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Ecological Research Series

EFFECTS OF COPPER AND ZINC ON SMOLTIFICATION OF COHO SALMON

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EFFECTS OF COPPER AND ZINC ON SMOLTIFICATION OF COHO SALMON

by

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FOREWORD

Effective regulatory and enforcement actions by the Environmental Protection Agency would be virtually impossible without sound scientific data on pollutants and their impact on environmental stability and human health. Responsibility for building this data base has been assigned to EPA's Office of Research and Development and its 15 major field installations, one of which is the Corvallis Environmental Research Laboratory (CERL).

The primary mission of the Corvallis Laboratory is research on the effects of environmental pollutants on terrestrial, freshwater, and marine ecosystems; the behavior, effects and control of pollutants in lake systems; and the development of predictive models on the movement of pollutants in the biosphere.

This report describes a potentially adverse effect of pollutants on fish such as salmon which must migrate from fresh water to sea water, and demonstrates that under certain conditions exposure to sublethal levels of pollutant can result in high mortality when fish subsequently enter sea water. Laboratory test methods are described which should detect this effect in screening tests and advance knowledge on the effects of pollutants on aquatic ecosystems.

A.F. Bartsch
Director, CERL

ABSTRACT

The 96-h LC50 values for copper ($CuCl_2$) for yearling coho salmon *(Oncorhynchus kisutch)* ranged from 74-60 µg/liter, depending on degree of smoltification. The 96-h LC50 of zinc ($ZnCl_2$) for yearling coho in April was 4600 µg/liter. All tests were conducted at 10 or 12 C in water with alkalinity and hardness ranging from 68-78 mg/liter and 89-99 mg/liter as $CaCO_3$, respectively.

Exposure of yearling coho for 144 h to sublethal concentrations of Zn in fresh water had little effect on the activity of Na^+, K^+-activated ATPase in gill microsomes or on the survival of fish when transferred to sea water. Exposure of yearling coho to sublethal concentrations of Cu (5-30 µg/liter) in fresh water (maximum of 172 days) had deleterious effects on downstream migration in a natural stream, lowered gill ATPase activity and reduced subsequent survival in sea water. Exposures >10 days had more severe effects than did 6-day exposures on downstream migration and survival in sea water, but not on gill ATPase. Fish ceased feeding after initiation of exposures to 20 and 30 µg/liter Cu and remained anoretic (loss of appetite) for several weeks to 4 months with the result that the mean lengths and condition factors of these fish were significantly lower than the controls at the end of the test.

Exposure of yearling coho to Cu (20 and 30 µg/liter) in fresh water affected their ability to maintain normal osmotic pressure and chloride concentrations in blood plasma. Similarly when these Cu intoxicated fish were transferred to sea water the plasma osmolality and chloride concentrations increased significantly compared to control fish. These responses are attributed, at least in part, to the suppression of Na^+, K^+-activated ATPase activity in the gills.

Coho yearlings given a "rest" (non-toxicant exposure in fresh water) following exposure to toxicant showed higher survival when tranferred to sea water than fish which were transferred immediately.

This report was submitted in fulfillment of Grant R 802468 by the Oregon Department of Fish and Wildlife under the partial sponsorship of the U.S. Environmental Protection Agency. This report covers the period October 1, 1973, to December 31, 1975.

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ACKNOWLEDGMENTS

This investigation was supported in part by the U.S. Environmental Protection Agency, Research Grant R-802468, and was funded through the National Water Quality Laboratory, Duluth Minnesota. Many contributed to this study and their assistance is gratefully acknowledged: Dr. G. R. Bouck provided valuable assistance in securing the grant. Dr. W. S. Zaugg and L. R. McLain, Western Fish Nutritional Laboratory, U. S. Fish and Wildlife Service provided physical facilities, technical assistance and help in the ATPase analyses run in 1974. Dr. G. A. Chapman, Project Officer, Western Fish Toxicology Station, EPA provided technical assistance and made arrangement with CERL for quarterly chemical water analysis as well as arranged the assistance of Mr. Joel McCrady (Chemist, WFTS) in analysis of copper and zinc concentrations used. Mr. Glen Gross, Mr. Will Beidler and Miss Lynda Smart assisted in the laboratory and field aspects of the work.

The authors gratefully acknowledge Drs. G. A. Chapman, J. D. McIntyre, A. V. Nebeker and H. H. Wagner who provided constructive criticism on the manuscript.

SECTION 1

CONCLUSIONS

1. The 96-h LC50 of Cu for yearling coho ranged from 60-74 µg/liter. The 96-h LC50 of Zn for yearling coho was 4600 µg/liter. All tests were conducted in water with an alkalinity of 68-78 mg/liter as $CaCO_3$.

2. No deaths were observed when yearling coho were exposed for 6 days to concentrations of 20 or 30 µg/liter Cu; however, following exposure to Cu for 172 days, mortalities of 12 and 38%, respectively, were recorded.

3. Coho yearling exposed to Cu concentrations $\geq$10 µg/liter showed anorexia (loss of appetite) and significantly lower mean lengths and condition factors than the control fish at the end of the exposure periods in 1974 and 1975 (37 and 172 days, respectively).

4. Gill microsomal Na^+, K^+-activated ATPase activity in yearling coho was decreased proportionally to Cu concentration by exposure to sublethal concentrations (10-30 µg/liter) whereas ATPase activity appeared unaffected in yearling coho exposed to Zn (100-5000 µg/liter).

5. Exposure of yearling coho to Cu in fresh water affected their ability to maintain normal blood plasma osmotic pressure and chloride concentration, and similarly when these Cu intoxicated coho were transferred to sea water an abnormal increase in plasma osmolality and chloride concentration was observed.

6. The percent survival of yearling coho in sea water decreased in proportion to the concentration of Cu in fresh water. Death in sea water was due to loss of osmoregulatory ability, and decreased Na^+, K^+-activated ATPase in the gills was probably one of the factors contributing to this loss.

7. Coho yearlings given a "rest" (nontoxicant exposure period) between Cu exposure and transfer to sea water showed improved survival over those transferred immediately to sea water.

8. The survival rate in sea water after a given exposure to Cu in fresh water was greater as coho became older, larger and transformed into smolts.

9. Long-term exposures to Cu had more severe effects than did short-term exposures on survival in sea water, but not on gill ATPase activity.

10. All concentrations of Cu tested (5-30 µg/liter) caused a reduction in percentage of downstream migrants compared to controls when the coho yearlings were released into a natural stream.

11. The 0.1 application factor for copper (6.1 x 96-h LC50) probably predicts "no effect" levels based on growth, direct mortality, latent seawater mortality, and ATPase activity, but a "no effect" level based on migratory success would fall below this level.

12. Two types of artificial sea water were found to be satisfactory substitutes for natural sea water in the testing survival of yearling coho after exposure to Cu.

13. Studies such as this that take into consideration critical points in the life history of fish (or other aquatic organisms) are essential in developing good water quality criteria.

SECTION 2

RECOMMENDATIONS

1. The sensitive techniques and methodology developed in this study are recommended and should assist EPA in developing more satisfactory water quality criteria to be used for setting water quality standards.

2. Prudence in applying the results of this study directly and quantitatively to field situations is recommended since variable factors (such as the proportion of "biologically available" Cu, the presence of other chemicals, disease, predators and food availability) could alter the reported response of yearling coho to a given concentration of Cu.

3. Additional research should be conducted to determine if the interference with smoltification of anadromous salmonids by sublethal levels of pollutants is a general phenomenon and to further validate the methodology.

4. Another area of research potential would be to determine if similar effects occur in euryhaline and stenohaline fish from estuarine and marine environments.

SECTION 3

INTRODUCTION

The problem of toxic effects of metals to fish as a result of water pollution has been recognized and investigated since the early part of this century. The early literature concerning this problem came primarily from England where metal pollution of streams occurred because of mining activity. A large volume of literature on the toxic effects of metals to fish has accumulated in the last fifty years, and there have also been many reviews prepared, of which Volumes 3 and 5 of the five volume Water Quality Criteria Data Book (1970-73) and the review of Eisler (1973) and Eisler and Wapner (1975) are among the most complete.

Considerable research has been done to determine water quality criteria for copper and zinc based on survival and growth of juvenile salmonids in fresh water (Chapman 1973; Hodson and Sprague 1975; Lloyd 1960; McKim and Benoit 1971; Sprague 1964; and Sprague and Ramsay 1965). However, there are essentially no data available for copper, zinc or any other pollutant which relates to effects on the migration of anadromous fish into sea water. There is a particular need for this type of data in the Pacific Northwest where large runs of anadromous salmonids are a valuable sport and commercial fishery resource and whose well-being is a significant factor in environmental impact considerations.

The seaward migration of juvenile coho salmon *(Oncorhynchus kisutch)* normally occurs during the spring of their second year of life. They are fully euryhaline several months earlier (Conte et al. 1966, Otto 1971) provided that they have achieved a threshold size of 9 cm (Conte et al. 1966). The experimental transfer of juvenile anadromous salmonids from fresh water to sea water is followed by a transient but marked disturbance of plasma water-electrolyte balance (Conte et al. 1966; Miles and Smith 1968). This osmotic disturbance is caused by the physiological changes necessary to adapt from freshwater osmoregulation (salt retention, water excretion) to seawater osmoregulation (salt excretion, water retention). These disturbances are minimized at the time of normal seaward migration or "parr-smolt transformation."

Zaugg and Wagner (1973) presented data showing that one of the physiological factors correlated with migratory behavior in steelhead trout *(Salmo gairdneri)* is an elevation of activity in Na^+, K^+-stimulated adenosine triphosphatase (ATPase) in the microsomes of gills. This enzyme activity doubles during the parr-smolt transformation of coho salmon and steelhead trout (Zaugg and McLain 1970; Zaugg and Wagner 1973). ATPase activity in salmonids increases rapidly during seawater exposure reaching a maximum after

about 30 days, and is thought to be an important component in maintaining salt (osmotic and ionic) balance in fish (Epstein et al. 1967; Zaugg and McLain 1970). Though no data concerning inhibition of Na^+, K^+-ATPase activity in fish by heavy metals was found in the literature, several workers have reported on "in vivo" inhibition of ATPase by chlorinated hydrocarbon insecticides and polychlorinated biphenyls (Campbell et al. 1974; Koch et al. 1972; Leadem et al. 1974). Jackim et al. (1970), however, reported on changes in activities of five liver enzymes after exposing fish or fish liver homogenates to salts of various toxic metals and these authors suggest that enzyme assay is a valid technique for diagnosing sublethal metal poisoning of fish.

This study was designed to determine the effects of freshwater exposure to sublethal concentrations of copper or zinc on the subsequent ability of yearling coho salmon to adapt to sea water, and to determine the effects of copper on downstream migration of yearling coho salmon. Growth, osmotic and ionic regulation and microsomal gill adenosine triphosphatase, were monitored during chronic exposure to copper in fresh water. A sub-objective of the study was to investigate and recommend methods that could be routinely utilized for measuring the effects of pollutants on the ability of anadromous salmonid to migrate to the ocean and adapt to sea water.

SECTION 4

METHODS

Experimental fish

All experiments were conducted on coho salmon between the ages of 10 and 18 months post-hatching. Coho salmon of the 1972 and 1973 year classes a/ originating from Fall Creek Salmon Hatchery (Alsea River, Oregon) were used in 1974 and 1975 experiments, respectively. Fish were hatched and reared from fertilized eggs brought into the laboratory and incubated at temperatures that ranged from 7.8 to 12.3 C. Rearing was done under natural light or simulated natural photoperiod in well water at a constant temperature of 12.3 C. In 1974, a constant temperature (12.3 C) was used throughout rearing and exposure to toxicant. In 1975, experimental fish (stock and exposure groups) were reared and exposed to toxicant at 12.3 C until February 1. Fish were then acclimated to 8.6 C over a period of 6 days and exposed to a simulated natural temperature regime, increasing in semi-monthly increments from 8.6 C on February 7 to 12.3 C on May 19. The temperature pattern used was based on mean semi-monthly maximal and minimal temperatures (6-year averages) on the North Fork of the Alsea River.

Fish were fed a commercially prepared moist pellet generally to repletion. Fish were not fed during the 24 h preceding exposure to toxicant, during acute ($\leq$144 h) toxicity experiments, nor during exposure to sea water. Feeding of fish was resumed in the chronic experiments after the first 144 h.

Exposures to toxicant

The fish were exposed to copper ($CuCl_2.2H_2O$) for periods ranging from 24 h to 4,128 h (172 days). Four 144-h tests were conducted with zinc ($ZnCl_2$). All 96-h LC50 tests were carried out in static water in 0.61 meter (2-foot) diameter fiberglass tanks. The water was continuously aerated and 85% of the 120 liters of test water was manually exchanged once per day. Toxicant solutions were mixed in a separate container prior to introductions to the tanks. Total metal concentrations of daily water samples were assayed by flame (Zn) or flameless (Cu) atomic absorption spectrophotometry and loss of metal from the water during any 24 h period was undetectable. Water temperature was maintained at 12 $\pm$ 1 C in 1974 and 10 $\pm$ 1 C in 1975 and all tests were conducted under simulated natural photoperiod. The above procedure was used for all Zn and static 144 h Cu exposures in 1974 and 1975.

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a/ *Year class refers to year of spawning. Coho normally spawn in the late fall and the eggs hatch several months later.*

All exposures of more than 144 h, as well as some ≤144 h, were conducted in a flowing water system. This system consisted of a gravity flow diluter (similar to Granmo and Kollberg 1972) that delivered 12 liters/min to each of 10 exposure tanks (Figure 1) at duplicated nominal concentrations of 30, 20, 10, 5 and 0 µg/liter Cu. The background concentration of Cu in the well water was less than 2 µg/liter. A volume of 1,000 liters was maintained in each of the ten 1.54 m (5-foot) diameter fiberglass exposure tanks, (Figure 2) and 95% of the water was replaced every 3.7 h (calculated from Sprague 1969, Figure 1). Submersible pumps were used in each tank to provide additional current, aeration and mixing (Figure 3).

Smaller groups of "stock" fish were exposed periodically to the flowing toxicant in the 0.61 m diameter fiberglass tanks. Flows (5 liter/min; 95% replacement every 1.5 h) to these 120-liter tanks were provided by a siphon from mid-depth in the 1.54 m chronic exposure tanks, with no difference in water quality between tanks. All tanks were covered with nets to prevent loss of fish and in 1975 a curtain was installed around the periphery of the tanks to minimize disturbance from external sources.

The concentrations of Cu used were chosen on the basis of prior static bioassays. Total Cu concentration was analyzed at least once per week from each exposure tank by flameless atomic absorption spectrophotometry. Water and toxicant flows in the diluter were checked at least once daily, and only occasional minor adjustments of flows were required. The levels of Cu in the static tests were more variable and therefore actual concentrations found are presented in those tables and figures whereas nominal concentrations are used in presentation of the diluter flow data.

Tolerance to sea water

Groups of 10 to 20 fish from each Cu exposure tank were periodically tested for tolerance to sea water and the percent survival of these juvenile coho provided a presumptive measure of the fish's osmoregulatory ability. Fish were placed into the 0.61 m diameter fiberglass tanks containing 120 liters of natural sea water. The water was continuously aerated and 85% of the 120 liters was manually exchanged once per day. Salinity of the water ranged from 29 to 33 °/oo in 1974 but was maintained at 30.0 ± 0.5 °/oo in 1975. The tanks were maintained at 12 ± 1 C in 1974 and 10 ± 1 C in 1975, under simulated natural photoperiod. The seawater exposure period was 15 days in 1974, but was reduced to 10 days in 1975 because deaths rarely occurred after the 8th day.

The source of natural sea water for these tests was the Depoe Bay Aquarium, which has been used by this and other laboratories for the past 10 years as a reliable source of sea water. The sea water was trucked to a 46,500 liter (12,000 gal) underground storage reservoir located at Oregon State University, and subsequently trucked to the laboratory in 3,800 liter (1,000 gal) lots and stored in covered fiberglass tanks. Two types of artificial sea water were also used; a commercial mixture of dry salts

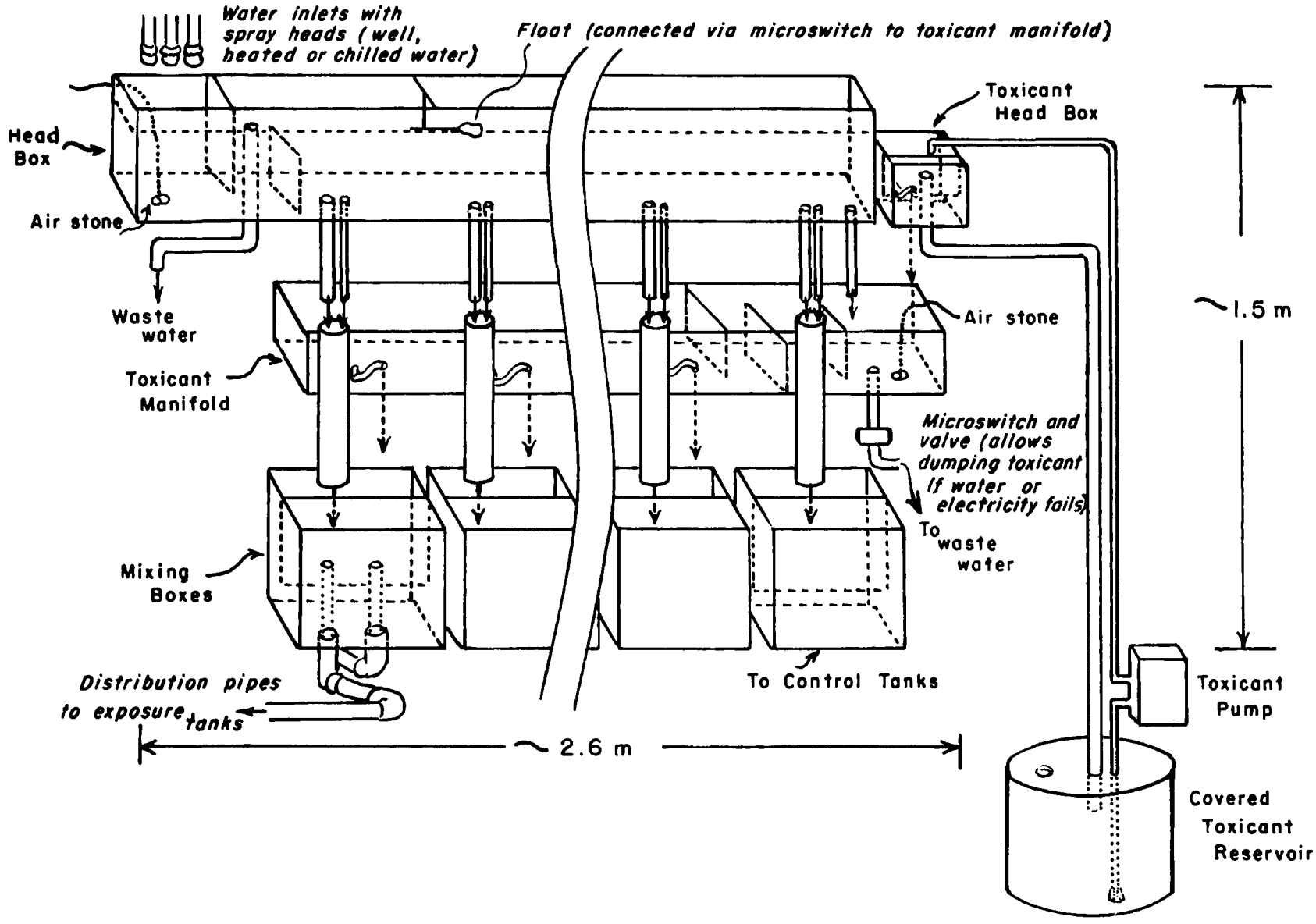

Figure 1. Diagrammatic sketch of flow-through diluter.

Figure 2. Exposure tanks with diluter in background.

Figure 3. Exposure tank with submersible pump for enhancing aeration, current and mixing.

(Rila Products of New Jersey) a/ to which water was added to achieve the desired salinity, and an artificial sea water prepared following the procedure of LaRoche et al. (1970). A batch of each artificial sea water was mixed daily in a separate container prior to introduction to the test tanks. During the seawater tests, dissolved oxygen content of the water was maintained at 6.4-8.5 mg/liter and pH at 7.4-8.1. The total ammonia ranged from 0.04 to 1.8 mg/liter NH3-N due to the daily exchange of 85% of the water in the test tanks.

Downstream migration

The effect of Cu on migration was assessed by releasing marked control and Cu exposed fish into a tributary of the North Fork of the Alsea River and monitoring their arrival at a trap 6.4 km downstream. The trap was built into a permanent weir and was usually checked daily. On the day prior to release, 50 to 100 fish from each exposure tank were anesthetized in MS-222, weighed, measured, and identified by freeze branding and a fin clip. Parr-smolt transformation is markedly size dependent and seasonal with wild coho juveniles normally spending 1 year in the natural stream before migrating seaward provided they attain a minimal size of 7 cm (Lorz and Mason, unpublished data). Under artificial propagation most coho reach a size of 11-15 cm in less than 1 year. No fish less than 10 cm were released. Seaward migration of wild juveniles normally begins in April, peaks in late May and ceases in late June. Releases of Cu exposed fish and their controls were made between April 8 and June 4, 1975. Trapping was terminated in early July, one month after the last release.

Gill ATPase activity

The activity of Na^+, K^+-activated ATPase in microsomes isolated from gill filaments was assayed by the procedure of Zaugg and McLain (1970). The activity was measured in three to six fish per metal concentration prior to each release into the stream and before most seawater tests. Assays of ATPase were also made on survivors of two seawater tests.

No inhibition of ATPase activity occurred when $CuCl_2.2H_2O$ was added to homogenized gill tissue from control fish. Final concentrations of Cu in the spiked homogenates (250 µg Cu/gm of gill filament tissue) were 100 times the concentration found in the whole gills of those coho exposed for 6 months to 20 µg/liter Cu in fresh water (Chapman, personal communication). This suggests that the decreased ATPase activity observed in gills from fish exposed to Cu was not an in vitro effect of Cu ions liberated during the homgenization procedure. Any free Cu ions would probably be bound by the EDTA (5mM) in the homogenizing solution (W.S. Zaugg, personal communication).

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a/ *Mention of commercial product does not constitute endorsement or recommendation for use.*

Osmotic and ionic regulation

Changes in osmolality and concentrations of Cl^- and Na^+ in blood plasma were followed by sacrificing groups of yearling fish for blood at various times after the start of toxicant or seawater exposure. The caudal peduncle was wiped dry, wrapped in a tissue to prevent dilution of blood by water or mucous and then severed. Blood flowing from the caudal artery was deposited onto a small piece of Parafilm^R and immediately transferred via polyethylene tubing into a small polyethylene microcentrifuge tube and centrifuged in a Beckman Microfuge (5500 g) for 1 minute. The supernatant plasma was transferred into a clean micro-centrifuge tube, centrifuged for 30 seconds and then frozen until such time as the micro-analysis could be done. The sodium concentration in plasma was measured by atomic absorption on 1/900 distilled water dilution of plasma utilizing a Perkin-Elmer Model 306 B atomic absorption spectrophotometer. Chloride ion was measured directly with a Corning Model 920 chloride meter. The osmolality of the plasma was measured on a Wescor Model 5100 vapor pressure osmometer calibrated with standard solutions of sodium chloride.

Assessment of coefficient of condition and growth

Parr-smolt transformation in several species of *Salmo* and *Oncorhynchus* is characterized by changes in coefficient of condition. A marked decrease in condition occurs for fish undergoing the transformation, followed by an increase in condition in fish reverting to a nonmigratory form (Hoar 1939; Malikova 1974; Vanstone and Markert 1968; Fessler and Wagner 1969; Pinder and Eales 1969; and Wagner 1974).

Each month, 20 to 50 fish were randomly selected from each exposure tank after a 24-h starvation period, anesthetized, weighed and measured. Individual fish $\geq$10 cm were weighed to 0.1 g, smaller fish were weighed to 0.01 g, and fork length was determined to 0.1 cm. The coefficient of condition (K) was determined for each fish in a sample using the formula $K = 100\ W/L^3$, where W denotes weight in grams and L denotes fork length in centimeters (Hile 1936, Hoar 1939).

Water quality

The source of water for the study was a well (approximately 1 mile east of Willamette River, Linn County, Oregon) and the chemical characteristics were measured quarterly by the Corvallis Environmental Research Laboratory (CERL) of the EPA and the data is presented in Table A-1. In addition alkalinity, hardness, dissolved oxygen, pH and ammonia in the exposure tanks were measured routinely at the laboratory and these chemical characteristics are presented in Table 1. Water temperatures were monitored continuously in both the static and flow-through systems with continuous recording thermographs.

Table 1. Chemical and physical characteristics of test water (flow-through and static water systems) a/

Characteristic		Year	Unit	Mean ± SD	Range	Number of analyses
Alkalinity		1974	mg/liter as $CaCO_3$	66 ± 4	57 - 70	28
		1975		72 ± 3	66 - 81	49
Hardness		1974	mg/liter as $CaCO_3$	95 ± 3	87 -100	30
		1975		93 ± 3	84 - 98	32
Dissolved oxygen	(static water)	1974	mg/liter	10.0 ± 0.7	8.1 - 10.7	43
	(flow-through)	1974		9.7 ± 0.3	9.2 - 10.2	18
	(static water)	1975		9.8 ± 0.3	9.4 - 10.1	8
	(flow-through)	1975		8.7 ± 0.9	6.2 - 10.9	52
Ammonia		1974	mg/liter NH_3-N	0.211	0.124- 0.297	2
		1975		0.387 ± 0.183	0.124- 0.570	7
pH	(static water)	1974		7.34 b/	6.74 - 7.96	56
	(flow-through)	1974		7.41 b/	7.27 - 7.62	19
	(static water)	1975		7.30 b/	6.81 - 7.54	6
	(flow-through)	1975		7.26 b/	7.07 - 7.54	42

a/ *Standard Methods for the Examination of Water and Wastewater, 11th Edition 1960.*

b/ *Median value.*

SECTION 5

RESULTS AND DISCUSSION

In the 20 months of study, six static bioassays with Cu and four with Zn were completed. These included determinations of the 96-h and 144-h LC50 values, effect of metal ion on gill microsomal ATPase, and ability of juvenile coho to adapt to sea water following exposure to toxicant in fresh water. Twelve tests of tolerance to sea water were completed using yearling coho exposed to Cu in a flowing freshwater toxicant system. In addition two tests comparing artificial sea salts to natural sea water; four tests of survival in sea water following periods of freshwater recovery from toxicant exposure; and one test of seawater survival after toxicant free acclimation to lower salinity were conducted. During the 1975 migratory period four releases of coho were made into a small coastal stream and the downstream movement of the migrants monitored.

96-h LC50 experiments

The 96-h LC50 of Cu for yearling coho was determined at different times during the parr-smolt transformation. In late November it was 74 µg/liter; in March it dropped to 70 µg/liter; and by late May had further declined to 60 µg/liter Cu (Figure 4, Table A-2). The greater sensitivity to acutely lethal effects of Cu in May was probably due to the onset of smoltification rather than increasing age or size of fish. This physiological transformation, which preadapts coho to seawater existence apparently increased their susceptibility to lethal effects of Cu in fresh water. The observed increase in susceptibility was small and may have been modified slightly in either direction because the earlier tests were conducted at a higher temperature (12 C) than the May test (10 C) and the interactions between temperature and metal toxicity are complex (Hodson and Sprague 1975).

Amounts of Cu greater than background were found in the controls and some of the test tanks containing the lower Cu concentrations during the determination of the 96-h LC50 values (Table A-2). The Cu level of the well water was normally 2 µg/liter but additional contamination from the brass head of the mixing chamber's water pump and inability to remove all Cu residue by rinsing the mixing chamber with water between concentration changes apparently occurred. When the "incipient lethal level" (ILL) was plotted following the technique of Sprague (1964), the ILL for Cu was close to 75 µg/liter for the November test and 65-70 µg/liter for the March tests (Figure 5). As noted by Sprague (1964), a sharp differentiation between lethal and non-lethal concentrations of Cu occurred and the line relating concentration to survival time broke and ran nearly parallel to the time axis. The longest LT50 at any concentration was 58 hours for one test tank of fish in March 1974. Generally the LT50 for Cu occurred from 19 to 35 h.

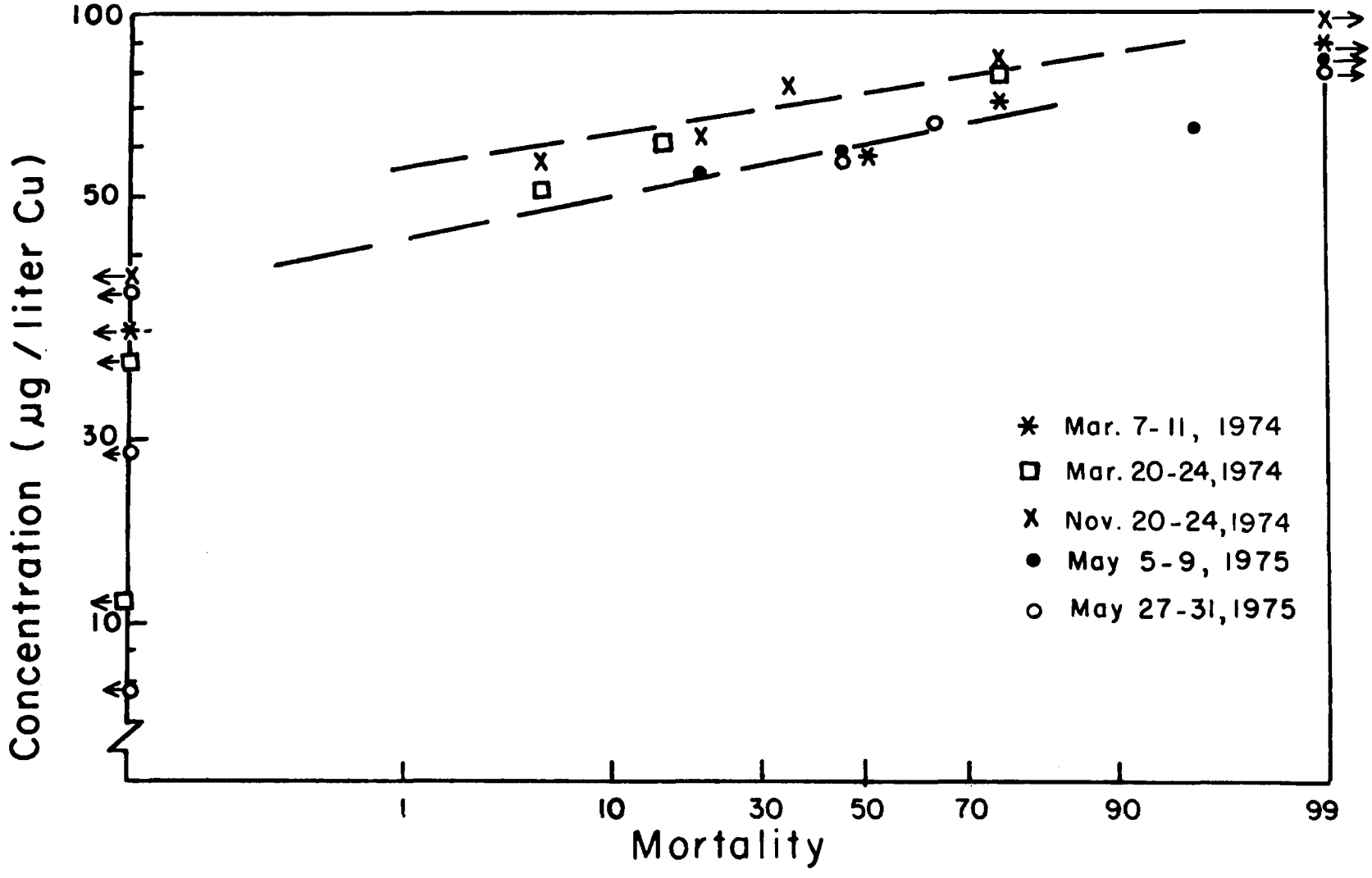

Figure 4. Effect of copper exposure on mortality of yearling coho salmon.

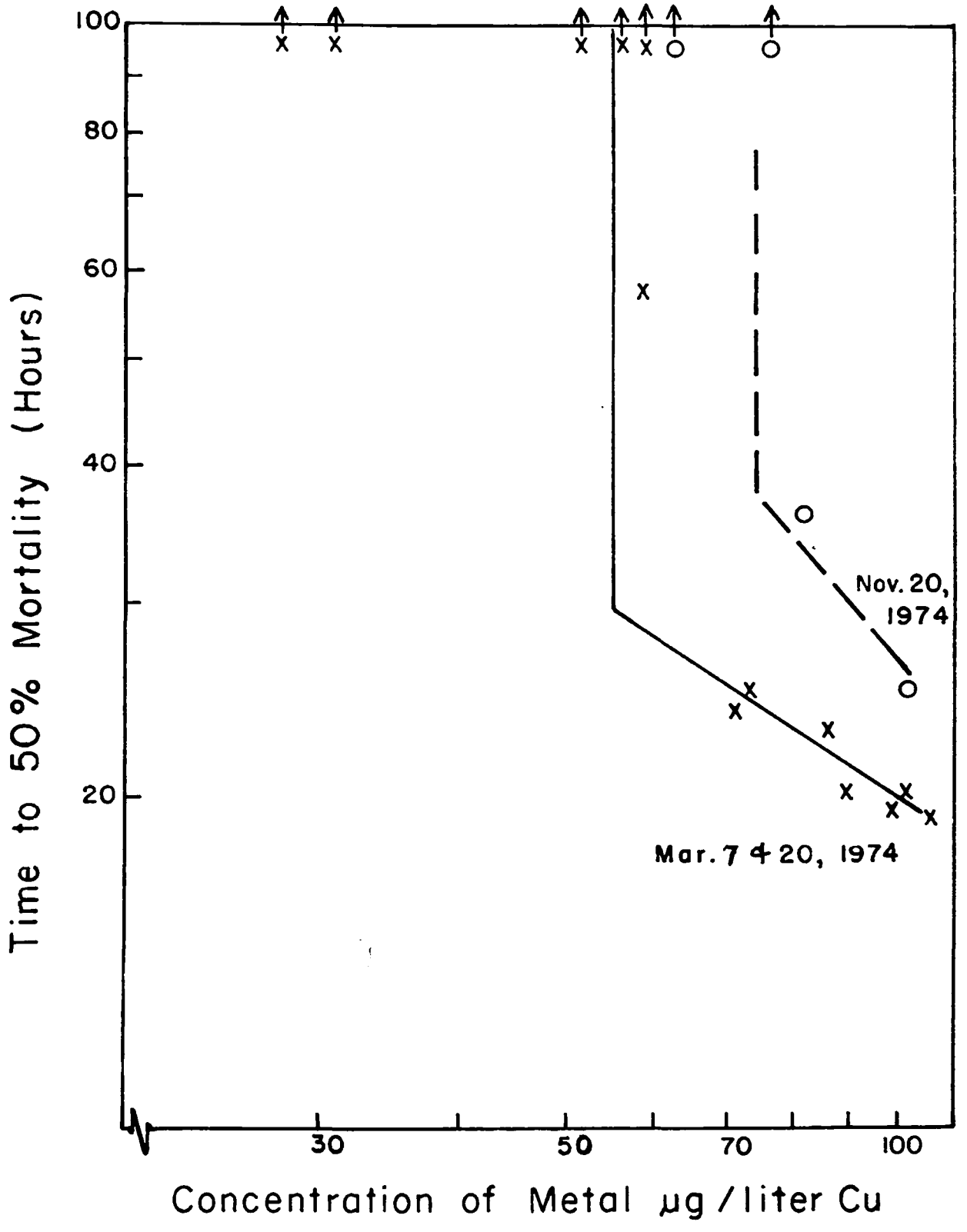

Figure 5. Median mortality-time of yearling coho salmon exposed to solutions of copper.

Our 96-h LC50 values of Cu for coho salmon are higher than those reported by Sprague (1964) for Atlantic salmon (ILL of 48 µg/liter) and Lloyd and Herbert (1962) for rainbow trout (ILL of 50 µg/liter). However, the alkalinity and hardness of our water, 66 and 95 mg/liter $CaCO_3$ respectively, were about four times greater, our fish were larger, and were of a different genus than those used in the cited studies. Chapman (unpublished data) found the 96-h LC50 of juvenile coho salmon from the same Alsea River stock to be 28-38 µg/liter Cu at the Western Fish Toxicology Station, Corvallis, Oregon, with a water hardness and alkalinity of 20-25 mg/liter $CaCO_3$.

The 96-h LC50 of Zn for juvenile coho was 4600 µg/liter at 12 C (Figure 6A, Table A-3). It appeared that there was increasing sensitivity to Zn as the fish underwent smoltification; however, as only a few Zn concentrations were run in May and June all data were combined for the single estimate. The 144-h LC50 of Zn for the juvenile coho was 4200 µg/liter at 12 C (Figure 6B). The longest LT50 in the three tests was 144 h for one test tank in June 1974. Generally the LT50 for Zn occurred from 30-85 h. Our 96-h LC50 value for Zn is again higher than that reported by other investigators (Sprague 1964, Chapman 1973, Hodson and Sprague 1975). However, our considerably greater alkalinity and water hardness may again account for this difference. Similarly the solubility of zinc salts decrease markedly as pH rises above 7.0 (Sprague 1964) and, therefore, our higher 96-h LC50 values could also be related to small pH differences.

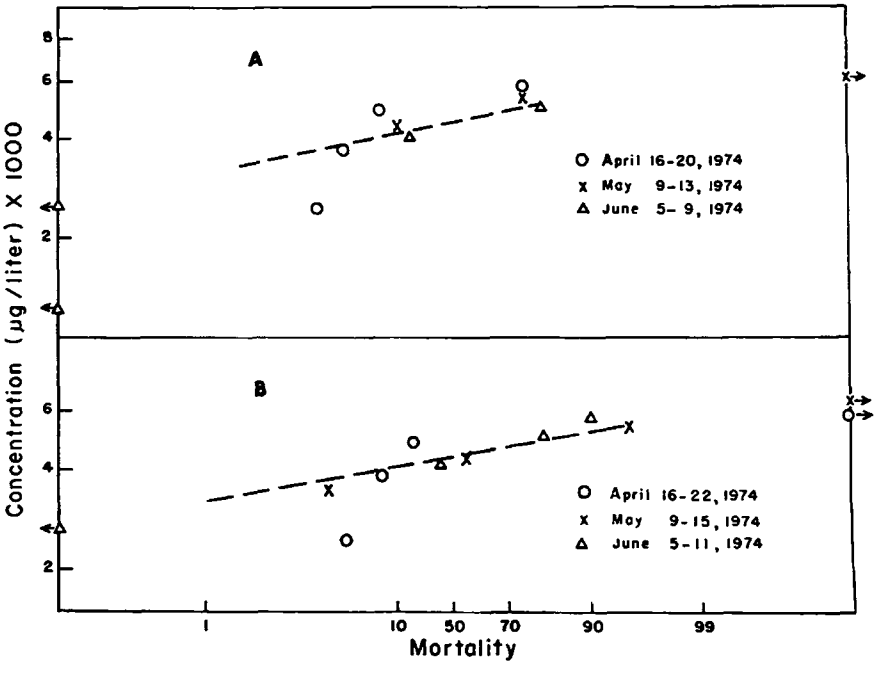

Figure 6. Effect of zinc exposure on mortality of yearling coho salmon.

Effect of copper on growth and survival

The longest period of juvenile coho exposure to Cu (in fresh water) during 1974 was 37 days (880 h). The exposure occurred in June and early July at a time after the parr-smolt transformation and majority of juvenile growth had occurred. The fish exposed to 20 and 30 µg/liter Cu immediately ceased feeding and only near the end of the 37-day exposure did they again begin to feed. Consequently, the mean lengths and the mean coefficient of condition (K) of the fish exposed to 20 and 30 µg/liter Cu were significantly lower than the control values at the end of the test (Table 2). In the control through 20 µg/liter Cu groups almost no mortality occurred through 37 days of exposure whereas the 30 µg/liter group incurred about 30% mortality (Table 2). The mortality in the 30 µg/liter groups occurred after the first 144 h of exposure.

In 1975 the effects of Cu exposure in fresh water on growth, survival and coefficient of condition of juvenile coho salmon showed a similar correlation with Cu concentration as it did in 1974. The 30 µg/liter groups showed the poorest survival, growth and coefficient of condition (Table 3). Survival of juvenile coho in the control through 20 µg/liter groups was similar after 59 days (1416 h) of toxicant exposure although K of the 20 µg/liter coho group was lower. After 6 months of exposure (Dec.-June) the 20 and 30 µg/liter Cu groups had incurred mortalities of 9 and 38%, respectively (Table 3). A second group of fish exposed for 10 weeks (March-June) to 30 µg/liter Cu exhibited a mortality of 29%. As noted in 1974, fish in the 20 and 30 µg/liter Cu groups again ceased feeding upon initiation of the toxicant exposure. Resumption of feeding in the higher toxicant concentration groups was not resumed until mid-May and then only with reduced intensity. Feeding was slightly inhibited by exposure to 10 µg/liter Cu. Fish in the control through 10 µg/liter groups were fed an amount equivalent to that eaten by the 10 µg/liter fish in an effort to minimize differences in size of fish among the test concentrations. Fish in the stock rearing tank exhibited the best growth as they were fed to repletion daily; whereas in the experimental tanks the controls exhibited the maximum growth and the others others grew according to the Cu concentration received (Figure 7).

The effect of Cu exposure which was best correlated to Cu concentration was the coefficient of condition (K) (Figure 8, Table A-4) with the fish receiving 20 or 30 µg/liter Cu losing condition rapidly after the initiation of the Cu exposure. The 20 and 30 µg/liter fish evidenced a recovery of feeding in late April and this was reflected in elevated K's in early June. The coefficient of condition of a group of juveniles exposed to 30 µg/liter Cu for 10 weeks (March-June) showed a rapid decline in April, leveled off in May and showed a very slight increase in June with a resumption of feeding.

Effect of copper and zinc on gill ATPase and survival in sea water

When fish were exposed to Cu for 144 h in static fresh water the ATPase activity was decreased in proportion to the concentration of Cu. The percent survival in sea water was also decreased in proportion to the concentration of Cu (Table 4, Table A-5). The decreased ATPase activity was

Table 2. Percent survival, mean length, weight and coefficient of condition of yearling coho salmon (1972 brood) exposed to copper for 37 days (880 h) in 1974

Nominal concentration µg/liter Cu	Percent survival in toxicant *a/*		Mean length *b/* ± SE		Mean weight *b/* ± SE		Mean condition *b/* ± SE	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
0	100.0	99.5	17.4 ± 0.184	17.5 ± 0.153	56.3 ± 1.91	56.0 ± 1.55	1.057 ± 0.0096	1.042 ± 0.0078
5	100.0	100.0	17.5 ± 0.184	17.4 ± 0.211	56.9 ± 2.17	55.6 ± 2.21	1.041 ± 0.0092	1.034 ± 0.0088
10	100.0	100.0	17.6 ± 0.181	17.7 ± 0.189	55.1 ± 1.80	57.2 ± 1.82	1.004 ± 0.0085	1.023 ± 0.0087
20	100.0	99.0	17.1 ± 0.212	16.9 ± 0.203	49.5 ± 1.93	48.4 ± 1.94	0.967 ± 0.0092	0.988 ± 0.0096
30	68.3	73.9	16.3 ± 0.180	16.2 ± 0.180	38.8 ± 1.55	38.6 ± 1.60	0.884 ± 0.0126	0.893 ± 0.0139

a/ Originally placed 225-250 fish in each tank. Survival calculated on number of fish remaining in tank following removal of fish for survival tests in sea water after exposures to toxicant of 144, 535, 880 h. Average survival calculated as the product of the percent survival during each time period.

b/ Sample size of 40 fish per test tank.

Table 3. Percent survival, mean length, weight and coefficient of condition of yearling coho salmon (1973 brood) exposed to copper for 59 and 165 days in 1975

Nominal concentration µg/liter Cu	Percent survival in toxicant a/ Rep. 1	Rep. 2	Mean length ± SE Rep. 1	Rep. 2	Mean length ± SE Rep. 1	Rep. 2	Mean condition ± SE Rep. 1	Rep. 2
A. 1416 h Cu exposure (59 d, December 20, 1974-February 17, 1975)								
0	99.6	99.9	15.1 ± 0.361 b/	15.3 ± 0.276 b/	40.9 ± 2.87	41.6 ± 2.30	1.160 ± 0.0174	1.149 ± 0.0175
5	99.9	100.0	14.7 ± 0.303 b/	15.3 ± 0.417 b/	35.5 ± 2.26	41.5 ± 3.46	1.102 ± 0.0145	1.118 ± 0.0185
10	99.9	99.6	14.5 ± 0.434 b/	14.3 ± 0.205 b/	36.6 ± 4.09	33.6 ± 2.10	1.132 ± 0.0248	1.127 ± 0.0330
20	98.6	99.5	14.6 ± 0.290 b/	13.7 ± 0.315 b/	33.0 ± 2.28	26.0 ± 1.90	1.028 ± 0.0199	0.988 ± 0.0167
30	83.7	74.3	13.3 ± 0.247 c/	12.8 ± 0.261 c/	22.7 ± 1.619	19.4 ± 1.371	0.957 ± 0.0227	0.915 ± 0.0213
B. 3960 h Cu exposure (165 d, December 20, 1974-June 3, 1975)								
0	99.4	99.4	17.2 ± 0.224 d/	17.9 ± 0.289 d/	56.2 ± 2.41	64.6 ± 3.51	1.083 ± 0.0099	1.096 ± 0.0120
5	97.0	96.8	17.1 ± 0.303 e/	17.4 ± 0.334 e/	54.1 ± 3.81	57.5 ± 3.81	1.021 ± 0.0119	1.031 ± 0.0184
10	98.4	97.9	16.1 ± 0.297 e/	15.5 ± 0.299 e/	43.1 ± 3.26	38.3 ± 2.85	0.959 ± 0.0209	0.953 ± 0.0216
20	90.8	92.3	15.3 ± 0.269 d/	14.6 ± 0.286 d/	36.0 ± 2.50	31.5 ± 2.64	0.959 ± 0.0262	0.943 ± 0.0294
30	61.9	71.2	f/14.4 ± 0.216 e/	16.7 ± 0.253 e/	26.8 ± 1.75	42.5 ± 2.12	0.851 ± 0.0247	0.900 ± 0.0207

a/ *Each tank originally contained 750-770 coho. Survival calculated on number of fish remaining in tank following removal for seawater tests or length-weight data.*
b/ *Sample size 15 fish.*
c/ *Sample size 10 fish.*
d/ *Sample size 30 fish.*
e/ *Sample size 40 fish.*
f/ *Fish exposed for 1632 h (68 d).*

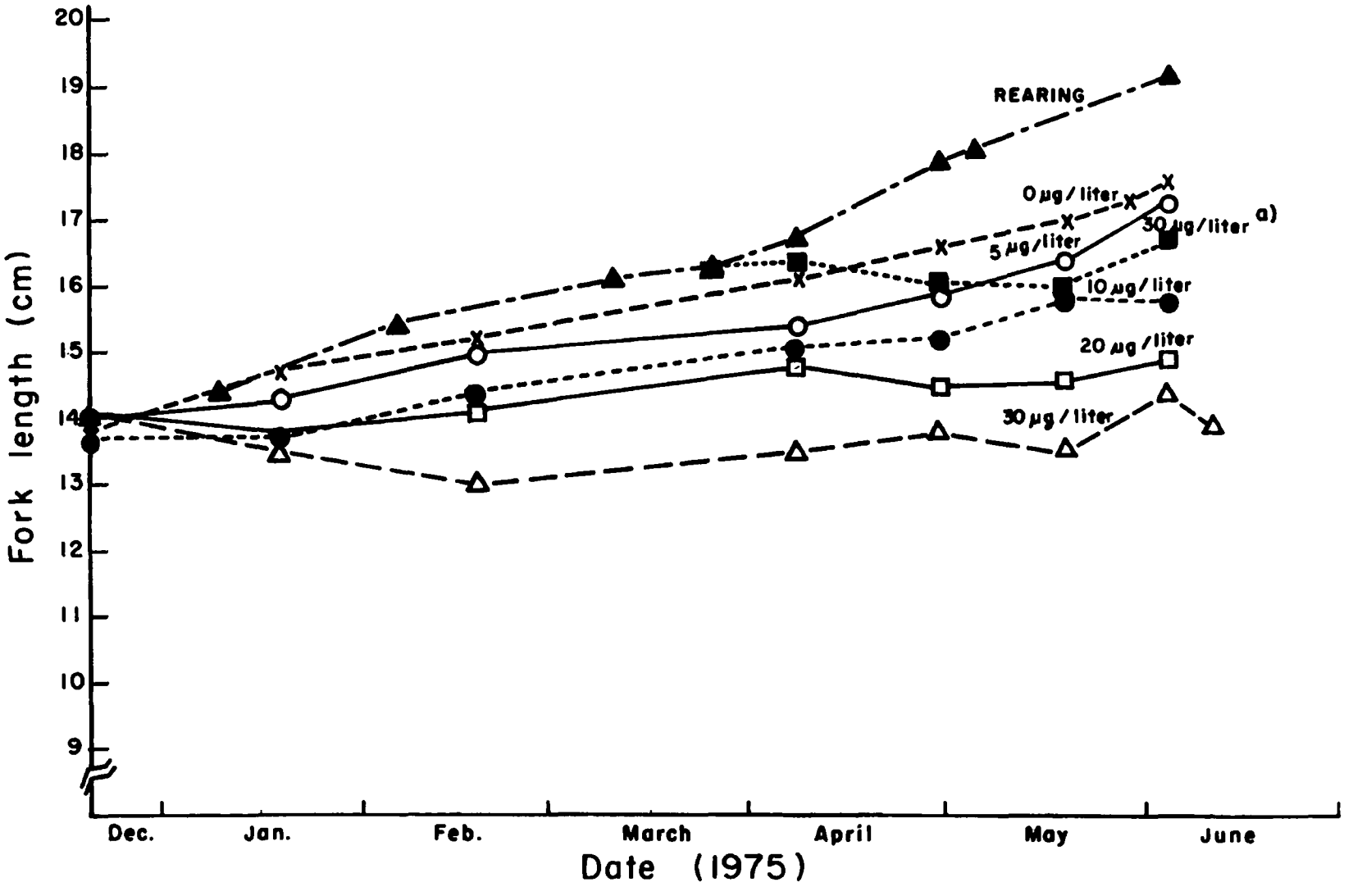

Figure 7. Effect of chronic copper exposure on growth of juvenile coho salmon. (*a/ Coho exposed to 30 µg/liter on March 27, 1975*).

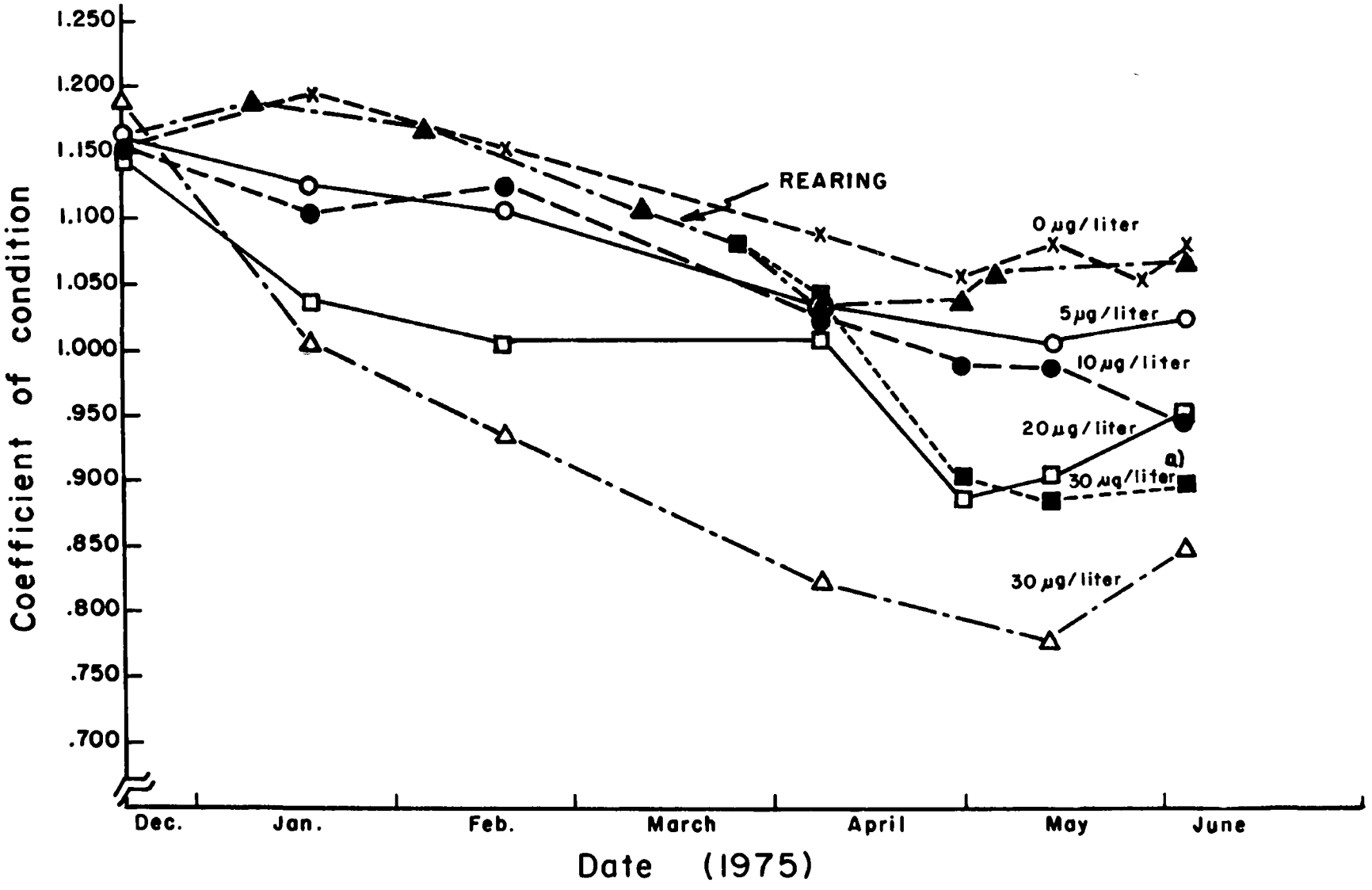

Figure 8. Effect of chronic copper exposure on coefficient of condition of juvenile coho salmon. (*a/ Coho exposed to 30 µg/liter on March 27, 1975*).

Table 4. Survival and gill ATPase activity of yearling coho salmon exposed to copper for 144 h in static fresh water and their subsequent survival and gill ATPase activity after transfer to sea water

Nominal concentration	Measured concentration ± SD	Percent survival	ATPase activity	Percent survival	ATPase activity
	µg/liter Cu	in copper *a/*	(fresh water) *b/*	in sea water	(sea water) *b/*
A. March 20-April 8, 1974 *c/*					
0	13.7 ± 6.1	100	12.9	94.0	51.4
20	27.6 ± 3.1	100	7.1	6.0	63.4 *d/*
30	34.7 ± 4.2	100	5.6	0.0	
50	51.8 ± 4.1	95	5.0	0.0	
60	60.7 ± 5.3	85	--	0.0	
80	81.4 ± 5.5	25	--	0.0	
B. April 16-May 6, 1974 *e/*					
0	15.7 ± 3.4	100	25.5	100.0	57.3
5	16.2 ± 2.4	100	16.3 *f/*	94.0	58.6
10	22.1 ± 5.3	100	9.8 *f/*	59.0	57.8
20	32.3 ± 2.7	100	6.6 *f/*	24.0	53.7 *f/*
30	42.8 ± 4.1	100	6.5 *f/*	6.0	70.2 *d/*
60	75.1 ± 3.9	30	4.0 *g/*	0.0	
C. May 9-31, 1974 *h/*					
0	7.9 ± 2.5	100	48.4	100.0	
5	11.5 ± 2.3	100	24.0	100.0	
10	15.4 ± 2.6	100	14.0	82.0	
20	23.1 ± 4.4	100	10.3	12.5	
60	62.9 ± 7.4	30	6.9	0.0	
D. June 5-26, 1974 *h/*					
0	14.4 ± 9.9	100	24.8	100.0	
5	16.3 ± 6.8	100	15.6	94.1	
10	22.0 ± 6.0	100	11.1	64.7	
20	28.0 ± 7.7	95	7.2	37.5	
30	37.4 ± 4.6	100	6.8	11.8	
50	52.7 ± 8.1	40	5.8	0.0	
60	61.2 ± 3.0	35	--	0.0	

a/ Twenty fish exposed per concentration.
b/ Gill microsomal Na^+, K^+-activated ATPase; µmoles ATP hydrolyzed/mg protein/h; mean of four fish at the end of exposure.
c/ Three hundred and twelve h seawater exposure.
d/ Only one fish.
e/ Three hundred and thirty-six h seawater exposure.
f/ Mean of three fish.
g/ Mean of two fish.
h/ Three hundred and sixty h seawater exposure.

probably one of the factors leading to loss of osmoregulatory ability and death in sea water. The ATPase activity of fish that survived exposure to sea water (Table 4, Table A-5) was increased two to threefold over the values obtained for control coho in fresh water, corroborating the results of Zaugg and McLain (1970).

Exposure of yearling coho for 144 h to sublethal levels of Zn ($\leq$2000 µg/liter) in static fresh water did not seriously affect survival in sea water (Table 5). The percent survival in sea water was not consistently related to the Zn concentration, perhaps reflecting the tendency of fish to become variably hypersensitive and hyperactive to stimuli during and after exposures of $\geq$1000 µg/liter Zn. These hyper-responsive episodes were sometimes followed by tetanic spasms and death. In another study Chapman (personal communication) exposed sockeye salmon to Zn for 19 months (egg through smolt) and then transferred the smolts to sea water and observed no difference in seawater survival between Zn exposed and control groups.

Gill ATPase was also inconsistently affected by Zn. It appeared to be stimulated by all concentrations in March but not by any concentrations in April or May (Table 5). The stimulation in March may have been an artifact of the fish analyzed as controls; the fish chosen may not have been representative, in addition to being subjected to a low Cu contamination (13.7 µg/liter). The difference in effects of Cu and Zn on gill ATPase and subsequent survival in sea water indicates that the two metals probably do not have a common mode of action.

The gill microsomal Na^+, K^+-activated ATPase activity of juvenile coho exposed to Cu in the flowing bioassay in June and July 1974 was reduced in a concentration dependent manner except that the 5 µg/liter group produced a slight stimulation in ATPase activity (Table 6, Table A-6) and was generally similar to that noted for exposures in the static tests (Table 4). The effects of Cu on seawater tolerance were greater after exposures to toxicant in the static water system (Table 4) than after exposures in the flowing water system (Table 6). This was probably due to the manual water changes (once/day) which caused excitation and stressing of the fish and fluctuations in water chemistry (Table 1) during the static exposures to toxicants.

The ATPase activity of coho exposed to 30 µg/liter Cu declined rapidly following the introduction of the toxicant (Figure 9, Table A-7). The ATPase activity declined to 72.6% of the control value after 56 h of toxicant exposure and continued to decline until 310 h when activity leveled off at 24.0% of the control value. Yearling coho exposed for as little as 24 h to 30 µg/liter Cu incurred some deaths upon transfer to sea water but the effect reached a maximum in those coho previously exposed for 96 to 120 h at 30 µg/liter Cu (Table 7). Additional exposure time to 30 µg/liter Cu did not appear to result in more deaths when the coho were transferred to sea water. The decline in ATPase activity with time of Cu exposure during the first 144 h correlated with subsequent mortality observed following transfer of the fish to sea water (Table 7).

Table 5. Survival and gill ATPase activity of yearling coho salmon exposed to zinc for 144 h in static fresh water and their subsequent survival and gill ATPase activity after transfer to sea water

Nominal concentration	Measured concentration ± SD	Percent survival in zinc a/	ATPase activity (fresh water)	Percent survival in sea water	ATPase activity (sea water) b/
µg/liter Zn					
A. March 20-April 8, 1974 c/					
0	29 ± 17	100	12.9 d/	100.0	51.4
100	115 ± 12	100	22.2	94.0	70.1
300	299 ± 9	100	--	100.0	65.7
600	555 ± 20	100	25.5	100.0	76.9
1000	924 ± 32	100	24.4	81.0	95.0
2000	1772 ± 46	100	29.8	81.0	70.5
2500	2271 ± 57	100	25.8	87.0	66.2
B. April 16-May 6, 1974 e/					
0	37 ± 29	100	25.5 d/f/	100.0	57.3
1000	937 ± 46	95	27.1 f/	100.0	50.9
2500	2495 ± 178	85	21.6 f/	100.0	48.6 f/
4000	3803 ± 207	75	21.8 f/	67.0	46.5 f/
5000	4833 ± 369	65	20.2 f/	80.0	58.3 f/
6000	5611 ± 172	10	--	50.0	--
C. May 9-31, 1974					
0	33 ± 30	100	48.4 d/	100.0	
4000	4261 ± 156	45	32.0	33.0	
5000	5245 ± 144	5	--	0.0	
6000	6158 ± 172	0	--		
D. June 6-26, 1974					
0	18 ± 13	100	--	100.0	
1000	1214 ± 62	100	--	100.0	
2500	2620 ± 56	100	--	100.0	
4000	4062 ± 76	55	--	70.0	
5000	4980 ± 231	20	--	66.7	

a/ *Twenty fish exposed per concentration.*
b/ *Gill microsomal Na^+, K^+-activated ATPase; µmoles ATP hydrolyzed/mg protein/h; mean of four fish at the end of exposure.*
c/ *Three hundred and twelve h seawater exposure.*
d/ *Control also served for copper exposed fish concurrently being run (see Table 4 for copper levels).*
e/ *Three hundred and thirty-six h seawater exposure.*
f/ *Mean of three fish.*

Table 6. Survival and gill ATPase activity of yearling coho salmon exposed to copper in fresh water and their subsequent survival after transfer to sea water

Nominal concentration µg/liter Cu	Measured concentration ± SD (n)		Percent survival in copper a/	ATPase activity (fresh water) b/		Percent survival in sea water c/	
A. June 5-26, 1974 (144 h exposure)							
0	1.8 ± 1.0	(10)	100.0	24.0	(5)	100.0	(40)
5	8.0 ± 3.9	(18)	100.0	26.5	(6)	100.0	(40)
10	9.9 ± 2.7	(18)	100.0	18.2	(6)	97.5	(40)
20	18.2 ± 2.9	(18)	100.0	11.4	(6)	70.0	(40)
30	31.3 ± 4.0	(15)	100.0	8.8	(6)	35.0	(40)
B. June 27-July 10, 1974 (525 h exposure)							
0	1.4 ± 0.9	(16)	99.7	32.3	(6)	100.0	(80)
5	7.7 ± 3.9	(31)	100.0	36.0	(8)	100.0	(80)
10	10.6 ± 3.3	(32)	100.0	25.9	(8)	100.0	(80)
20	20.3 ± 3.6	(32)	99.5	17.5	(8)	96.3	(80)
30	31.5 ± 3.8	(28)	81.9	14.5	(6)	35.0	(80)
C. July 10-24, 1974 (880 h exposure)							
0	1.4 ± 0.9	(24)	99.7	--		100.0	(40)
5	7.4 ± 3.5	(39)	100.0	--		100.0	(40)
10	10.8 ± 3.1	(40)	100.0	--		97.5	(40)
20	20.3 ± 3.5	(42)	99.5	--		67.5	(40)
30	31.3 ± 3.4	(36)	71.1	--		10.0	(40)
D. July 16-August 6, 1974 (144 h exposure) d/							
0	3.6 ± 1.8	(6)	100.0	26.1	(8)	97.5	(40)
5	9.7 ± 3.5	(8)	100.0	33.0	(10)	100.0	(40)
10	13.2 ± 2.7	(8)	100.0	18.8	(10)	60.0	(40)
20	26.0 ± 4.3	(7)	100.0	10.4	(10)	0.0	(40)
30	33.9 ± 2.0	(6)	100.0	7.5	(10)	0.0	(40)

a/ *Orginally placed 225-250 coho into each tank. Survival calculated on number of fish remaining in tank following removal of fish for survival tests in sea water.*

b/ *Gill microsomal Na^+, K^+-activated ATPase; µmoles ATP hydrolyzed/mg protein/h; sample size given in parenthesis.*

c/ *Three hundred and thirty to three hundred and sixty h seawater exposure; sample size given in parenthesis.*

d/ *Placed 26-30 coho into each tank.*

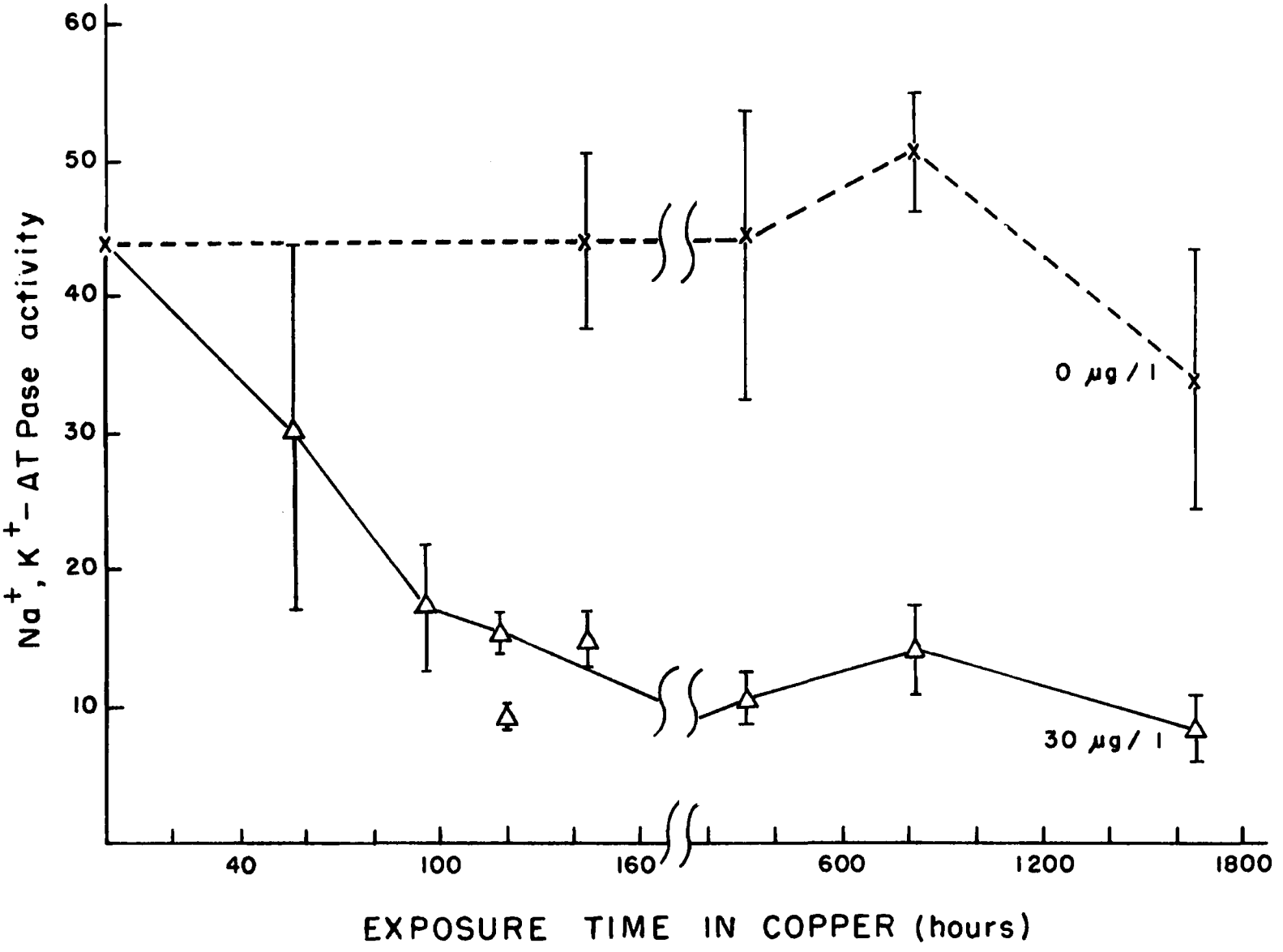

Figure 9. Influence of copper exposure in fresh water on gill microsomal Na^+, K^+-activated ATPase activity in juvenile coho salmon. Each point represents mean of four to six fish (µmoles ATPase hydrolyzed/mg protein/h ± SD).

Table 7. Gill ATPase activi., and survival in sea water of yearling coho salmon exposed to copper in fresh water for ≤144 h (tests conducted from March 6 to June 9, 1975)

Nominal concentration (µg/liter Cu)	Exposure time (h)	ATPase activity (fresh water) (mean ± SD) *a/b/*	Percent survival (240-312 h exposure) *b/*
0	144	46.3 ± 8.8 (18)	100.0 (103)
10	144	39.3 ± 8.0 (4)	98.8 (83)
20	144	--	60.7 (107)
30	144	13.4 ± 3.1 (8)	10.1 (99)
30	120	15.4 ± 1.5 (4)	9.5 (21)
30	96	17.2 ± 4.6 (4)	45.0 (20)
30	69	--	61.0 (59)
30	56	30.2 ± 13.5 (4)	--
30	41	--	78.0 (41)
30	24	--	75.6 (41)

a/ Gill microsomal Na^+, K^+-activated ATPase; µmoles ATP hydrolyzed/mg protein/h.
b/ Numbers of fish in parentheses.

The ATPase activity of juvenile coho chronically exposed to Cu starting December 20, 1975, is shown in Figure 10, (Table A-8). A peak in activity occurred in the control fish during April and May, corroborating the data of Zaugg and McLain (1970) that ATPase activity is highest during the parr-smolt transformation. Gill ATPase activity was suppressed by Cu (Figure A-1) and generally reflected decreased seawater tolerance but did not show any increase during the time an increasing tolerance of Cu-exposed coho juveniles to sea water occurred. The values for gill ATPase activity in 1974 (Tables 4 and 6) are lower than those in 1975 (Table 7) but may not represent biological differences. The discrepancies may also be due to the analysis being conducted at the Western Fish Nutrition Laboratory, Willard, WA in 1974 and at our laboratory in 1975.

The survival of juvenile coho in sea water following exposure to Cu in fresh water for 144 h at different times of the year (1975) is shown in Figure 11. The percent survival was inversely related to Cu concentration but there was a trend toward reduced effect with the onset of smoltification in the fish. Disturbance of osmotic balance in coho upon transfer to sea water is minimized during the smolting process (Conte et al. 1966). This physiological process of smolting similarly may have reduced the deleterious effects of Cu on survival of smolts in sea water, although increasing size and age may also have contributed.

From June to early August 1974, four survival tests in sea water were run on coho from the flowing toxicant system after 6 to 37 days (144 to 880 h) of

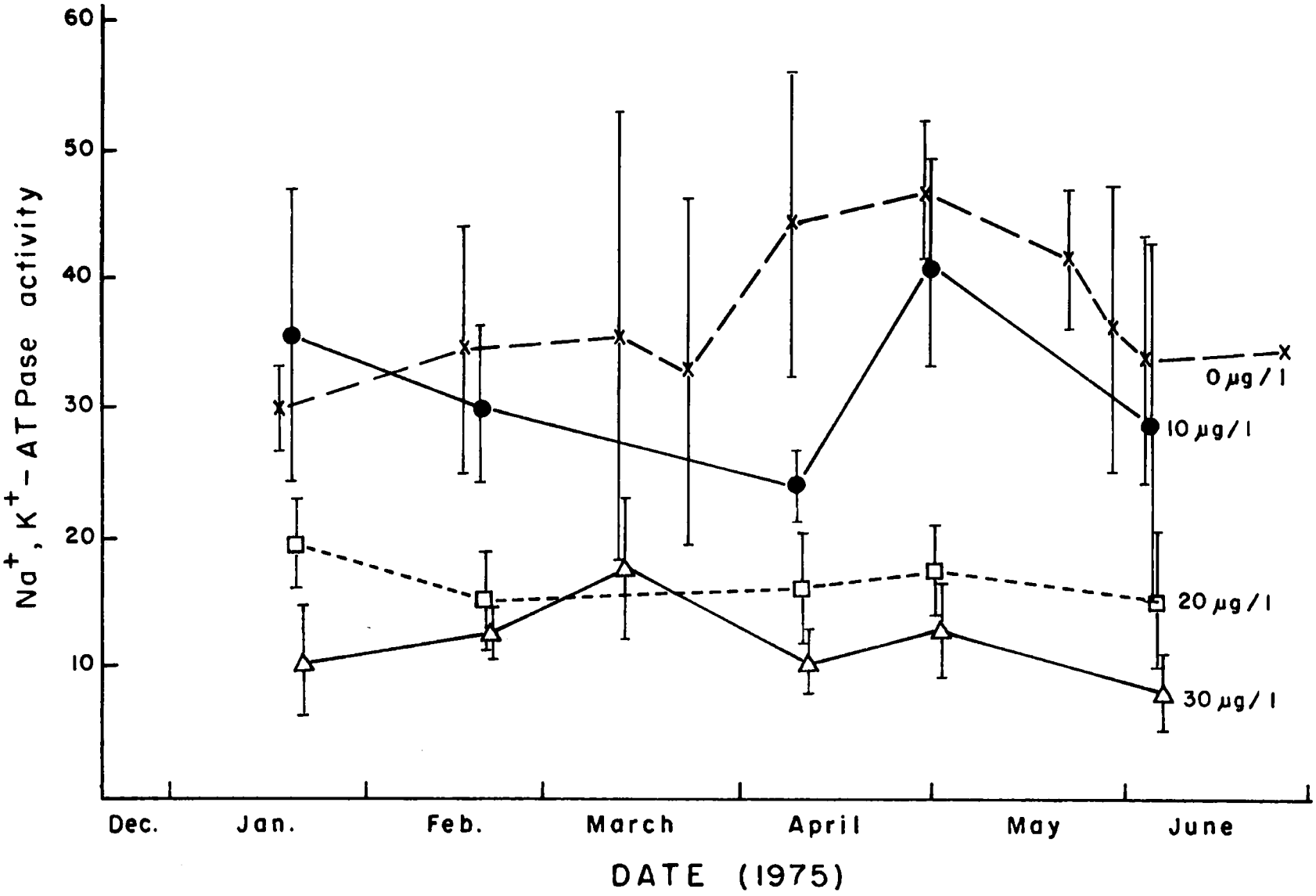

Figure 10. Influence of copper exposure in fresh water on gill microsomal Na^+, K^+-activated ATPase activity in yearling coho salmon chronically exposed to copper from December 20, 1974. Each point represents mean of four to six fish (µmoles ATPase hydrolyzed/mg protein/h ± SD.

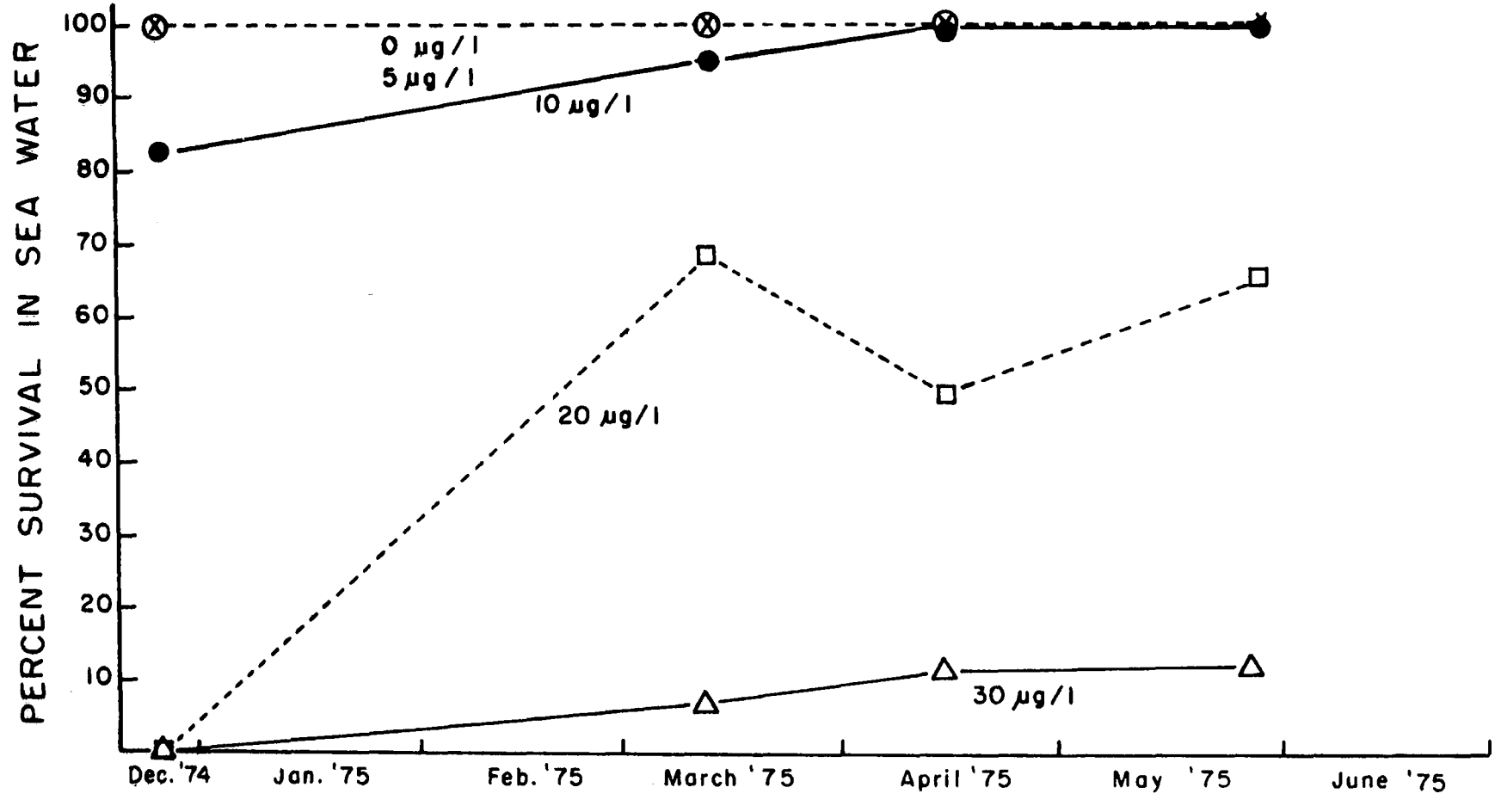

Figure 11. Survival of yearling coho salmon in sea water following exposure to copper in fresh water for 144 h at various times of the year. (Each point represents 16-45 fish).

Cu exposure (Table 6). The group receiving 30 µg/liter Cu suffered 29% mortality during the 37-day exposure, and 90% mortality of the toxicant survivors occurred upon transfer to seawater (Table 6). In the groups of coho that received 20 and 30 µg/liter Cu for 144 h in mid-July no mortality occurred during the freshwater exposure; however, total mortality occurred in sea water (Table 6). This higher mortality in sea water as compared to that observed earlier (Table 6) is probably due to desmoltification and the higher salinity of the sea water used. In the two prior tests the salinity averaged about 30 °/oo whereas in the mid-July test the salinity was 31.5 to 33 °/oo.

From mid-December 1974 to early June 1975, five survival tests in sea water were carried out on coho exposed to Cu for 6 to 172 days (144 to 4128 h, Table 8). Mortality during the 6 months of toxicant exposure in fresh water was 39% at 30 µg/liter, 12% at 20 µg/liter, less than 5% for the 5 and 10 µg/liter concentrations and less than 1% of the controls (Table 8). A group of fish exposed to 30 µg/liter Cu starting in late March showed a 29% mortality in fresh water following 74 days of exposure (Table 8). Data showing the survival in sea water of the various chronic exposure groups in 1975 are shown in Figure 12. A steady increase in tolerance to sea water occurred from February through June, and was similar to the increased tolerance observed for coho exposed to Cu for only 144 h (Figure 11) at various times throughout this period. However, the chronically exposed fish generally had higher mortality in sea water than did the 144-h exposed fish (Figure 13). This was partly due to the poorer condition and smaller size of the chronically exposed fish resulting from a Cu-induced concentration dependent suppression of feeding. These same factors precipitated a faster onset of mortality following transfer to sea water (Figure 13B).

A marked recovery of ability to survive in sea water was observed during May and June in the groups chronically exposed to 30 µg/liter Cu (Figure 10 and and 14C). Deaths during exposure to 30 µg/liter Cu in fresh water began to occur after 144 h and reached an asymptote after about 1800 h (Figure 13B). The fish that remained were Cu-tolerant and subsequently began to feed. Resumption of feeding led to improved condition and nutritional states, and consequently improved tolerance to sea water. In both groups chronically exposed to 30 µg/liter Cu, recovery of feeding began to occur in May, even though one group had been exposed for over 140 days and the other for less than 50 days. Therefore, during the April, May and June tests of survival in sea water, fish in the group exposed to Cu for the shorter time were larger and in better condition as well as having begun parr-smolt transformation before their exposure to Cu on March 27. Probably a combination of these factors resulted in the greater recovery of tolerance to sea water in this 30 µg/liter group (Figure 14C).

A comparison of survival in natural and artificially prepared sea water was also carried out with coho juveniles chronically exposed to Cu. The survival was generally comparable in natural and artificial sea water for all groups tested (Table 9).

Table 8. Survival and gill ATPase activity of yearling coho salmon exposed to copper in fresh water and their subsequent survival after transfer to sea water

Nominal concentration µg/liter Cu	Measured concentration ± SD µg/liter Cu		Percent survival in copper *a/*	ATPase activity (fresh water) *b/*		Percent survival in sea water *c/*	
A. Dec. 20, 1974-Jan. 5, 1975 (6 d exposure)							
0	2.8 ± 2.1	(12)	99.87	--		100.0	(40)
5	6.5 ± 1.3		100.00	--		100.0	(41)
10	11.4 ± 1.6		99.93	--		82.5	(40)
20	21.2 ± 3.0		99.87	--		0.0	(41)
30	31.3 ± 3.8		100.00	--		0.0	(41)
B. Feb. 25, 1975-Mar. 7, 1975 (67 d exposure)							
0	3.1 ± 1.5	(35)	99.73	34.5	(6)	100.0	(41)
5	7.3 ± 1.4		99.93	27.3	(6)	92.7	(41)
10	11.9 ± 1.4		99.72	30.2	(6)	75.0	(44)
20	21.7 ± 2.6		98.97	15.1	(6)	11.6	(43)
30	31.7 ± 2.5		77.90	12.7	(5)	0.0	(43)
C. April 14-24, 1975 (115 d exposure)							
0	2.6 ± 1.5	(55)	99.51	44.2	(6)	100.0	(40)
5	7.0 ± 1.5		99.46	--		100.0	(41)
10	11.6 ± 1.5		99.56	24.0	(5)	100.0	(41)
20	21.5 ± 2.8		97.99	16.1	(4)	25.0	(40)
30	31.4 ± 2.8		72.02	10.4	(4)	0.0	(40)
30 (18 d)			92.82	10.6	(5)	0.0	(40)
D. May 16-26, 1975 (147 d exposure)							
0	2.6 ± 1.5	(61)	99.41	46.9	(5)	100.0	(21)
5	7.1 ± 1.4		97.92	--		92.5	(40)
10	11.7 ± 1.5		99.18	41.3	(4)	87.5	(40)
20	21.7 ± 2.7		94.65	17.5	(4)	47.5	(41)
30	31.6 ± 2.8		67.70	13.0	(3)	4.4	(45)
30 (49 d)			73.50	14.2	(3)	17.5	(50)
E. June 10-20, 1975 (172 d exposure)							
0	2.6 ± 1.6	(68)	99.41	33.9	(5)	100.0	(20)
5	7.1 ± 1.5		96.37	--		92.3	(39)
10	11.7 ± 1.5		97.53	29.0	(4)	90.2	(41)
20	21.6 ± 2.6		88.30	15.3	(4)	55.0	(40)
30	31.7 ± 2.9		61.52	8.1	(3)	20.0	(20)
30 (74 d)			71.18	8.4	(3)	63.6	(11)

a/ Originally placed 750-770 fish into each tank. Survival calculated on number of fish remaining in tank following removal for survival tests in sea water, migration, etc.

b/ Gill microsomal Na^+, K^+-activated ATPase; µmoles ATP hydrolyzed/mg protein/hr; sample size in parenthesis, analysis run within a week of seawater tests.

c/ Two hundred and forty h seawater exposure; sample size in parenthesis.

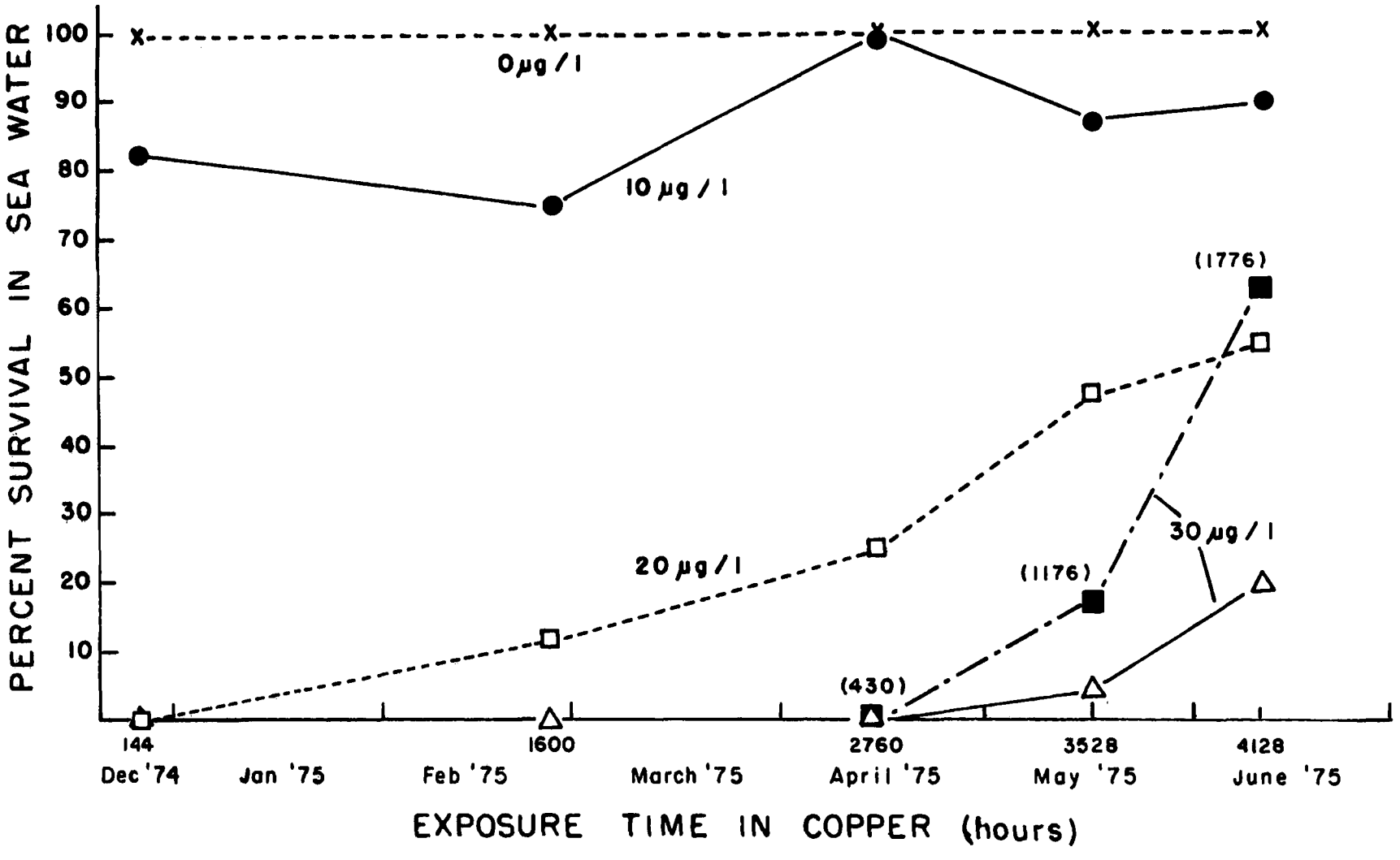

Figure 12. Survival of yearling coho salmon in sea water at various times of the year following exposure to copper in fresh water initiated on December 20, 1974. (Each point represents 20-45 fish. Numbers in parenthesis indicate exposure hours of coho placed in 30 µg/liter copper on March 27, 1975).

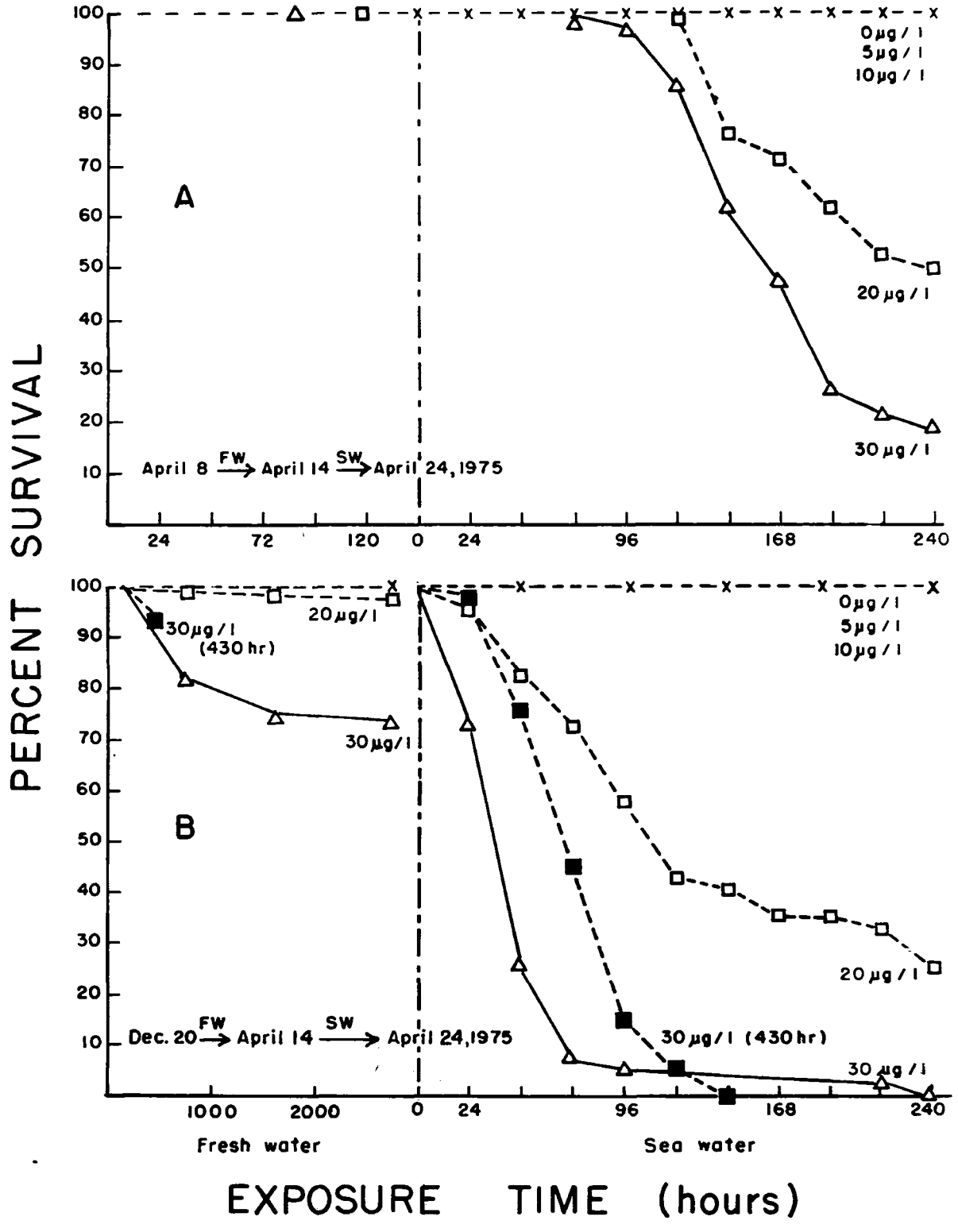

Figure 13. Survival curves of yearling coho salmon during exposure to copper in fresh water and subsequent survival when transferred to sea water. A. represents exposure to copper for 144 h; B. exposure to copper for 2760 h (also includes coho exposed to 30 µg/liter for 430 h prior to seawater test).

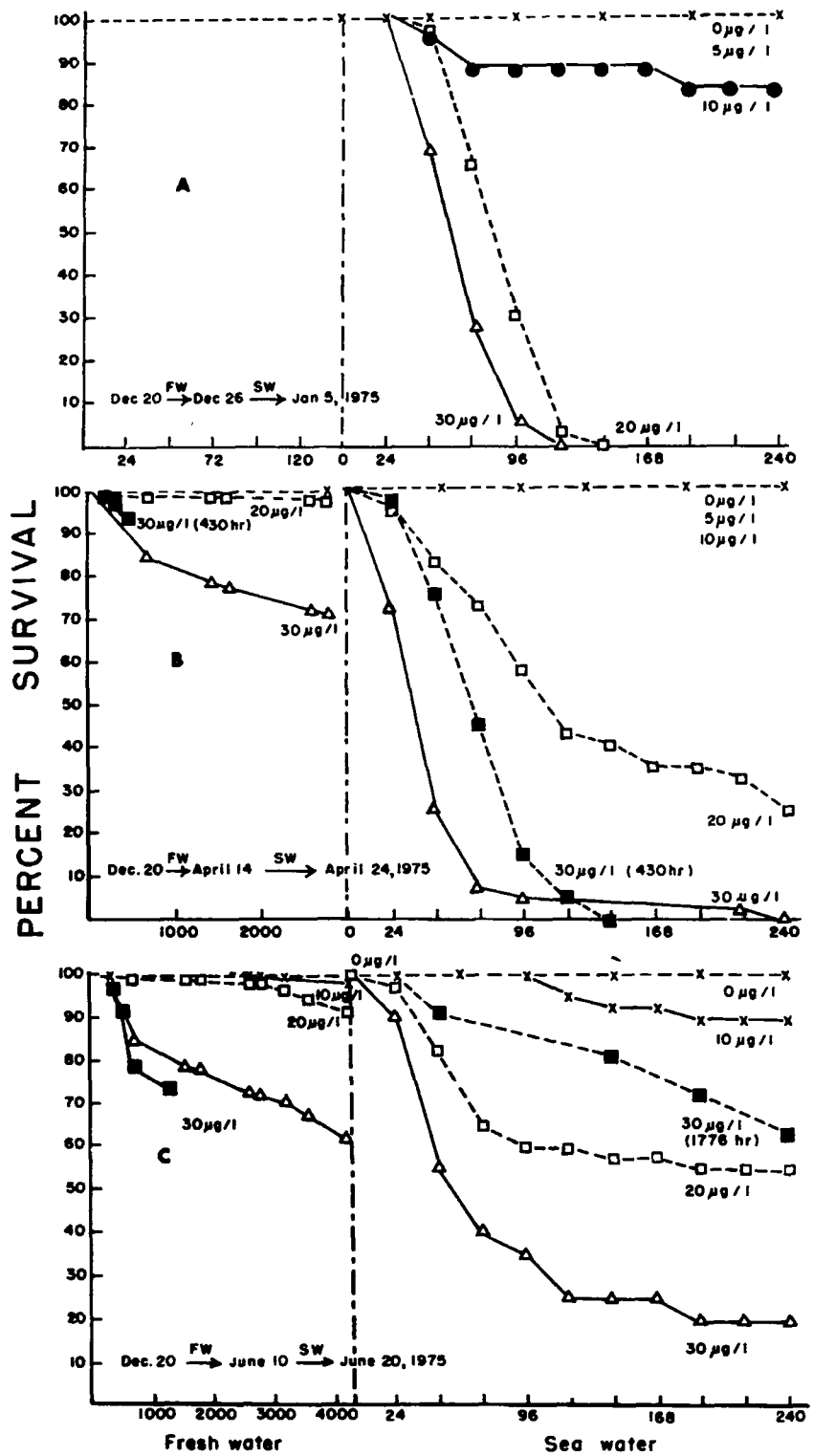

Figure 14. Survival curves of yearling coho salmon exposed to copper in fresh water and their subsequent survival upon transfer to sea water. A. represents exposure for 144 h; B. exposure for 2760 h; and C. exposure for 4128 h. B. and C. contain coho exposed to 30 ug/liter copper for 430 and 1776 h, respectively.

Table 9. Comparison of survival of fish exposed to natural and artificial sea water following 147 and 172 days of copper exposure in fresh water

Nominal concentration µg/liter Cu	Percent survival for 240 hours of exposure *a/*		
	Natural sea water	Artificial sea water A *b/*	B *c/*
A. 3528 h of copper exposure (147 d) May 16-26, 1975			
0	100.0 (21)	100.0 (21)	
5	92.5 (40)	95.2 (21)	94.8 (58)
20	47.5 (41)	45.2 (42)	50.0 (16)
30	4.4 (45)	2.3 (44)	
B. 4128 h of copper exposure (172 d) June 10-20, 1975			
0	100.0 (20)	100.0 (20)	100.0 (20)
5	92.3 (39)	100.0 (20)	
20	55.0 (40)	28.6 (42)	60.0 (20)
30	20.0 (20)	39.4 (33)	10.0 (20)

a/ Number of fish tested in parenthesis.

b/ LaRoche, G., R. Eisler, and C. M. Tarzwell. 1970. Bioassay procedures for oil and oil dispersant toxicity evaluation. J. Water. Poll. Cont. Fed. 42(11):1982-1989.

c/ Rila Marine Mix. Rila Products, P. O. Box 114, Teaneck, N. J. 07666.

Effect of periods of rest following copper exposure on survival in sea water

Cu-exposed yearling coho held in clean fresh water for 5 days prior to their transfer to sea water showed better survival (Table 10) than those transferred immediately to sea water following toxicant exposure. The survival in sea water of all static Cu-exposed groups was higher in rested fish than groups transferred to sea water immediately after toxicant exposure (Table 10). The increased survival may in part be accounted for by a two to fivefold increase in gill ATPase activity which occurred during the 5-d recovery period in clean fresh water.

Coho juveniles given a 5-d rest following toxicant exposure of 30 µg/liter Cu in the flowing system showed only a 10% mortality in sea water (Table 10) compared to 65% for those transferred immediately to sea water.

Table 10. Survival and gill ATPase activity of juvenile coho salmon exposed to copper for 144 h and subsequent survival in sea water with and without recovery periods

Nominal concentration µg/liter Cu	Measured concentration ± SD µg/liter Cu	Percent survival in copper	ATPase activity a/ (fresh water)	Percent survival b/ Fresh water	Percent survival b/ Sea water
A. Static toxicant exposure c/					
1. Direct transfer (April 16-May 6 1974)					
0	15.7 ± 3.4	100	25.5 (4)	--	100 (16)
10	16.2 ± 2.4	100	9.8 (3)	--	59 (17)
30	42.8 ± 4.1	100	6.5 (3)	--	6 (17)
60	75.1 ± 3.9	30	4.0 (2)	--	0 (4)
2. Five day recovery (April 25-May 21, 1974)					
0	13.9 ± 3.1	100	32.7 (3)	100 (6)	100 (10)
10	23.5 ± 3.9	100	20.3 (3)	100 (6)	90 (10)
30	44.1 ± 3.1	95	15.4 (3)	83 (6)	10 (10)
50	65.4 ± 2.7	75	19.3 (3)	--	45 (11)
B. Flowing toxicant exposure d/					
1. Direct transfer (June 5-26, 1974)					
0	1.8 ± 1.0	100	24.0 (5)	--	100 (40)
10	9.9 ± 2.7	100	18.2 (6)	--	97.5 (40)
30	31.3 ± 4.0	100	8.8 (6)	--	35.0 (40)
2. Five day recovery (June 5-July 1, 1974)					
0	1.8 ± 1.0	100	--	--	100 (20)
10	9.9 ± 2.7	100	--	100 (20)	100 (20)
30	31.3 ± 4.0	100	--	100 (20)	90 (20)

a/ *Gill microsomal Na^+, K^+-activated ATPase; µmoles ATP hydrolyzed/mg protein/h; sample size in parenthesis.*

b/ *Three hundred thirty-six to three hundred and sixty h seawater exposure; sample size in parenthesis.*

c/ *Twenty fish exposed per concentration.*

d/ *Originally placed 225-250 fish in each exposure tank.*

As the period of Cu exposure was considerably greater in 1975 than in 1974 it was important to again check survival in sea water following periods of rest or reduced toxicant exposure. The seawater survival of fish previously exposed for 1600 h to 20 and 30 µg/liter Cu was 12 and 0%, respectively (Table 11). When the fish were given a 5-d recovery or reduced Cu concentration exposure prior to challenge with sea water the survival of the 20 and 30 µg/liter Cu groups improved. However, the survival in sea water was lower than observed in 1974. This reduced survival may be a result of: (a) the poor condition of the fish following 1600 h of Cu exposure; (b) the coho had not reached smolt condition and the enzyme activity was still low making it more difficult for them to adapt to the osmotic stress; and (c) combination of the above plus other unknown factors important in adjustment to sea water. In the coho given a 15-d recovery prior to transfer to sea water the survival was almost equivalent to the control group (Table 11). Groups that received an exposure to reduced Cu concentration for 5 days and then 10 days of rest in fresh water prior to seawater challenge, incurred a mortality about 20-25% greater than the control fish (Table 11).

Coho given a 5-d recovery following 144-h exposure to 30 µg/liter Cu showed improved survival in sea water over the group challenged directly with sea water (Table 11). When the rest period was extended to 15 days the sea water survival improved threefold and was comparable to the control fish (Table 11). The reason for the longer recovery time required to achieve survival equivalent to the control groups in the 1975 tests is not known but might be related to lower ATPase levels at this time of year compared to fish tested in June 1974.

Coho yearlings given a 10 or 15 d-rest in fresh water following exposure to Cu for 172 d (4128 h) survived better in sea water than did fish challenged immediately or given a 5 d rest (Table 12). The lower survival of 20 µg/liter Cu following 5 days of rest is unknown but fish chosen for this seawater test might have been in poorer condition than others in the population. Lower seawater survival was also noted in the groups (20 and 30 µg/liter Cu) that were given a 10 d rest prior to testing. Coho held in subtanks for 5 days (12.3 C) and then transferred to test tanks but held another 5 days in static fresh water (10 C) prior to seawater exposure showed lower survival than fish rested in the test tanks (10 C) for the entire 10 days prior to seawater tests. The extra handling, different temperature and the low number of fish tested may account for the 20% differences in survival.

Coho given a 5 d rest in fresh water or acclimated to sea water of 20 °/oo after long exposure to Cu showed better survival when transferred to sea water (30 °/oo) than a group transferred immediately (Table 13). Fish from a group exposed to 20 µg/liter Cu showed nearly equal survival in sea water (30 °/oo) after being given either a 5-d rest in fresh water or in sea water of 20 °/oo. Fish from the 30 µg/liter Cu group, however, had better survival when rested in 20 °/oo sea water (Table 13). Similarly the coho exposed for 670 h to 30 µg/liter Cu showed almost a threefold better survival than those exposed for 3000 h. The exposure to 20 °/oo sea water should have been less stressful than fresh water to the fish as this salinity is closer to being isomotic with the blood and may have resulted in the better survival observed of the 30 µg/liter Cu groups.

Table 11. Survival of yearling coho salmon in sea water following exposure to copper for 1600 h or 144 h with or without recovery periods (February 25-April 6, 1975)

Nominal concentration µg/liter Cu	Rest period concentration µg/liter Cu	Percent survival in sea water a/
I. 1600 h of copper exposure		
A. Direct transfer to sea water		
0	--	100.0 (41)
20	--	11.6 (43)
30	--	0.0 (43)
B. Five-day rest prior to seawater transfer		
20	0	54.5 (33)
20	10	54.5 (33)
30	0	7.1 (42)
30	10	4.9 (41)
C. Fifteen-day rest prior to seawater transfer		
20	0	100.0 (15)
20	10 - 5 d, 0 - 10 d	78.6 (15)
30	0	95.3 (22)
30	10 - 5 d, 0 - 10 d	77.3 (22)
II. 144 h of copper exposure		
A. Direct transfer to sea water		
0	--	100.0 (42)
30	--	7.3 (41)
B. Five-day rest prior to seawater transfer		
30	0	30.0 (20)
30	10	25.0 (20)
C. Fifteen-day rest prior to seawater transfer		
30	0	100.0 (9)
30	10 5 d, 0 10 d	90.0 (10)

a/ *Two hundred and forty h seawater exposure; sample size in parenthesis.*

Table 12. Survival of yearling coho salmon in sea water following exposure to copper for 172 days (4128 h) with or without recovery periods (June 10-July 2, 1975)

Nominal concentration µg/liter Cu	Rest period concentration µg/liter Cu	Percent survival in sea water a/
A. Direct transfer to sea water		
0	--	100.0 (20)
20	--	55.0 (40)
30	--	20.0 (20)
B. Five-day rest prior to seawater transfer		
20	0 @ 12°	38.7 (31)
30	0 @ 12°	34.5 (29)
C. Ten-day rest prior to seawater transer		
20	0 @ 10° b/	85.7 (30)
20	0 - 5 d @ 12°, 5 d @ 10°	61.5 (13)
30	0 - 5 d @ 12°, 5 d @ 10°	67.7 (12)
D. Fifteen-day rest prior to seawater transfer		
20	0 @ 12°	94.7 (19)
30	0 @ 12°	90.0 (20)
30	0 @ 12° c/	-- (8)

a/ *Two hundred and forty h seawater exposure; sample size in parenthesis.*
b/ *Two fish (6.7%) died during freshwater holding phase.*
c/ *Eight fish held for 25 d in fresh water without mortalities.*

Table 13. Effects of freshwater rest or seawater acclimation (20 °/oo salinity) on survival of yearling coho salmon in 30 °/oo sea water following 115-125 d exposure to copper in fresh water (April 14-May 9, 1975)

Nominal concentration µg/liter Cu	Rest media	Percent survival in sea water *a/*	Percent survival *b/*
I. 2760-h copper exposure (115 d)			
A. Direct transfer to sea water			
0	--	100.0	(40)
20	--	25.0	(40)
30	--	0.0	(40)
30 *c/* (430 h)	--	0.0	(40)
II. 3000-h copper exposure (125 d)			
B. Five days rest in fresh water or 20 °/oo sea water			
0	20 °/oo	100.0	100.0 (21)
20	20 °/oo	77.5	75.6 (41)
30	20 °/oo	33.3	16.7 (42)
30 *c/* (670 h)	20 °/oo	90.0	42.9 (21)
20	Fresh water	88.9	80.0 (20)
30	Fresh water	5.0	5.0 (20)

a/ Two hundred and forty h seawater exposure.
b/ Survival based on total number of fish used; some died during the rest period in fresh water (≤10%) and 20 °/oo sea water (@ 50% mortality in each of the 30 µg/liter groups); sample size in parenthesis.
c/ Hours of exposure to copper.

Effect of copper on osmotic and ionic properties of blood plasma

Exposure of yearling coho to Cu in fresh water rapidly affected their ability to maintain normal osmolality and chloride concentrations in blood plasma (Figure 15, Figure A-2, respectively). Although the osmolality of plasma from control fish decreased 7% in the first 24 h (probably due to handling) and remained depressed, decreases in plasma osmolality of coho exposed to 10 and 30 µg/liter Cu were even greater during the 144 h test (9 and 16%, respectively). After 144 h of exposure, analysis of variance showed that the difference in osmolality of plasma between control fish and fish exposed to 30 µg/liter Cu was highly significant ($P < .01$) but the reduction caused by 10 µg/liter Cu was not significant ($P > .05$). The effect of 30 µg/liter Cu in reducing plasma osmolality below that of control fish was highly significant after only 8 h of exposure.

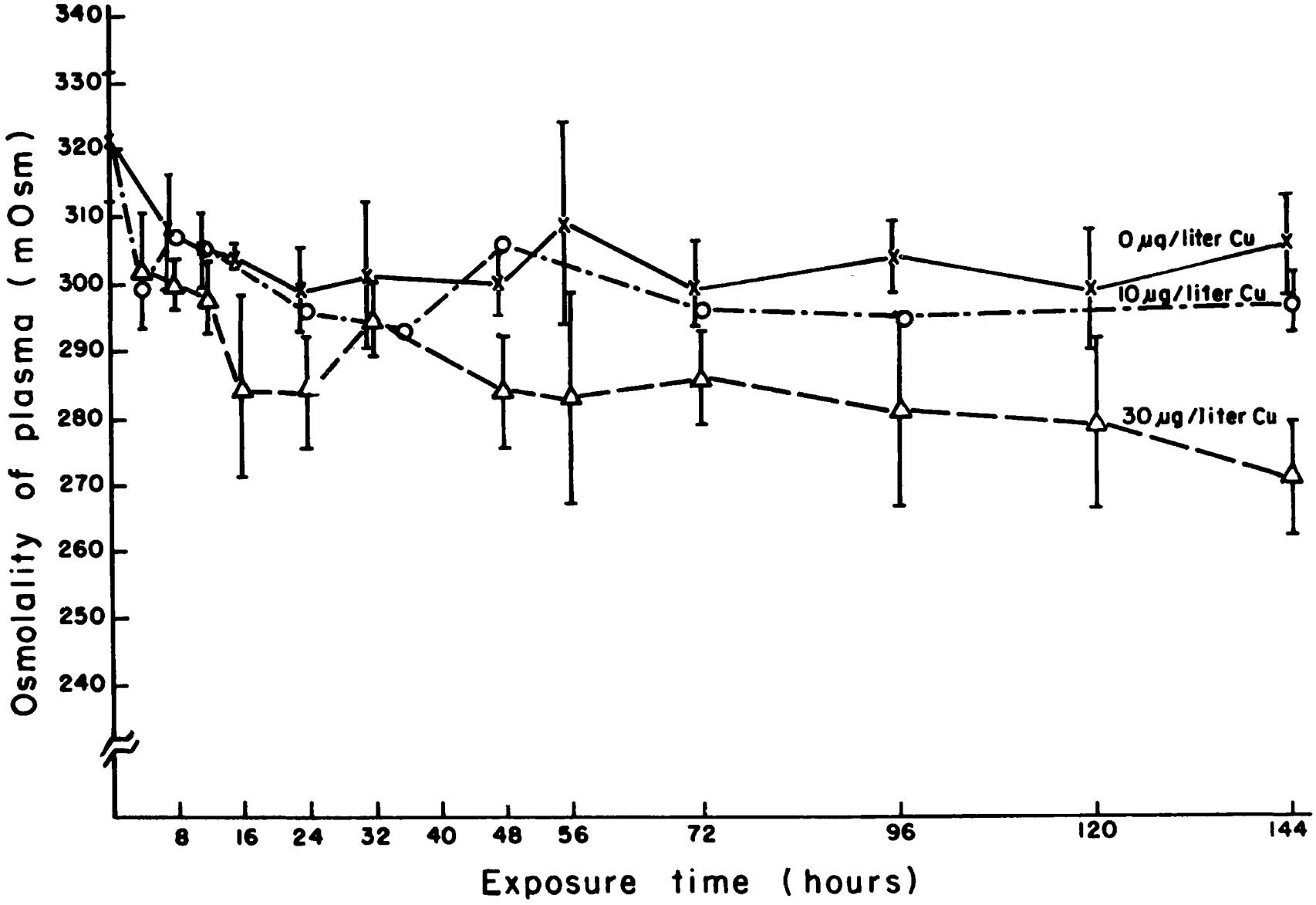

Figure 15. Effect of copper exposure in fresh water on the osmolality of the plasma of coho salmon during 144 h of exposure.

Chloride concentration (m Eq/liter) in the plasma followed a similar trend to osmolality (Figure A-2) which is supported by the highly significant correlation coefficient of 0.741 found when osmolality was regressed on Cl^- concentration for fish exposed to 30 µg/liter Cu. The correlation coefficient was least for control fish as a result of the small range of osmolality and Cl^- values but was still 0.550 ($P < .01$) for all groups combined.

A similar effect of sub-lethal levels on plasma osmolality and chloride concentration was observed in brook trout *(Salvelinus fontinalis)* by McKim, Christensen and Hunt (1970). They found 5 to 8% reductions in osmolality after 6 days (144 h) of exposure to 24 and 39 µg/liter Cu and 2 to 13% decreases after 21 days. Lewis and Lewis (1971) presented data showing that exposures of Cu and Zn to golden shiners and juvenile channel catfish for a maximum of 94 h reduced the plasma osmolality and was one of the probable causes of death.

The effects of 144-h exposure to Cu in fresh water on plasma osmolality and chloride concentration in coho smolts transferred to sea water are shown in Figure 16 and Figure A-3, respectively. Plasma osmolality was significantly lower for the coho exposed to 10 and 20 µg/liter Cu compared to control fish following the 144 h of toxicant exposure (zero time, Figure 16). Once in sea water, plasma osmolality of fish previously exposed to 20 µg/liter Cu increased rapidly so that after 24 h it was higher than that of control fish when tested at the 0.01 probability level. A mean maximum value of 432 mOsm (143% of the control) was reached after 120 h of exposure to sea water. Fish began to die in this 20 µg/liter Cu group after 98 h in sea water and the last death occurred before 193 h, similar to the pattern shown in Figure 13A for mortality in sea water of another group previously exposed to Cu for 144 h. The mean osmolality of plasma from survivors sampled after 240 h in sea water was only 111% of control fish sampled at the same time, correlated with the leveling-off of mortality.

Exposure of coho to 10 µg/liter Cu in fresh water for 144 h did not strongly affect their adaptation to sea water in the May 1975 test. Plasma osmolality and chloride concentrations were not significantly higher than control fish during the test, although they increased during the first 24 h while control values decreased (Figure 16 and Figure A-3). No deaths occurred nor were there deaths in the April and June seawater tolerance tests of smolts exposed to 10 µg/liter Cu for 144 h (Figure 11).

As observed for coho in fresh water, a close relationship between plasma osmolality and Cl^- concentration was also found in fish exposed to sea water. The correlation coefficient was again lowest for control fish and highest for the group exposed to the highest concentration of Cu (20 µg/liter). The correlation coefficients were 0.074 and 0.948, respectively, and 0.936 ($P < .01$) for all groups combined.

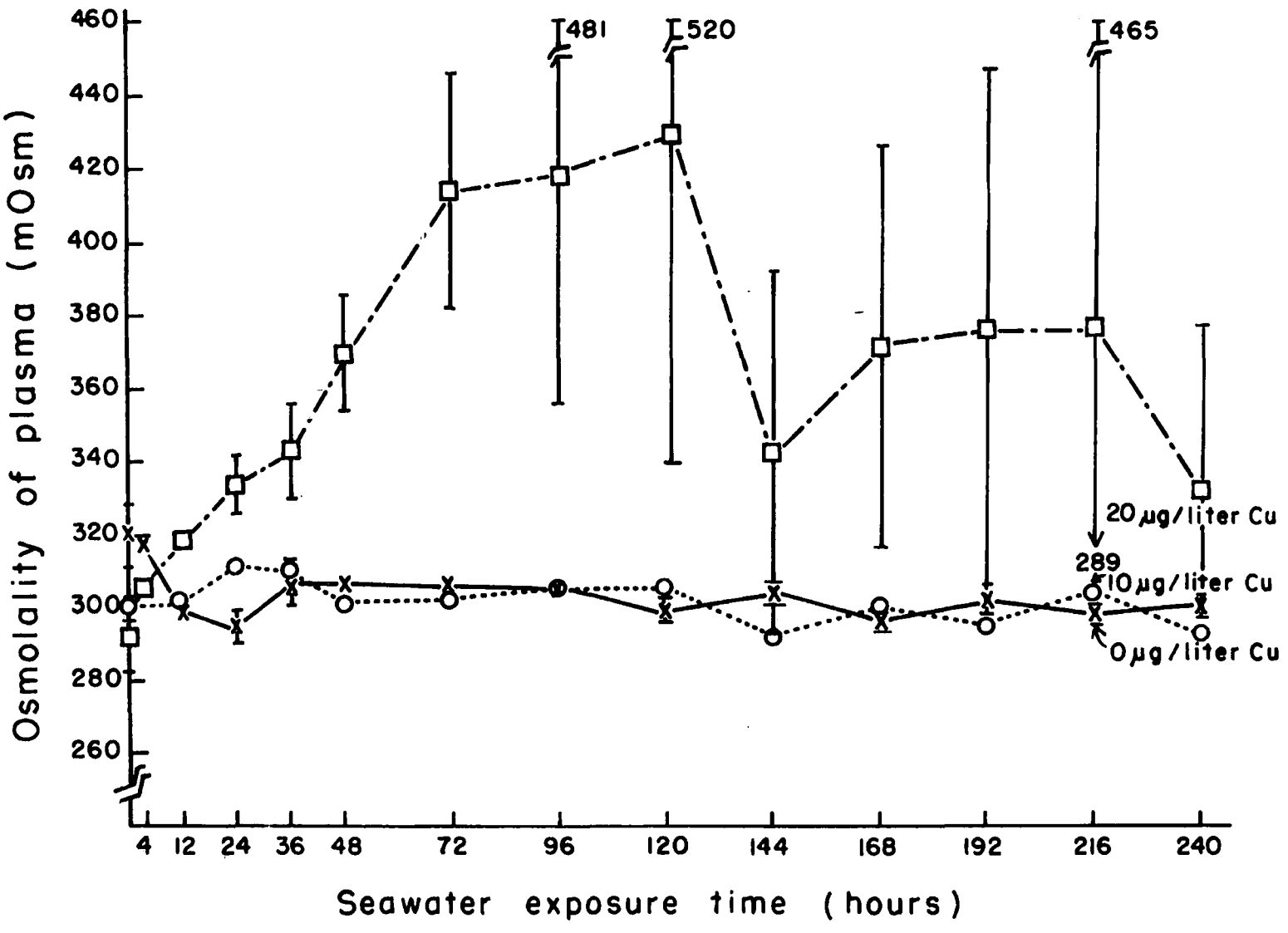

Figure 16. Effect of sea water exposure on the osmolality of the plasma of coho salmon previously exposed to copper (in fresh water) for 144 h.

The effects of chronic exposure to a sublethal concentration of Cu on the ability of coho to regulate osmotic and ionic properties of (Cl- and Na+) plasma during exposure to sea water were studied in July 1974 (Figure 17, Figure A-4 and Figure A-5). The fish were exposed for 792 h (33 days) to 0 and 20 µg/liter Cu before transfer to sea water. The sea water was only 28 °/oo during the first 24 h and ranged from 31.8 to 32.6 °/oo during the rest of the experiment.

The mean osmolality of plasma in control fish increased by 7% to 351 mOsm after 12 h and did not return to zero hour values until 36 h (Figure 17), probably a reflection of the poorer ability of these post-smolt coho to adapt to sea water as compared to smolting fish in the May test. Although the group exposed to 20 µg/liter Cu did not regulate osmolality, Cl-, or Na+ as well as the control fish, the values of osmolality and Cl- concentration did not reach the levels they did for fish exposed to 20 µg/liter Cu for 144 h (Figure 16, Figure A-3). This may indicate a lower tolerance to hyperosmotic or hyperionic blood condition in the chronically exposed fish. Deaths in sea water occurred as early as 24 h in fish chronically exposed to 20 µg/liter, at which time the mean osmotic and Cl- concentrations were well below the values observed in the 144-h exposed fish when they began dying after 98 h in sea water. The overall survival of the control and 20 µg/liter Cu groups of 100% and 76.7% compares equally with the survival of other chronically exposed groups (37 d) presented in Table 6.

These data show that exposure to Cu decreases the osmoregulatory ability of juvenile coho salmon in a concentration dependent manner leading to death in sea water. The data on effects of Cu upon the activity of gill microsomal Na^+, K^+-activated ATPase correlates with the osmoregulatory data. Suppression of this enzyme activity by Cu must be responsibile, in part, for the loss of osmoregulatory ability.

Effect of copper on downstream migration

Four releases of coho exposed to Cu since late December 1974 and two releases of coho exposed to Cu for 144 h were made into Crooked Creek, a tributary of the Alsea River. The releases were made during April, May and June 1975, during the normal period of coho smolt migration. The percent of released fish migrating to the trap was always greatest in control fish and percent migration was inversely related to Cu concentration (Table 14). Even the lowest concentration (5 µg/liter Cu), which had no measurable effect on gill ATPase activity or survival in sea water in the 1975 experiments, reduced the percentage of downstream migrants. The inhibitory effect of Cu on downstream migration was directly related to exposure time with acutely exposed fish having a higher percent migration than chronically exposed fish at any given Cu concentration (Table 14). Exposure to 30 µg/liter Cu for as little as 72 h caused a considerable reduction in migration as compared to the control fish (52% and 93%, respectively, Table 14).

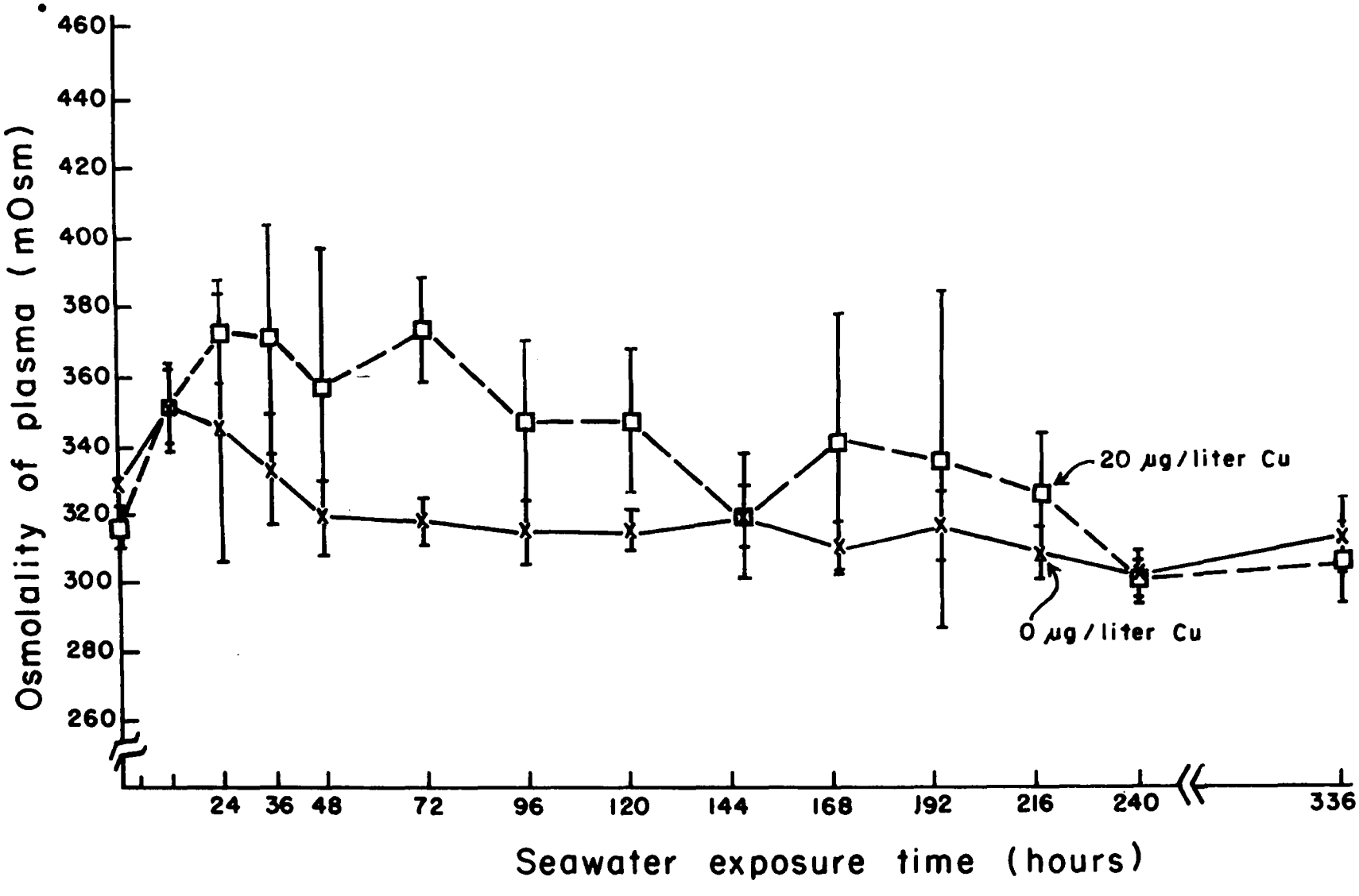

Figure 17. Effect of sea water exposure on the osmolality of the plasma of coho salmon chronically exposed to copper (in fresh water) for 792 h in July 1974.

Table 14. Percent migration through July 3, 1975, of coho salmon released into a small coastal stream following acute and chronic copper exposures

Nominal concentration µg/liter Cu	Release dates: April 8, 1975 *a/*	April 30, 1975 *b/*	May 14, 1975 *c/*	June 4, 1975 *d/*
A. Chronic exposures				
0	41.6	51.2	28.5	88.3
5	35.3	29.8	23.4	72.2
10	36.0	35.1	18.5	61.6
20	21.7	25.0	11.5	44.2
30	6.0	10.3	4.0	29.1
30 *e/*	10.9 (11)	32.1 (33)	8.9 (47)	60.3 (68)
B. Acute exposures (144 h or less) *f/*				
0 144	55.4	68.0	No release	92.7 *g/*
5 144	46.7	62.6		
10 144	52.4	58.6		
20 144	34.0	48.4		
30 *e/* (6)	14.6	17.5		65.9 *g/*
30 *e/* (4)				44.4 *g/*
30 *e/* (3)				52.0 *g/*

a/ One hundred and twenty to one hundred and fifty fish per concentration (108-d exposure)
b/ One hundred and twenty to one hundred and fifty fish per concentration (130-d exposure)
c/ One hundred and twenty to one hundred and seventy fish per concentration (145-d exposure).
d/ One hundred and twenty to one hundred and seventy fish per concentration (165-d exposure).
e/ Days of exposure to copper in parentheses.
f/ Ninety-one to one hundred and five fish per concentration (6-d exposure).
g/ Forty-five to fifty-five fish per concentration.

The migrational pattern of fish released in early June is shown in Figure 18. Similar trends of movement occurred in the acute and chronic exposure groups released earlier (Figure 19). The migration of the May 14 release was poorer than that observed for either earlier or later releases and is probably related to the low water flow and vandalism at the weir (Table 14). Most downstream migration occurred within the first 7 days of release; however, coho that had received 20 or 30 µg/liter Cu appeared to lag a little in their migration the first 2-3 days post-release but recovered rapidly and their movement was usually complete within 10 days. Only a few fish showed delayed migration and arrived at the weir after the 10th day following release (Figures 18, 19, Table A-9). A large percentage of the fish that did not migrate by early July, including control fish, are presumed to have died. In various migrational studies on this stream in prior years, electro-fishing above the weir following the normal migrational period (July) accounted for $\leq$5% of the fish that failed to migrate.

There is a possibility that the coho juveniles released in early June were in a better condition and nutritional state (because of resumed feeding in May than their counterparts released earlier. This improved condition was postulated as one of the factors important in the improved survival in sea water of the 30 µg/liter groups (Figures 12 and 14C, June test) and may also be the factor responsible for the observed increase in migrational tendency of the 30 µg/liter groups released June 4, 1975 (Table 14).

In conclusion, exposure of coho salmon yearlings to sublethal concentrations of Cu in fresh water resulted in significant mortality after these fish were placed in sea water, reduction of the migrational urge, and suppression of gill Na, K-ATPase thought to be important in sea water survival and an indicator of migrational readiness. However, caution is required in applying the results directly to field situations. The proportion of a total Cu concentration that is "biologically available" may vary among streams due to natural chelating agents, and there may be other antagonistic chemicals or factors that protect fish from Cu toxicity.

In the tests we conducted with freshwater recovery period of 5 to 15 days between Cu exposure and 30 °/oo seawater exposure, we noted better survival in the fish given a recovery than in Cu exposed fish that were transferred immediately to 30 °/oo sea water. Thus, fish that are affected by Cu pollutants in their headwater streams and have an extensive downstream migration before reaching the ocean may have a higher survival rate (provided fish migrate and have additional streams entering their waterways to dilute the pollutant) than fish subjected to Cu pollutants on short coastal streams. Also, Cu-induced seawater mortality would depend on rate of transition from fresh water to sea water. The exposure of sublethal levels of Cu for only several days just before the fish enter sea water could produce significant seawater mortality.

A stream may also have chemicals or other factors (such as temperature or disease) which increase the sensitivity of fish to Cu. Fish which survived and grew on a hand-fed diet in the laboratory might be incapable of foraging efficiently for natural foods. Therefore, we again recommend caution in

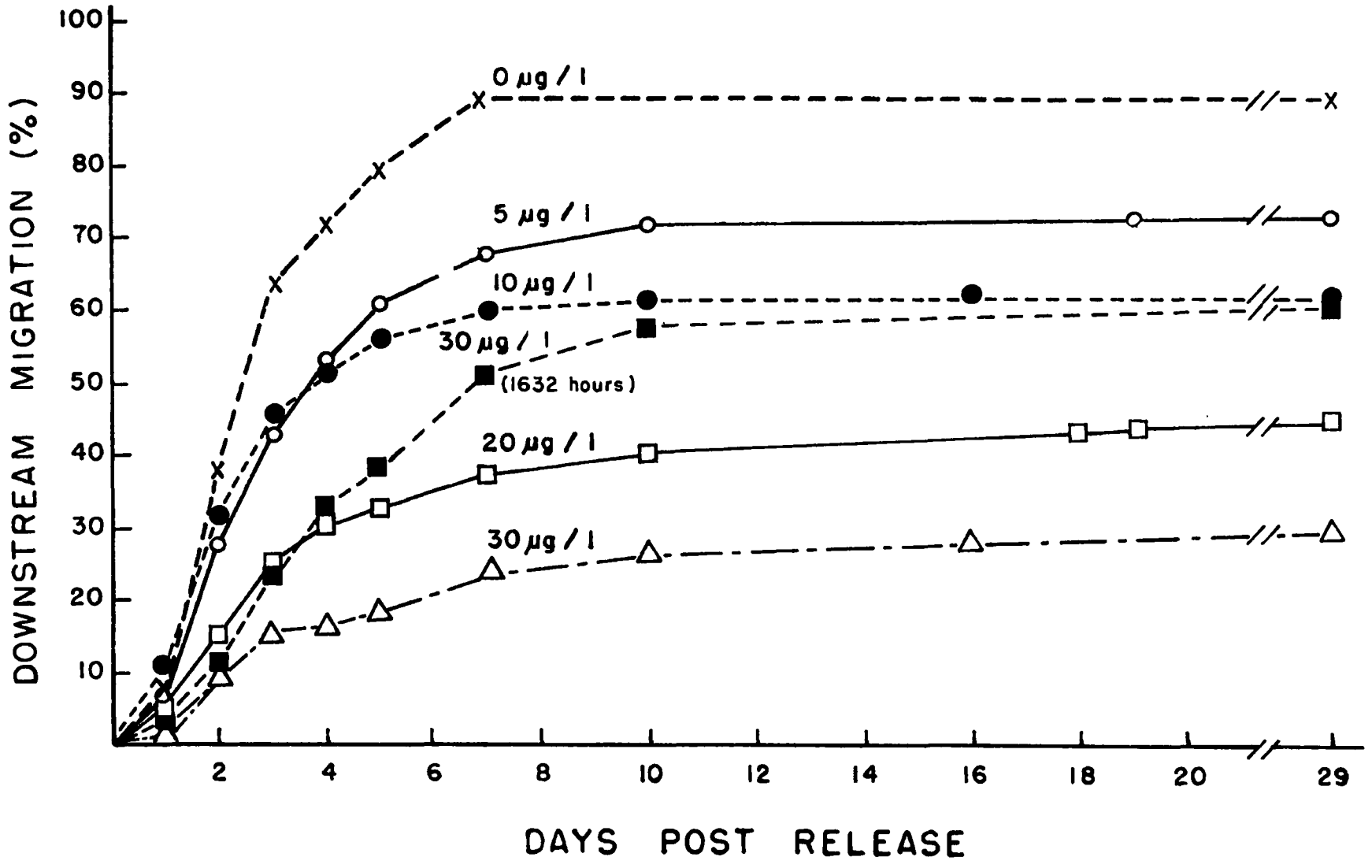

Figure 18. Influence of copper exposure for 3960 h (165 d) in fresh water on downstream migration of yearling coho salmon. Includes coho exposed to 30 µg/liter for 1632 h (68 d). Each line represents a release of 78-172 fish on June 4, 1975.

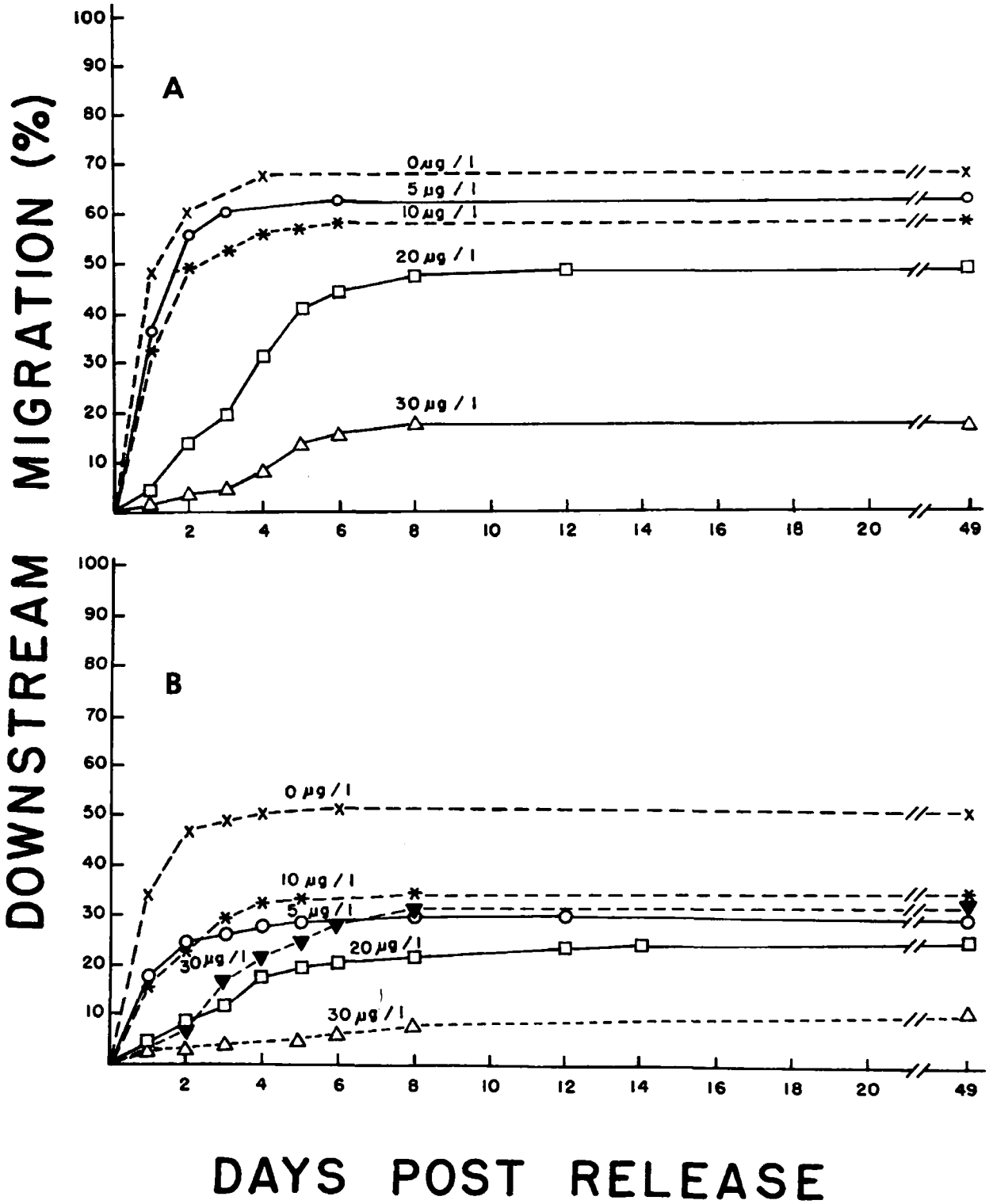

Figure 19. Influence of copper exposure in fresh water on downstream migration of yearling coho salmon released April 30, 1975. A. represents coho salmon exposed to copper for 144 h (6 d); B. represents copper exposure for 3120 h (130 d) and includes coho exposed to 30 µg/liter (▼) for 792 h (33 d).

applying laboratory results to natural situations because a given concentration of Cu in different streams could have somewhat different effects on migration and subsequent survival in sea water than the same concentration studied in the laboratory.

We conclude that studies such as this that take into consideration the life history of the fish are critical to the setting of water quality standards.

SECTION 6

REFERENCES

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APPENDICES

Table A-1. Chemical and physical characteristics of well water at the Oregon Department of Fish and Wildlife Laboratory a/, Corvallis, Oregon

Parameter	Units	10/18/73	3/1/74	3/15/74	5/10/74	6/20/74	8/8/74	11/7/74	2/4/75	6/3/75	Mean ± SD	Range	(N)
Cadmium	µg/liter	<1.0	--	--	<1.0	<1.0	<1.0	<1.0	<1.0	<3.0	0.6 b/ -	<1.0 - <3.0	(7)
Chromium	"	<1.0	--	--	1.0	3.0	<1.0	13.0	<2.0	<1.0	2.8 b/ -	<1.0 - 13.0	(7)
Cobalt		<1.0	--	--	<2.0	2.0	<1.0	<1.0	<1.0	<3.0	0.9 b/ -	<1.0 - <3.0	(7)
Copper		4.0	--	--	2.0	2.0	6.0	4.0	3.0	<3.0	3.2 ± 1.6 b/	2.0 - 6.0	(7)
Iron	"	48.0	--	--	22.0	20.0	16.0	110.0	28.0	21.0	38 ±34	16.0 -110.0	(7)
Lead	"	10.0	--	--	5.0	<5.0	<5.0	<1.0	8.0	6.0	4.9 b/ -	<1.0 - 10.0	(7)
Manganese	'	8.0	--	--	<1.0	1.0	1.0	5.0	--	<10.0	3.4 b/ -	<1.0 -<10.0	(6)
Mercury		<0.5	<0.5	--	12.0	<0.5	--	1.1	--	2.1	2.7 b/ -	<0.5 - 12.0	(6)
Nickel		1.0	--	--	<2.0	1.0	2.0	10.0	3.0	2.5	2.9 ± 3.2 b/	1.0 - 10.0	(7)
Zinc		4.0	--	--	5.0	1.0	4.0	1.0	2.0	< 3.0	2.6 ± 1.7 b/	1.0 - 5.0	(7)
Alkalinity as $CaCO_3$	mg/liter	76.0	--	61.0	--	66.0	71.3	65.0	71.5	73.5	69.0 ± 5.0	61.0 - 76.0	(7)
Calcium	"	21.0	24.0	27.0	22.0	16.3	17.0	24.0	--	12.6	20.0 ± 5.0	12.6 - 27.0	(8)
Chloride	"	6.0	6.0	6.0	6.0	7.0	7.0	7.7	--	7.0	7.0 ± 1.0	6.0 - 7.7	(8)
Dissolved oxygen	"	7.0	--	--	--	--	8.0	6.8	--	7.4	7.3 ± 0.5	6.8 - 8.0	(4)
Hardness as $CaCO_3$	"	101.0	85.0	95.0	93.0	95.0	100.0	99.0	92.0	96.0	95.0 ± 5.0	85.0 -101.0	(9)
Magnesium	"	12.0	13.0	14.0	11.0	10.7	10.4	18.0	--	10.2	12.0 ± 3.0	10.2 - 18.0	(8)
Nitrogen													
Free ammonia as N		<0.060	<0.040	<0.045	<0.090	<0.005	<0.085	<0.005	--	<0.005	0.022 - b/	<0.005- 0.090	(8)
Nitrate as N		3.1	8.6	7.5	7.8	6.8	6.72	6.1	--	--	6.7 ± 1.8	3.1 - 8.6	(7)
Nitrite as N	"	<0.001	<0.001	<0.001	0.004	<0.001	<0.001	<0.001	--	--	0.001 - b/	<0.001- 0.004	(7)
Potassium	"	0.9	1.1	1.0	0.95	1.0	1.0	1.1	--	1.0	1.0 ± 0.1	0.9 - 1.100	(8)
Sodium		6.9	6.1	8.7	6.6	7.2	6.4	10.0	--	8.7	7.6 ± 1.4	6.1 - 10.0	(8)
Solids													
Dissolved	'	173.0	174.0	180.0	203.0	176.0	192.0	--	--	--	183.0 ±12.0	173.0 -203.0	(6)
Suspended	'	<1.0	1.0	2.0	1.0	<1.0	2.0	--	--	--	1.2 - b/	<1.0 - 2.0	(6)
Sulfate		--	10.0	12.0	15.0	13.0	12.0	--	--	12.0	12.0 ± 2.0	10.0 - 15.0	(6)
pH		7.0	6.6	6.8	6.7	6.7	6.7	6.61	6.58	6.80	6.7 c/	6.61 - 7.0	(9)
Turbidity	JKSN	--	5.0	3.0	5.0	6.0	5.0	6.0	--	23.0	8.0 ± 7.0	3.0 - 23.0	(7)

a/ *Analysis run by Corvallis Environmental Research Laboratory.*
b/ *Values of <x were transformed to x/2 for calculations. Standard deviation was not calculated where more than one <x value was present.*
c/ *Median pH.*

Table A-2. Effect of copper in fresh water on the survival of yearling coho salmon (determination of 96-h LC50 values)

Nominal concentration µg/liter Cu	Measured concentration µg/liter Cu Mean ± SD	Range	n	Percent mortality (Range)
a) March 7-11, 1974 *a/*				
0	5.1 ± 1.4	(3.0- 7.0)	7	0
30	30.0 ± 4.3	(26.0- 34.5)	8	0
60	58.5 ± 4.7	(53.5- 67.8)	8	50 (40-60)
80	71.7 ± 5.7	(64.0- 78.8)	8	75 (60-90)
100	87.8 ± 2.9	(86.0- 92.0)	4	100
130	108.8 ± 5.0	(103.0-114.5)	4	100
b) March 20-24, 1974 *a/*				
0	11.7 ± 7.2	(2.4- 22.3)	6	0
20	27.4 ± 3.9	(21.7- 33.5)	8	0
30	35.2 ± 5.0	(28.5- 44.0)	8	0
50	50.9 ± 4.1	(45.5- 57.0)	8	5 (0-10)
60	61.0 ± 6.6	(53.3- 73.5)	8	15 (10-20)
80	80.5 ± 6.5	(71.3- 90.8)	8	75 (70-80)
c) November 20-24, 1974 *b/*				
0	7.8 ± 2.3	(5.0- 12.0)	8	0
30	36.3 ± 4.5	(32.2- 45.5)	8	0
50	56.7 ± 4.6	(52.1- 65.5)	8	5 (0-10)
60	62.7 ± 3.0	(58.0- 67.1)	8	20
70	75.5 ± 8.0	(62.5- 85.0)	8	35 (30-40)
80	81.8 ± 6.3	(76.0- 96.5)	8	75 (70-80)
100	102.9 ± 6.7	(96.0-112.0)	4	100
d/ May 27-31, 1975 *b/*				
0	19.1 ± 11.5	(4.0- 35.4)	8	0
45	54.3 ± 3.6	(49.2- 58.4)	8	20 (10-30)
50 *c/*	58.6 ± 14.6	(39.3- 69.8)	6	45 (40-50)
55	57.5 ± 2.6	(53.6- 60.4)	8	45 (40-50)
65	65.6 ± 2.2	(60.8- 67.2)	8	63.2 (40-88.9)
70 *c/*	65.0 ± 10.1	(51.6- 76.8)	6	95.0 (90-100)
80	81.2 ± 1.5	(79.8- 83.4)	4	100
100 *c/*	83.3 ± 7.5	(76.8- 93.4)	4	100

a/ Test run on coho of 1972 brood year.
b/ Test run on coho of 1973 brood year.
c/ Test run May 5, 1975; contamination of control with copper.

Table A-3. Effect of zinc in fresh water on the survival of yearling coho salmon (determination of 96-h and 144-h LC50 values)

Nominal concentration µg/liter Zn	Measured concentration µg/liter Cu Mean ± SD	(Range)	n	Percent mortality Mean	Range
96-h exposures					
a) April 16-20, 1974					
0	48 ± 31	(10- 109)	7	0	
1000	933 ± 56	(859-1040)	7	0	
2500	2426 ± 186	(2150-2650)	7	10	
4000	3761 ± 253	(3480-4025)	7	15	(10-20)
5000	4837 ± 468	(4440-5750)	7	25	(20-30)
6000	5673 ± 231	(5465-6000)	4	75	(70-80)
b) May 9-13, 1974					
0	38 ± 34	(9- 94)	6	0	
4000	4286 ± 170	(4100-4550)	7	30	(10-50)
5000	5243 ± 169	(5050-5500)	7	75	(70-80)
6000	6158 ± 172	(5900-6350)	6	100	
c) June 5-9, 1974					
0	12 ± 4	(6- 17)	6	0	
1000	1230 ± 66	(1170-1340)	5	0	
2500	2637 ± 47	(2570-2695)	5	0	
4000	4051 ± 63	(3990-4140)	5	0	
5000	4953 ± 260	(4465-5125)	6	60	
144-h exposures (continuation of 96-h run)					
a) March 20-26, 1974					
0	29 ± 17	(8- 56)	10	0	
100	115 ± 12	(99- 139)	12	0	
300	299 ± 9	(286- 314)	11	0	
600	555 ± 20	(530- 605)	12	0	
1000	924 ± 32	(905-1018)	11	0	
2000	1772 ± 46	(1718-1870)	12	0	
2500	2271 ± 57	(2168-2361)	12	0	
b) April 16-22, 1974					
0	37 ± 29	(6- 109)	11	0	
1000	937 ± 46	(859-1040)	11	5	(0-10)
2500	2495 ± 178	(2150-2700)	11	15	(10-20)
4000	3803 ± 207	(3480-4025)	11	25	(10-40)
5000	4833 ± 369	(4440-5750)	11	35	(30-40)
6000	5611 ± 172	(5465-6000)	8	90	(80-100)
c) May 9-15 1974					
0	33 ± 30	(9- 94)	8	0	
4000	4261 ± 156	(4100-4550)	9	55	(30-80)
5000	5245 ± 144	(5050-5500)	10	95	(90-100)
6000	6158 ± 172	(5900-6350)	6	100	
d) June 5-11, 1974					
0	18 ± 13	(6- 40)	8	0	
1000	1214 ± 62	(1150-1340)	7	0	
2500	2620 ± 56	(2530-2695)	7	0	
4000	4062 ± 76	(3990-4180)	7	45	(40-50)
5000	4980 ± 23	(4465-5230)	9	80	

Table A-4. Effect of copper exposure on average length-weight and coefficient of condition

Date of sample	Days of Cu exposure	Nominal concentration µg/liter Cu	Fork length cm	± SE	Weight g	± SE	Coefficient of condition K	± SE	n
12/18/74	0	0	13.8	0.160	31.0	1.197	1.156	0.00990	50
	0	5	14.0	0.196	32.3	1.342	1.163	0.02551	50
	0	10	13.7	0.162	30.7	1.147	1.158	0.00904	50
	0	20	14.1	0.153	32.3	1.150	1.145	0.01456	50
	0	30	14.1	0.172	33.8	1.286	1.188	0.01759	50
1/17/75	28	0	14.7	0.274	39.6	2.316	1.196	0.01102	39
	28	5	14.3	0.232	34.4	1.741	1.128	0.01116	40
	28	10	13.7	0.165	28.8	1.110	1.105	0.01194	40
	28	20	13.8	0.197	28.0	1.301	1.039	0.01103	40
	28	30	13.5	0.184	25.0	1.176	1.007	0.00975	30
2/17/75	58	0	15.2	0.224	41.3	1.810	1.155	0.01217	30
	58	5	15.0	0.257	38.4	2.080	1.109	0.01156	31
	58	10	14.4	0.236	35.1	2.276	1.129	0.02029	30
	58	20	14.1	0.228	29.5	1.587	1.008	0.01330	32
	58	30	13.0	0.183	21.1	1.108	0.937	0.01593	21
3/25/75	0	30	16.3	0.127	47.8	1.200	1.084	0.00574	120
4/17/75	108	0	16.1	0.186	46.3	1.670	1.091	0.00735	60
	108	5	15.4	0.145	38.5	1.205	1.036	0.00720	80
	108	10	15.1	0.170	36.9	1.451	1.027	0.00989	80
	108	20	14.8	0.183	33.8	1.460	1.012	0.01647	60
	108	30	13.5	0.171	21.0	0.922	0.825	0.01126	40
	11	30	16.4	0.237	47.4	2.247	1.041	0.01075	40
4/29/75	130	0	16.6	0.205	49.3	1.895	1.058	0.00770	60
	130	5	15.9	0.188	41.6	1.625	0.992	0.00990	80
	130	10	15.2	0.164	36.1	1.396	0.992	0.01059	80
	130	20	14.5	0.203	28.9	1.471	0.890	0.01449	60
	130	30	13.8	0.208	22.9	1.509	0.829	0.01952	40
	33	30	16.0	0.203	37.7	1.656	0.905	0.00823	40
5/13/75	144	0	17.0	0.170	54.3	1.766	1.084	0.00787	60
	144	5	16.4	0.206	46.3	1.783	1.009	0.00936	80
	144	10	15.8	0.173	41.2	1.728	0.993	0.01182	80
	144	20	14.6	0.194	30.3	1.785	0.907	0.01913	60
	144	30	13.5	0.155	20.0	0.975	0.782	0.01785	40
	47	30	16.0	0.234	37.5	1.781	0.889	0.00697	40
6/3/75	165	0	17.6	0.187	60.4	2.179	1.080	0.00776	60
	165	5	17.3	0.225	55.8	2.539	1.026	0.01091	80
	165	10	15.8	0.213	40.7	2.169	0.956	0.01495	80
	165	20	14.9	0.201	33.7	1.824	0.956	0.01955	60
	165	30	14.4	0.216	26.8	1.747	0.851	0.02472	40
	68	30	16.7	0.253	42.5	2.188	0.900	0.02068	30

Table A-5. Activity of microsomal Na^+, K^+-activated ATPase in gills of coho following exposure to copper for 144 h and subsequent seawater exposure

Concentration µg/liter Cu		Exposure time		ATPase activity in toxicant			Exposure time		ATPase activity in sea water		
Nominal	Measured	in copper	Date	Mean ± SD	Range	n	in sea water	Date	Mean ± SD	Range	n
0	13.7	144	3/26/74	12.9 ± 5.2	9.3 - 20.3	4	312	4/8/74	51.4 ± 14.7	26.7 - 72.2	5
20	27.6	144	3/26/74	7.1 ± 1.0	6.3 - 8.2	4	312	4/8/74	63.4		1
30	34.7	144	3/26/74	5.6 ± 1.1	5.1 - 7.1	4	No survivors				
50	51.8	144	3/26/74	5.0 ± 0.7	4.1 - 5.7	4	No survivors				
0	15.7	144	4/22/74	25.5 ± 5.7	18.2 - 32.2	4	336	5/6/74	57.3 ± 6.3	52.0 - 65.3	4
5	16.2	144	4/22/74	16.3 ± 4.6	11.4 - 20.6	3	336	5/6/74	58.6 ± 6.8	53.5 - 68.4	4
10	22.1	144	4/22/74	9.8 ± 2.1	7.9 - 12.0	3	336	5/6/74	57.8 ± 9.6	44.9 - 66.7	4
20	32.3	144	4/22/74	6.6 ± 2.1	4.6 - 8.8	3	336	5/6/74	53.7 ± 1.1	52.9 - 54.9	3
30	42.8	144	4/22/74	6.5 ± 1.3	5.7 - 8.0	3	336	5/6/74	70.2		1
60	75.1	144	4/22/74	4.0	3.2 - 4.8	2	No survivors				
0	7.9	144	5/15/74	48.0 ± 7.3	40.0 - 57.3	4					
5	11.5	144	5/15/74	23.9 ± 4.8	16.7 - 26.5	4					
10	15.5	144	5/15/74	14.4 ± 6.9	9.6 - 24.6	4					
20	23.1	144	5/15/74	10.3 ± 2.0	8.1 - 12.9	4					
60	62.9	144	5/15/74	6.9 ± 0.3	6.7 - 7.3	3					
0	14.4	144	6/11/74	24.8 ± 7.9	16.2 - 31.8	3					
5	16.3	144	6/11/74	15.6 ± 2.8	12.6 - 18.1	3					
10	22.0	144	6/11/74	11.1 ± 2.2	8.6 - 12.5	3					
20	28.0	144	6/11/74	7.2 ± 1.5	5.5 - 8.5	3					
30	37.4	144	6/11/74	6.8 ± 0.6	6.3 - 7.4	3					
50	52.7	144	6/11/74	5.8 ± 1.3	4.6 - 7.2	3					

Table A-6. Activity of microsomal Na^+, K^+-activated ATPase in gills of coho exposed to copper for various lengths of exposure in 1974

Concentration µg/liter Cu		Exposure time		ATPase activity in toxicant		
Nominal	Measured	in copper	Date	Mean ± SD	Range	n
0	1.8	144	6/11/74	24.0 ± 5.8	19.0 - 32.5	5
5	8.0	144	6/11/74	26.5 ± 3.8	20.1 - 31.4	6
10	9.9	144	6/11/74	18.2 ± 6.4	11.2 - 29.9	6
20	18.2	144	6/11/74	11.4 ± 1.3	10.0 - 13.6	6
30	31.3	144	6/11/74	8.8 ± 1.6	6.4 - 10.7	6
0	1.4	645	6/11/74	32.3 ± 8.2	22.7 - 47.1	6
5	7.7	645	6/11/74	36.0 ± 8.8	17.2 - 45.6	8
10	10.6	645	6/11/74	25.9 ± 5.2	17.9 - 33.4	8
20	20.3	645	6/11/74	17.5 ± 2.9	13.1 - 22.7	8
30	31.5	645	6/11/74	14.5 ± 4.5	8.0 - 21.6	8
0	3.6	144	7/22/74	26.1 ± 5.6	17.6 - 33.3	8
5	9.7	144	7/22/74	33.0 ± 9.7	25.0 - 58.2	10
10	13.2	144	7/22/74	18.8 ± 3.5	15.1 - 24.9	10
20	26.0	144	7/22/74	10.4 ± 2.3	7.2 - 13.2	10
30	33.9	144	7/22/74	7.5 ± 7.9	5.1 - 10.7	10

Table A-7. Activity of microsomal Na^+, K^+-activated ATPase in gills of coho exposed to copper for various time periods

Concentration µg/liter Cu		Exposure time		ATPase activity in toxicant		
Nominal	Measured	in copper (h)	Date	Mean ± SD	Range	n
0	3.0	744	1/20/75	29.9 ± 3.2	26.4 33.8	5
30	31.5	744	1/20/75	10.4 ± 4.3	6.3 - 15.7	4
0	3.1	1416	2/17/75	34.5 ± 9.5	23.4 - 46.1	6
30	31.7	1416	2/17/75	12.7 ± 1.9	10.6 - 15.6	5
0	2.8	1968	3/12/75	35.6 ± 17.2	17.8 58.8	4
30	31.5	1968	3/12/75	17.7 ± 5.4	12.4 - 23.6	4
0	2.6	2640	4/9/75	44.2 ± 11.7	32.3 - 59.1	6
30	31.6	312	4/9/75	10.6 ± 1.9	8.6 - 13.2	5
0	2.6	144	4/25/75	53.3 ± 12.4	42.9 69.6	4
30	31.6	144	4/25/75	12.1 ± 3.6	9.4 - 17.2	4
0	2.6	3144	4/30/75	46.9 ± 5.4	41.9 - 56.0	5
30	31.6	816	4/30/75	14.2 ± 3.3	11.1 - 17.7	3
0	2.6	3648	5/21/75	41.6 ± 5.4	33.6 - 44.5	4
30	31.7	56	5/21/75	30.2 ± 13.5	13.5 42.7	4
30	31.7	96	5/21/75	17.2 ± 4.6	12.6 22.2	4
30	31.7	118	5/21/75	15.4 ± 1.5	14.1 17.6	4
0	2.6	3984	6/4/75	33.9 ± 9.5	22.9 45.9	5
30	31.7	1656	6/4/75	8.4 ± 2.4	6.4 11.1	3
0	Rearing tank		6/25/75	34.6	34.4 - 34.8	2
30	31.7		6/25/75	9.3 ± 0.8	8.6 - 10.1	3

Table A-8. Activity of microsomal Na^+, K^+-activated ATPase in gills of coho exposed to copper for various time periods in 1975

Concentration µg/liter Cu		Exposure time		ATPase activity in toxicant			
Nominal	Measured	in copper (h)	Date	Mean ± SD	Range		n
0	3.0	744	1/20/75	29.9 ± 3.2	26.4	33.8	5
10	11.6	744	1/20/75	35.7 ± 11.3	24.4	50.2	4
20	21.3	744	1/20/75	19.5 ± 3.5	16.8	24.6	4
30	31.5	744	1/20/75	10.4 ± 4.3	6.3	15.7	4
0	3.1	1416	2/17/75	34.5 ± 9.5	23.4	46.1	6
10	11.9	1416	2/17/75	30.2 ± 6.1	21.6	37.2	6
20	21.7	1416	2/17/75	15.1 ± 3.6	10.2	19.2	6
30	31.7	1416	2/17/75	12.7 ± 1.9	10.6	15.6	6
0	2.8	1968	3/12/75	35.6 ± 17.2	17.8	58.8	4
30	31.5	1968	3/12/75	17.7 ± 5.4	12.4	23.6	4
0	2.6	2232	3/23/75	32.8 ± 13.3	19.7	44.4	4
0	2.6	2640	4/9/75	44.2 ± 11.7	32.3	59.1	6
10	11.6	2640	4/9/75	24.0 ± 2.7	19.8	27.1	5
20	21.6	2640	4/9/75	16.1 ± 4.3	12.4	22.2	4
30	31.6	2640	4/9/75	10.4 ± 2.4	8.0	13.3	4
0	2.6	3144	4/30/75	46.9 ± 5.9	41.9	- 56.0	5
10	11.7	3144	4/30/75	41.3 ± 8.0	42.8	54.0	4
20	21.7	3144	4/30/74	17.5 ± 3.5	14.4	22.5	4
30	31.6	3144	4/30/75	13.0 ± 3.8	9.8	17.2	3
0	2.6	3648	5/21/75	41.6 ± 5.4	33.6	45.5	4
0	2.6	3816	5/28/75	36.2 ± 11.1	25.3	51.6	4
0	2.6	3984	6/4/75	33.9 ± 9.5	22.9	45.9	5
10	11.7	3984	6/4/75	29.0 ± 13.9	19.2	49.5	4
20	21.6	3984	6/4/75	15.3 ± 5.2	10.7	20.6	4
30	31.7	3984	6/4/75	8.1 ± 2.9	6.4	11.5	3

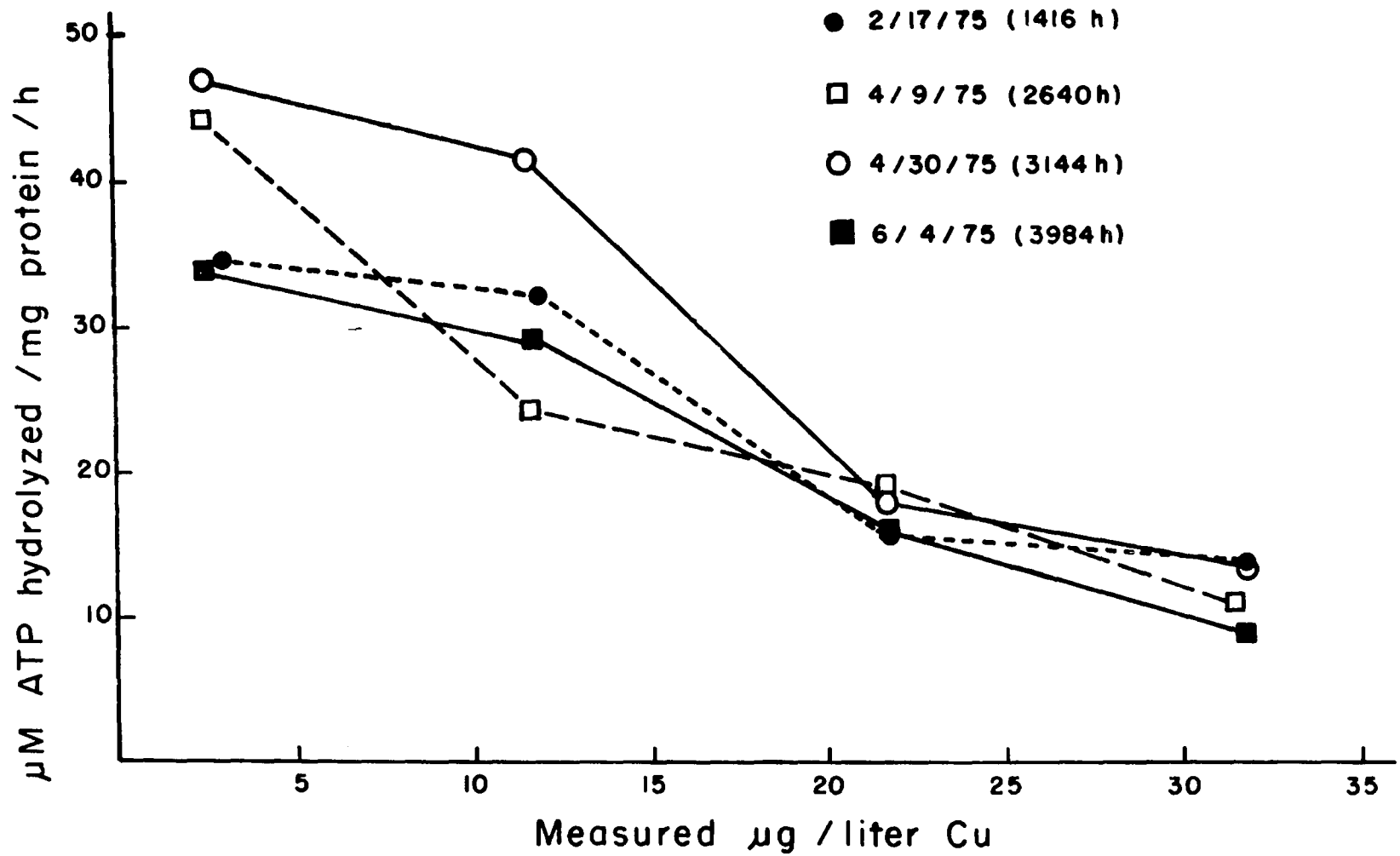

Figure A-1. Effect of copper exposure (in fresh water) on gill microsomal Na^+, K^+-activated ATPase activity of yearling coho salmon.

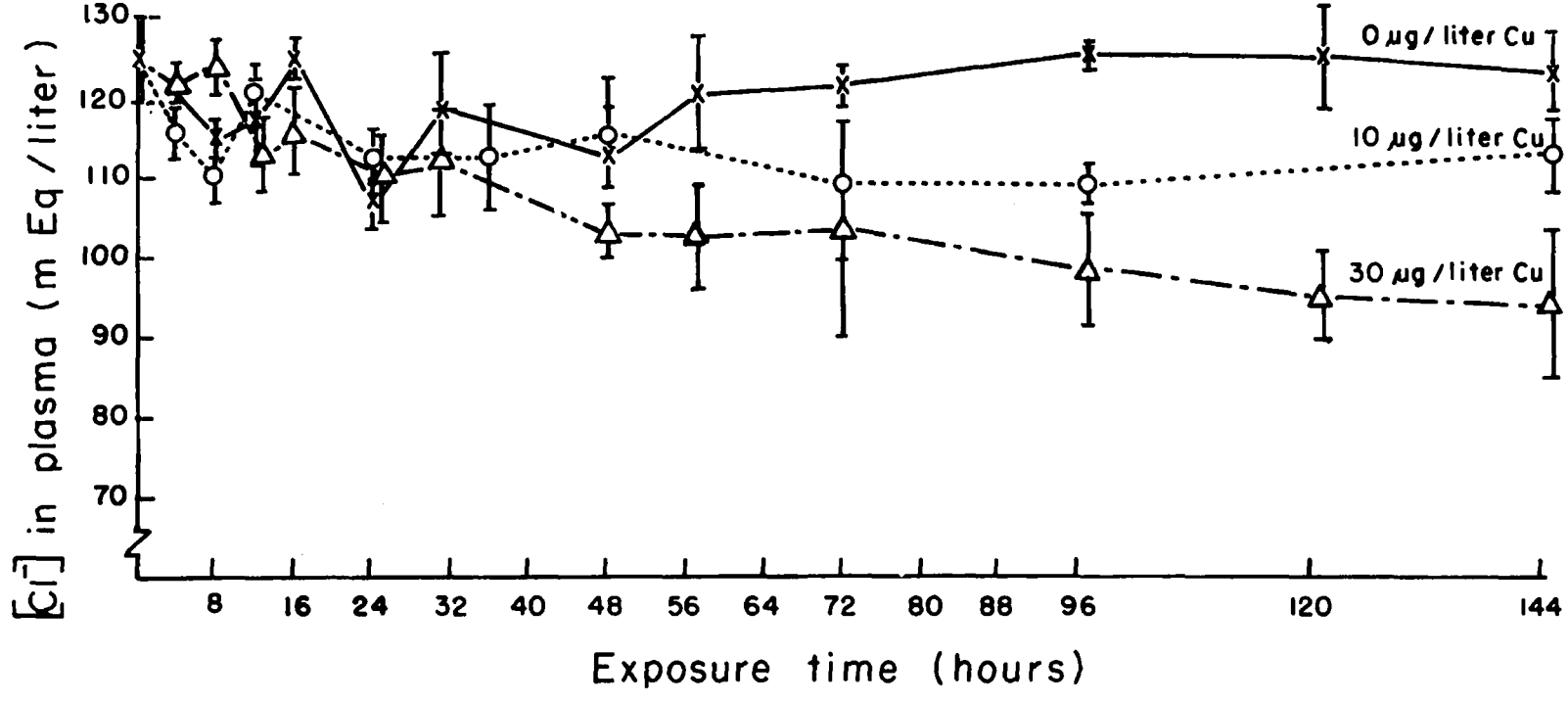

Figure A-2. Effect of copper exposure in fresh water on the chloride ion concentration of the plasma of coho salmon serially sampled during 144 h of exposure.

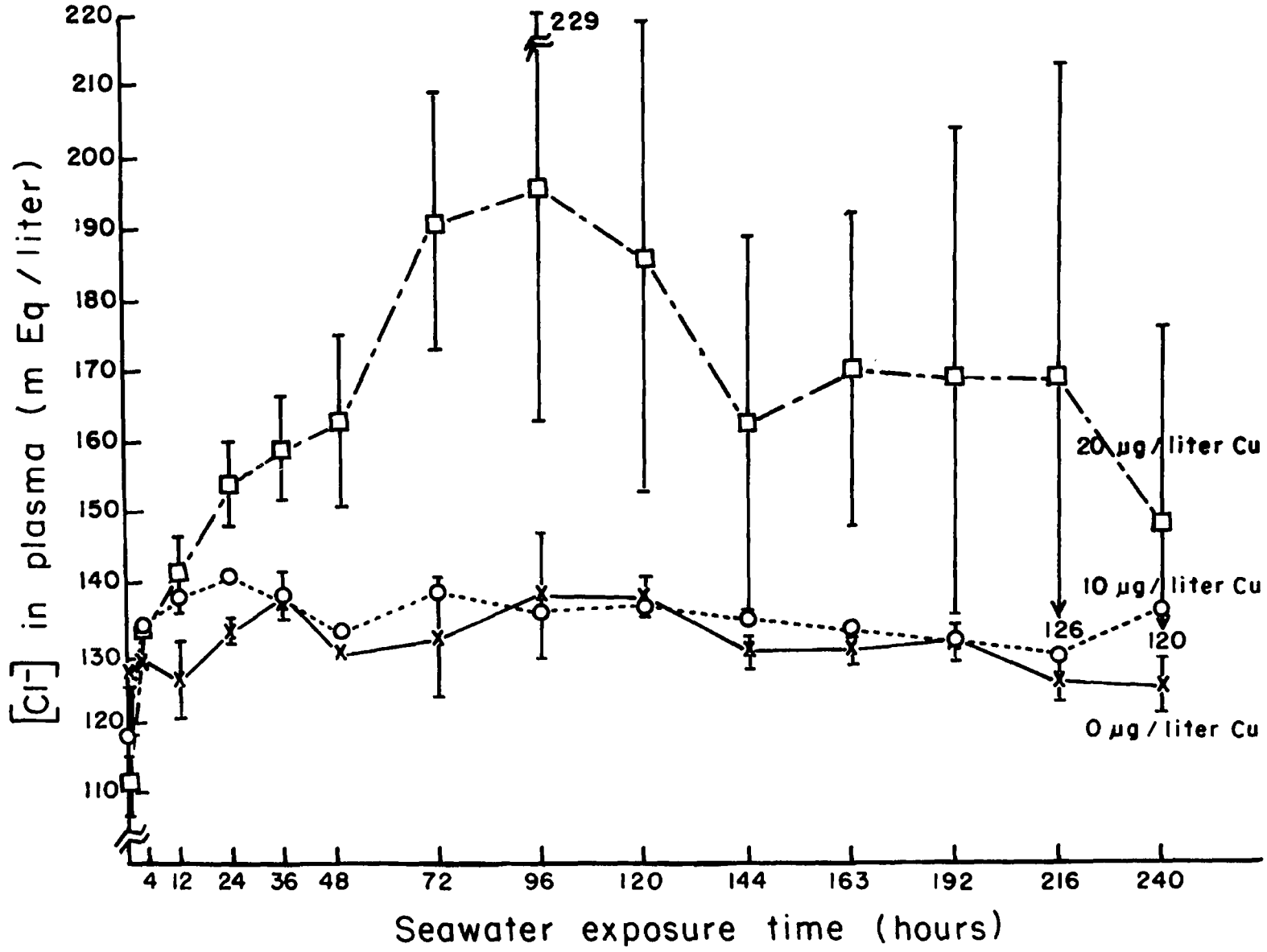

Figure A-3. Effect of seawater exposure on the chloride ion concentration of the plasma of coho salmon previously exposed to copper (in fresh water) for 144 h.

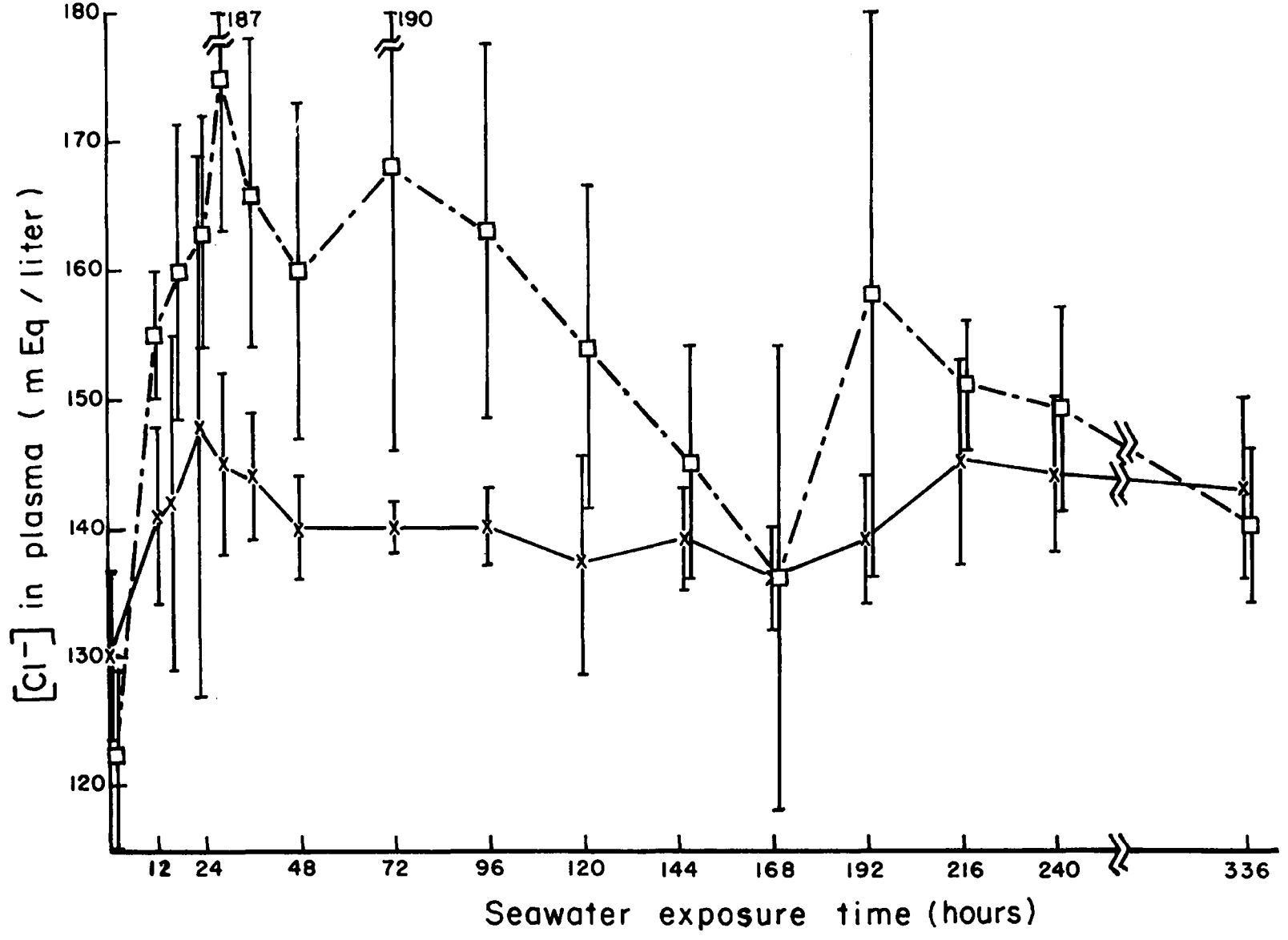

Figure A-4. Effect of seawater exposure on the chloride ion concentration of the plasma of coho salmon previously exposed to copper (in fresh water) for 792 h (x - control, □ - 20 µg/liter Cu).

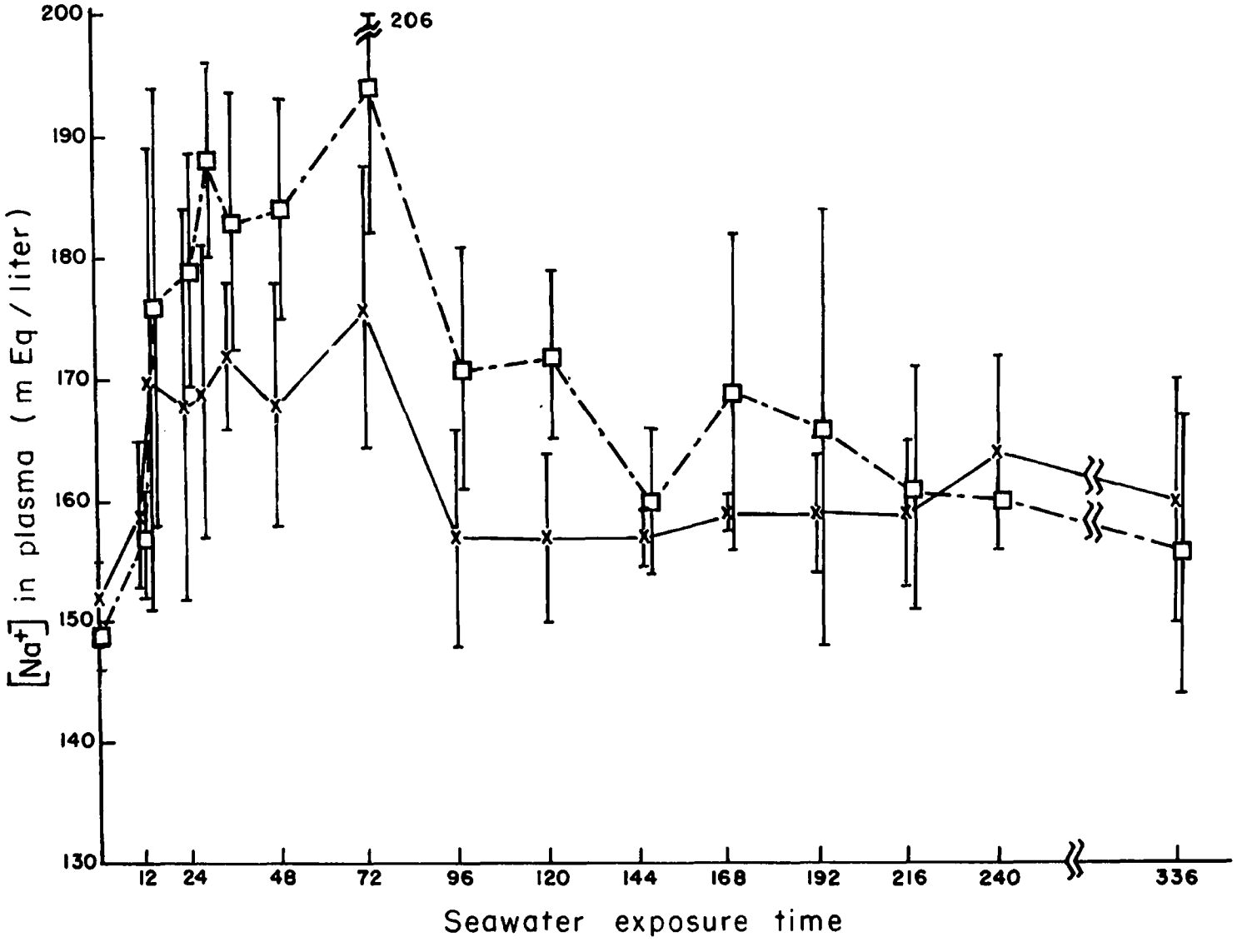

Figure A-5. Effect of seawater exposure on the sodium ion concentration of the plasma of coho salmon previously exposed to copper (in fresh water) for 792 h (x - control, □ - 20 µg/liter Cu).

Table A-9. Percent migration (to July 3, 1975) of yearling coho salmon released into a small coastal stream following acute and chronic copper exposure

Nominal concentration µg/liter Cu	Percent migration, Days post release: April 8, 1975 *a/* 1-5	6-10	11-20	21-30	31-40	41+	April 29, 1975 *b/* 1-5	6-10	11-20	21-30	31-40	41+
A. Chronic exposures												
0	39.2	40.8	41.6	--	--		50.4	51.2	--	--	--	--
5	30.7	33.3	34.0	34.6	35.3		28.5	29.1	29.8	--	--	--
10	28.7	30.6	34.0	36.0	--		33.1	34.4	34.4	34.4	34.4	35.1
20	15.8	18.3	19.2	21.7	--		19.2	21.7	24.2	25.0	--	--
30	3.0	4.0	5.0	5.0	6.0		5.2	7.2	7.2	7.2	10.3	--
30 *c/*	6.8	10.9	10.9	--	--	(11d)	24.4	30.8	30.8	30.8	30.8	32.1 (33d)
B. Acute exposure (144 h) *d/*												
0	54.5	54.5	55.4	--	--	--	68.0	68.0	--			
5	40.9	44.8	46.7	--	--	--	60.4	62.6	--			
10	49.5	49.5	52.4	--	--	--	57.6	58.6	--			
20	31.1	33.0	34.0	--	--	--	40.9	47.3	48.4			
30	7.8	10.7	12.6	12.6	13.6	14.6	13.4	17.5	--			

Nominal concentration µg/liter Cu	Days post release: May 14, 1975 *e/* 1-5	6-10	11-20	21-30	31-40	June 4, 1975 *f/* 1-5	6-10	11-20	21-30
C. Chronic exposures									
0	26.8	28.5	--	--	--	79.2	88.3	--	--
5	20.4	22.2	23.4	--	--	60.4	71.6	72.2	--
10	15.9	17.9	18.5	--	--	55.8	61.0	61.6	--
20	3.3	4.9	8.2	10.6	11.5	32.5	40.0	43.3	44.2
30	1.0	2.0	3.0	3.0	4.0	18.4	26.2	27.2	29.1
30 *c/*	2.5	6.3	7.6	8.9	(47d)	38.5	57.7	57.7	60.3 (68d)
D. Acute (144 h or less) *g/*									
0	No release					89.1	92.7	--	
30 (72h)	--					36.0	52.0	--	
30 (96h)	--					20.0	42.2	44.4	
30 (144h)	--					27.7	65.9	--	

a/ One hundred and twenty to one hundred and fifty fish per concentration (108-d copper exposure).
b/ One hundred and twenty to one hundred and fifty fish per concentration (130-d copper exposure).
c/ Days of copper exposure in parenthesis.
d/ Ninety-one to one hundred and five fish per concentration (6-d copper exposure).
e/ One hundred and twenty to one hundred and seventy fish per concentration (145-d copper exposure).
f/ One hundred and twenty to one hundred and seventy fish per concentration (165-d copper exposure).
g/ Forty-five to fifty-five fish per concentration.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

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EPA-600/3-77-032		

4. TITLE AND SUBTITLE	5. REPORT DATE
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Harold W. Lorz and Barry P. McPherson	

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15. SUPPLEMENTARY NOTES

16. ABSTRACT Many species of trout and salmon spend their early life in freshwater and then migrate to the sea. Transition from freshwater to marine existence requires physiological changes which are involved in the development of the migratory smolt stage. Sublethal exposure to pollutants in freshwater could theoretically disrupt smoltification and indirectly cause the dealth of smolts.

In this study, exposure of smolt age coho to sublethal levels of copper in freshwater interfered with normal osmotic and ionic control in blood plasma; when the copper exposed fish were transferred to seawater the plasma osmolality and chloride concentrations increased significantly, compared to controls, and many died. These responses were attributed in part to an observed suppression of Na^+, K^+ - activated ATPase activity in the gills of copper exposed fish. The most sensitive latent effect of exposure to sublethal levels of copper was the failure of copper exposed coho smolts to migrate successfully following release into a natural stream.

All copper concentrations tested (5-30 µg/l) produced adverse effects and were well below the 96-hr LC50 (60-74 µg/l). Exposure to sub-lethal levels of zinc produced no similar adverse effects.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Salmon, Copper, Zinc	Smoltification, Migration Osmoregulation	06/A,F,T

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