# EFFECTS OF VARIOUS CONCENTRATIONS OF DISSOLVED ATMOSPHERIC GAS ON JUVENILE CHINOOK SALMON AND STEELHEAD TROUT

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## ABSTRACT

Bioassays in shallow tanks (25 cm deep) with dissolved nitrogen and argon gas concentrations ranging from 100 to 125% of saturation in water at 15°C were conducted to determine lethal and sublethal effects on juvenile chinook salmon, *Oncorhynchus tshawytscha*, and steelhead trout, *Salmo gairdneri*. Significant mortality of both species commenced at 115% saturation of nitrogen and argon (111% saturation of total dissolved atmospheric gas pressure). Over 50% mortality of both steelhead and chinook occurred in less than 1.5 days in water at 120 and 125% of saturation. Significant differences in swimming performance, growth, and blood chemistry were measured in groups of fish tested at sublethal exposures in various concentrations of dissolved gases. Sublethal stress for 35 days at 110% dissolved nitrogen (106% total atmospheric gas) decreased normal swimming ability of chinook. Growth of both steelhead and chinook was affected by sublethal exposures in water saturated with atmospheric nitrogen and argon at 105, 110, and 115%. Blood chemistry was affected at sublethal exposures in water at 115% saturation.

Supersaturation of atmospheric gas (mainly nitrogen) in waters of the Columbia and Snake rivers—caused by spillway discharges from dams—has been well documented as a serious problem to valuable stocks of Pacific salmon, *Oncorhynchus* spp., and steelhead trout, *Salmo gairdneri*. Gas bubble disease resulting from this supersaturation causes both direct and indirect mortalities. Direct mortality results from air emboli in the heart and gill filaments, destruction of vital organs, or characteristic red blood cell hemolysis (Marsh and Gorham 1905; Pauley and Nakatani 1967; Bouck et al. 1970[2]). Indirect mortality is a consequence of later invasion by disease organisms (Coutant and Genoway 1968[3]) or of increased predation due to reduced performance capabilities of the fish as the result of sublethal exposure to supersaturation.

The lowest level of nitrogen supersaturation at which juvenile salmon or steelhead trout can be exposed continually with no detrimental effects is not known. Several investigators have recorded the lowest level observed during various experiments where mortalities occurred from gas bubble disease; however, very little attention has been given to determining the effect of sublethal exposure on physiological and behavioral performance. Harvey and Cooper (1962) indicated 108-110% saturation produced gas bubble disease and subsequent mortalities in sockeye salmon alevins, *O. nerka*; Rucker and Tuttle (1948) indicated a level somewhere between 110 and 115% as being the critical range for trout. Shirahata (1966) conducted the most comprehensive study to date on the effects of various levels of nitrogen gas on rainbow trout (rainbow trout is the nonanadromous form of *S. gairdneri*, whereas the steelhead trout is the anadromous) from hatching to the swim-up stage, but such detail is lacking for other species of salmonids. In many experiments on gas bubble disease, either the water temperatures, nitrogen gas concentrations, or life stages of the test fish were omitted from record, thus making the results incomplete for critical applications.

Costs involved in alleviating the supersaturation problem in the Columbia and Snake rivers will be considerable. The extent of these costs will depend on the degree of protection required to afford a safe environment for the aquatic biota. It is imperative, therefore, that regulatory measures established to govern the level of saturation be

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[2]Bouck, G. R., G. A. Chapman, P. W. Schneider, Jr., and D. G. Stevens. 1970. Observations on gas bubble disease in adult Columbia River sockeye salmon (*Oncorhynchus nerka*). Pac. Northwest Water Lab. [Fed. Water Qual. Adm., Corvallis, Oreg.], June 30, 1970. Unpubl. manuscr., 19 p.

[3]Coutant, C. C., and R. G. Genoway. 1968. Final report on an exploratory study of interaction of increased temperature and nitrogen supersaturation on mortality of adult salmonids to U.S. Bur. of Commercial Fisheries, Seattle, Washington. Battelle Mem. Inst. Pac. Northwest Lab. Richland, Wash., November 28, 1968, 28 p.

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based upon a thorough understanding of the effects of dissolved gases on aquatic organisms.

This paper describes the results of dissolved gas bioassays with juvenile steelhead trout and spring chinook salmon, *O. tshawytscha*, conducted by the National Marine Fisheries Service during the spring of 1972. These experiments were designed to assess lethal and sublethal effects of supersaturation of atmospheric gases on test fish at levels found in the Columbia and Snake rivers during the spring freshet. Atmospheric nitrogen concentrations[1] were of major concern and test levels ranged from 100 to 125% of saturation. Special note is made of testing procedures and ramifications of the effects of these on the outcome of our tests.

## METHODS

Bioassays were carried out in the laboratory in shallow tanks (25-cm water depth) to negate the effects of hydrostatic pressure compensation. These facilities were similar to those described by Ebel et al. (1971). Water flow into each test tank was maintained at 3 liters/min at a temperature of 15° ± 0.5°C. Test tanks were partitioned with perforated fiberglass plates to form four sections –in-flow area, test area A, test area B, and outflow section (Figure 1).

Supersaturated water was produced by metering 0.7 liter/min air into the suction side of a centrifugal pump which recirculated water through a 197-liter (52-gallon) closed receiver at a rate of about 190 liters/min (50 gal/min). Water pressure throughout the system was at 1.4 kg/cm$^2$ (20 psi) except in a short section of pipe on the discharge side of the pump where it was increased to 3.2 kg/cm$^2$ (45 psi) by use of a valve for additional back pressure necessary to achieve the required supersaturation. Water remained in the recirculatory system for about 10 min before passing to the test tanks. This arrangement supersaturated the water to about 145% of air saturation. Water was then piped to the test tanks where it passed over a series of perforated fiberglass plates into an inlet box with air bubbling through a bottom plate of porous polyethylene. The number of fiberglass plates and volume of air were adjusted to yield the various levels of saturation. An increase of air to water interface directly decreased the excess dissolved gas content.

Water samples for dissolved gas analyses were collected throughout the tests near the center of each test tank directly in front of the partition between A and B testing areas and in some tests at the center of each section of the tank. Frequency of analysis varied from once an hour to once a day depending on duration of test. Procedure for analysis of dissolved nitrogen was from Van Slyke and Neill (1924) using manometric blood gas apparatus; dissolved oxygen was analyzed using modified Winkler procedures

[1]Atmospheric nitrogen–nitrogen gas (98.8% by vol) plus argon gas (1.2% by vol) hereafter referred to as nitrogen or $N_2$ + Ar.

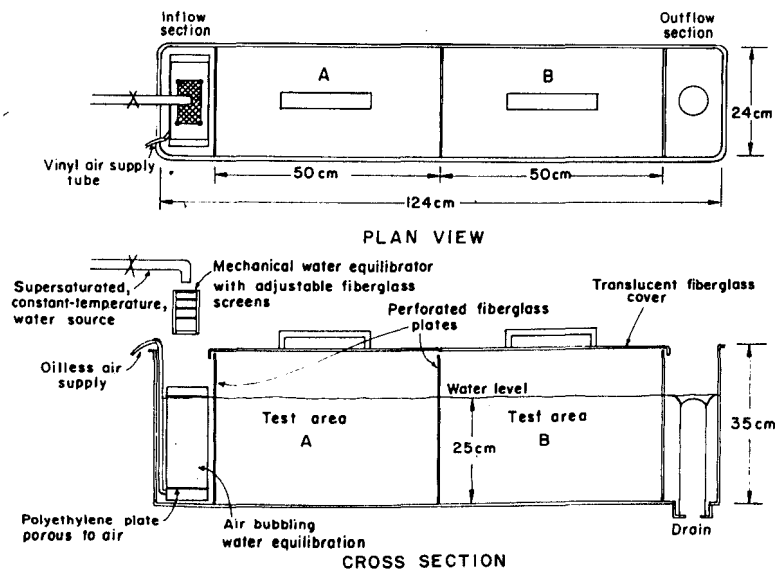


FIGURE 1.–Plan and cross-sectional views of test tank used for bioassay of dissolved gas.

(American Public Health Association et al. 1971). Gas concentrations at saturation (100%) were taken from Weiss (1970).

To obtain the dissolved gas levels for the various tests, we adjusted the water equilibrators of each tank (screens plus air bubbling boxes) until the nitrogen concentration remained within ±2% of the desired value. The oxygen concentration was then measured and we found that the saturation value was 5 to 10% lower than that of $N_2$+Ar for each tank. This did not differ appreciably from prevailing oxygen saturations in the Columbia and Snake rivers which are usually 5 to 10% lower than dissolved nitrogen values (Beiningen and Ebel 1971; Ebel 1971). After introducing fish, however, we noted that the oxygen concentration dropped further (presumably because it was consumed), resulting in values from 8 to 28% of saturation below that of $N_2$ + Ar, particularly in test area B and the outlet area of the tank. Due to large numbers of fish required for experiments on the survivors of these bioassays, and the complexity of changing the dissolved gas ratios of the water source, we did not alter the $O_2$ concentrations in the tests but carefully documented the mid-tank gas concentrations. Data affected by this drop in oxygen partial pressure are discussed later in this report.

One-year-old spring chinook salmon from Leavenworth National Fish Hatchery, Leavenworth, Wash., and steelhead trout from the Washington Department of Game Hatchery at Aberdeen, Wash., were used in the tests. Test populations were acclimated to laboratory water at 15°C with normal dissolved gas concentrations for at least 2 wk before testing. Groups of 30 or 60 fish were placed simultaneously in control (100% atmospheric nitrogen saturation) and test tanks set at 105, 110, 115, 120, and 125% of $N_2$ + Ar saturation and one to four replicates of each test were made, depending on test level. When 60 fish were being tested, 30 were in each of the two test sections A and B. Fish were randomized before introduction into individual test tanks. Mean sizes of the fish at completion of the tests are indicated in Table 1. Measurement of size at the beginning of the tests was omitted to avoid placing additional stress on the test animals. Feeding of fish during the test period began 48 h after introduction to test tanks; thereafter they were fed to satiation once each weekday.

Lethal exposure times to 10 and 50% mortality ($LE_{10}$ and $LE_{50}$) were averaged for lots of test fish held in tank sections A and B during the same time period, and the mid-tank gas concentrations were used for analysis with the exception of the steelhead groups stressed at 115% nitrogen; in these tests, exposure times and gas concentrations were measured separately for A and B sections of the tanks. In addition, lethal exposure times to 100% mortality ($LE_{100}$) for chinook and steelhead at all levels of supersaturation were taken only from groups held in the A section of the tanks.

Observations of behavior, progression of external signs of gas bubble disease, and mortality were recorded continuously for the first 6 h then every ½ h for 24 h and every 3, 6, or 12 h thereafter—depending on test concentration—until termination of the bioassay at 35 days. Observations of change in degree of external disease signs among test fish after a recovery period in normally saturated (100%) water also were made from selected groups.

Sublethal effects of supersaturation were as-

TABLE 1.—Comparison of mean weights and lengths of surviving test and control fish held in 15°C water with $N_2$ + Ar levels at 100 to 125% of saturation, February-April 1972.

| Test level (% of saturation of $N_2$ + Ar) | Testing period[1] (mo/day) | Duration (individual tests) | Test fish | | Control fish (100% $N_2$ + Ar) | |
|---|---|---|---|---|---|---|
| | | | Weight (g) | Length (mm) | Weight (g) | Length (mm) |
| | | Spring chinook salmon | | | | |
| 105 | 2/8-3/14 | 35 days | 13.6 | 115 | 15.5 | 119 |
| 110 | 3/7-4/11 | 35 days | 17.5 | 125 | 17.9 | 126 |
| 115 | 2/8-3/14 | 35 days | 13.6 | 115 | 15.5 | 119 |
| 120 | 2/8-3/3 | ≤55 h | 16.2 | 120 | 18.0 | 122 |
| 125 | 2/8-3/1 | ≤38 h | 16.8 | 117 | 16.8 | 118 |
| | | Steelhead trout | | | | |
| 105 | 4/3-5/8 | 35 days | 18.8 | 130 | 22.8 | 135 |
| 110 | 4/10-5/15 | 35 days | 20.0 | 130 | 22.0 | 132 |
| 115 | 4/13-5/13 | ≤35 days | 19.8 | 130 | 20.9 | 132 |
| 120 | 4/3-4/18 | ≤53 h | 20.6 | 124 | — | — |

[1]Replicates of tests at 115-125% levels were made at various time intervals throughout the indicated test period; others lasted the full indicated period.

sessed by using measurements of maximal swimming performance, blood chemistry, and photic response. Measurements were made on groups of survivors from lethal exposure tests immediately after the $LE_{10}$ and $LE_{50}$ points were reached or following a 2-wk recovery period in 100% saturated water. Swimming performance was measured by distance gained and time of swimming against a constant water current of 1.25 m/s within a U-shaped inclined trough (14 m long and 8 cm wide). Blood samples were analyzed on a Technicon Sequential Multiple Analyzer (SMA 12/60).[5] Pooled serum samples were analyzed for Ca, Na, $PO_4$, K, Cl, albumin, total protein, cholesterol, alkaline phosphatase, glucose, urea, uric acid, total bilirubin, lactic dehydrogenase and serum glutamic oxaloacetic-acid transaminase. Photic response was evaluated by electrophysiological monitoring of the optic tectum during retina stimulation with flickering light. A more detailed description of the methods used in the swimming performance and blood chemistry measurements appear in reports by Schiewe (1974) and by Newcomb (1974),[6] respectively.

## RESULTS

### Relationships Among Mortality, Exposure Time, and Gas Concentration

Mean exposure times at which 10, 50, and 100% mortality occurred at 120 and 125% $N_2$ + Ar saturation indicate no substantial difference between susceptibility of juvenile chinook and steelhead trout (Table 2). However, at 115% $N_2$ + Ar saturation, steelhead appeared to be more susceptible than chinook; i.e., steelhead reached the 50% mortality level within 35 days, whereas $LE_{50}$ was never reached in test groups of chinook.

Mortalities of control fish for all tests (105-125%) ranged from 0 to 3.3% throughout the 35-day test periods. Because of the comparatively minor losses of controls, data from test groups are given as observed (not compensated for loss of controls). Mortalities observed in tests at 105 and 110% of nitrogen saturation were 5% or less for both species, and gas bubble disease was not the apparent cause of death.

The onset of mortality attributable to gas supersaturation occurred at about 115% dissolved nitrogen among both steelhead and chinook.

At about 120% nitrogen saturation the means of lethal exposure times to 50% mortality ($LE_{50}$) were 26.9 and 33.3 h for chinook and steelhead, respectively. $LE_{50}$'s for chinook and steelhead at 125% nitrogen saturation were 13.6 and 14.2 h, respectively, which are similar to those (11.3 and 14.0 h) observed in earlier tests by Ebel et al. (1971) at test concentrations of 125 to 130% $N_2$+Ar. Test fish stocks used previously were from different hatcheries and earlier brood years and were slightly larger (spring chinook–23 g and 135 mm, steelhead–54 g and 179 mm).

TABLE 2.–Mean values of lethal exposure time for juvenile steelhead and chinook acclimated to 15°C and then subjected to various levels of gas saturation[1] from 100 to 125% in shallow tanks (25-cm depth).

| Percent saturation ($N_2$ + Ar) | Percent mortality | Exposure time (h) | |
|---|---|---|---|
| | | Steelhead | Chinook |
| 125 | 10 | 10.3 | 10.6 |
| | 50 | 14.2 | 13.6 |
| | 100 | [2]23.0 | [2]32.1 |
| 120 | 10 | 26.0 | 19.3 |
| | 50 | 33.3 | 26.9 |
| | 100 | [2]40.0 | [2]55.0 |
| 115 | 10 | [2]258.0 | (7% mortality in 792 h) |
| | 50 | [2]486.0 | Not reached |
| | 100 | Not reached | Not reached |
| 110<br>105<br>100 | Mortality of 5% or less recorded for either steelhead or chinook after 35 days at these concentrations. Gas bubble disease was not apparent cause of deaths. | | |

[1]Percentage saturation of nitrogen and argon was set as indicated in the table (± 2%). Oxygen concentrations ranged between 87 and 98% saturation in tanks set at 100-110% nitrogen plus argon saturation; in tanks set a 115-125% nitrogen saturation, $O_2$ levels ranged between 98 and 115%.

[2]Exposure times indicated for test replicates of section A only. Mortality in section B had not reached indicated level at termination of test.

### Effect of Oxygen Concentrations on Time to Death Measurements

The role of atmospheric gases other than nitrogen (particularly oxygen) in causing gas bubble disease has been questioned by several investigators. Arguments for and against the assumption that dissolved atmospheric nitrogen is the exclusive cause of gas bubble disease are prevalent throughout the literature (Marsh and Gorham 1905; Doudoroff 1957; Egusa 1959, 1969; Shirahata

[5]Trade names referred to in this publication do not imply endorsement of commercial products by the National Marine Fisheries Service, NOAA.

[6]Newcomb, T. W. 1974. Changes in juvenile steelhead (*Salmo gairdneri*) blood chemistry following sublethal exposure to various levels of nitrogen supersaturation. Northwest Fish. Cent., Natl. Mar. Fish. Serv., NOAA, Seattle, Wash. Unpubl. manuscr.

1966; Bouck 1972[7]; Rucker 1972). Most of the comprehensive studies, however, have been analyzed in terms of nitrogen concentration, assuming it to be the controlling influence upon the effects of gas bubble disease (even greater than indicated by the 80/20 ratio of the partial pressures $N_2$ /$O_2$ ). This assumption was based upon supposed biochemical decrease of the effective oxygen partial pressure within the fish.

In comparing data from this experiment with that from past research we should acknowledge that our primary criterion during planning and set-up stages was dissolved nitrogen + argon concentration. At the outset of these experiments, oxygen levels were monitored primarily for documentation of overall water quality rather than for use in analysis of their effect upon the test organisms. However, upon examination of initial results derived from each of the tests carried out to lethal exposures, we found that the times for $LE_{10}$ and $LE_{50}$ were consistently less in test section A than in Section B. Analyses of individual gas pressures in each of the two sections of the tanks were made to determine whether variations occurred among the component gases. We found that nitrogen concentrations were constant in both areas, but oxygen concentrations remained consistently lower (5-10%) in section B than in section A. The lower oxygen concentrations–thus lower (1-2%) total dissolved gas (TDG) saturations–appeared directly correlated with the lower mortality rates in section B of the test tanks. For example, when we examined mortality rates of individual groups of steelhead from A and B test sections at 115% $N_2$ + Ar, we found: $N_2$ + Ar saturation (in section B) of 116.0% and 88.2% of $O_2$ saturation (TDG at 110.0% of saturation) caused no mortality in 35 days for one replicate of 30 fish, whereas $N_2$ + Ar saturation (in section A) of 116.0% and 98.8% $O_2$ (TDG at 112.1%) caused 50% mortality in an average of 20 days for two replicates.

## Effect of Supersaturation Stress on Growth

Exposure to sublethal concentrations (concentrations at which no substantial mortality occurred within 35 days) of $N_2$+Ar appeared to affect growth of both juvenile chinook and steelhead. Mean weights and lengths of test fishes after 35 days in dissolved nitrogen concentrations of 105, 110, and 115% of saturation (Figure 2) were in each instance less than those of controls.

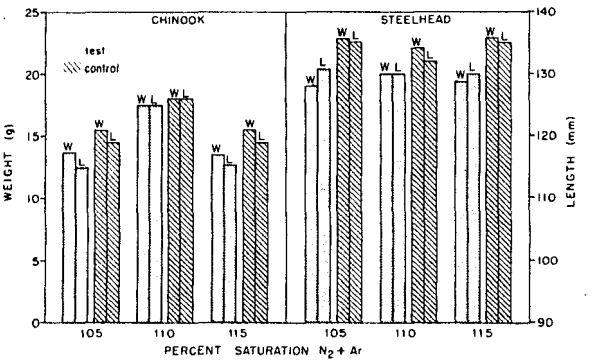


FIGURE 2.–Comparison of mean weights (W) and lengths (L) for test and control groups of juvenile chinook salmon and steelhead trout after 35 days at saturation levels of 100% (control), 105%, 110%, and 115%.

A statistical test of the hypothesis–that the slopes of the regression of mean weight of control fish groups and mean weight of test fish groups were equal–yielded a value of $t = 4.938$ ($P<0.02$ at 4 df). The same statistical test of mean lengths of control vs. test groups yielded $t = 1.36$ ($P<0.25$ at 4 df). The lower $t$ value calculated from length data is attributable to the duration of the test not being long enough to significantly overcome the variation in lengths between individuals within groups. We attribute the difference between size of test and control lots to the effect of supersaturation on the normal growth of the test fish.

After 30 days of testing at the 115% level, feeding response of the chinook fingerlings became lethargic. Many of the test fish had spinal flexures, exophthalmia, and large buccal cavity gas blisters and were unable or unwilling to move and accept food when made available. By contrast, control fish exhibited aggressive feeding behavior throughout the tests. Gross gas bubble disease signs and behavioral changes were less evident at 110% $N_2$ +Ar and nonexistant at 105%.

Testing for changes in the condition factor of juvenile fall chinook and steelhead during long-term (2-4 mo) holding in water saturated 100 to 127% $N_2$+Ar is currently underway. Results of these tests may provide further information on effects of gas supersaturation on growth rate.

[7]Bouck, G. R. 1972. Effects of gas supersaturation on salmon in the Columbia River. West. Fish. Toxicol. Stn., Environ. Prot. Agency, Corvallis, Oreg. Paper presented at Ecol. Soc. Am. Symp. Aug. 1972, 29 p.

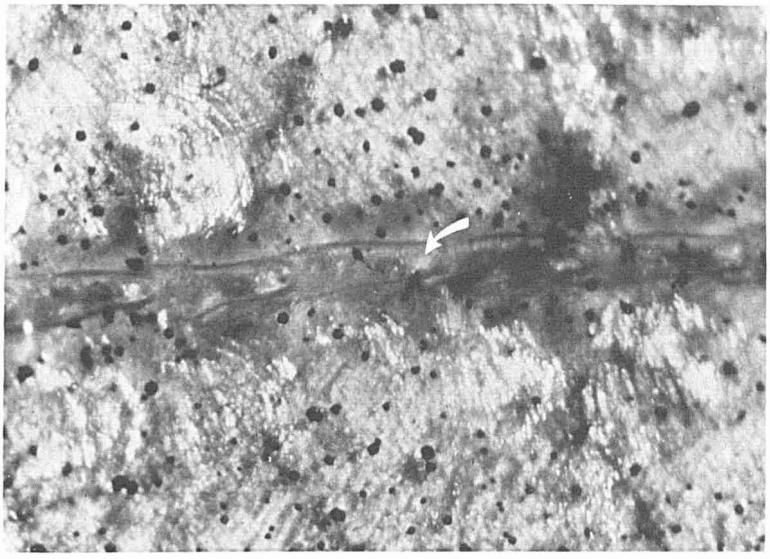

FIGURE 3.—Gas bubbles (arrow) in lateral line of juvenile chinook salmon.

## Progression of Gas Bubble Disease

Observations on the progression of external signs of gas bubble disease in spring chinook exposed to various levels of supersaturation revealed that the first developments such as bubble formation in the lateral line (Figure 3) appear within 2 h of exposure at 125%. Subcutaneous gas blisters between fin rays of at least one fin were present on each of the test animals before 11.5 h at 125%, and before 55 h at the 120% level. Several days' exposure were required before these signs occurred on fish tested at 115%. After 35 days, 56% of the fish at 110% had lateral line bubbles but only 4% had fin bubbles. Exophthalmia or "popeye," hyphema, cutaneous blisters of the head and buccal cavity, and spinal flexures were absent among fish tested at 120 and 125% but began appearing after 6 days on fish held at 115% and after 11 days on those held at 110% of nitrogen saturation. Apparently at the higher saturation levels, the fish died from cardiac occlusion or branchial artery occlusion (Figure 4) before development of these signs. By the end of 35 days, fish held at 115%

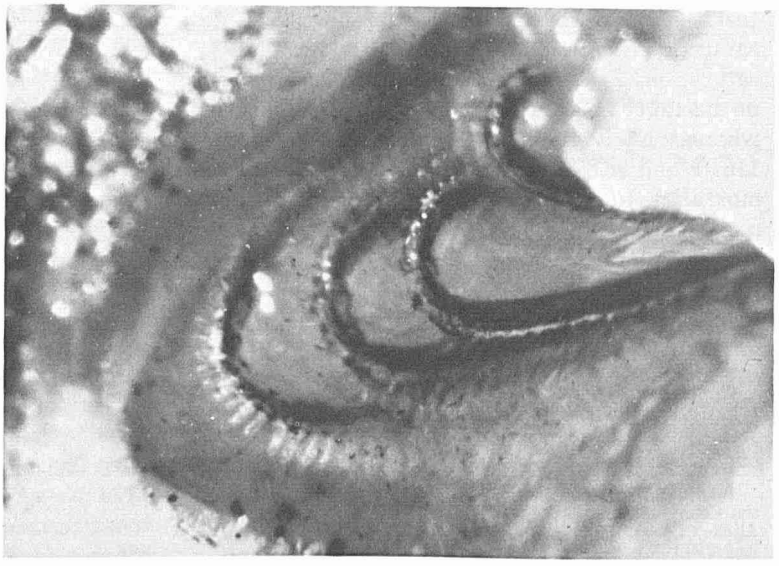

FIGURE 4.—Gas emboli occluding gill filaments and branchial artery of chinook salmon held in 125% nitrogen saturation for 20 h.

exhibited more than a 75% incidence of exophthalmia, 20% of the fish had spinal flexures, and 25% of the fish in section A became more or less immobile. After 35 days of exposure at 110% $N_2$ + Ar, only 12% of the test fish exhibited signs other than the lateral line bubbles. No apparent signs of gas bubble disease were observed in fish tested at 105% nitrogen.

Development of gas bubble disease signs in steelhead was similar to that of chinook—the signs occurred in the same sequence but the exposure time required to produce the signs was slightly less.

## Recovery From Gas Bubble Disease

Observations on disappearance of gas bubble disease signs and delayed mortality following tests were made on groups of survivors of fish stressed to the $LE_{50}$ level at 120, 125, and 130% of saturation. These survivors were placed in water at 100% gas saturation for up to 15 days. No delayed mortality could be attributed to prior exposure to supersaturation in either the chinook or the steelhead. The only significant mortality in any recovery group was a 10% loss of one replicate of steelhead subjected to 125% $N_2$+ Ar until $LE_{50}$, followed by a burst swimming performance test. Some mortality occurred after 102 h of recovery time, but the only observable disease sign was the presence of lateral line bubbles on one fish. Other mortalities during recovery were less than 3% of the fish held; no gas bubble disease signs were found. All external symptoms that were readily visible at the time the fish were removed from the recovery tanks had disappeared after 15 days in both species.

Steelhead that had undergone 16, 24, and 35 days' exposure at 115% nitrogen saturation still showed gas bubbles after being held 3 days in normally (100%) saturated water. After 1.5 days' recovery, 64% exhibited lateral line bubbles or fin ray gas blisters and one fish (7%) retained unilateral exophthalmia; after 2 days' recovery, 88% of another group retained signs of lateral line bubbles and fin gas blisters; at 3 days, 54% of the third group retained like signs of gas bubble disease. After 15 days' recovery, no gas bubble disease signs were observed on groups of test fish examined.

## Effect of Supersaturation Stress on Survivors

Burst swimming performance and blood chemistry were examined as potential indices of stress from sublethal exposures to supersaturated water.

Swimming performance (Schiewe 1974) of chinook that survived from tests at 110-125% was significantly lower than that of control fish. Visual observations of behavior during swimming performance tests indicated genuine debilitation (inability to swim in some cases) which in turn resulted in lower swimming performance (i.e. less distance gained and less swimming time against a constant water current stimulus). No difference was apparent between performance of chinook salmon tested at 105% saturation and the control fish.

Swimming performance of steelhead trout that survived tests at 105-125% was not significantly different from the performance of control fish. Performance of test and control lots of steelhead trout was highly variable. Fish stressed by exposure to supersaturation often responded in an irritated or stimulated fashion, which often resulted in a high measure of performance. Further tests with steelhead are needed to determine whether swimming performance is a useful index of stress from supersaturation and, if so, whether test results in the laboratory apply to survival of fish in the river.

Blood serum from groups of chinook and steelhead surviving supersaturation tests to $LE_{10}$ and $LE_{50}$ were analyzed (Newcomb see footnote 6) using a SMA 12/60. A 5% decrease in serum calcium was noted in chinook exposed to 115% nitrogen plus argon when compared to those exposed to lower levels of supersaturation. Steelhead exposed to 115% nitrogen showed a 10 to 17% decrease in serum calcium and a decrease in serum albumin, total protein, serum chloride, cholesterol, and in alkaline phosphatase activity when compared to controls and those exposed to lower saturations. No significant changes in blood serum components were observed in samples taken from test groups exposed to levels of 105 and 110% of saturation when these were compared with controls.

Measurements of photic response of salmonids failed to provide any consistent evidence of stress-related phenomena due to supersaturation so these tests were discontinued.

## DISCUSSION

Data from these tests indicate that the critical level of supersaturation of nitrogen where juvenile spring chinook and steelhead began to show mortality was about 115% $N_2$+Ar when $O_2$ saturations were about 95% (111% TDG). These data agree closely with the findings of Shirahata (1966), who indicated that the critical level for 2-mo-old rainbow trout was about 111.3%, $N_2$+Ar and 99.7% $O_2$ (109% TDG).

Although mortality from supersaturation did not occur until fish were exposed beyond 110% (± 2%) $N_2$ + Ar, swimming performance measurements with juvenile chinook showed some effect from stress caused by exposure to supersaturation at levels as low as 110% $N_2$ + Ar (106% TDG). We believe that one can infer from the results of these tests, that something less than normal survival will result when juvenile chinook and steelhead are exposed for 35 days or longer at or above 110% $N_2$+Ar (106% TDG).

Results of our testing program indicate that oxygen as well as nitrogen is responsible for causing gas bubble disease, even when $O_2$ concentrations are below saturation. The immediate conclusion drawn from this observation would be that total dissolved gas is the cause rather than any one or combination of component atmospheric gases. However, fish tolerance research by Egusa (1969) and by Rucker (1975) with various ratios of dissolved gas indicate that mortality from gas bubble disease is not necessarily in linear correlation with TDG. Egusa showed that oxygen saturation values of 400 to 500% were required to produce initial mortality of goldfish, *Carassius auratus*, and an eel *Anguilla japonica* when nitrogen concentrations were near 100% (TDG 160-180%). In earlier work with the same two species, however, Egusa (1959) recorded high mortality of goldfish with $N_2$+Ar at 132% and $O_2$ at 75% of saturation (TDG 123%), and of eel with $N_2$+Ar at 124%, $O_2$ at 66% (TDG 112). Rucker found that mortality rate of juvenile salmon declines considerably if the ratio of oxygen to nitrogen is increased even though the same TDG pressure is maintained.

It is apparent from our tests and those of Egusa and Rucker that the ratio of $O_2$ and $N_2$ must be considered as well as TDG when assessing possible effects from supersaturation.

Additional information is needed to quantify the effects of various gas ratios (nitrogen to oxygen) on tolerance limits of fish in general. It is probable that most fish could tolerate higher total gas pressure if the major portion of the excess gas were oxygen.

Dissolved gas measurements and resulting percentage saturations for the Columbia and Snake rivers (Ebel 1969, 1971; Beiningen and Ebel 1971) have been based on surface or atmospheric pressure plus vapor pressure. Corrections for the hydrostatic pressure (or depth) at which a sample was taken were not made. Thus, the calculations of percentage saturation were made as though the samples were collected at the surface. This is convenient when limnologists or oceanographers wish to compare values taken at various depths, but leads to confusion when attempting to assess how a given saturation measurement will affect a fish at depth.

The depth that populations of fish travel must be considered when one attempts to determine the effects of an exposure to supersaturated levels of dissolved gases. Bubble formation in the circulatory system or tissues of fish is directly dependent on the external hydrostatic pressure. For example, a fish traveling at a depth of only 1 m will be provided with enough hydrostatic pressure to compensate for a gas pressure in excess of 10% (110% saturation at surface pressures). A fish traveling at 3 m can compensate for 30%, or 130% saturation at surface pressures; a fish traveling at 10 m can compensate for an excess of 100% of saturation and so on. These tests were conducted in shallow tanks at essentially zero hydrostatic pressure with only a few centimeters depth compensation possible. The lethal exposure times we measured could only be applied directly to fish populations that could not compensate by sounding. Much more information is needed to determine how a given gas level in a river affects the population inhabiting the river. Information regarding the behavior of fish is obviously essential. We believe, however, that data from our tests support the 110% maximum allowable limit established by the Environmental Protection Agency primarily because significant mortalities did not occur until concentrations exceeded 110% TDG.

Gas bubble disease signs either singly or in combination with one another did not correlate well with mortality. Those generated from stress conditions of 120% saturation and higher seemed to be nearly the same at $LE_{10}$ as at $LE_{100}$ (gas blisters in the fins and lateral lines of most live and

dead fishes). Signs that developed at lower levels (110-115%) were obviously different from those appearing at the higher saturations; i.e., gas blisters in and around the eye, exophthalmia, cutaneous gas blisters on the head and in the mouth, and spinal flexures. Neither set of signs (low-level or high-level types) correlate by percent of incidence or severity, with accumulative mortality. But they showed that one could determine with reasonable accuracy, whether fish observed in the river had been exposed to supersaturation for a long or short duration. Populations with signs of chronic exposure (exophthalmia, spinal flexures, etc.) could have been either 1.5 to 2.0 m deep in highly supersaturated water (130-135%) or near the water surface at near 115% saturation.

## SUMMARY AND CONCLUSIONS

Bioassays in shallow tanks (25 cm) with dissolved nitrogen and argon gas concentrations ranging from 100 to 125% of saturation were conducted to determine lethal and sublethal effects on juvenile chinook salmon and steelhead trout.

Juvenile steelhead (130 mm fork length) reached the $LE_{50}$ level within 35 days when exposed to 115% of nitrogen and argon saturation (112% TDG), whereas mortality of juvenile chinook (115 mm) did not exceed 7%. There appeared to be no substantial difference between susceptability of chinook and steelhead at 120 or 125% saturation $N_2$ + Ar. No mortality related to supersaturation occurred in either juvenile chinook or steelhead trout exposed to 110 or 105% saturation $N_2$ + Ar. Signs of gas bubble disease (such as bubbles in lateral line and exophthalmia) were evident on both species, however, after 35 days exposure to 110%.

Time to death decreased in test tanks with higher oxygen concentrations (thus higher TDG) even though nitrogen and argon concentrations were identical, indicating that oxygen as well as nitrogen and argon concentrations must be considered when time to death values are compared.

The first notable sign of gas bubble disease was appearance of bubbles in the lateral line which appeared in some degree at all gas concentrations tested. Exophthalmia, dermal gas blisters of the buccal cavity and cephalic regions, and spinal flexures did not occur with short-term exposure (6 days) or at the higher levels (120 and 125%) but was prevalent after long exposure at both 115 and 110% saturation $N_2$ + Ar. External gas bubble disease signs disappeared within 15 days when fish were placed in normally saturated water (100%).

Fish stressed with supersaturation at sublethal levels for 35 days grew less than controls and the swimming performance of juvenile chinook exposed for sublethal periods to 110-125% nitrogen saturation was significantly lower than controls. Blood chemistry measurements indicated that significant differences occurred between blood samples taken from test and control chinook and steelhead after they were exposed to levels of 115% saturation. Serum calcium, for example, was 10-17% lower in samples taken from test groups of steelhead.

We concluded from these experiments that:

1. Significant mortality of both juvenile chinook and steelhead trout commences at about 115% saturation of nitrogen and argon (111% TDG).

2. Sublethal exposures to various concentrations of dissolved gas significantly affects swimming performance, growth and blood chemistry of chinook, and growth and blood chemistry of steelhead trout.

3. The first externally evident sign of gas bubble disease on juvenile chinook and steelhead trout exposed to supersaturation occurs as bubbles in pores of the lateral line.

4. Fish returned to normally (100%) saturated water appear to recover within 15 days from exposure to supersaturated water.

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