EPA-600/3-76-056
July 1976

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SALMONID BIOASSAY OF SUPERSATURATED DISSOLVED AIR IN WATER

Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Duluth, Minnesota 55804

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SALMONID BIOASSAY OF SUPERSATURATED DISSOLVED AIR IN WATER

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EPA-IAG-0155(D)

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ABSTRACT

Tests were conducted in shallow (.25 m) and deep (2.5 m) tanks of water at 10°C with concentrations of dissolved atmospheric gas ranging from 100 to 127% of saturation to determine the lethal and sublethal affects of the dissolved gas on juvenile fall chinook salmon, *Oncorhynchus tshawytscha*, and steelhead trout, *Salmo gairdneri*.

Fall chinook salmon (average fork length of 42 mm) were much more resistant to supersaturation than juvenile steelhead trout (average fork length of 180 mm). Salmon tested in the shallow tanks at 120% of saturation incurred 50% mortality after 22 days whereas trout tested at the same level, incurred 50% mortality in 30 hrs. Signs of gas bubble disease were noted on dead fish and on subsamples of live fish from deep water tests at 110% saturation and above and in shallow water tests at 105% and above. Vertical distribution of both salmon and trout in the deep tanks appeared to compensate for about 10% and 10 to 15% respectively of effective saturation. Average depths of the fish in deep tanks increased with increased gas concentration. Significant differences in growth and condition factor of the salmon and trout were not found between stressed and control fish during the test period.

CONTENTS

		Page
Abstract		iii
List of Figures		vi
List of Tables		viii
Acknowledgments		ix
Sections		
I	Conclusions	1
II	Recommendations	2
III	Introduction	3
IV	Methods and Materials	4
V	Results	13
VI	Discussion	30
VII	Literature Cited	36

FIGURES

No.		Page
1	Schematic drawing of system used to produce water supersaturated with dissolved atmospheric gas in shallow water tanks.	8
2	Mortality versus time curves for juvenile fall chinook salmon exposed to various concentrations of dissolved atmospheric gas (% of saturation T.D.G.) in shallow (0.25 m) and deep water (2.5 m) tanks at 10°C.	14
3	Mortality versus time curves for juvenile steelhead trout exposed for 7 days to various concentrations of dissolved atmospheric gas (% of saturation T.D.G.) in shallow (0.25 m) and deep (2.5 m) water tanks at 10°C.	15
4	Frequency (%) of dead juvenile chinook salmon with signs of gas-bubble disease for shallow (0.25 m) test tanks with different concentrations of dissolved gas.	17
5	Exposure times of 25% mortality of juvenile chinook salmon held at different concentrations of dissolved gas in deep (2.5 m) and shallow test (0.25 m) tanks.	19
6	Frequency (%) of dead juvenile chinook salmon with selected signs of gas-bubble disease for shallow (0.25 m) and deep (2.5 m) test tanks with different concentrations of dissolved gas.	20
7	Mean depth during daylight hours of groups of juvenile chinook salmon held in 2.5 m deep tanks at dissolved gas concentrations of 100, 105, 110, 115, 120, 124, and 127% of saturation--averaged for periods of 10 days over 127 days.	21

No.		Page
8	Mean depths of groups of juvenile chinook salmon in 2.5 m deep tanks at dissolved gas concentrations of 100, 105, 110, 120, 124, and 127% of saturation--averaged for 3 periods of the day over 30 days.	22
9	Mean depth of groups of juvenile steelhead trout in 2.5 m deep tanks at dissolved gas concentrations of 110, 115, 120, and 127% of saturation--averaged for 2 periods of the day over 15 days.	23
10	Mortality versus time curves for fall chinook salmon at various size (40-67 mm forklength) when exposed to dissolved gas at 110, 112, and 115% of saturation T.D.G. in shallow tanks (0.25 m or less). Tests at 112% T.D.G. were conducted by Meekin and Turner (1974).	33

TABLES

No.		Page
1	Length, weight, and condition factor of randomly sampled fall chinook salmon and steelhead trout taken from test populations before testing.	5
2	Chemical features of the water in the test tanks, 20 February to 25 June 1973 and 6 to 21 May 1974.	10
3	Mean concentrations of dissolved gas in test tanks over test periods of 4 mo. for chinook salmon and 7 days for steelhead trout.	12
4	Estimated values for model parameters, 60-day survival probability and expected life for a 25% survival proportion.	29

ACKNOWLEDGMENTS

We extend our sincerest appreciation to the Washington State Department of Ecology, especially Stephen Robb for the efforts taken to solve many water quality problems encountered before these tests and for the monthly analysis of our water system.

Also, thanks to Dr. Gerald Bouck for his tolerance with the problems that arose during the study and for concern and help given during organization and implementation.

SECTION I

CONCLUSIONS

(1) At 115% of saturation in shallow tanks (where hydrostatic compensation is not possible), substantial (80%) mortality to fall chinook populations occurred within 60 days of exposure. After 60 days of exposure, significant (13%) mortality occurred among salmon in the shallow tanks at 110% of saturation whereas in the deep tanks at 120% of saturation 4% mortality occurred.

(2) Substantial (75%) mortality of steelhead trout tested in the shallow tanks occurred at about 115% of saturation and in deep tanks at about 127% of saturation, 25% mortality occurred after 7 days exposure.

(3) Tolerance to supersaturation of atmospheric gas decreased with age and growth in both species.

(4) Emboli in branchial arteries, gill filaments, and the heart were rarely observed on live subsamples but were prevalent on dead fish, indicating these signs are directly associated with the death of the animal.

(5) The average depth maintained by salmon and trout when allowed to sound compensated for about 10% and 10 to 15% (respectively) of the saturation value measured and computed on the basis of surface (760 mm Hg) pressure.

(6) Salmon and trout with signs of gas-bubble disease were able to recover from exposure to supersaturation.

(7) Exposure to various concentrations of supersaturated gas does not seem to affect the ability of steelhead trout to adjust to salt water; data on the ability of fall chinook to adjust to salt water were inconclusive.

SECTION II

RECOMMENDATIONS

On the basis of the information contained in this report we recommend:

(1) that the provisional dissolved atmospheric gas standards remain as it is (110%) unless substantial depth distribution information on wild stocks of fish indicates that the level can be safely raised.

(2) that the dissolved atmospheric gas level be held below 110% of saturation whenever fish are confined in shallow water less than .25 m deep.

(3) that additional studies be done to determine whether the tendency of chinook and steelhead trout to sound when exposed to supersaturation occurs in natural waters.

SECTION III
INTRODUCTION

Effects of supersaturation of dissolved atmospheric gases on fresh water fishes have been studied by many investigators since the late 1800's. The current problem of supersaturation in the Columbia River and its large tributary the Snake River (Ebel, 1969; Beiningen and Ebel, 1970; Ebel, 1971; Meekin and Allen, 1974) has resulted in renewed interest in effects of supersaturation on fish, and a great deal of research on the subject has recently been accomplished by fishery and other agencies in the Pacific Northwest. Information on the resistance of indigenous fish species to high gas concentrations is well-documented for exposure in shallow water for short periods of time (Rucker and Tuttle, 1948; Harvey and Cooper, 1962; Coutant and Genoway, 1968; Bouck *et al.*, 1970; Ebel, Dawley, and Monk, 1971; Bouck, 1972; Blahm, McConnell, and Snyder, 1973; Fickeisen, Montgomery and Schneider 1973; Dawley and Ebel, in press) but there are still many unanswered questions regarding the effects of chronic low level exposure on survival. Fish may be subjected to low levels of supersaturation in two ways: they may inhabit water areas where they cannot compensate for gas saturation by sounding or they may inhabit deep water areas where hydrostatic pressure offsets the effects of high gas levels.

The National Marine Fisheries Service, funded in part by the Environmental Protection Agency (EPA) in 1972, began investigations of chronic effects of long term exposure of juvenile fall chinook to various low levels of supersaturation. In this report we describe those effects observed from deep and shallow water tanks on juvenile fall chinook salmon *Oncorhynchus tshawytscha*, and juvenile steelhead trout, *Salmo gardneri*.

SECTION IV
METHODS AND MATERIALS

Chinook salmon and steelhead trout are valuable species in the Columbia River system, and particularly in the Snake River and were used in the following study. Juvenile fall chinook were acquired for our experiment as buttoned up fry from the Spring Creek National Fish Hatchery in early February 1973. Juvenile steelhead were captured during their seaward migration down the Snake River on April 30, 1974. Steelhead were one plus years of age. Chinook and steelhead populations were acclimated to our laboratory water system at 10°C, for 19 and 6 days respectively prior to testing. Before beginning the tests, random samples were taken from each population to obtain average weights, lengths and condition factors (Table 1). Fish were fed an Oregon Moist Pellet ration 5 days a week, at a rate of 4% of body weight/day. Rations for each test tank were corrected daily for numbers of surviving fish and corrected each 28 days for weight change (calculated from size of fish subsampled every four weeks).

Two series of tests on effects of dissolved gas were conducted at the Northwest Fisheries Center (Seattle, Washington). The first was completed in 1973 using the fall chinook salmon as experimental animals, and the second in 1974 using the steelhead trout. The series consisted of 20 simultaneous tests of chinook and 18 simultaneous tests of steelhead, in fresh water at 10°C, with various concentrations of dissolved gas in deep and shallow water tanks. At termination of the tests, surviving fish were divided into two groups; one group was transferred directly to salt water to assess the effects of stress from supersaturation on their ability to acclimate to salt water; a second group was examined for signs of gas-bubble disease. Groups of chinook salmon and 1 group of steelhead exhibiting signs were then placed in equilibrated water (100% of saturation total dissolved atmospheric gas) for a 2 week recovery period and subsequently re-examined for signs.

Table 1.--LENGTH, WEIGHT, AND CONDITION FACTOR OF RANDOMLY SAMPLED FALL CHINOOK SALMON AND STEELHEAD TROUT TAKEN FROM TEST POPULATIONS BEFORE TESTING.

Species	No. of fish	Ave. weight of fish (g)	s.d.	Ave. length of fish (mm)	s.d.	Ave. cond. factor 1/
		FALL CHINOOK				
Chinook salmon	60	.43	.06	40	1.3	.67
	84	.42	.06	40	1.2	.65
		STEELHEAD				
Steelhead trout	24	56.5	12.7	180	14	.922
	26	54.5	13.3	180	15	.890
	29	53.7	13.7	180	15	.907
	28	54.8	14.6	180	16	.917

1/ Condition factor = $\frac{w \text{ (weight in grams)} \times 10^5}{L^3 \text{(fork length in millimeters)}}$

Water in the deep tanks was 2.44 m (8 feet) which provided a maximum hydrostatic compensation from excess dissolved gas of 0.24 atm. or 24% of saturation total dissolved gas (T.D.G.); and in shallow tanks 0.25 m (10 inches) deep providing only 0.025 atm. of pressure compensation or 2.5% of saturation. The shallow water tests on both chinook salmon and steelhead trout consisted of two replicates at 120, 115, 110, 105 and 100% (control) of saturation T.D.G. Deep water tests with chinook salmon included tests at 127% (1 tank), 124% (1 tank), 120% (2 tanks), 115% (2 tanks), 110% (2 tanks), 105% (1 tank) and 100% (1 tank). Deep water tests with steelhead trout consisted of two replicates at 127%, 120%, 115% and at 110% T.D.G. (Previous studies indicated that tests at 110% of saturation in deep tanks could serve as a quasi-control.

The tests with chinook salmon began February 20, 1973 with the introduction of 220 fish per tank and was terminated on July 8, (127 days of exposure to concentrations of excess dissolved gas plus 13 days of subsequent tests). Steelhead tests began May 6, 1974 with the introduction of about 80 fish per tank; these were terminated after 21 days (7 days of exposure to supersaturation of dissolved gas plus 14 days of subsequent tests).

Once testing began, each tank was examined 4 times daily for the first 4 days followed by three, two, or one times daily throughout the remainder of the test period. During each observation dead fish were removed, their length and weight recorded, and signs of gas-bubble disease noted. Vertical distribution of the fish in each deep tank was also noted in percentage of total population at four levels of depth; 0-0.6, .6-1.2 m, 1.2-1.8 m, and 1.8-2.5 m. Subsampling of each test group for condition factor and disease signs was done each 28 days at a rate of 10% (but not less than 5 individuals) of the surviving population. Fish from each subsample were weighed, measured, and examined for signs of gas-bubble disease (none were returned to the tests).

Dechlorinated water from the public water system of Seattle--which came from the Cedar and Tolt Rivers--was used in these tests. Temperature was maintained at 10° ± .5°C, by mixing hot (27°C) and cold (7°C) water in a reservoir tank. Water for the shallow tank system was supersaturated by injecting 0.5 l/min air and 0.23 l/min O_2 into the suction side of two centrifugal pumps which were plumbed with a recirculation loop to 2 closed receivers, [197 liters (52 gal) each]. Hydraulic pressure within the receivers was maintained at 2.1 kg/cm^2 (30 psi) where dissolved gas content was increased to about 122% of saturation T.D.G. (Fig. 1). Water for the deep tank system was recirculated by a pump through an open reservoir tank 9 m deep x 3 m in diameter which was tapped at the bottom for distribution to the test tanks. Air and oxygen were injected into the recirculating pump at about 2.0 l/min and 0.2 l/min respectively. This resulted in a stable saturation level of 128% T.D.G. Both sources supplied individual test tanks through polyvinyl chloride (PVC) pipes which directed the supersaturated water to a vertical stack of aluminum trays (each 28 x 41 cm) placed 5 to 10 cm above one another. One half of each tray was perforated with 500 - 3 mm holes and the perforated ends alternated to produce a back and forth flow of water from tray to tray. Water was then collected in a plexiglass box (46 x 46 x 25 cm deep) with a false bottom of porous polyethylene plate through which air was passed. The level of supersaturation desired for each test tank was maintained by regulating the number of perforated trays and the amount of air supplied to the collection boxes (greater contact with air at atmospheric pressure decreased the level of supersaturation). Water from each box was gravity fed to a test tank through a vinyl tube, ending at the water surface. A flow rate of 7.5 l/min was maintained which created a circulation of about 0.2 m/sec within the test tank.

Test tanks were made of green tinted fiberglass, 1.2 m in diameter and of two heights, 0.6 m and 3.0 m (shallow, deep) holding about 270 and 2700 liters of water, respectively. A plexiglass window

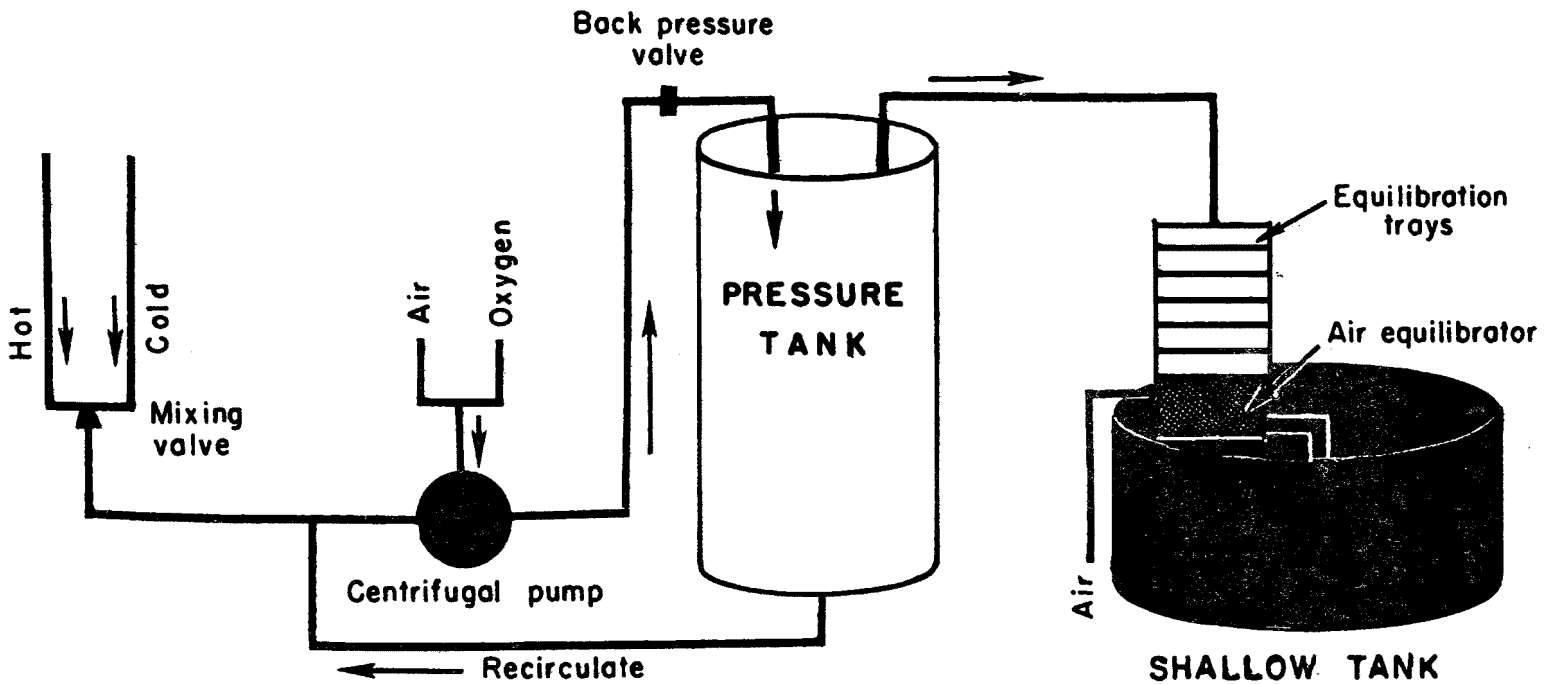

Fig. 1--Schematic drawing of system used to produce water supersaturated with dissolved atmospheric gas in shallow water tanks.

extended from the top to the bottom of the deep tanks allowing observations to be made over the entire water column. Curtains covered the windows and were removed only at times of observation. Water drained from the bottom of these tanks through an external stand pipe.

Lighting was controlled with time clocks to simulate natural sunrise and sunset and light intensity at the surface of each test tank was from 108 to 216 lumen/sq meter during full intensity periods.

Data on water quality (other than information on dissolved oxygen and nitrogen plus argon) were obtained before testing began and once each week--or once every 4 weeks depending on the chemical feature measured (Table 2). Procedures followed during analysis of water were those of the American Public Health Association, et al., (1971). The monthly measurements were made by personnel of the State of Washington Department of Ecology using a Perkin - Elmer 303 atomic absorption spectraphotometer.* All concentrations of heavy metals or other potentially dangerous compounds fell far below danger levels to chinook salmon and steelhead trout (McKee and Wolf, 1963).

Dissolved gas analyses were made on water from each tank at least once each weekday for the first two weeks, then a minimum of twice each week for the rest of the test period. Procedures were identical to Dawley and Ebel (in press); they employed gas chromatography calibrated for nitrogen plus argon using a modified manometric blood gas analysis apparatus (Van Slyke and Neill, 1924). The modified Winkler procedure (American Public Health Association et al., 1971) was used for analysis of oxygen concentrations. Water samples were collected by use of a siphon tube from the middle of the water column

*Trade names referred to in this publication are not an endorsement of commercial products by the Natl. Mar. Fish. Serv.

Table 2.—CHEMICAL FEATURES (CONCENTRATIONS IN mg/L) OF THE WATER IN THE TEST TANKS, 20 FEBRUARY TO 25 JUNE 1973 and 6 TO 21 MAY 1974.[1/]

CHEMICAL FEATURE	Shallow tanks (gas concentration)					Deep tanks (gas concentration)						
	100%	105%	110%	115%	120%	100%	105%	110%	115%	120%	125%	128%
Weekly Measurements												
Tot. Hard.	18-21					20	19-21					
Tot. Alk.	10-15					12-13	10-13					
pH	7.0-7.3	6.9-7.1	6.8-7.1	6.8-6.9	6.9-7.1	6.9-7.3	7.0-7.3	6.9-7.1	6.8-7.1	6.7-6.9	6.7-7.1	6.7-7.
NH_3	<.05 [2/]	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
Cl_2	<.02					<.02	<.02					
Monthly Measurements												
Zn	n.d. [3/]					n.d.						
Cu	n.d.					n.d.						
Cd	n.d.					n.d.						
Pb	n.d.					n.d.						
Ca	3.3-4.4					3.3-4.3						

1/ Other parameters measured prior to testing: CO_2 (1.2-2.0), chloride (4-6), cyanide (<.02), floride (.9-1), iron (.1-.8), nitrate (.03), nitrite (.003), phenol (.01), potassium (.2-1), sulfate (3). Measurements made by Environmental Protection Agency, Redmond, Wash.

2/ <--below minimum detectable levels indicated using colorimetric techniques.

3/ n.d.--nondetectable on Perkin-Elmer 303 atomic absorption spectrophotometer. Detection limits were Zn <0.005, Cu <0.01, Cd <0.025, and Pb <0.05. Measurements made by Wash. State Dept. of Ecology.

in the shallow tanks and from the surface of the deep tanks (clear vinyl tubes were used because some latex tubing produced a degassing effect as the water was siphoned). Gas concentrations remained steady throughout the test periods and mean values for each tank did not change more than 1% on a weekly basis with standard deviations for both tests less than 2.6% T.D.G. over all (Table 3). Samples were taken from the top, middle and bottom of the water column of the deep tanks several times and gas concentrations were found to be uniform throughout the tank.

TABLE 3.--MEAN CONCENTRATION OF DISSOLVED GAS IN TEST TANKS OVER TEST PERIODS OF 4 MONTHS FOR CHINOOK SALMON AND 7 DAYS FOR STEELHEAD TROUT.

Type of tank	Test no.	Chinook tests (mean % of saturation)				Steelhead tests (mean % of saturation)			
		O_2	N_2 + Ar	T.D.G. — —	sd	O_2	N_2 + Ar	T.D.G. — —	sd
Shallow	1	122.5	120.0	120.4	1.2	134.6	120.0	122.7	1.5
	2	120.9	120.0	119.9	1.3	132.9	119.1	121.6	1.3
	3	115.3	115.6	115.2	1.1	121.1	114.4	115.6	1.0
	4	115.6	115.9	115.4	1.4	119.3	114.1	114.9	1.4
	5	108.3	110.9	109.8	1.2	108.6	109.6	109.2	1.1
	6	107.7	109.9	109.3	1.1	108.1	110.2	109.5	1.2
	7	102.2	104.9	104.1	1.3	104.0	107.2	106.5	1.6
	8	102.5	105.3	104.4	1.0	100.8	107.1	105.7	2.2
	9	98.0	100.4	99.8	1.1	88.3	99.8	97.3	1.5
	10	98.6	100.5	100.0	1.0	86.0	99.7	96.9	1.0
Deep	11	101.7	98.6	100.8	1.1	-	-	-	-
	12	101.7	105.3	104.2	1.2	-	-	-	-
	13	108.1	111.2	110.3	0.8	105.6	110.2	109.1	1.4
	14	107.7	110.8	110.0	0.9	108.5	111.0	110.3	1.0
	15	115.3	116.0	115.6	1.1	114.0	114.1	113.8	1.5
	16	114.6	115.9	115.4	1.0	116.3	115.0	115.0	0.9
	17	118.6	119.7	119.2	1.2	126.7	120.4	121.4	0.8
	18	118.7	119.6	119.2	1.1	125.5	119.6	120.5	2.1
	19	124.8	124.4	124.1	1.6	133.4	125.2	126.6	2.5
	20	126.8	127.1	126.8	1.8	133.0	125.9	127.0	2.0

SECTION V
RESULTS

LETHAL EFFECTS OF DISSOLVED GAS

Juvenile chinook salmon held at 120% and 115% of saturation in the shallow tanks and at 127% and 124% in the deep tanks sustained substantial mortality (67-97%) after 60 days of exposure. By the same time, 15% mortality had occurred on fish held at 110% in shallow tanks and 5% had occurred in those held at 120% in deep tanks (Fig. 2). Mortality was insignificant for salmon held at lower gas concentrations. Curves of accumulative mortality of fish in the control tanks was minimal (3%) for the first 60 days, but by day 127 it had sharply increased to 26.3% in the shallow tanks and 13.6% in the deep tanks.

A change in normal feeding response and swimming behavior of chinook salmon in deep and shallow tanks was noticed on day 64 of the test. We believe these changes resulted from an infection caused by *Cytophaga psychrophila*. All fish (test and control) were taken from tanks and bathed in a 10 ppm solution of terramycin for 1 hour. A supplement of .5% oxytetracycline was added to the daily ration of food for the following 10 days, and after a 2 week interval another .5% supplement was added for 10 days. After the second treatment (day 100) the fish in all tanks behaved normally.

Steelhead trout held at 120% and 115% of saturation in shallow tanks developed substantial mortality--100% and 75% respectively--within 7 days and the two groups of fish at 127% in the deep tanks averaged 25% mortality (Fig. 3). Mortality of control fish was 2%, this value was small in relation to the test groups and was not used to adjust mortality curves of test groups.

PROGRESSION OF GAS-BUBBLE DISEASE

The frequency of occurrence of most signs of gas-bubble disease increased with increasing levels of supersaturation. The most fre-

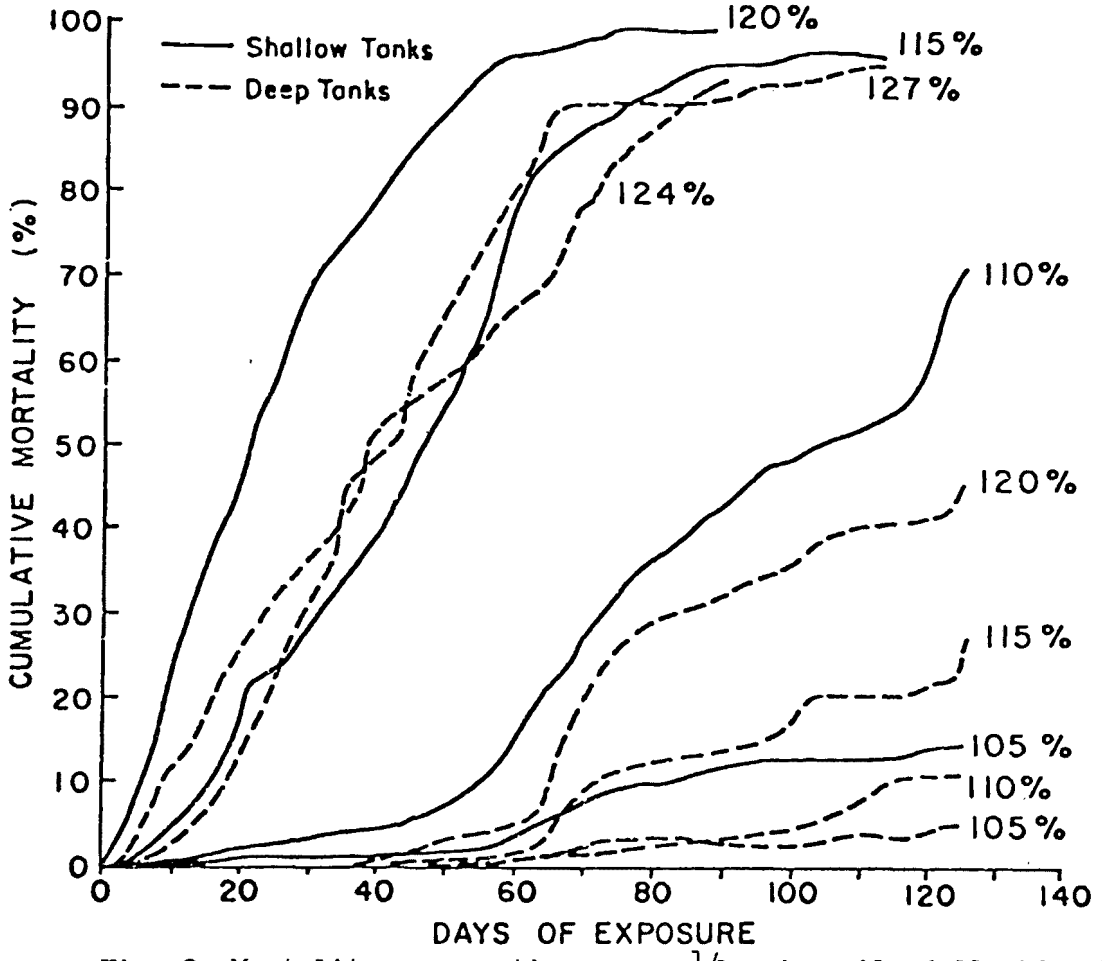

Fig. 2--Mortality versus time curves[1/] for juvenile fall chinook salmon exposed to various concentrations of dissolved of dissolved atmospheric gas (% of saturation T.D.G.) in shallow (0.25 m) and deep water (2.5 m) tanks at 10° C.

1/ Combined replicates corrected daily for mortality of control tests by subtracting percentage of mortality. Cold water disease affected the test fish on day 64 and ensuing treatment lasted through day 100.

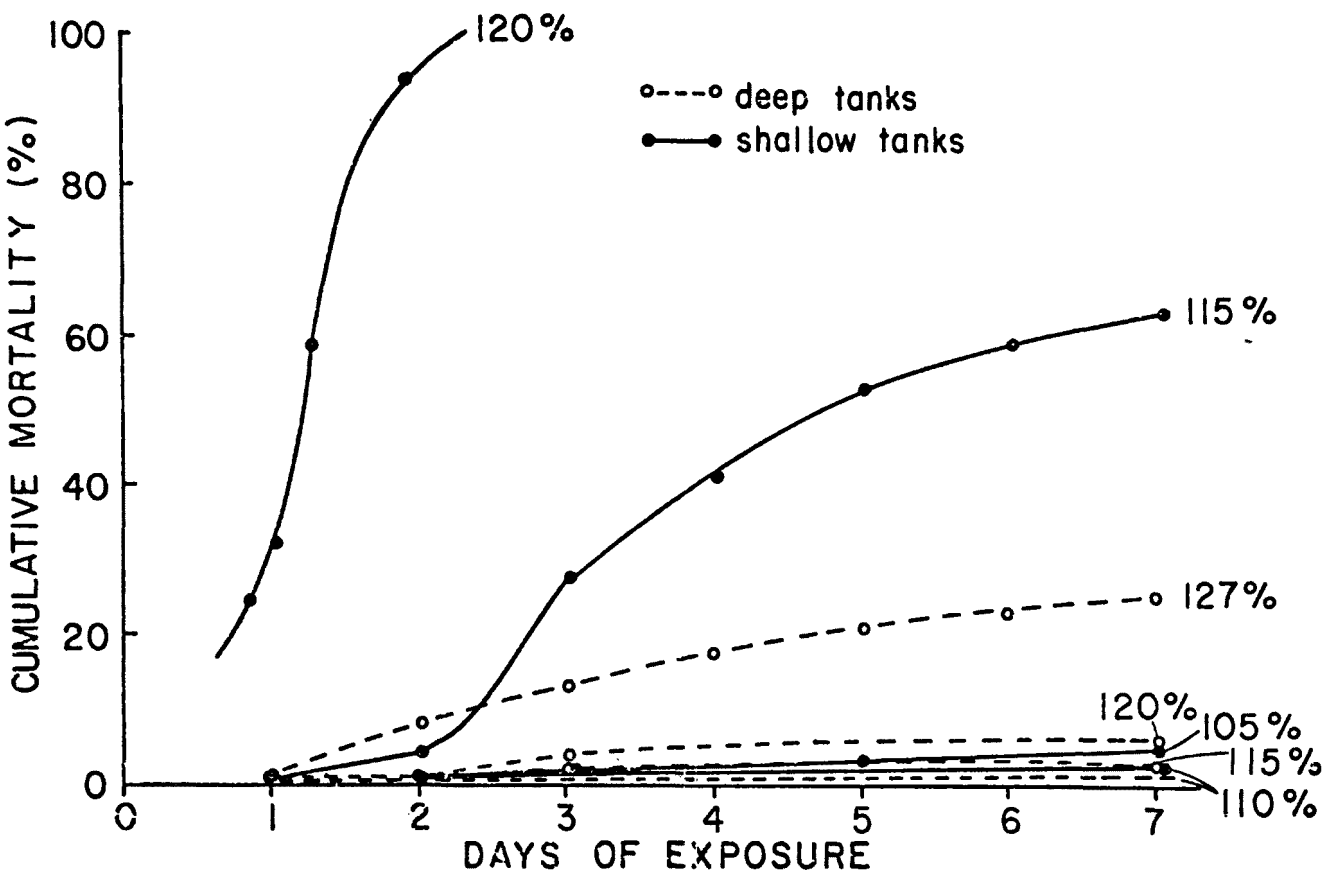

Fig. 3--Mortality versus time curves for juvenile steelhead trout exposed for 7 days to various concentrations of dissolved atmospheric gas (% of saturation T.D.G.) in shallow (0.25 m) and deep (2.5 m) water tanks at 10° C.

quently occurring signs of gas-bubble disease were cutaneous gas filled blisters on the head and mouth and occlusions of gill filaments on salmon which had succumbed to high gas levels. Dead fish from 120% and 115% shallow, and from 127% deep tanks showed 40-70% incidence of those signs. Other signs which increased with increasing levels of supersaturation but with lower frequencies of occurrence were: heart occlusions (14 to 34%), gas-blisters in the connective tissue surrounding the eye, and blisters between the fin rays (Fig. 4).

Research by Dawley and Ebel (in press) showed that the appearance of gas bubbles in the lateral line was the first external sign of gas-bubble disease to develop on juvenile spring chinook salmon exposed to high concentrations of dissolved gas. We, however, observed that gas bubbles in the lateral line of dead fall chinook and steelhead trout was not prevalent within any group, but did appear in a high percentage (50-100%) of live fish in subsamples from all test groups. Scattered bubbles (covering less than 15% of the lateral line) also appeared on most dead fish from the control groups. We cannot account for these bubbles on the control fish; and for that reason, only ascribed this sign to gas-bubble disease when more than 15% of the lateral line on test fish appeared occluded.

Disease signs on live fish generally appeared in rates and patterns similar to those recorded on dead fish removed from the same gas concentrations. However, emboli in branchial arteries, gill filaments, and the heart, were rarely observed on live subsamples, but were prevalent on fish examined immediately after death, indicating these signs are directly associated with the death of the animal.

The trends in gas-bubble disease signs noted on steelhead trout differed from those recorded during tests with the fall chinook salmon. Two differences were:

1. Heart and gill embolism occurred in almost 100% of the dead steelhead, whereas these signs were significantly fewer in dead chinook,

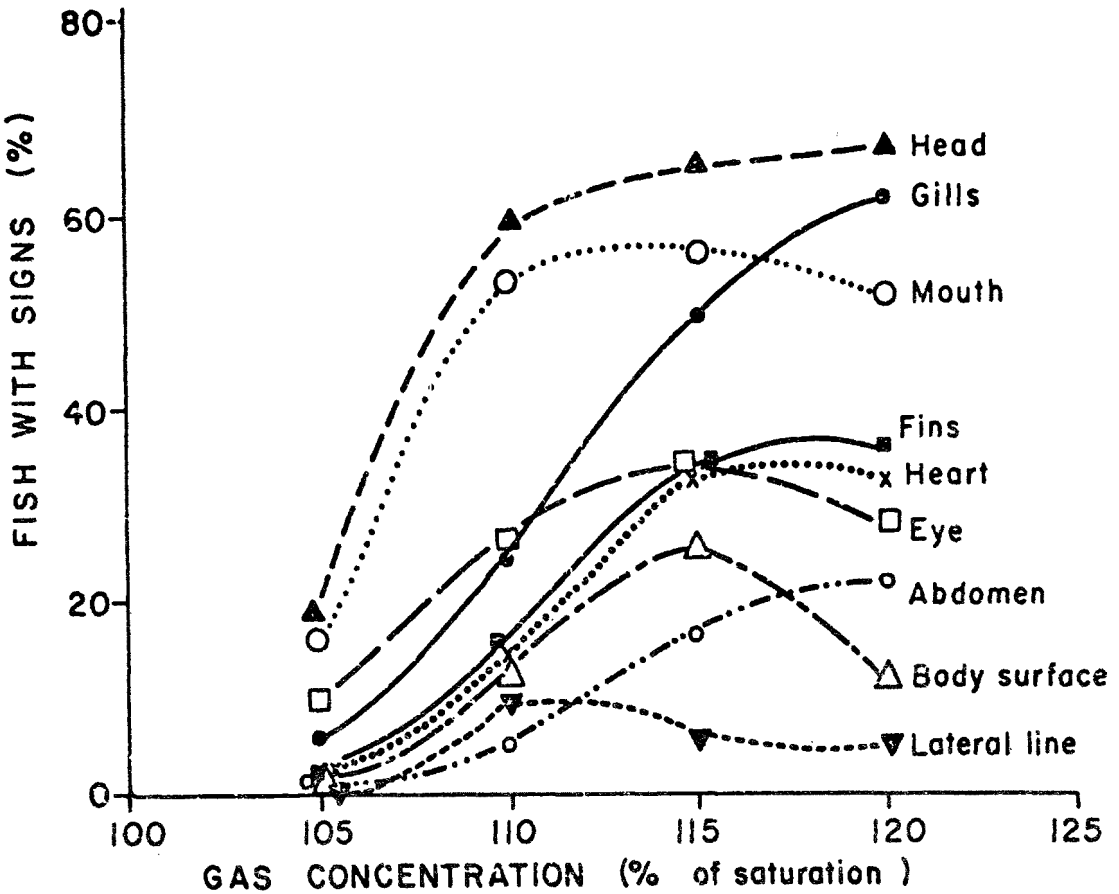

Fig. 4--Frequency (%) of dead juvenile chinook salmon with signs of gas-bubble disease for shallow (0.25 m) test tanks with different concentrations of dissolved gas.

suggesting that decomposition of the fall chinook quickly masked the emboli (due to their small size);

2. Rates of incidence of certain signs were directly associated with duration of the test. At termination of the tests live and dead steelhead showed light incidence of exophthalmia, cutaneous gas-blisters on the head and in the buccal cavity, and no signs of blisters of the body surface, however, the chinook subjected to supersaturation for a much longer duration, showed high incidence of those signs. Also, gas blisters between the fin rays were not prevalent on fall chinook, yet showed very high incidence on steelhead mortalities and live subsamples after 7 days exposure to high supersaturation.

EFFECTS OF WATER DEPTH

The average depth of the chinook and steelhead groups in the deep tanks appears to have compensated for about 10% and 10-15% of the dissolved gas content respectively; this observation is based on comparison of rates of mortality between deep and shallow tanks (Figs. 2 and 3). Figure 5 shows that the time to 25% mortality of fall chinook at various levels of dissolved gas concentrations in the deep tanks were comparable to times in the shallow tanks at a 9.5 to 10% lower effective saturation (i.e., 25% mortality was reached at 30 days of exposure in the deep tanks at 124% and in the shallow tanks at 115%). Also a comparison of incidence and degree of gas-bubble disease signs on dead chinook, between deep and shallow tanks indicates an effective decrease in supersaturation of 12 to 15% of the total dissolved gas concentration (Fig. 6).

Vertical distributions of chinook salmon groups for the first 3 days of testing were variable and not significantly different between different levels of saturation. After 3 days, however, the fish held at high saturation test levels maintained a greater average depth than those at the lower gas levels and maintained this difference thru the entire test period (Fig. 7). Tests with steelhead trout showed similar results, i.e., mean depth of fish increased with

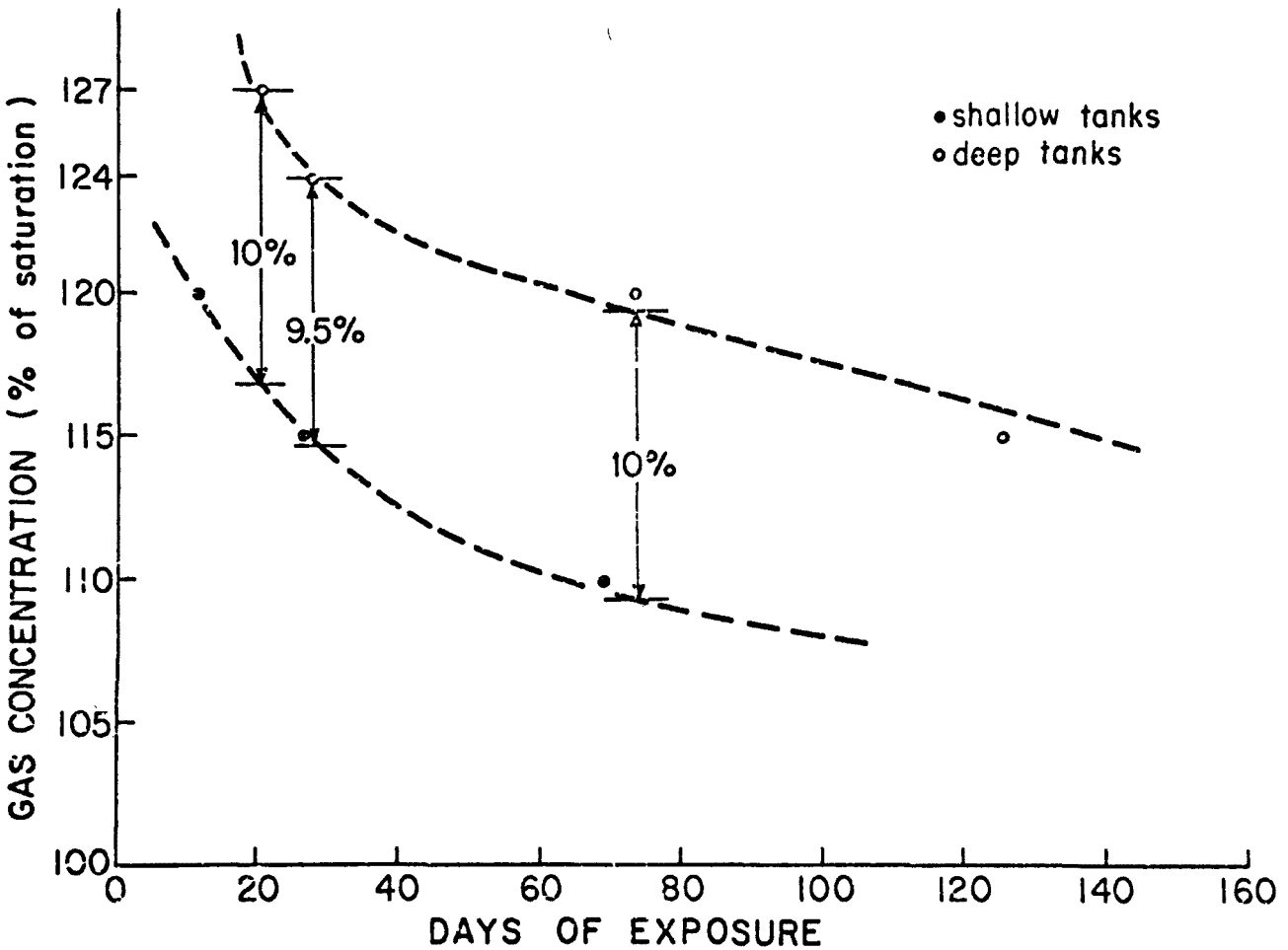

Fig. 5--Exposure times of 25% mortality of juvenile chinook salmon held at different concentrations of dissolved gas in deep (2.5 m) and shallow test (0.25 m) tanks.

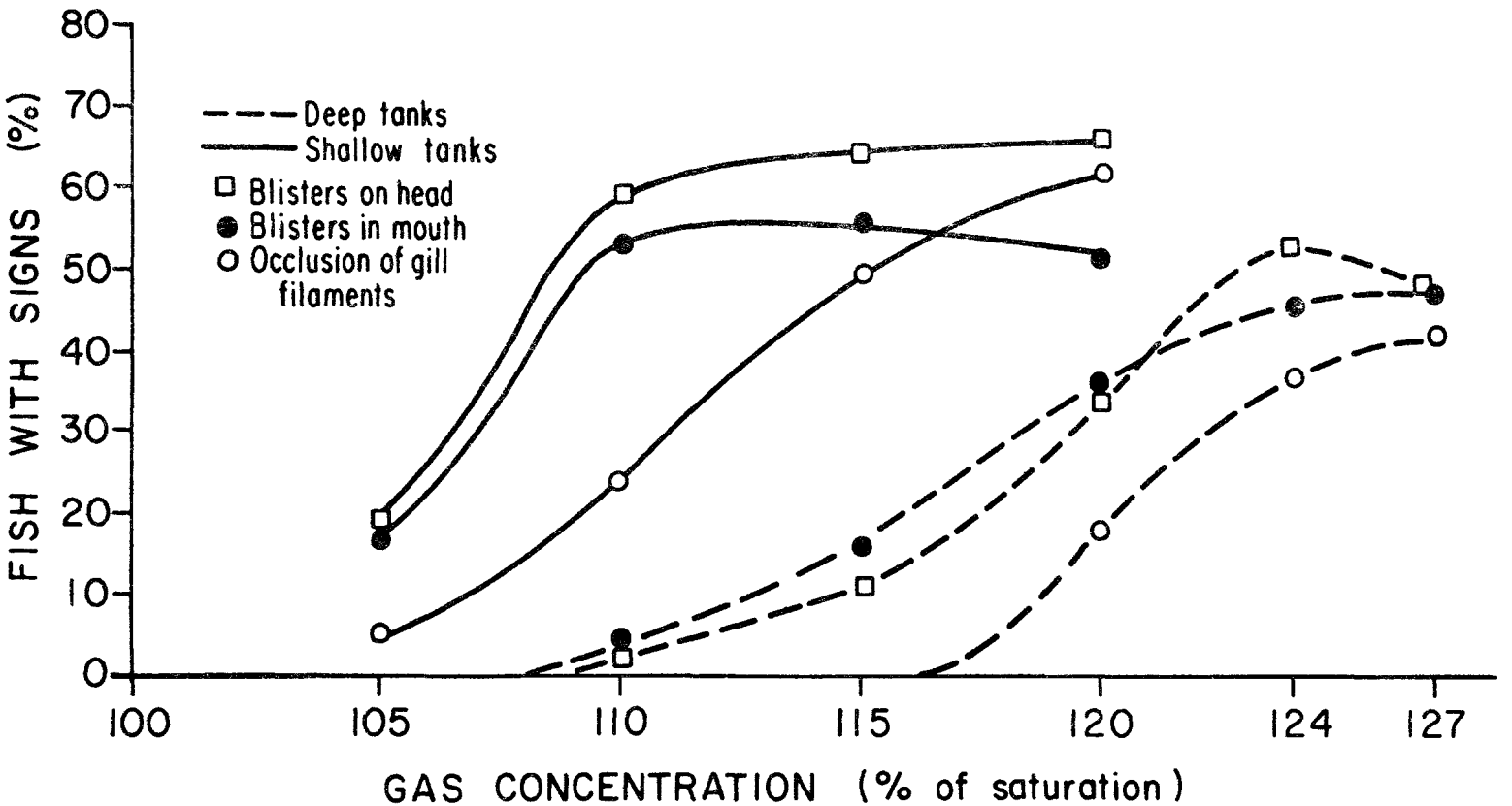

Fig.6--Frequency (%) of dead juvenile chinook salmon with selected signs of gas-bubble disease for shallow (0.25 m) and deep (2.5 m) test tanks with different concentrations of dissolved gas.

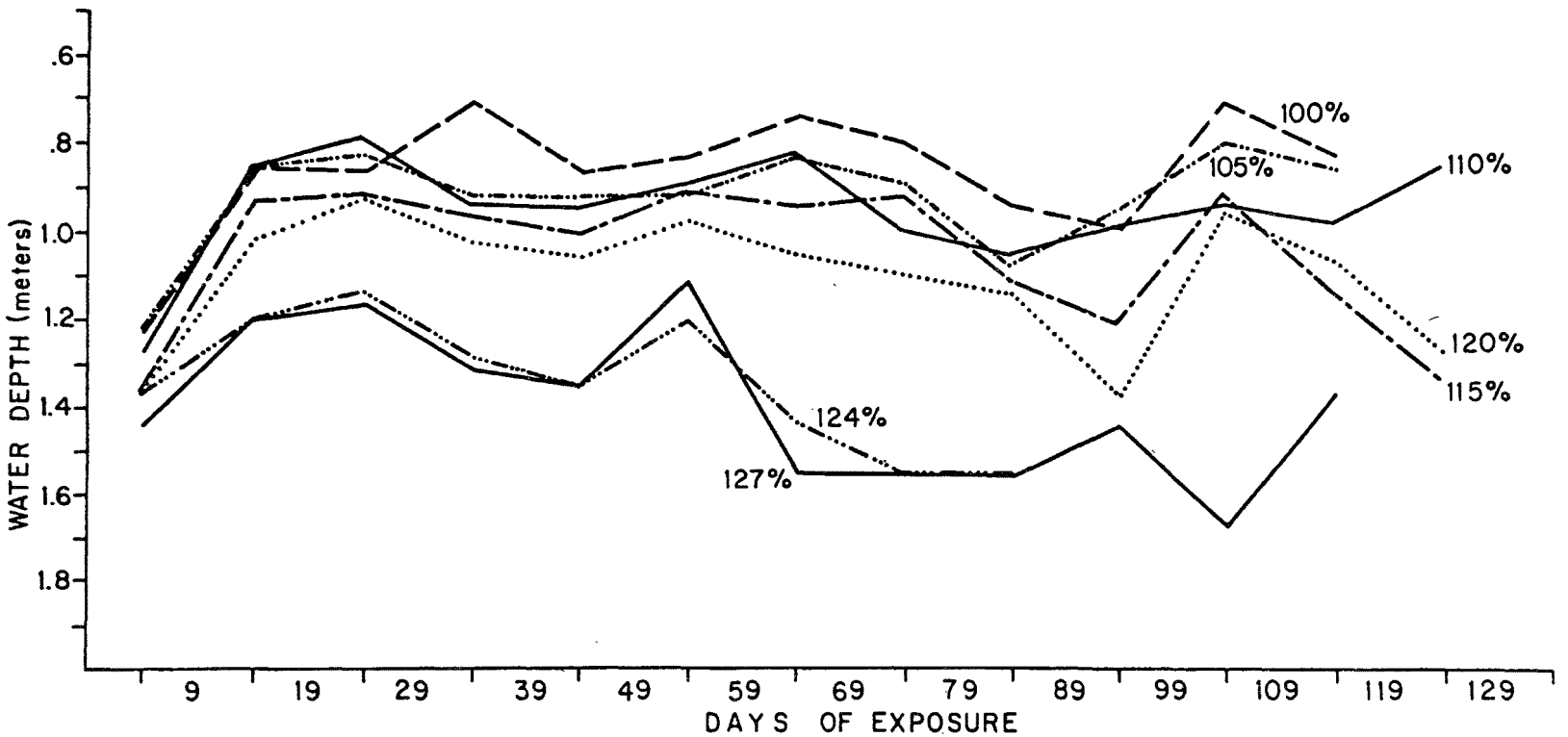

Fig. 7--Mean depth during daylight hours of groups of juvenile chinook salmon held in 2.5 m deep tanks at dissolved gas concentrations of 100, 105, 110, 115, 120, 124, and 127% of saturation--averaged for periods of 10 days over 127 days.

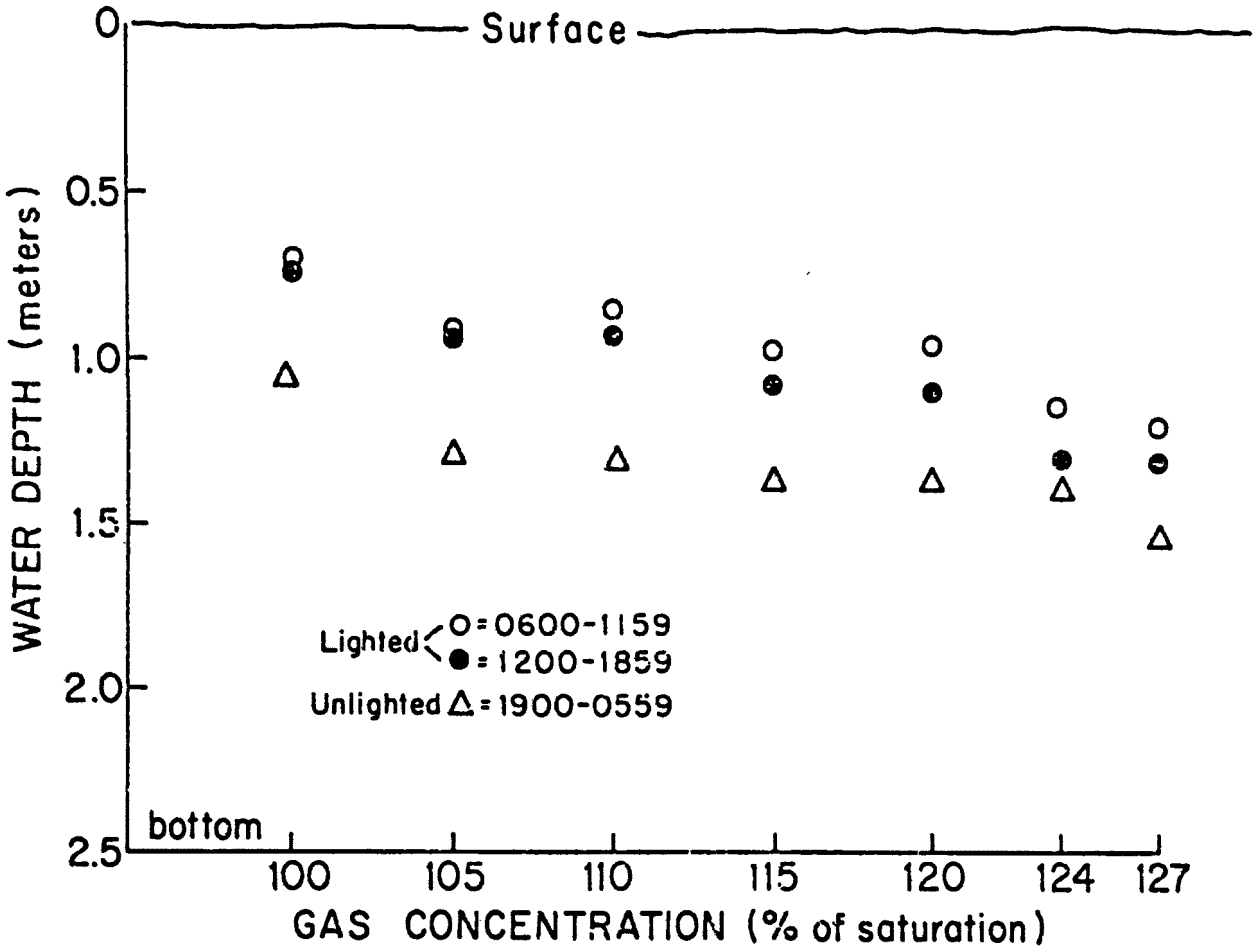

Fig. 8--Mean depths of groups of juvenile chinook salmon in 2.5 m deep tanks at dissolved gas concentrations of 100, 105, 110, 120, 124, and 127% of saturation--averaged for 3 periods of the day over 30 days.

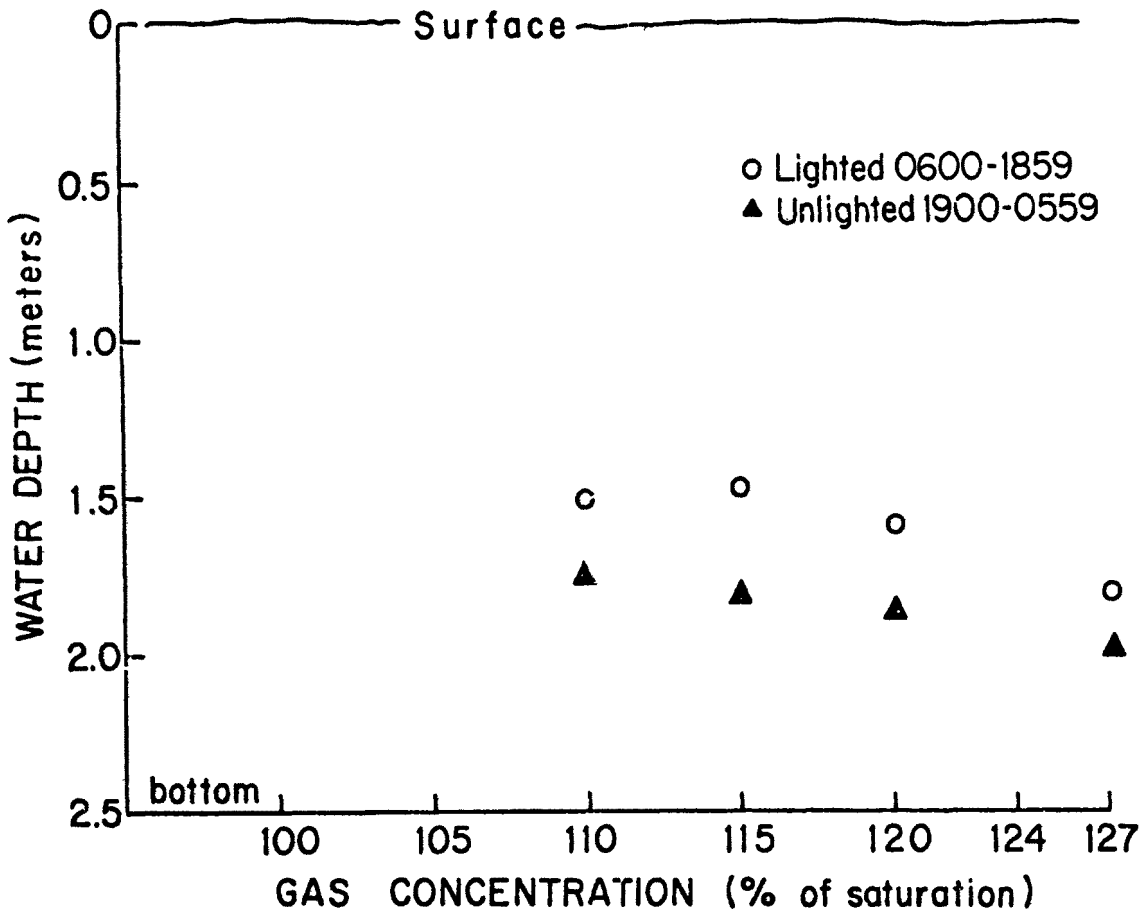

Fig. 9--Mean depth of groups of juvenile steelhead trout in 2.5 m deep tanks at dissolved gas concentrations of 110, 115, 120, and 127% of saturation--averaged for 2 periods of the day over 15 days.

increasing gas concentrations. Observations at night on distribution of fish in the deep tanks showed a downward shift of about 0.3 m soon after dark (Figures 8 & 9). The increase in average depth of fish with increasing levels of dissolved gas concentration, which was noted during daytime observations was also maintained at night.

EFFECT OF GAS SUPERSATURATION ON CONDITION FACTOR

Weight and length data obtained from subsampled fish and freshly dead test fish were used to calculate a condition factor "K" where:

$$K = \frac{w \quad \text{(weight in grams)} \times 10^5}{L^3 \quad \text{(fork length in millimeters)}}$$

These data were examined to determine if condition factors were related to susceptibility of fish to gas-bubble disease and death from supersaturation. Statistical comparisons were made to detect: 1) differences between condition factors of dead fish and those of live fish within each test population and; 2) differences among condition factors caused by chronic exposure to the various levels of supersaturation.

Condition factors and fork length data for salmon that died within 8 days of the monthly subsampling date were compared with those of live fish within the subsamples. Comparisons were not made if less than five fish had died within the specified time period. Condition factors of dead fish from shallow tanks at 110, 115, and 120% saturation and from deep tanks at 120, 124, and 127% saturation were examined (943 fish in the comparison). Mortalities from 11 test groups were significantly different in condition factor at the 90% probability level (Students T test) than their live fish counterparts for that test and three groups were not significantly different. Means for 12 of the 14 groups were larger for dead fish than for live fish. Comparisons of fork length measurements for the same fish groups showed that in 8 of 14 groups dead fish were significantly different from the live fish, however, the mean lengths of the mortalities (12 of 14 groups) were shorter than those of the live fish groups. Condition factor data may have been extensively biased by weight change of dead fish from water absorption after death. Some dead fish

remained in the test tanks as long as 16 hrs before being weighed and measured. Due to the consistency of these length data we believe that the smaller fish died at a higher rate than the larger fish, and that condition factor comparisons were invalid. Steelhead comparisons of lengths, weights, and condition factor showed no difference between mortalities and survivors of the 7 day test.

A wide range of condition factors of live fish examined each month was found for groups of chinook salmon tested at the same level of supersaturation (i.e., between replicates of tests at one gas concentration). Because of this variability between replicates we could not determine whether there was a difference in average condition factors (relating to differences in growth rates) among groups exposed to the various test levels of supersaturation.

RECOVERY FROM GAS-BUBBLE DISEASE

Upon termination of the tests at various levels of supersaturation, portions of the groups of chinook salmon and steelhead trout that exhibited signs of gas-bubble disease were placed in equilibrated water (100% T.D.G.) to determine if recovery was possible. Subsamples of salmon tested at 110% of saturation in shallow tanks at 110, 115, and 120% in deep tanks were used in this phase of the experiment. A portion of each of these test groups had sustained significant mortalities from gas-bubble disease. All subsamples (group size 27-48 fish) sustained mortalities from 10-16% in the two week recovery period, but these could not be attributed to gas bubble disease. After 2 weeks, the survivors no longer exhibited outward signs with the exceptions of one fish with a hemmorrhaged eye, and another with bubbles in the orbit.

Eleven steelhead trout that had survived in the deep tank tests with a gas concentration of 127% saturation were placed in a shallow tank at 100% of saturation. After 3 days, the cutaneous blisters on the trout had decreased in size and number; for example; gas-blisters 5 mm in diameter had decreased in size to 2 mm and 20 blisters on

the operculum decreased to 4. These fish were subsequently placed in water at 105% of saturation and all signs remained the same after another 4 days, at which time fish were released. There were no mortalities in the 7 day recovery period.

EFFECTS OF TRANSFER TO SALT WATER

To determine whether prior exposure to various levels of dissolved gases affected the ability of fish to make the transition to salt water, subsamples of survivors from combined replicates of all test groups of chinook salmon and steelhead trout were placed into salt water at 25 ppt salinity at 10°C. Samples of about 50 chinook salmon from each saturation level in deep and shallow tanks were transferred on test day 127 and observed for 13 days. A combined total of 98% mortality occurred in 3 days. Only 8 salmon survived for the entire period; 1 fish from the 105% shallow tank and 7 fish from the 110% deep tank. Results of a statistical comparison of fork lengths of the survivors ($\bar{X}$= 67.3 mm) to those that had succumbed ($\bar{X}$= 52. mm) indicates a definite size correlation with ability to survive transfer (T= 5.73, 46 df, P 0.01). The majority of the experimental stock of salmon had not yet reached normal smolting size and their ability to withstand transfer to saltwater may have been severely lessened.

Subsamples of 10 to 20 steelhead trout from previous tests were likewise observed after transfer to saltwater. Mortality varied from 0-16%, but there was no correlation to previous dissolved gas stress. However, a size comparison between dead and live trout indicated that the dead fish were significantly smaller (T= 1.925, 51 df $P < .06$) which suggests that the dead fish may not have been up to smolting size.

MATHEMATICAL MODEL OF MORTALITY

In these tests the fish were subjected to a continuous challenge until death or the termination of the experiment. The occurrance of a death was considered an "event" or "point" and the lapses in time between points constitute a General Point Process. Within this general theory

there are several event rate functions which could be considered candidates to represent the data. The most general event rate function can accomodate decreasing, constant, and increasing death rates. This represents the situation where the mortality intensity is changing with time. In these tests salmon exhibited conditions which could be characterized by progressively accumulating damage with a consequent increasing death rate (Figures 2, 4 and 6). If the mortality intensity is represented as an appropriate power function of time one may obtain the Weibull distribution (Mann, Schafer, Singpurivalla, 1974). This distribution will represent cases where the mortality intensity changes with time and it has been shown by Pike (1966), Peto and Lee (1973) and Murthy (1974) that experiments of the type conducted here can best be analysed by fitting an appropriate Weibull distribution. Upon this basis a three-parameter Weibull distribution was fitted to the mortality data from these experiments and used to compare the survival of the various test groups of salmon. It is also possible, within the context of the analysis of a Weibull distribution, to accomodate the case where there is Type I censoring of the data, in which the test was terminated at a specified time before all fish had died. In this case the number of deaths and the times-to-death are random variates. Also the case where there is progressive censoring or withdrawls of live fish from the test throughout the duration of the test can be analysed.

The probability density function for the three-parameter Weibull distribution is given by

$$f(t) = \begin{cases} \frac{b}{c}(t - G)^{b-1} \exp\left[-\frac{1}{c}(t - G)^{b}\right] & b, c \quad 0;\ t \quad G \quad 0 \\ 0 & t \quad G \end{cases}$$

Where t is the time to death.

The parameter G is sometimes called the threshold parameter, since a death occurs before time G with probability 0. The parameter C, usually

called the characteristic life, is a scale parameter. It is the 100 x [1-exp (-1)]th = 63.2 percentile of the distribution for any value of the shape parameter. The parameter b determines the shape of the distribution. If b=1, the distribution is unimodal and the ratio of mortality is increasing with time. Further information on the properties of the Weibull distribution is given in Mann (op. cit.) and Murthy (op. cit.).

The parameters of the Weibull distribution were estimated using maximum likelihood procedures as outlined in Mann (op. cit.). Table 4 lists the estimates of these parameters for each test. This distribution was also used to calculate the probability of survival to a specified time, t = 60 days, for each test condition (Table 4). The estimation formula is:

$$R_t = \exp\left[-(t/c)^b\right].$$

The expected life (days survival) corresponding to a 25% survival proportion was estimated by the formula:

$$P_{.25} = c\left[\log(1/.25)\right]^{1/b}$$

and the results for each test are shown in Table 4.

Table 4.--Estimated values for model parameters, 60-day survival probability and expected life for a 25% survival proportion.

Type of Tank	Test No.	T.D.G.	MODEL PARAMETERS Threshold (G)	Shape (b)	Characteristic Life (C) (days)	60-day survival probability (R_{60})	Expected life for a 25% survival proportion (P.25) (days)
Shallow	1	120	0.5	1.335	23.4	0.03	9.2
	2	120	2.4	1.335	30.1	0.08	11.8
	3	115	4.6	1.587	46.1	0.22	21.0
	4	115	6.5	1.088	47.9	0.28	15.3
	5	110	3.9	1.607	107.9	0.68	49.7
	6	109	6.8	1.169	156.2	0.72	53.8
	7	104	0.5	1.107	368.5	0.87	119.6
	8	104	12.8	1.054	371.0	0.86	113.8
	9	100	5.5	1.506	221.2	0.87	96.7
	10	100	8.9	1.151	491.7	0.92	166.6
Deep	11	101	21.7	1.039	831.6	0.94	250.7
	12	104	22.1	1.337	496.1	0.95	200.7
	13	110	12.0	1.517	384.0	0.94	168.9
	14	110	25.0	1.406	414.4	0.94	170.8
	15	116	2.9	1.112	211.6	0.78	69.0
	16	115	2.8	1.376	315.7	0.90	127.7
	17	119	5.9	1.216	151.3	0.72	54.3
	18	119	12.6	1.955	169.4	0.88	89.6
	19	124	2.5	1.450	40.8	0.17	17.3
	20	127	2.5	1.357	40.0	0.18	16.0

SECTION VI

DISCUSSION

The mortality curves (Fig. 2 & 3) may be affected synergistically by infection of fish with C. psychrophila after day 64, however, incidence rate and types of signs of gas-bubble disease on dead fish showed no apparent difference between the first 64 days and the last 63 days--indicating that the effect of C. psychrophila was not large. Signs which we attribute to the death of the test fish (heart and branchial artery embolism) were observed in about the same percentage of dead individuals before day 64 as after that time. We, therefore, assume the mortality curves (adjusted for control mortality) are generally representative of death rates caused by gas-bubble disease at the dissolved gas concentrations indicated. The first 64 days have no qualifications, but the last 63 days may represent a fish stock with less than normal tolerance to excess dissolved gas pressure.

As shown in figure 10, the rates of mortality and shape of curves derived from our studies correlate well with experiments done by Meekin and Turner (1974), in which they exposed juvenile fall chinook salmon (about 67, 53 and 40 mm long) to 122% N_2 + Ar plus 74% O_2 (112% T.D.G.). They showed a definite inverse correlation between size of fish and time to death in supersaturated conditions. Larger fish (53 mm, 67 mm) succumbed much more rapidly than 40 mm fish, tested at the same level of saturation (T.D.G.). Also, this same trend was shown by Shirahata (1966), testing rainbow trout from hatching to fry stage. Meekin and Turner (1974) also found that when testing fish for long period of time in supersaturated water, fish that died were smaller than the survivors which they concluded may have been caused by retarded growth of the fish that were overcome by stress. These same general trends appear in our data: i.e., 1) juvenile salmon that die from long term stress due to supersaturation are smaller than their surviving counterparts and; 2) mortality rates increased commensurate with aging and growth.

Certain observations made during these tests have important implications relative to the experience of natural and hatchery reared populations of juvenile salmonids. Most areas in the Columbia River system where salmon and steelhead trout incubate and develop naturally are located above dams--which cause high concentration of dissolved gas by heavy spilling of water--and, would be little affected by long term-low level supersaturation of water, however; spring chinook salmon and steelhead trout from tributaries of the Willamette River, a major tributary of the Columbia, make heavy use of areas below dams where supersaturation is prevalent. Also water for hatcheries in some instances is either taken from the river below dams where spilling occurs or from wells that have high concentrations of dissolved gas. In these instances effects of exposure to supersaturation during the early stages of life, such as the period of incubation, become important.

Significant changes in tolerance to dissolved gas occur at various life stages. Data from our bioassays and others--Shirahata (1966) and Meekin and Turner (1974) indicate that (1) yolk sac fry in water with low levels of supersaturation sustain injuries which become fatal as the yolk is nearly absorbed; and (2) after fry have "buttoned up" tolerance to supersaturation becomes quite high but decreases gradually thereafter until time of seaward migration. Equilibration of hatchery water sources is thus extremely important at certain stages of fish development. Also spillway discharges at certain times will have more effect on survival of downstream juvenile migrants than at others; management policies should consider the changing effects of these discharges.

Seaward migration of juvenile salmon and steelhead trout coincides with the spring freshet in the lower Columbia and Snake rivers. Freshet conditions are variable from year to year, but heavy spilling at dams usually occurs every year for some duration. This creates high gas concentrations of from 120% to 140% of saturation. During years of high flow, these high concentrations persist throughout long stretches of the river (650 km and more), resulting in long term exposure for some stocks of fish. Rates of migration of juvenile fish indicate that at least 28 days is required for travel from Little Goose Dam to the Columbia River estuary during the highest flows (Howard Raymond, personal communication*). Even if some fish are compensating for supersaturation by sounding, a significant portion of the population is probably exposed to concentrations of dissolved gas exceeding 120% saturation.

Data on depth distribution of migrating juvenile fish within the Snake River near Lower Monumental Dam (Smith,1974) indicate that 58% of the chinook salmon and 36% of the steelhead trout were in the upper 3.7 m of the water column. Mean depths for these portions of the migrating stocks were 1.30 m and 1.33 m respectively. This would compensate for 14.5 - 14.8% effective saturation, which means that at concentrations of 135% of saturation--or more--both stocks of fish would be exposed to gas concentrations equal to--or above--120% of saturation for at least 28 days during periods of high flow.

Our studies have shown that, although both the fall chinook salmon and steelhead trout tend to remain at greater depths with increasing levels of supersaturation, they are unable to totally compensate for dissolved gas concentrations above 120%. Therefore, it is imperative that corrective measures to reduce supersaturation be implemented as soon as possible to reduce mortality.

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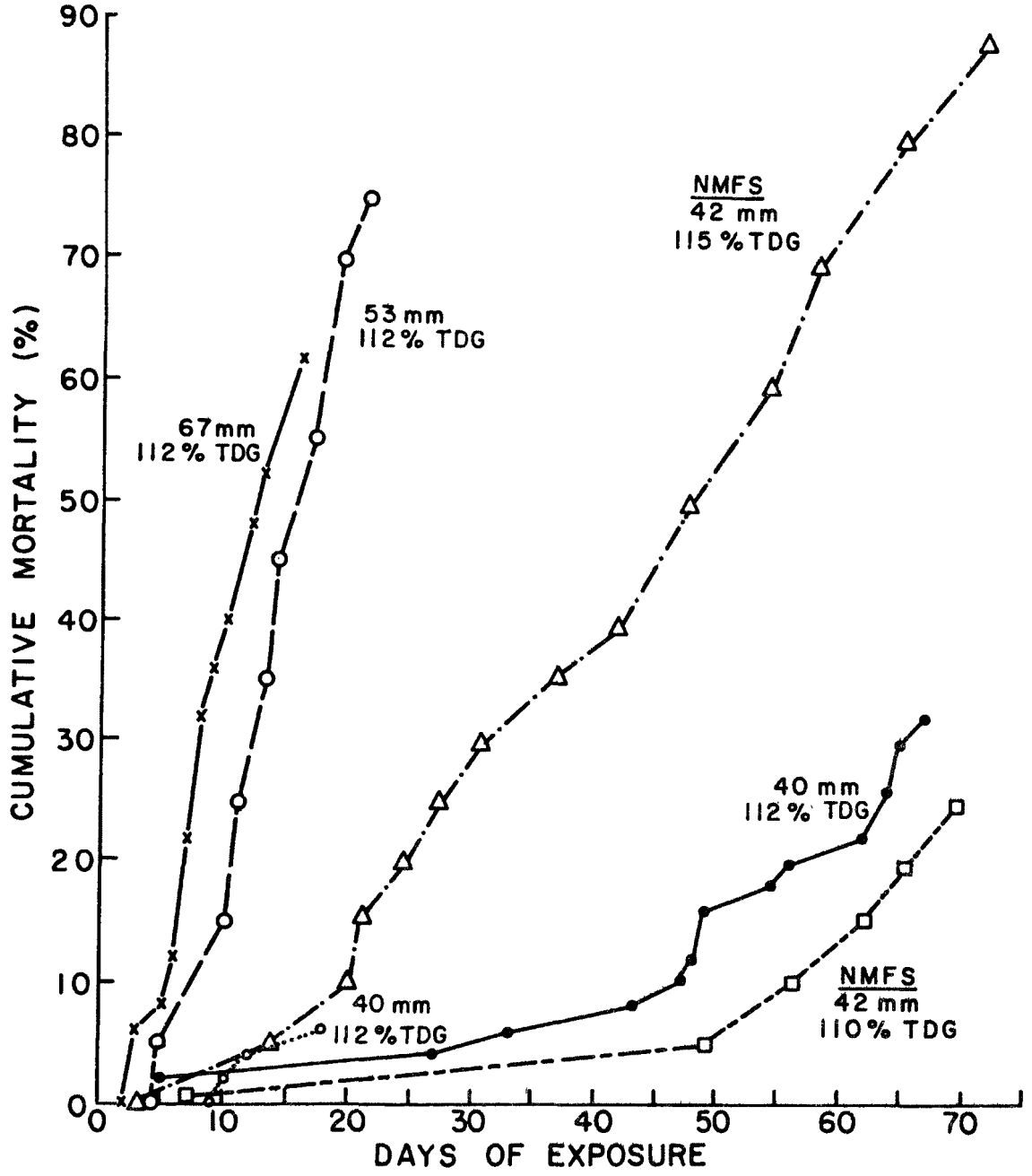

Fig. 10--Mortality versus time curves for fall chinook salmon at various size (40-67 mm forklength) when exposed to dissolved gas at 110, 112, and 115% of saturation T.D.G. in shallow tanks (0.25 m or less). Tests at 112% T.D.G. were conducted by Meekin and Turner (1974).

In tests by Dawley and Ebel (in press) the resistance times of steelhead trout in shallow water tanks at 115% supersaturation was 400% longer than in our tests with steelhead at the same saturation level. Fish in the earlier tests were hatchery reared and smaller than those used in our study; this is probably the reason for their greater resistance. This difference in resistance, however, is small compared to the one to two month differences in resistance times we observed between fall chinook salmon and steelhead trout. This greater difference correlated well with data from Meekin and Turner (1974) which indicated that fall chinook salmon were more tolerant to exposure to supersaturation than were steelhead trout of comparable size and age. This same order of ranking was noted by Ebel et al. (1971) and Dawley and Ebel (in press).

Some prominent signs of gas-bubble disease were observed on dead chinook salmon in association with certain stages in physical development or stress experience. Cutaneous blisters in the buccal cavity and on the body surface and hemorrhages in and around the eye required more time to develop than other signs, thus did not exist on mortalities from the higher test levels because of shorter time duration. Therefore, incidence rate was lower but is entirely dependent on the duration of stress. Exophthalmia appeared frequently in the 3rd and 4th months, similar in incidence to blisters on the head. Blisters at the midline of the vertical surface occurred frequently on dead salmon taken from the deep and shallow tanks of the highest saturation levels. This frequency decreased, however, as testing progressed and by the 4th month there was no evidence of this sign; it appeared to be related to recent yolk absorbtion. There was a very low incidence (40%) of blisters between the fin rays at the highest saturation levels compared to what had been previously observed by other investigators in tests with larger salmonids of other races and species (near 100%).

Although a portion of the test groups in the deep tanks remained at sufficient depth to increase their resistance time, the depth did not provide sufficient compensation to prevent mortality, particularly at levels above 120%. The mortality rates and signs of gas-bubble disease of both species also indicated that less hydrostatic compensation was derived due to depth disposition than expected when the mean depth of the fish groups are considered. Thus, individual fish apparently moved substantially from the observed mean depth of the test lot. If this did not occur, the effect of hydrostatic compensation would have resulted in a calculated reduction in effective supersaturation of 12-16% for salmon and 17-20% for trout. Since the actual mortality rates indicated that only a 10% and 10-15% (chinook, steelhead respectively) reduction occurred, we assume that the fish were moving randomly about within their observed vertical distribution.

SECTION VII

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TECHNICAL REPORT DATA
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1. REPORT NO. EPA-600/3-76-056	2.	3. RECIPIENT'S ACCESSION NO.
4. TITLE AND SUBTITLE SALMONID BIOASSAY OF SUPERSATURATED DISSOLVED AIR IN WATER		5. REPORT DATE July 1976 (Issuing Date)
		6. PERFORMING ORGANIZATION CODE N/A
7. AUTHOR(S) Earl Dawley, Bruce Monk, Michael Schiewe, Frank Ossiander, and W. Ebel		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS National Marine Fisheries Service Northwest Fisheries Center 2725 Montlake Boulevard East Seattle, Washington 98112		10. PROGRAM ELEMENT NO. 1BA608
		11. CONTRACT/GRANT NO. EPA-1AG-0155(D)
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Duluth, Minnesota 55804		13. TYPE OF REPORT AND PERIOD COVERED
		14. SPONSORING AGENCY CODE EPA-ORD

15. SUPPLEMENTARY NOTES

16. ABSTRACT

Tests were conducted in shallow (0.25 m) and deep (2.5 m) tanks of water at 10C with concentrations of dissolved atmospheric gas ranging from 100% to 127% of air saturation to determine the lethal and sublethal effects on juvenile fall chinook salmon (Oncorhynchus tshawytscha) and steelhead trout (Salmo gairdneri).
Fall chinook salmon (average fork length of 42 mm) were much more resistant to supersaturation than juvenile steelheas trout (average fork length of 180 mm). Salmon tested in the shallow tanks at 120% of saturation incurred 50% mortality in 22 days whereas trout tested at the same level incurred 50% mortality in 30 hours. Signs of bas bubble disease were noted on dead fish and on subsamples of live fish from deep water tests at 110% saturation and in shallow water tests at 105% or above. Vertical distribution of both salmon and trout in the deep tanks appeared to compensate for about 10% and 10-15% respectively of effective saturation. Average depth of the fish in deep tanks increased with increased gas concentration. Significant differences in growth and condition factor of the salmon and trout were not found between stressed and control fish during the test period.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
animal physiology, salmon, trout, environments, gas dynamics, pathophysiology, water pollution, fresh water	gas bubble disease supersaturation Pacific salmon	06/c/f 07/b 13/1/k

18. DISTRIBUTION STATEMENT Unlimited release	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 49
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA Form 2220-1 (9-73)

☆ U.S. GOVERNMENT PRINTING OFFICE: 1976— 657-695/5457