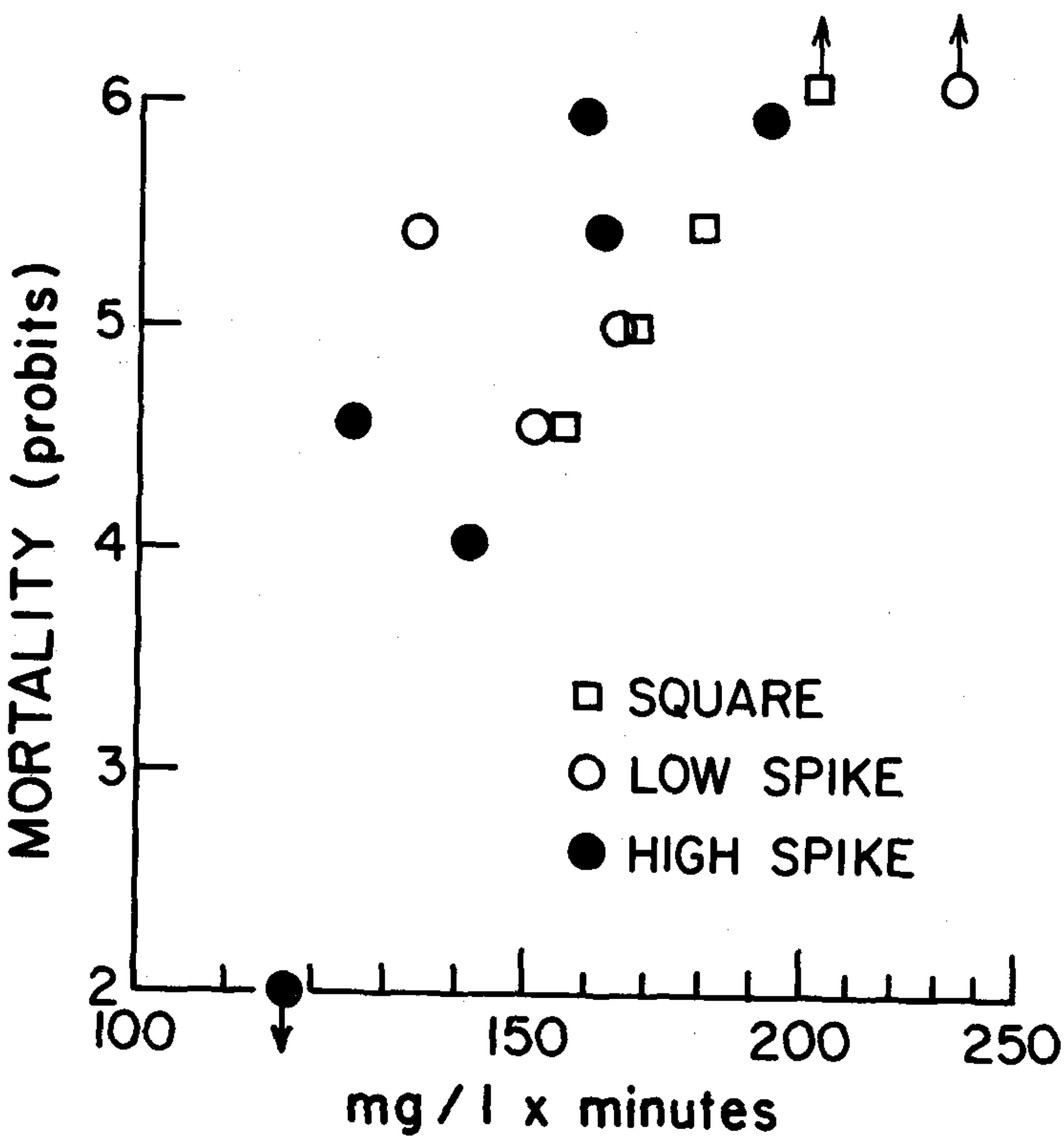

Figure 5. Relationships between mortality of largemouth bass (in probits) and the area under the time-concentration curve (expressed as mg/l x minutes) for fish subjected to square and high and low spike exposures to free residual chlorine (TRC). Data points with arrows pointing up indicate that all of the fish died; those with arrows pointing down indicate that no fish died during the test.

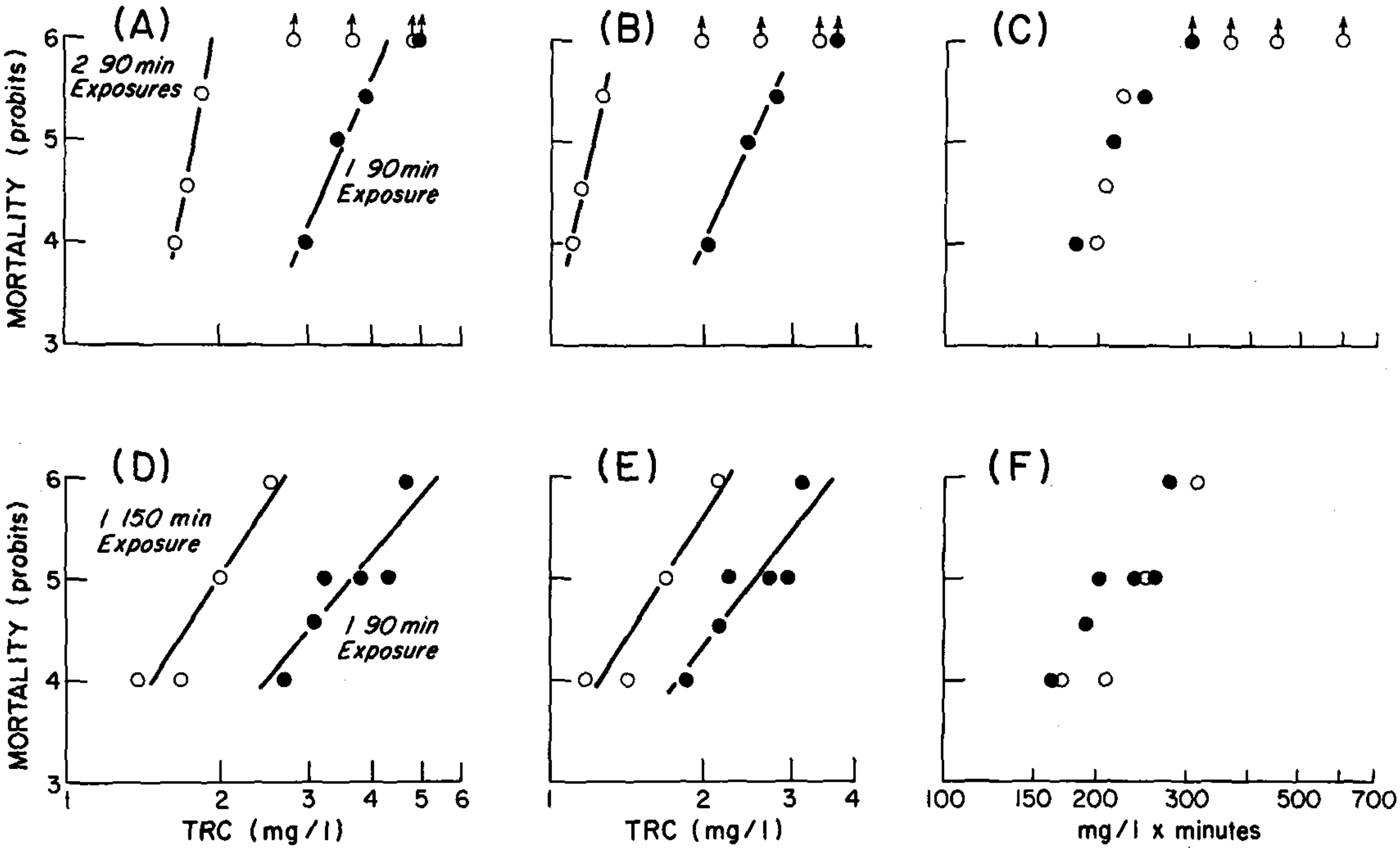

Figure 6. Relationships between mortality of largemouth bass (in probits) and the mean plateau concentration of free residual chlorine (A and D), mean concentration for the entire exposure (B and E), and area, expressed as mg/l x minutes under the time-concentration curve (C and F) for bass subjected to one or two 90-minute square patterns of exposure, or to one 90-minute or one 150-minute square pattern of exposure. Data points with arrows pointing up indicate that all of the fish died during the test.

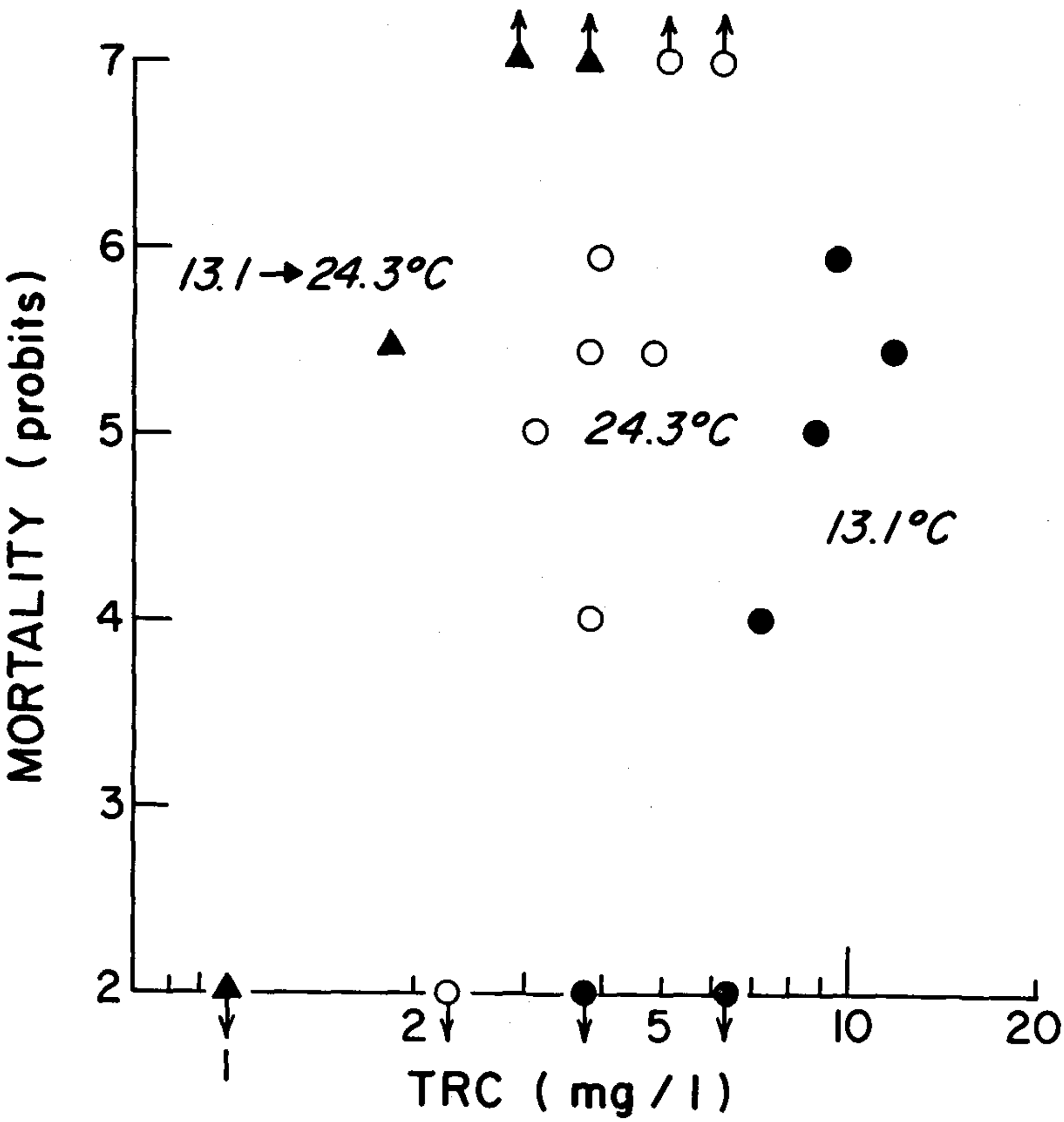

Figure 7. Relationships between mortality of largemouth bass (in probits) and the mean plateau concentrations of free residual chlorine (TRC) in 90-minute square patterns of exposure for bass acclimated and exposed to the toxicant at either 13.1 or 24.3 C and for bass acclimated to 13.1 C and exposed to the toxicant at 24.3 C. Data points with arrows pointing up indicate that all of the fish died; data points with arrows pointing down indicate that no fish died during the test.

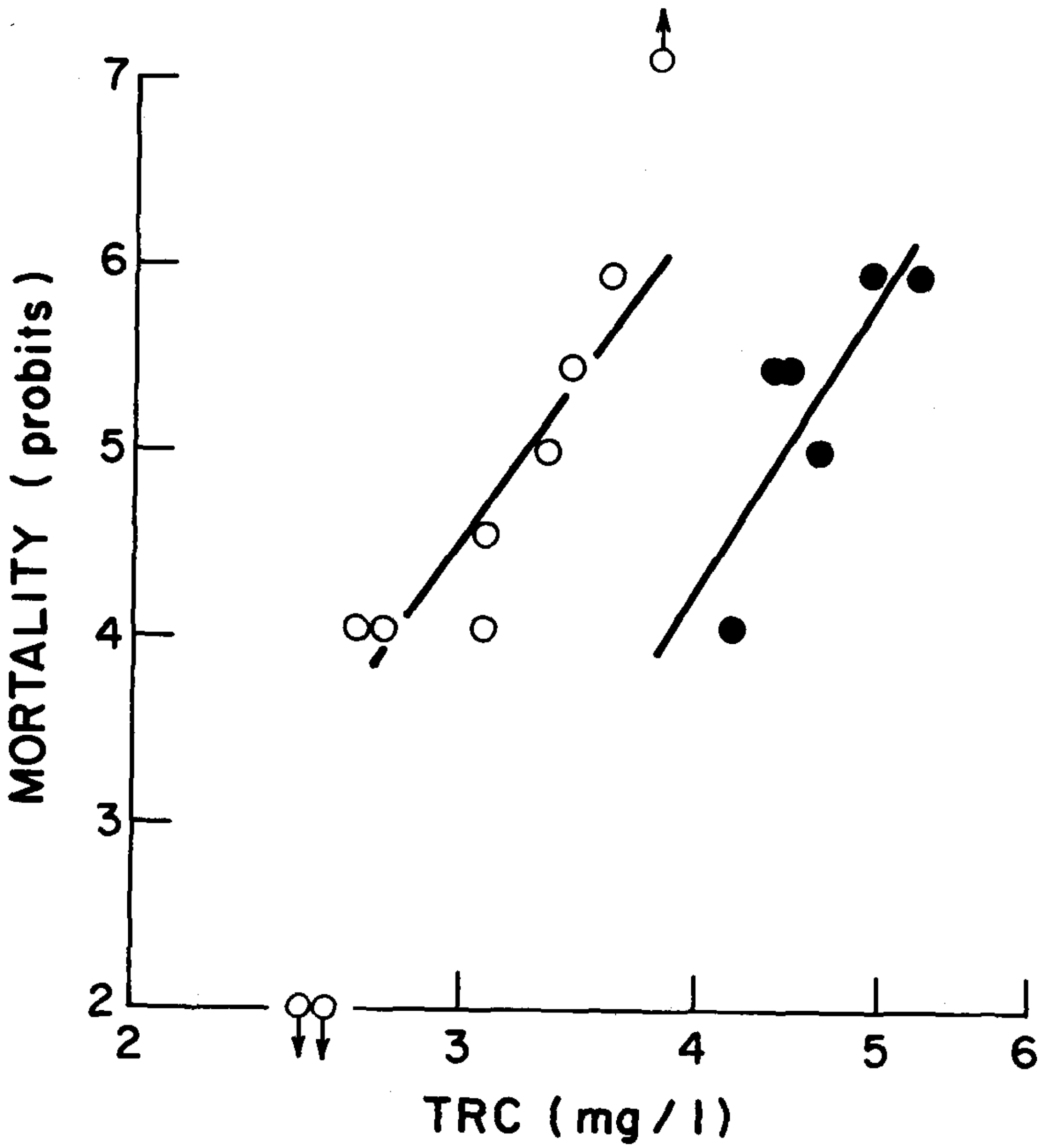

Figure 8. Relationships between mortality of largemouth bass (in probits) and the mean plateau concentrations of free residual chlorine (TRC) in 90-minute square *patterns of exposure for* bass weighing either 3.75 g (○) or 5.93 g (●) mean dry weight per fish. Data points with arrows pointing down indicate that no fish *died; data points with arrows pointing up indicate* that all of the fish died during the test.

For 53 minute exposures once each day for four days, mortality occurred at mean plateau concentration of about 745 μg/l, (Table 15). Effects of chlorine intoxication can apparently be cumulative under conditions of intermittent exposure, even when the interval between exposures is as long as one day.

The median lethal concentrations, based on deaths occurring within 96 hours after a single square pattern exposure of 90 minutes, were determined for bluegill, redside shiner, and blackside dace. Determined in this way from the mean plateau concentrations, the LC50 for the bluegill at a temperature of 25.2 C was 2.32 mg/l chlorine, and for the redside shiner at 20.6 C it was 1.6 mg/l (Fig. 9). Blackside dace having a mean weight of 0.33 gram had a LC50 of approximately 700 μg/l at 19.6 C, and those having a mean weight of 0.02 gram had a LC50 of about 400 μg/l, the large dace being more tolerant to chlorine under these conditions. The estimated mean concentrations of TRC for the entire exposure at the LC50's for bluegill, shiners, 0.33 gram, dace and 0.02 gram dace were 1.62, 1.13, 0.55 and 0.27 mg/l, respectively. Similarly, the estimated areas (expressed as mg/l x minutes) under the time-concentration curves at the LC50's for these fishes, in the same order, were 145.5, 101.5, 41.9, and 25.1. The median lethal concentration for brown bullheads acclimated at 24 C and tested at 22.5 C was about 4100 μg/l, no deaths occurred when the fish were acclimated at 12.5 C and tested at 22 C and a chlorine concentration of about 4800 μg/l (Table 16).

CHRONIC TOXICITY OF CHLORAMINES : PARTIAL CHRONIC TEST CONDITIONS

In the vicinity of sewage treatment plants, fish and other aquatic organisms may be exposed continuously to varying concentrations of chloramines. This, of course, raises the question of whether or not there are chronic effects of these chlorine compounds on the survival, reproduction, development, and growth of fish exposed to relatively low concentrations over rather long periods of time. Experiments in which adult aquatic organisms are maintained in constant concentrations of toxicants during gonadal maturation and reproduction and then the embryos and early juvenile stages develop and grow under these conditions have been called "partial chronic toxicity tests." In a "full chronic test," the resulting young would be held under the same conditions until they matured and reproduced, which would require experiments several years in duration for trout and crayfish, with which we performed only partial chronic tests. Continuous exposure to nearly constant concentrations of toxicants throughout the life history of fish probably rarely if ever occurs in nature. But, for some species of organisms, such tests do provide a means for determining some of the possible chronic effects of low levels of toxicants.

Two partial chronic tests were conducted with brook trout, two with cutthroat trout, and two with crayfish. The periods of these six experiments have already been presented in Table 5. Means and standard deviations of chloramine concentrations present during these experiments were given in Table 6. Neither species of trout spawned under the chloramine test or control conditions. But eggs and sperm were collected from the exposed brook

TABLE 15. MEAN DRY WEIGHT PER FISH AND THE NUMBER OF DEATHS FOR CUTTHROAT TROUT JUVENILES SUBJECTED TO DIFFERENT INTERMITTENT SQUARE EXPOSURES OF FREE RESIDUAL CHLORINE. MEAN PLATEAU CONCENTRATIONS WERE CALCULATED FROM 10 TO 30 MINUTES AND FROM 20 TO 60 MINUTES FOR THE 53- AND 90-MINUTE EXPOSURES, RESPECTIVELY. MEAN CONCENTRATIONS REPRESENT THOSE FOR THE ENTIRE EXPOSURES. AREAS UNDER THE TIME-TOXICANT CONCENTRATION CURVES ARE EXPRESSED AS μg/1 TRC X MINUTES.

Test	Replicate aquarium	Exposure regime	Mean plateau concentration of TRC (μg/1)	Mean concentration of TRC (μg/1)	Area (mg/1 x minutes)	Mean dry weight per fish (g)	Number of fish in each aquarium	Number of deaths
1	A	one 53-minute exposure per day for 4 days	744[*]	389[#]	82.72[+]	2.05	5	2
	B	one 53-minute exposure per day for 4 days	743	422	89.50	1.82	5	3
2	A	one 53-minute exposure	1067	631	33.39	2.46	5	0
	B	one 53-minute exposure	1077	615	32.54	2.34	5	2
3	A	one 90-minute exposure	987	606	54.58	2.16	5	1
	B	one 90-minute exposure	985	609	54.75	2.41	5	1

* For test 1, concentration given is the mean for all exposures.

For test 1, concentration given is the mean for all exposures.

+ For test 1, area given is the sum of all exposures.

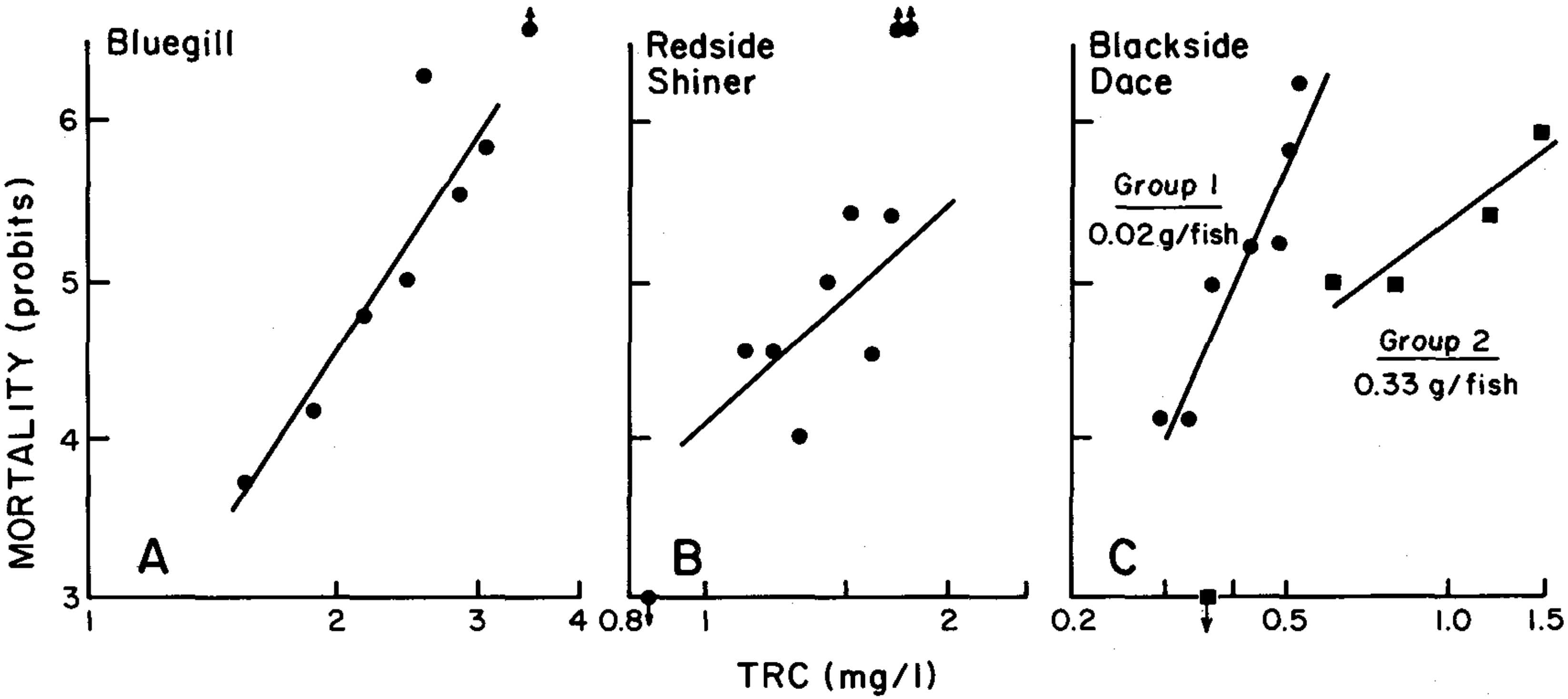

Figure 9. Relationships between mortality of fish (in probits) and the mean plateau concentration of free residual chlorine (TRC) during 90-minute square patterns of exposure of bluegill (A), redside shiner (B), and blackside dace (C). Data points with arrows pointing up indicate that all of the fish died; those with arrows pointing down indicate that none of the fish died during the tests.

TABLE 16. MEAN DRY WEIGHT PER FISH, TEMPERATURE OF TEST SOLUTIONS, TOXICANT EXPOSURES, AND THE NUMBER OF DEATHS OF BROWN BULLHEAD SUBJECTED TO 90-MINUTE SQUARE EXPOSURES OF FREE RESIDUAL CHLORINE.

Test	Acclimation temperature (C)	Test temperature (C)	Mean plateau concentration of TRC (μg/l)	Mean concentration of TRC (μg/l)	Area (mg/l x minutes)	Mean dry weight per fish (g)	No. of fish in each aquarium	Number of deaths
1	12.5	22.6	4108	2481	223.23	0.45	4	0
2	12.5	22.1	4815	2885	259.67	0.40	4	0
3	24.0	22.6	3274	2038	183.40	0.41	4	0
4	24.0	22.4	4123	2766	249.00	0.31	4	2
5	24.0	22.5	4823	2774	249.67	0.37	4	3
6	24.0	22.4	4580	2503	225.27	0.35	4	3

trout, and fertilized eggs were held through hatching and then alevin growth under the same test conditions. Some of the crayfish spawned, but hatching was not successful, because fungus developed on egg masses under both control and test conditions. In spite of our inability to complete partial chronic tests according to specifications, information on survival of adult trout and crayfish and development of reproductive products during such long exposures to relatively constant concentrations of chloramine is of considerable value. Of the total residual chlorine present in all tests, about 90 percent was in the monochloramine form.

The first test with adult brook trout began on December 4, 1973 (Table 5), at which time the fish were already in spawning condition, this test thus not meeting specifications for a "partial chronic test." The fish did not spawn on the provided substrate, and their gametes were found to be overripe in mid-January, when the test was terminated. Mortality during this 38 day experiment was quite low, even at the highest test concentrations of 21.7 and 44.7 µg/l total residual chlorine (TRC) and was probably not caused by the toxicant.

The second test with brook trout began on August 9, 1974, and extended to March 12, 1975. Mortality was higher during this test, but the pattern of mortality indicated that even at 50 µg/l survival was little affected by the toxicant (Table 17). Fish in all test groups except those held at 50 µg/l TRC were in spawning condition by early December. Two females from the 50 µg/l test group were ripe by early February, but sucessful fertilization was not accomplished with eggs collected from this test group of fish.

TABLE 17. CONCENTRATIONS OF INORGANIC CHLORAMINES AND THE NUMBER OF DEATHS OF ADULT BROOK TROUT DURING THE 1974-75 PARTIAL CHRONIC EXPERIMENT.

Concentration TRC (µg/l)	Replicate aquaria	No. of fish in each aquarium	Number of deaths
0 (control)	A	7	5
	B	7	0
5.4	A	7	2
	B	7	5
10.7	A	7	0
	B	7	2
21.8	A	7	1
	B	7	5
50.1	A	7	2
	B	7	1

Although some digging in the spawning gravel was observed in early December, no egg deposition took place. When it became apparent that successful spawning behavior was not likely to occur, eggs were stripped from two or three females from each test concentration (except 50 μg/l) and control group, and these were fertilized with milt from males from the same test conditions. Fish that were held under replicate conditions, and were ripe but were not artificially spawned, did not spawn in the test chambers even though they were held there until March 12, 1975. Variation in the number of eggs recovered from females under different test conditions appeared to be more related to the size of the females than to toxicant concentration (Table 18).

Fertilization was 100 percent for eggs from control fish and from those at 5.4, 10.7, and 21.8 μg/l TRC, but embryo mortality was relatively high in all groups (Table 19). Mortality of alevins--after hatching--was low in controls (4.5 percent), at 5.4 μg/l (0.4 percent), and at 10.7 μg/l (0 percent), but was 17.4 percent at 21.8 μg/l TRC (Table 19). And yolk utilization and growth of alevins at 21.8 μg/l was lower than for the controls and those at 5.4 μg/l (Table 19). Results very similar to these were obtained when fertilized eggs of brook trout from our brood stock were introduced into the test chambers for incubation and alevin growth (Table 19). Embryo and alevin mortality in the latter tests was higher at 21.8 and 50.1 μg/l than for controls and those at 5.4 and 10.7 μg/l TRC. And alevin yolk utilization and growth were lower at 10.7 μg/l and 21.8 μg/l than in the control group (Table 19). All alevins died at 50.1 μg/l.

The two extended tests with cutthroat trout, much as those with brook trout, failed to achieve the specifications and objectives of "partial chronic tests." Only a few of the cutthroat trout came into breeding condition in late April, in both tests, and these failed to spawn. Cutthroat mortality was quite high but apparently was unrelated to toxicant concentration up to about 50 μg/l TRC (Table 20). Much as with the brook trout, we obviously failed to provide suitable conditions for spawning or even for survival over very extended periods. This is, perhaps, not surpising, in view of the close confinement of about seven rather large fish, in each aquarium, even though others have reported some success with brook trout under specified partial chronic conditions (McKim and Benoit, 1971).

In the brook trout tests, fish held at the highest concentration of chloramine were observed to consume less food. This was also observed in the extended experiments with cutthroat trout. Food consumption of the cutthroat trout was measured on three different days, and this confirmed the casual observations that consumption was much lower at concentrations of chloramines near 50 μg/l (Table 21).

TABLE 18. LENGTH AND WET BODY WEIGHT AND THE NUMBER OF EGGS WHICH WERE STRIPPED FROM FEMALE BROOK TROUT EXPOSED TO INORGANIC CHLORAMINES DURING THE 1974-75 PARTIAL CHRONIC EXPERIMENT. THE EGGS WERE STRIPPED ON DECEMBER 16, 1974, EXCEPT FOR THOSE FISH EXPOSED TO 50.1 μg/l WHOSE OVA WERE NOT RIPE UNTIL FEBRUARY 7.

Concentration TRC (μg/l)	Length (cm)	Wet weight	No. of eggs*
0 (control)	21.0	103	260
	20.5	94	187
5.4	23.0	123	214
	16.2	38	46
	16.3	40	60
10.7	18.9	61	122
	17.3	43	24
21.8	16.0	45	66
	18.0	53	98
50.1	19.2	51	220

* At least two males were used to fertilize the eggs in each case except the 50.1 μg/l fish which were necessarily fertilized with milt from only 1 control male since there were no ripe males in the 50.1 μg/l aquaria on February 7.

TABLE 19. INFLUENCE OF CONTINUOUS EXPOSURE TO INORGANIC CHLORAMINES IN THE PERCENT FERTILIZATION, EMBRYO SURVIVAL AND HATCH, AND ALEVIN SURVIVAL AND GROWTH OF BROOK TROUT PROGENY FROM ADULTS KEPT UNDER PARTIAL CHRONIC TEST CONDITIONS OR FROM OUR BROOD STOCK. NINETY-FIVE PERCENT OF EACH GROUP OF EMBRYOS FROM ADULTS IN THE PARTIAL CHRONIC TEST HATCHED BY FEBRUARY 7, BUT RANGED FROM FEBRUARY 7 TO 9 FOR GROUPS OF EMBRYOS FROM THE BROOD STOCK.

Concentration TRC (μg/l)	No. of eggs	Percent fertilized	Embryo mortality		Embryo hatch		Alevin mortality			Alevins (March 12)	
			No.	%	No.	%	No.	Percent of hatch swim-up state (March 7-10)	Percent of hatch (March 12)	mg dry wt per alevin (w/o yolk)	Percent yolk (wet wt)
						Off-spring from partial chronic test					
0 (control)	447	100	226	50.6	221	49.4	10	4.5	4.5	10.1	2.0
5.4	320	100	56	17.5	264	82.5	1	.4	.4	8.0	1.7
10.7	146	100	104*	71.2	42	28.8	0#	0	-	-	-
21.8	164	100	72	43.9	92	56.1	16	17.4+	17.4	7.0	15.8
50.1	220	0	-	-	-	-	-	-	-	-	-
						Off-spring from brood stock					
0 (control)	52	100	12	23.1	40	76.9	2	5.0	5.0	14.0	16.7
5.4	36	100	7	19.4	29	80.6	0#	0	-	-	-
10.7	27	100	11	40.7	16	59.3	0	0	0	12.0	20.3
21.8	64	100	32	50.0	32	50.0	9	28.1+	28.1	7.0	42.4
50.1	79	100	32	40.5	47	59.5	47**	100.0	100.0	-	-

* Embryos were infested with fungus.

\# Until swim-up stage (March 7-10), alevins then escaped from test chambers.

\+ Alevins were lethargic and usually remained either near or on the bottoms of the test chambers.

**Before dying these alevins were lethargic and remained on the bottoms of the test chambers

TABLE 20. NUMBER OF DEATHS OF ADULT CUTTHROAT TROUT EXPOSED TO INORGANIC CHLORAMINES DURING THE 1974 AND 1974-75 PARTIAL CHRONIC TESTS.

Concentration TRC (μg/l)	Replicate aquaria	No. of fish in each aquarium	Number of deaths
	1974		
0 (control)	A	6	2
	B	6	4
4.3	A	6	2
	B	6	4
9.2	A	6	1
	B	6	2
23.1	A	6	2
	B	6	1
54.9	A	6	5
	B	6	5
	1974-75		
0 (control)	A	6	2
	B	6	4
5.9	A	6	4
	B	6	5
10.5	A	6	2
	B	6	5
18.8	A	6	6
	B	6	4
48.4	A	6	5
	B	6	3

TABLE 21. INFLUENCE OF INORGANIC CHLORAMINES ON THE FOOD CONSUMPTION (EXPRESSED AS PERCENT WET BODY WEIGHT OF EACH GROUP OF FISH) OF ADULT CUTTHROAT TROUT FOR THREE DAYS DURING THE 1974 PARTIAL CHRONIC EXPERIMENT. A SEVEN PERCENT FOOD RATION WAS FED DAILY TO EACH GROUP OF FISH (A & B) AT EACH TOXICANT CONCENTRATION AND CONTROL. FOOD WAS AVAILABLE FOR 24 HOURS.

TRC (μg/l)	March 27		April 3		April 5	
	A	B	A	B	A	B
0	3.4	0.7	3.4	1.1	3.1	2.1
4.3	1.5	2.9	2.5	2.5	1.3	1.1
9.2	2.2	1.3	2.8	2.8	2.2	1.8
23.1	1.4	0.3	1.4	2.1	1.5	1.4
54.9	0.1	0.2	0.2	0.2	0	0

Fungal infection of developing embryos prevented successful completion of the two "partial chronic tests" we performed with crayfish. But, in some respects, the crayfish experiments were more successful than those conducted with trout. Egg deposition, spermatophore placement, and fertilization of eggs occurred at all concentrations of chloramines tested except the highest, 373 μg/l TRC (Table 22). And mortality data provided good evidence that levels of chloramines lethal to crayfish subjected to long-term exposure are certainly no higher and probably are lower than those for trout (Table 22), even though crayfish are very much more tolerant to chloramines than are trout when exposures is for 96 hours (Table 14). Mortalities of crayfish at relatively low concentrations of chloramines were often associated with molting, especially at the chloramine concentration of 48.6 μg/l (Table 23).

During the first experiment conducted from August 20, 1974 to May 1, 1975 (254-day exposure) all crayfish died that were exposed to 170 and 373 μg/l TRC (Table 22). Those exposed to the latter concentration died within a few weeks. As these animals (group 1) died, they were replaced (group 2), and the replacements also died after a few weeks of exposure (Table 22). Extensive mortalities occurred among crayfish exposed to 80.1 and 38.7 μg/l TRC, 75 and 60 percent respectively. Three control crayfish died, one death resulting from an unsuccessful molt; males killed the other two females during mating.

TABLE 22. SPAWNING PERFORMANCE AND DEATHS OF ADULT CRAYFISH EXPOSED TO INORGANIC CHLORAMINES DURING THE 1974-75 AND 1975-76 PARTIAL CHRONIC EXPERIMENTS. A AND B REFER TO REPLICATE AQUARIA AT EACH TOXICANT CONCENTRATION AND CONTROL.

	Inorganic chloramine concentrations (TRC, μg/l) during Test 1 (1974-75)												Inorganic chloramine concentrations (TRC, μg/l) during Test 2 (1975-76)									
	0		38.7		80.1		170.3		373.0 Group 1		373.0 Group 2		0		25.2		48.6		99.4		194.0	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
No. crayfish per tank	10	10	10	10	10	10	10	10	10	10	10	10	8	8	8	8	8	8	8	8	8	8
Spermatophore depositions	5	5	5	5	5	5	5	5	4	3	0	3	4	4	4	3*	4	3*	4	2*	4	4
Egg depositions	5	3#	5	5	5	4	5	5	1	1	1	0	4	4	4	3	4	3	4	2	3+	4
Adult mortality																						
254 days of exposure																						
Males	0	0	3	3	3	2	5	5	5	5	5	5	0	0	0	0	0	0	2	4	4	4
Females	1**	2	2	2	2	4	4	5	5	5	5	5	0	0	0	1	0	1	2	4	4	4
365 days of exposure																						
Males	-	-	-	-	-	-	-	-	-	-	-	-	1	0	0	1	4	4	4	4	4	4
Females	-	-	-	-	-	-	-	-	-	-	-	-	0	2	1	2	3	4	4	4	4	4

* 1 female died after an unsuccessful molt before the spawning period.

2 females killed by males during mating.

+ 1 female killed by male during mating.

** Unsuccessful molt.

TABLE 23. NUMBER OF MOLTS AND THE NUMBER OF DEATHS ASSOCIATED WITH MOLTING FOR CRAYFISH EXPOSED TO INORGANIC CHLORAMINES DURING THE 1975-76 PARTIAL CHRONIC TEST. DEATHS OCCURRED EITHER DURING MOLTING OR PRIOR TO HARDENING OF THE NEW CARAPACES.

Concentration TRC (μg/l)	Replicate aquaria	No. of crayfish in each aquarium	No. molting	Deaths associated with molting
0 (control)	A	8	8	0
	B	8	5	0
25.2	A	8	8	1
	B	8	6	2
48.6	A	8	8	7
	B	8	7	7
99.4*	A	8	1	1
	B	8	0	0
194.0*	A	8	1	0
	B	8	1	0

* Most of these crayfish had died prior to the major molting period (spring, 1976).

During the first 254 days of the second test--conducted from August 14, 1975 to August 14, 1976 (365-day exposure)--all the animals exposed to 194 μg/l died, 75 percent died at 99.4 μg/l, and 6 percent died at 48.6 and 25.2 μg/l (Table 22). No control crayfish died. For the entire 365 days of exposure, however, all the animals exposed to 99.4 μg/l died, 94 percent died at 48.6 μg/l and about 19 percent died at 25.2μg/l and in the controls (Table 22). The days of exposure to 50 percent mortality of the crayfish decreased with increasing toxicant concentrations in both tests, as is clearly shown in Figure 10.

During the first experiment, one control crayfish and two crayfish exposed to 38.7 μg/l TRC molted in September. The former died but the latter molted successfully. During the second experiment, nearly all molting occurred in the spring of 1976, after 254 days of exposure. Most crayfish exposed to 48.6 μg/l TRC died during the molting process, while crayfish exposed to 25.2 μg/l had fewer mortalities that could be associated with molting (Table 23). Deaths among control animals were not attributable to molting.

In both experiments, nearly all the males successfully deposited spermatophores (Table 22). Only those exposed to 373 μg/l TRC during the first experiment had a reduction in performance--particularly the second group--as compared to the controls. Two males of the second group deposited spermatophores on the heads of females. Egg depositions occurred about 10

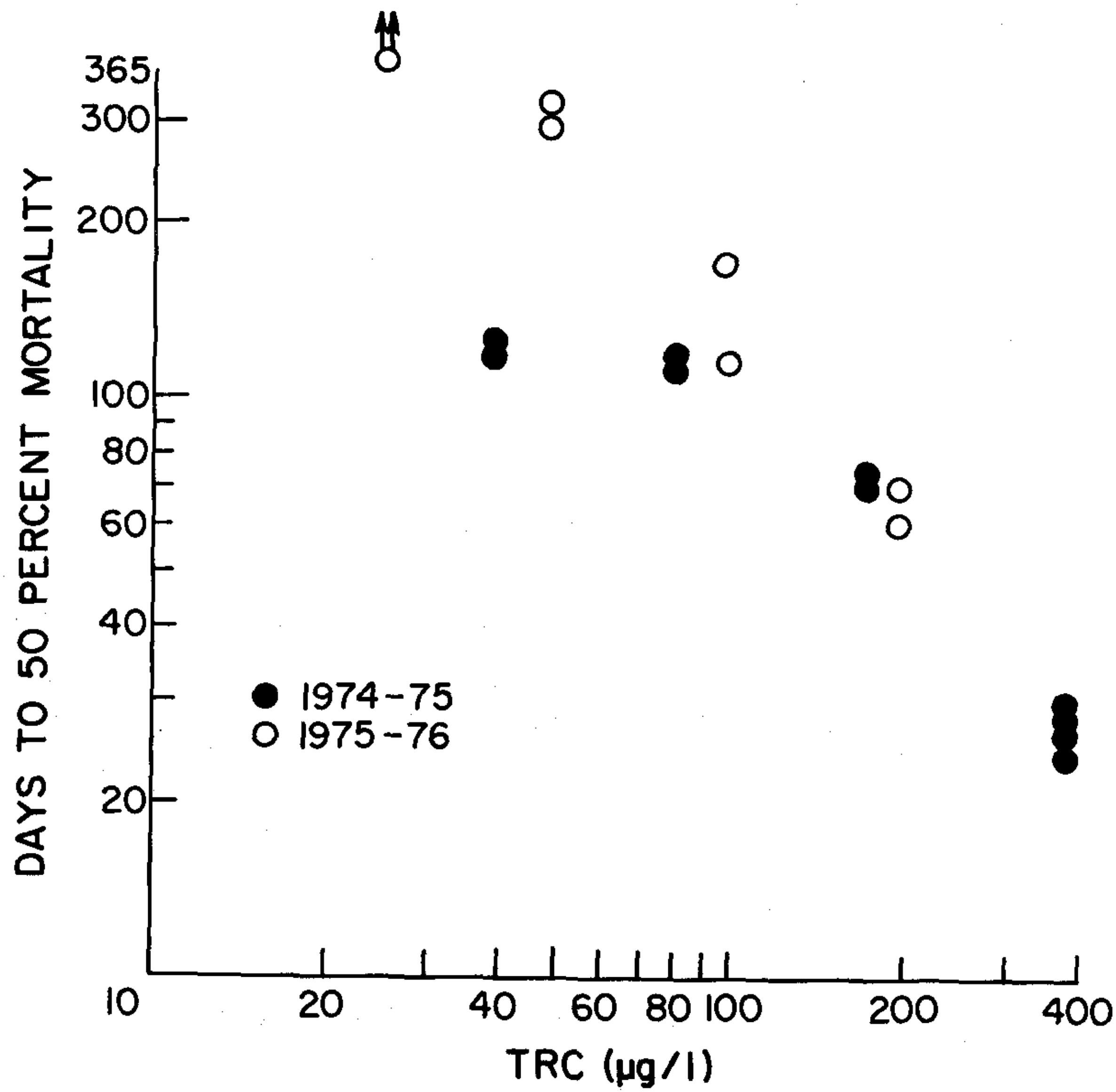

Figure 10. Relationship between the mean concentration of inorganic chloramines (TRC) and the days of exposure until 50 percent mortality of adult crayfish kept under partial chronic conditions during tests conducted in 1974-75 and 1975-76. The two data points with arrows pointing up indicate that fewer than 50 percent of the crayfish had died at that concentration by the end of the 365 day exposure (1975-76).

days after spermatophore depositions in each experiment and were apparently not affected by the toxicant at concentrations between 25.2 and 194.0 µg/l (Table 22). Only those females exposed to 373 µg/l TRC performed poorly, most dying prior to egg deposition. Embryo survival was poor for all test and control groups of crayfish. In nearly all cases, the embryos became infested with fungus and died, even those of control crayfish.

CHRONIC TOXICITY OF CHLORAMINES: DEVELOPMENT, SURVIVAL, AND GROWTH

Successful "partial chronic tests" permit some evaluation of the effects of toxicants on gonadal maturation, reproductive behavior, embryonic development, growth, and subsequent maturing and reproduction of progeny. But successful reproductive behavior of adult fish cannot always be expected under conditions specified for partial chronic tests, this often vitiating the approach. And, of course, partial chronic tests are hardly appropriate for anadromous species such as Pacific salmon. For these species, studies should be conducted on the effects of toxicants on life history stages likely to be exposed to toxicants.

We conducted experiments with embryos, alevins, and juveniles of coho salmon, an anadromous species. Effects of relatively low, constant concentrations of chloramines on embryo and alevin survival and on alevin and juvenile growth were determined. Fish remaining from the experiment on embryo and alevin survival and alevin growth were retained for studies on the effects of different concentrations of chloramines on growth of juveniles at different ration levels. Control fish from the embryo-alevin experiment were separated into groups to be tested at different chloramine concentrations, so that growth of juveniles having had no prior exposures to chloramines could be studied. In addition, the growth of juveniles developing from embryos and alevins exposed to various concentrations of chloramines was studied at these levels of toxicant exposure. Some juveniles from each prior test condition were held in water containing no toxicant in order to determine whether or not there were residual effects on growth. A second experiment on the effects of different concentrations of chloramines on the growth of juvenile coho salmon fed different ration levels was conducted with juveniles raised at our hatchery and not previously involved in any experiment.

The experiment on the effects of continuous exposure to constant concentrations of chloramines on embryonic survival and hatching and on the survival and growth of alevins of coho salmon was begun on November 22, 1973. The hatching process was about 95 percent complete under all test conditions by January 7, 1974. At the test concentrations of 0 (control A), 0 (control B), 5, 11, 23, and 47 µg/l TRC, survival of embryos was apparently unaffected by chloramines, total mortality among 600 embryos in each control and toxicant concentration being 75, 26, 32, 40, 54, and 26, respectively. Hatching did not appear to be affected by the presence of chloramines at these concentrations.

The activity and behavior of alevins was affected by exposure to chloramine concentrations of 23 and 47 μg/l but not by exposure to concentrations of 5 and 11 μg/l. Within one week after hatching, alevins exposed to 47 μg/l became lethargic, and many were lying on their sides on the chamber bottoms. By January 22, most of these alevins were lying on their sides and exhibited an abnormal, spiral, swimming behavior when disturbed. This syndrome began to appear in alevins exposed to 23 μg/l TRC by January 22 and was general in this group by January 28.

Mortality among alevins at all test conditions was very low during the first two weeks after hatching, as is shown in Figure 11. But by January 28, mortality of alevins at 47 μg/l TRC had increased markedly, an increase that continued and reached 70 percent by February 7. During this entire time, mortality of controls and those alevins held at chloramine concentrations up to and including 23 μg/l remained very low (Fig. 11). Substantial mortality occurred in all groups including controls on February 9, as a result of water flow interruption and low concentrations of dissolved oxygen (Fig. 11). Little or no further mortality occurred in any of the test groups by February 11, except among alevins at 47 μg/l where mortality reached 97 percent.

The growth of alevins was severely affected by chloramines at concentrations of 23 and 47 μg/l but not at 5 and 11 μg/l, as shown in Figure 12. On January 7, at which time hatching was approximately 95 percent complete in all test groups, the sizes of alevins in all groups were very nearly the same, about 0.01 gram dry weight per individual, exclusive of yolk. This, of course, is strong evidence that embryonic growth had not been affected by chloramines at the concentrations tested. But, as already noted, subsequent alevin growth was severely affected at 23 and 47 μg/l, weights of these fish being only about 80 and 45 percent those of controls by February 11 (Fig. 12).

The relative growth rates (instantaneous coefficients of growth) of fish and other animals are generally a negative function of body weight. Thus, as body weight increases with growth through time, relative growth rates decline. The relationships between relative growth rate and body weight (exclusive of yolk materials) for alevins tested at chloramine concentrations of 5 and 11 μg/l were not significantly different from those of control groups (Fig 13). Relative growth rates at given body weights (reached at different times) of alevins tested at 23 and 47 μg/l TRC were much lower than those of the other groups.

Determination and comparison of the caloric or heat value of alevin tissue and yolk material permitted evaluation in equivalent energy terms of yolk utilization for growth and for metabolism. Caloric values per gram of alevin tissue (exclusive of yolk materials) were essentially the same for all test conditions (Table 24). But total caloric value in grams of alevin tissue and yolk together was higher at 23 and 47 μg/l TRC than at the lower test concentrations and for the controls. Total yolk utilization, yolk utilization for growth, and yolk utilization for metabolism were clearly less for alevins held at 47 μg/l TRC than for those held under control conditions (Table 25).

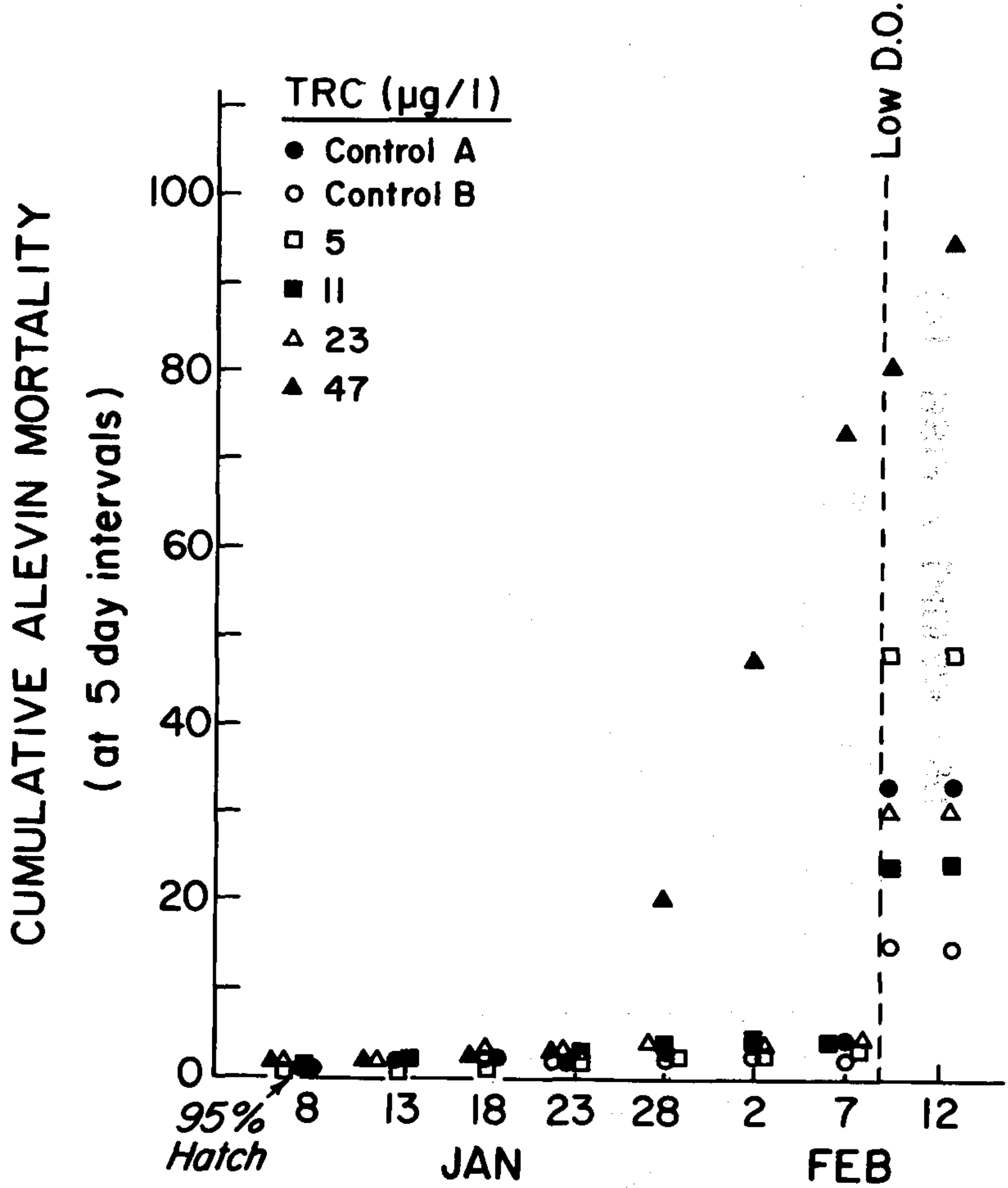

Figure 11. Cumulative alevin mortality (sum of replicates) at 5-day intervals at different concentrations of inorganic chloramines (TRC) in the coho salmon embryo-alevin experiment. Increased alevin mortality occurred at indicated time of low dissolved oxygen. Cumulative mortality in percent.

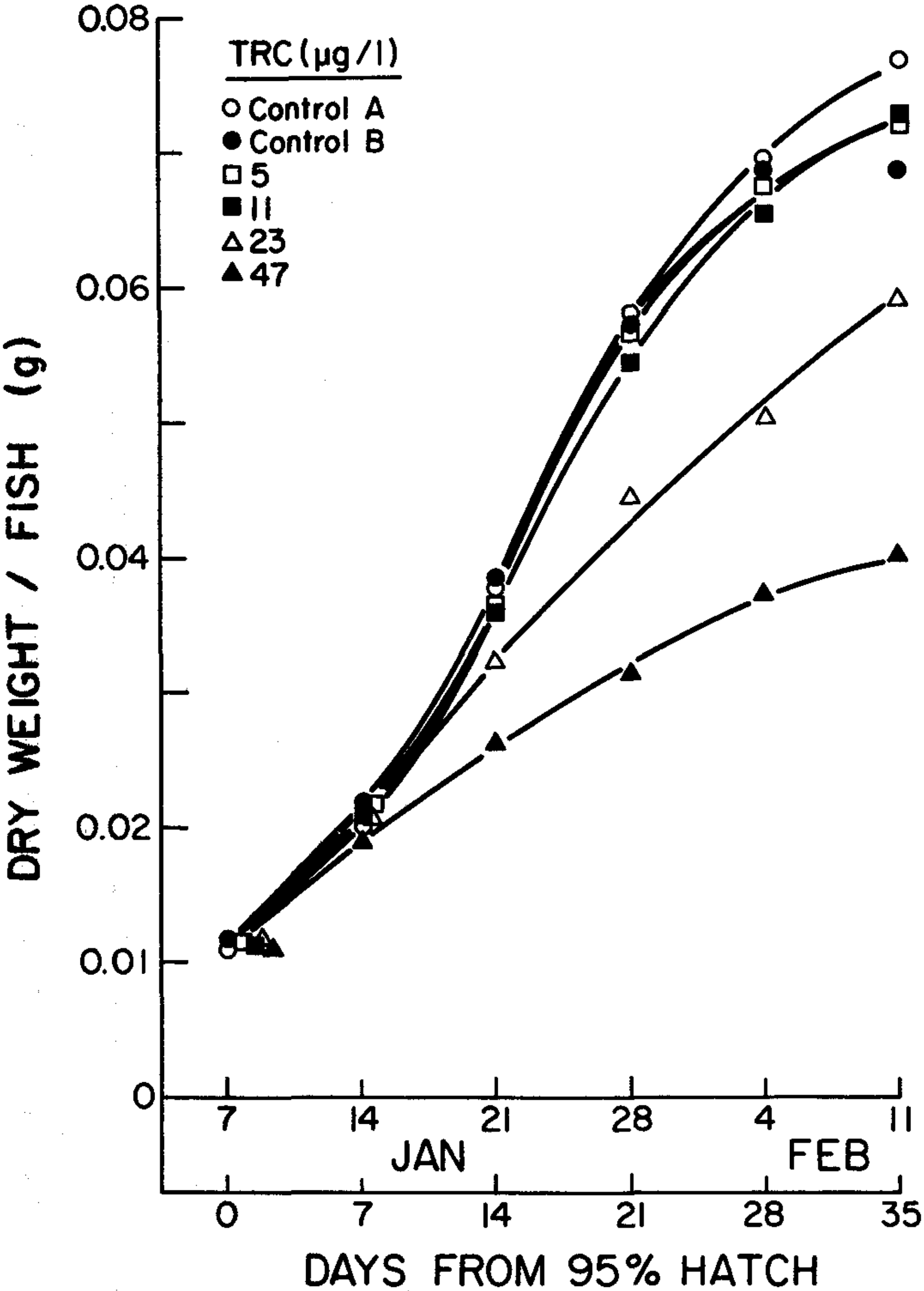

Figure 12. Relationships between the mean dry weight of alevins and the days from 95 percent alevin hatch at different concentrations of inorganic chloramines (TRC) in the coho salmon embryo-alevin experiment.

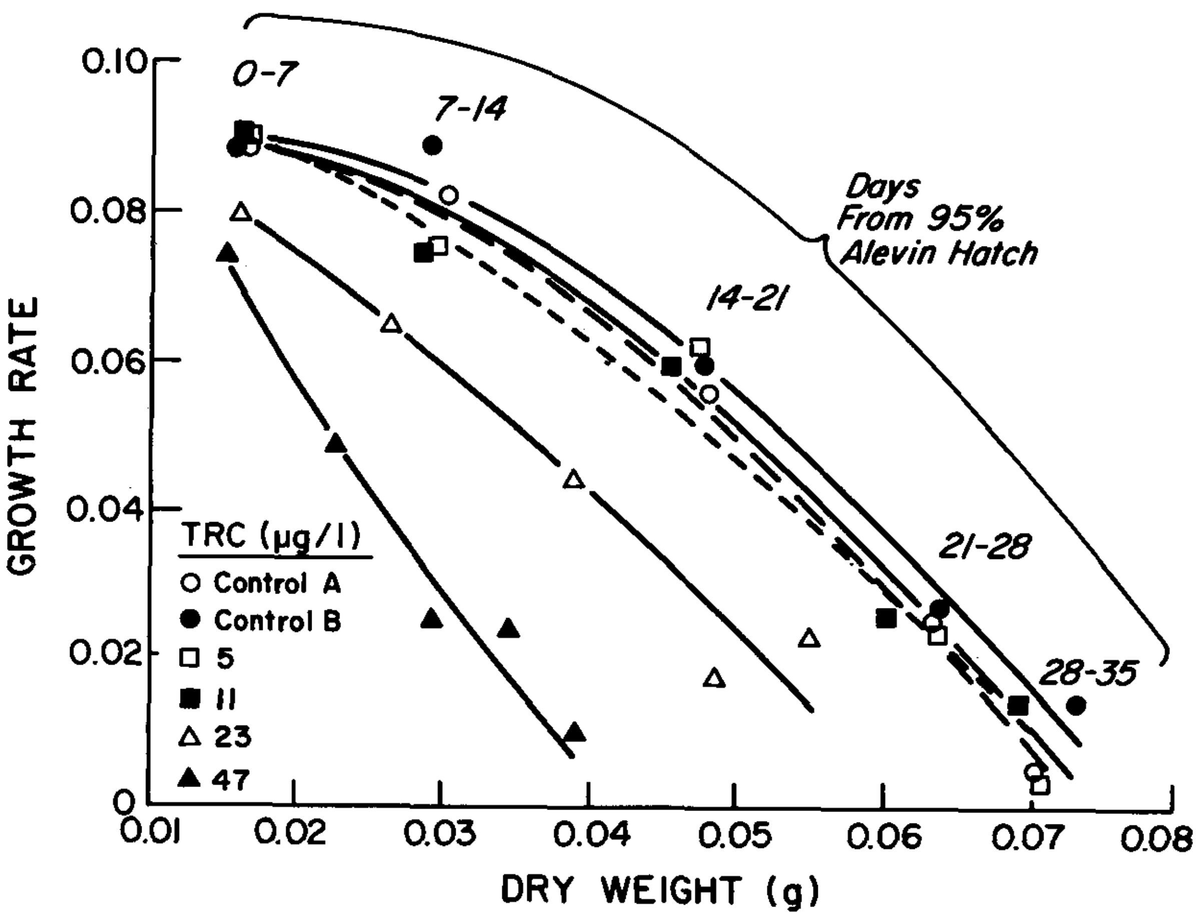

Figure 13. Relationships between instantaneous growth rate and mean dry weight of alevins at different concentrations of inorganic chloramines (TRC) in the coho salmon embryo-alevin experiment. The days from 95 percent hatch are indicated.

TABLE 24. INFLUENCES OF CONTINUOUS EXPOSURES TO INORGANIC CHLORAMINES ON THE CALORIC VALUE (CALORIES/G) FOR ALEVINS WITH YOLK AND WITH THE YOLK REMOVED DURING THE COHO SALMON EMBRYO-ALEVIN EXPERIMENT. SAMPLES WITH YOLK CONSISTED OF 5 ALEVINS WHEREAS SAMPLES WITHOUT YOLK CONSISTED OF 25 ALEVINS.

Concentration TRC (μg/l)	January 7	January 28		February 4	
	without yolk	with yolk	without yolk	with yolk	without yolk
0 A	5447	5770	5302	5432	5390
0	5242	5648	5254	5404	5440
0 B	5320	-	-	5501	5462
0	5279	-	-	5553	5389
5	5253	-	-	5436	5504
5	5308	-	-	5456	5423
11	5297	-	-	5536	5445
11	5320	-	-	5325	5540
23	5237	-	-	5698	5521
23	5227	-	-	5720	5618
47	5332	5949	5406	5944	5553
47	5368	5935	5589	5883	5509

In the first experiment on the growth of juvenile coho salmon at different concentrations of chloramines and at different ration levels, fish previously involved in the embryo and alevin experiment were used. In one part of this growth experiment, the growth of fish originally developing under control conditions of no exposure to the toxicant was then determined under different concentrations of the toxicant as well as under continued control conditions. In this series of groups, some effect of 11 μg/l TRC was apparent on the growth of juveniles fed at the 8 percent ration level, of which these fish consumed only about 83 percent (Fig. 14). Juveniles in this group having no previous exposure to chloramines and then exposed to 22 μg/l consumed much less food at both ration levels than did those in any of the other treatments. These fish used for growth with much less efficiency the food they did consume and exhibited much less growth than did those in the other groups (Fig. 14). The growth of juveniles having originally developed at 5 and 11 μg/l TRC was not affected when these fish were further tested at these concentrations, some selection or acclimation apparently having occurred (Fig. 14). Growth was high in those groups of fish that had been exposed to 0, 5, 11, or 23 μg/l inorganic chloramines (TRC) during development and then were transferred to water containing no toxicant during the first growth experiment (data not shown).

TABLE 25. COMPARISON OF YOLK METABOLISM FROM JANUARY 28 to FEBRUARY 4 BY CONTROL ALEVINS AND ALEVINS EXPOSED TO 47 µg/l INORGANIC CHLORAMINES IN THE COHO SALMON EMBRYO-ALEVIN EXPERIMENT. YOLK UTILIZATION RATE, GROWTH RATE AND TOTAL METABOLIC RATE ARE EXPRESSED AS CALORIES PER KILOCALORIE OF ALEVIN PER DAY.

Total residual chlorine (µg/l)	Total yolk utilized (cal)	Increase in calories per alevin (cal)	Mean calories per alevin (kcal)	Efficiency of yolk utilization for growth (%)	Yolk utilization rate (cal/kcal/d)	Growth rate (cal/kcal/d)	Total metabolic rate (cal/kcal/d)
0 A	143	58	.347	40.6	58.8	23.9	35.0
0 B	141	75	.335	53.2	60.1	32.0	28.2
47	69	39	.191	56.5	51.6	29.1	22.5
47	63	29	.190	46.0	47.4	21.8	22.3

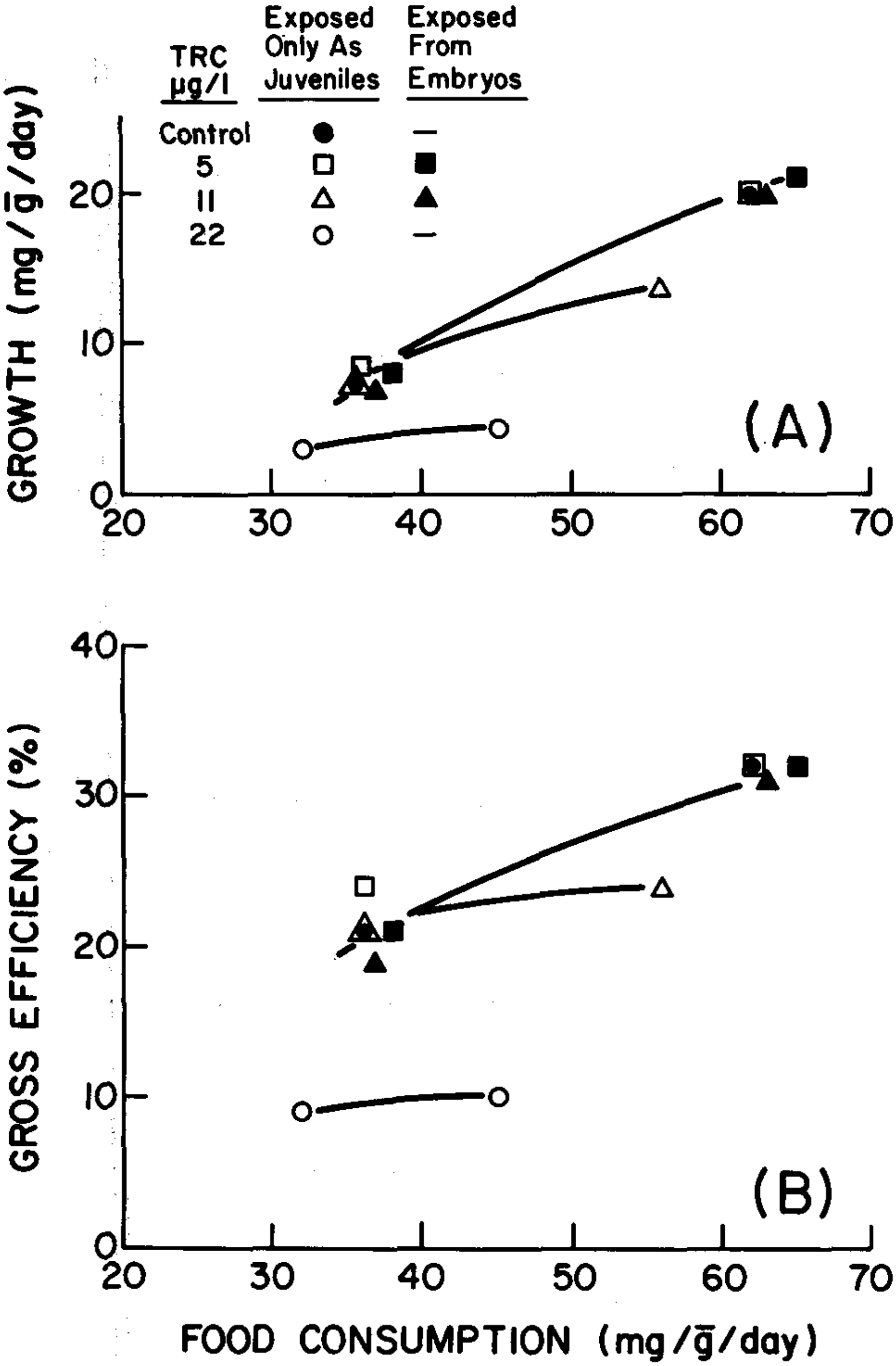

Figure 14. Relationships between food consumption rate and relative growth rate (A) and gross efficiency (B) for coho salmon juveniles (from the embryo-alevin experiment) at different concentrations of inorganic chloramines (TRC). Some groups of fish had been exposed to the toxicant since the embryo life stage, others were exposed only during the growth experiment.

In a second experiment on the effects of chloramines on the growth of juvenile salmonids at different ration levels, the fish used had not been previously involved in any experiment and thus had no history of exposure to chloramines. In this experiment, marked effects of 23 μg/l TRC on the food consumption, growth, and efficiency of food utilization for growth were observed (Fig. 15). This experiment tended to confirm the effects on food consumption, growth, and efficiency of food utilization for growth observed at 22 μg/l TRC in the previous experiment. No effects of concentrations of 5 and 10 μg/l TRC on food utilization and growth were demonstrated in this experiment.

BEHAVIORAL EFFECTS OF CHLORINE AND CHLORAMINES

The behavior of bass and other species subjected to intermittent exposure to both lethal and nonlethal concentrations of the chlorine was observed to be markedly modified. Other laboratory and field investigations have shown behavioral changes to occur in fish subjected to intermittent exposure to chlorine, and it is of importance to describe such behavioral changes and relate them to effective chlorine concentrations.

In aquarium tests, largemouth bass swam slowly and deliberately, had slow opercular movement, and seldom "coughed," during the 30-minute acclimation period before chlorine was introduced in each test. The behavior of the fish changed when chlorine was introduced at concentrations near median lethal ones. Behavioral changes usually occurred in the following sequence: (a) increased rates of swimming, opercular movement, and coughing; (b) reduced swimming activity with the animals "nearing the surface;" (c) rapid swimming with "thrashing" at the water surface and some jumping; (d) "lethargic swimming" and frequent collisions with aquaria walls and other fish; (e) dorsal portion of heads "bobbing" into the atmosphere; (f) resting "on the bottoms" of the aquaria, accompanied by heavy and pulsating opercular movement; and (g) turned over or belly up. Most of the behavioral changes occurred in all the tests, even when exposures to test solutions were not lethal. But despite the consistency of the order in this behavioral sequence, particular behavioral changes often failed to occur. For example, behaviors (f) and (g) did not occur when the fish were subjected to test solutions that were not acutely toxic. And even at the highest concentrations, some of the behaviors (e.g., bobbing) did not always occur. In such cases, the fish appeared to pass through the behavioral sequence so rapidly that some behavioral changes were skipped. Fish not dying from exposure to chlorine usually exhibited normal behavior within 24 hours after exposure.

Judging the time to first occurrence of each kind of behavior was rather subjective, but this time appeared to decrease with increasing toxicant concentration. Only the first occurrence of a behavior in each group of fish was recorded. It was not possible to relate times to occurrence to areas under the time-concentration curves, because the bass exhibited a particular response at different times when the cures were of different height, even when areas under the cures were the same. Thus our preliminary results were expressed as mean exposure concentration to the time of first occurrence of a behavior. When largemouth hass were exposed to two 90-minute square patterns of exposure to chlorine, with a two-hour

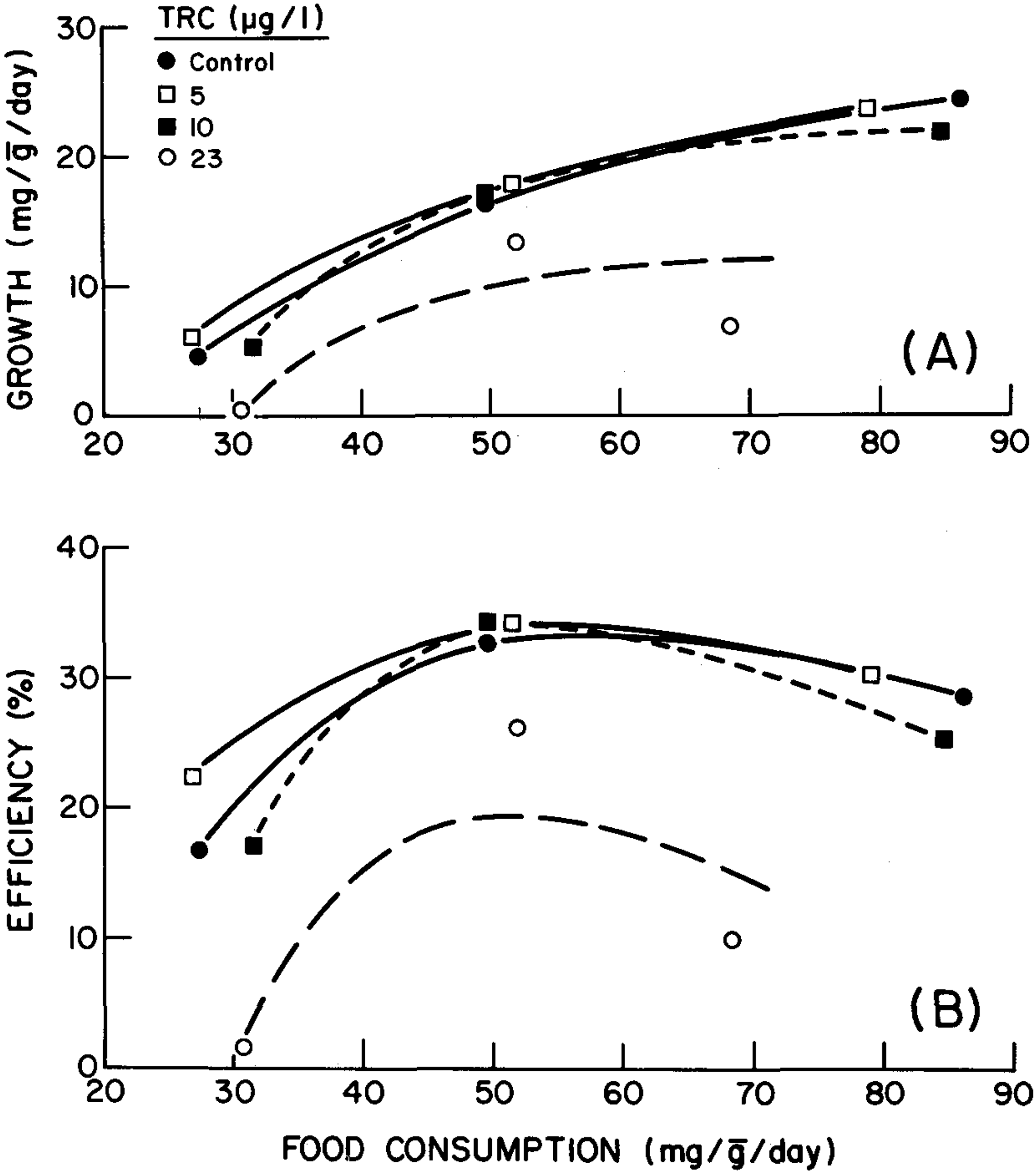

Figure 15. Relationships between food consumption rate and relative growth rate (A) and gross efficiency (B) for coho salmon juveniles at different concentrations of inorganic chloramines (TRC).

intervening period, the time to "bobbing" decreased as the toxicant concentration increased in the first exposure (Fig. 16). In the second exposure, bobbing occurred earlier than in the first, at nearly equal toxicant concentrations. Two groups did not exhibit bobbing in the second exposure. These had been exposed to acutely toxic concentrations in the first exposure, and some of the fish were on the bottoms of the aquaria at the start of the second exposure. The other fish in the aquaria were active, but their behavioral changes progressed quickly through the sequence, and bobbing behavior appeared to be skipped during the second exposure.

As in the tests with bass, the sequence of behavioral changes for each of the other species subjected to intermittent exposures to chlorine was not always complete, particularly at concentrations at which no fish died. The following description of the behavior of each species during the tests represents behavior occurring at or near the lethal concentration, or in the case of the bullhead, the highest toxicant concentrations used.

The behavioral changes of the trout were similar to those of the bass, "thrashing" and "bobbing" frequently occurring. The bluegill also responded in much the same manner as did the bass, except the bluegill did not thrash or jump. The shiner and dace differed from the above in their behavioral changes. During exposure, the swimming activity of these normally active swimmers continuously declined. Neither species exhibited "nearing surface," thrashing, or jumping, and only the shiner exhibited the bobbing behavior. For the shiner, resting on the bottom of the aquarium did not occur until they were very near "turnover;" in the dace this behavior occurred well in advance of turnover. Some of the behavioral changes of the bullhead were markedly different from those of the other species. Prior to toxicant introduction, bullhead swam actively throughout the aquaria. In each test with toxicant, behavioral changes of bullhead acclimated to 24 C and tested at 22 C usually occurred in the following sequence: (1) active swimming near the bottom of the aquaria; (2) active swimming near the surface of the water; (3) lethargic swimming followed by maintaining a vertical (head-up) position at the water surface; (4) resting on the bottom; (5) sporadic swimming, and (6) again resting on the bottom, accompanied by either stopped or greatly reduced opercular movement. The toxicant concentrations tested were not sufficiently high to induce "turnover" during the exposures. Those bullhead acclimated to 12.5 C and tested at 22 C exhibited the same sequence of behavior, except the vertical position did not occur. These fish appeared to progress through the sequence faster than the groups acclimated to 24 C and tested at 22 C.

Some behavioral observations were made on largemouth bass held in a current of flowing water and exposed to chlorine. During acclimation periods and control tests, the bass spent nearly all of their time under the cover provided. Within a few minutes after the addition of the toxicant, however, the fish typically began swimming throughout the test chamber but would return to the cover for short periods. If the concentration of chlorine was sufficiently high, the fish first "bobbed" and then "rested" with their caudal peduncle pressed against the downstream screen. Control fish were not observed to do this. Occurrence of the behavioral patterns outlined for the aquarium

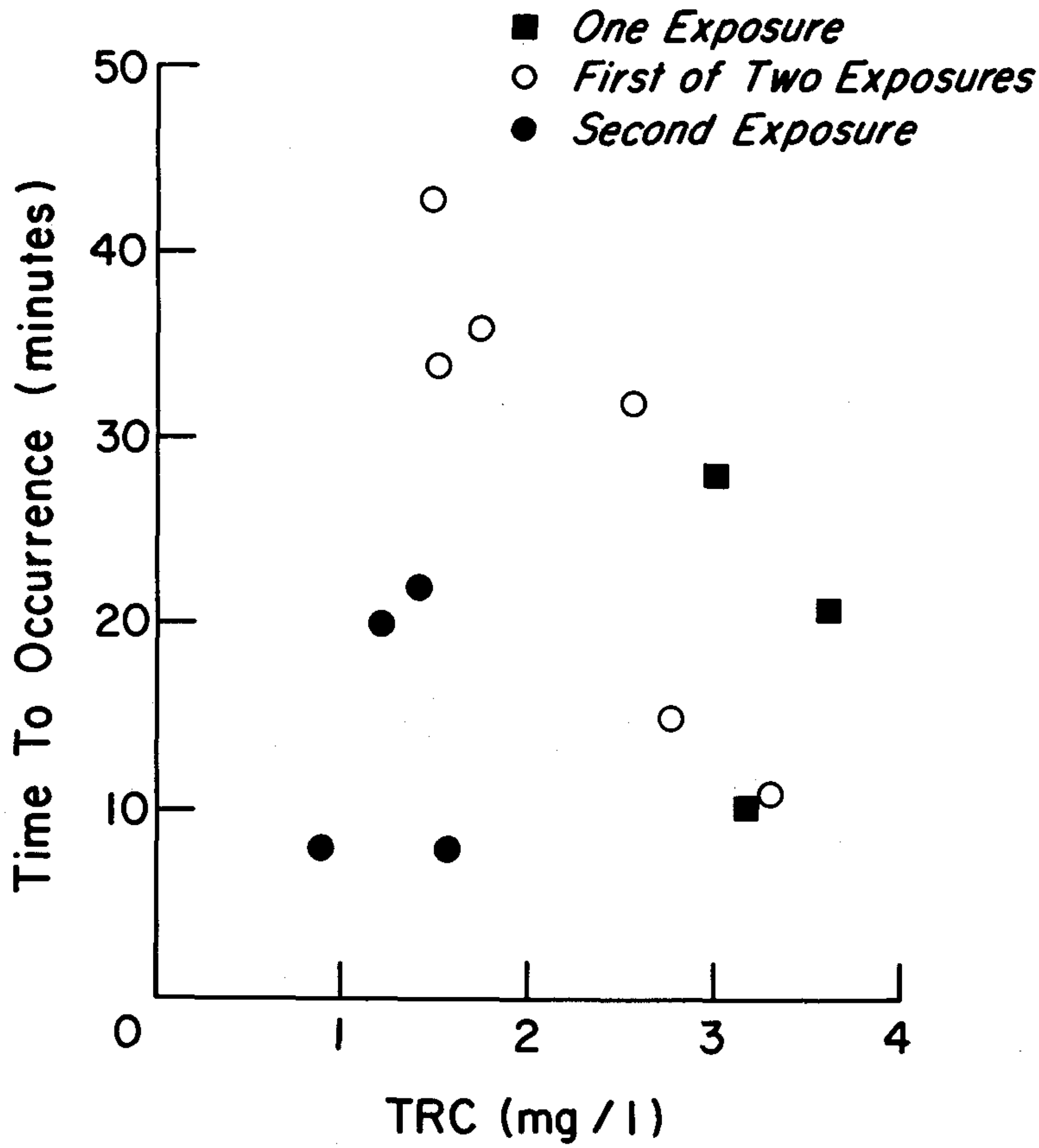

Figure 16. Relationships between the time to first occurrence of bobbing behavior of largemouth bass and the mean concentration of free residual chlorine (TRC) in the aquaria. Mean concentration calculated for the period until first occurrence, during one and two 90-minute square patterns of exposure.

studies was difficult to judge, bobbing and resting being the easiest to detect. Bobbing appeared to occur at about the same exposure (mg/l x min) in the square and spike patterns of exposure, but resting appeared to occur at a lower exposure in spike than in square patterns (Table 26).

Most of the behavioral changes that occurred at acutely toxic concentrations of chlorine also occurred at some sublethal concentrations. These results suggest that chlorinated discharges that are not acutely toxic could adversely affect the behavior and thus affect survival of bass. Unfortunately, very little is known about the extent to which fish are exposed to residual chlorine in the chlorinated discharge plumes of power generation plants. Certainly the location of the fish in such plumes could influence their ability to escape before serious modification of their behavior occurred. In view of these potential impacts of chlorine on the behavior of fish distributed at different locations in plumes of chlorinated discharges, we conducted two additional behavioral experiments with bass subjected to intermittent exposure to residual chlorine.

In one experiment, we attempted to determine the threshold concentrations of chlorine at which particular behavioral changes occurred in bass unable to avoid exposure. In a second experiment, we determined if bass would move out of or avoid water containing chlorine or chloramines. We also determined the extent to which such avoidance was correlated with the concentrations and species (inorganic chloramines or free residual chlorine) of total residual chlorine.

In each test in the first experiment, two bass of different mean dry weights (7.37 and 14.63 g/fish) were subjected together to either the square or the spike pattern of exposure to sublethal concentrations of chlorine. At the highest exposures, expressed as mg/l x minutes (Table 27), the sequence of behavioral changes was consistent with that occurring in the acute toxicity tests described earlier, except that none of the fish turned over (belly up) or died (Fig. 17). The threshold concentrations at which each behavior occurred were difficult to determine, but "nearing the surface" occurred at a lower exposure than did the other behaviors, in each type of exposure for each size group of fish. Nonetheless, an important aspect of these results is that the five behaviors occurred when the fish were subjected to the square and spike patterns of exposure at sublethal levels. For fish of the sizes used here, the median lethal level would have been at an exposure of more than 148 mg/l x minutes. In these tests, however, most of the changes of behavior occurred from exposures of less than about 102 mg/l x minutes. Based upon these results, it seems possible that bass, of the sizes tested here, could undergo considerable changes in behavior, if they were not soon able to escape from chlorinated discharge plumes. This could be so even for discharges that are not acutely toxic to the fish.

Before the results of the second experiment are presented, it is important that we make clear what we will mean by the expressions "move out" and "avoid." In each test, the bass showed a definite preference to position themselves in the toxicant discharge area (Fig. 1), so long as toxicant was not being introduced. During tests without toxicant, all of the fish (which

TABLE 26. INFLUENCE OF SQUARE AND SPIKE EXPOSURES OF FREE RESIDUAL CHLORINE ON THE OCCURRENCE OF THE BOBBING AND RESTING BEHAVIORAL RESPONSES AND DEATH BY LARGEMOUTH BASS IN THE FLOWING WATER INTERMITTENT EXPOSURE EXPERIMENT.

Square Exposure						Spike Exposure					
Mean plateau concentration TRC (μg/l)	Mean concentration TRC (μg/l)	Area (mg/l x minutes)	Occurrence (+ or -) Bobbing	Resting	Death	Mean plateau concentration TRC (μg/l)	Mean concentration TRC (μg/l)	Area (mg/l x minutes)	Occurrence (+ or -) Bobbing	Resting	Death
0	0	0	-	-	-	0	0	0	-	-	-
0	0	0	-	-	-	0	0	0	-	-	-
267	160	14.41				940	352	22.21	-	+*	-
368	254	22.88	-	-	-	1330	484	30.51	-	-	-
564	395	35.60	+	-	-	1660	619	38.99	+	-	-
759	565	50.85	+	-	-	1800	673	42.38	+	-	-
986	735	66.11	+	-	-	2070	807	50.85	+	+	-
1095	800	72.04	+	+	-	2100	834	52.55	+	+	-
1224	895	80.51	+	+	-	2790	1090	68.65	+	+	-
1297	970	87.29	+	+	-	3280	1291	81.36	+	+	-
1492	1092	98.31	+	+	-	3440	1332	83.90	+	+	-
1493	1074	98.48	+	+	-	3880	1534	96.62	+	+	-
1510	1121	100.85	+	+	-	4800	1910	120.35	+	+	-
1560	1177	105.94	+	+	-	5250	2072	130.52	+	+	+
1689	1252	112.72	+	+	-						
1850	1375	123.74	+	+	+						
1968	1469	132.21	+	+	+						

* Fish was extremely active during the early portion of the test and appeared to be exhausted during the rest of the test.

TABLE 27. CONCENTRATIONS OF FREE RESIDUAL CHLORINE AND AREAS UNDER THE TIME-CONCENTRATION CURVES WITH SQUARE AND SPIKE EXPOSURES IN THE BEHAVIORAL EXPERIMENT WITH LARGEMOUTH BASS WEIGHING EITHER 7.37 OR 14.65 G PER FISH.

Square Exposure			Spike Exposure		
Mean plateau concentration TRC (μg/l)	Mean concentration TRC (μg/l)	Area (mg/l x minutes)	Mean plateau concentration TRC (μg/l)	Mean concentration TRC (μg/l)	Area (mg/l x minutes)
140	94	8.48	400	121	7.63
280	198	17.80	855	283	17.80
351	254	22.88	1260	431	27.12
431	311	27.97	1650	565	35.60
542	396	35.60	2030	686	43.22
705	509	45.77	3010	1090	68.65
1081	791	71.19	5980	2072	130.52
1230	942	84.75	6980	2246	141.53
1437	1073	96.62			
2138	1554	139.84			
2130	1563	140.69			

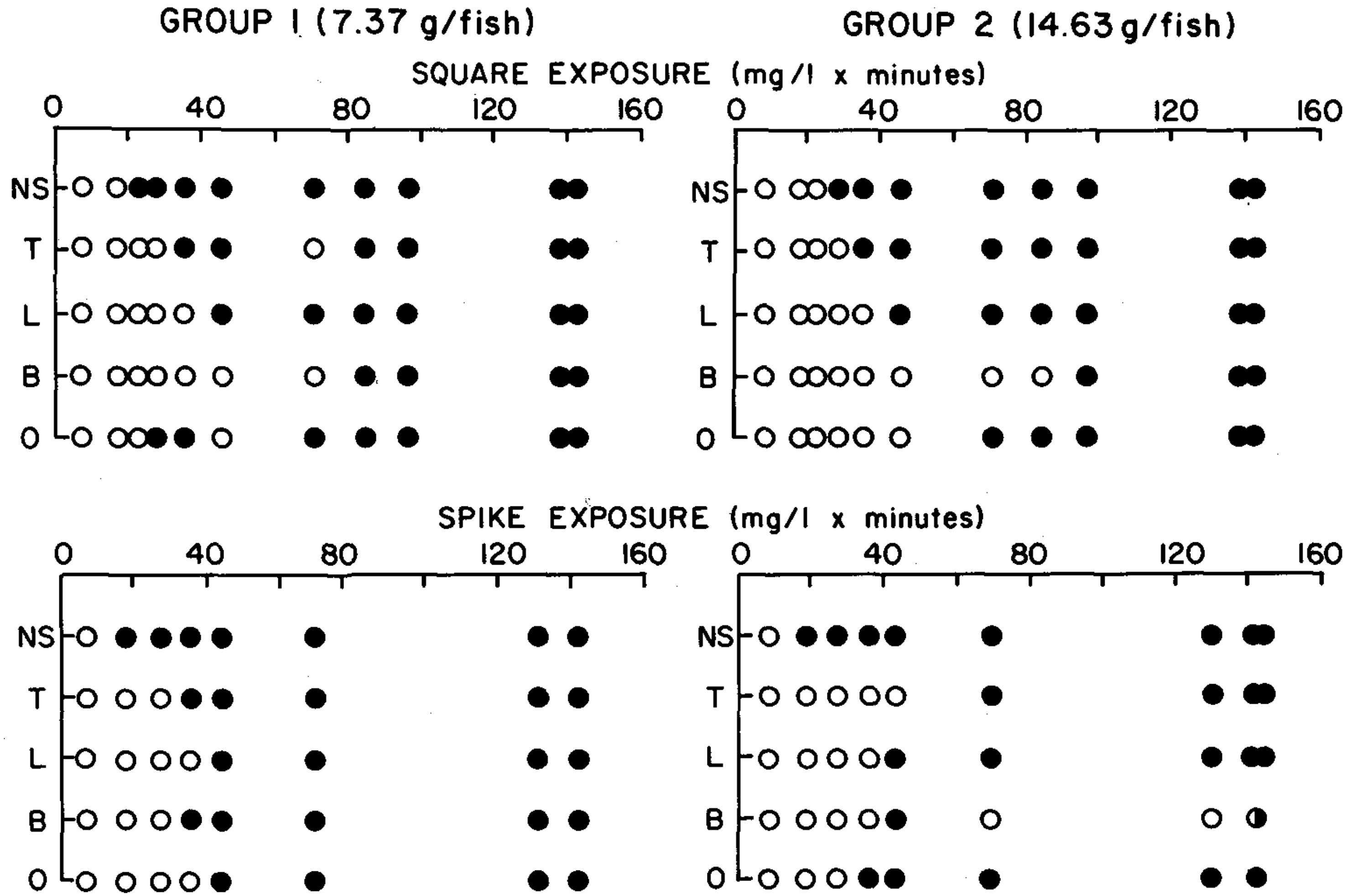

Figure 17. Relationships between the areas under the time-concentration curves, expressed as mg/l x minutes, and the occurrence of five behavioral changes in two weight groups of bass subjected to square and spike patterns of exposure to free residual chlorine (TRC). Open circles indicate that the behaviors did not occur; dark circles indicate that the behaviors occurred. Symbols: NS -near the surface; T - thrashing; L -lethargic swimming; B - bobbing; and O -on aquarium bottom.

were tested individually) spent nearly all of the time in the discharge area of the testing apparatus. During most toxicant tests, the fish moved out of this area, only to return at irregular intervals for short periods of time before moving out again. In nearly all tests, the fish returned to the toxicant discharge area within 15 minutes after toxicant introduction was terminated. Thus, in the experiment reported here, we determined the effects the toxicant had on keeping the bass out of the discharge area. We did not determine avoidance in the same way as did Sprague and Drury (1969).

In this experiment we found that bass spent less time in the discharge area when exposed to free residual chlorine than when exposed to inorganic chloramines, at similar concentrations expressed as total residual chlorine (Fig. 18). The amount of time spent out of the discharge area increased significantly with increasing concentration of either toxicant (regression analysis, $P < 0.05$). The slopes of the two regression lines were not significantly different (analysis of covariance, $P > 0.05$), but the Y-intercepts were significantly different ($P < 0.05$). Although the exposed bass moved out of the discharge area at all concentrations of free residual chlorine tested, the threshold for such movement when exposed to inorganic chloramines was about 0.09 mg/liter total residual chlorine (Fig. 18). Furthermore, none of the fish in these tests died or showed any of the behavioral changes described for the above experiment, even though some of the concentrations would have been acutely toxic to the fish, if they had not moved out of the discharge area.

In each toxicant test, the bass did not move out of the discharge area immediately after the toxicant was introduced. These delays and the repeated returns to the discharge area mentioned above resulted in greater exposures (expressed as mg/l x minute) to the toxicants as the concentrations increased (Fig. 19). The slopes of the regression lines for inorganic chloramines and free residual chlorine were significant ($P < 0.05$). But neither the slopes nor the Y-intercepts of the two lines were significantly different ($P > 0.05$). Thus the data were pooled and a single regression line was calculated (Fig. 19).

CONTINUOUS EXPOSURE OF LABORATORY STREAM COMMUNITIES TO CHLORAMINES

Twelve laboratory stream communities were maintained from May 1, 1975 through June 24, 1976. For purposes of conduct of experiments and analysis and representation of results, this entire period was separated into nine shorter experimental periods, which will be referred to by numbers 1 through 9. These periods are shown in Table 28, which presents data on laboratory stream temperatures for the various experimental periods. The twelve laboratory stream communities were divided into four groups of three each, our intention being that one group would provide replicate controls and each of the other three groups would provide replicates at chloramine concentrations near 25, 12, and 6 μg/l. It is our belief that the experiments were less than satisfactory, for a number of reasons. First, we experienced difficulty in developing stream communities that were as productive for coho salmon juveniles as we had wished. And variance in productivity among replicates at particular treatment levels was high. Further, it was extremely difficult to maintain total chlorine residuals

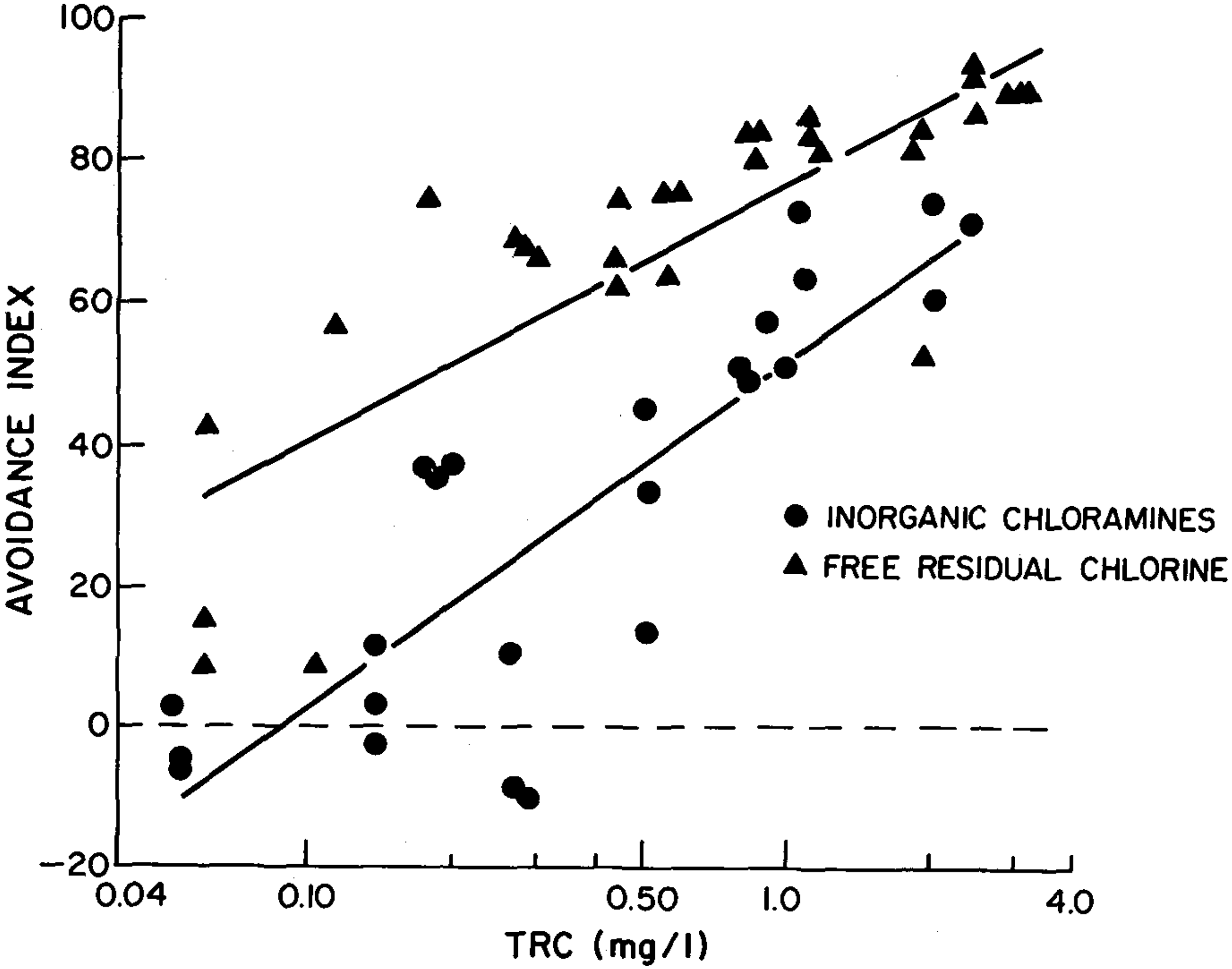

Figure 18. Relationships between the mean toxicant concentration in the toxicant discharge area of the avoidance apparatus and the avoidance index for largemouth bass subjected to 60-minute exposures to free residual chlorine or inorganic chloramines, expressed as total residual chlorine (TRC).

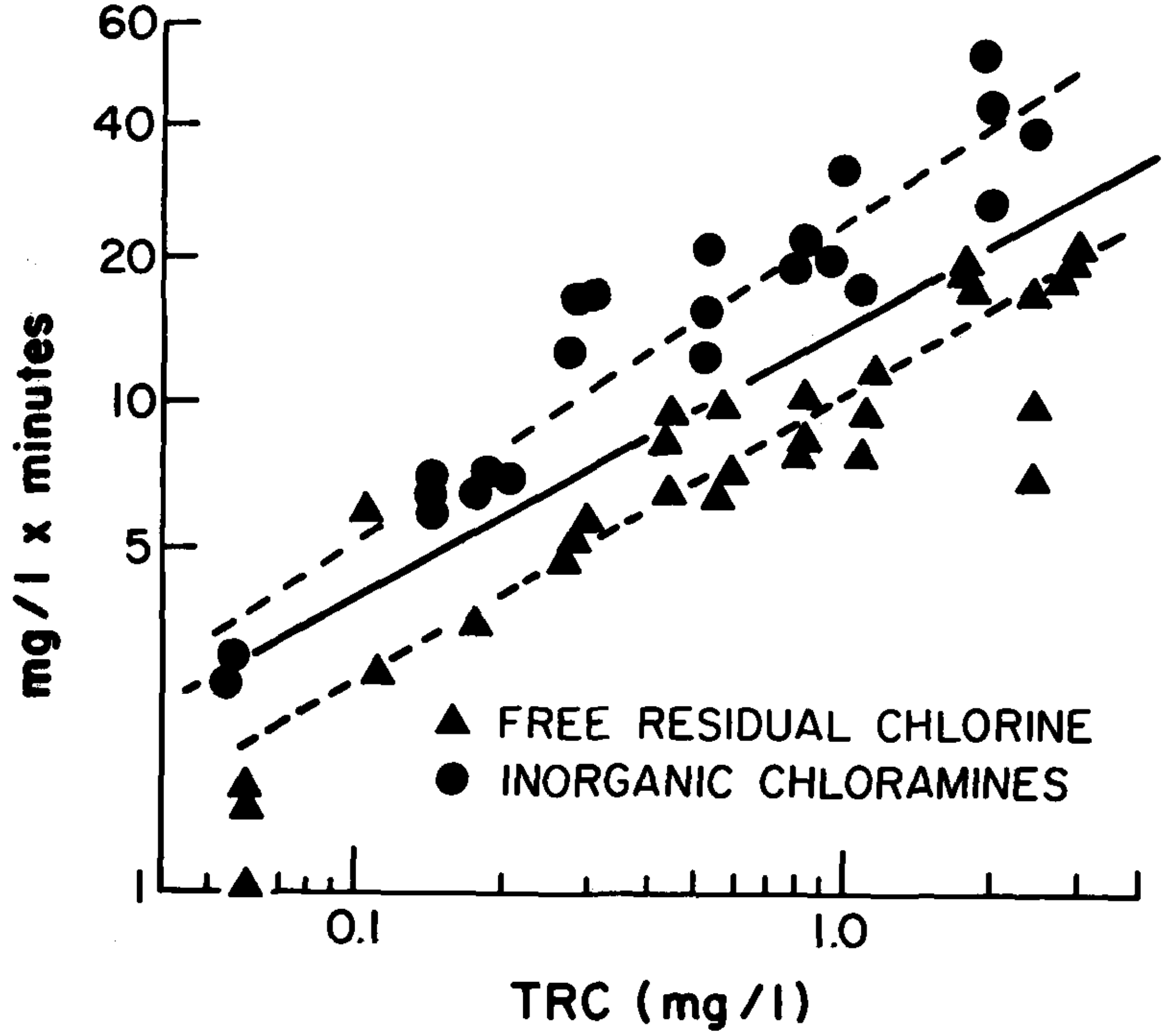

Figure 19. The amount of exposure, expressed as mg/l x minutes, to which bass were subjected when exposed to either free residual chlorine or inorganic chloramines in the avoidance experiment. The dashed lines represent the relationship for each toxicant. The solid line represents the relationship for the pooled data since neither the slopes nor the Y-intercepts of the individual toxicant lines were different significantly ($P > 0.05$).

near the values we sought for particular treatments, as can be seen from Table 29. Finally, it is not at all clear that the total residual chlorine, as measured by amperometric titration, represented inorganic chloramines. Around 50 percent of the total residual chlorine appeared as dichloramine, even though we had expected nearly all the residual chlorine to appear as monochloramine under the test conditions. As we will later note, this supposed dichloramine fraction, in these streams having high amounts of organic materials, may have been organic chloramines, of which little is known regarding toxicity.

TABLE 28. DAILY MEAN HIGH AND MEAN LOW TEMPERATURES (AND STANDARD DEVIATIONS) IN THE LABORATORY STREAMS FOR EACH EXPERIMENTAL PERIOD IN 1975 AND 1976.

Experimental period	Inclusive dates	High temperature C	Low temperature C
1975			
1	5-1 to 5-28	14.03 (1.67)	9.47 (1.17)
2	6-6 to 6-26	16.29 (2.56)	12.01 (1.11)
3	6-27 to 8-5	18.34 (2.35)	13.54 (1.71)
4	8-17 to 9-8	15.59 (1.12)	12.64 (1.12)
5	9-10 to 9-30	16.06 (0.76)	12.89 (1.06)
6	10-8 to 10-31	11.79 (1.39)	10.10 (1.24)
1976			
7	4-12 to 5-10	13.19 (2.83)	9.58 (2.10)
8	5-11 to 6-2	14.44 (1.67)	9.67 (1.19)
9	6-4 to 6-24	16.72 (1.44)	13.06 (0.54)

But in spite of these difficulties, a summary of the results is probably worthwhile. Relatively large amounts of chloramines were introduced into these laboratory stream communities over a long period of time, and yet there appeared to be little if any effect on the stream communities. The variability, even within treatments, that so plagued us experimentally was surely less than occurs in stream communities exposed to chlorine compounds in nature. And it is with nature, or something very like nature, that we must be most concerned in setting stream standards for the protection of aquatic life.

The concentrations of chloramines we found necessary to introduce with the exchange water to maintain residual concentrations near those we desired in the stream varied greatly among the different seasons of the year. In August and September, introduced concentrations ranged from about 400 to 800 μg/1 TRC (Fig. 20). But in May, June, October, and December, these introduced concentrations ranged from about 100 to 400 μg/1. As we will show, these periods roughly coincided with periods during which large amounts

TABLE 29. MEAN CONCENTRATIONS (AND STANDARD DEVIATIONS) OF RESIDUAL CHLORINE COMPOUNDS IN EACH LABORATORY STREAM DURING EACH EXPERIMENTAL PERIOD. SAMPLES WERE TAKEN DAILY IN MIDMORNING.

Experimental period		Low concentration				Medium concentration				High concentration				Control		
		S3	S5	N5	Grand mean	S2	N2	N3	Grand mean	S1	S4	N4	Grand mean	N1	N6	S6
1	TRC	1.2	1.0	4.8	2.3	4.9	5.3	3.6	4.6	12.9	10.9	7.2	10.3	0	0	0
	S.D.	(1.7)	(2.1)	(4.4)		(4.0)	(3.2)	(4.0)		(9.8)	(8.6)	(7.8)		-	-	-
2	TRC	3.0	3.0	3.0	3.0	9.7	9.4	8.1	9.1	24.8	20.8	21.5	22.4	0	0	0
	S.D.	(2.2)	(2.1)	(2.9)		(4.5)	(6.0)	(5.1)		(11.1)	(11.2)	(11.2)		-	-	-
3	TRC	1.5	1.1	1.5	1.4	5.4	6.3	6.3	6.0	17.7	16.9	15.1	16.6	0	0	0
	S.D.	(2.1)	(1.6)	(1.4)		(4.1)	(4.6)	(4.4)		(12.0)	(10.9)	(9.4)		-	-	-
4	TRC	1.2	1.0	1.4	1.2	3.4	5.7	2.5	3.9	17.9	10.7	11.9	13.5	0	0	0
	S.D.	(2.1)	(1.5)	(2.0)		(3.2)	(6.6)	(2.6)		(15.2)	(11.0)	(11.1)		-	-	-
5	TRC	4.0	1.9	2.1	2.7	6.6	7.4	6.6	6.9	26.2	23.2	23.4	24.3	0	0	0
	S.D.	(5.2)	(2.5)	(2.6)		(6.5)	(6.6)	(6.5)		(16.1)	(15.1)	(14.1)		-	-	-
6	TRC	3.1	4.1	2.8	2.9	12.4	13.1	13.0	12.8	26.7	26.3	41.7	31.5	0	0	0
	S.D.	(1.4)	(2.8)	(1.7)		(3.7)	(6.5)	(5.6)		(8.0)	(12.8)	(50.0)		-	-	-
7	TRC	2.2	4.0	3.3	3.2	8.9	7.8	7.6	8.1	20.8	20.6	18.1	19.8	0	0	0
	S.D.	(1.7)	(2.7)	(2.4)		(4.7)	(4.2)	(4.4)		(9.2)	(8.8)	(8.1)		-	-	-
8	TRC	4.8	4.0	3.6	4.1	11.0	8.5	7.7	9.1	24.7	22.2	21.8	22.9	0	0	0
	S.D.	(2.0)	(1.8)	(1.8)		(4.1)	(2.9)	(2.8)		(7.6)	(7.8)	(9.3)		-	-	-
9	TRC	5.5	4.6	5.0	5.0	11.4	9.7	8.1	9.7	22.4	25.9	23.1	24.5	0	0	0
	S.D.	(3.6)	(2.5)	(2.7)		(4.0)	(3.7)	(4.0)		(8.2)	(9.3)	(8.3)		-	-	-

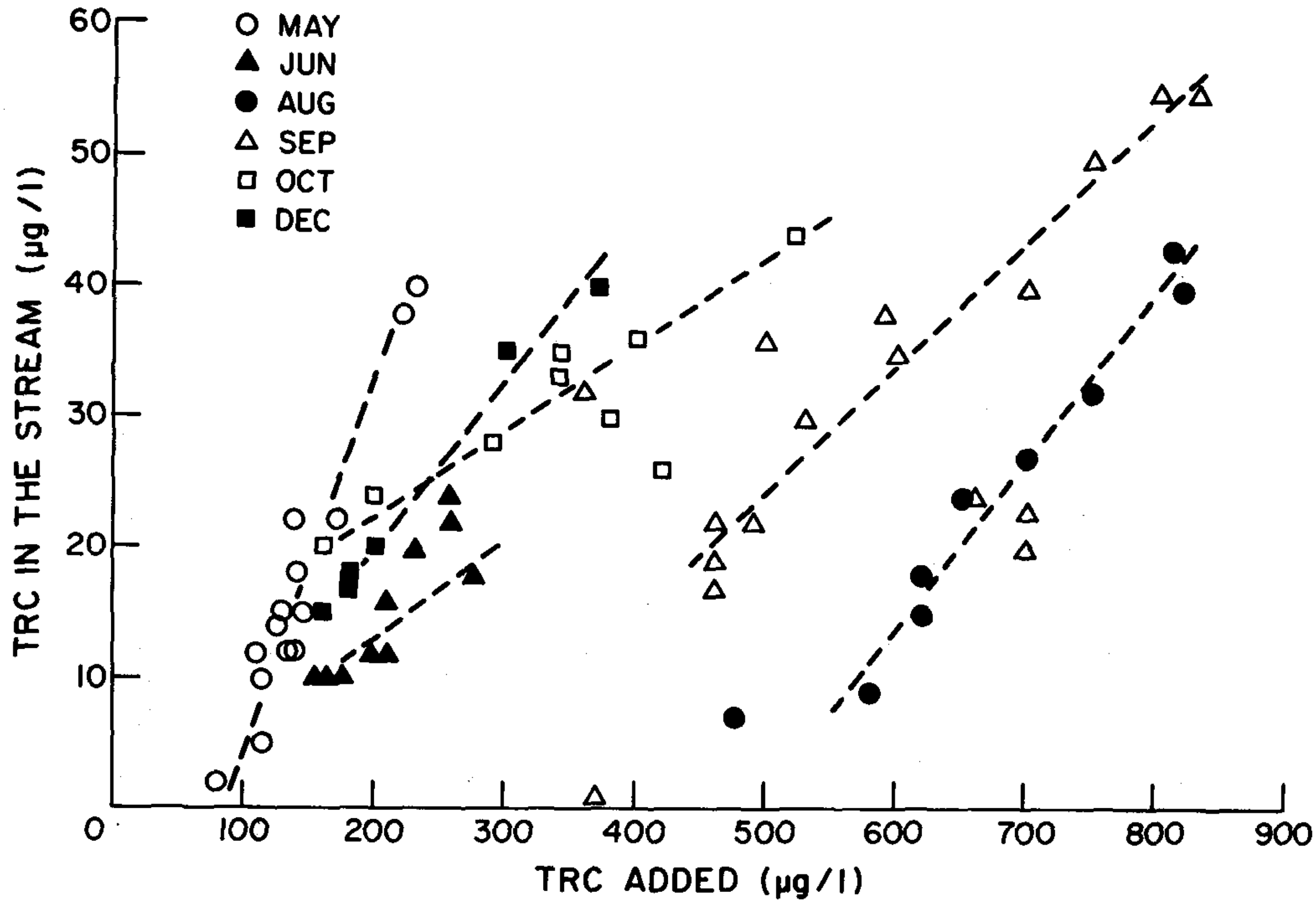

Figure 20. Relationships between the mean daily concentrations of chloramines (TRC) in laboratory stream renewal water and the concentrations of the toxicant in one of the streams, for selected periods in 1972.

and lesser amounts, respectively, of organic matter were present in the streams.

Even over a 24-hour period in any one stream, during which the concentration of chloramines introduced was maintained quite constant, the concentration of total residual chlorine changed dramatically in a cyclical manner, as can be seen in the example given in Figure 21. For different months, the amount of chloramines necessarily introduced to maintain a desired concentration of total residual chlorine in a particular stream was roughly correlated with the amounts of organic matter and chlorophyll present (Fig. 22). Mean concentrations of total residual chlorine maintained at the three treatment levels, for the entire experimental period, were 20.6, 7.8, and 2.9 μg/l (Table 29), and these are the treatment levels to which we will refer in the ensuing discussion.

Total organic matter, exclusive of macroinvertebrates and fish, varied considerably between replicates within each treatment (Table 30). But the major variation in organic matter was between seasons, the highest mean values being maintained during experimental periods 3 and 4 (late June to early September), as can be seen from Figure 23. The stream communities exposed to the highest concentrations of total residual chlorine (20.6 μg/l) tended to have the lowest amounts of organic matter (Table 30; Fig. 23), whatever may have been the causal relationships. The major differences and changes in total chlorophyll concentration (chlorophylls a, b, and c) were also seasonal and did not appear to be consistently associated with differences in chloramine treatments (Fig. 24). None of this is to say that chlorine treatment did not have effects on the algal communities, such as effects on species composition. But we did not determine algal species composition.

A reasonably diverse assemblage of species of macroinvertebrates colonized all streams, the major tendency being an increase in their total biomass throughout the entire period of experiments (Table 31). All major groups eventually appeared in all streams, and differences in their abundances were not clearly associated with differences in concentration of total residual chlorine. Very high biomasses of an isopod, Asellus, developed in one stream at the high concentration of total residual chlorine--the first stream to be colonized by this species. But eventually all streams were to be colonized by this species, which became moderately abundant in most streams. It is unlikely that abundance of this organism was closely related to the presence or absence of chlorine compounds.

The same major groups of insects appeared in all streams: Chironomidae, Coleoptera, Ephemeroptera, Odonata, Plecoptera, and Trichoptera. With the possible exception of the Ephemeroptera, their occurrence and abundance was not clearly related to the concentration of total residual chlorine. Ephemeroptera appeared less often in samples from treatment streams than in those from control streams. Within each treatment level and control, 33 benthic samples were taken, 11 from each stream. Of these 33 samples from each treatment and control level, Ephemeroptera were absent from 9, 9, 6, and 0 samples from high, medium, and low concentration treatment and control streams, respectively.

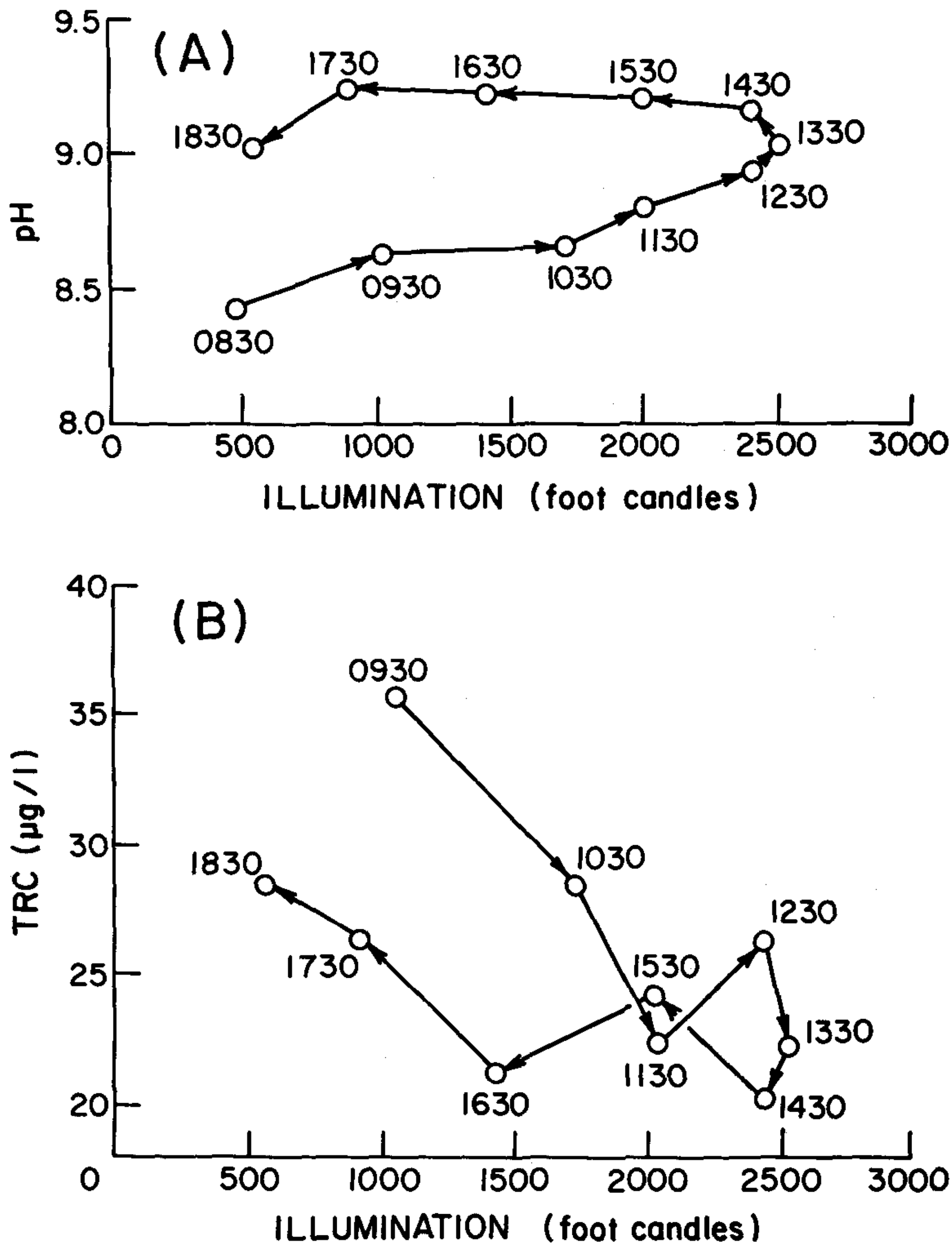

Figure 21. Relationships between the amount of illumination (in foot-candles) at the surface of a laboratory stream and the pH of the stream water (A) and the concentration of residual chlorine compounds (TRC) in the water (B) from approximately 0830 to 1830 hours on July 26, 1976.

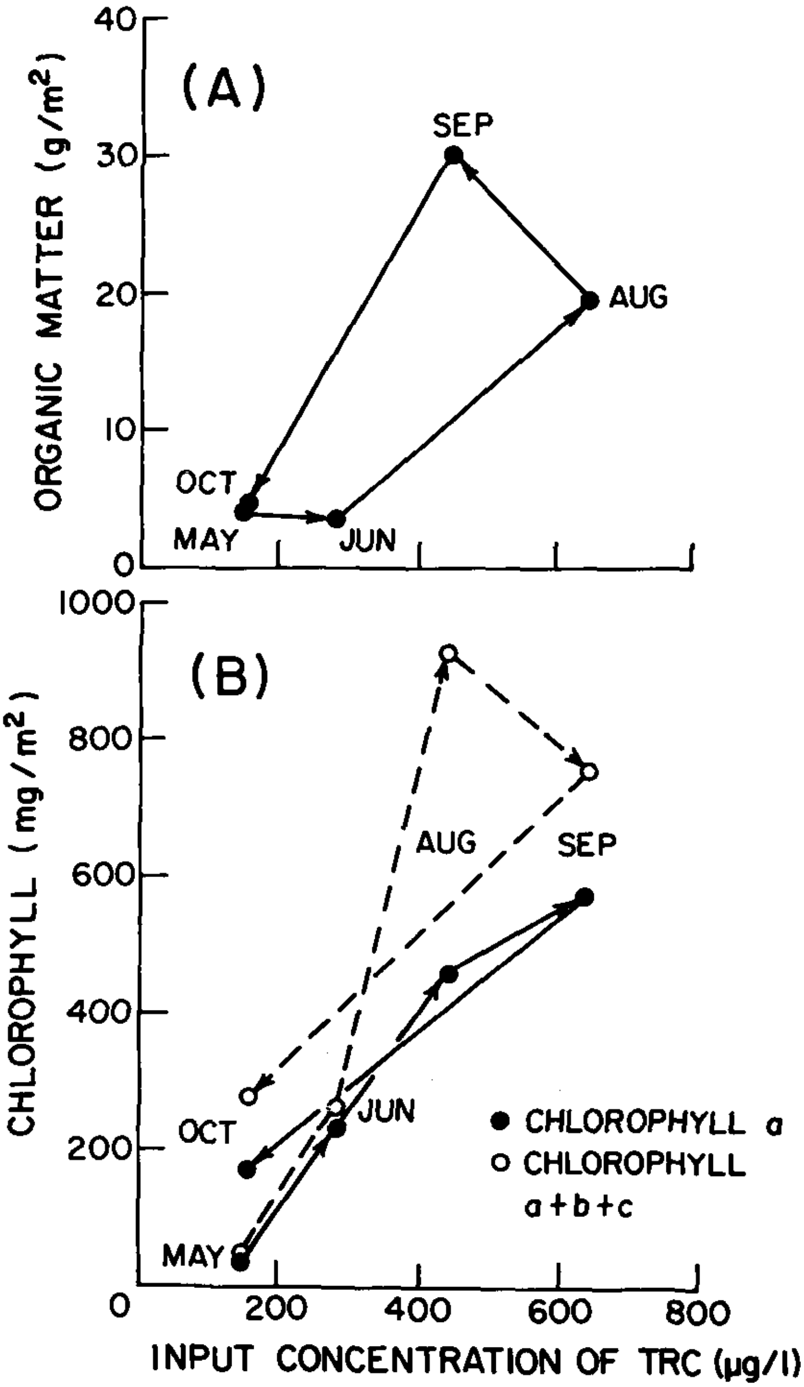

Figure 22. Relationships between the concentration of inorganic chloramine (TRC) in renewal water that was necessary to maintain a residual concentration of about 20 µg/l and the amounts of organic matter (A) and chlorophyll (B) present in the stream.

TABLE 30. DENSITY OF ORGANIC MATTER (G/M^2) IN THE LABORATORY STREAMS AT THE END OF EXPERIMENTAL PERIODS 1 THROUGH 6.

Treatment	Stream	Experimental period and date of sample					
		1 May 28	2 June 26	3 August 5	4 September 8	5 October 3	6 November 3
Control	S6	20.03	10.38	16.80	15.54	16.67	7.40
	N1	4.49	15.17	37.88	43.46	20.31	15.43
	N6	8.77	11.21	57.12	34.18	14.14	17.36
	Mean	11.10	12.25	37.27	31.06	17.04	13.40
2.9 µg/1 TRC	S3	9.09	40.87	24.84	25.40	25.62	8.15
	S5	4.49	5.76	35.27	57.12	14.70	5.24
	N5	10.08	11.13	37.30	54.57	26.41	16.82
	Mean	7.89	19.25	32.47	45.70	22.24	10.07
7.8 µg/1 TRC	S2	6.08	12.60	25.68	61.01	11.13	5.16
	N2	10.34	23.43	27.03	37.64	16.07	8.47
	N3	9.95	10.87	28.61	47.53	14.77	8.15
	Mean	8.82	15.63	27.11	48.73	13.99	7.26
20.6 µg/1 TRC	S1	4.07	3.94	19.90	30.37	4.86	3.04
	S4	3.98	19.71	29.68	20.86	7.62	8.26
	N4	8.34	3.36	36.36	24.84	19.30	6.63
	Mean	5.46	9.00	28.65	25.36	10.59	5.98

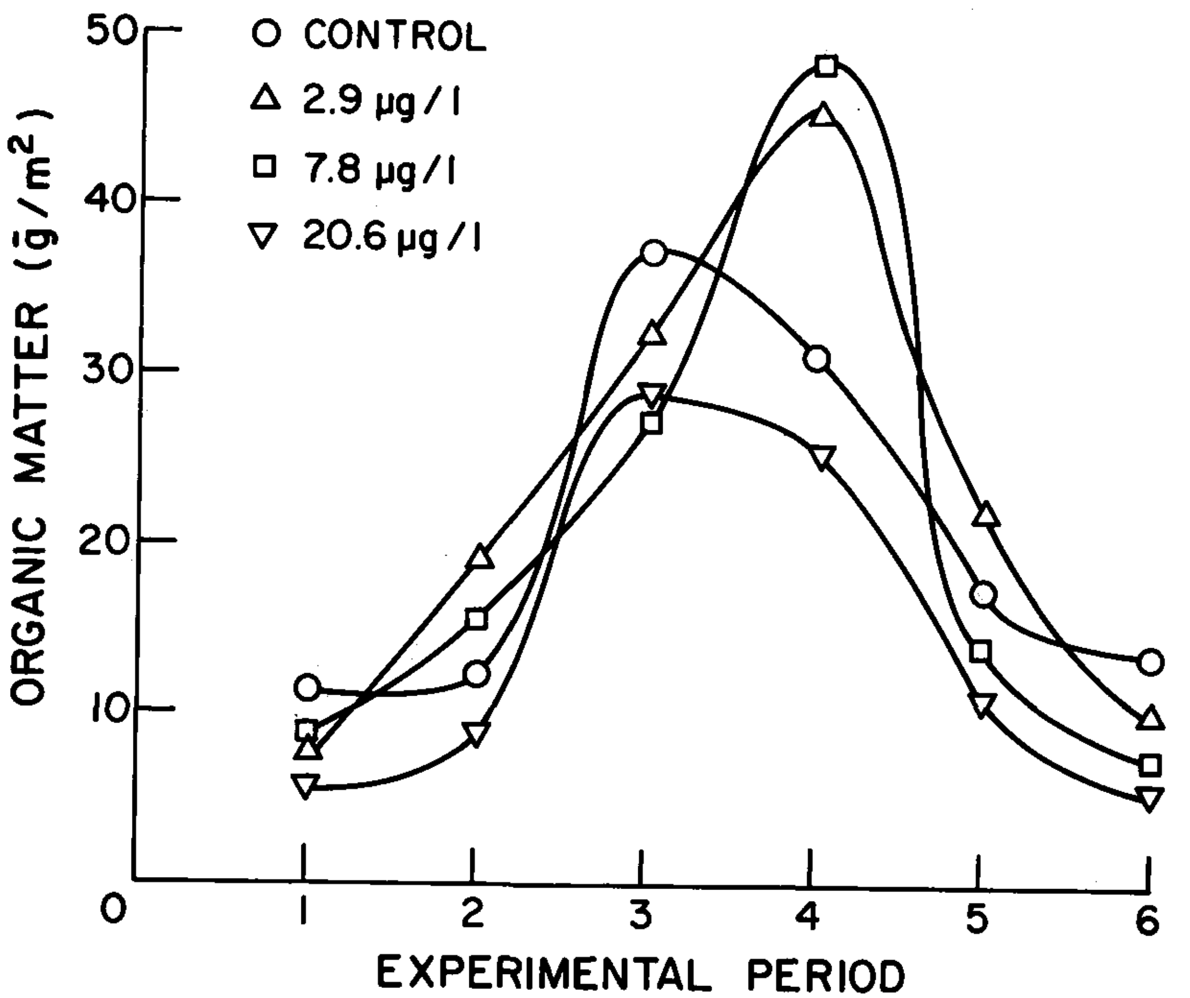

Figure 23. Densities of organic ($\bar{g}/m^2$) in the laboratory streams at the end of each experimental period, from 1 through 6. Each plotted value is a mean for three streams at a given concentration (TRC) and time.

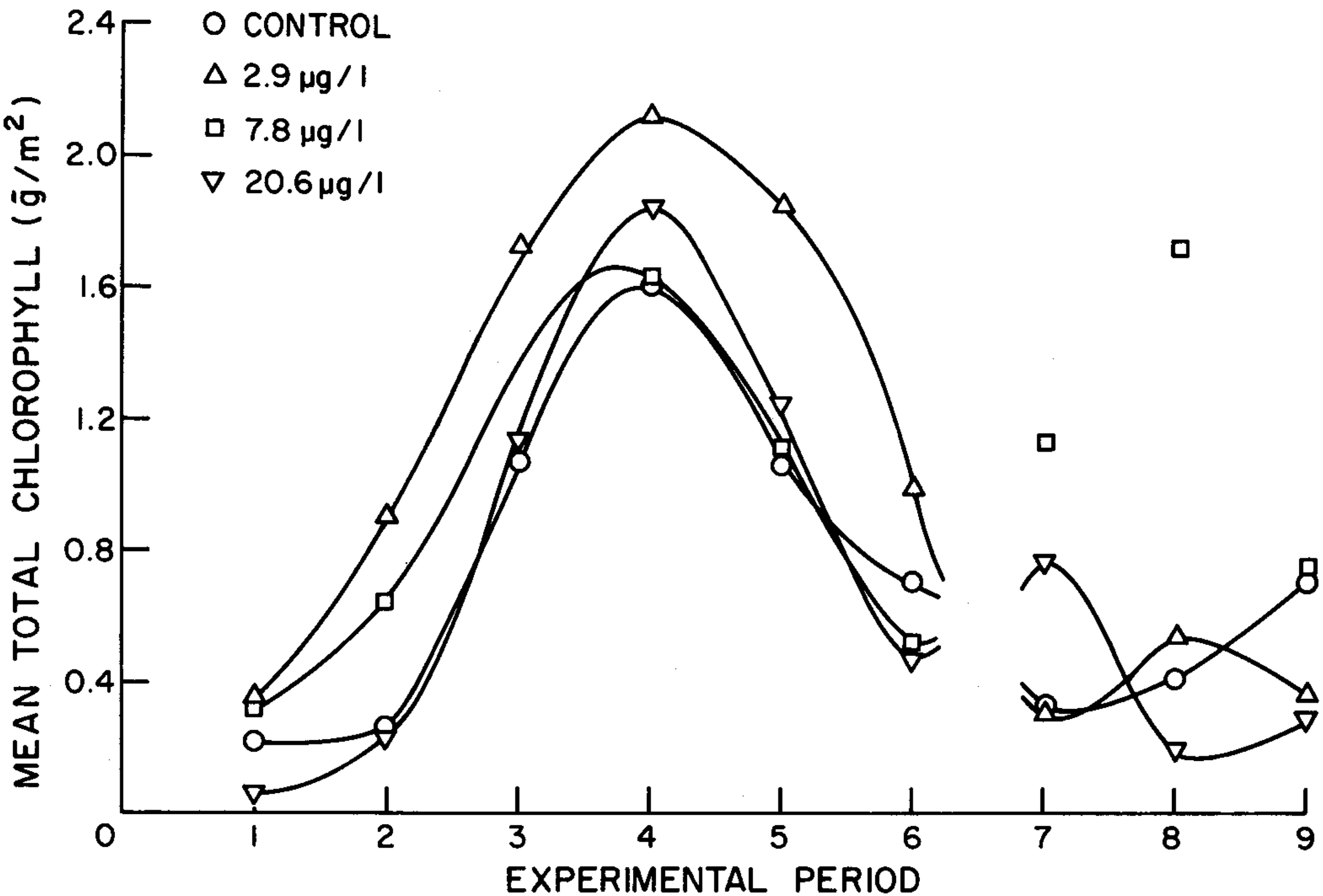

Figure 24. Densities of total chlorophyll ($\bar{g}/m^2$) in the laboratory streams at the end of each experimental period, 1 through 9. Each plotted value is a mean for three streams at a given concentration (TRC) and time.

TABLE 31. DENSITY OF INVERTEBRATES (G/M^2) IN THE LABORATORY STREAM AT THE END OF EACH EXPERIMENTAL PERIOD, INCLUDING TWO PRESAMPLES.

Taxa	Concentration of residual chlorine compounds (TRC, μg/l)															
	0	2.9	7.8	20.6	0	2.9	7.8	20.6	0	2.9	7.8	20.6	0	2.9	7.8	20.6
	April 24, 1975 (Presample)				*May 28, 1975 (Period 1)*				*June 25, 1975 (Period 2)*				*August 5, 1975 (Period 3)*			
Insects	9.50	7.52	4.94	6.71	5.04	7.27	10.48	4.83	2.73	3.12	3.51	3.46	3.15	3.18	2.78	3.08
Gastropoda (Physa)									3.07	0.03	0.17	<0.02	0.73	0.85	0.15	2.27
Isopoda (Asellus)							<0.01	0.20				0.09				1.47
Ostracoda							0.02						0.06	0.30	0.12	0.23
Tubificidae (Tubifex)					0.02	0.25	<0.01	0.03						0.12	0.14	0.02
Miscellaneous								<0.01						0.23		
Total	9.50	7.52	4.94	6.71	5.06	7.52	10.50	5.06	5.80	3.15	3.68	3.57	3.94	4.68	3.19	7.07
	September 8, 1975 (Period 4)				*October 3, 1975 (Period 5)*				*November 3, 1975 (Period 6)*				*April 24, 1976 (Presample)*			
Insects	5.17	4.53	4.87	4.66	3.35	5.06	4.51		3.56	4.27	2.81	0.20	5.80	3.70	2.24	3.03
Gastropoda (Physa)	0.45	2.88	0.65	2.64	1.34	5.79	1.60		1.16	1.93			0.26	0.43	4.16	0.55
Isopoda (Asellus)				5.62			0.09			0.01	2.20	9.31		1.27	0.07	10.65
Ostracoda	1.10	2.35	3.22	2.30	9.34	10.02	2.84		3.21	1.18		3.04	3.87	1.08	2.36	0.21
Tubificidae (Tubifex)	0.13	0.02	0.04	0.32	0.10		0.18		0.01	0.64	0.30	0.23		0.04		0.05
Miscellaneous				0.28	<0.01											
Total	6.85	9.78	8.78	15.82	14.13	20.87	9.22		7.94	8.03	5.31	12.78	9.93	6.52	8.83	14.49
	May 12, 1976 (Period 7)				*June 8, 1976 (Period 8)*				*June 29, 1976 (Period 9)*							
Insects	1.71	6.48	7.99	2.21	1.28	2.32	1.40	0.76	2.39	1.10	1.84	2.62				
Gastropoda (Physa)	6.69	6.08	5.62	1.80	6.94	7.18	7.25	5.18	1.50	0.74	8.06	3.12				
Isopoda (Asellus)	0.15	1.03	0.81	3.20	0.76	1.76	1.61	56.75	1.31	3.02	2.59	9.13				
Ostracoda	2.50	0.83	1.48	1.22	1.07	1.17	4.26	2.12	11.92	12.96	0.41	0.23				
Tubificidae (Tubifex)		0.02	0.10	2.40			0.31	0.52				0.28				
Miscellaneous		0.02			<0.01	<0.01	<0.01	<0.01			0.22					
Total	11.05	14.46	16.00	10.83	10.05	12.43	14.83	65.33	17.12	17.82	13.12	15.38				

Juvenile coho salmon were maintained in the laboratory streams throughout most of the entire experimental period. These fish were dependent for their food upon organisms produced in the streams. And, of course, they were continuously exposed to all other stream conditions, including the presence of residual chlorine compounds in the streams. Coho salmon biomasses, production rates, and relations with their food organisms were quite variable, but probably no more so than in natural streams. And the interpretation of such complex data requires the application of productivity theory, which we will do in a very cursory way. But, however the data were to be interpreted, it is extremely doubtful that effects of chloramine compounds at concentrations near those we sought to maintain could be shown to have had much if any direct or indirect effect on the juvenile salmon.

The mean biomasses of salmon in the 12 laboratory streams were similar during periods 1 through 5, although the high concentration streams supported slightly less fish biomass during periods 1, 2, and 3 than did the other streams (Table 32). No data are shown for the high chloramine concentration streams for period 5, because many of the fish were killed by the short-term presence of free chlorine due to the failure of the tubing pump supplying ammonium to the mixing chambers of the diluter. Fish were restocked in all streams during the winter (period 6), but because of the seasonally low invertebrate biomasses, and the large size of available juvenile salmonids of the current year class, the streams would not support a sufficient number of fish for a good experiment. The period 7 experiment was delayed until higher invertebrate biomass levels were present in May 1976. During periods 7, 8, and 9, the mean fish biomass increased rapidly in the low and medium concentration streams, but it declined or remained nearly constant in the control and high concentration streams (Table 32). Mean salmon biomasses in control streams were well below those in treatment streams during these 1976 periods.

The production rate of fish has been shown to increase to some maximum as fish biomass increases from zero to some intermediate level, but then production rate declines with further increase in biomass (Warren, 1971). Although such relationships are not always well defined, their examination is usually instructive. Each production curve is descriptive, to some extent, of a general level of productivity of the system--its capacity to produce salmon, in the present instance. Two major production relations can be roughly defined for the stream experiments on the effects of chloramines (Fig. 25). Curve A, in Figure 25, describes the high spring-early summer production relation occurring during the beginning of the study (periods 1 and 2). Only the streams having a high concentration of total residual chlorine exhibited lower production during period 2. Fish in the low concentration streams during period 9 were at this high level of production. As summer progressed into fall, production dropped in all streams to around the level described by curve B. Control streams were even lower during periods 7, 8, and 9, as were the high concentration streams during periods 7 and 8. Fish production in medium and low concentration streams increased during the final period 9.

TABLE 32. MEAN BIOMASS, GROWTH RATE AND PRODUCTION OF COHO SALMON EXPOSED TO RESIDUAL CHLORINE COMPOUNDS IN THE LABORATORY STREAM FOR EXPERIMENTAL PERIODS 1 THROUGH 9.*

Mean concentration (μg/l)		Stream	Mean biomass (g/m^2)	Growth rate (mg/g/day)	Production ($mg/m^2/day$)
			Experimental Period 1		
0		N1	5.82	26.4	154
		N6	4.86	22.8	111
		S6	5.30	23.2	123
	Mean		5.33	(24.4)	129
2.3		S3	5.26	21.1	111
		S5	6.39	29.2	184
		N5	4.87	15.8	77
	Mean		5.51	(22.5)	124
4.6		S2	5.60	22.5	126
		N2	5.59	23.0	129
		N3	4.68	17.7	83
			5.29	(21.3)	113
10.3		S1	6.33	27.4	174
		S4	5.16	20.3	105
		N4	3.77	22.0	83
	Mean		5.09	(23.7)	121
			Experimental Period 2		
0		N1	7.32	20.7	159
		N6	7.14	18.9	142
		S6	6.66	14.5	102
	Mean		7.04	(19.1)	134
3.0		S3	5.99	3.8	25
		S5	7.21	19.2	146
		N5	7.88	26.4	219
	Mean		7.03	(18.5)	130
9.1		S2	6.54	12.2	84
		N2	7.59	24.7	197
		N3	7.41	22.3	174
	Mean		7.18	(21.1)	152
22.4		S1	6.22	7.7	50
		S4	6.61	12.5	89
		N4	5.95	5.7	36
	Mean		6.26	(9.3)	58

TABLE 32. CONTINUED

Mean concentration (μg/l)	Stream	Mean biomass (g/m^2)	Growth rate (mg/g/day)	Production ($mg/m^2/day$)
		Experimental Period 3		
0	N1	9.68	4.8	46
	N6	8.66	0.7	7
	S6	8.91	7.1	63
Mean		9.08	(4.3)	39
1.4	S3	6.59	3.2	20
	S5	9.48	4.9	47
	N5	9.29	5.9	57
Mean		8.45	(4.9)	41
6.0	S2*	7.38		
	N2	11.05	7.7	85
	N3	8.90	- 1.7	- 15
Mean		9.98	(3.5)	35
16.6	S1	7.23	2.7	20
	S4	8.89	8.9	80
	S5	7.61	10.4	13
Mean		7.91	(4.7)	38
		Experimental Period 4		
0	N1	7.67	9.5	73
	N6	7.16	3.4	25
	S6	6.99	-3.0	- 8
Mean		7.27	(4.1)	30
1.2	S3	6.57	-0.9	- 6
	S5	6.95	3.1	23
	N5	7.16	1.9	17
Mean		6,89	(1.6)	11
3.9	S2	7.09	-0.7	- 2
	N2	7.71	10.8	82
	N3	7.31	10.5	78
Mean		7.37	(7.2)	53
13.5	S1	7.49	7.6	60
	S4	8.06	16.1	133
	N4	7.02	2.9	25
Mean		7.52	(9.7)	73

TABLE 32. CONTINUED

Mean concentration (µg/l)	Stream	Mean biomass (g/m^2)	Growth rate (mg/g/day)	Production ($mg/m^2/day$)
		Experimental Period 5		
0	N1	8.26	-2.9	-16
	N6	7.45	0.3	2
	S6	6.79	-2.3	- 8
Mean		7.50	(-1.0)	- 7
2.7	S3	6.18	-8.3	-33
	S5	8.27	13.0	108
	N5	7.21	-4.9	-23
Mean		7.22	(2.4)	17
6.9	S2#			
	N2	8.74	2.2	50
	N3	8.15	-3.0	- 2
Mean		8.45	(2.8)	24
24.3	S1#			
	S4#			
	N4#			
		Experimental Period 6		
0	N1#	10.53		
	N6#	11.16		
	S6#	10.93		
Mean		10.87		
2.9	S3#			
	S5#	11.48		
	N5#	10.42		
Mean		10.95		
12.8	S2#			
	N2#			
	N3#			
Mean				
31.5	S1#			
	S4#			
	N4#			
Mean				

TABLE 32. CONTINUED

Mean concentration (μg/1)	Stream	Mean biomass (g/m^2)	Growth rate (mg/g/day)	Production ($mg/m^2/day$)
		Experimental Period 7		
0	N1	5.37	5.6	30
	N6	4.58	-3.8	-17
	S6	4.71	-0.4	- 1
Mean		4.89	(0.7)	4
3.2	S3	5.75	12.7	73
	S5	6.05	13.0	79
	N5	4.97	1.8	9
Mean		5.59	(9.6)	54
8.1	S2	5.85	10.7	63
	N2	5.05	1.9	10
	N3	5.72	10.2	58
Mean		5.54	(7.9)	44
19.8	S1	5.26	5.2	27
	S4	4.72	1.4	7
	N4	4.85	-2.7	-13
Mean		4.94	(1.4)	7
		Experimental Period 8		
0	N1	5.41	-6.8	-37
	N6	3.70	-1.5	- 5
	S6	4.48	-3.8	-17
Mean		4.53	(-4.3)	-20
4.1	S3	7.86	-1.5	-12
	S5	7.46	2.7	20
	N5	4.68	-7.9	-37
Mean		6.67	(-1.4)	-10
9.1	S2	7.52	8.4	63
	N2	5.09	-1.1	- 1
	N3	6.90	3.8	26
Mean		6.50	(4.5)	29
22.4	S1	5.24	-7.2	-38
	S4	4.74	5.1	26
	N4	4.54	-2.1	-10
Mean		4.84	(-1.5)	- 7

TABLE 32. CONTINUED

Mean concentration (μg/l)		Stream	Mean biomass (g/m^2)	Growth rate (mg/g/day)	Production ($mg/m^2/day$)
			Experimental Period 9		
0		N1+	3.53	-29.3	-139
		N6+	2.35	-29.6	- 69
		S6	4.71	8.4	40
	Mean		3.53	(-15.9)	- 56
5.0		S3	8.59	9.5	82
		S5	10.01	22.2	222
		N5	4.50	18.6	84
	Mean		7.70	(16.8)	129
9.7		S2	8.95	7.6	68
		N2	5.88	12.6	74
		N3	8.37	13.3	112
	Mean		7.73	(11.0)	85
24.5		S1	5.45	20.1	109
		S4	5.22	7.4	38
		N4	4.88	8.7	43
	Mean		5.18	(12.5)	63

* Mean growth rate for fish in the three streams at each toxicant concentration and control for each experimental period = mean production/ mean biomass.

Insufficient number of fish recovered from stream.

* 2 or more fish not recovered from stream.

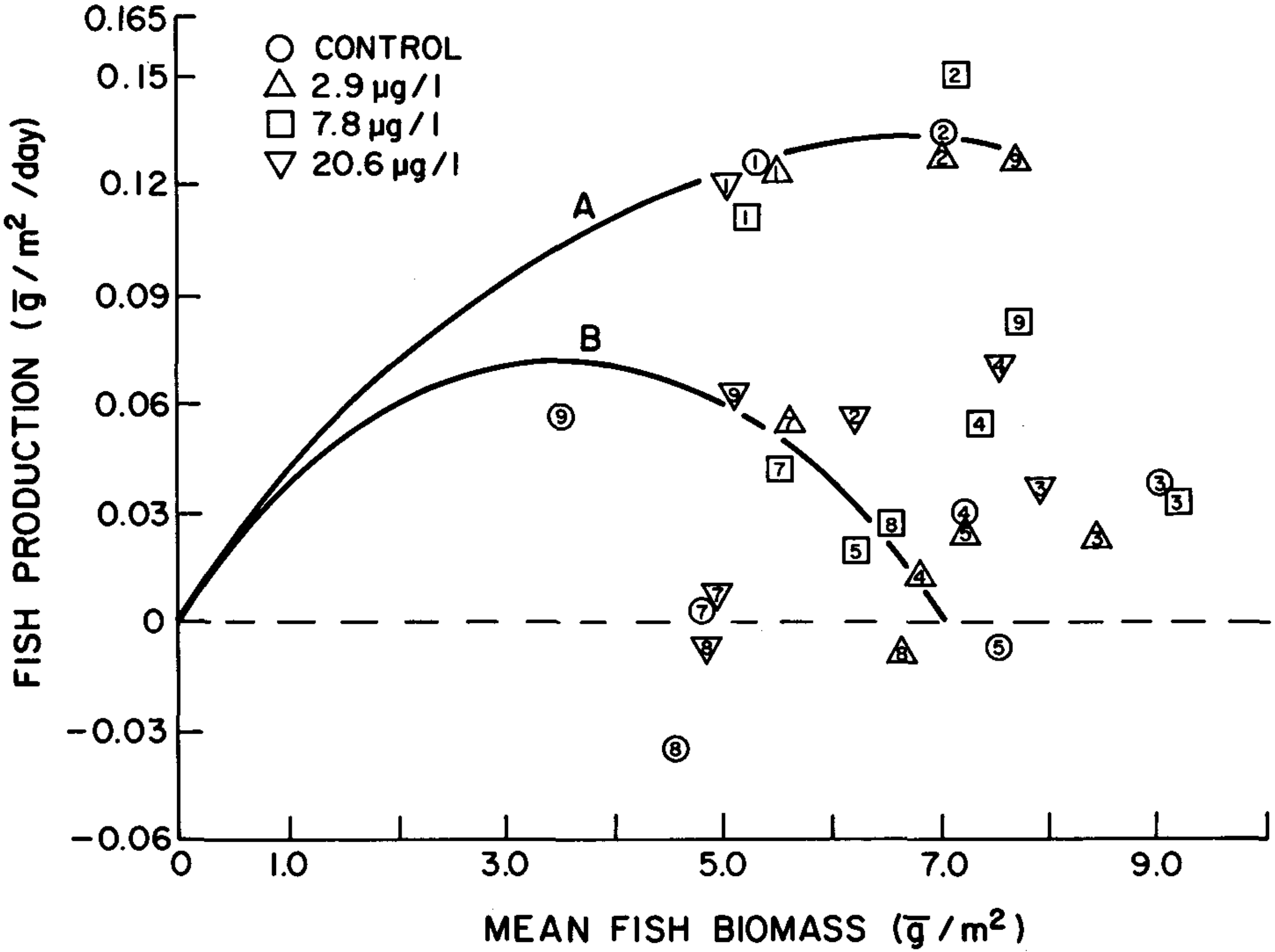

Figure 25. Relationships between the production and biomass of coho salmon in the laboratory streams. The enclosed numbers (1 to 9) refer to the experimental periods (see text). Two possible levels of productivity are indicated by curves A and B.

Except for period 2, salmon production in the three high concentration streams was as high or higher than that in control streams. Production in low and medium concentration streams was higher than in other streams during periods 7, 8, and 9. The presence of some ammonium derived from the introduction of the toxicant able to enrich these systems could be speculated.

Relationships between a predator species and its prey--coho salmon and invertebrates in the present case--are extremely complex. Generally, for fixed values of some external variable or variables (organic matter in this instance) prey density is related to predator density by a "prey isocline" defined by a series of possible equilibrium points. Other levels of organic matter can theoretically be shown to parameterize other relationships of this sort. Another set of isoclines, "predator isoclines," defining relations between predator and prey can be shown to be parameterized by predator loss terms. Theory for this has been explicated by Booty and Warren (MS), but this is too involved to go through here. Even so, some representation of fish and invertebrate data in terms of such relations may be worthwhile, for it shows that even with more sophisticated interpretations, no consistent effects of residual chlorine on fish and invertebrate could be shown (Fig. 26).

The descending "prey isoclines," P_1 through P_4, are identified with values for organic matter, as estimates of the energy and material availability to the predator-prey systems. Mean values for organic matter for stream data points along the lines P_4, P_3, P_2, and P_1 are respectively, 25.7, 19.3, 12.8 and 8.6 g/m , isocline P_4 partially defining high level of productivity for the salmonids. The "predator isoclines," M_1 through M_4, can be parameterized by different rates of mortality of the predator, salmon, M_1 being at a low mortality rate, M_4 being at a high rate. Examining mortalities during the experiment for data points along the predator lines, mean values of fish deaths per period per stream for M_1, M_2, M_3, and M_4 were 0.2, 0.4, 0.7 and 3.6 respectively. M_4 included some points for the late fall when mortality was too high to allow a meaningful calculation of fish production, as noted earlier.

Such a theoretical interpretation allows us to relate densities of salmonids to densities of invertebrates by two series of relationships, the first parameterized by organic matter, the second by fish mortalities. Although streams having low concentrations of total residual chlorine tended to have coordinate values for invertebrates and salmonids located about P_3 or above, and about M_3 or above, the distribution of coordinate values does not clearly show residual chlorine to have had a definite impact on the stream community.

In the growth studies of juvenile coho salmon in aquaria, which we reported earlier, growth was clearly depressed at concentrations of chloramines near 20 μg/l TRC. We might then have expected growth and production of juvenile salmonids in the laboratory streams to have been depressed at mean concentrations near 20 μg/l TRC, but this does not appear

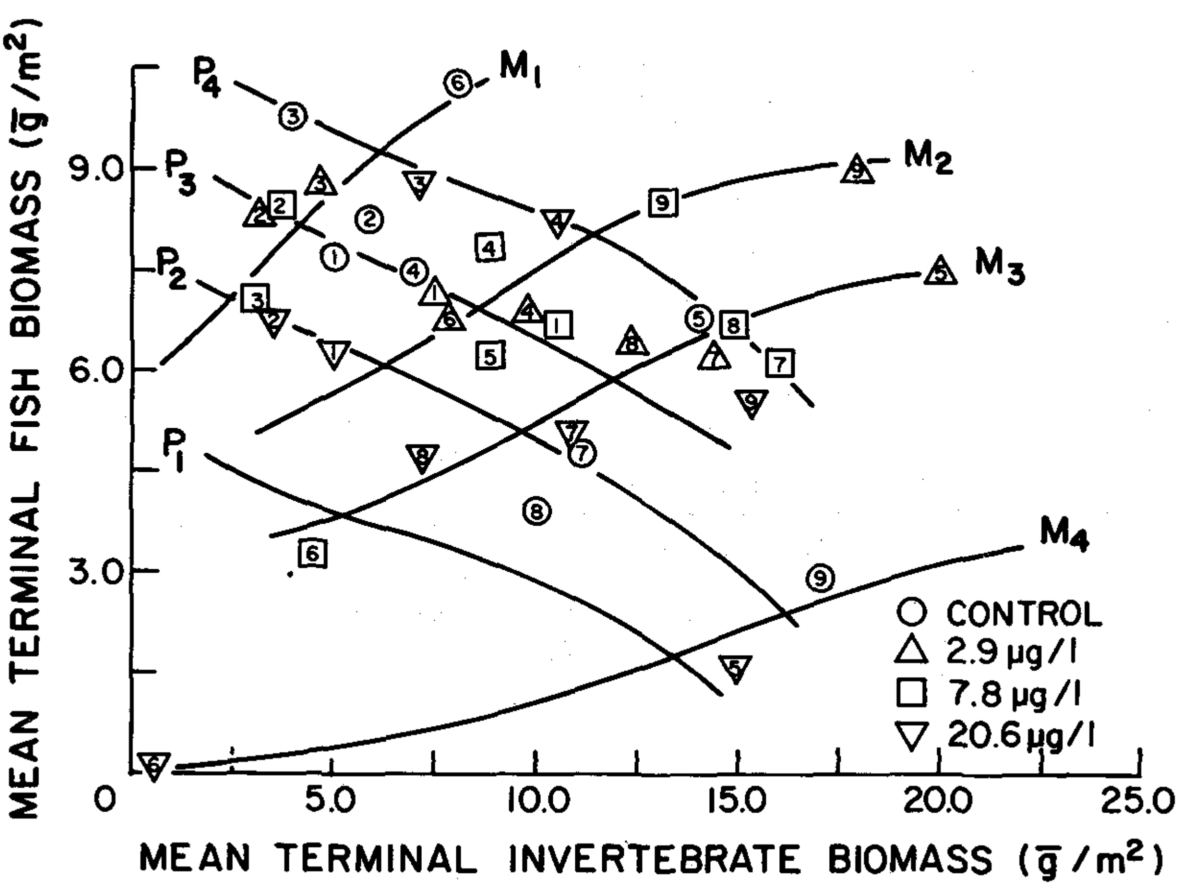

Figure 26. A graphical analysis, using the isocline method (Booty and Warren, MS) of the relationship between the mean terminal invertebrate biomass and fish biomass for each experimental period. The identity of the prey isoclines (P) is organic matter. The identity of the predator isoclines (M) is *mortality of fish. The enclosed numbers (1 to 9) refer to the experimental* periods. Each plotted value is a mean for three streams at a given concentration and time.

to have occurred. Variability in stream conditions and data may have been sufficient to obscure such a depression of growth and production, were it to have occurred. But we are more inclined to believe that no appreciable differences in production owing to the presence of residual chlorine compounds occurred. Differences in stream conditions make any simple extrapolation of the aquaria data hazardous. But beyond this, uncertainty of the meaning of the values for total residual chloramines--which were determined by amperometric titration--in the streams may be the biggest consideration. The concentrations of inorganic chloramines may have been considerably lower than the analyses suggest. Even so, it is of considerable interest that exchange flows of water containing up to about 800 μg/l TRC had little if any effect on the invertebrates and salmonids in the streams, whatever may have been the residual concentrations maintained in the streams.

DISCUSSION

Chlorine is exceedingly important for the disinfection of water supplies and sewage effluents and has important applications in controlling undesirable biological growth in power generation cooling facilities and other industrial applications. But the properties of chlorine and some of its compounds that make it valuable in such applications also make it a potentially serious toxic hazard to aquatic organisms in receiving waters. The setting of standards to protect aquatic life from toxic effects of chlorine and chlorine derivates, standards that still permit necessary uses of chlorine, presents an exceedingly complex problem, but perhaps no more so than setting standards for most other toxic materials.

Ward (1974) has estimated that about 200,000 tons of chlorine are used annually in wastewater treatment. It is our impression that unnecessarily large amounts of chlorine are used in sewage treatment and in slime control in power generation facilities. More chlorine is often used in sewage treatment than has been shown to be necessary for effective disinfection. Entirely too little effort has been expended to determine the minimum levels of chlorine application that would effectively control slime. Use of such knowledge could result in considerable economic savings as well as minimize the danger to aquatic life. It is our present belief that effective application of chlorine and the protection of aquatic life can usually be achieved. But more yet needs to be known about chlorine application technology, chemistry, and effects on aquatic life. Still, even now, enough is known to permit much better use and control of chlorine.

What is toxic about chlorinated effluents? This is not an easy question, and it has no single answer. The chemistry of chlorine in effluents and in receiving waters is highly complex, and it is not very well understood. Some investigators report that free residual chlorine is more toxic than inorganic chloramines (Merkens, 1958), but others believe the reverse to be true (Holland et al., 1960). Differences in water quality, such as pH, temperature, organic load, ammonia content, affect the chemistry of the chlorinated effluents. In addition to this, chlorinated effluents may contain small amounts of numerous chlorine containing organic compounds (Jolly, 1975). Very little is known about these compounds or their toxicity. The point we are making is that the conditions under which fish and other aquatic organisms are exposed in nature are complex, poorly understood, and probably make impossible any single adequate universal standard for chlorine.

Determination of levels of residual chlorine not likely to deleteriously affect aquatic life is further complicated by different patterns of exposure to the toxicants, different responses of various species of fish and different life stages of particular species, and the many possible effects on other organisms in aquatic communities. It is a problem faced in setting standards for any toxic material, and it is not one for which we are likely to develop entirely satisfactory solutions.

We have already mentioned that there is ample evidence that residual chlorine can be acutely toxic to aquatic life, even at very low concentrations, under conditions of continuous exposure (Brungs, 1976; Arthur et al., 1975). Tsai (1973) has shown in the field that chlorination of sewage effluent has caused great reductions in the species diversity of fishes, his index of diversity falling to zero where the mean concentration of residual chlorine was about 250 μg/liter. He also observed no fish in streams where the mean residual was near or above 370 ug/liter. And he found brook trout to disappear from streams receiving chlorinated sewage discharges, the mean concentration of residual chlorine in these streams being about 20 μg/l.

The work presented in this report was an attempt to determine the responses--survival, development, growth, behavior, and reproduction--of fishes to residual chlorine as influenced by life stage, water quality, and patterns of exposure. Possible effects of residual chlorine on the structure of laboratory stream communities and the production of salmon in the streams were also studied. As originally planned, a wide variety of approaches was employed. This prevented as much replication of the research as we would have liked. Nevertheless, taken as a whole, the work does elucidate important problems of chlorine and chloramine toxicity to fish and other aquatic organisms.

At acutely toxic concentrations of chloramines under conditions of continuous exposure, we showed the tolerance of salmonids to be functions of life stage and body weight. Others have also shown that body weight can affect the median lethal levels for fish exposed to residual chlorine (Rosenberger, 1971; Wolf, 1976). It is important that all life stages of species likely to be exposed to residual chlorine be taken into account in establishing standards for their protection, special emphasis being placed on the most sensitive. It is not going to be generally adequate to base standards only on the results of experiments on particular life history stages of species of concern.

Based upon the complex chemistry of chlorine in water and effluents, variations in water quality are apt to result in shifts in species of chlorine present, shifts that could alter the toxicity of the solutions, with or without change in concentration of total residual chlorine. Merkens (1958) discussed this with respect to chlorinated effluents discharged into rivers. In our experiments, we do not know why the toxicity of test solutions to coho salmon juveniles increased with pH increase from 7.5 to 8.1. The expected increase in monochloramine did not occur with the increase in pH, the percentage of monochloramine being 83 at each pH. Certainly much more needs to be known about the chemistry of chlorine and about entry and activity of residual chlorine compounds in fish. Some suggest that the primary action of chlorine is gill damage (Bass, 1975), but others believe this not to be the case (Fobes, 1971). The action and effects of chlorine in fish are probably much more complex than now thought. Buckley et al. (1976) found a decrease of hemoglobin and hematocrit to levels indicative of anemia in coho salmon exposed for 12 weeks to 30 μg/l. total residual chlorine in municipal sewage.

We found no difference in the acute toxicity of inorganic chloramines to coho salmon juveniles at 10 and at 15 C, when the fish were acclimated to these temperatures. Thatcher et al. (1976) obtained a similar result with brook trout exposed to 10 and 15 C, and they concluded that this was not a range of temperatures likely to cause thermal stress influencing the toxicity of residual chlorine compounds. But they did find that increasing the test temperature to 20 C, when acclimation temperatures had been 7, 10, and 15 C, did increase the sensitivity of the trout to residual chlorine. Sudden changes in temperature are likely to greatly influence the toxicity of chlorine and derivative compounds; and this can be an important problem when these are present in thermal discharges.

Chlorination of complex industrial effluents presents extremely difficult problems of chemistry and toxicology. We found stabilized pulp and paper mill effluents, which were not usually acutely toxic, to become acutely toxic after chlorination with about 1 mg/liter of total residual chlorine. Yet no residual chlorine was detectable in the test solutions in the aquaria. Much work would be necessary to identify the toxic compounds. Reduction of toxicity of such effluents may be possible through increased retention time, as found by Watkins (1973), or by other means, even when the toxic compounds are not known.

For a given species of fish, we would expect that the median lethal levels of acutely toxic solutions under conditions of intermittent introduction of residual chlorine to be higher than those for 96-hour continuous exposure, because of the shorter duration of exposure. But analysis and interpretation of results of intermittent exposure experiments present problems. The usual method of representing toxicity in terms of toxicant concentration does not permit results obtained from particular patterns of concentration and time of exposure to be applied to any other patterns to which organisms might be exposed. Results of experiments in which fish are exposed to patterns of given form, duration, and frequency, when reported in terms of peak or mean concentrations, are applicable only to those conditions of exposure. Those investigating spike patterns of exposure tend to report peak concentrations (i.e., Heath, in press), and those investigating square patterns of exposure tend to report mean plateau concentrations (i.e., Brooks and Seegert, 1977). But the precise patterns and all patterns occurring in receiving waters can never be duplicated and studied. We believe that further representation of data resulting from intermittent exposure experiments on the basis of the areas (expressed as mg/liter x time) under concentration-time curves could reduce problems of analysis, interpretation, generalization, and application. We showed, for the range of chlorine concentrations and shapes of curves that we studied, that the area under the curves was a general representation of the toxicity of different patterns of exposure. But the general usefulness of this sort of representation should be investigated for a greater variety of patterns, concentrations, and frequencies of exposure, so as to make boundary conditions of its applicability clear. Intermittently discharged effluents generally contain toxicants other than chlorine (Dickson et al., 1974), and this may influence the general usefulness of this approach. Furthermore, we used solutions containing mostly free residual chlorine, and work should be done with inorganic chloramines, the latter being predominant in some intermittent discharges (G. Nelson, EPA, personal communication).

Not only the toxic responses of fish to different patterns and durations of exposure but also to different frequencies of exposure may be usefully represented by total areas under time-concentration curves. In our one and two 90-minute square patterns of exposure, we found that bass did not recover much, if any, during the two-hour interval between the exposures. Using this approach, the amount of recovery between exposures can be directly determined when the duration between exposures increases. This approach could add much generality to work on intermittent exposure to residual chlorine compounds, and make it unnecessary to study all possible patterns, durations, and frequencies of exposures of fish and other organisms.

Our work and that of others (Brooks and Seegert, 1977; Greg, 1974; Stober and Hanson, 1974) indicate that temperature under conditions of intermittent chlorination can have substantial effects on the toxicity of residual chlorine compounds to aquatic organisms. This is especially true if there is considerable difference between the temperature of effluents and the temperature to which fish are acclimated (Stober and Hansen, 1974), for the fish may move from cooler waters into heated effluent plumes. Brooks and Seegert (1977) showed for alewife, after acclimation for up to two weeks at 10, 20, 25, or 30 C, that the median lethal level for one 30-minute exposure to residual chlorine decreased with increasing acclimation temperature. Our own work and that of Thatcher et al. (1976) indicate that a sudden increase in temperature over the acclimation temperature decreases the tolerance of fish to residual chlorine compounds. This must be taken into account in research and application of results to problems associated with the intermittent discharge of heated effluents containing residual chlorine compounds.

The empirical rule that warm-water fishes are less sensitive to residual chlorine than are cold-water species needs to be evaluated critically (Brungs, 1976). There appears to be a continuum of sensitivity between these two groups of fishes. But some species of minnows, not all of which are actually "cold-water" species, may be at least as sensitive to intermittent exposures to residual chlorine as are salmonids, as our results for the blackside dace suggest.

The significant feature of the partial chronic test is that the toxicity of relatively low concentrations of toxicants can be determined for fish and other organisms, when exposure is continuous throughout development of life history stages from gonadal maturation through juvenile growth. The success of partial chronic tests, as they have been defined, is largely dependent upon successful reproduction of the test species under test conditions. Arthur and Eaton (1971) exposed fathead minnows (96-hour TL50 between 85 and 154 μg/l total residual chlorine) for 21 weeks to residual chlorine. They found no effects on adult survival at concentrations of 43 μg/l and less, but spawning was reduced somewhat at 43 μg/l total residual chlorine. No effects on reproduction were observed at 16 μg/l. In tests we conducted with cutthroat trout, brook trout, and crayfish, reproduction was not successful. But we did establish that the trout could tolerate very long exposures to concentrations as high as 50 μg/l total residual chlorine (96-hour LC50 for juveniles was less than 100 μg/l). We did notice, however, that at the highest test concentration, about 50 μg/l, the trout did not eat as well as those fish exposed to lower concentration of the toxicant and to control conditions. In the tests with crayfish (96-hour LC50 greater than 749 μg/l), those exposed to about 50 μg/l lived for many months, but then most died during molting. The sensitivity of trout and crayfish to residual chlorine may not be very different under long-term exposure, even though median lethal levels determined only for 96 hours are very different for most life history stages.

When it is not possible or appropriate to conduct partial chronic tests on a species, it may be useful to determine growth and other responses of particular life history stages to relatively low concentrations of toxicants. In some of our tests with coho salmon, we examined the effects of concentrations of about 50 μg/l and less of total residual chlorine (mainly monochloramine) on the survival, development, and growth of embryos and alevins. The highest concentration had no measurable effect on embryo survival or the size of the alevins at hatching. The high concentration was, however, lethal to the alevins. The survival of alevins was not affected at about 23 μg/l total residual chlorine, but their growth rates were substantially reduced as compared to alevins exposed to concentrations of about 11μg/l and less and to control conditions. Such reductions of growth could delay the emergence of fry from gravel beds and could reduce their ability to compete for food and space in streams. Similarly, the growth of juveniles was reduced at 22-23 μg/l, the threshold being between 11 and 22-23 μg/l. Such reductions in growth rate could have a substantial impact on the size of juveniles at smolting. Reduced size of seaward migrants has been shown to be correlated with increased mortality rates of salmon (Ricker, 1972). Such studies provide useful data upon which to establish effects on fish of sublethal concentrations of residual chlorine known to occur in natural streams (Tsai, 1973).

A number of investigators have reported behavioral changes in fish subjected to either continuous or intermittent exposure to residual chlorine compounds (Dandy, 1972; Basch and Truchan, 1976; Brooks and Seegert, 1977). Although it is difficult to relate these behavioral changes to the persistence and status of fish populations in nature, such changes at least indicate the fish are stressed. We found the behavior of coho salmon alevins to be altered substantially when they were exposed to about 23 μg/l of total residual chlorine. The changes were probably sufficiently great to interfere with intragravel bed movement and later emergence of fry. Lethargic swimming and bobbing of largemouth bass, as a result of their exposure to free residual chlorine, could be detrimental to their survival by affecting their ability to avoid obstacles and predators and escape from chlorinated discharge plumes. Basch and Truchan (1976) noted that in some cases salmonids exhibited considerable distress at the water surface when exposed to intermittent chlorinated discharges from power generation plants. Others have observed birds feeding on fish floundering at the water surface below outfalls of chlorinated discharges from power plants (Brungs, 1977). Such behavioral responses as lethargic swimming, thrashing, and bobbing observed in our work with bass may be indicative of those noted in field studies. It is important to point out that in our studies bass exhibited these behavioral changes as a result of sublethal as well as lethal intermittent exposures to residual chlorine. Changes of behavior could serve as a sensitive index of sublethal exposure of fish to residual chlorine compounds, and the responses themselves may lead to reduction in the survival of fish.

Avoidance responses could have much to do with the actual exposure of fish to chlorine compounds in nature. Unfortunately, laboratory studies of avoidance, for dimensional and other reasons, may not be at all appropriate and are certainly difficult to interpret. And adequate field studies of avoidance behavior would be difficult to conduct. Sprague and Drury (1969) found that rainbow trout avoided as little as 1 μg/l total residual chlorine. Yet, the trout did not avoid the much higher concentration of 100 μg/l, and this could be given the rather unlikely interpretation "preference." Fava and Tsai (1976) noted a similar response of blacknose dace to free residual chlorine but not to inorganic chloramines. Meldrin et al., (1974) did not observe such an apparent "preference" response, nor did we. This illustrates a problem of all laboratory studies of avoidance behavior. Design of apparatus, conduct of experiments, and recording of behavior, as well as the ways in which observed behavior could be interpreted offer so many possibilities as to make extrapolation to nature extremely doubtful. Even so, such studies do indicate that fish do have the ability to avoid chlorinated waters under some conditions.

One aspect of this work needs attention. Tsai and Fava (1975) and we have shown that tested fish delay in moving out of chlorinated solutions. We showed that this resulted in bass receiving increased exposures as the toxicant concentration increased. Yet, even at the highest acutely toxic concentration of residual chlorine tested, the fish moved out of the solution soon enough not to receive lethal exposures to the toxicant. Delayed response behavior by fish in the field could be deleterious to their survival, even if conditions were such as to make avoidance possible. If the fish were exposed in an area over which chlorine compounds were widely distributed, or if exposures were such as to adversely affect their behavior, delay in avoidance behavior could lead to increased mortality. In our avoidance study with largemouth bass we found a greater response to free residual chlorine than to inorganic chloramines at the same concentration of total residual chlorine. Tsai and Fava (1975) found just the reverse situation when working with the blacknose dace.

In our laboratory stream studies, we found it necessary to introduce 100 to 800 μg/l total residual chlorine, in an exchange flow of two liters per minute, so as to maintain desired concentration of about 20 μg/l or less in the streams. Seasonal variation in the needed rate of introduction of chloramines was correlated with the amount of organic matter present in the streams. Our amperometric titration results indicated that from 50 to 80 percent of the residual chlorine compounds detected in the streams was dichloramine. But J. D. Johnson (personal communication) believes that dichloramines should not have been present at the stream pH levels we recorded and that what we detected as dichloramines could have been organic chloramines.

That streams containing as much organic matter as did our laboratory streams should have such a high chlorine demand is not particularly surprising. But attempts to account for this demand, without extensive empirical and theoretical investigation, are not apt to be very revealing. Loss of chloramines to break-point effects does not appear likely on the basis of present understanding of this difficult chemistry, because the chlorine/ammonia ratio of solutions added to the streams was slightly less than 1:1 (Sawyer, 1967). The demand of the laboratory streams for chloramines may have resulted from the formation of inorganic salts and chlorine containing organic compounds. Some losses of residual chlorine could have occurred through the volatilization of trichloramine, but monochloramines and dichloramines are not very volatile (J. D. Johnson, personal communication.) We can only conclude that, even though relatively high concentrations of residual chlorine were introduced into the streams to obtain desired residuals of 20 μg/l or less, the forms in which detected residuals were present and thus their possible toxicity to organisms in the streams was quite uncertain.

Our studies of organic matter, chlorophyll, macroinvertebrates, and juvenile salmon growth and production in the stream communities did not demonstrate any marked effects of introduction of chloramines. We would have expected, on the basis of our aquarium studies, some effects of residual chlorine concentrations near 20 μg/l on the growth and production of the salmon. But in the aquarium studies, the fish were exposed to residual chlorine mainly in the form of monochloramine, and we know practically nothing about what residual compounds actually constituted the determined stream concentrations. The 24-hour cycle in residual chlorine we observed in the streams may also have made conditions there less deleterious to the fish. The difficulty we found in applying simple aquarium experiments even to controlled laboratory stream experiments raises some question as to how adequately we can generally apply laboratory results to natural stream conditions, which must usually be still more complex. Along with more laboratory investigation of problems of chlorine toxicity, much more extensive and detailed investigation of the forms of residual chlorine present in stream waters under different conditions and of the apparent effects of these on populations of fish and other stream organisms would certainly be well advised. Only with more information of this sort can we expect to set standards adequate for the protection of aquatic life and still permit legitimate use of chlorination.

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16. ABSTRACT

Laboratory studies on the acute and chronic toxicity of chlorine and inorganic chloramines to trout, salmon, minnows, bullhead, largemouth bass, and bluegill were conducted. Acute toxicity under continuous and intermittent patterns of exposure as well as behavioral, reproduction, development, and growth responses to low level exposures to residual chlorine compounds were determined. But not all patterns of toxicant exposure or all responses of all fish species were studied. Acute and chronic toxicities of chloramines to crayfish were investigated. Algae, invertebrates, including insects, and juvenile salmon were exposed continuously to relatively low levels of residual chloramine compounds in laboratory stream communities. The acute toxicities of inorganic chloramines, as measured by 96-hour LC50 values, were less than 100 μg/l for salmonids and were a function of life history stage, body size, and some water quality conditions. Whereas adult trout may live indefinitely at concentrations near 50 μg/l, the LC50 values for late developmental stages--fry and very small juveniles--were not much above this concentration. Effects on growth of alevins and juveniles had threshold concentration values between about 10 and 22 μg/l, effects being quite marked at 22 μg/l. In intermittent exposure to relatively high concentrations of free residual chlorine, mortality was found to be a rather consistent function of the area under the time-concentration curves of exposure, for different forms, durations, and frequencies of such patterns of exposure. Behavioral responses of fish, such as avoidance of chlorinated water which could be advantageous in nature and lethargic swimming, surfacing, and sinking to the bottom which would probably be harmful were studied. Such behaviors occur not only at acutely toxic concentrations but also at lower ones. It was necessary to introduce concentrations ranging from 100 to 800 μg/l of chloramine into laboratory stream communities to maintain mean residual concentrations near 20 μg/l. No marked effects on algal or insect abundances or on survival and production of juvenile salmon were observed at this and lower concentrations in the laboratory streams. It is doubtful that the amperometrically determined residual concentrations of chloramines in the streams consisted predominantly of inorganic chloramines, organic chloramines perhaps being an important constituent under the stream conditions. Little is known of the toxicity of organic chloramines. The amount of inorganic chloramines introduced to maintain desired residual concentrations appears to have been a function of the amount of organic material in the stream communities.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS		b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Chlorination		Aquatic bioassays	57F
Chlorine	Condenser tubes	Chronic bioassays	57H
Toxicity	Cooling water	Toxicity tests	57Y
Bioassay	Cooling towers	Brocides	
Aquatic animals	Fouling prevention		
Fishes	Sewage		
Disinfection	Sewage treatment		

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