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Title: SOME EFFECTS OF LIFE STAGE, TEMPERATURE, PH, AND ALKALINITY ON THE ACUTE TOXICITY OF INORGANIC CHLORAMINES TO YOUNG COHO SALMON (ONCORHYNCHUS KISUTCH)

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Acute toxicity bioassays (96-hr TL_{50}) were employed to determine the tolerance of coho salmon (*Oncorhynchus kisutch*) to inorganic chloramines at the alevin (yolk sac fry), yolk absorption, and early juvenile life stages. Any influence of changes in temperature, pH, and total alkalinity on the tolerance of juvenile coho for chloramines was also investigated.

The least tolerant stage occurred shortly after yolk absorption. No change in juvenile coho was found when temperature was increased from 10.8 to 15.0 C. Increasing alkalinity from 135 to 320 mg/l was not shown to affect tolerance of the juvenile stage. Increasing pH from 7.0 to 7.5 apparently did not change tolerance at this stage, but an increase from 7.5 to 8.1 resulted in a significant decrease in tolerance.

Some Effects of Life Stage, Temperature, pH, and Alkalinity on the Acute Toxicity of Inorganic Chloramines to Young Coho Salmon (*Oncorhynchus kisutch*)

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INTRODUCTION

When chlorine is added to water that contains ammonia,[1] the chlorine and ammonia combine to form the inorganic chloramines monochloramine and dichloramine:

$$NH_4^+ + HOCl \rightarrow H^+ + H_2O + NH_2Cl$$

$$NH_2Cl + HOCl \rightarrow H_2O + NHCl_2$$

The proportions of monochloramine and dichloramine present are dependent upon the pH of the water. As pH increases from 5 to 9 the amount of monochloramine increases while the amount of dichloramine decreases, the reverse occurring when the pH decreases from 9 to 5 (Moore, 1951). This relationship is described by the equation:

$$2NH_2Cl + H^+ \rightleftharpoons NH_4^+ + NHCl_2$$

The presence of combined residual chlorine[2] in chlorinated domestic sewage and in wastewater from industries using chlorine

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[1] Organic nitrogen in water is converted to ammonia by aerobic saprophytic bacteria; this situation is most evident in secondary treated sewage effluent (Sawyer and McCarty, 1967; Warren, 1971).

[2] Combined residual chlorine is the sum of the concentrations of chloramines (mono- and di-) and other chloro derivatives.

for disinfection, bleaching, and antifouling of water lines creates conditions toxic to fish fauna inhabiting receiving waters (Brungs, 1973). From studies conducted in natural streams that received chlorinated effluents, the Michigan Department of Natural Resources (1971) found the 96-hr TL_{50}[3] for rainbow trout (Salmo gairdneri) to be 22.8 μg/l total residual chlorine;[4] concentrations high enough to cause mortality persisted for 0.8 mile downstream from the point of discharge. Further evidence that chlorination created the toxic condition was that the effluent when not chlorinated was not toxic to the trout. The 96-hr TL_{50} for fathead minnows (Pimephales promelas) was between 80 and 190 μg/l total residual chlorine (3.20-7.04 percent effluent) in chlorinated sewage effluent; no mortality of fathead minnows exposed for 96 hours to dechlorinated sewage effluent occurred in 100 percent effluent (Zillich, 1969). Growth and survival of fathead minnow larvae were reduced at 108 μg/l total chloramine[5] (Arthur and Eaton, 1971). Tsai (1973) reported 50 μg/l total residual chlorine approximately 5 miles below the point of discharge of chlorinated sewage into a stream.

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[3]The 96-hr TL_{50} is the 50 percent tolerance limit for the specified exposure time, i. e., the concentration at which 50 percent of the test fish die in 96 hours.

[4]Total residual chlorine is the sum of the concentration of free chlorine and combined residual chlorine.

[5]Total chloramine is the sum of the concentrations of monochloramine and dichloramine.

Because the tolerance of an organism for a lethal factor is dependent on the organism's genetic constitution and environmental history, tolerance may vary between individuals, between life history stages, and according to previous exposure to the lethal and other factors. Also, the level at which a lethal factor causes death often depends on the level of other factors (physical, chemical, and biological) in the organism's environment (Warren, 1971). The objectives of this research were to determine any differences in the tolerance of coho salmon between the alevin, yolk absorption, and early juvenile life stages and any effects of changes in temperature, pH, and total alkalinity on the tolerance of juvenile cohos for chloramines.

METHODS AND MATERIALS

A flowing water dilution system constructed of three $1\frac{1}{2}$ inch PVC pipes (manifolds) delivered the various concentrations of toxicant to the bioassay aquaria (App. 1). Two water manifolds delivered water of either different temperature, pH, or alkalinity to the bioassay and control aquaria. Toxicant was delivered to a toxicant head box from a system beginning with two Mariotte bottles (App. 1). One Mariotte bottle contained sodium hypochlorite (bleach) and the other contained ammonium chloride. Sodium hypochlorite and ammonium chloride entered a retention chamber where chloramine formation took place. The retention time required for this process was three hours, and the molar ratio of chlorine to ammonia was 0.7:1.0. Since ammonia was added in excess in order to prevent the presence of free chlorine, ammonia concentration was monitored to insure that toxic levels were not reached.

From the retention chamber, chloramine solution was added to the toxicant head box where it was mixed with water from an adjacent head box to give a desired total chloramine concentration. This diluted solution then flowed into the toxicant manifold. Desired flow rates of water and toxicant into the test chambers were attained by means of glass tubular spouts (located on the manifolds), which could be raised or lowered to adjust flow. Glass funnels conveyed water and toxicant to separate mixing chambers from where dilutions having the desired toxicant concentrations flowed to the bioassay aquaria. Glass aquaria containing either 15 or 30 l of water were used. Ten fish were placed in each aquarium. Fish smaller than 0.8 g dry weight were tested in 15 l of water; fish larger than 0.8 g dry weight were tested in 30 l of water. Exchange rates were maintained at 500 ml/minute, this giving a 95 percent replacement time of 1.5 and 3.0 hours for 15 and 30 l of water, respectively. Because organic matter adsorbs chlorine and chloramines, well water was used for making both sodium hypochlorite and ammonium chloride stock solutions as well as for dilution and exchange water.

Coho salmon (Oncorhynchus kisutch) from the Alsea River Salmon Hatchery (Fish Commission of Oregon) were used as the test organism. This is an important sport and commercial species in Pacific Northwest rivers, some of which now receive and others of which may in the future receive chlorinated effluents.

Acute toxicity bioassays were performed at 15 C,[6] pH 7.5, and a total alkalinity of 135 mg/l (as $CaCO_3$) in order to define the 96-hr TL_{50} for coho salmon at the alevin, yolk absorption (button-up), and early juvenile life stages. To test the effect of temperature on

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[6] Alevin and button-up bioassays were performed at 10 C to simulate a condition which would be encountered naturally at these two life stages.

the acute toxicity of chloramines, fish were exposed simultaneously to chloramine concentrations at 10 and 15 C at pH 7.5 and an alkalinity of 135 mg/l. Bioassays were performed to determine the effect of pH, these being conducted simultaneously at pH 7.0, 7.5, and 8.1 at 15 C and an alkalinity of 135 mg/l. Ambient pH was about 7.5. To lower pH to 7.0, carbon dioxide gas was bubbled into the water; pH was increased to 8.1 by the addition of 0.2 N sodium hydroxide. To test the effect of alkalinity, simultaneous bioassays of chloramine toxicity were performed at alkalinities of 135 and 320 mg/l at 15 C and pH 7.5. Ambient alkalinity and pH were about 135 mg/l and 7.5, respectively. Alkalinity was increased by the addition of a saturated solution of sodium bicarbonate to the water, the pH was then reduced from 8.0 to 7.5 with carbon dioxide.

Salmon were acclimated for at least seven days to water having the temperature, pH, and alkalinity to which they would be exposed during a bioassay. During acclimation, fish were fed Oregon moist pellet once every two days and denied food two days prior to an experiment. Alevin bioassays were conducted in the dark to simulate light conditions in stream gravels in which they normally develop. Photoperiod during acclimation and bioassays with button-up and juvenile stages was the same as that recommended by McKim and Benoit (1971). Upon completion of a bioassay, test fish were placed in a drying oven at 70 C for seven days to obtain dry weights.

Total chloramine concentration was measured as micrograms per liter (μg/l) of combined resiudal chlorine (chlorine combined with ammonia) using an amperometric titrator (American Public Health Association, 1971). Because of the chloramine concentration instability experienced, samples were measured every four hours during the four day bioassays. If the desired chloramine concentration varied more than ±3 μg/l, the toxicant flow rate was adjusted. An Orion Model 801 Ionalyzer was used to measure pH. Total alkalinity was measured by the bromcresol green - methyl red method (American Public Health Association, 1971).

The 96-hr TL_{50} was 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 total chloramine on a base ten log scale. To test separate logit regression lines for significant differences in slopes and y-intercepts, a computer program designed by Pierce (1975) was utilized.

RESULTS AND INTERPRETATION

Tolerance of coho salmon to chloramines varied between life stages (Table 1; Fig. 1). In the first alevin bioassay, all test animals lived at all chloramine concentrations tested, the 96-hr TL_{50} thus being greater than 83 μg/l. In the second bioassay, all test animals died at all concentrations of toxicant, and the 96-hr TL_{50} must have been less than the lowest test concentration, 80 μg/l. These data suggest that alevins exposed to chloramines in the second bioassay were less tolerant than those exposed in the first bioassay.

Tolerance at button-up (yolk absorption nearly complete) appears to be less than the tolerance of alevins (having visible yolk sacs) in bioassay A-1, but greater than the tolerance of the very small juvenile coho tested in bioassays J-1, J-2, and J-3 (Table 1; Fig. 1).

Tolerance was lowest for juvenile coho in bioassay J-2, as indicated by a 96-hr TL_{50} of 57 μg/l (Table 1). Thus it appears that coho in the early alevin stage were more tolerant than coho in the early juvenile stage, and increasing tolerance appears to be a trend as juveniles develop (Table 1; Fig. 1).

Juvenile coho salmon were tested at a series of chloramine concentrations at 10.8 and 15.0 C. Mean dry weight per fish was 0.114 g (range 0.102-0.125 g dry weight). The logit regression lines

Table 1. Three coho salmon life stages with corresponding mean dry weights and 96-hr TL_{50} values.

Bioassay Number	Life Stage	Mean dry weight per fish (g)	96-hr TL_{50} of Total Chloramine (ug/l)
A-1	Alevin	0.0238*	>83
A-2	Alevin	0.0549*	<80
B-1	Button-up	0.0727	69
J-1	Juvenile	0.123	62
J-2	Juvenile	0.114	57
J-3	Juvenile	0.227	64
J-4	Juvenile	0.210	72
J-5	Juvenile	0.234	72
J-6	Juvenile	0.240	71
J-7	Juvenile	0.944	74
J-8	Juvenile	1.15	82
J-9	Juvenile	1.55	82
J-10	Juvenile**	1.53	81

*Mean dry weight of alevins without yolk.

**These juveniles are 1973 year-class salmon; all other salmon are 1974 year-class.

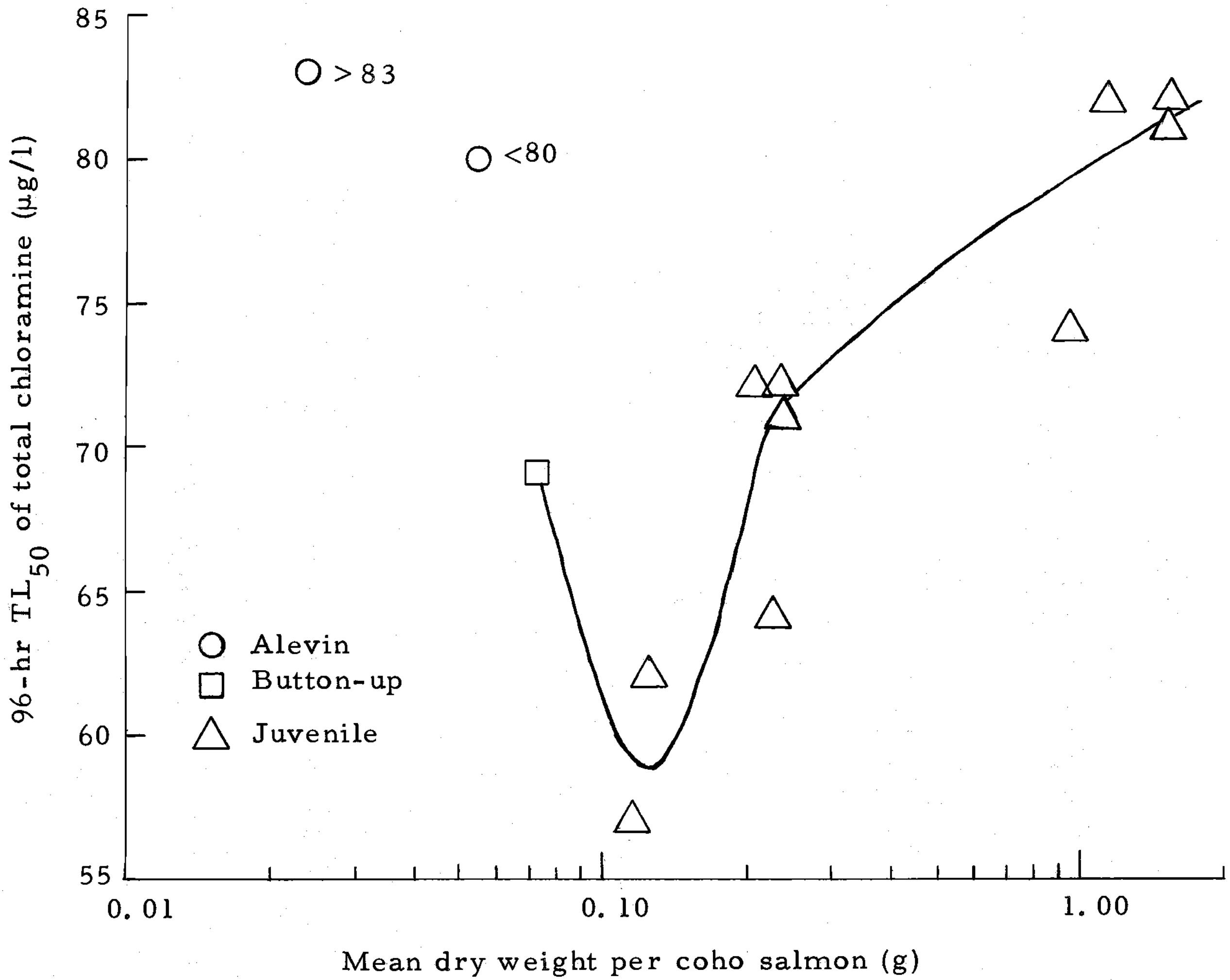

Figure 1. Relationship between 96-hr TL_{50} of total chloramine for coho salmon and mean dry weight of coho at different life stages.

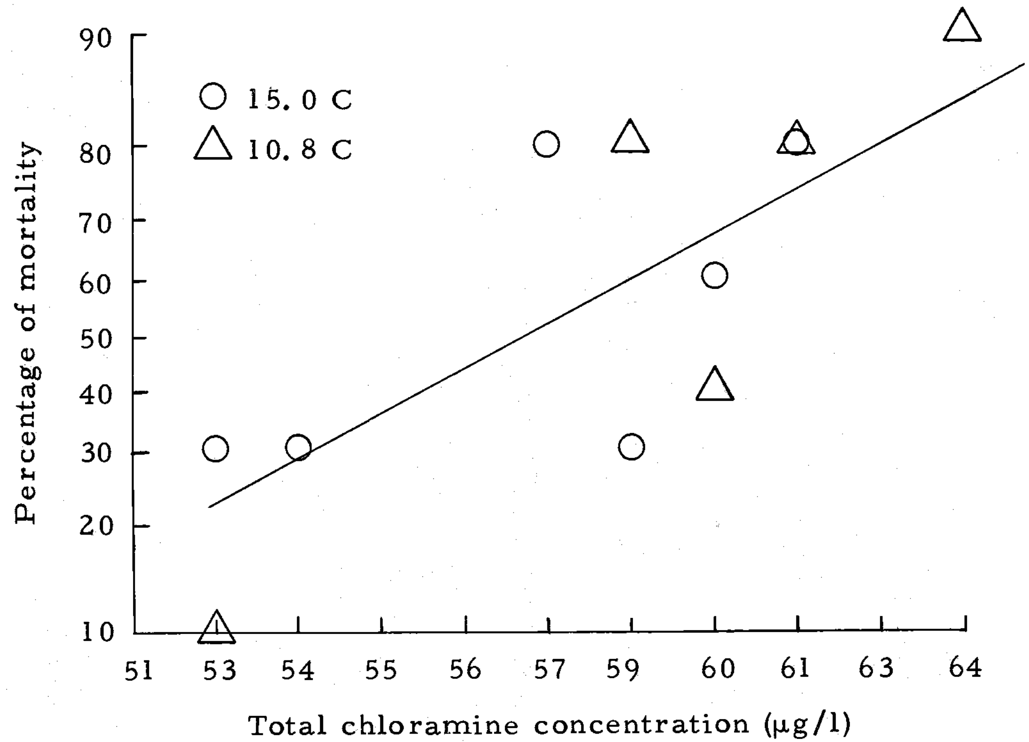

Figure 2. Relationship between percentage of mortality of coho salmon for 96 hours and total chloramine concentration at two temperatures.

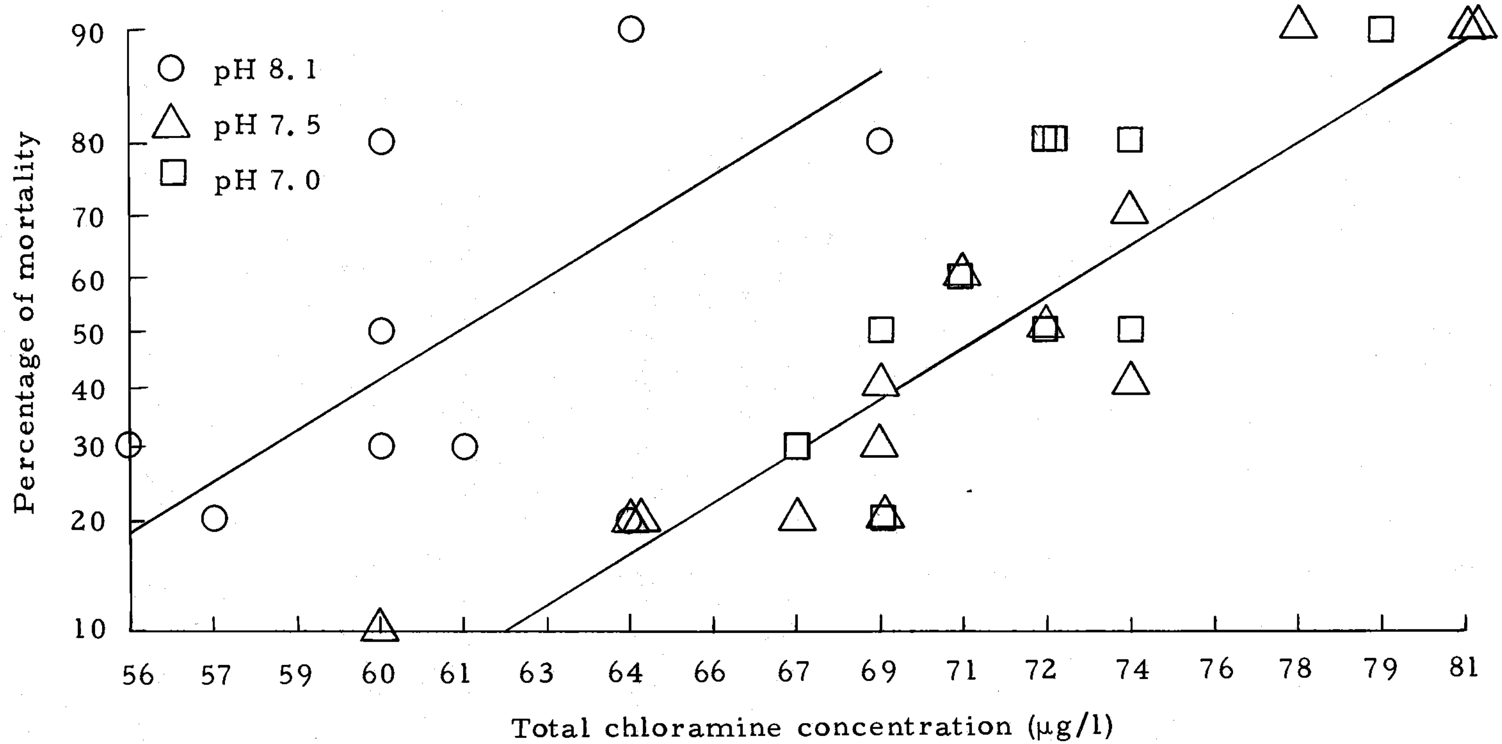

Figure 3. Relationship between percentage of mortality of coho salmon for 96 hours and total chloramine concentration at three levels of pH.

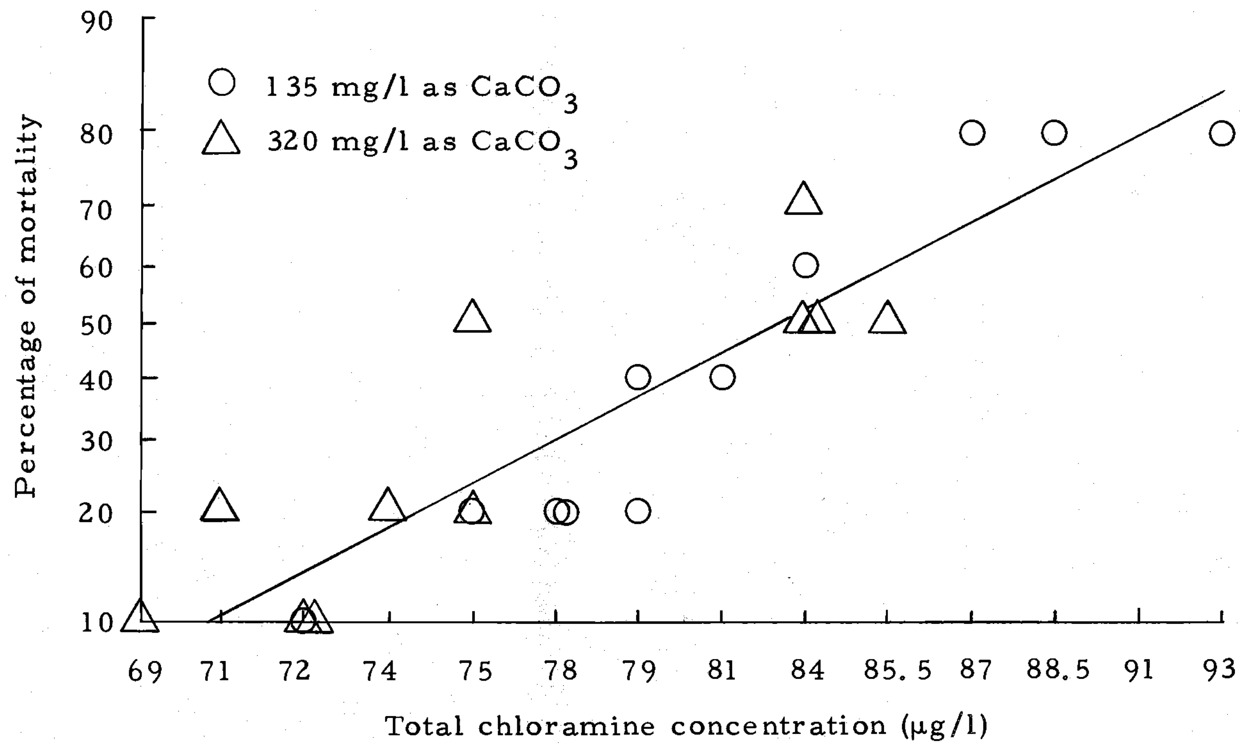

Figure 4. Relationship between percentage of mortality of coho salmon for 96 hours and total chloramine concentration at two levels of alkalinity.

for the resulting data were determined separately for each of the two temperatures but were not significantly different ($P > 0.99$). Thus the data of both temperature groups were combined, the single regression line giving a 96-hr TL_{50} of 57 μg/l (Fig. 2).

Juvenile coho salmon were tested at a series of chloramine concentrations at pH 7.0, 7.5, and 8.1, simultaneously during three separate series of bioassays. Mean dry weight per fish was 0.228 g (range 0.201-0.257 g dry weight). The logit regression lines, calculated separately for pH 7.0 and 7.5, were not significantly different ($P > 0.99$). Thus the data for these pH values were combined, the single regression line giving a 96-hr TL_{50} of 71 μg/l (Fig. 3). The logit regression line for pH 8.1 was significantly different ($P > 0.99$) from both the pH 7.0 and 7.5 regression lines and yielded a 96-hr TL_{50} of 61 μg/l (Fig. 3).

Juvenile coho salmon were tested at a series of chloramine concentrations at total alkalinities of 135 and 320 mg/l (as $CaCO_3$) at pH 7.5. In the first bioassay coho weighing 1.15 g mean dry weight per fish (range 1.05-1.29 g dry weight) were used and in the second bioassay coho weighing 1.55 g mean dry weight per fish (range 1.41-1.69 g dry weight) were used. The logit regression lines, calculated separately for each alkalinity, were not significantly different ($P > 0.99$). Thus the data for both alkalinity groups combined, the regression line yielding a 96-hr TL_{50} of 83μg/l (Fig. 4).

DISCUSSION

Acute toxicity bioassays demonstrated the tolerance of young coho salmon for inorganic chloramines was different for the alevin, button-up, and early juvenile life stages. Chadwick and Shumway (1969) found that steelhead were less tolerant to dieldrin shortly after button-up than during the alevin stage. This decrease in tolerance shortly after button-up may be linked to the absence of yolk lipids in which the dieldrin could be stored. The increased susceptibility of coho to chloramines shortly after button-up probably cannot be so simply explained. When considering deleterious effects of toxicants on coho populations, consideration should be given to all life history stages.

Increased temperature apparently did not alter the tolerance of coho salmon for chloramines. Explanation of this would, of course require further studies, as one would expect increase in metabolic rate associated with higher temperatures to decrease tolerance at concentrations acutely toxic.

Lloyd (1961) demonstrated that the tolerance of rainbow trout to ammonia decreased as bicarbonate alkalinity increased when pH remained constant. No effect of alkalinity on the acute toxicity of chloramines was apparent in the present study.

According to Moore (1951), at pH 8.0 chloramines are present as 85 percent monochloramine and 15 percent dichloramine, and at pH 7.0 as 65 percent monochloramine and 35 percent dichloramine. Nevertheless, when mono- and dichloramine concentrations in the coho bioassays presented here were measured at pH 7.0, 7.5, and 8.1 the percentage of monochloramine was always 83 percent. Thus the increase in toxicity of chloramines at pH 8.1 cannot be attributed to a shift in the equilibrium of mono- and dichloramine.

Merkens (1958) demonstrated that as pH increased from 6.3 to 7.0, the toxicity of a mixture of free chlorine and mono- and dichloramine increased. He attributed this increase in toxicity to a greater concentration of free chlorine comprising the total residual chlorine at pH 7.0. No free chlorine was detected in the coho bioassays presented here.

At acutely toxic concentrations, changes in temperature and differences in alkalinity, within the ranges presented here, appear not to alter the toxicity of chloramines to young coho salmon, whereas increases in pH above 7.5 may do so. Such conclusions may or may not apply to toxic effects of chloramines during longer exposures at lower concentrations. Differences in the tolerance of different life history stages of coho salmon for chloramines at acutely toxic concentrations are considerable.

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Appendix 1. Diagram of diluter used for chloramine bioassays.