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OSMOTIC AND IONIC REGULATION IN EMBRYOS, ALEVINS  
AND FRY OF THE FIVE SPECIES OF PACIFIC SALMON

ABSTRACT

The purpose of this study was to examine the physiological basis of the differences in the early life histories of pink Oncorhynchus gorbuscha, chum O. keta, coho O. kisutch, chinook O. tshawytscha and sockeye salmon O. nerka. To this end the following working hypotheses were tested: (i) the embryo, alevin and fry of pink and chum salmon are euryhaline, whereas the same life stages of coho, chinook and sockeye are stenohaline and (ii) the euryhalinity of pink and chum is due not to high tissue tolerance but to their ability to regulate the osmotic and ionic concentrations in their blood, whereas the stenohalinity of coho, chinook and sockeye stems from their inability to osmoregulate and ion regulate.

The LD<sub>50</sub> values and the osmoregulatory data indicated that pink and chum embryos are not euryhaline as hypothesized but like coho, chinook and sockeye embryos are stenohaline. However, pink and chum embryos showed significantly greater salinity resistance and osmoregulatory ability than embryos of the other species.

Contrary to the working hypothesis, all five species of alevins were found to be stenohaline; again, pink and chum showed greater salinity resistance and osmoregulatory abilities than coho and sockeye alevins.

Although chinook alevins survived considerably longer in 31.8 o/oo sea water than coho and sockeye alevins their ability to osmoregulate and ion regulate was not significantly different from that of coho and sockeye alevins. It was concluded, therefore, that the greater salinity resistance of chinook alevins was due to high tissue tolerance.

The data obtained from fry supported the working hypotheses. As in the case of alevins, chinook fry, due to higher tissue tolerance, manifested greater salinity resistance than coho and sockeye.

When the data for all species of embryos and fry were grouped and compared to the alevins taken as a group, the LD<sub>50</sub> values of embryos and fry were found to be significantly greater than those of the alevins. The blood osmotic concentrations of embryos and fry exposed for the same duration in 31.8 o/oo sea water were significantly lower than the blood osmotic concentrations for alevins. It was concluded, therefore, that embryos and fry have greater salinity resistance than alevins because of the greater osmoregulatory ability of embryos and fry.

On the basis of these results as well as information obtained from the literature, speculations were made on the phylogenetic relations within the genus Oncorhynchus.

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*by*

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B.Sc., University of Toronto, 1961

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF  
THE REQUIREMENTS FOR THE DEGREE OF  
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We accept this thesis as conforming to the  
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Date April 28, 1967

### ABSTRACT

The major purpose of this study was to examine the physiological basis of the differences in the early life histories of pink Oncorhynchus gorbuscha, chum O. keta, coho O. kisutch, chinook O. tshawytscha and sockeye O. nerka.

To this end, the following working hypotheses were tested:

- (i) the embryo, alevin and fry of pink and chum salmon are euryhaline, whereas the same life stages of coho, chinook and sockeye are stenohaline and
- (ii) the euryhalinity of pink and chum is due not to high tissue tolerance but to their ability to regulate the osmotic and ionic concentrations in their blood, whereas the stenohalinity of coho, chinook and sockeye stems from their inability to osmoregulate and ion regulate.

The results did not completely support these hypotheses. The LD<sub>50</sub> values and the osmoregulatory data obtained from embryos indicated that pink and chum are not euryhaline as hypothesized but like coho, chinook and sockeye embryos are stenohaline. However, pink and chum embryos showed significantly greater salinity resistance and osmoregulatory ability than embryos of the other species.

The data obtained from alevins also did not support the working hypothesis for alevins of all five species were found to be stenohaline. But, as in the case for embryos, pink and chum alevins showed greater salinity resistance and osmoregulatory abilities than coho and sockeye alevins. This ability of pink and chum was correlated with better ionic regulation of sodium and chloride. Although chinook alevins survived considerably longer in 31.8 ‰ sea water than coho and sockeye alevins, their ability to osmoregulate and ion regulate

was not very different from that of coho and sockeye alevins. It was concluded, therefore, that the greater salinity resistance of chinook alevins was due to high tissue tolerance.

Contrary to the results with embryos and alevins, the data obtained from fry supported the working hypotheses. The results showed that pink and chum were euryhaline and that this condition was due to their ability to osmoregulate and control the levels of sodium and chloride in the blood. Coho, chinook and sockeye fry were found to be stenohaline and were unable to regulate the osmotic and ionic concentrations in their blood. As in the case of alevins, chinook fry, due to higher tissue tolerance, manifested greater salinity resistance than coho and sockeye.

When the data for all species of embryos and fry were grouped and compared to the alevins taken as a group, the  $LD_{50}$  values of embryos and fry were found to be significantly greater than those of the alevins. The blood osmotic concentrations of embryos and fry exposed for the same duration in 31.8‰ sea water were significantly lower than the blood osmotic concentrations for alevins. It was included, therefore, that embryos and fry have greater salinity resistance than alevins because of the greater osmoregulatory ability of embryos and fry.

On the basis of these results as well as information obtained from the literature, speculations were made on the phylogenetic relations within the genus Oncorhynchus.



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## INTRODUCTION

Pacific salmon are migratory fish which spend a year or more in the ocean and then migrate in the fall usually to the freshwater system in which they hatched. The eggs are laid in a nest and are covered with gravel after fertilization. Hatching occurs after several months of development at which time the yolk-laden embryos are referred to as alevins; after yolk-sac absorption, they are referred to as fry.

The six species of Pacific salmon all belong to the genus Oncorhynchus. Pink O. gorbuscha, chum O. keta, coho O. kisutch, chinook O. tshawytscha and sockeye O. nerka are found along the American and Asiatic coasts of the North Pacific Ocean as well as in the Arctic Ocean (Nikol'skii, 1961). The masou O. masou, however, occur exclusively along the Asiatic coast (Nikol'skii, 1961).

The five North American species show several similarities during their life histories. As young fry their appearance is such that the recognition of each species can be made accurately only by an expert. In the ocean several species have been caught in a single net haul and during the fall spawning migration several species have been observed spawning very close together (Neave, 1958). In addition, Brett (1952) has reported very similar preferred temperatures and ultimate incipient lethal temperatures for the five species.

Although there are these similarities in morphology, ecology and physiology, important differences exist among the five North American species of Pacific salmon. Pink and chum salmon frequently spawn in close proximity to the sea (Clemens and Wilby, 1961) or in the intertidal zone in which the

eggs are subjected to sea water for part of the day (Skud, 1954). In addition, pink and chum embryos which, soon after hatching, return to the sea as alevins or fry, lack the cryptic parr marks associated with life in fresh water. These circumstances contrast sharply with the greater distance from the sea travelled by many chinook and sockeye populations during the spawning migration, the longer residency in fresh water of many coho, chinook and sockeye fry and the cryptic parr marks of these salmon as alevins and fry. These differences, along with the ethological differences described by Hoar (1958) and those described by Neave (1958), support the hypothesis that Pacific salmon have evolved towards a more marine life. It was speculated that examination of the physiological basis of these differences in the early life stages might bring further evidence to bear on the theory. To this end, the following working hypotheses were tested:

- (i) the embryo, alevin and fry of pink and chum salmon are euryhaline; whereas the same life stages of coho, chinook and sockeye are stenohaline and
- (ii) the euryhalinity of pink and chum is due not to high tissue tolerance but to their ability to regulate the osmotic and ionic concentrations in their blood, whereas the stenohalinity of coho, chinook and sockeye stems from their inability to osmoregulate and ion regulate.

To test these hypotheses, groups of fish were transferred from fresh water into 31.8 ‰ sea water and the time of death of the median fish ( $LD_{50}$ ) determined. Since Gordon (1959) and Parry (1961) have shown that a large portion of the osmotic concentration in the blood can be attributed to sodium and chloride, the concentrations of these two ions along with



the melting points were measured in the plasma or serum of fish maintained in fresh water or of fish transferred abruptly into 31.8<sup>0</sup>/oo sea water. As supplementary information, the potassium and magnesium concentrations in serum or plasma also were determined.

Because of the short period in which material was available, large numbers of animals could not be used to test the working hypotheses. Therefore, the data was analyzed by orthogonal contrasts in which pink and chum results were compared, in most cases, as a group, to the coho, chinook and sockeye results taken as a group. When the differences between the two groups were large, often individual species differences also were observed.

## MATERIALS AND METHODS

### Sources and Maintenance of Fish

The experiments have been carried out on hatchery reared embryos, alevins and fry of the five species of Pacific salmon obtained from various sources during the 1964-65 and 1965-66 spawning seasons as outlined in Table I.

Eggs from each species were maintained in flowing fresh water in separate incubation tanks (with an overflow standpipe at one end) measuring 110 X 35 X 30 cm. Under these conditions embryos and alevins were kept in darkness but with the approach of complete yolk absorption, late alevins were exposed to natural photoperiod and fed finely chopped frozen brine shrimp. Fry were fed frozen brine shrimp and wet food preparation consisting of 65% beef liver, 21% canned salmon, 8% Pablum (Mead Johnson of Canada Ltd., Toronto), 6% Brewer's yeast and 5 g of table salt per 300 g of mixture.

The average weekly water temperatures in which the 1964-65 and 1965-66 material were incubated are shown in Figure 1.

### Transfer Experiments

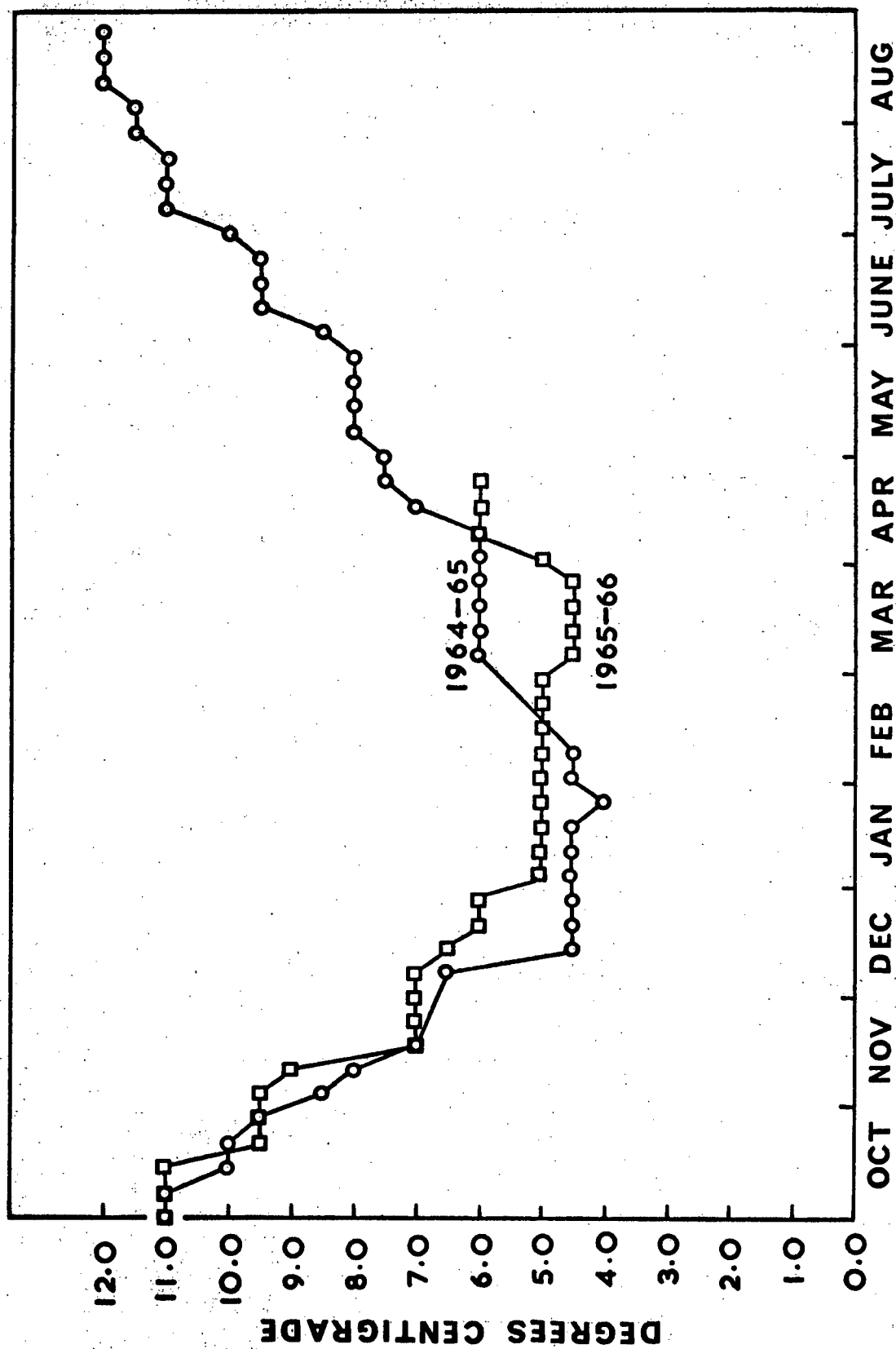
The basic design of all experiments was to transfer eggs, alevins or fry directly from fresh water to sea water. All fish used in such experiments were moved from the incubation tanks to glass dishes 21 cm in diameter and 2 litres in capacity. The dishes were immersed in a 183 X 61 cm trough maintained at  $9^{\circ} \pm 1^{\circ}\text{C}$  for embryos and alevins and  $11^{\circ} \pm 1^{\circ}\text{C}$  for fry. The dishes were filled with fresh water and were supplied with air which had been washed previously in water. After about 24 hr, the fish were transferred to glass dishes filled with sea water which had a salinity of 31.8 ‰ and

TABLE I General information on the fish.

<u>Species</u>	<u>Stage of Development When Incubation was Begun at University</u>	<u>Source</u>	<u>Date Fertilized</u>	<u>Date Hatched</u>
Chinook	a few hours after fertilization	Samish State Salmon Hatchery, Washington	October 13, 1964	December 18, 1964
Pink	eyed	Bear River, B.C.	October 22, 1964	January 1, 1965
Sockeye	eyed	Cultus Lake, B.C.	-	March 17, 1965
Chum	eyed	Qualicum River, B.C.	-	April 13, 1965
Coho	eyed	Hunts Creek, B.C.	-	April 19, 1965
Sockeye	eyed	Nanika Hatchery, B.C.	September 11, 1965	November 11, 1965
Sockeye	alevins	Lakelse Lake, B.C.	September 1, 1965	-
Chinook	eyed	Skykomish State Hatchery, Washington	-	December 10, 1965
Pink	a few hours after fertilization	Sweltzer Creek, B.C.	October 19, 1965	January 7, 1966
Coho	eyed	Qualicum River, B.C.	-	March 16, 1966
Chum	eyed	Qualicum River, B.C.	December 11, 1965	March 20, 1966

FIGURE 1

Average weekly temperatures in which  
1964-65-66 embryos, alevins and fry  
were incubated.



the following ionic concentrations:

	Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>++</sup>	Mg <sup>++</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>--</sup>
mEq/l	433.0	10.1	25.6	97.9	497.2	68.6

This sea water was made by the addition of salts to brackish water (27<sup>o</sup>/oo) from Burrard Inlet.

All animals placed in the glass dishes filled with either sea water or fresh water were kept in total darkness except during sampling of blood. Under these conditions, fish were transferred directly from fresh water to 31.8<sup>o</sup>/oo sea water and the time to death of the median fish (LD<sub>50</sub>) determined. In conjunction with these experiments, the osmolal, Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>++</sup> and Cl<sup>-</sup> concentrations of the serum or plasma were measured from fish exposed to sea water for 0, 4, 8, 12 and sometimes 24, 45 and 48 hr.

#### Collection of Blood

Each embryo was freed from its chorion by tearing open the chorion with a pair of fine watchmaker's forceps. The embryo was then quickly dried on cellucotton and its epidermis was removed near the caudal peduncle. There an incision was made with the forceps and the blood collected (approximately 0.001 ml) in a small glass capillary tube (#40090, Central Scientific Co., Vancouver) connected to rubber tubing through which mouth suction could be applied. The capillary tube containing blood was plugged at one end with paraffin and centrifuged at approximately 550 X g for 2 min to obtain serum.

A group of six to eight alevins was removed from fresh water or sea water, quickly dried on paper towelling and cellucotton, and their blood collectively sampled (approximately 0.002 ml per animal) into a cold (0<sup>o</sup>C) glass tube (#9530 Pyrex, Corning Glass Co., N.Y.) by mouth suction. Blood was taken near the caudal peduncle of the alevins and serum prepared in a

manner similar to that for embryos. While the tube was spinning in the centrifuge, the collection of blood was repeated with another group of six to eight alevins. When sufficient blood had been obtained, the serum, which was separated from the cells in each tube, was pooled into one tube and subsamples of serum obtained for measurement of each blood parameter.

In the case of fry, blood was obtained by severing the caudal peduncle and permitting the blood to run down a heparinized capillary tube. The tubes were sealed with paraffin wax and centrifuged for 2 min at 550 X g. Plasma and cells were separated and subsamples taken as in the case of serum from alevins.

#### Analyses of Blood

Melting points of serum and plasma were determined with a Melting Point Determination Apparatus designed by Ramsay and Brown (1955). Small samples of serum or plasma were drawn into fine glass capillary tubes (#40090, Central Scientific Co., Vancouver). These samples were enclosed by paraffin oil to prevent evaporation and were kept on dry ice until analysed. Melting points ( $^{\circ}\text{C}$ ) of blood were determined on the day of collection and the values converted to milliosmoles.

Concentrations of the cations  $\text{Na}^{+}$  and  $\text{K}^{+}$  were analysed by emission flame photometry using a Unicam SP900A Spectrophotometer (Unicam Instruments Ltd., Cambridge, England). Table II indicates the dilutions used in determining these ions. The dilutions were made in polyethylene vials (10 ml) using Drummond Microcaps (Drummond Scientific Co., Broomall, Pa.). The vials were stored at  $-20^{\circ}\text{C}$  prior to analysis. Care was taken to analyse samples only when they had reached room temperatures. As a check on the quantitative accuracy of these cation determinations, dilutions of Harleco

TABLE II

Dilutions used in analysing  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Mg}^{++}$  by flame photometry.

<u>Ion</u>	<u>Volume of Serum or Plasma</u>	<u>Dissolved In</u>	<u>Dilution</u>
$\text{Na}^+$	0.001 ml	5 ml of $\text{H}_2\text{O}$	5000 X
$\text{K}^+$	0.002 ml	3 ml of 100 ppm $\text{Na}^+$	1500 X
$\text{Mg}^{++}$	0.005 ml	3 ml of 8 ppm $\text{Na}^+$	600 X



Serum Control (#64098 Harlman - Leddon Co., Philadelphia, Pa.) were also analysed by flame photometry.

Chloride concentrations were measured potentiometrically on 1 microlitre (0.001 ml) serum or plasma samples diluted in 20 microlitres of 50% acetic acid following the method of Ramsay et al. (1955). A Radiometer pH Meter 25SE (Radiometer, Copenhagen, Denmark) and a Misco Vibrating Stirrer (#1385 Microchemical Specialities Co., Berkeley, California) with the single modification of a 60 ohm resistor between the transformer and solenoid proved very suitable.

#### Statistical Methods

The material used in the experiments about to be described are available for only a short period each year. Because of this and the time required to perform microtechniques, only three replicates for each species were used in measuring the blood parameters at each age and at each exposure time to sea water. Two- and three-way analyses of variance were carried out on the data. Each analysis was considered to be of the Model I type and therefore F tests were run with the error mean square as denominator (Steel and Torrie, 1960). Tukey's w, which was determined from each analysis of variance, permitted all comparisons of treatment means. Because of the relative insensitivity of Tukey's w, orthogonal contrasts were also performed. However, orthogonal contrasts do not permit all possible questions to be asked. Therefore data were lumped so as to test the working hypotheses. For example, the serum osmotic concentrations of pink and chum embryos at 0, 4, 8 and 12 hr sea water have been taken as the group, pink/chum and compared to the coho/chinook/sockeye group. If these two groups are statistically different from each other and members within each group are not statistically different from other members

in the group, then pink and chum are different from coho, chinook and sockeye.

Regression equations were calculated for each species at each age and for each parameter measured. If the curvilinear equation  $Y = A + BX + CX^2$  fit the data better than the straight-line equation  $Y = A + BX$ , the curvilinear was used in describing the regression. The regression equations are included in the appendix.

Student's t-tests were considered to be two tailed. Unless stated otherwise, the term "significantly different" has a probability level less than or equal to 0.05.

## RESULTS

### A. Embryos

#### LD<sub>50</sub> Values

Two weeks prior to hatching five embryos with intact chorions and five dechorionated embryos from each species (1965-66 progeny) were transferred from fresh water to 31.8 ‰ sea water and the LD<sub>50</sub> determined for each species. The high salinity of the sea water proved to be a lethal factor in all cases. The analysis of variance of the LD<sub>50</sub> values in Table III, indicates that there is at least one significant difference among the five species (Table IV). Tukey's w of 42 hr calculated from this analysis of variance indicates that pink and chum LD<sub>50</sub> values are significantly higher than coho and sockeye values. In addition, orthogonal contrasts, also calculated from the analysis of variance in Table III, reveal that pink/chum\* LD<sub>50</sub> values are significantly higher than chinook values. Since the LD<sub>50</sub> value for pink is not significantly different from chum, pink and chum have significantly higher LD<sub>50</sub> values than chinook.

The presence of the chorion results in increased salinity resistance on the part of all embryos ( $P < 0.05$ ). This increased salinity resistance cannot be attributed to impermeability of the chorion for the perivitelline fluid of eggs immersed 4 hr or more in 31.8 ‰ sea water is slightly hyperosmotic with the external environment ( $P < 0.05$ ).

---

\* Pink/chum refers to pink and chum data treated as a unit. For example, if pink/chum are significantly different from chinook, it does not necessarily follow that chum are significantly different from chinook.

TABLE III.

LD<sub>50</sub> (hr) of embryos transferred directly from fresh water to 31.8‰ sea water.

	<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
Normal	102	118	47	81	52
Dechorionated	95	94	33	65	50

TABLE IV

Analysis of variance of Embryo LD<sub>50</sub> values.

<u>Source<sup>1</sup></u>	<u>df<sup>2</sup></u>	<u>SS<sup>3</sup></u>	<u>MS<sup>4</sup></u>	<u>F<sup>5</sup></u>	<u>F<sub>0.05</sub>(4,5)</u>
Species	4	6618.5	1654.6	15.3	5.19
Error	5	541.5	108.3		

Since  $F = 15.3$  is greater than 5.19, the null hypothesis of no difference among the five species of embryos must be rejected.

- 1 source of variation
- 2 degrees of freedom
- 3 sum of squares
- 4 mean sum of squares
- 5 F test

### Serum Osmotic Concentrations

The greater salinity resistance of pink and chum embryos may be the result of a greater ability of their tissues to function in a wide range of salinities or be the result of greater osmoregulatory abilities. To evaluate these possibilities, embryos of each species were transferred directly from fresh water to 31.8‰ sea water. Blood was sampled from embryos immersed for 0, 4, 8, 12, 24, 45 and 48 hr in sea water and the melting points of the serums determined. The results of this experiment are shown in Figure 2. Every point is the mean of three determinations and each determination was obtained from one embryo. When salmon embryos are abruptly transferred from fresh water to 31.8‰ sea water, a rapid rise in the osmotic concentration of the serum occurs. In fact, the largest increase takes place in the pinks which are smaller than the chum, coho and chinook. At 12 hr, the osmotic concentration of the serum has levelled in the pink, chum and chinook but continues to increase in the coho and sockeye. At 24 hr, differences among the five species widen considerably; after 24 hr the differences, between pink and chum on the one hand and chinook on the other, increase still further.

Tukey's  $w$  was calculated from the analysis of variance (Table V) of the five species exposed to 0, 4, 8, 12 and 24 hr of sea water. If the difference between any two treatment means exceeds the calculated value of Tukey's  $w$ , in this case 120 mOsm, then this difference is significant. On this basis, no significant differences occur among the five species in 0, 4, 8 and 12 hr exposure to sea water. However at 24 hr, the osmotic concentration of sockeye and coho embryo serum is significantly different from that of pink and chum embryos. Also, chinook, at this time, are significantly different from sockeye.

FIGURE 2

Serum osmotic concentrations of Pacific  
salmon embryos plotted against exposure  
times to 31.8<sup>o</sup>/oo sea water.

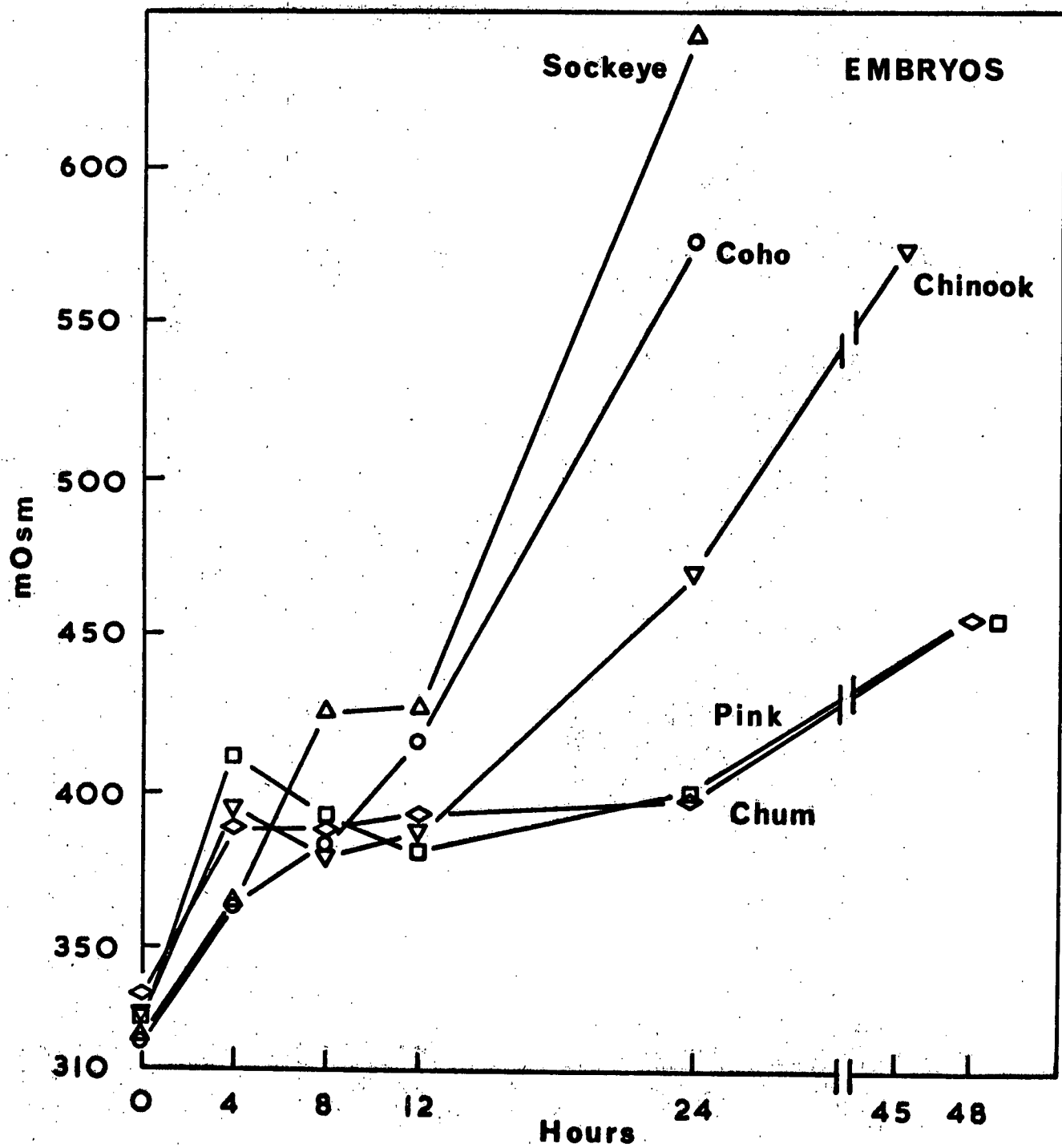




TABLE V

Analysis of variance of the osmotic concentrations of the five species of embryos exposed to 0, 4, 8, 12 and 24 hr of sea water.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
Species	4	34,457	8,614	5.965*
Exposure	4	228,745	57,186	39.602*
Interaction	16	124,051	7,753	5.369*
Error	50	72,178	1,444	

\* Highly significant ( $P < 0.005$ ). Therefore, the null hypotheses of no difference among the five species of embryos, of no difference among the five exposures and of no significant interaction between species and exposures must be rejected.

Orthogonal contrasts, calculated from the same analysis of variance used in deriving Tukey's w, point to more subtle differences among the five species than are indicated by Tukey's w. The difference in the osmotic concentration of pink/chum serums between 0 and 24 hr exposure to sea water is significantly different from the difference of chinook serums between 0 and 24 hr sea water (Table I, Appendix). However, the difference between 0 and 24 hr for coho is not significantly different from the difference for sockeye (Table II, Appendix).

The coho, chinook and sockeye data fit straight-line regressions better than curvilinear, whereas the pink and chum data are more accurately described by curvilinear regressions (Table III, Appendix).

In summary, the higher salinity resistance of pink and chum embryos has been correlated with their greater ability to osmoregulate and the lower salinity resistance of coho, chinook and sockeye embryos correlated with their inferior ability to osmoregulate.

## B. Alevins

### LD<sub>50</sub> Values

Nine alevins from each species (1965-66 progeny) were placed in 31.8‰ sea water and the LD<sub>50</sub> values determined. These alevins were approximately 2 weeks post hatching. Table VI indicates that pink alevins, in both years, had a higher resistance to the lethal factor than the chum, coho, chinook and sockeye alevins. When the experiment was repeated with alevins exposed to 10‰ sea water for 2 days followed by 20‰ for a subsequent 2 days, the LD<sub>50</sub> values of these alevins from all five species in 31.8‰ sea water were the same as for alevins transferred directly from fresh water to 31.8‰ sea water.

Tukey's w of 43 hr, calculated from the analysis of variance shown in Table VII, indicates that pink LD<sub>50</sub> values are significantly higher than coho and sockeye. Orthogonal contrasts further reveal that pink LD<sub>50</sub> values are higher than chum/chinook values and that chum are not significantly different from chinook. If, instead of comparing LD<sub>50</sub> values of pink and chum/chinook, the latter group had been compared to coho/sockeye, the LD<sub>50</sub> values of chum/chinook would have been found significantly greater than those of coho/sockeye. However, once the former comparison has been made, the latter is not orthogonal. Therefore, it is not possible to show that coho/sockeye LD<sub>50</sub> values are significantly lower than chum/chinook. Nevertheless, inasmuch as it was possible to obtain samples of blood for chum and chinook alevins exposed to 24 hr of sea water while it was not possible to do so for coho and sockeye because of considerable mortality, the evidence points to greater salinity resistance of chum and chinook over that of sockeye and coho. Therefore, the LD<sub>50</sub> values of chum and chinook are probably intermediate

TABLE VI

LD<sub>50</sub> (hr) of alevins transferred from fresh water directly into 31.8 ‰ sea water.

	<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
1964 - 65 *	85	45	< 24	22	< 22
1965 - 66 **	69	56	26	49	27

\* cessation of opercular movements

\*\* cessation of blood flow in vitelline vein

TABLE VII

Analysis of variance of the  $LD_{50}$  values of the five species of alevins transferred from fresh water directly into 31.8<sup>o</sup>/oo sea water.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u><math>F_{0.05}(4,5)</math></u>
Species	4	3,867.0	966.75	8.5	5.19
Error	5	568.5	113.7		

Since  $F = 8.5$  is greater than 5.19, the null hypothesis of no difference among the five species must be rejected.

between that for pink alevins on the one hand and coho and sockeye on the other hand.

The greater salinity resistance of pink alevins may be the result of a greater ability of their tissues to function in a wide range of salinities, or, as in the case of pink and chum embryos, greater osmoregulatory ability. To evaluate these possibilities, alevins (1965-66) of each species were transferred directly from fresh water to 31.8 ‰ sea water. Blood was sampled from these alevins immersed in sea water 0, 4, 8, 12 and 24 hr and the melting points and concentrations of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  of the pooled serum were determined. In Figures 3, 4, 5 and 6 which display the results of these measurements, every point is the mean of three determinations and each determination was made from the pooled blood of 20 to 30 individuals.

#### Serum Osmotic Concentrations

The osmotic concentrations of serums determined in this experiment are shown in Figure 3. As in the case of the embryos, a large increase in serum osmotic concentrations occurs within the first 4 hr of exposure to sea water. The serum osmotic concentration of pink alevins decreases between 4 and 12 hr exposure to sea water, while the serum osmotic concentration of chum alevins decreases between 4 and 8 hr. In contrast, serum osmotic concentrations of sockeye and chinook alevins continue to rise rapidly as the exposure time to sea water increases. Although serum osmotic concentrations of coho alevins show no levelling as observed for pink and chum, the coho, nevertheless, do not show the same rapid rise as sockeye and chinook.

On the basis of Tukey's  $w$  of 59 mOsm, calculated from the analysis of variance of the five species exposed to 0, 4, 8 and 12 hr of sea water (Table VIII), no significant differences occur among the alevins in fresh water. At

FIGURE 3

Serum osmotic concentrations of Pacific salmon alevins  
plotted against exposure times to 31.8<sup>o</sup>/oo sea water.

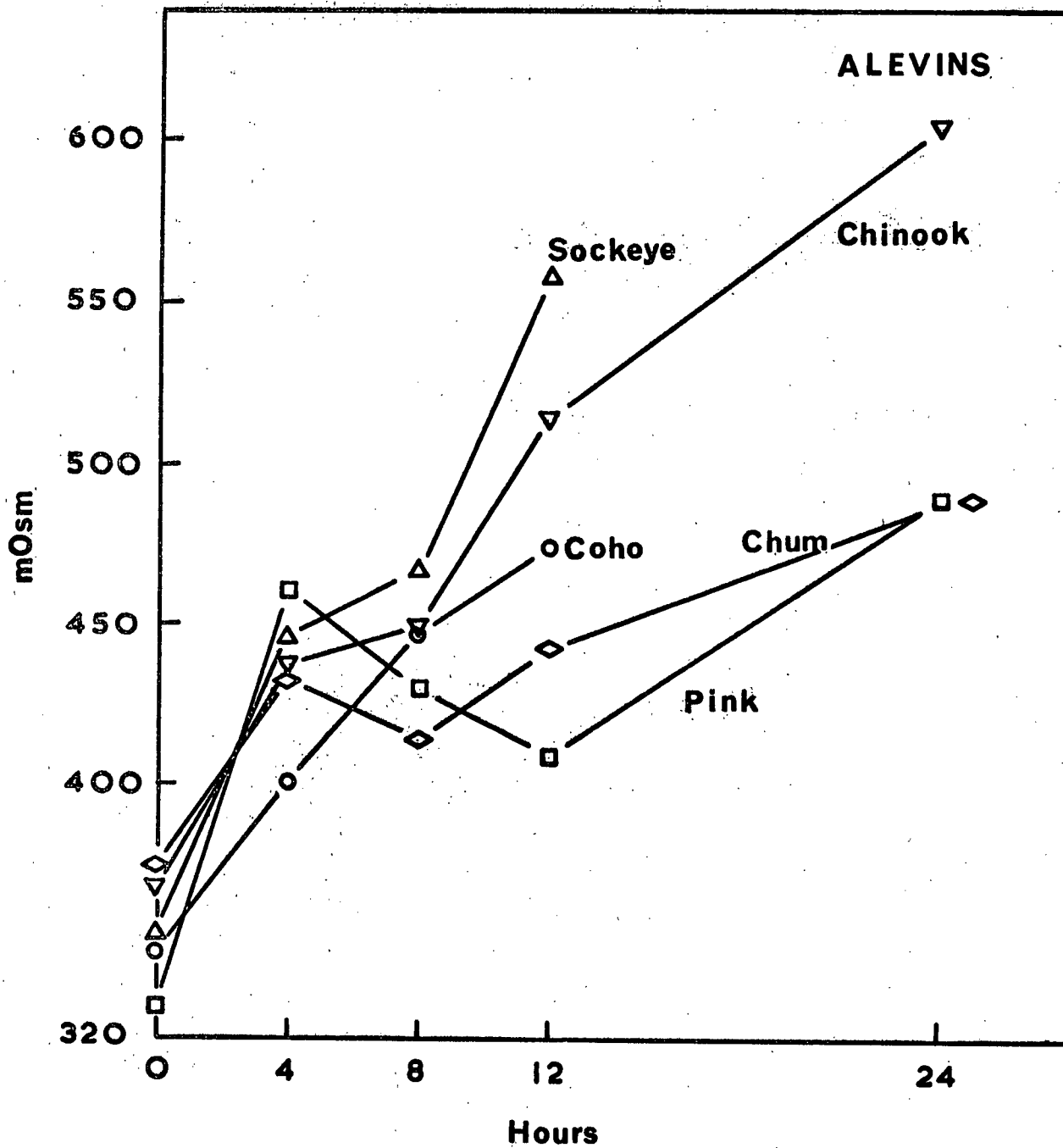




TABLE VIII

Analysis of variance of the serum osmotic concentrations of the five species of alevins exposed to 0, 4, 8 and 12 hr of sea water.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	
Species	4	20,004	5,001	13.96	$F_{0.005}(4,40)$ 4.37
Exposure	3	124,261	41,420	115.63	$F_{0.005}(3,40)$ 4.98
Interaction	12	35,760	2,980	8.32	$F_{0.005}(12,40)$ 2.95
Error	40	14,328	358.2		

Therefore Species, Exposure and Interaction are highly significant.

4 hr exposure to sea water, pinks show a significantly higher serum osmotic concentration than coho alevins. At 8 hr, no significant differences are found and at 12 hr, the serum osmotic concentrations of pinks are significantly lower from that in coho, chinook and sockeye, whereas chum, at this time, is significantly different from chinook and sockeye but not from pink and coho. By the Student's t-test, the serum osmotic concentration of pink and chum alevins at 24 hr is significantly different from chinook.

Orthogonal contrasts show that the difference in response to sea water between pink/chum and coho as measured by serum osmotic concentrations at 0, 4, 8 and 12 hr is not significant. Similarly, there is no significant difference in response between pink and chum alevins, and no significant difference between chinook and sockeye alevins. However, the interactions between species and exposure times are more interesting. The difference in serum osmotic concentrations of pink/chum in fresh water and at 12 hr in sea water is significantly different ( $P < 0.01$ ) from the difference observed for coho alevins (Table IV, Appendix). Similarly, chinook alevins are significantly different ( $P < 0.01$ ) from sockeye alevins (Table V, Appendix) whereas pinks are not significantly different from chums (Table VI, Appendix).

Except for the pinks, the data of the four other species individually fit straight-line regressions better than curvilinear (Table VII, Appendix). The slope of the chum regression line is considerably less than that for the coho, chinook and sockeye regressions.

In summary, the resistance of chum/chinook alevins to high salinities is intermediate between pink alevins on the one hand and coho/sockeye on the other. Nevertheless, pink and chum have very similar osmoregulatory abilities whereas chinook abilities differ significantly from pink and chum.

Although chinook survive longer in sea water than coho and sockeye alevins, the osmoregulatory abilities of chinook are not considerably greater than those of coho and sockeye. Therefore, the ability of chinook to survive longer in sea water than coho and sockeye is not due to osmoregulation but to high tissue tolerance.

#### Serum Na<sup>+</sup> Concentrations

The measurements of serum Na<sup>+</sup> concentrations are shown in Figure 4. A rapid rise in Na<sup>+</sup> concentration occurs for all species within the first 4 hr of exposure to sea water. Both pink and chum salmon alevins show decreased serum Na<sup>+</sup> concentrations between 4 and 8 hr and increased concentrations between 8 and 12 hr. However, coho, chinook and sockeye show increased levels of serum Na<sup>+</sup> throughout the first 12 hr in sea water, although coho do not manifest the same rapid rise as sockeye and chinook. Beyond 12 hr, the serum Na<sup>+</sup> concentrations in pink, chum and chinook are increasing at about the same rate.

On the basis of Tukey's w of 31 mEq/l Na<sup>+</sup>, calculated from the analysis of variance of the five species exposed to 0, 4, 8 and 12 hr of sea water (Table IX), pink alevins in fresh water are significantly different from chum alevins in fresh water. At 4 hr in sea water, no significant differences are found among the five species. At 8 hr, pinks are significantly different from chinook and sockeye but are not significantly different from coho ( $0.10 < P < 0.05$ ). However, chums at 8 hr are not significantly different from any of the other four species. At 12 hr, pink and chum are significantly different from chinook and sockeye but not from coho. In addition, coho are significantly different from chinook and sockeye. Thus coho, on the basis of Tukey's w, are not significantly different from pink and chum at any exposure

FIGURE 4

Serum  $\text{Na}^+$  concentrations of Pacific salmon  
alevins plotted against exposure times to  
31.8 $^{\circ}$ /oo sea water.

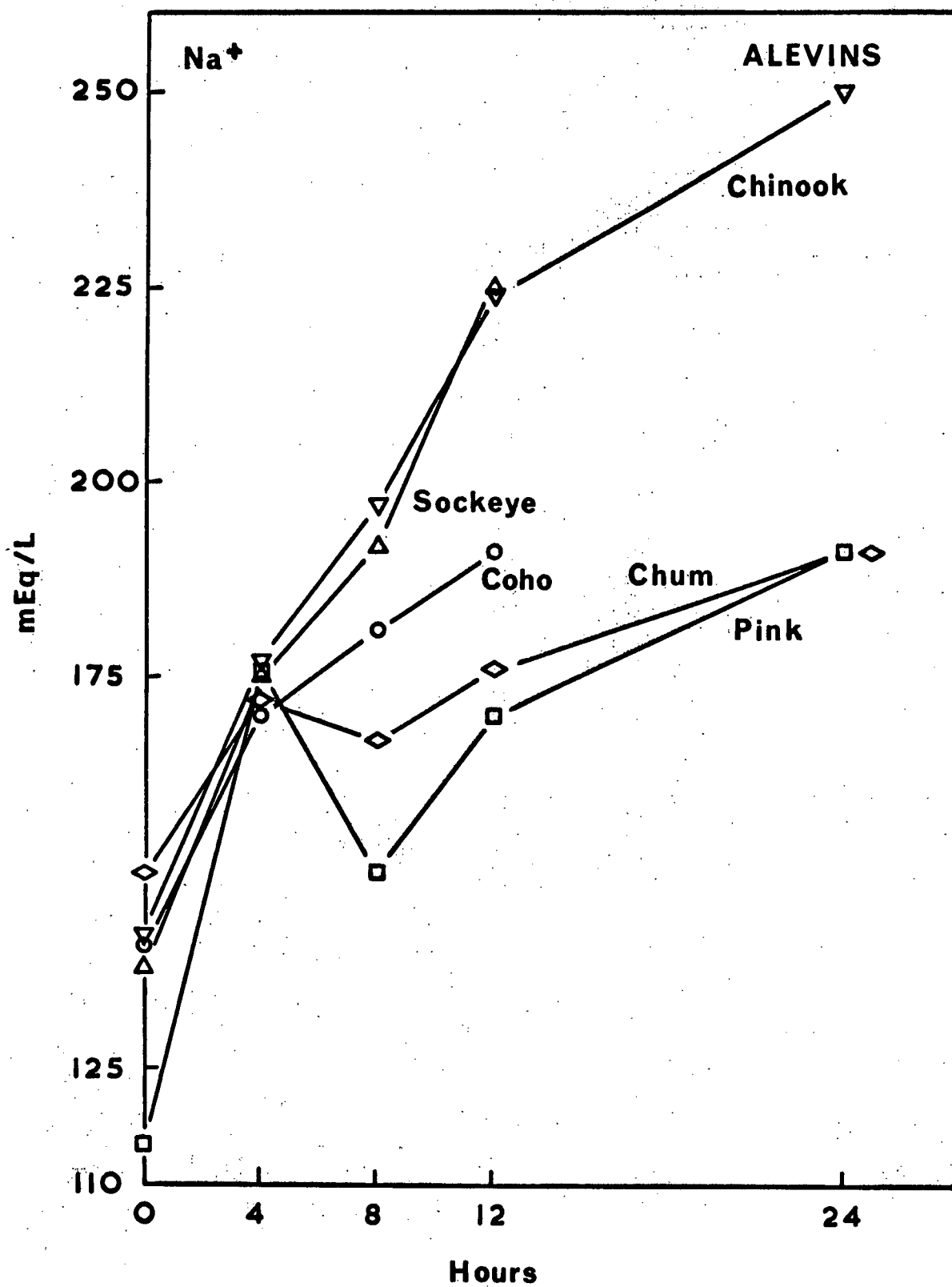


TABLE IX

Analysis of variance of the serum  $\text{Na}^+$  concentrations of the five species of alevins exposed to 0, 4, 8 and 12 hr of sea water.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	
Species	4	12,209	3,052	30.5	$F_{0.005}^{(4,40)} 4.37$
Exposure	3	28,054	9,351	93.5	$F_{0.005}^{(3,40)} 4.98$
Interaction	12	2,480	202	2.02	$F_{0.05}^{(12,40)} 2.00$
Error	40	4,012	100		

Therefore Species and Exposure are highly significant and Interaction is just significant at 0.05 level.

time. But orthogonal contrasts, which are more sensitive than Tukey's w, show pink/chum significantly different from coho when the levels of serum  $\text{Na}^+$  are compared collectively at 0, 4, 8 and 12 hr. Since pinks are significantly different from chums, we may not conclude that either one is significantly different from coho but that both taken together are different (see footnote, page 12).

The interactions between species and exposure times also have been analysed by orthogonal contrasts. The difference between pink/chum serum  $\text{Na}^+$  levels at 4 and 8 hr is significantly different from the difference in coho  $\text{Na}^+$  levels (Table VIII, Appendix). This same comparison of serum  $\text{Na}^+$  levels at 4 and 8 hr is not significantly different between pink and chum (Table IX, Appendix) but the difference between 0 and 12 hr is significantly different (Table X, Appendix). The difference in serum  $\text{Na}^+$  levels of coho alevins between 0 and 12 hr exposure is not significantly different from the difference observed for pink/chum (Table XI, Appendix).

For all five species, linear regressions fit the blood  $\text{Na}^+$  data better than do curvilinear regressions but the slopes of pink and chum regressions are less than those for coho, chinook and sockeye (Table XII, Appendix).

In summary, the ability of pink and chum alevins to regulate their serum  $\text{Na}^+$  concentrations is greater than that of chinook and sockeye. The ability of coho alevins in this regard is intermediate between these two groups.

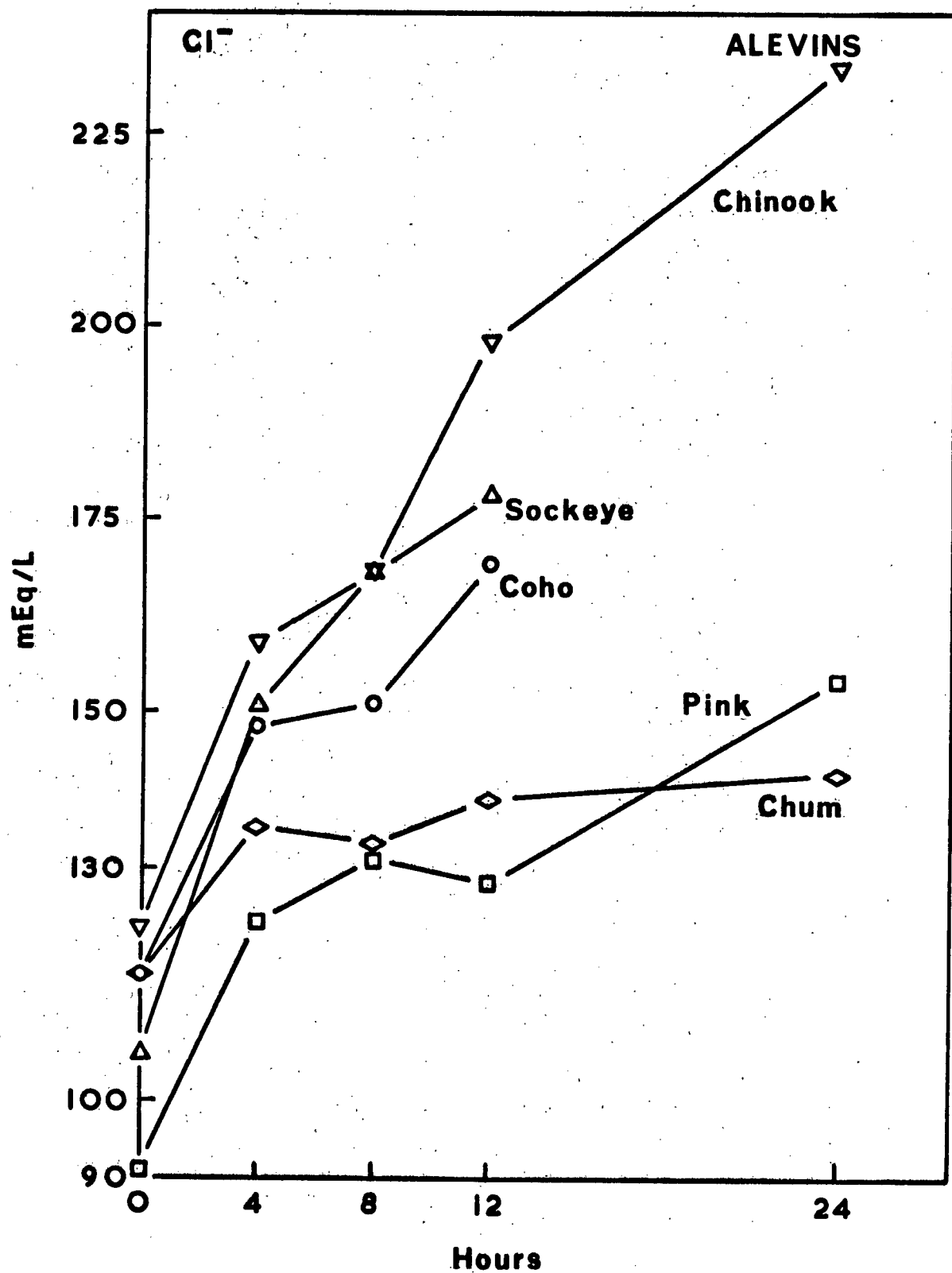
#### Serum $\text{Cl}^-$ Concentrations

The measurements of serum  $\text{Cl}^-$  concentrations are shown in Figure 5. Serum  $\text{Cl}^-$  levels rise rapidly for all species in the first 4 hr of exposure to sea water. Between 4 and 12 hr, the serum  $\text{Cl}^-$  levels in pink and chum

FIGURE 5

Serum  $\text{Cl}^-$  concentrations of Pacific salmon  
alevins plotted against exposure times to  
31.8 ‰ sea water.





alevins do not change appreciably while coho, chinook and sockeye show increasing levels. Beyond 12 hr,  $\text{Cl}^-$  levels in pink and chinook increase at about the same rate, while chum show little increase.

On the basis of Tukey's  $w$  of 16 mEq/l  $\text{Cl}^-$ , calculated from the analysis of variance of the five species exposed to 0, 4, 8 and 12 hr of sea water (Table X), several significant differences occur in fresh water: pink alevins are significantly different from chum, coho and chinook but not sockeye ( $0.1 < P < 0.05$ ). At 4 hr of exposure to sea water, pink alevins are significantly different from coho, chinook and sockeye but not from chum. Chum alevins at 4 hr are also significantly different from chinook but not from sockeye ( $0.1 < P < 0.05$ ) or coho. At 8 hr, pink and chum are both significantly different from coho, chinook and sockeye; coho are significantly different from chinook and sockeye; but pink alevins are not significantly different from chum. Similarly, at 12 hr, pink and chum are significantly different from the other three species, but not from one another. Coho and sockeye are significantly different from chinook but not from one another. At 24 hr, pink and chum are significantly different from chinook alevins.

Curvilinear regressions fit the pink and sockeye data better than linear regressions, while the reverse holds for chum, coho and chinook (Table XIII, Appendix).

In summary, the ability of pink and chum alevins to regulate their  $\text{Cl}^-$  concentrations is greater than that of coho, chinook and sockeye.

#### Serum $\text{K}^+$ Concentrations

The results of serum  $\text{K}^+$  measurements are shown in Figure 6. Serum  $\text{K}^+$  levels fall rapidly in the first 4 hr of exposure to sea water for all species except chinook. Between 4 and 8 hr all species except chinook show

TABLE X

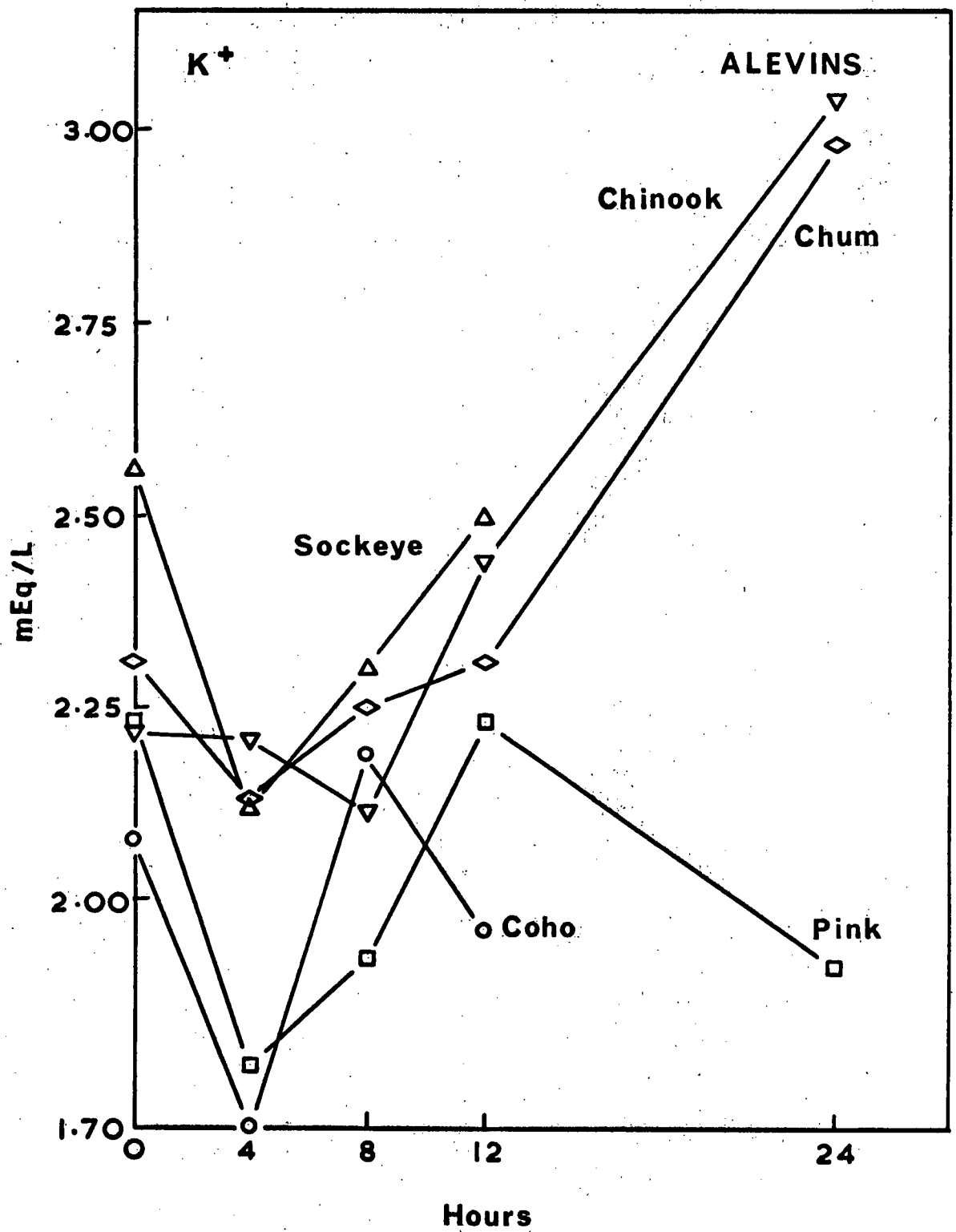
Analysis of variance of the serum  $\text{Cl}^-$  concentrations of the five species of alevins exposed to 0, 4, 8 and 12 hr of sea water.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	
Species	4	14,056	3,514	135	$F_{0.005}^{(4,40)} 4.37$
Exposure	3	22,776	7,592	292	$F_{0.005}^{(3,40)} 4.98$
Interaction	12	3,904	325	12	$F_{0.005}^{(12,40)} 2.95$
Error	40	1,051	26		

Therefore Species, Exposure and Interaction are highly significant.

FIGURE 6

Serum  $K^+$  concentrations of Pacific salmon alevins  
plotted against exposure times to 31.8<sup>o</sup>/oo sea  
water.



a rapid rise in serum  $K^+$  levels bringing them near freshwater levels. Between 8 and 12 hr all species except coho show increases in serum  $K^+$  levels. Between 12 and 24 hr  $K^+$  levels in chum and chinook diverge considerably from those of pink alevins. However, an analysis of variance of the data for the five species at 0, 4, 8 and 12 hr (Table XI), show no significant differences among the five species, among the four exposures and between the interaction of species and exposures.

TABLE XI

Analysis of variance of the serum  $K^+$  concentrations of the five species of alevins exposed to 0, 4, 8 and 12 hr of sea water.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	
Species	4	1,279	319.7	1.76	$F_{0.05}(4,40)$ 2.61
Exposure	3	872	290.7	1.60	$F_{0.05}(3,40)$ 2.84
Interaction	12	547	45.6	.25	$F_{0.05}(12,40)$ 2.00
Error	40	7,263	181.6		

Therefore there are no significant differences.

### C. Fry

#### LD<sub>50</sub> Values

Approximately five months after hatching, nine fry from each species (1964-65 progeny) were transferred from fresh water to 31.8<sup>0</sup>/oo sea water and the LD<sub>50</sub> determined for each species. The sea water proved lethal for coho, chinook and sockeye fry but not pink and chum (Table XII).

In conjunction with the LD<sub>50</sub> determinations, fry of each species were transferred directly from fresh water to 31.8<sup>0</sup>/oo sea water and the blood was sampled following immersion for 0, 4, 8, 12, 24 and 48 hr. Subsamples were taken from the plasma obtained from individual fish and the melting points and concentrations of Na<sup>+</sup>, Mg<sup>++</sup> and Cl<sup>-</sup> were determined. In Figures 7, 8, 9 and 10, which display the results of these measurements, every point is the mean of three determinations and each determination was made from the blood of one individual.

#### Plasma Osmotic Concentrations

The measurements of plasma osmotic concentrations are shown in Figure 7. Similar to the results of embryos and alevins, a large increase in plasma osmotic concentrations of fry occurs within the first 4 hr of exposure to sea water. Between 4 and 12 hr exposure to sea water, the plasma osmotic concentrations of pink and chum fry do not change appreciably whereas coho, chinook and sockeye fry show increasing levels. Between 12 and 24 hr pink and chum manifest declining plasma osmotic concentrations; and between 24 and 48 hr essentially no change in pink or chum levels occurs.

On the basis of Tukey's w of 45 mOsm, calculated from the analysis of variance of the five species exposed to 0, 4, 8 and 12 hr of sea water (Table XIII), it may be concluded that no significant differences occur



TABLE XII

LD<sub>50</sub> (hr) of 1964-65 fry transferred directly from fresh water  
to 31.8 ‰ sea water.

<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
> 336	> 336	32	120	18

FIGURE 7

Plasma osmotic concentrations of Pacific  
salmon fry plotted against exposure times  
to 31.8<sup>o</sup>/oo sea water.

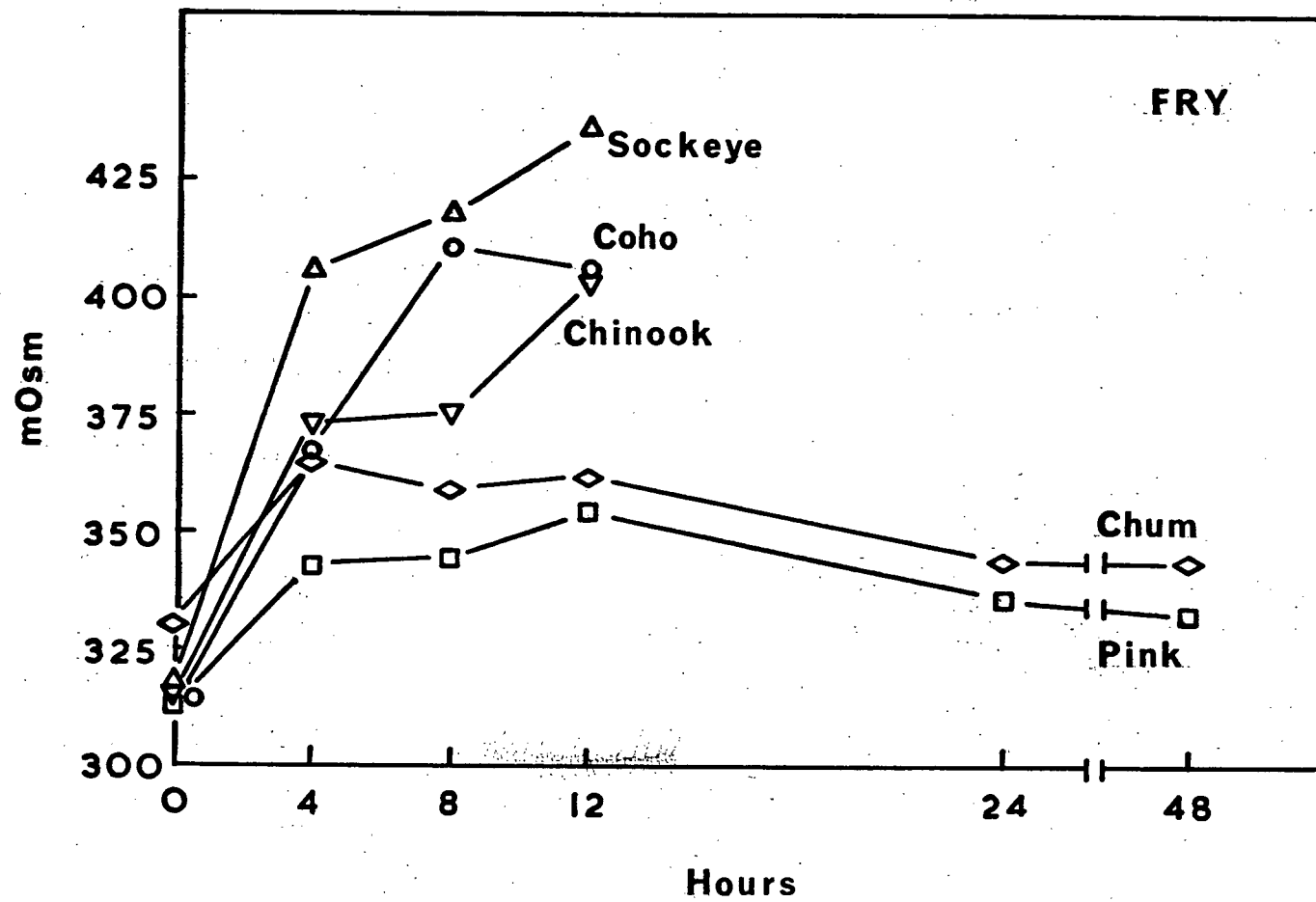


TABLE XIII

Analysis of variance of plasma osmotic concentrations of the five species of fry exposed to 0, 4, 8 and 12 hr of sea water.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	
Species	4	21,301	5,325.3	25.60	$F_{0.005}(4,40)$ 4.37
Exposure	3	49,314	16,438.0	79.01	$F_{0.005}(3,40)$ 4.98
Interaction	12	11,726	977.15	4.70	$F_{0.005}(12,40)$ 2.95
Error	40	8,321.6	208.04		

Therefore Species, Exposure and Interaction are highly significant.

among the five species in fresh water. At 4 hr exposure to sea water, pinks show a significantly lower plasma osmotic concentration than sockeye. At 8 hr, pink and chum levels are significantly lower than coho and sockeye. And finally, at 12 hr of exposure, pink fry are significantly different from coho, chinook and sockeye while chum are significantly different from sockeye but not from coho ( $0.10 < P < 0.5$ ).

Orthogonal contrasts show that the difference in response to sea water between pink/chum and coho/chinook/sockeye as measured by plasma osmotic concentrations at 0/4/8/12 hr is significant. However, the difference between pink and chum fry is highly significant ( $P < 0.005$ ). Similarly coho/chinook respond significantly differently from sockeye but coho fry do not differ from chinook. In addition, the difference in plasma osmotic concentration of chum at 8 and 12 hr exposure is not significantly different from the same difference for chinook (Table XIV, Appendix).

The pink and chinook data fit straight-line regressions better than curvilinear; the slope of the pink regression line is considerably less than that of the chinook (Table XV, Appendix). In contrast, the data of coho and sockeye fry are better described by curvilinear regressions. Chum data cannot be fitted to either the straight line or the curvilinear regression.

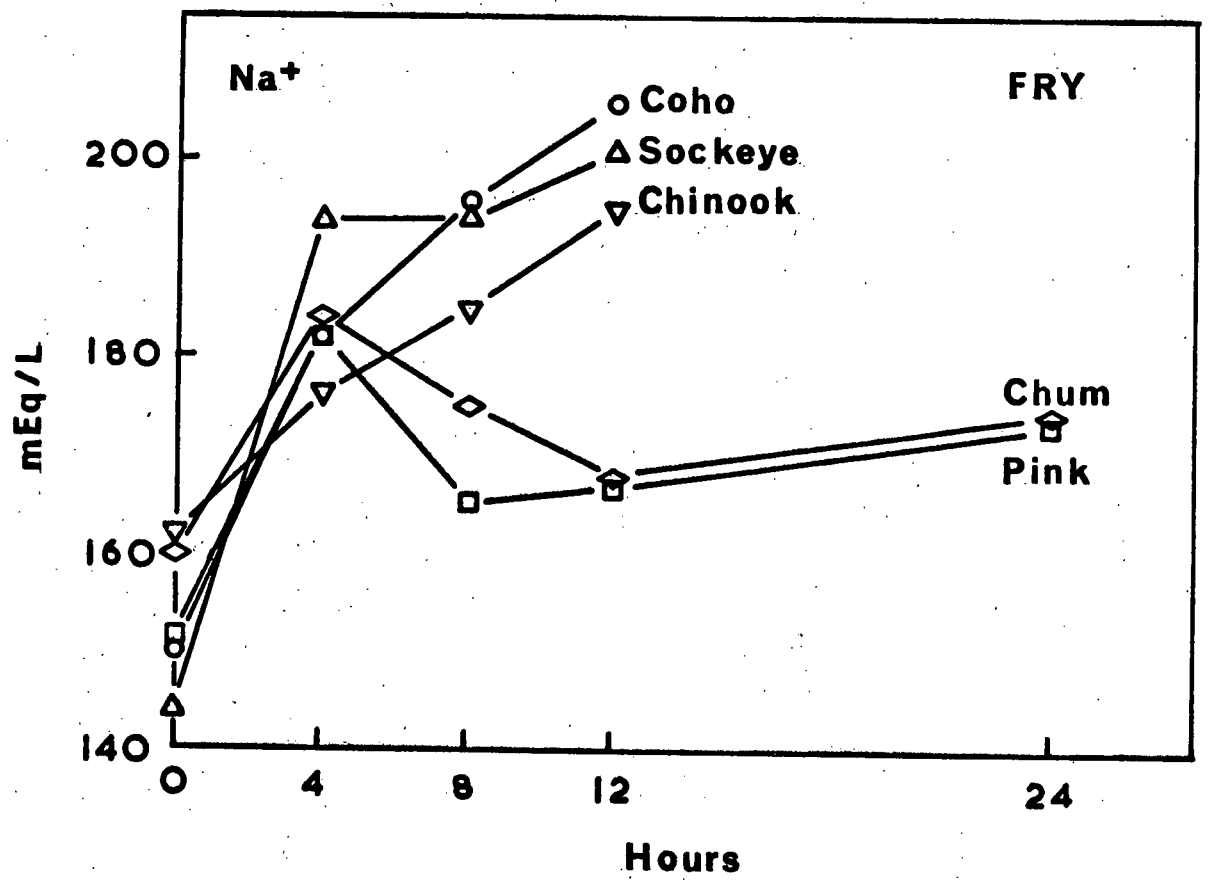
In summary, pink and chum fry manifest an ability to osmoregulate which is greater than that shown by sockeye. The abilities of coho and chinook fry to osmoregulate are intermediate. These capacities correlate with the tolerance of sea water by pink and chum fry and the greater salinity resistance of coho and chinook over that of sockeye.

#### Plasma $\text{Na}^+$ Concentrations

The measurements of plasma  $\text{Na}^+$  concentrations are shown in Figure 8. A

FIGURE 8

Plasma  $\text{Na}^+$  concentrations of Pacific salmon  
fry plotted against exposure times to 31.8<sup>o</sup>/oo  
sea water.



rapid rise in  $\text{Na}^+$  concentration occurs for all species within the first 4 hr of exposure to sea water. Between 4 and 12 hr both pink and chum fry show decreased levels of  $\text{Na}^+$  whereas coho, chinook and sockeye manifest increased levels. Between 12 and 24 hr only small changes occur in pink and chum fry levels.

On the basis of Tukey's w of 34 mEq/l, calculated from the analysis of variance of the five species exposed to 0, 4, 8 and 12 hr of sea water (Table XIV), no significant differences are present among the five species at 0, 4 and 8 hr sea water. However, at 12 hr, pink and chum fry have plasma  $\text{Na}^+$  levels which are significantly lower than coho levels but are not significantly different from sockeye levels ( $0.01 < P < 0.05$ ). Thus, chinook, on the basis of Tukey's w is not significantly different from pink and chum at any exposure time. But orthogonal contrasts, which are more sensitive than Tukey's w, show pink/chum significantly different from coho/chinook/sockeye when the levels of plasma  $\text{Na}^+$  are compared collectively at 0, 4, 8 and 12 hr. When pink and chum are compared collectively at 0, 4, 8 and 12 hr, they are not significantly different from each other. Similarly, coho are not significantly different from sockeye and coho/sockeye are not significantly different from chinook. Therefore, pink and chum are significantly different from coho, chinook and sockeye.

The chum and sockeye data fit curvilinear regressions better than straight-line regressions; coho and fry data are better described by straight-line regressions; pink data cannot be fitted to either the straight-line or the curvilinear regression (Table XVI, Appendix).

In summary, the abilities of pink and chum fry to regulate their plasma  $\text{Na}^+$  concentrations is greater than the abilities of coho, chinook and sockeye.



TABLE XIV

Analysis of variance of plasma  $\text{Na}^+$  concentrations of the five species of fry exposed to 0, 4, 8 and 12 hr of sea water.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	
Species	4	2,718.4	679.61	5.53	$F_{0.005}(4,40)$ 4.37
Exposure	3	10,787	3,595.8	29.27	$F_{0.005}(3,40)$ 4.98
Interaction	12	4,499.1	374.93	3.05	$F_{0.005}(12,40)$ 2.95
Error	40	4,914.0	112.85		

Therefore Species, Exposure and Interaction are highly significant.

### Plasma $\text{Cl}^-$ Concentrations

The measurements of plasma  $\text{Cl}^-$  concentrations are shown in Figure 9. A rapid rise in plasma  $\text{Cl}^-$  concentrations occurs for all species and particularly Sockeye within the first 4 hr of exposure to sea water. Between 4 and 8 hr, pink, chum and sockeye plasma  $\text{Cl}^-$  levels do not change appreciably, while coho and chinook  $\text{Cl}^-$  levels continue to increase. Between 8 and 12 hr, pink and chum  $\text{Cl}^-$  levels decrease and coho, chinook and sockeye  $\text{Cl}^-$  levels increase. At 24 and 48 hr, pink and chum plasma  $\text{Cl}^-$  appear to have stabilized at levels slightly higher than fresh water values.

On the basis of Tukey's w of 21 mEq/l, calculated from the analysis of variance of the five species exposed to 0, 4, 8 and 12 hr of sea water (Table XV), no significant differences occur in the plasma  $\text{Cl}^-$  levels among the five species at 0 and 4 hr sea water. At 8 hr sea water, pink plasma  $\text{Cl}^-$  levels are significantly lower than coho levels. At 12 hr, pink levels are significantly lower than coho, chinook and sockeye, whereas chum levels are significantly lower than coho but not from any of the other species.

Orthogonal contrasts show that pink/chum are significantly different from coho/chinook/sockeye as measured by plasma  $\text{Cl}^-$  concentrations at 0/4/8/12 hr in sea water. Since pink plasma  $\text{Cl}^-$  levels are not significantly different from those of chum at 0/4/8/12 hr, nor are coho/sockeye levels significantly different from chinook levels or coho levels from sockeye levels, the plasma  $\text{Cl}^-$  concentrations in pink and chum are significantly different from those in coho, chinook and sockeye at 0/4/8/12 hr in sea water.

Curvilinear regressions fit the pink, chum and sockeye data better than linear regressions, whereas the reverse holds for coho and chinook (Table :

FIGURE 9

Plasma  $\text{Cl}^-$  concentrations of Pacific salmon fry  
plotted against exposure times to  $31.8^\circ/\text{oo}$  sea  
water.

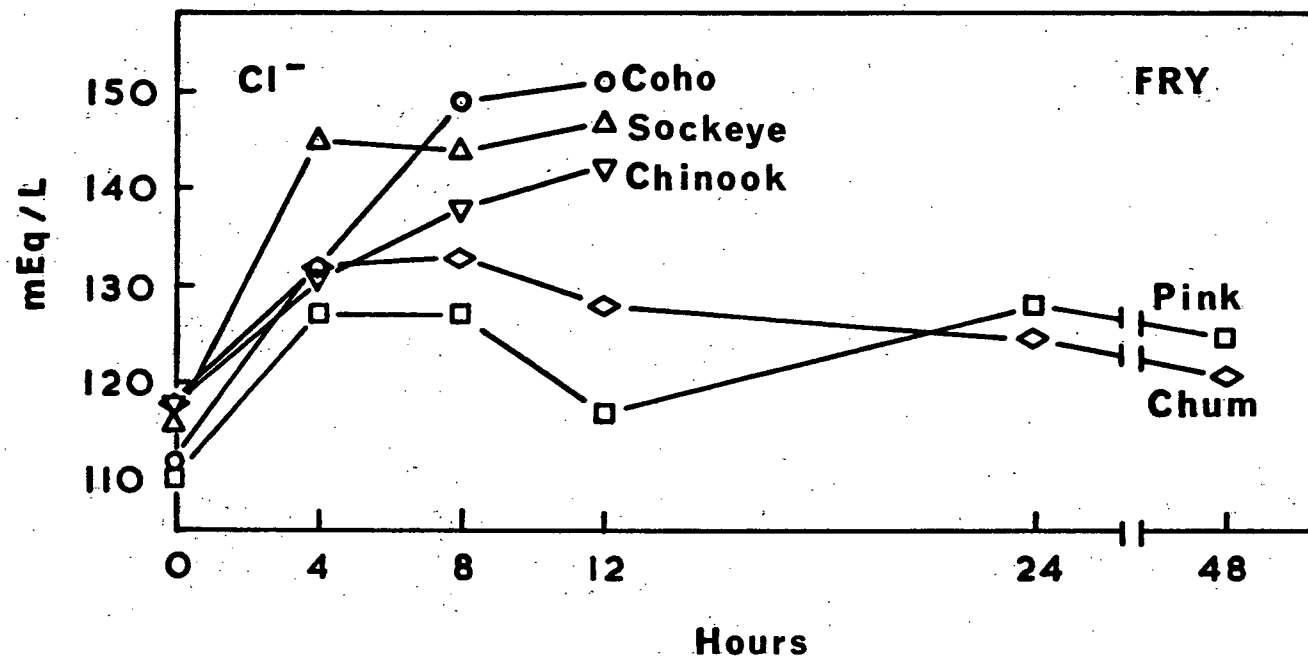


TABLE XV

Analysis of variance of plasma  $\text{Cl}^-$  concentrations of the five species of fry exposed to 0, 4, 8 and 12 hr of sea water.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
Species	4	2,416.4	604.11	12.96 $F_{0.005}(4,40)$ 4.37
Exposure	3	5,337.5	1,779.2	38.16 $F_{0.005}(3,40)$ 4.98
Interaction	12	1,594.3	132.86	2.85 $F_{0.01}(12,40)$ 2.66
Error	40	1,864.7	46.618	

Therefore Species and Exposure are highly significant and Interaction is significant.

XVII, Appendix).

### Plasma $Mg^{++}$ Concentrations

The measurements of plasma  $Mg^{++}$  concentrations are shown in Figure 10. A very large increase in plasma  $Mg^{++}$  concentration occurs for all species within the first 4 hr in sea water. Between 4 and 8 hr pink, chum and chinook plasma  $Mg^{++}$  levels decline while coho and sockeye levels rise. Between 8 and 12 hr, pink and coho levels decline while chum, chinook and sockeye levels increase. Between 12 and 48 hr, pink plasma  $Mg^{++}$  levels increase slightly while between 12 and 24 hr, chum levels decrease considerably and after 24 hr increase moderately.

On the basis of Tukey's w of 2.92 mEq/l, calculated from the analysis of variance of the five species exposed to 0, 4, 8 and 12 hr of sea water (Table XVI), no significant differences are present among the five species at 0, 4, 8 and 12 hr with the single exception of the sockeye plasma  $Mg^{++}$  concentration at 12 hr which is significantly higher than the plasma  $Mg^{++}$  concentrations of pink, chum and chinook fry in fresh water.

Orthogonal contrasts show that the difference between plasma  $Mg^{++}$  concentrations of pink fry and chum/coho/chinook fry at 0/4/8/12 hr is highly significant ( $P < 0.005$ ). However, the difference between chum and coho/chinook at 0/4/8/12 hr is not significant nor is the difference between coho and chinook.

There is no significant interaction between species and exposure times (Table XVI).

The data for pink fry fit a curvilinear regression better than a straight-line whereas the reverse is true for sockeye data (Table XVIII, Appendix). The chum, coho and chinook data cannot be fitted to the

FIGURE 10

Plasma  $Mg^{++}$  concentrations of Pacific salmon fry  
plotted against exposure times to 31.8 ‰ sea  
water.

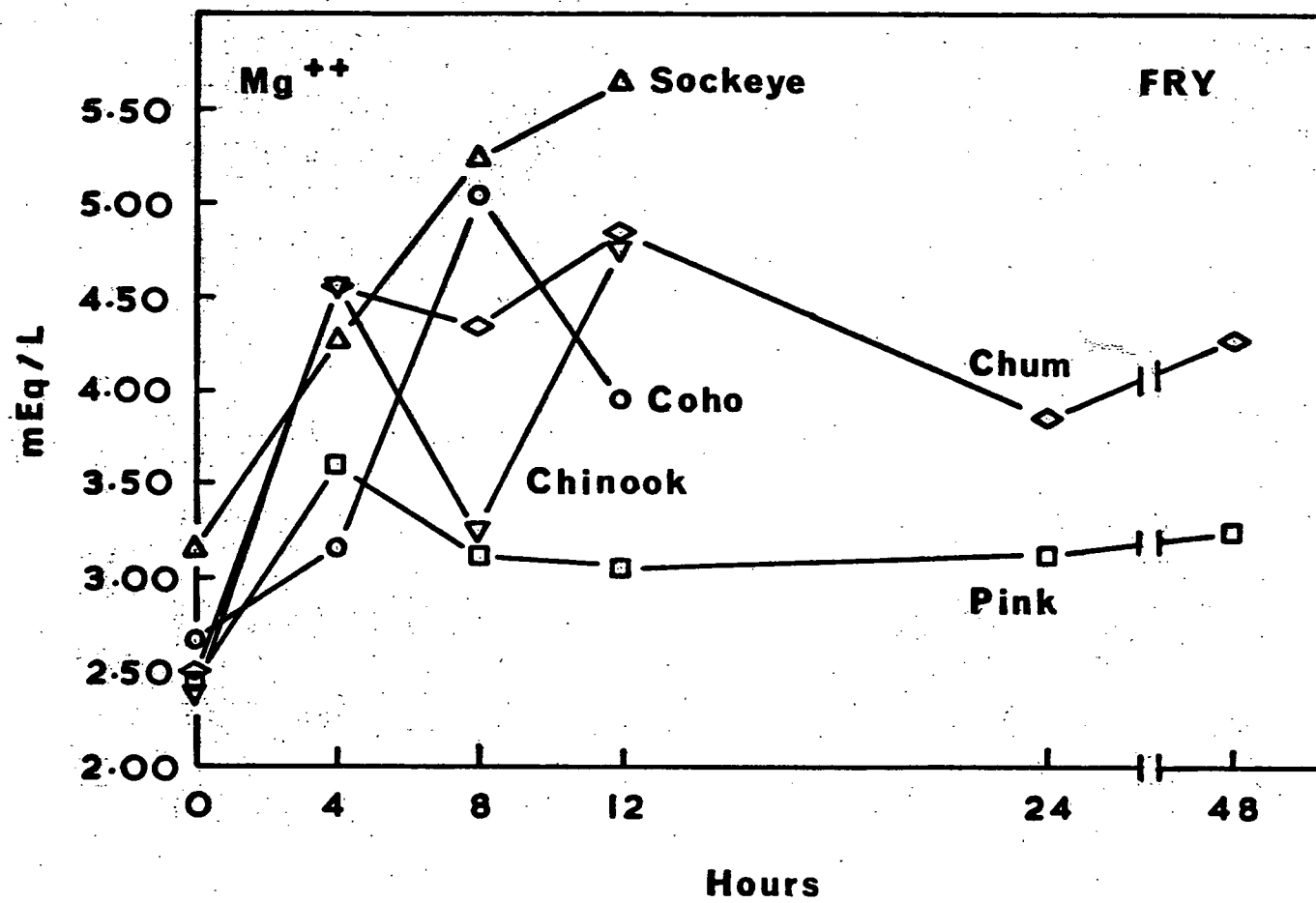




TABLE XVI

Analysis of variance of plasma  $Mg^{++}$  concentrations of the five species of fry exposed to 0, 4, 8 and 12 hr sea water.

<u>Source</u>	<u>df</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	
Species	4	14.785	3.6963	4.14	$F_{0.01}(4,40)$ 3.83
Exposure	3	30.052	10.017	11.23	$F_{0.005}(3,40)$ 4.98
Interaction	12	14.238	1.1865	1.33	$F_{0.10}(12,40)$ 1.71
Error	40	35.676	.89190		

Therefore Species is significant, Exposure is highly significant and Interaction is not significant.

curvilinear or the straight-line regressions.

In summary, pink fry can regulate the  $Mg^{++}$  concentration in their plasma whereas sockeye fry do not manifest this ability. The data for chum, coho and chinook are inconclusive.

#### D. Comparison of Embryo, Alevin and Fry Data

##### LD<sub>50</sub> Values

The salinity resistance times which have been outlined previously for each species at each age are brought together in Table XVII for purposes of comparison. The five species, as shown by their LD<sub>50</sub> values, fall into three groups. Pink and chum at all three ages are considerably more resistant to sea water than the coho and sockeye at the same ages. The salinity resistance of chinook is intermediate between these two groups. In addition, embryos and fry of all five species (except sockeye) manifest greater salinity resistance than their respective alevins.

Because the criterion for death of embryos and alevins was the arrestment of blood flow in the vitelline veins while the criterion for death of fry was the cessation of opercular movements, the salinity resistance of fry must be compared to that of embryos with some reservation. Since opercular movements are more rapidly inhibited by high salinities than is heart beat, the LD<sub>50</sub> values of fry would be higher than those in Table XVII if the cessation of blood flow had been used as the criterion of death in fry. Therefore, the survival of coho and sockeye fry may not be less than but equal to or greater than their respective embryos. However, without doubt, pink and chum fry survive considerably longer in 31.8‰ sea water than pink and chum embryos and alevins; in addition, chinook fry survive longer than chinook embryos.

##### Osmotic Concentrations

The question that arises from the LD<sub>50</sub> data is whether the measurements of blood osmotic concentration correlate with the differences outlined. Before answering this question, it must be noted that the method of

TABLE XVII

LD<sub>50</sub> (hr) of embryos, alevins and fry of Pacific salmon, transferred directly from fresh water to 31.8 ‰ sea water.

	<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
Embryos <sup>1</sup>	102	118	47	81	52
Alevins <sup>2</sup>	69	56	26	49	27
Fry <sup>3</sup>	> 336	> 336	32	120	18

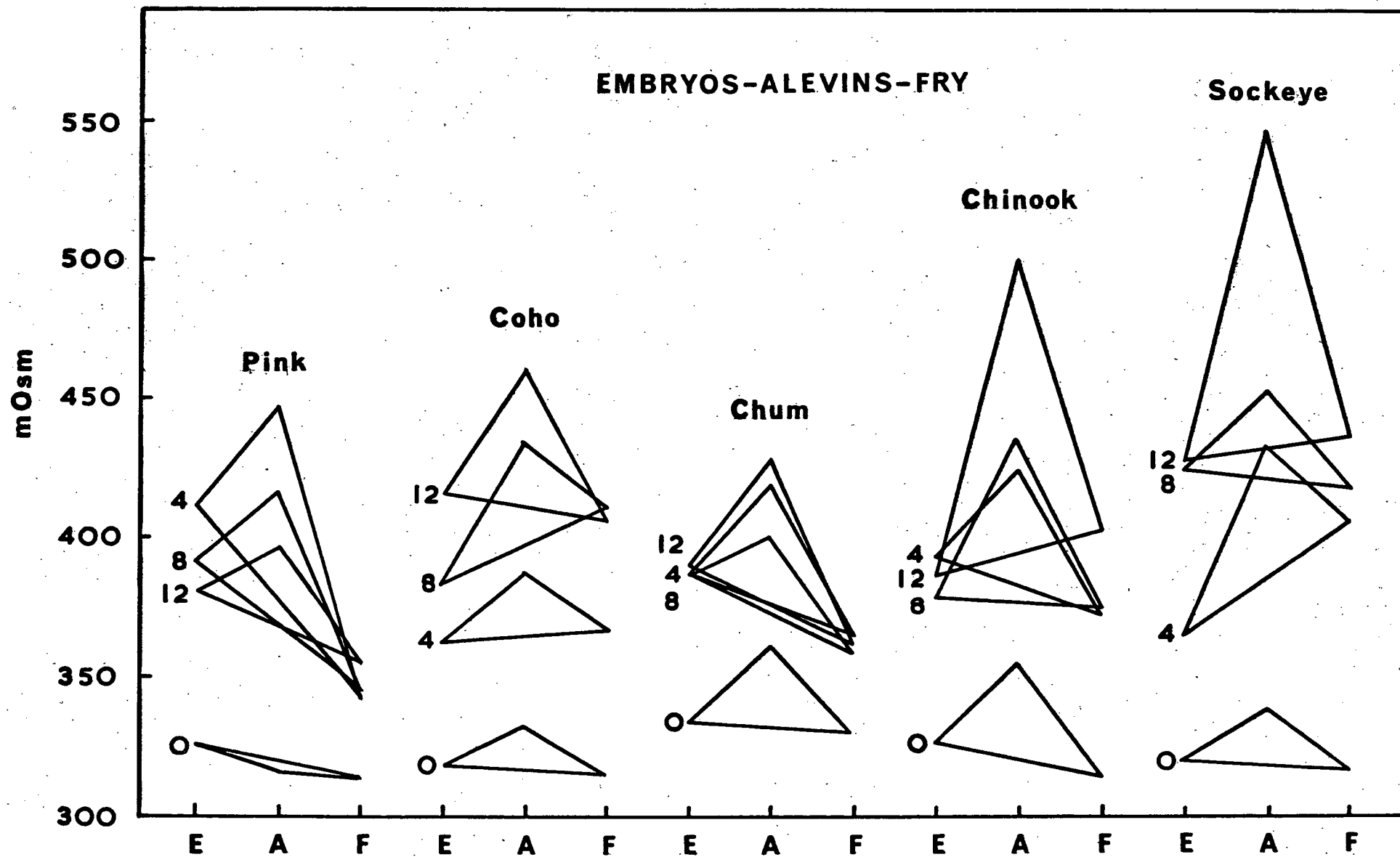
- 1 1965-66 embryos
- 2 1965-66 alevins
- 3 1964-65 fry

collecting blood was different for all three ages. As a result a test was made to determine if the blood collecting technique influenced the osmotic concentrations. When fry were sampled by the embryo collecting method (serum obtained) and by the usual fry method (plasma obtained) and the osmotic concentrations determined, no significant differences were observed between the two techniques. The reverse experiment, that is, comparing embryo and fry blood collecting methods on embryos was not possible. However, when blood from fry was collected by the alevin method (pooled serum obtained) and the fry method, the former resulted in osmotic concentrations which were significantly higher than the latter. The mean value obtained by the alevin method was 333 mOsm and the mean by the fry method was 320 mOsm. It was not possible to use the alevin blood collecting method for fry because of more rapid clotting in fry and the reverse was impossible because of hemolysis and clotting, both of which were not abetted by heparin.

Therefore, for comparative purposes, osmotic concentrations of embryo serums and fry plasma have not been altered but osmotic concentrations of pooled alevin serums have been decreased by 13 mOsm. On this basis, the blood osmotic concentrations of the five species of Oncorhynchus, at four exposure times to sea water and at three ages are compared in Figure 11. For any one species, blood osmotic concentrations of alevins are higher than those of embryos or fry at all exposures. However, within any one of the five species, the blood osmotic concentrations of alevins at 12 hr in sea water not only are higher than those of embryos and fry but by amounts which are greater than the difference between alevins and embryos or fry in fresh water. Osmotic concentrations of pink and chum fry are considerably below both embryo and alevin levels at 4, 8 and 12 hr but only slightly below

FIGURE 11

The blood osmotic concentration of Pacific salmon embryos (E), alevins (A) and fry (F) exposed to 0, 4, 8 and 12 hr of 31.8<sup>o</sup>/oo sea water. Each triangle is the result of joining the mean blood osmotic concentrations of embryos, alevins and fry exposed for the same duration to sea water.



embryo levels in fresh water.

With Tukey's  $w$  of 56 mOsm, calculated from the analysis of variance of the five species exposed to 0, 4, 8 and 12 hr of sea water as embryos, alevins and fry (Table XVIII), the following conclusions may be made:

- (1) The serum osmotic concentrations of pink, chum and coho embryos at 0, 4, 8 and 12 hr in sea water are not significantly different from those of the respective alevins, whereas chinook embryos at 8 hr are significantly different from chinook alevins at 8 hr, and sockeye embryos at 4 and 12 hr are significantly different from sockeye alevins at 4 and 12 hr.
- (2) The serum osmotic concentrations of chum, coho, chinook and sockeye alevins at 12 hr sea water are significantly different from the plasma osmotic concentrations of the respective fry at 12 hr; in addition, pink alevins at 4 and 8 hr are significantly different from pink fry at 4 and 8 hr, and chinook alevins at 8 hr are significantly different from chinook fry at 8 hr.
- (3) The serum osmotic concentration of pink embryos at 4 hr is significantly different from the plasma osmotic concentration of pink fry at 4 hr; otherwise no other significant differences are observed between embryos and fry of the same species.

Broader comparisons have also been made using orthogonal contrasts. The blood osmotic concentrations of pink/chum salmon as embryos/alevins/fry at 0/4/8/12 hr in sea water are highly significantly different from coho/chinook/sockeye osmotic concentrations as embryos/alevins/fry at 0/4/8/12 hr ( $P < 0.005$ ). Similarly, the difference between coho/chinook and sockeye is highly significant ( $P < 0.005$ ), but pink are not significantly different from chum, and coho are not significantly different from chinook.

The interaction between species and ages has also been analysed by



TABLE XVIII

Analysis of variance of blood osmotic concentrations of the five species of Pacific salmon embryos, alevins and fry exposed to 0, 4, 8 and 12 hr sea water.

Source	df	SS	MS	F	Table $F_{0.005}$
Species	4	28,566	7,141.6	25.89	3.92
Age	2	80,500	40,250	145.92	5.54
Exposure	3	213,240	71,080	257.68	4.50
Species X Age	8	14,220	1,777.6	6.44	2.93
Species X Exposure	12	40,754	3,396.1	12.31	2.54
Age X Exposure	6	12,902	2,150.3	7.80	3.28
Species X Age X Exposure	24	19,431	809.62	2.94	2.09
Error	120	33,101	275.84		

Therefore all main effects and all interactions are highly significant.

orthogonal contrasts. The difference between embryo and fry osmotic concentrations of pink/chum is highly significant ( $P < 0.005$ ) from the difference between embryo and fry of coho/chinook/sockeye (Table XIX, Appendix). Similarly, pink are not significantly different from chum (Table XX, Appendix); coho/chinook are not significantly different from sockeye (Table XXI, Appendix) and coho are not significantly different from chinook (Table XXII, Appendix). Therefore, the differences in blood osmotic concentrations between embryos and fry of pink and chum are significantly different from those of coho, chinook and sockeye.

The difference between embryos/fry and alevins of pink/chum is significantly different from the difference between embryos/fry and alevins of coho/chinook/sockeye (Table XXIII, Appendix). However, the difference between embryos/fry and alevins of pink is not significant from that of chum (Table XXIV, Appendix). Similarly, coho/chinook is not significantly different from sockeye (Table XXV, Appendix), but the difference between coho and chinook is highly significant ( $P < 0.005$ ; Table XXVI, Appendix).

Returning to more general comparison, orthogonal contrasts indicate that embryos/fry of pink/chum/coho/chinook/sockeye at 0/4/8/12 hr are highly significantly different from alevins ( $P < 0.005$ ) and that embryos are significantly different from fry.

And finally, the three-way interaction (Table XVIII) has been analysed by orthogonal contrasts and the following interaction found to be significant ( $P < 0.01$ ). The difference between 0 and 12 hr in sea water for embryos/fry vs alevins of pink/chum is significantly different from the difference between 0 and 12 hr for embryos/fry vs alevins of coho/chinook/sockeye (Table XXVII, Appendix).

Therefore, embryos and fry in sea water have lower blood osmotic concentrations than alevins in sea water. Since the embryos and fry (except sockeye) survive longer in sea water than their respective alevins, the results on blood osmotic concentrations correlate positively with the  $LD_{50}$  values. Therefore, embryos and fry probably survive longer in sea water than alevins due to greater osmoregulatory abilities.

#### E.. Relationship Between Osmotic and Ionic Concentrations

The contributions that  $Na^+$  and  $Cl^-$  concentrations make to the serum and plasma osmotic concentrations are shown in Figures 12 and 13 respectively. It is assumed that  $Na^+$  and  $Cl^-$  in serum or plasma are in ideal solutions in which one milliequivalent per litre of  $Na^+$  or  $Cl^-$  has an osmotic concentration of one milliosmole. In alevin serum,  $Na^+$  and  $Cl^-$  contribute 63 to 82% of the osmotic concentration which is significantly lower than the 80 to 90% in fry plasma ( $P < 0.005$ ). In addition, sodium-to-chloride ratios in alevin serum average 1.22 which is significantly different from the same ratio for fry plasma which averages 1.35 ( $P < 0.005$ ). No significant differences were observed in these ratios between alevins in fresh water and alevins in sea water; similar results were obtained with fry.

FIGURE 12

Osmotic, sodium and chloride concentrations of  
Pacific salmon alevins plotted against exposure  
time to 31.8 ‰ sea water.

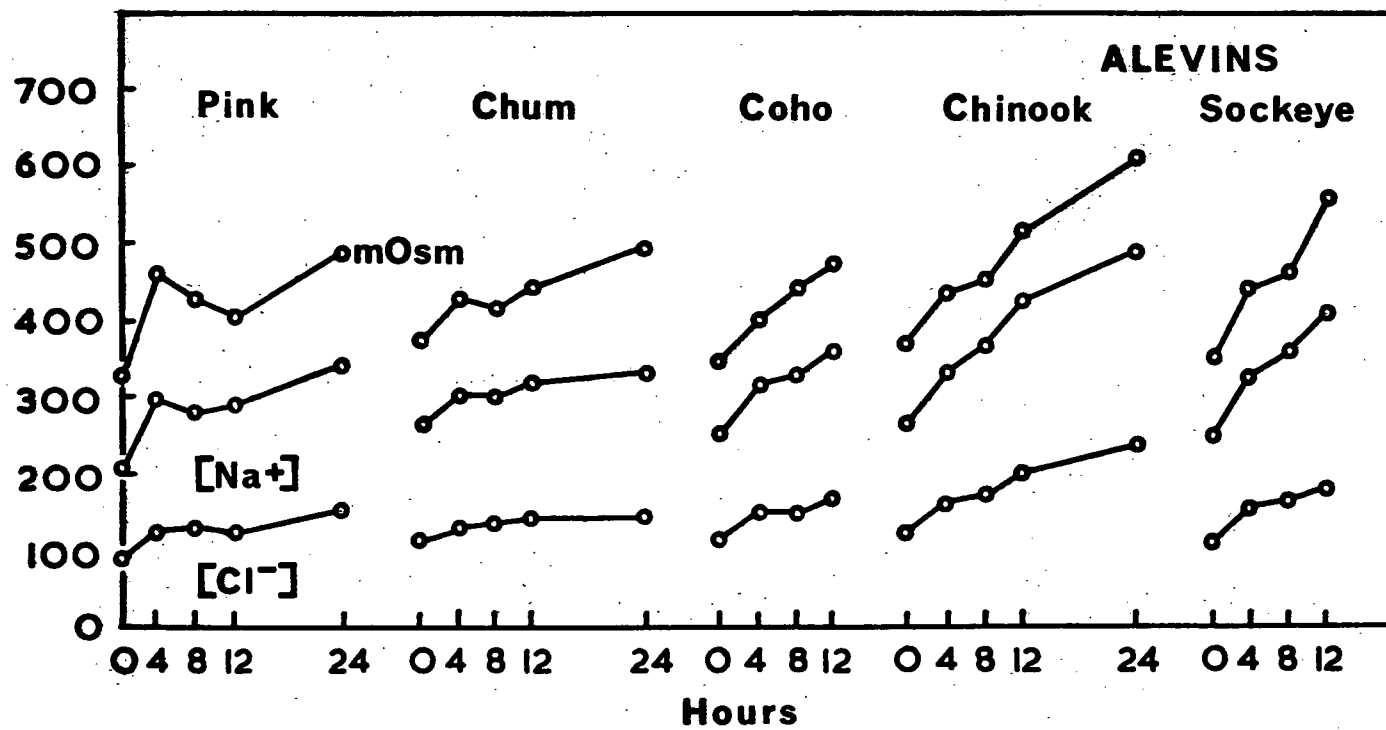
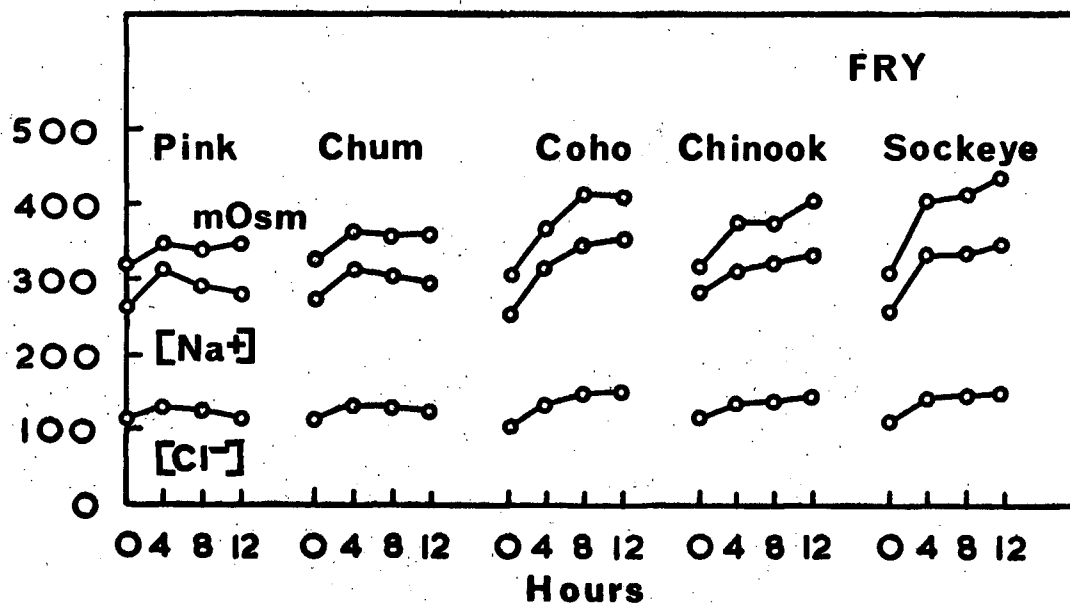


FIGURE 13

Osmotic sodium and chloride concentrations of  
Pacific salmon fry plotted against exposure time  
to 31.8 ‰ sea water.



## DISCUSSION

### A. Examination of Working Hypotheses

Differences in ecology and behaviour among the five species of Pacific salmon are particularly evident in the early life stages (Neave, 1958; Hoar, 1958). Based on this fact, it was postulated that (i) the embryo, alevin and fry of pink and chum salmon are euryhaline, whereas the same life stages of coho, chinook and sockeye are stenohaline and (ii) the euryhalinity of pink and chum is due not to high tissue tolerance but to their ability to regulate the osmotic and ionic concentrations in their blood, whereas the stenohalinity of coho, chinook and sockeye stems from their inability to osmoregulate and ion regulate.

The LD<sub>50</sub> values and the osmoregulatory data obtained from embryos indicate that pink and chum are not euryhaline as hypothesized; that is, they cannot survive in a wide range of salinities. Therefore, embryos of pink and chum, like coho, chinook and sockeye, are stenohaline. However, the LD<sub>50</sub> values and osmotic concentrations of pink and chum embryos indicate greater salinity resistance and osmoregulatory ability than embryos of the other species.

The data obtained from alevins also does not support the working hypotheses for alevins of all five species are stenohaline. But, as in the case for embryos, pink and chum alevins have greater salinity resistance and osmoregulatory abilities than coho and sockeye alevins. This ability of pink and chum has been correlated with better ionic regulation of sodium and chloride. Although chinook alevins survived considerably longer in 31.8‰ sea water than coho and sockeye alevins, their ability to osmoregulate and



ion regulate is not very different from that of coho and sockeye alevins. Therefore, the greater salinity resistance of chinook alevins is due to high tissue tolerance. Their organs are able to function in a wider range of osmotic and ionic concentrations of the milieu interieur than is the case for coho and sockeye.

Contrary to the results with embryos and alevins, the data obtained from fry supports the working hypotheses. The data indicate that pink and chum fry are euryhaline and that this condition is due to their ability to osmoregulate and control the levels of sodium and chloride in the blood. Coho, chinook and sockeye fry are stenohaline and are unable to regulate the osmotic and ionic concentrations in their blood. As in the case of alevins, chinook fry, due to higher tissue tolerance, manifest greater salinity resistance than coho and sockeye.

In summary, the working hypotheses have been shown to be only partially correct. Pink and chum fry are euryhaline whereas embryos and alevins of all five species are stenohaline. However, pink and chum embryos and alevins manifest greater abilities to regulate the osmotic and ionic concentrations in their blood than coho, chinook and sockeye. The abilities of pink and chum fry to osmoregulate and ion regulate support the hypothesis that their euryhalinity is not due to high tissue tolerance. Although chinook alevins and fry are stenohaline and therefore support the hypothesis, they survive longer in sea water than coho and sockeye. The greater survival is not the result of better osmoregulation but is due to higher tissue tolerance.

#### B. Mechanisms of Osmotic and Ionic Regulation in Embryos

Coelomic salmonid eggs, which are slightly hypo-osmotic to the maternal blood (Hayes, 1949), are permeable to water (Zotin, 1965), but after

fertilization, no net gain or loss of water occurs in normally developing eggs. Kao et al. (1954) believes that high pressure of the perivitelline fluid formed at fertilization may cause reduction in the size of pores in the vitelline membrane and thereby reduce permeability to water, whereas Zotin (1965) believes that "eggs (teleost) contain water under special conditions and that as a result it possesses properties somewhat differing from those of water in more usual physicochemical systems".

Fertilization of salmonid eggs is possible in dilute sea water but not in high concentrations. Rockwell (1956) found that a salinity of 18 ‰ or less does not inhibit fertilization of pink and chum eggs. In fact, dilute salt water prolonged the period of egg and sperm fertility (Rutter, 1902; Ellis and Jones, 1939; Rucker, 1949). This enhancement of fertility is not due to ions but the osmotic conditions of the dilute solutions (Ellis and Jones, 1939).

Just after entrance of a spermatozoan into the micropyle of the ovum, cortical alveoli break down releasing a colloid which imbibes water between the vitelline membrane and the chorion thereby forming the perivitelline space (Bogucki, 1930; Hayes and Armstrong, 1942; Zotin, 1958). As a result of this uptake of water, there is a 20% increase in weight of Salmo salar eggs (Hayes and Armstrong, 1942). The net uptake of water in Salmo salar is complete soon after fertilization while hardening of the chorion reaches its peak much later (Hayes and Armstrong, 1942; Zotin, 1958). While Na, K, Ca, Sr and Mg ions inhibit the release of cortical alveoli and also the release of hardening enzyme (for chorion) at least one of these ions is necessary in the first 5 min after fertilization for the hardening enzyme to function. The hardening enzyme is not present in the cortical alveoli (Zotin, 1958) but its normal activity may require some of the ions probably released from the

breakdown of the cortical alveoli. Although 0.1 N NaCl blocks release of cortical alveoli and hardening enzyme (Zotin, 1958), normal development of soft eggs in fresh water is possible (Rockwell, 1956).

The views of Kao et al. (1954) and Zotin (1965) have already been outlined as explanations of the fact that soon after fertilization 100% of the total volume of salmonid embryos is osmotically inactive. However, this does not preclude the possibility of water movement into or out of salmonid embryos for at least four different phenomena of water movement exist and have been defined by Zotin (1965):

- (1) entry or exit of water as determined by osmosis
- (2) water renewal without net change in water content of embryo
- (3) unilateral water transport dictated by needs of the embryo
- (4) redistribution of water within the embryo

Krogh and Ussing (1937) showed that soon after fertilization, penetration of heavy water ceased and did not resume until after formation of the eye. These results are in agreement with those of Svetlov (1929) who showed that the freezing point depression of the trout yolk did not change during development and those of Gray (1932) who showed no change in trout egg weight in hypertonic solutions. Later, Hayes and Armstrong (1942) observed no change in wet weight of embryos and no change in weight of water in eggs up to hatching and Busnel et al. (1946) found that the percent water of developing Salmo salar up to hatching remains constant. These results suggest that the penetration of heavy water for a short period after fertilization is indicative of Zotin's Type (2) water movement, that is, water renewal. In addition, the duration of this phenomenon after fertilization is species-specific for while chinook and rainbow trout eggs lose the ability to exchange

water soon after fertilization, eggs of Salmo salar require as long as ten days (Zotin, 1965).

One is therefore left with the impression from these results that the salmonid egg from fertilization to hatching (excluding the perivitelline space) does not take up net amounts of water. Winnicki (1958) and Domurat (1961) however, have shown that trout eggs cannot develop normally in a waterless environment for under such conditions a prolongation of embryonic development occurs. These results may be due to an accumulation of metabolites, to limiting oxygen concentrations or, as Winnicki (1958) and Domurat (1961) have argued, to a need for water from the external medium during the process of morphogenesis. Since there is no net change in water content of the embryo, the water requirement, if Winnicki and Domurat are correct, must be of Zotin's Type (2).

In addition to water renewal, salmonid embryos obtain water from the yolk. The water content of the yolk of Salmo salar eggs decreased from 63.5% at fertilization to 59% at hatching (Hayes and Armstrong, 1942), even though, as stated previously, the freezing point depression of the yolk remains constant throughout embryonic development (Svetlov, 1929).

Although eyed salmonid embryos may renew their water and obtain water from their yolk, they do not make net gains or losses of water. Busnel et al. (1946) found that the percentage of water in Salmo salar embryos remains around 63 to 64% when the eggs, near hatching, are immersed in fresh water, 10‰, 15‰, 20‰ or 25‰ saline water despite the fact the osmotic concentration of the milieu interieur increases from 318 to 712 milliosmoles. Therefore, since no net water gains are made by eggs before hatching (Hayes and Armstrong, 1942), the embryos must be capable of making

net gains or losses of salts but not water. This conclusion is supported by Hayes (1949) in that he observed net losses of phosphorus and potassium from the salmon egg before hatching.

Finally, pink and chum embryos, about to hatch, are not prevented from doing so by salinities up to 29.67<sup>0</sup>/oo; in addition, brackish water tends to accelerate hatching and improve the percentage of successful hatching (Rockwell, 1956). Enhanced and accelerated hatching in brackish water is probably due to the ionic strength effect of such solutions on the hatching enzyme, chorionase, and the more alkaline conditions in the perivitelline fluid which would increase the activity of the enzyme (Kaighn, 1964).

If the above interpretations are correct, then embryos of Oncorhynchus near hatching (2 weeks) can take up or lose net amounts of ions, but not water, and the greater ability of pink and chum embryos to osmoregulate must be due to their ability to ion regulate. If the differences among the five species of Pacific salmon were due to the surface-to-volume ratios, then sockeye embryos should have the least resistance to sea water and be the poorest osmoregulator while the reverse should hold for chinook embryos. My results agree with the contention for sockeye but not for chinook. In fact, although pink eggs are smaller than chinook or chum eggs, pink embryos osmoregulate better than chinook and as well as chum. Size, then, at the interspecific level is not as important as some other properties, but the other characters involved can only be conjectured at this point. The roles of the incompletely differentiated alimentary canal in extracting ions and water from sea water, and of the pronephros and gills in secreting excess ions into the environment have not as yet been studied; nor has the role of the epidermis.

### C. Mechanisms of Osmotic and Ionic Regulation in Alevins

While the evidence indicates that the salmonid embryo can make a net gain or loss of salts, but not water, the situation after hatching in the salmonid alevins is not as clear. Hayes and Armstrong (1942 and Busnel et al. (1946) have reported increased hydration of Salmo salar alevins in fresh water which Hayes and Armstrong showed was due to a net uptake of water, although the alevin was also obtaining water from the yolk. In addition, Rockwell (1956), Busnel et al. (1946) and Willer and Trahms (1942) reported dehydration of pink and chum, Atlantic salmon and brown trout alevins, respectively in sea water. Rockwell (1956) argued that because the salinity-time curves are smooth, a single cause of death was probable and that dehydration "with salt penetration possibly playing a minor role" was the main cause. However, as observed in my experiments and those of Busnel et al. (1946) for Salmo salar, the osmotic concentration of the blood or body fluids of the alevins increases shortly after hatching. Parry (1961) reports that the osmotic concentration of Salmo trutta eggs is considerably less than that of parr when both are maintained in fresh water. In addition, Hayes (1949) and his co-workers have observed a considerable loss of phosphorus and a smaller loss of potassium from Salmo salar alevins as well as no net gain or loss of calcium and sodium before hatching, but a considerable gain of these two ions after hatching. The evidence, therefore, suggests that osmoregulation in salmonid alevins is due to both water and ion regulation whereas in the salmonid embryo ion regulation alone is important in regulating the osmotic concentration of the body fluids.

If the above conclusions are valid then the greater ability of pink and chum alevins to osmoregulate may be due to either superior water or ion

regulation or both. As in the case of embryos, the surface-to-volume ratio phenomenon does not appear to be important interspecifically, for while sockeye alevins are considerably smaller than chinook alevins, their poor osmoregulatory abilities are only slightly worse than that of chinook alevins.

The concentrations of sodium and chloride in the serum, assuming an ideal solution, contribute 63 to 82% of the osmotic concentration. To my knowledge there are no data available from other species at this stage with which comparisons could be made. However, it is known that in older fish sodium and chloride ions make up a large part of the osmotic concentration of the blood (Gordon, 1959; Conte and Wagner, 1965; Conte et al., 1966).

Correlated positively with the greater ability of pink and chum alevins to osmoregulate is their greater ability to regulate the levels of sodium and chloride ions in the blood. The mechanisms which account for these differences in ionic and osmotic regulation among the five species of Oncorhynchus probably include the alimentary canal, kidney, gills and skin. With respect to the skin, it is of interest to note that Holliday and Blaxter (1960) have shown that herring larvae (alevins) have a wider salinity tolerance than adults despite the fact the larvae lack kidneys and gills. In this regard, Holliday and Jones (1965) have postulated that the epidermis of the larvae has the ability to osmoregulate. However, more recently, Jones et al. (1966) have observed no ultrastructural differences in the epidermis from larvae reared in various salinities. Preliminary observations that I have made indicate that Pacific salmon embryo and alevin skin is richly endowed with mucous cells (unpublished). Parry (1960) who has also observed that newly hatched Salmo salar alevins have an actively secreting mucous epithelium, believes that "the mucus produced cannot by itself maintain an osmotic barrier".

However, Munz and McFarland (1964) have suggested that the slime produced by hagfish aids in cation regulation in that the slime contains high concentrations of calcium, magnesium and potassium.

The low osmotic, sodium and chloride concentrations of pink alevins in fresh water may be indicative of the electrolyte depletion which Fontaine (1960) and his co-workers have postulated as stimulating seaward migration. The low chloride levels which have also been observed by Callamand and Fontaine (1940) for eel, Kubo (1955) for Oncorhynchus masou smolts and Fontaine (1951) for Salmo salar suggests that chloremia is characteristic of anadromous fish. Houston (1959) however, puts forth the view that "the 'dimineralization' of pre-migrants may be considered more plausibly to be a correlative of parr-smolt transformation rather than as an initiator of downstream migration". The drop in chloride levels may more likely be the result of the initiation of chloride cell secretory activity as suggested by the work of Hoar (unpublished) and Nishida (1953) (both cited by Black, 1957). However, chloride secretory activity of the acidophil cells found in fish gills is far from universally accepted (Parry, 1966), although recent unpublished findings of F. P. Conte (personal communication) strongly support the chloride cell theory.

#### D. Mechanisms of Osmotic and Ionic Regulation In Fry

When salmon fry are five months old they possess many of the definitive characteristics which are either lacking or incompletely developed in the embryos and early alevins. In particular, the opisthonephros is well formed, the alimentary canal is more highly differentiated and the number of chloride secretory cells may be greater.

The results indicate that pink and chum fry are able to survive a direct



transfer from fresh water to sea water while the coho, chinook and sockeye cannot, although the chinook do show greater salinity resistance than coho and sockeye. Correlated positively with these results are the osmotic and ionic concentrations of the plasma. The pink and chum fry manifest an adjustive phase in osmoregulatory adaptation of about 24 hr in which the osmotic and ionic concentrations rise rapidly and then begin to return to near freshwater levels and a regulatory phase in which osmotic and ionic levels appear to be maintained slightly higher than in fresh water (Figures 7, 8 and 9). These results are in agreement with the findings of Black (1951) for chum fry but contrast sharply with Parry's (1960) results. She observed that nine month old parr of Salmo gairdneri, S. salar and S. trutta in 17°/oo sea water have adjustive phases of 150, 200 and 300 hr respectively. The adjustive phase of five month old pink and chum fry, which is therefore considerably shorter than for nine month old species of Salmo, indicates the greater osmoregulatory abilities of these Pacific salmon.

Pink and chum fry results also support the view that euryhaline fish in the regulatory phase of seawater adaptation maintain plasma osmotic concentrations slightly hyperosmotic to freshwater individuals (Table XIX).

With regard to the coho, chinook and sockeye fry the results indicate the lack of ability to reach the regulatory phase. These fry are unable to survive in sea water and their poor osmotic and ionic regulatory abilities support this fact. The chinook which have a considerably higher salinity resistance than coho and sockeye also do not, however, manifest considerably greater osmotic and ionic regulatory ability. The results for coho fry are in agreement with those of Conte et al. (1966) who observed very poor survival of 5 - 6 cm coho fry approximately five months old and those of

TABLE XIX

Blood osmotic concentrations (milliosmoles) of fish in fresh water and in sea water.

<u>Species</u>	<u>F. W. Blood mOsm</u>	<u>S. W. Blood mOsm</u>	<u>Reference</u>
<u>Oncorhynchus gorbuscha</u>	313	333	Present study
<u>Oncorhynchus keta</u>	330	343	Present study
<u>Anguilla anguilla</u>	346	428	Parry, 1966
<u>Gasterosteus aculeatus</u>	290	340	Lange and Fugelli, 1965
<u>Pleuronectes flesus</u>	304	364	Lange and Fugelli, 1965
<u>Salvelinus alpinus</u>	328	431	Parry, 1966
<u>Platichthys stellatus</u>	306	380	Parry, 1966
<u>Salmo salar</u>	328	344	Parry, 1966
<u>Salmo trutta</u>	326	356	Gordon, 1959

Black (1951) who observed high mortality in coho fry transferred to 27 - 31<sup>o</sup>/oo sea water which she correlated with high body chloride concentrations at 24 hr.

E. Comparison of Mechanisms of Osmotic and Ionic Regulation in Embryos,

Alevins and Fry

When the osmotic concentrations and LD<sub>50</sub> values are compared among the five species at three ages, several significant observations can be made. The alevins of all five species have LD<sub>50</sub> values about half those of their respective embryos. This observation is contrary to the generally held belief that osmotic and ionic regulatory ability increases with age. These results are explained by the probable fact that embryos can take up net amounts of ions but not take up or lose net amounts of water, whereas alevins can take up both net amounts of ions and water. It may in fact be argued that the net loss of water and the net gain of ions proceed at approximately equal rates or have equal lethal effects since two of these factors cause the median death to occur about twice as fast as one factor. In addition to this difference, the pronephros of the late embryo may be better able to handle the osmotic and ionic problem in sea water than the mesonephros of the early alevin.

The notion of increasing salinity tolerance with age is well established in the literature, but evidence for it between late embryos and early alevins is lacking. In fact, the existing evidence for alevins is contradictory. Moore (1900), for example, observed that resistance times of Salmo trutta larvae (alevins) in saline solutions did not increase with age as expected and Rockwell (1956) found that in 32<sup>o</sup>/oo sea water, pink alevins, two days after hatching, have an LD<sub>50</sub> of 216 hr but an LD<sub>50</sub> of 84 hr 40 days after hatching. Although herring larvae lack gills and kidneys, the larvae manifest a wider salinity tolerance than adults (Holliday and Blaxter, 1960).

Similarly, Parry (1960) found that Salmo salar alevins one week after hatching had an LD<sub>50</sub> of 8 hr in 100% sea water, but at six weeks the LD<sub>50</sub> was 0.5 hr, whereas Auvergnat and Secondat (1941) observed greater osmoregulatory abilities in late S. salar alevins than in early alevins. And finally, the work of Rutter (1902), as shown in Table XX, indicates that while the time to 100% mortality increases with age, LD<sub>50</sub>, calculated from his results, decreases or remains the same for chinook alevins 6, 12 and 19 days after hatching. We, therefore, have the anomaly of increasing salinity resistance by some members of a population but decreasing or unchanging salinity resistance by the median individual of that population. The increase in LD<sub>100</sub> values with increasing age may be due to rapid growth of only a few individuals while decreasing LD<sub>50</sub> values may be the result of the inherent periodicity of salinity physiology present in salmonidae as postulated by Hoar (1965). However, it is doubtful that the phenomenon of decreased alevin salinity resistance over that of embryos can be explained by this hypothesis, since the experiments on Pacific salmon were run from December 1965 to April 1966 (Table I).

There is considerable evidence for increased tolerance of post-alevin fish with increasing age (Black, 1951; Parry, 1958, 1960; McInerney, 1963 as cited by Hoar, 1965; Conte and Wagner, 1965 and Conte et al., 1966). The work of Parry (1960), Conte and Wagner (1965) and Conte et al. (1966) has clearly shown that the increase in salinity tolerance is not due to chronological age per se but to increased size. Conte and Wagner have suggested that there is a critical size, 14 - 15 cm at which steelhead trout become tolerant of sea water. This critical size appears to be 12 - 15 cm for Salmo salar (Parry, 1960) and 7 - 8 cm for Oncorhynchus kisutch (Conte et al., 1966) and less than 6 cm for Oncorhynchus keta and O. gorbuscha but

TABLE XX

Approximate LD<sub>50</sub> and LD<sub>100</sub> (days) values of chinook alevins in 50%, 75% and 100% sea water as calculated from the data of Rutter (1902).

Age*	50% S.W.		75% S.W.		100% S.W.	
	LD <sub>50</sub>	LD <sub>100</sub>	LD <sub>50</sub>	LD <sub>100</sub>	LD <sub>50</sub>	LD <sub>100</sub>
6	-	4	2	3	1	2
12	5	8	1	4	1	2
19	2	12				

\* days after hatching

greater than 7 cm for Oncorhynchus tschawytscha (personal observations). If the chloride secretory cell theory is correct, there should be increasing numbers of acidophil cells in the gills of larger fish (Parry, 1966). In fact, Threadgold and Houston (1964) have shown that there is an increase in activity of these cells during heightened plasma electrolyte levels. Hoar (unpublished) and Nishida (1953) (both cited by Black, 1957) observed the development of acidophil cells (chloride secretory cells) before seaward migration of coho and masou respectively. The development and functional capacity of these cells may be determined for each species at a particular size and account for some of the differences in osmoregulatory ability observed in the five species of Pacific salmon. Although the ability of the acidophil cells of the gills to secrete chloride actively against a concentration gradient has not been absolutely established (Strauss, 1963; Parry, 1966), the evidence of Threadgold and Houston (1964), Philpott (1965) and F. P. Conte (personal communication) strongly support the theory.

The gut, as well, must play some role in absorbing water and ions from the sea water (House and Green, 1965; Aull, 1966) which marine fish drink (Black, 1957). However, there is no evidence that this adaptation is dependent on the size or age of the fish.

The increase in blood osmotic concentration of freshwater alevins over that of freshwater embryos has been observed for all chum, coho, chinook and sockeye but not pink salmon. This difference may lie in the fact that the chloride secretory cells may have developed early in the pinks as previously suggested. The phenomenon of increased blood osmotic concentration has been discussed. It has also been observed in embryos of S. salar (Busnel et al., 1946), of S. trutta (Parry, 1961) as well as in chick embryos (Howard, 1957).

The fact that sodium-to-chloride ratios in fresh water and sea water are the same is contrary to the results of Houston (1959) who observed lower ratios in sea water fish. However, the average sodium-to-chloride ratio of 1.22 for alevin serum lies within the range 1.18 to 1.28 that Gordon (1959) observed for sexually mature sea trout S. trutta while the average ratio of 1.35 for fry plasma lies outside this range. This difference may be indicative of the increased ability of fry to excrete chloride rather than increased concentrations of other anions such as bicarbonate.

It has previously been shown that longer survival of embryos and fry than alevins in 31.8 ‰ sea water was probably due to greater osmoregulatory abilities of embryos and fry. On the basis of the foregoing discussion, this situation can be explained further. The greater osmoregulatory abilities of embryos is probably due to the fact that embryos take up net amounts of ions but not water whereas alevins can take up both net amounts of water and ions. In the case of fry, their greater osmoregulatory ability is due to increased size and possibly to the presence of chloride cells in the gills as well as a more highly differentiated gut.

#### F. Phylogenetic Interralations In Oncorhynchus

It was speculated that testing of the working hypotheses would provide further information on the phylogenetic relations within the genus Oncorhynchus. Such speculation has been based previously on behavioural, ecological and morphological studies (Hoar, 1958; Hikita, 1962). To these studies may be added the following: Jones (1963) observed species specific differences in electrophoretic blood protein patterns of adult salmon; Baggerman (1960) reported differences in salinity preferences of young salmon fry; McInerney (1963) found species differences in the salinity tolerances of summer fry

and Tsuyuki and Roberts (1966) observed species-specificity in electrophoretic myogen patterns of Oncorhynchus. These studies and those of Foerster (1935), Brett (1952) and Kobayasi (1963) are included in Table XXI.

It is assumed that the ancestral oncorhynchid may have been a steelhead trout-like descendent of Salmo (Neave, 1958), which had already acquired an obligatory marine life. This ancestral oncorhynchid was a stream or river dweller much like the masou and coho are today. From such a habitat, evolution may have taken place in two other directions, that is, towards a more marine life (Neave, 1958) and towards a more freshwater existence. It is suggested that both directions have been exploited.

The electrophoretic myogen patterns of pink and sockeye salmon are very similar, but are most unlike those of coho and masou. While pink embryos are better osmoregulators than coho embryos, sockeye embryos are far worse. In other characteristics such as osmoregulation by alevins, schooling behaviour, elongate bodies as fry and numerous gill-rakers, pink and sockeye salmon are at considerable variance with coho. Pink and sockeye, therefore, appear to represent the two extremes so far reached in the evolution of Oncorhynchus. On the one hand, pink embryos, alevins and fry show the greatest adaptation to marine life while sockeye embryos, alevins and fry on the other hand show the greatest adaptation to freshwater life. The presence of intertidal spawning populations of pink salmon contrasts sharply with the incipient kokanee species of sockeye and its lacustrine habit.

On this basis, the evidence in Table XXI has been used to draw a family tree of the genus Oncorhynchus (Figure 14). The masou, coho and chinook share common characteristics such as parr marks and deep bodies as fry whereas the pink, chum and sockeye share different common features such as



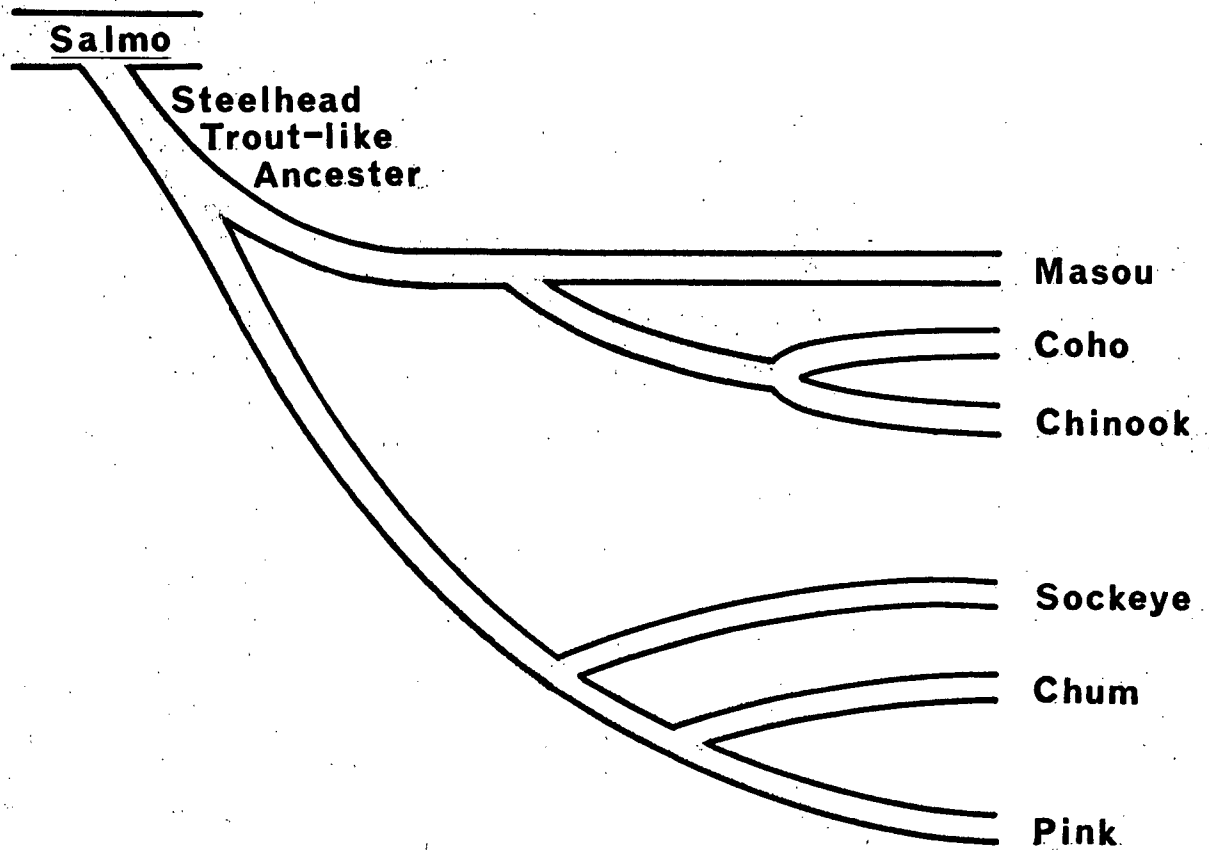
TABLE XXI Comparison of Pacific salmon. Presence of a character is indicated by + mark while its absence by 0. Intensity of activity is rated according to the number of + marks.

Character	Pink	Chum	Sockeye	Coho	Chinook	Masou	Reference:
Salinity Resistance of Embryos	++++	++++	++	++	+++		Present Study
Osmoregulation by Embryos	+++	+++	0	+	++		Present Study
Salinity Resistance of Alevins	+++	++	+	+	++		Present Study
Osmoregulation by Alevins	++	++	0	+	0		Present Study
Na <sup>+</sup> Regulation by Alevins	++	++	0	+	0		Present Study
Cl <sup>-</sup> Regulation by Alevins	++	++	+	+	0		Present Study
Salinity Resistance of Fry	*	*	+	+	++++		Present Study
Osmoregulation by Fry	++++	++++	+	++	++		Present Study
Na <sup>+</sup> Regulation by Fry	++++	++++	+	+	++		Present Study
Cl <sup>-</sup> Regulation by Fry	++++	++++	+	+	++		Present Study
Intertidal Spawning	+	+	0	0	0		Rockwell, 1956
Salinity Tolerance of Summer Fry	++++	++++	++	+	++		McInerney, 1963
Salt Preference of Young Fry	+++	+++	+++	+			Baggerman, 1960
Similarity of Myogens to <u>S. gairdneri</u>	0	+	0	+++	++	++++	Tsuyuki and Roberts, 1966
River Residents	0	0	0	+			Hoar, 1958
Lake Residents	0	0	++	+			Hoar, 1958
Fry Prefer Sea Water	++	++	+	0			Hoar, 1958
Fry Hide Under Stones	+	++	+++	+++			Hoar, 1958
Nipping Behaviour	0	+	++	+++			Hoar, 1958
Territorial Behaviour	0	0	+	++			Hoar, 1958
Schooling Behaviour	+++	++	+++	+			Hoar, 1958
Smolt Transformation	0	0	+	+	+		Hoar, 1958
Increased Activity at Night	+	+	+	0			Hoar, 1958
Parr Marks: Alevins	0	+	+++	+++	+++	+++	Hikita, 1962
Parr Marks: Fry	0	+	+++	+++	+++	+++	Hikita, 1962
Fry Body Elongate	+	+	+	0	0	0	Hikita, 1962
Number of Gill Rakers	+	0	+	0	0	0	Hikita, 1962
Number of Pyloric Caeca	+	+	0	0	+		Hikita, 1962
Blood Proteins	++	+	+	+	0		Jones, 1963
Hybridization	++	++	++	+			Foerster, 1935
Hybridization	++++	++++					Kobayasi, 1963
High Temperature Tolerance	+	+	++	+++	+++		Brett, 1952

\* Pink and chum fry do not resist sea water, they tolerate it.

FIGURE 14

Phylogenetic relationships of Oncorhynchus



schooling behaviour, electrophoretic myogen patterns and elongate bodies as fry. Therefore, the pink-chum-sockeye group has been shown as diverging from the masou-coho-chinook group.

Within the masou-coho-chinook group, the salinity resistance, osmotic and ionic regulation of fry, salinity tolerance of young fry, smolt transformation, high temperature tolerance and electrophoretic myogen patterns suggest that coho and chinook are very closely related but that chinook are somewhat more highly evolved. In addition, the electrophoretic myogen patterns of masou are more similar to Salmo gairdneri than are coho and chinook patterns.

Separation within the pink-chum-sockeye group is more difficult for Foerster (1935), Kobayasi (1963) and Jones (1963) observed great similarities among this group. The data on osmotic and ionic regulation indicate that sockeye embryos, alevins and fry are more poorly adapted for marine life than are coho and chinook. These results differ from those of Hunter (cited by Hoar, 1958) who found that sockeye fry could be readily adapted to dilute sea water and from its earliest stages, was much more resistant to sea water than coho. McInerney (1963) also observed greater salinity tolerance in sockeye fry over that of coho fry. In addition, Baggerman (1960) has shown that the salt water preference of sockeye fry is maintained from April until the end of August (except in June, when no preference was shown) and that in September the preference changed to fresh water. If this were the case for my sockeye, it seems probable that as alevins they would have preferred sea water. However, because of their poor salinity resistance, a preference for sea water seems unlikely. Therefore, on the basis of physiological criteria, including salinity resistance as observed in the present study and high

temperature tolerance as reported by Brett (1952), sockeye is shown in Figure 14 as diverging earlier than chum from the pink-chum-sockeye group. This arrangement, however, does not agree with the evidence of Tsuyuki and Roberts (1966) who observed that the electrophoretic myogen patterns of pink salmon are more similar to those of sockeye than those of chum salmon. This discrepancy cannot be explained at this time.

Because pink salmon manifest the greatest adaptation to marine life among the Pacific salmon and, therefore, the greatest divergence from the steelhead trout-like ancestor, they are accorded the position on the tree furthest from the masou. Figure 14, thus, derived in part from the physiological data obtained in the present research, agrees with the phylogenetic speculations of Hoar (1958). Therefore, the results of testing the working hypotheses have provided further information on the phylogenetic relations within the genus Oncorhynchus.

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# APPENDIX

TABLE I

Orthogonal contrast integers for the comparison of the difference in serum osmotic concentrations of pink/chum in 0 and 24 hr sea water against the difference in chinook levels at 0 and 24 hr sea water.

	<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
0	+1	+1	0	-2	0
4	0	0	0	0	0
8	0	0	0	0	0
12	0	0	0	0	0
24	-1	-1	0	+2	0

TABLE II

Orthogonal contrast integers for the comparison of the difference in serum osmotic concentrations of coho in 0 and 24 hr sea water against the difference in sockeye levels at 0 and 24 hr sea water.

	<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
0	0	0	+1	0	-1
4	0	0	0	0	0
8	0	0	0	0	0
12	0	0	0	0	0
24	0	0	-1	0	+1

TABLE III

Regression equations of serum osmotic concentrations of embryos plotted against exposure time in 31.8‰ sea water. P values indicate the significance of the regression slopes.

<u>Species</u>	<u>Regression Equation</u>	<u>P</u>
Pink	$Y = 347.14 + 7.23X - 0.22X^2$	0.05
Chum	$Y = 343.33 + 7.34X - 0.22X^2$	0.01
Coho	$Y = 310.30 + 10.55X$	0.005
Chinook	$Y = 342.01 + 5.09X$	0.005
Sockeye	$Y = 309.14 + 13.23X$	0.005

TABLE IV

Orthogonal contrast integers for the comparison of the difference in serum osmotic concentrations of pink/chum in 0 and 12 hr sea water against the difference in coho levels at 0 and 12 hr sea water.

	<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
0	+1	+1	-2	0	0
4	0	0	0	0	0
8	0	0	0	0	0
12	-1	-1	+2	0	0

TABLE V

Orthogonal contrast integers for the comparison of the difference in serum osmotic concentrations of chinook in 0 and 12 hr sea water against the difference in sockeye levels at 0 and 12 hr sea water.

	<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
0	0	0	0	+1	-1
4	0	0	0	0	0
8	0	0	0	0	0
12	0	0	0	-1	+1

TABLE VI

Orthogonal contrast integers for the comparison of the difference in serum osmotic concentrations of pink in 0 and 12 hr sea water against the difference in chum levels at 0 and 12 hr sea water.

	<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
0	+1	-1	0	0	0
4	0	0	0	0	0
8	0	0	0	0	0
12	-1	+1	0	0	0

TABLE VII

Regression equations of serum osmotic concentration of alevins plotted against exposure time in 31.8‰ sea water. P values indicate the significance of the regression slopes.

<u>Species</u>	<u>Regression Equation</u>	<u>P</u>
Pink	$Y = 338.97 + 33.24X - 2.34X^2$	0.005
Chum	$Y = 388.47 + 4.58X$	0.025
Coho	$Y = 353.23 + 10.64X$	0.005
Chinook	$Y = 374.80 + 11.24X$	0.005
Sockeye	$Y = 359.57 + 16.02X$	0.005

TABLE VIII

Orthogonal contrast integers for the comparison of the difference in serum Na<sup>+</sup> concentrations of pink/chum alevins in 4 and 8 hr sea water against the difference in coho alevin levels at 4 and 8 hr sea water.

	<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
0	0	0	0	0	0
4	-1	-1	+2	0	0
8	+1	+1	-2	0	0
12	0	0	0	0	0

TABLE IX

Orthogonal contrast integers for the comparison of the difference in serum Na<sup>+</sup> concentrations of pink alevins in 4 and 8 hr sea water against the difference in chum alevin levels at 4 and 8 hr sea water.

	<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
0	0	0	0	0	0
4	-1	+1	0	0	0
8	+1	-1	0	0	0
12	0	0	0	0	0

TABLE X

Orthogonal contrast integers for the comparison of the difference in serum Na<sup>+</sup> concentrations of pink alevins in 0 and 12 hr sea water against the difference in chum alevin levels at 0 and 12 hr sea water.

	<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
0	+1	-1	0	0	0
4	0	0	0	0	0
8	0	0	0	0	0
12	-1	+1	0	0	0

TABLE XI

Orthogonal contrast integers for the comparison of the difference in serum Na<sup>+</sup> concentrations of pink/chum alevins in 0 and 12 hr sea water against the difference in coho alevins at 0 and 12 hr sea water.

	<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
0	+1	+1	-2	0	0
4	0	0	0	0	0
8	0	0	0	0	0
12	-1	-1	+2	0	0

TABLE XII

Regression equations of serum Na<sup>+</sup> concentrations of alevins plotted against exposure time in 31.8‰ sea water. P values indicate the significance of the regression slopes.

<u>Species</u>	<u>Regression Equation</u>	<u>P</u>
Pink	$Y = 136.75 + 2.46X$	0.005
Chum	$Y = 155.23 + 1.85X$	0.005
Coho	$Y = 146.97 + 3.99X$	0.005
Chinook	$Y = 145.53 + 6.59X$	0.005
Sockeye	$Y = 141.40 + 6.89X$	0.005

TABLE XIII

Regression equations of serum Cl<sup>-</sup> concentrations of alevins plotted against exposure time in 31.8‰ sea water. P values indicate the significance of the regression slopes.

<u>Species</u>	<u>Regression Equation</u>	<u>P</u>
Pink	$Y = 91.67 + 9.63X - 0.55X^2$	0.005
Chum	$Y = 120.30 + 1.70X$	0.005
Coho	$Y = 121.97 + 4.08X$	0.005
Chinook	$Y = 125.90 + 5.98X$	0.005
Sockeye	$Y = 107.10 + 12.44X - 0.55X^2$	0.005

TABLE XIV

Orthogonal contrast integers for the comparison of the difference in plasma osmotic concentrations of chum fry in 8 and 12 hr sea water against the difference in chinook levels at 8 and 12 hr sea water.

	<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
0	0	0	0	0	0
4	0	0	0	0	0
8	0	+1	0	-1	0
12	0	-1	0	+1	0

TABLE XV

Regression equations of plasma osmotic concentrations of fry plotted against exposure time in 31.8‰ sea water. P values indicate the significance of the regression slopes.

<u>Species</u>	<u>Regression Equation</u>	<u>P</u>
Pink	$Y = 319.93 + 3.11X$	0.025
Chum	No significant regression	
Coho	$Y = 313.07 + 18.56X - 0.88X^2$	0.005
Chinook	$Y = 326.07 + 6.73X$	0.005
Sockeye	$Y = 320.85 + 22.59X - 1.11X^2$	0.005

TABLE XVI

Regression equations of plasma Na<sup>+</sup> concentrations of fry plotted against exposure time in 31.8‰ sea water. P values indicate the significance of the regression slopes.

<u>Species</u>	<u>Regression Equation</u>	<u>P</u>
Pink	No significant regression	
Chum	$Y = 161.42 + 6.19X - 0.48X^2$	0.05
Coho	$Y = 156.40 + 4.52X$	0.005
Chinook	$Y = 164.37 + 2.30X$	0.005
Sockeye	$Y = 146.78 + 12.45X - 0.68X^2$	0.01

TABLE XVII

Regression equations of plasma Cl<sup>-</sup> concentrations of fry plotted against exposure time in 31.8‰ sea water. P values indicate the significance of the regression slopes.

<u>Species</u>	<u>Regression Equation</u>	<u>P</u>
Pink	$Y = 110.42 + 5.48X - 0.41X^2$	0.005
Chum	$Y = 118.43 + 4.36X - 0.30X^2$	0.05
Coho	$Y = 116.23 + 3.31X$	0.005
Chinook	$Y = 120.50 + 2.00X$	0.005
Sockeye	$Y = 117.37 + 7.30X - 0.42X^2$	0.05

TABLE XVIII

Regression equations of plasma Mg<sup>++</sup> concentrations of fry plotted against exposure time in 31.8‰ sea water. P values indicate the significance of the regression slopes.

<u>Species</u>	<u>Regression Equation</u>	<u>P</u>
Pink	$Y = 2.529 + 0.2884X - 0.0210X^2$	0.05
Sockeye	$Y = 3.2828 + 0.2134X$	0.005
Chum, coho and chinook data cannot be fitted to either the straight line or curvilinear regressions.		

TABLE XIX

Orthogonal contrast integers for the comparison of the difference between embryo and fry osmotic concentrations of pink/chum against the difference of embryo and fry levels of coho/chinook/sockeye.

	<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
Embryo	+3	+3	-2	-2	-2
Alevin	0	0	0	0	0
Fry	-3	-3	+2	+2	+2

TABLE XX

Orthogonal contrast integers for the comparison of the difference between embryo and fry osmotic concentrations of pink against the difference of embryo and fry levels of chum.

	<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
Embryo	-1	+1	0	0	0
Alevin	0	0	0	0	0
Fry	+1	-1	0	0	0

TABLE XXI

Orthogonal contrast integers for the comparison of the difference between embryo and fry osmotic concentrations of coho/chinook against the difference of embryo and fry levels of sockeye.

	<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
Embryo	0	0	-1	-1	+2
Alevin	0	0	0	0	0
Fry	0	0	+1	+1	-2



TABLE XXII

Orthogonal contrast integers for the comparison of the difference between embryo and fry osmotic concentrations of coho against the difference of embryo and fry levels of chinook.

	<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
Embryo	0	0	-1	+1	0
Alevin	0	0	0	0	0
Fry	0	0	+1	-1	0

TABLE XXIII

Orthogonal contrast integers for the comparison of the difference between embryo/fry and alevin osmotic concentrations of pink/chum against the difference of embryo/fry and alevin levels of coho/chinook/sockeye.

	<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
Embryo	+3	+3	-2	-2	-2
vin	-6	-6	+4	+4	+4
Fry	+3	+3	-2	-2	-2

TABLE XXIV

Orthogonal contrast integers for the comparison of the difference between embryo/fry and alevin osmotic concentrations of pink against the difference of embryo/fry and alevin levels of chum.

	<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
Embryo	+1	-1	0	0	0
Alevin	-2	+2	0	0	0
Fry	+1	-1	0	0	0

TABLE XXV

Orthogonal contrast integers for the comparison of the difference between embryo/fry and alevin osmotic concentrations of coho/chinook against the difference of embryo/fry and alevin levels of sockeye.

	<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
Embryo	0	0	+1	+1	-2
Alevin	0	0	-2	-2	+4
Fry	0	0	+1	+1	-2

TABLE XXVI

Orthogonal contrast integers for the comparison of the difference between embryo/fry and alevin osmotic concentrations of coho against the difference of embryo/fry and alevin levels of chinook.

	<u>Pink</u>	<u>Chum</u>	<u>Coho</u>	<u>Chinook</u>	<u>Sockeye</u>
Embryo	0	0	+1	-1	0
Alevin	0	0	-2	+2	0
Fry	0	0	+1	-1	0

TABLE XXVII

Orthogonal contrast integers for the comparison of the difference between embryos/fry vs alevin osmotic concentrations of pink/chum in 0 and 12 hr sea water against the difference between embryos/fry vs alevin levels of coho/chinook/sockeye in 0 and 12 hr sea water.

	<u>Pink</u>			<u>Chum</u>			<u>Coho</u>			<u>Chinook</u>			<u>Sockeye</u>		
	<u>E<sup>1</sup></u>	<u>A<sup>2</sup></u>	<u>F<sup>3</sup></u>	<u>E</u>	<u>A</u>	<u>F</u>	<u>E</u>	<u>A</u>	<u>F</u>	<u>E</u>	<u>A</u>	<u>F</u>	<u>E</u>	<u>A</u>	<u>F</u>
0	+3	-6	+3	+3	-6	+3	-2	+4	-2	-2	+4	-2	-2	+4	-2
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	-3	+6	-3	-3	+6	-3	+2	-4	+2	+2	-4	+2	+2	-4	+2

- 1 E ≡ embryo  
 2 A ≡ alevin  
 3 F ≡ fry