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Pressure in the early life history of sockeye salmon

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General discussion of the effect of pressure

Discussion of the effect of pressure on behavior

Discussion of the effect of pressure on the swim-bladder with the results of different measurements on the swimbladder

Pressure tolerance of sockeye

Decompression effects

Effects of changes in the rate of increase and decrease in pressure

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PRESSURE IN THE EARLY LIFE HISTORY OF SOCKEYE SALMON

by

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B.Sc. University of Manitoba, 1953

M.Sc. University of Manitoba, 1956

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

in the

DEPARTMENT OF ZOOLOGY

We accept this thesis as conforming to the standard required from candidates for the degree of DOCTOR OF PHILOSOPHY

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Members of the Department of Zoology

The University of British Columbia

August, 1963

ABSTRACT

Young sockeye salmon (Oncorhynchus nerka) may occupy the epilimnion, thermocline or hypolimnion during lacustrine residence and may make extensive vertical migrations. Residence and migration over a range of pressure presents fish with certain physiological problems. Sockeye salmon meet these problems by adaptation, compromise and fortuity. Sockeye evidenced a tolerance to pressure in excess of 20 atmospheres, equivalent to a depth of water of 680 feet. Sockeye fry showed no behavioral response to pressure prior to initial filling of the swimbladder, but thereafter pressure induced compensatory swimming. Young sockeye proved to be dependent on atmospheric air for inflation of the swimbladder. The restrictions to vertical movement imposed by the swimbladder are minimized in sockeye by a relatively small bladder volume, little excess pressure within the bladder, the bladder being thin-walled and extensible and the inability of these fish to secrete gas into the bladder. When frightened, young sockeye sounded and expelled gas from the swimbladder. Gas expulsion was found to be under adrenergic control and retention of gas in the swimbladder under cholinergic control. During decompression with upward movement through thermally stratified water, gas disease or the "bends" is obviated

by the rapid clearance of dissolved nitrogen from the blood stream. Young sockeye showed a tolerance to rapid decompression except under conditions permitting swimbladder gas to appear as emboli in the blood stream.

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This study was supported financially by the International Pacific Salmon Fisheries Commission. For this support the author is deeply grateful.

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CHAPTER I

INTRODUCTION

The sockeye, Oncorhynchus nerka, in several respects is unique among the Pacific salmon. The adult fish typically spawn in streams flowing into lakes but may utilize outlet streams or the bottom of the lake itself. The eggs are deposited in the autumn within a nest or redd in the stream gravel, hatch during the winter and emerge from the gravel as fry in the spring. The fry most commonly are carried downstream into a lake, but may swim upstream if spawned in the outlet; or if spawned on the lake bottom, they stay within the lake. Within the Fraser River system, young sockeye usually remain one year in lake residence; occasionally they will remain two years but rarely zero or three years. Typically sockeye migrate from the lake environment in the spring of the year at a length of perhaps three inches. On leaving fresh water, the young sockeye enter the ocean and remain there generally two but occasionally one or three years. The adult fish return to fresh water late in their third year, spawning four years after having been deposited in the gravel.

The period of lake residence is unique to young sockeye and as a consequence sockeye frequently face problems quite unlike those of other species of salmon. The lake

habitat, for example, makes available to sockeye a vertical dimension in fresh water not accessible to stream-dwelling coho (O. kisutch) and chinook (O. tshawytscha) salmon, or to pink (O. gorbuscha) and chum (O. keta) salmon which leave fresh water as fry. Within this vertical and sometimes stratified habitat, sockeye are capable of occupying the surface (Foerster, 1925), the epilimnion (Ricker, 1937), the region of the thermocline (Ricker, 1937), or the hypolimnion (Krogius and Krokhin, 1948) as food supply dictates. Living over a wide range of depths requires concurrently living over a range of pressures. Pressure and pressure changes accompanying vertical movements present sockeye with a number of problems. These include: tolerance to positive pressure, tolerance to decompression, initial filling of the swimbladder, maintenance of neutral buoyancy or plane of least effort, gain and loss of swimbladder gas, regulation of compensatory swimming, manner of escape response and gaseous equilibration with the environment. The purpose of this study was to determine how these problems are met by young sockeye salmon.

There exists now an extensive and widely scattered literature on the perception and response of fish to pressure and the effects of pressure on fish. This literature was assembled and reviewed concommitant to the experimental

study and is included here. This will be presented following the experimental study on sockeye salmon.

The thesis is advanced that sockeye salmon meet the problems accompanying residence and movement over a range of pressure by adaptation, compromise and fortuity, by means that are anatomical, physiological and ethological.

CHAPTER II

METHODS

General

The study of pressure in the early life history of the sockeye salmon, Oncorhynchus nerka, was conducted in the main at the field laboratory of the International Pacific Salmon Fisheries Commission, at Cultus Lake, British Columbia. Cultus Lake (Fig. 1.) has an endemic race of sockeye salmon which for many years has been enumerated both during downstream smolt and upstream adult migrations. The counting weir is located on Sweltzer Creek close to the lake outlet. Seaward migrating smolts enter a trap in the weir, usually within minutes after leaving the lake. Experimental animals were obtained from this trap. The bulk of the downstream migration takes place during the months of April and May but lesser numbers of smolts were trapped during March, June and July (Fig. 2). For this reason most of the experimental studies were conducted between March and July during 1959, 1960 and 1961.

Certain aspects of the study did not require actively migrating fish and hence a number of smolts were held beyond the time of downstream migration, to extend the period of investigation. Sockeye eggs were hatched in the Cultus Lake Trout Hatchery and reared to smolt size. Studies on the

effects of pressure from fry to smolts were conducted on these fish. Studies involving dissolved nitrogen in fish blood were performed on rainbow trout, Salmo gairdneri, from the Cultus Lake Trout Hatchery.

Much of this study was conducted using a large pressure chamber (Fig. 3), illustrated diagramatically (Fig. 4). This consisted of a steel cylinder three feet in length and one foot in diameter. One end was fitted with a removable flange secured by bolts. Water of predetermined temperature was drawn from a reservoir beside the apparatus. Pressure was applied by means of a pump and regulated with valves and a by-pass over the range of 0 to 300 psi (pounds per square inch) above atmospheric pressure, the equivalent of 0 to 680 ft of hydrostatic head.

Pressures below atmospheric were investigated by means of a smaller cylinder, connected to the top of the pressure chamber, in which a vacuum was produced using a second pump. This provided negative pressures over a range of 0 to 29.5 in Hg of vacuum, or 12 to 760 mm Hg absolute pressure. Positive and negative pressures were measured with a Statham Pressure Transducer (range 0 to 500 psi absolute) installed in the wall of the chamber. Magnitude of positive and negative pressure and rate of change of

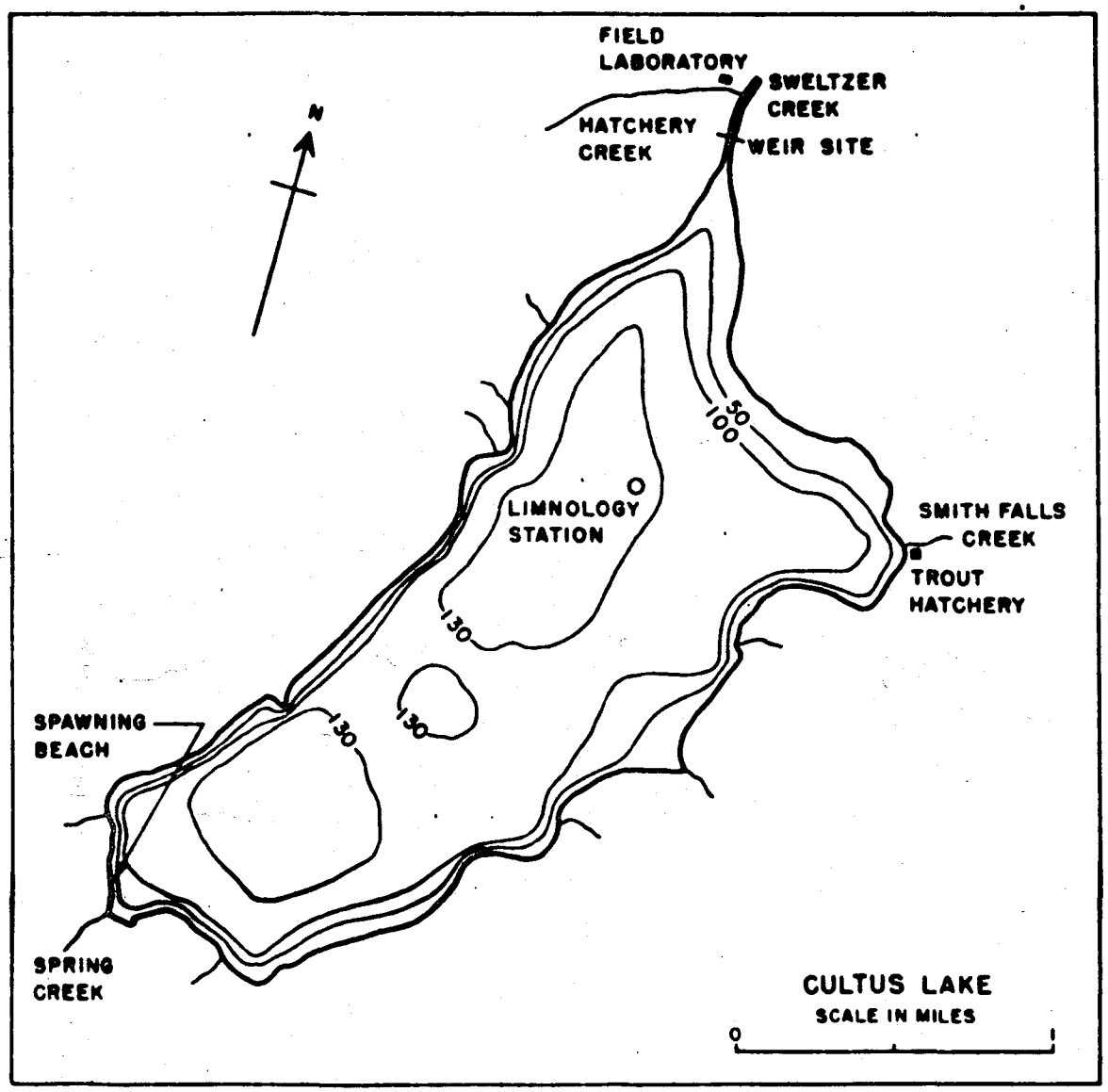


Figure 1. Cultus Lake, British Columbia, site of the investigation of pressure in the life history of the sockeye. After Ricker (1937).

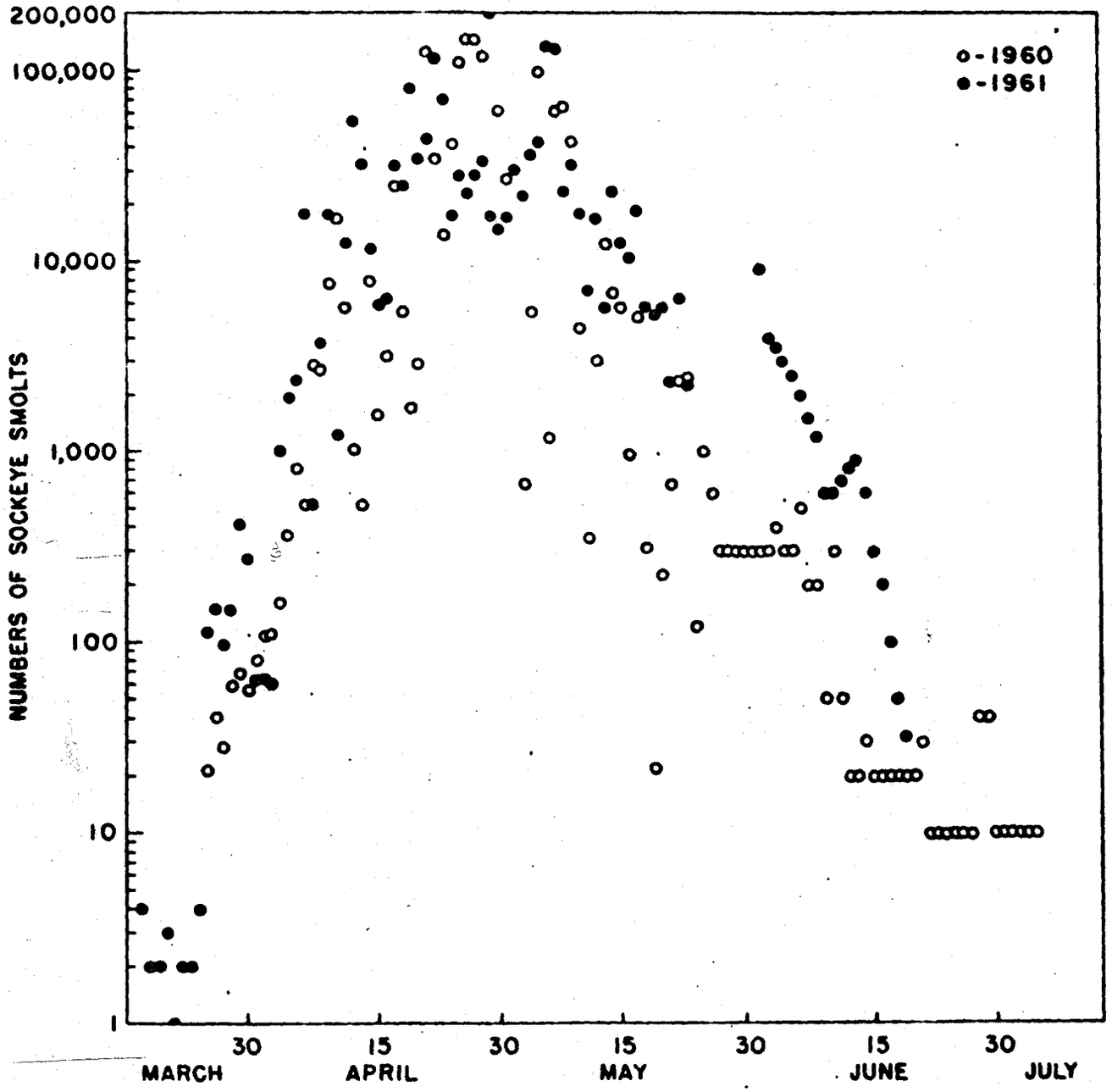


Figure 2. Daily migration of sockeye smolts from Cultus Lake during two years of the study. Note: Identical values in 1960 are estimates based on partial counts.

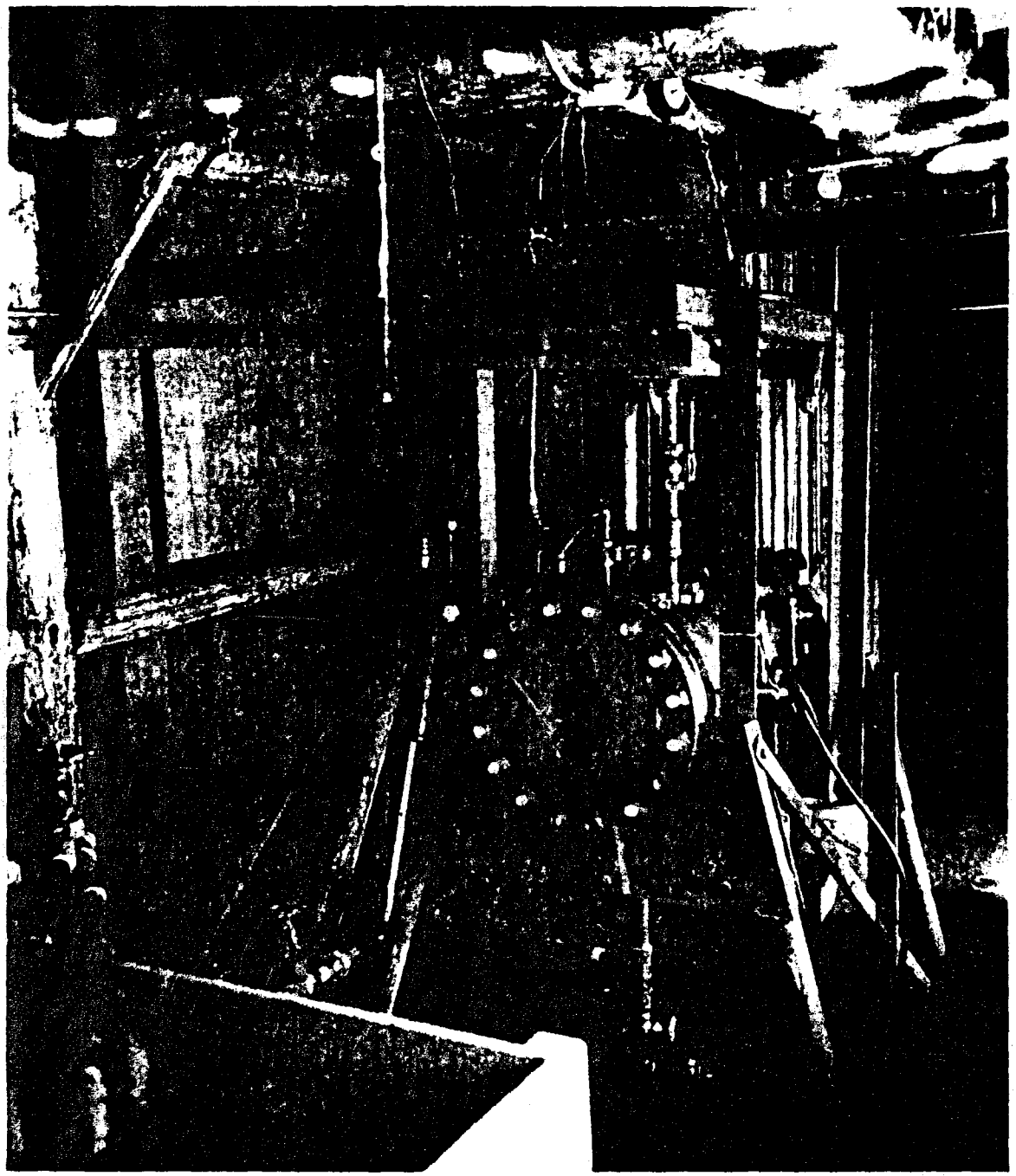


Figure 3. Pressure chamber located close to the weir on Sweltzer Creek.

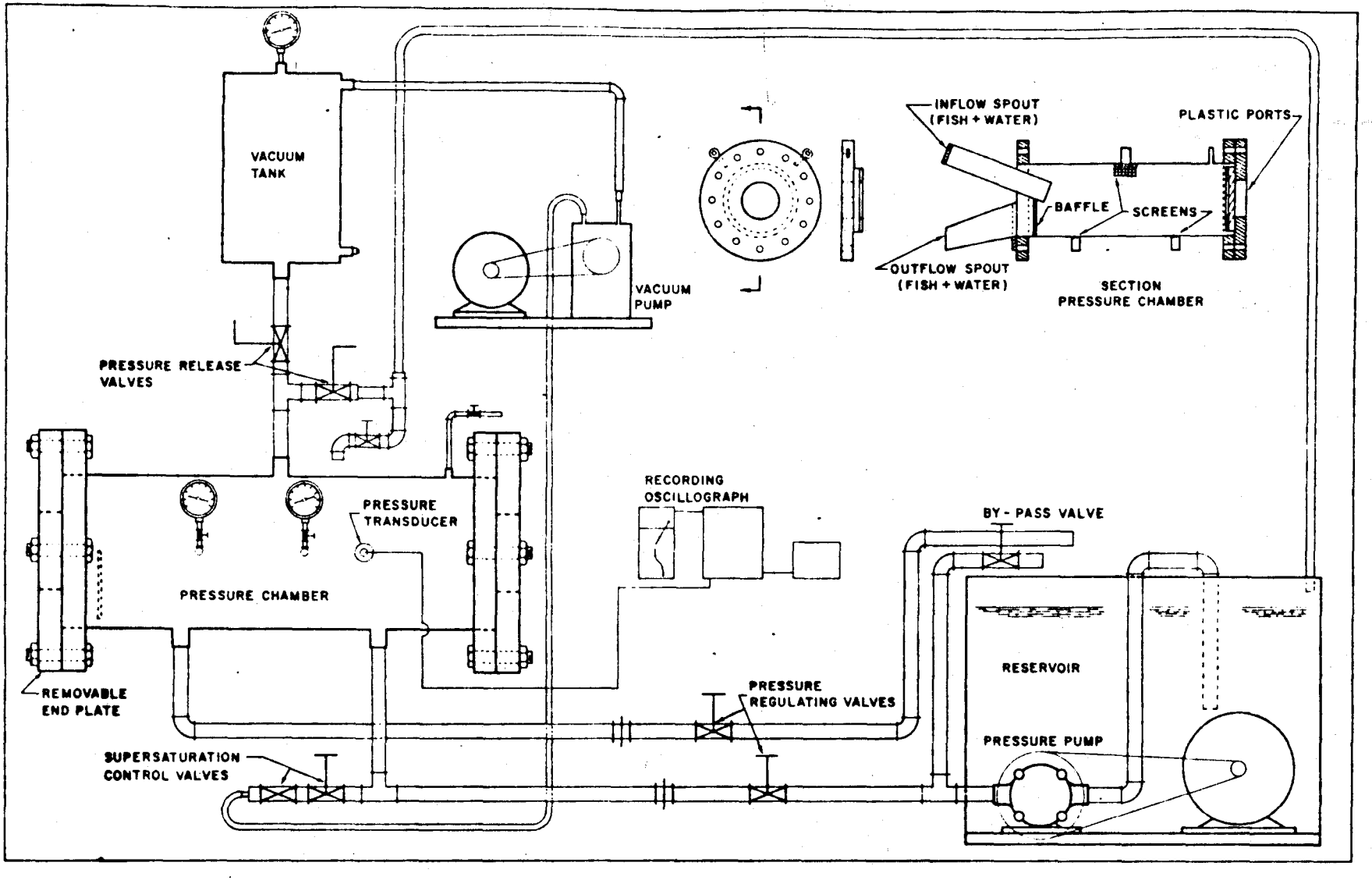


Figure 4. Diagrammatic representation of pressure apparatus.

pressure were recorded on a Brush Oscillograph linked through an amplifier to the pressure sensitive transducer.

Fish were poured into the opened chamber through a removable spout. The chamber was held half-filled with water during this procedure by a low, hinged baffle close to the removable flange. Fish were kept free of inflow and outflow vents by fine screens. Once the fish were inside the chamber the removable flange was secured in place. Pressure within the chamber was observed on one of three gauges: 0 to 15 psi, 0 to 50 psi or 0 to 400 psi, depending on the magnitude of pressure under study. The apparatus permitted conditions of either static pressure or water flowing through the chamber under pressure. The volume of the chamber (67 liters) was sufficient to allow a small number of smolts to be held several hours under static water conditions. Also under static conditions the gas content of the water in the chamber could be increased to 140 per cent of air saturation by pumping air under pressure into the bottom of the chamber.

The ends of the chamber were fitted with plastic ports 6 in in diameter, making possible observations on the fish within the chamber. Some difficulty was experienced in viewing fish smaller than smolts. Such fish were placed in a 2- or 4-liter glass beaker, the mouth of which was covered

with a fine screen and the beaker inserted into the chamber. With the base of the beaker against the viewing port it was possible to observe small fish close at hand. Fish were removed from the pressure chamber by first draining the water to the level of the internal baffle, removing the end flange, attaching a second spout and raising the hinged baffle. Water plus fish poured out into a container held at the base of the spout.

Fish-holding facilities consisted of troughs and ponds at the Cultus Lake Trout Hatchery, 36 holding boxes floated in Sweltzer Creek, portable holding cages placed in nearby Hatchery and Spring Creeks, and a number of 40- and 75-liter plastic tubs located at the field station. The latter were placed in a large, temperature-regulated water bath.

Pressure in Relation to Behavior

Pressure above atmospheric induced compensatory swimming, that is, increased upward swimming in response to negative buoyancy. This was apparent from the marked change in posture of the animal (Figs. 5 & 6). The angle of inclination to the horizontal increased with increasing pressure. Concurrently young sockeye made increased use of the pectoral fins in maintaining position. This beating of the pectorals was

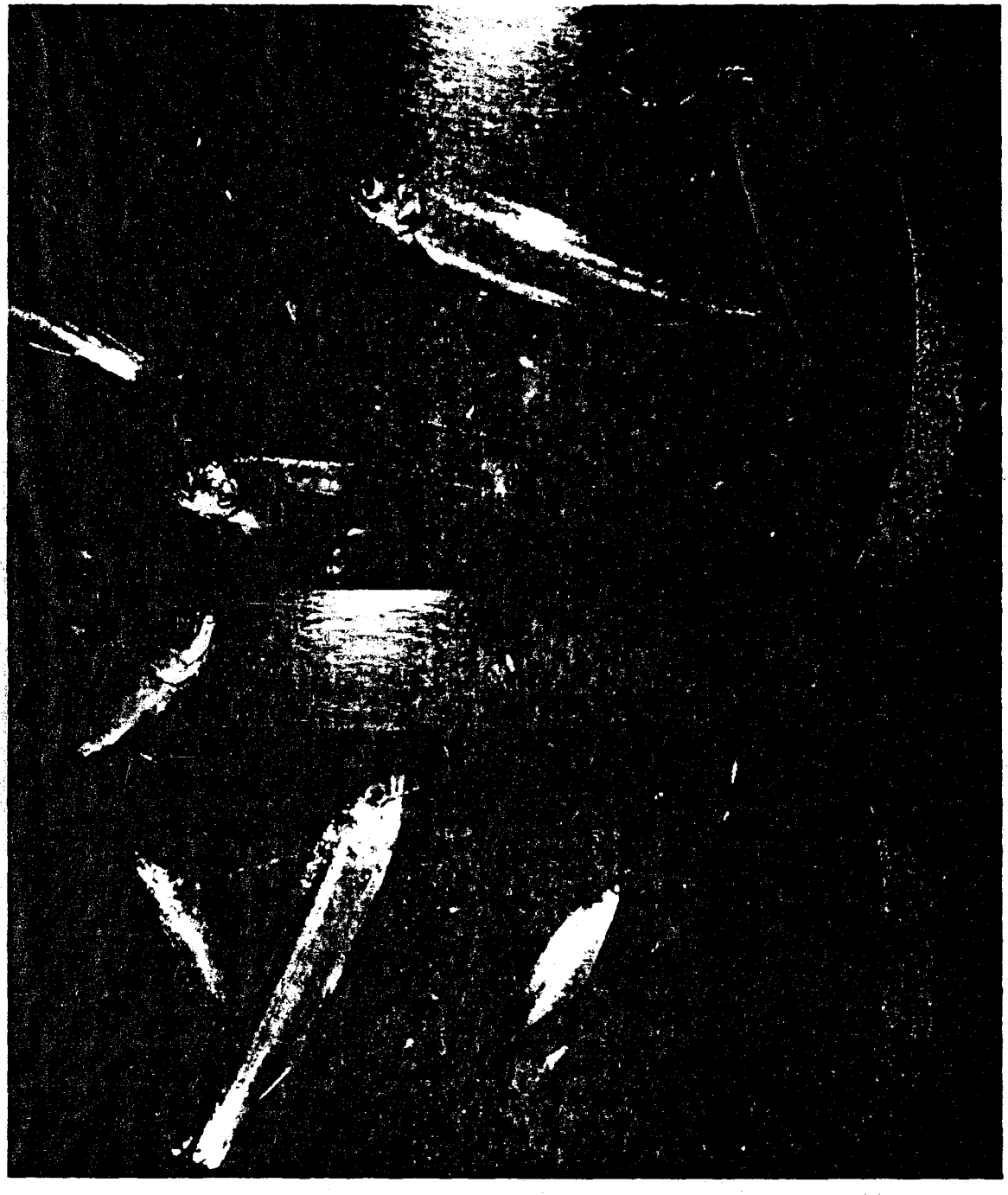


Figure 5 Characteristic posture of sockeye salmon smolts at atmospheric pressure (upper) and at a pressure of 50 psi above atmospheric (lower).

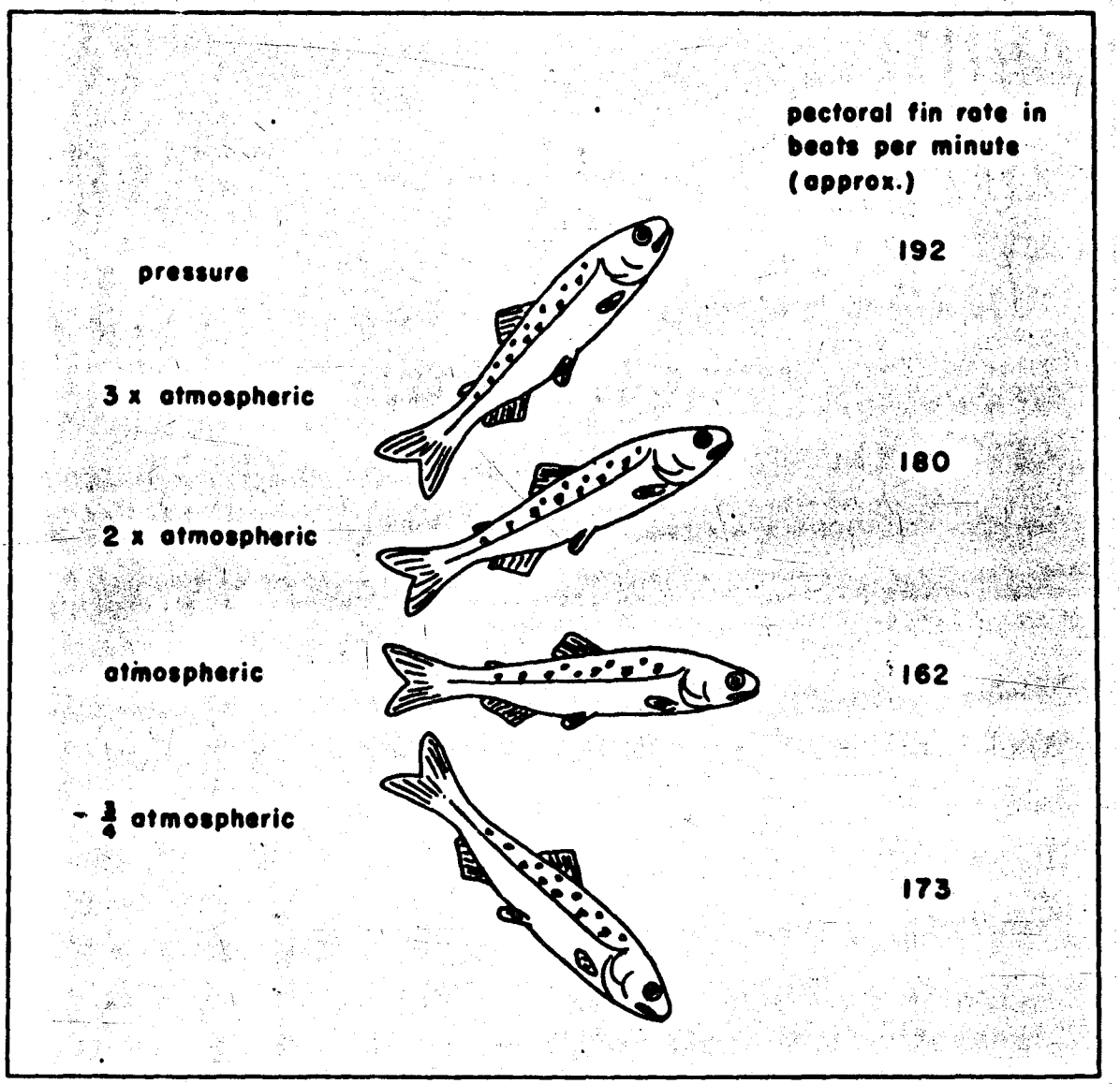


Figure 6. Posture and rate of pectoral fins of a smolt at pressures above and below atmospheric. Note: this smolt failed to emit gas at pressures below atmospheric and swam downward in response to positive bouyancy.

quantified by timing with a stop watch, to the nearest 0.1 sec, the time required for 20 strokes of the pectoral fins of a given fish. Fish were tested in samples of 2 or 3 easily identifiable individuals. Pectoral fin counts were made at 5 psi intervals from 0 to 50 psi, at 10 psi intervals from 50 to 100 psi and at 50 psi intervals from 100 to 250 psi. Counts were made only when individual fish were actively swimming upward. Nevertheless considerable variation was observed among repeated counts on the same fish. In practice the pressure was raised after pectoral counts had been made on each fish in the group. This required 5 to 20 min at each pressure and the total series required 2 to 3 hr. At the end of a series the pressure was returned to atmospheric conditions and the pectoral activity of the fish re-determined. Tests were conducted on artificially incubated fry and on fry dug from the spawning grounds before filling of the swim-bladder, fingerlings from fry to smolt stages, on one-year-old smolts, eighteen-month-old fingerlings and two-year-old smolts. Tests were conducted at temperatures ranging between 47 to 51° F. The tests commenced within the range of 47 to 49° F, but the temperature increased slightly at high pressures due to pumping energy appearing as heat.

The capacity of the experimenter to count the movements of the pectoral fins was established in two ways. The

ability to count a flashing light was determined using a Strobotac Type 1531-A stroboscope. The rate of flashing of the instrument was selected by an assistant and the experimenter timed 20 flashes by stop-watch. With practice, little difficulty was experienced counting rates up to 4 per sec (240 per min). At rates above this, counting soon became impossible. The approximate ranges of pectoral fin activity were: fry 200 to 250 beats per min, fingerlings 150 to 220, one-year smolts 150 to 180 and two-year smolts 100 to 140 beats per min. Thus only sockeye fry proved difficult to count. The accuracy of the counting methods was verified by means of the strobe light. Due to the small size of the pectoral fins it was not possible to use the instrument as a stroboscope in the dark in the usual fashion and hence have the moving object "stand still" when the two rates were synchronous. However with regular counting illumination in the chamber it was possible to synchronize the flashing light with the beating of the fins and hence use the stroboscope as a device for measuring frequency. This method agreed closely with fin rates obtained by visual counts timed by stop-watch.

Pressure in Relation to the Swimbladder

Measurements on the Fish

The measurement of pressure-induced changes in

density of sockeye was based on a knowledge of the density of the fish and the volume of the swimbladder. The calculation of the volume of the swimbladder required an accurate measure of the volume and weight of the fish.

Determination of volume. The volumes of the fish were determined by a variation of the displacement method. An individual smolt was allowed to drain for approximately 10 sec with the nose on blotting paper while held by the tail. The fish then was lowered into a glass tube, 2.5 cm in diameter and partly filled with water. The tube was filled until the center of the meniscus just touched a needle-point suspended from the top. The fish was removed and again allowed to drain for 10 sec with the nose touching the surface of the meniscus. The volume of water displaced by the fish was determined by filling the tube from a micro burette. Ten determinations were conducted on a single fish, the animal being returned to experimental holding conditions between tests. For a mean volume of 5.242 ml, 1 standard deviation was $\pm$0.065 ml or 1.25 per cent and for 2 standard deviations $\pm$0.127 ml or $\pm$2.42 per cent.

Determination of weight. The sockeye under study were weighed to the nearest 5 mg. Ten determinations on the same fish employed in the measurement of volume resulted in a mean weight of 5.233 g with $\pm$0.038 g or $\pm$0.73 per cent for 1

standard deviation and ±0.074 g or ±1.41 per cent for 2 standard deviations.

Determination of density. Initially the densities of the fish were calculated from the data on weights and volumes as found above. Ten calculations of the density of the same fish yielded a mean value of 0.999 g per ml, with ±0.014 g per ml or ±1.40 per cent for 1 standard deviation and ±0.027 g per ml or ±2.70 per cent for 2 standard deviations.

In view of the relative inaccuracy and the slowness of the weight-volume method, an alternative technique was tested. The density of the young sockeye was determined indirectly by establishing the point of neutral buoyancy in salt water baths of known density. A series of solutions of salt water was prepared by means of oceanographic hydrometers and the tables of Zerbe and Taylor (1953). Salinity increments of 2.5 parts per thousand were used over the range of densities studied. This corresponds to density differences of approximately 0.002 g per ml between baths. Some further refinement was possible by observing the sinking or buoying of a fish in adjoining baths and interpolating. Occasionally fish showed densities slightly below that of water and these were determined in 0.5 and 1.0 per cent solutions of ethanol.

In practice an electrically stunned or anesthetized fish was transferred to the salt baths and moved up or down the series to the nearest neutral buoyancy. In the studies of sockeye sounding behavior it was possible to estimate quite closely the density of the fish during the period of gas loss and relatively few transfers from bath to bath were necessary. Contamination was minimized by draining excess fluid from the fish before transferring, by having the volumes of the baths large (1 liter) in relation to the fish (5 g) and by keeping the baths covered to reduce evaporation. Nevertheless it was necessary periodically to test the specific gravity of the solutions and replace them as change took place. The specific gravity of salt water changes appreciably with temperature and that of the baths was corrected to the nearest 5° F.

This method of density determination was more rapid than the weight-volume procedure and did not necessitate killing the experimental fish. Ten determinations on the same fish yielded a mean density of 1.0003 g per ml with $\pm$0.0004 g per ml or $\pm$0.04 per cent for 1 standard deviation and $\pm$0.0008 g per ml or $\pm$0.08 per cent for 2 standard deviations. In the range of densities close to fresh water, solutions of intermediate values were inserted between the 0.002 g per ml

increments. This resulted in a higher degree of accuracy than was true of the density series over the complete range. As the fish described fell within the range of 0.001 g per ml increments, the accuracy expressed is approximately twofold too great. It is probable that the method is consistent to within ±0.1 to ±0.2 per cent, for 2 standard deviations, over the complete range of densities studied.

Measurements on the swimbladder

Volume of the bladder. A knowledge of the volume of the swimbladder was required for the calculation of pressure within the swimbladder and the extensibility of the bladder. Measurement of the volume of the swimbladder in the physostomous sockeye was complicated by the tendency of these fish to lose a portion of the gas from the swimbladder when disturbed. This property of the fish required swimbladder volume to be calculated in two ways, - one, where bladder volume was employed in the calculation of pressure within the swimbladder, and another in the calculation of distensibility of the bladder. In the former the statistic required was the actual volume of gas in the bladder, regardless of the degree of inflation this represented. In the latter method it was necessary to calculate extensibility of the bladder from the volume the swimbladder would have been, had the fish been at neutral buoyancy.

Both methods of calculation of volume of the bladder are based on the definition of density:

$$\text{density} = \frac{\text{weight}}{\text{volume}}$$

As the weight of the fish is constant, for all practical purposes, with the swimbladder empty or inflated with gas, density may be related directly to volume:

$$D_I \times V_{FI} = D_D \times V_{FD}$$

where D_I is the density of the fish, swimbladder partially inflated.

D_D is the density of the fish, swimbladder deflated.

V_{FI} is the volume of the fish, swimbladder partially inflated

V_{FD} is the volume of the fish, swimbladder deflated

but $V_{FD} = V_{FI} - V_B$

where V_B is the volume of the swimbladder at the measured density and volume.

Substituting: $D_D \times (V_{FI} - V_B) = D_I \times V_{FI}$

$$D_D \times V_{FI} - D_D \times V_B = D_I \times V_{FI}$$

$$-D_D \times V_B = D_I \times V_{FI} - D_D \times V_{FI}$$

$$V_B = \frac{D_D \times V_{FI} - D_I \times V_{FI}}{D_D}$$

$$V_B = \frac{V_{FI} \times (D_D - D_I)}{D_D}$$

The value "deflated density" (D_D) was determined on 60 smolts. The procedure was to remove the gas from the swimbladder of the intact fish by means of a fine hypodermic needle and small syringe. The volume and density of the fish was determined and the fish then was dissected open mid-ventrally under water. If any gas remained in the swimbladder it was removed and the density re-determined. The mean gas-free density was 1.0634 g per ml with $\pm$0.0015 g per ml for one standard deviation. In view of the consistency of the method this mean density was subsequently used throughout for the calculation of volumes of swimbladder. Thus initial density and initial volume of the fish were all that remained to be measured before the volume of the swimbladder could be calculated. For example, where the initial density of the fish was 1.0177 g per ml and the initial volume of the fish was 6.812 ml, the volume of the swimbladder was:

$$V_B = \frac{6.812 \times (1.0634 - 1.0177)}{1.0634}$$

$$V_B = 0.293 \text{ ml}$$

The above value represents the volume of gas in the swimbladder at the described conditions of volume and density, ignoring pressure for the moment. Thus this is the volume

most appropriate in any comparison of experimental and theoretical (Boyle's Law) expansion of gas in the swimbladder.

However, because of the tendency of the sockeye to lose some of the gas from the swimbladder, volumes as derived above are not applicable in the determination of extensibility of the swimbladder. That is, if a smolt lost one-half of the gas from its swimbladder, a subsequent reduction in pressure to one-half the former level would merely 'take up the slack' and restore the bladder to its original size. Thus it was necessary to calculate also the volume the swimbladder would have been were the fish at neutral buoyancy. This is based on the formula as derived above:

$$V_{BN} = \frac{V_{FN} (D_D - D_N)}{D_D} \qquad (1)$$

where V_{BN} is the volume of the swimbladder at density 1.0000 g per ml (neutral buoyancy)

V_{FN} is the volume of the fish at density 1.000 g per ml

D_D is the density of the fish, swimbladder deflated to density 1.0634 g per ml

D_N is the density of the fish, swimbladder inflated to density 1.0000 g per ml

$$V_{FN} - V_{FI} = V_{BN} - V_B \qquad (2)$$

where V_B is the volume of the partially-inflated swimbladder as determined above.

or $$V_{FN} = V_{BN} - V_B + V_{FI} \qquad (3)$$

Substituting V_{FN} of equation (3) to (1) we obtain the formula:

$$V_{BN} = \frac{(V_{BN} - V_B + V_{FI}) \times (D_D - D_N)}{D_D}$$

from which may be calculated the volume the swimbladder would have occupied had it been inflated to provide the fish with neutral buoyancy. Employing the values of volume and density as used in the example above:

$$V_{BN} = \frac{(V_{BN} - 0.293 + 6.812) \times (1.0634 - 1.000)}{1.0634}$$

$$V_{BN} = 0.0560\ V_{BN} + 0.3885$$

$$V_{BN} = 0.412 \text{ ml}$$

Ambient pressure. Alexander (1959) described a method for determining the ambient pressure within the swimbladder of live, anesthetized physostomes. In principle the technique permits a comparison of gas compressed within the swimbladder with the same gas compressed outside the bladder and hence free of any confining influence due to the swimbladder itself. In the present study the apparatus (Fig. 7) was patterned closely after that of Alexander. Individual sockeye were permitted to fill their swimbladders to neutral buoyancy in shallow water, then were exposed gradually to an anesthetizing dose of

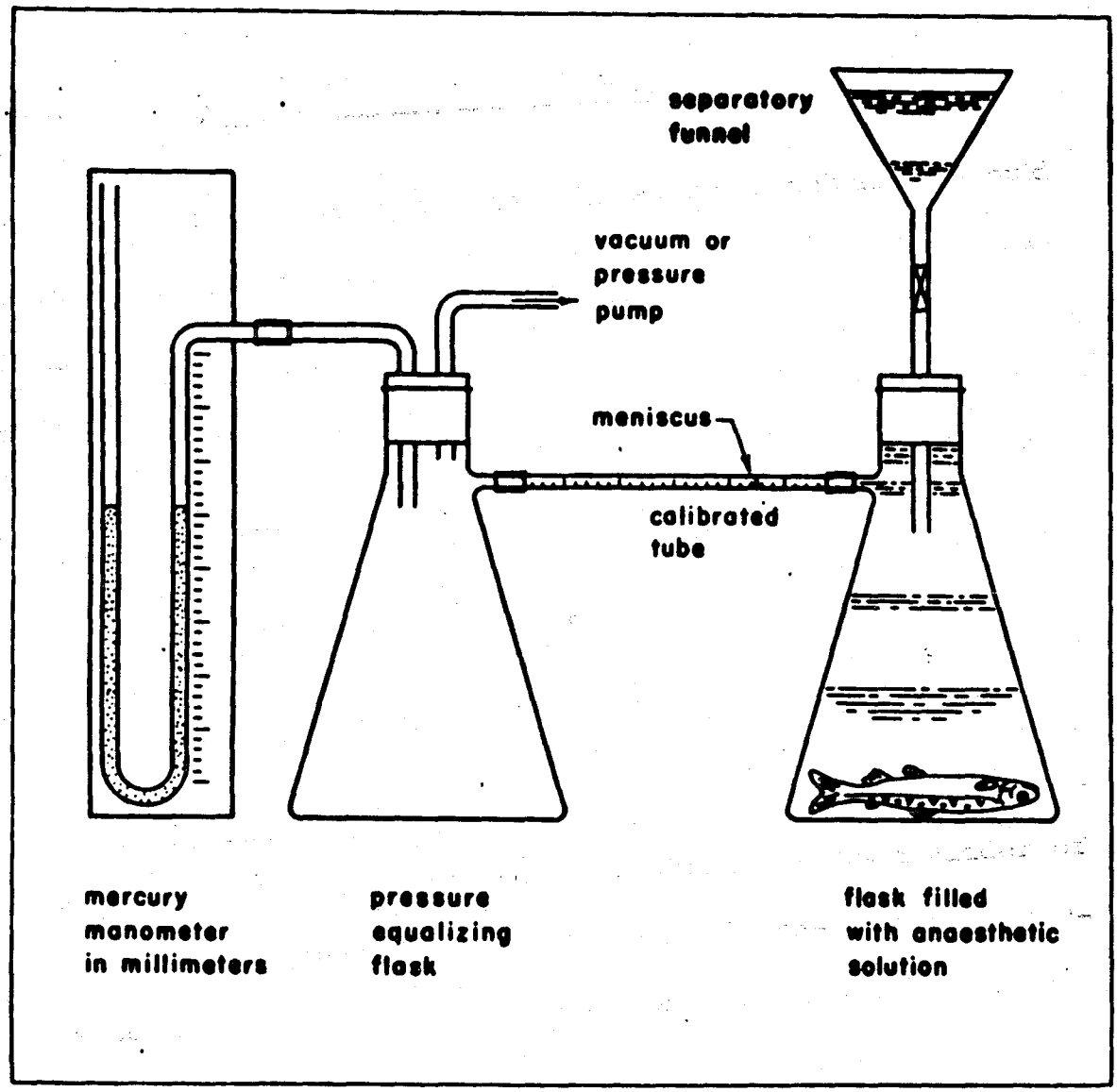


Figure 7. Apparatus for the measurement of ambient pressure within the swimbladder. (After Alexander (1959).

tricaine methane-sulfonate (Sandoz MS - 222). This procedure was essential to avoid fright-induced loss of gas from the swimbladder. The density of the anesthetized fish was determined prior to placing it in the apparatus and fish which had lost gas were not tested. With the fish inside, the apparatus was sealed and the volume of water adjusted until the meniscus was located satisfactorily in the calibrated tube. Pressure in the flask and hence on the fish was increased in increments of 30 mm Hg above atmospheric pressure and the displacement of the meniscus recorded. On reaching 360 mm Hg, the pressure was lowered to atmospheric and thence in similar increments to 360 mm Hg below atmospheric or until one-third to one-half of the gas had escaped from the swimbladder. This gas was trapped in the top of the flask. The pressure was returned to atmospheric and the increments of positive and negative pressure were repeated.

The apparatus was standardized after each determination for both the positive and negative range and the experimental values were corrected for this distortion of the apparatus. The gas content of the water used in the apparatus was reduced artificially by prolonged exposure to a partial vacuum to minimize the tendency of the gas to leave solution when the pressure was reduced below one atmosphere.

There was little evidence of gas leaving solution under the test conditions nor was there any evidence of gas in the top of the chamber entering solution at pressures above atmospheric.

Blank-corrected values of compression and expansion of the swimbladder were plotted against pressure (Fig. 8). Excess pressure within the swimbladder at atmospheric pressure was represented by the horizontal difference between the two curves.

Extensibility of the bladder. Extensibility was measured in an apparatus (Fig. 9) based on that which Jones (1951) employed on physoclistous perch. The tendency of physostomous sockeye to lose gas from the swimbladder through fright necessitated use of fish just killed either by anesthetic or electric shock. Another problem was upward escape of gas through the apparatus. This required the calibrated tube to be of sufficient diameter such that bubbles of gas could pass up the tube without forcing liquid out through the top. This requirement limited the minimum diameter of the tube and as a consequence calibration could not be read more closely than 0.02 ml.

In practice an individual fish was killed and its density, volume and weight determined. The fish was placed in the apparatus and exposed to a gradual reduction in pressure. Volume of the fish was recorded for increments of pressure reduction of 2.5 in Hg (63.5 mm Hg) from atmospheric

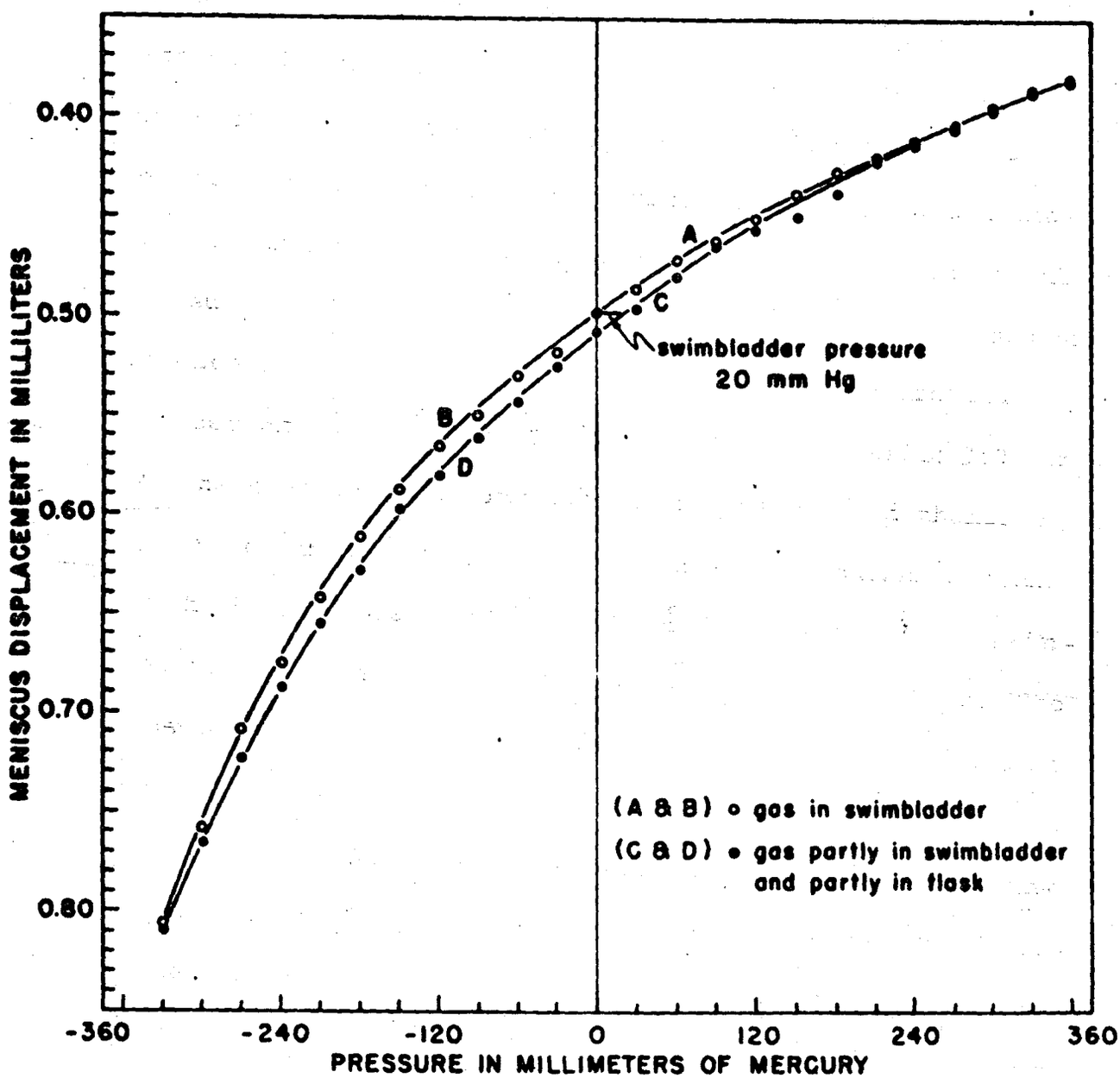


Figure 8. Calculation of excess pressure within the swimbladder.

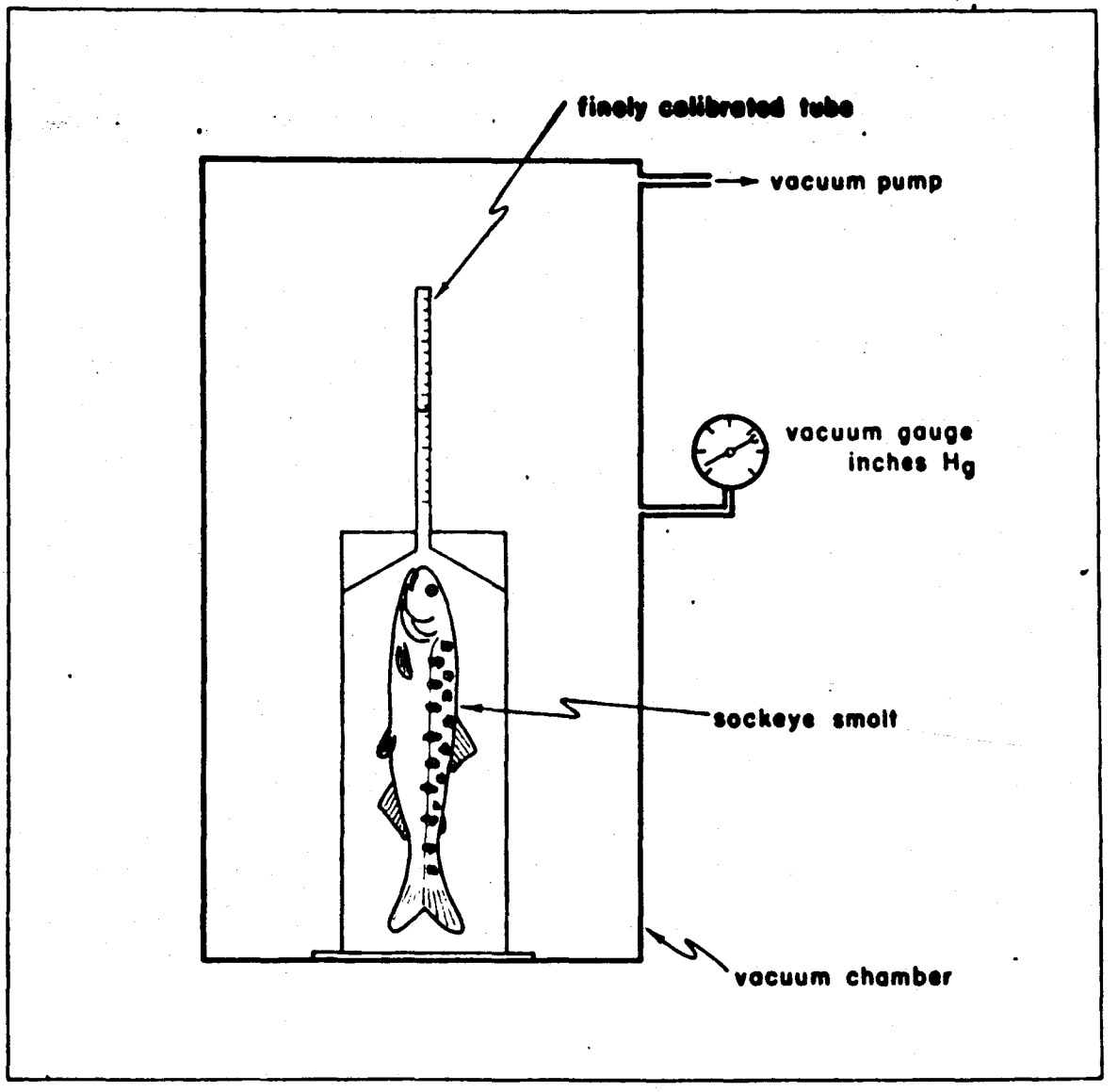


Figure 9. Apparatus for the measurement of the pressure required to force gas out through the pneumatic duct. After Jones (1951).

pressure to a vacuum of 27.5 in Hg (698.5 mm Hg). Observations on first evidence of gas loss, size and frequency of bubbles and rising and sinking of the fish were recorded. The apparatus was calibrated regularly over the range of vacuum studied and individual values for a fish were corrected for vacuum expansion by the apparatus.

Volume of the swimbladder was derived as explained previously and the theoretical expansion of the gas was calculated using Boyle's Law.

$$P_1 \times V_1 = P_2 \times V_2$$

where P_1 is atmospheric pressure or 29.92 in Hg

V_1 is the volume of the swimbladder at atmospheric pressure

P_2 is the pressure in in Hg at any given reading

V_2 is the calculated, theoretical volume of the swimbladder gas would occupy at the new pressure, P_2

Thus for example, if the initial volume of the swimbladder was 0.412 ml, then at a vacuum of 2.5 in Hg (693 mm Hg absolute pressure) the theoretical volume of the swimbladder would be:

$$\frac{29.92}{29.92 - 2.5} = 0.412 = 0.449 \text{ ml}$$

The theoretical volumes were calculated for each of the 12 increments of pressure reduction and together with the observed values were plotted as in Fig. 14.

Extensibility of the swimbladder was calculated as a per cent of the volume the swimbladder would occupy at atmospheric pressure and for a density of 1.0000 g per ml. This was calculated for individual fish up to the point of release of gas from the pneumatic duct or rupturing of the swimbladder. Thus the fish described in previous examples with swimbladder volume of 0.412 ml (at density 1.00003 g per ml and atmospheric pressure) lost gas from the swimbladder at an expanded volume of 0.473 ml or at 115 per cent of neutral-buoyancy volume.

The technique described above was employed on three series of fish under control conditions comprising 10 individual smolts in each series. The influence of the body wall was explored in a series of 10 fish in which a mid-ventral incision permitted unrestricted expansion of the swimbladder.

Contractibility of the bladder. Loss of an appreciable quantity of gas from the bladder by sockeye smolts when frightened suggested two possibilities. Firstly, that gas loss may be through active expulsion by contraction of the bladder. Secondly, being a fright response loss of gas may be under autonomic control. These two possibilities were examined

concurrently through the use of pharmacological agents on isolated strips and intact swimbladders.

Autonomic drugs do not act on autonomic nerves, as the name suggests, but rather on effector cells such as muscle or gland cells (Goodman & Gilman, 1955). Thus the nerves to the duct or bladder need not be intact for study with autonomic agents. Four types of autonomic drugs were utilized: sympathomimetics, drugs such as adrenaline and ephedrine which stimulate structures innervated by adrenergic nerves; adrenergic blocking agents such as "dibenzyline"; parasympathomimetics, drugs such as acetylcholine and pilocarpine stimulating structures with cholinergic innervation; and cholinergic blocking agents such as atropine. In general these agents were tested at concentrations of 10 mg per ml, abbreviated to 10^{-5} or 1 in 10^{5}. This is the standard method of expressing the concentration of drugs in baths (Lewis, 1960).

Isolated swimbladders initially were suspended longitudinally within aerated frog Ringer's solution in a muscle-preparation apparatus. Subsequently cross-sectional loops were cut out from the swimbladder and suspended within the muscle-bath such that a reduction in cross-sectional area would operate the writing lever. These tissues were tested with adrenaline (10^{-5}) and ephedrine (10^{-5}) before and after adrenergic blockade was attempted with "dibenzyline" (10^{-5}). By-passing

of "dibenzyline" blockade was attempted with monoacetin at concentrations of 10^{-5}, 10^{-4} and 10^{-3}, followed by adrenaline (10^{-5}). Pilocarpine and atropine were tested on fresh tissues at doses of 10^{-5}. All tests were conducted on the swimbladders of 18-month-old sockeye fingerlings. Bath temperatures were maintained between 45 and 50°F.

The response of the intact swimbladder was investigated using the same pharmacological agents as above. Individual sockeye fingerlings were stunned electrically and then killed in a strong dose of anesthetic. The fish were opened midventrally under physiological saline, exposing the swimbladder. With the abdominal walls held apart, a small amount of drug such as adrenaline was added to the saline in the region of the swimbladder. The response of the swimbladder was noted plus any movement of gas through the pneumatic duct and out the mouth. The adrenaline was washed away and the tissues exposed to "dibenzyline", re-washed and re-treated with adrenaline. Additional fish were tested with the drugs pilocarpine and atropine. In these intact swimbladder studies it was essential that the fish be kept under water (physiological saline) to detect the movement of gas out of the swimbladder. This made impossible close definition of the concentration of drug in contact with the swimbladder.

Gas secretaion. Fish in general respond to increased pressure and hence increased density by secretion of gas into the swimbladder whereas decompression and positive buoyancy are followed by resorption of gas. The extent of these processes in young sockeye was investigated at atmospheric pressure and pressures above atmospheric. In such studies on sockeye salmon it was essential to preclude access to atmospheric air to be certain air had not entered the swimbladder via the pneumatic duct. That is, there fish utilize atmospheric air for swimbladder inflation where possible. It was possible to deny atmospheric air to experimental fish and hence one could infer that any gain in volume of gas would be due to the activity of the swimbladder. The converse, of course, was not readily possible in physostomous sockeye. Gas lost from the swimbladder by way of the pneumatic duct would tend to mask gas resorption through the swimbladder.

A group of 10 smolts was placed in a 75 liter container and induced to sound to the bottom through a disturbance on the surface. This fright-induced sounding response was accompanied by loss of gas from the swimbladder. The density of the fish increased accordingly. A screen was placed over the fish, halfway down the container, thus preventing the smolts from utilizing atmospheric air (Fig. 10). A second group of fish was treated similarly but these fish were stunned

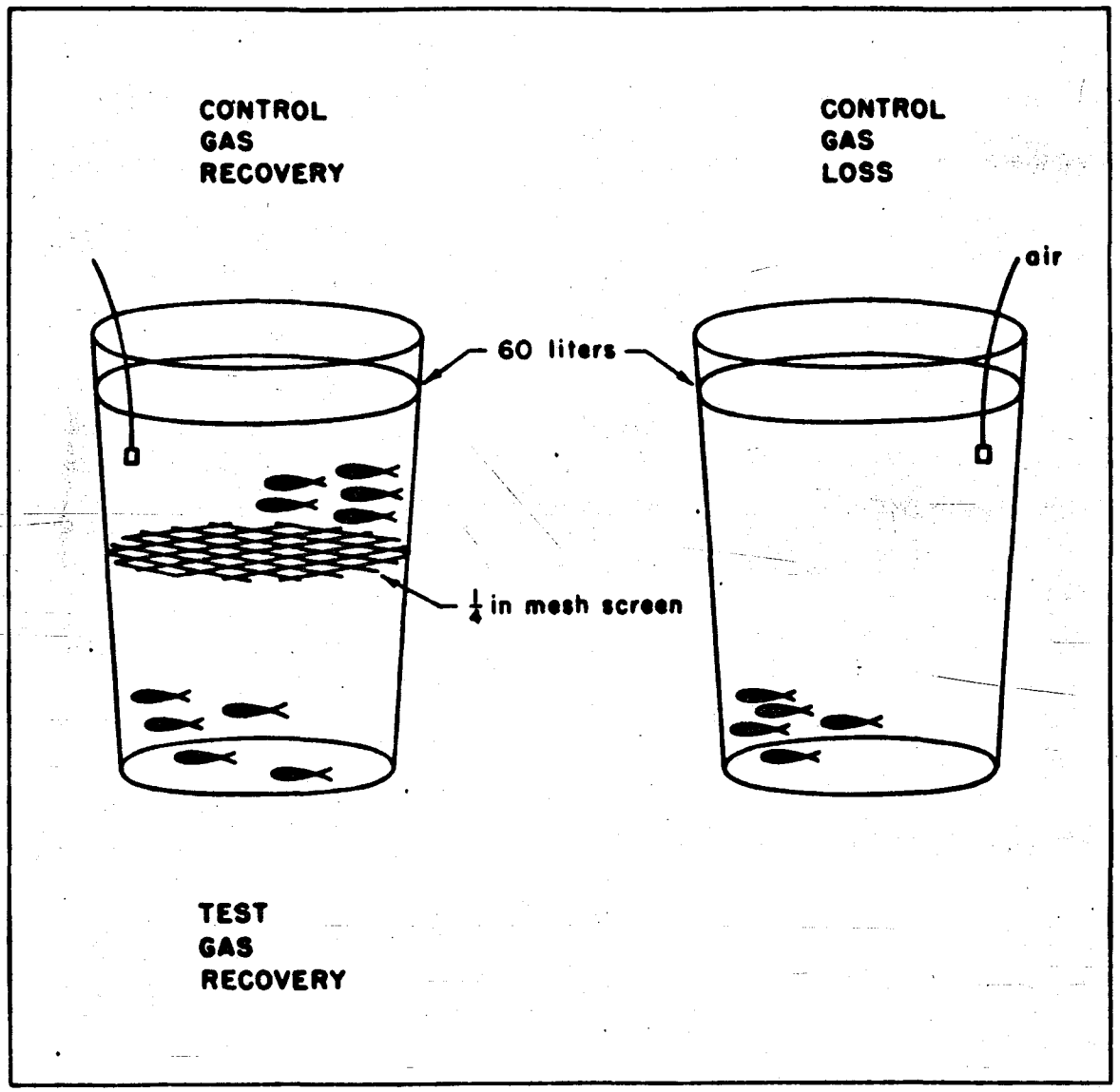


Figure 10. Investigation of inflation of the swimbladder with and without access to atmospheric air.

electrically after covering with a screen. In this case the density of the sockeye was determined immediately and these fish served as a check or control on the gas lost by the group held below the screen. A third group of 10 sockeye was placed above the screen, similarly induced to sound and then allowed free access to atmospheric air during the period of holding. Groups of sockeye were held in this way for periods of 0, 2, 4 and 8 days. At the end of these periods the fish were stunned electrically and the density determined.

After studying effects of atmospheric pressure as described above, the possibility remained that higher pressures may induce secretion of gas by the swimbladder. Holding fish under pressure in the apparatus, as described under general methods, presented no difficulty. There was however the serious problem of determining the density of the fish before and after prolonged holding under pressure. For this purpose the Cartesian Diver principle was employed, patterned after the method of Tait (1959). Initially it was necessary to relate smolt density to vacuum. This was done by determining the density of an individual, freshly killed smolt. The fish was placed in the chamber and the pressure reduced until the fish was suspended in mid-water. The vacuum necessary to bring the fish to neutral buoyancy was recorded. This procedure was repeated for a range of fish densities and plotted

as in Fig. 11. The curve is considered reliable to a vacuum of approximately 20 in Hg, beyond which gas leaving solution tends to form bubbles on the surface of the fish. Thus the density of a fish could be determined by applying a vacuum of sufficient magnitude to expand the gas within the swimbladder and suspend the fish in the chamber. This was most readily done with the fish immobilized. The steel chamber conducted away any electric current applied within it and thus made impossible electrical stunning of the fish. The use of anesthetics was tested but proved costly and difficult to apply because of the flow pattern within the pressure chamber.

It was decided finally to apply the Cartesian Diver technique to conscious fish when they were resting quietly. Thus an individual sockeye was placed in the apparatus, the chamber was filled with water and the fish was allowed time to become quiet in familiar surroundings. Because swimbladder gas was lost with excitement on entering the chamber, the fish were slightly denser than water and the fish were not permitted atmospheric air for re-inflation of the swimbladder. The initial density of the fish was determined by a reduction in pressure and subsequent buoying. The fish was then put under a pressure of 1 or 3 atmos above atmospheric and held 24 hr. At the end of this time the pressure was reduced to atmospheric and then gradually below that. The point of

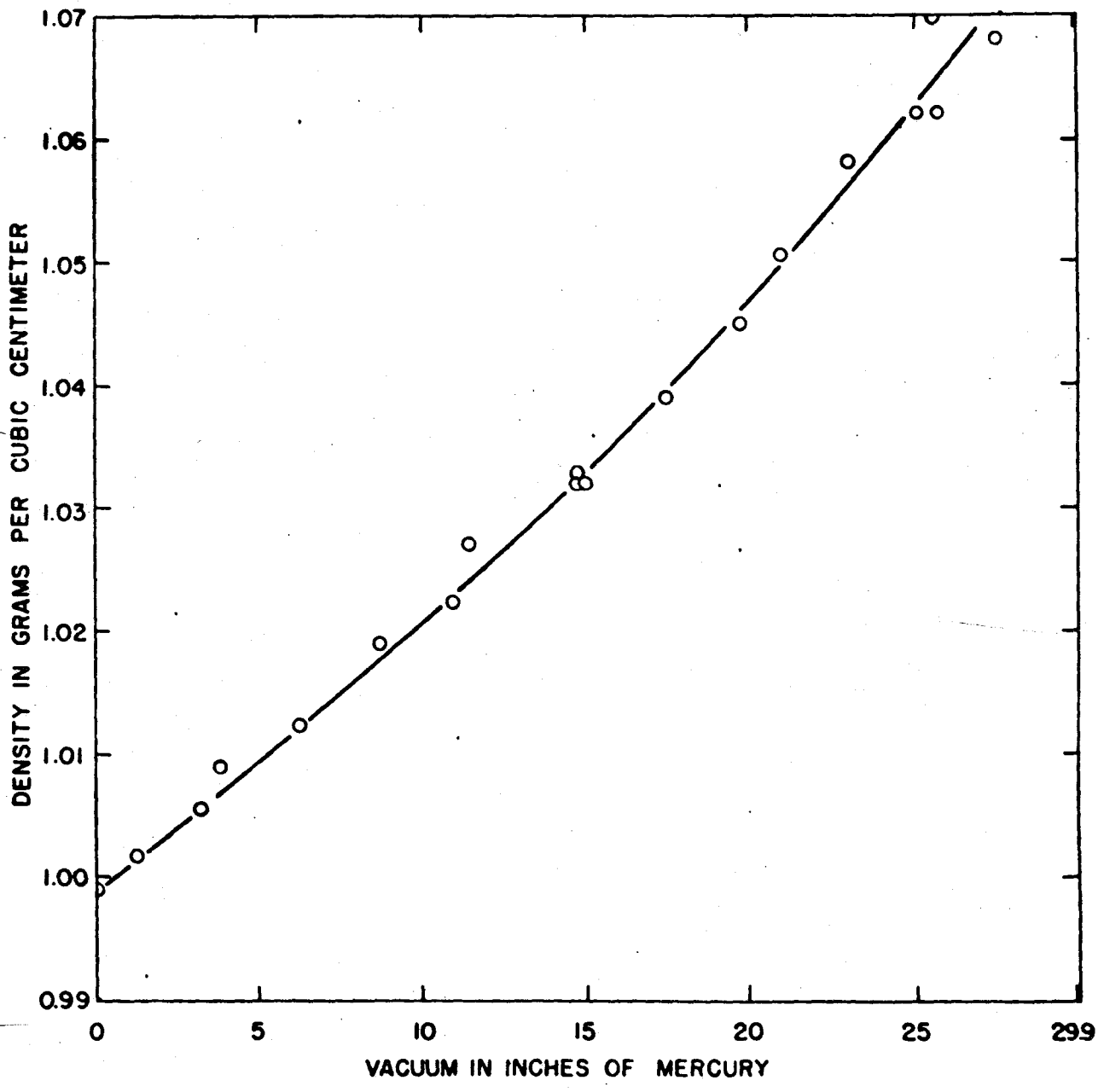


Figure 11. Smolt density and magnitude of vacuum necessary to affect neutral buoyancy.

neutral buoyancy of the fish was recorded and gain or loss of gas was interpreted from any change in density.

Measurements on the pneumatic duct

Gain and loss of swimbladder gas through the pneumatic duct could be expected to have important consequences for the fish. There is firstly the value of the sounding response as a mechanism of escape. Secondly, mortality of fish on decompression may be due to the entry of gas of swimbladder origin into the blood stream (Jones and Marshall, 1953). Thus the effects of decompression may be dependent on the degree of inflation of the bladder. Thirdly, the compensatory swimming of young sockeye would be expected to relate to the density of the fish, which is a function of degree of inflation of the bladder and pressure. For these reasons an attempt was made to quantify loss of gas on sounding and to establish the nature of the regulation of entry and loss of gas through the pneumatic duct.

Loss of gas on sounding. The sounding response of sockeye salmon smolts was readily apparent. When startled the fish dived immediately leaving an obvious trail of gas bubbles. This loss of gas from the swimbladder resulted in a corresponding increase in density. The sounding response was quantified by measuring the density of the fish at the end of gas emission.

The stimulus to sounding was standardized by having the fish sound in a 4-liter-cylindrical-glass jar (Fig. 12). A smolt so tested always sounds to the bottom, all the while releasing small bubbles of gas. Loss of gas is completed usually within 30 sec. In the exploratory tests fish were stunned electrically after 2 min and then removed for density determination. Subsequently it was found simpler to use an anesthetic solution of MS-222 in the jar, the strength being adjusted to produce anesthesia in about 2 min. Fish densities were measured in the series of salt baths described previously.

Once the sounding response had been quantified, it was possible to explore the mechanism of loss of gas from the swimbladder. Several pharmacological agents, with known autonomic effects in the higher vertebrates, were tested on sockeye smolts in an attempt to establish the nature of the control of the swimbladder and pneumatic duct. Sympathomimetic and parasympathomimetic agents plus sympatholytic and parasympatholytic drugs were tested. Relatively stable agents such as ephedrine and pilocarpine were used in preference to epinephrine and acetylcholine respectively. In practice, the agents were tested in simple solution, over a range of concentrations up to the level of initial mortality. Following this preliminary screening, drug doses well below the lethal level

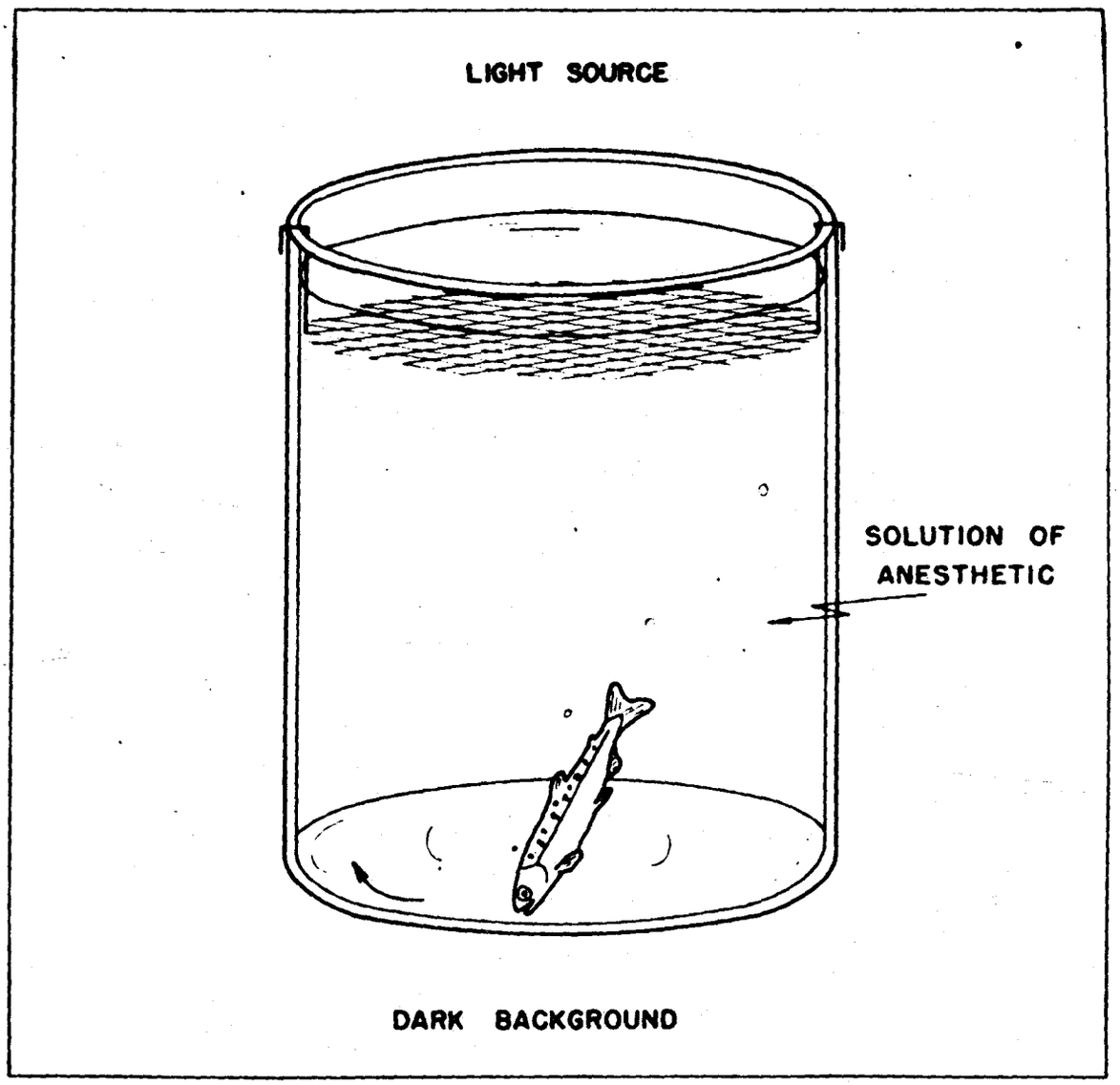


Figure 12. Apparatus used to induce the sounding response in sockeye smolts.

were explored. Where an effective dose was found, a time-response curve was established for the period of 1 to 12 hr. Fish were tested in groups of 10 after periods of 1, 2, 3, 4, 8, and 12 hr of exposure to a drug in solution. Control fish were held in similar containers of water and were tested concurrently for sounding response. In so far as was possible, the 60 test and 60 control fish were drawn from the migration as one large sample and treated identically but for the addition of the drug to the holding water of the test group.

The drug ephedrine, an effective sympathomimetic agent, was tested and the response determined at concentrationsoof 100 and 200 mg per liter. "Dibenzyline", a potent adrenergic blocking agent (Nickerson and Goodman, 1947), is accredited with a solubility in water of approximately 0.1 mg per liter. This concentration showed no effect on sockeye smolts and the dose was raised tenfold to 1.0 mg per liter. Some change in gas loss was evident at this concentration. The drug was re-tested at 5.0 mg per liter with the effects described under results. No explanation is offered for the effectiveness of this drug at a concentration of almost 50 times its supposed solubility. A second, otherwise unrelated adrenergic blocking agent "hydergine" (a mixture of three ergot alkaloids, Goodman and Gilman, 1955) was tested at

concentrations up to 10 mg per liter.

The effect of cholinergic stimulation on the sounding response was investigated using the parasympathomimetic drug pilocarpine. The cholinergic blocking agents "darbid" (isopropamide iodide) and atropine were tested at concentrations of 100 and 200 mg per liter respectively. A discussion of the pharmacology of "darbid" may be found in Proddsdiji-Hartzema, Janssen and Jongh (1955).

Gulping atmospheric air. The filling of the swimbladder by gulping of air at the surface, the "Luftschluchen" reflex, was examined first in relation to pressure changes and subsequently in regard to nervous control. The methods employed in the study of the nervous control of emptying of the swimbladder were not applicable readily to the study of inflation of the swimbladder due to the complication of fright-induced loss of gas. Two alternative methods of study were devised.

In the first approach, groups of 10 smolts each were placed in 70 liter containers, induced to sound by means of a disturbance on the water surface, then denied access to the surface by means of a screen, as described previously. A drug could now be put in solution in the water containing the test fish and the fish exposed to it for some period of time. At the end of this time the control group was stunned electrically, still under the screen and their individual densities

determined. This established the extent of loss of gas from the swimbladder which had occurred on sounding. The screens were then removed from the remaining containers, permitting the test and second control groups access to the water surface. After 5 to 10 min these fish were stunned electrically and their densities determined. The second control group, the individuals of which almost invariably returned to neutral buoyancy in the few minutes air was available, provided the basis of comparison for the drug-exposed fish. The periods of holding under the screens ranged from 1/2 to 4 hr. Holding such fish for one or more days resulted in a much slower return to the use of atmospheric air when the screens were withdrawn. The drugs tested in this study were the same doses of adrenergic and cholinergic stimulants and blocking agents used in the examination of loss of gass on sounding.

A second method of investigating the gulping of atmospheric air and inflating of the swimbladder was devised to verify the results of the under-the-screens studies. In principle the swimbladder was provided with an accessory opening to the exterior of the fish, such that air which entered the swimbladder escaped via the auxiliary duct. Individual smolts were anesthetized with MS-222, then the anterior half of the fish was isolated from the posterior half by means of a rubber diaphragm. The anterior end of the fish was

maintained in a narcotizing solution of anesthetic, leaving the posterior half of the fish available for surgery. A short length of fine polyethylene tubing was inserted in the swimbladder through the left side of the fish slightly anterior to the vent and below the lateral line. The catheter was secured in place by a small stitch through the adjacent musculature and terminated in a tie around the circumference of the fish. Whether or not the catheter ended within the lumen of the swimbladder was apparent by the response of the fish on recovery from the anesthetic.

Properly catheterized fish rose to the surface of the water, mouthed a bubble of air and angled downward toward the bottom of the aquarium. Concurrent to the change in position from head up to one of head down, a series of bubbles, about six in number, issued from the external end of the catheter. This procedure of swimming up to the surface, mouthing a bubble of air, swimming downward and passing the gas into the swimbladder (only to lose it to the outside) was repeated every few minutes for two to three days. At the end of this time the fish took to residing on the bottom of the tank and ceased making excursions to the surface. This behavior is illustrated in Fig. 13.

During the post-operative period when the fish were actively surfacing, gulping air and losing it through the catheter,

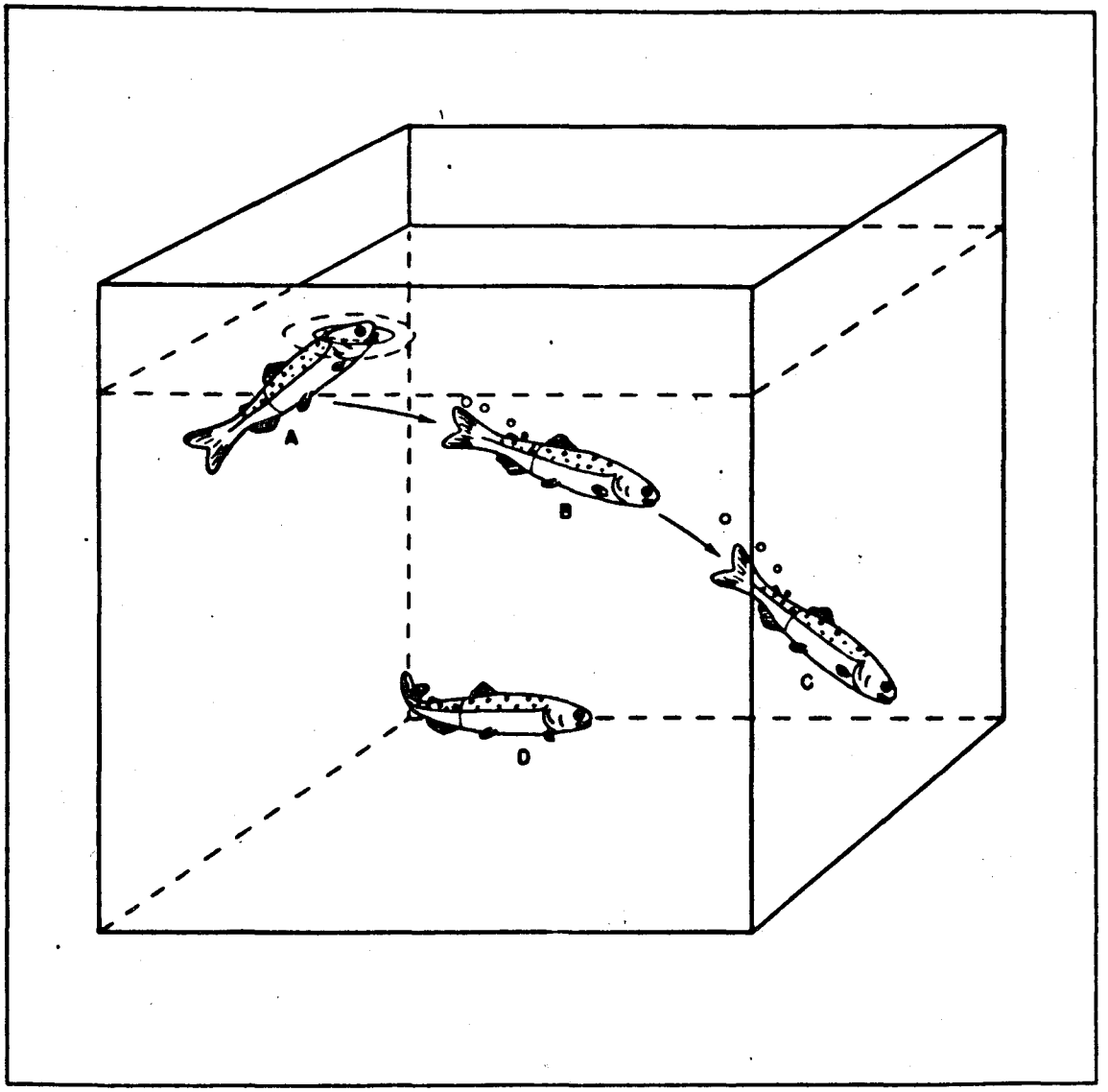


Figure 13. Response of sockeye smolts with a catheterized swimbladder. Fish attempt to inflate bladders for initial two to three days (A, B and C), thereafter residing on tank bottom (D).

some aspects of the fishes' air-introducing mechanism were amenable to study. In particular the nervous control of the pneumatic duct was explored by means of pharmacological agents in solution, especially the cholinergic and anticholinergic drugs pilocarpine and atropine. The fish were observed most readily while held in replicas of the salinity-preference chambers of Baggerman (1957).

Duct-release pressure. The excess pressure within the swimbladder necessary to force gas out through the pneumatic duct, hereafter termed the 'duct-release pressure' was calculated from the data on extensibility of the swimbladder. In this case the volume of the swimbladder was obtained using the formula:

$$V_B = \frac{V_{FI} \times (D_D - D_I)}{D_D}$$

as described previously. The observed expansion of the gas in the swimbladder was plotted, as in Fig. 14, at each increment of reduction in pressure of 2.5 in Hg (63.5 mm Hg). Theoretical volume of the swimbladder was calculated for each pressure reduction. For example, at a vacuum of 2.5 in Hg the volume of gas, obeying Boyle's Law, becomes

$$\frac{29.92}{29.92 - 2.5} \times 0.293 = 0.320 \text{ ml}$$

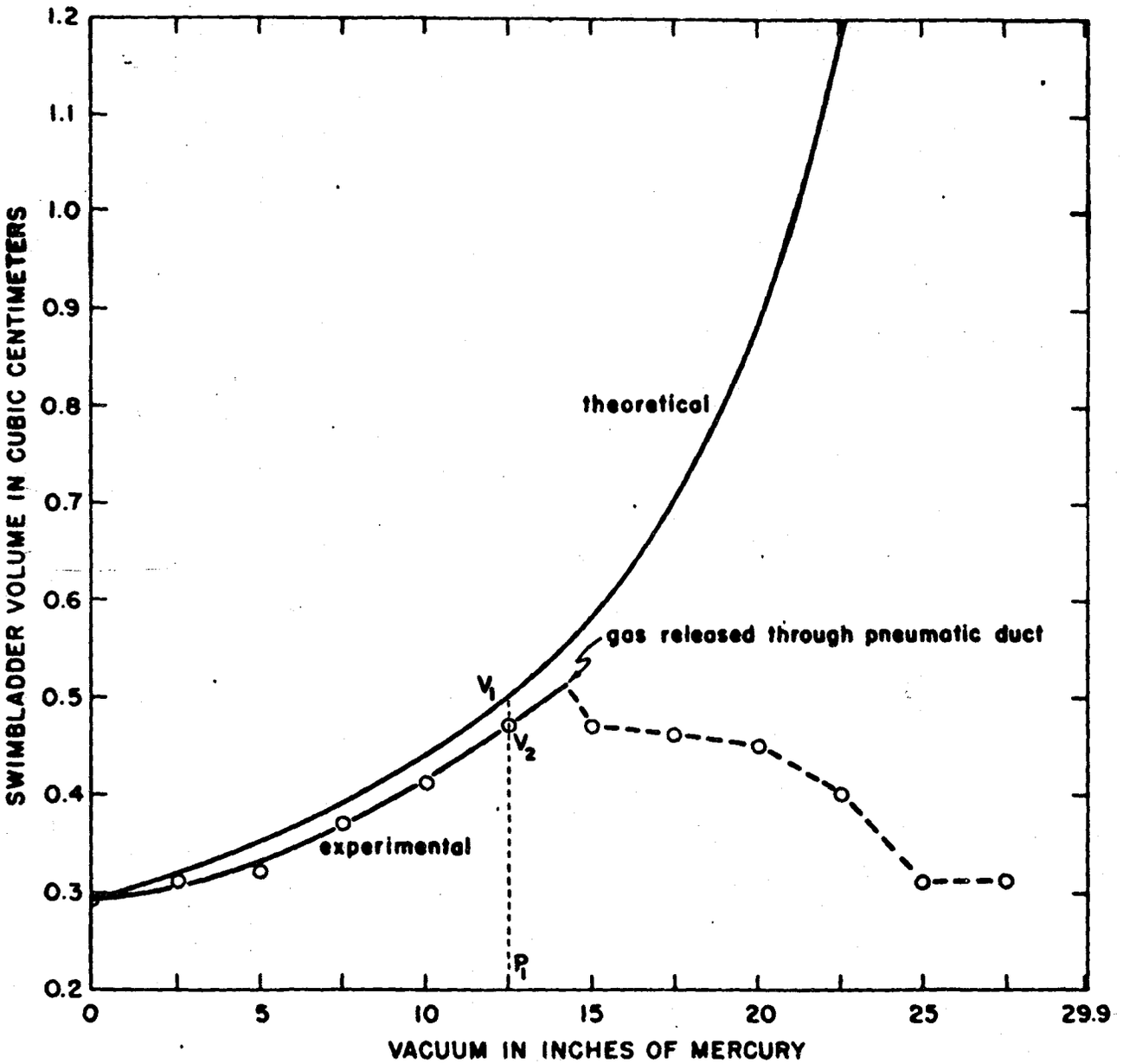


Figure 14. Calculation of pneumatic duct-release pressure from theoretical and observed expansion of gas in the swimbladder up to time of gas escape.

Pressure within the swimbladder similarly was calculated from Boyle's Law:

$$P_1 \times V_1 = P_2 \times V_2$$

where P_1 is absolute pressure in mm Hg at the point of observation.

V_1 is calculated theoretical volume of the swimbladder.

P_2 is pressure within the swimbladder.

V_2 is observed volume of the swimbladder.

For example, in the fish referred to above, gas escaped from the swimbladder under conditions of:

$$P_1 = 442 \text{ mm Hg}$$

$$V_1 = 0.503 \text{ ml}$$

$$V_2 = 0.473 \text{ ml}$$

$$442 \times 0.503 = P_2 \times 0.473$$

$$P_2 = 470 \text{ mm Hg}$$

Having derived P_2, the excess pressure within the swimbladder or the net pressure exerted against the duct is simply:

$$P_1 - P_2$$

Thus the duct-release pressure is approximately:

$$470 - 442 = 28 \text{ mm Hg}$$

The method described above was originally intended as a means of measuring the extensibility of the sockeye swimbladder and was subsequently adapted to the study of the

pressure required for the release of gas through the pneumatic duct. One difficulty with the method was that the pressure within the swimbladder cannot be calculated for the precise moment of escape of gas from the duct. This necessitated the use of the observed and calculated values immediately prior to release of gas.

The technique outlined was used in the investigation of the nature of the pneumatic duct. Evidence was sought for the presence of a sphincter or some form of constriction along the duct. Based on the known tendency of sphincters to relax following the death of an animal, fish were killed and the duct-release pressure was determined at various times after the death of the fish. Drug treatments which had been tested for their effects on the sounding response were further tested for their action on the pneumatic duct. Three series of control fish, comprising 10 sockeye smolts each, were tested during the course of the study. These groups showed average duct-release pressures of 28.9, 28.6 and 26.9 mm Hg.

In all of these studies on the release of gas through the duct it was not possible to use live fish, for when exposed to a gradual reduction in pressure, conscious sockeye simply released gas and returned to neutral buoyancy. For this reason it was necessary to use as experimental animals fish just killed by anesthetic.

Histological examination. The swimbladders and pneumatic ducts of experimental and control fish were examined histologically. The condition of the pneumatic ducts in particular was investigated in fish treated with sympathomimetic and parasympathomimetic agents plus adrenergic and cholinergic blocking agents. In addition to the examination of fish held in drug solutions, yearling sockeye of 10 g were injected with 0.1 mg of these agents. These fish were anesthetized and the tissues fixed for histological examination 1 hr after injection.

In all fish care was given to the fixation of the tissues of the duct and bladder. Fish were opened mid-ventrally and the gastro-intestinal tract turned out through the slit, permitting the fixative Bouins picro-formol, rapid access to the region of the pneumatic duct. Bladder and duct material was routinely embedded in paraffin, sectioned and stained with eosin and hematoxylin.

Pressure in Relation to Gases

As outlined in the introduction, upward movements in a stratified lake present fish with the problem of an increase in nitrogen saturation internally as a result of reduced gas-content of the water, increased temperature, reduced pressure and hence increased absolute saturation of the water. The

investigation of this problem required methods of gas analysis a study of the limnology of the lake environment and a measure of the tolerance of fish to decompression. Ultimately the study came to include a measure of the rate at which fish equilibrate with the dissolved nitrogen of their environment.

The theoretical considerations relevant to the study of dissolved gases are summarized in Appendix A. Included in Appendix A are the tables of oxygen and nitrogen solubilities used in the calculation of per cent of air saturation.

The methods of analysis of dissolved oxygen and nitrogen in fish blood appear in Appendix B.

Limnological investigations

Site of study. Limnological investigations in relation to the smolt migration were begun in 1960 at three stations along the length of the lake. Because results were similar these were reduced to a single station in the north-central part of Cultus Lake (Fig. 1). A second was established on Sweltzer Creek, 200 yd downstream from the lake outlet. In 1960, temperature and dissolved oxygen were determined vertically at irregular intervals during the spring smolt migration. In 1961 these measurements were made every two weeks during the downstream migration and at three- to four-week intervals beyond this time. Dissolved nitrogen was measured vertically every two weeks during the smolt migration and irregularly

after that.

Measurement of temperature. The vertical temperature structure of the lake was measured to within a few feet of the bottom by means of an oceanographic bathythermograph. The bathythermograph was placed in operation at a depth of one foot below the surface and a research thermometer read concurrently at that depth. The smoked slide from the bathythermograph was located in the reading grid on the basis of the thermometer temperature. The instrument was calibrated by the Pacific Oceanographic Group, Nanaimo, B.C. prior to commencement of the study.

Measurement of dissolved gases. Water samples were collected at depths of 0, 5, 10, 15, 20, 25, 30, 40, 50 and 130 ft in one-liter limnological water bottles. The samples were transferred to 300 ml BOD bottles. Dissolved oxygen was fixed on station and the titration performed in the laboratory. Water samples were taken separately for nitrogen analyses, transferred to previously cooled BOD bottles and during transport held at temperatures below that of the coldest water sampled. In this way the possibility of gas leaving solution as a result of warming was avoided. Nitrogen analysis was conducted in the laboratory employing the modification of the Scholander method described in Appendix B. The amount of water required for nitrogen analysis was very small

and oxygen determinations were repeated once on the sample remaining.

Calculations. Oxygen and nitrogen were expressed as mg per liter (Tables III and VI, Figs. 25 and 28). Using the theoretical solubilities as prepared from the absorption coefficients, Tables IX and XI, the per cent saturation was calculated from conditions of atmospheric or surface pressure (Tables IV and VI, Figs. 26 and 29) for oxygen and nitrogen respectively.

If the fish however were to migrate vertically from temperatures at depth to surface, conditions at a rate faster than gaseous equilibration was taking place, then saturation of the fish would be expressed more correctly as percent of saturation at surface temperature and pressure. For this reason oxygen and nitrogen saturations have been expressed as such (Tables V and VI, Figs 27 and 30). Finally, no attempt has been made to calculate hypolimnion saturations to the corresponding periods of air-water equilibrium. As Hutchinson (1957) pointed out, few workers have regarded this refinement as worthwhile.

Measurement of tolerance to pressure

During the downstream migrations of 1959 and 1960, groups of sockeye smolts, numbering 200 or 300 per sample, were tested in the pressure apparatus as described under

general methods. The tolerance of these fish to pressure was explored with respect to degree, duration and rates of change. Following testing, the groups of fish were held in one of the 36 cages situated in Sweltzer Creek. The fish were kept under post-test observation for two weeks during which time any injuries were noted and mortalities recorded. Control tests of a similar number of smolts were conducted after every fourth or fifth test. These fish were handled in an identical manner, being placed in the apparatus for the appropriate time and temperature, but maintained at atmospheric pressure. These control groups similarly were held for two weeks for observation.

In the study of tolerance to pressure and the succeeding study of factors influencing pressure tolerance, a total of 143 tests involving 19,849 smolts were conducted in 1959 and in 1960 an additional 117 tests on 13,002 smolts were carried out.

Magnitude of positive and negative pressure. Groups of 100 or 200 smolts were exposed to conditions of positive pressure ranging from atmospheric to 400 psi (27 atmos) above atmospheric. Routinely, pressures of 15, 20, 50, 100, 200 and 300 psi were tested. The pumping equipment was designed to operate at pressures of up to approximately 300 psi and only on a single occasion was the pressure raised to 400 psi.

The pressure range of 0 to 300 psi had been explored previously in the studies of sockeye behavior. However in the tolerance tests the larger samples of 200 fish were held in cages for two weeks of post-test observation as described above. Pressure was raised at the rate of 1 psi per sec, thus pressures of 300 psi were reached in 5 min.

Groups of smolts were exposed also to a range of pressures below atmospheric. An attempt was made to test these fish at vacuums of 0, 5, 10, 15, 20, 25 and 29.5 in Hg. Due to the nature of the apparatus, in particular the small water column between the vacuum and pressure chambers, it was not possible to achieve precisely the desired vacuum. However, the exact vacuum attained was recorded via the transducer mounted in the wall of the pressure chamber.

Duration of positive and negative pressure. The effect of prolonged exposure to pressure was tested only at relatively low pressures. Samples of smolts were subjected to a pressure of 15 or 20 psi above atmospheric for periods of 1, 3, 6, 12, 24 and 48 hr. At 50 psi groups were tested for short durations, such as 5, 30 and 60 min and for longer periods of 24 and 48 hr. The pressure pump was not well suited to prolonged operation at high pressures and smolts were not exposed to 300 psi for more than one hour.

The effect of exposure to prolonged negative pressures was tested at vacuums of 10 and 15 in Hg. Periods of exposure to vacuum were 0.1, 1.0, 10 and 100 sec. In various experiments dealing with the swimbladder, smolts were exposed to vacuums of 5, 10, 15, 20 and 25 in Hg for periods of 2 to 5 min. In these latter experiments however, the fish were not subjected to post-test holding and observation.

Rate of increase and decrease in pressure. A limited number of studies were conducted on rates of pressure increase. Pressure was developed by a motor-driven roller-pump, as described previously and this equipment was not suited to very quick application of pressure. The most rapid rate of increase possible was approximately 300 psi in 30 sec or 10 psi per sec.

Groups of 200 smolts were exposed to a range of rates of reduction in pressure from 50 and 300 psi to atmospheric conditions. In one series, for example, pressure was reduced from 300 psi to atmospheric pressure at intervals of 0.02, 0.2, 2.0, 20 and 200 sec, yielding rates of change of 15,000, 1,500, 150, 15 and 1.5 psi per sec.

Conditions altering resistance to decompression

Seasonal effect. Tests were conducted routinely during the downstream migration of 1959. A sudden change in tolerance of sockeye smolts appeared late in the migration.

This change focussed attention on the possibility that seasonal factors were involved in the tolerance of these fish to changes in pressure. This was explored in the succeeding years, 1960 and 1961. Samples of smolts drawn from the migration at one- to two-week intervals from March to June were subjected to the standardized decompression test and observed, post-test, as described.

This standard test consisted of placing a sample of sockeye smolts, trapped at the outlet of the lake during their downstream migration, into the pressure chamber and raising the pressure to 50 psi from atmospheric in 50 sec and holding the fish under this pressure for 5 min. This 5 min holding period merely provided time for adjusting valves and operating the recorder. Thereupon the pressure was released in 0.02 sec to a negative pressure consisting of a vacuum of 29 in Hg and the vacuum was maintained for 1.0 sec. Previous studies had shown that the brief exposure to positive pressure did not influence the resulting injury or mortality. This phase of the test was retained in order to make the results comparable with earlier applied studies and to provide a more uniform rate of change of pressure. The magnitude of vacuum was set at a high level to maximize mortality and hence better separate experimental groups. The mechanism of action of this change was explored as outlined in the succeeding sections.

Holding smolts at lake surface and depth. The seasonal change in pressure tolerance of migrating sockeye was observed in 1959 to be influenced also by the time elapsed between taking the smolts from the migration and subsequently subjecting these fish to the standard decompression test. In 1960 and 1961 the effect of this delay before testing was explored. Samples of smolts migrating out of the lake late in the season (June) were held in the pens located in Sweltzer Creek, essentially in temperatures equal to the surface water of the lake. Samples of smolts were tested after 0, 3, 6, 18, 48 and 72 hr of holding.

In 1960, a sample of 100 late-migrating smolts was tested and the resulting mortality recorded. A second sample of 200 smolts was held for 24 hr in Sweltzer Creek, then 100 of these were subjected to the standard test; the remaining sample of 100 fish was transferred, within a cage, to a depth of 35 ft in the lake and there suspended for an additional 24 hr. This depth coincided with the lower portion of the lake thermocline. At the end of this period the fish were retrieved and subjected to the standardized decompression test in Sweltzer Creek water.

The following year this experiment was repeated in a slightly more elaborate form and with longer periods of holding at the surface of the lake and at depth. In this experiment

a sample of 400 sockeye smolts was drawn from the migration in mid-June. A sub-sample of 100 of these fish was tested immediately as a control or index of the mortality associated with the seasonal effect. The remaining 300 fish were held three days in lake surface waters, at the end of which time 100 were tested as a measure of the extent of the reversal of the seasonal effect. The remaining 200 sockeye were then held at a depth of 35 ft in the lake for a period of seven days. One hundred of these were then tested for the return of the seasonal effect. The remaining 100 were held two days in lake surface conditions and then similarly tested. This study is illustrated diagramatically in Fig. 36.

Increasing content of dissolved gases. The apparatus was modified to permit the air saturation of the water in the chamber to be increased by bubbling air into the chamber under pressure. In practice, the pressure chamber was filled with water and air was pumped copiously in through the bottom at 8 psi. The air so entering was allowed to escape through the small valve at the top of the chamber. Pressure within the chamber was maintained by the pressure pump and was regulated with the by-pass valve and the air-exit valve. The level of saturation within the chamber was thus a function of the initial temperature and saturation of the water and the duration of bubbling air through under pressure. Temperature

and saturation were kept relatively constant at approximately 50°F and 11 ppm and hence it was possible to control gas content of the water over the range of 100 to 140 per cent of air saturation by bubbling air into the water for 0 to 60 min. A nomogram was constructed permitting the duration of aeration to be estimated for any given level of desired saturation.

Nitrogen analyses were conducted on one occasion to establish whether oxygen and nitrogen were increasing at the same rate, that is, in relation to the products of their partial pressures and solubilities. Harvey and Smith (1961) demonstrated that under pressure these two gases may increase in solution in relation to their partial pressures alone where the gas phase is small. Oxygen and nitrogen analyses were carried out initially and after every 10 min up to 60 min of aerating under pressure. Four analyses were conducted for each gas initially and at the end of 60 min and single analyses at each of the intervening times. The results of this study, as shown in Table I, indicate the two gases were increasing in solution at approximately the same rate. The grossest discrepancy, after 30 min of aerating under pressure, is still within the limits of the accuracy of the methods of analysis. In view of this agreement between oxygen and nitrogen saturation, thereafter air saturation within the chamber was determined solely by the much simpler and speedier Winkler method.

TABLE I

Increase in oxygen and nitrogen saturation during aeration under pressure.

Time in minutes	Oxygen per cent saturation	Nitrogen per cent saturation
0	96*	98*
10	121	119
20	129	132
30	133	137
40	135	137
50	136	138
60	137*	138*

* averages of four determinations

In operation, a sample of 50 or 100 smolts was placed in the apparatus, the chamber sealed, filled with water and air bubbled in at 8 psi for the time appropriate to the desired level of saturation. At the end of this time the air flow was stopped, the excess gas bled from the top of the chamber and the oxygen sample drawn. The pressure was raised to 15 psi and the fish were held there for some period of time exposed to the higher gas tension of the water. The periods of holding tested were 5 min, 1, 3, 6, 12, 24 and 48 hr. The

ranges of saturation tested were 100 to 110, 110 to 120 and 130 to 140 per cent of air saturation. At the end of the holding period the fish in the chamber were subjected to the standardized decompression test.

In these holding experiments, the water in the chamber was static and hence the oxygen in the water was consumed by the fish over a period of 8 to 12 hr. For this reason the water in the chamber was changed and recharged every 4 hr. Thus oxygen saturation did not fall below approximately 50 per cent of air saturation; a level which should have permitted these fish to maintain blood saturation. (Itazawa, 1959). As before, the fish were held under observation for two weeks following testing. In these saturation experiments, the series conducted at 100 to 110 per cent of air saturation served as controls for those conducted at the higher gas tensions.

Changes in temperature. Before determining the effect on fish of rapid changes in temperature and hence saturation, in relation to pressure, it was necessary firstly to measure the rate of change of temperature of the fish themselves following a change in the temperature of their environment. For this purpose, individual sockeye were anesthetized and a thermistor probe inserted through a small hole near the vent and located in the region of the head kidney. The lightly-

anesthetized fish were brought to a constant temperature of 45°F, then transferred to 60°F and the temperature recorded every 30 sec. The results obtained for five fish ranging from 3.8 to 42 g are shown in Fig. 15. With this knowledge of the rapidity of the change in temperature by the fish, it was possible to proceed with the temperature studies, allowing only 5 min for the smolts to equilibrate with temperature of their environment.

Following this, samples of 100 smolts were held at two temperatures, 45 and 55°F. After two to three days of holding at these temperatures the fish were transferred to the pressure chamber and subjected to sudden temperatures increases of 0 to 19°F before being exposed to the standard pressure test.

Swimbladder gas in relation to pressure

The possibility existed that the gas within the swimbladder was responsible in part or in total for the embolism-induced mortalities observed following pressure reduction. Five types of experiments were designed to explore this possibility.

Increasing and decreasing gas content of the bladder. A sample of 50 sockeye smolts was placed in the pressure apparatus and the fish held 90 min at a pressure of 15 psi. A large air lock was present in the top of the chamber to permit these fish to inflate their swimbladders to neutral

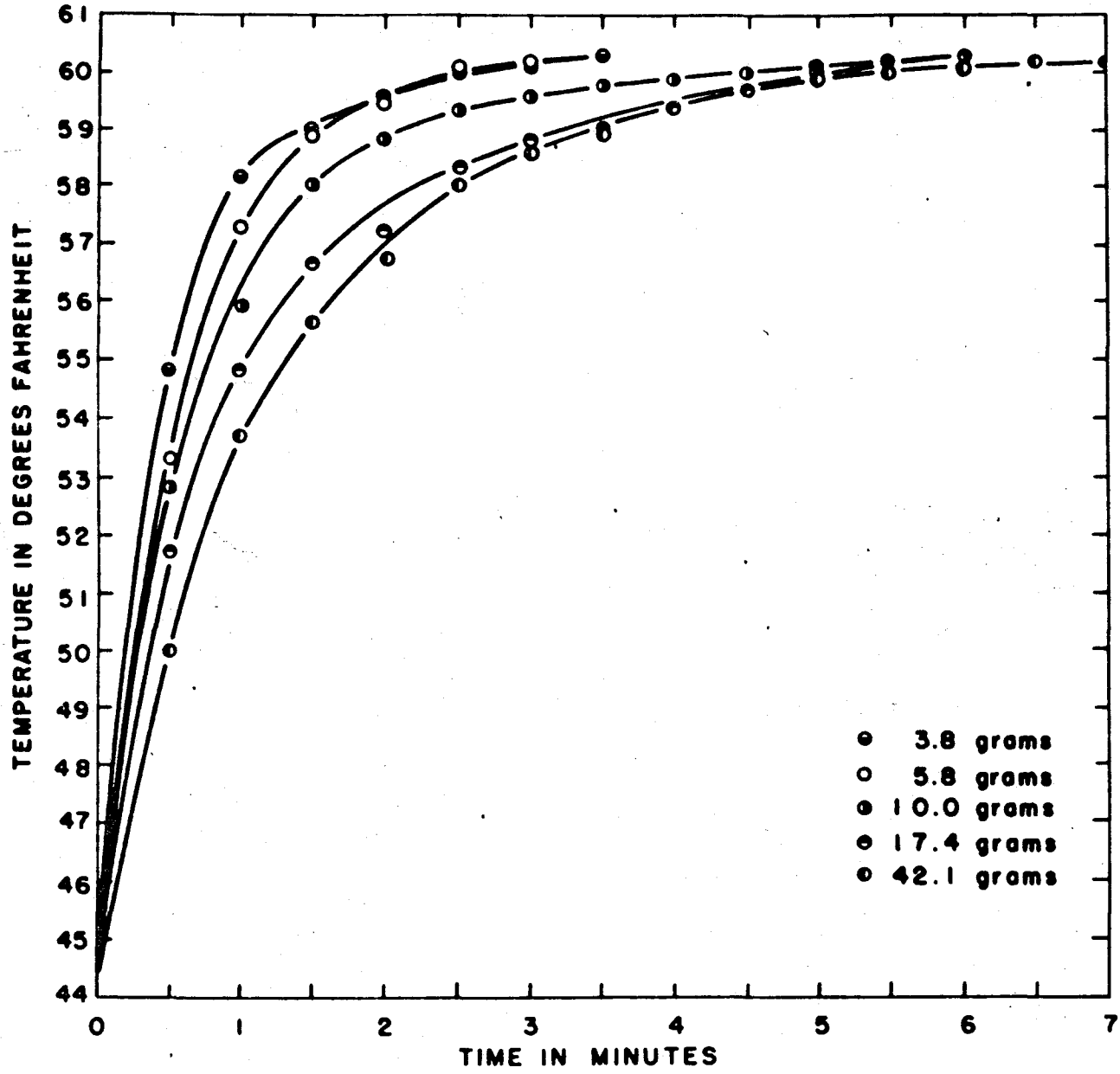


Figure 15. Change of temperature internally of fish transferred from 45 to 60° F water.

buoyancy. At the end of the holding time the air lock was bled off and the chamber completely filled with water, still at a pressure of 2 atmos. The pressure was reduced gradually and the pressure of neutral buoyancy of the fish noted. The smolts were then subjected to the standardized decompression test and post-test observation.

This experiment was repeated with a longer period of holding at a pressure of 30 psi above atmospheric. Even with a strong flow of water through the chamber the water still supersaturated appreciably. At the end of 24 hr the buoyancy of the fish was determined and the group subjected to the decompression test.

A 50-smolt sample was placed in the pressure chamber and exposed to increasing negative pressures. The pressure was reduced below atmospheric in increments of 2.5 in Hg and the buoyancy of the fish observed after each reduction. The vacuum reached 27.5 in Hg at the end of 15 min the pressure then being returned to atmospheric. These fish then were subjected to the decompression test.

Bladderless and catheterized-bladder fish. Groups of 20 prickly sculpins (Cottus asper) were subjected to the same experimental conditions which had influenced the mortality of sockeye. Sculpins were acquired from the sockeye migrant trap in Sweltzer Creek and held in a cage in the stream.

A sample of 20 sculpins was placed in the pressure apparatus and subjected to the standardized decompression test. A second group of 20 was held 24 hr at a nitrogen tension elevated to approximately 130 per cent of air saturation. The excess gas was held in solution by holding the fish and water at a pressure of 15 psi. As before, the sculpins were then subjected to the pressure-reduction test. A third group of 20 sculpins was similarly treated but the holding period in the higher nitrogen tension water was increased to 96 hr to assure fish-environment equilibrium before decompression.

As a check against possible species differences between the bladderless sculpins and sockeye, it was decided to remove the influence of the sockeye bladder. This was done by inserting a catheter into the bladder, as described previously, such that gas in the bladder was free to escape to the exterior. Thus reduction in pressure could not increase the pressure of gas within the bladder and hence increase the diffusion gradient between the bladder and the remainder of the fish, nor would the pressure be so great that damage to the bladder would result and gas bubbles enter the blood stream. Both of these two possibilities were postulated by Jones and Marshall (1953).

Ten such catheterized test fish and ten control smolts were placed in the pressure apparatus and subjected to a 24

hr exposure to high nitrogen tension (130 per cent of air saturation). At the end of this time the fish underwent the standardized decompression test followed by post-test observation.

Duct-release pressure and holding sockeye at depth. The studies on bladderless fish implicated the swimbladder in the mortalities associated with pressure reduction and the experiments on catheterized smolts suggested retention of gas in the swimbladder was involved in the mechanism of embolism formation. For this reason the pneumatic ducts of fish held at the surface of the lake were compared with those of sockeye held at a depth of 40 ft in the lake. These fish were compared as to the relative patency of the pneumatic duct, using the method for measuring duct-release pressure as described previously. Ten fish held at depth for 7 days were compared with 10 fish held the same period at atmospheric pressure in lake water pumped to the surface from depth.

Equilibration of fish with dissolved nitrogen of the environment

The purpose of this study was to establish the rate at which fish equilibrate internally with the dissolved nitrogen of their environment. More precisely the rate at which fish clear excess nitrogen from blood and tissues. The original approach was to measure nitrogen in venous and arterial

blood following transfer of the fish from water of high nitrogen to that of a lesser content of dissolved nitrogen. From a knowledge of cardiac output the rate of nitrogen clearance could be calculated.

Rate of change of nitrogen in venous and arterial blood. Adequate blood samples for gas analysis dictated fish of approximately 100 g. For this purpose hatchery rainbow trout, Salmo gairdneri were chosen. Individual fish were exposed to increased gas tensions within the pressure apparatus. Nitrogen content was raised to 17.5 ml per liter (an average of 127.9 per cent of air saturation, ± 3.3 per cent for 1 standard deviation) by bubbling air into the chamber for 30 min at a pressure of 8 psi. The temperature of the water within the chamber was held within a few degrees of 57°F. The trout were held in this water of increased gas content for 60 min at a pressure of 15 psi above atmospheric. The fish were then removed quickly from the chamber and transferred to water of 13.2 ml per liter nitrogen (approximately 100 per cent of air saturation). Individual fish were held 0, 5, 10, 15, 20, 30 or 60 min in air-saturated water and nitrogen in venous blood was determined at the end of that time. Five test and 5 control fish were employed for each of the seven periods of holding.

The absence of arterial blood in the fish heart required blood be drawn from the dorsal aorta (conducting oxygenated blood from the gills) if clearance of nitrogen across the gills was to be measured. Attempts to collect arterial blood without interfering with respiration, an essential to the measure of gill clearance, were uniformly unsuccessful. Alternatively the rate was investigated at which all dissolved nitrogen in the fish was equilibrating with that of the environment.

Rate of equilibration of total dissolved nitrogen. As internal nitrogen could not be measured directly, an attempt was made to measure the nitrogen given up by a fish when transferred to an environment of lower dissolved nitrogen. The problem was to measure accurately the nitrogen content of the medium water, preferably at intervals until fish-water equilibrium was reached. The nitrogen analysis was consistent to only ± 2.1 per cent for one standard deviation. This required that the initial content of dissolved nitrogen in the water be kept small relative to the nitrogen content of the fish. This was done by reducing the volume of the water and lowering the nitrogen concentration by boiling, cooling and oxygenating the water. In operation, individual yearling sockeye ranging from 7 to 11 g were acclimated for one week to water averaging 14.4 ml of nitrogen per liter, then transferred

to water averaging 1.64 ml per liter of dissolved nitrogen. The shortcoming of this method is unfortunate: the gradient of nitrogen between fish and environment is several times greater than that of the blood and pressure studies. Water samples were drawn immediately the fish were introduced into the low-nitrogen water and at intervals of 5, 10, 20 and 30 min. It was not possible to keep fish for longer periods under these test conditions.

Control series, in which only the fish was omitted, failed to show any change in nitrogen content of the water in the closed system over the 30 min of testing.

The nitrogen lost from the fish was calculated from the nitrogen gained by the water, the volume of water being corrected as each 0.6 ml sample was drawn. At the end of 5 min the nitrogen gained by the water was:

$$\frac{(47.4 - 0.6)}{1000} \times (2.17 - 1.66) = 0.024 \text{ ml}$$

where 47.4 is the initial volume of water in ml

0.6 is the volume of the sample drawn at time zero

2.17 is the nitrogen tension in ml per liter at the end of 5 min

1.66 is the nitrogen content at time zero

The potential loss of nitrogen was calculated from the size of the fish and difference between the nitrogen content of the acclimation water and closed-system water. Without

a precise measure of the solubility of nitrogen in the fluids and tissues of a fish, the fish were arbitrarily assigned a nitrogen solubility equal to that of water. Thus the potential loss of nitrogen by the fish in this example:

$$\frac{7.5}{1000} \times (14.30 - 2.17) = 0.091 \text{ ml}$$

where: 7.5 is the weight of the fish in g

14.30 is the nitrogen content in ml per liter of the water to which the fish was acclimated

2.17 is the nitrogen tension of the closed-system water at the end of 5 min

The nitrogen lost by the fish was expressed as per cent of potential loss at each time interval as an index to the rate of equilibration of nitrogen between fish and environment. Thus at the end of 5 min nitrogen loss was:

$$\frac{0.024}{0.091} \times 100 = 26 \text{ per cent of the dissolved nitrogen of the fish.}$$

CHAPTER III

RESULTS

Pressure in Relation to Behavior

The behavioral responses of young sockeye to pressure were readily apparent. Obvious changes in compensatory swimming and angle of inclination resulted as pressure was increased above atmospheric. Most apparent was the absence of a behavioral change with slight increase in pressure (but appreciable increase in density). Further increase in pressure soon evoked maximal compensatory swimming, suggesting young sockeye have a wide range of preferred density but when this is exceeded the fish respond maximally. Also noticeable was the absence of compensatory swimming in fry, its presence in fingerlings and gradual reduction with increasing size from one-year to two-year-old smolts.

Response of one-year-old smolts. Ten sockeye smolts, ranging in size from 2.3 to 6.4 g were tested. Pectoral fin rates ranged from 133 to 174 beats per min at atmospheric pressure (Fig. 16). This wide range was maintained throughout the pressures tested. It was due primarily to the size of the fish, the smaller fish showing the more rapid fin movements. As the pressure was raised from atmospheric

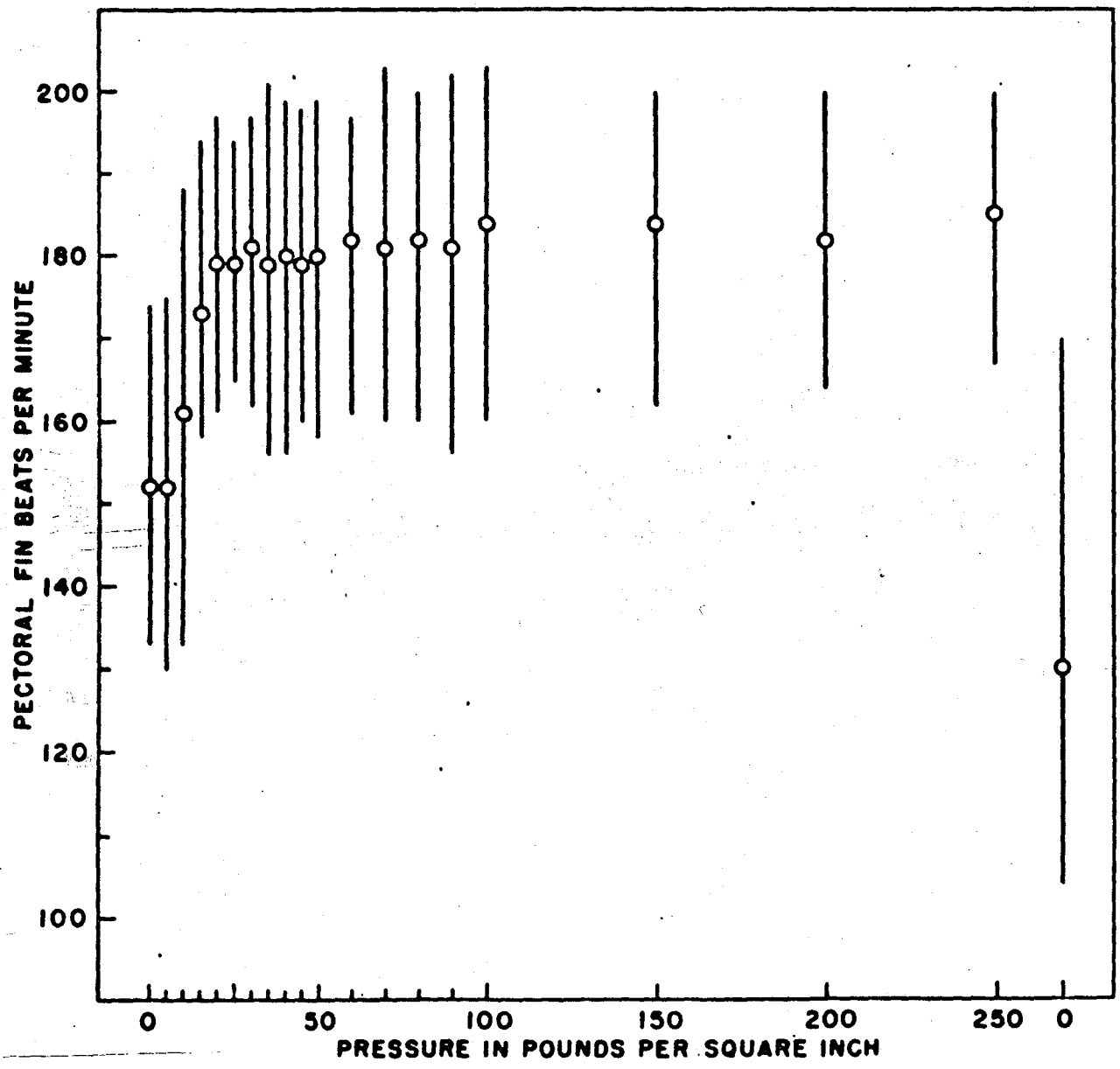


Figure 16. Average and range of pectoral fin beats for ten sockeye smolts from atmospheric pressure to 250 psi above atmospheric.

CHAPTER IV

DISCUSSION

Young sockeye, unlike other species of Pacific salmon, spend a year or more in lake residence before migrating seaward. The utilization of the vertical dimension of the lacustrine environment requires young sockeye to live over a range of depths and to make vertical migrations. Pressure is related directly to depth and is thus an environmental factor in the lake habitat. Residence and migration over a range of pressures present fish with certain physiological problems. The nature of these problems and how they are met will be discussed in the sequence of stages in the life history of the sockeye salmon.

The problem of pressure stimulating alevins and fry. Adult sockeye may deposit their eggs in redds on the lake bottom. This occurs in shallow water at Cultus Lake and at depths of a hundred feet or more in Great Central Lake, British Columbia. After hatching, sockeye alevins are subjected to the accompanying hydrostatic pressure until they emerge as fry from the redd. Experimentally, fry showed no response to increased pressure prior to initial filling of the swimbladder. (Fig. 17). That is, increased pressure does not result in increased swimming in the fry and probably

to 5 psi above atmospheric, equivalent to 10 ft of water depth, the fish evidenced no change in behavior. Thereafter, raising the pressure to 10, 15 and 20 psi resulted each time in an increase in the rate of movement of the pectoral fins. Concurrently the smolts assumed a progressively steeper angle (Fig. 5 and 6) from the horizontal reaching approximately 60 degrees at 20 psi. This increased upward swimming permitted the smolts to maintain a position between the top and bottom of the pressure chamber. From this central area of the chamber, individual fish periodically rose to probe the top of the chamber. Occasionally individuals ceased swimming actively and reclined on the bottom of the chamber.

Compared by means of the t-test, significant differences did not occur between the fin rates measured for 5 and 10, 10 and 15 or 15 and 20 psi. For example, comparing fin rates at 5 and 10 psi, $t = 1.22$ ($t_{0.3} = 1.1$). Comparing the combined rates at 0 and 5 psi with those recorded at 10 and 15 psi, the two were significantly different with $t = 3.22$ ($t_{0.0025} = 3.17$). Again the rates at 10 and 15 psi compared with those at 20 and 25 psi yielded a t value of 2.91($t_{0.005} = 2.86$). Finally, the difference between the minimal movements of the pectoral fins at 0 and 5 psi and maximal values at 20 and 25 psi were highly significant with $t = 6.77$. At the end of the test series when the pressure was reduced

from 250 psi to atmospheric conditions, there was a sharp reduction in swimming activity. Pectoral rates most commonly were lower than the initial values, possibly reflecting fatigue. Comparing intitial and final rates at atmospheric pressure $t = 2.38$ ($t_{0.05} = 2.26$) thus the final rate was slower possibly significantly, than the initial rate.

Response of fry and fingerlings. Preliminary studies in 1960 showed a marked difference in the behavior of young sockeye fry and fingerlings three months of age. The former showed no increase in pectoral fin movements, no tendency to swim upward and no increase in swimming activity. The latter in contrast evidenced the accelerated finning with the pectorals which was so characteristic in sockeye smolts. In 1961 this study was repeated commencing with fry dug from the spawning redds (prior to inflation of the swimbladder) and replicated at monthly intervals. The results, averages of five fish of each age are shown in Fig. 17. The change in behavioral response, as quantified by pectoral rates, was largely completed at the end of one month and at the age of two months the rather characteristic smolt pattern was established. Three-, six- and nine-month-old fingerlings showed essentially the same curves of increasing pectoral fin activity with increasing pressure up to 20 or 25 psi.

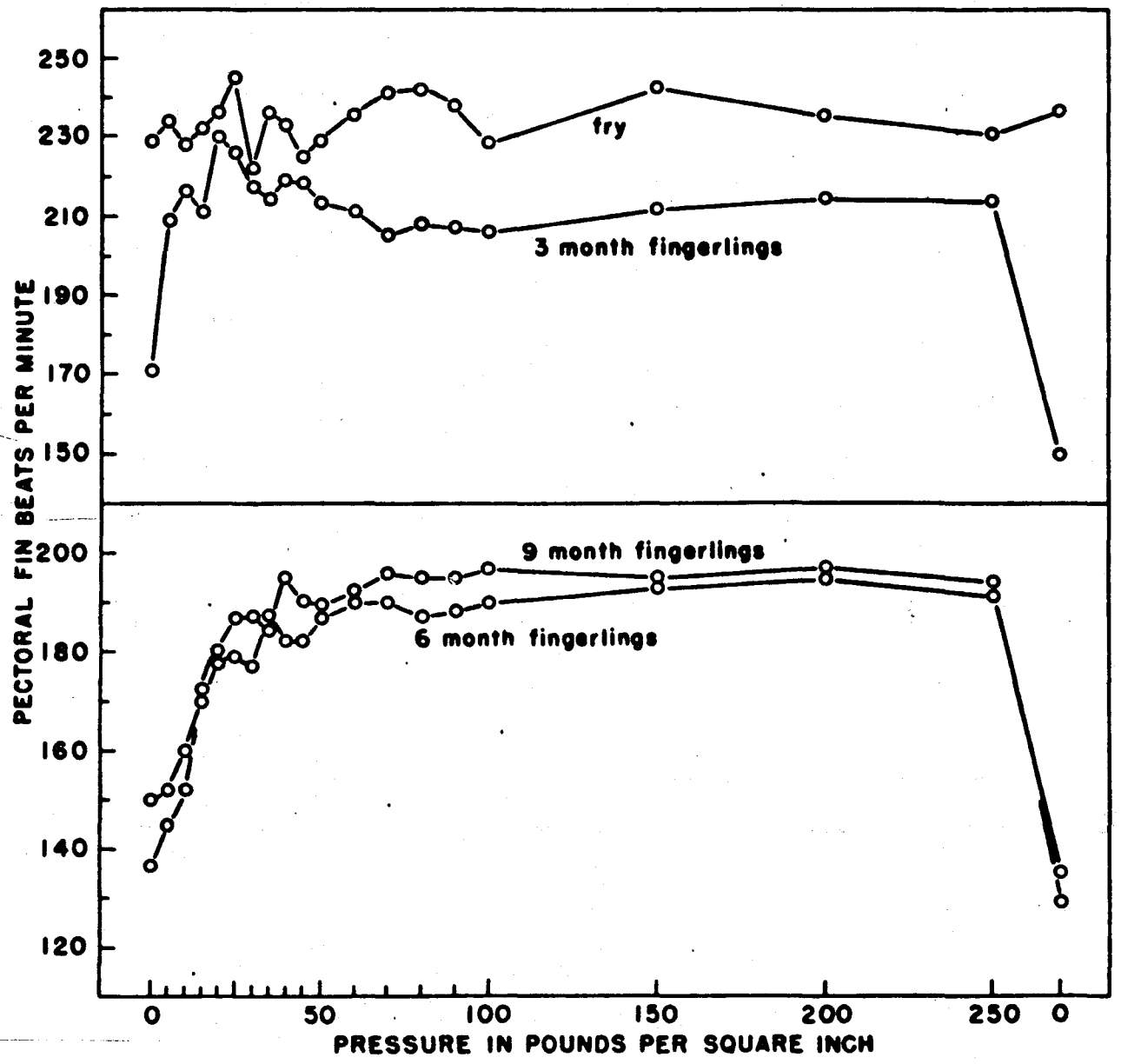


Figure 17. Average pectoral fin rates of fry and three-, six- and nine-month-fingerlings.

Response of yearlings and two-year-old smolts.

Sockeye of one and one-half and two years in fresh water showed a somewhat altered response. The larger fish did not maintain position in the chamber by simply swimming upward. These larger individuals instead swam to and fro rapidly along the long axis of the test cylinder planing upward on the pectoral fins. This altered behavioral response is largely responsible for the much more gradual increase in the pectoral fin beats of the two-year-old sockeye. The average pectoral rates for five fish in each of the two groups are shown in Fig. 18. The response of these larger fish (20 g for the two-year-olds) suggest sockeye in the oceanic environment could compensate for negative buoyancy through sustained swimming.

Pressure in Relation to the Swimbladder

Volume of the bladder. Young sockeye proved to have a relatively small swimbladder. At neutral buoyancy in fresh water the swimbladder volume was 5.96 per cent of the volume of the fish. Jones and Marshall (1953) gave expected values of 7 per cent for freshwater and 5 per cent for marine teleosts. The sockeye at 5.96 per cent is intermediate between these values, perhaps due to the presence of cartilage rather than denser bony tissue.

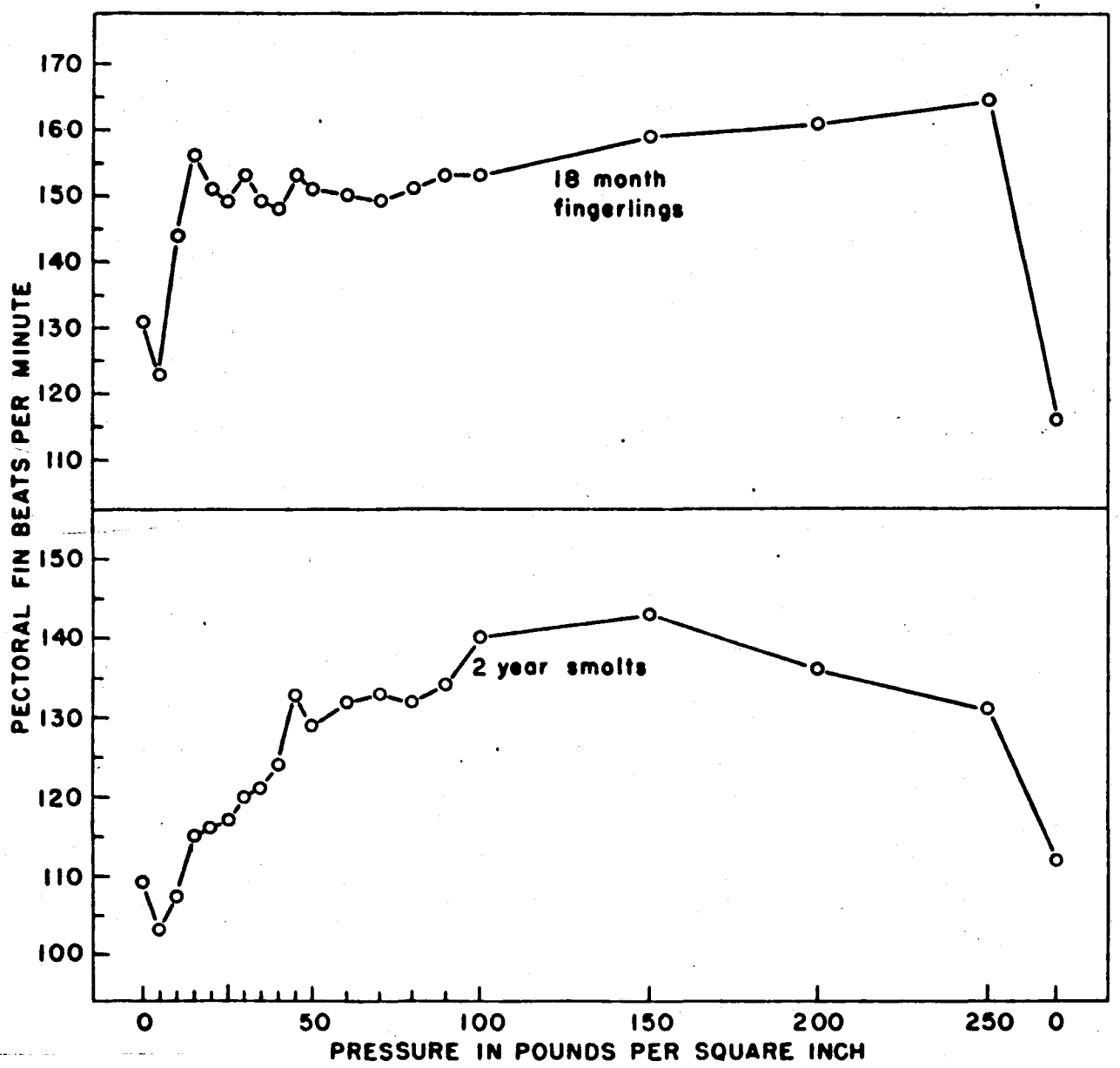


Figure 18. Average pectoral fin rates of eighteen-month and two-year-old sockeye.

Ambient pressure. The method of measuring ambient pressure was based on Alexander (1959a) and for 10 sockeye smolts yielded an average excess pressure of 0.2 mm Hg. That the method could not be employed with a high degree of precision is apparent from the variability about the mean, with ±5.5 mm Hg for one standard deviation. In dealing with so low an excess pressure in the swimbladder, there was little or no separation of the curves of gas expansion. That is, the curve for gas confined within the swimbladder and the curve for that gas outside the swimbladder were very nearly identical. The most extreme individual was chosen (Fig. 8) to illustrate the measurement of excess pressure. The purpose of measuring excess pressure in the bladder was to make possible calculation of duct-release pressure. In this regard the method was sufficiently accurate to show that the pressure within the swimbladder did not exceed appreciably the external pressure on the fish.

Extensibility of the bladder. The vertical movements of sockeye and inflation of the swimbladder to neutral buoyancy at some depth below the surface are both related to the extensibility of the bladder. That is, the ability of sockeye to move upward to the level of positive buoyancy and undergo bladder extension rather than gas loss. This could occur both in the course of vertical movements and possibly during over-

inflation of the bladder at the surface to achieve neutral buoyancy at some depth below the surface. For these considerations the statistic sought was the expansion of the bladder possible before gas escaped through the pneumatic duct. Thus the term extensibility as used here is different from that of Alexander (1959a).

Ten control smolts anesthetized to death and tested immediately showed an average expansion of 183 per cent (bladder volume at neutral buoyancy equalling 100 per cent) before gas escaped through the pneumatic duct. The fish ranged from 115 to 277 per cent with one standard deviation ±62 per cent.

Ten sockeye smolts were provided with an abdominal slit the length of the body cavity, thereby eliminating the confining influence of the abdominal wall. These fish showed, before gas release, an average expansion of 289 per cent, ranging from 242 to 311 per cent with ±20 per cent for one standard deviation. The body wall thus appears to limit the extent of bladder expansion to something between doubling and tripling. In three of these fish the swimbladder ruptured before gas escaped through the pneumatic duct.

Contractibility of the bladder. Isolated swimbladders suspended longitudinally in Ringer's solution showed little or no reduction in length, as interpreted from the kymograph record,

when exposed to adrenaline or acetylcholine (10^{-5}). Cross-sectional loops of bladder similarly tested evidenced a marked reduction in length when tested with adrenaline or ephedrine (10^{-5}). The shortening of the loop of bladder was one-quarter to one-third of its length, approximately equal to a one-half reduction in the cross-sectional area of the lumen of the swimbladder. Considering the sockeye swimbladder to be a cylindrical figure, the gas expulsion accompanying a contraction of this magnitude would be one-half or more of the volume at neutral buoyancy.

Such loops exposed to "dibenzyline" (10^{-5}) evidenced a gradual relaxation and could not be stimulated to contract with adrenaline or ephedrine. The "dibenzyline" blockade could not be by-passed with monoacetin over the range of concentrations 10^{-5} to 10^{-3}. In non-blocked loops of swimbladder adrenaline-induced contraction was relatively slow, lasting 2 to 3 min, in contrast to loss of gas on sounding which was complete usually within 1/2 min.

Little or no response accompanied treatment of isolated loops with acetylcholine, pilocarpine or atropine (10^{-5}). This suggests parasympathetic control was not involved in the contraction of the bladder and hence the expulsion of gas from the swimbladder.

Gas secretion. Fish held above the screens in the 75-liter tanks returned to the surface within a few minutes after sounding and commenced gulping atmospheric air. The density of these fish was very close to neutral buoyancy during the course of the eight-day experiment (Fig. 19). Fish held below the screens continued to lose gas from the swimbladder with the density of the fish increasing over the period of 8 days. There was thus no evidence of inflation of the swimbladder by means of gases dissolved in the holding water.

Fish held under pressure fo 24 hr similarly failed to increase the volume of gas in the swimbladder. In the first test three smolts showed an average density increase of 0.005 g per ml and in the second an average increase of 0.007 g per ml. Thus holding sockeye smolts under pressure for 24 hr did not result in the addition of gas to the swimbladder, on the contrary there occurred a slight gas loss as evidenced by an increase in density.

Loss of gas on sounding. Sixty sockeye smolts held 2 days in water 50 cm deep showed a mean density of 0.9995 g per ml with $\pm$0.0009 g per ml for one standard deviation. Fish similarly held, but startled into sounding by disturbance on the water surface, showed a mean density of 1.0143 g per ml with $\pm$0.0086 for one standard deviation. This was an average loss of 23 per cent of the gas from the swimbladder

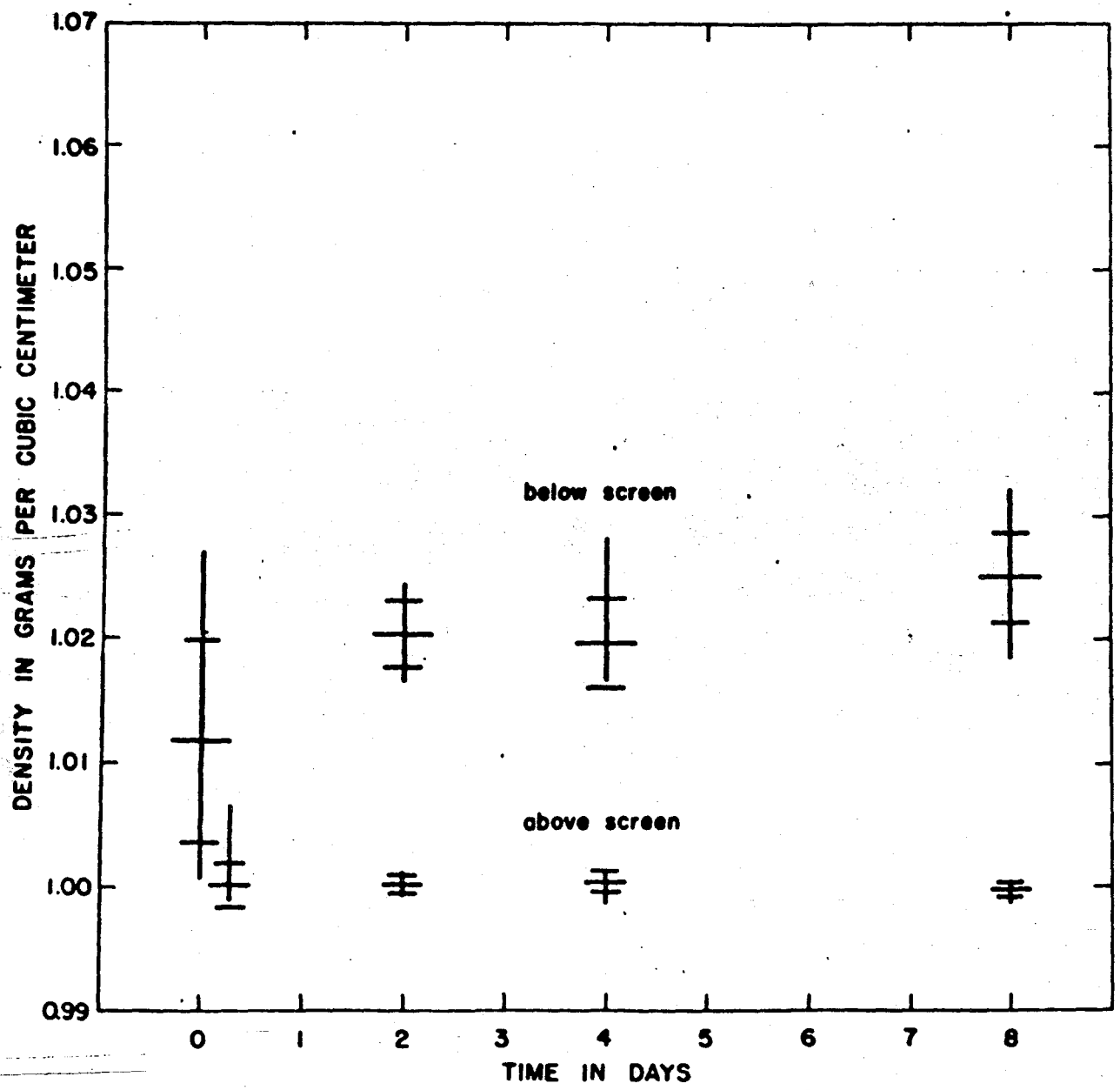


Figure 19. Density of sockeye smolts held above and below screens. Range shown by vertical bar, mean by long horizontal and one standard deviation by short horizontal bars.

gas on sounding. Smolts induced to sound wildly in an agitated glass cylinder had a mean density of 1.0244 g per ml with ±0.0090 for one standard deviation. This was an average loss of gas from the swimbladder of 38 per cent. The greatest loss of gas by any fish was 72 per cent. In the drug-evaluation studies, referred to below, 720 control smolts sounded to a mean density of 1.0214 g per ml, an average loss of gas of 34 per cent.

Sockeye salmon smolts held in a solution of the sympathomimetic ephedrine evidenced the same sounding response as control fish when tested. However, significantly more gas was lost from the swimbladder on sounding. The fish in the two series of tests with exposure to 100 mg per liter of ephedrine, sounded to a mean density of 1.0280 g per ml (Fig. 20), an average loss of gas of 44 per cent. The density or gas loss of ephedrine treated and control fish differed significantly, with t-test values of 3.93 and 2.92 ($t_{0.0025} = 2.91$). The density of smolts held in 200 mg per liter of ephedrine before testing, differed even more from the control fish on sounding with t values of 5.60 and 7.22 for the two series. That loss of gas on sounding was enhanced by adrenergic stimulation suggests the drug ephedrine was acting to lower the threshold of response.

Ephedrine treated sockeye showed marked blanching

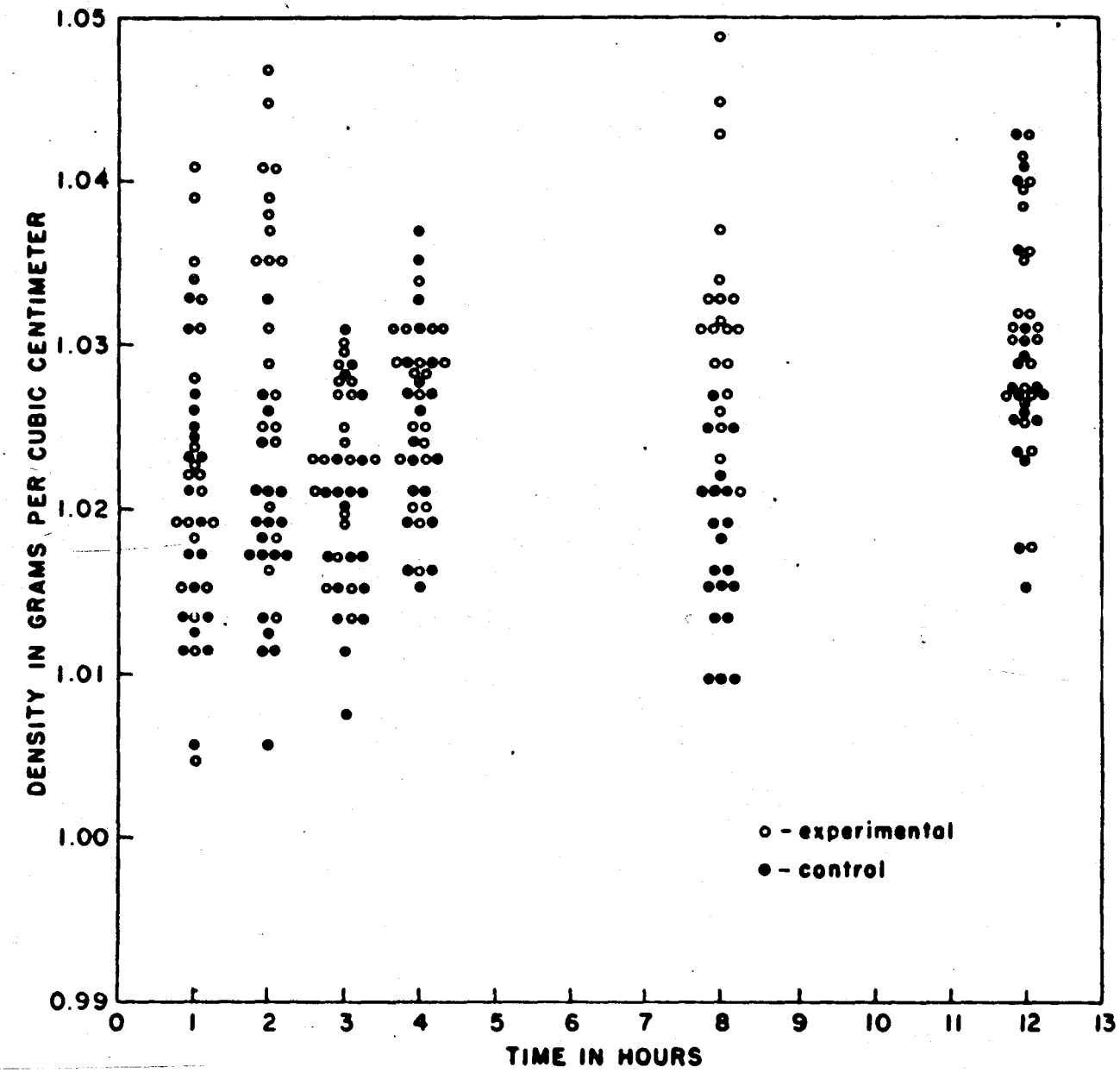


Figure 20. Density of sockeye smolts on sounding. Test fish exposed to 100 mg per liter of ephedrine for up to 12 hours.

of the chromatophores after one-half to one hour of exposure.

In the first "dibenzyline" series, the densities of test and control groups did not differ significantly during the initial 2 hr of drug exposure, with $t = 0.67$ ($t_{0.5} = 0.69$). Beyond 2 hr of holding in "dibenzyline", gas loss on sounding declined rapidly until after 4 hr treatment no loss of gas was observed on sounding. The density difference between treated and control fish was highly significant with $t = 12.10$. The sounding response was unaltered except for the failure of gas to escape from the swimbladder. The study was replicated at the same dose of 5 mg per liter and in the second series loss of gas was retarded even in the one- and two-hour treated groups. The combined results appear in Fig. 21.

A marked chromatophore response was apparent following 1 to 2 hr of exposure to "dibenzyline" with the fish becoming noticeably darker.

The adrenergic blocking agent "hydergine" also resulted in significantly less gas being lost on sounding among treated fish than in the corresponding controls; comparing densities $t = 3.87$ ($t_{0.0025} = 2.91$). At the tested dose of 10 mg per liter, a chromatophore response was apparent following 3 to 4 hr of exposure.

Cholinergic stimulating and blocking agents yielded less consistent results than the adrenergic drugs described above.

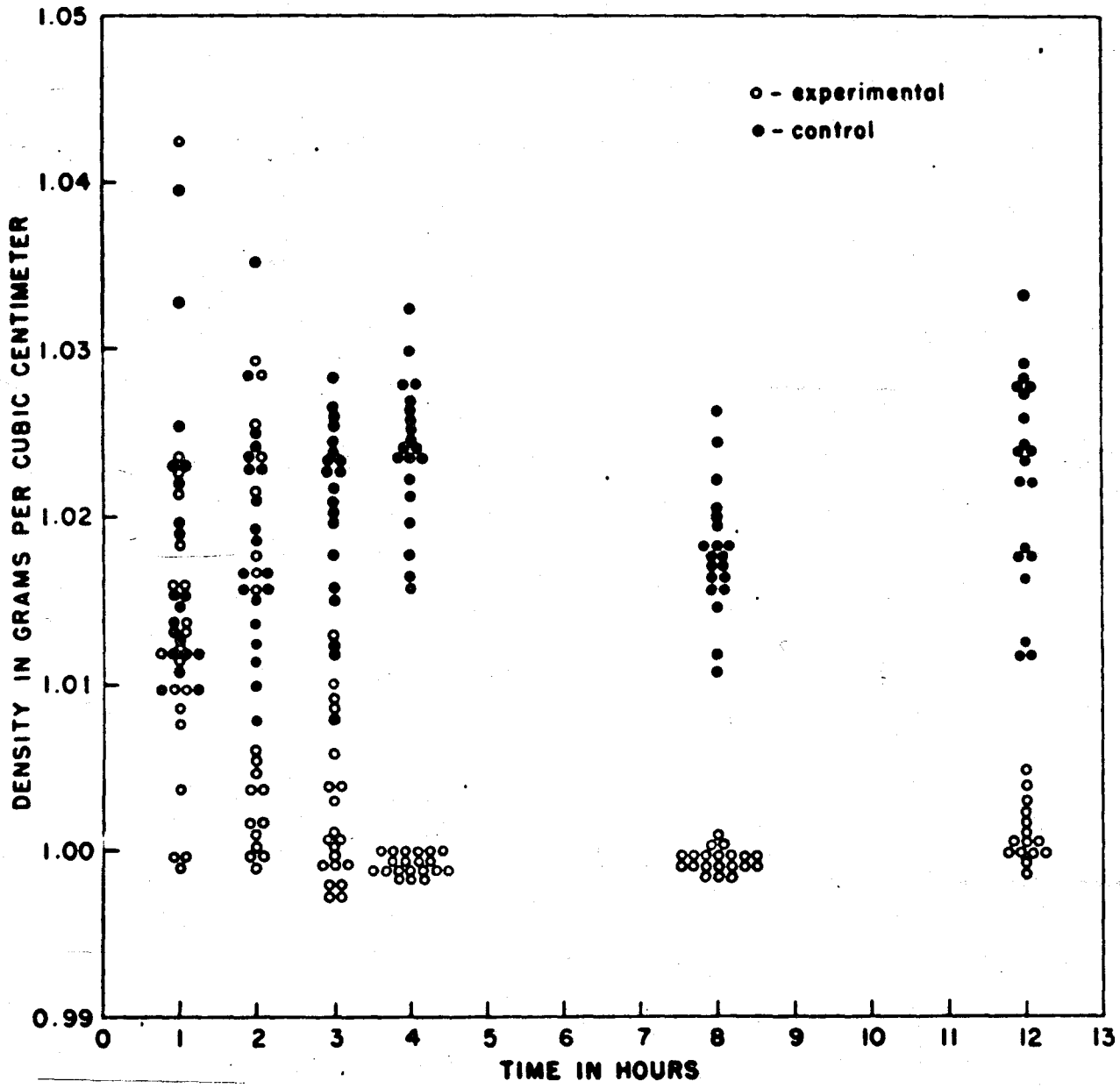


Figure 21. Density of sockeye smolts on sounding. Test fish exposed to 5 mg per liter of "dibenzyline".

Smolts held in 100 mg per liter of pilocarpine lost 42 per cent of their swimbladder gas on sounding, significantly ($t = 2.76$, $t_{0.005} = 2.66$) more than the 36 per cent lost by the corresponding control fish. Fish treated with atropine at a concentration of 200 mg per liter lost only 29 per cent of swimbladder gas on sounding, significantly ($t = 4.12$, $t_{0.0025} = 2.97$) less than the 38 per cent of the corresponding controls. In contrast the cholinergic blocking agent "darbid" did not alter significantly loss of gas on sounding.

Gulping atmospheric air. Smolts catheterized and then held in "dibenzyline" solution (4 hr at 5 mg per liter) continued to surface, mouth air and pass the gas into the swimbladder. There was thus no evidence that this drug was influencing the ability of the fish to inflate their swimbladders with atmospheric air. This was confirmed on non-operated fish which were induced to sound, occluded from the water surface by means of a screen and in this partly degassed condition exposed to "dibenzyline" for 4 hr. On removing the screen at the end of this time, treated and control fish prompty returned to the surface and inflated their swimbladders to neutral buoyancy.

Catheterized smolts treated with atropine similarly continued to attempt to complete inflation of the swimbladder. As was the case with "dibenzyline", atropine did not interfere with the process of air passing into the swimbladder. The response

however was visibly slower in the atropine treated as compared to the control fish.

Duct-release pressure. During the course of the studies on the pressure necessary to affect release of gas through the pneumatic duct, three control series were conducted with ten fish in each. These series showed average pressures at release of 28.9, 28.6 and 26.9 mm Hg and did not differ significantly. Smolts tested at intervals after death showed a progressive reduction in duct-release pressure up to 30 min. A regression line of $Y = 27.18 - 0.78X$ was calculated for this time period (Fig. 22). Fish tested over the period of 5 to 30 min after death differed significantly from control fish at time zero, with $t = 4.15$ ($t_{0.0025} = 3.50$). Beyond 30 min after death, duct-release pressure increased rapidly and in 3 fish exceeded that of the controls. This increase in duct-release pressure coincided with the onset of rigor.

Ten smolts exposed to "dibenzyline", for the time and concentration effective in stopping loss of gas on sounding, showed a mean duct-release pressure of 26.8 mm Hg and did not differ significantly from the control groups. Ten smolts held in a solution of 100 mg per liter of atropine for 12 hr showed an average duct-release pressure of 0.2 mm Hg. Atropine treated fish differed significantly from control and "dibenzyline" fish with $t = 5.32$ ($t_{0.0025} = 3.69$).

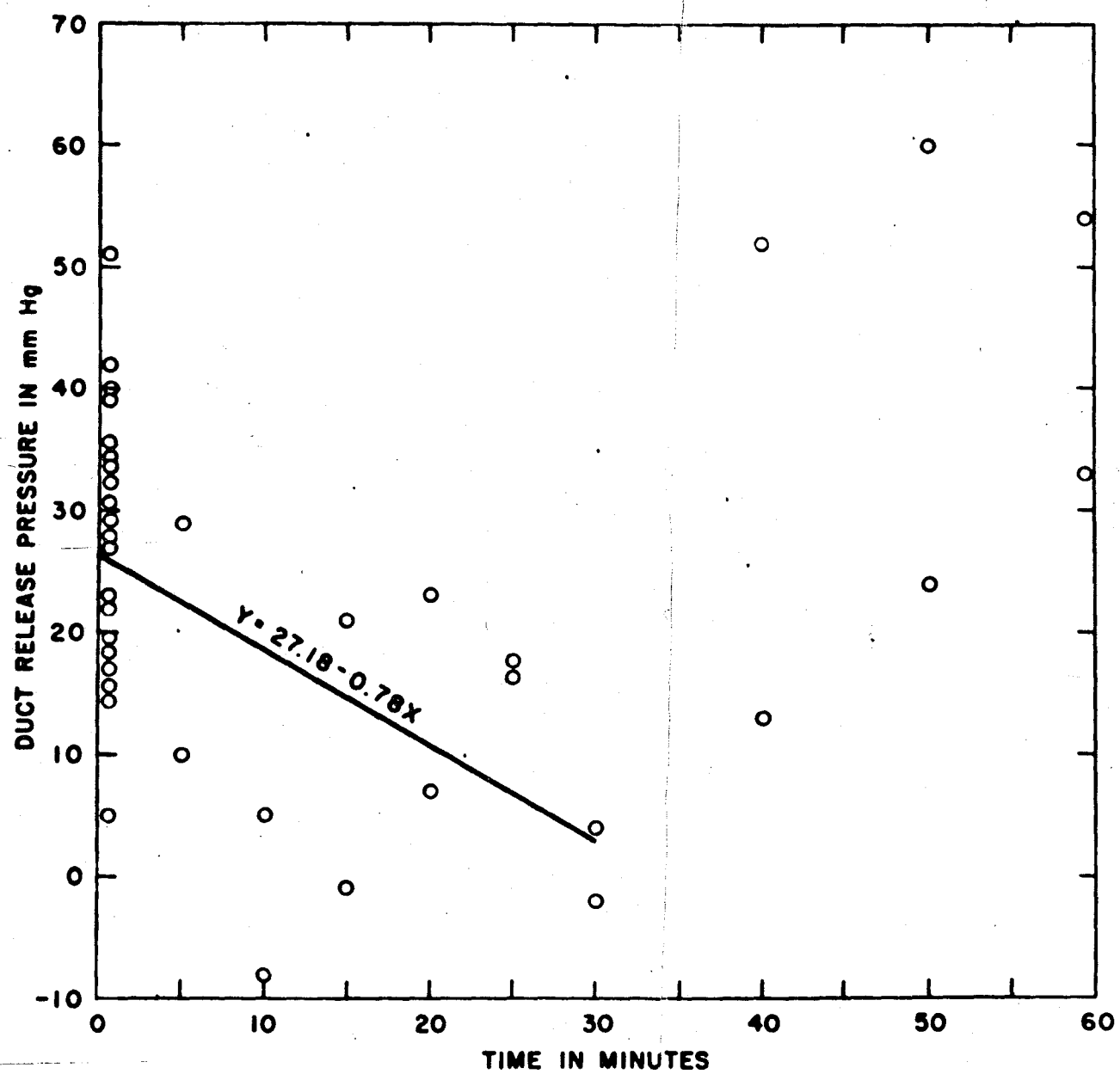


Figure 22. Change in pressure required to force gas out through the pneumatic duct following the death of the animal.

Histological examination. The pneumatic duct proved to be a convoluted connection between oesophagus and the anterior end of the swimbladder. This is apparent in Fig. 23, the oesophagus, lower left, connecting to the swimbladder, upper right. In the upper photomicrograph of Fig. 23, the connection cannot be traced due to the lateral curvature of the duct and the duct appears to terminate in an oesophageal bulb. In the lower photomicrograph the lumen of the duct may be traced from bulb to anterior swimbladder. No obvious sphincter is apparent but the musculature of the oesophagus could conceivably function in this way.

The pneumatic ducts were examined from smolts treated with sympathomimetic and parasympathomimetic drugs plus adrenergic and cholinergic blocking agents. Drug doses effective in altering the loss of gas on sounding had no visible affect on the pneumatic duct. Sockeye injected with 0.1 mg of acetylcholine, however, evidenced a marked constriction of the duct and bulb lumen. Fish injected with atropine showed the more or less typical lumen as illustrated.

Pressure in Relation to Gases

Limnological investigations

The freshwater environment, such as Cultus Lake, presents certain problems to the vertical movements of young



Figure 23. Photomicrographs of the pneumatic duct of the sockeye smolt. Lumen of the duct may be traced from oesophagus through bulbs and thence to anterior end of swimbladder.

sockeye. One of these is the problem of internal supersaturation accompanying temperature increase and decompression. The definition of the temperature and gas content of the lacustrine environment thus became an integral part of the study of the pressure problems of young sockeye. Continuous limnological investigation was limited to the calendar year 1961, the results of which are presented below.

Temperature. During the winter of 1960-61 Cultus Lake remained free of ice and cooled to a minimum temperature of 41°F in late February (Table II, Fig. 24). Considerable heating and mixing took place before thermal stratification, with bottom temperatures reaching 43.8°F and surface temperatures 46°F in late April. Stratification was established by mid-May and surface heating continued into August. During the period May to August there was warming to deeper layers. At 40 ft the temperature increased 7°F to 53°F with only partial loss of the excess gas. From August to mid-September the temperature at 40 ft increased an additional 6.5°F to 59.5°F, due in part to downward mixing of warmer water. Cooling and downward mixing continued from August to December. Complete turnover was achieved in early December as evidenced by isothermal conditions and the sudden increase in oxygen at a depth of 130 ft.

TABLE II

Temperature in degrees Fahrenheit
of Cultus Lake, 1961

Depth in ft.	Jan. 27	Feb. 24	Mar. 17	Apr. 6	Apr. 26
0	42.8	41.0	41.9	45.5	46.9
5	42.8	41.5	41.9	45.0	46.9
10	42.8	41.5	41.9	44.4	46.9
15	42.8	41.5	41.9	44.2	46.9
20	42.8	41.5	41.8	43.2	46.8
25	42.8	41.5	41.7	42.9	46.5
30	42.8	41.5	41.7	42.9	46.3
40	42.8	41.5	41.7	42.8	46.0
50	42.8	41.5	41.7	42.5	45.9
130	42.8	41.5	41.5	41.8	43.8

Depth	May. 17	June 1	June 13	June 30	July 18
0	56.5	65.3	69.5	69.0	72.3
5	53.0	60.0	63.5	66.2	72.0
10	52.5	58.0	63.0	65.5	71.0
15	49.8	55.0	62.5	65.1	69.5
20	49.0	53.0	60.0	63.0	68.0
25	47.7	50.0	55.0	57.5	64.0
30	47.2	48.0	52.0	54.0	60.0
40	46.3	46.0	48.5	50.0	53.0
50	45.5	45.0	46.5	47.0	48.0
130	43.5	43.4	43.6	42.2	42.3

TABLE II (continued)

Depth in ft.	Aug. 4	Sept. 7	Sept. 18	Oct. 8	Oct. 24
0	73.0	66.9	65.9	58.5	54.0
5	73.0	66.7	65.8	58.5	54.0
10	73.0	66.5	65.7	58.5	53.9
15	72.5	66.5	65.6	58.5	53.9
20	71.0	66.5	65.5	58.5	53.9
25	65.0	66.5	65.4	58.4	53.9
30	63.0	65.5	65.3	58.4	53.9
40	53.0	56.0	59.5	58.1	53.9
50	48.0	50.6	51.0	51.4	53.8
130	42.3	44.0	44.2	43.8	43.6

Depth in ft.	Nov. 9	Nov. 24	Dec. 11	Jan. 4/62	Jan. 25/62
0	49.6	46.4	44.2	42.6	40.5
5	49.6	46.4	44.2	42.6	40.4
10	49.6	46.4	44.2	42.6	40.3
15	49.6	46.4	44.2	42.6	40.2
20	49.6	46.4	44.2	42.6	40.1
25	49.6	46.4	44.2	42.6	40.0
30	49.6	46.4	44.2	42.6	40.0
40	49.6	46.4	44.2	42.6	40.0
50	49.6	46.4	44.2	42.6	40.0
130	44.8	45.8	44.2	42.6	40.2

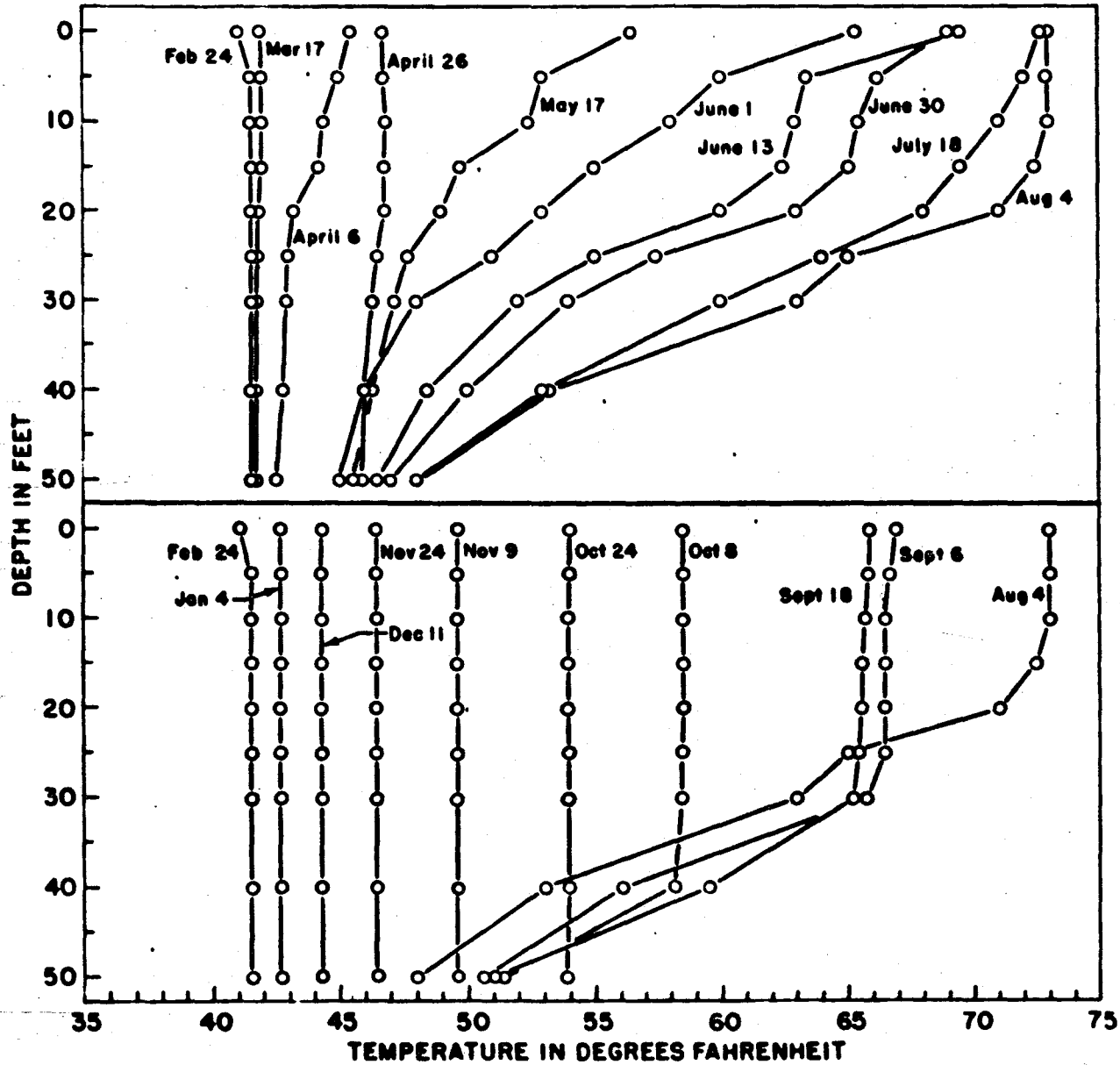


Figure 24. Temperature of the upper 50 feet of Cultus Lake during 1961.

TABLE III

Dissolved oxygen in milligrams per liter of Cultus Lake, 1961

Depth in ft.	Jan. 27	Feb. 24	Mar. 17	Apr. 6	Apr. 26
0	11.5	11.9	12.1	12.2	12.0
5	11.5	11.9	11.8	12.3	12.0
10	11.4	11.8	11.9	12.4	12.0
15	11.4	11.9	12.0	12.3	12.1
20	11.3	11.9	11.9	12.3	12.0
25	11.4	11.9	11.9	12.2	11.9
30	11.4	11.9	12.0	12.1	11.8
40	11.5	11.8	11.8	12.0	11.8
50	11.5	11.7	11.9	12.0	11.8
130	11.9	11.6	11.9	9.5	10.1

Depth in ft.	May 17	June 1	June 13	June 30	July 18
0	11.6	10.0	9.8	9.7	9.0
5	12.0	10.3	10.1	9.5	9.1
10	12.1	10.7	10.2	9.6	9.1
15	12.3	11.5	10.3	9.6	9.2
20	12.7	12.0	10.5	11.2	9.3
25	12.6	12.6	12.5	12.6	10.5
30	12.4	12.3	12.6	12.8	12.6
40	12.0	11.7	12.0	12.3	12.4
50	11.6	11.1	11.2	12.2	11.9
130	10.8	8.5	9.4	9.4	9.3

TABLE III (continued)

Depth in ft.	Aug. 4	Sept. 7	Sept. 18	Oct. 8	Oct. 24
0	8.9	9.6	9.7	10.0	10.2
5	8.8	9.6	9.6	10.1	10.3
10	8.8	9.5	9.6	10.3	10.5
15	8.9	9.4	9.7	10.1	10.4
20	9.8	9.4	9.6	10.2	10.4
25	11.6	9.6	9.8	10.2	10.5
30	12.4	10.0	11.3	10.2	10.5
40	12.6	12.6	12.0	11.1	10.4
50	11.7	12.0	10.5	9.8	10.0
130	10.1	8.1	5.3	7.5	7.9

Depth in ft.	Nov. 9	Nov. 24	Dec. 11	Jan. 4/62	Jan. 25/62
0	10.4	10.0	10.7	11.5	12.2
5	10.5	9.7	10.9	11.5	12.3
10	10.4	9.8	10.7	11.4	12.1
15	9.7	9.6	10.6	11.2	12.0
20	9.8	9.6	10.6	11.2	12.1
25	10.1	9.7	10.6	11.4	12.1
30	10.3	9.7	10.6	11.2	12.2
40	10.3	9.7	10.6	11.2	12.2
50	9.8	9.7	10.5	11.2	12.2
130	7.4	6.6	10.6	11.1	12.0

TABLE IV

Oxygen expressed as per cent of air saturation

Depth in ft.	Jan. 27	Feb. 24	Mar. 17	Apr. 6	Apr. 26
0	93	93	95	100	101
5	93	93	93	101	101
10	92	93	94	101	101
15	92	93	95	100	102
20	91	93	94	99	101
25	92	93	94	98	100
30	92	93	94	97	99
40	93	93	93	96	98
50	94	92	94	95	98
130	95	91	93	75	82

Depth in ft.	May 17	June 1	June 13	June 30	July 18
0	110	106	109	107	103
5	110	103	105	102	104
10	110	105	106	102	103
15	108	108	106	102	102
20	110	110	105	116	102
25	108	111	117	122	110
30	105	106	114	118	126
40	100	97	104	108	114
50	95	92	95	95	102
130	88	69	76	75	74

TABLE IV (continued)

Depth in ft.	Aug. 4	Sept. 7	Sept. 18	Oct. 8	Oct. 24
0	102	104	104	98	95
5	101	103	102	99	96
10	101	102	102	101	98
15	102	101	101	99	97
20	111	101	102	100	97
25	122	103	104	100	98
30	127	107	120	100	98
40	115	119	118	109	97
50	100	106	94	88	93
130	81	66	43	61	64

Depth in ft.	Nov. 9	Nov. 24	Dec. 11	Jan. 4/62	Jan. 25/62
0	92	85	88	92	94
5	93	82	89	92	95
10	92	83	88	91	93
15	85	81	87	90	92
20	86	81	87	90	93
25	89	82	87	91	93
30	91	82	87	90	94
40	91	82	87	90	94
50	86	82	86	90	94
130	65	55	87	89	93

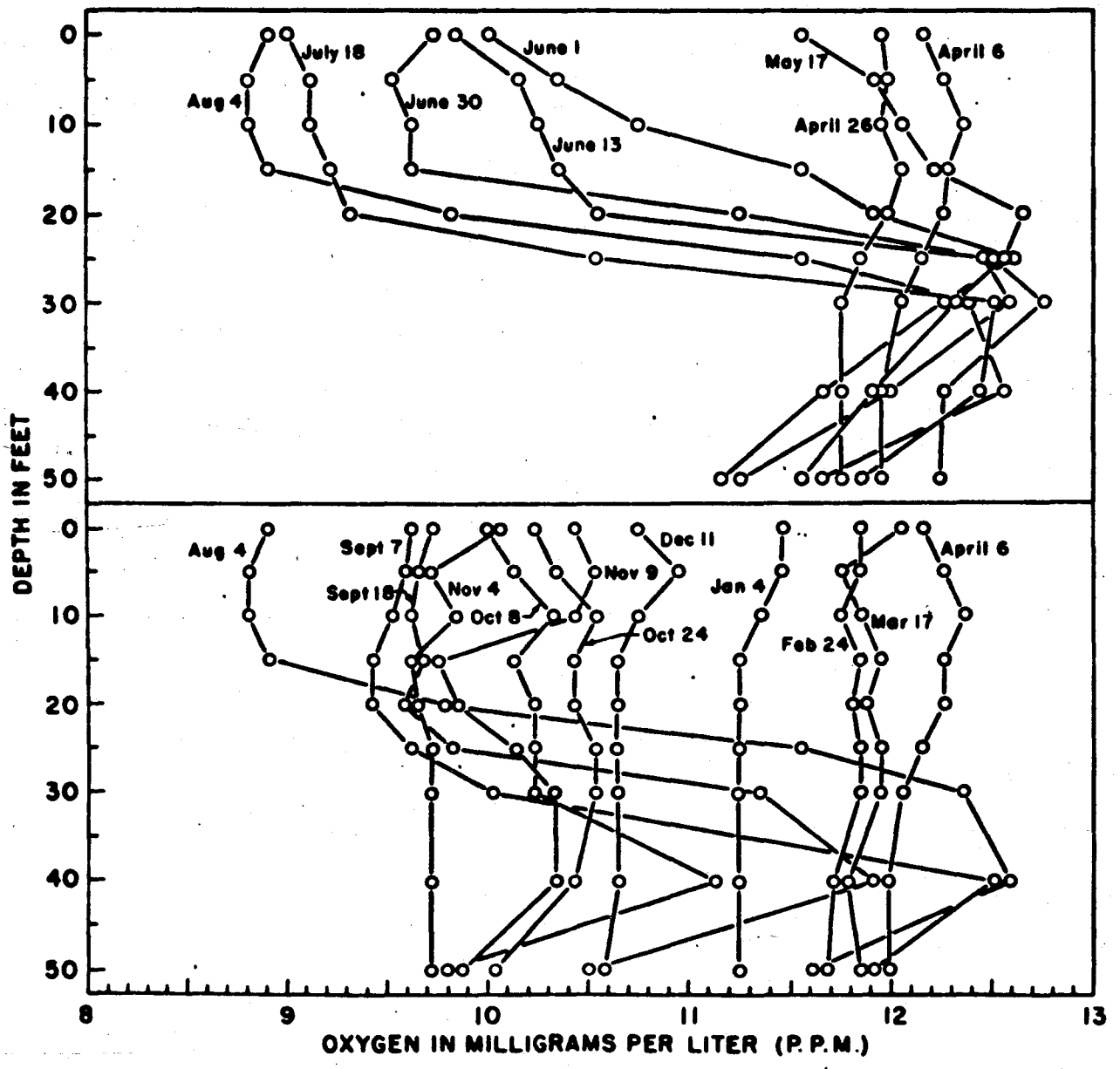


Figure 25. Dissolved oxygen content in milligrams per liter, Cultus Lake, 1961.

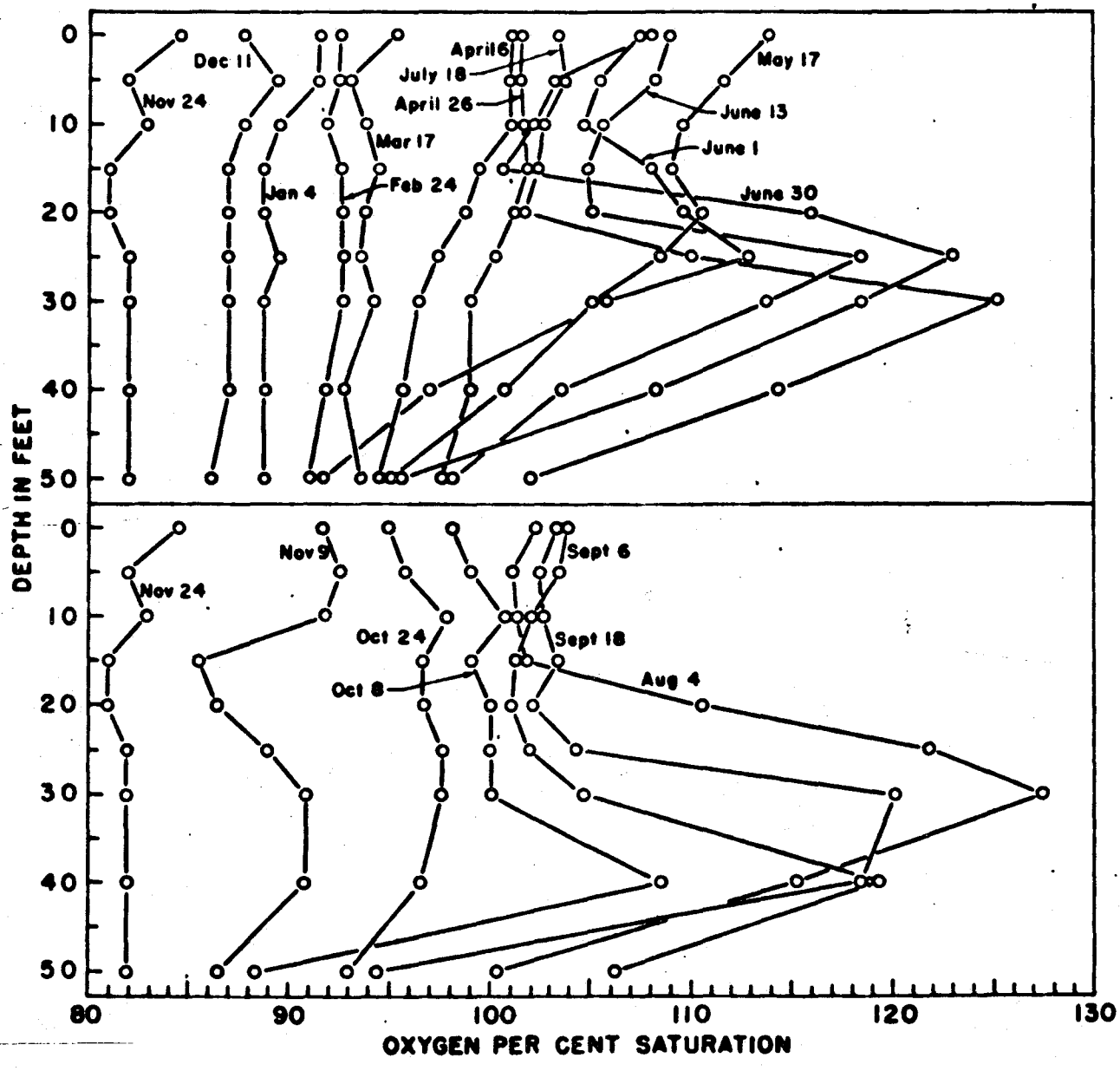


Figure 26. Oxygen content expressed as per cent of air saturation at surface pressure ard temperature in situ.

TABLE V

Oxygen expressed as per cent of air saturation at surface temperature

Depth in ft.	Jan. 27	Feb. 24	Mar. 17	Apr. 6	Apr. 26
0	93	93	95	100	101
5	93	93	93	101	101
10	92	92	94	102	101
15	92	93	95	101	102
20	91	93	94	101	101
25	92	93	95	100	101
30	92	93	95	99	100
40	93	92	93	99	100
50	92	91	94	99	100
130	95	91	93	75	82

Depth in ft.	May 17	June 1	June 13	June 30	July 18
0	110	106	109	107	103
5	114	110	112	105	105
10	115	114	113	106	105
15	117	121	115	106	106
20	121	127	117	124	107
25	120	133	138	139	121
30	118	130	139	141	144
40	114	123	132	135	143
50	110	118	125	124	136
130	88	69	76	75	74

TABLE V (continued)

Depth in ft.	Aug. 4	Sept. 7	Sept. 18	Oct. 8	Oct. 24
0	102	104	104	98	95
5	101	104	102	99	96
10	101	103	102	101	98
15	102	102	104	99	97
20	113	102	102	100	97
25	133	104	105	100	98
30	142	108	121	100	98
40	144	135	127	109	97
50	134	129	112	96	93
130	81	66	43	61	64

Depth in ft.	Nov. 9	Nov. 24	Dec. 11	Jan. 4/62	Jan. 25/62
0	92	85	88	92	94
5	93	82	89	92	95
10	92	83	88	90	93
15	85	81	87	89	93
20	86	81	87	89	93
25	89	82	87	90	93
30	91	82	87	89	94
40	91	82	87	89	94
50	86	82	86	89	94
130	65	55	87	89	92

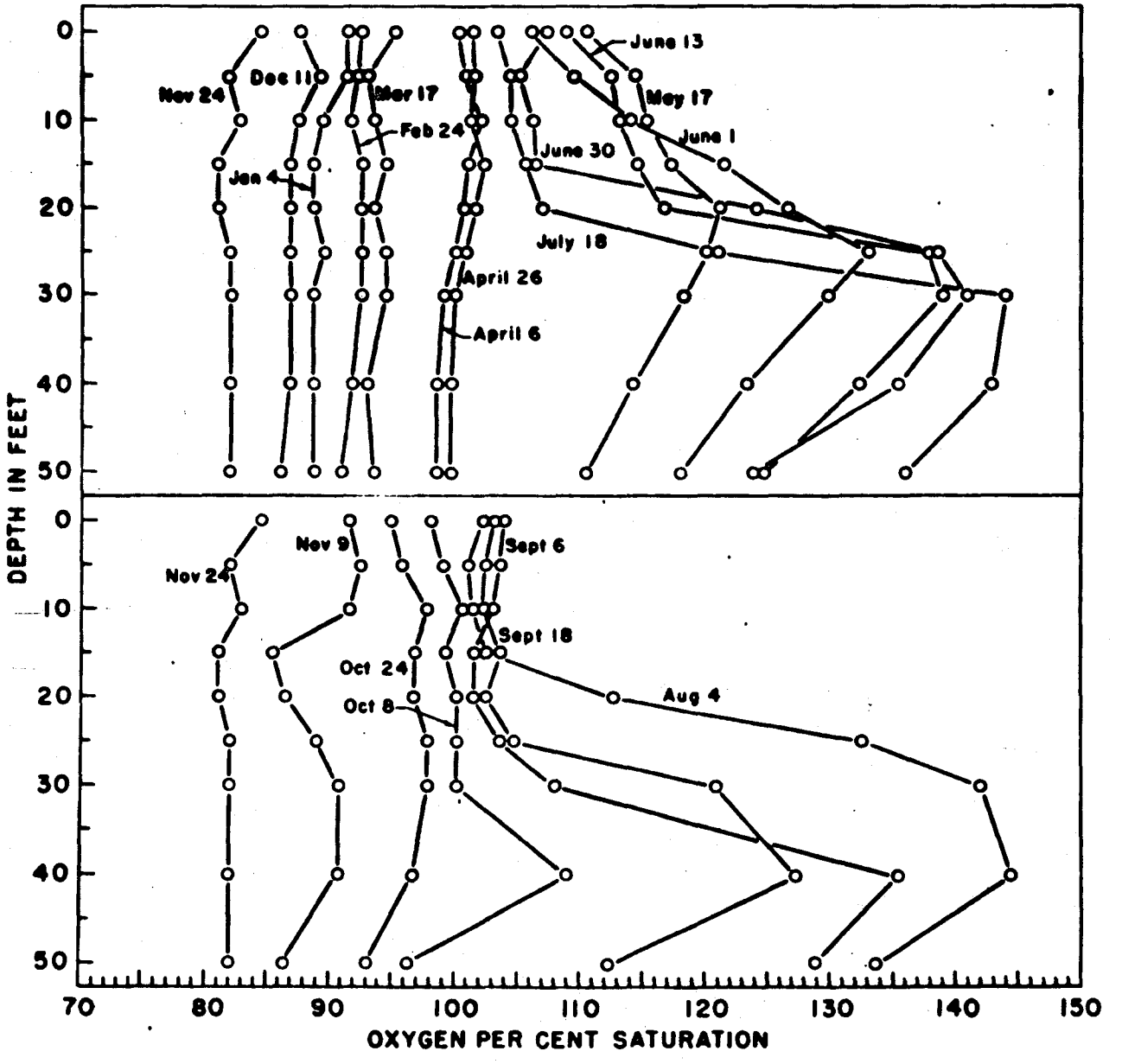


Figure 27. Oxygen expressed as per cent of air saturation at surface temperature and pressure.

TABLE VI

Dissolved nitrogen content, per cent of air saturation and per cent of air saturation at surface temperature

Depth in ft.	Apr. 6	May 17	June 13	June 30	July 18	Sept. 18	Nov. 9	Jan. 25/62
				Nitrogen in milligrams per liter				
0	18.9	17.7	15.8	14.9	14.6	15.3	18.4	20.3
5	20.0	18.7	15.9	15.0	14.5	15.2	18.4	19.8
10	19.6	18.7	16.2	15.0	14.5	15.5	-	19.8
15	20.0	18.8	16.2	15.6	14.8	15.5	17.9	20.0
20	19.9	19.4	17.9	15.6	15.0	15.2	18.2	20.2
25	-	19.2	18.9	17.9	16.3	15.9	18.2	20.1
30	20.0	20.0	19.4	19.1	18.5	17.8	18.4	20.3
40	20.0	19.4	19.7	19.3	19.0	19.3	18.8	20.1
50	20.0	20.1	19.7	19.1	19.4	19.6	18.3	20.5
130	19.4	20.4	19.8	20.1	19.7	20.1	19.5	19.5
				Nitrogen as per cent of saturation				
0	98	105	107	101	103	100	101	99
5	103	107	102	99	101	100	101	96
10	100	106	103	98	100	101	-	96
15	102	103	103	101	101	101	98	97
20	100	105	105	99	101	100	100	98
25	-	102	111	108	105	104	100	97
30	101	106	109	110	114	116	101	98
40	101	102	106	106	108	118	103	97
50	100	104	104	101	104	110	100	99
130	96	103	100	100	98	100	100	94

TABLE VI (continued)

Depth in ft.	Apr. 6	May 17	June 13	June 30	July 18	Sept. 18	Nov. 9	Jan. 25/62
Nitrogen as per cent of saturation at surface temperature								
0	98	105	107	101	103	100	101	99
5	104	111	108	102	102	100	101	96
10	102	111	110	102	102	102	-	96
15	104	112	110	105	104	102	98	97
20	104	115	115	106	105	100	100	98
25	-	114	129	121	114	104	100	97
30	104	119	132	129	130	117	101	98
40	104	116	134	131	134	126	103	98
50	104	119	134	130	136	129	100	100
130	101	121	134	136	138	132	107	95

Oxygen content and saturation. In early January of 1961, dissolved oxygen was 11.2 mg per liter or approximately 90 per cent of air saturation (Table III and IV, Fig. 25 and 26). Dissolved oxygen continued to increase until early April, beyond which surface oxygen declined and the absolute level of oxygen in the thermocline increased. Oxygen saturation in the region of the thermocline increased both through heating of water in situ and through oxygen produced. The excess gas was present below the zone of mixing and was retained in solution by the hydrostatic pressure. By September, oxygen tension at 50 ft was below the April maximum values and saturation below 100 per cent in spite of slight warming.

Autumn and winter cooling and mixing reduced oxygen saturation to a minimum of 82 per cent in late November. Thereafter oxygen uptake exceeded the rate of cooling and relative saturation increased.

In the section devoted to the vertical movement of fish, sockeye migration will be discussed in relation to relative temperature and saturation changes. For this reason oxygen saturation is expressed also as per cent of saturation at surface temperature and pressure (Table V, Fig. 27).

Nitrogen content and saturation. In late January, nitrogen content was approximately 20 mg per liter or 97 per cent of air saturation (Table VI, Fig. 28 and 29). By early April no net change had taken place in the nitrogen tension of the water but increasing temperature had raised the saturation to 100 per cent. Surface water saturation exceeded 100 per cent briefly April to June. At depths of 30 to 50 ft, temperature increased markedly May to September while nitrogen content declined little and hence the relative saturation was increased considerably above 100 per cent. Cooling and mixing of lake water did not lower nitrogen saturation as drastically as that of oxygen, with nitrogen saturation at approximately 100 per cent during incomplete mixing in November. As in the case of oxygen, nitrogen has been calculated also as per cent of saturation of surface temperature and pressure (Fig. 30).

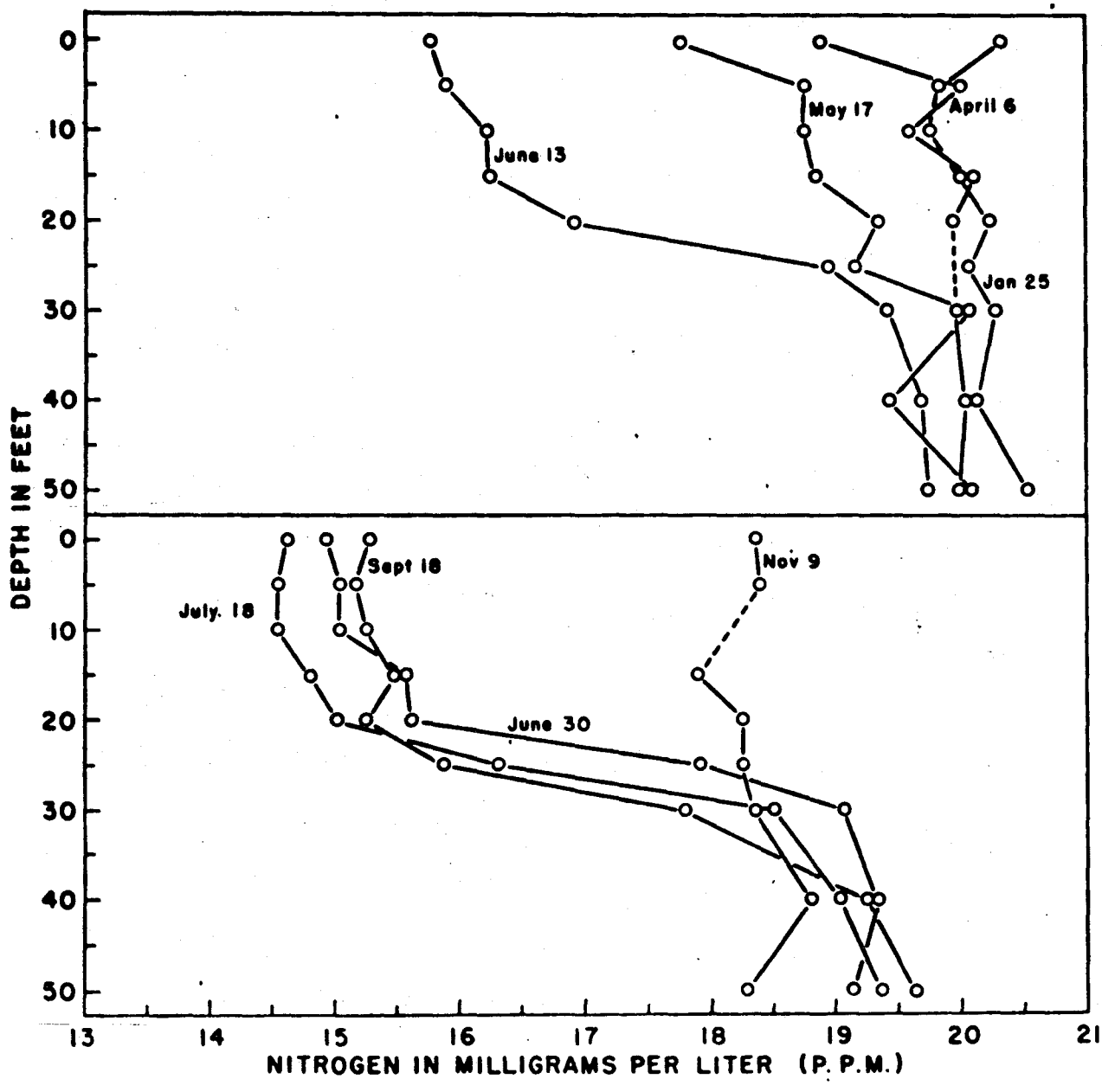


Figure 28. Dissolved nitrogen content in milligrams per liter Cultus Lake, 1961.

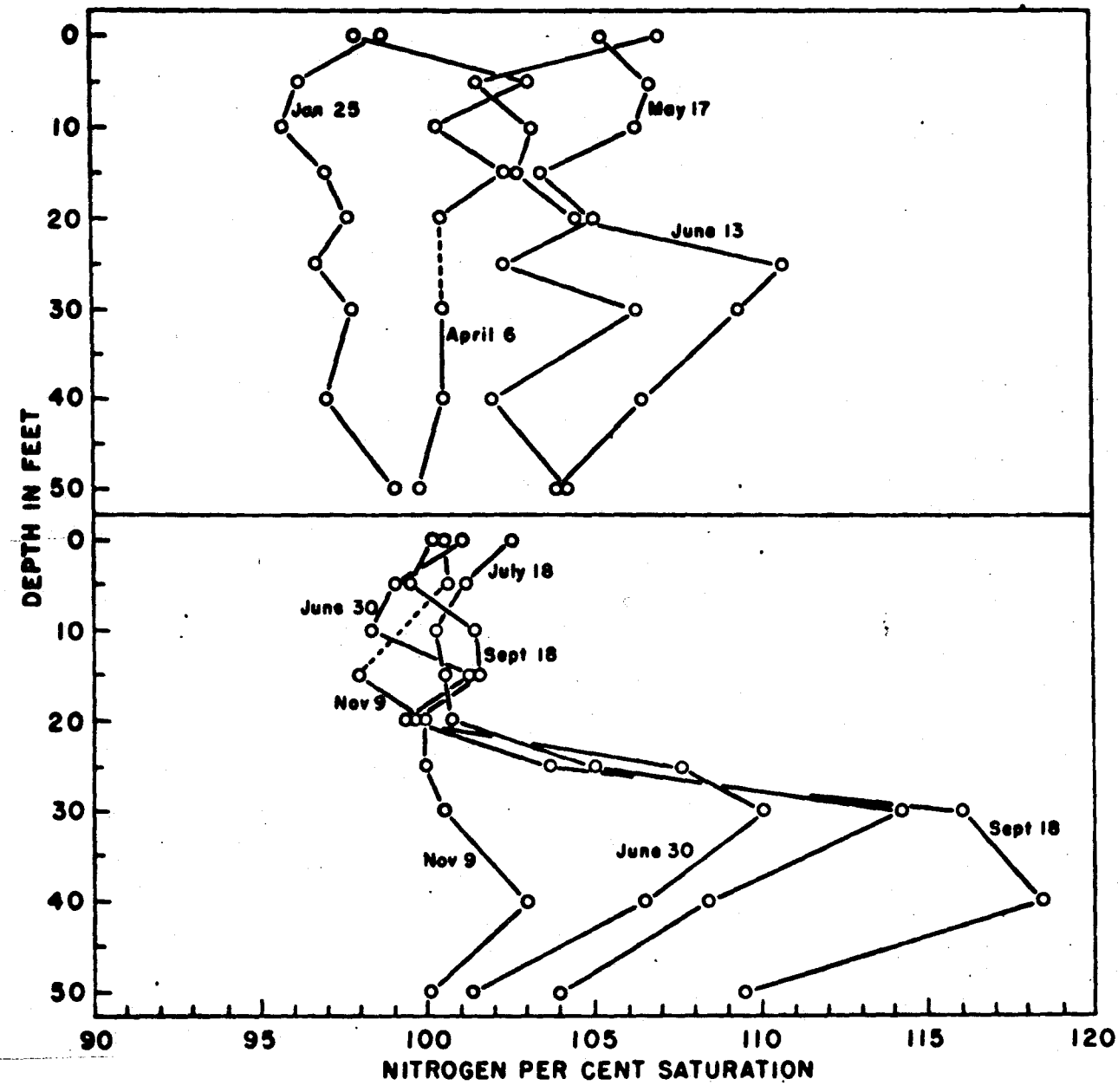


Figure 29. Nitrogen content expressed as per cent of air saturation at surface pressure and temperature in situ.

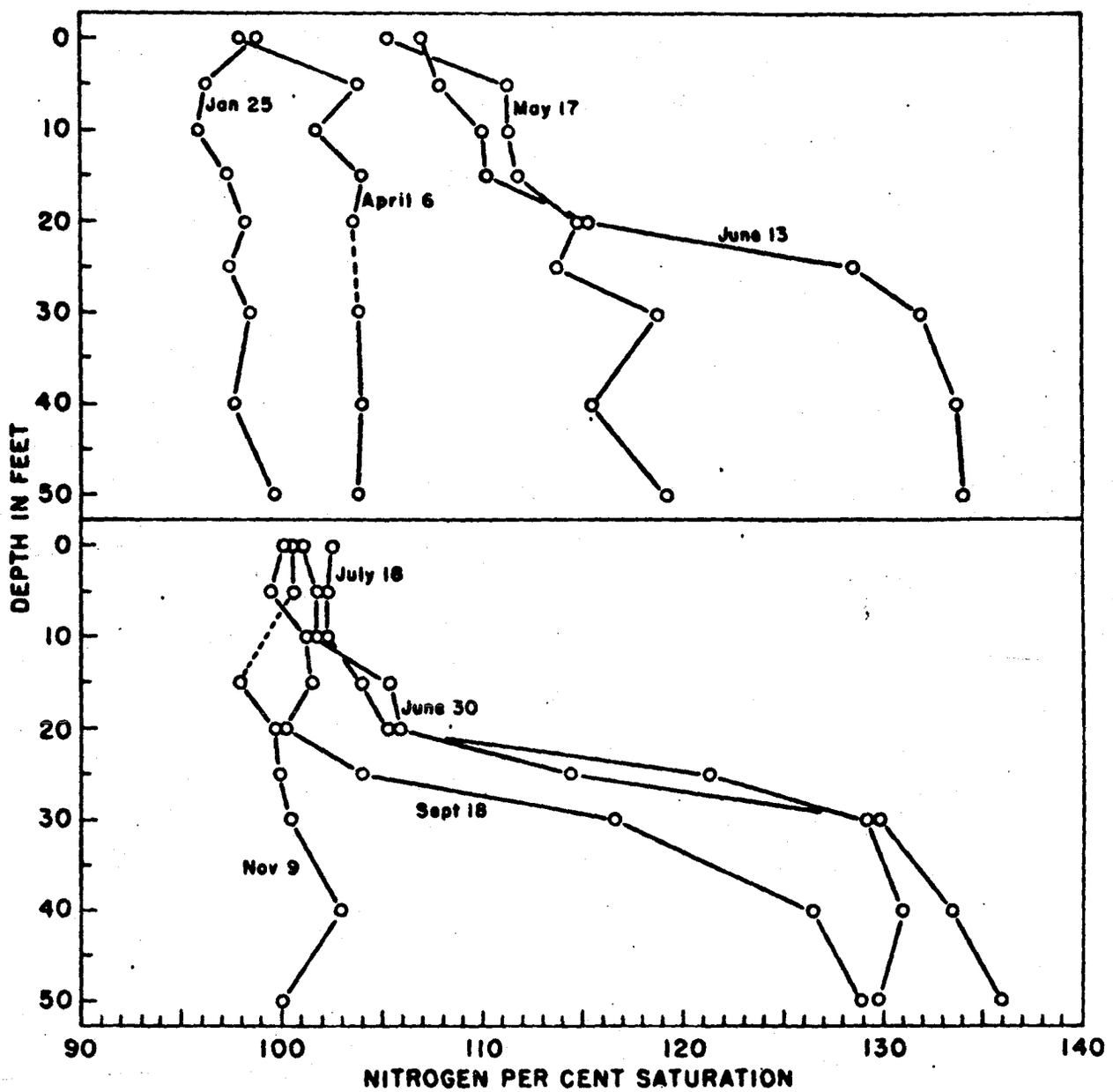


Figure 30. Nitrogen expressed as per cent of air saturation at surface temperature and pressure.

Tolerance to pressure and resistance to decompression

By no means all of the 143 tests of 1959 and 117 tests of 1960 were concluded successfully. Sudden, severe flooding of holding boxes in 1959 resulted in the loss of one series of experiments. A second complete series was lost in 1960 as a result of copper sulfate treatment of the near-by lake-shore. Finally, smolts held in Sweltzer Creek at high temperatures were prone to disease and in both 1959 and 1960 studies on tolerance to pressure were terminated prematurely for this reason.

During the course of the studies on resistance to decompression, a total of 2,800 control fish consisting of four groups of 300 each and eight groups of 200 fish were used. Mortality among these control fish ranged from 0 to 1 per cent per week and averaged 0.45 per cent per week during the 2 weeks of post-test observation.

Magnitude of positive and negative pressure. Sockeye smolts exposed to positive pressures as high as 300 psi evidenced little or no apparent ill effects. Samples of smolts raised to 300 psi and returned to atmospheric pressure evidenced mortalities of 0 to 1 per cent per week (Fig. 31 and 32), during post-test observation and hence were indistinguishable from the mortality rates of the control groups.

The effects of negative pressures on sockeye smolts

varied seasonally and are discussed further in a separate section. During the bulk of the studies, conducted from late March to early May, exposure to negative pressure resulted in a mortality which increased with increasing vacuum (Fig. 31 and 32). Groups of smolts were exposed suddenly to vacuum conditions from atmospheric, 50 psi and 300 psi above atmospheric pressure. The magnitude of the positive pressure had little or no effect on the resulting mortality. The two test series showed similar slopes (0.058 and 0.073) and intercepts (0.81 and 0.41). At pressures greater than 5 in Hg of vac.. vacuum (absolute pressure 633 mm Hg) mortalities were of the same order of magnitude as the control groups.

Duration of positive and negative pressure. As explained under methods, the apparatus would not permit the maintenance of high pressures for long periods of time. Smolts exposed to 50 psi for 24 hr then returned to atmospheric conditions evidenced 0.5 per cent per week mortality during post-test observation. Similarly goups of smolts held for 7 days at a depth of 40 ft in the lake (18 psi) showed no adverse effects when examined on return to atmospheric conditions.

Duration of exposure to negative pressure was investigated at a vacuum of 10 in Hg (506 mm Hg absolute pressure) and periods of 0.1 to 100 sec. This series was terminated on the appearance of disease-induced mortalities in some

of the groups. During the initial period of post-test observation, prior to the onset of the disease, there was no indication of any difference in the mortality rate among the test groups. Similarly, smolts gradually exposed to a reduction in pressure below atmospheric showed no ill effects immediately apparent at vacuum conditions of 20 to 25 in Hg (125 mm Hg absolute pressure). This was true only if the rate of reduction of pressure and hence expansion of gas did not exceed the rate at which the fish expelled gas from the swimbladder. Vacuum conditions were not maintained sufficiently long to reduce oxygen tension to the lethal level.

Rate of increase and decrease in pressure. Pressure was routinely increased at the rate of 1 psi per sec apparently without ill effect on the fish. The most rapid rate of pressure increase tested was 10 psi per sec which is the equivalent to a descent through water at the rate of 20 ft per sec and this also was without adverse effect.

The effect of the rate of reduction of pressure was tested over the range of 1.5 to 7,500 psi per sec. Two series were conducted in 1959 and 1960 in which groups of 200 smolts were lowered from 300 psi to atmospheric conditions. The results are shown in Fig. 33. There is some suggestion of slightly higher mortalities at the most rapid rate of

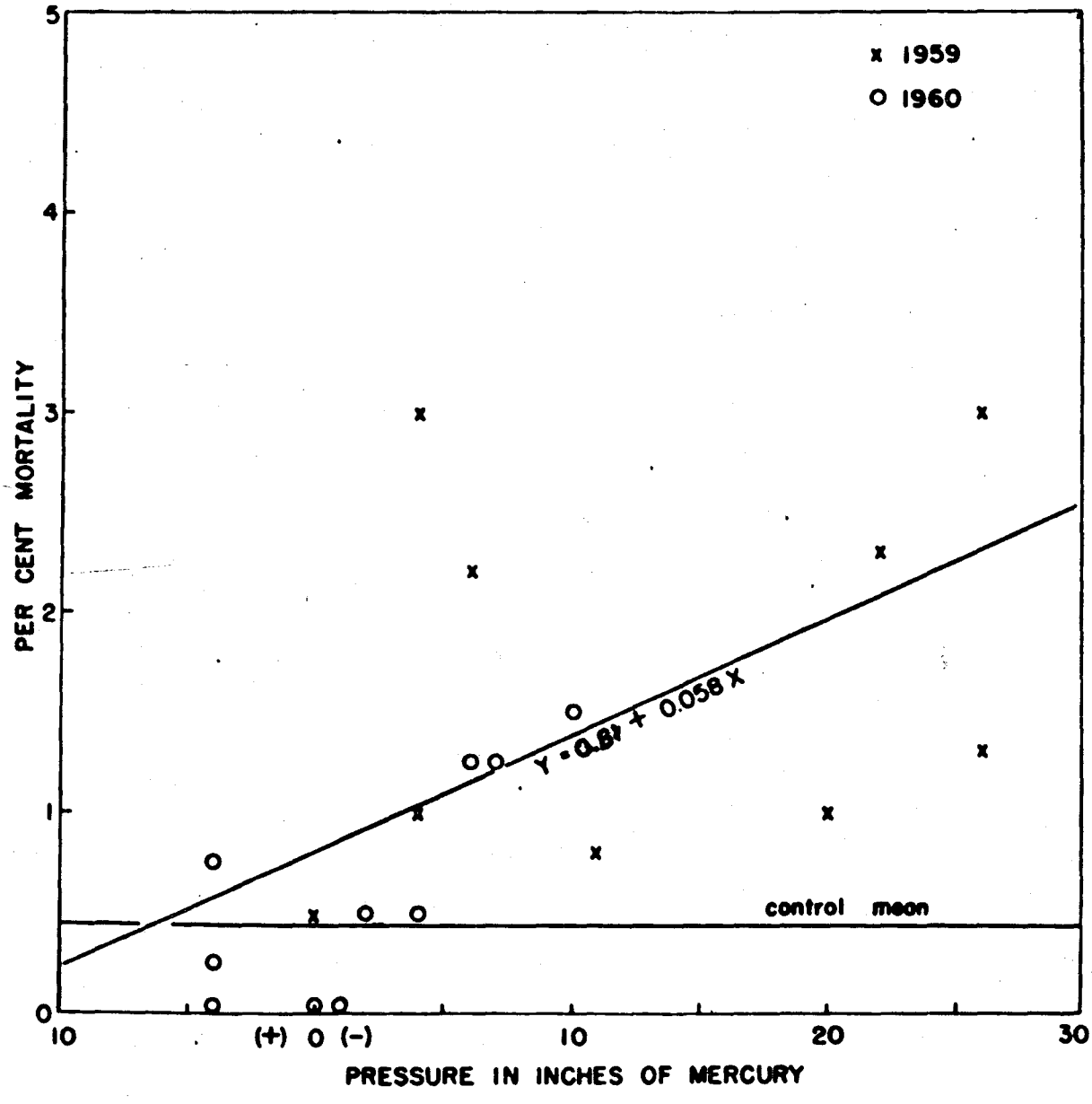


Figure 31. Mortality per week following decompression from 50 psi to positive and negative pressure.

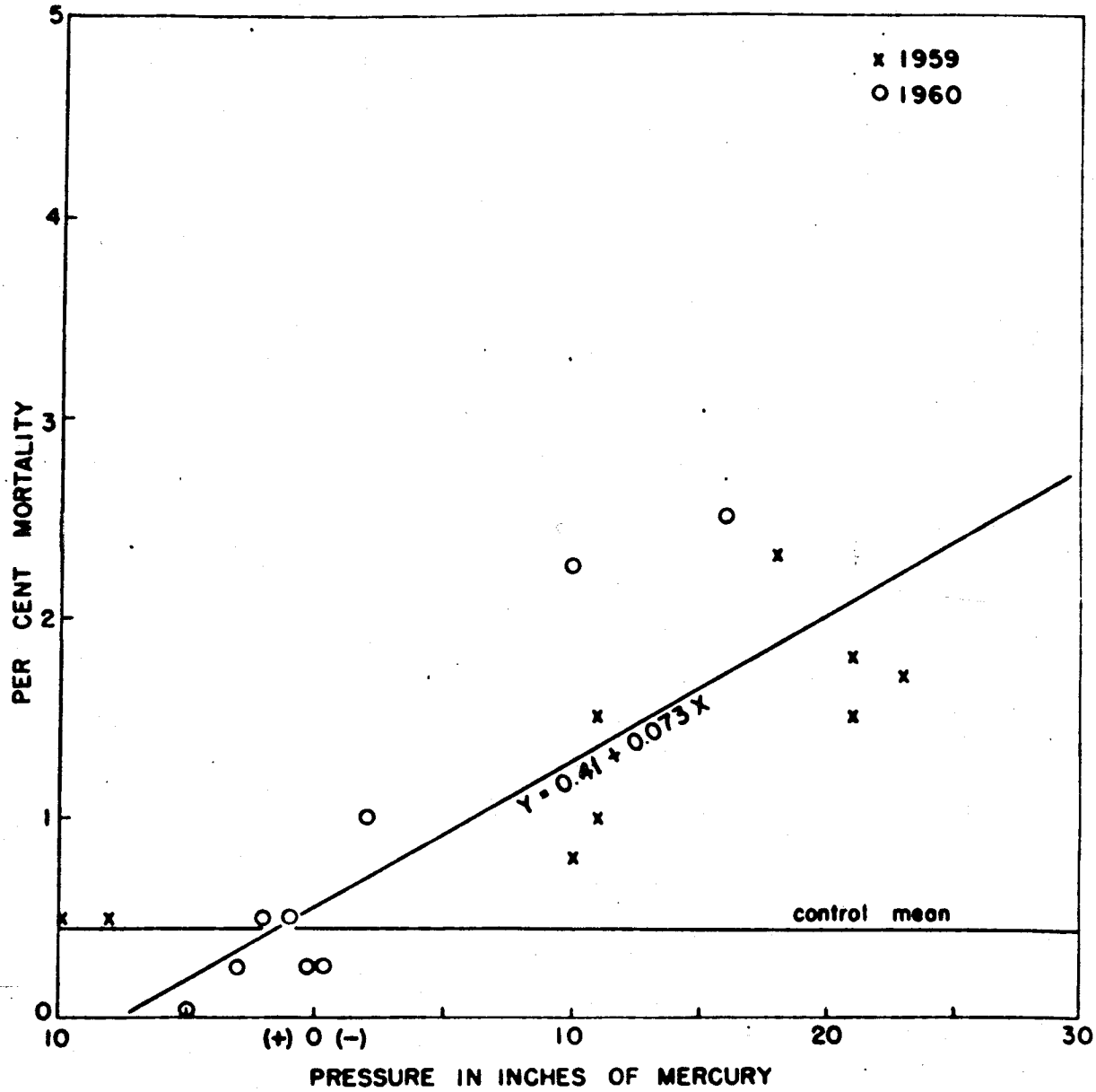


Figure 32. Mortality per week following decompression from 300 psi to positive and negative pressure.

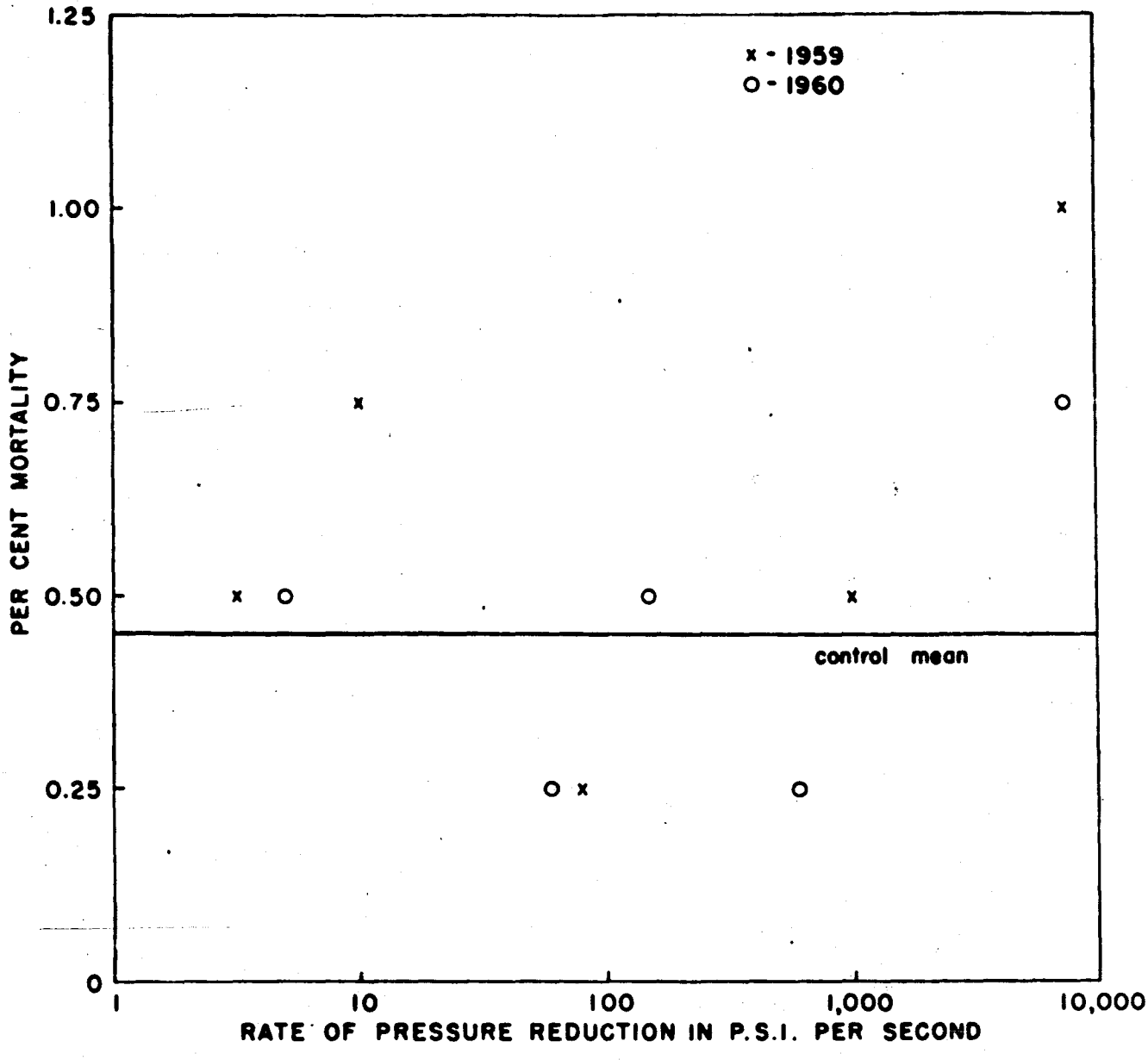


Figure 33. Mortality per week accompanying various rates of reduction of pressure.

decrease in pressure, but the maximum mortality of 1 per cent per week is within the extreme range of the control mortalities.

It should be noted the above series of tests were conducted on smolts migrating from Cultus Lake early in the season (late March to early May). Decompression tests yielded consistently low mortalities during this period, in contrast to the increasing mortalities late in the season (late May to late June), as described below.

Conditions altering resistance to decompression

Seasonal effect. The relatively small proportion of mortalities and injuries resulting from decompression changed suddenly during the course of the season. This change coincided with a temperature of 51°F in Sweltzer Creek, occurring about mid-May and coincident with the termal stratification of Cultus Lake. Among smolts tested migrating from the lake, the mortality increased rapidly with increasing temperature as shown in Fig. 34. The smolts died inside the pressure chamber within a few minutes of being exposed to a sudden reduction in pressure below atmospheric conditions. In the fish examined, death was due to minute gas emboli most commonly lodged in the heart or ventral aorta. This phenomenon was first observed during the studies of the spring of 1959.

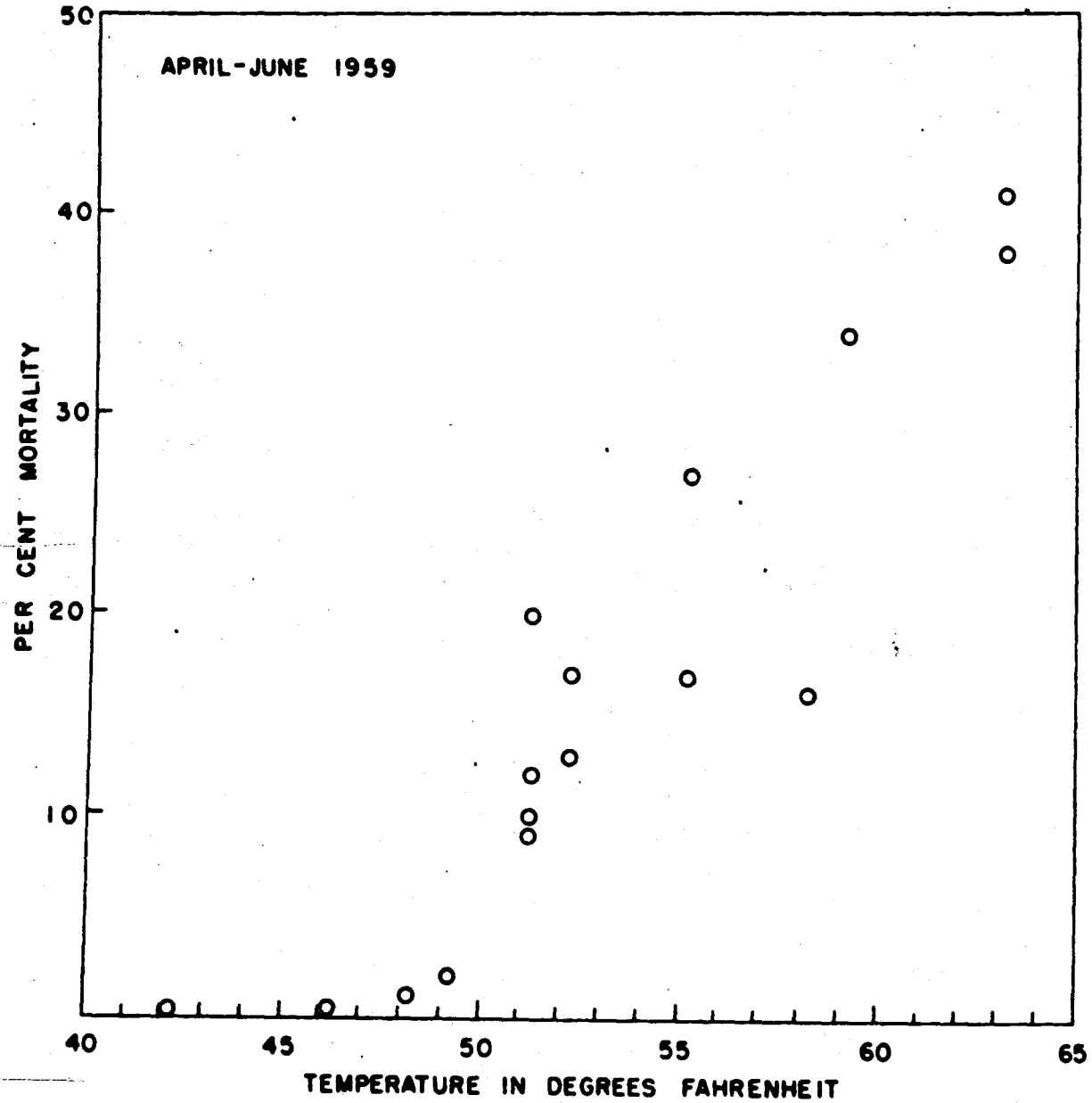


Figure 34. Mortality of sockeye smolts tested at various temperatures during the course of the migration 1959.

Similar series of tests were conducted in 1960 and 1961 yielding comparable results.

The highest mortalities recorded in this way were 35 per cent. Surviving fish were in an abnormal condition for some time following testing with numerous fish lying on their sides for several hours. When disturbed and induced to swim about, such fish occasionally died also.

Two possibilities exist for the sudden appearance of embolism mortalities. The excess gas may have originated through internal supersaturation; this supersaturation resulting from fish rising into the warm waters of the lake surface from depth immediately prior to downstream migration, thereafter being trapped and tested before achieving gaseous equilibration with their new environment. The second possibility was that of gas embolism due to the entry of swimbladder gas into the blood stream on decompression.

Smolts held at lake surface and depth. The relative permanence of the lethal factor was examined by holding samples of smolts varying periods of time between trapping and testing. Smolts trapped and tested with a few minutes evidenced a mortality of 19 per cent. The mortality declined rapidly when smolts were held in warm lake-outlet water prior to testing (Fig. 35). Mortality declined to 2 per cent within 3 days of pre-test holding.

The gradual loss of the lethal factor described above raised the possibility that it was due to an environmental pressure-conditioning that could be replicated experimentally. In order to test this, 400 smolts were trapped migrating from Cultus Lake in mid-June. One hundred tested immediately showed a mortality of 19 per cent. A second sample of 100 held in warm lake-surface water (Fig. 36) for 3 days evidenced a mortality of 2 per cent. Following 3 days of residence in warm surface water the remaining 200 fish were held at a depth of 40 ft in the lake for 7 days. A sample of 100 of these fish was recovered from depth and tested immediately, resulting in a mortality of 21 per cent. The fourth sample of 100 fish was held an additional 2 days in warm lake-surface water and showed an 11 per cent mortality on testing. The lethal factor thus was lost under conditions of lake-surface residence and recovered with residence at depth.

Increased content of dissolved gases. The gas content and hence saturation change, corresponding to that which smolts would experience migrating from thermocline to surface, was duplicated in the pressure chamber. Groups of smolts exposed to air saturation of 100 to 110 per cent at a pressure of 2 atmos (absolute saturation 50 to 55 per cent) showed a slightly increased mortality on testing following a 24 hr exposure to such water. Similarly, sample of smolts exposed

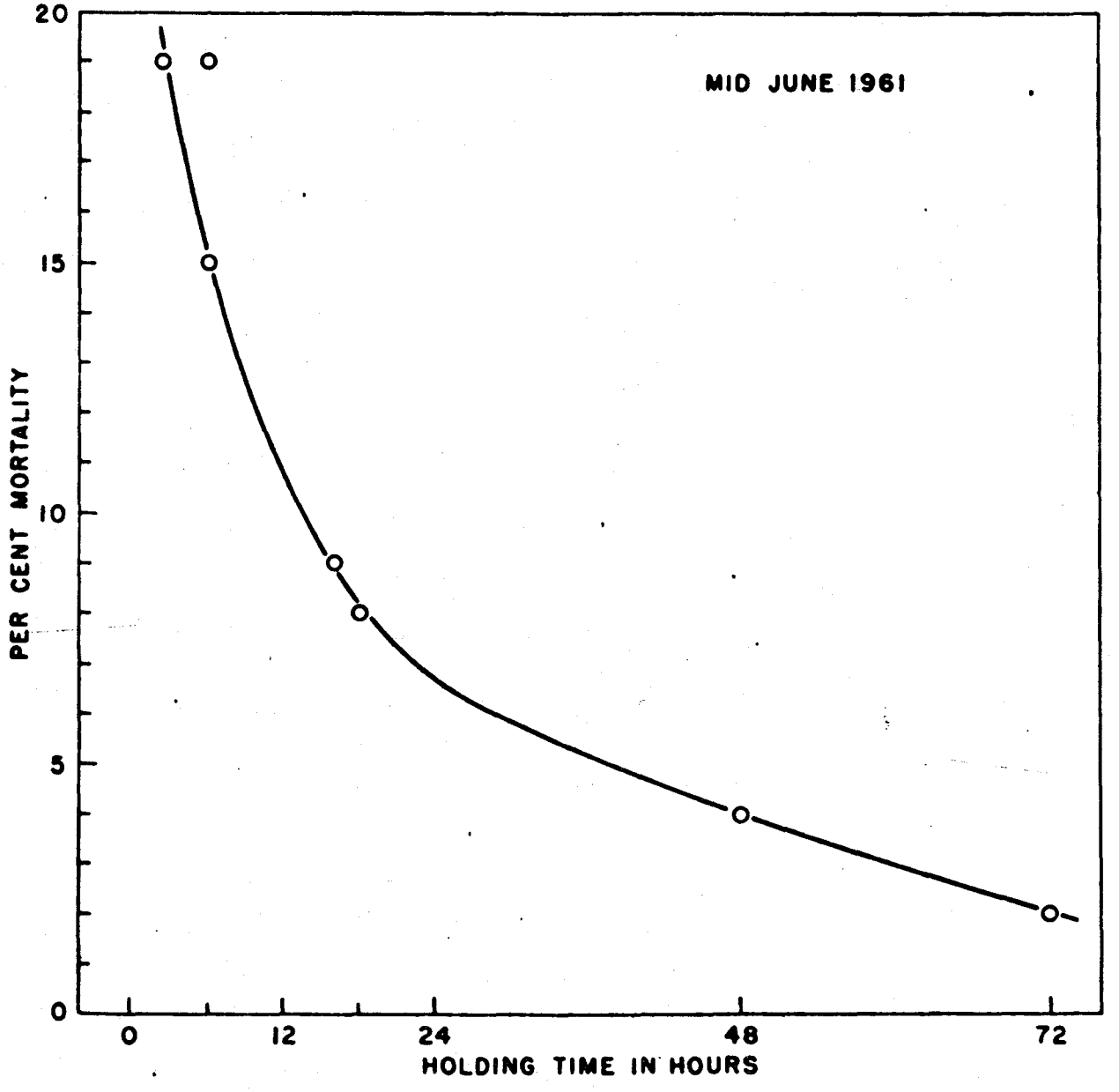


Figure 35. Decline in mortality among groups of smolts held up to 72 hours in Sweltzer Creek before testing,

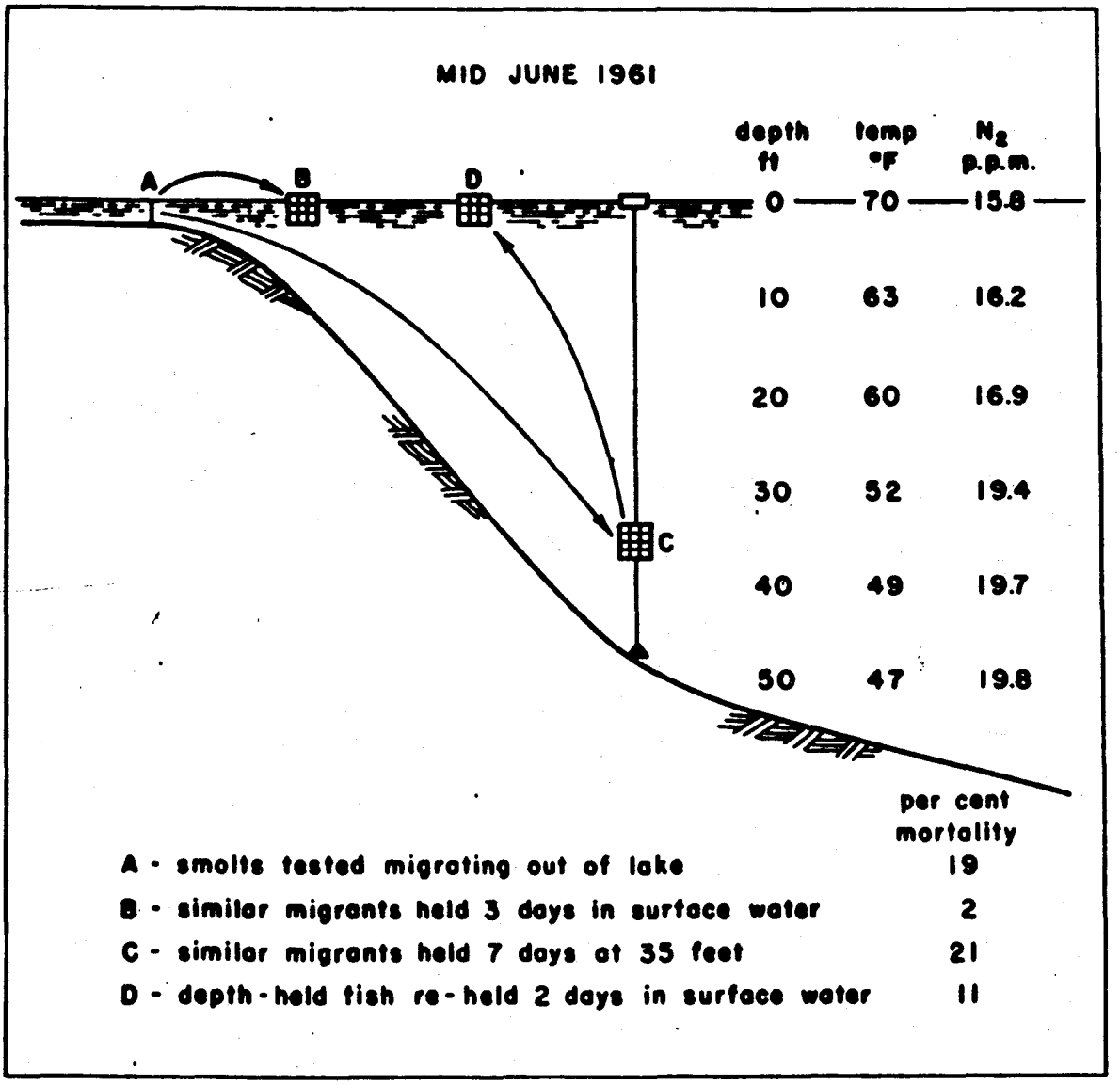


Figure 36. Mortality among smolts accompanying residence in surface and thermocline waters.

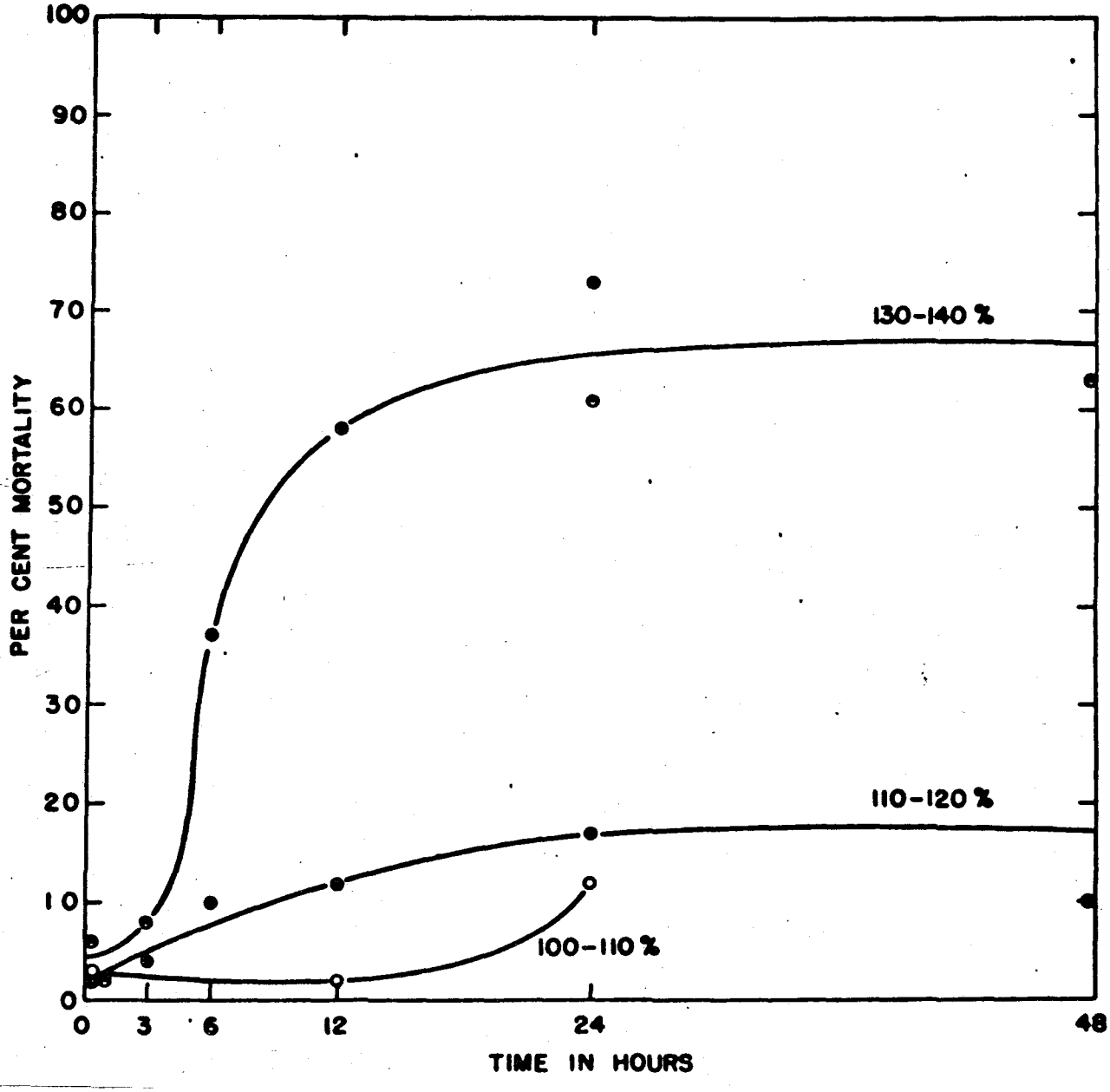


Figure 37. Mortality among smolts following exposure to water of increased air content. Gas tensions expressed as per cent of saturation at atmospheric pressure.

to air saturation of 110 to 120 per cent evidenced a gradually increasing mortality on testing with increased duration of exposure. Finally, markedly increased gas tensions in which the air saturation was 130 to 140 per cent resulted in sharply increased mortality on testing following 3 hr of exposure (Fig. 37). Maximum mortality appeared after 12 to 24 hr of holding in such water of increased gas content.

Changes in temperature. The saturation change due to increased temperature accompanying movement upward from the thermocline to the surface also was duplicated in the pressure chamber. Groups of 100 smolts were acclimated for 2 days to a constant temperature then subjected to a sudden increase 5 min before conducting the standardized decompression test. The mortality remained below 6 per cent for increments of increase of less than 13°F. For increases of 14 to 19°F mortalities of 10 to 20 per cent were recorded (Fig. 38).

The acclimation waters were not always at air saturation and hence the increments of increase in temperature were corrected to per cent saturation at the increased temperature. No clear trend is apparent (Fig. 39) between mortality on testing and temperature-induced increase in saturation.

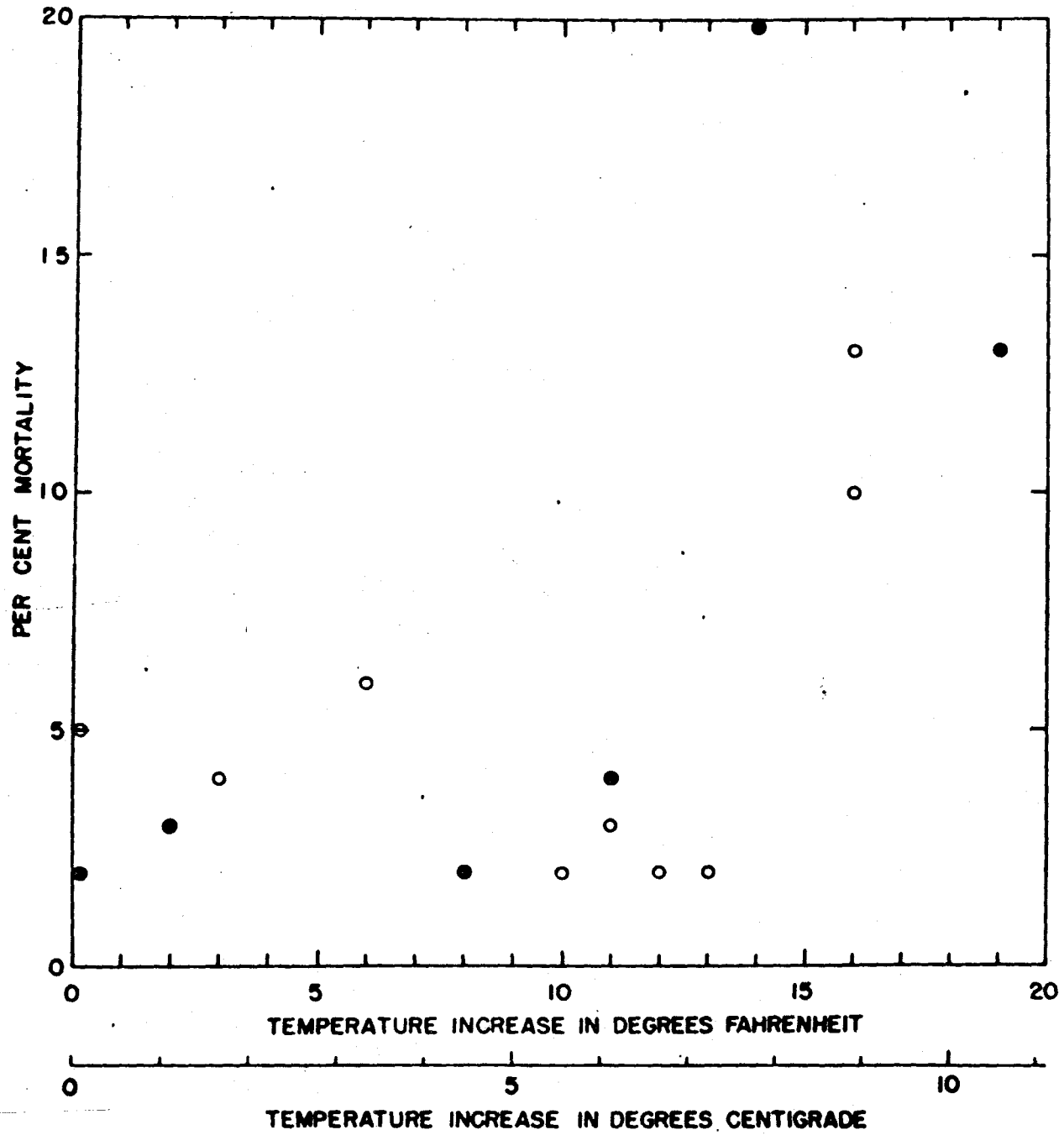


Figure 38. Mortality on decompression following sudden increase in temperature. Open circles increments above 45°F, solid circles above 55°F.

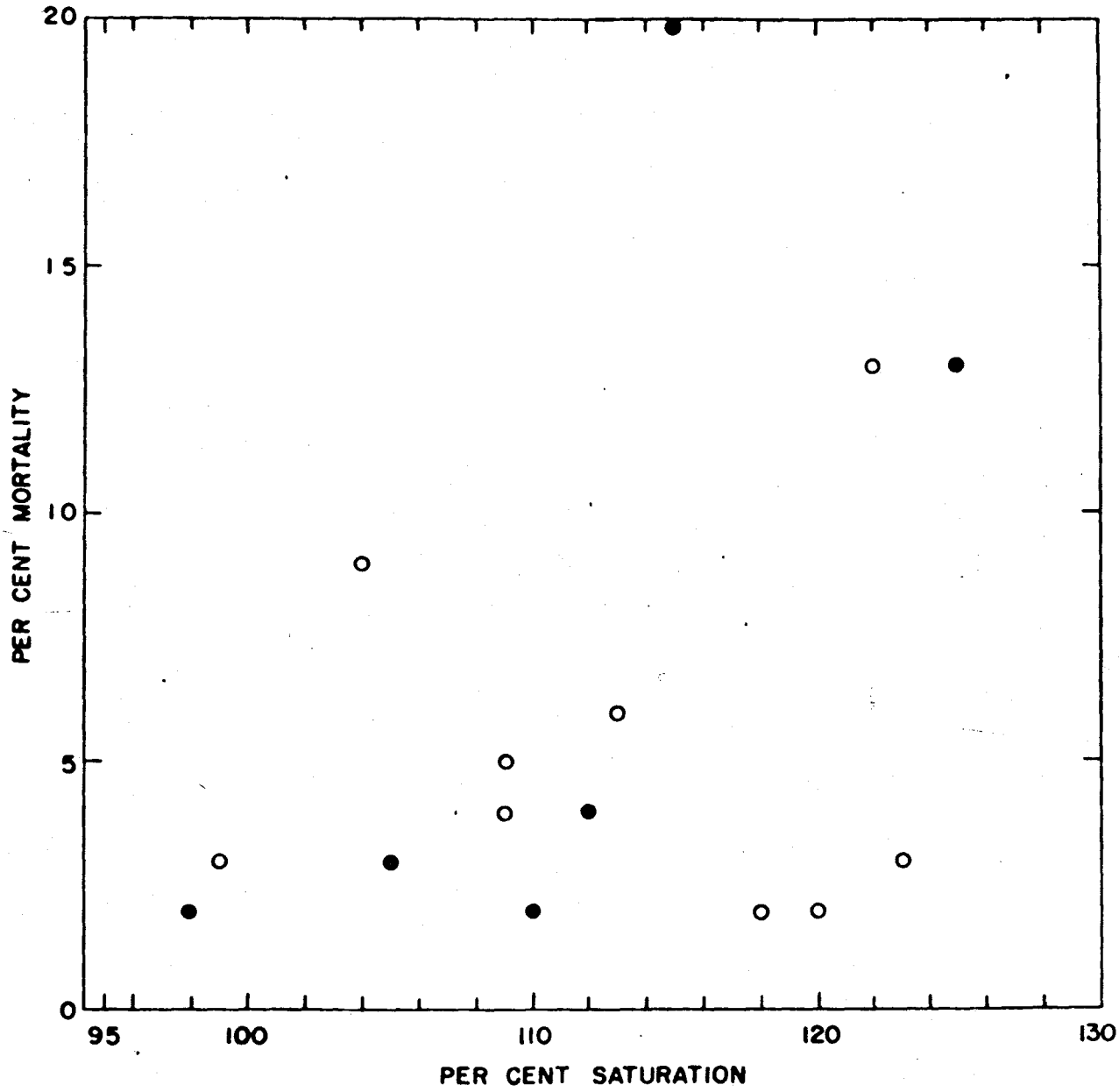


Figure 39. Temperature increases expressed as increases in per cent saturation.

Swimbladder gas and resistance to decompression

Increased and decreased gas content of the bladder. The smolts held 48 hr at 30 psi above atmospheric pressure with an air lock above the water surface quickly inflated their swimbladders to neutral buoyancy. On testing with the standardized decompression test, these fish showed a mortality of 25 per cent, compared with zero mortality among negative-buoyancy fish (below). Unfortunately the air lock supersaturated the water flowing through the chamber up to a maximum of 118 per cent, thus complicating the experiment. The experiment was repeated at a lower pressure (15 psi above atmospheric) and a shorter period of exposure of 90 min as before, the smolts came to neutral buoyancy utilizing atmospheric air. The decompression test resulted in a mortality of 12 per cent of the fish. Two groups of 50 smolts were tested under conditions of swimbladders inflated to atmospheric pressure and these samples had an average mortality of 2 per cent.

The group of smolts exposed to gradually decreasing pressure below atmospheric conditions lost gas from the swimbladder and attained neutral buoyancy at a vacuum of 25 in Hg (an absolute pressure 125 mm Hg). On being subjected to the standardized decompression test no mortality was observed among the 50 smolts in the sample.

Bladderless and catheterized-bladder fish. A sample of 20 prickly sculpins subjected to the standardized decompression test evidenced little discomfort on sudden exposure to vacuum conditions and no subsequent mortality. Groups of sculpins exposed to water of 130 per cent of air saturation at 2 atmos (absolute saturation 65 per cent) for 24 hr and 96 hr also showed no mortality when exposed suddenly to vacuum conditions.

Ten control sockeye and 10 smolts with catheterized swimbladders were subjected to the standardized decompression test following 24 hr of holding at 2 atmos pressure in water at 130 per cent of air saturation. These test conditions were identical to those described under the section on changes in water saturation. Control sockeye showed a mortality of 5 fish on exposure to vacuum conditions. The catheterized sockeye lost gas from the swimbladder through the catheter on decompression and evidenced no mortality.

Duct-release pressure and sockeye held at depth. Yearling sockeye held for 7 days at a depth of 40 ft in the lake showed an average duct-release pressure of 19.6 mm Hg with ±13.2 mm Hg for one standard deviation. Control fish held at the same temperature and surface pressure had an average pressure at release of 21.3 mm Hg with ±17.5 mm Hg for one standard deviation and were not different from the

fish held at depth. Thus the appearance of swimbladder gas as emboli appears not to be due to increased constriction of the pneumatic duct following residence at depth.

Equilibration of fish with dissolved nitrogen of the environment

Rate of change of nitrogen in venous blood. The reduction in nitrogen in venous blood of fish following transfer from water of nitrogen content of 17.5 to 13.0 ml per liter is shown in Fig. 40. It is clear that dissolved nitrogen in venous blood very quickly declines to the level of the control fish, with slightly less than half of the excess nitrogen being lost during the first 5 min after transfer. Complete equilibration was attained between 30 and 60 min after transfer from water of high nitrogen tension.

The attempt to measure nitrogen in arterial blood under the same experimental conditions was unsuccessful as the arterial sample could not be drawn without interfering with respiration. Thus it was not possible to calculate the rate of clearance of dissolved nitrogen from blood by measurement of nitrogen in arterial and venous blood and cardiac output.

Rate of equilibration of total dissolved nitrogen. The dissolved nitrogen given up by an individual fish was determined at 0, 5, 10, 20 and 30 min (Fig. 41). Approximately 30 per cent of the excess dissolved nitrogen was lost

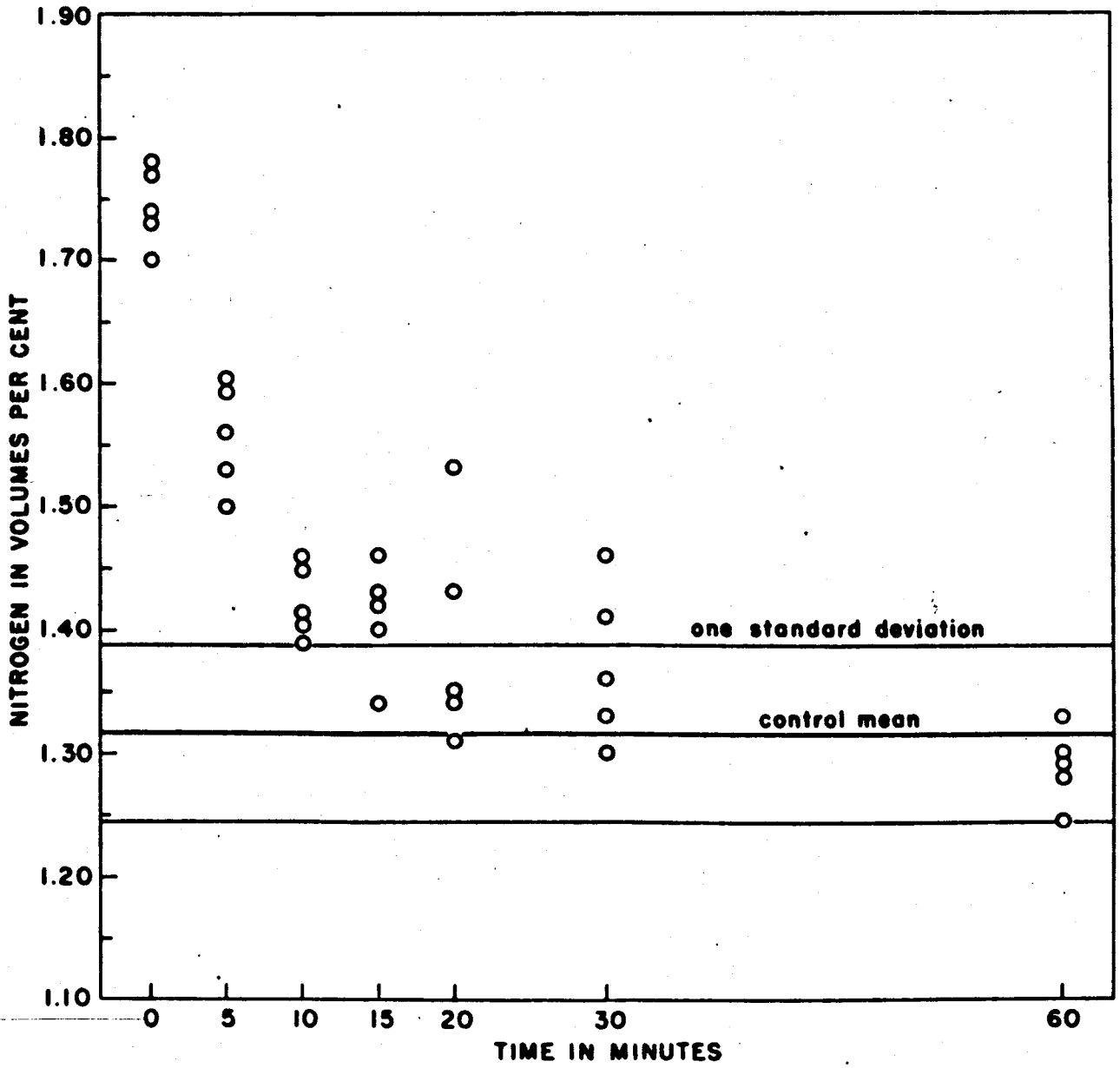


Figure 40. Decrease in nitrogen in venous blood following transfer of fish from water of 17.5 ml per liter nitrogen to 13.0 ml per liter.

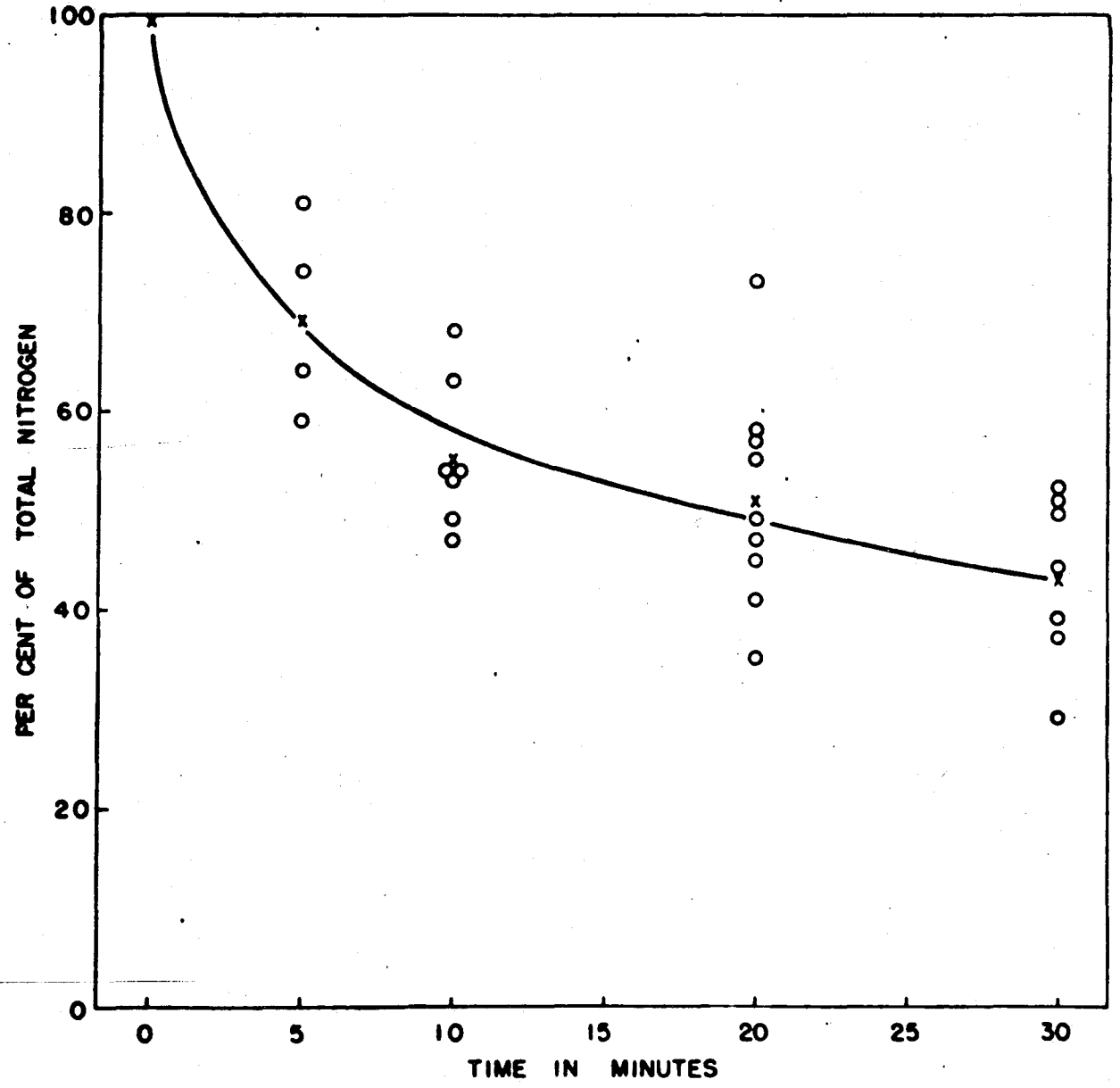


Figure 41. Per cent of excess nitrogen retained by fish following transfer from water of 14.4 to 1.6 ml per liter nitrogen.

in the first 5 min after transfer to water low in nitrogen and 60 per cent by the end of 30 min. These values may be slightly low as the solubility of nitrogen in the intact fish was arbitrarily placed at that of water. Thus the test fish may have given up a slightly larger percentage of their dissolved nitrogen than the calculations indicate. The date suggest that one-half of excess dissolved nitrogen is lost in approximately 20 min and the remaining one-half takes considerably longer, possibly 1 to 2 hr.

Discussion

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alevins before the swimbladder has been inflated. Alevins and fry, lacking a gas phase, do not respond to increased density as is the case with fingerlings. Nor is there evidence either in the literature or in this study that sockeye have a true sense of pressure. Thus alevins and fry may be able to reside in the spawning redd at some depth in the lake, without undue stimulation due to hydrostatic pressure.

Young Salmonidae initially fill their swimbladders by gulping atmospheric air at the water surface (Vogt, 1842). This is true also of certain physoclists (Ledebur, 1928). The air-gulping instinct in response to negative buoyancy is lost subsequently in physoclists but retained throughout life in many physostomes (Frisch and Stetter, 1932). Swimbladder filling takes place approximately at the time Atlantic salmon fry emerge from the gravel (Hoar, 1937). Chum salmon alevins reared in hatcheries may gulp air prematurely (Disler, 1953). Disler's observation was confirmed by the author (unpublished) on sockeye alevins which had access to atmospheric air during hatchery incubation. Alevins inflated their swimbladders midway in development between hatching and emergence.

Fry emerging from the gravel swim upward to the water surface and swallow air into the swimbladder. The question arises whether fry develop a sensitivity to pressure and hence swim upward, or whether the compensatory-

swimming response to pressure follows swimbladder inflation, the latter being a response to increased density. For fry emerging in shallow water, pressure stimulation would be very slight. The evidence for premature filling of the bladder also tends to argue against pressure being involved. If a developing pressure sensitivity induced fry to swim upward, then swimbladder filling should have appeared closer to time of emergence. For these reasons, the upward swimming of emergent fry is regarded as compensatory swimming in response to negative buoyancy. This activity tends to carry fry to the water surface where instinctive gulping of air and bladder inflation follows.

The problem of maintaining preferred depth. In contrast to fry, sockeye fingerlings and smolts showed a characteristic compensatory swimming in response to pressure (Fig. 16 and 17). This compensatory swimming provides some indication as to the pressure preferred by young sockeye and how the corresponding depth is maintained. It should be noted that this compensatory swimming is in response to increased density and not pressure per se. This was demonstrated by reducing the gas content of the swimbladder and inducing compensatory swimming at atmospheric pressure. Also by making air available to sockeye under pressure, the fish soon reached neutral buoyancy and compensatory swimming

ceased in spite of the increased pressure.

As compensatory swimming is a response to negative buoyancy, the significance of this response is more apparent if it is expressed in relation to the density of the fish. In water, the greatest percentage change in the volume of a gas occurs close to the surface (Fig. 42). Correcting this change in gas volume to change in density of a smolt (Fig. 43) it is obvious that the most rapid change in density takes place as the fish descends from or rises to atmospheric conditions. Expressing the rate of beating of the pectoral fins in relation to smolt density (Fig. 44), there was no apparent response for almost one-third of the potential increase in density. Thereafter movement of the pectoral fins increased rapidly and reached maximum frequency coincident with approximately one-half of maximum density.

This density increase with depth is altered profoundly by the sounding behavior of the fish (Fig. 45). For this reason it was essential that sockeye fingerlings and smolts to be tested be permitted to inflate their swimbladders to neutral buoyancy at atmospheric pressure.

The compensatory swimming of sockeye smolts in response to pressure would suggest these fish prefer the range from lake surface to a depth of perhaps 40 ft. A number of observations have been made on the vertical distribution of

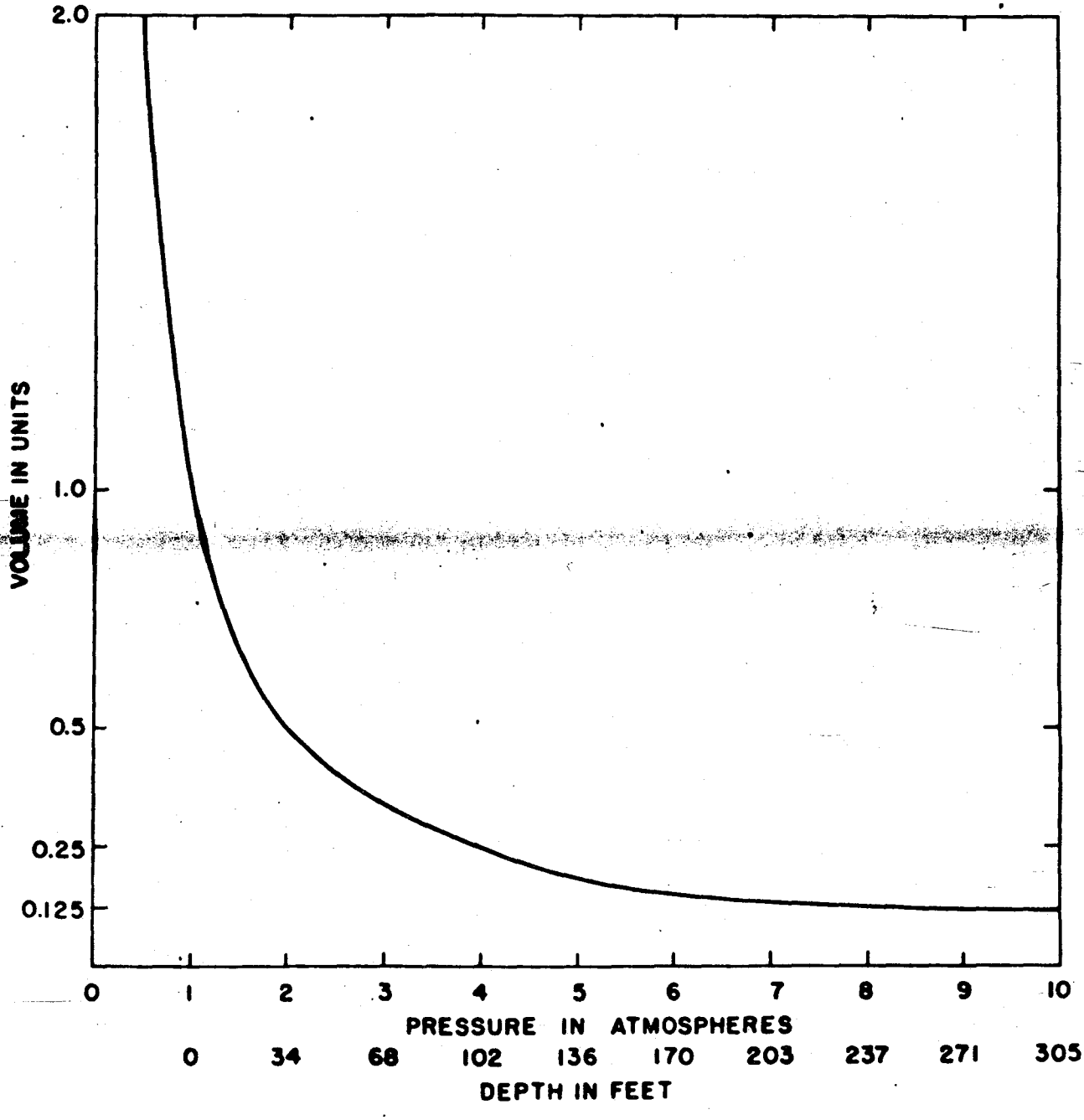


Figure 42. Change in unit volume of gas in relation to depth and pressure.

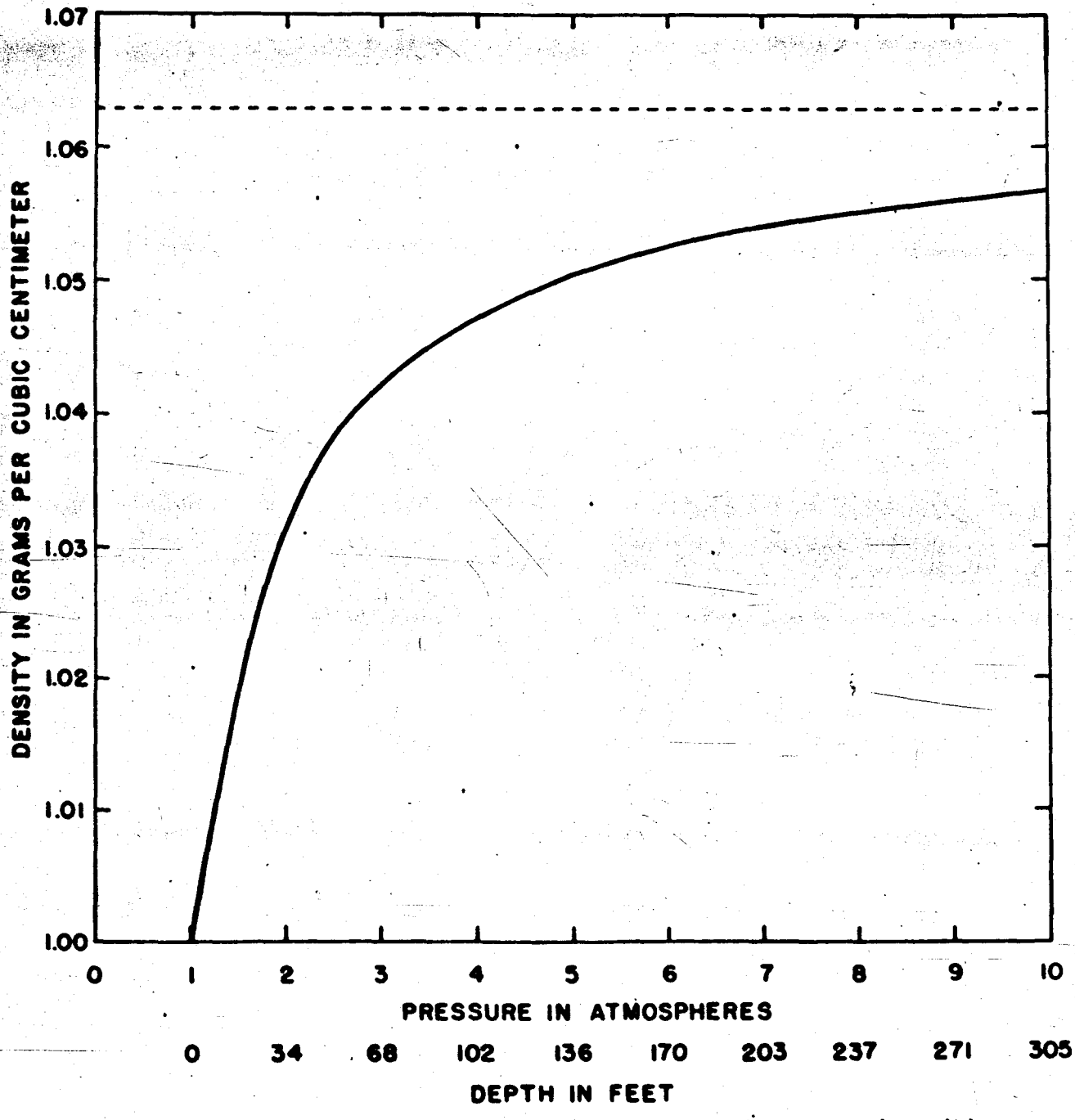


Figure 43. Change in the density of a sockeye smolt with increasing depth and pressure.

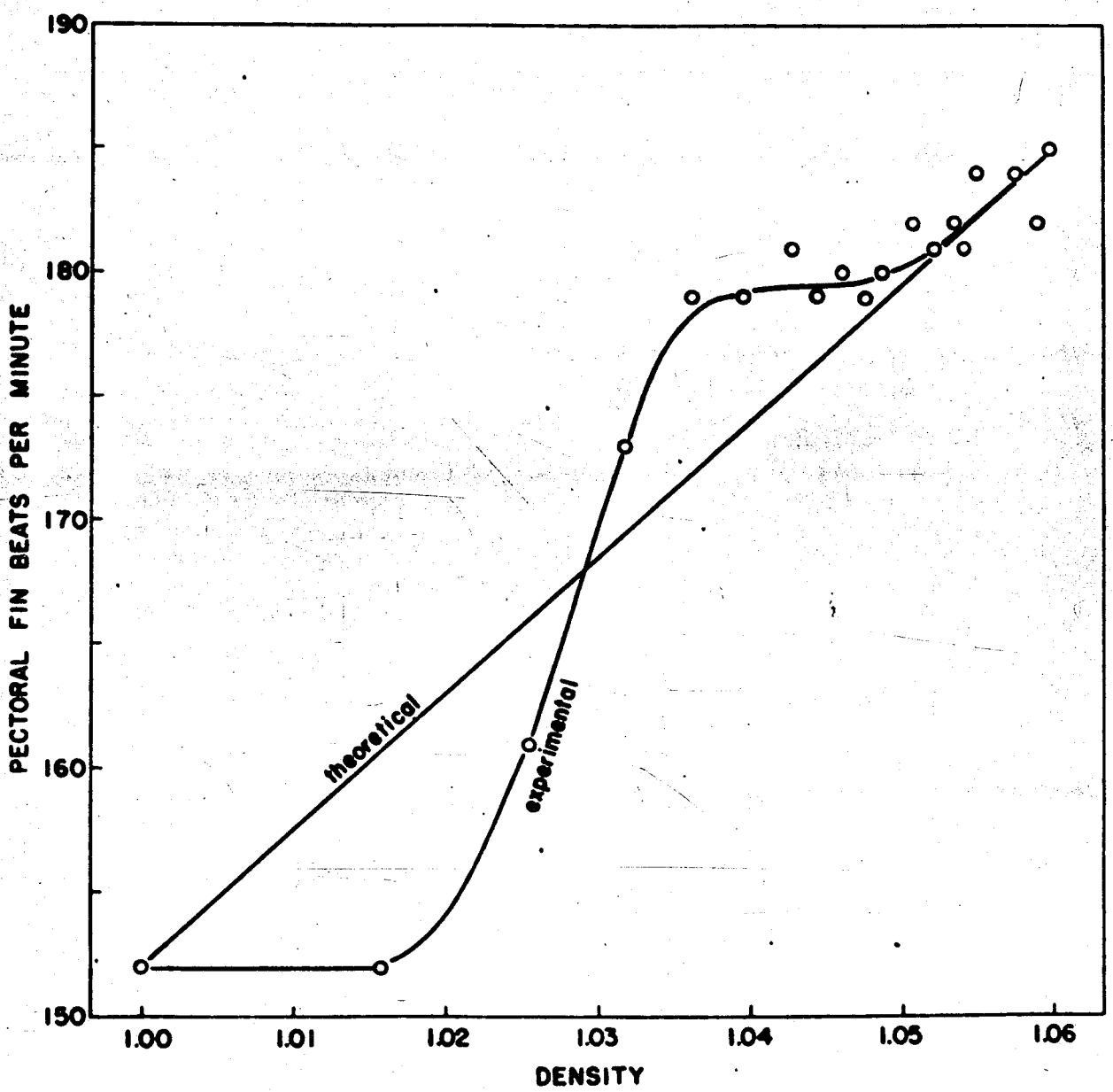


Figure 44. Pectoral fin beats in relation to sockeye smolt density. Theoretical line joins first and final values.

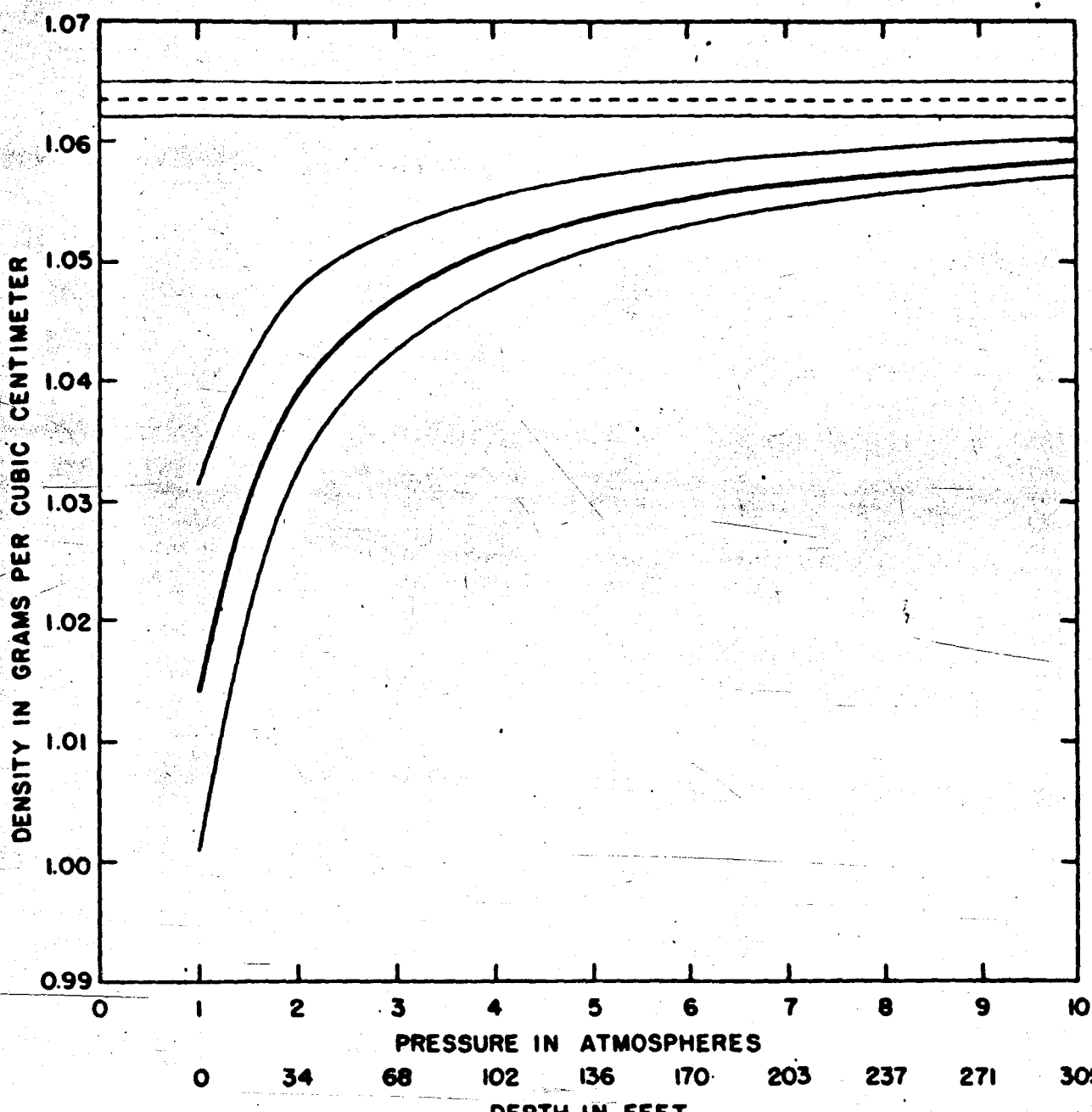


Figure 45. The effect of sounding on pressure-induced change in density. Heavy line mean and light lines range of response. Upper broken line maximum density, swim-bladder free of gas, upper light lines two standard deviations.

of young sockeye in the lake environment and these may be compared with preferred depth determined experimentally. Chamberlain (1907) caught young sockeye at depths of 15 to 60 ft during daylight and at the lake surface during darkness. Foerster (1925) noted the absence of young sockeye from the surface of Cultus Lake in contrast to the surface habits of this species reported to him for Crawford Lake. Foerster caged hatchery sockeye at depths of 2 to 30 ft and noted the fish held at 30 ft obtained more food than those in shallower water. Ricker (1937) found food was sparse in the epilimnion and believed light diminshed in the hypolimnion. He suggested accordingly feeding was in the layer between 5 to 15 m.

Krogius and Krokhin (1948) conducted vertical sampling in Lake Dalnee. They observed sockeye fry remained in shallow water for about one month after emergence from the gravel before moving into deeper water. During the summer sockeye fingerlings were in the epilimnion with up to 100 per cent of the fish at the surface. The young sockeye spread deeper during autumn and by winter were distributed almost uniformly to a depth of 50 m. Krogius and Krokhin believed the vertical distribution of the young sockeye was related to the distribution of food organisms.

Johnson (1956) described an intense distribution of young sockeye at the lake surface at first darkness.

Johnson (1958) noted the disappearance of the fish from the surface in late October, coinciding with lake overturn and changes in the behavior of the fish. Johnson (1959) found young sockeye were distributed at depths of 0 to 5 m during daylight, corresponding to the depth of maximum abundance of zooplankton. During darkness the fish spread downward to depths of 15 m.

Young sockeye salmon are concentrated in the upper 15 ft of Baker Lake during the spring (Rees, 1957) but are distributed as deep as 200 ft during fall and winter (E. Quistorff, personal communication). Burgner (1962) noted the movement of fingerlings offshore and changes in the vertical and horizontal distribution of these fish annually. Gregory and Mathisen (1963) found the greatest concentration of young sockeye at depths of 7 to 13 m with some fish as deep as 31 m.

Thus the depth preference of young sockeye, as shown experimentally by Hoar (1954) and in this study, is in keeping with the vertical distribution found by various investigators. Compensatory swimming probably plays an important role in maintaining young sockeye at their preferred depth.

The problem of maintaining buoyancy. The swimbladder must be regarded as an asset for the maintenance of buoyancy Delaroche (1809). Sockeye appear to have compromised to

some extent the advantages of the swimbladder. Sockeye smolts showed a bladder volume of only 5.96 per cent of the volume of the fish. This is similar to the volume of 5.1 per cent in Salmo trutta (Alexander, 1959c). The small volume of the swimbladder in sockeye indicates these fish are less dependent on the swimbladder for buoyancy than are the higher, bony fishes. The perch, for example, has a bladder volume of 7.5 per cent (Jones, 1951), 28 per cent greater than that of the sockeye. This difference is even more marked comparing sockeye with cyprinids such as the roach having a swimbladder volume of 9.9 per cent (Alexander, 1959b), 66 per cent larger than that of the sockeye.

The relative dependence of young sockeye on their swimbladders may be shown by the method Lowndes (1937) described for quantifying the density of an aquatic animal in relation to that of its environment. His statistic was the density of the animal divided by that of the environment, times 1000. This Lowndes termed the sinking factor. Young sockeye in fresh water with access to atmospheric air showed virtually neutral buoyancy (Fig. 19). Thus with swimbladders inflated to neutral buoyancy young sockeye would have a sinking factor of 1000. Relative dependence on the swimbladder is shown by the sinking factor of such fish free of bladder gas. At a gas-free density of 1.0634 g per ml in fresh water

young sockeye would have a sinking factor of 1063. Sinking factors of this magnitude are representative of bladderless or fish with a much reduced bladder (Jones and Marshall, 1953) and invariably bottom dwelling. The densest of the pelagic fishes appears to be the mackerel, with a sinking factor of 1043, for which it may be compensating by rapid swimming (Jones and Marshall).

There seems little doubt, then, that young sockeye are dependent, in spite of their low density, on the swimbladder for maintenance of buoyancy. Precise neutral buoyancy may not be achieved frequently in fresh water but some degree of swimbladder inflation is probably adequate to meet the needs of these fish.

This dependence on the swimbladder for buoyancy is much reduced in the marine environment, due to the greater density of sea water and the high fat-content of sockeye. Taylor (1921) calculated a fish (fat-free) of density 1.076 g per ml would require a fat content (fat density 0.925 g per ml) of 29.34 per cent for neutral buoyancy in sea water of density 1.026 g per ml. The fat-free density of 1.076 g per ml for bony fishes is probably too high for sockeye. Employing the density obtained for young sockeye, 1.0634 g per ml (admittedly not fat-free) a fat content of 24.3 per cent would bring sockeye to neutral buoyancy in sea water in the

absence of swimbladder gas. Idler and Clemens (1959) found a fat content of 15.1 per cent in eviscerated, maturing female sockeye in sea water. It is apparent that such fish are much less dependent on bladder gas for buoyancy, with more than half of their negative buoyancy compensated for by fat.

For an assumed fat-free density of 1.0634 g per ml and fat content of 15 per cent, the density of a maturing sockeye would be approximately 1.042 g per ml. At an environment density of 1.026 g per ml the sinking factor becomes 1015. Thus maturing sockeye in the marine environment have perhaps one-quarter of the sinking tendency of young sockeye in fresh water. It is suggested such fish are dependent very little or not at all on the swimbladder for maintenance of buoyancy.

It is noteworthy that maturing sockeye evidenced the small and thickened swimbladder Hoar (1937) described in adult Atlantic salmon.

The problem of restrictions imposed by the swimbladder. The rapid change in volume of a gas with depth, particularly near the surface, places restrictions on the vertical movements of fish possessing a swimbladder. Swimming downward below their level of neutral buoyancy, fish become progressively more dense and are obliged to add gas to the swimbladder,

if buoyancy is to be maintained. Swimming upward, fish become progressively less dense and if the fish was adapted to some depth, gas must be removed from the bladder or the fish may be buoyed helplessly to the surface. Jones (1951 and 1952) has described these restrictions for the physoclistous perch.

In sockeye salmon the restrictions of the swimbladder are minimized in a number of ways. The percentage volume of the bladder is small and hence there is less gas, relative to the size of the fish, to expand. Other factors being equal, this small volume of gas would permit sockeye a greater vertical range than that enjoyed by fish such as cyprinids, having a relatively large swimbladder.

The upward movement of sockeye is facilitated by the possession of an extensible swimbladder. In the study of bladder extensibility, the volume of the bladder increased an average of 83 per cent beyond the volume at neutral buoyancy, before gas escaped. These results suggest young sockeye are tolerant of considerable expansion of the swimbladder, almost doubling the volume without gas loss. Accordingly, sockeye may be capable of making vertical migrations without concern for swimbladder expansion. It remains to be demonstrated that sockeye do in fact ever permit the volume of the swimbladder to exceed neutral buoyancy.

In the physostomous sockeye the restrictions imposed by the swimbladder are lessened further by the tendency of this fish to emit gas ("Gasspuck" reflex) on reduction in pressure. Gradual decompression to a vacuum of 25 in Hg was accompanied by the periodic emission of bladder gas by the fish, such that a slightly negative buoyancy was maintained. It is suggested therefore, that sockeye would experience little difficulty in releasing excess gas from the swimbladder during ascent. This would be necessary only if sockeye inflated their swimbladders in excess of neutral buoyancy relative to surface pressure.

Rapid decompression may not permit the escape of swimbladder gas. Hence the swimbladder damage and mortalities among physostomous sockeye resulting from passage through turbines.

The restriction of vertical movement by fish is related also to the pressure of gas within the swimbladder. In sockeye, pressure within the bladder was found to be equal that of the environment. In contrast, Alexander (1959a) found an excess pressure of 108 mm Hg in the swimbladder of the bream. Alexander regards excess pressure in the cyprinid bladder as an adaptation for more precise maintenance of buoyancy over the narrow range of depth to which the fish is adapted. That is, excess pressure within the bladder

reduces the significance of the pressure of the environment. The maintenance of this excess pressure requires a relatively tough and inelastic bladder, restricting the vertical range of the fish. Sockeye possess a thin-walled and extensible swimbladder, incapable of maintaining an excess pressure and of restricting the vertical migrations of the fish.

The restrictiveness of the swimbladder is increased by the secretion of gas into the bladder. That is, during ascent, excess gas must be removed from the bladder either by resorption or emission. Young sockeye showed no ability to secrete gas, either when held beneath screens or under pressure. Thus sockeye fingerlings may be free to migrate to the surface without being required to emit gas from the bladder.

The problem of escape from predators. When frightened, the fry of chum and coho salmon seek cover and pink salmon fry scatter at the surface (Hoar, 1958). In response to fright, sockeye fingerlings and smolts sound into deeper water. This fright response is regarded as an adaptation to the lacustrine environment, sockeye thus making use of the vertical dimension available to them.

The sounding response of sockeye is accompanied by the partial expulsion of gas from the swimbladder. This usually approximates one-third of the bladder gas, but may

approach three-quarters. The function of the gas expulsion while descending (in contrast to herring which expel gas on ascending, Braun, 1962) remains open to speculation. The The accompanying increase in density perhaps accelerates the rate at which sockeye are able to swim downward. The exact value of gas expulsion perhaps can be determined through blockade of this adrenergically controlled mechanism with "dibenzyline" followed by measurements of sounding rates of treated and control fish.

Young sockeye in Cultus Lake are the object of intense predation (Foerster, 1938; Foerster and Ricker, 1941; Ricker, 1941). The important predators in the lake are squawfish, trout and char. These fish are relatively large in relation to under-yearling sockeye, and as swimming speed is a function of fish length, should have little difficulty in outperforming the smaller sockeye. For young sockeye living in the region of the thermocline, the sounding response would tend to carry these fish into the poorer light of the hypolimnion and perhaps some protection from predators.

It is noteworthy that the sounding response continues to be present in adult sockeye. When trapped in purse seines, sockeye attempt to sound under the net and the fishermen anticipate their catch by the extent of the bubbles rising to the surface.

A more thorough discussion of the sounding response may be found in Harvey and Hoar (unpublished manuscript).

The problem of gas disease in sockeye during vertical migrations in stratified lakes. There is some evidence that young sockeye salmon make vertical migrations from cold thermocline to warm surface waters. Ricker (1937) has suggested yearling sockeye may be making excursions from the thermocline into the epilimnion, even to the extent of taking insects from the surface. Harvey (1962) presented evidence that late-migrating smolts commenced their migration from the depth of the thermocline or greater. Such fish would thus pass upward through markedly warmer water, then exit from the lake in a flow of warm surface-water.

The upward movement of young sockeye in a thermally stratified lake has several consequences. The fish experience a rapid increase in temperature, a decrease in dissolved gas in the environment, a rapid decrease in pressure and through this, an increase in absolute saturation of both fish and environment. Thus sockeye rising from a depth of 50 ft to surface conditions in August of 1961 would experience an increase in temperature from 48 to 73°F (Fig. 24), an increase of 25 Fahrenheit degrees. Based on Brett (1952) a temperature of 73°F may be slowly lethal to sockeye. The more immediate consequences would include a sharply increased

saturation of nitrogen internally. From the nitrogen data of July, 1961, fish arriving at the surface would have an internal saturation of nitrogen approaching 136 per cent (Fig. 30). Absolute saturation would not exceed 100 per cent until the fish were within approximately 12 ft of the surface. Thereafter absolute saturation would increase rapidly (Fig. 46) toward 136 per cent. As described previously, the possible consequence of this decompression, plus saturation increase, is the "bends". This is obviated through the rapid rate of nitrogen equilibration between fish and environment (Fig. 40 and 41). This is not regarded, of course, as a unique adaptation of sockeye salmon. Rapid equilibration of dissolved nitrogen is a process of passive diffusion and thus should be common to fish in general. It does explain, however, how pelagic fishes such as sockeye are able to pass upward through a thermally-stratified body of water without developing gas disease.

The rapid clearance of dissolved nitrogen described above does not protect fish from developing gas disease in supersaturated water. Surface water saturations in excess of 100 per cent are relatively common in lakes during the spring period when water temperature is rising more quickly than gas is being lost. Ricker (1937) found surface saturations of 104 per cent during spring and Harvey (1962)

measured oxygen saturations of 110 and nitrogen of 107 per cent. Saturations as low as these are not usually lethal to adult fish (Harvey and Smith, 1961) although sockeye alevins are prone to gas disease at low levels of supersaturation (Harvey and Cooper, 1962). Foerster (1925) reported oxygen saturation as high as 127 per cent (133 per cent calculated to the theoretical solubilities, Handbook of Chemistry and Physics) for Cultus Lake in August, 1923. Such saturations would approach lethal conditions for surface dwelling fish. These high saturations in the epilimnion occurred the day following intense mixing by storm action and the oxygen values presented by Foerster are considerably higher than would be expected following air-equilibration.

A less common but more serious form of lake-surface supersaturation is that resulting from photosynthesis. Woodbury (1941) measured oxygen saturations on the surface of Lake Waubesha (Wisconsin) ranging from 171 to 306 per cent and attributed to this the deaths of a variety of fishes. In the present study of dissolved gases in Cultus Lake, oxygen supersaturation through photosynthesis was limited to the depths of 15 to 50 ft. The absolute saturation remained below 100 per cent and hence mortalities such as described by Woodbury could not occur.

The problem of tolerance to pressure and resistance to decompression. The utilization of the vertical dimension of the lake environment requires young sockeye to have a tolerance to pressures much in excess of atmospheric. The ascent of sockeye from depth requires these fish to tolerate decompression.

In the pressure apparatus, young sockeye tolerated pressures up to 20 atmos, equivalent to a depth of 680 ft. This is considerably greater than the depths which young sockeye are known to inhabit: 200 ft, Quistorff (personal communication) and 50 m, Krogius and Krokhin (1948). Neave (1960) reported sockeye were netted as deep as 200 ft during sampling in the eastern Pacific. It is possible therefore, that pressure does not limit the lower range of sockeye salmon. Other factors such as light, food or temperature may determine vertical distribution of sockeye.

Resistance to decompression was variable, depending on whether or not sockeye were decompressed to pressures below atmospheric (Fig. 31 and 32). Resistance to decompression varied seasonally (Fig. 34). Mortality of smolts was associated with residence at some depth in the lake (Fig. 36). Mortality on decompression was induced experimentally through increased content of dissolved gases (Fig. 37) and increased temperature (Fig. 38). In spite of these environmental

factors influencing mortality, there is little doubt that the embolism-induced deaths were due to emboli originating from swimbladder gas.

As these mortalities appeared only with rapid decompression, it is unlikely that the relatively gradual decompression accompanying ascent in lakes would result in comparable mortalities. Furthermore, decompression below atmospheric pressure is equivalent, in so far as expansion of swimbladder gas is concerned, to the ascent from depth of sockey having their swimbladders inflated to provide buoyancy at depth. Thus the exposure of sockeye (swimbladders inflated to neutral buoyancy at atmospheric pressure) to a pressure of $\frac{1}{2}$ atmos (15 in Hg vacuum), is equivalent to sockeye migrating to the surface from a depth of 34 ft (swimbladders inflated to neutral buoyancy at that depth, a pressure of 2 atmos). Until it has been demonstrated that sockeye do inflate their swimbladders at depth, this problem may be assumed not to arise naturally in the lake habitat. Decompression of fish to pressures below atmospheric does occur during passage through turbines.

The problem of the temperature barrier to the downstream migration of sockeye smolts. Chamberlain (1907) in his study of young salmon in the Naha River (Southeastern Alaska) observed sockeye yearlings commenced migrating in

large numbers at about 45°F (1903) and the migration ended at 50°F (1904).

Ward (1927) noted young sockeye remain in the reservoir, Lake Shannon, behind the then newly constructed Baker Dam. After the lake surface warmed, Ward believed these fish retired below the thermocline and spent the summer in deeper water. He did not know whether these sockeye continued their migration in the fall or became landlocked. Ward (1932) expanded on this idea. He observed smolts entered the reservoir during filling and hence at a time when water was not necessarily being spilled from the surface. Concurrently the surface waters warmed and when temperatures rose above 50°F the migrants were no longer seen and appeared to Ward to have deserted the surface for deeper layers. In doing so the smolts were barred from leaving the lake and were described by Ward as being physiologically landlocked.

Foerster (1937) termed this cessation of smolt migration by epilimnial temperatures a "temperature barrier or blanket". Foerster suggested the cessation of migration of sockeye smolts from Cultus Lake was related to the formation of such a temperature blanket in the epilimnion. Foerster's evidence for this included the relative frequency of smolts remaining on in the lake for a second year and

then migrating as two-year-old fish. The yearling migration of 1934, the end of which coincided with exceptionally high temperatures, was followed in 1935 by an exceptionally large fraction of two-year-old smolts. Precise temperatures could not be given for the thermal barrier; the migration was 80 per cent complete at 51°F and terminated at an average of 63.5°F.

Parker and Vincent (1956) described the migration of sockeye smolts out of Kitoi Lake (southeastern Alaska). The major portion of the smolt migration passed the outlet at temperatures ranging between 44 and 50°F. The migration ceased when surface waters reached 55°F or above and warm water extended to a depth of 16 ft. Parker and Vincent advanced the hypothesis sockeye avoid temperatures below 40° F and above 55°F. A small migration of stragglers continued at temperatures of 55 to 60°F.

Burgner (1962) reported the relationship of temperature to smolt migration from Lake Nerka, Alaska. For five of the six years of study, 90 per cent of the smolt migration coincided closely with water temperature reaching 50°F. Burgner concluded the cessation of migration was associated probably with a change in epilimnion temperatures. Burgner noted however the greatest concentrations of sockeye fingerlings on Lake Nerka were observed after lake surface

temperatures exceeded 50°F, the largest single surface catch being made at 61°F.

Lest the above discussion tends to convey the impression that sockeye migrations only terminate through high surface-temperatures, it might be useful to offer an example where a migration stops at a relatively low temperature. At Chilco Lake, B.C. (elevation 3,845 ft) sockeye smolt migrations commence at about 38°F and cessation coincides with a temperature of approximately 45°F. Nevertheless some yearling sockeye stay on in the lake for a second year. One might postulate that had he studied the migration from Chilco Lake rather than Lake Shannon, Ward might have concluded young sockeye avoid temperatures in excess of 45°F, rather than 55°F.

Roos (personal communication) observed sockeye smolt migrations out of Chignik Lake, Alaska, commenced at temperatures of approximately 38°F, were 95 per cent complete at 46°F and almost complete at temperatures of 50°F or less.

In the present study, the results of the decompression tests on migrating smolts bore directly on the concept of a thermal barrier to migration. Also pertinent is the smolt migration and temperature data collected concurrently at the outlet of Cultus Lake. The smolt migration, plotted chronologically in Fig. 2, differed considerably in relation to

temperature. The 1960 migration commenced at approximately 46°F, was 56 per cent complete at 50°F and 99.7 per cent complete at 55°F. Thus for the 1960 migration it appears rather that the migration was very nearly complete before the barrier commenced to exist. In 1961 the migration was considerably the more prolonged. It was 94 per cent complete at 55°F, 95 per cent at 60°F and 98.7 per cent at 65°F. In effect the migration proceeded without interruption during the period of warming of the lake surface.

Decompression studies indicated the late migrating smolts were not originating from surface water but rather from some depth, perhaps the region of the thermocline. The sudden increase in mortality of smolts on decompression coincided with lake-surface water warming to 51°F (Fig. 34). This may indicate that 51°F is the minimum temperature tending to depress sockeye from surface water. The relatively rapid decline in mortality with holding in surface water places a time limit on the period of surface residence before the fish pass through the lake outlet and hence are trapped and tested. These results suggest smolts are leaving the depth of the thermocline (or greater) and very soon thereafter entering the outlet stream, Sweltzer Creek. The results indicate also the thermal barrier is by no means an absolute barrier to sockeye in condition to migrate. On the contrary,

the barrier appears to be relatively permeable, as shown by the migration of more than 9,000 fish June 1, 1961. On that date lake temperatures increased uniformly from 48°F at a depth of 30 ft to 65°F at a depth of 1 ft below the lake surface.

CHAPTER V

SUMMARY AND CONCLUSIONS

Summary

1. Pressure did not influence the behavior of sockeye fry prior to initial filling of the swimbladder.

2. Sockeye fingerlings and smolts evidenced compensatory swimming in response to pressure. Compensatory swimming was invoked at a density approximately one-third of the maximum for the fish.

3. Yearling and two-year-old sockeye made less use of the pectoral fins and compensated for increased density by more rapid swimming.

4. The volume of the sockeye swimbladder was 5.9 per cent of the volume of the fish.

5. The excess pressure within the sockeye swimbladder was 0.2 mm Hg. The pressure within the bladder did not differ significantly from that of the external environment.

6. The swimbladder of young sockeye proved very extensible. Among smolts killed by an overdose of anesthetic, bladder volume increased an average of 83 per cent before gas escaped through the pneumatic duct.

7. Isolated loops of swimbladder, taken from yearling sockeye, contracted in response to adrenergic drugs. This response was blocked irreversibly by the adrenergic blocking agent "dibenzyline".

8. Young sockeye held beneath screens and under pressure failed to increase the gas content of the swimbladder. There was thus no evidence that sockeye were capable of secreting gas into the swimbladder.

9. When frightened, sockeye smolts sounded and lost gas from the swimbladder. Gas loss on sounding was enhanced by prior exposure to sympathomimetics and prevented by prior treatment with adrenergic blocking agents.

10. Young sockeye maintained buoyancy by gulping atmospheric air. Inflation of the swimbladder was retarded among fish treated with the cholinergic blocking agent, atropine.

11. Among smolts anesthetized to death, an average pressure of 28 mm Hg was necessary to force bladder gas out through the pneumatic duct. Atropine treatment reduced duct-release pressure to 0.2 mm Hg.

12. Histologically, the pneumatic duct of sockeye was found to be a convoluted connection between oesophagus and the anterior end of the swimbladder.

13. A limnological study of Cultus Lake was conducted in relation to the downstream migration of sockeye smolts. The temperatures and dissolved gases of the lake were measured during the year 1961.

14. Young sockeye salmon tolerated pressure up to the maximum tested, 300 psi, equal to pressure at a depth of 680 ft.

15. Sockeye smolts tolerated decompression from high pressures to atmospheric conditions. Resistance to decompression below atmospheric pressure was variable. Mortality of fish was due to emboli originating from swimbladder gas.

16. Smolts migrating out of Cultus Lake after the lake had stratified thermally were particularly prone to embolisms on decompression.

17. Mortalities due to embolisms were induced by rapid decompression of sockeye previously held at the depth of the thermocline in Cultus Lake.

18. Embolism mortality on decompression was increased by pre-test increase in the gas content of the swimbladder and decreased by reducing the gas content of the bladder before testing.

19. Bladderless fish and sockeye with a catheterized swimbladder did not succumb to embolisms on decompression.

20. No change was observed in the patency of the pneumatic duct such as to explain the variability of embolism-induced mortalities on decompression.

21. Rainbow trout evidenced a rapid reduction in dissolved nitrogen in venous blood following transfer from water of 17.5 to 13.2 ml per liter of nitrogen.

22. Sockeye yearlings transferred from a water of high to one of low dissolved-nitrogen content, showed a rapid equilibration with the nitrogen of their environment, approximately 60 per cent of excess nitrogen being lost in 30 min.

Conclusions

Pressure in the aquatic environment presents fish with certain physiological problems. Sockeye salmon meet these problems by adaptation, compromise and fortuity. Prior to initial filling of the swimbladder, sockeye alevins and fry are not induced to swim upward. Following inflation of the swimbladder, sockeye fingerlings respond to increased density due to pressure by compensatory swimming. Compensatory swimming probably plays a part in the vertical distribution of young sockeye. In fresh water, young sockeye have a reduced but nevertheless definite dependence on the swimbladder for the maintenance of buoyancy. In salt water,

the swimbladder is probably of little importance in maintaining buoyancy. In sockeye, the restrictions imposed by the swimbladder are minimized by the small percentage volume of the bladder, the marked extensibility, the "Gasspuck" reflex, the absence of an appreciable excess pressure in the bladder and the inability of sockeye to secrete gas into the bladder. Sockeye utilize the vertical dimension of the lacustrine environment for escape, sounding into deeper water when frightened. Sounding is accompanied by the expulsion of gas from the swimbladder. This is due to the contraction of the circular muscles of the bladder under control of the adrenergic component of the autonomic nervous system. Young sockeye are able to make vertical migrations in stratified lakes. The possibility of gas disease is obviated by the rapid equilibration of dissolved nitrogen between fish and environment. Sockeye have a tolerance to pressure and under certain conditions, to decompression.

In general, sockeye appear well suited to residence and migration over a range of pressures in the lacustrine environment. Such fish may be described eurypressural, in contrast to stenopressural fish having a narrow range of vertical distribution and movement.

CHAPTER VI

REVIEW OF RELATED LITERATURE

There exists now an extensive and scattered literature pertinent to a discussion of fish in relation to pressure. The swimbladder of fish has been the subject of most such research. Swimbladder reviews by Jones and Marshall (1953) and Qutob (1962) include discussions of some aspects of pressure. Other aspects, notably the response of fish to barometric pressure warranted critical examination. The effects of compression and decompression on fish similarly had not been reviewed to date.

Pressure Perception and Response in Fish

Pressure and gas secretion

The original observation that pressure induced secretion of gas appears to be that of Biot (1807), who found that the fraction of oxygen in the swimbladder increases in direct proportion to the depth at which the fish is found. Moreau (1876) measured gas secretion and resorption in response to pressure increase and decrease. Moreau demonstrated gas secretion following partial removal of bladder gas by puncture, thus establishing fish were secreting gas as a function of density and not due to pressure <u>per se</u>. Moreau

found also secretion of oxygen increased following sympathetic section; vagal section produced no change. Bohr (1894) expanded the innervation studies of Moreau. Bilateral section of the intestinal branch of the vagus supplying the swimbladder resulted in a decreased oxygen fraction in the bladder and when the swimbladder was emptied it remained emptied, thus secretion of gas had ceased. From his own experiments and those of Moreau, Bohr concluded bladder filling was controlled by the vagus and emptying by the sympathetic nerves.

That gas secretion was due to a perception of altered density, as opposed to a perception of change in pressure, is indicated by the experiments of Woodland (1912). Gas secretion was induced in artificially weighted fish. Conversely, physoclistous fish artificially buoyed by attached corks (Kuiper, 1915) decreased the tension of gas in the bladder. In the case of a "corked" carp, the fish continued to fill its swimbladder in excess of neutral buoyancy, leading Kuiper to conclude the reaction is independent of the density of the fish. He suggested the inflation of the bladder is probably controlled by the degree of bladder tension.

Hall (1924) confirmed experimentally the changes in oxygen content with pressure observed by Biot and Moreau. Perch held at atmospheric pressure showed a swimbladder oxygen content of 12.1 per cent whereas those maintained

under a hydrostatic pressure of 60 ft for 10 hr had 18.5 per cent of oxygen in the bladder.

Pressure-induced secretion of gas in the certain physostomes was shown by Evans and Damant (1928). The cyprinoids carp, roach and goldfish, lacking the "gas glands" or "red bodies" of physoclists, achieved neutral buoyancy in 6 hr under a pressure of 5 ft of hydrostatic head. Roach clearly were secreting oxygen which rose from 8.9 to 25.7 per cent of bladder gas.

Gas secretion in physoclists was investigated by Jacobs (1930) who observed that immobilized perch failed to refill previously emptied swimbladders. Jacobs (1934) confirmed the observation of Evans and Damant that certain physostomes were capable of secreting gas into the swimbladder. The cyprinids tested and Esox were capable of secreting gas but salmonids Hucho and Salmo were not.

Frisch (1934) removed the utriculus from blinded and control minnows and concluded the labyrinth played no part in gas secretion. This would rule out swimbladder pressure controlling secretion of gas via the Weberian apparatus.

Brown (1939) demonstrated the physoclistous guppy was capable of increasing the gas content of the swimbladder in response to pressure increase. Small pressure reductions resulted in gas resorption, but a decrease to 625 mm Hg

absolute pressure or less resulted in an increase in bladder gas. Brown attributed this to reduced pressure in the bladder favoring the passage of dissolved gases into the bladder. Rostorfer (1942) exposed the rock-bass to pressures ranging from 540 to 2,223 mm Hg, the fish reaching equilibrium at each pressure. This was achieved through rapid secretion of oxygen and carbon dioxide, the latter gas then gradually diffusing from the bladder and being replaced by oxygen.

Meesters and Nagel (1934) induced gas secretion and resorption in the perch through increase and decrease in pressure. These processes went on only so long as the fish was actively moving its fins, suggesting swimming activity was reflexly controlling inflation of the bladder. Changes in resorption were rapid due to quick opening of the oval and the carbon dioxide constituent of the gas.

Franz (1937) investigated the nervous control of gas secretion and confirmed vagal section reduced the production of gas. Pressure stimuli within the bladder were conducted to the central nervous system by the vagus and sympathetic (splanchnic) nerves. Franz concluded secretion of gas was regulated by the spanchnic nerve. Spinal cord section, extirpation of the cerebrum or diencephalon did not influence formation of gas but Franz located a center of gas secretion in the mid-brain.

Ledebur (1937) in his review of gas secretion and resorption repeated the observation of Meesters and Nagel that these processes may be related reflexly to the characteristic movement of the fins, which Jacobs had shown were released through the rising or sinking of the fish. Ledebur concluded gas secretion or resorption should occur only if these movements could be carried out.

Experiments testing this reflex control of swimbladder filling were conducted by Copeland (1952). Fundulus were maintained in very shallow water, such that they protruded above the surface and a control group allowed to swim freely in an aquarium. Deprived of swimbladder gas, the control group recovered buoyancy whereas the shallow-water fish did not. In a second experiment, fish forced continuously to swim upward increased the volume of gas in the bladder and conversely those forced to swim downward decreased it relative to control fish. On this basis, Copeland advanced the hypothesis that the reflex receptors controlling the volume of bladder gas are located in the musculature of the pectoral fins, these being the structures used in achieving a new orientation in the vertical plane.

The hypothesis of Copeland was promptly upset by Fänge (1953) who denervated the pectoral fins in the cod by sectioning the brachial plexus bilaterally. Gas removed from

the swimbladder of such fish by puncture was soon replaced, with test and control fish returning to neutral buoyancy within 24 hr and evidencing an increased bladder oxygen content. Fänge concluded movement of the pectoral fins is not necessary for the inflatory reflex. It is unfortunate that Copeland's hypothesis was not accompanied by observations on the response of the pectoral fins. It was essential that he demonstrate a difference in the rate or nature of fin movements between fish forced to swim upward and downward. Jones (1952) showed the pectoral fins in perch accelerated both in response to buoying and sinking, although the direction of the power stroke was reversed. In the present study, sockeye evidenced increased movement of the pectoral fins both in swimming upward in response to sinking under increased pressure, and while swimming downward on pressure reduction, before or during gas emission. In both cases the fish were angled steeply from the horizontal and the power stroke appeared to be in the same direction. It is difficult to imagine how increased use of the pectorals could lead to gas secretion in some instances and resorption in others.

Jacobs (1934) was unable to demonstrate gas secretion in either the brook or rainbow trout. Deprived of swimbladder gas and denied access to atmospheric air these fish failed to replace lost gas. This is not surprising, the glandular tissue

being poorly developed and retia mirabilia absent (Jones and Marshall, 1953). Jacobs' conclusions were in general acceptance until the experiments of Wittenberg (1958). The latter worker similarly emptied the swimbladders of rainbow and brown trout and maintained these fish away from atmospheric air. Both species achieved up to 50 per cent refilling in 8 days and up to 100 per cent in 13 days. Tait (1959) testing a number of salmonids was unable to verify these results. Lake char, for example, maintained under pressure showed a net loss of swimbladder gas, despite observations that among trout brought up from depth swimbladders were inflated appreciably in excess of atmospheric pressure.

In the present study, sockeye smolts frightened into expelling swimbladder gas then held under screens, showed no tendency to inflate their swimbladders (Fig. 19). Indeed such fish apparently continued to lose gas from the swimbladder during the 8 days of study. Similarly smolts held under pressure for 24 hr showed a slight increase in specific gravity at the end of this time. There was thus no evidence that young sockeye salmon were capable of secreting gas into their swimbladders, either in response to an increase in density or to an increase in pressure.

Decompression and gas resorption

Moreau (1876) observed Serranus brought to the surface from 25 m had a much distended bladder. The distention diminished slowly over several hours. Investigating this experimentally, Moreau held Labrus at a depth of 7 m for several days. Returned to surface pressure the fish floated but restored its initial volume after 3 days. Bohr (1894) confirmed these observations on cod. Brought up from a depth of 14 m the fish were buoyed to the extent of being inverted at the surface. Within a day the size of the fish had diminished and oxygen had declined from 52 to 13 per cent of bladder gas. Bohr did not experiment himself with the control of swimbladder emptying but concluded from Moreau's studies that the sympathetic nervous system controlled gas resorption. Baglioni (1908) showed resorption of gas was due to positive buoyancy. Attaching floats to Balistes the fish were buoyed to the surface. On removal of the floats a day later the fish sank to the bottom of the tank and evidenced a reduction in girth.

The site of gas resorption, Jaeger (1903) suggested, was the oval. Reis and Nusbaum (1906) described the opening and closing of the oval in Ophidium. Woodland (1913) showed the oval of the pollack was closed tightly during active secretion of gas induced by weighting. Ledebur (1929) proved

experimentally the oval was the site of gas resorption by means of a ligature around the bladder, isolating oval from red gland. Examined later the chamber with the oval was free of gas.

Following these early studies on the stimulus and site of gas resorption, subsequent investigations have been directed at rate and nervous control of resorption. Jacobs (1932) measured volume fluctuations in the perch due to swimbladder gas. He concluded secretion and resorption of gas were both rapid and precise enough to maintain the density of the fish constant during variations in barometric pressure. Meesters and Nagel (1934) repeated Jacobs' studies on the perch. A pressure reduction of 5 cm Hg resulted in a corresponding reduction in volume which was achieved in 10 min. The authors attributed rapid gas resorption to quick opening of the oval. Meesters and Nagel described gas loss from the bladder as diffusion through the oval but were uncertain whether this was an active or passive process. The rapidly absorbed component of swimbladder gas proved to be carbon dioxide, in the absence of which resorption was relatively slow.

Brown (1939) subjected guppies to decreased pressures. At a reduction of 100 mm Hg from atmospheric pressure, gas was resorbed from the swimbladder restoring

neutral buoyancy within 2 hr. Larger pressure reductions favored entry of gas into the swimbladder and the resulting positive buoyancy became progessively greater. Jones (1952) measured the rate of gas resorption in the perch and calculated decompression rates for these fish. A 20 per cent reduction in pressure required 1 hr and 50 hr were necessary for a 90 per cent decrease in pressure. From this Jones showed diurnal migrations with ascents of 5 to 7 hr could bring perch to the surface from depths not greater than 6 to 7 m.

Fänge (1953) investigated the nervous control of gas resorption from the swimbladder. The movable muscosa of the oval formed a mechanism regulating the permeability of the bladder wall to gases. This resorbent mucosa contracted with acetylcholine and relaxed with atropine and adrenaline. Fänge introduced carbon dioxide into the swimbladder of Ctenolabrus and observed its disappearance in 5 to 10 min, thus demonstrating the high permeability of the resorbent mucosa.

Brief reviews of swimbladder gas resorption have appeared in Plattner (1941), Jones and Marshall (1953) and Jones (1957).

Sockeye salmon smolts induced to sound and expel swimbladder gas, then held under screens, failed to secrete

gas and return to neutral buoyancy. Indeed the density of the fish continued to increase due either to gas resorption or to further gas loss through the pneumatic duct. Sockeye smolts denied access to surface air and held in water of low oxygen tension (2 ppm) showed a rapid reduction in swimbladder oxygen content. Thus gaseous exchange across the wall of the swimbladder cannot be ignored in these fish. However the tendency of the fish in the samples to become more alike in density with prolonged holding under screens argues in favor of gas loss through the pneumatic duct. Thus repeated sounding during the period of holding would tend to bring the group to a common maximum of gas expulsion. The question of resorption of gas being involved could be answered perhaps by prolonged holding of fish without access to atmospheric air. If the density of the sockeye eventually exceeded that possible through gas expulsion, then gas resorption across the wall of the bladder would appear to be involved.

Pressure and air gulping in physostomes

Baer (1835) apparently first observed young fish gulping atmospheric air at the surface and inflating their swimbladders. Mortalities among those which failed to do so

led Baer to conclude this phenomenon was involved in respiration. Jaeger (1904) decompressed tench thus partially emptying their swimbladders and observed swallowed air entered the bladder and under certain conditions the gut.

Thilo (1906) emphasized the importance of air gulping and concluded this was the main function of the pneumatic duct. Thilo also evacuated the bladder of tench by decompression. Allowing these fish access to air at atmospheric pressure, swimbladders were re-filled in 3 to 8 hr. Thilo concluded this was too rapid to be due to secretion and air entry was by way of the pneumatic duct. Woodland (1911) noted the ease with which physostomes were able to renew their swimbladder gas by rising to the surface of the water and passing air through the pneumatic duct.

Evans and Damant (1928) studied air gulping in carp, roach and goldfish. Goldfish which were partly deflated through decompression, on return to atmospheric pressure began gulping air at the surface within 5 min and returned to neutral buoyancy within 1 1/2 hours. Goldfish placed under an additional pressure of 8 ft of water in the presence of an air-lock, inflated to neutral buoyancy within 5 min. No gas was observed in the stomach or intestine. Evans and Damant examined histologically the pneumatic duct and termed the fleshy enlargement described by Guyénot (1909), the

pneumatic bulb. Guyénot was of the opinion the duct was suited only to the exit of gas from the bladder. Evans and Damant demonstrated gas entry was possible and suggested the pneumatic bulb served to pump swallowed air into the bladder.

Jacobs (1934) studied air gulping in brook and rainbow trout, several cyprinids and the pike. Swimbladders emptied by puncture were re-filled by gulping within 2 to 3 hr in the case of rainbow trout and brook trout required 6 to 12 hr. Rainbow trout similarly deflated by puncture and occluded from the surface by screens continued to show negative buoyancy after 27 days. Franz (1937) observed air gulping in the minnow increased with pressure and with weighting of the fish and concluded the response was due to increased specific gravity. Moehres (1940) reported air gulping was less marked in the bottom-dwelling cyprinid, the gudgeon.

Plattner (1941) decompressed tench, inducing them to emit gas from the swimbladder. On return to atmospheric pressure such fish gulped air at the surface. Bilateral section of the vagus in Leuciscus stopped secretion and also paralysed the function of the pneumatic duct. The duct remained closed, the bladder empty and air swallowed by the fish passed into the intestine. The same result was obtained by ligaturing the pneumatic duct.

Sockeye salmon readily evidenced air gulping in the

present study. This occurred, for example, shortly after fright-induced expulsion of gas. The fish returned to the surface and commenced to gulp atmospheric air within 5 min of having sounded. Sockeye smolts exposed to pressures in excess of atmospheric in the presence of an air lock, commenced to gulp air almost immediately pressure was increased. At a pressure of 3 atmos absolute, sockeye smolts increased the volume of swimbladder gas almost three-fold.

The mechanics of air entry into the sockeye swimbladder may not be quite the same as Evans and Damant (1928) have suggested for cyprinids. These authors described a thick-walled oesophagus within which was located the inflating mechanism, the pneumatic bulb. Such a muscular bulb may be necessary to inflate the roach bladder to the measured pressure of 62 mm Hg. In sockeye, the duct penetrates the wall of the oesophagus and passes through a bulbous thickening before becoming continuous with the anterior end of the bladder. The bulb is much less muscular and this may be in keeping with the very slight excess pressure measured within the sockeye bladder (0.2 mm Hg). From observations on the behavior of young sockeye it would appear air is passed into the swimbladder with considerable ease. In the case of the catheterized fish (Fig. 13) air gulped at the surface issued from the bladder with a few seconds.

Decompression and gas emission in physostomes

Moreau (1876) noted the emission of gas bubbles by the tench accompanying a reduction in pressure of 4 to 6 cm Hg below atmospheric. Guyénot (1909) explored the control of this loss of gas. He concluded the pneumatic duct functioned to release excess gas to the exterior. Loss of gas was regulated in cyprinids by a sphincter which Guyénot termed the pneumatic sphincter. The sphincter being closed was inhibited reflexly by impulses originating through an increase in bladder volume and conducted to the labyrinth along the Weberian ossicles. Guyénot showed a greater reduction in pressure was necessary to evoke gas emission following interruption of the Weberian apparatus.

Guyénot's gas-releasing-reflex explanation of the function of the Weberian ossicles was soon tested and his experiments have been repeated many times since. Kuiper (1916) took exception to Guyénot's technique and was able to reproduce the latter's results only if the fish was in poor condition and suffering from post-operative shock. Kuiper concluded neither interruption of the Weberian ossicles nor removal of the labyrinth, both essential to Guyénot's reflex, influenced the reduction in pressure necessary to bring about the emission of gas. Kuiper experimented with vagal section and concluded from his results that sphincter tone is maintained by the

sympathicus and inhibited by the vagus. It is interesting that Kuiper observed that his fish emitted a few bubbles when confined and hence frightened but apparently he failed to recognize the conflict between this observation and the innervation he was proposing.

Kokas (1932), acknowledging the work neither of Guyénot nor Kuiper, repeated their experiments on the interruption of the Weberian apparatus with the removal bilaterally of the mallei. Before the operation a reduction in pressure of 30 mm Hg evoked gas emission, after the operation a reduction of 120 mm Hg was required, the latter pressure forcing open the pneumatic duct. Kokas concluded from these results the Weberian apparatus formed the afferent part of the mechanism regulating pressure.

Jacobs (1934) acknowledged it has long been known the pneumatic duct permits gas to escape on reduction in pressure. He quotes Jaeger's (1903, 1904) view that the pneumatic duct is to the physostome what the oval is to the physoclist, namely a mechanism for removing gas from the swimbladder.

Franz (1937) repeated the studies of Guyenot and Kokas, but could find no hydrostatic function for the Weberian apparatus and concluded the ossicles were not involved in the "Gasspuck" reflex. In the case of the minnow, the site

or perception of pressure was the wall of the swimbladder and gas expulsion was due to contraction of the musculature of the bladder. In addition, both the utriculus and eyes were involved in gas emission through perception of buoyancy. Franz found, as had Kuiper, that vagal section resulted in the disappearance of resistance to the loss of gas from the bladder.

Moehres (1941) investigated the pressure sensitivity of the gudgeon as evidenced by gas emission with a reduction in pressure. Moehres found removal of the pars superior of the labyrinth resulted in a temporary increase in the threshhold of pressure necessary for gas release. Extirpation of of pars inferior, however, resulted in a greater reduction in pressure being required to evoke spitting; from 13 cm of water on non-operated fish to more than 40 cm post-operatively. Moehres concluded the Weberian apparatus serves a hydrostatic function in the gudgeon. His results agreed with Guyénot and Kokas, and Moehres felt the objections to their techniques expressed by Franz were not applicable here, since the Weberian apparatus was inactivated quite differently. Moehres limited his conclusions to the function of the Weberian apparatus in the bottom-dwelling gudgeon and suggested his results need not necessarily be true of surface-dwelling fish.

Plattner (1941) reviewed the conditions evoking gas emission in physostomes. These included a reduction in external pressure, an increase in internal pressure, artificial lightening of the fish and psychic excitation. He confirmed sphincter opening was inhibited by vagal section or the application of anesthetics. Plattner agreed gas loss through the pneumatic duct was an active reflex but did not commit himself to the nature of the pathway.

Dijkgraaf (1941) trained minnows to respond to changes in hydrostatic pressure of 0.5 to 1 cm of water. Following interruption of the Weberian apparatus through removal of both mallei, this response decreased to the extent that an excess pressure of 40 cm elicited no response. Unilateral extirpation of the malleus did not impair the response. Dijkgraaf concluded accordingly the Weberian apparatus served not only in an acoustical capacity, as shown by Frisch and Stetter, but also in a hydrostatic capacity. Dijkgraaf (1942), using blinded minnows, measured the decrease in pressure necessary to induce gas emission in both intact and fish in which the incus was removed bilaterally. The result was a slight increase in the threshhold of gas emission. Dijkgraaf concluded expansion of the swimbladder can cause emission of gas without the participation of the Weberian apparatus. The threshhold of gas emission was raised about 30 per cent

on removal of the incus and thus the Weberian ossicles were concerned only to a limited extent in the release of the gas-spitting reflex. Dijkgraaf (1950) investigated the role of direct perception of expansion of the swimbladder by sensory nerve-endings in the release of the spitting reflex. The exteroceptive stimuli and change in buoyancy were eliminated through removal of the pars superior and permitting the animals to orient to a horizontal light, changes in buoyancy thus appearing as current. In such fish the reduction in pressure necessary to induce gas emission was almost the same before and after interruption of the Weberian apparatus. Dijkgraaf concluded the Weberian apparatus perceives the finer variations in pressure whereas the bladder wall is responsible for the coarser variations, perhaps above 30 mm Hg.

Fänge (1953) observed acetylcholine increased the pressure necessary to open the sphincter of the pneumatic duct. He suggested the pneumatic-sphincter mechanism is to some extent homologous to the smooth muscles of the resorbent area of the euphysoclist. Fänge pointed out the similarities between the deflatory reflex in euphysoclists and the "Gasspuck" reflex in physostomes.

In the present study, investigation of the responses of sockeye smolts to pressure was complicated frequently by the fright response of the fish. In this the fish dived

or sounded and emitted gas from the swimbladder. Harvey and Hoar (unpublished) have termed this the sounding response and as a fright reaction have divorced it from the "Gasspuck" reflex. This latter term they reserve for gas loss accompanying pressure reduction. There is a marked difference in the nature of gas emission in the two responses. On sounding, sockeye smolts lost up to 72 per cent of swimbladder gas and increased significantly in density in so doing. In the "Gasspuck" reflex, fish emit gas in response to a reduction in pressure only to the extent necessary to restore neutral buoyancy.

Loss of gas on sounding was not the simple release of gas held under pressure in the swimbladder. This was apparent both from the negligible excess of pressure measured in the bladder (0.2 mm Hg) and from the extent of gas lost on sounding. A loss of 72 per cent of bladder gas would require an excess pressure internally of almost 4 atmos. This implied gas was expelled forcibly from the swimbladder by the wall of the bladder or the body wall of the fish.

The adrenergic control of this expulsion was evident from the effects of the sympathomimetic, ephedrine and the adrenergic blocking agents, "dibenzyline" and "hydergine". The former slightly enhanced gas loss on sounding, the latter prevented it entirely. The behavioral response was otherwise

unaffected by these agents. Expulsion of gas was due to the contraction of the swimbladder and hence a reduction in its cross-sectional area. This was clear from studies on both isolated and intact swimbladders. Anesthetized smolts held under water emitted gas on application of adrenaline in the region of the swimbladder. Smolts in which the abdominal musculature was opened mid-ventrally showed a marked contraction of the bladder with adrenaline.

Parasympathomimetics and cholinergic blocking agents caused slight changes in the amount of gas lost on sounding. Because of the possible action of these agents at synapses in sympathetic nerves, limited significance is attached to these results.

The pressure necessary to force gas out through the pneumatic duct averaged 28.1 mm Hg for 30 smolts. That this is an active constriction is indicated by the rapid decline in duct-release pressure following the death of the animal (Fig. 22). The duct-release pressure of "dibenzyline" blocked fish (26.8 mm Hg) did not differ significantly from control fish. Hence sphincter contraction was not maintained by sympathetic nerves as suggested by Kuiper (1916) and Franz (1937). Atropine on the other hand lowered duct-release pressure to an average of 0.2 mm Hg, indicating sphincter constriction was under cholinergic control. These

results agree closely with Fänge's demonstration of sphincter closure with acetylcholine.

Reviews of gas emission accompanying pressure reduction may be found in Jones and Marshall (1953) and Qutob (1962). The argument of Rabaud and Verrier with Guyénot and Plattner, as to the origin of gas emitted with pressure reduction, is discussed in the section on the effects of decompression on fish.

Anatomical basis of pressure perception

Hasse (1873) first suggested that the function of the Weberian ossicles was to acquaint fish with the varying amount of gas pressure within the swimbladder. Bridge and Haddon (1893) agreed with Hasse on the basis of their extensive anatomical studies and the experiments of Charbonnel-Salle (1887). Deineka (1904) examined the wall of the carp swimbladder and described nerve endings within the connective tissue. Eissele (1922) described the histology of the bladder wall of trout, perch, catfish and carp but did not stain specifically for nervous tissue. Evans (1925) found three layers of nerve cells and fibers in the roach swimbladder wall. Fibrils penetrated into an inner layer of elastic tissue and possibly to the epithelium. These being afferent fibers, Evans suggested this was a receptor organ for the perception of pressure changes. Dotterweich (1932) suggested the

ampullae of Lorenzini on the heads of sharks were hydrostatic-pressure organs, but as Dijkgraaf (1941) pointed out, there is no experimental data to support such a suggestion.

More recently four studies have appeared describing the innervation of the swimbladder. Scevola (1938) described it briefly in the carp and Stefanelli (1946) in more detail for the goldfish. The latter author concluded the afferent fibers would be stimulated through distortion of the bladder wall. Terio (1948) confirmed the presence in the bladder wall of numerous nerve ganglia and nerves of somatic, parasympathetic and sympathetic origin.

Abraham and Stammer (1954) described a double innervation in the wall of the carp swimbladder. They recognized thin, sympathetic nerves with bipolar ganglia and concluded these were vasomotor to the rich network of blood vessels. The thicker, vagal sensory-fibres terminated as pressure receptors in connective tissue of the submucosa. As Qutob (1962) observed, Abraham and Stammer have not demonstrated that these nerve endings are in fact pressure receptors.

Jones (1958) concluded there was no evidence for a pressure-sensory function in the swimbladder of physoclists. In physostomes such a function may be involved in the release of gas bubbles. In the Ostariophysi, the Weberian apparatus

may increase sensitivity of the swimbladder to changes in pressure and thus make possible better control of gas entry and loss.

It is noteworthy that other animals demonstrating a pressure sensitivity may or may not make use of a gas phase in doing so. Thus certain pressure-sensitive insects do (Thorpe and Crisp, 1946) and marine crustaceans do not utilize a gas bubbles in pressure perception, as shown by Enright (1961) for the amphipod Synchelidium. This form Enright found to be sensitive to 0.01 atmos, equal to a hydrostatic head of 4 in of water.

Neurophysiological studies

The pressure-receptor function of the swimbladder was demonstrated by Koshtojanz and Vassilenko (1937). They showed an increase in pressure in the carp swimbladder, equal to 60 to 80 mm of water, induced strong swimming movements by the fins plus reactions from respiratory muscles and heart. The same response was elicited through stimulation of the afferent vago-sympathicus nerve. Following section of the nerve, increased pressure in the bladder did not result in any of these responses. Vasilenko and Livanov (1936) extended the study of Koshtojanz and Vassilenko, recording the impulses transmitted along the vago-sympathicus. Presumably they continued to use carp and bladder pressure changes

were of the same order of magnitude. The recordings of Vassilenko and Livanov indicated rhythmic fluctuations in potential and these changed with pressure increase or decrease within the swimbladder. Increasing bladder pressure resulted in an increase in high-frequency fluctuations. The authors concluded the swimbladder was a receptive organ.

Long (1959) obtained changes in swimbladder volume equal to 10-15 mm Hg, through electrical stimulation of the tecti optici. These contractions were not due to the wall of the swimbladder but rather by means of the musculature of the body cavity. The findings of Long are not in agreement with Franz (1937) who showed gas emission in the minnow was due to bladder contraction. In the present study there appears little doubt that sockeye expell gas through a reduction in the cross-sectional area of the swimbladder.

Qutob (1962) explored the sensitivity of the swimbladder and pneumatic duct by probing the surface and recording action potentials along the vago-sympathicus. He concluded receptors of some kind were present along the wall of the duct and some areas of the swimbladder in Leuciscus. Qutob repeated the experiment of Vassilenko and Livanov, again measuring action potentials along the vago-sympathicus accompanying increase or decrease in pressure. Discharges increased during pressure increase but the frequency of these

impulses returned to normal when increase stopped. Pressure release temporarily depressed discharges, followed by the normal pattern. Qutob (1962) repeated these measurements on Scardinius exposed to pressure changes within a closed container. Pressure increase of 25 mm Hg or decrease of 18 mm Hg resulted in doubling and halving respectively the rate of spontaneous discharge. These altered rates gradually returned to the original level. Qutob concluded pressure perception is achieved via the swimbladder wall.

Response to changes in barometric pressure

Fish culturists have long been aware of the changes in the behavior of some fish accompanying changes in the weather. Bert (1873) acknowledged the sensitivity of fish to atmospheric pressure and reported Gobitus tenia may be kept in an aquarium and serve as a barometer. Sagemehl (1895) examined the swimbladder in a number of catfishes in which the bladder extended through the lateral musculature and terminated against the skin. He concluded that this arrangement would limit the usefulness of the bladder in a hydrostatic role but would increase its sensitivity for perception of pressure. Consequently Sagemehl advanced the hypothesis that the Weberian apparatus was not concerned with reporting pressure and hence depth to the fish, but rather with variations in atmospheric pressure which indicate weather

changes. Sagemehl reserved proof of this hypothesis for a later study on the Weberian apparatus. The author has been unable to locate any such subsequent study.

Philippsen (1913) presented a superficial but poetic account of fish responding to weather changes. He noted, for example that pike, eels, perch and bream like to bite before a thunderstorm, coincident with a fall in the barometer. Philippsen also reported the erratic behavior of flounders in estuaries of North Sea streams before poor weather or thunderstorms and associated this with low barometric pressure.

Scheuring (1922) examined the swimbladder studies of Müller, Weber, Hasse, Sagemehl, and others and concluded that the two-compartment bladder represented the beginning of a functional change. That is, a change from a more primitive, hydrostatic apparatus to a purely hydrostatic or better, barometric-perception organ. Scheuring noted the swimbladder apparatus of the Cobitidae would permit these fish to perceive the finest pressure variation and constitute "weather fish". He obtained specific reactions from these fish with pressure changes of 1/20 atmos.

Kyle (1926) noted fish such as loaches, with a "drum" apparatus connected to the swimbladder, are very sensitive to changes in atmospheric pressure and have been called

"weather fishes". He described anterior prolongations of the clupeid swimbladder extending into the semicircular canals and thus conveying differences in swimbladder pressure to the endolymph. Kyle concluded this apparatus served as a manometer or barometer.

Wunder (1936), in reviewing the functions of the swimbladder, repeated the general observation that certain fish react to barometric variations. He described as well known the sensitivity of sheat fish and cyprinids to barometric changes. Wunder added his personal observations of fish in aquaria rising from the bottom and swimming about actively on the approach of a thunderstorm. He was concerned with the biological value of this sensitivity of bottom fish to changes in barometric pressure and suggested it was associated with food through the response of insects.

Recently two studies have appeared in which the response of fish is related to barometric pressure. In each case the relationship is supported, unlike earlier observations, by data. Allen (1959) correlated the movement of chinook and coho salmon into the fishway (University of Washington) with eight environmental factors. Allen found a significant, negative correlation for the entry of 82 chinook salmon with barometric pressure; the value of -0.50 accounting for 25 per cent of the variability in the data. There are several

objections to Allen's treatment of the data. Firstly, it is not acceptable to conduct a large number of correlations and then attach importance to one which proves significant. The laws of chance permit occasional spurious correlations and the chances of a 'significant' result increase with the number of correlations attempted. Thus for eight correlations the possibility of obtaining 1 per cent significance is not 1:100 but rather 1:12 1/2. Secondly, the correlation is not limited to the period when fish were entering the fishway, but rather includes the 3 days of high barometric pressure before any fish entered. Thirdly, Allen has not made clear whether he considers all of the fish to be present when the first fish enters the fishway or whether the fish are arriving at the fishway in a normal distribution. If the former is the case, then the number of fish migrating must be weighted as to the number available to migrate in response to changes in barometric pressure. If the latter is the case, then barometric pressure should be correlated with the numbers migrating expressd as a proportion of the current accumulated number of fish available. Fourthly, while it is not completely clear from the text, it appears that Allen correlated barometric pressure and fish migration of the same day. The fish migrated during the early morning hours and barometric pressure was recorded at 0400, 1000, 1600 and 2200 hr and averaged.

Thus the correlation would be fish movement with barometric pressure in the succeeding 24 hr. Fifthly, barometric pressure does not fluctuate randomly from one observation to the next and Allen does not appear to have eliminated this effect of barometric pressure in determining subsequent barometric pressure. As Moran (1949) pointed out, this invalidates the use of "simple correlation". Sixthly, Allen does not described the demands which interpretation of pressure change place on the fish. For example the peak migration followed a fall in barometric pressure of 0.21 in Hg or a reduction of 3 in or less in hydrostatic head. How does the fish measure this? Because of the effects of waves, tides, seiches, etc., pressure measurements of such small magnitude could not be made at depth, but would have to be made at the surface with the fish some fixed distance below the meniscus. Also the fish would require an absolute pressure sense on which to base a comparison. Response to falling barometric pressure places even greater demands on the fish, that is, measuring the rate of change in pressure. Thus for Allen's data, the pressure decrease of 0.21 in Hg took place over 42 hr, requiring the fish to measure a rate of reduction of 0.006 ft water per hr. That trout or salmon are capable of doing so has yet to be demonstrated experimentally. The available experimental evidence, to be sure,

is to the contrary. Dijkgraaf (1943) showed blinded trout could be conditioned to changes in hydrostatic pressure of 10 to 15 cm of water, providing the fish was in physical contact with the sides or bottom of the aquarium. Free-swimming trout could not be conditioned to changes in pressure. Dijkgraaf concluded the trout lacked a sense of hydrostatic pressure.

More recently, Westman and Hoff (1962) examined angling results for the bluefish (Pomatomus saltatrix), the fluke (Paralichthys dentatus) and the porgy (Stenotomus chyrsops) in the New York Bight. The authors concluded that in all cases a singificantly greater number of fish per angler were caught during days of below-average barometric pressure than on days of above-average pressure. An objection must be raised here to what Westman and Hoff termed 'average' barometric pressure. In this case they chose 29.92 in Hg, the long-term meteorological average at sea level. This had the effect of separating the data for the bluefish into 9 values of below and 43 above average. This implies 29.92 in Hg has some significance to the fish. Separating catch per angler on the basis of the average barometric pressure during the period of study (cf Westman and Hoff) no difference was found between above and below-average barometric pressure days ($t = 0.58$, $t_{0.50} = 0.69$).

This was found also to be the case with the fluke and porgy catches per angler. The significant differences claimed by Westman and Hoff, using the lop-sided distribution about 29.92, are not significant for the average pressure over the period of study ($t = 1.66$, $t_{0.05} = 2.03$).

In summary, the oft-repeated observations of fish responding to change in barometric pressure leaves little doubt that this may be possible for certain species, particularly those with a Weberian apparatus and living in shallow aquaria. As yet, however, there appear to be no quantitative measurements to establish this phenomenon beyond doubt.

Perception of the small and gradual variations in barometric pressure places important demands on fish. In view of this, considerable caution is warranted in granting all species of fish the ability to respond to the slight changes in pressure taking place in the atmosphere.

An examination of the problem of fish responding to barometric pressure may be found in Bridge and Haddon (1893).

Pressure and Compensatory Swimming

Fishes with a swimbladder may undergo an appreciable change in density with change in pressure. Pressure increase results in compression of bladder gas, increased density and

a tendency for the fish to sink, which is countered through the fish swimming upward. The converse is true of a reduction in pressure. Baglioni (1908) noted this swimming response in fish he pressurized experimentally. He concluded the swimbladder wall was the sense-organ reflexly controlling the described swimming movements. He based this opinion on the knowledge that the swimbladder, being a gas-filled sac, would undergo volume variations in relation to pressure, and secondly Deineka (1904) had demonstrated already the presence of nerve endings in the bladder wall. As Jones (1958) remarked, this is a very reasonable hypothesis but there is little evidence to support it.

Kuiper (1915) adapted a perch to a pressure decrease of 10 cm Hg. Returned to atmospheric pressure, such a fish then swims upward to avoid sinking. With an attached cork the fish was drawn to the surface and attempted to swim downward, the opposite direction which would be expected on the basis of Baglioni's hypothesis. Qutob (1962) quotes Kuiper (1914) as having demonstrated that carp, tench, perch and loach show a fin response on pressure decrease which occurred before passive displacement. This of course would tend to support Baglioni's hypothesis.

Remotti (1924) claimed to have proved the hypothesis of Baglioni that tension in the bladder wall controlled reflexly

the swimming activity. Remotti emptied physoclist swimbladders of air and refilled them with paraffin oil or water. With an excess of pressure within the bladder, these fish swam downward when of course they ought to have swum upward. Remotti increased the hydrostatic pressure and enhanced the downward swimming response, which result he attributed to increased nerve impulses following bladder expansion. Franz (1937) noted this contradiction, pointing out any change in response would require the swimbladder to be partly filled with gas initially (presumably because of the incompressible nature of liquids). If there was in fact gas within the bladder, Franz continued, then increased pressure would decrease the volume, relax the wall of the bladder and thus induce the fish to swim upward. Franz offered the explanation that the downward swimming observed by Remotti was a flight response. Franz concluded Remotti's experiments scarcely can be considered as support for Baglioni's hypothesis.

Franz (1937) quoted numerous authors who removed swimbladder gas or the bladder itself and still observed normal swimming movements; he concluded that the swimbladder alone could not be responsible for compensatory swimming. Franz decompressed perch experimentally and recorded the pressure reduction necessary to evoke compensatory swimming,

then weighted the same fish and repeated the decompression. Following weighting, compensatory swimming commenced at a much greater reduction in pressure and hence greater expansion of the bladder. Franz felt this argued against perception by way of the bladder wall. Switching to minnows, Franz adapted the fish to a pressure reduction of 400 mm Hg,these physostomous fish expelling gas to achieve buoyancy. When atmospheric pressure was restored the fish were too dense but were rendered buoyant by air injected into the body cavity. Thus in spite of the swimbladder volume being greatly diminished the fish evidenced no compensatory swimming or tendency to gulp air at the surface. Franz concluded this ruled out the swimbladder wall of the minnow as well as the perch as the site of perception involved in compensatory swimming.

Koshtojanz and Vassilenko (1937) recorded compensatory-swimming movements in response to pressure increases in the swimbladder of carp. They describe the downward movement of the tail and rotary movement of the pectorals, typical of fish attempting to swim upward. Jones and Marshall (1953) pointed out the inconsistency in these observations. An increase in bladder pressure would be perceived by the fish as a decrease in hydrostatic pressure and hence the fish should tend to swim upward. Jones and Marshall

noted the fish were inverted and out of water and stimuli from the labyrinth may have influenced the response.

Compensatory swimming accompanying a reduction in pressure was quantified by Jones (1952) for the perch. The reduction in pressure to which these fish were able to compensate provided Jones with a measure of the upward movement such fish can make without being buoyed to the surface. Jones noted unpublished experiments indicating perch do not respond to the stretching of the bladder wall nor did blinding abolish compensatory swimming. Jones concluded this left the labyrinth, sense organs of the skin or the lateral line as sites of stimulation.

McCutcheon (1958) described compensatory swimming in the pinfish (Lagodon rhomboides) for both increased and decreased buoyancy. Due to the position of the swimbladder in this fish, pressure reduction resulted in the head tilting upward. Compensatory movement of the pectoral fins coincided with a tilt of 5 to 15°, rapid decompression evoking a response at a lesser angle that a gradual reduction in pressure. At neutral buoyancy pectoral fin beats were nil, but on decompression rose to a maximum of 88 per min with an increased amplitude. Increased pressure and hence negative buoyancy induced pectoral rates as high as 114 per min with the direction of pectoral thrusts opposite that with positive

buoyancy. Fish in hydrostatic equilibrium and resting on the bottom commenced compensatory movements of the pectoral fins with a pressure change of 1-2 cm water and before buoyancy drift. Changes in swimbladder volume suggested a behavioral sensitivity to pressure of 14.5 cm water. The high sensitivity of bottom-resting fish may be the result of tactile stimuli, although McCutcheon appears to favor tension receptors in the bladder. McCutcheon (1958, 1962) describes small volume changes in the swimbladder of the pinfish resulting from muscular contraction and which he terms "compensatory compensation".

In the present study, the results of the investigation into compensatory swimming in young sockeye are discussed in relation to the behavior and vertical distribution of this fish.

Pressure conditioning of fish

Dijkgraaf (1941) was able to train the minnow, Phoxinus laevis either to an increase or decrease in pressure of 0.5 to 1.0 cm of water. This response was lost following removal of the mallei bilaterally, with the minnows failing to respond to pressure increases of as much as 40 cm of water. Dijkgraaf concluded the Weberian apparatus serves a hydrostatic function through perception of pressure change, in

addition to the accoustical function described by Frisch and Stetter (1932) and others. Dijkgraaf (1943) attempted to condition the physostomous trout to changes in hydrostatic pressure. These fish responded to pressure increase or decrease of 10 to 15 cm of water only if they were in physical contact with some fixed object. Free-swimming trout failed to respond to changes in pressure. Dijkgraaf concluded trout lack a true hydrostatic pressure sense, as he had expected, trout lacking the Weberian apparatus essential to pressure sense. Qutob (1962) trained blinded minnows (P. laevis) without mallei to respond to pressure changes of as little as 7 to 8 cm of water. Based on the behavior of the fish, Qutob believed the threshhold of perception of pressure change was 5 to 6 cm of water. This value agrees closely with the pressure change of 5 cm of water necessary to elicit a spontaneous response in non-conditioned fish. Comparing his results on operated minnows with those of Dijkgraaf on intact fish, Qutob concluded the Weberian apparatus made possible perception of changes in pressure of 0.5 or 1 to 5 cm of water. He concluded, nevertheless that the Weberian apparatus was not involved essentially in the maintenance of hydrostatic equilibrium through perception of changes in hydrostatic pressure.

Similar pressure conditioning experiments have yet to be described for any of the species of Oncorhynchus. However, the inability of trout to train to a pressure change over as many as 131 tests, as reported by Dijkgraaf (1942), casts doubt on the possibility that Pacific salmon are capable of detecting small changes in pressure.

The Effects on Fish of Positive and Negative Pressure

The effects of positive pressure

That some fishes are tolerant of high pressure is obvious from the great depths at which fish have been recorded. For example, Marshall (1960) examined fish from a depth of 5,600 m which is equivalent to a pressure of approximately 8,500 psi. Experimental evidence suggests pressure tolerance is not constant, but varies among species of fish. Bert (1873) placed eels under a pressure of 10 to 15 atmos but observed their death only under conditions of increased oxygen content. From the brief description of his experiments it is doubtful that pressure per se caused the death of the fish. Carbonnier (1873) examined the depths and hence pressures inhabited by freshwater fish. The Chicago Field (1879) carried an anonymous report of trout passing through an inverted siphon "without inconveniences" under a maximum pressure of 376 psi. Regnard (1884a)

observed fish could be subjected to 100 atmos with impunity if lacking a swimbladder or if the bladder has been emptied. A pressure of 200 atmos induced a reversible narcosis, and 300 atmos caused death. Regnard (1884b) extended his observations to marine fish and demonstrated narcosis in flatfish after 10 min exposure to high pressures, prolonged to an hour, death resulted. Gorham (1899), investigated the causes of gas-bubble disease in fish and described the pressures at which deep-sea fishes live. Melamphaes beanii, for example was found at a depth of 2,949 fathoms and hence a pressure of 3.9 tons per sq in. Fontaine (1929 a and b) investigated the effect of pressure on oxygen consumption by Pleuronectes platessa. Oxygen consumption increased 58 per cent at a pressure of 100 kg per cm (97 atmos) and fish were killed at 145 atmos. Ebbecke (1935) studied the effect of high pressure on a variety of marine animals. The rock-fish (Gobius niger) became more active at 50 atmos swam wildly at 100 atmos and soon died at a pressure of 200 atmos. A pressure of 500 atmos immediately killed the rock-fish, flounder (P. platessa) and stickleback (Spinachia vulgaris). The fish were stiff and crooked, the gills red, flared and congested. Ebbecke suggested respiratory failure was due to disturbance of the central nervous system and stiffening was due to direct muscle stimulation. Very brief and incomplete reviews

of the effects of pressure on fish may be found in Cattell (1936) and Hoff (1948). Hubbs and Rechnitzer (1952) in studying the effect of explosion-induced pressures on fish, subjected "fish" to positive pressures of 68 atmos briefly and 34 atmos for 12 hr with little immediate and no lasting effect. Rowleg (1955) exposed rainbow trout to hydrostatic pressures ranging from 50 to 200 psi for periods up to 48 sec. The fish were immobilized under pressure but resumed activity after decompression and showed no deterimental effects during post-test observation. Muir (1959) in studying the effects of pressure and decompression on coho fingerlings, subjected fish to pressures of up to 110 psi, apparently without ill effect.

Recently three studies have appeared by Bishai (1961 a,b,c) reporting the effects of pressure on fish. Bishai (1961a) after experimenting with Salmo salar and S. trutta concluded the effects of pressure depended on the age of the fish. Yolk-sac alevins tolerated 5 atmos. Fingerlings of both species, at an age of 98 to 179 days, died within 24 hr when subjected to an increase in pressure of more than 15 cm Hg. This observation by Bishai was based on seven experiments totalling eleven fish and hence requires verification. No suggestion was made as to the mechanism of action of such slight (1/5 atmos) increase of pressure in causing

the death of the fish. Bishai (1961 b and c) was able to double, without effect, the pressure to which various Nile fishes were adapted.

In the behavior portion of the present study, young sockeye salmon were exposed to 300 psi (20 atmos) as emergent fry and again at 3-month intervals during their first year in fresh water. No lethal-pressure period was found such as described by Bishai (1961a) for the Atlantic salmon and brown trout. The series of tests in which sockeye smolts were placed under a pressure of 50 or 300 psi then released to atmospheric pressure resulted in a mortality similar to that of the control groups (Fig. 31 and 32). Thus little or no adverse effect may be associated with brief exposure to these relatively high pressures.

The effects of negative pressures

The study of decompression probably began with the classical experiments of Robert Boyle on pressure and volume, respiration and combustion. Boyle (1670) reduced the pressure on freshly drawn blood and noted the gas evolved. He questioned whether the same was true of animals subjected to rapid decompression:

> Note, that the two foregoing Experiments were made with an Eye cast upon the inquiry, that I thought might be made; Whether, and how far the destructive operation of our Engin upon the included Animal, might be

> imputed to this, that upon the withdrawing of the Air, besides the removal of what the Airs presence contributes to life, the little bubbles generated upon the absence of the Air in the Bloud, juyces, and soft parts of the Body, may by their Vast number, and their conspiring distention, variously streighten in some places, and stretch in others, the Vessels, especially the smaller ones, that convey the Bloud and Nourishment; and so by choaking up some passages, and vitiating the figure of others, disturb or hinder the due circulation of the Bloud?

Boyle decompressed small animals and observed the appearance of gas bubbles, thus confirming his suspicions.

Since Boyle, an extensive medical literature on decompression has grown out of the injuries and mortalities accompanying the use of caissons, diving gear and in connection with high-altitude aviation. This has been reviewed in detail by Hoff (1948). Two decompression effects in humans are common in principle to fish and hence are described here. "Lung burst" may result from the expansion of lung gas on decompression. In this, alveoli are distended to the point of rupture and air bubbles may enter broken blood vessels causing air embolism (Duffner, 1960). Jones and Marshall (1953) suggested this was a possible explanation of the symptoms shown by fish raised to the surface from some depth. That is, blood vessels of the swimbladder may rupture with distention and gas bubbles enter the blood stream. The second analogous category of decompression effects is that of excess nitrogen leaving solution within the animal and forming embolisms.

The associated pain and injury is known as "the bends" or caisson disease (Behnke, 1955). Jones and Marshall examined this possibility also and suggested the blood could become supersaturated with swimbladder gas by way of the oval and that subsequent pressure reduction would result in gas leaving solution and forming emboli.

Historically, the effects of decompression on fish have been investigated by a number of workers. Bert (1873) noted eels decompressed after 3 days at 10 atmos soon died. This he attributed to gas leaving solution in the blood and filling the heart. Regnard (1884a) reported fish lacking a swimbladder or first having the bladder emptied can be submitted to a pressure of 100 atmos and decompressed with impunity. He observed, however, when this precaution was not taken the gas within the swimbladder dissolved in the blood under this high pressure and was liberated on decompression. The resulting foam stopped the circulation and the animal died.

Gorham (1898, 1899) investigated the cause of gas disease in marine fishes taken from deep water and placed in an aquarium. He postulated the condition was due to swimbladder gas being forced out into the tissues or to dissolved gases of the tissues leaving solution. Gorham was able to induce the symptoms through decompression and aleviate the disease by holding afflicted fish under a pressure of 20 ft of

water. Gorham concluded the disease was understandable in terms of simple physical laws.

Progress in the understanding of the effects of decompression was interrupted by the work of Rabaud and Verrier. Verrier (1931) disputed the swimbladder was of value in swimming by experiments on bladder extirpation in carp, tench and catfish. She ligatured the pneumatic duct of two carp and two tench, making, as she described it, physoclists from physostomes; when decompressed these fish continued to release gas. These results led Verrier to suggest the swimbladder was not involved in the response to variations in pressure, in equilibrium and in vertical displacement as was believed generally. These studies were repeated with the same result on other species (Rabaud and Verrier, 1931), on fish from which the swimbladder had been removed (Rabaud and Verrier, 1934) and on bladderless fish (Rabaud and Verrier, 1932,1934). Faced with the difficulty of explaining the damage along the junction of swimbladder and musculature, Rabaud and Verrier (1935) concluded gas diffused through the wall of the swimbladder, filled the abdominal cavity and thus stretched the lateral musculature on decompression.

The ligaturing of the pneumatic duct was promptly repeated by Meierhans (1935 a,b), who found no such escape of gas on decompression, but carp and roach in which the

duct was not tied released bladder gas on slight reduction in pressure. In this latter case the bladder was partially deflated when examined. Meierhans was of the opinion that the bubbles appearing on the surface of the fish in the decompression experiments of Rabaud and Verrier did not originate from the tissues and blood but represented rather gas leaving solution under reduced pressure.

Plattner (1937) also repeated these experiments and concluded gas bubbles did originate from the swimbladder and passed out through the pneumatic duct. No bubbles were emitted when the duct was ligatured and the bladder was deflated following decompression if the duct was not ligatured (Plattner, 1938b). Guyénot and Plattner (1938, 1939) raised a number of objections to the experimental procedures of Rabaud and Verrier particularly in respect to the removal of the swimbladder and the tendency of fish to swallow air into the body cavity following such an operation. Rabaud and Verrier (1939) replied to Guyénot and Plattner but the latter authors had the last word in the review of swimbladder function by Plattner (1941). This debate as to the function of the swimbladder has been reviewed in detail by Jones and Marshall (1953).

Baudin (1937a) described an increase in erythrocyte number in the perch, accompanying slight decompression while

oxygen content was held constant. Baudin (1937b) related this to changes in swimbladder pressure and was able to induce the erythrocyte change by altering the volume of bladder gas. As yet there is no explanation of these results. Brown (1939) investigated the effect of decompression on swimbladder inflation in the guppy. He found that below a critical range, decompression favored the diffusion of gas into the swimbladder, resulting in bloating. Hogan (1940) examined fish which had passed through a siphon at a vacuum of 18 to 26 in Hg for 30 sec. Physostomous carp, catfish, minnows and gar suffered little or no damage but physoclistous crappies, bass and bluegills evidenced ruptured bladders and hemorrhages.

Jones (1949) reported the frequency of ruptured swimbladders among perch trapped at depths to 60 ft, then rapidly decompressed to atmospheric conditions. This was followed (Jones, 1951) by a discussion of the restrictions a swimbladder imposes on the vertical movements of a fish and expressed in an equation as the rate at which a physoclist is able to decompress without injury. Jones (1952) quantified these restrictions for the perch and showed that this fish was restricted to a narrow zone above its plane of neutral buoyancy equal to a one-sixth reduction in pressure.

Recently Bishai (1961a) reported the effects of decompression on young salmonids. He concluded alevins are

unaffected by decompression, the swimbladder being incompletely developed at this stage. At an age of 56 days, decompression did not result in gas emission and the bladder bloated. This Bishai attributed to incomplete development of the sphincter mechanism. Death of the young salmonids was associated with the gaseous supersaturation of the experimental water, bubbles of gas externally covering the fish and causing asphyxia. Bishai raised the possibility of true decompression sickness causing the death of the fish. Critically examining Bishai's study, a number of objections must be raised to the procedure employed. Fish were held in static water and pressure was maintained by means of an air lock over the water. This had the effect of supersaturating the water, relative to atmospheric pressure. Bishai occasionally measured the oxygen content but this would provide a poor approximation of nitrogen saturation (Harvey and Smith, 1961). While under pressure the fish would tend to come to equilibrium internally with the nitrogen content of their environment.

Thus on decompression there would be the marked tendency of the now unstable nitrogen to leave solution within the fish, coalesce to form bubbles and ultimately appear as emboli. Also inherent in the design of the experiment was the residence of the fish in supersaturated water on decompression. This leads, although seldom very rapidly, to the

classical symptoms of gas disease (Marsh and Gorham, 1905, reviewed by Harvey and Smith, 1961). Thus Bishai's fish could have been afflicted in this way. Yet another cause of gas disease is that already referenced to Jones and Marshall (1953), that is, the entry of swimbladder gas into the circulation of the fish on decompression either by diffusion while in solution or directly as bubbles entering ruptured vessels. In view of the air lock available under pressure and the tendency of such physostomes to inflate their swimbladders to neutral buoyancy, the latter explanation appears the most likely for Bishai's results. Bishai (1961) repeated the decompression studies on several Nile River fishes and in this series he attributed the death of the fish to gas disease brought on by the supersaturated water at the end of decompression. The symptoms accompanying death, darting and bending, resemble much more those accompanying emboli originating from swimbladder gas.

Cyclical pressure changes

Under certain conditions fish may be subjected to both pressure increase and decrease within a short period of time. This occurs during the passage of fish through hydro-electric turbines and where fish are in close proximity to underwater explosions. That these cyclical pressure changes may be harmful to fish is well known. However, the complexity of the

pressure increase and decrease and other pressure-related phenomena, such as cavitation, make difficult the isolation of the deleterious component. In the subsequent two sections the literature is reviewed for fish passage through turbines and underwater explosions and discussed in relation to the effects of pressure increase and decrease on fish, as found in the present study.

Discussion here will be limited to the reaction-type turbines, as opposed to impulse turbines, passage of fish is not a consideration with the latter. Generally forebay water enters the penstock at some appreciable depth below the surface. The penstock leads downward terminating at the runner under conditions of maximum positive pressure. Pressure falls rapidly as the water passes across the blade of the runner, the decrease taking place in a fraction of a second. Within the draft tube below the runner the pressure may be above or below atmospheric depending on the height of the tailrace water. Thus fish passing through turbines may be subjected to very different conditions of pressure increase and decrease, depending on:

i. the height of the dam and hence depth of the reservoir.

ii. the depth of the penstock below the surface.

iii. the magnitude of the positive or negative pressure in the draft tube.

iv. the efficiency at which the turbine is operating.

v. the propensity of the design to produce cavitation.

In addition the effects of these factors will vary depending on the condition of the fish:

i. whether the fish are physostomous or physoclistous.

ii. whether the fish were residing at depth or had sounded from the surface to enter the penstock.

iii. the degree of swimbladder inflation.

iv. the dissolved gas content of the fish passing through the turbine.

v. for species of physostomous fish, the morphology of the pneumatic duct and its relative patency for gas emission.

vi. the age, size and relative healthiness of the fish.

The increase in positive pressure accompanying passage through the penstock has seldom been considered of importance to the fish. Thus for low-head dams of 100 ft or so, the depth and pressure, would be in keeping with the known vertical distribution of species such as sockeye. Schoeneman and Junge (1954) assessed the mortality at a 100 ft (lower Elwha) and 194 ft (Glines) dam. The latter showed a mortality of 33 per cent compared to nil for the former, other conditions being relatively equal. The authors concluded the

difference in survival rates was a function of hydrostatic head and hence pressure differences, the critical head being between 100 and 200 ft. In support of Schoeneman and Junge, measurements at low-head dams (Big Cliff, Bonneville, McNary) have shown relatively low mortalities, whereas studies at high dams (Baker, Puntledge, Shasta) have evidenced mortalities similar to that of the Glines. In the present study fish subjected to 680 ft of hydrostatic head (Fig. 31) and 100 ft of head (Fig. 32), then released to above or below atmospheric pressure, showed very similar effects and losses. It is concluded therefore that some factor associated with hydrostatic head, but other than positive pressure, is responsible for the variable death of fish in the turbines of low and high-head dams.

That negative pressures in the draft tube could injure or kill fish is apparent from the literature on the effects of decompression on fish (Bert, 1873; Gorham, 1898; Baudin, 1937; Hogan, 1941). Experimentally this was shown to be the case by Cramer (1960) in model studies using physoclistous smallmouth bass. Mortalities increased as draft-tube pressures were reduced below atmospheric. Muir (1959) on exposing fingerling coho salmon to a sudden reduction in pressure from as high as 110 psi to a 29 in Hg vacuum, found little mortality. He concluded from this significant

mortality is unlikely to result from exposure to a partial vacuum in the absence of cavitation. Corps of Engineers (1960) decompression tests on salmon fingerlings resulted in "heavy mortality" only if the pressure reduction was rapid and the fish were allowed an air lock under pressure. Such fish were described as being "pressure accommodated". From witnessing a subsequent test it was apparent that the fingerling salmon were inflating their swimbladders to neutral buoyancy with atmospheric air while under pressure. On sudden reduction in pressure such fish were unable to emit the then excess gas. The result was the rapid death of the fish, probably due to embolisms resulting from swimbladder gas passing into the tissues or ruptured blood vessels, as described by Jones and Marshall (1953). In addition the water containing the fish was greatly in excess of air saturation. The fish would be in gaseous equilibrium with this water and on pressure reduction would become supersaturated temporarily. Thus the fish could have died from gas disease or "the bends" quite independent of swimbladder gas. The present study has shown decompression may or may not have serious consequences, depending on the magnitude of the vacuum, the condition of the fish and physical factors in the environment of the fish.

An aspect of pressure changes within turbines is the phenomenon of cavitation. In this condition, water-vapor

bubbles form, then collapse or implode, primarily in the area of low pressure along the runner. This is accentuated by a negative pressure in the draft tube. Experimental cavitation studies by the Corps of Engineers (1957), employing a venturi with pressures as low as a 28 1/2 in Hg vacuum, showed a maximal mortality of 8 per cent. Muir (1959), on the results of his experimental studies of decompression and cavitation, was led to conclude that cavitation in turbines was the more important factor involved in fish mortalities. Cavitation tends to increase as runner efficiency declines, leading to the common belief that Kaplan (adjustable blade) turbines would result in lower mortalities than the Francis type. Cramer's (1960) model studies showed no difference in mortalities between the two types. Cramer and Oligher (1961) concluded (in so far as pressure considerations are concerned) the adverse effects of turbines could be minimized by operation at high efficiency and having the runner submerged in the tailwater. This would reduce cavitation on the runner and negative pressures in the draft tube.

Physical and biological factors involved in the mortality of fish in relation to dams have been analyzed recently by Andrew and Geen (1960) and Lucas (1962)

Underwater explosions may be considered a special case of pressure and pressure change and are discussed here

only insofar as they apply to the effects of pressure on fish. The complex physics of underwater explosions has been described in detail by Cole (1948). The magnitude of the pressure wave depends on the size and nature of the explosion and diminishes as the third power of the distance from the center. The wave of rarefaction is more marked close to the water surface. Gowanloch and McDougall (1946) measured a compression-wave duration of five one-thousands of a second followed by a negative-pressure phase of twenty-five one-thousands of a second. These authors concluded damage occurs at a critical pressure of 500 psi, when this wave crosses the phase boundary of fluid tissues to swimbladder gas. Under these conditions "shredding" takes place and results in hemorrhagic lesions. Alpin (1947) confirmed the rupturing of the bladder and noted bladderless sculpins were unaffected by underwater explosions. Fitch and Young (1948) concluded each species of fish has a specific resistance to shock pressure. Barracuda have a cylindrical body, a tough, heavy-walled swimbladder and have more resistance to pressure than laterally compressed fish, such as the saltwater perch, with a thin-walled bladder. Coker and Hollis (1950) examined fish injured or killed in marine underwater explosions and invariably found the swimbladder ruptured with some degree of vascular hemorrhage within the bladder. Hubbs and Rechnitzer (1952)

established the lethal threshhold for dynamite explosions at 40 to 70 psi, the lower threshhold being due to the very rapid rate of pressure change. Fish mortalities were greater close to the water surface, possibly due to the rarefaction wave. This is formed as a compression wave arrives at the air-water interface, is reflected downward and soon cancelled by the increasing hydrostatic pressure.

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APPENDICES

APPENDIX A

Theoretical Considerations

The solubility of a gas in a liquid has a finite value dependent on the nature of the gas, of the liquid and on the pressure and temperature. The solubility of a gas is usually expressed as the absorption coefficient, which is defined as the volume of gas, reduced at 0°C and 1 atmos dissolved by unit volume of solvent at the temperature of the experiment and under a partial pressure of the gas of 1 atmos (Gladstone, 1946). Where V_D is the volume of dissolved gas reduced to conditions of standard temperature and pressure, V is the volume of the liquid and P is the partial pressure of the gas in atmospheres, then the absorption coefficient (α) is equal to

$$\alpha = \frac{V_D}{V \quad P}$$

Pressure is the most important factor affecting the solubility of gases. This is stated in Henry's Law: the mass of gas dissolved by a given volume of solvent, for a constant temperature is proportional to the pressure of the gas with which it is inequilibrium. This may be expressed by the formula

$$M = k \times P$$

where M is the mass of gas dissolved by unit volume of liquid,

k is a constant and

P is the pressure in atmospheres.

The effect of pressure in reducing oxygen saturation is shown in Fig. 46.

Temperature is another factor influencing the solubility of gases in liquids. In the case of oxygen and nitrogen, solubility in water decreases rapidly with increasing temperature. The change in solubility is not quite linear as shown in Fig. 47, nor is it the same for the two gases.

The partial pressure of a gas is defined as the pressure each gas would exert if it alone occupied the volume of a mixture of gases at the same temperature. This is expressed in Dalton's Law of partial pressures: the total pressure of a mixture of gases is equal to the sum of the partial pressures of the constituent gases. Dalton showed that in a mixture of gases each constituent dissolves according to its own partial pressure, or simply that Henry's Law applies to each gas independent of the pressure of the other gases. For dry air free of carbon dioxide and a total pressure of 760 mm Hg, the fractional composition and partial pressures of the principle gases are:

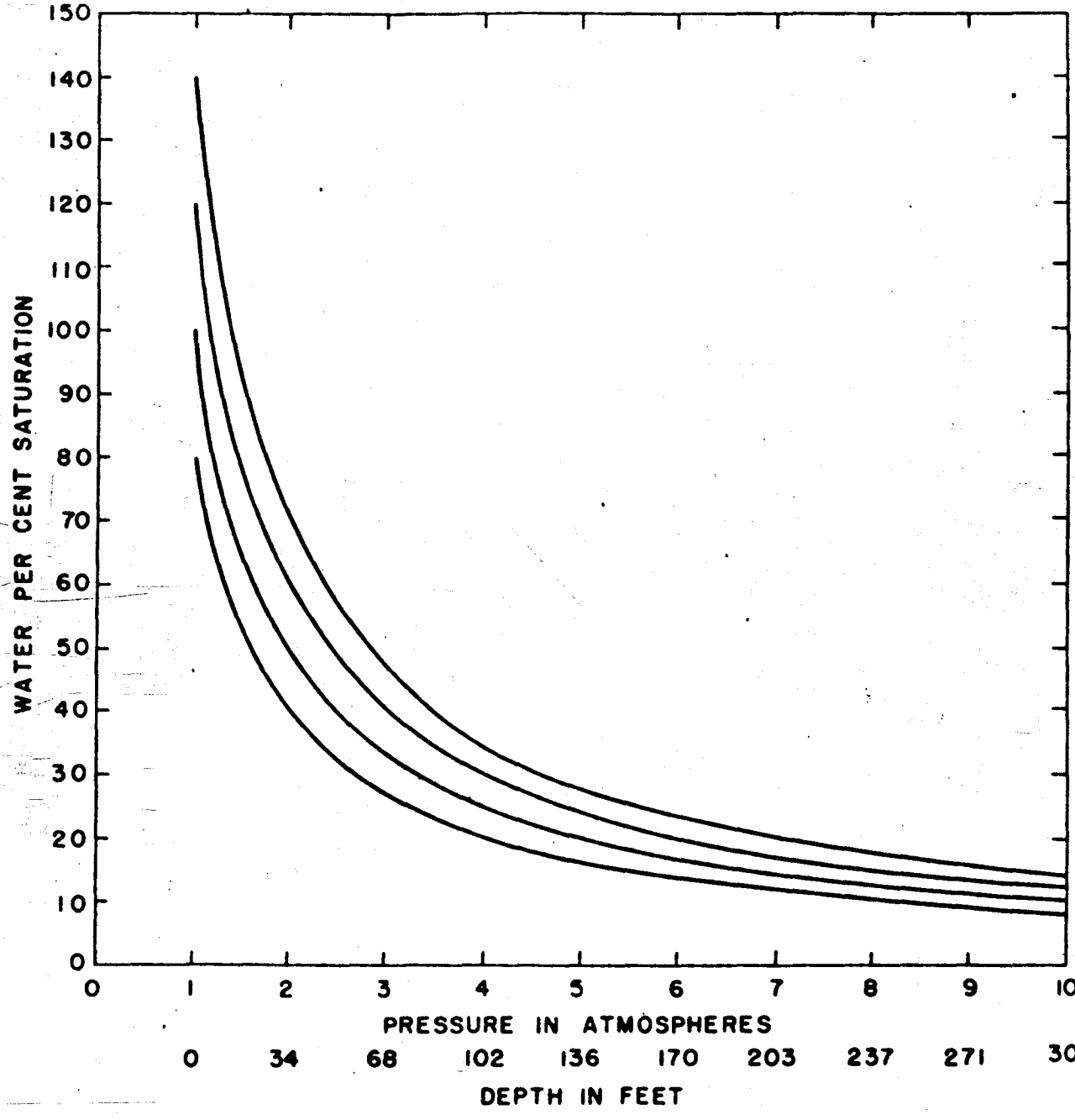


Figure 46. Per cent saturation in relation to pressure and depth. Absolute saturation of Ricker (1934).

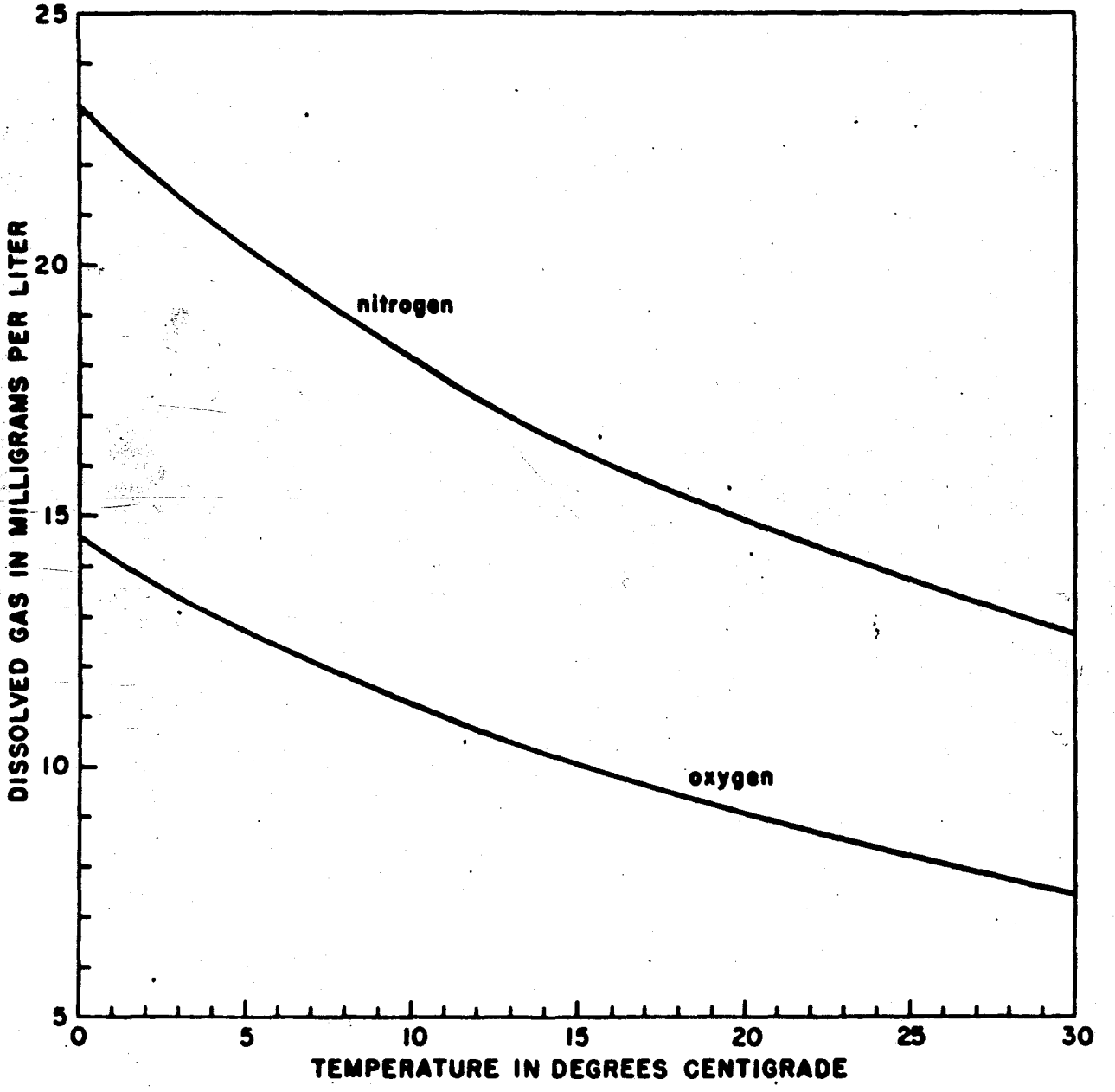


Figure 47. Oxygen and nitrogen content of air-equilibrated water, 0 to 30°C.

nitrogen	0.7811	593.6 mm Hg
oxygen	0.2095	159.2 mm Hg
argon	0.0094	7.2 mm Hg

These gases dissolve in relation to solubilities, as expressed by the absorption coefficients and in relation to the partial pressures. Thus at a temperature of 0°C, the three gases will enter solution in water from air in the ratios of:

nitrogen	0.7811 x 0.02354 = 0.018387
oxygen	0.209 x 0.04889 = 0.010242
argon	0.0094 x 0.0578 = 0.000543

Thus oxygen, which is only one-quarter as plentiful as nitrogen in air, being twice as soluble as nitrogen, becomes one-half as plentiful as nitrogen in water which is in equilibrium with a large volume of air. Hence the percentage composition of oxygen increases from 21 per cent in air to approximately 34 per cent in water. Hereafter argon will not be treated separately but rather included with nitrogen, as is commonly done, under the term "atmospheric nitrogen" consisting by volume of 98.815 per cent nitrogen and 1.185 per cent argon (Handbook of Chemistry and Physics, 1958). The air fraction of "atmospheric nitrogen" becomes 0.7905 and exerts a partial pressure of 600.8 mm Hg.

In considering a gas or mixture of gases in equilibrium with a solvent it is necessary to have regard for the solvent

which is in the gaseous state. In the case of air over water, the total pressure of the mixture of gases is equal to the partial pressures of the individual gases plus the partial pressure of the water vapor. At any given temperature the pressure of the water vapor in equilibrium with liquid water is a constant quantity, termed the aqueous or water vapor pressure. The vapor pressure of a liquid increases with rising temperature and the increase becomes rapid as the temperature approaches the boiling point. The aqueous vapor pressure over water is given for temperature from 0 to 100°C in Table VII. At 0°C water vapor pressure is 4.6 mm Hg or 0.6 per cent of 1 atmos, thus failing to correct for water vapor pressure would invoke an error of 0.6 per cent at this temperature. At 25°C the water vapor pressure is 23.8 mm Hg and constitutes 3.1 per cent of atmospheric pressure. Thus an appreciable error results if water vapor pressure is not taken into account in calculating gas tensions.

In the present study, oxygen and nitrogen solubilities were calculated from the absorption (Bunsen) coefficients (α) as found in the Handbook of Chemistry and Physics. The oxygen fraction of air was taken as 0.2095 as recommended by Hutchinson (1957). Correcting for water vapor pressure the solubility of oxygen is air-equilibrated water was calculated for each 0.1°C from 0 to 30°C. Thus oxygen

TABLE VII

Water vapor pressure in mm Hg for temperatures of 0 to 100 degrees Centigrade

Temp.	W.V.P.	Temp.	W.V.P.	Temp.	W.V.P.	Temp.	W.V.P.
0	4.6						
1	4.9	26	25.2	51	97.2	76	301.4
2	5.3	27	26.7	52	102.1	77	314.1
3	5.7	28	28.3	53	107.2	78	327.3
4	6.1	29	30.0	54	112.5	79	341.0
5	6.5	30	31.8	55	118.0	80	355.1
6	7.0	31	33.7	56	123.8	81	369.7
7	7.5	32	35.7	57	129.8	82	384.9
8	8.0	33	37.7	58	136.1	83	400.6
9	8.6	34	39.9	59	142.6	84	416.8
10	9.2	35	42.2	60	149.4	85	433.6
11	9.8	36	44.6	61	156.4	86	450.9
12	10.5	37	47.1	62	163.8	87	468.7
13	11.2	38	49.7	63	171.4	88	487.1
14	12.0	39	52.4	64	179.3	89	506.1
15	12.8	40	55.3	65	187.5	90	525.8
16	13.6	41	58.3	66	196.1	91	546.1
17	14.5	42	61.5	67	205.0	92	567.0
18	15.5	43	64.8	68	214.2	93	588.6
19	16.5	44	68.3	69	223.7	94	610.9
20	17.5	45	71.9	70	233.7	95	633.9
21	18.7	46	75.7	71	243.9	96	657.6
22	19.8	47	79.6	72	254.6	97	682.1
23	21.1	48	83.7	73	265.7	98	707.3
24	22.4	49	88.0	74	277.2	99	733.2
25	23.8	50	92.5	75	289.1	100	760.0

solubility was calculated in ml per liter from:

$$\alpha \times 0.2095 \times \frac{760 - w.v.p.}{760}$$

The resulting values appear in Table VIII. The solubility of oxygen in mg per liter (parts per million) was calculated as above and an oxygen density of 1.429 mg per ml.

$$\alpha \times 0.2095 \times \frac{760 - w.v.p.}{760} \times 1.429$$

The resulting values appear in Table IX.

Nitrogen solubilities were calculated similarly from the absorption coefficients, Handbook of Chemistry and Physics. The nitrogen fraction of the air, inclusive of the noble gases, was 0.7905. Correcting for water vapor pressure, the solubility of nitrogen in ml per liter of air-equilibrated water becomes:

$$\alpha \times 0.7905 \times \frac{760 - w.v.p.}{760} \quad \text{(Table X)}.$$

Nitrogen solubility in mg per liter (parts per million) was obtained by correcting for the density of the gas (1.251 mg per ml).

$$\alpha \times 0.7905 \times \frac{760 - w.v.p.}{760} \times 1.251 \quad \text{(Table XI)}$$

TABLE VIII

Solubility of oxygen, from a wet atmosphere at a pressure of 760 mm Hg, in ml per liter at temperatures from 0 to 30°C

Temp.	0.0	0.1	0.2	0.3	0.4	0.5
0	10.17	10.14	10.11	10.08	10.06	9.93
1	9.90	9.85	9.85	9.82	9.79	9.76
2	9.64	9.61	9.59	9.56	9.53	9.50
3	9.38	9.35	9.33	9.30	9.28	9.25
4	9.13	9.10	9.08	9.05	9.03	9.01
5	8.89	8.87	8.85	8.82	8.80	8.77
6	8.66	8.64	8.62	8.60	8.59	8.56
7	8.47	8.45	8.43	8.40	8.38	8.35
8	8.24	8.23	8.21	8.19	8.17	8.15
9	8.05	8.03	8.01	7.99	7.98	7.96
10	7.86	7.84	7.82	7.80	7.79	7.77
11	7.69	7.67	7.66	7.64	7.62	7.60
12	7.52	7.50	7.48	7.46	7.45	7.43
13	7.34	7.32	7.31	7.29	7.28	7.26
14	7.19	7.17	7.16	7.14	7.13	7.11
15	7.04	7.02	7.01	6.99	6.98	6.96
16	6.89	6.87	6.86	6.84	6.83	6.81
17	6.74	6.72	6.71	6.69	6.68	6.67
18	6.60	6.58	6.57	6.56	6.55	6.53
19	6.47	6.46	6.45	6.43	6.42	6.41
20	6.35	6.34	6.33	6.31	6.30	6.28
21	6.21	6.20	6.19	6.18	6.17	6.15
22	6.10	6.08	6.07	6.06	6.05	6.04
23	5.97	5.96	5.95	5.94	5.93	5.91
24	5.86	5.85	5.84	5.82	5.81	5.80
25	5.74	5.73	5.72	5.71	5.70	5.69
26	5.63	5.62	5.61	5.60	5.59	5.58
27	5.53	5.52	5.51	5.50	5.49	5.48
28	5.42	5.41	5.40	5.39	5.38	5.38
29	5.33	5.31	5.30	5.29	5.28	5.27
30	5.23	5.22	5.21	5.20	5.19	5.18

TABLE VIII (continued)

Temp.	0.6	0.7	0.8	0.9
0	10.01	9.98	9.95	9.92
1	9.74	9.71	9.69	9.66
2	9.48	9.45	9.43	9.40
3	9.22	9.19	9.17	9.15
4	8.99	8.96	8.94	8.91
5	8.75	8.73	8.71	8.68
6	8.54	8.52	8.51	8.49
7	8.33	8.31	8.29	8.27
8	8.13	8.11	8.10	8.07
9	7.94	7.92	7.90	7.88
10	7.76	7.74	7.73	7.71
11	7.59	7.57	7.55	7.53
12	7.41	7.39	7.38	7.36
13	7.25	7.23	7.21	7.20
14	7.10	7.08	7.07	7.05
15	6.95	6.93	6.91	6.90
16	6.79	6.78	6.77	6.75
17	6.66	6.64	6.63	6.61
18	6.52	6.51	6.50	6.48
19	6.40	6.39	6.38	6.36
20	6.27	6.25	6.24	6.22
21	6.14	6.13	6.12	6.11
22	6.03	6.01	6.00	5.98
23	5.90	5.89	5.88	5.87
24	5.79	5.77	5.76	5.75
25	5.68	5.66	5.65	5.64
26	5.57	5.56	5.55	5.54
27	5.47	5.45	5.44	5.43
28	5.37	5.36	5.35	5.34
29	5.27	5.26	5.25	5.24
30	5.17	5.17	5.16	5.16

TABLE IX

Solubility of oxygen, from a wet atmosphere at a pressure of 760 mm Hg, in mg per liter at temperatures from 0 to 30°C

Temp.	0.0	0.1	0.2	0.3	0.4
0	14.53	14.49	14.45	14.41	14.37
1	14.14	14.10	14.07	14.03	13.99
2	13.77	13.74	13.70	13.66	13.62
3	13.40	13.36	13.33	13.29	13.26
4	13.04	13.00	12.97	12.94	12.91
5	12.71	12.68	12.64	12.61	12.58
6	12.38	12.35	12.32	12.30	12.27
7	12.10	12.07	12.04	12.01	11.98
8	11.79	11.76	11.73	11.71	11.68
9	11.51	11.48	11.45	11.43	11.40
10	11.23	11.21	11.18	11.16	11.13
11	10.99	10.97	10.94	10.92	10.89
12	10.74	10.72	10.69	10.67	10.64
13	10.49	10.47	10.45	10.42	10.40
14	10.27	10.25	10.23	10.21	10.19
15	10.06	10.04	10.02	9.99	9.97
16	9.84	9.82	9.80	9.78	9.76
17	9.63	9.61	9.59	9.57	9.55
18	9.43	9.41	9.39	9.38	9.36
19	9.25	9.23	9.22	9.20	9.18
20	9.08	9.06	9.04	9.02	9.00
21	8.88	8.86	8.85	8.83	8.82
22	8.72	8.70	8.68	8.66	8.64
23	8.53	8.51	8.50	8.48	8.47
24	8.37	8.35	8.34	8.32	8.30
25	8.20	8.19	8.17	8.16	8.14
26	8.05	8.04	8.02	8.01	7.99
27	7.90	7.89	7.87	7.86	7.84
28	7.75	7.74	7.72	7.71	7.69
29	7.61	7.60	7.58	7.57	7.55
30	7.47	7.46	7.44	7.43	7.42

TABLE IX (continued)

Temp.	0.5	0.6	0.7	0.8	0.9
0	14.34	14.30	14.26	14.22	14.18
1	13.96	13.92	13.88	13.84	13.81
2	13.59	13.55	13.51	13.47	13.44
3	13.22	13.18	13.15	13.11	13.08
4	12.88	12.84	12.81	12.78	12.74
5	12.55	12.51	12.48	12.45	12.41
6	12.24	12.21	12.18	12.16	12.13
7	11.95	11.91	11.88	11.85	11.82
8	11.65	11.62	11.59	11.57	11.54
9	11.37	11.34	11.31	11.29	11.26
10	11.11	11.09	11.06	11.04	11.01
11	10.87	10.84	10.82	10.79	10.77
12	10.62	10.59	10.57	10.54	10.52
13	10.38	10.36	10.34	10.31	10.29
14	10.17	10.14	10.14	10.10	10.08
15	9.95	9.93	9.91	9.88	9.86
16	9.74	9.71	9.69	9.67	9.65
17	9.53	9.51	9.49	9.47	9.45
18	9.34	9.32	9.30	9.29	9.27
19	9.17	9.15	9.13	9.11	9.10
20	8.98	8.96	8.94	8.92	8.90
21	8.80	8.78	8.77	8.75	8.74
22	8.63	8.61	8.59	8.57	8.55
23	8.45	8.43	8.42	8.40	8.39
24	8.29	8.27	8.25	8.23	8.22
25	8.13	8.11	8.10	8.08	8.07
26	7.98	7.96	7.95	7.93	7.92
27	7.83	7.81	7.80	7.78	7.77
28	7.68	7.67	7.65	7.64	7.62
29	7.54	7.53	7.51	7.50	7.48
30	7.41	7.39	7.38	7.37	7.35

TABLE X

Solubility of nitrogen, from a wet atmosphere at a pressure of 760 mm Hg, in ml per liter at temperatures from 0 to 30°C

Temp.	0.0	0.1	0.2	0.3	0.4
0	18.50	18.45	18.40	18.36	18.31
1	18.03	18.00	17.95	17.91	17.86
2	17.59	17.55	17.51	17.47	17.42
3	17.15	17.11	17.07	17.03	16.99
4	16.73	16.69	16.65	16.61	16.57
5	16.34	16.30	16.26	16.22	16.18
6	15.94	15.90	15.87	15.83	15.80
7	15.57	15.54	15.50	15.47	15.43
8	15.21	15.17	15.14	15.10	15.07
9	14.86	14.83	14.79	14.76	14.72
10	14.52	14.49	14.46	14.43	14.40
11	14.23	14.19	14.16	14.13	14.10
12	13.92	13.89	13.86	13.93	13.80
13	13.62	13.59	13.57	13.54	13.52
14	13.36	13.33	13.30	13.27	13.25
15	13.09	13.07	13.05	13.02	12.99
16	12.84	12.81	12.79	12.76	12.74
17	12.60	12.57	12.55	12.52	12.50
18	12.37	12.34	12.32	12.39	12.27
19	12.13	12.11	12.09	12.07	12.05
20	11.91	11.89	11.87	11.85	11.84
21	11.73	11.71	11.69	11.67	11.65
22	11.51	11.49	11.48	11.46	11.45
23	11.33	11.31	11.29	11.27	11.26
24	11.15	11.13	11.12	11.10	11.09
25	10.99	10.97	10.95	11.93	10.91
26	10.80	10.78	10.77	10.75	10.74
27	10.64	10.62	10.61	10.59	10.58
28	10.48	10.46	10.45	10.43	10.42
29	10.31	10.29	10.28	10.26	10.25
30	10.16	10.14	10.13	10.11	10.10

TABLE X (continued)

Temp.	0.5	0.6	0.7	0.8	0.9
0	18.27	18.22	18.17	18.13	18.08
1	17.82	17.77	17.73	17.68	17.64
2	17.38	17.33	17.29	17.24	17.20
3	16.95	16.90	16.86	16.82	16.77
4	16.54	16.50	16.46	16.42	16.38
5	16.14	16.10	16.06	16.02	15.98
6	15.76	15.72	15.68	15.64	15.61
7	15.40	15.36	15.32	15.28	15.25
8	15.03	15.00	14.96	14.93	14.89
9	14.69	14.65	14.68	14.59	14.55
10	14.37	14.34	14.31	14.28	14.25
11	14.07	14.04	14.01	13.98	13.95
12	13.77	13.74	13.71	13.68	13.65
13	13.49	13.46	13.43	13.41	13.38
14	13.22	13.20	13.17	13.13	13.12
15	12.96	12.93	12.91	12.89	12.86
16	12.71	12.69	12.67	12.65	12.62
17	12.47	12.45	12.43	12.41	12.39
18	12.24	12.20	12.20	12.18	12.16
19	12.02	12.00	11.98	11.96	11.93
20	11.82	11.80	11.78	11.76	11.74
21	11.62	11.60	11.58	11.56	11.53
22	11.43	11.41	11.39	11.37	11.35
23	11.22	11.22	11.20	11.19	11.17
24	11.07	11.06	11.04	11.02	11.00
25	10.89	10.88	10.86	10.84	10.82
26	10.72	10.70	10.68	10.67	10.65
27	10.56	10.54	10.52	10.51	10.49
28	10.40	10.38	10.36	10.34	10.32
29	10.23	10.22	10.20	10.19	10.17
30	10.08	10.07	10.05	10.04	10.02

TABLE XI

Solubility of nitrogen, from a wet atmosphere at a pressure of 760 mm Hg, in mg per liter at temperatures from 0 to 30°C

Temp.	0.0	0.1	0.2	0.3	0.4
0	23.14	23.08	23.02	22.97	22.91
1	22.56	22.51	22.45	22.40	22.34
2	22.01	21.96	21.90	21.85	21.79
3	21.46	21.41	21.35	21.30	21.25
4	20.93	20.88	20.83	20.78	20.73
5	20.44	20.39	20.34	20.29	20.24
6	19.94	19.89	19.85	19.80	19.76
7	19.48	19.54	19.39	19.35	19.30
8	19.03	18.99	18.94	18.90	18.85
9	18.59	18.55	18.50	18.46	18.42
10	18.16	18.12	18.09	18.05	18.02
11	17.80	17.76	17.72	17.68	17.64
12	17.41	17.37	17.34	17.30	17.26
13	17.04	17.01	16.97	16.94	16.91
14	16.71	16.68	16.64	16.61	16.58
15	16.38	16.35	16.32	16.28	16.25
16	16.06	16.03	16.00	15.97	15.94
17	15.76	15.73	15.70	15.67	15.64
18	15.47	15.44	15.41	15.38	15.35
19	15.18	15.15	15.12	15.10	15.07
20	14.90	14.88	14.85	14.83	14.81
21	14.67	14.64	14.62	14.59	14.57
22	14.41	14.39	14.36	14.34	14.32
23	14.18	14.16	14.13	14.11	14.09
24	13.95	13.93	13.91	13.89	13.87
25	13.75	13.73	13.70	13.68	13.65
26	13.51	13.49	13.47	13.45	13.43
27	13.31	13.29	13.27	13.25	13.23
28	13.11	13.09	13.07	13.05	13.03
29	12.90	12.88	12.86	12.84	12.82
30	12.71	12.69	12.67	12.65	12.63

TABLE XI (continued)

Temp.	0.5	0.6	0.7	0.8	0.9
0	22.85	22.79	22.73	22.68	22.62
1	22.29	22.23	22.18	22.12	22.07
2	21.74	21.68	21.63	21.57	21.52
3	21.20	21.14	21.09	21.04	20.98
4	20.69	20.64	20.59	20.54	20.49
5	20.19	20.14	20.09	20.04	19.99
6	19.71	19.66	19.62	19.57	19.53
7	19.26	19.21	19.17	19.12	19.08
8	18.81	18.77	18.72	18.68	18.63
9	18.38	18.33	18.29	18.25	18.20
10	17.98	17.94	17.91	17.87	17.84
11	17.61	17.57	17.53	17.49	17.45
12	17.23	17.19	17.15	17.11	17.07
13	16.88	16.84	16.81	16.78	16.74
14	16.55	16.51	16.48	16.45	16.41
15	16.22	16.19	16.16	16.12	16.09
16	15.91	15.88	15.85	15.82	15.79
17	15.62	15.59	15.56	15.53	15.50
18	15.33	15.30	15.27	15.24	15.21
19	15.04	15.01	14.98	14.96	14.93
20	14.79	14.76	14.74	14.72	14.69
21	14.54	14.51	14.49	14.46	14.44
22	14.30	14.27	14.25	14.23	14.20
23	14.07	14.04	14.02	14.00	13.97
24	13.85	13.83	13.81	13.79	13.77
25	13.63	13.61	13.58	13.56	13.53
26	13.41	13.39	13.37	13.35	13.33
27	13.21	13.19	13.17	13.15	13.13
28	13.01	12.98	12.96	12.94	12.92
29	12.81	12.79	12.77	12.75	12.73
30	12.62	12.60	12.58	12.56	12.54

APPENDIX B

Methods of Gas Analysis

Oxygen in water. Dissolved oxygen content of water was determined by the Winkler procedure as described in the Standard Methods for the Examination of Water and Wastewater. Water samples were drawn into 300 ± 0.5 ml BOD bottles. Two ml quantities of manganous sulfate, alkaline iodide and sulfuric acid were used throughout to assure fixation of oxygen at high gas tensions. A 200 ml aliquot was titrated with 0.02N sodium thiosulfate. The titration volume was corrected for the 4 ml of water displaced by the first two reagents by multiplying by the factor 1.013. Thiosulfate solutions were standardized periodically and corrected to within ± 1 per cent.

Nitrogen in water. Water analysis for dissolved nitrogen was conducted by means of the apparatus of Scholander, van Dam, Claff and Kanwisher (1955). The technique described by these authors was altered somewhat to replace the reagent alkaline pyrogallol with commercially available oxygen absorber (Oxorbent, Burrell Corp., Pittsburgh, Pa.). This oxygen absorber was incompatible with the alkaline citrate normally used in the gas analyzer. Alternatively the analyzer was filled with a saturated solution of sodium chloride as

described by Hoar (1960) for use in gas analysis. Water nitrogen was calculated after the method of Scholander et al. (1955), correcting for nitrogen in the reagents by means of blank determinations, for nitrogen remaining in the liquid phase in the extractor and for water vapor pressure, temperature and barometric pressure, thus reducing the gas volume to conditions of standard temperature and pressure. The accuracy of the dissolved nitrogen method was such that twenty analyses on air-equilibrated, distilled water averaged 100.5 per cent of air saturation, with one standard deviation $\pm$ 2.1 per cent.

Dissolved nitrogen in fish blood. Dissolved nitrogen was determined by a modification of the techniques of Edwards, Scholander and Roughton (1943) and Sundes (1960), utilizing the extractor and gas analyzer of Scholander, van Dam, Claff and Kanwisher (1955).

The reagents were prepared and utilized as follows:

Heparin solution: 10 mg per ml of sodium heparin

Occlusion fluid: saturated sodium chloride colored with methyl red indicator and barely acidified with concentrated hydrochloric acid

Caprylic alcohol: (2-octanol)

Acid sulphate solution: 150 g anhydrous sodium sulphate dissolved in 500 ml of distilled water and acidified with 25 ml concentrated sulphuric acid

Ferricyanide solution: 62.5 g potassium ferricyanide, 30.0 g potassium bicarbonate, and 2.5 g of saponin dissolved in 250 ml of water

Potassium hydroxide solution: 100 g potassium hydroxide dissolved in water to a volume of 500 ml

Oxygen absorber: Oxorbent

The acid sulphate and ferricyanide solutions are essentially those of Sundes (1960) and the occlusion fluid that of Hoar (1960). The oxygen absorber was stored under oil in a separatory funnel and delivered into the gas analyzer by means of a short length of polyethylene tubing. The other solutions were held in reagent bottles and permitted to equilibrate with air. The blood, caprylic alcohol, saturated sodium chloride, and ferricyanide were injected into the extractor by means of 0.5 or 1.0 ml tuberculin syringes fitted with no 16 needles and short lengths of polyethylene tubing, the 20 per cent potassium hydroxide was injected into the extractor with a 5 ml syringe similarly fitted.

The extractor and gas analyzer were cleaned with acid permanganate or acid dichromate solution and rinsed thoroughly. The extractor was next flushed with 0.5 ml of acid sulphate solution by drawing that volume of solution into the analyzer

then turning it tip up and running the plunger up and down. The acid sulphate was expelled and this step repeated, removing any air. The plunger was pushed home leaving the syringe extension filled with acid sulphate (approximately 0.1 ml).

The fish were stunned (but not killed) electrically and a blood sample obtained by heart puncture. The blood was drawn into a heparinized 1 ml syringe, care being taken to avoid excessive negative pressure. The blood sample was large enough to permit the transfer of 0.4 ml of blood and still leave 0.2 ml or more of blood in the syringe with the small volume of heparin from the needle. If any free gas appeared in the sample either from gas leaving solution in the blood or from air contamination, the sample was discarded.

Following transfer of the blood sample to the extractor, a small quantity of caprylic alcohol was necessary to reduce frothing. It was desirable to use the smallest amount possible. This was gauged by injecting the caprylic alcohol into the extractor to the point of bore enlargement 1.5 cm from the tip (a volume of approximately 0.015 ml) of the syringe extension.

The clotting problem was overcome by next injecting 0.5 ml of occlusion fluid into the extractor after the caprylic alcohol. The tip of the extractor was closed with a

rubber cap and the contents mixed by shaking for a half-minute. Promptness in the addition of the caprylic alcohol and sodium chloride to the blood in the extractor appeared to lessen further the tendency of the blood to form large clots.

The cap was removed and 0.3 ml of ferricyanide solution injected into the extractor. The cap was replaced and the extractor contents again shaken for one-half minute. The result was a gas phase (carbon dioxide, nitrogen and oxygen) of 3.5 ml overlying 0.9 ml of liquid.

The carbon dioxide of the gas was reabsorbed by injecting 3.0 ml of 20 per cent potassium hydroxide into the extractor. If the blood had broken up properly it settled to the bottom of the liquid in fine particles, floating clots indicated trapped gas bubbles and the sample was discarded. The gas bubble was rolled back and forth once over the potassium hydroxide then forced up into the syringe extension.

The gas bubble was transferred to the gas analyzer which previously had been cleaned and filled with occlusion fluid. The bubble was drawn through the capillary into the analyzer barrel then slowly returned to the capillary. The excess fluid was removed from the cup at the end of the analyzer, the length of the total-gas column recorded and the bubble returned to the barrel. Oxygen absorber was run

into the cup and drawn through the analyzer until the barrel was filled (approximately 0.3 ml). The gas bubble was rolled about in the absorber then slowly returned to the capillary. The length of the gas column, the temperature of the gas analyzer, and the barometric pressure were recorded.

The nitrogen content was corrected to a dry gas at standard temperature and pressure and expressed as volumes per cent. The calculation were based on the original work of Peters and Van Slyke (1932) and on the Scholander, Van Dam, Claff, and Kanwisher (1955) description for water.

1. Blank determinations for nitrogen in the reagents were averaged and the mean value subtracted from the measured length of the sample nitrogen column. The average of 10 blanks was 12.8 mm. Thus for fish no.1 the nitrogen column was:

$$34.3 - 12.8 = 21.5 \text{ mm}$$

2. The remaining gas column was corrected to a blood sample size of 1.0 ml by multiplying by the inverse of the sample size:

$$21.5 \times \frac{1.0}{0.4} = 53.75 \text{ mm}$$

3. The length of the gas column in mm was converted to cu mm with a calibration correction factor for the gas analyzer. In this case the value was very close to 0.283 thus:

53.75 x 0.283 = 15.21 cu mm

4. The recorded barometric pressure was temperature corrected from the table of values to be found in the 40th Edition of the Handbook of Chemistry and Physics. At 19°C and a barometric pressure of 767 mm Hg the corrected value was:

767 - 2.4 = 764.6 mm Hg

5. The temperature-corrected barometric pressure was corrected to a dry gas by subtracting the water vapor pressure at the temperature of the gas analyzer (19°C):

764.6 - 16.5 = 748.1 mm Hg

6. The nitrogen volume as a dry gas was corrected to the standard pressure of 760 mm Hg

$$15.21 \times \frac{748.1}{760} = 14.97 \text{ cu mm}$$

7. The nitrogen volume at standard pressure was corrected to the standard temperature of 0°C from the temperature (19°C) of the gas analyzer:

$$14.97 \times \frac{273}{292} = 14.00 \text{ cu mm}$$

8. A small correction was made for nitrogen remaining in solution in the syringe analyzer. The nitrogen in solution was a function of the partial pressure and solubility of the gas. The partial pressure of the nitrogen in the gas phase:

$$\frac{N_2 \text{ vol.}}{CO_2 + N_2 + O_2 \text{ vol.}} \times 760$$

In the example at hand the nitrogen column in the gas analyzer was:

$$34.3 \times 0.283 = 9.71 \text{ cu mm}$$

The total gas phase was approximately 3.5 ml or 3500 cu mm. Thus the partial pressure of the nitrogen in the gas phase:

$$\frac{9.71}{3500} \times 760 = 2.1 \text{ mm Hg}$$

The solubility of nitrogen at 19°C from the absorption coefficient, Handbook of Chemistry and Physics, was 15.70 cu mm per ml of water. For a liquid phase of 0.9 ml at a partial pressure of 760 mm Hg:

$$15.70 \times 0.9 = 14.13 \text{ cu mm}$$

Correcting to the nitrogen partial pressure of 2.1 mm Hg (from above) the volume of nitrogen remaining in solution was:

$$\frac{2.1}{760} \times 14.13 = 0.039 \text{ cu mm}$$

Thus the nitrogen volume of 9.71 cu mm was increased by a factor of:

$$\frac{9.71 \times 0.039}{9.71} = 1.004$$

Correcting the volume from step no. 7, the nitrogen volume was:

$$14.00 \times 1.004 = 14.06 \text{ cu mm}$$

of dry gas at standard temperature and pressure for a blood sample of 1.0 ml, or 1.41 volumes per cent.

9. Theoretical values were calculated from the table of absorption coefficients, Handbook of Chemistry and Physics.

NOTE: The absorption coefficient should not be confused with the coefficient of solubility, the latter being calculated to the temperature and pressure at which the gas is measured. A fuller explanation may be found in Glasstone (1946).

At the sample temperature of 11.6°C the solubility of dry nitrogen reduced to STP was 18.01 ml per liter, obtained by interpolation between values in the table of absorption coefficients. The recorded barometric pressure was temperature corrected as above:

767 - 2.4 = 764.6 mm Hg

This corrected pressure was reduced to conditions of a dry gas by subtracting the water vapor pressure at the temperature of the sample:

764.6 - 10.2 = 754.4 mm Hg

In the dry air the partial pressure of nitrogen (the noble gases in the air were also considered as nitrogen here) was:

0.7905 x 754.4 = 596.4 mm Hg

Correcting the absorption coefficient value to this nitrogen partial pressure, at 11.6°C the theoretical nitrogen content of air-equilibrated water was:

$$18.01 \times \frac{596.4}{760} = 14.14 \text{ ml per liter}$$

or 1.41 volumes per cent.

10. The percentage of air saturation was calculated form the corrected experimental value, no. 8, and the corrected theoretical nitrogen, no. 9:

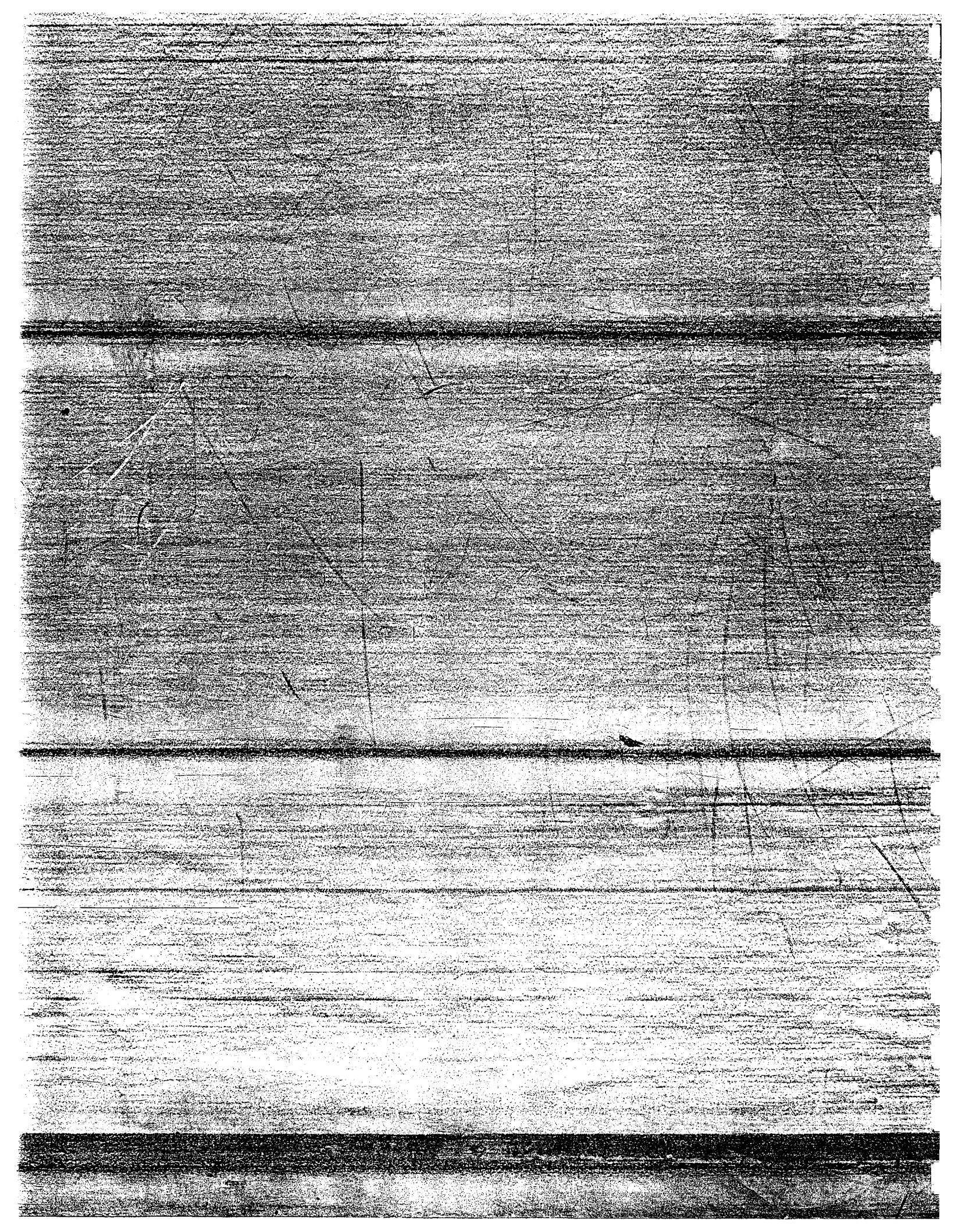
$$\frac{14.06}{14.14} \times 100 = 99.4 \text{ per cent}$$

11. Corrections were not made for certain lesser inaccuracies. There was a difference in the size of the liquid phase and hence a difference in the amount of nitrogen remaining in solution in the blank and blood determinations. The solubility of nitrogen in the reagents of the liquid phase was less than that of the value for water used in the calculation no. 8. This was opposed by the higher solubility of nitrogen in blood than in water. A measure of this may be found in Van Slyke, Dillon and Margaria (1934).

Ten blank determinations averaged 12.8 mm with one standard deviation $\pm$ 0.4 mm. The average of 10 determinations on distilled water, air-equilibrated in a rotating flask was 101.0 per cent of air saturation with $\pm$ 2.1 per cent for one standard deviation. Ten blood

determinations on rainbow trout held in a pond at 99 to 101 per cent of air saturation averaged 99.4 per cent with ± 2.8 per cent for one standard deviation. The technique described was employed successfully over the range of 100 to 135 per cent of air saturation (1.31 to 1.77 volumes of per cent).

A slightly more detailed description of this method may be found in Harvey (1961).



Ebel. 1970. Dissolved nitrogen surveys of the Columbia and Snake Rivers. (Monthly Narrative Report)

The nitrogen surveys on the Snake and Columbia Rivers on May 5 and May 19 are in table form with written summary.

KB Jan 1970

Monthly Narrative Report

May 1970

Dissolved Nitrogen Surveys of the Columbia and Snake Rivers

Prepared for

U. S. Corps of Engineers, Portland District

Portland, Oregon

by

Wesley J. Ebel

May 28, 1970

U. S. Bureau of Commercial Fisheries
2725 Montlake Boulevard East
Seattle, Washington 98102

Oxygen and nitrogen saturation values of samples collected during aircraft surveys on May 5 and 19 are shown in table 1; mean daily discharge through spillways and powerhouses at the various dams are given in table 2.

Nitrogen saturation levels in the Columbia River on May 5 were low because practically no spill occurred during or prior to the survey. Levels in the Snake River were quite high (132.8 below Little Goose Dam) because of rather consistent spilling at dams on the Snake. Juvenile salmon examined in gatewells at Ice Harbor and McNary dams indicated no symptoms of gas bubble disease at this time.

The results of the second survey on May 19 definitely indicated that nitrogen levels were rising in the Columbia and Snake Rivers. All dams in the sample area were spilling substantial amounts of water which, no doubt, accounts for the rise. Highest levels in the Columbia were recorded in the tailraces at McNary (127%) and Bonneville (134%) dams; in the Snake River, the highest levels were in the tailraces at Little Goose (135%) and Lower Monumental (136%) dams.

Twenty-five percent of the juvenile salmon examined during the latter period at Ice Harbor Dam had obvious external symptoms of gas bubble disease. No external symptoms of gas bubble disease have been noted in adult chinook handled at Ice Harbor Dam.

Comparable surveys were made in the Columbia River from Grand Coulee Forebay to below Priest Rapids Dam by the Washington Department of Fisheries. This information is contained in table 3. Spilling prevailed at Grand Coulee, Chief Joseph, Rocky Reach, and Rock Island Dams during these surveys.

Our next survey of nitrogen levels will be completed on June 3.

Wesley J. Ebel
May 28, 1970

Table 1.--Saturation of dissolved oxygen and nitrogen in samples collected in the Columbia and Snake Rivers on May 5, and 19, 1970

May 5, 1970

Station	Location	Depth	°C Temp.	Oxygen		Nitrogen	
				P.p.m.	% Sat.	P.p.m.	% Sat.
Little Goose Dam	Forebay	0'	12.1	12.5	116.6	18.3	102.2
		33'	11.2	11.8	107.8	18.3	100.4
	Tailrace	0'	10.8	14.2	128.4	24.4	132.8
Lower Monumental Dam	Forebay	0'	11.4	14.0	128.4	21.7	119.6
		33'	10.6	14.1	126.9	22.3	120.6
	Tailrace	0'	10.7	12.8	115.5	21.2	115.2
Ice Harbor Dam	Forebay	0'	12.7	13.6	128.7	20.7	116.9
		33'	10.8	13.3	120.2	20.7	112.7
	Tailrace	0'	10.7	12.6	113.7	21.6	117.4
Columbia River	Above Snake mouth	0'	11.4	12.6	115.6	19.2	105.8
McNary Dam--Wash. side	Forebay	0'	12.0	12.8	119.0	18.2	101.4
		33'	11.2	12.6	115.4	19.6	107.5
	Tailrace	0'	11.1	12.6	114.7	19.4	106.2
McNary Dam--Ore. side	Forebay	0'	11.7	13.1	121.1	18.6	103.1
		33'	10.4	12.6	112.9	19.8	107.0
	Tailrace	0'	11.0	12.5	113.6	18.1	99.0
John Day Dam	Forebay	0'	10.2	12.9	114.8	18.5	99.5
		33'	9.9	12.8	113.3	18.2	97.3
	Tailrace	0'	10.2	11.6	103.2	18.4	99.0
The Dalles Dam	Forebay	0'	10.6	11.6	104.4	17.8	96.5
		33'	10.6	11.7	105.6	18.6	100.9
	Tailrace	0'	10.7	11.7	105.6	17.7	96.2
Bonneville Dam	Forebay	0'	10.8	12.1	109.4	18.2	99.1
		33'	10.8	12.1	109.4	18.3	99.7
	Tailrace	0'	11.0	11.6	105.4	18.3	100.0
Washougal		0'					
Portland	Above Willamette	0'					
Longview	BCF barge	0'	11.5	10.9	100.3	17.9	98.8
Astoria	Pilot Rock	0'	10.2	11.1	98.7	17.5	94.1

Table 1 (continued)

May 19, 1970

Station	Location	Depth	°C Temp.	Oxygen P.p.m.	Oxygen % Sat.	Nitrogen P.p.m.	Nitrogen % Sat
Little Goose Dam	Forebay	0'	14.0	10.6	103.3	18.5	107.1
		33'	13.2	10.8	103.3	19.0	108.3
	Tailrace	0'	13.3	13.4	128.5	23.7	135.3
Lower Monumental Dam	Forebay	0'	13.0	13.7	130.5	24.0	136.4
		33'	12.2	13.5	126.2	23.5	134.0
	Tailrace	0'	12.2	13.5	126.0	23.8	135.7
Ice Harbor Dam	Forebay	0'	12.0	13.5	125.6	23.5	131.0
		33'	11.7	13.4	123.8	22.9	126.9
	Tailrace	0'	11.8	13.4	124.2	22.6	125.4
Columbia River	Above Snake mouth	0'	12.5	11.3	106.2	18.7	107.2
McNary Dam--Wash.side	Forebay	0'	13.7	12.0	116.2	18.8	108.2
		33'	13.0	11.8	112.4	19.8	112.5
	Tailrace	0'	13.6	13.0	126.0	22.3	126.8
McNary Dam--Ore. side	Forebay	0'	14.0	12.7	123.8	20.3	117.5
		33'	12.9	12.8	121.7	21.4	116.7
	Tailrace	0'	13.1	12.2	116.6	19.6	111.5
John Day Dam	Forebay	0'	13.4	11.3	108.6	18.3	104.7
		33'	13.4	11.5	110.6	19.5	111.5
	Tailrace	0'	13.6	12.0	116.0	20.7	119.0*
The Dalles Dam	Forebay	0'**	--	--	--	--	--
		33'**	--	--	--	--	--
	Tailrace	0'	13.1	12.1	115.5	20.9	118.9
Bonneville Dam	Forebay	0'	12.5	11.6	109.2	19.3	110.7
		33'	12.6	11.8	111.3	19.6	110.5
	Tailrace	0'	12.8	13.1	124.2	23.7	134.4
Washougal	Wash. side	0'	12.8	11.7	111.1	20.3	114.7
Portland	Above Willamette	0'	13.0	12.1	114.4	19.7	111.9
Longview	BCF barge	0'	13.5	11.0	106.0	18.5	106.1
Astoria	Pilot Rock	0'	13.8	10.7	104.0	18.2	105.0

* Taken in shallow water near shore due to high winds.
** Couldn't land due to high winds.

Table 2.--Mean daily spillway and powerhouse discharges during surveys of nitrogen saturation levels on May 5 and 19, 1970

May 5, 1970

Dam	Spill discharge	Powerhouse discharge	Total
	1000's - cfs	1000's - cfs	1000's - cfs
Little Goose	46.4	19.9	66.5
Lower Monumental	.07	58.1	58.9
Ice Harbor	14.4	42.3	57.0
McNary	1.5	167.6	171.8
John Day	0.0	157.2	157.9
The Dalles	0.0	151.3	152.8
Bonneville	16.0	127.1	139.2

May 19, 1970

Little Goose	129.9	21.5	151.4
Lower Monumental	110.5	43.2	153.7
Ice Harbor	104.9	46.5	151.4
McNary	155.6	144.9	300.5
John Day	121.4	182.6	304.0
The Dalles	125.0	169.1	294.1
Bonneville	168.7	129.9	298.6

Table 3.--Saturation of dissolved nitrogen and oxygen, Grand Coulee to Priest Rapids, May 19, 1970

Sampling area	Water temp.	Dissolved nitrogen		Dissolved oxygen	
		ppm	saturation	ppm	saturation
Grand Coulee forebay	49.5	18.64	99.3	11.42	100.5
1.5 miles below Grand Coulee	49.0	23.50	124.4	13.96	122.0
Chief Joseph forebay	49.5	22.91	122.0	13.62	119.9
2 miles below Chief Joseph	48.5	22.91	120.7	13.62	118.3
Wells forebay	52.5	22.43	123.6	13.59	124.7
1 mile below Wells	51.5	22.23	121.0	13.13	118.7
Wells stretch 1	50.0	19.86	106.4	11.53	102.3
Wells stretch 23	54.5	17.91	100.8	11.85	111.5
Rocky Reach forebay	51.0	19.88	107.6	12.64	113.5
Rocky Reach stretch 2	52.0	19.69	107.9	11.51	104.8
1 mile below Rocky Reach	51.5	21.83	118.9	13.25	119.8
Priest Rapids forebay	53.0	18.53	102.7	11.10	102.6
2 miles below Priest Rapids	53.0	19.23	106.6	12.26	113.3
Priest Rapids stretch 1	53.0	19.40	107.6	11.44	105.7
Priest Rapids stretch 24	54.0	18.68	104.5	11.44	106.9

Roesner and Norton. 1971. A nitrogen gas (N_2) model for the lower Columbia River.

This report describes the theory of modelling supersaturation in the Columbia River and the use of this computer program for predicting the effects of various flow patterns on supersaturation in the Columbia River.

A NITROGEN GAS (N_2) MODEL FOR THE LOWER COLUMBIA RIVER

FINAL REPORT

L. A. Roesner
W. R. Norton

prepared for
The Portland District, North Pacific Division
U.S. ARMY CORPS OF ENGINEERS

JANUARY 1971

REPORT No. 1-350

WRE
WATER RESOURCES ENGINEERS, INC.
WALNUT CREEK, CALIFORNIA • 94596

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I. INTRODUCTION

BACKGROUND

The Columbia River, illustrated in Figure 1, is the fourth largest river on the North American continent (1)*. Only the Mississippi, the MacKenzie, and the St. Lawrence Rivers travel farther and carry more water. However, as a source of power, the Columbia is the mightiest stream on the continent, possessing one third of the hydroelectric potential of the United States. The river drains an area of 259,000 square miles, 15 percent of which is in Canada, and has a mean annual flow of about 239,000 cfs at its mouth.

In its upper reaches in Canada, the river is almost as wild and untamed today as it was when the first white explorers saw it. By contrast, the river on the United States side is highly developed. This is particularly true of the lower Columbia. Here, the development of the river for hydropower and navigation has culminated in a series of four low head dams which have created slack water from the Columbia Estuary to the mouth of the Snake River. Furthermore, with the completion of a similar series of four dams on the lower Snake River, this slack water will extend all the way to the Lewiston-Clarkston area on the Washington-Idaho border, a distance of 464 miles above the Columbia River mouth.

While the development of the lower Columbia and lower Snake Rivers has produced many benefits, it has had an adverse effect on the salmon and steelhead trout resource in those rivers. One particular effect has been fish mortalities resulting from the "gas bubble" disease

*Numbers in parenthesis refer to References following Chapter IV.

FIGURE 1

LOCATION MAP - COLUMBIA RIVER BASIN

or nitrogen embolism. The "gas bubble" disease is the direct result of spillway operation at the dams during the snowmelt or high runoff period which extends from April through July.

As large volumes of water spill over these dams into the stilling basins below, air bubbles are entrained and plunged with the main flow to the bottom of the stilling basin. Here, the nitrogen gas under high pressure is driven into solution in the water causing a supersaturated condition to exist. In the subsequent passage of this water through the downstream reservoir pool, there is insufficient circulation to liberate the excess nitrogen; consequently, the supersaturated condition exists through the entire slackwater pool. The nitrogen embolism that accompanies this condition occurs when a fish swims near the surface of the river or reservoir where the hydrostatic pressure is less than the pressure required to keep the excess N_2 in solution. As a result, the gas comes out of solution in the gills and bloodstream causing small bubbles to form in the circulatory system of the fish. If the embolism is sufficiently severe, the fish will die directly from the gas bubble disease. In milder cases, the fish often dies from secondary infections which set in in the damaged tissues.

The Corps of Engineers is cognizant of this problem and anxious to take positive steps both toward its possible alleviation in the Columbia and Snake Rivers, and toward its prevention at future projects. While the documentation of nitrogen levels and associated fish mortalities has already been initiated by the Bureau of Commercial Fisheries (2), it is necessary that these data be complemented by engineering studies on the physics of nitrogen absorption and desorption at these projects. Information emanating from such studies could be used by the Corps in its reservoir planning, design and operation to alleviate or control nitrogen supersaturation at its projects.

The most direct way of acquiring this information is in the form of a mathematical nitrogen (N_2) model which will simulate the process of entrainment in the spillway and stilling basin and subsequent deaeration in the downstream reservoir. Such a model could be used to investigate alternative operational control measures and the effectiveness of various project design alterations.

OBJECTIVES AND SCOPE

The objective of the study reported herein was to develop a mathematical model of the behavior of dissolved nitrogen (N_2) in the lower Columbia and Snake Rivers. The model was to be formulated on physical principles to the maximum possible extent, and to be specifically designed to simulate the absorption and desorption of dissolved nitrogen gas in the lower Columbia and Snake River systems. The mathematical model was to be incorporated into a computer program written in the FORTRAN IV programming language for numerical solution. Results from the mathematical model were to be compared to dissolved nitrogen observations taken from a prototype situation operating under a known set of hydraulic and hydrologic conditions. This comparison was to be made on the basis of available sampling and engineering data for John Day Dam and The Dalles Reservoir and was to be used to establish the accuracy and confidence which can be placed in the model.

This report describes the formulation and testing of the N_2 model. In Chapter II which follows, the mathematical model is formulated and the basic elements of the computer code are presented. Chapter III describes model testing and the evaluation of empirical coefficients. The results of the model application to John Day Dam and The Dalles Reservoir are also presented and discussed. Chapter IV contains a discussion of the model including its general applicability, limitations,

and possible extention and modifications. Conclusions and recommendations complete the chapter.

In addition to this report, a Computer Supplement and User's Guide has been prepared under separate cover. This document contains the detailed documentation of the computer program and instructions for its use. The program documentation includes descriptions of input data requirements, flow charts depicting program logic, definitions of program variables, and definitions of output.

AUTHORITY AND RESPONSIBILITY

The work reported herein was performed under Contract No. DACW57-70-C-0075 dated 16 February 1970, between the U. S. Army Corps of Engineers, Portland District, and Water Resources Engineers, Inc. All aspects of the technical work were conducted by the staff of WRE under the supervision of Dr. Gerald T. Orlob, President.

Dr. Larry A. Roesner coordinated the project and was responsible for the theoretical development. Mr. W. R. Norton developed the computer simulation package and User's Guide.

Technical representatives for the Corps of Engineers were:

Peter B. Boyer
Edward M. Mains
Philbin F. Moon

ACKNOWLEDGMENTS

WRE has enjoyed the close liaison and cooperative attitude that prevailed between itself and the Corps of Engineers during the performance

of this work. The establishment of this rapport is attributable in large part to Mr. Peter B. Boyer who has been closely associated with this study from its start. The attitude and enthusiasm of Mr. Boyer materially aided the smooth and successful completion of this study.

II. MODEL DEVELOPMENT

The processes that govern nitrogen concentrations in the reservoir system of the lower Columbia and Snake Rivers embody two distinct and separable phenomena: air entrainment and nitrogen absorption in the spillway-stilling basin combination, and desorption in the reservoir reaches between dams. In this chapter models are formulated for each of these components and the basic outline of the computer program that links them is described. Since both of these models are derived from the basic physical relationships governing gas transfer across gas-liquid interfaces, these concepts are presented briefly below as a prelude to the model development itself.

CONCEPTS OF GAS TRANSFER

Two concepts of gas transfer are important to understanding the gas absorption and desorption phenomena that are observed in the lower Columbia and Snake Rivers. These are the concepts of *equilibrium concentration* and of *the transfer mechanism* itself.

Equilibrium Relationships

When a gas that is soluble in a liquid is brought into contact with that liquid, molecules of the gas will diffuse across the gas-liquid interface into the liquid. Concurrently, gas molecules in solution will leave the liquid phase and enter the gas phase. The direction and rate of the net transfer depends upon the difference in gas concentration between the liquid and gas phases. If the two phases are left in contact for a sufficient period of time, the rate at which gas molecules enter

solution becomes equal to the rate at which they return to the gas phase and the system is said to be in *equilibrium*.

For gases such as N_2 and O_2 that are only slightly soluble in water and do not react chemically with the water, the equilibrium concentration, C_E, of the gas in the water is given by Henry's law as

$$C_E = \frac{1}{H} p \tag{1}$$

where p is the partial pressure of the gas in the gas phase and H is Henry's constant. The constant is different for each gas and varies with temperature.

To determine C_E in equation 1, the partial pressure of the gas must be known. If the gas phase is air, p can be determined as follows. The <u>total</u> pressure P of the gas phase is the sum of the partial pressures of its component gases. Since air is composed principally of N_2, O_2, and A (78, 21 and 1 percent by volume, respectively), the total air pressure is the sum of the partial pressures of N_2, O_2, and A, i.e.

$$P = p_{N_2} + p_{O_2} + p_A. \tag{2}$$

The partial pressure of water vapor in the air has been neglected in equation 2. Now, for our purposes air can be assumed to follow the perfect gas laws. Hence, the ratios of the partial pressures of the component gases to the total pressure of the gas mixture may be taken as equal to the volume ratios. Using the volume ratios for air given above,

$$\frac{p_{N_2}}{P} = 0.78 \, , \frac{p_{O_2}}{P} = 0.21 \, , \frac{p_A}{P} = 0.01 \tag{3}$$

Substituting these relationships into equation 2 gives

$$P \simeq 0.78\,P + 0.21\,P + 0.01\,P = 1.00\,P \qquad (4)$$

As long as the volume percentages of the air remain constant, equations 3 and 4 hold whether we are considering pressures at the water surface or pressures existing at the air-water interface of an air bubble at a depth d below the water surface. Thus, we can write equation 1 for nitrogen and oxygen as

$$C_E = \frac{0.78}{H_{N_2}} P \qquad \text{for nitrogen} \qquad (5A)$$

and

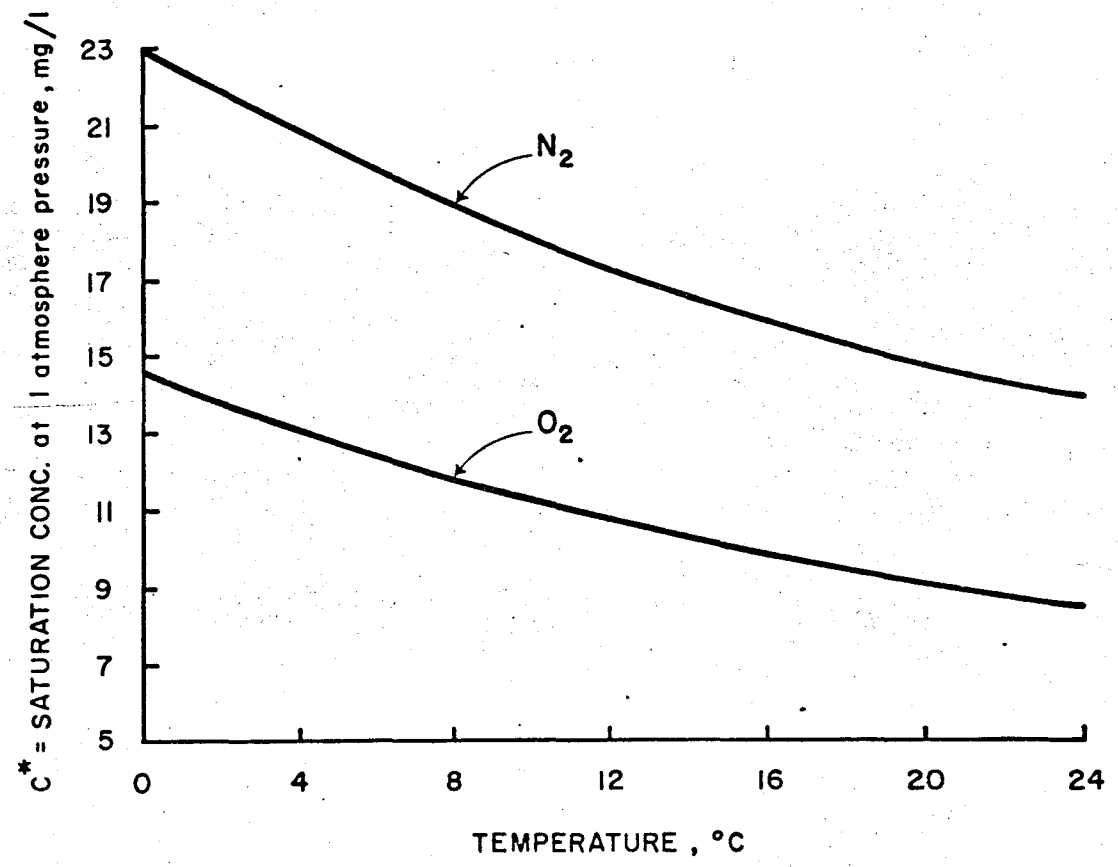
$$C_E = \frac{0.21}{H_{O_2}} P \qquad \text{for oxygen} \qquad (5B)$$

where P is the total pressure of the air at the air-water interface.

If we now assign units of atmospheres to the total air pressure P, we can write equation 5 as

$$C_E = C^*P \qquad (6)$$

where C^* is $0.78/H_{N_2}$ for nitrogen and $0.21/H_{O_2}$ for oxygen, and has units of (mg/l)/atmosphere. In other words, C^* is the equilibrium (or saturation) concentration of the gas at the surface of the water under one atmosphere of pressure. A plot of C^* versus temperature is shown in Figure 2 for nitrogen and oxygen. These values are based on solubility data for nitrogen and oxygen in the 40th edition of the *Handbook of Physics and Chemistry*.



Based on solubility data for nitrogen and oxygen in the 40th edition of the *Handbook of Chemistry and Physics*

SATURATION CONCENTRATIONS OF N_2 AND O_2 FOR DRY AIR OVER WATER UNDER ONE ATMOSPHERE OF PRESSURE

FIGURE 2

Where gas transfer is occurring between the water and air bubbles at a depth d below the water surface the equilibrium concentration of the gas in the water at that depth is expressed as

$$C_E = C^* (P_o + \alpha d) \qquad (7)$$

where P_o is the atmospheric pressure in atmospheres and α is the specific weight of water in atm/foot of depth. We will use equation 7 as the defining equation for the equilibrium or saturation concentration of nitrogen at a depth d in water.

The Transfer Mechanism

Two theories have been advanced to explain the mechanism of the exchange of gases that occurs when a liquid containing dissolved gases is brought into contact with an atmosphere of gas other than that with which it is in equilibrium. These are called the *penetration theory* and the *film theory*.

The penetration theory was first proposed by Higbie in 1935 (3). According to this theory, eddies originating in the turbulent bulk of the liquid migrate to the gas-liquid interface, where they are exposed briefly to the gas before being displaced by other eddies arriving at the interface. During their brief residence at the interface, the eddies absorb molecules from the gas phase. Upon return of the eddies to the liquid bulk, the molecules are distributed by turbulence. The rate of transfer is considered to be a function of the diffusivity, of a concentration gradient, and of a surface renewal factor.

The film theory, introduced by Lewis and Whitman in 1924 (4), is based on a physical model in which one or two ficticious films exist at the gas-liquid interface; a liquid film is always assumed to exist

while the presence of a gas film may or may not be postulated depending on the details of the theory. These films are considered to be stagnant and furnish all resistance to gas transfer. They are thought of as persisting regardless of how much turbulence is present in the gas and liquid, the turbulence serving only to reduce the film thickness. Details of both the film and the penetration theories can be found in references (5), (6), (7) and (8).

Regardless of the theory used, it is assumed that the liquid phase controls the rate of gas transfer between the two phases. The gas phase is assumed to supply sufficient molecules of gas to keep the liquid phase saturated at the air-water interface. One of the two exchange theories is then used to represent the rate of transfer of the gas molecules from this interface, which has a concentration C_E as given in equation 7, into the bulk liquid phase which has a concentration C. In both theories, however, the transfer rate is assumed to be directly proportional to the concentration difference $(C_E - C)$, and the area of the air-water interface across which the transfer is occurring. Furthermore, the rate at which the gas is transferred from the interface into the bulk phase must be equal to the rate at which gas molecules are replaced on the water surface; hence

$$N = \frac{dM}{dt} = K_L A (C_E - C) \qquad (8)$$

where

N = the local rate at which the gas is transferred across the air-water interface,

M = the mass of the gas diffusing across the interface,

t = time,

K_L = the overall mass transfer coefficient, and

A = the total interfacial area over which the gas transfer is occurring.

Equation 8 is known as Fick's first law.

When equation 8 is applied to gas transfer between the atmosphere and the water, A is the surface area of the water. When gas transfer between submerged air bubbles and the water is being computed, A is the surface area of the air bubbles.

The manner in which K_L is theoretically defined depends upon whether the penetration theory or the film theory is used to describe the gas transfer phenomenon. In practice, however, K_L reflects the degree of turbulence in the system. Its minimum value, which is the molecular diffusivity of the gas, is approached as the system approaches quiescence. Its maximum value, which is several orders of magnitude greater than the molecular diffusivity, occurs when mixing within the system is violent.

We are now ready to develop the actual model components for the spillway-stilling basin combination and the reservoir reach. Equations 7 and 8 will be used to describe the basic gas transfer relationships in both components of the model.

SPILLWAY-STILLING BASIN REPRESENTATION

The spillway and stilling basin form the first model component whereby nitrogen gas is driven into solution. As the surface spill plunges into the stilling basin below, the physical entrainment of air bubbles occurs. These bubbles are carried with the main flow to the bottom of the stilling basin where they are subjected to the hydrostatic pressure of the overlying water. Under this excess pressure, nitrogen (and oxygen) is driven into solution resulting in gas concentrations in excess of the saturation concentration under one atmosphere of pressure and the so-called "supersaturated" condition is observed.

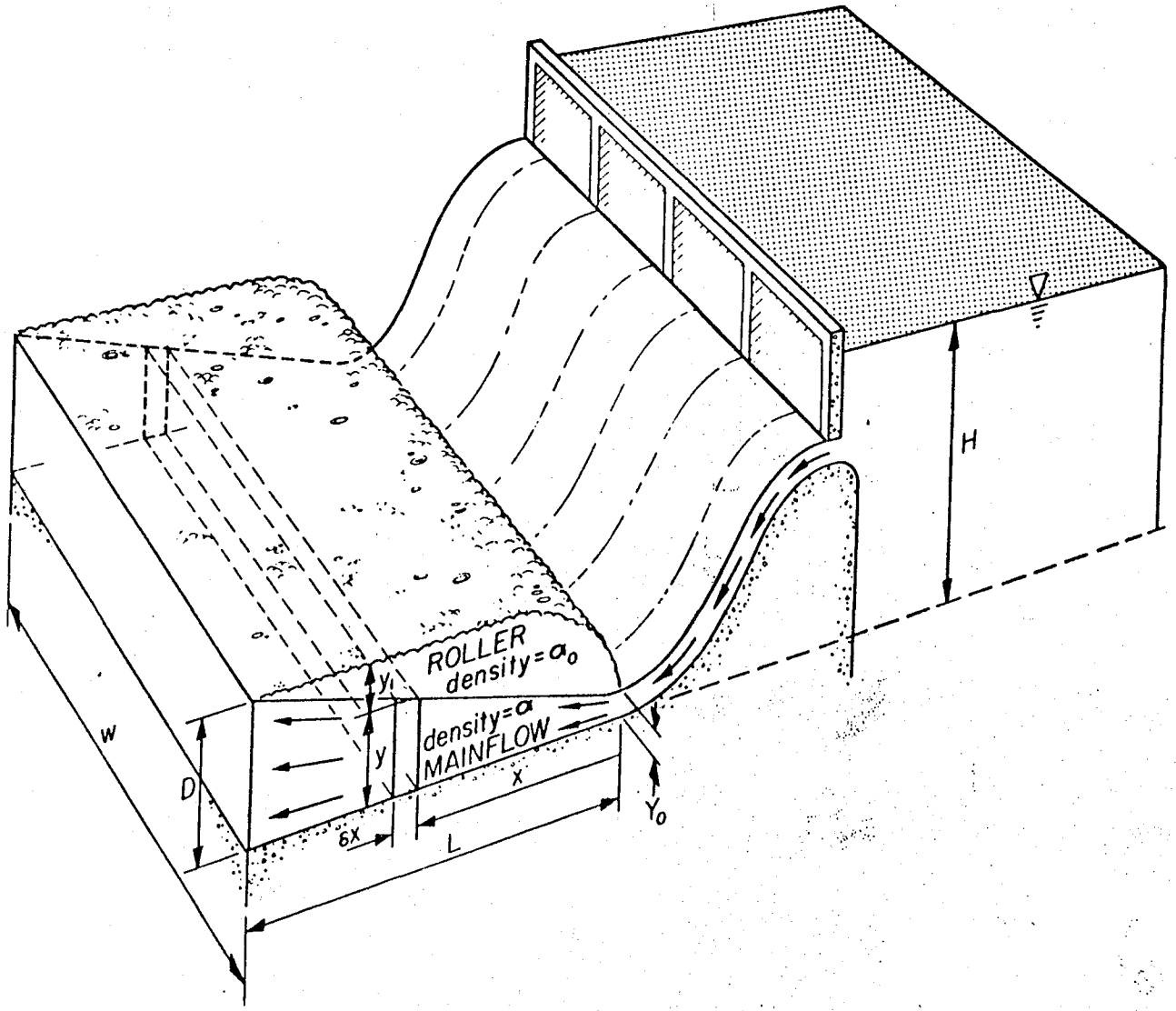
Conceptual Representation

The hydraulics of the spillway-stilling basin combination and the air entrainment phenomena are exceedingly complex and their exact mathematical description is virtually impossible. Hence, it is necessary to make some simplifying assumptions regarding the behavior of these processes, i.e., it is necessary to develop a *conceptual representation* of the prototype. The conceptual representation of the spillway-stilling basin combination envisioned for this study is shown in Figure 3.

It is presumed that the spill is uniform over the entire width of the spillway and that it enters the stilling basin at the point $x = 0$ at a depth Y_o. The jump that occurs is assumed to take place in a linear fashion, transforming the main flow from its initial depth Y_o to its sequent depth D which is the tailwater depth in the stilling basin. The energy loss on the spillway is assumed to be zero. The length L of the jump is taken as the length of the stilling basin. The width of the stilling basin w is an *effective width* that varies with the operation of the spillway. It is taken as the total effective width of spillway that is in operation at any given time.

The aerated roller which overlies the jump is assumed to have a specific weight α_o which is less than or equal to the specific weight of water α. Furthermore, it is assumed that there is no transfer of mass between the roller and the underlying main flow so that the only effect of the roller is to increase the hydrostatic pressure on the main flow by an amount $\alpha_o y_1$.

The water parcel shown in Figure 3 moves through the stilling basin decelerating and increasing in height y as it passes from $x = 0$ to $x = L$. The lateral dimension δx also varies as the parcel moves through the basin so that the volume of the parcel is maintained at a



CONCEPTUAL REPRESENTATION
OF THE SPILLWAY-STILLING BASIN COMBINATION

FIGURE 3

constant value $wy\delta x$. At the point $x = 0$, it is assumed that a mass of air M_A is entrained into the water as discrete bubbles of various sizes. The bubbles, which are assumed to remain uniformly distributed within the water parcel as it moves through the basin, are responsible for the nitrogen gas absorption that occurs in the stilling basin. The addition or removal of N_2 by biological or chemical processes is assumed to be insignificant.

Functional Representation

It is now possible to apply the gas transfer equations 7 and 8 to the conceptual water parcel described above. The fully developed mathematical description that results is called the *functional representation* of the prototype. For the sake of brevity, the development of the functional representation will be outlined only briefly here. The detailed derivation is presented in Appendix A.

Writing equation 7 again for reference,

$$\frac{dM}{dt} = K_L A (C_E - C) \tag{7}$$

We now apply this expression to the water parcel as it travels through the stilling basin. In the water parcel, the mass of the nitrogen M in solution is the product of the concentration C in the water parcel times the volume of the parcel, i.e.,

$$M = (wy\delta x)C \tag{9}$$

Since the equilibrium concentration varies linearly with depth, the effective value of C_E for the water parcel is its value at the mid-depth of the parcel, i.e. at the depth $d = y_1 + y/2$. However, since there is water of two different densities overlying this point, the pressure term $(P_o + \alpha d)$ in equation 8 must be written as $[P_o + \alpha_o y_1 + \alpha(y/2)]$.

Substituting this pressure term into equation 8 and writing y_1 in terms of D and y the expression for C_E becomes

$$C_E = [P_o + \alpha_o D - (\alpha_o - \tfrac{\alpha}{2})y]C^* \qquad (10)$$

The air bubble surface area also varies with depth since air is compressible. However, this variation is not linear, hence a value based on mid-depth properties is incorrect. Equation A-16A in Appendix A gives the proper expression for the total surface area A of the air bubbles in the water parcel as

$$A = (w\delta x)K_A \left\{ [P_o + \alpha_o D + (\alpha - \alpha_o)y]^{1/3} - [P_o + \alpha_o D - \alpha_o y]^{1/3} \right\} \qquad (11)$$

where K_A is system constant reflecting the total air mass entrained and its distribution among the various bubble sizes. The temperature of the water also affects this constant (see the expression for K_A in equation A-16B in Appendix A).

If equations 9, 10, and 11 are substituted into equation 7, and the resulting expression is simplified, there results

$$\frac{dC}{dt} = \frac{K}{y} \left\{ [P_o + \alpha_o D + (\alpha - \alpha_o)y]^{1/3} - [P_o + \alpha_o D - \alpha_o y]^{1/3} \right\} \left\{ [P_o + \alpha_o D - (\alpha_o - \tfrac{\alpha}{2})y]C^* - C \right\} \qquad (12)$$

where $K = K_L K_A$. The solution of equation 12 as shown in Appendix A, yields the functional representation for nitrogen entrainment in the spillway-stilling basin combination as

$$C_S = \overline{P}C^* - (\overline{P}C^* - C_F)e^{-\frac{K}{q} L \overline{\Delta P^{1/3}}} \qquad (13)$$

where

C_S = N_2 concentration of water leaving the stilling basin, mg l^{-1},

C_F = N_2 concentration in the forebay, behind the dam, mg l^{-1},

C^* = equilibrium concentration of N_2 in water under air at one atmosphere of pressure, mg $l^{-1}atm^{-1}$,

K = empirical coefficient, ft sec^{-1} $atm^{-1/3}$,

L = stilling basin length, ft,

q = discharge per unit width of spillway, ft^2 sec^{-1},

$\overline{P}$ = average hydrostatic pressure in the main flow in the stilling basin, atm,

$$= P_o + \frac{\alpha_o}{2}(D-Y_o) + \frac{\alpha}{4}(D+Y_o),$$

P_o = atmospheric (or barametric) pressure at the site, atm,

$$\overline{\Delta P^{1/3}} = [\overline{P} + \frac{\alpha}{4}(D+Y_o)]^{1/3} - [\overline{P} - \frac{\alpha}{4}(D+Y_o)]^{1/3}, \text{ atm}^{1/3},$$

α = density of the mainflow, atm ft^{-1},

α_o = density of the roller, atm ft^{-1},

Y_o = $q/\sqrt{2gH}$ ft, and

H = assumed total energy head at entrance to stilling basin (see Figure 3), ft.

We can define the *effective equilibrium concentration*, $\overline{C}_E$ for the spillway-stilling basin combination as $\overline{P}C^*$. If the forebay concentration C_F is less than $\overline{P}C^*$, nitrogen will be driven into solution as the spill passes through the stilling basin. However, if C_F is greater than $\overline{P}C^*$ the stilling basin actually drives the gas out of solution. Some idea of the value of the *effective equilibrium concentration* associated with the stilling basin can be acquired by evaluating $\overline{P}$ at its extreme values, i.e.

$$\overline{P} = P_o + \frac{3}{4}\alpha D - \frac{1}{4}\alpha Y_o \qquad \text{for } \alpha_o = \alpha, \text{ and} \qquad (14A)$$

$$\overline{P} = P_o + \frac{\alpha}{4}(D + Y_o) \qquad \text{for } \alpha_o = 0. \qquad (14B)$$

For the purpose of the discussion here, P_o can be taken as 1.0 atmosphere, α as one atmosphere per 34 feet of water, and Y_o as zero. Thus, from equation 14,

$$\overline{P} \simeq 1 + \frac{3}{4}\left(\frac{D}{34}\right) \qquad \text{for } \alpha_o = \alpha \qquad (15A)$$

and

$$\overline{P} \simeq 1 + \frac{1}{4}\left(\frac{D}{34}\right) \qquad \text{for } \alpha_o = 0 \qquad (15B)$$

Now, noting that $\overline{C}_E/C^* = \overline{P}$, the term ($\overline{P}$ x 100) could be defined as the *saturation potential* of the stilling basin. That is to say, irrespective of the N_2 concentration in the forebay, the N_2 concentration of the water leaving the stilling basin tends toward a value that is ($\overline{P}$ x 100) percent saturated with respect to the saturation concentration at the water surface. Furthermore, this value is a function of the tailwater depth and the density of the roller only. Thus, if the tailwater in a stilling basin is 45 feet, the saturation potential of that basin would be between 133 and 199 percent, corresponding to values of $\alpha_o = 0$ and $\alpha_o = \alpha$, respectively. This idea will be pursued further in Chapter 3 where the results of testing the spillway-stilling basin model are discussed.

The empirical coefficient K will be defined as an *entrainment coefficient*. It has been defined under equation 12 as the product of the *overall mass transfer coefficient* K_L and the *air entrainment coefficient* K_A. Its expanded form is given by equation (A-19) in Appendix A as

$$K = \frac{3}{\alpha}\left(\frac{6\sqrt{\pi}R}{28.9}\right)^{2/3}\left\{K_L T^{2/3} M_A \int_0^1 n_b^{-1/3}\, dB\right\} \qquad (16)$$

where

R = universal gas constant,

T = absolute temperature,

M_A = mass of air entrained per unit volume of main flow, and

B = that fraction of the total air mass having a mass per bubble less than or equal to n_b.

The term $\frac{3}{\alpha}\left(\frac{6\sqrt{\pi}R}{28.9}\right)^{2/3}$ is a constant, independent of time and of system configuration. The term in braces, however, is dependent upon both the physical configuration and operation of the spillway-stilling basin system. It is also temperature dependent.

The overall mass transfer coefficient, K_L, was discussed earlier, and essentially accounts for the increased rate of mass transfer across the air-water interface with increased turbulence in the system. It is also temperature dependent. Reasonable estimates of its value can be made for systems of rather simple geometric configuration; however, to determine its value in the stilling basin, empirical tests would be required.

The term $M_A \int_0^1 n_b^{-1/3}\, dB$ reflects both the total mass (per unit volume) of air entrained in the main flow and the distribution of that mass among the various bubble sizes. It is to be expected that for a given set of hydraulic conditions the term would vary among the various dams as a result of variations in their physical geometry. Moreover, variations are also to be expected at a given dam as the hydraulic operation is varied. The term is probably affected also by temperature. Since these variations cannot be described by first principles, this term would have to be evaluated empirically also.

6) Thus we see that both K_L and $M_A \int_0^1 n_b^{-1/3} dB$ must be evaluated empirically; however, since they appear as a product in equation 16, an independent evaluation of each term is impossible. Hence the product must be evaluated empirically. Moreover, since both terms are affected by the water temperature, the functional dependence of equation 16 on water temperature cannot be analytically described either. Thus, all the terms in equation 16 are lumped together in the *entrainment coefficient K*, which must be evaluated for each spillway-stilling combination. On the basis of the discussion above, we would expect K to vary with the turbulence level in the stilling basin, water temperature, and the physical configuration of the spillway-stilling basin combination. The empirical evaluation of K for the projects investigated is described in Chapter III.

RESERVOIR REPRESENTATION

Conceptual Representation

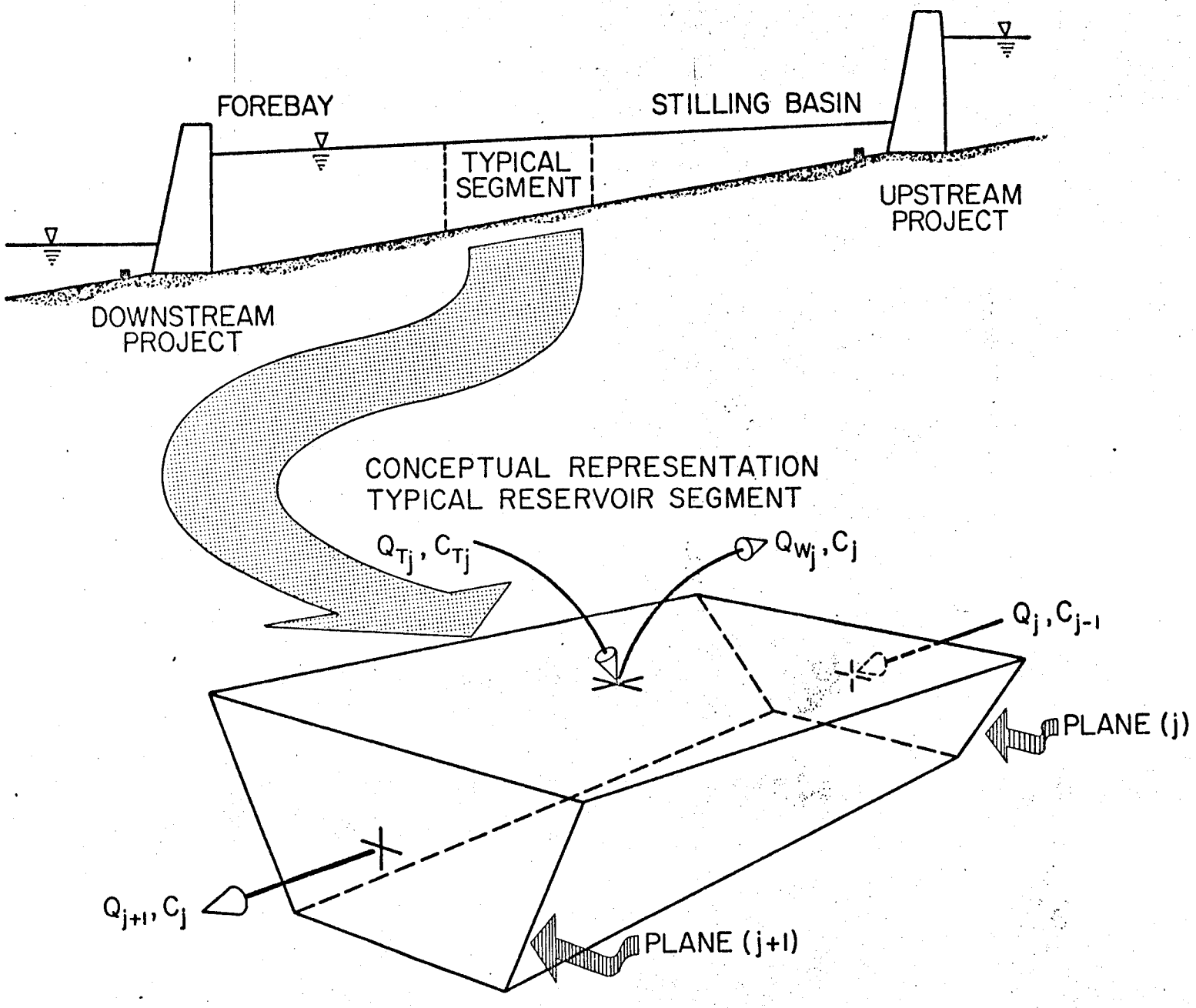
As a general proposition, the dams and reservoirs found in the lower Snake and Columbia system are of the low head, run-of-river type. These dams have been purposefully designed to take maximum advantage of available hydraulic head, and thus are positioned such that the tailwater of an upstream project is coincident with the headwater of the next project downstream. In the majority of the projects, the average flow-through time is quite short, typically on the order of days rather than weeks or months. This means that the hydraulic velocities are of sufficient magnitude in the reservoir bodies to make them quite similar to slowly moving streams. The dams in this system have been constructed to a height such that the reservoirs fill most of the preproject stream channel; hence, there is relatively little opportunity for major flood control with these structures. The difference in the water surface elevations

of the forebay and tailwater at the projects remains relatively constant over many days of system operation. Compared to the flow in the main stream of the Snake and Columbia, tributary inflows are minor; thus, any particular reservoir can be considered to be non-branching in form.

With the above considerations in mind, the reservoirs of the lower Snake and Columbia have been conceptually envisioned to be a slowly moving stream which is fully mixed in its vertical and transverse dimensions. At any instant in time the reservoir's water surface can be considered to be a plane surface, although it need not be horizontal. The upstream boundary of each reservoir is considered to be just below the stilling basin of the upstream project, and the lower boundary is located in the forebay of the downstream project. The reservoir is assumed to have a trapezoidal cross section, with a uniform ratio of bottom to surface width at all pool elevations. At any instant in time the reservoir is treated as if it has a constant width over its entire length, and the reservoir's bottom is considered to be a planar surface which slopes uniformly over the length of the reservoir. The planes of the water surface and the bottom need not be parallel. A pictorial representation of the ideas embodied in the conceptual representation of the reservoirs of the lower Snake and Columbia systems is shown in Figure 4.

Functional Representation

The functional representation used to describe the behavior of dissolved nitrogen in the subject reservoirs is obtained by describing the continuous system implied by Figure 4 as a collection of smaller segments of finite dimension. Within each segment all system state variables are assumed uniform, and it is tacitly assumed that an investigation of the behavior of the collection of segments gives an adequate description of the complete system. A pictorial representation of one of the system segments is also shown in Figure 4.



CONCEPTUAL REPRESENTATION TYPICAL SYSTEM RESERVOIR

FIGURE 4

The procedure actually employed for the mathematical description of dissolved nitrogen consists of writing a mass balance for nitrogen around each reservoir segment, and then, through an appropriate numerical approximation, to solve the resulting sets of equations for nitrogen concentrations. Appendix B contains a complete description of the equations actually used; for the more casual reader the following brief explanation is presented to give an overview of the processes included in the simulation.

Again, referring to Figure 4, consider an arbitrary finite segment, j, defined and bounded by vertical planes j and $j+1$. This segment is assumed to be bounded on both its upstream and downstream sides by segments of the same type as segment j.

A differential equation for the mass balance of nitrogen can be written around segment j in the form:

$$\left(\frac{dM}{dt}\right)_j = F_{A_j} + F_{X_j} + F_{S_j} + F_{D_j} \qquad (17)$$

where

$\left(\frac{dM}{dt}\right)_j$ = the time rate of change of mass of nitrogen associated with segment j,

F_{A_j} = the net mass flux of nitrogen for segment j by the internal advection process (flow between segments),

F_{X_j} = the net mass flux of nitrogen for segment j by external advection processes (external additions or withdrawals of flow),

F_{S_j} = the net mass flux of nitrogen for segment j by exchange across the air-water interface, and

F_{D_j} = the net mass flux of nitrogen for segment j by the eddy diffusion process.

As opposed to equation 7, which was applied to mass transfer around a water parcel moving through the stilling basin, equation 17 is

written for a segment that is fixed with respect to the stream channel and has water flowing through it. The term F_{S_j} is defined by the right-hand side of equation 7 (with $C_E = C^*$), and describes the rate of nitrogen loss across the air-water interface as flow moves through a segment. Both this term and the other terms on the right-hand side of equation 17 can be expressed in terms of the state variables of segment j, and its bonding segments, j-1 and j+1. To clarify the situation, consider the following definition for segments j-1, j, and j+1.

A_j = the cross sectional area at plane j,

A_{j+1} = the cross sectional area at plane j+1,

A_{S_j} = the effective surface area of segment j,

C_j = the nitrogen concentration of segment j,

C_{j-1} = the nitrogen concentration of segment j-1,

C_{T_j} = the nitrogen concentration associated with flow Q_{T_j},

C^*_j = the saturation concentration for dissolved nitrogen at the local stream temperature,

D_j = the eddy diffusivity coefficient at plane j,

D_{j+1} = the eddy diffusivity coefficient at plane j+1,

D_m = the molecular diffusivity for nitrogen at the local stream temperature,

$\frac{dV}{dt}$ = the rate of change of volume for segment j,

H_j = the average depth of segment j,

Q_j = the flow through plane j,

Q_{j+1} = the flow through plane j+1,

Q_{T_j} = the flow tributary to segment j,

Q_{W_j} = the flow withdrawn from segment j,

U_j = the stream velocity associated with segment j,

V_j = the volume of segment j, and

Δx = the length of each system segment in the direction of flow (all segments are equal in length).

Employing these definitions, the terms in equation 17 can be defined

$$\left(\frac{dM}{dt}\right)_j = V_j \left(\frac{dC}{dt}\right)_j + C_j \left(\frac{dV}{dt}\right)_j \tag{18A}$$

$$F_{A_j} = Q_j C_{j-1} - Q_{j+1} C_j \quad , \tag{18B}$$

$$F_{X_j} = Q_{T_j} C_{T_j} - Q_{W_j} C_j \quad , \tag{18C}$$

$$F_{S_j} = K_{L_j} A_{S_j} (C_j^* - C_j) \quad , \text{ and} \tag{18D}$$

$$F_{D_j} = \frac{A_j D_j}{\Delta x} (C_{j-1} - C_j) - \frac{A_{j+1} D_{j+1}}{\Delta x} (C_j - C_{j+1}) \tag{18E}$$

The overall mass transfer coefficient, K_{L_j} has been defined as

$$K_{L_j} = \left(\frac{D_m U_j}{H_j}\right)^{1/2} \tag{19}$$

This expression was developed by O'Connor and Dobbins (9) and is based on a rationally conceived physical model of the mechanism of gas transfer across the air-water interface. It is dimensionally correct for any consistent set of units.

The area term A_{S_j} is the true water surface area of the segment and in general will be greater than the planar projection of the surface area of the segment A_{P_j}. Thus we define A_{S_j} as

$$A_{S_j} = (1 + B_a')A_{P_j} \tag{20}$$

where B_a' is a coefficient that accounts for the increased surface area of the water as the result of wind, turbulence, channel irregularities, etc. Now, combining equations 17, 18, 19 and 20, and simplifying, the nitrogen mass balance for segment j can be written in the form:

$$V_j\left(\frac{dC}{dt}\right)_j = C_{j-1}\left(Q_j + \frac{A_jD_j}{\Delta x}\right) - C_j\left(Q_{j+1} + Q_{W_j} + \frac{dV}{dt_j} + (1 + B_a')\,A_{P_j}\left(\frac{D_mU_j}{H_j}\right)^{1/2} + \frac{A_jD_j + A_{j+1}D_{j+1}}{\Delta x}\right) + C_{j+1}\left(\frac{A_{j+1}D_{j+1}}{\Delta x}\right) + Q_{T_j}C_{T_j} + (1 + B_a')A_{P_j}\left(\frac{D_mU_j}{H_j}\right)^{1/2} C_j^* \tag{21}$$

In the actual computer solution, equation 21 is written for each segment in the reservoir. The resulting set of equations is then combined with a suitable numerical approximation, (see Appendix B), providing a necessary second set of equations. This set of equations is then solved simultaneously, yielding a description of segment concentrations at a particular instant in time. Through recursive operation the process is repeated, moving forward in time, to produce a complete time and space description of nitrogen behavior in the system reservoir.

The reservoir model contains two system parameters that must be specified prior to prototype simulation. The first is the average ratio

of the surface width to the bottom width for the reservoir. This parameter specifies the shape of the trapezoidal cross section of the segments. The second parameter is the *effective surface area factor* B_a' as defined in equation 20.

It becomes immediately apparent that the two parameters are interrelated. If the width ratio used to describe the reservoir cross section is too small, the channel representation will be overly rectangularized causing the surface width to be smaller in the model representation than in the prototype. This causes the planar area A_{P_j} used in the model to be smaller than in the prototype. If the model is to produce a true simulation in this case, the parameter B_a' will have to correct for misrepresentation of channel geometry in addition to reflecting the non-planar nature of the reservoir's actual water surface. This interrelationship should cause no difficulty, however, as long as one is aware of its existence. The best way to avoid this situation is to fit trapezoids to several typical reservoir cross sections and derive an average width ratio from these fitted sections.

The value of B_a' required to adequately account for the non-planar nature of the reservoir's surface will require a certain amount of experience and analysis. Contained in the Computer Supplement and User's Guide is a summary of the present level of experience, with suggestions for values of B_a'.

COMPUTER MODELING AND SYSTEM SIMULATION

Coupling For Total System Simulation

The simulation of a complete system, consisting of one or more dams and reservoirs, is accomplished through successive applications of

the previously described spillway-stilling basin and reservoir models. As should be clear from the model descriptions, each dam and reservoir is considered to behave as if it were functionally independent of all other dams and reservoirs of the system. The coupling of system components is brought about by the so-called *downstream coupling process*, whereby the output from an upstream simulation is retained at the time of its generation and later used as input to the subsequent simulation of the next project downstream. This process is perfectly valid for the lower Snake and Columbia systems; moreover, it is easy to employ, interpret, and understand. Simply stated, then, the complete simulation of a system of dams and reservoirs is accomplished through the successive application of the individual simulation procedures developed for dams and reservoirs.

The downstream coupling processes has been a primary concern in the design and development of the computer codes for this project. The present version of the computer program is coded in a form such that the simulation routine for the reservoir operates independently from the routine for spillway-stilling basin simulation. The two independent routines are directed and called from an executive routine that is coded to make alternate reservoir/spillway-stilling basin simulation calls regardless of the beginning condition. The retention of data for downstream coupling is handled through an intermediate, on line, storage device such as a magnetic tape or disk. The computer program is described in detail in the Computer Supplement and User's Guide.

Data Requirements

As is to be expected, a certain amount of physical and operational data is required for nitrogen simulation in stilling basins and reservoirs. The following paragraphs outline the data that are required for the simulation. Data requirements are subdivided into time independent and time dependent categories. Normally, time dependent data are specified as mean hourly or mean daily values.

Spillway-Stilling Basin Simulation Data Requirements

Time Independent Data

1. Length of the stilling basin.
2. Elevation of the stilling basin floor.
3. Average spillway gate width.
4. The specific gravity of the air-water mixture over hydraulic jump in the stilling basin.
5. Two empirical coefficients, a and b, which are used to describe the nitrogen exchange in the stilling basin. These coefficients are described extensively elsewhere in this report.

Time Dependent Data

1. Total hydraulic discharge at the project.
2. Spillway discharge.
3. Water surface elevation in the project forebay.
4. Tailwater elevation in the stilling basin.
5. Number of spillway gates open.
6. Nitrogen concentration in the forebay.
7. Water temperature in the forebay.

Reservoir Simulation Data Requirements

Time Independent Data

1. The total reservoir length.
2. The volume-stage relationship for the reservoir.
3. The reservoir's effective bottom elevation at its upstream and downstream boundaries.
4. The ratio of top to bottom width for the assumed reservoir trapezoidal cross section.
5. The system parameter B_a' relating the effective water surface area to the planar projection of the reservoir's surface area.
6. The initial longitudinal nitrogen profile in the reservoir.

Time Dependent Data

1. Total reservoir inflow.
2. Total reservoir outflow.
3. The water surface elevation at the upstream boundary of the reservoir.
4. The water surface elevation at the downstream boundary of the reservoir.
5. The average water temperature in the reservoir.
6. The concentration of nitrogen entering the reservoir by upstream inflow (concentration of combined spillway and turbine discharge).

The above data items constitute the data base necessary for dissolved nitrogen simulation in the lower Snake and Columbia river system.

III. MODEL TESTING AND DEMONSTRATION

The spillway-stilling basin model developed in the previous chapter was tested with prototype data taken in 1967, 1968, and 1969 at Bonneville, The Dalles, and Ice Harbor Dams and with data taken in 1968 and 1969 at John Day Dam. The data were taken over the spill period each year and represent most of the prototype operational conditions that would be encountered under the present operating rules for the dam-reservoir system.

The reservoir model was tested and calibrated on The Dalles Reservoir with data taken in 1969. These model components were then coupled and the Columbia River reach from the forebay of John Day Dam to the forebay of The Dalles Dam was simulated for the period 1 May 1969 through 28 July 1969. The details of this testing and demonstration are presented in this chapter.

CALIBRATION AND TESTING OF THE SPILLWAY-STILLING BASIN MODEL

The spillway-stilling basin model defined by equation 13 contains three parameters that require definition:

1. α, the density of the main flow,
2. α_o, the density of the roller, and
3. K, the entrainment coefficient.

The following sections describe how these parameters are determined.

Evaluation of Roller and Mainflow Densities

Until now, the density of the mainflow has been specified as a parameter in order to preserve generality in the model development. However, there is little reason to expect it to be significantly different from the density of water in prototype facilities; hence, we now set the value of α as a constant equal to the density of water in all applications of the model. This value is

$$\alpha = 0.0295 \text{ atmospheres/ft. of water} \tag{22}$$

In contrast to the density of the mainflow, it would be expected that the density of the roller would be less than that of water because of the large quantity of air entrained within it. Since prototype data on the density of the roller did not exist, prototype simulations were made using values of α and $\alpha/2$ for the density of the roller, α_o. No advantage could be found for using a value of α_o different from α especially since there was no rational method for selecting it. Thus, we suggest that the value of α_o be taken equal to that of water, i.e. $\alpha_o = \alpha$. There is one exception to equation 22.

When the model was tested on the prototype data, with $\alpha_o = \alpha$, good results were obtained for the Bonneville, John Day and Ice Harbor projects; however, The Dalles project could not be simulated satisfactorily. The data indicated that nitrogen desorption often occurs in the stilling basin, a phenomenon that could not be reproduced with the model. This meant that the "saturation potential" as defined by equation 15A was too large. The only way to reduce the saturation potential is to reduce α_o (see the definition of $\overline{P}$ under equation 13). A review of operational data and some available literature (10) on the Columbia River dams revealed that The Dalles has the lowest tailwater of the four dams tested and that it is the only one of the four dams where the jump is

not submerged during the periods of spring runoff (this is probably because of the low tailwater). When the jump is not submerged, the roller degenerates from the triangular wedge shape indicated in Figure 3 to a thin rectangular sheet overlying the mainflow and its effect on the total pressure $\overline{P}$ in the mainflow becomes negligible. Setting α_o equal to zero is equivalent to specifying this phenomenon for the strong jump, and it produces a significant decrease in the saturation potential as seen from equation 15B. The use of $\alpha_o = 0$ resulted in a satisfactory representation of The Dalles project.

On the basis of the experience described above, we recommend that α_o be defined as follows:

$$\alpha_o = \alpha = 0.0295 \text{ atmos/ft. for submerged jumps} \qquad (23A)$$

$$\alpha_o = 0 \qquad \text{for unsubmerged jumps} \qquad (23B)$$

Determination of the Entrainment Coefficient

The discussion of the *entrainment coefficient K* in Chapter II revealed that it is a function of the turbulence level in the stilling basin and of the air entrainment characteristics of the stilling basin. It was also noted that the definition of *K* for the various stilling basins would have to be obtained empirically.

It is well known that the turbulence level in the stilling basin varies directly with the rate of energy loss in the basin. It also seems reasonable to expect that the air entrainment characteristics of the stilling basin would vary with the turbulence level and thus with the rate of energy loss. As the turbulence level increases, the mass of air entrained into a water parcel traveling through the stilling basin would

be expected to increase and the average bubble size to decrease. It can be shown that the effect of increasing the mass of entrained air and decreasing the bubble size is to increase the term $M_A \int_0^1 n_b^{-1/3} dB$ (see equation 16) and thus to effect an increase in K. Therefore, it is concluded that the entrainment coefficient varies directly with the turbulence level in the stilling basin and hence with the rate of energy loss.

If equation 13 is solved for K, there results

$$K = \frac{q}{L\ \overline{\Delta P}^{1/3}}\ ln\left(\frac{\overline{PC}^{*}-C_F}{\overline{PC}^{*}-C_S}\right) \qquad (24)$$

Before equation 24 could be used to back calculate K from operational data, it was necessary to make some assumptions regarding the composition of the nitrogen samples collected downstream from the dams. These samples were taken one-fourth to one-half mile below the dam and thus contained a mixture of spillway and turbine discharge. In order to compute C_S for use in equation 24 from the nitrogen concentration C of the sample, the following two assumptions were made:

1. For all dams except Bonneville (and John Day in 1968 when there was no turbine discharge) the sample represented a complete mix of spillway and turbine discharges.

2. The nitrogen concentration in the turbine discharge was equal to the nitrogen concentration in the forebay.

With these assumptions, the dissolved nitrogen concentration C_S of water leaving the stilling basin was computed as

$$C_S = \frac{QC - (Q-Q_S)C_F}{Q_S} \qquad (25)$$

where

C = N_2 concentration measured at the downstream sampling location,

C_F = N_2 concentration measured in the project forebay,

Q = total river discharge, and

Q_S = discharge over the spillway.

The value of C_S computed by equation 25 was used in the calculation of K by equation 24 except at Bonneville where C_S was measured.

Back computations of K were made at Bonneville, The Dalles, John Day, and Ice Harbor dams. These values were further reduced to the common temperature base, 20°C by the relationship

$$K = K_{20}\,(1.028)^{T-20} \tag{26}$$

where K_{20} is the equivalent value of K at 20°C and T is the water temperature in °C. This expression is commonly used to describe the temperature effect on the overall mass transfer coefficient K_L and is assumed to be applicable to the entrainment coefficient K. The constant 1.028 was rather arbitrarily selected, but it falls at about the midpoint of reported values for the constant which ranges from 1.015 to 1.047.

The rate of energy loss was computed as the total headloss through the stilling basin divided by the residence time of the flow in the stilling basin and was defined as

$$E = \frac{\text{Total Headloss}}{\text{Residence Time}} = \frac{q(H-D)}{DL} - \frac{q^3}{2gD^3L} \tag{27}$$

where

$$\text{Total Headloss} = H - \left(D + \frac{V_L^2}{2g}\right) = H - D - \frac{q^2}{2gD^2}\text{, ft, and}$$

$$\text{Residence Time} = \frac{DL}{q}\text{, sec.}$$

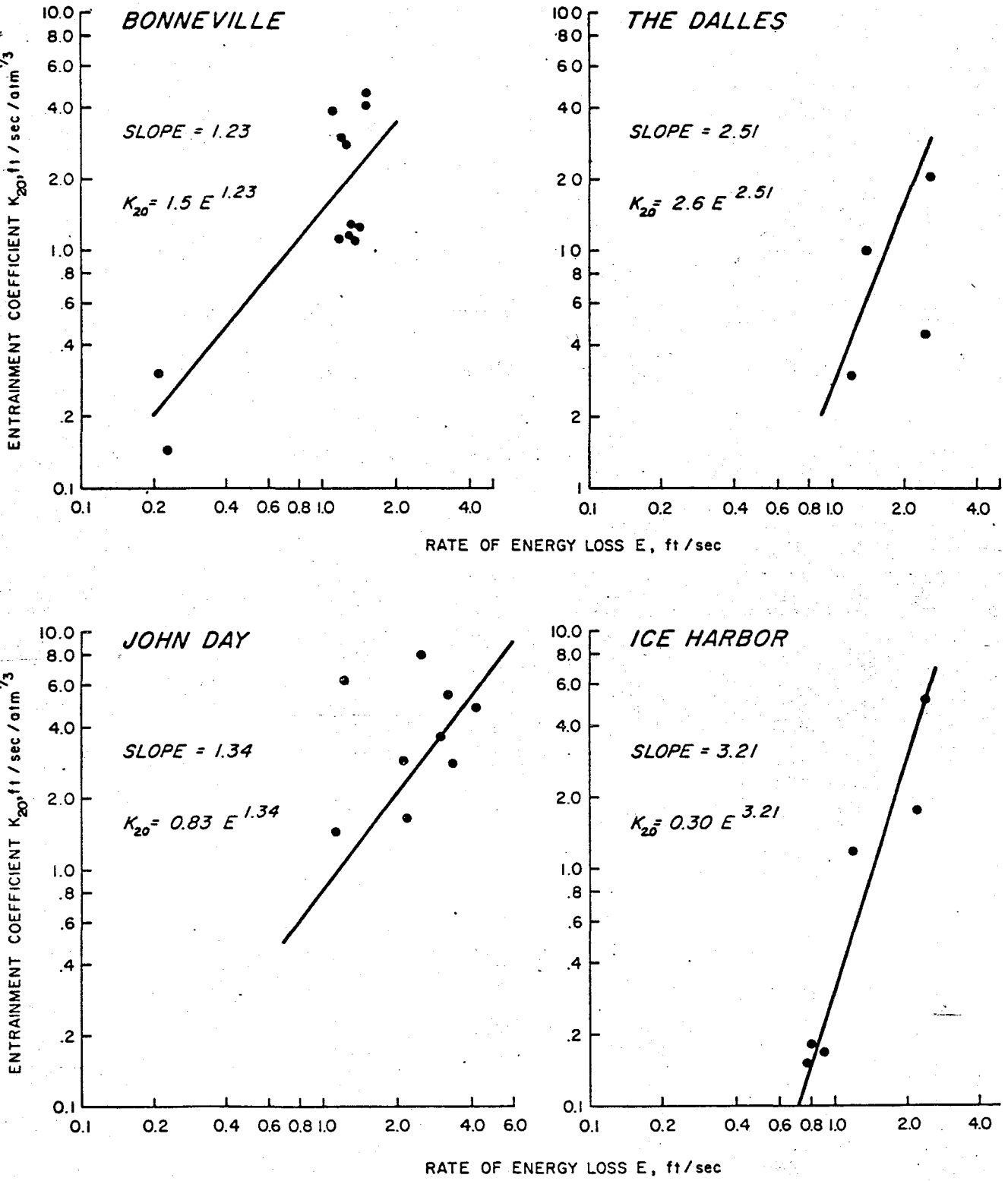
Definitions of the variables can be found on pages 17 and 18 under equation 13.

A log-log plot of the computed entrainment coefficient K_{20} versus the rate of energy loss E in the stilling basin is shown in Figure 5 for each of the four dams tested. Also shown is the log-log relationship that we believe best fits the plotted points. These curves were positioned on the basis of our judgment regarding the validity of the individual points. Mathematical curve fitting procedures were applied initially, but were discarded because they placed too much weight on extreme points.

It is our opinion that the scatter in the plots is due largely to the use of assumption one on page 36 for computing C_S from the measured concentration C downstream. While mixing of the spillway discharge and the turbine discharge certainly occurred, it is possible that the mixing was not complete, especially since the samples were collected on the spillway side of the river channel. Moreover, the degree of mixing probably varied with the relative magnitudes of the two discharges. Thus, the assumption of complete mixing, which was made for lack of better information, probably produced errors in the computed values of C_S.

On the basis of available data, the second assumption appears valid.

One other factor that probably affected the computed values of K is that the samples were collected at the water surface and at a point downstream where "pinpoint bubbles could no longer be observed in the water" (11). This observation implies that the gas absorbed in the stilling basin could be effervescing upstream of the sample point, i.e. the "pinpoint bubbles" are <u>not</u> air bubbles, but rather, they are pure N_2 gas bubbles coming out of solution near the water surface where the pressure is insufficient to keep it in solution. If this is true, the



TEMPERATURE COMPENSATED ENTRAINMENT COEFFICIENT, K_{20}
VS
RATE OF ENERGY LOSS, E

FIGURE 5

values of C_S computed from equation 27 are probably lower than the true values. We simply note here that it would be desirable to have data collected at the stilling basin or as close thereto as possible without endangering human life. Recommendations with regard to future data sampling are contained in Chapter IV.

Returning again to the empirical description of the entrainment coefficient, the fitted relationships between K and E are shown on the plots in Figure 5. In all cases the relationship is

$$K_{20} = aE^b \tag{28}$$

Values for a and b are given in Table 1 for each of the dams.

TABLE 1
COEFFICIENTS FOR K-E RELATIONSHIP*
IN THE STILLING BASIN

DAM	a	b
Bonneville	1.5	1.23
The Dalles	2.6	2.51
John Day	0.83	1.34
Ice Harbor	0.30	3.21

*$K_{20} = aE^b$

$K = K_{20}(1.028)^{(T-20)}$

The units of K are ft. sec^{-1} $atm^{-1/3}$

The units of E are ft. sec^{-1}

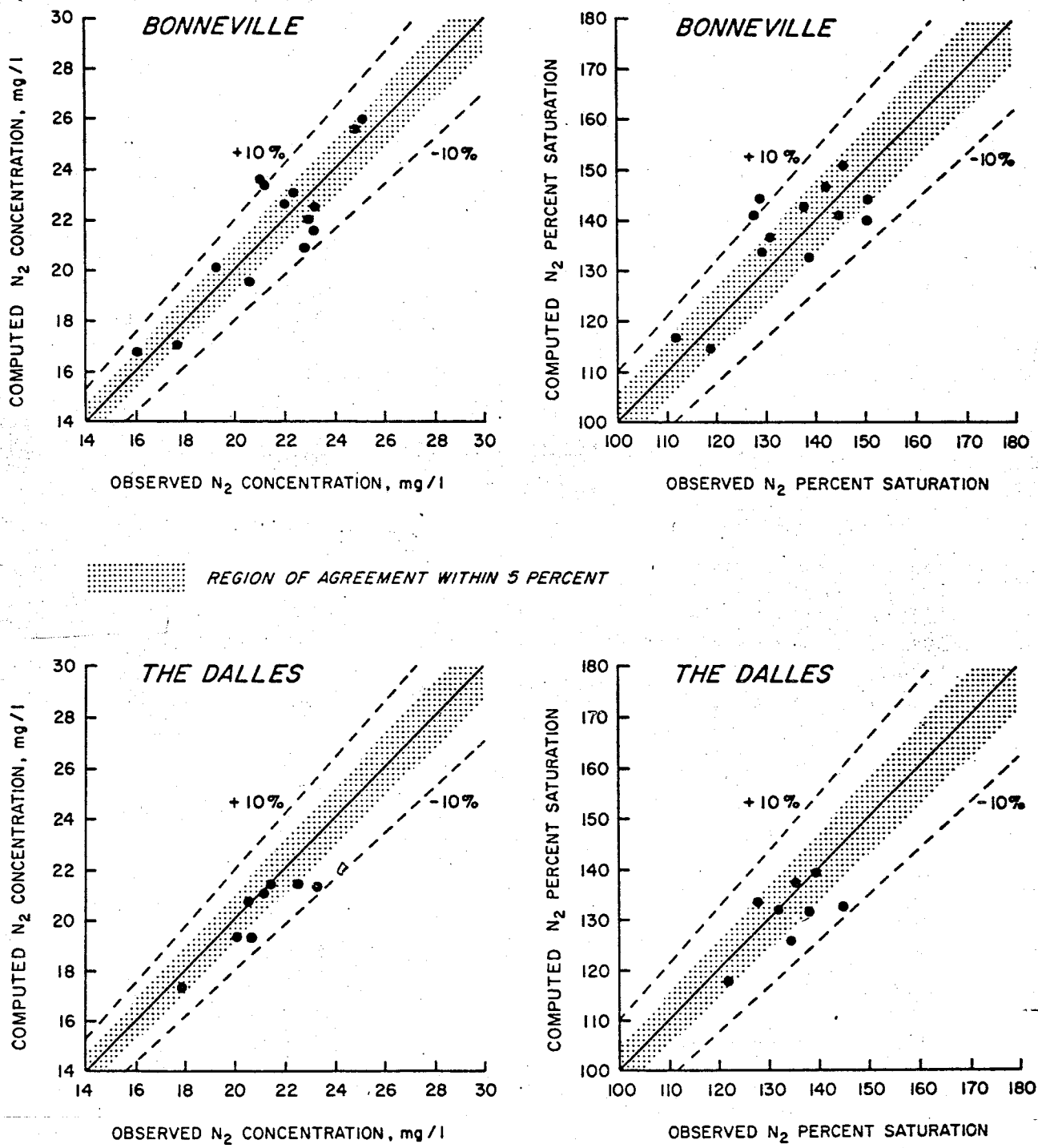
The units of T are °C

No apparent relationship is observed between the coefficients for the various dams, and none should be expected except, perhaps, between John Day and Ice Harbor. The hydraulic characteristics of the stilling basins are all significantly different. At Bonneville, for example, the spillway crest is submerged during the high runoff period. The Dalles, as noted previously, has a strong jump whereas the jumps at the other projects are submerged. John Day and Ice Harbor dams are most similar hydraulically, but the stilling basin floors are different. At John Day, jump control is maintained by an abrupt rise at the end of the concrete apron while two rows of blocks are used for this purpose in Ice Harbor. Perhaps the analysis of the other dams on the lower Snake and Columbia rivers would give more insight into the relationship of these parameters among the various dams. Also, better dissolved nitrogen data for the stilling basins would allow better estimates to be made of these coefficients.

Reliability of the Spillway-Stilling Basin Model

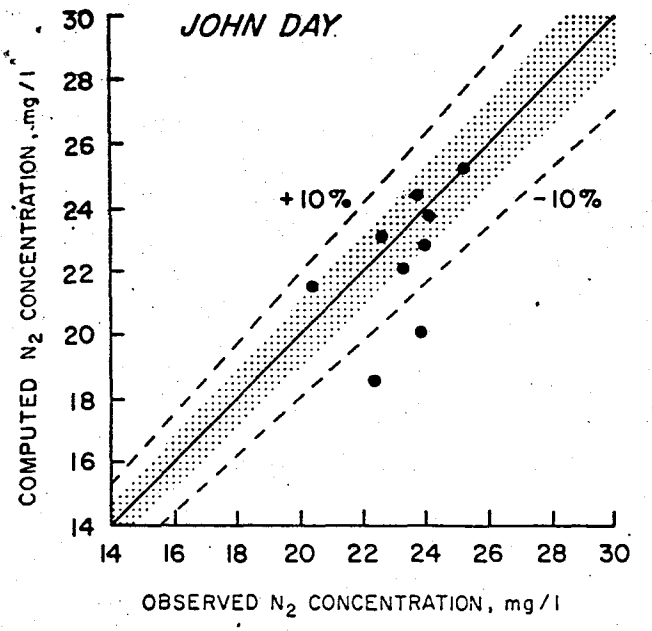
Employing the coefficients given in Table 1 for K_{20}, and equation 25 for computing K, the spillway-stilling basin model was solved for C_S by equation 13, using observed hydraulic data and forebay concentrations. Computations were made at each dam for every day on which nitrogen samples were taken in 1968 and 1969. These computed values are compared to the measured values at each of the dams in Figures 6-A and 6-B. The computed values have been corrected by equation 25 to account for mixing with the turbine discharge. Comparisons are shown for both concentration and percent saturation (i.e. 100 x (C/C^*)).

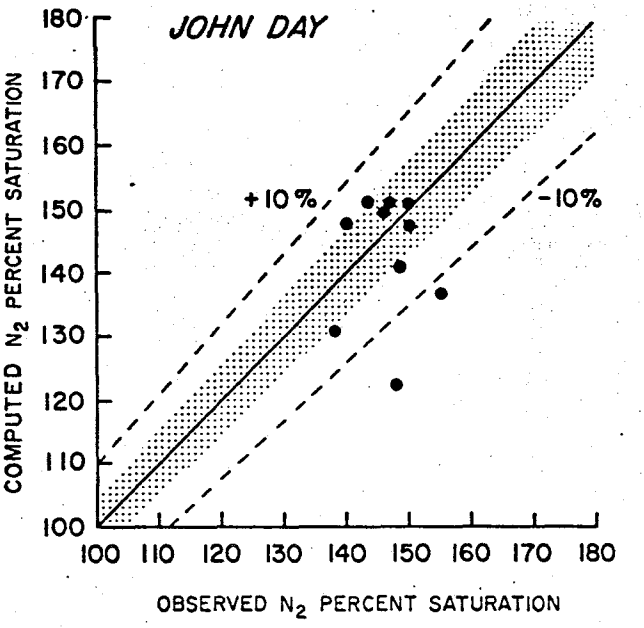
The agreement is very good especially in view of the difficulties encountered with the data measured downstream from the projects. In the forward simulation, 68 percent of the computed values are within 5 percent of the observed values, and 85 percent of the computed values are within 10 percent of the observed values.



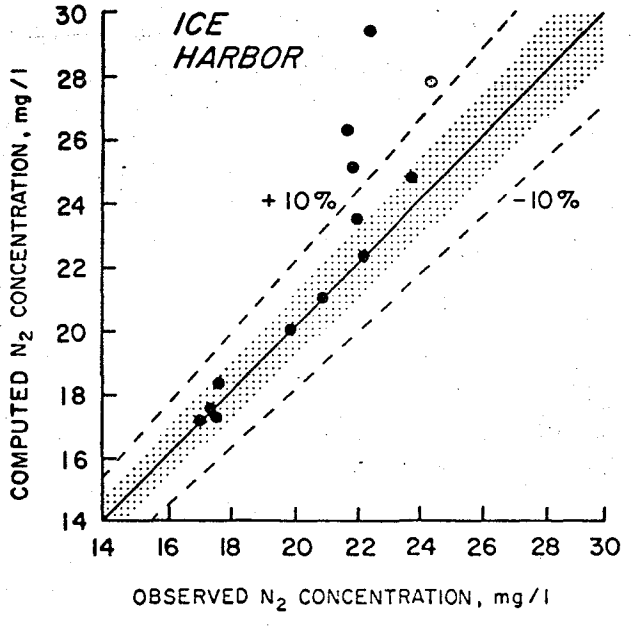
COMPARISON OF OBSERVED DISSOLVED NITROGEN VALUES WITH VALUES COMPUTED WITH SPILLWAY - STILLING BASIN MODEL
(BONNEVILLE AND THE DALLES DAMS)

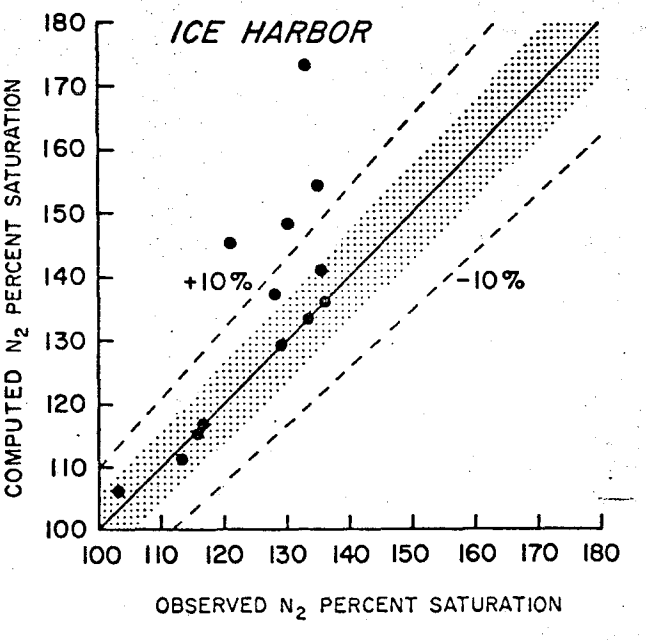
FIGURE 6-A





REGION OF AGREEMENT WITHIN 5 PERCENT





COMPARISON OF OBSERVED DISSOLVED NITROGEN VALUES WITH VALUES COMPUTED WITH SPILLWAY - STILLING BASIN MODEL
(JOHN DAY AND ICE HARBOR DAMS)

FIGURE 6-B

CALIBRATION OF THE RESERVOIR MODEL

The reservoir model contains two system parameters that must be specified for operation of the program. These are the *width ratio*, which is the average ratio of the surface and bottom widths as determined from typical cross sections of the reservoir, and the *effective surface area factor* B_a'. These parameters and their interrelationships were discussed in Chapter II.

All calibrating and testing of the reservoir model was done using physical and hydraulic data from The Dalles Reservoir as inputs. Since we were unable to obtain cross sections of the reservoir, it was necessary to estimate the *width ratio*. On the basis of our knowledge of the Columbia River, a value of 2.0 was selected. Using this ratio, the volume-stage curve for the reservoir, and the water surface elevations at each end of the reservoir, the trapezoidal representation of the reservoir had a surface width of 4170 feet on the average. This surface width varied slightly as the stage changed at the reservoir ends.

Using a width ratio of 2.0, several preliminary test runs were made with the reservoir model using different values of B_a'. After examining the results of these test runs, it was concluded that a value of 2.0 would produce the best representations.

The values selected for the width ratio and B_a' both seem reasonable (although the area factor may be larger than necessary) and they produce satisfactory results in the reservoir model.

MODEL APPLICATION TO JOHN DAY DAM AND THE DALLES RESERVOIR

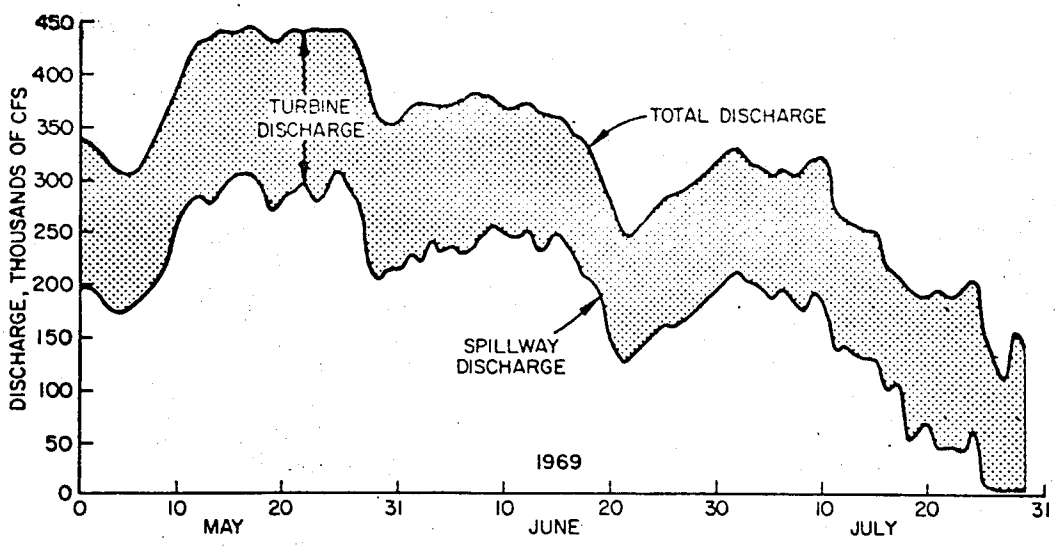
Having tested and calibrated the spillway-stilling basin and reservoir components of the dissolved nitrogen model, the computer

simulation model was applied to John Day Dam and The Dalles Reservoir (see Figure 1 in Chapter I). The simulation period extended from 1 May through 28 July 1969. This period was chosen because it contained the most measured N_2 data at the two projects.

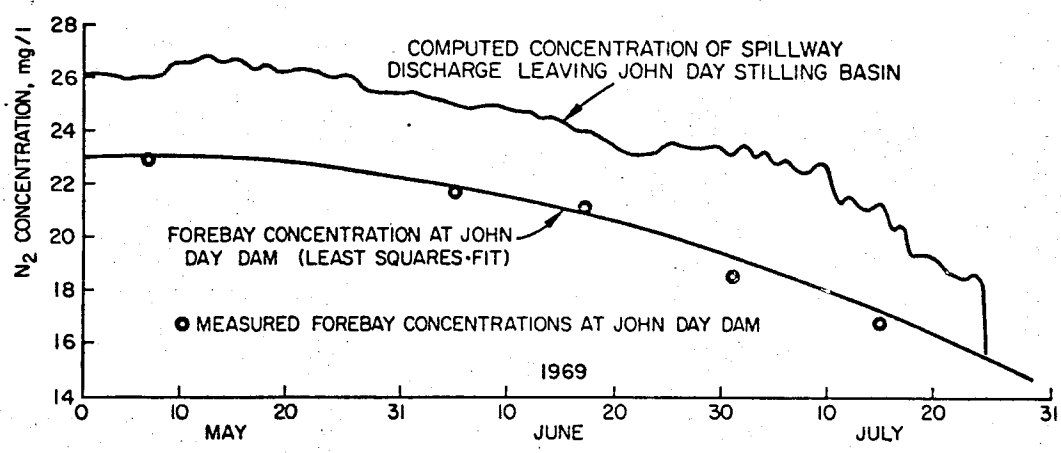
Mean daily total and spillway discharges at John Day Dam are shown in Figure 7A for the simulation period. The difference between the two curves is the turbine discharge. The total discharge curve for The Dalles project was not significantly different from that shown for John Day and thus has not been illustrated. Other pertinent operational data and modeling data are contained in Table 2.

Dissolved nitrogen concentrations in the forebay of the John Day Dam were supplied as input to the model. These values were then routed through the dam and through The Dalles reservoir downstream. Since there were only five measured N_2 concentrations in the John Day forebay, it was necessary to estimate values for the period between sampling days. A second order polynomial was fitted to these points and is illustrated in Figure 7B. The results of routing these input concentrations is illustrated in Figure 7B and 7C.

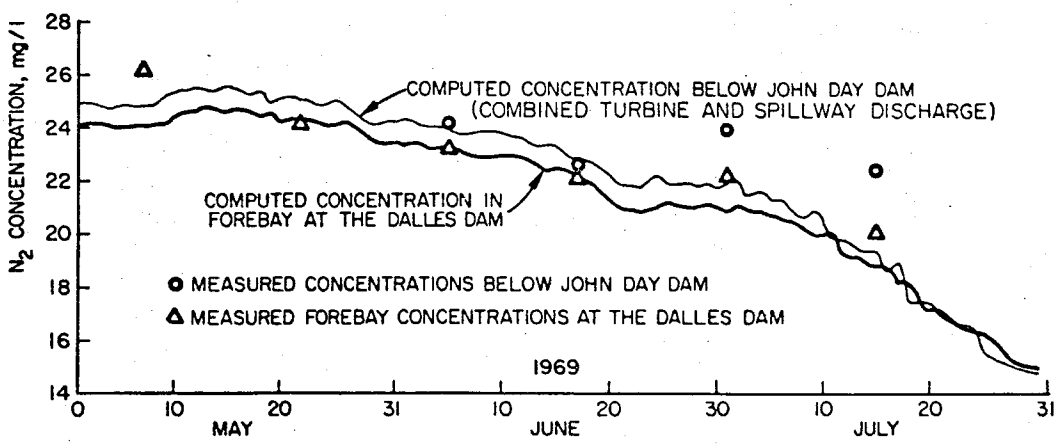
The upper curve in Figure 7B gives the computed N_2 concentrations of the spillway discharge as it leaves the stilling basin. Combining this flow with the turbine discharge and assuming complete mixing, produces the computed N_2 concentration at the point of measurement downstream from the dam. These values and the measured values are shown in Figure 7C. Finally, routing these values through The Dalles reservoir produces the computed N_2 concentrations in the forebay of The Dalles Dam. This value is shown as the lower curve in Figure 7C. Measured values in the forebay are also shown. It is interesting to note that the concentration curves in Figure 7C cross near the end of the simulation period. This does not mean that the reservoir model



A. DISCHARGE AT JOHN DAY DAM



B. N_2 ROUTING THROUGH JOHN DAY SPILLWAY AND STILLING BASIN



C. N_2 ROUTING THROUGH THE DALLES RESERVOIR

COMPARISON OF COMPUTED AND OBSERVED NITROGEN CONCENTRATION

(JOHN DAY AND THE DALLES PROJECTS)

FIGURE 7

TABLE 2

HYDRAULIC CHARACTERISTICS AND SYSTEM DATA FOR
JOHN DAY DAM - THE DALLES RESERVOIR SIMULATION
(Simulation Period 1 May - 28 July 1969)

	Maximum	Minimum	Average During Simulation Period
WATER SURFACE ELEVATIONS, FT.			
John Day Forebay	267.8	258.3	262.8
John Day Tailwater	167.0	162.9	164.3
The Dalles Forebay	160.0	156.0	158.2
TOTAL HYDRAULIC HEAD AT JOHN DAY DAM, FT.	104.6	91.4	98.5
WATER TEMPERATURE, °C	20.6	10.6	16.0
NUMBER OF GATES OPEN ON SPILLWAY AT JOHN DAY DAM	20 (all)	12	14

OTHER DATA

John Day Dam

Spilling Basin Length, ft.	185.0
Spilling Basin Floor EL, ft.	114.0
Spillway Gate Width, ft.	62.0
1st Entrainment Coefficient, a	0.83
2nd Entrainment Coefficient, b	1.34
Specific Gravity Over Roller	1.00
Simulation Interval, hrs.	24.0

The Dalles Reservoir

Upstream Bottom Elevation, ft.	145.0
Downstream Bottom Elevation, ft.	80.0
Total Reservoir Length, miles	20.0
Effective Surface Area Factor, B_a'	2.0
Ratio of Surface/Bottom Width	2.0
Number of Reservoir Segments	10.0
Simulation Interval, hrs.	24.0

is predicting N_2 concentration increases as the water travels through the reservoir, but rather, the result is caused by the time of travel through The Dalles reservoir, i.e. there is a time lag of about two days between the time a water parcel leaves John Day Dam and the time it reaches The Dalles Dam. To illustrate, water arriving at The Dalles Dam on the 26th of July with a N_2 concentration of 16.1 mg/l, actually left John Day Dam on the 24th with a N_2 concentration of 16.8 mg/l. The amount of nitrogen lost to the atmosphere during its trip through the reservoir (0.7 mg/l) was less than the amount by which the N_2 concentration in the discharge leaving the John Day Project dropped during the same period. Consequently, the nitrogen concentration in The Dalles forebay is greater on the 26th than that below John Day Dam.

On the basis of this simulation alone it is difficult to make a judgment with regard to the general validity of the system simulation model. For example, there are only five measured values downstream from John Day Dam with which to compare the values computed in the spillway-stilling basin component of the model. Moreover, two of the measured values are considerably higher than the computed values; these values are, unfortunately, the same two values that fell outside the ten percent agreement limits for John Day Dam in Figure 6-B. Considering the agreement between the computed and measured results below John Day Dam for the other eight points shown in Figure 6-B, we conclude that the reported values of the N_2 concentrations measured on 1 July and 15 July were either too low in the forebay or too high below the dam. It is also possible that the river was not completely mixed at the point of measurement below the dam as assumed. Such an event would also account for the discrepencies.

In view of the problem described above, and considering the general problems associated with the measurements taken below the dam, as discussed earlier in this chapter, a better assessment of the

capabilities of the model can be made by comparing the end result of the simulation, i.e., by assessing the ability of the model to route N_2 concentrations from the forebay of John Day Dam to the forebay of The Dalles Dam, which is a complete dam-reservoir unit. This comparison is tabulated below.

Difference Between Measured and Computed N_2 Concentrations in THE DALLES Forebay	Number of Points
less than 0.1 mg/l	3
less than 1.3 mg/l	5
less than 2.2 mg/l	6 (all)

Considering the small amount and the type of data available for comparison, we do not wish to press the argument for verification further. We simply state that, based on the results shown in Figure 6 for the spillway-stilling basin model, and on our general experience with the reservoir model, we believe that the dissolved nitrogen simulation model truly represents the prototype behavior in the lower Snake and Columbia River systems. However, we strongly suggest that the model be tested further with the nitrogen data obtained in the 1970 sampling program on the Columbia and Snake Rivers. We also suggest that supplemental data be gathered nearer the stilling basins of the dams for additional testing and calibration of the spillway-stilling basin model. This topic is discussed more fully in the following chapter under Recommendations.

IV. CONCLUSIONS AND RECOMMENDATIONS

GENERAL APPLICABILITY OF THE MODEL

The dissolved nitrogen simulation model developed in Chapter II was formulated from the basic physical laws of gas transfer and hydraulics. As such, it accounts for all significant geometric and hydraulic factors that affect nitrogen absorption and desorption in the system and thus can be used to study the efficacy of alternative operating rules and project designs for controlling nitrogen levels at Corps projects. On the basis of the testing and calibration reported in Chapter III, we believe that the model is applicable to all the Corps' projects on the lower Snake and lower Columbia Rivers with possible extensions to other projects as discussed below.

Applicability and Limitations of the Spillway-Stilling Basin Model

The spillway-stilling basin component of the model should be applicable to all low head dams whose geometric configurations are similar to those of the dams studied. Since the model formulation does not explicitly include the spillway geometry, we expected that the model could also be applied to high head dams as long as the stilling basin can be characterized hydraulically by the conceptual representation shown in Figure 3. The model would not be valid for flip bucket spillways, for example, since they have hydraulic characteristics that are basically different from those of the dams studied herein.

There are, of course, limitations on the generality of the spillway-stilling basin model. In addition to the requirement of geometric similarity just mentioned, there is the matter of selecting the

empirical coefficients a and b which are required to describe the entrainment coefficient (see equation 28 and Table 1). The results of testing the model on Bonneville, The Dalles, John Day, and Ice Harbor Dams indicate that these parameters may depend upon particular characteristics of the spillway-stilling basin such as

1. Whether or not the outlet gates are submerged in the tailwater.
2. The presence or absence of blocks in the stilling basin.
3. Whether the jump is submerged or unsubmerged.
4. Spillway geometry.

Assessing the importance of these factors will require additional testing of the model on other existing prototypes in the Columbia and Snake Rivers.

Another factor, which is actually a problem rather than a limitation, is specifying the number of spillway gates that are open at any given time. The problem revolves around the assumption of uniform gate openings. This is rarely true. The typical situation is that all or nearly all of the gates are open with the gates near the center of the spillway being open significantly more than those at the ends of the spillway. It is our judgment that the overall behavior of the stilling basin is best described by taking the effective stilling basin width w as that portion which is receiving discharge from the gates that are opened significantly farther than the rest. About one-half of the time the number of gates to be used is obvious; the other half, the number could vary by two or three gates depending on an individual's judgment. Since there are 10 to 14 gates open on the average, the model is not overly sensitive to these variations. The model could be modified to accept individual gate discharges without a great amount of difficulty; however we do not believe that such an extension is warranted until supplemental N_2 data are gathered with which to test a model so modified. Data requirements for this testing are outlined under RECOMMENDATIONS in this chapter.

Applicability and Limitations of the Reservoir Model

Given the scope and specifications of the present work, a working mathematical model has been formulated to simulate the behavior of dissolved nitrogen in reservoirs at a level of sophistication that is consistent with present needs and requirements. The model and its associated computer code have been designed in a manner such that additional features and capabilities can be added to the model as needs arise. We believe that the reservoir model is applicable throughout the lower Snake and Columbia systems. In fact, this model and its mathematical description should be valid and applicable wherever a prototype situation approximates the model's conceptual representation as outlined in Chapter II. This includes streams as well as reservoirs. The major considerations which preclude more general applicability are the assumptions regarding the hydraulic behavior of the reservoir, i.e. that the reservoir system is completely mixed in the vertical and transverse planes. As long as these assumptions are met, the model is valid even though some geometrical modifications might be required in extreme situations. Adherence to these assumptions means, of course, that stratified reservoirs cannot be investigated with this model. While this restriction is not important in the lower Snake and Columbia systems, there is little doubt that the quality problems of stratified reservoirs are of importance and will merit more attention in the future.

Potential Modification and Extension of the Reservoir Model

As mentioned above, the reservoir model has been designed and structured such that additional features and capabilities can be added. A list of what are seen to be the most likely extensions of the model is given below. It is emphasized, however, that the model is adequate under the present problem specifications and that the modifications indicated below should be implemented only as specific requirements arise.

1. As the model now stands, only gross system inflow and outflows are specified by input. A logical model extension would be to allow the introduction and withdrawal of flow at any point along the reservoir's axis.

2. The present version of the model assumes no longitudinal temperature gradient within the reservoir. Under certain conditions it may prove desirable to modify the model to represent such thermal gradients in order to more realistically predict nitrogen behavior. As an even more flexible and desirable method of temperature specification, thought should be given to incorporating a dynamic, first principles temperature computation into the overall model framework.

3. Present experience suggests that advection is the most important mechanism of in-stream mass transport, especially when longitudinal concentration gradients are small as are the N_2 concentration gradients. With this in mind, the diffusion coefficients in the present computer model have been set to zero internally in the program. Should a situation be encountered where eddy diffusion is thought to be a significant component of the mass transfer, the data input routine should be modified to allow non zero values for the coefficient of eddy diffusivity to be read in.

4. With the model's present formulation, a certain amount of uniformity has been imposed on a reservoir's geometrical configuration. Uniform segment length and width are examples of this condition. Should a prototype be encountered where system geometry varies widely along the reservoir's axis, it would be a straightforward process to modify the model to allow completely variable geometrical descriptions for each segment in the reservoir.

5. It was stated in the model development that a description of the hydraulic behavior (water surface elevations and flows) must be supplied externally as input data to the model. While the model structure presented is primarily designed for quality behavior, it would not be too difficult to adapt any of several existing one dimensional hydrodynamic simulation techniques to the basic model framework. The inclusion of a hydrodynamic simulator would be very useful for the investigation of transients within the reservoir.

6. Finally, a model has been presented for the simulation of a particular quality characteristic of a reservoir: dissolved nitrogen. There is nothing in the model, however, which limits its use to this specific item, and in fact many other quality parameters can be simulated using a structure very similar to the one presented here. Virtually all conservative and many non-conservative substances could be investigated with this basic model structure. A most important quality parameter, dissolved oxygen, would be particularly suitable for this model, and would require a relatively small amount of effort to introduce.

The above list gives some indication of the potential of the reservoir model. We are hopeful that this work will serve the Corps as a logical and sound starting point for more ambitious quality modeling efforts that will follow.

SUMMING UP

It was the stated objective of this study to develop a mathematical simulation model for dissolved nitrogen in the lower Columbia and Snake Rivers. We believe that this objective has been satisfactorily achieved and that the dissolved nitrogen model presented in this report will serve as a valuable tool for identifying ways to control N_2 levels in the river system. The accuracy of the model is commensurate with the data that were available for testing and calibration. The availability of supplemental data will allow many of the refinements and extensions noted above to be included in the model should it become desirable to do so.

This study has not, by any means, solved the nitrogen embolism problem. The salmon and steelhead trout are still being subject to stress and death at Corps projects and will continue to be until a change is made in the operation or construction of the system. Moreover,

with the construction of Lower Granite Dam, the problem can be expected to worsen as one more free stream reach is eliminated from the system and another potential entrainment device is added. The solution to the nitrogen embolism problem is to control the amount of nitrogen absorption at the dams. Which control measures would be most effective can be determined by sensitivity analysis of the dissolved nitrogen model, particularly the spillway-stilling basin component. Sensitivity analysis would indicate those parameters which exert the greatest influence on the dissolved nitrogen concentration in the water. Sufficient funds were not available in this study to carry out such testing; however, on the basis of our experience with the model, and with the data, we believe that the tailwater depth and whether or not the jump is submerged are extremely important in determining the N_2 concentrations below the dams. Both of these variables are included in the *saturation potential* parameter $\overline{P}$ (see equation 15) and it could be that this is the most influential parameter in the model affecting N_2 concentrations below the dams. Sensitivity analysis will show whether or not this is true.

Another benefit of the sensitivity analysis is that it would indicate what modifications, if any, are required in the models as presented. It would also supply information that would aid the design of a supplemental data collection program. For example, if sensitivity analysis shows that the number of gates open is an important parameter, the spillway-stilling basin model should be modified to reflect the variability in the discharge of the individual gates, and supplemental N_2 data should be collected to test and calibrate the model so modified.

Finally, on the basis of the results from the sensitivity analysis, a number of alternative control plans should be devised and tested with the simulation model to determine the most feasible plan for controlling N_2 levels in the river system. The tests would be primarily on different ways of operating the system, but some of the plans could include

modifications in the physical structures. The plan selected from the alternatives would be based on both its costs and its effectiveness in maintaining N_2 concentrations in the system within the required limits.

RECOMMENDATIONS

On the basis of the results of model testing and calibration reported in Chapter III and the discussion and conclusions of this chapter, we make the following recommendations:

1. That the dissolved nitrogen simulation model be used to simulate the 1970 N_2 concentrations between the forebay of John Day and the forebay of The Dalles dams and that these values be compared with measured data gathered by the U.S. Bureau of Commercial Fisheries during the same period.

2. That the spillway-stilling basin model be calibrated on other projects to which the model is applicable and that a relationship be sought between the empirical coefficients a and b and the characteristics of the stilling basin as discussed above.

3. That the model, especially the spillway-stilling basin component, be subjected to rigorous sensitivity testing to determine those parameters that exert the greatest influence on the N_2 concentration in the water. Results of such an analysis should be used to develop alternative N_2 control plans for the lower Columbia and Snake River system, and to design a supplemental data collection program that will provide information for more precise testing and calibration of the model.

4. That a supplemental data collection program be designed to meet the requirements established in item 3. This program should include at least the considerations outlined below.

Suggestions for Supplemental Data Gathering

It is fairly evident from the references to data in this report that better nitrogen data is required below the projects. In particular, data is required that gives the N_2 concentration of the spillway discharge as it leaves the stilling basin. Suggestions for a supplemental data gathering program were submitted to the Corps on 18 August 1970 as a data Addendum to the second report of progress on this study. That addendum is attached to this report for reference as Appendix C.

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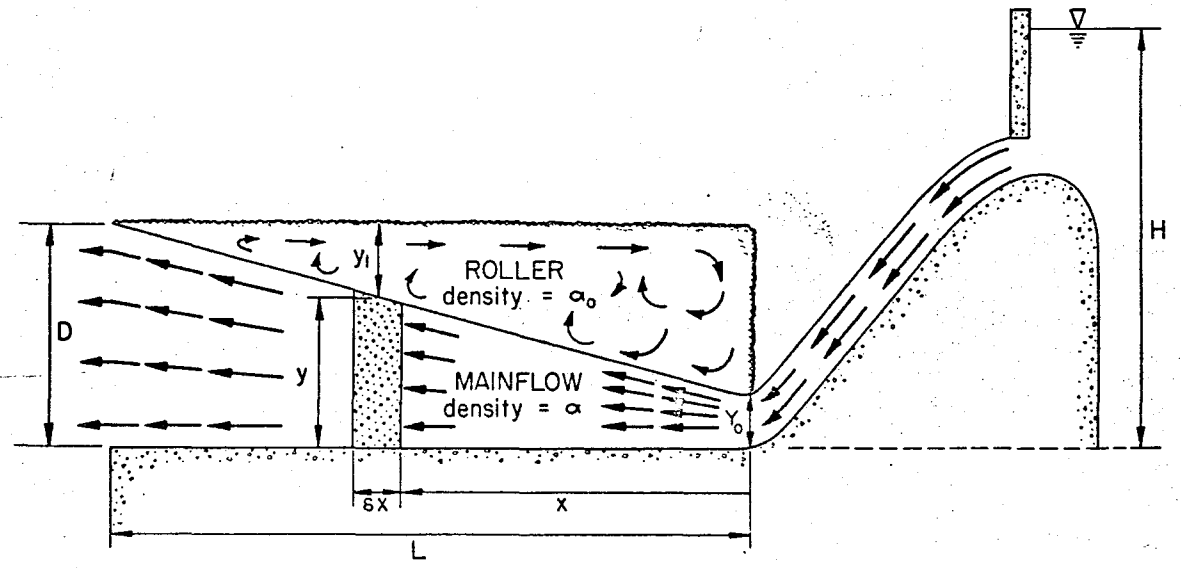
APPENDIX A

DERIVATION OF THE SPILLWAY-STILLING BASIN MODEL

APPENDIX A

DERIVATION OF THE SPILLWAY-STILLING BASIN MODEL

Consider the conceptual representation of the stilling basin shown below.



CONCEPTUAL REPRESENTATION OF SPILLWAY-STILLING BASIN COMBINATION

The water parcel indicated in cross-section by the shaded area moves through the stilling basin, decelerating and increasing in height. It extends laterally the full effective width, ω of the stilling basin as illustrated in Figure 3 of the main report.

We now make the following assumptions for the water parcel and stilling basin:

1. For that length of spillway that is in operation at a given time, the discharge is uniform along the

crest (this is equivalent to assuming that the properties of the water parcel are constant along any line parallel to the spillway crest).

2. The value Y_o is the initial depth of the spill before the jump. It is computed as:

$$Y_o = \frac{q}{V_o} = \frac{q}{\sqrt{2gH}} \qquad \text{(A-1)}$$

where

q = discharge per foot along the crest

H = total reservoir head above the stilling basin floor.

3. The only effect of the roller which overlies the main flow is to increase the static pressure within the water parcel by an amount $\alpha_o y_1$.

4. A given mass of air M_A is entrained as discrete bubbles into the water parcel at the point $x = 0$ and remains uniformly distributed within the water parcel as it passes through the stilling basin.

5. The distribution of the mass of air among the various bubble sizes remains unchanged during the water parcel's journey through the stilling basin.

6. The dissolved nitrogen within the water parcel is uniformly distributed.

7. Rate of nitrogen dissolution $\frac{dM}{dt}$ in the water parcel is governed by Fickian diffusion as:

$$\frac{dM}{dt} = K_L A (C_E - C) \qquad \text{(A-2)}$$

where

M = the mass of dissolved nitrogen in the water parcel,

K_L = rate coefficient,

A = total surface area of the air bubbles contained in the water parcel,

C_E = effective saturation concentration of dissolved nitrogen in the water parcel, and

C = actual concentration of dissolved nitrogen in the water parcel.

With these assumptions, we can now define the parameters M, A, and C_E in equation A-2 as functions of the location of the water parcel in the stilling basin.

Assumption 6 allows us to write the mass M as the product of the concentration C and the volume of the water parcel,

$$M = (wy\delta x)C \qquad \text{(A-3)}$$

where w is the effective width of the stilling basin, i.e., w = (number of gates open) x (width per gate).

The saturation concentration of a gas such as N_2 or O_2 that is only slightly soluble in water is governed by Henry's Law which states that the equilibrium or saturation concentration of the gas in solution is directly proportional to the pressure existing at the gas-liquid interface. In the water parcel the pressure P at an elevation z above the stilling basin floor is

$$P = P_o + \alpha_o y_1 + \alpha(y-z) \qquad \text{(A-4)}$$

where P_o is the atmospheric (or barometric) pressure, and the α-parameters are the densities of the roller and main flow as shown in Figure A-1. Hence, the saturation concentration at any elevation z in the parcel is given as:

$$C_{sat} = [P_o + \alpha_o y_1 + \alpha(y-z)]C^* \qquad \text{(A-5)}$$

where C^* is the saturation concentration under one atmosphere of pressure. In equation A-5, the pressure term has units of atmospheres of pressure. From equation A-5, it is seen that C_E varies linearly with z. It follows that the average or *effective* saturation concentration, C_E in the water parcel is the value of C_{sat} at mid-depth, or at $z = y/2$: Thus,

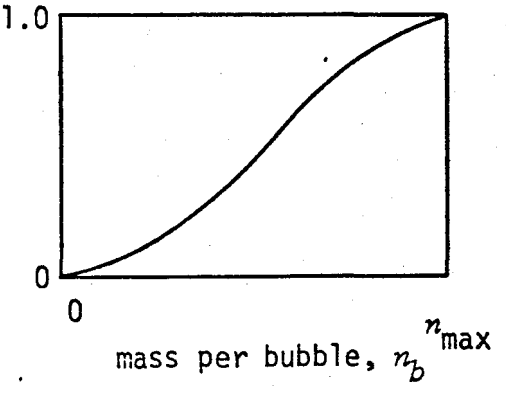
$$C_E = [P_o + \alpha_o y_1 + \alpha(y/2)]C^* \qquad \text{(A-6)}$$

Noting that $y_1 = D-y$ gives the final form of C_E as

$$C_E = [P_o + \alpha_o D - (\alpha_o - \tfrac{\alpha}{2})y]C^* \qquad \text{(A-7)}$$

The total surface area A of the air bubbles in the water parcel depends upon the total mass of air entrained and, upon the bubble size distribution. It is not unreasonable to expect that the entrained mass of air will be distributed among the various bubble sizes in a manner similar to that shown below.

B = fraction of total air mass in the water parcel with bubbles having a mass less than or equal to n_b



The volume V_b of an air bubble with mass n_b can be found from the ideal gas law:

$$V_b = \frac{mRT}{P} \tag{A-8}$$

where

m = number of moles of air in the bubble,
R = universal gas constant,
T = absolute temperature, and
P = the total pressure in the bubble.

In equation A-8, m can be replaced by $n_b/28.9$ where 28.9 is the molecular weight of air. The diameter d_b and the area A_b of a sphere are given by:

$$d_b = \left(\frac{6}{\pi} V_b\right)^{1/3} \tag{A-9a}$$

$$A_b = \pi d_b^{\,2} \tag{A-9b}$$

Now, combining equations A-8 and A-9, the following expression results for the surface area A_b of an air bubble with mass n_b:

$$A_b = \left(\frac{6\sqrt{\pi}RT}{28.9}\right)^{2/3} \left(\frac{n_b}{P}\right)^{2/3} \tag{A-10}$$

Thus, if the total air mass entrained per unit volume of water at Y_o is M_A, the total air bubble surface areas A', per unit volume of water is found from the bubble size distribution and equation A-10 as

$$A' = \int_0^{n_{max}} A_b \frac{M_A \frac{dB}{dn_b}\, dn_b}{n_b} \tag{A-11}$$

or

$$A' = \left(\frac{6\sqrt{\pi}RT}{28.9P}\right)^{2/3} M_A \int_0^1 n_b^{-1/3}\, dB \tag{A-12}$$

Finally, to get the total bubble surface area in the water parcel it is necessary to integrate equation A-12 over the volume of the parcel $wy\delta x$, i.e.,

$$A = \int_{\delta x} \int_{w} \int_{z} A' \, dx \, dw \, dz \qquad \text{(A-13)}$$

Applying assumptions 4 and 5 and substituting for A' from equation A-12 gives

$$A = w\delta x \left(\frac{6\sqrt{\pi RT}}{28.9}\right)^{2/3} M_A \int_0^1 n_b^{-1/3} dB \int_{z=0}^{y} \frac{dz}{P^{2/3}} \qquad \text{(A-14)}$$

Replacing P with equation A-4 and integrating,

$$A = 3\left(\frac{6\sqrt{\pi RT}}{28.9}\right)^{2/3} M_A \int_0^1 n_b^{-1/3} dB \quad (w\delta x) \frac{\left(P_o + \alpha_o y_1 + \alpha y\right)^{1/3} - \left(P_o + \alpha_o y_1\right)^{1/3}}{\alpha} \qquad \text{(A-15)}$$

Substituting $(D-y)$ for y_1 gives the final form as

$$A = K_A \, (w\delta x) \left[\left[P_o + \alpha_o D + (\alpha-\alpha_1)y\right]^{1/3} - \left[P_o + \alpha_o D - \alpha_o y\right]^{1/3}\right] \qquad \text{(A-16)}$$

where

$$K_A = \frac{3}{\alpha} \left(\frac{6\sqrt{\pi RT}}{28.9}\right)^{2/3} M_A \int_0^1 n_b^{-1/3} dB$$

If the expressions for M, C_E, and A from equation A-3, A-7 and A-15 respectively are substituted into the rate expressions given in equation A-2, there results

$$(y\omega\delta x)\frac{dC}{dt} = (\omega\delta x)\ K_L K_A \left\{[P_o + \alpha_o D + (\alpha-\alpha_o)y]^{1/3} - [P_o + \alpha_o D - \alpha_o y]^{1/3}\right\}$$
$$\left\{[P_o + \alpha_o D - (\alpha_o - \frac{\alpha}{2})y]C^* - C\right\} \qquad \text{(A-17)}$$

We can now write rate expression $\frac{dC}{dt}$ in terms of the location in the stilling basin by using the relationship

$$\frac{dC}{dt} = \frac{dx}{dt}\ \frac{dC}{dx} = v\ \frac{dC}{dx} = \frac{q}{y}\ \frac{dC}{dx} \qquad \text{(A-18)}$$

where v is the velocity of the parcel and q is the discharge per unit width of the stilling basin. In addition, we define a system parameter K, which we will call the *entrainment coefficient*, as

$$K = K_L K_A = \frac{3}{\alpha}\left(\frac{6\sqrt{\pi R}}{28.9}\right)^{2/3}\ [T^{2/3} K_L\ M_A \int_0^1 n_b^{-1/3} dB] \qquad \text{(A-19)}$$

Substituting equation A-18 and A-19 into A-17 gives the expression for the concentration change in the water parcel as

$$\frac{dC}{dx} = \frac{K}{q}\left\{[P_o + \alpha_o D + (\alpha - \alpha_o)y]^{1/3} - [P_o + \alpha_o D - \alpha_o y]^{1/3}\right\}$$
$$\left\{[P_o + \alpha_o D - (\alpha_o - \frac{\alpha}{2}\ y)]C^* - C\right\} \qquad \text{(A-20)}$$

The solution is obtained as follows. Evaluate the pressure terms at the midpoint of the stilling basin $y = \frac{D+Y_o}{2}$ to obtain

$$\frac{dC}{dx} = \frac{K}{q}\left\{[\overline{P} + \frac{\alpha}{4}\ (D + Y_o)]^{1/3} - [\overline{P} - \frac{\alpha}{4}\ (D + Y_o)]^{1/3}\right\}\left\{\overline{P}C^* - C\right\} \qquad \text{(A-21)}$$

where $$\overline{P} = P_o + \frac{\alpha_o}{2}(D - Y_o) + \frac{\alpha}{4}(D + Y_o)$$

Now let

$$\overline{\Delta P^{1/3}} = [\overline{P} + \frac{\alpha}{4}(D + Y_o)]^{1/3} - [\overline{P} - \frac{\alpha}{4}(D + Y_o)]^{1/3} \quad \text{(A-22)}$$

Rewriting equation A-21, with these substitutions gives

$$dC + C\frac{K}{q}\overline{\Delta P^{1/3}}\,dx = \frac{K}{q}\overline{\Delta P^{1/3}}\,\overline{PC^*}\,dx \quad \text{(A-23)}$$

which has the solution

$$C = \overline{PC^*} + ke^{-\frac{K}{q}\overline{\Delta P^{1/3}}\,x} \quad \text{(A-24)}$$

Evaluating equation A-24 at $x = 0$, where C equals the forebay concentration C_F, and at $x = L$ where C equals the stilling basin concentration C_S, yields the spillway-stilling basin model as

$$C_S = \overline{PC^*} - (\overline{PC^*} - C_F)\,e^{-\frac{K}{q}L\,\overline{\Delta P^{1/3}}} \quad \text{(A-25)}$$

APPENDIX B

DERIVATION OF THE EQUATIONS FOR THE SIMULATION OF DISSOLVED NITROGEN IN RESERVOIRS

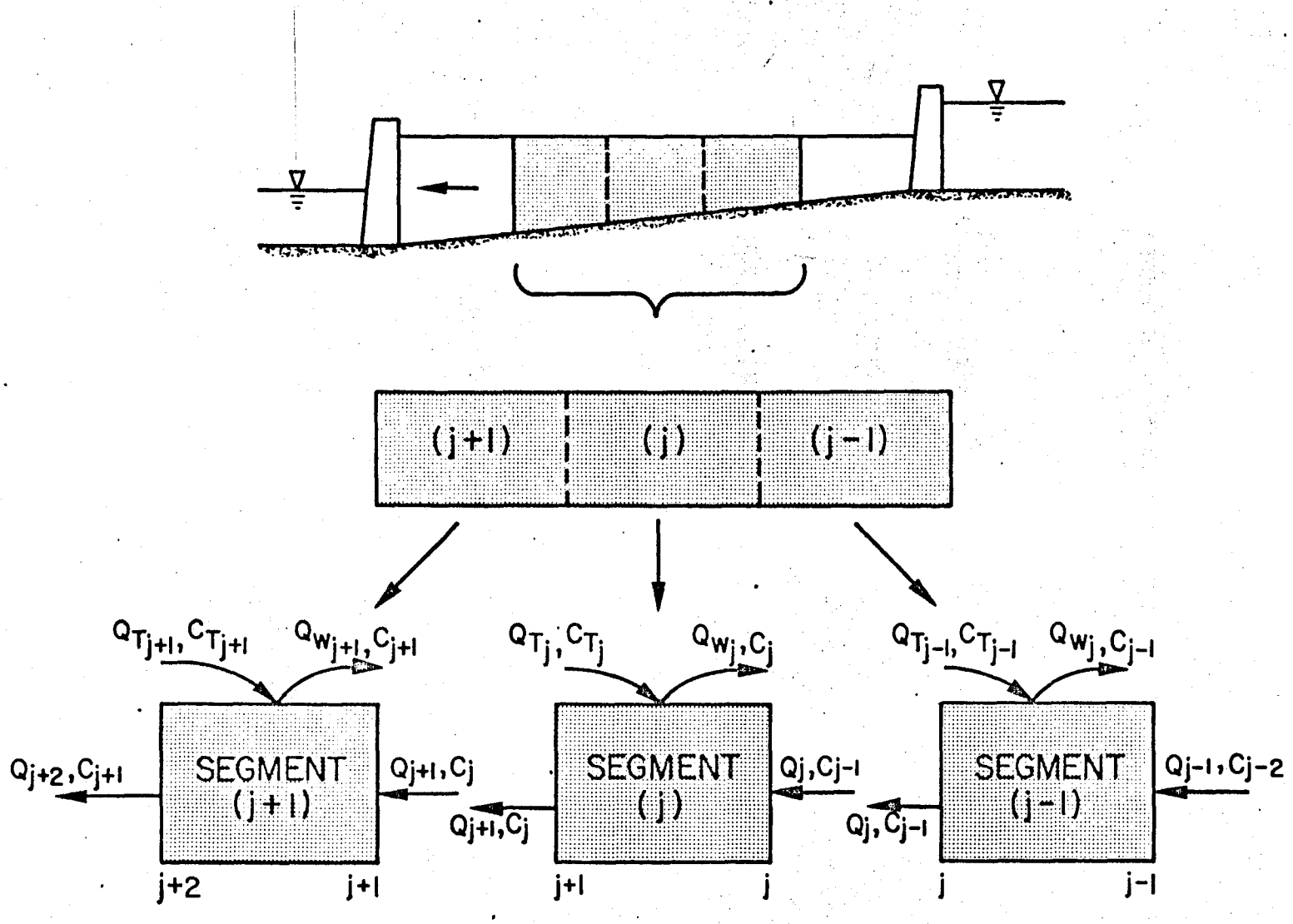
APPENDIX B

Derivation of the Equations For the Simulation of Dissolved Nitrogen In Reservoirs

The following paragraphs give a derivation of the equations and techniques suitable for simulating the dissolved nitrogen behavior in a reservoir. For the purposes of this derivation, it is assumed that the hydraulic behavior of the reservoir is known, and that all pertinent hydraulic variables are available for input to the nitrogen formulation. The method of approach for this derivation is a combination of a first principles, mass balance formulation, coupled with a numerical approximation of system behavior. The reservoir is conceptually divided into a large, but finite, number of relatively small, homogeneous, isotropic pieces, called segments. When taken together, these segments represent the complete reservoir to be simulated. A set of differential equations, in finite form, is written for the nitrogen mass balance in each segment, and a solution to these equations provides the simulation of the reservoir's behavior. An implicit solution scheme is suggested, and a recursive procedure for moving forward in time is outlined.

In order to write the required equations, consider an elevation view of any three segments within the reservoir as suggested pictorially by Figure B-1. If we denote a particular segment by subscripting all its geometric and state variables, for the center segment of Figure B-1 we can write the following nitrogen mass balance at any instant in time:

$$\left(\frac{dM}{dt}\right)_j = F_{A_j} + F_{X_j} + F_{S_j} + F_{D_j} \qquad \text{(B-1)}$$



CONCEPTUAL REPRESENTATION SEGMENTED RESERVOIR SYSTEM FIGURE B-1

where $\left(\frac{dM}{dt}\right)_j$ = the time rate of change of mass of nitrogen associated with segment (j),

F_{A_j} = the net mass flux of nitrogen for segment (j) by the internal advection process,

F_{X_j} = the net mass flux of nitrogen for segment (j) by processes of external advection,

F_{S_j} = the mass flux of nitrogen for segment (j) across the air-water interface, and

F_{D_j} = the net mass flux of nitrogen for segment (j) by the instream eddy diffusion process.

Now, it is also possible to write the well known identity for mass, volume and mass concentration as:

$$M_j = V_j C_j \tag{B-2}$$

where

M_j = total mass of nitrogen contained in segment (j),

V_j = volume of segment (j), and

C_j = the mass per unit volume of nitrogen in segment (j).

And, differentiating equation (B-2) with respect to time yields

$$\left(\frac{dM}{dt}\right)_j = V_j \left(\frac{dC}{dt}\right)_j + C_j \left(\frac{dV}{dt}\right)_j \tag{B-3}$$

Substituting equation (B-3) into (B-2) and rewriting gives the expression

$$V_j \left(\frac{dC}{dt}\right)_j = F_{A_j} + F_{X_j} + F_{S_j} + F_{D_j} - C_j \left(\frac{dV}{dt}\right)_j . \tag{B-4}$$

Now, again referring to Figure B-1, it is possible to express the net effects of the internal and external advection processes as

$$F_{A_j} = Q_j\, C_{j-1} - Q_{j+1}\, C_j \qquad \text{(B-5)}$$

and

$$F_{X_j} = Q_{T_j}\, C_{T_j} - Q_{W_j}\, C_j \qquad \text{(B-6)}$$

where Q_j = the total flow across plane (j) into segment (j),

C_{j-1} = the nitrogen concentration in segment $(j-1)$,

Q_{j+1} = the total flow across plane $(j+1)$ leaving segment (j),

C_j = the nitrogen concentration currently existing in segment (j),

Q_{T_j} = the total flow introduced into segment (j) by external advection,

C_{T_j} = the nitrogen concentration associated with the flow Q_{T_j}, and

Q_{W_j} = the total flow withdrawn from segment (j) by external advection.

Substituting equations (B-5) and (B-6) into (B-4), there results

$$V_j \left(\frac{dC}{dt}\right)_j = Q_j C_{j-1} - Q_{j+1} C_j + Q_{T_j} C_{T_j} - Q_{W_j} C_j + F_{S_j} + F_{D_j} - C_j \left(\frac{dV}{dt}\right)_j . \qquad \text{(B-7A)}$$

Or rearranging and grouping terms

$$V_j \left(\frac{dC}{dt}\right)_j = C_{j-1} Q_j - C_j \left(Q_{j+1} + Q_{W_j} + \left(\frac{dV}{dt}\right)_j\right) + F_{S_j} + F_{D_j} + Q_{T_j} C_{T_j} . \qquad \text{(B-7B)}$$

In accordance with our earlier conceptual representation, it is possible to express the transfer of nitrogen across the air-water interface by the Fickian diffusion analogy. This can be expressed as

$$F_{S_j} = A_{S_j} K_{L_j} (C_j^* - C_j) \qquad \text{(B-8)}$$

where

F_{S_j} = the mass flux of nitrogen for segment (j) across the air-water interface,

A_{S_j} = the effective surface area of segment (j),

K_{L_j} = a transfer coefficient determined from system parameters as given below,

C_j^* = the saturation concentration for nitrogen at the temperature of segment (j), and

C_j = the concentration of nitrogen in segment (j).

In order to complete the necessary formulation for equation (B-8), introduce the following expression which is taken from Reference 9.

$$K_{L_j} = \left(\frac{D_m U_j}{H_j}\right)^{1/2} \qquad \text{(B-9)}$$

where

D_m = the molecular diffusivity of nitrogen at 20°C,

U_j = the hydraulic velocity in segment (j), and

H_j = the depth of segment (j).

Also, introduce the following expression relating the effective area of water surface of segment (j) to the planar surface area given by the idealized situation suggested in Figure B-1.

$$A_{S_j} = A_{P_j} B_a \qquad \text{(B-10)}$$

where A_{S_j} = the effective area of water surface for segment (j),

A_{P_j} = the surface area of segment (j) as given by the product of its length and width,

B_α = $1.0 + B_\alpha'$, and

B_α' = a coefficient depending upon wind, turbulence, channel irregularities, etc.

If equations (B-9) and (B-10) are substituted into equation (B-8) the resulting expression is:

$$F_{S_j} = B_\alpha A_{P_j} \left(\frac{D_m U_j}{H_j}\right)^{1/2} (C_j^* - C_j) \qquad \text{(B-11A)}$$

or

$$F_{S_j} = B_\alpha A_{P_j} \left(\frac{D_m U_j}{H_j}\right)^{1/2} C_j^* - B_\alpha A_{P_j} \left(\frac{D_m U_j}{H_j}\right)^{1/2} C_j \qquad \text{(B-11B)}$$

which, when substituted into equation (B-7B) gives

$$V_j \left(\frac{dC}{dt}\right)_j = C_{j-1} Q_j - C_j \left\{ Q_{j+1} + Q_{W_j} + \left(\frac{dV}{dt}\right)_j + B_\alpha A_{P_j} \left(\frac{D_m U_j}{H_j}\right)^{1/2} \right\}$$
$$+ F_{D_j} + Q_{T_j} C_{T_j} + B_\alpha A_{P_j} \left(\frac{D_m U_j}{H_j}\right)^{1/2} C_j^* . \qquad \text{(B-12)}$$

The quantification of the diffusion term, F_{D_j}, is also accomplished by resorting to a Fickian diffusion analogy. At any time the mass transfer across plane (j) can be estimated from the standard diffusion equation as

$$\left(\frac{dM}{dt}\right)_{D_j} = - A_j D_j \left(\frac{dC}{dx}\right)_j \qquad \text{(B-13)}$$

where $\left(\frac{dM}{dt}\right)_{D_j}$ = the mass transfer of nitrogen across plane (j) by diffusion,

A_j = the cross sectional area of plane (j),

D_j = the coefficient of eddy diffusion at plane (j), and

$\left(\frac{dC}{dx}\right)_j$ = the longitudinal concentration gradient of nitrogen across plane (j).

Now, estimating the concentration gradient across plane (j) by the first difference expression as

$$\left(\frac{dC}{dx}\right)_j = \frac{C_j - C_{j-1}}{\Delta x} \qquad \text{(B-14A)}$$

and the gradient across plane (j+1) as

$$\left(\frac{dC}{dx}\right)_{j+1} = \frac{C_{j+1} - C_j}{\Delta x} \qquad \text{(B-14B)}$$

where

Δx = the distance between mid points of any two adjacent segments.

The net flux can be expressed as:

$$F_{D_j} = \frac{A_j D_j}{\Delta x}(C_{j-1} - C_j) - \frac{A_{j+1} D_{j+1}}{\Delta x}(C_j - C_{j+1}) \qquad \text{(B-15A)}$$

or

$$F_{D_j} = C_{j-1}\frac{A_j D_j}{\Delta x} - C_j\left(\frac{A_j D_j + A_{j+1} D_{j+1}}{\Delta x}\right) + C_{j+1}\frac{A_{j+1} D_{j+1}}{\Delta x} \qquad \text{(B-15B)}$$

Now, substituting equation (B-15B) into (B-12), there results the final nitrogen mass balance equation for segment (j) as

$$V_j\left(\frac{dC}{dt}\right)_j = C_{j-1}\left(Q_j + \frac{A_jD_j}{\Delta x}\right) - C_j\left\{Q_{j+1} + Q_{W_j} + \frac{dV}{dt}_j + B_\alpha A_{P_j}\left(\frac{D_m U_j}{H_j}\right)^{1/2}\right.$$

$$\left. + \frac{A_jD_j + A_{j+1}D_{j+1}}{\Delta x}\right\} + C_{j+1}\left(\frac{A_{j+1}D_{j+1}}{x}\right) + Q_{T_j}C_{T_j}$$

$$+ B_\alpha A_{P_j}\left(\frac{D_m U_j}{H_j}\right)^{1/2} C_j^* \qquad \text{(B-16)}$$

In the actual process of simulating nitrogen behavior, an equation of the form (B-16) must be written for each segment in the system. Obviously, certain terms in equation (B-16) must be modified for the first and last segment in the system, but these changes are straightforward and will not be included here.

As expedient, and to provide a more concise description of the system mass balance equations, certain substitutions shall now be made and all equations expressed in more compact matrix notation.

First, define the elements of a matrix [S] with the terms of equation (B-16) which multiply the nitrogen concentrations such that

$$S_{j,j-1} = \left(Q_j + \frac{A_jD_j}{\Delta x}\right),$$

$$S_{j,j} = \left(Q_{j+1} + Q_{W_j} + \frac{dV}{dt}_j + B_\alpha A_{P_j}\left(\frac{D_m U_j}{H_j}\right)^{1/2} + \frac{A_jD_j + A_{j+1}D_{j+1}}{\Delta x}\right),$$

$$S_{j,j+1} = \left(\frac{A_{j+1} D_{j+1}}{\Delta x}\right)$$

This matrix will be tridiagonal in form, with entries on and to either side of the diagonal, and zero elsewhere.

Next, complete the matrix definition of the terms of equation (B-16) by assigning the following notation:

Define a diagonal matrix $[V]$ such that

$$V_{ij} = V_j \text{ if } i = j$$

and

$$V_{ij} = 0 \text{ if } i \neq j \ .$$

Define a column vector of the time rates of concentration change, $\{\dot{C}\}$, such that

$$\dot{C}_j = \left(\frac{dC}{dt}\right)_j$$

Define a column vector, $\{P\}$, of the constant terms such that

$$P_j = Q_{T_j} C_{T_j} + B_a A_{P_j} \left(\frac{D_m U_j}{H_j}\right)^{1/2} C_j^*$$

Define a column vector, $\{C\}$, of the nitrogen concentrations such that

$$C_j = C_j$$

Then, equation (B-16) can be written in matrix form as:

$$[V]\ \{\dot{C}\} = [S]\ \{C\} + \{P\} \qquad \text{(B-17)}$$

Prior to going further, it is worth while to stress the importance of equations (B-16) and (B-17), for it is primarily on these formulations that the simulation of dissolved nitrogen relies. It is most important to understand that while these equations express the relationships which exist at a particular instant in time, the instant to which it applies is not integral to the formulation. Great use shall be made of this fact in what follows.

We now wish to establish a procedure for the numerical integration of equation (B-17). In order to accomplish this, assume that we are at some point in time (t), and we wish to move forward a finite amount, Δt. Further, assume that we have complete knowledge of our present condition, and that all existent state variables are known values. As a reasonable method of numerical integration, assume that the <u>rate</u> of change of the nitrogen concentration varies in a linear fashion from its value at (t), $C\ (t)$, to its value at ($t + \Delta t$), $C\ (t + \Delta t)$. Under this assumption, we can write the following expression relating our final state, $C\ (t + \Delta t)$, to our initial state, $C\ (t)$, and our rates of change, $\frac{dC}{dt}\ (t)$ and $\frac{dC}{dt}$ $(t + \Delta t)$.

$$C(t + \Delta t)_j = C(t)_j + \frac{\Delta t}{2}\left(\frac{dC}{dt}\ (t)_j + \frac{dC}{dt}\ (t + \Delta t)_j\right) \qquad \text{(B-18A)}$$

or

$$C(t + \Delta t)_j = C(t)_j + \frac{\Delta t}{2}\frac{dC}{dt}\ (t)_j + \frac{\Delta t}{2}\frac{dC}{dt}\ (t + \Delta t)_j \qquad \text{(B-18B)}$$

Under the condition that we have knowledge of all state variable at the initial point, we can write equation (B-18B) as:

$$C_j = B_j + \frac{\Delta t}{2}\left(\frac{dC}{dt}\right)_j \qquad \text{(B-19)}$$

where

C_j = the nitrogen concentration for segment (j) at the *end* of the interval Δt,

$\left(\frac{dC}{dt}\right)_j$ = the time rate of change of C_j at the *end* of time interval,

B_j = $C(t)_j + \frac{\Delta t}{2}\frac{dC}{dt}(t)_j$, and

Δt = the time interval.

It should be recognized that equation (B-19) is general in nature, and completely independent of equation (B-17). Thus, we have two equations, (B-17) and (B-19), and two unknowns, C_j and $\frac{dC}{dt}(t + \Delta t)_j$. We shall now set out to solve these equations and establish a recursive scheme suitable for machine computation.

First, write equation (B-19) in the following matrix form:

$$\{C\} = \{B\} + \frac{\Delta t}{2}\{\dot{C}\} \qquad \text{(B-20)}$$

where

$\{C\}$ = a vector of the nitrogen concentrations in each segment at the end of a time interval,

$\{B\}$ = a vector of the term $\left(C_j + \frac{\Delta t}{2}\left(\frac{dC}{dt}\right)_j\right)$ for each segment at the beginning of the time interval,

$\{\dot{C}\}$ = a vector of the time rates of change of C for each segment at the end of time interval, and

Δt = the time interval.

Now, substitute equation (B-20) into equation (B-17) which gives:

$$[V]\ \{\dot{C}\} = [S]\ \{B\} + \frac{\Delta t}{2}\ [S]\ \{\dot{C}\} + \{P\} \qquad \text{(B-21)}$$

which can be simplified to:

$$[S^*]\ \{\dot{C}\} = \{P^*\} \qquad \text{(B-22)}$$

where $[S^*] = [V] - \frac{\Delta t}{2}\ [S]$ (B-23)

and $\{P^*\} = [S]\ \{B\} + \{P\}$ (B-24)

Equation (B-22) forms the basis for our solution, as we now have only one unknown in the equation, $\{\dot{C}\}$. With this equation, we can define the following recursive scheme for the numerical solution of equation (B-22).

1. Form the vector $\{B\}$ from the initial conditions or the solution just completed.
2. From the known hydraulic solution (assumed to be computed externally), and known boundary conditions, define the conditions which will exist over the interval Δt. With this knowledge form $[V]$, $[S]$ and $\{P\}$.
3. Form $[S^*]$ and $\{P^*\}$.
4. Solve for $\{\dot{C}\}$ at time $(t + \Delta t)$.
5. Compute $\{C\}$ by substitution in equation (B-20).
6. Go to Step 1.

The above recursive scheme is that used in the computer code, and has proved to be very stable and efficient.

As a final comment, it should be noted that the Thomas Algorithm (12) is employed for the solution of equation (B-22). This is an extremely fast and simple procedure which is uniquely suited to solving equations of this general form.

APPENDIX C

DATA ADDENDUM

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DATA ADDENDUM

Comments on the Adequacy of the Present Nitrogen Sampling Program and Its Results With Regard to the Development of a Mathematical Model for the Columbia System

At the outset of the current work, it was understood by all concerned that field data would provide a very important input to the development of dissolved nitrogen model for the Columbia River system. Since the initiation of the modeling project, it has become increasingly clear that the data available leaves quite a bit to be desired with regard to model development and verification. The more important shortcomings from this point of view are the four given below.

1. All samples taken below the stilling basins were taken quite a long distance below the basins. It would be more advantageous from our point of view to move the sampling point upstream and nearer the end of the stilling basins.

2. In the case of all sampling stations, excepting Bonneville, the samples were taken such that the nitrogen concentration leaving the stilling basin was obtainable only after making a computation involving assumptions of complete mixing, and of the nitrogen concentration of the turbine discharge. This, of course, is not nearly as desirable as having directly measured values.

3. While the data taken in the forebays is probably adequate, we have no information to indicate if any transverse nitrogen

concentrations occur in the reservoirs. We doubt their existence, but would like to know.

4. It has come to our attention that pinpoint bubbles have been observed in the rivers below the stilling basins, and this is at least partially the reason that the sampling stations are located so far below the dams. If these small bubbles are actually nitrogen and/or oxygen coming out of solution, the data reported are further degraded for modeling purposes.

What would we like? In order to adequately sample for model verification, a sampling program could be specifically designed for model verification. This program would be of short duration and high intensity and would include at the least the following:

1. Complete time history of all important flows, heads, and gate openings.

2. Vertical and transverse nitrogen, oxygen, and temperature profiles in the dam forebays, below the stilling basins and below the turbine discharges.

3. Vertical and transverse profiles of nitrogen, oxygen and temperature several thousand feet below the dams.

4. Identification of the composition of the downstream, pinpoint bubbles, if any are observed.

Wold. 1973. Surface agitators as a means to reduce nitrogen gas in a hatchery water supply.

This article describes the use of mechanical surface agitators, originally installed at Dworshak Hatchery as aerators, to reduce nitrogen to levels acceptable to fish culture purposes.

SURFACE AGITATORS AS A MEANS TO REDUCE NITROGEN GAS IN A HATCHERY WATER SUPPLY

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NITROGEN SUPERSATURATION AND ITS EFFECT on fish (gas bubble disease) has been a problem in some fish hatchery operations for a number of years [*5, 7, 8, 9, 10, 11*]. Gas bubble disease in fish is characterized by small emboli in the vascular elements of the fins, gills, and skin. The bubble disease may not kill the fish outright, but may cause small ruptures in the skin making the fish more susceptible to secondary disease infections.

Wood [*10*] considered the following nitrogen saturation levels as detrimental or lethal for salmon (*Oncorhynchus* sp.): 103 to 104 percent for yolk-sac fry and young fingerlings; 105 to 113 percent for older fingerlings and yearlings; and 118 percent for adults. Westgard [*9*] showed that adult chinook salmon (*O. tshawytscha*) held in water with nitrogen concentrations at 116 percent saturation developed definite symptoms of gas bubble disease. Harvey and Smith [*5*] reported that saturation levels of 108 percent produced gas bubble disease in trout fingerlings. Wyatt and Beiningen [*11*] reported that 152 percent nitrogen saturation in an Oregon hatchery killed all juvenile salmon and steelhead (*Salmo gairdneri*) in 5 hours.

At Dworshak National Fish Hatchery, steelhead yolk-sac fry held in water with 107 percent nitrogen saturation developed gas bubble disease causing them to float upside down in the water. When the nitrogen level was reduced to 105 percent the fry acclimated to the new environment and returned to normal swimming activities.

Since high nitrogen saturation levels are harmful to fish, some means to reduce the level to less than 105 percent in hatchery water supplies is necessary to maintain a healthy hatchery stock.

Several methods for aerating fish hatchery water supplies have been used successfully [*3, 4, 7, 8*]. Rucker and Tuttle [*8*] found that nitrogen saturation was reduced from 144 to 101 percent when water was dropped in a series of troughs to create thin sheets of water. Harvey and Cooper [*4*] used a structure with baffles set at right angles to reduce nitrogen saturation from 119.5 to 102 percent. Erdman [*3*] used a spray to aerate a heated water supply. Rucker and Hodgeboom [*7*] used a baffled flume to reduce nitrogen gas saturation from 120 to 115 percent.

At Dworshak National Fish Hatchery, located 1.6 miles below Dworshak Dam on the North Fork of the Clearwater River in Idaho, a water treatment facility was constructed to provide a settling basin and aeration facility for low oxygenated reservoir water. Since dams have different characteristic effects on nitrogen saturation [*2*], the effect of Dworshak Dam on the water quality of the hatchery supply was unknown. During the spring of 1972, 2,000 to 30,000 cubic feet per second water was released down the spillway from the low level outlet (elevation 1,230) at Dworshak Dam. This water plunged into the stilling basin (water surface elevation 973, bottom elevation 931) entrapping air and creating nitrogen levels ranging from 115 to 130 percent saturation. During this time surface mechanical agitators originally installed for use as aerators successfully reduced nitrogen to levels acceptable for fish culture purposes.

DESCRIPTION OF FACILITIES

The water treatment facility at Dworshak National Fish Hatchery is a large concrete structure with a total volume of 47,676 cubic

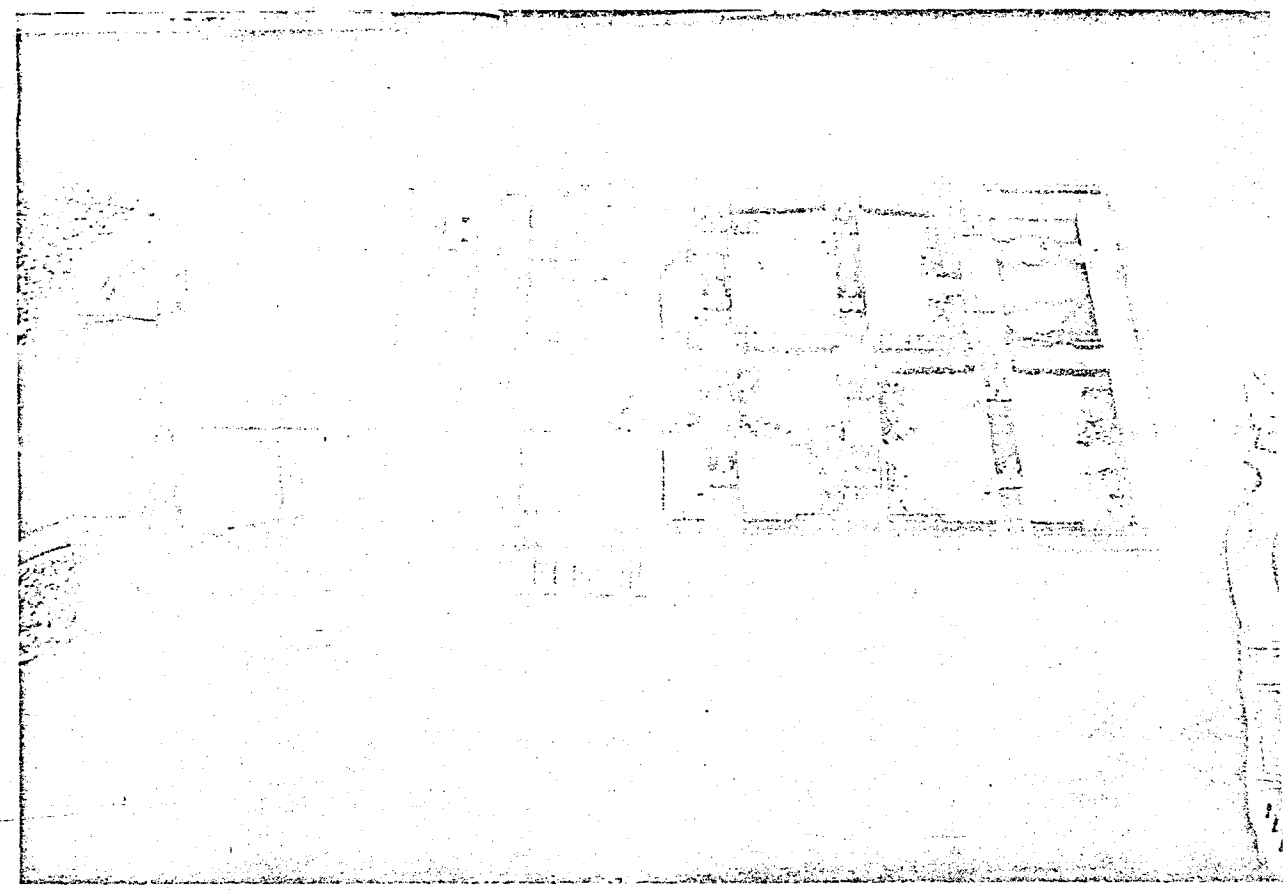


Figure 1.—Water treatment facility with aerators 2, 4, 5, 9, 10, and 12 in operation.

feet (356,641 gallons) (fig. 1). Twelve 30-horsepower surface aerators with a gear ratio of 25.6:1 are platform mounted and supported on steel bridges (fig. 2). The impeller which rotates at 75 rpm is an inverted cone with blades radiating outward from a boss at the center. As the impeller turns, it draws the water upwardly towards the boss; it is propelled outwardly in a low trajectory as a fine spray.

The hatchery water supply is delivered to the water treatment facility from the river by any combination of three 15,500-gpm and two 8,000-gpm pumps. The aerators are installed in three rows with four aerators in each row (fig. 3). The water level is maintained by a weir to provide a continuous depth for best aerator efficiency.

CONSTRUCTION, OPERATION, AND MAINTENANCE COSTS

The water treatment facility described in this report costs $477,527 to construct in 1968 with each aerator costing $6,000. The pumping facility to deliver water from the river to the aerators cost an additional $581,000. With a rate of 3.6 mills/kilowatt-hour the cost of operating each aerator is $64.80 per month. The cost of labor and supplies is approximately $60 a year with six man hours of labor required for each aerator.

METHODS

Nitrogen and oxygen levels were measured from samples of water obtained from the North Fork of the Clearwater River and the effluent of the water treatment facility. Analysis for dissolved nitrogen was made using a Van Slyke manometric blood gas analyzer modified for water determinations [*6*]. Dissolved oxygen was analyzed using the Azide modification of the Winkler Method [*1*].

Analyses of water samples were made with combinations of 2 to 12 aerators in use with flows of 19,500 and 37,000 gpm.

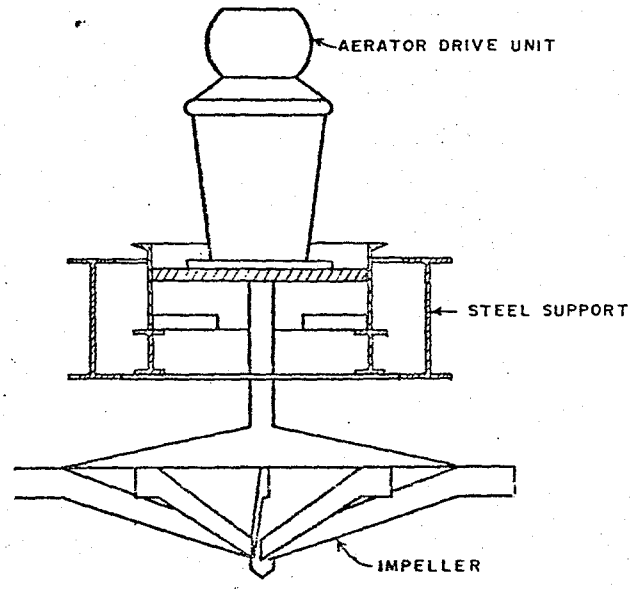


Figure 2.—Platform-mounted aerator used in water treatment facility.

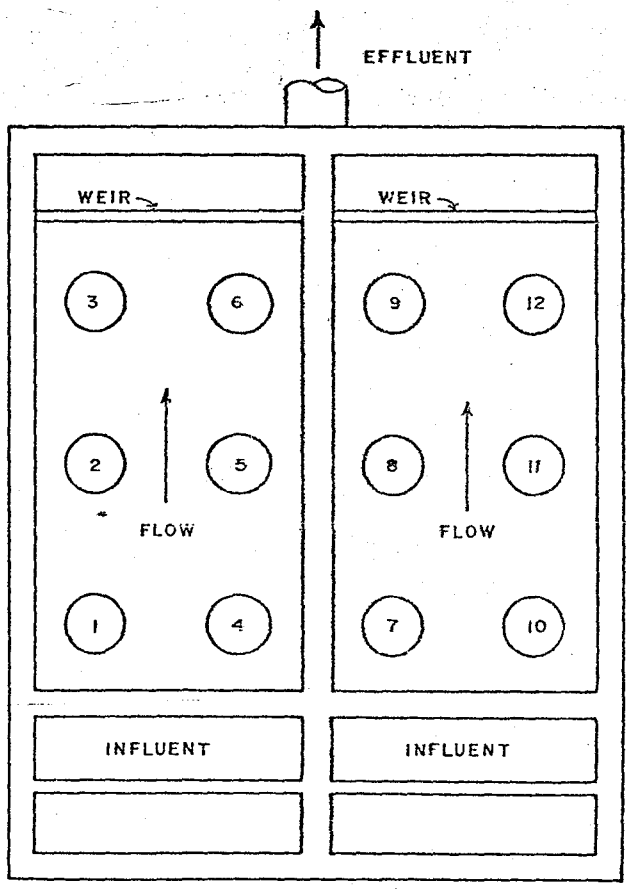


Figure 3.—Location of aerators in water treatment facility as indicated by numbered circles.

RESULTS

A minimum of four agitators was necessary to reduce the nitrogen levels from 115 to 130 percent saturation to an acceptable level. Some combinations using four agitators were not as efficient as others and only reduced the nitrogen levels to 108.8 percent. This decrease in efficiency was due to location and spacing of agitators.

Four corner agitators were less efficient than four agitators set in a line perpendicular to the flow. Efficiency of the aerators increased with increased flow rates. The results of nitrogen and oxygen determinations with regards to flow, temperature, and number of aerators operating are shown in the table.

Enough data have been obtained from tests run at Dworshak National Fish Hatchery to demonstrate that agitation provided by surface aerators provides an excellent method for reducing supersaturation of nitrogen to levels acceptable for fish propagation.

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Summary of nitrogen and oxygen analyses with several combinations of flows, temperatures, and numbers of aerators operating

Aerators operating	Flow (gpm)	Temperature (°C)	Percent saturation			
			Nitrogen (Van Slyke Method)		Oxygen (Winkler Method)	
			Influent	Effluent	Influent	Effluent
1 2 3 4 5 6 7 8 9 10 11 12	37,000	4.9	127.0	101.0	122.0	100.6
1 2 4 5 7 8 10 11	37,000	4.9	127.0	102.6	122.0	102.2
2 3 5 6 8 9 11 12	37,000	5.5	116.9	104.0	114.8	103.5
1 3 4 6 7 9 10 12	37,000	5.5	118.5	104.3	117.0	102.8
1 3 5 8 10 12	37,000	5.5	118.5	102.8	117.0	99.8
1 2 3 10 11 12	37,000	6.8	118.6	103.4	117.1	101.0
1 4 7 10	37,000	6.8	118.6	101.4	117.1	99.4
2 4 8 11	37,000	6.0	116.9	102.8	114.8	99.7
3 6 9 12	37,000	6.0	116.9	108.8	114.8	105.6
1 3 10 12	37,000	6.0	118.5	107.1	117.0	104.2
5 8	37,000	6.0	116.9	110.4	114.8	108.4
1 10	37,000	5.7	116.8	112.3	114.7	110.1
1 2 4 7 8 10	19,500	9.2	117.3	102.8	121.9	102.9
1 4 7 10	19,500	9.2	119.8	101.8	117.9	103.1

Newcomb. 1974. Changes in blood chemistry of juvenile steelhead trout, Salmo gairdneri, following sublethal exposure to nitrogen supersaturation.

This paper describes the effects of nitrogen supersaturation on blood chemistry of fish with no changes noted at saturations of 110% or less.

K.B.
10/1979

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

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NEWCOMB, T. W. 1974. Changes in blood chemistry of juvenile steelhead trout, *Salmo gairdneri*, following sublethal exposure to nitrogen supersaturation. J. Fish. Res. Board Can. 31: 1953–1957.

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

NEWCOMB, T. W. 1974. Changes in blood chemistry of juvenile steelhead trout, *Salmo gairdneri*, following sublethal exposure to nitrogen supersaturation. J. Fish. Res. Board Can. 31: 1953–1957.

Des groupes de jeunes truites steelhead (*Salmo gairdneri*) ont été exposées durant 35 jours à diverses saturations sublétales (103, 105, 110 et 116%) d'azote plus argon. Sur des échantillons de sérum combinés, nous avons déterminé Ca, Na, PO_4, K, Cl, albumine, protéines totales, cholestérol, phosphatase alcaline, glucose, urée, acide urique, bilirubine totale, lactate déshydrogénase et glutamique oxalacétique transaminase sérique. Nous avons observé une augmentation du potassium et du phosphate sériques, et une diminution, dans le sérum, de l'albumine, calcium, cholestérol, protéines et phosphatase alcaline chez des truites exposées à une saturation d'azote (N_2+Ar) de 116% (110% de saturation des gaz atmosphériques totaux). Nous n'avons pas observé de changements majeurs dans la chimie du sang à des saturations d'azote de 110% ou moins.

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

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

A number of mortality studies with nitrogen gas supersaturation have been made on juvenile salmonids, but little work has been published on the sublethal effects (Dawley and Ebel 1974). Sublethal effects from gas supersaturation may cause alterations in the fish's blood chemistry which can be used as tools to determine the degree of stress. These blood chemical measurements may help pinpoint potential long-range problems encountered by the juvenile salmonid such as ingestion and assimilation of food, predator avoidance, locomotion, homing behavior, fecundity, and the quantity and quality of subsequent fertilized eggs and fry. In addition, measuring these blood chemistry characteristics may allow in situ evaluation of nitrogen gas supersaturation stress response both singly and in combination with other normally occurring stressors (e.g. passage through turbines) which occur to the juvenile salmonid on its down-stream migration.

This blood chemistry experiment represents an extension of a bioassay by Dawley and Ebel (1974)

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which was designed to provide a variety of lethal and sublethal biological criteria which would help form the basis for an EPA provisional water quality standard. The primary goal was the establishment of a series of LE_{50} values. Secondary goals were changes in voluntary swimming performance and blood chemistry characteristics which might give some estimate of sublethal stress response to gas-bubble disease.

Materials and methods — The fish used in these experiments were steelhead trout, *Salmo gairdneri*, provided by the Washington State Department of Game Hatchery at Aberdeen, Wash. They weighed 20.5 ± 6.3 g and measured 132 ± 17 mm in fork length at the completion of these tests. Fish were acclimated to water temperatures of 15.0 ± 0.5 C (5 C/wk with 2 wk at 15 C) in acclimation tanks described by Ebel et al. (1971). Fish were fed once each day a maintenance ration of Oregon Moist Pellets.

Fish were exposed to various nitrogen supersaturation levels in shallow rectangular tanks (Dawley and Ebel 1974) with water 23 cm deep from the same source as used in the acclimation tanks. Tests were performed at 15.0 ± 0.5 C at nitrogen gas (N_2 + Ar) saturations of 103.5, 105.4, 110.1, and 116.0 ± 2.0% as calculated from Weiss (1970). These nitrogen saturations corresponded to total gas pressure (TGP) saturations (N_2 + Ar + O_2) of 99.9, 102.0, 105.9, and 110.2%. Mean oxygen saturations ranged between 87.5 and 90.5%. Fish were continued on the same maintenance ration of Oregon Moist Pellets during these tests.

Sixty fish were taken concurrently from the acclimation tanks and placed in each of three experimental and three control tanks (102.0 vs. 99.9; 105.9 vs. 99.9; and 110.2 vs. 99.9% TGP). Tests at each saturation level were continued until 35 days had elapsed (the 35-day test period was chosen to parallel the average exposure time of seaward migrant steelhead passing downstream through the nitrogen supersaturated waters of the lower Snake and Columbia rivers). At this time, each fish was given an individual voluntary swimming performance test (2–5 min) in an inclined vee trough against a water current of 1.28 m/s (15.0 ± 0.5 C) (Schiewe 1974). A variety of experimental limitations dictated that these fish must be used in the swimming performance test before being used in the present blood chemistry work. Therefore, to minimize short-term blood chemistry changes due to the swimming challenge, each experimental and control group was placed in a control tank for 24 h before blood sampling (Grant and Mehrle 1973).

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

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

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

Water analysis for nitrogen and oxygen were performed daily in each test tank using the methods of Dawley and Ebel (1974). Weekly measurements of total alkalinity (16–20 ppm), total hardness (10–21 ppm), carbon dioxide (1.2–2.0 ppm), chlorine + chloramine (<0.02 ppm) and pH (6.8–7.3) were made according to Tarus et al. (1971). Calcium (6.4–6.7 ppm) and potassium (0.3–1.0 ppm) were determined monthly by flame spectrophometer.

Results — Alteration from control mean values ($>3\sigma$) were observed in 7 of 16 blood characteristics in juvenile steelhead exposed to 116.0% nitrogen saturation, while meaningful changes in these 16 substances were not noted at lower saturations (Fig. 1). An increase in concentrations of potassium and phosphate was noted at 116.0% nitrogen saturation, while a decrease was noted in albumin, calcium, cholesterol, AP-ase, and total protein. Decrease in serum calcium was positively correlated with decreases in cholesterol ($r = .86$; $P<0.05$) and albumin ($r = .95$; $P<0.01$), and negatively correlated with an increase in potassium ($r=-.77$; $P<0.05$).

In addition to potassium, a decrease in albumin was negatively correlated with an increase in phosphate ($r=-.82$; $P<0.05$), and positively correlated with decreases in cholesterol ($r=.97$; $P<0.01$) and total protein ($r=.90$; $P<0.01$).

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

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

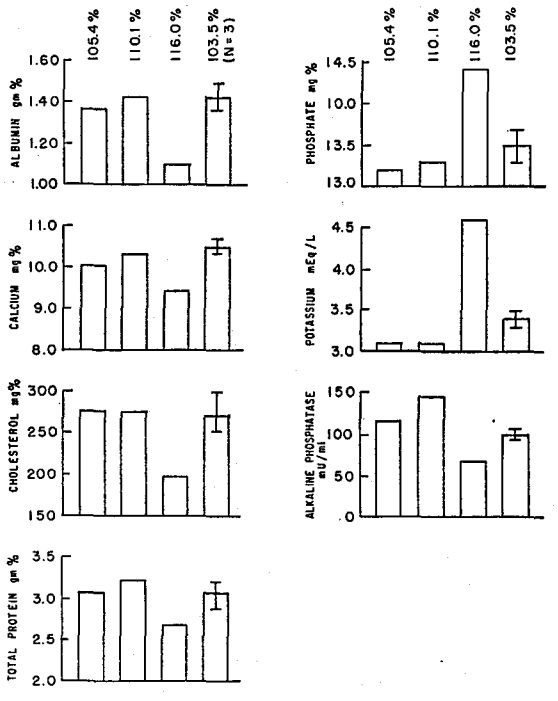


FIG. 1. Major serum chemistry parameters with increasing N_2 + Ar saturations. Control (103.5% N_2 + Ar) values are mean and range.

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

Two mechanisms have been invoked in an attempt to explain gas-bubble disease. These mechanisms are gas embolism of the heart, gills, or other vital portions of the blood system (Marsh and Gorham 1905; Dawley and Ebel 1974), and starvation due to blebs in the lining of the mouth preventing ingestion of food (Dawley and Ebel 1974). A 50% reduction in serum glucose was indicated by Robertson et al. (1963), when rainbow trout were starved. Similarly, albumin (Booke 1964) and total protein (Phillips et al. 1960) decreased in brook trout, *Salvelinus fontinalis*.

Emboli may be envisioned to block arteries and veins in a variety of critical tissues and organs, which would result in local or systemic hypoxia, respiratory acidosis and necrosis from the buildup of toxic wastes. The gills, brain, heart, and kidney are four such critical sites. Short-term hypoxia was shown to cause severe hemoconcentration, a doubling of serum phosphate, a 60% increase in serum glucose, and an uncompensated respiratory acidosis in Atlantic menhaden, *Brevoortia tyrannus* (Hall 1928). Extreme hypoxia in the brown bullhead, *Ictalurus nebulosus*, and the American eel, *Anguilla rostrata*, in fresh water, resulted in severe albuminemia (Bieter 1931). Hunn (1969) found that rainbow trout subjected to hypoxic stress showed a doubling of plasma phosphate.

Juvenile steelhead stressed for 35 days at 116.0% nitrogen saturation show no significant decrease in mean weight or length when compared to other test groups. These data would tend to support the hypoxia argument at the expense of the starvation proposition. Hypoxia should show an increase in serum cholesterol (Wedemeyer 1970a), but the present data suggest that cholesterol in fact declines

in steelhead exposed to 116.0% nitrogen saturation. While steelhead exposed to 116.0% nitrogen saturation alter their serum concentrations of phosphate (Hunn 1969) and calcium (Wedemeyer 1971) in a manner consistent with the hypoxia thesis, comparable data for the starvation interpretation is missing. In reciprocal fashion decreases in albumin and total protein are known (Phillips et al. 1960) following a period of starvation, but comparable data for chronic hypoxia is needed. Steelhead serum potassium and AP-ase levels need to be determined for both chronic hypoxia and starvation.

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

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Lethal and sublethal effects of dissolved nitrogen and argon gas concentrations ranging from 100 to 125% of saturation in water at 15° C in shallow tanks (25 cm deep) are presented in this paper. Sublethal effects monitored included change in swimming ability, growth rates and blood chemistry.

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

EARL M. DAWLEY AND WESLEY J. EBEL[1]

ABSTRACT

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

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

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

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

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

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

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

METHODS

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

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

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

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

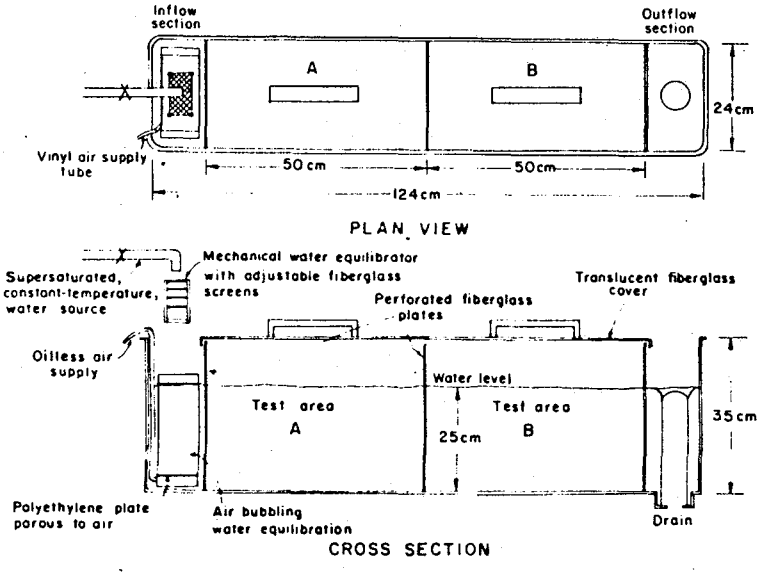


FIGURE 1.–Plan and cross-sectional views of test tank used for bioassay of dissolved gas.

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

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

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

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

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

Sublethal effects of supersaturation were as-

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

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

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

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

RESULTS

Relationships Among Mortality, Exposure Time, and Gas Concentration

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

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

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

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

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

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

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

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

Effect of Oxygen Concentrations on Time to Death Measurements

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

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

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

1966; Bouck 1972[1]; Rucker 1972). Most of the comprehensive studies, however, have been analyzed in terms of nitrogen concentration, assuming it to be the controlling influence upon the effects of gas bubble disease (even greater than indicated by the 80/20 ratio of the partial pressures N_2 /O_2). This assumption was based upon supposed biochemical decrease of the effective oxygen partial pressure within the fish.

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

Effect of Supersaturation Stress on Growth

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

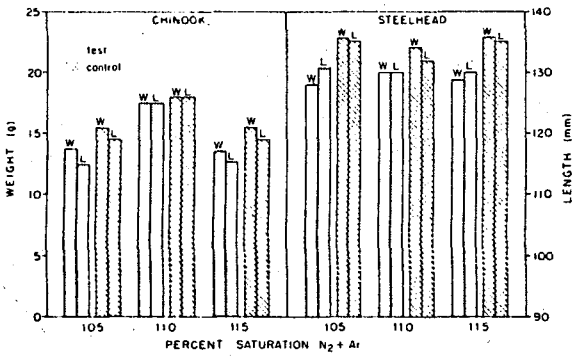


FIGURE 2.—Comparison of mean weights (W) and lengths (L) for test and control groups of juvenile chinook salmon and steelhead trout after 35 days at saturation levels of 100% (control), 105%, 110%, and 115%.

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

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

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

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

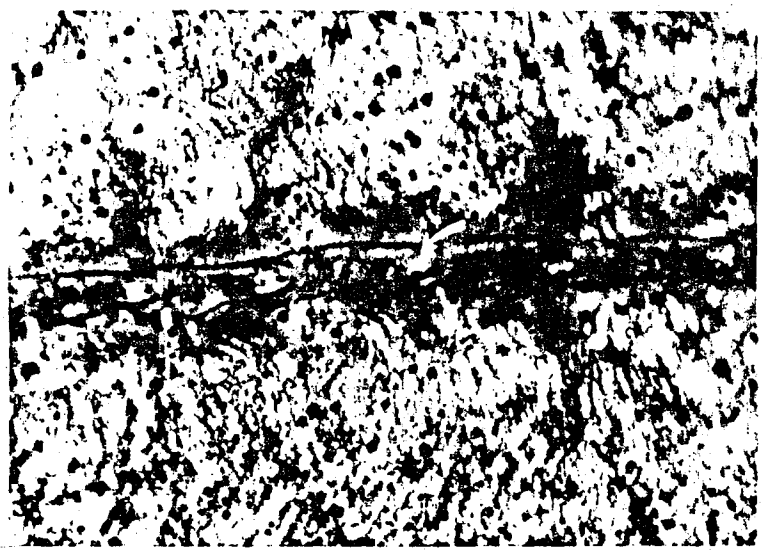


FIGURE 3.–Gas bubbles (arrow) in lateral line of juvenile chinook salmon.

Progression of Gas Bubble Disease

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



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

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

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

Recovery From Gas Bubble Disease

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

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

Effect of Supersaturation Stress on Survivors

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

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

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

SUMMARY AND CONCLUSIONS

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

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

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

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

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

We concluded from these experiments that:

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

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

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

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

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Discusses or describes:

- Effects of supersaturation on juvenile salmonids
- Laboratory studies done to define the effects of various levels of dissolved gases on several species of fish
- Sublethal effects of supersaturated water
- Field studies
- Live cage studies
- Migration rate
- Survival estimates
- Effects of supersaturation on adult fish and associated field studies.

MFR PAPER 1191

Effect of Atmospheric Gas Supersaturation on Salmon and Steelhead Trout of the Snake and Columbia Rivers

WESLEY J. EBEL and HOWARD L. RAYMOND

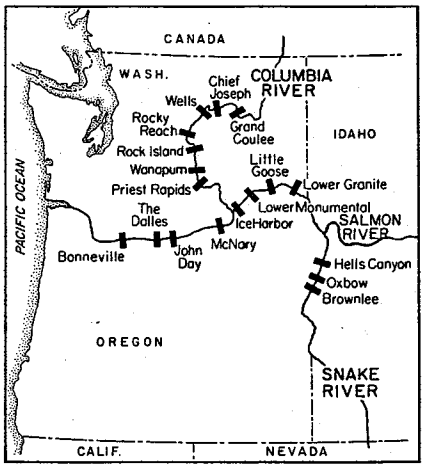


Figure 1.—Main stem dams in the Columbia River Basin.

ABSTRACT—Laboratory and field research data defining the effects of supersaturation of atmospheric gas on fish (primarily salmonids) in the Columbia River are summarized. Recent bioassay information regarding effects of various levels of supersaturation on several species of salmonids are compared with observations obtained in the river. The severity of the effect of supersaturation was dependent on level of supersaturation, the duration of exposure, water temperature, general physical condition of the fish, and the swimming depth maintained by the fish. Mortality caused by supersaturation was estimated for each of several years from 1966-74.

INTRODUCTION

Dams constructed on the Columbia and Snake Rivers in the past decade have impounded most of the free flowing sections of these rivers and created a water condition that in high-flow years is deadly to valuable stocks of anadromous Pacific salmon, *Oncorhynchus* spp., and steelhead trout, *Salmo gairdneri*. With high spills, the water becomes supersaturated with atmospheric gases to a level that is lethal to fish. In response to this problem, a major research effort is in progress which seeks to define the effects of supersaturation on fish and to develop methods of reducing supersaturation caused by dams. This report summarizes the present status of the research and the relation of our current information to the anadromous resources of the Columbia River.

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Salmon and steelhead resources of the Columbia River have in the past produced as much as 50 million pounds of fish in one year for consumption. Today, production is estimated at between 25 and 30 million pounds. Even in today's depressed state, the contribution of the Columbia River resources to our commercial and recreational fisheries is of a magnitude far greater than most people realize.

Columbia River salmon and steelhead represent an annual value of $22.5 million to commercial fishermen, and nearly $77.2 million at the retail level. An average of 42 percent of the chinook salmon, *O. tshawytscha*, and 73 percent of the steelhead trout taken annually by the United States commercial fisheries are of Columbia River origin.

The great majority of Columbia River fish are affected by the huge dams built for hydroelectric power, navigation, irrigation, and flood control. The upper river runs, because they must pass through a long sequence of dams (Fig. 1), are most severely affected. This review addresses itself to the salmon and steelhead trout runs of the Snake River where we have our most complete information, but it is logical that the conclusions apply as well to the stocks of the upper Columbia River and to a lesser degree, to the runs entering the Columbia River below the Snake River.

SUPERSATURATION OF NITROGEN: ITS SOURCE AND ITS EFFECT ON FISH

Supersaturation of nitrogen (atmospheric gas) was first recognized as a potential problem to anadromous fish in the Columbia River in 1965 when levels as high as 125 percent of saturation were recorded. A comprehensive study (Ebel, 1969) of dissolved gas levels done in 1966-1967 throughout the Columbia from Grand Coulee Dam to the estuary at Astoria, Oreg. substantiated that high levels of dissolved gases occurred throughout the study area (Fig. 2) during the spring and early summer when high flows occur. The study also showed that water plunging over spillways was the main cause of supersaturation and that little equilibration occurred in the reservoirs associated with the dams.

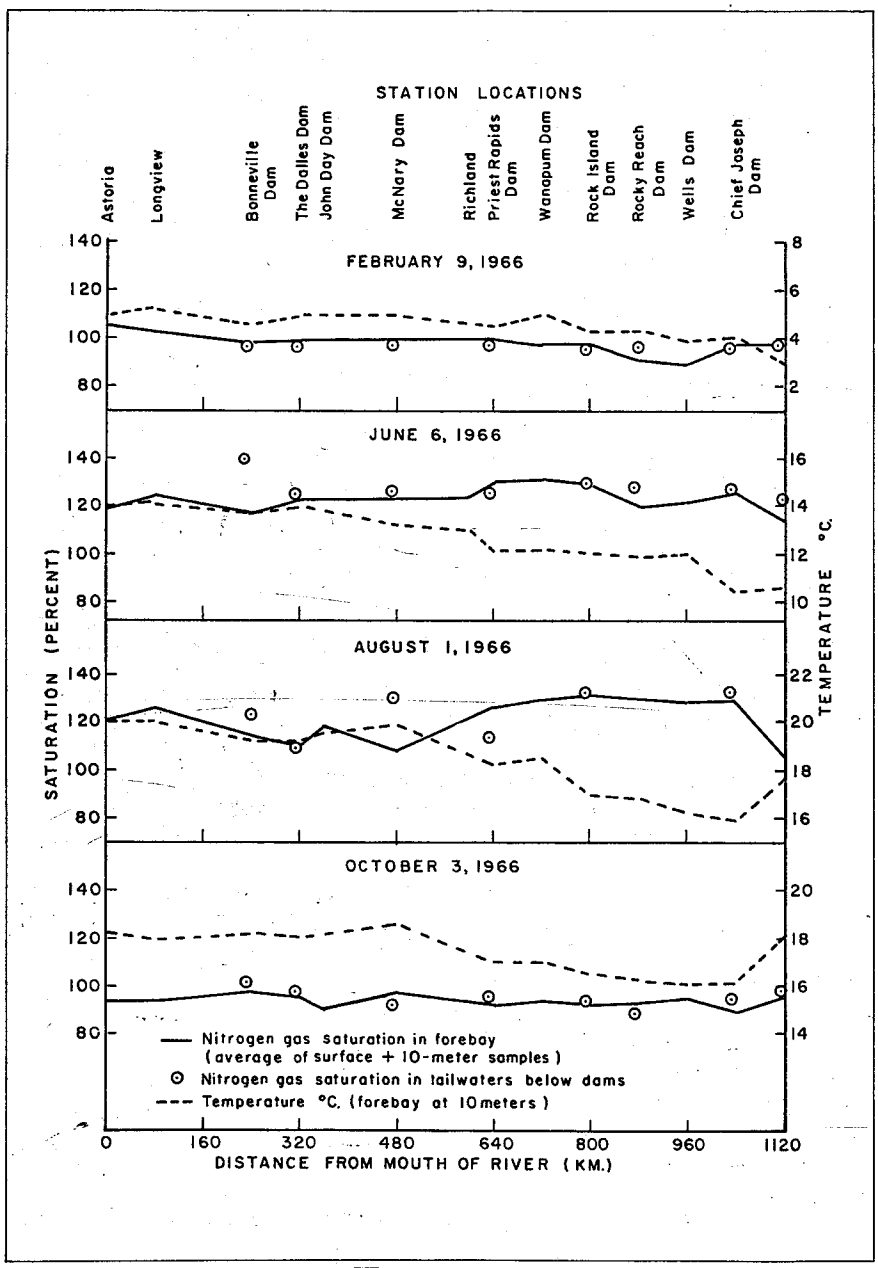


Figure 2.—Saturation of dissolved nitrogen and temperature of samples in Columbia River from Astoria to Grand Coulee Dam, February to October 1966. (Source: Ebel, 1969.)

Subsequent studies conducted by Roesner and Norton [1] (1971) and Meekin and Allen (1974) substantiated the earlier data of Ebel. Sufficient data are now available to establish air entrainment coefficients and rating curves for most of the dams on the Columbia and Snake Rivers; reasonably accurate predictions of the level of supersaturation that might occur downstream from a given dam during a specified flow are now possible. Thus, the mechanisms involved in creating supersaturation in the Columbia and Snake Rivers are at this time well understood.

A substantial impact from supersaturation of N_2 on fish populations, however, was not documented until John Day Dam was placed in operation in 1968. During the spring of that year heavy spillway discharge at the dam caused abnormally high (123-143 percent) supersaturation downstream

[1]Roesner, L. A., and W. R. Norton. 1971. A nitrogen gas (N_2) model for the lower Columbia River. Water Resources Engineers, Inc., Walnut Creek, Calif., Final Rep. to Portland Dist., U.S. Army Corps of Engineers, 88 p.

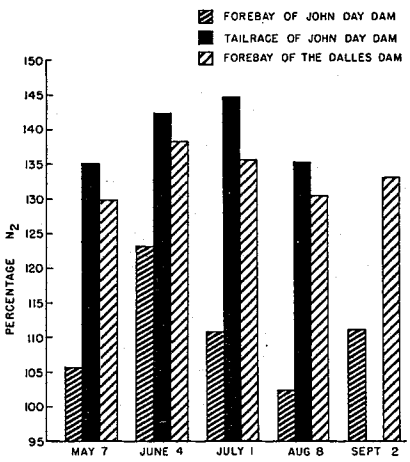


Figure 3.—Comparison of percentage saturation of dissolved nitrogen in forebay of John Day Dam with tailrace of John Day Dam and The Dalles Dam forebay, May-September 1968. (Source: Beiningen and Ebel, 1970.)

(Fig. 3) and substantial mortalities of both adult and juvenile salmonids were recorded (Beiningen and Ebel, 1970). Continued evidence that a substantial problem for both juvenile and adult salmon and steelhead existed in the Columbia drainage (Ebel, 1971; Raymond [2]) prompted a large scale research effort to 1) measure the effects of supersaturation of dissolved gases on fish and 2) develop methods of reducing levels of supersaturation caused by dams. This report summarizes most of the recent research on effects of supersaturation of dissolved gases on fish.

Effects of Supersaturation on Juvenile Salmonids

Supersaturation of atmospheric gas (mainly nitrogen) in waters of the Columbia and Snake Rivers was well documented by 1970 as a serious problem to valuable stocks of salmon and steelhead. There was sufficient evidence that gas bubble disease resultling from this supersaturation caused both direct and indirect mortalities. However, precise data on tolerance of various species of salmonids to various levels of supersaturation of atmospheric gases was unknown.

Several investigators recorded the

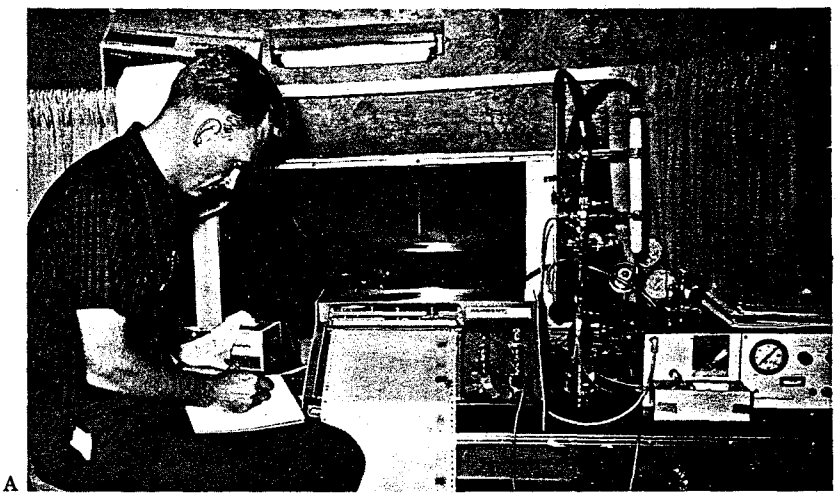
[2]Raymond, Howard L. 1970. A summary of the 1969 and 1970 out-migration of juvenile chinook salmon and steelhead trout from the Snake River. Bur. Commer. Fish. Biol. Lab., Seattle, Wash., Progress Rep. 11 p. Processed.

lowest level observed during various experiments where mortalities occurred from gas bubble disease; however, very little attention was given to determining precise tolerance levels or effect of sublethal exposure on performance. Harvey and Cooper[3] (1962) indicated 108-110 percent saturation produced gas bubble disease and subsequent mortalities in sockeye salmon alevins, *O. nerka*; Rucker and Tuttle (1948) indicated a level somewhere between 110 percent and 115 percent as being the critical range for trout. Shirahata (1966) conducted the most comprehensive study to date on the effects of various levels of nitrogen gas on rainbow trout (rainbow trout is the resident freshwater form of *S. gairdneri*, whereas the steelhead trout is the anadromous form of the species) from hatching to the swim-up stage, but such detail is lacking for other species of salmonids. In many experiments on gas bubble disease, either the water temperatures, nitrogen gas concentrations, or life stages of the test fish were omitted from the record, thus making the results incomplete for critical applications. The extent of the costs involved in alleviating the supersaturation problem in the Columbia and Snake Rivers will depend on the degree of protection required to afford a safe environment for the aquatic biota. It was imperative, therefore, that regulatory and corrective measures established to govern the level of saturation be based upon a thorough understanding of the effects of dissolved gases on aquatic organisms.

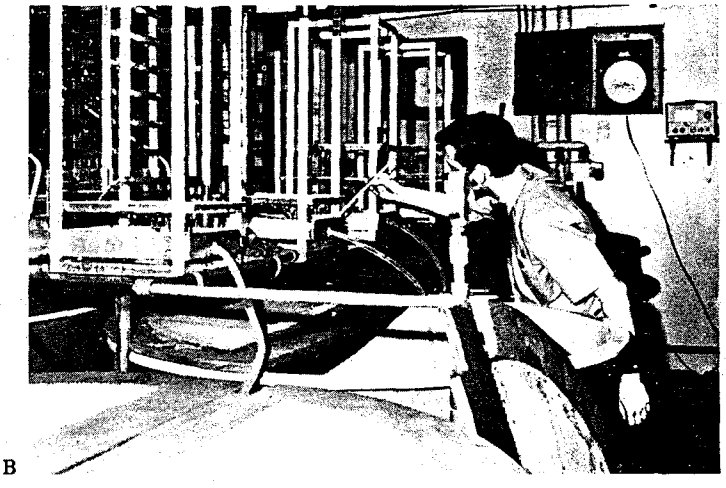
Presently available laboratory and field research data defining the effects of supersaturation on juvenile salmonids and some nonsalmonids present in the Columbia drainage are summarized here.

Laboratory Studies

Research (Fig. 4) was undertaken to define the effects of various levels of dissolved gases on several species of fish (primarily juvenile salmonids) by the National Marine Fisheries Service (NMFS), Bureau of Sport Fisheries and



A



B



C

Figure 4.—Research on effects of gas supersaturation on juvenile fish at National Marine Fisheries Service laboratories: A) Analysis of dissolved atmospheric gas; B) supersaturation tolerance test in shallow tanks; and C) supersaturation tolerance test in deep tanks.

[3] Harvey, H. H. and A. C. Cooper. 1962. Origin and treatment of a supersaturated river water. Int. Pacific Salmon Fish Comm. Prog. Rep. 9. 19 p.

Wildlife (BSFW)[4], Battelle Northwest Laboratories (BNW), and the Environmental Protection Agency (EPA).

NMFS centered their research on smolting size juvenile spring chinook and steelhead, fall chinook fry, and juvenile and adult squawfish, *Ptychocheilus oregonensis*. Dawley and Ebel (in press) found that exposure of juvenile spring chinook and steelhead to 120 percent saturation for 1.5 days resulted in over 50 percent mortality; 100 percent mortality occurred in less than 3 days. They also determined that the threshold level where significant mortalities begin occurring (Table 1) is at 115 percent saturation nitrogen and argon (111 percent total gas saturation).

Table 1.—Mean values of lethal exposure time for juvenile steelhead and spring chinook acclimated to 15°C and then subjected to various levels of gas saturation[1] from 100 to 125% in shallow (25 cm depth) tanks. (From Dawley and Ebel, 1975.)

Percent mortality	Percent saturation (N_2 + Ar)	Hours exposure	
		Steelhead	Chinook
10	125	10.3	10.6
50		14.2	13.6
100		[2]23.0	[2]32.1
10	120	26.0	19.3
50		33.3	26.9
100		[2]40.0	[2]55.0
10	115	[2]58.0	7% mortality in 792 h
50		[2]486.0	Not reached
100		Not reached	Not reached
110, 105, 100		Mortality of 5% or less recorded for either steelhead or chinook after 35 days at these concentrations. Gas bubble disease was not apparent cause of deaths.	

[1] Percentage saturation of nitrogen and argon was set as indicated in the table (±2%). Oxygen concentrations ranged between 87 and 98% saturation in tanks set at 100-110% nitrogen plus argon saturation; in tanks set at 115-125% nitrogen saturation, O_2 levels ranged between 98 and 115%.
[2] Exposure times indicated for test replicates of section A only. Mortality in section B had not reached indicated level at termination of test.

Similar results on mortality rates of juvenile coho salmon, *O. kisutch*, were obtained in bioassays conducted by Nebeker[5] and Rucker (1975). Rucker also determined that smaller coho (3.8-6 cm) were more resistant than larger coho (8-10 cm) at the same gas concentration. In addition, he determined the effect of individual ratios of oxygen and nitrogen and established that a drastic decrease in lethal effect occurred when the nitrogen content fell below 109 percent saturation even though total gas saturation remained at 119 percent saturation, indicating the importance of determining the concentration of the individual components (O_2 and N_2) of the atmospheric supersaturation.

An additional study was done by NMFS in 1974 to more accurately define the benefit that stocks of fish might receive by sounding into deeper water levels (hydrostatic compensation). An experiment was conducted where juvenile fall chinook salmon and steelhead mortality rates could be compared when they were exposed to various levels of N_2 in deep and shallow tanks. This study (Dawley, Schiewe, and Monk, 1976) showed that by providing chinook and steelhead the option to sound in deep tanks, their ability to survive was increased, particularly at the lower levels of supersaturation. However, substantial mortality still occurred in fish tested in deep tanks when the concentrations exceeded 120 percent (Fig. 5). It is apparent from this study and similar field studies that depth of migration or hydrostatic pressure must be considered when estimating probable loss from N_2 for fish populations migrating in the Columbia or Snake River. Calculations of estimated mortality discussed later in this report take this into consideration.

Sublethal Exposure

Experiments were conducted by Schiewe (1974), Weber and Schiewe (1976), and Newcomb (1975) to determine the effect of sublethal exposure of juvenile steelhead and chinook to supersaturated water. Effect of sublethal exposure on swimming performance, growth, lateral line function, and blood chemistry was measured. These experiments showed that swimming performance of chinook and lateral line function of steelhead were adversely affected by sublethal exposure to various levels of supersaturated atmospheric gas. Blood chemistry measurements showed altered characteristics in albumen, calcium, phosphates, potassium, and chloride levels after exposure to supersaturated water. These data suggest that survival of juvenile populations stressed to a sublethal level by exposure to supersaturated water would be lower than normal, particularly in a river environment like the Columbia where numerous hazards are encountered.

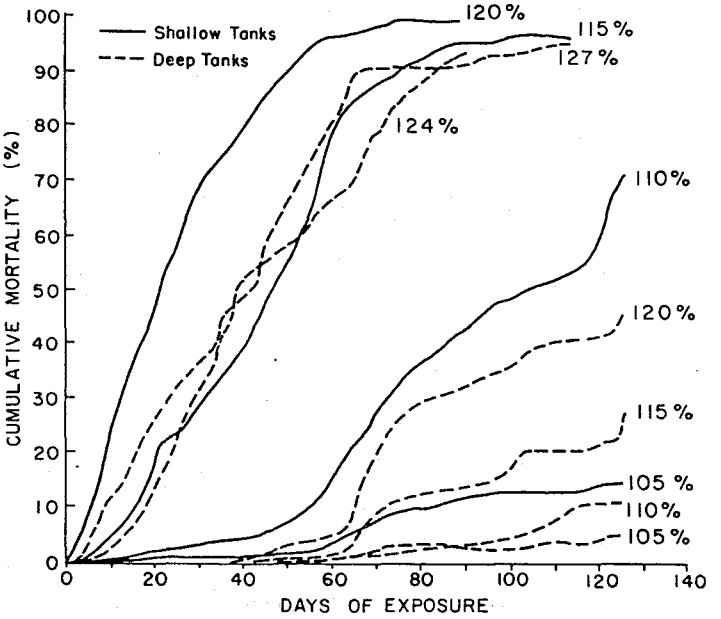


Figure 5.—Mortality versus time curves for juvenile fall chinook salmon exposed to various concentrations of dissolved atmospheric gas (percent of saturation TDG) in shallow (0.25 m) and deep water (2.5 m) tanks at 10°C. (Source: Dawley et al., 1976.)

[4] This agency is now the U.S. Fish and Wildlife Service (FWS).
[5] Nebeker, Alan V. 1973. Effect of nitrogen (air) supersaturation on Columbia River fishes. Environ. Prot. Agency, Western Fish Toxicol. Sta., Corvallis, Oreg. Annu. Prog. Rep. 1973, ROAP 21 AKG.

Field Studies

Numerous field studies have been conducted by state, federal, and private fishery research groups to obtain information on effect of supersaturation of atmospheric gas on juvenile salmonids in the Columbia and Snake Rivers. Live cage, migration rate, timing, and survival studies were of particular significance because they have been done over a range of river conditions for several consecutive years. Incidence of symptoms of gas bubble disease in juvenile migrants arriving at Ice Harbor and The Dalles Dams was also determined for several years. Data are therefore available from studies done during periods of low, intermediate, and high levels of supersaturation.

Live Cage Studies. The first live cage studies conducted in the middle Columbia River (Ebel, 1969) indicated that 6-16 percent mortality occurred in populations of juvenile coho and chinook held in a deep (surface to 6 m) cage when concentrations of N_2 ranged from 118 to 143 percent. Mortality ranged from 10 to 100 percent in fish held in shallow cages (surface to 1 m deep). Similar tests done in the lower Columbia River (The Dalles forebay) by Beiningen[6] and Ebel in 1969 resulted in a mortality range for juvenile fall chinook of 25-30 percent in the deep cage and 95-100 percent in shallow cages. At this time N_2 concentrations ranged from 126 to 145 percent of saturation. Another live cage test done later by Ebel (1971) in the forebay of Ice Harbor Dam on the Snake River resulted in a range of mortality from 45 to 68 percent in the deep cage where fish could sound to 4.5 m. In these tests both hatchery and wild stocks of juvenile spring and fall chinook were used as test animals. Nitrogen concentrations ranged from 127 to 134 percent of saturation.

Finally, a similar live cage test was again conducted in the Wanapum area of the Columbia in 1974 by Weitkamp[7]. No mortality occurred in his deep cage, but nitrogen levels never exceeded 126 percent during these tests.

It is obvious from these live cage studies that various levels of mortality occur depending on the location, level of nitrogen supersaturation, and stock of fish used. Of particular significance, however, is the fact that substantial mortalities occurred even in the deep cages, which allowed fish to sound when concentrations exceeded 125 percent saturation. Nearly 100 percent mortality occurred in surface cages used in all the tests.

Similar studies were conducted by Blahm, McConnell, and Snyder[8], 1973. In these studies, ambient Columbia River water was pumped into 1-m and 3-m deep tanks, and mortality was recorded as dissolved gas concentrations in the river changed from a low of about 106 percent in April to a high of 130 percent in June. Substantial mortality of both juvenile chinook and coho salmon occurred in the shallow (1-m) tank; in the deep (3-m) tank, mortality did not begin until dissolved nitrogen concentrations approached 125 percent near the termination of the test in June. Nitrogen concentrations did not exceed 125 percent saturation except for about one day at the termination of the test. These data substantiate other laboratory and field data where high

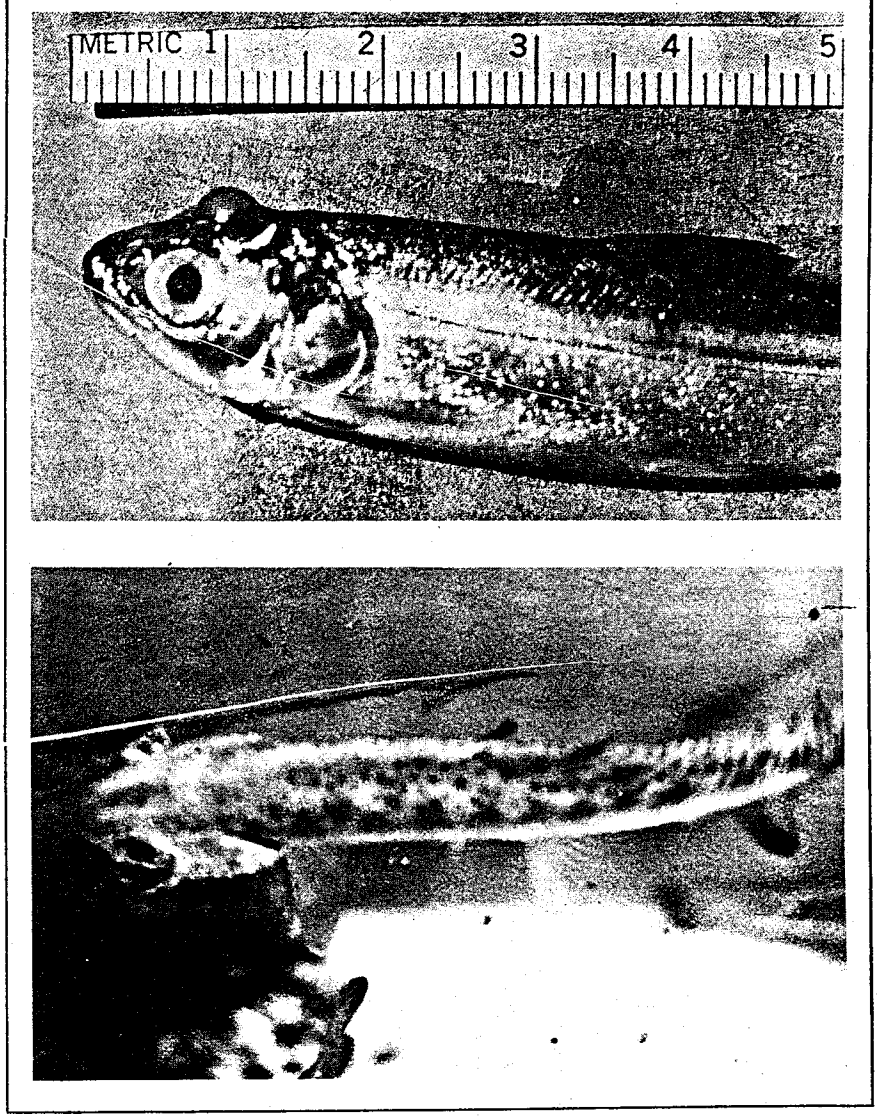


Figure 6.—Gas bubble disease symptoms observed on juvenile fish sampled at The Dalles Dam. Above, gas blister on juvenile coho salmon (photo from Beiningen and Ebel, 1970). Below, bilateral exophthalmia on juvenile chinook salmon.

[6]Kirk T. Beiningen, presently employed by the Oregon Dept. of Fish and Wildlife, Clackamas, Oreg.

[7]Donald E. Weitkamp, Parametrix Inc., Seattle, Wash. Unpubl. data.

[8]Blahm, Ted H., Robert J. McConnell, and George R. Snyder. 1973. Effect of gas supersaturated Columbia River water on survival of juvenile salmonids April-June 1972. Natl. Mar. Fish. Serv., NOAA, Prescott, Oreg. Final Rep., Pt. I. 61 p. Processed.

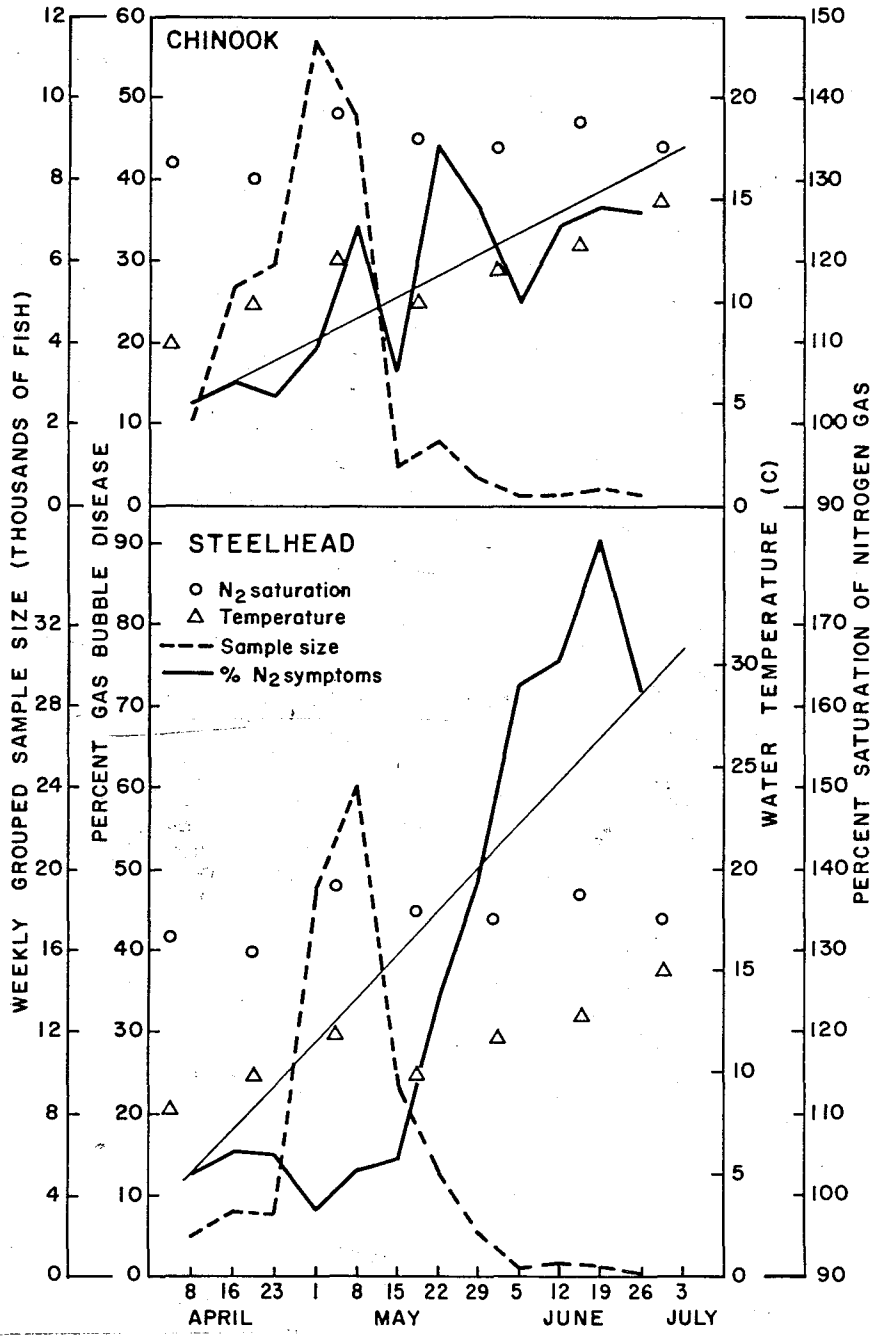


Figure 7.—Percentage gas bubble disease symptoms in juvenile steelhead and chinook in relation to nitrogen concentrations and temperature of tailrace water samples at Lower Monumental Dam. Sample size reflecting the magnitude of the migrating populations is also shown. (Source: Ebel et al., 1971, footnote 12.)

levels of mortality were recorded in fish held in shallow water at dissolved gas levels as low as 115 percent, and substantial mortality does not occur where juvenile salmon are allowed the option to sound until concentrations exceed 120-125 percent of saturation.

Observations of gas bubble disease symptoms in juvenile migrants (Fig. 6) in the Columbia River were first noted in 1966 (Ebel, 1969) from samples of fall chinook and coho netted from gatewells. These symptoms persisted throughout July and August when levels of nitrogen as high as 137 percent were recorded. Subsequent observations of symptoms were made from samples taken in gatewells at The Dalles Dam in 1968 and at Ice Harbor Dam each year since 1969 (Ebel, 1971; Ebel, Krcma, and Raymond[9]; Ebel, Raymond et al.[10,11]. These data generally show that during periods when nitrogen levels are high the incidence of symptoms is also high, particularly on the later migrants (Fig. 7). Incidence of symptoms cannot be directly transferred into percentage mortality, but we believe they are an indication of the degree of stress a population is suffering from exposure to supersaturation of atmospheric gas. For example, in 1970 the incidence of symptoms of gas bubble disease on chinook migrants examined at Ice Harbor Dam ranged from 25 to 45 percent; the survival of chinook (Raymond[12]; footnote 2) passing from the Salmon River to Ice Harbor Dam was estimated to be 30 percent. In 1971, incidence of gas bubble disease ranged from 10 to 32 percent during the main portion of the migration; survival was estimated at 50 percent.

Relationship Between Survival Estimates (Juvenile Chinook and Steelhead) and Supersaturation of Atmospheric Gases. Research on the migrations of juvenile salmonids from the Salmon River (the main spawning tributary of the Snake River) as far downstream as The Dalles Dam has

[9]Ebel, Wesley J., Richard F. Krcma, and Howard L. Raymond. 1973. Evaluation of fish protective facilities at Little Goose Dam and review of other studies relating to protection of other salmonids in the Columbia and Snake Rivers, 1973. Northwest Fish. Cent., Natl. Mar. Fish. Serv., NOAA, Seattle, Wash., Prog. Rep. to U.S. Army Corps of Engineers, contract no. DACW68-71-0093, 62 p. Processed.

[10]Ebel, Wesley J., Howard L. Raymond, Clifford W. Long, Willman M. Marquette, Richard Krcma, and Donn Park. 1971. Progress report on fish-protective facilities at Little Goose Dam and summaries of other studies relating to the various measures taken by the Corps of Engineers to reduce losses of salmon and steelhead in the Columbia and Snake Rivers. Northwest Fish. Cent., Natl. Mar. Fish. Serv., NOAA, Seattle, Wash. Prog. Rep. to U.S. Army Corps of Engineers, contract no. DACW68-71-C-0093, 58 p. Processed.

[11]Ebel, Wesley J., Howard L. Raymond, Clifford W. Long, Willman M. Marquette, Richard Krcma, and Donn Park. 1973. Evaluation of fish protective facilities at Little Goose Dam and review of other studies relating to protection of juvenile salmonids in Columbia and Snake Rivers, 1972. Northwest Fish. Cent., Natl. Mar. Fish. Serv., (NOAA), Seattle, Wash. Prog. Rep. to U.S. Army Corps of Engineers, contract no. DACW68-71-0093, 83 p. Processed.

[12]Raymond, Howard L. 1974. Snake River runs of salmon and steelhead trout: trends in abundance of adults and downstream survival of juveniles. Northwest Fish. Cent., Natl. Mar. Fish. Serv., NOAA, Seattle, Wash., 12 p. Processed.

been conducted since 1966. The object of the research has been to measure the effects of new dams and impoundments on juvenile timing, migration rate, and survival. A general decline in survival of downstream migrants has occurred since 1967. This decline is attributed to four main factors: passage through turbines, delays in migration, predation, and supersaturation of nitrogen.

In this section of the report, losses or decrease in survival of juvenile salmonid populations attributable to excessive exposure to supersaturation of atmospheric gases (N_2) will be defined. The results obtained from data collected in 1966-67 provided a basis for comparing migrations after John Day (1968), Lower Monumental (1969), and Little Goose Dams (1970) were completed. Details of methods, experimental design, and statistical calculations are contained in annual progress reports (Raymond[13,14], footnote 2, and 1968; Park and Bentley[15]). Fish at each of the sampling sites from the Salmon River to The Dalles Dam were given separate marks to identify the sampling site and the time of release. Subsequent recovery of these marked fish (Fig. 8) at dams provided the necessary data for the measurement of survival. Juvenile chinook salmon and steelhead trout, which represent 95 percent of the outmigration from the Snake River, were the primary species studied.

Migration Rate. Travel time or migration rate is related to river velocity; i.e., the higher the water velocity the higher the rate of fish migration. Raymond (1968 and 1969) determined that juvenile chinook moved only one-third as fast through McNary and John Day reservoirs as through free-flowing stretches of river. Calculations made for impoundments in the Snake River indicated similar delays. Migration rates of both chinook and steelhead are similar. These are shown (Table 2) for low, moderate, and high river flows through both free-flowing and impounded sections of the Snake and Columbia Rivers. These data become particularly important when estimating the length of time fish are exposed to supersaturation of atmospheric gas.

Timing of Peak Migration. Peak migrations generally occur between

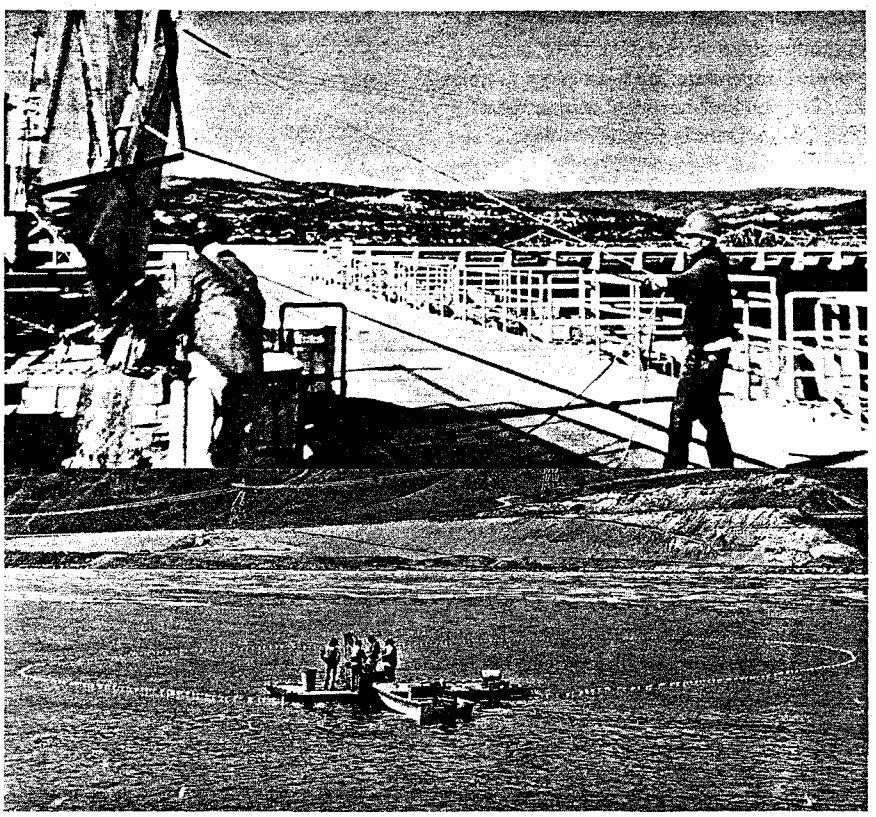


Figure 8.—Above, sampling for juvenile migrants at the turbine intake gatewell of a dam with a specially designed dip net. Below, purse seining for juvenile migrants near Little Goose Dam.

Table 2.—Travel time[1] estimates in days for Snake River juvenile chinook and steelhead trout to travel from the Salmon River to the estuary.

Section of river	Flow		
	Low[2]	Moderate[3]	High[4]
Salmon River to Lewiston (115 miles) free-flowing	8	5	3
Lewiston to Lower Granite Dam (35 miles) impounded	7	4	2
Lower Granite to Little Goose Dam (40 miles) impounded	8	5	3
Little Goose to Ice Harbor Dam (63 miles) impounded	13	8	4
Total Snake River	36	22	12
Ice Harbor to The Dalles Dam (143 miles) impounded	29	18	10
The Dalles Dam to the Estuary (192 miles) like free flowing	13	8	6
Total Columbia River 335 miles	42	26	16
Grand total	78	48	28

[1]Travel time based on the following migration rates. Free-flowing: Low, 15 m/day; moderate, 25 m/day; high, 34 m/day. Impounded: Low, 5 m/day; moderate, 8 m/day; high, 15 m/day.
[2]Snake River, 30-50,000 cfs; Columbia River, 150-180,000 cfs.
[3]Snake River, 80-100,000 cfs; Columbia River, 200-300,000 cfs.
[4]Snake River, 120-180,000 cfs; Columbia River, 350-500,000 cfs.

[13]Raymond, Howard L. 1967. A summary of the 1966 outmigration of juvenile salmonids in the Columbia Basin. Bur. Commer. Fish., Biol. Lab., Seattle, Wash., 20 p. Processed.
[14]Raymond, Howard L. 1968. A summary of the 1968 outmigration of juvenile salmon and steelhead trout from the Snake River. Bur. Commer. Fish., Biol. Lab., Seattle, Wash., 12 p. Processed.
[15]Park, Donn L., and Wallace W. Bentley. 1968. A summary of the 1967 outmigration of juvenile salmonids in the Columbia Basin. Bur. Commer. Fish., Biol. Lab., Seattle, Wash., 14 p. Processed.

late April and late May with the chinook peaks preceeding the steelhead peak outmigration by 3-14 days (Table 3). The arrival of peak migrations to The Dalles Dam is now 10-20 days later than it was before the recent dams (John Day, Little Goose, and Lower Monumental) impounded large stretches of the river. This later arrival of peak migrations is caused by a reduction in the migration rate in the impounded stretches of the river to about one-third of that measured in free-flowing stretches. The late arrival of the peak migrations of Snake River stocks has resulted in increased exposure to supersaturation during some high flow years. In years prior to 1968, the peak migration of juvenile chinook from the Snake River usually entered the Columbia River before flows in the Columbia had peaked and hence were not subjected to the highest levels of supersaturation in the Columbia. With a 10-day later arrival they often become subjected to the highest levels of supersaturation in both rivers. Recent differences (since 1970) in timing between years and between species are attributable to annual variability in water temperature, stream flow, and—particularly in the case of steelhead smolts—time of release from hatcheries.

Survival Estimates. Survival of juvenile populations of chinook and steelhead from the Salmon River to Ice Harbor Dam was about equal and was estimated to be about 90 percent (Fig. 9) in the years prior to 1968. Survival between Ice Harbor and The Dalles dams was reduced to about 60 percent during the years prior to 1968, largely we believe, because of fish passage through the turbines of Ice Harbor and McNary Dams (N_2 levels were not critical through this stretch of river until 1969). After 1968, survival rate of the two species declined and was no longer equal. The data indicate that the decline in survival of both species during high-flow years can be attributed primarily to increased exposure to supersaturated water. Knowledge of the timing and migration rate, depth distribution (Fig. 10), and dissolved gas concentrations makes it possible to estimate the percentage loss that can be attributed to exposure to supersaturation. Figure 11 provides a pictorial representation of the relationship between surface supersaturation, exposure time, depth of fish, and hydrostatic compensations. Table 4 provides an example of the method used to calculate the percentage loss of chinook attributable to exposure to supersaturation of atmospheric gas (nitrogen) in 1970. Similar calculations were used to estimate losses attributable to supersaturation in other years.

Survival estimates from the upper Snake River to The Dalles Dam for juvenile populations of chinook and steelhead migrating down the Snake and Columbia Rivers are shown in Figure 12. Survival differences noted between the Snake River stretch of the migration and the Columbia River stretch between Ice Harbor and The Dalles Dams are indicated in Figure 8. Both figures are referred to frequently throughout this section.

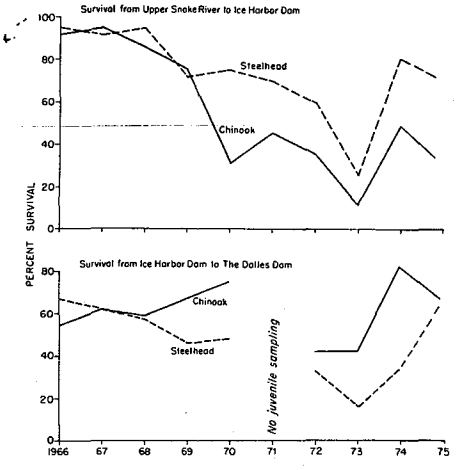


Figure 9.—Survival of juvenile chinook salmon and steelhead from the upper Snake River to Ice Harbor Dam and The Dalles Dam.

Table 3.—Comparison of timing of the migration peak of juvenile chinook salmon and steelhead trout at Ice Harbor Dam on the Snake River 1966-74.

Year	Chinook		Steelhead		Timing difference in days
	Peak of migration	River flow at peak	Peak of migration	River flow at peak	
1966	5/ 4	65,000	5/11	108,000	7
1967	5/10	93,000	5/22	148,000	12
1968	5/10	53,000	5/21	90,000	11
1969	4/28	107,000	5/12	165,000	14
1970	5/13	88,000	5/25	175,000	12
1971	5/4	188,000	5/8	204,000	4
1972	5/12	122,000	5/22	186,000	10
1973	5/21	87,000	5/24	78,000	3
1974	5/6	157,000	5/13	140,000	7

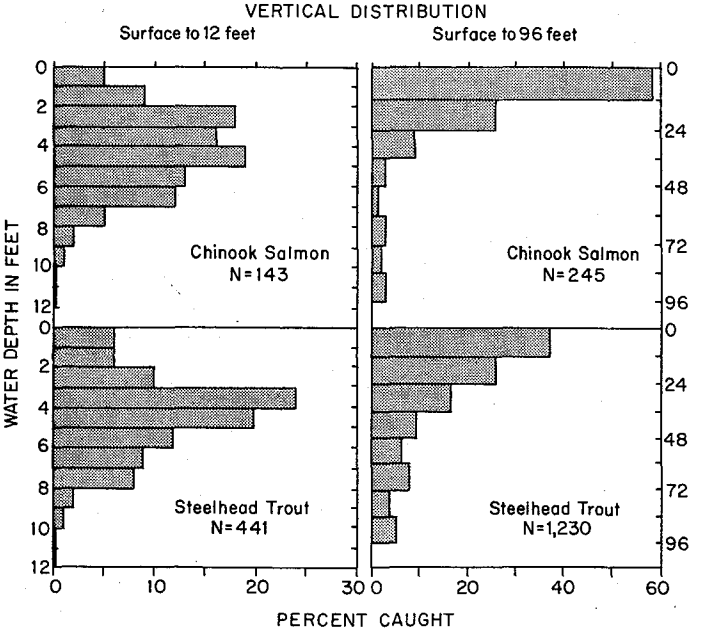


Figure 10.—Vertical distribution of juvenile chinook salmon and steelhead caught in the forebay of Lower Monumental Dam, 1973. (Source: Ebel et al., footnote 13.)

Survival of both chinook and steelhead smolts during migration down the Snake River to Ice Harbor Dam declined from over 90 percent through 1968 to about 70-75 percent in 1969. It is believed that N_2 levels above 130 percent, appearing for the first time in the Snake River after completion of the Lower Monumental Dam, were largely responsible for the decline. Survival of chinook smolts from Ice Harbor Dam to The Dalles Dam in 1969 were slightly higher than in the previous years (67 percent). Conversely, steelhead survival through this stretch of river dropped significantly to 46 percent. The lower steelhead survival was attributed to increased exposure to higher N_2 supersaturation in the Columbia River. Most of the chinook migrated downstream in April and early May when flows were 80-100,000 cfs in the Snake River and about 300,000 cfs in the Columbia River. Steelhead migrations were nearly 2 weeks later when flows exceeded 166,000 cfs in the Snake and 440,000 cfs in the Columbia River. Thus, even though a larger portion of the steelhead migrated deeper they were exposed longer to a higher level of supersaturation and consequently suffered a higher mortality than the chinook in this stretch of the river in 1969.

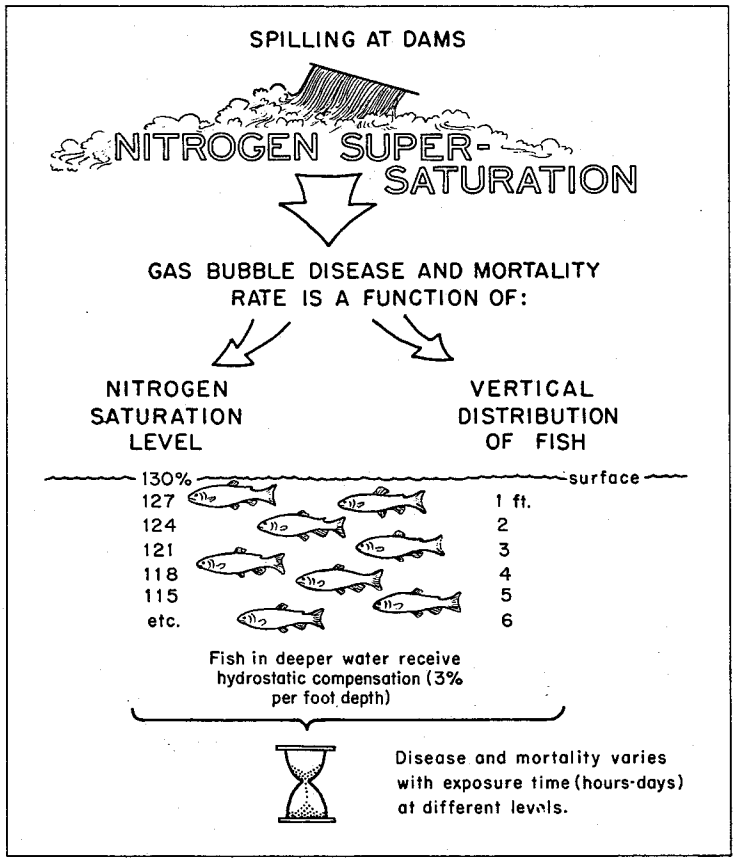


Figure 11.—Generalized representation of cause and effect relationship in gas supersaturation-gas bubble disease-fish mortality.

Estimated survival of juvenile chinook and steelhead from the upper Snake River to The Dalles Dam in 1970 was 24 and 40 percent, respectively. However, with respect to area of loss, survival of juvenile chinook and steelhead was markedly different in 1970 for the two species. Most chinook mortalities occurred in the Snake River while most of the steelhead did not succumb until reaching the Columbia River. Differences can be related to length of exposure and differences in depth (vertical) distribution and tolerance to N_2 supersaturation. Most chinook migrated during Snake River flows of 50-100,000 cfs during the first 20 days in May. Travel time was 8-13 days from Little Goose to Ice Harbor Dam, and N_2 levels exceeded 130 percent saturation. Survival of chinook smolts to Ice Harbor Dam was 32 percent.

Table 4.—Estimated mortality of juvenile chinook salmon from exposure to supersaturated atmospheric gas in the Snake River, 1970 (from Ebel, 1971).

Percentage migration	Depth distribution (feet)	Gas level (% sat.)	Depth[2] compensated level (% sat.)	Exposure[3] time (days)	Percentage[4] mortality	Total mortality of group (%)	Portion[5] of total migration
34	0-5	135	120	3	100	34.0	
11	5-7	135	114	3	0	0.0	
					Subtotal	34.0	1/3
34	0-5	135	120	14+	100	34.0	
11	5-7	135	114	14+	30	3.3	
					Subtotal	37.3	2/3

Summary of estimates:

One-third of migration, 34.0% loss
Two-thirds of migration, 37.3% loss

Weighted average, 35.2% total estimated mortality

[1]Determined from depth distribution study.
[2]Computed from prevailing gas concentration minus hydrostatic pressure compensation as determined by average depth of migrants.
[3]Determined from migration rate and timing data. The first one-third of the migration was subjected to 3 days exposure to levels of 135% or greater; the remaining two-thirds of the migration was subjected to 135% or greater for 14 days.
[4]Percentage mortality as determined from exposure time and bioassay research.
[5]Portion of migration subjected to this loss as determined by timing of migration.

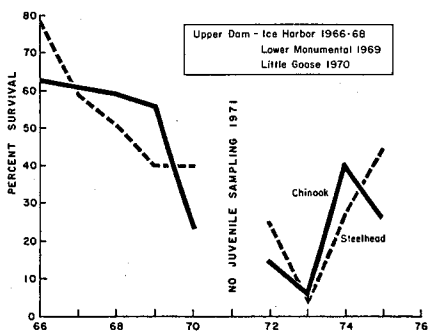


Figure 12.—Survival of Snake River juvenile chinook salmon and steelhead from the upper dam down to The Dalles Dam, 1966-74. (Source: Raymond, 1974, footnote 14.)

Data by Ebel (1971) indicate that losses from nitrogen are directly related to exposure time. He found that fish held in surface cages all died within 48 hours; 50 percent of the fish held in a 0-4.5 m volitional cage, where fish were allowed to seek their own depth, died in 7 days (127-134 percent saturation). If chinook were traveling near the water surface as indicated from depth distribution studies (Smith, 1974) and were exposed to high nitrogen from 10 days to 2 weeks as indicated by the data on timing of migration, most of the 68 percent mortality measured could be attributed to gas bubble disease caused by prolonged exposure to high levels of supersaturated nitrogen as fish migrated down the Snake River to Ice Harbor Dam. In contrast to high losses in the Snake River, mortality of chinook between Ice Harbor and The Dalles Dams was only 25 percent; most of this was believed to have been turbine related. There was little spilling at Columbia River dams during this period which resulted in relatively low levels of supersaturation in the Columbia River during the chinook outmigration.

On the other hand steelhead smolts migrated between 15 May and 5 June; this was 2 weeks later than chinook and in river flows of 160-200,000 cfs in the Snake River and 350-400,000 cfs in the Columbia River. During their migration, N_2 levels increased to over 140 percent in the Snake River and 127-139 percent in the Columbia River. With the higher flows, travel time was much faster—4 days in the Snake River and 10 days in the Columbia River. Estimated survival was 75 percent down the Snake River, sharply declining to 48 percent in the Columbia River. Although gas concentrations were higher in the Snake River during this period, exposure time (4 days) was short; and since a larger portion of the steelhead migrate at deeper levels, the loss to Ice Harbor Dam was only 25 percent. However, a 10-day additional exposure in the Columbia River from Ice Harbor to The Dalles Dam in supersaturated water was sufficient to cause an additional 52 percent loss, resulting in a 60 percent total loss.

Measurements of survival of juvenile chinook and steelhead to The Dalles Dam were not conducted in 1971 because sampling of juvenile populations for marks was discontinued that year. Measurements of survival from the Salmon River to Ice Harbor Dam indicated that survival of chinook was 45 percent and steelhead, 70 percent. The Snake River flow was extremely high in 1971 (180-200,000 cfs during the peak of the migration of both steelhead and chinook), and the majority of the mortality was attributed to exposure of the populations to supersaturation of nitrogen gas. The relationship between time (exposure), supersaturation of nitrogen, and survival is shown in Table 5. Slower moving fish migrating during the lower flows in 1970-71 survived at a lower rate (25-37 percent) than those traveling at a faster rate during higher flows (50-52 percent). The table also indicates that a flow control effort for 3 days between 21 April and 30 April, which reduced dissolved gas concentrations to 109 percent for about 3 days, increased survival from 25 to 37 percent.

Table 5.—Relationship between travel time, supersaturation of nitrogen, and survival of juvenile chinook salmon from the Salmon River to Ice Harbor Dam 1970-71.

Year	Travel time (days)	Period of migration	Nitrogen[1] % saturation	Percent survival
1970	25	4/19-5/13	130	25
	12	5/14-5/31	136	50
1971	26	4/7 -5/31	[2]109-131	37
	13	5/4 -5/19	135	50

[1] N_2 levels—Lower Monumental Dam Forebay.
[2] N_2 109 percent during flow control period 4/27-29.

Survival of Snake River juvenile chinook and steelhead populations to The Dalles Dam in 1972 was 15-25 percent, respectively. Survival to Ice Harbor Dam in the Snake River was 35-60 percent, respectively. We believe differences in timing between chinook and steelhead in 1972 accounts for the higher survival calculated for steelhead down the Snake River. Slotted bulkheads installed in skeleton bays to reduce N_2 in the river caused significant losses to juveniles (Long and Ossiander[16]). Most of the chinook migrated when the bulkheads were in place, whereas, the peak steelhead migration occurred 10 days later—after many of the bulkheads had been removed. After removal of the bulkheads, N_2 levels exceeded 130 percent through most stretches of the Snake and Columbia Rivers. A total of 14 days exposure (based on migration rate) to nitrogen levels of this magnitude would have caused at least 36 percent of the 75 percent loss of steelhead measured from Little Goose to The Dalles Dam in 1972.

Catastrophic losses in 1973 were primarily turbine-predator related and resulted from passage of nearly the entire juvenile migration through the powerhouses of dams. Net survival to The Dalles Dam was only 4-5 percent for both species. Supersaturation of nitrogen was not a factor in 1973 because there was little or no spilling at most dams.

Downstream survival of chinook and steelhead in 1974 to The Dalles Dam increased from the low of 4-5 percent in 1973 to 40 percent for chinook and 27 percent for steelhead. Nearly two-thirds of the 60 percent loss for chinook in 1974 was attributed to exposure to supersaturation of atmospheric gas. The major factor causing the loss of steelhead in 1974 cannot be estimated because of a disease problem[17] in stocks of steelhead released from Dworshak Hatchery, which now accounts for 50-75 percent of the juvenile steelhead migrating down the Snake River. A combination of loss from exposure to supersaturated atmospheric gases and disease no doubt occurred.

Estimated survival of juvenile steelhead and chinook from Lower Granite Dam to The Dalles Dam in 1975 was 42 and 25 percent, respectively. Steelhead survival was the highest measured since 1969. The lower survival of chinook was attributed to dominance (60 percent) of the 1975 outmigration by hatchery fish (mostly Rapid River) which survived at a much lower rate (17 percent) than native stocks (38 percent). In previous years less than 30 percent of the outmigration were of hatchery origin and their impact on overall survival was not significant. The 38 percent survival of native

[16] Long, Clifford W., and Frank J. Ossiander. 1974. Survival of coho salmon fingerlings passed through a perforated bulkhead in an empty turbine bay and through flow deflectors (with and without dentates) on spillway at Lower Monumental Dam, Snake River April-May 1973. Northwest Fish. Cent., Natl. Mar. Fish. Serv., NOAA, Seattle, Wash., Rep. to U.S. Army Corps of Engineers, contract no. DACW68-72-C-0101, 20 p. Processed.

[17] Verbal communication, Einer Wold, former fish pathologist at Dworshak Hatchery, Ahsahka, Idaho.

chinook was nearly as high as that measured for steelhead. Several factors contributed to the higher survival of steelhead and native chinook in 1975.

These included: 1) the installation of traveling screens which diverted about 1.5 million fingerlings from turbine intakes of Lower Granite and Little Goose Dams and; 2) installation of spillway deflectors at Lower Granite and Lower Monumental Dams which reduced dissolved gas levels. Losses attributable to exposure to supersaturation were not estimated in 1975.

Experiments to measure the effects of supersaturation of atmospheric gas on juvenile fall chinook migrants in the lower Columbia River have also been conducted and these show that losses as high as 40 percent[18] have been attributed to exposure of these stocks to high levels of supersaturation. For example in 1970, fall chinook (Sims, 1975) released from Spring Creek Hatchery in April before supersaturation was high survived at a rate of about 90 percent. Releases in late May and June after gas saturation levels increased suffered a 50 percent loss.

The Fish Commission of Oregon conducted similar experiments in 1969 and concluded that losses exceeding 50 percent[18] occurred during periods of supersaturation.

In summation, losses of juvenile chinook and steelhead migrating from the Salmon River to The Dalles Dam have ranged from about 40 to 95 percent since 1968. The proportion of this loss that can be attributed to exposure of fish to supersaturation of nitrogen varies from zero in low-flow years as in 1973 to 80 percent in high-flow years as in 1971.

Effect of Supersaturation on Adult Fish

Spawning migrations of steelhead trout and chinook and sockeye salmon take place in the Columbia River Basin when levels of dissolved gases are at critically high levels. Evidence of detrimental consequences are found in both laboratory experiments and field observations.

Laboratory Studies

The most comprehensive laboratory study conducted to date was by Nebeker (footnote 5). In this study, groups of adult salmon and steelhead trout were held at various levels of supersaturation while the water temperature was maintained at 10°C. The fish were observed and the resistance times until 50 percent mortality were determined.

Table 6.—Hours to median mortality (50 percent dead) of various adult salmonids at various levels of supersaturation (From Nebeker, 1973, footnote 5).

Species	Sex	Level of supersaturation			
		115% hr	120% hr	125% hr	130% hr
Spring chinook	Female	526	46;80	17.5	7.8
Spring chinook	Male	526	40;68.5	16.3	8.8
Sockeye	Not spec.	30% in 192	82	18	—
Coho	Female	166	45;51	21	—
Coho	Male	264	74;86	20	—
Winter steelhead	Female	10% in 350	92	—	—
Winter steelhead	Male	30% in 350	76	—	—
Summer steelhead	Not spec.	—	118	31	—

Coho salmon were the most susceptible of the adults tested (Table 6), with 50 percent mortality occurring after slightly less than 7 days exposure at 115 percent saturation. Spring chinook were the most tolerant, requiring about 22 days exposure at 115 percent to reach the 50 percent level of mortality.

Coutant and Genoway[19] held groups of fish at one of two levels of nitrogen supersaturation (low 110 percent, high 118 percent) for 5 days and then transferred them into either high or low level nitrogen conditions with the water elevated 5°C (from approximately 17° to 22°C). The objective was to determine if a 5°C raise in temperature (that was not in itself lethal) would result in mortality when combined with effects of supersaturated gases.

In this study, they found that fish held for the 5-day period in the high nitrogen level water at control temperatures showed visible symptoms of gas bubble disease (bubbles in the dorsal fin) within 24 hours. Coloration and alertness were affected after 4 days. Fish held at the low nitrogen level water at control temperatures showed no gas bubble disease symptoms and retained good coloration, skin texture, and alertness throughout the 27-day test period. All fish, regardless of nitrogen acclimation level, died within 24 hours when placed into high nitrogen water with the water temperature elevated 5°C. Those fish stressed at the high nitrogen level died sooner. External symptons of gas bubble disease that were evident on fish disappeared rapidly after death; nearly all symptoms were lost within 24 hours.

Bouck, Chapman, Schneider, and Stevens[20] confirmed gas bubble disease among wild adult sockeye salmon migrating upstream through the Columbia River in 1968 and 1969. Adult sockeye salmon were taken from the river and examined in July 1968 and July 1969 when dissolved nitrogen levels were in excess of 120 percent throughout the lower 600 km of the Columbia River.

In July 1968, seven live sockeye salmon were collected near the south entrance to the Oregon shore fishway at John Day Dam (river km 345). These fish were examined for external symptoms of gas bubble disease and then immediately killed so histological and other internal examinations could be carried out. Three of the seven fish had external symptoms of gas bubble disease (gas bubbles in skin and on fins and eye damage). Histological studies revealed microscopic sized vesicles in the extravascular spaces of the spleen and other tissues, but it was not possible to state whether the vesicles existed during life or evolved during fixation. Aneurysms filled with blood

[18]Verbal communication, Kirk Beiningen, Oregon Dept. of Fish and Wildlife, 17330 S.E. Evelyn St., Clackamas, Oreg. 97015.

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

[20]Bouck, G. R., G. A. Chapman, P. W. Schneider Jr., and D. G. Stevens. 1970. Observations on gas bubble disease in adult Columbia River sockeye salmon (*Oncorhynchus nerka*). Environ. Prot. Agency, Western Fish Toxicol. Sta., Corvallis, Oreg., June 30, 1970. Unpubl. manuscr., 19 p.

were found in the gill lamellae of all fish that had macroscopic gas bubbles in the skin or fins.

In July 1969, 123 adult sockeye salmon collected from the Washington shore fishway at Bonneville Dam (river km 235) were held at the Oregon Fish Commission's hatchery at Bonneville Dam. A cursory external examination of these fish revealed gas bubble disease symptoms in 13 fish. The 123 fish were divided into four groups, and each group was held in water of a different temperature but with the nitrogen level maintained at approximately 100 percent. Visible symptoms of eye damage developed that were compatible with the eye damage observed in the sockeye salmon at John Day Dam in 1968. The results of the study are summarized in Table 7 taken directly from Bouck et al. (Footnote 22). As a result of their work in 1968 and 1969, the authors concluded that dissolved nitrogen gas causes significant damage to salmon in the Columbia River.

Field Studies

In 1962, during an investigation of a high rate of prespawning mortality among chinook salmon at the McNary spawning channel, it was noted that a high percentage of the dead fish were blind. Subsequent investigation (Westgard, 1964) revealed that the problem was a result of gas bubble disease caused by high levels (up to 130 percent) of dissolved gases in the water. In addition, test lots of chinook salmon were placed in live boxes located in water having one of two levels of nitrogen supersaturation: 1) low, 104-107 percent; and 2) high, 116-130 percent. Thirty-four percent of the fish held in the high nitrogen level water were blind within 10 days. Eighty-eight percent of the blinded fish died before spawning (only 6 percent of the non-blind died before spawning). In addition, blinded females did not dig redds and blinded males were unable to successfully cover eggs with milt.

Surveys were conducted during 1967 to determine the levels of supersaturation of dissolved nitrogen in the Columbia River and its effect on salmon and steelhead (Ebel, 1969). During periods of high nitrogen, adult salmon and steelhead were examined for symptoms of gas bubble disease. Saturation levels of dissolved nitrogen gas were found to be high enough and persisted for a long enough period in a large enough area to be potentially dangerous to adult salmon and steelhead trout. In July, symptoms of gas bubble disease were observed in 10 out of 1,000 sockeye salmon examined at McNary Dam. No symptoms were noted in 1,762 chinook salmon and 1,461 steelhead trout. Field evidence

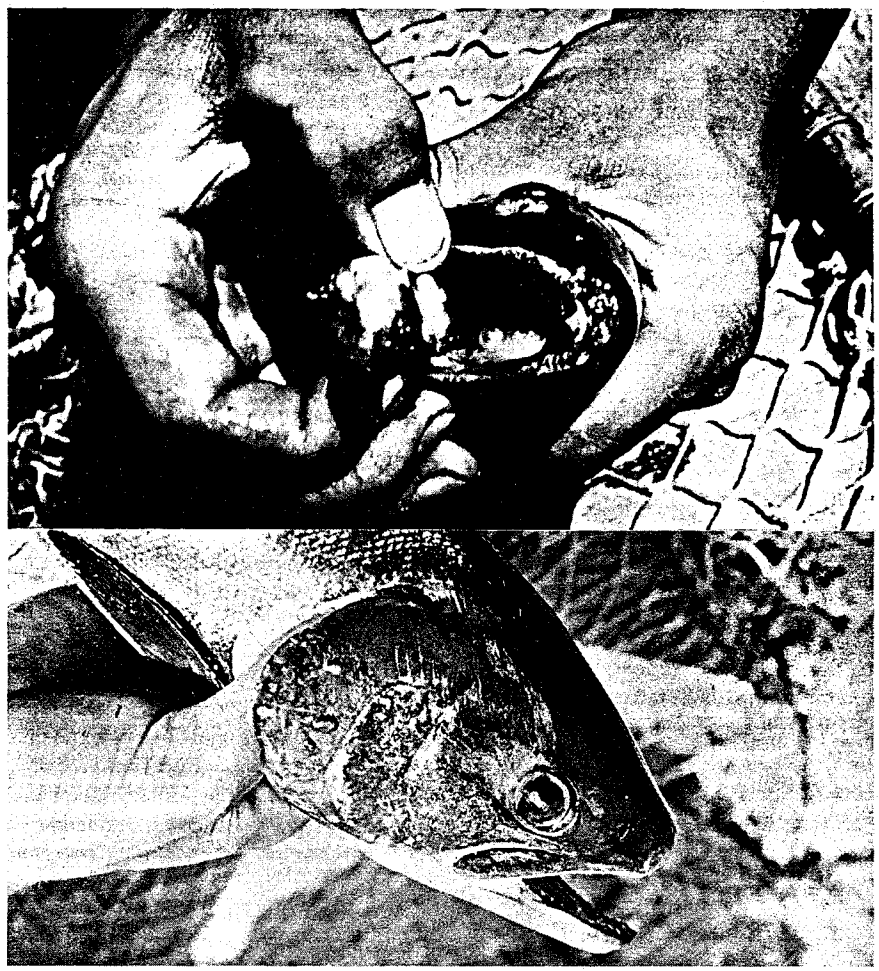


Figure 13.—Above, vesicles in roof of mouth and distended eyes of adult sockeye salmon captured at entrance to south ladder at John Day Dam, 20 July 1968. Below, large gas bubble in eye tissue and numerous gas bubbles on operculum of adult sockeye salmon captured at entrance to south ladder at John Day Dam, 20 July 1968. (Photo source: Beiningen and Ebel, 1970.)

Table 7.—Incidence of eye damage and mortality among adult Columbia River sockeye salmon, *Oncorhynchus nerka*, during a post-capture holding period at various temperatures (from Bouck et al., 1970, footnote 22).

	Tank #1	Tank #2	Tank #3	Tank #4
Test temperature (C°)	10	16 (ambient)	20	22.5
Number of specimens	31	31	30	31
Incidence of eye damage				
Left eye only	19%	10%	33%	35%
Right eye only	3%	0%	7%	10%
Both eyes	13%	19%	13%	19%
Blindness in at least one eye	35%	29%	53%	65%
Total mortality	19% in 44 days [1]	32% in 44 days [1]	100% in 31 days	100% in 12 days

[1]Experiment terminated on the 44th day.

did not positively indicate that high nitrogen in the Columbia River in 1967 caused substantial mortality of adult salmon but the possibility cannot be discounted.

The first substantial loss of adult salmon and steelhead was documented in 1968 by Beiningen and Ebel (1970). Concentrations of dissolved nitrogen gas were measured in the Columbia River from April to September 1968 to determine the effect of newly constructed John Day Dam on nitrogen saturation. Adult salmon were also observed for symptoms of gas bubble disease and mortality. Nitrogen levels of 123-143 percent saturation were measured below the dam. These concentrations were considerably higher than measured previously, and they persisted throughout the salmon migration. During the period of high nitrogen levels, a high incidence of injury and death to adult fish from gas bubble disease was observed near John Day Dam. As many as 13 sockeye and 365 chinook salmon were recovered in one day below John Day Dam. Fish captured at the entrance to the ladder at John Day Dam exhibited obvious symptoms of gas bubble disease: eyes distended and hemorrhaged; bubbles in the subcutaneous layers surrounding the eyeball (Fig. 13); and large vesicles in the roof of the mouth and on the outside of the opercula. Reports from fish counters observing fish at the underwater viewing chamber in the south fishway at John Day Dam are replete with the observations of typical gas bubble disease symptoms. Tissue specimens of salmon and steelhead trout collected by Gerald R. Bouck, EPA, indicated definite tissue damage from gas bubble disease in spleen and gill lamellae of sockeye salmon as well as tentative indications of gas emboli in gill filaments of chinook salmon.

Based on the recovery of dead salmon, the Fish Commission of Oregon estimated that over 20,000 summer chinook were missing in the area of concern.

Investigations were carried out in the upper Columbia River from 1965 to 1969 by Meekin and Allen (1974) to determine the effect of Grand Coulee and Chief Joseph dams on dissolved nitrogen gas levels and their effect on spawning salmon. They found that nitrogen levels of from 125 to 135 percent occurred each year as a result of spilling at Grand Coulee Dam. The supersaturated water did not equilibrate while passing through Chief Joseph Reservoir and subsequent spilling at the dam maintained or increased the high levels. Boat searches and aerial surveys from 1967 between Chief Joseph and Wells dams revealed that mortalities of unspawned summer chinook and sockeye salmon occurred during the spill season and terminated after spilling stopped.

In 1970, spilling was greatly reduced and nitrogen levels were considerably lower than 1967 to 1969 (generally less than 118 percent). Field surveys during this time showed that both adult chinook and sockeye salmon mortalities were substantially reduced from previous years.

Estimated losses of adult sockeye and summer chinook below Chief Joseph Dam, 1967-70.

Year	Percent loss		Nitrogen level
	Chinook	Sockeye	
1967	68	46	High
1968	44	—	High
1969	56	—	High
1970	5	30	Low

We have examined the relationship between dissolved gas supersaturation in the Snake River and the number of spring and summer chinook redds on the spawning grounds in the Salmon River system. Redd count index number was calculated by dividing the number of redds counted during spawning ground surveys (Idaho Fish and Game Department) by the number of spring and summer chinook salmon counted over Ice Harbor Dam minus the subsequent sports catch and hatchery returns. In general, we found a sharp reduction in the redd count index number after 1968 when nitrogen levels sharply increased. However, in 1972 when the nitrogen levels were reduced through the use of slotted bulkheads and in 1973 when very low flows eliminated spilling, there was a return to the higher redd count index numbers.

Relation of nitrogen supersaturation in the Snake River to spring and summer chinook redd counts in the Salmon River system.

Period	N_2 Level (Mid-April through May)	Redd Count Index No.[1] (Range)
1963-1968	Low (100-110%)	11-19
1969-1971	High (125-135%)	6- 8
1972-1973	Low (100-110%)	10-13

[1]Index no. = Redd counts (spring, summer chinook) in Salmon River system. / Number of spring and summer chinook over Ice Harbor Dam minus sport catch and hatchery return.

SUMMARY AND CONCLUSIONS

In summation, there is ample evidence, both in laboratory and field studies, that adult and juvenile salmon and steelhead are jeopardized by gas bubble disease in the Columbia River Basin. The severity of the disease and its consequences depend on the level of supersaturation, the duration of exposure, water temperature, general physical condition of the fish, and the swimming depth maintained by the fish.

During spill, levels of dissolved gases measured at and between major dams are well above critical levels. Unfortunately, even with maximum utilization of turbine capacities, the dissolved gas levels during average and high flow years will continue to be high enough to cause problems for upstream and downstream migrants. Because all reaches of the Columbia and Snake Rivers through which adult and juvenile salmon and steelhead must migrate are significantly supersaturated, the total time of exposure is serious, and any undue delays that fish may encounter could prove disastrous.

Information currently available on depth distribution of juvenile salmonids (Mains and Smith, 1964; Smith, Pugh, and Monan, 1968; Monan, McConnell, Pugh, and Smith, 1969; and Smith, 1974) all indicate that the largest percentage of downstream migrants are found in the top 5 feet of water. This means that the average hydrostatic compensation achieved is about 7.5 percent of saturation—insufficient to compensate for levels as high as 135-140 percent which occur over wide areas during high flow years.

Even if migrants are able to gain relief by traveling deep in the river, adults are forced to utilize restricted depths when entering and negotiating fishways at dams. Radio tracking

studies (Monan and Liscom[21]) have shown that the amount of time adult salmon spend negotiating fishways can be a substantial period that varies from dam to dam. For example, a group of spring chinook salmon tracked through the fishways at Bonneville Dam took from 4 to 57 hours each (average 22 hours), while the same fish tracked through the fishways at The Dalles Dam took 3 to 23 hours (average 7 hours). During the time the fish are in the fishways, they are restricted to a maximum depth of about 7 feet. Observations at various dams indicate the fish are frequently near the surface in the fishways. Even though there is some reduction in the dissolved gas levels in the ladder, the restricted depth places an additional stress on fish previously equilibrated to high levels of gas supersaturation.

Several conclusions regarding the effect of supersaturation of atmospheric gas on fish in the Columbia River can be made from the laboratory and field data presented. The main conclusions we reached are:

1) Supersaturation of atmospheric gas has exceeded 130 percent over long stretches of the Columbia and Snake Rivers during several years since 1968.

2) Juvenile and adult salmonids confined to shallow water (one meter) suffer substantial mortality at 115 percent (TDG) saturation after 25 days of exposure.

3) Juvenile or adult salmonids allowed the option to sound and obtain hydrostatic compensation either in the laboratory or in the field, still suffer substantial mortality when saturation levels (TDG) exceed 120 percent saturation after more than 20 days exposure.

4) On the basis of survival estimates made in the Snake and Columbia Rivers from 1966 to the present, we conclude that juvenile fish losses ranging from 40 to 95 percent do occur and a major portion of this mortality can be attributed to fish exposure to supersaturation of atmospheric gases during years of high flow.

5) Juvenile salmonids subjected to sublethal periods of exposure to supersaturation can recover when returned to normally saturated water, but adults do not recover and generally die from direct and indirect effects of the exposure to supersaturation.

[21]Monan, Gerald E., and Kenneth L. Liscom. 1973. Radio tracking of adult spring chinook salmon below Bonneville and The Dalles Dams, 1972. Northwest Fish. Cent. Natl. Mar. Fish. Serv., NOAA, Seattle, Wash., Rep. to U.S. Army Corps of Engineers, delivery order DACW57-72-0398, 37 p. Processed.

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Nebeker, Bouck, and Stevens. 1976. Carbon dioxide and oxygen-nitrogen ratios as factors affecting salmon survival in air-supersaturated water.

This article discusses the effect of different O_2/N_2 ratios at different levels of air-supersaturated water on juvenile sockeye salmon.

Carbon Dioxide and Oxygen-Nitrogen Ratios as Factors Affecting Salmon Survival in Air-Supersaturated Water

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ABSTRACT

Juvenile sockeye salmon (*Oncorhynchus nerka*) were exposed to lethal levels of air-supersaturated water (120 percent, 125 percent, 130 percent total gas saturation) containing different oxygen-nitrogen ratios and different carbon dioxide concentrations. Fish mortality was not significantly different at different carbon dioxide levels (1.7 to 22.0 mg/liter CO_2) when tested at the same total gas saturation concentrations. Total gas saturation levels are much more important than the O_2 N_2 ratios, as fish mortality will not occur unless total gas saturation exceeds 100 percent, regardless of the O_2/N_2 ratios. There was a significant decrease in mortality when the ratio of oxygen to nitrogen was increased while holding the total percent saturation constant. Much more extensive and severe signs of gas bubble disease developed at high O_2 N_2 ratios than at low O_2/N_2 ratios, indicating that oxygen plays a significant part in forming external emphysema and lesions.

Dissolved gas composition in surface waters can vary for several reasons including photosynthetic activity, biochemical oxygen demand, aeration activity at dams, and upwellings. The concentrations of inert gases tend to change rather slowly in water while carbon dioxide and oxygen, which are acted upon biologically, will fluctuate significantly. Surface and underground water can have widely different gas saturation levels depending upon water source, temperature, turbulence, pressure, presence of oxygen-consuming materials, man's activities such as dams, and other factors. Recent studies (Nebeker and Brett 1976) have shown that juvenile sockeye salmon die rapidly at total gas levels of 120 percent–130 percent. Other studies with salmon and other fish species are summarized in the literature reviews of Rucker (1972), Weitkamp and Katz (1973, 1975), and Bouck (1973). However, the literature contains little information on how carbon dioxide or oxygen-nitrogen ratios affect the lethality of gas-supersaturated water to freshwater or marine fish or invertebrates.

Carbon dioxide can cause adverse physiological effects in fish at levels as low as 30 ppm, and most effects occur at levels less than 10 percent of saturation, far below levels which could be expected to cause formation of bubbles (Harvey 1975). Mrsic (1933) attributed gas bubble formation to CO_2, but no other reports have been found that confirm his studies.

Rucker (1972) and Harvey (1975) have reviewed the literature on effects of oxygen causing gas bubble disease. Rucker states that oxygen can cause gas bubble disease from the effect of the partial pressures of oxygen and nitrogen. If excess oxygen is present in water with a deficiency of nitrogen the total gas pressure may be too low to cause a problem, but as Egusa (1969) indicates, excess oxygen can cause a problem if nitrogen is maintained normal. The sum of the partial pressures must be greater than the ambient pressure to cause gas bubble disease. Harvey (1975) in his review summarizes by stating that there is no known naturally occurring case of gas disease due to oxygen alone: in reported cases attributed to oxygen only oxygen was measured. Oxygen supersaturation was probably superimposed on air-saturated or supersaturated waters, in many cases caused by increased water temperatures. Whether nitrogen was supersaturated or not, bubble formation and growth would involve both oxygen and nitrogen.

Many studies (Weitkamp and Katz 1973, 1975) have shown that water supersaturated with nitrogen causes severe damage and

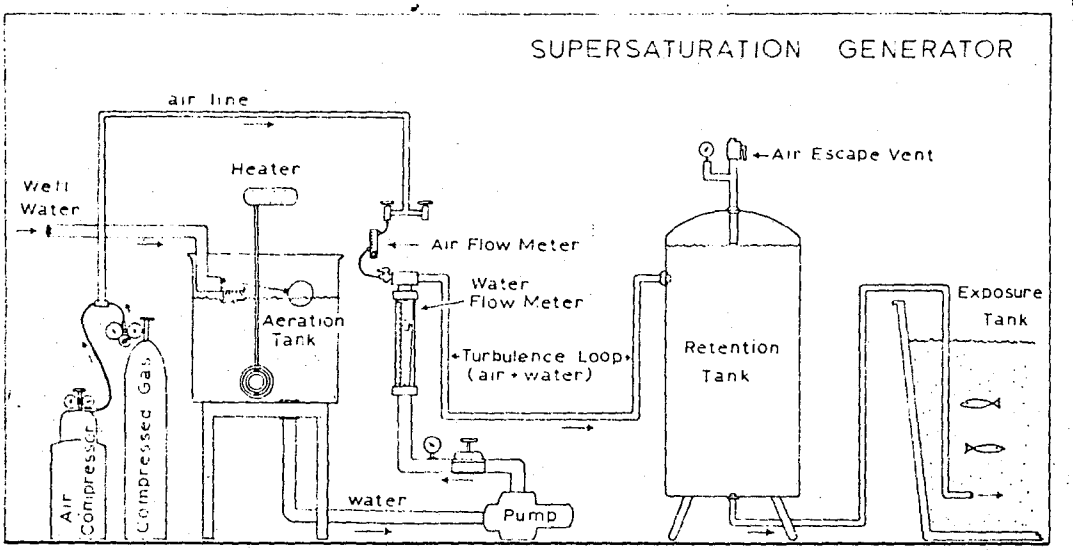


FIGURE 1.—*Diagram of system for adding oxygen or carbon dioxide to air-supersaturated water generator facility.*

death to a variety of fish species. However, only Rucker (1976) has conducted detailed studies to show the effect of varying oxygen-nitrogen ratios on fish while maintaining a constant total gas pressure. Rucker held the total gas saturation constant at 119 percent and varied the oxygen-nitrogen ratio, using juvenile coho salmon as the test species. The oxygen-nitrogen ratios varied from 50 percent/138 percent to 229 percent/90 percent. His data indicated that total gas supersaturation had to occur before fish damage occurred and that oxygen plays a more significant role above 110 percent than below 110 percent total gas saturation. A drastic decrease in lethal effect of individual ratios of O_2 to N_2 occurred between 159 percent O_2/109 percent N_2 and 173 percent O_2/105 percent N_2 at the same total gas pressure (119 percent).

The purpose of this study was to determine if (1) increased concentrations of carbon dioxide within normal extremes found in the field, and (2) different oxygen-nitrogen ratios, would cause differences in survival and signs of gas bubble disease in young sockeye salmon (*Oncorhynchus nerka*) at constant levels of air-supersaturated water.

METHODS

Physical Conditions

The test facilities used during these studies were the same as those described by Nebeker et al. (1976), where four 6,000-liter fiberglass tanks were used as test containers and individual supersaturation generators produced test water for each tank. One generator was modified to permit addition of CO_2 or O_2 gas to the compressed air which is added to the test water. Compressed CO_2 or O_2 at 2.8 kg/cm^2 was carefully metered into the compressed air line (2.7 kg/cm^2). This gas mixture was then metered and injected into the water supply which was pumped at a pressure of 1.7 kg/cm^2. The mixture of compressed air, CO_2 (or O_2) and water was then passed through a detention tank where excess gas was vented at the top and the supersaturated water was drained from the bottom and delivered to the test tanks (Fig. 1).

Two test tanks were used for control tanks with supersaturated water but without the addition of extra CO_2 or O_2. One tank was modified for the addition of extra CO_2 or O_2 into supersaturated water, and the fourth

tank was used for an unsupersaturated water control tank. Fish were tested either in the open tank or in four cages set inside the tank. Four groups of fish could be tested simultaneously in each of the four tanks.

The test water was obtained from two wells located about 30 m from the Willamette River. The water was chilled to just below test temperature, aerated to saturation to stabilize the dissolved gas concentrations, and then maintained at final test temperature with electric immersion heaters. Well water had low dissolved oxygen concentrations (3.0–5.0 mg/liter) making it very easy to get low oxygen to nitrogen rations without adding N_2 gas in addition to O_2 gas. This was accomplished by decreasing aeration of the well water. Photoperiod was natural for the time of year because the tanks were shaded and out-of-doors.

Chemical and Gas Analyses

The well water used for tests was relatively soft with the following average chemical characteristics: hardness, 34 mg/liter; alkalinity (as $CaCO_3$), 31 mg/liter; pH, 6.7–7.3 (depending upon aeration time). Complete chemical characteristics of the test water can be found in the publication by Samuelson (1976). All chemical analyses were conducted according to the American Public Health Association (1971). Dissolved oxygen was measured by the azide modification of the Winkler method. Hydrogen ion (pH) concentration was measured with glass electrode pH meters. Hardness was determined using the EDTA titrametric method, and alkalinity was determined potentiometrically with 0.02 N H_2SO_4 and colorimetrically. Carbon dioxide (as free CO_2) was calculated nomographically, and determined potentiometrically and colorimetrically. The means of these results were used as final CO_2 values.

Percent total air saturation was determined with a Weiss saturometer (Nebeker et al. 1976), calibrated with the Van Slyke gas analyser and the Winkler method for oxygen. The formula

$$\frac{BP + \Delta P - VP}{BP} \times 100 = \% \text{ saturation},$$

where BP = barometric pressure, ΔP = saturometer reading, and VP = water vapor pressure, was used to calculate total percent saturation.

Percent oxygen saturation was determined by converting the oxygen in mg/liter from the Winker analysis to ml/liter, and then dividing by the O_2 saturation at the given temperature obtained from tables by Weiss (1970):

$$\frac{O_2 \text{ mg/liter} \times 0.7}{\text{Sat. } O_2 \text{ @ Temp.}} \times 100 = \% \text{ } O_2 \text{ saturation.}$$

Percent carbon dioxide saturation was determined using 2,260 mg/liter CO_2 at 15 C as saturation and dividing that into the actual mg/liter CO_2 determined in test water.

Percent nitrogen (including argon) saturation was determined following the method of Lambert and Rulifson (1972):

$$\text{Step 1. } PO_2 = \frac{O_2 \text{ mg/liter } (0.7)}{BO_2} \times 0.76$$

$$= O_2 \text{ in mm Hg.}$$

$$\text{Step 2. } PN_2 = BP + \Delta P - (PO_2 + PH_2O)$$

$$= N_2 \text{ in mm Hg.}$$

$$\text{Step 3. } \%\ N_2 = \frac{PN_2}{0.79\,BP} \times 100$$

$$= \% \text{ nitrogen (+ argon)},$$

where PO_2 = oxygen pressure in mm Hg; BO_2 = Bunson coefficient for oxygen (Weiss 1970); PN_2 = nitrogen (+ argon) pressure in mm Hg; BP = barometric pressure in mm Hg; ΔP = gauge reading on saturometer in mm Hg; PH_2O = water vapor pressure in mm Hg (Weiss 1970); and 0.79 = % N_2 + Ar in dry air.

Fish Test Methods

Young sockeye salmon (*O. nerka*) nine to twelve months old, were tested with normal and high levels of CO_2 and various O_2/N_2 ratios at 120 percent, 125 percent, and 130 percent total gas saturation. They were chosen as the test fish because sockeye are sensitive to supersaturation (Nebeker and Brett 1976) and they are a different species

than tested by Rucker (1976). The fish were hatched and reared from eggs taken from adults collected at the north ladder of Bonneville Dam on the Columbia River and artificially fertilized at Western Fish Toxicology Station, Corvallis, Oregon. The fish hatched in December, 1972, and were tested September–December, 1973. Fish were fed Oregon Moist Pellets during testing. Mean fork-length and weight of fish during testing were 8.6 cm and 7.7 g, respectively.

Five tests were conducted, each using 160 fish. Two tests were conducted with a high O_2/N_2 ratio and three tests were completed with a high CO_2 level. Ten fish were placed in each chamber of the four-chambered cage in each tank, totalling 40 fish at each gas level (each tank) and O_2 or CO_2 level. Test water was 60 cm deep and held at 16 ± 1 C for the CO_2 tests and 15 ± 1 C for O_2/N_2 ratio tests. All fish were randomly assigned to test chambers and acclimated to test facilities for two weeks prior to testing in test 1, three days in tests 2 and 4, and placed directly into supersaturated water in tests 3 and 5.

Time to 50 percent death at each concentration was determined and the t test of significance ($P = 0.05$) was used to compare survival of young sockeye at different CO_2 levels and O_2/N_2 ratios.

RESULTS AND DISCUSSION

Effects of CO_2

Fish tested at 10.0, 2.8, and 2.1 mg/liter free CO_2 at 125 percent total gas saturation showed no significant difference ($P = 0.05$) in survival times, although the fish at 10 mg/liter did not die as rapidly. Time to 50 percent death did not differ significantly for fish tested at 20.0, 2.6, and 1.7 mg/liter CO_2 with 120 percent total gas saturation and essentially identical nitrogen saturation levels. Fish held at 22 mg/liter CO_2 with 120 percent total gas saturation also showed no significant difference in survival from those held at 3.3 and 2.1 mg/liter CO_2. No deaths occurred in the control fish during testing (Table 1).

TABLE 1.—*Effect of CO_2 on survival of juvenile sockeye salmon in supersaturated water at 125 percent and 120 percent and 16 ±1 C.*

Total percent saturation Mean ± SD	Mean CO_2 percent saturation	Mean O_2 percent saturation / Mean N_2 percent saturation (O_2/N_2 ratio)	Mean CO_2 (mg/liter)	Mean time to 50% death[a] (hours)
127.1 ± 0.6	0.4	126.5 / 127.9	10.0	12.1
127.2 ± 1.5	0.1	120.3 / 129.7	2.8	10.0
127.8 ± 1.4	0.1	126.4 / 128.9	2.1	9.2
120.0 ± 0.6	0.9	109.6 / 123.3	20.0	57.8
120.5 ± 0.5	0.1	112.6 / 123.1	2.6	48.5
120.0 ± 1.4	0.1	114.6 / 122.0	1.7	52.8
119.8 ± 0.9	1.0	116.5 / 121.2	22.0	73.2
119.9 ± 0.8	0.1	114.6 / 121.8	3.3	51.3
119.5 ± 1.0	0.1	119.7 / 119.9	2.1	56.3

[a] No significant differences ($P = 0.05$) between survival at high and low CO_2 levels.

Effects of O_2/N_2 Ratios

Total gas saturation levels are much more important than the O_2/N_2 ratios, as fish mortality due to gas bubble disease will not occur unless total gas saturation exceeds 100 percent, regardless of the O_2 N_2 ratios. There was a significant ($P = 0.05$) decrease in mortality when the ratio of oxygen to nitrogen was increased while holding the total percent saturation constant. Fifty percent died after 71 hours at 120 percent when the O_2 N_2 ratio was 117.2/121.3 percent. Only 7 percent of the fish died after 167 hours when the O_2/N_2 ratio was changed to 170.5/107.0 percent. No deaths occurred in the control fish.

Fish tested at 130 percent total gas saturation also exhibited significant differences ($P = 0.05$) in time to 50 percent death at different O_2/N_2 ratios. Mean time to 50

TABLE 2.—*Effect of O_2/N_2 ratios on survival of juvenile sockeye salmon in supersaturated water at 130 percent and 120 percent at 15 C.*

Total percent saturation Mean ± SD	Mean O_2 percent saturation / Mean N_2 percent saturation (O_2, N_2 ratio)	Mean O_2 (mg liter)	Mean time to 50% death (hours)
129.4 ± 1.5	186.2 / 114.6	18.7	37.0[a]
129.9 ± 1.0	129.5 / 130.1	13.1	22.3
129.7 ± 1.4	127.5 / 130.5	12.8	23.3
120.1 ± 0.3	170.5 / 107.0	17.2	[a,b]
120.3 ± 0.8	117.2 / 121.3	11.8	71.6
120.1 ± 0.4	114.5 / 121.8	11.5	65.8

[a] There was a significant difference ($P = 0.05$) between survival at high and low O_2/N_2 ratios.

[b] Seven percent dead after 167 hours.

percent death was 37.0 hours at 186.2/114.6 percent and 22.3 hours at 129.9/130.1 percent (Table 2).

External Signs of Gas Bubble Disease

External signs of gas bubble disease did not appear until several hours after initial exposure to gas-supersaturated water and their severity was closely correlated with percent saturation, O_2/N_2 ratios, and exposure time. At 125 percent total gas saturation most external signs of GBD consisted of bubbles (emphysema) in the tail. Some bubbles occurred on the paired, dorsal, and anal fins, but most of the fish died before extensive emphysema developed. The fish held at 120 percent saturation exhibited a greater variety of signs because they lived longer and thus had a longer exposure to supersaturated water. Most of these fish had bubbles in the tail, and many had emphysema in the fins and the opercles. The cause of death is due to emboli (bubbles) in the blood stream blocking the capillaries and preventing normal blood flow through the gills and other tissues.

Much more extensive and severe signs of gas bubble disease developed at high O_2/N_2 ratios indicating that oxygen plays a significant role in forming external emphysema and lesions. Even those fish exposed to 130 percent total gas saturation (186.2 percent O_2/114.6 percent N_2) and dying within as little at 12 hours, had massive bubble formations on the head, opercles, tail, and fins when tested in supersaturated water with abnormally high oxygen levels. Exophthalmia, or popeye, where the eyes are driven out of the sockets from gas behind the eye, was also much more prevalent when the O_2/N_2 ratios were high.

ACKNOWLEDGMENTS

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Nebeker and Brett. 1976. Effects of air-supersaturated water on survival of Pacific salmon and steelhead smolts.

This article discusses the laboratory results of super-saturation levels on sockeye, coho, and steelhead smolts conducted in 12° C water in tanks 60 cm deep.

Effects of Air-Supersaturated Water on Survival of Pacific Salmon and Steelhead Smolts

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ABSTRACT

Coho (*Oncorhynchus kisutch*) and sockeye (*O. nerka*) salmon smolts and steelhead trout (*Salmo gairdneri*) smolts were exposed to several concentrations of air-supersaturated water in the laboratory from March through June, 1974, the normal fish migration period in the Columbia River and the time when the river water is usually supersaturated. Tests were conducted at 12 C in water 60 cm deep. Time to 20 percent death was determined and lethal threshold concentrations of 114.5 percent saturation for coho, 113.5 percent for sockeye, and 114.0 percent for steelhead were calculated. Ninety-six hour LC50 values were 120.5 percent for coho, 116.7 percent for sockeye, and 116.0 percent total gas percent saturation for steelhead. Thirty-day LC50 values were 116.2 percent for coho, 113.9 percent for sockeye, and 114.0 percent for steelhead. Severe emphysema (bubbles) occurred on fins, in the mouth, and on the head and opercles of the fish exposed to 120 percent and 115 percent saturation. Emphysema was not as severe at 110 percent but occurred in 60 percent of the coho, 20 percent of the sockeye, and 80 percent of the steelhead.

One of the critical periods in the life cycle of Pacific salmon and steelhead trout migrating through the Columbia River is in the spring when high water spilling over the dams supersaturates the river with dissolved gases to lethal levels near the water surface. Smolts, the juvenile salmon and steelhead that migrate to the ocean, move down river during this period of highly supersaturated water. Recent acute tests (Bouck et al. 1976) have shown that fish are very sensitive to supersaturated water and will die rapidly at 125 percent saturation and above. Nitrogen levels of 120 to 130 percent were found to be lethal to goldfish, eel, bitterling, killifish, and carp within a two-week period (Egusa 1959). Pauley and Nakatani (1967) described the histopathology of gas bubble disease in chinook salmon fingerlings from the Columbia River but no analyses of dissolved gas levels were given. Detailed pathology of gas bubble disease in adult and juvenile salmonids is described and illustrated by Stroud et al. (1975). Rucker (1976) and Nebeker, Bouck, and Stevens (1976) showed that gas bubble disease is due to total gas pressure rather than just dissolved nitrogen, but that the nitrogen oxygen ratio is important in determining severity of symptoms and rate of mortality. Water depth can compensate for supersaturation, but Durkin et al. (1970) found the majority of downstream-migrating juvenile salmon in the surface waters of Brownlee Reservoir. Similar studies on the vertical distribution of chinook and steelhead juveniles in the forebay of Lower Monumental Dam on the Snake River showed 58 percent of the chinook in the top 3.5 m, with most of those in the top 1 m (Beiningen 1973 in Harvey 1975). Thus, according to Harvey (1975), the behavior of these fish during migration would expose the majority to supersaturation without appreciable relief through hydrostatic pressure. Mains and Smith (1964) also showed that 15–50 percent of juvenile chinook salmon migrating downstream in the Columbia were in the top meter of water.

The purpose of this study was to expose sockeye salmon (*Oncorhynchus nerka*), coho salmon (*O. kisutch*), and steelhead trout (*Salmo gairdneri*) smolts to air-supersaturated water in the laboratory at the same time of year and for the same lengths of time that they would be migrating down the Columbia River, to determine saturation levels that smolts can tolerate during their migration.

METHODS

The fish were tested using the facilities described by Bouck et al. (1976). Water in five 6,000-liter tanks, 60 cm deep, was held

TABLE 1.—*Nominal and measured test levels of total gas supersaturation.*

Test number	Test tank	Nominal % total gas saturation[a]	Measured total gas saturation (%)		
			N	Mean ± SD	Range
1	1	120	19	119.6 ± 0.8	118.3 – 121.3
	2	115	27	115.0 ± 0.9	113.7 – 117.8
	3	115	27	114.8 ± 0.7	113.7 – 116.5
	4	110	26	109.4 ± 0.9	107.9 – 111.1
2	1	120	3	120.3 ± 0.9	119.7 – 121.3
	2	115	18	115.1 ± 1.0	113.7 – 117.8
	3	115	18	114.9 ± 0.7	114.0 – 116.5
	4	110	18	109.3 ± 1.0	107.9 – 111.1
3	1	120	17	119.8 ± 0.6	118.6 – 121.0
	2	115	25	114.7 ± 0.5	113.6 – 115.9
	3	115	24	114.5 ± 0.5	113.2 – 115.1
	4	110	24	109.1 ± 0.8	107.7 – 111.0
4	1	120	5	119.4 ± 0.6	118.6 – 120.3
	2	115	25	114.7 ± 0.5	113.6 – 115.9
	3	115	24	114.5 ± 0.5	113.2 – 115.1
	4	110	24	109.1 ± 0.8	107.7 – 111.0

[a] Control tanks (Tank 5) remained near 100 percent at all times.

at 120 percent, 115 percent, 115 percent (replicate), 110 percent, and 100 percent total air saturation. Two separate cages within each tank permitted replicate groups of fish to be tested concurrently in the same water. Four tests were conducted, with the five different total gas (air) saturation concentrations for each test. Tests No. 1 and 2 with sockeye smolts lasted for 43 days and 35 days, respectively. Test No. 3 with coho smolts ran for 26 days, and test No. 4 with steelhead lasted for 27 days. Times to 20 percent and 50 percent death at each concentration were determined and lethal threshold concentrations were obtained using the methods of Sprague (1969). Unpublished acute data were also included to calculate threshold concentrations. Ninety-six hour and 30-day LC50 values (lethal concentration where 50 percent died) were also calculated. Data were analyzed according to the American Public Health Association (1971). Sublethal effects of supersaturated water on the fish and signs of gas bubble disease (GBD) were recorded.

Test fish were reared from eggs at the Western Fish Toxicology Station and were fed Oregon Moist pellets. The mean fork-length of coho was 16 cm. The sockeye length was 14 cm, and steelhead length was 20 cm. The coho, sockeye, and steelhead had mean weights of 51 g, 36 g, and 74 g, respectively. Fish were transferred directly from holding tanks to test tanks already set at test supersaturation levels, then checked daily for mortality and signs of GBD. Fish were not fed for three days prior to or during testing.

Water temperature was maintained at 12 ± 1 C. Alkalinity, hardness, and pH values remained near 20 mg/liter, 19 mg/liter (as $CaCO_3$), and 7.3, respectively throughout the test period. Samuelson (1976) has described detailed chemistries of test water.

Total dissolved gas saturation (Table 1) was measured with the Weiss Saturometer (Nebeker, Stevens, and Brett 1976), calibrated with the Van Slyke gas analyzer and Winkler dissolved oxygen analyses. The formula used to calculate total percent saturation was:

$$\% = \frac{BP + \Delta P - VP}{BP} \times 100$$

where BP = barometric pressure, ΔP = the saturometer gauge reading, VP = water vapor pressure. Normal atmospheric O_2/N_2 ratios were maintained by injecting compressed air into the test water under pressure, then returning the water to normal atmospheric pressure in the test tanks.

RESULTS AND DISCUSSION

Sockeye and steelhead smolts died rapidly at 120 percent total gas saturation with 50 percent of the deaths occurring at 38 and 43 hours respectively. Coho smolts were more tolerant with 50 percent of the deaths occur-

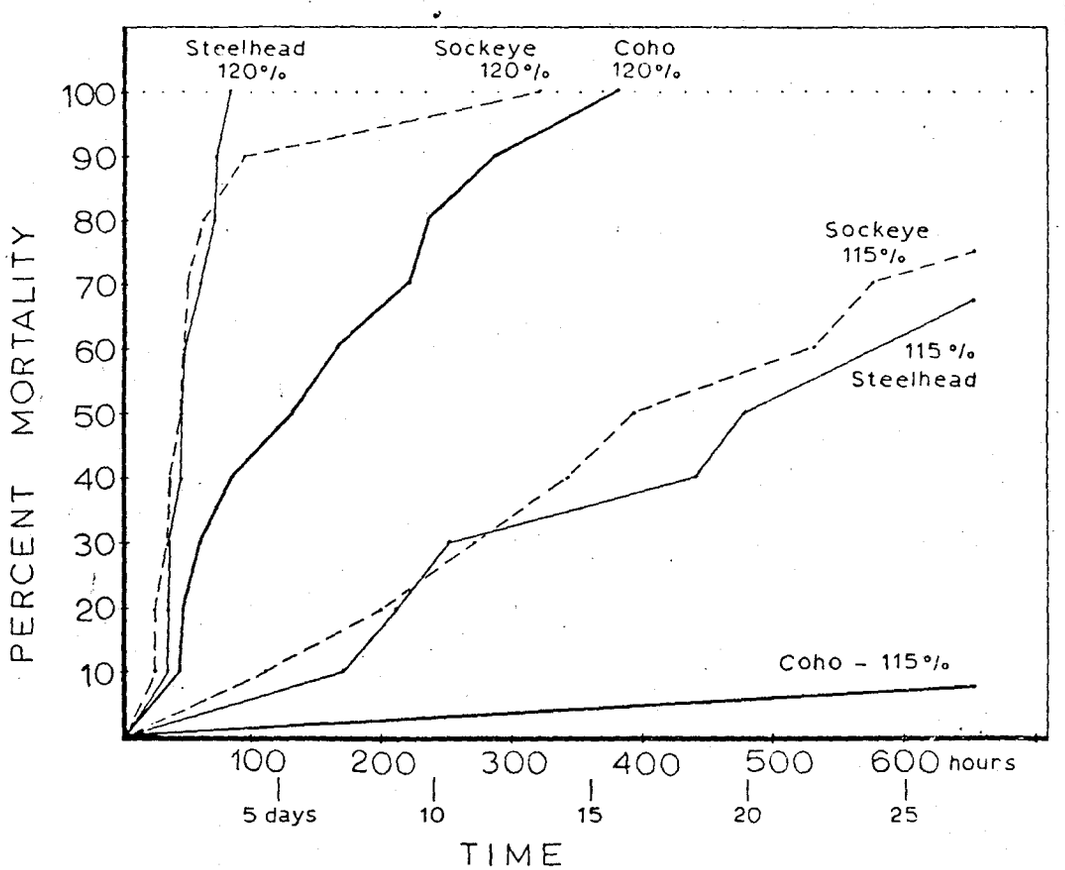


FIGURE 1.—*Percent mortality of steelhead trout, sockeye and coho salmon smolts at 120 percent and 115 percent total gas supersaturation in water 60 cm deep at 12 C.*

ring after 101 hours. At 115 percent, 50 percent of the sockeye had died at 360 hours and 50 percent loss of steelhead occurred at 456 hours. Less than 10 percent of the coho died after 27 days at 115 percent (Table 2). No sockeye or coho and only 3 percent of the steelhead died at 110 percent (Fig. 1). Lethal threshold concentrations (those concentrations

TABLE 2.—*Mean time to 50 percent and 20 percent death for juvenile (smolt) coho and sockeye salmon and steelhead trout.*

Species	Nominal percent saturation	Mean time to 50% death (h)	Mean time to 20% death (h)
Coho salmon	120	104	47
	115	na[a]	na
	110	na	na
Sockeye salmon	120	38	24
	115	359	168
	110	na	na
Steelhead trout	120	43	30
	115	382	142
	110	na	na

[a] Not achieved—insufficient number of deaths.

TABLE 3.—*Summary of acute and chronic effects of air-supersaturated water on coho and sockeye salmon smolts and steelhead smolts at 12 C in test water 60 cm deep.*

Species	96-hour LC50[a] (total % sat.)	30-day LC50[a] (total % sat.)	Lethal threshold conc. (%)[b]
Coho	120.5	116.2	114.5
Sockeye	116.7	113.9	113.5
Steelhead	116.0	114.0	114.0

[a] LC50 = Lethal concentration where 50 percent died after 96-hour (or 30-day).

[b] Concentration where significant mortality no longer occurs, calculated from time to 20 percent death at each concentration.

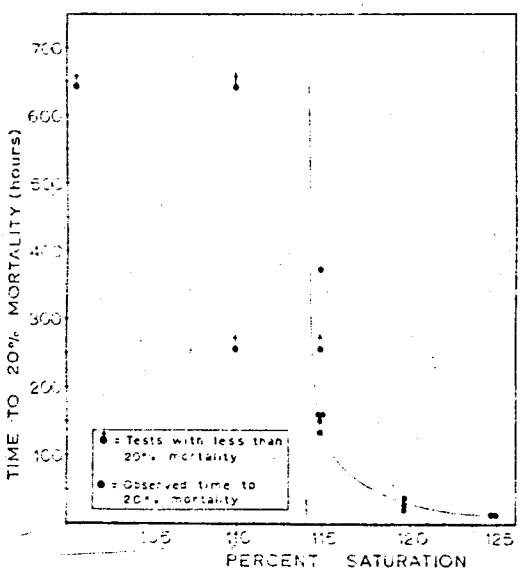


FIGURE 2.—*Lethal threshold concentration determined for steelhead trout smolts at 12 C in 60 cm water (unpublished acute data included).*

where significant mortalities no longer occur) were 114.5 percent for coho, 113.5 percent for sockeye, and 114.0 percent for steelhead (Fig. 2).

All but the first fish to die in each test exhibited many signs of GBD, and the severity of GBD symptoms directly correlated with the length of exposure and the gas concentration. Severe emphysema (bubbles) occurred on the fins and tail (Fig. 3), in the mouth (Fig. 4), and on the head and opercles (Fig. 5) of the fish exposed at 120 percent and 115 percent. Emphysema was not as severe at 110 percent, but still occurred in 20 percent of the sockeye, 60 percent of the coho (mostly in the



FIGURE 4.—*Subcutaneous emphysema within the mouth of a juvenile salmon, exposed to supersaturated water.*

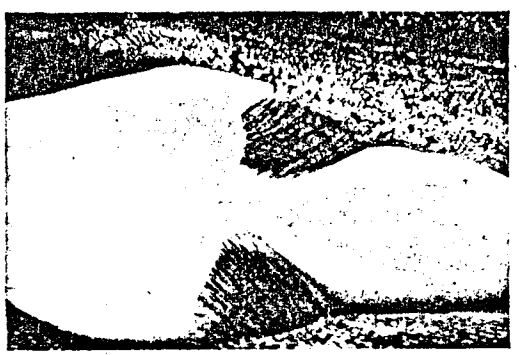


FIGURE 3.—*Anal fins of juvenile steelhead trout, one with emphysema in the interray tissue (above) and one normal (below).*

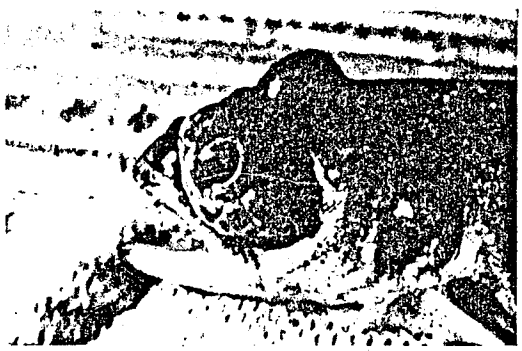


FIGURE 5.—*Subcutaneous emphysema on the dorsal aspect of the head and exophthalmia due to emphysema of tissue surrounding the eye in a juvenile salmon exposed to sublethal levels of supersaturation.*



FIGURE 6.—*Dorsal view of juvenile steelhead trout showing exophthalmia or popeye.*

tail), and 80 percent of the steelhead. Exophthalmia or "popeye" occurred but only in a small percentage of the fish (Fig. 6). The tolerance of individual fish varied widely within a species; some appeared very sensitive and died rapidly, while others appeared unaffected during the tests. Some were grossly distorted by bubbles and twisted out of normal body shape while others had few external signs or sublethal behavior modifications.

The 96-hour LC50 value (lethal concentration which killed 50 percent after 96 hours) for coho smolts was 120.5 percent. The 96-hour LC50 values for sockeye and steelhead were 116.7 percent and 116.0 percent, respectively. Thirty-day LC50 values were 116.2 percent for coho, 113.9 percent for sockeye, and 114.0 percent for steelhead (Table 3). As indicated, the 96-hour LC50 represents a concentration which kills 50 percent of the fish in a relatively short period of time and is a value that should not be exceeded for even a few hours at most. The thirty-day LC50 values and lethal threshold concentrations are very similar and indicate conditions that smolts migrating through the Columbia River probably would be able to tolerate.

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Garton and Nebeker. 1977. A multiple approach to solving the gas supersaturation problem.

Laboratory and field studies to determine sources, effects, persistence and prevention of supersaturation in the Columbia and Snake Rivers are discussed in this report.

Shallow (10-60 cm in depth) bioassay tanks were used to determine classic TL 50 data for salmonid fishes (eggs, embryos, young fish through smolt stage).

The article includes a table on the comparative sensitivity of juvenile and adult salmonids and bass to air supersaturated water and a figure useful in determining the time to 20% mortality at different saturation levels for various adult species of salmon.

A MULTIPLE APPROACH TO SOLVING THE GAS SUPERSATURATION PROBLEM

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ABSTRACT

Gas supersaturation of water was first recognized as a serious problem in the Snake and Columbia rivers of the Pacific Northwest. To solve the problem, a multiple approach was used combining laboratory and field studies to determine sources, effects, persistence, and prevention of the supersaturation. Classical bioassays were used to determine effect, but additional tests were needed because of the unique nature of supersaturation. These tests included assessment of avoidance capability of fishes, assessment of depth compensation and temperature effects, and field surveys of aquatic organism distribution in the affected areas. Data from the combined approaches were used to set safe levels for aquatic organisms. In addition, engineering expertise from other groups was applied in an attempt to prevent or mitigate the effects of supersaturation.

INTRODUCTION

The purpose of this paper is to demonstrate how a cooperative effort by a number of agencies was used in an attempt to solve the problem of gas supersaturation of water in the Columbia River Basin and other rivers and coastal waters of the U.S. The multiple approach combined classic toxicity studies, newly-designed special effect studies, field studies, and engineering expertise. The desire is not so much to present data on any particular part of the study as to chronicle the overall effort and to show how toxicity studies are an integral part of the problem definition and solution.

THE PROBLEM

Air supersaturation of water was first noted in hatchery and aquarium facilities in the early 1900's (Gorham 1901), and was ascribed as causing the condition in fish known as gas bubble disease. Based on Gorham's conclusions and the more precise analytical methods of Van Slyke and Neill (1924) for nitrogen determination, dissolved nitrogen gas was postulated to be the cause of gas bubble disease and received primary research emphasis. Supersaturation caused by entrainment of air in water spilled over dams first became a problem on the Columbia River when Bonneville Dam was constructed in 1938, although it was apparently undetected at that time. As more dams were constructed on the river the problem increased; water was supersaturated by each dam with the result that the entire river could be supersaturated during some periods of the year (Ebel 1969; Weitkamp and Katz 1973, 1975; Rucker 1972). Considering only the Snake River chinook salmon and steelhead stocks, Ebel et al. (1975) forecast a total loss of 2 million adult fish during the period 1976 to 2000 if no remedial actions were taken to reduce the hazards of supersaturation. In economic terms (1974 dollars), this loss would range between $47.2 and $126.9 million. These figures are very conservative since they do not take into account stock from the Columbia River and its tributaries, nor other species such as sockeye and coho salmon.

ORGANIZATION FOR SOLVING THE PROBLEM

As supersaturation on the Columbia and Snake rivers became more severe the U. S. Secretary of the Interior and the Tri-State Governors Conference (Idaho, Washington, and Oregon) formed a Nitrogen Task Force to work out solutions to the problem. The task force was made up of representatives from 23 public and private agencies. One of their first duties was to organize a division of labor between various agencies for research on the problem. The Environmental Protection Agency, represented by the Western Fish Toxicology Station, was detailed to carry out laboratory studies to determine safe levels of supersaturation for both adult and juvenile salmonids. This study was later expanded to include food organisms as well as predators and competitors of these fishes. The National Marine Fisheries Service and the states of Washington, Oregon, and Idaho were detailed to conduct field studies to determine effects and persistence of supersaturation in the Columbia River Basin. They also conducted some laboratory studies on effects.

The U. S. Army Corps of Engineers, in cooperation with the Bonneville Power Administration, were already operating the dams and regulating the flow on the lower river; their responsibility for flow manipulation and control of the power plants continued. In addition the Corps funded fisheries research through the National Marine Fisheries Service laboratories and conducted engineering and modeling studies to provide solutions to the problem.

The Northwest Utility Cooperative, together with public and private research organizations such as Parametrix, Inc., and Battelle Northwest, provided funding and cooperation for additional research (Weitkamp and Katz 1973, 1975).

DEVELOPMENT OF MEASUREMENT TECHNIQUES

At the beginning of the supersaturation study, measurement techniques had to be developed for use in both laboratory and field experiments. Previously two standard techniques had been employed, the standard Winkler determination for dissolved oxygen (APHA 1971) and the Van Slyke method for nitrogen determination (Van Slyke and Neill 1924). The oxygen determination method was quick and easily used, but did not determine nitrogen or total gas saturation levels. The Van Slyke method measured O_2, N_2 and total gas, but was relatively cumbersome and difficult for field use. A third technique, the gas chromatograph, was also available for determining nitrogen, oxygen, or carbon dioxide. It is relatively quick and precise but requires expensive equipment which is not readily portable (Fickeisen et al. 1975). The development of the Weiss saturometer in 1970 significantly changed the method of analysis, and made field and some laboratory determinations relatively easy to accomplish. The saturometer, developed by Ray Weiss (1970, unpublished data, Scripps Inst. Oceanog., La Jolla, Cal.) and adapted for field use by Robert Rulifson (S. Lambert and R. L. Rulifson, 1972, unpublished report, U. S. Environmental Protection Agency, Seattle), consists of a metal framework upon which is wound a length of about 100 ft (the length is variable) of semi-permeable, medical-grade, silastic tubing. The tubing is permeable to gas when submerged but is not permeable to water; thus, the gas in the water goes through the wall of the tubing until gas pressure within the water and the tubing becomes equilibrated. The gas pressure within the tubing is then measured by a manometer to determine the difference between gas pressure in the water and gas pressure in the atmosphere. With a known atmospheric pressure one can calculate percent total gas pressure in the water as compared to that in the atmosphere. This device does not differentiate between nitrogen pressure and oxygen pressure. However, it can be used in conjunction with the Winkler method for dissolved oxygen. The saturometer determines total gas pressure, and by subtraction of oxygen, nitrogen pressures can be obtained.

FIELD WORK TO DETERMINE EXTENT AND MAJOR PROBLEMS

Intensive field work on the Columbia River was begun in 1966 to determine causes of supersaturation and its effect on salmon and steelhead trout (Ebel 1969). These studies showed that the primary cause of supersaturation was the spilling of water at dams on the river with a direct correlation between amount of spill and saturation levels. As the water spilled over the dams it entrained air, carried it to great depths where it was held under pressure and dissolved into the water. This water, upon return to lesser depths and pressure, became supersaturated with both oxygen and nitrogen.

To illustrate the effect of a change in pressure, at 10 C (50 F) one liter of air-saturated water will hold 36.25 cc of air at a depth of 6.1 m (20 ft) of water exerting a total pressure of 1208 mm of mercury. It the water is returned to the surface with a pressure of only 760 mm of mercury pressure, one liter of air-saturated water will hold only 22.8 cc of air. Thus, the water will be supersaturated to 159%.

Since passage of water through the turbines did not cause supersaturation at the dams on the Columbia and Snake rivers, there was an initial tendency on the part of researchers to ignore turbines from other sites as possible sources of supersaturation. However, studies conducted by Western Fish Toxicology Station and Bureau of Reclamation personnel in Colorado (Garton et al. 1973) found supersaturation as high as 130% produced in the turbine structures at Morrow Point Dam on the Gunnison River. This supersaturation was the result of insertion of air into the penstock to cushion the fall of water against the turbine blades. The air dissolved into the water in the deep draft tube between turbine blades and the outlet to the river. This same situation was found by MacDonald and Hyatt (1973) on the Mactaquac River in New Brunswick.

Thermal power plants were also identified as sources of supersaturation; water at a low temperature can hold more air than water at a higher temperature and when heated in a thermal power plant it becomes supersaturated. Depending upon the temperature, water will increase in saturation from 2.0 to 2.8% for every 1 C rise in temperature.

To illustrate the effect of temperature increase, at 3 C (37.4 F) and at 760 mm of mercury pressure, one liter of air-saturated water will hold 26.9 cc of air. In a power plant with a 16 C (28.8 F) temperature rise (ΔT), the effluent temperature would be 19 C (66.2 F). At this effluent temperature, a liter of air-saturated water would hold only 19.02 cc of air. If air is not released back to the atmosphere to compensate, the water will be supersaturated to 142%. A ΔT of 16 C is high for a power plant but not unreasonable and, of course, lower ΔT's also cause supersaturation but in correspondingly lesser amounts.

At the Pilgrim Plant in Massachusetts, thousands of menhaden were killed by supersaturation when they chose, because of temperature preference, the warmed but supersaturated discharge canal (Marcello et al. 1975). Since that time additional sources of supersaturation from thermal power plants have been identified in both salt and fresh water sites, such as the Green River in Wyoming (Roy Hamilton, personal communication).

Along with the question of source of supersaturation also comes the question of determining persistence of the condition. Persistence is largely determined by the ratio of surface area to volume of the body of water and by the amount of turbulence at the air-water interface. The Snake and Columbia rivers, which are deep, slow-moving river-reservoir systems, do not easily lose dissolved gases. The supersaturated condition may, at times, persist from upstream dams in Idaho and central Washington all the way to the Pacific Ocean (Ebel 1969), making supersaturation an especially serious problem. Garton et al. (1973) found that supersaturation in the Gunnison and Frying Pan rivers in Colorado was not nearly so persistent. A saturation level of 130% in the turbulent Gunnison River was reduced to 100% in less than eight miles of flow. The small turbulent Frying Pan River reduced supersaturation levels from over 115% to 100% in less than three miles of flow. May and Huston (1975) in Montana found that supersaturation in the Kootenai River (average peak flows of 65,000 cfs) persisted for 30 miles downstream. High flows (above 20,000 cfs) kept gas concentrations above 125% saturation as far

as 20 miles downstream from the dam, and at times gas levels had not reached equilibrium 100 miles downstream. The Columbia River below Bonneville Dam at times remained supersaturated above 110% all the way to Astoria; while the Snake River, below Hells Canyon Dam was still supersaturated at the mouth of the Salmon River.

Measurement of effects of supersaturation on fish in the field is often difficult because of the problems of sampling for dead or injured fish in the large bodies of water affected. However, Ebel et al. (1975) and others (Weitkamp and Katz 1975) noted obvious effects on returning fishes at the ladders on the Snake and Columbia rivers. Gas bubbles were observed in both adult and juvenile fish, and mark and recapture studies demonstrated high mortality in downstream migrants. In addition, high supersaturation levels have reduced return rates of fish released for downstream migration.

Excess spilling of water over the newly-completed dam on the Kootenai River near Libby, Montana, supersaturated the water with air and killed many fish in the river downstream (May and Huston 1975). There was a marked reduction of whitefish numbers, in part from mortality of juveniles due to gas bubble disease. Large-scale suckers in the first 10-15 miles below the dam had a high incidence of gas bubble disease, but their numbers remained high, indicating that they were able to tolerate high gas concentrations, possibly, in part, due to their preference for deeper water. Complete mortality of cutthroat trout and mountain whitefish occurred in 1-5 to 3-8 days when held within two feet of the water surface at total gas levels of 131-139% saturation. In volition cages extending to 10 ft the trout still suffered 55% mortality and the whitefish suffered 67% mortality after 24 days.

"TOXICITY" STUDIES TO DETERMINE EFFECTS AND SAFE LEVELS

Although supersaturation is not a toxic substance in the classic sense, traditional toxicity studies (bioassays) were used to determine effects of supersaturation and safe levels for aquatic organisms. Additional experiments were designed along with the toxicity tests to study effects such as acclimation, avoidance, and recovery from supersaturation.

Before supersaturation could be studied in the laboratory a system had to be developed to produce supersaturated water in the test tanks. Such systems were developed simultaneously at the Western Fish Toxicology Station (Bouck et al. 1976; Nebeker et al. 1976) and by the National Marine Fisheries Service (Dawley et al. 1976). These systems attempted to simulate pressure change much like the source of supersaturation at dams. Here atmospheric air of bottled gases such as nitrogen, oxygen, or carbon dioxide were injected into water under pressure where they were dissolved to saturation at high pressures. When the pressure was relieved by release of the water into the test aquaria or tanks the water became supersaturated with the gas.

The first approach was to determine classic TL50 data for salmonid fishes of all ages including eggs, embryos, young fish through smolt stage, and the adult returning to spawn. These studies were conducted in shallow tanks (10-60 cm in depth) of varying sizes, with precise temperature control, where the fish could be exposed to carefully monitored levels of

supersaturation and closely observed for effect. Both short-term and long-term chronic studies were conducted. Longer-term experiments also made possible detailed pathology studies (Figures 1, 2, 3) to determine and document the development of the "gas bubble disease" in the fish (Stroud et al. 1975). Salmonid eggs are seldom exposed to supersaturated conditions in the field. Because of the importance of supersaturation to hatcheries in the Pacific Northwest, especially where the water is heated to speed up growth of young fish, egg through swim-up studies were conducted at the Western Fish Toxicology Station. Nebeker et al. (1977a) determined that 125% supersaturation was a safe level for salmonid eggs and young sac-fry larvae in shallow tanks in the laboratory; however, when the swim-up stage developed they died at gas levels as low as 113% saturation (Figure 4). Studies conducted by Lorz and McPherson (1976) suggested that the ability of smolts to migrate and adapt to sea water may be especially sensitive to some pollutants. Nebeker et al. (1977c) conducted similar smolt studies using supersaturation as a toxicant and found that sublethal levels of supersaturation which caused gas bubble disease had no discernible effect on ability of salmonids to smolt and to acclimate to salt water.

Because salmonid stocks could be affected by the effects of supersaturation on their predators, competitors, and food organisms, other tests in addition to TL50 studies were conducted. Studies conducted at the Western Fish Toxicology Station (Bouck et al. 1976) with a predator, the largemouth bass, and juvenile salmonids in the same tank of supersaturated water demonstrated that bass could tolerate supersaturation levels that killed or injured young salmonids. Similar studies conducted with squawfish by Meekin and Turner (1974) showed that squawfish were more tolerant than salmonids but ceased to feed at higher saturation levels. Food organism studies with *Daphnia*, crayfish, and aquatic insects were conducted at the Western Fish Toxicology Station utilizing acute, long-term, and full-life cycle studies (Nebeker 1976). These studies showed that, in general, invertebrates (with the possible exception of *Daphnia magna*) were more tolerant to supersaturated water than fishes.

Because of the special nature of supersaturation, use of the classic TL50 experiments alone did not provide sufficient data to propose safe levels for aquatic life. For any given level of gas in the water the percent saturation is dependent upon both the pressure and the temperature of the body of water. A rise in water temperature or a decrease in pressure increases supersaturation. The pressure phenomenon is especially important in rivers such as the Snake or Columbia because water which is saturated to 130% at the surface, for example, will be saturated to only 100% at a depth of 10 ft. Fish staying in deeper water escape effects of supersaturation. Dawley et al. (1976), on a grant from the Environmental Protection Agency, tested juvenile chinook and steelhead trout in both shallow and deep tanks and found that fish tended to move to the slightly deeper levels with increased levels of supersaturation. Similar results were obtained by Blahm et al. (1976). However, both studies left a serious question unanswered. It was not known whether the fish stayed in the deeper areas of the tank to escape supersaturation or because the configuration of the tank made them prefer the security of the deeper water during increased stress. M. D. Knittel and coworkers at the Western Fish Toxicology Station (unpublished



Figure 1. Adult sockeye salmon showing gas blisters in the mouth and on the left opercle.



Figure 2. Gill arch of adult chinook salmon showing gas blisters on the gills and rakers.



Figure 3. Cross section of adult chinook salmon flesh showing cavities (swiss cheese effect) in the muscle.



Figure 4. Steelhead trout fingerling with gas bubble disease.

manuscript) held fish in cages (30 inches in diameter by 8 inches deep) at different levels in 10 ft deep tanks. They determined that fish are able to compensate for supersaturation by moving to deeper levels in the water but that the compensation by the fish is not quite as great as one would predict. From the gas laws we assumed compensation of 5% per 20 inches increase in depth. However, they found that the compensation was only 3.7% per 20 inches increase in depth. They also found that the longevity of fish was increased by submersion to greater depths after exposure to lethal levels of gas supersaturation.

Even though it was determined that fish compensated for supersaturation by seeking greater depths there was still the question as to whether fish can sense supersaturation and whether the fish will voluntarily go to a level where supersaturation is lower. Stevens et al. (1977 unpublished manuscript) in their avoidance studies showed that the ability to detect and avoid supersaturated water seems to vary among species. Salmon were able to differentiate between different percentages of supersaturation in a pie-shaped avoidance chamber. However, trout were not clearly able to do so consistently. Avoidance of supersaturation might be secondary to behavior such as increased activity due to aggression and territoriality, a need for cover and lower light intensities, or choice of a particular current velocity. Schiewe and Weber (1976) found that bubbles in the lateral line of fish exposed to supersaturated water diminished or completely blocked the ability of the sensory units to respond to stimuli. The loss of ability to respond to stimuli decreases the fish's capability to detect objects or locate predators. Chapman and Nebeker (1977 unpublished manuscript) considered the possibility of synergism between supersaturation and the heavy metals copper and zinc, but were unable to detect any such effect. Nebeker et al. (1977b) detected effects of temperature on fish survival in supersaturated water. With juvenile steelhead trout, tested from 9 to 18 C at 116% saturation, each one degree (C) increase decreased time to 50% death by about 30 hours, from 330 hours at 9 C to about 50 hours at 18 C. Increased temperatures significantly decreased survival time of steelhead and chinook salmon, but no significant effect was apparent for sockeye or coho salmon.

One of the results of the toxicity studies was the determination of total-air-saturation water quality criteria for the protection of fish and other aquatic life. These criteria were published in the 1976 document, "Quality Criteria for Water," published by the Environmental Protection Agency in compliance with Public Law 92-500. In this document, 110% total-air-saturation was stated as a safe level for salmonid fishes in shallow water. These criteria were established with full knowledge that fish would be able to tolerate higher levels of supersaturation at greater depths. However, sublethal effects of supersaturation were noted at 110%, and lethal levels are found to be not far above 110% (Nebeker and Brett 1976). Thus, it was determined that the safe level is near 110%, especially in shallow waters of hatcheries or fish-rearing areas where depth compensation is not possible, but that deviations from this may be justified in some specific cases (Table 1, Figure 5).

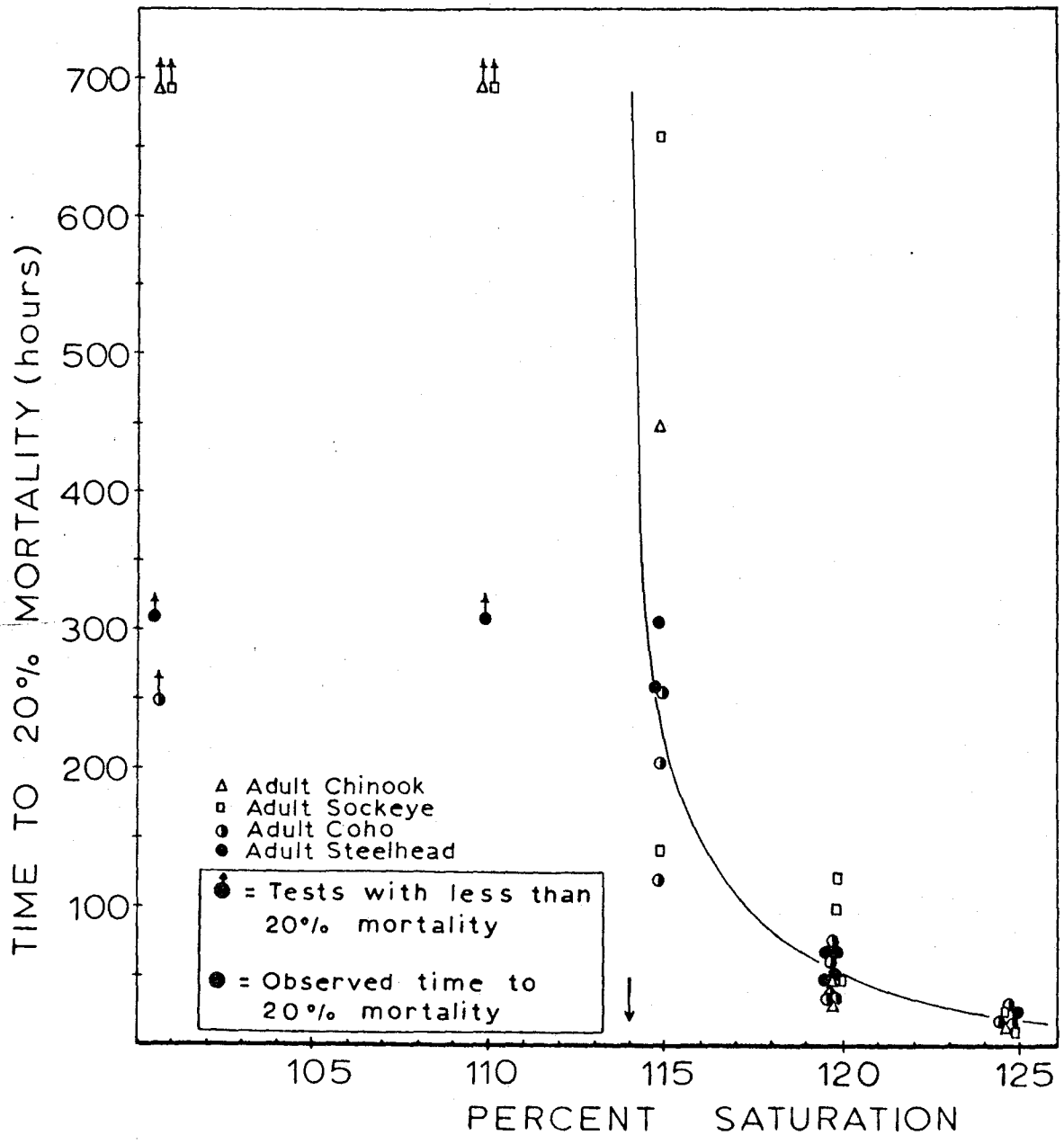


Figure 5. Determination of threshold concentration (114%) for adult salmonids (● = 10 fish/test).

TABLE 1. COMPARATIVE SENSITIVITY OF JUVENILE AND ADULT SALMONIDS AND BASS TO AIR-SUPERSATURATED WATER.

Fish	Threshold (% sat.)*
Sockeye smolts	113.6 %
Juvenile steelhead	113.8
Juvenile sockeye	114.0
Adult sockeye	114.2
Steelhead smolts	114.2
Adult coho	114.4
Adult steelhead	114.6
Adult chinook	114.7
Coho smolts	114.8
Juvenile coho	118.0
Adult bass	126.8
Juvenile bass	128.0

*based on time to 20% mortality (as determined using methods shown in Fig. 1)

SOLUTIONS

Solutions to the problem in the Pacific Northwest, in the form of structural modifications of the dams, were determined and are being implemented primarily by the Corps of Engineers. National Marine Fisheries Service personnel, funded by the Corps of Engineers, also constructed screening structures for trapping and hauling downstream migrants around problem areas. Solutions to the problem can be based either on initial planning to avoid causing supersaturation or by reduction of supersaturation when it cannot be avoided due to design of existing facilities. Reduction or elimination of supersaturation is sometimes a feasible alternative at hatcheries or other areas where a flow of waters is involved. However, reduction of supersaturation in rivers such as the Columbia is an almost-impossible task and the problem should be attacked at the start, if possible, by avoidance of supersaturation production.

Prevention of supersaturation in the Snake and Columbia river system is approached in two different ways. The first is by manipulation of river flow to avoid spilling at dams where supersaturation may be produced. The second is by physical changes in the structure of the dams themselves, such as the flip lip (Boyer 1974). Another method used to help the fishery resource is to avoid the supersaturated water completely by collecting fish with traveling screens at dams, such as Little Goose or Lower Granite on the Snake River, and trucking the fish in tank trucks to the Lower Columbia River below Bonneville Dam. This precaution has the advantage of avoiding a large part of the supersaturated river for downstream migrant fishes; however, it has the disadvantage of high trucking costs. This solution is currently under study by the Corps of Engineers and by the National Marine Fisheries Service to determine feasibility and effect of trucking, or air freighting

young fish downstream. Hopefully, continuation of the field studies on young fishes will determine whether the trucking, the flow manipulations, or the physical changes are actually doing the job in reducing supersaturation effect on migrating salmonids in the Columbia-Snake river system.

SUMMARY

The supersaturation research project is an example of a case where bioassays (classic TL50 and specially designed depth compensation studies) were used in conjunction with other field and engineering research methods to set criteria and solve a specific problem. In this case "toxicity" studies were used as a definite part of the problem-solving technique. Similar studies have also been conducted on polychlorinated biphenyls, mirex, and other pollutants. This approach is likely to continue in the future when a pollutant becomes known as important, and an all-out effort is mobilized to solve the problem. Solution of the problem depends upon identifying the source and effects of the pollutant, and determining safe levels through laboratory studies. The supersaturation study was different from many in that it has not resulted in enforcement proceedings. In this case it was a cooperative effort coordinated by the Nitrogen Task Force between state pollution control agencies and the U. S. Environmental Protection Agency, and between the fishery resource agencies of the states of Oregon, Washington, and Idaho, and the National Marine Fisheries Service. The Corps of Engineers, the Bureau of Reclamation, and the Public Utility Districts shared responsibility for dam modification and other methods used to decrease the problem or its effect. In this case there was a cooperative effort to cure mistakes in design which were carried over from the past when the problem of supersaturation was well understood.

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