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ESTIMATED SAFE ZINC AND COPPER LEVELS FOR CHINOOK SALMON, *ONCORHYNCHUS TSHAWYTSCHA*, IN THE UPPER SACRAMENTO RIVER, CALIFORNIA [1]

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Long-term (83-d) and short-term (96-h) toxicity tests with combined copper and zinc solutions were conducted on chinook salmon, *Oncorhynchus tshawytscha*, eggs, alevins, and swim-up fry to determine tolerance levels to copper and zinc in the acid-mine waste from Spring Creek, a tributary to the Sacramento River. The ratio of copper-to-zinc in the waste can vary between 1:2 and 1:12. Eggs were just as tolerant or more tolerant than alevins and fry to combined concentrations of copper and zinc. The 83-d LC50 values (median lethal) for the period of eggs-to-fry to 1:3, 1:6, and 1:11 copper-to-zinc ratio stock solutions were 44 μg/l Cu and 160 μg/l Zn, 27 μg/l Cu and 206 μg/l Zn, and 17 μg/l Cu and 253 μg/l Zn, respectively, demonstrating that the toxicities of copper and zinc were "additive". The 96-h LC50 values for fry not previously exposed to the stock solutions were 37 μg/l Cu and 132 μg/l Zn, 29 μg/l Cu and 213 μg/l Zn, and 20 μg/l Cu and 279 μg/l Zn, for the same copper-to-zinc ratio stock solutions, respectively, demonstrating that an acclimation to these metals had occurred in the 83-d tests during embryonic and alevin development at the higher (1:3) copper-to-zinc ratio but not at the lower (1:6 and 1:11) copper-to-zinc ratios. Based on reduced growth of exposed fry for the period of eggs-to-fry, estimated "safe" levels of copper and zinc for chinook salmon would be below 11 and 83 μg/l, respectively.

INTRODUCTION

The acid-mine waste from the Spring Creek drainage (Figure 1) has caused numerous kills of anadromous and resident salmonid fishes in the upper Sacramento River between Keswick Reservoir and Cottonwood Creek, California (U.S. Fish and Wildlife Service 1959; Prokopovich 1965; Nordstrom 1977). Of the fishes affected by the waste, anadromous chinook salmon are of particular concern since they are both a recreationally and commercially valuable species, and the carrying capacity of the spawning areas in the upper Sacramento River below Keswick Dam fail to reach their anticipated levels. Since 1963, the waste has collected in Spring Creek Reservoir and been metered into Keswick Reservoir. The flow of the waste through the Spring Creek Diversion Dam is determined by the amount of "dilution" water originating from Shasta Lake. From 1963 through 1978 this water quality management program was based on dilution factors calculated by Lewis (1963) using waste copper concentrations as criteria. Finlayson and Ashuckian (1979) listed six theoretical reasons why these dilution factors had not provided for adequate protection of salmonid resources in the upper Sacramento River and further demonstrated that these factors could potentially allow very toxic concentrations of zinc to enter the Sacramento River. The factors did, however, provide borderline protection for steelhead trout, *Salmo gairdneri*, against acute copper toxicity.

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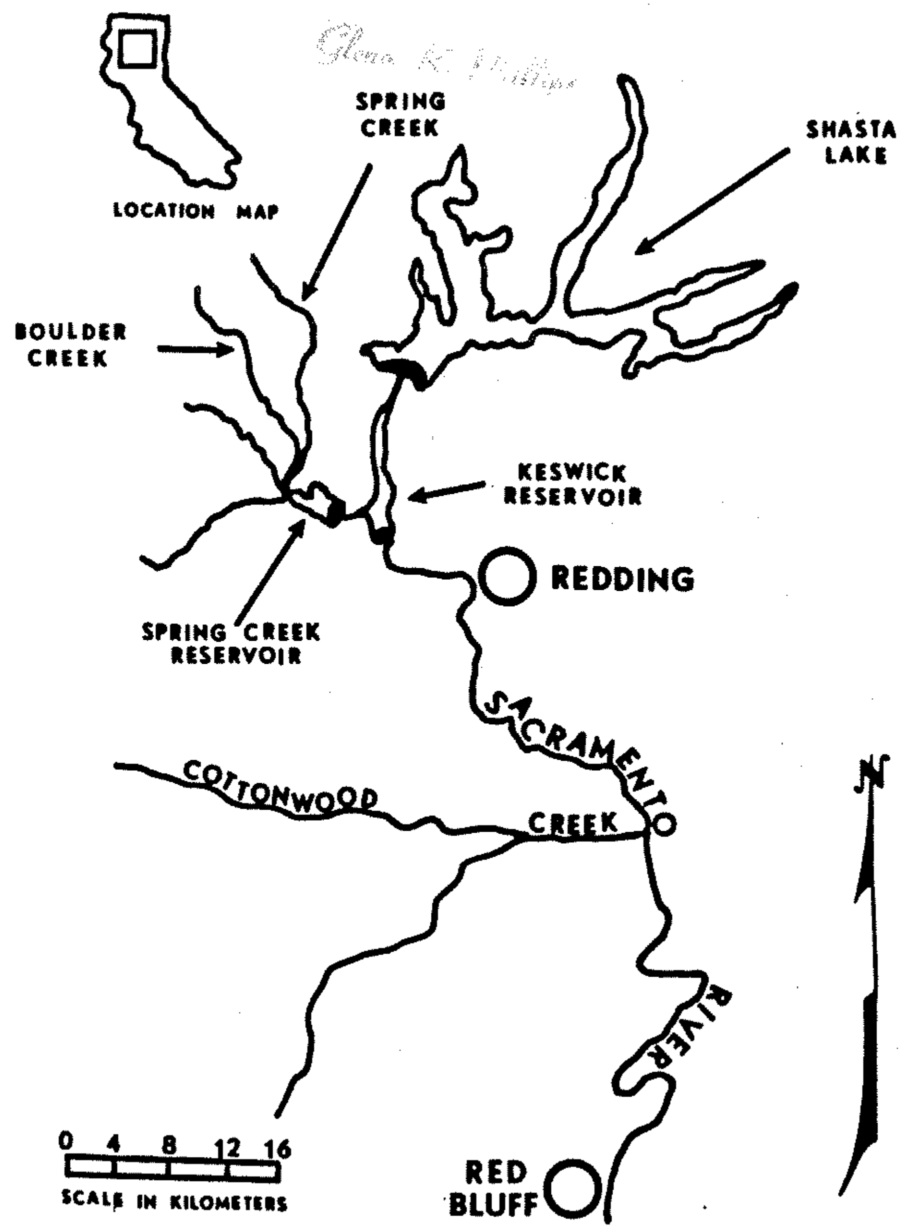

FIGURE 1. Location of Spring Creek drainage, upper Sacramento River basin, California

Finlayson and Ashuckian (1979) investigated the toxicity of the Spring Creek waste to steelhead trout eggs, alevins, and swim-up fry, and concluded that the toxicity of the waste was entirely due to its copper and zinc content and not to the other metals (aluminum and iron) present. Much information is available regarding the toxicities of zinc (Nehring and Goettl 1974; Sinley, Goettl, and Davies 1974; Lorz and McPherson 1977; Chapman 1978; Chapman and Stevens 1978; Lorz, Williams, and Fustish 1978), copper (Hazel and Meith 1970; McKim and Benoit 1971; Chapman and McCrady 1977; Lorz and McPherson 1977; Chapman 1978; Chapman and Stevens 1978; Lorz et al. 1978), and their combinations (Lloyd 1961; Sprague 1964; Sprague, Elson, and Saunders 1965; Sprague and Ramsey 1965; Thomsen, Hazel, and Meith 1970; Lorz et al. 1978) to salmonids. However, no one has investigated the combined toxicities of copper and zinc to chinook salmon eggs, alevins, or swim-up fry. It is the dissolved fractions of zinc (Sinley et al. 1974) and copper (Chapman and McCrady 1977) (Zn^{++} and Cu^{++} in acid waters; $ZnOH^+$ and $CuOH^+$ in soft waters), which are believed to produce toxic effects in fish (Davies et al. 1979).

Much of the copper contained in the Spring Creek waste now can be removed by several copper cementation plants located at the mine-adit openings (Nordstrom 1977). However, during periods of precipitation, the leachate from exposed tailings results in an increase in the ratio of copper to the other metals present. Hence, the copper-to-zinc ratios of the waste leaving the Spring Creek Diversion Dam fluctuate between 1:2 and 1:8 for wet and dry periods, respectively (D. Wilson, Calif. Dept. of Fish and Game, pers. commun.). Successful operation of the copper cementation plants could result in ratios as low as 1:12 during future dry periods (D. Wilson, pers. commun.). The other metals in the waste remain in the same ratio to each other throughout the year even though their concentrations decrease during storm periods and increase during dry periods (B. Finlayson, unpublished data, 1979).

Wilson (1978) developed new interim release schedules for the Spring Creek diversion dam to replace those of Lewis (1963). The new schedules use ratios of copper-to-zinc as criteria for releases and should eliminate the potential of toxic zinc concentrations in the upper Sacramento River. Recent investigations (Finlayson and Ashuckian 1979) have supported this new approach. However, additional toxicological information is needed before new Sacramento River Basin Plan copper and zinc objectives and permanent Spring Creek release schedules can be calculated and implemented by the State Regional Water Quality Control Board.

The objectives of this study were to gather accurate and relevant toxicological information on copper-to-zinc ratios that were not considered by Lewis (1963), to evaluate the new release schedules of Wilson (1978), and to complement the toxicity data on steelhead trout (Finlayson and Ashuckian 1979).

MATERIALS AND METHODS

We tested the toxicities of combined copper and zinc solutions rather than the acid-mine waste from Boulder Creek (Figure 1) which was done by Finlayson and Ashuckian (1979). This alternative was toxicologically valid because the toxicity of the waste had been demonstrated to be due entirely to its copper and zinc content. The combined copper and zinc stock solutions were prepared using reagent grade copper ($CuSO_4 \cdot 5H_2O$) and zinc ($ZnSO_4 \cdot 7H_2O$) sulfates in de-ionized water. All of the metals present in the Spring Creek acid-mine waste are in the form of sulfates (Nordstrom 1977).

Continual-flow acute and chronic toxicity tests using the methods of Peltier (1978) and Finlayson and Ashuckian (1979) were carried out at the Department's Water Pollution Control Laboratory (WPCL) with freshly fertilized chinook salmon eggs and the resulting alevins and swim-up fry. All concentrations were tested in replicate. Water from the American River, a tributary to the Sacramento River, was sand-filtered and used for dilution. Since the American and Sacramento rivers are of similar pH and hardness, similar dissolved fractions of both metals would be expected in the Sacramento River and in our toxicity test water.

Three copper-to-zinc ratio (1:3, 1:6, and 1:11) stock solutions were used in both chronic (83-d) and acute (96-h) toxicity tests. The concentrations of zinc in the solutions were held constant at approximately 1525 mg/l and the copper concentrations were adjusted by adding copper to the appropriate ratio. The solutions were acidified to pH 1.8 with sulfuric acid (H_2SO_4) to keep the metallic ions in solution and to keep the metals from plating-out on the walls of the 20-l glass toxicant reservoirs. During the tests, the stock solutions were replenished every 7 to 14 d, and each new solution was analyzed for copper and zinc as a quality control measure (Table 1).

TABLE 1. Chemical Composition of Copper and Zinc Stock Solutions Used in Toxicity Tests.

Metal	Copper-to-zinc ratios 1:3	1:6	1:11
Copper (mg/l)	548±21* n=8	266±12 n=13	145±10 n=15
Zinc (mg/l)	1503±61 n=8	1543±101 n=13	1543±75 n=15

*Mean ± SD

The solutions were diluted in a geometric series of six concentrations (100, 56, 32, 18, and 10%, and control) and continually delivered to the developing eggs, alevins, and fry using the predilution systems and modified Mount and Brungs (1967) type proportional diluters designed and constructed by Finlayson and Ashuckian (1979). Immediately prior to entry into the proportional diluters, the concentrated stock solutions were prediluted to 0.042, 0.049, and 0.081% of original strength for the 83-d chronic toxicity tests and to 0.035, 0.053, and 0.052% of original strength for the 96-h acute test for the 1:3, 1:6, and 1:11 copper-to-zinc ratio solutions, respectively. These initial dilutions constituted the 100% concentrations used in the geometric series of dilutions.

The 83-d chronic toxicity tests began on 6 December 1978 with fertilized chinook salmon eggs obtained by dry spawning four adult female and two male fish at the Department's Nimbus Hatchery on the American River and ended on 27 February 1979 when the individuals were at the swim-up fry stage. At the end of the 83-d tests, the control fry had a mean weight of 400 mg, a mean total length of 36 mm, and had the yolk-sac absorbed to the diameter of 1 to 2 mm. The 96-h acute tests began on 5 March 1979 with the 90-d old unexposed swim-up fry of similar size and weight and ended on 9 March 1979. Continuous low-level illumination was provided by two, 40 W incandescent lamps during the 83-d tests. A continual 8-h light and 16-h dark photoperiod was provided during the 96-h tests by eight, 60 W fluorescent lights which were switched on and off with a rheostat.

Following fertilization, the eggs were allowed to water-harden in the different concentrations of the combined copper and zinc solutions. The control eggs were water-hardened in filtered American River water. Water-hardening lasted 3 h and took place in 4-l glass jars that previously had been acid-rinsed, followed by several rinses with de-ionized water. Following hardening, the eggs were volumetrically measured and floated into perforated, polyethylene egg baskets (50 eggs per basket; 3 baskets per replicate) contained inside of the 12-l plexiglass troughs used by Finlayson and Ashuckian (1979). Eggs not used in the chronic toxicity tests were water-hardened in filtered American River water, deposited in several square, 10-l, overflowing plexiglass aquaria, placed in the dark with a supply of filtered flowing water, and allowed to hatch and develop into swim-up fry. These unexposed swim-up fry were used in the acute toxicity tests, with 25 fry placed in each 12-l plexiglass trough (50 fry per concentration).

During the tests, water samples from the troughs were collected twice a week for analyses of both total and dissolved copper and zinc. Water samples (10 ml) were collected in 15-ml polycarbonate Nalgene® centrifuge tubes which were first rinsed twice in 1*N* nitric acid (HNO_3) followed with two rinses of de-ionized water and then followed by a sample rinse. Samples (10 ml) for dissolved metals were collected in a SESI® nylon syringe and filtered through a Gelman® type A-E 25-mm glass fiber filter held in a Nuclepore® 25-mm plastic valve. Before taking each sample, the syringe, filter, and valve were rinsed with 1*N* nitric acid and then with de-ionized water. All samples were preserved with 0.3 ml of 6*N* nitric acid and capped until analyzed. Copper concentrations $\geq$ 50 µg/l and all zinc concentrations were determined by air-acetylene flame atomic absorption analysis; copper concentrations <50 µg/l were determined by carbon-rod atomization atomic absorption analysis. Analytical precision (2σ) was determined from the modified Shewhart equation:

$$\sigma = \sqrt{\Sigma\,(\bar{x} - x)^2/N\text{-}1}$$

where the absolute value of $x = [A_1 - A_2]/[A_1 + A_2]$, and A_1 and A_2 are paired observations. Analytical precision for zinc was ± 4.4%, for copper concentrations $\geq$ 50 µg/l it was ± 3.5%, and for copper concentrations < 50 µg/l it was ± 18%. Dissolved oxygen, pH, and temperature were measured in each test trough three times weekly, whereas water hardness was measured once every 3 wk. The hardness of American River water varies throughout the year between 19 and 26 mg/l $CaCO_3$ (California Department of Fish and Game, unpublished data, 1970–78).

Deaths were not recorded in the chronic tests until the eggs had become "eyed", approximately 3 wk after fertilization. The dead (opaque, white) eggs were recorded weekly and carefully suctioned away to avoid disturbing the other developing embryos. When hatching began 7 wk after fertilization, dead (absence of ventilation and movement) alevins and fry were recorded and removed three times weekly. Mortalities were calculated for the life-history periods of eggs-to-hatch (M_e), hatch-to-swim-up fry (M_a), and for the total 83-d exposures of eggs-to-swim-up fry (M_t). A sample of 20 fry or those remaining (whichever was less) was measured and weighed in each replicate at the end of the 83-d test to determine the effects of copper and zinc on fry growth. Mortalities were also calculated for the 90-d old unexposed swim-up fry (M_f)

for the 96-h exposures. Log-logit (metal concentrations vs. mortality estimates) analyses (Finley 1971) were used to calculate the $LC50_e$, $LC50_a$, $LC50_t$, and $LC50_f$ (50% lethal concentration) and the $LC10_e$, $LC10_a$, $LC10_t$, and $LC10_f$ (10% lethal concentration) by least square regression. The mortality estimates used in log-logit analyses were first normalized using Abbott's formula: $m = (1 - S_x/S_c)\,100$ where m = normalized mortality, S_x = survival in concentration x, and S_c = survival of controls.

RESULTS AND DISCUSSION

The toxicity test concentrations had a pH range of 6.6 to 7.4 with the lowest and highest values corresponding to the highest and lowest solution concentrations, respectively. Water hardness of the test concentrations varied with time between 21 and 27 mg/l $CaCO_3$. Water temperatures during the toxicity tests varied with time between 8.5 and 11.0 C, and dissolved oxygen concentrations varied with the time between 8.5 and 12.2 mg/l.

Mortalities of the eggs (M_e), alevins (M_a), and for the total 83-d exposures (M_t) increased with increased total and dissolved copper and zinc concentrations in the 1:3 ratio (Appendix 1), 1:6 ratio (Appendix 2), and 1:11 ratio (Appendix 3) toxicity tests. For the period of eggs-to-fry (M_t), 100% mortality occurred in the highest concentrations while minimal mortality (means = 8.5 to 19.5%) occurred in the lowest concentrations tested. Mortalities of the controls from eggs-to-fry were also minimal (means = 5.5 to 8.6%). Dissolved copper concentrations in the controls were consistently <2 µg/l and dissolved zinc concentrations ranged from 2 to 5 µg/l. All copper-to-zinc ratio solution concentrations tested produced 83-d old fry which had lower mean weights and lengths than the control fry.

Mortalities of the unexposed 90-d old fry (M_f) increased with total and dissolved copper and zinc concentrations in the 1:3 ratio, 1:6 ratio, and 1:11 ratio tests (Appendix 4). Complete mortality occurred at the highest concentrations while minimal mortality (means = 0.0 to 2.6%) occurred at the lowest concentrations tested. Mortalities of the control swim-up fry were also minimal (means = 0.0 to 5.3%). Dissolved copper and zinc concentrations in the control concentrations ranged from 1 to 2 µg/l and <5 to <7 µg/l, respectively.

The toxicity test data indicate that the swim-up fry at the end of the 83-d exposures (Table 2) were more tolerant to copper and zinc in the 1:3 copper-to-zinc ratio than the previously unexposed swim-up fry in the 96-h exposures (Table 3), almost as tolerant to copper and zinc in the 1:6 copper-to-zinc ratio, and less tolerant to copper and zinc in the 1:11 copper-to-zinc ratio. For example, the $LC50_t$ values of the 1:3, 1:6, and 1:11 copper-to-zinc ratio solutions during the 83-d exposures were 44 µg/l Cu and 160 µg/l Zn, 27 µg/l Cu and 206 µg/l Zn, and 17 µg/l Cu and 253 µg/l Zn, respectively, while the $LC50_f$ values during the 96-h exposures were 37 µg/l Cu and 132 µg/l Zn, 29 µg/l Cu and 213 µg/l Zn, and 20 µg/l Cu and 279 µg/l Zn, respectively.

The apparent acclimation to copper and zinc during the 83-d test in the higher (1:3) copper-to-zinc ratio, lack of this acclimation in the middle (1:6) ratio, and the greater toxicity of copper and zinc during the 83-d test in the lower (1:11) ratio may indicate that chinook salmon were able to acclimate to copper with relatively less amounts of zinc present (1:3 ratio) but that acclimating to zinc with relatively less amounts of copper present (1:11 ratio) was not possible. Chinook salmon have been reported to acclimate to copper during early life-history stages but copper acclimation has not been investigated in the presence of zinc (G. Chapman, Research Aquatic Biologist, U. S. Environmental Protection Agency, pers. commun.).

TABLE 2. Summary of Statistics and 95% Confidence Limits (In Parentheses) for Chinook Salmon Exposed for 83 Days to 1:3, 1:6, and 1:11 Copper-to-Zinc Stock Solutions.

	Eggs-to-hatch					
	LC50$_e$			*LC10$_e$*		
Metal	*1:3*	*1:6*	*1:11*	*1:3*	*1:6*	*1:11*
Dissolved zinc (μg/l)	174 (141–207)	280 (210–380)	477 (377–631)	145	224	396
Total zinc (μg/l)	207 (169–242)	326 (248–438)	524 (418–681)	175	254	437
Dissolved copper (μg/l)	48 (39–58)	37 (28–50)	31 (25–41)	40	29	26
Total copper (μg/l)	84 (69–99)	63 (48–85)	58 (46–78)	70	50	49
	Hatch-to-swim-up fry					
	LC50$_a$			*LC10$_a$*		
Metal	*1:3*	*1:6*	*1:11*	*1:3*	*1:6*	*1:11*
Dissolved zinc (μg/l)	182 (159–234)	205 (146–298)	253 (187–314)	119	156	208
Total zinc (μg/l)	214 (190–266)	243 (173–351)	283 (212–347)	146	187	234
Dissolved copper 'μg/l)	49 (43–63)	27 (20–38)	17 (13–20)	32	20	14
Total copper (μg/l)	89 (75–136)	45 (33–63)	32 (24–41)	56	37	27
	Total					
	LC50$_t$			*LC10$_t$*		
Metal	*1:3*	*1:6*	*1:11*	*1:3*	*1:6*	*1:11*
Dissolved zinc (μg/l)	160 (112–196)	206 (157–256)	253 (180–317)	131	165	200
Total zinc (μg/l)	192 (148–232)	245 (187–301)	283 (206–351)	170	197	226
Dissolved copper (μg/l)	44 (34–54)	27 (21–33)	17 (12–21)	38	21	13
Total copper (μg/l)	77 (59–94)	46 (36–56)	32 (22–39)	64	36	25

In toxicity test exposures of both 83 d and 96 h, it was apparent that the toxicities of copper and zinc were additive. The additive nature of copper and zinc was expressed by the fish becoming more tolerant of zinc concentrations as the copper concentrations decreased. This additive nature of copper and zinc toxicity is probably not of a completely additive concentration nature (i.e. ½ Cu LC50 plus ½ Zn LC50 producing 50% mortality), although we have no separate copper and zinc LC50 values to substantiate this suspicion. The 83-d LC50$_t$ values indicate that as the copper-to-zinc ratio decreased from 1:3 to 1:11, there was a 58% increase in zinc tolerance coupled with 61% reduction in copper tolerance. This is in comparison to the 96-h LC50$_f$ values which indicate there

was a 111% increase in zinc tolerance coupled with a 46% reduction in copper tolerance between the same copper-to-zinc ratio solutions. Finlayson and Ashuckian (1979) found additive toxicity between copper and zinc to steelhead trout. Because there is substantial evidence that the toxicities of copper and zinc are additive, and by reducing the copper-to-zinc ratio from 1:3 to 1:11 will make the combined copper and zinc solutions 58% to 111% more tolerable to chinook salmon, the continued and efficient operation of the copper cementation plants in the Spring Creek drainage, which have the capability to decrease the copper-to-zinc ratio of the acid-mine waste, are of beneficial water quality value.

TABLE 3. Summary of Statistics and 95% Confidence Limits (In Parentheses) for Chinook Salmon Swim-up Fry Exposed for 96 Hours to 1:3, 1:6, and 1:11 Copper-to-Zinc Ratios of Stock Solutions.

	90-d swim-up fry					
	LC50$_f$			*LC10$_f$*		
Metal	*1:3*	*1:6*	*1:11*	*1:3*	*1:6*	*1:11*
Dissolved zinc (μg/l)	132 (103–160)	213 (181–245)	279 (243–321)	111	180	234
Total zinc (μg/l)	144 (112–173)	245 (209–282)	318 (274–373)	120	210	271
Dissolved copper (μg/l)	37 (25–48)	29 (25–35)	20 (18–24)	30	25	17
Total copper (μg/l)	48 (37–58)	40 (35–45)	31 (26–38)	40	33	26

The chinook salmon eggs in our tests were more tolerant to copper and zinc concentrations in the two lower (1:6 and 1:11) copper-to-zinc ratio solutions than were the resulting alevins and fry (Table 2). However, the alevins and fry were as tolerant to copper and zinc as the eggs in the higher (1:3) copper-to-zinc ratio solution. Finlayson and Ashuckian (1979) found steelhead trout eggs were more tolerant of copper and zinc than the resulting alevins and fry in two copper-to-zinc ratios (1:4 and 1:12), and Hazel and Meith (1970) found chinook salmon eggs were more tolerant of copper than the resulting alevins and fry. Hence, the eggs of both chinook salmon and steelhead trout are as tolerant or more tolerant to solutions containing copper and zinc than are the resulting alevins and fry.

Lethal levels of copper and zinc for our chinook salmon agree reasonably well with those of other researchers. Our LC50$_a$ and LC10$_a$ values varied from 17 to 49 μg/l Cu and 14 to 32 μg/l Cu and from 182 to 253 μg/l Zn and 119 to 208 μg/l Zn, respectively while Chapman (1978), in water hardness of 22–24 mg/l $CaCO_3$, found the separate copper and zinc 200-h LC50 and LC10 values for non-acclimated chinook alevins to be 20 and 15 μg/l Cu and >661 μg/l and 364 to 661 μg/l Zn, respectively. Hazel and Meith (1970) found about a 10% mortality to chinook salmon alevins and fry at 21 μg/l Cu. Our 96-h LC10$_f$ values for previously unexposed fry varied from 17 to 30 μg/l Cu and 111 to 234 μg/l Zn while Chapman (1978) found the separate copper and zinc 200-h LC10 values for non-acclimated fry at 14 μg/l Cu and 68 μg/l Zn. Chapman (1978) concluded that chinook salmon and steelhead trout alevins were more tolerant to copper and zinc than were swim-up fry. Additionally, he found that chinook salmon and steelhead trout tolerances to copper and zinc increased with development past the swim-up fry stage. Therefore, we must conclude that swim-up fry of chinook salmon and steelhead trout are probably the most sensitive life-history stage to copper and zinc, and hence, recommended "safe" levels based on this life-history stage should be protective for all developmental stages.

Based on our acute tests, estimated safe levels for copper and zinc at copper-to-zinc ratios of 1:3 to 1:11 for chinook salmon would be below 17 μg/l and 111 μg/l, respectively. However, other criteria besides death affect the success of an organism. When compared to our control fish, exposed fry showed reduced growth (weight and length) at the end of the 83-d exposures in all concentrations of all copper-to-zinc ratio solutions. The lowest mean concentrations in the 83-d tests were 24 μg/l Cu and 88 μg/l Zn, 12 μg/l Cu and 86 μg/l Zn, and 11 μg/l Cu and 161 μg/l Zn for the 1:3, 1:6, and 1:11 copper-to-zinc ratio solutions, respectively. The lowest copper (11 μg/l) and zinc (86 μg/l) concentrations affecting a reduction of growth in chinook salmon fry were below the lowest copper (17 μg/l) and zinc (111 μg/l) concentrations at the $LC10_f$ values for previously unexposed chinook salmon swim-up fry. Since our response criteria to combined copper and zinc solutions consisted of growth from fertilized eggs to the most sensitive life-history stage (swim-up fry) for chinook salmon, the copper and zinc concentrations of 11 and 86 μg/l, respectively, should approximate the maximum acceptable toxicant concentrations (MATC) of Mount and Stephan (1967). MATC values are between the toxicant concentration having no effect and the toxicant concentration having a measurable effect on the organism. However, even lower copper concentrations have been reported to have an effect on anadromous salmonids. For example, Lorz and McPherson (1977) found that as little as 5 μg/l Cu (7% of Cu 96-h LC50), in water hardness of 68–78 mg/l $CaCO_3$, caused a reduction (below that of control fish) in the percentages of downstream yearling migrants of coho salmon, *Oncorhynchus kisutch*. Additionally, Lorz et al. (1978) found a greater effect on downstream migration when copper and zinc were present together at sublethal levels (10 μg/l Cu and 800 μg/l Zn) than when copper (10 μg/l) or zinc (800 μg/l) were present alone. We would recommend safe levels for chinook salmon in the Sacramento River below 11 μg/l Cu and 86 μg/l Zn until further information is obtained on the possible chronic effects of copper and zinc concentrations on smoltification and downstream migration.

Our toxicity data and those of Finlayson and Ashuckian (1979) are based on dissolved concentrations of copper and zinc. The term "dissolved" refers to copper and zinc concentrations which can pass through a 0.30 μm glass fiber filter. The filtering process removes 90% of the metal-containing particles and complexes which are greater than 0.30 μm in diameter and therefore, probably removes all suspended particulate matter containing copper and zinc. These particles and complexes are bound by adsorption onto particulate matter and complexed in large carbonate, hydroxide, and oxide colloids or precipitates, and are believed to be biologically unavailable. In natural waters relatively free of suspended particulate matter, the fractions of copper and zinc concentrations which compose these biologically unavailable complexes would be expected to increase with increased water pH and alkalinity. The relationship of these water quality parameters to copper (Chapman and McCrady 1977) and zinc (Sinley et al. 1974) toxicity has been expressed by fish becoming increasingly more tolerant of both metals with increased water pH and alkalinity. Since our toxicity data are based on dissolved concentrations of copper and zinc, they should better reflect the concentrations of these metals actually biologically available to cause fish toxicity.

There are two advantages with presenting copper and zinc toxicity levels in dissolved concentrations. First, it allows for a more accurate comparison of the toxicities of various metals to various organisms and secondly, it allows for the toxicity data to be applied to a variety of waters regardless of suspended particulate matter content and possibly water pH, alkalinity, and hardness. Other investigators (Sinley et al. 1974; Chapman 1978) have instead based their toxicity data on total metal concentrations and have listed the pH, hardness, and alkalinity (sometimes the suspended particulate matter content) of the water in which the data were obtained.

Finlayson and Ashuckian (1979) calculated that the old release schedules of Lewis (1963) would allow for theoretical maximum dissolved copper concentrations below Keswick Dam of 10 and 17 μg/l during periods May through December, and January through April, respectively. Likewise, these schedules allowed for maximum dissolved zinc concentration ranges of 20 to 910 μg/l, and 40 to 1600 μg/l for the same periods, respectively. Our present study indicates that safe levels of copper and zinc for chinook salmon are approximately 11 and 86 μg/l, respectively. Thus, the release schedules of Lewis (1963) provided borderline protection from copper toxicity and little, if any, protection from zinc toxicity. The new interim release schedules proposed by Wilson (1978) allow for maximum dissolved copper and zinc concentrations of 5 μg/l and 64 μg/l, respectively. Both the maximum copper and zinc criteria proposed by Wilson (1978) are below the levels determined by us to have a small adverse effect on the growth of chinook salmon fry.

In conclusion, this investigation has demonstrated that the chinook salmon eggs are as tolerant or more tolerant to combined concentrations of copper and zinc than alevins and fry, and that chinook salmon appear to be more easily acclimated to copper rather than to zinc during the period of eggs-to-fry. Additionally, this investigation has demonstrated that the toxicities of copper and zinc are additive to chinook salmon eggs, alevins, and fry. Finally, this investigation suggests safe copper and zinc levels below 11 μg/l and 86 μg/l for chinook salmon in the upper Sacramento River.

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REFERENCES

Chapman, G. 1978. Toxicities of cadmium, copper, and zinc to four juvenile stages of chinook salmon and steelhead. Am. Fish. Soc., Trans., 107:841–847.

Chapman, G., and J. McCrady. 1977. Copper toxicity: A question of form; Pages 134–151 *in:* Recent advances in fish toxicology: A symposium held in Corvallis, Oregon on January 13–14, 1977. Oregon State University, Corvallis, Oregon and Oregon Dept. of Fisheries and Wildlife, July 1977. U.S. Environ. Prot. Agency, Ecological Res. Ser. EPA-600/3-77-035: 203 p.

Chapman, G., and D. Stevens. 1978. Acutely toxic levels of cadmium, copper, and zinc to adult male coho salmon and steelhead. Am. Fish. Soc., Trans., 107:837–840.

Davies, P., A. Calabrese, J. Goettl, P. Hodson, J. Kent, J. McKim, J. Pearce, D. Reish, J. Sprague, and W. Sunda. 1979. Cadmium, pages 51–66 *in:* Revew of the EPA Red Book: Quality criteria for water. R. V. Thurston, R. C. Russo, C. M. Fetterolf, T. A. Edsall, and Y. M. Barber eds. Water Quality Section, American Fisheries Society, Bethesda, MD.

Finlayson, B., and S. Ashuckian. 1979. Safe zinc and copper levels from the Spring Creek drainage for steelhead trout in the upper Sacramento River, California. Calif. Fish Game, 65(2): 80–99.

Finley, D. 1971. Probit analysis. 3rd ed. Syndics of Cambridge University Press, New York. 265 p.

Hazel, C., and S. Meith. 1970. Bioassay on king salmon eggs and sac fry in copper solutions. Calif. Fish Game, 56 (2):121–124.

Lewis, R. 1963. Recommended flow releases from Spring Creek Debris Dam for the protection of salmonid fishes in the Sacramento River. Calif. Dept. Fish and Game, Region 1, Redding, CA. Unpublished MS. 8 p.

Lloyd, R. 1961. The toxicity of mixtures of zinc and cooper sulfates to rainbow trout (*Salmo gairdneri*, Richardson). Ann. Appl. Biol., 49:535–538.

Lorz, H., and B. McPherson. 1977. Effects of copper and zinc on smoltification of coho salmon. U.S. Environ. Prot. Agency, Ecological Res. Ser. EPA-600/3-77-032: 69 p.

Lorz, H., R. Williams, and C. Fustish. 1978. Effects of several metals on smolting of coho salmon. U.S. Environ. Prot. Agency, Ecological Res. Ser. EPA-600/3-78-090: 85 p.

McKim, J., and D. Benoit. 1971. Effects of long-term exposures to copper on survival, growth, and reproduction of brook trout (*Salvelinus fontinalis*). Can., Fish. Res. Bd., J. 28:655–662.

Mount, D. and W. Brungs. 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1:21–29.

Mount, D., and C. Stephan. 1967. A method for establishing acceptable toxicant limits for fish-malathion and the butoxyethyanol ester of 2,4-D. Am. Fish. Soc., Trans., 96(2):185–193.

Nehring, B., and J. Goettl. 1974. Acute toxicity of a zinc-polluted stream to four species of salmonids. Bull. Environ. Contam. and Tox. 12:464–469.

Nordstrom, D. 1977. Hydrogeochemical and microbiological factors affecting the heavy metal chemistry of an acid mine drainage system. Ph.D. Dissertation. Stanford University, Palo Alto, CA. 210 p.

Peltier, W. 1978. Methods for measuring the acute toxicity of effluents to aquatic organisms. U.S. Environ. Prot. Agency, Environ. Monitoring Ser. EPA-600/4-78-012: 52 p.

Prokopovich, N. 1965. Siltation and pollution problems in Spring Creek, Shasta County, California. J. Am. Water Works Assoc. 57:986–995.

Sinley, J., J. Goettl, and P. Davies. 1974. The effects of zinc on rainbow trout in hard and soft water. Bull. Environ. Contam. and Tox. 12:193–201.

Sprague, J. 1964. Lethal concentrations of copper and zinc for young Atlantic salmon. Can., Fish. Res. Bd., J. 21:1–17.

Sprague, J., P. Elson, and R. Saunders. 1965. Sub-lethal copper-zinc pollution in a salmon river-field and laboratory study. Int. J. of Air and Water Poll. 9:531–543.

Sprague, J., and A. Ramsey. 1965. Lethal levels of mixed copper-zinc solutions for juvenile salmon. Can., Fish. Res. Bd., J. 22:425–432.

Thomsen, W., C. Hazel, and S. Meith. 1970. Toxicity of copper and zinc ions to steelhead in Mokelumne River water. Calif. Dept. Fish and Game, Water Projects Branch, Sacramento, CA. Lab. Rep. 50 p.

U.S. Fish and Wildlife Service. 1959. Effects of mine-waste pollution on anadromous and resident fish in Upper Sacramento River, Shasta County, California. 15 p. + Attachments.

Wilson, D. 1978. Proposed interim release flows from Spring Creek Debris Dam for the protection of salmonid fishes in the Sacramento River. Calif. Dept. Fish and Game, Region 1, Redding, CA. Memorandum to California Regional Water Quality Control Board—Central Valley Region, August 31, 1978. 5 p. + Attachments.

APPENDIX 1—Zinc and Copper Concentrations in Toxicity Testing Troughs, Mortalities for Chinook Salmon Eggs Raised to Swim-up Fry Stage for 83 Days, and Weights and Lengths of Surviving Swim-up Fry at 83 Days in Five Concentrations and the Controls of the 1:3 Copper-to-Zinc Stock Solutions.

			Percent mortality				
Replicate	*Zn* (*μg/l*)	*Cu* (*μg/l*)	*Eggs* (M_e)	*Alevins* (M_a)	*Total* (M_t)	*Wt* (*mg*)	*L* (*mm*)
100-1	700 ± 50* (751)	220 ± 27 (310)	100.0	—	100.0	—	—
100-2	710 ± 58 (760)	210 ± 25 (310)	100.0	—	100.0	—	—
56-1	383 ± 48 (430)	120 ± 19 (220)	100.0	—	100.0	—	—
56-2	382 ± 24 (421)	111 ± 19 (182)	100.0	—	100.0	—	—
32-1	214 ± 29 (240)	58 ± 9 (110)	70.7	63.9	89.4	330	29
32-2	200 ± 14 (242)	52 ± 10 (92)	65.0	63.4	87.2	330	29
18-1	150 ± 20 (178)	41 ± 6 (70)	37.3	21.7	50.9	340	31
18-2	141 ± 15 (173)	39 ± 6 (67)	35.7	40.3	61.6	340	31
10-1	88 ± 14 (111)	24 ± 4 (45)	2.4	3.3	5.6	350	34
10-2	89 ± 0 (112)	24 ± 3 (40)	4.9	8.6	13.1	350	34
Control-1	4 ± 3 (23)	2 ± 2 (4)	2.4	3.3	5.7	400	37
Control-2	4 ± 4 (22)	1 ± 2 (3)	6.7	1.8	8.4	395	37

*Mean dissolved zinc and copper concentrations ± SD; mean total zinc and copper concentrations in parentheses.

APPENDIX 2—Zinc and Copper Concentrations in Toxicity Testing Troughs, Mortalities for Chinook Salmon Eggs Raised to Swim-up Fry Stage for 83 Days, and Weights and Lengths of Surving Swim-up Fry at 83 Days in Five Concentrations and the Controls of the 1:6 Copper-to-Zinc Stock Solution.

Replicate	Zn (μg/l)	Cu (μg/l)	Percent mortality: Eggs (M_e)	Alevins (M_a)	Total (M_t)	Wt (mg)	L (mm)
100-1	800±82[a] (900)	110±15 (190)	100.0	—	100.0	—	—
100-2	820±90 (900)	122±17 (216)	100.0	—	100.0	—	—
56-1	480±67 (530)	64±6 (112)	91.9	100.0	100.0	—	—
56-2	447±40 (510)	63±13 (111)	88.2	86.7	98.4	300	27
32-1	263±33 (302)	32±5 (53)	12.3	30.7	39.2	300	32
32-2	250±50 (300)	27±6 (46)	9.3	29.9	36.4	300	32
18-1	147±16 (180)	20±14 (33)	3.2	17.4	20.0	330	34
18-2	150±31 (180)	19±4 (33)	9.0	13.1	20.9	330	34
10-1	86±14 (106)	12±2 (19)	2.4	2.5	4.8	360	35
10-2	91±25 (112)	12±3 (21)	9.4	3.4	12.2	360	35
Control-1	3±3 (6)	1±0 (2)	2.4	3.3	5.6	400	36
Control-1	5±4 (10)	1±0 (2)	3.9	1.6	5.5	390	36

[a] Mean dissolved zinc and copper concentrations ± SD; mean total zinc and copper concentrations in parentheses.

APPENDIX 3—Zinc and Copper Concentrations in Toxicity Testing Troughs, Mortalities for Chinook Salmon Eggs Raised to Swim-up Fry Stage for 83 Days, and Weights and Lengths of Surviving Swim-up Fry at 83 Days in Five Concentrations and the Controls of the 1:11 Copper-to-Zinc Stock Solution.

Replicate	Zn (μg/l)	Cu (μg/l)	Percent mortality: Eggs (M_e)	Alevins (M_a)	Total (M_t)	Wt (mg)	L (mm)
100-1	1240±77[a] (1340)	87±9 (143)	100.0	—	100.0	—	—
100-2	1250±121 (1340)	81±13 (149)	100.0	—	100.0	—	—
56-1	662±62 (701)	37±6 (80)	82.7	100.0	100.0	—	—
56-2	643±59 (702)	41±7 (78)	79.9	100.0	100.0	—	—
32-1	383±33 (442)	21±5 (42)	11.9	49.5	55.6	330	33
32-2	383±39 (426)	24±5 (49)	12.0	40.0	47.2	330	33
18-1	225±24 (224)	17±2 (31)	0.8	9.2	9.8	360	35
18-2	225±18 (268)	16±2 (30)	4.6	25.8	29.2	360	35
10-1	161±16 (184)	11±2 (20)	15.9	5.2	20.3	390	35
10-2	161±16 (190)	11±2 (20)	13.9	15.3	18.6	390	35
Control-1	2±1 (7)	1±0 (2)	5.9	4.5	10.0	400	36
Control-2	4±3 (5)	1±2 (2)	6.4	0.9	7.2	400	36

[a] Mean dissolved zinc and copper concentrations ± SD; mean total zinc and copper concentrations in controls.

APPENDIX 4—Zinc and Copper Concentrations and Mortalities for 90-Day Old Chinook Salmon Swim-up Fry Exposed for 96 Hours to Five Concentrations and the Controls of 1:3, 1:6, and 1:11 Copper-to-Zinc Stock Solutions.

	Copper-to-zinc ratios								
	1:3			1:6			1:11		
Replicate	Zn (µg/l)	Cu (µg/l)	Percent mortility (M_t)	Zn (µg/l)	Cu (µg/l)	Percent mortality (M_t)	Zn (µg/l)	Cu (µg/l)	Percent mortality (M_t)
100-1	532 ± 6*	150 ± 14	100.0	741 ± 27	120 ± 0	100.0	813 ± 61	50 ± 7	100.0
	(543)	(205)		(850)	(160)		(873)	(71)	
100-2	513 ± 21	180 ± 14	100.0	796 ± 29	131 ± 21	100.0	806 ± 35	65 ± 3	100.0
	(571)	(200)		(817)	(162)		(844)	(99)	
56-1	302 ± 22	84 ± 8	100.0	430 ± 23	60 ± 5	100.0	425 ± 7	35 ± 5	95.0
	(348)	(140)		(442)	(94)		(439)	(51)	
56-2	298 ± 28	73 ± 8	100.0	439 ± 19	63 ± 10	100.0	420 ± 9	30 ± 5	93.3
	(306)	(115)		(487)	(89)		(449)	(51)	
32-1	168 ± 7	56 ± 1	85.0	243 ± 0	31 ± 11	73.7	243 ± 13	18 ± 4	68.4
	(174)	(63)		(277)	(48)		(269)	(27)	
32-2	156 ± 13	47 ± 9	80.0	248 ± 11	36 ± 6	72.7	232 ± 12	17 ± 3	68.4
	(183)	(69)		(293)	(47)		(259)	(25)	
18-1	113 ± 10	34 ± 1	57.2	151 ± 6	22 ± 2	31.2	145 ± 14	10 ± 3	5.3
	(126)	(50)		(183)	(32)		(181)	(15)	
18-2	118 ± 9	34 ± 1	25.0	160 ± 18	22 ± 1	5.0	160 ± 9	12 ± 3	11.8
	(121)	(40)		(208)	(33)		(176)	(15)	
10-1	75 ± 3	18 ± 3	5.3	89 ± 3	11 ± 1	0.0	134 ± 34	7 ± 1	0.0
	(83)	(21)		(109)	(13)		(136)	(12)	
10-2	78 ± 11	24 ± 4	0.0	92 ± 0	12 ± 1	0.0	107 ± 6	7 ± 1	5.0
	(84)	(24)		(100)	(14)		(120)	(14)	
Control-1	7 ± 5	2 ± 2	0.0	6 ± 5	1 ± 1	0.0	7 ± 3	2 ± 1	10.5
	(10)	(4)		(8)	(1)		(7)	(2)	
Control-2	6 ± 3	2 ± 1	0.0	5 ± 3	2 ± 1	0.0	5 ± 4	2 ± 1	0.0
	(12)	(3)		(10)	(4)		(8)	(4)	

* Mean dissolved zinc and copper concentrations ± SD; mean total zinc and copper concentrations in controls.

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AGE AND GROWTH OF WHITE STURGEON COLLECTED IN THE SACRAMENTO-SAN JOAQUIN ESTUARY, CALIFORNIA: 1965–1970 AND 1973–1976 [1]

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Ages of white sturgeon, *Acipenser transmontanus*, collected in the Sacramento-San Joaquin Estuary from 1965 to 1970 and from 1973 to 1976 were estimated from transverse sections of pectoral fin rays. Sturgeon were difficult to age and there was considerable disagreement between individuals interpreting ages. Estimates of male and female growth rates were not significantly different. A von Bertalanffy growth curve was calculated for ages 0–21 from the 1973–1976 collection. A length-weight relationship was estimated from 1965–1970 data. Estimated growth rate was similar in 1965–1970 and 1973–1976, but was lower than in 1954. It was not possible to determine if a recent decrease in growth rate was real or was due to different aging techniques. Possible causes of reduced growth are discussed.

INTRODUCTION

White sturgeon occur mainly in estuaries and rivers along the Pacific Coast of North America. Presently they support a small but important sport fishery in the Sacramento-San Joaquin Estuary and its tributaries. Sturgeon were common here in the mid-1800's but commercial exploitation severely reduced the population by 1900. Sturgeon fishing was prohibited from 1901 to 1910 and from 1917 to 1954, when sport fishing was again permitted. The sport fishery expanded with the discovery in 1964 that shrimp (*Crangon* spp. and *Palaemon macrodactylus)* were effective bait. Both catch and effort peaked in 1967 and have generally declined since (unpublished data).

Initiation of the sport fishery in 1954 stimulated investigations of sturgeon biology to provide information to manage the fishery. Pycha (1956) reported on sturgeon age, growth, size composition, migrations, and abundance. Chadwick (1959) and Miller (1972*a*, 1972*b*) estimated harvest rates and described migrations. Young (Schreiber 1962) and adult (McKechnie and Fenner 1971) sturgeon food habits also were described. Stevens and Miller (1970) and Kohlhorst (1976) determined time and location of spawning.

This report presents new information about white sturgeon age composition and growth from 1965–1970 and 1973–1976 and compares the recent growth rate to that estimated by Pycha (1956).

METHODS

Data were gathered during two collection periods: by the junior authors from spring 1965 to spring 1970 and by the senior author from spring 1973 to summer 1976. Samples from the two periods were analyzed separately since we wanted to compare growth rates between periods. In general, sampling was not systematic; we examined most available fish.

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