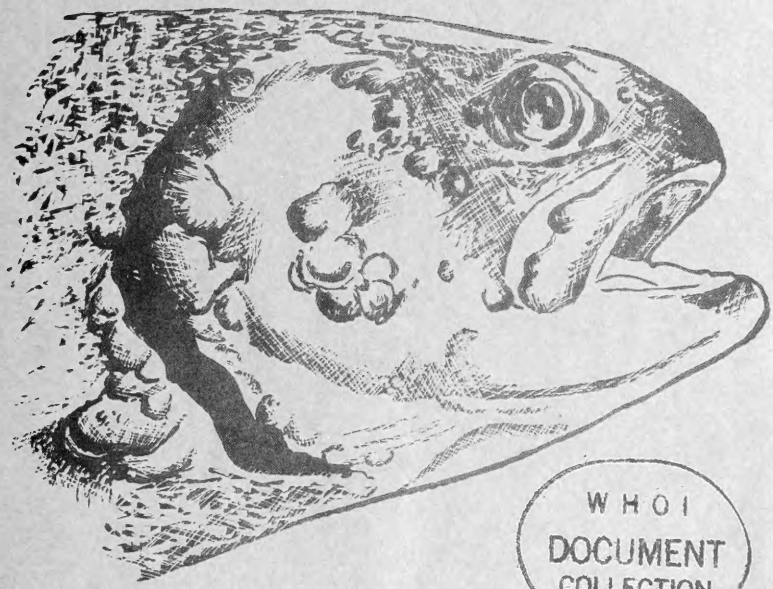
Gas Bubble Disease



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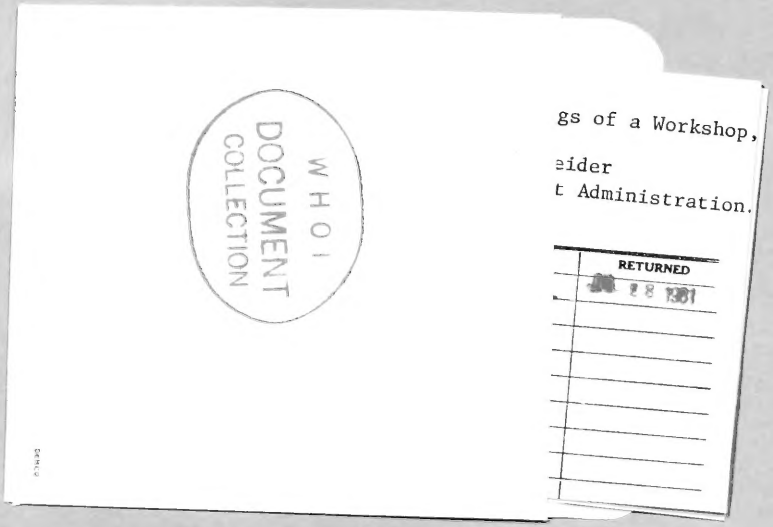

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Gas Bubble Disease

Proceedings of a Workshop held at Richland, Washington,
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Editors

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and

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Foreword

We are happy to host the Nitrogen Task Force and other interagency participants of this workshop on gas bubble disease. Six years ago, Battelle first participated in a tripartite research program of the Environmental Protection Agency (EPA), the National Marine Fisheries Service, and the Atomic Energy Commission involving many of the people here today. One needs only to compare the state of knowledge on gas bubble disease at the time of the tripartite study* with this workshop proceedings in order to fully appreciate the extent to which refinement and accurate delineation of the supersaturation problem has taken place. In 1971, the effects of gas bubble disease seemed to be confounded with those of other stressors; in the present series of papers, we now see it as a quite lethal factor initiated at a fairly critical level of supersaturation. We also know a good deal about its pathology. With the limited resources available to investigators of gas supersaturation effects, the findings here represent significant accomplishments.

Several participants have identified areas of uncertainty requiring continuing research during the years ahead. I would like to comment on several points. In listening to the past two days' deliberations, I believe that four areas require early attention. They require early attention because data useful in practical applications to minimize gas bubble mortality will depend in a key way on our understanding of the underlying processes. First, it seems to me that determinations are needed of the vertical distributions of fishes with respect to gas supersaturation, especially at periods of high runoff in the river system of the Columbia, for example. To too large an extent, models in use are approximations that need fairly systematic validation for each application. Appropriate field effort, as was described yesterday, is time-consuming and hence expensive when applied to validation purposes. It is also necessary, considering the confoundment caused by possible habitat preferences of fish.

Second, field effort should be directed to establishing systematically the population pressures and habitat preferences of various fish. Fish seem to have limited ability to distinguish levels or to detect critical levels of gas supersaturation, *per se;* thus, it is not difficult to see how mortality in salmonids exposed to low levels of gas supersaturation might be greatly aggravated by flight to avoid predation by squawfish, for example.

Third, pressure-equilibration relationships in fish need to be better defined at physiological levels. We need to keep in mind the experience of hyperbaric physiologists in other fields; namely that tissue gas equilibration, while varying inversely with pressure and time, is probably a multi-compartmental process showing widely differing rate constants. Thus, a small compartment, slow in equilibrating, may trigger a neurological incapacitation during decompression even though the body fluids, generally, seem to be equilibrated at the lower pressure. Certain delayed effects, also described in the past two days, may have a similar explanation.

In the papers and the round table discussions that follow, a number of related ideas are developed. If past progress is a guide, I look forward in our future meetings to definitive explanations of these problems affecting hydroelectric power development.

Burton E. Vaughan, Manager
Ecosystems Department
Battelle, Pacific Northwest Laboratories

*EPA-Columbia River Thermal Effects Study
Vol. 1 - Biological Effects Studies, January 1971
Vol. 2 - Temperature Prediction Studies, January 1971

Preface

Gas bubble disease resulting from exposure of aquatic organisms to water supersaturated with dissolved gas was described some 100 years ago, but only in the past decade has a serious problem been recognized in a natural river system. Operation of spillways at hydroelectric generating facilities on the Columbia River and its major tributaries results in entrainment of air into the river water, increasing the dissolved gas content to supersaturated levels. The resulting gas bubble disease is a factor contributing to observed declines in salmonid fish stocks. Even more recently, a widespread gas bubble disease problem has been realized with fish kills in steam generating station discharge plumes, both at freshwater and marine sites. In these cases, supersaturation is caused by the decrease in gas solubility which accompanies heating of water used to cool condensers.

In the past several years, intensive research efforts have been undertaken by several agencies in the Pacific Northwest. These projects have been broadly scoped with major goals of prediction of gas levels and their effects, reduction of impacts on populations of aquatic biota, and development of national water quality criteria and standards. Many of these studies have progressed to a point of having a sufficient data base to begin drawing major conclusions and we have been encouraged by work in other regions. The Nitrogen Task Force has served as an informal forum for exchange of ideas and development of plans for future work, but we felt a need for a Gas Bubble Disease Workshop to draw together on-going research within the Pacific Northwest as well as other regions. The workshop format included formal presentation of papers printed herein and a series of informal round table discussions charged with determining research needs in specific areas of interest. Notes of the round table discussions are also included herein. It is with the hope of stimulating further research efforts and providing a comprehensive view of on-going projects that we present these proceedings.

The success of the Gas Bubble Disease Workshop was certainly due to the efforts of participants for which we thank them. Those who accepted our invitations to co-chair round table discussions contributed significantly to the workshop. Additionally, many Battelle-Northwest staff members contributed significantly to the workshop, and their aid is also appreciated. In particular, J. C. Montgomery, R. W. Hanf, Jr., J. C. Mourich, and J. L. Helbling deserve special recognition for their hard work.

Duane H. Fickeisen
Mark J. Schneider
Richland, Washington
November 1975

For Battelle-Northwest and the Division of Biomedical and Environmental Research of the Energy Research and Development Administration.

Contents

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

E. M. Dawley
M. Schiewe
B. Monk

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 tschawytscha* 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 tschawytscha*, 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 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).

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

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

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 O_2 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-

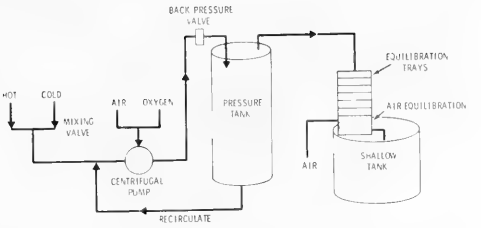

FIG. 1 Schematic drawing of system used to produce water supersaturated with dissolved atmospheric gas in shallow water tanks.

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/ft^2 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

	Test tanks											
	Shallow					Deep						
Parameter	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[B] atomic absorption spectrophotometer. Measurements made by Washington State Department of Ecology, Olympia, Washington.

NOTE: Other parameters measured prior to testing: CO_2(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

	Chinook				Steelhead			
Tank #	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

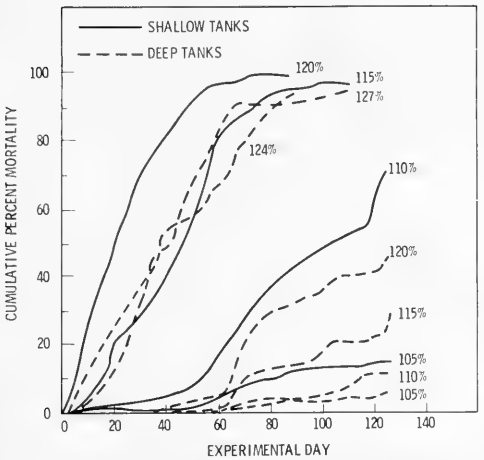

FIG. 2 Mortality versus time curves (combined replicates corrected for mortality of control tests) for juvenile fall chinook exposed to various concentrations of dissolved atmospheric gas in shallow (0.25 m) and deep (2.5 m) water tanks at 10°C.

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

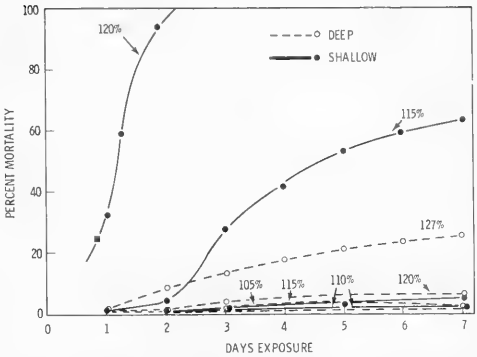

FIG. 3 Mortality versus time curves for juvenile steelhead exposed to various concentrations of dissolved atmospheric gas in shallow (0.25 m) and deep (2.5 m) water tanks at 10°C.

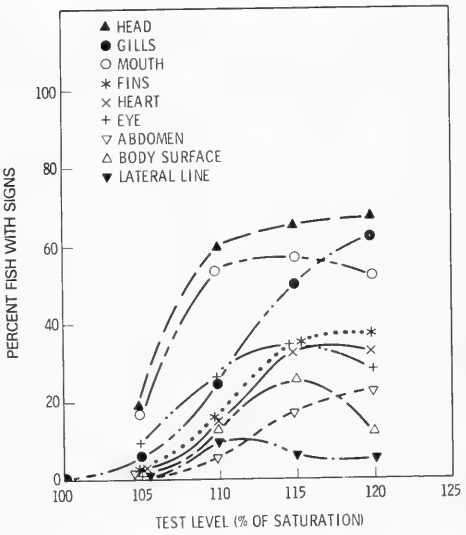

FIG. 4 Frequency (%) of dead juvenile chinook bearing gas bubble disease signs from shallow (0.25 m) test tanks at various levels of dissolved gas.

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

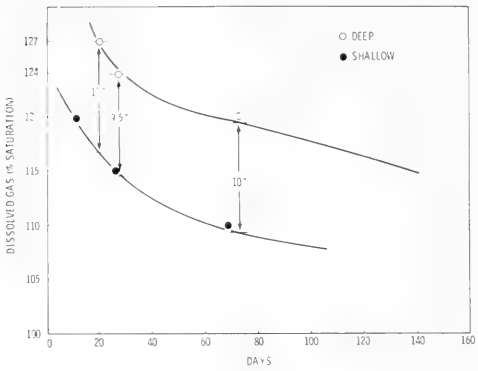

FIG. 5 Exposure times to 25% mortality of juvenile chinook held at various levels of gas in deep (2.5 m) versus shallow water (0.25 m) tanks.

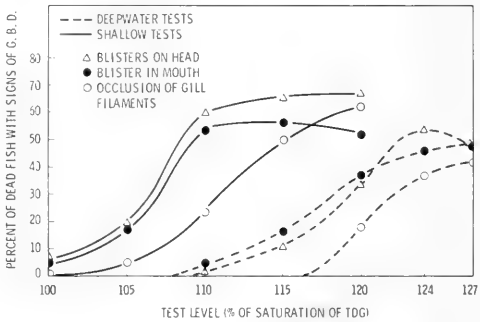

FIG. 6 Frequency (%) of dead juvenile chinook bearing selected gas bubble disease signs from shallow (0.25 m) versus deep (2.5 m) test tanks at various levels of dissolved gas.

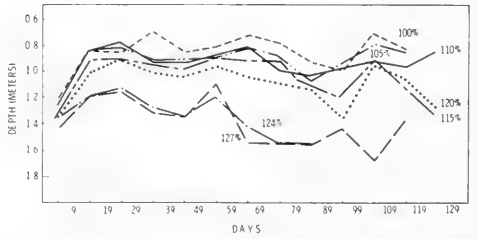

FIG. 7 Mean depth during daylight hours of groups of juvenile chinook held in 2.5 m deep tanks at concentrations of 100, 105, 110, 115, 120, 124, and 127% of saturation—averaged for periods of 10 days over 127 days.

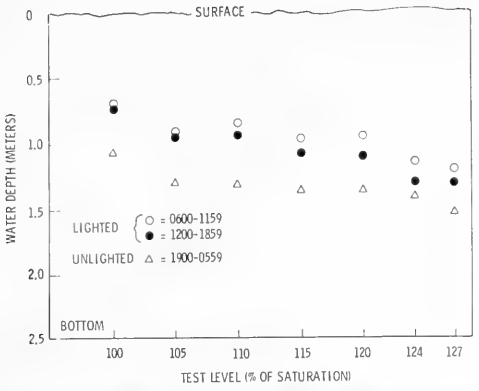

FIG. 8 Mean depths of juvenile chinook groups in 2.5 m deep tanks at dissolved gas concentrations of 100, 105, 110, 115, 120, 124, and 127% of saturation—averaged for 3 segments of the day over 30 days.

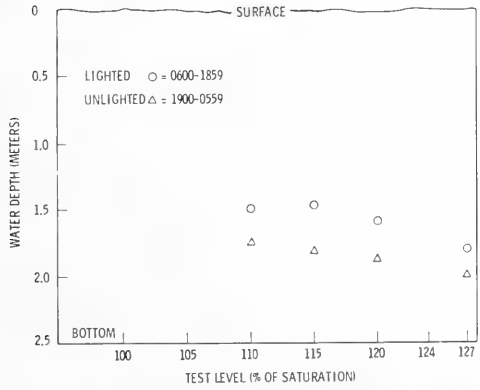

FIG. 9 Mean depth of juvenile steelhead groups in 2.5 m deep tanks at dissolved gas concentrations of 110, 115, 120, and 127% of saturation—averaged for 2 segments of the day over 15 days.

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 ± 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.

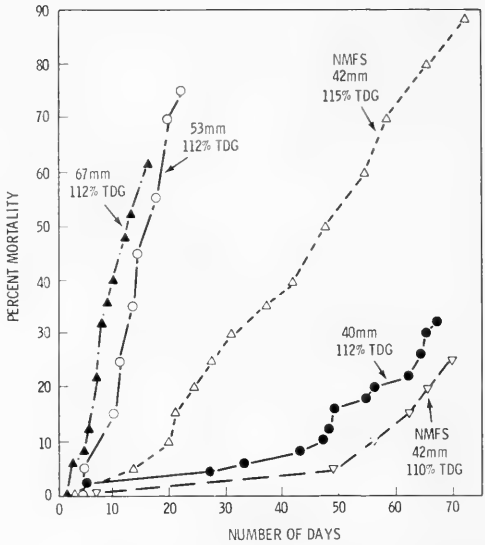

FIG. 10 Mortality versus time curves for bioassay of dissolved gas in shallow tanks (0.25 m or less) at 122% N_2 + Ar and 74% of saturation O_2 (resulting in 112% T.D.G.) with fall chinook at various sizes (Meekin and Turner 1974) and curves at 115 and 110% T.D.G. with fall chinook at 42 mm (NMFS data).

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 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 supersatuation 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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Gas Supersaturation Research National Marine Fisheries Service Prescott Facility—1971 to 1974

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B. McConnell
G. R. Snyder

ABSTRACT

In 1969, the NMFS constructed a field facility for "on-site" environmental testing. The facility is housed on two 110 x 32 foot barges moored on the Columbia River near Prescott, Oregon (RM 72). Research on the effects of nitrogen supersaturation was begun in 1971. Survival is better in the 2.5-m deep tanks than in 1-m deep tanks. Results of tests done in "shallow" test tanks are not representative of what might occur in the river, as fish are not restricted to "shallow" depths. Intermittent exposure to high (130, 120, and 110%) and equilibrated (110%) levels of N_2 saturation generally enhanced test fish survival over that recorded for fish held in constant high levels. Preliminary tests indicate that the fish are not able to detect and avoid lethal conditions of supersaturation. Dissolved gas levels have been monitored at Prescott since 1971.

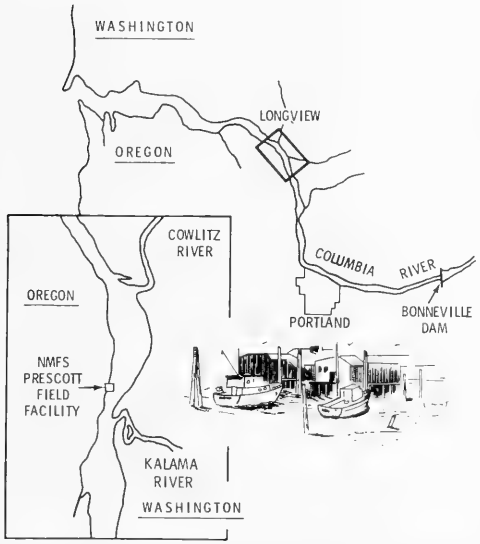

FIG. 1 Lower Columbia River and locale of Prescott Field Facility.

In 1969 the National Marine Fisheries Service initiated "on-site" environmental research on the lower Columbia River. The research facility (Snyder, Blahm, McConnell, 1970) is housed on two 33.5 x 10 m (110 x 32 ft) barges moored near Prescott, Oregon. (Fig. 1).

Research on the effect of nitrogen supersaturation was begun in 1971. The primary emphasis has been on bioassay of prevailing Columbia River water at the site; however, several other types of tests have been conducted e.g. avoidance and detection of gas supersaturation, vertical depth distribution of fish, intermittent exposure to supersaturated and equilibrated N_2 levels. In addition to the biological tests, daily N_2 monitoring at Prescott has been done since 1971; also in relation to monitoring, a study was initiated (in 1974) to determine the gas equilibration characteristics in the Columbia River between The Dalles Dam and Prescott, Oregon. Exploratory tests have been done, and will continue, on O_2 consumption and stamina of fish in relation to N_2 saturation. The effects of hydrostatic pressure on the survival of N_2 stressed fish is also being examined. Results of the exploratory tests will not be included in this report.

The objective of this summary report is to outline representative tests and results to demonstrate the types and diversity of studies completed at the Prescott Facility.

Test results included herein are brief descriptions of general samplings from the total effort; more detailed information will be made available on request. A list of published and non-published data will be included in this report. Following is the sequence in which the various projects will be discussed:

Blahm, McConnell, and Snyder: Environmental Conservation Division, National Marine Fisheries Service, Seattle, Washington.

1. Biological
   A. Intermittent exposure to supersaturated and equilibrated N_2 levels
   B. Detection and avoidance of N_2 supersaturated water
   C. Bioassay of prevailing Columbia River conditions
      1. deep versus shallow tanks
      2. artificially created N_2 levels
   D. Description of the vertical distribution of fish using depth sounding gear
2. Physical Monitoring
   A. Daily gas saturation levels at Prescott
   B. Gas equilibration characteristics in the Columbia River between The Dalles Dam and Prescott, Oregon

INTERMITTENT EXPOSURE

The intermittent exposure tests were planned to assess the effect of intermittently exposing fish to either 130, 120, 110 or 100% N_2 saturation. Information from these tests would help determine if manipulation of water flows (spill) at dams could possibly afford the fish some relief from gas supersaturated water conditions during critical N_2 periods.

Groups of at least 10 fish were held in separate 175 ℓ tanks in which the N_2 levels 130, 120, 110, and 100% saturated were alternately switched as per the following diagram:

Percent N_2

130	120	110
↕	↕	↕
100	100	100

The time cycle, in two test tanks, was based on 24 hr, e.g. one tank, 8 hr at 130% and 16 hr at 100% and the other, 16 hr at 130% and 8 hr at 100% N_2; the same pattern was used for the 120 and 110% N_2 tests. The switching was done by valving, which eliminated handling the fish. Test temperatures were between 10 and 13°C. The alternating cycle was continued for 192 hr (8 days). For this report the results of the 130% to 100% N_2 tests are used to present the general results. Table 1 summarizes the results of intermittent exposure to 130 and 100% N_2. Compiled in Table 1 is the time (hr) to 50 and 100% mortality when the fish were exposed to 130% N_2 for 24, 16, and 8 hr.

With the exception of steelhead and whitefish the 16- or 8-hr exposure enhanced survival time to 50% mortality over that recorded for a constant (24-hr) exposure to 130% N_2. At the 100% mortality level the 16- or 8-hr exposure enhanced survival of all species over that recorded for fish held constantly at 130% N_2 (Table 1).

DETECTION AND AVOIDANCE OF N_2 SUPERSATURATION

Juvenile salmonids are being tested at the Prescott Facility to determine if they can detect and/or avoid N_2 supersaturation. Homogenous groups of fish are introduced into the end of a test tank (Fig. 2) which provides a lateral choice of two channels, one containing dissoived nitrogen at 130% saturation and the other at 102% saturation. Water depth in the channels is maintained at 0.33 m to eliminate the effects of hydrostatic pressure. External influences are minimized by placing curtains around the test tanks and by limiting inspection and water sampling to twice daily. Test duration is 192 hr or until 50% mortality occurs. A

TABLE 1 Time in hr to 50 and 100% Mortality for Fish Subjected to 130% N_2 Saturation for Either 24, 16, or 8 hr of Each 24-hr Cycle During 192-hr Intermittent Exposure Test. The fish were alternately exposed to 130 and 100% N_2.

	50% Mortality			100% Mortality		
	Number of hr during each 24-hr cycle that fish were subjected to 130% N_2					
Species	24	16	8	24	16	8
	Time - hr			Time - hr		
Largemouth bass	93.5	*	*	173.5	*	*
Rainbow	47.0	69.5	*	117.5	141.5	*
Chinook	24.0	120.0	*	48.0	*	*
Cutthroat	24.0	72.0	103.5	39.0	*	*
Whitefish	23.5	23.5	*	23.5	47.5	*
Coho	22.0	*	*	78.0	*	*
Steelhead	16.0	16.0	*	31.0	*	*

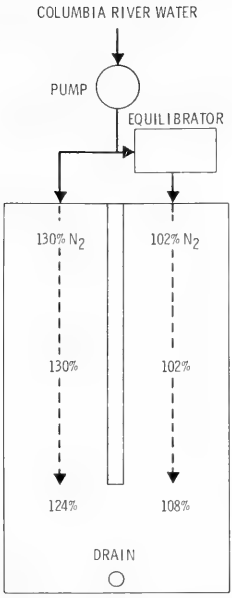

FIG. 2 Plan view of 1.5 x 3.3 m tank used to determine if juvenile fish can detect and avoid N_2 supersaturated water.

replicate test is conducted (using fish from the same population) by switching the "high" N_2 and "low" N_2 channels. A sample of 20 fish is used for each test.

Preliminary tests have been conducted on two species of fish; juvenile steelhead trout and fall chinook salmon. During the first test 50% of the steelhead died in 42.5 hr; in the replicate test a 50% mortality was reached in 43 hr. The survivors from both tests had external gas bubble disease symptoms. No mortalities occurred in the two replicate chinook tests (within 192 hr) and only 10% showed external N_2 symptoms. These two preliminary tests indicate that the juvenile steelhead did not avoid the high gas concentration while fall chinook salmon did. Results from the intermittent exposure tests in Table 1 of this report show juvenile steelhead to be less tolerant to concentrations of dissolved nitrogen than chinook.

N_2 BIOASSAY TESTS

Two types of N_2 bioassays have been conducted at the Prescott Facility since 1971: 1) tests using deep (1.8 x 2.5 m) and shallow (1.8 x 1 m) test tanks at prevailing river levels of N_2 and 2) tests using small 175 ℓ tanks (0.33 m water depths) at constant N_2 levels of 130, 120, 110, and 100% saturation.

Mortality in Deep and Shallow Tanks

The primary objective of these tests was to determine if water depth enhanced fish survival. Table 2 summarizes the species used, number of fish in each test, test duration, range and average nitrogen levels and the total percent mortality that occurred during the test. The N_2 levels, as indicated in Table 2, were either created artificially or were

TABLE 2. Summary of the Numbers of Each Species Held in the Three Tanks (Two Each 1-m Deep and a 2.5-m Deep Tank) Used for Bioassay Tests at the Prescott Facility. Included in the table is the number of days the fish were held and the N_2 saturations. The percent mortality that occurred in each tank is shown for each species.

Species	Number of fish	Test tank water depth			Duration	Percent N_2	
		1 m[1]	1 m	2.5 m		Range during test	Average
		Percent Mortality					
Cutthroat	50	10	60	40	59	119 - 136	124
Cutthroat	50	8	40	27	49	112 - 130	120
Steelhead	80	10	80	6	55	112 - 129	120 R[2]
Chinook	95	0	80	11	55	112 - 129	120 R
Smelt	75	30	100	40	12	119 - 122	121
Smelt	50	24	100	23	5	117 - 121	119 R
Crappie	50	0	0	0	20	117 - 123	120 R
Squawfish	20	0	0	0	35	115 - 124	120

[1]Control tank gas equilibrated
[2]R = naturally occurring river levels

the naturally prevailing Columbia River levels during the test. The shallow control tank was supplied with gas equilibrated Columbia River water. The following is a list of water quality parameters that were monitored in each test tank during the N_2 bioassay studies:

1. Dissolved oxygen-O_2
2. Nitrogen gas-N_2
3. Carbon dioxide-CO_2
4. Ammonia-NH_3
5. Conductivity
6. Alkalinity
7. Turbidity
8. pH

With the exception of N_2, all parameters remained within safe biological ranges throughout test period.

Examining Table 2, we see that survival was better in the deep tanks. This is what one would expect knowing that as hydrostatic pressure increases (with water depth) the percent of nitrogen decreases. In these tests the crappie and squawfish were the most tolerant while the smelt were the least tolerant. Within the salmonids tested, the cutthroat were slightly more tolerant than either the chinook or steelhead, while the latter two species showed comparable tolerance. These general conclusions apply only to the N_2 levels as indicated in Table 2.

Small Tanks—130, 120, 110, and 100% N_2 Saturation

The tests in the 175 ℓ tanks were designed to provide added information on the effect of nitrogen on fish survival. Thirteen bioassay tests have been done with nine species. Groups of from 5 to 20 fish were held in separate tanks at 130, 120, 110, and 100% N_2 saturation. Each test was continued for 192 hr during which mortality was recorded. As with the preceding tests (deep versus shallow tanks) water quality parameters remained in acceptable biological ranges with the exception of N_2. Test temperatures were between 10 and 13°C. Table 3 summarizes the species of fish used and the time (hr) to 50% mortality at 130 and 120% N_2 saturation. The 50% death level was not reached in any of the 110% N_2 saturation tests nor did any mortality occur in the 100% N_2 saturated control tests. Bass and crappie were the most tolerant of the species used in these tests while smelt were the least tolerant. At the 130% N_2 level the rainbow and steelhead trout were the most and least tolerant, respectively, of the salmonid species. The remaining salmonids, including whitefish, seemed to be grouped at around 24 hr survival for 50% of the test animals subjected to 130% N_2 saturation. The ranking changes at the 120% level, but not drastically (Table 3). These tests reflect general trends, and one could surmise that the synergistic effect of various stresses (temperature, disease, maturity, etc.) could alter the pattern derived from these series of tests.

TABLE 3 Species and Time, in hr, to 50% Mortality for Groups of Fish Held at 130, 120, 110, and 100% N_2 Saturation. Included is a ranking from most to least tolerant.

Species	Percent N_2 saturation			
	130	120	110	100
	Time to 50% mortality - hr			
Largemouth bass	93.5 (1)[1]	* (1)	*	*
Crappie	55.0 (2)	* (2)	*	*
Rainbow	47.0 (3)	141.5 (5)	*	*
Chinook	24.0 (4)	* (3)	*	*
Cutthroat	24.0 (5)	119.5 (6)	*	*
Whitefish	23.0 (6)	50.5 (8)	*	*
Coho	22.0 (7)	* (4)	*	*
Steelhead	16.0 (8)	72.0 (9)	*	*
Smelt	5.5 (9)	30.0 (7)	*	*

* = No 50% mortality level

[1] Number in parenthesis indicates ranking of tolerance

DESCRIPTION OF VERTICAL DISTRIBUTION OF FISH USING DEPTH SOUNDING GEAR

In 1972 a Benmar depth sounder was modified for use in a 1.8 x 3 m redwood test tank. Preliminary tests in the tank indicated that the sounding gear would work satisfactorily to determine depth distribution of fish. After examining the resulting tapes of several 24-hr tests we found that the fish were generally below 1 m water depth (this would enhance their survival from that in a 1 m deep tank). Two transducers were used in the test tank; one at the water surface and one on the bottom. A printer/counter system provided a fish count for each 0.6 m interval of water in the 2.5 m tank. (A description of the technical aspects of the system is attached to this report.)

The next step was to test this equipment in the river. The two-transducer arrangement was modified to a 10-transducer array (Fig. 3) which could be placed on the bottom of the river at gently sloping beaches. This configuration was used at two locations near Prescott for a total of 75 hr during day and night. Fig. 4 summarizes the results: of 776 fish approximately 72% were detected between 0.9 to 2.1 m (3 and 7 ft). Many more fish were detected during darkness than daylight. While species could not be differentiated by the sounder, a minimum seine effort (2 sets) on the

FIG. 3 Photo of 10-transducer sonic array and recorder.

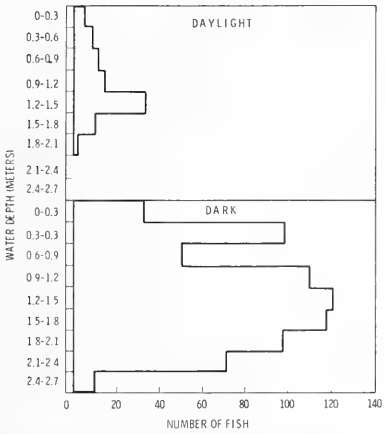

FIG. 4 Numbers of fish detected (at 0.33 m intervals) by sonic gear during 75 hr of operation at Columbia River beaches near Prescott, Oregon.

beaches netted 37 juvenile chinook, 21 crappie, 17 perch, 16 stickleback, 9 flounder, 2 peamouth chub and a whitefish. This indicates that the "sounder" results can be approximately quantified for species composition with a minimum beach seining effort. We feel this method of determining vertical fish distribution will eliminate some of the problems associated with gill netting.

GAS SATURATION LEVELS AT PRESCOTT, OREGON—1971-1974

Since January 1971 approximately 1,000 Columbia River water samples have been analyzed for gas content. During this period samples have been taken twice weekly from the Oregon side of the Columbia River (RM 72)*; daily samples were taken when the N_2 level exceeded 110%. The highest level recorded at Prescott was 136.6% N_2 (132.6% total gas saturation) on June 25, 1974. Nitrogen concentrations in the lower Columbia River exceeded the provisional standard (110% N_2) each month from November 1973 through August 1974. Additional information was obtained from samples taken weekly on a horizontal transect of the river. N_2 saturations on the Washington side of the river are usually higher than on the Oregon side; highest levels are generally recorded from the ship channel.

The saturation levels at Prescott, Oregon, are influenced by the volume of water being spilled at Bonneville Dam, for example, when weekly average spillway discharges at Bonneville reaches 4.247 KCMS (150 KCFS) the N_2 saturation levels at Prescott exceed 115% (Fig. 5). Gas saturation levels during the first 8 months of 1972 and 1974 are comparable (Fig. 6); both being associated with 40-yr high-water flows. In 1973 N_2 levels were considerably less than in previous years; outflow was 69% of a 15-yr average.

The data collected from this sampling program is tabulated and stored in the Corps of Engineers, North Pacific Divisions' ADP system.

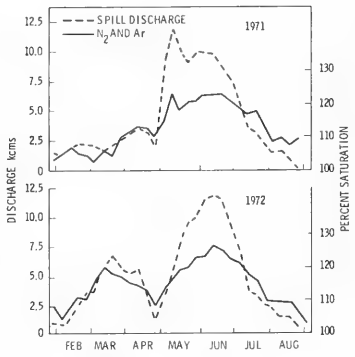

FIG. 5 Weekly averages of Bonneville "spill" and percent N_2 saturation at Prescott, Oregon.

*River mile (RM), rather than river kilometer, is used in this report because most current references do not include the metric equivalent.

FIG. 6 Ten-day average N_2 gas level at Prescott, Oregon, January through August, 1972, 1973 and 1974.

GAS EQUILIBRATION CHARACTERISTICS IN THE COLUMBIA RIVER BETWEEN THE DALLES DAM AND ASTORIA, OREGON

Two surveys were done during 1974 to determine the mixing characteristics and rate of gas equilibration in the Columbia River between The Dalles Dam and Astoria, Oregon. One survey was done between Bonneville Dam and Prescott on April 9 and 10 and another between The Dalles Dam and Astoria during June 11 to 14, 1974.

Methods

An 18-ft survey boat floated with the water mass following a 2.4-m long buoy which was submerged except for the top 0.6 m; the buoy was equipped with a light for night drifting. Transectional and/or single water samples were taken at least every 10-12 river miles (Fig. 7) and returned to Prescott for analysis. Time delay on the samples, between collection and analysis, was never more than 12 hr.

Mixing and Equilibration Between Bonneville and Prescott—April 1974

Table 4 is a summary of the results of the survey. The "float" time between Bonneville and Prescott was approximately 26 hr. The average total outflow from Bonneville Dam was 8.1 KCMS (286 KCFS) during this period. Bonneville forebay and power house samples each showed 115% N_2 saturation at the beginning of the survey. The N_2 saturation in the spillrace (3 transect samples) ranged from 123 to 130%. Table 4 shows side-to-side mixing (within error of analysis) at Rooster Rock (RM 130), approximately 15 miles down river from Bonneville Dam. Below Vancouver the N_2 values were apparently influenced (decreased) by the inflow from the Willamette River. The N_2 level decreased approximately 10% between Bonneville and Prescott.

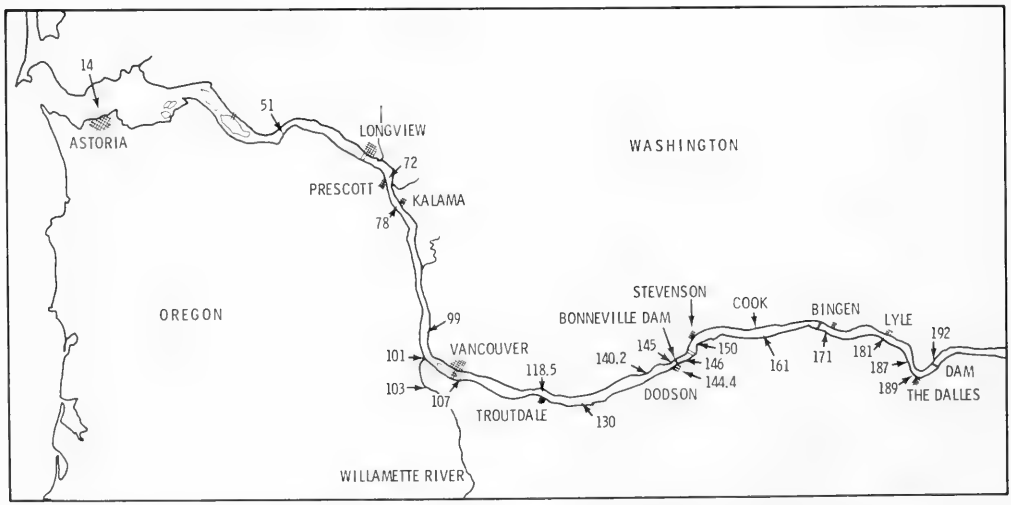

FIG. 7 Sampling locations used during a study to determine gas equilibration characteristics in the Columbia River between The Dalles Dam and Astoria, Oregon.

TABLE 4. Percent N_2 Saturation of Water Samples Taken Between Bonneville Dam and Prescott, Oregon; the Survey was Done on April 9-10, 1974. The average water flow past Bonneville Dam during this period was 8.1 KCMS (286 KCFS).

Sample site	River mile	Location in river	Time of sample	Temp.	N_2 ml/ℓ	N_2 %
April 9						
Bonneville Forebay	146.0	Center	07:35	8.0	17.9	114.9
Bonneville Power House	145.0	Center	08:10	7.8	17.95	114.7
Bonneville Spillway	145.0	Ore.	08:20	7.9	19.2	123.0
		Center		7.8	19.9	127.1
		Wash.	08:25	7.9	20.2	129.5
Tanner Creek	144.4	Ore.	08:35	7.8	17.95	115.0
		Center		7.8	19.1	122.1
		Wash.		8.0	19.8	127.1
Dodson	140.2	Ore.	09:05	7.8	18.1	115.7
		Center		7.8	18.3	117.0
		Wash.		8.0	19.3	124.0
Rooster Rock	130.0	Ore.	11:15	8.0	18.4	118.2
		Center		7.9	18.8	120.5
		Wash.		7.9	18.85	120.8
Troutdale	118.5	Center	17:00	8.1	18.4	118.5
Vancouver	107.0	Ore.	19:40	8.0	18.4	118.2
		Center		8.1	18.4	118.5
		Wash.		8.0	18.4	118.2
Above Willamette River	101.0	Center	21:50	8.1	17.7	114.0
Morgan Turn	99.0	Ore.	23:05	8.2	17.5	112.9
		Center		7.9	18.1	116.0
		Wash.		7.9	18.3	117.3
April 10						
Sauvie	88.0	Ore.	02:35	7.9	18.1	116.0
Kalama	78.5	Center	06:20	8.0	18.0	115.6
Prescott	72.0	Ore.	09:00	8.4	17.3	112.2
		Center		8.0	17.3	115.0
		Wash.		7.6	17.7	112.7

Mixing and Equilibration Between The Dalles and Astoria—June 1974

This sampling effort was begun on June 11 at The Dalles (RM 192) and completed on June 14 at Astoria, Oregon (RM 14). No significant equilibration occurred in the 46-mile reservoir between The Dalles and Bonneville Dams (Table 5). Spillway discharge at The Dalles was held constant at 6.8 KCMS (239 KCFS) for a 7 hr period prior to the start of the survey; total outflow during this period was 10.07 KCMS (379 KCFS). The average total flow at The Dalles on June 10-11 was 10.1 KCMS (386 KCFS) while the average spill was 4.8 KCMS (170 KCFS). In reference to nitrogen level the flow seems to be mixed (side-to-side) immediately below the dam and remains so to the Bonneville forebay.

After a 22-hr time lapse the survey was continued from Bonneville to Astoria on June 12. The Bonneville forebay and power house samples

were approximately the same, 119 and 120% N_2, respectively (Table 5); while the three transect spillrace samples ranged from 135 to 142% N_2. Side-to-side mixing had apparently occurred at Rooster Rock (RM 130). As with the April survey there was an approximate 10% decrease in N_2 level between Bonneville and Prescott and about 18% between Bonneville Dam and Astoria, Oregon. The Bonneville average total flow during June 12-14 was 11.7 KCMS (413 KCFS) while the average spill was 8.1 KCMS (286 KCFS).

In summary it was noted that: 1) during both surveys side-to-side mixing, in reference to N_2 level, occurred at Rooster Rock, 15 river miles below Bonneville Dam, 2) both surveys showed approximately 10% equilibration between Bonneville Dam and Prescott, Oregon, 3) 18 to 20% equilibration occurred between Bonneville Dam and Astoria, Oregon, 4) no significant gas equilibration occurred between The Dalles and Bonneville Dams, and 5) side-to-side mixing was characteristic in all transects between The Dalles and Bonneville forebay. These conclusions should be considered valid only in relation to flows (total and spill) which occurred during the surveys.

TABLE 5. Percent N_2 Saturation of Water Samples Taken Between The Dalles and Astoria, Oregon. The survey was done on June 11-14, 1974. The average water flow past both The Dalles and Bonneville Dams was approximately 11.3 KCMS (400 KCFS).

Sample site	River mile	Location in river	Time of sample	Temp.	N_2 ml/l	N_2 %
June 11						
The Dalles	192.0	Wash.	06:50	13.5	17.6	127.2
Docks	189.0	Ore.	06:55	13.4	17.6	126.9
		Center		13.4	17.8	128.3
		Wash.	07:00	13.4	17.7	127.6
Crates Point	187.0	Ore.	07:10	13.4	17.7	127.6
		Center		13.4	17.6	126.9
		Wash.	07:15	13.4	17.7	127.6
Lyle 160' marker "56"	181.0	Ore.	07:35	13.5	17.0	122.8
		Center		13.4	17.5	126.2
		Wash.	07:45	13.4	17.0	122.6
Bingen	171.0	Ore.	08:05	13.6	17.5	126.7
		Center		13.5	17.5	126.4
		Wash.	08:10	13.5	17.5	126.4
Cook	161.0	Ore.	08:30	13.5	17.4	125.7
		Center		13.5	17.5	126.4
		Wash.	08:40	13.4	17.5	126.2
Stevenson	150.0	Ore.	09:05	13.6	17.1	123.8
		Center	09:10	13.5	17.6	127.2
		Wash.	09:15	13.4	17.6	126.9
Bonneville Forebay	146.0	Ore.	09:35	13.3	17.6	126.7
		Center		13.2	17.6	126.4
		Wash.	09:25	13.4	17.6	126.9
			Approximately 22-hr time lapse			
June 12						
Bonneville Forebay	146.0	Center	07:40	13.6	16.4	118.8
Bonneville Power House	145.0	Center	08:50	13.8	16.5	120.0
Bonneville Spillway	145.0	Ore.	08:55	13.8	18.6	135.3
Tanner Creek	144.4	Ore.	09:10	13.8	16.9	122.9
		Center		13.8	17.4	126.5
		Wash.	09:15	13.8	19.7	143.3

TABLE 5. (Continued)

Sample site	River mile	Location in river	Time of sample	Temp.	N_2 ml/ℓ	N_2 %
Dodson	140.2	Ore.	09:45	13.8	18.0	130.9
		Center		13.8	18.5	134.5
		Wash.	09:55	13.8	19.1	138.9
Rooster Rock	130.0	Ore.	13:10	14.6	18.6	137.5
		Center	13:15	14.2	18.6	136.4
		Wash.	13:20	14.2	18.7	137.4
Troutdale	118.5	Ore.	16:05	14.8		
		Center	16:10	14.4	18.5	136.1
		Wash.	16:15	14.4	18.7	137.6
Vancouver	107.0	Ore.	18:15	15.0	18.1	134.8
		Center	18:17	14.6	18.4	136.0
		Wash.	18:20	14.7	18.5	136.9
Above Willamette River	101.0	Center	19:55	14.8	18.2	135.0
Willamette River	103.0	Center	20:10	15.8	16.9	127.8
Morgan Turn	99.0	Ore.	20:50	14.6	17.9	132.3
		Center	20:55	14.6	18.1	133.8
		Wash.	21:00	14.4	18.3	134.6
Sauvie[1]	88.0	—	—	—	—	—
June 13						
Kalama	78.0	Ore.	06:25	14.4	17.3	127.3
		Center	06:20	14.2	17.5	128.3
		Wash.	06:15	14.3	16.9	124.2
Prescott	72.0	Ore.	08:35	14.5	16.6	128.4
		Center	08:25	14.4	17.4	128.0
		Wash.	08:20	14.3	17.5	128.6
Eagle Cliff	51.0	Ore.	15:00	14.9	16.8	124.9
		Center	14:55	14.6	16.8	124.2
		Wash.	14:50	14.5	16.6	122.4
June 14						
Astoria	14.0	Ore.	07:50	15.0	15.5	115.4
		Center	08:10	14.4	15.3	112.6
		Wash.	08:20	14.3	15.7	115.4

[1]Sample omitted by error

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Equipment and Techniques for Monitoring the Vertical Distribution of Fish in Shallow Water

W. Marshall

ABSTRACT

A recording echo sounder was modified to scan multiple custom transducers to record the depth distribution of fishes. The system has been used in tanks as well as the Columbia River and eliminates the objections that other sampling methods influence depth distribution.

Bioassay tests conducted at the NMFS Prescott Facility in 1972 revealed that salmonids tested in 8-ft deep wooden tanks survive the stress of gas supersaturated water more readily than fish tested in shallower tanks (Blahm, 1972). The hydrostatic pressure of the water at depths effectively prevents gas bubbles from forming in the organs of the exposed fish. Therefore, few mortalities occur.

This paper describes an acoustic counting system developed to provide information on the vertical distribution of Columbia River fishes. Because of the river's turbidity and various practical problems involved with employing fishing gear, an acoustic system was considered a desirable alternative to possible optical or direct capture methods. The acoustic system was initially developed to record the vertical distribution of fish in the Prescott Laboratory's 8-ft test tanks. Later, equipment was developed to monitor the vertical distribution of fish occurring along river beaches near Prescott.

ECHOSOUNDER LIMITATIONS

Depth sounding equipment has been used successfully for locating fish for both sport and commercial fishing. However, the use of conventional recording depth sounders to indicate the depth of fish in shallow water (0 to 10 ft) is difficult because of mechanical limitations. The minimum full chart depth displayed by a single-stylus sounder is typically 100 ft. If 5-in. wide chart paper is used, fish at depths to 10 ft will be displayed in a 0.5-in. wide section of the chart (Fig. 1). If there are many fish recorded in this 0.5-in. space, it is difficult to differentiate individual fish. To spread this 0- to 10-ft depth display to the full 5 in., the stylus must move across the paper at a speed 10 times faster than before. Construction of a mechanical train to move the stylus this fast is practically impossible.

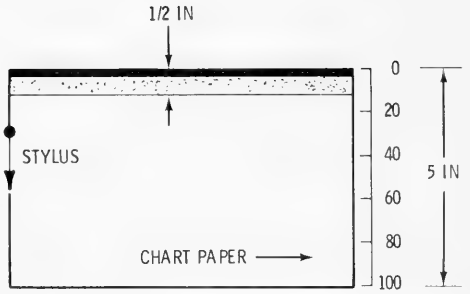

FIG. 1 A typical moving-stylus depth sounder echogram when the transducer is used inside a 10-ft deep test tank.

The Benmar DR-680® sounder eliminates problems associated with a moving-stylus recorder by using 320 individual non-moving stylii. A 400 kHz carrier frequency provides high resolution of small targets; individually detected fish are precisely recorded (Fig. 2). Custom-made wide beam transducers were obtained from Webster Transducers and provided a detection cone with a base diameter of 3 ft in an 8-ft deep by 6-ft diameter test tank (Fig. 3).

PRELIMINARY TESTS

In May 1973, a series of 24-hr tests was conducted with juvenile coho salmon in the 8-ft deep

Marshall: Marine Fish and Shellfish Division, National Marine Fisheries Service, Seattle, Washington.

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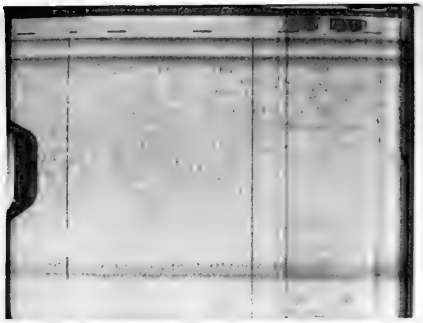

FIG. 2 An echogram from the Benmar DR-680 showing individual targets of 3-in. long coho salmon. Water depth is 8 ft.

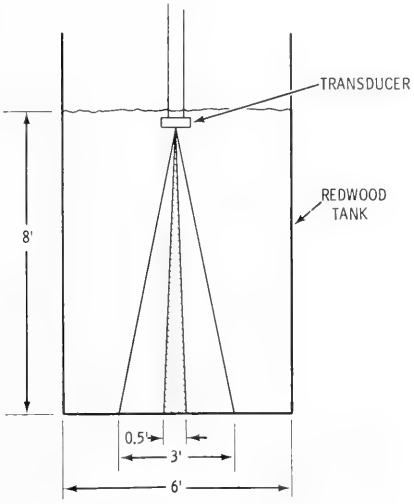

Fig. 3 Transducer in test tank showing conical detection volumes of original transducer (shaded) and the Webster wide beam transducer.

test tank. The transducer was mounted at the bottom of the tank so that as much as possible of the water at the top of the tank could be monitored for fish. The sounder was activated for approximately 2.5 min once each hour by an electrical timer. The resulting echograms revealed two distinct depth behavior patterns: 1) during daylight hours the fish were quite active and homogenously distributed below 2 ft, 2) at night the fish concentrated at mid-depth (2 to 6 ft deep) and were inactive.

Individual targets of fish could have been manually counted to quantify the percentages of fish in specific depth intervals; however, this proved to be difficult because there were approximately 300 fish in the test tank.

COUNTER/PRINTER SYSTEM

An electronic target echo counter and tape printer system were designed and constructed so that the numbers of fish at specific depth intervals could be quantified and recorded automatically. This system was constructed with standard transistor-transistor-logic gates on printed circuit boards manufactured at the Prescott Facility. The printer used was the Model B5-102 Moduprinter® manufactured by Practical Automation, Inc.

Two transducers were used with the counter system so that a maximum volume of water inside the tank could be sampled. One transducer was mounted at the bottom of the tank; the other just beneath the water surface. Four detection areas were defined in the intersecting detection cones (Fig. 4). Because the entire volume of the tank is not sampled, all results of the tests are based on the assumption of homogenous horizontal distribution of fish in the tank.

Basic operation of the counter/printer system is illustrated by the timing diagram in Fig. 5. The instrument can be programmed to generate a trigger pulse once every 5, 10, 20, or 60 min. Whenever this pulse occurs, it turns on the sounder, automatically switches to the top transducer, and initiates the control logic for the counting of a sample. Forty sec after this pulse occurs, the control logic activates electronic counters #3 and #4. During a sampling period of 1 sec, all fish echos

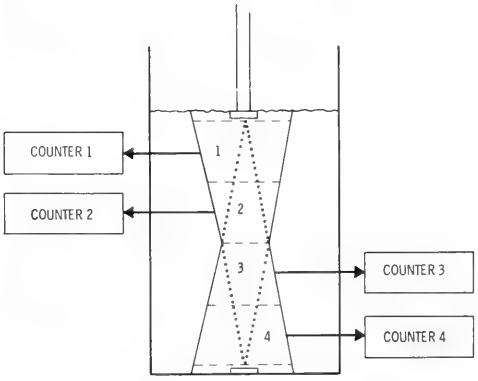

FIG. 4 Detection areas inside the intersecting conical detection volumes.

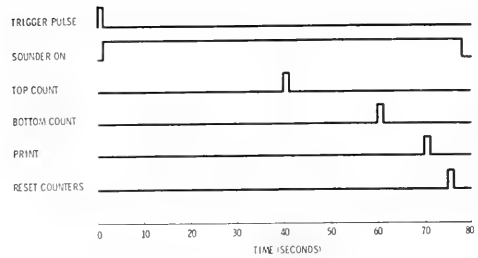

FIG. 5 Basic timing diagram of the electronic counter/printer system.

detected by the sounder in the lower half of the tank are counted and stored in the counters (Fig. 4). Sixty transmit pulses occur during this sample period; therefore, a single fish in detection area #3 for the sampling period would result in the number 60 being stored in counter #3. Two fish in the area would result in the number 120 being stored in the counter. The sounder transmit pulse width is 50 μsec. At a velocity of sound in water of 5000 ft/sec, this represents a distance of 3 in. Therefore, two fish closer together than 3 in. could possibly be detected as a single fish by the sounder. Fifty sec after the trigger pulse, the bottom transducer is automatically switched to the sounder and after a 10-sec delay the second sampling period is used for counting the number of fish echos in detection areas #1 and #2 (Fig. 4). Seventy sec after the trigger pulse the data stored in the counters is printed on the chart paper. The counters are then reset to zero and the sounder and control circuits are turned off. When the next trigger pulse occurs, the sequence is repeated.

RESULTS OF TANK TESTS

During 1974, separate tests were conducted continuously for a week to monitor the diel depth behavior of squawfish, smelt, crappie, and cutthroat trout in the test tank. For these tests, the trigger pulse was programmed to count a sample once an hour, day and night. Fish depth data were recorded by the automatic counter/printer system; the task of manually counting fish echos was eliminated. Data from the tapes were tabulated and standardized to compensate for the unequal water volumes of the detection areas (Fig. 4). Although some variation was observed in the results of these tests, all species generally avoided the top 2 ft of the tank and tended to concentrate in the middle areas. The test tank environment is obviously different from that of the river; however, we did not have to contend with the problem of false echos caused by other fish or debris.

These tests demonstrated that the Benmar® sounder could be used effectively for monitoring the depth of fish in shallow water.

MONITORING THE VERTICAL DISTRIBUTION OF FISH IN THE LOWER COLUMBIA RIVER

An attempt to use the sounder from a boat in the river was unsuccessful. The presence of the boat seemed to "spook" the fish and very few were detected. Subsequently an array of 10 transducers, mounted on an aluminum sled (Fig. 6), was placed on the bottom of the river to overcome this problem.

An electronic sequencer, with transistor-transistor-logic gates and relays (Fig. 7), was designed to serially switch the sounder output to each transducer. Fig. 8 is a block diagram of this unit.

FIG. 6 Photograph of the 10-transducer array.

FIG. 7 The electronic sequencer used with the 10 transducers.

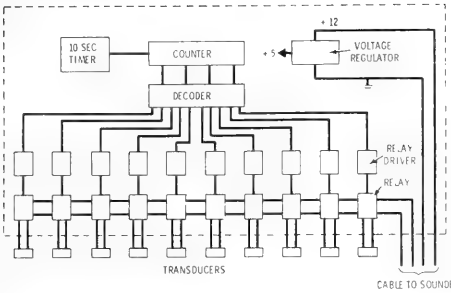

FIG. 8 Schematic diagram of the electronic sequencer.

The sled is anchored underwater on a gently sloping beach with the transducers "looking" upwards (Fig. 9). Cables from each transducer are connected to the sequencer on the beach. A single coaxial cable connects the sequencer to the sounder.

After the gear is in position on the beach, the sounder is set to record for 5 min once an hour. Each transducer is gated on for 10 sec. The resulting echogram is a series of "stair step" patterns because of the different depth of each transducer (Fig. 10). The counter/printer system has not been adapted for automatically recording data from the transducer array and the fish echos must be counted manually.

This system proved to be reliable and relatively trouble free. Data on the vertical distribution of fish in a natural environment was collected and reported on by the staff of the Prescott Facility (Blahm, McConnell, and Snyder, 1974).

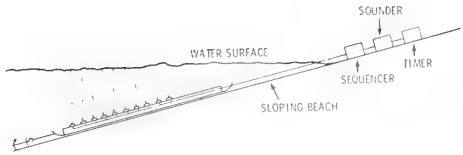

FIG. 9 Transducer array in position underwater with the electronics equipment on the beach.

FIG. 10 An echogram from the transducer array installed underwater on a sloping beach.

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Dissolved Gas Supersaturation: Live Cage Bioassays at Rock Island Dam, Washington

D. E. Weitkamp

ABSTRACT

Three live cage bioassays using juvenile chinook salmon *(O. tshawytscha)* were conducted in supersaturated Columbia River water at the Rock Island Dam forebay. The tests of 10 and 20 days' duration utilized volition, specific depth, and intermittent exposure cages which were suspended between the surface and a depth of 4 m. The volition cages extended from the surface to depths of 2, 3, and 4 m. The four specific depth cages were 1 m deep cages suspended at 1 m intervals between the surface and a depth of 4 m. Intermittent exposures were achieved by raising and lowering three 1 m deep cages in the water column daily to change the actual level of supersaturation experienced by the test fish.

At total dissolved gas supersaturations of about 120% in the reservoir water, significant mortalities were encountered only in fish held within 1 m of the surface. Fish held within 2 m of the water's surface for a period of 16 hr per day suffered significant mortalities only when the supersaturation rose to about 125% and above. The effects of supersaturations above about 125% appear to be much greater than supersaturations below this level. Fish allowed to seek the depth of their choice in the 4 m deep volition cage did not suffer mortalities at saturations of 119% and 128%, although some developed gas bubble disease lesions. Most fish showing slight to severe gas bubble disease lesions were able to recover when placed at a depth of 3 to 4 m in the supersaturated water.

One of the obvious means of determining the effect of a given water quality parameter on fish is the bioassay experiment. This technique has been applied to the investigation of the effects of dissolved gas supersaturation on fish. Bioassays in gas supersaturated water have been conducted by many workers under both artificial laboratory and natural field conditions. Most of the experimental work dealing with the effects of dissolved gas supersaturation has been conducted in the laboratory using artificially supersaturated water or, in some cases, supersaturated Columbia River water in shallow tanks. Several live cage bioassays studying the effects of depth in relation to supersaturation have been conducted in the Columbia and Snake Rivers by Ebel (1969 and 1970) and Meekin and Turner (1974).

The live cage bioassays conducted by Ebel at Priest Rapids and Ice Harbor Dams tested varying high levels of supersaturation. The nitrogen levels during the three Priest Rapids tests in 1967 ranged from a high of 143% at the start of the first test to 118% at the end of the third test. The 1970 Ice Harbor tests exposed juvenile chinook for 7 days to nitrogen levels of 127% to 134%. At some time during most of these tests, supersaturations of 130% or greater were experienced. It is therefore difficult to determine if the mortalities resulted from the highest levels encountered or from continuous levels above 120% or 125%.

The following study was therefore designed to test juvenile chinook under similar field conditions at lower, but more consistent levels of supersaturation. The snowpack of the 1973-74 winter was sufficient to ensure at least moderate levels of supersaturation in the mid region of the Columbia River during the 1974 runoff period. The study was conducted at Rock Island Dam on the mid Columbia. Favorable weather and water conditions allowed the levels of supersaturation to remain quite constant at the test location during test periods.

The study was also designed to test the effect of intermittent daily exposures to gas supersaturation. It is believed that under natural conditions juvenile salmonids and other fish are most likely exposed to high gas levels on an intermittent basis. In some areas this is due, in part, to the intermittent production of supersaturation conditions, as the spill may occur intermittently at dams on smaller rivers. In certain areas, such as the mid and lower Columbia River, however, supersaturation can be present continuously for several months at a time. Fish are intermittently exposed to this continous supersaturation by their vertical movements in the water column. The live cage bioassay

Weitkamp: Parametrix, Inc., Environmental Services Section, Seattle, Washington.

tests were designed to investigate this type of intermittent exposure that fish experience as they undergo diel migrations in continuously supersaturated reservoirs. Intermittent exposure due to daily changes in depth should, at least theoretically, reduce the effects of supersaturation.

PROCEDURES

Location of Study

A major objective of the dissolved gas bioassays was to test the survival of fish in water containing moderate, but relatively constant, levels of supersaturation under natural conditions. The Rock Island Dam forebay on the mid region of the Columbia River was chosen as a site where these conditions could be obtained during the 1974 runoff period. This dam provided a location where the live cages could be placed in water at least 20 ft deep and with a desirable current (0.1 to 0.5 m/sec). The moderate current ensured good circulation of water through the live cages without stressing the test fish.

Submerged Cages

The cages used to conduct the supersaturation bioassays were of three basic types, volition, fixed-depth, and intermittent-exposure. All of the cages were constructed with horizontal dimensions of 4 ft by 4 ft. The volition cages extended from the surface to depths of 2, 3, and 4 m. The four fixed-depth cages were each 1 m deep and were placed at depths of 0 to 1 m, 1 to 2 m, 2 to 3 m and 3 to 4 m. Following the first test, the 3 to 4 m cage was used for testing the recovery of fish showing signs of gas bubble disease (GBD). The intermittent-exposure cages were also 1 m deep, but were constructed so that they could be placed at desired depths between 0 to 1 m and 3 to 4 m. In the first test, the upper depth of the intermittent-exposure cages was 1 to 2 m. The upper depth was raised to 0 to 1 m for Tests II and III. The lower depth was 3 to 4 m for all three tests. A schematic of the various live cages is shown in Fig. 1.

The cages were constructed with a framework of 1 in. angle aluminum held together by stainless and cadmium plated bolts and nuts. Diagonals of 1/2 in. aluminum conduit were used to stabilize the deeper cages. The frames for the volition and fixed-depth cages were constructed with 6-ft cross pieces at the top to suspend the cages within the floats. Suspension of the intermittent-exposure cages was accomplished by placing removable 6-ft pieces of aluminum tubing horizontally under the cross pieces which were located at the appropriate depths.

Knotless nylon netting of 1/2 in. stretched mesh was used to enclose the cages. Each cage had

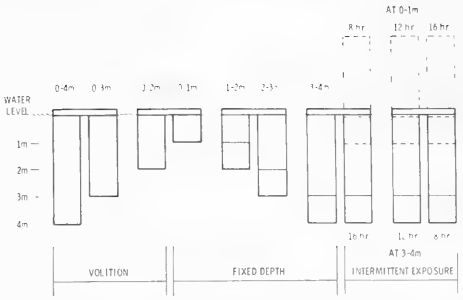

FIG. 1 Schematic of fish live cages used in supersaturation bioassays showing the depths occupied by the various cages.

a zippered opening at the bottom of one side. When the cages were raised each day, these openings permitted removal of dead fish from the bottom of the cage. These openings also permitted observation of the test fish in shallow water when the cages were raised. The specific depth and intermittent-exposure cages were provided with additional zippered openings on the top for introducing the fish into the cages. The assembled frames and nets are shown in Fig. 2.

Two cages were suspended within each float. This provided a minimum distance of 1-1/2 ft between cages in the same float and about 6 ft between cages in adjacent floats. Fig. 3 shows the cage and float assemblies in position adjacent to a barge in the Rock Island forebay.

Test Periods

The first test was conducted for a period of 10 days while the second and third tests were extended to 20 days each. Because Ebel's (1969 and 1970) previous experiments were conducted for

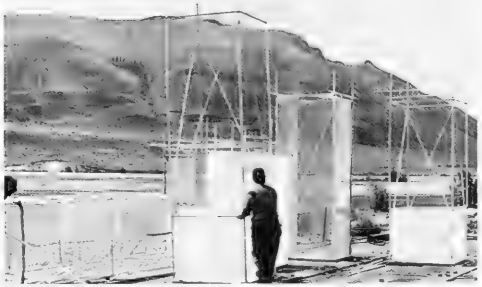

FIG. 2 Live cages being assembled at the study site; 1 m intermittent exposure and fixed depth cages, and 3 m volition cage are shown.

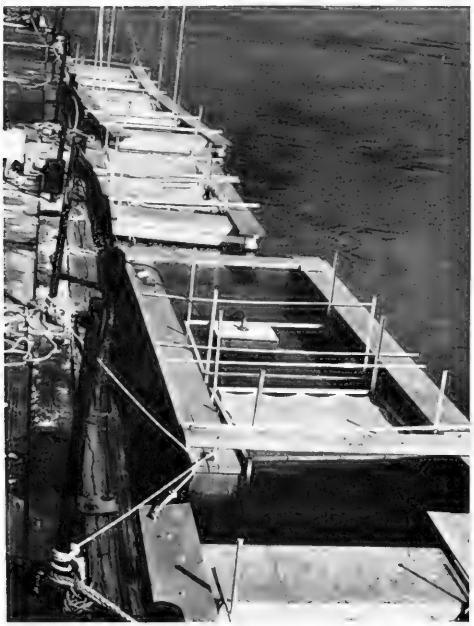

FIG. 3 Assembled live cages in floats. Note extended frame of raised intermittent-exposure cage in background.

periods of 7 to 12 days, it was felt that tests of 10 days' duration would be practical. However, the mortality rates of the first test were much lower than originally anticipated, so the second and third tests were lengthened to 20 days.

Fish Used

The juvenile chinook salmon used in these tests were provided by the Washington Department of Fisheries from its hatchery facilities at the Wells Spawning Channel. Much of the success of these tests can be attributed to the excellent condition of these upper Columbia River summer run chinook salmon. The fish were free of complicating diseases and appeared very healthy when placed in the live cages. The fish showed no signs of smolting at the time they were placed in the live cages. The fish had an average fork length of 97 mm in Test I, 105 mm in Test II, and 112 mm in Test III. One hundred fish were placed in each of the 1 m deep cages. Between 100 and 200 fish were placed in the deeper volition cages, depending on the number of fish provided.

Feeding of Fish

No food was given to the fish during Tests I and III. The fish used in Test II were fed from the 11th day to the end of the test period. It was decided to begin feeding the Test II fish with Oregon Moist Pellets® when some began to show signs of starvation. They were fed at times when the cages were brought to the surface to check mortalities. It was hoped that feeding the fish only when the cages were raised would prevent an artificial depth distribution in the volition cages that might occur with surface feeding when the cages were at their normal depth. Although feeding with the cages near the surface was successful, it did appear to cause at least some of the fish to spend more time near the surface. For this reason no feeding was attempted in Test III.

Mortalities

Each live cage was raised and checked for mortalities between 0800 and 1100 daily for each test. During this period the cage was held with its bottom several inches below the water's surface so that the fish could be observed for signs of gas bubble disease (GBD) or any other problems that might occur. This procedure permitted close observation of the fish each day without unduly stressing the fish. The procedure also permitted cleaning and inspection of the cage netting. Dead fish floating in the cages that extended to the surface were also removed at 1600, 2000, and 2400 hr when the depth of the intermittent-exposure cages was changed. All three of the intermittent-exposure cages were lowered to a depth of 3 to 4 m at 0800 each day. One of the cages was then raised to the surface position at 1600, 2000, and 2400 hr to provide the surface exposures of 16, 12 and 8 hr, respectively.

The mortalities were checked immediately following their removal from the cages for external signs of GBD. The locations of external emboli and hemorrhages were recorded as well as the fish length. The abdominal cavities of all mortalities were checked for internal signs of GBD and the presence of food in the digestive tract.

Dissolved Gas Analysis

During the morning inspection of the live cages, the dissolved gas levels present in the forebay were measured using a Weiss saturometer. The measured saturations given in this report are most likely lower than the levels actually present in the forebay water. An on-site comparison of the saturometer used in this study was made with several other saturometers, indicating readings were 1% to 3% low. Routine monitoring by the Chelan County PUD indicated that supersaturation in the study area ranged from 120% to 131% during the test periods. The saturometer readings were also taken at a time of day when river supersaturations were near their lowest level. A 24-hr monitoring

study conducted in the Rock Island forebay by the Chelan County PUD in August 1974, showed that dissolved gas levels change by about 3% of saturation during a 24-hr period. The lowest gas levels during this 24-hr period occurred between 0300 and 0900 hr, which includes the time that the gas content was measured during the live-cage tests. Some daily variation in the gas levels observed in the live-cage bioassay is due to differences in the water temperature which varied according to the time of day during which measurements were taken.

A number of times during the tests, dissolved gas supersaturations were measured inside some of the cages. This was done to detect any measurable effect of the cages on the gas content of the water as it passed through the cages. The gas content of the water inside the cages was always lower than that of the water outside of the cage by less than 1% of saturation.

Dissolved oxygen levels were measured by the Winkler method. Water temperatures were measured at the surface using a mercury thermometer accurate to ±0.1°C. Light penetration readings were taken daily adjacent to the cages using a Secchi disc.

An attempt was made to record the fish depths inside the 0 to 4 m volition cage. A Benmar DR-681® echo sounder with a minimum range of 0 to 50 ft was used to make the recordings. The DR-681 was the only shallow water echo sounder that could be acquired for these tests. No useful recordings were obtained with the echo sounder due to apparent interference from the cages.

As the depth distribution of the fish in the volition cages was of considerable importance, an attempt was also made to observe the fish using scuba gear. Even with a strong underwater light, the diver was unable to observe any of the fish due to the turbidity of the river water.

RESULTS

Test I

The fish for the first live cage bioassay were placed into the cages in the Rock Island forebay on May 24, 1974. At this time, the total dissolved gas saturation in the river water was 122%. During the first 4 days of the study the saturations remained near 122%. The saturations then dropped to about 120%, and remained near 120% for the remaining 6 days of the test. The total dissolved gas levels measured at the study location are shown in Fig. 4. Saturometer readings were also taken inside the cages during all three tests. Saturations inside the cages were not more than 1% of saturation lower than the readings for the surrounding water.

The water temperatures which were recorded daily during Test I ranged from 10.4°C to 10.9°C.

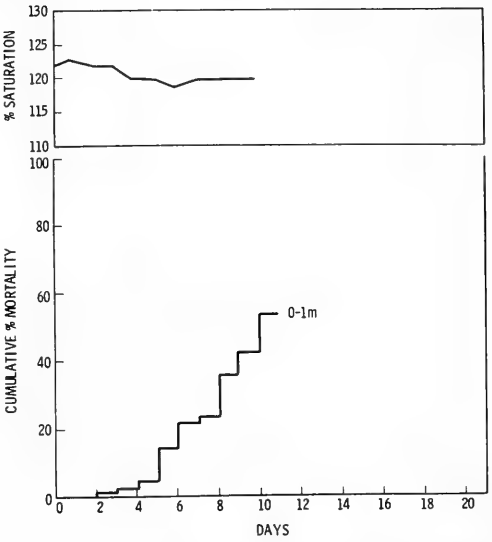

FIG. 4 Test I, total dissolved gas levels and % cumulative mortalities for the 0 to 1 m cage, May 24 to June 3, 1974. No mortalities occurred in the other cages.

These temperatures were recorded between 0800 and 1000 hr. Some days surface water temperatures were a degree or more higher later in the day. Secchi disc readings taken during this test ranged from 0.91 to 1.22 m.

In Test I no fish were lost due to handling stress. The first mortality occurred during the 2nd day in the 0 to 1 m fixed-depth cage. The mortality rate increased somewhat in the 0 to 1 m cage after the 4th day, reaching a cumulative mortality of 53% at the end of 10 days. A single mortality occurred in the 2 to 3 m fixed-depth cage during this test. This single mortality was obviously due to trauma, not to GBD. No mortalities occurred in any of the other cages during this 10-day test. The percent cumulative mortality during this test is shown in Fig. 4, and Table 1 lists the daily cumulative mortality for Test I.

The surviving fish were examined for signs of GBD at the end of the test. Ninety percent of the survivors in the 0 to 1 m cage showed some signs of GBD. These varied from a few bubbles in one fin to bubbles in most fins, the head, and mouth, as well as hemorrhaging at the base of the fins. In the 1 to 2 m cage 15% of the fish showed signs of GBD. Only 3% of the fish in the 0 to 2 m cage showed signs. In both cages the signs of GBD were restricted to bubbles in the caudal fins.

TABLE 1 Test I, Percent Cumulative Mortality Due to Gas Bubble Disease in Juvenile Chinook Salmon Exposed to 119% to 123% Supersaturation at Rock Island Forebay, May 24 to June 3, 1974.

Holding time (days)	Cage depth (meters)									
								1-2 m and 3-4 m[1]		
	0-4 m	0-3 m	0-2 m	0-1 m	1-2 m	2-3 m	3-4 m	↑16 ↓8	↑12 ↓12	↑8 ↓16
1										
2				1						
3				2						
4				4						
5				14						
6				21						
7				23						
8				35						
9				42						
10	0	0	0	53	0	0	0	0	0	0
Survivors with GBD lesions (%)	0	0	3	90	15	0	0	0	0	0

[1] ↑indicates hr/day at 1-2 m depth, ↓indicates hr/day at 3-4 m depth.

Most of the surviving fish with evidence of GBD were placed in the 3 to 4 m cage to determine if they could recover. A few of the severely affected fish died before the cage was submerged. The remainder were held as part of the second experiment. All surviving fish with no indication of GBD were released in the Rock Island forebay.

Test II

The total dissolved gas saturation of the river water at the beginning of the second test (June 5) was down to 120% (Fig. 5) as measured at the test site. By the 4th day (June 9), the gas saturation had risen to 122%, and remained constant until the 10th day when it rose to 123%. During the last 11 days (June 15 to 25) of the test, the saturation varied between 123% and 126%.

The water temperatures at the beginning of the second test remained near 10.7°C for the first 3 days. During the remainder of the test the water temperatures showed a steady increase of about 0.2°C per day, reaching 14°C by the end of the test. The light penetration decreased considerably during the last half of the second test. For the first 8 days, the Secchi disc readings ranged from 0.91 m to 1.22 m. During the last half of the test, Secchi disc readings decreased to 0.33 to 0.61 m.

Two fish were lost at the beginning of this test, apparently due to handling stress. The other test fish showed no signs of GBD at the time of these mortalities. The mortalities occurring due to GBD during the second test did not begin until the 6th day. As in the previous test, mortalities occurred first in the 0 to 1 m cage (Table 2). The daily mortality rate in Test II (Fig. 5) was similar, but not as consistent as that of Test I. The rate at which mortalities occurred in the 0 to 1 m cage between

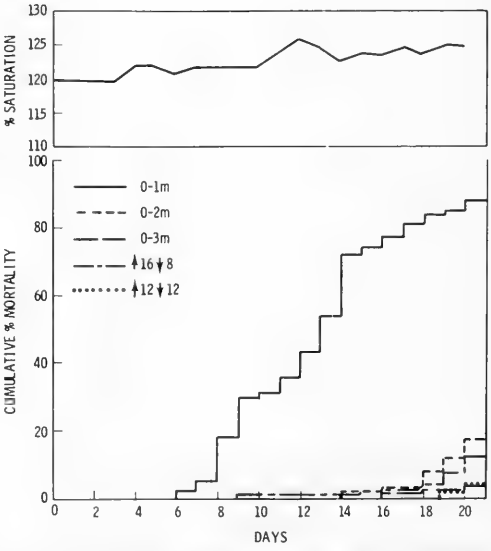

FIG. 5 Test II, total dissolved gas levels and % cumulative mortalities greater than 1% for all cages, June 5-25, 1974.

TABLE 2 Test II, Percent Cumulative Mortality Due to Gas Bubble Disease in Juvenile Chinook Salmon Exposed to 120% to 126% Supersaturation at Rock Island Forebay, June 5-25, 1974.

Holding time (days)	Cage depth (meters)									
							Recovery	0-1 m and 3-4 m[1]		
	0-4 m	0-3 m	0-2 m	0-1 m	1-2 m	2-3 m	3-4 m	↑16 ↓8	↑12 ↓12	↑8 ↓16
1										
2										
3										
4										
5										
6				2						
7				5			2			
8		0.5		18			4			
9				30			7	1		
10				31			7	1		
11				36			7	1		
12				43			10	1		
13				54		1	10	1		
14			2	72		1	10	1		
15			2	74		1	10	2		
16		1	3	77		1	10	2		
17		1	3	81		1	10	2		
18	*	2	8	84		1	10	4		
19		2	11	85		1	10	7	1	1
20		3	17	88	1	1	10	12	4	1
Survivors with GBD lesions (%)	38	64	77	91	0	2	9	69	24	15

[1] ↑indicates hr/day at 0-1 m depth, ↓indicates hr/day at 3-4 m depth

*Approximately 1/2 of the fish lost through small tear in cage.

days 5 and 14 during Test II was very close to that of the same cage between days 2 and 10 during Test I. The mortality rate in the 0 to 1 m cage of Test II appeared essentially the same as that of the same cage in Test I, except that it began about 3 days later. A cumulative mortality of 53% was reached on day 10 of Test I, and a similar mortality of 54% was reached on day 13 of Test II.

The mortality rate in the 0 to 1 m cage decreased during the last 6 days of Test II. From day 14 to the end of the test, the cumulative mortality only increased from 72% to 88% All but one of the fish remaining in the 0 to 1 m cage at the end of the test showed signs of GBD.

Unlike the first 10-day test, mortalities occurred in a number of the other cages during Test II. These mortalities began to occur prior to 10 days, but at a very low rate (Table 2). The 0 to 3 m cage lost one of 200 fish on the 8th day. No other mortalities occurred in this cage until the 16th day. No mortalities occurred in the 0 to 4 m cage during Test II. The size of the test group in the 0 to 4 m cage was reduced around the 18th day when slightly more than half of the group was lost through a small tear in the netting. The 0 to 3 m cage fish experienced a few mortalities during the last 5 days with a final cumulative mortality of 3%. Mortalities began to occur in the 0 to 2 m cage on the 14th day and increased to 17% at the end of Test II.

A portion of the survivors in each of the three volition cages showed signs of GBD. In the 0 to 4 m cage 38% of the survivors showed indications of GBD, as did 64% of those in the 0 to 3 m cage and 77% in the 0 to 2 m cage.

Only a single mortality occurred in each of the two fixed-depth cages between 1 and 3 m. In the 1 to 2 m cage a fish died on the last day, and in the 2 to 3 m cage a mortality occurred on the 13th day. No survivors in the 1 to 2 m cage showed signs of GBD; however, 2% of those in the 2 to 3 m cage had GBD.

Some mortalities occurred in each of the intermittent-exposure cages which were raised to a depth of 0 to 1 m in Test II rather than to 1 to 2 m as in Test I. In the cage spending 16 hr/day at the

surface and 8 hr/day at 3 to 4 m the first mortality occurred on the 9th day with the cumulative mortality reaching 12% by the end of the 20-day test. Mortalities did not occur until the 18th day in the other two intermittent-exposure cages. The cumulative mortalities at the end of the test were 4% in the cage spending 12 hr/day at the surface and 1% in the cage spending 8 hr/day at the surface.

The portion of the survivors showing signs of GBD was related to the length of the surface exposure. Signs of GBD were found in 69% of the survivors in the cage spending 16 hr/day at the surface, 24% in the 12 hr/day exposure cage, and 15% in the 8 hr/day exposure cage.

In Test II the 3 to 4 m cage was used to test the recovery of fish from Test I that showed signs of GBD. These fish began to suffer mortalities on the 7th day, and had a 10% cumulative mortality after 20 days. None of the fish in the recovery cage died after the 12th day.

These mortalities appeared to be due to severe fungal infections resulting indirectly from GBD. All dead fish had the caudal fin completely eroded, and were covered with fungus as far forward as the anus and dorsal fin. Most of the fish placed in the recovery cage lost all signs of GBD by the end of Test II. Only 9% of the surviving fish in this cage showed any signs of GBD when they were released. The remaining signs were slight cases of exophthalmia. Bubbles and hemorrhages were not observed in any of these fish at the time of their release.

Test III

The supersaturation of the water at the test site in Rock Island forebay at the initiation of the third test was much higher than at the beginning of the two previous tests. During the first 9 days the supersaturation dropped slightly and ranged from 120% to 125% at the end of the test.

During the third test, water temperatures were also higher than they had been during the two previous tests. At the beginning of Test III, the surface water was 14.3°C. The temperature continued to show a steady increase throughout the test, reaching 16.1°C at the end. Unlike the previous tests, light penetration showed a steady increase through the third test. Secchi disc readings were at 0.76 m at the beginning and reached 1.89 m near the end of the test.

The initial mortalities occurring in most of the cages during the third test were considerably higher than in the two previous tests. In the 0 to 1 m cage 6% of the fish had died with signs of GBD (Fig. 6). Eighty-three percent were dead by the end of the 2nd day, and all test fish had died with GBD lesions by the 3rd day.

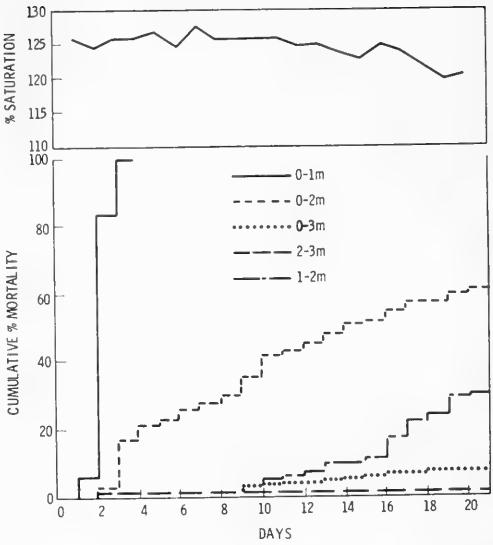

FIG. 6 Test III, total dissolved gas levels and % cumulative mortalities for the volition and fixed depth cages, June 28 to July 18, 1974.

Mortalities also occurred in the two fixed-depth cages between 1 and 3 m, but at a much lower rate than in the 0 to 1 m cage. In the 1 to 2 m cage mortalities first occurred on the 9th day of the test (Table 3). In this cage the mortalities then remained constant giving a cumulative mortality of 70% at the end of 20 days. In the 2 to 3 m cage, a single fish died with signs of GBD on the 2nd day. No other mortalities occurred in this cage. Thirty-three percent of the survivors in the 1 to 2 m cage showed signs of GBD, while none of the survivors in the 2 to 3 m cage showed GBD lesions.

Fish in the shallowest volition cage began to suffer mortalities on the 2nd day (Fig. 6). After a high mortality of 17% between the 2nd and 3rd day, the mortality continued at a relatively constant rate. The cumulative mortality at the end of the test was 61% in this cage (Table 3). In the 0 to 3 m cage, the first mortality did not occur until the 5th day, and only 7.5% had died at the end of the test. No mortalities of any kind occurred in the 0 to 4 m cage during the 20-day test period. Signs of GBD were present in 85% of the survivors in the 0 to 2 m cage, 35% in the 0 to 3 m cage, and in only 8% of the survivors in the 0 to 4 m cage. The 8% with GBD lesions in the 0 to 4 m cage had only slight signs, usually only a few bubbles in a single location.

TABLE 3 Test III, Percent Cumulative Mortality Due to Gas Bubble Disease in Juvenile Chinook Salmon Exposed to 120% to 128% Supersaturation at Rock Island Forebay, June 28 to July 18, 1974.

Holding time (days)	Cage depth (meters)									
							Recovery	0-1 m and 3-4 m[1]		
	0-4 m	0-3 m	0-2 m	0-1 m	1-2 m	2-3 m	3-4 m	↑16 ↓8	↑12 ↓12	↑8 ↓16
1				6				3		
2			3	83		1		21	9	
3			17	100		1		45	20	1
4			21			1		48	22	1
5		0.5	23			1		52	23	1
6		0.5	26			1	2	57	27	3
7		0.5	28			1	2	61	27	3
8		1.0	30			1	2	64	28	3
9		3.0	35		3	1	2	68	35	5
10		4.0	42		5	1	2	68	35	7
11		4.5	43		6	1	2	68	38	7
12		4.5	45		7	1	5	68	38	7
13		5.0	48		10	1	5	68	38	7
14		5.5	51		10	1	5	68	38	7
15		6.0	52		11	1	5	68	38	7
16		7.0	55		17	1	6	68	38	7
17		7.0	57		22	1	7	68	38	7
18		7.5	57		24	1	8	68	38	7
19		7.5	60		29	1	10	68	38	7
20	0	7.5	61		30	1	13	70	39	7
Survivors with GBD lesions (%)	8	35	85	--	33	0	5	4	0	0

[1] ↑indicates hr/day at 0-1 m depth, ↓indicates hr/day at 3-4 m depth

The fish in the intermittent-exposure cages suffered much higher mortalities during the third test than during the two previous tests. Almost all of these mortalities occurred during the first half of the test. In the cage occupying the 0 to 1 m depth for 16 hr/day, the fish began to die with evidence of GBD on the 1st day (Fig. 7). This mortality reached 45% by the 3rd day and then leveled off. At 9 days the mortality in this cage was 68% and this only increased to 70% at the end of 20 days. The mortality in the 12 hr/day cage was similar to that of the 16 hr/day cage, but at a lower rate. In the 12 hr/day cage, the mortality was 20% by the 3rd day and 35% by the 9th day. The final cumulative mortality in this cage was 39%. The first death in the 8 hr/day exposure cage occurred on the 3rd day. This cage suffered a much lower mortality than the other intermittent-exposure cages at only 7% in the first 10 days. No additional mortalities occurred in the 8 hr/day exposure cage.

A number of wild fish residing in the area of the live cages were examined for signs of GBD. One or more juvenile chinook salmon about 40 to 80 mm long were trapped on top of the deeper live cages each day as the cages were raised to check for mortalities. These small chinooks were residing around the live cages from late May to the end of the 3rd test on July 18. Three-spined sticklebacks *(Gasterosteus aculeatus)* were also common around the live cages. These fish were occasionally trapped on top of the cages, but many were captured with a dip net when they were at the reservoir's surface. As many as 15 sticklebacks were captured at one time in the evening when they were normally found at the surface. None of the juvenile chinooks or the sticklebacks captured adjacent to the live cages showed any signs of GBD. It is uncertain how many fish were checked as the fish were returned to the reservoir and some were undoubtedly captured more than once.

DISCUSSION

Objective

The major objective of this study was to test the effects of naturally occurring moderate levels of dissolved gas supersaturation (120 to 130%) on juvenile salmonids. This objective was achieved in field experiments consisting of one 10-day test and

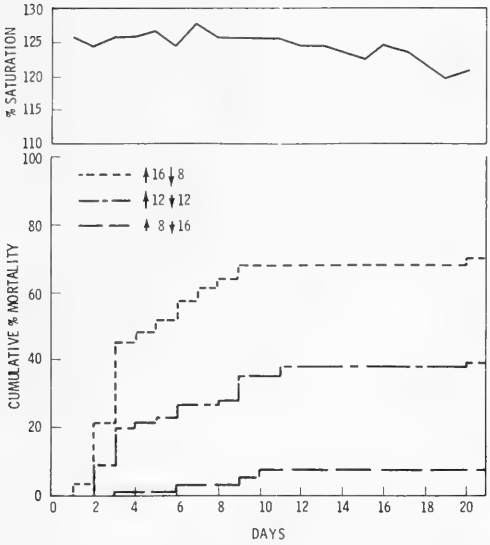

FIG. 7 Test III, total dissolved gas levels and % cumulative mortalities for the intermittent-exposure cages, June 28 to July 18, 1974.

two 20-day tests. The presence of nearly constant levels of supersaturation in the 120% to 130% range, combined with the use of healthy test fish, permitted the accurate determination of the biological effects of a narrow range of supersaturation, rather than those resulting from a wide range of supersaturation. The gas levels tested were also of particular significance as they were approximately equal to the highest levels suggested in any previous reports as permissible without producing GBD (Egusa, 1959 and Shirahata, 1966).

This study concentrated on the mitigating effect that a fish's depth in the water column has on the level of supersaturation which it can tolerate. Since most fish in a reservoir occupy depths greater than 1 m most of the time, they should theoretically be able to withstand higher levels of supersaturation than indicated for depths of less than 1 m. The increase in pressure associated with this increase in depth of 1 m reduces the in situ supersaturation value by about 10% (Leman, 1971). Thus, fish deeper than 1 m would be subjected to no more than 110% saturation when the surface saturation is 120%. This apparently explains why fish continue to survive in reservoir areas experiencing high levels of supersaturation well above 130% in surface waters.

Depth Effect

The distinct mitigating effects of small differences in depth was demonstrated by these three live cage tests. In Tests I and II, at supersaturations of 119% to 123%, only fish restricted within 1 m of the surface suffered mortalities during the first 10 days. In Test II the fish held within 2 and within 3 m of the surface experienced 17% and 3% mortalities, respectively, during the last 10 days of the 20-day test when the supersaturation varied between 123% and 126%. The number of survivors with signs of GBD was likewise directly related to the depth at which the fish were restricted, and thus their exposure to supersaturation. Those fish exposed to effectively decreased supersaturation levels, due to the greater depth of their cage, had both more survivors and a smaller percentage of survivors with signs of GBD.

It is highly significant that no fish were killed by supersaturation in the 0 to 4 m volition cage during any of the three tests at saturations of 120% to 128%. The number of fish in this cage with signs of GBD was low, ranging from 0% at the end of the first test to 33% at the end of the second test. The lack of mortalities and low incidence of GBD in this cage is of particular importance, as these are the test conditions most representative of what actually occurs in the reservoirs with unrestricted fish movement. Yet it should be noted that even in this 0 to 4 m cage, fish are confined in shallower water than they normally occupy in the reservoirs according to available depth distribution data. If juvenile chinooks are able to tolerate supersaturations between 120% and 128% in this cage for a period of 20 days, it is highly likely that they would do at least as well without the restraints of the cage during their normal migration.

Intermittent-Exposure

The occurrence of GBD was also directly related to the daily duration of exposure to supersaturation resulting from raising and lowering fish in the water column in the intermittent-exposure cages. As with the deeper volition cages, both mortalities and the percentage of survivors with signs of GBD were lower than those occurring in the 0 to 1 m cage. In both of the 20-day tests, the 16 hr/day near-surface exposure cage had cumulative mortalities and percentages of survivors with signs of GBD that were more than twice those of the fish receiving only 12 hr/day of exposure at the surface. Those fish receiving only 8 hr/day of exposure experienced mortalities and showed evidence of GBD only when the supersaturation ranged from 123% to 128%, as occurred during the last 10 days of Test I and during the first 18 days of Test II.

Effect Above and Below 125% Saturation

Although quite constant levels of supersaturation were experienced during the three tests, the slight variations in the supersaturation of the reservoir were significant, as demonstrated by the resulting mortalities. During the first test and the first half of the second test, the supersaturation remained below 123%. Almost all mortalities during this time occurred in the 0 to 1 m fixed-depth cage. For the second half of Test II and the first half of Test III, the supersaturation rose to between 124% and 128%, dropping to 123% on only one day. During this period of higher gas content, mortalities occurred in most of the cages. Again during the second half of Test III, few mortalities occurred in any cage except the 1 to 2 m fixed-depth cage. The level of supersaturation during this last period had again dropped to between 120% and 125%.

These results indicate that juvenile chinook remaining below a depth of 1 m can only be expected to suffer significant mortalities over a 20-day period when supersaturation is above 123%. Fish held within 1 m of the surface for no more than 16 hr/day also suffered significant mortalities only when the supersaturation rose to about 125% or higher. At supersaturations of about 125% and higher there was a marked increase in mortalities of fish which spent 8 hr/day or more within 1 m of the surface, and also in fish which were held continuously between 1 and 2 m. These mortalities were reduced in fish which received not more than 16 hr/day of surface exposure when the supersaturation dropped below 125%. Fish held between 1 and 2 m appear to suffer mortalities only after exposures of 9 or 10 days at supersaturations near 125%. This is indicated by comparing the mortalities in the 1 to 2 m fixed-depth cage of Tests II and III. In Test II only a single mortality occurred in the 1 to 2 m cage on the last day after 10 days of exposure to supersaturations between 124% and 126%. In Test III the mortalities in the 1 to 2 m cage began on the 9th day following exposures to supersaturations of 125% to 128%.

The percentage of survivors having signs of GBD in the intermittent-exposure cages at the end of Tests II and III is another indication of the effects of supersaturations below 125% as contrasted to those above 125%. At the end of Test II, GBD lesions were present in 15%, 24%, and 69% of the survivors from the 8 hr/day, 12 hr/day and 16 hr/day near-surface exposure cages, respectively. The supersaturation was low (120 to 123%) during the first 10 days of Test II, but higher (124 to 126%) during the last 10 days of this test. At the end of Test III, signs of GBD were absent in the survivors from the 8 hr/day and 12 hr/day exposure cages, and signs were present in only 4% of the survivors from the 16 hr/day exposure cage. The supersaturation was between 125% and 128% during the first 10 days of this test, but dropped to between 120% and 125% during the last 10 days. A comparison of these two tests indicates that fish spending any appreciable time in water less than 1 m deep are affected by supersaturation above 125%, but recover rapidly when it drops below 125%.

As indicated above, there appears to be a significant difference in the effects of supersaturations above and below about 125%. Above 125% the effects of supersaturation are much greater producing GBD faster in a greater percent of test fish and in fish held in deeper water. Supersaturations below about 125% appear to produce GBD only in fish held within 1 m of the surface at least for exposure periods of about 10 days.

Severe Mortality in 0 to 1 Meter Cage

The severe mortality that occurred in the 0 to 1 m fixed-depth cage of Test III cannot be fully explained. This 100% mortality within 3 days occurred at supersaturations (125 to 128%) that were only slightly higher than the supersaturations (123 to 126%) present during the last 10 days of Test II. The final cumulative mortality for the same cage in Test II was 88%.

There are several factors that may have influenced the sensitivity of the Test III fish. They may have been stressed more than the fish used in previous tests due to high atmospheric temperatures during their transport to the test site. There was, however, no indication that the fish were stressed when they were placed in the cages. The fish in Test III were slightly larger than those of Test II. It is doubtful that this slight size difference was sufficient to alter the sensitivity of the test fish to produce the great differences between Tests II and III mortalities.

The only real differences observed between the two tests are the supersaturations at the beginning of each test and the water temperatures during the tests. The water temperatures during the first few days of Test III were less than 1°C higher than during the last few days of Test II. It seems unlikely that this slight increase in temperature would have been responsible for the much higher mortality rate during the first few days of Test III. Test II had lower supersaturation initially (120%), rising to the higher levels only during the last half of the test; higher levels of supersaturation (125 to 126%) were present at the beginning of Test III. The fish may have been seeking a way out of the cage during the first few days, and were therefore spending more time near the top of the cage at the time of the high supersaturations in Test III, whereas by the time the highest supersaturations occurred in

Test II, the fish may have adjusted to the cage. The Test III population was also reduced by about 50% by the time of the highest supersaturation, enabling the population to occupy a smaller space, perhaps near the cage bottom.

Depth Distribution in 0 to 4 Meter Cage

It was hoped that some additional information on the depth distribution of juvenile salmonids could be gained by recording the depths occupied by the fish in the 0 to 4 m volition cage. However, the depth distribution of the fish in the 0 to 4 m cage could not be determined by either electronic means or by direct observation. The depth distribution can be estimated by comparing the cumulative mortalities and the percent of survivors with signs of GBD in this volition cage to the mortalities and survivors in the fixed-depth and intermittent-exposure cages. The comparisons of mortalities strongly suggest that the fish in the 0 to 4 m cage remained somewhat below a depth of 1 m most of the time during all three tests. In the third test, both the mortalities and the percent of survivors with GBD in the 1 to 2 m cage were greater than those in the 0 to 4 m cage. Both mortalities and the percentages of survivors with signs of GBD were greater in all of the intermittent-exposure cages than in the 0 to 4 m cage during Tests II and III. These data indicate that the fish in the 0 to 4 m volition cage spent something less than 8 continuous hr/day above a depth of 1 m.

Gas Bubble Disease Lesions

An attempt was made to correlate the obvious external GBD lesions (symptoms, signs) with the severity of the disease in each of the three tests. The appearance of bubbles along the lateral line was not used due to the difficulty of detecting this lesion in the field. In most fish the gas bubbles almost always appeared first between the rays of the caudal fin (Fig. 8). Fish with bubbles in this fin only were considered to have slight GBD lesions. Bubbles appeared next most frequently in the dorsal fin, followed by the anal (Fig. 9) and paired fins. Bubbles were also, in several instances, observed in the adipose fin. Fish with bubbles in several fins and/or a few bubbles on the head, or with a mild case of exophthalmia were considered to have moderate GBD lesions. Bubbles on the head (Fig. 10) generally occurred only after several of the fins were involved. In the area of the head, bubbles appeared on the opercles, jaws, around the eyes and inside the mouth. In very severe cases, the head appeared to be a foamy mass due to the bubbles covering all surfaces (Fig. 11).

Exophthalmia has often been considered to be a classic indication of GBD. In these live cage tests,

FIG. 8 Bubbles in the caudal fin of a juvenile chinook. This is usually the first indication of gas bubble disease.

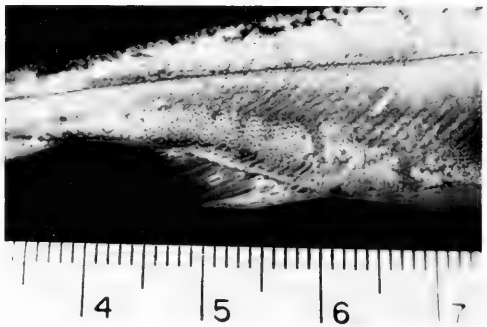

FIG. 9 Numerous bubbles in the anal fin of a juvenile chinook.

FIG. 10 The head of a juvenile chinook showing numerous bubbles around the eyes, mouth, and the opercle.

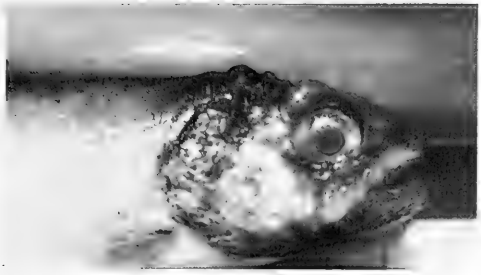

FIG. 11 Severe signs of gas bubble disease with exophthalmia of the left eye, accompanied by hemorrhaging and bubbles around the eye.

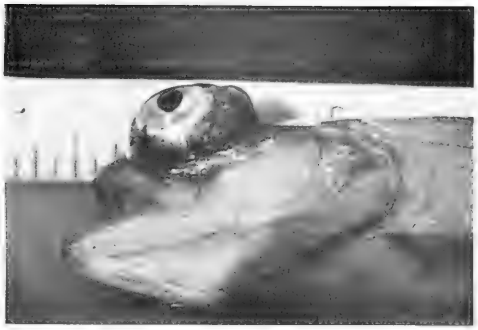

FIG. 12 Exophthalmia, gas bubbles, and hemorrhaging around the eye of a juvenile chinook salmon with severe signs of gas bubble disease.

exophthalmia was present in about 20% of the fish with GBD lesions. Meekin (1974) found exophthalmia in about 5% of the juvenile chinook succumbing to GBD in a similar study. It occurred both as the only sign of GBD, and with other signs of GBD such as hemorrhages and bubbles (Fig. 12). Hemorrhages occurred most frequently at the base of the paired fins, but also occurred around the eyes, in the jaws, gills and opercles, and occasionally at the base of the anal and dorsal fins. Hemorrhage at the base of the caudal fin was not observed. Those fish possessing hemorrhages, exophthalmia and/or extensive bubbles on the head were considered to have severe GBD lesions.

Although many of the mortalities displayed severe GBD lesions, it was not uncommon for fish with only slight or moderate indications of GBD to die. It appears that the severity of the external lesions is not a good indicator of the length of survival or of probable mortality. Some fish with extremely severe external signs of GBD survived the 20-day test and recovered.

Recovery from GBD

The apparent ability of fish exhibiting GBD lesions to recover has been reported in several studies (Gorham, 1901; Rucker and Hodgeboom, 1953; Rukavina and Varenika, 1956, and Ebel et al., 1971). Pauley and Nakatani (1967), however, questioned the apparent ability of fish to recover stating that recovery was probably related to the level of supersaturation, duration of exposure, and water temperatures, as well as the size and species. The destructive tissue changes that Pauley and Nakatani described as part of the early stages of GBD are inconsistent with recovery of the fish.

Due to the significance of recovery of fish with GBD, the 3 to 4 m fixed-depth cage was converted to a recovery cage in Tests II and III. The fish placed in this cage during each test possessed a variety of GBD lesions ranging from a few bubbles in one fin to extensive bubbles in most fins and the head, as well as hemorrhages and exophthalmia. Most of the fish placed in the recovery cage displayed at least moderate GBD lesions (bubbles present in several fins).

The recovery of fish with evidence of GBD is also indicated by the survivors of the 0 to 4 m volition cage in Tests II and III. At the end of Test II, following 10 days of supersaturation between 123% and 126%, GBD lesions were present in 33% of the survivors. During Test III saturations ranged from 125% to 128% during the first 10 days and then dropped to between 120% and 125%. Only 8% of the survivors of the 0 to 4 m cage showed evidence of GBD at the end of Test III. If saturations of 123% to 126% for 10 days produced GBD lesions in 33% of the 0 to 4 m cage fish during Test II, it is likely that a similar portion of the fish were affected during the first 10 days of Test III when saturations were even higher. If a third of the fish were affected during the first half of Test III, then most of them subsequently recovered and lost all external evidence of GBD during the last 10 days of this test when saturations did not exceed 125%.

A few mortalities did occur in the recovery cage during both Test II and III (10% and 13%, respectively). The dead fish for the most part did not appear to have died as a direct result of GBD as did the majority of mortalities in the other cages. Most of the mortalities in the recovery cage developed fungal infections of the caudal fin (Fig. 13) which were apparently secondary infections due to gas bubble damage. The remaining mortalities in the recovery cage either showed hemorrhage in the

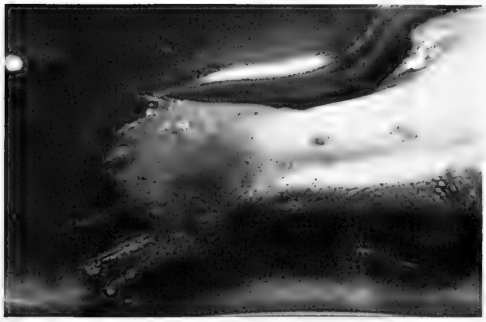

FIG. 13 Eroded caudal fin and fungal growth typical of fish that did not recover from gas bubble disease.

area of the gills and eyes or no signs of GBD. Some of the mortalities still showed bubbles in the roof of the mouth although all other external indications of GBD had disappeared.

The loss of only about 10% of the fish with GBD in the recovery cage indicates most of the fish showing GBD lesions are able to survive and recover within a 20-day period. This recovery appears to be complete in most cases. Only 9% and 5% of the fish in the recovery cage of Tests II and III, respectively, showed any indication of GBD after 20 days. This experiment indicates recovery of the majority of fish with GBD lesions when they are subjected to increased water pressure. It is not known if the same effect would be derived by placing the sick fish in saturated water at the same depth or pressure as they occupied when they acquired the disease. No attempt was made to correlate survival with the severity of the GBD lesions at the beginning of these tests. The presence of hemorrhages in some of the survivors from the recovery cage is an indication that some of the fish that began the test with severe signs of GBD had lost some of the signs of GBD and recovered and survived for 20 days.

Previous Live Cage Studies

The results of this live cage study are quite similar to the results of Meekin and Turner (1974). Meekin and Turner tested juvenile chinook in river water with nitrogen supersaturation at 124% and an oxygen supersaturation of 117% (about 120% total dissolved gas). In their study most fish held within 0.61 m (2 ft) at the surface died with GBD lesions within 7 days. Juvenile chinook held at 1.52 to 2.13 m (5 to 7 ft) for 14 days had few mortalities (4 to 16%) and the fish held at 2.44 to 3.05 m (8 to 10 ft) had no mortalities or survivors with GBD lesions.

Ebel's Priest Rapids (1967) and Ice Harbor (1971) live cage studies indicated juvenile chinook must remain below 2.5 m to be free of GBD symptoms. The difference between Ebel's results and the results of this study appear to be due to the higher levels of supersaturation experienced in Ebel's studies. In Ebel's studies nitrogen supersaturation reached 127% to 143% during the various tests. These higher nitrogen supersaturations would provide total dissolved gas saturations of about 125% or higher. The higher supersaturations in Ebel's tests would account for the greater mortalities, according to the more severe effects of supersaturations of 125% and higher indicated by our 1974 study.

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Supersaturation and Fishery Observations in Selected Alpine Oregon Streams

G. R. Bouck

ABSTRACT

Several Alpine Oregon streams were sampled and found to contain excess levels of dissolved gas tension. Effects of this naturally supersaturated water on hatchery operations and results of sampling of natural populations of aquatic organisms are presented.

Hyperbaric dissolved gas pressures may occur naturally in streams for one or more of several reasons. These reasons include geothermal heating of groundwater (Egusa, 1959), solar or seasonal heating of lakes and reservoirs (Harvey, 1967), water falls (Harvey and Cooper, 1962), photosynthesis (Renfro, 1963) and high stream velocity (Lindroth, 1957). While these sources of supersaturation have existed since time began and have been studied to some extent by researchers, there has been relatively little study of the biological impact of naturally occurring supersaturation on wild aquatic organisms. Conversely most of the supersaturation bioassay research has been done in hatchery, livebox, or laboratory circumstances. Therefore, it seemed prudent to investigate the limnology and fishery biology of naturally occurring supersaturation, as is described in this report.

Although supersaturation may be a new water quality parameter to many people, it is not a new phenomenon to fish or aquatic invertebrates in general. During eutrophic periods of our geological history, photosynthetically produced high oxygen supersaturations must have occurred countless times and impacted the evolving aquatic fauna. In this regard, it is interesting to note that Rucker's (1974) study provides strong evidence that fishes tolerate oxygen supersaturation much better than they tolerate nitrogen supersaturation (given isobaric total dissolved gas pressures in either case). Likewise, many trout and salmon streams are derived completely from springs that discharge geothermally heated, hence usually supersaturated groundwater. Such streams present a naturally existing opportunity for studying the impact of supersaturation on aquatic life, and as such, provide an opportunity for a much needed field verification of laboratory bioassay data.

Several years experience with the lethality of supersaturation has led the author to the conclusion that the typical laboratory testing program is not sufficiently robust to meet its ultimate purpose. For example, the laboratory testing circumstance typically uses the "worst possible" conditions that a fish might conceivably experience, such as crowded conditions, shallow water, continuous exposure and frequent disturbances. Moreover, the nutritional, immunological, and acclimation state of the test fish is typically unknown. All of these reasons emphasize the need for evaluating the problem in situ.

Attempts to determine the impact of supersaturation in the Columbia and Snake Rivers have been frustrated, if not stymied, by the magnitude of the required resources and efforts needed for its accomplishment. Detection limits for supersaturation mortality therein are frightful, because ocean mortality kills upwards of 98% of the salmon; hence a slight change in marine survival can have major effects on the adult salmon run. Mortality detection limits are still bad in the Columbia River where a flow of 250 x 10^3 cfs discharges about 1.35 trillion lb of water per day; this would require a daily kill of 13,500 lb of fish to achieve a 1 ppm detection limit. Adult fish passage records at dams help reduce the detection limit, but the accuracy of these records has been disputed and in any event they fail to indicate the cause of death. Thus, it would seem that the circumstances in a small supersaturated stream might present the required blend of natural circumstances and appropriate size which might overcome most of the previously listed objections.

A fortuitous fish kill and subsequent investigation in the Klamath Basin revealed that several Alpine Oregon streams were naturally supersaturated. This in turn generated further studies which are still in progress. Therefore, this is a preliminary report.

Bouck: U.S. Environmental Protection Agency, Western Fish Toxicology Station, Corvallis, Oregon.

DESCRIPTION OF STUDY AREA

The upper Klamath Basin of Oregon is the general study area (Fig. 1) with surrounding peaks ranging to about 10,000 ft above sea level. The basin floor is situated at about 4100 above sea level. This area is located between Crater Lake and Klamath Lake at about 42° 40′ north latitude and 121° 56′ west longitude. The terrain was formed mainly by volcanic activity which resulted in various depositions of ash, lava, and pumice and was modified by glaciation and errosion. Most of the watershed does not appear to have noteworthy amounts of organic material in the soil and this is covered primarily by lodgepole pine at upper elevations and ponderosa pine at lower elevations. Altitude increases rapidly from the valley (basin) floor to the top of steep-sided foothills. Human habitation is very scarce except on the valley floor where a few cattle ranches or summer homes dot the area. The nearest settlements are Chiloquin and Ft. Klamath.

Many springs can be found at the base of these steep-sided hills. Typically the springs have a basin area 10 m or more across, and collectively discharge several hundred gpm of crystal clear water. These discharges form rivers which flow swiftly across the valley floor into Agency Lake or Klamath Lake. The water is alkaline ranging from pH 7.4-8.0, but is poorly buffered with only about 30-40 ppm total alkalinity (as $CaCO_3$). Dissolved oxygen in the spring water is typically above 90% of the saturation value for that altitude. Water temperatures are rather variable between the springs and in one instance ranges from 6°C to 11°C (42-51°F) within 100 m of each other.

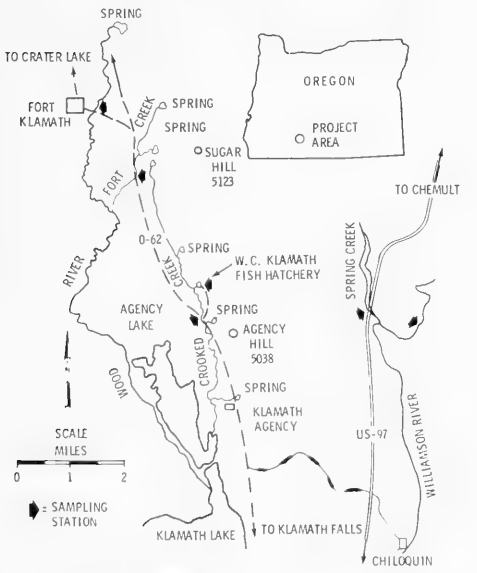

FIG. 1 Project area map.

There are many spring-fed rivers in this basin, but only five are being monitored at present. These are Spring Creek, Williamson River, Crooked Creek, Fort Creek and Wood River. Additionally, the springs supplying water to the Klamath Hatchery are being monitored. Each of these is discussed below, and the data are presented in Table 1.

Klamath Hatchery Spring and Crooked Creek

Circumstances at the Klamath Hatchery have been of concern for some time because several fish kills have occurred previously and were attributed to gas bubble disease. Another kill began in early August of 1974; Eagle Lake rainbow trout of fry size were dying and emboli were evident in the afferent arteries of the gills. Few emphysema were evident. Dying fish were cultured for common infectious disease agents but the results were all negative. The possibility of involvement with chemical toxicity was not ruled out, but its likelihood was remote. Therefore, it was concluded that the fish were dying of gas bubble disease from supersaturation.

The suspect water supply is drawn from the North settling basin which is uncovered and contains several springs. Total dissolved gas pressure was about 50 mm Hg above air pressure (ΔP) or about 1.076 atm. In the raceways where fish were dying, the ΔP was only 35 mm Hg above air pressure or about 1.053 atm. Possibly as many as 25% of these fry died at these relatively low hyperbaric levels.

Remedial action was taken to degas the water by cascading it into buckets and almost immediately thereafter the fish kill abated.

Many springs contribute to Crooked Creek in addition to the hyperbaric hatchery effluent. Dissolved gas pressures were measured about 1 mile below the hatchery and showed a ΔP of 46 mm Hg or about 1.070 atm. As noted above, a slightly lower level of supersaturation was found in the hatchery where fish were dying of gas bubble disease.

Gas pressures were measured again at about monthly intervals. The August and September data indicate that total dissolved gas pressure in the spring is dropping gradually, i.e., $\Delta P = 50 \rightarrow 48 \rightarrow 36$. Possibly this trend will prove to be a cyclic pattern because fish kills here have occurred both earlier and later than August. Whatever the case, these results indicate that the dissolved gas pressure in this spring water can vary significantly without an equally evident change in temperature.

Damage to fish or invertebrates in this stream was not readily apparent by qualitative observations. Trout were feeding in the stream at the surface and their light color did not indicate stress. Examination of the benthic fauna revealed sculpins, larval

TABLE 1 Levels of Dissolved Gases[1] in Various Alpine Rivers of the Klamath Basin, Oregon

Location	Date	Time	Water temp. C	Barometric pressure (mmHg)	ΔP (mm Hg-P_{H_2O})	Total dissolved gas pressure atm.	Dissolved oxygen ppm	Dissolved oxygen mmHg	Dissolved nitrogen %Sat	Dissolved nitrogen mmHg
Klamath Hatchery Spring and Crooked Creek										
North Spring	Aug. 31, 74	1100	10.0	658	+51	1.076	—	—	—	—
Basin outlet	Aug. 21	1100	10.0	659	+48	1.072	8.7	106	113.6	585
	Aug. 21	1630	10.0	658	+51	1.063	—	—	—	—
	Sept. 24	1520	10.0	657	+36	1.055	9.1	110	105.2	540
Raceway No. 1	Aug. 13	1130	10.0	658	+36	1.055	—	—	—	—
	Aug. 21	1045	10.0	659	+25	1.024	9.5	116	107.3	552
	Sept. 24									
Mile below	Aug. 13	1500	10.5	658	+46	1.070	—	—	—	—
Hatchery	Aug. 21	1500	9.7	659	+41	1.062	9.7	118	111.7	575
	Sept. 24	1545	11.0	657	+36	1.055	9.9	123	102.8	527
Spring Creek Area										
Basin Outlet	Aug. 20	1700	9.0	656	+69	1.107	—	—	—	—
	Sept. 24	1040	6.5	657	+38	1.058	10.8	120	103.3	530
	Sept. 24	1600	9.0	656	+62	1.094	11.4	134	100.7	516
US-97 Bridge	Aug. 20	1600	9.0	656	+66	1.101	—	—	—	—
	Aug. 21	0930	6.0	658	+15	1.012	10.5	115	105.8	544
	Sept. 24	1040	6.5	657	+25	1.038	10.8	121	103.1	529
Wood River										
Fort Klamath	Aug. 21	1215	8.0	659	+32	1.042	10.2	117	109.2	562
Bridge	Sept. 24	1315	8.5	657	+31	1.036	10.7	126	103.3	530
Fort Creek										
Hiway 62	Aug. 21	1330	10.5	658	+36	1.055	10.8	113	108.2	556
Bridge	Sept. 24	1415	11.0	657	+27	1.041	11.0	136	100.1	514
Williamson River										
Above confluence with Spring Creek	Aug. 21	0830	13.0	658	- 5	0.922	8.7	112	103.5	540

[1]Dissolved gas levels estimated by calculations on Weiss saturometer reading.

lamprey, and abundance of invertebrates including stonefly and mayfly nymphs, caddis fly larvae, midge larvae, snails, and sponges.

Fort Creek

This stream originates from springs similar to Crooked Creek. The August samplings showed dissolved gas levels (ΔP) of 36 mm Hg (1.055 atm) and this was 27 mm Hg (1.043) in September. These levels may indicate a pattern of declining gas levels.

Fort Creek has a reputation for being a good trout stream, but neither the fish nor the invertebrate fauna were sampled.

Wood River

This is a stream which is both too swift and deep to wade safely. Its total gas saturation level has been about 1.050 atm in both August and September. The fish and invertebrates in it were not sampled.

Spring Creek

This stream arises from springs in a basin about 2 km long and perhaps 100 m wide. At its outlet, the discharge is at most a few hundred cubic feet per second and like Wood River, it is generally too deep and swift to wade safely. It flows less than 1 km and joins the Williamson River.

The situation in this large but shallow spring basin is somewhat similar to that of a lake and it has a diurnal change both in temperature and in dissolved gas pressure. During August and September, early morning water temperatures were about 6°C, and the saturation level was hardly above air pressure (1.012 atm). As the sun rose, water temperatures climbed rapidly to 9°C and the dissolved gas level reached 78-80 mm Hg (1.108 atm or higher). Shortly after the sun set, the water cooled and gas levels returned to normal.

Much of the spring basin is rather shallow and trout of all sizes can be seen. Dead fish have been seen on the basin bottom, but fishermen are the suspected cause because it is a popular place to fish. Trout fry in shallow water were observed to be light colored in the morning and darkened by late afternoon, indicating stress. Some of these fry were "pinheaded" indicating possible fasting. However, human residents report that trout have been seen spawning in this basin each fall (brook trout?) and spring (rainbow trout?).

The invertebrate fauna was extremely abundant in this apparently very productive stream. Bottom samples contained mayfly nymphs, stonefly nymphs, caddis larvae, midge larvae, limpets, snails, leeches, and oligocheates. Apparently, this potentially lethal supersaturation is not having a devastating effect in its diurnal form.

Williamson River

This stream was sampled only once in August and it was slightly hyposaturated at that time. It is famous for its good trout fishing and it has a prodigious benthic invertebrate population.

DISCUSSION

Supersaturation in the Klamath Basin streams begins with cold rain and melting snow percolating into the ground thus recharging aquifers, but in so doing, the water is geothermally warmed enough to supersaturate most of its dissolved gases. Dissolved oxygen levels were near saturation values which according to Rucker (1974) may diminish the likelihood of gas bubble disease. Even so, the result of supersaturation has been significant mortality from gas bubble disease in the hatchery (at 105%). Even higher levels of supersaturation (107%) occur continuously below the hatchery in Crooked Creek and still higher levels (110%) occur diurnally in Spring Creek. The impact to the wild fish is still undetermined but wild trout are present and feed during supersaturation apparently reproducing (judging from the presence of trout fry). Also, freshwater invertebrate populations were well represented with desirable types being present in abundance.

It is possible that the low saturation levels which killed fish in the hatchery are being aggravated by solar heating of their bodies as well as by heating of the water. These waters are crystal clear and since sunshine can be very intense, sunburning of the fish has been a significant problem in previous years. Since it is theoretically possible to raise the gas pressure within the fish by solar heating, sunshine may convert an otherwise tolerable gas pressure into an internally lethal gas pressure. If so, it might account for the fish mortality in the hatchery raceway.

This leaves the unanswered question as to why the wild fish (and invertebrates) seem to be thriving at higher gas levels than killed their hatchery counterparts. One possibility is that present bioassay methods result in hypersensitivity among the test fish. For example, it seems rather unlikely that a wild fish could expose itself continuously for 10 days to a given uncompensated lethal hyperbaric dissolved gas level. One can think of other possible factors, but whatever they may be, one sees a significantly different problem when they compare hatchery and laboratory bioassays to instream conditions. After these phenomena are given further study the results may shed some much needed light both on "background" levels of supersaturation and on how much supersaturation is too much in nature.

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Some Effects of Excess Dissolved Gas on Squawfish, *Ptychocheilus oregonensis* (Richardson)

W. W. Bentley
E. M. Dawley
T. W. Newcomb

ABSTRACT

In the spring of 1974, large numbers of squawfish were encountered in the Snake River between Lower Monumental and Little Goose Dams. Squawfish exhibited gas bubble disease symptoms within 1 week after the onset of 125 to 135% saturation. A 12-day bioassay in shallow tanks to determine tolerance levels and resistance times at various gas concentrations was conducted. We found squawfish to be similar to juvenile salmon and steelhead trout in their resistance to supersaturated concentrations of dissolved gas. Feeding response changed after stress to high concentrations of dissolved gas. Average daily food consumption of test groups decreased with increased supersaturation. Squawfish captured in the field during periods of high supersaturation were less abundant and only a small portion of them had been feeding compared with survey results taken during lower supersaturation. Nitrogen supersaturation could be an important factor in assessing the effects of predation on juvenile salmonid migrants in the Columbia River system.

Squawfish have attracted the interest of many investigators in the past, primarily because they have been regarded as an efficient predator. Our attention was focused on them as being one of the possible causes of mortality of seaward migrating juvenile salmon and steelhead trout in the Snake River.

In the spring of 1974 large numbers of squawfish were encountered in the Snake River between Lower Monumental and Little Goose Dams (Fig. 1). At this time, an abnormally high runoff occurred, resulting in high nitrogen gas concentrations of prolonged duration (Table 1). The squawfish exhibited gas bubble disease symptoms within 1 week after the onset of high gas saturation. Laboratory experiments and field observation were conducted to determine the tolerance of squawfish to supersaturation of dissolved gas and how it affected their food intake or predation rate. We wish to report on these aspects and discuss problems that may require further clarification.

Predation and eating habits have been examined by several investigators. Squawfish in the Columbia River were determined to be opportunists in their eating habits and, by and large, the availability of prey influences their selectivity of daily food intake (Thompson, 1959). Thompson found that approximately 63% had empty stomachs and only 7.5% showed any evidence of eating juvenile salmon. Hamilton et al. (1970), in the Lake Merwin investigation, found that 70% of the stomachs examined were empty, but concluded at the end of his study, that predation precluded the use of the Lake Merwin Reservoir as a rearing area for coho salmon. Brett and McConnell (1950) used an estimated consumption rate of 140 salmon fingerlings per squawfish per year, a figure which was accepted as reasonable, to account for calculated losses of sockeye juveniles from Lakelse Lake, British Columbia. If we use these figures for the Snake River during a 45-day juvenile outmigration, each adult squawfish might consume

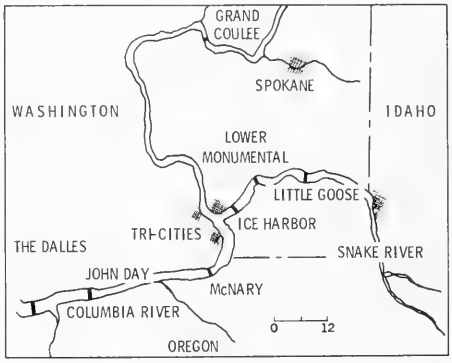

FIG. 1 Vicinity map showing location of Lower Monumental and Little Goose Dams.

Bentley, Dawley, and Newcomb: National Marine Fisheries Service, Seattle, Washington.

TABLE 1 Temperature, Dissolved Gases, Spill and Total Water Flow (in Thousands of c.f.s.) at Lower Monumental and Little Goose Dams from April to August, 1974. Data obtained by biweekly airplane flights and analyzed by staff in Seattle.

	Little Goose Dam forebay						Lower Monumental Dam forebay					
Date	Temp°C(F)	$\%O_2$	$\%N_2$	T.D.G.	Spill	Tot. flow	Temp°C(F)	$\%O_2$	$\%N_2$	T.D.G.	Spill	Tot. flow
4/19	8.3(46.9)	99.6	101.4	101.0	86.0	131.0	8.5(47.3)	117.8	122.1	120.9	83.0	150.0
4/23	9.6(49.1)	102.5	106.8	105.8	61.0	130.0	9.9(49.8)	130.8	134.6	133.3	59.0	126.0
5/7	11.6(52.9)	105.9	110.3	109.2	96.0	162.0	12.1(53.8)	130.8	135.8	134.2	95.0	164.0
5/21	11.4(52.5)	98.5	104.1	102.8	17.0	83.0	11.3(52.3)	114.2	121.5	119.7	15.0	80.0
6/4	12.2(54.0)	99.2	102.9	102.0	92.0	161.0	11.9(53.4)	127.9	141.1	137.7	90.0	159.0
6/19	12.8(55.0)	102.4	111.5	109.4	228.0	300.0	12.5(54.5)	128.7	143.8	140.0	220.0	294.0
7/2	16.9(62.4)	99.5	106.8	105.1	0.0	126.0	16.7(62.1)	124.6	131.8	129.8	66.0	133.0
7/16	17.4(63.3)	103.4	106.7	105.9	19.0	63.0	19.0(66.2)	102.4	106.4	105.5	3.0	68.0
7/30	24.5(76.1)	117.7	110.3	111.5	0.0	47.0	23.2(73.6)	110.3	106.7	107.2	0.0	44.0
8/13	21.7(71.1)	99.4	104.9	103.7	0.0	44.0	21.7(71.1)	101.8	105.8	104.9	0.0	43.0

17.4 salmonids causing a loss of 5.2 million fingerlings if a population of 300,000 squawfish exists for the stretch of the river between Ice Harbor to Little Goose Dam.

The effects of dissolved gas on squawfish had been examined by Meekin and Turner (1974) and Blahm (1974). Blahm's results indicate that squawfish were more resistant than juvenile salmonids when stressed with supersaturation. Meekin and Turner indicated slightly less tolerance than salmonids and that predation ability was substantially reduced when they were in supersaturated conditions. We attempted to place specific values on the dissolved gas tolerance of the squawfish, to substantiate and enumerate changes in the predation rate, and to discern if any correlations exist between laboratory experiments and field observations.

TECHNIQUES AND MATERIALS

Squawfish for our laboratory experiment in Seattle were captured in a Lake Merwin trap installed in the Palouse River arm at Lyons Ferry, Washington. This unit was identical to those described by Hamilton et al. (1970) in the Lake Merwin study in the Lewis River. Purse seining at Little Goose Dam was accomplished with a seine 15-ft deep and 525-ft long operated from a power-driven barge similar to that described by Durkin and Park (1969). This shallow net was employed because of the depth limitations near the navigation locks and spill gates where we concentrated much of our effort.

All squawfish were tagged with a Floy anchor tag, FD 67®, and released. Besides the tag, some were branded using the liquid nitrogen technique described by Mighell (1969); none were fin-clipped or operculum-perforated.

We purse seined at Little Goose Dam tailrace under the assumption that squawfish would be moving upstream to potential spawning areas of rip-rap dikes adjacent to the dam. In addition to learning something of their movements, we wished to gain some knowledge regarding the numbers of squawfish in that section of the river. Also, purse seining at the dam during and after spill might tell us whether there would be any change in behavior or response to high nitrogen levels. In this area, seining began on April 24 and concluded on August 8. Since we wished to tag and recover the fish in this area of the dam, we did not sacrifice the fish for stomach analysis but examined the stomachs by firmly pressing the lower ventral area and working forward to the pectoral area.

Water samples were taken biweekly by aircraft; dissolved gas values were determined in our Seattle laboratory by techniques described by Ebel (1969) and Beiningen and Ebel (1970).

Six laboratory tests were conducted where dissolved gas concentrations averaged 126.1, 120.4, 117.2 and 99.8% of saturation of total dissolved gas (T.D.G.). Average variation from the desired test concentration was ± 1.1% of T.D.G. Test duration was 12 days. Simultaneous replicates were made of tests at 117, 110 and 100% saturation.

All test tanks were 1.2 m in diameter with water depths of 25 cm (hydrostatic compensation 0.025 atm of pressure, or about 2.5% of saturation decrease). Water flow was maintained at 7.5 ℓ/min at 10° ± 1°C. Tests were conducted with about 10 fish per tank over 12 days starting April 17, 1974. Mean size of the test fish at introduction was 364 mm and 534 g. Test fish were starved for 16 days prior to testing. A sample population was fed to determine the maximum weight of food an unstressed fish might consume in a 2-week period. A mixed diet of live steelhead (average size - 21 g, 80 mm) and dead smelt (average size - 30 g, 170 mm) was used. On the basis of food intake of the sample

population we established a daily ration for test groups of four dead smelt and one live steelhead fingerling. One-half of the smelt introduced as food were cut in half to accommodate the smaller squawfish.

RESULTS AND DISCUSSION

Laboratory Bioassay

Substantial squawfish mortality occurred in tests at 126, 120 and 117% saturation. One hundred percent mortality occurred in 20 hours at the 126% level; 60% loss occurred within the 12-day test period at the 120% level; and 32% at 117% saturation. Mortality from gas bubble disease did not occur at 110, 107 or 100% T.D.G. saturation. Lethal exposure times for 10 and 50% mortality (LE_{10} and LE_{50}) for squawfish are compared in Table 2 with LE_{10} and LE_{50} values established (Dawley and Ebel, 1974 and Dawley et al., 1975) for potential salmonid prey. It is evident that squawfish are somewhat more tolerant than juvenile steelhead and spring chinook, but have much less resistance than fall chinook fry.

Gas bubble disease signs were found in all fish exposed to 126, 120 and 117% T.D.G. saturations. Eighty-nine percent of the squawfish exposed to 110% saturation also had gross signs of gas bubble disease; one of ten fish exposed to 107% T.D.G. saturation exhibited signs. No signs were noted at 100% T.D.G. saturation (Fig. 2). Gross gas bubble disease signs included hemorrhage and subcutaneous blisters present over large areas of their bodies. All fish showing signs of gas bubble disease exhibited grossly distended blisters between the fin rays. Exophthalmia ("pop-eye") occurred in only two experimental fish. Gaseous emboli were noted in the blood vessels of at least one gill arch in all gas bubble disease mortalities. Emboli were also observed in the gills of all squawfish surviving the 120% saturation test and many surviving the 117% T.D.G. tests. Gill emboli were not detected in squawfish exposed to lower saturation.

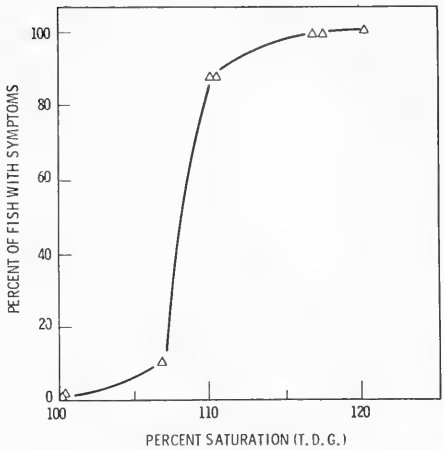

FIG. 2 Gas bubble disease symptoms in northern squawfish with increasing T.D.G. saturation.

Active feeding decreased from an average of 14.3 g food per fish per day for squawfish exposed to 100% saturation down to 2.3 g food per fish per day for squawfish exposed to 120% T.D.G. saturation (Fig. 3). These data indicate that feeding would be reduced by 50% at about 115% saturation. Below test saturations of 120%, squawfish showed a preference for live steelhead over the dead smelt (Fig. 4). The number of test days the ration of live steelhead was consumed was reduced about 40% when test fish were exposed to 117% T.D.G. saturation. Squaw-

TABLE 2 A Comparison of Lethal Exposure Times for Squawfish and Potential Salmonid Prey with 2.5% Hydrostatic Compensation

Percent saturation (T.D.G.)	Squawfish 364 mm 534 g 10°C		Juvenile steelhead 135 mm 20 g 15°C		Juvenile S. chinook 120 mm 15 g 15°C		Juvenile F. chinook 42 mm 0.4 g 10°C	
	LE_{10}	LE_{50}	LE_{10}	LE_{50}	LE_{10}	LE_{50}	LE_{10}	LE_{50}
110	>12 day	>12 day	>35 day	>35 day	>35 day	>35 day	57 day	106 day
115							20 day	47 day
117	4.8 day	>12 day	26 hr	33 hr	19 hr	27 hr		
120	41 hr	9.7 day	10 hr	13 hr	11 hr	14 hr	7 day	22 day
126	19 hr	20 hr						

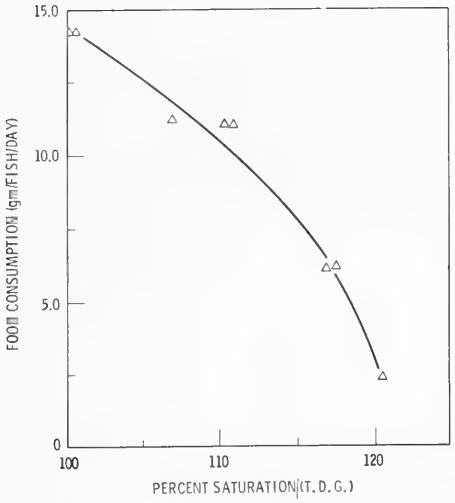

FIG. 3 Feeding inhibition of northern squawfish on a weight of food basis.

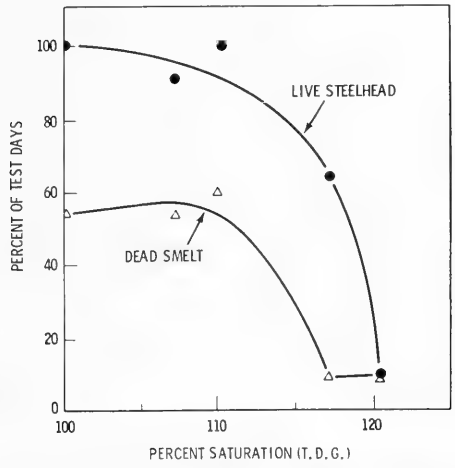

FIG. 4 Selective feeding inhibition of northern squawfish—% of test days when ration is completely consumed.

fish held at 120% saturation ate live steelhead only on the first test day.

Lethargy exhibited by the highly stressed fish may be of significance in regard to squawfish predation on juvenile salmonids migrating down the Columbia and Snake Rivers. However, the average water depth inhabited by squawfish may compensate for the effects of supersaturation. For example, in the reservoir of Lower Monumental Dam on the Snake River the dissolved gas level remained near 140% for an extended period of time this year and if the predatory squawfish had maintained an average daily depth of 2.76 m (9 ft), the effective saturation would have been 110%—low enough to allow the squawfish safe sojourn with no effective curtailing of predatory capacities. During experiments done earlier at the Prescott Field Station (Blahm, 1974), as well as those reported here, the squawfish tended to reside on the bottom of the tanks. However, this behavior may be an artifact due to the unnatural conditions of the laboratory test tanks. Nevertheless, the behavior of remaining on the bottom of the tanks changed the effective saturation value of the Prescott 1 m depth test from 119.7 to 109.1% T.G.P. and the 2.5 m test from 119.8 to 95.8% T.G.P. saturation. As a result, no mortality was reported in tests at Prescott, but gas bubble disease signs were apparent on all fish from the 1 m test after 35 days.

Seattle and Prescott data suggest that knowledge of the depth distribution is needed on the squawfish before its role as a juvenile salmonid predator can be correctly defined.

Merwin Trap - Purse Seine

Fish taken in our Merwin trap located in the Palouse River arm from January 1 to August 12, 1974, numbered 80,060 (Table 3). Squawfish totaled 16,626, with the majority taken in April, May and June, when dissolved gas saturation, temperatures and water flows were increasing (Table 1). Large numbers of squawfish may have concentrated in the Palouse River arm to escape the high dissolved gases in the Snake River. The Palouse River arm was sampled on July 2, 1974, and showed 106.8% nitrogen saturation at 21.7° C (71.1° F). Recaptures of marked fish released in the vicinity of the trap were 1,590, indicating that a high percentage remained in the area. Purse seining the navigation locks and spill area at Little Goose Dam captured 2,101 squawfish which were tagged and released. Fifty-five of those marked at the purse seine were subsequently recaptured in the seine at Little Goose Dam; and 97 that had been marked at the Merwin trap, over 11 miles downriver, were also taken in the seine at Little Goose Dam. Thirteen that were tagged at the dam appeared in the trap. One 370 mm squawfish marked at the trap made a round trip to the dam and back to the trap. Approximately 300 of the 1,590 recaptures at the trap were multiple recaptures. One 359 mm squawfish appeared in the trap 10 times.

Tagged recoveries showing movements between Lyons Ferry and Little Goose Dam indicate that high nitrogen values in surface waters appear to be

TABLE 3 Summary of Merwin Trap Catch at Lyons Ferry in the Snake River, January to August, 1974

Species	Jan. 30 to Apr. 4	Apr. 5 to May 28	May 29 to June 26	June 27 to July 24	July 30 to Aug. 12	Totals
Chinook, *Oncorhynchus tschawytscha*	1	1	4	0	0	6
Coho, *Oncorhynchus kisutch*	0	0	0	0	0	0
Sockeye, *Oncorhynchus nerka*	0	1	1	0	0	2
Steelhead, *Salmo gairdneri*	21	14	10	0	1	46
Shad, *Alosa sapidissima*	0	0	0	0	0	0
Squawfish, *Ptychocheilus oregonensis*	1475	6685	7944	240	282	16626
Whitefish, *Prosopium williamsoni*	6	16	0	0	0	22
Yellow bullhead, *Ictalurus natalis*	692	488	320	380	92	1972
Sucker, *Catostomus macrocheilus*	340	5288	14343	2617	4192	26780
Crappie, *Pomoxis annularis*	6273	11655	3764	3136	1347	26175
Yellow perch, *Perca flavescens*	125	123	21	138	20	427
Sunfish, *Lepomis* sp.	5	6	5	25	15	56
Shiner, *Notropis* sp.	51	58	73	15	6	203
Madtom, *Noturus gyrinus*	11	1	0	8	0	20
Bluegill, *Lepomis* sp.	18	6	42	17	9	92
Chiselmouth, *Acrocheilus alutaceus*	195	950	1211	64	182	2602
Carp, *Cyprinus* sp.	209	201	816	293	272	1791
Channel catfish, *Ictalurus punctatus*	59	504	884	1212	462	3121
Dolly varden, *Salvelinus malma*	4	1	1	0	0	6
Chub, *Hybopsis* sp.	20	0	1	0	0	21
Bass L.M., *Micropterus salmonides*	1	0	0	0	0	1
Bass S.M., *Micropterus dolomieui*	0	4	5	0	2	11
Lamprey, *Entosphenus tridentatus*	1	0	0	0	0	1
Sturgeon, *Acipenser* sp.	0	2	0	2	0	4
Peamouth, *Mylocheilus caurinus*	0	1	48	11	8	68
Sucker, Unident., *Catostomus* sp.	0	5	7	1	0	13
Total fish						80060

avoided during transience, but whether the squawfish were able to detect and avoid this area is unknown. The depth they normally inhabit may be sufficient to adequately compensate for the levels of supersaturation which occurred.

Length frequencies of those taken in the trap were different from those captured in the purse seine. In Fig. 5 a bimodal frequency is shown for fish sampled at Little Goose Dam in July and August with the lesser mode which is similar to the length frequency of fish sampled at the trap (Fig. 6). In the upper mode a group of squawfish, between 300 and 460 mm, had not been encountered before, suggesting that they may represent an entirely different population. One population may be reservoir spawners and the other tributary spawners. We have had tags turned in from the Palouse and Tucannon Rivers, the mouth of the Snake River and the Columbia River at Kennewick, Washington.

Purse Seine - Feeding

Purse seine catch at Little Goose Dam is shown in Table 4. When dissolved gas saturation was high, 176 squawfish were taken; five showed evidence of feeding. The numbers of squawfish taken per purse seine set were 11.7, indicating that there were few available. When dissolved gas saturation returned

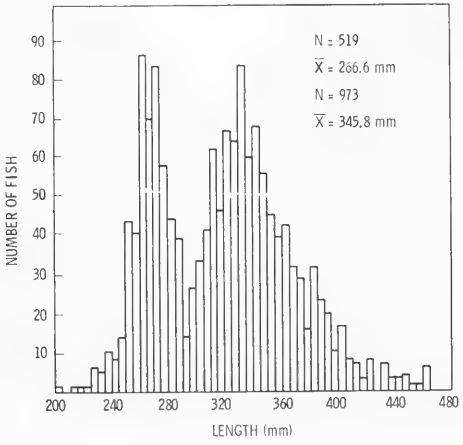

FIG. 5 Bimodal length frequency of squawfish taken by purse seine in the tailrace at Little Goose Dam from July 31st to August 8, 1974.

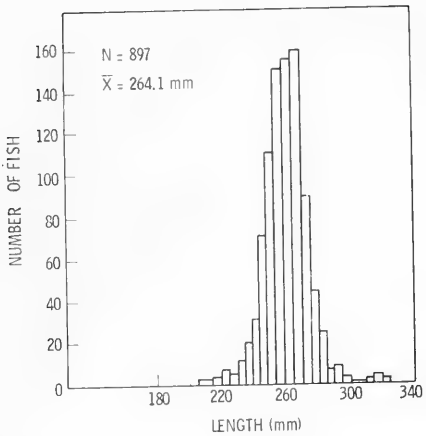

FIG. 6 Length frequency of squawfish taken in Merwin trap at Lyons Ferry in June, 1974.

TABLE 4 Purse Seine Results From Catches in the Tailrace of Little Goose Dam, 1974

Date		Sets	Sample size	Food
April	24	2	3	none
	25	4	26	1 unident.
May	3	1	22	none
	16	2	12	none
	30	3	17	2 unident.
	31	2	18	none
July	12	1	78	2 unident.
		15	176	5
		11.7 per set		
July	17	2	26	lamprey
	18	3	398	lamprey
	31	4	125	lamprey
August	2	1	542	lamprey and
	6	1	699	unident.
	8	1	145	fish
		12	1935	
		161.2 per set		

to normal, catches increased to 1,935 in 5 days of seining with an increase to 161.2 fish per set. All fish checked in the latter sample showed evidence of feeding heavily on lamprey ammocetes *Entosphenus tridentatus*, (Gairdner).

We might interpret the presence or absence of squawfish in the tailrace at Little Goose Dam as being influenced by high dissolved gases. Saturation in the spill and adjacent areas around the dam tended to keep squawfish away; and after gas saturation returned to normal they moved in to feed on whatever was available, which was mainly lamprey ammocetes. On the other hand, it is possible that large numbers of squawfish may have been present, but below the depth of our net where they would also be at sufficient depth to compensate for gas supersaturation and thus be safe from the disease. We do not know if they are at this depth. Thus, what appeared to be a response to high nitrogen levels could be the result of normal behavior patterns in the vicinity of spill gates and turbine discharge draft tubes at dams. It is evident, however, that those squawfish taken during high dissolved gas saturation were not effective predators.

CONCLUSION

Laboratory studies indicated that adult squawfish are susceptible to supersaturation of atmospheric gas at or exceeding 117% and exposure to these levels significantly reduced their food intake.

Field studies indicated that exposure of squawfish to supersaturation could be an important factor in assessing the effects of predation on juvenile salmonid migrants in the Columbia River but more information is needed on their movement, behavior and depth distribution before an accurate assessment can be made.

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Responses of Coho Salmon *(Oncorhynchus kisutch)* to Supersaturation at One Atmosphere

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B. G. D'Aoust
L. Smith

ABSTRACT

While saturation limits of 110-120% have been established as minimum lethal levels, mortalities may not be entirely related to gas saturation or desaturation rates. To compare maximum saturation times and capacities for inert gas with bubble formation, groups of small coho salmon (60-100 mm) were exposed to supersaturations that were induced either internally (by decompression) or externally (by placing fish in supersaturated water). A minimum of 1 hr at depth was required to obtain maximum lethality from decompression. Assuming maximum lethality was associated with maximum gas absorbed for any one decompression, it was concluded that for any given gas pressure, saturation was completed within 60 to 90 min. Thus, the well-documented lethal times of 24 hr or more for an over-saturation of 122% of 1 atm (Meekin and Turner, 1974) indicate a time lag in achieving maximum effect which cannot be related to gas saturation or desaturation rates. The external supersaturations showed that 250 to 500% total gas pressure would result in complete mortality in 10 to 30 min.

Since Marsh and Gorham (1905) implicated excess dissolved gas as the causative agent of gas bubble disease in fish, most studies of the problem have centered around: 1) defining the critical levels at which problems due to supersaturation arise, and 2) describing occurrences of and solutions to specific outbreaks of the disease. It seems, however, that some very interesting and important studies may have been overlooked. These other studies involve the actual dynamics of gas bubble formation within fish tissues and the relationship of bubbles to gas uptake and elimination rates. One only needs to look at the example of fish dying due to supersaturations of around 110 to 120% total gas pressure. Such saturations in human divers are readily tolerated and according to current practice (U.S. Navy, 1973), staged decompression from saturations of less than 200% of 1 atm (dives of less than 30 ft)* is not needed. Therefore, the sensitivity of fish to gas bubble disease appears to be somewhat anomalous to the response of air breathing animals (including man) to gas supersaturation imposed by decompression, primarily because the fish are affected at such low levels.

In our studies, we have been investigating the responses of salmonids to acute supersaturations (greater than 150% saturation) to determine the relationships among supersaturation, gas uptake, and death due to bubble formation.

To study these relationships, we have exposed fish to three separate types of supersaturation conditions (internal, external, and a combination of both). The direction of net gas movement (air in these tests) in and out of the fish and the initial site of supersaturation imposed on the fish were varied in each condition. Internal supersaturations were produced by decompression from saturation where a net outward movement of gas occurred as the fish was desaturated. In the external tests, fish were placed in supersaturated water, and thus the net movement of gas was inward. When the internal and external conditions were combined, the supersaturation occurred both inside and outside the fish and there was initially no significant net movement of gas except that allowed by bubble formation in the pressure chamber. Our preliminary studies have centered on the differences in the occurrence of bubble formation among these three types of supersaturations ("treatments").

MATERIALS AND METHODS

Pressure Chamber

A 4-ℓ stainless steel pressure chamber, capable of withstanding 10 atm test levels, was used for our tests (Fig. 1). The chamber has viewing endplates, sampling ports, and attached pressure

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*In these studies, the terms depth, atmosphere (atm), and pressure are interrelated as follows: 1 atm = 34 ft of fresh water = 14.7 lb/in².

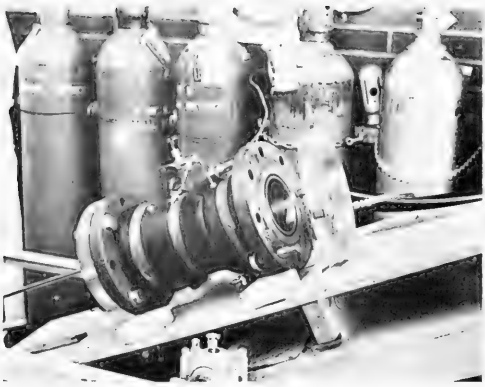

FIG. 1. Four-ℓ pressure chamber used to test the responses of salmonids to various supersaturation conditions. Chamber is mounted on tilting table which facilitates handling of fish.

gauges and flowmeters for monitoring and regulating the conditions to which the fish are exposed. The chamber is mounted on a tilting table which facilitates the transfer of fish in and out of the chamber.

Test Evaluation

The procedure to estimate time to complete saturation of critical tissues and organ systems (tissues and organs that are of vital importance to the fish, e.g. heart) has been an acute bioassay technique where coho salmon (60 to 100 mm) were exposed to a certain supersaturation condition and a dive score taken of the response. The dive score was calculated as follows: 2 points for a dead fish, 1 point for loss of equilibrium and 0 for neither of these. Signs of distress such as increased irritability, excitement, and rapid ventilation were not counted. Most of the fish responded to the given stress either within 30 min or at some time much later (hrs). Therefore, dive scores were recorded at 15 and 30 min after any particular exposure, and an average of the two scores was used for data.

A gross autopsy of mortalities was conducted to locate the bubbles within the fish tissues to determine if there were differences in the pathologies caused by the different procedures used to impose supersaturations in the fish.

Internal Supersaturation

To create internal saturation, the fish were placed in the chamber and saturated at various depths (by bubbling gases under pressure through the chamber at 2 ℓ/min).* After various lengths of time (exposure) at depth, the fish were rapidly decompressed to the surface at 100 ft/min, removed from the chamber, placed in water containing gas at 1 atm, and a dive score recorded.

External Supersaturation

In this series of tests, coho (at surface saturation) were placed directly into supersaturated water and dive scores recorded. Initial saturation levels ranged from 200 to 700% of one surface value, but due to mechanical manipulation of the chamber and oxygen consumption by the fish, these levels decreased during the test. In these preliminary tests, no additional gas was added to compensate for the decrease.

Combined External and Internal Supersaturation

During the internal supersaturation tests, the gas in the fish was allowed to diffuse outward into fresh water when the fish was removed from the chamber. If this outward diffusion was eliminated for short periods of time, more gas should remain in the fish and the severity of bubble formation and its effects should increase. To minimize this outward movement of gases, fish were saturated at depth (100 ft only in these tests) as in the internal supersaturation tests, but instead of placing the fish in water containing gases at 1 atm, the fish were held in the chamber (in the supersaturated water) for various lengths of time (up to 15 min) and a dive score recorded.

RESULTS AND DISCUSSION

Internal Supersaturation

As the pressure of saturation and resulting supersaturation after decompression were increased, the response increased as reflected by the dive scores (Fig. 2). No signs of bubble disease were noted after decompressions from 66 ft (300% saturation). At 100 ft no scores were recorded; however, these fish were on the threshold of distress because any additional stress such as an induced fright response applied to the fish after decompression resulted in signs of bubbles. At pressures greater than 100 ft, dive scores increased at each level until 100% mortality was reached at decompressions from 200 ft (700% saturation). From 60 to 90 min exposure was required to obtain maximum lethality from decompression from any one depth (e.g., no significantly greater increase in percent

*As pressure is increased, water and tissues can keep greater amounts of gas in solution (according to C (concentration) = P (pressure) x α (solubility), if gas is bubbled through fresh water at 34 ft, the water will take up approximately twice the concentration of gas found in water at the surface).

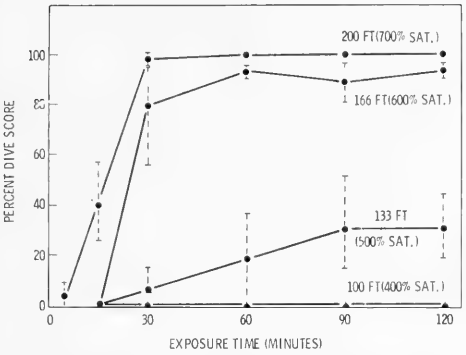

FIG. 2 Responses of coho salmon to decompressions (internal supersaturations) from various depths. Exposure time indicates the length of time the fish were maintained at any particular depth before decompression. The initial saturation is indicated in parentheses. N = 15 fish (3 tests x 5 fish per test), ranges indicated by vertical broken lines.

dive score was observed after 60 to 90 min at 133 ft; a stress producing a 35% dive score). Theoretically, it takes the same time period for a tissue to reach equilibrium at 1 atm (total gas pressure) as it does at 5 atm. This arises from the fact that although a greater amount of gas must go into solution in the fish's tissues, the rate increases proportionally to the gradient and thus the total saturation time remains the same. Therefore, assuming maximum lethality is associated with maximum gas absorbed for any given gas pressure, it was concluded that saturation of critical tissues was completed within 60 to 90 min for this size fish. Since this time of equilibration, as indicated above, should apply to any saturation level, the well-documented lethal times of 1 day or more for a saturation of 122% (Meekin and Turner, 1974) indicates a time lag in achieving maximum effect which cannot be related to gas saturation or desaturation rates per se, but also involves the amount of gas transfer. In other words, the critical tissues of fish that die from long-term chronic bioassays (greater than 10 hr) are saturated after only 60 to 90 min of exposure. Therefore, other factors related to gas bubble formation, growth, and effects within the tissues are causing the mortalities. Other potential mechanisms are unclear at this time but additional studies by our group (Casillas, Smith, and D'Aoust, 1975) are investigating possibilities such as alterations in the blood clotting mechanism and/or disseminated intravascular coagulation which has been recently implicated in the sequelae of decompression sickness in man (DCIEM Conference, 1973).

Bubbles were found in relation to the degree of perfusion of a particular tissue, indicating the importance of total gas transport. The frequency of occurrence was much higher in the blood than in the white muscle and fat. As the depth of exposure was increased, there was obvious increase in frequency of bubble formation in all tissues. Bubbles in the blood were probably the most critical to the fish because these bubbles cut off circulation to other organs and systems, occluded the gills, and frequently filled the chambers of the heart.

External Supersaturation

Fish were more susceptible to external than internal supersaturation (Table 1) with fish dying at 250% saturation (a concentration approximately equal to water saturated at 50 ft) in 30 min. Most of our results with this method were preliminary, but it appears that a slightly different mechanism related to the direction of the supersaturation gradient and the total volume of gas available accounts for this increased response. Also, in contrast to the internal supersaturations, bubbles were primarily found in the dorsal aorta, coronary artery, and in the heart. Very few bubbles were found within the organs and tissues, mainly because the bubbles in the circulatory system were effective in causing death long before any bubbles were able to form in the other tissues.

TABLE 1 Responses of Coho Salmon to Acute External Supersaturations. N = 20 fish (10 fish x 2 tests at each saturation).

	Initial level of supersaturation			
	200%	250%	400%	700%
%Dive Score	0	66%	100%	100%

External and Internal Supersaturation

When the supersaturation gradient between the fish and the water was eliminated by simultaneously internally and externally supersaturating the fish, the response was increased (Fig. 3). These results indicate that the longer the fish are held in supersaturated water after decompression, the greater the dive score. The occurrence of bubbles in this series of tests was similar to the internal supersaturation with bubbles found throughout the tisssues.

COMMENTS AND SUMMARY

From the results of these acute tests, it is clear that bubble formation can be induced by two

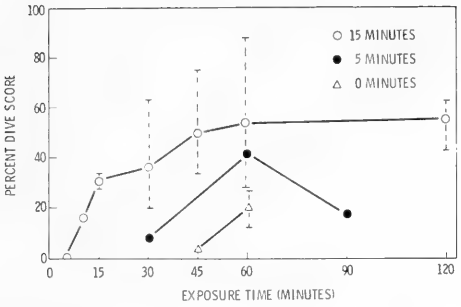

FIG. 3 Preliminary results of internally and externally combined supersaturations. Exposure time indicates the length of time the fish were maintained at depth (100 ft only in these tests) before decompression. Zero, 5, and 15 min indicate the length of time the fish were held in supersaturated water after decompression. Most data points represent 30 fish (10 fish x 3 tests) and ranges are indicated by vertical broken lines. Where no vertical lines appear, only one test was conducted and N = 10 fish.

separate procedures* and the combination of the two is somewhat more effective than either taken alone. Use of these different procedures explains the anomaly between human diving physiology and the problems fish encounter naturally. It is the internal saturation that parallels human diving decompressions. The interesting and significant difference between man and fish is that fish of this size are actually more tolerant of supersaturation (400 versus 200%) than are humans. As long as a fish is allowed the opportunity and sufficient time to eliminate the gas, it can survive high internal supersaturation.

The fish has no opportunity to eliminate gas when either exposed to external supersaturation or exposed simultaneously to internal and external supersaturation. Thus, the effects of the latter treatment are synergistic. Furthermore, the bubbles do not necessarily have to be found throughout the fish, *nor does the fish have to be anywhere near fully saturated;* bubbles in the coronary arteries are sufficient to cause death.

To summarize our studies and put the results into perspective, several statements can be made:

1. Equilibration of highly perfused tissues of coho salmon (60-100 mm) occurs in 60 to 90 min at any saturation. Therefore, below a certain saturation level (less than 150%) mortalities cannot be directly related to equilibration time only because death in these chronic tests occurs hours or even days after equilibration is reached. The total amount of gas necessary to cause mortality is a critical factor but it is unclear at this time what other factors are involved in the chronic tests.

2. Fish can withstand high supersaturations for short periods as long as they are able to eliminate the excess gas.

3. Depth is critical in keeping gas in solution. If a fish in a river sounds he can redissolve bubbles. Water often has the same gas concentration throughout the water column (due to turbulent mixing). Therefore, a sounding fish may redissolve the bubble, but will not be able to eliminate the gas and will still be supersaturated on surfacing again. If, however, the fish (at the surface) swims into an area of non-supersaturated water (e.g., another stream) he can desaturate quite easily although some sublethal effects may be incurred due to the previous supersaturation (e.g., fish that we have exposed to sublethal supersaturations are extremely susceptible to disease). If the fish stays at depth all of the time, no bubbles should appear.

FUTURE STUDIES

The main emphasis in our future studies will be to investigate further the relationship of gas saturation rates and bubble formation to different sizes, temperatures, activity rates, and other gases; resolve the differences between our acute test results and chronic tests (the time lag): and explore the additional mechanisms (blood clotting, etc.) that could be related to this time lag.

ACKNOWLEDGMENTS

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*Temperature-induced bubbles will be examined in future studies.

Effects of Gas Supersaturated Water on Freshwater Aquatic Invertebrates

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D. G. Stevens
J. R. Brett

ABSTRACT

Tests with the stoneflies *Acroneuria californica, Acroneuria pacifica,* and *Pteronarcys californica; Daphnia magna;* crayfish *(Pacifastacus leniusculus);* and young steelhead trout *(Salmo gairdneri)* were conducted to determine the sensitivity of freshwater insects, crustacea, and fish to gas supersaturated water. Stoneflies and crayfish were tolerant of supersaturation levels (125%) that killed trout; however, survival was similar in Daphnia and trout. Crayfish died at 150% and 140%; some deaths and sublethal signs occurred at 130%. Stoneflies were immobilized at 135% and exhibited buoyancy problems at 125%, but were unaffected at 115%. Daphnia were killed at 120% and exhibited partial mortality at 115%; air in the gut caused food blockage and subsequent starvation. Bubbles were observed in body fluid and tissues, and general body distention occurred before death in Daphnia, crayfish and stoneflies. The open circulatory system of invertebrates, relatively simple compared to fish, appeared to be the main reason for the greater tolerance of insects and crustacea to gas bubble disease. They do not have the complex capillary blood vessel system of fish which is rapidly blocked by bubbles, or emboli, that form in the blood.

The effects of supersaturated water on fish, the so-called gas bubble disease (GBD), have been familiar to aquarium and hatchery workers for many years (Marsh and Gorham, 1905; Embody, 1934; Rucker and Hodgeboom, 1953). Supersaturated water (up to 150% total dissolved gas) created by dams on the Columbia River, and its effect on fish, have recently been described by Westgard (1964), Pauley and Nakatani (1967), Ebel (1969), and others. Three recent literature reviews (Rucker, 1972; Weitkamp and Katz, 1973; and Bouck, 1974) adequately summarize previous work on gas bubble disease and supersaturated water problems. Laboratory studies have been conducted recently by Blahm et al. (1973) and Bouck et al. (1973) to determine comparative sensitivity of various fish species to supersaturated water, but little work has been completed with fish-food organisms, especially freshwater invetebrates.

This study was conducted to determine the effects of various levels of supersaturated water on the following freshwater aquatic insects, crustacea, and fish: *Daphnia magna;* western crayfish, *Pacifastacus leniusculus;* three stoneflies, *Acroneuria californica, Acroneuria pacifica,* and *Pteronarcys californica;* and juvenile steelhead, *Salmo gairdneri.*

MATERIALS AND METHODS

Physical Conditions

Five 6000-ℓ fiberglass tanks, 60 cm deep, were used for maintaining four different levels of supersaturated water and one saturated control (Fig. 1). A supersaturation generator system (Fig. 2) was used to control the gas level in each tank. Test water was obtained from two wells located about 30 m from the Willamette River. Water was chilled, aerated to saturation, heated to a given temperature with immersion heaters, pumped under

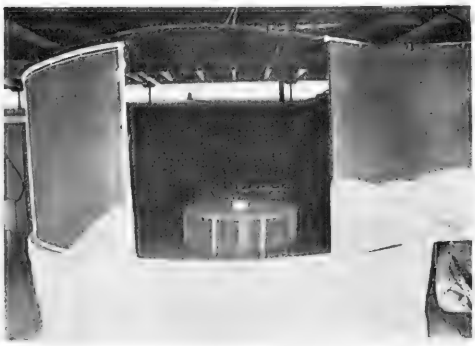

FIG. 1 View of interior of test tank showing open tank and net cages for isolating crayfish.

Nebeker, Stevens, and Brett: U.S. Environmental Protection Agency, Western Fish Toxicology Station, Corvallis, Oregon.

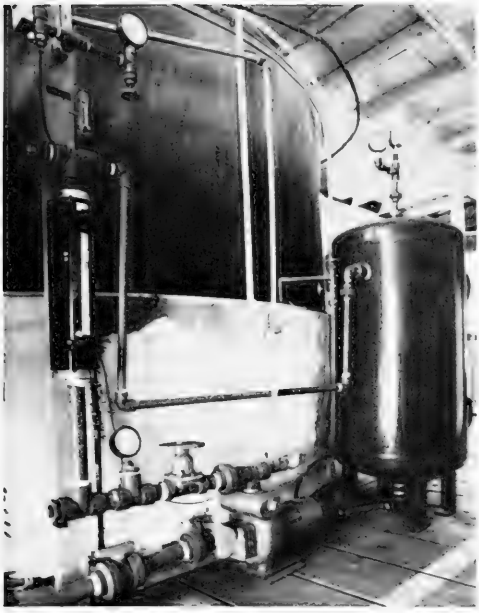

FIG. 2 Supersaturation generator system where air and water are mixed under pressure to produce required percentage of total gas supersaturation.

pressure, and mixed with compressed air (Fig. 3). Water and any remaining air not in solution flowed through the retention tank where excess air was vented off. Test water then flowed into the exposure tank, where it became supersaturated when released back to normal atmospheric pressure. The degree of supersaturation was controlled by the amount of compressed air added to the water.

Test animals were either allowed to move about freely in the open tank or were restricted to four net cages (Fig. 1) or to stainless steel wire cages (Nebeker and Lemke, 1968) suspended in the water. Two sizes of wire cages, 7.5 cm in diameter by 12.5 cm high, and 20 cm in diameter by 25 cm high, were used. They were shaped like a standard cylindrical tin can with a screen bottom on the large cage and one set of small cages and a petri dish bottom on the other set of small cages.

The water in Tank 1 was supersaturated at 125% total gas saturation and was used for testing directly and also as a water source for siphoning into other smaller tanks and aquaria holding additional test animals. Water flowing at a rate of 9 ℓ/min (Tank 1a - 500 ℓ), and 6.3 ℓ/min (Tanks 1b, 1c, 1d - 19 ℓ) through the siphon lines from Tank 1 maintained the smaller tanks at 125%.

Gas, Chemical, and Data Analyses

A Weiss saturometer (Fig. 4), modified and adapted as a routine analytical laboratory instrument, was used for measuring total dissolved gas pressures in the exposure tanks (Table 1). It was calibrated and checked periodically with the Van Slyke Gas Analyzer and the Winkler method for dissolved oxygen. Several saturometer sensors, gauges, and a mercury manometer were compared periodically to ensure accuracy. The formula: BP + ΔP - VP/BP x 100 = % saturation, where BP = Barometric Pressure, ΔP = Saturometer reading, and VP = Water Vapor Pressure, was used to calculate total percent saturation.

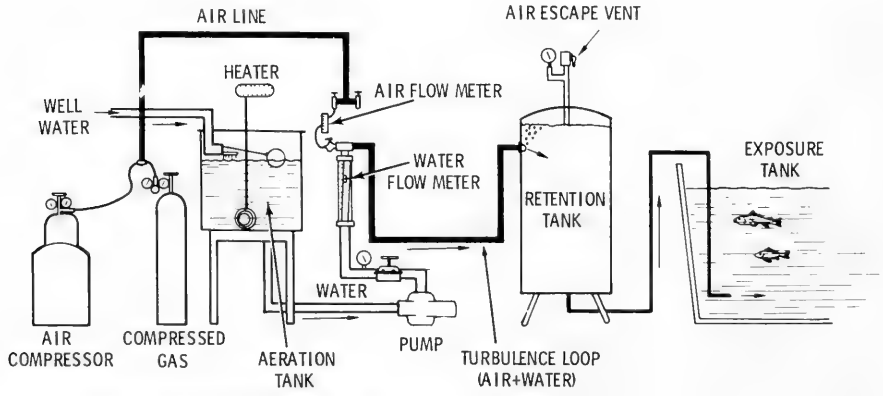

FIG. 3 Diagram of supersaturation generator system.

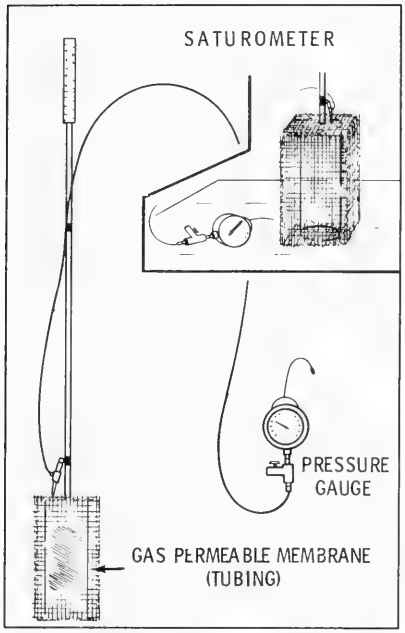

FIG. 4 Diagram of modified Weiss saturometer.

The well water was relatively soft with the following mean chemical characteristics: hardness, 34 mg/ℓ, alkalinity (as $CaCO_3$), 31 mg/ℓ; pH, 6.7. The pH increased to 7.2 to 7.4 after water aeration. Complete analyses of test water were summarized by Samuelson (1975). Flow rates into the test tanks were set at 38 ℓ/min, and water velocity varied from less than 3.0 cm/sec inside the cages to up to 21 cm/sec in the open tank.

Deaths and signs of GBD were observed and recorded daily, or more often during Daphnia exposures, and data were analyzed according to methods modified from Sprague (1969). Time to death was plotted on graph paper. For Daphnia each point represented the average of duplicate tests at the given concentration. For crayfish each point represented an individual crayfish. Time to 50% death was determined by using straight-line graphical interpolation on log-probit paper, where each death in each test replicate was plotted, a line fitted through the points, and 50% death determined where the line crossed 50% mortality. Lethal threshold mortality concentrations were determined by plotting time to 50% and 20% death at each test concentration for Daphnia and time to 20% death for crayfish.

TABLE 1 Nominal and Measured Test Levels of Total Gas Saturation

Test No.[1]	Test chamber	Nominal total gas saturation (%)	Measured total gas saturation (%)[2] N	Mean ± SD		Range
1	1	140[3]	5	140.6	1.4	139.0 to 142.0
	2	130	5	130.5	1.1	129.0 to 131.8
	3	120	5	119.4	0.5	118.6 to 120.0
2	1	140	7	139.8	1.1	138.0 to 141.3
	2	130	10	128.6	1.4	126.6 to 130.7
	3	120	10	119.1	0.8	117.6 to 120.0
	4	150	5	150.2	0.9	148.8 to 151.2
3	1	120	3	119.2	1.1	118.0 to 120.0
	2	115	3	115.4	0.6	114.8 to 115.9
	3	115	3	114.8	0.4	114.4 to 115.3
	4	110	3	108.7	0.6	108.1 to 109.3
4	1	125	45	124.7	0.7	123.2 to 126.3
5	1	150	5	148.8	1.1	147.3 to 150.4
6	1	140	21	141.4	1.5	139.0 to 143.6
	2	130	25	130.2	1.2	128.0 to 131.8
	3	120	29	119.4	0.8	117.6 to 120.9
	4	150	3	150.4	1.2	149.1 to 151.2
7	1	140[4]	10	139.9	0.9	138.0 to 141.3
	3	120	12	119.3	0.8	117.6 to 120.9
	4	150	11	150.9	1.1	148.8 to 152.6
8	1	125	45	124.7	0.7	123.2 to 126.3
9	1	135	4	135.5	0.4	135.0 to 135.9

[1]Test No. = WFTS Test No.: 1 = 31, 2 = 34, 3 = 38, 4 = 8, 5 = 29, 6 = 30, 7 = 32, 8 = 8, 9 = 39.
[2]Analysis with Weiss saturometer.
[3]Control tanks remained near 100% at all times.
[4]130% animals were accidentally killed.

Daphnia Test Methods

Known-age *Daphnia magna* from cultures maintained at the Western Fish Toxicology Station (WFTS) were used for testing. Rearing cultures were maintained at 15 ± 2°C on a 12-hr photoperiod with one white-light and one Grow-Lux fluorescent bulb. They were held in 3.8-ℓ glass jars and fed a combination of Oregon Moist Pellets®, pulverized in water, and a mixed algae culture. Water was changed biweekly. All Daphnia were transferred with a large dropper from their rearing jars into a transfer beaker and then gently poured into the test cages. They were fed twice daily during testing and had food in their guts when placed in the supersaturated water. Lack of a heart beat or movement when disturbed was used as the criterion for death.

The small screen cages with petri dish bottoms were ideal for Daphnia as they provided minimal current, and food retention was not a problem. Water flow through the cages was sufficient to circulate the test water and maintain gas levels but not disturb the Daphnia. The cages were hung on the outer lip of the tank in the open current (Test 1), sheltered from the current (Test 2), and on the net cages in the center of the tank (Test 3) where the water velocity was less than 5 cm/sec.

Crayfish Test Methods

Crayfish used in Test 4 were collected from Beaver Creek in Benton County, Oregon; those in Tests 5, 6, and 7 were collected from the Alsea River in Benton County, Oregon. Before testing, crayfish were held in aquaria with aeration and continual flow-through of water and were fed young salmon that had died in other supersaturation tests. The test gas levels were set at the desired percentage, and the crayfish were taken directly from the holding tanks (11 to 12°C) and placed in the open tanks, in the net cages, in the large screen cages, or in Tank 1a, depending on test needs.

Observations on the Branchiobdellid Annelid worms living on the crayfish exoskeleton were recorded but no detailed tests were conducted with them.

Fish Test Methods

Twenty young steelhead trout (*Salmo gairdneri*) were tested for 2 weeks with crayfish and aquatic insects at 125%. Time to death was determined, and weight, length, and sublethal signs of GBD were recorded. Five young sockeye salmon were also tested with crayfish at 140%.

Insect Test Methods

The stoneflies used were collected from the Calapooia River, 5 miles upstream from Holly, Linn County, Oregon, and were placed in holding tanks with flowing water and adequate water movement. They were acclimated to test water and temperature (12°C) for at least 24 hr before testing. Three 19-ℓ aquaria, 17.5 cm deep, were used as test chambers for Test 8, two containing water supersaturated at 125% and one with saturated (100%) water as a control. Siphon lines delivered water to each aquarium, maintaining adequate water movement to simulate stream flow required by stoneflies. Rocks and sticks were placed on the aquaria bottoms for substrate.

Test 9 was conducted in the large tanks. Two cylindrical stainless steel wire cages (20 cm in diameter by 25 cm high) were suspended near the water surface in the open current (21 cm/sec) in each tank so water movement through the cage would be adequate. The screen served as a suitable substrate so no additional material was placed in the cages.

RESULTS

Daphnia Tests

Test 1 Young non-egg-carrying adults were tested for 96 hr at 140%, 130%, 120%, and 100% total dissolved gas at 12°C (Table 1) and were held in the 20 by 25 cm wire cages. Ten Daphnia were placed in each cage, with one cage immersed in each of the four exposure tanks.

Time to 50% death (Table 2) determined at 140% total dissolved gas was 71 hr. When the test was

TABLE 2 Mean Time to 50% and 20% Death for Daphnia, Steelhead, Crayfish, and Insects

Test number (test animal)	Nominal percentage saturation	Mean time[1] to 50% death (hr)	Mean time[1] to 20% death (hr)
1 (Daphnia)	120	91	38
	130	65	45
	140	71	48
2 (Daphnia)	120	210	131
	130	130	92
	140	123	72
	150	101	82
3 (Daphnia)	110	*	*
	115	*	137
	120	93	49
4 (Steelhead)	125	50	22
4 (Crayfish)	125	*	*
5 (Crayfish)	150	---	35
6 (Crayfish)	120	*	*
	130	*	454
	140	330	130
	150	94	40
7 (Crayfish)	120[2]	*	*
	140	165	122
	150	123	66
8 (Insects)	125	*	*
9 (Insects)	115	*	*
	120	*	*
	135	*	*

*Not achieved—Insufficient number of deaths.
[1]Determined from straight-line plots on log-probit graph paper.
[2]130% animals were accidentally killed.

terminated 80% were dead after 96 hr (Table 3), and 50% had observable bubbles in the gut or brood pouch (Fig. 5). Time to 50% death at 130% was 65 hr, and 80% were dead after 96 hr. Ten percent at 140% and 130% were carrying eggs. The time at which 50% died at 120% saturation was 91 hr. Sixty percent were dead after 96 hr at 120% and all were carrying young, indicating much better reproduction at the lower gas level (Fig. 6).

Test 2 Young non-egg-carrying adults were tested at 150%, 140%, 130%, 120%, and 100% total dissolved gas at 12°C for 11 days and were held in the 7.5 by 12.5 cm screen cages sheltered from direct current by a larger screen cage (Fig. 6). Ten Daphnia were placed in each cage, and two cages were immersed in each of the five exposure tanks.

At 150% all were alive after 24 hr but many had air in the gut and brood pouch and were swimming at the surface. The first deaths occurred at 52 to 53 hr at 150% and 140%, indicating a delayed lethal effect not directly attributable to blockage of vital fluids, as in fish (Table 4). Mean time for 50% death at 150% was 101 hr. Mean time to 50% death was 123 hr at 140% saturation, and all had died at 198 hr. Fifty percent were dead after 130 hr at 130%, and many had air in the gut and were held at the water surface (Table 4). Mean time to 50% death at 120% was 210 hr (Table 3), and 70% were dead when the test was terminated after 264 hr (Fig. 7).

Test 3 Daphnia were tested at 120%, 115%, 110%, and 100% saturation at 12°C for 1 week. Two 7.5 by 12.5 cm screen cages with petri dish bottoms

TABLE 3 Daphnia Mortality After 96 hr at 140%, 130%, 120%, and 100% Total Gas Saturation at 12°C, and Observed Signs of Gas Bubble Disease[1] (Test 1).

Percentage saturation	Daphnia No.	Time to death (hr)	Signs of gas bubble disease (GBD) (B = Bubbles)
140%	1	40	One B inside carapace - body fungused
	2	40	No B - lying on cage bottom
	3	64	B in brood pouch - fungused
	4	64	B in gut and carapace
	5	64	3-4 B under carapace
	6	70	No bubbles
	7	70	One B under carapace
	8	88	Body badly fungused
	9	96*	No heart beat - no B, slight movement, half out of molt
	10	96**	Food in fore- and midgut, B filling hindgut, B in brood pouch crowding 3 young
130%	1	40	No internal B, 3-4 clinging externally
	2	40	One B inside carapace - body badly fungused
	3	40	One B inside carapace - fungused
	4	64	Fungused
	5	64	Fungused
	6	64	Fungused
	7	70	No bubbles or fungus
	8	88	Gut full of air - fungused
	9	96*	Gut full of air, no heart beat, slight movement of appendages
	10	96**	Gut full of air, heart beating well, carrying ephyppia (2 eggs)
120%	1	40	No bubbles apparent
	2	40	4 small B clinging externally
	3	64	Many B - in gut, brood pouch and under carapace
	4	88	2 B under carapace, some fungus
	5	88	No B - carrying 2 small eggs
	6	91	Gut full of air
	7	96**	No B, carrying embryos
	8	96**	Gut full of air, B in brood pouch with 2 young
	9	96**	Gut 4/5 full of air, carrying several young
	10	96**	Gut 1/2 full of air, carrying several young
100% (Control)	1-10	Controls	2 dead, but with live young, no bubbles

[1]WFTS Test No. 31.
*Barely Alive - 96 hr
**Alive and Active - 96 hr.

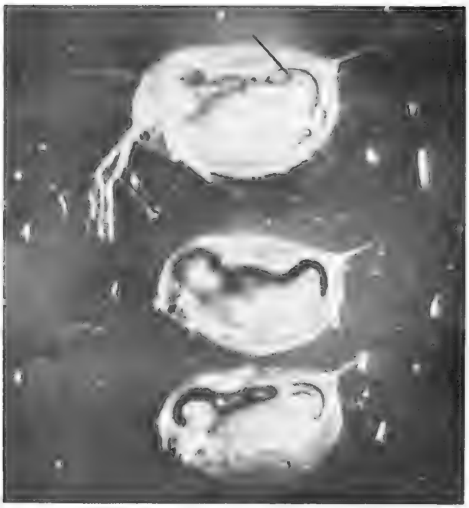

FIG. 5 Daphnia with air bubbles in the gut (upper and lower animals); center animal without air bubbles is control.

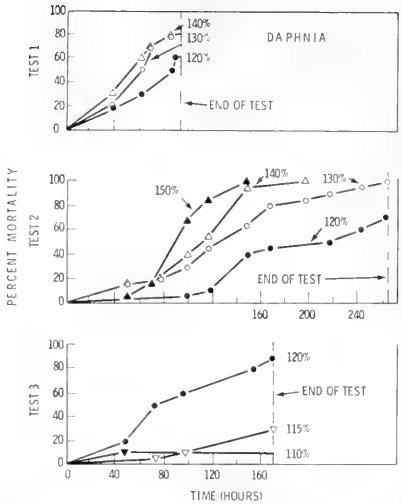

FIG. 6 Time to death for *Daphnia magna* exposed to 110%, 115%, 120%, 130%, 140%, and 150% total dissolved gas saturation (Test 1, 2 and 3).

TABLE 4. Daphnia Mortality After 11 Days at 150%, 140%, 130%, 120%, and 100% Total Gas Saturation of 12°C, and Observed Signs of Gas Bubble Disease[1] (Test 2).

Percentage saturation	Daphnia No.	Time to death (hr)	Signs of gas bubble disease (GBD) (B = Bubbles)
150%	1	72	No B, gut with food
Cage A	2	102	Gut full of air, B under carapace
Tank 4	3	102	B in brood pouch and under carapace
	4	102	B in gut and brood pouch
	5	102	3 B under carapace
	6	102	B under carapace
	7	102	B uncer carapace
	8	102	B under carapace
	9	119	Small B under carapace
	10	150	No apparent GBD
150%	1	52	No obvious B, some fungus[2]
Cage B	2	72	B in gut and brood pouch
Tank 4	3	102	B under carapace, fungus
	4	102	B in gut and brood pouch
	5	102	B in gut
	6	102	B under carapace
	7	119	Gut 3/4 full of air, B in brood pouch and under carapace
	8	119	4-5 small B under carapace
	9	150	5-6 small B under carapace
	10	150	No apparent GBD
140%	1	53	Large B filling brood pouch, some fungus
Cage A	2	53	Large B in brood pouch
Tank 1	3	102	Gut full of air, large B in brood pouch, B under carapace
	4	102	B in brood pouch and under carapace
	5	102	2 small B under carapace
	6	150	No apparent GBD
	7	150	Gut and brood pouch full of air
	8	150	1 B under carapace, some fungus
	9	150	No apparent GBD
	10	198	2 small B under carapace

TABLE 4 (continued)

Percentage saturation	Daphnia No.	Time to death (hr)	Signs of gas bubble disease (GBD) (B = Bubbles)
140%	1	53	No apparent GBD, fungus
Cage B	2	77	B in gut and brood pouch, fungus
Tank 1	3	102	B under carapace
	4	119	Brood pouch full of air
	5	119	Gut full of air
	6	119	No apparent GBD, fungus
	7	150	No apparent GBD
	8	150	No apparent GBD, tissues fragmented
	9	150	No apparent GBD, tissues fragmented
	10	150	No apparent GBD, tissues fragmented
130%	1	77	No apparent GBD, fungus
Cage A	2	77	No apparent GBD, fungus
Tank 2	3	102	Air in gut, 1 B under carapace, fungus
	4	150	No apparent GBD
	5	150	No apparent GBD, tissues fragmented
	6	169	No apparent GBD, tissues fragmented
	7	169	No apparent GBD, tissues fragmented
	8	217	No apparent GBD, tissues fragmented
	9	243	No apparent GBD, tissues fragmented
	10	lost	Apparently disintegrated[2]
130%	1	78	No apparent GBD, fungus
Cage B	2	78	No apparent GBD
Tank 2	3	102	Gut full of air
	4	119	No apparent GBD
	5	119	No apparent GBD
	6	119	No apparent GBD
	7	150	Big B in brood pouch separating carapace halves, gut 1/2 full of air
	8	150	Large B separating carapace, gut full of air
	9	169	No apparent GBD
	10	198	No apparent GBD
120%	1	150	No B, fragmented, fungus
Cage A	2	150	No B, fragmented, fungus
Tank 3	3	150	No B, fragmented, fungus
	4	150	1 small B under carapace
	5	243	B in gut and brood pouch
	6	264	No apparent GBD
	7-10	264*	No apparent GBD
120%	1	102	No B, fungus
Cage B	2	119	No B
Tank 3	3	150	No B, carapace torn
	4	150	No B, fragmented
	5	169	3 small B under carapace
	6	217	No apparent GBD
	7	243	2 small B under carapace
	8	264	No apparent GBD
	9-10	264*	No apparent GBD
100%	1	150	Carapace torn
Control	2-10	264*	No apparent GBD
Cage A			
Tank 5			
100%	1-3	150	3 lost or fragmented
Control	4-10	264*	No apparent GBD
Cage B			
Tank 8			

[1]WFTS Test No. 34
[2]Fungus begins growing on Daphnia soon after death
#Alive after 11 days.

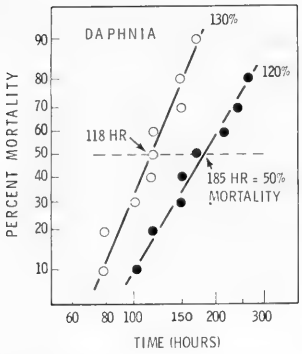

FIG. 7 Time to 50% death of *Daphnia magna* at 130% and 120% total dissolved gas saturation.

were suspended in each exposure tank, and five Daphnia, 10 days old, were placed in each cage. About half the Daphnia were beginning to develop eggs in the brood pouch.

The first death occurred after 48 hr at 120% (Table 5), and air was observed in the gut. One Daphnia, the only one observed during these tests, had a small bubble in the body fluid of the head next to the eye. Fifty percent were dead after 93 hr at 120%; bubbles were observed in the gut and on the carapace of several survivors. The food eaten by Daphnia was unable to pass through the gut when air bubbles were present, and many Daphnia apparently died of starvation. At 115% saturation 30% had died when the test was terminated after 170 hr of exposure. Eighty-five percent were carrying eggs or young after 96 hr. Mean time to 20% death was determined to be 137 hr.

At 110% the first and only death occurred after 48 hr, but there was no evidence of GBD and it had young in the brood pouch. Sixty percent were carrying young after 96 hr of exposure. Only 10% were dead when the test was terminated after 170 hr, but one was at the surface with a large bubble in the brood pouch.

Lethal threshold concentrations for Daphnia in gas supersaturated water were determined by using the time to 50% and 20% death. The saturation percentage at which 50% were no longer killed was near 128%. The level at which less than 20% of the Daphnia were killed, a possible safe level, was near 111% total dissolved gas (Fig. 8).

Crayfish Tests

Test 4 The water in Tank 1 was supersaturated at 125% total gas saturation and used both for testing and as a water source for siphoning into Tank 1a

TABLE 5 Daphnia Mortality After 1 Week at 120%, 115%, and 110% Total Gas Saturation at 12°C, and Observed Signs of Gas Bubble Disease[1] (Test 3).

Percentage saturation	Daphnia No.	Time to death (hr)	Signs of gas bubble disease (GBD)
120%	1	72	**
Cage A	2	72	**
Tank 1	3	154	Air in hindgut
	4	**	Gut full of air
	5	Lost	------
120%	1	48	Fragmented
Cage B	2	48	Air in gut - fungus
Tank 1	3	72	Fragmented
	4	96	**
	5	154	Air in gut
115%	1	72	Much fungus
Cage A	2	96	Air in gut
Tank 2	3	*	Air in gut
	4	*	**
	5	*	**
115%	1	170	Fungus
Cage B	2	*	**
Tank 2	3	*	**
	4	*	**
	5	*	**
115%	1	170	Fungus
Cage A	2	*	**
Tank 3	3	*	**
	4	*	**
	5	*	**
115%	1	170	Air in gut
Cage B	2	*	**
Tank 3	3	*	**
	4	*	**
	5	Lost	-----
110%	1	*	Large bubble in brood pouch
Cage A	2	*	**
Tank 4	3	*	**
	4	*	**
	5	Lost	-----
110%	1	48	**
Cage B	2	*	**
Tank 4	3	*	**
	4	*	**
	5	*	**

[1]WFTS Test No. 38.
*Alive after 170 hr.
**No apparent GBD.

where crayfish were tested with young steelhead. Tank 2 was used as a control and contained saturated water. All tanks were maintained at 12°C. Crayfish were placed in the net cages in Tank 1 (Fig. 1) and in Tank 1a; water flowing at a rate of 9 ℓ/min maintained Tank 1a at 125%. Four large

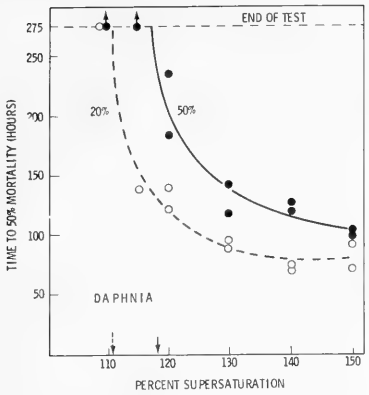

FIG. 8 Threshold concentration determined for *Daphnia magna* by using times to 50% and 20% mortality to plot the curves.

crayfish (30 to 40 g) were placed in net cage C of Tank 1 and 10 medium-sized crayfish (20-30 g) were placed in net cage D. Three medium-sized and 11 small crayfish (10 to 15 g) were placed in Tank 1a (with steelhead). They were exposed for 12 days.

Test 4 was a survey test at 125% total gas saturation to compare the susceptibility of crayfish, insects, and fish. No signs of GBD were observed in crayfish, but the fish died rapidly at 125% (Table 6) and all but one were dead after 12 days.

Test 5 Crayfish were tested at 150% total gas saturation to determine their tolerance and to expose 10 animals at a high gas level and observe signs of GBD in individual animals. The test exposure lasted 6 days, at which time all animals had died.

One male died after 23 hr and was necropsied to determine signs of GBD. Gills were slide-mounted along with those of a control animal that was sacrificed. Bubbles were readily apparent in the gills of crayfish from 150%, but no bubbles were observed in gills of the control animal. Three more male crayfish (No. 2, 3, 4) were moribund after 40 hr. They were removed from supersaturated water and placed in saturated water to observe them for possible recovery. The others remaining in the tank were sluggish and unresponsive. Bubbles were in the body fluids of the moribund crayfish and they could be seen easily through membranous joints (Fig. 9) and the ventral abdominal sternites. There were no bubbles in the control animals. The three moribund crayfish (No. 2, 3, 4) showed no recovery

TABLE 6 Time to Death of Young Steelhead Trout Tested Concurrently with Crayfish and Insects to Verify Lethality of Test Water at 125% Total Gas Saturation (Test 8)[1]

	Time to death	
Fish	Tank 1 chamber B	Tank 1a with crayfish[2]
1	10	10
2	14	14
3	18	18
4	38	18
5	48	25
6	48	27
7	50	27
8	54	30
9	54	30
10	75	32
11	75	38
12	95	38
13	118	48
14	121	54
15	125	119
16	142	120
17	142	121
18	166	142
19	190	195
20	288 (alive)	224

[1]WFTS Test No. 8.
[2]No crayfish or insect deaths occurred during the time period of this test at 125%.

in saturated water after 24 hr; one was dead, but there were still a few slight movements of abdominal appendages in the other two.

Crayfish No. 5 was moribund after 47 hr and was sacrificed for necropsy. There were bubbles under the membrane between the carapace and abdominal tergites (Fig. 9). The body cavity was opened, and many bubbles were found in the body fluids, and lodged between the internal membranes, organs, and muscles; there were also bubbles in the heart. Bubbles were very conspicuous in the gills (slide mount) and the main gill rib (Fig. 10). Bubbles were also obvious in the body fluid of the plates protecting and lying with the gills.

FIG. 9 Dorsal view of crayfish. Left: with bubbles under the distended membrane between the carapace and the abdominal segments. Right: control.

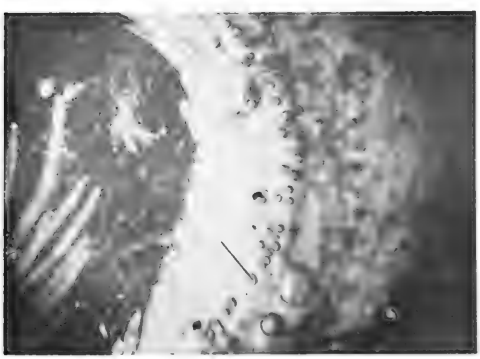

FIG. 10 Crayfish gills showing bubbles inside the gill mid-rib and filaments.

Crayfish No. 6 was alive after 47 hr, but was unable to control chelapods or to pinch. Crayfish No. 7 was alive and, with difficulty, was able to manipulate chelapods. Crayfish No. 8, 9, and 10 were alive and able to swim with good coordination. Another large male, necropsied after 64 hr, was turgid and swollen from internal pressures of gas and from water taken up because of osmotic imbalance (generalized stress reaction in invertebrates). Many bubbles were present in the body fluid, gills, and associated structures; bubbles could also be seen through the abdominal sternites. One crayfish died after 88 hr; three remained alive. Bubbles were conspicuous in the body fluid of the dead crayfish and could be seen through the body wall. They could be observed through the thin portions of the exoskeleton, legs, ventral abdominal sternites, and membranous joints. The last three crayfish were dead after 6 days.

Water supersaturated at 150% total dissolved gas, acutely lethal to trout in less than an hour (Nebeker, unpublished data), was acutely debilitating to crayfish, though not immediately lethal, as they remained in an advanced state of immobility with no coordinated movements for several days after a possible "ecological death point" was reached.

Test 6 Crayfish were tested at 150%, 140%, 130%, 120%, and 100% total dissolved gas saturation at 12°C for 30 days (Table 7). Ten crayfish were exposed to each gas level, five each in two of the four net cages in each tank. An additional 10 crayfish were placed in the open tank at 150% saturation. Two stainless steel wire cages were also suspended in the test water in each tank, and one crayfish was placed in each cage.

Thirty percent were dead after 40 hr (Fig. 11) at 150%, and many bubbles were readily apparent in the body fluid, gills and other tissues. There was no correlation between crayfish size and time to death in any of the tests. All were dead after 96 hr at 150%. Fifty percent were dead after 330 hr (Table 2) at 140%, and most exhibited some degree of bubbles in body fluids and tissues (Fig. 9 and 10). There was an obvious difference in feeding behavior between crayfish at 140% and controls (100%) after 96 hr. Crayfish at 140% moved slowly or hardly at all, whereas the controls jumped and swam when startled and moved about freely when disturbed. All crayfish tested at 140% were dead after 595 hr (24 days).

Two crayfish died at 130%, one after 215 hr and one after 453 hr (Table 7); both had bubbles in their body fluid. No crayfish died at 120% during the 30-day test, and no changes were apparent in feeding behavior when compared to control animals. Water supersaturated at 120% was apparently safe for crayfish over a 30-day period.

Bubbles were abundant in the small branchiobdellid worms living on the carapace of the test crayfish from 140%, but were not present in those from control water. The five young sockeye salmon placed in 140% were immediately affected; one had died, one had lost equilibrium, and one had hemorrhage popeye after 4 hr of exposure.

Test 7 Crayfish were tested at 150%, 140%, 130%, 120%, and 100% total dissolved gas saturation at 12°C for 2 weeks. Males and females were tested separately with five males in one net cage and five females in another, giving a total of 10 crayfish per gas level.

TABLE 7 Crayfish Mortality After 30 Days at 150%, 140%, 130%, 120%, and 100% Total Gas Saturation at 12°C, and Observed Signs of Gas Bubble Disease[1] (Test 6)

Percentage saturation	Crayfish No.	Time to death (hr)	Weight (g)	Carapace length (cm)	Symptoms of gas bubble disease
150% Tank 4	1	40	26.6	4.2	Many bubbles in body fluid and tissues
	2	40	33.3	4.9	
	3	40	15.5	3.7	
	4	94	14.8	3.6	
	5	94	23.2	4.3	
	6	94	14.1	3.7	
	7	94	10.2	3.1	
	8	94	22.7	4.4	
	9	94	10.1	3.2	
	10	94	-	-	
140% Tank 1	1	94	20.8	3.8	Many bubbles in body fluid and tissues
	2	120	36.3	4.5	
	3	185	36.3	4.6	
	4	279	37.5	4.7	
	5	303	22.4	4.1	
	6	447	44.3	4.8	
	7	447	10.2	3.1	
	8	524	11.7	3.4	
	9	572	15.9	3.5	
	10	595	36.4	4.6	
130% Tank 2	1	215	11.4	3.5	Bubbles in body fluid
	2	453	34.6	4.8	
	3	alive	17.0	3.8	
	4	alive	12.3	3.2	
	5	alive	33.5	4.6	
	6	alive	40.7	5.0	
	7	alive	29.1	4.4	
	8	alive	9.1	3.1	
	9	alive	34.3	4.6	
	10	alive	9.7	3.2	
120%	-	Size similar to others (all unaffected after 30 days)			
100%	-	Size similar to others (all unaffected after 30 days)			

[1]WFTS Test No. 30.

The first death occurred after 49 hr of exposure at 150%, and many bubbles were observed in the body fluids. Fifty percent were dead at 150% after 123 hr (Table 2), and all had bubbles in the body fluids (Table 8). There was no difference in the susceptibility of males and females and all were dead after 262 hr of exposure. Fifty percent had died at 140% after 165 hr and all had bubbles in the tissues and body fluids. All had died after 329 hr (2 weeks) when the test was terminated. Crayfish exposed to 130% were accidentally killed and no data were

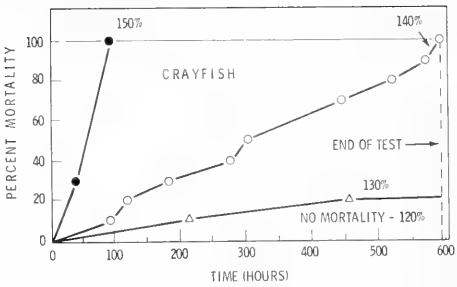

FIG. 11 Time to death for the crayfish *Pacifastacus leniusculus* **exposed to 120%, 130%, 140%, and 150% total dissolved gas (Test 6).**

obtained. No deaths occurred at 120%, and no apparent signs of GBD were observed during the 2-week exposure period.

A lethal threshold concentration for crayfish in supersaturated water was determined by using time to 20% death as the criterion (Fig. 12). The threshold concentration where crayfish were apparently safe, at least for 30 days, was near 127% total dissolved gas.

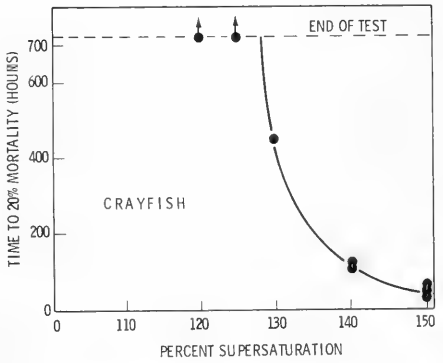

FIG. 12 Threshold concentration determined for the crayfish *Pacifastacus leniusculus.*

Insect Tests

Test 8

The stonefly species *Pteronarcys californica* and *Acroneuria pacifica* were tested at 125% total dissolved gas saturation for 12 days at 12°C to determine their comparative sensitivity with crayfish and young steelhead trout. Ten stoneflies, five

TABLE 8 Crayfish Mortality After 2 Weeks at 150%, 140%, 120%, and 100% Total Gas Saturation at 12°C, and Observed Signs of Gas Bubble Disease[1] (Test 7)

Percentage saturation	Crayfish No.	Sex	Time to death (hr)	Weight (g)	Carapace length (cm)	Symptoms of gas bubble disease (B = Bubbles)
150% Tank 4	1	male	49	12.9	3.5	Many B in body fluid
	2	female	69	6.8	2.7	Many B in body fluid
	3	female	69	14.8	3.5	Many B in body fluid
	4	male	92	7.8	2.5	Many B in body fluid
	5	female	113	16.8	4.0	Many B in body fluid body turgid from B
	6	male	165	11.6	3.6	Many B in body fluid
	7	female	192	10.1	3.1	Body turgid w/many B in body fluid
	8	female	233	4.3	2.1	—
	9	male	262	19.2	4.0	—
	10	male	lost	—	—	—
140% Tank 1	1	female	112	14.6	3.6	Many bubbles in body fluid and tissues
	2	male	112	5.0	2.5	
	3	male	114	17.4	3.7	
	4	male	166	17.7	3.7	
	5	male	168	24.5	4.1	
	6	female	168	16.4	4.0	
	7	male	192	7.7	2.9	
	8	female	192	15.8	3.8	
	9	female	262	14.0	3.6	
	10	female	329	6.0	2.8	
120% Tank 3	-	No deaths or apparent signs of gas bubble disease				
100%	-	Controls - No deaths				

[1]WFTS Test No. 32 (crayfish at 130% accidentally killed).

late instar nymphs of each species, were placed in each test chamber. All stoneflies appeared well adjusted to the test system. Bubbles were seen adhering to stoneflies during the first few hours after supersaturated water was introduced into the aquaria. Three hours after the test began two *Acroneuria* were floating and covered with bubbles; the other animals also had bubbles adhering to their bodies. They were checked every 4 hr during the next 2 days and were apparently unaffected. However, *Acroneuria* had a few bubbles clinging externally. One *Acroneuria* successfully molted after 24 hr at 125% and appeared normal. Most *Acroneuria* had some bubbles clinging to them, and they would periodically rise to the surface when they lost hold of the substrate, but would manage to get back to the bottom as many bubbles would burst when they struck the water surface. *Pteronarcys* had few bubbles on the external body wall, but did have many small bubbles adhering to the ventral tracheal gill masses (Fig. 13); however, bubbles did not appear to greatly affect behavior or movements.

FIG. 13 Ventral view of stonefly with bubbles on the gills.

All stoneflies were on the rock substrates and were alive after 96 hr with no abnormal behavior patterns, abdominal distention, or signs of stress; only one *Acroneuria* was observed with a few bubbles on it. All were apparently unaffected after 8 days, but on day 11 one *Acroneuria* was at the surface and was unable to get to the bottom because of gas bubbles. Its body was partially distended and had external bubbles among the gills. On the 12th day one *Acroneuria* in Tank 1b and one in Tank 1c were at the water surface and their bodies were partially distended. Bubbles buoyed them to the surface and they became trapped in the aquaria corners. This would not have occurred in streams where substrate would be more suitable than the slick glass surface. However, they would be more vulnerable to predators in nature as they would float to the surface more frequently because of their additional buoyancy. All stoneflies were alive and apparently unharmed after 12 days' exposure at 125% saturation.

Test 9 The stonefly species *Acroneuria californica* was tested at 135%, 120%, 115%, and 100% total dissolved gas saturation. Ten late instar nymphs were tested for 11 days at 12°C at each gas level.

No effects on stoneflies were observed after 96 hr at 120%, 115%, and 100% saturation. A few bubbles were observed on the stoneflies, but caused no difficulties. At 96 hr the gas in Tank 1, 120%, was reset to 135% and left at that level for the remaining 7 days of the test. One stonefly molted successfully at 100 hr when the gas (130%) was in transition between 120% and 135%.

The stoneflies appeared unaffected after 48 hr at 135%. However, after 120 hr of exposure, two *Acroneuria* in cage A had distended bodies but were active when disturbed. Two stoneflies in cage B also had distended bodies and one was having difficulty moving normally. Controls were normal and showed no signs of GBD. Stoneflies were held at 115% for 10 days without any observable effect. Those at 135% were more sluggish than the controls and those at 115% and had bubbles adhering to their external body wall. Two stoneflies in cage A, 135%, were full of air bubbles; the bubbles were concentrated in the body fluids of the thorax at the base of the legs and gills. Their bodies were fully distended, appearing expanded like balloons. A small midge living on one stonefly was filled with air, also looking like a tiny balloon. Two stoneflies in cage B, 135%, also had bubbles in the body fluids, and their bodies were distended to the capacity of the body wall. Bubbles were visible through the body wall at the base of the legs, the gills, and also scattered throughout the body fluids, such as in the mandibles, etc.

The stoneflies in 135% were removed from the supersaturated water after 7 days' exposure and placed in control water. After 4 hr no bubbles could be found in the body fluids and the insects were no longer distended, although they were still somewhat sluggish. They were able to recover from short periods of relatively high levels of supersaturated water. Thus stoneflies are much less susceptible to gas-supersaturated water than are fish, especially salmon and trout, but the floating and unnatural buoyancy may be important in increasing drift and predation.

DISCUSSION

Insects and crayfish were more tolerant of gas supersaturated water than any of several fish species tested (Nebeker, unpublished data). Daphnia, although able to withstand short exposures, were unable to avoid the problem of food blockage by air in the gut and died at levels similar to those that affected young salmon. Only one instance was observed where an air bubble was seen in the body fluid of Daphnia, and the heart was never observed to be impaired by emboli. The circulatory system of Daphnia is so simple that the problem of capillary blood-vessel blockage by air, crucial to fish, was nonexistent. The open circulatory systems of the insects and crayfish, though more complex than that of Daphnia, are relatively simple compared to fish and appeared to be the main reason for the greater tolerance of invertebrates to supersaturation. The external exoskeleton apparently prevents much of the surface injury and secondary infections that are common to fish exposed to supersaturated water (Nebeker and Brett, 1975).

The few reports of GBD in invertebrates that have been found are generally incidental observations of bubbles on or in the tissues, and little or no quantitative gas data are given. Gorham (1901) reported that scallops, hydroids, and squids showed signs of GBD. Evans and Walder (1969) used the shrimp *Crangon crangon* to study bubble formation under decompression because its transparent exoskeleton allowed any bubbles formed to be immediately seen. The shrimp, when subjected to 400 kg/cm^2 for 10 min and then removed, were not as active as before exposure but rapidly recovered without apparent harm. The stoneflies in the present study responded similarly, although exposure conditions and purposes were quite different. Hughes (1968) reported gas bubble disease in lobsters when exposed to water supersaturated by air leaking into the hatchery water supply, but no gas levels were given. The occurrence of GBD in three species of bivalve molluscs was described by Malouf et al. (1972), but dissolved gas levels again were not given. Massive blisters were formed on the

valves of oysters, and bubbles were observed in gill filaments. The cause, increased water temperature with subsequent supersaturation of dissolved gas, is a common occurrence and surely is responsible for much more damage to aquatic animals than is documented in the literature. Gas bubble disease in larval and juvenile brown shrimp (*Penaeus aztecus*) was recently described by Lightner et al. (1974). Stage II protozoeal, larval shrimp developed the disease after being placed in water warmed in a closed heater that did not allow excess gas to escape. Ten percent of the shrimp were affected (5% died) and had gas bubbles under the carapace and either in the gut or the hemocael surrounding the gut. No saturation levels were given. Most freshwater invertebrates are probably less sensitive to GBD than fish, although there are exceptions, like Daphnia. In general, if fish are protected by reasonable water quality standards, the invertebrates will probably be protected also. However, further work should be done with important freshwater and marine invertebrate species to determine their comparative tolerance and to define their most sensitive life stages.

CONCLUSIONS

Daphnia magna was affected by gas-saturated water ≥115%. Signs of gas bubble disease ranged from no obvious effects to massive air bubbles in the gut, brood pouch, and under the carapace. Bubbles were rarely observed in the body fluid. Death in most cases was due to physical blockage of the gut by air emboli, with subsequent starvation. The most obvious sign of gas bubble disease was the buoyancy created by bubbles, which caused the Daphnia to float at the water surface. The heart was apparently unaffected.

Crayfish were tolerant of supersaturated water and were alive with no apparent effects at 120% and 125%, levels that were lethal to the young steelhead trout tested with them. Bubbles were found in body fluids, gills and other tissues of crayfish that died at 140% and 150% total gas pressure. They were more resistant to supersaturated water than any of the 12 fish species tested at the Western Fish Toxicology Station (Nebeker, unpublished data).

Aquatic insects, represented by the three stonefly species, were also comparatively tolerant of gas-supersaturated water. No deaths occurred at 125%, but some insects were immobilized and had air bubbles in the body fluids. They recovered rapidly (4 hr) when transferred from supersaturated to saturated water. Increased buoyancy was observed and could increase mortality of insects from predation.

The open circulatory system of invertebrates, relatively simple compared to fish, appeared to be the main reason for the greater tolerance of insects and crustacea to gas bubble disease. They do not have the complex capillary-blood-vessel system of fish, which is rapidly blocked by the bubbles or emboli that form in the blood.

The tough exoskeleton of insects and crustacea prevents much of the surface injury and secondary infections that are common to fish exposed to supersaturated water.

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A Study of the Pathogenesis of Gas Bubble Disease in Steelhead Trout *(Salmo gairdneri)*

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ABSTRACT

Steelhead trout were exposed to supersaturated water and randomly removed for necropsy at predetermined intervals Lesions included emphysema of fins and opercular, gas emboli, exophthalmia, and hemorrhaging. The hypothesis that death from gas bubble disease is due to hemostasis due to blockage of blood flow by emboli was supported by the necropsy results. A "cascading bubble effect" is described to explain bubble formation.

Although gas bubble disease (GBD) in salmonid fish has been studied extensively in connection with supersaturation of the Columbia River by hydroelectric projects, few studies of the pathogenesis of GBD have been reported. Observations of lesions such as emphysema of the fins and body, exophthalmia, gas emboli within the circulatory system, gill hemorrhage, and others have been made on fish exposed to supersaturated water in hatcheries, under experimental conditions, and in natural situations (Bouck et al., 1970; Ebel, 1969; Harvey and Smith, 1961; Marsh and Gorham, 1905; Rucker and Hodgeboom, 1953; Rucker, 1972; Shirahata, 1966; Wyatt and Beiningen, 1971). Observations in these studies were primarily made on fish that had died or were near death.

This study attempts to document the events leading to the death of fish and describe the sequence of development and incidence of certain gross lesions in juvenile steelhead trout (*Salmo gairdneri*) continuously exposed to three levels of supersaturated water in shallow tanks.

MATERIALS AND METHODS

Steelhead trout obtained as eyed-eggs from the Alsea Hatchery of the Oregon Wildlife Commission were raised for experimental purposes at the Western Fish Toxicology Station, Corvallis, Oregon. These fish ranged from 17 to 24 cm fork length, with the majority of fish in the 18 to 20 cm range at the time of use.

The experimental apparatus consisted of four 12-ft (3.75 m) diameter circular tanks containing well water supersaturated to various levels by controlled air injection. Water in the tanks was maintained at a depth of 24 in. (60 cm) and 10°C. A more detailed description of the experimental tanks and method of supersaturation has been reported elsewhere (Nebeker and Stevens, 1975). Supersaturation levels were monitored daily with a Weiss saturometer. Supersaturation levels were maintained within ±1% of 120%, 115% and 110% which were the three exposure levels used in this experiment. A 100% saturated tank was used as a control.

A four-compartment submersible cage was placed in the center of each of the four tanks. Thirty-eight randomly selected fish were divided into four lots and placed in the four compartments of the cage in the 120% supersaturated water. Twenty-nine fish were similarly caged in 115% water, 29 in 110% and 12 in 100%. Although caging fish in this manner prevented access to peripheral areas of the tank where the water current was greatest, it did facilitate sampling of fish.

Random samples consisting of five fish were taken from each tank at predetermined intervals to check the development of lesions associated with GBD. The fish were immediately placed in a high concentration of MS222. Complete anesthesia was achieved in less than 2 min. The water used as a vehicle for the MS222 was dipped from the same tanks from which the fish were taken. This procedure kept the fish continually exposed to the same level of supersaturation until they were necropsied. Sampling intervals varied according to previously

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acquired LC50 data (that level at which 50% died) for the various saturation levels so that specimens could be obtained which would be in progressive stages of the syndrome.

Each fish was measured and examined for external gross lesions. A gill arch was cut to observe if gas emboli were present within the conus arteriosis and the ventral aorta. An entire gill arch was removed and examined under a dissecting microscope for evidence of gas emboli in the afferent lamellar gill vessels. The body cavity was opened and the heart, kidneys, liver, and internal vessels were examined for the presence of gas emboli and other pathology. Samples of the fins, gills, various internal organs, and the head were preserved in Bouin's solution for histological examination. These observations are the subject of another report and are not included here.

At 120% supersaturation, 19 fish died during the 54-hr experiment. Those fish which died during the day were examined in the afternoon of that day. Fish that died during the night were examined the following morning. There was no more than a 12-hr maximum time between death and necropsy. All dead fish remained in the supersaturated water until necropsied. Necropsy procedures were the same as for the sampled fish.

RESULTS

Occurrence of major gross lesions at selected time intervals is presented in Tables 1, 2, and 3. The lesions in fish still alive when sampled are compared to lesions in fish that died. Two fish sampled while still alive after 20-hr exposure were in terminal convulsions. These fish contained gas emboli within the afferent gill vessels, heart and major vessels. Only one other fish sampled while alive in 120% supersaturated water contained gas emboli. This fish did not appear to be in terminal convulsions. Gas emboli were in the gills, heart and major vessels in all fish dying of GBD, but were not observed in fish sampled alive except for the three mentioned above. Because mortality did not occur in the groups exposed to 115% and 110% supersaturation (after

TABLE 1 Occurrence of Lesions in Live Steelhead (*Salmo gairdneri*) Smolts Exposed to 120% Supersaturation Level and Sampled After 8, 20, 30, and 42 hr Compared with Fish Dead from the Effects of 120% Supersaturation at 18-, 30-, 40-, and 54-hr Exposure

	Fish alive					Fish dead				
Time sampled, hr	**8**	**20**	**30**	**42**	**Total**	**18**	**30**	**40**	**54**	**Total**
Number of fish in sample	**5**	**6**	**3**	**4**	**18**	**7**	**3**	**4**	**5**	**19**
Number with emphysema of:										
tail		1	2	3	6	2	3	4	4	13
anal fin		1	1	3	5		1	3	1	5
pelvic fin	1		3	1	5	4	2	2	1	9
pectoral fin		2	1		3	4	2	1	2	9
dorsal fin										
operculum						1		2	1	4
Number having gas emboli in:										
afferent gill vessels		2*	1		3	7	3	3	5	18
conus arteriosis		2*			2	6	3	3	4	16
atrium						3	1	1	2	7
other vessels or tissues						2	1	1		4
Number with:										
exophthalmia						1				1
hemorrhage from gill							2	1	2	5
fin hyperemia		1		1	2					
stomach filled with food	4	4	3	1	12	4	3	1	3	11
intestine filled with food			2	2	4	1	1	2	2	6
excess mucus in intestine			1		1	1	1	2	3	7
other eye pathology										

*Fish in terminal convulsions when sampled.

TABLE 2 Occurrence of Lesions in Steelhead (*Salmo gairdneri*) Smolts Exposed to 115% Supersaturated Water and Sampled After 45, 93, 143, 216, 255, and 336 hr

	Number of hr of exposure						
	45	93	143	216	255	336	Total
Number of fish in sample	**5**	**5**	**5**	**5**	**5**	**4**	**29**
Number with emphysema of:							
tail	1	3	4	2	5	1	16
anal fin			1	2	1	3	7
pelvic fin			1	1	1	1	4
pectoral fin		1	1	2	2	1	7
dorsal fin			1				1
operculum	1		1	1	1	3	6
Number having gas emboli in:							
afferent gill vessels							
conus arteriosis							
atrium							
other vessels or tissues				1*			1
Number with:							
exophthalmia		2	1	1	2		6
hemorrhage from gills				1			1
fin hyperemia	2			1		2	5
stomach filled with food	2	1					3
intestine filled with food	2	3					5
excess mucus in intestine	1		1	3			5
other eye pathology							

*Under peritoneum along kidney.

TABLE 3 Occurrence of Lesions in Steelhead (*Salmo gairdneri*) Smolts Exposed to 110% Supersaturated Water and Sampled After 69, 142, 213, 310, 360, and 408 hr

	Number of hr of exposure						
	69	142	213	310	360	408	Total
Number of fish in sample	**5**	**5**	**5**	**5**	**5**	**4**	**29**
Number with emphysema of:							
tail							
anal fin							
pelvic fin							
pectoral fin							
dorsal fin							
operculum		2	1	3			6
Number having gas emboli in:							
afferent gill vessels							
conus arteriosis							
atrium							
other vessels or tissues							
Number with:							
exophthalmia							
hemorrhage from gills		2	3	4	5	3	17
fin hyperemia							
stomach filled with food							
intestine filled with food	3						3
excess mucus in intestine		3			4	3	10
other eye pathology		1*	3**		1*	1*, 1**	7

*Corneal opacity.
**Hemorrhage in anterior chamber of at least one eye.

336 and 408 hr, respectively), direct comparisons could not be made with those that died after exposure to 120%. However, visible gas emboli were not found in the heart, gills, or major vessels of any fish exposed to 115% and 110% supersaturated water. Emboli were absent in the controls.

At 120% and 115% supersaturation, gas accumulations in the interray membranous tissue (emphysema) and within the venules adjacent to the cartilagenous rays appeared more often in the tail than in similar locations in the anal, dorsal, and paired fins. Gross emphysema did not develop in the fins of fish exposed for over 408 hr (17 days) to 110% supersaturation. Subcutaneous emphysema along the opercula was found in a significant number of fish from all three levels and apparently is associated with longer periods of exposure than that necessary to produce emphysema of the fins. Fin and tail emphysema is apparently a more acute lesion and is associated with high levels of supersaturation.

Exophthalmia, a lesion frequently associated with GBD, occurred in six fish after 93 hr exposure to 115% supersaturation. Exophthalmia associated with GBD is caused by the accumulation of gas within the fatty tissues of the periorbital space, resulting in abnormal protrusion of the eye. Other ocular lesions including blood in the anterior chamber were seen only at 110% supersaturation after long periods of exposure.

The entire group of fish were fed immediately prior to the experiment, but not during the experiment. This afforded an opportunity to observe the effects of supersaturation stress on intestinal motility. According to Klontz,* food should pass through the stomach and intestinal tract of trout in 8 to 10 hr depending on the water temperature. Food consisting of Oregon Moist Pellets® was retained in the stomach for up to 54 hr at 120% and 93 hr at 115%. Food observed in the stomach appeared to have undergone little change due to the digestive process. A similar delay in the emptying of the intestine was also observed. Controls and fish held at 110% did not have undigested food in the stomach when necropsied. The intestines of many fish also contained an excess of thick bile-stained mucus.

DISCUSSION

The cause of death of fish by acute GBD has been reported by many investigators as hemostasis. This is caused by blockage of blood flow through the heart and gills from the accumulation of gas emboli within the capillaries of the gills and results in anoxia and death. This study supports this conclusion. All fish that died at 120% supersaturation level had large accumulations of gas emboli in the heart, ventral aorta, and gills. However, with the exception of two fish in terminal convulsions and one fish that appeared normal, fish exposed to 120% and sampled prior to death did not have visible accumulations of gas within major vessels, heart or gills. Visible emboli were not seen in any fish at 115% or 110% levels. Based on these observations, the formation and/or migration of macroscopic emboli within the blood vascular system appears to be an acute terminal or near terminal phenomenon. Otherwise, gradual accumulation of gas emboli would be visible in the gill filaments of fish still alive under supersaturated conditions. No such lesions were found in this experiment.

The data indicate that initiation of bubble growth from pre-existing nuclei and/or bubble dislodgement from the periphery of the body may occur under certain physiological conditions that can trigger a "cascading bubble effect" of emboli into the gill capillaries with resultant hemostasis. The physiological conditions involved in the initiation of this apparently irreversible cascading effect are unknown for fish. Many studies have been conducted on mammalian systems in connection with decompression sickness or the "bends." Similar studies should be done to define the physiological processes occurring in fish dying of GBD and findings should be considered in the determination of acceptable levels of supersaturation.

For example, supersaturation levels as high as 120% may be tolerated by some salmonid fish under certain circumstances, whereas levels as low as 115% may be acutely lethal under a different set of conditions. Circumstances that force fish to swim rapidly while in sublethal supersaturated water such as excessive water flow, flight from predators, etc., may initiate the "cascading bubble effect" through increased muscular activity. Muscular activity is known to contribute greatly to the development and release of visible gas emboli in cats during decompression experiments (Harvey, 1944b). The effect of muscular activity was especially important at the lower levels of pressure change. The explanation given by Harvey is that muscular contraction favors bubble formation by further reducing the hydrostatic pressure causing the formation of large vapor cavities into which gas can diffuse. If the vapor cavity persists long enough, a visible gas bubble can be formed in liquids with low gas tension. The bubble may gradually dissolve, but before this happens, it might move into the general circulation. Gas accumulations are known to form in muscle even at relatively low levels of supersaturation or decompression (D'Aoust, 1974; Stroud et al., 1975).

*Personal communication, George W. Klontz, Professor of Fisheries, University of Idaho, Moscow, Idaho.

Another explanation of bubble formation related to muscular activity is that an increase in blood temperature as it passes from the gills to the systemic circulation causes dissolved gas to come out of solution (Marsh and Gorham, 1905). Increased muscular activity causes a rise in body heat even in poikilothermic animals.

This experiment and others have shown that fish in supersaturation experiments frequently die of gas embolism shortly after disturbances such as netting live fish, removing dead fish, taking supersaturation measurements with the Weiss saturometer, etc. This is probably due to the "cascading bubble effect" initiated by muscular activity or excitement as a response by the fish to the disturbance of the environment.

If muscular activity or stress precipitates the formation and/or release of gas nuclei from peripheral vessels, it can be assumed that gas emboli may be present in the blood vascular system prior to the activity. Gas-filled venules were seen in the fins of fish exposed to 120% and 115% levels of supersaturation prior to the appearance of emboli in the gills. At 110% supersaturation, similar observations have been made in previous experiments, but were not seen in this experiment. Undoubtedly, gas emboli exist in venules throughout the rest of the body, but are most easily demonstrated histologically in the fin and tail.

Supersaturation levels causing tissue emphysema and the development of gas emboli in peripheral locations such as the fins may vary from 130% to 110% or below. However, the severity and rate of development of such external lesions as bubbles in the fins and tail and subcutaneous emphysema on the body surface seem to have a direct relationship to time of exposure as well as the level of supersaturation (i.e., lesions develop more rapidly at 120% than at 115%). Fish dying of gas embolism at 120% supersaturation had a greater incidence of fully developed lesions than surviving fish even though exposure times were approximately equal. This indicates that fish which were more susceptible to external lesion development may also be more prone to the "cascading bubble effect."

The incidence of external bubbles in steelhead trout is greater in the tail than in the fins. It has been reported from physical models that bubbles tend to form along lines of material stress (Harvey et al., 1944a). Material stress may be related to swimming movement. The tail has a greater frequency of movement than the fins when fish are in moving water. Therefore, it is possible that increased evidence of gas blisters in the tail is due to increased movement. Similarly, the high incidence of emphysema on the opercula may be related to mobility.

If muscular activity is as important a factor in emboli formation and release from peripheral locations in fish as Harvey indicates it is for mammalian species, then the results of static water supersaturation bioassays should be carefully examined and compared to bioassays done in systems requiring fish to swim against a current. Direct comparison of supersaturation tolerances of salmonids in static water versus situations where fish are forced to swim have not been reported. Fig. 1 shows the results of such a study in largemouth bass (*Micropterus salmoides*) where a significant difference in tolerance to supersaturated water developed (Bouck et al., 1975). Such experiments on salmonids would provide data more representative of GBD in wild fish and would be useful to determine permissible supersaturation levels.

Another interesting aspect of the pathogenesis of GBD indicated by this data is that exposure to sublethal levels of supersaturated water may cause decreased peristaltic movement of the gastrointestinal tract. Food was held in the stomach of test fish (up to 54 hr at 120% and 93 hr at 115%) much longer than could normally be expected. In mammals, stimulation of the sympathetic nervous system by stress factors can slow passage of food in the gastrointestinal tract (Guyton, 1966). High protein food may tend to "ferment" during this period of delayed passage through the gut. This process may permit the abnormal buildup of gas, pathogenic bacteria, toxic products, heat, or other causes of irritation which could damage the intestinal lining. Excess dark bile-stained mucus with some hyperemia of the intestinal surface which was seen at necropsy in this experiment indicates intestinal irritation. Such damage may

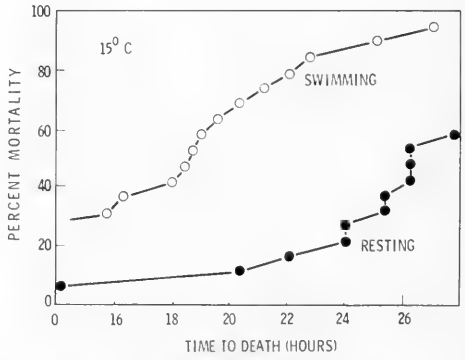

FIG. 1 Effects of swimming activity on the mortality of largemouth bass *(Micropterus salmoides)* **in water supersaturated to 140%.**

result in an increased chance of systemic bacterial infection from foci of infection in devitalized areas of the gut.

Although the sample size is small, data from this experiment indicate that several aspects of the pathogenesis of GBD need further investigation. A study of factors contributing to the acute death of fish through blockage of the circulatory system by gas emboli is needed to determine if proposed permissible supersaturation levels will assure survival of fish concurrently stressed by multiple factors. A second study designed to investigate disease susceptibility in fish surviving sublethal supersaturation levels is needed. Vibriosis, a septisemic bacterial disease of salmon, is present within the saltwater estuaries of the Oregon Coast and may cause high mortalities in salmonid fish (Cisar and Fryer, 1969). Present knowledge indicates that this disease invades the fish through the gastrointestinal tract. If the integrity of the gastrointestinal tract is significantly disturbed by sublethal supersaturation levels, vibriosis or other fish pathogens may cause increased losses.

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Effect of Temperature on Tolerance to Dissolved Gas Supersaturation of Black Bullhead, *Ictalurus melas*

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J. C. Montgomery
R. W. Hanf, Jr.

ABSTRACT

Black bullhead, *Ictalurus melas*, were acclimated to 8, 12, 16, and 20°C in Columbia River water, and were tested at each of the acclimation temperatures to determine acute tolerance to dissolved atmospheric gas tensions in excess of equilibrium saturation. The data were subjected to probit analysis and mean 96-hr TI'_{50} values were 126.7% of equilibrium saturation at 8°C, 125.1% at 12°C, 123.8% at 16°C, and 124.4% at 20°C, indicating a slightly elevated tolerance at the lowest test temperature. These values, while indicating a statistically significant difference in tolerance, do not indicate an ecologically significant effect of temperature on acute tolerance of black bullhead in the range of temperatures tested.

Preliminary bioassays of tolerances of several selected freshwater teleost species to dissolved atmospheric gas supersaturation which were conducted at 20°C (Fickeisen, Schneider, and Montgomery, 1973) demonstrated an apparently lower tolerance for the species having "cold water" preferenda than for the "warm water" species tested. In addition, for several years the literature has held that increased temperature decreases tolerance to supersaturated dissolved gases, but little experimental evidence has been available, especially for non-salmonids (Anonymous, 1971; Weitkamp and Katz, 1973). We therefore began an extensive series of acute bioassays of the effect of excess atmospheric gas tension at different temperatures on black bullhead (*Ictalurus melas*), pumpkinseed sunfish (*Lepomis gibbosus*), and rainbow trout (*Salmo gairdneri*). Tests were to be conducted under non-shock conditions, that is, the fish were to be acclimated to the test temperature prior to exposure to supersaturation. This paper is a progress report of studies that are continuing, and describes the effects of temperature on tolerance of black bullhead.

MATERIALS AND METHODS

Adult black bullhead were collected by beach seine and by hook and line from a backwater pond of the Columbia River in the McNary Wildlife Refuge at Burbank, Washington. The common stock was held in circular tanks receiving flowing Columbia River water and were fed trout pellets. As required for prophylaxis, the stocks were treated with malachite and with Diquot as well as fed pellets containing antibiotics. Prior to being tested, the required number of fish were brought to the test temperature at a rate of 1°C per day. They were held at the test temperature for a period of at least 10 days. Temperature fluctuation during this acclimation period was less than 1°C. Throughout the holding period all of the stock tanks were heavily aerated and experience has shown that under these conditions gas tensions range generally between 95 and 105% of equilibrium value.

Water supersaturated with atmospheric gases was generated in a pressure vessel which received an air-water mixture at about 40 psig. Control of the dissolved gas tension was provided by an adjustable standpipe with a float valve to release excess gas, thereby permitting an adjustable head space of air over the water by adjusting the backpressure and turnover time, and by adjusting the rate of air inflow.

The testing facility consisted of four rectangular tanks, each 1.17 by 0.46 m with the water level maintained at a depth of 35 cm to minimize hydrostatic pressure compensation for excess gas tension. One of the tanks was supplied with water from the supersaturation system. Two of them received supersaturated water mixed with normally saturated water to control gas tension, and the fourth tank was a control and received normally saturated water. Turnover time in each tank was calculated to be less than 20 min based on the flow rate. Temperature in all tanks was maintained within 0.5°C

Fickeisen, Montgomery, and Hanf: Battelle-Northwest, Ecosystems Department, Richland, Washington.

of the desired test temperature and was continuously recorded.

During a test 10 fish were placed in each of the four troughs receiving artificially supersaturated water generated by injecting compressed air into water under pressure in a receiver. The tanks were covered with black plastic to minimize disturbing effects of wet lab activity. Troughs were checked at least daily for mortalities which were removed. About one-third of the mortalities were randomly selected for gross necropsy to determine cause of death. At least three times during each 4 day test dissolved gas tension was measured using the Weiss saturometer with a mechanical shaker. Water temperature was also recorded as was barometric pressure using a mercurial barometer. From these data the percentage of equilibrium saturation was computed for total dissolved gases. In addition, dissolved oxygen was measured by Winkler titration, and pH was measured once during each test. At each temperature it was necessary to run replicate tests varying the gas tensions somewhat in order to obtain a sufficient data base for probit analysis to determine TL_{50} values. In most cases, three tests were required at each temperature.

Mean gas tensions and mortalities at the end of 96-hr were analyzed by computer probit analysis to determine fiducial limits about the fitted curve of proportional mortality versus total gas saturation.

RESULTS AND DISCUSSION

Gross necropsies demonstrated that in nearly all cases examined the cause of death was clearly gas bubble disease, manifest by massive cardiac blockage by emboli. Emphysema and petechial hemorrhaging were commonly observed as was the presence of emboli in blood vessels, gills, and organs. Cases of not clearly gas bubble disease mortalities were due to indeterminant causes, but were likely due to unobserved gas embolism. In no case were there any mortalities in the control tank and at the termination of the test control fish showed no external signs of gas bubble disease while they were common among survivors which were exposed to supersaturated water.

Plots of proportional mortality against total gas tension for each of the test temperatures are shown in Fig. 1 through 4. It is important to note that the curves are quite steep, indicating a narrow range between non-lethal and lethal levels of gas tension. This factor could be significant in planning mitigation activities and in defining water quality standards as even a relatively small decrease in dissolved gas content might have a large effect on resulting mortality, provided that the new, lower gas tension was below the lethal threshold. Species differences in tolerance and depth distribution as it relates to hydrostatic pressure compensation for excess gas tension may, however, dictate that each potentially affected area be independently assessed prior to instigating mitigation plans.

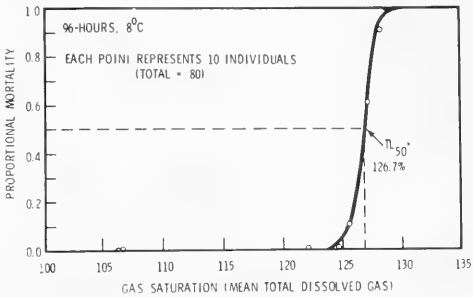

FIG. 1 Acute bioassays of dissolved gas tolerances of *Ictalurus melas* **(Black Bullhead) at 8°C.**

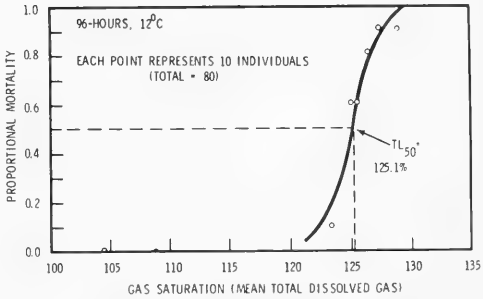

FIG. 2 Acute bioassays of dissolved gas tolerances of *Ictalurus melas* **(Black Bullhead) at 12°C.**

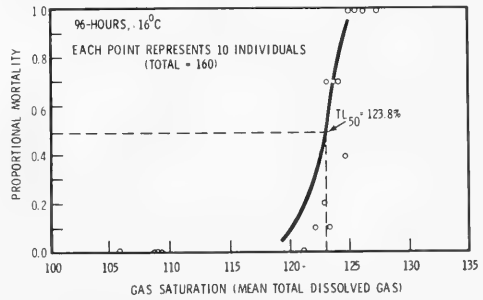

FIG. 3 Acute bioassays of dissolved gas tolerances of *Ictalurus melas* **(Black Bullhead) at 16°C.**

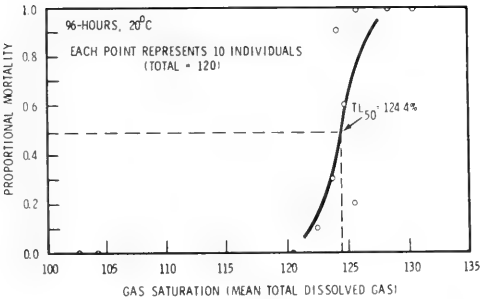

FIG. 4 Acute bioassays of dissolved gas tolerances of *Ictalurus melas* (Black Bullhead) at 20°C.

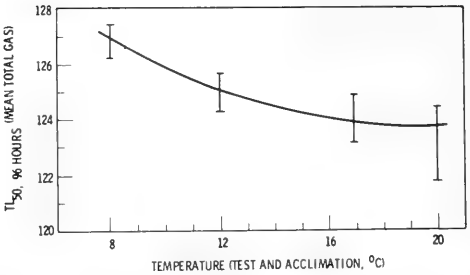

FIG. 5 Effect of temperature on tolerance of *Ictalurus melas* (Black Bullhead) to excess dissolved gas tension. Bars represent fiducial limits about the TL_{50} value.

Fig. 5 is a plot of the 96-hr TL_{50} values with their fiducial limits at each of the test temperatures. The tolerance at the lowest test temperature is statistically significantly greater than that at the higher temperatures. It is important to note that the differences are small and not of ecological significance as greater perturbations in gas tension take place over short periods of time in nature. An active fish moving vertically in the water column will also experience greater changes in the compensatory effect of hydrostatic pressure.

Tests with rainbow trout are continuing, but we are able to report that at 20°C the TL_{50} is about 118% total gas supersaturation, a value significantly lower than that for black bullhead, both from a statistical and an ecological basis.

To summarize our findings, the effect of temperature within the range tested has little effect on tolerance of black bullhead acclimated to the test temperature. Future tests are planned at temperatures ranging up to 30°C. However, if exposed to the combined effects of a thermal shock (acclimation temperature not equal to test temperature), and supersaturation, it is probable that a different effect would be observed.

ACKNOWLEDGMENTS

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Gas Bubble Disease Mortality of Atlantic Menhaden, *Brevoortia Tyrannus*, at a Coastal Nuclear Power Plant

R. A. Marcello, Jr.
R. B. Fairbanks

ABSTRACT

A substantial mortality of Atlantic menhaden, *Brevoortia tyrannus*, occurred in the discharge channel and discharge plume area of the Boston Edison Company's Pilgrim Nuclear Power Station Unit 1 during the period April 8 through April 24, 1973. Gas bubble disease was implicated as the cause of their death. Measurements of dissolved gas concentration of the station's intake and discharge water during this fish mortality are presented. Observations on the behavior and results of the pathological examination of menhaden afflicted with gas embolism are discussed.

Water supersaturated with dissolved gases can have detrimental effects on fish and other aquatic organisms. The manifestation of these effects in fish is generally referred to as gas bubble disease. While fish appear to tolerate mild cases of gas bubble disease, extreme cases have resulted in mortalities (Marsh and Gorham, 1905; Woodbury, 1942; Renfro, 1963; Ebel, 1969; Beiningen and Ebel, 1970; DeMont and Miller, 1971; and others).

Gaseous supersaturation of natural waters has been attributed to increased photosynthetic activity (Woodbury, 1942; Renfro, 1963), the falling of water into plunge basins below dam spillways (Harvey and Cooper, 1962; Ebel, 1969; Beiningen and Ebel, 1970), and the drawing in of air at water pump intakes, leaky pipelines, and similar situations (Marsh and Gorham, 1905; Harvey and Smith, 1961). More recently the passage of cooling water through the circulating water systems of electric power generating stations has also been identified as having the potential for creating gas-supersaturated conditions in the cooling water discharge (DeMont and Miller, 1971).

Dissolved gas supersaturation in the thermal effluent of a power plant can occur due to the inverse relationship between temperature and gas solubility in water. When natural waters are at or near saturation levels with dissolved gases and the water is subjected to a substantial temperature increase upon passage through a power plant condenser system without allowing equilibration with the atmosphere, the water will become gas supersaturated. The return to normal saturation levels under such conditions by gaseous diffusion across the air-water interface appears to be a slow process and usually does not take place before the discharge water is again cooled to ambient by entrainment mixing and heat loss to the atmosphere. Fishes attracted to heated effluents supersaturated with dissolved gases may develop gas bubble disease and are subject to possible mortality.

Mortality of fish due to gas bubble disease in the heated effluent of power plants has been observed (DeMont and Miller, 1971; Marcello and Strawn, 1973). However, with only a few reported cases of gas bubble disease mortality of fish in the thermal effluents of power generating stations, documentation of all such incidents is needed to enhance our understanding of what environmental conditions and power plant design features and modes of operation may lead to gas bubble mortality of fish. This paper documents a substantial mortality of Atlantic menhaden, *Brevoortia tyrannus*, that occurred in the discharge channel and thermal plume of Boston Edison Company's Pilgrim Nuclear Power Station Unit 1 during April 8 through 24, 1973.

PILGRIM NUCLEAR POWER STATION

Pilgrim Nuclear Power Station is situated on the western shore of Cape Cod Bay in the town of

Marcello: Boston Edison Company, Nuclear Engineering Department, Environmental Sciences Group, Boston, Massachusetts; Fairbanks: Massachusetts Department of Natural Resources, Division of Marine Fisheries, Sandwich, Massachusetts.

Plymouth, Massachusetts (Fig. 1). The Pilgrim nuclear unit is a direct cycle boiling water reactor with a design power rating of 1998 MWt and a net power output of 655 MWe. The station uses once-through cooling with an open channel surface jet discharge system. Water used for condenser cooling is drawn from Cape Cod Bay at a normal rate of about 310,000 gpm which removes about 4.5 x 10^9 Btu/hr of heat from the condenser resulting in a water temperature increase of about 29°F above the water temperature at the intake. The cooling water is returned to Cape Cod Bay via a 900-ft discharge channel at velocities varying from about 2 fps (MHW) to 8 fps (MLW). The general configuration of the intake, discharge channel and breakwater jetties is shown in Fig. 1.

MENHADEN MORTALITY

Following a several-day station outage, the Pilgrim reactor was restarted and the generator phased to grid on April 6, 1973. During startup operation and until full power level was achieved, the temperature of the discharge water increased an average 0.5°F/hr above that at the intake. Small numbers of live menhaden were seen in the discharge channel by Pilgrim Station personnel on April 6, 1973. It was not until April 8, 1973, however, that Pilgrim personnel began to observe several moribund menhaden in the discharge effluent. By April 9, 1973, the number of menhaden observed in the discharge channel dying and exhibiting signs of stress characterized primarily by erratic and uncoordinated movements had increased substantially, and as a result the Massachusetts Division of Marine Fisheries was notified that a fish kill was in progress at Pilgrim Station. With the exception of two adult menhaden collected on the station's intake screens on March 15, 1973, no menhaden had been recorded prior to April 6, 1973, in the vicinity of the station during routine biological sampling conducted by the Massachusetts Division of Marine Fisheries throughout the winter months. Menhaden do not usually appear in the Cape Cod Bay region until May and generally depart by November.

Based on visual observations it was estimated that several thousand menhaden were in the discharge channel and an additional 75,000 to 100,000 were schooling immediately off the discharge channel in the thermal plume where water temperatures ranged between 50°F and 62°F. The fish were

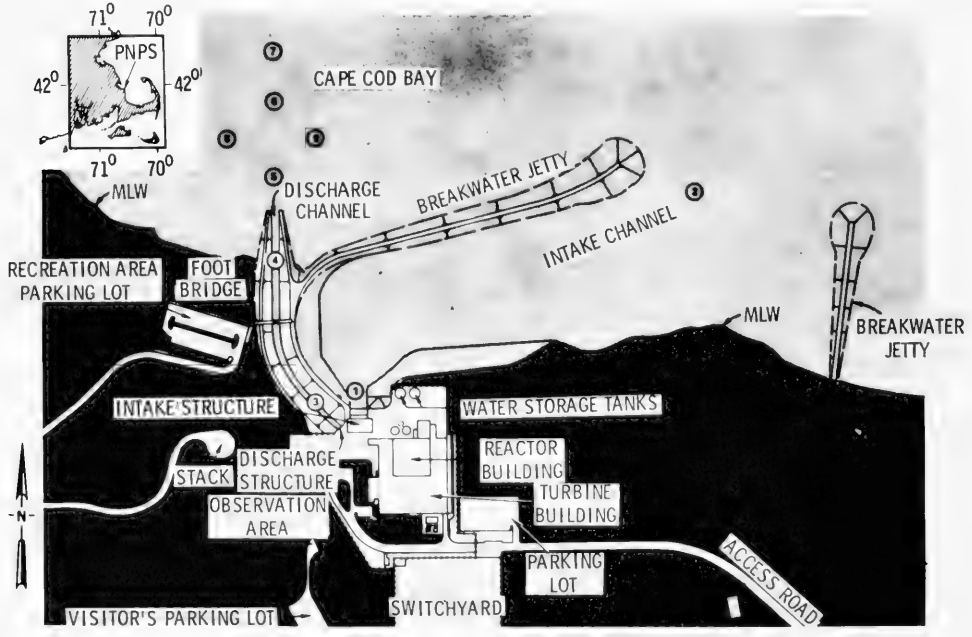

FIG. 1 Pilgrim Nuclear Power Station Unit 1 site plan. Arabic numerals indicate water sampling stations.

approximately 30 to 40 cm in total length and about 500 g in weight.

Menhaden from the discharge effluent displayed two apparent external lesions, i.e., hemorrhage and subcutaneous emphysema. Subcutaneous emphysema were evident between the fin rays of all body fins especially the dorsal and pectorals (Fig. 2) and within the oral cavity. Subcutaneous ecchymoses (0.5 to 2 cm in size) were evident in various body locations but particularly frequent about the head. In addition, some evidence of pronounced exophthalmos was also observed. Frequently menhaden in the thermal effluent were observed jumping clear of the water and propelling themselves rapidly along the water surface on their sides. Just prior to death, the fish became disoriented and gyrated below or on the water surface. On the basis of the external symptoms displayed and the aberrant behavior observed, gas bubble disease was diagnosed as the cause of the menhaden mortalities. At least 90% of the menhaden in the discharge channel and thermal plume displayed external symptoms of gas bubble disease.

On April 11, 1973, several moribund menhaden were transported to the University of Rhode Island, Sea Grant Marine Pathology Laboratory for confirmation of the gas bubble disease diagnosis and to ensure that no other infectious disease processes were involved in the menhaden mortality. Cultures of kidney, liver, and skin lesions from moribund and recently dead menhaden were made in Zobells Marine Broth and cultured at room temperature; no growth was observed after 48 hr. Sections of gill, heart, liver, spleen, kidney, pancreas, stomach and intestine were made and were within normal ranges. Decalcified cross sections of the cephalic area were examined for olfactory and lateral line changes and no significant lesions were noted. Sections of the brain and meninges revealed congestion and foci of hemorrhage compatible with vascular damage. Blood cells were also found free within the ventricles. Based on these findings, Wolke (1973) concurred with the gas bubble disease diagnosis and concluded that the menhaden died from gas emboli resulting in cerebral anoxia and hemorrhage.

An attempt was made to obtain measurements of dissolved oxygen and nitrogen of Pilgrim Station intake and discharge water by gas chromatography. Dissolved gas levels in replicate samples varied substantially, however. In addition, comparison of dissolved oxygen measurements determined by gas chromatography with those determined by Winkler titration (which showed good agreement between replicate samples) revealed such large discrepancies that the validity of analyses by gas chromatography in this instance are questionable and are not presented.

Dissolved oxygen concentrations of intake and discharge waters were measured throughout the period menhaden mortality was occurring. Sampling

FIG. 2 Subcutaneous emphysema between rays of dorsal fin of adult menhaden (photo courtesy of R. E. Wolke, University of Rhode Island, Kingston, Rhode Island).

stations are shown in Fig. 1. Water samples were collected just beneath the surface with a Van Dorn bottle, chemically fixed and analyzed for dissolved oxygen by the Winkler method (APHA, 1965). Water temperatures at the time of sample collection were obtained using an ARA electronic thermometer. Saturation levels were calculated using a solubility curve from the data of Weiss (1970).

In general, all dissolved oxygen measurements showed that Pilgrim Station intake waters during the menhaden mortality were near saturation (Table 1). Dissolved oxygen saturation levels in the discharge channel and thermal plume usually ranged from 120% to 140%.

Mortality of menhaden continued until April 24, 1973. However, after April 19, 1973, an apparent decline in mortality occurred. Estimates of menhaden mortality rates were made on several occasions during the observed fish kill. Severely stressed and dead menhaden were collected on the surface of the thermal plume during 15-min periods. Time collections of menhaden averaged 10.7 fish per period (range of 5 to 17 fish per period) or about 0.7 fish per min. For each fish collected at the water surface at least two additional menhaden were observed in distress beneath the water surface that were not collected. Applying an approximate minimum mortality rate of about 2.7 fish per min, an estimated 43,000 menhaden died due to gas bubble disease during the period April 9 through April 19, 1973.

An attempt was made to decrease gas saturation levels in the discharge water to those believed to be non-lethal to menhaden (i.e., less than about 120% and 130% saturation for nitrogen and oxygen, respectively) based on the tolerance observations of a related species, Atlantic herring (*Clupea harengus harengus*), reported by Stickney (1968). The Massachusetts Water Resources Commission (Division of Water Pollution Control) requested that beginning April 20, 1973, Boston Edison Company reduce Pilgrim Station power level by approximately 50% so that the temperature differential between intake and discharge water would not exceed 15°F. During the load reduction, studies of the dissolved gas concentration and saturation of the station intake and discharge water would be conducted. In compliance with the Water Resources Commission's request, Pilgrim Station power level reduction began at 2400 hr EST on April 20, 1973, and by 0600 hr EST on April 21, 1973, the station output was 300 MW gross (Δt condenser = 14.85°F) and remained approximately the same until 2145 hr on April 22, 1973, when the Water Resources Commission permitted Boston Edison Company to begin increasing the station power level back to full capacity. During the station power reduction, discharge dissolved

TABLE 1 Dissolved Oxygen Concentration and Percent Saturation of Pilgrim Nuclear Power Station Intake and Discharge Water During the April 1973 Menhaden Mortality

Date	Station	Time (hr)	Water temperature (F)	Dissolved oxygen concentration (mg/ℓ)	Dissolved oxygen saturation (%)
April 12	1	No sample			
	3	1340	69.0	10.2	136[1]
	4	1350	68.0	7.2	95
	5	1530	60.0	10.4	125
April 13	1	1227	41.0	10.6	101
	3	1250	68.0	10.0	132
	4	1300	68.0	10.2	134
	5	1400	52.0	10.2	112
April 17	1	1130	43.0	11.0	108
	3	1110	68.0	9.8	129
	4	1115	68.0	9.6	126
	5	1215	54.0	10.5	118
April 18	1	1230	42.5	10.6	103
	3	1233	62.0	10.0	123
	3	1400	59.5	10.2	122
	4	1220	69.0	10.2	136
	4	1250	63.0	9.8	122
April 20	1	1140	48.5	10.6	112
	1	1530	50.9	10.0	109
	3	1145	73.0	10.3	143
	3	1500	74.7	9.8	138
	4	1123	73.0	9.9	137
	4	1450	73.4	10.1	141
April 21	1	1132	44.4	10.4	104
	2	1140	43.9	10.9	104
	3	1110	57.2	10.5	123
	4	1055	57.2	10.6	124
	6	1150	50.0	11.2	120
April 22	1	1420	43.9	9.7	96
	2	1430	42.8	9.9	97
	5	1435	55.0	9.7	110
	7	1445	49.5	9.9	105
	8	1450	54.1	9.7	109
	9	1445	49.1	10.2	108
April 23	1	1351	44.5	9.5	95
	3	1345	65.5	9.9	127
	4	1335	65.5	9.5	122
April 24	1	1308	52.5	11.7	129
	3	1300	70.0	10.6	142
	4	1253	70.0	10.1	136
April 26	1	1323	46.0	9.2	94
	3	1330	74.0	8.8	123
	4	1315	73.0	8.8	122
April 27	1	0845	44.7	9.4	94
	3	0850	72.0	9.0	125
	4	0840	71.5	9.1	125
April 30	1	1230	47.0	9.8	101
	3	1225	74.0	9.1	127
	4	1213	73.0	9.2	128

[1]Saturations calculated from solubility data reported by Weiss (1970) for a salinity of 30 ppt.

oxygen saturation levels were reduced. Menhaden in the thermal plume still displayed external symptoms of gas bubble disease, but very little mortality was observed.

Field observations of the menhaden continued on April 23 and 24, 1973, and although the majority of fish still displayed symptoms of gas bubble disease, they appeared to be more responsive and in somewhat better condition.

The area around and under the surface thermal plume was examined on April 23, 1973, utilizing SCUBA. A large school of pollock (*Pollachius virens*) was observed along the interface between the ambient water and the thermal effluent. Temperatures where the pollock concentrated ranged between 44° and 48°F. The pollock appeared in good condition, were actively feeding, and showed no external symptoms of gas bubble disease. Other fishes observed and showing no sign of gas bubble disease were striped bass (*Morone saxatilis*), shorthorn sculpin (*Myoxocephalus scorpius*), and sea raven (*Hemitripterus americanus*). No lobster (*Homarus americanus*) were sighted but cancer crabs (*Cancer* spp.) and green crabs (*Carcinus maenas*) were common and feeding upon decomposing menhaden on the ocean bottom.

Attempts to remove the school of menhaden from the discharge area by commercial fisherman using purse seines were unsuccessful due to the presence of several large boulders in the discharge area and also the high velocity of the cooling water discharge which interferred with normal methods of purse seining. The school of menhaden was last observed in the thermal plume area on April 27, 1973.

Another large school of adult menhaden (estimated 200,000 to 300,000 fish) was observed in the Pilgrim Station thermal plume in early July 1973. The menhaden remained in the vicinity of the station discharge for about 1 day. During the 1-day observation, no menhaden mortality was noted, although a few menhaden were observed propelling themselves on their sides on the water surface and at times jumping clear of the water. None of the several menhaden examined showed external signs of gas bubble disease. The menhaden were concentrated in the thermal plume where water temperatures ranged from 68°F to 82°F. Dissolved oxygen concentrations and percent saturation at the periphery of the school of fish ranged from 9.6 to 9.9 mg/ℓ and 124% to 146%, respectively.

As of the end of September 1974, no large schools of adult menhaden had been sighted in the immediate vicinity of the station discharge. It should also be noted, however, that from October 1973 until August 1974, Pilgrim Station has been at reduced power or not operating due to refueling, maintenance and contested licensing hearings regarding a change in the fuel design.

DISCUSSION

Since the Pilgrim Station menhaden mortality, concern over the potential problem of gas bubble disease at coastal electric generating stations has increased. Recognizing that fishes are attracted to power plant thermal discharges which may be supersaturated with dissolved gases, it has become obvious that the tolerance of important species to gas supersaturation needs to be determined.

Such studies on the tolerance of menhaden to supersaturation at varying water temperatures are currently being conducted (Clay et al., 1974). Subsequent experiments will attempt to determine the recovery rates of menhaden after exposure to supersaturation as well as determining the importance of other factors (e.g. physiological stress due to extreme activity levels) in modifying the tolerance of menhaden to gas supersaturation.

Other considerations include the ability of some fishes to detect and avoid water supersaturated with dissolved gases. Meldrim, Gift and Petrosky (1973a and 1973b) found that the behavioral responses of some freshwater fish to supersaturated conditions varied with the species. They noted that the yellow perch (*Perca flavescens*) showed no definitive response to supersaturated water while the silvery minnow (*Hybognathus nuchalis*), golden shiner (*Notemigonus crysoleucas*), and the satinfin shiner (*Notropis analostanus*) were very responsive to supersaturated conditions. Stickney (1968) observed that the Atlantic herring (a species related to menhaden) definitely tended to avoid gas-supersaturated water, but only when saturation levels were high enough to produce gas bubble disease. It is possible that menhaden are also capable of detecting and avoiding supersaturated environments.

The ability of fish to detect and avoid supersaturated environments may be altered by other factors, however. Meldrim et al. (1973b) have reported that the behavioral response of the golden shiner to supersaturated conditions may change depending on the water temperature associated with the exposure. They noted that the golden shiner usually avoided gas supersaturations that exceeded 110%. However, when temperature increases of 5°C and 10°C are associated with the supersaturated conditions, temperature preference of golden shiner overrides avoidance of the supersaturation. It seems likely that similar behavior occurred during the menhaden incident described in this paper, i.e., the preference for above ambient temperatures within the discharge channel and

thermal plume may have negated a menhaden avoidance of the gas-supersaturated cooling water discharge.

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Observations on the Effects of Gas Embolism in Captured Adult Menhaden

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S. Testaverde
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ABSTRACT

The problems of entrapped fish in effluent water of power plants prompted a study of the parameters that induce gas embolism in adult menhaden. Adult menhaden were captured by purse seining in early summer on route to the warmer headwaters of Boston Harbor and maintained in tanks at the New England Aquarium. Supersaturation of the waters in experimental tanks was deliberately induced and the behavior and certain histological indices studied at a range of temperature, salinity, and supersaturation.

The occasion of menhaden mortality in the effluent of the Pilgrim Nuclear Power Plant in Plymouth, Massachusetts (Marcello and Fairbanks, 1974), prompted an investigation of the cause, and a search for practical solutions to avoid future destruction of adult menhaden. If, indeed, supersaturation of gases in the discharge canal of power plants induces gas embolism in fish, a number of engineering solutions are possible. An initial step in developing such solutions is to rigorously define the range of environmental conditions that induce gas bubble disease in a menhaden population.

Unfortunately, investigations of the environmental conditions that induce gas embolism have been hampered by an inability to hold adult menhaden in captivity. This may seem strange in view of the numbers of Atlantic menhaden caught and processed each year, but realistic in view of the fact that menhaden are considered a trash fishery whose main use is for fertilizer and cat food. Thus, there has not been any emphasis or effort to maintain or stock menhaden as a potential sports fishery, nor even to hold adult menhaden for display or research purposes. Consequently, a reliable capture and maintenance program had to be developed. A project was undertaken that incorporated three phases: to capture and maintain a stock of menhaden for testing purposes; to determine lethal levels of supersaturation at various temperatures; and to identify pathological symptoms at these saturation levels.

The Atlantic menhaden, *Brevoortia tyrannus*, is a pelagic, euryhaline species. It ranges along the entire eastern United States coastline from Maine to Florida. The general migration pattern includes a northward migration in the spring and summer months from the southern waters. In the early fall, the schools begin to move from the northern areas to the southern portions of the coast. Tremendous schools appear off North Carolina in November and December, where they remain until late spring (Nicholson and Higham, 1966).

CAPTURE AND MAINTENANCE

Adult Atlantic menhaden were captured by commercial purse seiners in Boston Harbor during July 1974. Since menhaden are an easily excitable fish and once entrapped may become extensively damaged by contact with the seine, observations were made aboard a carrier vessel to determine the best means of collection.

The purse seine set consists of a carrier vessel and a purse boat. A spotter plane locates the school and directs the purse boat in setting the net. When the school is encircled, lead weights are released to close the net and trap the fish. The net is hauled aboard the purse boat with the aid of a hydraulic power block until the school is concentrated into the heavily constructed pocket of the seine, called the bunt. Then the carrier vessel comes alongside the bunt, the fish are further "dried up," and brailed into the fish hold of the carrier vessel.

From our observations, it was determined that the best time to transfer the menhaden was as soon as the carrier vessel was secured adjacent to the bunt. The fish were then transferred from the bunt with a dip net into an aerated, 2.4-m diameter tank

Clay, Barker, Testaverde: New England Aquarium Corporation, Boston, Massachusetts; with Marcello and McLeod: Boston Edison Company, Boston, Massachusetts.

aboard our collecting vessel, R. V. Shrock. Approximately 150 fish were transported to the holding facility at the New England Aquarium.

The holding facility (Fig. 1) incorporated three biological filters to break down nitrogenous waste products and a mechanical filter to remove particulate matter. Water was gravity siphoned into the biological filters, and then air lifted back into the tank. The mechanical filter pumped water from the bottom center of the tank through a sand filter and back into the tank at about 60 gal/min flow. This return line helps in aeration of the tank as well as creation of a current which appears to aid in the schooling behavior of the fish. The walls of the holding facility were painted black, a technique which reduces the likelihood of fish colliding with the walls (Hettler). The fish were fed finely grained food 4 to 5 times a day; total daily intake was approximately 5% of their body weight. Experimentation with various food sizes indicated that Purina® tropical fish food gave the most efficient feeding since it remained in the water column long enough for maximum consumption.

No immediate losses were noted due to handling and transfer. However, during the first 2 weeks a total of 34 fish, or 22%, died, possibly from the effects of net damage. Since that time only three additional fish have died. Standard water quality parameters were measured on a regular basis. During July and August the temperature remained about 20°C, but as cold weather set in the temperature steadily dropped to 13°C, and an abrupt color change occurred in some fish, from bright silver to black, possibly indicating thermal stress. Reduced feeding behavior and sluggishness were also noted. Salinity remained between 30 and 32 ppt, pH was maintained between 7.5 and 7.8, and dissolved oxygen between 6 and 7 ppm. Ammonia levels rose for 3 weeks to a maximum of 3.5 ppm until the bacterial population in the biological filter was established. Values then steadily declined to a stable level of 0.225 ppm and the nitrate levels subsequently rose from 0.5 ppm to a maximum of 2.5 ppm. Nitrite also rose from 0.006 ppm to 1.5 ppm during the same period. Water changes were made as necessary to keep nitrate and nitrite levels below 3 ppm NO_3^- and 1.5 ppm NO_2^-.

TESTING PROCEDURE

Fish were removed as needed to perform tests, and a control was run at each saturation. Twelve fish were used in each experiment, six in the test tank and six in the control. The test and control tanks were 145-gal capacity with the test tank attached to a 50-gal pressure chamber detailed in Fig. 2. The pressure chamber was constructed of 60 cm diameter PVC pipe. A 3/4 hp pump provided water circulation through the pressure chamber and a ball valve on the return line to the test tank provided control of pressure and water flow. Original experiments used a venturi method of aeration in the pressure chamber; however, in later experiments an air compressor was added to allow for higher saturations.

The temperature, dissolved oxygen, and dissolved gas pressure were measured at regular intervals during the experiment in both the control

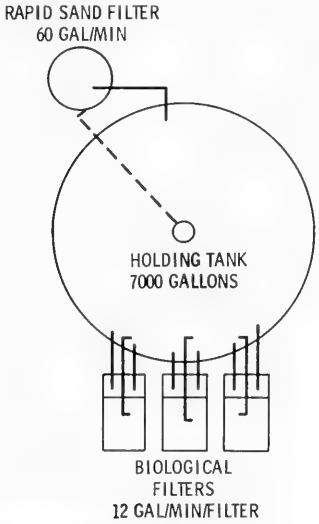

FIG. 1 Menhaden holding facility.

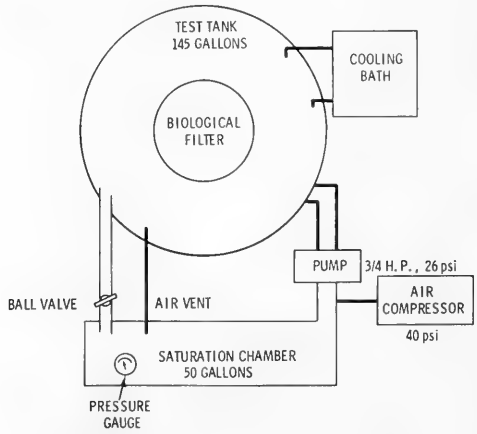

FIG. 2 Supersaturation testing system.

and test tanks. Dissolved gas pressure was measured with a Weiss saturometer made by Eco Enterprises, and the nitrogen saturation and total dissolved gas saturation calculated according to the equations:

$$\text{Total dissolved gas \% saturation} = \frac{P_{atm} + \Delta P}{P_{atm}}$$

where P_{atm} = atmospheric pressure at time and altitude of saturometer reading

and ΔP = saturometer reading

$$\%\ N_2\ \text{sat} = \left\{(P_{atm} + \Delta P) - \left[\frac{O_2}{\beta O_2}\left(\frac{22.41\ \text{ml/m mole}}{32.00\ \text{mg/m mole}}\right)\cdot(0.76)^T\right] - P_{H_2O}\right\} \Big/ (P_{atm} - P_{H_2O})\ (0.7902)$$

where O_2 = oxygen concentration in mg/ℓ

βO_2 = Bunsen solubility coefficient for oxygen

P_{H_2O} = partial pressure of water vapor as a function of temperature

T = observed temperature in °C.

As the test fish died, necropsies were performed as soon as possible. Weight, fork length, sex and sexual maturation were determined and age estimated using a modification of a graph by C. E. Richards (1969) (Fig. 3). Of the 77 which have died since capture, fish length ranged from 225 to 310 mm, the average weight was 304 g, 46% were males and 54% were females. The majority of fish, 60%, were age two, 20% age three, 17.3% age four, and 2.7% age five.

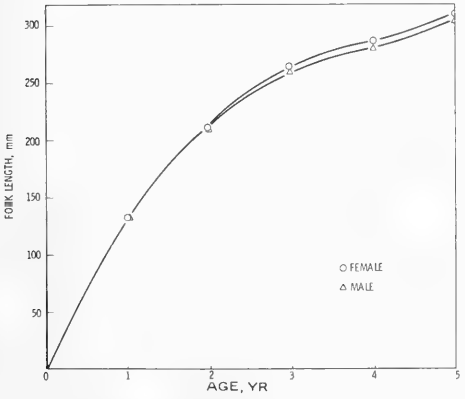

FIG. 3 Menhaden age determination.

RESULTS

The necropsies of control specimens showed no apparent external or internal symptoms of gas bubble disease. However, as can be seen in Table 1, increased nitrogen saturation increased the evidence of gas emboli at 22°C. At 105% saturation nitrogen, all test specimens lived for 96 hr with no pathological evidence of gas bubble disease. However, observations conducted during the course of the experiment showed definite behavioral changes in the test fish in comparison with the control fish. The test fish were excreting mucus, swimming erratically, and more excitable than the control fish. Body color change was also apparent on some test fish.

TABLE 1 Etiology of Gas Bubble Disease in Menhaden at 22°C

% Saturation			Observations	Pathology
N_2	Total	O_2		Appearance of gas bubbles in:
100	95	70	None apparent.	None apparent.
105	95	75	Some mucus, erratic swimming, body color change noted in 1 fish, no deaths in 96 hr.	None apparent.
110	107	102	Some mucus, erratic swimming, body color change noted in 2 fish, 2 died within 96 hr.	Eyes, intestines, pyloric caeca, and mesentery.
120	110	85	Mucus, erratic swimming, death in 24 hr.	Eyes, intestines, pyloric caeca, roof of mouth, bulbus arteriosis, fins, operculum, gill arterioles.

At 110% saturation nitrogen, behavioral changes similar to those observed at 105% were noted. Necropsies showed some evidence of emboli in the intestines, the pyloric caeca, the operculum, and the eye. Two fish died within 96 hr, one during the 3rd day and one between the 3rd and 4th days.

At 120% nitrogen saturation, death occurred within 24 hr with classic symptoms of gas bubble disease displayed. Evidence of exophthalmia was

apparent in some of the fish. Emboli were also apparent in all high saturation test specimens in the pyloric caeca, intestines, eye, operculum, roof of mouth, epithelium of dorsal fin, mesentery, and gill arterioles. In two cases the bulbus arteriosis was greatly distended, apparently by the presence of an embolus.

CONCLUSIONS

Preliminary results indicated that adult menhaden could be maintained, with care, for an extended period of time. Results also showed that gross symptoms of gas bubble disease could be induced in menhaden at high levels of nitrogen saturation. A probable threshold toxic effect for adult menhaden exists between 115% and 120% nitrogen saturation at 22°C. Further testing at other temperatures will be necessary to define a relationship between nitrogen saturation, toxicity and temperature. We also intend to explore the tolerance and recovery capabilities of menhaden to the effects of intermittent exposure to supersaturation levels of 120% to 130% nitrogen.

ACKNOWLEDGMENTS

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Gas Bubble Disease of Salmonids: Variation in Oxygen-Nitrogen Ratio with Constant Total Gas Pressure

R. R. Rucker

ABSTRACT

Coho salmon fingerlings were subjected to a total gas pressure of 119% at 13.6°C with the O_2/N_2 varying from 50%/138% to 229%/90%. The small fish (3.8 to 6 cm) were the most resistant and the larger fish (8 to 10 cm) the least resistant to gas bubble disease at the gas concentrations used. A drastic decrease in lethal effect of individual ratios of O_2 to N_2 occurred between 159% O_2/109% N_2 and 173% O_2/105% N_2 at the same total gas pressure (119%).

A review of the literature regarding gas bubble disease can be found in a recent publication by Rucker (1972); one in press by the National Academy of Science (1972); and an unpublished report by Weitkamp and Katz (1973). Most discussions on gas bubble disease have dealt with the inert gas, nitrogen; oxygen was given a secondary role. It is important to know the relationship of nitrogen and oxygen when we are concerned with the total gas pressure in water. Where water becomes aerated at dams or falls, both oxygen and nitrogen are about equally saturated when expressed as a percentage. When oxygen is removed from water by metabolic and chemical action, or when oxygen is added to the water by photosynthesis, there is a definite change in the ratio of oxygen and the inert gases (mainly nitrogen with some argon, etc.). This present study shows the effect of varying the oxygen and nitrogen ratio in water on fingerling coho salmon *(Oncorhynchus kisutch)* while maintaining a constant total gas pressure.

GENERAL EXPERIMENTAL OBJECTIVES AND METHODS

The primary purpose of these experiments was to determine differences in lethality of various gas ratios of oxygen and nitrogen at a constant total gas pressure of 119%. I also wished to determine whether there was a difference in susceptibility between sizes and stocks of juvenile coho. Also to be examined was the effect of reducing the oxygen while holding the nitrogen constant.

Juvenile coho salmon averaging 6 cm in length, obtained from the Quilcene National Fish Hatchery, Quilcene, Washington, and the Montlake Laboratory of the National Marine Fisheries Service, Seattle, Washington, were used during all the tests concerning differences in lethality of O_2/N_2 ratios. During these tests temperatures were 13.6°C ± 0.1°C. Gas concentrations usually varied very slightly from the desired ratios. The tank facility consisted of six troughs, two of which were used to hold experimental fish at normal saturation (100%) and two pairs of troughs used to test fish at different gas ratios.

Control of gas concentrations and the test apparatus is described in a subsequent section. During initial testing of the gas control system, I determined that a ratio of 114% O_2 to 121% N_2 could be achieved by merely allowing air to be sucked into the intake side of the recirculation pump. Since this gas ratio did not require injection of either oxygen or nitrogen, the resultant concentration (114% O_2 and 121% N_2) was used as a quasi control for comparison with the other gas ratios. Several replicates were completed at this concentration. This ratio and concentration were also used to test for differences in size and stock and to provide base line data in determining effect of reduced oxygen concentration while maintaining a constant nitrogen level.

In all the tests free carbon dioxide was found near normal, about 2 ppm. Oxygen is expressed as "O_2" and the inert gases as "N_2".

Rucker: Coastal Zone and Estuarine Studies Division, National Marine Fisheries Service, Seattle, Washington.

The number of days to kill 25% of the fish at the different gas levels is expressed as the lethal exposure—LE_{25}, and to kill 50%—LE_{50}.

TEST APPARATUS

Apparatus shown in Fig. 1 was used to produce water with a definite oxygen and nitrogen content. The tank (1) was divided so that two experiments could be carried on simultaneously with the same equipment. Water was circulated by a centrifugal pump (2) with a valve (3) on the effluent side to cause a controlled back pressure as read on a gauge (4). This created a vacuum on the inflow side (5) so that air could be introduced into the water with either oxygen or nitrogen (6) through a "Y" tube. Circulation of the water caused an increase in temperature which was maintained at approximately 13.6°C by means of a refrigeration system (7) and recorded on a thermograph (8). Water level in the tank was maintained by float valves (9). Each trough was supplied with 1 ℓ per min of water regulated with flowmeters (10). The water used was from the municipal supply, was quite soft, and was passed through activated charcoal to remove the chlorine. A greater depth of water was needed for absorption of the gases than was afforded by the tank (1), so two towers (11) were added to the system. The spout (12) at the top of the towers was to direct possible overflow water back into the system.

Inside dimensions of troughs in the fish holding area (13) were 104.5 x 23.5 x 20 cm high. Water depth was maintained at 14 cm. Each trough could be separated into three compartments with screens —"A" was at the inflow end of the trough, "B" middle, and "C" outflow end. In a few cases a compartment was divided longitudinally so that two groups of fish could be subjected to almost identical conditions.

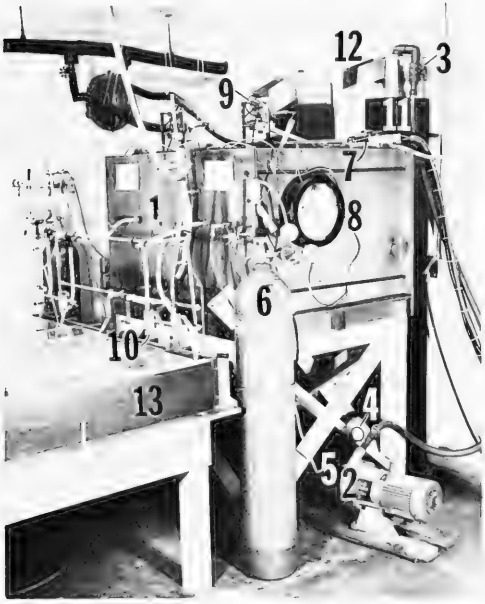

FIG. 1 Apparatus for subjecting fish to constant-temperature, flowing water with a definite oxygen and nitrogen content.

Effect of Variation in O_2/N_2 Ratios

Times to death (LE_{25} and LE_{50}) of juvenile coho salmon at various concentrations of O_2 and N_2 during constant total gas saturation of 119% appear in Table 1 and are shown graphically in Fig. 2. With one exception (192% O_2 and 100% N_2), all increases in ratio of O_2 to N_2 resulted in increased tolerance to the total gas saturation. A marked increase in tolerance to total gas pressure occurred between concentrations of 159/109 and 173/105% saturation of O_2 and N_2 (Fig. 3).

TABLE 1 Time to Death (Days) of Juvenile Coho Salmon (6 cm) Exposed to 119% Total Gas Supersaturation at Different Levels of O_2 and N_2 in 13.6°C Water

Gas concentration, % saturation		Time to death (days)			
		LE_{25}		LE_{50}	
O_2	N_2	Range	Average	Range	Average
50	138	1.8 to 1.9	1.9	3.2 to 4.0	3.7
75	131	1.8 to 2.7	2.3	3.5 to 4.3	3.9
114	121	3.2 to 4.1	3.8	6.3 to 7.3	6.9
159	109	3.2 to 5.3	4.5	6.5 to 9.1	8.2
173	105	33.5 to 35.3	34.4	-	[1]
192	100	-	32.0[2]	-	[3]
229	90	-	[4]	-	[4]

[1]Not reached, 28% mortality in 39 days.
[2]One replicate reached, 24% mortality in 30 days, the other 25% in 32 days.
[3]Not reached, test concluded at 33 days.
[4]Not reached, 20% mortality in 35 days.

Effect of Size and Stock

A number of tests were carried out in the water containing 114% O_2 and 121% N_2 to determine effect of size and stock of fish on susceptibility to gas supersaturation (Table 2). Two groups of 3.8 cm coho from the Montlake Laboratory which had just

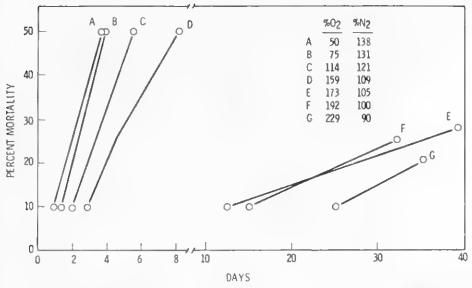

FIG. 2 Mortality pattern of 6 cm coho salmon reared at different O_2/N_2 levels at 13.6°C with a 119% total gas pressure.

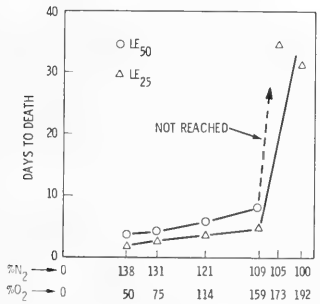

FIG. 3 Relationship between O_2/N_2 levels and time to death of 6 cm coho salmon fingerlings at 13.6°C and total gas concentration of 119%.

TABLE 2 Resistance of Juvenile Coho Salmon to Gas Supersaturation (114% O_2 + 121% N_2) in Relation to Size and Stock of Fish. Lethal exposure levels (LE_{25} and LE_{50}) in days to death, water temperature 13.6°C + 0.1°C and test duration 30 days

Lethal exposure	Days to death by size and stock			
	3.8 cm Montlake	4.6 cm Quilcene	10 cm Montlake	10 cm Quilcene
LE_{25}	16.9	16.7	2.1	2.9
LE_{50}	Not reached in 30 days	27.4	2.6	4.2

started feeding were initially tested. One group of 96 fish reached LE_{25} in 22.9 days and LE_{30} after 30 days. The other group of 50 fish reached LE_{25} in 10.9 days. No further losses occurred until the 27th day. Loss at 30 days was 34%. Averages of the two groups place LE_{25} at 16.9 days. Average loss at 30 days was 32%.

Two groups of 4.6 cm fish from the Quilcene National Fish Hatchery were also tested. These tests produced LE_{25}'s of 15.1 and 18.3 days. LE_{50} was reached in 24.7 and 30 days. Averages of the above place LE_{25} at 16.7 and LE_{50} at 27.4 days.

Five groups of fish, approximately 10 cm long, from the Montlake Laboratory were then tested. The average for all groups gave an LE_{25} of 2.1 days and an LE_{50} of 2.6 days.

Three groups of 10 cm fish from the Quilcene National Fish Hatchery were similarly tested. Averages were 2.9 days for LE_{25} and 4.2 days for LE_{50}.

These results indicate that the larger fingerlings are definitely more subject to harm from excess air in the water than the smaller fish. These data agree with those of Meekin and Turner (1974).

Although the data are limited, there appears to be little difference between susceptibility of the Montlake and Quilcene stocks.

Effect of Reduced O_2 Concentration on Mortality Rate

Fish held in compartments in a trough utilize oxygen so that the water in compartment "C" (outflow end) would have less oxygen than in compartment "A" (inflow end). Compartment "B" in the central part of the trough would have O_2 levels somewhere between those in "A" and "C". Nitrogen levels in these compartments, however, were the same. To demonstrate the effect of reduced oxygen in relation to gas bubble disease, 48 coho of 8.5 cm fork length were randomly distributed into compartments "A", "B", and "C". Two additional replicates of the "C" compartment tests were run using 32 coho (8.5 cm) in each trial. These are listed as C_1 and C_2 in Table 3.

TABLE 3 Mortality of 8.5 cm Coho Salmon Subjected to a Constant Temperature (13.6°C) and 121% N_2 with a Variation in Oxygen Tension

	Trough compartments				
	A	B	C	C_1	C_2
Percent oxygen	113	110	105	105	105
Percent total gas pressure	119	118	117	117	117
LE_{25} in days	2.5	3.6	3.8	4.2	5.4
LE_{50} in days	3.3	5.3	5.3	6.6	6.6

Inspection of these data indicates that when 121% N_2 is maintained, oxygen plays a more significant role above 110% than below 110%.

Some of the data obtained when the oxygen-nitrogen ratio tests were done also illustrated the

effect of reduced oxygen on the mortality rate. This was apparent in the experiment using 173% O_2 and 105% N_2. At 173% O_2 there were losses of 26% and 30% in 39 days, whereas slightly larger fish at the lower end of the troughs subjected to 169% O_2 had losses of only 7% in 39 days.

PATHOLOGY

Generally, the fish died quite suddenly in the higher nitrogen concentrations. Never was tissue damage or any progressive pathology demonstrated. The fish always seemed to die from gas embolism restricting the flow of blood through the gills. When the nitrogen was near normal and the oxygen quite high, the fish were moribund for many days before succumbing. These fish had blebs in the mouth which interfered with feeding and caused emaciation.

SUMMARY

Coho salmon fingerlings were subjected to a total gas pressure of 119% at 13.6°C with the O_2/N_2 varying from 50%/138% to 229%/90%. The small fish (3.8 to 6 cm) were the most resistant and the larger fish (8 to 10 cm) the least resistant to gas bubble disease at the gas concentrations used. A drastic decrease in lethal effect of individual ratios of O_2 to N_2 occurred between 159% O_2/109% N_2 and 173% O_2/105% N_2 at the same total gas pressure (119%).

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The pathology was studied by William T. Yasutake, pathologist, Western Fish Disease Laboratory.

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Effect of Gas Bubble Disease on Lateral Line Function in Juvenile Steelhead Trout

M. H. Schiewe
D. D. Weber

ABSTRACT

Normal lateral line response of juvenile steelhead trout, *Salmo gairdneri*, to a standardized set of stimuli was compared with the response of fish affected by gas bubble disease. Electrophysiological monitoring of individual afferent nerve fibers showed that as gas emboli formed in the scale pockets of the trunk lateral line of stressed fish, the ability to respond to stimuli was either diminished or completely disappeared. Further testing demonstrated that this sensory loss is reversible and that upon return to equilibrated water, accompanied by the disappearance of the gas emboli, normal function was regained. This sublethal effect of gas bubble disease on the lateral line sensory system may be an important element contributing to indirect mortality.

Mortality of fish resulting from gas bubble disease has been extensively studied and documented (see review by Rucker, 1972). Death results from occlusion of major vessels by gas emboli when the total dissolved gas pressure of the water environment ($P_{N_2} + P_{O_2} + P_{Ar}$) exceeds approximately 110% of atmospheric saturation. In addition, some recent studies suggest the possibility of gas bubble disease contributing indirectly to mortality (Coutant and Genoway, 1968; Newcomb, 1974; Schiewe, 1974).

Under close observation, the first visible sign of gas bubble disease in fish is the presence of gas emboli in the continuous scale pockets that form the trunk lateral line. These scale pockets contain the hair cell receptor mechanism of this sensory system. Although controversy still exists concerning the exact function of the lateral line system, it is generally recognized that it responds to near field water displacements (Harris and Van Bergeijk, 1962). Behavioral studies (Disler, 1960; Dijkgraaf, 1962) tend to support the theory that the lateral line serves as a "distant touch" sensory modality for predator-prey relationships, schooling, and obstacle localization.

Impairment of a sensory system with such an important role in fish behavior could adversely affect survival. This paper reports electrophysiological studies designed to assess the effect of gas bubble disease on lateral line function.

MATERIALS AND METHODS

Experimental fish were hatchery-reared juvenile steelhead trout, *Salmo gairdneri*, from a uniform population with a mean length and weight of 202 mm and 74 g respectively. All fish were acclimated and tested at a temperature of between 13°C and 15°C. The limited temperature range was used because of the dependency of saturation on temperature.

The fish support system, for monitoring both control and stressed fish, is shown in Fig. 1. Equilibrated water was supplied from a dechlorinated tap water source. Supersaturated water was generated by passing water from the same source through a high pressure pump operated under back pressure.

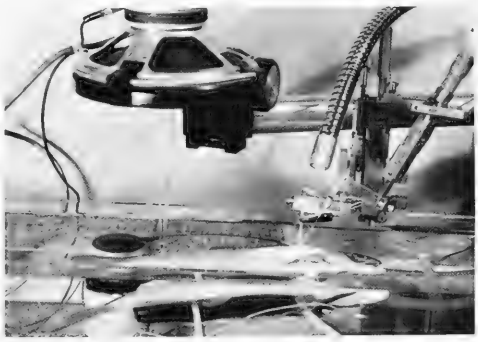

FIG. 1 Testing apparatus for monitoring lateral line response of steelhead trout. The black disc adjacent to the glass bulb stimulator is a monitoring hydrophone; and the two electrodes beneath the head of the fish are for volume conduction recording of EKG.

Schiewe and Weber: National Marine Fisheries Service, Seattle, Washington.

Compressed air was metered into the intake side of the pump to create the desired saturation level. Supersaturated water was maintained at 118 ± 4% of atmospheric saturation in the test range of 13°C to 15°C. Analysis procedures were the gas chromatographic methods of Swinnerton et al. (1962) modified by Ebel (1969).

Anesthesia was accomplished with tricaine methanesulfonate followed by gallamine triethiodide at the rate of 0.6 mg/10 g body weight. At opercular arrest the fish was moved to the testing apparatus where water (equilibrated or supersaturated) was forced over the gills at the rate of 1 ℓ/min. If the heart rate, monitored by volume conduction EKG, fell below normal for a given temperature, the experiment was terminated.

Water level in the testing apparatus was adjusted to provide an unsubmerged area 2 to 3 cm posterior to the operculum. In this area the lateral line nerve bundle was dissected out and placed over a silver, spoon-shaped indifferent electrode. Phosphate-buffered saline was used to both bathe the exposed nerve and to fill the depression of the spoon. Individual nerve fibers were then teased out and draped over an uninsulated, electrolytically sharpened, stainless steel hook electrode for recording.

Initial recording of nerve activity during isolation was done with a Tektronix® model 565 dual beam oscilloscope coupled with a loudspeaker. Further study and documentation were accomplished by transfer of the recording to a Tektronix® model 5103N storage oscilloscope with a Polaroid® camera attachment.

Normal response pattern was based on the ability of the associated receptors to respond to varying degrees of stimulation. Once a fiber was isolated, the corresponding receptor response was measured by application of light touch, surface waves, and pulsed water displacements. The pulsed water displacements were generated by a 1 cm glass bulb attached to the cone of an 8-in. loudspeaker driven by a Waveteck® model 144 HF sweep generator. During testing the bulb was positioned 5 mm above the appropriate receptors.

RESULTS AND DISCUSSION

Our initial studies indicated that individual afferent lateral line nerve fibers would innervate receptors in 1 to 26 scale pockets to form a sensory unit. The resting or spontaneous activity rate of an isolated fiber was directly dependent on the number of scales comprising that sensory unit (Fig. 2). This fact became a useful criteria in support of our findings.

Activity from over 150 fibers was recorded in 20 control fish (maintained on equilibrated water) and 10 stressed fish which were maintained on equilibrated water for a short acclimation period and then shifted to supersaturated water. Signs of gas bubble disease began to appear in the stressed fish within 2 to 6 hr. This protocol enabled us to characterize nerve fibers of fish whose lateral line showed three distinct stages of gas bubble occurrence: no bubble formation, partial bubble formation, and complete bubble formation.

None of the control fish maintained in equilibrated water developed gas emboli in lateral line scale pockets. All spontaneously active fibers were responsive to light touch, surface waves, and pulsed water displacements generated by the glass bulb stimulator. Corresponding sensory units were easily located and spontaneous activity correlated with number of scales in the sensory unit.

The scale pockets of the trunk lateral line of stressed fish would progressively fill with gas emboli until complete occlusion occurred. All fibers isolated and characterized under a state of complete occlusion did not respond to any form of stimuli except firm pressure on a limited number of scales. The number of scales reacting to stimulation did not correspond to the observed spontaneous activity (Fig. 3).

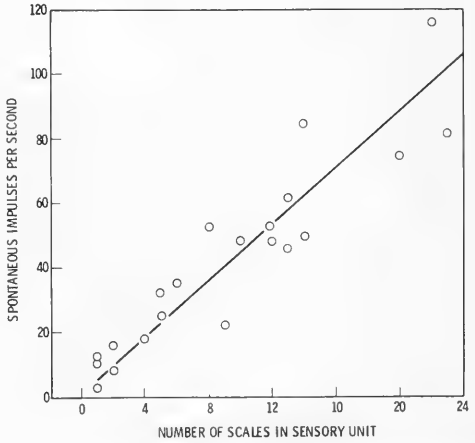

FIG. 2 Spontaneous activity of single lateral line nerve fibers and number of scales innervated. Data derived from fish acclimated and tested at 13°C to 15°C. Regression line ($y = 2.49 + 4.29X$, $N = 21$) parameters estimated using reduced major axis techniques from Miller and Kohn (1962).

®Trade names are used to simplify description; no endorsement is implied.

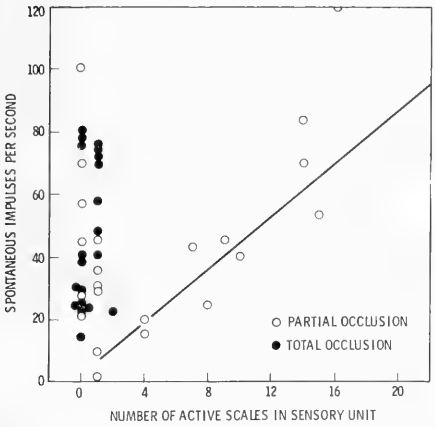

FIG. 3 Spontaneous activity of single lateral line nerve fibers from gas stressed fish and corresponding number of scales eliciting neural activity. Regression line represents spontaneous activity of fibers from control fish at 13°C to 15°C and is the same as shown in Fig. 2.

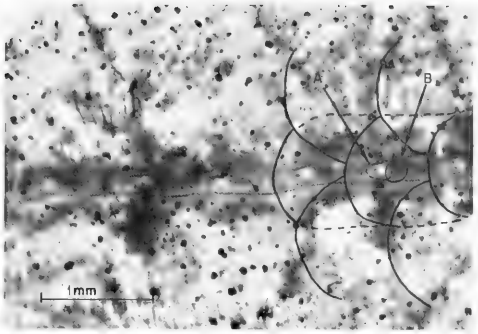

FIG. 4 Juvenile steelhead trout trunk lateral line showing complete gas bubble occlusion. A. Anterior suprascalar pore. B. Posterior infrascalar pore.

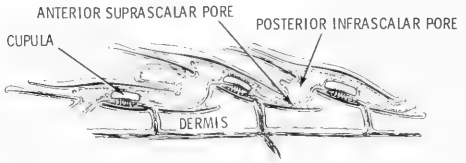

FIG. 5 Longitudinal section through a totally occluded lateral line.

Twenty-two fibers were isolated and characterized under conditions of partial bubble formation. Fifty percent responded normally in all respects, and upon localization of the sensory units no emboli were visible. The remaining fibers behaved as did those fibers isolated when emboli formation was complete. In addition to the inability to match spontaneous activity to the number of scales in the sensory unit, the limited number of scales that did respond to firm pressure either contained or were adjacent to scales with gas emboli.

We additionally isolated and characterized nerve fibers from three fish that were maintained 3 consecutive days in the test apparatus. On days 1 and 3 support was with equilibrated water and day 2 was with gas supersaturated water. This procedure provided for an evaluation of any residual effects the gas emboli may have on the functioning of the sensory units. Results showed a return to normal response patterns as the emboli disappeared 16 to 20 hr after return to equilibrated water.

In fish showing signs of gas bubble disease, the reduced ability of the trunk lateral line sensory units to respond to stimuli appears to be mechanical in nature. Fig. 4 is a photomicrograph of a section of the trunk lateral line of a juvenile steelhead trout that exhibits complete occlusion with gas emboli. A longitudinal section through this area would appear as in Fig. 5. The physical presence of the gas emboli probably acts directly on the cupula rendering it immobile. This cupular immobility prevents the shearing displacement action necessary to stimulate the hair cell receptors. Although we never monitored nerve fibers from the numerous cephalic branches of the lateral line, morphological and visual observations indicate that these receptors are equally affected. This being the case, the ability of an affected fish to detect and localize predators, or stationary objects may be impaired. This would have a definite adverse affect on survival.

From mark and recovery experiments, Raymond (1970) estimated that survival of downstream migrating salmonids in the Snake River basin between 1964-68 was nearly 100%. Following dam construction and resultant high concentrations of dissolved gas (during periods of heavy spilling at dams) in the water of the lower Snake River, survival of downstream migrants dropped to 30%. Considering factors of fish tolerance, behavior, and gas concentration, Ebel (1973) estimated that 60% of the total 1970 mortality was due to direct effects of gas bubble disease. Predation and mortality associated with dam passage accounted for the remaining 40% of the loss. We believe that a large proportion of the mortality attributed to these nonspecific causes was probably an indirect effect of gas bubble disease through impairment of the lateral line sensory system.

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Effects of Stress on Salmonid Blood Clotting Mechanisms

E. Casillas
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B. G. D'Aoust

ABSTRACT

Enhancement of blood clotting functions is a possible factor which could kill fish following stress resulting from exposure to supersaturation. During examination of various hematological parameters in rainbow trout which were subjected to stress from exercise, blood coagulation times were found to decrease to 45% of the original pre-stress values within a half hour after the termination of the stress period. Thrombocyte counts were found to increase three- to fourfold in the same period. Hematocrits and blood plasma glucose also rose significantly with respect to the stress applied. Red blood cell and white blood cell counts, however, did not increase in response to the stresser. The degree of the responses observed were compared between members of a wild trout population from Chester Morse, Washington, and hatchery-reared Donaldson-strain rainbow trout. The wild strain showed a more rapid return to pre-stress conditions than the hatchery-reared trout. Based on very preliminary experiments, the responses of the clotting mechanisms in Pacific salmon are similar and probably change even more rapidly than in rainbow trout. This variability in the blood coagulation rate is proposed as a mechanism to avoid disseminated intravascular coagulation (D.I.C.) in the poorly perfused muscles of fish. Experiments in this area are continuing.

Most of the bioassays regarding gas bubble disease have reported that the incipient lethal dose of supersaturation for salmonids kept in shallow water is in the vicinity of 120% saturation for exposures of more than 24 hr (Meekin and Turner, 1974). Work reported by Beyer, D'Aoust, and Smith (this conference) indicated that 6 cm fish come to equilibrium with the gas content of its environment in less than 2 hr. Based on these results, maximum lethality should occur soon after 2 hr of exposure to any supersaturation level, which however, is only the case at high level supersaturation. It therefore seems reasonable to propose that some other mechanism(s) in addition to or instead of simple bubble formation operate to cause mortality in fish during long-term exposure to low levels of supersaturation. One such factor—blood clots—will be presented in this report as a possible contributing factor in making low-level supersaturations eventually lethal.

METHODS

Rainbow trout from the University of Washington hatchery (Donaldson strain) and from Lake Chester Morse (an undisturbed watershed 30 miles east of Seattle) were used in all of the experiments. A typical trout from the hatchery was about 700 g in weight, 12 to 14 months of age, and reared on a commercial diet of Oregon Moist Pellets®. The trout from Lake Chester Morse were of similar size and weight, but of unknown age and diet. They were captured by hook and line and transported to our university laboratory in oxygenated tanks. They were kept in the lab in running lake water for at least 2 weeks before being used in experiments.

To perform an experiment, a plastic tube was inserted into the dorsal aorta and anchored in the nasal cartilages using the method of Smith and Bell (1964). MS-222 anesthetic, but no anticoagulants, was used during the surgery, and then a recovery period of 48 hr was allowed before beginning an experiment. Stress was produced by placing a hook in the caudal peduncle and producing vigorous activity by the fish for 2 min, after which the fish exhibited strong signs of fatigue. One blood sample was taken before the stress period and additional samples were taken for up to 5 hr afterwards. Blood samples were replaced with an equal volume of physiological saline, with the total blood volume removed by sampling being less than 5% of the fish's total blood volume. Blood samples used to determine clotting time were collected into non-heparinized capillary tubes, while those samples used for other analyses were collected into heparinized tubes. Analyses included determination of red and white cell counts, thrombocyte count, hematocrit, and plasma glucose concentration.

Control fish were treated exactly the same as the experimental fish except for the omission of the period of stress.

RESULTS

The decrease in blood clotting times for the stressed fish are shown in Fig. 1 and are nearly the same in both strains of fish, except that the

Casillas and Smith: University of Washington, Seattle, Washington; and D'Aoust: Virginia Mason Research Center, Seattle, Washington.

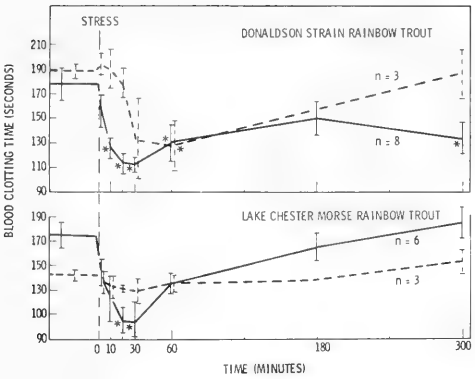

FIG. 1 Effect of stress and repeated sampling on blood clotting times in a hatchery trout (top) and a wild trout (bottom); —— stressed group; ------ control group. Each point represents the mean ± s.e. Asterisk (*) indicates significant difference from initial levels (P = 0.05).

hatchery trout maintain a hypercoagulable condition for at least 5 hr. The corresponding increase in numbers of thrombocytes is shown in Fig. 2 and 3. While the hematocrits increased (Fig. 4 and 5), neither the counts of red cells nor white cells increased, suggesting that the increased hematocrits resulted from increased cell volume. This phenomenon has been observed before (Sovio and Nyholm, 1973) when red cells were exposed to low O_2 levels.

Increases in plasma glucose have been identified as an indicator of the degree of stress in humans and also in rainbow trout (Wedemeyer, 1972). The

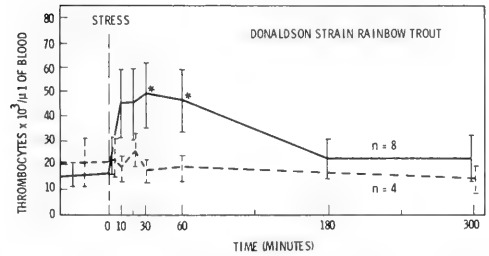

FIG. 2 Effect of stress and repeated sampling on the number of circulating thrombocytes in a hatchery trout; —— stressed group; ----- control group. Each point represents the mean ± s.e. Asterisk (*) indicates significant difference from initial levels (P = 0.05).

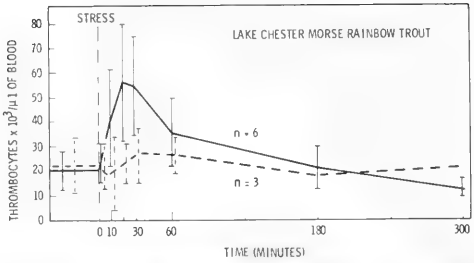

FIG. 3 Effect of stress and repeated sampling on the number of circulating thrombocytes in the wild trout; —— stressed group; ----- control group. Each point represents the mean ± s.e.

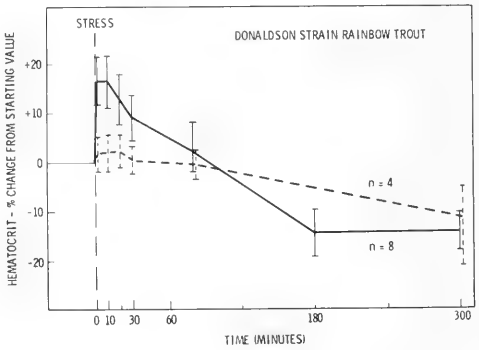

FIG. 4 Percent change of hematocrits from initial levels in response to stress for a hatchery trout; —— stressed group; ------ control group. Each point represents the mean ± s.e.

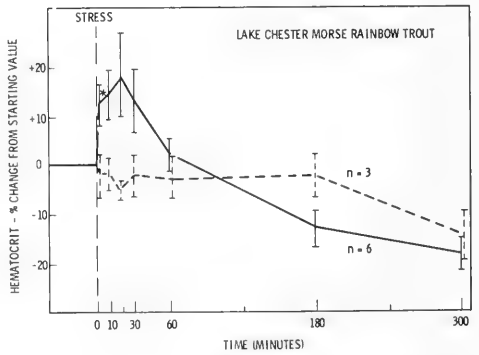

FIG. 5 Percent change of hematocrits from initial levels in response to stress for a wild trout; —— stressed group; ------ control group. Each point represents the mean ± s.e. Asterisk (*) indicates significant difference from control level (P = 0.05).

changes in plasma glucose are shown in Fig. 6 and verify that a physiologically significant stress was imposed on the fish. Another difference between the hatchery and wild fish was that the blood glucose in the wild fish returned to normal after 3 hr and stabilized there, while the blood glucose for the hatchery trout remained unstabilized for the duration of the sampling period (5 hr).

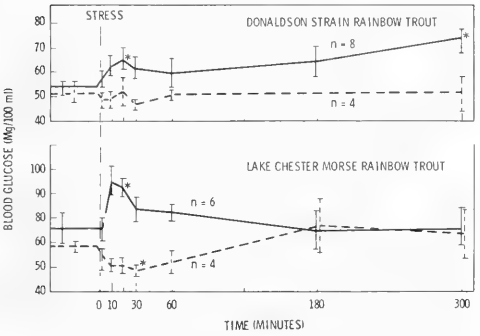

FIG. 6 Effect of stress and repeated sampling on blood glucose levels in a hatchery trout (top) and a wild trout (bottom); —— stressed group; —— control group. Each point represents the mean ±s.e. Asterisk (*) indicates significant difference from initial level (P = 0.05).

DISCUSSION

Our results clearly demonstrate the presence of increased blood clotting rates in salmonids after periods of stress. The presence of increased numbers of thrombocytes at the same time was also shown, but whether or not they cause the change in clotting function is not clear. However, their greater availability would enhance the ability of the clotting system to produce the initial "plug" of cells at a wound site to minimize any blood loss. The increases in blood glucose during the experiments showed that the degrees of stress imposed on the fish were physiologically significant. The changes in blood glucose may also indicate that hatchery fish could be less resistant to stress than wild fish.

The significance of the blood clotting system in relation to the gas bubble problems of fish is still speculative and largely extrapolated from what is known about clotting problems in people. It is highly probable that the major immediate effect of bubbles arising from external supersaturation is to block blood vessels. Depending on the importance of the tissues supplied by the vessel and the duration of the bubble, the fish may die quickly, at the one extreme, or not die at all, at the other extreme. Once a blood vessel is blocked by a bubble, it is also highly probable that a clot will form there. The likelihood of clot formation and having the clot spread further once it has begun would be greatly increased, we believe, by the presence of the clotting enhancement response induced by the shock of bubble formation. The problem caused by clotting is further aggravated by the slow rate of blood flow found in the white muscle of fish. Once a clot is formed, the severity of the resulting damage to tissue due to intravascular coagulation increases. Such clots might take a week or more to be removed. Once the blood vessel is blocked long enough, the tissue downstream from the blockage dies, with the degree of importance depending again on which tissue is involved. We believe it is significant that many of the juvenile coho which were used in our decompression studies and which survived the decompression with few or no symptoms of gas bubble disease died a week later with severe tail rot.

Finally our results suggest that the mechanisms involved in the pathology and mortality associated with formation of bubbles in fish chronically exposed to low-level supersaturation are more complex than can be simply explained by simple bubble formation. The role of sublethal changes by the fish in response to supersaturation may eventually be shown to be as important to the survival of Columbia River salmon as the lethal effects which have been the subject of most recent studies.

ACKNOWLEDGMENTS

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Changes in Blood Chemistry of Juvenile Steelhead, *Salmo gairdneri*, Following Sublethal Exposure to Nitrogen Supersaturation

T. W. Newcomb

ABSTRACT

Groups of juvenile steelhead trout (*Salmo gairdneri)* were exposed for 35 days to various (103, 105, 110, and 116% sublethal nitrogen plus argon saturations. Pooled serum samples were analyzed for Ca, Na, PO_4, K, Cl, albumin, total protein, cholesterol, alkaline phosphatase, glucose, urea, uric acid, total bilirubin, lactate dehydrogenase and serum glutamic oxalacetic transaminase. An increase in serum potassium and phosphate, and a decline in serum albumin, calcium, cholesterol, total protein and alkaline phosphatase were noted in steelhead exposed to 116% nitrogen (N_2 + Ar) saturation (total atmospheric gas saturation 110%). No major changes in blood chemistry were observed at nitrogen saturations of 110% or less.

One major water pollution problem of the Columbia and Snake Rivers is atmospheric gas supersaturation which produces gas bubble disease in fish.

Gas emboli form in the fish's supersaturated blood in some as yet undefined manner (D'Aoust,* personal communication). Gas emboli may then lodge at a variety of sites throughout the fish's body. These emboli can then limit delivery of oxygen and removal of carbon dioxide and toxic metabolites from the affected tissues and organs. Persistence of this physiological condition will lead to the ultimate death of the fish.

A number of mortality studies with nitrogen gas supersaturation have been made on juvenile salmonids, but little work has been published on the sublethal effects (Dawley and Ebel, MS). Sublethal effects from gas supersaturation may cause alterations in the fish's blood chemistry which can be used as tools to determine the degree of stress. These blood chemical measurements may help pinpoint potential long-range problems encountered by the juvenile salmonid such as ingestion and assimilation of food, predator avoidance, locomotion, homing behavior, fecundity, and the quantity and quality of subsequent fertilized eggs and fry. In addition, measuring these blood chemistry characteristics may allow in situ evaluation of nitrogen gas supersaturation stress response both singly and in combination with other normally-occurring stressors (e.g. passage through turbines) which occur to the juvenile salmonid on its downstream migration.

This blood chemistry experiment represents an extension of a bioassay by Dawley and Ebel (MS) which was designed to provide a variety of lethal and sublethal biological criteria which would help form the basis for an EPA provisional water quality standard. The primary goal was the establishment of a series of LD_{50} values. Secondary goals were changes in voluntary swimming performance and blood chemistry characteristics which might give some estimate of sublethal stress response to gas bubble disease.

MATERIALS AND METHODS

The fish used in these experiments were steelhead trout, *Salmo gairdneri*, provided by the Washington State Department of Game Hatchery at

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*B. G. D'Aoust, Research Biologist, Hyperbaric Laboratory, Virginia Mason Research Center, Seattle, Washington.

Aberdeen, WA. They weighed 20.5 ± 6.3 g and measured 132 ± 17 mm in fork length at the completion of these tests. Fish were acclimated to water temperatures of 15.0 ± 0.5°C (5°C per week with 2 weeks at 15°C) in acclimation tanks described by Ebel et al. (1971), and Dawley and Ebel (MS). Fish were fed once each day a maintenance ration of Oregon Moist Pellets®.

Fish were exposed to various nitrogen supersaturation levels in shallow rectangular tanks (Dawley and Ebel, MS) with water 23 cm deep from the same source as used in the acclimation tanks. Tests were performed at 15.0 ± 0.56°C at nitrogen gas (N_2 + Ar) saturations of 103.5, 105.4, 110.1, and 116.0 ± 2.0% as calculated from Weiss (1970). These nitrogen saturations corresponded to total gas pressure (TGP) saturations (N_2 + Ar + O_2) of 99.9, 102.0, 105.9 and 110.2%. Mean oxygen saturations ranged between 87.5 and 90.5%. Fish were continued on the same maintenance ration of Oregon Moist Pellets® during these tests.

Sixty fish were taken concurrently from the acclimation tanks and placed in each of three experimental and three control tanks (102.0 versus 99.9; 105.9 versus 99.9; and 110.2 versus 99.9% TGP). Tests at each saturation level were continued until 35 days had elapsed (the 35-day test period was chosen to parallel the average exposure time of seaward migrant steelhead passing downstream through the nitrogen supersaturated waters of the lower Snake and Columbia Rivers). At this time, each fish was given an individual voluntary swimming performance test (2 to 5 min) in an inclined vee trough against a water current of 1.28 m/sec (15.0 ± 0.5°C) (Schiewe, in press). A variety of experimental limitations dictated that these fish must be used in the swimming performance test before being used in the present blood chemistry work. Therefore, to minimize short-term blood chemistry changes due to the swimming challenge, each experimental and control group was placed in a control tank for 24 hr before blood sampling (Grant and Mehrle, 1972; Wells, 1932; Nakano and Tomlinson, 1967; and Hill and Fromm, 1968).

Fish were then individually anesthetized with neutralized MS-222 (tricaine methanesulfonate) (Wedemeyer, 1970a) in a concentration sufficient to reach plane three anesthesia (Klontz and Smith, 1964), [in about 5 min]. The fork length of each fish was measured. Blood was then collected from the severed caudal peduncle and processed after the method of Miles and Smith (1968) except that plain hematocrit tubes were used to obtain serum. These fish were then weighed and examined for signs of gas bubble disease (Dawley and Ebel, MS). Individual serum samples were pooled within each experimental or control group and immediately sealed and frozen at 0°C until chemical analysis was performed. Pooling was necessary to make up the minimum sample volume needed for the following analysis.

A Technicon® SMA 12-60 (Sequential Multiple Analyzer) was used to determine serum calcium, phosphate, glucose, urea, uric acid, cholesterol, total protein, total bilirubin, alkaline phosphatase (AP-ase), lactate dehydrogenase (LDH) and glutamic oxalacetic transaminase (SGOT). All samples were run sequentially with a reference synthetic serum (General Diagnostic's Calibrate Automated "Lock-in"), while three control synthetic sera (General Diagnostic's Calibrate) were used to calibrate the output of each of the 12 channels. Measurement error was less than 1.0% with the exception of cholesterol and LDH which were 1.2%. Serum calcium was determined by the method of Kesler and Wolfman (1964) as modified by Technicon®. Inorganic phosphate was detected by a Technicon® modification of the method of Fiske and Subbarow (1925). Glucose was detected by a modification of the procedures of Brown (1961), and Bittner and McCleary (1963). Urea nitrogen was determined by a modification of the method of Marsh et al. (1965). Uric acid is measured by a Technicon® modification of the cupric-neocuproine procedure originally described by Bittner et al. (1963). A Technicon® modification of the Lieberman-Burchard reagent was used in the direct determination of serum cholesterol. Total protein was quantitated by the Technicon® modification of the biuret reaction. The HABA anionic dye {[(2-(4' - hydroxyazobenzene) benzoic acid)]} used in the Technicon® SMA 12/60 was unusable for salmonid albumin, and a manual procedure using the bromcresol green method of Doumas et al. (1971) was substituted in these experiments. The method for the estimation of total bilirubin is based on a Technicon® modification of the procedure of Jandrassik and Grof (1938). Alkaline phosphatase was determined by a modification of the King-Armstrong procedure developed by Marsh et al. (1959). LDH was measured by a method based on the procedure of Hochella and Weinhouse (1965). SGOT was quantitated after the procedure of Morgenstern et al. (1966).

Serum sodium and potassium were measured on a Corning Model 170 digital flame photometer, while serum chloride was determined by the method of Schales and Schales (1941). Correlation coefficients were calculated for all of the above blood characteristics along with oxygen, nitrogen and TGP saturations, and mean wet weight and fork length.

Water analysis for nitrogen and oxygen were performed daily in each test tank using the methods

of Beiningen (1973) and Ebel (1969). Weekly measurements of total alkalinity (16 to 20 ppm), total hardness (10 to 21 ppm), carbon dioxide (1.2 to 2.0 ppm), chlorine + chloramine (<0.02 ppm) and pH (6.8 to 7.3) were made according to Tarus et al. (1971). Calcium (6.4 to 6.7 ppm) and potassium (0.3 to 1.0 ppm) were determined monthly by flame spectrophometer (Dawley and Ebel, MS).

RESULTS

Alteration from control mean values (>3σ) were observed in 7 of 16 blood characteristics in juvenile steelhead exposed to 116.0% nitrogen saturation, while meaningful changes in these 16 substances were not noted at lower saturations (Fig. 1). An increase in concentrations of potassium and phosphate were noted at 116.0% nitrogen saturation, while decreases in concentration were noted in albumin, calcium, cholesterol, AP-ase and total protein. Decrease in serum calcium was positively correlated with decreases in cholesterol ($r = 0.86$; $P < 0.05$) and albumin ($r = 0.95$; $P < 0.01$), and negatively correlated with an increase in potassium ($r = -0.77$; $P < 0.05$).

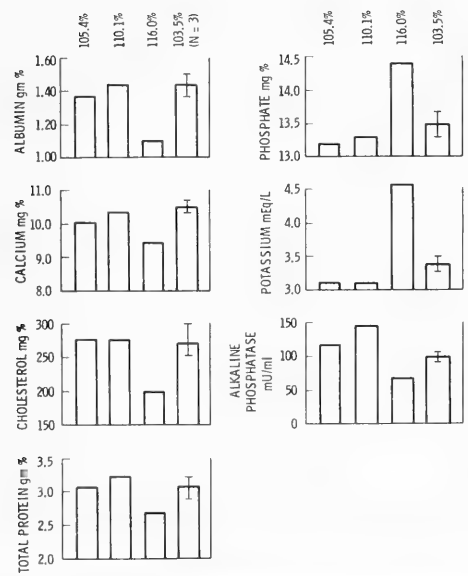

FIG. 1 Major serum chemistry parameters with increasing N_2 + Ar saturations. Control (103.5% N_2 + Ar) values are mean and range.

In addition to potassium, a decrease in albumin was negatively correlated with an increase in phosphate ($r = -0.82$; $P < 0.05$), and positively correlated with decreases in cholesterol ($r = 0.97$; $P < 0.01$) and total protein ($r = 0.90$; $P < 0.01$).

No correlation ($P < 0.5$) could be drawn between oxygen, nitrogen or TGP saturation and mean weight or length.

A 46% incidence of external gas bubble disease signs were noted in steelhead exposed to 116.0% nitrogen saturation, while no external signs were observed at lower saturations. These signs were largely in the form of lateral line bubbles with a lower incidence of blebs appearing in the dorsal and caudal fins. No mortalities occurred during these tests (Dawley and Ebel, MS).

DISCUSSION

It is important to distinguish between blood chemistry changes due to *chronic* as opposed to *acute* (short-term) stressors (Wedemeyer, 1970b). Much of the literature on salmonid blood chemistry is concerned only with acute stressors; those applied over 1 hr to 1 week (Cardwell et al., 1970; Miles and Smith, 1968; and Wedemeyer, 1970, 1971, and 1972). Because no mortality occurred over the 35-day test at 116% nitrogen (110% TGP), the author only considered chronic stressor responses in this discussion. The chronic stress noted at 110% TGP is particularly significant because 110% TGP saturation is the level presently suggested by EPA as a provisional water quality standard (Rulifson,* personal communication).

Two mechanisms have been invoked in an attempt to explain gas bubble disease. These mechanisms are gas embolism of the heart, gills or other vital portions of the blood system (Marsh and Gorham, 1905; and Dawley and Ebel, MS), and starvation due to blebs in the lining of the mouth preventing ingestion of food (Dawley and Ebel, MS). A 50% reduction in serum glucose was indicated by Robertson et al., 1962, when rainbow trout were starved. Similarly, albumin (Booke, 1964) and total protein (Phillips et al., 1960) decreased in brook trout, *Salvelinus fontinalis*.

Emboli may be envisioned to block arteries and veins in a variety of critical tissues and organs, which would result in local or systemic hypoxia, respiratory acidosis and necrosis from the buildup of toxic wastes. The gills, brain, heart and kidney are four such critical sites. Short-term hypoxia was

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shown to cause severe hemoconcentration, a doubling of serum phosphate, a 60% increase in serum glucose and an uncompensated respiratory acidosis in menhaden, *Brevoortia tyrannus* (Hall et al., 1926). Extreme hypoxia in the brown bullhead, *Ictalurus nebulosus*, and the American eel, *Anguilla rostrata*, in fresh water, resulted in severe albuminemia (Bieter, 1931). Hunn (1969) found that rainbow trout subjected to hypoxic stress showed a doubling of plasma phosphate.

Juvenile steelhead stressed for 35 days at 116.0% nitrogen saturation show no significant decrease in mean weight or length when compared to other test groups. These data would tend to support the hypoxia argument at the expense of the starvation proposition. Hypoxia should show an increase in serum cholesterol (Wedemeyer, 1970a), but the present data suggest that cholesterol in fact declines in steelhead exposed to 116.0% nitrogen saturation. While steelhead exposed to 116.0% nitrogen saturation alter their serum concentrations of phosphate (Hunn, 1969) and calcium (Wedemeyer, 1971) in a manner consistent with the hypoxia thesis, comparable data for the starvation interpretation is missing. In reciprocal fashion, decreases in albumin and total protein are known (Phillips et al., 1960) following a period of starvation, but comparable data for chronic hypoxia is needed. Steelhead serum potassium and AP-ase levels need to be determined for both chronic hypoxia and starvation.

Further work will be necessary to ascertain whether these signs reflect hypoxia and tissue necrosis, starvation or some generalized stressor response.

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Continuous Monitoring of Total Dissolved Gases, a Feasibility Study

T. F. Jenkins

ABSTRACT

A preliminary investigation was undertaken to determine if a continuous analyzer could be configured to monitor dissolved gases in natural waters. A three-component system was designed consisting of a pumping system, a continuous stripper, and a detector. Prototypes of the first two components were assembled and evaluated under field conditions. Based upon these results, it is possible to configure an unattended, near-continuous monitor to measure total dissolved gas concentration in natural waters.

The symptoms of gas bubble disease were first observed in fish by Gorham (1901) at Woods Hole, Massachusetts. These symptoms include the formation of gas bubbles in the blood vessels, behind the eyes, and in the fins. Death can be caused directly by gas blockages within the circulatory system or indirectly by blindness or infections that enter the system through associated breaks in the skin.

In a later paper, Marsh and Gorham (1905) explored the cause of this disease and associated it with supersaturated levels of dissolved gases in the aquaria waters. Gas bubbles occurring in diseased fish were found to contain primarily nitrogen. They concluded that supersaturated levels of nitrogen gas were primarily responsible for the development of the aforementioned symptoms. Subsequent research has generally substantiated their conclusions. Under certain unusual circumstances, however, an extremely high level of oxygen (Plehn, 1924; Woodbury, 1941) is also capable of producing these effects. It is the common view today that, while nitrogen is undoubtedly the major contributor to this problem, its effect will be felt only when both it and the total gas concentration* are in supersaturation.

Recently the Environmental Protection Agency has published its proposed water quality guidelines (Vol. 1, 1973). The proposed maximum acceptable value for dissolved gas pressure is 110% of existing atmospheric pressure. While this value will be discussed and may be changed, EPA's concern in this area is clear. The Army Corps of Engineers' recent water quality directive will require all districts to maintain adequate surveillance on all pertinent water quality parameters. This will undoubtedly apply to dissolved gas levels in the Pacific Northwest where potential problems have already been identified.

The three state-of-the-art methods currently used to measure dissolved gases are: the Van Slyke method, gas chromatography using a Swinnerton stripping chamber (Swinnerton, 1962), and the saturometer. The Van Slyke method is a well-known technique in which water samples are introduced, the dissolved gases liberated by a combination of chemical and physical means, and the pressure change measured with a manometer. From the combination of this pressure change and the known volume of the system, a total dissolved gas concentration can be calculated. Individual dissolved gases can also be found by the addition of chemicals which specifically remove individual components of the released gas, thereby reducing the pressure.

The gas chromatographic method involves the use of a Swinnerton stripping chamber. A known volume of water is injected into the chamber, a continuous supply of helium gas strips the dissolved gases from the water, and the stripped gases are directed onto a gas chromatographic column. The column most frequently used is molecular sieve 5A, which separates the individual components by molecular size. Each component elutes individually from the column and passes through a thermal conductivity detector whose output is recorded on a strip chart recorder. The area under each peak on the

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*Because of the equilibrium solubility of atmospheric gases, total gas concentration may be closely approximated by considering only nitrogen and oxygen.

chart is proportional to the concentration of each component in the original water sample.

The saturometer, unlike the other two methods, is designed to measure total gas pressure in situ and consists of a length of semipermeable silastic tubing which is attached to a pressure gauge. The tubing is inserted in the river and after gas equilibration across the tubing is achieved, the gas pressure is measured directly.

While the Van Slyke and gas chromatographic methods of measuring dissolved gas levels are appropriate for small-scale research studies, they are not suitable for long-term monitoring of dissolved gas levels. Water samples must be collected, returned quickly and carefully to the laboratory, and analyzed by highly trained individuals at a relatively sophisticated laboratory. Routine monitoring at a large number of locations using either of these methods would be both inconvenient and expensive.

The saturometer, however, has been designed primarily for routine monitoring. It is manually operated, relatively inexpensive and designed for use by individuals with limited training. However, investigators are somewhat skeptical about the quality of the data acquired using this device. This skepticism results primarily from the variability of the results obtained using different devices. This may be due in part to allowing an insufficient amount of time for this system to come to equilibrium. Fifteen to 30 min is commonly required, with the last small change in pressure being the most time consuming. High pressure leaks are also frequent occurrences and are difficult to detect and eliminate. If these problems are satisfactorily solved, this instrument may be the most useful of the currently available devices for routine monitoring. The three methods described above are manual techniques which do not lend themselves to unattended operation as would be required to interface with automatic data collection equipment. No device capable of unattended monitoring of dissolved gases is currently available. Should such a device become available, it would certainly be the most convenient and perhaps the most cost effective way of maintaining a large monitoring program. As a result of this need, the Seattle District, Corps of Engineers, asked CRREL to investigate the possibility of configuring a monitoring device capable of unattended operation and incorporation in a data collection network.

EXPERIMENTAL APPROACH

Basically, two different approaches can be envisioned with regard to constructing an unattended monitoring system. One could either measure total gas content of the water in situ or the gas could be removed quantitatively from the water and analyzed in the gaseous state. While the first approach would be ideal, the number of applicable methods are quite limited. Since any device used must be capable of measuring both oxygen and nitrogen the in situ dissolved oxygen electrodes in common use are not sufficient. In addition the possibility of monitoring dissolved nitrogen in a similar manner is very remote. In fact, the only current in situ approach which merits consideration for unattended operation is one which physically measures total gas pressure, like the saturometer.

On the other hand, if the gases were removed from the water matrix, many current state-of-the-art chemical detection techniques are applicable. These include many common techniques such as gas chromatography, mass spectroscopy, and optical spectroscopy, as well as various less expensive specific gas detectors. However, the use of any of these methods requires a satisfactory solution to two problems: 1) River water must be supplied on a continuous basis to an external device while sample integrity is maintained with respect to total gas concentration; 2) Dissolved gases must be quantitatively stripped from the continuously supplied water sample.

Faced with two alternatives, a limited time schedule and a small budget, a decision was required on whether to pursue the in situ or gas phase monitoring approach. While the in situ method of directly sensing total gas pressure as in the saturometer has some obvious advantages, the problems identified with manual operation of the saturometer would be even more significant for unattended use. While these problems are potentially solvable, the more novel second alternative, with a wider choice of detectors, seemed to have a higher probability of success and was thus chosen for additional study. With this in mind, the FY-74 feasibility study was directed toward developing prototype systems to solve the two problems previously mentioned. Although time did not allow for the development of a prototype detector system, it was hoped that a specific detector, which was both applicable and relatively inexpensive, could be identified for future development.

SYSTEM CONFIGURATION

Pumping System

Initial discussions with Corps personnel at Libby Dam, Montana, indicated that significant problems had been encountered in attempting to pump river water supersaturated with dissolved gases. Bubble formation within the pump lines was so intense that the water supplied became effervescent. After discussions with CRREL engineers, a

pumping system was configured based upon a centrifugal type submersible pump. The system included a control valve at the farthest downstream point where sample integrity was required. The back pressure in the lines caused by this control valve would maintain a high equilibrium gas solubility and thus reduce the tendency toward bubble formation. This valve would also serve to maintain control over the flow rate of water continuously supplied by the pump.

Stripper Unit

While attempts were being made to devise a system to remove dissolved gases quantitatively from a continuously supplied water sample, a device developed by Dr. D. Williams and R. R. Miller (1962) at the Naval Research Laboratory was discovered. This device was an adaptation of the spinning disc oxygenator used in the medical field to oxygenate blood during open heart surgery. A device configured for medical application was obtained from Mary Hitchcock Hospital, Hanover, New Hampshire, and tested. The results were so encouraging that a stripper device specific to our application was constructed based on the design parameters published by Williams and Miller (1962) (Fig. 1).

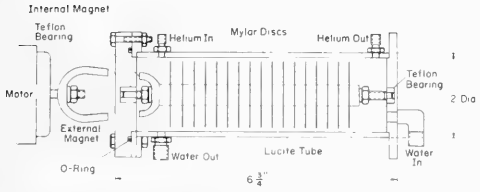

FIG. 1 Diagram of stripper unit.

The principle of operation is as follows. A continuous water sample is introduced to the device (Fig. 1). A water level approximately 1/3 of capacity is maintained in the device prior to outflow. A continuous supply of helium carrier gas circulates through the stripper. During operation, the motor, magnetically coupled to the internal shaft, rotates the internal Mylar discs at several hundred rpm. As the discs rotate through the water, a thin film is spread over the surface and carried into the helium headspace. This thin film rapidly equilibrates with the headspace, which is essentially devoid of air. Since this thin film and the air involved are continuously removed, rapid and efficient gas stripping is achieved.

RESULTS AND DISCUSSION

Following construction, preliminary testing of the stripper was conducted at CRREL. Tap water containing 10 to 12 ppm of dissolved oxygen was continuously passed through the device. Water at the outflow was collected under an inert atmosphere and analyzed for residual dissolved oxygen content by the classical Winkler method. Levels consistently less than 0.5 ppm were found. While this trace of dissolved oxygen was always present, it could have been introduced by dissolved oxygen within the reagents or explained by problems in maintaining a totally oxygen-free environment during the 10-min period required to collect the sample.

The pumping system was also configured within the laboratory and under visual inspection did not seem to produce bubbles within the pump lines. Complete checkout was impossible, however, since a large supply of water supersaturated with gases was not readily available.

Final checkout of both systems was conducted in the field at the National Marine Fisheries Service Environmental Research Laboratory at Prescott, Oregon. This facility (Fig. 2) is located on a barge in the Columbia River, 35 miles downstream from Portland, with easy access to river water having high gas levels. The field configuration included a submersible pump which was maintained about 1 ft beneath the water, the stripping chamber, and a portable gas chromatograph to be used as a detection system (Fig. 3 and 4).

While the system was in operation, water samples were withdrawn with a liquid syringe from a septum cap (Point A, Fig. 3) located on the system just upstream from the water control valve, and from the river near the location of the submersible pump. The dissolved gas content of these samples

FIG. 2. Prescott environmental field station.

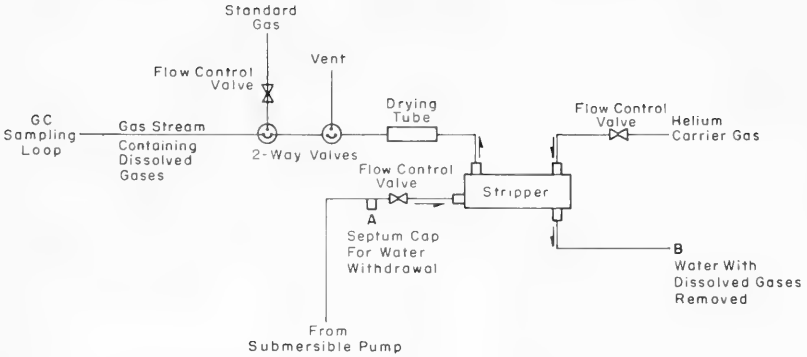

FIG. 3 Field instrumental configuration.

FIG. 4 System in operation.

was analyzed with a gas chromatograph (Hewlett Packard Model 5700A®) equipped with a Swinnerton stripping chamber (Swinnerton, Linnenbom, and Cheek, 1962). The results are shown in Fig. 5. Both the strip charts and a digital integrator on-line to the gas chromatograph indicated the samples to be identical with respect to dissolved nitrogen and oxygen. Hence the pumping system was indeed supplying water to the stripper with dissolved gas concentrations equal to those in the river itself.

The next step in the field test was to determine the stripper's efficiency in dissolved gas removal. Published information (Williams and Miller, 1962) regarding operation of the stripper unit indicated that 100% stripping efficiency was found when the flow rate ratio of helium/water was maintained between 10 and 0.5 for flow rates up to 100 cc/min. Flow rates used in these tests were approximately 8 ml/min of water and 22 ml/min of helium. When the system was operating under these conditions, samples were taken from the output of the stripper (Point B, Fig. 3) and analyzed for residual dissolved gas content with the Hewlett Packard® gas chromatograph previously described. Generally, no peaks were detectable on the strip chart. In a few cases, an extremely small peak was observed but was too small to be detected by the digital integrator. These results indicate that the stripper was quantitatively removing dissolved gas from the continuous water sample and transferring this gas to the helium carrier stream.

The final phase of the field test was to determine whether a detector could sense the amount of dissolved gases in the carrier stream and from this accurately quantitate the dissolved gas level in the river. The majority of the experimental difficulties were encountered in this phase. Since time did not permit fabrication of a detector specific for this task, a portable gas chromatograph (Analytical

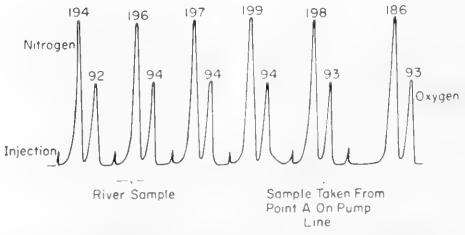

FIG. 5 Chromatogram of dissolved gases in river water and continuously pumped sample.

Instrument Development, Model 512) was used. Quantitative results were obtained using a sampling loop and a thermal conductivity detector and comparing the output to that obtained when running a calibration gas.

The major problem encountered in obtaining accurate measurements involved maintaining precise knowledge of the water and gas flow rates in the stripper unit. Specifically the value obtained from the output of the gas chromatograph must be adjusted using a factor based upon the ratio of the water and gas flow rates. If the gas and water flow rates can be maintained at an identical value, the concentration of dissolved gas found in the carrier stream is equivalent to that in the river water sample.

The difficulty encountered maintaining constant flow rates resulted from changes of resistance in the gas flow system. These changes occurred as the drying tube picked up water, resulting in a higher gas head pressure in the stripper and a lower water flow rate through the system. The second problem was encountered when the valve on the gas chromatograph sampling loop was switched, sending a gas sample into the GC column. When this was done, the resistance on the gas flow system was also changed, resulting in a different head pressure in the stripper. This caused a change in water and gas flow rates as well as a change in the water level in the stripper.

In spite of these difficulties, when the system was manually operated with extreme care, reasonably constant flow rates could be maintained. Values obtained under these conditions were compared with those found by injecting river water samples into the Swinnerton chamber of the gas chromatograph. The values obtained for dissolved nitrogen were 21.1 ppm by the stripper system and 20.7 ppm by the Swinnerton method. While we were unable to compare these methodologies over an extended period, the results are quite encouraging.

RECOMMENDATIONS

The results of this investigation confirm the feasibility of developing an unattended monitoring system for dissolved gases. The next step is to actually configure a prototype system for field validation. A diagram of a specific system, which utilizes a thermal conductivity detector for total gas measurement, is shown in Fig. 6. Prior to fabrication of this system the following areas must be investigated: 1) The detector design must be refined, a prototype assembled, and the system validated under field conditions; 2) The electronic systems required for detector operation, temperature control, flow cycling, and signal processing must be designed and configured; and 3) Systems to maintain a higher degree of control on flow rates within the stripper unit must be designed and implemented.

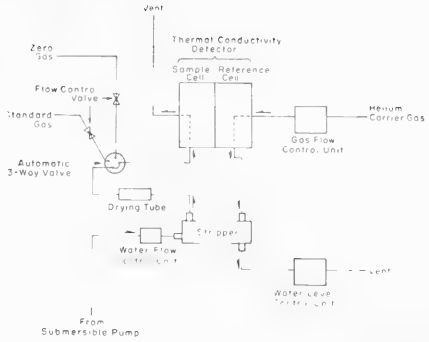

FIG. 6 Proposed monitor configuration.

Should a system be required to quantitate a single component of the gas matrix, a more specific detector would be required but the remainder of the system would be largely applicable.

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An Electronic Monitor for Total Dissolved Gas Pressure

B. G. D'Aoust
R. White
H. Seibold

ABSTRACT

The environmental and biomedical problem of supersaturation of dissolved gas and the research related to it has produced a need for a more efficient means of measuring and monitoring total dissolved gas pressure than those now in use. A modification of the Weiss saturometer is described which equilibrates within 8 min, is portable and can be operated remotely in a recording mode. The basic unit is inexpensive, easily constructed out of available components and allows many options in design so that such units can be custom-made to specific needs. It has been field-tested and is currently in use.

The environmental problem of supersaturation of rivers, lakes and streams due to naturally or man-induced excess runoff and/or temperature increases has created an urgent need for a less expensive and more efficient and reliable means of monitoring total dissolved gases then is presently available. In this paper we describe the design and function of a basic instrument which involves several modifications of the original Weiss saturometer which has been used and evaluated for the past 3 yr in the northwestern United States. It is based on the use of a watertight plastic tube across which dissolved gases exchange according to their partial pressures and the total pressure is measured manometrically (as described by Enns et al., 1965, in studies of the effect of hydrostatic pressure on dissolved gases). We have replaced the large dead-space Bourden tube gauge with a low dead-space solid state electronic pressure transducer to facilitate both portability and remote monitoring. This ability to monitor a particular location inexpensively should provide more useful and meaningful information at much less public and private expense.

PRINCIPLE OF OPERATION

Since the device measures total dissolved gas tension or partial pressure, we propose the term "tensionometer" as a more accurate description of what is actually being measured. The basic principle uses the fact that most gases and vapors diffuse rather quickly through thin layers of most plastics, one of which (silicon rubber in the form of Dow-Corning medical grade silastic® tubing, catalogue #602-105) is particularly suitable for this application since its small diameter provides optimal surface area/volume ratios across a 0.006 in. thickness. With one end of a length of tubing blinded and the other communicating with the diaphragm or sensitive element of a low dead-space pressure transducer, the pressure of all gases and vapors in the water contacting the tubing will, at equilibrium, be measured. A most important consideration in design, then, is elimination of dead-space. This involves both the choice of the appropriate pressure transducer and the design of a low dead-space connection of the tubing to the pressure transducer.

DESIGN

The unit described has been designed for minimal expense, simplicity of fabrication, and ease of operation. Its basic design also allows individual modifications according to the needs and/or preferences of the user.

Fig. 1a indicates the complete parts and materials necessary for construction of the unit. Catalogue sources and numbers are given as the part is described.

Pressure Transducer and Probe Assembly

A National Semiconductor® LX 1601 AF integrated-circuit pressure transducer which measures from 10 psia to 20 psia appears ideal for the range in dissolved gas pressure likely to be encountered in the field. However, there are many additional models and makes to choose from. The pressure transducer is shown (Fig. 1b) mounted on a 3/16 in. brass Swagelock® O-seal connector fitting (B-300-1-20R) using two O-rings (Parker 1/16 in.-008) instead of the metal ferrules which are normally used. The Swagelock fitting (Fig. 2a) is adapted to the pressure transducer nipple diameter by drilling with a #7 (0.201) drill. In addition it is advisable,

D'Aoust, White and Seibold: Virginia Mason Research Center, Seattle, Washington.

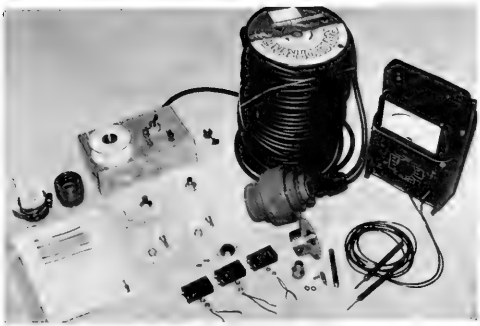

FIG. 1a Complete parts and materials to construct electronic tensionometer. VOM meter on right can be used to read output (V_O) of device. Parts and supplies are described individually in text.

FIG. 1b PVC threaded pipe union which serves as submersible probe housing shown with brass insert containing Swagelock fitting in which pressure transducer (LX 1601AF) is mounted.

FIG. 1c Unit completed with approximately 30 ft of cable. R1 and R2 (see text) are on lower two corners of aluminum box; switch (S1) is in center below readout (V_O) jacks. Hair curler spool to hold silastic is shown without perforated metal protector.

FIG. 1d Same as 1C but with digital VOM meter connected to output V_O.

though not essential, to machine a part of the Swagelock fitting insert to extend into the nipple of the pressure transducer as shown in Fig. 2a so it occupies most of the dead space of the pressure transducer nipple. The other end of the machined rod holds a 2 in. length of #25 hypodermic stock to connect to the silastic tubing. Care must be taken when constructing and assembling this part of the unit to avoid the risk of stressing or puncturing the silastic fluid-isolating membrane of the pressure transducer (Fig. 2a).

The Swagelock fitting is screwed into a brass insert (Fig. 1b and 2a) which is constructed of 1/8 in. plate 2-3/4 in. in diameter, soldered or brazed to a 1 in. length of 1-5/8 O.D. brass bar stock which has been machined to an I.D. of 1 in. to receive the Swagelock fitting.

This brass adapter fits inside a schedule 80 PVC 1-1/2 in. threaded plastic pipe union as shown in Fig. 2a and 1d. Approximately 10 ft of silastic tube is wound around a plastic hair curler (TipTop®) #5840/X, Faberge Inc., Omaha, Nebraska) and one end is tightly knotted and/or plugged with a short, smooth length of nylon fishing line. The other end of the silastic tubing is strain-relieved on the side of the hair curler by a loose knot or RTV adhesive

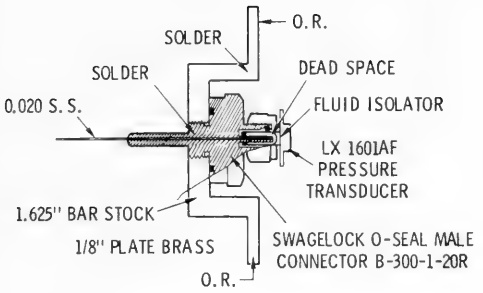

FIG. 2a Detail of mounting of pressure transducer in Swagelock fitting and brass insert. The latter is most easily constructed by first machining the bar stock to correct dimensions and then adding the 1/8 in. plate. The center adapter of the Swagelock fitting can be machined from 1/4 in. brass rod and the 0.020 s.s. capillary soldered inside it.

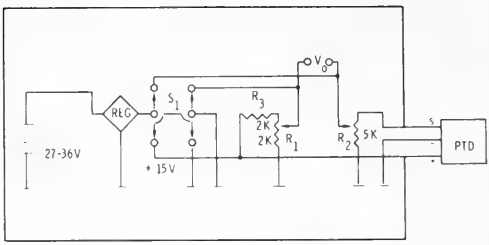

FIG. 2b Circuitry showing battery operation: Reg.; LM 340-15 National Semiconductor 15 volt regulator; S1, three position switch for on/off and battery or regulated voltage reading; R1, 2000 ohm 10 turn helipot; R2, 5000 ohm 10 turn helipot. R3, 2000 ohm (1/4 watt) resistor; P_D, pressure transducer; V_O, jacks where output or battery voltage is measured.

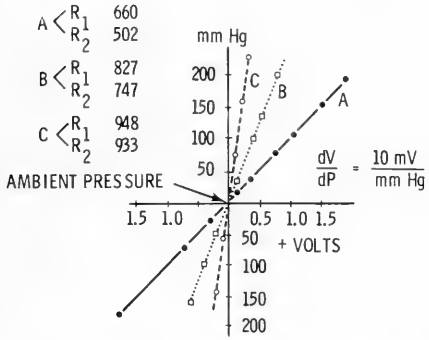

FIG. 2c Alternative calibration slopes for V_O. A, to read in mm Hg where 1 mm Hg = 10 mV; B, to read in inches of mercury where 1 in. mercury = 100 mV and inches of mercury = V_O x 10; C, to read in percent supersaturation where 10% supersaturation = 100 mV, the percentage excess of undersaturation = V_O x 100. The latter calibration "throws away" most of the pressure sensitive output and is accordingly less accurate.

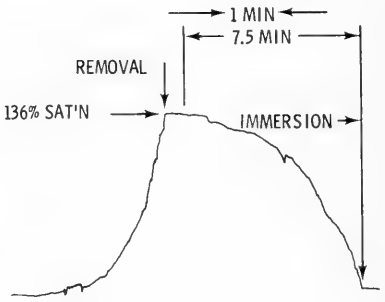

FIG. 2d Recording of approach to maximum reading taken in the N.M.F.S. Montlake Laboratory in Seattle. Final pressure measured (referred to surface) was 136% saturation reached in 7.5 min and was reproduced in three consecutive trials. Notice the more rapid return to equilibrium following removal of the probe because of the physically different (gas/membrane/gas) diffusion situations.

which does not occlude the lumen of the tube and the other end is attached to the protruding end of the stainless steel capillary. The hair curler is fastened (with a hose clamp) to the machined end of a PVC pipe plug which threads directly into the PVC union. A cylinder of perforated metal is in turn hose-clamped directly to the latter to protect the silastic tube. This construction facilitates carrying several prewound "spare membranes" in field studies so that replacement, if necessary, can be accomplished in minutes.

The terminals of the pressure transducer are connected to the waterproof three-wire neoprene-covered cable (Belden 19229, 18-3 SJO Ranchoprene®) by constructing a small five-wire clip from one side of an in-line integrated circuit IC socket. Only the ground (pin 2), supply (pin 5) and output (pin 1) terminals of the chip are used in this configuration and are connected to the cable by 5 in. of small flexible #20 hookup wire. The cable attachment to the PVC union is both waterproofed and strain-relieved by means of a plastic cord grip

connector (1/2 in. male pipe, catalogue #2672, Thomas and Betts, Elizabeth, New Jersey) which screws into the 1-1/2 in. to 1/2 in. PVC reducing bushing (Fig. 1) and firmly strain-relieves the heavy cable. Teflon tape is used in all of the threaded connections to assure a watertight seal.

ELECTRONICS

There are many options available in the power supply and the readout circuitry used. That which is described is offered only as a starting point but provides a reasonably inexpensive, efficient unit.

Fig. 2b indicates the circuitry used, all of which, save the cable and the pressure transducer, is contained in a cast aluminum box (BUD #CU-247) as shown in Figs. 1a, 1c, and 1d. The unit is powered by a DC source between 18 and 30 volts. This can be from two to four 9-volt transistor batteries or two larger 12-volt batteries. A DC power supply is desirable for laboratory calibration since it saves battery life.

The DC source shown in Fig. 2b supplies a regulator (LM 34015 National Semiconductor) which maintains a constant 15 volts. This voltage is further dropped by resistors R3 and R1 to vary the offset voltage of the transducer against which the output voltage of the pressure transducer—itself attenuated by R2—is measured. Any VOM multimeter of at least 20,000 ohms/volt will suffice to measure V_O. Ten-turn small helipots are used for R1 (2K) and R2 (5K). It is desirable to have either the regulator voltage or the battery voltage readable by means of the two position switch S1.

Calibration

Fig. 2c is a curve showing several different ways the device can be calibrated. The voltage measured can accurately indicate (by shifting a decimal) either millimeters of mercury pressure, inches of mercury pressure, or percent saturation, referred to barometric pressure (B); a reading of zero volts is equivalent to ambient pressure. Approximate helipot dial settings are given for R1 and R2 associated with the three calibration graphs assuming B = 760 mm Hg. These will vary slightly with the altitude and ambient pressures prevailing during use, and it is recommended that the instrument be thoroughly calibrated with a number of different slope and pressure settings prior to use. The pressure transducer itself is sensitive enough to act as a barometer and/or altimeter.

Maximum accuracy is obtained when dV/dP is maximal; that is, maximum voltage per unit pressure. In the experience with the unit thus far it appears most feasible to calibrate in terms of 10 mV/mm of mercury so that millimeters of mercury can be read directly by multiplying by 100. It is of course feasible to integrate into the unit a digital voltmeter for readout. However, this involves considerably greater expense not only for the DVM but also for the more sophisticated power supplies to run it. Applications oriented toward monitoring and telemetry over long time periods may justify such further sophistication.

Use of the Unit

Since the measurement of supersaturation always suffers from the uncertainty that a physically unstable situation is being measured, several notes of caution are in order for the use of these instruments. As with other units, agitation is necessary to remove bubbles from the silastic tubing to provide the pressure reading. However, when supersaturation is not present, equilibrium occurs without agitation within 8 min. Fig. 2d is a curve showing the time course of pressure buildup in the tube following immersion of the probe in approximately 30 ft of supersaturated water in the tower operated by the National Marine Fisheries Service in Seattle. The reading shown in Fig. 2d is 136% saturation and required 7.5 min to reach this value, which was reproduced on three consecutive measurements. It is clear that if the depth of water which is to be measured is at least 10 ft it should be possible to measure supersaturations as high as 33% relative to the surface without having to agitate the probe. It appears that in most field situations it would be sufficient to measure at a depth of 15 ft and, provided the assumption of vertical mixing was valid, agitation should not be necessary. This should give data of greater reliability than that afforded by using existing units in a large sample of water. Further, these units are not efficient because of the large amount of tubing necessary to counteract their large dead space. Since the tubing also acts as growth sites for bubbles, adding more of it to counteract a large dead volume is of questionable benefit. Comparison of the present unit with that commercially available on single samples of supersaturated tap water has shown that the latter can under-read by as much as 40% of the maximum value shown by the unit described here.

It is important in comparing these devices to other quantitative modes of analysis such as the Van Slyke and/or gas chromatography to keep in mind that, where supersaturation exists in the water being analyzed, tensionometers can *underestimate* the total dissolved gas *content* and quantitative methods can *overestimate* the dissolved gas *tension* because of the possible presence of microbubbles. Use of either measurement to estimate the other with solubility tables (Weiss, 1970) must be interpreted with caution. It is also emphasized that the only reliable measurements where supersaturation

is present are those taken under conditions of steady-state where the readings stabilize and are reproducible. For purposes of natural water management it appears most desirable to more continuously monitor dissolved gas tension at different locations to accurately quantify and delimit the problem.

ACKNOWLEDGMENTS

This research was supported by USPHS grants #HL 12015, #HL 14801, to the Virginia Mason Research Center, Seattle, Washington, and Career Award KO4 HL 70543 to B. G. D'Aoust.

We thank Bruce Monk of the National Marine Fisheries Service, Montlake Laboratory, for the use of the supersaturation test tower. The author's original interest in this problem was sparked by a meeting in January 1971 between Dr. Robert Rucker (1972) of the Western Fish Disease Lab and Dr. Merrill Spencer, then director of Virginia Mason Research Center.

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Round Table Discussions

Informal round table discussions were held dealing with several topic areas related to gas bubble disease. The co-chairmen of each discussion were asked to establish an agenda and lead the discussion with goals of determining the current state of knowledge and identifying research needs in the subject area. A diagram of research areas (Fig. 1) was presented as a basis for discussion.

Participants were asked to select from four topic areas: biological studies, physical and engineering studies, analytical problems, and water quality criteria. The biological studies were subdivided into groups dealing with field and laboratory oriented studies that were held concurrently as were sessions on analytical problems and physical and engineering studies.

The following summaries of each of the discussions were prepared from notes and tapes of the sessions and we hope they accurately represent a general concensus of the participants' opinions.

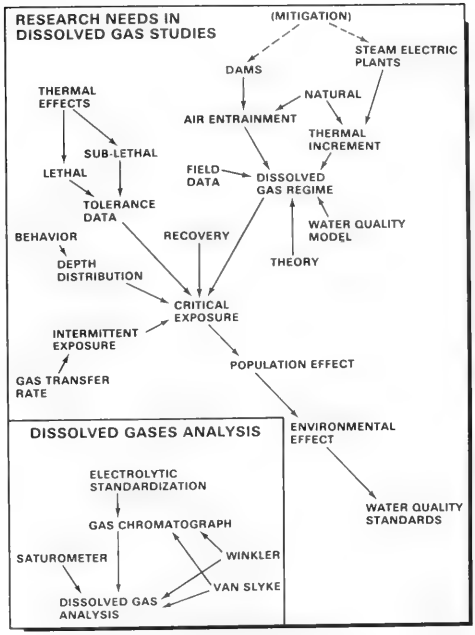

FIG. 1 Research areas in dissolved gas studies

Biological Studies: Laboratory Orientation

Co-Chairmen
A. V. Nebeker
D. H. Fickeisen

Laboratory studies of the effects of dissolved gas supersaturation on aquatic organisms have been designed primarily to determine acute tolerance using death as an endpoint.

Gas supersaturation is an immediate and urgent problem in the Columbia River system, particularly with regard to salmonid resources, requiring management decisions on a time scale which precludes long-term or basic studies of the effects of dissolved gas supersaturation on aquatic biota. We have by necessity focused on short-term experiments designed to answer questions of lethal levels of gas saturation, in most cases during acute experiments. These tests assume that once exposed to excess dissolved gas an organism becomes and remains supersaturated. However, two other contingencies exist: the gas solubility may be increased (i.e., by sounding to increase hydrostatic pressure or by decreasing the temperature) or the tissue dissolved gas content may be decreased (by re-equilibration with water having a lower dissolved gas tension). If neither of these occurs, the excess gas will leave solution and form gas-phase bubbles causing gas bubble disease.

Hydrostatic pressure increases gas phase partial pressures such that the gas saturation level of an organism decreases as it moves to a deeper location. Most river systems subject to artificial supersaturation are deep enough to permit this pressure compensation, but the behavior of fishes exposed to supersaturation is poorly understood. Factors other than gas levels (e.g., light, temperature, prey density, pressure) may cause a depth-selective response. Trauma due to gas bubble disease may be another stimulus affecting depth distribution. Diadromous species are forced to surface when passing over dams, decompressing, and perhaps producing gas bubble disease when the hydrostatic pressure decreases.

Dissolved gases move down a gradient of gas tension: the external gas tensions must be less than the internal tension at the exchange surface for a net outward flux of gas to take place.

There is some evidence that the rate of equilibration between organisms and water is rapid, with equilibration nearly complete in a matter of 1 to 2 hr. Re-equilibration is also rather rapid; however, in the Columbia River system re-equilibration during the spillway operating season is not feasible under present operating schemes as essentially the entire system is artificially supersaturated for extended periods.

During acute tolerance tests we have recognized that additional information on swimming depth distribution, behavioral responses to gas supersaturation, and effects of intermittent exposure to excess dissolved gases is needed. Long-term and basic studies dealing with sublethal and chronic effects at the population and ecosystems level rather than limited to the individual level should also be undertaken. Specific research needs identified during the discussion include:

- Sublethal and chronic effects of exposure to gas supersaturated water. Effects on fecundity, predator-prey relationships, and sensory physiology require further study.
- Combined stressor effects. More information is needed detailing effects of temperature, fish disease, and operations of hydroelectric facilities acting with gas supersaturation. It is quite possible that these and other factors act synergistically to increase the magnitude of their individual effects.
- Gas bubble formation trigger mechanisms. Initial experiments and a review of available data indicate that mechanisms triggering bubble formation are multiple and differ in acute and chronic tests. Basic physiological research is required to define trigger mechanisms, effects of internal body pressures and nucleation sites, and factors resulting in bubble size stabilization. Differences in individual gases also require attention.
- Detection of and response to gas supersaturation. Evidence regarding the ability of fishes to respond to supersaturation is conflicting. Some

Nebeker: U.S. Environmental Protection Agency, Corvallis, Oregon; and Fickeisen: Battelle-Northwest, Ecosystems Department, Richland, Washington.

estuarine species appear to avoid water containing dissolved oxygen tensions in excess of 110% of atmospheric pressure, while other species do not appear to respond to elevated gas tensions. Simple selection chambers should be employed in additional tests with several species.

In addition, the following acute tests are needed:

- Intermittent exposure. Selective spilling such that only a portion of a river system is supersaturated might alleviate the effect of gas supersaturation on diadromous fishes. About 2 yr ago the Nitrogen Task Force recommended that the feasibility of spilling "slugs" of water and the effect of the anticipated intermittent exposure be investigated. Additionally, diadromous species are subject to decreases in hydrostatic pressure when they pass over dams. Although some intermittent exposure data is being collected, additional studies are needed.
- Acute tolerance. Data are available describing the tolerance of freshwater fishes to dissolved gas supersaturation; however, tolerances of aquatic invertebrates and marine organisms to acute exposure are poorly defined at present.

The session closed with a plea for basic research aimed at determining long-term population and ecosystems effects and for some immediate cooperative solution to maintain the Columbia River system's salmonid resources.

Biological Studies: Field Orientation

Co-Chairmen
W. Ebel
R. McConnell

During the review of past research and throughout the discussion of current research, a consensus of opinion was developed concerning several points. A general summary of these points is as follows:

- Both adult and juvenile fish populations suffer substantial mortalities if exposed for a sufficient duration at levels exceeding 120% saturation even though they have the option to sound and compensate for supersaturation.
- Adult mortalities that have occurred at Bonneville Dam since 1955 have been related to gas bubble disease and, in 1968, 20,000 adult chinook were estimated lost in the vicinity of John Day Dam.
- Spawning ground surveys in the Columbia and Snake Rivers have indicated a recent decline in numbers of redds and these can be directly related to years when supersaturation was high.
- Percentage adult return of steelhead and spring and summer chinook to the Snake River have steadily declined since 1970, in spite of increased production by hatcheries.
- Mortality of juvenile populations of salmonids varies between dams and the cause of mortality changes, depending on the flow and the type of dam the fish must pass through. During high flow years the majority of the mortalities can be attributed to N_2 supersaturation. However, in low flow years such as 1973, all mortality had to be the result of delays in migration rate, predation, and passage through turbines. It was the consensus that an extremely low flow year such as 1973 created much greater mortality to juveniles than high flow years when supersaturation is present. For example, Raymond (NMFS) estimated the juvenile loss of chinook from the Salmon River in Idaho to Ice Harbor Dam was about 50% in 1971 and 70% in 1972 (both high flow years) while in 1973 (a low flow year with no supersaturation) mortality was about 88%.
- Resident fish species and invertebrate population also are affected by gas bubble disease. Eighteen species of fish in the lower Columbia River have been observed with gas bubble disease and invertebrate species diversity below Libby Dam has been reduced leaving primarily one species (*Chironomus* sp.).
- Knowledge of depth distribution of fish species is important when attempting to assess mortality that might be caused by exposure to supersaturation of N_2. When levels of 130% are recorded at surface pressures, juvenile salmon in the Snake River are still subjected to levels of at least 118%, even after accounting for the hydrostatic compensation indicated by their average depth distribution. Studies of depth distribution by Mains and Smith (1954-55) indicated that 44% of the outmigrant chinook were in the top 2.5 ft of water and 68% were near shore. Studies of depth distribution at Lower Monumental by Smith (1973) indicated 34% of the juvenile chinook and 27% of the steelhead were in the top 5 ft of water. Preliminary tests to determine depth distribution in the lower Columbia by use of a sonic fish detector indicated 58% were in the top 5 ft.

All three studies indicated more fish movement occurred during the night hours.

- Live cage studies done by Parametrix were in agreement with past live cage studies. No mortality occurred in a vertical cage even though levels rose to 125% saturation. It was pointed out that other live cage studies done earlier resulted in substantial mortalities in the volitional cage (as high as 60% in the Snake River) but levels of saturation were as high as 135 to 140%.
- Intermittent exposure of juvenile salmon increases survival over that recorded for constant exposure to high levels of supersaturation.
- Recovery of exposed juveniles with obvious symptoms of gas bubble disease does occur but nonlethal exposure of adults usually results in death from secondary infection from some other disease such as Columnaris.
- Studies to evaluate devices for reducing N_2 concentrations indicate that spillway deflectors are the best solution to date. All data obtained indicate that they reduce N_2 levels substantially from what would occur with the standard spillway and no adverse effects to either juveniles or adults passing through or in the vicinity of the devices could be detected.

Ebel: National Marine Fisheries Service, Seattle, Washington; and McConnell: National Marine Service Fisheries, Longview, Washington.

- Transportation studies indicate that survival of chinook and steelhead can be increased from 10 to 400% by collecting juveniles at the upper dam and transporting them to locations below Bonneville Dam.

The following areas were indicated by the group as areas where research should be continued or, if not already in progress, should be started.

- Transportation studies should continue.
- Survival data should be continually obtained to determine changes in survival rates that occur at various flows as spillway deflectors are installed.
- Additional horizontal and vertical distribution data are needed for both salmonid and resident species to estimate what happens to the food chain and predator-prey relations when the biomass is subjected to varying degrees of exposure to supersaturation of N_2.
- Additional data on tolerance of adult salmonids to supersaturation of N_2 are needed. Several species have not been tested and additional bioassays should be conducted.
- Observations of the Kootenai River below Libby Dam should continue to obtain data on degree of recovery of the ecosystem after N_2 levels are reduced.
- Studies to define effect of long-term sublethal exposures on survival and productivity are needed.
- Synergistic effects of other factors on tolerance to supersaturation are unknown and some investigation is needed.

A final general comment was made and generally agreed upon, and that was: "We have enough information now to proceed with correction of the problem and spillway deflectors should be installed as soon as possible. There is little need to delay installation while additional nebulous information is obtained."

Analytical Methods

Co-Chairmen
M. J. Schneider
B. G. D'Aoust

Methods of dissolved gas quantification were discussed and compared. Presently available methods report data for a single discrete sample and each has unique features making the selection dependent on intended use of the data, operator skill, and equipment cost. In addition, the two methods being developed for continuous and semi-continuous unattended monitoring were discussed as was the need for such capabilities. Each of the methods will be treated separately below.

The Weiss saturometer or tensionometer measures total dissolved gas tension directly. Dissolved gases are permitted to diffuse across a semi-permeable membrane into a closed, gas-filled space connected to a pressure sensor. The design most commonly used consists of a coil of Silastic® tubing inside a protective cage which is submerged in the water being analyzed. The tubing is connected to a pressure gauge providing total dissolved gas pressure including water vapor relative to the surface atmospheric pressure. A positive pressure, therefore, represents a supersaturated condition.

Three different formulae have been used to compute the percentage of equilibrium saturation. If the degree of total dissolved gas saturation (including water vapor pressure) relative to moist air is desired, use the formula:

$$\text{Saturation} = \frac{\text{barometric pressure} + \text{saturometer pressure}}{\text{barometric pressure}} \times 100 \quad (1)$$

For total gases excluding water vapor relative to moist air, use

$$\text{Saturation} = \frac{\text{barometric pressure} + \text{saturometer pressure} - \text{water vapor pressure}}{\text{barometric pressure}} \times 100. \quad (2)$$

And for the degree of total gas saturation excluding water vapor relative to dry air, use:

$$\text{Saturation} = \frac{\text{barometric pressure} + \text{saturometer pressure} - \text{water vapor pressure}}{\text{barometric pressure} - \text{water vapor pressure}} \times 100 \quad (3)$$

Equation (2) correlates with values normally reported in the literature for other methods; however, the appropriate formula to use depends on the intended use of the data. In any case, the barometric pressure and water temperature should be reported with data obtained with the saturometer to permit conversion between values.

Advantages of the saturometer include low cost and capability of use in the field. It is readily portable and provides data on site. It does not, however, provide data for individual gases and requires running a simultaneous Winkler titration to determine oxygen concentration so that separate gas data may be computed. Several participants were critical of the instrument due to problems arising from the relatively long equilibration time (approximately 20 min) and the need to vigorously agitate the instrument during this equilibration time. It was agreed that the operator must be familiar with potential problems including leaks and the equilibration time and must conscientiously use the saturometer.

Dr. Brian D'Aoust suggested that the concept of the saturometer has not been thoroughly explored through radical design changes including reducing the volume of the dead space in the tubing and pressure sensor. As little as 2 in. of tubing may be

Schneider: Battelle-Northwest, Ecosystems Department, Richland, Washington; and D'Aoust: Virginia Mason Research Center, Seattle, Washington.

successfully used rather than the 100 to 300 ft commonly used. Tubing dead space may be reduced by inserting stainless steel wire. Use of a pressure transducer in place of the gauge adapts the saturometer for continuous monitoring (see paper by D'Aoust, this conference). In addition, a technique for pre-pressurization of the saturometer to the expected final reading was discussed. This aids in reducing the equilibration time.

The microgasometric method of Scholander et al. (*Biol. Bull. 109:*328-334; 1955) was mentioned as being intermediate in difficulty between the saturometer and Van Slyke methods. The apparatus is portable and can be used in the field; however, it does not appear to have been widely used in gas bubble disease research and may deserve further investigation.

The Van Slyke manometric method has been widely used in conjunction with a Winkler titration to report data on oxygen and nitrogen saturation. It is generally agreed to require high-level competence to operate and is not readily portable. In addition, mercury is used in relatively large quantities presenting a health-safety problem. Participants agreed, however, that it is a highly accurate method and is useful for calibration of other methods, as well as for primary data collection.

Gas chromatography offers several advantages including rapid analysis of multiple samples. It is not portable and is relatively costly although it was reported that Carle offers a suitable chromatograph for less than $1000 (without integration). Use of electronic peak area integration greatly increases accuracy and precision. A disadvantage is the requirement for calibration with each use; however, development of a simplified electrolytic calibration is continuing and a recently developed ultrasonic detector offered by Tracor is claimed to eliminate external calibration for gas analysis.

Finally, a technique of continuous monitoring devices indicated that additional research in this area is needed and that the capability would be useful for determining the dissolved gas regime in the river.

Physics of Dissolved Gases and Engineering Solutions

Co-Chairmen
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PHYSICS OF NITROGEN SUPERSATURATION

The condition of supersaturation occurs when water falling over a spillway entrains large volumes of air as it plunges into the stilling basin. Atmospheric gases are driven into solution by the high pressure of the impacting water. Because the condition of supersaturation is a chemically unstable condition, a natural degasification process occurs which releases the excess gas in solution. The rate of gas release across the air-water interface is generally controlled by atmospheric pressure and water temperature. The degasification rate can be estimated from the relation:

$$S_r = k(C - C_s) \quad (1)$$

where C is the concentration of dissolved nitrogen (N_2), C_s the equilibrium saturation level and k is a rate coefficient. The saturation concentration is principally a function of temperature. At 1 atm pressure, the nitrogen solubility data is adequately described by the relation:

$$C_s = 23 - 0.55808\ T + 0.00763\ T^2 \quad (2)$$

where the temperature, T, is given in degrees centigrade. The rate coefficient, k, is a function of temperature, the degree of turbulence and the interfacial area over which the gas transfer occurs. One of numerous correlations for k proposed in the literature is the relation:

$$k = \sqrt{\frac{D_z U}{h^3}}\ 1.028^{(T-20)} \quad (3)$$

where D_z is the film diffusion coefficient, U is the average flow velocity and h the mean water depth.

MODELING OF GAS REGIMES

There are two distinct gas regimes which require modeling, namely the near-field and far-field regimes. The near-field program involves describing the processes of entrainment and supersaturation which occur in the stilling basin. This problem represents a considerable challenge to any modeling effort because it involves a description of a highly turbulent phenomenon. The far-field problem refers to the problem of describing motion and distribution of nitrogen-supersaturated water given the level of supersaturation at the stilling basin. This problem has been modeled with considerable success using one-dimensional transport models.

MITIGATION ACTIVITIES

Consideration of methods to reduce the levels of dissolved gas supersaturation in the Columbia-Snake River systems caused by operation of hydroelectric facilities led the Corps of Engineers to two approaches: A reduction in spill volume and modification of spillways to reduce the degree of air entrainment to stilling basin depths. Several methods of achieving these goals were discussed, including:

- Additional upstream storage reservoirs to reduce the flow during the run-off season and release the water later in the year. However, there is much opposition to construction of additional dams due to their anticipated environmental impact.
- Increase water flow through power houses, thereby reducing the volume passing over the spillway. There are two methods to do this; either by installing additional generators in skeleton bays of the Snake River dams or by passing water through the skeleton bays which have no turbines. Both approaches have been used, but present plans are to install a full complement of turbines by 1979. The use of slotted bulkheads to break the force of the water was tested and successfully aided in re-

Richardson: Corps of Engineers, Walla Walla Washington; and Baca: Battelle-Northwest, Richland, Washington.

ducing levels of supersaturation; however, fish passage through the bulkheads resulted in a high (50%) mortality rate and their use was discontinued during periods of downstream salmonid migration.

- Spillway deflectors to reduce plunging were designed and tested. These have resulted in a substantial reduction of saturation levels below the spillways, and the deflectors are planned for installation at several dams in the next few years. The deflectors are designed to break the fall of spilling water and deflect the water and entrained air across the surface of the stilling basin rather than permitting it to plunge to depths where hydrostatic pressure forces the air to dissolve into the water. A constraint on the design was to permit normal plunge basin operation during very high flow years to dissipate the energy of the spilled water and prevent structural damage to the dam. Thus, the deflectors were designed for a 1-in-10-yr flood. Tests indicated up to a 15% reduction in saturation values when the forebay was normally saturated and a 10 to 20% degasification when the forebay was supersaturated at Lower Monumental Dam. Tests of coho, chinook, and steelhead survival passing over normal spillway bays and those with deflectors indicated results ranging from no significant difference to greatly improved survival with the deflectors.

A final method of mitigation which was discussed was transportation of salmonids around the dams. In this scheme, which has been tested with good results, screening devices are installed before the turbines of an upstream dam and downstream migrants transported by truck to a location below Bonneville Dam. Initial tests are favorable with a greatly increased rate-of-return. Estimates are that 40% of the migrants could be captured at a given Snake River dam, meaning that about two million chinook and two to three million steelhead would be transported. Research on screening configuration is continuing to minimize capture damage (primarily scaling).

Water Quality Standards

Co-Chairmen
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Based on a review of 20 papers dealing with the biological effects of total dissolved gas supersaturation on aquatic organisms, a group consisting of Rob Rulifson, Ron Pine, Ed Quan, and Gene Ralston representing EPA and state regulatory agencies of Washington, Oregon and Idaho, respectively, concluded that total dissolved gas supersaturation is only one of many factors affecting the fisheries of a river system where hydroelectric projects are involved. During high flow years supersaturation is a predominant factor causing juvenile mortalities. Prototype tests with spillway deflectors indicate they contribute minimally to total losses; however, it is generally agreed that the 110% level of total dissolved gas supersaturation will probably never be reached. A level of 115% supersaturation can be tolerated by juvenile salmonids considering the compensatory effects of hydrostatic pressure and travel time in the river system which will act to minimize mortality. Therefore, the group concluded, "the total dissolved gas standard in the Columbia and Snake Rivers could be raised to 115% without causing significant mortalities."

Comments and discussion were solicited from the round table participants and the resulting discussion raised several questions concerning the appropriateness of the value 115% as a criterion to provide reasonable protection of the most desirable water use and the riverine ecosystem as well as attributes of a strong enforceable water quality standard.

There was no substantial opposition to the recommendation of 115% as a criterion for protection of juvenile salmonids from direct lethal effects, but it was suggested that the proposed value would not adequately protect shallow water resident organisms, in particular, invertebrate benthos which are important food chain organisms, and that 110% would provide reasonable protection for them. In addition, levels below 115% may result in as yet undefined sublethal effects. On the other hand, several suggested that even higher levels would protect fishes in some locations given sufficient hydrostatic pressure compensation, but detailed knowledge of depth distribution of fishes is not yet available.

Provisions suggested for inclusion in an enforceable standard included:

- Methodology to be employed in measuring dissolved gas levels. This should include computations, sampling locations, and exclusion of water vapor pressure from total gas tension calculations.
- The area or extent of permitted levels of excess dissolved gas tension should be defined, including the zone allowed for mixing of powerhouse and spillway waters.
- Deviations from a basic enforcement level might be permitted for a specified duration.
- Temperature might be a factor involved in a standard if studies of combined effects indicate a synergistic effect of supersaturation and temperature.
- A flexible system might be developed which would provide a separate enforcement level for each site based on species composition, water depth, and minimum practicable dissolved gas levels.
- Relevancy to regions beyond the Pacific Northwest should be considered carefully as the standard or criteria may become nationally accepted.

A general consensus was that a level of 115% would protect salmonid fishes migrating through the Columbia-Snake River systems but that a lower level (110%) should be adopted as a criterion for protection of shallow-water benthos. As an enforceable standard, 115% total gas saturation appeared to be agreeable for the Columbia-Snake River system as a realistically enforceable value except during particularly high flow years.

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