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EFFECTS OF SEVERAL METALS ON SMOLTING OF COHO SALMON

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EFFECTS OF SEVERAL METALS ON SMOLTING OF COHO SALMON

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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 freshwater to seawater, and demonstrates that under certain conditions exposure to sublethal levels of pollutants can result in mortality when fish subsequently enter seawater. 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 value for cadmium ($CdCl_2$) for yearling coho salmon *(Oncorhynchus kisutch)* was 10.4 µg/liter in January but appeared to have increased in smolting fish in May. The 96-h LC50 of mercury ($HgCl_2$) for yearling coho salmon was 240 µg/liter. All static bioassay tests were conducted at 10°C in water with alkalinity and hardness ranging from 70-83 mg/liter and 85-93 mg/liter as $CaCO_3$, respectively.

Exposure of yearling coho salmon for $\leq$144 h to sublethal levels of Cd, Ni ($NiCl_2$), Cr ($K_2Cr_2O_7$) or Zn ($ZnCl_2$) in freshwater had no apparent affect on the (Na, K)-stimulated ATPase activity of the gill. Fish exposed to concentrations >4 µg/liter Cd died in a dose dependent manner following transfer to seawater. Coho salmon given a 5-day rest (non-toxicant exposure in freshwater) between Cd exposure and transfer to seawater exhibited seawater survival comparable to control fish. Few deaths occurred following transfer of the Ni, Cr or Zn exposed fish into seawater.

Yearling coho salmon exposed to $\leq$4.5 µg/liter Cd for 144 h did not migrate as well as the control fish following release into a natural stream. However, fish chronically exposed to $\leq$4.5 µg/liter Cd (47 and 70 days) migrated as well or occasionally better than control fish following their release.

The (Na, K)-stimulated ATPase activity of the gills of coho salmon chronically exposed to Cd or Zn was suppressed and the downstream migration was reduced when 10 µg/liter Cu was added with the Cd or Zn. Deaths also occurred when Cd-Cu or Zn-Cu exposed fish were challenged with seawater. Neither Cd nor Zn appeared to affect feeding activity, growth or survival during exposures of 70 and 27 days respectively; however, the addition of 10 µg/liter Cu for only 6 days to Zn exposed fish significantly reduced condition factor.

Histological examination of liver, kidney and gill tissue of coho salmon exposed to Cd or Zn (singly or in combination with Cu) showed no aberrations in these organs. Cadmium was found to accumulate in the gills, liver and kidney, but only minor amounts occurred in muscle tissue.

This report was submitted as partial fulfillment of Grant R-804283 by the Oregon Department of Fish and Wildlife under the partial sponsorship of the U. S. Environmental Protection Agency. This report covers the period January 5, 1976 to December 20, 1976.

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SECTION I

CONCLUSIONS

1. Cadmium was the most toxic of the metals tested to yearling coho salmon; 96-h LC50 of 10.4 µg/liter in January.

2. Few deaths occurred when yearling coho salmon previously exposed to ≤ 4 µg/liter Cd were challenged with seawater, whereas fish exposed to concentrations >4 µg/liter Cd died in a dose-dependent manner following transfer to seawater.

3. Coho salmon yearlings given a 5-day rest (non-toxicant exposure period) between Cd exposure and transfer to seawater exhibited survival comparable to the control groups when challenged with seawater.

4. Yearling coho salmon previously exposed to salts of nickel or chromium (≤ 5 mg/liter) survived the seawater challenge, whereas fish exposed to mercury (50-300 µg/liter) died in a dose-dependent manner upon transfer to seawater.

5. Gill (Na, K)-stimulated ATPase activity in yearling coho salmon was not affected by cadmium, chromium, nickel or zinc salts.

6. Yearling coho salmon exposed to ≤ 4.5 µg/liter Cd for 144 h did not migrate as well as control fish following a release into a natural stream. However, fish chronically exposed to Cd (47 to 70 days) and Zn (27 days), respectively, showed acclimation to the toxicants and exhibited migration similar to the controls.

7. The (Na, K)-stimulated ATPase activity of the gills of chronically exposed yearling coho salmon was suppressed and downstream migration of the released fish was reduced when 10 µg/liter Cu was combined with the Cd or Zn. Low levels of mortality also occurred when the Cd-Cu or Zn-Cu exposed fish were challenged with seawater.

8. Neither Cd nor Zn exposures appeared to affect feeding, growth or survival during exposures of 70 and 27 days respectively; however, addition of 10 µg/liter Cu for only 6 days to the Zn-exposed fish significantly reduced their condition factor.

9. No histological changes were noted in examination of liver, kidney, or gill tissues of yearling coho salmon chronically exposed to Cd or Zn either singly or in combination with Cu.

10. Exposure of yearling coho salmon to Cd in freshwater did not affect their ability to maintain normal blood plasma osmotic pressure and chloride ion concentration.

11. Copper produced a marked dose-dependent cortisol stress response. Cadmium, even at concentrations that produced death, did not elicit a plasma cortisol stress response in yearling coho salmon. Cadmium exposed fish were capable of a stress response as noted by handling or seawater challenge tests.

12. Cadmium was found to accumulate in the gills, liver, and kidneys of yearling coho salmon but only a minor amount was found in the muscle tissue. The bioaccumulation appeared to be dependent both on duration of exposure and exposure concentration.

SECTION II

RECOMMENDATIONS

1. The techniques and methodology developed in this and the prior study (Lorz and McPherson 1977) are recommended for the determination of pollutant effects on anadromous fishes. The Seawater Entry Test (Appendix 1) is a quick method for determining if pollutants in freshwater could be potentially harmful to the downstream migration and subsequent seawater life history phases.

2. Prudence in applying the results of this study directly and quantitatively to field situations is recommended since other factors such as acclimation, water quality, presence of other chemicals, disease, organic content of the water, etc., could alter the reported responses of yearling coho salmon to a given toxicant concentration.

3. Additional research should be conducted to determine if the interference observed with smolting of anadromous salmonids by sublethal levels of metals or their combination (Cu, Cd-Cu, Zn-Cu) is a general phenomenon. Research on affects of toxicant exposure to earlier life stages and subsequent effects on smolting are also needed.

4. Another potential area of research would be to determine if application of toxicants to a natural stream and its fish population would produce results similar to those noted in the laboratory study.

SECTION III

INTRODUCTION

Potential metal contamination of waterways may occur from industrial, thermal-electric power generation, agricultural, municipal, mining and natural sources. Considerable research has been done to determine safe limits of various metals for survival and growth of juvenile salmonids in freshwater, but there is little information on effects of metals affecting migration and survival of fish in seawater. The need for this information is recognized in the Pacific Northwest where large populations of anadromous salmonids are a valuable commercial and sport fishery resource.

Studies carried out in Eastern Canada indicated that copper-zinc mining pollution interrupted the upstream migration of adult Atlantic salmon, *Salmo salar*, and caused considerable numbers to return downstream (Sprague, Elson and Saunders 1965, Saunders and Sprague 1967). Laboratory studies by Sprague (1964a, 1964b) and Sprague and Ramsay (1965) determined not only the lethal levels of copper and zinc, (singly and in combination) but also the concentrations that produced avoidance behavior in juvenile Atlantic salmon. From the laboratory data, Sprague, Elson and Saunders (1965) postulated that the combined affect of severe pollution in most years, plus the necessity of a 3 year juvenile residency, resulted in lowered salmon smolt production in the Miramichi River. Similarly, Södergren (1976) suggested the decline of the Atlantic salmon population from the Ricklea, a small salmon river in northern Sweden, was due to heavy metal pollution of the stream's ecosystem.

This report is the second of a three part study by the Oregon Department of Fish and Wildlife and funded in part by the Environmental Protection Agency. In the previous study, Lorz and McPherson (1977) noted deleterious effects of copper exposure (sublethal levels in freshwater) on yearling coho salmon survival when transferred to seawater, a decrease in the (Na, K)-stimulated ATPase activity of the gill, and a reduced migratory tendency when copper-exposed fish were placed into a natural stream. The present study examined similar parameters (tolerance to seawater, (Na, K)-stimulated ATPase activity, and migratory disposition) following freshwater exposure to the metals cadmium, zinc, and combinations of cadmium-copper and zinc-copper. In addition, tolerance of yearling coho salmon to seawater was determined after exposure to chromium, nickel, and mercury salts.

SECTION IV

METHODS

EXPERIMENTAL FISH

All experiments were conducted on coho salmon between ages of 12 and 17 months. Coho salmon of the 1974 year class[1] from Fall Creek Salmon Hatchery (Alsea River, Oregon) were used. Fish were hatched and reared from fertilized eggs brought into the laboratory under conditions similar to those reported by Lorz and McPherson (1977).

EXPOSURE TO TOXICANT

Yearling coho salmon were exposed to cadmium ($CdCl_2 \cdot 2.5\ H_2O$) for periods ranging from 6 to 70 days, zinc ($ZnCl_2$) for up to 28 days, mixtures of cadmium-copper for 6 days, and mixtures of zinc-copper for up to 21 days. Five 96-h LC50 static tests were conducted in 0.61 m diameter fiberglass tanks with four metallic compounds: two tests were run with cadmium (Cd) and one test each was run with chromium ($K_2Cr_2O_7$), mercury ($HgCl_2$), and nickel ($NiCl_2 \cdot 6H_2O$). In the static tests (144-h exposure) the water was continuously aerated, and 85% of the 120 liters of test water was exchanged once each 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 WFTS) by flame or flameless atomic absorption spectrophotometry and there was no detectable loss of metal from the water during any 24-h period. Results of the AA analysis taken during the continuous exposure indicated that the mean weekly measured concentration approximated the nominal concentrations (Table A-1 to 3). Water temperature was maintained at $10 \pm 1^{o}C$ in all static tests.

All exposures of ≥ 144 h were conducted in a flowing water system. This system consisted of a gravity flow diluter (Fig. 1) that delivered 12 L/min to each of ten 1.54 m diameter fiberglass tanks (five concentrations replicated, Fig. 2). The background concentration of cadmium and zinc in the well water was <1 µg/L and <4 µg/L, respectively. A volume of 1000 L was maintained in each of the tanks, and 95% of the water was replaced every 3.7 h. Submersible pumps were used in each tank to provide additional current, aeration and mixing.

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[1] *Year class refers to year of spawning.*

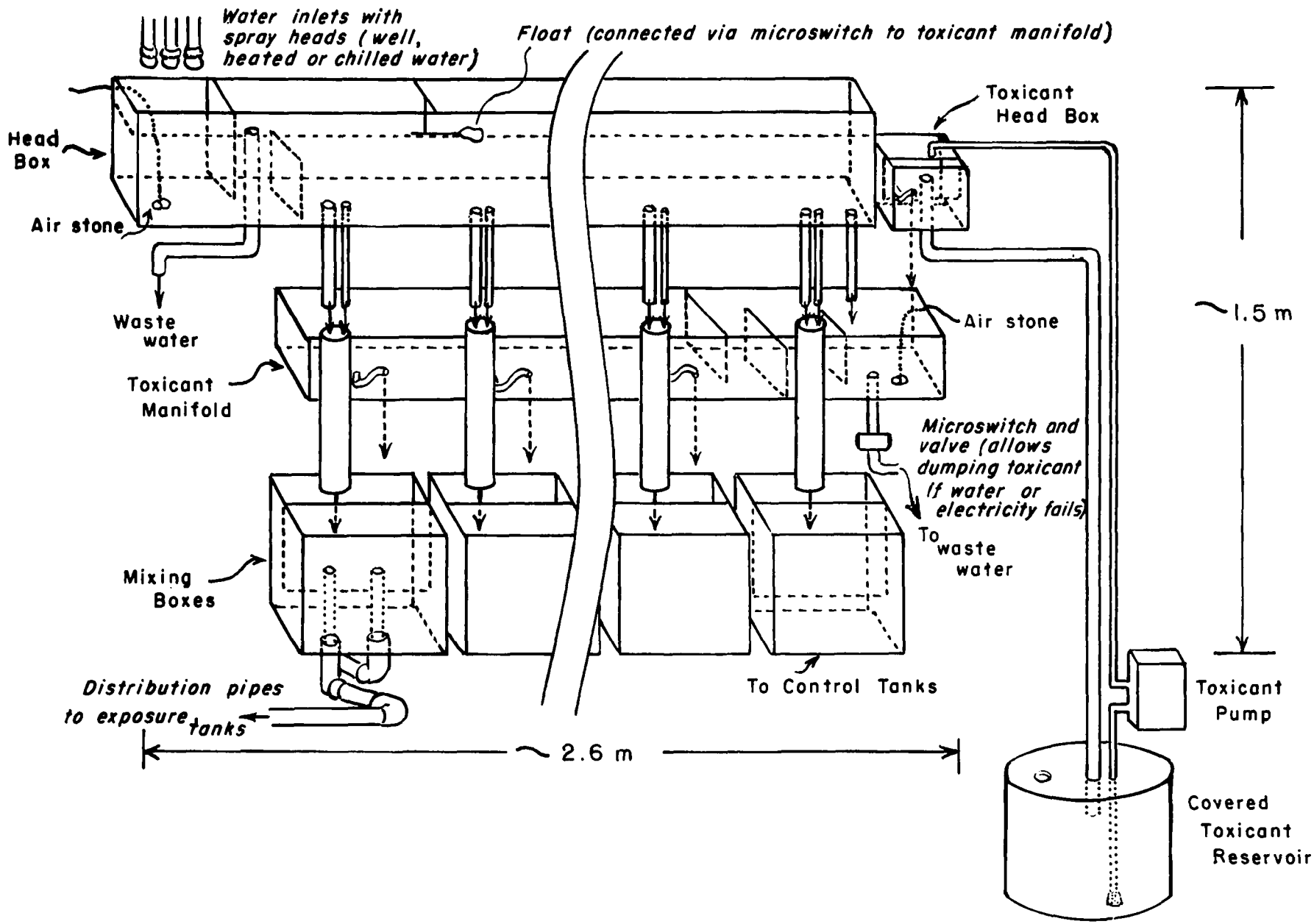
Water inlets with spray heads (well, heated or chilled water)
Float (connected via microswitch to toxicant manifold)
Toxicant Head Box
Head Box
Air stone
Waste water
Toxicant Manifold
Air stone
1.5 m
Microswitch and valve (allows dumping toxicant if water or electricity fails)
To waste water
Mixing Boxes
Distribution pipes to exposure tanks
To Control Tanks
2.6 m
Toxicant Pump
Covered Toxicant Reservoir

Figure 2. Exposure tanks with diluter in background.

Smaller groups of fish were exposed periodically to the flowing toxicant in 0.61 m diameter fiberglass tanks. Water flow (5 L/min; 95% replacement every 1.5 h) to these 120-liter tanks was provided by a siphon from mid-depth in the 1.54 m chronic exposure tank, with no difference in water quality between tanks. All tanks were covered to prevent loss of fish.

Starting in April 1976, 10 µg/L Cu was added for 6 days (with a Mariotte bottle) to four of the tanks of fish that were receiving Cd and one control tank. Similarly, four of the zinc exposure tanks received 10 µg/L Cu for up to 21 days in May and June 1976.

Cadmium and zinc concentrations chosen for chronic study were determined from prior static toxicity tests. Total metal concentration was analyzed at least once per week from each exposure tank (from a composite water sample) by atomic absorption spectrophotometry. Water and toxicant flows in the diluter were checked at least once daily and only occasional minor adjustments of flow were required.

TOLERANCE TO SEAWATER

Ten to 20 fish from each exposure tank (containing concentrations of Cd, Zn, Cd-Cu, Zn-Cu or well water) were periodically tested for tolerance to seawater and percent survival provided a presumptive measure of osmoregulatory ability. Fish were transferred directly from the exposure tanks to 0.61 m diameter fiberglass tanks containing 120 L of natural seawater and held for at least 10 days. Eighty-five percent of the 120 L was exchanged daily and the seawater was continuously aerated. Water was maintained at a salinity of 30 $\pm$ 0.5 ^{o}/oo and a temperature of 10 $\pm$ 1°C. Procedure for a seawater challenge test that can be used to survey the possible detrimental effect of any chemical (in freshwater) is given in Appendix I.

GILL ATPASE ACTIVITY

(Na, K)-stimulated ATPase activity was measured by the whole gill method (Johnson et al. 1977). Conditions for optimal enzyme activity were determined from appropriate characterization curves (Appendix II). In addition, (Na, K)-stimulated ATPase in microsomes isolated from gill filaments was assayed by the procedures of Zaugg and McLain (1970). The (Na, K)-stimulated ATPase activity was measured on four to six fish per metal concentration. The whole homogenate assay procedure was a more convenient assay because it required no centrifugation and thus allowed for a larger number of samples to be run as well as allowing freezer storage of the samples for up to 2 weeks prior to analysis.

DOWNSTREAM MIGRATION

The effect of exposure to metals (Cd, Zn, Cd-Cu and Zn-Cu) on the coho salmon smolt's migratory disposition was assessed by releasing marked control and metal-exposed fish into Crooked Creek, a tributary of the North Fork of the Alsea River and monitoring their migration to a trap 6.4 km downstream. The trap was built into a permanent weir and was checked daily for the first 10 days following release. 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. To insure that all fish in the migration studies had attained the smolt size no fish smaller than 11 cm were released. Seaward migration of wild coho salmon juveniles began at the trap in late April, peaked in late May and ceased by late June. Releases of metal-exposed and control fish were made between April 14 and June 9, 1976. Trapping was terminated July 6, 1976 almost one month after the last release. The stream was electrofished on July 6 and 7, 1976 from above the release points down to the weir, in an attempt to collect fish that had not migrated.

OSMOTIC AND IONIC REGULATION

Changes in osmolality and chloride ion (Cl^-) concentration in blood plasma were monitored by sacrificing groups of yearling fish at various times after the initiation of toxicant exposure. The methods and equipment used in the analysis were outlined by Lorz and McPherson (1977).

ASSESSMENT OF COEFFICIENT OF CONDITION AND GROWTH

Each month, approximately 40 fish were randomly selected from each exposure tank after a 24-h starvation period, anesthetized, weighed, and measured. Individual fish were weighed to 0.1 g and fork length was determined to 0.1 cm. The coefficient of condition was determined for each fish in the sample using the formula $K = 100\ W/L^3$, where W denotes weight in grams, and L denotes fork length in centimeters (Hoar 1939).

HISTOLOGICAL EXAMINATION AND TISSUE CADMIUM LEVELS

Histological sections of several tissues were prepared from fish previously exposed to metals and an assessement of histopathological effects was made (Appendix III). Similarly, levels of cadmium and mercury accumulation in various fish organs were determined (Appendix IV, Table A-4).

WATER QUALITY

The source of water for the study was wells located approximately one mile east of the Willamette River, Linn County, Oregon and the chemical characteristics were measured by Corvallis Environmental Research Laboratory (CERL) (Lorz and McPherson 1977). 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 recording thermographs.

TABLE 1. CHEMICAL AND PHYSICAL CHARACTERISTICS OF TEST WATER (STATIC AND FLOWING-WATER SYSTEMS).

Characteristic	Unit	Year	Mean ± SD	Range	n
Alkalinity	mg/L as $CaCO_3$	1975	72 ± 3	66 - 81	49
		1976	76 ± 4	70 - 83	13
Hardness	mg/L	1975	93 ± 3	84 - 98	32
		1976	90 ± 3	85 - 93	13
Dissolved oxygen	mg/L	1975 static	9.8 ± 0.3	9.4 - 10.1	8
		1975 flowing	8.7 ± 0.9	6.2 - 10.9	52
		1976 static	9.9 ± 0.9	7.8 - 11.1	34
		1976 flowing	9.5 ± 0.8	6.6 - 10.6	48
Ammonia	mg/L NH_3-N	1975	0.39 ± 0.18	0.12-0.57	7
		1976	0.15 ± 0.07	0.08-0.21	8
pH		1975 static	7.30	6.81-7.54	6
		1975 flowing	7.26	7.07-7.54	42
		1976 static	7.59	7.00-7.82	16
		1976 flowing	7.03	6.48-7.37	7

SECTION V

RESULTS AND DISCUSSION

From January to July 1976, five static toxicity tests using four metals were completed. These tests included acute lethality determinations, effects on gill (Na, K)-stimulated ATPase activity, and effects on the ability of juvenile coho salmon to adapt to seawater following exposure to metals in freshwater. Additionally, 10 tests of tolerance to seawater were completed using yearling coho salmon previously exposed to either Cd or Zn in the flowing freshwater toxicant system. Included in these seawater tolerance tests were groups of Cd exposed fish that had received a freshwater recovery period prior to the seawater and also groups of yearling coho salmon exposed to mixtures of Cd-Cu or Zn-Cu. During the 1976 migratory period four releases of yearling coho salmon were made into a small coastal stream and the downstream movement of migrants monitored.

ACUTE LETHALITY TESTS

Cadmium

In January-February 1976, the 96-h LC50 was about 10.4 µg/L Cd; but in May it appeared to be considerably higher as only a 10% mortality was noted at a concentration of 10.3 µg/L Cd (Fig. 3). This decrease in sensitivity may have been related to increasing size and age of the coho salmon. Unlike the results with Cu (Lorz and McPherson 1977) the parr-smolt transformation did not appear to cause the coho salmon to be more sensitive as smolts to Cd. The reason for lack of 100% deaths at the 30 µg/L group in the January test is unknown, however Chapman, (personal communication) noted the occasional similar occurrence of incomplete kill at high concentration in his studies. When the "incipient lethal level" (ILL) was plotted following the technique of Sprague (1964a), the ILL for Cd was about 9.0 µg/L (Fig. 4). A sharp differentiation between lethal and non-lethal concentrations for Cd occurred, and the line relating concentration to survival time breaks and runs nearly parallel to the time axis (Fig. 4). The longest LT 50 (time to 50% mortality) in any test was 138 h for one test tank. Generally the LT 50 for Cd occurred from 82 to 130 h (Fig. 4). Time to 50% death of coho salmon was considerably greater with Cd than previous studies with Cu or Zn (Lorz and McPherson 1977).

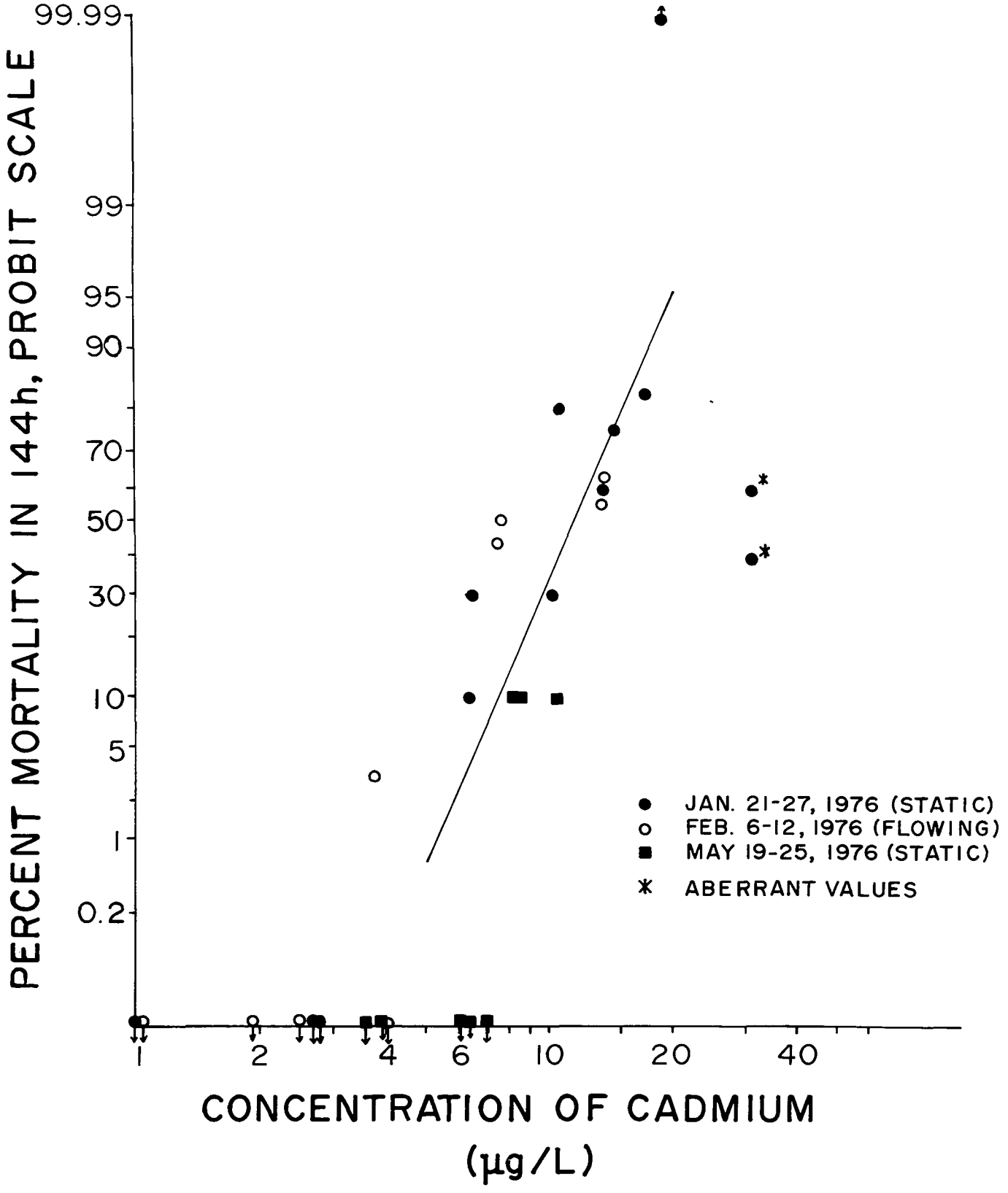

Figure 3. Mortality of yearling coho salmon exposed to various cadmium concentrations (line fitted by eye with aberrant values excluded).

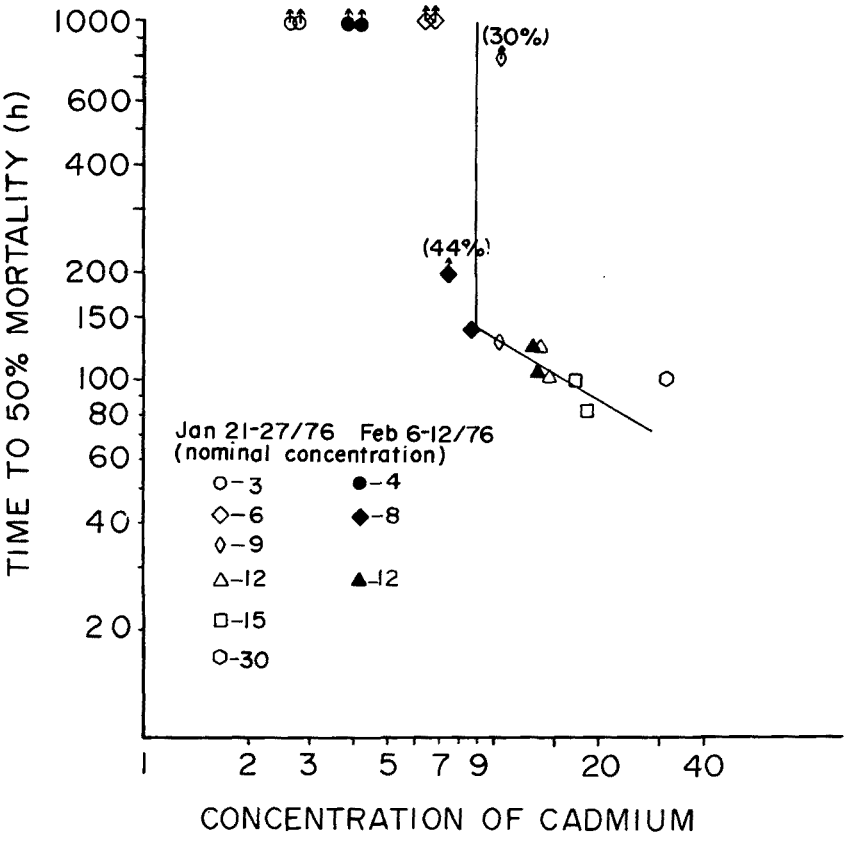

Figure 4. Median mortality-time of yearling coho salmon exposed to solutions of cadmium.

Our 96-h LC50 values for Cd (10.4 µg/L) for yearling coho salmon are higher than those reported by Chapman (1975) for coho salmon of the same stock (2.7 µg/L for 6 mo parr). Our water alkalinity and hardness were approximately four times greater than those reported in Chapman's study. Our LC50 values are similar to that reported by Ball (1967) for rainbow trout, *Salmo gairdneri* (15 µg/L) in water with a hardness of 290 mg/L as $CaCO_3$. Other researchers have recorded LC50 concentrations of 400-8000 µg/liter Cd for various warm water and spiny-rayed fish (Chapman 1973). As noted by other researchers, despite the high toxicity of cadmium, the response of the fish is initially very slow, even at high Cd-concentrations. Sauter et al. (1976) found brook trout fry survival (in soft water) through 30 days post hatch was not affected by exposures to Cd concentrations as high as 47 µg/L, but total length of fry (at these concentrations) was significantly reduced. However survival was significantly reduced during the 60-day exposure to test concentrations of cadmium $\geq$6 µg/L. In hard water, Sauter et al. (1976) found brook trout fry survival significantly reduced during 60 days of exposure to 21 and 12 µg/L Cd, indicating a cumulative effect during the 30-60 day exposure period.

Chromium

No coho salmon mortalities occurred during our chromium toxicity test, even at 5 mg/L, a concentration 50 times the recommended acceptable concentration (0.10 mg/L, U.S.E.P.A. 1976). Benoit (1976) noted that the 96-h LC50 values of hexavalent chromium for rainbow trout and brook trout were 69 and 59 mg/L Cr, respectively and believed the difference between species was due to difference in age of fish used. Warm water fish species also are relatively insensitive to hexavalent chromium as noted by their 24- and 96-h LC50 concentrations of 40-280 and 20-130 mg/L chromium, respectively (Trama and Benoit 1960, Pickering and Henderson 1966, Ruesink and Smith 1975).

Holland et al. (1960) found that trivalent chromium was more toxic to coho salmon than hexavalent chromium, although chromium sulfate (trivalent) precipitated to form non-ionized chromous carbonate at concentrations <50 mg/L. Mills (1975) noted very steep mortality curves for trivalent Cr with yearling coho salmon in seawater. Although the above cited authors note significant mortalities to juvenile-yearling salmonids (LC50's from 30-70 mg/L), Olson and Foster (1956) noted that survival and growth of alevin chinook salmon and rainbow trout were affected by concentrations as low as 0.02 mg/L Cr (hexavalent) during 4 months of exposure and this agreed closely to the findings of Benoit (1976) who used rainbow and brook trout. Strik et al. (1975) noted that 25 percent of the rainbow trout exposed to 10 mg/L Cr (hexavalent) for 15 days showed decreased activity and food intake. They also observed swollen blood vessels in the intestine and evidence of liver damage and impaired gill and kidney function. Buhler et al. (1977) reported on tissue accumulation and effects of hexavalent chromium on enzmatic activity in two strains of rainbow trout. Benoit (1976) suggests an application factor (MATC/96-h LC50) for brook trout and Cr would be between 0.003 and 0.006. However, Sauter et al. (1976) suggest that the current recommendations for chromium (100 µg/L, U.S.E.P.A. 1976) are not stringent enough, and the maximum permissible concentration of chromium in the aquatic environment should be reduced.

Nickel

Doudoroff and Katz (1953) noted that toxic concentrations of nickel ranged from 1.0 mg/L to 10 mg/L depending on fish species, pH, exposure time, synergism, etc. Pickering (1974) found the 96-h LC50 of nickel for fathead minnows was 32 mg/L Ni. However, he noted that a nickel concentration of 0.73 mg/L caused a significant reduction (following chronic exposure) in both the number of eggs per spawning and the hatchability of the eggs. Rehwoldt et al. (1971, 1972) noted that Ni was less toxic (96-h LC50 6.2-46.2 mg/L) than copper or zinc to several fish species from the Hudson River. In our study no mortalities occurred in yearling coho salmon following 144-h exposure to Ni even at concentrations of 5 mg/L.

Mercury

The estimated 96-h LC50 for coho salmon yearlings exposed to a series of different inorganic mercury ($HgCl_2$) concentrations was 240 µg/L Hg

(Table 2). Similar values were reported for juvenile rainbow trout by MacLeod and Pessah (1973) who found 96-h LC50 values for $HgCl_2$ of 220, 280, and 400 µg/L, at 20, 10 and 5°C, respectively.

TABLE 2. SURVIVAL OF YEARLING COHO SALMON EXPOSED TO MERCURIC CHLORIDE IN FRESHWATER FOR 144 h (APRIL 20-26, 1976).

Nominal concentration µg/L Hg	Percent survival (144-h exposure)[a]	
0	100	(20)
30	100	(19)
50	100	(22)
100	100	(20
200	61.9	(21)
300	4.8	(21)
400	0	(20)
500	0	(21)

[a]*Number of fish exposed in parentheses.*

Due to microbial action, inorganic mercury is known to change chemical form when released into the natural aquatic environment. It has been shown that microorganisms present in bottom sediments, fish intestine and the external mucus of fish synthesized methylmercury from inorganic mercury (Jernelov and Lann, 1971). Fish can absorb both inorganic and organic forms of mercury, although metabolic pathways, accumulation and excretion rates are quite different (Jensen and Jernelov 1969, Jernelov and Lann 1971, Hannerz 1968). Buhler et al. (1973) reported that 60-80% of the total mercury present in freshwater and estuarine fish is in the methylmercury form. The 96-h LC50 concentration of methylmercury for juvenile coho salmon was found to be 38.9 µg/L (McPherson 1973), which is six times more toxic than the $HgCl_2$ used in this study.

Tissue analysis for Hg showed a typical pattern of absorption-accumulation as described by Jernelov and Lann (1971). Our analysis revealed highest total Hg concentrations occurred in the gills, while kidney and liver tissue contained lesser but significant amounts. Very small amounts of total Hg were found in the muscles and gonads (Table A-4).

Zinc

No acute lethality tests were carried out for zinc this year, however, tests carried out in 1974 (Lorz and McPherson 1977) gave 96-h LC50 values of 4600 µg/L Zn for yearling coho salmon. There was also an indication that fish became increasing sensitive to Zn during smolting. Zitko and Carson (1977) noted that the incipient lethal level (ILL) of zinc to juvenile Atlantic salmon (in freshwater with a hardness of 14 mg/L) varied from 150 to 1000 µg/L as a function of season and developmental stage of the fish.

They noted that the ILL increased from 500 to 1000 µg/L Zn during the first year but reduced to 150 µg/L Zn the following spring. Zitko and Carson suggest that the most sensitive stage in the Altantic salmon's life history, as evidenced by decreased ILL, coincides with and is probably related to the parr-smolt transformation.

Herbert and Wakeford (1964) noted that the resistance of yearling rainbow trout and Atlantic salmon smolts to zinc sulphate increased with salinities up to 30-40% seawater, in which these species could withstand concentrations of zinc sulphate 13 times greater than those in freshwater during 48 h of exposure. If the salinity was increased to 72% seawater then a reduction in tolerance to the zinc salts occurred. Salmon were more susceptible to zinc poisoning than trout in freshwater and at all salinities tested.

EFFECT OF METAL EXPOSURE ON GROWTH AND SURVIVAL

Cadmium

Coho salmon in the higher concentrations of Cd did not appear to feed quite as actively as the controls. However, even yearling coho salmon held under control conditions for 70 days starting in late February 1976 experienced little additional growth, and the Cd concentrations tested did not appear to reduce growth (Table A-5).

Yearling coho salmon subjected to 10 µg/L Cu for the last 5 days of 68-day exposure to Cd showed no apparent ill effects on growth or coefficient of condition (Table A-5) as had been noted previously with coho salmon (Lorz and McPherson 1977) or goldfish and carp (Ozaki et al. 1970) exposed to just Cu. Almost no mortality occurred during 68 days of exposure to Cd although accumulation of Cd occurred in some tissues (Appendix IV).

Zinc

Yearling coho salmon were exposed to Zn ($ZnCl_2$) for 27 days in May and June with half of the tanks also receiving 10 µg/L Cu for the last 20 days. No mortalities occurred during the exposure. Coefficient of condition was significantly lower in groups that received only Cu or Zn-Cu mixtures as compared to the controls or the Zn-only exposed fish (Fig. 5, Table A-6). Coho salmon receiving Cu or Zn-Cu exposures did not feed as readily as controls or Zn-only groups but did feed better than coho salmon exposed to higher Cu concentrations in a previous study (Lorz and McPherson 1977). The effect of Cu and Zn-Cu mixtures in lowering the coefficient of condition was evident after only 6 days of Cu exposure (Table A-6). It appears that Cu may not only reduce the feeding response, but also may increase the metabolic rate when combined with Zn, causing a reduction in condition factor.

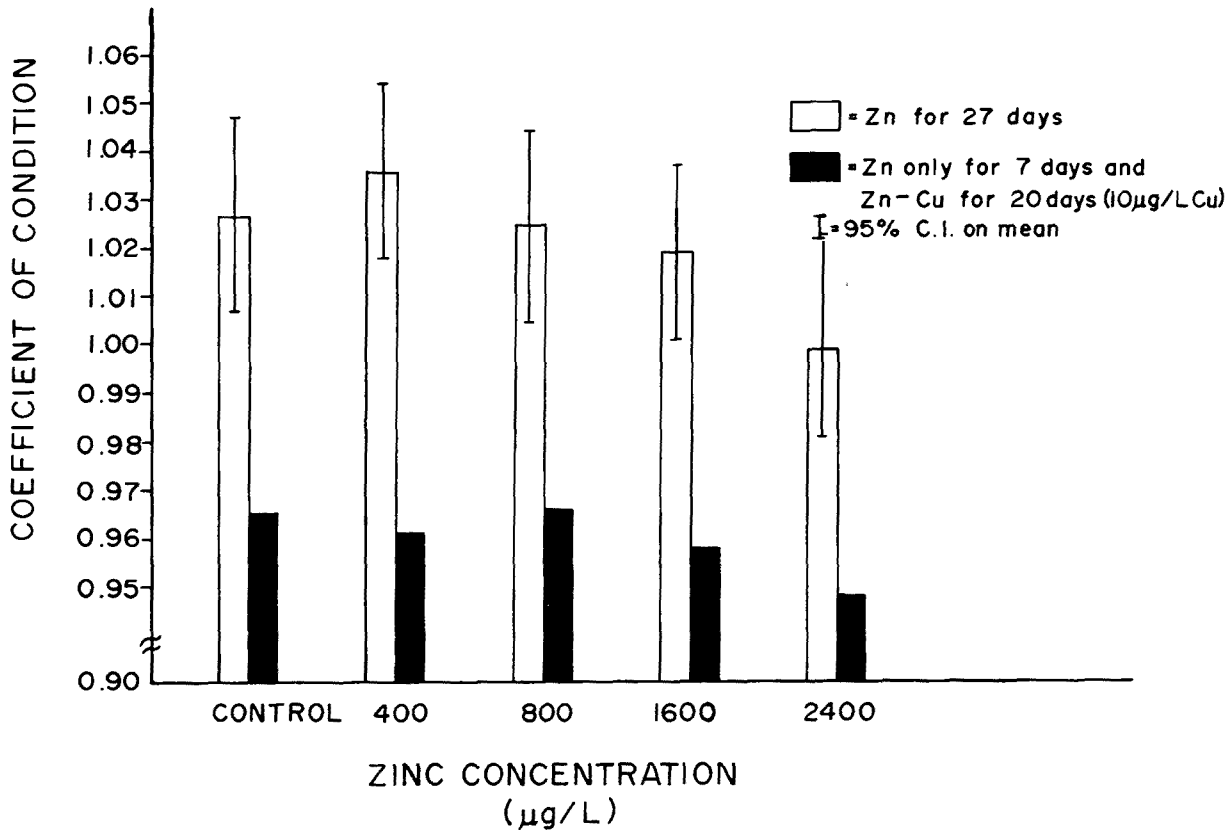

Figure 5. Effect of zinc, copper and zinc-copper mixtures on coefficient of condition of yearling coho salmon.

EFFECTS OF METAL EXPOSURE ON GILL ATPASE ACTIVITY AND SURVIVAL IN SEAWATER

Cadmium

Exposure of coho salmon during January and February to concentrations of Cd >4 µg/L usually caused death of at least 20% of the test fish during the toxicant exposure. When the survivors of these 144-h static and flow-through tests were transferred directly to seawater (30 °/oo), at least 60% of the transferred fish died (Fig. 6 and 7). Deaths normally occurred within the first 72 h following transfer to seawater; this result contrasts with the effects observed for Cu exposed fish where deaths occurred predominantly after 72 h in seawater (Lorz and McPherson 1976).

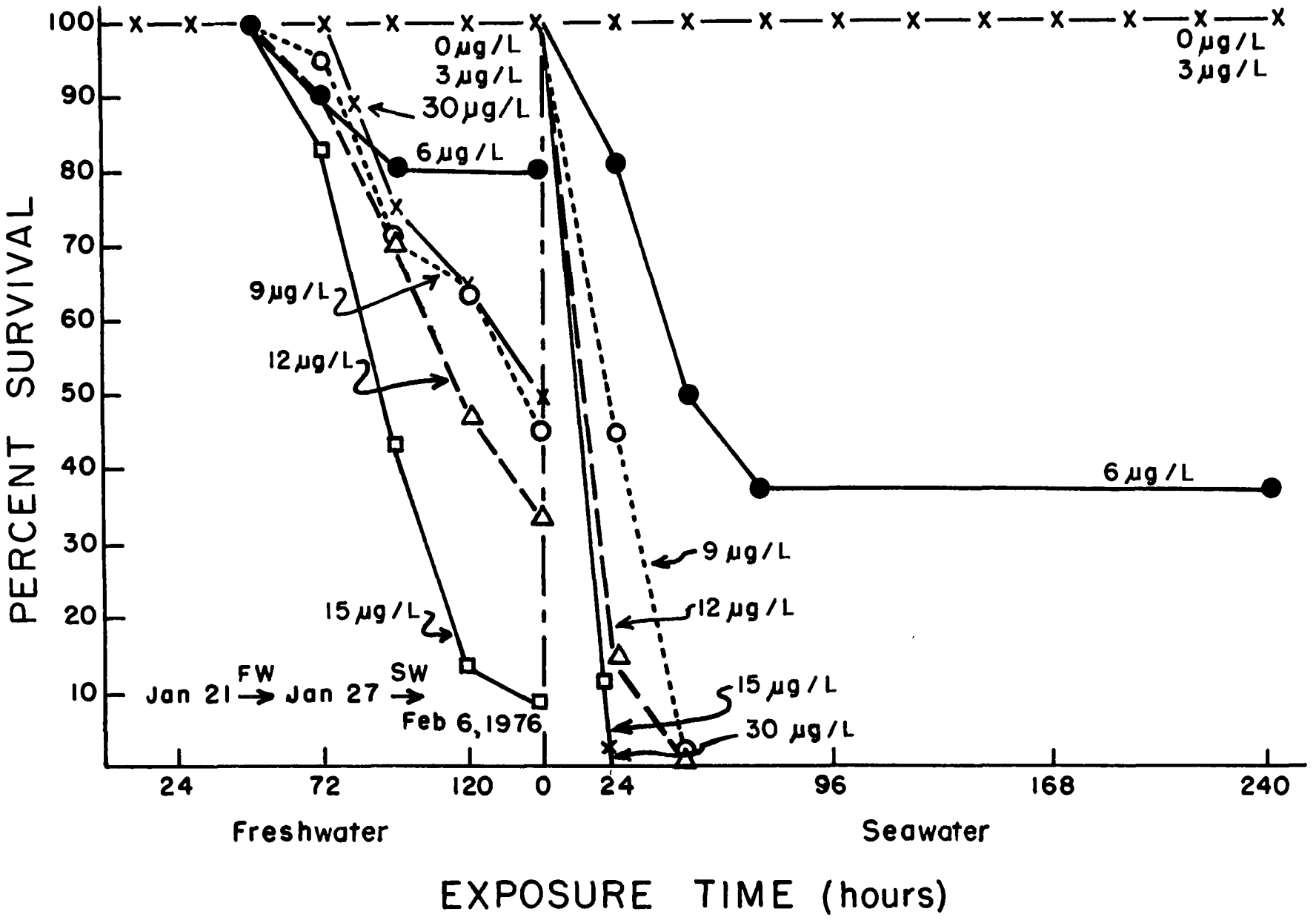

Figure 6. Percent survival of yearling coho salmon exposed to cadmium in freshwater (static) and subsequent survival upon transfer to seawater.

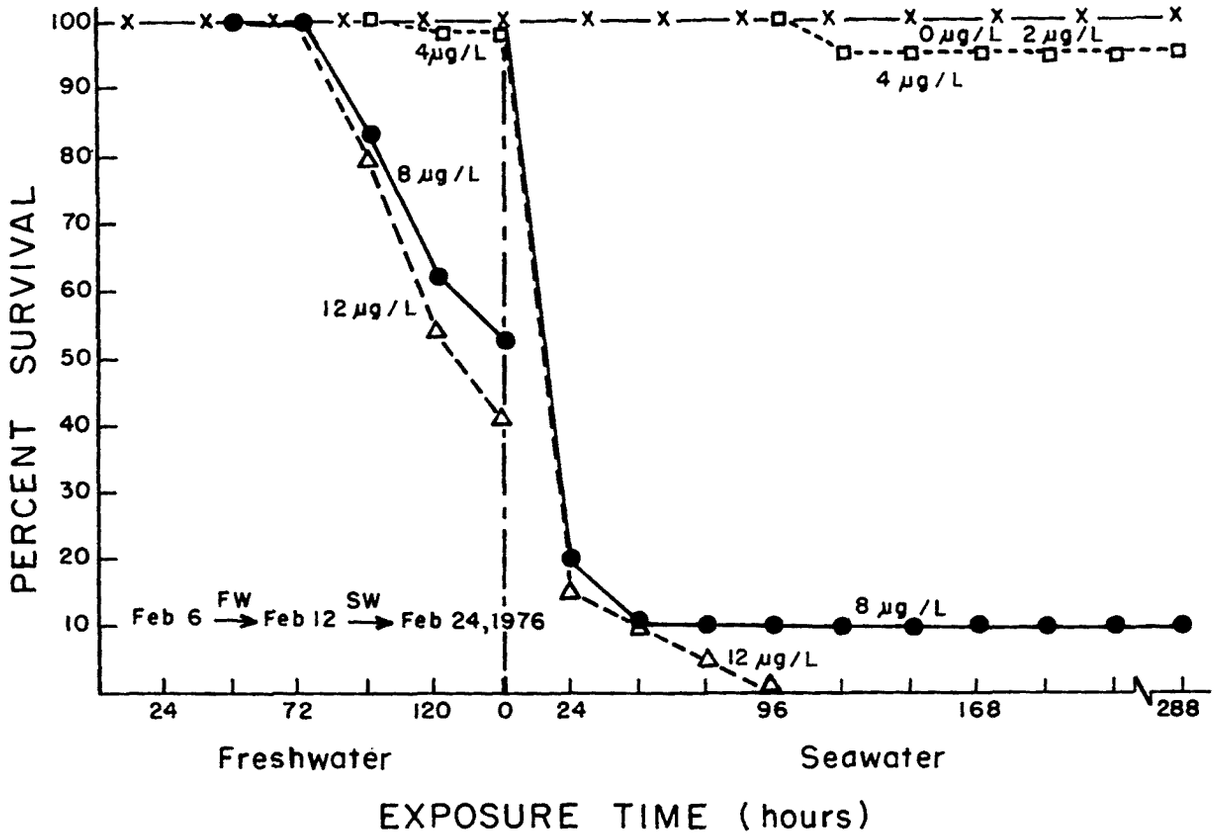

Figure 7. Percent survival of yearling coho salmon exposed to cadmium in freshwater (flow-through) and subsequent survival upon transfer to seawater.

Yearling coho salmon given a 5-day rest (non-toxicant exposure) following Cd exposure did not die when transferred to seawater, whereas, similar groups transferred directly showed considerable mortality (Table 3). It appeared that during the rest period the coho salmon were able to adjust their body burden of the metal and thus avoid the immediate detrimental effects observed in seawater.

TABLE 3. SURVIVAL OF YEARLING COHO SALMON WITH EITHER REST OR IMMEDIATE TRANSFER TO SEAWATER FOLLOWING EXPOSURE TO CADMIUM FOR 144 h.

Nominal concentration (µg/L)	Percent survival in FW (144-h exposure)	Percent survival in FW (120-h "rest")	Percent survival in SW (280-h exposure)
A. Direct transfer to seawater			
0 (56)[a]	100		100
2 (32)	100		100
4 (55)	98.2		93.3
8 (82)	52.4		10
12 (82)	40.2		0
B. Five days rest in freshwater prior to seawater exposure			
0 (19)		100	100
4 (26)		100	100
8 (18)		100	100
12 (9)		88.9	100

[a]*Number of fish exposed in parentheses.*

Deaths of fish chronically exposed to Cd and then transferred to seawater occurred only in the groups exposed to 4.5 µg/L Cd. Coho salmon chronically exposed to Cd did not exhibit greater mortality in seawater than those acutely exposed (Tables 4 and 5). Thus, there appeared to be no cumulative effect of chronic exposure to Cd either during exposure to the toxicant or upon subsequent transfer to seawater. These results were considerably different from those observed during chronic Cu exposure, where many yearling coho salmon died during exposures to 20 and 30 µg/L Cu and also upon subsequent exposure to seawater (Lorz and McPherson 1976).

Following 1660 h of Cd exposure, 144 h of Cu exposure at 10 µg/L, or combined exposure to both metals fish transferred to seawater incurred 13-43% mortality in the groups receiving Cu only or the Cd-Cu mixture, while the 4.5 µg/L Cd-only exposed fish exhibited <7% mortality (Table 5).

The yearling coho salmon exposed to Cd in May (Fig. 8) were more tolerant to both Cd and seawater than were groups challenged in January (Fig. 6). The deaths that did occur in seawater following Cd exposure were dose dependent. The improved survival in May may have been related to the increased age and size of fish tested or to physiological changes that occur during the parr-smolt transformation. In a previous study, Lorz and McPherson (1977) noted an increased tolerance to seawater but a decreased tolerance to Cu during the parr-smolt transformation.

TABLE 4. SURVIVAL OF YEARLING COHO SALMON EXPOSED TO CADMIUM IN FRESHWATER AND THEIR SUBSEQUENT SURVIVAL AFTER TRANSFER TO SEAWATER.

Nominal concentration (µg/L Cd)	Percent survival in freshwater	Percent survival in seawater
A. Feb. 26, 1976-Mar. 19, 1976 (22-day exposure)		
0	100[a]	100 (41)[b,c]
0.75	100	100 (42)
1.5	100	100 (42)
3.0	100	100 (41)
4.5	99.3	95.1 (41)
B. Mar. 12-18, 1976 (6-day exposure)		
0 (21)[b]	100	100 (16)[b,d]
3.0 (36)	100	96.7 (30)
4.5 (37)	100	96.7 (30)
C. Feb. 26, 1976 - April 9, 1976 (43-day exposure)		
0	100	100 (31)[b,e]
0.75	100	100 (42)
1.5	100	100 (42)
3.0	100	100 (45)
4.5	99.2	95.0 (40)

[a]*Originally placed 300-325 fish into each of 10 tanks.*
[b]*Number of fish exposed in parentheses.*
[c]*13 days exposure in SW.*
[d]*11 days exposure in SW.*
[e]*10 days exposure in SW.*

TABLE 5. SURVIVAL OF YEARLING COHO SALMON EXPOSED TO CADMIUM OR A CADMIUM-COPPER MIXTURE IN FRESHWATER AND THEIR SUBSEQUENT SURVIVAL AFTER TRANSFER TO SEAWATER.

Nominal concentration (µg/L Cd)	Percent survival in freshwater	ATPase activity[a] (freshwater)	Percent survival in seawater
A. April 28, 1976 - May 4, 1976 (6-day exposure)			
3.0	100		100 (31)[b,c]
4.5	100		93.5 (31)
B. Feb. 26, 1976 - May 6, 1976 (70-day exposure)			
0	100[d]	3.75	100 (41)[e]
0.75	100		100 (44)
1.5	100		100 (45)
3.0	100		100 (43)
4.5	99.2	4.80	97.7 (44)
C. Feb. 26, 1976 - May 5, 1976 (63-day of Cd; last 6 days Cd + Cu)			
0	100	3.75	100 (41)[f]
10 Cu	100	2.0	82.9 (41)
0.75 Cd + 10 Cu	100		57.1 (42)
1.5 Cd + 10 Cu	100		75.6 (41)
3.0 Cd + 10 Cu	100		87.2 (39)
4.5 Cd + 10 Cu	99.2	1.70	70.0 (50)

[a] *(Na, K)-stimulated ATPase activity (µmoles ATP hydrolyzed/mg protein/h).*
[b] *Number of fish exposed in parentheses.*
[c] *13 days exposure in SW.*
[d] *Originally placed 300-325 fish into each of 10 tanks.*
[e] *12 days exposure in SW.*
[f] *14 days exposure in SW.*

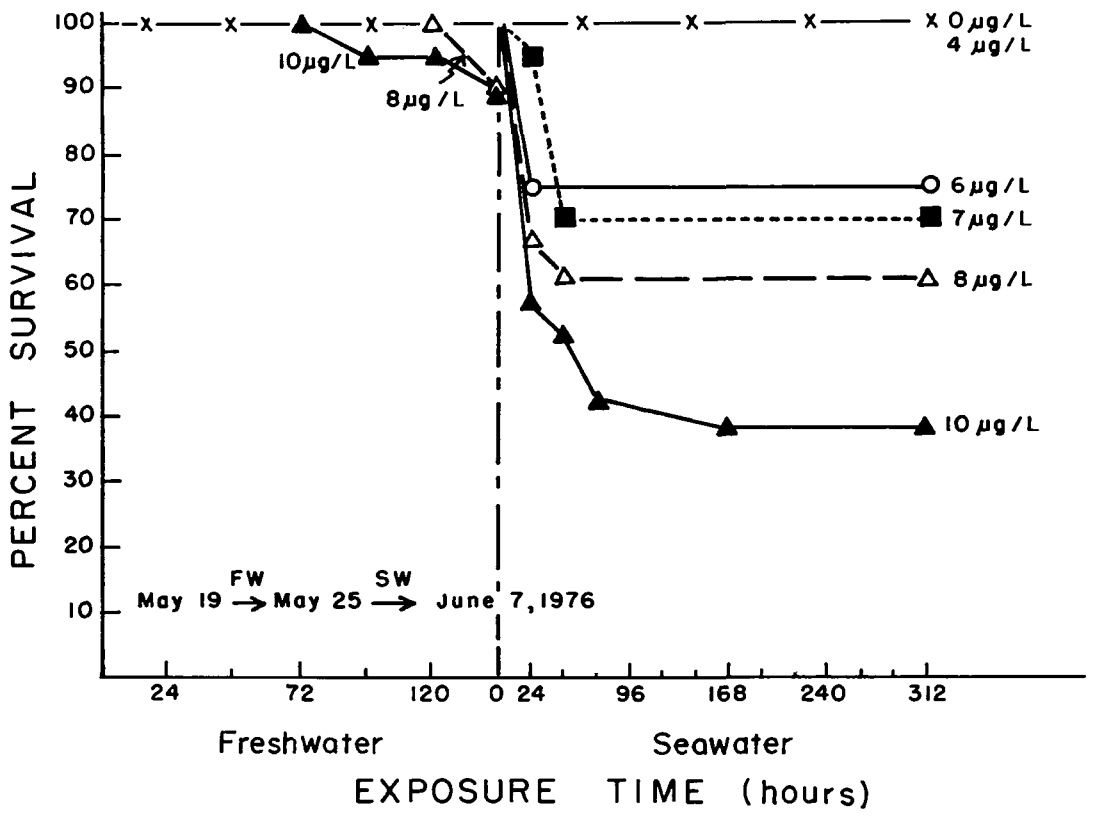

Figure 8. Percent survival of yearling coho salmon exposed to cadmium in freshwater (static) and subsequent survival upon transfer to seawater.

Fish exposed to 8 and 12 µg/L Cd in early February had mortalities of 48 and 60% respectively in the toxicant and subsequent mortalities of 90 and 100% respectively when transferred to seawater, (Table 3), however, only the 12 µg/L Cd fish had (Na, K)-stimulated ATPase activities significantly lower than the control fish (Table A-7). Since Cd-exposed fish died quickly when transferred to seawater, perhaps important metabolic systems other than osmoregulation were being affected. In coho salmon yearlings previously exposed to copper in freshwater, mortalities in seawater usually did not occur until the third or fourth day after transfer (Lorz and McPherson 1977). Osmoregulatory effects due to copper (increased osmolality of plasma following transfer to seawater) took 12-36 h to develop and about 200 h to return to normal.

The (Na, K)-stimulated ATPase activity peaked in mid-April and remained high through May in both the control fish and yearling coho salmon chronically exposed to Cd since February (Fig. 9a). There was no significant difference in specific activity in the groups that received 4.5 µg/L Cd as compared to controls. The group of fish exposed to 10 µg/L Cu for 6 days and the group exposed to Cd-Cu mixture exhibited significantly lower ATPase specific activity than the controls or the 4.5 µg/L Cd groups (Fig. 9b,

Table A-8). The whole g and microsomal methods of gill preparation showed similar patterns of ATPase activity (Table A-8).

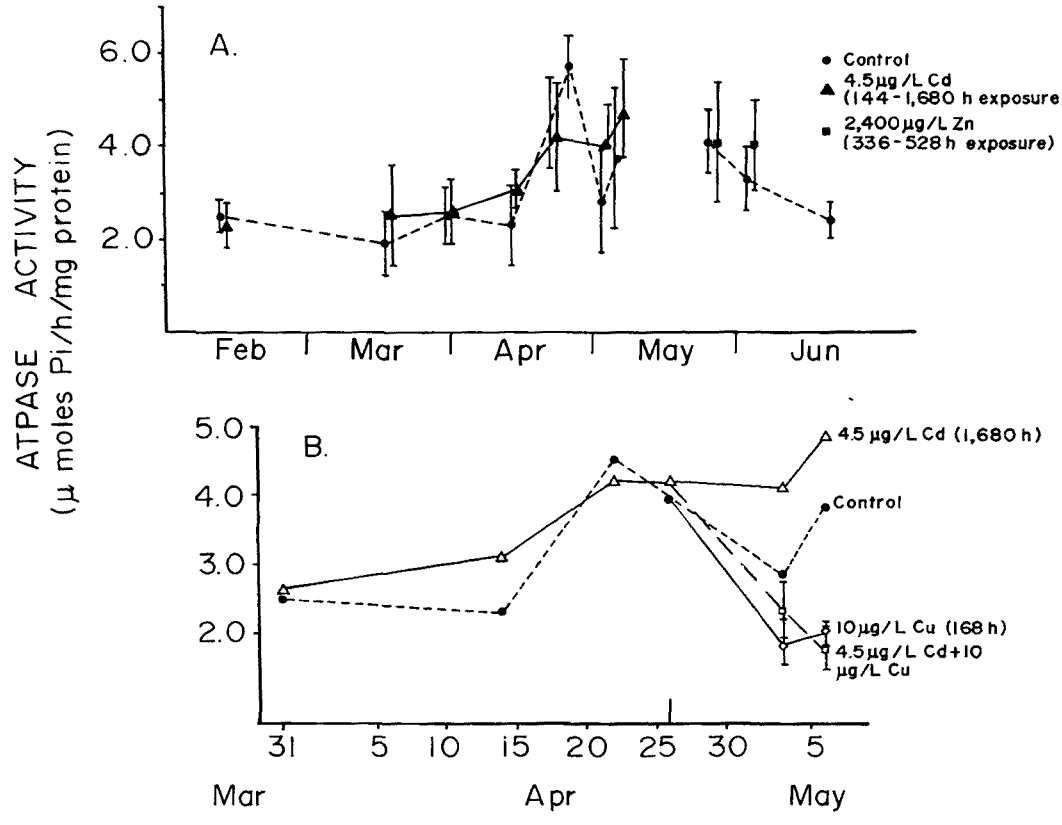

Figure 9. Gill (Na, K)-stimulated ATPase activity of yearling coho salmon during exposure to cadmium, zinc and cadmium-copper mixture.

Zinc

Fish chronically exposed to Zn for 13 and 28 days and then challenged with seawater had no mortalities in seawater (Table 6). However, most of the groups that received the Zn-Cu mixture (13 days) exhibited some mortaility when exposed to seawater, but when the Zn-Cu groups were exposed for a longer period (28 days) and then challenged with seawater only minimal mortality occurred (Table 6). The Na, K)-stimulated ATPase activity of yearling coho salmon exposed to Zn showed no significant difference from control fish while the groups receiving Cu or Zn-Cu exhibited significantly lower ATPase activity than the controls (Table 6, Table A-9). Considering that both the ATPase activity and coefficient of condition of the Zn-Cu exposed fish (Table 6, Fig. 5) following 28 days of exposure are significantly lower than controls, it is difficult to explain why so few fish died during the second seawater challenge (Table 6).

TABLE 6. SURVIVAL OF YEARLING COHO SALMON EXPOSED TO ZINC OR A ZINC-COPPER MIXTURE IN FRESHWATER AND THEIR SUBSEQUENT SURVIVAL AFTER TRANSFER TO SEAWATER.

Nominal con-concentration µg/L Zn	Percent survival in freshwater	ATPase activity[a] (freshwater)	Percent survival in seawater (310-h exposure)
A. Zinc (315 h May 12-26 SW June 7, 1976).			
0	100	4.09	100 (20)[b]
400	100		100 (20)
800	100		100 (21)
1600	99.4		100 (21)
2400	99.9	4.09	100 (21)
B. Zinc and copper (171 h Zn + 144 h Zn + Cu)			
10 Cu	100	2.55	100 (20)
400 Zn + 10 Cu	100		90 (20)
800 Zn + 10 Cu	100		100 (20)
1600 Zn + 10 Cu	100		90 (20)
2400 Zn + 10 Cu	100	1.42	77.8 (18)
C. Zinc (675 h May 12-June 9, 1976 SW June 22, 1976)			
0	100	3.3	100 (39)
400	100		100 (21)
800	100		100 (40)
1600	99.4		100 (40)
2400	99.0	4.1	100 (40)
D. Zinc and copper (171 h Zn + 504 h Zn + Cu)			
10 µg/l Cu	100	1.6	100 (40)
400 Zn + 10 Cu	100		100 (20)
800 Zn + 10 Cu	100		95.1 (41)
1600 Zn + 10 Cu	100		100 (40)
2400 Zn + 10 Cu	100	1.5	100 (39)

[a] *(Na, K)-stimulated ATPase activity (µmoles ATP hydrolyzed/mg protein/h).*
[b] *Number of fish in parentheses.*

Chromium and Nickel

Little mortality occurred during the seawater challenge of yearling coho salmon previously exposed to chromium or nickel for 144 h in freshwater (Table 7). The maximum level of Cr tested (5 mg/L) was 50 times the acceptable concentration (100 µg/L Cr - U.S.E.P.A. 1976). Thus, the water quality recommended for Cr appears adequate for coho salmon entering seawater. Sauter et al. (1976), however, recommended the maximum permissible concentration of chromium in the aquatic environment should be reduced. Similarly, the effect of interaction of metals or the effects of chronic exposure of these metals on the salmonids migratory disposition or subsequent tolerance to seawater is not known.

TABLE 7. SURVIVAL OF YEARLING COHO SALMON EXPOSED TO CHROMIUM OR NICKEL IN FRESHWATER AND THEIR SUBSEQUENT SURVIVAL AFTER TRANSFER TO SEAWATER.

Nominal concentration mg/L	Percent survival in freshwater (144-h exposure)	Percent survival in seawater	
A. Chromium (March 9-15 SW -March 25, 1976)			
0	100	100	(20)[a]
0.03	100	100	(19)
0.05	100	100	(20)
0.08	100	100	(20)
0.10	100	100	(20)
0.30	100	100	(20)
0.50	100	100	(20)
3.0[b]	100	100	(11)
5.0[b]	100	91.7	(12)
B. Nickel (April 2-8 SW - April 19, 1976)			
0	100	100	(20)
0.25	100	100	(20)
0.50	100	100	(20)
0.75	100	95	(20)
1.0	100	100	(20)
2.5	100	100	(21)
4.0	100	100	(20)
5.0	100	100	(20)

[a]*Number of fish exposed in parentheses*
[b]*Exposed to chromium for 96 hours prior to transfer to seawater.*

Mercury

Coho salmon yearlings previously exposed to mercuric chloride for 144 h were transferred to seawater and their survival monitored. Deaths occurred in all concentrations ≥50 µg/L Hg in a dose dependent manner (Fig. 10). The mortalities in seawater occurred within the first 72-h exposure, which is similar to the results observed for Cd-exposed fish (Fig. 7 and 8), but dissimilar to that noted for Cu-exposed fish (Lorz and McPherson 1976). The (Na, K)-stimulated ATPase activity of coho salmon exposed to Hg (≥50 µg/L) was suppressed but the decrease was not statistically significant (Table A-10).

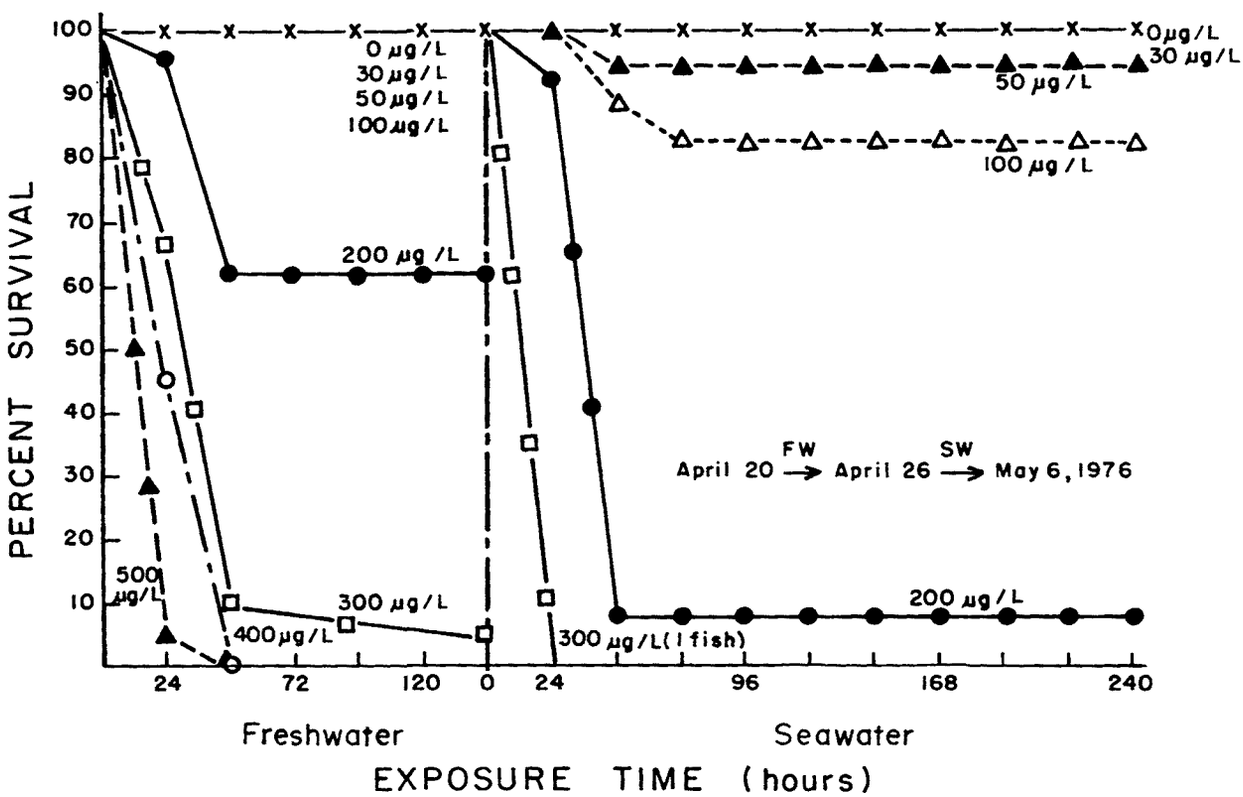

Fig. 10. Percent survival of yearling coho salmon exposed to inorganic mercury ($HgCl_2$) in freshwater (static) and subsequent survival upon transfer to seawater.

EFFECT OF METAL EXPOSURE ON PLASMA OSMOTIC PROPERTIES

Exposure of yearling coho salmon to Cd in freshwater did not appear to affect their ability to maintain either normal osmolality or chloride concentrations of blood plasma although a statistically non-significant decrease in osmolality and chloride ion concentration was noted following 96-h exposure to 12 µg/L Cd (Figs. 11 and 12 Table A-11). Larsson (1975) noted that Cd had only slight effects on the major plasma electrolytes, sodium and chloride, in flounders but found the levels of other plasma ions (K, Ca and Mg) were remarkably changed after Cd exposure. The osmolality of the plasma of fish exposed to Zn, or 10 µg/L Cu did not differ statistically from controls. Similarly, Skidmore (1970) found that the osmotic concentration and the concentrations of Na, K, Ca, Mg and Zn in blood were largely unaffected in rainbow trout immobilized by zinc sulfate. Coho that had received the Zn-Cu (2400 µg/L Zn + 10 µg/L Cu) mixture for 480 h exhibited reduced osmolality (Fig. 11). The lack of effect of Cd on plasma osmolality and plasma chloride is in contrast to the changes in blood parameters noted in coho, brook trout and brown bullhead exposed to sublethal copper concentrations (Christensen et al. 1972, Lorz and McPherson 1977, McKim et al. 1970). However, McCarty and Houston (1976) found found that goldfish exposed to sublethal levels of cadmium (44.5 and 380 µg/L for a period of 25 days showed significant changes in plasma chloride. Goldfish held in 44.5 µg/L had apparently compensated for most of the initial cadmium effect after 50 days exposure while those exposed to 380 µg/L exhibited significant deviations in plasma sodium and chloride levels.

EFFECT OF METAL EXPOSURE ON CORTICOSTEROID RESPONSE

To determine the feasibility of using plasma cortisol levels as an indicator of heavy metal toxicity, yearling coho salmon were exposed to Cu and Cd and the cortisol level of the plasma measured. The work was carried out cooperatively with Dr. C. B. Schreck, Oregon Cooperative Fishery Research Unit, Oregon State University. The basic findings of the study were as follows: Cu produced a marked, dose-dependent cortisol stress response, but Cd did not elicit a cortisol elevation, even in moribund fish. Handling and confinement produced similar cortisol elevation in controls and Cu or Cd treated fish, but Cu exposure reduced the salmon's ability to survive the stress of handling and confinement (Schreck and Lorz 1978). Two groups of coho salmon were treated to determine the potential additive or synergistic effects of low levels of Cu and Cd. Both groups received Cu at 10 µg/L as $CuCl_2$ on April 29, 1976. One group, however, had been exposed continuously since February 26, 1976 to 4.5 µg/L Cd, and thus received both Cu and Cd simultaneously. Following 96 h of Cu exposure, serum was collected from 10 fish for each treatment. The mixture of the two metals did not produce an elevation in cortisol [$\bar{x}$ = 46.6 $\pm$ 9.1 (SE)]; their cortisol level was similar to that of fish exposed to 10 µg/L Cu only [$\bar{x}$ = 51.5 $\pm$ 8.6 (SE)].

Groups of coho salmon exposed to Hg as $HgCl_2$ for 120 h were sampled for cortisol. Although the data were not conclusive, it appeared that the Hg caused an elevation in plasma cortisol levels; mean titers ($\pm$ SE) were 37.4 $\pm$ 5.9 for the control and 66.9 $\pm$ 9.0, 51.0 $\pm$ 14.1, and 85.5 $\pm$ 6.7 for

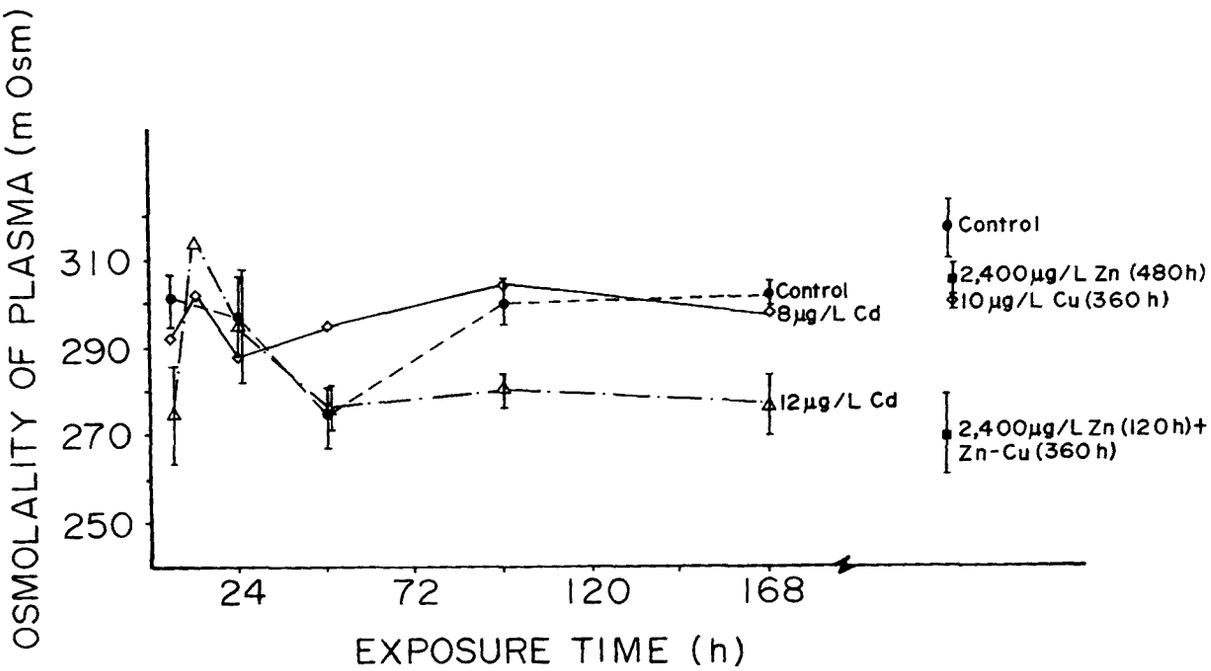

Figure 11. Effect of cadmium exposure in freshwater on the plasma osmolality of coho salmon. Mean ± SE (effect of zinc, copper and zinc-copper exposure on plasma osmolality). n = 5.

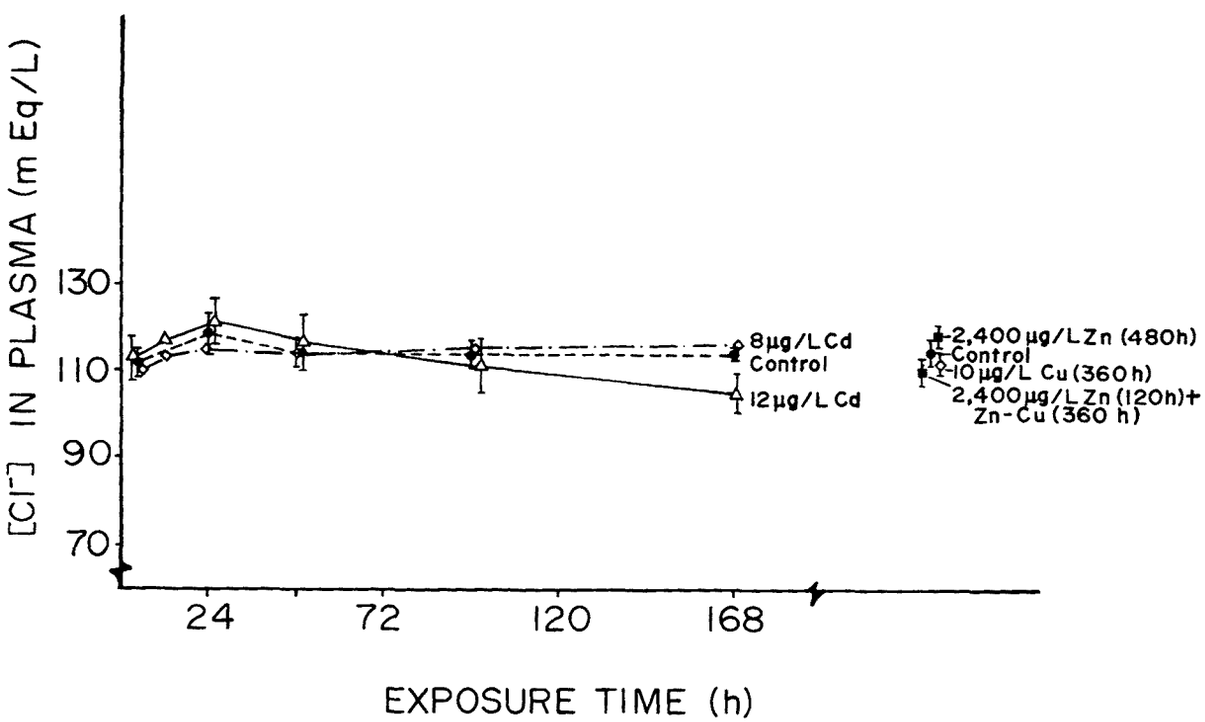

Figure 12. Effect of cadmium exposure in freshwater on the chloride ion concentration of plasma of coho salmon. Mean ± SE (effect of zinc, copper, and zinc-copper exposure on chloride ion concentration). n = 5.

the 30, 50, and 100 µg/L Hg treatments, respectively. The mean level produced by the 100 µg/L Hg was not only higher than the control groups, but also higher than any mean of a control found in the other experiments. We concluded (Schreck and Lorz 1978) that cortisol and other characteristics of the general adaptation syndrome of stress should not be universally applied as indicators of heavy metal stress.

EFFECT OF METAL EXPOSURE ON DOWNSTREAM MIGRATION

The proportion of released test fish that migrated downstream in Crooked Creek was almost always greatest for control fish (Table 8), but there was not a dose-dependent response as noted for Cu exposed fish (Lorz and McPherson 1977). In the fish chronically exposed to Cd or Zn some groups migrated better than the controls but not in a consistent manner (Table 8). Most downstream migration occurred within the first 10 days of release and often the major portion was completed in 5 days (Table A-12).

A 7 km section of the stream was electrofished from above the release sites to the weir in an attempt to collect non-migrants. Only two yearling coho that had been released earlier were found, although native cutthroat and rainbow of comparable size to the released fish were caught. There were only two small areas in the stream where our electrofishing gear appeared inadequate because of the depth of water. Thus, fish that had not migrated by early July apparently died as a result of predators, stress, natural causes or other indirect effects of the toxicant exposure.

Cadmium

The first release of Cd-exposed fish was made in mid-April. The controls of the acutely exposed fish migrated best, although there was only 7% difference in rates of migration of the five release groups (Fig. 13A). The chronically exposed fish (47 days) did not migrate as well as the acutely exposed groups but there was no suggestion of a dose response (Fig. 13B). It appeared that Cd had little effect on migratory disposition.

A second release of 15 groups of yearling coho salmon (5 groups exposed for 6 days; 5 groups exposed for 67 days; and 5 groups exposed to Cd for 67 days plus 10 µg/L Cu for the last 5 days prior to release) was made into Crooked Creek in early May. There appeared to be little effect of Cd on the downstream migration of either acutely or chronically exposed fish, although the former groups exhibited better migration (Fig. 14, Table 8). In the acutely exposed groups the controls exhibited the best migration and although there was no overall dose-response exhibited, the 4.5 µg/L group showed 16% less migratory tendency than the controls (Fig. 14A). The chronically exposed groups that received Cu for 5 days in addition to the Cd showed a decrease in their migratory disposition (Table 8, Fig. 14B). The Cd-Cu mixture appeared to act synergistically, as migration was inhibited to a greater extent than noted for fish exposed to Cu alone (Fig. 14B).

TABLE 8. PERCENT MIGRATION THROUGH JULY 6, 1976 OF YEARLING COHO SALMON RELEASED INTO A SMALL COASTAL STREAM FOLLOWING ACUTE OR CHRONIC METAL EXPOSURE.

Exposure and nominal concentration µg/L	Percent Migration 4/14/76[a]	5/5/76[a]
A. Chronic exposure[b]		
Control	60.2	67.0
10 Cu		62.3
0.75 Cd	68.7	69.9
0.75 Cd + 10 Cu		47.0
1.5 Cd	62.4	55.6
1.5 Cd + 10 Cu		51.4
3.0 Cd	65.9	69.3
3.0 Cd + 10 Cu		56.7
4.5 Cd	59.4	66.3
4.5 Cd + 10 Cu		54.8
B. Acute exposure (144 h)		
Control	80.2	85.1
0.75 Cd	77.8	71.8
1.5 Cd	73.5	67.6
3.0 Cd	73.5	80.7
4.5 Cd	73.7	69.0
	5/26/76[a]	6/9/76[a]
C. Chronic exposure[c]		
Control	80.2	71.2
10 Cu	63.7	65.4
400 Zn	75.9	73.1
400 Zn + 10 Cu	52.5	58.1
800 Zn	71.0	67.5
800 Zn + 10 Cu	47.0	61.9
1600 Zn	82.0	67.6
1600 Zn + 10 Cu	34.0	63.5
2400 Zn	66.0	74.5
2400 Zn + 10 Cu	37.7	64.5
D. Acute exposure (144 h)		
Control	No release	81.5
10 Cu	"	66.1
400 Zn + 10 Cu	"	72.0
800 Zn + 10 Cu	"	67.2
1600 Zn + 10 Cu	"	53.1
2400 Zn	"	62.1
2400 Zn + 10 Cu	"	58.3

[a]*Date of release.*
[b]*Exposed to Cd since Feb. 26, 1976.*
[c]*Exposed to Zn since May 10, 1976*

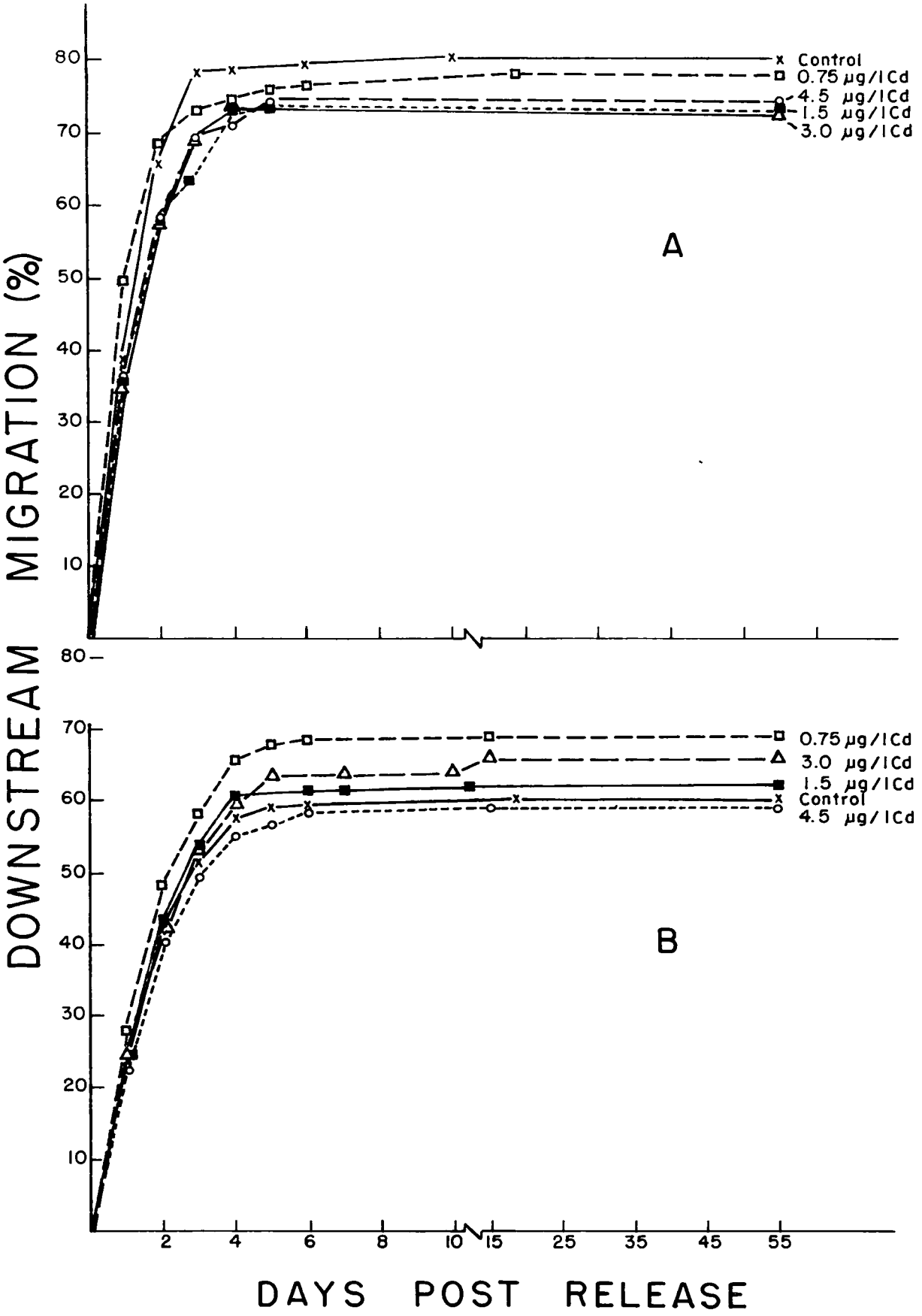

Figure 13. Percent downstream migration of yearling coho salmon previously exposed to cadmium in freshwater and released April 14, 1976. A. represents 94-102 fish per group exposed for 144 h. B. represents 180-192 fish per group exposed for 47 days.

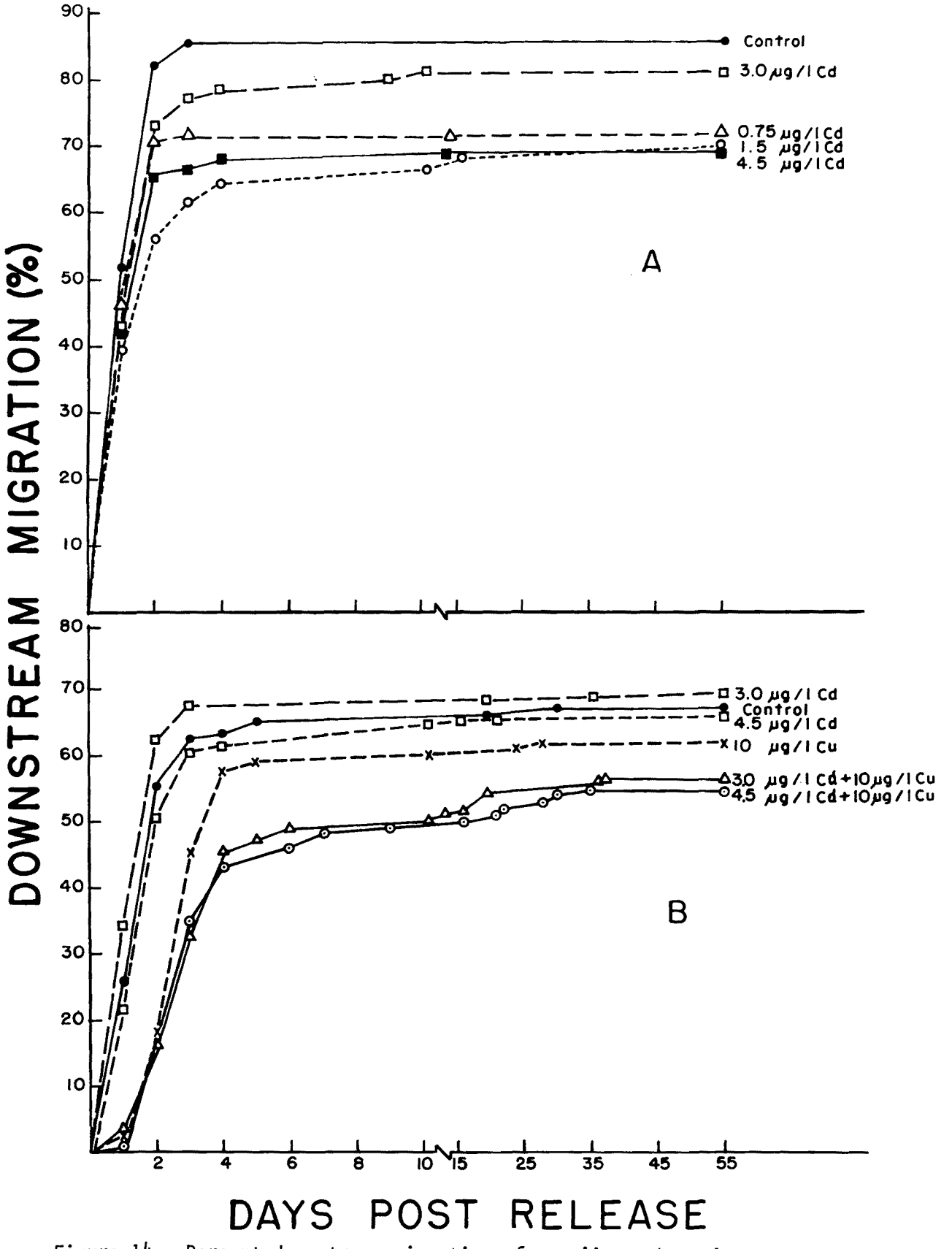

Figure 14. Percent downstream migration of yearling coho salmon previously exposed to cadmium, copper, and cadmium-copper mixtures in freshwater and released May 5, 1976. A. represents 67-88 fish per group exposed for 144 h to cadmium. B. represents 100-114 fish per group exposed to cadmium (67 days), copper (5 days), or cadmium-copper mixture (62 days Cd + 5 days Cd-Cu).

Zinc

A release made in late May of 10 groups of yearling coho salmon showed that movement of the groups exposed to Zn was similar to that of the controls, except in the highest Zn group where fewer migrants were observed (Fig. 15, Table 8). In the group exposed to Cu and the groups exposed to a Zn-Cu mixture fewer migrants were observed than in the controls or the corresponding Zn (only) groups (Fig. 15, Table 8). The reduced movement of the groups exposed to the Zn-Cu mixture again showed the apparent synergistic action of the combined metals as well as indications of a dose-dependent response.

Another release of yearling coho salmon exposed to Zn, Cu, or Zn-Cu mixtures was made in early June. In the acutely exposed groups (6 days) the controls showed the best migration while those receiving 2400 µg/L Zn or 10 µg/L Cu migrated at a lesser intensity (Table 8, Fig. 16A). The groups receiving the highest Zn-Cu mixtures (1600 and 2400 µg/L Zn + 10 µg/L Cu) migrated with considerably less intensity than controls (Fig. 16A), and this was similar to the migration patterns exhibited in the earlier release (Fig. 15). In the chronically exposed groups (27 days), controls were intermediate in their movement compared to the four Zn groups (Table 8, Fig. 16B). The fish from the highest Zn concentrations which had previously exhibited less movement than controls (Fig. 15) exhibited slightly better migration than the controls in the June release. Whether the yearling coho salmon had acclimated during the extended exposure or whether the downstream movement was complicated by the lower June streamflow is not known. The groups that received only Cu or the Zn-Cu mixture migrated better than comparable groups released in late May although their migration was still 6 to 13% less than the controls or corresponding groups exposed only to Zn (Fig. 16B). Similarly, the groups that received the longer exposure to Zn or Zn-Cu mixture (27 days) appeared to have acclimated and responded with greater survival in the seawater challenge test than the groups that had been exposed to Zn for 13 days plus Cu for the last 6 days (Table 6). Sprague (1970) reviewed several studies that reported acclimation to zinc. Lloyd (1960) found increased survival times for trout following two weeks of acclimation, however Sprague (1968a) found that the avoidance threshold, a sublethal response of rainbow trout, was not influenced by acclimation. Chapman (1978) found that chinook salmon fry acclimated to Cu and Zn (but not to Cd) survived concentrations of Cu and Zn which were lethal to non-acclimated salmon. However, he found there was an optimal concentration for Cu acclimation (9-16 µg/L). In avoidance studies Chapman (1978) noted that 80% of the control fish (steelhead trout fry) avoided Cu at 10 or 20 µg/L, but none of the acclimated fish avoided any tested concentration, even ones that was lethal. Thus, Chapman stated, "the development of acclimation may be a mixed blessing; it allows existence in a marginal environment but interferes with behavioral defense mechanisms."

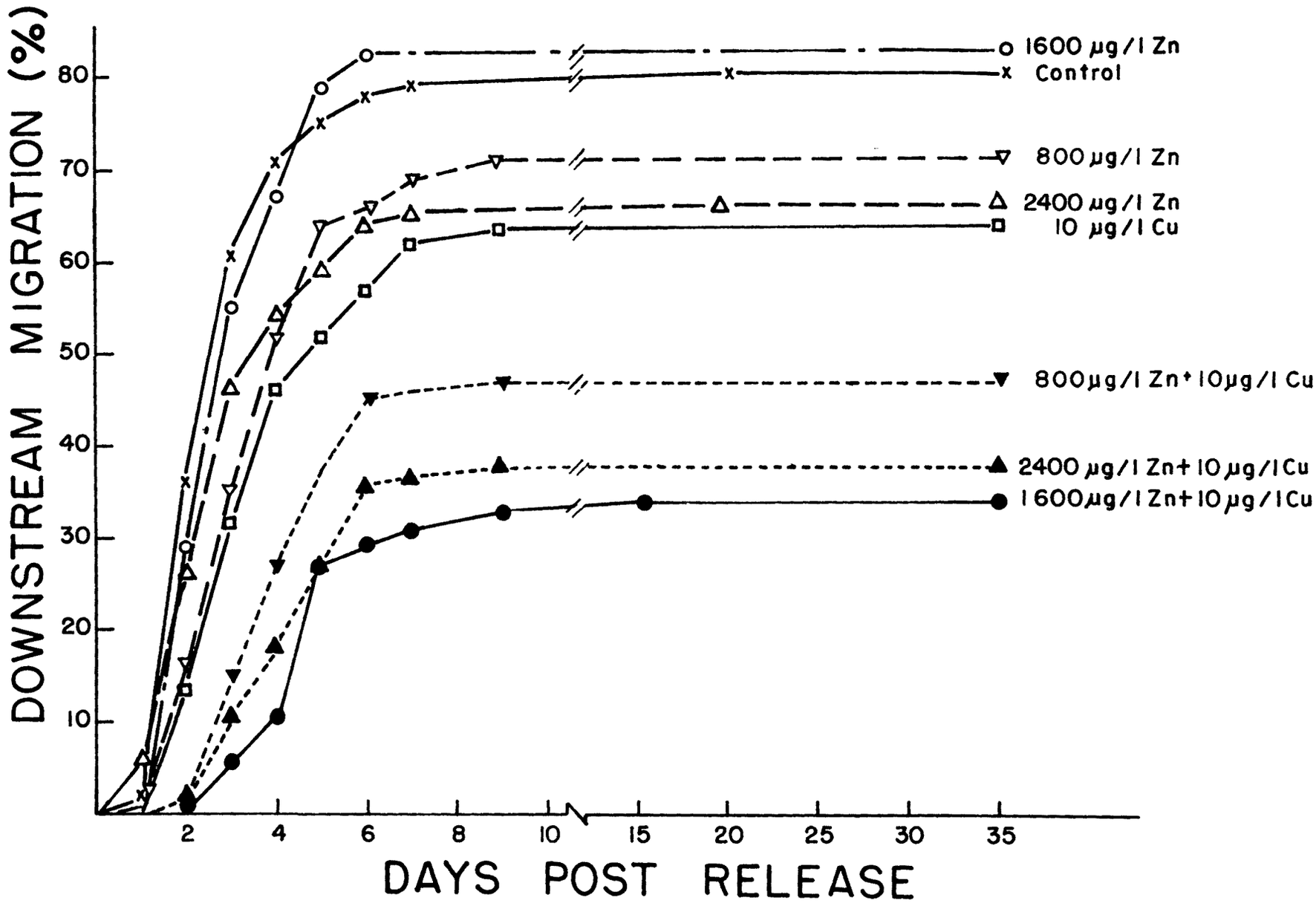

Figure 15. Percent downstream migration of yearling coho salmon previously exposed to zinc, copper, and zinc-copper mixtures in freshwater and released May 26, 1976. Each line represents a release of 100-106 fish previously exposed for; a) 13 days to zinc, b) 6 days to Cu, and c) 7 days to Zn + 6 days Zn-Cu.

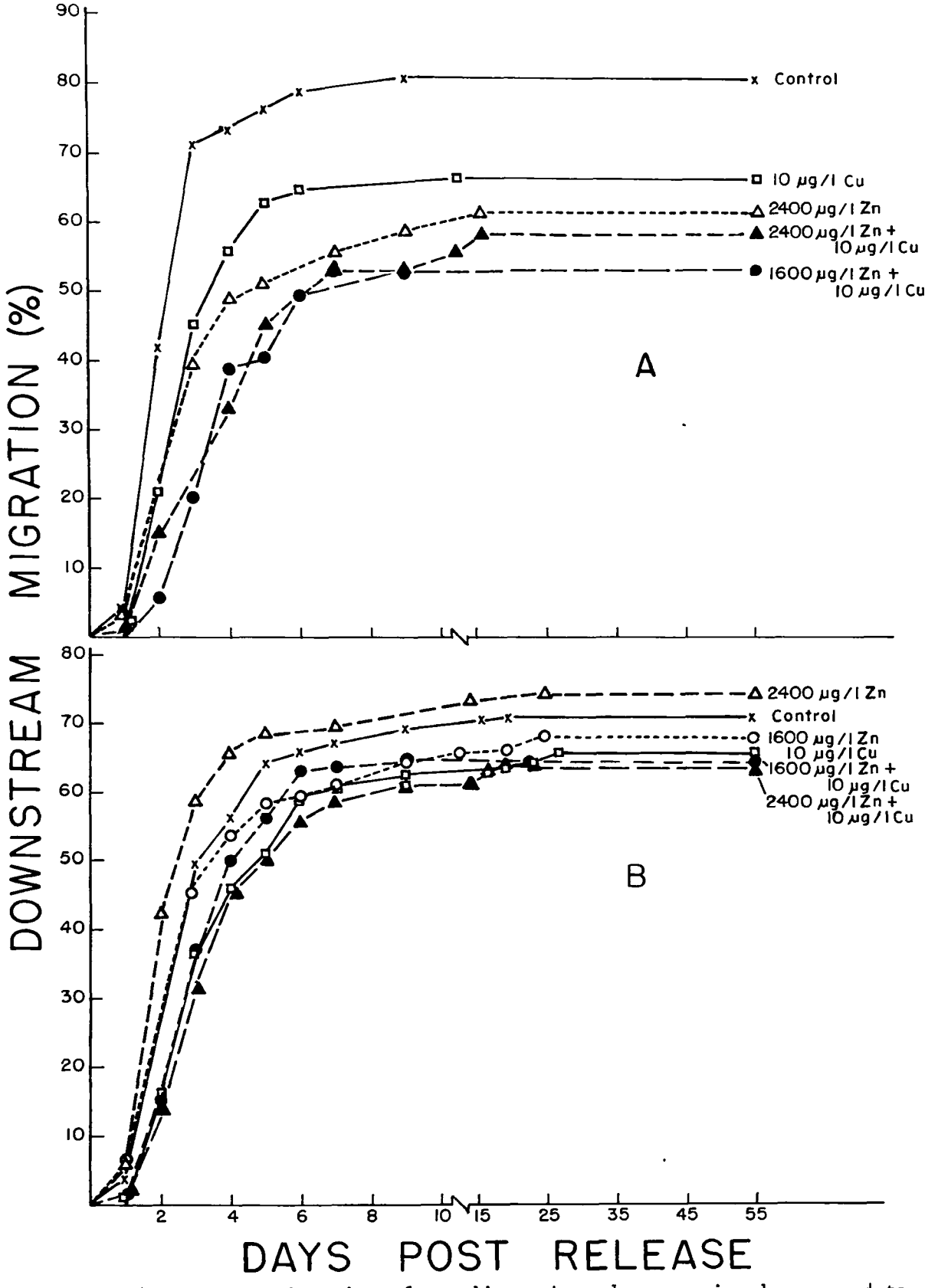

Figure 16. Percent migration of yearling coho salmon previously exposed to zinc, copper and zinc-copper mixtures in freshwater and released June 9, 1976. A. represents 50-65 fish exposed for 144 h to zinc, copper, or zinc-copper mixture. B. represents releases of 102-110 fish exposed for; a) 27 days to Zn, b) 20 days to Cu, and c) 7 days to Zn + 20 days Zn-Cu.

SECTION VI

DISCUSSION

As mentioned in the introduction, considerable laboratory research has been done to determine water quality criteria for cadmium, copper, and zinc based on survival and growth of juvenile salmonids in freshwater (Chapman 1973, 1975, Christensen 1975, Hodson and Sprague 1975, Lloyd 1960, McKim and Benoit 1971, 1974, Sauter et al. 1976, Sinley et al. 1974, Sprague 1964a, and Sprague and Ramsey 1965). However, only a few field studies are available where contaminants in the natural environment have been related to effects on fish populations (Geckler et al 1976, Goettl et al. 1973, Grande 1967, Herbert et al. 1965, Nehring and Goettl 1974, Södergren 1976, and Sprague et al. 1965). There are also several laboratory studies available where researchers working with mixtures of metals have described their findings. Lloyd (1961) noted that copper and zinc were additive in their toxicities, in acute threshold mortality tests, in both hard and soft water. Sprague and Ramsay (1965) also observed summation of copper and zinc toxicity to Atlantic salmon in soft water whereas; Anderson (1973) working with guppies *(Poecilia reticulata)* described his results of copper and zinc mixtures as "supra-additive" while mixtures of copper and nickel were concentration additive. Brown (1968), Brown et al. (1969), and Brown and Dalton (1970) described a method of predicting toxicities of mixtures of substances based on 48-h LC50 determinations and assuming direct summation. Their 48-h LC50 values were thought to provide a "reasonable estimate" of acute threshold concentrations. However, Eaton (1973) studied the effects of chronic toxicity of cadmium, copper and zinc mixtures to fathead minnows *(Pimephales promalas* Rafinesque), and he concluded that the toxicity of the trimetal mixture could not have been predicted closely from the results of single-toxicant chronic tests by assuming they were totally additive in their toxic units. Eaton noted (acute tests) that it took considerably less metal in combination to obtain a threshold value and therefore suggested that some toxicity-increasing interaction was occurring. Similarly, Eisler and Gardner (1973) working with the estuarine teleost *(Fundulus heteroclitus)* noted that mixtures of Cu and Zn (96-h bioassay studies) produced more deaths than expected on the basis of toxicities of individual components. If concentrations of Cd not ordinarily lethal were added, they exerted a negative effect on survival of fish previously intoxicated by salts of Cu, Zn, or both. Spehar et al. (1978) found that when flagfish *(Jordanella floridae)* were exposed to sublethal levels of cadmium and zinc that the action of the toxicants was not additive, but a joint action was indicated. Effects on survival showed that the toxicity to cadmium and zinc mixtures was little if any greater than the toxicity of zinc alone.

The early research studies have served to determine the relative sensitivities of aquatic organisms to various metals or their mixtures and the effects of water quality parameters such as alkalinity, hardness, pH, and dissolved oxygen concentration. However, in the last 10 years considerable progress has been made toward determining the relationship of toxicity of heavy metals to their aqueous chemistry:i.e., toxicity of concentrations of ionic and molecular forms in solution, physiological mechanism involved, and application of the results to field situations. Andrew (1976) reviewed some of the literature that demonstrated chelation of copper by organic reagents effectively reduced its toxicity and summarized EPA's recent experiments that attempted to clarify the relationship between copper toxicity and water chemistry. Sprague (1968b) found that use of the trisodium salt of NTA (nitrilotriacetate) increased the tolerance of Atlantic salmon to copper up to 33 times the lethal threshold. Similarly, Biesinger et al. (1974) noted that chelates of copper or zinc with NTA are relatively non-toxic to *Daphnia magna*. Wilson (1972) observed that addition of pulp mill spent sulphite liquor, allowed Atlantic salmon parr to survive at copper concentrations considerably greater than their 96-h LC50 values. Wilson found that chelation of copper by sulphite liquor greatly increased the difficulty in developing a simple predictive model for copper toxicity in receiving waters. Similarly, Zitko et al. (1973) noted that the addition of humic acid to solutions of copper or zinc increased the incipient lethal level of copper by binding cupric ions but did not affect the ILL of zinc to juvenile Atlantic salmon. Chynoweth et al. (1976) noted that bound copper was less toxic than unbound copper to guppies *(Lebistes reticulatus)* when several organic compounds were added to copper bioassays. An inverse relationship was observed between the degree of copper binding and copper toxicity. Similarly, Brown et al. (1974) noted that the toxicity of a given total concentration of copper to rainbow trout was reduced with additions of sewage effluent, an amino acid, humic substances, or suspended organic matter. These authors concluded that data from toxicity tests with copper in which natural surface waters are used for dilution purposes cannot define the true toxicity of copper or have application to other natural waters except when the concentration of the toxic chemical species is known.

Stiff (1971), using equilibrium equations investigated the formation of complex carbonate species in copper-bicarbonate solutions and suggested that the difference in toxicity of copper to fish with differences in water hardness might be dependent upon carbonate complexation. Stiff proposed that the free cupric ion was the primary toxic form. This conclusion is supported by Pagenkopf et al. (1974) who examined published copper toxicity data, developed an equilibrium model, amd postulated that $CuOH^+$ might also be involved in the toxicity. Chapman and McCrady (1977) using a computer program computed the free copper concentrations for a variety of alkalinities and pH values and obtained results similar to Stiffs', however they suggested that pH may be an important factor in copper toxicity in addition to its usual association with alkalinity.

Hara et al. (1976) noted that, "chemosensory receptors are immediate detectors of environmental chemical stimuli. Environmental changes are detected by transducer elements of the receptors and information is subsequently carried along nerve fibers to various integrating centers of the

body. Olfaction plays an important role in the survival of fish. It mediates such diverse phenomena as feeding, recognition of prey and predator, sexual and social behavior, orientation, and migration. Since the olfactory membranes are not protected by external barriers, man-made alterations in water quality could easily interfer with their functioning, the consequences being a breakdown in communication among fish and the environment." Earlier, Hara (1972) found that pretreatment of the nasal cavity of sockeye or coho salmon with solutions of $HgCl_2$ or $CuSO_4$ for as little as 10 seconds completely blocked the olfactory response. However, the effect was reversible. Hara et al. (1976) continued the studies and found that the olfactory responses to a standard stimulant in rainbow trout could be depressed by mercury and copper at sublethal concentrations. The lowest concentration of mercury and copper to cause appreciable effects within 2 h was estimated at 100 and 8 μg/L, respectively. The authors speculated that these metals may possibly interfere with the perception of the normal homestream odors that guide salmon to their spawning tributary. Exposure of 0.5-5.0 mg/L Cu for 6-24 h permanently damaged the olfactory mucosa of some estuarine fishes (Gardner and LaRoche 1973), and Hara et al. (1976) suggest that persistent binding of heavy metals to receptor sites at sublethal concentrations may also result in irreversible damage to the receptor cells. Voyer et al. (1975) noted tissue damage in nasal passages and buccal cavities of mummichogs exposed to 28 mg/L Cd for 6.5 hours.

The insecticides, DDT and Sumithion (fenitrothion) have been shown to modify salmonid physiology, behavior and learning ability following exposure to sublethal concentrations (Anderson 1971, Bull and McInerney 1974, Hatfield and Anderson 1972, Symons 1973, and Warner et al. 1966). Similarly, studies on sublethal effects of metal pollutants have elicited changes in the physiology and behavior of fishes (Kania and O'Hara 1974, Kleerekoper 1973, and Seller et al. 1975). Recently, Sullivan et al. (1978) discussed changes in prey-fish behavior resulting in selective predation of stressed prey at concentrations of the toxicant well below lethal levels. Sullivan et al. (1978) were able to demonstrate increased prey vulnerability of the fathead minnow *(Pimephales promelas)* exposed to acute (24 h) and subacute (21 day) sublethal cadmium levels prior to interacting with largemouth bass *(Micropterus salmoides)*. Prey exposed to cadmium displayed altered behavior patterns including abnormal schooling behavior at concentrations of cadmium previously suggested to be safe for fathead minnows (Pickering and Gast 1972). Sullivan et al. recommend that behavioral studies be an integral part of future bioassay studies where water quality criteria are being determined. Similarly, Solbe and Flook (1975) observed a behavioral change in the stone loach, *Noemacheilus barbatulus* (L), the loss of instinct to hide in daylight, following exposure to cadmium. They suggested this behavioral change made the stone loach more vulneralbe to predation and thus may explain the stone loach's absence from parts of the River Tean.

In a recent study. Woltering et al. (1978) found that predator-prey interactions of largemouth bass and the mosquitofish *(Gambusia affinis)* were altered depending on the density of the prey species and ammonia concentration. In the study both species were simultaneously and continuously exposed to

the toxicant (ammonia) for the duration of the experiment. Woltering et al. found that the largemouth bass were more sensitive than the mosquitofish to ammonia and at high ammonia and high prey densities the harassment of the predator by the prey was generally lethal to the bass.

From this brief review of the literature and the results obtained in our studies it is apparent that additional research is necessary to determine whether metal exposures in the early life stages or during smolting in salmonids could result in permanent damage to the population. In our studies we observed reduced seawater tolerance and migratory response in some metal intoxicated fish, however, we do not know if the effects would result in lowered adult returns.

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TABLE A-1. MEASURED CONCENTRATIONS OF CADMIUM AND COPPER DURING EXPOSURE TESTS.

Tank No.	Nominal concentration µg/L	Measured concentration µg/L Mean ± SD[a]	Range
Cadmium exposure (Feb. 27-May 6, 1976) n = 10			
E- 1	0	0.09 ± 0.19	0 1.6
E- 2	0	0.02 ± 0.35	0 - 1.0
E- 3	1.5	1.41 ± 0.15	1.2 - 1.7
E- 4	1.5	1.41 ± 0.10	1.3 - 1.6
E- 5	3.0	2.73 ± 0.21	2.3 - 3.0
E- 6	3.0	2.69 ± 0.17	2.4 - 2.9
E- 7	0.75	0.91 ± 0.38	0.6 - 1.6
E- 8	0.75	0.78 ± 0.24	0.6 - 1.4
E- 9	4.5	3.90 ± 0.31	3.6 - 4.5
E-10	4.5	4.05 ± 0.39	3.6 - 4.9
Copper exposure (April 30-May 6, 1976) n = 1			
E- 1	0	1.7[b]	
E- 2	10	29.3[c]	
E- 4	10	17.0	
E- 6	10	13.1	
E- 8	10	10.4	
E-10	10	10.5	

[a]*Results of weekly composite water samples.*
[b]*Results of a 6-day composite water sample.*
[c]*Suspected contamination of sample container.*

TABLE A-2. MEASURED CONCENTRATIONS OF ZINC AND COPPER DURING CHRONIC EXPOSURE TESTS.

Tank No.	Nominal concentration µg/L	Measured concentration µg/L Mean ± SD[a]	Range
Zinc exposure (May 12-June 9, 1976) n = 4			
E- 1	0	4.8 ± 5.1	0 - 12
E- 2	0	7 ± 6.5	0 - 13
E- 3	800	698 ± 67.0	613 - 768
E- 4	800	721 ± 26.1	687 - 746
E- 5	1600	1384 ± 95.1	1273 - 1482
E- 6	1600	1391 ± 96.0	1285 - 1507
E- 7	400	354 ± 18.0	331 - 369
E- 8	400	359 ± 16.3	340 - 374
E- 9	2400	2027 ± 202.9	1737 - 2180
E-10	2400	2087 ± 187.1	1810 - 2210
Copper exposure (May 19-June 9, 1976) n = 3			
E-1,3,5,7,9	0	2.5 ± 1.2	1.1 - 4.5
E- 2	10	13.2 ± 3.1	10.9 - 16.8
E- 4	10	12.4 ± 2.1	10.1 - 14.3
E- 6	10	12.3 ± 1.0	11.5 - 13.4
E- 8	10	12.5 ± 1.1	11.3 - 13.4
E-10	10	12.1 ± 0.6	11.4 - 12.6

[a]*Results of weekly composite water samples.*

TABLE A-3. MEASURED CONCENTRATIONS OF THE METALS; CHROMIUM, NICKEL, AND MERCURY DURING ACUTE EXPOSURE TESTS.

Tank no.	Nominal concentration µg/L	Measured concentration µg/L Mean ± SD[a]
Chromium (March 9-15, 1976) n = 6		
21, 31	0	12 ± 0
22, 23	30	32 ± 14
24, 25	100	113 ± 14
26, 27	80	95 ± 13
28	5,000	-
32, 33	500	543 ± 48
34, 35	300	349 ± 73
36, 37	50	62 ± 10
38	3,000	-
Nickel (April 2-8, 1976) n = 6		
21, 22	0	0
23, 24	1,000	1118 ± 76
25, 26	500	530 ± 87
27, 35	750	828 ± 34
31, 32	250	285 ± 40
33, 34	5,000	5275 ± 413
36, 37	4,000	4145 ± 97
28, 38	2,500	2610 ± 65
Mercury (April 20-26, 1976) n = 6		
21, 22	50	28 ± 24[b]
23, 24	400	317 ± 47
25, 26	0	0
27, 28	300	284 ± 53
31, 32	500	500 ± 50
33, 34	30	22 ± 8
35, 36	100	67 ± 12
37, 38	200	193 ± 10

[a]*Results of daily grab water samples.*
[b]*Due to excessive storage time these values may not be accurate.*

TABLE A-4. MERCURY CONTENT OF SELECTED TISSUES OF COHO SALMON EXPOSED TO VARIOUS CONCENTRATIONS OF MERCURIC CHLORIDE.

Nominal concentration µg/L	Exposure time (h)	Tissue	ng Hg/mg tissue[a] (wet weight)
300	96	Gill	37.6
300	96	Liver	8.0
300	96	Kidney	22.9
300	96	Muscle	0.8
400	48	Gill	43.4
400	48	Liver	5.3
400	48	Kidney	17.0
400	48	Muscle	0.8
400	48	Testes	0
500	24	Gill	62.8
500	24	Liver	4.5
500	24	Kidney	15.6
500	24	Muscle	0.3

[a]*One fish per concentration analyzed with Coleman Mercury Analyzer.*

TABLE A-5. EFFECTS OF CADMIUM OR CADMIUM-COPPER EXPOSURE ON AVERAGE LENGTH, WEIGHT, AND CONDITION FACTOR OF COHO SALMON.

Date of sample	Days of exposure	Nominal concentration µg/L Cd	Cu	Fork length[a] cm ± SE	Weight[a] g ± SE	Condition factor[a] KFL ± SE
1/13/76	0	0	-	14.7 ± 0.14	40.8 ± 1.25	1.232 ± 0.005[b]
2/24/76	0	0	-	15.3 ± 0.19	41.8 ± 1.58	1.141 ± 0.009[c]
"	0	0	-	15.4 ± 0.24	42.1 ± 1.90	1.124 ± 0.008[c]
"	0	0.75	-	15.2 ± 0.21	40.9 ± 1.76	1.139 ± 0.007
"	0	0.75	-	15.0 ± 0.19	39.2 ± 1.53	1.134 ± 0.007
"	0	1.5	-	15.2 ± 0.23	41.4 ± 2.00	1.141 ± 0.010
"	0	1.5	-	15.5 ± 0.23	43.4 ± 2.00	1.149 ± 0.010
"	0	3.0	-	14.8 ± 0.20	37.3 ± 1.62	1.132 ± 0.010
"	0	3.0	-	15.1 ± 0.18	40.7 ± 1.46	1.151 ± 0.008
"	0	4.5	-	15.1 ± 0.23	40.7 ± 1.86	1.141 ± 0.007
"	0	4.5	-	15.7 ± 0.22	45.2 ± 1.99	1.143 ± 0.003
4/14/76	48	0	-	15.8 ± 0.24	42.7 ± 2.17	1.043 ± 0.009
"	48	0	-	16.8 ± 0.27	50.3 ± 2.54	1.025 ± 0.011
"	48	0.75	-	16.1 ± 0.29	45.5 ± 2.68	1.056 ± 0.012
"	48	0.75	-	15.4 ± 0.22	39.0 ± 1.77	1.057 ± 0.018
"	48	1.5	-	16.3 ± 0.28	46.8 ± 2.67	1.049 ± 0.012[c]
"	48	1.5	-	16.2 ± 0.23	45.6 ± 2.05	1.039 ± 0.011
"	48	3.0	-	15.6 ± 0.22	40.5 ± 1.87	1.028 ± 0.009
"	48	3.0	-	16.3 ± 0.24	47.3 ± 2.11	1.064 ± 0.017
"	48	4.5	-	15.6 ± 0.26	40.7 ± 2.22	1.044 ± 0.014
"	48	4.5	-	16.3 ± 0.28	46.9 ± 2.56	1.039 ± 0.012
5/4/76	68	0	0	16.5 ± 0.26	46.2 ± 2.23	0.997 ± 0.009
"	68	0	10	16.2 ± 0.34	44.5 ± 3.36	0.989 ± 0.013[d]
"	68	0.75	0	16.2 ± 0.28	43.9 ± 2.39	0.993 ± 0.011
"	68	0.75	10	16.6 ± 0.22	46.3 ± 1.99	0.988 ± 0.013[d]
"	68	1.5	0	16.2 ± 0.25	43.4 ± 2.22	0.988 ± 0.013
"	68	1.5	10	16.9 ± 0.29	50.2 ± 2.97	0.995 ± 0.012[d]
"	68	3.0	0	16.1 ± 0.26	43.4 ± 2.30	1.011 ± 0.011
"	68	3.0	10	16.5 ± 0.23	45.8 ± 2.11	0.988 ± 0.008[d]
"	68	4.5	0	15.7 ± 0.24	39.4 ± 1.92	0.985 ± 0.009
"	68	4.5	10	16.3 ± 0.24	44.0 ± 2.15	0.988 ± 0.008[d]

[a]*Sample size 40 except where noted,* [b]*Sample size 199,* [c]*Sample size 39,* [d]*5 days of copper exposure.*

TABLE A-6. EFFECTS OF ZINC-COPPER EXPOSURE ON AVERAGE LENGTH, WEIGHT, AND CONDITION FACTOR OF COHO SALMON.

Date of sample	Days of exposure Zn	Days of exposure Cu	Nominal concentration µg/L Zn	Nominal concentration µg/L Cu	Fork length[a] cm ± SE	Weight[a] g ± SE	Condition factor[a] KFL ± SE	95% C.I. on mean KFL
5/25/76	13	0	0	0	19.1 ± 0.25	70.1 ± 2.90	0.989 ± 0.007	0.9749-1.0031
	13	6	0	10	17.6 ± 0.27	55.3 ± 3.07	0.973 ± 0.009	
	13	0	400	0	18.6 ± 0.30	67.6 ± 3.39	1.027 ± 0.011	1.0058-1.0492
	13	6	400	10	18.0 ± 0.29	59.8 ± 3.39	0.984 ± 0.008	
	13	0	800	0	18.2 ± 0.24	62.4 ± 2.50	1.018 ± 0.009	0.9998-1.0362
	13	6	800	10	17.3 ± 0.14	51.6 ± 1.33	0.990 ± 0.009	
	13	0	1600	0	18.1 ± 0.28	61.8 ± 2.96	1.017 ± 0.008	1.008-1.0332
	13	6	1600	10	17.7 ± 0.21	55.7 ± 2.37	0.984 ± 0.009	
	13	0	2400	0	17.9 ± 0.22	57.4 ± 2.07	0.990 ± 0.010	0.9698-1.0102
	13	0	2400	10	17.2 ± 0.24	50.4 ± 2.18	0.968 ± 0.007	
6/8/76	27	0	0	0	18.8 ± 0.25	69.2 ± 2.93	1.027 ± 0.010	1.0068-1.0472
	27	20	0	10	18.6 ± 0.29	64.3 ± 3.45	0.965 ± 0.010	
	27	0	400	0	19.2 ± 0.32	75.8 ± 4.17	1.036 ± 0.009	1.0178-1.0542
	27	20	400	10	18.1 ± 0.24	58.6 ± 2.72	0.961 ± 0.010	
	27	0	800	0	18.9 ± 0.36	73.1 ± 4.39	1.025 ± 0.010	1.0048-1.0452
	27	20	800	10	17.7 ± 0.26	54.5 ± 2.56	0.966 ± 0.010	
	27	0	1600	0	17.9 ± 0.28	60.3 ± 3.03	1.019 ± 0.009	1.0008-1.0372
	27	20	1600	10	17.8 ± 0.28	56.2 ± 2.84	0.958 ± 0.008	
	27	0	2400	0	17.8 ± 0.26	57.6 ± 2.55	0.999 ± 0.009	0.9808-1.0233
	27	20	2400	10	17.9 ± 0.24	55.9 ± 2.49	0.948 ± 0.007	

[a]*Sample size equal to 40.*

TABLE A-7. GILL (NA,K)-STIMULATED ATPASE ACTIVITY OF COHO SALMON EXPOSED TO CADMIUM FOR 144 h.

Nominal concentration µg/L	Sample date	n	Specific activity Mean ± SD	95% C.I.
0	2/12/76	6	2.49 ± 0.39	2.09-2.90
8	2/12/76	6	2.32 ± 0.54	1.75-2.89
12	2/12/76	4	1.89 ± 0.23	1.50-2.26
0	3/22/76	5	1.90 ± 0.78	0.93-2.87
3	3/22/76	6	1.85 ± 0.75	1.06-2.64
4.5	3/22/76	7	2.53 ± 1.10	1.51-3.55

TABLE A-8. GILL (NA, K)-STIMULATED ATPASE ACTIVITY OF COHO SALMON CHRONICALLY EXPOSED TO CADMIUM OR CADMIUM-COPPER MIXTURE. WHOLE GILL TECHNIQUE USED EXCEPT WHERE NOTED.

Nominal concentration µg/L	Exposure time (h) Cd	Cu	Sample date	n	ATPase specific activity Means ± SD	95% C.I.
0	816	0	3/31/76	6	2.5 ± 0.6	1.8-3.1
4.5	816	0	3/31/76	6	2.6 ± 0.7	1.9-3.3
0	816	0	3/31/76	6	49.4 ± 20.3	26-72[a]
4.5	816	0	3/31/76	6	55.3 ± 20.6	34-77[a]
0	1152	0	4/14/76	15	2.3 ± 0.9	1.8-2.8
4.5	1152	0	4/14/76	15	3.1 ± 0.4	2.6-3.6
0	1344	0	4/22/76	6	4.5 ± 1.0	3.5-5.6
4.5	1344	0	4/22/76	6	4.2 ± 1.3	2.9-5.6
0	1344	0	4/22/76	6	100 ± 21.5	77-123[a]
4.5	1344	0	4/22/76	6	101.1 ± 25.9	74-128[a]
0	1608	0	5/3/76	5	2.8 ± 1.1	1.4-4.1
4.5	1608	0	5/3/76	6	4.1 ± 0.9	3.2-5.0
0/10	1608	96	5/3/76	6	1.8 ± 0.5	1.2-2.5
4.5/10	1608	96	5/3/76	6	2.3 ± 0.6	1.7-3.0
0	1680	0	5/6/76	6	3.8 ± 1.6	2.1-5.4
4.5	1680	0	5/6/76	6	4.8 ± 1.1	3.7-6.0
0/10	1680	168	5/6/76	6	2.0 ± 0.3	1.7-2.3
4.5/10	1680	168	5/6/76	6	1.7 ± 0.4	1.3-2.3
0	1680	0	5/6/76	6	29.6 ± 11.0	18-41[a]
4.5	1680	0	5/6/76	6	37.0 ± 4.6	32-42[a]
0/10	1680	168	5/6/76	6	15.4 ± 2.0	12-17.5[a]
4.5/10	1680	168	5/6/76	6	16.9 ± 4.2	12.5-21.2[a]

[a]*Microsomal preparation.*

TABLE A-9. GILL (NA, K)-STIMULATED ATPASE ACTIVITY OF COHO SALMON CHRONICALLY EXPOSED TO ZINC AND ZINC-COPPER MIXTURE, WITH SUBSEQUENT SALTWATER CHALLENGE AND FRESHWATER RECOVERY. WHOLE GILL TECHNIQUE USED EXCEPT WHERE NOTED.

Nominal concentration µg/L	Exposure time (h) Zn	Cu	Sample date	n	ATPase specific activity Mean ± SD	95% C.I.
0	0	0	5/26/76	6	4.09 ± 0.7	3.3 - 4.8
2400	336	0	5/26/76	6	4.09 ± 1.4	2.6 - 5.5
10	0	168	5/26/76	6	2.56 ± 0.6	1.9 - 3.2
2400; 10	336	168	5/26/76	6	1.42 ± 0.3	1.1 - 1.7
0	528	0	6/3/76	6	3.27 ± 0.7	2.5 - 4.0
2400	528	0	6/3/76	6	4.08 ± 0.9	3.1 - 5.1
10	0	456	6/7/76	6	1.55 ± 0.5	1.0 - 2.1
2400; 10	624	456	6/7/76	6	1.52 ± 0.4	1.0 - 1.9
0	528	0	6/3/76	6	41.10 ± 15.4	26.0 -56.2[a]
2400	528	0	6/3/76	6	41.10 ± 9.6	31.6 -51.8[a]
10	0	456	6/7/76	6	17.60 ± 6.6	10.7 -24.5[a]
2400; 10	624	456	6/7/76	6	12.88 ± 1.3	11.5 -14.3[a]

	Zn;	Cu;	SW	FW				
0	0	0	288	0	6/21/76	10	4.20 ± 0.6	3.8 - 4.6
2400	675	0	288	0	6/21/76	10	4.70 ± 0.3	4.5 - 4.9
10	0	500	288	0	6/21/76	10	4.61 ± 0.6	4.2 - 5.0
2400; 10	675	500	288	0	6/21/76	10	4.49 ± 0.9	3.8 - 5.1
0	0	500	0	288	6/21/76		2.40 ± 0.4	1.9 - 2.9
10	0	500	0	288	6/21/76		1.86 ± 0.9	0.7 - 2.9
2400; 10	675	500	0	288	6/21/76		1.44 ± 0.3	1.0 - 1.8

[a] *Microsomal preparation.*

TABLE A-10. GILL (NA, K)-STIMULATED ATPASE ACTIVITY OF COHO SALMON EXPOSED TO MERCURY FOR 144 h.

Nominal concentration µg/L	n	Specific activity Mean ± SD	95% C.I.
0	3	5.7 ± 0.7	3.9-7.4
50	5	3.3 ± 0.9	2.2-4.4
100	3	4.5 ± 2.0	0.5-9.5

TABLE A-11. PLASMA SERUM OSMOLARITY AND PLASMA CHLORIDE LEVEL OF COHO SALMON EXPOSED TO CADMIUM.

Nominal concentration (µg/L)	Exposure time (h)	x̄ osmolarity ± SD; SE (mOsm)	x̄ chlorinity ± SD, SE (m Eq/liter)
Control	6	301.0 ± 14.3, 6.4	112.0 ± 6.6, 2.9
	24	295.8 ± 19.8, 8.9	118.8 ± 10.1, 4.5
	48	273.7 ± 15.9, 7.1	114.3 ± 7.5, 3.3
	96	300.5 ± 12.4, 5.6	113.7 ± 5.7, 2.9
	168	302.4 ± 3.4, 1.9	114.1 ± 1.4, 0.6
8	6	292.4 ± 4.28, 1.9	111.0 ± 6.4, 2.8
	12	302.2 ± 6.34, 2.0	112.9 ± 5.1, 1.6
	24	288.2 ± 7.23, 3.2	115.3 ± 2.9, 1.3
	48	294.9 ± 10.72, 3.4	114.4 ± 6.5, 2.9
	96	304.0 ± 6.10, 2.7	114.4 ± 2.5, 1.1
	168	298.2 ± 3.88, 1.7	115.0 ± 3.4, 1.5
12	6	275.0 ± 25.2 11.3	113.1 ± 10.9, 4.8
	12	314.0 ± 11.2, 5.0	117.8 ± 5.5, 2.5
	24	295.0 ± 28.7, 12.8	121.5 ± 10.1, 4.5
	48	276.5 ± 12.8, 5.7	117.2 ± 15.8, 7.0
	96	280.0 ± 8.4, 3.8	111.3 ± 12.9, 5.7
	168	277.3 ± 16.5, 7.4	105.2 ± 9.6, 4.3

TABLE A-12. PERCENT MIGRATION (TO JULY 6, 1976) OF YEARLING COHO SALMON RELEASED INTO A SMALL COASTAL STREAM FOLLOWING ACUTE AND CHRONIC EXPOSURE TO SEVERAL METALS.

Nominal concentration µg/L	Percent migration (Days post release) (1-5)	(6-10)	(11-20)	(21+)	(1-5)	(6-10)	(11-20)	(21+)
A. Chronic exposure	April 14, 1976[a]				May 5, 1976[b]			
0 Cd	58.1	59.1	60.2	60.2	65.1		66.0	67.0
10 Cu					59.4	-	60.4	62.3
0.75 Cd	67.7	68.2	68.7	68.7	65.0	68.0	69.9	69.9
0.75 Cd + 10 Cu					42.0	43.0	45.0	47.0
1.5 Cd	60.7	61.8	62.4	62.4	47.8	48.6	53.0	55.6
1.5 Cd + 10 Cu					44.9	49.5	50.5	51.4
3.0 Cd	63.2	64.3	65.9	65.9	67.5	-	68.4	69.3
3.0 Cd + 10 Cu					47.1	49.2	54.8	56.7
4.5 Cd	56.7	58.9	59.4	59.4	61.4	-	65.3	66.3
4.5 Cd + 10 Cu					43.2	49.0	50.0	54.8
B. Acute exposure (144 h)[c]								
0 Cd	78.7	80.9	-	80.9	85.1	-	-	85.1[d]
0.75 Cd	75.8	76.8	77.8	77.8	71.8	-	-	71.8
1.5 Cd	73.5	-		73.5	64.7	-	67.6	67.6
3.0 Cd	73.5			73.5	78.4	79.5	80.7	80.7
4.5 Cd	73.7	-		73.7	67.9	-	69.0	69.0
C. Chronic exposure	May 26, 1976[e]				June 9, 1976[f]			
	(1-5)	(6-10)	(11-20)	(21+)	(1-5)	(6-10)	(11-20)	(21+)
0	75.5	79.2	80.2	80.2	64.4	69.2	71.2	71.2
10 Cu	51.9	63.7	-	63.7	51.9	62.5	63.5	65.4
400 Zn	72.2	75.9	-	75.9	64.1	67.9	71.8	73.1
400 Zn + 10 Cu	48.8	52.5	-	52.5	51.4	56.8	58.1	58.1
800 Zn	64.0	71.0	-	71.0	59.3	63.8	66.7	67.5
800 Zn + 10 Cu	37.0	47.0	-	47.0	50.4	59.0	61.9	61.9
1600 Zn	79.0	82.0	-	82.0	57.8	64.7	66.7	67.6
1600 Zn + 10 Cu	27.2	33.0	34.0	34.0	50.5	60.7	63.5	63.5
2400 Zn	59.2	65.0	66.0	66.0	68.6	69.6	73.5	74.5
2400 Zn + 10 Cu	27.3	37.7	-	37.7	56.4	64.5	64.5	64.5
D. Acute exposure (144 h)[g]								
0					76.9	81.5	81.5	
10 Cu					62.9	64.5	66.1	
400 Zn + 10 Cu					66.0	72.0	72.0	
800 Zn + 10 Cu					55.7	67.2	67.2	
1600 Zn + 10 Cu					40.6	53.1	53.1	
2400 Zn					51.7	58.6	62.1	
2400 Zn + 10 Cu					45.0	53.3	58.3	

[a]*Release of 180-192 fish per group (47 days Cd).*
[b]*Release of 100-114 fish per group (67-days Cd; 5-days Cu; 62 days Cd + 5 days Cd-Cu).*
[c]*Release of 94-102 fish per group (6-days Cd).*
[d]*Release of 67-88 fish per group (6-days Cd).*
[e]*Release of 100-106 fish per group (13-days Zn; 6-days Cu; 7 days Zn + 6 days Zn-Cu).*
[f]*Release of 102-110 fish per group (27-days Zn; 20 days Cu; 7 days Zn + 20 days Zn-Cu).*
[g]*Release of 50-65 fish per group exposed for 6 days.*

APPENDIX I. RECOMMENDED SEAWATER-ENTRY TEST PROCEDURE

Sublethal exposure to toxic chemicals can produce aberrations in physiology which diminish the organism's ability to grow, reproduce, or otherwise adequately perform its overall biological function. Detection of these aberrations usually requires the use of testing procedures beyond those necessary to produce routine acute toxicity data. Juvenile anadromous salmonids undergo a series of physiological changes, collectively known as smolting, which enable and cause the fish to migrate from freshwater to the ocean. Toxic chemicals are capable of disrupting normal smolting by interfering with osmoregulatory processes or downstream migration (Lorz and McPherson 1976, 1977, Bouck and Johnson MS). Procedures which detect interferences in smolting have been developed using enzyme assays, field migration studies, or blood sodium levels, but these procedures are often complex, limited to specific toxicants, or insufficiently documented for use in routine screening methods for toxic wastes or chemicals. One test procedure which lends itself to routine toxicity screening methods is "the seawater-entry test" (SET). This procedure involves a routine freshwater acute toxicity test followed by immediate transfer of survivors to 30 $^{o}/oo$ seawater and holding for one week. Interference with smolting is frequently manifested in osmotically related mortality of toxicant-exposed fish following transfer to seawater. The SET is recommended for use with all chemicals or wastes likely to be discharged into rivers or estuaries having populations of anadromous fish. Like any toxicity test, SET provides an index of probable environmental damage and an effect level for use in risk assessment.

Test Procedure

The acute toxicity test comprising the first phase of SET should be conducted according to the procedures outlined in Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians (EPA, 1975). It is recommended that SET be conducted with either coho salmon *(Oncorhynchus kisutch)*, Atlantic salmon *(Salmo salar)*, or steelhead trout (anadromous *Salmo gairdneri*). Smolting characteristics of these species are such that smolts are normally larger than 7 cm in length; however, the sole criterion recommended for suitability of test fish for SET is a greater than 90% survival of control fish in seawater.

At the termination of the 96-h acute toxicity test in freshwater the survivors are randomly split into two groups of equal numbers, with one group transferred into 30 $^{o}/oo$ seawater and the other into normal (non-toxicant containing) freshwater. This procedure enables the investigator to separate seawater-related, toxicant-induced mortalities from direct residual effects of the toxicant exposure. Both freshwater and seawater temperatures should be 12°C.

Fish loading for the holding period following the acute toxicity test should not exceed 5 g/L. Since no toxicant is involved in this latter phase, static tests and aeration of the water are acceptable. The dissolved

oxygen concentration should not fall below 75% saturation and concentrations of unionized ammonia should not exceed 20 µg/L. The test organisms should not be fed throughout the test.

A salinity of 30 °/oo is recommended. This salinity allows the use of near-shore supplies of natural seawater which usually do not achieve the approximately 35 °/oo salinity of full strength seawater. Dilution of more saline seawater to 30 °/oo is achieved with any good quality freshwater suitable for acute toxicity testing. Artificial seawater is acceptable as a substitute for natural seawater and can be reconstituted with the composition given in Table 1.

The salinity must be adjusted to 30 ± 0.5 °/oo and the pH of the seawater measured. Dissolved oxygen should be determined daily in each test tank during the second phase of static tests to assure that aeration is sufficient.

The overall duration of the test is 11 days, with an initial 96-h freshwater acute toxicity phase followed immediately by a 7-day seawater exposure.

The number of dead organisms in each test chamber must be counted at least every 24 hours after the beginning of the test and dead organisms should be removed as soon as they are observed.

Following a freshwater acute toxicity test only one or two toxicant concentrations may have enough survivors for the SET; if these concentrations result in mortality of seawater-exposed fish, it is necessary to repeat the test procedure starting with a second acute toxicity test in freshwater using lower concentrations of the toxicant.

TABLE 1. RECOMMENDED PROCEDURE FOR PREPARING RECONSTITUTED SEAWATER[a]

Add the following technical grade chemicals[b] and water[c] to make one liter of seawater. It is important that the listed order of ingredient additions be followed and that each ingredient be dissolved prior to adding the next one; this is best accomplished by constant mixing in the course of preparation.

1. Add 890 ml of tap water to a suitable container
2. Add 2.85 mg NaF
3. Add 19.5 mg $SrCl_2 \cdot 6H_2O$
4. Add 30.0 mg H_3BO_3
5. Add 100.0 mg KBr
6. Add 699.0 mg KCl
7. Add 1.1 g $CaCl_2 \cdot 2H_2O$
8. Add 3.99 g Na_2SO_4
9. Add 5.0 g $MgCl_2 \cdot 6H_2O$
10. Add 23.48 g $NaCl$
11. Add 19.5 mg $Na_2SiO_3 \cdot 9H_2O$
12. Add 0.6 mg ethylenediaminetetraacetate (EDTA) tetrasodium salt
13. Add 199.5 mg $NaHCO_3$. The pH should be 8.0 $\pm$ 0.2
14. Fill to one-liter mark, the salinity should be 30 $\pm$ 0.5 $^{o}/oo$. If a lower test salinity is desired, dilute with additional tap water.

[a]*From Zaroogian et al. 1969 and LaRoche et al. 1970.*
[b]*Reagent grades of EDTA are used because of unavailability of technical grades.*
[c]*Good practice to determine chemical characteristics of water being used in making seawater.*

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APPENDIX II. PARTIAL CHARACTERIZATION OF GILL (NA, K)-STIMULATED, OUABAIN SENSITIVE ADENOSINE TRIPHOSPHATASE FROM COHO SALMON, *Oncorhynchus kisutch*.

INTRODUCTION

An enzyme system composed of a (Na, K)-stimulated, ouabain sensitive adenosine triphosphatase has been shown to be a fundamental mechanism for active ion transport across cell membranes (Maetz 1969, Post et al. 1960, Skou 1960, 1972). Numerous investigators have found that the gill (Na, K)-stimulated ATPase system plays an important role in saltwater adaptation of euryhaline fishes (Epstein et al. 1967, Jampol and Epstein 1970, Kamiya and Utida 1968, 1969, Sargent and Thomson 1974). Parr-smolt transformation and migratory behavior of several salmonid fishes have been shown to correlate with an increase in gill (Na, K)-stimulated ATPase (Giles and Vanstone 1976, Lorz 1974, Zaugg and McLain 1970, 1972, Zaugg and Wagner 1973).

Changes in enzyme activities of vertebrates have been reported due to exposures of heavy metals. Donaldson et al. (1971) found inhibition of (Na, K)-stimulated ATPase in rat brain preparations by several metals. Activities of five liver enzymes of a cyprinodont fish changed either by exposing the liver homogenate, or the fish itself to salts of various metals (Jackim et al. 1970). Lorz and McPherson (1976) were able to detect gill (Na, K)-ATPase inhibition in coho salmon exposed to sublethal levels of copper. Consequently, during the present study periodic monitoring of gill (Na, K)-stimulated ATPase activities was undertaken to reveal any changes attributable to heavy metal exposure. Significant change in this biochemical mechanism in an anadromous fish may disturb the normal migratory behavior or the ability of the fish to adapt to a hypertonic environment.

In past studies at this laboratory, an enzyme assay was used for gill microsomal (Na, K)-ATPase which involved detergent extraction and centrifugation as portions of a lengthy procedure (Zaugg and McLain 1970). The complexity of the procedure limited the number of fish sampled to the extent that statistical analysis of the data was difficult. In addition, these procedures were never characterized sufficiently to reveal the extent of variability of enzyme activity if chemical components of the assay were varied. An assay method was developed to determine (Na, K)-stimulated, ouabain-inhibitable ATPase activity quantitatively in whole homogenates of gill filaments (Ewing and Johnson 1976). All centrifugation and detergent treatments were eliminated. The reduction in analytical time, as well as the versatility of the whole gill homogenate technique, allows the investigator the advantage of a larger number of samples and field or laboratory application. Johnson et al. (1977) described in detail a characterization of spring chinook salmon, whole gill (Na, K)-stimulated ATPase. Several of their observations were assumed to be consistent in the coho salmon enzyme system, and therefore, were not investigated in this characterization. These included ion requirements, stability upon storage with 2-mercaptoethanol, fractionation with and without deoxycholate, and reaction time.

METHODS

Coho salmon, *Oncorhynchus kisutch*, from the Fall Creek Hatchery, Oregon, were reared for approximately 14 months in well water at 12°C. Fish were fed at recommended levels (ODFW-hatchery procedures) with Oregon Moist Pellet (Bioproducts, Inc.). Fish chosen for analysis were pre-smolts and approximately 15 cm in length.

Procedures for enzyme analysis followed those described by Ewing and Johnson (1976) with a few changes in concentrations of components. Gill filaments were homogenzied in a Potter-Elvehjem homogenizer containing a solution of 0.1 M imidazole buffer, 20 mM Na_2EDTA, 0.3 M sucrose, and 10 mM 2-mercaptoethanol. adusted to pH 7.2. Fifteen mg wet weight of gill filament per mL of homogenizing media (HMM) was used routinely. To initiate the reaction, 0.1 mL of the homogenate was added to 0.4 mL of a reaction mixture containing; 100 mM imidazole buffer, 10 mM Na_2ATP, 20 mM $MgCl_2$, 90 mM KCL, and 270 mM NaCl, adjusted to pH 7.2. Incubation was carried out at 37°C (without shaking) for 20 min. The reaction was stopped with 3.0 mL of 1.66 N H_2SO_4 and liberated phosphate was measured by the method of Ernster et al. (1950). Protein was measured by a modification of the method of Lowry et al. (1951). (Na, K)-stimulated activity was calculated as the difference between rates of inorganic phosphate liberated in the presence or absence of 0.5 mM ouabain and divided by the protein value.

RESULTS

Whole gill homogenates of coho salmon may contain numerous enzymes capable of hydrolyzing ATP. The activities of these enzymes can be stimulated by Mg^{2+}, Na^+, K^+, possibly Ca^{2+} and other ions, while significant inhibition of some activity occurs in the presence of ouabain. The (Na, K)-stimulated ouabain sensitive portion is the component responsible for the (Na, K) ion transfer phenomenon (E.C. 3.7.6.2) and is of interest here. The ouabain insensitive portion appears to represent a complex of activities and was not considered of primary importance in this study.

PROPERTIES OF OUABAIN SENSITIVE ATPASE

The optimal pH for the reaction was 7.3, with sharp declines in activity on either side of this value (Fig. 1A).

Effects of tissue concentration and the chelators EDTA or EGTA in the HMM, were tested for their influence on activity. Figure 1B shows a definite suppression of activity by EGTA, while in the presence of EDTA the activity was proportional to the tissue concentration. A tissue concentration of 15 mg/mL was considered adequate. Although inhibition of the enzyme has been reported by concentrations of EDTA in excess of 1 mM (Wallick et al. 1973), this problem didn't appear in our assays. The 20 mM of $MgCl_2$ in our reaction mixture may have alleviated the inhibitory effect of EDTA.

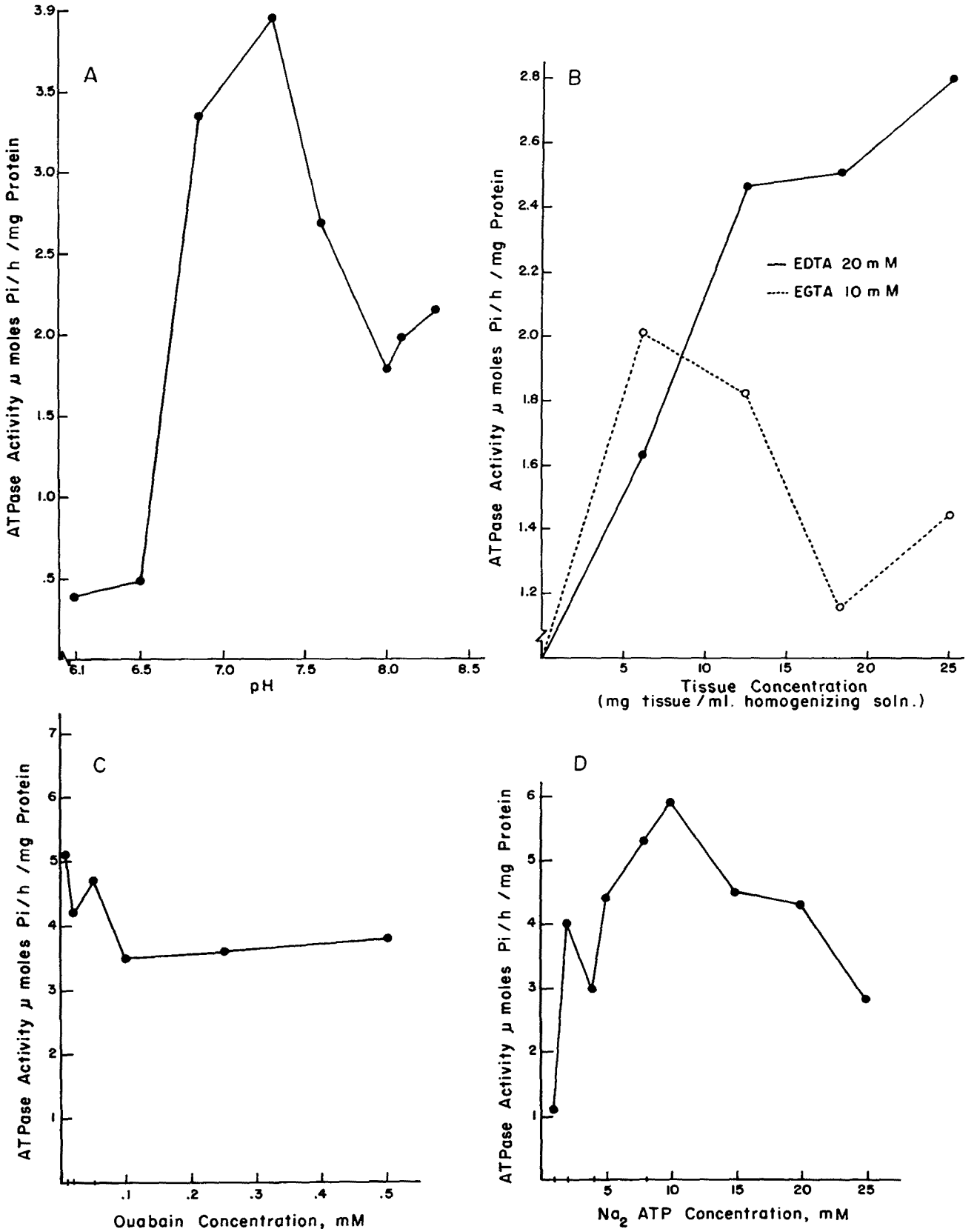

Figure 1. Relationship of gill (Na, K)-stimulated ATPase activity of coho salmon to: A. pH of reaction mixture; B. tissue concentration and the chelators EDTA and EGTA; C. ouabain concentration; D. disodium ATP concentration.

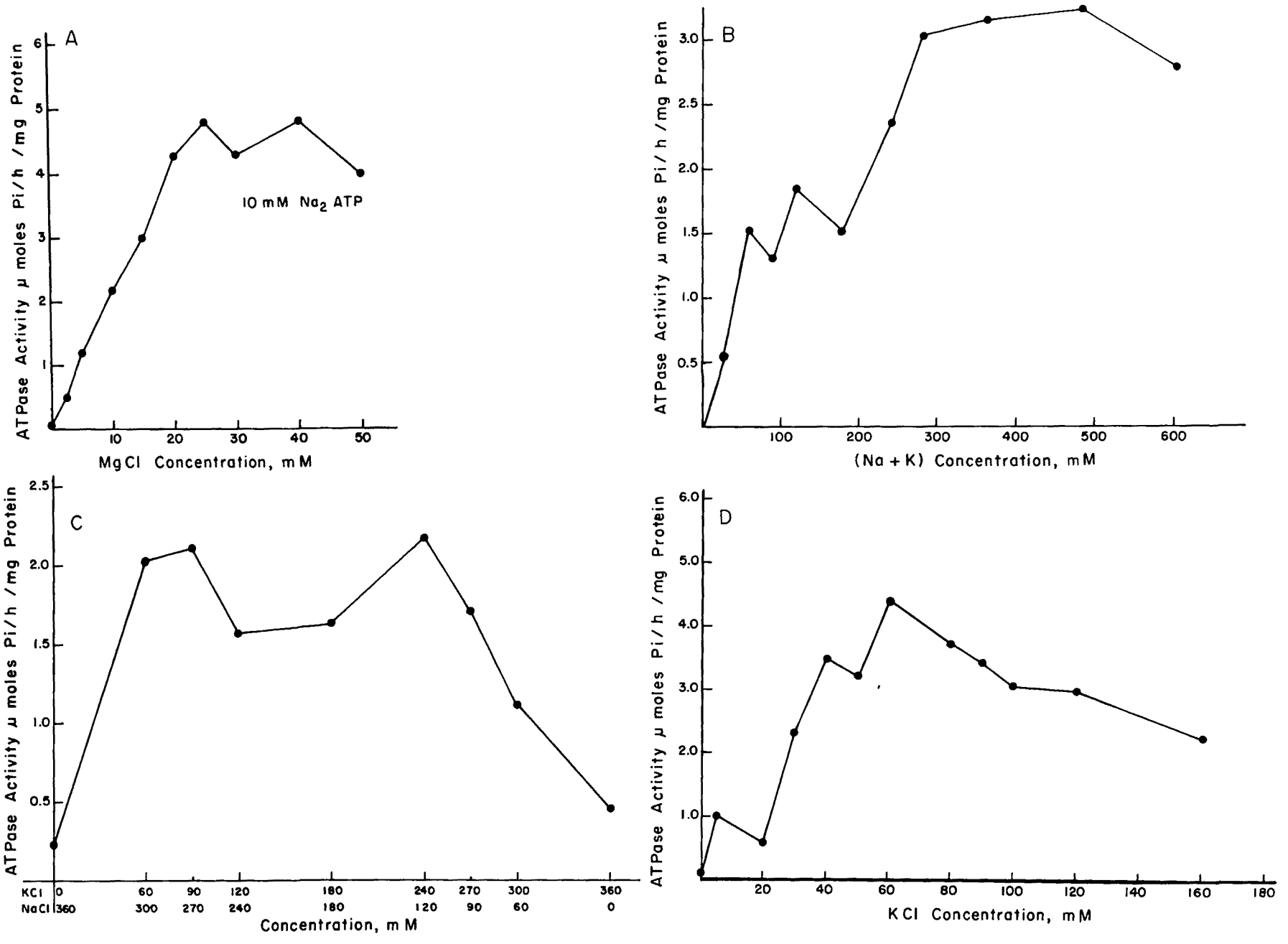

Figure 2. Relatinship of gill (Na, K)-stimulated ATPase activity of coho salmon to: A. magnesium chloride concentration; B. combined concentrations of NaCl and KCl; C. ratios of NaCl/KCl in reaction mixture; D. potassium chloride concentration.

The inhibitory effect of ouabain is shown in Fig. 1C. Maximal inhibition occurred at a concentration of 0.1 mM, while half maximal inhibition occurred at 0.025 mM. Disodium ATP concentrations were varied (Fig. 1D) and maximal reaction rates were observed at 10 mM ATP in the presence of 20 mM $MgCl_2$, 270 mM NaCl, and 90 mM KCl. Optimal activity occurred at $MgCl_2$ concentration of 20 mM in the presence of 10 mM Na_2ATP (Fig.2A).

The activity of the ouabain sensitive enzyme complex was not only dependent upon the concentrations of Na^+ and K^+ but also to the ratio of these ions and their combined ionic strength in the reaction mixture. Four tests were performed to define the influence of these variables in promoting enzyme activity. Optimal activities occurred when the ionic strength was between 360-450 mM (Fig. 2B). Using 360 mM (Na+K) ionic strength as our standard, the Na^+:K^+ ratio was varied. Although a complex relationship exists between the ratios and enzyme activity (Fig.2C), a 3:1 ratio (270 mM Na:90 mM K) gave consistently maximal activities. In the presence of 20 mM $MgCl_2$ and 270 mM NaCl maximal activity occurred at a KCl concentration of approximately 60 mM (Fig. 2D). The sodium concentration curve was more complex but revealed an optimal activity at 270 mM NaCl (Fig. 3).

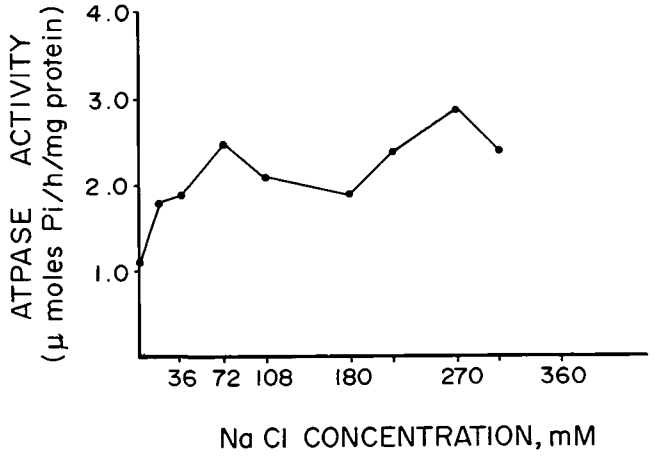

Figure 3. Relationship between NaCl concentrations and gill (Na, K)-stimulated ATPase activity.

DISCUSSION

An analytical procedure utilizing a whole gill homogenate of coho salmon *(O. kisutch)*, required reaction conditions different from those previously reported for microsomal isolates. Whereas Jorgensen and Skou (1971) found maximum activation of (Na, K)-ATPase by addition of deoxycholate (DOC) to microsomal preparations of rabbit kidney, a loss of activity in whole gill homogenates of chinook salmon *(O. tshawytscha)* occurred from exposure to DOC (Johnson et al. 1977). Although the effects of DOC on whole gill homogenates from coho salmon were not specifically tested it was assumed that a reduction in enzyme activity would occur similar to that found in chinook salmon. Other conditions which differed from various commonly used (Na, K)-ATPase analyses were: ionic strength; Na:K ratio; Mg^{2+}; and ATP concentrations. Reasons for the differences are not known.

Since the subcellular units in the homogenate were not fractionated, sedimentation occurred upon standing. Considerable variation in the assay resulted if thorough homogenization and resuspension of all particles was not accomplished.

It appeared that the enzyme activity determined by ouabain inhibition was the best method in which to calculate the (Na, K)-stimulated ATPase activity using the whole gill homogenate assay. Tests conducted during this study with 0-age class coho salmon parr reveal an inhibition of total ATPase activity (Mg^{2+} only stimulated), by the addition of extraneous NaCl and KCl to the reaction mixture. If further testing substantiates this observation, it will have definite implications on the manner in which the Na^+, K^+-transport enzyme activity is calculated. At this time we believe that determination of the Na^+, K^+-transport enzyme activity in coho salmon is best calculated by the difference between reactions involving: substrate + enzyme + (Mg^{2+}, Na^+, K^+) and substrate + enzyme + (Mg^{2+}, Na^+, K^+, ouabain).

In any given population of fish, the (Na, K)-stimulated ATPase activities are quite variable between individuals. This is illustrated often by large standard deviations in specific activities (Fig. 4). The coefficients of variation (C.V.) were calculated for all assays run on control fish for the period February 12 to June 21, 1976. Using these C.V. a brief comparison was made between the two assay methods used at our laboratory; a) gill microsomal technique (Zaugg and McLain 1970), and b) whole gill homogenate technique (Ewing and Johnson 1976). The results (Table 1), indicated slightly greater average C.V. occurred with the microsomal preparation. However, the C.V. experienced in our whole gill homogenate assays of coho salmon, were approximately 12% higher than those of 14 spring chinook salmon assayed by the same procedure (Johnson et al. 1977). Differences are probably inherent in the variation of enzyme activities between individuals, and not due solely to the technique used.

Several investigators have found seasonal variations in (Na, K)-ATPase using microsomal preparations of coho salmon gill (Giles and Vanstone 1976, Zaugg and McLain 1972). We felt it would be of interest to determine if this

seasonal variation occurred when whole gill preparations were used. Using coho salmon of the 1975 brood, 30 fish from "stock" tanks were sampled monthly. The whole gill homogenates were analyzed for (Na, K)-ATPase as described. Figure 4 shows the seasonal fluctuation in the (Na, K)-ATPase activities. A distinct rise in activity occurred in the spring (February thru May) of their second year of life. This rise in activity coincided with migratory movements we have observed in both wild and experimental groups from this stock. Zaugg and McLain (1972) found a similar rise in activities of yearling coho salmon, while Giles and Vanstone (1976) reported increased activities occurred in mid-winter and decreased activities in the spring.

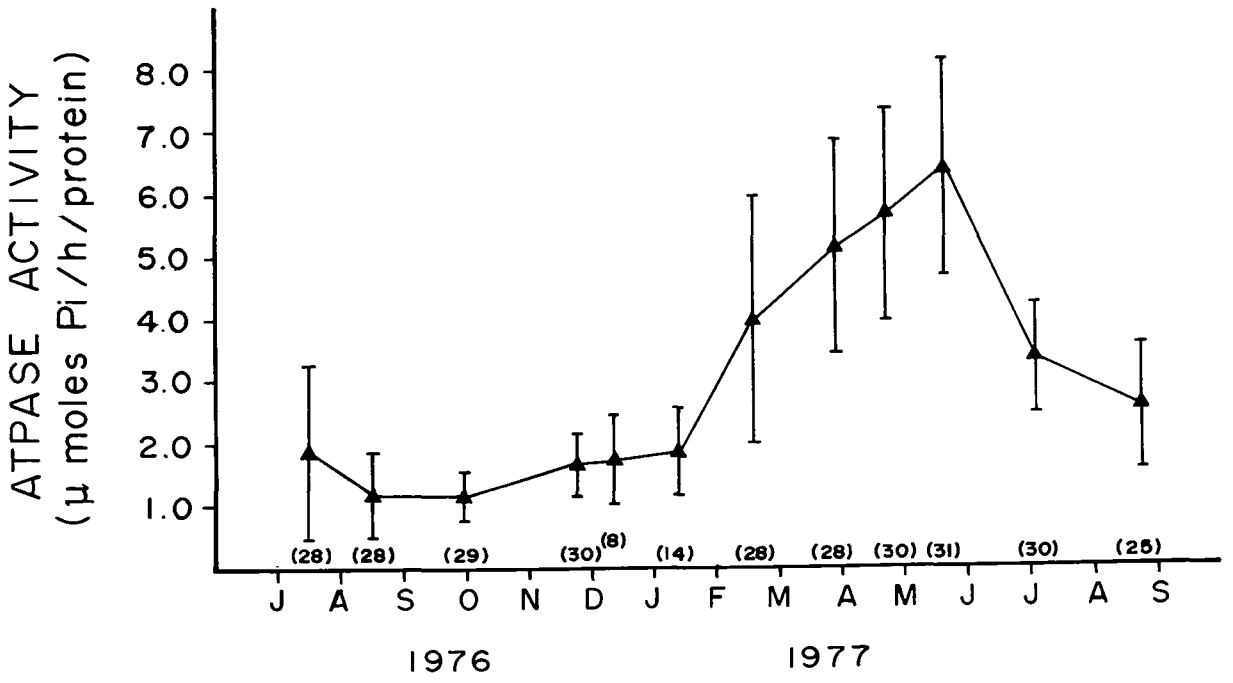

Figure 4. Gill (Na, K)-stimulated ATPase activity: mean ± SD of coho salmon from 7 month parr to post smolts. Sample size in parentheses.

TABLE 1. COEFFICIENTS OF VARIATION OF MEAN SPECIFIC ACTIVITIES OF GILL (NA, K) ATPASE USING TWO DIFFERENT ASSAY TECHNIQUES.

Preparation	No. of assays	Total No. of fish	Mean C.V.	Range
Microsomal (coho)	4	24	34%	22%-41%
Whole gill homogenate (coho)	11	69	27%	12%-43%
Whole gill homogenate (spring chinook)[a]	1	14	14.5%	-

[a] *Data from Johnson et al. 1976.*

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APPENDIX III. HISTOLOGICAL EFFECTS OF SEVERAL METALS IN SELECTED TISSUES OF YEARLING COHO SALMON.

INTRODUCTION

In an attempt to explain observed differences in migratory behavior, (Na, K)-stimulated ATPase levels and saltwater tolerance, histological sections were prepared from selected tissues of fish exposed to several metals.

METHODS

Five exposed fish and 5 control fish were taken at each sampling. The following metals, exposure periods and concentrations were used:

Cadmium

12 µg/L for 48 h
12 µg/L for 120 h
3 µg/L for 21 days
4.5 µg/L for 70 days
4.5 µg/L for 70 d + 10 µg/L copper for the last 6 days

Copper

10 µg/L for 6 days
10 µg/L for 20 days (3 fish)

Mercury

500 µg/L for 24 h

Zinc

2400 µg/L for 28 days
2400 µg/L for 28 days + 10 µg/L copper for the last 20 days.

Fixing, mounting and staining methods recommended by Galigher and Kozloff (1971) were followed. Gills, liver, gonads, head kidney, and a portion of the middle kidney were taken from each fish. Tissues were imbedded in paraplast on an Autotechnicon and 10 micrometers (µm) sections were cut. Mayers' egg albumen was used as an affixative. Slides were stained with Harris' hematoxylin with eosin as a counterstain. Sections were mounted from the beginning, middle and end of each band of parafin.

RESULTS AND DISCUSSION

Tissues from fish exposed to metals were compared to control fish to determine differences in organ and cell structure, staining properties, and the consideration of the nucleus. No apparent histological aberrations resulted from the metal exposures (Table 1). Specific data for tissues examined are given below.

Gills No major structural damage from any of the metals or combination of metals tested. Post-mortem decomposition destroyed the gills of fish exposed to mercury.

Gonads No necrosis was observed that could be attributable to metals. There was some discoloration and tissue necrosis in ovaries that came into contact with bile fluid. Due to the prevalence of the burst gall bladders in control fish, we assumed that the necrosis observed were not caused by the metals but by of the bile. The caustic action of bile has been shown to cause destruction of liver tissue (Hendricks et al. 1976). Sangalang and O'Halloran (1973) found that the testes of mature brook trout when exposed to 25 µg/L Cd for 24 h showed extensive hemorrhagic necrosis and disintegration of lobule-boundary cells. In a second experiment with fish exposed for a maximum of 30 days to 10 µg/L Cd similar injury to the testes was noted but the damage was not as extensive. In our experiments fish were sexually immature, our concentrations of Cd <2.5 times Sangalang and O'Halloran's experiment. This may explain the lack of histopathological effects.

Kidneys No necrosis was noted that could be directly attributable to heavy metal intoxication. Differences between samples and controls were suspect, because of fixation procedure. The kidney should be, but was not excised prior to fixation. If formalin is used as a fixative, it should be fresh and changed every two weeks during the preservation period. Formalin degrades naturally to formic acid which is caustic and could cause decomposition of fixed tissues.

Liver Liver sections showed two forms of necrosis, but neither one could be attributed to metal intoxication. Sections showed subcapsilar necrosis where gall bladders became ruptured or appeared to leak. Secondly, fish used in static bioassays were not fed and did not have as many glycogen deposits in liver cells as did the well-fed chronically tested fish.

The levels of metals used in these studies were too low or the exposure too short to produce detectable tissue necrosis. Voyer et al. (1975) found no histopathology in the (freshwater) mummichog *(Fundulus heteroclitus)* exposed to cadmium at concentrations of 3 mg/L and suggest this level may have been too low to stimulate morphological change. Other authors also have presented evidence that histopathological changes occur only when the fish have been exposed to metals above a minimal threshold concentration: Lloyd (1960) found gill damage in rainbow trout subjected to 20 mg/L Zn for 2.5 h but absent in specimens exposed to 3 mg/L Zn, a sublethal concentration, for 2 days; similarly no histopathology was apparent in mummichogs exposed to 5 mg/L Cd for one year, although intestinal lesions and pathological

TABLE 1. SUMMARY OF HEAVY METALS HISTOPATHOLOGY.

Metal	Nominal concentration µg/L	Sample N	Exposure period	Tissue	Histopathology
Cd	12	5	48 h	gills	None
		5	48 h	liver	None
		5	48 h	gonads	None
		5	48 h	kidney	None
	12	5	120 h	gills	None
		5	120 h	liver	None
		5	120 h	gonads	None
		5	120 h	kidney	None
	3	5	21 days	gills	None
		5	21 days	liver	None
		5	21 days	gonads	None
		5	21 days	kidney	None
	4.5 + 10 µg/L Cu	3	70 days + Cu	gills	None
		3	last 6 days	liver	None
		3		kidney	None
Cu	10	8	6 days	gills	None
		8	20 days	liver	None
		8	20 days	kidney	None
		5	20 days	gonads	None
Hg	500	3	24 h	gills	Post-mortem decomp.
		3	24 h	liver	None
		3	24 h	kidney	None
Zn	2400	3	28 days	gills	None
		3	28 days	liver	None
		3	28 days	kidney	None
Zn	2400 + 10 µg/L Cu	3	28 days	gills	None
		3	+ Cu for	liver	None
		3	last 20 days	kidney	None

changes of the kidney and gills developed within 1 h at 50 mg/L (Gardner and Yevich 1970). Skidmore and Tovell (1972) studied the histological changes that occurred in gill tissue of rainbow trout following exposure to 40 mg/L Zn. They found the epithelium covering the secondary lamellae lifted away from the pillar cell system, thus increasing the diffusion distance from water to blood. Eisler and Gardner (1973) observed renal pathology in the estuarine mummichog subjected to either 1 or 10 mg/L Cd in combination with copper; lesions were always present in fish exposed to 10 mg/L Cd, but were sometimes missing in fish exposed to 1 mg/L Cd. All of Eisler and Gardner's (1973) metal concentrations that showed histopathological damage also caused some level of mortality during the exposure period. Newman and MacLean (1974) noted histopathological effects in cunner, *Tauggolabrus adspersus*, following exposure to cadmium concentrations of 48 mg/L for 96 h but noted few changes in fish receiving lesser concentrations. Similarly, Gardner and LaRoche (1973) found no visible evidence of hepatic alteration in the mummichog or Atlantic silverside *(Menidia menidia)* exposed to copper for up to 21 days. Some lesions were present in some mummichog receiving paired injections of 100 mg/L copper, and the liver was marked by focal areas of necrosis in these cases. Gardner and LaRoche suggest that liver cell functions could be affected long before a cellular alteration can be identified with a light microscope.

Thus, from the tissues sampled it appears that the mortalities or behavioral changes observed following exposure to several metals were probably due to biochemical rather than tissue lesions.

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APPENDIX IV: CADMIUM UPTAKE AND ACCUMULATION IN COHO SALMON.

INTRODUCTION

The phenomenon of accumulation of toxic substances by aquatic organisms is of concern not only to fisheries biologists, but also to the general public as users and consumers of these organisms. It has been shown that metals can accumulate in aquatic organisms exposed to sublethal doses and in fact can become more concentrated in the tissues of the animal than in the environment (Clearly and Coleman 1974, Hannerz 1968, Mount and Stephan 1967). This poses two potential problems, deleterious effects upon the individual or its offspring via gradual accumulation of metal in the tissue, and trophic level bioconcentration of the toxicant.

The primary objective of this study was to investigate cadmium accumulation from the water in tissues of Cd exposed fish.

METHODS

Fish were killed at the end of the chronic Cd exposure test from the control and 4.5 µg/L (1685 h) and from 8 and 12 µg/L Cd concentrations after 144 h. Fish were killed by a sharp blow on the head and frozen for approximately 2-3 months prior to processing.

Tissue was dissected, weighed and dried for 24 h at 90°C. Dry weight was taken and 10-200 mg of the tissue was placed in glass Kimax 20 mL test tube with a teflon lined cap. Digestion procedures were modified from Adrian (1971). Since only milligram portions were used, the acid-mix volume was 1.0 mL. Heating was done in a Gilson water bath at 80°C for 8 h.

After cooling, the volume was brought up to 10 mL by the addition of glass distilled water. Analysis of total cadmium was conducted by flameless atomic absorption spectrophotometry (Perkin-Elmer 305B) with a Hollow Graphite Atomizer 2000.

RESULTS AND DISCUSSION

Concentrations of Cd found in tissue of yearling coho salmon are listed in Table 1. Values obtained for control fish tissues are similar to values in fish from natural waters (Lovett et al. 1972, Lucas et al. 1970) and salmonids from Oregon hatcheries (McCrady, WFTS personal communication). The distribution of Cd in various tissues follows a typical pattern with the kidney containing the highest level in both control and Cd exposed fish. Concentrations of Cd in the kidney have been reported to increase with increasing exposure times (Kumada et al. 1973, Smith et al. 1976). This phenomenon does not appear to be universal. Eaton (1974) found no increase in Cd concentration in hepatic or renal tissue with longer periods of exposure. Similarly, Mount and Stephan (1967) found no direct correlation between exposure time and absolute liver Cd concentration in the bluegill *(Lepomis macrochirus)*. Rehwoldt and Karixian-Teherani (1976) studied the

uptake and effect of Cd in the zebrafish *(Brachydanio rerio)* and found that there was a period of rapid accumulation of Cd followed by a period of reduced uptake which gradually reached a plateau.

In our study, a general trend of increasing tissue Cd concentrations was seen between control fish and those exposed to cadmium chloride. The trend is well illustrated in the chronically exposed group where the accumulation is of the following order: liver 2.2x; kidney 18x; and gill 43x the level measured from control fish (Table 1). This trend in Cd accumulation is typical of fishes and has been shown to occur in other species as well (Clearly and Coleman 1974, Eisler 1971, Eisler and Gardner 1973, Kumada et al. 1973, Lander and Jernelov 1969, Mount and Stephen 1967, Rowe and Massaro 1974). It is apparent that the internal organs begin accumulating Cd within 24 h at exposures of 8 ug/L Cd, and in all concentrations (4, 8, 12 µg/L) within 96 h. Muscle tissues did not accumulate noticeable amounts of Cd in the acute exposure tests.

Kumada et al. (1973) found that juvenile rainbow trout accumulated the highest levels of Cd in the kidney with a steady-state level being reached in 10-20 weeks after which the concentration was essentially constant. They also found that the liver, but not the kidney of rainbow trout showed a reduction in Cd (in Cd-free water) following a 10-week exposure of Cd. Analysis of gill tissue prior to exposure to the clean water revealed a considerable accumulation of Cd. The hypothesis was made that the gills were the site of absorption of Cd from the water and the metal was excreted through the kidneys.

TABLE 1. CONCENTRATIONS OF CADMIUM IN VARIOUS TISSUES OF COHO SALMON SMOLTS EXPOSED TO LETHAL AND SUBLETHAL CONCENTRATIONS OF CADMIUM CHLORIDE.

Type of exposure	n	Concentration of cadmium µg/L				
		Liver	Kidney	Gill	Muscle	Ovary
		Chronic exposure 2/26-5/7/76				
Control group with 7 days 10 µg/L Cu	3	0.24	0.62	0.18	-	-
4.5 µg/L Cd with 7 days 10 µg/L Cu	3	0.52	11.2	7.8	-	0.70*
		Acute exposures 6/21-7/2/76				
Control group - 24 h	3	0.16	0.46	0.26	-	0.48*
Cd 4 µg/L - 24 h	4	-	-	0.44	0.09	-
Cd 4 µg/L - 96 h	6	0.11*	0.80***	1.15	0.02	0.13*
Cd 8 µg/L - 24 h[a]	6	0.26	1.0	0.43	<0.01	0.09***
Cd 8 µg/L - 96 h[a]	6	0.40	0.78	0.70	0.02	0.09***
Cd 12 µg/L - 96 h[b]	6	0.68	1.78	0.87**	-	0.35**

* n = 1
** n = 2
*** n = 3

[a]*Approximate LC50 value*
[b]*Lethal dose >LC50 value*

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Report contains 94 references and a recommended seawater-entry test procedure.

16. ABSTRACT

Exposure to sublethal levels of copper in freshwater reduces Na,K-activated gill ATP'ase in coho salmon and results in latent effects such as poor migration and poor survival in seawater (EPA-660/3-75-009). Similar tests with cadmium, chromium, mercury nickle and zinc indicated that only mercury produced similar latent effects. Additional tests with cadmium and zinc plus 10 ug/liter of copper produced latent effects considerably more severe than those produced by 10 ug Cu/liter by itself. Histological examination of liver, kidney and gill showed no effect of cadmium or zinc, either singly or in combination with copper. Cadmium was found to accumulate in gill, liver and kidney, but only minor amounts occurred in muscle tissue. A seawater-entry test (SET) is recommended for detecting latent effects of toxicants on salmonid smolts.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Salmon, Copper, Zinc, Cadmium, Nickle, Chromium, Mercury	Smolting, Migration, Osmoregulation	06/A,F,T

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