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PRESSURE IN THE EARLY LIFE HISTORY OF SOCKEYE SALMON

by

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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.

ACKNOWLEDGMENTS

The author wishes to express a deep-felt gratitude to Dr. W.S. Hoar through whose inspiration and patient direction this work was conducted. The author is indebted to Dr. P.A. Larkin for advice on statistical problems. The manuscript was examined cirtically by Dr. D.H. Copp, Dr. B. McK. Bary and Dr. P.A. Larkin to whom the author expresses his appreciation.

The pressure chamber was designed by Mr. J. Pyper, engineer, of the International Pacific Salmon Fisheries Commission. Technical assistance was required in the operation of the equipment and this was ably offered by Mr. L.W. Johnston and Mr. E. Stone. The author is indebted to Miss E. Haskell and Mr. Johnston for assistance with the illustrations.

Thanks are due to Dr. F.R.H. Jones, whose incredulity in no small measure contributed to the extent to which the work was pursued. Thanks are due also to Mr. J. Terpenning for the use of facilities and fish at the Cultus Lake Trout Hatchery.

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

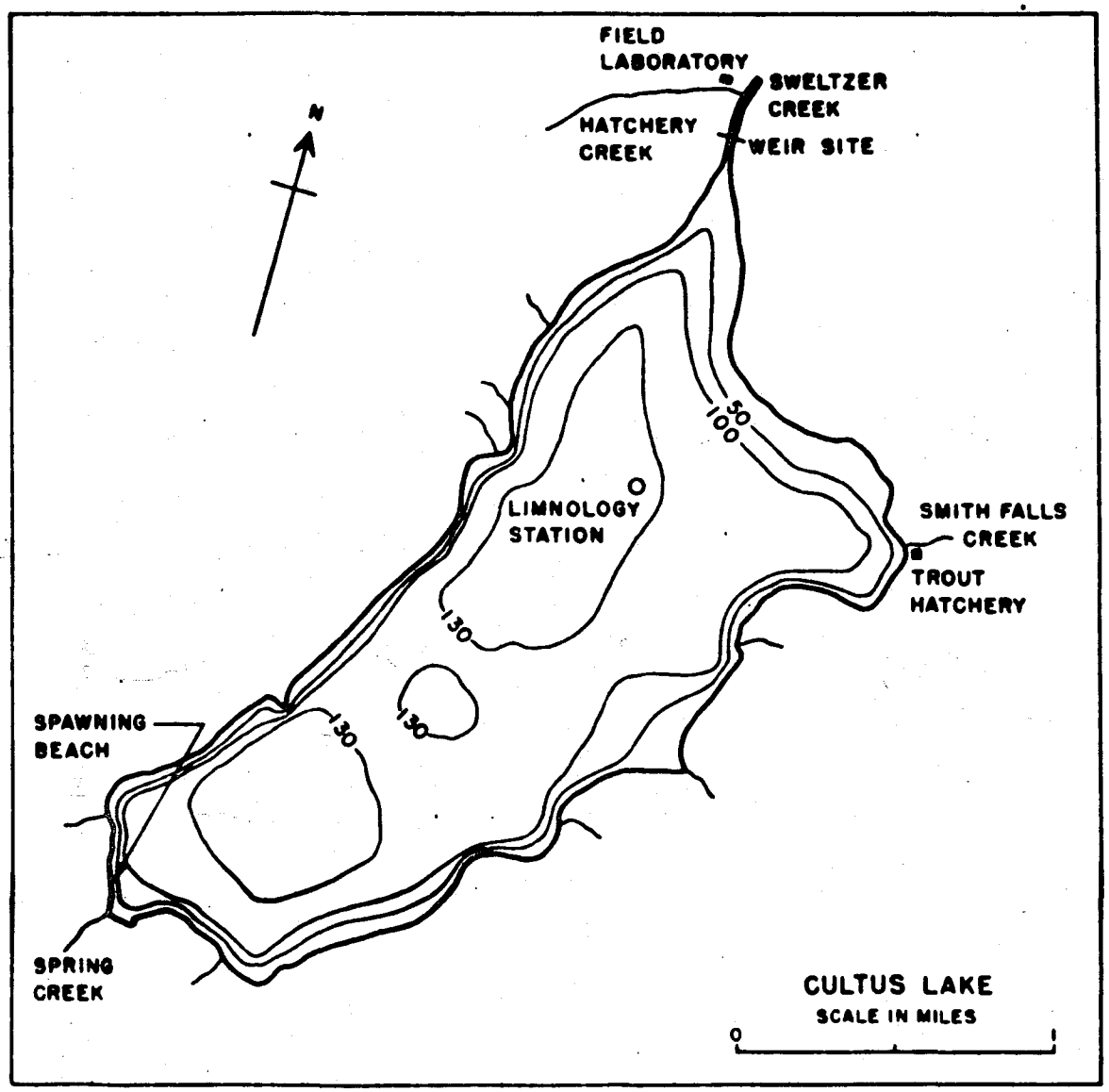

Figure 1. Cultus Lake, British Columbia, site of the investigation of pressure in the life history of the sockeye. After Ricker (1937).

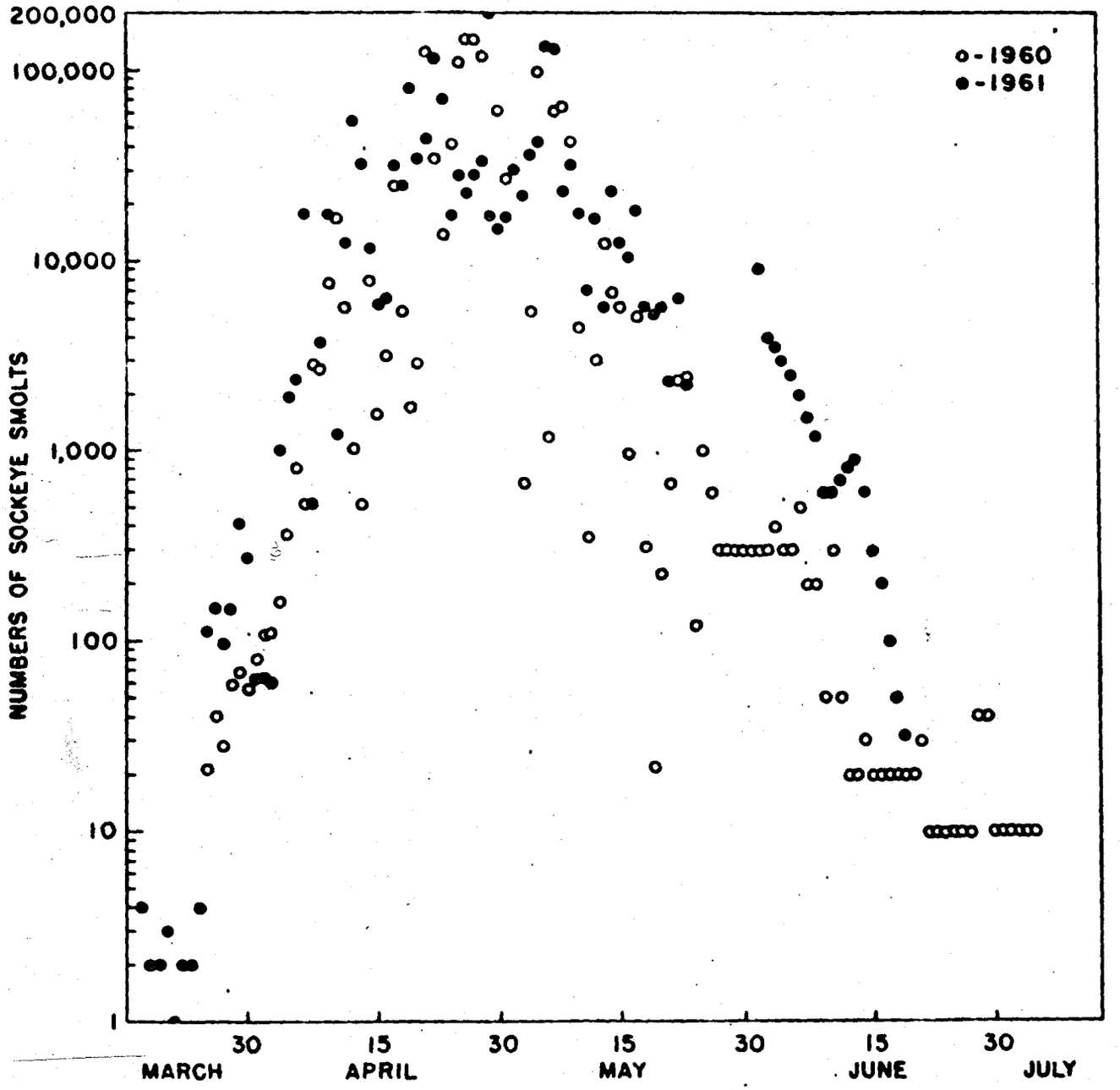

Figure 2. Daily migration of sockeye smolts from Cultus Lake during two years of the study. Note: Identical values in 1960 are estimates based on partial counts.

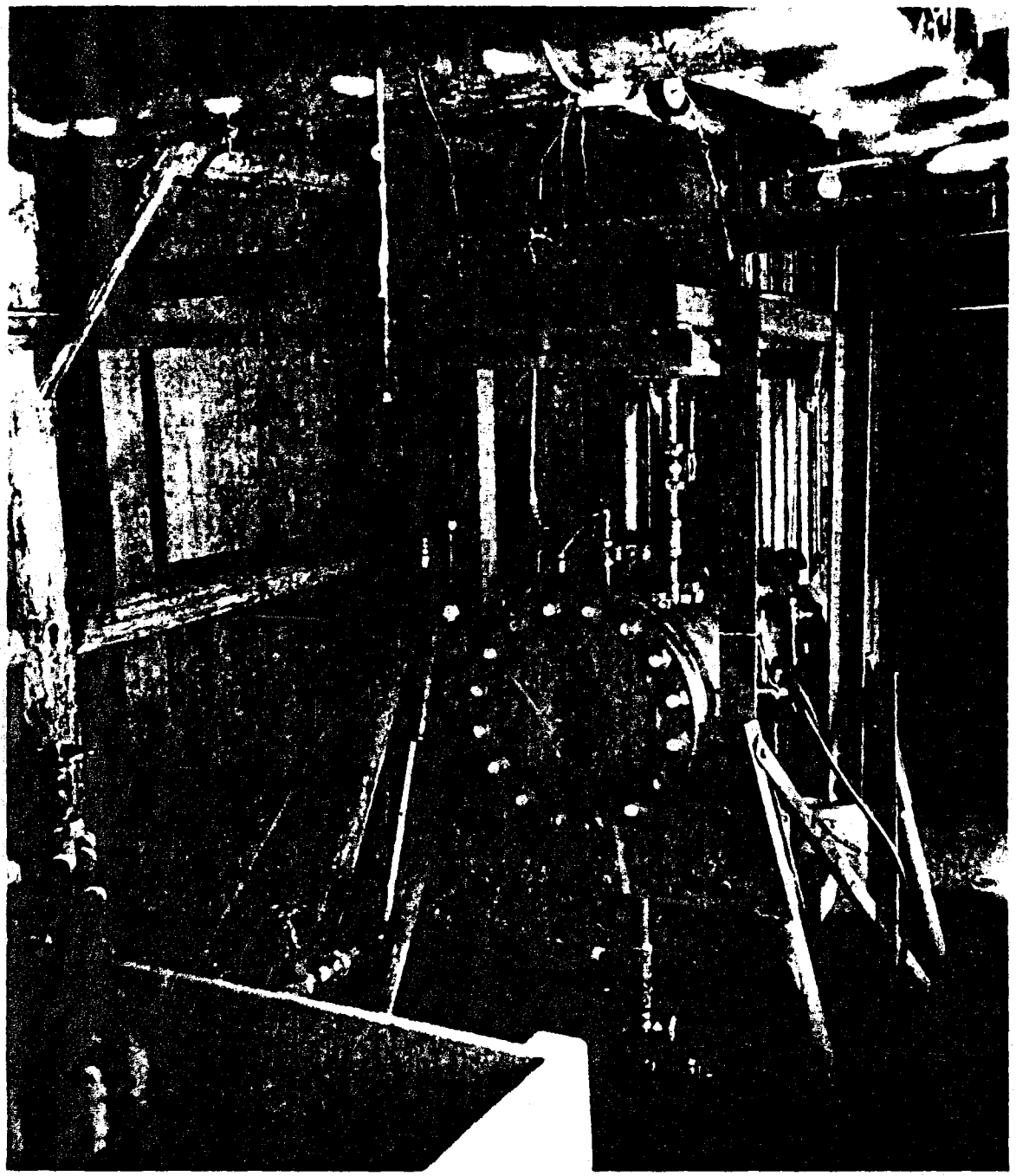

Figure 3. Pressure chamber located close to the weir on Sweltzer Creek.

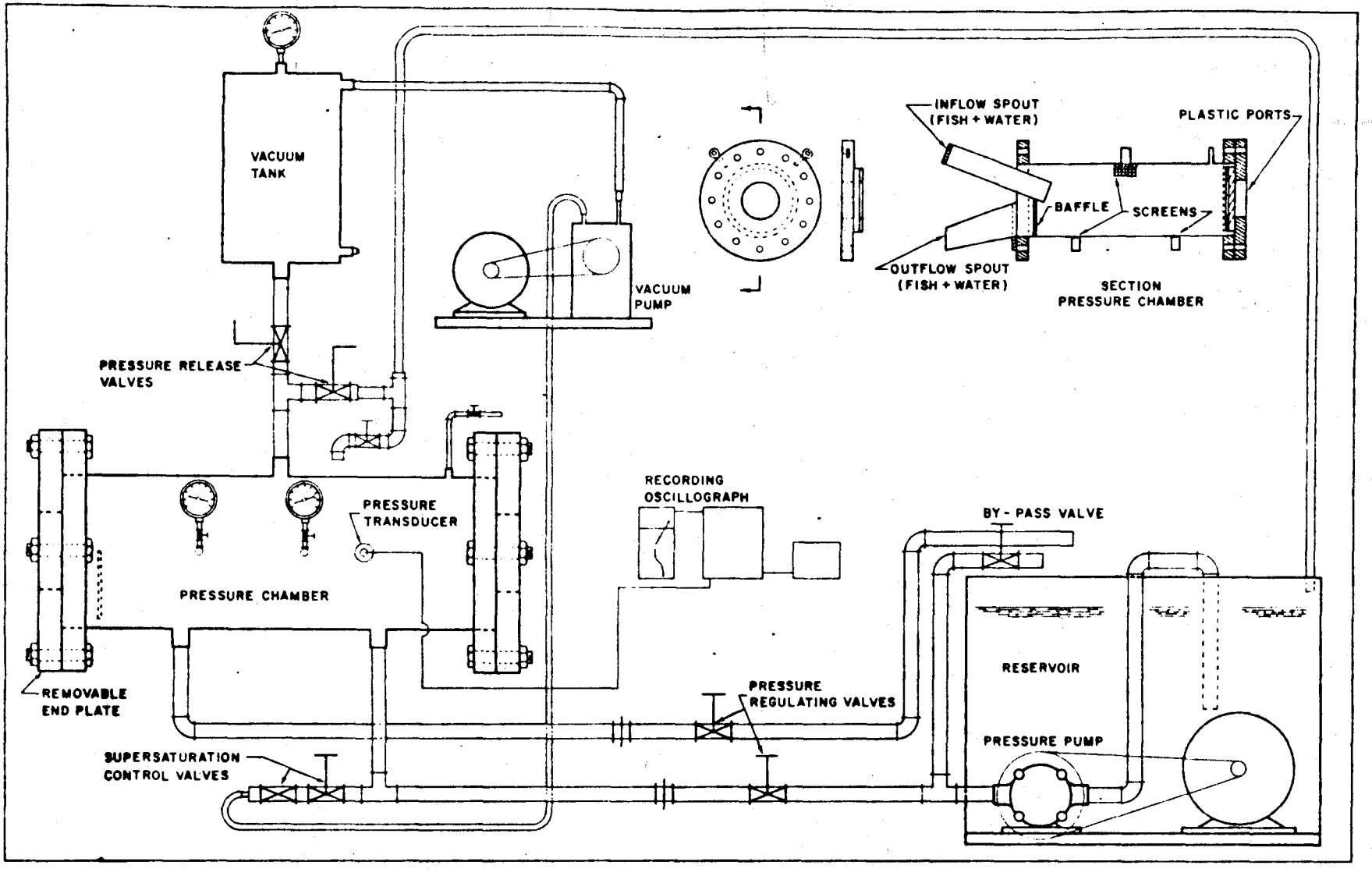

Figure 4. Diagrammatic representation of pressure apparatus.

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

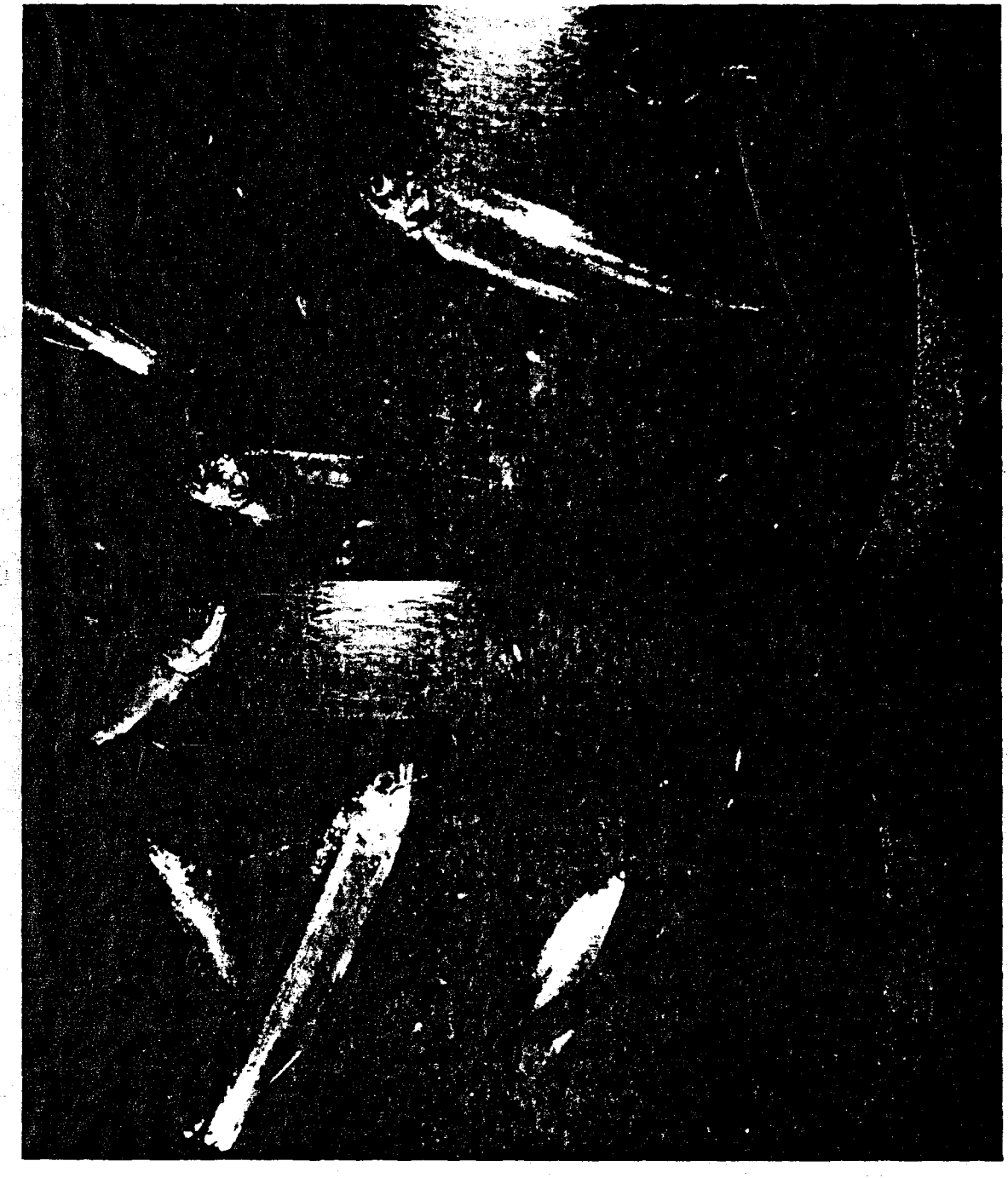

Figure 5 Characteristic posture of sockeye salmon smolts at atmospheric pressure (upper) and at a pressure of 50 psi above atmospheric (lower).

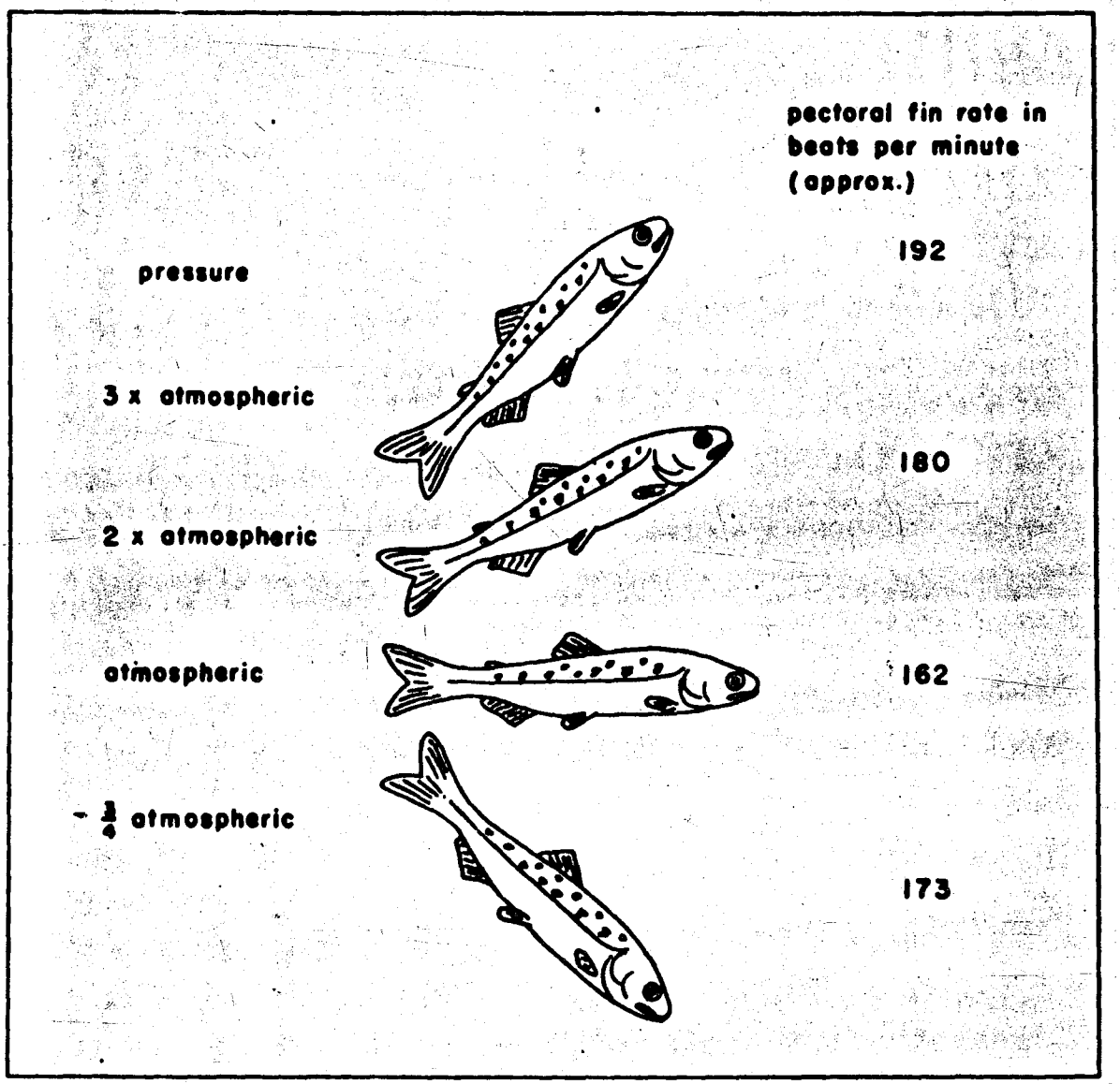

Figure 6. Posture and rate of pectoral fins of a smolt at pressures above and below atmospheric. Note: this smolt failed to emit gas at pressures below atmospheric and swam downward in response to positive bouyancy.

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

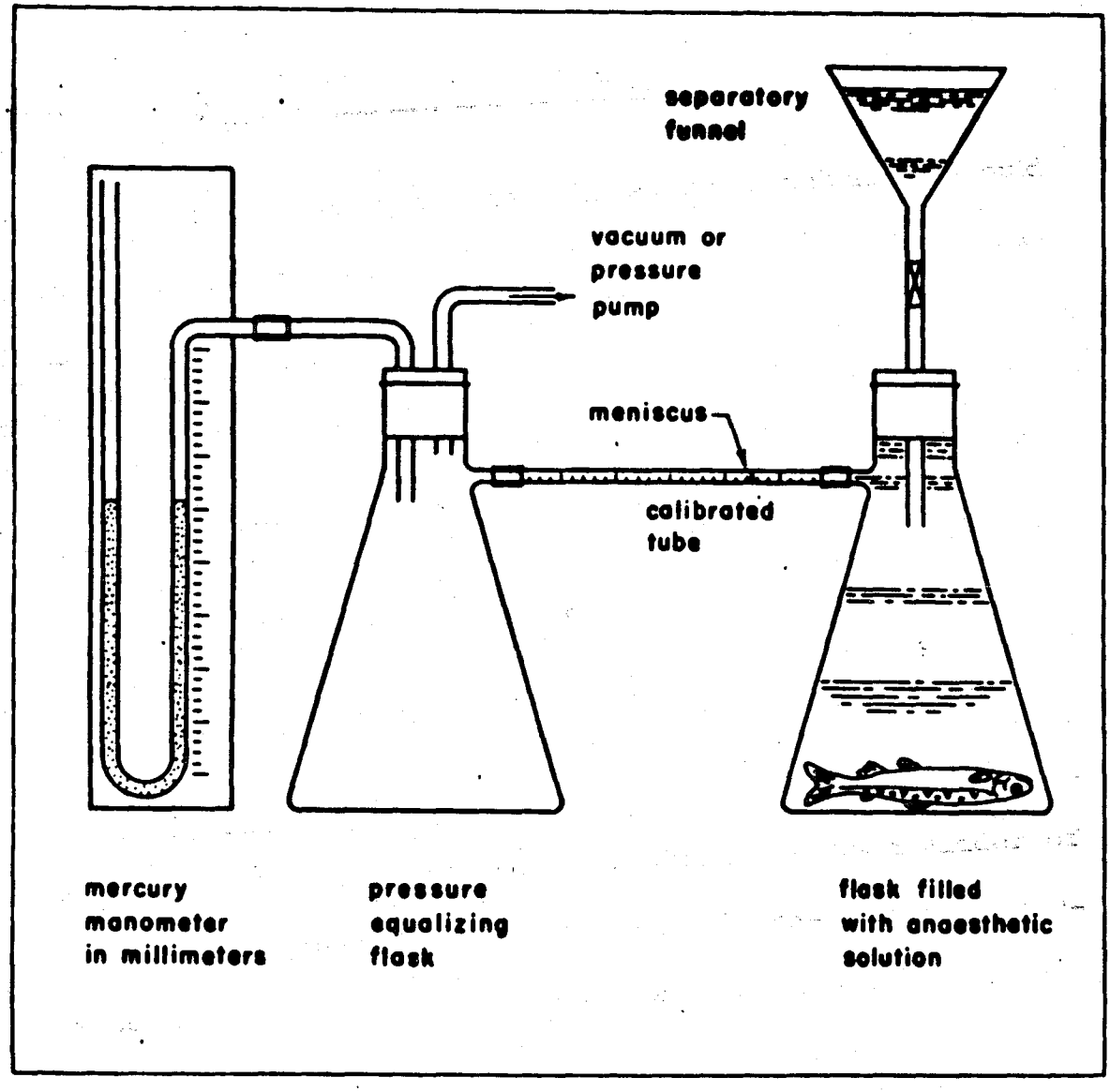

Figure 7. Apparatus for the measurement of ambient pressure within the swimbladder. (After Alexander (1959).

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

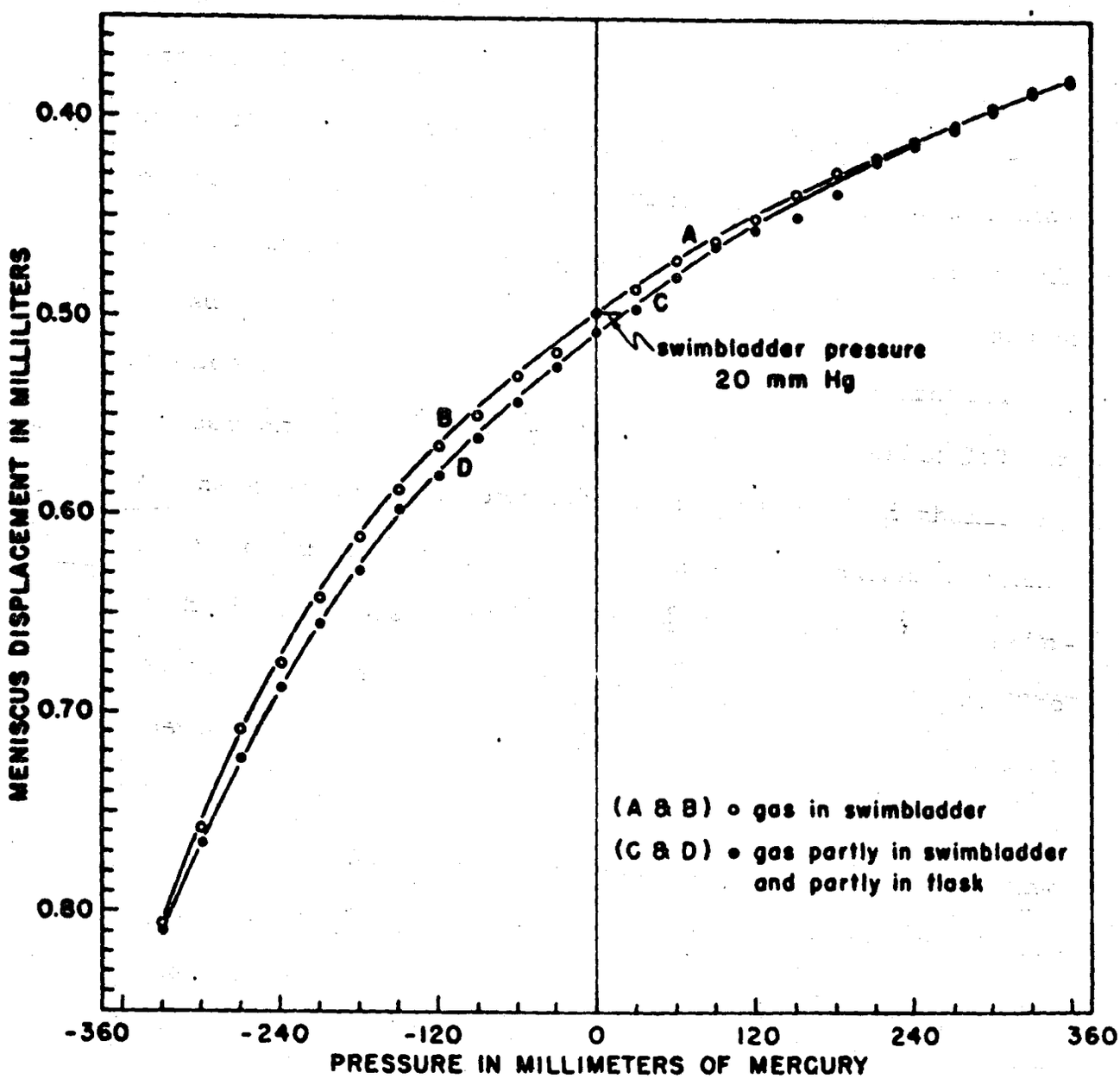

Figure 8. Calculation of excess pressure within the swimbladder.

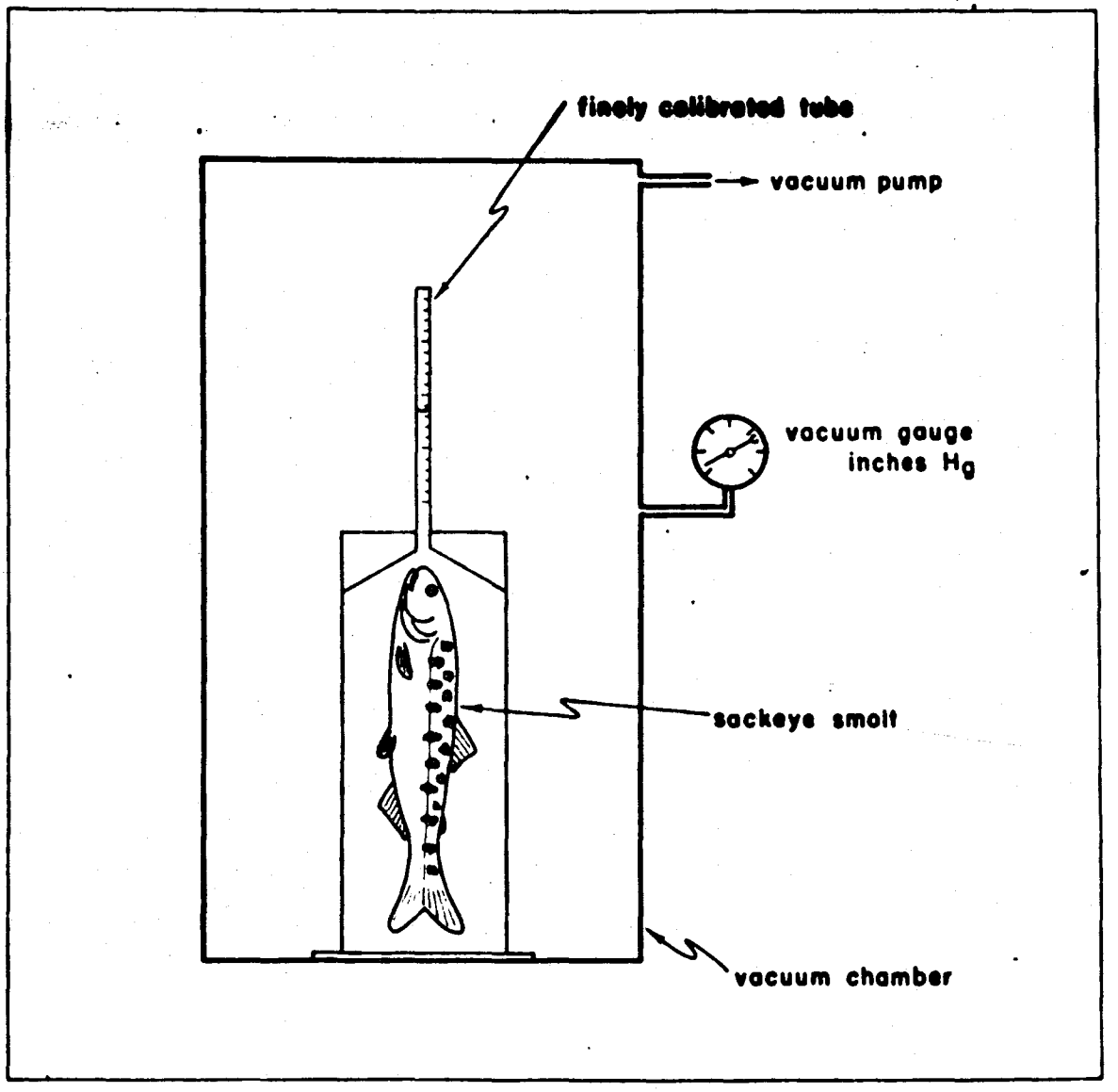

Figure 9. Apparatus for the measurement of the pressure required to force gas out through the pneumatic duct. After Jones (1951).

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

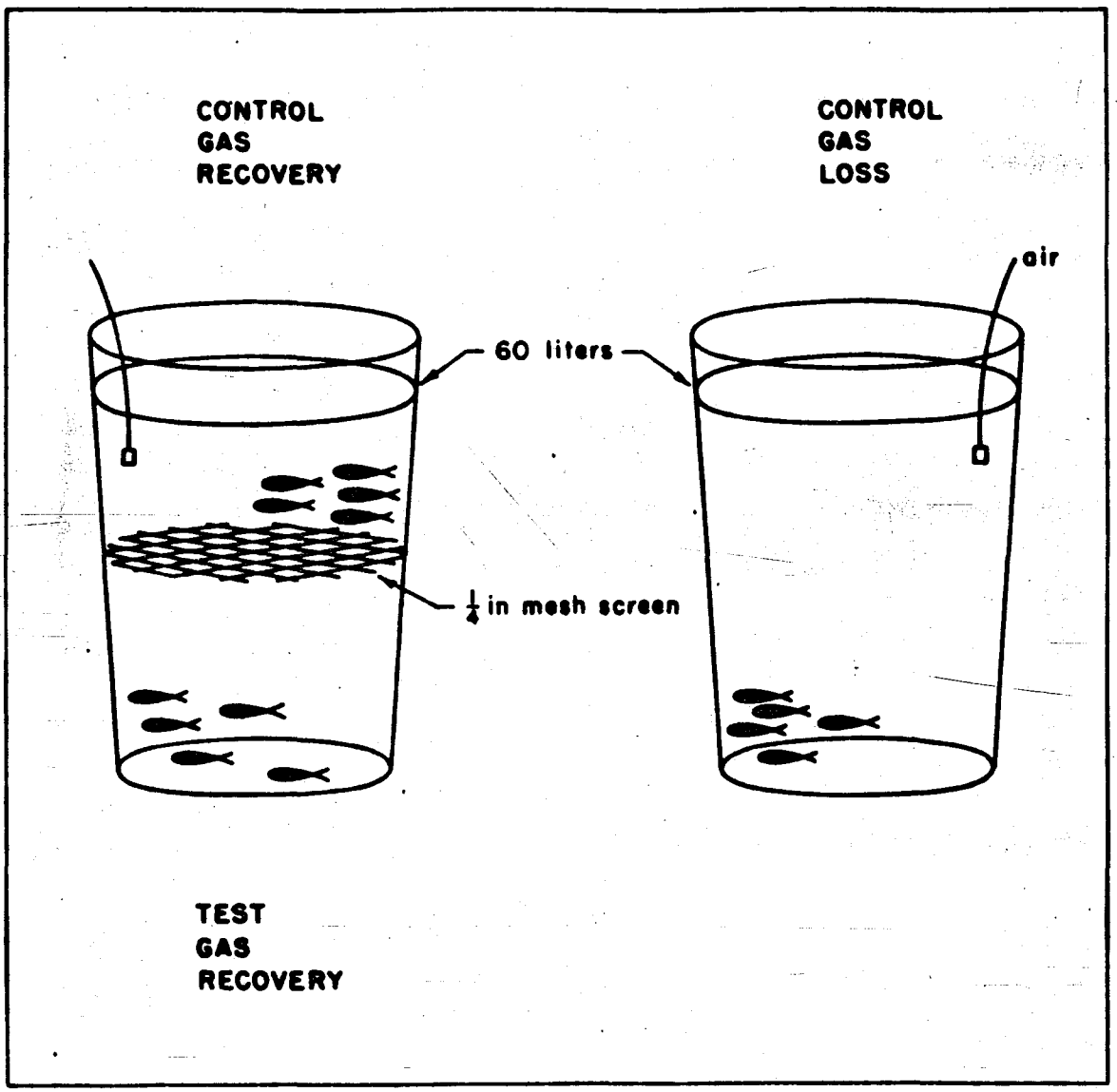

Figure 10. Investigation of inflation of the swimbladder with and without access to atmospheric air.

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

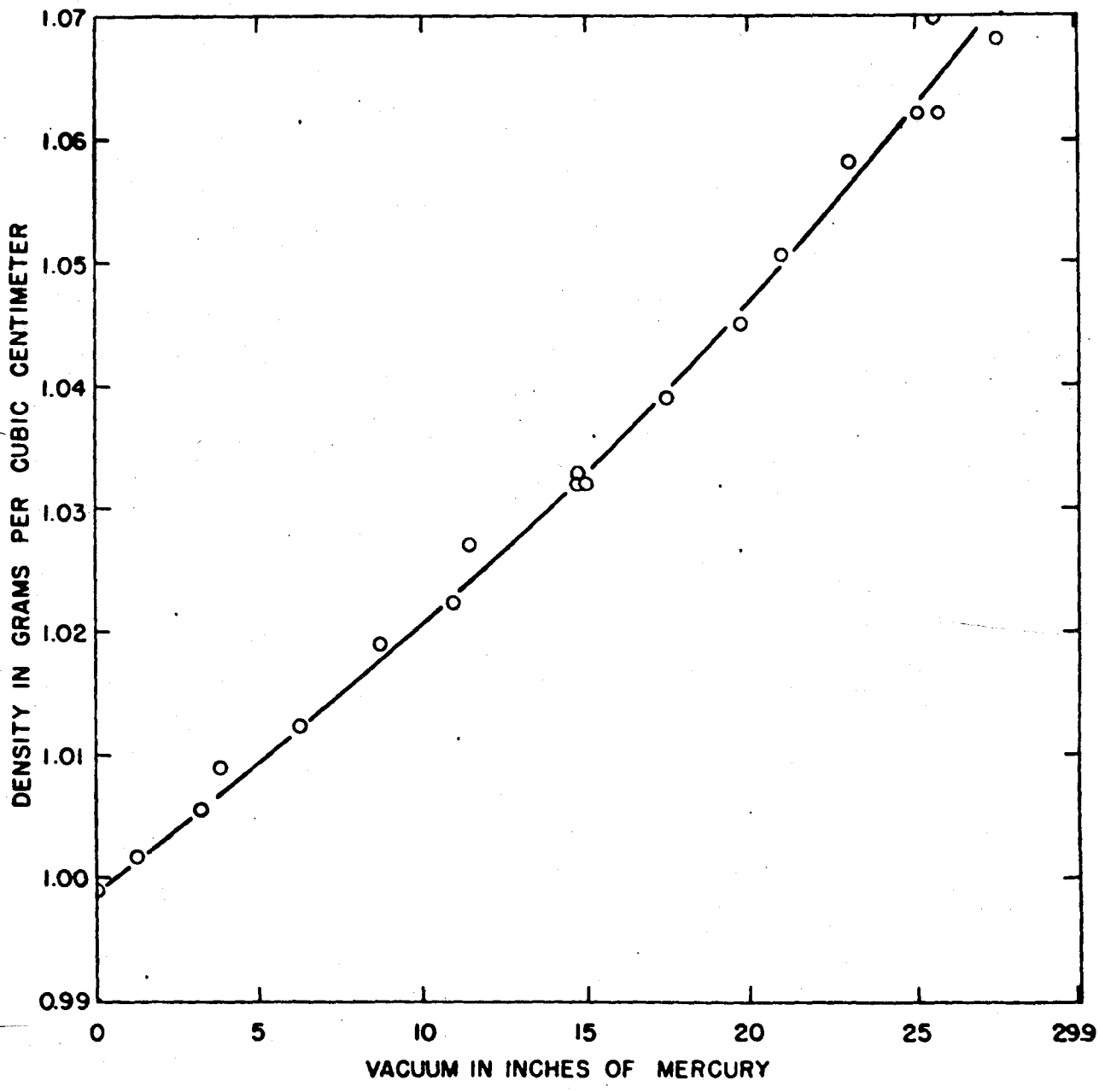

Figure 11. Smolt density and magnitude of vacuum necessary to affect neutral buoyancy.

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

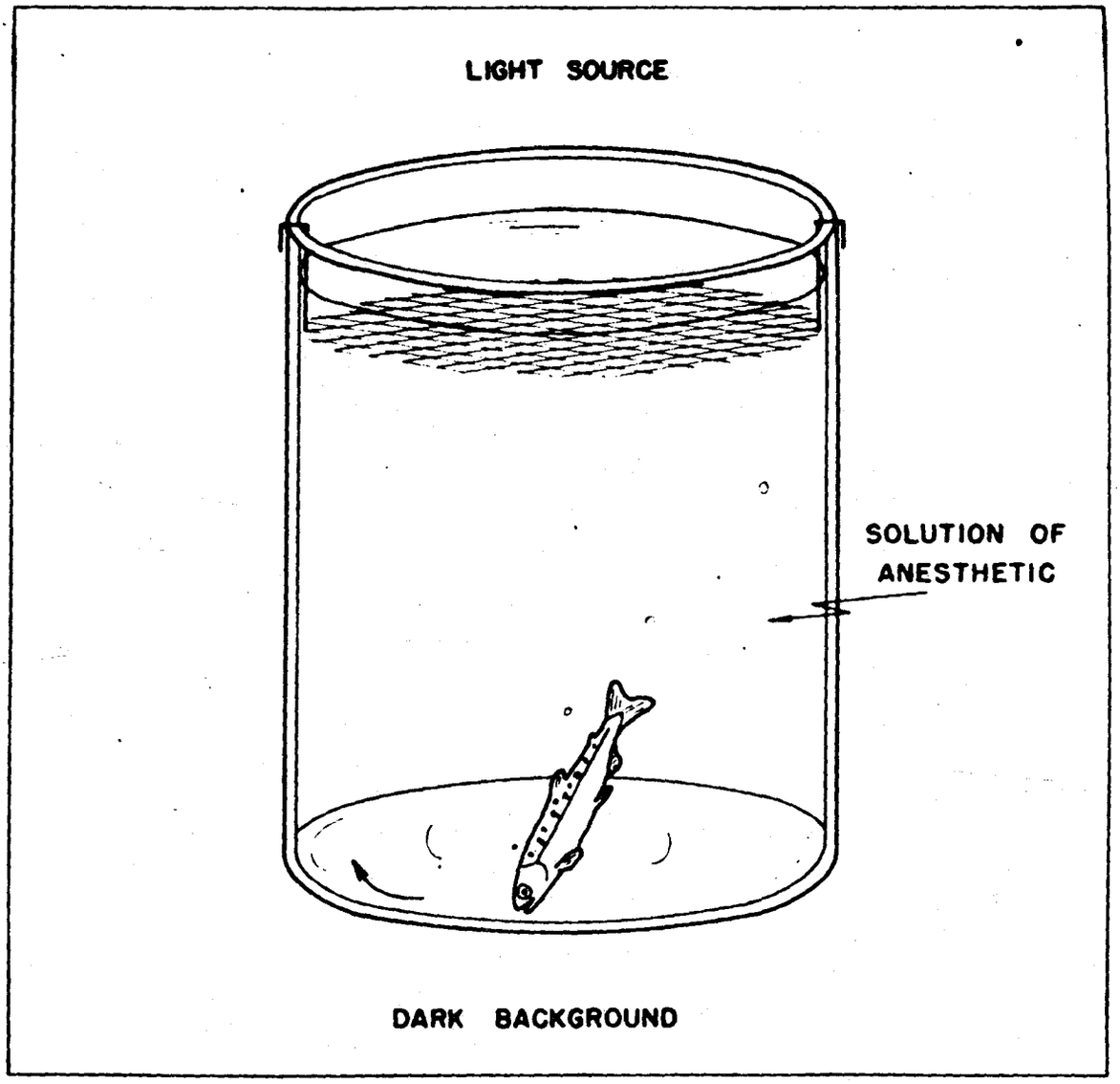

Figure 12. Apparatus used to induce the sounding response in sockeye smolts.

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 concentrations of 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,

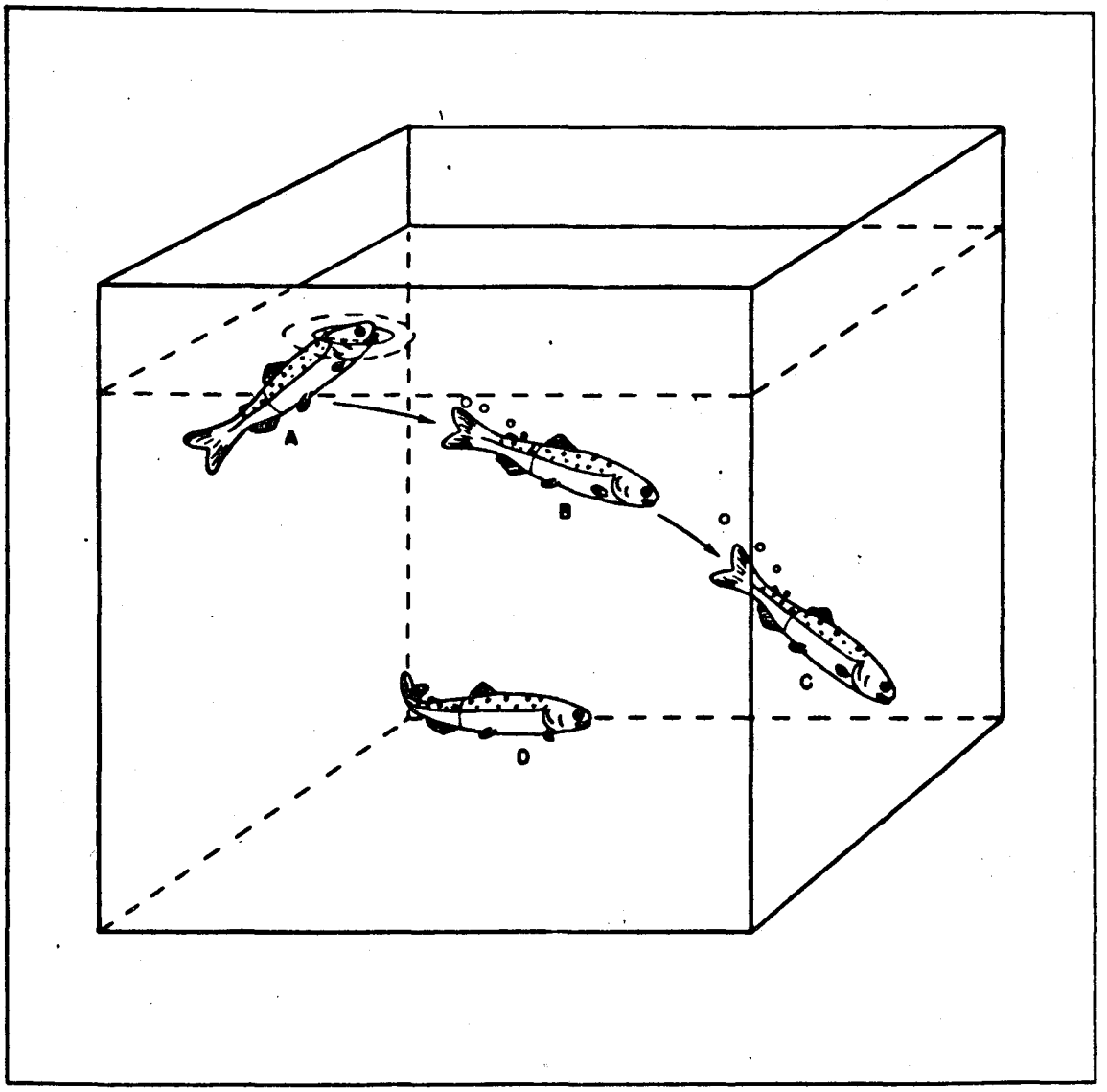

Figure 13. Response of sockeye smolts with a catheterized swimbladder. Fish attempt to inflate bladders for initial two to three days (A, B and C), thereafter residing on tank bottom (D).

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

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

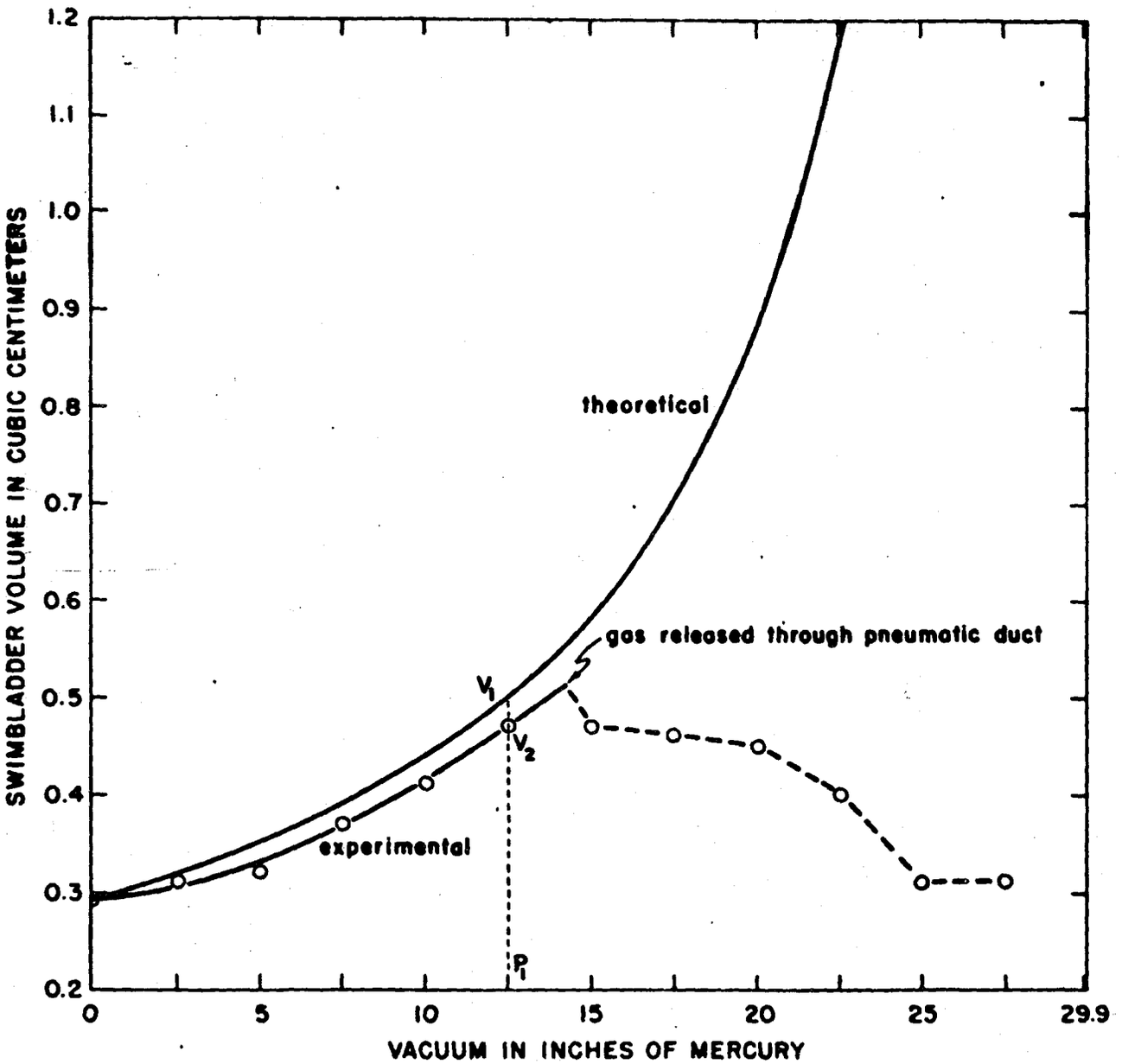

Figure 14. Calculation of pneumatic duct-release pressure from theoretical and observed expansion of gas in the swimbladder up to time of gas escape.

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

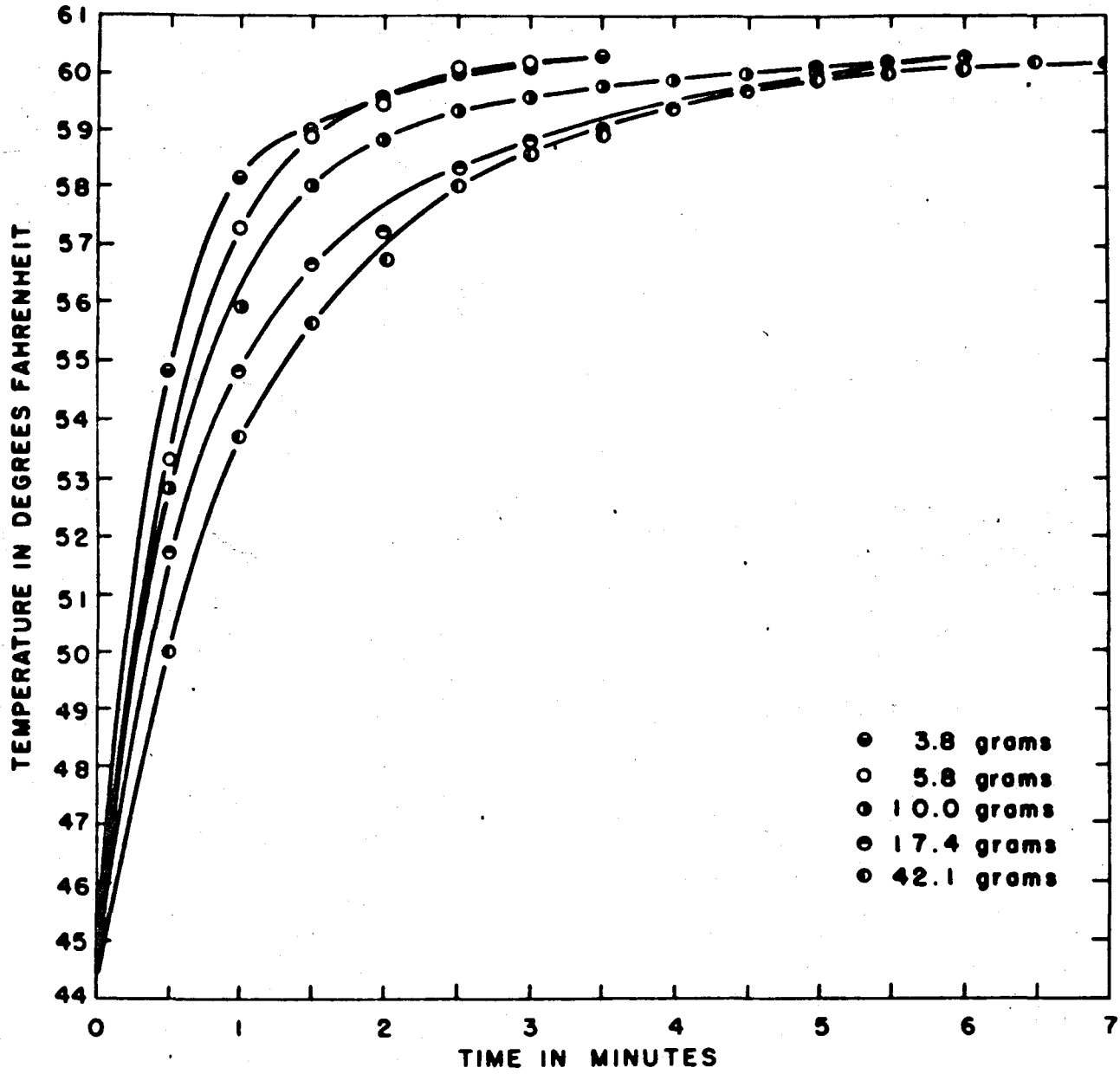

Figure 15. Change of temperature internally of fish transferred from 45 to 60° F water.

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

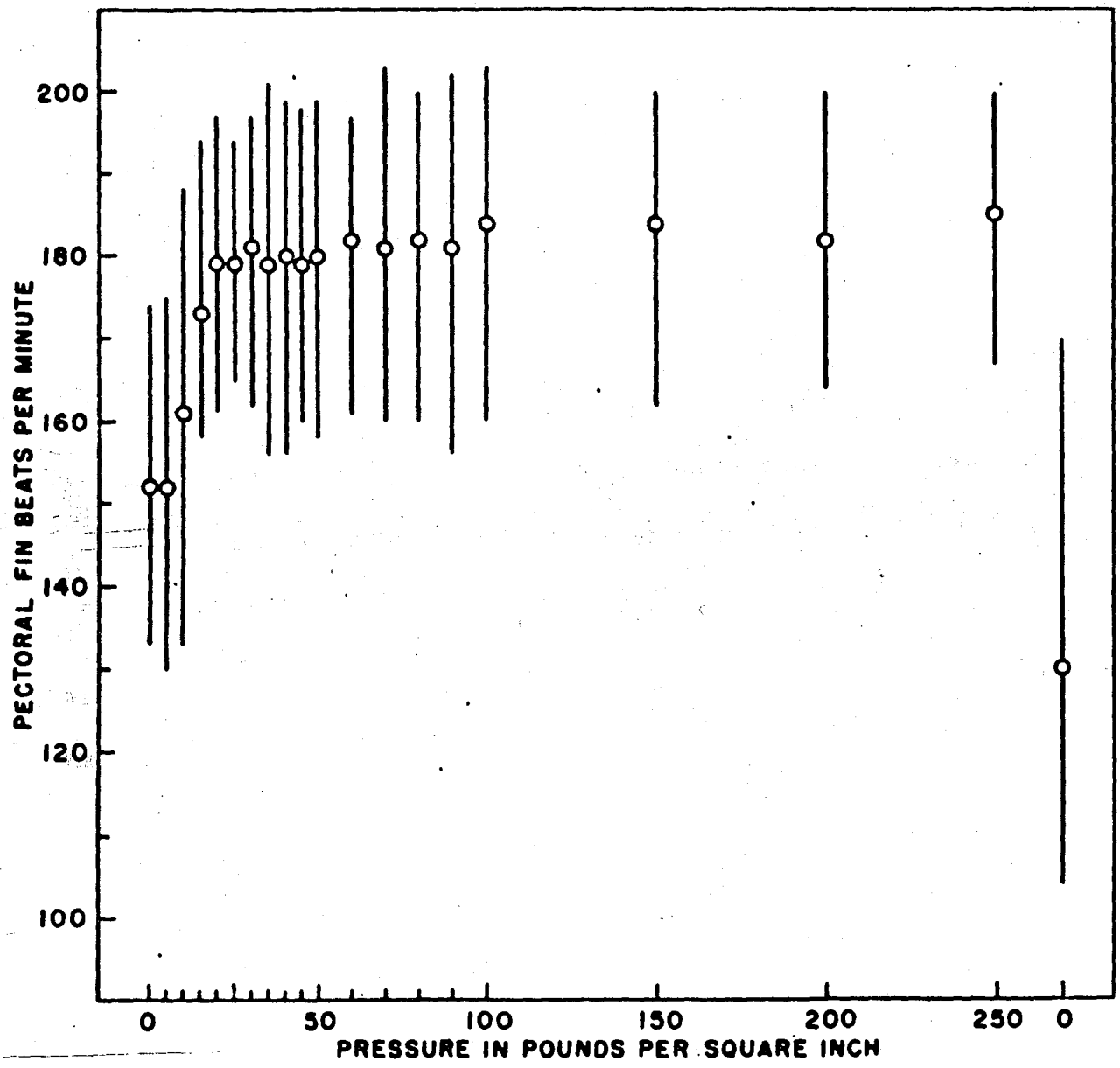

Figure 16. Average and range of pectoral fin beats for ten sockeye smolts from atmospheric pressure to 250 psi above atmospheric.

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.

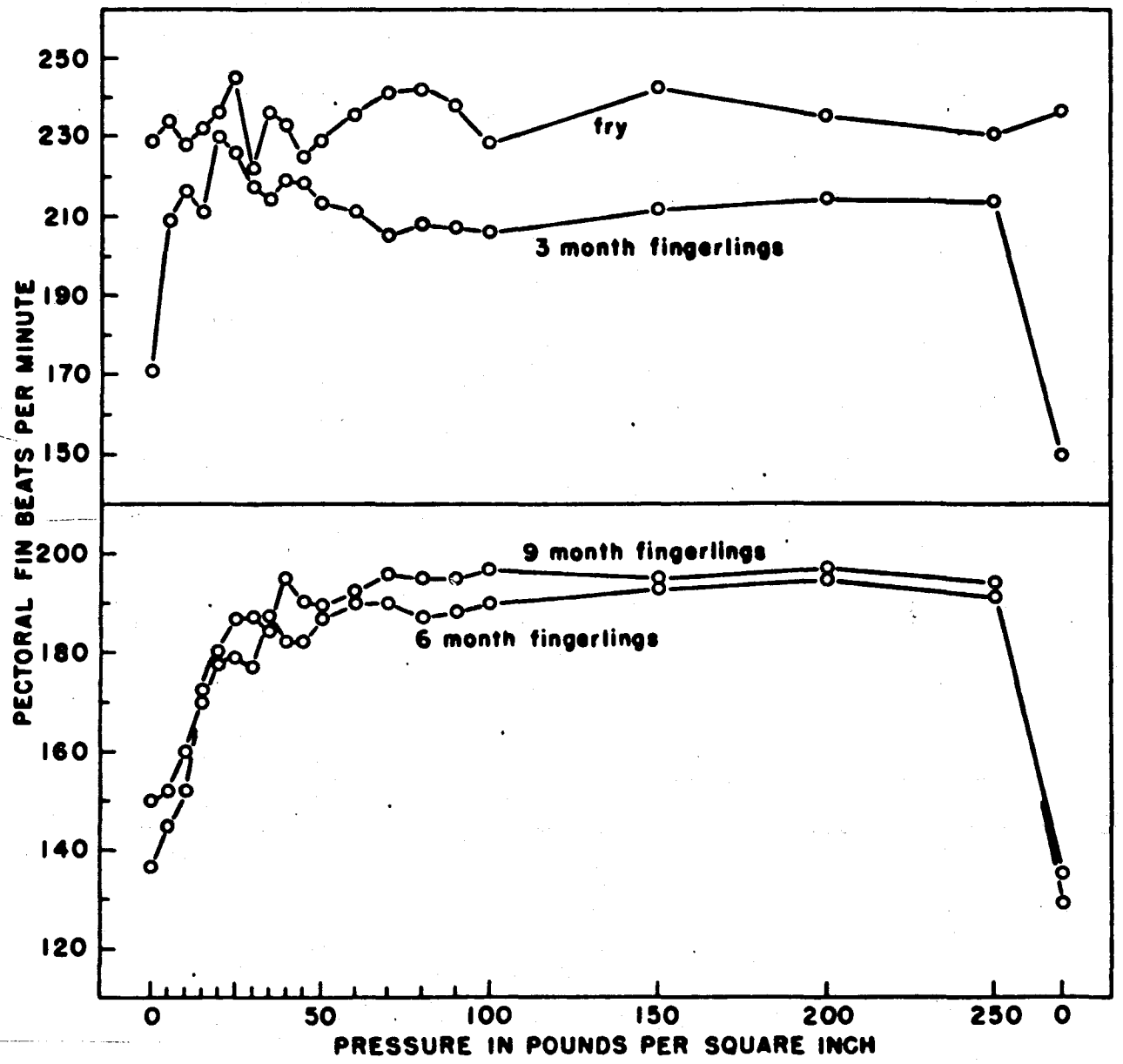

Figure 17. Average pectoral fin rates of fry and three-, six- and nine-month-fingerlings.

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.

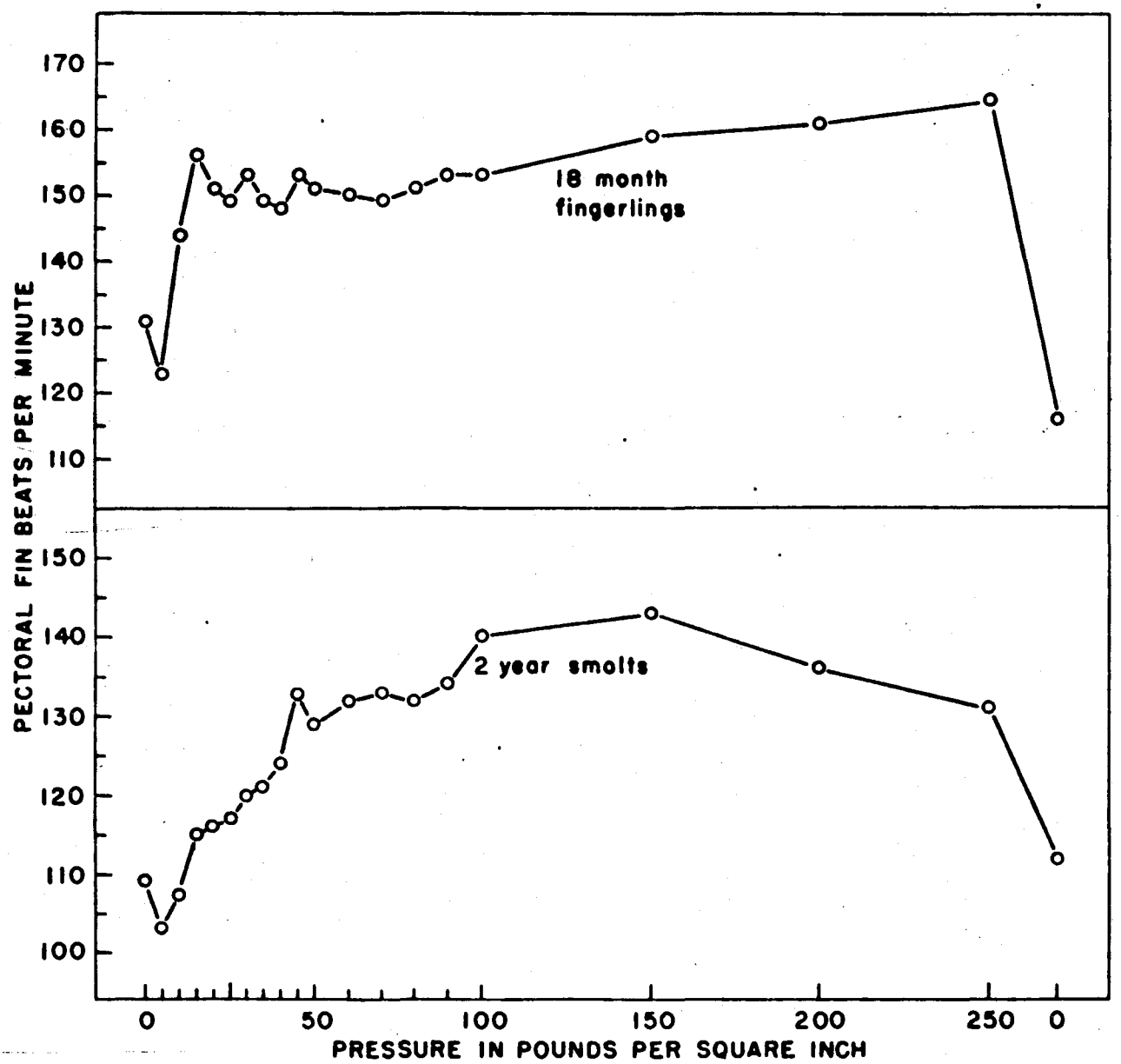

Figure 18. Average pectoral fin rates of eighteen-month and two-year-old sockeye.

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

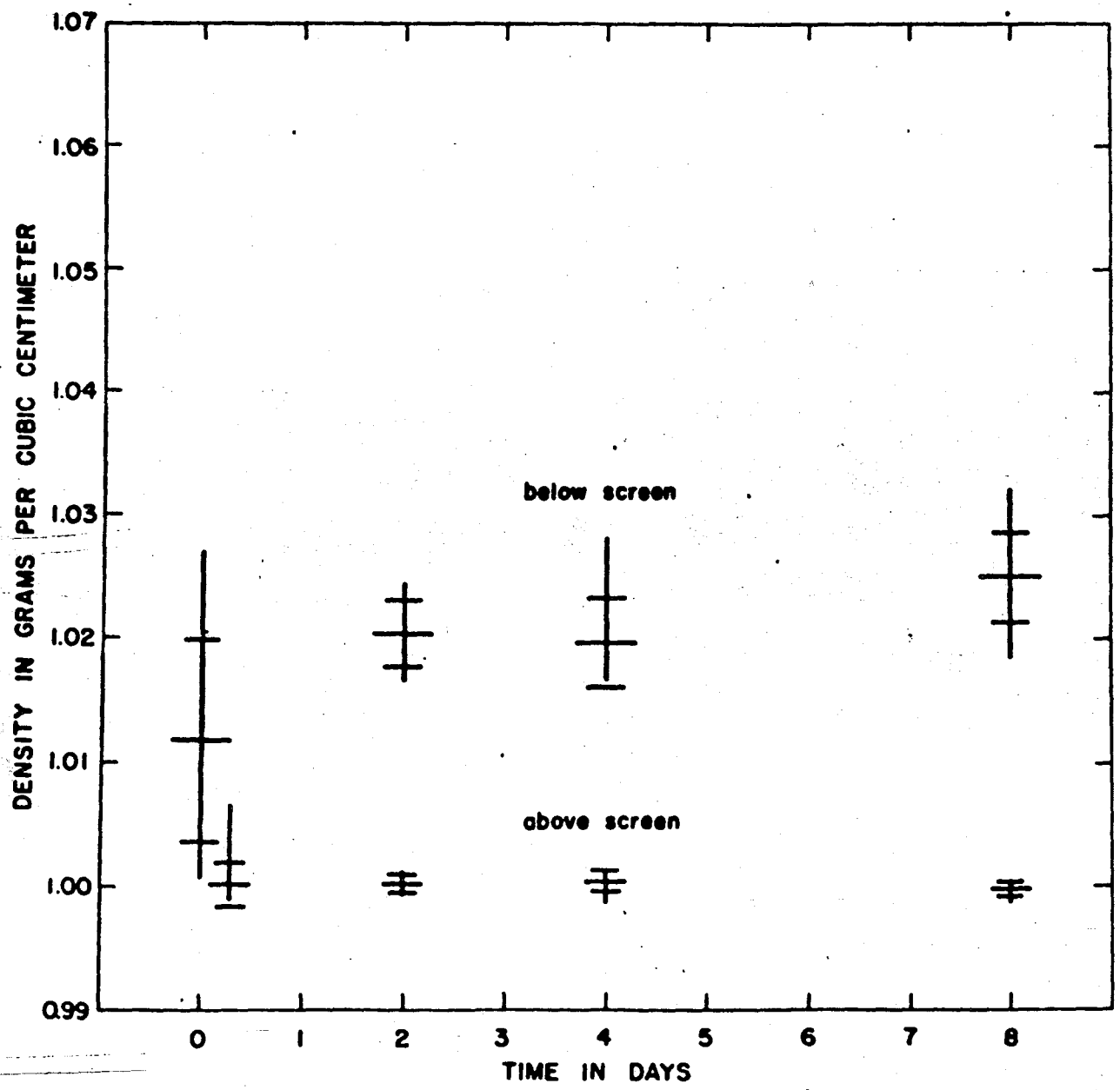

Figure 19. Density of sockeye smolts held above and below screens. Range shown by vertical bar, mean by long horizontal and one standard deviation by short horizontal bars.

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

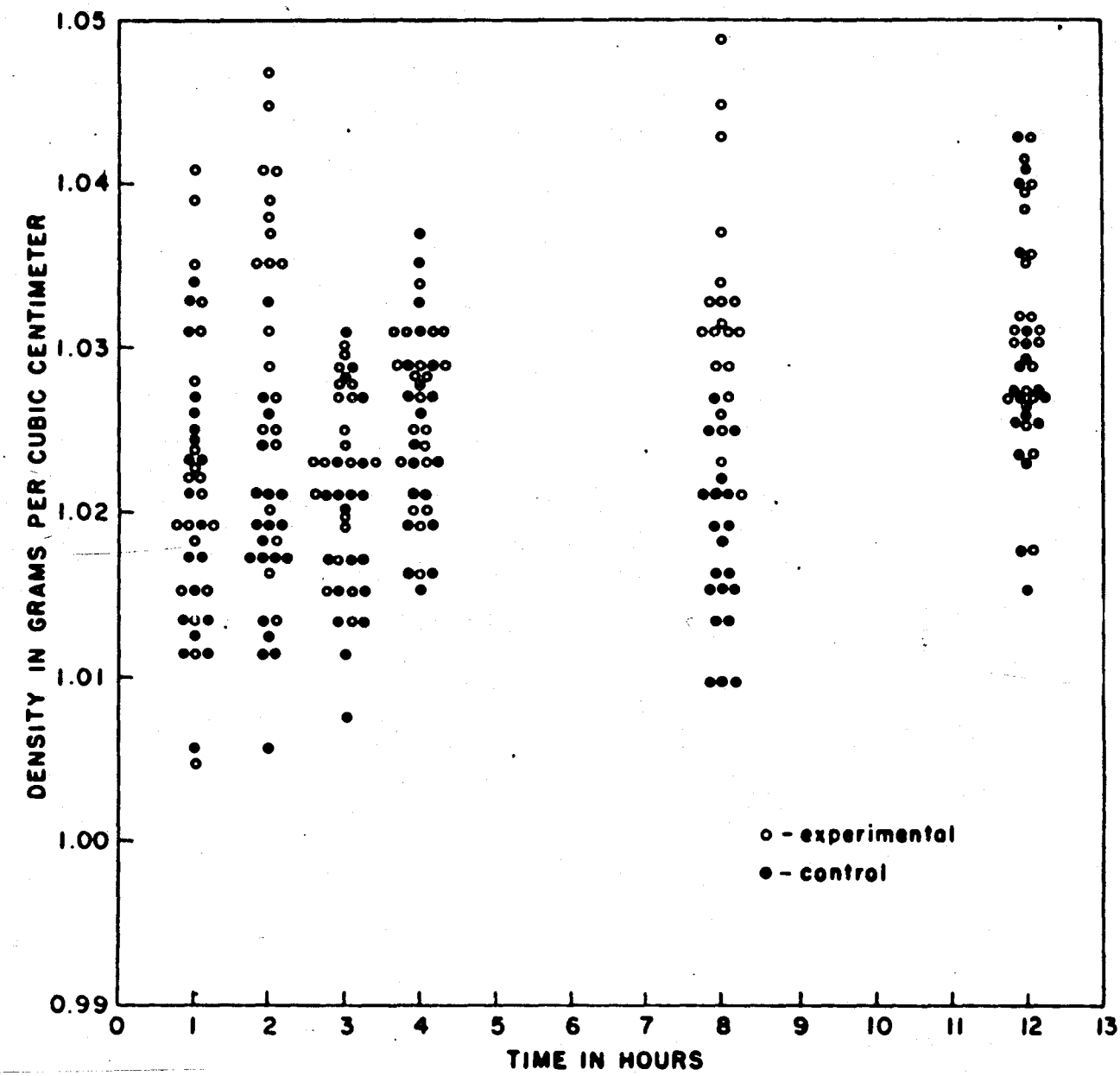

Figure 20. Density of sockeye smolts on sounding. Test fish exposed to 100 mg per liter of ephedrine for up to 12 hours.

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.

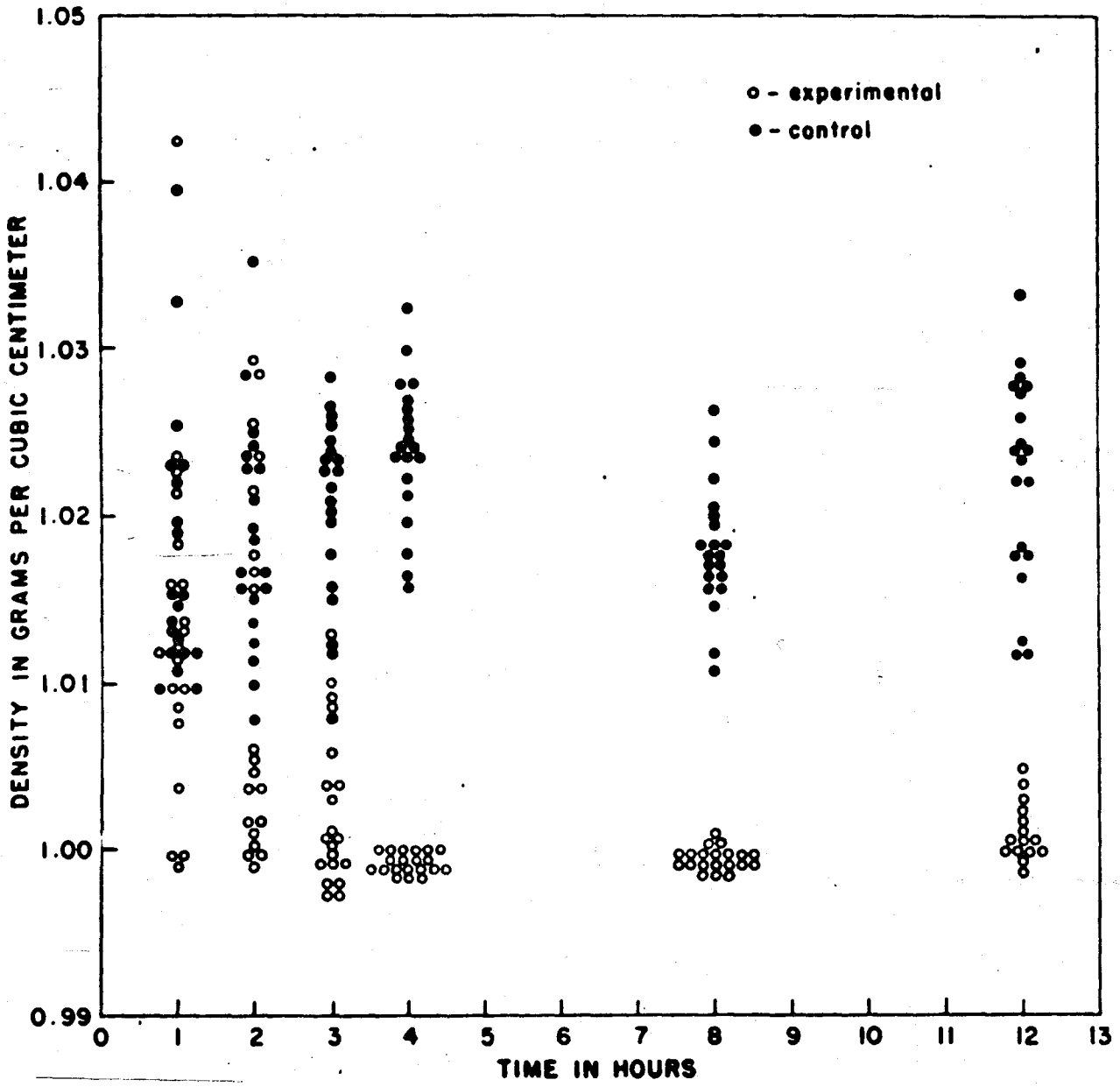

Figure 21. Density of sockeye smolts on sounding. Test fish exposed to 5 mg per liter of "dibenzyline".

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 $t = 5.32$ ($t_{0.0025} = 3.69$).

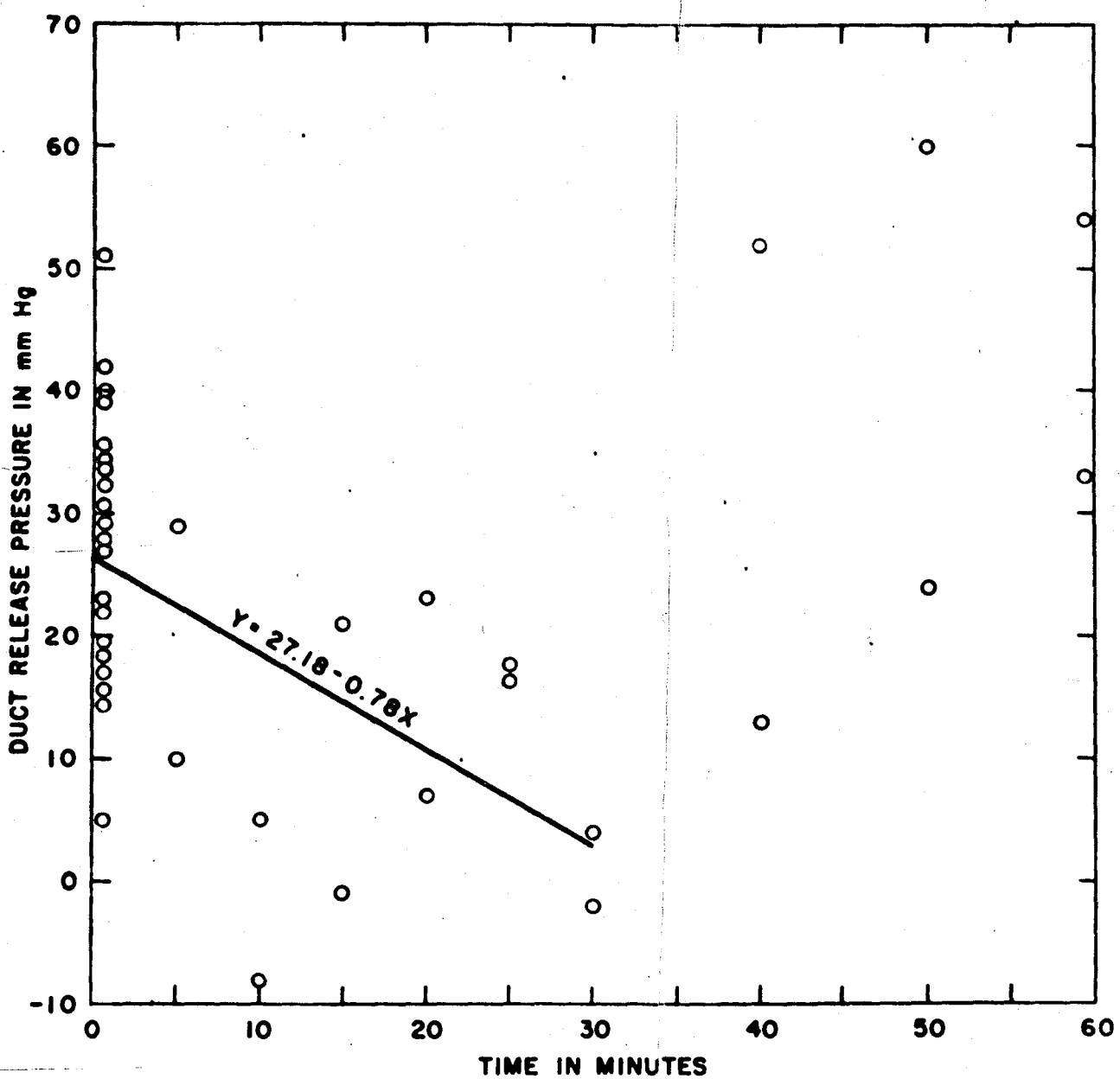

Figure 22. Change in pressure required to force gas out through the pneumatic duct following the death of the animal.

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

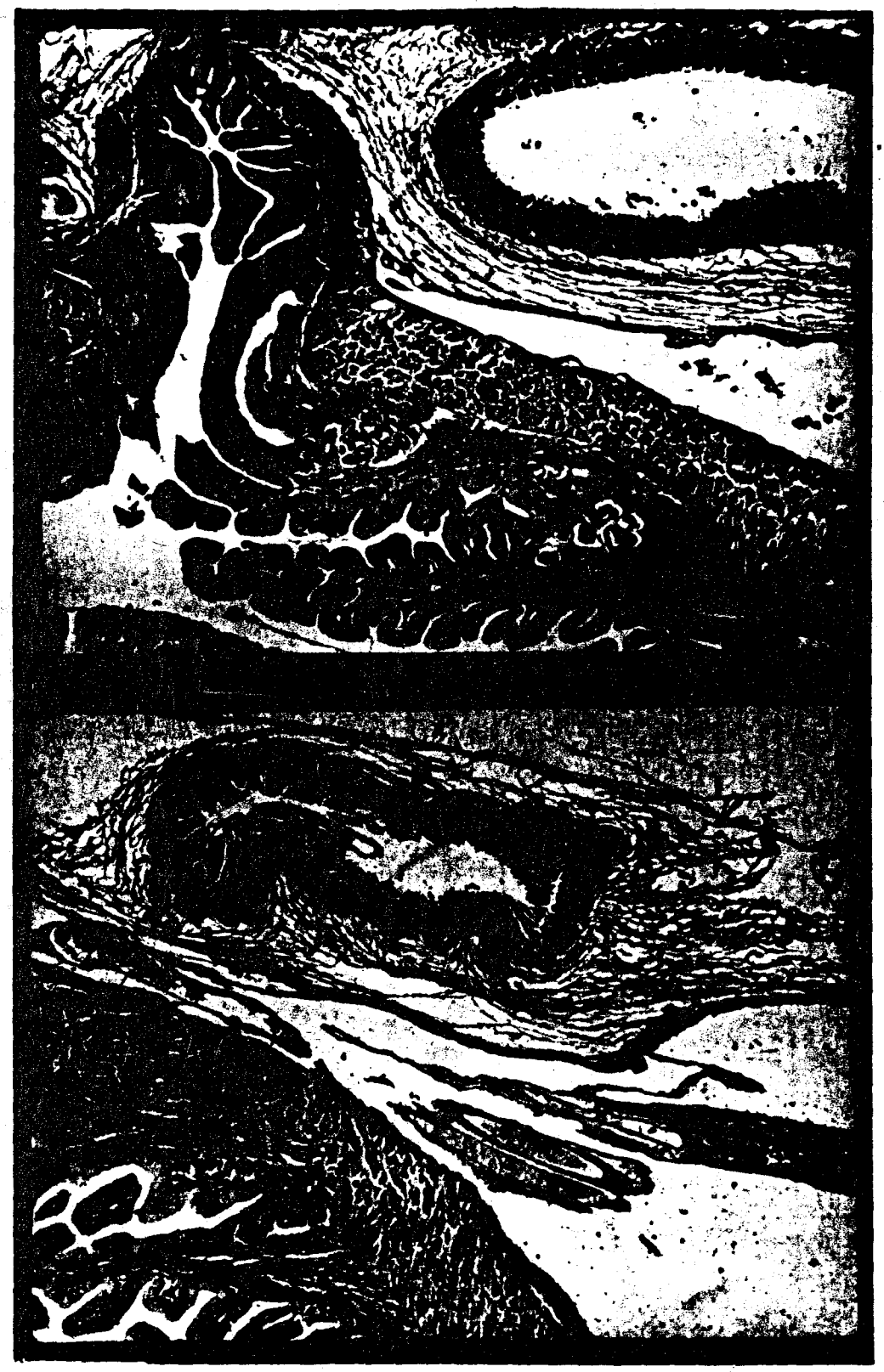

Figure 23. Photomicrographs of the pneumatic duct of the sockeye smolt. Lumen of the duct may be traced from oesophagus through bulbs and thence to anterior end of swimbladder.

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

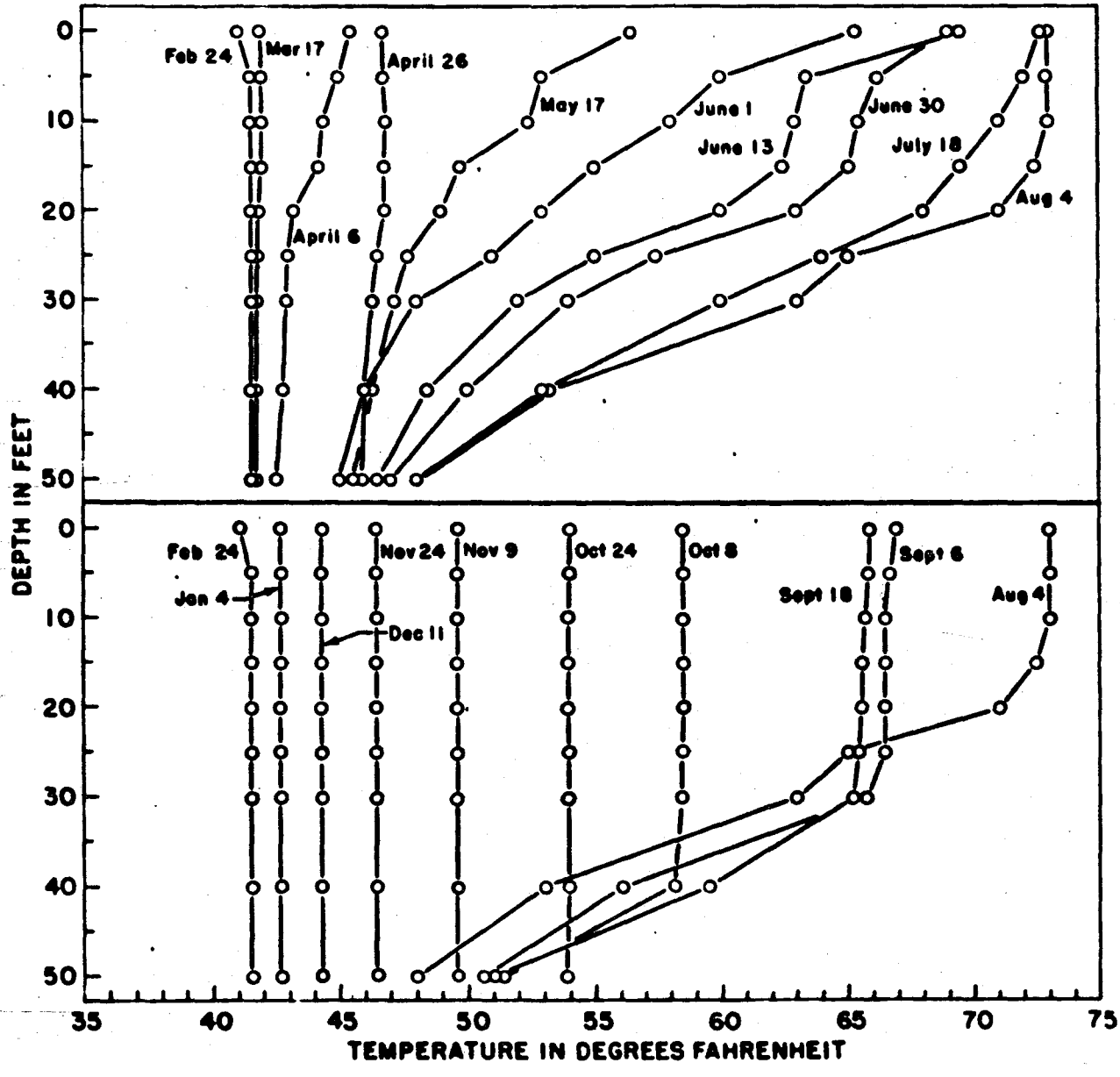

Figure 24. Temperature of the upper 50 feet of Cultus Lake during 1961.

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

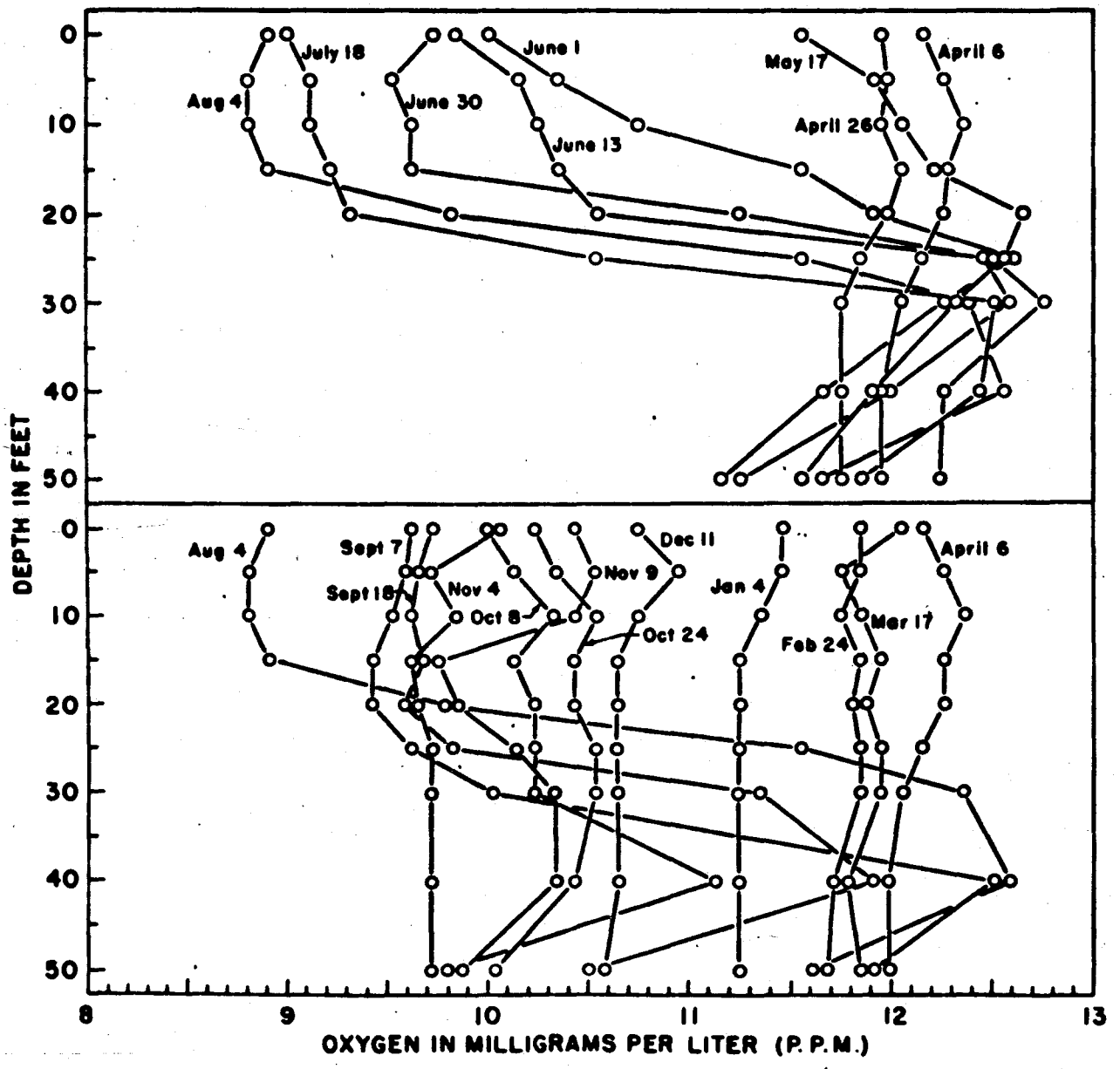

Figure 25. Dissolved oxygen content in milligrams per liter, Cultus Lake, 1961.

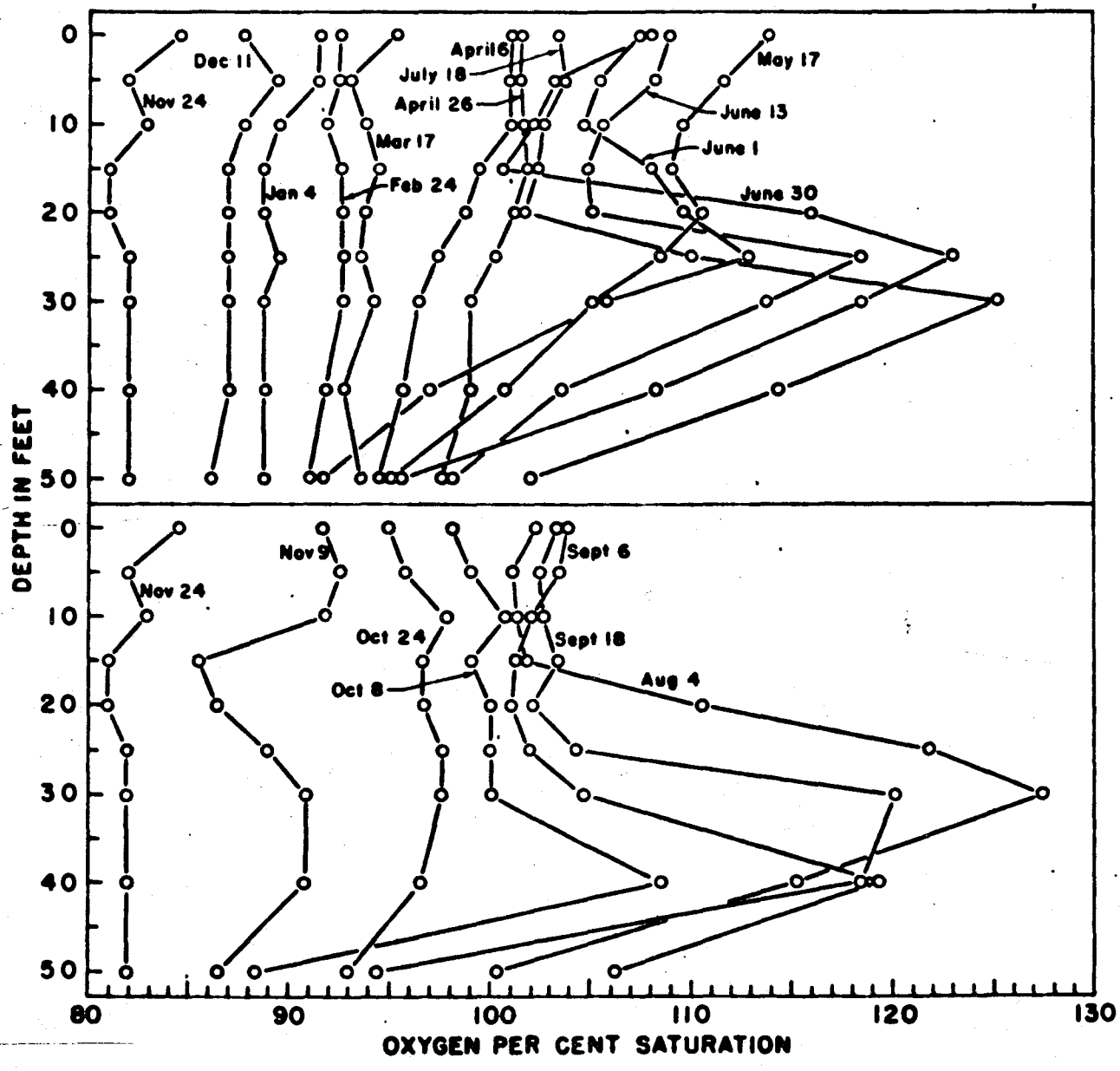

Figure 26. Oxygen content expressed as per cent of air saturation at surface pressure ard temperature in situ.

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

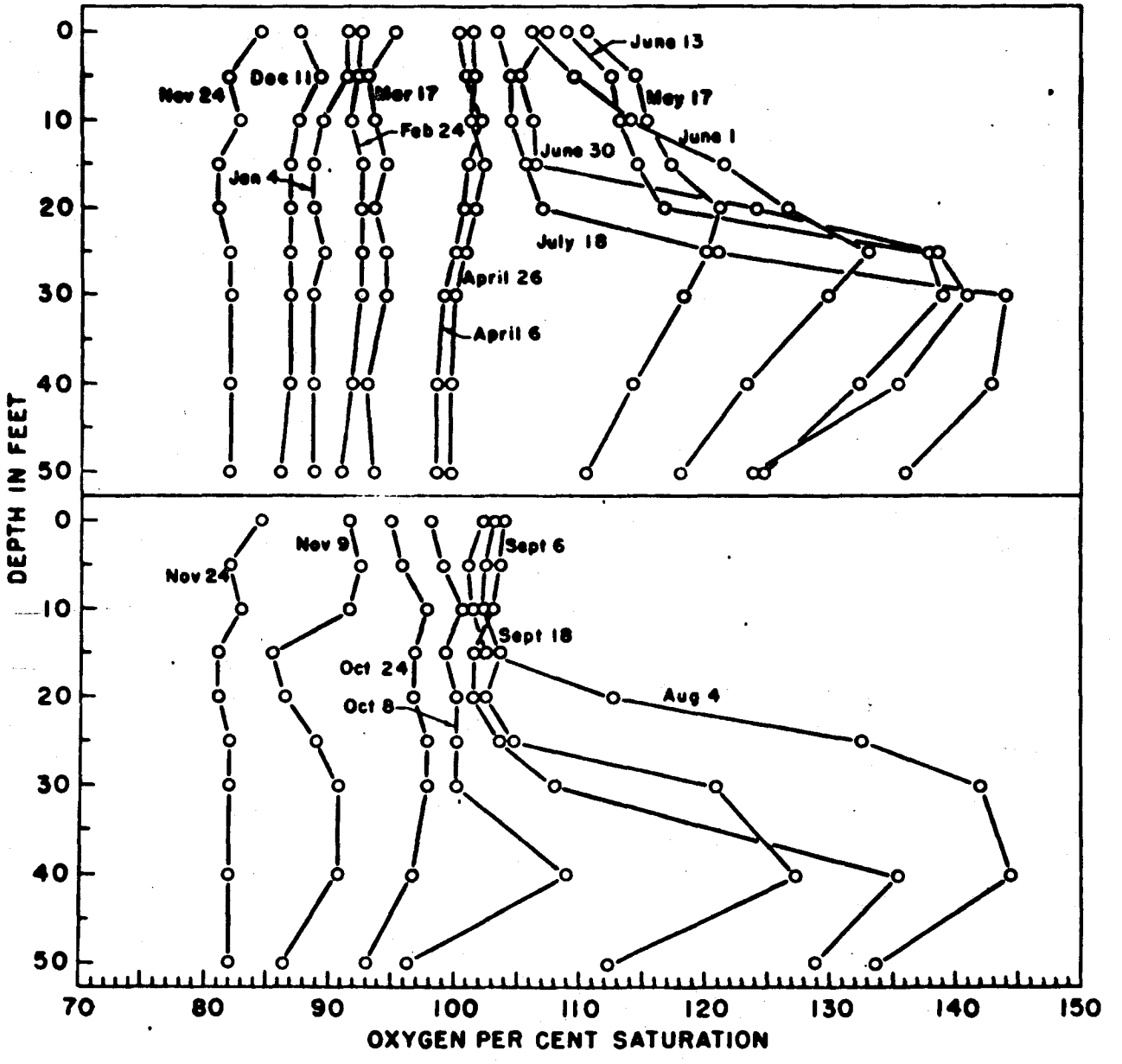

Figure 27. Oxygen expressed as per cent of air saturation at surface temperature and pressure.

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).

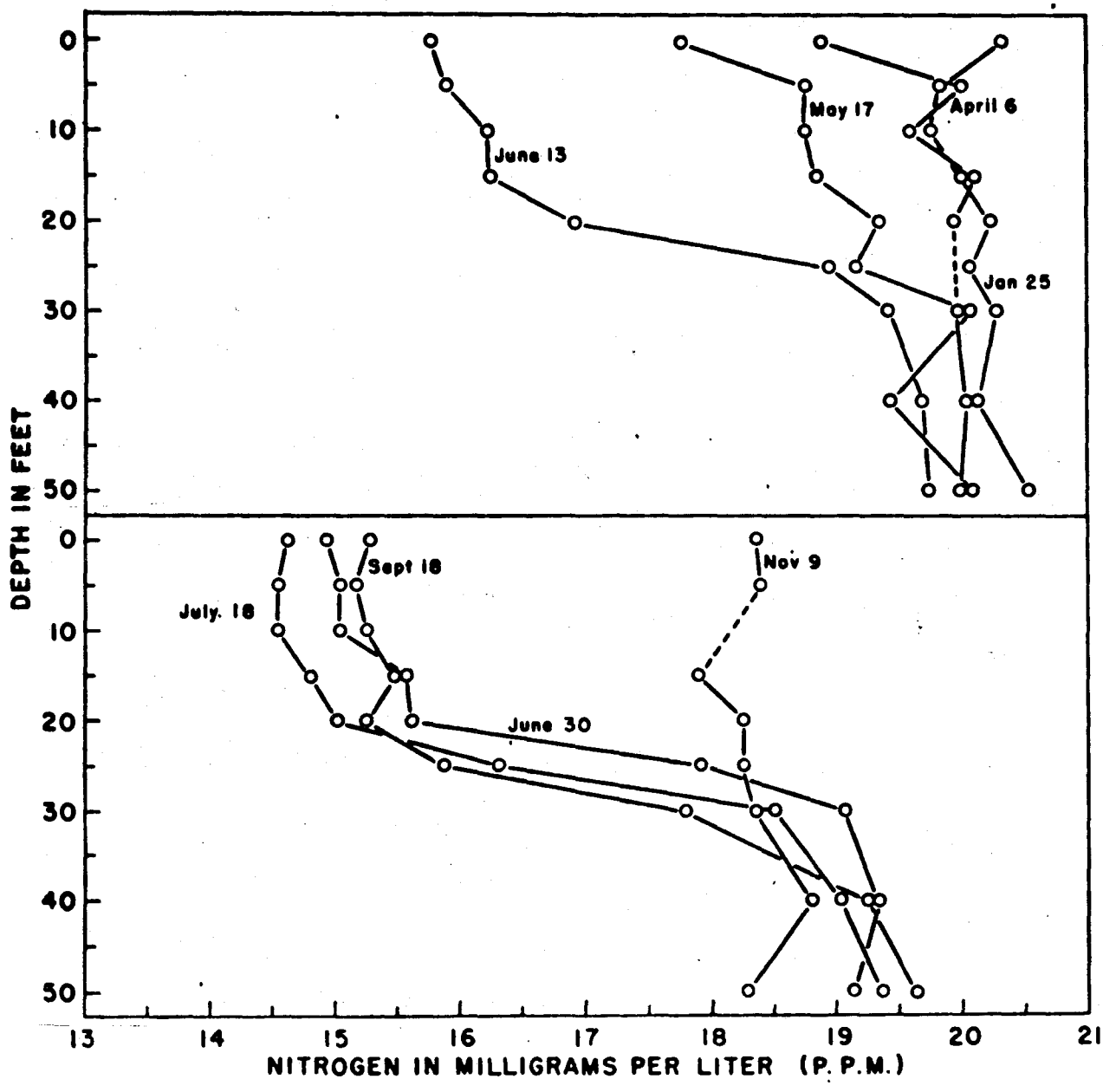

Figure 28. Dissolved nitrogen content in milligrams per liter Cultus Lake, 1961.

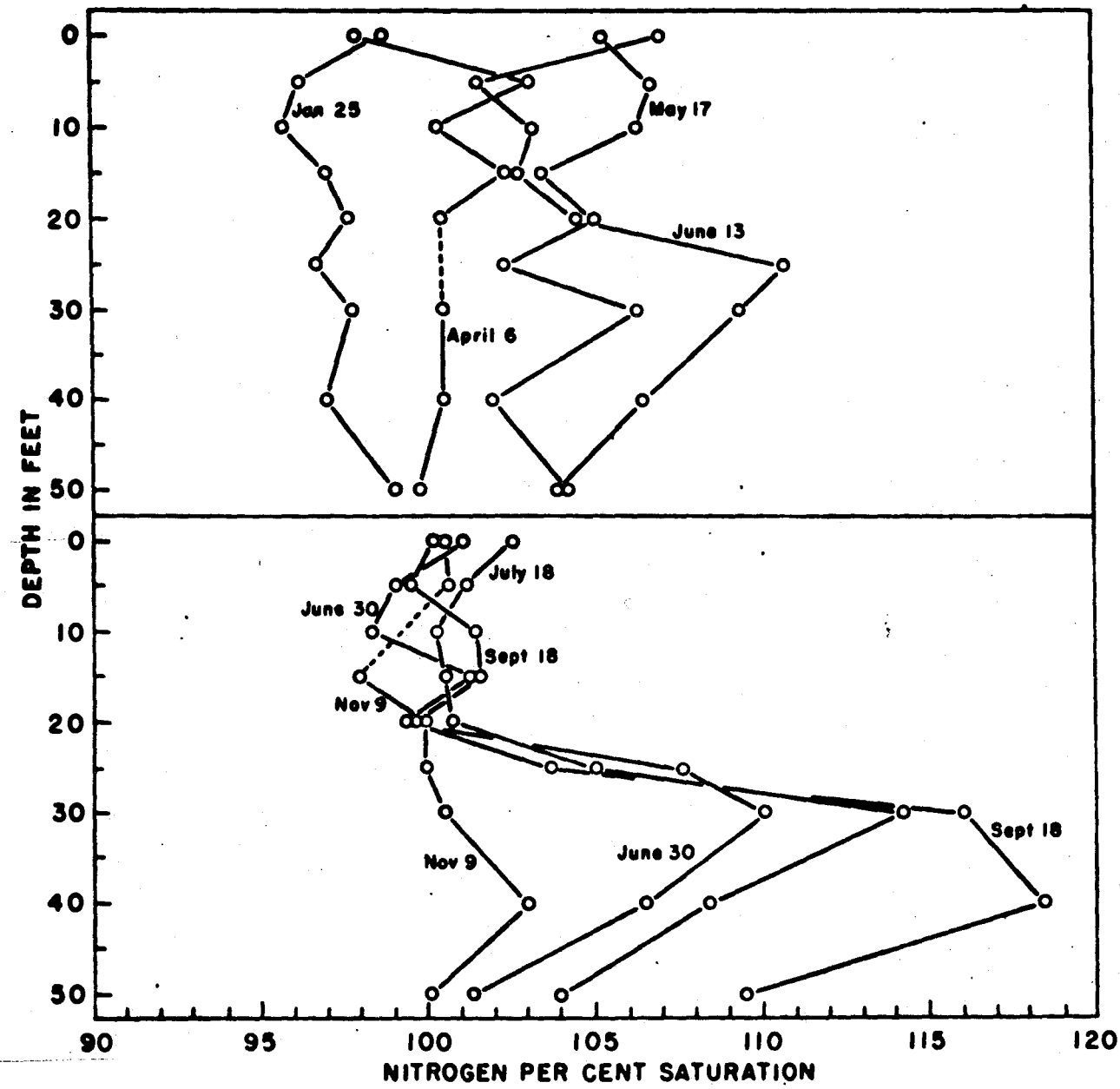

Figure 29. Nitrogen content expressed as per cent of air saturation at surface pressure and temperature in situ.

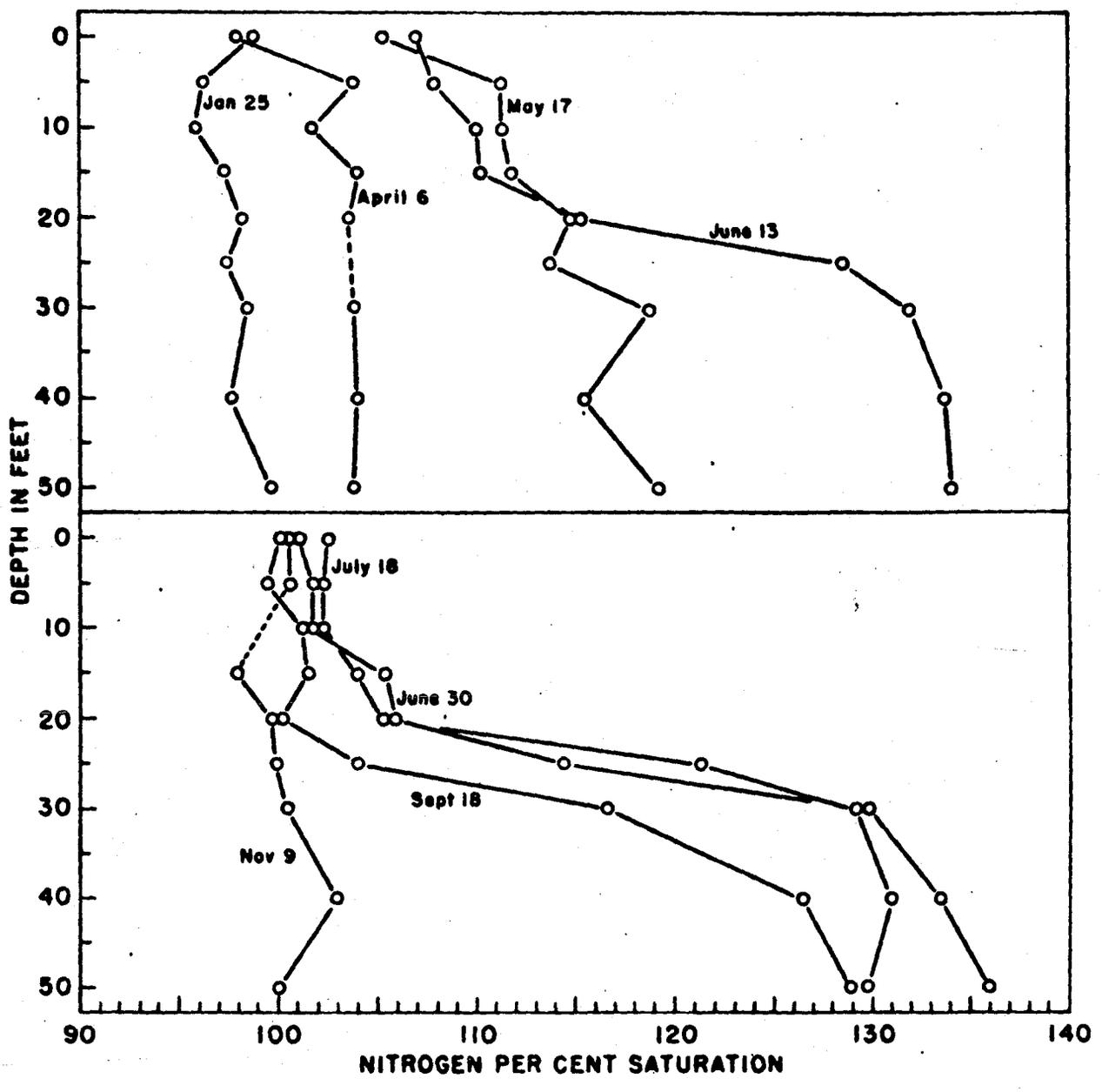

Figure 30. Nitrogen expressed as per cent of air saturation at surface temperature and pressure.

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

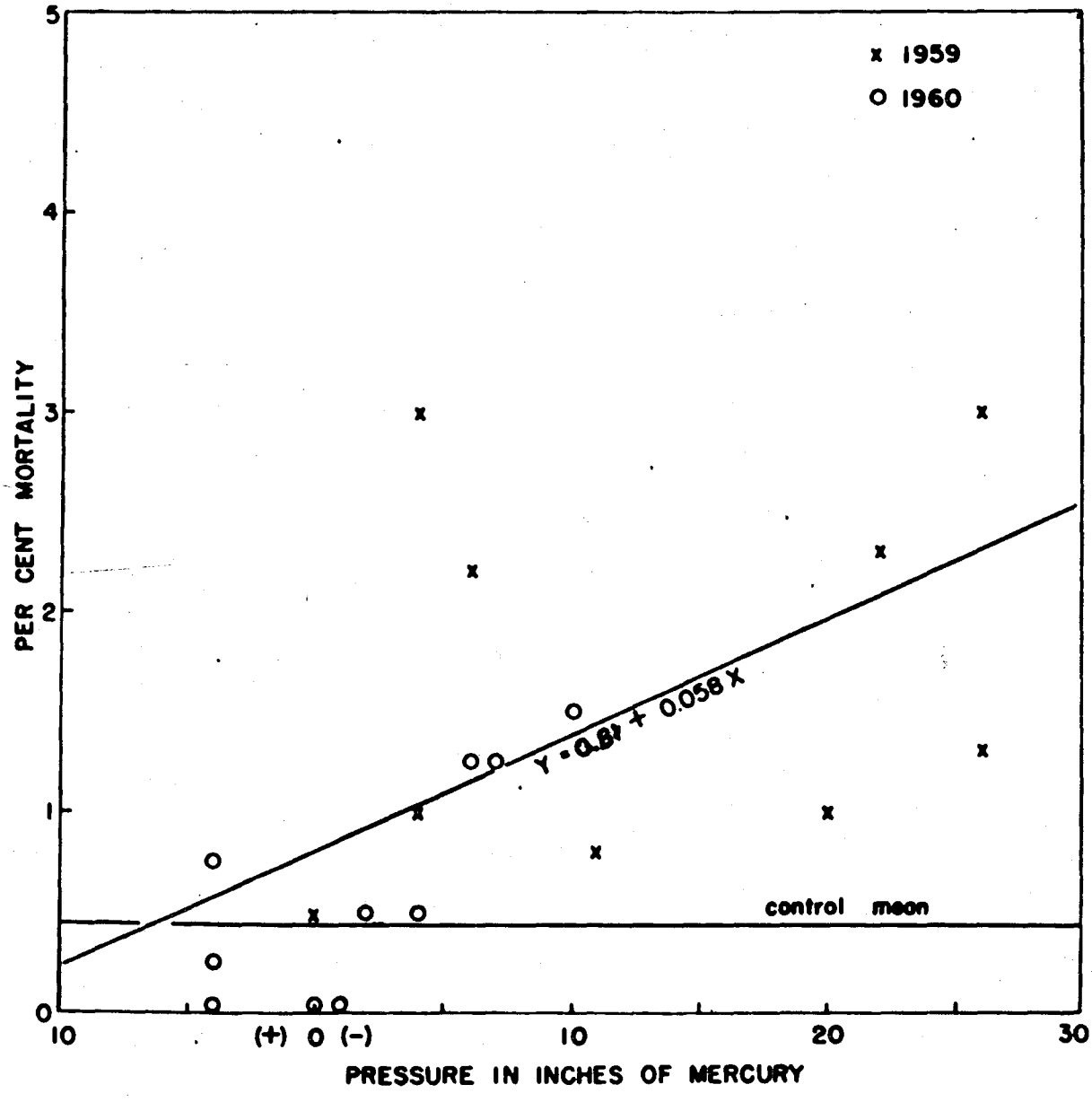

Figure 31. Mortality per week following decompression from 50 psi to positive and negative pressure.

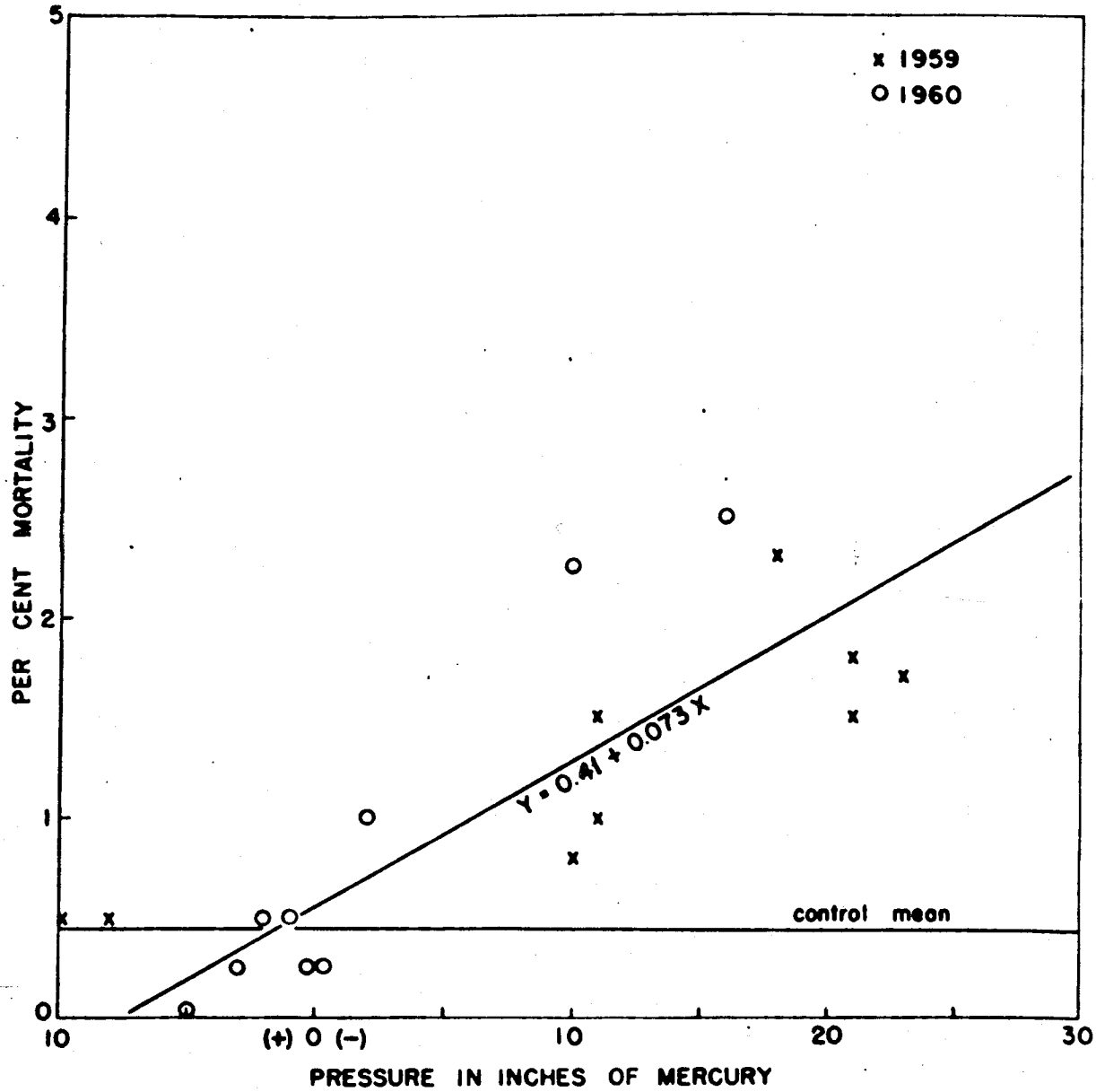

Figure 32. Mortality per week following decompression from 300 psi to positive and negative pressure.

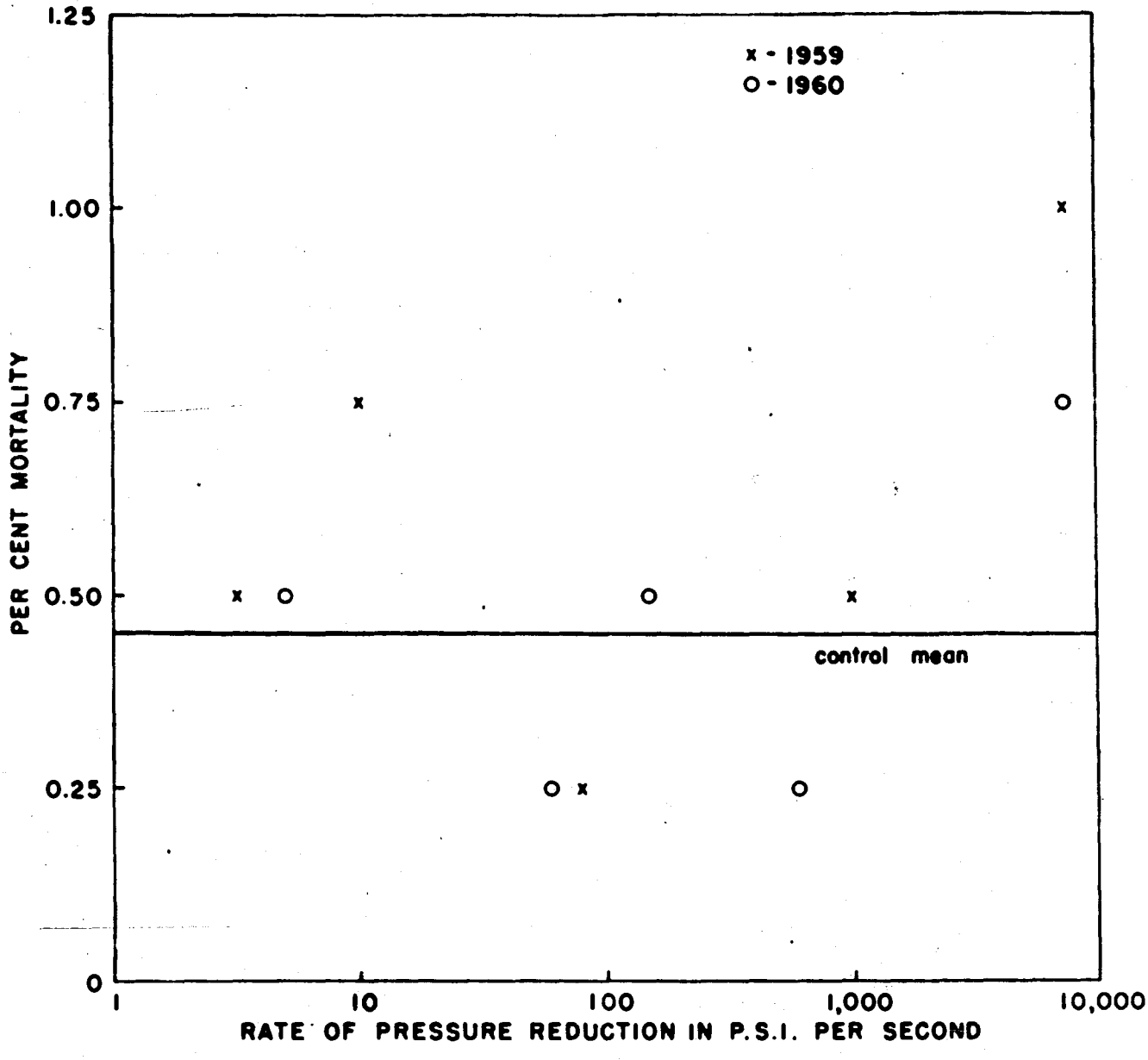

Figure 33. Mortality per week accompanying various rates of reduction of pressure.

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.

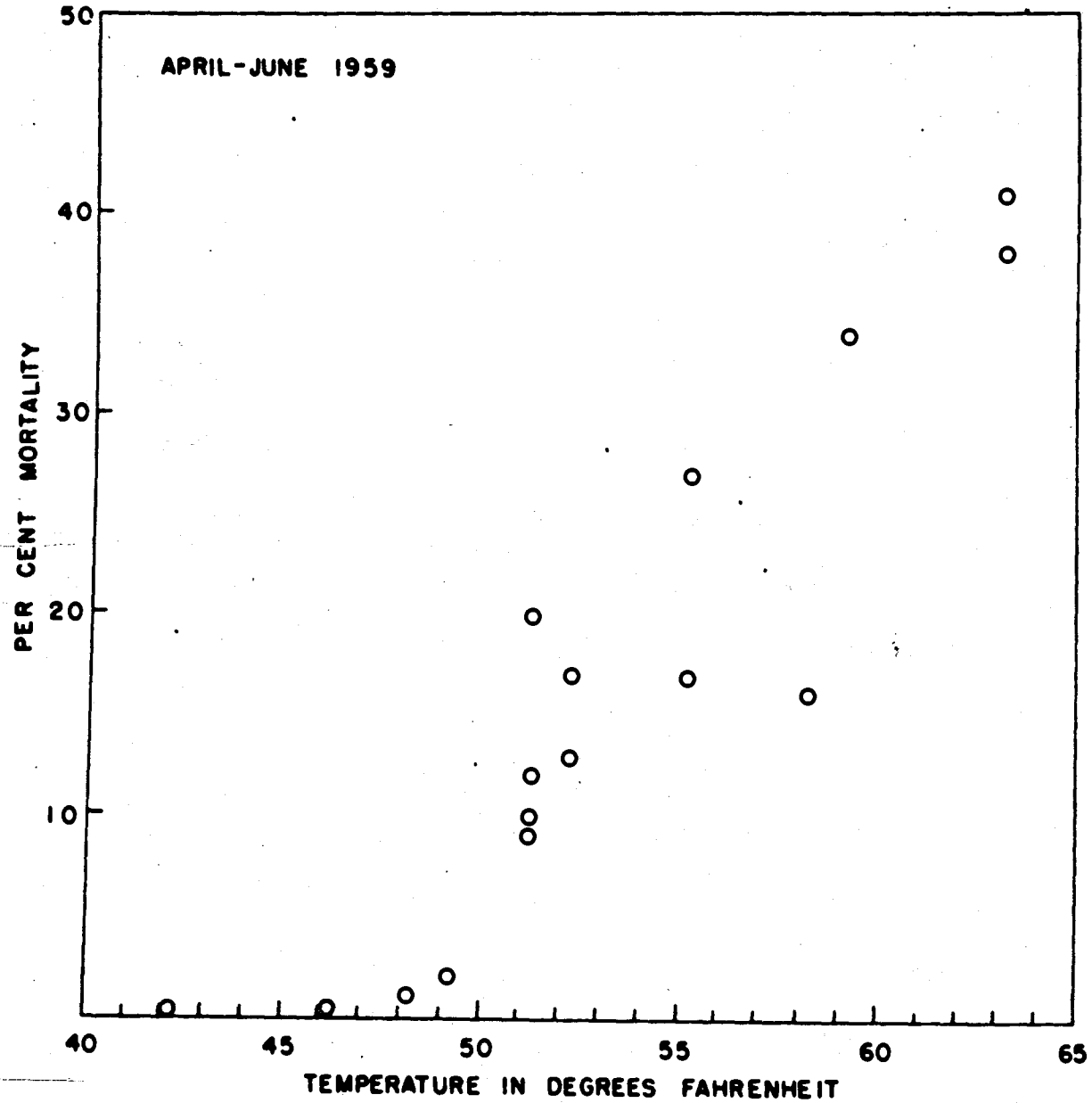

Figure 34. Mortality of sockeye smolts tested at various temperatures during the course of the migration 1959.

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

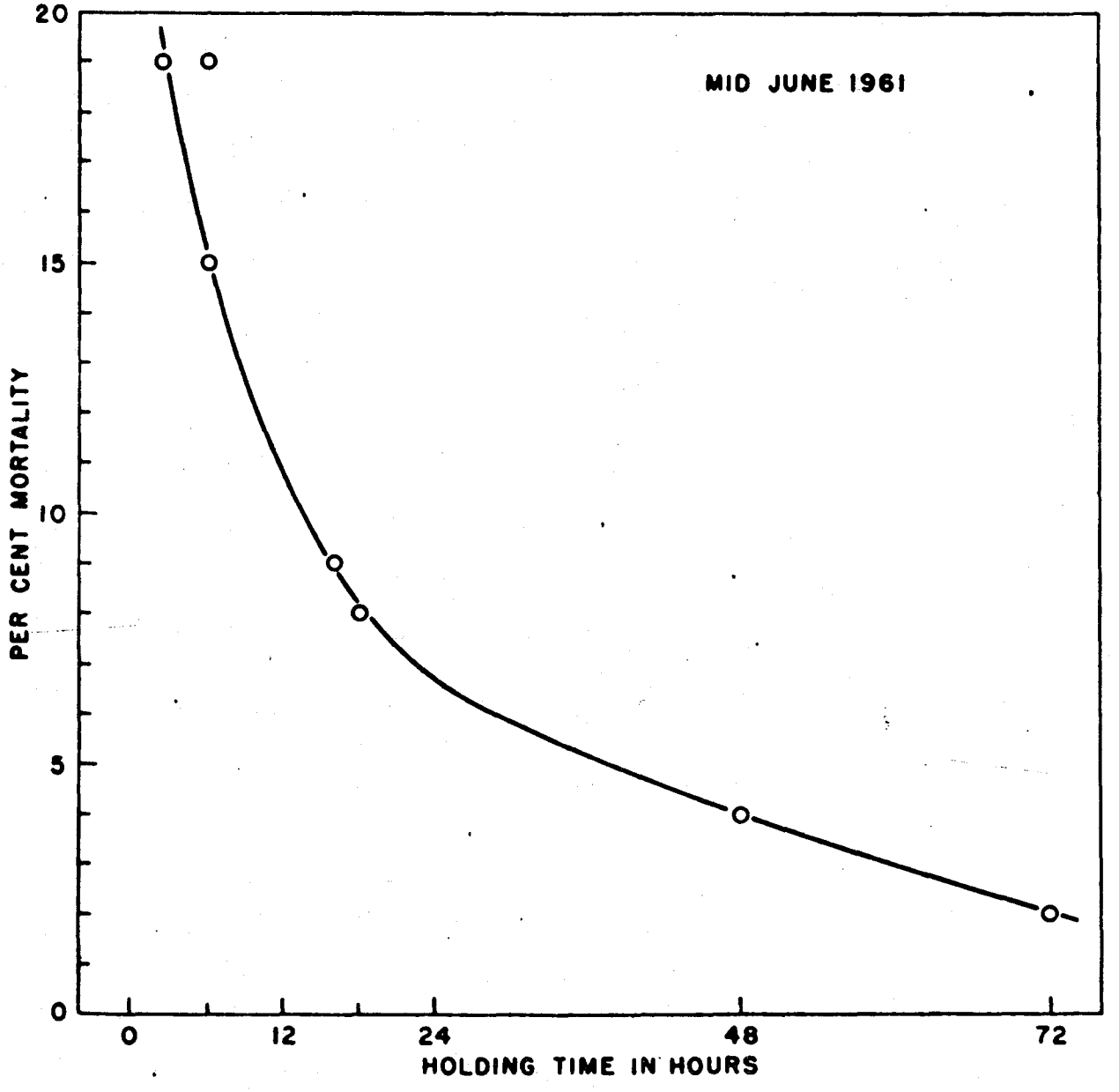

Figure 35. Decline in mortality among groups of smolts held up to 72 hours in Sweltzer Creek before testing,

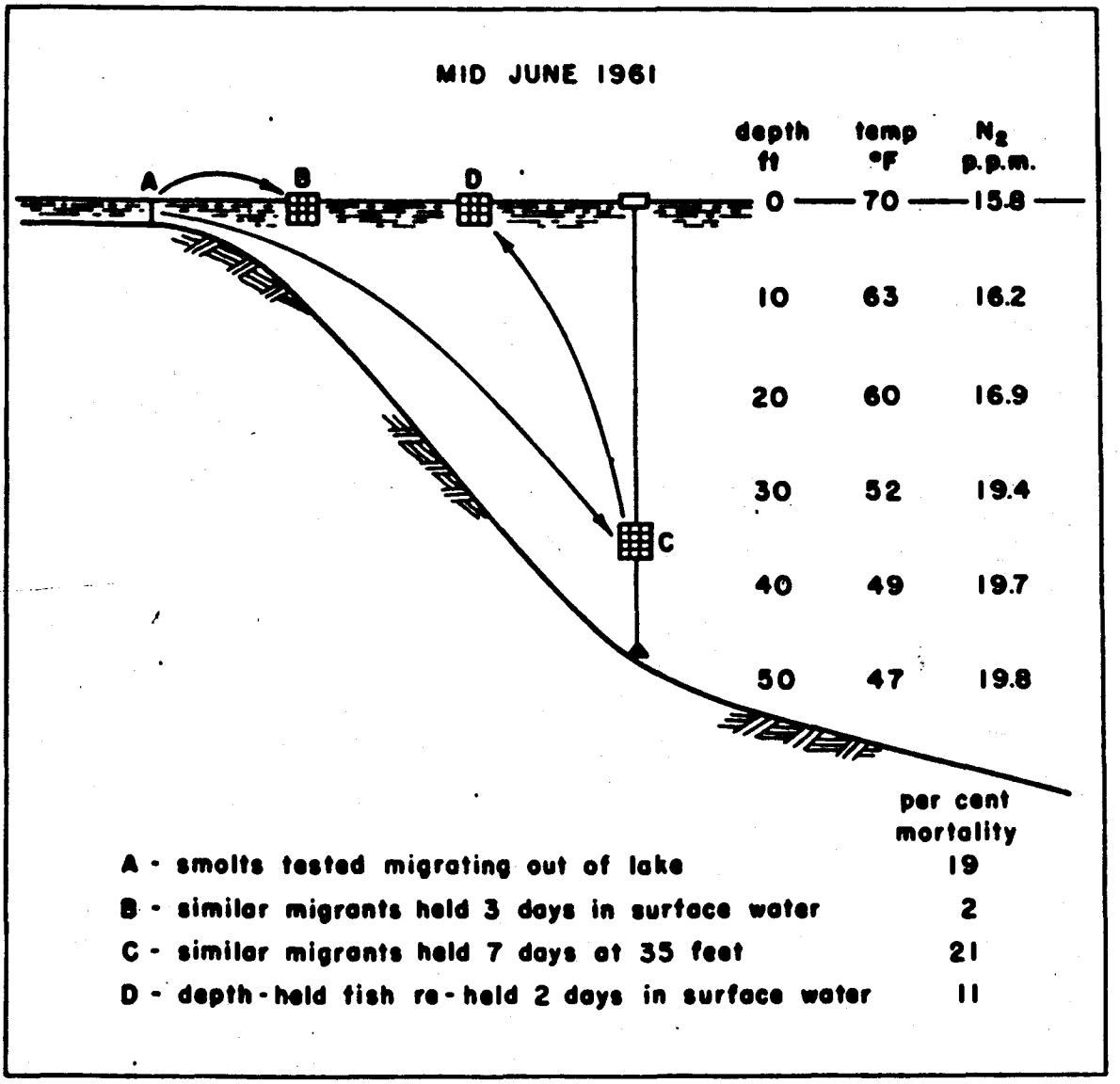

Figure 36. Mortality among smolts accompanying residence in surface and thermocline waters.

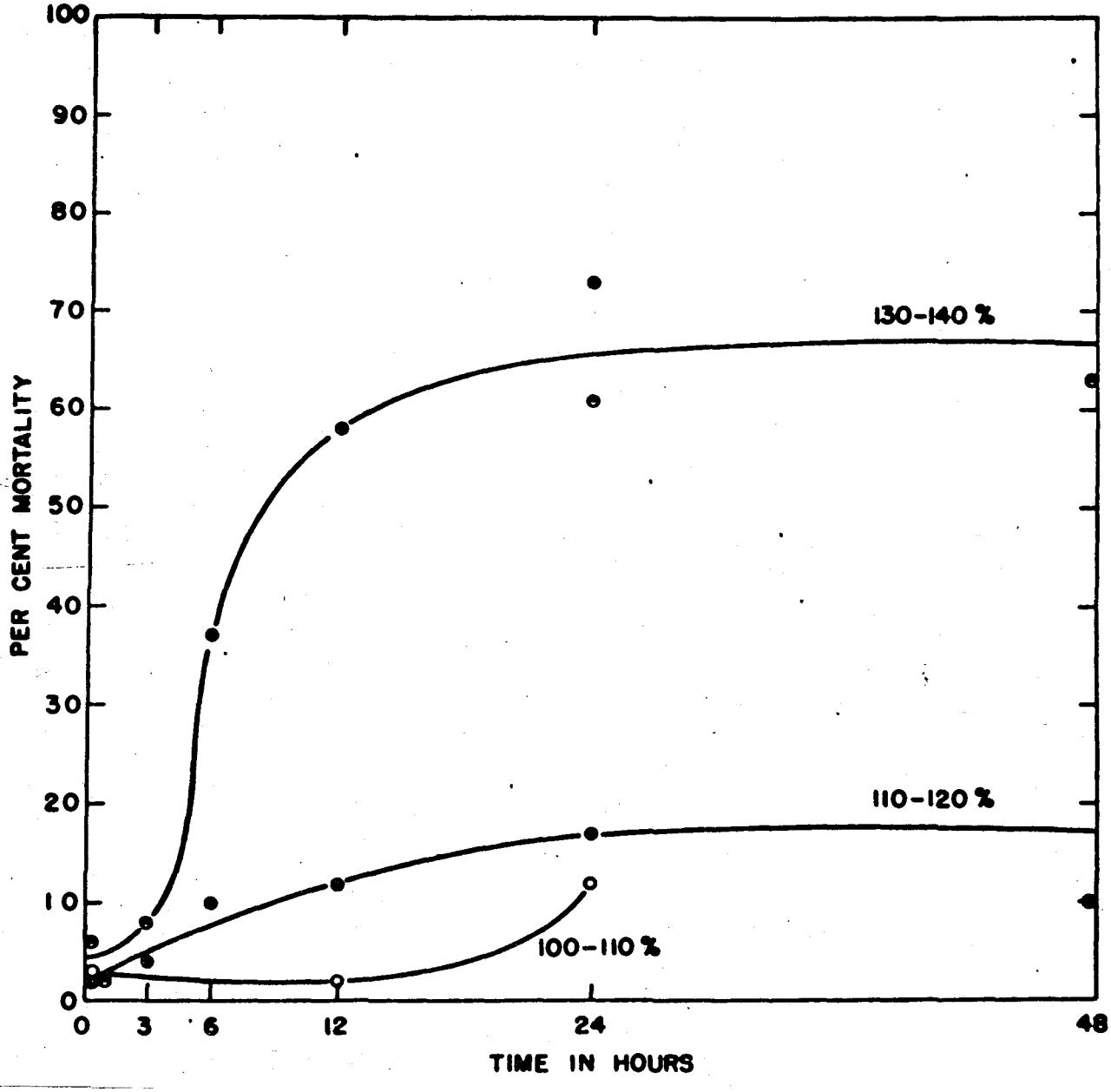

Figure 37. Mortality among smolts following exposure to water of increased air content. Gas tensions expressed as per cent of saturation at atmospheric pressure.

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.

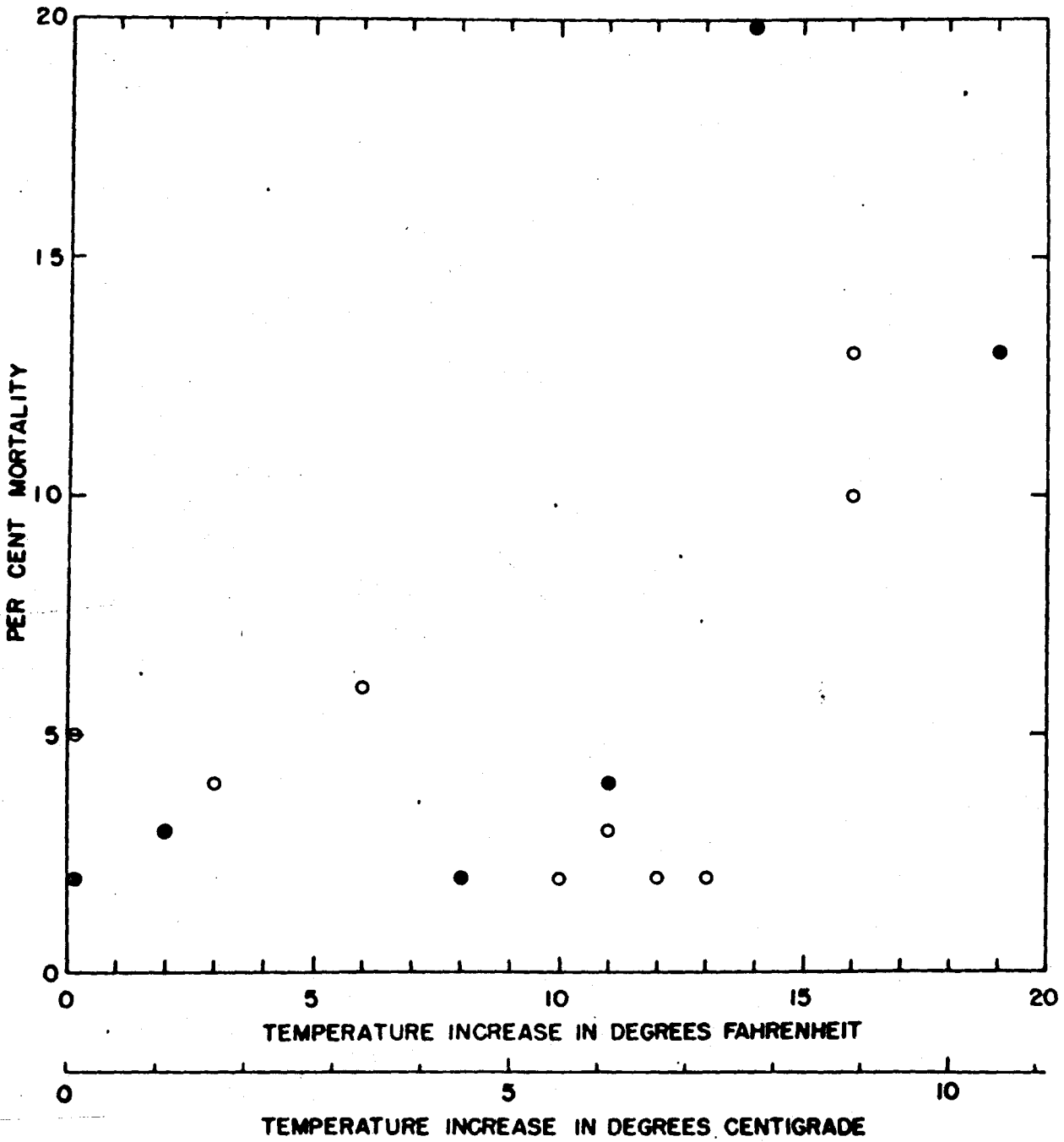

Figure 38. Mortality on decompression following sudden increase in temperature. Open circles increments above 45°F, solid circles above 55°F.

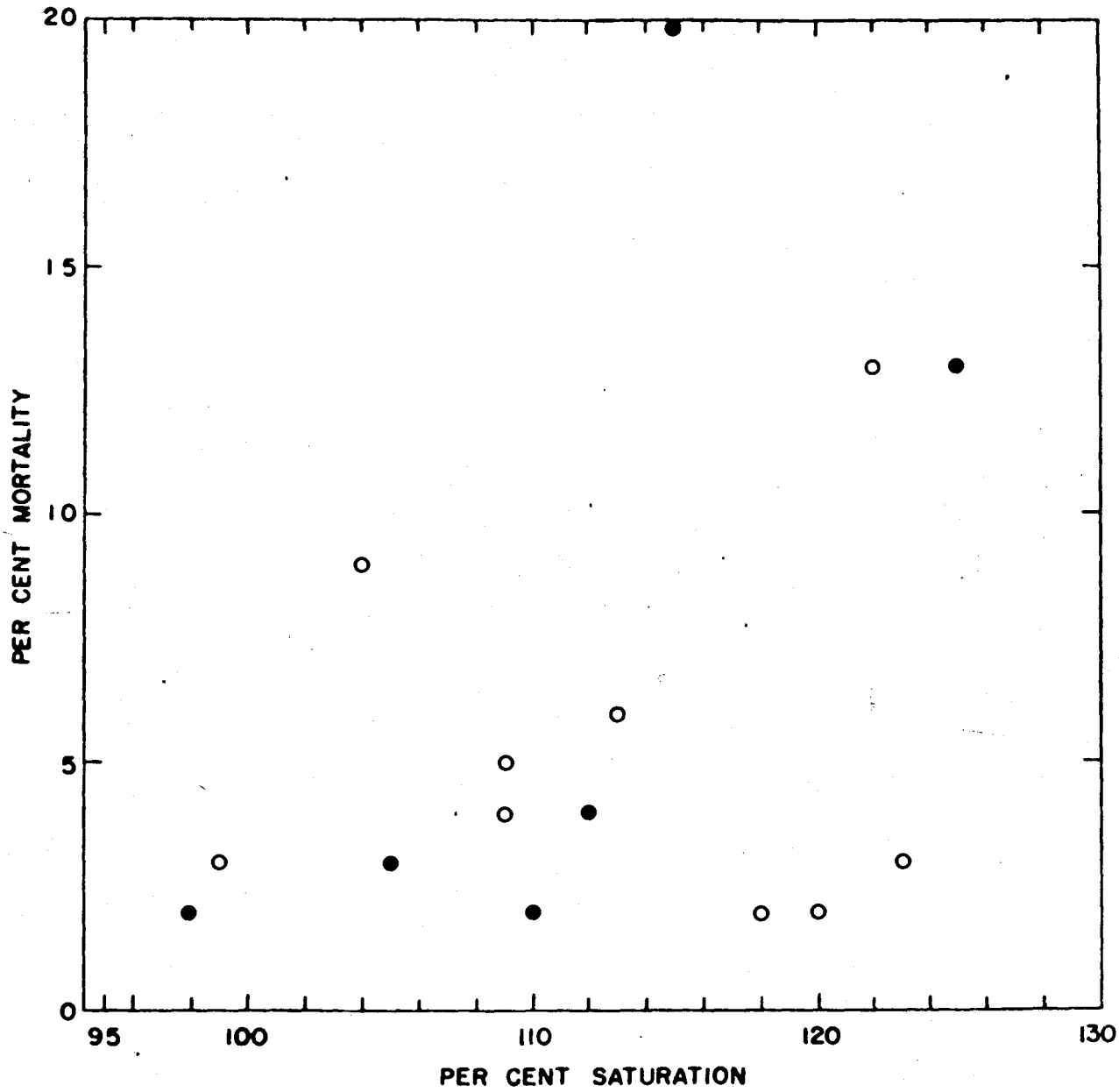

Figure 39. Temperature increases expressed as increases in per cent saturation.

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

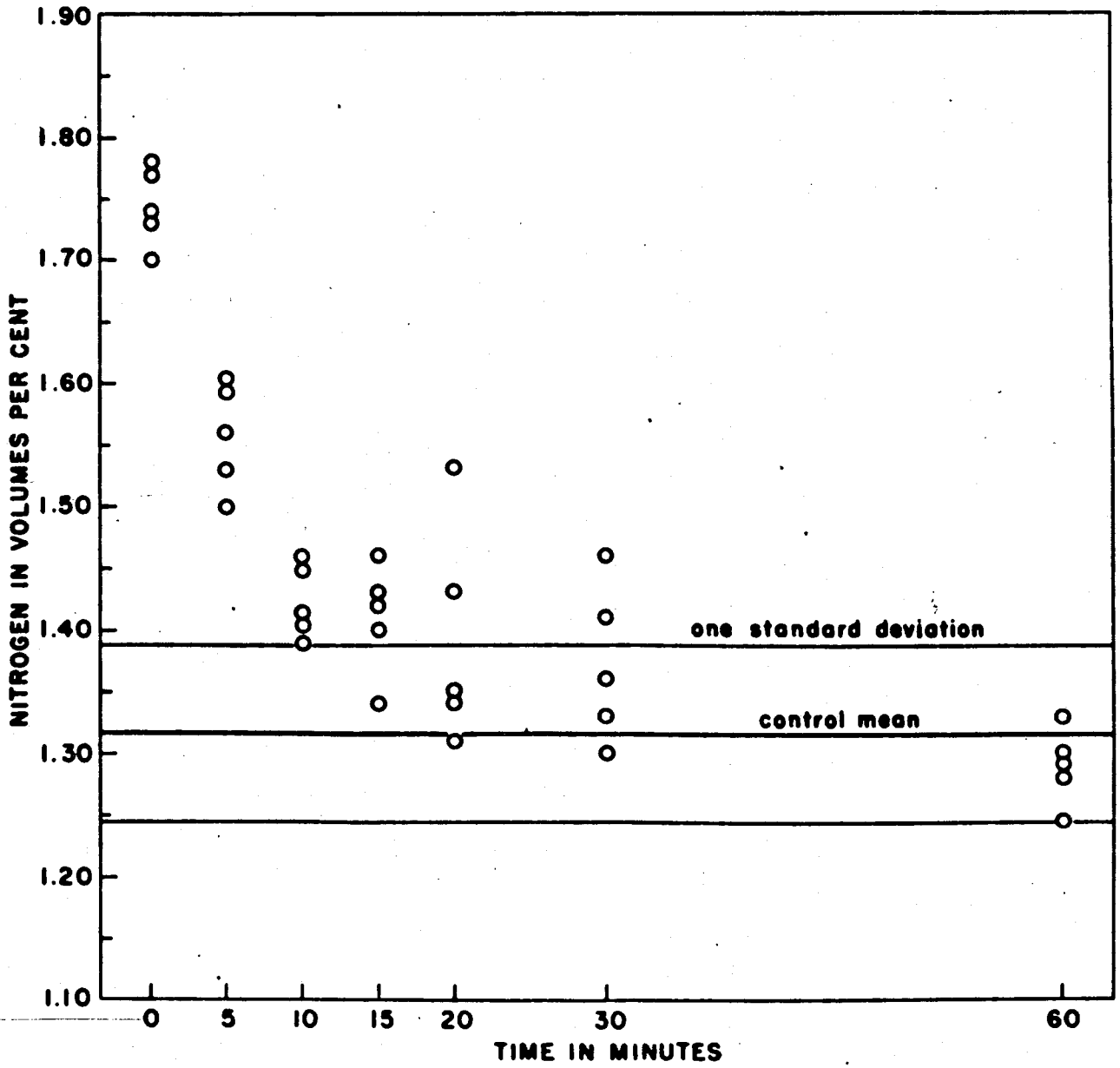

Figure 40. Decrease in nitrogen in venous blood following transfer of fish from water of 17.5 ml per liter nitrogen to 13.0 ml per liter.

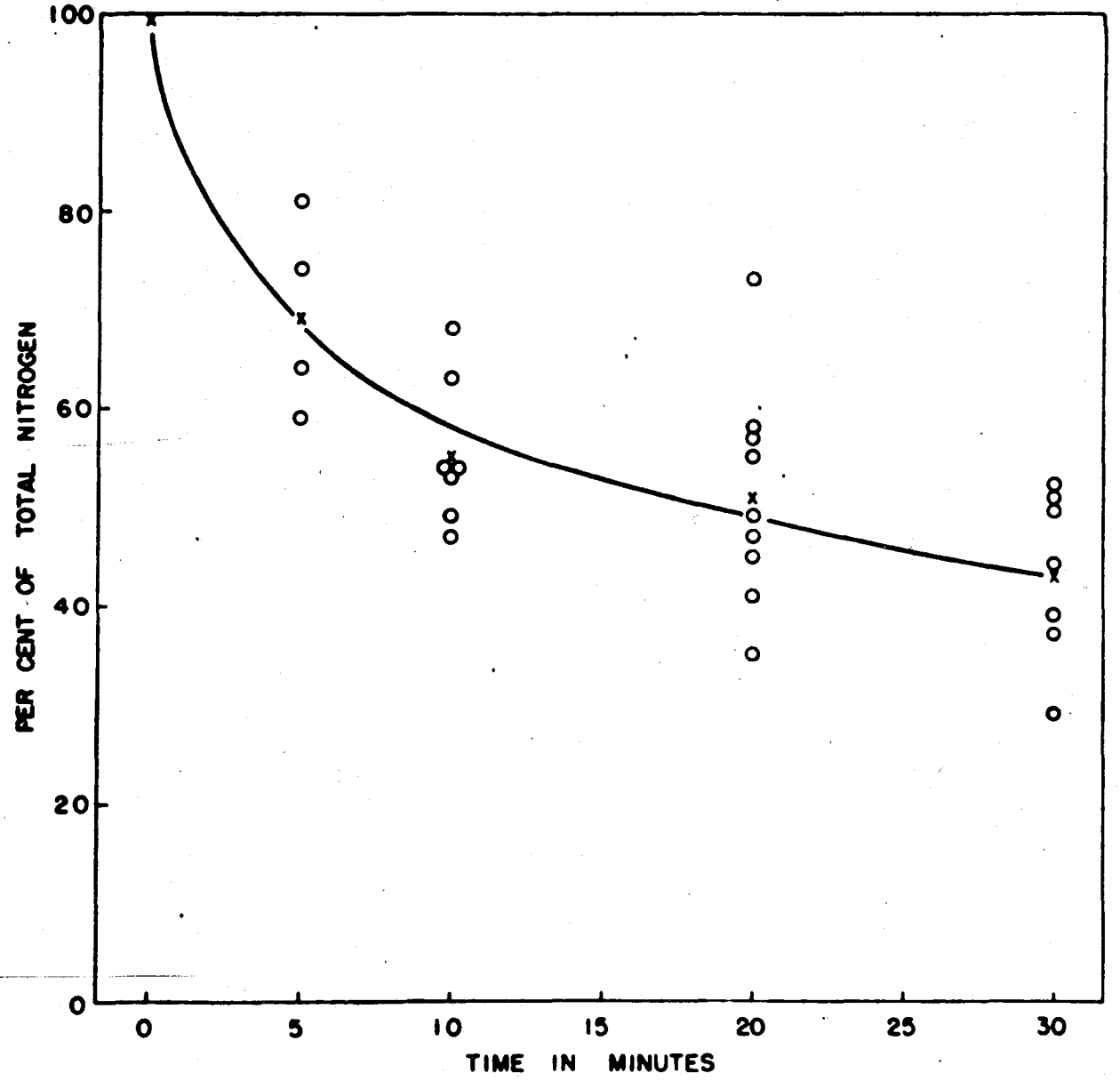

Figure 41. Per cent of excess nitrogen retained by fish following transfer from water of 14.4 to 1.6 ml per liter nitrogen.

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

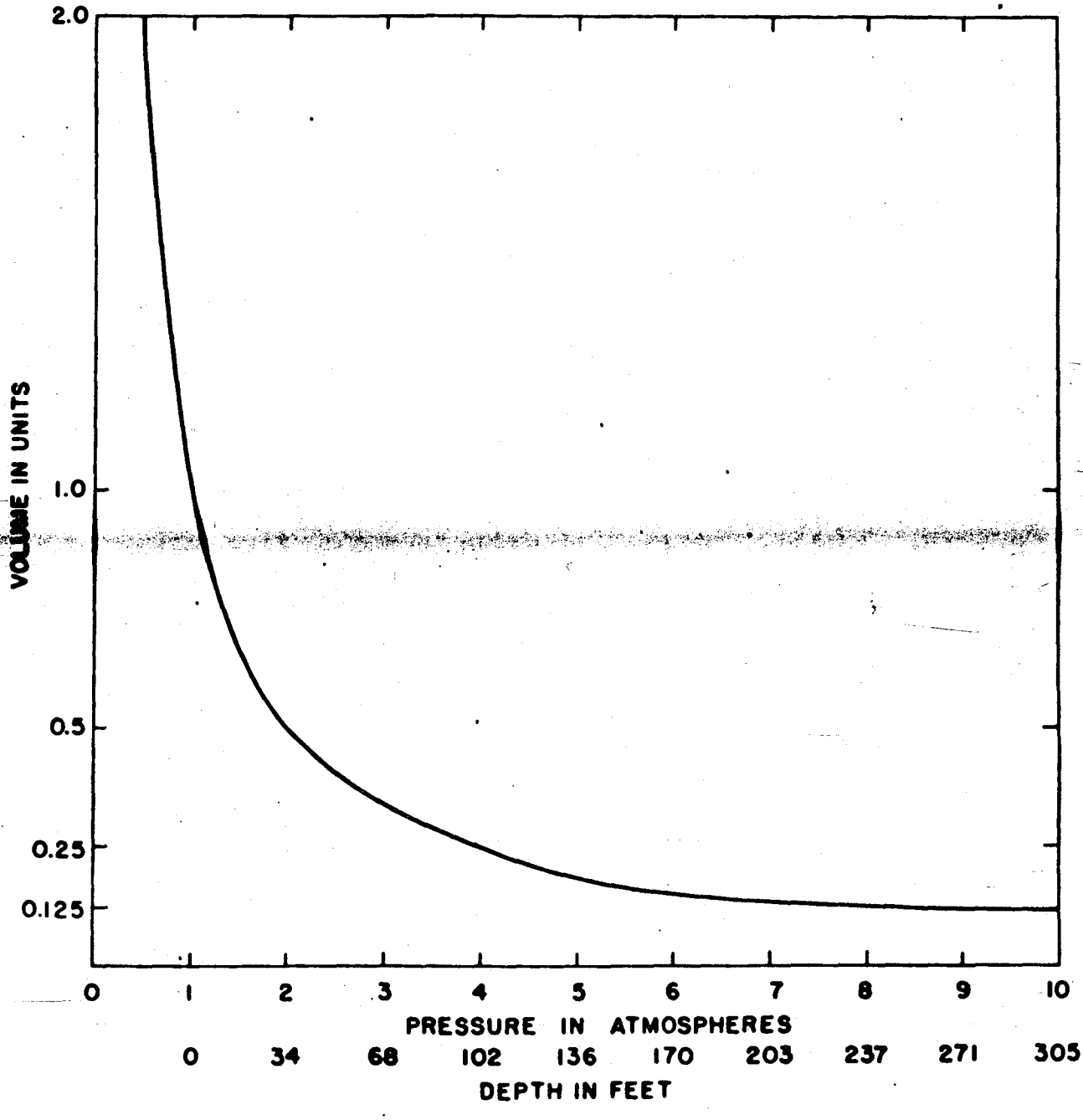

Figure 42. Change in unit volume of gas in relation to depth and pressure.

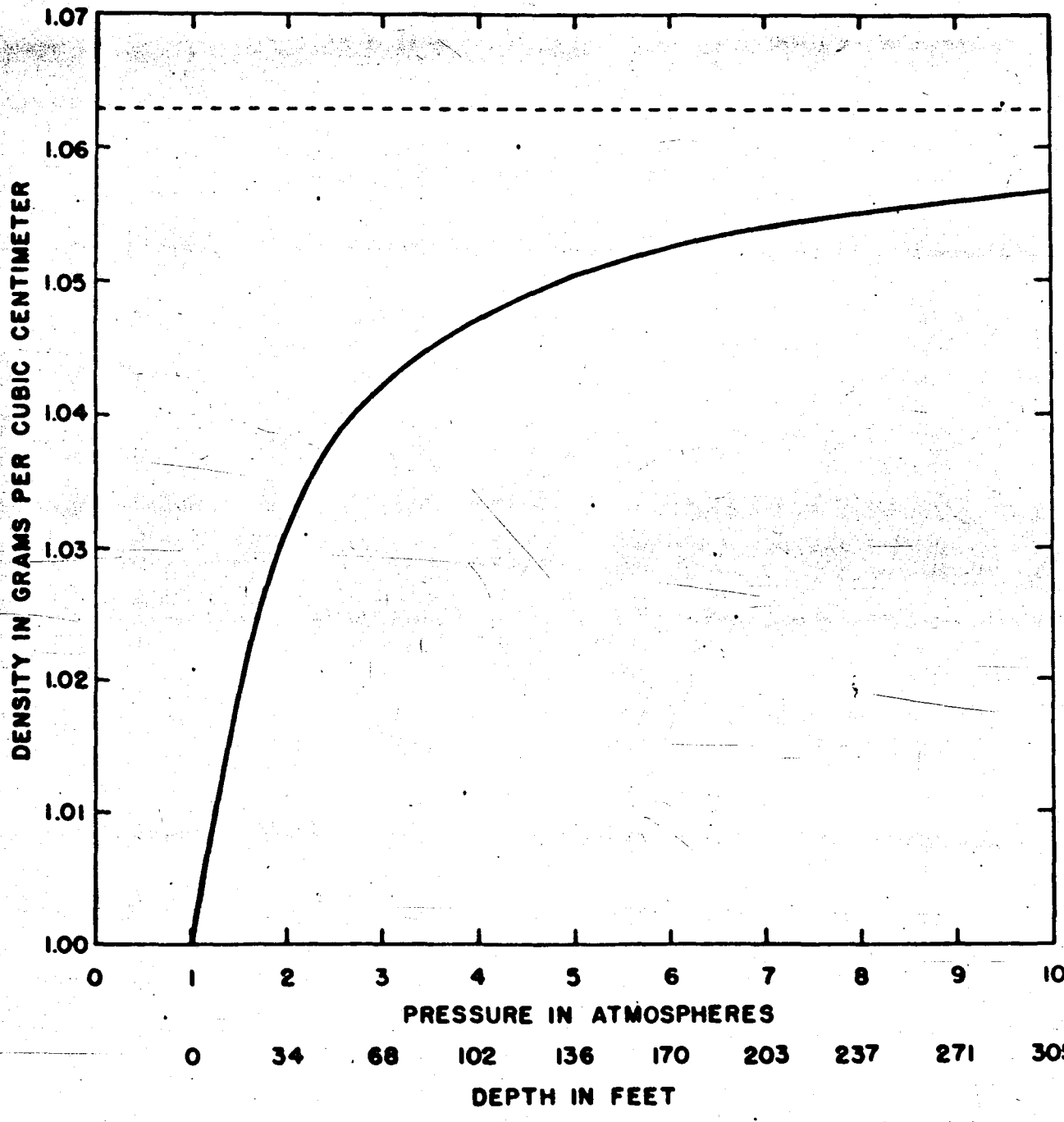

Figure 43. Change in the density of a sockeye smolt with increasing depth and pressure.

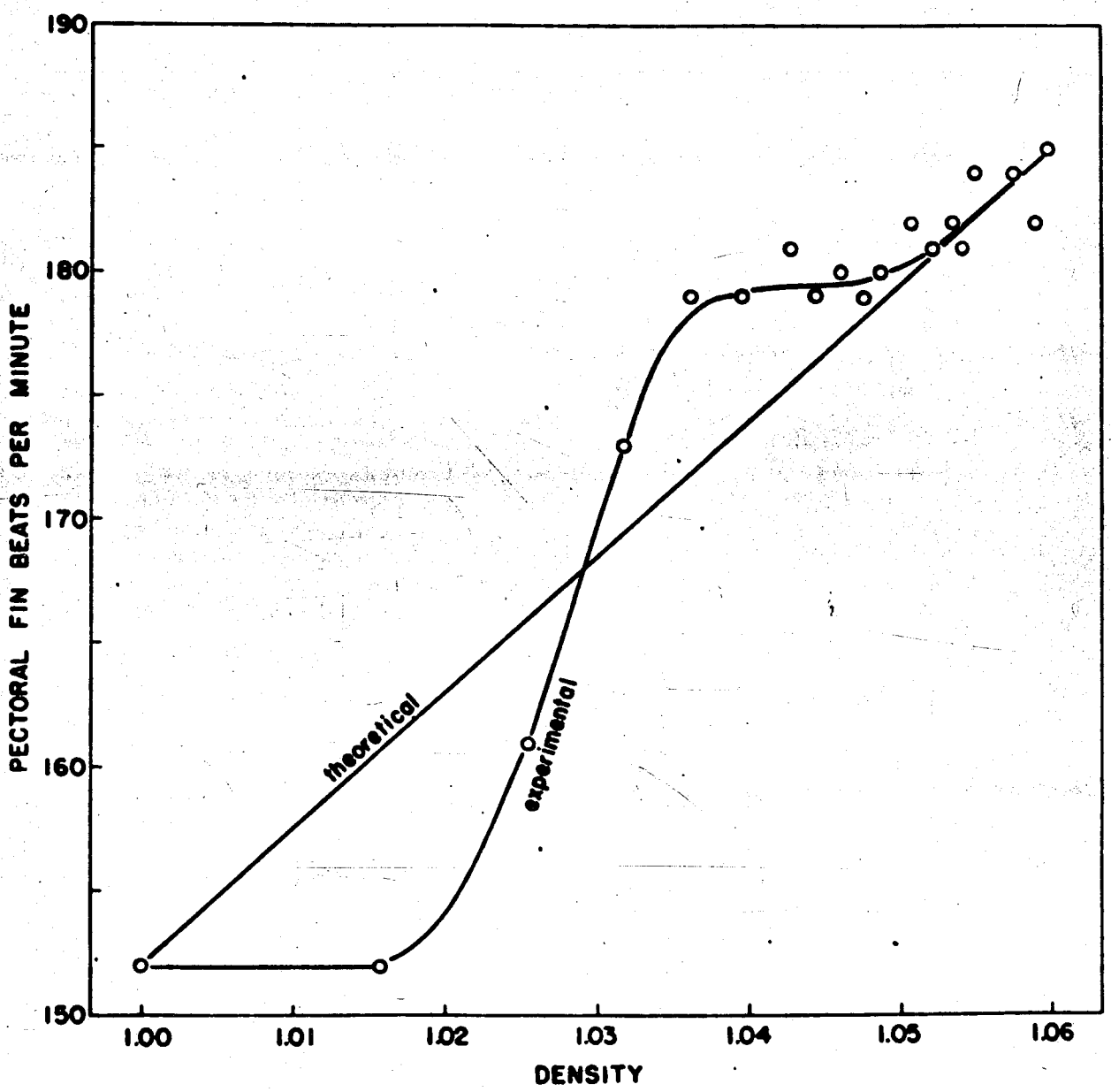

Figure 44. Pectoral fin beats in relation to sockeye smolt density. Theoretical line joins first and final values.

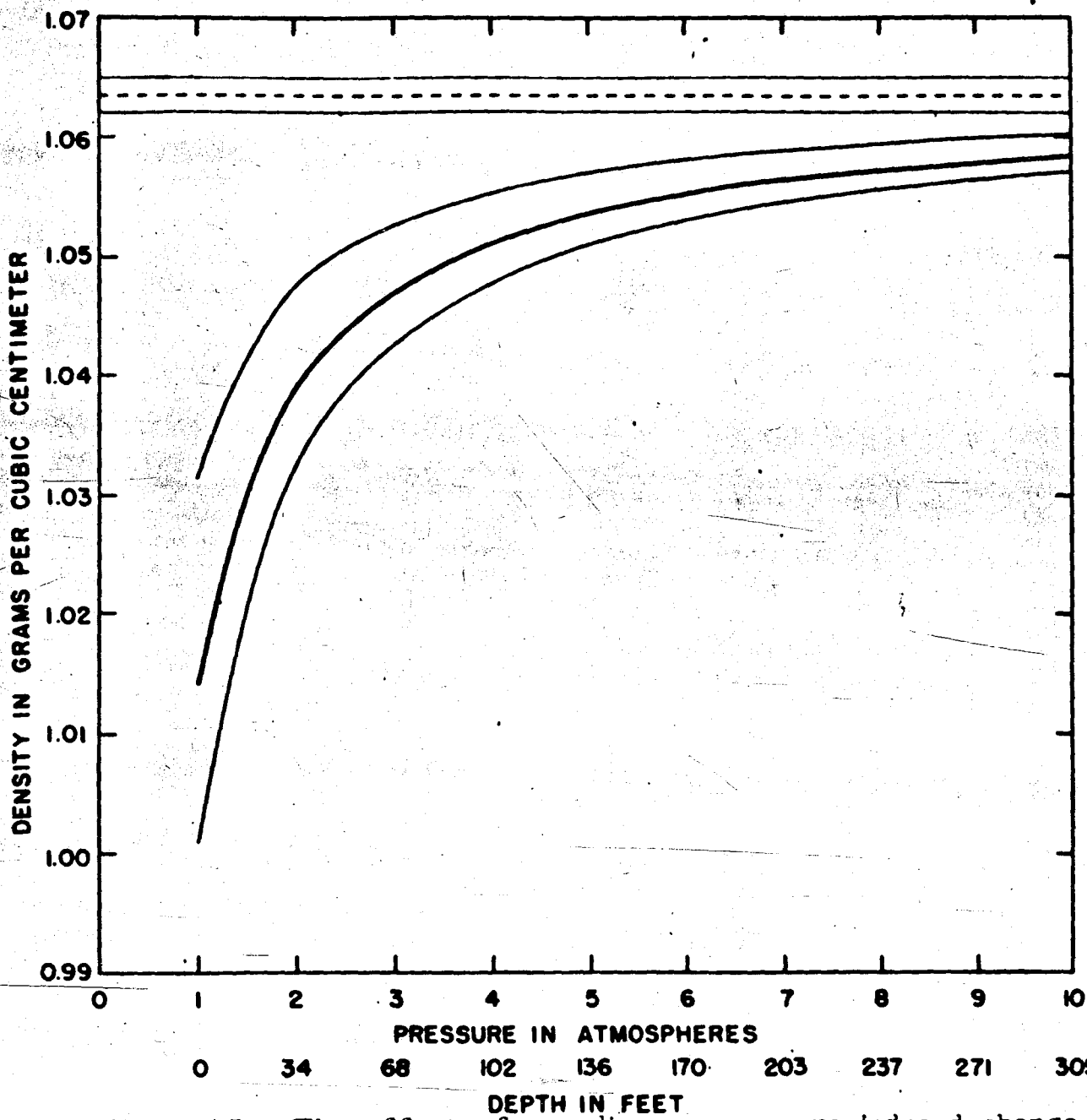

Figure 45. The effect of sounding on pressure-induced change in density. Heavy line mean and light lines range of response. Upper broken line maximum density, swim-bladder free of gas, upper light lines two standard deviations.

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:

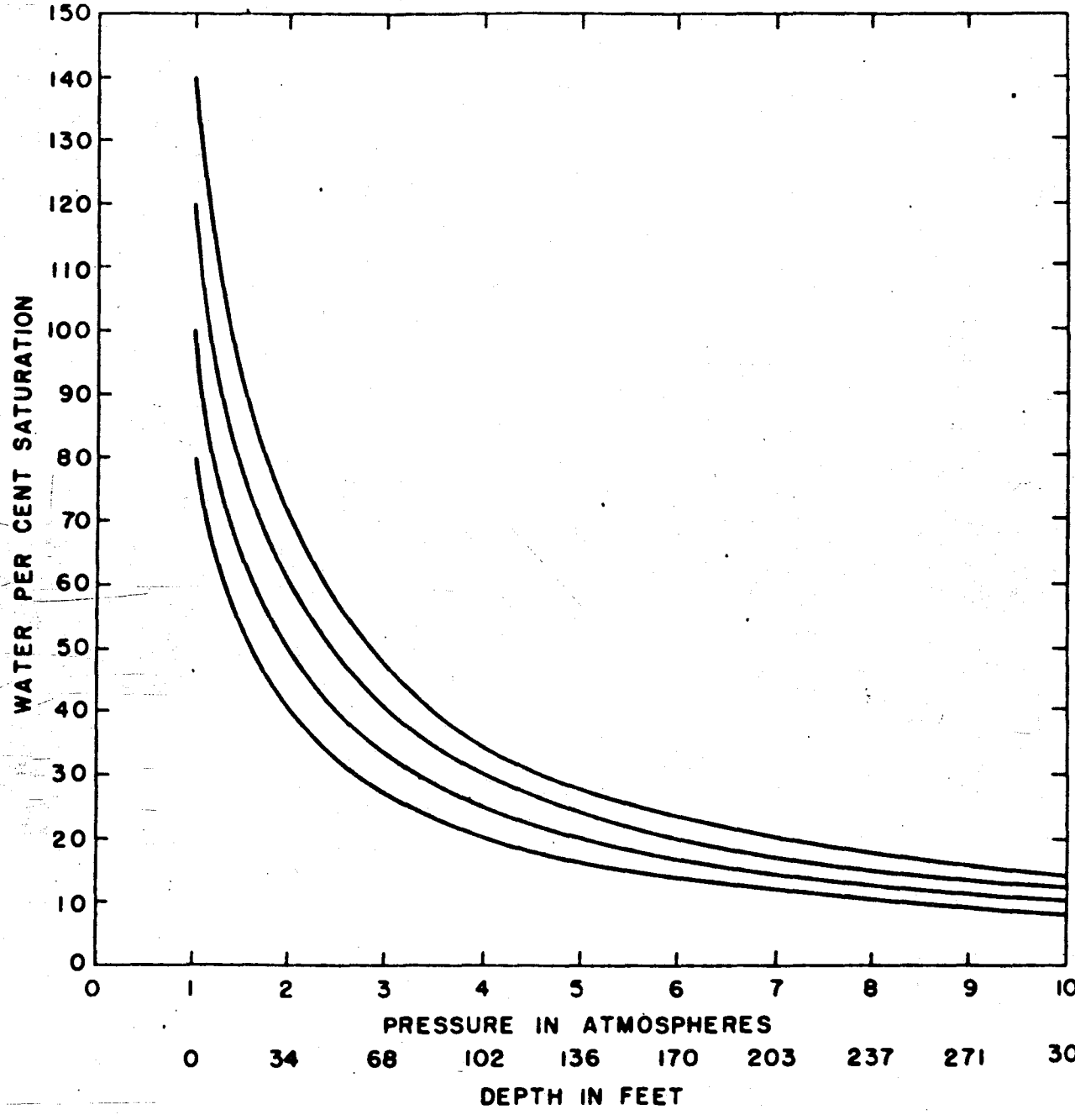

Figure 46. Per cent saturation in relation to pressure and depth. Absolute saturation of Ricker (1934).

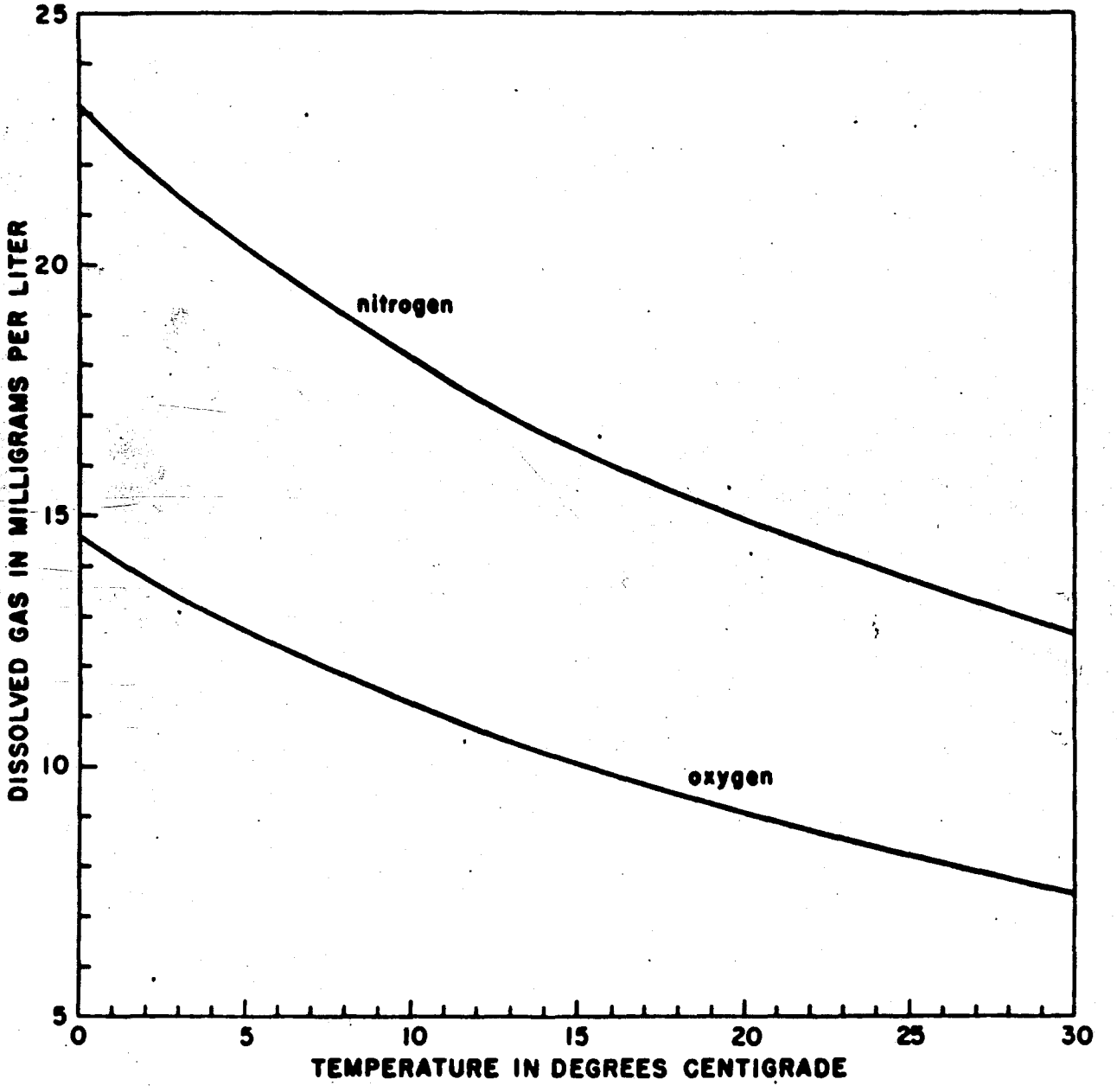

Figure 47. Oxygen and nitrogen content of air-equilibrated water, 0 to 30°C.

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 o f 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:

$53.75 \times 0.283 = 15.21$ 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:

$$767 - 2.4 = 764.6 \text{ mm Hg}$$

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

$$764.6 - 10.2 = 754.4 \text{ mm Hg}$$

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

$$0.7905 \times 754.4 = 596.4 \text{ mm Hg}$$

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).