THE AVOIDANCE REACTIONS OF CHINOOK SALMON, *ONCORHYNCHUS TSHAWYTSCHA* (WALBAUM), AND COHO SALMON, *ONCORHYNCHUS KISUTCH* (WALBAUM), TO PAPER MILL EFFLUENTS

by

BENJAMIN FRANKLIN JONES

A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1955

APPROVED:

Redacted for privacy

Head of Department of Fish and Game Management

In Charge of Major

Redacted for privacy

Chairman of School Graduate Committee

Redacted for privacy

Dean of Graduate School

Date thesis is presented May 11, 1955

Typed by Lenora J. Bond

ACKNOWLEDGMENT

This opportunity is taken to express acknowledgment to those individuals who have helped me in this study. To Assistant Professor Charles E. Warren the writer owes a debt of gratitude for assistance in the organization of research and guidance and suggestions in the preparation of this thesis. Special thanks are extended to Professor R. E. Dimick for his constant encouragement during the past six years. Sincere appreciation is expressed to Dr. Peter Doudoroff for his constructive criticism during the course of this study. Thanks are hereby given to Dr. Max Katz for his invaluable advice and for his assistance in collecting and caring for the fish used in the investigations.

This study was supported by a gift from the National Council for Stream Improvement.

TABLE OF CONTENTS

		Page
I.	INTRODUCTION	1
II.	REVIEW OF LITERATURE	4
	General	4
	Preliminary Studies at Oregon State College	7
III.	MATERIALS AND METHODS	14
	Materials	14
	Study Apparatus	14
	Water Supply	18
	Holding Tanks	18
	Test Animals	19
	Chemical Pump	20
	Methods	21
	Preparation of Waste Liquors	21
IV.	AVOIDANCE AND TOXICITY OF SULFATE WASTE LIQUOR	24
	Avoidance	24
	Synthetic Waste Liquor	24
	Whole Waste Liquor	31
	Toxicity	34
V.	AVOIDANCE AND TOXICITY OF SULFITE WASTE LIQUOR	38
	Avoidance	38
	Toxicity	39
VI.	SUMMARY	44
VII.	BIBLIOGRAPHY	47

LIST OF TABLES

Table		Page
1.	Reactions of juvenile salmonid fish to a concentration gradient of sulfate waste	9
2.	Reactions of Coho salmon to a concentration gradient of methyl mercaptan	13
3.	Ratios of water to sulfate waste and concentrations produced in channels . .	23
4.	Preparation of sulfite waste liquor . . .	23
5.	Avoidance of synthetic sulfate waste liquor by Chinook salmon	29
6.	Avoidance of synthetic sulfate waste liquor by Coho salmon	30
7.	Avoidance of whole sulfate waste liquor by Chinook salmon	32
8.	Avoidance of whole sulfate waste liquor by Coho salmon	33
9.	Toxicity to Coho salmon of a synthetic sulfate waste liquor as found by Amberg	36
10.	Avoidance of sulfite waste liquor by Chinook salmon	42
11.	Avoidance of sulfite waste liquor by Coho salmon	43

LIST OF FIGURES

Figure		Page
1.	Avoidance reaction apparatus	15
2.	Chinook salmon avoidance of synthetic sulfate waste liquor	28
3.	Chinook salmon avoidance of sulfite waste liquor	41

THE AVOIDANCE REACTIONS OF CHINOOK SALMON, ONCORHYNCHUS TSHAWYTSCHA (WALBAUM), AND COHO SALMON, ONCORHYNCHUS KISUTCH (WALBAUM), TO PAPER MILL EFFLUENTS

I. INTRODUCTION

This report presents the findings of a study made at the Fisheries Research Laboratory at Oregon State College during the summer and fall of 1954 on the avoidance reactions of two species of Pacific salmon to paper mill effluents. The purpose of the study was to determine whether or not juvenile Chinook salmon, Oncorhynchus tshawytscha (Walbaum), and juvenile Coho salmon, Oncorhynchus kisutch (Walbaum), would react by avoiding different concentrations of sulfate and sulfite paper mill wastes.

Work was started on this problem in 1952 when Carl E. Bond, Assistant Professor of Fish and Game Management, began a series of preliminary experiments on the reactions of Chinook salmon, Coho salmon, and Steelhead trout, Salmo gairdneri gairdneri Richardson, in a gradient tank containing sulfate waste liquor. Since he demonstrated varying degrees of avoidance reactions of these species of fishes in his preliminary study, it was decided that a more extensive investigation was warranted which might establish definite limits for the avoiding reactions exhibited by the two species of salmon under consideration. This study was therefore planned to obtain

information on the degrees of avoidance that Chinook salmon and Coho salmon would exhibit to different concentrations of sulfate and sulfite paper mill effluents and to relate this information to the possible toxicity of these effluents.

Since the destruction of fish populations may come about through means other than the direct toxic action of a waste, it was considered to be of importance to determine whether the presence of non-lethal concentrations of paper mill effluents might cause fish to leave or not enter a contaminated area. Further, knowledge concerning the avoidance or lack of avoidance by fish of lethal or non-lethal concentrations of a waste is extremely important in determining the best means of dispersing that waste in the receiving stream. For example, if a species is known to avoid a lethal concentration of a particular waste, that waste might best be channeled in high concentrations down one bank of a river, leaving a clean channel for fish movement, rather than dispersing the waste over the entire stream bed and possibly entirely preventing fish movement. Conversely, if a species is known to exhibit no avoidance to a particular waste, that waste might best be disposed of in the most dilute state possible over the entire stream bed.

This paper will consider in some detail the apparatus

devised to carry out these avoidance studies. The avoidance reactions exhibited by Chinook salmon and Coho salmon to synthetic sulfate waste liquor, whole sulfate waste liquor, and sulfite waste liquor will be discussed. Some information on the toxicity of these wastes to young salmon will be presented, and this will be considered in relation to the avoidance of these wastes by the salmon.

II. REVIEW OF LITERATURE

General

The reactions exhibited by several kinds of fish when confronted with various substances have been studied by a number of investigators. Among the early investigations was that on which Shelford published in 1917 (15, pp.381-412). In this study of the effects of gas waste on certain fishes of the families Centrarchidae, Catostomidae, Cyprinidae, and Ameiuridae, he found that fish will enter water containing certain pollutants from pure water readily and will turn back to the polluted water when fresh water is encountered. From this it is evident that fish may exhibit an apparent preference for several kinds of polluted water, whatever its mechanism.

However, other investigators have found that fish will exhibit avoidance to pollutants in their environment. Jones (10, pp.22-34) found that sticklebacks, Gasterosteus aculeatus Linnaeus, reacted negatively by avoiding 0.001 N solutions of sodium sulfide. The reaction was almost immediate at this concentration, while at greater dilutions the reaction time increased. Using the minnow, Phoxinus phoxinus Linnaeus, Jones (11, pp.261-270) found that over the range of 0.04 to 0.0004 per cent the fish appeared to have little or no power of discrimination between phenol

and water, and would swim into the solution of phenol with no hesitation. At concentrations of 0.03 per cent to 0.04 per cent the minnows avoided para-cresol and ortho-cresol solutions immediately.

Temperature has been regarded as having an important influence on the migration and distribution of fishes (4, pp.79-118) (17, pp.235-254) (6, pp.246-262). Wells (18, pp.48-59), Shelford and Powers (14, pp.315-334), and Chidester (3, pp.373-380) found that fish are very sensitive and will react to the stimulus of temperature.

Jones (12, pp.403-415) observed that sticklebacks had no immediate appreciation of water of low oxygen concentration and would swim into it without hesitation. Respiratory distress soon developed, however, and this was accompanied by uneasiness and random movement. If the random movement took the fish out of the area of low oxygen concentration, there was a tendency for the random movement to cease and for the fish to recover.

Collins (5, pp.375-396) investigated the preference of an anadromous fish, Pomolobus sp., for water having specific physical and chemical characteristics. He found that Pomolobus showed selectivity when presented with the choice of two channels, one with warm and one with cold water. These fish also exhibited an avoiding reaction to the water containing the highest of two carbon dioxide

concentrations when the difference exceeded 0.3 parts per million. He also found that the velocity and turbulence of the water were factors influencing the reactions of these fishes.

The recent studies by Smith and Saalfeld (16, pp.3-26) on the Columbia River smelt, Thaleichthys pacificus Richardson, show that these fish exhibit a definite preference for clean water in contrast to water containing certain concentrations of paper mill wastes. They found that the smelt preferred a channel of unaltered water to a channel which contained dilutions of mixed sulfite-base and sulfate-base paper mill waste liquor in concentrations ranging from 1:103 to 1:400. Avoidance of the sulfate waste liquor alone was exhibited for concentrations ranging from 1:50 to 1:800.

The investigations by MacKinnon and Hoar (13, pp.523-528) in British Columbia demonstrated that Chum salmon fry, Oncorhynchus keta (Walbaum), and Coho salmon fry respond to differences in velocity of water flow. Both species selected the stronger of two currents; but, after a time, Coho failed to respond to small differences while the Chums continued to select the greater flow.

Brett and MacKinnon (2, pp.21-22), using Coho salmon, Chinook salmon, and Sockeye salmon, Oncorhynchus nerka (Walbaum), found these fishes were repelled apparently,

by the odors, coming from human skin. Merely placing a hand in the water of a fish-ladder caused a marked decrease in the numbers of adult fish moving up the ladder. Successive rinses of the hand showed a reduced activity, suggesting that the material may have been accumulated on the skin. These same workers (1, pp.10-12) demonstrated that Coho and Chinook salmon are apparently able to detect extracts from the skin of hair seals, sea lions, and dogfish. It was thought that the substance detected and avoided was an excretory or secretory product associated with the lower skin layer.

Still other investigators have demonstrated that fish are capable of detecting pollutants in their environment. Hasler and Wisby (7, pp.64-70) have shown that bluntnose minnows, Hyborhynchus notatus Rafinesque, can detect and discriminate between phenols and p-chlorophenols at concentrations as low as 0.0005 parts per million. The fish were trained to associate the odor of phenol with food and the odor of p-chlorophenol with punishment. At the end of a training period the fish were able to distinguish between the two odors and also to distinguish them from a third pollutant, o-chlorophenol.

Preliminary Studies at Oregon State College

Preliminary investigations on the avoidance reactions

of salmonoid fishes to paper mill effluents were begun by Bond in 1953 at the Oregon State College Fisheries Research Laboratory. His experiments were conducted with a rectangular trough which was divided into four compartments by a series of baffles. Water was introduced at one end of the trough and desired concentrations of water and waste liquor at the opposite end. Both the water and the diluted waste flowed toward the center of the trough, where a standpipe drain was located. A concentration gradient of waste was thus established. Fish were able to swim around the baffles and select compartments at will. The responses of juvenile Chinook salmon, Coho salmon, and Steelhead trout to concentration gradients of whole sulfate waste and of methyl mercaptan were investigated.

Twenty fish were used for each experiment and observations were made several times. Each entry in table 1 represents the total number of fish observed in each compartment in several observations. Concentrations of whole sulfate waste in the compartments ranged from 1:5 to 1:40 and were determined photometrically by means of the Pearl-Benson test.

The results of seventeen experiments with sulfate waste in which Chinook salmon, Coho salmon, and Steelhead trout were used as test animals are shown in table 1. The concentrations given are the mean concentrations in the

TABLE 1

REACTIONS OF JUVENILE SALMONID FISHES TO A CONCENTRATION GRADIENT OF SULFATE WASTE AS FOUND BY BOND IN 1953.

Compartment region			A	B	C	D
Mean percent concentration			0.0	1.5	4.3	4.8
Standard deviation of mean percent concentration			0.00	0.20	0.84	0.80
Species	**Trial**	**Date**	**Numbers of fish observed**			
Chinook	1	5/28-29/53	36	7	0	0
	2	6/4/53	23	19	1	5
	3	6/11-12/53	93	16	0	0
	4	6/13/53	32	2	1	1
	5	6/18-19/53	46	3	1	0
Total observations			230	47	3	6
Percent observations			80.4	16.4	1.1	2.1
Coho	1	5/26-27/53	21	15	8	8
	2	6/19/53	0	38	3	5
	3	6/29-30/53	27	11	1	13
	4	7/1-2/53	27	12	7	13
	5	8/5-6/53	72	61	3	11
	6	8/8-9/53	28	40	8	14
	7	9/11-12/53	11	49	81	3
	8	9/14-15/53	6	27	20	4
Total observations			192	253	131	71
Percent observations			29.7	39.1	20.2	11.0
Steelhead	1	7/21/53	3	14	3	4
	2	7/23-24/53	4	3	4	15
	3	9/3-4/53	17	46	49	15
	4	9/16-17/53	3	42	103	9
Total observations			27	105	159	43
Percent observations			8.1	31.4	47.6	12.9

four compartments of the trough. The standard deviation of the waste concentration for each compartment is given. The compartment totals and percentages for all experiments with each species are also presented.

Chinook salmon showed a marked avoidance of sulfate waste. In five experiments, only 2.1 per cent of the total number of observations were in the 4.8 per cent concentration of waste. Of the total observations, only 1.1 per cent occurred in the compartment having a waste concentration of 4.3 per cent, and 16.4 per cent occurred in the compartment having a concentration of 1.5 per cent. A total of 80.4 per cent of the observations occurred in the waste-free compartment. Generally, Chinook salmon seemed to prefer the end compartments of the trough to the center compartments. The waste inlets were changed to the opposite end of the trough at frequent intervals to make certain that the fish did not develop a preference for any one compartment.

Coho salmon exhibited avoidance to sulfate waste liquor. However, the avoidance was not as sharply defined as that exhibited by Chinook salmon. Of the total observations, 11.0 per cent were made in the 4.8 per cent concentration, 20.2 per cent in the 4.3 per cent concentration, 39.1 per cent in the 1.5 per cent concentration, and 29.7 per cent in the waste-free compartment. It seems

significant that with the Coho salmon approximately 69 per cent of the observations were in compartments with concentrations of waste of 1.5 per cent and below. This would indicate that Coho salmon are not entirely insensitive to pollutants occurring in their environments, which tends to substantiate other findings presented in this paper. An examination of the groupings of the test-animals as shown in table 1 indicates that the Coho salmon preferred the center compartments of the trough to those at the ends.

Steelhead trout exhibited no avoidance of the wastes in concentrations ranging from 1.5 per cent to 4.5 per cent. This is interesting as they are closely related to the two other species used as test-animals. An inspection of the results shows that the observations in compartment C, which had a waste concentration of 4.3 per cent, were much more numerous than in any of the other three compartments. This probably indicates an insufficient number of experiments, there being no reason to assume the fish were exhibiting a preference for the waste. If the fish were exhibiting no avoidance, the high numbers should be distributed randomly over the compartments and additional experiments would probably result in an equalizing of these percentages. As did Coho salmon, Steelhead trout preferred the center compartments.

Since methyl mercaptan is one of the compounds

present in sulfate mill waste, and since it has a strong odor, the reactions of fish confronted with this compound were considered of interest. Coho salmon were exposed to a concentration gradient of 0.00 to 1.9 parts per million of methyl mercaptan. In a series of three experiments, Coho salmon exhibited a definite preference for the water having the lowest concentrations of the pollutant. Of the total number of observations, 12.0 per cent were in the compartment with a concentration of 1.4 to 1.9 parts per million, 13.7 per cent in the concentrations of 0.6 to 1.7 parts per million, 31.0 per cent in the concentrations of 0.2 to 0.4 parts per million, and 43.3 per cent in the concentration of 0.00 to 0.2 parts per million.

Haydu, Amberg, and Dimick (5, pp.545-549), in experiments on the effects of Kraft Mill waste components on salmonid fishes, found that the minimum lethal concentrations of methyl mercaptan were as follows: 1.75 parts per million for Coho salmon, 1.20 parts per million for Cutthroat trout, Salmo clarki clarki Richardson, and 0.9 parts per million for Chinook salmon. The minimum lethal concentration was defined as the least amount of toxic agent capable of producing a 100 per cent mortality of the test fish. Thus, concentrations highly toxic to the Coho salmon were present in a large part of the avoidance reaction trough. It is of interest that the Coho salmon

TABLE 2

REACTIONS OF COHO SALMON TO A CONCENTRATION GRADIENT OF METHYL MERCAPTAN AS FOUND BY BOND IN 1953.

Compartment region	A	B	C	D
Range of concentration in p.p.m.	0-0.2	0.2-0.4	0.6-1.7	1.4-1.9
Trial	**Date**	**Numbers of fish observed**		
1	7/29-30/53	54	41	15	14
2	8/13-14/53	25	22	12	10
3	8/16/53	44	25	12	10
Total fish observed		123	88	39	34
Percent of entry		43.3	31.0	13.7	12.0

continued to enter the regions of high concentration, though high mortalities resulted. However, this might be expected, since in close quarters the minimum movement of the fish might carry them into toxic zones which they would normally avoid.

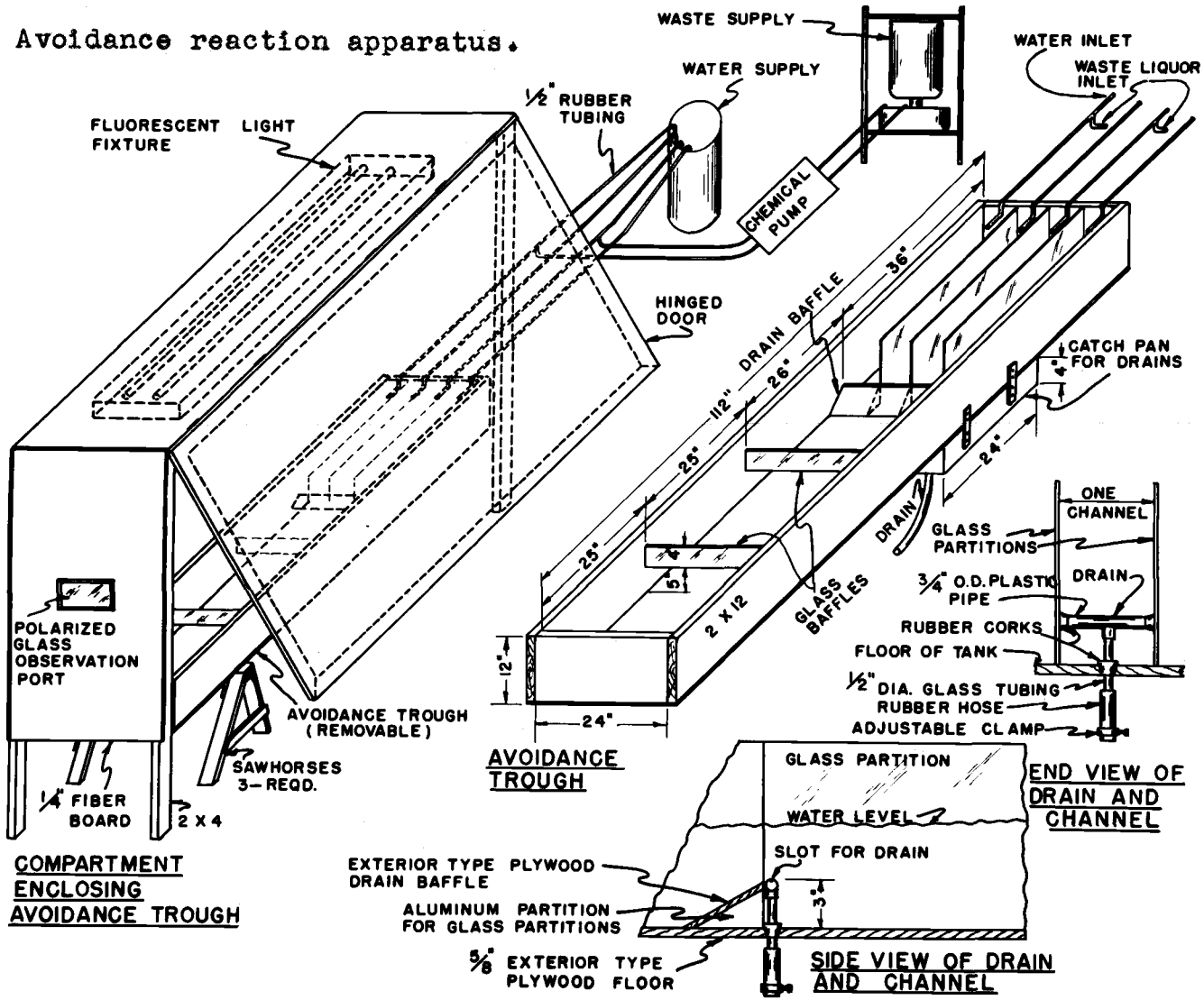
III. MATERIALS AND METHODS

Materials

Study Apparatus

The basic apparatus, figure 1, consisted of a rectangular tank, nine feet long by two feet wide by one foot deep, placed on three waist-high trestles. For one-third of its length the interior of the tank was divided into four channels by sheets of heavy plate-glass. An individual drain was placed at the entrance of each of the four channels. Water and waste liquor were introduced into the channels through four glass tubes leading from the water and waste supplies located above the avoidance apparatus. The trough was shielded from outside disturbances by partitions which extended from the ceiling of the room to the bottom edge of the tank on three sides and by a wall on the fourth side. The interior surfaces of the compartment were painted with a flat-white paint to prevent glare, and a two-tube fluorescent light was suspended above the tank to give uniform illumination over the entire area. An observation port was located at the end of the compartment opposite the channeled end of the tank to permit observation of the fish movements. One wall of the compartment was hinged

Figure 1. Avoidance reaction apparatus.



across the top so that it could be swung upwards and suspended from the ceiling to permit access to the tank.

Since the effective functioning of the study apparatus depended largely on the efficiency of the drain, this system will be discussed in greater detail. The construction of the drains is shown in figure 1. The four drains were designed to operate in conjunction with a baffle placed on the floor of the tank and abutted against the ends of the glass partitions at a 45 degree angle. The design of the drains was such that all water flowing down the channels was diverted down the drains and not permitted to flow beyond the channels into the open end of the tank. Each drain was constructed of one-inch plastic pipe so as to form a "T", the horizontal portion being slotted so as to allow the water to flow in and then down the vertical portion. The vertical portion of the "T" was forced through a drilled rubber stopper which was inserted in a drain hole in the floor of the tank at the entrance to each channel. A short section of rubber tubing was stretched over the lower end of the "T" protruding below the bottom of the tank, and a screw-clamp was utilized to adjust the flow of water from the individual drain. The top edge of the baffle, as shown in figure 1, was at the same level as the tops of the drains. This arrangement served to divert the flow of water in each channel up and

over the slot in the top of the drain. By properly adjusting the inlet tubes to the channels and the outlet tubes beneath the channels, it was possible to maintain any desired depth of water in the tank, and yet to divert all of the incoming water down the drains and prevent its mixing with the water in the open portion of the tank.

At first it was thought some strong stimulus would be required to force the fish to leave the clean zone of water in the one end of the tank and choose between the polluted and clean channels at the opposite end. Two methods of raising the water temperature were tried, and although no difficulty was encountered in heating the water, it was found that due to stratification; counter currents were created which caused water from the channels to be drawn past the drains and out into the open portion of the tank. The mixing of the polluted water with the clean water created a polluted zone through which the fish had to pass in order to reach the channels. It was believed that exposure of the fish to the waste before they had an opportunity to choose between it and clean water would defeat the purpose for which the apparatus was designed. The search for methods of stimulation was abandoned when it was found that the fish would enter the channels in preference to remaining in the still water of the open end of the tank if the current in the channels

was great enough.

Water Supply

Two sources of water were utilized during the course of this study. Corvallis city water was used for the first two months. The water was first passed through a filter of activated charcoal to remove chlorine. The use of this water was discontinued because of low pressures during the summer months. For the remainder of the study, water was pumped from nearby, Mary's River.

A fifty-gallon tank fitted with a constant-head device supplied water from a one-thousand-gallon storage tank to the overflow jar located on a shelf directly above the avoidance apparatus. Four siphons carried water from the overflow jar to the channel inlets.

Water temperatures were in the range of 17 to 19 degrees Centigrade throughout the summer and into the fall months. In October, temperatures began to drop, and by January they had reached a minimum of 5 degrees Centigrade.

Holding Tanks

Two types of tanks were employed for holding fish. During the summer months, when young fish were readily available, these were collected in small numbers and used within five or six days. For holding the fish for such

short periods, two one-hundred-gallon stainless steel tanks were used and from fifty to one hundred fish were held in each. The water was aerated continuously. The tanks were cleaned and the water was changed approximately once each week.

During the latter part of the study, the young fish in the streams began to migrate seaward, and it became more difficult to secure them in adequate numbers. Rather than to be forced to discontinue studies for lack of fish, it was decided to stock considerable numbers for future use. For this purpose, two two-hundred-fifty-gallon wooden tanks were constructed. Each was provided with a drain near the bottom and inlet and overflow pipes near the top. Water was replaced in the tanks at the rate of approximately one gallon per minute, and aeration was not required. Five hundred to seven hundred juvenile salmon could be held in these tanks for extended periods.

Test Animals

Juvenile Chinook salmon were collected from two tributaries of the Willamette River system in western Oregon during the summer and fall of 1954. Fish were taken from the Santiam River at several points above the town of Lebanon in Linn County. On the McKenzie River, collections were made at two stations above Eugene in

Lane County.

Juvenile Coho salmon were taken from two smaller streams in western Oregon. Collections were made in the Yaquina River above Nashville and in Beaver Creek near Newport, in Lincoln County.

The fish were captured by means of one-half-inch stretched mesh seines and were transported to the holding tanks at the Corvallis laboratory in five-gallon wide-mouth jars wrapped in burlap. Loss of fish during the transfer was negligible. These fish were in the size range of 40 to 160 millimeters total length, and all appeared to be in good physical condition.

During the fall of 1954, it became difficult to secure fish from the streams in the desired numbers. Through the cooperation of the Oregon Fish Commission, some 300 young Chinook salmon were obtained from the Marion Forks Hatchery in eastern Marion County. The size range of these fish was from 80 to 130 millimeters total length, and all were in excellent condition.

Chemical Pump

Waste liquor was injected into the water lines leading to the channels by a low-capacity chemical pump available commercially as a "Sigmamotor". This device consists of an electric motor, a speed reducer, and a

pump, all mounted on a rectangular steel plate. The pump is constructed in the form of a square box inside of which are mounted two rows of finger-like steel rods which press against a metal plate placed between the two rows. The rods are caused to press against the plate in an undulating fashion by the action of a camshaft in the lower part of the unit. Rubber tubing is placed in the pump between the fingers and the plate; and the movements of the fingers exert a milking movement against the tubing, causing the liquid inside to flow away from the pump. The rate of flow is controlled by a calibrated lever on the speed reducer.

Methods

Preparation of Waste Liquors

Sulfate waste was obtained from the Springfield, Oregon, plant of the Weyerhauser Timber Company. Whole waste was drawn from the main disposal pipeline which emptied into the McKenzie River. The various components of the whole waste, when desired, were taken at suitable sampling points within the plant.

Synthetic sulfate waste was prepared by diluting ninety milliliters of combined condensates and two milliliters of black liquor to one liter with Mary's River

water. The synthetic waste was made as needed in quantities of eighteen liters. In order to obtain the desired dilutions of waste in the channels of the study apparatus, measured amounts of waste were injected into the water inlets by the chemical pump. Table 3 shows the ratios in which synthetic and whole sulfate waste were mixed with water.

Calcium-base sulfite waste liquor was procured from the Publisher's Paper Company mill at Oregon City, Oregon. Since the concentration of the raw waste liquor varied from day to day, it was necessary to prepare stock solutions with uniform solids content for use in the experiments. Table 4 shows the ratio in which the stock solutions were mixed with water, the concentrations of bone-dry solids present in the stock solutions, and the concentrations of waste liquor which were introduced into the channels. Since both the water and the waste liquor could be accurately metered, it was possible to obtain the desired concentrations in the channels with only small errors.

TABLE 3

RATIOS OF WATER TO SULFATE WASTE
AND CONCENTRATIONS PRODUCED IN CHANNELS.

Ml. sulfate waste	Ml. water	Sulfate waste in channel: approximate % by volume
120	1200	10.00
60	1200	5.00
30	1200	2.50
15	1200	1.25

TABLE 4

PREPARATION OF SULFITE WASTE LIQUOR

ppm of bone-dry solids in stock solution SWL.	Ml. stock soln.	Ml. water	Channel conc. of SWL: ppm solids	Channel conc. of SWL: ppm of SWL of 10% solids content
2600.0	100	1200	200.0	2000
1300.0	100	1200	100.0	1000
750.0	100	1200	50.0	500
375.0	100	1200	25.0	250
187.5	100	1200	12.5	125

IV. AVOIDANCE AND TOXICITY OF SULFATE WASTE LIQUOR

Avoidance

Synthetic Waste Liquor

The direct application of the results of laboratory studies to natural situations is usually difficult if not impossible. Under the controlled conditions of studies such as this one, the responses of the fish may vary greatly from those occurring under natural conditions. However, it was thought that experiments carried out under controlled conditions might produce results which would give an indication of the responses of salmonid fishes confronted by these pollutants in streams.

Per cent avoidance, as used in this study, was derived from the formula $100(E - A)/E$, where E equals the total number of entries of fish into clean channels and A equals the total number of entries of fish into polluted channels. The per cent avoidance thus determined may have no absolute or actual relationship to the reactions of these fish under stream conditions. However, the concentrations at which the fish show zero avoidance of a waste are of considerable importance.

The behavior of fish when exposed to wastes under controlled conditions is of interest. At low concentrations

of waste the fish would often enter the polluted channels readily and usually would swim to the extreme ends and congregate around the water and waste inlet tubes. Rarely would the fish remain more than several minutes in this area. Usually they would turn and swim out of the channels into the open end of the tank. Occasionally a fish would leave one channel and, instead of swimming back out into the open area of the tank, would immediately turn and enter an adjacent channel. When the higher concentrations of wastes were used, fish would often start to enter a polluted channel, then pause as they encountered the polluted water, turn, and swim back out of the channel into the open zone. Occasionally a fish would enter a channel of high waste concentration and remain for several minutes in the strong currents created by the inlet tube. However, in most instances, the fish did not remain in the polluted channels at the higher concentrations. Reactions of the fish were much the same in clean channels. The fish would congregate around the inlet tubes at times, but their constant movement was such that no aggregation of animals ever occurred in the channels.

Preliminary experiments with both Coho salmon and Chinook salmon using only unaltered water in all four channels showed that the fish moved about the tank in a random manner. No preference was shown for any one

channel or pair of channels.

As a control measure during the actual experiments, the waste inlet tubes and water inlet tubes were moved to alternate channels several times each week. In addition, the test animals were never used more than two times with any one kind of effluent.

The random movement of the test-animals during a sixty minute observation period was usually sufficient to allow for at least several hundred entries into the four channels. One entry was defined as the movement of one fish completely past the ends of the glass partitions and the drain and into the channel. In the event that the fish entered only partly into the channel, no entry was recorded.

Chinook salmon were found to avoid synthetic sulfate waste in certain concentrations. The degree of avoidance appears to be, in part, dependent on the concentration of the waste liquor. These results are presented in table 5. At a concentration somewhere between 1.25 per cent and 2.50 per cent synthetic sulfate waste, the fish cease to exhibit an avoiding reaction as measured by the previously described method. A significant avoidance was noted at the 2.50 per cent concentration and zero avoidance was exhibited at the 1.25 per cent level. At concentrations of 10.00 per cent, a mean avoidance of 86.89 per cent was

exhibited, the 95 per cent confidence limits of the mean being 78.74 per cent and 95.05 per cent. From 2.50 per cent to 10.00 per cent, increasing avoidance of the polluted channels was exhibited. A mean avoidance of 28.46 per cent was shown at the 2.50 per cent concentration, with the 95 per cent confidence limits of the mean being -3.60 per cent and 61.48 per cent. At the 5.00 per cent level, a mean avoidance of 69.09 per cent was recorded, with the 95 per cent confidence limits being 48.48 per cent and 89.70 per cent. The relationship of the per cent avoidance to the per cent concentration of the waste liquor is shown in figure 2. When the waste concentration is plotted on a logarithmic scale the curve is straightened considerably. Theoretically, the per cent avoidance should be plotted on a probit scale, since such values are expected to follow a normal distribution. However, the zero values obtained at the 1.25 per cent concentration of waste caused the line to be distorted, so no attempt was made to fit the line mathematically. The line was drawn by using the mean per cent avoidance of each grouping at a particular concentration.

Although the variability within the arrays is rather great, the overlap between the arrays is reasonably small, as is shown in table 5 and figure 2. This variability was probably largely due to variation in the characteristics of the waste used. This is illustrated by the results of

Figure 2.

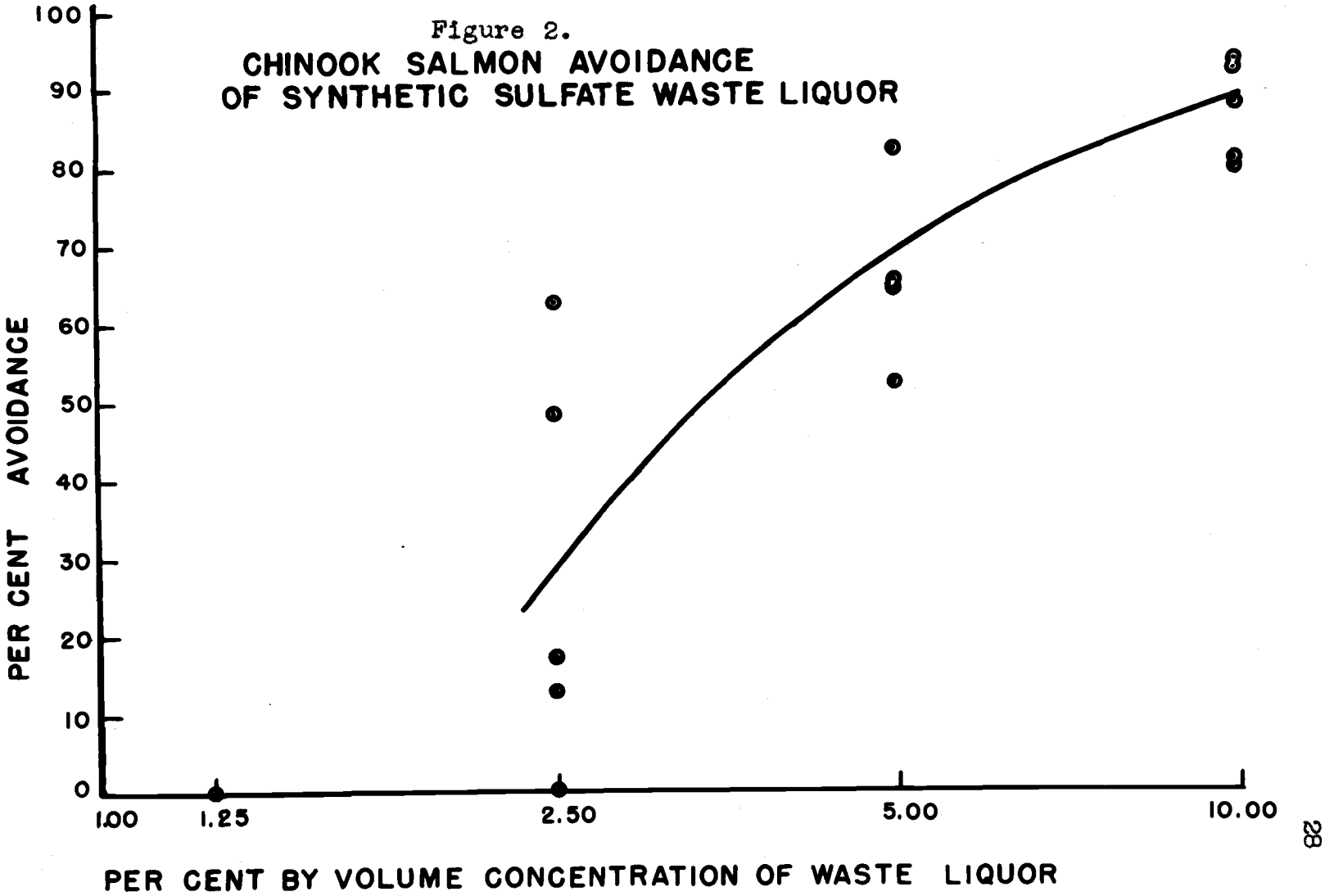

CHINOOK SALMON AVOIDANCE
OF SYNTHETIC SULFATE WASTE LIQUOR
PER CENT AVOIDANCE
100
90
80
70
60
50
40
30
20
10
0
1.00
1.25
2.50
5.00
10.00
PER CENT BY VOLUME CONCENTRATION OF WASTE LIQUOR

TABLE 5.

AVOIDANCE OF SYNTHETIC SULFATE WASTE LIQUOR BY CHINOOK SALMON.

Concentration of waste: % by volume	Trial number	Date	Number of Entries				% Avoidance	Est. mean % avoidance	95% Confidence limits of mean
			Waste	Control	Waste	Control			
10	1	8/17	9	197	16	196	93.69		
	2	8/31	2	47	5	43	92.22		
	3	9/7	1	16	3	17	87.88		
	4	11/19	122	471	46	344	79.39		
	5	11/26	43	377	89	292	80.26	86.89	78.74%; 95.04%
5	1	8/16	48	128	53	156	64.43		
	2	8/30	6	31	4	26	82.45		
	3	9/8	31	89	35	102	65.45		
	4	11/30	45	111	48	83	52.06	69.09	48.48%; 89.70%
2.5	1	8/10	145	262	81	352	63.19		
	2	8/12	42	80	30	60	48.57		
	3	8/25	52	89	81	65	13.64		
	4	9/9	23	28	31	37	16.92		
	5	12/3	109	77	136	90	00.00	28.46	-3.60%; 61.48%
1.25	1	8/18	35	34	79	60	00.00		
	2	8/24	26	19	21	27	00.00		
	3	12/11	152	66	74	115	00.00	0.00	----

TABLE 6.

AVOIDANCE OF SYNTHETIC SULFATE WASTE LIQUOR BY COHO SALMON.

Concentration of waste: % by volume	Trial number	Date	Number of entries				Per Cent Avoidance
			Waste	Control	Waste	Control	
10	1	12/29	157	207	187	181	11.34
5	1	12/8	326	277	339	337	0.00

bioassays made by Amberg which are discussed below. Using waste components to make up stock synthetic wastes, he found considerable variation in the samples collected on different days. This could be demonstrated by the fact that the amounts required to produce comparable mortalities varied greatly.

Coho salmon exhibited considerably less avoidance of synthetic sulfate waste liquor than did Chinook salmon (table 6). This is of interest since the two species are so closely related. In a single trial at a 10 per cent concentration of waste, Coho salmon exhibited only 11.34 per cent avoidance. In one trial at the 5 per cent concentration, zero avoidance was shown.

Whole Waste Liquor

The avoidance shown by the two species of salmon to whole mill effluent was markedly less than the avoidance shown to synthetic effluent (tables 7 and 8). At a concentration of 10 per cent whole mill effluent, Chinook salmon exhibited a mean avoidance of 37.66 per cent, whereas an avoidance of 86.89 per cent was shown by the same species exposed to the 10 per cent concentration of synthetic waste. Only 33.33 per cent avoidance was shown by Chinook salmon exposed to whole waste at the 5 per cent level, this being less than one-half the avoidance

TABLE 7.

AVOIDANCE OF WHOLE SULFATE WASTE LIQUOR BY CHINOOK SALMON.

Concentration of waste: % by volume	Trial number	Date	Number of entries				Per Cent avoidance	Mean per cent avoidance	95% Confidence limits of mean
			Waste	Control	Waste	Control			
10	1	7/19	84	163	123	168	37.44		
	2	7/20	157	305	197	302	41.68		
	3	7/21	92	113	88	242	49.29		
	4	12/22	197	289	237	269	22.22	37.66	19.53%; 55.78%
5	1	7/28	76	162	90	148	46.45		
	2	8/5	90	179	99	229	53.54		
	3	12/15	178	168	174	169	00.00	33.33	-8.97%; 75.63%
2.5	1	7/23	26	25	34	48	17.81		
	2	7/26	50	37	47	61	1.02		
	3	7/28	77	111	90	106	19.32		
	4	8/6	53	60	72	84	13.19	12.84	0.3522%; 26.03%

TABLE 8.

AVOIDANCE OF WHOLE SULFATE WASTE LIQUOR BY COHO SALMON

Concentration of waste: % by volume	Trial number	Date	Number of entries				Per cent avoidance
			Waste	Control	Waste	Control	
10	1	12/22	142	116	113	99	0.00

exhibited when synthetic waste was used. At the 2.5 per cent concentration, a mean avoidance of 12.84 per cent was demonstrated by the Chinook salmon.

As with synthetic waste, Coho salmon showed less avoidance of the whole waste than did the Chinook salmon (table 8). In one trial using 10 per cent concentration of whole waste no avoidance was exhibited by the Coho salmon. Bond's results (table 1) showing species differences between Chinook salmon and Coho salmon are supported by the evidence obtained in this study.

Toxicity

The toxicity of sulfate waste liquor is highly variable. Amberg (unpublished results) found that a synthetic waste prepared from one set of waste samples by using 5000 millliliters of combined condensates and 30 millliliters of black liquor diluted to 18 liters produced 50 per cent mortality in juvenile Coho salmon in 34.30 hours at a concentration of 8.0 per cent. He also demonstrated that a synthetic waste made from a second set of waste samples by using only 1620 millliliters of combined condensates and 25 millliliters of black liquor diluted to 18 liters would effect a 30 per cent mortality of young Coho salmon in 35.5 hours at 7.5 per cent concentration. With a synthetic waste made from a third set of waste samples by

using 3000 millliliters of combined condensates and 30 millliliters of black liquor diluted to 18 liters, Amberg obtained results comparable to those obtained with the above-mentioned synthetic wastes. The results obtained with this waste made from the third set of samples are given in table 9. The large differences necessary in volumes of different condensates used in the synthetic wastes to produce wastes of comparable toxicity is rather striking evidence of the highly variable nature of these condensates and hence both synthetic and whole wastes.

The toxicity of the third set of samples might be assumed to be about average, since in order to produce comparable mortalities, approximately twice as much of the first condensate sample was necessary and only one-half as much of the second condensate sample was necessary as was required in the third set of tests. The synthetic sulfate waste used for the avoidance studies was prepared by using 1620 milliliters of combined condensates and 36 milliliters of black liquor. Thus, considering only the condensate concentrations, this waste was prepared at approximately one-half the strength of Amberg's third set of samples. If the toxicity of this third set of samples is assumed to be approximately average, then the average toxicity of the synthetic wastes used for the avoidance studies was possibly about one-half the toxicity of the waste shown in

TABLE 9

TOXICITY TO COHO SALMON AT 17°C. OF A SYNTHETIC SULFATE WASTE OF 3000 MILLILITERS COMBINED CONDENSATES AND 30 MILLILITERS BLACK LIQUOR DILUTED TO 18 LITERS AS FOUND BY AMBERG (UNPUBLISHED DATA).

Concentration, % by volume	2.7	4.0	6.0	8.0
Time in hours	Per Cent Mortality (among 10 fish)			
45	0	0	20	40
100	0	0	20	100
120	0	0	20	
200	--	--	50	

table 9.

No toxicity to Chinook salmon was found with a synthetic waste made from one set of samples used in the avoidance studies at either 10 per cent or 5 per cent concentrations over a 48 hour period. If this set of samples had been average, mortality might have been expected at the 10 per cent level since Amberg found 20 per cent mortality in 45 hours at a 6 per cent level of his third set of samples (table 9). This was, however, a low mortality, and differences in concentration could account for the lack of mortality in the samples used for avoidance studies. Coho salmon show some avoidance to 10 per cent concentrations. Chinook salmon avoid

concentrations of synthetic waste as low as 2.5 per cent and it is unlikely that these would prove toxic to the fish in five days. At the 10 per cent concentration, it is possible that mortality would result to both species in five days or less. Thus, while Chinook salmon apparently exhibit marked avoidance to concentrations well below the acutely toxic level, Coho salmon exhibit only slight avoidance of potentially toxic concentrations.

It is of interest that Haydu, Amberg, and Dimick (5, pp.545-549) found the Chinook salmon more sensitive to certain constituents of sulfate waste liquor than were Coho salmon. Whether or not the greater sensitivity of Chinook salmon is in some way related to their greater tendency to avoid these wastes would be an interesting subject for further investigation.

V. AVOIDANCE AND TOXICITY OF SULFITE WASTE LIQUOR

Avoidance

Chinook salmon exhibit avoidance to some concentrations of sulfite waste liquor. The results of nine experiments are presented in table 10. Figure 3 indicates that when probit values for per cent avoidance are plotted against the logarithms of the sulfite waste liquor concentration, a straight-line relationship exists. The regression line shown was determined from the probit and logarithm values by using the least squares method. The 95 per cent confidence limits for the means of the mortalities at particular concentrations estimated from the regression line were also calculated.

At concentrations of 2000 parts per million the Chinook salmon exhibited a mean avoidance of 81.38 per cent, the 95 per cent confidence limits being 57.18 per cent and 95.36 per cent. A mean avoidance of 65.81 per cent with 95 per cent confidence limits of 35.74 per cent and 87.68 per cent was shown at 1000 parts per million of sulfite waste liquor. At the concentration of 500 parts per million the mean avoidance was 44.51 per cent with 95 per cent confidence limits of 20.47 per cent and 70.87 per cent. A mean avoidance of 25.07 per cent with 95 per cent confidence limits of 7.09 per cent and 54.99 per cent

was demonstrated at 250 parts per million. At the lowest concentration tested, 125 parts per million, the Chinook salmon exhibited a mean avoidance of only 11.38 per cent, the 95 per cent confidence limits being 2.52 per cent and 32.38 per cent.

Concentrations of 125 parts per million of sulfite waste liquors containing 10 per cent solids are not unusual in water receiving this waste in the Pacific Northwest. In general, however, concentrations as high as 2000 parts per million would result in severe oxygen depletion. Coho salmon showed avoidance of sulfite waste liquor only at 2000 parts per million (table 2). In two experiments at this concentration, a mean avoidance of 14.02 per cent was demonstrated. At 1000 parts per million, the fish showed no avoidance. Here, again, the rather marked difference in the responses of these two species to paper mill wastes is demonstrated.

Toxicity

Investigators of the Washington State Department of Fisheries (6, p.104) found that the "threshold index" (estimate of approximately a 5 per cent kill) was dependent not only on the species of juvenile salmon, but also on the age. Juvenile Chinook salmon in long-term experiments were found to have threshold indices of 1,175 parts per

million when 38 days old, 600 parts per million when 280 days old, and 560 parts per million when 305 days old. Juvenile Coho salmon had threshold indices of 1,230 parts per million when 351 days old and 1,015 parts per million when 412 days old (6, p.104). Sulfite waste liquor appears to be more toxic to Chinook salmon than to Coho salmon. This is of interest since Coho salmon show far less avoidance of the waste than do the Chinook salmon. While Chinook salmon showed some avoidance of sulfite waste liquor at concentrations as low as 125 parts per million, Coho salmon exhibited no avoidance at a concentration of 1000 parts per million (tables 10 and 11).

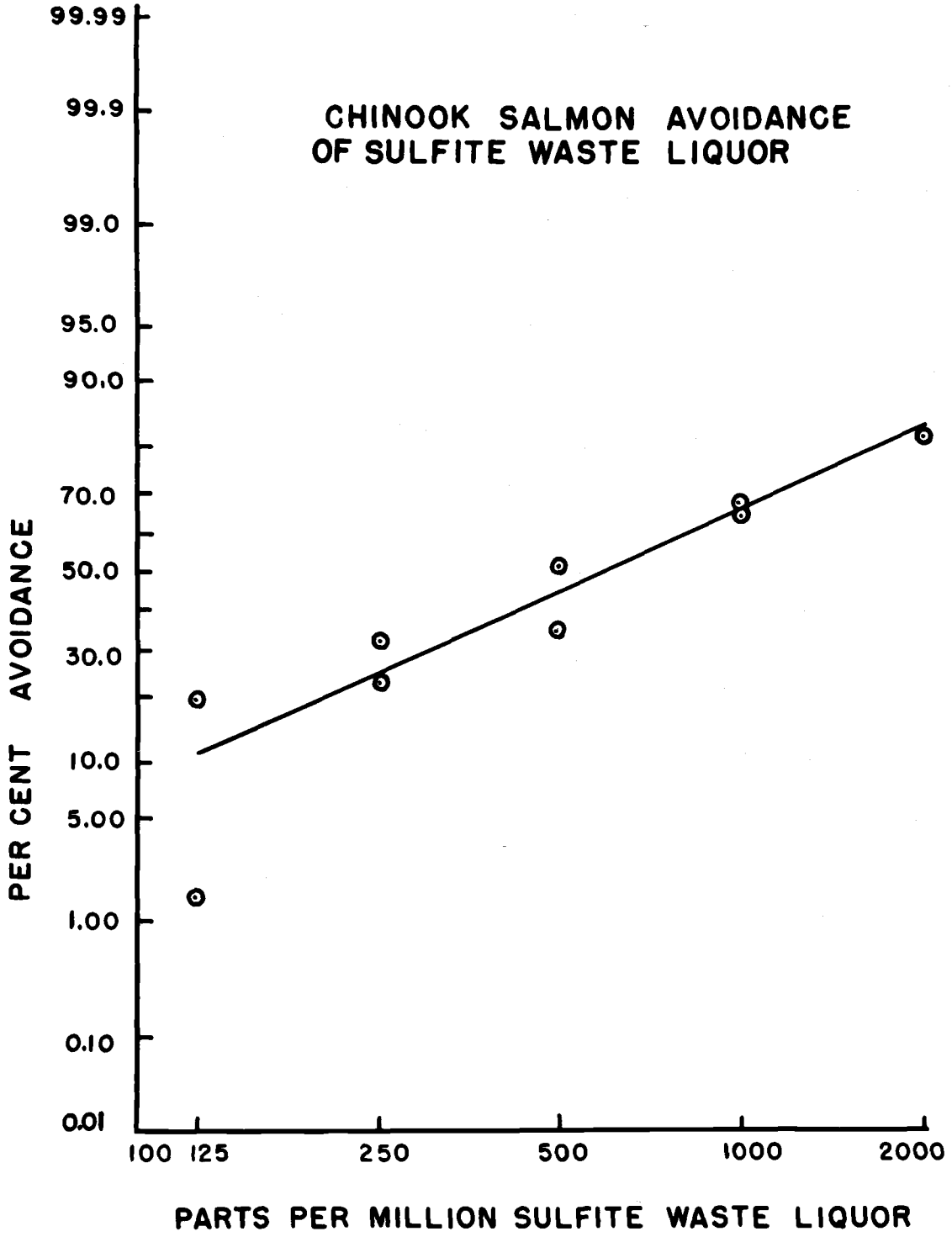

CHINOOK SALMON AVOIDANCE
OF SULFITE WASTE LIQUOR
99.99
99.9
99.0
95.0
90.0
70.0
50.0
30.0
10.0
5.00
1.00
0.10
0.01
PER CENT AVOIDANCE
100
125
250
500
1000
2000
PARTS PER MILLION SULFITE WASTE LIQUOR

Figure 3.

TABLE 10

AVOIDANCE OF SULFITE WASTE LIQUOR BY CHINOOK SALMON

Concentration of waste: p.p.m.	Trial number	Date	Number of entries				Per Cent avoidance	Estimated mean per cent avoidance	95% confidence limits of mean
			Waste	Control	Waste	Control			
2000	1	9/23	27	175	70	346	81.38	82.41	57.18%; 95.36%
1000	1	10/16	102	345	169	427	65.15		
	2	11/6	40	217	108	229	66.81	65.41	35.74%; 87.68%
500	1	10/23	131	250	175	392	52.34		
	2	10/9	74	156	82	83	34.73	44.51	20.47%; 70.87%
250	1	10/23	226	285	207	278	23.09		
	2	11/13	106	178	97	127	33.44	25.07	7.09%; 54.99%
125	1	10/23	189	219	211	281	20.00		
	2	11/13	244	316	212	162	4.60	11.38	2.52%; 32.28%

TABLE 11

AVOIDANCE OF SULFITE WASTE LIQUOR BY COHO SALMON

Concentration of waste: p.p.m.	Trial number	Date	Number of Entries				Per cent avoidance	Mean per cent avoidance
			Waste	Control	Waste	Control		
2000	1	9/21	151	332	248	145	16.35	
	2	9/22	280	320	336	224	11.69	14.02
1000	1	10/9	148	194	218	165	0.00	

VI. SUMMARY

This paper covers studies made on the avoidance reactions of Chinook salmon, Oncorhynchus tshawytscha (Walbaum), and Coho salmon, Oncorhynchus kisutch (Walbaum), to paper mill effluents. Preliminary work was begun on this problem in 1953 by Assistant Professor Carl E. Bond at the Oregon State College Fisheries Research Laboratory. His studies showed that certain salmonid fishes exhibited a preference for water having lower waste concentrations in a concentration gradient of paper mill effluents. As a result of these findings a study was begun in 1954 to ascertain what concentrations of paper mill effluents would be avoided by Chinook salmon and Coho salmon.

A rectangular trough, nine feet long, two feet wide, and one foot deep, was constructed. For one-third of the total length, the trough was divided into four channels. Waste liquors of desired concentrations and clean water were introduced into alternate channels. Fish were placed into the trough and their movements during a set period of time were observed. The random movements of the fish during a sixty-minute observation period were usually sufficient to allow for the recording of several hundred entries into the channels. Avoidance of the polluted channels was calculated from the formula $100(E - A)/E$ where E is the total number of entries of fish into clean

channels and A is the total number of entries into polluted channels.

Effluents used in this study were sulfite waste liquor, whole sulfate waste liquor, and synthetic sulfate waste liquor. Since the whole sulfate liquor varied in concentration from day to day, it was thought that a standard synthetic waste would make possible the more accurate evaluation of the results of the avoidance experiments. The synthetic waste was made from the constituents of the whole effluent in the ratio of 90 millimeters of combined condensates, 2 milliliters of black liquor, and 908 milliliters of water.

Waste concentrations of 10 per cent, 5 per cent, 2.5 per cent, and 1.25 per cent by volume were used in the sulfate waste experiments. Using synthetic sulfate waste, it was found that Chinook salmon would exhibit an avoiding reaction to concentrations above 1.25 per cent. At the 2.5 per cent concentration, a mean avoidance of 28.45 per cent was exhibited. A maximum avoidance of 93.69 per cent and a mean avoidance of 86.89 per cent were shown at the 10 per cent concentration.

Using Coho salmon and synthetic sulfate waste, only 11.34 per cent avoidance was noted at the 10 per cent concentration. No avoidance was exhibited at the 5 per cent concentration.

Experiments with Chinook salmon and whole sulfate waste liquor showed that less avoidance was exhibited to the whole mill effluent than to the synthetic waste. At 10 per cent concentrations of whole waste a mean avoidance of 37.66 per cent and a maximum of 49.29 per cent were exhibited. A mean of only 12.84 per cent avoidance was shown at the 5 per cent level.

Coho salmon showed no avoiding reactions to the whole sulfate liquor.

When sulfite waste liquor was used, avoiding reactions were exhibited by Chinook salmon at all concentrations tested over the range of 125 to 2000 parts per million. At the lowest concentration tested, 125 parts per million, the Chinook salmon showed a mean avoidance of 11.38 per cent. At the highest concentration, 2000 parts per million, 81.38 per cent avoidance was exhibited.

A mean avoidance of 14.02 per cent was demonstrated by Coho salmon at 2000 parts per million of sulfite waste liquor. No avoiding reactions were exhibited to a concentration of 1000 parts per million.

It appears that Chinook salmon are more sensitive to pulp mill wastes in their environment than are Coho salmon. Over the range of concentrations tested, Coho salmon exhibited considerably less avoidance to the wastes in all the experiments with the three wastes.

VII. BIBLIOGRAPHY

1. Alderdice, D. F., et al. Further observations on olfactory perception in migratory adult Coho and Spring salmon - properties of the repellant in mammalian skin. Fisheries research board of Canada. Progress reports of the Pacific Coast stations 98:10-12. 1954.

2. Brett, J. R. and Dixon MacKinnon. Some observations on the olfactory perception in migratory adult Coho and Spring salmon. Fisheries research board of Canada. Progress reports of the Pacific Coast stations 90:21-22. 1952.

3. Chidester, F. E. Studies on fish migration, II. The influence of salinity on the dispersal of fishes. American naturalist 56:373-380. 1922.

4. Chidester, F. E. A critical examination of the evidence for physical and chemical influences on fish migration. British journal of experimental biology 2:79-118. 1924.

5. Collins, Gerald B. Factors influencing the orientation of migrating anadromous fishes. Fishery bulletin of the U. S. fish and wildlife service 52:375-396. 1952.

6. Creaser, C. W. Relative importance of hydrogen-ion concentration, temperature, dissolved oxygen, and carbon dioxide tension, on habitat selection by brook trout. Ecology 11:246-262. 1930.

7. Hasler, Arthur D. and Warren J. Wisby. Use of fish for the olfactory assay of pollutants (phenols) in water. Transactions of the American fisheries society 79:64-70. 1949.

8. Haydu, E. P., H. R. Amberg, and R. E. Dimick. The effect of Kraft mill waste components on certain salmonoid fishes of the Pacific Northwest. Tappi 35:545-549. 1952.

9. Holland, G. A., ed. Toxic effects of sulfite waste liquor on young salmon. Olympia, 1953. 111p. (Washington. Department of fisheries. Research bulletin no. 1)

10. Jones, J. R. Erichsen. A further study of the reactions of fish to toxic solutions. Journal of experimental biology 25:22-34. 1948.

11. Jones, J. R. Erichsen. The reactions of the minnow, Phoxinus phoxinus Linnaeus, to solutions of phenol, ortho-cresol, and para-cresol. Journal of experimental biology 28:261-270. 1951.

12. Jones, J. R. Erichsen. The reactions of fish to water of low oxygen concentration. Journal of experimental biology 29:403-415. 1952.

13. MacKinnon, Dixon and William S. Hoar. Responses of Coho and Chum fry to current. Journal of the fisheries research board of Canada 10:523-528. 1952.

14. Shelford, V. E. and E. B. Powers. An experimental study of the movements of herring and other marine fishes. Biological bulletin 28:315-334. 1915.

15. Shelford, V. E. An experimental study of the effects of gas waste upon fishes, with special reference to stream pollution. Bulletin of the Illinois state laboratory of natural history 11:381-412. 1917.

16. Smith, Wendell E. and Robert W. Saalfeld. Studies on Columbia River smelt, Thaleichthys pacificus (Richardson). Washington. Department of fisheries. Fisheries research papers 1:3-26. 1955.

17. Ward, H. B. Some of the factors controlling the migration and spawning of the Alaska red salmon. Ecology 2:235-254. 1921.

18. Wells, M. M. The reactions and resistance of fishes to temperature. Illinois academy of science 7:48-59. 1914.