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THE EFFECTS OF SALMON CANNERY WASTE ON
JUVENILE SALMON IN A CLOSED SYSTEM

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SUMMARY AND CONCLUSIONS

- (1) Salmon wastes held in a laboratory under static bioassay conditions can cause mortalities of selected juvenile salmonids after prolonged exposure at concentrations greater than 3,300 ppm (wet weight of salmon waste to volume of sea water). These concentrations are rarely, if ever, found in receiving waters near salmon cannery discharges because of the rapid dilution and dispersion of wastes due to tidal action.
- (2) In the static system, significant changes in certain water quality parameters were apparent. These included dissolved oxygen (DO) decreases, increases in turbidity, and pH decreases. Similar changes are not found around discharges (due to dilution) which accounts for the lack of any significant ecological damage to the environment at most cannery locations.
- (3) Two major factors caused mortalities during the bioassays. The first was a decrease in dissolved oxygen below minimum levels due to the high biochemical oxygen demand (BOD) of the wastes despite addition of air to the aquaria. Mortality was somewhat alleviated when oxygen was maintained at supersaturated values; however, delayed mortalities still occurred. The second factor was probably some product or products of decomposition. Comparisons of mortality curves of fresh, frozen, fresh-frozen, and stored waste held for 16 hr provided some evidence for this. The longer the waste was held before testing, the more toxic it became.
- (4) pH data and ammonia concentrations tended to substantiate the increased decomposition-increased mortality relationship. Ammonia, a product of decomposition, is toxic in the unionized form, but at the pH levels observed in these experiments, most of the ammonia was probably in the ionized form and therefore should not have been a direct contributor to the mortalities. However, further studies are needed to confirm this idea.
- (5) Screening to remove solids, activated charcoal and glass wool filtering, and Whatman No. 4 filter paper were all ineffective in removing the toxicant from the waste. The critical factor was associated with the liquid portion of the wastes rather than with the solids.
- (6) Cotton filtering and alum flocculation showed a marked reduction in mortalities with a large delay occurring with cotton and complete elimination of mortalities with alum flocculation when pH was controlled.

THE EFFECTS OF SALMON CANNERY WASTE ON
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INTRODUCTION

Salmon canneries commonly discharge their waste materials directly into estuarine or marine waters. These wastes consist of heads, tails, viscera, and blood. Several types of discharge practices exist. They range from untreated discharge to complete removal of solids with only liquid discharge. Whenever it is economically feasible, some of this waste is reclaimed. Typical examples are the grinding and freezing of salmon cannery waste solids for later conversion to fish or mink food, oil extraction from heads, and use of eggs for caviar by the Japanese. Complete recovery, better known as the total utilization concept (TUC) (vaa Haagen, 1972) is still in the developmental stages for the salmon canning industry. Currently, the TUC involves recovery of protein in wastes for use as an animal feed or human use. Eventually it is hoped that all wastes can be recovered and processed as saleable products. However, in many areas such as Alaska, transportation costs, operating costs, short canning seasons, and poor keeping qualities of salmon waste make total utilization impractical at present.

Field studies on the impact of salmon cannery wastes on the water quality and biota of the marine receiving water were reported for canneries at Petersburg, Kodiak (Port Bailey, Alitak, and Larsen Bay) and Naknek, Alaska (University of Washington, 1971 and Nakatani et al., 1972). No significant damage from a biological or ecological aspect was observed in the field for these canneries. Despite this lack of demonstrable damage in the field studies, these salmon canneries may be required to treat their

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wastes, possibly by primary treatment or even by secondary treatment, before discharge to marine waters. One method of treatment being explored is to screen the solids and discharge only the liquid effluent (Cornell et al., 1972). With this method, the larger solids are removed. However, the liquids are discharged and hence, the need to evaluate this liquid effluent from a biological aspect.

The scope of the work in this study was to obtain, in the laboratory, the upper or critical concentration values of salmon wastes which would elicit a detrimental response from test fish (juvenile salmon). Although the fresh salmon wastes being discharged by operating canneries are not toxic to aquatic life, the intent in the laboratory was to create or simulate the "worst" conditions. Because the actual field situation near a salmon cannery outfall is often complex and unique, the laboratory work here does not attempt to simulate a particular field condition. However, the laboratory study does provide a better and much needed understanding of the problem. Also, it provides some reference data needed for improved assessments of field situations and provides an evaluation of different waste treatments being explored.

Specific Objectives

The field work in Alaska and some preliminary laboratory work indicated that at least two major factors, low dissolved oxygen (DO) and toxic products of decomposition of salmon wastes, might be critical for aquatic organisms subjected to salmon wastes. Thus, this study focused its effort on these two factors as related to effects of salmon wastes, and used the technique of bioassays to reach a better understanding of the problem of assessing the biological effect of salmon waste discharge.

As the bioassays progressed, they developed into several areas of emphasis, namely to determine the:

- (1) effect of various concentrations of waste on fish,

- (2) effect of dissolved oxygen concentrations on critical concentrations of salmon wastes (those concentrations at which mortalities are observed),
- (3) effect of quality (state of decomposition) of waste,
- (4) effectiveness of screening and filtering the waste (simulated waste treatment) in reducing mortalities, and
- (5) ammonia concentrations and pH changes in relation to decomposition of the waste.

METHODS AND MATERIALS

The static bioassay (American Public Health Association, 1971) was the most practical method for the available facilities. The continuous flow system was not utilized primarily because the amount of waste required was prohibitively high at the concentrations that were needed to elicit a response.

Locations

The bioassays were conducted on the R/V *Kumtuks*, the College of Fisheries' floating laboratory, between April 13 and August 28, 1972. During the first part of the study designated as series A, the *Kumtuks* was anchored in sea water adjacent to the northern shore of Kiket Island, Washington. The *Kumtuks* was returned to the University on July 16 and further tests, series B, were conducted at this location. A summary of information pertaining to series A and B is presented in Table 1.

Test Species - Juvenile Salmon

Juvenile chum salmon [*Oncorhynchus keta* (fork lengths: 42 to 60 mm, body weights: 0.2 to 3 g)] from the Quilcene National Fish Hatchery, chinook salmon [*O. tshawytscha* (51 to 70 mm, 0.9 to 3.1 g)] from the Washington Department of Fisheries Skagit Fish Hatchery, and coho salmon [*O. kisutch* (59 to 88 mm, 3.3 to 5.4 g)] from the Washington Department of Fisheries Issaquah Fish Hatchery represented the test fish for the bioassays.

Table 1. Summary of series A and B of bioassays

	Series A	Series B
Location	R/V <i>Kumtuka</i> - Kiket Island, Washington	R/V <i>Kumtuka</i> - College of Fisheries
Dates	April 13 - July 15, 1972	July 16 - August 28, 1972
Wastes tested	Frozen	Frozen, fresh, fresh-frozen and fresh stored (16 hr at 4 C)
Temperature	Variable (ambient) at 9 to 13 C	Constant 10.5 ± 0.5 C
Salinity	Variable between bioassays (ambient) at 15 to 32 ppt	Constant at 30 ppt (from College of Fisheries storage tanks)
Fish, type of bioassay	Chum - Wide ranged concentration tests Chum - simulated treatment tests including: a. Stainless steel screens b. Solids versus liquids Chinook - wide ranged concentration tests Chinook - simulated treatment tests including: a. Cotton b. Activated charcoal c. Glass wool d. Glass wool and activated charcoal e. Alum flocculation f. Whatman No. 4 filter paper (1)	Coho - Wide ranged concentration test - Effect of quality of waste bioassay

Salmonids were selected because they are relatively sensitive to pollutants. Also, they are economically important fish found in canning areas.

The chums, utilized for tests between April 13 and June 14, were held in freshwater in a 4-ft diameter continuous-flow fiberglass tank (120 gal capacity) for several days prior to departure of the *Kumtuks* from the University. As the *Kumtuks* was towed to Kiket Island, the water in the tank was changed from fresh to sea water (up to 30 ppt). No mortalities occurred as a result of this salinity increase. The fish were maintained at ambient temperatures (8 to 13 C) and salinities (17 to 30 ppt).

The chinooks, tested between June 1 and June 29, were transferred directly from a hatchery truck (freshwater) to floating pens (sea water) that were moored alongside the *Kumtuks*. After 4 days, some of these fish were transferred to the 4-ft circular tank inside the laboratory and were used as necessary for the bioassays.

The coho were tested after the *Kumtuks* returned to the University (series B). The fish were transported from the hatchery in freshwater tanks, held for 3 days in freshwater, and then transferred to a 4-ft circular tank in the College of Fisheries sea water room (30 ppt salinity, 13 ± 1 C). They were later transferred to the test aquaria on the *Kumtuks* 24 hr prior to the bioassays. All three species of fish were maintained on Oregon Moist Pellet with no feeding 24 hr before each series of bioassays.

Bioassay Apparatus

Test containers were 5 gal glass aquaria with 15 liters of sea water as the test volume. At first, turbidity due to the wastes obscured observations of the fish and made removal of dead fish difficult. Therefore, a plastic liner made of non-toxic "Vexar" and nylon monofilament line was placed into each aquarium. This liner was raised to count and remove

the dead fish at each observation. Control fish in sea water with no wastes were raised at the same time to subject them to handling conditions similar to the experimental fish.

The aquaria were placed in a water bath which ranged (during series A) from 9 to 13 C and varied ± 1 C for a specific test according to ambient conditions. During series B, a cooling unit was necessary to keep temperatures at a constant 10.5 ± 0.5 C. All temperatures were similar to those found in canning areas in the Pacific Northwest and Alaska.

Depending on the particular test, either air or pure oxygen was used for aeration. The source of air was aquarium pumps (designated as regular aeration) whereas pure oxygen was obtained from an oxygen cylinder with regulators (supersaturation). Delivery to the aquaria was through tubing and diffusion was by air stones.

Sources and Quality of Salmon Wastes

Fresh waste which was not available until series B was obtained from a salmon cannery operation at La Conner, Washington. This waste was taken from adult sockeye salmon (*O. nerka*) that were on ice in the cannery's fish bins. The fish were hand-butchered to obtain viscera, blood, fins, and kidneys. The waste was kept in plastic buckets surrounded by ice during the trip to the University. Testing started immediately after arrival at the University (approximately 3 hr after removal of waste from the fish) to keep the waste as fresh as possible.

Because fresh salmon wastes were initially not available, frozen salmon wastes consisting of viscera, fins, and bones were used as test materials for some of the bioassays. This waste was obtained from a fish-food processing plant. It was gathered from various canneries in Washington in 1971, frozen, and stored in 50-lb bags for at least

9 months. Clearly, the quality of this frozen salmon waste was not representative of salmon cannery wastes, but the lack of fresh wastes and the need of test materials for working out the experimental protocol provided no alternative but to work with frozen wastes during the early tests. Two other types of wastes, fresh-stored and fresh-frozen, were also bio-assayed.

All wastes tested were passed through a hand-operated meat grinder (1/4 inch openings) before each test, except in the case where frozen wastes were ground prior to freezing. It should be emphasized that none of the wastes, either fresh or frozen, were obtained from the discharge of a fish processing plant, as such, because of the need to control concentrations by taking a known amount of waste and diluting it to the appropriate concentrations.

In further discussions of types of waste, the following terms will apply:

<u>Term</u>	<u>Waste source</u>	<u>Type of handling</u>
Fresh	Salmon cannery operation, La Conner, Washington	Obtained fresh from fish
Fresh-stored	"	Fresh waste refrigerated for a known length of time (16 hr at 4 C)
Fresh-frozen	"	Fresh waste frozen for a known length of time (1 to 2 weeks)
Frozen	Fish food plant, La Conner, Washington	Frozen for at least 9 months but exact time unknown; thawed for 12-15 hr at 10 C

Water Quality

Salinity, temperature, pH, turbidity, DO, and ammonia concentrations were monitored during the bioassays. Following is a brief description of the methods or instrumentation used to determine each parameter:

Salinity - modified Goldberg refractometer, Model 10423 (precision of ± 0.5 ‰),

Temperature - glass - mercury thermometer with a precision of ± 0.1 C,

pH - Corning research pH meter and glass electrode (precision of ± 0.1 pH units),

Turbidity - Hach turbidometer,

DO - Yellow Springs Instrument Corporation oxygen meter and stirring probe (precision of ± 0.2 ppm),

Ammonia (as NH_3) - modified method of Burnett (1965) which is available from the Food and Drug Administration.

The ammonia was measured in the fresh and frozen forms of undiluted waste and the values obtained were used as an index of decomposition (Burnett, 1965).

Critical Concentrations and Quality of Waste

To establish the critical concentrations of fresh waste needed to cause mortalities, a wide range of concentrations was tested. These ranged from 20,000 ppm (wet weight of salmon waste to volume of sea water) to 300 ppm and zero for controls. The highest concentration of 20,000 ppm was based on one of the higher concentrations estimated in a cannery effluent by Brooks et al. (1970) and was approximately 2/3 of the peak concentration observed at Petersburg, Alaska in 1971 at one of the cannery outfalls. Juvenile salmon representing test fish species were placed in the aquaria 24 hr prior to testing. Initial agitation to disperse the waste was accomplished by gently raising and lowering all aquaria liners. Continued dispersion of the waste during the bioassay was maintained by the action of the aeration systems. Both

types of aeration, regular and supersaturation, were used. At each observation, dead fish were removed, recorded, and lengths and weights measured.

Four types of wastes, frozen, fresh-frozen, fresh-stored (16 hr at 4 C) and fresh, were tested to determine the effects of the quality of the wastes. The frozen and fresh-frozen wastes were thawed for 12 to 15 hr at 10 C and then utilized for bioassay. Although only the fresh waste was representative of most salmon cannery wastes, the other three types of waste were expected to elicit a response at lower concentrations than fresh waste, and hence, show that the quality of wastes, in addition to concentration, must be considered.

Wide ranged critical concentration tests were also conducted with the chum and chinooks. These tests were conducted with frozen waste only.

Screening and Filtering of Wastes

Simulations of screening to remove suspended solids were tested to determine if this would improve the quality of the waste water as related to the time-mortality curves. In these tests, waste water (20,000 ppm) was thoroughly mixed in one aquarium and drained through a stainless steel screen into a second aquarium. The residual solids were removed from the screen to test the differences in mortalities between the solids and the screened liquids. Screen sizes used were 20, 40, and 60 mesh. A 5-mesh "Vexar" plastic screen was also tested.

Filtrations of liquid wastes, screened by 60 mesh to remove the solids, included filters made of absorbent cotton, activated charcoal, glass wool, and an activated charcoal and glass wool combination. Also tested by bioassay was filtration through Whatman No. 4 filter paper and flocculation with alum (AlKSO_4). (For techniques or proportions of the filtration materials, see Appendix 1). A preliminary alum bioassay indicated that the pH dropped to 4.8, a level considered critical for salmonids (State of California, 1957), so a

further bioassay with pH adjustment (by adding NaOH) to 7.1 was necessary. For the screening and filtering tests, two control groups, namely, an untreated "control" (straight waste) and a control without any waste were bioassayed simultaneously with the waste treatment groups. Only frozen waste was used in these screening and filtration tests.

RESULTS

Critical Concentrations of Wastes

Despite the hazards of providing "critical" concentrations by bioassays, these reference data do have their utility if used with caution. Figure 1 shows the cumulative mortality curves of juvenile coho salmon subjected to fresh salmon wastes ranging in concentration from 20,000 ppm to 1,300 ppm. The effect of low DO was eliminated by keeping the test solution super-saturated with oxygen; and thus, the fish were presumed to be responding to toxic substances in solution. As expected, the mortality curves were in **order** of concentration, i.e., a higher concentration of waste caused earlier mortality. Also, the results showed that the initial mortality, even at 20,000 ppm, did not occur until after 20 hr, well beyond the time of a tidal cycle. This was an important finding, mainly because most salmon wastes are "washed" to sea with each tidal cycle. The lowest concentration that elicited a response after 48 hr exposure was 3,300 ppm waste or approximately 1/9 of the peak discharge observed at Petersburg. However, the only concentration at which 100 per cent mortality was observed was 20,000 ppm. Complete mortalities were not observed at the lower concentrations.

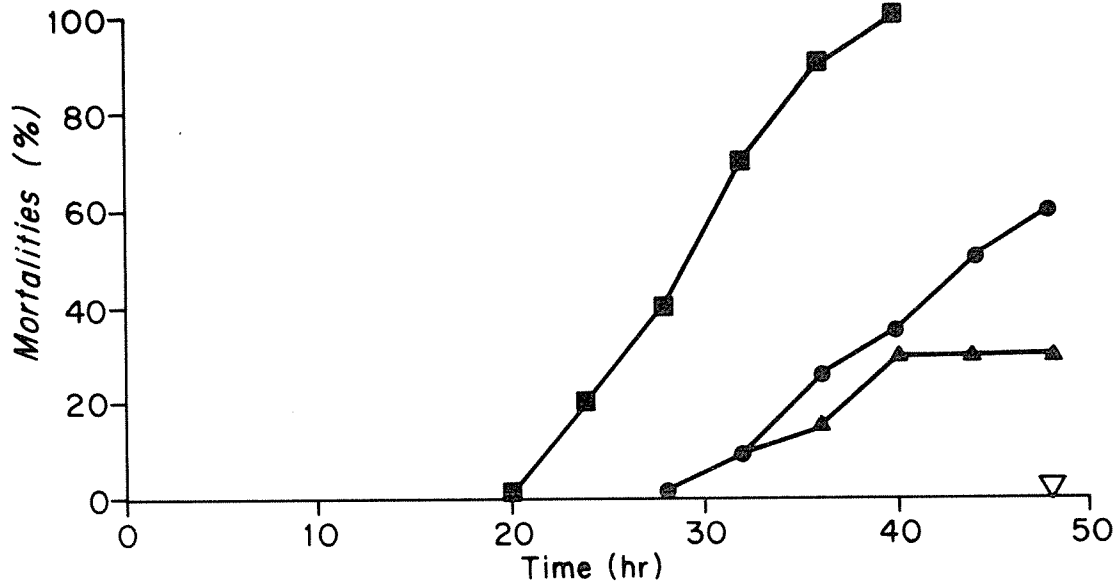


Fig. 1. Effects of fresh salmon waste on juvenile coho salmon. Tests conducted August 10-12 and 23-25, 1972. N=20 fish/concentration, supersaturation aeration.

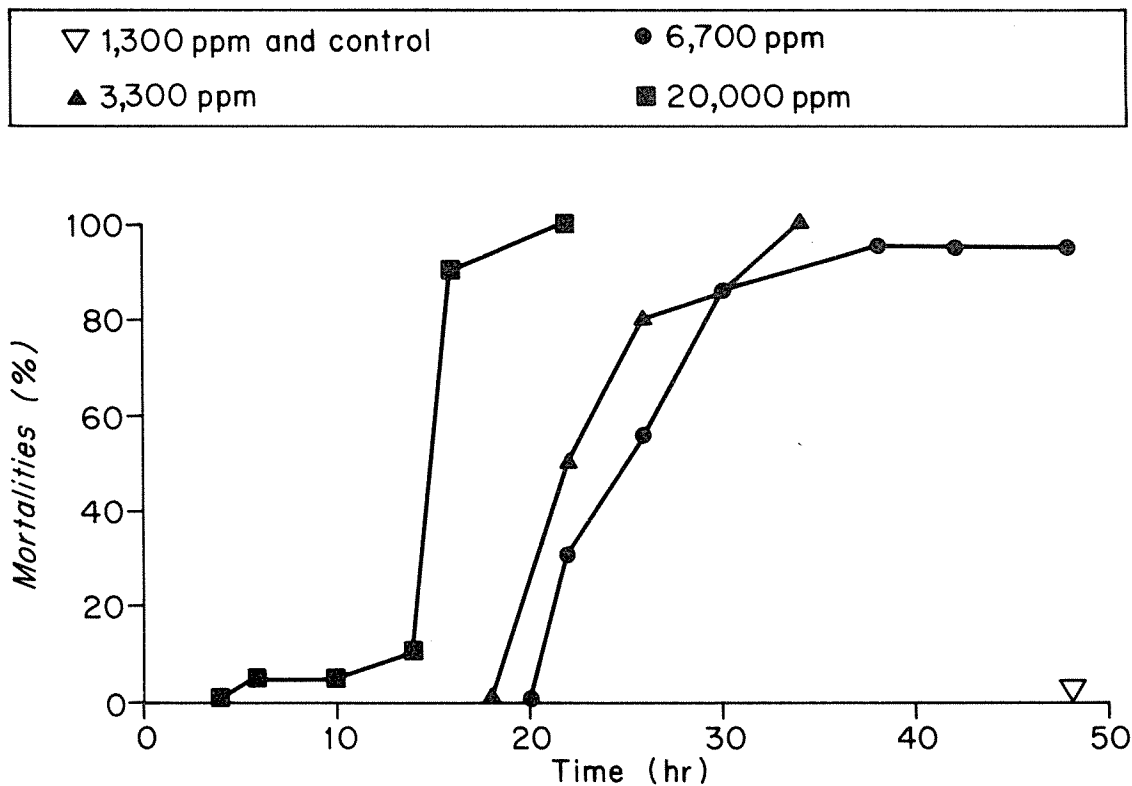


Fig. 2. Effects of concentration of fresh cannery waste on juvenile coho salmon, August 1-3, 1972. N=20 fish/concentration, regular aeration.

Effect of Low DO

When regular aeration was used with juvenile coho, a definite shift of the mortality curves to the left was observed (Fig. 2). All fish at 20,000 ppm were dead before 20 hr exposure compared to a minimum of 20 hr needed to elicit a response in the previous tests with supersaturation. At the lower concentrations of 6,700 and 3,300 ppm, mortalities with regular aeration were almost 100 per cent compared to 60 and 30 per cent, respectively, after 48 hr exposure, with supersaturation. Most of the mortalities with regular aeration were directly related to decreases in DO to subminimal concentrations [less than 5 ppm (State of California, 1957)]. Figure 3 shows the DO concentrations and mortalities that occurred in the individual aquariums. An inverse relationship was obvious at the higher waste concentrations with mortalities observed soon after the DO declined to 5 ppm. A crossing of curves between the 6,700 and 3,300 ppm concentrations was observed (Fig. 2) and was probably related to one fish that survived at 6,700 ppm waste. Also, inefficient aeration by some air stones that resulted in unexpected DO decreases and premature mortalities, may have been a factor in the crossing of curves.

Effect of Quality of Waste

Figure 4 illustrates the results of the test comparing frozen, fresh-frozen, fresh-stored, and fresh wastes, each at a concentration of 20,000 ppm. Dissolved oxygen was maintained at 20 ppm (supersaturation) and, thus, was not a limiting factor in this test. The results were as expected. Increased storage time or frozen wastes resulted in mortalities within a shorter period of exposure than for fresher wastes. As a result, the test fish, juvenile coho, were believed to be responding to products of degradation of the salmon wastes.

The chinook and chum bioassays (series A) with frozen waste and regular aeration showed that the mortality curves of these species (Fig. 5) were

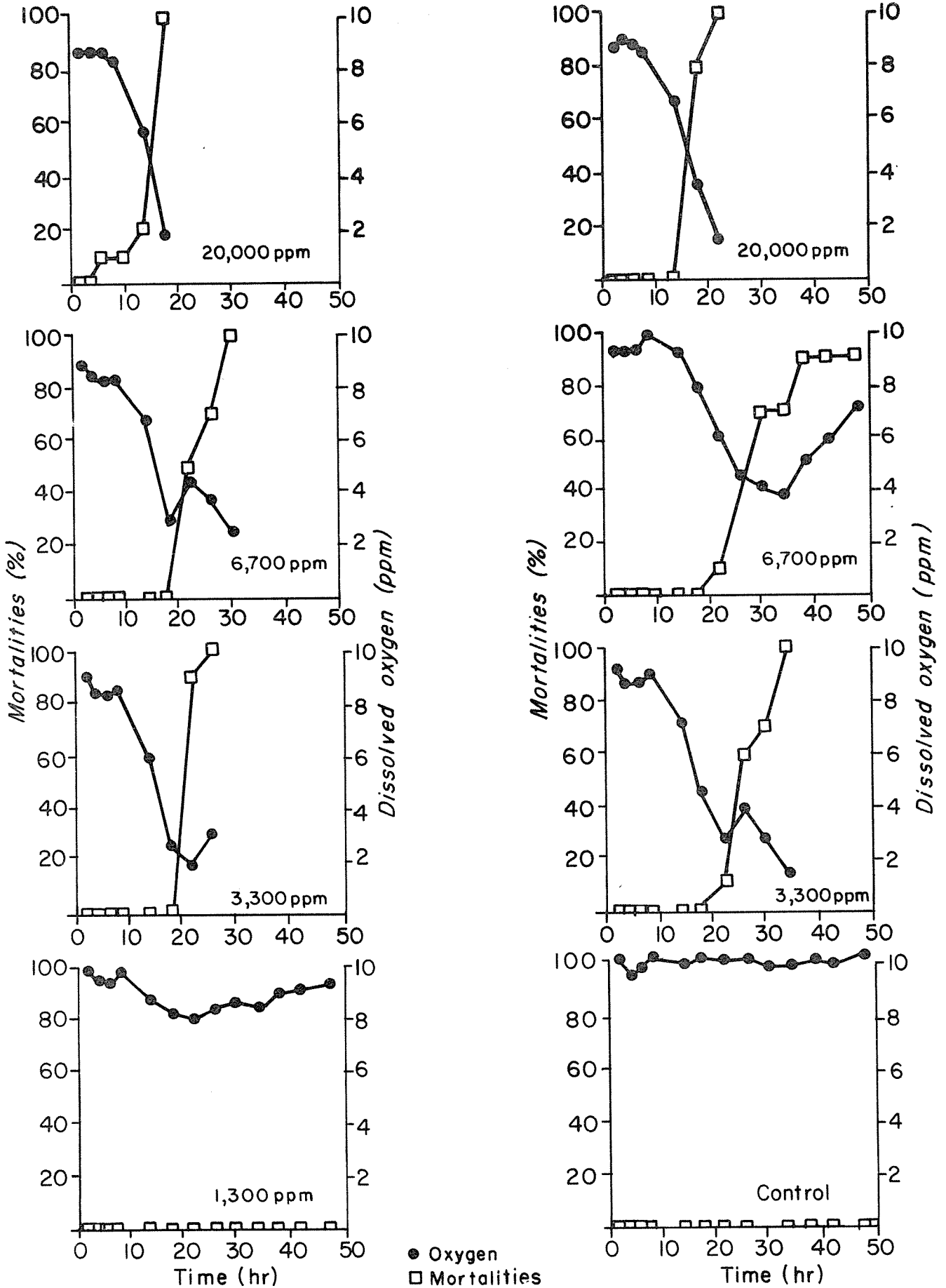


Fig. 3. Mortalities in each individual aquarium as related to dissolved oxygen during coho bioassay (Fig. 2), August 1-3, 1972.

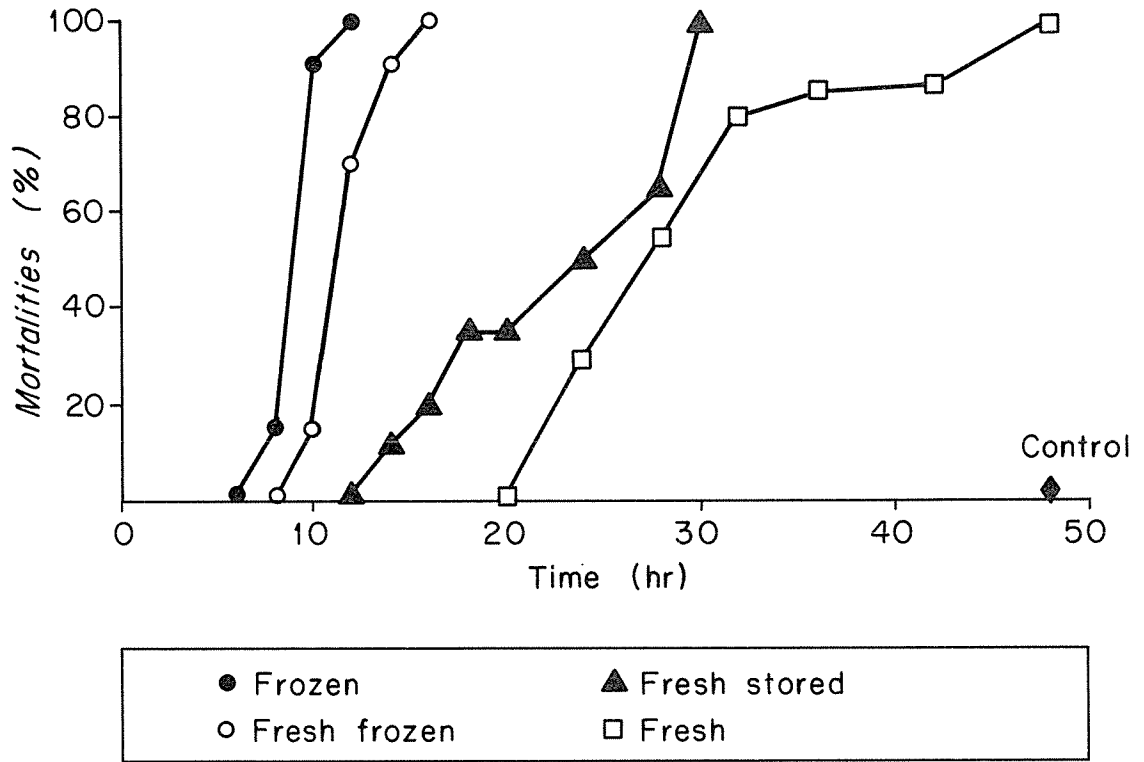


Fig. 4. Effects of quality of salmon waste (20,000 ppm) on juvenile coho salmon. Tests conducted August 10-12 and August 23-25, 1972. N=20 fish/test, supersaturation aeration.

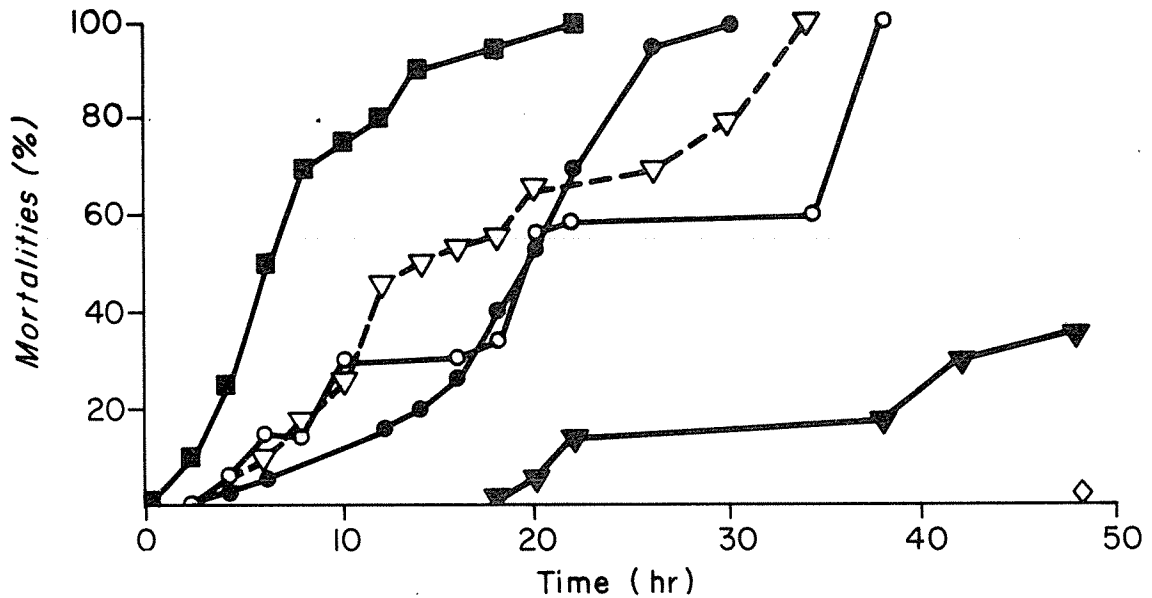


Fig. 5-a. Cumulative time-mortality curves of the effects of concentration of frozen salmon waste on juvenile chum salmon, May 3-5, 18-20, and 22-24, 1972. N=35 fish/concentration, regular aeration.

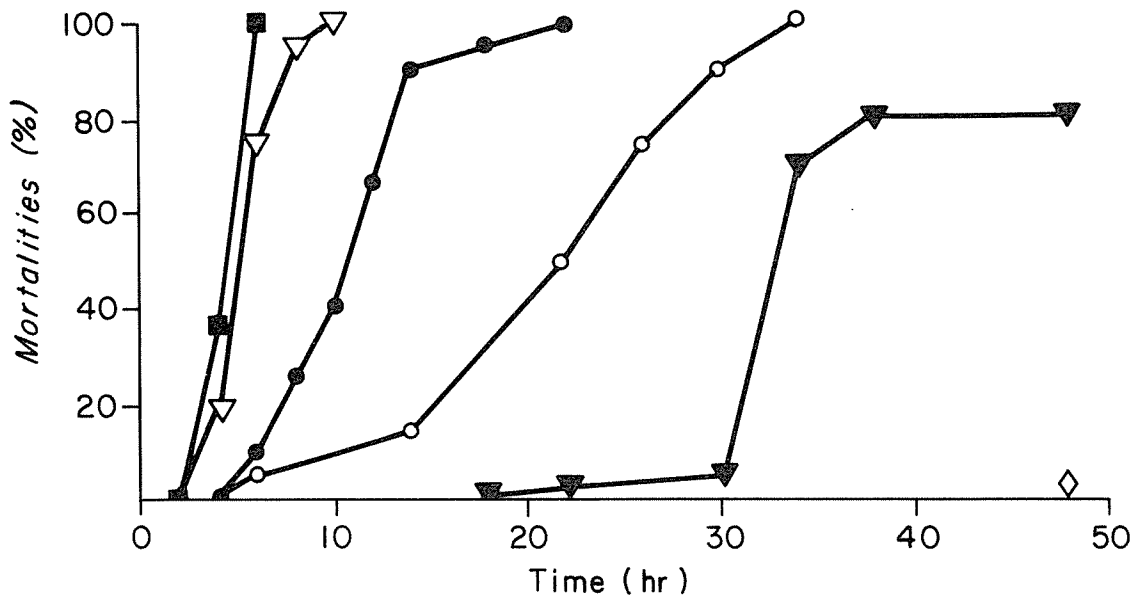
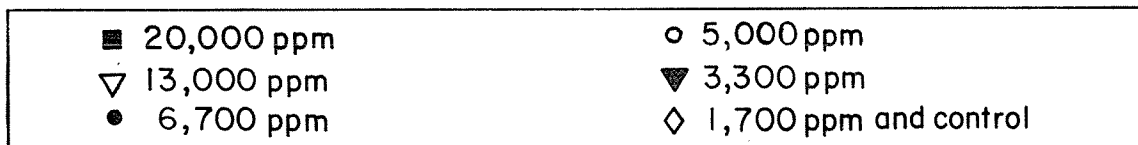


Fig. 5-b Cumulative time-mortality curves of the effects of concentration of frozen salmon waste on juvenile chinook salmon, June 21-23, 1972. N=20 fish/concentration, regular aeration.

similar to the curves of coho exposed to fresh waste. However, mortalities occurred earlier. This follows the relationships established during the tests on quality of wastes and effects of oxygen. If fresh waste and supersaturation were used with the chinook and chum salmon, the time mortality curves would be expected to be very similar to the coho (Fig. 1). The mortalities in the chinook and chum bioassays were directly related to oxygen depletion which the regular aeration system could not overcome. Also, decomposition products probably contributed to the mortalities but their direct effects were masked by the oxygen depletion. The crossing of curves between concentrations, previously observed in the coho (regular aeration) bioassay, was again observed with the chum salmon.

Screening and Filtering of Wastes

Screening of 20,000 ppm frozen waste, at least to the 60-mesh size, was not effective in reducing mortalities (Fig. 6) with all fish in the screened waste dying within 8 hr of exposure. Dissolved oxygen was apparently not limiting since monitoring the oxygen showed values of 8.6 ppm or higher. The bioassay of the residue, or the solids removed by screening of 20,000 ppm waste, showed no mortalities within 48 hr. Therefore, the critical components of the wastes were contained primarily in the soluble fraction or liquid portion of the waste. Following this finding, the remaining treatments were applied to the screened liquid (from 20,000 ppm frozen waste) portion to find a possible means of removing the critical component (Fig. 7).

Filtration of wastes by using activated charcoal, glass wool, and the combination of the two did not remove the critical component in the liquid wastes, at least not at the 20,000 ppm concentration (Fig. 7-a). Also, there were no apparent differences in mortality rates among the three waste treatments.

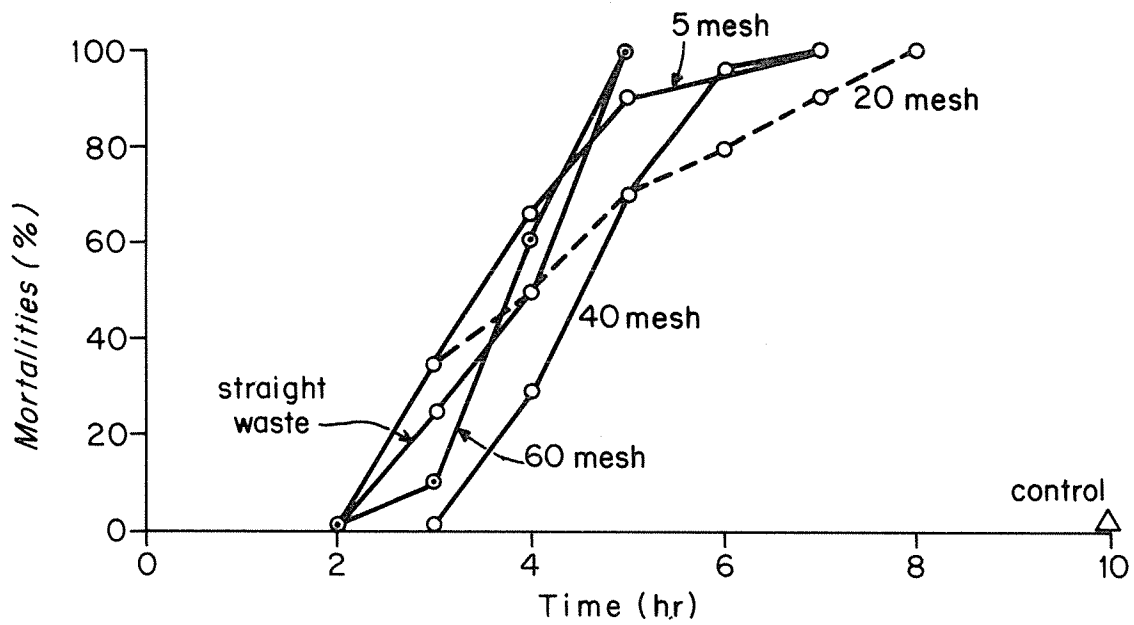


Fig. 6. Effects of screening of frozen salmon waste (20,000 ppm) on juvenile chum salmon, May 31 and June 1, 1972. N=20 fish/test, regular aeration.

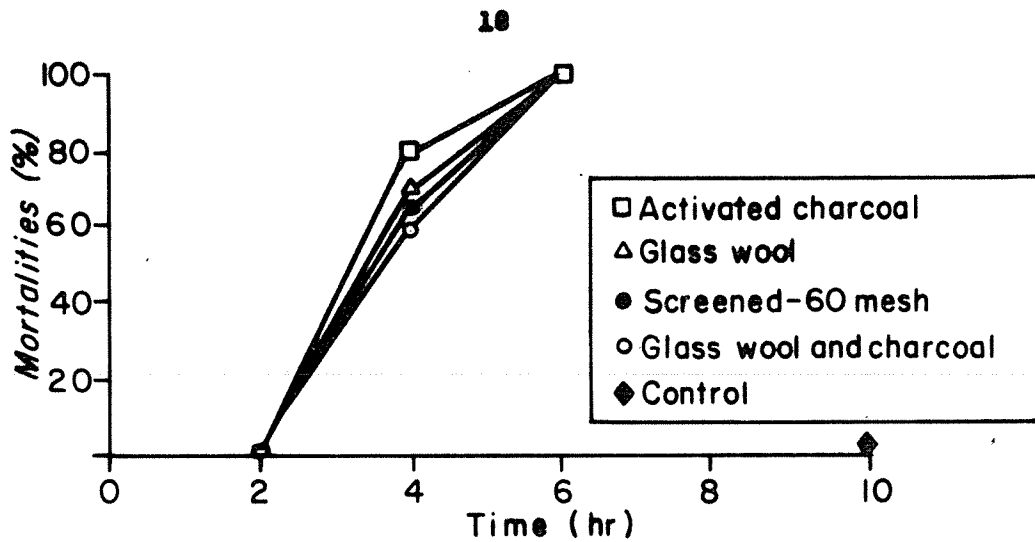


Fig. 7-a. Effects of various simulated treatments utilized during bioassays of frozen (20,000 ppm) salmon cannery wastes with juvenile chinook salmon, June 26, 1972. N=20 fish/test, regular aeration.

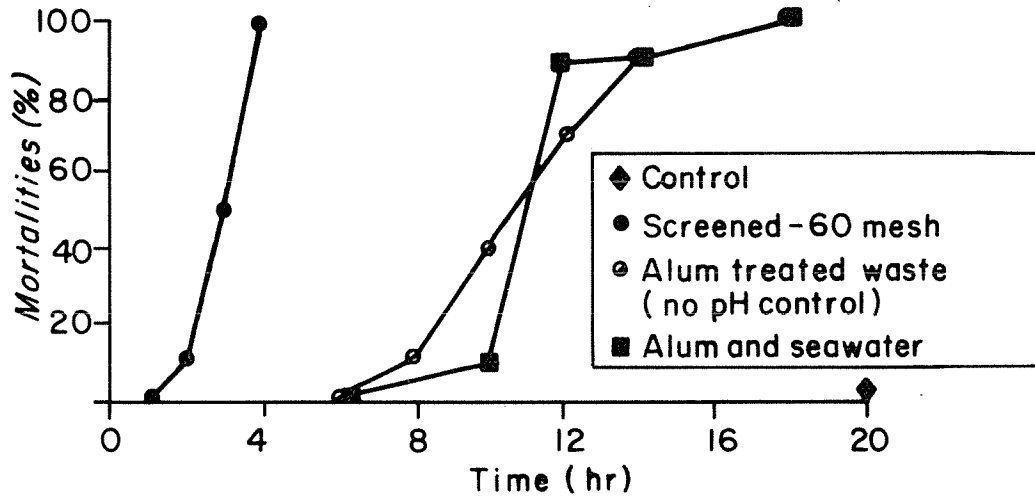


Fig. 7-b. Effects of uncontrolled alum treatment utilized during bioassays of frozen (20,000 ppm) salmon cannery wastes with juvenile chinook salmon, June 28, 1972. N=10 fish/aquaria, regular aeration.

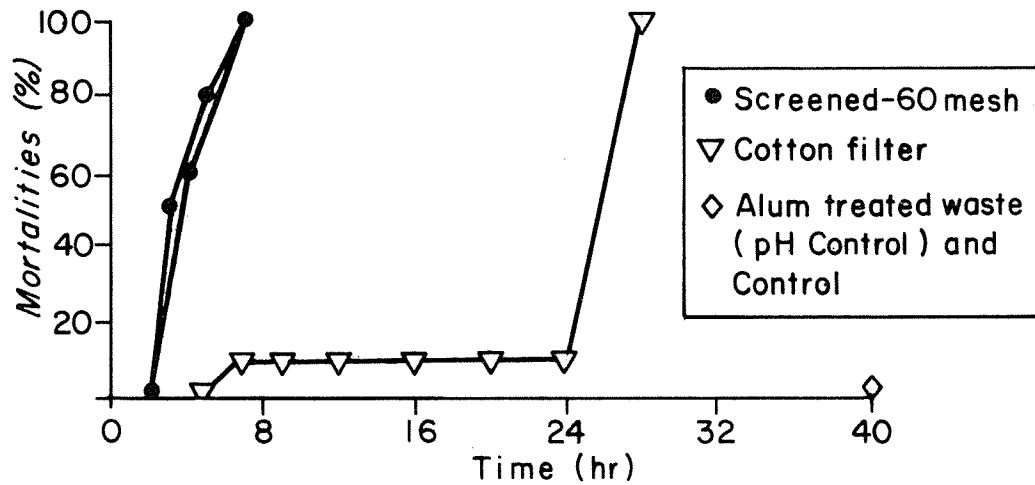


Fig. 7-c. Effects of cotton filter and controlled pH alum treatment utilized during bioassays of frozen (20,000 ppm) salmon cannery wastes with juvenile chinook salmon, June 29-July 1, 1972. N=10 fish/aquaria, regular aeration.

Turbidity was unaffected (Table 2) by these treatments and may have slightly increased with the activated charcoal due to particles of carbon added to the waste during filtering.

Alum flocculation without pH control showed encouraging results because the mortalities were delayed (Fig. 7-b). Turbidity decreased from 350 to 115 Jackson Turbidity Units (JTU's), thus indicating some removal of suspended materials. Among all waste treatments, alum flocculation with pH controlled at 7.1 was most effective with no mortalities recorded in a 48-hr exposure period (Fig. 7-C). In both the uncontrolled and the controlled pH tests, oxygen levels remained above 6.2 ppm.

Filtration through cotton also delayed mortality (Fig. 7-C). However, low levels of oxygen (1.8 ppm) were encountered during the period of mortalities and probably were the primary causes of the high rates of death observed. Turbidity decrease due to the cotton was almost identical to the alum treatment with a reduction from 350 to 118 JTU's.

Filtration through Whatman No. 4 filter paper was ineffective in reducing mortalities (Table 3).

Ammonia Concentrations of Wastes

Table 4 shows the concentrations of ammonia (total $\text{NH}_4^+ - \text{NH}_3$) that were present in the wastes. The levels increased as the wastes became older. This was expected due to the increased period of decomposition (Fernandez-Flores and Salwin, 1968). The frozen waste was especially high as compared to the others. The proportion of unionized ammonia (NH_3), the toxic form, varies with pH. By adjusting the pH of the waste to a high value, the method utilized in this study converted all of the ammonium (NH_4^+) ions to unionized ammonia. Therefore, to determine the unionized proportion in the aquaria, the values obtained from this method had to be converted back

Table 2. Turbidity changes due to simulated treatments of frozen salmon cannery waste (20,000 ppm)

Type of treatment	Initial JTU's	JTU's after treatment
Activated charcoal	600 ¹	675 ¹
Glass wool	480 ¹	490 ¹
Combination of wool and activated charcoal	600 ¹	675 ¹
Alum flocculation	350 ¹	115 ¹
Cotton	350	118

¹Value represented is an average of two test aquaria.

Table 3. Mortalities during bioassay comparing screened (60 mesh) frozen salmon cannery waste (20,000 ppm) with Whatman No. 4 filtration, June 27, 1972. N = 11 fish/aquaria, salinity = 25 ‰, temperature = 10.6-11.6 C

Hours of exposure Treatment	0	2	4	6	8	10	12
Whatman No. 4	0	0	6	9	9	10	11
Screened waste	0	0	4	8	8	10	11
Control	0	0	0	0	0	0	0

Table 4. Ammonia concentrations of various types of wastes

Type of waste	Ammonia (as total $\text{NH}_4\text{-NH}_3$) ppm in 20,000 ppm salmon cannery waste	pH	Ammonia (as NH_3) ppm in 20,000 ppm salmon cannery waste at specific pH
Frozen	15.3	6.68	1.3×10^{-2}
Fresh-frozen (1 week)	4.3	6.82	5.5×10^{-3}
Fresh waste	2.5	7.19	7.4×10^{-3}

to the pH in the aquaria. This conversion was made by using the dissociation formula:

$$\frac{(\text{NH}_4^+)(\text{OH}^-)}{(\text{NH}_3 \cdot \text{H}_2\text{O})} = K_b$$

where the dissociation constant for ammonia, K_b , at 10 C = 1.570×10^{-5} (Weast and Shelby, 1967) and the ionization constant for water, K_w , at 10 C = 2.92×10^{-15} . No allowance was made for effects of salinity but at such low concentrations of unionized NH_3 , this influence would be minimal. The final values (Table 3) showed that the unionized proportion was insignificant.

pH Changes Due to Wastes

Table 5 presents the average pH values at the beginning of all bioassays with frozen wastes. The ranges of decrease were often quite large (e.g., 1.15 to .45 at 20 ppt waste) but due to the difference of waste source and the biological activity in decomposing these wastes, wide variation might be expected.

Table 6 shows the changes in pH values during the fresh and frozen waste comparison. The effects of freezing and storage were to decrease pH. The frozen waste caused the largest decrease of almost one full pH unit. Fresh-frozen waste and the fresh waste stored for 16 hr also caused significant decreases. The fresh waste, however, caused a relatively smaller change of .47 units.

The solids from the screening test caused a smaller pH change (Table 7) than the liquid portion or the unscreened waste. The liquid and unscreened wastes were quite similar.

Table 8 shows the pH values in the fresh waste and the stored wastes as they were monitored initially and at 26 hr of exposure. In most cases,

Table 5. Differences in pH values obtained at the beginning of each test involved with frozen salmon cannery waste

Concentration of frozen salmon waste (ppm)	No. of bioassays or tests	Average pH of undiluted sea-water used as dilution water	Range of pH decrease due to wastes	Average decrease	Resultant pH
20,000	7	7.69	.72 - 1.15	.92	6.78
13,300	7	7.69	.72 - .90	.69	7.00
6,700	14	7.69	.15 - .77	.55	7.14
5,000	4	7.69	.44 - .63	.50	7.19
3,300	6	7.69	.30 - .51	.42	7.27
1,700	5	7.69	.21 - .42	.30	7.39
700	4	7.69	.10 - .22	.16	7.53
300	3	7.69	.03 - .17	.09	7.60

Table 6 . Differences in pH values recorded at the beginning of each bioassay involved with fresh and frozen wastes comparisons

Type of salmon waste (20,000 ppm)	Undiluted pH	Resultant pH due to wastes	Decrease due to wastes
Frozen	7.66	6.68	.98
Fresh-frozen 1 week	7.69	6.82	.87
frozen 2 weeks	7.58	6.72	.86
fresh waste stored 16 hr	7.66	6.81	.85
fresh waste	7.66	7.19	.47

Table 7. Changes in pH values due to screening (60 mesh) of 20,000 ppm salmon cannery waste (frozen)

Form of waste	Undiluted pH	Decrease due to wastes	Resultant pH
Unscreened waste	7.54	1.08	6.46
Liquid portion	7.54	1.00	6.54
Solids	7.54	.33	7.21

Table 8. Changes in pH values in aquaria containing fresh waste and stored waste

Concentration, ppm	pH values		Changes
	Initial value	26 hr	
20,000	7.16	6.43	-0.73
6,700	7.31	6.85	-0.46
3,300	7.40	7.28	-0.12
1,300	7.68	7.71	+0.03
20,000 held 16 hr	7.07	6.47	-0.60
Control	7.69	7.78	+0.08

except at 1.3 ppt, the pH decreased with time and in proportion to waste concentration and eventually approached levels found in the frozen wastes.

DISCUSSION

The various bioassays with fresh waste demonstrated that prolonged periods of exposure are required for salmon cannery wastes to cause mortalities of selected juvenile salmonids exposed to static bioassay in a laboratory. The data from the bioassays only provide "bench-marks" of critical concentrations under the "worst" conditions. Therefore, this bioassay data should not be taken out of context and applied indiscriminantly to a field situation. In the field, dilution is high, dispersion is rapid, and the prolonged exposure of fish to cannery effluent is unlikely because in most cannery discharge areas, tidal exchange renews the water every 12 hr. The differences between the static system and the actual environment can be shown with results from the Petersburg study (Nakatani et al., 1971). During the "controlled studies" phase of the Petersburg investigation, experiments showed a reduction of DO from 7.5 ppm to about 3 ppm and a decrease of pH from 7.8 to 7.0 in 6 hr with a subsample of discharged waste held in an enclosed system. At the same time, no significant changes due to similar waste concentrations were observed in the receiving waters.

Observations of mortalities during the bioassays showed that after the initial mortality in an aquarium occurred, the mortality rate was rapid and total mortality occurred within a relatively short period. Two major factors, namely, low DO and toxic products of waste decomposition, are believed to have caused these mortalities. The decrease in DO to critical levels was caused by the high BOD of the wastes and the toxic products of decomposition were considered to be largely the result of biological activities of micro-organisms in the wastes. Field observations of depressed DO near

salmon canneries have shown a wide range from no decrease at Petersburg to a significant decrease for short periods of time at a Bristol Bay cannery.

At no time, however, have the field measurements reached the prolonged severity of low DO observed in the test aquaria. Therefore, the critical considerations are the size of the affected area and the duration of low DO.

The second factor, a toxic product or products, was only inferred largely from the persistent mortalities observed in tests which had continued high levels of DO (Fig. 1). Identification of the toxic substance was of academic interest and much work would be required for proper identification; however, some evidence to support the presence of a toxic product was shown by the pH decreases and increases in ammonia levels of low quality wastes. The pH decrease due to fresh waste was somewhat reduced initially but eventually reached levels similar to the frozen waste. When this occurred, mortalities began to occur. In the field situation, the majority of wastes are fresh when discharged. Therefore, no problems are observed in the environment because these wastes are well dispersed before they reach the stage of decomposition that can be toxic to fish.

If ammonia is toxic only in its unionized form, then the values found in these experiments were substantially below any toxic concentrations cited in the literature. Therefore, unless some reactions were occurring at the gill surface where ammonium ion (NH_4^+) could be changed to unionized ammonia (NH_3) in spite of the pH levels in the solution, ammonia was probably not a significant contributor to mortality. Nevertheless, sublethal effects (Burrows, 1964) should not be disregarded. Brickell and Goering (1971) showed an increase of NH_4^+ -N from cannery wastes. However, if only the unionized NH_3 is considered then no acute toxicity to salmonids would be apparent at the concentrations that they observed because most

tests show that NH_3 is toxic only at levels greater than approximately 0.4 ppm (Ball, 1967). The results of the ammonia concentration tests suggest that more investigation will be needed if the critical substance is to be determined.

The time period involved to cause mortalities with fresh waste was beyond 24 hr which is almost two full tidal cycles after discharge. Therefore, only in situations such as an enclosed bay or other areas where the wastes are held in concentration and allowed to decompose, would a serious problem arise. To put the results into perspective, if fresh wastes were diluted to 3,000 ppm, or 1/10 of a typical peak concentration at the point of discharge (based on figures observed at Petersburg) in a time period of up to 24 hr, then no effects from degradation products of the waste would be apparent. It is unlikely that, in most canning areas, any salmon wastes would need such a long period of time to be diluted. The critical factor of DO remains, but the field studies show that this decrease is insignificant if there is adequate dispersion of the waste.

Solids, if allowed to accumulate, could cause problems not only with oxygen depletion but with eventual decomposition product toxicity. However, the canners are presently changing from direct discharge to grinding of their wastes which, again, aids in dispersion of the wastes. The time span required for these ground particles to reach toxic levels is greater than 48 hr as indicated by the lack of mortalities during the liquid-solids bioassay. Therefore, by the time that these particles are decomposed to a toxic level, the critical concentration is probably nonexistent.

Screening bioassays and the Whatman No. 4 filter bioassay demonstrated that the second factor causing mortalities was in solution rather than

associated with the particulate wastes. As a result, these treatments were ineffective in reducing short-term toxicity under laboratory conditions. However, solids such as heads, tails, and fins do tend to accumulate near canneries (unless ground prior to discharge). Therefore, screening may be an alternate treatment to grinding which would also help to reduce long-term effects due to decomposition of the solids.

The other simulated treatments (activated charcoal, glass wool, and a combination of the two) were also ineffective in reducing toxicity at the amounts of materials used for filtering. Activated charcoal did not remove the toxic decomposition products and glass wool, being chemically inert, could not remove any of the compounds in solution, only the particulate materials.

The cotton filter and alum flocculation were the most promising of all treatments. Cotton, an organic compound, may have absorbed some of the toxicants and alum either precipitated these critical factors out of solution or altered their form. Further investigations will be needed to confirm or reject these ideas.

The simulated waste treatments were conducted with frozen waste only. As a result, all values on the time mortality curves would have been expected to occur after longer periods of exposure if fresh wastes and supersaturation had been used. However, regardless of the positions of the curves the results would have been the same, i.e., either the treatment would or would not have been effective in eliminating mortalities.

The results from the bioassays support the rationale of why no ecological damage is observed in many of the field studies. In most canning areas the wastes are diluted to noncritical concentrations long before any critical exposure time is reached. Therefore, the only real problems with wastes in many areas are accumulations of large solids, which should be

eliminated by grinding, and limited areas of turbidity increases around discharges that may be esthetically unfavorable.

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Appendix 1. Proportions of filtration materials or techniques used in the simulated treatment bioassays

<u>Treatment</u>	<u>Proportion or techniques used</u>
Filtration through:	
Absorbent cotton	15 liters of 20 ppt salmon waste per 31 g of cotton
Activated charcoal	15 liters of 20 ppt salmon waste per 17 oz activated charcoal
Glass wool	15 liters of 20 ppt salmon waste per 2 oz glass wool
Activated charcoal and glass wool combination	15 liters of 20 ppt salmon waste per 17 oz activated charcoal and 2 oz glass wool
Whatman No. 4 filter paper	15 liters of 20 ppt salmon waste filtered through paper (once the paper became clogged, a new paper was inserted into the filtering apparatus)
Flocculation with alum	10 g of alum were placed in 30 liters of screened waste (20 ppt) and 15 liters of the nonsettled liquid were siphoned into a 5 gal aquarium.