INTERNATIONAL PACIFIC SALMON FISHERIES COMMISSION

APPOINTED UNDER A CONVENTION
BETWEEN CANADA AND THE UNITED STATES FOR THE
PROTECTION, PRESERVATION AND EXTENSION OF
THE SOCKEYE AND PINK SALMON FISHERIES
IN THE FRASER RIVER SYSTEM

PROGRESS REPORT

No. 30

PRELIMINARY SURVEY OF TOXICITY OF CHLORINATED SEWAGE TO SOCKEYE AND PINK SALMON

BY

J. A. SERVIZI and D. W. MARTENS

NEW WESTMINSTER, B.C.
CANADA
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INTRODUCTION

Population growth and public demand for a cleaner environment have necessitated planning and construction of sewage treatment facilities at many centers. Sewage treatment processes reduce or eliminate many deleterious constituents with the degree of purification dependent upon the degree of treatment (primary, secondary or tertiary) employed. Since sewage contains pathogenic as well as many harmless organisms, disinfection to protect public health is often required before discharge. Disinfection is generally achieved by chlorination (chlorine gas or hypochlorite). Although chlorine is a very effective bactericide it is also very toxic to fish and other aquatic life.

Since chlorine may play a significant role in the toxicity of domestic sewage, the fate of chlorine added to sewage is of interest. Addition of chlorine gas or hypochlorite to sewage results in a mixture of hypochlorous acid (HOCL) and hydrochloric acid (HCL). Chlorine and hypochlorous acid further react with a variety of substances in sewage. Reactions with ammonia and subsequent formation of chloramines as indicated in the following equations are probably most important with respect to toxicity.

$$NH_3 + HOCL \longrightarrow NH_2CL + H_2O \quad \text{(monochloramine)}$$

$$NH_2Cl + HOCL \longrightarrow NHCL_2 + H_2O \quad \text{(dichloramine)}$$

$$NHCl_2 + HOCL \longrightarrow NCL_3 + H_2O \quad \text{(trichloramine or nitrogen trichloride)}$$

The relative amounts of mono-, di- and trichloramines depend upon pH, temperature and ammonia concentrations (Merkens 1958; McKee and Wolf 1963). Since trichloramines will not be formed, except at low pH values, mono- and dichloramines usually occur in chlorinated municipal sewage (McKee and Wolf 1963).

The toxicity of chlorine residuals to rainbow trout (Salmo gairdneri Richardson) and fathead minnows (Pimephales promelas) respectively, have been reported by Merkens (1958) and Zillich (1972). Merkens reported 0.08 mg/l residual chlorine (primarily as monochloramine in water) killed about half of a group of trout in seven days and suggested that a safe level of residual chlorine may be as low as 0.004 mg/l. Using chlorinated sewage from Michigan treatment plants, Zillich (1972) reported toxicity to fathead minnows at effluent concentrations of 2.0 to 4.0% v/v in river water and estimated the average threshold concentration for sublethal stress at 0.04-0.05 mg/l residual chlorine. Arthur and Eaton (1971) reported 0.108 mg/l chloramines in tap water reduced survival of fathead minnow larvae while spawnings were reduced at 0.043 mg/l.

The magnitude of potential sewage pollution problems in relation to Fraser River salmon can be appreciated in the light of fish kill data reported by the United States Environmental Protection Agency (1972). This report states that of the 21 major sources of pollution causing major fish kills (100,000 or more fish per kill), municipal sewage discharges killed about 29% of the total and were the single most serious cause of fish kills. Pearson, et al. (1970) concluded that acute toxicity was the most significant pollutant discharged to San Francisco Bay and about 56% was from municipal sewer systems.

Owing to the hazard of chlorinated sewage to fish, preliminary studies were conducted in 1972 and early 1973 to assess the toxicity of sewage from three sewage treatment plants to freshwater life stages of sockeye salmon (Oncorhynchus nerka) and pink salmon (O. gorbuscha).

DESCRIPTION OF STUDY SITES

Site I

Wastewater primarily of domestic origin from a population of approximately 2,800 persons, at a mean flow of approximately 200,000 USgpd, was

treated in a primary plant (Treatment plant staff). Following sedimentation, sewage flowed into one of two chlorine contact chambers where design retention time was 22 min. Chlorinated effluent was discharged alternately from each of the two chambers through a 600-ft long pipeline to a side channel of receiving stream I. Observations at the outfall indicated flow of sewage was intermittent with no visible discharge for 10 to 15 min followed by a surge for an approximately equal time. Thus, sewage was not uniformly diluted in the receiving stream with respect to time.

In-stream bioassays were conducted and grab samples collected at control and test station at increasing distances downstream of the outfall (FIGURE 1). Studies at this site commenced in February 1972, were halted with the onset of high river levels during the spring 1972 freshet but resumed in autumn. Stream flow was restricted from outfall to a point 400 ft downstream during autumn 1972 owing to shifting of gravel bars during the 1972 spring freshet. Thus in autumn, flow in the side channel consisted mainly of treated sewage and dilution with river water commenced at about 425 ft.

Site II

This sewage treatment facility (FIGURE 2) served about 550 households and had an average daily flow of approximately 96,000 USgpd (Treatment plant operator). The sewage, primarily of domestic origin, was treated in an activated sludge plant, chlorinated (chlorine gas) and discharged to one of two large effluent holding lagoons. During the study each lagoon had a retention time of 30 to 60 days before it was drained. Flow from the lagoons was similar to the average flow entering the activated sludge plant. Lagoons were emptied alternately, one being drained while the other was being filled. Thus the final effluent consisted of treated sewage which had been impounded for considerable time following chlorination.

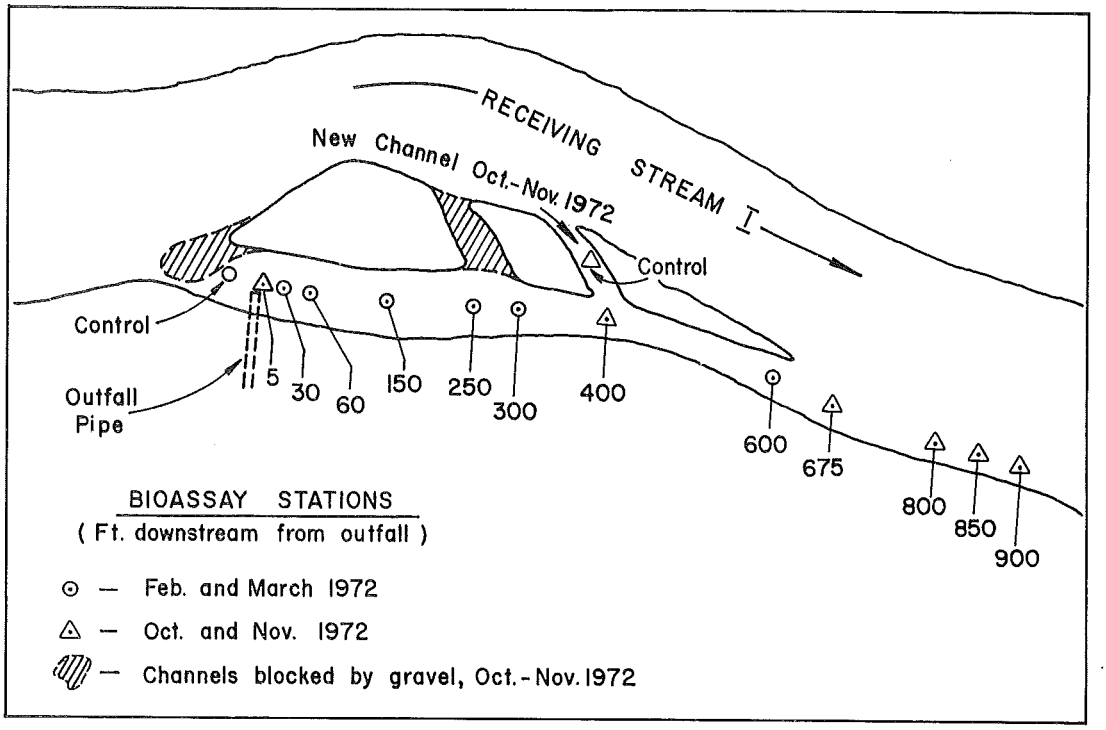

FIGURE 1 - Schematic Diagram of Site I.

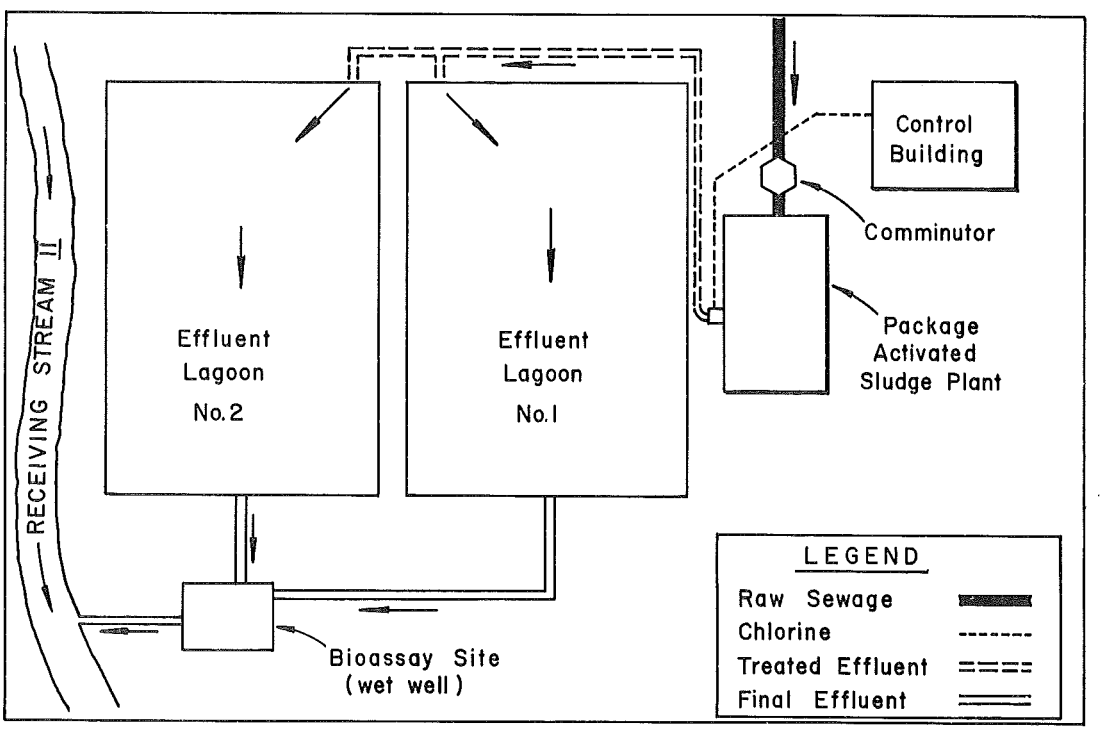

FIGURE 2 - Schematic Diagram of Site II Sewage Treatment Plant.

Effluent from the lagoons entered a wet-well before discharge to receiving stream II. The wet-well, which was free from excessive turbulence, was the bioassay and grab sample collection site. Studies at this site commenced in March 1972 and were completed in May, with the exception of 2 samples collected for analysis in December.

Site III

During the 1972 period of study, sewage primarily of domestic origin from a community of approximately 12,000 persons was entering the treatment facility at about 1 MUSGD. However, sewage flow increased in winter 1973 as a new trunk sewer began collecting sewage from additional homes. The increase in flow was not known since sewer hookups were added steadily.

Sewage was treated in an aerated lagoon followed by retention in a non-aerated lagoon. Effluent from the latter was chlorinated and passed through a contact chamber of about 23 min detention before discharge via a 10 ft long pipe and a 30 ft long outfall channel to receiving stream III (FIGURE 3). A small stream approximately equal in size to receiving stream III, joined it 700 ft downstream of the outfall, providing additional dilution.

Bioassays were conducted in the outfall channel, two locations in the receiving stream and at two control locations. Studies were conducted between March and May 1972, in November 1972 and February 1973.

METHODS

Analyses of Sewage and Receiving Water

Since the toxicity of sewage is a reflection of its physical and chemical characteristics, a number of pertinent parameters were measured in grab samples in both sewage and receiving water at bioassay stations. Temperature was measured to the nearest 0.2°C. Dissolved oxygen was measured to the nearest 0.1 mg/l using a YSI Model 54 oxygen meter.

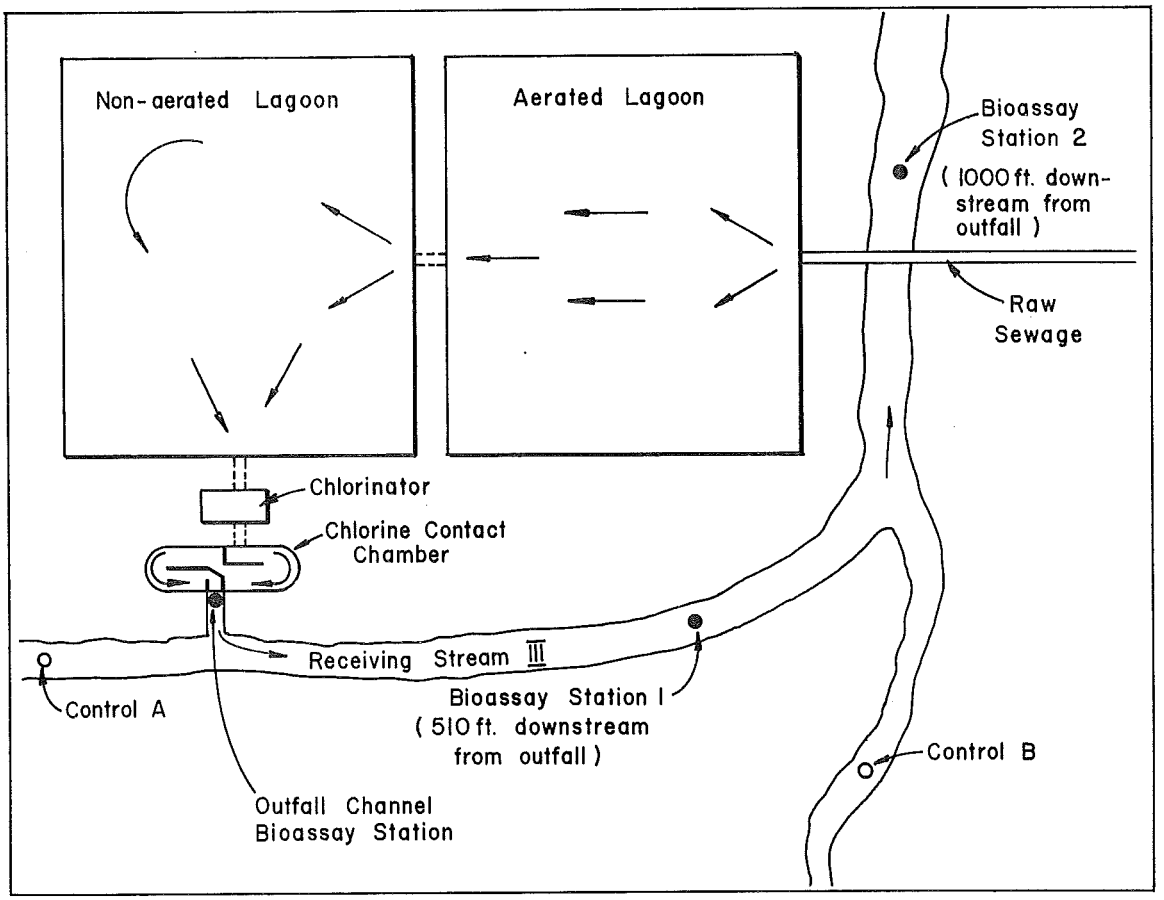

FIGURE 3 - Schematic Diagram of Site III.

Hydrogen ion content was measured with a Radiometer Model 29 pH meter. Since the effluents were primarily of domestic origin, heavy metals were not suspected as toxic constituents, but zinc and copper were measured on one occasion at Site III. Zinc was measured using the Dithizone Method (Standard Methods 1971) and copper was measured according to the method of Abbott and Harris (1962). Ammonia nitrogen was determined using the method described by Harwood and Kühn (1970). Methylene Blue Active Substance (MBAS, "detergents") was measured with a Hach Kit when it became available in the latter part of the study. Total chlorine residual was measured using both the amperometric and the orthotolidine (O.T.) methods (Standard Methods 1971). Although the O.T. method of measuring chlorine residual is relatively insensitive in sewage or sewage polluted water it was employed for comparison since it was in common use at sewage treatment plants. Tests indicated the amperometric method had accuracy and detection limits of $\pm$ 0.02 mg/l and 0.02 mg/l, respectively.

Since it is generally accepted that chlorine residuals dissipate with time and reaction with constituents in the receiving water, tests were conducted to determine the length of time chlorine residuals persisted in samples of chlorinated sewage from Sites I and III. Forty liter volumes of sewage and mixtures of sewage and lower Fraser River water from Port Mann were held in polyethylene containers. Contents were mixed well and samples withdrawn periodically for measurement of chlorine residual, dissolved oxygen, pH, ammonia nitrogen, MBAS and temperature.

Bioassays

The following fish were used in bioassays: adult Cultus Lake sockeye[a], Adams River and Cultus Lake sockeye fingerlings reared at Cultus Lake Laboratory (ave. wt. 7.2 gm and 1.5 gm, respectively), Cultus Lake

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[a] Adult sockeye were trapped at the Sweltzer Creek counting weir approximately one month prior to spawning.

wild sockeye smolts (ave. wt. 5.46 gm), and alevins of Cultus Lake sockeye and Sweltzer Creek pink salmon.

Generally five or ten fingerlings or smolts, 10 alevins, or five adults were used in each test. Numbers of fish used in specific tests appear in the RESULTS.

Fingerlings, smolts and alevins were held in floating metal screen cages (epoxy coated) measuring 22 x 6 x 4 inches. The front of each cage was covered with Formica to exclude excessive current and net floats were attached to maintain buoyancy. Fingerlings and smolts were held in the main body of the cages separated from fry and alevins which were held in small cylindrical nylon screen baskets (4 inches dia. x $2\frac{1}{2}$ inches deep) within the larger cages. Adult sockeye were held in wooden live-boxes measuring 2 ft x 2 ft x 8 ft.

The duration of bioassays was variable with some tests terminated at observation of first mortalities, while others were continued until mortality was complete or prolonged survival indicated acute toxicity was not a factor (up to 35 days). Since all bioassays could not be checked daily, times to death in some tests where fish appeared to have been dead for some time when checked are recorded as less than ($<$) the exposure time.

Some surviving test fish and control fish were placed in a histological fixative (Bouin's solution or formal saline) for later histopathological examination of gills. These included both moribund and normal appearing fish. In addition, histological samples were taken from sockeye fingerlings and adults on a predetermined sampling schedule of time exposures to chlorinated and unchlorinated sewage.

RESULTS

Site I

February - March, 1972

Receiving Water Characteristics

Measurements of dissolved oxygen and temperature in the receiving stream indicate these characteristics were favorable for fish survival (TABLE 1A). Ammonia nitrogen was present at a very low level (0.03 mg/l) in the natural stream water but was significantly higher downstream from the outfall with a maximum measured concentration of 0.20 mg/l 60 ft downstream. With the exception of four samples, the orthotolidine method of chlorine measurement indicated chlorine was not present. On the other hand, amperometric measurements showed chlorine residuals existed as far as 600 ft downstream from the outfall. Chlorine residuals ranged from barely detectable (0.02 mg/l) to 0.26 mg/l. Variability in chlorine residual on a given day was believed caused by intermittent discharge of effluent, as explained earlier. River water levels began rising late on February 27 and dilution increased in the side channel where effluent was discharged. In spite of greater dilution, chlorine residuals were in the 0.02 mg/l to 0.07 mg/l range on March 2 at 60 ft to 150 ft downstream from the outfall. Owing to the intermittent character of sewage discharge, a more extensive sampling program would be required to accurately define variation of chlorine residual with time downstream of the outfall.

Acute Toxicity

Bioassays indicated that conditions lethal to salmon existed downstream of the outfall. In two successive exposures at 30, 60 and 250 ft downstream from the outfall, 100% of the test fish died in less than one day of exposure (TABLE 1A). A further bioassay at 250, 300 and 600 ft downstream of the outfall resulted in 100% mortality at the sites nearest

TABLE 1A - Characteristics of receiving water and mortality of caged sockeye fingerlings in vicinity of Site I Sewage Treatment Plant outfall. Feb.-Mar. 1972.

Distance from Outfall feet	Sample Date	Temp. °C	D.O. mg/l	NH_3-N mg/l	Chlorine O.T.[a] mg/l	Chlorine Amp. mg/l	Bioassay Fish per Cage	Bioassay Exposure Time Hr	Bioassay Mortality %
Control	Feb. 23	5.0					10	96	0
	25	5.7	12.6		N.D.[c]	N.D.			
	26				N.D.	N.D.			
	Mar. 1			.03	N.D.	N.D.			
30	Feb. 17					0.09			
	23	5.0					10	<24	100
	24	5.1					10	<24	100
60	Feb. 23						10	<24	100
	24						10	<24	100
	Mar. 2				N.D.	0.02			
	2			.20	N.D.	0.06			
150	Mar. 2				N.D.	0.04			
	2				N.D.	0.07			
250	Feb. 23	5.7	12.3				10	<24	100
	24						10	<24	100
	25	5.7	12.3		trace	0.16	5	<24	100
	26				0.15	0.26			
	Mar. 1			.14	N.D.	0.03			
	2				N.D.	<0.02			
300	Feb. 25	5.5	12.3		trace	0.07	5	<48	100
	26			.14	trace	0.07			
600	Feb. 25	5.5	12.3		N.D.	0.02	5	48	20
	26				N.D.	0.03			
	26				N.D.	0.04			
	Mar. 1			.03					
	2				N.D.	<0.02			
	3				N.D.	0.02			

[a] O.T. - orthotolidine measurement of chlorine.
[b] Exposure commenced on water sampling date.
[c] N.D. - not detected.

the outfall and 20% mortality at the site furthest downstream. Bioassays were halted late on February 27 since it was evident that rising river levels would create excessive current in the bioassay cages. Currents were not excessive in cages at sites from the control to 300 ft when bioassays were stopped, but at the 600 ft station there may have been more current than the fish were able to withstand. Thus the roles which chlorinated sewage and current played in mortalities at the 600 ft station are unknown. However, the data suggest that mortalities can be expected at locations where chlorine residuals are 0.02 mg/l or more in grab samples.

On February 24, two dead rainbow trout (S. gairdneri), approximately 4 inches long, were found in shallow water 250 ft downstream from the outfall and it was presumed that they were killed by chlorinated sewage since test fish were also dead at that site.

Site I

October - November, 1972

Receiving Water Characteristics

Temperature of the receiving water during October - November, 1972 was favorable for fish survival (TABLE 1B). However, less dilution of sewage in a zone to a point 425 ft downstream from the outfall was reflected in less dissolved oxygen during this period compared with dissolved oxygen concentrations in the same vicinity during February - March, 1972. The oxygen concentrations measured (3.6 mg/l and 5.2 mg/l) may have imposed an added stress on test fish.

Ammonia nitrogen concentrations were greater in October - November, 1972 than in February - March, 1972.

MBAS (detergent) concentration was 4 mg/l at bioassay stations 5 ft and 400 ft downstream from the outfall.

TABLE 1B - Characteristics of receiving water and mortality of caged sockeye fingerlings and adults in vicinity of Site I Sewage Treatment Plant outfall. October - November, 1972.

Distance from Outfall ft.	Sample Date	Temp. °C	D.O. mg/l	pH	NH_3-N mg/l	MBAS mg/l	Chlorine O.T.[a] mg/l	Chlorine Amp. mg/l	Bioassay[d] Fish Per Cage	Bioassay[d] Exposure Time[b] Hr	Bioassay[d] Mortality %
Control	Oct.31	7.2	11.9	7.6			N.D.[c]	N.D.	10(5)	144(144)	0(0)
	Nov.27[e]					N.D.	N.D.	N.D.	10	4	0
5	Nov.23						0.15	1.20	10	0.2	100
	Nov.27[e]	13.0	3.6	7.6	9.0	4	N.D.	N.D.	10	3	90
400	Nov. 3	13.0	5.2		7.9		0.1	1.02	10	0.3	100
	Nov.27[e]	13.0	3.6	7.6	8.6	4	N.D.	N.D.	10	>3<4	100
675	Nov. 2	10.0	11.3				N.D.	0.04	10(5)	3 (4)	100 (60)
800	Oct.31	8.0	9.6				N.D.	0.23	10(5)	18(<18)	100(100)
	Nov. 2	10.0	11.5				N.D.	0.02	10(5)	4 (4)	20 (0)
850	Nov. 1		9.1	7.3	2.0			0.16	10(5)	1 (1)	100(100)
	1							0.09			
	1							0.18			
900	Oct.31	8.0	10.1		1.8		N.D.	0.24	10(5)	30 (30)	100 (40)

[a] O.T. - orthotolidine measurement of chlorine.

[b] Exposure commenced on water sampling date.

[c] N.D. - not detected.

[d] Condition pertaining to adult sockeye in brackets.

[e] Chlorinator off.

Chlorine was detected in only 2 of 10 determinations by the ortho-tolidine method and these 2 samples were taken 5 ft and 400 ft downstream from the outfall before sewage was appreciably diluted with receiving water. The amperometric method detected chlorine in all samples taken downstream from the outfall on days when sewage was being chlorinated.

Acute Toxicity

Sewage was lethal to sockeye at all bioassay stations downstream from the outfall (TABLE 1B). As in the previous study at this site, mortalities occurred when grab samples indicated chlorine residuals were 0.02 mg/l or greater. Survival times were more than 10 times longer at 5 ft and 400 ft when the chlorinator was off on November 27 than when it was on, indicating that chlorinated sewage was much more toxic than primary effluent without chlorine. Toxicity of the latter was probably related to MBAS and ammonia (Esvelt, Kaufman and Selleck 1973) with an added stress from low dissolved oxygen (Anon. 1962).

Histopathology

Histological examination of gills of fingerlings exposed to unchlorinated primary effluent on November 27 at the same time and place as those for acute bioassays indicated a progression in pathology. Although fingerlings were neither moribund nor lethargic after one hour exposure, comparison of gill tissue with those from control fish revealed swelling of epithelial cells (FIGURES 4 and 5). This effect was most pronounced in gills of fingerlings held 5 ft downstream from the outfall but was also apparent in fingerlings held at 400 ft. Gills of moribund fingerlings held for 3 hr at the above mentioned stations had severe epithelial swelling and widespread necrotic areas in the gill epithelium (FIGURE 6).

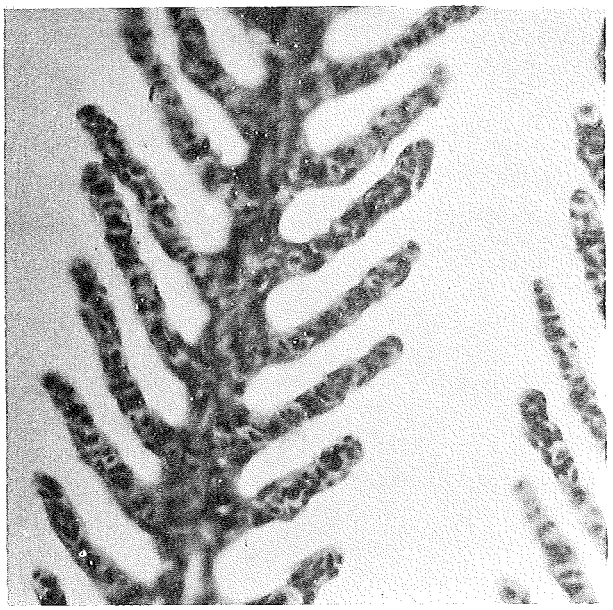

FIGURE 4 - Gill tissue from fingerling sockeye salmon control Site I. X 320.

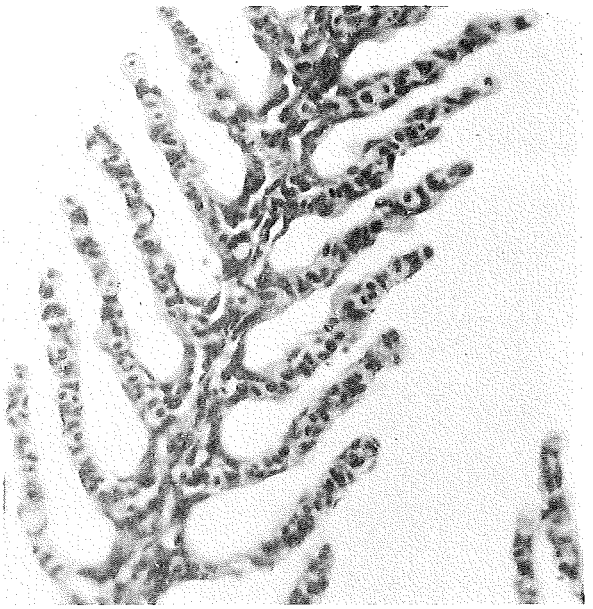

FIGURE 5 - Gill tissue from fingerling sockeye salmon exposed to unchlorinated primary treated sewage for 1 hr Site I. Epithelial cells swollen. X 320.

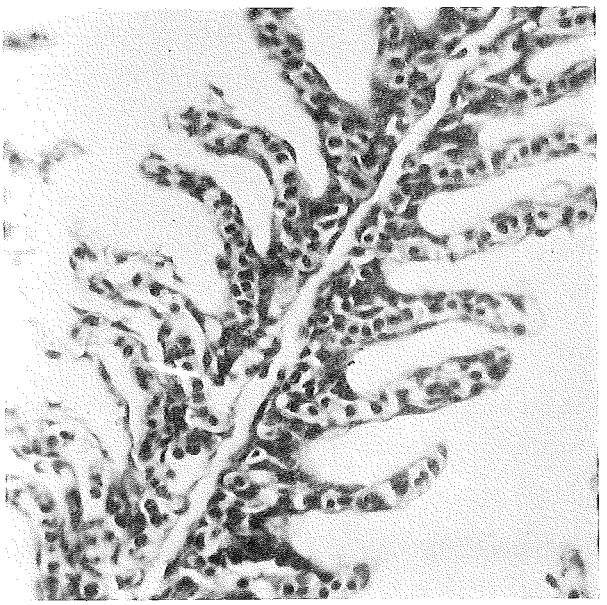

FIGURE 6 - Gill tissue from fingerling sockeye salmon exposed to unchlorinated primary treated sewage for 3 hr Site I. Epithelial swelling and necrosis. X 320.

Site II

Lagoon Effluent Characteristics and Toxicity

Temperature and dissolved oxygen in lagoon effluent were favorable for fish survival during the early period of the study (TABLE 2). Dissolved oxygen reached high levels as a result of algal photosynthesis but decreased to 1.3 mg/l in mid-May owing to algal decay. Ammonia nitrogen levels ranged from 1.35 mg/l to 6.00 mg/l, while pH ranged from 7.30 to 8.65. Chlorine was not detected in the effluent with either the amperometric or orthotolidine methods. Two measurements of MBAS subsequent to the study revealed concentrations of 0.5 mg/l and 0.8 mg/l.

TABLE 2 - Characteristics of Site II Sewage Treatment Plant lagoon effluent.

Date	Temp. °C	D.O. mg/l	pH	NH_3 - N mg/l	Chlorine O.T.[a] mg/l	Chlorine Amp. mg/l
March 15	9.0	16.2	8.65	5.10	N.D.	N.D.[b]
March 17	9.5	14.9	8.45	5.40		N.D.
March 21	8.5	13.6		4.80		N.D.
March 29	7.7	7.3	7.40	6.00	N.D.	N.D.
April 7	7.8	12.8	7.60	4.50	N.D.	N.D.
May 12	15.5	1.3	7.30	1.35	N.D.	N.D.

[a] O.T. - orthotolidine determination of chlorine.

[b] N.D. - not detected.

Sockeye fingerlings and alevins and pink salmon alevins were exposed to lagoon effluent without mortality (TABLE 3). Exposures were halted April 24 as algae growths began clogging cages. Lagoon effluent was not acutely toxic during the bioassay period but since dissolved oxygen decreased to 1.3 mg/l in May, fish would have died from lack of oxygen if bioassays had been conducted at that time.

TABLE 3 - Survival of young salmon in Site II Sewage Treatment Plant lagoon effluent.

	Sockeye			Pink
	Fingerlings		Alevins	Alevins
Date Exposure Commenced	March 15	March 29	April 17	April 6
Number Exposed	5	5	10	10
Days Exposure	14	26	7	18
Survival, %	100	100	100	100

Survival of test fish was evidence that lethal concentrations of chlorine, detergents and ammonia did not occur. Histopathological examination of gills from 2 sockeye exposed 14 days (starting March 15) to lagoon effluent indicated slight epithelial swelling, which may have been caused by irritation owing to ammonia (Burrows 1964).

Site III

Water Analyses and Bioassays at Control Stations A and B

Dissolved oxygen, temperature, pH and ammonia nitrogen at control stations A and B were within a range satisfactory for survival of salmon (TABLE 4). Chlorine was not detected at either station by the orthotolidine or amperometric methods of measurement.

TABLE 4 - Characteristics of water at Site III control stations A and B.

Date	STATION A						STATION B					
	Temp. °C	D.O. mg/l	pH	NH_3-N mg/l	Chlorine O.T.[a] mg/l	Chlorine Amp. mg/l	Temp. °C	D.O. mg/l	pH	NH_3-N mg/l	Chlorine O.T.[a] mg/l	Chlorine Amp. mg/l
Mar. 9	7.0			0.04	N.D.	N.D.	7.0			N.D.	N.D.	N.D.
10	8.7	9.5				N.D.	10.0	10.0				N.D.
15	8.5	10.4	7.00	N.D.[b]		N.D.	8.5	10.3	7.00	N.D.		N.D.
16	10.8					N.D.	10.8					N.D.
17	9.6	10.0	7.00	N.D.	N.D.	N.D.	10.5	11.1	7.10	N.D.		N.D.
21	8.0			N.D.		N.D.	8.0			N.D.		N.D.
28	8.0	10.2	7.05	N.D.	N.D.	N.D.	7.5	11.3	7.20	N.D.	N.D.	N.D.
Apr. 7	9.0	11.0		N.D.	N.D.	N.D.	9.0	13.0		N.D.	N.D.	N.D.
12	8.2				N.D.	N.D.	9.0				N.D.	N.D.
17							9.8				N.D.	N.D.
19	8.5				N.D.	N.D.	8.5				N.D.	N.D.
21	9.2				N.D.	N.D.	9.5				N.D.	N.D.
24	9.0				N.D.	N.D.	9.0				N.D.	N.D.
26	9.5				N.D.	N.D.	10.4				N.D.	N.D.
28	10.5				N.D.	N.D.	12.0				N.D.	N.D.
May 2	11.0				N.D.	N.D.	11.0				N.D.	N.D.
5	12.9				N.D.	N.D.	15.0				N.D.	N.D.
12	14.0	10.6	7.40	N.D.	N.D.	N.D.	14.5	9.2	7.40	N.D.	N.D.	N.D.
25	13.0	10.5	7.25	N.D.	N.D.	N.D.	13.0	9.7	7.25	N.D.	N.D.	N.D.
Nov. 8	7.8				N.D.	N.D.	7.5					
9	7.7	11.0			N.D.	N.D.	7.5	9.2			N.D.	N.D.
10	7.5	10.8	7.30				7.5	9.0	7.35			
14	6.3	11.0	7.25				6.3	10.0	7.30			
15	6.3	10.2	7.20	N.D.	N.D.	N.D.	6.5	6.9	7.30			
16	6.5	10.6	7.05	0.06			5.9	10.4	7.0	N.D.		
				N.D.								
16	MBAS = N.D.						MBAS = N.D.					

[a] Orthotolidine method of measuring chlorine.

[b] N.D. - not detected.

No mortalities occurred among fish maintained at control stations A and B (TABLE 5). There was no histological evidence of pathological change to gills of fingerlings or to gills and olfactory rosettes of adult sockeye.

Analyses of Sewage and Bioassays in the Outfall Channel.

Samples of sewage were collected at the outlet weir of the chlorine contact chamber before it entered a 10 ft long discharge pipe. Temperature of the sewage varied widely during the study (2.0 to 20.4°C) as a result of changes in ambient temperature (TABLE 6). Dissolved oxygen also varied widely during the study (8.9 to 19.3 mg/l) with the high dissolved oxygen concentration probably a result of oxygen production associated with algal photosynthetic activity. Algae content of the final effluent was high at this time, coloring it green. The pH of the sewage ranged from 5.0 to 9.1. Zinc and copper concentrations were less than 0.1 mg/l and 0.02 mg/l, respectively, and were less than levels found acutely toxic to sockeye or pink salmon (Servizi and Martens M.S.). Ammonia nitrogen concentrations averaged 14.0 mg/l and ranged from 8.2 to 30.25 mg/l.

Chlorine was measurable by the orthotolidine method (av. 0.09 mg/l) at the outlet of the chlorine contact chamber. Amperometric measurement of residual chlorine indicated an average of 0.74 mg/l or more than eight times the average residual chlorine measured using orthotolidine.

Fifty percent mortality of sockeye fingerlings occurred in 0.8 hr when chlorine residual was 0.85 mg/l but was 13 hr when the chlorinator was not operating (TABLE 7), demonstrating again the high toxicity of chlorine residuals.

Treated sewage was more toxic in February 1973 than in November 1972 based upon survival time when the chlorinator was not operating (TABLE 7). Greater toxicity in February coincided with highest concentrations of MBAS and ammonia which may have been caused by poor treatment performance owing to low temperature and greater load on the treatment lagoon associated with addition of a trunk sewer to the collection system.

TABLE 5 - Survival of salmon at Site III, control stations A and B.

Test Specimens	Date Exposure Commenced	Exposure Hour	Exposure (Days)	No. Exposed	Mortality %
	CONTROL A				
Sockeye Fingerlings	March 8	168	(7)	5	0
Sockeye Fingerlings	15	552	(23)	5	0
Sockeye Fingerlings	April 7	456	(19)	5	0
Sockeye Smolts	26	528	(22)	5	0
Sockeye Fingerlings	May 18	168	(7)	5	0
Sockeye Alevins	April 17	600	(25)	10	0
Pink Alevins	6	672	(28)	10	0
Sockeye Adults	Nov. 8	48	(2)	5	0
Sockeye Fingerlings	Nov. 8	48	(2)	10	0
Sockeye Adults	Nov. 10	48	(2)	5	0
Sockeye Fingerlings	Nov. 10	48	(2)	10	0
Sockeye Adults	Nov. 15	9	(37)	5	0
Sockeye Fingerlings	Nov. 15	9	(37)	10	0
Sockeye Adults	Nov. 16	9	(37)	5	0
Sockeye Fingerlings	Nov. 16	9	(37)	10	0
	CONTROL B				
Sockeye Fingerlings	March 8	168	(7)	5	0
Sockeye Fingerlings	15	840	(35)	5	0
Sockeye Fingerlings	April 19	168	(7)	5	0
Sockeye Smolts	26	384	(16)	5	0
Sockeye Fingerlings	May 12	144	(6)	5	0
Sockeye Fingerlings	18	168	(7)	5	0
Sockeye Alevins	April 17	600	(25)	10	0
Sockeye Alevins	May 12	144	(6)	10	0
Sockeye Alevins	18	168	(7)	10	0
Pink Alevins	April 6	864	(36)	10	0
Chum Fry	19	360	(15)	10	0
Sockeye Fingerlings	Nov. 8	48	(2)	10	0
Sockeye Fingerlings	Nov. 10	96	(4)	10	0
Sockeye Fingerlings	Nov. 15	9	(37)	10	0
Sockeye Fingerlings	Nov. 16	9	(37)	10	0

TABLE 6 - Characteristics of Site III effluent at outlet of chlorine contact chamber.

Date	Temp °C	D.O. mg/l	pH	NH_3-N mg/l	MBAS mg/l	Chlorine O.T.[a] mg/l	Chlorine Amp. mg/l
1972							
March 9	7.0		7.5	11.3		0.1	0.53
10	8.0	8.9	7.5				0.34
15	9.5	10.4	7.55	11.3			0.68
16	10.5						0.50
17	11.5	10.0	7.55	12.4			0.72
21	8.5		7.50	11.9			0.72
28	8.5	13.0	7.50	11.3		0.15	0.90
April 7	10.5	10.2	7.50	10.7		0.1	0.78
17	10.5					0.1	0.80
21	10.5					0.15	0.70
24	10.0					0.15	0.70
26	13.0					0.15	0.79
28	13.9					0.15	0.70
May 2	14.3					0.15	0.83
5	18.6					0.15	0.97
12	20.4	19.3	9.1	8.2		0.0	< 0.02
30	19.0	9.0	8.1	9.9		0.1	0.68
Nov. 8	7.5		7.6	15.1		<0.1	0.75
							0.75
							0.67
						0.1	0.80
10[b]	7.5	8.4	7.6			0.0	N.D.
12				15.5			
				15.9			
14	5.0	9.4	7.5	14.5			
15	6.0	6.9	7.4	14.1		0.10	0.86
				19.5		0.10	0.86
							0.83
							0.96
16	7.5	8.1		17.5	N.D.	0.0	N.D.
				16.4	N.D.		
				17.5			
				18.7			
1973							
Feb. 8[b]	3.0	7.0	7.2	25.0	3	trace	0.86
12[b]	2.0	6.3	7.2	25.0	3	0.0	N.D.
15[b]	3.0	5.5	7.3	30.3	4	0.0	N.D.

[a] Orthotolidine determination of chlorine.

[b] Chlorinator not operating.

TABLE 7 - Mortality of sockeye salmon in chlorine contact chamber outlet, Site III.

Date Exposure Started	Test Fish and No. ( )	Exposure Hr	Mortality %
1972			
Nov. 10[a]	Adults (5)	48	80
10[a]	Fingerlings (10)	48	20
1973			
Feb. 8	Fingerlings (10)	0.8	50
12[a]	Fingerlings (10)	<16	100
15[a]	Fingerlings (10)	13	50

[a] Chlorinator off.

Water Analyses, Bioassays and Histopathology at Station 1

A float study on April 17 indicated the flow time from the sewage discharge point to Station 1 was about 9 min. Water temperatures, dissolved oxygen and pH were within a range suitable for survival of salmon (TABLE 8). Ammonia nitrogen concentrations of the receiving stream were significantly increased by sewage discharge; with measured values between 1.05 and 7.60 mg/l. Chlorine residual was detected on only one occasion by the orthotolidine method but analyses using the amperometric method indicated chlorine was usually present, with detectable concentrations ranging from 0.02 to 0.79 mg/l (TABLE 8).

The chlorinator was not operating during the periods May 20 to 26, November 10 to 14, and November 16 and as expected, chlorine was not detected during these periods. On four occasions chlorine was not detected (less than 0.02 mg/l) by amperometric measurement although the chlorinator was operating. Measurements of chlorine residual were made at the contact chamber outlet (TABLE 6) on three (March 16, April 24 and April 28) of the four aforementioned days and there was a significant residual at that point. Thus, in some instances chlorine may have been dissipated by reaction with dissolved, suspended or benthic material in the receiving stream, but the typical situation was one in which chlorine residuals were significant.

Bioassays using caged fingerling and adult salmon indicated that diluted, chlorinated sewage was acutely toxic (TABLE 9). Although time was not available to examine test fish every day, it was often evident from their decayed state upon inspection that some fish had died much before the stated exposure. However, correlation between mortality and chlorine concentration was evident on November 15 when successive measurements indicated chlorine was between 0.20 and 0.22 mg/l and adult and juvenile sockeye died within 3.8 and 6 hr, respectively. It is noteworthy that although toxic conditions existed at Station 1, control fish located upstream of the sewage discharge survived without mortality for periods of one to four weeks (TABLE 5).

TABLE 8 - Analytical results at Site III, Station 1.

Date	Temp °C	D.O. mg/l	pH	NH_3-N mg/l	MBAS mg/l	Chlorine O.T.[a] mg/l	Chlorine Amp. mg/l
March 9	7.0		7.30	3.30		N.D.	0.02
10	8.7	9.0					0.21
15	8.5	9.8	7.35	1.56		N.D.	0.05
16	10.8						< 0.02
17	10.0	9.6	7.35	1.70			0.07
21	8.5			1.92			0.07
27	8.0	10.4	7.30	1.78		N.D.	0.08
April 7	9.0	11.0		1.42		N.D.	0.16
12	9.0					N.D.	0.12
17	9.0					N.D.	0.10
19	8.5					N.D.	< 0.02
21	9.5					N.D.	0.03
24	9.0					N.D.	< 0.02
26	10.5					0.15	0.79
28[b]	10.8					N.D.	< 0.02
May 26[b]	11.0					N.D.	N.D.
30	13.0	10.6	7.60	1.05		N.D.	0.02
Nov. 8				5.10		< 0.10	0.10
							0.08
							0.06
9		9.8					0.05
	8.5	9.3				< 0.10	0.11
							0.05
10[b]	7.5	9.4	7.30			N.D.	N.D.
14	6.2	10.2	7.40	6.10		N.D.	N.D.
15	6.5	9.1	7.30	7.60	N.D.	< 0.1	0.22
							0.21
							0.20
							0.21
16[b]	7.2	9.9	7.10	5.20	N.D.	N.D.	N.D.
				7.00	N.D.		
				6.90			
				3.40			

[a] Orthotolidine determination of chlorine.

[b] Chlorinator not operating May 20 to 26, November 10-14 and November 16.

[c] N.D. - not detected.

TABLE 9 - Mortality of salmon at Site III, Station 1.

Date Exposure Started	SOCKEYE								PINK	
	Adults (5)[a]		Fingerlings (5)[a]		Smolts (5)[a]		Alevins (10)[a]		Alevins (10)[a]	
	Exposure Hr	Mort. %	Exposure Hr	Mort. %	Exposure Hr	Mort. %	Exposure Hr	Mort. %	Exposure Hr	Mort. %
March 8			24	80						
9			24	40						
10			120	100						
15			144	100						
21			144	100						
28			216	100						
April 6			72	100					72	100
10			48	60					48	0
12			120	100					120	20
17			48	100			96	40	48	40
19			48	100					48	30
21			72	100			168	100	72	40
24			48	80					96	20
26					<48	100				
May 20[b]					144	0	144	0	144	0
Nov. 8	<24	100	<24	100[c]						
9	16	100	15	100						
10[b]	48	0	48	0						
15	3.8	100	6	100						
16[b]	9	0	9	0						

[a] Numbers in brackets indicate the number of fish exposed at a time.

[b] Chlorinator not operating May 20-26, November 10-14 and November 16.

[c] November 8-16, 10 fingerlings exposed at a time.

The chlorinator was not operating during the periods May 20 - 26, November 10 - 14 and November 16 which afforded an opportunity to conduct bioassays at Station 1 in the absence of chlorine. During these periods adult and fingerling salmon survived without mortality thereby implicating chlorine as the major cause of acute toxicity at Station 1.

Histopathological examination of two surviving sockeye fingerlings from the April 10 to 12 exposure period at Station 1 indicated obvious changes in gill epithelia, including hyperplasia and some necrosis of epithelial cells (FIGURE 7). Pathological changes similar to, but less severe than those noted above for sockeye fingerlings, were seen in gills of each of two surviving sockeye and pink alevins with exposures starting April 17 and 19, respectively.

In order to assess some sublethal toxic effects, adult and fingerling sockeye were exposed for predetermined intervals at Station 1, parallel to bioassays on November 15 and 16. Fish were sacrificed, tissues preserved and examined histologically. Gill epithelia of adult and fingerling sockeye were swollen and separation of the epithelium from underlying pillar cells was evident among fish sacrificed after 1 hr exposure on November 15 (FIGURES 8 and 9). The pathology mentioned above was more severe among fish exposed for 3 hr (FIGURE 10) although olfactory rosettes appeared unaffected according to histological examination. The pathology observed coincided with 100% mortality in bioassay groups in 3.8 and 6 hr for adults and fingerlings, respectively (TABLE 9).

In a similar experiment of 9 hr duration on November 16, conducted when the chlorinator was not operating, gills of juveniles and adults and olfactory rosettes of the latter were unaffected. Fish in bioassay groups survived without mortality (TABLE 9).

Minimum concentrations of chlorine causing mortality or gill pathology could not be accurately estimated from the data because chlorine residuals varied widely during the study. However, mortalities were common when chlorine residuals exceeded 0.02 mg/l.

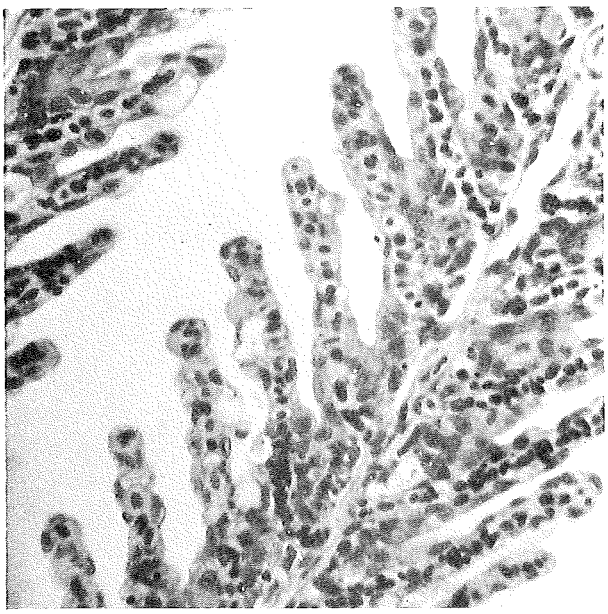

FIGURE 7 - Gill tissue from fingerling sockeye salmon exposed 48 hr at Site III Station 1. Hyperplasia and some necrosis of epithelial cells. X 320.

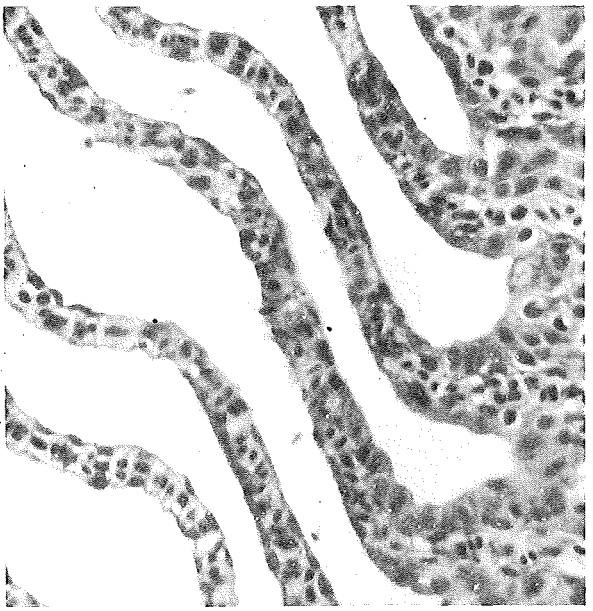

FIGURE 8 - Gill tissue from adult sockeye salmon control Site III. X 320.

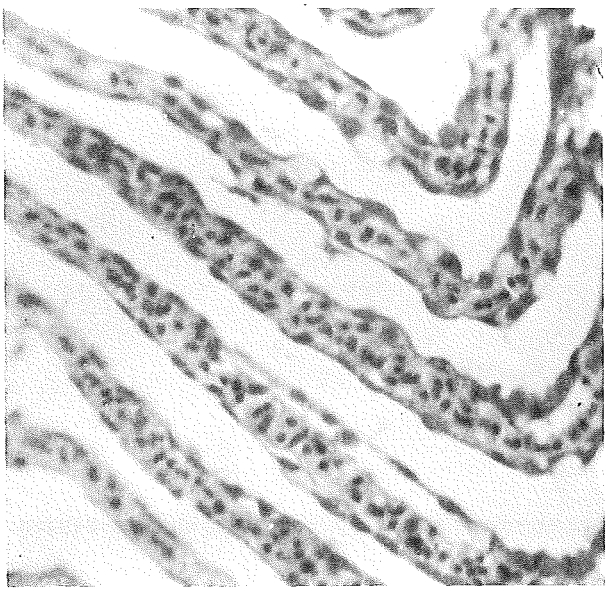

FIGURE 9 - Gill tissue from adult sockeye salmon exposed 1 hr at Site III Station 1. Epithelial cells swollen and separated from pillar cells. X 320.

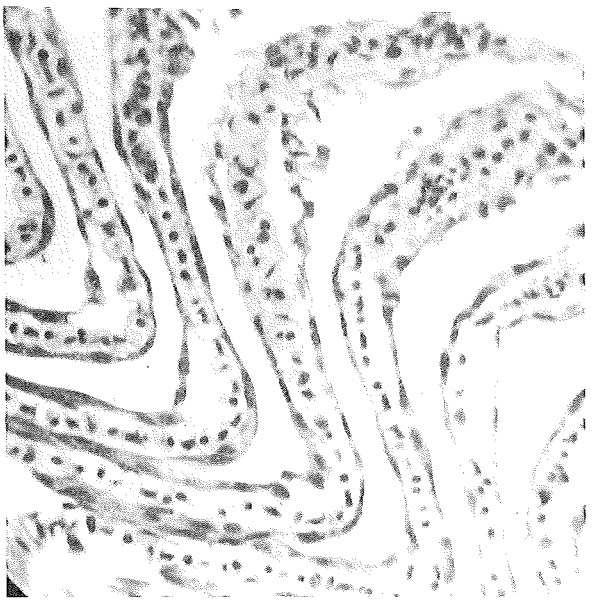

FIGURE 10 - Gill tissue from adult sockeye salmon exposed 3 hr at Site III Station 1. Severe separation of epithelium from pillar cells. X 320.

Water Analyses, Bioassays and Histopathology at Station 2

A float study on April 17 indicated flow time from the sewage discharge point to Station 2 was about 19 min. Temperature, dissolved oxygen and pH were satisfactory for survival of test fish (TABLE 10). The combined effect of time, distance and additional dilution by the tributary stream was evident on ammonia nitrogen and chlorine concentrations. A trace of chlorine was detected by the orthotolidine method on April 7. Chlorine was detected in 11 of 31 samples using the amperometric method.

Survival of salmon at Station 2 was variable, with mortality ranging from 0 to 60% (TABLE 11). No mortalities occurred among test fish when the chlorinator was not operating in the periods May 20 - 26, November 10 - 14 and November 16. Sublethal effects were observed when gills of two sockeye fingerlings from the group exposed 23 days starting March 15 were examined histologically. There was evidence of hyperplasia and some necrosis of epithelial cells but effects were both less severe and extensive than those noted among surviving fish at Station 1. As at Station 1, adult and fingerling sockeye were exposed up to 9 hr at Station 2 parallel to bioassays on November 15 and 16. Gills and olfactory rosettes of adult sockeye and gills of fingerling sockeye appeared free of histopathological effects on both November 15 and 16. Thus it appears from comparison with results obtained during 23 days starting March 15 that histopathological effect developed at Station 2 during prolonged but not brief exposure.

Comparison of mortalities with amperometric measurement of chlorine at Station 2 (TABLE 10) shows that chlorine was not always present in grab samples collected during intervals when mortalities occurred. When considering such results, account must be taken of the fact that chlorine residuals may vary considerably as seen in measurements made at Station 1 (TABLE 8) and at the chlorine contact chamber (TABLE 6). Thus grab samples probably failed to document chlorine residuals high enough to be

TABLE 10 - Analytical results at Site III, Station 2.

Date	Temp. °C	D.O. mg/l	pH	NH_3-N mg/l	MBAS mg/l	Chlorine O.T.[a] mg/l	Chlorine Amp. mg/l
March 9	7.0		7.15	0.52		N.D.[c]	<0.02
10	9.5	9.4				N.D.	<0.02
15	8.5	9.6	7.15	0.33			<0.02
16	10.8						<0.02
17	10.5	10.0	7.20	0.30			<0.02
21	8.5			0.29			<0.02
28	7.5	10.3	7.30	0.32		N.D.	<0.02
April 7	9.0	11.0		0.50		<0.1	0.16
12							0.03
17	9.5					N.D.	0.04
19	8.5					N.D.	<0.02
21	9.5					N.D.	0.03
24	9.0					N.D.	<0.02
26	10.5					N.D.	0.03
28	11.0					N.D.	<0.02
May 2	11.0					N.D.	<0.02
5	14.6					N.D.	<0.02
12	14.1	10.1	7.50	0.20		N.D.	<0.02
30	13.0	9.6	7.30	0.23		N.D.	<0.02
Nov. 8				0.92		<0.1	<0.02
							0.02
							<0.02
9	8.2	9.3				<0.1	0.02
		9.2					<0.02
							<0.02
10[b]	6.9	9.0	7.2			N.D.	N.D.
	7.5	9.1	7.3				
14	6.0	10.1	7.3				
15	9.0	9.3	7.2	0.95	N.D.	<0.1	0.02
					N.D.	<0.1	0.02
							0.03
							0.55
16[b]	6.8	10.4	7.1	1.05	N.D.	N.D.	N.D.
				0.95	N.D.		
				1.05			

[a] Orthotolidine measurement of chlorine.

[b] Chlorinator not operating Nov. 10 through 14 and Nov. 16.

[c] N.D. - not detected.

TABLE 11 - Mortality of salmon at Site III, Station 2.

Date Exposure Started	SOCKEYE								PINK	
	Adults (5)[a]		Fingerlings (5)[a]		Smolts (5)[a]		Alevins (10)[a]		Alevins (10)[a]	
	Exposure Hr	Mort. %	Exposure Hr	Mort. %	Exposure Hr	Mort. %	Exposure Hr	Mort. %	Exposure Hr	Mort. %
March 8			168	40						
15			552	0						
April 6									840	0
7			120	20						
12			120	20						
17			168	60			600	0		
19										
24			48	0						
26					384	20				
May 20[b]					144	0	144	0	144	0
Nov. 8	40	0	40	10						
10[b]	4	0	96	0						
15	9	0	9	0						
16[b]	9	0	9	0						

[a] Numbers in brackets indicate the number of fish exposed at a time.

[b] Chlorinator not operating May 20-26, November 10-14 and November 16.

lethal and it is evident that occasional grab samples may not be sufficient to monitor chlorine residuals in receiving streams. However, based upon results at Station 2, lethal conditions could be expected from time to time if some chlorine residuals in a series are 0.02 mg/l or more.

Effect of Time on Chlorine Residuals

Residual chlorine is known to decrease with time owing to reaction with substances in sewage and the receiving stream. In order to determine the relationship between chlorine residual and time, samples of chlorinated sewage from Sites I and III were held under aerobic conditions with and without dilution and chlorine residuals were measured.

Temperatures of the sewage and sewage-water mixtures were near ambient (40-50°F) and pH ranged from 7.35 to 7.90. Dissolved oxygen was about 7.4 mg/l in sewage and increased to 9.8 near end of the test. Ammonia nitrogen concentrations were a function of dilution and remained relatively constant in sewage and sewage-water mixtures.

Residual chlorine in primary sewage (Site I) decreased significantly during the first 10 hr and assumed a virtually constant value of 0.2 mg/l which persisted beyond 50 hr (FIGURE 11). Residual chlorine was measurable at 28 hr in a mixture of 10 parts sewage and 90 parts Fraser River water.

The chlorine residual in lagoon effluent (Site III) declined to about 0.12 mg/l in 50 hr (FIGURE 12). Chlorine residual decreased to 0.02 mg/l between 5 and 10 hr in 5% v/v and to nil in 22 hr. The decrease in chlorine residual was slower in lagoon effluent than in primary effluent probably because the latter had undergone less treatment and contained more reactive matter. These results are not unlike those reported by Monroe and Phillips (1972) who found chlorine residuals ranged between 0.5 mg/l and 4 mg/l in trickling filter effluent after 3 hr depending upon chlorine dosage.

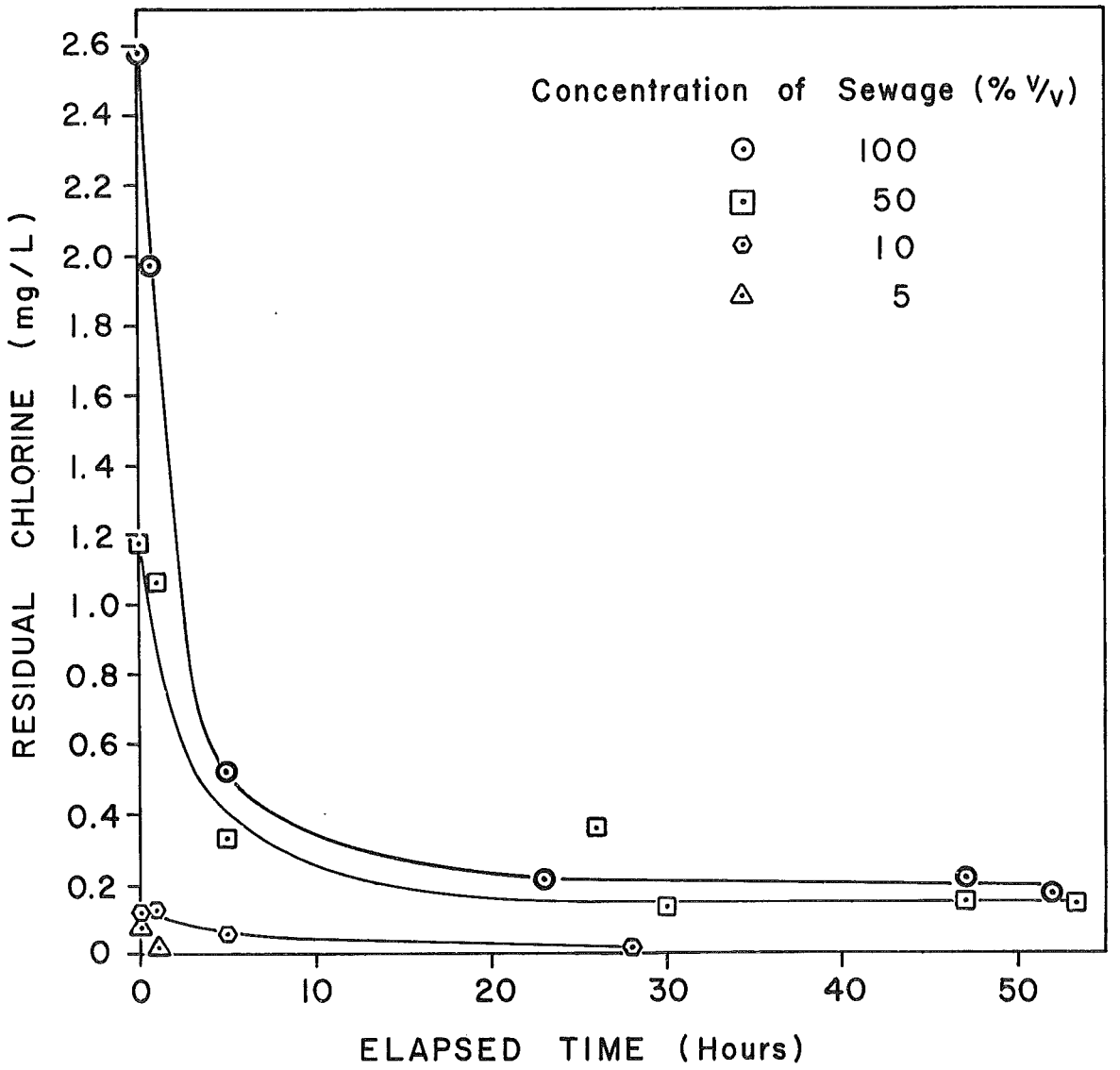

FIGURE 11 - Chlorine residuals in sewage and mixtures of sewage and Lower Fraser River water Site I.

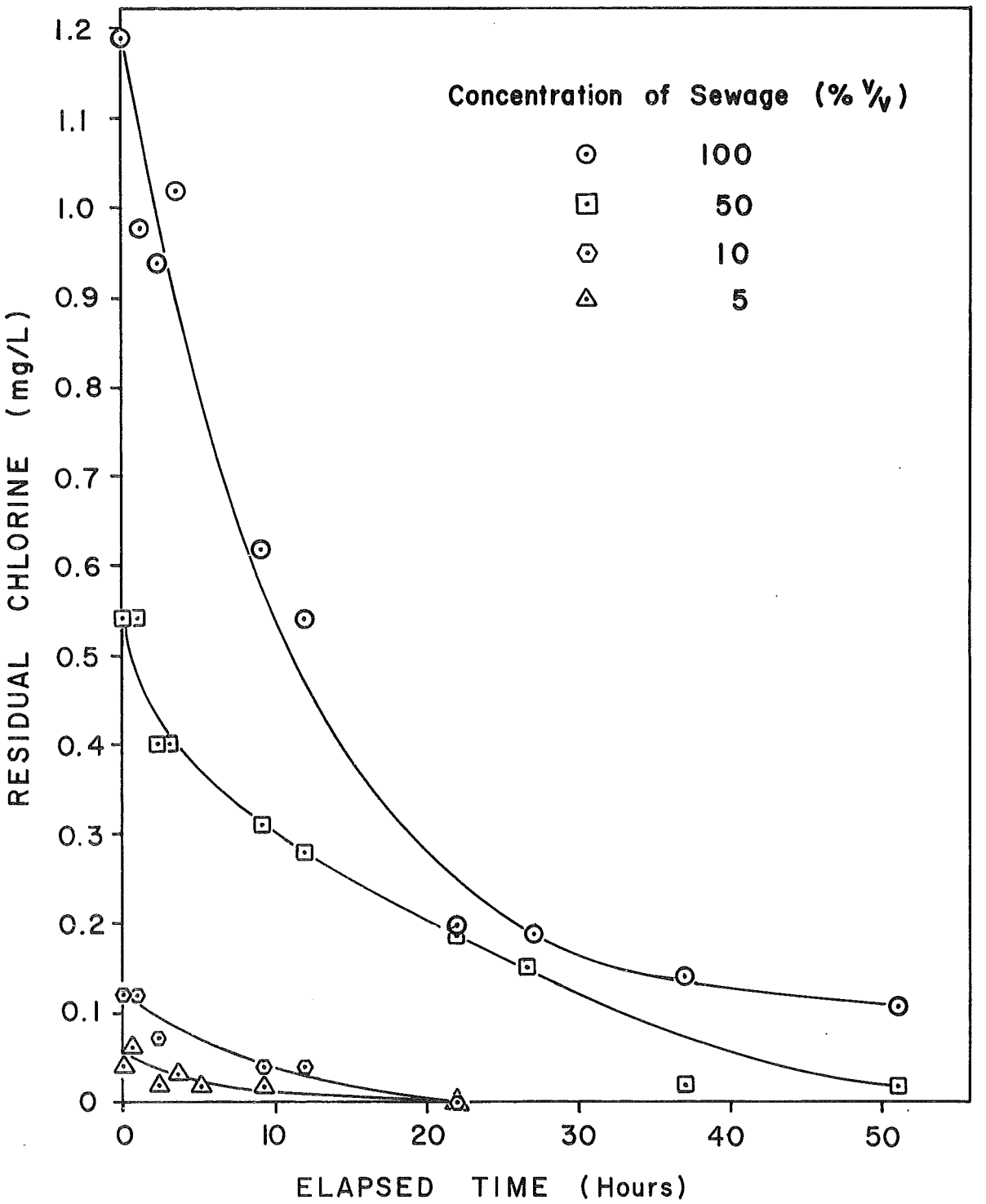

FIGURE 12 - Chlorine residuals in sewage and mixtures of sewage and Lower Fraser River water Site III.

These results demonstrate that chlorine residuals remain in the toxic range for a considerable time. Thus significant aging is required to dissipate chlorine induced toxicity. Similar results were noted in studies by Esvelt, Kaufman and Selleck (1973).

DISCUSSION

Primary sewage (Site I) and aerated lagoon effluent (Site III) were lethal to salmon when bioassayed without chlorination. In one study acute toxicity of primary sewage was correlated with MBAS and ammonia (Esvelt, Kaufman and Selleck 1973) and the values of MBAS reported herein for sewage at Sites I and III were within the range cited as lethal (2 to 6 mg/l), except on November 16 at Site III. In the latter case, ammonia nitrogen concentrations were near the mean of 18.5 mg/l reported by Esvelt, Kaufman and Selleck (1973) and thus may have contributed to toxicity. At Site I, dissolved oxygen was 3.6 mg/l during the aforementioned bioassays and may have increased toxic effects of ammonia (Anon. 1962).

Although MBAS and ammonia have been cited as contributing to acute toxicity of pre-chlorinated sewage, these constituents may not be the only ones responsible for acute toxicity. Esvelt, Kaufman and Selleck (1973) suggested that heavy metals and perhaps reduced substances contributed to toxicity. Furthermore, Martens and Servizi (1974) noted that calculated values of un-ionized ammonia in municipal sewage were less than those believed lethal by Esvelt, Kaufman and Selleck (1971) and suggested that additional factors may influence toxicity of ammonia.

At Sites I and III, treated sewage contained residual chlorine when discharged but measurements using the orthotolidine method for chlorine residual gave consistently low values in effluent and in the receiving water when compared with results obtained using the amperometric method. For example, at Site III Station 1, the orthotolidine method was virtually

unable to detect chlorine residuals in the receiving water whereas residuals measured using the amperometric method were significant and important in view of the fact that mortalities occurred there consistently. The lack of sensitivity of the orthotolidine method for measuring residual chlorine in sewage or water containing sewage is well known. Sawyer (1957) measured chlorine concentrations in sewage using amperometric and orthotolidine methods and reported the former measured up to 9.5 times as much as the latter. Monroe and Phillips (1972) concluded that the orthotolidine method was an unreliable indicator of chlorine residual in trickling filter effluent when chlorine concentration was 5 mg/l or less. It is evident from results reported herein and the work of others that the orthotolidine method of chlorine measurement is not suitable for monitoring effluents or receiving waters for the purpose of protecting aquatic life.

Treated sewage was chlorinated at all three study sites, but at Site II effluent was dechlorinated in lagoons prior to discharge while at Sites I and III, effluent was discharged following retention in a chlorine contact tank. The benefits of dechlorination were demonstrated by the fact that no mortalities occurred during prolonged exposure of juvenile salmon to undiluted lagoon effluent at Site II. On the other hand, chlorinated sewage caused mortalities of juvenile salmon following dilution at points downstream of Site I and Site III sewer outfalls. Evidence that toxicity of sewage was much less without chlorine was obtained at Sites I and III. In the latter case no mortalities occurred at Station 1 when the chlorinator was not operating whereas mortalities were commonplace when chlorine was applied.

In addition to the two wild juvenile rainbow trout reported dead at Site I on February 24, 1972, 15 wild juvenile coho (O. kisutch) were found dead in the side channel on December 14, 1965 and one dead coho was noted on January 10, 1973. Chlorine concentration was 1.26 mg/l in the side channel when the 15 dead coho were found. These observations suggest

that fish will not necessarily avoid lethal concentrations of chlorinated municipal sewage when free to do so.

Results reported herein are in agreement with other field studies. Comparison of water quality and fish populations in the vicinity of secondary sewage treatment plants showed that fish populations were adversely affected by chlorine residuals downstream of plants where chlorination was the final process (Tsai 1971). On the other hand, dechlorination of effluent in lagoons before discharge safeguarded the general fish population.

In-stream bioassays using rainbow trout in the vicinity of 4 municipal sewage treatment plants where effluents were normally chlorinated showed that lethal conditions prevailed downstream of 3 plants (Basch 1972). No mortalities were noted in 4 days downstream of the fourth plant, apparently owing to considerable dilution. Bioassays were repeated without chlorination of effluent and fish survived in each case.

Laboratory studies demonstrated that chlorine induced toxicity was removed by chemical dechlorination using sodium thiosulfate (Zillich 1972) or sodium bisulfite (Esvelt, Kaufman and Selleck 1973). Full-scale dechlorination is practiced at Sacramento and Burlingame, California, using SO_2, while sewage treatment plants at Palo Alto, San Jose and Hayward were awaiting installation of dechlorination equipment (H. F. Collins pers. com.).

A threshold concentration of chlorine for acute toxicity or sub-lethal effect could not be accurately established from this study since chlorine residuals varied widely with time at bioassay stations. In general, acute toxicity was common at stations where chlorine residuals were 0.02 mg/l or greater. This result could be expected since Merkens (1958) demonstrated that 0.08 mg/l chlorine was acutely toxic to rainbow trout in seven days. Coventry (1935) reported chlorine was toxic to trout fry in 48 hr at 0.06 mg/l and Westfall reported chloramines lethal to

trout fry at 0.06 mg/l chlorine. Sprague and Drury (1969) reported 0.01 mg/l chlorine lethal to rainbow trout in 12 days. Tsai (1971) reported brook trout (Salvelinus fontinalis) and brown trout (Salmo trutta) were absent where mean chlorine exceeded 0.02 mg/l in the receiving stream. Chlorine concentrations averaging 0.014 mg/l were lethal to rainbow trout downstream of a primary treatment plant and mean chlorine concentrations of about 0.02 to 0.05 mg/l were lethal downstream of 2 secondary treatment plants (Basch 1972).

It was apparent in this study that absence of chlorine in grab samples analyzed by the amperometric method was not assurance that lethal concentrations were not occurring at times when samples were not being collected since mortalities occurred under such circumstances during continuous bioassays (Site III, Station 2). Consequently, receiving waters where chlorine residuals are detected even occasionally by amperometric measurement (0.02 mg/l) may prove hazardous to salmon part of the time.

Although a limited number of fish were examined histologically, evidence of damage to gill tissues by unchlorinated, chlorinated and dechlorinated sewage effluents was detected in this study. Ammonia and surfactants probably caused damage to gills of fish exposed to unchlorinated effluent (Burrows 1964, Schmidt and Mann 1961). Gill damage was most severe and rapid among fish exposed to chlorinated effluents. Irritation to gills was least among fish exposed to effluent dechlorinated in a lagoon.

It is evident that further study would be needed to establish the amounts of chlorine and chlorinated sewage which could be tolerated by salmon in various stages of freshwater residence without harm during short or long-term exposure. The study reported herein concerned effluents composed primarily of domestic sewage but sewage from a district with diverse manufacturing and commerical establishments may contain materials such as heavy metals and waste chemicals which are toxic to fish. Therefore these additional factors cannot be excluded from the general problem of toxicity of sewage to fish.

Chlorination Requirements and Practices

Sewage is chlorinated to kill pathogenic bacteria and viruses as a public health protection practice. Unfortunately, reliable, routine methods are not available for measuring the presence of pathogens following chlorination and thus chlorination practice is based upon empirical data.

Regulatory agencies develop standards associated with disinfection by chlorination. These standards commonly specify that MPN's (Most Probable Number of coliform bacteria per 100 mls of sewage) shall not exceed certain levels and where applicable, sewage treatment plant operators add sufficient chlorine to meet standards. A chlorine residual of 0.1 to 1.0 mg/l (measured by O.T.) has been found satisfactory in many cases. In the interest of protecting fish it has been common practice not to exceed 0.2 mg/l chlorine (measured by O.T.) in the vicinity of the discharge. However the results of this study have shown such a chlorine concentration is rapidly lethal to salmon.

The method of measuring chlorine residual has been left to choice of those concerned but data presented herein indicate that the orthotolidine method would not detect concentrations which would otherwise prove rapidly lethal. It is apparent that if the orthotolidine method is used to measure chlorine residual, as is typically the case, the amount of chlorine present in the effluent and receiving water may be considerably in excess of that measured. Therefore, in the interest of fish protection and accuracy the amperometric method of measuring chlorine residual is recommended.

CONCLUSIONS

1. Effluents from a primary sewage treatment plant and an aerated lagoon were lethal to salmon.

2. Chlorination caused a substantial increase in toxicity of effluents and created conditions acutely toxic to captive salmon in two receiving streams.

3. Domestic sewage when dechlorinated by lagooning following secondary treatment was not acutely toxic to juvenile salmon.

4. Chlorine, surfactants and ammonia in domestic sewage were believed responsible for damage to gill tissue of captive juvenile salmon but damage caused by the former was most severe.

5. The orthotolidine method of measuring chlorine residual in sewage or the receiving water was not adequate and the amperometric method is recommended.

6. Present standards concerning chlorine residuals in receiving waters should be re-evaluated.

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