LOCOMOTOR PERFORMANCE AND OSMOREGULATION IN JUVENILE ANADROMOUS SALMONIDS FOLLOWING ABRUPT ENVIRONMENTAL SALINITY CHANGE

by

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B.Sc., McMaster University, 1954
M.A., University of British Columbia, 1956

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

DOCTOR OF PHILOSOPHY

in the Department of

Zoology

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

Members of the Department.

THE UNIVERSITY OF BRITISH COLUMBIA

June, 1958
ABSTRACT

The relationship between osmoregulatory stress and locomotor performance has been investigated in juvenile salmonids during their adjustment to sea water. Transfer from fresh water to sea water produced a statistically significant depression of the maximum swimming speed of chum salmon fry (Oncorhynchus keta). The effect of sea water was immediate and reached a maximum fourteen hours after transfer. From thirty-six to eighty hours (the duration of the experiment) relatively stable performance levels were recorded. Some recovery from the initial effects of sea water was apparent but the swimming speeds of "recovered" fish were significantly lower than those of fry in fresh water. Statistically significant correlations between swimming speed changes and changes in total body chloride and water concentrations have been demonstrated.

Since chum fry were too small to allow separate sampling of plasma and tissue, the Steelhead trout (Salmo gairdneri gairdneri) was used to investigate the sequence of events in the osmoregulatory adjustment of salmonids to sea water. Transfer into sea water was accompanied by increases in plasma concentrations of chloride, sodium and potassium, but not of calcium. Cellular dehydration resulted from transfer of cellular fluids to the extracellular phase, and from loss of water to the environment. Cellular levels of calcium and sodium rose markedly. Smaller increases in cellular potassium and chloride were noted. Changes in cellular sodium and calcium were primarily due to ion uptake. Increases in cellular potassium were the result of cellular dehydration since tissue levels of this cation fell slightly in sea water.

The data indicate that impairment of the efficiency of physiological
processes sensitive to altered electrolyte concentrations, and the utiliz-
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Body size was shown to influence the extent and duration of changes
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In Steelhead trout efficiency of osmoregulation was highest at certain
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The operation of plasma electrolyte homeostatic mechanisms was indicat-
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from the circulating fluids by complex formation and by active excretion
have been considered.
The University of British Columbia
Faculty of Graduate Studies

PROGRAMME OF THE
FINAL ORAL EXAMINATION
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

of

ARTHUR HILLIER HOUSTON
B.Sc., McMaster University, 1954
M.A., University of British Columbia, 1956

IN ROOM 187A, BIOLOGICAL SCIENCES BUILDING
MONDAY, JUNE 30, 1958 at 2:30 p.m.

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The operation of plasma electrolyte homeostatic mechanisms was indicated in the Steelhead trout and the possible roles of the withdrawal of ions from the circulating fluids by complexation and by active excretion have been considered.
PUBLICATIONS


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ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude to Professor W.S. Hoar, F.R.S.C., Department of Zoology, for his supervision of this study. Other members of his doctoral committee, Professors I. McTaggart-Cowan, F.R.S.C., W.A. Clemens, F.R.S.C., J.R. Adams, P.A. Larkin, and P.A. Dehnel, all of the Department of Zoology, gave freely of their time and advice. Professor E.E. Daniel, Department of Pharmacology, provided the flame spectrophotometer used in sodium and potassium analyses, and critically read the manuscript. Professors D.H. Copp, E.C. Black, F.R.S.C., C.F. Cramer, and H. McLennan, Department of Physiology, were also most generous in their assistance.

Professor M. Darrach, Department of Biochemistry, supplied the Klett-Summerson photocolorimeter used in plasma calcium analyses. Mr. T.G. Northcote, British Columbia Game Commission, loaned the apparatus used for determinations of swimming speed, while Mr. L. Royal of the International Pacific Salmon Commission, made available constant temperature apparatus.

The author is indebted to Mr. S.B. Smith and Mr. F. Pells of the British Columbia Game Commission for supply and maintenance of Steelhead trout. Dr. Ferris Neave, Pacific Biological Station, Nanaimo, British Columbia, provided chum salmon fry.

To his fellow graduate students, Dr. M.A. Ali, Mr. H.H. Harvey and Mr. C.P. Hickman, the author wishes to extend thanks for stimulating discussion and constructive criticism.

Financial assistance from the National Research Council in the form of three Studentships enabled the author to carry out this study.
INTRODUCTION

The organization of inanimate materials into the condition of life runs counter to the normal operation of physical laws (Blum, 1955). The animate condition may be maintained therefore, only by the continuous expenditure of energy by the living organism in excess of that tending to produce disorganization. Living creatures must, however, do more than produce energy just sufficient to ensure their structural integrity - an excess must be available for the multiplicity of behavioural activities essential for individual survival and effective reproduction. Considered from this point of view energy expenditure may be roughly classified into two categories; "energy of maintainance", and "energy of behavioural activity".

In most, and perhaps all, types of organisms "maintainance" involves the expenditure of some energy for the establishment and regulative control of conditions within the organism which are more favourable for metabolic efficiency than those of the external environment. Thus, by diversion of a portion of its total energy production into homeostatic processes the organism safeguards metabolic efficiency and becomes, to some degree, independent of fluctuations in external environmental conditions. If the assumption can be made, however, than an upper limit exists for the rate at which energy sources can be converted into utilisable energy it follows that any environmental situation which requires energy expenditure for the maintainance of either structure or favourable metabolic conditions, or which produces an impairment in metabolic efficiency may result in a decrease in the fraction of total energy available for behavioural activity. In the words of Fry (1947) the animals' "scope for activity" will be reduced.

The life histories of anadromous salmonid fishes provide a natural
situation in which these animals must meet conditions of the type suggested in the preceding paragraph. During their initial fresh water phase juvenile salmonids are adapted to a particular physiological situation - that of maintaining a constant internal environment in the face of conditions which tend to deplete levels of diffusible electrolytes and increase those of water. This they accomplish by production of a voluminous hypotonic urine, and absorption of salts to replace those lost by diffusion (Krogh 1939, Black 1951a, 1957). In the course of their seaward migration these fish must meet, within a relatively short period of time, environmental conditions exactly the reverse of those to which they were previously adjusted. Survival under such conditions demands the reversal of existing osmoregulatory processes and re-establishment of regulated osmotic and ionic conditions within the internal milieu. In a word, the fish must suddenly become hypo-osmoregulators. That salmonids and other diadromous species have the ability to rapidly accomplish this adjustment has been abundantly verified (see Keys 1933, Krogh 1939, Fontaine & Koch 1950, Black 1957 for reviews). Conversion from fresh water to marine osmoregulation is however invariably attended by a period of adjustment. The latter is accompanied by changes in the total osmotic pressure, ion concentrations, and relative ion ratios of tissues and of body fluids. Under such conditions behavioural activity might be expected to be reduced due to demands upon the energy pool of the organism for the re-establishment and maintenance of new equilibria between external and internal environments. In addition transitory losses of metabolic efficiency during the period of adjustment as a result of changed electrolytic conditions may occur. Indeed, several authors, including Leovy (1938), Huntsman and Hoar (1939), Shepard (1948), Fontaine, Callamand and Vibert (1950) and Houston (1957) have observed decreased activity in fishes subjected to abrupt var-
Variations in environmental salinity.

The studies reported herein have centered around an examination of some aspects of the relationship between osmoregulatory stress and activity of juvenile anadromous salmonids immediately following their transfer from fresh water into sea water. Three interrelated aspects of this problem have been considered. Changes in activity (measured as maximum swimming speed) attendant upon experimental transfer of chum salmon fry into sea water have been investigated. An attempt has been made to correlate variations in activity with changes in total body electrolyte and water levels. A statistically valid correlation between these variables having been demonstrated, a detailed study of the sequence of events in the adjustment of salmon to sea water has been carried out, and an attempt made to suggest mechanisms which may aid in accounting for observed changes in activity. Since data not previously present in the literature on osmoregulation of salmonids has been derived from this study an attempt to make a fuller description of their adjustment to sea water has been included in this thesis.
II. MATERIALS AND METHODS

A. Materials

Two species of salmonids indigenous to British Columbia have been used in this study, the chum salmon, *Oncorhynchus keta* (Walbaum), and the Steelhead trout, *Salmo gairdneri gairdneri*, Richardson. Both are anadromous in habit. Chum salmon migrate to sea soon after emergence from their redds. Steelhead trout, however, tend to remain in fresh water for at least one year, moving seaward in late April and early May, after parr-smolt transformation.

Chum fry were obtained from the Pacific Biological Station, Nanaimo, British Columbia, and originated in the Oyster River, Vancouver Island. Steelhead trout juveniles were supplied by the British Columbia Game Commission from stocks held at their Smith Falls Hatchery, Cultus Lake, British Columbia.

Both stocks were maintained at the University immediately prior to use and were fed a dry commercial fish food. The water supply at the University was dechlorinated city water. Water quality at both points of origin was undetermined. Hatchery temperatures varied from 5 to 17°C over periods of maintenance, and the fish were subjected to normal day lengths.

B. Methods

(1) Locomotor Performance Studies on Juvenile Chum Salmon

(a) Apparatus

An artificial stream constructed by Mr. T.G. Northcote of the British Columbia Game Commission was used in studies on locomotor performance. The general construction of this apparatus is indicated in Figure 1. Temperature was maintained at 10.0±0.50°C, and light at 0.8 foot-
Fig. 1. Apparatus used in determination of swimming speeds.

(1) Intake
(2) Control Valve
(3) Outlet
(4) Refrigerating Equipment
(5) Turning Vanes
(6) Performance Chamber
(7) Rheotactic Cues
(8) Mirrors
(9) Lights
candles (measured at the water surface). An overhead arrangement of lights and mirrors allowed observation of the fish with a minimum of disturbance. The apparatus was painted throughout with a non-toxic commercial marine enamel.

An inset channel measuring 56 x 20 x 5 cm. and screened at both ends with a coarse mesh plastic screening served as the performance chamber. Visual cues for rheotactic response consisted of vertical and horizontal bars painted down the sides and across the floor of the channel. Current velocities were determined with a Stevens midget current meter (Leupold & Stevens Instruments, Inc., Portland, Oregon).

A system of blocks inserted at the upstream end of the turning vanes (Fig. 1-5) was found by experiment to give a relatively uniform current pattern at depths of 2 and 4 cm. from the surface. This pattern did not vary markedly with distance from the upstream screen.

(b) **Procedure**

Fish were acclimated to sea water (salinity 22 - 24 °/oo) in five gallon all-glass aquaria at hatchery trough temperatures (12 - 17°C). The acclimation times were such that, allowing 3 1/2 to 4 hours for each determination, the total intervals in sea water fell into the ranges 3 1/2 - 4, 9 - 10, 13 - 14, 20 - 22, 26 - 28, 34 - 36, 48 - 54, and 76 - 78 hours.

The apparatus was filled with sea water of the same salinity to a depth of 5 cm. Single fish were transferred in water from the acclimation tanks into the performance chamber. A period of 1 hour was allowed for recovery from any shock of handling. During this interval the water velocity was held at a level just sufficient to ensure adequate
temperature control (2 - 3 cm/sec.), and evoked only mild and transitory positive rheotactic reactions.

Following the recovery period, water velocity was raised 1 - 2 cm. each five minutes. At velocities of 6 - 10 cm/sec. a strong positive rheotaxis was invariably elicited in fish of good condition. Individuals failing to respond within this current range were discarded. Water velocity was then further increased until the fish was swept downstream. A period of 5 minutes was allowed for fish to work themselves off the downstream screen. Animals failing to do this were stimulated by tapping on the caudal peduncle with a glass rod. Electrical stimuli were generally found inadequate for this purpose. Velocities were again increased until no further amount of stimulation produced escape from the downstream screen. The end point was taken as that velocity at which the fish could no longer maintain position at a distance of two body lengths from the downstream screen for 10 seconds. This was termed the "maximum swimming speed".

Fork lengths were recorded, and the fish discarded.

(c) Treatment of Performance Data

Swimming speeds were expressed in cm/sec. For each experimental group swimming speeds were plotted against fork length and regression equations and standard errors of regression calculated (Snedecor, 1946). Analysis of covariance (Snedecor, 1946) was used to test for significant variations in swimming speeds among different groups of fish.

(2) Water and Electrolyte Studies on Juvenile Steelhead Trout

(a) Acclimation to Sea Water

The water-electrolyte system of fishes is subject to spontaneous disturbance by a variety of factors. Jorgensen and Rosenkilde (1956)
have demonstrated spontaneous changes in absolute water and chloride levels in starving goldfish. Meyer (1948, 1951) has shown marked changes in sodium and chloride metabolism resulting from handling. Forster (1953) and V.S. Black (1957) have ably reviewed the many investigations on the course and effects of "laboratory diuresis" in fish. This condition has been particularly described for marine species in which it may be initiated by a variety of traumatic conditions and is characterized by increased glomerular filtration rates and abnormal losses of body water and salts. "Laboratory diuresis" may be of less importance as a factor in the experimental handling of fresh water species since these forms in all probability chronically are diuretic. Its avoidance in studies on the adjustment of fresh water species to sea water would, however, seem to be a necessary precaution. Furthermore, E.C. Black in a series of publications (1955, 1956, 1957 a, b) has outlined the effects of disturbance and forced activity on plasma levels of lactate ion. These studies suggest that changes in lactate levels may be associated with variations in plasma and tissue levels of other electrolytes, and with the establishment of a condition of acidosis in the fish.

Accordingly, the method of acclimating fish to sea water was designed to avoid direct handling, exposure to air, increased activity and starvation. Four wooden boxes of approximately 50 litre capacity were weighted, and immersed in a constant temperature bath (10°C ± 0.2°C). Dark painted covers reduced visual stimulation while the surrounding water bath dampened auditory and vibratory stimuli. The tanks were aerated throughout acclimatization and the fish fed at twelve hour intervals.

Twenty litres of water were placed in each box and time allowed for temperature equilibration. Six to ten Steelhead were then
transferred in water from the hatchery troughs, and sufficient water added to bring the volume to thirty litres. The boxes were covered and an interval of 24 - 36 hours allowed for the fish to recover from any shock of handling, and to acclimate to the experimental temperature. By means of a fixed siphon water was removed to the 8 cm. mark, and the tanks refilled with sea water from an overhead reservoir. Sea water concentrations were adjusted such that a final salinity of 22 - 24 °/oo was achieved. Little disturbance was noted among the animals during this operation, and it was hoped that disturbances in electrolyte balance had been obviated or reduced.

(b) Sampling

Prior to sampling, fish were anesthetized with tricainemethanesulphonate (M222 Sandoz Co., Basle, Switzerland). No standard concentration of anesthetic was used, water or additional anesthetic being added as required to maintain the desired level of narcosis. Individual fish were wrapped in damp towelling and loosely mounted with rubber bands, ventral side uppermost, upon a frame. An open rather than blind cardiac puncture was used to prevent the possibility of contamination of blood samples with pericardial fluid. A mid-ventral slit was made from a point near the insertion of the pectoral fins forward through the isthmus and the latter were separated from the ventral portions of the lower jaw. Hemostats clamped on either side of the isthmus held the isthmus flaps down, exposing the heart. Little haemorrhage resulted from this operation. The pericardial membrane was then slit and drawn aside, and blood samples drawn from the bulbus arteriosus. Mild massage from the caudal region forward aided in expressing blood into heparinized syringes. Chilling, followed by immediate centrifuging tended to prevent haemolysis. Slight sample haemolysis produced no significant variation in plasma chloride levels. The potassium concentrations
of such samples were however, from two to ten times as great as those of normal plasma, and were discarded.

Tissue samples were excised from the dorsal fin area of the epaxial muscle band, grossly dissected free of cartilage, skin, bones, and fat, and immediately transferred to air-tight moist chambers. Plasma samples were capped with Parafilm to avoid evaporation, and both they and tissue samples refrigerated until used.

(c) Analytical Techniques

(1) Plasma Chloride

Plasma chloride concentrations were determined by the technique of Schales and Schales (1941) as modified by Asper, Schales and Schales (1947). Samples of 0.10 ml. volume were used with a measured volume (0.03 ml.) of diphenylcarbazone indicator. Earlier work indicated the necessity of accurate measurement of the amount of indicator used, as variations of 0.02 to 0.04 ml. in the amount added produced changes of as much as 5 - 7 % in endpoint. Samples were titrated in duplicate whenever possible. Comparisons of duplicate samples indicate a mean variation of 1.34 % from the average, a value within the 2 % limits suggested by the authors. Chloremia was expressed as millimoles per litre plasma (mM/L).

(2) Tissue Chloride

A modified "open Carius" procedure described by Manery (pers. comm.) was used in tissue chloride estimations. In past years there has been much controversy as to the most reliable technique for this analysis. The method originally described by Van Slyke (1923) involved the digestion of tissue in a solution of nitric acid with added silver nitrate. Chloride yields were improved by Wilson and Ball (1928) who
added nitric acid and silver nitrate separately. Subsequently Hastings and Eichelberger (1937) demonstrated that long periods of soaking in silver nitrate prior to nitric acid digestion gave values 10 to 20% higher than those obtained by the original procedure. Usage of potassium hydroxide as the digesting agent had earlier been shown by Sunderman and Williams (1933) to give increases in yield of about the same magnitude.

The sources of error in the open Carius technique have recently been clarified by Shenk (1954) who demonstrated that chloride ion in the presence of hot nitric acid reacts with tyrosine, and possibly also phenylalanine, to produce the volatile compound chloranile (tetrachloroquinone).

The open Carius technique as modified by Manery utilized samples of 0.5 to 1.0 gm. fresh tissue which were dried to constant weight, and soaked overnight in silver nitrate. Digestion in nitric acid was carried out over a water bath, the tubes being covered with glass bulbs with a downward projecting tip. This modification overcomes chloride loss by volatilization and probably accounts for the high values obtained. Digestion periods of longer than six hours were found to produce no increase in maximum yield of chloride. This technique was slightly modified by the author who found that powdering dried tissue prior to digestion gave more consistent results. Small additions of hydrogen peroxide reduced the content of fat and connective tissue during digestion without significantly affecting chloride yields. Subsequent to digestion, samples were decolourized with potassium permanganate, and residual silver nitrate titrated with sodium thiocyanate, using ferric alum as an indicator. Duplicate determinations were carried out in most cases and indicated an average variation from the mean of 3.46%.
Tissue chloride levels were expressed in millimols per kilogram fresh tissue (mM/kgm).

(3) **Plasma and Tissue Water**

Small samples of plasma (0.05 - 0.2 ml.) were pipetted into hanging drop slides, covered with cover slips to reduce evaporation, weighed, and dried to constant weight at 103 °C. In only a few cases were plasma samples large enough to permit duplicate estimations to be made. Average deviation of tissue water content estimated by the same means was only 0.26 %. Plasma and tissue water concentrations were expressed in grams per kilogram fresh tissue or grams per litre plasma (gm/kgm, gm/L).

(4) **Plasma Calcium**

Plasma calcium determinations were made by the technique of Lehmann (1953). Samples were titrated with disodium ethylenediaminetetraacetate, a chelating agent, at a pH of 12 - 13. Ammonium purpurate was used as an indicator and the end point graphically calculated from light transmittancy readings made with a Klett-Summerson photoelectric colorimeter and 500 second order interference filter. Plasma calcium values were expressed as millimols per litre (mM/L).

(5) **Tissue Calcium**

Tissue calcium was estimated by a modification of the method outlined by Comar et al. (1951). Samples of 2 - 3 gm. fresh tissue were dried, powdered, digested in nitric acid with the addition of hydrogen peroxide, and quantitatively transferred into heavy wall centrifuge tubes. One ml. of carrier calcium (10 mg.%) was added. Following addition of 1.0 ml. of saturated ammonium oxalate and adjustment of the pH to 5.5, samples were refrigerated over-night to allow precipitation of calcium as oxalate. They were then centrifuged, washed twice with dilute ammonium hydroxide, 1 ml. of 20 % sulphuric
acid added, and the oxalate titrated with potassium permanganate at 85° C.
Duplicate determinations were performed in most cases and indicated an average
variation of 3.4% from the mean. Recoveries of added calcium averaged 93.5
\(^{\circ}/o\). Tissue calcium values were expressed as millimols per kilogram fresh
tissue (mM/kgm.).

(6) Plasma Sodium and Potassium

Plasma sodium and potassium levels were determined flame-
spectrophotometrically using a Jahnke lithium-internal standard flame spectro-
photometer. Samples of 1.0 or 0.5 m. of plasma were diluted 1/200 with double-
distilled water containing 10% lithium nitrate, and read against bracketting
standard solutions of sodium and potassium chloride. Sodium and potassium
were expressed as millimols per litre (mM/L.). Plasma samples were, in general,
not large enough to allow duplicate determinations.

(7) Tissue Sodium and Potassium

Tissue sodium and potassium concentrations were estimated
in the same way. Samples of 0.08 to 0.20 gm. fresh tissue were dried to con-
stant weight, powdered, and digested with hydrochloric acid in pyrex test tubes.
They were then diluted to about 1/200 with double-distilled water containing
10% lithium nitrate, and read as above, against bracketting standards. Sodium
and potassium concentrations were expressed as millimols per kilogram fresh
tissue (mM/kgm.).

(d) Treatment of Data

Values for water, chloride, sodium, and potassium have been plotted
against body weight, coefficient of condition and opercule to fork length ratio
as necessary. Regression lines and standard errors of regression were deter-
mined (Snedecor 1946) and "standard-sized" fishes compared by
picking points from the regression lines.

The data allowed for the indirect calculation of certain quantities as outlined below.

(1) Extracellular Fluid Space

The extracellular fluid phase, space or volume (ECP, ECS, ECV) contains those portions of the body tissue which are not within the cells. Since this space may act as a buffer element in any responses of fish to changes in environmental tonicity its estimation is of some value in an investigation of this type.

Conway in a recent review (1957) has cited three main approaches in estimating extracellular volumes. The first involves the use of substances such as sucrose, inulin, etc., which are believed to penetrate into all portions of the extracellular phase, but not into cells. The second utilizes substances which penetrate the extracellular phase rapidly and enter the cells more slowly so that extrapolation of the linear portion of the cellular entrance may be used in the estimation of the space. Finally the total extracellular phase plus adjacent compartments which resemble the extracellular phase (eg. intravascular space, interstitial space, connective tissue space and possibly nerve space) may be determined and the various components partitioned and corrected for, to give an estimate of the extracellular volume.

The approach used in this investigation has been influenced by certain assumptions. The findings of Meyer (1948, 1951) and Forster (1953) concerning the effects of handling on electrolyte balance in fish have been cited. Several authors have shown such conditions to pertain when serial blood sampling has been attempted (eg. Maetz, 1956). Thus all techniques included in Conway's second class would seem to be obviated, as would all
those requiring the initial injection into the fish of some foreign sub-
stance. Moreover, the latter approach produces unreliable results which
turn upon the non-penetration of some of the compounds used into the whole
extracellular phase, and/or their actual utilization by the cells. Various
aspects of this question have been recently reviewed by Nichols et al (1953),
Manery (1954), Elkington and Danowski (1955), Conway (1957) and others.

It was decided therefore, to use a material already known to be
present in the animals, which was readily analyzable, and which on the basis
of the current literature appeared to give reasonably reliable estimates.
These requirements were met by the chloride ion.

The use of the chloride space as an estimate of extracellular
muscle volume is predicated upon several assumptions; that chloride penetrates
all parts of the true extracellular space, that chloride concentrations are
similar in all possible subcompartments of the extracellular phase, that ion
activities are equal throughout this phase, and that no chloride is present
in, or subsequently enters muscle cells. A further assumption in comparing
chloride spaces of fish in fresh water and in sea water is that these relat-
ionships do not change under the varying plasma electrolyte conditions ex-
hibited by the trout in their adjustment to sea water.

The investigations of Nichols et al (1953), Cotlove (1954) and
Cheek et al (1957) indicate that chloride is present in all parts of the
extracellular phase. Nichols et al (loc. cit.) attributed the relatively
greater volume of chloride as compared to inulin spaces to the fact that
the larger molecule does not readily penetrate the connective tissue phase
of the extracellular compartment. On the basis of their data this con-
clusion appeared valid, but does not include the possibility of chloride
penetration into cells.
Manery et al (1938) have reported that connective tissue had an electrolyte pattern more like that of a plasma ultrafilterate than that of cellular fluid. For purposes of estimating the extracellular phase it was felt by these authors, and Manery (1954), that both the connective tissue and interstitial fluid phases could be considered as equalling a plasma ultrafilterate in concentration.

Since chloride ion has been shown to exhibit little protein binding under physiological conditions (see Manery 1954, for review) the assumption of equal activities appears valid.

The employment of the chloride technique seems to depend primarily upon the assumption of an extracellular position for all muscle chloride. Overton (1902) apparently was the first to suggest that this might be the case. This view was supported by Ernst and Takacs (1931), Mond and Netter (1932), Fenn et al (1934), Fenn (1936), Peters (1936), Eggleton et al (1937) and others. Harrison et al (1936) extended the hypothesis to include all tissue, while Truax (1939) presented data suggesting an extracellular position for liver chloride. Gersh (1938) attempted to localize muscle chlorides by histochemical techniques and came to the conclusion that all chloride lay outside the cells. This opinion was criticized by Scott and Packer (1939) on the basis that preparation of the materials must have so disorganized it as to make any conclusions regarding electrolyte distribution most tenous.

Amberson et al (1938) replaced normal cat blood with sulphate-substituted blood, changing the plasma chloride levels over the range 6% to 100% of normal. Calculation of plasma-tissue ratios indicated the presence of non-diffusible (i.e. intracellular) chloride fractions in lung, nerve and connective tissue but not in muscle. This view was supported by the work of Eggleton et al (1937), Fenn et al (1934), and Hiatt (1940) on frog muscle in
vitro. Yannet and Darrow (1940), on the other hand, used isotonic glucose to deplete cat plasma chloride levels and reached the conclusion that a small intracellular fraction existed.

Studies by Conway and his co-workers (reviewed by Conway, 1957) and by Fisher and Subrahmanyan (1937) on chloride diffusion in muscle also suggest that low concentrations of chloride may be present in muscle cells (1.5 - 5.0 mM/L cell water). Assuming tissue chloride concentrations of about 20 mM/kgm, and an extracellular phase of 124 gm/kgm, the contribution of cellular chloride to tissue chloride becomes 1.3 to 8.8 mM/kgm, a level sufficiently great to influence chloride space estimates of extracellular phase. Data available on comparative estimates of extracellular volume by the chloride space technique and that proposed by Conway (see below) do not indicate which approach is more reliable. Conway's hypothesis however, since it depends upon only one assumption, that extracellular and intracellular chloride and potassium concentrations are related by a Donnan equilibrium, is very attractive.

The generally low blood volumes of trout sampled in this investigation precluded any routine estimate of extracellular volume by both techniques. In most cases, therefore, chloride space has been used to estimate the volume of this entity, with the knowledge that the space described in this way might overlap to a greater or lesser extent than of the true cellular phase. That this overlap was slight was indicated by the fact that intracellular spaces calculated on the basis of both types of estimate varied only by 3.6%. It was also of interest to note that while absolute levels of extracellular space varied somewhat when both techniques were employed the changes in phase volume resulting from transfer into sea water were essentially the same.
Using Manery and Hastings's definition (1939) of the chloride space; "the volume required to distribute all the tissue chloride at the concentration of a plasma ultrafiltrate", the extracellular phase may be estimated as follows:

\[ ECV = \frac{(tCl)}{(pCl)} \times (pH_2O) \times (Cl) \times \text{gm/kgm} \]

where: \( t = \) tissue concentration
\( p = \) plasma concentration

\( Cl = 0.977 \)

The relationship proposed by Conway for the estimation of extracellular depends upon the relationship:

\[ (ecs K)(ecs Cl) = (ics K)(ics Cl) \]

where; \( ecs = \) extracellular space
\( ics = \) intracellular space

and is summarized in equation 2 below:

\[ ECS = \frac{(tk) \times (tCl) - (H_2O) \times (ecsK)(ecsCl)}{(ecsK)(ecsCl) + (tk) \times (tCl) - 2(H_2O) \times (ecsK)(ecsCl)} \]

\[ \text{gm/kgm} \]

where: \( t = \) tissue.

A simplification of this formula has been developed by Conway who stated that this approximation gave "..... results so good that no advantage would appear to be gained by the use of any more elaborate formula, particularly when one considers the inevitable small variations and not quite exact assumptions involved in the most detailed calculations ..." This simplified formula is given below:

\[ ECS = 0.90 \times \frac{(tCl)}{(ecsCl)} \]

\[ \text{gm/kgm} \]

When equation 1 is reduced to the form of equation 3 by the assumption of a plasma water content of 940 gm/kgm it becomes:
The close correspondence between these two forms indicates that a relatively small difference would result from comparison of spaces estimated in the two ways.

(2) Derivations Based Upon the Extracellular Fluid Volume

(a) Intracellular Fluid Space

An estimate of the intracellular fluid space, volume of phase (ICS, ICV, ICF) may be made by considering it to equal the difference between the total tissue water (tH₂O) and the chloride space (ECV) thus:

\[ \text{ICV} = (tH₂O) - \text{ECV} \]

(b) Extracellular Fluid Space Electrolyte Concentrations

Concentrations of electrolytes in plasma ultrafilterates do not generally equal those in plasma proper. This is largely due to ion binding by protein, and the restrictions upon diffusion imposed by fluid flow. An estimate of the extracellular electrolyte concentration may be made as follows (Manery, 1954):

\[ \text{(eB)} = \frac{(pB) \times (vB) \times 1000}{(pH₂O)} \]

Where: \( A \) = Anion concentration
\( B \) = Cation concentration
\( p \) = Plasma concentration
\( vA, vB \) = Donnan factors
(Manery, 1954)

\[ \text{(eA)} = \frac{(pA) \times 1000}{(pH₂O)(vA)} \]

Donnan Factors:

\[ \frac{(pCl)}{(eCl)} = 0.977 \]
\[ \frac{(eNa)}{(pNa)} = 0.942 \]
\[ \frac{(eK)}{(p/K)} = 0.943 \]
\[ \frac{(eCa)}{(pCa)} = 0.714 \]
(c) **Intracellular Fluid Space Electrolyte Concentrations**

Knowing the total electrolyte content of a tissue, the extracellular and intracellular fluid spaces and the extracellular fluid space concentration of the electrolyte, an estimate of the amount of the electrolyte associated with the cells of the tissue can be made.

\[
(cB) = (tB) - (eB) \times (ECV)
\]

Where: \( c \) = cellular cation

Many authors express cellular cation per litre cell water. However, recent investigations (eg. Conway and Cruess-Callaghan 1937, Tabour and Hastings 1945, Holland and Auditore 1955, Gilbert and Fenn 1957), suggest that much intracellular electrolyte is protein bound and hence not in the free distribution assumed by the above expression. Recently Lilienthal et al (1950) have suggested the use of non-collagenous nitrogen as an accurate reference standard. In this study intracellular electrolytes have been reported per gm. solid material or per litre cell water in the tissue. No correction has been made for the solid content of the extracellular phase.

(e) **Studies Carried Out**

As was indicated in the Introduction this study has been centered around three topics; the effect of introduction into sea water on the motor performance of juvenile anadromous salmonids, the correlation of changes in motor performance with those in total body levels of electrolytes and water, and the sequence of events in the adaptation of migratory salmonids to sea water insofar as these may be related to changes in performance. The investigations carried out with regard to each aspect are outlined below.

(1) Determination of the maximum swimming speed of groups of ten to twenty chum salmon fry (*Oncorhynchus keta*) have been made on fish in fresh water, and at intervals of 3, 9, 14, 22, 28, 36, 50, and 80 hours after
(2) Total body chloride levels and total water contents of the same species under similar conditions have been determined on groups of 15 fish held in sea water for 3, 10, 15, 22, 28, and 36 hours.

(3) Since chum fry were so small as to preclude separate sampling of plasma and tissue studies on the processes of adjustment to sea water were carried out on a related species of a similar life history, the Steelhead trout (*Salmo gairdneri gairdneri*). Three sets of investigations have been made:

(i) Variations in plasma chloride and water, tissue chloride and water, and chloride and intracellular spaces have been determined on groups of fish in fresh water, and at intervals of 4, 10, 15, 22, 28, 86, 126, and in the case of plasma chloride only, 168 hours after their transfer into the sea water.

The assumption in using the Steelhead trout as a test animal has been made that both species undergo essentially the same change in their adaptation to sea water, and that results obtained with the two species are comparable. On this basis peak chloride contents have been taken as indicators of similar physiological state.

(ii) Changes in plasma and tissue calcium have been determined in groups of fish in fresh water and after 15, 26, 52, 126, 168, and 240 hours in sea water. Values obtained from the preceding studies (i) have been utilized in estimating variations in extra-, and intracellular concentrations of calcium.

(iii) Variations in plasma and tissue concentrations of water, chloride, sodium, and potassium have been determined simultaneously, whenever possible, on groups of fish in fresh water and after 36 hours in sea water. The latter interval was chosen as that representing maximum deviations from
fresh water and electrolyte levels. Values for extracellular space arising from estimates of chloride and chloride-potassium spaces were used as a basis for the estimation of intracellular volumes, and thereby cellular sodium and potassium levels.
III. RESULTS

Data will be presented in three sections each dealing with one of the major aspects of this investigation. Thus Section One includes studies on motor performance of chum fry in fresh water and following their transfer into sea water. Section Two is concerned with changes in total body levels of chloride and water in the same species under similar experimental conditions, and with the correlation between changes in swimming speed and changes in water and electrolyte concentrations. Section Three consists of a more detailed study of the course of events in the adaptation of Steelhead trout to sea water.

In general, the data presented in Figures summarise the results of particular aspects of this study. More detailed data may be found in the Appendix.

SECTION ONE: Motor Performance of Chum Salmon Fry in Fresh Water and Following Transfer into Sea Water

(a) Fresh Water

Data on swimming speeds of chum salmon fry in fresh water and sea water are present in Figure 2 together with representative data on other salmonid species. Swimming speeds in fresh water appeared to be related directly to fork length (Appendix Table I, $b = + 1.051$), and in this feature resemble those reported by Davidson (1948) for Atlantic salmon fry ($\text{Salmo salar}$) of about the same size. The data obtained in this investigation are in good agreement with previously determined values. The somewhat lower swimming speeds obtained by Davidson (loc. cit.) probably do not represent maximum performance levels since no stimulus requiring forced response on
Fig. 2. Swimming speeds of chum salmon fry and other salmonid species in fresh water.
the part of the fish was incorporated in the experimental design.

(b) Sea Water

For purposes of graphical presentation the mean fork lengths and swimming speeds of each experimental group have been taken as the best established points. Swimming speeds of fish of similar size in fresh water have been calculated from the fresh water regression line, and the performance of each experimental group expressed as a percentage of its fresh water potential (Fig. 3, Appendix Table II).

A decrease in performance occurred immediately after the transfer of fry, and reached a maximum 14 1/2 hours after their introduction into sea water. At this time swimming speed was 68.2% of the fresh water level. Recovery from the effects of transfer was apparent at 22 hours and from 36 to 80 hours after transfer no consistent increase in performance was apparent. During this period the overall mean swimming speed was 95.2% of the fresh water potential (average of points at 36, 50, and 86 hours).

Analysis of covariance (fork length x swimming speed x interval in sea water) carried out on the total body of data gave an F value of 24.37*, significant at the 0.005 level. The decreases noted were, therefore, highly significant. Similar analysis of data from groups in fresh water and after 36, 50 and 80 hours in sea water gave an F value of 4.22. This value was significant at the 0.05 level, and almost significant at the 0.01 level. Since no further recovery appeared to take place after 36 hours, it may be concluded that over the duration of this experimental period, sea water had not only an immediate effect on motor performance, but also a continuing effect. The fish never fully regained their fresh water potential. Since fish were fed during acclimation, and since Black (1955 pers. comm.) has shown that they recover more rapidly from fatigue in sea water these two possibilities may be ruled
To follow page 23.

Fig. 3. Changes in swimming speed of chum salmon fry following transfer into sea water; swimming speeds expressed as percentage of corresponding fresh water value.
% FRESH WATER MOTOR PERFORMANCE

HOURS IN SEA WATER

Fresh water

80

100

90

80

70

60

10

20

30

40

50

60

70

80
out as influencing factors.

Some indication of a size influence on the effects of transfer may be seen in Appendix Table I. The samples were, however, too small to preclude any but tentative conclusions. The absolute mean rates of decrease in swimming speed over the interval zero to 14 1/2 hours in sea water were - 0.68, - 0.67, and - 0.66 cm/sec/hour for 4.0, 4.5, and 5.0 cm. fish, and were not significantly different. Taken as percentages of the original swimming speed however, the decreases became - 3.14, - 2.49, and - 2.08%/hour indicating a more rapid effect of sea water on smaller fish. This is consistent with the results of Huntsman and Hoar (1939) on the resistance of Atlantic salmon to sea water. These authors have related the low resistance which small parr exhibit towards sea water to their relatively high surface area to body mass ratios.

SECTION TWO: Total Body Chloride and Water of Chum Salmon Fry in Fresh Water and Following Transfer into Sea Water: Correlation of Variations in Swimming Speed, Total Chloride and Total Water

(a) Total Body Chloride Concentrations

(1) Fresh Water

Data relative to chloride levels in fresh water, and following transfer from fresh to sea water, are summarized in Figure 4 and Appendix Table III.

Some size influence on total body chloride levels of fry in fresh water was apparent (Appendix Table III, b = - 0.6024). Heilbrunn (1952) has noted that in general both plasma and tissue water and electrolyte levels fall gradually with growth and/or maturation. In the case of chloride ion which is predominantly extracellular in position decreases in tissue concentration probably reflect decreases in the volume of the extracellular compartment.
Fig. 4. Total body chloride concentrations of chum salmon fry in fresh water, and following transfer into sea water; vertical bar equals one standard error of regression.

Fig. 5. Total body water levels of chum salmon fry in fresh water, and following transfer into sea water.
180

HOURS IN SEA WATER

HOURS IN SEA WATER

BLACK (1951) X
Barlow and Manery (1954) have observed decreases in the chloride space of chicken muscle with growth, while Daniel and Daniel (1955) noted that similar variations with maturity were found in the chloride space of rat muscle. Evidence to be presented in Section Three indicated much the same sort of situation in Steelhead trout, save that maturity could not be implicated as an operating factor.

(2) Sea Water

Entry into sea water was accompanied by a massive uptake of chloride which probably reflected a large increase in the size of the extracellular phase (Fig. 4). Maximum levels were found 14 hours after transfer. Levelling off of chloride concentration occurred after 24 hours to 28 hours in sea water, although the stable level was somewhat above the fresh water concentration. Black (1951 a) has provided comparable data on the same species, and indicated much the same pattern of events. Marked increase in chloride content occurred immediately after transfer into water of about the same salinity (27.7 0/oo). Peak concentrations were found 12 to 15 hours after transfer, and relatively stable concentrations were reached within 36 hours. Again, the final concentrations were higher than in fresh water level. Extreme differences are apparent, however, between the chloride concentrations found by this author, and those recorded in the present investigation. Black noted an increase from 50 mM/kgm in fresh water to a maximum of about 70 mM/kgm after 15 hours in sea water. The corresponding values found in this study were 74 - 86 mM/kgm, and 108 - 165 mM/kgm respectively.

The source of this discrepancy may lie in the technique used for tissue chloride analysis. Shenk (1954) has compared various techniques used for tissue chloride estimation, including the Van Slyke procedure utilized by Black, and Parr bomb, and micro-Carius methods. The latter are chiefly
characterized by ashing of samples in closed, rather than open containers. The average ratio of chloride yield by Van Slyke analysis as compared to Parr-bomb and micro-Carius analyses was 0.403. Shenk suggested that chloride loss might be related to the formation and subsequent loss of chloranile, a volatile organic substance arising from the union of chloride ion and tyrosine, and possibly phenylalanine, in the presence of hot nitric acid. Since closure of the digestion tubes in the procedure outlined by Manery (see Methods, c - 2) presumably precluded loss of volatile substances it seems reasonable to attribute to this source of error, the variations between the earlier and present estimations. The mean ratio between the estimates of Black and the present author was 0.52.

Size as measured by fork length had a marked effect on both the net uptake rate of chloride ion and the percentage increases in chloride exhibited by the fish. Higher rates of uptake (estimated as the mean net uptake rate over the initial 14 1/2 hours in sea water) were characteristic of smaller animals. A mean rate of 5.45 mM/kgm/hour was calculated for fish of 4.0 cm. fork length. The corresponding value for 6.0 cm. fish was 2.37 mM/kgm/hour. Similarly uptake rate was inversely correlated with weight and surface area, and directly with surface area to body weight (Appendix Table IV). Again, these data bear out the conclusions of Huntsman and Hoar (loc. cit.) regarding the relation of salinity tolerance to body size in Atlantic salmon.

In a similar manner the percentage change in chloride content was much greater for smaller animals (Appendix Table V). Fry of fork lengths 3.0 cm. exhibited a maximum increase of 91.6 % over their fresh water chloride level. The chloride concentration of 6.0 cm. animals increased only 46.4 %.

As will be pointed out under Section Three the ability of the Steelhead trout to regulate in sea water appears to be keyed to specific stages in
their growth and maturation. These points were indicated by inflections in length-weight and other body part relationships, and are believed to be related to parr-smolt transformation. No such inflection was seen in the length-weight relation of chum fry, or in that of a related species, the pink salmon (Skud, 1955). On this basis it seems likely that the enhanced ability of larger fish to adjust to sea water is a function of surface area to body mass relations, rather than one of growth or particular physiological condition.

(b) Total Body Water Levels

Data on total body water concentrations of fry in fresh water and sea water are summarized in Figure 5 and Appendix Table VI.

Some size effect on moisture concentration was indicated in both fresh and sea water fish. This influence was however, very much less than that seen for chloride levels, \( b = -0.0695 \) in fresh water, \( b = -0.2904 \) after 14 1/2 hours in sea water.

Entry into sea water was accompanied by a decrease in total body water. The maximum effect was again found at 14 1/2 hours, and amounted to about 5% of the original fresh water level. Water loss by animals entering sea water has frequently been described (see Keys 1933, Krogh 1939, Black 1951b, 1957 for reviews). Body water levels reported by Black for the same species (1951a) are almost exactly comparable (Fig. 5).

(c) Correlation of Changes in Performance and Total Chloride and Water Levels

Maximum body chloride and minimum body water concentrations coincided with the occurrence of minimum motor performance. In the correlation of changes in these three variables chloride and water levels have been related to the
same size groups upon which Figure 3 was based. Variation in chloride content with time has been expressed as percentage increase relative to the body level found in fish of equivalent size in fresh water (Appendix Table VII). Similarly changes in body water have been calculated as percentages of fresh water levels (Appendix Table VIII).

The coefficient of correlation (Snedecor, 1946) between percentage changes in swimming speed and body chloride was 0.8152 (significant between the 0.03 and 0.01 levels). The coefficient of correlation for percentage changes in water levels and swimming speed was 0.8594 (significant at the 0.05 level). These correlations are indicated in Figures 6 a, b.

These data with those of Section One indicate that: (1) statistically significant decreases in motor performance are attendant upon transfer of chum salmon fry into sea water and (2) that these decreases in performance may be significantly correlated with chloride uptake and water loss under the same experimental conditions.

SECTION THREE: Studies on Osmoregulation in Steelhead Trout

(a) Plasma Chloride

Sixty Steelhead trout were sampled to establish variation in plasma chloride levels in fresh water. Two groups drawn from the same population were used. The first, sampled in June and July 1957, consisted of fish varying in weight from 25 to 70 gm. The second, sampled during February and March 1958, included fish in a weight range of 70 to 250 gm. Evidence of parr-smolt transformation was present in the characteristic silvery appearance of some of the fish.

Marked variation in plasma chloride concentration was evident in fish of the first group (Fig. 7). Between 25 and 40 gms chloride levels fell
To follow page 28.

Fig. 6a. Correlation of percentage changes in total body water and swimming speed.

Fig. 6b. Correlation of percentage changes in total body chloride and swimming speed.
% FRESH WATER BODY WATER

% FRESH WATER SWIMMING SPEED

% INCREASE BODY CHLORIDE

% FRESH WATER SWIMMING SPEED
sharply from 160 to about 140 mM/L. An additional slight decrease was seen from 50 to 250 gms. The possibility that the unusually high chloride contents in fish weighing less than 40 gms. might have been due to water evaporation from plasma samples was obviated by the fact that the plasma water levels of the fish in question were as high, or higher, than those of animals exhibiting lower chloremias.

Kubo (1955) has noted similar variations in plasma chloride levels of the Japanese Sakuramasu (Oncorhynchus masou). Early smolt stages were characterized by high chloremias relative to late stage parr. Late smolts exhibited falling chloride levels. The data reported above resemble those of Kubo in many respects. The unusually high values probably correspond to Kubo's early smolt stages. Unfortunately no early or late stage parr were sampled in this programme.

(b) Plasma Chloride Levels Following Transfer Into Sea Water

Figure 8 summarizes changes in the chloremia of 55 gms. fish following transfer into sea water. Additional data on 40 and 70 gms. animals may be found in Appendix Table IX. An immediate increase in concentration occurred and a peak was reached at 36 hours. If the assumptions stated in the Introduction are correct, and peak chloride levels may be used as indicator points of similar physiological condition, it may be predicted that at this period the swimming speed of Steelhead trout would be maximally depressed. Variations in plasma chloride concentrations described an unusual course inasmuch as double peaks were found 15 and 36 hours after transfer into sea water. Hickman (1958) has described a similar situation in changes in plasma osmotic pressure and radiiodine uptake rates of flatfish transferred from brackish water into sea water. The variation in sample chloride levels was, however, so great that little significance can be placed on these changes. Removal
Fig. 7. Weight-related variation in plasma chloride levels of Steelhead trout in fresh water.

Fig. 8. Plasma chloride levels of 55 gm Steelhead trout following transfer from fresh to sea water; vertical bars equal one standard error of regression.
SAMPLED JUNE-JULY 1957
SAMPLED FEBRUARY-MARCH 1958
of the point at 28 hours, which was based on a relatively small sample of fish, would convert the course of changes into a relatively smooth curve. From 36 to 168 hours after transfer chloride levels fell smoothly and had returned to approximately the fresh water level at the last sampling time.

(c) The Influence of Weight and Body Proportions on Changes in Plasma Chloride Concentrations Following Entry Into Sea Water

In many cases the effects of environmental variation upon fish have been shown to be size-dependent. Keys (1931) studied the relation of weight and relative head length upon the resistance of *Fundulus* to asphyxiation and dilution of their medium. Larger fish were found more resistant to hypoxia, while smaller animals were more tolerant of dilution. Shepard (1955), working with *Salvelinus fontinalis*, has corroborated Key's findings with regard to size-dependent resistance to low oxygen tensions. Job (1955) also working on the latter species demonstrated a similar influence of size on the relationship of temperature to changes in basal and active metabolism. Fontaine (1930), Huntsman and Hoar (1939), Black (1951 a), and Morris (1956) have provided data relative to the adjustment of the diadromous species *Petromyzon marinus*, *Salmo salar*, *Oncorhynchus keta* and *O. kisutch*, and *Lampetra fluviatilis* respectively, to transfer between fresh and sea water. Finally Hickman (1958) has shown a size effect on changes in plasma osmotic pressure and thyroid activity in flatfish moved from fresh to sea water, and from brackish water to concentrated sea water.

To investigate the influence of size and body proportions upon changes in chloride content of the extracellular fluids of Steelhead trout shifted from fresh to sea water, measurements have been made of weight, fork length, and operculum lengths. Changes in chloremia have been related to weight,
Fig. 9a. Influence of condition upon percentage changes in chloremia of Steelhead trout following transfer from fresh to sea water.

Fig. 9b. Influence of weight upon percentage changes in chloremia of Steelhead trout following transfer from fresh to sea water.

Fig. 9c. Influence of operculum to fork length ratio upon percentage change in chloremia of Steelhead trout following transfer from fresh to sea water.
coefficient of condition, and operculum-fork length ratios. Coefficient of condition has been taken as a measure of the "leaness" or "fatness" of individual fishes, while the operculum-fork length ratio has been considered to give a rough approximation of relative branchial areas. Plasma chloride concentrations have been regressed upon these three variables and values calculated for standard weights (40, 70 gm.), coefficients of condition (0.7, 0.9 gm/cm$^3$) and operculum to fork length ratios (1.08, 1.20). Figure 9 summarizes changes in chloride concentration as percentages of fresh water levels. Appendix Tables X, XI, XII, include the original chloremias for standard animals.

A clear divergence between both degree and duration of the hyperchloremic condition of animals of low and high coefficients of condition is shown in Fig. 9 a. (Appendix Table X). The former group exhibited an increase of 14.8% over their fresh water chloride level, while the "fatter" fish underwent an increase of 26.3%. The duration of the hyperchloremic state was about 100 hours in the "lean" animals, while the second group still displayed relatively high chloride levels at the last period of observation (168 hours).

The effect of weight is shown in Fig. 9 b. Except in one case 22 hours after transfer, the chloride curves follow each other closely, that of the 70 gm. fish being about 5% higher than that of the 40 gm. animals. A somewhat greater divergence is apparent after 86 hours, suggesting either a levelling off in a somewhat hyperchloremic state, or greater duration of this condition. Achievement of chloride regulation in the smaller fish was essentially complete by 168 hours. At this time the 70 gm. animals still exhibited chloremias 8.4% above fresh water levels.

The effects of relative gill area are somewhat less obvious than those of weight and condition (Fig. 9 c, Table XII). The maximum increase seen in
low ratio fish was 30.4%, and took place 36 hours after transfer. Fish with an operculum fork length of 1.20 exhibited a maximum increase of 23.8% 15 hours following their introduction into sea water. Return to pre-transfer chloride levels was complete after 168 hours in this group. Low ratio animals had not achieved regulation at this time and points at 126 and 168 hours suggest that plasma chloride concentrations may have levelled off at about 110% of the fresh water level.

In summarizing these findings it would appear that all three factors, weight, condition, and relative gill areas affect the extent to which chlorides are taken up and retained by the fish. Since fish of low weight and coefficient of condition, and relatively high operculum-fork length ratio appeared to be best suited for adjustment to sea water it was of some interest to determine whether these conditions are characteristic of any one phase of the species life history.

A plot of fork length against weight revealed a gradual change in this relationship, with the most abrupt variation occurring between 30 and 40 gms. (Fig. 10 a). Similarly, changes in coefficient of condition with weight indicated a decrease in condition within the same weight range (Fig. 10 c).

Hoar (1939) has investigated variation in condition of Atlantic salmon at various ages and phases of development. Parr exhibited relatively high coefficients while parr-smolt transformation was accompanied by a distinct decrease in condition. Weight-length relations in Steelhead trout appear to resemble those of Atlantic salmon. The smallest fish (20 - 30 gm) exhibited relatively high coefficients, while those in the 30 - 40 gm. weight range had the smallest coefficients of condition. For weights over 40 gm. a steady increase in condition was seen.

Changes in weight-branchial area relation were somewhat different,
Fig. 10a. Length-weight relationship of Steelhead trout.

Fig. 10b. Coefficient of condition-weight relationship of Steelhead trout.

Fig. 10c. Operculum to fork length ratio (x 10) - weight relationship of Steelhead trout.
(Fig. 10c), as the most marked changes occurred between 50 and 60 gm.

Price (1941, reviewed by Fry 1957) has shown an inverse relationship between weight and branchial area in Micropterus dolomieu. This was related to inter-lamellar growth, a phenomenon producing a decrease in the number of lamellae per unit branchial filament.

These data indicate the occurrence of growth stanzas in the development of Steelhead trout. Martin (1949) has studied growth in salmonid fishes, and describes five such stanzas. The inflection points coincided with the eyed-egg stage, hatching, ossification, and sexual maturity. In this case, however, the inflection point seems more likely to be associated with parr-smolt transformation.

Maher and Larkin (1954) have provided additional support for this conclusion. In investigations of Steelhead trout populations of the Chilliwack River, British Columbia, they found 60% of the juvenile fish to migrate in March and April of their second year, at an average length of 16.49 cm. Interpolation of this value into the length-weight curve for the population used in this study gave a mean weight of about 35 gm., almost exactly the inflection point of the curve.

(d) Tissue Chloride

(1) Fresh Water

A sample of sixty Steelhead trout was used to establish normal tissue chloride levels in fresh water. This group was the same as that used for plasma chloride analysis and hence consisted of two sub-groups; one including 25 to 70 gm. animals, sampled in June and July, 1957; and the second consisting of fish weighing from 70 to 250 gm., sampled during February and March, 1958. Inspection of the data (Fig. 11) indicated no variation in chloride level which could not be attributed to size. Seasonal variations
Fig. 11. Weight-related variation in muscle chloride levels of Steelhead trout in fresh water.

Fig. 12. Muscle chloride levels of 55 gm. Steelhead trout following transfer from fresh into sea water; vertical bars equal one standard error of regression.
SAMPLED JUNE-JULY 1957
SAMPLED FEBRUARY-MARCH 1958

WEIGHT, GMS

CL⁻, mM/KGM

FRESH WATER

HOURS IN SEA WATER

CL⁻, mM/KGM
in tissue chloride level were not indicated.

Smaller animals contained considerably more chloride per kgm muscle than did larger animals ( \( b = -0.1827 \) over the range 30 to 70 gm.), but unlike the situation with plasma levels of the same ion no abrupt variations with weight were apparent. A steady decrease from about 28 to 10 mM/kgm occurred between the range 30 to 110 gm. Thereafter tissue levels were relatively steady at about 8 to 13 mM/kgm.

Inasmuch as chloride ion in muscle is confined largely to the extracellular phase (see Methods, D-1), and chloride analyses were made primarily as a means of estimating this entity, a discussion of size-related variation in fresh water tissue chloride levels will be reserved for a later section (Section Three - C). It may be mentioned here however, that values obtained in this investigation are in reasonably good agreement with those recorded by Fontaine (1951) for a related species, \( S. \) salar. Tissue concentrations of 9.37, 8.94 - 8.97, and 7.28 - 7.33 mM/kgm fresh tissue were indicated for early and late parr, and migrating smolts respectively. Unfortunately, Fontaine provided neither the weights of his experimental animals nor the analytical procedure used. Malcolm (1957) reported values of 3 to 5 mM/kgm fresh tissue for another salmonid, Salvelinus alpinus, in fresh water. These however, appear to have been obtained by use of the open digestion procedure and are probably low (see Section Two a - 2). Better agreement was found with the value of 24.7 mM/kgm reported by Nishie and Harris (1955) for the muscle of \( E. \) electricus.

(2) Sea Water

Transfer into sea water was accompanied by an immediate increase in tissue chloride content. Figure 12 summarizes changes in tissue concentrations of 55 gm. animals. Additional values for 40 and 70 gm. fish are
A peak chloride level of 38.8 mM/kgm was recorded after 36 hours in sea water, and represented an increase of 78.0%. This peak coincides, as might be expected, with that for plasma chloride, and is believed to be the reference point for similar physiological condition of chum salmon fry (14 hours) and Steelhead trout smolts (36 hours) during their adaptation to sea water.

Two phases of uptake were apparent; a rapid initial phase between entry and 4 hours in sea water during which the net tissue chloride uptake rate was 2.49 mM/gm/hour, and a slower phase from 4 to 36 hours after transfer during which the net uptake rate was 0.18 mM/kgm/hour. Stable tissue levels were reached 86 hours after transfer, and were about 12% above pre-transfer concentrations. Malcolm (1957) reported values for Arctic char in sea water ranging from 6 to 22 mM/kgm (mean, 14.8) on five fish. Comment has already been made regarding the probable validity of these estimates. Fontaine (1951) obtained values of 10.8 and 11.1 mM/kgm on two specimens of Atlantic salmon two to three days after passage into sea water. These levels were approximately 20% above his fresh water records, but probably indicate that the fish were still in the process of regulation, rather than at stable marine levels.

Size influenced both net rate of uptake and percentage increase in concentration in much the same way as was seen for chloremias. Smaller fish (40 gm.) exhibited smaller maximum percentage increase in tissue chloride (75.4%) than did either 55 gm. and 70 gm. trout (78.0%, 95.3% respectively). Similarly over the initial rapid phase of chloride uptake 70 gm. fish displayed uptake rates of 3.58 mM/kgm/hour while 55 gm. fish took up chloride at 2.49 mM/kgm/hour. The stable chloride levels of 40 and 55 gm. animals were 13.1% and 18.4% above pre-transfer concentrations respectively (mean of 86
and 126 hour points).

Data on tissue chloride changes in sea water, therefore, tend to bear out the generalizations made concerning changes in chloremias. Smaller animals exhibited less rapid uptake, and did not undergo as great percentage changes as did larger animals.

(e) Body Fluids

Body fluids may be partitioned into two major phases; intracellular and extracellular. The intracellular fluids include the water and accompanying solutes of the cell proper and are therefore the site of the bulk of the organisms' metabolic activity. The extracellular fluids are all those lying outside the cellular boundary. This phase must have an anatomical distribution, but it has proven more useful, from the dynamic point of view to consider the extracellular fluid as an entity with physiological boundaries.

As commonly defined (Gamble 1951, 1958, Nichols et al 1953, Manery 1954, Elkington and Danowski 1955, Cheek et al 1957) the extracellular fluids are considered to include the plasma water, an extravascular compartment which rapidly equilibrates with large molecules such as inulin (interstitial fluids) and a second extravascular phase which equilibrates slowly with such substances (connective tissue). In many cases, however, transcapillary transfer rates of inorganic electrolytes are sufficiently rapid that the intravascular and interstitial components, and possibly the connective tissue phase as well, may be considered dynamically as a single unit. (Armstrong et al 1952, Thomas et al 1952, Manery 1954, Comar 1955, Copp 1957).

The extracellular space comprises the "milieu interieur" within which cellular processes operate. The chief function of this entity appears to lie in the maintenance of relatively constant internal environmental conditions, and in the provision of points of contact between most of the cells of
the body, and the organs of exchange and the external environment. The intravascular and interstitial components together make up an elastic and expansible compartment which may function in regulating the size and osmotic pressure of the cellular phase. In the words of Manery (1954) "... One must recognize the possibility that very large volumes (of water) must move extremely rapidly between compartments when osmotic imbalances occur ...".

In this study variations in plasma water content and in the volumes of chloride space and intercellular phases (total muscle-chloride space) have been investigated following the entry of fish into sea water.

(1) **Plasma Water**

Plasma water levels of Steelhead trout in fresh water were established simultaneously with plasma and tissue chlorides. As was the case with the former determinations, some size effect was apparent (Fig. 13). Plasma water content fell as body weight increased ($b = -0.2194$ Appendix Table XIV). Size-related variations in plasma levels of water did not resemble those in chloride content, inasmuch as no sharp break was seen between 30 and 40 gm. A steady downward trend from 30 to about 100 gm. was apparent. In fish heavier than 100 gm. plasma water concentration was relatively size-stable, and varied from 920 to 945 gm/kgm.

Figure 14 a indicates variations in the water content of the plasma of 55 gm. Steelhead trout following their transfer into sea water. Appendix Table XIV summarizes data for 40 and 70 gm. fish. Unlike the situation seen with changes in chloride levels under the same conditions there was no immediate rise in plasma water. Following an abrupt increase in environmental tonicity plasma water content changes must be the net of two processes; depletion due to osmotic transfer from circulating fluids to environment, and increases resulting from transfer from cells to extracellular
Fig. 13a. Weight-related variation in plasma water levels of Steelhead trout in fresh water.

Fig. 13b. Weight-related variation in muscle water content of Steelhead trout in fresh water.

Fig. 13c. Weight-related variation in intracellular phase (muscle water - chloride space) of Steelhead trout in fresh water.

Fig. 13d. Weight-related variation in chloride space of Steelhead trout in fresh water.
fluids. The level seen after 4 hours in sea water suggested that the two processes were in equilibrium at that period. Subsequent increases in plasma water may indicate more rapid transfer than loss.

Unlike the condition with regard to plasma chloride, plasma water did not return to the pre-transfer level. The mean water content of samples taken 86 hours and 126 hours after transfer was 959.6 gm/kgm, 1.39% above fresh water levels. This increase was not, however, statistically significant.

(2) Chloride Space

Figure 13 summarizes variations in the chloride space of the muscle of trout in fresh water. Few estimates of either chloride space or any other means of defining the extracellular phase have been made in fish. Nishie and Harris (1955) have indicated values of 12.3 and 12.6% for the chloride spaces of the muscle of *Electrophorus electricus*. Chloride spaces of the electric organs were much greater (46.7 - 76.9%). Data provided by Fontaine, Callamand and Vibert (1950) and Fontaine (1951) for Atlantic salmon allow the estimation of chloride spaces by Conway's procedure (see equation 3, Methods, D-1). The values calculated from these data, 63.4 to 83.3 gm/kgm. muscle, were in reasonable agreement with those found in the larger Steelhead trout used in this investigation. A space of similar volume (96 gm/kgm.) has been suggested by the work of Hayes and Joudry (1952) on phosphorus metabolism in *Salvelinus fontinalis*.

Weight-related variation in the volume of chloride space was marked (Fig. 13 c, Appendix Table XV, b = - 1.2748). Chloride space volumes fell from about 165 gm/kgm. in animals weighing less than 40 gm. to about 130 gm/kgm. in 70 gm. fish. Volumes of between 50 and 90 gm. were characteristic of trout heavier than 100 gm. A similar decrease, although less marked,
Fig. 14a. Plasma water levels of 55 gm Steelhead trout in fresh water and following transfer into sea water; vertical lines equal one standard error of regression.

Fig. 14b. Intracellular (muscle water - chloride space) phases of 55 gm Steelhead trout in fresh water and following transfer into sea water; vertical lines equal one standard error of regression.

Fig. 14c. Chloride spaces of 55 gm Steelhead trout in fresh water and following transfer into sea water; vertical lines equal one standard error of regression.
was implied by recalculation of data provided by Fontaine (1951) and Fontaine, Callamand and Vibert (1950) for plasma and tissue chloride concentrations of Atlantic salmon. Early parr had chloride spaces of 83.3 gm/kgm. Mean values for late parr ranged from 79.4 to 79.7 gm/kgm., while those of actively migrating smolts were 63.8 gm/kgm.

Findings relative to growth-or maturation-related decreases in chloride space have been reviewed in Section One (Barlow and Manery 1954, Daniel and Daniel 1955). The changes noted here may be considered from this point of view. An alternate explanation should also be considered. Fontaine and his co-workers have suggested that migration of anadromous and catadromous fishes may be initiated by decreases in fresh water osmoregulatory efficiency, leading to or resulting in a generalized "demineralization." This in turn was thought to lead to increased activity which subsequently brought the migrants seaward where their osmoregulatory difficulties were alleviated by increased salinity. This theory is far from established, and will be considered in the Discussion.

Figure 14 c summarizes variations in the volume of the chloride space following transfer of 55 gm. Steelhead trout from fresh water to sea water. Additional data for 40 and 70 gm. fish may be found in Appendix Table XV. Changes in chloride space occurred in three stages; an initial rapid increase in volume immediately following entry-into sea water, a second stage during which continued increase in volume continued at a slower rate, and finally, return of the chloride space to approximately pre-transfer levels. The mean of the last two observations indicated a value of 158.8 gm/kgm., a level of 8.1% above the fresh water level.

Comment upon the effects of weight upon changes in plasma chloride concentration has already been made. Size-dependent differences were present
in the percentage variation of chloride volume of 40, 55, and 70 gm. fish. The maximum increase in 40 gm. trout was 42.6%, that in 70 gm. fish 83.3%. Percentage increases in smaller animals were consistently less than those in heavier fish, and return of the chloride space to fresh water levels was more rapidly attained by lighter fish. These data again bear out the conclusion that adjustment of migrants to sea water is keyed to specific stages in their physiological development.

Figure 15 indicates more clearly the two phases of volume change in 55 gm. Steelhead trout during their initial 40 hours in sea water. The line-of-best-fit through points taken between 4 and 36 hours in sea water can be described by the equation: \( Y = 214.12 + 0.1153X \). On the basis of values interpolated from this equation, the rate of change of the chloride space, \( \frac{d\text{(ecs)}}{dt} \), during the initial rapid phase was +17.85 gm/kgm/hour. The rate of change over the slower phase was much less, +0.12 gm/kgm/hour, but nevertheless indicated continued recruitment of volume. The source of this additional water will be shown in the following section to have been in the intracellular compartment.

(3) Intracellular Phase (Tissue Water - Chloride Space)

Variations in the volume of the intracellular phase (total muscle water - chloride space) of trout in fresh water are indicated in Figure 13 d. Despite considerable scatter a trend towards higher cellular volume with increases in weight is apparent. As was the case noted previously for size-related variations in chloride space these changes are consistent both with the data provided by Barlow and Manery (1954) on changes in chick muscle and that of Daniel and Daniel (1955) on mammalian muscle, and the "demineralization" hypothesis of Fontaine. The problem of age-related changes in water distribution will be considered in the Discussion.
Fig. 15. Rapid and slow phases of change in extracellular and intracellular compartments of 55 gm Steelhead trout following transfer into sea water.
\[
\frac{d(\text{ics})}{dt} = -16.86 \text{ GM/KGM/hr}
\]
\[
\frac{d(\text{ics})}{dt} = -0.67 \text{ GM/KGM/hr}
\]
\[
\frac{d(\text{ECS})}{dt} = +0.12 \text{ GM/KGM/hr}
\]
\[
\frac{d(\text{ECS})}{dt} = +17.85 \text{ GM/KGM/hr}
\]
Figure 14b summarizes changes in the intracellular volume of 55 gm. trout following their transfer into sea water. Additional data for 40 and 70 gm. animals is recorded in Appendix Table XV. The sequence of changes is divisible into three components: an initial rapid loss of water, a secondary continuing loss at a lower rate, and finally restoration of the intracellular volume to approximately fresh water levels. The mean of points at 86 and 126 hours was 2.1% below the fresh water level.

Figure 15 indicates changes over the first two phases of loss. Data taken from 4 to 36 hours after transfer may be described by the equation,

\[ Y = 598.39 - 0.8322X \]

Using values interpolated from this equation, the rate of changes of intracellular volume over the rapid phase of loss was \(-16.86\) gm/kgm/hour. The rate of loss over the second period was considerably smaller, \(-0.67\) gm/kgm/hour.

Loss of water from the intracellular phase may be attributed to (1) transfer from the intracellular compartment to the extracellular compartment in the restoration of isomolarity, (2) overall loss of water to the environment. The initial rates of loss from the cells and increase in body fluids (\(-16.86\) and \(+17.85\) gm/kgm/hour) are in close agreement and suggest that overall water loss was slight during this phase. During the second phase of change the discrepancy between rates was much greater (\(d(ics)/dt = -0.67\) gm/kgm/hour, \(d(ecs)/dt = +0.12\) gm/kgm/hour), and suggested that much of the water transferred from the cells into the extracellular fluid was eventually lost to the environment. The rate of water loss estimated as the difference between \(d(ics)/dt\) and \(d(ecs)/dt\) was \(-0.55\) gm/kgm/hour. Assuming an initial water content of 790 gm/kgm. muscle (from Fig. 13b) this rate of loss corresponded to 0.7% /hour.
(4) Chloride-Potassium Space

The assumptions underlying the usage of the chloride-potassium space as a means of calculating extracellular volume and the method involved in its determination have been outlined under Methods.

Trout sampled in February and March, 1958, were in some cases sufficiently large that both plasma chloride and potassium concentrations could be determined on individual fish. Comparisons have been made, therefore, on the extent and direction of the shifts in phase volume indicated by the two approaches.

The mean chloride space of fresh water trout was 87.8 gm/kgm., while that of the chloride-potassium space was 63.1 gm/kgm. The discrepancy between these two values may be attributed to the inclusion of chloride in the intracellular phase by the latter technique. Cellular water volumes predicted on the basis of the two values differed by 3.6%. The corresponding volumes for trout which had been in sea water for 36 hours were 169.3 gm/kgm. (chloride space) and 138.1 gm/kgm (chloride-potassium space). The variation in intracellular volumes was 5.5%.

These data indicate that some variation in extracellular volume estimates arises from the inclusion of part of the tissue chloride concentration in the cellular phase. This result might be expected, but the results nevertheless indicate that the pattern of shifts described by the two approaches was essentially the same. Moreover, the changes in extracellular volume resulting from transfer of the fish into sea water were nearly the same (81.5 and 76.0 gm/kgm.). The conclusion can be drawn that variations in chloride space adequately outlined the pattern of changes in phase volume taking place in fish following their entry into sea water.

As was noted under Methods comparison of extracellular volumes in
fish in fresh and sea water estimated by the chloride space technique depends upon the assumption that increases in cellular chloride concentrations do not parallel those in extracellular concentration. Usage of chloride-potassium space values allowed the estimation of intracellular chloride levels. The concentration derived for cellular chloride of fresh water fish was 5.5 mM/L., a level in good agreement with those described by Conway (1957) for mammalian muscle (1.5 - 5.0 mM/L). The value for fish in sea water was somewhat higher, 7.0 mM/L. The total amounts of cellular chloride, however, did not vary significantly in these two cases due to the decrease in intracellular volume (3.9 mM/kgm. in fresh water, 4.1 mM/kgm. in sea water). The author felt therefore that valid comparisons could be made of chloride space changes.

(f) Sodium and Potassium

Plasma and tissue levels of sodium and potassium, chloride and water were determined simultaneously, whenever the volume of plasma samples was sufficiently large, on two groups of trout, one of which was held in fresh water and the other acclimated to sea water for a period of 36 hours. The latter interval was chosen as representing the period during which maximum deviations from fresh water levels and electrolytes might be expected.

Of the 60 fish sampled in this programme values have been obtained as follows; fresh water, plasma chloride - 23, plasma water - 24, plasma sodium and potassium - 24, tissue chloride - 21, tissue sodium and potassium - 12, sea water, plasma chloride - 23, plasma water - 25, plasma sodium and potassium - 22, tissue chloride - 35, tissue sodium and potassium - 18. In fish for which plasma and tissue chloride, water and cation levels were available cellular cation concentrations were calculated. Potassium concentrations
are reported for 11 fish in fresh water, and 13 fish in sea water, sodium levels for 7 trout in fresh water and 11 in sea water.

These fish constituted the second group of Steelhead referred to under Section Three, a - 1, and were sampled during February and March, 1958. Reference to Figures 7, 11, 13 a, b, c, d, and to Figures 16 and 18 indicates that, in general, little size-related variation was present in tissue and plasma electrolyte and water levels. Sample means have therefore been recorded for this group of fish rather than values interpolated from regression lines for standard weight animals as was the case for trout sampled in June and July, 1957.

Seasonal variations in the effects of environmental variation on fish have frequently been demonstrated (eg. Brett 1944, Hoar 1955 on seasonal variations in temperature resistance). The possibility existed, therefore, that a difference in either type and/or extent of stress induced by transfer into sea water might be present between the first and second groups of Steelhead. The data presented below suggest that some discrepancies between the two groups did exist, but that the general pattern of events induced by sea water was essentially the same in both cases. Plasma chloride levels of February-March fish held in fresh water agreed reasonably well with those recorded in the earlier group, and constituted a prolongation of the chloremia-weight relationship described in Figure 7. A similar situation was apparent for tissue chloride concentrations as well (Fig. 11). Following transfer into sea water chloremia rose to 162.5 mM/L. The corresponding value for 70 gm. June-July trout was 168.2 mM/L. The mean tissue chloride level after 36 hours in sea water was 29.3 mM/kgm. The effects of sea water on tissue and chloride levels would seem therefore to have been similar in both cases. The chloride spaces of the older fish did not have a linear relationship with
To follow page 44.

Fig. 16. Weight-related variation in plasma and tissue sodium and potassium levels of Steelhead trout in fresh water.
those calculated for the June-July sample. A flattening of the chloride space-weight curve occurred between about 90 and 110 gm. (Fig. 13 c). Similarly the intracellular spaces (total muscle water - chloride space) of the February-March trout are not strictly comparable to those of younger fish for the same reason (Fig. 13 d). These discrepancies between phases are believed to be related to the demineralization of the fish in fresh water and will be considered in the Discussion. The increase in chloride space of fish held in sea water for 36 hours was of the same magnitude in both cases (81.5 gm/kgm. for February-March fish, 69.9 gm/kgm. for 70 gm. fish sampled in June and July). Similarly the change in chloride-potassium space was 75.0 gm/kgm. Much the same changes are also apparent in the intracellular spaces of both groups after equal periods in sea water; chloride space, 70 gm. June-July fish, 93.9 gm/kgm., chloride space, February-March fish, 94.1 gm/kgm., chloride-potassium space, February-March fish, 87.7 gm/kgm. Decreases in muscle water content were also of the same magnitude in both sets of trout. The most marked variation between the results of transfer into sea water was found in changes in plasma water content. The level of water in the plasma of 70 gm. fish went from 943.1 gm/kgm. to 971.3 gm/kgm. after 36 hours in sea water. In the February-March sample plasma water concentration decreased slightly from 932.1 to 912.9 gm/kgm.

Notwithstanding these last discrepancies the bulk of the data indicate that despite growth and possible changes in water and electrolyte balance in the older fish transfer into sea water invoked comparable variations in electrolyte levels and water distribution in both groups of trout.

(1) Sodium Levels in Fresh Water and Sea Water

The mean sodium level of the plasma of Steelhead trout was 162.5 mM/L., a level in good agreement with those reported by Field, Elvehjem and
Juday (1943, 156 mM/L.) and Phillips and Brockway (1958, 154.7 mM/L.) for a related species, the brown trout (_S. trutta_). This value also falls well within the range of sodium concentrations recorded by Vinogradov (1953) for a variety of fresh water species.

The mean sodium-chloride ratio of Steelhead was 1.202. Data from the studies by Field, Elvehjem and Juday, and Phillips and Brockway (loc. cit.) were 1.37 and 1.36 respectively. The former authors also give values for these ions in _Cyprinus carpio_ which indicate a ratio of 1.15 in this species. Recalculation of concentrations recorded by Robertson (1954) and Forster and Berglund (1956) gave sodium-chloride ratios for several marine and fresh water species as follows: _Lampetra fluviatilis_ - 1.24, _Muraena helena_ - 1.12, _Coregonus clupeoides_ - 1.21, and _Lophius americanus_ (= _L. piscatorius_) - 1.12. The ratio recorded here was not therefore unusually low for fish, although somewhat less than that seen in some higher vertebrates.

Following transfer into sea water mean sodium concentration rose slightly to 170.0 mM/L. The mean sodium-chloride ratio, however, fell from 1.202 to 1.046. Since under normal conditions the sum of plasma anions and cations must be equal to maintain electroneutrality a sharp departure from this condition is suggested by these data. A concomitant fall in bicarbonate (the second most prevalent plasma anion) would alleviate this suggested condition. Such a situation has been recorded by Fontaine and Boucher-Firly (1943) who found that the alkaline reserve of the plasma of _Anguilla vulgaris_, when measured as bicarbonate carbon dioxide, fell following the movement of this species into sea water. A decrease in bicarbonate ion of this magnitude while decreasing the possibility of a plasma anionic excess may result in a condition of acidosis. This may be a true acidosis resulting from a decrease
in the bicarbonate concentration of the blood, or it may be a compensated acidosis in which the fall in bicarbonate ion is paralleled by a fall in carbonic acid as well. In the latter case no "clinical" effects would be apparent. The possible effects on acidosis in fish adjusting to sea water must, however, be considered.

Since plasma sodium concentrations did rise in sea water fish the conclusion may be drawn that this ion is not in some way excluded. The variation in sodium-chloride ratio may then be accounted for in a number of ways, all of which turn upon various mechanisms whereby fish may reduce the amount of circulating sodium in their extracellular compartments. Active excretion of sodium via branchial components has been described frequently (Krogh 1939, Meyer 1951, Black 1951 b, 1957). Active transport of chloride may be initiated more slowly, or proceed at a lesser rate than that of sodium, and should be considered as a possible explanation. Sodium may also be withdrawn from the circulating fluids by complex-formation with either soft tissues or with osseous elements.

Data available upon the sodium concentrations of muscle suggest the likelihood that considerable amounts of sodium are actually taken up by muscle cells. The tissue levels of sodium in fresh water and sea water trout were 81.4 and 97.8 mM/kgm. fresh tissue respectively. These values were considerably higher than the levels commonly noted for skeletal and myocardial muscle of mammals (Daniel, 1958), and the value reported for Electrophorus by Nishie and Harris (1955). Better agreement was found with the range of values recorded by Daniel (loc. cit.) for various types of smooth muscle (70.7 - 110.4 mM/kgm.). Fish muscle also resembled uterine muscle inasmuch as it was found to contain a relatively high content of water. By contrast chloride values were closer to those of striated than smooth muscle.
Utilizing values for "extracellular" space derived from measurements of chloride and chloride-potassium spaces estimates were made of cellular sodium content. Using chloride space values cellular concentrations of 77.8 and 114.1 mM/L; cell water were indicated for fish in fresh and sea water respectively. Corresponding levels based upon the chloride-potassium estimate of the volume of the extracellular phase were 99.2 and 117.6 mM/L. Due to the high sodium content of the tissues the values derived for cellular concentrations are unusually high when compared to those reported by Manery (1954), Nishie and Harris (1955) and Daniel (1958) for skeletal muscle. Concentrations indicated by Daniel for smooth muscle, however, include those reported here. Despite the discrepancies noted above, the data indicate the sequestration of sodium in cells. That the values recorded above were not solely the result of concentrations of existing cellular sodium by cellular dehydration was indicated by recalculation of cellular sodium concentrations on the basis of fresh water intracellular volumes. Based upon chloride space estimates the level obtained in this way was 99.6 mM/L; that based upon chloride-potassium estimate of extracellular space was 103.1 mM/L. In neither case can cellular dehydration account for the enhanced cellular sodium levels exhibited by fish in sea water.

An estimate may be made of the role of increases in extracellular space in accounting for the "sodium load" of fish transferred into sea water. Since increases of about the same amount were found in both chloride and chloride-potassium spaces no distinction will be made between the two types of estimate. The muscle "load" of sodium taken up by Steelhead trout after 36 hours in sea water was 16.6 mM/kgm. Of this 0.7 mM/kgm was taken up in the concentration of the fresh water chloride space to the level noted in fish in sea water. An additional 13.2 mM/kgm. may be attributed to the
increased volume of the extracellular space, leaving 2.7 mM/kgm. to be accounted for by cellular uptake. Thus, increases in extracellular space must have accounted for the bulk of the sodium (about 80%) taken up by the fishes, and this illustrates the important role played by this entity in the internal regulation of electrolytes.

(2) Potassium Levels in Fresh Water and Sea Water

The mean potassium concentration of the plasma of Steelhead trout was 6.04 mM/L., a level comparing favourably with those reported by Field, Elvehjem and Juday (1943, 5.1 mM/L.) and Phillips and Brockway (1958, 5.01 mM/L,) for brown trout. Representative values for fresh and marine species indicate that low plasma levels of this ion are characteristic of fish as well as most of the higher vertebrates, e.g., Anguilla, 6.8 mM/L. (Drilhon, 1943), Cyprinus carpio, 4.6 - 5.1 mM/L. (Pora, 1935), 6.3 mM/L., (Field, Elvehjem and Juday, 1943), Lophius piscatorius, 6.88 - 9.0 mM/L. (Vinogradov, 1953).

Mean tissue and intracellular concentrations in fresh water trout were 125.2 mM/kgm and 182.0 mM/L, respectively. These values were somewhat higher than those reported by Nishie and Harris (1955) for Electrophorus, but the tissue levels were in good agreement with those reported by McBride and McLeod (1956) for various species of Pacific salmon. Similarly the range of values recorded by Manery (1954) for skeletal muscle and by Daniel and Daniel (1957) for uterine muscle of mammals includes the concentrations noted here.

Transfer from fresh water to sea water results in a much greater enrichment of sodium than of potassium in the environment of the fish. Elkington and Danowski (1955) have noted sodium-potassium ratios of 100:34-45 for river water and 100:2 for sea water. Similarly Sverdrup, Johnson and
Fleming (1942) report ratios of 100:40 for river water, and 100:3 for sea water. Since both ions enter fish under appropriate concentration gradients, a relatively greater enrichment of sodium than of potassium may also be expected to occur in the body fluids of trout following their transfer from fresh water into sea water. Under such circumstances it has frequently been noted that cellular sodium concentrations increase, while those of potassium fall. Gamble (1951) for instance, has reported that ingestion of sea water by mammals produced increases in sodium and magnesium levels, while those of potassium, phosphate and sulphate decreased. After 36 hours in sea water the mean tissue level of trout had fallen from 125.2 to 116.2 mM/kgm, which is in accordance with the findings of Gamble. The intracellular potassium concentration, however, rose during the same interval from 182.0 mM/L. cell water to 192.0 mM/L. cell water. This apparently anomalous situation may be clarified by recalculation of cellular potassium levels on the basis of fresh water intracellular volumes. The value obtained in this way using chloride space as a measure of extracellular volume in fresh water was 168.1 mM/L. which is in line with the observed decrease in the total tissue level changes of this cation. The noted rise in cellular potassium may therefore be attributed to the concentration of potassium remaining in the cells through cellular dehydration. The gain in cell sodium, 2.7 mM/kgm., bears no obvious relationship to the loss of potassium, 9.6 mM/kgm., and does not indicate linked transfer of these ions.

Concomitant with transfer into sea water the plasma level of potassium rose slightly from a mean of 6.04 mM/L. to one of 7.61 mM/L. This plasma enrichment may probably be attributed to recruitment from either the environment or from the cellular pool.
A marked disparity was found between the sum of cations in extracellular and intracellular phases of fish in both fresh water and sea water. Dependent upon whether cellular volume estimates were based upon chloride or chloride-potassium spaces the differences in fresh water amounted to 89.6 - 103.0 mM/L, and 118.0 - 127.4 mM/L in sea water (Appendix Table XVIII).

If these values for intracellular cation concentrations can be accepted as valid estimates, which seems reasonable, some means of accounting for the discrepancies must be advanced. The simplest hypothesis would seem to be that the volume of distribution for cellular cations was too small, i.e., estimates of extracellular volume included part of the intracellular phase. Since such a criticism has been directed against the chloride space an attempt has been made to redetermine cellular concentrations using a smaller estimate of this critical entity. Cheek, West and Golden (1957) have provided comparative estimates of extracellular volume by inulin, chloride and sodium spaces. The average ratio between inulin and chloride spaces was 0.852. Accepting tentatively that inulin provided a better estimate of extracellular volume than do either chloride or chloride-potassium spaces cellular cation levels have been recalculated on the basis of extracellular volumes of 74.8 and 144.2 gm/kgm. for fresh water and sea water fish respectively. The cation differences arising from this approach were 119.6 and 104.7 for sea water and fresh water fish. A further simple calculation would show that the volume of water necessary to distribute the cellular sodium and potassium in a free solution at the concentration of extracellular fluid is greater than the volume of the tissue in which it is contained. The probability that cation excesses in the intracellular fluid arise from inadequate means of estimating the volume.
in which they are distributed may therefore be regarded as unlikely.

If these values are accepted and reasonable and if it is postulated that they are in free solution within the cells, a condition of hyper-osmolarity of cells relative to extracellular fluids must be accepted. This in turn implies a system for active water transport, since free water transfer into cells would either alleviate the osmotic gradient or result in cytolysis. Robinson (1954) has reviewed literature pertinent to the topic of water transport and while providing data suggesting that such systems do operate in animal cells notes that "... The weakest point in this dynamic theory of the water exchanges of cells is that it has still not been demonstrated directly that intracellular fluids are hypertonic...". Reviews by Conway (1957) and Harris (1956) also lead to the conclusion that cellular and extracellular fluids are essentially isomolar in concentration.

If a condition of cellular hyperosmolarity cannot be accepted the final alternative is that osmotic concentrations of cellular fluids are reduced by the withdrawal of intracellular cations from the cellular osmotic pool. This may be accomplished if a portion of intracellular anion is polyvalent, such that electrical neutrality is accomplished with a relatively slight contribution by intracellular anion to the total intracellular osmotic pool. Alternatively a portion of intracellular cation may be bound and therefore osmotically inactive. The possibility that the latter may be the case has been demonstrated by Gilbert and Fenn (1957) for calcium. Repeated leaching of frog muscle in calcium free solutions and in solutions of EDTA, a calcium chelating agent, indicated that 0.9 mM/kgm. or about 40% of the total calcium in the muscle was non-exchangeable. Similarly Daniel and Daniel (1957) showed that residual sodium amounting to about half the concentration of living muscle was present in mammalian uterine muscle following several hours of soaking in an
isotonic sodium-free solution. In a later publication Daniel (1958) indicated that substantial amounts of both sodium and potassium were bound, or in some other way made unleachable, in smooth muscle. Experiments of the same sort reviewed by Conway (1957) provided evidence that only about one-half of the sodium actually thought to be inside fibers was readily exchangeable with the environmental fluids. The amount of sodium existing within fibers as free ion was estimated as about 1 mM/kgm, or less than 10% of the total tissue concentration.

Earlier work by Stanbury and Mudge (1953) and Mudge (1955) indicated that a considerable fraction of the potassium of liver mitochondria could not be removed with repeated washing in potassium-free leaching solutions.

Finally Klotz (1953) has reviewed data on the question of ion binding by cellular components and come to the conclusion that carboxyl, sulphate and phosphate groups of naturally occurring polysaccharides, proteins and nucleic acids have the ability to combine with sodium and potassium at physiological levels of cation concentration.

An acceptable explanation for the intracellular cation excess noted for the cells of Steelhead trout in both fresh water and sea water must then include the binding of sodium and potassium such that the actual number of osmotically active cations is considerably below the number indicated by the intracellular concentration predicated in the expression of cation concentrations as mM/L. cell water. Since the cation excesses of fish in sea water were some 15 - 40% greater than those recorded for fish in fresh water it is also necessary to postulate the occurrence in the cells of reactive groups capable of binding more cation than they are required to hold in that medium. Klotz (loc. cit.) has provided evidence that certain naturally
occurring carboxylated compounds have the ability to bind sodium and potassium in direct relationship with external cation concentration. Additional binding was, however, found to result in the transformation of molecular structure from a coiled to an extended form, with resultant increases in viscosity and other physical properties. Klotz has suggested that such effects may be related to the extension of protein such as actomyosin during the contraction cycle, and his data are suggestive that cation excesses within cells may result in "fixation" of structure, and a possible resultant decrease in contractile efficiency. Further consideration to this possible influence of cations on muscle efficiency will be given in the Discussion. Engels, Joseph and Catchpole (1953) have verified the ability of tissue "colloid" (defined as including mucoproteins, collagen, and intra- and extracellular protein) to combine with more calcium than they are normally exposed to. Serum proteins, for instance, were found to be about 12% saturated with calcium under normal serum levels of this element. The corresponding values for dense and loose connective tissue were 8% and 5% respectively. Thus it would appear that some tissue components, at least, have the ability to carry much larger amounts of cation than they normally hold.

Results reported in this section are summarized in Appendix Table XVII and in the Yannet-Darrow diagrams of Figure 17. Values for intracellular and extracellular calcium levels in fresh water and after 26 hours in sea water have been appended to this Figure as have those for intracellular and extracellular chloride concentrations.

(g) Calcium

Of the data recorded in this section plasma and tissue calcium were analyzed directly. Extracellular and cellular concentrations have been
Fig. 17. Yannet-Darrow diagrams describing changes in extracellular and intracellular levels of sodium, potassium, chloride and calcium following transfer of Steelhead trout into sea water: solid lines - values for fresh water, dashed lines - values for sea water (36 hours); X-axis - gm/kgm., left side extracellular phase, right side intracellular phase, Y-axis - mM/L.
derived on the basis of volume estimates of extra and intracellular phases by means of the chloride space technique.

(1) Fluid Phase Calcium

(i) Fresh Water

A sample of 24 trout was used to establish calcemias of fresh water animals, (Fig. 18, Appendix Table XVIII). The values obtained were notable for their agreement with those seen in mammals, i.e. 2.2 - 2.7 mM/L, (Elkington and Danowski, 1955) and fall into the range of values reported by earlier workers for fresh water fishes (Vinogradov 1953). The standard error of estimate for the group was 0.32 mM/L, a considerably lower degree of variation than was found for either chloride (8.39 mM/L) or sodium (5.35 mM/L). The influence of size was less marked than that noted for other plasma electrolytes (i.e. b = - 0.0041 for calcium, - 0.3044 for chloride). Unfortunately, the size range of the animals used (47 to 116 gms.) did not extend low enough to indicate any abrupt variations at parr-smolt transformation. The work of Fontaine, Callamand and Vibert (1950) suggests that variations occur at this stage in Atlantic salmon.

(ii) Sea Water

A slight, but statistically insignificant, rise in calcemia occurred 52 to 168 hours after transfer of the fish into sea water (Fig. 19, Appendix Table XVIII). Despite the doubtfulness of this rise it is interesting to note that it may be correlated with the restoration of the extracellular space to pre-transfer levels.

Changes in extracellular space calcium concentrations differed from those in plasma in that a very slight decrease took place between 15 and 26 hours after transfer. Although again of doubtful statistical significance this decrease may be correlated with the rapid increase of the
Fig. 18. Weight-related variation in plasma calcium levels of Steelhead trout in fresh water.

Fig. 19. Plasma and extracellular calcium levels of 55 gm Steelhead trout following transfer from fresh water to sea water; vertical bars equal one standard error of regression.

Fig. 20. Tissue and cellular calcium levels of 55 gm Steelhead trout following transfer from fresh water to sea water; vertical bars equal one standard error of regression.
extracellular volume, as this would tend to produce some degree of dilution (Appendix Tables XV, XIX). Following this initial decrease there was a slight increase in concentration, although on a percentage basis the changes in extracellular calcium concentration were less than those of any other phase (Appendix Table XIX).

The amount of calcium in the extracellular phase varied from 6.4 to 9.4% of the total tissue level. The value in fresh water (8.8%) is in reasonable agreement with the estimation made by Gilbert and Fenn (1957) for the leg muscles of the frog (12%).

The constancy exhibited by fluid phase calcium concentration following transfer into sea water differed markedly from the situation seen in the case of sodium and chloride concentrations. Since transfer into sea water of the salinity used in these experiments (22 - 24°/oo) involved a change in environmental calcium of less than 1 to between 10 and 12 mM/L. (Barnes 1954), two possible explanations exist; either calcium was excluded in some way from the animals, or an extremely efficient extracellular phase calcium homeostatic mechanism operated in the animals. Data to be presented in the following section indicate that calcium exclusion did not occur. The second alternative, homeostasis, will be considered in the Discussion.

(2) Cellular Phase Calcium

(i) Fresh Water

Tissue calcium levels were determined simultaneously with calcemias, and in common with most other tissue electrolytes, calcium varied inversely with weight (b = -0.015 Appendix Table XX). Since there was also some decrease in extracellular volume with weight increase, the possibility existed that the lowering of tissue levels in larger animals might have been a reflection of a smaller contribution of the extracellular phase to the total
tissue level.

An estimate of cellular calcium may be made by correcting tissue values for the amount contained in the extracellular space. This correction may be further refined by partitioning the extracellular space into intra and extravascular components, as calcium concentrations in the former are nearly twice as great as those in the latter. However, even if the total extracellular compartment is considered to be intravascular (which is impossible since the fish were essentially exsanguinated during blood sampling) the correction factor does not in most cases, exceed the standard error of estimate of tissue calcium (Appendix Table XX). This additional refinement has not, therefore, been made.

Cellular calcium values have been expressed in two ways: per kilogram tissue solids, and per litre cell water. Neither method is ideal. The former assumes all tissue solids to be intracellular, and all cellular calcium bound. The latter assumes all calcium to exist free in solution. The work of Gilbert and Fenn (1957) indicates the existence of at least three discrete cellular phases, the surface, intracellular, and non-exchangeable fractions. The two representations chosen, are however, at least useful indications of variations in cellular concentrations.

When calculated in this manner the suspected decrease in cellular calcium with size is made more apparent (Appendix Table XXI). No explanation can be advanced to account for this situation.

(ii) Sea Water

Following transfer into sea water a sharp rise in tissue calcium (Appendix Table XX, Fig. 20) took place. Net exchange rates varied, dependent upon the mode of expression of cellular concentrations, but were uniformly high and positive over the interval 0 to 26 hours (Appendix Table XXII). Thereafter, a decrease occurred, and all net rates were negative from 100 hours onward. Cellular calcium when expressed per litre cell water peaked at 26 hours, and fell steadily from that point onward. When expressed per
kilogram fresh tissue and per kilogram tissue solids, the peak occurred at 100 hours, and had fallen somewhat by 168 hours.

To some extent, and particularly when cellular concentrations are given in terms of cell water, the increased concentration must be due to decreases in intracellular volume. Recalculation of cellular water concentrations of fish in sea water on the basis of an intracellular phase of 651.8 gm/kgm. (fresh water level) gave an indication of the extent to which concentration by cellular dehydrations had taken place. The data suggest that this effect would not account for more than 15% of the observed rise in concentration. An actual, as opposed to an apparent, increase in cellular calcium must, therefore, have occurred.
IV. DISCUSSION

Transfer of juvenile Pacific salmonids from fresh water into sea water produced an immediate, and marked depression in motor performance. Essentially complete recovery from this initial depressive action of sea water was apparent, but over the duration of observations the fish did not again attain the level of performance displayed in fresh water. The relatively stable values recorded from 36 to 80 hours after transfer suggest a permanent depression of locomotor ability.

These changes in performance were associated with massive increases in total body electrolyte levels and with some loss of body water. Correlation was particularly good during the downward trend of performance (i.e. from 0 to 14 hours in sea water). Sustained high levels of chloride and low values for performance tended to flatten the correlation during the recovery phases. More detailed studies on the sequence of events in the osmoregulatory adaptation of salmonids to sea water indicated that the initial response to sea water was primarily adjustive (Prosser 1955) at the cellular as well as at the total body level. Extracellular concentrations of chloride, sodium and potassium rose to varying extents. Cellular dehydration occurred presumably as an alleviatory response to the tendency toward hyperosmolarity of body fluid relative to cells caused by increase in extracellular concentrations. This was accompanied by raised cellular cation concentration. The latter was primarily due to cellular dehydration in case of potassium, but was correlated with actual cation uptake in the cases of sodium and calcium. The data also infer that decreases in extracellular bicarbonate and a possible metabolic acidosis were characteristic of this adjustive phase of osmoregulatory adaptation. In addition the enrichment of the magnesium environment of the fish, and the
studies of Gamble (1951), strongly suggest that increases in extracellular and cellular concentrations of this ion may have accompanied adjustment to sea water. The extent and duration of adjustment to raised internal electrolyte concentrations appeared to be influenced by size in both species studied.

These data suggest the thesis that a causal relationship existed between changes in locomotor ability and changes in electrolyte concentration and distribution. In other words, it is indicated that the "scope for activity" of these fish was depressed by the exigencies of initial adaptation to sea water. The continuing depressive effect of sea water on performance implied by non-recovery of the fish over a long interval of acclimation furthermore suggested that the energy costs of marine osmoregulation were higher than those in fresh water. Finally the effect of size on the successful adaptation of trout to sea water indicated that normal seaward migration might be keyed to rather sharply limited phases in species life history.

(a) The Inhibition Of Motor Performance During Adjustment To Sea Water

The various components of the locomotor apparatus have frequently been shown to be sensitive to variations in their electrolyte environment. Investigations by Heppel (1939), Fenn (1940), Szent-Gyorgyi (1947, 1951), Mammaerts (1950) and Steinback (1953, 1954) indicate that the efficiency of the muscles of intact animals, of excised muscles and of the subcellular components of muscle is profoundly influenced by variations in potassium levels. Moreover the influence of internal and external concentrations of this cation on resting membrane potentials suggests that changes in potassium concentration may produce increases and decreases in neuramnuscular irritability (Green, Giarman and Salter 1952, Hoffman and Suckling 1956, Del Castillo

A considerable body of data also exists which suggests that variations in sodium levels can inhibit the contractile mechanism. Heppel (loc. cit.), Hadju (1953), Steinback (loc. cit.), Regan et al (1955), and Hercus et al (1955) have provided data correlating sodium enrichment in intact animals and in excised muscle with depressed muscular efficiency. However procedures which induce sodium uptake usually also result in potassium loss. Since the latter factor produces decreased muscular effectiveness it is difficult to attribute changes in muscular efficiency solely to increased sodium levels in these cases. While the above studies on whole animals do not permit firm conclusions, investigations carried out on extracted muscle components indicate that relatively slight changes in electrolyte concentration may markedly influence contractile efficiency. In this regard the investigations of Heilbrunn (Heilbrunn 1940, Heilbrunn and Wiercinski 1947) on the precipitation of muscle protein by calcium may be cited. The same author has also shown that under certain conditions sodium ion may decrease muscular irritability (Heilbrunn and Ashkenaz 1941). Bowen (1952) has found that small increases in calcium ion concentration profoundly reduce the shortening of myosin fractions. Ranney (1955) has studied the effects of calcium, magnesium, and sodium on adenosinetriphosphate induced contraction of glycerol-extracted myocardial fibers. Magnesium was found to stimulate tension development within a small range of concentrations (approximately 2.5 to 3.5 mM/L.) and to sharply inhibit it at all others. Similar results were obtained with calcium. At all concentrations exceeding 0.5 mM/L. this ion reduced contraction in the preparation. Sodium at all concentrations produced inhibition of tension development. These findings indicate that virtually all variations in cellular levels of free cation may invoke depression of
contractile efficiency. The work of Szent-Gyorgyi (1951) suggests that this conclusion may be extended to potassium as well since departures from a concentration of 0.16 M/L caused a loss of response to adenosinetriphosphate.

Variation in bicarbonate ion may also produce loss of muscular efficiency. Creese (1949) has shown that under conditions of either constant, or varying pH, loss of bicarbonate from the environment of mammalian muscle decreases its ability to develop and maintain tension. This author attributed this effect to losses in enzymatic efficiency, either through a direct effect, or through a generalized increase in the acidity of intracellular conditions.

Variations in cellular cation levels may reduce muscular efficiency through inhibition of enzyme action. It is generally felt that the breakage of high energy phosphate bonds by the action of the adenosinetriphosphatase enzyme complex on ATP is an important first step in the contractile cycle (see Sumner and Somers 1953, Baldwin 1952, for reviews). Some of the components of this complex are known to be sensitive to changes in cation concentration, particularly those of magnesium and calcium (Szent-Gyorgyi 1947, 1951, Dubuisson 1954, Sumner and Somers 1953, Skou 1957). The work of the latter author also suggests that variation in intracellular ratios of sodium to potassium may influence adenosinetriphosphatase activity, since potassium activation of these enzymes was decreased by increasing sodium levels.

The formation and release of the pre-synaptic transmitter substance, acetylcholine, may be affected by changes in cation concentration (see reviews by Del Castillo and Katz 1956, Eccles 1957). The direct influence of potassium is slight. Similarly the effect of sodium has been difficult to consistently ascertain, and is believed to be related to the influence of
this ion on action potentials (Hodgkin and Katz, 1947). Decreased calcium levels may suppress acetylcholine release (Harvey and MacIntosh 1940, Hutter and Kostial 1954). Increased magnesium levels invariably block this process.

These data then support the hypothesis that some components of the locomotor apparatus are sensitive to variations in electrolyte levels. Observations made in this study, however, tend to eliminate the likelihood of some of the possible inhibitory effects. The absolute changes in cellular (+9.8 to 15 mM/L.) and extracellular (+0.79 mM/L.) potassium concentrations do not appear large enough to seriously affect muscular efficiency. Similarly changes in the ratio (icsK)/( ecsK) were not marked enough to alter resting potentials to any extent (25.7 - 26.9 in fresh water, 24.3 - 25.9 in sea water).

The rise in extracellular space sodium concentration (11.3 mM/L.) did not appear to be sufficiently marked to invoke significant changes in the magnitude of action potentials and subsequent recovery of muscle and nerve.

On the other hand cellular sodium and calcium levels increased by 47.8 and 77.9 % respectively. Assuming some accompanying increase in intracellular free ion concentrations the likelihood of direct calcium and/or sodium inhibition of contractile components seems reasonable. The inhibition of adenosinetriphosphatase may also have contributed to a decrease in muscular efficiency. Both direct ion effects and the antagonism by sodium of potassium activation of this enzyme complex may be considered possible (fresh water icsNa/icsK = 0.51, sea water icsNa/icsK = 0.64). The data of Creese (1947) also suggest that falling bicarbonate levels may have reduced muscular effectiveness.

Magnesium concentrations were not determined in this investigation.
Nevertheless data provided by Elkington and Danowski (1955) indicate a marked enrichment of this ion in sea water, and the concentration gradients existing between sea water and extracellular fluids (approximately 20:1) would tend to promote uptake of magnesium by fish adjusting to sea water. Magnesium inhibition of acetylocholine release is, therefore, a probable though unestablished contributor to the depression of motor activity.

(b) Variations In Scope For Activity

The arguments cited above suggest that inhibition of various components of the locomotor system sensitive to changes in electrolyte concentrations may be an important factor in the depression of activity in sea water. Consideration may also be given to the effects of conversion from fresh water to marine osmoregulatory processes on the general availability of energy required in locomotor activity.

The investigations of Fry (1947, 1957, 1958) suggest that the amount of metabolic energy available for behavioural activities such as swimming is a function of the difference between the maximum rate at which energy sources can be converted into utilizable energy (active metabolic rate) and the rate at which it must be expended in vegetative functions (standard or basal metabolic rate). In several experimental studies on the cruising speed of fish Fry has shown a direct relationship between performance and "scope for activity" (see Fry, 1957, for review). From this point of view depression of locomotor performance in fish adjusting to, or adjusted to sea water may be a reflection of enhanced energy expenditure in marine osmoregulation.

No data are at present available on changes in the "scope for activity" displayed by euryhaline fish during their adjustment to sea water. Consideration of the processes involved in osmotic regulation in fresh water and
sea water may, however, give some indication of their relative energy requirements.

Fresh water fish are faced with the problem of maintaining regulated water and electrolyte concentrations in the face of environmental conditions which tend to deplete the latter while raising the former. Water excesses are reduced primarily by the production of relatively large quantities of hypotonic urine (Smith 1932, Krogh 1939, Forster 1953). The main energy requirements of this process are supplied by the circulatory system, but an unknown amount of energy may also be expended in the outward secretion of water. Salt depletion may arise from two sources; urinary salt loss, and outward diffusion of ions through semipermeable membranes. Tubular reabsorption of anions and cations may decrease salt loss by the first factor (Black, 1957) but electrolyte deficits must necessarily arise in urine production since the process of reabsorption is not 100% efficient. Deficits arising in this manner are alleviated by two processes; dietary intake of salts, and the active absorption of salts from the environment (Keys 1932, McCay et al 1936, Krogh 1937, 1938, 1939, Wikgren 1953, Black 1957).

In sea water, the central osmoregulatory problem is that of restricting water loss and salt uptake. In the alleviation of osmotic water loss from exposed membranes, euryhaline and marine fish customarily ingest and absorb considerable quantities of sea water (Smith 1930, 1953, Keys 1932). Since fish cannot produce a hypertonic urine the renal reduction of the "salt load" incurred in this process would result in a net loss of body water (Potts, 1954). This restriction is circumvented by extrarenal salt excretion. Accepting the values of Elkington and Danowski (1955) for the relative osmolarities of sea water and human extracellular fluid as reasonably representative it becomes obvious that marine fish must actively excrete or in some other way reduce the concentration of ingested sea water by approximately
800 millimols per liter in order to avoid increases in internal tonicity. Data assembled by Black (1957) indicate that the rate of depletion of total body chlorides by urinary loss and outward diffusion in two typical fresh water species, *Salmo irideus* and *Carrasius auratus*, is between 0,1 and 0,3 mM/kgm. body weight/hour. The net muscle chloride increase rate of Steelhead trout during their initial hours in sea water provides a gross estimate of the rate at which chloride is taken up from the marine environment, and therefore of the rate at which it must be excreted in the maintenance of regulated extracellular conditions. The value obtained in this way was 4,2 mM/kgm/hour. Comparison of these rates suggests that the amount of this ion which must be excreted in marine osmoregulation is greater than the amount which must be absorbed by fresh water fish in carrying out the same function. Since Ussing's recent studies (1958) indicate that energy expenditure in electrolyte transport is a function of the number of ions moved rather than the direction of their transport relative to electrochemical gradients it would appear that the energy requirements of marine osmoregulation are greater than those of fresh water osmoregulation. This conclusion is substantiated by the recent investigations of Hickman (1958) on the basal metabolic rates of euryhaline flounder in fresh and sea water.

Increases in the rate of energy expenditure in regulative functions may be paralleled by adaptive increases in maximum metabolic rate, such that "scope for activity" is unchanged by transfer into sea water. Certain observations, however, mediate against this possibility. Fry (1957) indicates that fish, and particularly salmonids, have a relatively restricted ability to increase their active metabolic rates. In addition Graham (1949) and Job (1955) have shown a direct dependancy of maximum metabolic rate upon the available oxygen content of the medium in *Salvelinus fontinalis*.
Analyses carried out in an earlier investigation (Houston 1957) corroborated the many observations that the oxygen content of sea water is less than that of fresh water (9.4 ± 0.1, as compared to 11.2 ± 0.3 gm/L.). Therefore, without extensive resort to anaerobic processes, for which no evidence has been found in fish, (Fry 1957) it seems unlikely that an adaptive increase in metabolic rate could counteract the decrease in "scope for activity" which probably arises in osmoregulation in sea water. In addition many of the enzyme systems associated with primary metabolic energy conversions are known to be sensitive to relatively slight changes in cation levels (see Sumner and Myrback 1950, Sumner and Somers 1953, for reviews). Enhanced cellular cation levels might therefore, also tend to inhibit any adaptive increases in metabolic rate, particularly during the adjustment phase of adaptation to sea water.

Migratory movements of salmonids from fresh water into sea water would seem on the basis of the data presented in this study to be disadvantageous to the fish. Adaptation to sea water evokes osmoregulatory stresses of marked and continuing influence on the locomotor ability of the migrants. Depressed activity enhances the likelihood of successful predatory action and marked predation of salmon stocks off river mouths has been frequently observed (Brooks, 1958). On the other hand entry into the sea opens greater food stocks to exploitation of successful migrants, allowing better growth as may be seen by comparison of sea run and land-locked forms of some salmonid species (Salmo salar sebago, Oncorhynchus kenoerli). In addition the selective action of predators while depleting stocks initially may act to improve the general fitness of the population as a whole.

(c) Influence Of Size On Adaptation To Sea Water

Size has been shown to influence adaptation to sea water in both species
studied. In chum fry, over the size range investigated, the extent and duration of hyperchloremia varied inversely with fork length, and weight. The data suggest that the limiting factor in ion uptake was the relative area of permeable surfaces to mass. This corroborates the conclusions of Huntsman and Hoar (1939) regarding salinity resistance in Atlantic salmon parr.

In Steelhead trout on the other hand adaptation with minimum departures from fresh water electrolyte concentrations was most rapidly accomplished by fish within a narrow range of lengths, weights, coefficients of condition and operculum to fork length ratios. Since these factors were characteristic of migrants (Maher and Larkin 1955) it would appear that not only do the animals move seaward at a specific period in their life history, but also that they do so at a stage at which they are best fitted to make the necessary osmoregulatory adjustment.

Plasma chloride levels have been shown to undergo abrupt decreases over the weight range characteristic of downstream migrants. Kubo (1955) and Pickford and Atz (1957) have indicated that pre-migratory decreases in chloremia were characteristic of diadromous fishes. Fontaine and his co-workers have observed this condition in Atlantic salmon and in European eels (Anguilla vulgaris, A. anguilla) and have suggested that electrolyte depletion plays a role in the initiation of seaward migration (Fontaine 1943, 1948, 1951, Fontaine and Callamand 1951, Callamand 1943, Callamand and Fontaine 1940 a, b). The main tenets of this "demineralization theory" are as follows; prior to migration the ability of the fish to osmoregulate in fresh water is diminished and results in a loss of electrolytes, this in some undetermined manner stimulates activity, and subsequent downstream movement leads the migrants into the sea where osmoregulatory difficulties
are alleviated by the increased tonicity of this medium.

It is difficult to relate Fontaine's theory to observed behavioural variations since the mild water intoxification implied by his observation does not, at least in mammals, promote increased activity (Bard, 1956). Nevertheless pre-migratory decreases in chloremia may be related to factors other than growth and/or maturation, as suggested by Barlow and Manery (1954), Daniel and Daniel (1955) and Elkington and Danowski (1955).

The main chloride excretory apparatus of marine salmonids is the bran­chial-secretory unit (Keys and Willmer 1932, Copeland 1948, 1950). Such cells develop in salmonids immediately prior to migration (Nishida 1953, Hoar 1951). It seems likely, therefore, that decreasing chloremias may be a function of the development and operation of these cells. Alternately hormonal suppression of the ion-absorbing mechanism may also occur as a pre­adaptation to marine osmoregulation. Depletion in the latter case might then be primarily due to urinary salt loss. In either case it seems more plausible to consider the "demineralization" of pre-migrants as a correlative of parr-smolt transformation rather than as an initiator of downstream mig­ration.

On the other hand high initial chloremias may function in rapid adjust­ment to sea water. Keys (1933) and Copeland (1950) have observed that activ­ation of the chloride-secretory mechanism is dependent upon increases in internal chloride concentration. Since chloride uptake in smaller fish probably occurs more rapidly than in larger fish this mechanism may be brought into activity following relatively small percentage changes from fresh water electrolyte concentration. This would in turn result in a more rapid ach­ievement of marine osmoregulation.
A correlation may exist between falling chloremias and the abrupt weight-related decrease in chloride space. Inorganic electrolytes provide the bulk of the osmotically active material of the extracellular phase. Any loss of electrolyte from this compartment tends to enhance the role of osmotically active, but non-diffusible particles such as proteins. Since the latter are chiefly intracellular it follows that loss of inorganic electrolytes from the extracellular fluid tends to increase their contribution to the total osmotic pool. In the maintenance of a condition of isomolarity water must then move from the extracellular to the intracellular phase. As a result the volume of the former compartment falls, while the latter rises.

(d) Homeostatic Mechanisms

The order of magnitude of the concentration changes in the extracellular fluids varied markedly although regulative control was apparent in all cases, except possibly potassium.

As chloride ion is predominantly extracellular in distribution uptake of chloride resulted in an increase in the concentration of this compartment. Some chloride was calculated to have entered the cellular phase but this fraction amounted to only 1.9% of the "chloride load" taken up by the fish (16.1 mM/kgm.) during their initial 36 hours in sea water. Since osmotic inactivation by complex formation does not readily occur under physiological conditions (Manery 1954) a tendency for hyperosmolarity of extracellular fluids relative to the cellular phase results. Alleviation of this imbalance is correlated with the transfer of water from the intracellular phase to the extracellular compartment. Restoration of isomolarity is accomplished, however, only with the increase in the tonicity of both phases, and with what
what amounts to a condition of cellular dehydration.

The main mechanisms in chloride homeostasis appear to be active and extrarenal since the urine of marine animals is particularly low in chloride content (Berglund and Forster 1958, Forster and Berglund 1956). Reference has been made to the ion-transporting cells of the gills. The chloride-secretory mechanism is activated by increases in internal electrolyte concentrations (Keys 1933, Copeland 1950). The activation time varies with species but cytological evidence of secretory activity has been observed in Fundulus within one half to three hours after transfer into sea water (Copeland 1948, 1950) and within six hours in Anguilla rostrata (Getman 1950). Maximum activity was noted in either case within 15 - 24 hours.

The primary adjustive and regulative responses to chloride uptake seem, therefore, to be expansion of the extracellular phase and subsequent extrarenal chloride excretion.

The response to increases in extracellular levels of sodium and calcium was somewhat different. While the loads of sodium and chloride after 36 hours in sea water were approximately the same (16.6 mM/kgm. sodium, 16.1 mM/kgm. chloride) the observed expansion of the chloride-potassium space accounted for only 83.7% of this cation. Similarly only 68.3% of the increase in tissue calcium could be attributed to its inclusion in this compartment. The data suggest, therefore, the withdrawal of a considerable fraction of the cation "load" from the extracellular fluid. In the sense that this process reduces the free ion content of the extracellular pool by the formation of physiologically and osmotically inactive complexes it may be considered a component, although a passive one, of the homeostatic mechanism. It was of some interest to note an inverse relationship between percentage increases in the extracellular levels of chloride (22.6%), sodium
(6.8 %), and calcium (0 %) and the amount of the electrolyte "load" accounted for by the cellular phase (1.9 %, 16.3 %, 31.7 %).

While the soft tissues may, by reversible complex formation withdraw cations for the extracellular phase, bone probably serves as a more important cation acceptor. Boroughs et al (1957) have shown that the skeleton of *Tilapia mossambica* accounts for over 40 % of administered loads of calcium-45, while only 4 % was found in muscle. Strontium-89 sequestration in bone was similar. Rosenthal (1956) reported much the same ratio of uptake for the skeletal and soft parts of *Libestes* - 60 % in the bones, and 3 to 5 % in muscle. Lovelace and Podoliak (1952) suggest similar distributions of calcium-45 by *Salvelinus fontinalis*. The conclusion may be drawn that bone has the ability to sequester at least ten times as much calcium as muscle, despite the disparity in their contributions to body mass. Bone may also serve as a sodium acceptor in cases of hypernatremia (Neuman and Neuman 1958).

The biological half-life of calcium in bone is extremely high (Rosenthal 1956). This essentially irreversible combination of calcium with bone has been studied by Neuman and Weikel (1955). These authors believed that excess cations in the extracellular pool might enter bone by falling into unoccupied lattice positions ("faults") in individual bone crystals. Further exchanges between crystals plus overgrowth might then serve to bury such ions still further from the readily exchangeable areas.

Since Neuman and Weikel (loc. cit.) have noted that "faulty" bone crystals are characteristic of relatively young animals, and those suffering cation insufficiencies, the ossification of unsaturated bone may operate in the homeostasis of plasma cation levels during the initial adjustment of salmonids to sea water. This type of "fixation" within bone crystals has the advantage over complex formation that ions so removed are less readily
available to the extracellular phases.

The reactions outlined above do not result in a diminution of the total ion "load" incurred in transfer into sea water although they may transfer considerable quantities of ion to physiologically and osmotically inactive sites. Therefore any hypothesis concerning the homeostatic mechanisms of fish must include a consideration of processes reducing total electrolyte levels.

Meyer (1951, 1952) and Sexton and Russell (1955) have indicated a branchial involvement in sodium excretion in fish. The enzyme succinic hydrogenase has been implicated in this process by these authors, although the cellular site of its activity has not as yet been determined. Since Maetz (1956) has recently demonstrated the presence of the enzyme carbonic anhydrase, which is believed to operate in chloride transport in gills the possibility exists that the two systems are coexistent in cells previously described having "chloride secretory" functions (Keys and Willmer 1932, Liu 1942, Copeland 1948, 1950, Getman 1950, Burden 1956, Morris 1957).

Berglund and Forster (1958) make the generalization that the most important function of the agglomerular renal tubule is the active elimination of inorganic divalent ions. The kidney of the salmon is glomerular in nature but the possibility of renal tubular excretion of calcium in these fishes should be considered. Normally only small amounts of urine are produced by marine fishes (see Black 1957 for review) but Forster and Berglund have demonstrated the ability of Lophius americanus to put out urine with a calcium concentration of up to 30.0 mM/L. Thus even with relatively low urine flows enhanced active tubular secretion or decreased tubular resorption of calcium might account for the excretion of significant amounts of calcium.

A second reasonable extrarenal route for calcium elimination would
seem to be the gut. Active calcium transport has been demonstrated by several authors (Lansing and Rosenthal 1952, Berglund and Forster 1958, Gilbert and Fenn 1957) while secretion of calcium into the gut has been reported by Nicolaysen et al. (1953). The conditions of the hind gut of fishes would seem to be correct for the precipitation of such secreted calcium as insoluble salts and their elimination with fecal material (Barrington 1957, Black 1957).

In summary it would appear that homeostasis of extracellular phase electrolytes during the adaptation of salmonids to sea water is not solely dependent upon the activity of excretory mechanisms. Withdrawal of ions from this phase by the activity of bone and soft tissues may aid in relieving the necessity of immediate outward transport. Furthermore bone may serve as a permanent "sink" for considerable quantities of calcium and sodium, permanently withdrawing portions of the incurred "loads" of these ions from the body fluids.
V. SUMMARY AND CONCLUSIONS

(1) Transfer of juvenile anadromous salmonids into sea water evokes depression of their locomotor ability. Depression of performance correlated well with the uptake of electrolytes and loss of water characteristic of the adjustive response of these fish to sea water, but was also present in fish adapted to marine osmoregulation.

(2) The adaptive phase of adjustment to sea water was accompanied by increases in extracellular electrolyte levels, transfer of water from cellular to extracellular compartments, and the enhancement of cellular cation levels.

(3) The data suggest a causal relationship between observed changes in performance and changes in cellular electrolyte levels. Both the cation inhibition of components of the locomotor apparatus, and the increased utilization of metabolic energy in marine osmoregulation may contribute to observed variations in motor performance.

(4) Size has been shown to influence the successful adaptation of both chum salmon fry and Steelhead trout smolts to sea water. In the former species the extent and duration of adjustment to sea water bore a simple relationship to surface area-mass ratios. In Steelhead trout adaptive success is keyed to particular growth stages. The data suggest that premigratory changes in water and electrolyte metabolism control the course of adjustment to sea water.

(5) Consideration of changes in extracellular anion and cation levels during adjustment to sea water indicates that the primary response to chloride
uptake was an expansion of the extracellular compartment. Cation homeostasis was in part due to this factor, but withdrawal of cation from the extracellular phase by cellular uptake, and possibly by sequestration in bone, may sharply restrict variations in body fluid electrolyte levels. Such passive activities must, therefore, be considered adjunctive to secretory mechanisms in the adjustment of salmonids to sea water.
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+ Original reference not consulted.
### TABLE I

**Motor Performance of Chum Salmon Fry in Fresh Water and Following Transfer Into Sea Water**

<table>
<thead>
<tr>
<th>Situation</th>
<th>Sample Size</th>
<th>Ave. Fork Length, cm.</th>
<th>Regression Equations Swimming Speed vs F.L.</th>
<th>Mean Swimming Speed, cm/sec.</th>
<th>Sy</th>
<th>Calculated Swimming Speeds +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Water</td>
<td>17</td>
<td>44.2</td>
<td>$Y = 26.0 + 1.051 X$</td>
<td>26.0</td>
<td>3.1</td>
<td>21.6 26.8 32.1</td>
</tr>
<tr>
<td>3.0</td>
<td>10</td>
<td>45.7</td>
<td>$Y = 23.6 + 1.199 X$</td>
<td>23.6</td>
<td>1.9</td>
<td>16.8 22.7 28.7</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>46.2</td>
<td>$Y = 22.1 + 1.1061 X$</td>
<td>22.1</td>
<td>1.3</td>
<td>15.5 20.8 26.1 31.4</td>
</tr>
<tr>
<td>14</td>
<td>10</td>
<td>46.1</td>
<td>$Y = 19.1 + 2.391 X$</td>
<td>19.1</td>
<td>3.1</td>
<td>21.7 28.5</td>
</tr>
<tr>
<td>22</td>
<td>10</td>
<td>52.0</td>
<td>$Y = 27.5 + 0.825 X$</td>
<td>27.5</td>
<td>2.5</td>
<td>21.8 25.9 30.1</td>
</tr>
<tr>
<td>28</td>
<td>10</td>
<td>52.0</td>
<td>$Y = 28.0 + 1.011 X$</td>
<td>28.0</td>
<td>4.7</td>
<td>20.9 26.0 31.0</td>
</tr>
<tr>
<td>37.5</td>
<td>3</td>
<td>51.0</td>
<td>$Y = 32.3$</td>
<td></td>
<td></td>
<td>30.5</td>
</tr>
<tr>
<td>50</td>
<td>10</td>
<td>51.8</td>
<td>$Y = 31.6 + 1.416 X$</td>
<td>31.6</td>
<td>5.6</td>
<td>21.9 29.0 36.1</td>
</tr>
<tr>
<td>80</td>
<td>10</td>
<td>54.7</td>
<td>$Y = 35.7 + 0.626 X$</td>
<td>35.7</td>
<td>4.1</td>
<td>32.8 35.9</td>
</tr>
</tbody>
</table>

+ 40, 45, 50, 55 mm. Fish.
TABLE II

Percentage of Fresh Water Swimming Speed Exhibited by Chum Salmon Fry Following Transfer From Fresh Water Into Sea Water

<table>
<thead>
<tr>
<th>Time In Sea Water</th>
<th>F.L., cm.</th>
<th>Swimming Speed cm/sec.</th>
<th>Fresh Water Swimming Speed Of Similar Sized Fish</th>
<th>Percentage Fresh Water Swimming Speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Water</td>
<td>4.4</td>
<td>26.0</td>
<td>26.0</td>
<td>100.0</td>
</tr>
<tr>
<td>3.0 hours</td>
<td>4.6</td>
<td>23.6</td>
<td>27.8</td>
<td>84.9</td>
</tr>
<tr>
<td>9.0 hours</td>
<td>4.6</td>
<td>22.1</td>
<td>28.1</td>
<td>78.6</td>
</tr>
<tr>
<td>14 1/2 hrs.</td>
<td>4.6</td>
<td>19.1</td>
<td>28.0</td>
<td>68.2</td>
</tr>
<tr>
<td>22.0 hours</td>
<td>5.2</td>
<td>27.5</td>
<td>34.2</td>
<td>80.4</td>
</tr>
<tr>
<td>28.0</td>
<td>5.2</td>
<td>28.0</td>
<td>34.2</td>
<td>81.9</td>
</tr>
<tr>
<td>37.5 hours</td>
<td>5.1</td>
<td>32.3</td>
<td>33.2</td>
<td>97.3</td>
</tr>
<tr>
<td>50.0 hours</td>
<td>5.2</td>
<td>31.6</td>
<td>34.0</td>
<td>92.9</td>
</tr>
<tr>
<td>80.0 hours</td>
<td>5.5</td>
<td>35.7</td>
<td>37.0</td>
<td>96.5</td>
</tr>
</tbody>
</table>
TABLE III

Total Body Chloride Levels of Chum Salmon Fry in Fresh Water and Following Transfer into Sea Water

<table>
<thead>
<tr>
<th>Situation</th>
<th>Sample Size</th>
<th>(Cl&lt;sub&gt;total&lt;/sub&gt; / F.L.)*</th>
<th>Sy</th>
<th>Standard Values **</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Water</td>
<td>15</td>
<td>Y = 110.27 - 0.6024 X</td>
<td>3.94</td>
<td>86.2 80.2 74.1</td>
</tr>
<tr>
<td>S.W. - 4 1/2 hrs.</td>
<td>15</td>
<td>Y = 262.60 - 2.9302 X</td>
<td>-9.32</td>
<td>145.4 116.1 86.8 57.5</td>
</tr>
<tr>
<td>S.W. - 10 1/2 hrs.</td>
<td>15</td>
<td>Y = 240.86 - 2.3702 X</td>
<td>10.02</td>
<td>146.1 122.4 98.7 75.0</td>
</tr>
<tr>
<td>S.W. - 14 1/2 hrs.</td>
<td>12</td>
<td>Y = 278.50 - 2.8337 X</td>
<td>6.34</td>
<td>165.2 136.8 108.5 80.14</td>
</tr>
<tr>
<td>S.W. - 24 hrs.</td>
<td>12</td>
<td>Y = 269.65 - 3.1271 X</td>
<td>5.34</td>
<td>144.6 113.3 82.0 50.8</td>
</tr>
<tr>
<td>S.W. - 28 hrs.</td>
<td>14</td>
<td>Y = 220.39 - 2.2221 X</td>
<td>7.19</td>
<td>131.5 109.3 87.1 64.8</td>
</tr>
<tr>
<td>S.W. - 37 hrs.</td>
<td>14</td>
<td>Y = 229.25 - 2.3274 X</td>
<td>7.41</td>
<td>- 112.9 89.6 66.3</td>
</tr>
</tbody>
</table>

* F.L., cm

** Standard Values 40.0, 50.0, 60.0, 70.0 mm. Fish.
TABLE IV
Size-Related Variation In Mean Net Total Body Chloride Uptake
Rate Of Chum Salmon Fry Following 14 1/2 Hours In Sea Water

<table>
<thead>
<tr>
<th>Fork Length cm</th>
<th>Weight gm</th>
<th>Surface Area cm²</th>
<th>Sₐ/Wt. cm²/gm</th>
<th>Uptake Rate mM/kgm/hr</th>
<th>%/kgm/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>0.368</td>
<td>0.514</td>
<td>1.397</td>
<td>5.45</td>
<td>6.31</td>
</tr>
<tr>
<td>5.0</td>
<td>0.81</td>
<td>0.869</td>
<td>1.073</td>
<td>3.90</td>
<td>4.87</td>
</tr>
<tr>
<td>6.0</td>
<td>1.51</td>
<td>1.316</td>
<td>0.872</td>
<td>2.37</td>
<td>3.20</td>
</tr>
</tbody>
</table>
### TABLE V

Size-Related Percentage Increases in Total Body Chloride Levels of Chum Salmon Fry Transferred from Fresh Water Into Sea Water

<table>
<thead>
<tr>
<th>Fork Length, cm.</th>
<th>Time In Sea Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 1/2</td>
</tr>
<tr>
<td>4.0</td>
<td>68.7</td>
</tr>
<tr>
<td>5.0</td>
<td>44.8</td>
</tr>
<tr>
<td>6.0</td>
<td>17.1</td>
</tr>
</tbody>
</table>
TABLE VI

Total Body Water Content Of Chum Salmon Fry In Fresh Water And Following Transfer Into Sea Water

<table>
<thead>
<tr>
<th>Situation</th>
<th>Sample Size</th>
<th>$H_2O/F.L. \ +$</th>
<th>Standard Values $\ +$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Water</td>
<td>18</td>
<td>$Y = 833.6 - 0.0695 X$</td>
<td>830.8  830.1  829.4  828.7</td>
</tr>
<tr>
<td>Sea Water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.5 hours</td>
<td>18</td>
<td>$Y = 822.2 - 0.0551 X$</td>
<td>820.0  819.4  818.9  818.3</td>
</tr>
<tr>
<td>10 hours</td>
<td>17</td>
<td>$Y = 820.7 + 0.0576 X$</td>
<td>823.0  823.6  824.2  824.7</td>
</tr>
<tr>
<td>15 hours</td>
<td>11</td>
<td>$Y = 813.2 - 0.2904 X$</td>
<td>801.6  798.7  795.8  792.9</td>
</tr>
<tr>
<td>23 hours</td>
<td>16</td>
<td>$Y = 825.6 - 0.0128 X$</td>
<td>825.1  825.0  824.8  824.7</td>
</tr>
<tr>
<td>36 hours</td>
<td>14</td>
<td>$Y = 842.0 - 0.0791 X$</td>
<td>838.8  838.0  837.2  836.5</td>
</tr>
</tbody>
</table>

+ Fork length, cm.

++ Standard values; 40.0, 50.0, 60.0, 70.0 mm Fry.
TABLE VII

Percentage Changes in Total Body Chloride Content of Chum Salmon Fry in Sea Water, and Their Correlations With Percentage Changes in Performance

<table>
<thead>
<tr>
<th>Time in Sea Water</th>
<th>Percentage Fresh Water Swimming Speed</th>
<th>Percentage Change in Chloride Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 - 4 1/2 hrs.</td>
<td>- 84.9</td>
<td>+ 49.38</td>
</tr>
<tr>
<td>9 - 10 1/2 hrs.</td>
<td>- 78.6</td>
<td>+ 54.08</td>
</tr>
<tr>
<td>14 - 14 1/2 hrs.</td>
<td>- 68.2</td>
<td>+ 73.15</td>
</tr>
<tr>
<td>22 - 24 hrs.</td>
<td>- 80.4</td>
<td>+ 35.60</td>
</tr>
<tr>
<td>28 hrs.</td>
<td>- 81.9</td>
<td>+ 32.81</td>
</tr>
<tr>
<td>37 - 37 1/2 hrs.</td>
<td>- 97.3</td>
<td>+ 38.97</td>
</tr>
</tbody>
</table>

Regression of Percentage Fresh Water Swimming Speed and Percentage Change in Total Body Chloride Concentration = 181.60 - 1.6696 X (Y =)

Coefficient of Correlation = 0.8152, P 0.03 0.01
TABLE VIII

Percentage Changes In Total Body Water Content Of Chum Salmon Fry
In Sea Water, And Their Correlations With Percentage Changes In
Performance

<table>
<thead>
<tr>
<th>Time in Sea Water</th>
<th>Percentage Fresh Water Swimming Speed</th>
<th>Percentage Of Fresh Water Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 - 3 1/2 hrs.</td>
<td>84.9</td>
<td>98.20</td>
</tr>
<tr>
<td>9 - 10 hrs.</td>
<td>78.6</td>
<td>97.88</td>
</tr>
<tr>
<td>14 1/2 hrs.</td>
<td>68.2</td>
<td>97.27</td>
</tr>
<tr>
<td>22 - 23 hrs.</td>
<td>80.4</td>
<td>98.67</td>
</tr>
<tr>
<td>37 - 38 hrs.</td>
<td>97.3</td>
<td>100.76</td>
</tr>
</tbody>
</table>

Regression of Percentage Fresh Water Swimming Speed and Percentage Fresh Water Body Water Concentration: \( Y = 90.04 + 0.1032 \times \)

Coefficient of Correlation = 0.8594, \( P \) 0.05 0.01.
### TABLE IX

Plasma Chloride Levels of Steelhead Trout (June - July 1957)

In Fresh Water, And Following Transfer Into Sea Water

<table>
<thead>
<tr>
<th>Situation</th>
<th>Sample Size</th>
<th>pCl/wt</th>
<th>Sy</th>
<th>Standard Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Water</td>
<td>39</td>
<td>$Y = 152.8 - 0.3044 X$</td>
<td>6.78</td>
<td>140.5 136.0 131.5**</td>
</tr>
<tr>
<td>Sea Water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 - 4 hrs.</td>
<td>17</td>
<td>$Y = 159.8 - 0.2408 X$</td>
<td>8.39</td>
<td>150.2 146.5 142.9</td>
</tr>
<tr>
<td>9 - 11 hrs.</td>
<td>14</td>
<td>$Y = 172.0 - 0.2474 X$</td>
<td>5.28</td>
<td>162.1 158.4 154.7</td>
</tr>
<tr>
<td>14 - 16 hrs.</td>
<td>12</td>
<td>$Y = 172.7 - 0.2258 X$</td>
<td>11.44</td>
<td>163.7 60.3 156.9</td>
</tr>
<tr>
<td>21 - 23 hrs.</td>
<td>16</td>
<td>$Y = 163.3 - 0.0719 X$</td>
<td>7.70</td>
<td>160.4 159.4 158.8</td>
</tr>
<tr>
<td>27 - 28 hrs.</td>
<td>5</td>
<td>$Y = 217.1 - 1.1171 X$</td>
<td>9.81</td>
<td>172.4 144.7 138.9</td>
</tr>
<tr>
<td>35 - 36 hrs.</td>
<td>15</td>
<td>$Y = 177.6 - 0.1336 X$</td>
<td>14.57</td>
<td>172.2 170.2 168.2</td>
</tr>
<tr>
<td>84 - 88 hrs.</td>
<td>19</td>
<td>$Y = 170.6 - 0.1446 X$</td>
<td>6.89</td>
<td>164.8 162.7 160.5</td>
</tr>
<tr>
<td>122 - 126 hrs.</td>
<td>15</td>
<td>$Y = 147.9 + 0.0247 X$</td>
<td>7.59</td>
<td>148.9 149.3 149.6</td>
</tr>
<tr>
<td>166 - 169 hrs.</td>
<td>7</td>
<td>$Y = 136.9 + 0.0805 X$</td>
<td>4.18</td>
<td>140.1 141.3 142.5</td>
</tr>
</tbody>
</table>

+ Standard Values; 40, 55, 70 gm. Fish.

++ mEq/L.
### TABLE X

Effect Of Coefficient Of Condition Upon Changes In Plasma Chloride Concentration Of Steelhead Trout Transferred From Fresh Water Into Sea Water

<table>
<thead>
<tr>
<th>Situation</th>
<th>pCl/Coefficient of Condition</th>
<th>Coeff. = 0.700</th>
<th>% Change</th>
<th>Coeff. = 0.900</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Water</td>
<td>Y = 226.68 - 105.82 X</td>
<td>152.6</td>
<td>0</td>
<td>131.4</td>
<td>0</td>
</tr>
<tr>
<td>S.W. - 4 hrs.</td>
<td>Y = 176.12 - 37.52 X</td>
<td>149.9</td>
<td>-1.81</td>
<td>142.4</td>
<td>+8.30</td>
</tr>
<tr>
<td>S.W. - 10 hrs.</td>
<td>Y = 108.62 + 63.69 X</td>
<td>153.2</td>
<td>-0.61</td>
<td>165.9</td>
<td>+26.25</td>
</tr>
<tr>
<td>S.W. - 15 hrs.</td>
<td>Y = 78.07 + 97.77 X</td>
<td>146.5</td>
<td>-4.00</td>
<td>166.1</td>
<td>+26.24</td>
</tr>
<tr>
<td>S.W. - 22 hrs.</td>
<td>Y = 157.47 + 2.29 X</td>
<td>159.1</td>
<td>4.23</td>
<td>159.5</td>
<td>+21.37</td>
</tr>
<tr>
<td>S.W. - 36 hrs.</td>
<td>Y = 213.59 - 54.77 X</td>
<td>175.3</td>
<td>14.84</td>
<td>164.3</td>
<td>+25.00</td>
</tr>
<tr>
<td>S.W. - 86 hrs.</td>
<td>Y = 159.69 + 43.55 X</td>
<td>162.7</td>
<td>6.64</td>
<td>163.6</td>
<td>+24.48</td>
</tr>
<tr>
<td>S.W. - 126 hrs.</td>
<td>Y = 136.18 + 21.17 X</td>
<td>151.0</td>
<td>-1.05</td>
<td>155.2</td>
<td>+18.10</td>
</tr>
<tr>
<td>S.W. - 168 hrs.</td>
<td>Y = 138.46 + 4.67 X</td>
<td>146.7</td>
<td>11.59</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE XI

Effect Of Weight Upon Changes In Plasma Chloride Concentration Of
Steelhead Trout Transferred From Fresh Water Into Sea Water

<table>
<thead>
<tr>
<th>Situation</th>
<th>pCl/wt.</th>
<th>40 gm pCl</th>
<th>% Change</th>
<th>70 gm pCl</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Water</td>
<td>( Y = 152.8 - 0.304 \times X )</td>
<td>140.5</td>
<td>-</td>
<td>131.5</td>
<td>-</td>
</tr>
<tr>
<td>S.W. - 4 hrs.</td>
<td>( Y = 159.8 - 0.241 \times X )</td>
<td>150.2</td>
<td>6.90</td>
<td>142.9</td>
<td>8.67</td>
</tr>
<tr>
<td>S.W. - 10 hrs.</td>
<td>( Y = 172.0 - 0.247 \times X )</td>
<td>161.1</td>
<td>15.37</td>
<td>154.7</td>
<td>17.64</td>
</tr>
<tr>
<td>S.W. - 15 hrs.</td>
<td>( Y = 172.7 - 0.226 \times X )</td>
<td>163.7</td>
<td>16.51</td>
<td>156.9</td>
<td>19.32</td>
</tr>
<tr>
<td>S.W. - 22 hrs.</td>
<td>( Y = 163.3 - 0.072 \times X )</td>
<td>160.4</td>
<td>14.16</td>
<td>158.8</td>
<td>20.76</td>
</tr>
<tr>
<td>S.W. - 36 hrs.</td>
<td>( Y = 177.6 - 0.134 \times X )</td>
<td>172.2</td>
<td>22.56</td>
<td>168.2</td>
<td>27.91</td>
</tr>
<tr>
<td>S.W. - 88 hrs.</td>
<td>( Y = 170.6 - 0.145 \times X )</td>
<td>164.8</td>
<td>17.30</td>
<td>160.5</td>
<td>22.05</td>
</tr>
<tr>
<td>S.W. - 126 hrs.</td>
<td>( Y = 147.9 - 0.025 \times X )</td>
<td>148.9</td>
<td>5.98</td>
<td>149.6</td>
<td>13.76</td>
</tr>
<tr>
<td>S.W. - 168 hrs.</td>
<td>( Y = 136.9 - 0.081 \times X )</td>
<td>140.1</td>
<td>-0.28</td>
<td>142.5</td>
<td>8.37</td>
</tr>
</tbody>
</table>
TABLE XII

Effect Of Operculum To Fork Length Ratio Upon Changes In Plasma Chloride Concentration Of Steelhead Trout Transferred From Fresh Water Into Sea Water

<table>
<thead>
<tr>
<th>Situation</th>
<th>pCl / (OL/F.L. x 10)</th>
<th>pCl</th>
<th>% Change</th>
<th>pCl</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Water</td>
<td>Y = 90.25 + 39.16 X</td>
<td>132.5</td>
<td>0</td>
<td>137.2</td>
<td>0</td>
</tr>
<tr>
<td>S.W. - 4 hrs.</td>
<td>Y = 311.41 - 147.74 X</td>
<td>151.9</td>
<td>14.57</td>
<td>134.1</td>
<td>-2.26</td>
</tr>
<tr>
<td>S.W. - 10 hrs.</td>
<td>Y = 84.26 + 64.29 X</td>
<td>153.7</td>
<td>15.96</td>
<td>161.4</td>
<td>17.60</td>
</tr>
<tr>
<td>S.W. - 15 hrs.</td>
<td>Y = 6.26 + 136.34 X</td>
<td>153.5</td>
<td>15.82</td>
<td>169.9</td>
<td>23.78</td>
</tr>
<tr>
<td>S.W. - 22 hrs.</td>
<td>Y = 127.07 + 29.44 X</td>
<td>158.9</td>
<td>19.87</td>
<td>162.4</td>
<td>18.37</td>
</tr>
<tr>
<td>S.W. - 36 hrs.</td>
<td>Y = 216.26 - 40.19 X</td>
<td>172.9</td>
<td>30.41</td>
<td>168.0</td>
<td>22.45</td>
</tr>
<tr>
<td>S.W. - 86 hrs.</td>
<td>Y = 106.94 + 48.04 X</td>
<td>158.8</td>
<td>19.83</td>
<td>164.6</td>
<td>19.93</td>
</tr>
<tr>
<td>S.W. - 126 hrs.</td>
<td>Y = 66.44 + 72.47 X</td>
<td>144.7</td>
<td>9.18</td>
<td>153.4</td>
<td>11.78</td>
</tr>
<tr>
<td>S.W. - 168 hrs.</td>
<td>Y = 194.77 - 45.14 X</td>
<td>146.0</td>
<td>10.2</td>
<td>140.6</td>
<td>2.45</td>
</tr>
</tbody>
</table>
TABLE XIII

Tissue Chloride Levels of Steelhead Trout (June - July 1957) In Fresh Water, And Following Transfer Into Sea Water

<table>
<thead>
<tr>
<th>Situation</th>
<th>Sample Size</th>
<th>tCl/wt.</th>
<th>Sy</th>
<th>Standard Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Fresh Water</td>
<td>35</td>
<td>$Y = 31.9 - 0.1827 X$</td>
<td>2.97</td>
<td>24.4 21.8 19.1++</td>
</tr>
<tr>
<td>Sea Water</td>
<td></td>
<td></td>
<td></td>
<td>Standard Values</td>
</tr>
<tr>
<td>3 - 4 hrs.</td>
<td>13</td>
<td>$Y = 24.6 + 0.1523 X$</td>
<td>4.95</td>
<td>33.0 35.2</td>
</tr>
<tr>
<td>9 - 11 hrs.</td>
<td>14</td>
<td>$Y = 44.1 - 0.1653 X$</td>
<td>3.96</td>
<td>37.5 35.0 32.5</td>
</tr>
<tr>
<td>14 - 16 hrs.</td>
<td>12</td>
<td>$Y = 56.1 - 0.4019 X$</td>
<td>5.18</td>
<td>40.0 34.0 27.9</td>
</tr>
<tr>
<td>21 - 23 hrs.</td>
<td>16</td>
<td>$Y = 37.8 - 0.0066 X$</td>
<td>7.77</td>
<td>37.5 37.4 37.3</td>
</tr>
<tr>
<td>27 - 28 hrs.</td>
<td>5</td>
<td>$Y = 54.8 - 0.3188 X$</td>
<td>7.19</td>
<td>42.0 37.3 32.4</td>
</tr>
<tr>
<td>35 - 37 hrs.</td>
<td>15</td>
<td>$Y = 53.5 - 0.2667 X$</td>
<td>5.89</td>
<td>42.8 38.8 34.8</td>
</tr>
<tr>
<td>84 - 88 hrs.</td>
<td>19</td>
<td>$Y = 30.3 - 0.1046 X$</td>
<td>4.47</td>
<td>26.1 24.5 23.0</td>
</tr>
<tr>
<td>122 - 126 hrs.</td>
<td>15</td>
<td>$Y = 34.5 - 0.1357 X$</td>
<td>5.39</td>
<td>29.1 27.1</td>
</tr>
</tbody>
</table>

+ Standard values: 40, 55, 70 gm. Fish
++ mEq/kgm Fresh Tissue
TABLE XIV

Plasma Water Levels Of Steelhead Trout (June - July 1957)
In Fresh Water And Following Transfer Into Sea Water

<table>
<thead>
<tr>
<th>Situation</th>
<th>Sample Size</th>
<th>pH (0 \div \text{wt.} ) ( \frac{\text{gm}}{\text{kg}} )</th>
<th>(\text{Sy})</th>
<th>Standard Values +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Water</td>
<td>39</td>
<td>( Y = 958.4 - 0.2194 \times X )</td>
<td>11.13</td>
<td>947.7 946.4 943.1</td>
</tr>
<tr>
<td>Sea Water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 - 4 hrs.</td>
<td>15</td>
<td>( Y = 973.1 - 0.5056 \times X )</td>
<td>11.06</td>
<td>945.3 937.7</td>
</tr>
<tr>
<td>9 - 11 hrs.</td>
<td>14</td>
<td>( Y = 993.1 - 0.4863 \times X )</td>
<td>8.98</td>
<td>973.5 966.4 959.1</td>
</tr>
<tr>
<td>16 - 16 hrs.</td>
<td>12</td>
<td>( Y = 996.3 - 0.4400 \times X )</td>
<td>10.08</td>
<td>978.7 972.1 965.5</td>
</tr>
<tr>
<td>21 - 23 hrs.</td>
<td>16</td>
<td>( Y = 953.6 + 0.1461 \times X )</td>
<td>7.06</td>
<td>959.4 961.6 963.8</td>
</tr>
<tr>
<td>27 - 28 hrs.</td>
<td>5</td>
<td>( Y = 903.4 + 0.9470 \times X )</td>
<td>9.16</td>
<td>941.3 955.5</td>
</tr>
<tr>
<td>35 - 37 hrs.</td>
<td>14</td>
<td>( Y = 982.0 - 0.1529 \times X )</td>
<td>8.92</td>
<td>975.9 973.6 971.3</td>
</tr>
<tr>
<td>84 - 88 hrs.</td>
<td>20</td>
<td>( Y = 955.1 + 0.0305 \times X )</td>
<td>9.10</td>
<td>956.3 956.7</td>
</tr>
<tr>
<td>122 - 126 hrs.</td>
<td>17</td>
<td>( Y = 943.8 + 0.3380 \times X )</td>
<td>6.08</td>
<td>962.4 967.4</td>
</tr>
</tbody>
</table>

+ Standard Values: 40, 55, 70 gm. Fish.
++ gm/kgm.
TABLE XV

Chloride Spaces Of Steelhead Trout (June - July 1957) In
Fresh Water And Following Transfer Into Sea Water

<table>
<thead>
<tr>
<th>Situation</th>
<th>Sample Size</th>
<th>Ecs/wt.</th>
<th>Sy</th>
<th>Standard Values +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Water</td>
<td>33</td>
<td>$Y = 217.1 - 1.2748 X$</td>
<td>18.04</td>
<td>166.1 146.9 127.8</td>
</tr>
<tr>
<td>Sea Water</td>
<td></td>
<td></td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>3 - 4 hrs.</td>
<td>10</td>
<td>$Y = 268.0 - 0.8582 X$</td>
<td>17.57</td>
<td>220.8 207.9</td>
</tr>
<tr>
<td>9 - 11 hrs.</td>
<td>14</td>
<td>$Y = 249.8 - 0.7492 X$</td>
<td>22.82</td>
<td>219.9 208.6 197.4</td>
</tr>
<tr>
<td>14 - 16 hrs.</td>
<td>10</td>
<td>$Y = 446.37 - 4.2361 X$</td>
<td>19.97</td>
<td>319.2 213.3 149.8</td>
</tr>
<tr>
<td>21 - 23 hrs.</td>
<td>14</td>
<td>$Y = 146.0 + 1.2619 X$</td>
<td>35.09</td>
<td>196.5 215.4 234.3</td>
</tr>
<tr>
<td>27 - 28 hrs.</td>
<td>5</td>
<td>$Y = 222.0 + 0.0095 X$</td>
<td>18.71</td>
<td>222.4 222.6 222.7</td>
</tr>
<tr>
<td>35 - 37 hrs.</td>
<td>15</td>
<td>$Y = 289.1 - 1.3058 X$</td>
<td>33.60</td>
<td>236.9 217.3 197.7</td>
</tr>
<tr>
<td>84 - 88 hrs.</td>
<td>17</td>
<td>$Y = 186.9 - 0.7580 X$</td>
<td>31.71</td>
<td>156.6 145.2 133.8</td>
</tr>
<tr>
<td>122 - 126 hrs.</td>
<td>16</td>
<td>$Y = 190.6 - 0.2948 X$</td>
<td>31.61</td>
<td>178.8 174.4 169.9</td>
</tr>
</tbody>
</table>

+ Standard Values: 40, 55, 70 gm. Fish.

++ gm/kgm, Fresh Tissue.
TABLE XVI

Intracellular Water (Muscle Water - Chloride Space) Of Steelhead Trout (June - July, 1957) In Fresh Water And Following Transfer Into Sea Water

<table>
<thead>
<tr>
<th>Situation</th>
<th>Sample Size</th>
<th>Ics/wt.</th>
<th>Sy</th>
<th>Standard Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Water</td>
<td>35</td>
<td>Y = 628.3 + 0.4283 X</td>
<td>21.8</td>
<td>641.1 651.8 658.2</td>
</tr>
<tr>
<td>Sea Water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 - 4 hrs.</td>
<td>12</td>
<td>Y = 643.7 - 0.9240 X</td>
<td>26.97</td>
<td>592.8 579.0</td>
</tr>
<tr>
<td>9 - 11 hrs.</td>
<td>14</td>
<td>Y = 718.5 - 2.3629 X</td>
<td>34.30</td>
<td>624.0 588.5 533.1</td>
</tr>
<tr>
<td>14 - 16 hrs.</td>
<td>12</td>
<td>Y = 492.1 + 1.9409 X</td>
<td>30.89</td>
<td>569.8 598.9 628.0</td>
</tr>
<tr>
<td>21 - 23 hrs.</td>
<td>14</td>
<td>Y = 631.2 - 1.2223 X</td>
<td>29.33</td>
<td>582.3 564.0 533.4</td>
</tr>
<tr>
<td>27 - 28 hrs.</td>
<td>5</td>
<td>Y = 684.8 - 1.8122 X</td>
<td>18.71</td>
<td>585.2</td>
</tr>
<tr>
<td>35 - 37 hrs.</td>
<td>15</td>
<td>Y = 571.2 - 0.0996 X</td>
<td>33.60</td>
<td>567.2 565.7 564.3</td>
</tr>
<tr>
<td>84 - 88 hrs.</td>
<td>19</td>
<td>Y = 611.8 + 0.4969 X</td>
<td>31.71</td>
<td>631.7 639.2 646.6</td>
</tr>
<tr>
<td>122 - 126 hrs.</td>
<td>16</td>
<td>Y = 631.4 - 0.1262 X</td>
<td>31.61</td>
<td>626.4 624.4 622.5</td>
</tr>
</tbody>
</table>

+ Standard Values: 40, 55, 70 gm. Fish.

++ gm/kgm, Fresh Tissue.
TABLE XVII

Plasma, Extracellular, Tissue And Intracellular Concentration Of Water, Sodium, Potassium And Chloride Of Steelhead Trout In Fresh Water And Sea Water (36 Hours)

<table>
<thead>
<tr>
<th>Component</th>
<th>Fresh Water</th>
<th>Sea Water</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma, gm/kgm.</td>
<td>932.05</td>
<td>912.88</td>
</tr>
<tr>
<td>Extracellular space, gm/kgm.</td>
<td>87.8</td>
<td>169.3 +</td>
</tr>
<tr>
<td></td>
<td>63.1</td>
<td>138.1 ++</td>
</tr>
<tr>
<td>Tissue, gm/kgm.</td>
<td>776.9</td>
<td>764.2</td>
</tr>
<tr>
<td>Intracellular space, gm/kgm.</td>
<td>689.1</td>
<td>594.9 +</td>
</tr>
<tr>
<td></td>
<td>713.8</td>
<td>626.1 ++</td>
</tr>
<tr>
<td><strong>Sodium:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma, mM/L.</td>
<td>162.5</td>
<td>170.0</td>
</tr>
<tr>
<td>Extracellular space, mM/L.</td>
<td>164.2</td>
<td>175.4</td>
</tr>
<tr>
<td>Tissue, mM/kgm.</td>
<td>81.4</td>
<td>97.8</td>
</tr>
<tr>
<td>Intracellular space, mM/L.</td>
<td>77.8</td>
<td>114.4 ++</td>
</tr>
<tr>
<td></td>
<td>99.2</td>
<td>117.6 ++</td>
</tr>
<tr>
<td><strong>Potassium:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma, mM/L.</td>
<td>6.04</td>
<td>7.61</td>
</tr>
<tr>
<td>Extracellular space, mM/L.</td>
<td>6.77</td>
<td>7.56</td>
</tr>
<tr>
<td>Tissue, mM/L.</td>
<td>125.2</td>
<td>116.2</td>
</tr>
<tr>
<td>Intracellular space, mM/L.</td>
<td>182.0</td>
<td>196.0 +</td>
</tr>
<tr>
<td></td>
<td>174.8</td>
<td>184.0 ++</td>
</tr>
<tr>
<td><strong>Chloride:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma, mM/L.</td>
<td>135.0</td>
<td>162.1</td>
</tr>
<tr>
<td>Extracellular space, mM/L.</td>
<td>147.8</td>
<td>181.5</td>
</tr>
<tr>
<td>Tissue, mM/kgm.</td>
<td>13.15</td>
<td>29.25</td>
</tr>
<tr>
<td>Intracellular space, mM/kgm.</td>
<td>5.5</td>
<td>7.0 ++</td>
</tr>
</tbody>
</table>

* + Based on chloride space  
++ Based upon chloride-potassium space.
TABLE XVIII

Plasma Calcium Levels Of Steelhead Trout (June-July 1957) In Fresh Water
And Following Transfer Into Sea Water

<table>
<thead>
<tr>
<th>Situation</th>
<th>Sample Size</th>
<th>pCa/wt.</th>
<th>Sy</th>
<th>Standard Values +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Water</td>
<td>24</td>
<td>$Y = 2.71 - 0.0041 X$</td>
<td>0.315</td>
<td>2.55 2.49 2.43 2.35</td>
</tr>
<tr>
<td>Sea Water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 hours</td>
<td>11</td>
<td>$Y = 2.15 + 0.0079 X$</td>
<td>0.262</td>
<td>2.47 2.59 2.71 2.87</td>
</tr>
<tr>
<td>26 hours</td>
<td>12</td>
<td>$Y = 1.98 + 0.0093 X$</td>
<td>0.173</td>
<td>2.35 2.49 2.63 2.81</td>
</tr>
<tr>
<td>52 hours</td>
<td>12</td>
<td>$Y = 3.10 - 0.0054 X$</td>
<td>0.272</td>
<td>2.88 2.80 2.72 2.61</td>
</tr>
<tr>
<td>100 hours</td>
<td>11</td>
<td>$Y = 2.83 - 0.0003 X$</td>
<td>0.260</td>
<td>2.82 2.81 2.81 2.80</td>
</tr>
<tr>
<td>168 hours</td>
<td>7</td>
<td>$Y = 3.16 - 0.0032 X$</td>
<td>0.293</td>
<td>3.03 2.93 2.93 2.87</td>
</tr>
<tr>
<td>240 hours</td>
<td>8</td>
<td>$Y = 2.57 + 0.0034 X$</td>
<td>0.362</td>
<td>2.71 2.76 2.81 2.88</td>
</tr>
</tbody>
</table>

++ Standard Values: 40, 55, 70, 90 gm. Fish.
+++ mM/kgm. Fresh Tissue.
**TABLE XIX**

Absolute And Percentage Variations In Plasma, Extracellular, Tissue And Intracellular Levels

Of 55 gm. Steelhead Trout (June - July 1957) Following Transfer From Fresh Water To Sea Water

<table>
<thead>
<tr>
<th></th>
<th>Fresh Water</th>
<th>15 hrs.</th>
<th>26 hrs.</th>
<th>52 hrs.</th>
<th>100 hrs.</th>
<th>168 hrs.</th>
<th>240 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(pCa)</td>
<td>2.49</td>
<td>2.48</td>
<td>2.49</td>
<td>2.80</td>
<td>2.81</td>
<td>2.98</td>
<td>2.76</td>
</tr>
<tr>
<td>(ecsCa)</td>
<td>1.42</td>
<td>1.40</td>
<td>1.39</td>
<td>1.52</td>
<td>1.56</td>
<td>1.65</td>
<td></td>
</tr>
<tr>
<td>(tCa)</td>
<td>2.40</td>
<td>3.21</td>
<td>3.73</td>
<td></td>
<td>3.76</td>
<td>3.40</td>
<td></td>
</tr>
<tr>
<td>Ca, ecs ++</td>
<td>0.21</td>
<td>0.30</td>
<td>0.31</td>
<td>0.24</td>
<td>0.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cCa, kgm. fresh tissue +++</td>
<td>2.19</td>
<td>2.91</td>
<td>3.42</td>
<td>3.52</td>
<td>3.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cCa/L. cell water</td>
<td>3.36</td>
<td>4.86</td>
<td>5.98</td>
<td></td>
<td>5.55</td>
<td>5.03</td>
<td></td>
</tr>
<tr>
<td>cCa/kgm. tissue solids</td>
<td>10.71</td>
<td>14.59</td>
<td>16.16</td>
<td>16.93</td>
<td>15.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change (pCa)</td>
<td>-0.41</td>
<td>0.00</td>
<td>12.45</td>
<td>12.85</td>
<td>19.68</td>
<td>10.84</td>
<td></td>
</tr>
<tr>
<td>% change (ecsCa)</td>
<td>-1.41</td>
<td>-2.11</td>
<td>7.04</td>
<td>9.86</td>
<td>16.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change (tCa)</td>
<td>33.75</td>
<td>55.42</td>
<td></td>
<td>56.67</td>
<td>41.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change (cCa/L. cell water)</td>
<td>44.64</td>
<td>77.98</td>
<td>65.18</td>
<td>49.70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change (cCa/kgm. tissue solids)</td>
<td>36.23</td>
<td>50.89</td>
<td>58.08</td>
<td>49.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change, (ecsCa)</td>
<td>42.86</td>
<td>55.00</td>
<td>14.85</td>
<td>23.81</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ All values in mm. calcium.
++ (Ca,ecs): total calcium in extracellular phase/kgm. fresh tissue.
+++ (cCa): calcium associated with cell, (ie. tissue calcium - Ca,ecs)
TABLE XX

Tissue Calcium Levels Of Steelhead Trout (June - July 1957) In Fresh Water And Following Transfer Into Sea Water

<table>
<thead>
<tr>
<th>Situation</th>
<th>Sample Size</th>
<th>tCa/wt.</th>
<th>Sy</th>
<th>Standard Values +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Water</td>
<td>23</td>
<td>$Y = 3.24 - 0.015$ $X$</td>
<td>0.746</td>
<td>2.63 2.40 2.17 1.87 ++</td>
</tr>
<tr>
<td>Sea Water</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 hours</td>
<td>11</td>
<td>$Y = 3.22 + 0.001$ $X$</td>
<td>0.528</td>
<td>3.22 3.21 3.21 3.20</td>
</tr>
<tr>
<td>26 hours</td>
<td>13</td>
<td>$Y = 2.54 + 0.009$ $X$</td>
<td>0.771</td>
<td>3.40 3.73 4.05 4.48</td>
</tr>
<tr>
<td>100 hours</td>
<td>12</td>
<td>$Y = 7.09 - 0.061$ $X$</td>
<td>0.960</td>
<td>4.67 3.76</td>
</tr>
<tr>
<td>168 hours</td>
<td>7</td>
<td>$Y = 3.59 - 0.003$ $X$</td>
<td>0.296</td>
<td>3.45 3.40 3.35 3.29</td>
</tr>
</tbody>
</table>

+ Standard values: 40, 55, 70, 90 gm. fish.
++ mM/kgm, fresh tissue.
<table>
<thead>
<tr>
<th>Weight, gms.</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>(pCa), mM/L.</td>
<td>2.59</td>
<td>2.55</td>
<td>2.51</td>
<td>2.47</td>
<td>2.43</td>
<td>2.39</td>
</tr>
<tr>
<td>(tCa), mM/kgm., fresh tissue</td>
<td>2.78</td>
<td>2.63</td>
<td>2.48</td>
<td>2.33</td>
<td>2.17</td>
<td>2.02</td>
</tr>
<tr>
<td>(ecsCa), mM/L.</td>
<td>0.25</td>
<td>0.23</td>
<td>0.21</td>
<td>0.20</td>
<td>0.18</td>
<td>0.16</td>
</tr>
<tr>
<td>(ECS), gm/kgm.</td>
<td>178.8</td>
<td>166.1</td>
<td>153.3</td>
<td>140.6</td>
<td>127.8</td>
<td>115.1</td>
</tr>
<tr>
<td>ECS-CA, mM.</td>
<td>0.45</td>
<td>0.39</td>
<td>0.32</td>
<td>0.28</td>
<td>0.23</td>
<td>0.18</td>
</tr>
<tr>
<td>(cCa), mM/kgm. fresh tissue</td>
<td>2.33</td>
<td>2.24</td>
<td>2.16</td>
<td>2.05</td>
<td>1.94</td>
<td>1.84</td>
</tr>
<tr>
<td>Tissue solids</td>
<td>186.4</td>
<td>193.6</td>
<td>200.8</td>
<td>204.4</td>
<td>208.1</td>
<td>215.3</td>
</tr>
<tr>
<td>(ICS), gm/kgm.</td>
<td>641.1</td>
<td>646.1</td>
<td>649.7</td>
<td>654.0</td>
<td>658.2</td>
<td>662.5</td>
</tr>
<tr>
<td>cCa/kgm. tissue solids</td>
<td>12.23</td>
<td>11.57</td>
<td>10.76</td>
<td>10.03</td>
<td>9.32</td>
<td>8.55</td>
</tr>
<tr>
<td>cCa/L. cell water</td>
<td>3.62</td>
<td>3.47</td>
<td>3.33</td>
<td>3.14</td>
<td>2.95</td>
<td>2.78</td>
</tr>
</tbody>
</table>
TABLE XXII

Net Exchange Rates of Calcium In Plasma, Extracellular Fluid And Tissue

<table>
<thead>
<tr>
<th>Time in Sea Water</th>
<th>0 - 15 hrs.</th>
<th>15 - 26 hrs.</th>
<th>26 - 52 hrs.</th>
<th>52 - 100 hrs.</th>
<th>100 - 168 hrs.</th>
<th>168 - 240 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma +</td>
<td>- 0.007</td>
<td>+ 0.009</td>
<td>+ 0.119</td>
<td>0.000</td>
<td>+ 0.025</td>
<td>- 0.031</td>
</tr>
<tr>
<td>Extracellular Fluid +</td>
<td>- 0.014</td>
<td>- 0.009</td>
<td>+ 0.050</td>
<td>+ 0.013</td>
<td>+ 0.013</td>
<td></td>
</tr>
<tr>
<td>C. -A. Ca/L ++</td>
<td>+ 0.100</td>
<td>+ 0.102</td>
<td></td>
<td>- 0.058 +++</td>
<td></td>
<td>- 0.076</td>
</tr>
<tr>
<td>cell water</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. -A. Ca/kgm +++</td>
<td>+ .259</td>
<td>+ 0.143</td>
<td>+ 0.104</td>
<td></td>
<td></td>
<td>- 0.141</td>
</tr>
<tr>
<td>tissue solids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ mM/L/hour.
++ mM/L. cell water/hour.
+++ 26 - 100 hours
++++ mM/kgm. tissue solids/hour