FINAL REPORT

STUDIES ON EFFECTS OF SUPERSATURATION OF DISSOLVED GASES ON FISH

Prepared

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EXECUTIVE SUMMARY

Nitrogen supersaturation studies were conducted by the staff of the National Marine Fisheries Service during the three year period; 1972-1975. Research was selected from priorities established by a sub-committee representing water use and pollution control agencies. Results of findings are summarized as follows:

Detection and Avoidance

1. Lateral movement as an indication of an avoidance response to supersaturated water was investigated with two species of fish; steelhead trout and chinook salmon. Test fish were given a choice of inhabiting high or normally saturated water; 1/2 of a shallow trough area was supersaturated and 1/2 was not and free access was allowed to both areas. Tests indicated that chinook may be able to avoid high N_2 levels whereas steelhead may not.

2. Vertical movement as an indicator of an avoidance response to supersaturation was also investigated with steelhead trout and chinook salmon. Vertical positioning of the fish was observed hourly under varied conditions of turbidity, light, restricted and unrestricted depths.

Observations made from these and other studies indicate that both chinook and steelhead juveniles usually displayed an avoidance to supersaturation by sounding. However, the avoidance behavior was not sufficient to prevent mortality. The avoidance behavior changed when turbidity was introduced; i.e., fish remained in shallower water. Depth distribution also changed from night to day even when test tanks were lighted day and night.

Intermittent Exposure

1. Tests were conducted to determine differences in survival when fish were exposed intermittently or continuously to supersaturated water. Eight species (salmonids and non-salmonids) were exposed to constant or intermittent levels of 100, 110, 120 and 130% saturation N_2. Intermittent exposure resulted in higher survival than continuous exposure to supersaturated water.

2. Variation in concentrations of dissolved gas was investigated as it related to intermittent spilling at three Snake River dams to determine whether a significant reduction in supersaturation could be achieved to create an intermittent exposure condition. Decreased spill from 150,000 to 60,000 cfs was maintained as long as possible (average 4-hours) within a 24-hour period. Dissolved gas levels declined about 4% for a short period of time (3-7 hours). Therefore, it is doubtful whether fish survival could be significantly enhanced through the manipulation of spillways at Snake River dams.

Bioassays

1. To determine the effect of depth on fish survival, test tanks were supplied with Columbia River water at ambient dissolved gas levels. Seven species were tested (salmonid and non-salmonid). Test duration ranged from 12 to 59 days, normally each test was terminated hen 50% mortality had occurred. Dissolved N_2 fluctuated from 112 to 136 percent. Survival was better among all species tested in the deep water (2.5 meter) tanks. In general, the salmonids were less tolerant than the non-salmonids.

2. Nine species of fish were also used in tests conducted in shallow tanks where concentrations of dissolved gas in Columbia River water were controlled. Test conditions were 110, 120, 130% saturation levels with appropriate controls. Fish were ranked from least tolerant (smelt) to most tolerant (crappie). Steelhead were the least tolerant of the salmonids, however, 50% mortality level of all salmonids tested occurred near 24 hours exposure at 130% saturation. The rankings of salmonid tolerance changed slightly when tests at 130% were compared with tests at 120%. The 50% mortality level was not reached with any species tested at 110% saturation N_2.

3. The effects of supersaturation on survival and predation ability of squawfish was investigated at supersaturation levels ranging from 100 to 126% of saturation total dissolved gas (TDG). Live and dead steelhead fingerlings and eulachon were introduced into test tanks to measure food consumption when squawfish were exposed to various levels of saturation. Test fish (squawfish) exhibited mortality rates similar to that of salmonids i.e., 100% mortality at 125% TDG (total dissolved gases) in 20 hours and 32% mortality in 12 days at 117% TDG. Predation ability (food consumption) decreased with increasing gas levels. Squawfish held at 117% TDG levels and above did not consume the live food ration (steelhead fingerlings).

Depth Distribution

Depth distribution of juvenile salmonids was investigated in Lower Monumental reservoir during their downstream migration period. Parallel gill nets were fished at selected locations from surface to bottom. Fifty-eight percent of the chinook and 36% of steelhead trout were captured in the upper 12 feet of the reservoir; of these, 80% were in the upper 6 feet.

Physiological Effects

1. Stamina of juvenile chinook exposed to about 130% of saturation for 16 to 20 hrs. was compared to controls held in equilibrated (100% saturation) water. This was done by comparing the time that test and control fish were able to maintain their position in a constant water velocity of 1.5 feet per second. Average swimming endurance of fish held in supersaturated water was considerably less than the swimming endurance of control fish. Results indicated that performance is detrimentally effected when fish are stressed by supersaturation.

2. Oxygen consumption was established for three species of salmonids: coho, chinook and cutthroat trout. Oxygen consumption increased initially in all cases after supersaturated water was introduced into the environment; then declined until death of the test animals occurred. The decline in O_2 consumption is probably due to either inhibited gas exchange at the gill surface or numerous emboli forming in the gill arches. Dead fish exhibited signs normally associated with suffication.

3. Blood chemistry was measured to determine changes in fish exposed to supersaturated water. Chinook and steelhead were used in five separate laboratory tests. Field sampling of smolts at four different downstream sites were also sampled to determine degree of stress within a migrating population. A limited capability to predict mortality was demonstrated from blood chemistry analysis of samples taken from steelhead stressed at 120% TDG. A number of blood chemistry parameters show altered characteristics (albumen, calcium,

phosphates and potassium) and chloride decrease was the most characteristic change observed in both species. It is apparent that more baseline information is needed to accomplish the goal of indexing stress from supersaturation by blood chemical changes.

4. The effect of gas bubbles in the lateral line from exposure to supersaturated water was measured. To assess these effects, lateral line nerves from stressed and unstressed fish were attached to an EEG which measured nerve activity associated with water displacement produced by a pulse generator. Tests show that functions of the lateral line were impaired. Impairment of this sensory mechanism could adversly affect survival of migrating fish.

Effects of Oxygen/Nitrogen Ratio

Research conducted to determine the effect of differential ratios of O_2, N_2 at a lethal total dissolved gas level (119%) indicated that survival times decreased as the ratio of dissolved nitrogen to oxygen increased.

INTRODUCTION

In 1965, the Bureau of Commercial Fisheries (now National Marine Fisheries Service, NMFS) initiated a monitoring program for dissolved nitrogen gas (N_2) in the Columbia and Snake Rivers to determine seasonal variations in concentrations (Beiningen et al., 1971). These monitoring programs have continued to the present time, being funded in recent years by the U.S. Army Corps of Engineers. Nitrogen gas levels as high as 145% were noted (Beiningen and Ebel, 1970). Ebel (1969 and 1971) published observations of the effects of nitrogen supersaturation on salmonids in general in the Columbia Basin. Some specific tests, however, have been conducted on the effect of nitrogen saturation on various individual species of juvenile salmonids (Ebel, et al., 1971; Snyder and Craddock, 1972; Blahm et al., 1972). In 1972, tests conducted by the National Marine Fisheries Service were supported cooperatively by the Corps of Engineers; these tests were limited to a restricted range of conditions. A broader base of information was needed to establish sound regulatory criteria for survival of fish. Information was needed to determine whether design or operational changes at hydroelectric dams would reduce or alleviate the deleterious effects of supersaturation of dissolved gases on the fisheries of the Snake and Columbia Rivers. Additional biological information was needed to define the behavior response and survival levels of fish that are being subjected to seasonal variation of dissolved gas concentration. For example, how deep do juvenile fish travel (vertical distribution); are they able to detect and avoid supersaturation; can

survival be enhanced if they are exposed to downstream passage? What are the conditions necessary to provide safe passage of juvenile salmonids during periods when the river is supersaturated with dissolved atmospheric gas? The answer to these questions and many others were not available.

A committee of representatives from fishery and water management agencies of the Pacific Northwest termed the Nitrogen Task Force, was established during the Governor's Conference in 1970. The Task Force subsequently established a Sub-committee to examine research needs regarding the problem of dissolved gas supersaturation.

Several pressing questions needed to be answered. Some of the most important were:

1. What is the response of fish to gas supersaturation—can fish detect high dissolved gas concentrations and if so, will they avoid it?

2. Will intermittent exposure to "low" and "high" levels of dissolved gas enhance survival?

3. What is the effect on fish survival of various levels of supersaturation in combination with other naturally occurring water quality parameters that prevail in the Columbia during the period of the major fish migrations?

4. What is the extent of the indirect effects of high dissolved gas concentration during the same period, e.g., effect on performance, predator-prey relationship, etc.?

5. How do various ratios of the dissolved gases (N_2/O_2) affect fish survival?

In 1973 the Corps of Engineers, Bonneville Power Administration, and Bureau of Reclamation provided funds for research on effects of supersaturation of dissolved gas to be done by personnel of the National Marine Fisheries Service. The studies were funded for three years and were scheduled to terminate on June 30, 1975. The research was conducted within two divisions of the Northwest Fisheries Center: Coastal Zone and Estuarine, at the Seattle Laboratory and Environmental Conservation, at the Prescott Field Station (Figure 1). The objective of this report is to summarize the results of the three year study. Separate reports of many of the individual experiments completed during the study period have been published; these are also summarized.

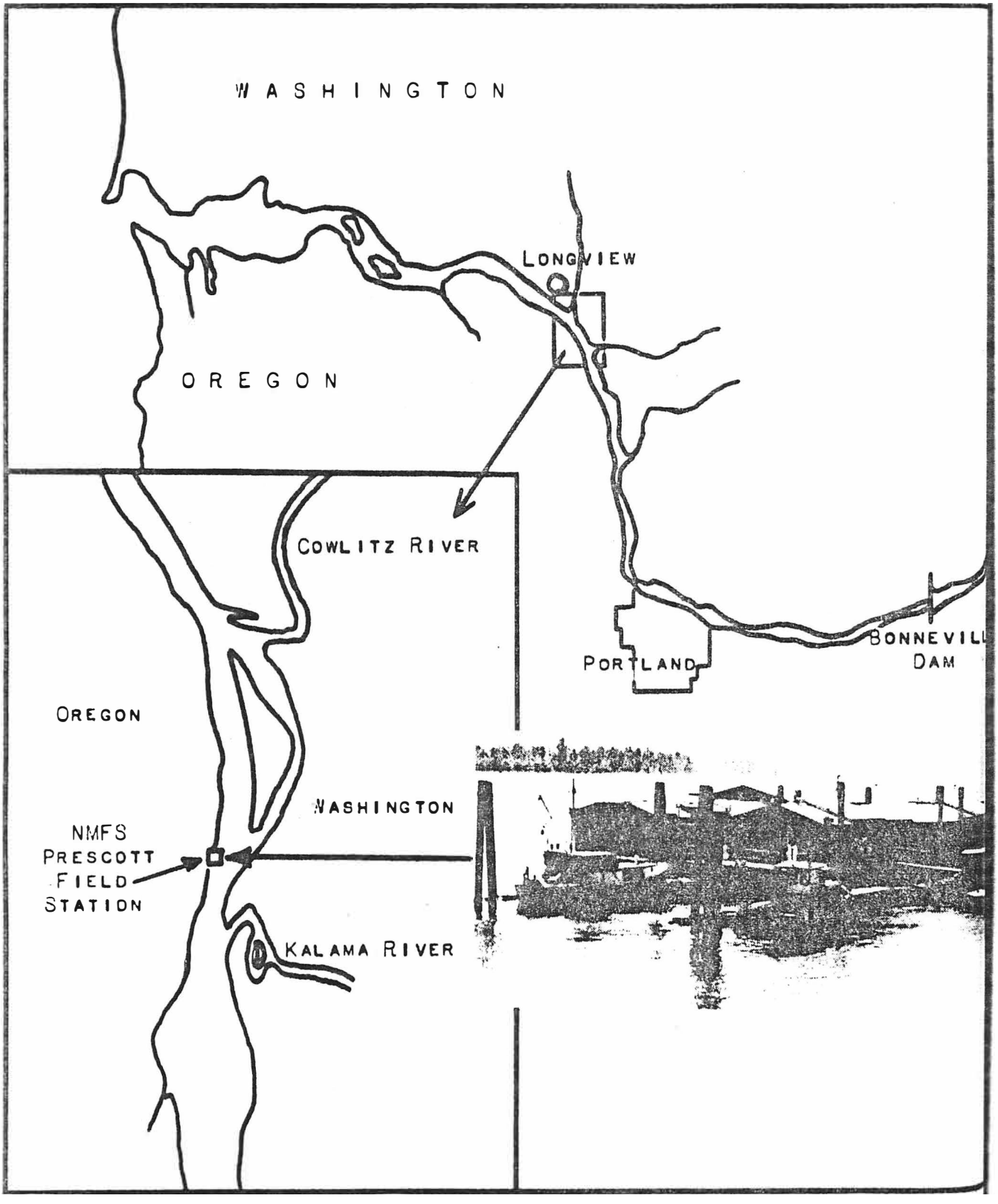

Figure 1.--Location of study area and National Marine Fisheries Service facility on the Columbia River near Prescott, Oregon.

DETECTION AND AVOIDANCE

Lateral Movement

These tests were designed to determine if juvenile salmonids would respond laterally to a choice of "high" and equilibrated nigrogen levels. Information of this nature would indicate if fish could detect, and attempt to avoid, areas in the river where gas supersaturation prevailed.

Two homogeneous groups of juvenile salmonids were introduced into a test tank (Figure 2) providing them a choice of channels carrying either supersaturated water (130%) with N_2 or with normal water (100%) saturation N_2. Water depth in the channels was maintained at .33 m to minimize the affects of hydrostatic pressure. Test duration was 192 hours or until 50% mortality occurred. Replicate tests were conducted, using fish from the same population, by switching the "high" N_2 and "low" N_2 channels. A twenty fish sample of either steelhead trout (Salmo garidneri) or chinook (Onchorhynchus tshawytscha) was used for each test.

During the first test with steelhead, (avg. 137 mm) 50% died in 42.5 hours; in the replicate test a 50% mortality was reached in 43 hours. The survivors from both tests had external gas bubble disease symptoms.

No mortalities occurred in the test (two replicates) when chinook (avg. 110 mm) were used as the test animals and only 10% showed external N_2 symptoms. Lateral movement of either the steelhead or chinook was not visually observed due to high turbidity, however, by examining survival and incidence of gas bubble disease it follows directly that the chinook were avoiding supersaturation. Time to 50%

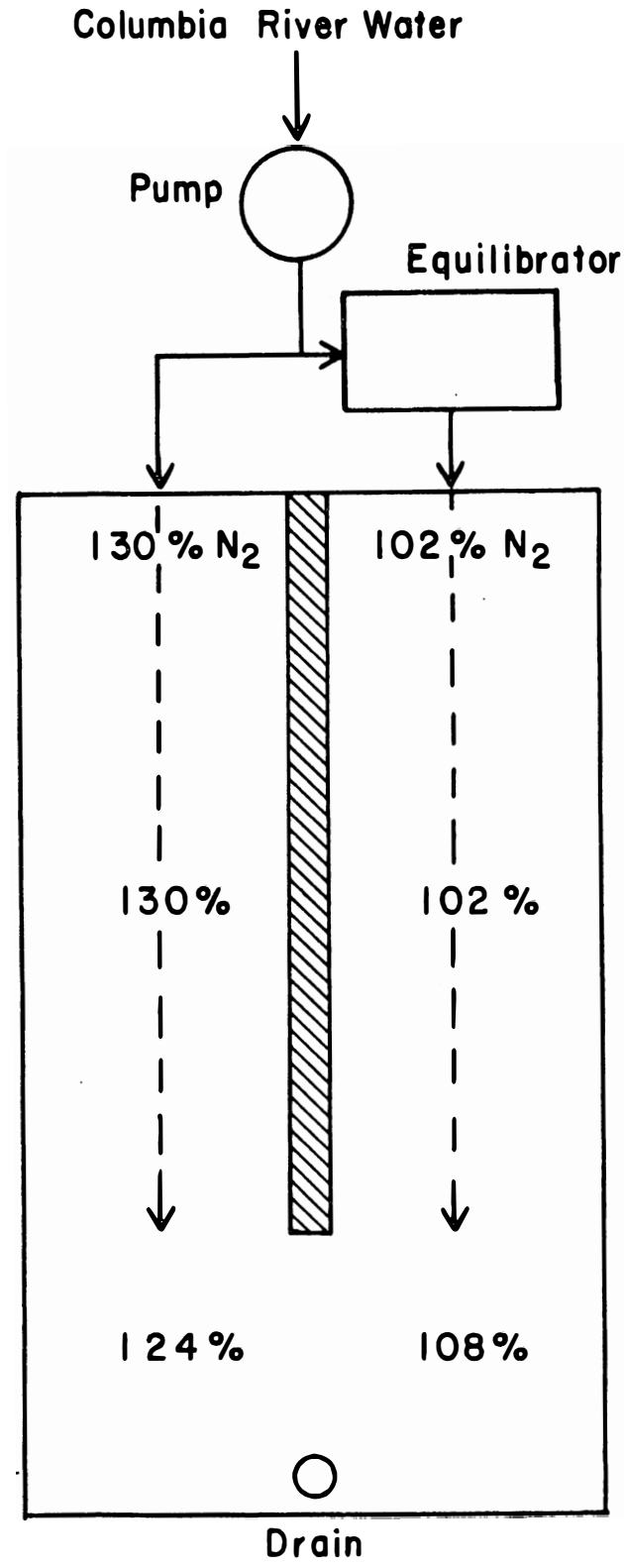

Figure 2.--Plan view of 1.5 x 3.3 m tank used to determine if juvenile fish can detect and avoid N_2 supersaturated water. Homogenous groups of fish are placed in the test tank which affords them a lateral choice of two channels; one containing dissolved N_2 at 130% saturation and the other at 102%.

mortality for juvenile chinook held in 130% water (as observed from bioassays) is about 65 hr, whereas these detection and avoidance tests lasted 192 hr.

Test results indicate that the juvenile steelhead did not avoid the high gas concentration while fall chinook salmon did. Results from other exposure tests shows juvenile steelhead to be less tolerant to concentrations of dissolved nitrogen than chinook. These tests indicate the possibility of species variation in lateral response to nitrogen; however, additional supporting tests should be done to confirm this conclusion.

Vertical Movement

In previous laboratory experiments at the N.W. Fisheries Center, we found that supersaturation of dissolved gases in water affected the depth distribution of salmon and trout. Two experiments had been conducted with deep tanks 1.2 m wide x 2.5 m deep, where 10 groups of fish were exposed to various levels of saturation ranging from 100 to 127% TDG. One experiment tested fall chinook salmon fry, the other, steelhead trout smolts. In each experiment the mean depth of the test groups was progressively deeper when correlated to increased supersaturation as shown in Figures 3 and 4 (Dawley, et al., processed report). Fish held at 120, 124, and 127% levels, however, did not reside deep enough to avoid mortality from gas bubble disease; in fact 100% mortality occurred in the chinook groups at 124 and 127%.

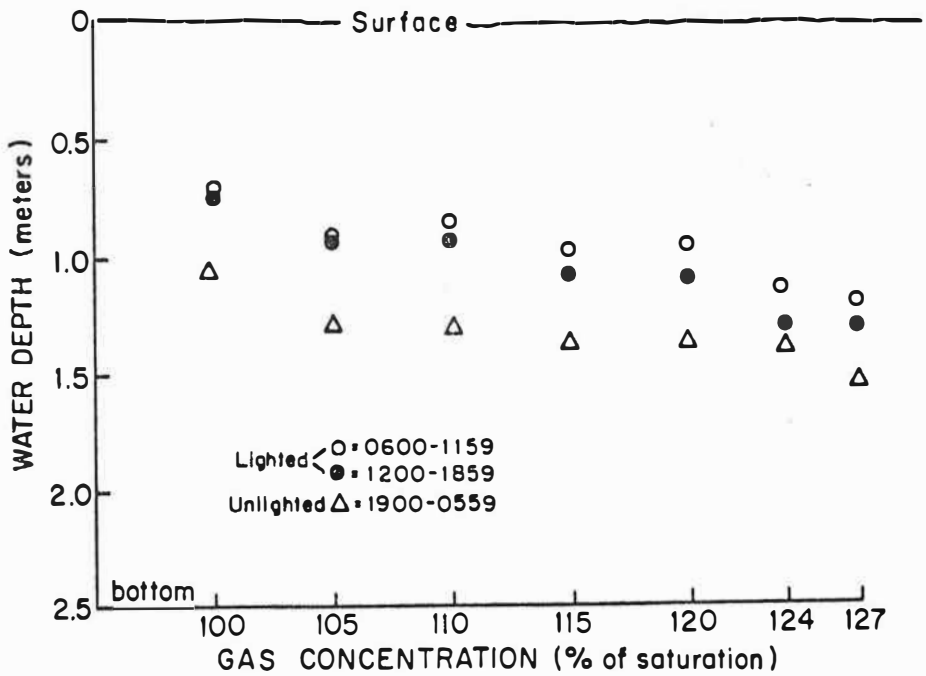

Fig. 3 --Mean depths of groups of juvenile chinook salmon in 2.5 m deep tanks at dissolved gas concentrations of 100, 105, 110, 120, 124, and 127% of saturation--averaged for 3 periods of the day over 30 days.

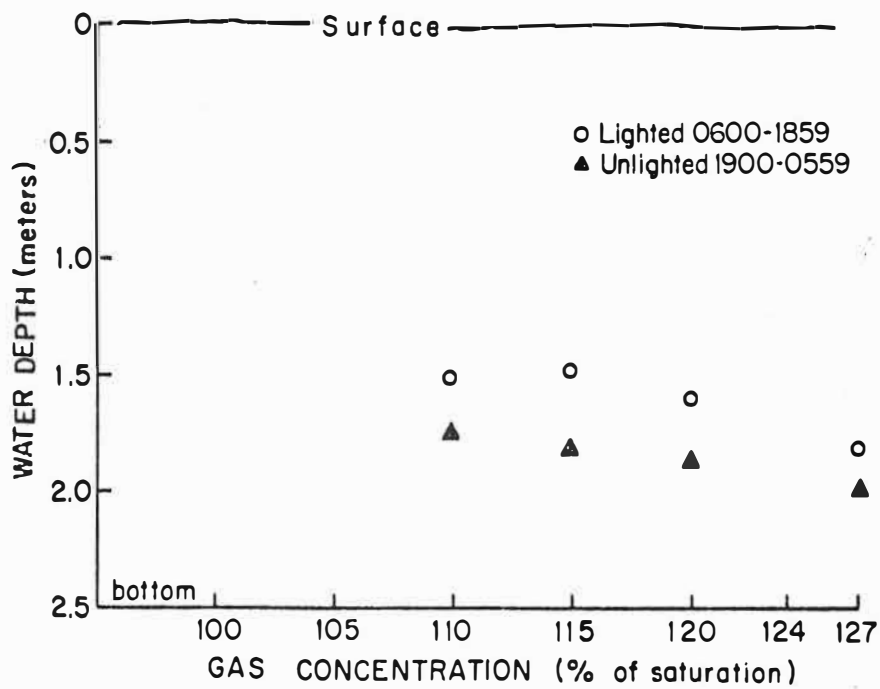

Fig. 4--Mean depth of groups of juvenile steelhead trout in 2.5 m deep tanks at dissolved gas concentrations of 110, 115, 120, and 127% of saturation--averaged for 2 periods of the day over 15 days.

We believe that the high density of fish to water and the limited depth of water may have affected this data. Therefore, we conducted an additional series of experiments to clarify the effects of supersaturation on depth distribution of salmon and trout.

Tests were conducted with spring and fall chinook salmon and steelhead trout in a large tank (3 m wide by 10 m deep) to minimize the affect of crowding and allow a more natural depth range.

Tests with steelhead trout

Two groups of 30 juvenile steelhead (205 mm avg.) were introduced into normally saturated water at 10°C for a control period of 7 days; the water was then supersaturated to 130% (TDG) and maintained at that level for 7 days. Visual observations of the depth distribution of the test group and movements of 10 individually marked fish (spaghetti tags) were made every hour throughout the test. Light intensities were controlled to simulate natural conditions. Fish were examined for signs of gas bubble disease at the termination of each test. One test was conducted using turbid water (bentonite was suspended in the water to decrease visibility from more than 10 m to about 1 m). During this test depth distributions could not be ascertained. Gas bubble disease signs and related mortality were recorded for comparison with data obtained from fish tested in similar conditions with no turbidity.

Examination of the behavior of the 10 marked fish during the first test with clear water showed a wide variation in individual behavior. For example, one individual changed depth frequently before

the water was supersaturated; after supersaturation, this individual remained at a fixed depth for several hours and varied little from that depth thereafter. A second individual showed no perceptible change in behavior following supersaturation. The mean depth of the entire population during the period of supersaturation was 3.8 m. At this depth the dissolved gas concentration was about 95% of saturation. Vertical movement of most individual fish was nearly continuous during the day; slight movement was noted during the night. Supersaturation did not seem to affect this movement. About 6% of the fish population (2 of 30) was observed in the upper 3 feet of the tank during the test, where gas saturation was 120% (TDG) or greater; however, since vertical movement of the population was continuous, no fish were subjected to high saturation for any extended duration. As a result, mortality did not occur, although 27% of the fish showed signs of gas bubble disease at termination of the test.

Detection of supersaturation and avoidance by sounding was not oberved in this test, mainly because of the continuous vertical migration of these fish.

Mortality (3%) did occur in the second test which was conducted with turbid water. Gas bubble disease signs were prevalent on 48% of the fish after receiving the same exposure to supersaturation as those from clear water tests (7 days). This test indicates that steelhead maintained a more shallow depth distribution in turbid water than in clear water thus were exposed to higher gas supersaturation.

Test with fall chinook salmon

In a test of juvenile fall chinook salmon (161 mm avg.) a false bottom was placed in the tank at 3.5 meters to confine the chinook to a smaller area. Depth changes made by individual fish could be detected with less difficulty and the area, in the tank, below the zone of lethal supersaturation was considerably less. Hourly observations of vertical distribution were made for 7 days in equilibrated water, then water in the tank was supersaturated to 130% TDG (133 N_2 and 117% O_2) and subsequent observations were made for 4 days.

After supersaturation of the test water the majority of the population moved downward. By the 3rd day all fish remained deeper than two meters (depth compensated saturation level of 110% or less) and the majority had moved to a depth (3-3.5 m) greater than had previously been occupied before the water was supersaturated (Figure 5). Fifty percent mortality occurred in 50 hours in spite of the downward shift of the population. No further mortality occurred after 57 hours and the resulting loss after 4 days totaled 53%. All survivors from the test showed signs of gas bubble disease.

Ten of the 30 chinook tested were marked with various colored spaghetti tags for visual identification. The average depth maintained by an individual, before exposure to supersaturation seemed to determine if the individual survived exposure. Fish that did not survive tended to stay near the surface (upper 1.5 meters) and either did not increase their depth, or did not increase it sufficiently to compensate for pressures from supersaturated gases. Vertical positions of two fish that died from gas bubble disease during a 24 hour

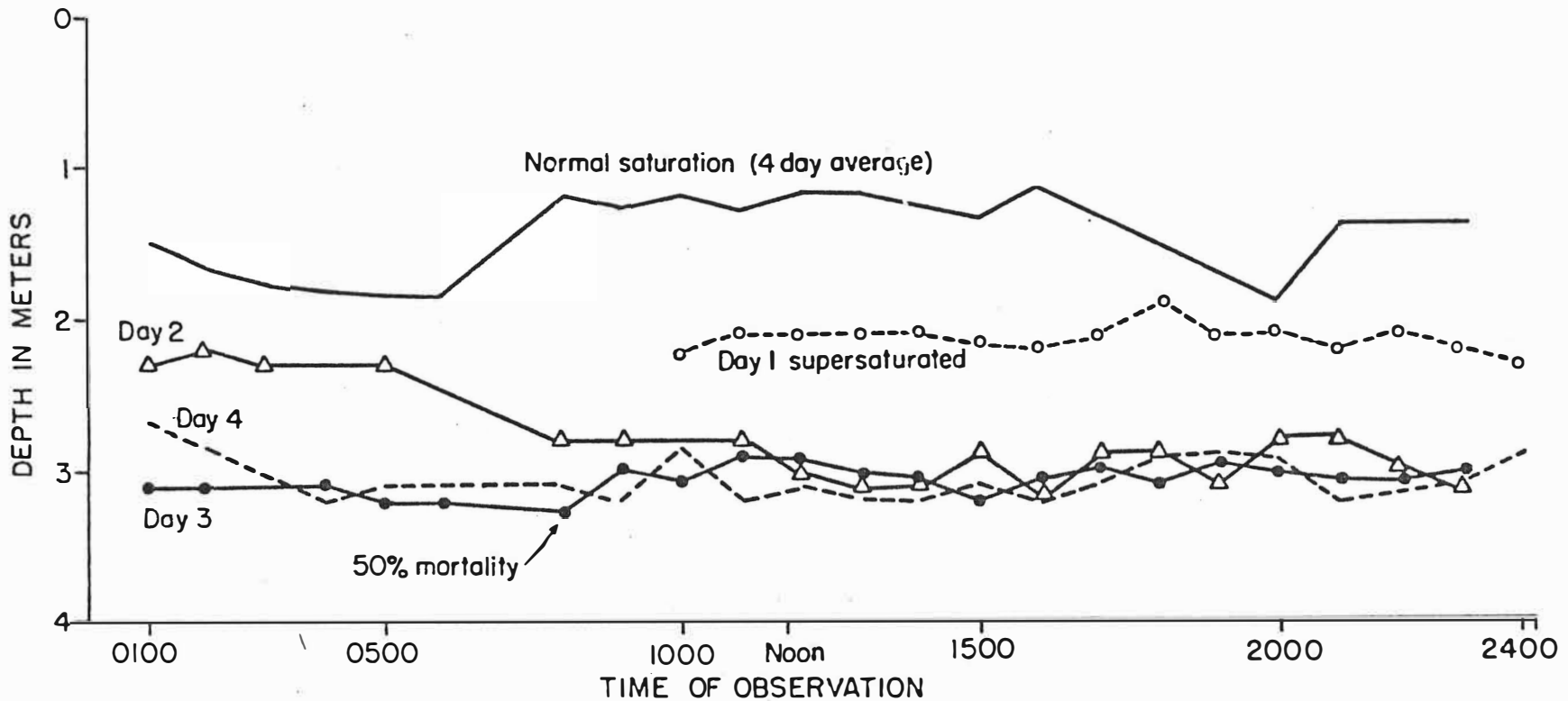

Figure 5 -- Hourly mean depths (meters) of juvenile fall chinook tested during normal gas concentrations (100% TDG) and supersaturated conditions (130% TDG). Depths at 100% TDG are an average of 4 days.

period are shown in Figure 6. The average depth of two marked fish that survived (Fish C&D in Figure 7) ranged from 1.5 to 2.5 meters in equilibrated water and from 2.5 to 3.35 meters in supersaturated water.

Territorial interaction between the test fish seemed to impede vertical movement. However, the change in depths of nearly all fish indicated that detection of high dissolved gas concentration was made and an avoidance reaction was exhibited. The reaction (sounding) was not always great enough to allow survival of the fish as was observed in earlier tests.

Test with spring chinook salmon

A group of 50 spring chinook (121 mm avg.) was similarily tested using the entire depth of the 9 m tank. However, to decrease the effects of diurnal movement the lights were left on (at an intensity equal to daylight) for 24 hours a day.

After 37 days of testing, in water averaging 133% N_2, there was one mortality (on the 5th day); it had no apparent signs of gas bubble disease. Mean depth maintained by the population was 3.6 meters (with a range of 2.4 to 5.1 m) the hydrostatic pressure at this depth compensated for approximately 35% saturation. About 10% of the population had gas blisters on the fins from day eight through the end of testing. Diurnal depth changes did not seem to be inhibited by the artificial lighting. The depth range of distribution was greater than in the fall chinook test (with a false bottom at 3.5 m); however, the same diurnal movement pattern was observed.

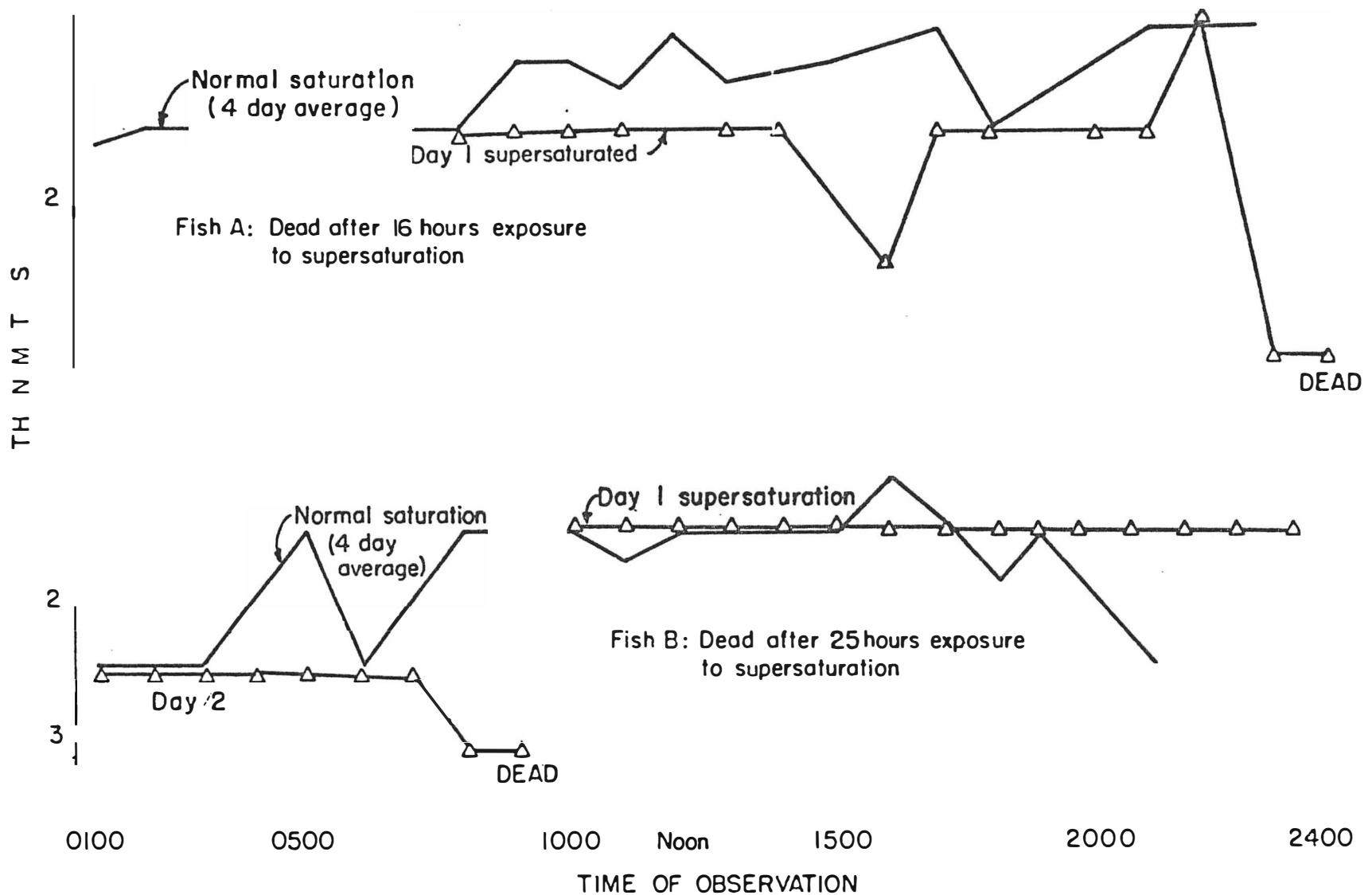

Figure 6 -- Hourly depth for two individual juvenile fall chinook tested at normal gas concentrations (100% TDG) and supersaturated conditions (130% TDG). Depths at 100% TDG are an average of 4 days.

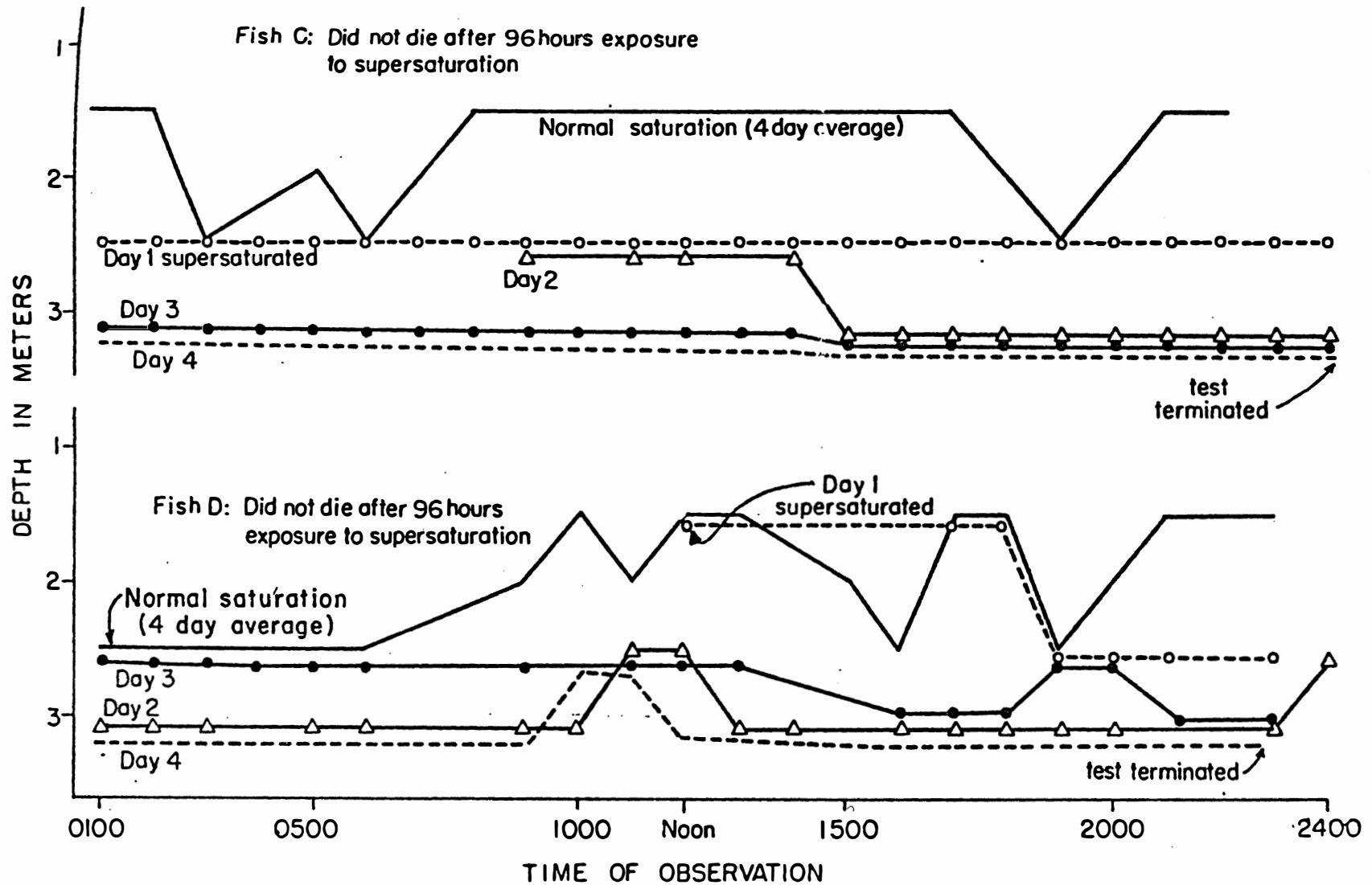

Figure 7 -- Hourly depth for two individual juvenile fall chinook tested at normal gas concentrations (100% TDG) and supersaturated conditions (130% TDG). Depths at 100% TDG are an average of 4 days.

About 5% of the test fish were observed at 0-1 m of depth (120% TDG or greater) throughout the test which was similar to the behavior observed in tests with steelhead.

Holding tests (in deep cages) conducted by Ebel, 1970, in the Snake and Columbia Rivers show high mortality rates of spring chinook in conjunction with elevated gas levels. However, gas concentrations, water quality and fish stocks differed from those used in laboratory tests.

The most significant observations made from these detection and avoidance tests are: (1) From past and present tests, where water depths were 3.5 m or less, both steelhead and chinook responded to supersaturation and displayed an associated avoidance reaction by sounding. However, the avoidance reaction in these tests was not sufficient to prevent mortality; mortality occurred even after the fish sounded. (2) Comparative observation of gas bubble disease incidence indicates that depth distribution of steelhead was altered upward when turbidity was increased.

INTERMITTENT EXPOSURE

As flow conditions change on the Columbia and Snake Rivers the dissolved gas content varies in relation to the volume of water spilled. Information was needed to determine if daily changes in dissolved gas content affects the survival of indigenous fishes. If significant changes in survival were apparent then coordination of water flows up and down the river could be accomplished to either

increase or decrease fluctuation of spill volume for the desired affect.

Survival in Laboratory Tests

Tests were planned and conducted to assess the effect of intermittently exposing fish to high (130, 120, and 110%) and then equilibrated levels (100% N_2) of saturation.

Groups of at least 10 fish (size of fish included in table 2) were held in separate 175 l flow through tanks (Figure 8) in which the N_2 level of 130, 120, 110, and 100% saturation were alternately changed. Figure 9 is a diagramatic representation of N_2 levels and the time base used for the tests. The time cycle was based on 24 hours e.g. one tank, eight hours at 130% and 8 hours at 100% N_2. The alternating cycle was continued for 192 hours (8 days). The changing of supersaturation levels in the test tanks was done by valving, which eliminated the handling of the fish. Water temperatures during tests ranged between 10 and 13°C.

Summarized in table 1 are the results of the intermittent exposure tests; compiled in the table is the time (in hours) to 50% mortality when test fish were exposed to 130% or 120% N_2 for 24, 16, and 8 hours. Tests at 110% are not included in the table because the 50% mortality level was not reached; however, some mortality did occur during tests with bass and whitefish (Appendix 1).

Tests at 130% indicated that a return to equilibrated water for either 16 or 8 hours increased time of exposure to 50% mortality--for six species tested--when compared to that recorded for a constant (24 hour) exposure to 130% saturation. Steelhead and

Figure 8 -- Flow-through bioassay tanks of 175 l capacity

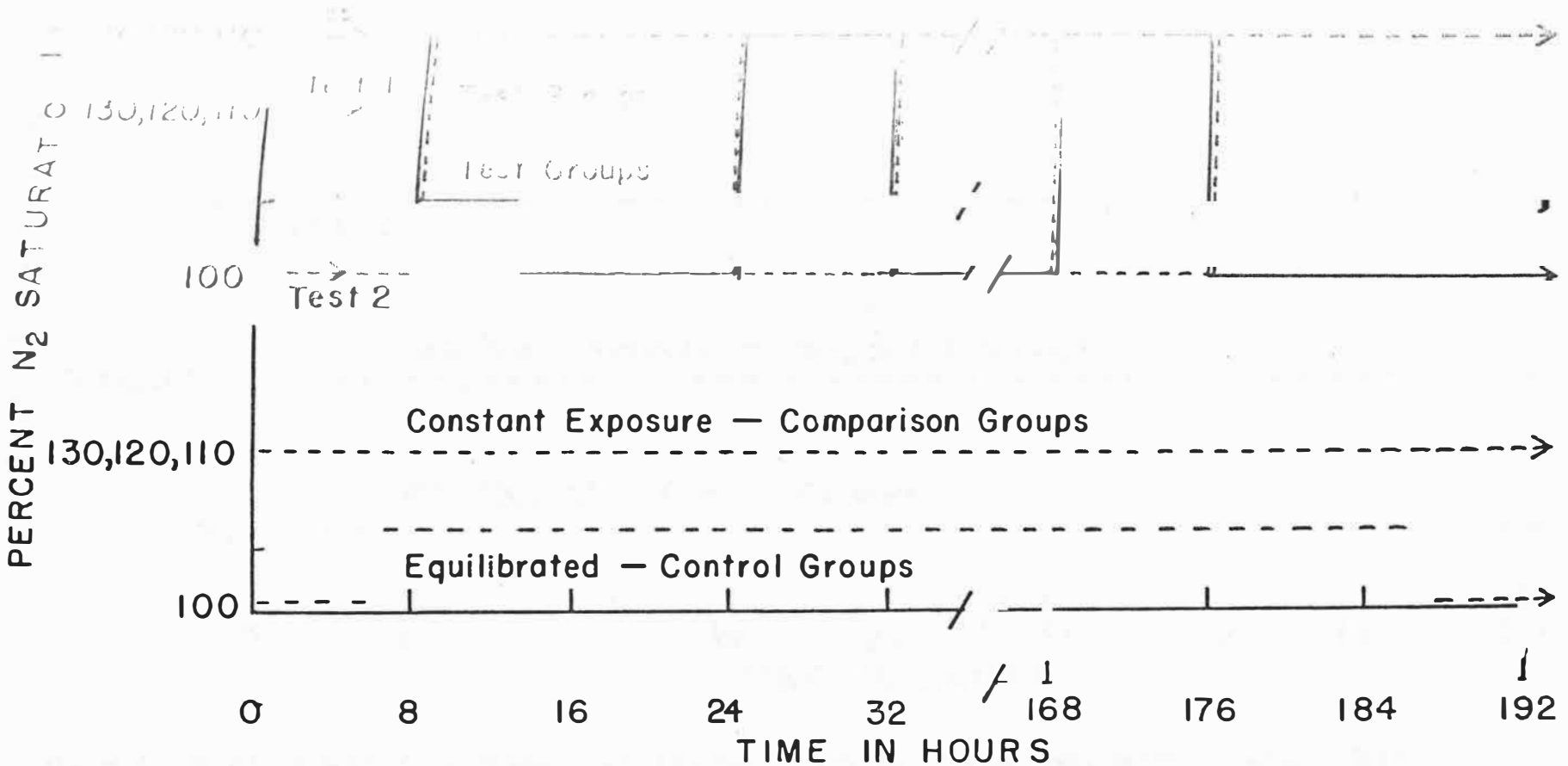

Figure 9.--Nitrogen percent saturation and time base used for intermittent exposure tests. Test groups were intermittently exposed to either 130, 120 or 110% N_2 for either 8 or 16 hrs then returned to 100% N_2 (equilibrated) for the remainder of each 24 hr period. Comparison groups were exposed continually to the supersaturated condition until 50% mortality occurred or until test termination at 192 hrs. Control groups were held throughout the test period in 100% N_2 water.

Table 1 -- Time in hours to 50% mortality for juvenile salmonids and non-salmonids subjected to 130 and 120% N_2 saturation for either 24, 16 or 8 hours of each 24 hour cycle during 192 hour intermittent exposure test. The fish were alternately exposed to the supersaturated condition and equilibrated water.

		PERCENT N_2 SATURATION					
		130			120		
		Number of hours during each 24 hour cycle fish were subjected to supersaturation					
Test Species	Average length of fish mm	24	16	8	24	16	8
		Time in hours to 50% mortality					
White crappie	66	*	*	*	*	*	*
Largemouth bass	72	93.5	*	*	*	*	*
Largemouth bass	72	102.0	*	*	*	*	*
Chinook	100	64.0	*	*	*	*	*
Chinook	102	24.0	120.0	*	*	*	*
Rainbow trout	77	47.0	69.5	*	141.5	*	*
Rainbow trout	61	31.0	*	*	*	*	*
Coho	79	26.0	46.0	102.0	59.0	*	*
Coho	82	22.0	*	*	*	94.0	*
Cutthroat trout	99	24.0	72.0	103.0	*	*	*
Whitefish	243	23.5	23.5	*	95.5	*	*
Whitefish	228	21.5	21.5	99.0	69.5	145.5	181.5
Steelhead	124	16.0	16.0	*	72.0	*	*

* = No 50% mortality

whitefish did not receive significant benefit by returning the population to equilibrated water. During the 8 days of testing at 120% N_2, four of the eight species reached 50% mortality when tested continuously with supersaturation. Two species (whitefish and coho salmon) reached 50% mortality in 8 days when exposed to 16 hours supersaturation followed by 8 hours equilibration and; only the whitefish test population reached 50% mortality in tests with 8 hour exposure to 120%. N_2 and 16 hrs. equilibrated water.

At the levels tested, and the time frame used, intermittent exposure to supersaturated and equilibrated water enhanced fish survival over that recorded for a constant (24 hour) exposure to the supersaturated conditions. However, as indicated by the following section of this paper it is doubtful if a time frame of intermittent spill at lower Snake River Dams could be created that would alter the gas saturation levels to the extent that would provide a dramatic increase in fish survival. The possibility of a more effective intermittent spilling might exist at other dams with a larger storage to power output ratios such a Libby or Brownlee Dams.

Variations in River Supersaturation Related to Intermittent Spilling

A monitoring program was initiated on the lower Snake River to determine how dissolved gas concentration varied during fluctuations of spillway discharge.

During a period when average stream flow was about 105,000 cfs,[1/] spill volume at Lower Granite Dam was decreased each morning during

1/ This report cfs, rather m^3/s, is used in deference to C of E usage in their reporting.

the 1975 fish migration season to facilitate passage of adult salmon and steelhead. Starting at 7 or 8 a.m. the spillway discharge was lowered to 60,000 cfs and maintained until the reservoir level rose from minimum to the maximum elevation. When maximum pool elevation was reached spill volume was increased to about 150,000 cfs; by the following morning pool elevation was again at the minimum. As a consequence the water flow rates at each of the other lower Snake River Dams (Little Goose, Lower Monumental and Ice Harbor) fluctuated within this general pattern.

During this period the variations in dissolved gas content created by the fluctuating spillway volumes was established. Monitoring was conducted for 24 hours in the Little Goose reservoir (below Lower Granite Dam); 40 hours in the Lower Monumental reservoir (below Little Goose Dam) and 42 hours in the Ice Harbor reservoir (below Lower Monumental Dam). Measurements of total dissolved gas were made every 1/2 hour for 24 hours and every hour thereafter.

Dissolved gas concentration varied less than 7% at all the reservoir sampling stations throughout the monitoring period. As a result of the operational procedure outlined, gas concentrations were: (1) below Lower Granite about 123.5% for 7 hours and 126.5% for 8 hours with intermediate values the rest of the 24 hour period (Figure 10) (2) below Little Goose, about 130% for 3 hours, and 134% for 6 hours (Figure 11) and (3) below Lower Monumental, about 125% for 6 hours and 127% for 8 hours (Figure 12). Mixing of high and low spill flows in the downstream reservoirs no doubt lowered the benefit that was achieved by reducing the spill volume.

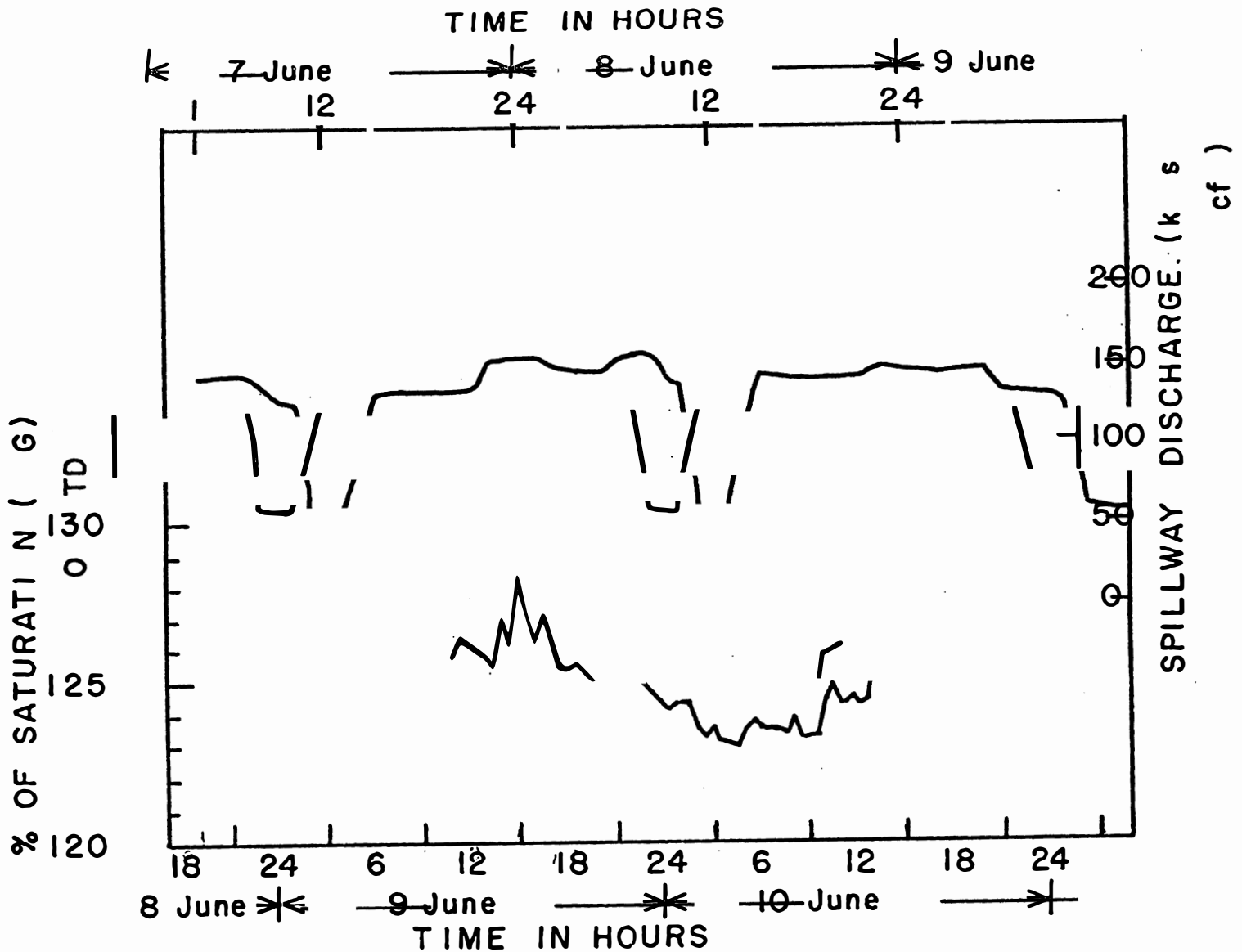

Figure 10 -- Dissolved gas measurements (% of saturation TDG) of Little Goose forebay vs. time in relation to previous spillway discharge (Kcfs) at Lower Granite Dam.

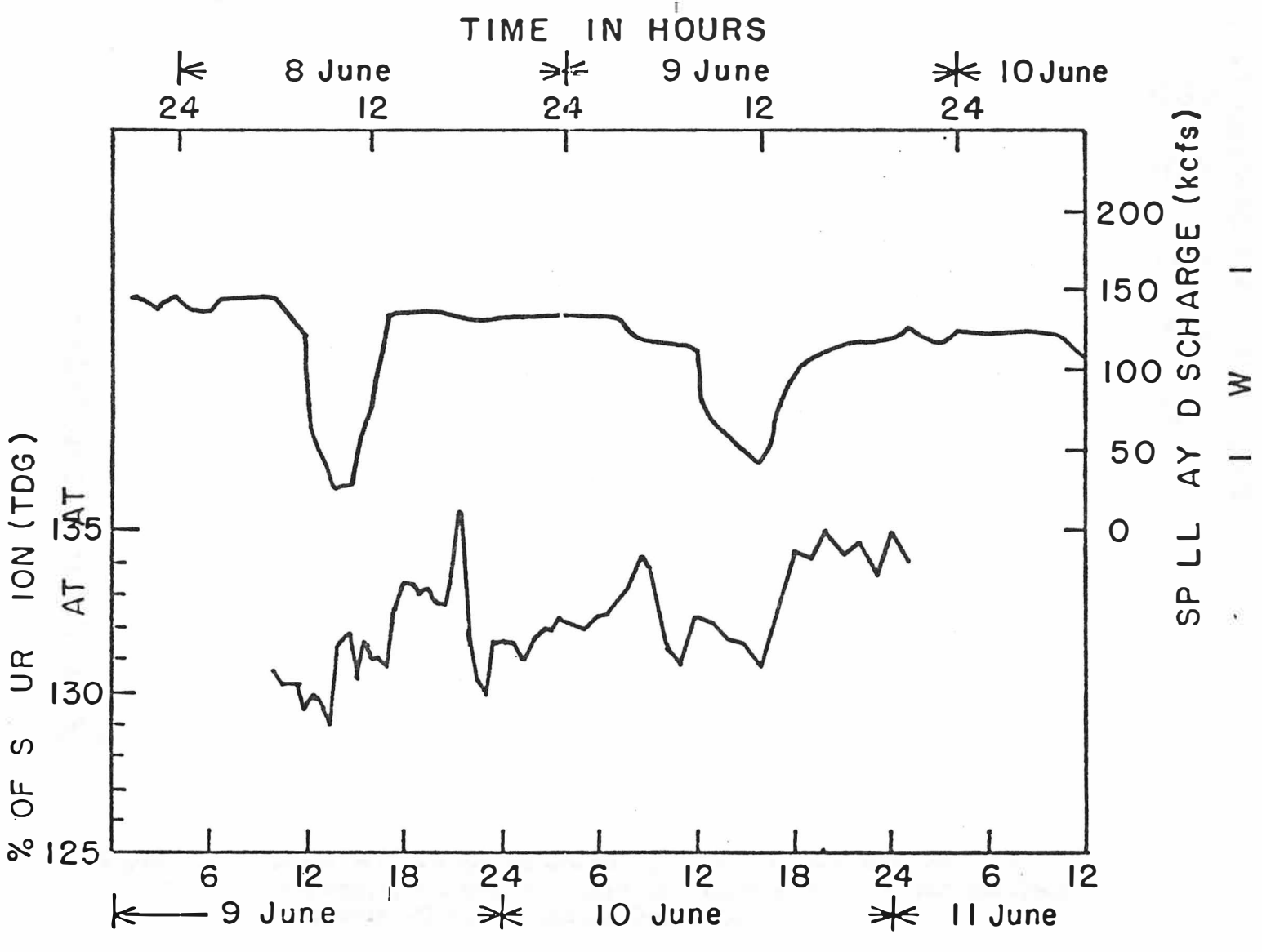

Figure 11 -- Dissolved gas measurements (% of saturation TDG) of Lower Monumental forebay vs. time in relation to previous spillway discharge (Kcfs) at Little Goose Dam.

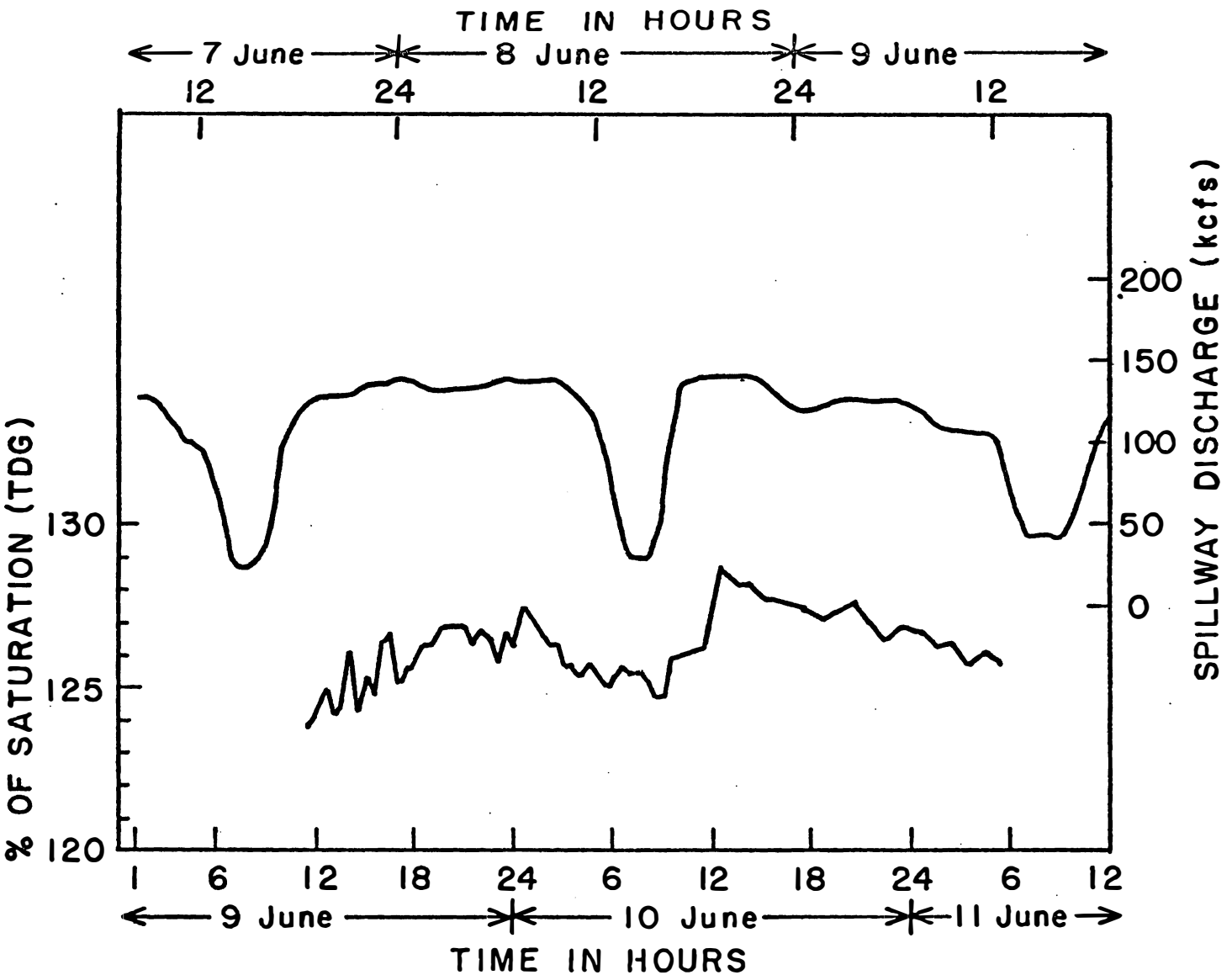

Figure 12 -- Dissolved gas measurements (% of saturation TDG) of Ice Harbor forebay vs. time in relation to previous spillway discharge (Kcfs) at Lower Monumental Dam.

It is doubtful whether fish survival could be significantly enchanced by manipulating the spill at the lower Snake River dams even at lower average flows because of limited reservoir capacity.

BIOASSAYS

Two types of dissoved gas bioassays have been done at the Prescott Facility: (1) a series using deep (2.5 m) and shallow (1 m) tanks (Figure 13) supplied with ambient (unaltered except for gas equilibrated controls) Columbia River water and (2) tests in 175 l tanks (Figure 8) with approximately .35 m water depth and artificially created nitrogen gas levels of 130, 120, and 110% saturation.

Deep and Shallow Tank Studies-Prevailing River Conditions

The primary objective of these tests was to determine whether fish survival would be increased if the option to sound in deeper water was provided by use of deep test tanks. The deep test tank was 1.8 m in diameter with a water depth of 2.5 m; the shallow tank was identical except for a water depth of 1 m. The water supply for the tank was pumped from the Columbia River. Summarized in Table 2 are the: (1) number, size and species of test fish, (2) number of days held during test and (3) ranges and average nitrogen levels. Also included in Table 2 is the total percent mortality that occurred in the tanks. The N_2 levels as indicated were the naturally prevailing Columbia River levels during the tests. The shallow control tank was supplied with gas equilibrated Columbia River water. Following is

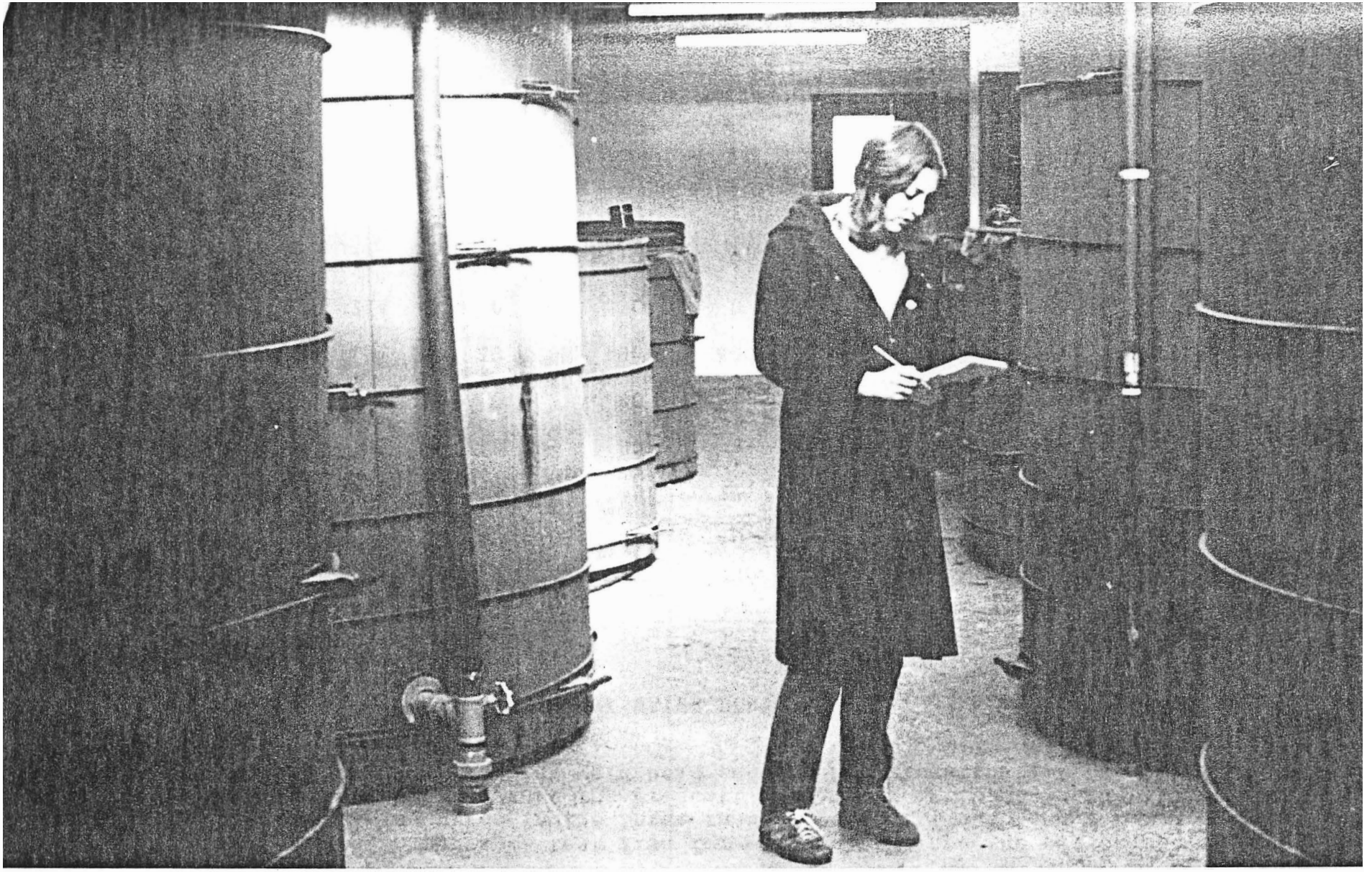

Figure 13 -- Fish holding tanks used for bioassay of prevailing Columbia River gas supersaturation and temperature conditions. The "tall" tanks are 1.8 m in diameter by 2.5 m deep, the "shallow" tanks are 1.8 m by 1 m deep.

Table 2 — Summary of the numbers of test fish for each species and the percentage of mortality that occurred in the three tanks (2 each 1 m deep and a 2.5 m deep tank) used for bioassays at the Prescott Facility. Included in the table is the number of days the fish were held and the N_2 saturations.

Species	Number of Fish	Average length of fish mm	TEST TANK WATER DEPTH (Equil) 1 m	1 m	2.5 m	Days Fish Held	PERCENT N_2 Range During Test	Avg.
			Percent Mortality					
Cutthroat	50	252	10	60	40	59	119 - 136	124
Cutthroat	50	250	8	40	27	49	112 - 130	120
Steelhead	80	191	10	80	6	55	112 - 129	120
Chinook	95	72	0	80	11	55	112 - 129	120
Smelt	75	181	30	100	40	12	119 - 122	121
Crappie	50	214	0	0	0	20	117 - 123	120
Squawfish	20	367	0	0	0	35	115 - 124	120
Suckers	58	365	15	33	2	46		116

a list of water quality parameters and the average deviation in the test tank from recorded levels in the Columbia River during the N_2 bioassay studies (1972-1975):

Parameter	Average percent deviation from river levels 1/
Dissolved oxygen - O_2	-5.1
Nitrogen gas - N_2	-1.4
Carbon dioxide - CO_2	+0.2
Ammonia - NH_4	+0.6
Conductivity	+2.5
Alkalinity	+0.8
Turbidity	+1.0
pH	+1.0
Water temperature	+0.1

1/ Caused by respiration of test fish.

With the exception of N_2, all parameters remained within safe biological ranges throughout the 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 supersaturation (calculated on a basis of zero hydrostatic pressure) decreases. In these test the crappie and squawfish were the most tolerant while the smelt were the least tolerant. However, this order of ranking for tolerance to supersaturation may have been affected to some extent by the difference in behavior of test animals. For example, as discussed later in this report, lethal exposure times for

squawfish tested with supersaturation in very shallow water were similar to salmonids. Squawfish tended to reside on the bottom of the test chambers in all tests compared to mid-depth distributions by other species.

With the smelt, a 30% mortality occurred in the control fish and 40% in the deep tank. The smelt were returning adults in spawning condition which probably accounts for the abnormal mortality in the control group.

Except for the smelt, the salmonids were the least tolerant of the species tested. The cutthroat were slightly more tolerant than either the chinook or steelhead, which showed comparable tolerance. These general conclusions apply only to the N_2 levels as indicated in Table 2.

Shallow Tanks - Induced Supersaturation

The tests in the 1751 tanks were designed to provide added biological information on the effect of nitrogen levels on fish survival. Nineteen 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 hours during which mortality was recorded. As with the preceeding tests (deep vs. shallow tanks) water quality parameters remained in exceptable biological ranges with the exception of N_2. Test temperatures were between 10 and 13°C. Table 3 summarizes the time (hours) to 50% mortality at 130, 120, and 110% N_2 saturation for indicated species and size. Mortality did not occur in the control tanks containing equilibrated (100% saturation) water.

Table 3 -- Time (in hours) to 50% mortality for groups of fish held at 130, 120, 110, and 100% N_2 saturation for 192 hours (species ranked in order of tolerance).

Species	Average fish length mm	PERCENT N_2 SATURATION 130	120	110	100
		Hours to 50% Mortality			
Crappie	66	(20)[1/] 192.0 (1)[2/]	*	*	*
Crappie	222	55.5	*	*	*
Largemouth bass	68	220.0 (2)	*	*	*
Largemouth bass	72	102.0	*	*	*
Largemouth bass	72	93.5	*	*	*
Rainbow	63	70.5 (3)	192.0	*	*
Rainbow	77	47.0	141.5	*	*
Rainbow	61	31.0	*	*	*
Chinook	100	64.0 (4)	*	*	*
Chinook	102	24.0	*	*	*
Coho	79	26.0 (5)	59.0	*	*
Coho	82	22.0	118.0	*	*
Cutthroat	99	24.0	119.0	*	*
Whitefish	218	23.0	50.5	*	*
Whitefish	243	23.5 (7)	95.5	*	*
Whitefish	228	21.5	69.5	*	*
Steelhead	131	16.0 (8)	72.0	*	*
Steelhead	124	16.0	N/T[3/]	N/T	N/T
Smelt	165	5.5 (9)	30.0	*	*

* = No 50% mortality
1/ 20 % mortality in 192.0 hours
2/ Number in parenthesis indicated ranking of tolerance
3/ NT = No Test

Bass and crappie were the most tolerant of the species tested while smelt were least tolerant. Of the salmonid species, rainbow trout were more tolerant at the 130% N_2 level; however, this was not true at 120% N_2 saturation. The steelhead were the least tolerant of the salmonids at 120%. The 50% level of mortality of remaining salmonids, including whitefish, seemed to be grouped at around 24 hours exposure when tested at 130% N_2 saturation. The ranking changes at the 120% level, but not drastically (Table 3).

Survival and Feeding Response in Shallow Tanks-Induced Supersaturation

The object of this study was to establish the effect of supersaturation of dissolved gas on predation rates and survival of the northern squawfish.

Nine simultaneous tests using adult squawfish were conducted during a 12 day period; the percent dissolved gas concentrations (TDG) averaged: (1) 126.1, (2) 120.4, (3) 2 tests at 117.2, (4) 2 tests at 110.3, (5) 107.2 and (6) 2 tests at 99.8. Water depth was 25 cm which produced a pressure compensation decrease of 2.5% of saturation. Nitrogen and oxygen concentrations were held to + 4% of the TDG saturation value and standard deviations of the TDG values ranged from 1.2 to 3.4% of saturation over the 12 day period. A daily food ration for each test group was established at 4 dead eulachon (smelt) (avg. 30 g, 17 cm) and 1 live steelhead fingerling (avg. 21 g, 8 cm).

Significant mortality occurred in tests at 117, 120, and 126% of 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% mortality occurred in 12 days in the tanks set at 117% saturation. All live and dead fish at these concentrations displayed gross signs of gas bubble disease. Hemorrhages and subcutaneous gas blisters were prevalent in large areas on the bodies of many test animals and the others showed large blisters between the fin rays. Exophthalmia (popeye) occurred in only two of the survivors; presumably it was of minor incidence due to the relatively short test period. Mortality associated with gas bubble disease did not occur at or below the 110% level of saturation. However, signs of gas bubble disease were apparent in 89% of the fish tested at the 110% level. Only one fish from the 107% test had signs of gas bubble after 12 days.

Feeding and predatory responses of fish held at the lethal concentrations of dissolved gas were inhibited. We commenced introducing food to the test tanks on day #2; all surviving test groups consumed the ration, but on the following days only partial rations were eaten by fish in the tests at 120 and 117% TDG. When a portion of the daily food ration was rejected in these test tanks 40% of the time, live steelhead remained in the tank, whereas in the tanks with lower saturation (110, 107, and 100%) steelhead were uneaten only 4% of the time. Average daily consumption rates for the combined replicates, adjusted for weights of test fish and mortality were: 2.3 g food/fish/day (g/f/d) at 120% level; 6.2 g/f/d 117% level; 10.9 g/f/d 110% level; 11.2 g/f/d 107% level; and 14.3 g/f/d for 100% level.

The decrease in food consumed as the saturation values increased indicated that predation by squawfish may change by as much as

50% in water at 115% of saturation TDG. The change in food from live fish to dead fish (when squawfish were stresses to a greater degree in the higher saturations) suggests they were debilitated to the extent that they were not capable of capturing the live steelhead.

Lethargy was exhibited by squawfish stressed by high gas concentrations and may decrease predation on juvenile salmonids migrating down the Columbia and Snake Rivers. However, the average water depth inhabited by squawfish may compensate for the effect of supersaturation. For example; in the Snake River the dissolved gas level remained at 140% (surface) for an extended period in 1974 and if the 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 residence without curtailing predatory capacities. The depth distribution of squawfish in the Snake River is not known, thus the overall effects of supersaturation on their survival and predatory ability remains in question.

During experiments done at the Prescott Field Station (page 34) as well as those reported here, the squawfish tended to reside near the bottom of the tanks. At Prescott the % of saturation value at the bottom of the 1 meter depth tanks was 109.1% and in the 2.5 m tank it was 95.8%. As a result, no mortality was reported in those tests; however, gas bubble disease signs were apparent on all fish from the 1 m test after 35 days; similar to our observations at 110% after 12 days.

Peaking studies being conducted[2/] in the reservoirs of the lower Snake River will compliment these data on the effects of supersaturation on squawfish. Information on survival, migration and diet of squawfish is being collected which may provide further insight into the effects of supersaturation in the river on squawfish predation.

DEPTH DISTRIBUTION - FIELD STUDIES[3/]

Knowledge of the distribution of migrating juvenile salmonids in rivers and impoundments is important to our understanding of their survival during passage to the sea. Dissolved gas concentrations of are lethal to salmonids migrating near the surface. Higher gas concentrations become progressively less damaging as a fish sounds to deeper waters: each foot of hydrostatic pressure enables a fish to compensate for about 3% excess dissolved gas (at levels above 100% saturation). Consequently the depth at which a fish travels becomes an important factor in determining overall effects of supersaturated waters on its survival.

The primary objective of this study, was to obtain information on swim depth of juvenile spring chinook salmon and steelhead trout during their seaward migration in the Snake River (deemed high priority research by the Nitrogen Task Force in 1973). Information obtained enabled us to gain further understanding of the effects of

2/ Wallace Bentley, NMFS Biological field station Tri-Cities Airport Bldg. 57, Pasco, WA 98301.

3/ A more detailed report has been published (Smith, 1974).

supersaturated gases on migrating juveniles.

Two sets of gillnets were placed in the reservoir of Lower Monumental Dam at two sites; one near shore in 15 m of water depth, and one at a water depth of 29 m. Three net sections 18.3 m (60 feet) long x 3.7 m (12 feet) deep were used at various depths throughout a one month period coinciding with smolt migrations. Depth distributions of these stocks, were examined on a daily and diurnal basis.

Combined catches (Table 4) at the deep and shallow stations show that 58% of the chinook salmon and 36% of the steelhead trout were traveling in the upper 3.7 m of the reservoir. A breakdown of catches in the upper 3.7 m of the reservoir at 0.3 m intervals (Figure 14) provides further insight of fish distribution in the most critical area of the water mass with regard to supersaturation.

Of fish caught in the upper 3.7 m, 80% of the chinook salmon and steelhead trout were in the upper 1.8 m of the nets. Thus, when applied to the total catch, we find that about 46% (0.80 x 58) of the chinook salmon and 29% (0.80 x 36) of the steelhead trout were migrating between the surface and 1.8 m of depth. This signifies that more chinook salmon (because of their tendency to migrate nearer the surface) than steelhead trout, would be subject to losses from the effects of supersaturation.

Data from catches in the upper 3.7 m and from 3.7 to 7.3 m were compared for diel variation. The proportion of chinook salmon catches in the surface nets (0-3.7 m) increases at night whereas steelhead trout declines. No chinook were taken from (0-3.7 m) during the day, but at night 60% of the total catch in the upper 7.3 m was taken in

Table 4.--Vertical distribution of juvenile chinook salmon and steelhead trout caught at shallow and deep stations in the forebay of Lower Monumental Dam, 1973.

Shallow station				
	Chinook		Steelhead	
Depth (meters)	Number of fish	%	Number of fish	%
0-3.7	50	69	260	51
3.7-7.3	20	28	135	26
7.3-11.0	1	1.5	70	14
11.0-14.6	1	1.5	46	9
Totals	72	100	511	100

Deep station				
	Chinook		Steelhead	
Depth (meters)	Number of fish	%	Number of fish	%
0-3.7	93	53	181	25
3.7-7.3	43	24	156	22
7.3-11	18	11	119	16
11-15	3	2	60	9
15-18	3	2	61	9
18-22	6	4	62	9
22-26	2	1	32	4
26-29	5	3	48	6
Totals	173	100	719	100

Shallow and deep stations combined				
	Chinook		Steelhead	
Depth (meters)	Number of fish	%	Number of fish	%
0-3.7	143	58	441	36
3.7-7.3	63	26	291	24
7.3-11	19	8	189	15
11-15	4	2	106	8
15-18	3	1	61	5
18-22	6	2	62	6
22-26	2	1	32	2
26-29	5	2	48	4
Totals	245	100	1,230	100

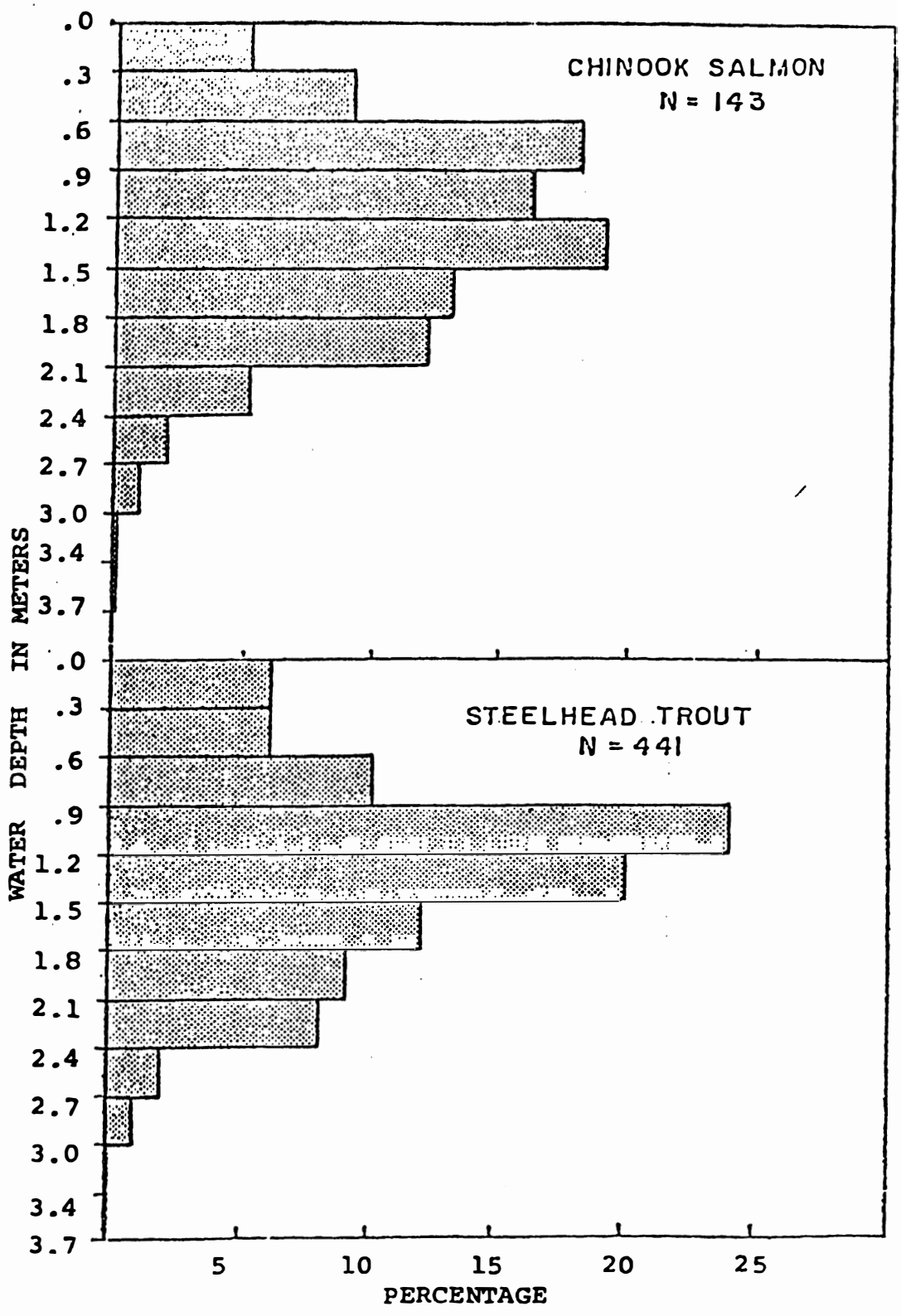

Figure 14.--Vertical distribution (in percent) of chinook salmon and steelhead trout caught in the upper 3.7 meters of Lower Monumental Forebay in 1973.

the surface nets. Steelhead catches in the surface nets declined from 74% during the day to 36% at night. We believe these catches reflect actual behavioral differences between species; chinook tend to be surface oriented at night whereas steelhead trout are the reverse--nearer to the surface during the day.

Effects of changes in the river temperature, flow turbidity and solar illumination on the horizontal and vertical distribution of juvenile salmon and trout were examined, but no specific correlations were found. This may due to the lack of substantial variations in the prevailing river conditions. Secchi disc readings ranged from 86 to 137 cm; Secchi disc readings in the Snake River during the spring freshet normally range as little as 15 cm but, owing to the low runoff in 1973, the river was consistently much clearer than the norm. Readings from the solar illuminance meter indicated that total darkness in the river ranged from 4.8 to 7.6 m. Water temperatures at the surface of the reservoir ranged from 10.5 to 15.8°C and generally varied between 1.1 and 2.8°C cooler at 29 m (maximum depth). Total river discharge at the dam ranged for 60,000 to 90,000 cfs which is far below the normal range of flow in the spring. The relatively narrow range of flow volume resulted in only minor changes in current velocity; no correlation between velocity change and catches was noted.

The unusual clarity of the river in 1973 could have influenced fish distributions during this study. Also fish behavior may be different in reservoirs of the Columbia River where light transmittance, water velocities and width of the water mass are different.

For these reasons, additional depth distribution studies are needed during different flow conditions and at various locations along the migration routes. A detailed report of this research has been published (Smith 1974).

Ebel (1973) published a paper titled "Relations between fish behavior, bioassay information and dissolved gas concentration on the survival of juvenile salmon and steelhead trout in the Snake River", in which he estimates mortality caused by supersaturation in the river; his calculations were based on vertical distribution from the Smith report.

PHYSIOLOGICAL EFFECTS

The lethal effect of supersaturation of dissolved gas on fish has been studied extensively; however, there was a need for studies of sublethal effects. In 1972 NMFS personnel completed a series of tests comparing the swimming performance and blood chemistry of unstressed juvenile chinook salmon and steelhead trout to those that had been stressed with supersaturation.

We found that salmon exposed to gas concentrations at or above 110% of saturation N_2 (106% TDG) gained less distance and swam for less time against a constant water current stimulus than did their unstressed counterparts. Steelhead tests showed no statistical difference between swimming abilities of stressed vs. unstressed fish (Schiewe, 1974).

These tests also indicated that changes in blood plasma concentrations of potassium, phosphate, albumin, calcium and alkaline

phosphates from steelhead trout tested at 116% N_2 (110% TDG) were directly related to stress from dissolved gas (Newcomb, 1975).

In order to further define effects of sub-lethal stress, we conducted the following research.

Fish Stamina

During February and March of 1975, 50 juvenile chinook (avg. 103 mm) were timed swimming against a water velocity of 1.5 fps in gas supersaturated (128 to 134% N_2) and equilibrated (100% N_2) water. The tests were done in a large scale environmental respirometer (Figure 15).

The test fish (in groups of 5) were held for 16 to 20 hours pre-test at each gas saturation condition before testing. Survivors from the pre-test holding were then tested.

The average swimming time for the fish held and tested in the supersaturated water was less than that recorded for the control (equilibrated N_2) group. An average swimming time of 17 min. was measured at supersaturated N_2 and 25 minutes at equilibrated N_2. The results indicate that fish stamina is detrimentally affected by gas supersaturation. The primary cause of decreased performance is probably the animals inability to efficiently utilize the available O_2 because of gas bubbles obstructing the blood flow within the circulatory system supplying the gills. The implications are that fish did not operate efficiently in an environment which was supersaturated with atmospheric gases, consequently, the ability of fish to feed, reproduce, and escape preditors would be adversely affected.

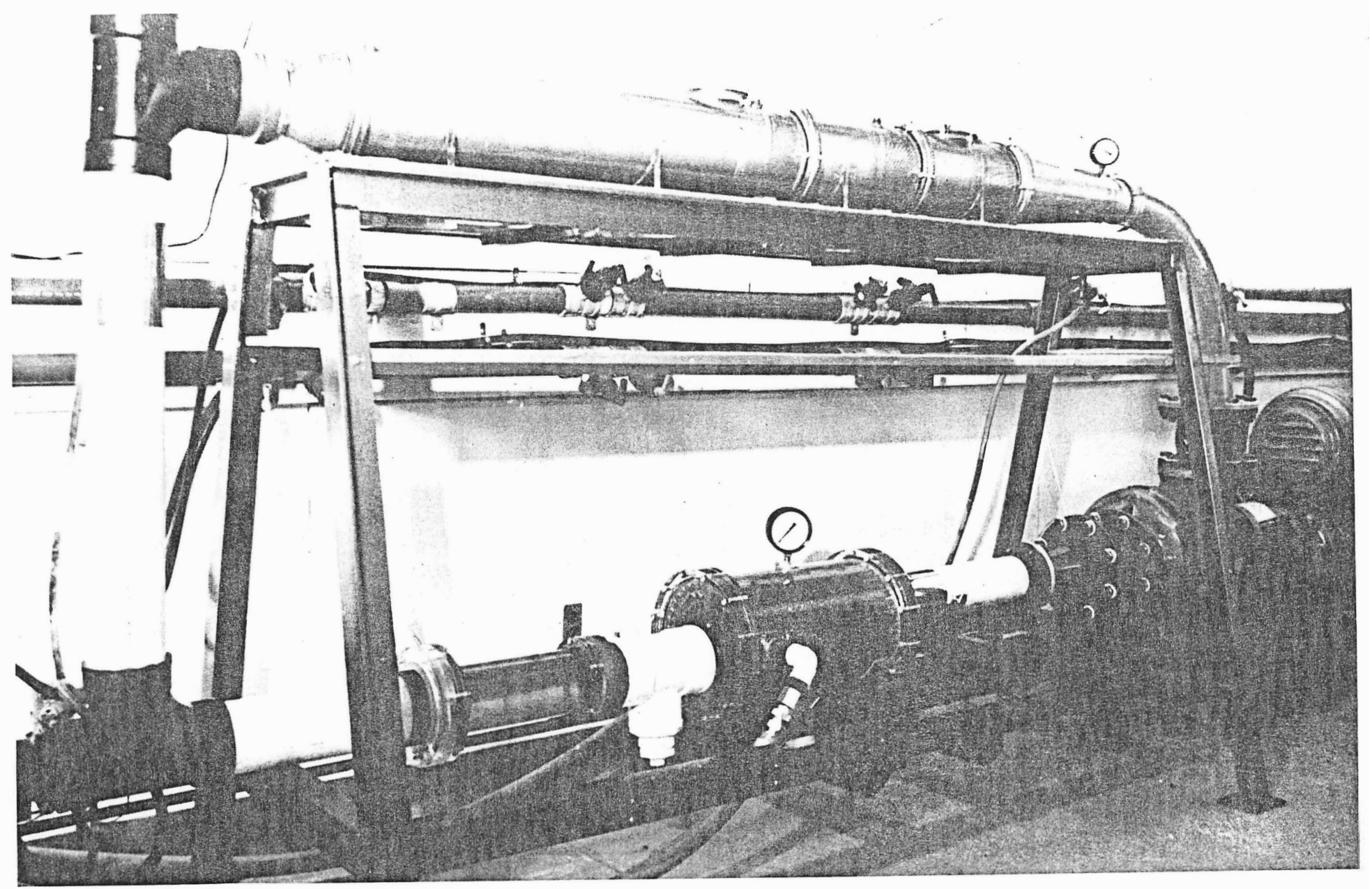

Figure 15 -- Controlled environmental chamber used for fish stamina tests at the Prescott Facility

Oxygen Consumption

Oxygen consumption of fish is used by many investigators as an index of stress: we used this parameter to indicate the indirect (sublethal) effects of nitrogen supersaturation on juvenile salmonids.

A flow through respirometer (Figure 16) was attached to constant head reservoirs which contained equilibrated and gas supersaturated Columbia River water; N_2 levels in the respirometer were controlled by valving. Water samples were drawn into hypodermic syringes through rubber diaphrams located at each end of the chamber. Differences in dissolved oxygen content between the inlet and outlet of the chamber was used to calculate the amount utilized by each gram of test fish per hour ($ccO_2/g/hr$). Sample analysis was either by the Micro Winkler technique or gas chromatography.

Three species of juvenile salmonids were tested: (1) coho salmon (2) chinook salmon and (3) cutthroat trout. A brief summary of one test for each species follows:

1. Oxygen consumption of eight coho salmon (avg. 12.5 g and 97 mm) in equilibrated water (103% N_2) averaged 0.121 cc O_2/g/hr and varied very little over a 98 hour exposure. When this same group of fish were subjected to saturated water with 123% nitrogen the oxygen consumption increased and averaged .181 cc/g/hr. over the next 67 hours. At the end of this period O_2 consumption decreased to 0.120 cc/g/hr. and one fish died; the remaining seven had external gas bubble disease signs.

2. Two groups of 10 each, juvenile chinook (avg. 56 g and 79 mm) were tested simultaneously; one in equilibrated water, the other

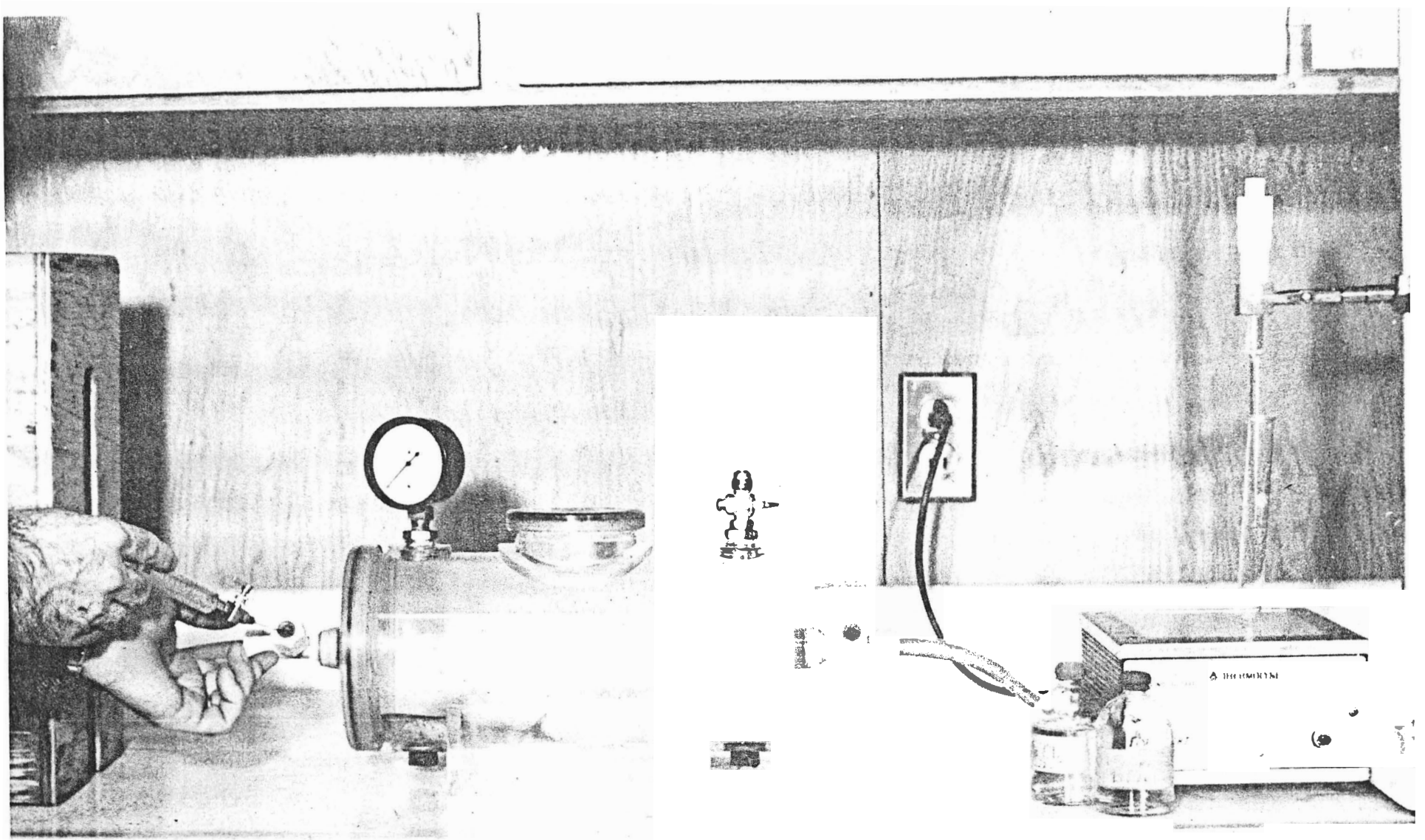

Figure 16 -- Flowthrough respirometer used for Oxygen Consumption tests at the Prescott Facility.

in N_2 supersaturated water. Oxygen consumption of the chinook in equilibrated water (control group) changed little and averaged 0.184 cc/g/hr. during 230 hours of exposure; no mortality occurred. Fifty percent mortality occurred within 50 hours in the test group which was held in supersaturated water (127% N_2). Dead fish were removed and consumption calculations were adjusted accordingly. Consumption reached a high of .276 cc/g/hr. after a 27 hour exposure, thereafter oxygen consumption decreased to .127 cc/g/hr.

3. Following is a brief table showing time, O_2 consumption and percent N_2 saturation for a group of 10 juvenile cutthroat trout (avg. 12.5 g and 97 mm) tested in a manner similar to that used during the tests with coho

Time	O_2 consumption cc/gram/hour	N_2 Saturation percent
0900	.149	109
1300	.142	108
1315 Initiated supersaturation with nitrogen		
1400	.310	133
1500	.112	149
1600	.076	152
1900 1/	.064	150

1/ One fish had lost equilibruim, died by 20:00

Increased O_2 consumption occurred in all tests subsequent to supersaturated water being introducted into the chambers; apparently

consumption increases, then declines until death occurs. The decline in O_2 consumption is probably due to inhibited gas exchange of the gills. The blood vessels supplying the gills were inundated with gas bubbles; dead fish exhibited suffication symptoms.

Characteristically, as the N_2 supersaturated water was introduced into the test chamber, the fish appeared to become excited and the O_2 consumption increased. The chambers were maintained in a darkened cover and manipulation of supersaturated water was done by remote valving so as to eliminate disturbing the fish during the test. Gas bubble disease was not evident in the controls; however, all survivors from the test groups exhibited external signs.

Blood Chemistry

The goal of this research was to discover changes in blood chemicals that can be used to establish the degree of stress from supersaturation that a population of juvenile salmon or trout has sustained and how much more they can tolerate. Two laboratory test designs were used in an attempt to identify blood chemicals indices. The first approach (completed in 1972) incorporated 35 bioassays at various levels of supersaturation, to measure changes in juvenile steelhead and spring chinook blood chemistry at the end of each test. The second approach was to periodically sub-sample juvenile salmonids throughout a test at one gas concentration. A sampling program was then undertaken to apply lab results to verify supersaturation stress in the fields (Snake and Columbia Rivers).

Thirty-five day stress of juvenile steelhead

Groups of 60 juvenile steelhead (avg. size, 20.5 g and 132 mm) were exposed for 35 days to various nitrogen saturations at 15°C. Experimental groups were exposed to 105, 110 and 116% of saturation nitrogen (102, 106, and 110% TDG) and three control groups were exposed to 103% N_2. Blood samples were taken and pooled then analyzed for calcium, sodium, phosphate, potassium, cholride, albumin, total protein, cholesterol, alkaline phosphatese (A.P-hase), glucose, urea, uric acid, total bilirubin, lactate dehydrogenase (LDH) and serum glumatic oxalacetic transaminase (SGOT) utilizing a SMA 12/60 Autoanalyzer[4/] (Table 5). An increase in serum potassium and phosphate, and a decline in serum albumin, calcium, chloresterol, total protein and A.P-hase were noted in steelhead exposed to 116% nitrogen saturation (110% TDG) (Figure 17); mortality did not occur.

A similar group of steelhead exposed to 116% nitrogen saturation, but a higher (112.6%) TDG saturation, suffered fifty percent mortality in 20 days. This group did not show the increase in serum potassium or phosphate seen in the 110 % TDG stressed steelhead, however, 112.6% TDG exposed steelhead did show similar decreases in cholesterol, total protein, albumin and A.P-hase. In addition, serium calcium was further depressed in the group stressed at 112.6% TDG than in the 110 % group. A significant decline in chloride was also noted in the groups of steelhead exposed to the higher TDG saturation. A slight decline in chloride was noted in the group stressed at 110 % TDG.

4/ Trade names referred to inthhis report are not an endorsement of commercial products by the National Marine Fisheries Service.

Table 5.--Blood chemistry levels of juvenile steelhead in response to 35 day exposure to various levels of nitrogen saturation (means and the standard deviation of the concentrations).

Blood characteristic	Units of measure	Dissolved Gas Saturation				
		103.5±2.0 % N_2 99.9±2.0 % T.D.G. n=3x60 35day	105.4 102.0 n=60 35day	110.1 105.9 n=60 35day	116.0 110.2 n=30 35day	116.0 112.6* n=30 20day
		$\bar{X}$±S.D.				
Calcium	(mg%)	10.51±0.17	10.05	10.33	9.45	8.75
Potassium	(mEq/L)	3.4±0.1	3.1	3.1	4.6	3.2
Sodium	(mEq/L)	157±2	155	155	158	164
Chloride	(mEq/L)	112.0±2.5	116	113	109	103
Phosphorus	(mg%)	13.5±0.2	13.2	13.3	14.4	13.5
Glucose	(mg%)	112±2	113	115	107	120
Urea	(mg%)	0.9±0.1	1.0	1.0	1.0	2.5
Uric Acid	(mg%)	0.4±0.1	0.3	0.5	0.4	0.5
Cholesterol	(mg%)	274±24	277	283	197	195
Total Protein	(gm%)	3.1±0.2	3.1	3.2	2.7	2.7
Albumin	(gm%)	1.4±0.1	1.4	1.4	1.1	1.1
Total Bilirubin	(mg%)	0.2±0.1	0.2	0.3	0.2	0.3
A.P-ase	(mU/ml)	100±7	117	145	67	53
LDH	(mU/ml)	551±64	646	740	567	630
SGOT	(mU/ml)	1000±69	1000	1060	940	900

* Survivors of LE_{50} (20day).

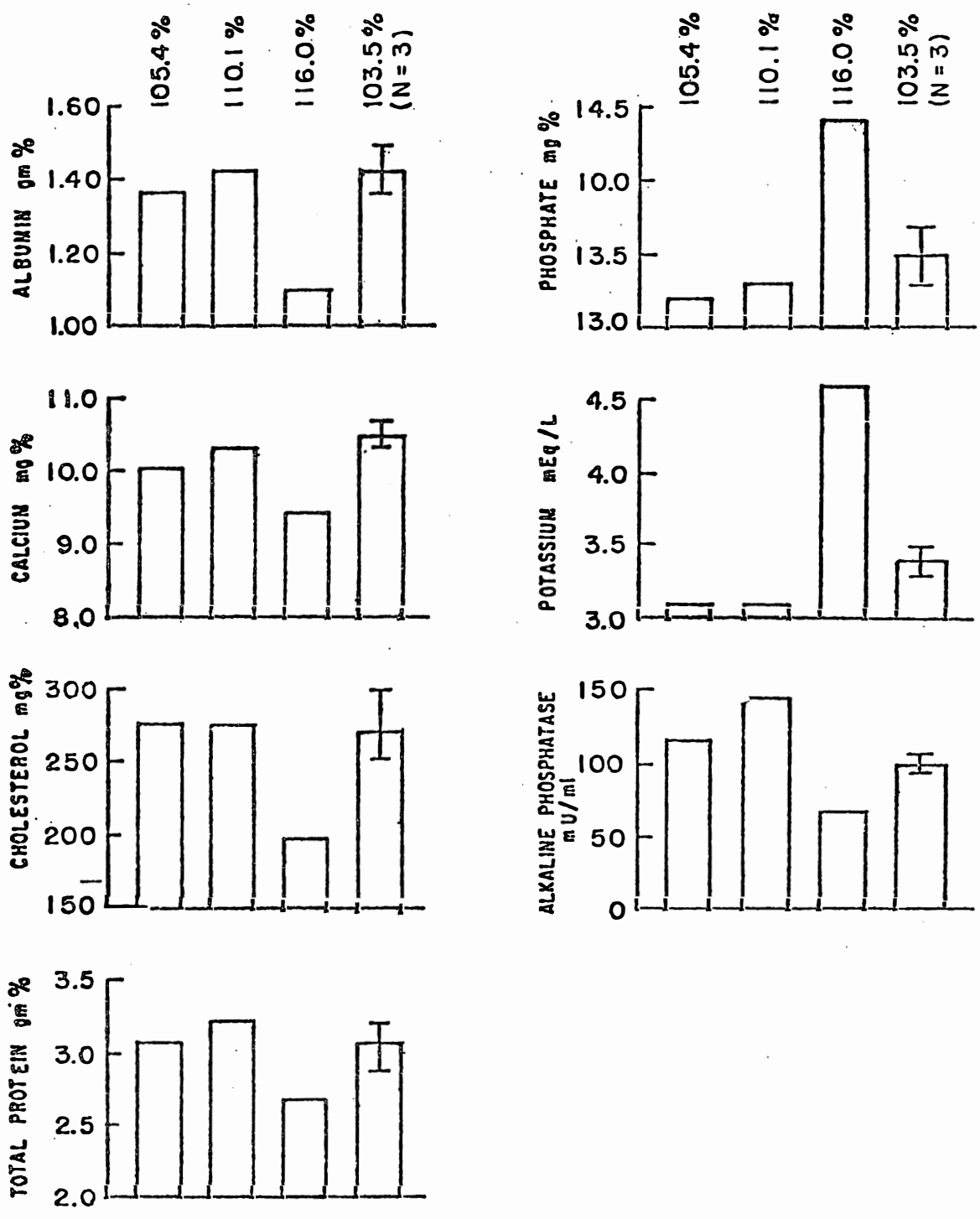

Fig.--17 Major serum chemistry parameters with increasing N_2 + Ar saturations. Control (103.5 % N_2 + Ar) values are mean and range.

No major changes in blood chemistry were observed at nitrogen saturations of 105%

Thirty-five day stress of juvenile spring chinook

A second bioassay was conducted on groups of 60 spring chinook (avg. size 16 g and 118 mm) exposed for 35 days to various (102, 106, 110, 116, and 121%) nitrogen saturations. Pooled serum samples were then similarly taken and analyzed (Table 6). A decrease in serum calcium was noted in fish exposed to 110, 116, and 121% saturation.

While serum calcium does show the same change in spring chinook as in steelhead, the changes, in general, of spring chinook differed at least in degree if not also in kind to the same stress situation.

Four month stress of fall chinook fry

Pooled samples of blood were collected each month from a sub-sample of up to 25 surviving button-fall chinook fry from each of 20 test groups of fish used in a four month bioassay with super-saturation at 10°C. Replicate groups of 250 fry were placed in shallow tanks (25 cm water depth) with dissolved gas concentrations of 100, 105, 110, 115 and 120% of saturation (TDG) and in deep tanks (2.5 m water depth) with levels of 110, 115, 120, and 125% plus individual deep tank groups held at 100 and 105% TDG. For blood chemistry purposes fish in the four lowest saturation tanks in each series (shallow and deep) were taken to be controls. The plasma samples were very small (10-30 ul) and only calcium and chloride were measured.

Differences in calcium levels between test and control were noted in fish from the 120% TDG shallow tanks on the first serial

Table 6.--Blood chemistry levels of juvenile spring chinook in response to 35 day exposure to various levels of nitrogen saturation (means and the standard deviation of the concentrations).

Blood characteristic	Units of measure	Dissolved Gas Saturation				
		101.8±2 % N_2 100.1±2 % T.G.P. n=3x40 35day	106.1 104.2 n=42 35day	109.8 105.9 n=58 35day	115.5 111.6 n=53 35day	120.8 116.6* n=30 1.2day
		$\bar{X}$±S.D.				
Calcium	(mg%)	9.13±0.13	9.05	8.78	8.65	8.80
Potassium	(mEq/L)	2.8±0.3	2.6	3.1	2.7	2.7
Sodium	(mEq/L)	--	--	--	--	--
Chloride	(mEq/L)	116±1	114	116	119	114
Phosphorus	(mg%)	<10.0	<10.0	<10.0	<10.0	<10.0
Glucose	(mg%)	74±5	75	87	73	115
Urea	(mg%)	2.9±2.3	1.0	6.0	0.7	5.0
Uric Acid	(mg%)	0.4±0.1	0.4	0.4	0.4	0.3
Cholesterol	(mg%)	160±20	188	172	168	242
Total Protein	(gm%)	2.5±0.2	2.6	2.5	2.6	2.9
Albumin	(gm%)	1.1±0.1	1.0	1.2	1.0	1.3
Total Bilirubin	(mg%)	0.2±0.0	0.2	0.2	0.2	0.2
A.P-ase	(mU/ml)	28±8	33	30	28	45
LDH	(mU/ml)	401±141	358	545	350	415
SGOT	(mU/ml)	803±180	640	856	520	832

* Sampled at LE_{50} (27 hours).

sub-sample (28 days) and in the fish from 115% TDG shallow tanks on the third and fourth sub-sample (at 84 days) (Table 7). Increased calcium concentrations were also measured in fish from deep tanks at 125% TDG in the sub-sample at 28 days. There was a slight calcium difference measured between deep tank control and 120% TDG saturation fish at 84 days. In general, all groups of button-up fry show a gradual decrease in plasma calcium with increasing age but high gas saturations tended to retard this change.

Significant differences in plasma chloride were not observed in the shallow or deep tank series of tests (Table 8). The initial high chloride value (169.3, mEq/l) followed by the declines observed after the 28 day sub-samples may represent an effect of increasing age.

Twelve day test of juvenile fall chinook salmon

A series of shallow tank (25 cm water depth) tests at 120 and 100% N_2 at 10°C, were carried out on juvenile steelhead trout and fall chinook salmon. Samples of surviving fish were taken periodically from the start of the test until LE_{100} (Time of exposure to 100% mortality) was reached then blood was analyzed from each (Figures 18 and 19).

Groups of 60 fish were used in the fall chinook test (avg. size, 35 g and 138 mm). Blood samples from surviving fish (n = 3 each time) were individually measured for plasma cholesterol, albumin, total protein, phosphate, calcium, potassium, magnesium, and chloride. No significant differences between experimental and control samples could be found in cholesterol, albumin, total protein, potassium,

Table 7.--Mean (±S.D.); Plasma Calcium (mg/100 ml). Pooled by tank for each saturation level for monthly subsamples of fall chinook fry surviving shallow (25 cm) and deep (2.5 m) water bioassays of supersaturation (n=no. of groups) with notes on cumulative mortality (corrected for control loss).

	Exposure Time			
	28 day	56 day	84 day	112 day
Shallow Water				
Control (100% TDG)	12.32±0.21 (n=4)	10.01±0.42 (n=4)	10.10±0.19 (n=4)	9.88±0.45 (n=4)
110% TDG	12.57 (n=1) 3% mortality	9.93±0.23 (n=2) 11% mortality	10.21±0.88 (n=2) 40% mortality	10.18±1.76 (n=2) 52% mortality
115% TDG	11.80±0.07 (n=2) 25% mortality	10.09 (n=1) 70% mortality	11.35 (n=1) 93% mortality	12.00 (n=1) 95% mortality
120% TDG	13.09 (n=1) 70% mortality	14.10 (n=1) 95% mortality		
Deep Water				
Control	11.21±0.21 (n=4)	10.65±0.19 (n=4)	10.44±0.20 (n=4)	
115% TDG	11.03 (n=1)	10.26±0.07 (n=2)	10.26±0.37 (n=2) 12% mortality	
120% TDG	11.18±0.07 (n=2)	11.87±1.56 (n=2) 4% mortality	10.99±1.25 (n=2) 30% mortality	
125% TDG	12.98 (n=1) 28% mortality	11.26±0.23 (n=2) 70% mortality		

Table 8.--Mean (±S.D.); Chloride values (mEq/L) of plasma pooled by tank (5 to 22 fish per tank) for monthly subsamples of fall chinook fry surviving shallow (10") and deep (8') water bioassays for control and experimental tanks (n = no. of groups).

	Exposure Time			
	28 day	56 day	84 day	112 day
Shallow Water				
Control (100 & 105% TDG as available)	170.6±2.8 (n=2)	143.7±4.4 (n=4)	142.8±4.2 (n=4)	140.0±2.5 (n=3)
Experimental (110, 115 & 120% TDG as available)	172.1±2.1 (n=2)	150.1±13.2 (n=3)	143.4±4.9 (n=2)	145.8±2.1 (n=2)
Deep Water				
Control (100, 105 & 110% TDG as available)	153.5±2.8 (n=2)	146.6±5.9 (n=3)	144.9±3.9 (n=4)	
Experimental (115, 120 & 125% TDG as available)	170.6 (n=1)	147.6±9.3 (n=5)	159.6±25.9 (n=6)	

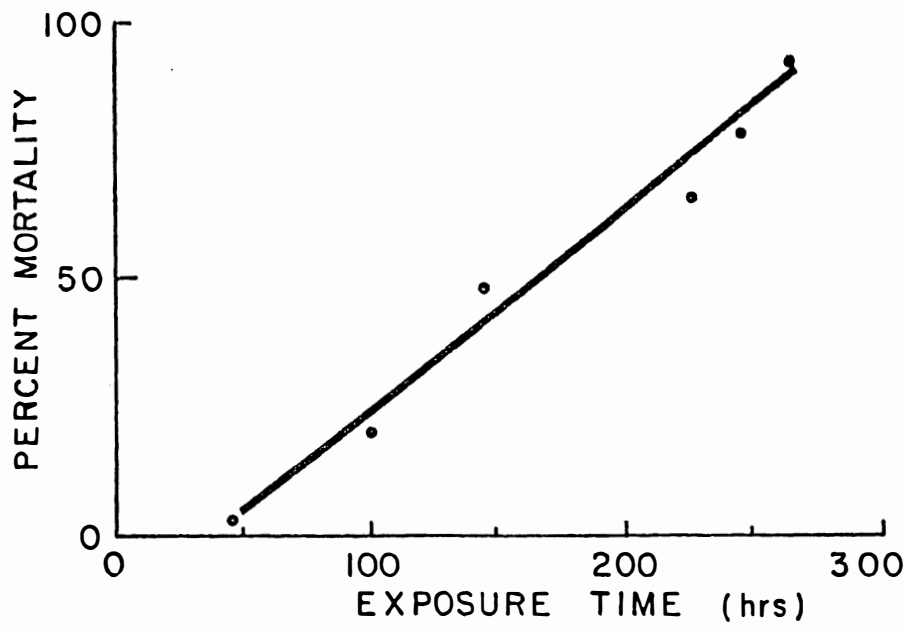

Figure 18.--Mortality versus time curves for juvenile fall chinook salmon exposed to 120% of saturation N_2 in shallow tanks (0.25 m) at 10°C.

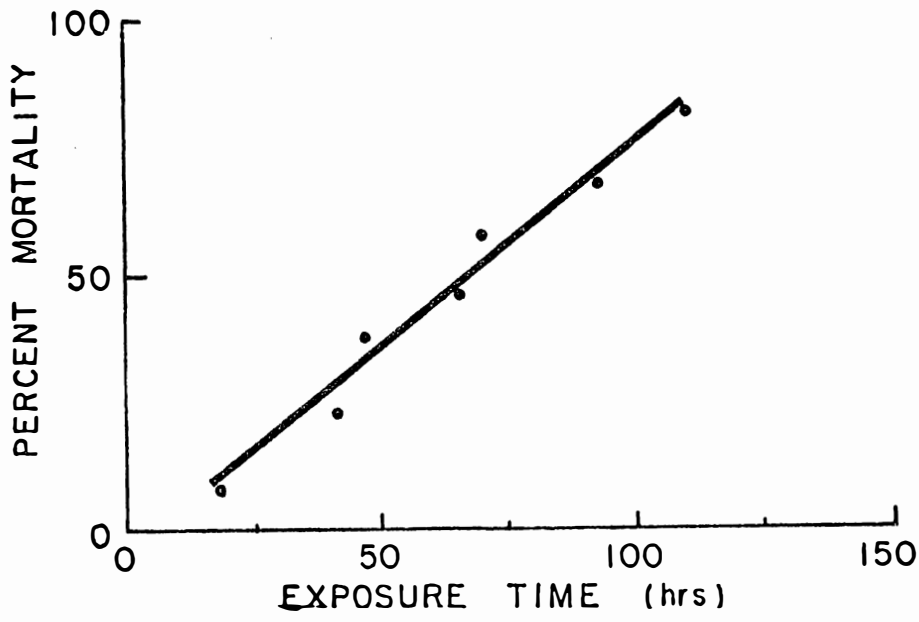

Figure 19.--Mortality versus time curves for juvenile steelhead trout exposed to 120% of saturation N_2 in shallow tanks (0.25 m) at 10°C.

calcium, magnesium or phosphate (Table 9). However, individual experimental fish sampled at the end of the bioassay did show lower values for cholesterol and albumin when compared to control fish. Calcium concentrations increased temporarily about thirty hours into the test then returned to control values by the fiftieth hour (Figure 20). This was probably the immediate response to resporatory acidosis from gas emboli lodged in the fish's circulatory system. Mean plasma chlorides of experimental fish was significantly lower than mean control values taken as a group (Table 9). Chlorides of experimental fish dropped dramatically in concentration after 50% mortality had been reached (Figure 21), and probably reflect the longer term compensation to respiratory acidosis by means of a "chloride shift" similar to that seen in man (Searcy, 1969).

Seven day test of juvenile steelhead

In a similar experiment with 30 juvenile steelhead (avg. size, 62 g and 171 mm) per tank, sub-samples (n = 3 each time) of surviving fish and all fish that lost equilibrium, were individually measured for calcium, chloride, albumin, potassium, phosphate and magnesium. There were significant changes in, chloride, phosphate, and potassium (Table 10). Chloride values were depressed after the 50% level of mortality was reached (Figure 22). As in the fall chinook test, a chloride shift caused by respiratory acidosis is suggested as the cause of the declining plasma chloride level. A similar depression at the same point in the experiment was noted in plasma albumin (Figure 23). Plasma calcium was depressed from initial values for most of the test (Figure 24). A significant and gradual increase in plasma

Table 9.--A statistical comparison (by group) of blood chemicals, experimental to control, for juvenile fall chinook exposed to 120% N_2 saturation.

Characteristic (and units of measurement)	Group	Mean	Range	Tukey's Quick Test Statistic N_2-N_1	N_1	T_3	Significance (α)
Chloride (mEq/L)	Control	137.5	123.4 to 150.2	5	19	9.5	.05
	Experimental	130.8	98.6 to 143.2				
Calcium (mg/100 ml)	Control	11.91	9.19 to 14.41	5	19	0	N.S.
	Experimental	11.73	8.64 to 15.52				
Potassium (mEq/L)	Control	5.66	4.73 to 7.06	2	15	3	N.S.
	Experimental	5.40	3.62 to 6.88				
Magnesium (mg/100 ml)	Control	2.16	1.67 to 3.20	1	12	5.5	N.S.
	Experimental	1.77	1.12 to 2.87				
Phosphate (mg/100 ml)	Control	14.07	10.47 to 16.77	5	19	0	N.S.
	Experimental	14.24	8.25 to 19.70				
Albumin (gm/100 ml)	Control	1.92	1.57 to 2.39	5	19	0	N.S.
	Experimental	1.81	1.29 to 2.81				
Total Protein (gm/100 ml)	Control	3.60	2.59 to 6.17	4	18	0	N.S.
	Experimental	3.38	2.49 to 7.29				
Cholesterol (mg/100 ml)	Control	315	207 to 417	5	19	0	N.S.
	Experimental	312	139 to 485				

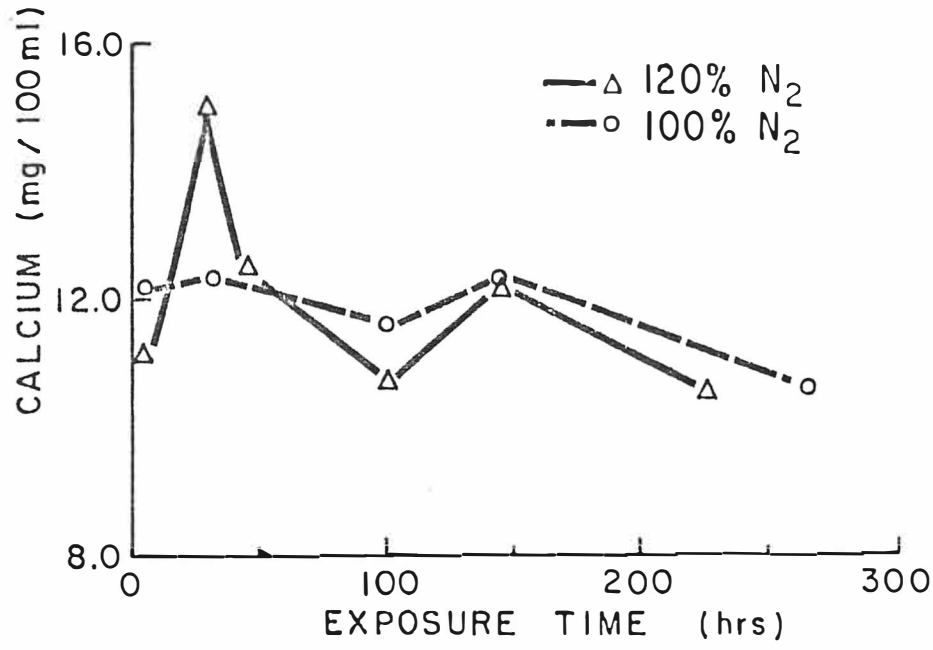

Figure 20.--Mean concentrations of Calcium versus time; for the blood of juvenile fall chinook salmon tested at 120% and 100% of saturation N_2.

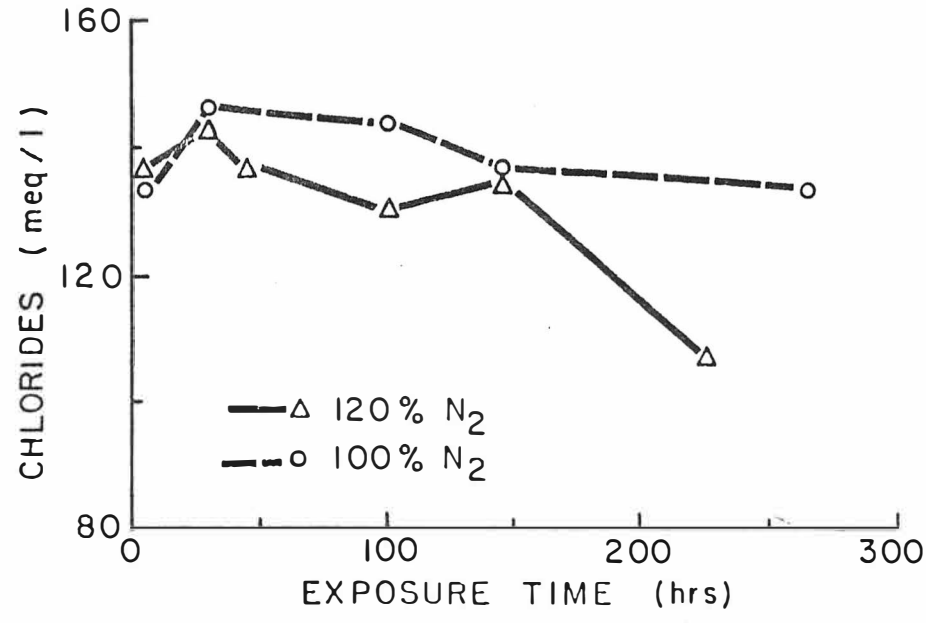

Figure 21.--Mean concentrations of chlorides versus time; for the blood of juvenile fall chinook salmon tested at 120% and 100% of saturation N_2.

Table 10.--A statistical comparison (by group) of blood chemicals, experimental to control, for juvenile steelhead exposed to 120% N_2 saturation.

Characteristic (and units of measure)	Group	Mean	Range	Tukey's Quick Test Statistic N_2-N_1	N_1	T_3	Significance (α)
Potassium (mEq/L)	Control	4.25	3.55 to 5.24	2	9	7.5	.05
	Experimental	4.90	4.08 to 5.98				
Magnesium (mg/100 ml)	Control	2.44	2.26 to 2.76	2	9	0	N.S.
	Experimental	2.46	2.24 to 2.77				
Chloride (mEq/L)	Control	137.6	133.0 to 143.5	2	9	7	.05
	Experimental	133.1	122.5 to 139.5				
Phosphate (mg/100 ml)	Control	13.87	11.46 to 15.82	2	9	7.5	.05
	Experimental	16.54	12.70 to 22.53				
Albumin (gm/100 ml)	Control	1.93	1.74 to 2.14	2	9	0	N.S.
	Experimental	1.83	1.08 to 2.40				
Calcium (mg/100 ml)	Control	9.50	8.93 to 10.07	2	9	0	N.S.
	Experimental	8.96	8.11 to 10.15				

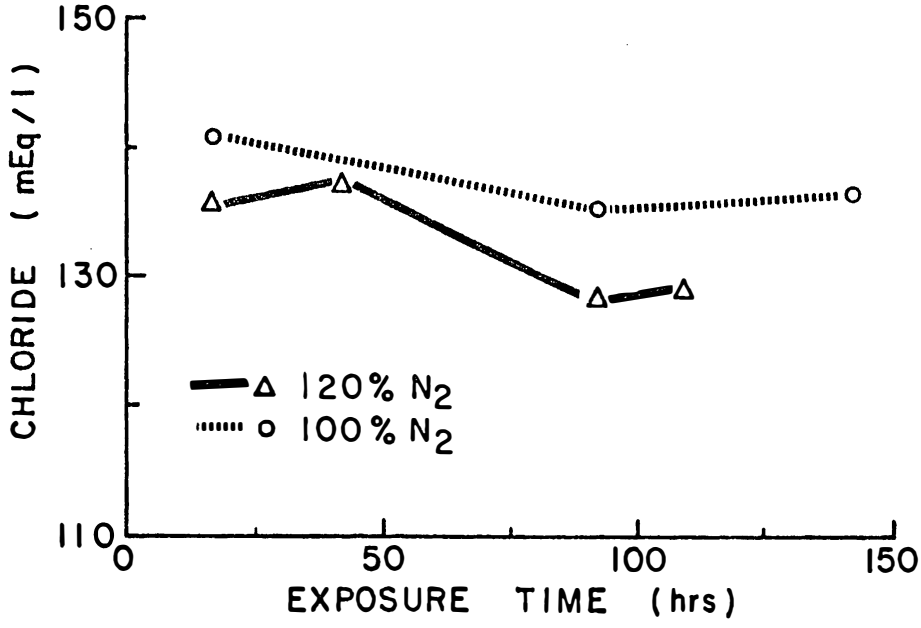

Figure 22.--Mean concentrations of Chlorides versus time; for the blood of juvenile steelhead trout tested at 120% and 100% of saturation N_2.

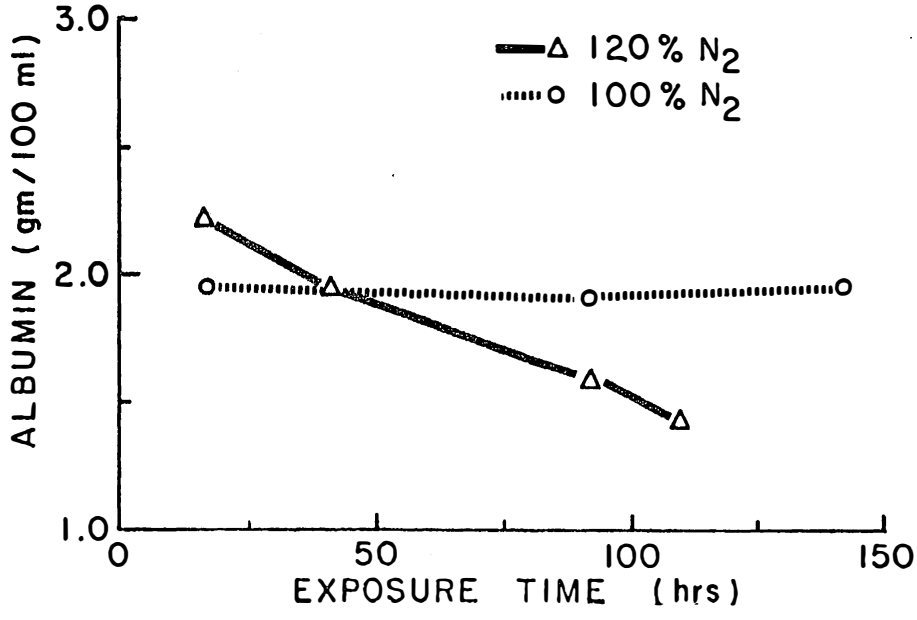

Figure 23.--Mean concentrations of Albumins versus time; for the blood of juvenile steelhead trout tested at 120% and 100% of saturation N_2.

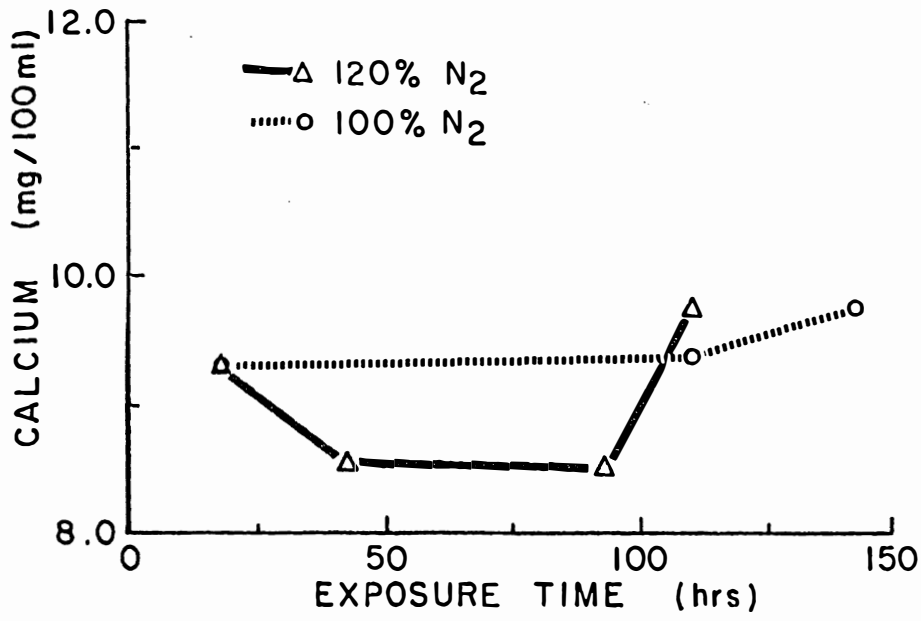

Figure 24.--Mean concentrations of Calcium versus time; for the blood of juvenile steelhead trout tested at 120% and 100% of saturation N_2.

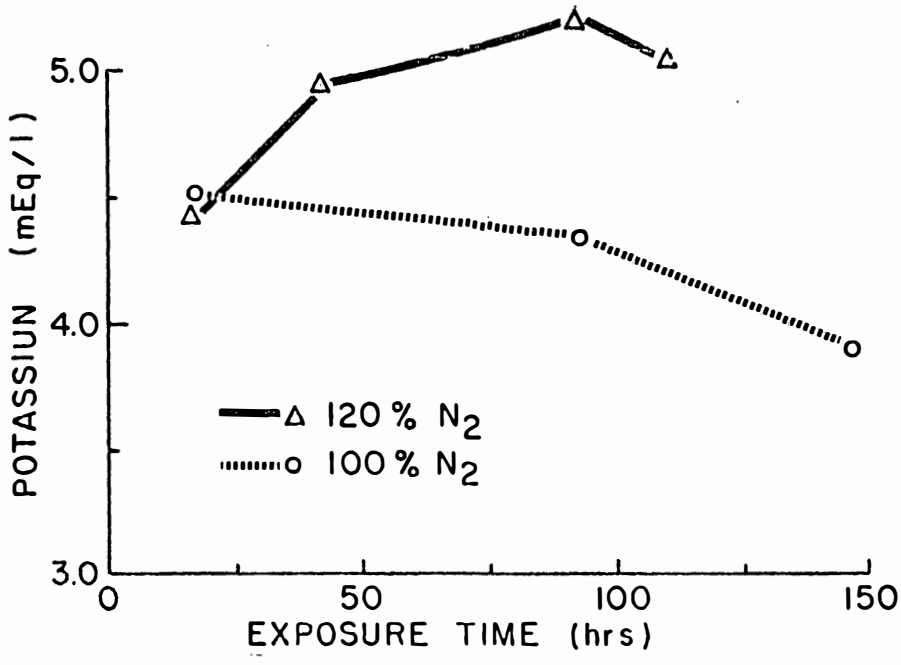

Figure 25.--Mean concentrations of Potassium versus time; for the blood of juvenile steelhead trout tested at 120% and 100% of saturation N_2.

potassium which also suggests respiratory acidosis, was noted throughout the first half of the test followed by elevated values persisting through the remaining half of the test (Figure 25).

An initial increase in phosphate was maintained throughout the first half of the test with a subsequent return to normal values (Figure 26).

Moribund steelhead (fish that lost equilibrium) were also sampled for blood. Moribund steelhead showed blood chemistry alterations including a 12% increase in albumin, a 50% increase in potassium, a 35% increase in phosphate, and a unique 40% increase in magnesium when compared to control steelhead (Table 11). They also showed a decrease in plasma chloride (5%) which was similar to that found in surviving steelhead exposed to 120% N_2. In similar fashion, increases in potassium and phosphate seen in experimental fish were also seen in the steelhead mortalities.

At this time several comparisons can be made between live juvenile steelhead and fall chinook in their blood chemistry responses to 120% N_2 saturation. A similar chloride response seems to be operating in both fall chinook and steelhead. The calcium response does not seem to be the same. It appears that fall chinook have a better homeostatic response to calcium depletion than do steelhead. Change in plasma albumin levels were only noted after long exposure; similar in both fall chinook and steelhead.

Observations of stress in the Snake and Columbia rivers

In 1974 we sampled the blood of juvenile steelhead and spring chinook as they passed through the Snake and lower Columbia River

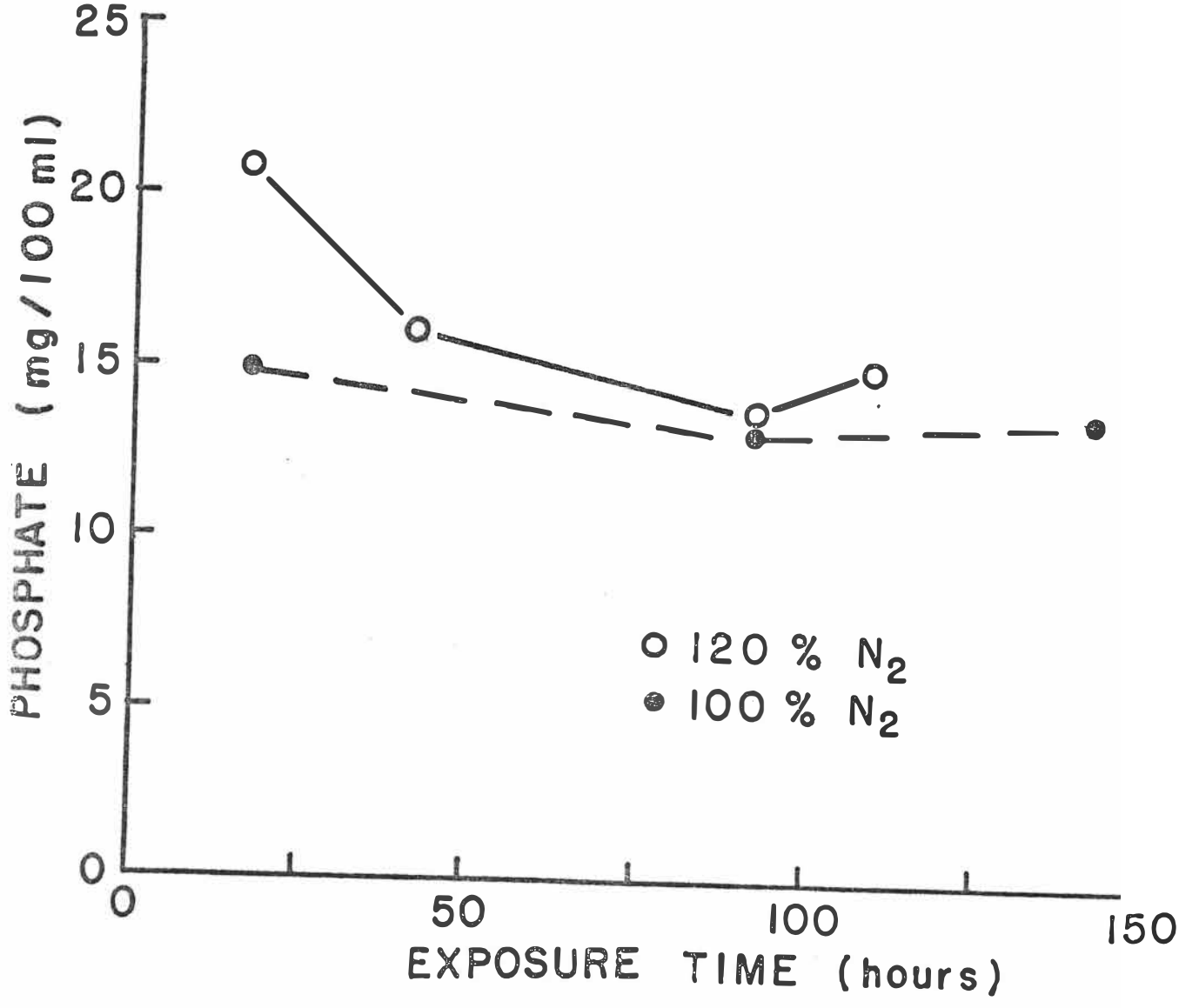

Figure 26.--Mean concentrations of Phosphate versus time; for the blood of juvenile steelhead trout tested at 120% of saturation N_2.

Table 11.--A statistical comparison (by group) of blood chemicals, experimental to control, for moribund juvenile steelhead exposed to 120% N_2 saturation.

Characteristic (and units of measure)	Group	Mean	Range	Tukey's Quick Test Statistic N_2-N_1	N_1	T_3	Significance (α)
Potassium (mEq/L)	Control	4.25	3.55 to 5.24	1	8	17	.001
	Moribund	6.57	5.29 to 8.19				
Magnesium (mg/100 ml)	Control	2.44	2.26 to 2.76	1	8	17	.001
	Moribund	3.47	3.09 to 3.92				
Chloride (mEq/L)	Control	137.6	133.0 to 143.5	1	8	7	.05
	Moribund	132.4	122.5 to 139.0				
Phosphate (mg/100 ml)	Control	13.87	11.46 to 15.82	2	7	14	.001
	Moribund	18.71	15.71 to 22.64				
Albumin (gm/100 ml)	Control	1.93	1.74 to 2.14	1	8	11.5	.01
	Moribund	2.49	1.92 to 2.77				
Calcium (mg/100 ml)	Control	9.50	8.93 to 10.07	1	8	0	N.S.
	Moribund	9.73	8.84 to 11.13				

dams to correlate chemistry changes with stress to response supersaturation.

Techniques of analysis were similar to those used in the laboratory. Blood chemistry changes in steelhead (Table 12) similar to those observed in the laboratory did occur. For example, serum calcium was depressed 22% in the Ice Harbor sub-sample relative to the Little Goose group. Subsequent downstream measurements of calcium show gradually increasing values. Phosphate show the same overall pattern as serum calcium, while A.P-ase shows a significant depression in the Ice Harbor sub-sample with values returning to the levels measured at Little Goose by the time the fish arrived at McNary and The Dalles. Potassium shows a 19% increase in the Ice Harbor group when compared to Little Goose fish. Downstream sub-samples at McNary and The Dalles show levels of potassium similar to those levels obtained at Little Goose Dam.

Blood chemistry changes were also observed in samples analyzed from migrating spring chinook. These changes also agreed with those observed in laboratory tests (Table 13); i.e., serum calcium and serum chloride decreased as fish migrated downstream.

Discussion

Our goal of using blood chemistry changes in juvenile salmonids to predict degree of stress from gas supersaturation has proven to be over ambitious. However, several points can be made on the basis of these tests.

First it is obvious that the stress (nitrogen supersaturation) response of juvenile steelhead is different in degree if not also

Table 12.--Juvenile Dworshak steelhead blood chemistry levels in response to various stressors including nitrogen supersaturation as they passed through the Snake & Lower Columbia River dams.

Blood characteristic	Units of measure	Dworshak hatchery 4-2-74	Little Goose 4-23-75	Ice Harbor 5-3-74	McNary 5-8-74	The Dalles 5-15-74
Calcium	(mg%)	11.97	10.45	8.20	9.10	9.65
Potassium	(mEq/L)	5.2	3.2	3.7	3.3	2.9
Sodium	(mEq/L)	156	162	151	145	130
Chloride	(mEq/L)	124	136	119	117	105
Phosphate	(mg%)	16.1	13.4	10.9	11.6	12.7
Glucose	(mg%)	124	118	165	195	333
Urea	(mg%)	2.0	2.5	5.0	3.0	0.5
Uric Acid	(mg%)	0.5	1.2	0.6	0.8	0.6
Cholesterol	(mg%)	265	158	145	205	195
Total Protein	(gm%)	3.7	2.5	2.4	2.8	2.9
Total Bilirubin	(mg%)	0.1	0.2	0.4	0.4	0.3
A.P-ase	(mU/ml)	248	78	45	83	93
LDH	(mU/ml)	450	460	1090	510	705
SGOT	(mU/ml)	474	< 600	< 600	< 600	< 600

Table 13.--Juvenile Rapid River spring chinook blood chemistry levels in response to various stressors including nitrogen supersaturation as they passed through the Snake & Lower Columbia River dams.

Blood characteristic	Units of measure	Rapid River Hatchery 4-4-74	Little Goose 4-23-74	Ice Harbor 5-2-74	McNary 5-7-74	The Dalles 5-14-74
Calcium	(mg%)	10.43	10.10	9.40	9.35	9.15
Potassium	(mEq/L)	3.4	2.8	2.6	3.4	3.5
Sodium	(mEq/L)	151	146	140	136	136
Chloride	(mEq/L)	122	119	101	108	115
Phosphate	(mg%)	12.3	11.4	11.2	11.1	10.1
Glucose	(mg%)	106	103	260	110	90
Urea	(mg%)	1.3	1.8	2.5	2.0	3.0
Uric Acid	(mg%)	0.4	0.8	0.5	0.7	0.2
Cholesterol	(mg%)	464	402	380	345	253
Total Protein	(gm%)	4.2	3.4	3.5	3.4	2.9
Total Bilirubin	(mg%)	0.1	0.5	0.5	0.5	0.3
A.P-ase	(mU/ml)	194	98	75	78	50
LDH	(mU/ml)	500	540	625	625	565
SGOT	(mU/ml)	600	$<$ 600	$<$ 600	$<$ 600	$<$ 600

in kind from that of juvenile chinook. The only change of blood chemistry which is common to most of the tests is the depression of chloride (Table 14). Even here, the response is not elicited in juvenile chinook exposed for 35 days to 116% nitrogen saturation, while it is demonstrated in juvenile steelhead under the same conditions. Chloride depression can be seen at 120% nitrogen saturation in both juvenile fall chinook and steelhead. As this "chloride shift" is usually the secondary result of the fish's body being unable to properly eliminate carbon dioxide from the gills, it is fair to assume that its occurrence implies a functional blockage of part or all of the gill capillaries, or some upstream portion of the fish's blood system such as the capillaries of the kidney, liver or musculature.

The second point is that there are other blood characteristics which consistently change in a particular direction in response to nitrogen stress. This is best shown in juvenile steelhead where albumin, calcium, phosphate and potassium responses are in the same direction, in both the 120% N_2 12 day and the 116% N_2 35 day tests. Potassium and phosphate have predictive value (Table 10). In laboratory tests chinook show calcium increase to nitrogen supersaturation stress, while spring chinook show the opposite response, but test design does not really allow a proper comparison.

Third, the blood chemistry responses to supersaturation measured in the laboratory can be certified in field experiments. This is best shown from Rapid River chinook, where serum chlorides were significantly depressed in samples from Ice Harbor and McNary dam and

Table 14.--A comparison of juvenile chinook and steelhead blood chemistry changes from laboratory and field analyses.

	Blood characteristic							
	Albumin	Calcium	Cholesterol	T. Protein	Phosphate	Potassium	A.P-ase	Chloride
Chinook:								
74 field season Rapid River stock Ice Harbor & McNary samples/Little Goose sample (135 to 120% TDG)		↓	—	—	—	↓	—	↓
120 % N_2 (117% TDG) F. Chinook Subsampled with time at LE_{50}	—	↑	—	—	—	—		↓
4 mo. bioassay F. Chinook Button-Up Fry 110 % N_2 @ 10" H_2O 115 % N_2 @ 8 ft. H_2O 1 mo. subsamples		↑						—
35 day exposure S. Chinook 116 % N_2 (110 % TDG)	—	↓	—	—		—	—	—
Steelhead:								
74 field Season Dworshak stock Ice Harbor & McNary samples/Little Goose sample (135 to 120% TDG)		↓	—	—	↓	↑	↓	↓
120 % N_2 (117% TDG) Subsampled with time at LE_{50}	↓	↓			↑	↑		↓
35 day exposure or LE_{50} 116 % N_2 (110 % TDG) terminal samples @ 112 % TDG only	↓ @	↓	↓	↓	↑	↑	↓	↓

——— No change relative to control or non-nitrogen related change.

slightly depressed in The Dalles sample (relative to branded Rapid River chinook collected at Little Goose Dam), Table 13. The diagnosis of gas bubble disease on the basis of a depressed serum cholride is confirmed by cutaneous fin blisters, which is one of the first signs of gas bubble disease. Rapid River chinook also show depressed serum calcium values in the Ice Harbor, McNary, and The Dalles dams samples (relative to branded Rapid River chinook taken at Little Goose Dam). This compares favorably with serum calcium changes noted in spring chinook at the end of 35 days exposure at 116% N_2 supersaturation (Table 6).

The fourth point is that decrease in chloride and calcium were caused by an increase in TDG (110 to 113%) at the same nitrogen supersaturation (116%) (Table 5) which is corroborated by mortality of juvenile steelhead in these tests.

Fifth, while insufficient experiments have been done with blood chemistry to correlate change in a given blood chemistry characteristic with increasing stress to all levels of supersaturation some bracketing of prior exposure can be attempted for juvenile steelhead challenged with 120% TDG. From figures 22 through 25, it can be seen that increases in potassium and possibly decreases in calcium occur by the fourteenth hour of the bioassay, while meaningful changes do not occur in serum albumin and chloride until later, possibly as late as the 93rd hour of the test. This allows us to discriminate between a mortality of 25% (40 hours exposure) and one of 70% (93 hours exposure) (Figure 19) at a saturation of 120% TDG.

Lateral Line Function[5/]

One of the early signs associated with gas bubble disease in salmonids is the appearance of bubbles in the trunk portion of the lateral line system. Shallow tank bioassays (Dawley and Ebel, in press) evaluating the effect of supersaturated atmospheric gas on survival indicate that bubbles form in lateral line scale pockets after as short an exposure as 2 hours at a concentration of 130% of saturation. It was additionally noted that bubble formation occurred at concentrations ranging as low as 105% of saturation after extended exposure (24 days).

The occurrence of bubbles in the lateral line of salmonids at dissolved gas concentration and exposure times determined to be less than lethal, has led to speculation as to the effect of bubbles on the functioning of the lateral line system.

In assessing the effects of gas bubbles on the lateral line we characterized the response of over 150 afferent nerve fibers in 13 test (fish that had been stressed in water supersaturated with dissolved gas) and 30 control fish.

Juvenile steelhead trout were anesthesized with tricaine methane-sulfonate and immobilized with gallamine triethiodide administered intramuscularly. At the point of opercular arrest, the fish were placed in the test apparatus and water was forced over the gills at a rate of 1 l/min.

5/ A detailed report of this research is in press - Weber, Douglas and Mike Schiewe (Journal of Fish Biology - Academic Press, London, New York, San Francisco).

Water in the test chamber was adjusted to provide an unsubmerged area of 2 to 3 cm posterior to the operculum. Within this area the lateral line nerve bundle was exposed and placed over a silver electrode. An individual nerve fiber was then isolated and placed over a stainless steel hook electrode for recording.

Basis monitoring of nerve activity was accomplished by visual observation on a dual beam oscilloscope coupled to a loudspeaker. During nerve fiber characterization, the impulses from the isolated fiber were photographed for documentation and more detailed analysis.

The quantifiable measurement we employed for assessing lateral line performance is the ability of a sensory unit to respond to water displacement produced by a pulsating sphere at a 5 mm distance. The sound pressure level was approximately 25 decibel (DB) above threshold and 50 db above ambient tank noise for frequencies greater than 60 hertz (Hz); ambient noise obscured measurement below 30 Hz.

Test fish were placed in water with a total gas pressure of 118% of saturation. Signs of gas bubble disease began to appear in the trunk lateral line of stress fish within 2-6 hours. This experimental procedure enabled characterization of fibers whose trunk lateral line showed 3 distinct stages of gas bubble occurrence; no bubble formation, partial bubble formation, and complete bubble formation.

Under conditions where trunk lateral line bublles were not present all spontaneously active fibers recorded responded to light touch, surface waves, and pulsed water displacements generated by the glass bulb stimulator. In addition, the sensory units were readily located and spontaneous activity correlated with the number of scales

in the sensory unit. When gas bubble formation was complete along the entire length of the trunk lateral line the fibers proved either nonresponsive or responded only to direct, firm, pressure on one or two scales which did not relate to the spontaneous activity observed.

Of the 22 fibers examined during partial bubble formation, 50% were respondent to all stimuli, and no gas bubbles could be seen in any of these sensory units. For the remaining fibers monitored during partial bubble formation, spontaneous activity could not be matched with any corresponding scale units, and their reaction to firm pressure was similar to those fibers assessed during complete gas bubble formation—indicating that these nonrespondent fibers innervated receptors located in the occuluded areas.

Three additional steelhead trout were monitored for 3 consecutive days during which the fish were held in equilibrated water (100% saturation) the first and third days and supersaturated water the second day. This allowed characterization of fibers before, during, and after the presence of lateral line bubbles, to evaluate any residual effects that gas bubbles may have on the lateral line. Deleterious effects of the gas bubbles disappeared before 16 hours in equilibrated water except possibly for one parameter. There was a slight indication that fibers monitored following exposure were retarded in their ability to follow on a 1:1 basis the pulse frequence generated by the glass bulb stimulator.

Gas bubbles in the lateral line of stressed fish appear to arise from within the scale pocket. Gas released from the neuromast capillary bed in molecular form coalesce as bubbles on the inner

surface of the lateral line canal, and the enclosed structure of the canal does not allow the formed bubbles to be released. Upon return of stressed fish to equilibrated water, the lateral line gas bubbles slowly redissolve either by reabsorption through the tissues or in fluid exchanged from the external environment through the external cannaliculi.

Our studies indicate that gas bubbles present in the lateral line mechanically block this sensory system from responding to stimuli. It appears that one of the more important functions of the lateral line is for detection of underwater objects, whether they be predators, prey, or stationary objects, and, this gas bubble disease impairment of the lateral line system would have an adverse effect on survival.

Piddington (1971-1972) in his studies on goldfish, (*Carassius auratus*) aditory response has shown that a fish giving a sudden tail filp will generate a rarefaction wave forward and a compression wave backward, suggesting that a "listening" fish with capabilities of indiscriminating between the compression or rarefaction phase of a wave could detect another fish approaching or recording. Stober (1969) noted that the sudden swimming movement of cutthroat trout, (*Salmo clarki*), resulted in and acoustic "thump" in the range of 100-150 Hz. This frequency is well within the range detectable by the lateral line, and if the receiver fish is within the near field area (e.g., approximately 3 m at 100 Hz, Tavolga, 1971) the lateral line may be a necessary sensory modality in predator-prey relationships.

We believe that occusion of the lateral line with gas bubbles causes more than normal predation on juvenile salmonid migrants in the Columbia and Snake Rivers. A manuscript of this research has been accepted for publication (Weber and Schiewe; in press).

EFFECTS OF OXYGEN/NITROGEN RATIO

Research was conducted by Rucker (1974) to determine the effects of differential O_2/N_2 ratios on juvenile coho salmon. He found that time of survival decreased as the ratio of dissolved nitrogen to oxygen increased. The TDG was maintained at 119% of saturation, a replicate series of tests was run with gas levels at: 90% N_2 and 229% O_2; 100% N_2 and 192% O_2; 105% N_2 and 73% O_2; 109% N_2 and 150% O_2; 121% N_2 and 114% O_2; 131% N_2 and 75% O_2 and 138% N_2 and 50% O_2. Time to 50% mortality drastically decreased when the N_2 gas concentration exceeded 109% saturation. We believe this study adequately covered the effects of varying ratios of N_2 and O_2 and further study was not pursued.

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Appendix I Results of bioassay tests done in 175 L tanks; including constant and intermittent exposure Prescott Field Station 1972 to 1975.

	PERCENT N_2 SATURATION									
	130			120			110			100
	(Hours exposure each 24 hour cycle)									
Species	24	16	8	24	16	8	24	16	8	24
	Time in hours to 50% Mortality									
Crappie	(20)[1/] 192.0	N.M.	N.M.	N.M.	N.M.	N.M.	N.M.	N.M.	N.M.	N.M.
Largemouth bass	120.0			N.M.			N.M.			N.M.
Largemouth bass	102.0	N.M.	N.M.	N.M.	N.M.	N.M.	N.M.	N.M.	N.M.	N.M.
Largemouth bass	93.5 (40)	165.5	N.M.	N.M.	N.M.	N.M.	N.M. (40)	141.5	N.M.	N.M.
Rainbow	70.5			3.13			N.M.			N.M.
Rainbow	47.0	69.5 (40)	149.5	141.5	N.M.	N.M.	N.M.	N.M.	N.M.	N.M.
Rainbow	31.0 (10)	24.0 (20)	30.0	N.M.	N.M.	N.M.	N.M.	N.M.	N.M.	N.M.
Chinook	64.0 (30)	184.0	N.M.	N.M.	N.M.	N.M.	N.M.	N.M.	N.M.	N.M.
Chinook	24.0	120.0 (10)	160.0	N.M.	N.M.	N.M.	N.M.	N.M.	N.M.	N.M.
Coho	26.0	46.0	102.0	59.0 (20)	190.0	N.M.	N.M.	N.M.	N.M.	N.M.
Coho	22.0 (40)	166.0 (30)	150.0 (10)	118.0	94.0	N.M.	N.M.	N.M.	N.M.	N.M.
Cutthroat	24.0	72.0	103.5	119.0 (20)	143.0	N.M.	N.M.	N.M.	N.M.	N.M.
Whitefish	23.5	23.5	N.M.	95.5	N.M. (10)	191.5 (30)	191.5 (30)	191.5 (10)	(31.5) (20)	191.5
Whitefish	23.0			50.5			N.M.			N.M.
Whitefish	21.5	21.5	99.0	69.5	145.5	181.5 (20)	45.5	N.M.	N.M.	N.M.
Steelhead	16.0	16.0	N.M.	72.0	N.M.	N.M.	N.M.	N.M.	N.M.	N.M.
Steelhead	16.0									N.M.
Steelhead	23.5									N.M.
Smelt	5.5			30.0			N.M.			N.M.

1/ Percent mortality that occurred in time shown

N.M. = No mortality