

Gas Bubble Disease

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Effects of Long-Term Exposure to Supersaturation of Dissolved Atmospheric Gases on Juvenile Chinook Salmon and Steelhead Trout in Deep and Shallow Test Tanks

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ABSTRACT

Bioassays in shallow (0.25 m) and deep (2.5 m) tanks with dissolved atmospheric gas concentrations ranging from 100 to 127% of saturation in water at 10°C were conducted to determine the lethal and sublethal effects on juvenile fall chinook *Oncorhynchus tshawytscha* and steelhead trout *Salmo gairdneri*.

Juvenile fall chinook (38.7 to 41.3 mm) were much more resistant to supersaturation than juvenile steelhead (164 to 196 mm). Chinook tested in the shallow tanks at 120% of supersaturation incurred 50% mortality after 22 days, whereas steelhead tested at the same level incurred 50% mortality in 30 hr. Gas bubble disease signs were noted on mortalities and on live subsamples taken every 28 days. Vertical distribution of both chinook and steelhead groups in the deep tanks appeared to compensate for about 10% and 10 to 15%, respectively, of effective saturation. Average depths of the fish tested in deep tanks increased with increased gas concentration. Significant differences in growth and condition factor were not found between stressed and control fish during the test period.

Effects of supersaturation of dissolved atmospheric gases on freshwater fishes have been studied by many investigators since the late 1800's. The current problem of supersaturation in the Columbia and Snake Rivers (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 has recently been accomplished by fisheries 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, et al., 1973;

Dawley and Ebel, 1974) 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 gairdneri*.

MATERIALS AND METHODS

Two bioassays of dissolved gas were conducted at the Northwest Fisheries Center (Seattle, WA). The first was completed in 1973 using fall chinook salmon as the test animals, and the second in 1974 using steelhead trout. Assays 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

Dawley, Schiewe, and Monk: National Marine Fisheries Service, Seattle, Washington.

stress from supersaturation on their ability to transfer to salt water; a second group was examined for signs of gas bubble disease. Groups of chinook and one group of steelhead exhibiting signs were then placed in equilibrated water (100% T.D.G.) for a 2-week recovery period and subsequently re-examined for signs of gas bubble disease.

Deep water tanks were 2.44 m (8 ft) deep which provided a maximum hydrostatic compensation of 0.27 atm or 27% of saturation, and shallow tanks were 0.24 m (10 in.) 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) total dissolved gas (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 work indicated that tests at 110% of saturation in deep tanks could serve as a quasi-control).

Juvenile fall chinook were acquired from the Spring Creek National Fish Hatchery in early February 1973 as buttoned up fry for use in the first experiment, and juvenile steelhead were captured during their seaward migration down the Snake River on April 30, 1974, for use in the second bioassay conducted in 1974. Steelhead were 1+ yr 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 initiation of tests, random samples were taken from each population to obtain average weights, lengths, and condition factors (Table 1).

The bioassay with chinook 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 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 concentrations of dissolved gases plus 14 days of subsequent tests).

Once testing began, each tank was examined four 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 mortalities 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 m, 0.6-1 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

TABLE 1 Means and Standard Deviations of Weights, Lengths, and Condition Factors of Randomly Sampled Fall Chinook and Steelhead Taken from Test Populations Before Testing

Fall chinook			
n	Wt. (g)	Ln (mm)	Condition factor
60	.43 ± .06	40 ± 1.3	.67
84	.42 ± .06	40 ± 1.2	.65
Steelhead			
n	Wt. (g)	Ln (mm)	Condition factor
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

than five 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). 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 4 weeks).

Dechlorinated water from the Seattle city water system which is supplied by the Cedar River was used in these tests. Temperature was maintained at 10° ± 0.5°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 O₂ into the suction side of two centrifugal pumps which were plumbed with a recirculation loop to two closed receivers (52 gal each). Hydraulic pressure within the receivers was maintained at 2.1 kg/cm² (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 PVC lines which directed the supersaturated water to a vertical stack of aluminum trays (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 per-

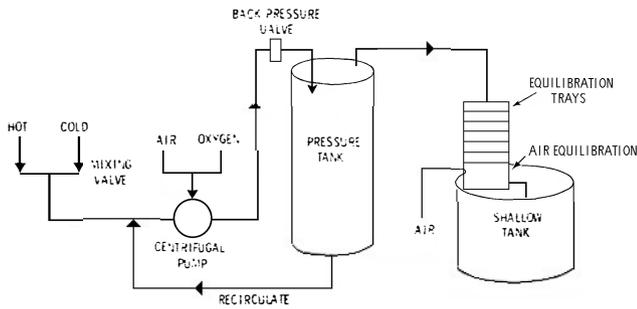


FIG. 1 Schematic drawing of system used to produce water supersaturated with dissolved atmospheric gas in shallow water tanks.

forated ends alternated to produce a back and forth flow of water from tray to tray. Water was then collected in a plexiglass box (18 in. x 18 in. x 10 in. 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. 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 at 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 l of water, respectively. A plexiglass window 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 standpipe.

Lighting was controlled with time clocks to simulate natural sunrise and sunset and light intensity at the surface of each test tank was from 10 to 20 lumens/ft² during full intensity periods.

Water quality determinations for parameters other than dissolved oxygen and nitrogen were made before testing began and once each week or once every 4 weeks depending on the parameter measured (Table 2). Analysis procedures were those of A.P.H.A., *Standard Methods for the Examination of Water and Waste Water*, 1971. The monthly measurements were made by personnel of the State of Washington Department of Ecology using a P & E 303[®] atomic absorption spectrophotometer. All concentrations of heavy metals or other potentially dangerous compounds fell below potential danger levels to salmon and steelhead (McKee and Wolf, 1969).

Dissolved gas analyses were made on each tank at least once each weekday for the first 2 weeks, then a minimum of twice each week for the rest of the test period. Procedures were identical to Dawley and Ebel (1974). A gas chromatograph was calibrated for nitrogen and argon using a modified manometric blood gas analysis apparatus (Van Slyke and Neill, 1924). The modified Winkler procedure (A.P.H.A., 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 in the shallow tanks and from the surface of the deep tanks. 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. overall (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.

RESULTS

Lethal Effects of Dissolved Gas

Chinook groups 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, 13% mortality had occurred in groups held at 110% in shallow tanks and 4% had occurred in groups held at 120% in deep tanks. Mortality was insignificant in groups held at lower gas concentrations. Curves of accumulative mortality for all test groups are shown in Fig. 2. Average cumulative mortality in the control tanks was minimal (3%) for the first 60 days, but by day 127 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 groups (both deep and shallow) was noticed on day 64 of the test. We believe these changes resulted from an infection caused by *Cytophaga psychrophila*. All groups (test and control) were taken from test tanks and bathed in a 10 ppm solution of terramycin for 1 hr. A supplement to the daily ration of 0.5% oxytetracycline was administered for the following 10 days, and after a 2-week interval another 0.5% supplement was added for 10 days. After the second treatment (day 100) the fish in all tanks behaved normally.

Steelhead groups held at 120% and 115% of saturation in shallow tanks developed substantial mortality within 7 days, 100% and 57% respectively,

* Trade names referred to in this publication are not an endorsement of commercial products by the National Marine Fisheries Service.

TABLE 2 Range of Concentrations in mg/ℓ of Water Quality Parameters Measured of Water from Testing Facilities During the Period February 20-June 25, 1973 and May 6-21, 1974

Parameter	Test tanks												
	Shallow					Deep							
	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	6.7-7.0	
NH ₃	.05	.05	.05	.05	.05	.05	.05	.05	.05	.05	.05	.05	
Cl ₂	.02					.02	.02						
Monthly measurements													
Zn	n.d.					n.d.							
Cu	n.d.					n.d.							
Cd	n.d.					n.d.							
Pb	n.d.					n.d.							
Cd	n.d.					n.d.							

n.d. = nondetectable on Perkins and Elmer 303® atomic absorption spectrophotometer. Measurements made by Washington State Department of Ecology, Olympia, Washington.

NOTE: Other parameters measured prior to testing: CO₂(1.2-2.0), chloride (4-6), cyanide (.02), fluoride (.9-1), iron (.1-.8), nitrate (.03), nitrite (.003), phenol (.01), potassium (.2-1), sulfate (3).

Measurements made by Environmental Protection Agency, Redmond, Washington.

TABLE 3 Mean Dissolved Gas Concentrations of 4-Month Period for Test Tanks Containing Groups of Juvenile Chinook Salmon and 15-Day Period for Steelhead Tests

Tank #	Chinook				Steelhead			
	Mean percent of saturation				Mean percent of saturation			
	O ₂	N ₂ +Ar	T.D.G.	sd	O ₂	N ₂ +Ar	T.D.G.	sd
Shallow tank (0.25 m depth)								
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 tanks (2.5 m depth)								
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

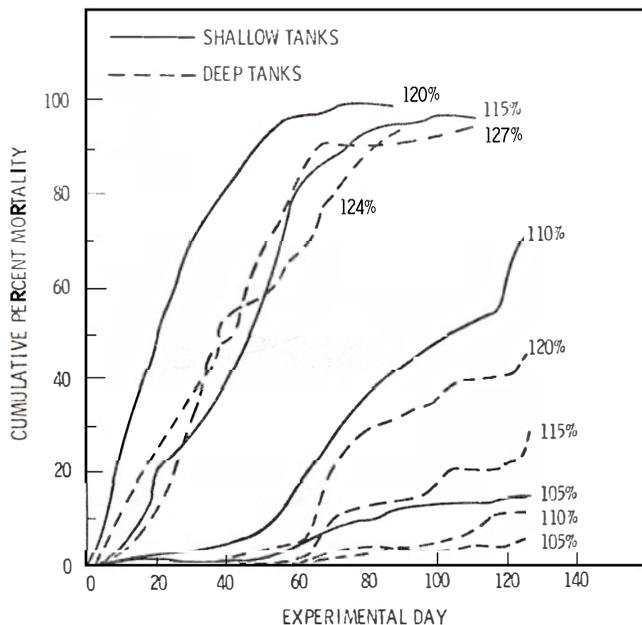


FIG. 2 Mortality versus time curves (combined replicates corrected for mortality of control tests) for juvenile fall chinook exposed to various concentrations of dissolved atmospheric gas in shallow (0.25 m) and deep (2.5 m) water tanks at 10°C.

and the two groups at 127% in the deep tanks averaged 25% mortality (Fig. 3). Control mortality was 2% and was not used to adjust test mortality curves.

Progression of Gas Bubble Disease

The frequency of occurrence of most gas bubble disease signs increased with increasing levels of supersaturation. The fall chinook mortalities incurred the highest incident rate for cutaneous blisters on the head and mouth and for occlusion of gill filaments. Mortalities from 120% and 115% shallow and from 127% deep tanks showed 40-70% incidence of these signs. Other signs which increased with increasing levels of supersaturation but with lower frequencies of occurrence were: heart occlusions (14 to 34%), blisters in the connective tissue surrounding the eye, and blisters between the fin rays (Fig. 4).

In research by Dawley and Ebel (1974), the appearance of gas emboli in the lateral line was the first external sign of gas bubble disease to develop on spring chinook exposed to various levels of supersaturation. We, however, observed that gas emboli in the lateral line of both fall chinook and steelhead trout mortalities were not prevalent within any group, but did appear in high percentage (50-100%) on the biological (live) subsamples from all test groups. Scattered bubbles (less than 15% of the lateral line) also appeared on most mortalities from the control groups. We cannot account for this observation and for that reason only

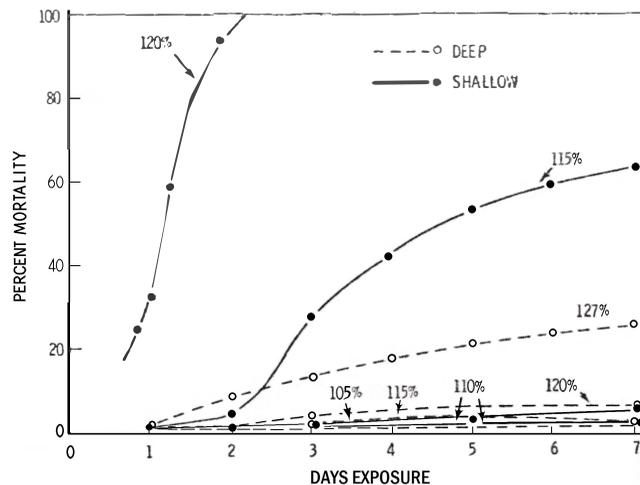


FIG. 3 Mortality versus time curves for juvenile steelhead exposed to various concentrations of dissolved atmospheric gas in shallow (0.25 m) and deep (2.5 m) water tanks at 10°C.

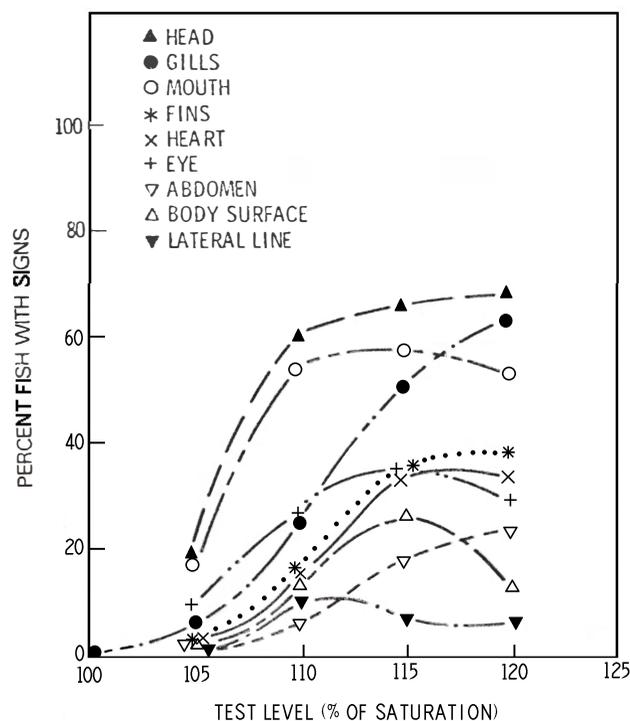


FIG. 4 Frequency (%) of dead juvenile chinook bearing gas bubble disease signs from shallow (0.25 m) test tanks at various levels of dissolved gas.

ascribe this sign to gas bubble disease when more than 15% of the lateral line appears occluded.

Disease signs on live fish sampled generally appeared in rates and patterns similar to those recorded on dead fish removed from the same test concentrations. However, emboli in branchial arteries, gill filaments, and the heart were rarely observed on live subsamples, but were prevalent

on mortalities 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 the steelhead differed from those recorded during tests with the fall chinook salmon. Two differences were:

1. Heart and gill emboli occurred in almost 100% of the dead steelhead, whereas these signs were rarely noted in dead chinook, suggesting that decomposition of the fall chinook quickly masked these signs.

2. Incidence rates of certain signs were directly associated with duration of the test. Live and dead steelhead at test termination showed light incidence of exophthalmia, cutaneous bubbles on the head and in the buccal cavity, and no signs of bubbles on the body surface, whereas the chinook, subjected to supersaturation for a much longer duration, showed high incidence of these signs. Also, gas bubbles 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

When mortality rates in the deep tanks are compared to those in the shallow tanks (Fig. 2 and 3), the average depth of the chinook and steelhead groups in the deep tanks appears to have compensated for about 10% and 10-15%, respectively, of effective saturation. Fig. 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 time 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 G.B.D. signs on dead chinook between deep versus shallow tanks indicates an effective decrease in supersaturation of 12 to 15% (Fig. 6).

Vertical distributions of chinook groups for the first 3 days of the test were variable and not significantly different from one gas level to the next. After 3 days, however, the fish groups at higher saturation levels maintained a greater depth than those at the lower levels and maintained this difference the entire test period (Fig. 7). Steelhead freshwater tests showed similar results, i.e., mean depth increased with increasing gas concentrations.

Night observations showed a depth shift downward of approximately 0.3 m (0.18-0.42 m) for each of the test species at each saturation level (Fig. 8 and 9). The increased depth trend with

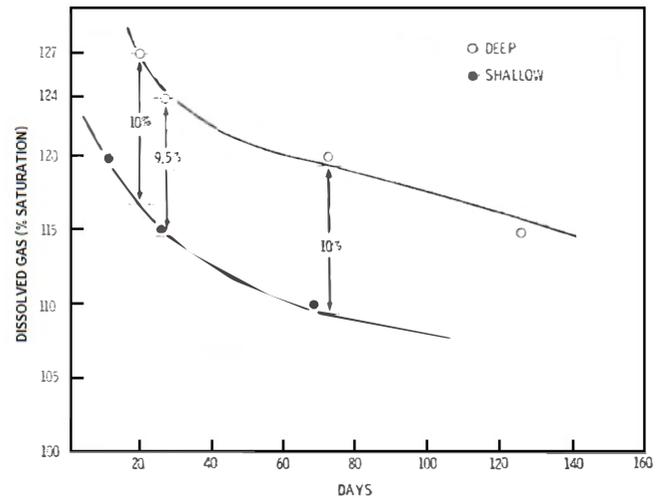


FIG. 5 Exposure times to 25% mortality of juvenile chinook held at various levels of gas in deep (2.5 m) versus shallow water (0.25 m) tanks.

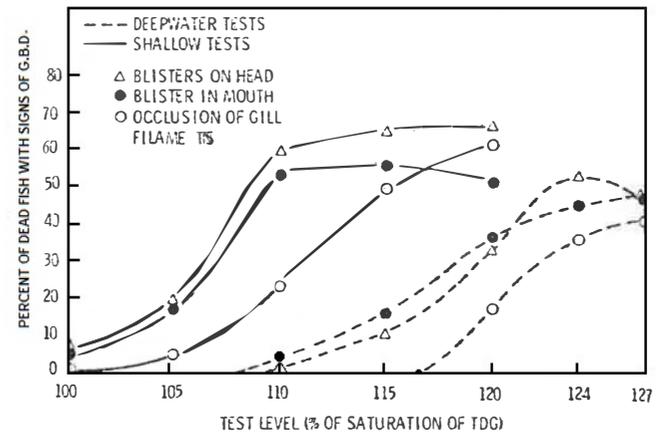


FIG. 6 Frequency (%) of dead juvenile chinook bearing selected gas bubble disease signs from shallow (0.25 m) versus deep (2.5 m) test tanks at various levels of dissolved gas.

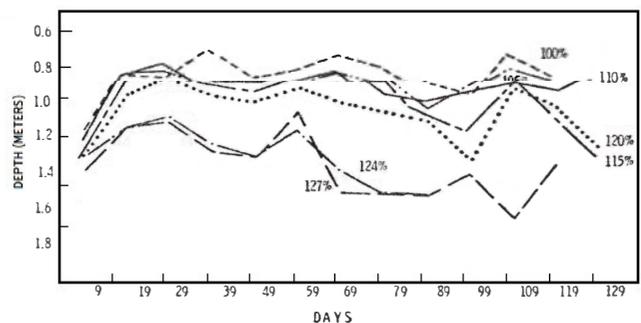


FIG. 7 Mean depth during daylight hours of groups of juvenile chinook held in 2.5 m deep tanks at concentrations of 100, 105, 110, 115, 120, 124, and 127% of saturation—averaged for periods of 10 days over 127 days.

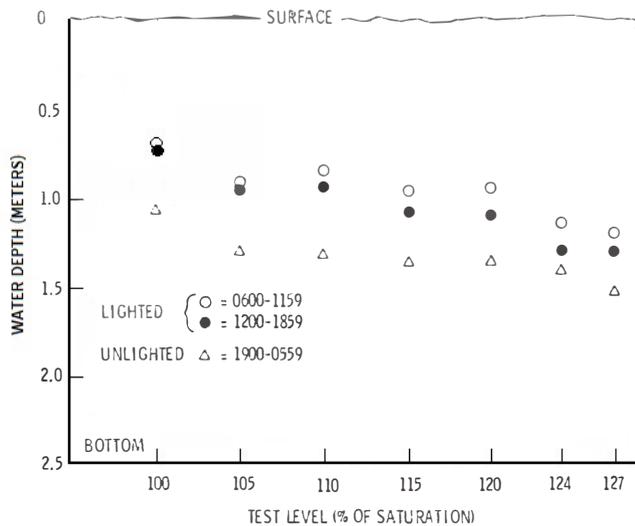


FIG. 8 Mean depths of juvenile chinook groups in 2.5 m deep tanks at dissolved gas concentrations of 100, 105, 110, 115, 120, 124, and 127% of saturation—averaged for 3 segments of the day over 30 days.

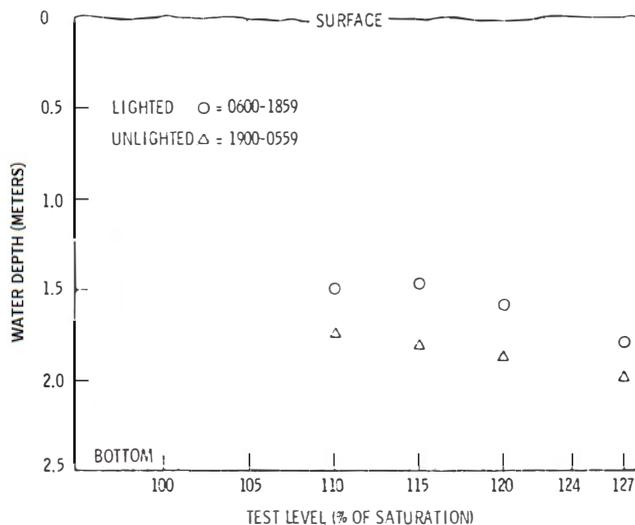


FIG. 9 Mean depth of juvenile steelhead groups in 2.5 m deep tanks at dissolved gas concentrations of 110, 115, 120, and 127% of saturation—averaged for 2 segments of the day over 15 days.

higher dissolved gas concentration noted during daytime observations also occurred at night.

Effect of Gas Supersaturation on Condition Factor

Weight and length data obtained from the live subsamples and the fresh mortalities were used to calculate a condition factor "K" where:

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

These were examined to 1) determine if certain size portions of the population were more susceptible to gas bubble disease and, 2) detect any variations in growth caused by chronic exposure to the various levels of saturation.

Condition factors of mortalities occurring within a 16-day range of the monthly subsamples (e.g., day of subsample \pm 8 days) were compared, by means of a student's T-test, with the mean condition factor of these same subsamples. The mortalities in the 110, 115 and 120% saturation shallow tanks and in the 124 and 127% deep tanks had condition factors significantly higher than the live subsamples ($t = 3.78$, 32 df, $P < 0.001$, $t = 3.87$, 27 df, $P < 0.001$; for deep and shallow tanks, respectively). Thus, the larger fish with higher condition factor died at a significantly higher rate at these concentrations.

Recovery From Gas Bubble Disease

At completion of the freshwater phase of testing, chinook and steelhead groups still surviving retained signs of gas bubble disease similar to those noted on the monthly subsamples (described earlier). Subsamples of chinook tested at 110% of saturation in shallow tanks and at 110, 115 and 120% in deep tanks were placed in fresh water at 100% saturation for recovery observations. A portion of each of these test groups had sustained significant mortalities from gas bubble disease, other groups not included in the recovery tests had no observable signs of gas bubble disease. All subsamples sustained mortalities from 10-16% in the 2-week recovery period (group size 27-48 fish). However, these mortalities could not be attributed to gas bubble disease. After 2 weeks, the survivors no longer exhibited outward signs with exception of one fish with a hemorrhaged eye and another with bubbles in the orbit.

Eleven steelhead surviving the deep test tanks set at 127% saturation were placed in a shallow tank at 100% saturation. After 3 days, examination indicated that cutaneous blisters had decreased both in size and number. (e.g., 5 mm blisters 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. Mortality did not occur in the 7-day recovery period.

Effects of Transfer to Salt Water

Subsamples of survivors from combined replicates of all test groups (chinook and steelhead) were placed into salt water at 25 ppt salinity at 10° C, to determine whether prior exposure to various levels of dissolved gases affected the

ability of these fish to make this transition. Chinook groups of 50 fish from each gas level and from each series (deep and shallow) were transferred on test day 127. A combined total of 98% mortality occurred in 3 days. Only 8 fish survived for a longer time; 1 fish from the 105% shallow tank and 7 fish (14%) from the 110% deep tank; these lasted the entire 13 days. Results of a statistical comparison of fork lengths of the survivors ($X = 67.3$ mm) to those of mortalities ($X = 52.5$ mm) indicate a definite size correlation with ability to make the transfer ($T = 5.73$, 46 df, $P < 0.001$). This suggests that the majority of the experimental stock had not yet reached smolting size and their ability to transfer to salt water was thus severely lessened.

Steelhead test groups were likewise subsampled and groups of 10 to 20 fish were placed into salt water. Mortality varied from 0-16 with no correlation to previous stress experience. However, a size comparison between mortalities and survivors indicated that mortalities were the smaller of the population ($T = 1.925$, 51 df, $P < 0.06$) again suggesting that the dead fish may not have been up to smolting size.

DISCUSSION

Test Results

The mortality curves (Fig. 2 and 3) may be affected by synergistic effect of *C. psychrophila* after day 64; however, incidence rate and types of gas bubble disease signs of dead fish showed no apparent difference for individual tests between the first 60 days and the last 67 days indicating that the effect was not large. 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 60 days have no qualifications, but the last 67 days may represent a fish stock with less than normal tolerance to excess dissolved gas pressure.

As shown in Fig. 10, the death rates and curve shapes correlate well with experiments done by Meekin and Turner (1974), in which they exposed 67, 53, and 40 mm fall chinook to 122% $N_2 + Ar$ plus 74% O_2 (112% T.D.G.). Fish tested by these researchers showed a definite inverse correlation between resistance times in supersaturated conditions and age and growth. Larger fish (53 mm, 67 mm) succumbed much more rapidly than 40 mm fish, tested at the same level of percent saturation (T.D.G.). This same trend was also shown by Shirahata (1966), testing rainbow trout from hatching to fry stage. From this evidence we conclude that the times to death at indicated gas concentrations presented here are typical for these species at this size, and that the increase in mortality rates of fall

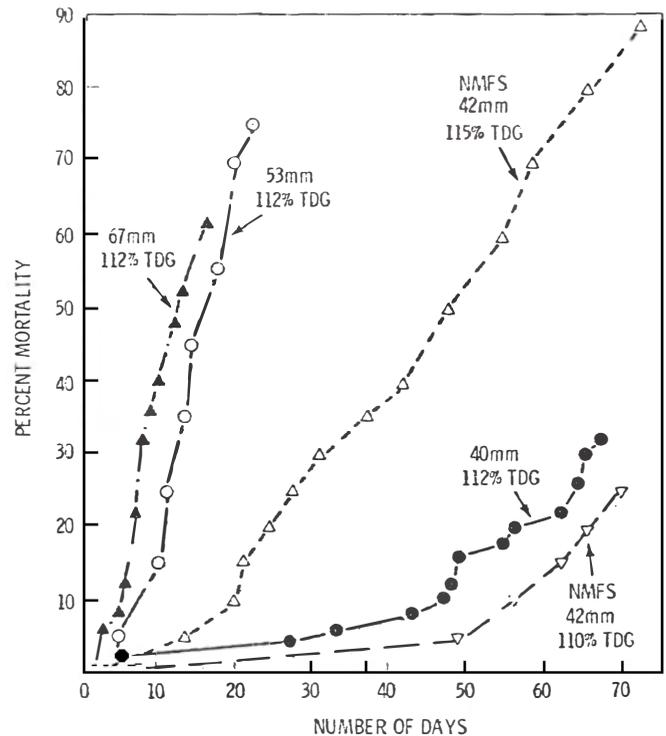


FIG. 10 Mortality versus time curves for bioassay of dissolved gas in shallow tanks (0.25 m or less) at 122% $N_2 + Ar$ and 74% of saturation O_2 (resulting in 112% T.D.G.) with fall chinook at various sizes (Meekin and Turner 1974) and curves at 115 and 110% T.D.G. with fall chinook at 42 mm (NMFS data).

chinook groups as the experiment progressed was mainly commensurate with aging and growth.

In tests done by Dawley and Ebel (1974) the resistance times of steelhead in shallow water tanks at 115% supersaturation was 400% longer than our tests with steelhead at the same saturation level. Fish tested at that time were hatchery reared and smaller (130 mm compared to 180 mm) which is probably the reason for their greater resistance.

The order of magnitude of this difference, however, is small compared to the 1- to 2-month differences in resistance times we observed between fall chinook and steelhead. This difference correlated well with data by Meekin and Turner (1974) which also indicates that fall chinook were more tolerant to exposure to supersaturation than were steelhead of comparable size and age. This same order of ranking was noted by Ebel, Dawley, Monk (1971) and Dawley and Ebel (1974).

Some prominent signs of gas bubble disease occurred on dead chinook 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 time under stress. Exophthalmia appeared frequently in the 3rd and 4th months, similar in incidence to blisters on the head. Blisters at the mid-line of the vertical surface occurred frequently on chinook mortalities taken from the deep and shallow tanks at the highest 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 absorption. Blisters between the fin rays occurred at a very low incidence (40%) at the highest test levels compared to what had been previously observed by other investigations in other tests with larger salmonids of other races and species.

Comparison of condition factors between live and dead fish seems to indicate that the larger fish were more susceptible to gas bubble disease. This agrees with earlier research by Shirahata (1966) and Meekin and Turner (1974). The species tested by these researchers (rainbow trout, salmon, and steelhead, respectively), became less tolerant with age and growth, starting as button up fry.

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 G.B.D. signs 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 is considered. Thus, individual fish must move 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 chinook and 17-20% for steelhead. Since the actual mortality rates indicated that only a 10% and 10-15% (chinook and steelhead, respectively) reduction occurred, we can assume that the fish were moving randomly about within the tank.

Application to the River Environment

Certain observations made during these bioassays have important implications relative to the experience of naturally migrating populations of juvenile salmonids. The Columbia River system is of major concern in our research efforts, thus the following discussion is centered on the implications to fish in the Columbia.

Most areas where salmon and steelhead incubate and develop in the Columbia River system are located above dams and, therefore, would be little affected by supersaturation. However, spring

chinook and steelhead on tributaries of the Willamette River, a major tributary of the Columbia, make heavy use of areas below dams. Also, hatchery water sources in some instances are either below dams that may produce supersaturation during the rearing period or are taken from wells yielding water with high dissolved gas content. In these instances the early stages of life such as the period of incubation become quite important.

Data from our bioassays and others—Shirahata (1969) and Meekin and Turner (1974)—indicate that: 1) yolk sac fry sustain injuries at low levels of dissolved gas which become fatal as the yolk is nearly absorbed; and 2) that after fry have “buttoned up” tolerance to supersaturation becomes quite high but decreases gradually thereafter until time of seaward migration. Nebeker (1973) indicated that tolerance of adult salmonids to supersaturation is slightly less than that of juvenile migrants. Significant changes in tolerance at various life stages obviously occur and the effect varies depending on the life stage. 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, thus management policies should consider the changing effects of these discharges.

The spring freshet on the lower Columbia and Snake Rivers coincides with juvenile salmonid outmigrations as well as some adult upstream migrations. Freshet conditions are variable from year to year, but heavy spillway discharges usually occur every year for some duration creating supersaturation from 120% to 140%. During years of high flow these levels occur throughout long stretches of the river (650 km and more), resulting in long-term exposure of some stocks. Rates of juvenile migration indicate that at least 28 days is required for travel from Little Goose Dam to the Columbia River estuary during the highest flows. Thus, even if fish are compensating for supersaturation by sounding a significant portion of the population is subjected to levels of dissolved gas supersaturation exceeding 120%.

Data on depth distribution of migrating juvenile fish within the Snake River near Lower Monumental Dam (Smith 1974) indicate that 58% of the chinook and 36% of the steelhead 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 higher levels of supersaturation (135% or greater) both stocks of fish would be exposed to levels of gas concentration above 120% for at least 28 days during periods of high flow.

These bioassays have shown that although both the fall chinook and steelhead 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.

SUMMARY AND CONCLUSIONS

Bioassays in shallow (0.25 m) and deep (2.5 m) tanks with dissolved nitrogen and argon gas concentrations ranging from 100 to 127% of saturation were conducted to determine lethal and sublethal effects on juvenile fall chinook salmon and steelhead trout. Throughout the test, mortalities and live subsamples were weighed, measured, and examined for signs of gas bubble disease. After exposures of 127 days (fall chinook) and 7 days (steelhead), remaining groups of fish were: 1) put into saltwater tanks to determine the ability to transfer to salt water; or 2) put into equilibrated water (100% T.D.G.) to determine the ability to recover from gas bubble disease.

We concluded from these experiments that:

1) Significant mortality of juvenile fall chinook commences at about 115% of supersaturation (T.D.G.) in shallow tanks where hydrostatic compensation is not possible and at about 124% in deep tanks where compensation is possible.

2) Significant mortality of juvenile steelhead commences at about 115% in shallow tanks and at about 127% in deep tanks where hydrostatic compensation is possible.

3) Tolerance to supersaturation of atmospheric gas of both fall chinook and steelhead decreases with age and growth.

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

5) The average depth maintained by chinook and steelhead groups 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) pressure.

6) Both fall chinook and steelhead with signs of gas bubble disease are able to recover from exposure to supersaturation.

7) Exposure to various levels of supersaturation does not seem to affect the ability of steelhead to transfer to salt water; data on effect of exposure to supersaturation on ability of fall chinook to transfer to salt water were inconclusive.

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