AN ABSTRACT OF THE THESIS OF

I	ERICK MICHA	EL TOKAR	for the	MASTER OF	SCIENCE
	(Name	:)		(Deg	ree)
in	FISHERIES (Major)	pre	esented on	Draw 7 (Date) /2 %
Title:	SOME CHRC	NIC EFFECT	SOFEXP	OSURE TO KE	AFT MILL
	EFFLUENT	ON JUVENIL			
Abstra	act approved:	Redact	ed for	privacy	
110 5 11 1	iet approved.	1170	George G.	Chadwick	

The effects of sublethal concentrations of kraft mill effluents (KME) on the growth, food consumption, and swimming ability of juvenile chinook salmon, <u>Oncorhynchus tschawytscha</u> (Walbaum), were studied from February 1966 to May, 1967.

The KME used in these studies was obtained from two pulp and paper mills producing paper from unbleached pulp. Samples were collected weekly during the experimental periods from settling lagoons of mill A, and waste outfalls of mill B. Acute toxicity bioassays of these wastes were performed periodically, and 96-hour median tolerance limits (TL_m) were estimated for the salmon and for guppies (<u>Poecilia reticulata</u> Peters). Water and wastes were introduced into 16 experimental chambers by a system of head boxes and siphons. In each experiment, growth rates of salmon continuously exposed to three waste concentrations and of controls were evaluated at four different feeding levels. The waste concentrations were adjusted so as to provide for nearly constant increments of biochemical oxygen demand (BOD). Tubificid worms were the food organism in all experiments. The feeding levels employed ranged from an unrestricted ration, through several restricted rations, to starvation.

The growth rates of salmon were found to be reduced at all concentrations of KME from mill A (0.5 to 4.0 mg/liter BOD) when the ration was unrestricted, and were reduced at all concentrations with BOD increments greater than 0.5 mg/liter when the next lower ration was fed. Some death occurred within 16 days at all feeding levels at concentrations having a BOD increment of 4.0 mg/liter. At low feeding levels, no consistent differences in growth attributable to KME were observed. Dilutions of waste from mill B had only small effects on the growth of chinook salmon.

Salmon held in clean water and fed worms which had been exposed to KME from mill A showed only slight decreases in growth rate, except when they were fed worms exposed to 100-percent effluent which reduced the efficiency of food utilization considerably. When salmon were exposed to two concentrations of KME from mill A and then tested for swimming ability, no differences of maximum sustained swimming speeds attributable to the wastes were found.

Except at the 4.0 mg/liter BOD level of waste from mill A,

no reduction in the appetite of salmon exposed to KME was found in the growth experiments. Any reductions in the growth rates observed can be attributed to KME-caused decreases of the efficiency of food utilization for growth. The higher metabolic rates of the fish kept on the high ration presumably were responsible for the increased effects of the KME on these fish.

Some Chronic Effects of Exposure to Kraft Mill Effluent on Juvenile Chinook Salmon

by

Erick Michael Tokar

A THESIS

submitted to

Oregon State University

in partial fulfillment of the requirements for the degree of

Master of Science

June 1968

APPROVED:

Redacted for privacy

Assistant Professor of Fisheries in charge of major

Redacted for privacy

Head of Department of Fisheries and Wildlife

Redacted for privacy

Dean of Graduate School

Date thesis is presented ______ Typed by Donna Olson for _____ Erick Michael Tokar

ACKNOWLEDGMENTS

I gratefully acknowledge the guidance and assistance of Mr. George G. Chadwick, Assistant Professor of Fisheries, in all phases of this research and in the preparation of this thesis. Throughout the conduct of this study the advice of Dr. Peter Doudoroff, Professor of Fisheries, was gratefully appreciated, and his critical review of this thesis was invaluable.

Appreciation is extended to Mr. Russell O. Blosser and Mr. Eben L. Owens of the National Council for Stream Improvement for providing technical information on the wastes used in the growth experiments. Mr. Owens contributed substantial help in the conduct of some of the growth experiments and acute toxicity bioassays.

I am also indebted to my fellow graduate students for the help freely given throughout these studies.

This investigation was financed by the Northwest Pulp and Paper Association and the National Council for Stream Improvement and by the Office of Water Resources, Research Project No. B-004-ORE.

TABLE OF CONTENTS

Page

INTRODUCTION	1
EXPERIMENTAL MATERIALS, METHODS, AND PROCEDURES	6
Experimental Materials	6
Experimental Apparatus	9
Experimental Procedures	14
Growth Experiments	14
Swimming Performance Tests	20
Acute Toxicity Bioassays	21
RESULTS	23
DISCUSSION	38
BIBLIOGRAPHY	44
APPENDIX	46

LIST OF TABLES

Table		Page
1.	Characteristics of the wastes used in the growth experiments.	8
2.	Dates and experimental conditions for the growth experiments.	18
3.	Waste concentrations used in the growth experiments.	19
4.	Results of acute toxicity bioassays conducted during growth experiments.	25
5.	Growth and food consumption data for growth experiment A3.	34
6.	Results of feeding KME-exposed worms to chinook salmon.	35
7.	Results of the sustained swimming performance tests.	36
8.	Percent of the 96-hour TL _m for each nominal BOD increment in every growth experiment.	41

LIST OF FIGURES

Figure		Page
1.	Photograph of the experimental apparatus.	10
2.	Diagram of the experimental apparatus.	12
3.	The relationship of normalized growth rates of chinook salmon kept on unrestricted rations to the BOD increments to which they were exposed.	28
4.	The relationship of normalized growth rates of chinook salmon kept on high restricted rations to the BOD increments to which they were exposed.	29
5.	The relationship of normalized food consump- tion rates of chinook salmon kept on unrestricted rations to the BOD increments to which they were exposed.	31
6.	The relationship of normalized food conversion ratios to BOD increments.	32
7.	The relationship of normalized growth rates of chinook salmon kept on unrestricted rations to KME concentrations expressed as percentages of the 96-hour median tolerance limit (TL _m).	43

SOME CHRONIC EFFECTS OF EXPOSURE TO KRAFT MILL EFFLUENT ON JUVENILE CHINOOK SALMON

INTRODUCTION

The Pacific Northwest is blessed with abundant timber and water resources, making this area well suited for paper manufacturing. Consequently, the kraft pulping industry has become a major economic asset to this region. With the gradual expansion of the industry, however, water pollution problems have increased, and despite the use of some modern waste treatment and control facilities, the large quantity of water used in the paper manufacturing processes is not always returned to the receiving water in a condition acceptable for other uses.

Undiluted and untreated kraft mill effluents (KME) are usually acutely toxic to fish and many other aquatic organisms, and even after dilution in receiving streams, the wastes can cause fish mortalities attributable to toxicity. These wastes can also cause a drastic reduction in the dissolved oxygen content of the receiving water and promote a blanketing growth of bacteria and other organisms on stream bottoms.

Concentrations of KME that are too low to produce the above conditions may, nevertheless, have deleterious effects on the production of fish and change the structure of aquatic communities. Little information is available concerning the long-term effects of persistent low concentrations of KME in the aquatic environment.

In the kraft (sulfate) process of pulp and paper manufacture, wood chips are digested with an alkaline liquor (sodium hydroxide and sodium sulfide). After the chips have been digested, the spent digesting liquor (black liquor) is sent to a recovery plant, where it is evaporated to approximately 35-percent solids before being burned, and the chemicals reclaimed from the ash are reused. The wood fibers from the digesters are subjected to a series of washings with water. Wash waters having a sufficiently high black liquor concentration are sent to the recovery plant. The final wash water, however, is generally too dilute for economical recovery of the chemicals, and this water becomes a portion of the mill waste.

Condensed relief vapors released from the digesters during the cooking cycle, and condensed vapors (evaporator condensates) from the chemical recovery plant also may become a portion of the mill waste. The dilute wash waters and the combined condensates are the major toxic components of the total effluent of the typical pulp mill manufacturing unbleached kraft pulp. The processes of manufacturing bleached and unbleached kraft pulp are similar, but because of the bleaching process, some of the major toxic components of the wastes from these mills may not be the same.

Because of diverse mill designs and methods of operation, the

variety of woods used and different waste disposal methods employed, the toxicity of the wastes and the proportions of the different toxic components of the wastes vary considerably from mill to mill. These differences in part account for the lack of agreement of the results of some of the acute toxicity studies that have been reported (Ebling, 1931; Bergstrom and Vallin, 1937; Cole, 1935; McHugh, 1954; Webb, 1958).

Relatively few studies have been undertaken to determine the effects of KME on the growth of fishes. Nightingale and Loosanoff (1930) reported that chinook salmon, <u>Onchorhynchus tshawytscha</u> (Walbaum), held for 14 days in a concentration of black liquor of 1/10,000 lost weight (9.8 percent), while control fish gained weight (5.1 percent). At a concentration of 1/15,000, no differences in growth were found.

Considerable research was conducted by the Washington State Department of Fisheries (1960) on the effects of bleached and unbleached KME on several species of Pacific salmon. It was found that after 30 days of exposure to lagooned KME (bleached) at dilutions as strong as 1:16.5, no deaths of chinook salmon occurred, but the growth of the salmon was significantly reduced in dilutions as weak as 1:169.

Servizi, Stone and Gordon (1966) studied the effects of neutralized bleached kraft pulp mill wastes on the growth and development

of sockeye salmon, <u>Onchorhynchus nerka</u> (Walbaum), and pink salmon, <u>Onchorhynchus gorbuscha</u> (Walbaum). They found that the growth of sockeye salmon alevins was reduced at concentrations as low as one percent, and that of pink salmon alevins was reduced at concentrations as low as two percent.

Fujiya (1961) investigated the physiological changes in the vital organs of a species of marine fish, <u>Sparus macrocephalus</u>, exposed to sublethal levels of KME. He undertook extensive histological examinations of the internal organs of these fish exposed to the waste for 12 and 24 hours in a bay near the point of KME discharge. His findings indicate that in a waste-water mixture having a chemical oxygen demand (COD) of more than 50 parts per million (ppm), there were harmful effects on protein synthesis, glycogen metabolism, and hepatic circulation. He suggested that the waste may impair the reproductive function and growth.

The study described here is a part of a continuing investigation of the effects of kraft pulping wastes on the production of fish and the structure of aquatic communities. The objective of this segment of the study was to determine the influence on the growth of juvenile chinook salmon of concentrations of KME that are neither acutely toxic nor seriously oxygen depleting.

This research was done during the winter, spring, and summer of 1966 and 1967 at the Oak Creek Laboratory of the Pacific

Cooperative Water Pollution Laboratories, located about five miles northwest of Corvallis, Oregon.

EXPERIMENTAL MATERIALS, METHODS, AND PROCEDURES

Experimental Materials

The chinook salmon, <u>Onchorhynchus tshawytscha</u> (Walbaum), was selected for use in these experiments because of its economic importance and its presence in many Oregon waters receiving pulping wastes.

Salmon used in experiments A1, A2, and A3 were obtained from the Oregon State Game Commission's Research Laboratory at Corvallis and averaged 44, 46, and 73 millimeters (mm) in total length, respectively. Fish used in experiments A4 and A5 were from the Oregon State Fish Commission's hatchery at Oakridge, and averaged 42 mm in total length in both experiments. The Eagle Creek National Fish Hatchery located near Estacada, Oregon, supplied the salmon used in experiments B1 and B2, and these fish averaged 74 and 89 mm in total length, respectively.

Before being used in the growth experiments, all fish were acclimated to the experimental water, temperature, and diet at the Oak Creek Laboratory for at least one week. Here they were held in a 50-gallon glass aquarium in constantly exchanged and aerated water.

Salmon in the holding tank and in the experimental chambers were fed tubificid worms (Tubifex sp.). These worms were

collected periodically from gravel-bottomed raceways at the Roaring River Trout Hatchery of the Oregon State Game Commission, and were held until needed at the laboratory in wooden troughs supplied with constantly flowing stream water.

The kraft pulping waste used in the growth experiments was collected each week from two western Oregon pulp and paper mills (hereafter referred to as mill A and mill B) manufacturing and processing unbleached kraft pulp. Waste from mill A was pumped from the settling lagoons into 55-gallon drums lined with polyethylene bags, hauled to the laboratory, and there stored in a large wooden tank.

Wastes from mill B were collected from the various points of discharge and pumped into separate transporting drums. At the laboratory they were pumped into the storage tank in proportions by volume approximating those characteristic of the total mill effluent. Table 1 summarizes the analyses of the effluents used in the growth experiments.

In experiment A3, a portion of the waste from mill A was biologically treated (stabilized) to reduce toxicity and biochemical oxygen demand (BOD) before being used. Stabilization was accomplished by using a bacterial floc suspended by aeration and supported by the minimal amount of nitrogen and phosphorus necessary to effect an 80 to 85 percent BOD reduction. The nitrogen and phosphorus source

Experiment	BOD	COD	Resin acid soaps	Total dissolved solids	Volitile solids
Al	329	844	39	752	204
A2	246	745	11	742	248
A 3	220	610	23	690	257
A4	208	570	1/	637	230
A 5	224	710	1/	725	261
Bl	122	275	8	542	115
В2	192	410	7	465	79

Table 1. Characteristics of the effluents used in the growth experiments. All values are in milligrams per liter and are means for the various batches of waste used in each experiment.

 $\frac{1}{1}$ Resin acid soap determinations were not made in experiments A4 and A5.

was dibasic sodium ammonium phosphate (NaNH₄HPO₄·4H₂O) which was added to the waste after the waste had been seeded with raw sewage to establish the bacterial floc.

Experimental Apparatus

The experimental apparatus (Figure 1) was constructed so that waste and water from head boxes could be mixed in the desired proportions and siphoned continuously into a 4-compartment distribution box and thence into 16 experimental chambers containing fish. The sixteen chambers made it possible to test the differences in growth rates of salmon at four waste concentrations and at four levels of feeding at each concentration.

A waste head box rested on a plywood platform suspended from the ceiling. This was an $8" \times 8" \times 6"$ acrylic plastic (Plexiglas) box having a partial cover and a standpipe drain. A constant head was maintained by allowing a small excess of inflowing waste to overflow into the drain. Waste entered the system by gravity flow from the storage tank through a 3/4" black polyethylene pipeline. A screening device at the head box removed any particulate matter. The flow of mill waste into the head box was adjusted with a glass stopcock and adjustable siphons were used to regulate the flow of KME to the distribution box.

Filtered water from a small spring-fed stream entered the

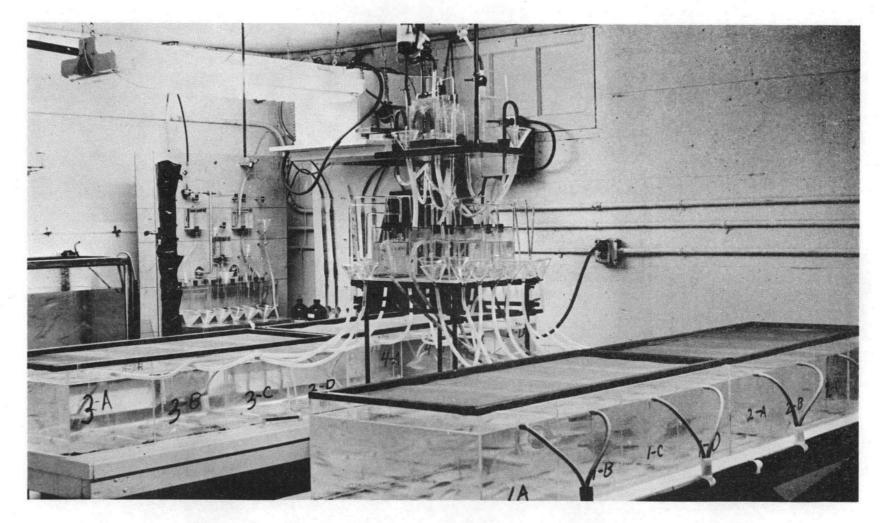


Figure 1. Photograph of the experimental apparatus.

system from a wooden head box equipped with an aerator and a thermostatically-controlled heater. The flow of water from this head box to the apparatus was measured by glass flowmeters and adjusted with stopcocks. This water flowed into funnels attached to the waste head box platform, and mixed there with the waste dripping from the siphon tubes (Figure 2). All water supply tubing was covered as much as possible with black plastic to prevent the growth of algae.

The distribution box was a 12" x 12" x 6" acrylic plastic box divided into four compartments. A stream of water or diluted waste from a mixing funnel entered each of these compartments. Constant head was maintained by allowing a small amount of the waste and water mixture from each compartment to overflow into a drain. The distribution box was covered with a 1/16" stainless steel plate which supported 16 glass siphon tubes. Four siphons were used to distribute and regulate the flows of diluted waste or control water from each compartment to four of the 16 experimental chambers.

Two banks of eight 10" x 18" x 9" acrylic plastic experimental chambers were arranged below and on opposite sides of the distribution box. Each chamber was fitted with an inflow and outflow orifice and with plastic screen covers to prevent the fish from jumping out.

Lights over the experimental apparatus were left on 24 hours

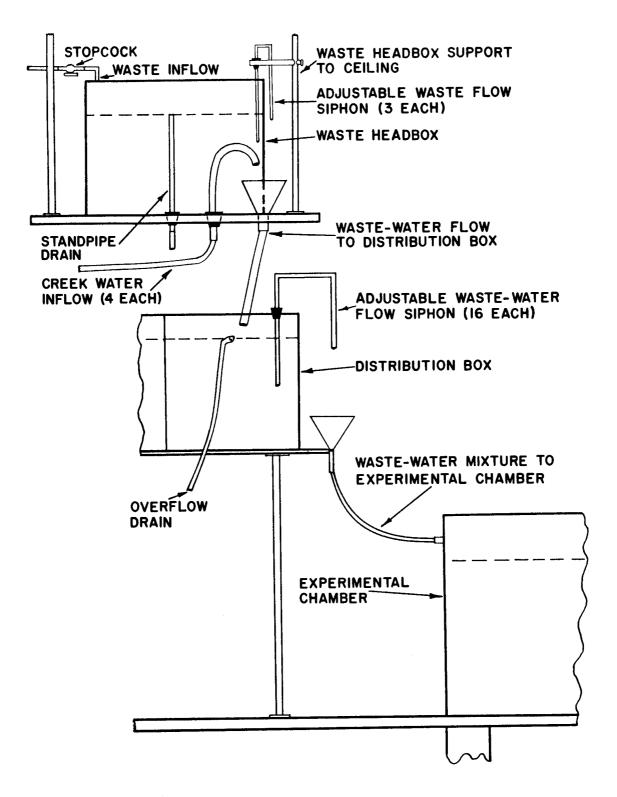


Figure 2. Diagram of the experimental apparatus.

a day during all experiments except experiment A5. In this experiment, there were several modifications of the apparatus: the lights were regulated with a timer to correspond to the daily outdoor illumination, the experimental chambers were partially covered with black plastic sheeting which allowed the fish some concealment, and the water temperature was allowed to vary between 8° and 9°C with the stream water temperature. This was done so that this growth experiment would complement certain laboratory stream studies which were proceeding concurrently.

The temperature of the room in which the apparatus was situated was controlled to within $0.5^{\circ}C$ of the desired temperature. In all experiments with the exception of A5, room and water temperatures were the same. These were $10^{\circ}C$ in the winter and spring (A1, A2, and A4) and $15^{\circ}C$ in the summer (A3, B1, and B2). During experiment A5, the room was maintained at $10^{\circ}C$, but the water temperature was allowed to fluctuate.

One special experiment was designed to test the effects on the growth of salmon held in clean water of feeding them worms which had been exposed to KME. Eight chambers, $8" \times 9" \times 10"$, were constructed of acrylic plastic for this experiment. A standpipe drain was used to regulate the depth of water in each, and stream water flowed through each chamber at a rate of 300 milliliters per minute. Worms were exposed to KME in plastic compartments which

were situated below the distribution box used for distributing diluted waste in the other growth experiments. The desired concentration of KME for each compartment was taken from the overflow drain of the appropriate chamber of the distribution box, or from the waste head box.

Maximum sustained swimming speed tests were made with an apparatus which has been described fully by Davis (1963) and Dahlberg (1963). The test chamber was a horizontal glass tube 60" long and 4" in diameter. Water could be circulated through this tube by means of a centrifugal pump at any desired velocity from 0.0 to 2.7 feet per second.

Experimental Procedures

Growth Experiments

Before each experiment, the fish to be used were sorted into 18 or 19 groups usually of ten fish each. In experiment B2, however, the fish were larger, and each group consisted of only five fish. Salmon nearly uniform in size were selected for each experiment; the differences in wet weight between groups were no more than 0.5 grams within any one experiment. Sixteen of the groups were then distributed among the test chambers. The remaining groups were killed in tricaine methanesulfonate (MS-222), and the wet weights and lengths of individual fish were taken. These initial individual fish samples were placed in a drying oven at 80[°]C for five days before dry weights were determined. The ten fish in each of the groups used in experiments A1 and A2 were combined, and the caloric content of a sample from each group was obtained using an oxygen bomb semimicro calorimeter.

The four feeding levels employed in experiments A1, A2, A3, and B1 were as follows: 1) Unrestricted ration. Worms were available at all times to the fish kept on this ration, and fresh worms were added each day. Periodically, any accumulation of uneaten worms was removed from the chambers. 2) High restricted ration. Here, fish held at the highest concentration of waste were fed to repletion once each day, the amount of food consumed was determined, and the fish at the other concentrations received the same amount. 3) Low restricted ration. This ration was selected to approximate the maintainance requirements of the fish. In experiments Al through A4, the low restricted ration was 20-percent of the high restricted ration. Later in the year, when the fish were larger, this ration was increased to 25-percent (A5) or 30-percent (B1 and B2) of the high restricted ration. 4) Starvation. No food was available to the starved fish.

In experiments A4, A5, and B2, the starvation level was not used, and a ration between the high and low restricted rations was substituted. This was called the <u>medium restricted ration</u>, and defined as one-half the high restricted ration.

Tubificid worms that were to be fed to the fish were blotted on an absorbent paper towel for about one minute before being weighed. Weights of the worms were recorded to the nearest 0.01 gram. To insure equal opportunity for all salmon to receive food, care was taken to distribute worms evenly throughout each test chamber.

The unrestricted and high restricted rations were fed daily in all experiments. At the lower rations, the fish could not be fed daily, as there would not have been enough worms to insure adequate distribution among the individual fish. For this reason, the lower restricted rations were computed and fed every second or third day.

Once each day, the flows of waste and water to the distribution box were measured and adjusted as necessary, and any dead fish were removed from the test chambers, weighed, and measured. Table 2 shows when each experiment was performed and the experimental conditions of each.

The BOD levels called for in the design of each experiment will be termed the "nominal BOD increments". These levels were decided upon before each experiment. The BOD value of each batch of waste was estimated from COD determinations made at the time of waste collection and rates of flow of waste from the waste head box to the distribution box were adjusted on the basis of these estimated BOD values to produce the desired nominal increments. Upon completion of an experiment, the "actual mean BOD increment" of each test dilution was computed from the daily flow measurements and the actual determined BOD values of samples of the whole waste used in the experiment. The low natural BOD of the dilution water was not measured or taken into consideration in making these calculations. Therefore, the "actual mean BOD increment" of waste dilution represents the increment of BOD due to the addition of waste and is slightly less than the true or total BOD value of the dilution. As the actual mean BOD increments did not vary by more than 0.2 mg/liter from the nominal BOD increments (Table 3), the nominal increments will be used throughout this thesis.

In the afternoon of the final day of feeding, all food was removed from the chambers, and the fish were removed for weighing about 24 hours later. This allowed one day for the fish to empty their intestinal tracts. The fish were then killed in MS-222 and their lengths and wet and dry weights obtained. Samples of the fish from experiments Al and A2 only were analyzed for caloric content.

Concurrently with experiment A5, the experiment was performed to test the effects on salmon of feeding them worms held in KME. Four groups of ten fish were held in clean water at each of the two feeding levels, namely, the unrestricted and the high restricted rations. One group at each feeding level was fed worms

Experiment Number	Dates	Waste source	Temperature ([°] C)	Flow rate (ml/min/chamber)	Exchange time (hours/chamber)	Fish per chamber	Days Fed
Al	February 18 - March 6, 1966	Mill A	10	200	2.0	10	16
A2	March 21 – April 7, 1966	Mill A	10	200	2.0	10	16
A3	July 7 - July 25, 1966	Mill A	15	150	2,7	10	16
A4	March 2 – March 17, 1967	Mill A	10	200	2.0	10	15
A 5	April 19 – May 3, 1967	Mill A	8-9	200	2.0	10	14
B1	August 18– September 1, 1966	Mill B	15	150	2.7	10	13
B2	October 11 - October 25, 1966	Mill B	15	150	2.7	5	13

Table 2. Dates and experimental conditions for the growth experiments.

Table 3. Waste concentrations used in the growth experiments. The nominal BOD increments are those desired, and the actual mean BOD increments are values computed from the daily flow measurements and BOD determinations.

Nominal BOD increment (mg/1)	Actual mean BOD increment (mg/1)	Mean percent by volume
1.0	1.0	0.30
2.0	1.8	0.56
4.0	3.9	1.19
0.5	0.4	0.15
1.0	0.9	0.33
2.0	1,8	0.66
3.0, /	3.0	1.36
	2.8	4.67
<u>1</u> /	0.7	1.19
1.0	1.0	0.48
	2.1	1.00
4.0	4.1	2.00
0.5	0.5	0.55
	2.1	0.94
3.0	3.2	1.43
	increment (mg/1) 1.0 2.0 4.0 0.5 1.0 2.0 $3.0\frac{1}{3.0\frac{1}{2.0}}$ 1.0 2.0 4.0 0.5 2.0 4.0 0.5 2.0 4.0	increment (mg/1)increment (mg/1)1.01.02.01.84.03.90.50.41.00.92.01.8 $3.0\frac{1}{2.0}$ 3.0 $3.0\frac{1}{2.8}$ $$ 0.71.01.02.02.14.04.10.50.52.02.1

 $\frac{l}{Biologically}$ stabilized effluent.

that had been held in clean water, and these fish served as controls. The remaining groups were fed tubificid worms that had been exposed to KME. Three different worm exposure levels were tested at each feeding level: 2.0 mg/liter BOD (nominal BOD increment) for 24 hours, 2.0 mg/liter BOD for 96 hours, and 224 mg/liter BOD (100 percent effluent) for 24 hours. The 2.0 mg/liter BOD waste dilution for exposing the worms came from the distribution box, and the 100 percent effluent was supplied from the overflow of the waste head box.

The relationship of dry weight to wet weight of worms held at each exposure level was obtained, and this ratio was used in computing the dry weights of the food consumed during the experiment.

Salmon used in this experiment were from the same source as those used in experiment A5. Water temperatures and lighting conditions and all weighing procedures were also the same in the two experiments.

Swimming Performance Tests

Salmon obtained from the same source as those used in experiment A2 were tested for maximum sustained swimming speed after being exposed for 12 days to waste from mill A. Two groups of fish were held in the growth apparatus at exposure levels of 1.0 mg/liter nominal BOD increment (six fish) and 2.5 mg/liter nominal BOD increment (five fish), and a third group (six fish used as controls) was held in clean water.

These salmon averaged 91 mm in total length. They were not fed for 24 hours before being tested, and a period of one hour was allowed for the fish to become accustomed to the swimming chamber; during this acclimation period a water velocity of 0.50 feet per second was maintained. The velocity was then progressively increased by increments of 0.15 feet per second at ten minute intervals until the fish were no longer able to swim against the current. As each fish failed, it was removed from the chamber and its total length in mm was recorded. The water velocity at the time of failure of the fish was recorded as its final swimming speed.

Acute Toxicity Bioassays

Periodically during the experiments, the acute toxicity of the KME samples was determined by bioassay. The methods employed were those of Doudoroff, <u>et al</u>. (1951) with minor modifications. Chinook salmon and young guppies, <u>Poecilia reticulata</u> Peters, were used in these bioassays.

The containers used in the salmon bioassays were cardboard ice-cream cartons lined with disposable polyethylene bags. Four to six cartons were each filled with six liters of the diluted waste. Five salmon from the same source from which those used in the concurrent growth experiment were obtained were then placed in each container. Oxygen was supplied by bubbling the pure gas through each container slowly enough to avoid raising the dissolved oxygen concentration much above the air-saturation level. Each salmon bioassay was performed at the temperature at which the concurrent growth experiment was performed.

Acute toxicity bioassays using guppies were all done at 20°C. Ten guppies, three to ten days old, were placed in each of several one-liter glass beakers containing one liter of the test solution (five inches deep), which was not artificially oxygenated.

RESULTS

In presenting the results of the growth experiments, all growth and feeding data will be reported in terms of the dry weights of salmon and food. Initial dry weights of the experimental fish were computed by multiplying the initial wet weight of each of the 16 groups of fish by the ratio of dry weight to wet weight obtained for groups of fish used as initial samples. Dry weights of the worms fed to the salmon during the experiments were computed in the same manner, the worms having been sampled at the beginning of each experiment.

A growth rate was computed by dividing the dry weight gained per fish in milligrams by the arithmetic mean of the initial and final dry weights of the fish in grams, and then dividing the quotient by the number of days the fish were fed during the experiment. A food consumption rate was computed by dividing the dry weight of the worms consumed per fish in milligrams by the mean weight of the fish in grams, and then dividing the quotient by the number of days the fish were fed. Thus, both of these rates are expressed as milligrams per gram per day (mg/g/day).

For purposes of graphical presentation of the experimental results, some of the growth rates have been normalized by dividing the growth rates of the experimental fish by the growth rates of their respective controls and multiplying by 100, thus equating the

mean growth rate of each of the control groups to 100. Consumption rates at the unrestricted rations were normalized in a similar manner.

Food conversion ratios were computed by dividing the mean dry weight gained by the fish by the mean dry weight of the worms consumed. Normalized food conversion ratios were computed in the same manner as were the normalized growth and food consumption rates.

The KME used in the growth experiments was tested periodically for acute toxicity to salmon, to guppies, or to both. Table 4 summarizes the conditions and results of the acute toxicity bioassays.

It was expected that the three waste concentrations used in experiment A1, namely, 1.0, 2.0 and 4.0 mg/liter nominal BOD increment, would all be below the lethal level. However, after four days it was evident that the fish at the 4.0 mg/liter BOD waste concentration were not feeding as well as the fish held at other concentrations. At this time, it was decided to base the high restricted rations on the amount of worms consumed by the fish held at the 2.0 mg/liter BOD level, rather than 4.0 mg/liter. By the last day of the experiment, 21 of the 40 fish held at the high concentration had died; most of these were fish held on the low restricted rations or unfed fish. Because of this heavy loss, no growth or food

Sample description	Test animals and experimental conditions	96-hour TL _m
Collection date 2/24/66 Mill A 332 mg/l BOD Experiment Al	5 salmon/6 liters Oxygen added 10°C	3.9 percent 12.9 mg/1 BOD
Collection date 2/24/66 Mill A 332 mg/l BOD Experiment Al	10 guppies/liter No oxygen added 20 ⁰ C	4.5 percent 14.9 mg/1 BOD
Collection date 3/4/66 Mill A 270 mg/l BOD Experiment A2	l0 guppies/liter No oxygen added 20 ⁰ C	7.5 percent 20.2 mg/l BOD
Collection date 4/4/66 Mill A 276 mg/l BOD Experiment A2	l0 guppies/liter No oxygen added 20 ⁰ C	7.0 percent 19.3 mg/l BOD
Collection date 3/1/67 Mill A 230 mg/l BOD Experiment A4	5 salmon/6 liters Oxygen added 10 ⁰ C	7.5 percent 17.2 mg/l BOD
Collection date 3/8/67 Mill A 200 mg/1 BOD Experiment A4	5 salmon/6 liters Oxygen added 10 ⁰ C	7.5 percent 15.0 mg/1 BOD
Collection date 3/15/67 Mill A 195 mg/1 BOD Experiment A4	5 salmon/6 liters Oxygen added 10 ⁰ C	13.0 percent 25.3 mg/1 BOD
Collection date 4/19/67 Mill A 235 mg/1 BOD Experiment A5	5 salmon/6 liters Oxygen added 10 [°] C	7.5 percent 17.6 mg/1 BOD

Table 4. Results of acute toxicity bioassays conducted during growth experiments.

Continued on next page

Table 4. Continued.

Sample description	Test animals and experimental conditions	96-hour TL _m
Collection date 4/26/67 Mill A 213 mg/l BOD Experiment A5	5 salmon/6 liters Oxygen added 10 ⁰ C	4.2 percent 8.9 mg/l BOD
Collection date 8/26/66 Mill B 140 mg/l BOD Experiment Bl	5 salmon/6 liters Oxygen added 15 ⁰ C	42.0 percent 50.8 mg/l BOD
Collection date 10/13/66 Mill B 192 mg/1 BOD Experiment B2	5 salmon/6 liters Oxygen added 15°C	35.0 percent 67.2 mg/l BOD

consumption data are reported for fish receiving little or no food at the highest waste concentration.

The normalized results of these experiments are presented in Figures 3 and 4. In Appendix 1, the complete data from these experiments are presented in tabular form, and in Appendix 2 are presented graphs relating growth rate to food consumption in each experiment.

When rations were unrestricted (Figure 3) fish held in dilutions of KME from mill A showed less growth than did the controls at all exposure levels in all experiments. Growth rates decreased steadily as the BOD increment increased up to 3.0 mg/liter. Between 3.0 and 4.0 mg/liter BOD the growth rate curve declines sharply, and at 4.0 mg/liter BOD some fish died. Fish held in dilutions of KME from mill B on unrestricted rations showed growth rates higher than those of the controls when the BOD increments were 1.0 and 2.0 mg/liter, and only slightly lower than those of the controls when the BOD increment was 4.0 mg/liter.

When the high restricted ration was fed (Figure 4), salmon held in waste from mill A once again showed a steady decline in growth rate with increasing concentration of KME. The effects at BOD increments of 0.5 to 3.0 mg/liter were less than those observed when rations were unrestricted and shown in Figure 3. Again, however, there was a sharp decline of growth rate between

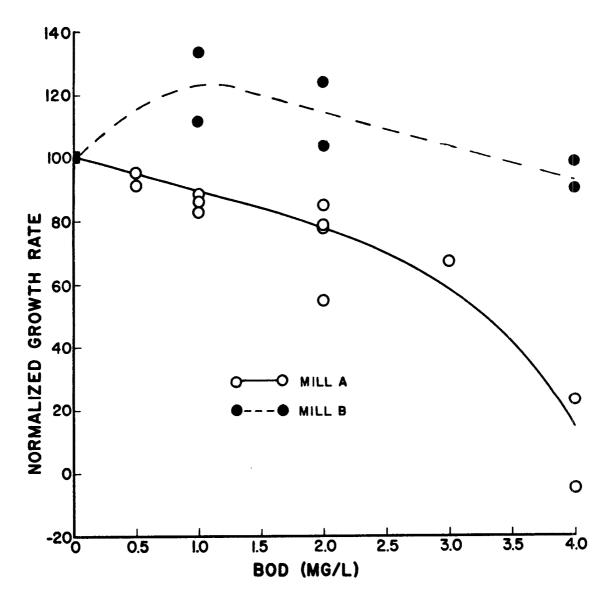


Figure 3. The relationship of normalized growth rates of chinook salmon kept on unrestricted rations to the BOD increments to which they were exposed.

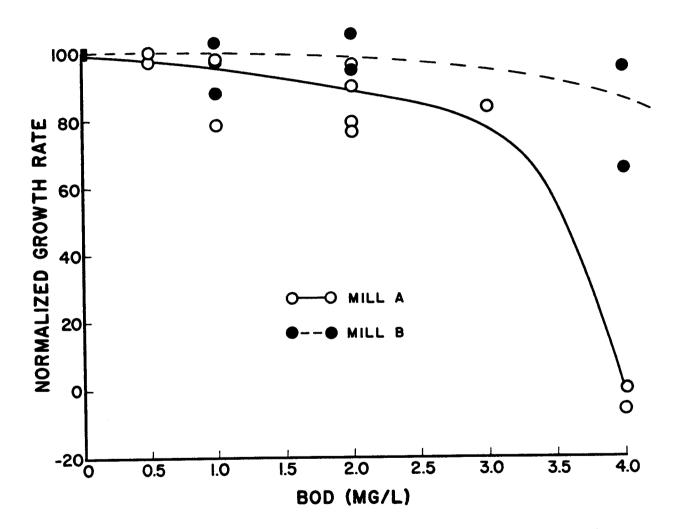


Figure 4. The relationship of normalized growth rates of chinook salmon kept on high restricted (daily repletion) rations to the BOD increments to which they were exposed.

the 3.0 and 4.0 mg/liter BOD levels. Fish fed the high restricted ration and exposed to wastes from mill B showed little effect on growth except a slight decrease at the 4.0 mg/liter BOD level.

Figure 5 presents the normalized food consumption rates recorded for each BOD increment when rations were unrestricted. When the fish were exposed to wastes from mill A, there was little effect on the food consumption rate except at 4.0 mg/liter BOD. Fish held in KME from mill B at the 1.0 and 2.0 mg/liter BOD levels consumed worms at a slightly greater rate than did the controls, and those exposed to the 4.0 mg/liter BOD level consumed food at a slightly lower rate than did the controls.

Figure 6 shows the relationship of normalized food conversion ratios to BOD increments. When the fish were kept on low and medium restricted rations or starved, little or no gain in weight was recorded, and conversion ratios could not be meaningfully computed.

In experiment A3, a portion of the waste tested had been biologically stabilized to reduce BOD and the acute toxicity. Acute toxicity bioassays of samples of treated waste showed that no computation of a TL_m was possible, as more than 50 percent of the fish survived for 96 hours in 100 percent treated effluent. All groups of fish that received food and were exposed to dilutions of KME, stabilized and unstabilized, had growth rates lower than those

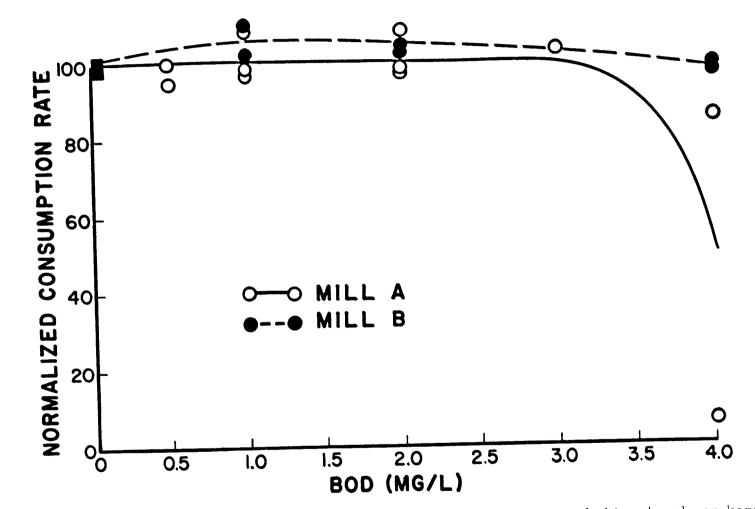


Figure 5. The relationship of normalized food consumption rates of chinook salmon kept on unrestricted rations to the BOD increments to which they were exposed.

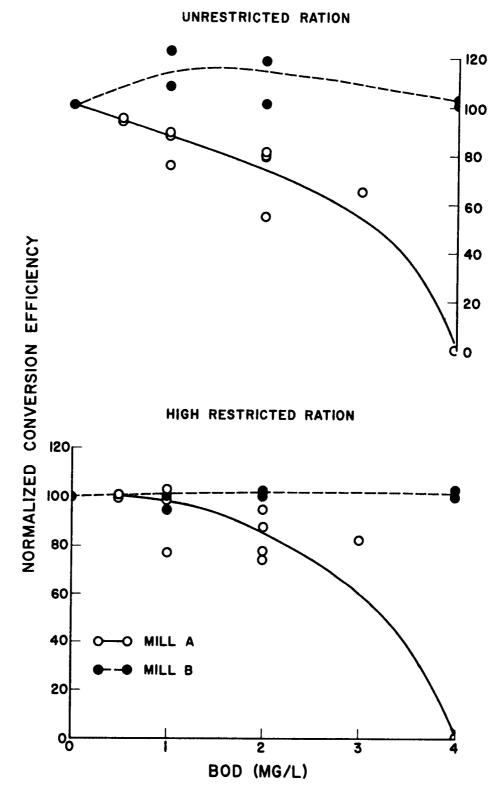


Figure 6. The relationship of normalized food conversion ratios to BOD increments.

of the respective controls kept on the same rations. Results obtained at the different KME exposure levels were variable and no consistent differences between stabilized and unstabilized waste were observed (Table 5).

In the experiment on the palatability and toxicity of KMEexposed worms, growth rates of fish that were fed worms exposed to BOD increments of 2.0 mg/liter for 24 and 96 hours were only slightly lower than those of the controls. Table 6 presents these data. A striking effect of efficiency of food utilization was noted where fish were fed worms exposed to 100 percent effluent. Fish fed unrestricted rations of these worms ate considerably more than did the other fish, but their growth rate was slightly depressed. When the high restricted ration was fed, so that the amount of food consumed was uniform, salmon fed worms exposed to 100 percent effluent had much lower growth rates than did the controls.

Chinook salmon were tested for sustained swimming ability after exposure for 12 days to wastes from mill A. Final swimming speeds were expressed as lengths per second, and were adjusted (size-corrected for a total body length of 92 mm, the mean fish length) to compensate for differences in fish size (Table 7). When the total length of a fish was greater than 92 mm, the adjustment was made by adding a value equal to 0.0027 times the difference between 92 mm and the actual length of the fish in mm to the

	non		Dry weigh	ts of fish		Dry wt . of food	Growth rate	Consump– tion rate	Food conversion	Number of fish
Ration	BOD increment	Initial	Final	Mean	Gain	Consumed	mg/g/day	mg/g/day	ratios	surviving
Unrestricted	0.0	598.5	1281, 2	939.8	682.7	2012, 1	45.4	134.4	0.338	10
	3.0	609, 9	1189.2	899.6	579.3	1913.7	40, 3	133.5	0, 302	10
	0.7*	596, 9	1174,6	885.7	577.7	1948.8	40, 8	138, 2	0, 295	10
	3.0*	607.9	1096, 5	852.2	488.9	1920, 3	35.7	141.5	0.252	10
High										
restricted	0.0	607.4	898.0	752.7	290 <u>.</u> 6	845.8	24.1	70 <u>,</u> 6	0,344	10
	3.0	601,2	840, 1	720, 6	238.9	845, 8	20, 7	73.7	0.281	10
	0, 7*	600, 2	770, 9	685 <u>,</u> 5	170.7	845, 8	15.6	77.5	0.201	10
	3.0*	603 . 9	822, 9	713.4	219.0	845.8	19.2	74. 5	0, 257	10
Low										
restricted	0.0	603 . 9	53 9, 8	571.8	-64.1	161.6	-7. 1	17.5		9
	3.0	606,4	488, 7	547.5	-117.7	16 1 <u>.</u> 6	- 13, 9	18.5		10
	0.7*	599, 8	479.1	539, 4	-120.7	161.6	-13.9	18.5		10
	3.0*	612.0	525 . 6	568.8	-86.4	16 1 <u>.</u> 6	- 9 . 4	17.6		10
Starvation	0.0	594.6	380, 2	487.4	-214.4	0	-27.5	0		9
	3.0	599, 8	393.0	496.4	-206.8	0	-25, 9	0		9
	0.7*	617.2	420, 4	518, 8	-196.8	0	-23 8	0		10
	3.0*	611.4	400. 3	505,8	-211.1	0	-26, 1	0		10

Table 5. Growth and food consumption data for growth experiment A3. All dry weights are the means for the surviving fish and are expressed in milligrams.

*Stabilized effluent.

	Treatment	of worms		Dry weig	hts of fish		Food	Growth rate	Consump- tion rate	Food conversion	No, of fish
Ration	(mg/1 BOD)	(duration)	Initial	Final	Mean	Gain	Consumed	(mg/g/day)	(mg/g/day)	ratios	surviving
Unrestricted	0.0		90.4	139, 5	114, 9	49.1	161.6	32,9	108, 1	0.303	10
	2.0	24 hrs	90,0	134, 5	112, 2	44, 5	159.8	30, 5	109, 5	0, 27,8	10
	2.0	96 hrs	89,9	134,4	112.1	44.5	131.6	30, 5	117.4	0, 337	10
	224	24 hrs	95 . 3	136, 1	115 . 7	40 <u>,</u> 8	220, 3	27.1	146.5	0.185	10
High									_		
restricted	0.0		96.5	142.4	119, 4	45.9	134.3	29.6	86,5	0,342	10
	2.0	24 hrs	96.3	137.2	116 . 7	40, 9	135.8	26.9	89.5	0, 301	10
	2.0	96 hrs	97.4	137.8	117,6	40, 4	127.5	26.4	83, 4	0, 318	10
	224	24 hrs	99 . 5	122, 2	110 <u>.</u> 8	22.7	130 <u>,</u> 5	15.8	90, 6	0, 175	10

Table 6. Results of feeding KME-exposed worms to chinook salmon. All dry weights are the means for the ten surviving fish and are expressed in milligrams.

	Total fish length (mm)	Observed final swimming speed (lengths/sec)	Corrected final swimming speed (lengths/sec)
Control	95	5.9	6.0
	91	7.2	7.1
	96	6.8	7.0
	92	7.1	7.1
	95	7.8	7.9
	99	8.0	8.3
mean		7.1	7.2
1.0 mg/l	87	7.0	6.8
BOD	89	6.8	6.7
	90	7.8	7.7
	94	7.4	7.5
	96	7.3	7.5
	92	8.1	8.1
mean		7.4	7.4
2.5 mg/l	91	7.2	7.1
BOD	85	7.7	7.4
	93	7.5	7.5
	90	7.8	7.7
	91	8.7	8.6
mean		7.8	7.7

Table 7. Results of the sustained swimming performance tests.

-

logarithm of the individual fish length, and then finding the antilogarithm of the sum. When the length of a fish was less than the mean, the adjustment was made by subtraction. The value of 0.0027 was used by Dahlberg, <u>et al.</u> (1968) for juvenile coho salmon, Oncorhynchus kisutch (Walbaum), 70 to 120 mm in total length.

The six control fish had an average adjusted final swimming speed of 7.2 lengths per second. The mean adjusted final swimming speed of the six fish exposed to 1.0 mg/liter BOD increment was 7.4 lengths per second, and that of the five fish exposed to the 2.5 mg/liter BOD increment was 7.7 lengths per second.

The apparent increase in the maximum sustained swimming speed with increasing BOD increments is largely attributable to the inferior performance of a single fish in the control group and the superior performance of a single fish at the 2.5 mg/liter BOD level.

Results of the caloric determinations on samples of fish from experiments A1 and A2 are presented in Appendix 3. These determinations should give an indication of differences in body fat content of the fish held at different rations and waste concentrations. Observed differences were not found to be statistically significant by a t test.

DISC USSION

Only that portion of the food consumed by a fish that is assimilated can be used for metabolism and growth, and the portion of the materials obtained from assimilated food that can be used for growth depends on the energetic costs of food transformation, standard metabolism, and necessary activity (Warren and Davis, 1967). Kraft mill effluents, or any pollutant, can affect growth of fish either by direct toxic action on the fish, which decreases the food conversion efficiency, or by damaging the food supply. Since food consumption rates of the salmon in these experiments at each feeding level were not affected by the KME (except near the lethal concentration level), effects of the wastes on the growth rates of these fish must be attributable to decreased efficiency of food utilization for growth. The relation between growth and food conversion efficiency is shown by the similarity of the normalized growth rate and normalized conversion efficiency curves (Figures 3, 4 and 6).

In these experiments, only when fish were kept on unrestricted or high restricted rations were definite decreases in growth rate observed. The fish kept on these rations would be expected to have metabolic rates higher than those of fish kept on rations close to the maintainance level (Davis and Warren, 1965). The effects of any stress placed on a fish by a toxicant can generally be expected to be

most evident when the metabolic rate is high.

The results of the swimming performance tests, which indicated that exposure to KME had no detrimental effects on the sustained swimming ability of salmon, confirmed earlier unpublished results obtained by George G. Chadwick with guppies. This suggests that there may be no impairment of food capture, escape from predators, or other activities in a natural environment.

The effect of reducing and stabilizing the BOD of KME on the waste's chronic toxicity to fish (as indicated by impairment of growth) is unclear. In the experiment described here as A3, there does not appear to be any difference attributable to biological treatment which reduced the BOD by approximately 73 percent, between the growth rates of salmon held in stabilized and unstabilized wastes from mill A; these results, however, are not deemed conclusive. The effect of biological treatment on the acute toxicity of the waste is, however, quite evident: bioassays on similar wastes (unpublished data of Eben Owens) showed that when the BOD was reduced 80-85 percent, fish lived in the undiluted effluent for 96 hours, whereas the same batch of effluent before treatment killed fish at concentrations as low as five percent in this time period. Ellis (1968) found that growth of salmon was reduced in laboratory streams with simplified plant and animal communities but no mortality of salmon occurred at waste concentrations which often proved fatal in

my experiments. He concluded that there was probably degradation of the waste and a reduction of its toxicity by the organisms in the streams. The conditions to which fish in most of my experiments were exposed can most nearly be compared to conditions that might exist immediately below the point of discharge of untreated waste into a stream, before any biological or chemical modification of the waste can occur.

It has been suggested (Henderson and Tarzwell, 1957) that "application factors" be used in estimating permissible concentrations of toxic wastes in receiving waters on the basis of acute toxicity bioassay results in order to insure adequate protection against chronic damage to fisheries resources in nature. The utility of such "application factors" is questionable, because no clear proof exists that the waste components and mechanisms of toxic action involved in acute toxicity are the same as those involved in chronic toxicity. Results of the growth experiments reported here give some indication that, in the case of the pulp wastes studied, there may be a general relationship between acute toxicity and effects on growth in salmon.

It can be seen (Table 8) that a 4.0 mg/liter BOD increment of waste from mill A corresponds to 21.2 to 30.0 percent of the 96-hour TL_m . At this level, some deaths occurred and little growth was recorded in 16 days. However, a 4.0 mg/liter BOD increment of

waste from mill B corresponds to only 7.2 to 9.2 percent of the 96hour TL_m , and substantial growth occurred at this concentration level. Comparable growth in waste from mill A occurred when 5.3 to 7.5 percent of the 96-hour TL_m was tested, which was at a BOD increment of 1.0 mg/liter.

Experiment number	BOD (mg/1)	Percent of 96-hour TL _m		
A1	1.0	7.5		
	2.0	15.0		
	4.0	30.0		
A4	1.0	5.3		
	2.0	10.6		
	4.0	21.2		
A 5	0.5	3.8		
	2.0	15.2		
	3.0	22.8		
Bl	1.0	1.8		
	2.0	3.6		
	4.0	7.2		
В2	1.0	2.3		
	2.0	4.6		
	4.0	9.2		

Table 8. Percent of 96-hour TL_m for each nominal BOD increment in every experiment in which toxicity bioassays with salmon were performed.

The growth rates of salmon kept on unrestricted rations and exposed to different waste concentrations were shown in Figure 3 to be higher at a given BOD level in dilutions of effluents from mill B than in dilutions of effluents from mill A. When the growth rates of these fish are plotted against degrees of toxicity of dilutions of the waste from the two mills expressed as percentages of the 96hour TL_m, the effects of the wastes from the two mills on growth appear to be similarly related to their acute toxicity (Figure 7). These results indicate that although the BOD may be a reliable index of the relative chronic toxicity of the wastes from a given mill, it does not provide a satisfactory basis for judging the relative chronic toxicity of wastes from different mills.

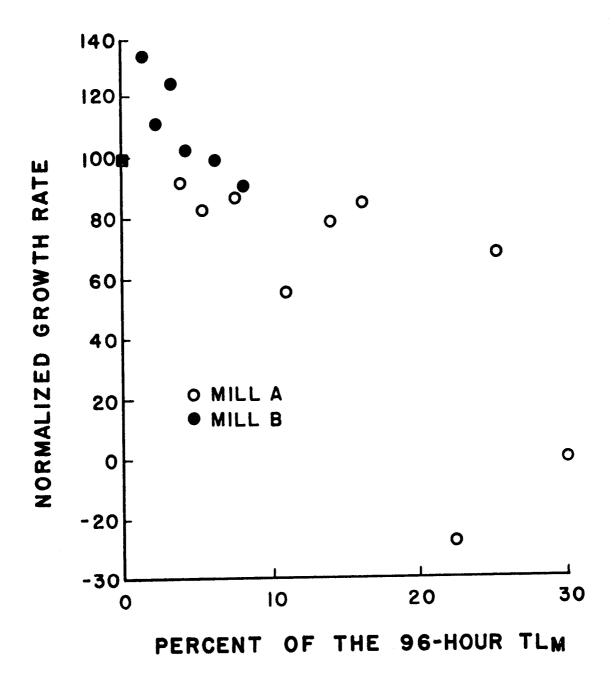


Figure 7. The relationship of normalized growth rates of chinook salmon kept on unrestricted rations to percent of the 96-hour median tolerance limit.

BIBLIOGRAPHY

- Bergstrom, Hilding and Sten Vallin. 1937. The contamination of water by the waste liquors of sulfate pulp mills. Meddelanden fran Statens Undersoknings-och Forsoksanstact for Sotvattensfisket, Kungl. Lantbruksstyrelsen 13:1-17. (Abstracted in Chemical Abstracts 34:2597. 1940)
- Cole, Arch E. 1935. Water pollution studies in Wisconsin. Effects of industrial pulp and paper mill wastes on fish. Sewage Works Journal 8:280-302.
- Dahlberg, Michael Lee. 1963. Influence of dissolved oxygen and carbon dioxide on the sustained swimming speed of juvenile largemouth bass and coho salmon. Master's thesis. Corvallis, Oregon State University. 57 numb. leaves.
- Dahlberg, M. L., D. L. Shumway and P. Doudoroff. 1968. Influence of dissolved oxygen and carbon dioxide on swimming performance of largemouth bass and coho salmon. Journal of the Fisheries Research Board of Canada. (In press)
- Davis, Gerald <u>et al</u>. 1963. The influence of oxygen concentration on the swimming performance of juvenile pacific salmon at various temperatures. Transactions of the American Fisheries Society 92:111-124.
- Davis, Gerald E. and Charles E. Warren. 1965. Trophic relations of a sculpin in laboratory stream communities. Journal of Wildlife Management 29:846-871.
- Doudoroff, Peter et al. 1951. Bioassay methods for the evaluation of acute toxicity of industrial wastes to fish. Sewage and Industrial Wastes 23:1380-1397.
- Ebling, G. 1931. Recent results of the chemical investigation of the effect of waste water from cellulose plants on fish. Vom Wasser 5:192-200. (Abstracted in Chemical Abstracts 26: 2262. 1932)
- Ellis, Robert Hewitt. 1968. Effect of kraft pulp mill effluents on the production and food relations of juvenile chinook salmon in laboratory streams. Master's thesis. Corvallis, Oregon State University. 55 numb. leaves.

- Fujiya, Masaru. 1961. Effects of kraft pulp mill wastes on fish. Journal of the Water Pollution Control Federation 33:968-977.
- Henderson, Croswell and Clarence M. Tarzwell. 1957. Bioassays for control of industrial effluents. Sewage and Industrial Wastes 29:1002-1017.
- McHugh, Robert. 1954. Preliminary report on a study of the factors responsible for the toxicity of wastes from a modern kraft pulp and paper mill. Oregon State College, Corvallis, Oregon. 10p. (Mimeographed)
- Nightingale, H. W., and V. L. Loosanoff. 1930. Effects of waste black liquor. Pacific Pulp and Paper Industry 4(11): 47.
- Servizi, J. A., E. T. Stone and R. W. Gordon. 1966. Toxicity and treatment of kraft pulp and bleach plant waste. New Westminster, B. C., Canada. International Pacific Salmon Fisheries Commission. 34p. (Progress Report 13)
- Warren, Charles E., and Gerald E. Davis. 1967. Laboratory studies on the feeding, bioenergetics, and growth of fish. In: The biological basis for freshwater fish populations, ed. by
 S. D. Gerking. Oxford, Blackwell Scientific Publications.
 p. 175-214. (In press)
- Washington. Department of Fisheries. 1960. Toxic effects of organic and inorganic pollutants on young salmon and trout. Seattle, Washington. 264p. (Research Bulletin No. 5)
- Webb, William Edward. 1958. Preliminary studies to determine the nature and principal toxic constituents of kraft pulp mill wastes. Master's thesis. Corvallis, Oregon State University. 53 numb. leaves.

Appendix 1. Growth and food consumption data for the growth experiments.	All values are the means for the surviving fish and are expressed in milligrams.
--	--

BOD Dry weights of fish Dry wt. Growth rate Food consumption rate Conversion ratios Number of increment of food fish Ration Final (mg/liter) Initial Mean Gain consumed (mg/g/day) (normalized) (mg/g/day) (normalized) (actual) (normalized) surviving 0.0 Unrestricted 99, 3 219.7 159.5 120.4 492.0 100 47.3 193.0 0.245 100 100 10 1.0 106.3 208.5 157.4 102, 2 470.0 40.7 86 187.0 97 0.217 88 10 2,0 182.8 98.9 140.8 83.9 428.0 37.2 190,0 79 98 0,196 80 10 4.0 101.8 101.6 101.7 -0, 2 -0.1 184.0 0 11.0 6 8 High restricted 0.0 97.7 190,6 144.1 92, 9 314.0 100 40.3 128,0 100 0,296 100 10 1.0 103,6 198.8 151.2 95, 2 39.4 314.0 98 121.0 94 0.303 102 10 2.0 106.7 176.9 141.8 70, 2 30,9 314.0 77 138.0 108 0.223 75 10 4.0 111,3 124.0 117.6 12.7 98.0 6.7 17 52.0 40 6 Low restricted 0.0 111, 3 114.0 112.6 2.7 63.0 1.5 35.0 10 1.0 96.3 110.3 103.3 14,0 62.0 8,5 38.0 9 2.0 98.4 97.0 97.7 -1.4 63.0 -0.9 40.0 10 4.0 99, 8 4 Starvation 0.0 97.9 81.3 64.7 -33.2 -25.5 0 0 9 1.0 83.9 102, 4 65.4 -37.0 -27.5 0 0 8 2.0 78.6 109, 4 94, 2 -31.7 0 -21.1 0 7 4.0 102.7 0 1

Experiment A1

	BOD increment		Dry weigh	ts of fish		Dry wt. of food	Growth	rate	Food consum	ption rate	Conver	sion ratios	Number of
Ration	(mg/liter)	Initial	Final	Mean	Gain	consumed	(mg/g/day)	(normalized)	(mg/g/day)	(normalized)	(actual)	(normalized)	fish surviving
Unrestricted	0.0	120, 8	209 . 2	165.0	88.4	544.0	33 . 5	100	206.0	100	0.162	100	10
	0.5	121.5	205.3	163.4	83.8	541.0	32, 1	96	207.0	100	0.155	96	10
	1.0	121.8	197.9	159,8	76.1	524,0	29.7	88	205.0	99	0.145	89	10
	2.0	125.4	192 <u>.</u> 2	158.8	66.8	514.0	26.2	78	202.0	98	0.130	80	10
High	0.0	124, 9	205.5	165 . 2	80,6	372.0	30.5	100	141.0		0.217	100	10
estricted	0, 5	124,4	205, 4	164.9	81.0	372.0	30.7	100	141.0		0.217	100	10
	1.0	124,4	204, 9	164,6	79.8	372.0	30,2	99	141.0		0.214	99	10
	2.0	124, 9	195,8	160 <u></u> 3	70 . 9	372.0	27.7	91	145.0		0.190	87	10
•													
Low	0.0	122.7	130, 1	126.4	7.4	74.0	4.0		37.0				10
restricted	0.5	122.3	119.4	120.8	-2, 9	74.0	- 1 . 5		38.0				10
	1.0	122, 7	119.0	120.8	-3.7	74,0	- 1, 9		39, 0				10
	2.0	122, 3	126.9	124,6	4.6	74.0	2, 3		37,0				10
Starvation	0.0	128,0	85, 1	106.5	-42,9	0	-25,2		0				10
	0 . 5	125.8	85, 4	105,6	-40, 4	0	-23.8		0				10
	1.0	125, 4	88.0	106.7	-38, 4	0	-22.5		0				9
	2.0	127.8	87.2	107.5	-40.6	0	-23.7		0				10 9

Experiment A2

	BOD	. <u></u>	Dry weigh	ts of fish		Dry wts.	Growt	h rate	Food consu	mption rate	Conversion ratios		Number of
Ration	increment (mg/liter)	Initial	Final	Mean	Gain	of food consumed	(mg/g/day)	(normalized)	(mg/g/day)	(normalized)	(actual)	(normalized)	fish surviving
Inrestricted	0,0	118.7	156.5	137.6	37.8	321.8	18.3	100	155.9	100	0.118	100	10
	1.0	119.2	149.5	134.6	30, 8	341.3	15.2	83	169.1	108	0.090	76	10
	2.0	119,9	139 <u>,</u> 6	129.8	19.7	300, 2	10, 1	55	154,2	99	0.065	55	10
	4.0	120, 2	112,4	116.3	-7.8	230, 9	-4.4	-24	132, 3	85	0.000	55	9
ligh	0.0	122, 2	147.4	134, 8	25.2	179, 4	12.5	100	88.6		0.141	100	10
estricted	1.0	121.5	141.1	131.3	19.6	179.4	10.0	79	91 . 1		0.109		10
	2.0	121.3	141.1	131.2	19,8	179.4	10.0	80	91.1		0.109	77	10
	4.0	127.8	126 <u>.</u> 5	127.1	-1.3	131.7	-0,6	-5	69.0		0.110	78	10 9
low	0.0	122, 6	133.5	1 27 •9	10, 7	89.7	5.6		46.7				10
estricted	1.0	125.7	128.0	126,9	22.7	89.7	1.4		49.7				10
	2.0	124.8	131.7	128, 3	6.9	89.7	4.2		49.9				10
	4. 0	123.1	116 <u>.</u> 9	120.0	-6.2	70, 9	-3.9		30, 1				10 10
Starvation	0.0	131, 1	123.2	127.2	-7.9	50, 1	-4. 5		28.1				10
	1.0	128, 7	118.4	123.5	-10, 3	50, 1	-5,9		28.9				10
	2.0	129, 1	118.2	123.6	-10, 9	50 . 1	-6.3		28, 9				
	4.0	122.8	109.0	115 <mark>.</mark> 9	-13.7	50, 1	-7.9		30, 9				10 4

Experiment A4

	BOD		Dry weig	hts of fish		Dry wt.	Grow	th rate	Food cons	umption rate	Conve	rsion ratios	Number of
Ration	increment (mg/liter)	Initial	Final	Mean	Gain	of food consumed	(mg/g/day)	(normalized)	(mg/g/day)	(normalized)	(actual)	(normalized)	fish surviving
Unrestricted	0.0	89.3	144.6	166 _• 9	55.3	279.6	33, 8	100	170, 8	100	0.197	100	10
	0.5	90.6	140.3	115,4	49.7	263.6	30.8	91	163.1	95	0.188	95	10
	2,0	89.6	134.7	112,1	45 , 1	290, 5	28.7	84	185, 1	108	0.155	79	10
	3.0	91.2	126 <u>.</u> 0	108.6	34.8	268.8	22, 9	68	176, 7	103	0.129	65	10
High	0,0	92.4	134,3	133 . 3	41.9	142.8	26.4	100	90, 0		0.293	100	10
restricted	0, 5	92, 9	133.7	113.3	40.8	142.8	25.7	97	90, 0	•	0.293	100	10
	2.0	91.9	131, 9	111.9	40.0	1 42. 8	25.5	97	91 <u>,</u> 1		0.279	95	10
	3.0	93.2	128,0	110.6	34.8	1 42 , 8	22 . 5	85	92 <u>,</u> 2		0.243	83	10
Low	0.0	94.8	115.0	104, 9	20.2	73.2	13.7		49, 8				10
restricted	0, 5	95.8	117.8	106,8	22.0	73 <u>.</u> 2	14.7		48, 9				10
	2.0	93.6	109, 2	101, 4	15.6	73 <u>.</u> 2	10, 9		51.6				10
	3.0	96 . 2	109 . 9	103.0	13.7	73 . 2	9 . 5		50 . 8				10
Starvation	0, 0	99, 8	100.4	100.1	0.6	37.0	0.4		26.4				10
	0, 5	98.4	101.1	99.7	2.7	37.0	1.9		26.5				10
	2.0	98,6	99.4	99,0	0,8	37.0	0.6		26.7				10
	3.0	99 . 5	94. 8	97.1	-4.7	37.0	-3.4		27.2				10

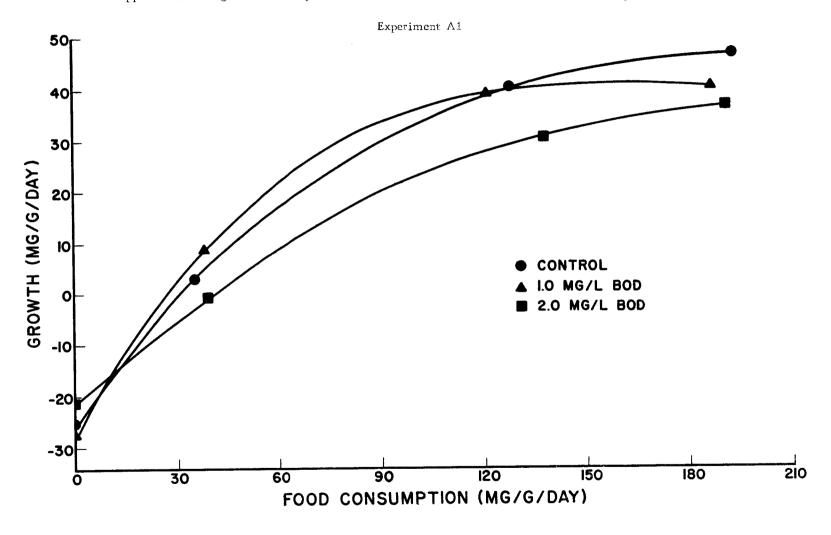
Experiment A5

	BOD	I	Ory weight	s of fish		Dry wt.	Growtl	n rate	Food con	sumption rate	Convei	rsion ratios	Number of
Ration	increment (mg/liter)	Initial	Final	Mean	Gain	of food consumed	(mg/g/day)	(normalized)	(mg/g/day)	(normalized)	(actual)	(normalized)	fish surviving
Unrestricted	0.0	657.9	999 . 8	837.8	323.9	1157.6	29.7	100	106, 3	100	0.278	100	10
	1,0	674,8	1143.4	909, 1	468.6	1376.8	39.6	133	116, 5	109	0.342	123	10
	2.0	673.6	1085, 7	879 . 6	419.1	1239 <mark>.</mark> 0	36.7	124	110, 5	104	0.332	119	10
	4. 0	676.7	99 4 , 5	835.6	317.8	1134, 1	29 . 3	99	104, 3	98	0.281	101	10
High	0,0	679 . 7	953.6	816.6	273 . 9	744, 9	25.9	100	70. 1		0,367	100	10
estricted	1.0	681, 1	923.0	802,0	241.9	696.1	23,2	89	66.7		0.348	95	9
	2.0	682, 8	978, 9	830.8	296, 1	788. 1	27.4	106	72, 9		0.376	104	10
	4. 0	665 . 7	923 . 7	794.7	258.0	744, 9	24,9	96	72.0		0.347	103	10
Low	0, 0	683 <u>.</u> 8	655 . 7	669 . 7	-28, 1	203, 9	-3,2		23.4				10
restricted	1.0	676.9	646.1	661.5	-30, 8	194, 7	-3,6		22, 7				10
	2,0	681, 3	699,2	690 <u>,</u> 2	17.9	203.9	2.0		22, 7				10
	4. 0	672 . 9	789 , 9	731,4	117.0	203, 9	12,2		21.5				10
Starvation	0.0	687.6	564.5	626.0	-122.0	0	-14.9		0				10
	1.0	688,0	552.0	620.0	-136.0	0	-16.9		0				10
	2.0	686, 1	617.3	651,7	-68,8	0	-8, 1		0				10
	4.0	692 <u>,</u> 8	559, 3	626.0	-133, 5	0	-16.7		0				10

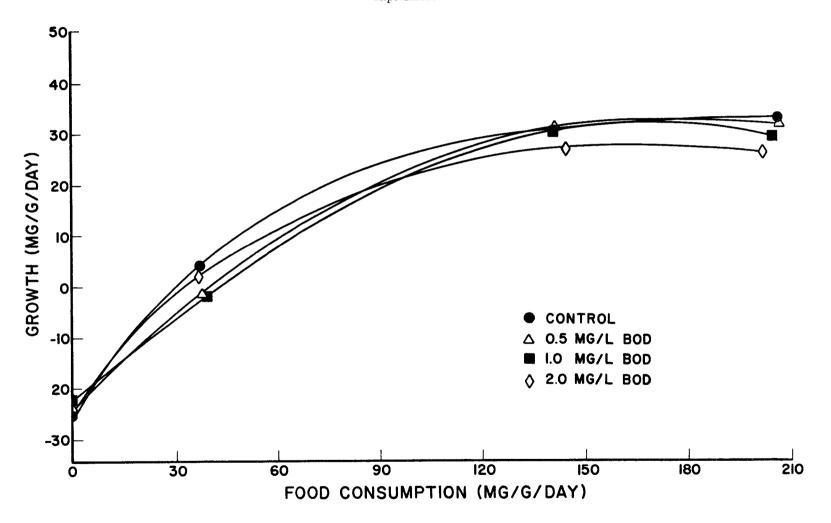
Experiment B1

	BOD	I	Ory weight	s of fish		Dry wt.	Growt	The Food consumption		mption rate	Conversion ratios		Number of fish
Ration	increment (mg/liter)	Initial	Final	Mean	Gain	of food consumed	(mg/g/day)	(normalized)	(mg/g/day)	(normalized)	(actual)	(normalized)	surviving
Unrestricted	0,0	1264, 4	1906.4	1585.4	642.0	2263.0	31, 1	100	109, 8	100	0.282	100	5
0/11/06/04/200004	1.0	1253, 9	1988, 6		734.1	2362.8	34.8	112	112.0	102	0.308	109	5
	2.0	1253.1		1585 , 3	664,5	2310 <u></u> 2	32,2	103	112.8	103	0.286	101	5
	4,0	1255 . 7	181 7. 4	1536 . 5	561 . 7	2141.5	28.1	90	107.1	97	0.292	103	3
High	0.0	1246.9	1745.6	1496.2	642.0	1469,2	25.6	100	75 .6		0.324	100	5
restricted	1,0	1234.8		1493,3	51 7, 0	1469,2	26.6	104	75.7		0.351	100	5
	2.0	1258.3		1494, 2	471.9	1469,2	24.3	95	75.7		0,320	100	5
	4,0	1334, 2	1656 . 4	1495 . 4	322, 2	1469,2	16.6	65	75.6		0.217	100	5
Low	0, 0	1247.8	1487.0	1367.4	239.2	786,2	13.4		44, 2				5
restricted	1.0	1251.3		1350.3	198, 1	786.2	11.3		44.8				5
	2.0	1286.2	1490,6		204.4	786.2	11.3		43.6				5
	4,0	1246,9		1319.1	144, 5	786.2	8.4		45 <u></u> 8				5
Starvation	0,0	1241 . 7	1252 _• 0	1246.8	- 10 . 3	445, 4	-0.6		27.4				5
o dat y woods it	1.0	1259.6	1342, 0		82.4	338, 3	4.8		2 0, 0				2
	2,0	1222.9	1189.6		-33, 3	445.4	-2,1		28, 4				5
	4.0	1285,7	1195,6		-90, 1	445, 4	- 5,6		27.6				5

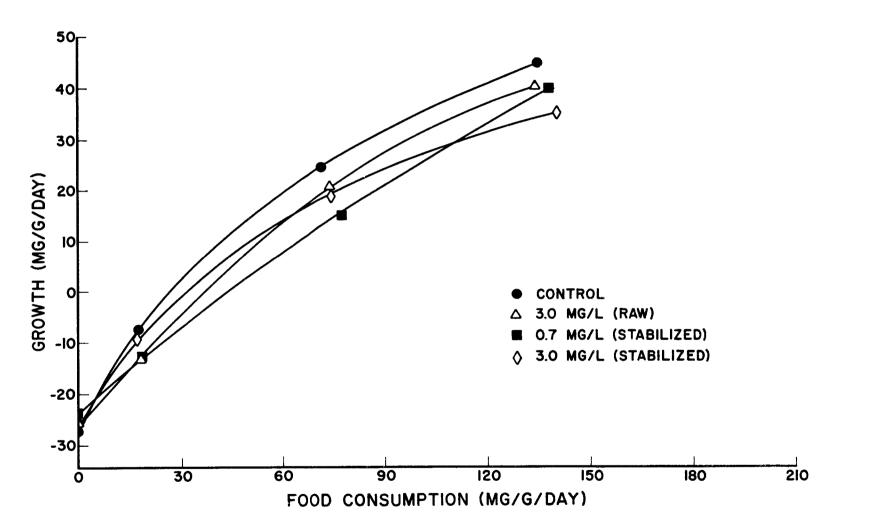
Experiment B2



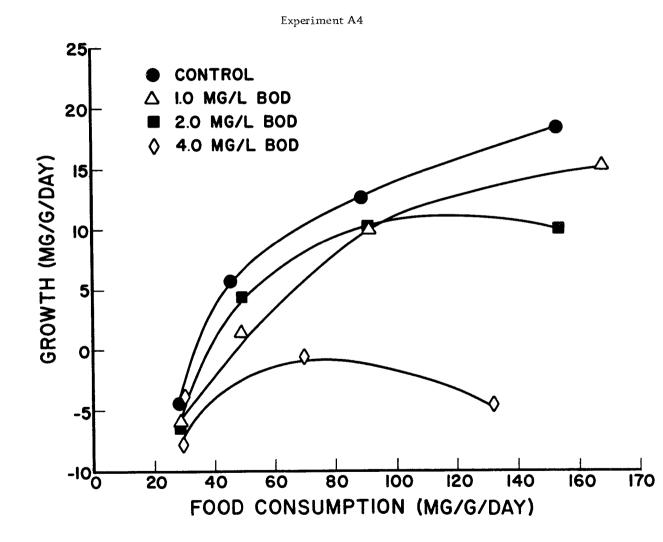
Appendix 2. The growth rates of juvenile chinook salmon in relation to their food consumption rate in the growth experiments,



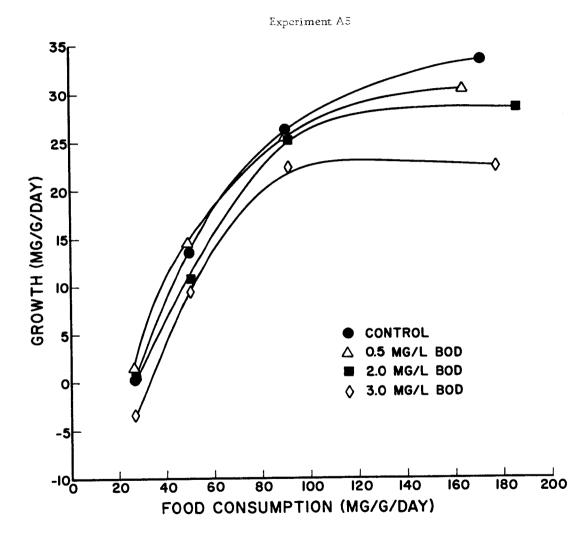


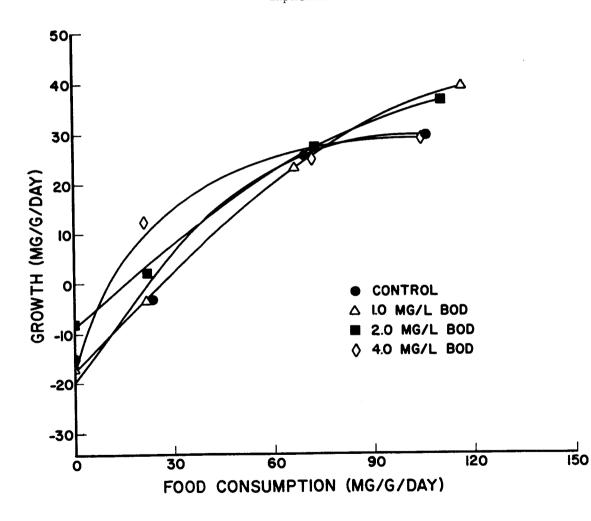


Experiment A3

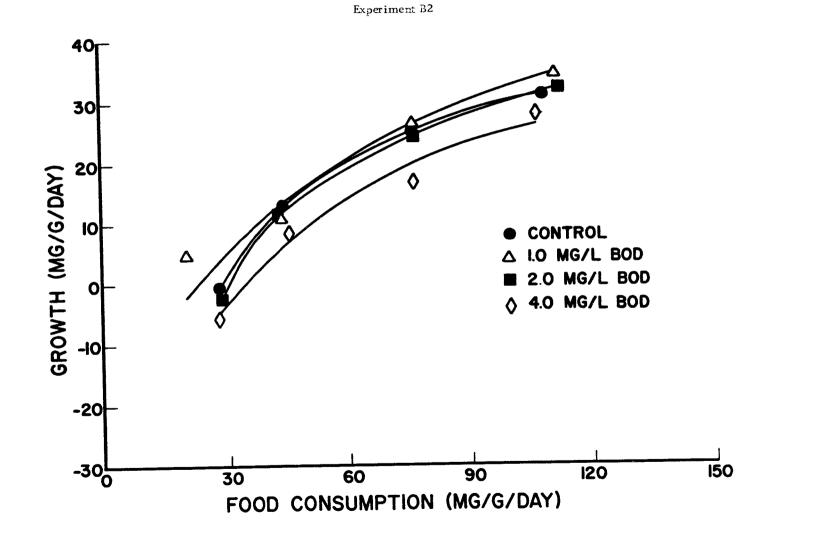


ე ე





Experiment B1



Experimer	nt A1	Feeding	Experiment A2			
BOD	Cal, /g	level	BOD	Cal./g		
Control	5222		Control	5121		
1.0 mg/l	5339	Unrestricted	0.5 mg/l	504 2		
2.0 mg/l	5382		1.0 mg/l	5016		
•8, -	-		2.0 mg/1	5359		
lontrol	5322		Control	4986		
0 mg/l	5392	High	0.5 mg/l	5041		
0 mg/l	5368	restricted	1.0 mg/l	5039		
			2.0 mg/1	5301		
Control	5094		Control	4830		
0 mg/l	4991	Low	0.5 mg/l	4609		
0 mg/l	4876	restricted	1_0 mg/l	4683		
			2.0 mg/l	5036		
ontrol	4755		Control	4545		
1.0 mg/l	4857		0.5 mg/l	4386		
0 mg/l	4983	Starvation	1.0 mg/l	4811		
0, -			2.0 mg/l	4506		

Appendix 3. Results of caloric determinations on the final samples from growth experiments A1 and A2. All values are in calories per gram of dry weight.