

THE EFFECTS OF SALINITY ON SURVIVAL, GROWTH, AEROBIC
METABOLISM AND ION REGULATION IN EARLY LIFE
STAGES OF PACIFIC SALMONIDS

by

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ABSTRACT

A series of laboratory experiments were conducted to test the hypothesis that the energetic requirements for ion regulation in early life stages of three Pacific salmonids would be minimal at an isotonic water salinity (8-10 ppt).

Hatching success, alevin survival, time to hatching, size, and metabolic rates were measured for steelhead (Oncorhynchus mykiss) and fall chinook salmon (O. tshawytscha) embryos incubated in four salinities (0, 4, 8 and 12 ppt) from the eyed stage. Egg hatchability was relatively high in all four salinities, but completion of hatch was delayed in the hypertonic (12 ppt) salinity. Newly hatched alevins were also smaller and showed decreased survival in 12 ppt salinity. Salinity effects on alevin survival and size were greater for steelhead trout than for chinook salmon. Metabolic rates of eyed steelhead trout eggs, and chinook salmon eggs and alevins were not significantly affected by the salinities tested. The metabolic rate of newly hatched steelhead trout alevins, however, was significantly elevated in 12 ppt salinity compared to the other treatments. The results indicated that a salinity of 8 ppt is the upper limit for the normal development of steelhead trout and chinook salmon eggs and alevins. The regulation of movements of ions and water in eggs and alevins of these salmonid species may rely on passive processes, such as low membrane permeabilities and tissue tolerances, rather than active mechanisms of ion and osmotic regulation.

Rainbow trout, steelhead trout and fall chinook salmon fry were acclimated to a range of salinities, with one near isotonic. Survival, growth, metabolic rate, plasma Na^+ and Cl^- concentrations, and seawater adaptability were measured for 5 to 12 weeks, depending on the

species. Growth of all three species was highest in fresh water and declined with increasing salinity. Metabolic rates increased with salinity and were inversely correlated with growth rates. Isotonic salinity, therefore, did not offer significant metabolic or growth advantages to rainbow, steelhead and chinook fry. While plasma Na^+ and Cl^- concentrations varied among groups, chinook fry tended to better maintain ionic homeostasis at higher salinities than the trout. Acclimation to the various dilute salinities did not influence the seawater adaptability of juvenile steelhead trout or chinook salmon. These results indicate that optimal salinities for growth and metabolic rates were influenced by species and life history stage. The metabolic rate data suggested that the energetic cost of ionic regulation increased with salinity, but attempts to quantify this cost were probably affected by other metabolic processes which responded to changes in salinity.

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GENERAL INTRODUCTION

Salmonids and other teleost fish maintain the salt content of their blood at approximately one-third the concentration of seawater (Eddy 1981). In marine fish, there is therefore a tendency for the fish to be dehydrated by osmosis and for salt to move passively along concentration gradients into the body. Salt and water movements occur principally across the permeable gill epithelia. Water losses are replaced by drinking seawater and excess salts (sodium and chloride ions) are actively excreted by mitochondria-rich chloride cells located in the gills (Foskett and Scheffey 1982). In freshwater fish, the blood and body fluids are more concentrated than the external medium and thus water enters the body by osmosis and salts are lost by passive diffusion. The excess water is removed by the copious production of a dilute (hypotonic) urine, while the salt losses are compensated by active ion uptake mechanisms in the gills (Payan et al. 1984). There have been several attempts to quantify the energy required for active ionic and osmotic (=ion-osmotic) regulation in fish. Estimates of the energetic cost of ion-osmotic regulation range widely from 0.5 to 29% of resting metabolic rate, depending on the species, environmental conditions and method of determination (Eddy 1982).

Many euryhaline fish species, including salmonids, spend a portion of their life history in estuaries, where the salinity of the external environment is approximately isotonic with the blood and body fluids of the fish. In theory, the energy associated with ion-osmotic regulation would be lowest when the fish are in an isotonic environment, because the ionic gradients between blood and water would be minimal. The purpose of this study was to determine whether the presumed energy savings in isotonic salinity associated with ion-osmotic regulation would be substantial enough to affect parameters such as growth and metabolism. Laboratory experiments

were designed to examine survival, growth, metabolic rates and ionic regulation in early life stages of Pacific salmonids acclimated to a range of water salinities, with one near the isotonic level (8-10 ppt). The main body of this thesis is divided into two parts. Chapter 1 deals with the effects of salinity on aerobic metabolism, survival and development of eggs and alevins of steelhead trout (Oncorhynchus mykiss) and chinook salmon (O. tshawytscha). Chapter 2 examines the effects of salinity on survival, growth, metabolism and ionic regulation in rainbow and steelhead trout and chinook salmon fry.

CHAPTER 1

Effects of Salinity on Aerobic Metabolism and Development of Eggs
and Alevins of Steelhead Trout (Oncorhynchus mykiss) and
Fall Chinook Salmon (Oncorhynchus tshawytscha)

INTRODUCTION

Although there have been many studies dealing with the effects of salinity on marine fish eggs and larvae (e.g. Holliday and Blaxter 1960; Holliday and Jones 1967; Alderdice and Forrester 1968, 1971a,b; Alderdice and Velsen 1971; Lee and Menu 1981), relatively little is known about the influence of salinity on embryonic stages of anadromous salmonid species. Notable exceptions to this include studies on salinity tolerance and ion-osmotic regulation in salmonid eggs and alevins by Rockwell (1956), Weisbart (1968), Shen and Letherland (1978a) and Groot (1989).

Differences in oxygen consumption rates in relation to salinity have been used extensively with juvenile and adult teleost fish in attempts to estimate the energetic cost of ion-osmotic regulation (e.g. Rao 1968; Farmer and Beamish 1969; Nordlie and Leffler 1975; Febry and Lutz 1987). This approach was adopted in the present study to assess the ion-osmotic regulatory capabilities of embryonic stages of development. In this study, I examined survival, rate of development, growth and metabolic rates in eggs and alevins of steelhead trout (Oncorhynchus mykiss) and fall chinook salmon (O. tshawytscha) acclimated to a range of water salinities. The steelhead trout is an anadromous form of the rainbow trout, which usually spends two years in fresh water before migrating to the sea (Scott and Crossman 1973). Fall chinook salmon are also anadromous, but spend a shorter period of time in fresh water than steelhead trout, migrating seaward within three months of emergence (Lister and Walker 1966).

MATERIALS AND METHODS

Egg Acquisition and Incubation

Steelhead trout and chinook salmon gametes were taken from fish returning to spawn in the Big Qualicum and Puntledge Rivers on Vancouver Island, British Columbia (B.C.) during April and November, 1989, respectively. The gametes were collected into separate plastic containers at each site, and transported in insulated containers with ice packs to the Pacific Biological Station, Nanaimo, B.C. Transit times from the collection sites to the laboratory were less than 8 h and temperatures during transport were 4°C.

The eggs were fertilized immediately upon arrival at the laboratory. Steelhead trout eggs were pooled from three females and fertilized by the dry method with the pooled milt of three males, at an egg to milt ratio of 500:1. Water-hardened steelhead eggs had an average weight of 137 mg and an average diameter of 5.9 mm (n=15). Chinook salmon eggs from a single Big Qualicum female were fertilized with milt from a single male. The eggs were 323 mg and 8.1 mm (n=20) after water-hardening.

The incubation system consisted of 1.2 L cylindrical perspex incubators, measuring 14 cm in diameter and 15 cm in height (Rankin 1979). The eggs sat in 1 mm nylon mesh baskets (11.5 cm in diameter and 2.5 cm in height) near the top of the incubator, and were perfused with water entering at the bottom and passing upwards through a plastic cone. The incubators, therefore, provided a flat velocity front and microturbulent flow, perfusing one layer of eggs normal to the direction of flow. Three incubators were connected in series using silicone tubing and were

submerged in a 40 L incubation tank, equipped with temperature control (Alderdice and Velsen 1968). Nominal water temperatures of 9 and 10°C were selected for incubation of steelhead and chinook eggs, respectively. Water circulation and aeration was maintained by placing an airstone across the back of the tank. Water was pumped from the bottom of the tank, through the incubators, and back into the tank using a peristaltic pump (Masterflex®, Cole-Parmer Instrument Co., Chicago, IL). Water flowed past the eggs at about 300 mL·min⁻¹, equivalent to an apparent perfusion velocity of 180 cm·h⁻¹. Four incubation tanks were set up as described to provide test salinities of 0, 4, 8 and 12 ppt. Flow-through fresh and salt water were provided to each incubation tank from overflowing reservoirs, and flow rates were controlled by metered pumps to achieve the desired salinities.

The newly fertilized eggs were placed in the incubators at loading densities of about 250 eggs per incubator. The steelhead trout eggs were maintained in fresh water until after completion of epiboly (117 accumulated thermal units or ATUs), while the chinook salmon eggs were kept in fresh water until after reaching the eyed stage (299 ATUs). The eggs were kept in fresh water until these stages of development because water-hardening is known to be inhibited in salinities greater than 3 ppt (Li et al. 1989), and ion-regulating chloride cells do not appear until the basic structure of the embryo has fully formed (Alderdice 1988). The embryos were then gradually acclimated to the test salinities at a rate of 1-2 ppt per day. Salinity, temperature, dissolved oxygen and pH in each tank were recorded on a regular basis throughout the incubation period (Table 1). Dissolved oxygen levels decreased with increasing salinity due to differences in oxygen solubility with increasing salt content, but they were maintained in excess of 93% of the air-saturated value. The minimum oxygen concentrations recorded were considered satisfactory to meet the dissolved oxygen requirements for the incubation of these species at the water

Table 1. Water quality parameters measured in the steelhead trout and chinook salmon embryo incubation tanks.

Species	Mean values \pm SE			
	Salinity (ppt)	Temp. ($^{\circ}$ C)	D.O. (mg·L ⁻¹)	pH
Steelhead trout ¹	0 \pm 0	9.0 \pm 0.01	11.3 \pm 0.3	7.0 \pm 0.1
	4.2 \pm 0.2	9.0 \pm 0.01	11.1 \pm 0.3	7.2 \pm 0.1
	7.9 \pm 0.1	8.9 \pm 0.02	10.8 \pm 0.3	7.4 \pm 0.1
	12.1 \pm 0.1	9.0 \pm 0.03	10.5 \pm 0.3	7.6 \pm 0.1
Chinook salmon ²	0 \pm 0	10.0 \pm 0.01	11.1 \pm 0.1	7.2 \pm 0
	4.0 \pm 0	10.0 \pm 0.02	10.9 \pm 0.1	7.4 \pm 0.1
	8.0 \pm 0	10.0 \pm 0	10.7 \pm 0.1	7.5 \pm 0.1
	11.8 \pm 0.2	10.0 \pm 0	10.4 \pm 0.1	7.7 \pm 0.2

1. N: salinity=7, temperature=13, DO=2, pH=2

2. N: salinity=8, temperature=20, DO=2, pH=2

temperatures and perfusion velocities used in the present study (Silver et al. 1963; Shumway et al. 1964; Davis 1975; Rombough 1986, 1988a).

All incubators were kept in darkness except during brief examination periods. Samples of approximately 55 eggs per salinity treatment were set aside in one of the incubators, using a plastic partition, to determine the effect of salinity on hatching success and development time. The incubators were opened every three to four days and any dead (opaque) eggs were removed and preserved in Stockard's solution to determine the embryonic stage at death (Ballard 1973). During hatching, the numbers of alevins that appeared were recorded daily and alevin survival was monitored for about one week post hatching. Hatching success was calculated as the percentage of eggs that survived from the beginning of salinity exposure to hatch. The time of hatching was taken as the time in days from fertilization to when 50% and 100% of the eggs hatched (Murray and McPhail 1988).

Respirometry

Routine metabolic rates of eyed eggs and newly hatched alevins incubated in the different salinity treatments were determined by monitoring rates of oxygen consumption in continuous-flow respirometers described by Rombough (1988b). The respirometers consisted of 71 mL cylindrical acrylic chambers, with nylon screens at either end to contain the embryos. The chambers were coated with water-soluble silicone (Clay Adams Co.) to inhibit bubble formation. Stainless steel tubing was used to connect the chambers to dissolved oxygen probe holders equipped with magnetic stirrers. A variable speed gear pump and a four-way valve allowed the respirometer to operate in an open (flow-through) or closed mode. Four respirometers were

immersed in a common 40 L bath, insulated with neoprene rubber, and regulated to $\pm 0.1^{\circ}\text{C}$ by an attached temperature control unit. Water temperatures were kept similar to those used in incubation. The bath was filled with different volumes of fresh and salt water to achieve the test salinities, and the dissolved oxygen concentration of the bath water was adjusted to saturation by supplying aeration in the temperature control unit. Dissolved oxygen levels in each chamber were monitored continuously using polarographic oxygen electrodes and a multichannel oxygen meter (Orbisphere Model 2710). The electrodes were calibrated using air-saturated distilled water.

Metabolic rates were determined at the eyed stage (steelhead trout: 240-252 ATUs, chinook salmon: 429-443 ATUs) and approximately one week post-hatch (steelhead: 438-450 ATUs, chinook: 640-653 ATUs). Only alevins which appeared vigorous and normal were used. Six replicate trials were conducted per salinity treatment at each stage tested. Two consecutive days were required to complete the respiration measurements for each developmental stage. Depending on the species and stage of development, between 25 and 100 individuals were placed into each of three respirometers and the flow rate past the eggs or alevins was adjusted to $60\text{ mL}\cdot\text{min}^{-1}$ (equivalent to a bulk water velocity of $510\text{ cm}\cdot\text{h}^{-1}$). The fourth respirometer was run without animals to serve as a control. The top of the respirometer bath was covered with black plastic sheeting and the eggs or alevins were acclimated to the respirometers in flow-through conditions for 30 min prior to measurement. The respirometers were then closed and the subsequent decline in oxygen concentrations (to the nearest $0.05\text{ mg}\cdot\text{L}^{-1}$) was monitored for 1 h, with values recorded every 5 min. One replicate during each respiration trial was also plotted continuously using a strip chart recorder for comparison to the manual readings. After a trial was completed, the eggs or alevins were removed and 10 individuals from each respirometer were

preserved in 10% neutral formalin for at least 120 d before size measurements were made. Samples of live chinook alevins were also weighed immediately after removal from the respirometers to provide an estimate of fresh alevin wet weights. The live alevins were anesthetized with MS-222 and gently blot dried before weighing. After preservation, the egg and alevin samples were measured for length and weight. Alevin fork length was measured to the nearest mm. The yolk and tissue components of dechorionated eggs and alevins were separated by dissection, and individuals from each replicate were pooled before weighing. Preservation in formalin is known to alter the weight of fishes (Heming and Preston 1981), and evaporation of formalin during the dissection procedure also affected wet weight measurements. Because of these errors associated with using preserved wet weights, only dry weights were determined. Tissue and yolk were oven dried at 60°C for 48 h and weighed to the nearest 0.1 mg. Percent embryonic tissue (P_T) was calculated for each development stage by dividing tissue dry weight by total embryo dry weight (Blaxter 1969). Tissue dry weights (TDW) were converted to tissue wet weight equivalents for the expression of metabolic rates on a wet weight (TWW) basis. Conversion factors for steelhead trout and chinook salmon were 0.133 (Rombough 1988c) and 0.20 (J.D. Morgan, unpublished data).

Dissolved oxygen concentrations in the respirometers initially declined in a curvilinear fashion for about 10-15 min, apparently as a result of pressure changes associated with closing the system (Rombough 1988b). Oxygen levels then decreased at a constant rate for about 30-45 min, dropping 2-4 mg·L⁻¹ during the linear portion of the measurements. Oxygen consumption rates were estimated over the linear portion using regression analyses (r^2 values: 96.9-99.9%). Metabolic rates were determined by multiplying the regression slope of oxygen uptake (corrected for the blank controls) by the respirometer volume, and were expressed in terms of the number

of individuals tested, and dry and wet tissue weights over a 1 h period (i.e. $\mu\text{g O}_2\cdot\text{h}^{-1}$ per individual, $\mu\text{g O}_2\cdot\text{g TDW}^{-1}\cdot\text{h}^{-1}$, and $\mu\text{g O}_2\cdot\text{g TWW}^{-1}\cdot\text{h}^{-1}$).

For alevins, the oxygen consumption curves eventually began to decline in slope, reflecting a decrease in oxygen uptake as dissolved oxygen became limiting. The point at which the curve deviated perceptibly from a straight line has been referred to as the critical dissolved oxygen level (P_C , Rombough 1988b). Agreement between the oxygen uptake slopes calculated by regression analyses and on the strip chart recordings was very good (average = 98%), therefore P_C values were estimated for alevin respiration trials where a paper chart recording was made.

Chloride Content of the Perivitelline Fluid

Samples of the perivitelline fluids (PVF) were taken from four to five chinook salmon eggs from each of the four salinity treatments. These eggs were well eyed (420 ATUs) and had been exposed to the test salinities for about 5 days. The eggs were removed from the incubators, gently rolled onto tissue paper to remove surface moisture, and were mounted into small plastic cups. Perivitelline fluid was sampled by making a small hole in the chorion and withdrawing the fluid into 10 μL capillary tubes (Microcap[®], Drummond Scientific Co., Broomall, PA). Chloride ion concentrations ($[\text{Cl}^-]$) were measured immediately by coulometric titration (Haake Buchler Instruments Digital Chloridometer). Water samples from each incubation tank were also measured for $[\text{Cl}^-]$ for comparison with the PVF values.

Statistical Analysis

Data are presented as means \pm standard error (SE) where appropriate. The P_T values were normalized using the arcsine transformation prior to statistical analysis. The metabolic rate and size data were subjected to analysis of variance. When significant effects were detected, Tukey's multiple comparison test (Steel and Torrie 1980) was used to identify significantly different treatment means ($P < 0.05$). The $[Cl^-]$ determined for chinook egg PVF and incubation water in similar salinity treatments were compared using unpaired t-tests ($P < 0.05$). All analyses were performed with the SYSTAT statistical program (Wilkinson 1988).

RESULTS

Survival and Development

Steelhead trout embryo survival from the beginning of salinity exposure to hatching was high in 0, 4 and 8 ppt but declined slightly in 12 ppt (Table 2). The majority of dead eggs in 12 ppt salinity were 'well eyed' (Stage 22b, Ballard 1973) and died just before hatching. Hatching success of chinook salmon eggs was high in all four salinity treatments. Times to 50% hatching for steelhead trout and chinook salmon embryos in fresh water were similar to predictive models relating temperature and incubation time of Pacific salmonids (Alderdice and Velsen 1978; Rombough 1986; Jensen 1988; Beacham and Murray 1990), and were not affected by incubating salinities up to 12 ppt. Completion of hatch for both species, however, was delayed 3-4 d in 8 and 12 ppt compared to 0 and 4 ppt salinity (Table 2).

Steelhead trout and chinook salmon alevin survival was high in all salinities except 12 ppt, where there was a slight decline in chinook and a sharp decline in steelhead alevin survival after a one week exposure period (Table 2). Alevins hatched in the highest salinity were not as vigorously active as those in lower salinity treatments, and some had abnormal yolk sacs with localized areas of coagulated yolk material. This condition was more prevalent in steelhead trout than chinook salmon alevins. Although alevin survival was not monitored to complete yolk sac absorption, it was considered unlikely that any steelhead would survive to this stage in 12 ppt salinity.

Table 2. Survival and development times for steelhead trout and chinook salmon embryos incubated in different water salinities.

Salinity (ppt)	Percent Hatch ¹	Development time (days)		7 d Alevin Survival (%)
		50% hatch	100% hatch	
<u>Steelhead trout</u>				
0	98.1	39	41	98.0
4	98.1	39	40	98.1
8	96.6	40	44	100
12	84.4	40	44	25.0
<u>Chinook salmon</u>				
0	100	53	55	100
4	100	53	55	100
8	98.0	54	58	100
12	98.1	53	58	90.4

1. N=55 eggs per salinity treatment

Metabolic Rates

Oxygen consumption rates of individual eyed eggs ($\mu\text{g O}_2\cdot\text{h}^{-1}$) were substantially lower than recorded for newly hatched alevins (Table 3), due to an increase in the mass of respiring tissue during development (Table 4). Routine metabolic rates ($\mu\text{g O}_2\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) of eyed steelhead trout eggs and chinook salmon eggs and alevins were not significantly affected ($P<0.05$) by incubating salinities up to 12 ppt (Table 3, Fig. 1). Metabolic rates of newly hatched steelhead alevins, however, were significantly lower in 8 ppt and higher in 12 ppt compared to the other salinity treatments. Mean P_C values for steelhead and chinook alevins ranged from 4.7-5.8 $\text{mg}\cdot\text{L}^{-1}$ and did not vary significantly ($P<0.05$) with salinity (Table 3).

Egg and Alevin Size

Total dry weights of steelhead and chinook eggs did not differ significantly ($P<0.05$) among the four salinity treatments (Table 4). A slight increase in P_T values with salinity reflected the chronological order of sampling, as eggs in 8 and 12 ppt were sampled one day later than those in 0 and 4 ppt salinity.

One week old steelhead trout alevins had similar total dry weights in all salinities, however the P_T values decreased with increasing salinity and were significantly lower ($P<0.05$) in 8 and 12 ppt than in 0 and 4 ppt salinity. Steelhead alevin fork lengths also decreased significantly in 8 and 12 ppt, reflecting the lower amounts of tissue in these salinity treatments. Chinook alevins showed a similar size response to salinity, but P_T values and fork lengths were significantly lower ($P<0.05$) only in 12 ppt salinity. Fresh wet weights obtained for chinook salmon alevins were

Table 3. Metabolic rates and critical oxygen levels for eyed eggs and newly hatched alevins of steelhead trout and chinook salmon in four salinities.

Species/stage Elapsed time	Salinity (ppt)	N	Metabolic rates (mean values ± SE) ¹			
			μg O ₂ ·h ⁻¹ ·ind ⁻¹	μg O ₂ ·g TDW ⁻¹ ·h ⁻¹	μg O ₂ ·g TWW ⁻¹ ·h ⁻¹	P _C (mg·L ⁻¹)
Steelhead trout:						
Eyed eggs	0	6	1.9 ± 0.1 ^a	1257.5 ± 45.4 ^a	167.2 ± 6.0 ^a	
640-672h	4	6	1.8 ± 0.1 ^a	1135.8 ± 61.8 ^a	151.2 ± 8.2 ^a	
	8	6	1.9 ± 0.1 ^a	1124.4 ± 49.1 ^a	149.6 ± 6.5 ^a	
	12	6	1.9 ± 0.1 ^a	1078.0 ± 19.2 ^a	143.4 ± 2.6 ^a	
Alevins	0	6	14.7 ± 1.2 ^a	1048.6 ± 87.3 ^a	139.5 ± 11.6 ^a	4.8 ± 0.2 ^a
1168-1200h	4	6	15.2 ± 0.6 ^a	1071.3 ± 37.7 ^a	142.5 ± 5.0 ^a	4.7 ± 0.2 ^a
	8	6	8.8 ± 0.1 ^b	779.0 ± 21.3 ^b	103.6 ± 2.8 ^b	5.0 ± 0.4 ^a
	12	2	12.0 ± 0.1 ^a	1454.0 ± 142.2 ^c	193.4 ± 18.9 ^c	-
Chinook salmon:						
Eyed eggs	0	6	3.5 ± 0.2 ^a	419.8 ± 25.0 ^a	84.0 ± 5.0 ^a	
1030-1063h	4	6	3.9 ± 0.3 ^a	489.8 ± 19.9 ^a	98.0 ± 4.0 ^a	
	8	6	3.9 ± 0.3 ^a	466.8 ± 28.6 ^a	93.4 ± 5.7 ^a	
	12	6	4.2 ± 0.2 ^a	460.0 ± 23.3 ^a	92.0 ± 4.7 ^a	
Alevins	0	6	16.0 ± 0.8 ^a	595.2 ± 23.0 ^a	119.0 ± 4.6 ^a	5.6 ± 0.6 ^a
1536-1567h	4	6	19.0 ± 0.6 ^b	661.0 ± 21.4 ^a	132.2 ± 4.3 ^a	4.9 ± 0.1 ^a
	8	6	15.5 ± 0.3 ^a	581.8 ± 17.9 ^a	116.4 ± 3.6 ^a	5.3 ± 0.1 ^a
	12	6	16.1 ± 0.8 ^a	662.8 ± 28.3 ^a	132.6 ± 5.7 ^a	5.8 ± 0.2 ^a

NOTE: TDW = Tissue Dry Weight, TWW = Tissue Wet Weight, P_c = critical oxygen level.

1. For each species and stage, mean values in a column with a common superscript letter are not significantly different ($P < 0.05$) by Tukey's test.

Table 4. Weights and lengths for eyed eggs and newly hatched alevins of steelhead trout and chinook salmon incubated in four salinities.

		Mean values \pm SE ¹						
Species/stage Elapsed time	Salinity (ppt)	Total embryo dry wt (mg) ²	Tissue dry weight (mg)	Yolk dry weight (mg)	P _T	Length (mm)	Fresh wet weight (mg)	
Steelhead trout:								
Eyed eggs	0	44.6 \pm 0.6 ^a	1.5 \pm 0.02 ^a	43.1 \pm 0.6 ^a	3.4 \pm 0.1 ^a			
640-672h	4	43.8 \pm 0.6 ^a	1.6 \pm 0.1 ^{ab}	42.2 \pm 0.6 ^a	3.6 \pm 0.2 ^{ab}			
	8	43.9 \pm 0.6 ^a	1.7 \pm 0.04 ^b	42.2 \pm 0.6 ^a	3.9 \pm 0.1 ^{bc}			
	12	43.1 \pm 0.3 ^a	1.8 \pm 0.1 ^b	41.3 \pm 0.3 ^a	4.2 \pm 0.1 ^c			
Alevins	0	38.9 \pm 0.5 ^a	14.0 \pm 0.1 ^a	24.9 \pm 0.5 ^a	36.0 \pm 0.5 ^a	22.0 \pm 0.1 ^a		
1168-1200h	4	40.9 \pm 0.6 ^a	14.2 \pm 0.2 ^a	26.7 \pm 0.6 ^{ab}	34.7 \pm 0.6 ^a	21.8 \pm 0.1 ^a		
	8	39.8 \pm 0.5 ^a	11.3 \pm 0.2 ^b	28.5 \pm 0.3 ^b	28.4 \pm 0.3 ^b	20.7 \pm 0.1 ^b		
	12	45.4 \pm 1.9 ^a	8.4 \pm 0.8 ^c	37.0 \pm 1.0 ^c	18.5 \pm 1.1 ^c	19.0 \pm 0.2 ^c		
Chinook salmon:								
Eyed eggs	0	110.2 \pm 0.4 ^a	8.4 \pm 0.1 ^{ab}	101.8 \pm 0.4 ^a	7.6 \pm 0.1 ^{ab}			
1030-1063h	4	110.8 \pm 0.4 ^a	8.1 \pm 0.5 ^a	102.7 \pm 0.8 ^a	7.3 \pm 0.5 ^a			
	8	110.0 \pm 0.3 ^a	8.4 \pm 0.2 ^{ab}	101.6 \pm 0.4 ^a	7.6 \pm 0.2 ^{ab}			
	12	110.5 \pm 0.3 ^a	9.2 \pm 0.1 ^b	101.3 \pm 0.3 ^a	8.3 \pm 0.1 ^b			
Alevins	0	99.9 \pm 0.5 ^{ab}	26.8 \pm 0.3 ^a	73.1 \pm 0.4 ^a	26.8 \pm 0.2 ^a	26.7 \pm 0.1 ^a	310.2 \pm 3.0 ^a	
1536-1567h	4	101.5 \pm 0.4 ^a	28.7 \pm 0.2 ^b	72.8 \pm 0.5 ^a	28.3 \pm 0.2 ^b	26.7 \pm 0.1 ^a	312.3 \pm 1.0 ^a	
	8	98.8 \pm 0.6 ^b	26.6 \pm 0.4 ^a	72.2 \pm 0.8 ^a	26.9 \pm 0.5 ^a	28.1 \pm 0.2 ^b	300.2 \pm 1.5 ^b	
	12	101.7 \pm 0.3 ^a	24.2 \pm 0.4 ^c	77.5 \pm 0.4 ^b	23.8 \pm 0.3 ^c	25.5 \pm 0.1 ^c	293.3 \pm 1.2 ^b	

NOTE: For each species and stage, mean values in a column with a common superscript letter are not significantly different (P<0.05) by Tukey's test.

1. For weights, n=6 (except for steelhead alevins in 12 ppt where n=2) with each replicate representing a pooled average of 10 individuals. For lengths, n=20 alevins per salinity treatment.
2. Dechorionated eggs.

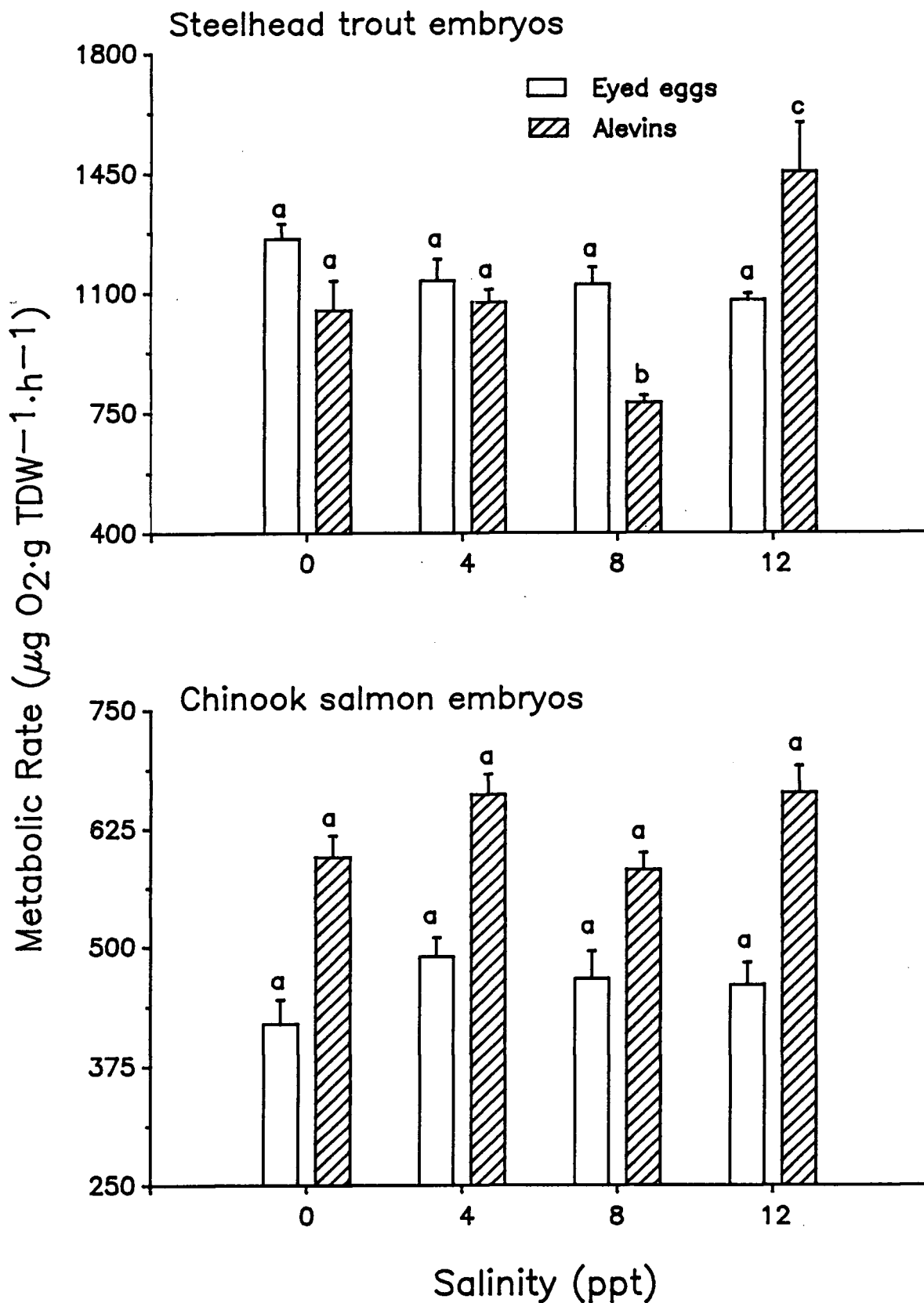


Figure 1. Metabolic rates of eyed eggs and newly hatched alevins of steelhead trout and chinook salmon in four salinities. Means (+ 1 SE) with a common superscript letter are not significantly different by Tukey's test.

highest in 0 and 4 ppt and decreased significantly ($P<0.05$) in 8 and 12 ppt salinity. Since total dry weights were not different between the four salinity treatments, these data suggest that the moisture contents of chinook alevins maintained in 8 and 12 ppt salinity were lower than the other two treatments.

Perivitelline Fluid Chloride Content

The $[Cl^-]$ of the PVF in eyed chinook salmon eggs was lowest in fresh water and increased with the salinity of the external medium (Table 5). There were no significant differences ($P>0.05$) found between the $[Cl^-]$ of the PVF and the incubation water for any given salinity treatment.

Table 5. Chloride content of the perivitelline fluid in eyed chinook salmon eggs acclimated to four salinities. Results are presented as mean values \pm SE (n).

Salinity (ppt)	Chloride (meq·L ⁻¹)	
	Perivitelline fluid	Incubation water
0	8.2 \pm 2.3 (4)	2.9 \pm 0.5 (3)
4	57.6 \pm 3.8 (5)	62.5 \pm 0.5 (2)
8	123.6 \pm 2.7 (5)	123.0 \pm 5.0 (2)
12	185.8 \pm 4.9 (5)	190.0 \pm 2.0 (2)

NOTE: Unpaired t-tests between the chloride content of the PVF and the incubation water at similar salinities revealed no significant differences ($P>0.05$).

DISCUSSION

The survival data suggest that a salinity of 8 ppt is the upper threshold limit for the normal development of steelhead trout and chinook salmon eggs and alevins. This salinity is approximately isotonic with the blood of salmon alevins (Weisbart 1968), and indicates that continuous exposures to hypertonic salinities are not tolerated. Previous studies on the salinity tolerance of salmonid embryos have also shown decreased survival and increased abnormalities in salinities above the isotonic level (Saunders 1966; Leatherland and Lin 1975; Shen and Leatherland 1978a; Veltishcheva 1983; Groot 1989). In our study, chinook salmon eggs and alevins had higher survival rates in 12 ppt salinity than were obtained for steelhead trout. This greater salinity resistance may have been due, in part, to a higher tolerance for increased salinity in the tissues of chinook salmon, as proposed by Weisbart (1968). Also, the chinook salmon eggs and alevins were larger than the steelhead trout and probably had lower, and thus more favorable, surface area:volume ratios. A similar explanation was given by Talbot et al. (1982) for the longer periods of survival observed for Atlantic salmon (Salmo salar) alevins in hypertonic salinities compared with smaller sea trout (S. trutta) alevins.

Salinity has been shown to either delay or advance hatching, depending on the species and direction of salinity change (Holliday 1969). Completion of hatch for chinook salmon and steelhead trout embryos in the present study was delayed for several days in 8 and 12 ppt compared to the lower salinities. In addition, many of the eggs which failed to hatch were fully developed embryos. The hatching period may therefore have been prolonged due to physical difficulties in breaking the chorion by weakened embryos which were stressed by the higher salinities. Although not examined in this study, it is also possible that the higher salinities

somehow interfered with the release or activity of the hatching enzyme, which is normally secreted by glands in the embryo to soften the chorion before the hatching process begins (Yamagami 1988).

Size differences of hatching larvae related to salinity have been reported by many workers (Holliday 1969; von Westernhagen 1988). In the current study, steelhead and chinook alevins were smaller in 12 ppt salinity compared to the other treatments. The lower P_T values in 12 ppt salinity suggest that the hypertonic salinity may have interfered with the conversion of yolk to body tissue. There are indications that large yolk sacs occur because of metabolic (i.e. mitochondrial) malfunctions or osmotic disturbances in the alevin which prevent normal use of the energy stored in the yolk (von Westernhagen 1988). The presence of coagulated yolks in steelhead alevins continuously exposed to 12 ppt salinity may therefore have reflected an osmotic imbalance which resulted in poor utilization of yolk reserves.

The metabolic rates and P_C levels determined for steelhead and chinook eggs and alevins in fresh water were comparable to values obtained by other workers for various salmonid species (Hayes et al. 1951; Alderdice et al. 1958; Carrick 1981; Wieser et al. 1985). Difficulties in comparing literature values arise from differences in stages of development, activity levels, temperatures and measurement techniques employed by different investigators. These factors can have large influences on the metabolic rates of eggs and alevins (Rombough 1988c). Rombough (1988a,b,c) conducted comprehensive studies on the aerobic metabolism and dissolved oxygen requirements of eggs and alevins of steelhead trout and chinook salmon, using the same apparatus and methodology employed in the present study. Comparisons to his predictive regression equations relating metabolic rates and P_C levels with incubation time and temperature indicated

similar values for steelhead trout eggs and alevins, while metabolic rates for chinook salmon eggs and alevins were higher than determined in this study.

Metabolic rates of steelhead trout and chinook salmon eggs and alevins were largely unaffected by constant salinities up to 12 ppt. These results are consistent with previous studies using marine teleost embryos. Lasker and Theilacker (1962; Pacific sardine), Holliday et al. (1964; Atlantic herring), Walsh and Lund (1989; cottids) and Walsh et al. (1989; striped mullet) all reported no significant differences in metabolic rates of marine fish eggs and larvae incubated in various salinities. The current study is the first to record a similar metabolic response to salinity for embryonic stages of anadromous salmonid species. It is not clear from these data whether energetic costs for ion regulation are negligible when compared to total metabolic rate, or if increased ion regulatory costs are compensated for by decreases in other metabolic processes (Holliday et al. 1964). Atlantic salmon embryos have shown low permeabilities of the vitelline membrane and overgrowing tissues to movements of ions and water (Potts and Rudy 1969; Rudy and Potts 1969; Loeffler and Lovtrup 1970). Numerous chloride cells responsible for ion excretion have been found in the yolk sac epithelium of marine fish eggs and larvae (Shelbourne 1957; Lasker and Threadgold 1968; Guggino 1980), however such has not been the case for salmonid embryos and alevins. Chloride cells could not be detected on the yolk sac epithelium of coho (Oncorhynchus kisutch) alevins maintained in different salinities (Leatherland and Lin 1975). Small numbers of chloride cells were found in the yolk sac epithelium of rainbow trout embryos just before hatching (Shen and Leatherland 1978b), however no difference in chloride cell abundance was apparent between different salinity treatments. It was therefore not clear whether they functioned for ion uptake or ion excretion. The available information suggests that internal ionic and osmotic balance in salmonid embryos is maintained mostly by the passive

regulation of ion concentrations by impermeable membranes, rather than by active, energy consuming, mechanisms of ionic and osmotic regulation (Alderdice 1988). The absence of a salinity effect on metabolic rates of steelhead trout and chinook salmon eggs and alevins in the present study supports this hypothesis. Low permeabilities of the vitelline membrane probably served to maintain ion gradients between body fluids and the external medium and minimized energy requirements for active ion regulation. Elevated respiration rates measured for steelhead trout alevins in 12 ppt salinity, accompanied by an increase in mortality, may have resulted from a change in membrane permeability at hatching and subsequent dysfunction of the vitelline membrane in a hypertonic medium. Groot (1989) also speculated that membrane permeability is increased by a lowering of the internal embryo pressure in response to increased tonicity of the external medium. Increased membrane permeability would allow ions and water to move more freely along prevailing concentration gradients. In 12 ppt salinity, sodium and chloride ions would move into the embryo and water would pass outward. Dehydration in dilute salinities observed in chinook salmon alevins in this study and in other salmonid alevin studies (Leatherland and Lin 1975; Shen and Leatherland 1978a; Talbot et al. 1982), indicates that water loss occurs in salinities above the isotonic level. Assuming that these alevins had limited active ion-osmotic regulation capabilities, cellular disruptions would occur and eventually lead to death. The increased rates of oxygen uptake observed for steelhead trout alevins in 12 ppt salinity, therefore, probably reflected a metabolic response to osmotic stress, rather than an energetic cost of ion regulation. The lack of a metabolic response in chinook salmon alevins to 12 ppt salinity and an associated lower mortality rate may indicate species-specific differences in membrane permeability and tissue tolerance to increased salinities. It appears likely, therefore, that salinity tolerance thresholds in salmonid eggs and alevins are determined by the capability of the vitelline

membrane to maintain ion gradients by its permeability characteristics, and the capacity of the internal tissues to withstand ionic and osmotic changes during development.

The $[Cl^-]$ of the PVF in eyed chinook salmon eggs closely matched the $[Cl^-]$ of the external medium. The chorion of salmon eggs has been shown to be permeable to sodium and chloride ions (Rudy and Potts 1969; Peterson and Martin-Robichaud 1987) and water (Potts and Rudy 1969) but not to large molecules such as colloids, which are released after spawning from the yolk cortex into the perivitelline space resulting in the osmotic uptake of water to form the perivitelline fluid (Eddy 1982). The perivitelline colloid has a net negative charge at normal pH levels (Peterson 1984) and can accumulate sodium ions at concentrations in excess of those present in fresh water (Rudy and Potts 1969). There were no indications in this study of significant accumulation of chloride ions in the PVF relative to the external medium. This was to be expected since the perivitelline colloids would provide an anion excess and attract cations rather than anions. There have been suggestions in the literature that the chorion and PVF may afford some degree of protection to the developing embryo from adverse environmental conditions, such as acidic water, compared to dechorionated embryos and newly hatched alevins (Daye and Garside 1979; Eddy and Talbot 1985). Weisbart (1968) further showed that dechorionated embryos of all five species of Pacific salmon had slightly decreased survival times in seawater compared to intact embryos. Why the chorion would impart increased salinity resistance to the embryo is unclear since, as mentioned above, the chorion is permeable to external salt and the PVF has an osmotic pressure similar to the external environment (Shen and Leatherland 1978a, Groot 1989). In the current study, alevins in 12 ppt showed an increase in mortality rates soon after hatching, which also suggests a decreased salinity resistance compared to intact embryos. This apparent decrease in salinity tolerance may be related to a temporal

effect of salinity exposure on mortality or to a change in membrane permeability at hatching, rather than a protective role of the chorion to high ambient salinities. Furthermore, hatching is a physically exhaustive process for the embryo and in combination with increased salinity may impose a stress from which it cannot recover. The role of the chorion in the salinity tolerance of salmonid embryos clearly warrants further study.

In addition to providing information on the effects of salinity on metabolism and development of salmonid eggs and alevins, the results of the present study may also have practical application. Salt has recently become a popular alternative to Malachite Green in fish culture as a fungicide for incubating salmon eggs. Current hatchery practices involves the immersion of eggs in a static, 20 ppt salinity bath for 1 h, 3 times per week from water-hardening to the eyed stage (P. Edgell, Robertson Creek Hatchery, Port Alberni, B.C., personal communication). In this study, constant incubation of steelhead trout and chinook salmon embryos in 4 ppt salinity following epiboly did not adversely affect survival, rate of development, growth or metabolism, compared with fresh water. Coastal hatcheries equipped with saltwater lines could therefore safely incubate salmonid eggs and alevins in a slightly enhanced salinity (i.e. <5 ppt), which would probably decrease the incidence of infections by stenohaline freshwater bacteria and fungi, and may increase the salinity tolerance of emerging fry (Alderdice 1988). As the present experiments were terminated when the alevins were only one week old, further testing would be necessary before implementation of such a strategy to ensure that the alevins were not affected in later stages of development.

CHAPTER 2

Effects of Salinity on Growth, Metabolism and Ion Regulation
in Rainbow and Steelhead Trout (Oncorhynchus mykiss) and
Fall Chinook Salmon (Oncorhynchus tshawytscha) Fry

INTRODUCTION

There are conflicting reports in the literature regarding the effects of isotonic salinity (i.e. 8-10 ppt; Brett 1979) on the growth and metabolic rate of salmonids. Both Canagaratnam (1959) and Otto (1971) found that growth rates of pre-smolt coho salmon (Oncorhynchus kisutch) were higher between 5 and 12 ppt than in fresh water. Measurements of oxygen consumption in rainbow trout (O. mykiss) further showed a reduction in metabolic rate (20-28%) in isotonic salinity relative to fresh water and seawater (Rao 1968). These studies support the hypothesis that the energetic cost of ion regulation is lowest in an isotonic environment, where the ionic gradients between blood and water are minimal, and that this energy savings is substantial enough to increase growth. Other studies, however, fail to show isotonic salinity as the point of maximal growth in salmonids; growth rates were highest in fresh water in studies by Shaw et al. (1975), Clarke et al. (1981), McKay and Gjerde (1985), and McCormick et al. (1989a). A theoretical estimate of the energetic cost of osmoregulation in salmonids suggests that it would be very low, being less than 1% of resting metabolism (Eddy 1982). Accordingly, changes in metabolic rate of that magnitude would be very difficult to measure accurately. Studies such as that of Bullivant (1961), who reported no significant differences between the metabolic rates of yearling chinook salmon (O. tshawytscha) reared in fresh water, half-strength seawater and full-strength seawater, support those theoretical estimates.

Few studies have made simultaneous measurements on growth and metabolic rates of salmonids in relation to salinity. To study those relationships, I examined growth, metabolic rates and ionic regulation in juvenile rainbow and steelhead trout and fall chinook salmon acclimated to a range of salinities, with one near isotonic. These species were chosen to investigate the

possible effects of different life history patterns on growth and metabolic responses to environmental salinity. The steelhead trout is an anadromous form of the rainbow trout, usually spending two years in fresh water before migrating to the sea (Scott and Crossman 1973). Fall chinook salmon are also anadromous, but spend a shorter period of time in fresh water than steelhead trout, migrating seaward within three months of emergence (Lister and Walker 1966).

MATERIALS AND METHODS

Fish Stocks

Domesticated rainbow trout fry were obtained from Sun Valley Trout Farms, Mission, B.C. in November, 1988. Steelhead trout and fall chinook salmon eggs were taken at the Big Qualicum River hatchery on Vancouver Island in April and November, 1989, respectively, and incubated to the swim-up stage at the Pacific Biological Station in Nanaimo. All three stocks were transferred to the E.V.S. Consultants Marine Laboratory in North Vancouver, and were held in fibreglass tanks with running fresh water for up to three weeks prior to experimentation.

Growth Experiments

The experimental design for each growth trial is shown in Table 6. At the start of the experiments, the fry were lightly anesthetized in 2-phenoxyethanol ($0.5 \text{ ml}\cdot\text{L}^{-1}$), measured for length (to the nearest mm) and weight (to the nearest 0.01 g), and added to 180 L fibreglass tanks supplied with both fresh water and seawater. One tank was prepared for each salinity treatment. Flow rates of fresh and salt water into common header mixing buckets were controlled by valves to achieve the desired salinities (± 1 ppt) in the rearing tanks. The fish were gradually acclimated to the test salinities in a stepwise manner at a rate of 1-2 ppt per day. Salinity, temperature, dissolved oxygen, pH and mortalities were recorded daily in each rearing tank. Water temperatures were kept constant ($\pm 1^\circ\text{C}$) using immersion heaters, and aeration was supplied to each tank to maintain dissolved oxygen levels near saturation. Dissolved oxygen levels ranged from $8.6\text{-}10.7 \text{ mg}\cdot\text{L}^{-1}$ due to differences in oxygen solubility with increasing salt

Table 6. Experimental conditions for juvenile salmonid growth trials in different water salinities.

Parameter	Rainbow trout	Steelhead trout	Chinook salmon
Timing	Nov - Dec, 1988	June - Sept, 1989	Feb - June, 1990
Duration	5 weeks	12 weeks	11 weeks
Salinities (ppt)	0, 9*, 18	0, 4, 8*, 12, 16	0, 5, 10*, 20, 28
Temperature (°C)	11	14	11.5
Photoperiod (h)	12 (constant)	14 (constant)	11-15 (natural)
No. of fish per tank	50	80	100
Initial mean weights (g)	1.0	0.4	1.0
Sampling frequency	5 weeks	3 weeks	3 weeks

* closest to isotonic value (i.e. 8-10 ppt)

content, but the minimum concentrations were not considered to be limiting to growth (Brett 1979). The fish were fed 4-5% of their total body weight daily by hand, using a diet of dry EWOS fry feed. The fish actively fed and there were no observable differences in swimming activity between the different salinity treatments.

Random samples of 20 steelhead trout and chinook salmon fry per treatment were measured for length and weight after the acclimation periods, and at 3 week intervals throughout the experiments, to determine growth rates and feeding rations. Percent moisture content was determined from a sample of 10 chinook fry per treatment at each sampling period after oven drying at 80°C for 24 h. Blood was also collected from 10 fish per treatment at several sampling periods for the determination of hematocrit values and ion concentrations. After anesthetizing the fish, the caudal peduncle was severed and blood was collected from the dorsal aorta into heparinized capillary tubes. Hematocrits (% red blood cells by volume) were determined after centrifugation at 10,000 rpm for 5 min. The separated plasma was withdrawn using Microcap[®] tubes and frozen at -20°C in 0.5 mL plastic vials prior to ion concentration analyses. Because of low plasma volumes in the smaller fish, it was necessary to pool samples in some cases to obtain sufficient volume for the ion analyses. Plasma chloride concentration ($[Cl^-]_p$) determinations were made by coulometric titration (Haake Buchler Instruments Digital Chloridometer), while plasma sodium concentrations ($[Na^+]_p$) were measured by flame photometry (Corning Flame Photometer Model 410). Appropriate reference standards were run every 10 samples to ensure measurement accuracy. Water was collected from each rearing tank mid-way through the growth trials and analysed for sodium, chloride and calcium ions for comparison with blood plasma values (Table 7).

Table 7. Chemical composition of water samples collected from the steelhead trout and chinook salmon fry rearing tanks.

Salinity (ppt)	Chemical parameter			
	Na ⁺ (meq·L ⁻¹)	Cl ⁻ (meq·L ⁻¹)	Ca ⁺⁺ (meq·L ⁻¹)	pH
<u>Steelhead trout</u>				
0 (FW)	3	<1	<1	6.0
4	71	63	1	7.1
8	146	120	1	7.3
12	189	170	2	7.4
16	213	240	3	7.5
28 (SW)	386	450	6	7.7
<u>Chinook salmon</u>				
0	3	<1	<1	6.0
5	81	71	1	7.1
10	163	156	3	7.3
20	273	288	5	7.5
28	371	456	7	7.6

At the end of each growth experiment, all remaining fish were measured for length and weight and 10 fish from each treatment were killed for hematocrit, $[\text{Na}^+]_{\text{pl}}$ and $[\text{Cl}^-]_{\text{pl}}$ determinations. Specific growth rates (expressed as percent change per day) were calculated for each treatment as $\ln(W_t \cdot W_i^{-1}) \cdot d^{-1} \cdot 100$, where W_i and W_t are the initial (after salinity acclimation) and final mean weights, respectively. Condition factors ($K = \text{wt} \cdot l^{-3} \cdot 100$; Ricker 1975) were also calculated from the length and weight data.

Respirometry Experiments

After the growth experiments were completed, oxygen consumption rates were measured for fish acclimated to the test salinities. The respirometers consisted of 1 L cylindrical glass chambers, 12 cm in diameter and 9 cm in height, and designed to sit on magnetic stir plates. Water from an aerated and temperature controlled reservoir was passed through the chambers using a Masterflex® pump. Thick-walled plastic tubing was used to connect the chambers to dissolved oxygen probes and a four-way valve assembly which allowed the respirometer to operate in an open (flow-through) or closed mode. Circular water currents within each respirometer chamber were generated by magnetic stirrers and speeds were controlled by calibrating digital rpm readouts on the stir plates with velocities determined for each chamber. Dissolved oxygen levels in each chamber were monitored continuously using polarographic oxygen electrodes and a multichannel oxygen meter (Orbisphere Model 2710).

Measurements of respiratory metabolism in fish can be influenced by several factors including water temperature, and fish size and activity (Brett and Groves 1979). To determine the effect of salinity alone, concerted efforts were made to control all other variables and therefore

minimize variation in test results. Depending on the species, two to five fish of similar size were placed into each respirometer through a ground-glass lid and the flow rate was adjusted to approximately $100 \text{ mL}\cdot\text{min}^{-1}$. The fish were acclimated to the respirometers for 24 h, and were not fed for 48 h prior to testing to ensure a postabsorptive digestive state (Brett 1964). To shield the fish from outside disturbances, the respirometers were covered with black plastic throughout acclimation and testing. After acclimation, the fish were given a 15 min swimming adjustment period before the respiration trial. In each trial, the swimming speed was set to 1 body length per second to standardize the level of activity (Brett 1962). This mild swimming challenge was necessary to reduce the variation in oxygen consumption caused by spontaneous activity at swimming speeds less than 1 body length per second (Brett and Glass 1973). The respirometers were then closed and the subsequent decline in water oxygen concentration (to the nearest $0.05 \text{ mg}\cdot\text{L}^{-1}$) was monitored for 1 h, with values recorded every 5 min. After a trial, the fish were removed and measured for length and weight. The trials were conducted at approximately the same time each day to minimize diurnal variation in metabolism due to entrainment to a feeding schedule or photoperiod (Brett and Zala 1975). Water temperatures were kept similar to those used in the growth experiments (Table 6). Background oxygen consumption, due to bacteria, was measured by running blanks (i.e. no fish) throughout the experimental period. A correction was applied to the fish respiration measurements assuming a linear increase of bacterial oxygen consumption.

Water oxygen concentrations initially declined in a curvilinear fashion for about 10-15 min, apparently as a result of pressure changes associated with closing the system (Rombough 1988b). Oxygen levels then decreased at a constant rate for about 30-45 min. Approximately 25% of the initial oxygen was consumed during the linear portion of the measurements. Oxygen

consumption rates were estimated over the linear portion using regression analysis. Metabolic rates were determined by multiplying the regression slope of oxygen uptake by the respirometer volume and were expressed in terms of wet weight over a 1 h period (i.e. $\text{mg O}_2 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$). Six replicate trials were conducted per salinity treatment for the steelhead trout and chinook salmon, and three for the rainbow trout fry.

Preliminary trials with rainbow trout fry in fresh water showed oxygen consumption to increase linearly with increasing fish weight when expressed on a double logarithmic grid (slope = 0.77). This is a well established relationship in studies on respiratory metabolism of fish (Brett and Groves 1979), and was used in the present study to validate the method of measuring oxygen consumption in the circular chambers.

Seawater Challenge Testing

At the end of the study, juvenile steelhead trout and chinook salmon (ave. weights = 7-10 g) were subjected to seawater challenge tests (Blackburn and Clarke 1987) to determine the effect of prior acclimation to the dilute salinities on subsequent adaptability to full-strength seawater. Random samples of 10-12 fish were transferred from the test salinities to 28-29 ppt (with the exception of the chinook 28 ppt treatment which had only four fish of small size remaining), and were sampled after 24 h of exposure for $[\text{Na}^+]_{\text{pl}}$ as described before. The criterion for proper seawater adaptation is the ability to maintain $[\text{Na}^+]_{\text{pl}}$ less than $170 \text{ meq} \cdot \text{L}^{-1}$ one day after transfer to seawater (Clarke et al. 1981).

Statistical Analysis

Data are presented as means \pm standard error (SE) where appropriate. When analysis of variance indicated significant effects, Tukey's multiple comparison test (Steel and Torrie 1980) was used to identify significantly different treatment means ($P < 0.05$). All analyses were performed with the SYSTAT statistical program (Wilkinson 1988).

RESULTS

The data presented as figures in this section are also provided in tabular form in the appendices.

Mortality

Mortality was less than 5% in all treatments except for chinook salmon fry reared in 28 ppt, which suffered a loss of 24 fish (Table 8). The majority of the mortalities were less than 5 cm in fork length and weighed less than 1.5 g.

Growth and Condition

Growth rates declined with increasing salinity for all three species (Fig. 2, Table 8). Rainbow trout fry growth decreased slightly from fresh water to isotonic salinity and decreased significantly in the hypertonic treatment. Steelhead trout fry showed a similar response, but final weights in 4 ppt were significantly lower than in fresh water (Fig. 3). Growth of chinook salmon fry was significantly higher in fresh water than all other treatments and showed a steady decline with increasing salinity to full-strength seawater. Specific growth rate values for chinook salmon fry in fresh water and seawater were similar to those reported by Clarke et al. (1981).

Condition factors of steelhead trout and chinook salmon fry did not differ significantly between salinity treatments for the first three sampling periods of the growth trials. Final condition factors varied among growth experiments, showing no consistent pattern between

Table 8. Mortality, specific growth rates, condition factors and moisture content of juvenile rainbow and steelhead trout and chinook salmon reared in various salinities.

Salinity Treatment (ppt)	Percent Mortality	Specific Growth Rate (% wt per day)	Final Mean Condition Factor (\pm SE)	Final Mean Percent Moisture Content (\pm SE)
<u>Rainbow trout</u>				
0	2	3.35	1.13 ± 0.01^a	
9	0	3.25	1.17 ± 0.01^a	
18	4	1.57	1.07 ± 0.01^b	
<u>Steelhead trout</u>				
0	3	3.27	1.12 ± 0.01^a	
4	0	2.67	1.15 ± 0.01^b	
8	0	2.98	1.19 ± 0.01^c	
12	0	3.11	1.17 ± 0.01^{bc}	
16	0	2.75	1.17 ± 0.01^{bc}	
<u>Chinook salmon</u>				
0	0	2.62	1.05 ± 0.01^a	78.0 ± 0.2^a
5	0	2.44	1.06 ± 0.01^a	78.1 ± 0.2^{ab}
10	0	2.32	1.06 ± 0.01^a	78.6 ± 0.2^{ab}
20	0	2.30	1.07 ± 0.01^a	79.1 ± 0.2^b
28	24	1.84	1.15 ± 0.01^b	81.4 ± 0.4^c

NOTE: Mean values with a common superscript letter are not significantly different ($P < 0.05$) by Tukey's test.

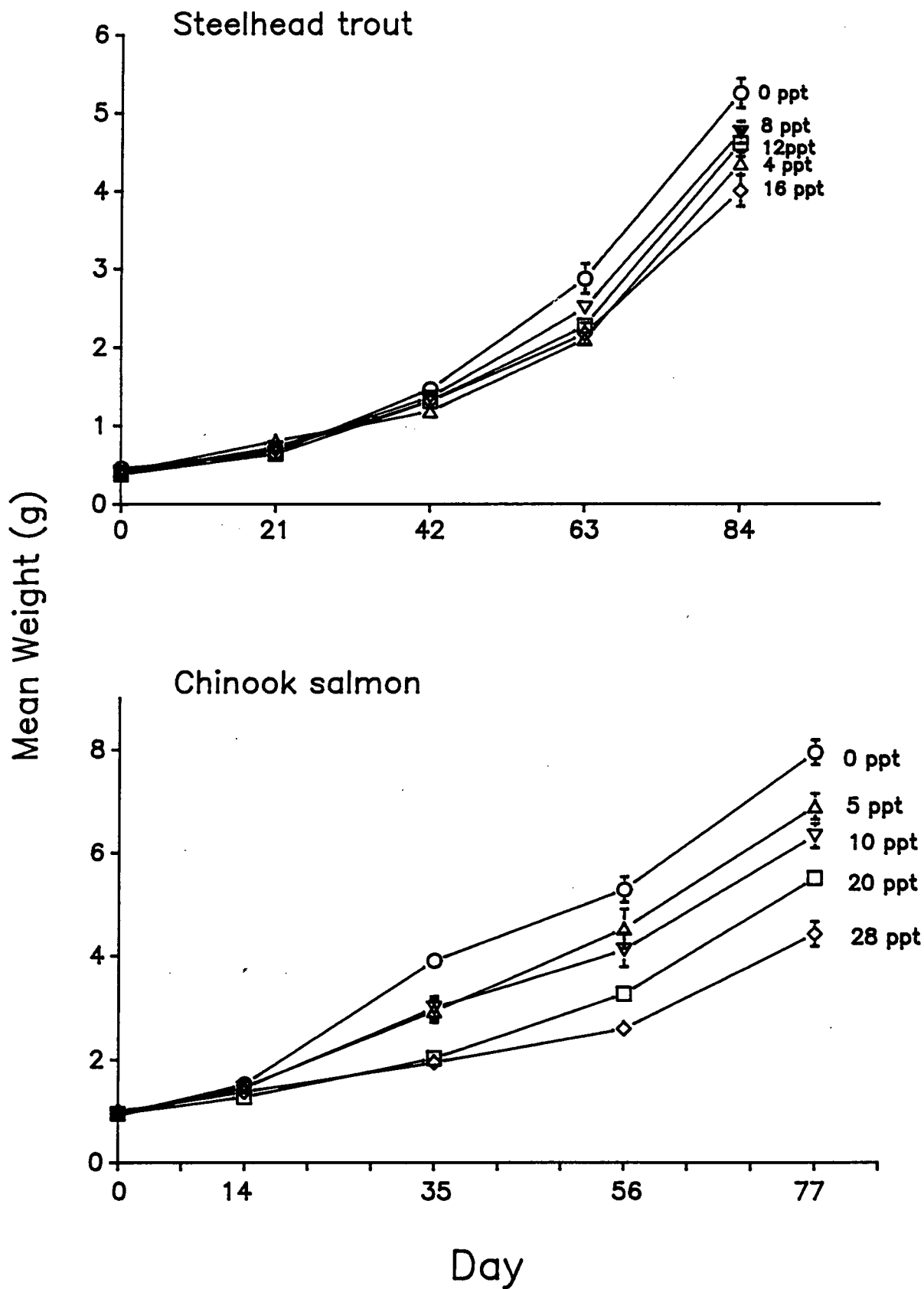


Figure 2. Growth curves for steelhead trout and chinook salmon fry reared in five different salinities. Absence of standard error bars indicates that the SE was smaller than the symbol.

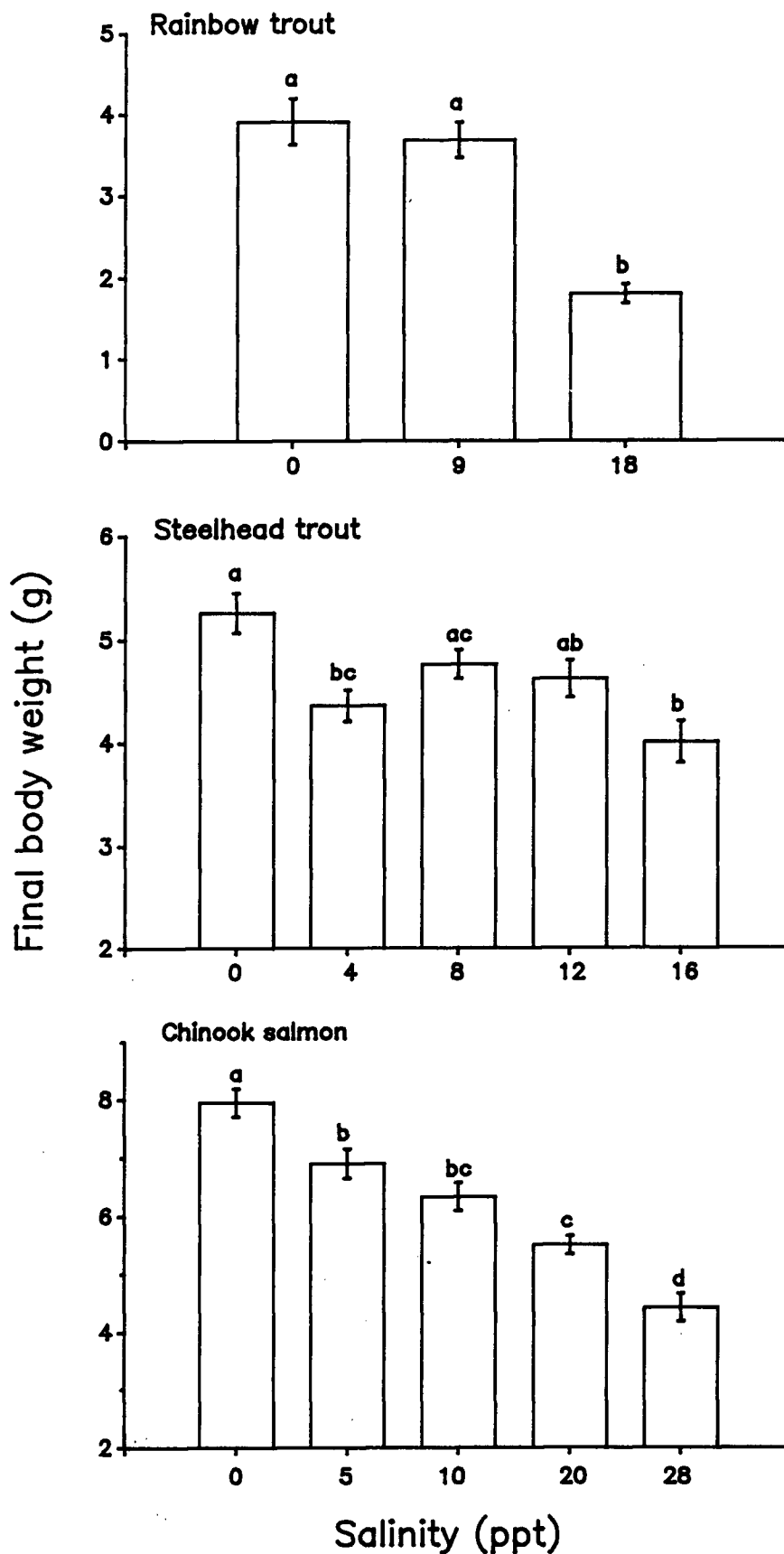


Figure 3. Effect of salinity on growth of juvenile rainbow trout, steelhead trout and chinook salmon (starting weights = 1.0, 0.4 and 1.0 grams, respectively). Means (± 1 SE) with a common superscript letter are not significantly different by Tukey's test.

species (Table 8). Rainbow trout fry in fresh water and isotonic salinity had significantly higher final condition factors than the hypertonic treatment, but the condition factor for steelhead trout fry in fresh water was significantly lower than the other treatments. Condition factors for the chinook salmon fry were similar up to 20 ppt, but were significantly higher in 28 ppt; fish in full-strength seawater tended to be deeper-bodied than the more slender body forms in the other treatments. The initial moisture content of the chinook salmon fry was 81% in all treatments and, as expected, declined with growth of the fry (Clarke et al. 1981). Differential growth rates between salinity treatments, however, resulted in size differences which masked the anticipated dehydration effect of salinity on moisture content (Table 8).

Hematocrit and Plasma Ion Concentrations

Hematocrit values for rainbow trout fry increased with salinity and were significantly higher in 18 ppt than in fresh water (Fig. 4). Hematocrit values for steelhead trout fry were also significantly higher in 16 ppt than in fresh water on day 63, but this pattern was not repeated at the other sampling times (Fig. 5). Hematocrit values for chinook salmon fry followed an opposite trend from the trout and decreased with increasing salinity, being significantly lower in 20 and 28 ppt than in fresh water on day 56 (Fig. 6). Plasma $[\text{Na}^+]$ measured in rainbow trout did not differ significantly among treatments, while $[\text{Cl}^-]_p$ were significantly higher in 18 ppt (Fig. 4). Plasma $[\text{Na}^+]$ in steelhead trout fry tended to increase with salinity and were significantly higher in 12 and 16 ppt compared to fresh water on day 84. Plasma $[\text{Cl}^-]$ did not differ significantly among treatments, although they tended to be higher at 12 and 16 ppt on days 42 and 63 (Fig. 5). Plasma $[\text{Na}^+]$ and $[\text{Cl}^-]$ in chinook salmon fry did not differ significantly among treatments at each sampling period (Fig. 6).

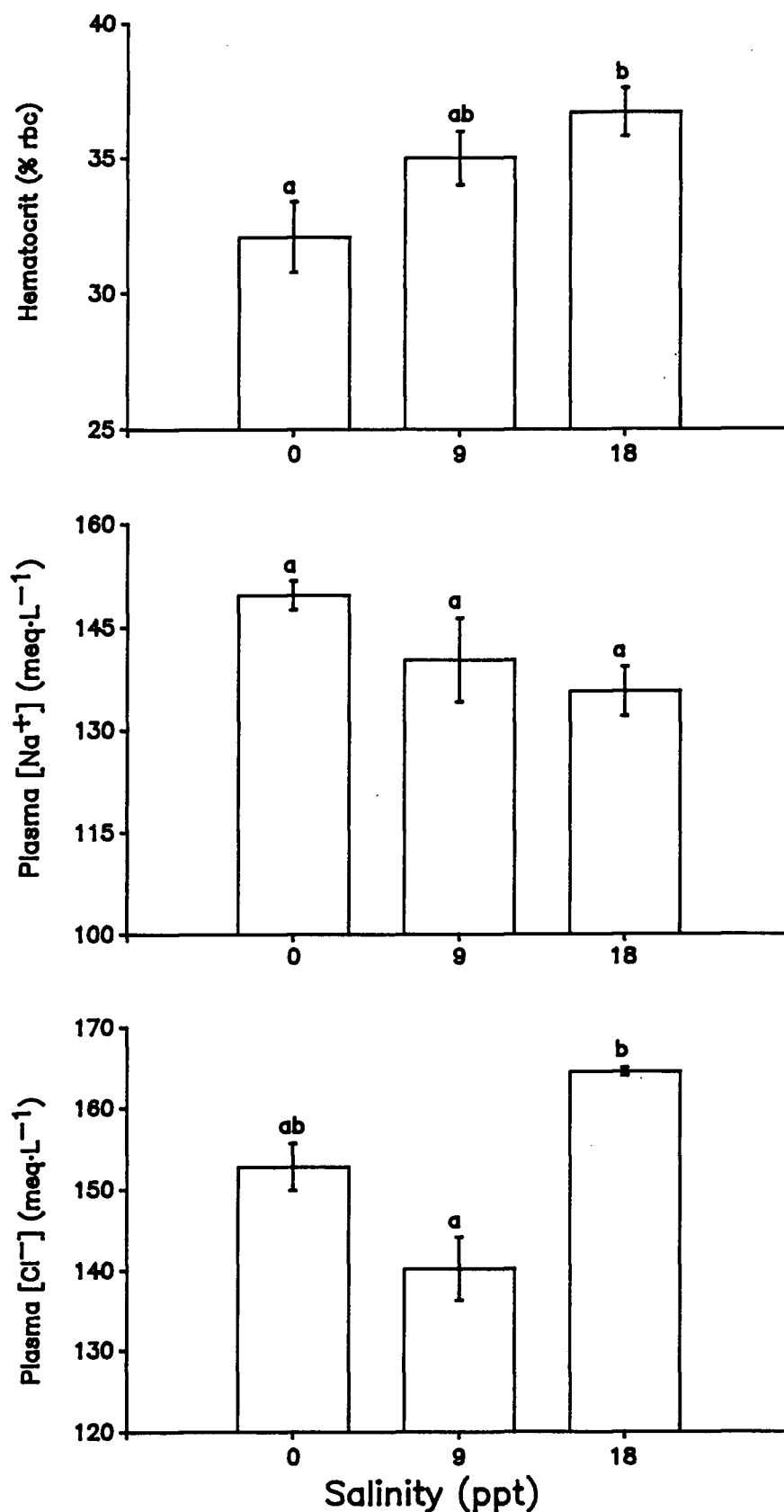


Figure 4. Mean hematocrit and plasma Na^+ and Cl^- concentrations for rainbow trout fry after five weeks in three different salinities. Means (± 1 SE) with a common superscript letter are not significantly different by Tukey's test.

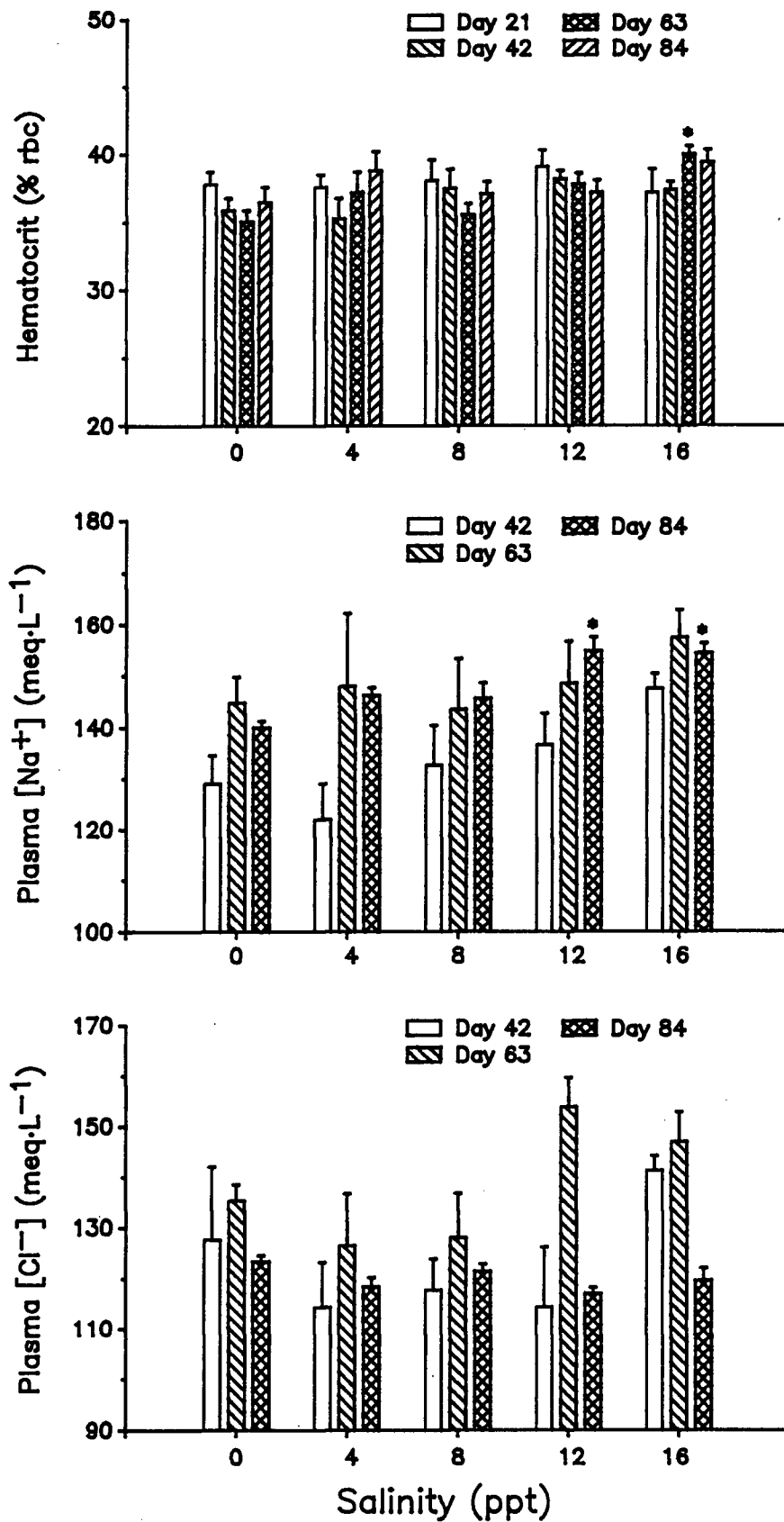


Figure 5. Mean hematocrit and plasma Na⁺ and Cl⁻ concentrations (+ 1 SE) for steelhead trout fry reared in five different salinities. Asterisk denotes salinity treatment means which are significantly different from the freshwater value.

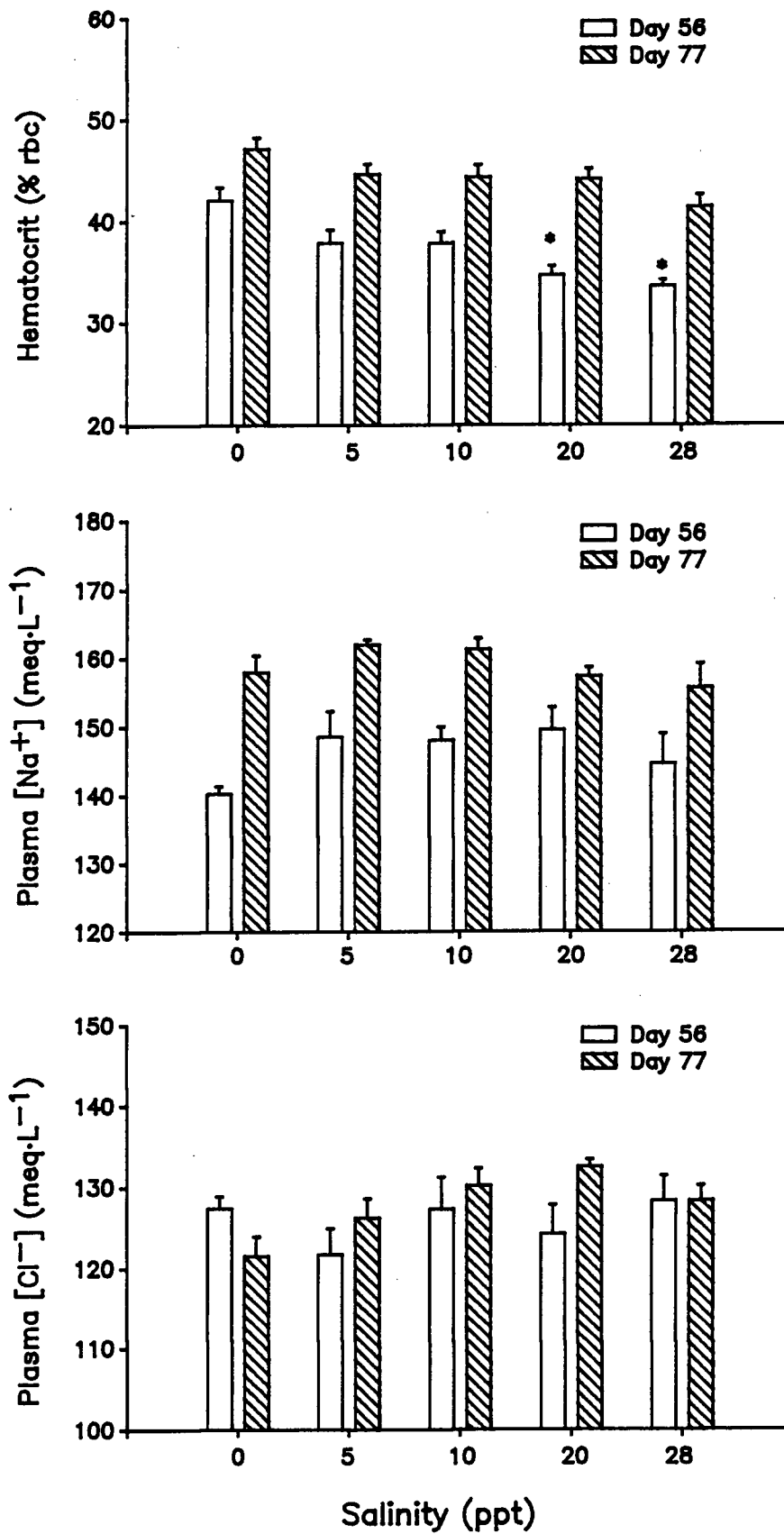


Figure 6. Mean hematocrit and plasma Na⁺ and Cl⁻ concentrations (+ 1 SE) for chinook salmon fry reared in five different salinities. Asterisk denotes salinity treatment means which are significantly different from the freshwater value.

Metabolic Rates

The average metabolic rates of juvenile salmonids in fresh water measured in the present study ranged from 207 to 270 mg O₂·kg⁻¹·h⁻¹, depending on species and temperature, and were comparable to values obtained by Brett (1965) for juvenile sockeye salmon of similar size and activity. Metabolic rates of all three species increased with salinity, being significantly higher in hypertonic treatments than in fresh water (Fig. 7). The magnitude of these changes in metabolic rate from fresh water to 16-20 ppt ranged from 25 to 30%. The following linear regression equations describe the relationship between metabolic rate and salinity, determined for the three species:

(1) rainbow trout: $\ln \dot{M}O_2 = 0.080 \cdot \ln (S+1) + 5.328 ; r^2 = 98.6\%$

(2) steelhead trout: $\ln \dot{M}O_2 = 0.064 \cdot \ln (S+1) + 5.581 ; r^2 = 75.2\%$

(3) chinook salmon: $\ln \dot{M}O_2 = 0.087 \cdot \ln (S+1) + 5.569 ; r^2 = 93.7\%$

where $\dot{M}O_2$ is metabolic rate (mg O₂·kg⁻¹·h⁻¹) and S is salinity (ppt). These equations provide good estimates of metabolic rates for the size of fish, water temperatures, activity level and range of salinities used in this study. The increases in metabolic rate also showed significant inverse linear correlation to the observed decreases in growth (r^2 values: rainbow=0.69, steelhead=0.63, chinook=0.89).

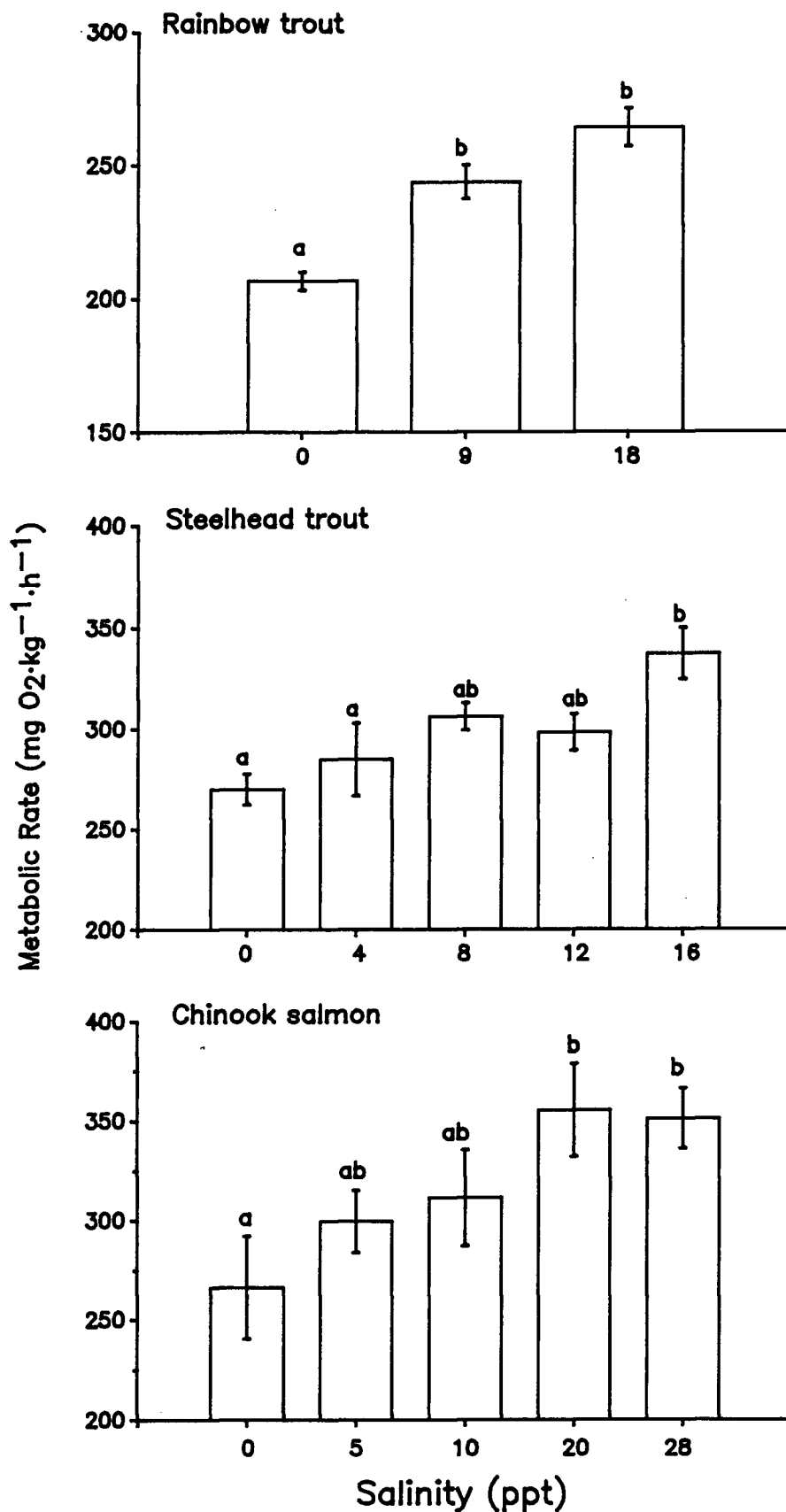


Figure 7. Metabolic rates of juvenile rainbow trout, steelhead trout and chinook salmon in different salinities. Means (\pm 1 SE) with a common superscript letter are not significantly different by Tukey's test.

Seawater Challenge Tests

No mortalities were recorded during the 24 h seawater challenge tests. Plasma $[\text{Na}^+]$ in juvenile steelhead trout and chinook salmon after 24 h of exposure to seawater were all below the $170 \text{ meq}\cdot\text{L}^{-1}$ threshold and did not differ significantly among treatments, with the exception of steelhead trout transferred from 4 ppt which averaged $177.4 \pm 3.6 \text{ meq}\cdot\text{L}^{-1}$ (Fig. 8). Acclimation to dilute salinities therefore had little influence on the seawater adaptability of juvenile steelhead trout and chinook salmon in the present study.

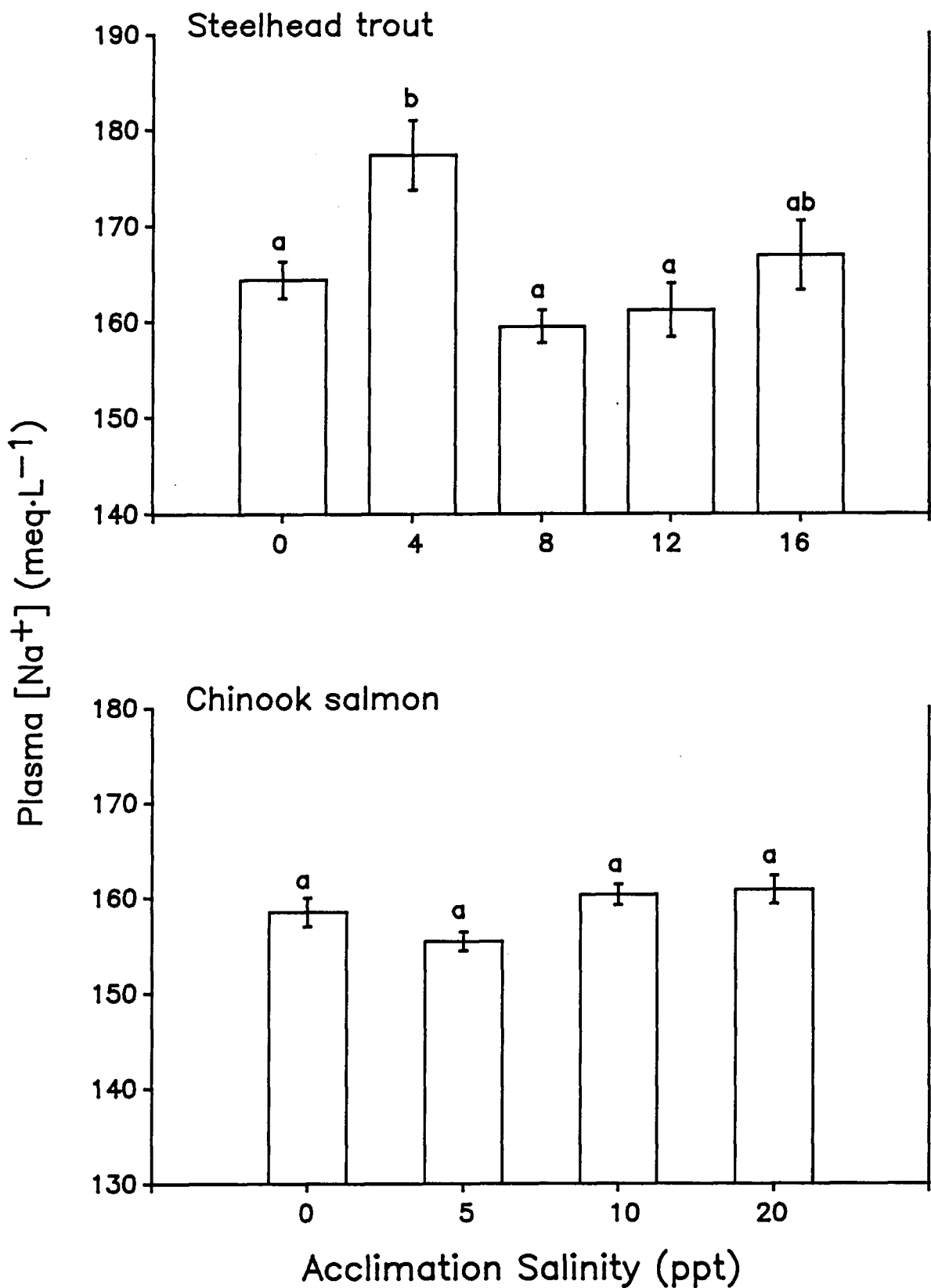


Figure 8. Plasma Na^+ concentrations of juvenile steelhead trout and chinook salmon following a 24 h seawater challenge test. Means (± 1 SE) with a common superscript letter are not significantly different by Tukey's test.

DISCUSSION

The mortality data indicate that the small fry stages (approx. 0.5-1.0 g) of these species can adapt to salinities of up to 20 ppt when a gradual acclimation procedure is followed. Previous studies on seawater adaptation have also shown increased survival of 1 g chinook salmon fry following gradual transition to seawater (Wagner et al. 1969; Kepshire and McNeil 1972), and 20 ppt salinity to be an upper threshold limit for the survival of slightly larger (10-30 g) rainbow trout (Landless 1976; Eddy and Bath 1979; Jackson 1981; Johnsson and Clarke 1988) and Atlantic salmon (Salmo salar) parr (Saunders and Henderson 1969a).

The measured values for both $[\text{Na}^+]_{\text{pl}}$ and $[\text{Cl}^-]_{\text{pl}}$ in fresh water are in good agreement with normal values for all three species (Conte and Wagner 1965; Wagner et al. 1969; Hille 1982). Plasma $[\text{Na}^+]$ and $[\text{Cl}^-]$ in rainbow and steelhead trout rose slightly when reared in salinities above the isotonic level, while plasma ion concentrations in chinook salmon fry did not change across all salinities (the ionic status of mortalities in 28 ppt could not be determined). Despite these slight increases (8-10%) in the trout, plasma ion concentrations for all three species were maintained within acceptable levels (i.e. $[\text{Na}^+]_{\text{pl}} < 180 \text{ meq}\cdot\text{L}^{-1}$, $[\text{Cl}^-]_{\text{pl}} < 160 \text{ meq}\cdot\text{L}^{-1}$; Eddy and Bath 1979; Johnston and Cheverie 1985; Stagg et al. 1989), indicating that the fish were regulating blood ions competently against concentration gradients (Table 7). It is likely, therefore, that the observed growth and metabolic rates reflect steady state in fish with respect to ionic status. The increase in calcium content of the external media at higher salinities may have assisted in regulating the passive movements of sodium and chloride ions, as calcium is known to alter the permeability of gill membranes to these ions (Eddy 1975). The variable responses of hematocrit to salinity observed in the present study have been reported previously

(Leatherland and McKeown 1974; Zeitoun et al. 1974; Bath and Eddy 1979), and may be explained by differential changes in red cell and plasma volumes which appear to be species-specific.

The results of the present laboratory experiment indicated that salinity had a negative effect on the growth of all three species, particularly at salinities above the isotonic level. These findings are in agreement with Shaw et al. (1975), Clarke et al. (1981), McKay and Gjerde (1985), and McCormick et al. (1989a), who also found that isotonic salinity was not the point of maximal growth in juvenile salmonids. The works of Canagaratnam (1959) and Otto (1971) with coho salmon pre-smolts are widely quoted in the literature as studies which support the notion of an optimum growth response in an isotonic environment. Subsequent experiments, however, have revealed conflicting results. Canagaratnam (1959) used small aquaria with static water to study the growth response of sockeye and chum as well as coho salmon fry to different salinities. This resulted in low overall specific growth rates (1.25 % per day for coho fry in fresh water), and very high mortalities in the sockeye and chum fry tests. It is possible, therefore, that confinement stress affected the study results. In the experiments conducted by Otto (1971), growth rates of coho salmon fry were actually higher in fresh water from June to September and increased in 10 ppt salinity only from October to February. This shift in salinity for optimal growth during the fall is consistent with other studies on the behaviour and physiology of coho salmon pre-smolts. Otto and McInerney (1970) found that the preferred salinity for the same stock of coho salmon fry changed during October and November to 10 ppt, corresponding with the growth results. Conte et al. (1966) also found that juvenile coho salmon were able to tolerate full-strength seawater in October, six months prior to the usual seawater migration period. Lasserre et al. (1978) further showed that the activity of gill $\text{Na}^+\text{-K}^+$ ATPase, the enzyme located

in chloride cells which is indicative of ion excretion capacity (see review by Folmar and Dickhoff 1980), increased during mid-October in coho salmon underyearlings in fresh water. This resulted in improved growth and survival of the fish when they were transferred to seawater netpens during autumn compared with other times of the year (Harache et al. 1980). These results are all consistent with the observation that coho salmon are sometimes found in estuaries as underyearlings (Tschaplinski 1987), and have therefore evolved physiological mechanisms to optimize growth in changing environments. A similar rise in gill $\text{Na}^+\text{-K}^+$ ATPase activity and estuarine residence during autumn has also been reported for Atlantic salmon parr (Langdon and Thorpe 1985; Cunjack and Chadwick 1989). Determinations of the optimal salinity for growth in salmonids must therefore consider the influences of species-specific differences, developmental stage, size, and season (Saunders and Henderson 1969b; McCormick and Naiman 1984; McCormick et al. 1989a).

The highest growth rates of juvenile salmonids in the present study were associated with fresh water, which is also the natural habitat for these species at this life stage. Growth of landlocked rainbow trout fry decreased 53% from fresh water to the hypertonic salinity of 18 ppt, while the growth of anadromous steelhead trout and chinook salmon fry declined only 16% and 12%, respectively, over a similar range. These data indicate that even at the fry stage, anadromous steelhead trout and chinook salmon adapt and grow better at hypertonic salinities than the freshwater resident rainbow trout. Although not examined in the present study, it is possible that the steelhead trout and chinook salmon fry possessed a greater number of, or more active, ion-excreting chloride cells on the gill, which are known to be poorly developed and fewer in number in strictly freshwater fish than in anadromous or marine species (Folmar and Dickhoff 1980; Burton and Idler 1984).

Contrary to our initial hypothesis, we found that oxygen consumption rates for all three species of salmonids were not lowest in isotonic salinity; they were lowest in fresh water. The higher metabolic rates at higher water salinities apparently reflected a significant energetic cost, as growth rates declined with increasing salinity, and correlated very well with the changes in oxygen consumption. Assuming that these increased energy demands were related solely to ionic and osmotic regulation, the metabolic rate data suggested that the energetic cost of ion-osmotic regulation for fry of these salmonid species was lowest in fresh water and increased to 12-18% in isotonic salinity.

It is difficult to reconcile the differences in the metabolic responses to changes in salinity found in the present study with those previously reported. In other studies, total metabolic rates measured in acclimated fish have either increased, decreased, or remained unchanged as salinity increased. From the recent literature (Table 9) we have compiled and categorized five patterns of metabolic responses of teleost fish to altered salinities, some of which are similar to those proposed by Nordlie (1978). These patterns include: I) metabolic rate does not change over a wide range of salinities, II) metabolic rate is minimum in isotonic salinity and increases at lower and higher salinities, III) metabolic rates are minimum in fresh water and increase at higher salinities, IV) metabolic rates are highest in fresh water and decrease to isotonic; higher salinities are not tolerated, and V) metabolic rates are lowest in seawater and increase in lower salinities.

Life habits will, to a certain extent, determine the type of metabolic response to changes in salinity (Table 9). Euryhaline fish which move freely through waters of varying salinity generally demonstrate a type I response, i.e. metabolic rates do not change. Euryhaline forms which are found in fresh water as adults or in estuaries as juveniles tend to illustrate a type II

Table 9. Metabolic response patterns of teleost fish to changes in environmental salinity, compiled from recent literature.

Metabolic response	Species and life stage	Habits ¹	References
I. No significant change in metabolic rate over a wide salinity range	<u>Oncorhynchus tshawytscha</u> (chinook salmon)-sub adult	Anad-Est/Mar	Bullivant (1961)
	<u>Kuhlia sandvicensis</u> (aholehole)-adult	Eur-Mar	Muir and Niimi (1972)
	<u>Aphanius dispar</u> (cyprinodont)-juv/adult	Eur-Mar	Skadhauge and Lotan (1974)
	<u>Gillichthys mirabilis</u> (gobie)-adult	Eur-Mar	Courtois (1976)
	<u>Ambassis interrupta</u> adult	Eur-Est	Nordlie (1978)
	<u>Fundulus heteroclitus</u> (mummichog)-adult	Eur-Bra	Nead and Buttner (1987)
II. Metabolic rate is minimum in isotonic salinity and increases at lower and higher salinities	<u>Girella nigricans</u> (percoid)-adult	Eur-Mar	Fang (1982)
	<u>Oncorhynchus mykiss</u> (rainbow trout)-adult	Eur-Fw	Rao (1968)
	<u>Tilapia nilotica</u> (tilapia)-adult	Eur-Fw	Farmer and Beamish (1969)
	<u>Brevoortia tyrannus</u> (Atlantic menhaden)-juv.	Eur-Est	Hettler (1976)
	<u>Ambassis interrupta</u> juvenile	Eur-Est	Nordlie (1978)
	<u>Mugil curema</u> (white mullet)-juvenile	Eur-Est	Fanta-Feofiloff et al. (1986)
	<u>Oreochromis niloticus</u> (tilapia)-juvenile	Eur-Est	DeSilva et al. (1986)
	<u>Oreochromis mossambicus</u> (tilapia)-adult	Eur-Fw	Febry and Lutz (1987)

Table 9 (Cont'd).

Metabolic response	Species and life stage	Habits ¹	References
III. Metabolic rate is lowest in fresh water and increases with salinity	<u>Platichthys stellatus</u> (starry flounder)-juv/adult	Eur-Est	Hickman (1959)
	<u>Mugil cephalus</u> (striped mullet)-adult	Eur-Est/Mar	Nordlie and Leffler (1975)
	<u>Cyprinodon salinus</u> (pupfish)-adult	Eur-Bra	Stuenkel and Hillyard (1981)
	<u>Oncorhynchus kisutch</u> (coho salmon)-juvenile	Anad-Fw	Nagibina (1983)
	<u>Leiostomus xanthurus</u> (spot)-juvenile	Eur-Est	Moser and Hettler (1989)
	<u>Oncorhynchus mykiss</u> (rainbow & steelhead trout)-juveniles <u>Oncorhynchus tshawytscha</u> (chinook salmon)-juveniles	Anad-Fw	This study
IV. Metabolic rate is highest in fresh water and decreases to isotonic; higher salinities are not tolerated	<u>Ctenopharyngodon idella</u> (grass carp)-juvenile	Sten-Fw	Maccina et al. (1980)
	<u>Ictalurus nebulosus</u> & <u>I. punctatus</u> (catfish)-adult	Sten-Fw	Furspan et al. (1984)
V. Metabolic rate is minimum in seawater and increases in lower salinities	<u>Mugil cephalus</u> (striped mullet)-adult	Eur-Mar	Marais (1978)
	<u>Mugil macrolepis</u> (mullet)-adult	Eur-Mar	Mathew (1978)
	<u>Cyprinodon variegatus</u> (sheepshead minnow)-juvenile/adult	Eur-Bra	Barton and Barton (1987)

1. Anad - anadromous Bra - brackish
 Eur - euryhaline Est - estuarine
 Sten - stenohaline Fw - fresh water
 Mar - marine

metabolic response (minimum in isotonic salinity), whereas fish which are found as juveniles in fresh water or in estuaries as adults display a type III response pattern (metabolism increases with salinity). Stenohaline freshwater fish show a type IV response, which probably reflects a depression of metabolic rate due to stress. Finally, fish species which are found primarily in marine habitats illustrate a type V response, which is in accordance with Prosser's (1973) general hypothesis that for euryhaline marine species, metabolic rates increase at reduced salinities. It is also apparent from this summary of metabolic patterns that juveniles frequently respond differently than do adults of the same species (e.g. Oncorhynchus tshawytscha, Ambassis interrupta) and, in general, are more influenced by salinity variations than are the larger adults, a point also recognized by Nordlie (1978). It is evident from the studies cited in Table 9, therefore, that the lowest metabolic rates in response to salinity are associated with the environments to which the species are most commonly found, and presumably most physiologically adapted, for a particular life stage. The strong correlation between optimum growth and metabolic rates in response to salinity observed in the present study supports this hypothesis.

Given that the mechanisms of ion-osmotic regulation are similar among fish species (Evans 1984), the variety of metabolic responses to different salinities depending upon species, habits and life stage suggest that these responses are being influenced by physiological processes in addition to the energy required for ion-osmotic regulation (Febry and Lutz 1987). McCormick et al. (1989b), for example, have recently reported that the changes in the metabolic capacity of isolated gill and kidney tissues of Atlantic salmon in different salinities did not account for the magnitude of changes seen in whole body metabolism. During acclimation to different salinities, hormonal changes occur in fish which, in addition to their role in ionic regulation, may also have

secondary effects on metabolism not directly related to ion exchange between blood and water. Cortisol, for example, is known to promote branchial ion excretion by stimulating chloride cell proliferation, differentiation and secretory activity (Foskett et al. 1983; McCormick et al. 1989c; Madsen 1990a,b), and shows peaks during the parr-smolt transformation of juvenile salmonids in fresh water (Specker and Schreck 1982). This hormone has also shown long-term increases in juvenile salmonids after entry into seawater (Redding et al. 1984; Young et al. 1989; Avella et al. 1990) and has been associated with increases in metabolic rate (Chan and Woo 1978; Barton and Schreck 1987). Therefore, the increase in metabolic rates observed for juvenile salmonids in the present study may also have been related to a more general metabolic response of the test species to increasing salinity, as opposed to a strictly energetic cost of ion-osmoregulation. The confounding effects of salinity changes on overall metabolism make it difficult to distinguish metabolic costs exclusively associated with ion-osmotic regulation. The estimates provided previously, therefore, probably overestimate the energetic cost of ion-osmotic regulation. More detailed and quantitative descriptions of the physiological responses of fish to changes in water salinity that account for differences in species, life habits and stages are necessary to accurately assess the energetic cost of ionic regulation. For example, comparison of gill oxygen consumption to total body metabolism in vivo may assist in separating energetic activities associated with ionic regulation from other metabolically active processes involved with adjustments to alterations in water salinity.

Acclimation to dilute salinities did not improve the osmoregulatory performance of juvenile steelhead trout and chinook salmon challenged with an acute (24 h) exposure to full-strength seawater. Similar results were obtained by Clarke et al. (1981) for coho and chinook salmon reared at 10°C. The use of this technique in a hatchery prior to seawater transfer is therefore

probably not necessary, once the fish have reached the critical size and developmental state for smolting. Freshwater rearing temperature, fish size, and time of transfer have been shown to be the most important factors influencing adaptability and growth in seawater for juvenile fall chinook salmon (Clarke and Shelbourn 1985). The absence of mortalities during the 24 h seawater challenge tests were unexpected for the 7-9 g steelhead trout, whose critical size for seawater adaptation is generally accepted to be about 35 g (Conte and Wagner 1965; Clarke 1982). A possible explanation for these findings may be that a constant spring photoperiod (14 h) was used throughout the steelhead experiment, and this has been shown to accelerate seawater adaptation in juvenile steelhead trout (Johnsson and Clarke 1988).

In summary, growth and metabolic rates of rainbow trout, steelhead trout and chinook salmon fry were optimum in fresh water, their natural habitat at this life stage. Isotonic salinity did not provide metabolic or growth advantages in the present study, despite the hypothesis that it would provide the lowest energetic demands for ion-osmotic regulation. Comparison to other studies indicated that optimal salinities for growth and metabolic rates were influenced by species, life stage and season. Although the oxygen consumption data suggested that the energetic cost of ion-osmotic regulation increased with salinity, attempts to quantify this cost were probably affected by other metabolic processes which respond to changes in salinity.

CONCLUDING REMARKS

The present study indicates that there are ontogenetic and phylogenetic differences in ion-osmotic regulation processes of Pacific salmonids. Since embryos and newly hatched alevins do not have fully developed organs for ion-osmotic regulation (i.e. gills, kidney and intestine), they may therefore rely on passive mechanisms such as low membrane permeabilities to maintain ionic and osmotic balance. As the fish develops into the juvenile stage, active mechanisms for achieving ionic and osmotic homeostasis become functional to compensate for an increase in permeable membranes, and the fish is able to tolerate higher salinities. During the parr-smolt transformation, these ion-osmotic regulatory capabilities are enhanced (e.g. chloride cell recruitment) enabling the fish to thrive in full-strength seawater. The results of this study also showed species-specific differences in salinity tolerance, with anadromous salmonids being more tolerant of high salinities than non-anadromous forms, and salmon having a higher salinity tolerance than equivalent stages of trout.

There are several aspects of this study which require further investigation. Embryos and alevins were not examined for the presence of chloride cells in this study and their response to salinity has only been examined in two species of salmonids to date (rainbow trout and coho salmon). A comparative search for chloride cells in embryos of all species of Pacific salmonids would be useful, particularly in pink and chum salmon which have the ability to ion regulate at a very small size (Weisbart 1968). Similarly, it was beyond the scope of this thesis to sample the salmonid fry in the different rearing salinities for chloride cells. Given the interactive roles of cortisol and chloride cells in ion regulation (Foskett et al. 1983) and the inferred effect of cortisol on metabolic rate in this study, future experiments of this type should make simultaneous

measurements of chloride cell abundance and osmoregulation hormones such as cortisol and prolactin. It would also be interesting to repeat the growth experiments using estuarine smolts of several salmonid species, which would presumably show higher growth rates in the isotonic salinity compared with the freshwater-dwelling fry used in this study. The role of calcium in decreasing membrane permeabilities and ion fluxes was mentioned only briefly, and it would be useful to determine whether altering the calcium content of fresh water and seawater would significantly affect parameters such as growth and metabolism. Finally, there have been suggestions in the literature that rearing salinity may influence food intake, protein absorption and conversion efficiencies in rainbow trout (MacLeod 1977). Other studies, however, have been unable to show differences in nitrogen assimilation rates or conversion efficiencies with changes in salinity (Smith and Thorpe 1976; McCormick et al. 1989a). No efforts were made in the present study to examine these factors, partly due to the difficulties of measuring these parameters in small fish (e.g. small food particle size, difficult to collect fecal material). The effects of salinity on the nutritional physiology of salmonids is therefore another area worthy of further study.

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APPENDICES

Appendix I

Sampling Summaries for Salmonid Fry Growth Trials

Species: Rainbow trout
Date: November 5, 1988
Trial Day 0

Tank No.	1	2	3
Mean Length (cm)	4.5	4.8	4.8
SE	0.1	0.1	0.1
N	50	50	50
Mean Weight (g)	1.04	1.19	1.21
SE	0.06	0.06	0.08
N	50	50	50
Condition Factor (K)	1.07	1.06	1.03
SE	0.02	0.01	0.01
N	50	50	50

Species: Rainbow trout
 Date: December 10, 1988
 Trial Day 35

Tank No.	1	2	3
Mean Length (cm)	5.4	6.7	6.8
SE	0.1	0.1	0.2
N	47	50	48
Mean Weight (g)	1.80	3.68	3.91
SE	0.12	0.22	0.28
N	47	50	48
Condition Factor (K)	1.07	1.17	1.13
SE	0.01	0.01	0.01
N	47	50	48
SGR (% wt per day) Day 0 - 35	1.57	3.23	3.35
Mean Hematocrit (% RBC)	36.7	35.0	32.1
SE	0.9	1.0	1.3
N	10	10	10
Mean Plasma Na ⁺ (meq·L ⁻¹)	135.6	140.2	149.7
SE	3.6	6.1	2.1
N	5	6	7
Mean Plasma Cl ⁻ (meq·L ⁻¹)	164.5	140.2	152.8
SE	0.5	3.9	2.9
N	2	6	6
<u>Water Quality : Day 0 - 35</u>			
Salinity (ppt)	17.5	8.7	0
SE (n)	0.2 (26)	0.2 (26)	0 (26)
Temp. (°C)	10.9	10.7	10.8
SE (n)	0.1 (36)	0.1 (36)	0.1 (36)
D.O. (mg·L ⁻¹)	9.6	10.2	10.7
SE (n)	0.1 (36)	0.1 (36)	0.1 (36)
pH	7.2	6.9	6.3
SE (n)	0.04 (36)	0.03 (36)	0.03 (36)

Species: Steelhead trout
 Date: June 17, 1989
 Trial Day 0

Tank No.	1	2	3	4	5
Mean Length (cm)	3.6	3.6	3.7	3.6	3.8
SE	0.02	0.02	0.02	0.02	0.03
N	80	80	80	80	80
Mean Weight (g)	0.40	0.39	0.42	0.42	0.46
SE	0.01	0.01	0.01	0.01	0.01
N	80	80	80	80	78
Condition Factor (K)	0.83	0.82	0.84	0.85	0.87
SE	0.01	0.01	0.01	0.01	0.01
N	80	80	80	80	78

Species: Steelhead trout
Date: July 8, 1989
Trial Day 21

Tank No.	1	2	3	4	5
Mean Length (cm)	4.2	4.0	4.2	4.3	4.1
SE	0.1	0.1	0.1	0.1	0.1
N	10	10	10	10	10
Mean Weight (g)	0.71	0.65	0.73	0.81	0.67
SE	0.03	0.03	0.03	0.07	0.07
N	10	10	10	10	10
Condition Factor (K)	0.98	0.98	0.96	0.98	0.92
SE	0.01	0.02	0.02	0.02	0.03
N	10	10	10	10	10
SGR (% wt per day) Day 0 - 21	2.73	2.43	2.63	3.13	1.79
Mean Hematocrit (% RBC)	37.2	39.1	38.1	37.6	37.8
SE	1.7	1.2	1.5	0.9	0.9
N	9	8	8	9	9
Mean Plasma Na ⁺ (meq·L ⁻¹)	120	103	123	120	90
SE	-	-	-	-	-
N	1	1	1	1	1
Mean Plasma Cl ⁻ (meq·L ⁻¹)	110	106	90	128	130
SE	-	-	-	-	-
N	1	1	1	1	1
<u>Water Quality : Day 0 - 20 (Acclimation period)</u>					
Salinity (ppt)	8.2	7.5	5.7	3.3	0
SE (n)	1.2 (21)	1.0 (21)	0.7 (21)	0.3 (21)	0 (21)
Temp. (°C)	14.0	14.1	13.9	13.6	13.7
SE (n)	0.2 (21)	0.1 (21)	0.1 (21)	0.1 (21)	0.1 (21)
D.O. (mg·L ⁻¹)	9.7	9.8	9.9	10.1	10.3
SE (n)	0.1 (21)	0.1 (21)	0.1 (21)	0.04 (21)	0.02 (21)
pH	7.1	7.1	7.0	6.9	6.1
SE (n)	0.1 (21)	0.1 (21)	0.1 (21)	0.1 (21)	0.02 (21)

Species: Steelhead trout
Date: July 29, 1989
Trial Day 42

Tank No.	1	2	3	4	5
Mean Length (cm)	5.0	5.0	5.1	4.9	5.3
SE	0.1	0.1	0.1	0.1	0.1
N	10	10	10	10	10
Mean Weight (g)	1.32	1.32	1.37	1.19	1.47
SE	0.06	0.05	0.08	0.07	0.06
N	10	10	10	10	10
Condition Factor (K)	1.05	1.03	1.01	1.03	1.00
SE	0.02	0.01	0.01	0.02	0.02
N	10	10	10	10	10
SGR (% wt per day)					
Day 21 - 42	2.95	3.37	3.00	1.83	3.74
Mean Hematocrit (% RBC)	37.4	38.2	37.5	35.3	35.9
SE	1.4	0.6	1.4	1.5	0.9
N	10	10	10	10	10
Mean Plasma Na ⁺ (meq·L ⁻¹)	147.3	136.7	132.7	122.0	129.0
SE	2.9	6.0	7.7	7.0	5.6
N	3	3	3	3	3
Mean Plasma Cl ⁻ (meq·L ⁻¹)	141.3	114.3	117.7	114.3	127.7
SE	2.9	11.9	6.2	9.0	14.4
N	3	3	3	3	3
<u>Water Quality : Day 21 -42</u>					
Ave. Salinity (ppt)	15.5	11.8	7.8	3.9	0
Ave. Temp. (°C)	14.4	14.3	14.2	14.1	14.1

Species: Steelhead trout
Date: August 19, 1989
Trial Day 63

Tank No.	1	2	3	4	5
Mean Length (cm)	5.8	5.9	6.1	5.7	6.4
SE	0.1	0.1	0.1	0.1	0.1
N	20	20	20	20	20
Mean Weight (g)	2.18	2.28	2.51	2.11	2.88
SE	0.14	0.10	0.10	0.12	0.19
N	20	20	20	20	20
Condition Factor (K)	1.11	1.11	1.10	1.10	1.07
SE	0.01	0.01	0.01	0.02	0.01
N					
SGR (% wt per day)					
Day 42 - 63	2.39	2.60	2.88	2.73	3.20
Mean Hematocrit (% RBC)	40.0	37.8	35.6	37.2	35.1
SE	0.6	0.8	0.8	1.5	0.8
N	10	10	10	10	10
Mean Plasma Na ⁺ (meq·L ⁻¹)	157.5	148.5	143.5	148.0	144.8
SE	5.3	2.2	9.8	14.2	5.0
N	4	4	4	4	4
Mean Plasma Cl ⁻ (meq·L ⁻¹)	147.0	153.8	128.0	126.5	135.3
SE	5.8	5.8	8.7	10.2	3.2
N	4	4	4	4	4
<u>Water Quality : Day 42 -63</u>					
Ave. Salinity (ppt)	16.0	11.9	8.0	4.0	0
Ave. Temp. (°C)	14.2	14.1	14.2	14.1	14.3

Species: Steelhead trout
Date: September 9, 1989
Trial Day 84

Tank No.	1	2	3	4	5
Mean Length (cm)	6.9	7.3	7.3	7.2	7.7
SE	0.1	0.1	0.1	0.1	0.1
N	47	47	48	45	40
Mean Weight (g)	4.01	4.62	4.76	4.36	5.26
SE	0.20	0.17	0.14	0.15	0.19
N	47	47	48	45	40
Condition Factor (K)	1.17	1.17	1.19	1.15	1.12
SE	0.01	0.01	0.01	0.01	0.01
N	47	47	48	45	40
SGR (% wt per day)					
Day 63 - 84	2.90	3.36	3.05	3.46	2.87
Day 21 - 84	2.75	3.11	2.98	2.67	3.27
Mean Hematocrit (% RBC)	39.4	37.2	37.1	38.8	36.5
SE	0.9	0.9	0.9	1.4	1.1
N	10	10	10	10	10
Mean Plasma Na ⁺ (meq·L ⁻¹)	154.4	154.8	145.6	146.2	140.0
SE	1.9	2.7	3.0	1.5	1.3
N	5	5	5	5	5
Mean Plasma Cl ⁻ (meq·L ⁻¹)	119.6	117.0	121.4	118.4	123.4
SE	2.4	1.2	1.5	1.8	1.2
N	5	5	5	5	5

Water Quality : Day 21 - 84

Salinity (ppt)	15.9	11.8	7.9	4.0	0
SE (n)	0.1 (64)	0.1 (64)	0.1 (64)	0.1 (64)	0 (64)
Temp. (°C)	14.2	14.1	14.1	14.1	14.1
SE (n)	0.1 (64)	0.1 (64)	0.1 (64)	0.1 (64)	0.1 (64)
D.O. (mg·L ⁻¹)	8.8	9.1	9.4	9.6	9.8
SE (n)	0.03 (64)	0.04 (64)	0.04 (64)	0.04 (64)	0.04 (64)
pH	7.5	7.4	7.3	7.1	6.0
SE (n)	0.01 (64)	0.01 (64)	0.01 (64)	0.01 (64)	0.01 (64)

Species: Chinook salmon
 Date: February 27, 1990
 Trial Day 0

Tank No.	1	2	3	4	5
Mean Length (cm)	4.7	4.7	4.7	4.7	4.7
SE	0.02	0.02	0.02	0.02	0.02
N	100	100	100	100	100
Mean Weight (g)	1.01	0.96	0.93	0.95	0.96
SE	0.02	0.01	0.01	0.01	0.01
N	100	100	100	100	100
Condition Factor (K)	0.97	0.93	0.90	0.90	0.90
SE	0.01	0.01	0.01	0.01	0.01
N	100	100	100	100	100

Species: Chinook salmon
Date: March 13, 1990
Trial Day 14

Tank No.	1	2	3	4	5
Mean Length (cm)	5.2	5.1	5.2	5.3	5.3
SE	0.1	0.1	0.1	0.1	0.1
N	20	20	20	20	20
Mean Weight (g)	1.39	1.29	1.47	1.48	1.53
SE	0.05	0.06	0.07	0.07	0.05
N	20	20	20	20	20
Condition Factor (K)	0.98	0.97	1.01	1.00	1.01
SE	0.01	0.01	0.02	0.01	0.01
N	20	20	20	20	20
SGR (% wt per day) Day 0 - 14	2.28	2.11	3.27	3.17	3.33
Moisture Content (%)	80.8	81.5	80.9	80.7	80.5
SE	0.2	0.1	0.2	0.1	0.2
N	10	10	10	10	10

Water Quality : Day 0 - 14 (Acclimation period)

Salinity (ppt)	13.3	11.2	7.9	4.4	0
SE (n)	2.4 (14)	1.8 (14)	0.9 (14)	0.4 (14)	0 (14)
Temp. (°C)	10.7	10.5	10.5	10.0	10.2
SE (n)	0.2 (14)	0.3 (14)	0.2 (14)	0.2 (14)	0.2 (14)
D.O. (mg·L ⁻¹)	9.9	10.3	10.5	10.8	11.1
SE (n)	0.2 (14)	0.2 (14)	0.1 (14)	0.1 (14)	0.1 (14)
pH	7.2	7.2	7.1	7.0	6.0
SE (n)	0.1 (14)	0.1 (14)	0.1 (14)	0.1 (14)	0.1 (14)

Species: Chinook salmon
Date: April 3, 1990
Trial Day 35

Tank No.	1	2	3	4	5
Mean Length (cm)	5.5	5.7	6.4	6.4	7.0
SE	0.1	0.1	0.1	0.1	0.1
N	20	20	20	20	20
Mean Weight (g)	1.95	2.03	3.00	2.94	3.91
SE	0.08	0.09	0.22	0.22	0.17
N	20	20	20	20	20
Condition Factor (K)	1.14	1.06	1.10	1.08	1.12
SE	0.01	0.01	0.02	0.02	0.02
N	20	20	20	20	20
SGR (% wt per day)					
Day 14 - 35	1.61	2.16	3.40	3.27	4.47
Moisture Content (%)	83.4	82.1	79.5	79.8	78.8
SE	0.2	0.2	0.3	0.3	0.3
N	10	10	10	10	10
<u>Water Quality : Day 15 - 35</u>					
Ave. Salinity (ppt)	28.1	19.4	10.0	5.0	0
Ave. Temp. (°C)	11.3	10.9	11.3	10.8	11.1

Species: Chinook salmon
Date: April 24, 1990
Trial Day 56

Tank No.	1	2	3	4	5
Mean Length (cm)	6.0	6.7	7.2	7.4	8.0
SE	0.1	0.1	0.1	0.2	0.1
N	20	20	20	20	20
Mean Weight (g)	2.60	3.27	4.11	4.52	5.28
SE	0.17	0.14	0.32	0.38	0.25
N	20	20	20	20	20
Condition Factor (K)	1.16	1.08	1.08	1.07	1.03
SE	0.01	0.01	0.01	0.01	0.01
N	20	20	20	20	20
SGR (% wt per day) Day 35 - 56	1.37	2.27	1.50	2.05	1.43
Mean Hematocrit (% RBC)	33.6	34.7	37.9	37.9	42.1
SE	0.6	0.9	1.1	1.3	1.3
N	10	10	10	10	10
Mean Plasma Na ⁺ (meq·L ⁻¹)	144.6	149.6	148.1	148.6	140.3
SE	4.4	3.2	1.9	3.7	1.1
N	5	5	7	7	8
Mean Plasma Cl ⁻ (meq·L ⁻¹)	128.3	124.2	127.3	121.7	127.4
SE	3.1	3.3	3.9	3.2	1.5
N	4	5	7	7	8
Moisture Content (%)	82.0	79.6	79.0	78.8	78.2
SE	0.5	0.3	0.3	0.2	0.2
N	10	10	10	10	10
<u>Water Quality : Day 36 - 56</u>					
Ave. Salinity (ppt)	28.3	19.6	10.1	5.0	0
Ave. Temp. (°C)	11.7	11.6	11.8	11.5	11.6

Species: Chinook salmon	Date: May 15, 1990			Trial Day 77	
Tank No.	1	2	3	4	5
Mean Length (cm)	7.2	8.0	8.3	8.6	9.1
SE (n)	0.1 (36)	0.1 (59)	0.1 (60)	0.1 (60)	0.1 (60)
Mean Weight (g)	4.42	5.50	6.33	6.90	7.95
SE (n)	0.24 (36)	0.16 (59)	0.24 (60)	0.25 (60)	0.24 (60)
Condition Factor (K)	1.15	1.07	1.06	1.06	1.05
SE (n)	0.01 (36)	0.01 (59)	0.01 (60)	0.01 (60)	0.01 (60)
SGR (% wt per day)					
Day 57 - 77	2.53	2.48	2.06	2.01	1.95
Day 14 - 77	1.84	2.30	2.32	2.44	2.62
Mean Hematocrit (% RBC)	41.4	44.2	44.4	44.7	47.2
SE	1.2	1.0	1.2	1.0	1.1
N	10	10	10	10	10
Mean Plasma Na ⁺ (meq·L ⁻¹)	155.6	157.3	161.3	162.0	157.9
SE	3.5	1.3	1.6	0.7	2.5
N	10	10	10	10	10
Mean Plasma Cl ⁻ (meq·L ⁻¹)	128.3	132.5	130.2	126.2	121.5
SE	1.9	0.9	2.1	2.4	2.4
N	9	10	10	9	10
Moisture Content (%)	81.4	79.1	78.6	78.1	78.0
SE	0.4	0.2	0.2	0.2	0.2
N	10	10	10	10	10
<u>Water Quality : Day 15 - 77</u>					
Salinity (ppt)	28.2	19.7	10.1	5.0	0
SE (n)	0.1 (63)	0.1 (63)	0.1 (63)	0.03 (63)	0 (63)
Temp. (°C)	11.6	11.5	11.7	11.4	11.5
SE (n)	0.1 (63)	0.1 (63)	0.1 (63)	0.1 (63)	0.1 (63)
D.O. (mg·L ⁻¹)	8.6	9.2	9.7	10.1	10.3
SE (n)	0.02 (63)	0.03 (63)	0.05 (63)	0.05 (63)	0.05 (63)
pH	7.6	7.5	7.3	7.1	6.0
SE (n)	0.01 (63)	0.01 (63)	0.01 (63)	0.01 (63)	0.01 (63)

Appendix II

Metabolic Rates of Juvenile Salmonids in Different Salinities

Summary of Metabolic Rates Determined for Juvenile Salmonids in Various Salinities

Species	Salinity (ppt)	N	Metabolic Rate \pm SE (mg O ₂ ·kg·h ⁻¹)
Rainbow trout	0	3	206.7 \pm 3.4
	9	3	243.7 \pm 6.1
	18	3	263.9 \pm 7.1
Steelhead trout	0	6	270.0 \pm 7.6
	4	6	285.0 \pm 18.2
	8	6	306.2 \pm 6.7
	12	6	298.4 \pm 9.1
	16	6	337.1 \pm 12.8
Chinook salmon	0	6	266.4 \pm 25.9
	5	6	299.6 \pm 15.6
	10	6	311.4 \pm 24.2
	20	6	355.3 \pm 23.3
	28	6	351.1 \pm 15.1

Appendix III

Data Summaries of 24 Hour Seawater Challenge Tests with Juvenile Steelhead Trout and Chinook Salmon

Species: Steelhead trout
 Date: October 3 - 4, 1989

Tank No.	1	2	3	4	5
Treatment Salinity (ppt)	16	12	8	4	0
Mean Length (cm)	8.6	8.9	8.9	8.8	9.7
SE	0.1	0.1	0.1	0.1	0.1
N	10	10	10	10	10
Mean Weight (g)	6.72	7.28	7.18	6.79	9.46
SE	0.23	0.30	0.05	0.27	0.39
N	10	10	10	10	10
Condition Factor (K)	1.05	1.04	1.04	1.00	1.02
SE	0.01	0.01	0.01	0.01	0.01
N	10	10	10	10	10
Mean Hematocrit (% RBC)	44.9	40.9	42.8	40.5	38.0
SE	1.2	1.4	1.6	1.0	1.1
N	10	10	10	10	10
Mean Plasma Na ⁺ (meq·L ⁻¹)	166.9	161.2	159.5	177.4	164.4
SE	3.6	2.8	1.7	3.6	1.9
N	10	10	10	10	10

Water Quality:

Salinity (ppt)	28	28	28	28	28
Temp. (°C)	13.5	13.5	13.5	13.5	13.5
D.O. (mg·L ⁻¹)	8.6	8.6	8.6	8.6	8.6
pH	7.8	7.8	7.8	7.8	7.7

Species: Chinook salmon
Date: June 2 - 3, 1990

Tank No.	1	2	3	4
Treatment Salinity (ppt)	20	10	5	0
Mean Length (cm)	9.3	9.5	9.5	10.0
SE	0.1	0.1	0.1	0.1
N	12	12	12	12
Mean Weight (g)	8.23	9.02	8.88	10.03
SE	0.32	0.44	0.34	0.31
N	12	12	12	12
Condition Factor (K)	1.03	1.05	1.03	1.00
SE	0.01	0.02	0.01	0.01
N	12	12	12	12
Mean Hematocrit (% RBC)	41.5	40.6	43.2	43.4
SE	1.0	0.8	0.8	0.9
N	12	12	12	12
Mean Plasma Na ⁺ (meq·L ⁻¹)	160.8	160.3	155.4	158.5
SE	1.5	1.1	1.0	1.5
N	12	12	12	12

Water Quality:

Salinity (ppt)	29	29	29	29
Temp. (°C)	12.5	12.5	12.5	12.5
D.O. (mg·L ⁻¹)	8.6	8.6	8.6	8.6
pH	7.7	7.7	7.7	7.7