INTERNATIONAL PACIFIC SALMON
FISHERIES COMMISSION

PROGRESS REPORT

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TOXICITY AND TREATMENT OF KRAFT PULP BLEACH PLANT WASTE

BY

J. A. SERVIZI, E. T. STONE
and R. W. GORDON

NEW WESTMINSTER, B. C.
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Appointed under a Convention
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the Sockeye and Pink Salmon Fisheries in
the Fraser River System

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ABSTRACT

Some lethal and sub-lethal effects of neutralized kraft pulp bleach waste (NBW) on sockeye and pink salmon in fresh water were investigated. Sockeye and pink salmon alevins suffered reduced growth at concentrations of NBW much lower than those found toxic to fingerling sockeye. Prolonged exposure to dilute NBW resulted in deaths of adult migrant sockeye but differences between adults and fingerlings in resistance to NBW was not demonstrated conclusively. Viability of sperm and ova was not reduced in the case of adult sockeye which survived exposure to NBW. Experiments with biological treatment of the waste indicated that, although the initial toxic and chemical strength of NBW varied widely, a reduction of BOD by about 60% would render the waste almost non-toxic to fingerling sockeye. Possible mechanisms by which lethal and sub-lethal toxic effects occur are discussed and recommendations are made for further research.

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TOXICITY AND TREATMENT OF KRAFT PULP BLEACH PLANT WASTE

INTRODUCTION

The pulp potential of the vast wood resources of the Fraser River watershed is now being exploited. The wood resources are suited to kraft pulping and the five mills in either the planning or construction stage will all produce bleached kraft pulps. Production could reach a total of 3,000 tons of pulp per day. Residual wastes produced in these operations will be carried away by the Fraser and one of its principal tributaries, the Thompson River. These mills therefore constitute the first large scale pollution threat to the Fraser River system, one of the world's largest producers of salmon. The annual wholesale value of Fraser River salmon for the twelve year period of 1951-1962, based upon 1965 prices was $37.3 million.

Wastes come from two primary sources in a bleached kraft pulp mill; first, from pulping and chemical recovery processes and second from bleaching operations. Pulping and chemical recovery wastes, composed largely of black liquor and various condensates, contain valuable chemicals and heat content which can be re-used within the mill. Through careful engineering design the mills under construction on the Fraser watershed will re-use pulping and chemical recovery wastes so that these will not be discharged. However, due to the large volume of water required during bleaching operations, the waste streams from the bleach plant cannot be re-used within the mill, but must be discharged. Bleach wastes contain soluble fragments of lignin, for example tetrachloroguaiacol (Sarkanen, 1962). Tetrachlorocatechol would also be a substance likely present in bleach waste due to further oxidation of chloroguaiacol. Both catechol and guaiacol are substances toxic to fish and it is possible that the chlorinated forms are highly toxic. Besides soluble lignin fragments, bleach wastes contain toxic resin acid soaps (Van Horn, et al., 1949) which are carried over from pulping operations. Since it was obvious that bleach wastes contain substances toxic to fish and since the International Pacific Salmon Fisheries Commission is responsible under Treaty between Canada and the United States for the protection, preservation and extension of the sockeye and pink salmon stocks of the Fraser River system, it was imperative that the Commission investigate the effects on salmon of these wastes.

It is generally recognized that a toxic material can have two principal effects on an animal. Firstly, at high concentrations the toxic material can have an acute or lethal effect in a relatively short time. Fishery workers have usually quantified this toxic property by determining, under specified conditions, the concentration of toxic material at which 50 per cent of a group of fish succumb during 96 hours of exposure (96 hr. TL_m). Secondly, at lower concentrations, a toxic material may have sub-lethal effects on growth or some other attribute important to the long-term survival of the animal. These more subtle effects are not so readily quantified and generally require investigation of growth or other capability of the animal during long-term exposure to low concentrations of the toxic material. A toxic material may also have indirect effects by promoting the growth of unwanted organisms which compete or interfere with the development of the animal under study. All of these possible deleterious effects of bleach wastes on sockeye and pink salmon are currently under study by the Commission.

The acute toxicity of bleach wastes has been studied by Howard and Walden (1965) using sockeye fingerlings. They found the 96 hr. TL_m was about 12% for raw bleach waste from a kraft pulp mill. When the high acidity of this waste was neutralized, the toxicity decreased and the 96 hr. TL_m ranged from 29% to 64%. Through acclimation procedures they were able to adapt sockeye fingerlings to concentrations of neutralized bleach waste which would otherwise be lethal.

Although the aforementioned tests indicate concentrations at which acute toxicity may occur to fingerling sockeye they cannot be extrapolated to indicate the effects on salmon of sub-lethal concentrations. Furthermore it is not advisable to apply acute toxicity data obtained on fingerling sockeye to other stages such as eggs, alevins and fry. Application of these data to adult migrating salmon, especially sockeye, may be particularly misleading since these fish are frequently under stress due to high water temperatures and velocities.

As the first steps in a long-term study of the possible effects of neutralized bleach wastes upon the freshwater life of pink and sockeye salmon, short and long-term bio-assays of an exploratory nature have been performed. In addition, some observations have been made on the growth of slimes in waters polluted with bleach wastes and on the effects of these slimes on the embryonic stages of salmon. Although the investigations are not complete, some of the findings to date are of considerable interest and are presented in this report.

An associated series of experiments has been concerned with methods of reducing the toxicity of bleach wastes. The active materials in bleach wastes are mainly organic compounds which, under suitable conditions, may be broken down by bacterial action into simpler and generally less active compounds. Since this breakdown involves a series of oxidative processes, a demand is made on the oxygen supplies of the receiving water. This oxygen demand of organic wastes is generally quantified in terms of biochemical oxygen demand (BOD), expressed as milligrams of oxygen per liter of fluid (mg/l) under specified conditions of time and temperature (e.g., 5 days, 20°C).

Generally, the harmful effects of organic wastes can be reduced by biological treatment, i.e., passing the wastes through devices which promote intense bacterial activity with consequent reduction in the BOD of the waste. However, with regard to bleach wastes, there is a dirth of information on the relationship between BOD reduction and toxicity to fish. For this reason, several waste treatment experiments were conducted to evaluate the effects of BOD reduction on short-term toxicity.

MATERIALS AND METHODS

Bleach Waste

The bleach waste used in these experiments was obtained from a kraft pulp mill when the mill was making full bleach pulp (brightness 89-92) from coniferous trees. The bleach waste consisted of the following separate wastes: Caustic bleach waste (24.4%), acid bleach waste (36.6%) and wash water from the final washing of pulp following recovery of black liquor (39%). This mixture was very acid, having a pH of about 3, but before use in any bio-assays it was neutralized to pH 7 to 7.4 with 10N KOH. The waste was neutralized because such treatment would normally be given wastes discharged to the Fraser River. The neutralized waste is abbreviated NBW in this paper.

Wastes were hauled from the pulp mill to the laboratory in wooden barrels and then mixed together in a 300 gallon fiberglass storage tank in a cold room (38°F). Waste was taken from the storage tank and neutralized as needed for each day's use. Depending upon the experiments being conducted 300 gallons of waste generally lasted from five to ten days.

After mixing in the storage tank the waste was analyzed for BOD, chemical oxygen demand, chloride and chlorine. Biochemical oxygen demand, chloride

(Mohr Method) and chlorine (Starch-Iodide Method) were measured according to Standard Methods (1960). Chemical oxygen demand was determined by the modified dichromate method (Dobbs and Williams, 1963) in which mercuric sulfate was added to precipate chloride. An analysis for soda loss was made, at the time the waste was obtained, by the pulp mill laboratory staff.

The mill from which the bleach waste was obtained normally adds two strong wastes to its acid bleach waste, they are: digester blow and relief gases and turpentine recovery underflow. This procedure is not typical, however, so when acid bleach waste was obtained for bio-assays these aforementioned strong wastes were prevented from entering the acid bleach waste sewer.

Acute Bio-assays Using Fingerling Sockeye

Acute short-term bio-assays, using sockeye fingerlings, of NBW and NBW which had been treated to reduce toxicity were conducted in 30 liter aerated glass aquaria each containing 10 fish. The fingerlings had an average length of about 9.6 cm. Temperatures were maintained at about 46°F and aeration was provided with oil free compressed air, which kept the dissolved oxygen at about 11.5 mg/l (96% saturation). Since bleach waste interferes with the modified Winkler method for determining dissolved oxygen a galvanic cell oxygen analyzer was used to determine dissolved oxygen. The fish were acclimated to the aquaria for 48 hours before starting the bio-assay and were not fed during the test. All control fish survived and were vigorous following the bio-assay which indicates that conditions within the aquaria were satisfactory.

Following acclimation, a series of concentrations, as per cent waste in Cultus Lake water, for example 10%, 20%, 30%, and 40% NBW, were set up for the 96 hour test. Dead fish were removed each day. The 96 hour median tolerance limit was determined by plotting per cent concentration versus per cent survivors after 96 hours on semi-logarithmic paper and drawing a straight line between two points which had survivals greater than 50% and less than 50%, respectively. The concentration which corresponded to 50% survival on the line drawn was the median tolerance limit. This method is set forth by Henderson and Tarzwell (1957). Fish which survived during bio-assay were not used in subsequent tests.

Bio-assay Using Adult Migrant Sockeye

A long-term bio-assay of NBW, using adult migrant sockeye bound for the Cultus Lake spawning ground, was conducted. Sockeye were taken from a trap on Sweltzer Creek, near the laboratory, and transferred to four 6 ft diameter by 3 ft high fiberglass tanks containing 300 gallons of Cultus Lake water. The tanks were fitted with lids; four-fifths of each tank was covered with a transluscent lid while one-fifth of each tank had an opaque lid. A continuous supply of water, pumped from Cultus Lake at the rate of 300 gpd, was provided for each tank during 12 days of acclimation, which allowed time for observation of the fish under captive conditions. After acclimation, NBW was added slowly so that the test concentrations of 1%, 2% and 5% were reached in 24 hours. A continuous supply of water and NBW was provided at the total flow rate of 300 gpd.

Tank water was recirculated, primarily to produce a current, with a cast iron centrifugal pump which forced water through an iron heat exchanger, fiberglass filter and plastic diffuser. All plumbing connecting these pieces of equipment was plastic and valves were either plastic or iron. Diffusers were positioned in each tank so that the four tanks had identical currents. Average peripheral velocities were about 1.7 fps (20 miles per day). Temperatures were kept near those at the natural Cultus Lake spawning ground (47^{o}F). Oil free compressed air was used to maintain dissolved oxygen at an average of 90% saturation in each tank and saturation values ranged from 85% to 95% during the course of the experiment. Ten fish were put in each tank, except in the control which contained nine. Two fish were taken from each tank for initial samples of blood and tissue before NBW was added. One fish was taken from each of the control, 1% and 2% lots thirteen days after NBW was added for analyses of blood and tissue.

To make handling easier and to prevent unnecessary disturbance of fish, mild anesthesia was used when handling fish for spawning or sampling purposes. The dosage, 0.2 ml/gal of 2-phenoxyethanol, was just enough to quiet the fish so that they could be handled easily (Sehdev, et al., 1963). Fish were observed through narrow openings in the transluscent portion of the covers and observing the fish in this manner never seemed to frighten them.

Bio-assays Using Salmon Eggs and Alevins

Two long-term bio-assays were conducted using pink and sockeye salmon eggs and alevins. Sockeye eggs were taken from Cultus Lake spawners, mixed together, separated into four lots and then fertilized. Several males were used during fertilization and, as much as possible, each male was used in fertilizing each lot of eggs. Eggs were fertilized in lake water (lot I) or in 1%, 2% or 5% concentrations of NBW (lots V, VI, VII, respectively). Lots V, VI and VII were incubated in 1%, 2% and 5% NBW, respectively, except that about 250 eggs from each of lots V, VI and VII were incubated in lake water to determine whether NBW caused any ill-effects during fertilization (lots II, III, IV, respectively). About 1,800 eggs from each lot were measured volumetrically and separated into thirds for incubation so that each basket contained about 600 eggs. Eggs, according to the foregoing schedule, were incubated in a continuous fresh flow of 0%, 1%, 2% or 5% NBW made up in Cultus Lake water.

Long-term bio-assays of pink salmon eggs (provided by the Canada Department of Fisheries from the Atnarko River, B.C.) were conducted in the same manner as those bio-assays on sockeye eggs except that each basket contained about 300 eggs. Eggs fertilized in water only were designated lot A, while eggs fertilized and incubated at 1%, 2% and 5% NBW were designated lots E, F, and G, respectively. Eggs from each of lots E, F, and G were incubated in water and these were designated lots B, C, and D, respectively.

Sockeye eggs were incubated at a temperature of about 47°F, which is the normal temperature for the spring-fed Cultus Lake spawning ground. Pink eggs were incubated at temperatures corresponding to those in the Atnarko River during the 1963-64 incubation period. Dissolved oxygen was maintained at 96% to 101% of saturation by using oil-free compressed air in all troughs. Light was excluded by covering all baskets during incubation. Eggs were incubated in 6 in. by 4 in. baskets, there being three baskets to a trough. Flow was upward through the eggs at 0.1 gpm, which gave an apparent velocity* of 146 cm/hr (1.337×10^{-3} fps).

Following blastopore closure, dead eggs were removed each day. Samples of these were cleared in saturated sodium chloride solution so that any embryonic development could be observed.

Upon eyeing, the sockeye eggs were reduced to 300 per basket, and the middle basket in each trough of lots I, V, VI and VII was converted to a gravel nest. This gave an arrangement of a basket with gravel positioned between two baskets, of the same egg lot, without gravel. The baskets containing gravel had an inch of gravel below the eggs and 1½ in. over them.

Alevins were incubated in the same baskets in which they were hatched and all baskets were cleaned regularly with a siphon.

*Computed as if passage of water were unobstructed by eggs (and gravel where applicable).

For measurements of embryo weight, samples of twenty or more alevins were preserved in 10% formalin. By careful dissection, the embryo was separated from the yolk, dried for 24 hours at 98°C and then weighed on an analytical balance. Pink salmon were sampled at intervals throughout development whereas the sockeye were sampled only at complete yolk utilization. Yolk utilization was considered complete when one-half the fish had traces of yolk and half did not.

Treatment of Bleach Waste to Reduce Toxicity

As described earlier, the toxicity of organic wastes can be reduced by bacterial oxidation and this would apply to bleach wastes, however, some persons were of the opinion that toxicity of pulping wastes could be reduced through atmospheric oxidation. For these reasons, three treatments were applied to different batches of NBW taken from a single load of bleach waste so that a comparison of toxicity reduction could be made. One treatment consisted solely of storing NBW for seven days without agitation at a temperature of 55°F in an open vessel measuring 30 in. diameter by 16 in. deep. In a second treatment, NBW was held under conditions similar to the first except that it was aerated and at intervals of 1, 2, 5, 7 and 13 days samples were taken for bio-assay. No attempt was made to encourage bacterial growth in this experiment. The purpose of the two foregoing treatments was to determine the reduction of toxicity achieved by atmospheric oxidation. Little success was expected with these treatments since Carpenter and Porter (1947) and Moggio (1948) found that oxidation of pulp wastes upon storage was due mainly to biological oxidation rather than to chemical oxidation by the atmosphere.

The third scheme consisted of aerobic biological treatment using a heterogeneous culture of bacteria adapted to oxidation of NBW. The bacterial culture was started and adapted by seeding 10 gallons of NBW with a gallon of sewage and 200 ml of supernatant from a soil-water suspension. The mixture was aerated for a couple of days until a flocculent culture of bacteria (biological solids) was observed. The air was turned off, the biological solids were allowed to settle and the supernatant fluid was decanted. Fresh NBW was added and the mixture was aerated for an additional 24 hours before the biological solids were again settled and the supernatant decanted. This procedure was repeated for about two weeks before the biological solids were tested for ability to reduce the toxicity of NBW. For comparison with the two atmospheric treatment schemes, two separate biological treatments were applied to NBW: (a) aeration of one batch of NBW for 24 hours with 100 mg/l of biological suspended solids and (b) aeration of a similar batch for 72 hours with 55 mg/l biological suspended solids.

After treatment, neutralized bleach waste was used in 96 hour acute bio-assays of fingerling sockeye (as described previously) and the results were compared with bio-assays of NBW which was not treated. In addition to bio-assay data, biochemical oxygen demand of the waste was determined before and after treatment.

To examine further the effect of BOD reduction on toxicity, additional trials of biological treatment were made using 24 hours aeration and various concentrations of biological suspended solids. To obtain biological suspended solids concentrations greater than 100 mg/l, nitrogen and phosphorous were added as nutrients.

RESULTS

Acute Toxicity, Chemical and Biochemical Characteristics of NBW

Analyses of bleach wastes indicated that chemical properties, biochemical oxygen demand and acute toxicity to sockeye fingerlings varied widely among loads (TABLE 1). The BOD fluctuated widely so that the maximum value was more than three times the minimum value. Chlorine, although it was present in acid bleach waste, was absent from bleach waste since there is a great deal of organic matter with which it can react when the three wastes making up the composite bleach waste are mixed. Chlorides are in high concentration as a result of the chlorine used in bleaching. Acute toxicity to sockeye fingerlings was determined as 96 hour TL_m (the per cent concentration at which 50% of the fish succumb in 96 hours, as described previously).

TABLE 1 - Analysis of some bleach plant waste characteristics.

Characteristic	No. of Loads Analyzed	Max.	Min.	Av.
Biochemical Oxygen Demand, 5 day, 20°C, mg/l	23	351	111	201
Chemical Oxygen Demand, mg/l	17	1,530	511	922
Chloride, mg/l	4	590	490	547
Chlorine, mg/l	6	nil	nil	nil
Soda Loss, lbs. per ton Air Dry pulp	19	66	6.6	31
96 hr. TL_m for sockeye fingerlings, per cent concentration NBW	9	43	12	22

For many organic wastes, BOD is a nearly constant fraction of chemical oxygen demand (COD), but no such correlation was found when data for individual loads of bleach waste (TABLE 2) were compared. In addition, toxicity expressed as 96 hr. TL_m to sockeye fingerlings appeared unrelated to BOD or COD. Such results suggest that the compounds responsible for NBW toxicity do not represent a constant fraction of all the compounds exerting BOD or COD. Furthermore, the ratio of organic compounds oxidizable in the BOD test to the compounds oxidized in the COD test does not appear to be a constant for bleach waste. The foregoing variations occurred even though the mill was producing pulp of uniform quality.

TABLE 2 - Biochemical Oxygen Demand, Chemical Oxygen Demand and Toxicity of 9 loads of neutralized bleach waste.

Load	Biochemical Oxygen Demand mg/l	Chemical Oxygen Demand mg/l	Toxicity as per cent Concentration NBW Required to Kill 50% of Fish in 96 hr. (96 hr. TL_m)
A	282	1,394	27
B	188	-	19
C	351	1,530	23
D	204	829	14
E	274	1,100	43
F	213	735	26
G	166	923	19
H	149	714	19
I	182	991	12

Reactions of Sockeye Fingerlings During Acute Toxicity Bio-assays

The criterion used in determining a median tolerance limit is mortality in a specified time. However, during a bio-assay, symptoms of distress occurred at concentrations in which no test fish died. In each aquarium a definite, identical current pattern was created by careful placement of the aeration diffusers. The control fish spread across the aquarium, near the bottom and maintained positions in the current. At 0.8 of the 96 hr. TL_m concentration some of the fish would maintain a position in the current for awhile while others would swim about (TABLE 3). Fish exposed to 1.2 times the 96 hr. TL_m concentration swam about the aquarium and never held a position.

TABLE 3 - Reactions of sockeye underyearlings to various concentrations of NBW.

96 hr. TL_m Concentration	Mortality Per Cent	Orientation to Current	Respiratory Movements	Alarm Response	Maintain Equilibrium
1.2	100	None	Severe gulping	None	No
0.8	10	Slight	Severe gulping	None	Yes
0.4	0	Yes	Moderate gulping	Slight	Yes
0.0 (Control)	0	Yes	No gulping, normal	Extreme	Yes

Fish exposed to NBW had a unique respiratory movement which could be described as a quick gulp or cough. Gulping was severest in the highest concentrations of NBW and reduced in intensity as the concentration decreased (TABLE 3). In addition to gulping, the mouths of test fish were always slightly open and this gape increased with higher concentrations of NBW.

In response to a light directed into the aquarium, a quick movement or a disturbance at the surface, many of the control fish reacted normally by attempting to sound. However, for fish in NBW, this alarm response was reduced considerably (TABLE 3). At 0.4 of the 96 hr. TL_m concentration some fish would swim away from the disturbance, but did not sound, while the remaining fish made no move whatsoever.

All fish which died during bio-assay of NBW were in severe distress for hours before dying. Before succumbing, the fish would repeatedly lose equilibrium, swim on their backs and then lie on their sides on the bottom for a few minutes before temporarily regaining the ability to swim again.

Due to darkness in the aquaria, caused by the opacity of NBW, it is felt that the symptoms of greatest significance are abnormal respiration and loss of equilibrium. The other abnormal reactions observed may have been influenced by darkness of the test solution as well as by toxic effect. Fish in biologically treated NBW (which also tends to be opaque) were also observed and, at concentrations which were non-toxic, their behavior was similar to that of the controls except that they did not orient in the aquarium as well as the controls but often swam near the aquarium wall. It is possible that because of the opacity of both NBW and biologically treated NBW, the fish were attracted by light outside the aquaria.

Toxicity of NBW to Adult Migrant Sockeye

Observations throughout the experiment indicated that adult sockeye in the control tank tended to school in the small portion of the tank covered with an opaque cover. They always oriented against the current and maintained their position for long periods. Fish in the tank containing 1% NBW behaved similarly.

Fish in 2% NBW schooled well and maintained position in the tank under the opaque cover during the early stages of the experiment. However, after about 13 days exposure to NBW, the fish spent a fair amount of time drifting with the current and they did not school as before.

Fish in 5% NBW began drifting with the current only a few days after the NBW was added. Five fish in 5% NBW died after 15 days of exposure and, since these mortalities occurred during the day, it was possible to observe four of these fish as they succumbed. The first symptom was loss of equilibrium: that is, a fish swam on its back or side and then laid on the bottom for several minutes before again swimming normally. After repeating this behavior a few times the fish finally died. This reaction was similar to that already described for sockeye fingerlings.

All of the fish in 5% NBW succumbed in 19 days and were followed by those in 2% NBW which died in 29 days (TABLE 4). Mortalities in the other two lots of fish did not commence until 39 days.

All of the fish which died in 5% NBW were immature (eggs or sperm not in spawning condition), while those mortalities in 2% NBW consisted of two immature, four mature and one partially mature fish.

Two fish which died had fungus on them. The female which died on the 29th day in 2% NBW had fungus on its gills and this could have been at least partially responsible for its death. The control fish which died on the 45th day was covered with fungus which, together with any associated disease organisms, undoubtedly caused its death. This fish had been used for spawning on the 41st day and probably contracted the fungus as a result of being handled.

As mentioned earlier 2-phenoxyethanol at a dosage of 0.2 ml/gal was used as an anesthetic. This dosage had been used several times before on adult sockeye without mortality. However, when it was used on the 41st day, a male could not be revived in the control tank and died. Possibly, through error, this fish received a concentrated dose of anesthetic before it was thoroughly mixed.

Three of the control fish (2♀, 1♂) and five of the fish in 1% NBW (3♀, 2♂) were matured and spawned. At spawning, all females and one male (from the 1% concentration) were sacrificed. Both males not sacrificed when spawned subsequently died. The male in the control tank became covered with fungus and died on the 45th day, probably as a result of handling. The male from the 1% tank which died on the 49th day, was in excellent condition and contained much milt.

In the typical bio-assay, the test animals are not undergoing rapid natural physiological changes and therefore all response can be attributed safely to

TABLE 4 - Mortalities of adult migrant sockeye during exposure to three concentrations of neutralized bleach waste.

Days After NBW Added to Experimental Tanks	Mortalities				Remarks
	Control	1%	2%	5%	
13			1		♂, immature, excellent appearance
14				1	♂, immature, excellent appearance
15				5	2♂, 3♀, all immature, excellent appearance
16				1	♂, immature, excellent appearance
19				1	♀, immature, excellent appearance
20			1		♂, immature, excellent appearance
23			1		♀, mature, excellent appearance
24			2		2♀, mature, excellent appearance
27			1		♂, partially mature, excellent appearance
29			1		♀, mature, fungus on gills
39	1				♂, partially mature, excellent appearance
40		1			♂, mature, excellent appearance
41		1			♀, immature, excellent appearance
41	1				♂, immature, died from anesthesia
45	1				♂, mature, covered with fungus
49		1			♂, mature, excellent appearance
Total Mortality	3	3	7	8	

the substance being assayed. However, sensitivity to a substance may vary with life stage and physiological state and it is necessary that such variation be investigated. As a consequence the experiment just described was conducted upon migrating, adult fish which were undergoing rapid physiological changes leading to spawning and eventual death. Because of this natural sequence of events in the life of these adults it is important that all observations be considered before attributing toxicity to the NBW.

Based upon behavior, condition and state of maturation of the fish it can be concluded that 5% NBW was lethal. Fish in 2% NBW followed the same behavior pattern as those in 5% and this pattern was similar to that of sockeye fingerlings in short-term bio-assays. With the exception of one individual (fungus on gills) the condition of the fish in 2% NBW appeared excellent at the time they died. Four of the fish were sexually mature when they died but it is considered unlikely that death was due to the normal degenerative processes associated with spawning. It appears likely that 2% NBW was lethal to most of the fish.

Ten days after the last fish in 2% NBW had succumbed a partially mature male in the control tank died for no obvious reason (TABLE 4). The other two mortalities in the control tank were due to anesthesia and fungus. Following the unexplained control mortality, two of the fish in 1% NBW died. These fish appeared to be in excellent condition (one was immature) and had not followed the abnormal behavior pattern of fish in 5% and 2% NBW. The last mortality in 1% NBW was a fish that had been used for spawning eight days before and it may have died from natural causes. In light of the unexplained death of a control fish and the behavior of the fish in 1% NBW it is not clear whether 1% NBW had any lethal effect on mature sockeye.

Spawn from the surviving fish was checked by incubation of the fertilized eggs and it was found that eggs and alevins developed normally. Mortality of eggs from control fish was 13% and 36% while mortality of eggs from fish in 1% NBW was 1% and 46% (eggs of two females combined in second case). Mortality of alevins was less than 1% for all groups. These data indicate that contact of the parents with 1% NBW apparently did not affect the spawn.

Mortality of Eggs and Alevins Exposed to NBW

Mortality of incubating eggs showed no relationship to concentration of NBW used during fertilization or incubation of eggs. For example, mortality of sockeye eggs which were fertilized and incubated in 5% NBW was 5% while the mortality of controls was 9%. Pink salmon egg mortalities were 81% for controls, 89% for eggs fertilized in 5% NBW and incubated in water but only 61% and 47% for eggs fertilized and incubated in 5% and 2% NBW, respectively. Examination of dead pink and sockeye eggs indicated that nearly all had not been fertilized and this may have contributed to the variable results. Both sockeye and pink eggs exposed to NBW hatched at the same time as did their respective control groups. Thus no direct effect of NBW on either pink or sockeye salmon eggs was indicated.

Pink salmon alevins exposed to 5% NBW all died (TABLE 5) within two weeks of hatching. At 2% NBW, pink salmon mortalities were also high but deaths occurred at a uniform rate over a two month period. At 1% NBW, mortalities were similar to those of the controls.

Sockeye salmon alevins, by contrast, showed little mortality at 2% NBW (TABLE 5). Unfortunately the group exposed to 5% NBW were lost two days after hatching because their water supply ceased and the NBW increased to 28%. Mortalities of sockeye and pinks reported in TABLE 5 exclude alevins incubated in baskets containing gravel.

TABLE 5 - Mortality of pink and sockeye alevins from hatching to complete yolk utilization.

Lot	Conditions		Mortality	
	Fertilization	Incubation	Number	Per Cent
		PINK SALMON		
A	Water	Water	2	1.5
B	1% NBW	Water	0	0
C	2% NBW	Water	0	0
D	5% NBW	Water	1	3.2
E	1% NBW	1% NBW	3	0.8
F	2% NBW	2% NBW	119	31.5
G	5% NBW	5% NBW	327	100
		SOCKEYE SALMON		
I	Water	Water	2	0.3
II	1% NBW	Water	1	0.3
III	2% NBW	Water	1	0.3
IV	5% NBW	Water	1	0.3
V	1% NBW	1% NBW	2	0.3
VI	2% NBW	2% NBW	10	1.6
VII	5% NBW	5% NBW	600*	100*

*Mortality due to accidental shock load of NBW.

A feature of all egg baskets exposed to NBW was the growth of slime. Visual comparisons indicated that the quantity of slime was roughly proportional to the concentration of NBW. However, except for those containing gravel, all baskets were cleaned before slimes smothered the eggs. At 2% NBW the eyed eggs placed in gravel were enveloped in clumps of slime and abnormally high mortalities occurred in this case (TABLE 6). As described above, eggs in 5% NBW were lost due to failure of their water supply.

TABLE 6 - Mortality of sockeye from the eyed stage to yolk utilization, with and without gravel.

Incubation Conditions	Mortality - Per Cent	
	With Gravel	Without Gravel
Water	6.3	5.0
1% NBW	0.3	1.0
2% NBW	35.7	6.0

Dry embryo weights indicated that pink salmon alevins in NBW developed at a slower rate than the controls (Lots A through D), as shown in FIGURE 1. Furthermore, they did not attain as great a dry embryo weight before their yolks were completely utilized and weight began to decline. To reach the complete yolk utilization stage, test fish required about 110 more thermal units than did control fish (a difference of about 15 days). The lag in yolk utilization was correlated with observations of "swim-up" activity. Swim-up by fry in Lots A, B, C and D preceded that in Lots E and F by about 14 days.

A statistical comparison (using Student's "t") of the maximum dry embryo weight of pink salmon control fry and the test fry, showed that at the 1% concentration of NBW the differences were barely significant ($P = 0.05$), while at the 2% concentration the difference in weights was highly significant ($P < 0.001$). Furthermore, there was a highly significant difference in weight between fry in 1% and in 2% NBW ($P < 0.001$).

Sockeye alevins, in contrast to pink salmon, showed no retardation of yolk utilization during exposure to NBW. Yolk absorption occurred at about 1706 thermal units at a temperature of about 46.5°F, which is normal for the Cultus stock when incubated under saturated dissolved oxygen conditions (Brannon, 1965). However, the mean dry weight of sockeye at complete yolk

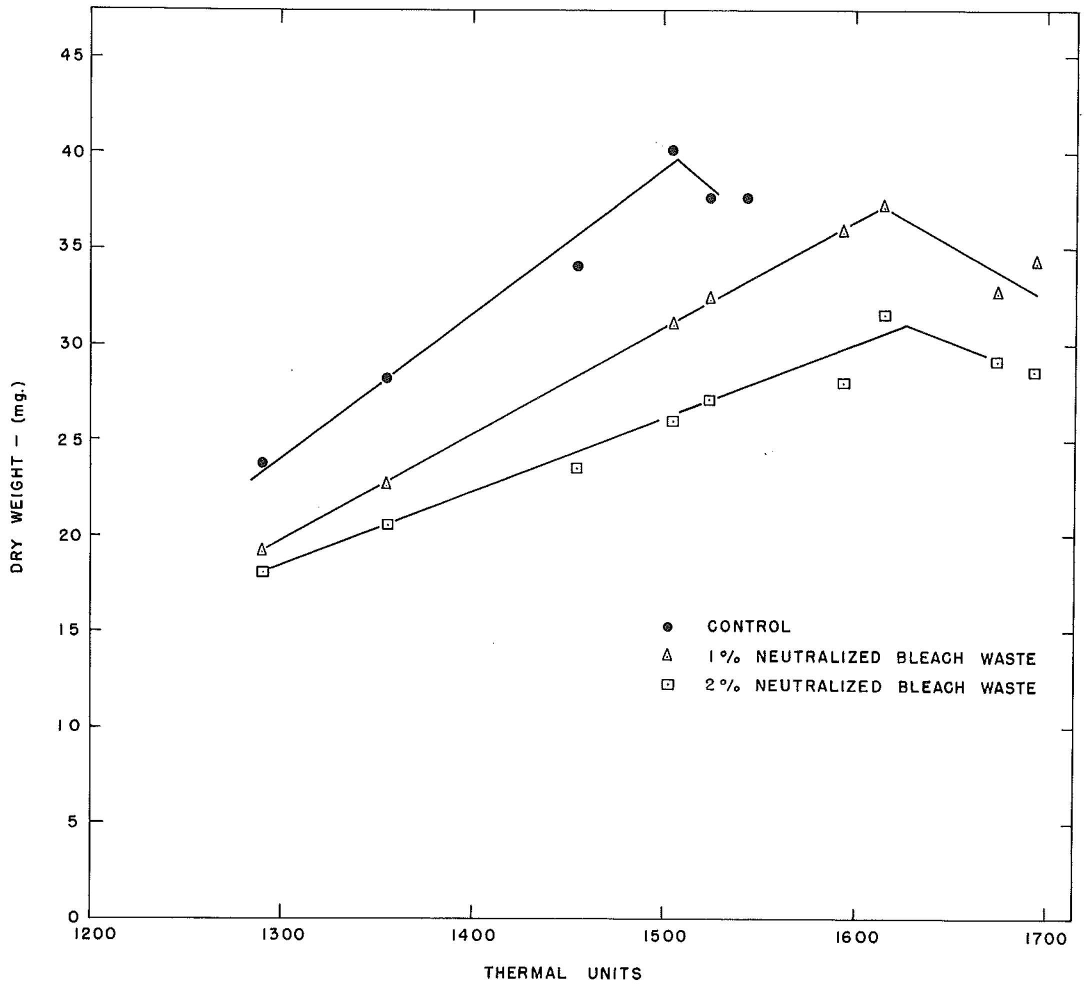

FIGURE 1 - Influence of neutralized bleach waste upon growth of pink salmon embryos between hatching and complete yolk utilization.

utilization decreased with increasing concentration of NBW (TABLE 7), indicating that growth of alevins was depressed by exposure to NBW.

TABLE 7 - Dry weight of sockeye fry at yolk absorption.

Lot	Incubation Conditions	Dry Weight, mg
Control	Water	17.95 ± 1.29
V	1% NBW	17.14 ± 1.48
VI	2% NBW	16.31 ± 1.31

Statistical comparison of dry weights in TABLE 7 showed that there was a highly significant difference ($P < 0.001$) between the control fry and those in 1% NBW, as well as between control fry and those in 2% NBW ($P < 0.001$). A comparison of fry in 1% and 2% NBW also showed a highly significant difference ($P < 0.01$).

Reduction of Neutralized Bleach Waste Toxicity by Treatment

Reduction of toxicity to sockeye fingerlings following storage of NBW in a vessel open to the air for one week was small; the 96 hour TL_m going from 19% to 25% (TABLE 8). Aeration of NBW for one day was no more effective than standing for one week without aeration. Continuous aeration for 13 days reduced NBW toxicity from a 96 hr. TL_m of 19%, to 44%. It is significant that during aeration the waste was undoubtedly contaminated with bacteria from equipment and the surroundings so that by the thirteenth day a light dispersed culture was evident. Although no measurement was made it was estimated that by the thirteenth day the concentration of biological solids would have been near 25 ppm. The bacteria undoubtedly played a major role in reducing BOD and toxicity during the aeration period. Biological treatment, in comparison to the foregoing methods of treatment, was the most effective means of reducing toxicity and BOD.

TABLE 8 - Reduction of BOD and of toxicity to sockeye fingerlings by various treatments of NBW.

Treatment	BOD ppm	96 hr. TL_m Per Cent Concentration NBW
None	188	19
Standing 7 days	-	25
Aeration: 1 day	162	25
2	-	35
5	144	35
7	140	37
13	117	44
Biological treatment:		
24 hrs., 100 ppm suspended solids	119	32 (approximate)
72 hrs., 55 ppm suspended solids	85	100% survival at 65% concentration in 96 hours.

To examine further the effect of BOD reduction on toxicity, additional trials of biological treatment were made using 24 hours aeration and various suspended solids concentrations. Initial BOD and toxicity (96 hr. TL_m) values were quite variable for the five samples of waste tested but in general the survival data (TABLE 9) indicated that with greater BOD reduction there was a pronounced trend toward lower toxicity. On the basis of these data, a BOD reduction of about 60 per cent could render the waste nearly non-toxic in a 96 hr. bio-assay.

The loading rate (ppm BOD reduction/ppm suspended solids/day) varied between 0.23 and 1.34 for the seven trials listed in TABLE 9. Ideally, the loading rate would be constant but, in these experiments, it may have varied because of changes in the ratio of rapidly metabolized compounds to slowly metabolized compounds in the waste. In addition, variation of the loading rate was undoubtedly influenced by build-up of the inert suspended solids fraction (dead bacterial cells and fiber from the waste) of the suspended biological solids (withdrawals of sludge were made when the build-up appeared excessive).

TABLE 9 - Reduction of BOD and NBW toxicity to sockeye fingerlings by biological treatment.[a]

Waste	Initial BOD ppm	BOD Reduction Per Cent	Suspended Solids ppm	Loading Rate[c]	96 hr. TL_m Before Treatment Per Cent Concentration NBW	Per Cent Survival in Various Concentrations of Treated NBW — Concentration-Per Cent: 30	50	65	75	85	100
A	282	8.5	106	0.23	27		95	10		0	
B	188	36	100	0.67	19	90	0				
C	351	39	102	1.34	23				70		0
D	204	45	274	0.34	14				100	90	
B	188	55[b]	55	0.63	19			100			
E	274	63	189	0.91	43					100	90
E	274	70	144	1.33	43					100	100

[a] 24 hour treatment except as noted.

[b] 72 hour treatment.

[c] ppm BOD reduction/ppm suspended solids/day.

DISCUSSION

Waste Variability

Results presented in TABLES 1 and 2 showed that bleach waste obtained from the mill varied in chemical, biochemical and toxic strength. These fluctuations occurred in spite of the fact that bleach waste was obtained when the mill was producing a constant grade of pulp. Furthermore, toxicity of waste declined slightly during storage in the laboratory (TABLE 8). Besides fluctuations in waste toxicity from a single mill there is undoubtedly variation among wastes from different mills; for example the kraft bleach waste used by Howard and

Walden (1965) was generally less toxic (96 hr. TL_m from 29% to 64%) than that which was used here. Variations of this type are to be expected since the toxicity of bleach waste will be related to type of wood, to severity of pulping and bleaching and to water use. There is a need to isolate the compounds responsible for toxicity so that concentrations of these compounds can be used as criteria during investigations of toxicity and regulation of pulp mill discharges.

It is important to realize that the results obtained in the present experiments are useful only insofar as they indicate a range of bleach waste concentrations which are likely to have lethal and sub-lethal toxic effects on salmon. It is not yet possible to designate precisely those concentrations which are safe since such concentrations are undoubtedly dependent upon environmental conditions, life stage of the fish as well as the characteristics of the particular wastes involved.

Waste Treatment

Toxicity was reduced very little by waste treatments which relied upon atmospheric oxidation or stripping with diffused air. These results are in agreement with the findings of Carpenter and Porter (1947) and Moggio (1948) cited earlier. Howard and Walden (1965) found only a negligible toxicity reduction with two days storage of neutralized bleach waste at $2^{\circ}C$. Five days storage increased the 96 hr. TL_m from 29 per cent for fresh waste to 46 per cent.

Although treatment by atmospheric oxidation was inadequate, biological treatment was able to reduce toxicity effectively. The results of seven trials made on five different loads of NBW indicated that toxicity reduction was related to BOD reduction. Biochemical oxygen demand reductions of approximately 60 per cent, obtained in 24 hours of biological treatment, rendered the waste nearly non-toxic in a four-day bio-assay of sockeye fingerlings. This result is in general agreement with the finding that biological treatment effectively removed toxicity of neutralized bleach waste to guppies (Howard and Walden, 1965).

Acute and Sub-Lethal Toxic Effects

The toxicity of a waste to fish or other water inhabiting organisms is often based upon the acute lethal toxicity in a short-term bio-assay, frequently 24 or 96 hours in duration. Realizing that for continuous exposure, lethal or sub-lethal toxic effects may occur at concentrations less than those found in short-term bio-assays, regulatory agencies have frequently assumed some fraction, often one-tenth, of the acute toxic concentration to be safe. This is a rule of thumb and may prove to be hazardous in some cases due to a variety of possibilities. Warren and Doudoroff (1958) point out that toxicity in the short-term may be of a different nature than that in the long-term experiment. Assumptions as to the safety of some fraction of the short-term lethal concentration may be expedient but there is general agreement that the results of detailed studies of long-term exposure experiments would be preferred information. Results from some exploratory long-term exposure experiments have been presented and it is interesting to compare these results with those from short bio-assays.

The 96 hr. TL_m of NBW using sockeye fingerlings was found to range from 12 per cent to 43 per cent while a 2 per cent concentration was found to be lethal to adult migrants in 20 to 29 days. Judging from the symptoms exhibited by fingerling and adult sockeye both groups of fish succumbed to the same type of toxic substance. Since the two loads of bleach waste used during the experiment with adult fish were not bio-assayed with fingerling sockeye, the toxicity of those wastes to fingerlings was unknown and therefore a direct comparison of susceptibility of fingerlings and adults is difficult. However, some known characteristics of the wastes enable a gross comparison. The BOD of the two wastes were 194 and 207 mg/l and the soda loss was 18.3 lbs/ton for both loads. From the standpoint of BOD the wastes used were about average while they were below average with respect to soda loss (TABLE 1). Although there is no correlation between BOD and toxicity of NBW to fingerlings it would appear from data in TABLES 1 and 2 that the two loads of waste used could have had a maximum acute toxicity corresponding approximately to a 96 hr. TL_m of 12%. It is important to note that although the minimum 96 hr. TL_m found was 12% (TABLE 2) there were no mortalities during that test at a 10% concentration, which correlates fairly well with results in TABLE 3 where mortalities were only 10% at a concentration which was 0.8 of the 96 hr. TL_m. Therefore, in order to obtain mortalities of fingerlings at a 2% concentration the 96 hr. TL_m should have been about 3%. Judging from characteristics of the wastes used, it is unlikely that toxicity to fingerlings would have been so great and consequently it appears that adults may be less tolerant to NBW. On the other hand, if the adults are not less tolerant than fingerlings the results indicate an even wider variation in NBW toxicity than is presented in TABLES 1 and 2.

If adults had a lower tolerance to NBW than fingerlings this may have been due to their physiological condition, the longer exposure or their greater activity. Herbert and Shurben (1963) found that toxicity of ammonium chloride and zinc sulfate to rainbow trout was increased by activity and they suspected that accelerated metabolic rate, resulting from increased activity, caused more of the toxic material to be absorbed by the fish. In the present experiments the adult fish swam against a much greater current than that which faced the sockeye fingerlings and this activity may have resulted in more uptake of toxicants and consequent mortality at a lower concentration.

Extended exposure beyond 96 hours, according to Howard and Walden (1965), did not increase mortalities of sockeye fingerlings exposed to neutralized bleach waste. In contrast to those results, adult sockeye were affected only after extended exposure. Of course, the adult fish were undergoing natural degeneration and this may have made them more susceptible. As pointed out earlier it cannot be concluded definitely, from the data at hand, whether or not adult fish were more susceptible to prolonged exposure to NBW than were fingerlings in a short exposure, but the data suggest that they might be and this possibility should be investigated.

Obviously the results have raised some questions concerning the effects of environment, age and physiological condition on the tolerance of fish to NBW and these questions must be considered in further research. With present knowledge it would be premature to assume that under natural conditions 1% NBW will not be harmful to adult sockeye or that 2% NBW will be harmful.

Both sockeye and pink salmon alevins were adversely affected by NBW at concentrations much below the average 96 hr. TL_m of 22%, although there were some differences in response. Pink salmon alevins suffered high mortalities at 2% concentration of NBW but there was no evidence that 2% concentrations affected the survival of sockeye alevins. Thus there is evidence that mortality may be species dependent. It is possible that the apparent greater susceptibility of pink salmon alevins was due to some undetected disease. This is considered to be unlikely since mortality of pink alevins was proportional to NBW concentrations. However, NBW might lower the resistance of fish to disease and in this way act to increase mortality. This is certainly a point worthy of further research.

Dilute NBW had no apparent ill effect on the process of fertilization nor upon embryos prior to hatching. However, pink salmon alevin development was retarded in NBW so that complete yolk utilization in 1% and 2% NBW was attained about 15 days after that of the controls. Furthermore, the maximum weight attained by the fry in 2% NBW was about 22% less than that attained by the controls. The effect on alevin growth of 1% NBW was not so pronounced but even at this concentration, maximum weight was about 7% less than that of the controls.

In contrast to pink alevins, complete yolk utilization in sockeye alevins incubated in NBW was not delayed. However, as with pink salmon, the weight at yolk absorption was depressed by long-term exposure of sockeye alevins to dilute NBW.

Since there is apparently some difference in response to NBW by these two closely related species of salmon, even greater differences in response might be expected between salmonids and more distantly related groups of fish. For the sake of convenience, bio-assays are often conducted on a species of fish which is easy to handle in the laboratory and the results applied, using an application factor, to another species. Such extrapolation obviously must be undertaken with great care and a thorough knowledge of the two species in question.

The foregoing results with pink salmon eggs and alevins are in general agreement with results of an experiment conducted earlier, using neutralized acid bleach waste. In the earlier experiment the growth of alevins in 1% and 2% concentrations was retarded and the maximum size was less than that of the controls. The acid bleach waste was obtained from the same pulp mill under the same pulp mill production conditions as the waste used in the current experiments, except that gases from digester blow and relief plus underflow from turpentine recovery were not excluded as in the present experiments. Due to the intermittent flow of these additional wastes into the acid sewer, the amount present in the waste undoubtedly varied during the earlier experiment. However, it is apparent that the exclusion of these portions of the waste in the present experiment did not prevent the deleterious effects on growth.

Mechanisms of Toxicity

The mechanisms causing limited growth, retardation and mortalities are not revealed in these experiments and much work must be done before mechanisms are elucidated. One necessary investigation is a thorough analysis of bleach waste to determine the constituent compounds. Model compound studies have indicated the possible presence of tetra-chlorocatechol. If tetra-chlorocatechol is present may not pentachlorophenol be present also? In metabolic systems, pentachlorophenol acts as an uncoupler of oxidative phosphorylation, which is a cause of inefficient metabolism and in high concentration, of mortality. The results presented here fit this pattern and it is interesting to speculate that uncoupling may play a role in the toxicity of bleach waste. However, uncoupling of oxidative phosphorylation is often accompanied by increased respiration and this does not fit well into the picture presented by the results because, if yolk absorption rate can be used as an index of metabolic rate, it appears that metabolism was depressed in pink salmon alevins and unaffected in sockeye alevins.

Some results from hatchery research suggest that dissolved oxygen transfer may be related to delayed yolk absorption. Brannon (1965) depressed the dissolved oxygen to 50% of saturation and found that growth was delayed but that the final weight at yolk absorption was equal to that of the fry grown under saturated dissolved oxygen conditions. In the experiments reported here, dissolved oxygen was kept very close to saturation at all times. However, if the extraction of oxygen by the alevins was depressed by NBW, the result might be to slow yolk absorption, as occurred with pink salmon alevins. It is not clear why yolk absorption was delayed in pink salmon alevins but not in sockeye.

Gravel Incubation

The high mortality (35.7%) of eggs and alevins which were incubated in gravel at 2% NBW was probably caused by suffocation. Although the total flow through the gravel incubation basket was the same as in the baskets without gravel, about $\frac{1}{2}$ in. to 1 in. additional head was required to maintain this flow when slime growths clogged the gravel in 2% NBW. Although the apparent velocity in these experiments was 146 cm/hr (1.337×10^{-3} fps), and good egg survival can be expected under natural conditions at apparent velocities of 20 cm/hr, slime growths completely enveloped some eggs and undoubtedly suffocated them. Since slimes metabolize organic compounds in NBW as the mixture passes through gravel, the amount of slime which develops is dependent upon the flow rate and temperature, as well as on the concentration of NBW in the water. A detailed study would be required in order to predict accurately the amount of waste that would cause detrimental slime growths in a river.

Further Research

The results presented in this report are the initial data in a program designed to study toxicity of NBW and its removal. Although preliminary, the results indicate that the short-term bio-assay is an inadequate index for evaluation of toxicity. It, of course, has its usefulness as a control method for discharge of a waste which has been studied for its long-term effects.

The studies reported here have not accounted for the fact that conditions in a natural environment may be less favorable than in laboratory conditions. For example, in the long-term experiments, temperature, dissolved oxygen and velocity were favorable to the fish, eggs and alevins. But, judging from the work of Alderdice (1963), it is conceivable that toxic effects would have been more severe or might have occurred at lower concentrations, if dissolved oxygen had been low or temperatures unfavorable. Migrating anadromous fish are sometimes faced with severe natural conditions which impose a considerable stress upon them. Additional stress created by a pollutant may prevent them from completing their journey. If spawning beds are naturally silted and water movement through the gravel were barely sufficient to ensure alevin development, then what would be the outcome of minor additional clogging of gravel due to the growth of slime?

It is obvious that further research is required to answer the many questions which come to mind concerning bleach wastes and salmon. Future studies will be made to determine whether biological treatment removes long-term toxic effects. The toxicity, and removal by treatment, of particular compounds found in bleach waste will also be investigated. The mechanisms by which lethal and sub-lethal factors operate must be determined. Finally, the influence of bleach waste upon the relationship between salmon and its variable natural environment must be elucidated.

CONCLUSIONS

1. The toxicity of NBW to sockeye salmon fingerlings can be substantially reduced by biological treatment.

2. The most significant sub-lethal effect found was poor growth exhibited by sockeye and pink salmon alevins incubated in concentrations of NBW about one-tenth to one-twentieth of the average 96 hr. TL_m for sockeye fingerlings.

3. Neutralized bleach waste promotes the growth of slime in gravel and this can result in increased mortality of incubating eggs and alevins.

4. Exposure of adult sockeye to neutralized bleach waste did not harm viability of ova and sperm.

5. Additional research is required to confirm whether or not adult migrant sockeye are more susceptible to toxicity of NBW than are fingerlings.

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