AN ABSTRACT OF THE THESIS OF

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Title: EFFECTS OF METHYLMERCURY EXPOSURE ON SEA WATER ADAPTATION OF JUVENILE SALMONIDS

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Laboratory experiments utilizing juvenile coho salmon (Oncorhynchus kisutch) and steelhead trout (Salmo gairdneri) were conducted between March and October, 1972, to determine some of the effects of methylmercury chloride exposures on anadromous salmonids. Toxic effects in the fish were measured during 96 hr exposures to the mercurial in fresh water and after transfer of the fish from methylmercury solutions to fresh water or 21 ppth S sea water solutions (free of mercury). Total mercury levels were also determined in tissues of juvenile coho salmon sampled from field locations in Oregon between May and September, 1971.

Measurement of mercury in water and tissue samples during laboratory experiments was facilitated by the use of ^{203}Hg-labeled methylmercury. The presence of the methyl form of mercury in the water was verified by gas chromatographic analysis. Mercurial

exposures were conducted in a modified static water bio-assay system in which fish were shifted to fresh methylmercury solutions every 24 hr to compensate for depletion of mercurial concentration in the water (accumulation of mercury in the fish accounted for most of the mercury loss from the water).

The 96 hr LC-50 of methylmercury chloride for 6.5 g coho salmon was estimated to be 38.9 ppb Hg (average initial concentration each 24 hr) at 15°C in dechlorinated city water. The average pH, EDTA hardness, and dissolved oxygen concentration in the water was pH 7.5, 24.2 ppm $CaCO_3$, and 7.9 ppm DO, respectively.

Mortality after transfer to saline water for seven days averaged 0, 57.5, and 95.0% for both fish species after 96 hr exposures to mercurial concentrations which approximated 25.7, 51.8, and 82.9%, respectively, of the 96 hr LC-50 for coho salmon. The respective concentrations of mercury in the whole body of coho salmon transferred from these mercurial solutions were 2.7, 6.5, and 9.2 ppm Hg. No mortality was observed in fish shifted from any of the mercurial solutions to fresh water for seven days.

Increased opercular beating and coughing reflex rates proportional to mercurial concentrations in the water, and hypersensitivity to light changes and noise were observed in fish during exposures to methylmercury. The liver, gills, kidney, brain, and muscle tissues contained 29.9, 25.5, 24.4, 5.0, and 1.9 ppm Hg, respectively, in

steelhead trout exposed for 96 hr to 32.2 ppb Hg. Histological damage was found in the gills, kidney and liver tissues of these fish.

The opercular beating rates usually remained high in fish moved into saline solutions, but decreased to near normal upon movement of fish from mercurial to fresh water solutions. The coughing reflex rate always decreased to near normal within 24-48 hr after removal of fish from mercurial solutions. Hypersensitivity to noise and light changes persisted or increased in fish shifted from mercurial to saline solutions, but disappeared in fish moved from mercurial to fresh water solutions.

The probable toxic mechanisms of methylmercury to fish in fresh water and during sea water adaptation are discussed.

Total mercury levels in selected tissues ranged from 2 to 200 ppb Hg in juvenile coho salmon sampled from field locations in Oregon. No correlations between mercury levels and geographic sampling locations were found, but fish from field locations appeared to have lower levels than fish held in captivity. An increase in mercury concentration proportional to age and size of the fish was noted.

Effects of Methylmercury Exposure on Sea Water Adaptation of Juvenile Salmonids

by

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF FIGURES (Cont.)

LIST OF TABLES

EFFECTS OF METHYLMERCURY EXPOSURE ON SEA WATER ADAPTATION OF JUVENILE SALMONIDS

INTRODUCTION

Environmental Contamination by Mercury

Interest in the abilities of fish and other aquatic organisms to accumulate inorganic and organic mercury compounds has risen sharply since the tragic mercury poisoning of people living on the shores of Japan's Minimata Bay in the late 1950's. The poisoning of humans, cats, and birds in that area was traced to the consumption of fish and aquatic invertebrates which had greatly concentrated mercury in their flesh from an industrial outfall of mercuric chloride and methylmercury salts (Kurland, Faro, and Seidler, 1960; Selikoff, 1971).

Subsequently, mercury pollution was indicated in Sweden by the relatively high levels of mercury found in tissues of aquatic and terrestrial animals from several areas of Sweden, and the loss of mercurials from some industrial and agricultural uses were implicated as the contributors to the high mercury levels in fish and wildlife (Hasselrot, 1968; Norén and Westöö, 1967). Areas where mercury content in animals is elevated have since been found in Canada and the U.S., including Oregon (Buhler, Claeys, and Shanks, 1973; Buhler, Claeys, and Rayner, 1973).

The natural occurrence of mercury in some geographical areas is likely to be responsible for relatively high mercury levels in surface waters (Wallace, et al., 1971). Principal sources of mercury to the environment attributed to man's activities have been chlor-alkali plants, industrial processes utilizing mercurial catalysts, burning of fossil fuels, mercurial seed treatment in agriculture, mercury mining, amalgamation of gold and silver with elemental mercury, and the use of mercurial slimicides in the pulp and paper industry (Fimreite, 1970; Harriss, 1971; U.S.G.S., 1970).

Norén and Westöö (1967) demonstrated that fish sampled from industrial mercury outfalls contained relatively high levels of mercury. They found 94-95% of the mercury in the skeletal muscle tissue of fish was methylmercury (CH_3Hg-X). The industries were not releasing methylmercury, which suggested conversion of the outfall mercury to methylmercury in the fish or elsewhere in the ecosystem. Buhler, Claeys, and Shanks (1973) found that 80.6 to 84.4% of the mercury in muscle tissue was methylmercury in resident fresh water fishes of the Pacific northwest.

Selikoff (1971) summarized the literature which indicates that all forms of mercury may be converted to methylmercury in stream and lake sediments. Methanogenic bacteria are strongly implicated as part of the mechanism. Therefore, it appears likely that methylmercury is one form, if not the predominant form, to which fish are

exposed in aquatic environments.

Mercury Toxicology in Fish

Inorganic mercury salts appear to be very toxic to fish when present in the water. Depending on the salts and fish species used, mercury salts have been shown to have toxicities approximating those of cadmium, silver, and copper salts (Akiyama, 1970; Doudoroff and Katz, 1953; and Schweiger, 1957). The toxicity of organic mercurials in aquatic organisms depends largely on the particular compound and concentration used but has been studied little. Based on TL_m or LC-50 values, organic mercurials appear to range from equally toxic to over ten times as toxic as inorganic mercury (Akiyama, 1970; F.R.B.C., 1971). No information on the LC-50 of methylmercury in any fish species over any time period has been found in the literature by this investigator.

Lethality occurring within a few days or less in fish exposed to metal salt solutions (including inorganic mercury) has generally been attributed to suffocation. This is thought to be the result of coagulation of mucus over the gills and/or damage to the epithelial cells of the gills (Doudoroff and Katz, 1953). Skidmore (1970) conclusively demonstrated that zinc sulfate could block the uptake of oxygen into the arterial blood of rainbow trout (Salmo gairdneri). This was correlated with histological damage to the gill epithelium.

Some organic mercurials appear to have similar effects on the gills. Amend, Yasutake, and Morgan (1969) found histological damage in the gills of rainbow trout exposed to ethylmercury phosphate and attributed lethality to suffocation. Akiyama (1970) observed mucus covering the gills of Oryzias latipes exposed to methoxyethyl mercuric chloride or phenylmercuric acetate and concluded that suffocation was the cause of death. Phenylmercuric hydroxide was observed to damage the gill epithelia of Leuciscus rutilis in less than one hour (Lindahl and Hell, 1970). Suffocation was even described as the lethal mechanism in rainbow trout given oral doses of methylmercuric nitrate because gill damage was observed (Miettinen et al., 1970). These investigators also observed gill damage in pike (Esox lucius) given oral doses.

The consequences of metal toxicity to the gills, other than interference with respiration, has not been examined very thoroughly. Some evidence of osmoregulatory disturbance in fish exposed to metals has been gathered, but the mechanisms are not clear. Meyer (1952) demonstrated a complete inhibition of Na^+ uptake from fresh water, and a marked increased Na^+ efflux in goldfish (Carassius auratus) exposed to mercuric chloride, but involvement of the gills was not proven. Lewis and Lewis (1970) reported that the osmolarity of blood serum in channel catfish (Ictalurus punctatus) decreased in fresh water and increased in saline water when sub-lethal concentrations of

zinc sulfate or copper sulfate were present in the solutions. Similar results were obtained when golden shiners (Notemigonus crysolencas) were exposed to copper sulfate solutions. Mucus secretion and coagulation was observed around the gill area. After exposing the head region and body region of catfish separately to zinc sulfate, the authors concluded that osmoregulatory changes resulted from damage to the head and gill areas of the fish.

Unlike some of the other metals, mercury compounds penetrate rapidly and achieve a wide distribution in the body of a fish. Bäckstrom (1969) demonstrated this point by administering nitrates of mercury, methylmercury and phenylmercury to salmonids and other fishes through ingestion, intravenous and intramuscular injections, or ambient water.

High concentrations of mercurials tend to accumulate and persist in the kidney and liver of salmonids (Rucker and Amend, 1969). Histological damage to these fish tissues has been observed, including damage to the nephrons of the posterior kidney by methylmercury nitrate (Miettinen et al., 1970). The posterior kidney is an important organ in maintaining ionic and osmotic homeostasis in the blood of fish. Therefore, toxicity due to mercury compounds in the kidney might disrupt these blood parameters. Certain organic mercurials which affect the mammalian kidney have been used as clinical diuretics (Mudge, 1970).

Neurotoxicity has been commonly found in mammals poisoned with mercurials, but has received little attention in fish. Disturbances of ionic and osmotic regulations by mercurials also suggests the possibility of an effect on the neural components of the regulating mechanisms.

Objectives of the Investigation

Based on the above information about mercury in the aquatic environment and the toxicology of mercury in fish and other animals, a theoretical model of mercury intoxication in juvenile anadromous salmonids was constructed. The model envisioned accumulation of methylmercury in rearing areas and/or along migratory routes where geological or human related sources of mercury might occur. The toxic action of the methylmercury on the osmotic and ionic regulating mechanisms was hypothesized to have a greater effect on fish attempting to migrate into saline estuaries than on fish remaining in fresh water.

The principle objective of this investigation was to determine if a previous exposure of juvenile anadromous salmonids to methylmercury in fresh water could have the hypothesized effects in fresh water and dilute sea water (saline water). The second objective was to compare levels of methylmercury exposure that resulted in inhibition of adaptation to saline water with levels of exposure that were

lethal in fresh water.

A third objective was to gain some insight on the mechanism of toxic actions observed. Histopathology of tissues, determination of accumulation and distribution of mercury in the fish, and visual observation of toxic symptoms were the means of gaining insight.

Visual observations of toxic symptoms included measurement of opercular beating rate and coughing reflex rate. Opercular beating rate can be used to estimate the relative rate of respiratory water flow over the gills of similar size fish of the same species (Heath, 1972). Bijtel (1947) described a "coughing reflex" as a reversal of water flow over the gills in response to particulate matter in the water. The reflex has since been observed in response to toxicants in the water (Schaumburg, Howard and Walden, 1967).

Finally, it was desired to assay concentrations of total mercury in selected tissues of anadromous salmonids from field locations. These could be compared with mercury concentrations in experimental laboratory fish that displayed toxic symptoms. Comparisons could also be made between locations and with other investigations to identify any "mercury problem" areas.

LABORATORY EXPERIMENTS

Methods

Coho salmon (Oncorhynchus kisutch) and steelhead trout (Salmo gairdneri) were the species chosen for this investigation because their anadromous life histories fit into our hypothetical model of methylmercury intoxication. These species are also widespread and important for recreation and commercial uses in the Pacific northwest, and they have been widely used in laboratory and field investigations.

Coho salmon used in this investigation were Alsea River stock (1971 brood) obtained as embryos from the Oregon Fish Commission's Fall Creek Hatchery). They were reared at the Averill facility of the O.S.U. Department of Fisheries and Wildlife and transferred to the Weniger Hall laboratory on July 31, 1972. All experiments with these fish were conducted between August 17 and October 6, 1972.

Steelhead trout were Siletz River (summer) stock of the 1970 brood. They were fed low rations at the O.S.U. Marine Science Center and transferred to the Weniger Hall laboratory on March 6, 1972. These fish were used in experiments between March 29 and May 18, 1972.

The basic experimental design involved exposing groups of ten fish for 96 hr in fresh water to a logarithmic series of methylmercury

chloride (CH_3HgCl) concentrations which were non-lethal in that time period. Each exposed group was then divided and half transferred to fresh water with no added mercury while the other half was transferred to mercury-free 21 ppth S sea water (approximately 2/3 sea water). Fish were observed for seven days in the fresh and saline water before termination of the experiments. A seven-day time period was selected because it has been demonstrated that ionic and osmotic concentrations in the blood stabilize after about seven days in juvenile coho salmon which adapt to 30 ppth S sea water (Conte et al., 1966) and the greatest mortality occurs within the first four days in fish that fail to adapt (Wagner, 1971).

Two replicates of these experiments were conducted with each fish species. The replicates were labeled GS-I and II and KS-I and II for steelhead trout and coho salmon, respectively. Steelhead trout were exposed in a third replicate (GS-III) to methylmercury at a concentration near the maximum used in the GS and KS replicates, but were sacrificed at 96 hr for mercury and histological analyses.

Before these experiments were conducted, the highest concentration of methylmercury chloride which was non-lethal to fish during 96 hr was determined by lethality experiments. With coho salmon and steelhead trout, the same maximum concentration of mercurial was selected for use in the salinity tolerance experiments although the preliminary experimental procedures differed for each species.

For the limited stock of steelhead trout, testing involved a 96 hr exposure of three groups of fish (three fish each) to three methylmercury solutions ranging from 10 to 100 ppb Hg. Coho salmon were exposed to ten concentrations of methylmercury chloride in groups of ten fish each. A logarithmic series of concentrations was selected (APHA, AWWA, and WPCF, 1965) so that the 96 hr LC-50 of methylmercury chloride could be determined for coho salmon.

Acclimation procedures for the fish began with their transfer to the constant temperature (15°C) laboratory in Weniger Hall. The acclimation tanks were either made of plywood painted with "hatchery white" or made of fiberglass covered with a white "gel" coat (Systems Manufacturing Corp., Albany, Oregon). Charcoal filtered city water containing less than 0.05 ppm total chlorine (chemical kit, Hach Chemical Co., Ames, Iowa) was delivered at 13-16°C through a constant flowing system. Pipes were made of polyvinylchloride or plastic tubing in all cases. The dissolved oxygen concentration was maintained above 8 ppm using filtered compressed air.

The fish were fed Oregon pellets (Bio Products, Inc., Warrenton, Oregon) until 60 hr preceding each experiment. Twelve to 18 hours before each experiment, fish were sorted, gently blotted on a damp cloth, and weighed in a tared beaker of water (fork lengths of fish were also determined when each fish expired or was terminated during the experiments). Fish were then divided into groups of

uniform number and weight and transferred to exposure chambers for the last phase of acclimation.

Groups of ten fish each were exposed to each concentration of the mercurial in the 96 hr LC-50 experiments and in all the KS and GS experiments except replicate GS-III. Eight fish per mercurial concentration were used in the latter replicate, but these fish were about 15% heavier than those used in the other replicates, so the total fish weight per chamber was similar in all experiments. The exposure chambers used in all phases of the experiments were five gallon glass jars filled with 15 l of solution and aerated with compressed air.

The 96 hr methylmercury exposure employed a modified static water procedure in which fish were transferred by dip-net to freshly prepared methylmercury solutions each 24 hr to compensate for toxicant depletion from the water. This procedure was chosen instead of a constant flowing system primarily due to the desirability of using radioactive methylmercury as a tracer and the prohibitive expense of using radioactive material in the flowing system. The radioactive compound was employed as a means of efficiently assaying toxicant concentrations in the fish tissue and water. Mercury-203 labeled methylmercury chloride with a specific activity of 2.24 mCi/mg Hg was procured from New England Nuclear (Boston, Mass.) for use in replicate GS-III. The same ^{203}Hg-labeled methylmercury was used in all coho salmon replicates. Non-radioactive methylmercury

chloride (M.P. 168.5°-170.5°C) was obtained from Alfa Inorganics, Inc. (Beverly, Mass.), for use in other experiments.

The first experiments using steelhead trout (GS-I and II) were conducted without the use of the labeled compound. In these two experiments, 0.150 to 0.474 ml of a stock solution of methylmercury (1 mg Hg/ml in 100% ethanol) was pipetted into 15 l of water in each chamber to achieve the proper concentration. The control chambers received 0.474 ml of 100% ethanol each day so that ethanol concentrations would be the same in all solutions. To achieve homogeneity of each solution before fish were added, each chamber was placed on a magnetic stirrer running at high speed for five minutes before all experiments.

The carrier for the radioactive stock solution used in GS-III was 29% ethanol. A volume of 1.42 ml of stock solution was pipetted into the 15 l of exposure solution each day giving a radioactivity concentration of 29.0 μCi/15 l. The control chambers received 0.5 ml of 100% ethanol each day (0.41 ml of 100% ethanol equals 1.42 ml of 29% ethanol).

In all coho salmon experiments, a stock solution of non-radioactive methylmercury in ethanol, a stock solution of radioactive methylmercury in ethanol, and a solution of pure ethanol were each pipetted into the 15 l solutions. This was done to achieve a nearly constant concentration of radioactivity (except for the control group)

and of ethanol in all exposures while the methylmercury chloride concentration was varied between different chambers. The radioactivity concentration was maintained between 3.9 and 4.5 μCi/15 l and the ethanol concentration at 2 ml/15 l.

To avoid concentrating mercury in the city water effluent, some of the residual mercury was removed from the solutions at the end of exposures by extracting it into a dithizone-chloroform solution. After evaporating to dryness under a hood, the dithizone-mercury residue was disposed of by the university with other toxic, solid wastes.

Sampling of solutions for mercury assay was done five to ten minutes before adding the fish and airstones to fresh solutions and 10-15 minutes after removal of the fish and airstones from "spent" solutions. Some samples were also taken at other times and were always taken near the center of the chamber.

Assay of methylmercury concentrations in the water of replicates GS-I and II was carried out exclusively by a gas chromatography procedure modified from Westöö (1968), using 35 ml of water per assay. A mean recovery of 84.4% with a range of 81.2 to 87.3% was demonstrated in water samples spiked with methylmercury chloride. The percent recovery was independent of the methylmercury concentrations between 10 and 40 ppb Hg. The same procedure was used on radioactive stock solutions and some water samples from all other replicates to assay the percent methylmercury represented by the

assayed radioactivity.

Radioactivity due to ^{203}Hg in five ml water samples and excised tissue was assayed by gamma ray spectroscopy in a Packard Auto-Gamma Model 5022 equipped with a NaI well crystal (Packard Instrument Co., Downers Grove, Ill.). The Model 5022 was connected to a Packard Model 3002 Tri Carb Scintillation Spectrometer. The radioactivity in the whole animal (frozen) was assayed in a Packard Model 446 Liquid Scintillation Detector (external sample) connected to the Model 3002 spectrometer. A plastic sample holder was constructed to hold the sample in the center of the well of the Model 446. The counting efficiency of these two systems for ^{203}Hg ranged from 26 to 36% and 18.5 to 25% for the Model 5022 and Model 446, respectively.

Temperature was measured during the experiments with a mercury thermometer and the pH of 15 ml water samples was assayed on an electronic pH meter. Water hardness was assayed by the EDTA titrimetric method (APHA et al., 1965). The dissolved oxygen concentration of solutions was measured by siphoning the solution through a glass chamber surrounding the probe of an electronic oxygen meter (YSI Co., Yellow Springs, Ohio). The siphoned solution was returned to the exposure chamber, enabling many assays to be made without a net volume reduction of the solution.

The initial concentrations of methylmercury and other water quality parameters that were measured during the 96 hr LC-50 experiments are listed in Appendix IIIa. These measurements were also made during the 96 hr mercurial exposures which preceded transfer of fish to the salinity tolerance experiments (Appendices IIIb and IIIc).

During the methylmercury exposures, fish were observed for toxic symptoms at least every 12 hours. The weight and fork length of mortalities from the 96 hr LC-50 experiments were measured before they were tightly wrapped in plastic bags and frozen for subsequent mercury assay. At the end of the LC-50 experiments, surviving fish were sacrified by a blow to the head and then were subjected to the same analytical procedures. The lengths and weights of fish used in these experiments are listed in Appendix IIa. Appendices IIb and IIc contain data on the lengths and weights of steelhead trout and coho salmon transferred to the salinity tolerance experiments.

The opercular beating rate (OBR) and coughing reflex rate (CRR) were measured by visual examination. For each analysis, OBR and CRR were observed in three to five fish in each chamber for 20-30 seconds per fish, using a stopwatch to measure the time period accurately. Mean rates for each chamber at each inspection time were calculated. During these observations, any other visual

toxic symptoms were also noted.

Fish sampled at 96 hr for histological examination were sacrificed with a blow to the head, weighed, measured, and fixed in buffered Bouin's solution. Histological preparation of tissues (gill, kidney, and liver from GS-III fish; gill and kidney from KS-I fish) was contracted to the O. S. U. Department of Food Science and the Department of Veterinary Medicine. The sectioned tissue (6μ) was stained with haematoxylin and eosin.

During the seven day exposures to fresh and saline water most of the same procedures for the assay of water quality and toxic symptoms were continued. However, fish were not transferred to fresh solutions every 24 hr. Instead, solutions were circulated through submerged charcoal filters by compressed air ("bubble-up filters"). In addition to charcoal, a marine filter mix (Aquarium Systems, Inc., Eastlake, Ohio) was added to the filters in saline water to maintain a pH near 8. 0.

The 21 ppth S sea water was prepared by addition of a sea salt mixture (Aquarium Systems, Inc.) to fresh water and mixing on a magnetic stirrer. The salinity was monitored with a calibrated sea water hydrometer. Fresh water was added as needed to the fresh and saline water chambers to compensate for evaporation.

During the fresh and saline water exposure periods the DO varied from 8. 5 to 10. 5 ppm and the temperature was 15. 0 $\pm$ 0. 2°C.

The salinity remained at 21.0 ± 0.5 ppth S, but the pH gradually decreased from about pH 9.0 to pH 7.9 in the first four days and then remained relatively constant during the last three days of all saline exposures. In freshwater exposures, hardness was constant while the pH began at values similar to those observed during the immediately previous methylmercury exposure and then increased a few tenths by the seventh day. No mercury was detectable in the fresh or saline water even after fish containing mercury were introduced to these solutions.

Observations of the fish were made at least every eight hr during the first 48 hr, at least every 12 hr during the second 48 hr, and at least every 24 hr during the last 72 hr of the seven day period. Mortalities were removed, weighed, measured, wrapped in plastic bags, and frozen. Seven-day survivors were treated similarly after killing them with a blow to the head.

Results

The 96 hr LC-50 of Methylmercury Chloride for Coho Salmon

Estimations of lethal toxicity to the fish were made for the mercurial in fresh water. These data were used to choose methylmercury concentrations for employment in subsequent salinity tolerance experiments and for comparison with mercurial exposures which produced toxicity in fish transferred to dilute sea water.

The concentration of radioactive methylmercury present in all of the exposure solutions decreased in a similar manner after fish were introduced into the test aquaria. Examples of this pattern of depletion are shown in Figure 1 for three of the ten exposure solutions used to determine the LC-50. Every 24 hr, each group of fish was transferred to freshly prepared solutions containing identical concentrations of the radioactive methylmercury. The mean of the measured methylmercury concentrations at the beginning of each 24 hr period was chosen to describe the concentration used for each 96 hr exposure period. The initial concentrations of methylmercury in the ten exposure solutions and the percent of methylmercury disappearance from the water are summarized in Table 1.

Most of the methylmercury disappearing from the solutions was accumulated in the fish as some form of mercury. An additional (but unquantified) amount of mercury was recovered in the mucus and other organic precipitates, helping to explain the location of the remaining 1.4 to 14.7% of mercury depleted from the solutions. In addition, a mean loss of 1.5% methylmercury was observed from aquaria containing no fish but aerated at a rate similar to that used in the exposure solutions. It is likely that this 1.5% loss was due to adsorption to the glass and/or volatilization of the methylmercury.

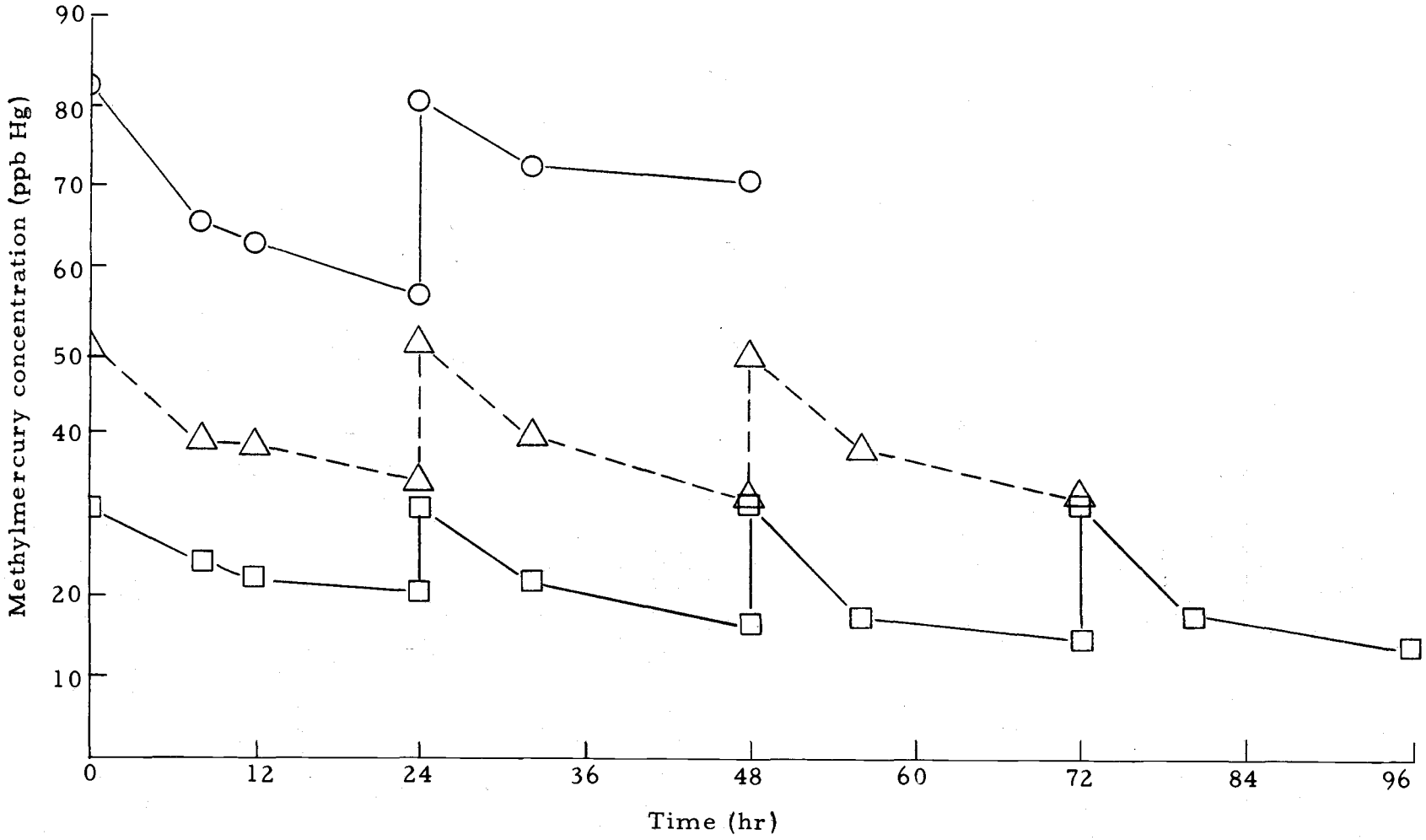


Figure 1. Reduction of methylmercury concentrations in the water reflecting the accumulation of mercury in fish during the 96 hr LC-50 experiments. Three of the ten exposures are shown (fresh methylmercury solutions were used each 24 hr).

Table 1. The fate of methylmercury introduced in the water during the 96 hr LC-50 experiments.

Initial concentration of methylmercury in water[a] (ppb Hg)	Mercury		
	Percent disappearance from water	Percent accumulated in fish	Percent unaccountable[b]
Control	---	---	---
31.0 ± 0.2	46.8	30.6	14.7
34.0 ± 0.4	29.4	22.1	5.8
37.2 ± 0.3	26.5	22.0	3.0
39.1 ± 0.6	29.5	21.8	6.2
39.3 ± 0.3	36.0	25.0	9.5
41.2 ± 0.6	41.7	25.5	14.7
43.2 ± 0.5	27.0	18.5	7.0
47.1 ± 0.6	18.9	16.0	1.4
50.2 ± 0.6	35.4	21.7	12.2
81.5 ± 1.0	18.4	11.0	5.9

[a] Mean ± S.D.

[b] Excluding a mean loss of 1.5% found in aerated water containing no fish. An unquantified amount of mercury was found in organic precipitates when fish were present.

Most investigators have assumed that degradation of methylmercury does not occur to any significant extent in fish. There does not seem to be any direct evidence, however, to support this assumption in the literature. Tonomura and Kanzaki (cited in Selikoff, 1971) have isolated a strain of Pseudomonas bacteria which is capable of degrading methylmercury chloride to produce metallic mercury. Norseth and Clarkson (1970) found that methylmercury could be transformed to inorganic mercury in the cecum of the rat intestine and postulated the involvement of microorganisms.

Since the results of these investigations suggest the possibility of microbial degradation of methylmercury in the intestines of fish, the radioactive mercury assayed in fish tissues is not designated as the methyl form in this investigation. It is assumed, however, that any degradation of the methyl form in the fish was slow and that the fish contained a high percentage of methylmercury, although gas chromatographic analysis was not performed to verify the presence of methylmercury in the tissues.

There did not appear to be any conversion of methylmercury to other forms of mercury in the water. All stock solutions, four samples from fresh exposure solutions, and four samples from mercurial solutions having contained fish for 24 hr were found to contain 100 $\pm$ 2% methylmercury by gas chromatographic and radioactivity analysis.

A 96 hr LC-50 value was estimated graphically from the relationship between percent mortality and initial concentration of methylmercury in the water (Figure 2). The 96 hr LC-50 of methylmercury chloride was estimated to be 38.9 ppb Hg (mean initial concentration) for 6.5 g Alsea River stock coho salmon at 15°C in dechlorinated Corvallis city water using the above described exposure system. The average pH, EDTA hardness and DO concentration of the water were pH 7.5, 24.2 ppm $CaCO_3$, and 7.9 ppm DO, respectively.

Approximately 33.5 ppb Hg appeared to be the highest mean initial concentration of methylmercury to which this size coho salmon could be exposed for 96 hr without the occurrence of mortality (Figure 2).

A much lower mortality than expected occurred at 41.2 ppb Hg and might be explained by the higher DO. concentration present in that exposure as compared to the other exposures. It is probable that at the lower DO. fish were stimulated to increase the rate of respiratory water flow over the gills which resulted in a faster rate of mercury accumulation and consequently greater toxicity to the fish (Amend et al., 1969). However, the 41.2 ppb Hg exposure was conducted more than one month later than the other exposures, using fish of a greater age and longer laboratory acclimation time. In addition, the pH had decreased and the water hardness increased over the

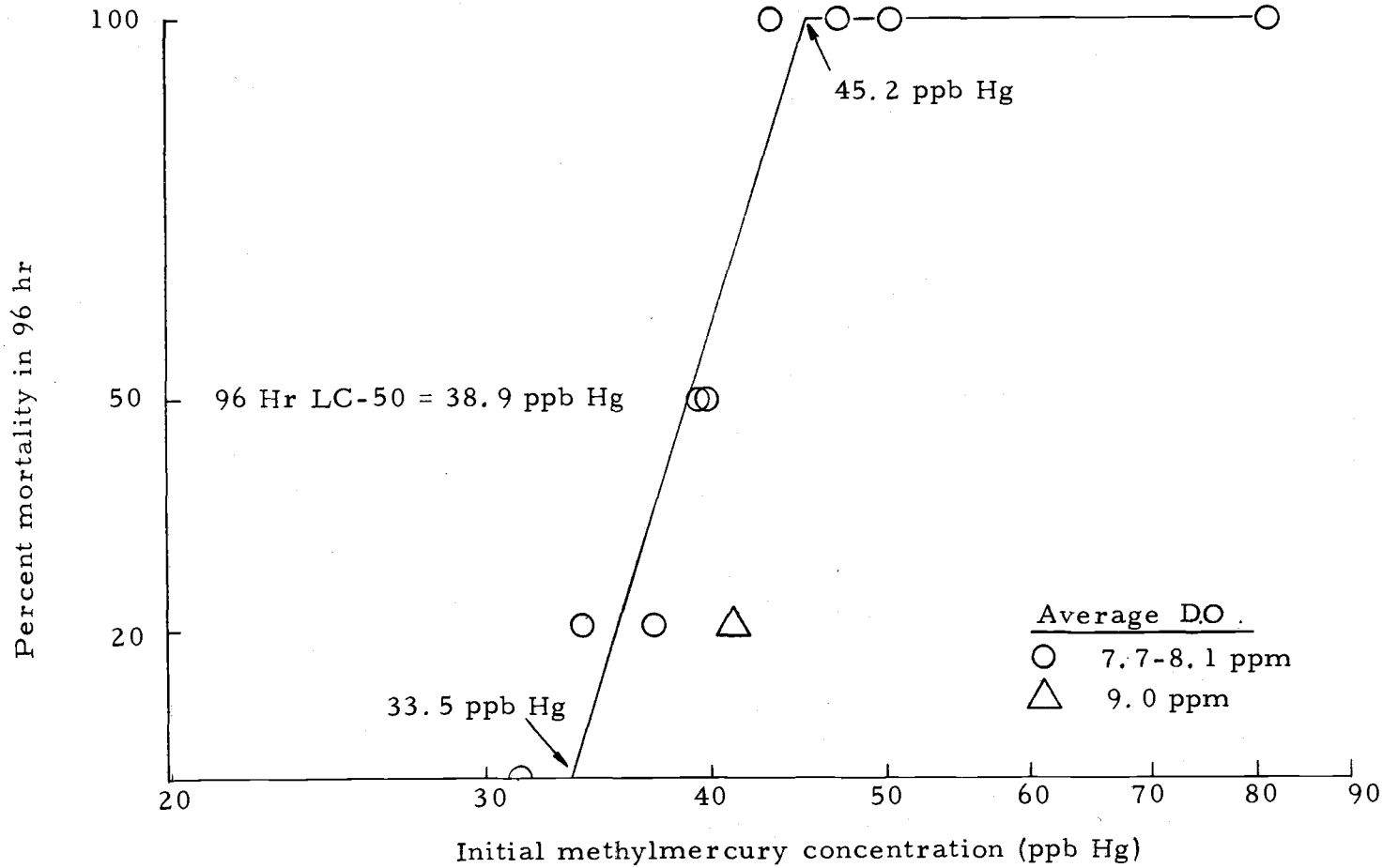


Figure 2. The relationships among percent mortality, initial concentration of methylmercury in the water, and dissolved oxygen concentration during 96 hr LC-50 experiments with coho salmon.

month (Appendix IIIa). Any of these factors or combination of these factors may have contributed to the decreased toxicity in the 41.2 ppb Hg group.

Within a few hours after immersion in mercurial solutions, increased rates of opercular beating and coughing were observed in the fish. Prior to death, the exposed fish also displayed vigorous darting in response to noise or sudden changes in light (hypersensitivity), quivering of fins and lateral muscles, and loss of equilibrium. Some of the fish darkened in color and the gills of fish exposed to 81.5 ppb Hg began to bleed prior to death.

The amount of mercury accumulated in fish surviving 96 hr was greatest when the concentration of methylmercury originally present in the water was highest (Table 2). Comparatively higher mercury levels found in fish following exposure to 31.0 ppb Hg probably resulted from the lower DO. concentration observed in that exposure.

The amount of mercury in the fish that died increased with increasing survival time and concentration of mercurial originally present in the water. Fish which lived until the fourth day in medium methylmercury concentrations accumulated more mercury than fish which lived for the same time period in lower concentrations or fish that died quickly in higher concentrations of methylmercury. These results, therefore, suggest the absence of a threshold concentration of mercury in the whole fish which was correlated with death.

Table 2. The relationships among total mercury concentrations in fish, time to death, and methylmercury concentrations in the water.

Initial concentration of methylmercury in water (mean ppb Hg)	Percent mortality in 96 hr	Time to death (hours)	Concentration of mercury in fish[a] (ppm Hg)	
			Survivors	Mortalities
31.0	0	--	8.6 ± 0.5	---
34.0	20	68	7.9 ± 0.6	5.0 ± 0.5
37.2	20	66-95	8.1 ± 0.4	6.7 ± 1.0
39.1	50	70-92	9.5 ± 2.1	7.6 ± 0.7
39.3	50	76-93	9.5 ± 1.0	8.4 ± 0.5
41.2	20	92-95	9.8 ± 0.8	10.2 ± 0.7
43.2	100	50-93	---	7.3 ± 1.6
47.1	100	19-69	---	5.3 ± 1.1
50.2	100	45-69	---	7.3 ± 1.4
81.5	100	10-32	---	3.8 ± 0.7

[a] Mean ± S.D.

Dilute Sea Water Adaptation Experiments

The salinity tolerance experiments were designed to test our hypothesis on the effects of methylmercury exposure to juvenile anadromous salmonids. Saline water was hypothesized to produce greater toxicity than fresh water if fish previously exposed to the mercurial were transferred to saline or fresh water free of mercury. Several toxicological parameters in the fish were measured during exposure to methylmercury and after transfer to fresh and saline water.

The opercular beating rate (OBR) of fish during exposure to methylmercury chloride in replicate GS-I was proportional to the initial concentration of methylmercury in the water (Figure 3). The OBR at 11.1 ppb Hg was not significantly different than that of controls and is hence not shown. Note that the OBR increased rapidly during the first six to eight hours of each 24 hr period when the fish were transferred to freshly prepared methylmercury solutions and then decreased again as the concentration in solution declined (the pattern of methylmercury decrease was similar to Figure 1 and the percent of decrease is given in Appendix IIIb). This OBR response pattern was similar in all replicates with both steelhead trout and coho salmon.

The coughing reflex rate (CRR) of fish during exposure to methylmercury chloride followed a pattern somewhat similar to that

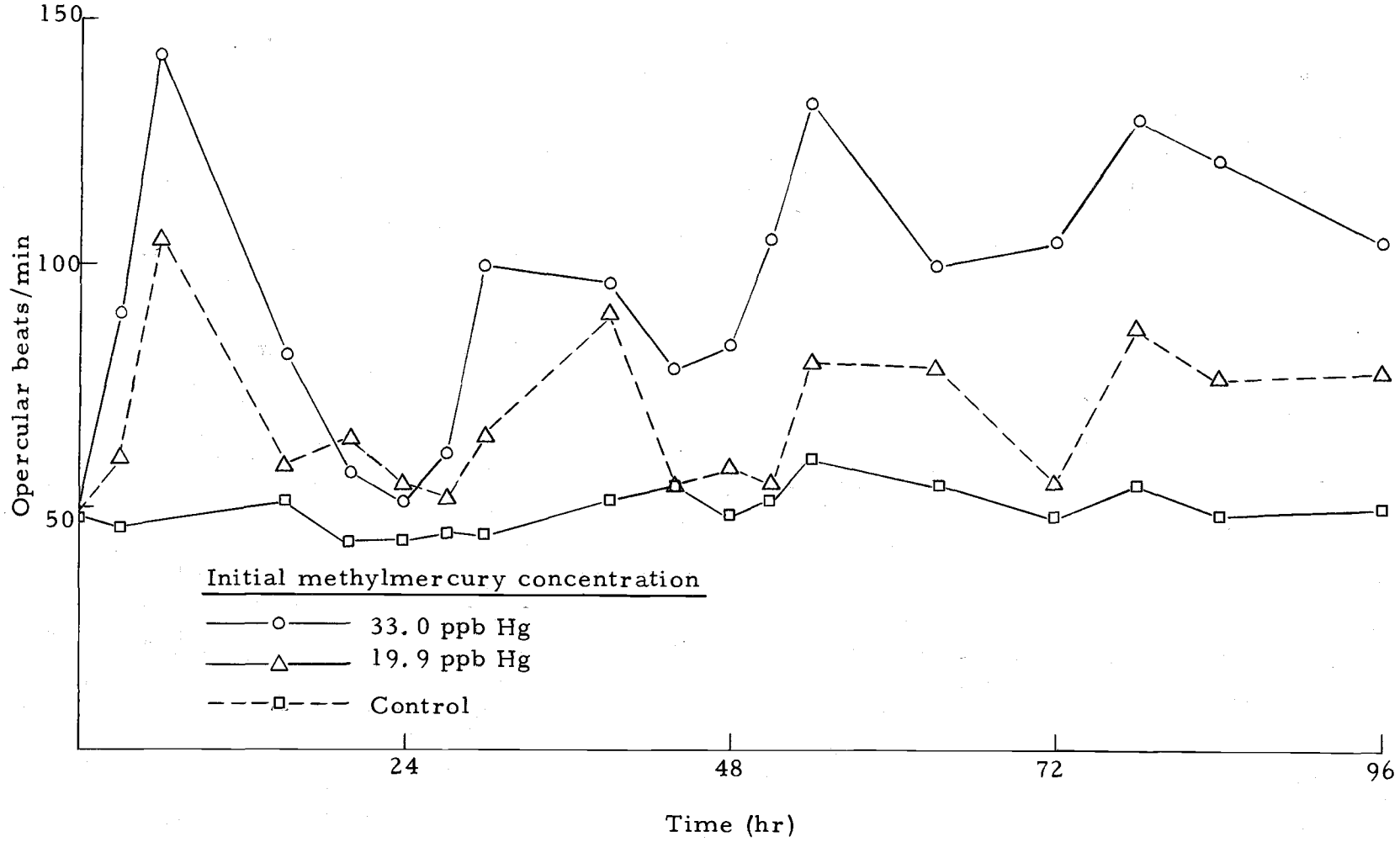


Figure 3. The relationships among the opercular beating rate of steelhead trout during 96 hr exposures, the methylmercury concentration in the water, and exposure time (replicate GS-I).

of the OBR (Figure 4). The CRR was proportional to the initial methylmercury concentrations and also reflected the 24 hr fluctuations in concentrations. The CRR was calculated as cough reflexes per 100 opercular beats to compensate for differences imposed by variations in the OBR. This facilitated comparison of CRR at different points of time and between different exposures. Thus, it can be seen that while the OBR response was most severe on the first day, the intensity of CRR response increased until the third day. All steelhead trout and coho salmon displayed this basic type of CRR pattern.

Although the patterns of OBR and CRR responses were similar in all replicates, the degree of response varied. To compare responses, the mean OBR and CRR rates at 8 and 23 hours after the introduction of fish to fresh solutions have been calculated for each entire 96 hr exposure (Figures 5 and 6). There was little difference in the OBR and CRR between controls and fish exposed to approximately 10 ppb Hg, but in the other exposures the responses increased in relation to the concentrations of methylmercury.

The responses in replicate GS-II at approximately 20 and 32 ppb Hg were markedly less than at the same concentrations in replicate GS-I. But the responses in KS-II fish were significantly greater than in KS-I fish in nearly every case. No consistent correlations were observed between the differences in responses and the DO in the solutions, the hardness of the water, or the age and laboratory

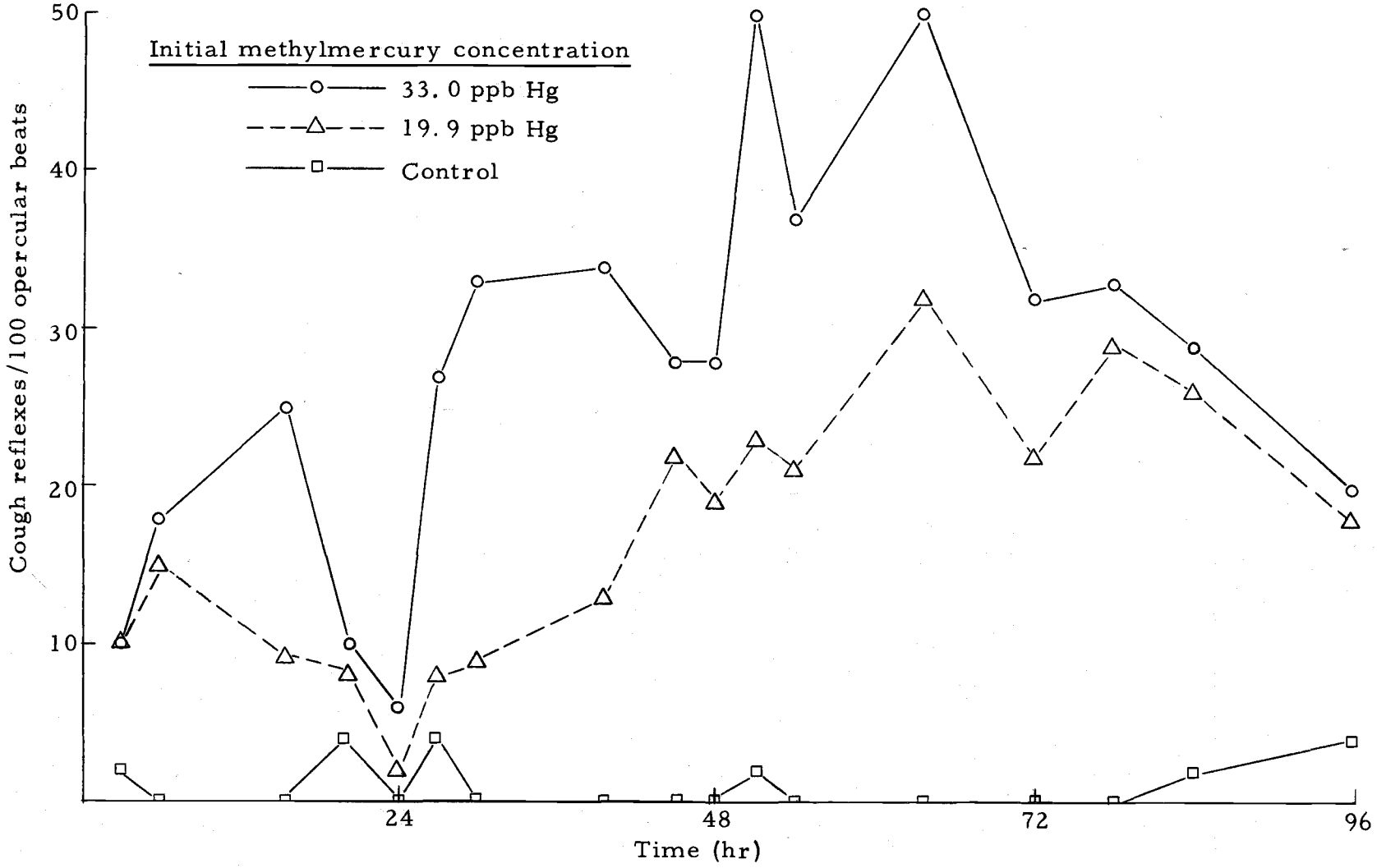


Figure 4. The relationships among the coughing reflex rate of steelhead trout during 96 hr exposures, the methylmercury concentration in the water, and exposure time (replicate GS-I).

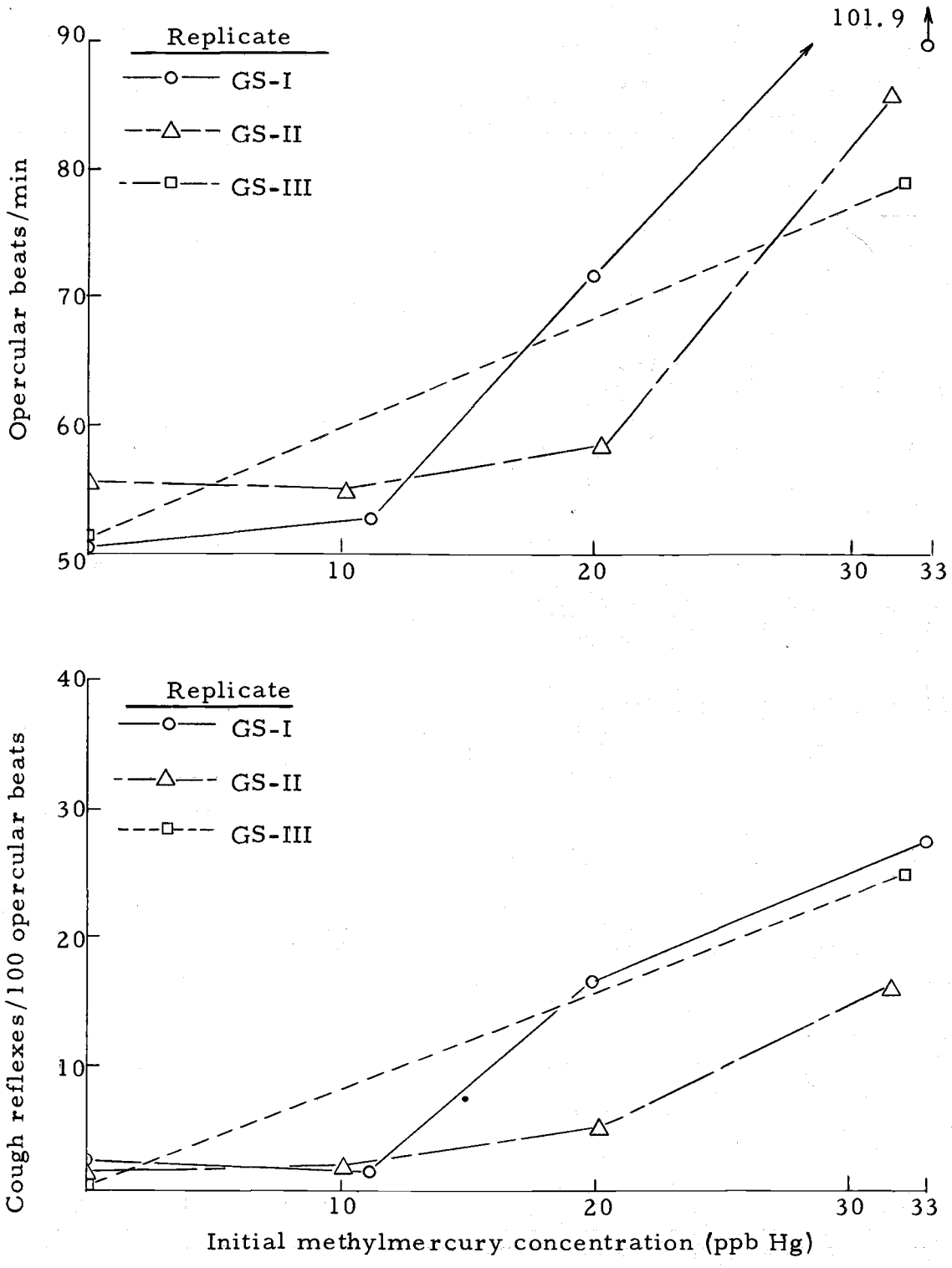


Figure 5. The effects of methylmercury exposure on opercular beating and coughing reflex rates of steelhead trout during 96 hr exposures.

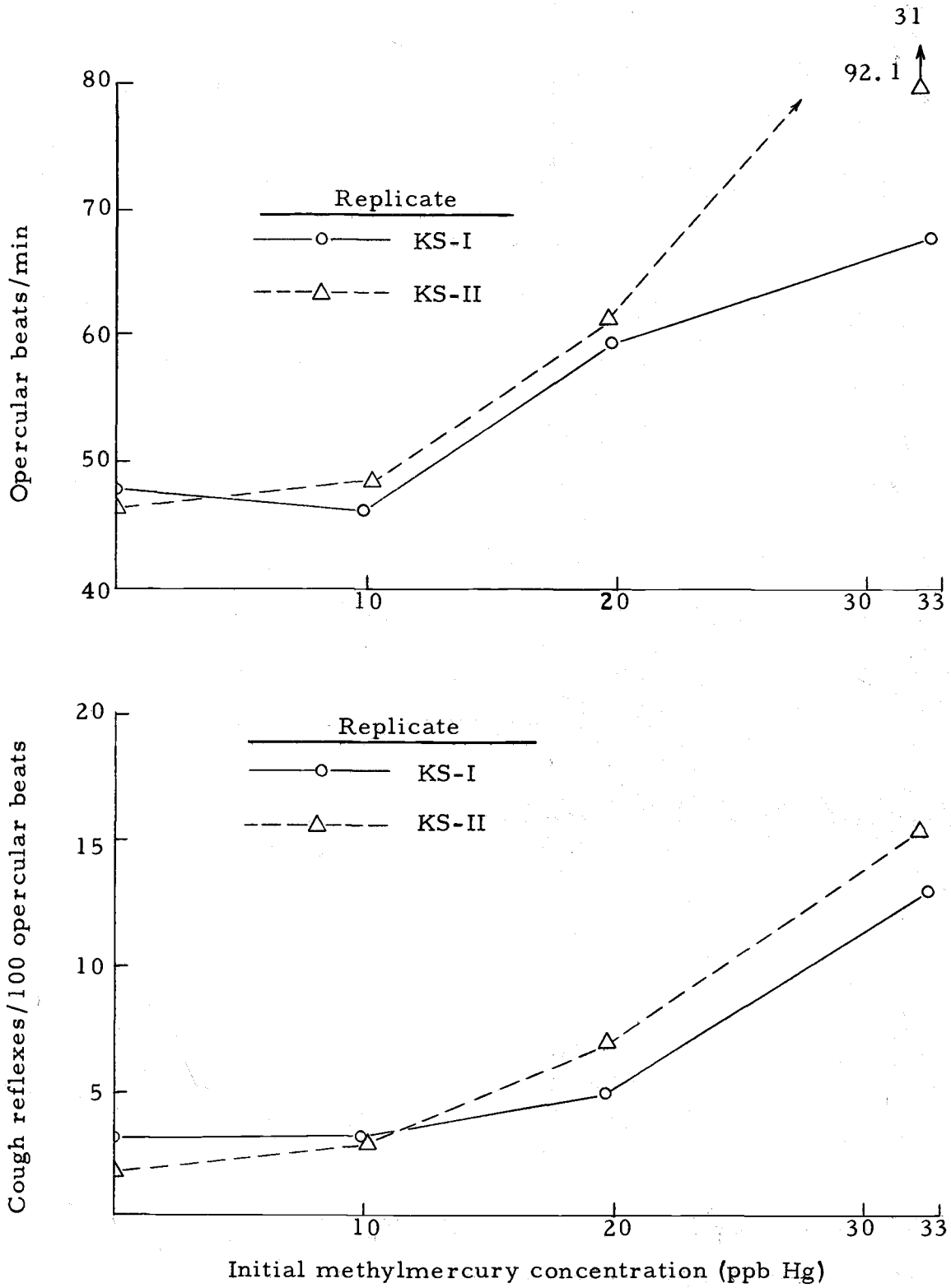


Figure 6. The effects of methylmercury exposure on opercular beating and coughing reflex rates of coho salmon during 96 hr exposures.

acclimation time of the fish. The slightly heavier fish in replicate GS-II than in replicate GS-I might explain the response differences in that case, but KS-I and II fish were nearly identical in weight. The pH of KS-I and II solutions was 7.4 and 6.9, respectively, but the pH of all GS-I and II solutions was 7.2. Since the pK of methylmercury chloride is 5.4 (Clarkson, 1972), there should have been a greater dissociation of the methylmercury cation and the chloride at pH 6.9 than at pH 7.4. It is not known, however, what effect an increased ratio of methylmercury cation/methylmercury chloride could have had on the OBR or CRR. Therefore, no clear explanations for the differences in responses are evident for either fish species.

Hypersensitivity to noise and sudden light changes were observed in many of the steelhead trout and coho salmon during the fourth day of exposure to approximately 32 ppb Hg. Some of these fish became darker in color, but loss of equilibrium and quivering that was observed in the 96 hr LC-50 experiments failed to occur.

Mercury that had accumulated in selected tissues of steelhead trout from replicate GS-III at the end of 96 hr was assayed (Table 3). Each fish used in replicate GS-III was about 15% heavier than fish used in replicates GS-I and II (Appendix IIb) since there were 20% fewer fish for the third replicate. Therefore, the total weight of fish in each exposure chamber was similar. Less methylmercury was depleted from the water in replicate GS-III than in replicates GS-I and

II (Appendix IIIb). Since most methylmercury depleted from the water was accumulated in the fish (Table 2), the concentration of mercury in the tissues of the GS-III fish were probably somewhat less than in fish exposed to approximately 32 ppb Hg in replicates GS-I and II.

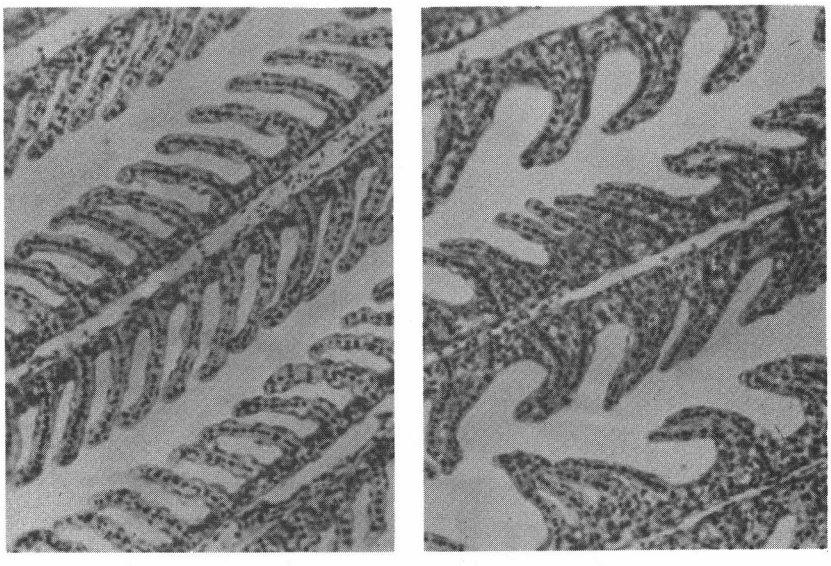
Table 3. Mercury concentrations in selected tissues of steelhead trout exposed for 96 hr to methylmercury chloride at a mean initial concentration of 32.2 ppb Hg (replicate GS-III).

Tissue	Number of fish sampled	Mercury concentration (ppm Hg $\pm$ S.D.)
Whole body	5	5.6 $\pm$ 0.4
Liver	3	29.9 $\pm$ 3.6
Gill and arch	3	25.5 $\pm$ 2.0
Kidney	3	24.4 $\pm$ 3.0
Brain	3	5.0 $\pm$ 0.6
Epaxial muscle	3	1.9 $\pm$ 0.6

Comparing mercury levels of different tissues indicates that the liver, gill and bony arch, and kidney tissues accumulated the highest concentrations in descending order. The concentration of mercury in the soft tissue of the gill filaments was probably somewhat higher than in the gill and arch combined since fish bone tends to accumulate less mercury than many other tissues (Hannerz, 1968). Therefore, the soft gill filament tissue appeared to have the highest concentration of any tissue examined.

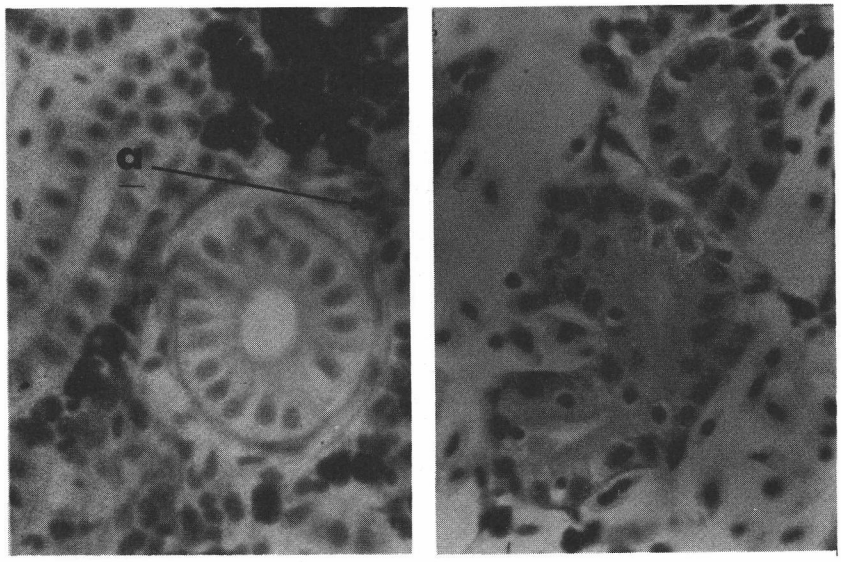
Histological analysis was performed on gill, kidney and liver tissues from the GS-III fish after the 96 hr exposure. Hyperplasia of interlamellar tissue in the gills of steelhead trout exposed to an initial methylmercury concentration of 32.2 ppb Hg was apparent when compared to control fish in Figure 7. These results are typical of the three fish examined at each exposure. The masses of tissue enlarged and filled in part of the spaces between the secondary lamellae where the blood was normally separated from the irrigating water by a thin epithelium. There were many cases where the lamellae of fish exposed to methylmercury appeared to be stuck together without the normal separation between them. The damage appeared to be more severe near the tips of the filaments than at the bases, perhaps as a result of greater water flow near the tips and therefore exposure of the tips to more methylmercury per unit time. Surprisingly, no histological damage was found in the gills of the coho salmon (two fish at each methylmercury concentration) examined at 96 hr in replicate KS-I at a methylmercury concentration similar to that used in replicate GS-III.

Histological examination revealed degenerated kidney tubules in the posterior kidneys of steelhead trout exposed to methylmercury (Figure 8). Areas were also present where interstitial hematopoetic cells were degenerated to a smaller size than normal or altogether absent. No histological damage was found in the kidneys of coho



Control Experimental

Figure 7. Photomicrographs of gill filaments from steelhead trout showing hyperplasia of interlamellar tissue and cohesion between secondary lamellae in fish exposed to methylmercury (32.2 ppb Hg, initial concentration) for 96 hr. These were wax imbedded, 6μ sections stained with haematoxylin and eosin (x 100).



Control Experimental

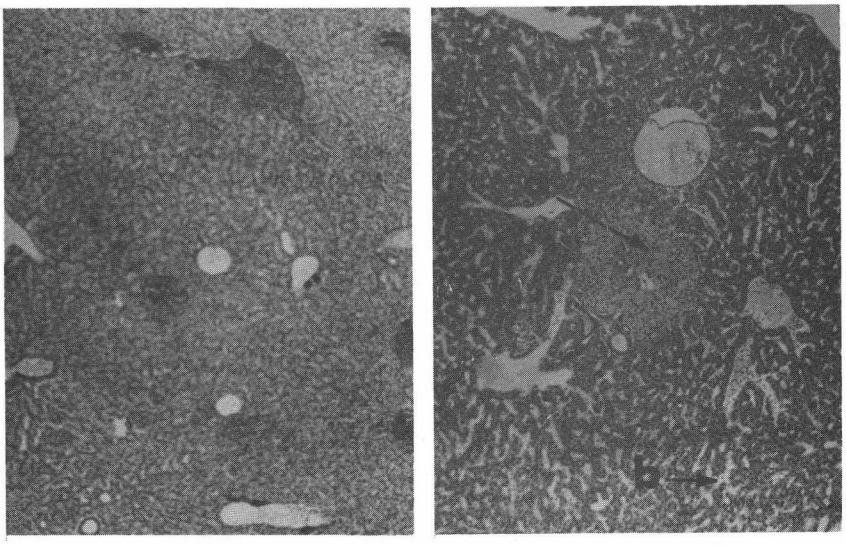
Figure 8. Photomicrographs of posterior kidney tissues from steelhead trout showing degenerated tubules and partial absence of interstitial hematopoetic tissue in fish exposed to methylmercury (32.2 ppb Hg, initial concentration) for 96 hr. These were wax imbedded, 6μ sections stained with haematoxylin and eosin (x 400). a = interstitial hematopoetic tissue

salmon from replicate KS-I.

Areas of necrotic hepatocytes were numerous in liver tissue from steelhead trout exposed to methylmercury (Figure 9). This condition was described as peribiliary necrosis by Wales (1972). In addition, the less dense appearance of the liver tissue from steelhead trout exposed to methylmercury was the result of depleted stored materials consequently leaving larger capillary spaces when compared to control fish. Liver tissue from coho salmon was not examined.

The effect of methylmercury exposure on dilute sea water adaptation of juvenile steelhead trout is shown in Figure 10. Regardless of the previous 96 hr exposure to concentrations of methylmercury as high as 33 ppb Hg, no mortality was observed when the fish were transferred for seven days in fresh water free of mercury. Control fish and fish exposed to approximately 10 ppb Hg were also able to survive in 21 ppth S water for seven days. But mortality was severe in 21 ppth S water among fish previously exposed to approximately 20 and 32 ppb Hg.

Mortality was less in the GS-II fish than in the GS-I fish which correlates with the lesser OBR and CRR responses of the GS-II fish during the 96 hr methylmercury exposure. All mortality occurred within 7.5 hr in GS-I fish previously exposed to 33.0 ppb Hg while mortality for all other fish occurred from 15 to 106 hr after transfer to 21 ppth S water.



Control Experimental

Figure 9. Photomicrographs of liver tissues from steelhead trout showing necrotic hepatocytes and depleted material stores in fish exposed to methylmercury (32.2 ppb Hg, initial concentration) for 96 hr. These were wax imbedded, 6μ sections stained with haematoxylin and eosin (x 40). a = necrotic area; b = enlarged capillary space due to partial absence of stored materials.

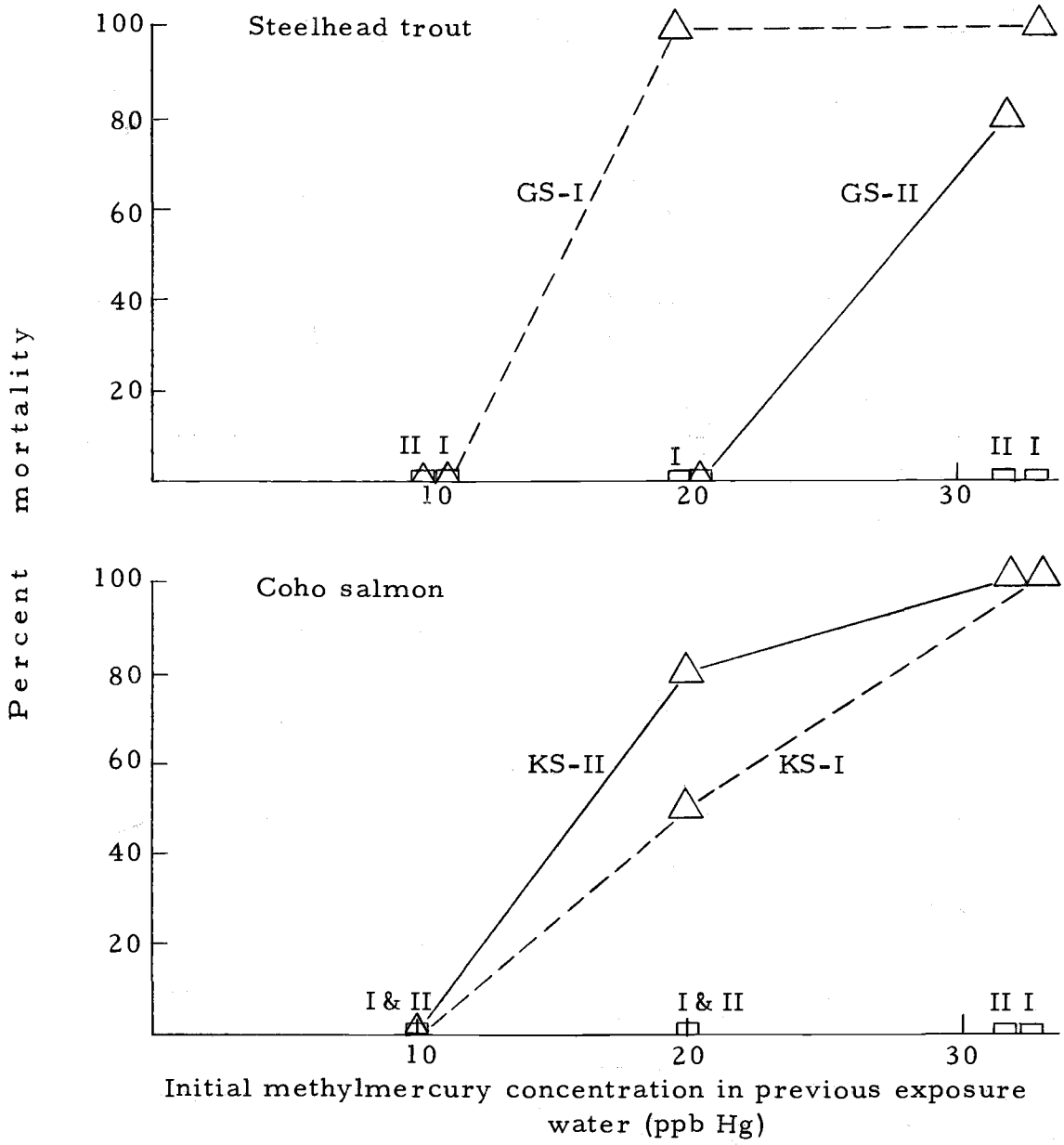


Figure 10. The effect of methylmercury exposure on the mortality of steelhead trout and coho salmon after transfer to fresh water or 21 ppth S sea water. □ = 7 days in fresh water; △ = 7 days in saline water.

The effect of methylmercury exposure on dilute sea water adaptation of juvenile coho salmon was similar to that of steelhead trout (Figure 10). The percent of mortality again correlated well with the OBR and CRR responses observed during the 96 hr methylmercury exposures. Deaths occurred within 15 hr after fish previously exposed to approximately 32 ppb Hg were transferred to saline water. For fish previously exposed to 20 ppb Hg, mortality occurred between 7 and 50 hours after transfer to the dilute sea water.

Measurements of OBR and CRR were made in fish held in fresh and saline water. The differences in OBR responses in steelhead trout from replicates GS-I and II after transfer to saline water are shown in Figures 11 and 12. The OBR in saline water was well correlated with the concentration of methylmercury in the previous exposure solutions and presumably with the concentration of mercury in the fish. The OBR rapidly decreased during the first 24 hr after transfer to fresh water in all cases.

Unfortunately, in coho salmon transferred to saline water, observations on the OBR of fish previously exposed to approximately 32 ppb Hg could not be made before the rapid death of the fish. However, the OBR of the fish previously exposed to approximately 20 ppb Hg decreased slowly to a level near that of control fish during the first three days and remained near that level during the last four days. Fifty to eighty percent of the fish died during this time period. The

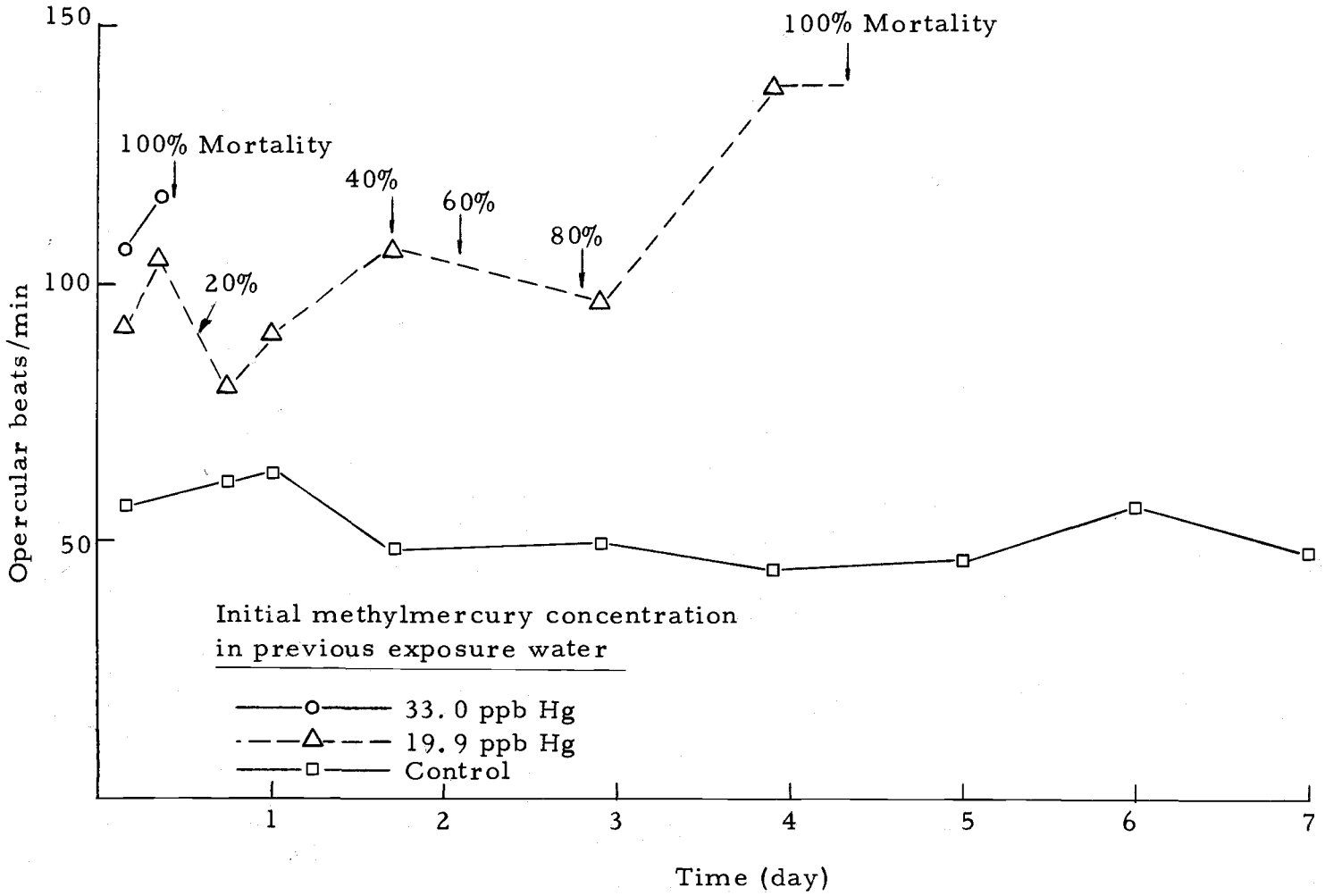


Figure 11. The effect of 21 ppth S sea water on the opercular beating rate of steelhead trout after exposure to methylmercury solutions (replicate GS-I).

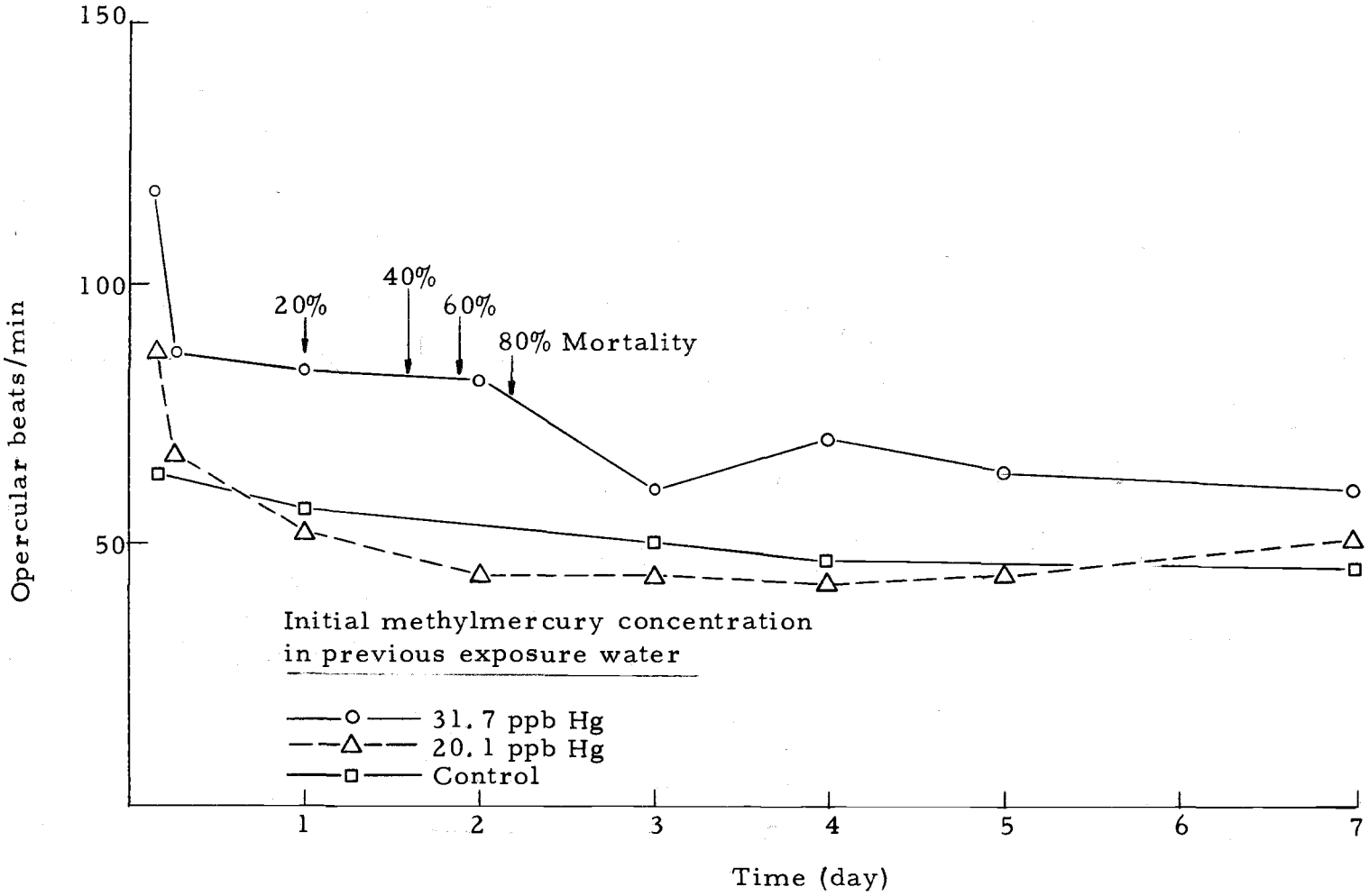


Figure 12. The effect of 21 ppth S sea water on the opercular beating rate of steelhead trout after exposure to methylmercury solutions (replicate GS-II).

OBR of coho salmon transferred to fresh water decreased rapidly in the first 24 hr.

It is interesting that the CRR of steelhead trout and coho salmon decreased rapidly in the first 24 hr of transfer to fresh or saline water regardless of their previous exposure to methylmercury or the CRR observed during the methylmercury exposure period. This was even true for fish which eventually died in the saline water.

Fish which appeared intoxicated in saline water after transfer from methylmercury solutions (fresh water) displayed symptoms similar to intoxicated coho salmon in the 96 hr LC-50 experiments. Hypersensitivity to noise and sudden light changes, quivering of fins and lateral muscles, darkening of the skin, and loss of equilibrium was observed in both steelhead trout and coho salmon previously exposed to approximately 20 or 32 ppb Hg. Many fish became thin, as if catabolizing considerable amounts of tissue or losing body water, before finally dying. The symptoms disappeared within four days in fish that survived.

Coho salmon from replicates KS-I and II were assayed for mercury at their time of death or seven days after transfer from the methylmercury solutions (Figure 13). Fish exposed to the same methylmercury solutions retained nearly identical accumulations of mercury after seven days in fresh water or saline water, suggesting that salinity did not significantly affect the retention of mercury over

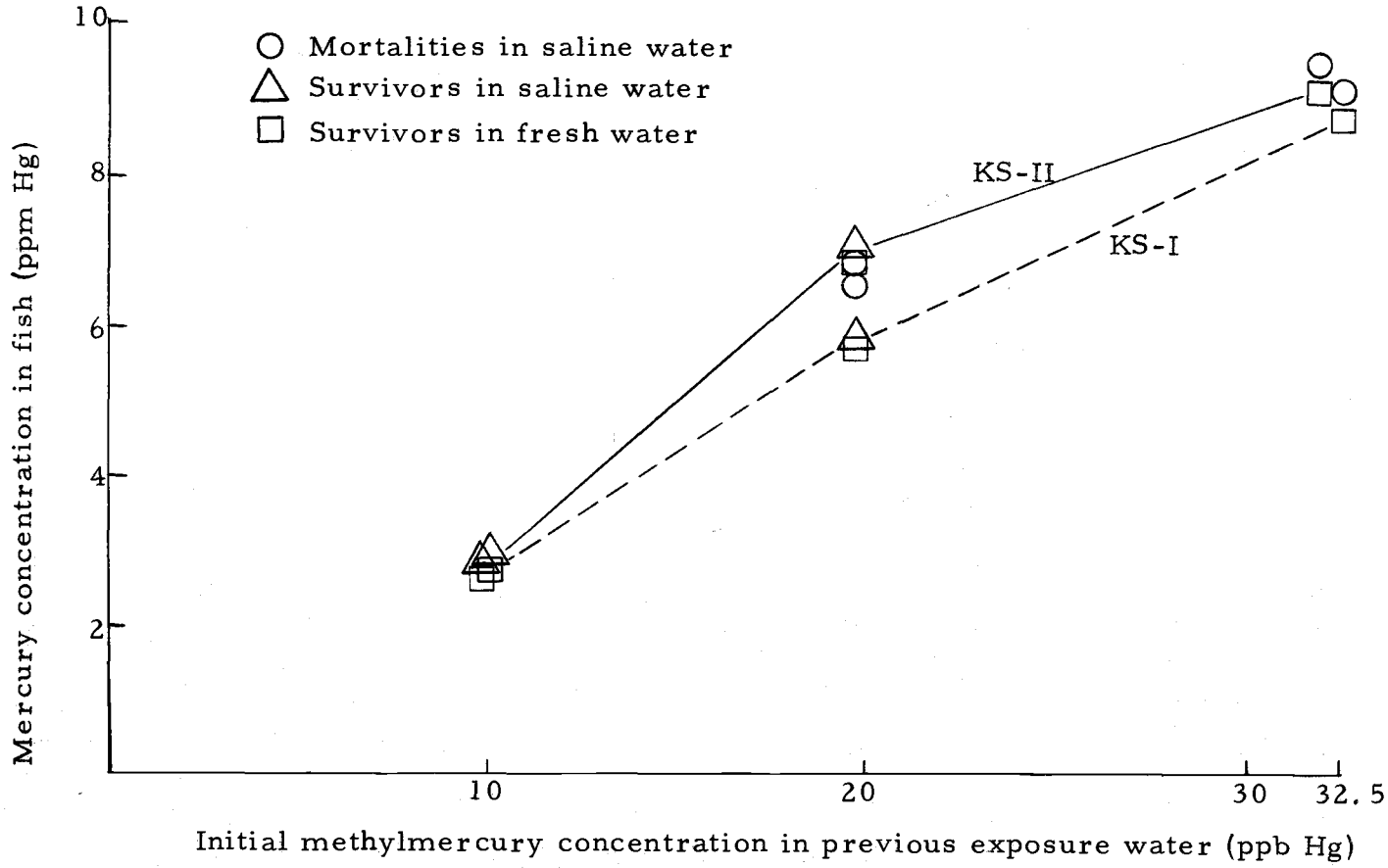


Figure 13. The effect of methylmercury exposure on the mercury content of coho salmon after transfer to 21 ppth S sea water or fresh water for seven days.

this time period. Although mercury was never detected in these solutions, some radioactive mercury was recovered in the charcoal filters. This indicates that small amounts of mercury were eliminated from the fish into the water and subsequently accumulated in the charcoal.

Some small differences are seen when comparing mercury accumulations in fresh water survivors and saline water mortalities from the same previous methylmercury exposure. Since it was observed that some mercury was eliminated from the fish, the differences may be due in part to the longer time period during which survivors could have excreted or lost mercury. However, since the mercury concentrations are calculated on a wet weight basis, the higher concentrations found in mortalities may have been due to dessication of the fish in saline water.

Differences in mercury concentrations in the coho salmon correlated well with relative toxic responses of the fish. The KS-II fish which had higher concentrations of mercury also had higher OBR and CRR responses during the period of methylmercury exposure, their mortality was higher in saline water, and their survival time was shorter when compared to KS-I fish.

MERCURY IN JUVENILE COHO SALMON OF OREGON

Methods

Resident and seaward migrating juvenile coho salmon were collected from western Oregon waters for analysis of total mercury content (the sum concentration of all forms of mercury) in muscle, gill, liver, and kidney tissues. All sampling locations were in coastal drainages between the Alsea River drainage (central coast) and the Sixes River drainage (south coast) except for two locations in the inland valley of the Willamette River drainage.

Coho salmon reared in captivity were also collected for measurement of mercury content. One captive population consisted of fingerlings (1970 brood) from the Oregon Fish Commission's Fall Creek Hatchery which were reared at the O.S.U. Averill facility until June, 1971, and then reared in the Weniger Hall laboratory until November, 1971. In the laboratory, these fish were kept in 15°C water and fed Oregon pellets. A second group consisted of 1969 brood fish reared at Fall Creek Hatchery until mid-April and then held captive (without feeding) until mid-May in aluminum cages anchored in Squaw Creek near O.S.U.

All collections of fish for mercury assay were made in May, June and September of 1971. Field collections were obtained by seining, electro-shocking, or from stream traps, depending on the

location and personnel involved. Fish were frozen with two to six hours after collection.

Analysis of the May and June collections was made between June and August, and analysis of the September collections was completed in October. The tissues were thawed, excised and weighed. Depending on the size of the fish, pooling of tissue from numerous fish was necessary to give an adequate aliquot for assay. Tissues were digested to oxidize all forms of mercury to ionic mercury by a method similar to that of Uthe, Armstrong, and Stainton (1970). The method is outlined in Appendix I.

The digest was assayed for total mercury content by flameless atomic absorption using a Jarrell-Ash Model 82 atomic absorption spectrometer equipped with a 25 cm quartz cell. The method was similar to that described by Jeffus, Elkins, and Kenner (1970), but nitrogen was used as the carrier gas for the mercury vapor. The percent recovery of methylmercury spikes that were introduced at the initiation of the digestion process was assayed using four replicates at each spike concentration. The percent recovery was found to be 92.8% $\pm$ 12.4% at 25 ppb Hg, 108.3 $\pm$ 8.0% at 50 ppb Hg, and 100.0 $\pm$ 3.1% at 1,000 ppb Hg.

Results

The concentrations of total mercury (all forms of mercury which are present) in selected tissues of juvenile coho salmon of Oregon are tabulated in Tables 4 and 5. The trend of increasing mercury concentration with increasing age and/or size of fish which have been collected from the field has been documented by Bache, Gutenmann and Lisk (1971), Hasselrot (1968), and Westöö (1967). In accordance with this trend, the largest fish in our study generally contained the highest concentration of mercury and the smallest fish contained the lowest, but this is stated cautiously, however, because of the limited data on different size fish from the same location at the same time of year.

Because of the size and age effect on mercury concentrations, comparisons of mercury levels in tissues of fish from different locations must be made with similar size and age fish in order to estimate differences in mercury exposure between locations. Significant geographical differences are not evident from the data presented in Table 4.

There appears to be significant differences in mercury concentrations in muscle tissue between fish reared in captivity and fish from the wild. Coho salmon from the Fall Creek Hatchery that had been raised in the Weniger Hall laboratory were sampled in September

Table 4. Concentrations of total mercury in muscle tissue of coho salmon from field locations in Oregon.

Sample Location (drainage)	Collection date	Wet weight[a] (g)	Fish per assay	Number of assays	Mercury[a] (ppb Hg)
Adams Cr. (Tenmile Lake)	6/71	1.5 ± 0.1	5	2	24 ± 4
Congdon Cr. (Triangle L.)	5/71	1.2 ± 0.1	10	2	20 ± 4
Crafton Cr. (Sixes R.)	5/71	1.3 ± 0.1	5	2	17 ± 5
Crooked Cr. (Alsea R.)	5/71	1.0 ± 0.1	10	2	8 ± 5
Crooked Cr. (Alsea R.)	5/71	13.6 ± 0.9	1	6	26 ± 9
Fall Creek (Alsea R.)	5/71	1.0 ± 0.1	11	2	13 ± 6
Park Cr. (Coquille R.)	6/71	1.8 ± 0.1	5	2	43 ± 4
Rock Cr. (Siletz R.)	5/71	1.2 ± 0.1	10	2	16 ± 2
S. Tenmile L.	5/71	21.3 ± 0.1	1	2	56 ± 6
S. Tenmile L.	5/71	35.0 ± 2.5	1	2	75 ± 27
Tobe Cr. (Alsea R.)	5/71	0.9 ± 0.1	13	2	9 ± 7
Tobe Cr. (")	5/71	6.2 ± 0.3	1	2	32 ± 5
Tobe Cr. (")	5/71	21.1	1	1	24
Tobe Cr. (")	9/71	1.6 ± 0.3	4	3	33 ± 4
Tobe Cr. (")	9/71	5.1 ± 0.4	2	3	27 ± 2
Tobe Cr. (")	9/71	8.2 ± 1.2	1	6	33 ± 4
Willamette R.[b]	5/71	21.9 ± 2.6	1	5	57 ± 7
Willow Cr. (Floras L.)	5/71	1.4 ± 0.1	5	2	39 ± 1

[a] Mean ± S.D.

[b] Near Selwood Bridge, Portland.

Table 5. Concentrations of total mercury in selected tissues of coho salmon.

Sample				Mercury[a] (ppb Hg)			
Location (drainage)	Collection date	Wet weight[a] (g)	Number of fish	Muscle	Liver	Gill & arch	Kidney
Crooked Cr. (Alsea R.)	5/71	13.6 ± 0.9	6	26 ± 9	15[b]	---	---
S. Tenmile L.	5/71	21.3 ± 0.1	2	56 ± 6	125[b]	---	---
S. Tenmile L.	5/71	35.0 ± 2.5	2	75 ± 27	120[b]	---	---
Tobe Cr. (Alsea R.)	9/71	1.6 ± 0.3	12	33 ± 4[c]	---	22[b]	37[b]
Tobe Cr. (Alsea R.)	9/71	5.1 ± 0.4	6	27 ± 2[d]	23[b]	25[b]	34[b]
Tobe Cr. (Alsea R.)	9/71	8.2 ± 1.2	6	33 ± 4	31[b]	22[b]	39[b]
Willamette R.[e]	5/71	21.9 ± 2.6	5	57 ± 7	134[b]	---	---
Weniger Hall (lab.)	6/71	6.7 ± 0.8	6	22 ± 4	64[b]	32[b]	73[b]
Weniger Hall (lab.)	9/71	6.5 ± 0.9	12	101 ± 6	105 ± 8[f]	58 ± 6[f]	168 ± 34[f]
Squaw Cr.[g]	5/71	27.4 ± 0.7	2	120 ± 6	151[b]	---	---

[a] Mean ± S.D.

[b] All fish were combined for one assay.

[c] Three groups of four fish each were assayed.

[d] Three groups of two fish each were assayed.

[e] Near Selwood Bridge, Portland.

[f] Two groups of six fish each were assayed.

[g] Held in Squaw Cr. near O.S.U. for one month after rearing at Fall Cr. Hatchery (Alsea River).

and found to have accumulated considerably higher mercury residues than those present when they were first sampled in June (Table 5). The levels of mercury were also greater than those found in any fish from field locations. Fish from the Fall Creek Hatchery stock that were collected from Squaw Creek in May also contained high mercury residues relative to fish from other locations. The Squaw Creek fish were planted one month earlier and were the only fish which had been reared for over one year in a hatchery.

Analysis of the fishmeal-based Oregon pellet diet fed to the Weniger Hall fish showed that four samples contained 48 $\pm$ 4 ppb Hg after reconstituting it to contain 80% water. Information on the mercury content of the hatchery diet or field diets was not obtained.

Gill and arch tissue of fish generally contained the lowest concentration of mercury of any tissue assayed in a particular group of fish (Table 5). Liver and muscle tissue generally contained intermediate concentrations (although liver tissue was often higher) and kidney tissue generally contained the highest concentrations of mercury. This is a typical distribution for mercury in fish from relatively unpolluted field locations (Amend et al., 1969, and Lockhart et al., 1972).

DISCUSSION

Laboratory Experiments

Fluctuations in the concentration of methylmercury were observed to follow a similar pattern during all 96 hr exposures. It is not known if this pattern is analogous to that found in the case of any specific stream or lake, but additional information on biological responses to fluctuating concentrations of a toxicant is valuable since conditions of polluted environments in the field are not likely to remain constant. Particularly in a stream, diurnal fluctuations in physical, chemical, and biological factors (including the activities of man) may lead to similar variations in toxicant concentrations.

The mean initial concentration of methylmercury in the fresh exposure solutions every 24 hr has been used to describe the concentration employed for each 96 hr exposure experiment. Based on this definition of exposure concentrations, most toxic responses in fish were reproducible in replicate methylmercury exposures.

The histology of gill and kidney tissue, however, was not comparable between steelhead trout and coho salmon. Damage was observed only in the tissues of steelhead trout, though coho salmon were similarly exposed to methylmercury. It appears that the latter species was either more resistant to damage from a given accumulation of mercury in the gills, or the species was able to prevent

concentrations of mercury from reaching the levels found in steelhead trout gills. Coho salmon gills were not assayed for mercury; however, concentrations in the whole body were greater in coho salmon than in steelhead trout similarly exposed to methylmercury (Table 3 and Figure 13). The steelhead trout were exposed to higher levels of radiation from ^{203}Hg-labeled methylmercury (Appendices IIIb and IIIc), but the damage was atypical of radiation damage to gills of aquatic animals and was more likely due to some action of methylmercury (Mix, 1972).

Comparison of results from this investigation with results where methylmercury concentrations are more constant or vary in a different pattern may present some difficulties. The toxicity of constant or fluctuating concentrations of zinc appear to be quite similar in some cases. This was demonstrated by Brown, Jordan and Tiller (1969) using rainbow trout and by Cairns, Waller, and Smrchek (1969) using goldfish. But Brown et al. (1969) found that the toxicity of ammonia to rainbow trout decreased if the ammonia concentration was not constant.

Several factors observed in this investigation suggest that the toxicity of methylmercury to coho salmon and steelhead trout will be greater in a constant concentration than in a daily fluctuating concentration of the toxicant. The rate of methylmercury depletion was greatest during the first few hours after transfer of the fish to a

fresh solution. Most of the depleted mercury was concentrated in the fish, so it was assumed that accumulation was most rapid during those first few hours. This high accumulation rate was correlated with an observed increase in the OBR during the same period. Since the OBR is an estimation of respiratory water flow over the gills, it is logical to assume that the gills would be exposed to more methylmercury solution per unit time when the OBR is increased and it follows that the rate of mercury accumulation would also be increased. The OBR was also found to increase with increasing concentrations of methylmercury in the water, consequently fish would probably accumulate mercury faster in a solution of the mercurial that is held at a given concentration X than in a solution where the mercury content of the water varied with a maximum concentration equal to X. This would likely lead to greater toxicity in the fish exposed to the solution with the constant level of mercurial.

Physiological, histopathological, and behavioral responses, and accumulation and distribution of mercury in the body have been used to provide insight on the mechanisms of methylmercury toxicity in this investigation. Knowledge of the mechanisms of toxic action may provide increased predictability of toxicity in relation to interaction of methylmercury with other factors within the fish's environment and life history. Conversely, by studying how these factors affect the toxicity of methylmercury to the fish, knowledge of toxic mechanisms can be gained.

Acutely lethal concentrations of various metallic compounds including inorganic mercury and some organo-mercurials are believed to block oxygen uptake in the gills of fish by causing damage to the gill tissue and/or coagulation of mucus on the filaments. Skidmore (1970) demonstrated that rainbow trout increased their rate of respiratory water flow in response to a blockage of oxygen uptake by zinc. Respiratory water flow over the gills has been suggested as a means of detecting zinc and other toxicants with "in-house" toxicant monitoring systems for industries (Sparks, Cairns, and Heath, 1972).

The visual technique of measuring OBR in this investigation has some limitations but it does provide a fair approximation of relative respiratory water flow (Heath, 1972). My results show that the rate of water flow across the gills increases in juvenile coho salmon and steelhead trout in relation to the concentration of methylmercury in the water. This is probably a response, in part, to interference with oxygen uptake and transport, but it could be partially due to an increased oxygen demand within the fish.

The area of the gills usable for normal gas exchange between the blood and ambient water appears to have been markedly reduced after exposure to methylmercury. But this was only true for steelhead trout. Also, the OBR increased within the first few hours in both species (perhaps before gill damage occurred in the steelhead trout), suggesting that something else was blocking oxygen uptake and

transport or else methylmercury caused an increased oxygen demand by the fish. Increased mucus secretion and coagulation over the gills has been suggested as a block to oxygen uptake, but either this did not occur, or was too slight to visually observe except in a few rare cases during this investigation.

Since methylmercury penetrated so rapidly into the fish's body, oxygen uptake and transport might have been inhibited by an interaction of the mercury with the blood. However, effects on oxygen uptake and transport have not been found in mammals even though 90 to 95. 5% of the methylmercury in the blood is bound to erythrocytes after injection of the mercurial (Selikoff, 1971). This does not eliminate the possibility of a direct effect of methylmercury in the fish's blood. Methylmercury may have disrupted osmoregulatory or iono-regulatory mechanisms in the fish which might have also led to decreased oxygen uptake and transport efficiency of the blood. Acidosis of the blood and resultant Bohr effect on oxygen binding efficiency of the hemoglobin are possibilities to consider.

The increased coughing reflex rate (CRR) observed in methylmercury solutions probably also made a small contribution to reducing oxygen uptake efficiency. This would be due to the momentary reversal of water flow over the gills during a cough reflex.

Evidence that increased oxygen demand was responsible for the increased OBR was indirect. The hypersensitive darting of the fish,

the muscle tremors, and even the increased muscular activity of the respiratory pump probably increased the oxygen demand of the fish. The depleted energy stores of liver tissue from steelhead trout is evidence that the metabolic rate and oxygen demand of these starved fish may have been increased. Another metal, copper, has been shown to increase the oxygen consumption rate of bluegills (Leponis macrochirus) at sub-lethal concentrations in the water and this measurement has been suggested as a rapid indicator of copper in industrial water (O'Hara, 1971).

Although suffocation due to interference with oxygen uptake and transport appears to have been the primary mode of lethal action by methylmercury, additional complicating actions were evident. Loss of blood from the gills was evident in coho salmon exposed to 82 ppb Hg. Damage to the gill and kidney of steelhead trout exposed to methylmercury suggest the possible occurrence of osmotic, ionic, or pH imbalances. Neurotoxicity of methylmercury in other animal species is well summarized in Selikoff (1971). Neurotoxic symptoms of tremors and hypersensitivity of the fish were evident in this investigation and could have contributed to the lethal action of methylmercury.

The distribution of mercury among selected tissues of steelhead trout and the histopathology of gills, kidney, and liver from these fish were correlated with results found by other investigators using mercurials. Miettinen et al. (1970) observed damage in the same

three tissues of rainbow trout given oral doses of methylmercury nitrate. Bäckstrom (1969) found these three tissues to contain high concentrations of mercury relative to other tissues in salmonids, regardless of the route of entry of methylmercury nitrate. The routes used were peroral, intramuscular, intravenous and ambient water. Histological damage of the kidney was also observed in this latter study. Amend et al. (1969) observed that the degree of histological gill damage increased as the concentration of mercury in the gills increased when rainbow trout were exposed to ethylmercury phosphate under differing conditions. Thus it appears that high concentrations of organo-mercurials in gills, kidney, and liver can cause damage to these tissues which can contribute to the lethal toxicity of the mercurials.

The coughing reflex rate (CRR) observed during exposure of fish to methylmercury seems to be an indicator of the presence or absence of methylmercury in the water. During 96 hr exposures to the mercurial, the CRR was observed to be proportional to the concentration of methylmercury whenever the concentration was 20 ppb Hg or greater. The CRR decreased to normal within 24 hr in fish transferred to fresh or saline water free of the mercury compound. However, the CRR has limited applicability as a bioassay for methylmercury in water because the response is not immediate, the degree of response increases with increasing exposure, and the response is

also elicited by other chemicals (Schaumburg et al., 1967). These limitations also apply to the suggested uses of respiratory water flow or oxygen consumption rate as bioassays for specific toxicants.

The marked lethality in dilute sea water after exposure to methylmercury was probably related to complications resulting from the inability of either species of fish to prevent salt concentrations from increasing in the blood. Although histological damage in the gills and kidneys was observed only in the steelhead trout exposed to methylmercury, this suggests that physiological changes could have occurred in these vital ion and water regulating organs in both species.

The physiological action of a number of mercurials on the mammalian kidney has led to their use as clinical diuretics. They have been shown to depress active sodium reabsorption and potassium excretion in the kidney tubules, possibly by inhibition of the enzyme Na^+/K^+ ATPase (Mudge, 1970). Inhibition of isolated Na^+/K^+ ATPase by mercurials has been demonstrated in vitro (Akera, Brody, and Leeling, 1970, and Mudge, 1970). These studies suggest the possibility of diuresis of the fish in 21 ppth S water which would result in dehydration of the animal. They also suggest the possibility of methylmercury inhibition of the Na^+/K^+ ATPase in the gills. This gill enzyme normally helps to excrete salt from the blood of the fish during sea water residency.

The damage observed in the gills of steelhead trout could have presented physical barriers to the active excretion of salts. There is the possibility that a physical barrier in the form of coagulated mucus was present on the gills, though it was not visually detected.

The neurotoxic symptoms observed in fish exposed to dilute sea water suggest the possibility of neurotoxic contributions to the lethal mechanism. One way this could have occurred would have been through methylmercury interference with neurological control of salt and water balance.

Since the OBR remained high or increased in fish exposed to 21 ppth S water after being exposed to methylmercury, it seems likely that oxygen uptake and transport was complicated, or increased oxygen demand within the fish was caused by the saline water. Changes in blood chemistry due to dehydration probably occurred. This could have reduced the oxygen carrying capacity of the blood. The histology of the gills was not examined, but if such damage developed, or if mucus coagulation on the gills developed undetected, blockage of oxygen uptake may have occurred in saline water.

However, the increased OBR may have been primarily due to an increase in metabolic rate. The hypersensitive darting, the tremors, and the metabolic work required to retain water and pump salt out of the fish probably increased the oxygen demands of the fish.

Whether the 8-9 ppm Hg concentrations remaining in the bodies of seven-day survivors from fresh water could lead to the mortality of these fish if transferred to saline water is an important question. Based on the investigation of Rucker and Amend (1969), the distribution of mercury among the tissues probably shifted slightly during the seven days. But mercury may have shifted to or from different tissues and/or sites of toxic action. Rucker and Amend (1969) found that the shift was away from the gills and blood and into the liver and kidney. Lockhart et al. (1972) found that the distribution of mercury in fish (Esox lucius) can remain constant in a natural lake even when the total mercury content of the fish decreases as much as 29%. The high percentages of the methyl form of mercury in all tissues examined did not change, either. The liver and kidney were found to contain the highest concentrations of mercury in nearly every fish.

These studies suggest that after exposure to methylmercury, the distribution of mercury in the body of a juvenile salmonid will slowly shift toward the liver and kidney. Based on the present investigation, a shift of methylmercury toward the kidney may impair the ability of the fish to adapt to saline water. Since the biological half-life of methylmercury in rainbow trout and pike appears to be 700-1,000 days (Giblin and Massaro, 1972, and Lockhart et al., 1972), juvenile salmonids from the present investigation may have been inhibited from adapting to saline water for many weeks after exposure to methylmercury.

Mercury in Juvenile Coho Salmon of Oregon

The range of total mercury concentrations in muscle tissue of juvenile coho salmon extended below the range of values found by Buhler et al. (1973) in adult anadromous salmonids (coho and chinook salmon, and steelhead and cutthroat trout) and resident rainbow trout from Oregon waters. This supports the generalization that increasing age and size in fish from field locations are correlated with increasing concentrations of mercury in the muscle tissue. No data could be found in the literature on mercury levels in fish similar in size to the juvenile salmon assayed in this investigation. It appears that most Oregon fresh water areas contain relatively low levels of mercury pollution by comparing the mercury levels in adult freshwater salmonids from Oregon with those from freshwater areas where mercury pollution is known to be relatively high (Johnels et al., 1967, and Wobeser et al., 1970).

The concentration of methylmercury in the juvenile fish from the field was not determined. Bache et al. (1971) found that the percent of total mercury present as the methyl form in homogenous samples of whole fish increased with the age of lake trout (Salvelinus namaycush) between 1 and 12 years of age. The mercury in the youngest and oldest groups averaged 33.5 and 77.2% methylmercury, respectively. Since Buhler et al. (1973) found that 80.6 to 84.4% of the

mercury was in the methyl form in muscle tissue of adult freshwater fishes (age unknown) of the Pacific northwest, the presence, but not the percentage, of methylmercury in the muscle tissue of juvenile coho salmon is assumed.

The ratio of mercury concentration in the liver to mercury concentration in the muscle has been suggested as an indicator of recent mercury exposure in fish (Buhler, 1971). Regardless of route of entry, several mercurials have been shown to accumulate more rapidly in liver tissue than in muscle tissue and both tissues are relatively easy to excise and large enough to assay. A high ratio in fish exposed to methylmercury is noticeable in replicate GS-III. Ratios varied in juvenile coho salmon sampled from the field, but the data are too limited to establish differences due to geographical locations in Oregon.

Concentrations of mercury in the muscle, liver, gill, and kidney tissues of juvenile coho salmon from the field were rarely over 0.1 ppm Hg, even for the largest fish of smolting size. Assuming that the distribution of mercury within the coho salmon that were exposed to methylmercury in the laboratory experiments was similar to the distribution observed in steelhead trout similarly exposed, then the lowest concentration in the above listed tissues of coho salmon which displayed toxic symptoms was approximately 2 ppm Hg. Therefore it is unlikely that fish sampled from the field would have displayed the

same toxic symptoms upon exposure to 21 ppth S water that were observed in the laboratory fish exposed to saline water after exposure to methylmercury.

The conditions which led to higher mercury levels in fish reared in captivity than fish from the field are unknown. It seems possible that if captive fish were fed diets derived from fishmeal (as laboratory fish were), they might have been subjected to a greater food chain magnification of mercury than fish from the field which probably fed primarily on herbivorous or first order predatory invertebrates. Also, crowded conditions in captive situations may have led to greater accumulations of mercury from the water. This might have occurred due to a lower factor of dilution for mercury excreted from each captive fish and subsequently absorbed from the water by neighboring fish.

Conclusions

The following are the principal conclusions drawn from this investigation.

1. The 96 hr LC-50 of methylmercury chloride was 38.9 ppb Hg (mean initial concentration) for 6.5 g coho salmon in dechlorinated city water using a modified static water bioassay system in which the methylmercury was replenished every 24 hours. The average temperature, pH, EDTA hardness,

and dissolved oxygen concentration of the water were 15.0°C, pH 7.5, 24.2 ppm $CaCO_3$ and 7.9 ppm DO, respectively.

2. Exposure of juvenile coho salmon and steelhead trout for 96 hr to methylmercury chloride in fresh water resulted in lethal inhibition of their adaptation to 21 ppth S sea water (approximately 2/3 sea water). This response was elicited after exposure to mercurial concentrations of 51.8 and 82.9% of the 96 hr LC-50 for juvenile coho salmon.

3. Asphyxia was probably the cause of death during 96 hr exposures to methylmercury, as suggested by the histological damage to the gills of steelhead trout, a proportional relationship between opercular beating rate and concentration of mercurial in the water, and an inverse relationship between lethality and dissolved oxygen concentration of the water. But the observations of histological damage in the kidney and liver of steelhead trout and the neurotoxic symptoms suggest that the mercurial acted at several tissue sites which complicated the lethal mechanism.

4. Inability to maintain salt and water balance in the tissues due to mercurial effects on the kidneys and gills appeared to be the cause of death in fish transferred to saline water from methylmercury solutions. The lethal mechanism appeared to be complicated and in some cases probably involved asphyxia

resulting from the combined effects of methylmercury and ion imbalance on oxygen uptake and transport. Complications from mercurial toxicity in the nervous system also appeared to be involved.

5. An increased coughing reflex rate in fish indicated the presence of methylmercury chloride concentrations above 20 ppb Hg in the water. Although the response was proportional to methylmercury concentration in the water, usefulness as a quantitative indicator is limited since the response lag-time was several hours and the response was also proportional to exposure time. The response is not a specific indicator of methylmercury because other chemicals in the water can elicit similar responses in fish.

6. Juvenile coho salmon sampled from field locations in Oregon generally had relatively low concentrations of mercury in their tissues, though accumulations tended to increase in proportion to the age and size of the fish. It was apparent that none of the fish were in danger of losing their ability to adapt to 21 ppth S sea water as a result of mercury accumulations in their tissues.

This investigation indicates the need to consider the effects of methylmercury on sea water adaptation when setting water quality standards for this compound to ensure maximum gross production of anadromous salmonids.

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APPENDICES

APPENDIX I. DIGESTION OF TISSUE SAMPLES FOR TOTAL MERCURY ANALYSIS BY ATOMIC ABSORPTION SPECTROSCOPY

The procedure is given for tissue aliquots ranging from 0.1 to 2.0 g. The reagent volumes are stated on the basis of 1.0 g of tissue and should be adjusted according to the weight of the aliquot being assayed.

1. Place the sample in the bottom of a 50 ml centrifuge tube equipped with a ground glass stopper.
2. Set the tube in an ice bath and add 1 ml of conc. nitric acid.
3. Place a wide rubber band vertically around the tube to hold the stopper on, set in a shaking water bath at 58°C for one hr.
4. Cool on ice and add 2 ml of conc. nitric and sulfuric acids in a 1:1 ratio. Seal and return to the shaking water bath at 58°C for three hr.
5. Cool on ice and add a drop of silicone anti-foaming agent. Slowly add 5 ml of 6% $KMnO_4$ with swirling, reseal tube and allow to stand overnight at room temperature.
6. If the red color has disappeared, add 0.5 ml aliquots of 6% $KMnO_4$ until the color persists for 30 min.
7. Add 2 ml of 6% $K_2S_2O_8$ and allow it to stand 30 min.
8. Add 10% H_2O_2 dropwise with swirling until the solution is clear of color.

9. Filter the solution through glass wool (to remove fat) and rinse several times with distilled water.

10. Dilute to an even volume of 25 ml or less with distilled water, depending on the weight of tissue sample assayed.

Appendix IIa. Lengths and weights of coho salmon in 96 hr LC-50 experiments.

Initial concentration of methylmercury in water (mean ppb Hg)	Final fork length[a] (mm)	Initial wet weight[a] (g)
control	86.7 ± 2.0	6.8 ± 0.5
31.0	87.1 ± 0.8	7.1 ± 0.2
34.0	83.9 ± 2.1	6.4 ± 0.4
37.2	85.6 ± 1.0	6.4 ± 0.4
39.1	84.5 ± 2.2	6.3 ± 0.4
39.3	85.9 ± 2.5	6.8 ± 0.6
41.2	86.1 ± 1.6	6.8 ± 0.4
43.2	85.0 ± 1.8	6.5 ± 0.5
47.1	84.6 ± 2.0	6.4 ± 0.4
50.2	86.4 ± 2.2	6.9 ± 0.3
81.5	87.5 ± 2.7	7.0 ± 0.5

[a] Mean ± S.D.

Appendix IIb. Lengths and weights of steelhead trout transferred to the salinity tolerance experiments.

Initial concentration of methylmercury in water[b] (mean ppb Hg)	Replicate number	Fork length[a] (mm)		Wet weight[a] (g)	
		Fresh water	21 ppth S	Fresh water	21 ppth S
control	GS-I	88.2 ± 2.3	88.4 ± 2.7	6.6 ± 0.6	6.6 ± 0.6
control	GS-II	88.6 ± 3.3	87.9 ± 2.4	6.5 ± 0.7	6.8 ± 0.6
control	GS-III[c]	94.9 ± 1.2	----	7.4 ± 0.5	----
11.1	GS-I	87.4 ± 2.9	88.8 ± 2.9	6.9 ± 0.6	6.8 ± 0.6
10.2	GS-II	87.6 ± 3.4	88.6 ± 1.9	6.6 ± 0.6	6.8 ± 0.6
19.9	GS-I	86.2 ± 0.7	86.2 ± 2.0	6.6 ± 0.5	6.9 ± 0.7
20.1	GS-II	88.0 ± 3.8	88.2 ± 3.5	6.8 ± 0.8	6.6 ± 0.8
33.0	GS-I	86.6 ± 2.4	83.9 ± 2.9	6.7 ± 0.7	6.7 ± 0.8
31.7	GS-II	88.4 ± 2.1	86.6 ± 3.1	6.5 ± 0.6	6.7 ± 0.7
32.2	GS-III[c]	94.9 ± 1.2	----	7.6 ± 0.7	----

[a] Mean ± S.D.
[b] Previous exposure solution.
[c] Eight fish only.

Appendix IIc. Lengths and weights of coho salmon transferred to the salinity tolerance experiments.

Initial concentration of methylmercury in water[b] (mean ppb Hg)	Replicate number	Fork length[a] (mm)		Wet weight[a] (g)	
		Fresh water	21 ppth S	Fresh water	21 ppth S
control	KS-I	86.0 ± 3.6	84.2 ± 0.5	6.0 ± 0.2	6.0 ± 0.2
control	KS-II	85.4 ± 1.5	84.6 ± 1.2	6.4 ± 0.4	6.4 ± 0.3
9.9	KS-I	84.8 ± 1.0	84.5 ± 1.3	6.0 ± 0.3	6.2 ± 0.4
10.1	KS-II	84.6 ± 1.6	85.0 ± 2.6	6.4 ± 0.4	6.3 ± 0.4
19.7	KS-I	84.8 ± 1.2	84.5 ± 1.3	6.1 ± 0.2	6.1 ± 0.2
19.7	KS-II	85.4 ± 1.6	82.2 ± 1.7	6.2 ± 0.4	6.2 ± 0.4
32.5	KS-I	85.0 ± 0.8	82.2 ± 2.0	6.0 ± 0.2	6.0 ± 0.2
31.9	KS-II	84.4 ± 0.8	83.2 ± 1.9	6.3 ± 0.3	6.2 ± 0.3

[a] Mean ± S.D.

[b] Previous exposure solution.

Appendix IIIa. Water quality during 96 hr LC-50 test for coho salmon.

Initial concentration of methylmercury[a] (ppb Hg)	Radioactivity per 15 l (mean μCi)	Dissolved oxygen[a] (ppm)	EDTA hardness (mean ppm $CaCO_3$)	Mean pH	Temperature[a] (°C)
control	---	7.9 ± 0.4	22	7.4	15.1 ± 0.2
31.0 ± 0.2	4.5	7.7 ± 1.1	22	7.4	15.1 ± 0.1
34.0 ± 0.4	4.4	7.8 ± 0.4	26	7.6	15.0 ± 0.1
37.2 ± 0.3	4.4	8.0 ± 0.1	26	7.6	15.0 ± 0.1
39.1 ± 0.6	4.4	8.0 ± 0.3	26	7.6	15.0 ± 0.1
39.3 ± 0.3	4.5	7.9 ± 0.6	22	7.4	15.1 ± 0.1
41.2 ± 0.6	4.3	9.0 ± 0.9	30	6.9	15.1 ± 0.1
43.2 ± 0.5	4.4	7.8 ± 0.6	26	7.6	15.0 ± 0.1
47.1 ± 0.6	4.4	7.9 ± 0.3	26	7.6	15.0 ± 0.1
50.2 ± 0.6	4.5	8.0 ± 0.2	22	7.4	15.1 ± 0.1
81.5 ± 1.0	4.5	8.1 ± 0.2	22	7.4	15.1 ± 0.1

[a] Mean ± S.D.

Appendix IIIb. Water quality during 96 hr exposure preceding salinity tolerance experiments for steelhead trout.

Initial concentration of methylmercury in water[a] (ppb Hg)	Replicate number	Percent mercury loss from water	Dissolved oxygen[a] (ppm)	EDTA hardness (mean ppm $CaCO_3$)	Mean pH	Temperature[a] (°C)
control	GS-I	---	8.6 ± 1.8	26	7.2	14.8 ± 0.7
control	GS-II	---	8.4 ± 1.7	29	7.2	14.8 ± 0.3
control	GS-III[c]	---	9.0 ± 0.9	31	7.3	15.1 ± 0.2
11.1 ± 0.2	GS-I	21.5	8.8 ± 1.6	26	7.2	15.0 ± 0.3
10.2 ± 0.3	GS-II	26.0	8.5 ± 1.5	29	7.2	14.9 ± 0.3
19.9 ± 0.2	GS-I	26.8	8.5 ± 1.9	26	7.2	14.8 ± 0.3
20.1 ± 0.4	GS-II	37.5	8.4 ± 1.6	29	7.2	14.8 ± 0.4
33.0 ± 2.2	GS-I	48.9	8.7 ± 1.7	26	7.2	14.8 ± 0.4
31.7 ± 0.4	GS-II	44.1	8.1 ± 1.9	29	7.2	14.8 ± 0.5
32.2 ± 1.1[b]	GS-III[c]	24.9	9.0 ± 1.0	31	7.3	15.2 ± 0.3

[a] Mean ± S.D.

[b] 29.0 μCi per 15 l.

[c] Eight fish only.

Appendix IIIc. Water quality during 96 hr exposure preceding salinity tolerance experiments for coho salmon.

Initial concentration of methylmercury in water[a] (ppb Hg)	Replicate number	Radioactivity per 15 l (mean μCi) mean	Percent mercury loss from water	Dissolved oxygen[a] (ppm)	EDTA hardness (mean ppm $CaCO_3$)	Mean pH	Temperature[a] (°C)
Control	KS-I	---	---	9.3 ± 0.4	23	7.4	15.1 ± 0.1
Control	KS-II	---	---	9.1 ± 0.9	30	6.9	15.1 ± 0.1
9.9 ± 0.2	KS-I	3.9	50.8	9.1 ± 0.4	23	7.4	15.1 ± 0.1
10.1 ± 0.1	KS-II	4.3	50.8	9.0 ± 1.0	30	6.9	15.1 ± 0.1
19.7 ± 0.3	KS-I	3.9	55.9	9.0 ± 0.7	23	7.4	15.1 ± 0.1
19.7 ± 0.2	KS-II	4.3	52.5	9.0 ± 1.0	30	6.9	15.1 ± 0.1
32.5 ± 0.7	KS-I	3.9	54.4	9.2 ± 0.5	23	7.4	15.1 ± 0.1
31.9 ± 0.1	KS-II	4.3	47.3	8.9 ± 1.1	30	6.9	15.1 ± 0.1

[a] Mean ± S.D.