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Title: EFFECTS OF SALINITY ON THE TOLERANCE, GROWTH, FOOD CONVERSION AND OXYGEN CONSUMPTION OF THE KELP GREENLINGS (*Hexagrammos decagrammus*)

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Richard A. Tubb

The osmoregulatory capabilities and tolerance of Kelp greenling, *Hexagrammos decagrammus*, were studied in a series of seven salinities ranging from fresh water to concentrated sea water of 50 ppt. Results indicated that they were tolerant over a wide range of salinities but were incapable of surviving below 5 ppt and above 45 ppt. In moderate salinities ranging from 7.5 to 45 ppt, the fish required approximately 48 hours for their plasma osmolarity to stabilize. Kelp greenling were hyperosmotic at 7.5 ppt but at higher salinities of 15 ppt and above, became hyposmotic, capable of maintaining the plasma osmolarity about 360 mOsm/Kg in sea water. Acclimation to low salinity (7.5 ppt) for more than two months had no effect on their survival or tolerance to challenges to sea water.

The effects of different salinities (7.5, 15, and 30 ppt) on growth, food conversion and oxygen consumption were studied. Fish reared in 15 ppt had the highest relative growth rate and lowest food conversion. There were no significant differences in relative growth rate and food conversion ratios between fish reared at 7.5 and 30 ppt.

Kelp greenling at 7.5 and 30 ppt consumed more oxygen than those at 15 ppt. The metabolic costs of maintaining water balance and ion exchange may account for the increased oxygen consumption.

EFFECTS OF SALINITY ON THE TOLERANCE, GROWTH, FOOD CONVERSION AND OXYGEN CONSUMPTION OF KELP GREENLING

(Hexagrammos decagrammus)

by

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Typed by Pamela A. Rogers for Camille Reda El Zahr

DEDICATION

This work is dedicated to my parents, Mr. Reda A. and Ms. Souad M. El Zahr, whose support and encouragement throughout my education are very much appreciated.

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(*Hexagrammos decagrammus)*

CHAPTER I.

INTRODUCTION

The success of any animal species ultimately depends upon its ability to obtain sufficient energy and materials to grow, reach maturity and to reproduce. Although readily observed and easily measured, growth is one of the more complex activities of the organism. It represents the net outcome of a series of behavioral and physiological processes that begin with food intake and terminate in tissue elaboration. The energy and materials that an animal may use for growth are available only after all metabolic costs are satisfied. Energy must be available for maintaining the vital processes of the body, and for the breakdown of food materials as well as for synthesis. Not all the food consumed by an animal is converted into useful energy. Some is lost in the form of nitrogenous compounds, some is lost as feces and some is lost through heat increment.

Within the genetic potential of any species to grow, many abiotic and biotic factors limit maximum growth (Brown 1957; Ivelev 1961). Among them, salinity is considered an important one.

Fish commonly inhabit waters varying in salinity. Some species of fish can tolerate a wide range of salinity and are termed euryhaline. Other are referred to as

stenohaline, surviving only within a narrow range of salinities, beyond which they are unable to control water and salt balance.

Salt concentration and plasma osmolarity in the blood of teleost fish is less than that of sea water. Thus in a marine environment water is lost at the gills and other body surfaces. Conversely, fresh water homeostasis is dependent on the elimination of absorbed water, because the solute concentration of the body fluids is greater than that of the environment (Black 1957). Whatever the mechanism, fish must expend energy for osmotic regulation. Thus salinity is classed as a masking factor constantly requiring some energy expenditure associated with active transport of ions to maintain homeostasis (Fry 1971).

Although a wide variety of species have been tested for the effect of salinity on growth, the results tend to be scattered. Fresh water species in general grow well within the isosmotic range, but maximum growth rate decreases above this concentration (Brett 1979). In long-term growth trials, channel catfish *(Ictalurus punctatus)* in 5 ppt grew almost as well as those in fresh water (Allen and Avault 1969). Canagaratham (1959) reported that euryhaline fish grew better in a saline medium, although they can thrive at different salinities. He also demonstrated that the percentage increase in body weight of Coho fry *(Oncorhynchus kisutch)*

was greater in saline water (16 ppt) than it was in fresh water. Throughout the pre-smolt period highest growth was observed at salinities of 5-10 ppt (Otto 1971). In grey mullets (*Mugil cephalus)* maximum growth was found to occur at 20 ppt (DeSilva and Perera 1976). It is thought that in the case of strictly marine species, growth rate tends to decrease at low salinities and this has been supported by studies done with *Paralicthys denatatis* (Peters 1971).

The species chosen for research was Kelp greenling, *Hexagrammos decagrammus*, which are distributed in the northeastern Pacific off the coast of North America from Alaska to southern California. It also occurs around the Aleutian Islands east of the Near Islands. Adults attain a length of 45-50 cm. The fish lives among stones, rocks and algae. Spawning takes place in October and November; their food consists of worms, crustaceans and small fishes. Adults exhibit sexual dimorphism. Males color is brownish olive with blue spots on head and anterior part of body. Females color is light brown with small reddish spots (Quast 1960, Gorbunova 1970, Hart 1973).

The flesh is very palatable, but is seldom brought to market by commercial fisheries. Little is known about the biology of this species. Preliminary rearing trials indicated that the fish can be easily reared in tanks under the artificial culture conditions;

furthermore, the fish grew well with satisfactory survival when fed formulated ration (Dr. J. Lannan, Oregon State University, personal communication).

The purposes of the study were to estimate some ecological requirements of Kelp greenling by determining their salinity tolerance and to measure the effects of salinity on growth and food conversion. Oxygen consumption was used as an index of metabolic rate to estimate the amount of energy spent by fish on osmoregulation.

CHAPTER II.

MATERIALS AND METHODS

Collection and Care of the Fish

The fish were collected by otter trawl from Yaquina Bay, Oregon, in 15 to 30 meters of water between buoys 7 and 9 (Figure 1). Collection of the kelp greenling started on September 27, 1983, and ended on October 12 of the same year, during which 35 trawls were made in six fishing trips (Table 1). Each trawl lasted approximately 10 minutes. On the average 17 kelp greenling were caught per trawl. The total number of live fish caught was 610, ranging in total length from 10.0 to 28.0 cm. The fish were transported live to the Oregon State University Hatfield Marine Science Center. The fish were selected according to size and distributed to three fiberglass circular tanks (175 cm in diameter with 2000 liters of sea water) supplied with running sea water from Yaquina Bay at a flow rate of 18-20 liters per minute. All tanks were well aerated. The fish were fed with chopped squid for the first two weeks, followed by a mixture of equal portions in weight of chopped squid and Oregon Moist Pellets for a week. Thereafter the fish were fed only Oregon Moist Pellets. Approximately 20% of the fish, mostly of the smaller size (<15 cm), did not accept any kind of food and died.

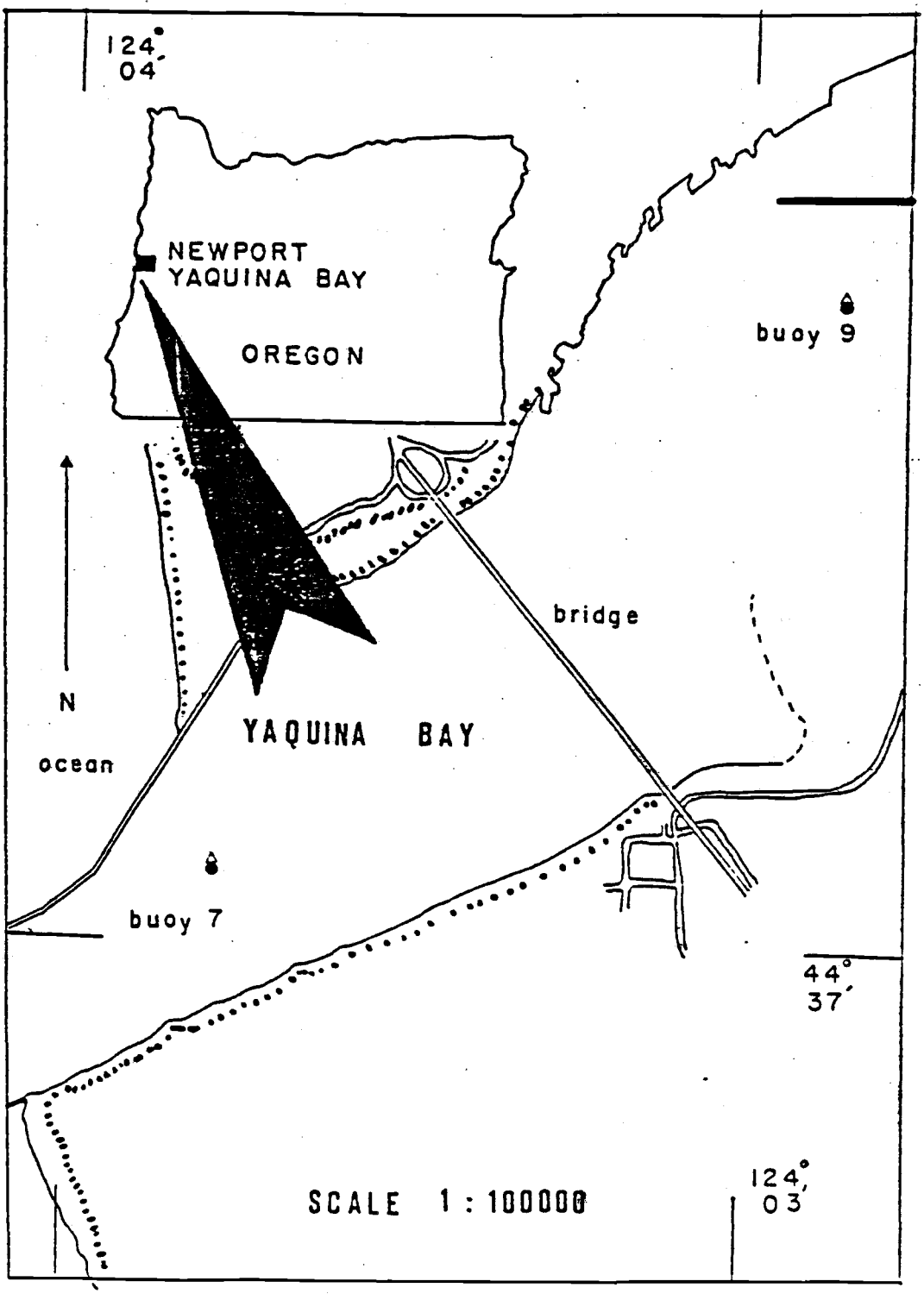

Figure 1. Yaquina Bay, Oregon, site of Kelp greenling collection

Table 1. Dates of fishing trips, number of trawls and number of Kelp greenling caught

	Fishing Trip #	Date	Number of Trawls	No. of Live Fish Caught
	1	Sept. 27, 1984	7	150
	2	Sept. 30, 1984	7	80
	3	Oct. 4, 1984	5	80
	4	Oct. 6, 1984	6	50
	5	Oct. 10, 1984	6	170
	6	Oct. 12, 1984	4	80
Total	6		35	610

Salinity Tolerance Limits of Kelp greenling

The experiment was conducted in January, 1984. The Kelp greenling used varied in size from 18.0 to 28.0 cm and were actively feeding. The fish were held for two weeks in a circular fiberglass tank (diameter 175 cm) supplied with running untreated sea water from Yaquina Bay. Acclimation was considered complete after two days in a salinity of 30 ppt at 13°C. The salinity and temperature were measured by hydrometer and thermometer respectively. The fish were deprived of food for one day prior to starting the experiment.

Twenty fish were randomly selected and allocated to each of the following salinities tested: 0 (fresh water), 7.5, 15, 30 (sea water) and 45 ppt . The fish maintained in 30 ppt sea water were considered controls. Low salinities were prepared by diluting sea water with dechlorinated fresh water while high salinities were prepared by addition of salt (Instant Ocean Sea Salts, manufactured by Aquarium Systems, Inc., Ohio). Four fish from each salinity studied were sampled after 6, 12, 24, 48 and 96 hours of the initiation of the experiment. Fish in fresh water died before the end of the sampling period while fish at 15 ppt were sampled only at 12, 24 and 48 hours. Each fish was blotted dry and measured for total length and body wet weight.

Blood samples were collected from the caudal

artery by heparinized natelson capillary tubes (length 150 mm - ID 1.5 mm) and transferred to Eppendorf microtest tubes (volume 0.5 ml). Each capillary tube contained 6 u.s.p. units of ammonium heparin. The plasma was separated then from the blood cells and fibrin by centrifuging the microtest tubes at 3200 rpm for five minutes (Brinkman centrifuge 3200). Osmolarity of the plasma was determined using Wescor vapor pressure osmometer model 5100C. The results were expressed in milliosmoles per kilogram of water.

Another sample of blood was collected by heparinized micro-haematocrit capillary tubes (length 75 mm - ID 1.2 mm). The capillary tube was sealed at one end and centrifuged at 4000 rpm for 10 minutes. The haematocrit was read using Spiracrit micro-haematocrit capillary tube reader which permits reading of packed cell volume directly in percentage.

The fish were dissected and their gonads removed and weighed. To determine the effects of salinity on the moisture content, the fish were dried at 80°C to a constant weight and placed in a dessicator for 24 hours prior to weighing.

A second experiment was conducted in June following the same experimental procedure except that the fish were acclimated for a week at 30 ppt instead of two days. Salinities tested were 5, 30 (control) and 50 ppt. Five fish were sampled for each salinity

studied after 12, 24, 48, 96 and 192 hours after the initiation of the experiment.

Effects of Salinity on Growth and Food Conversion

Growth and food conversion of the greenlings were studied at the following salinities: 7.5, 15 and 30 ppt. A total of approximately 200 fish were used for the experiment. A group of fish were acclimated to each of the three salinities to be tested for a week before the initiation of the experiment. Each treatment included three replicates. The experiment was carried out in a 600-liter circular fiberglass tank. The number of fish stocked per tank ranged from 21 to 24. Continuous aeration was provided to all tanks. The required salinities were made up by mixing dechlorinated fresh water with filtered, ultraviolet light-treated sea water from Yaquina Bay. Tanks were cleaned daily as far as possible. Approximately 80% of the water was changed every 48 hours. All tanks were fitted with translucent fiberglass covers to shield the fish from outside visual stimulation. During both acclimation and experimentation, photoperiod was determined primarily by natural lighting from the windows in the roof of the lab.

The fish were fed Oregon Moist Pellets twice a day. The feeding rate was maintained at 1.5% of wet body weight and was adjusted to accommodate for growth after each measurement. The approximate compositon

of the rations was analyzed by the OSU Seafood Laboratory in Astoria (Table 2).

Growth was estimated in terms of wet weight and total length, and was monitored by measuring the fish initially and after 17, 35, 45 and 60 days from the initiation of the experiment. Before measurements fish were anesthesized with MS222 (tricaine methanesulfonate, Sigma Chemical Co.).

Fish were individually dip-netted, blotted with absorbent paper and weighed in a tared container on a top-loading balance (Mettler PL300) to the nearest 0.01 g. Total length was measured to the nearest 0.1 cm. No detrimental effects of handling were apparent and all fish resumed feeding the following day after measurement. Prior to weighing, fish were deprived of food 24 hours to allow evacuation of food. Growth in length was calculated as the difference between initial and final lengths divided by the number of days of the experiment. Average relative growth was calculated using the formula given in Warren (1971):

$$\text{Growth rate} = \frac{W_2 - W_1}{0.5(W_1 + W_2)\ (t_2 - t_1)} \times 100$$

where W_1 was the average weight of fish at the beginning of the growth experiment, W_2 was the average weight of fish at the end of the growth experiment, and (t_2-t_1) was the number of days of the experiment. Food conversion

Table 2. Approximate composition of Oregon Moist Pellets

Replicate Number	Percent wet weight Moisture	Ash	Fat	Protein
1	27.12 ± 0.12	11.18 ± 0.00	13.57 ± 0.17	40.28 ± 0.06
2	28.04 ± 0.07	11.13 ± 0.07	13.55 ± 0.08	40.69 ± 0.04
Average	27.58	11.16	13.56	40.49

(Analyzed by Department of Food Science and Technology, Seafoods Laboratory, Oregon State University)

ratio was calculated as the dry weight of food ingested over the whole experimental period divided by the dry weight gained by fish, multiplied by 100. Survival rate was calculated by dividing the total number of fish at the end of the experiment by the initial total number of fish, multiplied by 100.

Water samples were collected from the tanks three times a week for determination of some basic environmental conditions during the course of the study. Salinity and water temperature were measured by hydrometer and mercury thermometer respectively. Measurements of the dissolved oxygen content were made using a dissolved oxygen meter (YSI model 51B). pH was determined by Corning 125 pH meter.

Long Term Effects of Salinity on Plasma Osmolarity, Moisture and Ash Content

The experimental fish were also utilized to estimate the long-term effects of salinity on plasma osmolarity, moisture and ash content. By the end of the growth experiment, the fish had been exposed for at least 60 days constantly to one of the following salinities: 7.5, 15 and 30 ppt. In order to evaluate the long-term effects of salinity on the osmoregulation capability of the Kelp greenlings, and to assess differences between sexes, 10 males and 10 females were sampled from each experimental group. Blood samples were collected. Haematocrit readings and plasma osmolarity were determined

as described earlier.

Each fish was dried to a constant weight in an oven at 80°C. The loss of weight was taken as moisture content which was expressed as percentage of the initial weight of the fish. The whole dry fish was placed in a crucible and ashed in a muffle furnace for seven days at 600°C, cooled in a dessicator and weighed.

Effects of Acclimation to Lower Salinity on Fish Tolerance to Fresh Water

At the end of the growth experiments, the fish of two tanks containing 23 fish each were assigned to the experiment. The fish were acclimated to salinities of 7.5 and 30 ppt for at least 70 days and no mortalities were noted. The fish in each tank were divided into two groups. One group which consisted of 12 fish was transferred directly to dechlorinated fresh water while the remaining 11 fish served as controls. The low salinity was obtained by diluting sea water with dechlorinated fresh water. The time of death of each fish was recorded. The survival time in fresh water was considered as a measure of tolerance.

Criteria for death were cessation of body, fin and opercular movements. The dead fish were measured for total length and body weight. The fish then were dried and ashed.

Effects of Salinity on Oxygen Consumption

Oxygen consumption was taken as a measure of metabolic expenditure and was determined by measuring

the loss of oxygen and rate of flow water through a chamber containing the fish.

Apparatus

The system was made up of a constant-head tank placed above a reservoir tank (Figure 2). To insure that air-saturated water was supplied to the test chamber, the reservoir tank was well aerated. The test chamber was a glass flask (volume 2.8 liters, base diameter 20 cm) sealed by a rubber stopper with two openings, inlet and outlet. To prevent stratification, water was stirred gently in the test chamber. Fish tested were protected from the magnet by enclosing it with a section of plastic pipe (10 cm in diameter - 2.5 cm in height) that had a plastic mesh cover. The flask was covered with black polyethylene sheets to reduce the possibility of frightening the test animal by outside movements. The fish were observed through a small window in the cover. To prevent air from being trapped in the system, the test chamber was always opened and sealed under water. Measurements of oxygen concentrations were made using a dissolved oxygen meter (YSI model 54A) that was connected to a chart recorder (YSI model 81A). Continuous recording allowed measurements to be made over relatively long intervals. The oxygen probe was mounted on a plexiglass structure referred to as a polarograph chamber (Figure 3). The polarograph chamber was made up of two connected compartments.

A . Supply tank

B Pump

C Constant head tank

D Reservoir tank

E Test chamber

F Stirrer

G Polarograph chamber

H Oxygen meter

I Chart recorder

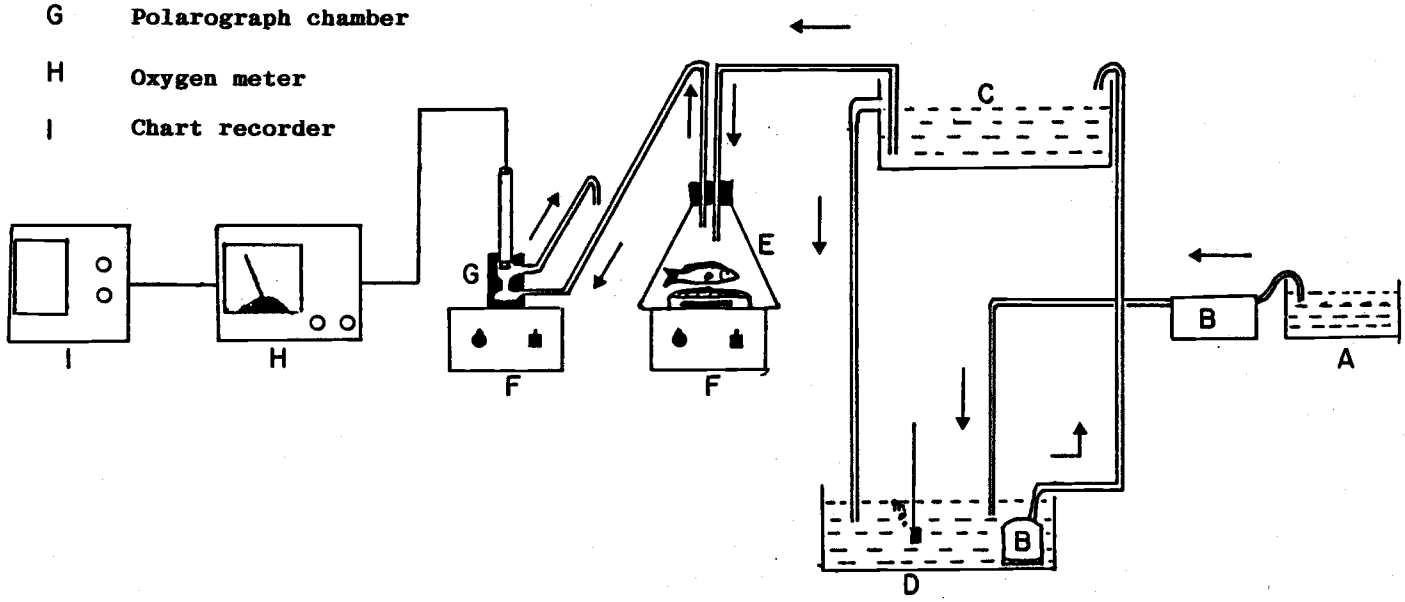

Figure 2. Diagram of the respirometry system. Arrows indicate the direction of water flow.

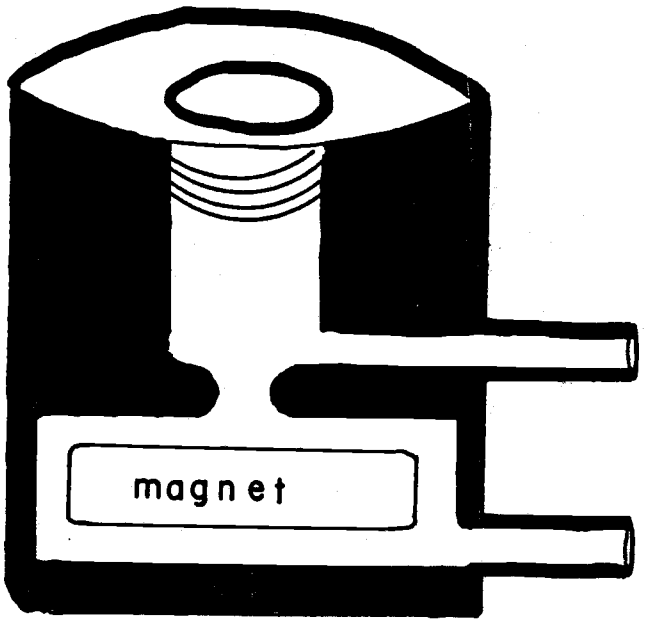

Figure 3. Polarograph chamber

Water coming from the test chamber was stirred by a magnet in the lower compartment before passing to the upper one where the probe was mounted. All connections from the constant-head tank to the polarograph chamber were made by polyethylene tubing. Rate of water flow through the respirometer was controlled by raising or lowering the polyethylene tube from the outlet of the polarograph chamber which determines the height of the water level in the constant-head tank. Flow rates were measured by collecting the outflow in graduate cylinders. Water lost from the system was replaced from the supply tank. The amount of water in the system remained more or less constant. The experiments were conducted in a temperature-controlled room to maintain the thermal environment of 9°C.

Experimental Procedure

Eight male fish of approximately 18.0 cm in total length were acclimated to one of the following salinities: 7.5, 15 and 30 ppt and a temperature of 9°C for at least a week before the initiation of the experiment. Following two days of food deprivation, a fish was placed in the test chamber. At rest, the fish depleted the oxygen supply of the inflowing water by a measured amount. The system required at least 3 hours after the initiation of a test run to equilibrate. Equilibrium was said to be reached when the difference in oxygen content between inflow and outflow water remained relatively

constant for about three hours. A number of preliminary tests showed that once the fish were sealed within the experimental chamber they lay quiescent and, under most conditions, the apparatus was equilibrated after approximately six hours. From the results of the preliminary tests, the following procedure was adopted. Fish were sealed in the test chamber and the apparatus allowed to equilibrate. As measured from continuous chart recordings, but irrespective of these readings, no measurements of oxygen consumption were taken within six hours of setting up the test. Following equilibrium, recording was continued for a further six hours and estimates of oxygen consumption were based on these readings. Fish were measured for total length and body weights at the end of each test run. The following experimental conditions were maintained during the experiment: darkness, quietness and constant temperature. The dissolved oxygen meter was calibrated before each test run using the Azide modification of the Winkler method (American Public Health Association, 1980).

Statistical Analysis

The data collected were subjected to the analysis of variance and Duncan's multiple range test, regression and covariance analysis, and student's t-test whichever applicable. The methods described by Duncan (1955), Sokal and Rohlf (1969), Snedecor and Cochran (1974) and Zar (1974) were used for these statistical analyses.

CHAPTER III.

RESULTS

Salinity Tolerance Limits of Kelp Greenling

The physiological responses of fish to changing environmental osmotic stress are most easily measured by following osmotic or ionic changes in the blood. In the first experiment conducted in January of 1984, the osmotic response of Kelp greenling was studied for the following salinities: fresh water, 7.5, 15, 30 and 45 ppt. The osmolarity of the serum of Kelp greenling at 30 ppt (sea water) averaged 375M osm/Kg, 6 hours after the initiation of the experiment (Figure 4. Table 3). At 12 hours the average serum osmolarity increased to 390 mOsm/Kg. The osmolarity dropped to 362 mOsm/Kg after 24 hours and remained relatively constant thereafter. Similar increase in osmolarity was observed after 12 hours in fish placed in salinities of 7.5 and 15 ppt. This sudden increase in osmolarity was thought to be due to stress caused by fish handling. Attention has recently been drawn to the mechanisms of osmoregulatory disturbances induced by stress which upset water and mineral balance. Maetz (1974) suggested that alternations in the concentration of adrenaline were probably responsible for the effect of handling on the water balance of the fish. Abrupt transfers to fresh water resulted in an immediate drop in serum osmolarity (Figure 4). The average

Table 3. Plasma osmolarity, haematocrit and moisture content of Kelp greenling at salinities of 0, 7.5, 15, 30 and 45 ppt at different times (each point represents 4 fish)

Salinity (ppt)	Time (hours)	Plasma osmolarity (mOsm/Kg) Mean	S.D.	Haematocrit (Percent) Mean	S.D.	Moisture Content (Percent) Mean	S.D.
0	2	304.50	5.80	57.75	4.75	74.91	0.79
	6	307.50	3.54	64.5	1.00	75.59	1.25
7.5	6	358.75	1.71	33.25	7.80	75.57	1.24
	12	365.50	4.51	35.75	9.78	77.25	1.83
	24	327.25	6.08	35.00	6.56	78.14	1.55
	48	333.00	8.29	32.5	6.40	79.31	3.03
	96	330.25	16.3	47.0	11.17	--	--
15	12	376.25	2.99	31.25	3.50	76.85	2.52
	24	324.75	8.06	26.75	0.96	78.82	2.10
	48	360.25	4.75	32.50	7.14	75.16	1.98
30	6	374.75	16.58	30.75	5.91	73.37	1.49
	12	389.75	5.06	35.00	9.97	75.18	2.26
	24	361.75	8.96	32.00	7.53	78.41	2.15
	48	361.50	5.07	37.00	6.27	75.38	1.90
	96	358.75	4.27	32.50	1.00	--	--
45	6	430.00	31.51	30.00	1.83	72.73	1.28
	12	453.50	8.23	34.75	10.21	72.42	1.29
	24	421.50	8.19	31.00	4.08	74.85	3.51
	48	388.50	8.89	32.00	4.24	74.49	3.89
	96	455.25	24.73	45.75	6.95	--	--

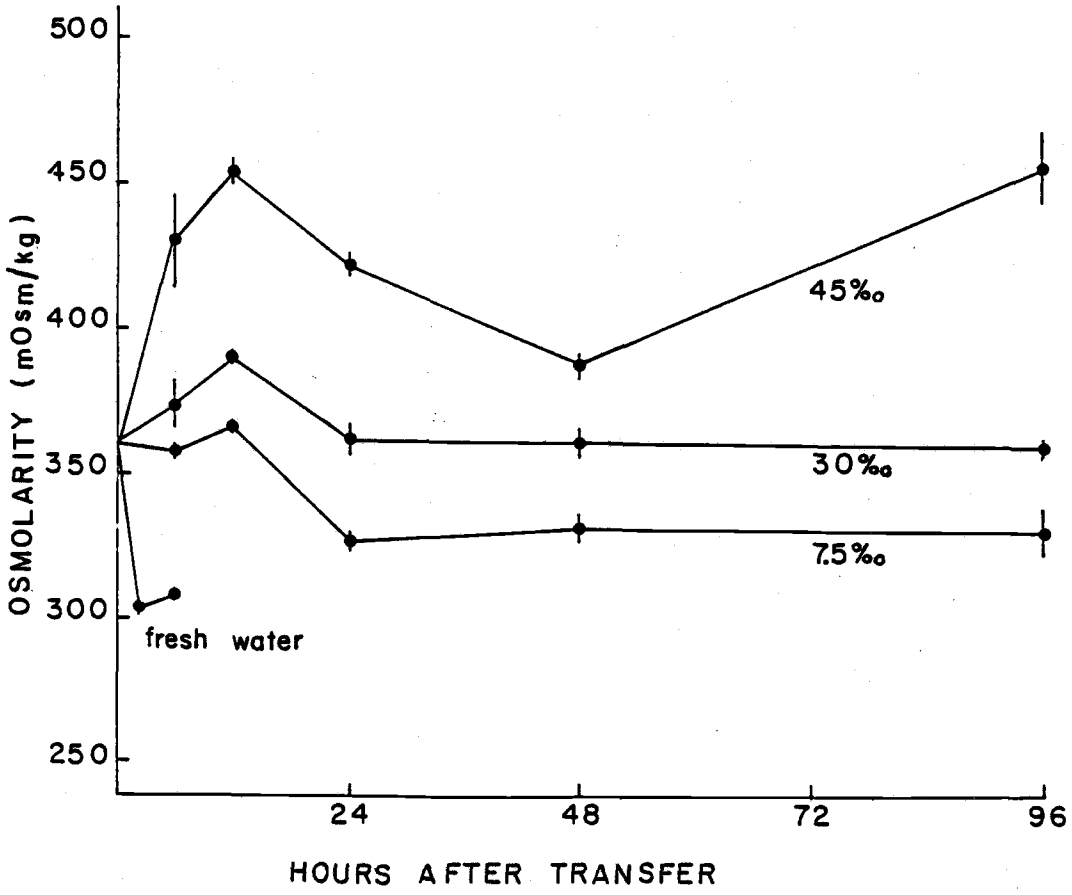

Figure 4. Plasma osmolarity of Kelp greenling as a function of time at 0, 7.5, 30 and 45 ppt. The circles represent the means. Bars indicate ± SE.

serum osmolartiy of greenling in fresh water was lower at 2 than at 6 hours, suggesting the initial influx of water into the extracellular space exceeded the capacity of the kidney to excrete the excess fluids. Although a brief recovery occurred after 6 hours, the fish could not tolerate the abrupt transfer from sea to fresh water and died within 10 hours. A noticeable edema occurred and was accompanied by stiffening of the body and a marked decrease in swimming ability. Collection of blood samples became increasingly difficult, suggesting cardiovascular disturbances which are characteristic of osmoregulatory failure.

The blood osmolarity quickly rose when greenling were abruptly transferred from the control salinity of 30 ppt (875 mOsm/Kg) to concentrated sea water of 45 ppt (1340 mOsm/Kg). No mortality occurred through the duration of the experiment (96 hours) at 45 ppt.

There was no marked pattern in haematocrit readings in the salinities tested. Large variations were observed among individual fish treated similarly. It is worth noting that the haematocrit of fish placed in fresh water was high. The blood was pinkish in color indicating hemolysis of blood cells.

Moisture content of fish was only monitored for 48 hours. The moisture content of fish placed in dilute sea water (7.5 ppt) increased with time and was significantly higher than those in concentrated

sea water (45 ppt) after 48 hours ($P < 0.05$). There were no marked differences in moisture content of fish subjected to moderate salinities of 15 and 30 ppt during the same period.

The average total length of fish used was 23.80 cm (Table 4). When fish were separated according to sex, there was no significant difference in body weight between males and females based on a t-test ($P > 0.05$). The gonads of males were significantly smaller than those of females. The gonadosomatic index of males and females were 0.02 and 0.24 respectively.

A second experiment was conducted in June, 1984, with the objective of further defining the salinity tolerance range of Kelp greenling more specifically. The results of the previous experiment indicated that the fish tolerated a salinity of 7.5 ppt for 4 days but could not survive in fresh water. On the other extreme the fish survived at 45 ppt. Therefore the greenling were tested in salinities of 5 and 50 ppt, with fish at 30 ppt being used as controls.

The plasma osmolarity and moisture content of Kelp greenling at sea water (30 ppt) remained relatively constant throughout the experiment. The average plasma osmolarity ranged from 359 to 367 mOsm/Kg while moisture content ranged only from 70.9 to 71.7%. Haematocrit values were 36% at 12 hours and dropped to about 32% thereafter (Table 5).

Table 4. Total length, body weight, gonad weight and gonadosomatic index of male and female Kelp greenling utilized in the experiment dated January 15, 1984 and tested at the following salinities: 0, 7.5, 15, 30 and 45 ppt

Sex	Number of fish	Total length (cm)		Body weight (g)		Gonad weight (g)		Gonadosomatic index (%)	
		Mean	S.D*	Mean	S.D.*	Mean	S.D.*	Mean	S.D.*
Male	27	23.6	1.7	145.5	39.3	0.03	0.02	0.02	0.01
Female	49	23.9	1.6	148.2	36.1	0.37	0.16	0.24	0.06

*Standard deviation

Table 5. Plasma osmolarity, haematocrit and moisture content of Kelp greenling at salinities of 5, 30 and 50 ppt (each point represents 5 fish)

Salinity (ppt)	Time (hours)	Plasma osmolarity (m Osm/Kg)		Haematocrit (Percent)		Moisture Content (Percent)	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
5	12	319.00	7.75	34.60	8.56	72.45	2.03
	24	310.80	6.94	36.00	4.06	71.60	1.46
	48	285.20	7.26	37.00	5.48	74.70	2.08
	96	264.80	2.17	33.80	5.26	72.78	1.34
	192	261.80	5.76	40.00	6.00	73.69	2.63
30	12	358.80	7.56	35.80	6.83	71.66	1.07
	24	363.00	9.92	31.60	4.56	71.01	2.45
	48	367.00	11.58	31.40	3.78	70.91	1.49
	192	358.80	3.70	32.40	0.89	71.57	1.11
50	12	437.40	40.73	35.60	6.84	68.53	2.26
	24	457.20	23.61	36.20	5.02	66.86	1.88
	48	476.00	25.50	31.00	4.36	67.22	2.56

Figure 5 shows that abrupt transfer to hypotonic media of 5 ppt (150 mOsm/Kg) resulted in immediate drop of serum osmolarity. This was accompanied by relatively high haematocrit and moisture readings. Equilibrium was reached after 4 days. Average plasma osmolarity at equilibrium was 265 mOsm/Kg and did not change much after 8 days. Kelp greenling tolerated a salinity of 5 ppt with no mortality for 8 days.

The average serum osmolarity rose to 437 mOsm/Kg after 12 hours of transferring the fish from control salinity of 30 ppt to concentrated sea water of 50 ppt. The average osmolarity of the serum further increased to 457 and 476 mOsm/Kg after 24 and 48 hours respectively. The fish were dehydrated. This was reflected in relatively low moisture content and low haematocrit values. The fish became extremely sluggish, some fish lost equilibrium after 50 hours of exposure and displayed erratic body movements. All fish died between 60 and 72 hours after initiating the experiment.

The average total length of fish used was approximately 29.0 cm. Average body size and gonad weights of both males and females were summarized in Table 6. There were no significant differences in body weight between males and females ($P > 0.05$). Male gonad weights were significantly smaller than those of females ($P < 0.05$).

Figure 6 is a plot of the average plasma osmolarity of fish placed at different salinities after 24 and

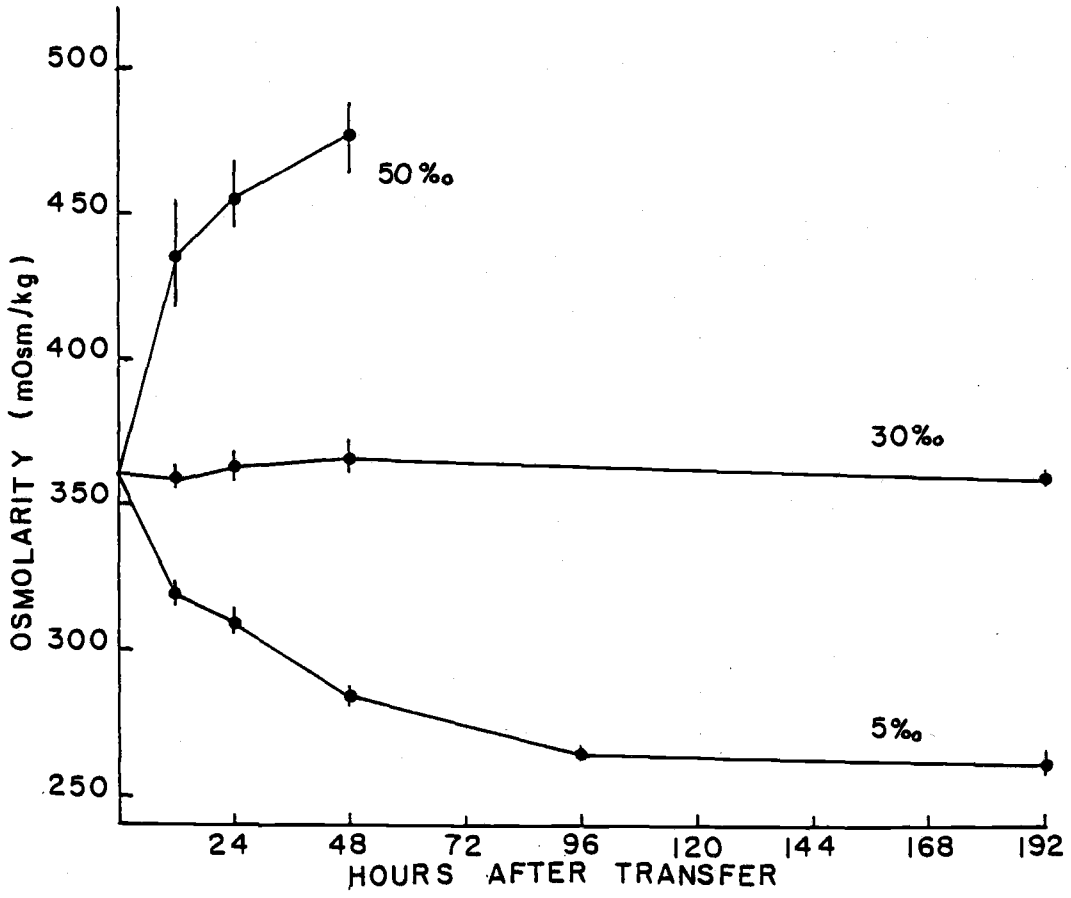

Figure 5. Plasma osmolarity of Kelp greenling as a function of time at 5, 30 and 50 ppt. The circles represent the means. Bars indicate ± SE.

Table 6. Total length, body weight, gonad weight and gonadosomatic index of male and female Kelp greenling utilized in the experiment dated June 25, 1984, tested at the following salinities: 5, 30 and 50 ppt

Sex	Number of Fish	Total length (cm)		Body weight (g)		Gonad weight (g)		Gonadosomatic index (%)	
		Mean	S.D.*	Mean	S.D.*	Mean	S.D.*	Mean	S.D.*
Male	33	28.7	1.9	296.2	67.7	0.06	0.02	0.02	0.01
Female	45	29.1	2.3	296.4	86.2	1.27	0.67	0.42	0.15

*S.D. = standard deviation

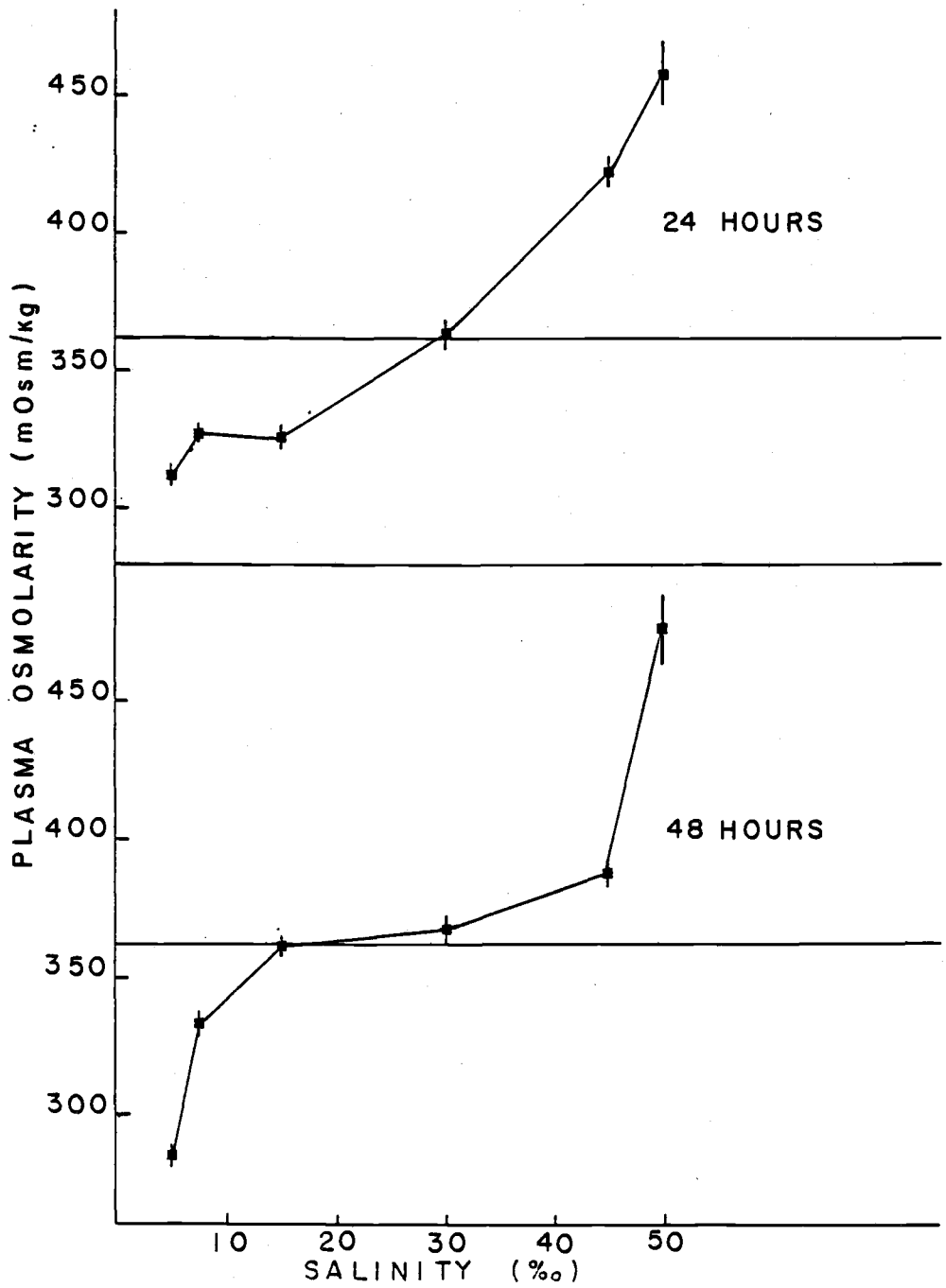

Figure 6. Relation between plasma osmolarity and salinity plotted after 24 and 48 hours of exposure to different salinities. The horizontal line is a reference line representing the plasma osmolarity of control fish placed in 30 ppt. Bars indicate ± SE.

48 hours of exposure. The horizontal lines are reference lines calculated by taking the average plasma osmolarity of fish at 30 ppt. The average plasma osmolarity of fish after 24 hours tends to be scattered away from the reference line. Fish exposed to salinities of 7.5 and 15 ppt had a higher plasma osmolarity than those exposed to 5 ppt but less than fish at 30 ppt. Average plasma osmolarity of fish at 50 ppt was higher than the reference line and the plasma osmolarity of fish at 45 ppt. After 48 hours of exposure plasma osmolarity of fish placed at 7.5, 15 and 45 ppt tend to come closer to the reference line in comparison to their previous position at 24 hours. Plasma osmolarity of fish at the extreme salinities of 5 and 50 ppt moved even further away from the reference line after 48 hours, suggesting that they were not osmoregulating as efficiently as the fish exposed to 7.5, 15 and 45 ppt.

Effects of Salinity on Growth and Food Conversion

The average of the mean body weights of fish reared at a salinity of 15 ppt was lower at the initial measurement and higher at the final measurement, than that of fish reared at salinities of 7.5 and 30 ppt (Table 7). The differences were statistically insignificant in both measurements ($P > 0.05$).

Within salinities, weight gain per fish increased with time in a linear fashion (Figure 7). Based on

Table 7. Initial and final total length and body weight measurements of fish utilized in the experiments to study the effect of salinity on the growth of Kelp greenlings. Growth in length, relative growth rate, food conversion and survival were calculated from pooled data of all fish in each tank. The duration of the experiment was 60 days.

Salinity (ppt)	Replication Number	Number of Fish Per Tank	Total length (cm)				Body weight (g)				Growth in length (cm /day)	Relative growth rate (%body weight /day)	Food conversion (%)	Survival (%)
			Initial		Final		Initial		Final					
			Mean	S.D.*	Mean	S.D.*	Mean	S.D.*	Mean	S.D.*				
7.5	1	22	16.2	1.3	17.5	1.0	41.7	10.4	57.4	10.9	0.022	0.529	6.1	100
	2	22	16.9	1.1	18.1	1.1	48.8	10.1	66.6	14.0	0.019	0.514	6.2	86.4
	3	24	16.4	0.4	17.5	1.2	42.9	13.4	59.0	13.1	0.023	0.527	6.0	100
15	1	22	16.6	1.6	18.2	1.5	46.5	14.7	64.4	16.4	0.026	0.536	5.9	100
	2	22	16.0	1.1	17.6	0.9	39.1	10.6	57.8	9.6	0.027	0.641	5.0	86.4
	3	22	16.5	1.4	18.2	1.2	45.7	13.8	66.0	14.2	0.027	0.608	5.3	95.3
30	1	21	16.7	1.6	18.1	1.5	46.5	14.4	63.3	16.4	0.023	0.510	6.1	100
	2	23	16.5	1.0	17.7	1.1	44.6	12.4	60.1	10.9	0.021	0.495	6.3	100
	3	23	15.9	1.3	17.3	0.4	41.5	8.6	54.3	10.7	0.023	0.444	7.0	100

*S.D. = standard deviation

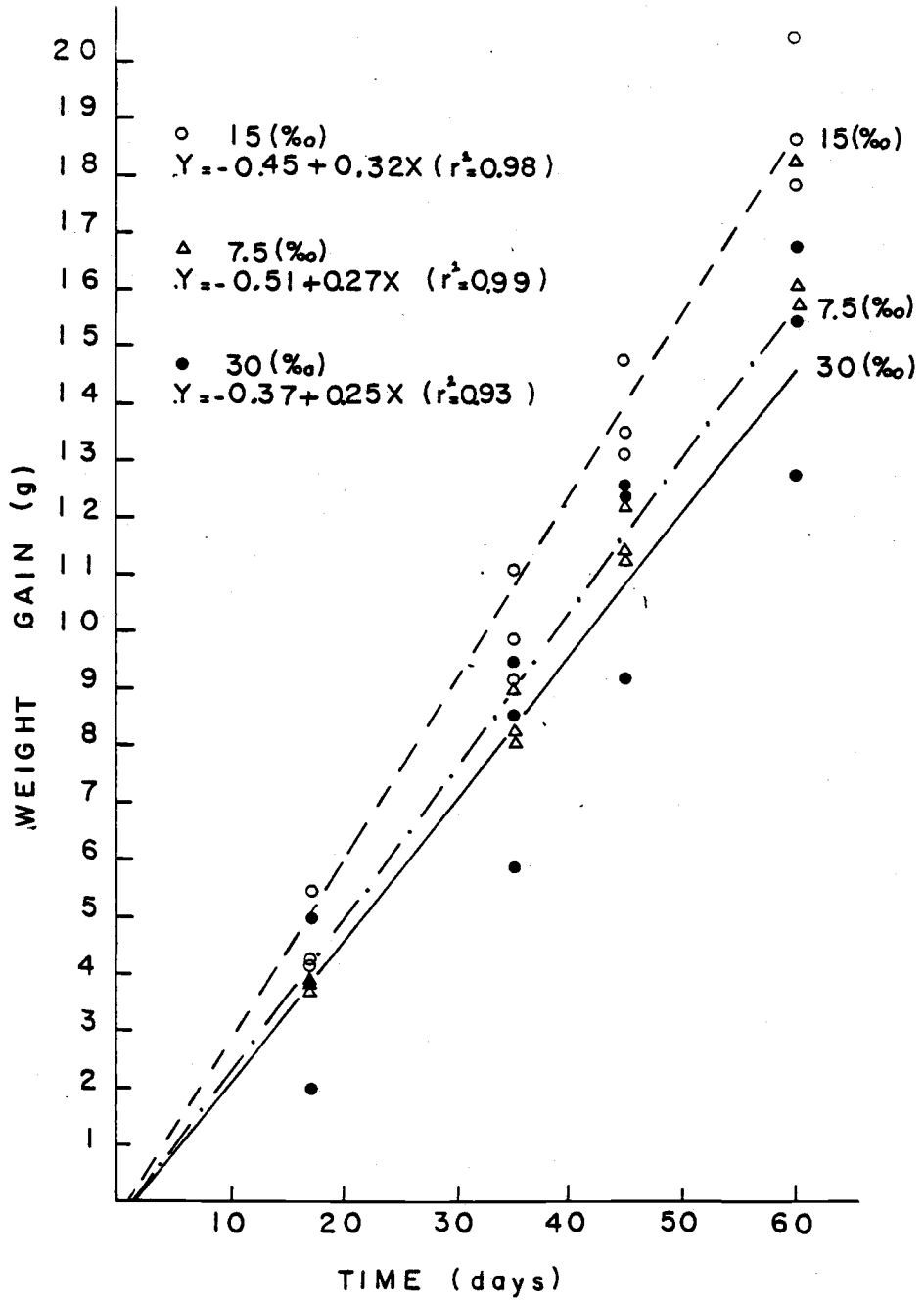

Figure 7. Weight gain of Kelp greenling at 7.5, 15 and 30 ppt.

a linear regression and covariance analysis, the weight gain per fish was significantly higher at 15 ppt than at salinities of 7.5 and 30 ppt.

The mean weight gain per fish in 60 days was 18.95 g at 15 ppt compared to 16.55 g and 15.45 g at 7.5 and 30 ppt respectively. Statistical analysis of the weight gain per fish obtained from the 3 different salinities tested showed no significant difference between fish exposed to 7.5 and those exposed to 30 ppt (Duncan's multiple range test, at 0.05 level).

Growth in length and relative growth rate showed similar patterns: they were both significantly higher at 15 ppt than at 7.5 and 30 ppt (Table 7, Figure 8).

There were no significant differences in growth in length or in relative growth rate, when statistical comparisons were performed, between salinities of 7.5 and 30 ppt.

The food conversion ratios varied from 6.04 to 6.22 at 7.5 ppt and from 6.08 to 6.97 at 30 ppt (Table 7, Figure 9). In neither salinity did the fish show much difference in their efficiency in food conversion ($P > 0.05$).

The food conversion ratios of fish were lowest at 15 ppt. At salinities higher or lower than 15 ppt, the efficiency of conversion was reduced significantly ($P < 0.05$).

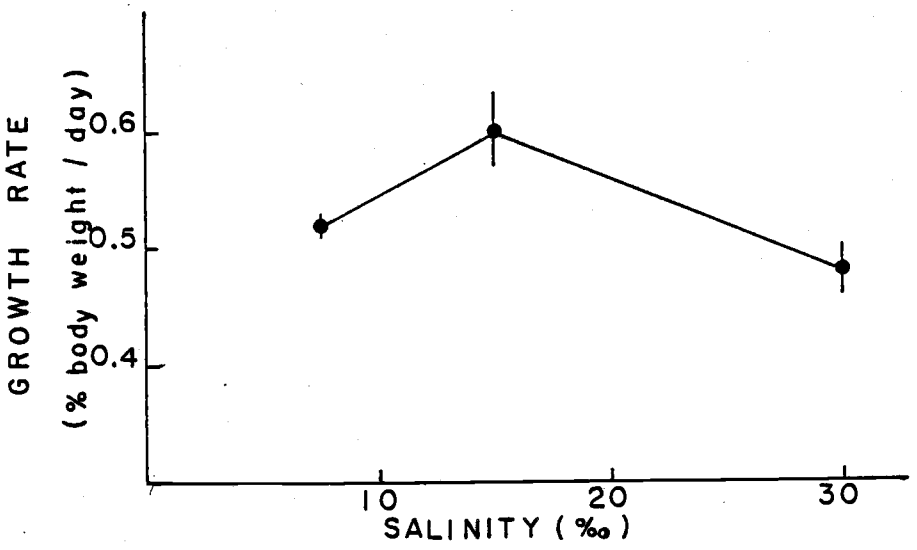

Figure 8. Relative growth rate of Kelp greenling as a function of salinity. The circles represent the means. Bars indicate ± SE.

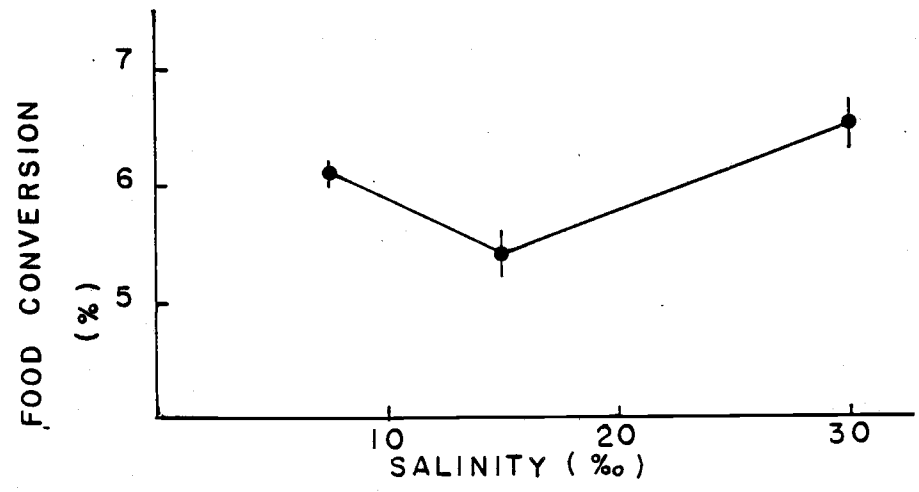

Figure 9. Food conversion of Kelp greenling as a function of salinity. The circles represent the means. Bars indicate ± SE.

There were no mortalities observed among fish reared at 30 ppt. At the end of the experiment, survival rates of fish at salinities of 7.5 and 15 ppt varied from 86.4 to 100%. The survival rates of fish reared at salinities of 7.5, 15 and 30 ppt were not significantly different from one another($P>0.05$).

Water temperature varied from 12.0 to 15.5°C during the experiments. The average water temperature in all tanks was 13.6°C (Table 8). The mean measured salinities were 7.7, 15.1 and 29.6 ppt, only slightly different than the experimentally designed salinities of 7.5, 15 and 30 ppt.

As the salinity increased the dissolved oxygen content in the water decreased (Table 8). pH varied from 7.1 to 9.0.

Long Term Effects of Salinitiy on Plasma Osmolarity, Moisture and Ash Content

The experimental fish from the growth experiment had been exposed constantly to one of the following salinities--7.5, 15 and 30 ppt--for at least 60 days. A sample of 10 males and 10 females from each experimental group were utilized to assess differences among sexes (Table 9). Although average body weights of females were larger than those of males, based on a t-test the difference was not significant ($P>0.05$). The average gonadosomatic indices of males were signficantly smaller than those of females. There were no significant differences in plasma osmolarity, haematocrit, moisture

Table 8. Changes in the water temperature, salinity, dissolved oxygen content and pH in the tanks used for the growth experiment. Each value is the mean of the measurements taken every 48 hours over the period of the experiment. The duration of the experiment was 60 days. Values in brackets indicate the ranges.

		Proposed Salinity (ppt)		
	Replication Number	7.5	15	30
Water temperature (°C)	1	13.6 (12.5-15.0)	13.6 (12.0-15.5)	13.6 (12.0-15.0)
	2	13.6 (12.5-15.0)	13.6 (12.5-15.0)	13.6 (12.0-15.0)
	3	13.6 (12.0-15.0)	13.6 (12.5-15.0)	13.6 (12.0-15.5)
Salinity* (ppt)	1	7.7 (7.0-8.5)	15.1 (14.5-15.5)	29.6 (28.0-32.0)
	2	7.7 (7.0-8.5)	15.1 (14.5-15.5)	29.6 (28.0-32.0)
	3	7.7 (7.0-8.5)	15.1 (14.5-15.5)	29.6 (28.0-32.0)
Dissolved oxygen content (cc/l)	1	8.8 (7.6-9.6)	8.4 (7.8-9.4)	7.9 (7.2-9.1)
	2	9.2 (8.0-9.8)	8.6 (8.0-9.1)	7.7 (7.0-9.1)
	3	9.4 (8.6-9.8)	8.6 (7.9-9.4)	7.5 (6.6-9.0)
pH	1	7.3 (7.1-7.7)	7.6 (7.3-7.9)	7.7 (7.3-8.0)
	2	7.4 (7.1-7.7)	7.6 (7.3-7.9)	7.7 (7.4-8.0)
	3	7.4 (7.1-7.7)	7.5 (7.2-7.9)	7.6 (7.4-8.0)

*Actual salinity as measured in the tanks

Table 9. Total length, body weight and gonadosomatic index of male and female Kelp greenling and long term effects of salinity on plasma osmolarity, haematocrit, moisture and ash content

Salinity (ppt)	Sex	Total length (cm)		Body weight (g)		Gonadosomatic index (%)		Plasma osmolarity (M osm/Kg)		Haematocrit (%)		Moisture (%)		Ash (%)	
		Mean	S.D.*	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
7.5	♂	17.4	1.1	56.25	11.45	0.021	0.007	329.50	17.42	28.10	5.45	73.72	0.83	15.39	0.70
	♀	17.1	1.0	58.14	10.04	0.180	0.048	319.20	5.12	27.20	7.19	74.00	0.67	15.54	0.79
15	♂	17.7	1.2	58.31	12.35	0.011	0.007	344.60	8.11	30.10	3.41	73.43	0.67	15.52	0.62
	♀	18.6	1.0	67.34	11.89	0.165	0.028	346.10	8.84	27.20	4.02	73.42	0.93	15.69	0.89
30	♂	17.9	1.3	59.11	14.24	0.010	0.006	359.20	11.64	30.80	2.82	73.17	0.66	15.89	0.55
	♀	18.5	1.2	65.95	14.49	0.164	0.032	363.10	8.39	29.40	4.06	72.98	0.93	15.90	0.81

*S.D. = standard deviation

content and ash percentage among males and females ($P > 0.05$), thus the results were combined and comparisons were made between salinities (Table 10).

As the salinity increased from 7.5 to 30 ppt, the average plasma osmolarity increased from 324 to 361 mOsm/Kg. Based on analysis of variance and Duncan's multiple range test, there were significant differences in the average plasma osmolarity between 7.5 and 15 ppt, 7.5 and 30 ppt, and between 15 and 30 ppt ($P < 0.05$).

There were tendencies for haematocrit readings and ash percentages to increase with an increase in salinity, but the differences were not significant when statistical comparisons were made among the salinities tested. Although the moisture percentage decreased only from 73.86 to 73.07 ppt with an increase in salinity from 7.5 to 30 ppt, this small difference was significant ($P < 0.05$).

Plasma osmolarity, haematocrit, moisture and ash values were plotted as percent differences from those of the control fish at 30 ppt (Figure 10). It was observed that as the salinity decreased plasma osmolarity, haematocrit and ash readings decreased while moisture percent increased in reference to fish at 30 ppt. The percent changes in plasma osmolarity and haematocrit readings were relatively large: at 7.5 ppt they were less than the values of fish at

Table 10. Long term effects of salinity on plasma osmolarity, haematocrit values, moisture and ash content of Kelp greenlings

Salinity	Plasma Osmolarity (mOs m/Kg)		Haematocrit (Percent)		Moisture (Percent)		Ash (Percent)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
7.5	324.35	15.58	27.65	6.23	73.86	0.75	15.46	0.73
15	345.35	8.29	28.65	3.92	73.43	0.79	15.61	0.75
30	361.15	10.07	30.10	3.48	73.07	0.79	15.89	0.67

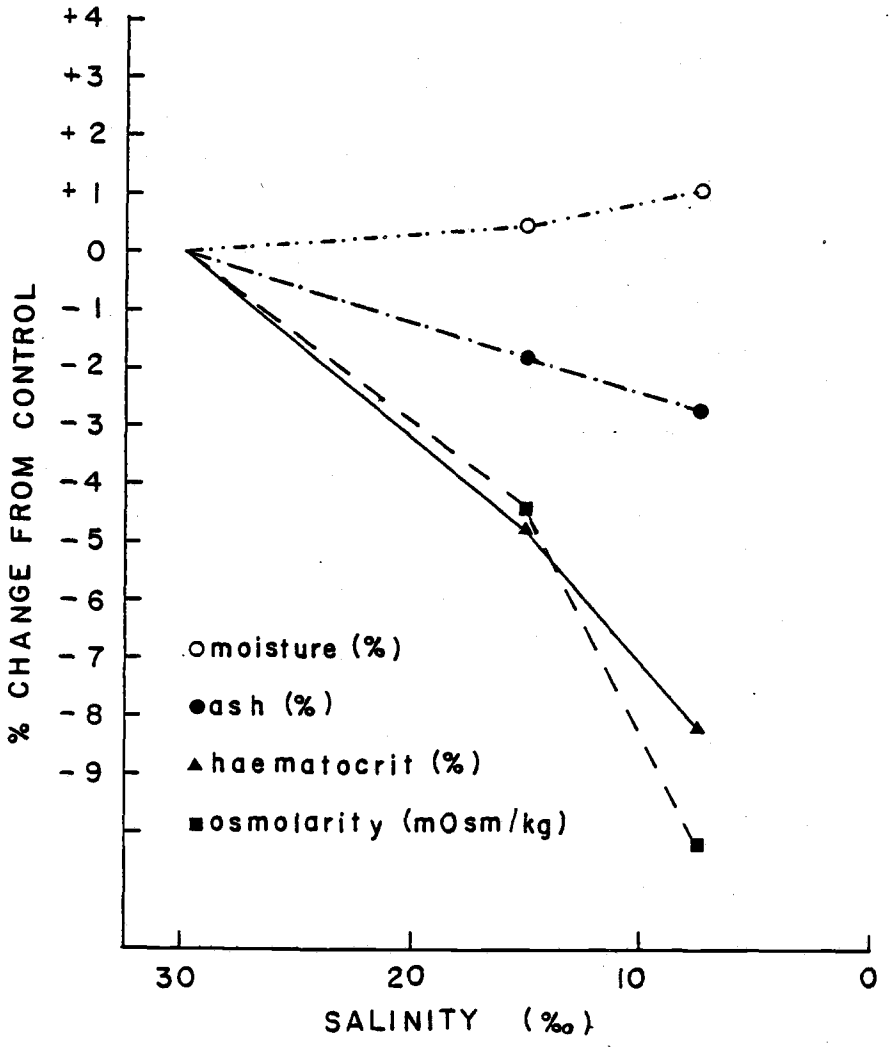

Figure 10. Percent change of moisture, ash, haematocrit and plasma osmolarity at 7.5 and 15 ppt from those at 30 ppt.

30 ppt by 10.2 and 8.1% respectively, while the percent changes in moisture and ash readings were less dramatic. At 7.5 ppt the ash content was less by 2.7% and the moisture content was more only by 1.1% than the values of the control fish.

When the plasma osmolarity was plotted against salinity and the isosomatic line drawn (Figure 11), it was found that Kelp greenling were hyperosmotic at 7.5 ppt (215 mOsm/Kg) but at the higher salinities tested became hyposmotic.

Effects of Acclimation to Lower Salinity on Fish Tolerance to Fresh Water

The experiment was designed to determine the effects of acclimation to a salinity of 7.5 ppt for 70 days on its tolerance in fresh water when compared to fish acclimated to 30 ppt for the same period of time. Two groups of fish served as controls. One group was assigned to each salinity. No mortalities were observed in either control throughout the duration of the experiment (48 hours). The total length of fish used in the experiment ranged from 15.0 to 20.0 cm. Body weight and total length measurements are summarized in Table 11. Kelp greenling did not tolerate transfer to fresh water. All fish in fresh water died with 265 minutes. The fish became sluggish, lost equilibrium and showed slow respiratory and body movements and eventually died. The relation between

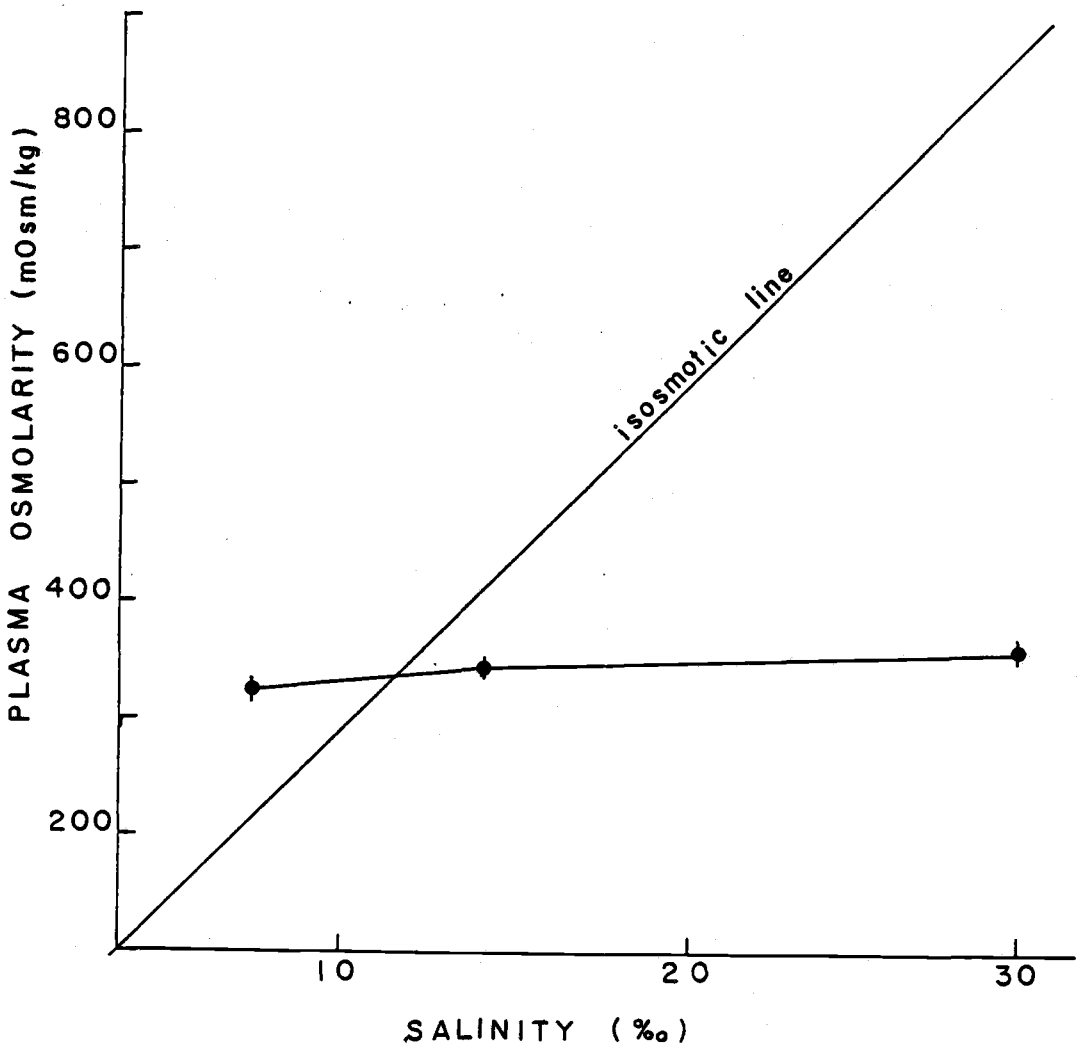

Figure 11. Plasma osmolarity of Kelp greenling as a function of salinity. The circles represent the means. Bars indicate ± SE.

Table II. Total length, body weight, moisture percent, ash percent and survival of Kelp greenling acclimated to 7.5 and 30 ppt after transfer to fresh water, 7.5 and 30 ppt.

Treatment		TL (cm)	Bwt (g)	Moisture (%)	Ash (%)	% Survival after 48 hours
7.5 → F.W.	x	17.0	59.80	75.27	15.78	0
	S.D.	1.0	13.20	0.94	1.16	
	n	12	12	12	12	
7.5 → 7.5 ppt	x	17.9	57.98	74.24	16.33	100
	S.D.	1.3	13.34	0.79	0.77	
Control	n	11	11	11	6*	
30 → F.W.	x	17.51	59.77	75.05	16.08	0
	S.D.	0.8	9.06	0.88	0.81	
	n	12	12	12	12	
30 → 30	x	18.1	59.66	73.08	16.49	100
	S.D.	1.1	12.24	5.79	1.02	
Control	n	11	11	11	11	

*5 samples were processed incorrectly.

cumulative survival in fresh water and time of death was plotted (Figure 12). No differences were noticed between fish that were acclimated to 7.5 ppt from those that were acclimated to 30 ppt. Time when 50% of fish died were 220 and 215 minutes after transfer to fresh water for fish acclimated at 7.5 and 30 ppt respectively. Moisture content of fish acclimated to 7.5 ppt was significantly higher than their control after transfer to fresh water ($P < 0.05$). The ash content was lower although the difference was insignificant ($P > 0.05$). The same holds true for fish acclimated to 30 ppt and their control after transfer to fresh water except that the differences in moisture content were insignificant ($P > 0.05$).

Effects of Salinity on Oxygen Consumption

Oxygen consumption was taken as a measure of metabolic expenditure and was determined by measuring the loss of oxygen and rate of flow water through a chamber containing the fish.

The oxygen consumption of Kelp greenling was found to be lowest at 15 ppt (Figure 13). Fish at 7.5 ppt consumed 20% more oxygen on the average than those at 15 ppt. At salinities higher than 15 ppt oxygen consumption also increased. The average oxygen consumption of fish at 15 ppt was 0.060mg/g/hr compared to 0.092mg/g/hr at 30 ppt. Analysis of variance

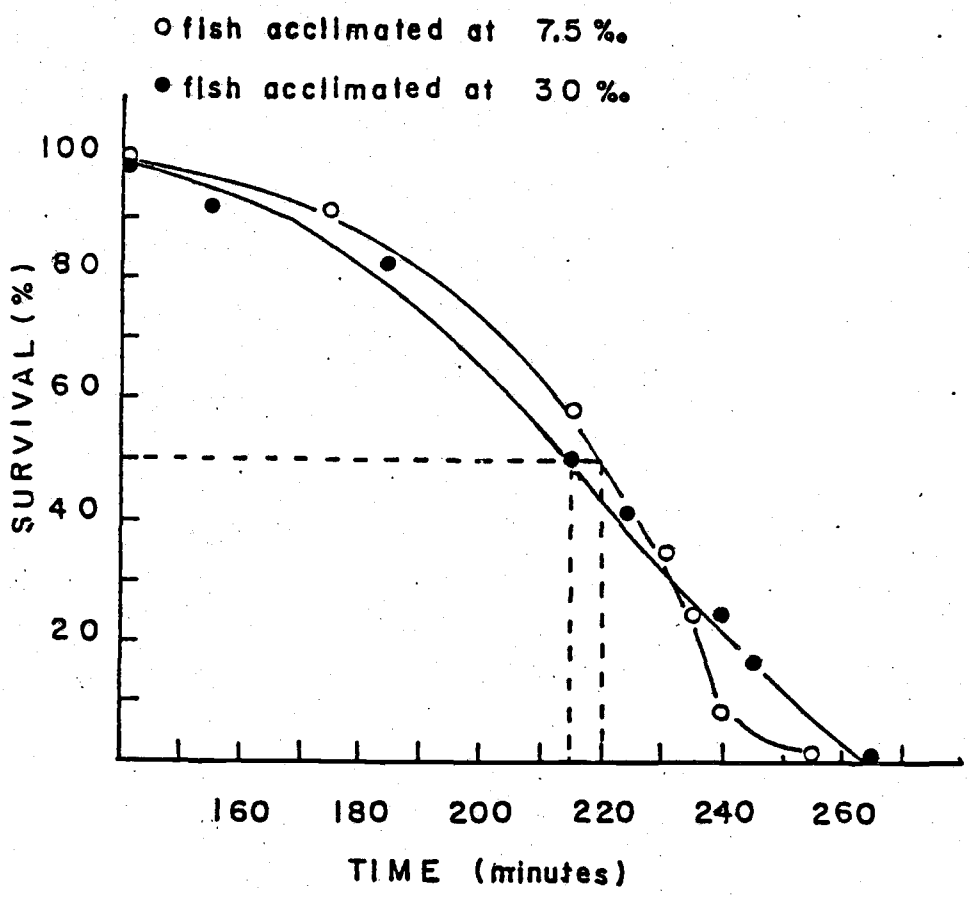

Figure 12. Survival time of Kelp greenling transferred to fresh water after being acclimated at 7.5 and 30 ppt.

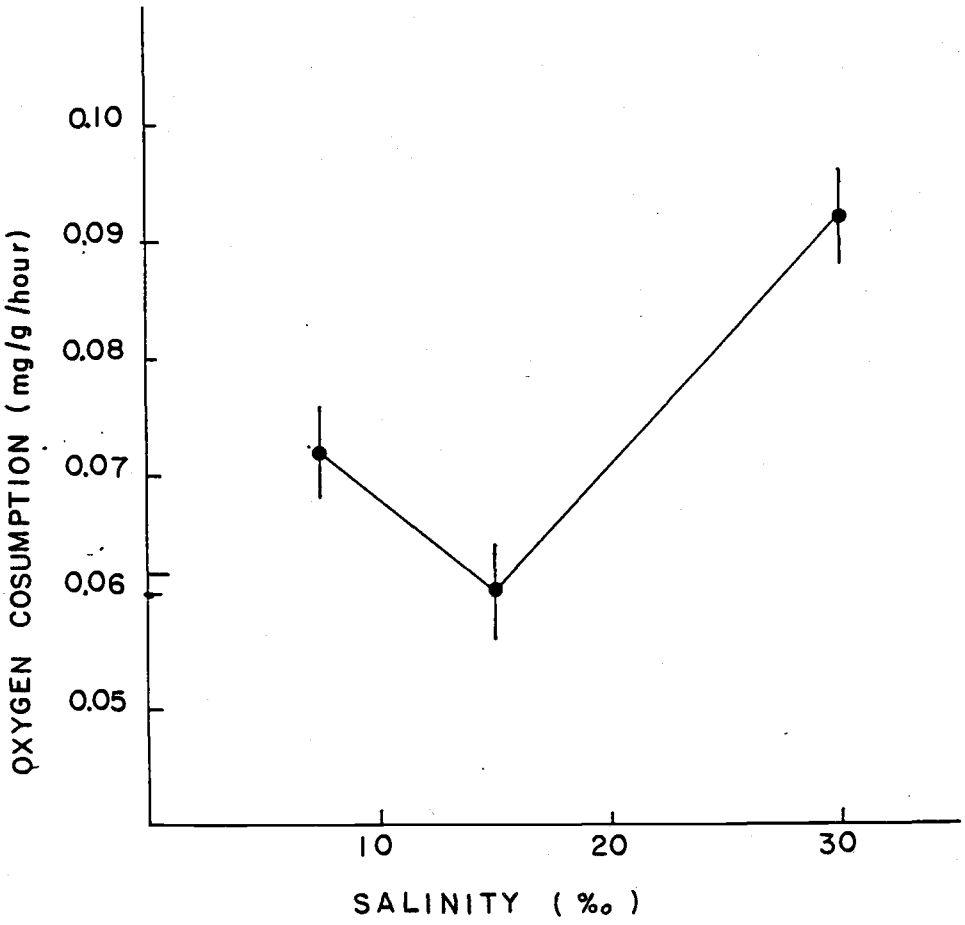

Figure 13. Oxygen consumption of Kelp greenling males at 7.5, 15 and 30 ppt. The circles represent the means. Bars indicate ± SE.

and Duncan's multiple range tests confirmed that all three means of oxygen consumption at the different salinities tested were significantly different from one another ($P < 0.05$).

Table 12 summarizes the total length and body weight measurements of fish utilized in determining oxygen consumption rates at different salinities. Their average total length and body weight were 18.2 cm and 65.60g respectively.

Table 12. Total length, body weight and oxygen consumption of Kelp greenling at different salinities

Salinity									
7.5 ppt			15 ppt			30 ppt			
Total length (cm)	Body weight (g)	Oxygen consumption (mg/g/hr)	Total length (cm)	Body weight (g)	Oxygen consumption (mg/g/hr)	Total length (cm)	Body weight (g)	Oxygen consumption (mg/g/hr)	
17.0	55.39	0.098	16.1	43.10	0.075	20.7	92.04	0.109	
18.7	69.63	0.074	20.6	101.25	0.068	18.1	64.29	0.102	
17.2	51.34	0.072	18.9	73.50	0.066	19.4	80.32	0.099	
18.8	73.85	0.071	18.4	67.50	0.063	17.1	50.36	0.090	
18.4	66.89	0.068	16.6	51.22	0.060	17.4	53.73	0.088	
17.1	56.00	0.065	17.4	64.99	0.053	19.7	82.42	0.087	
17.7	59.95	0.064	19.1	74.05	0.053	17.5	51.83	0.083	
17.2	55.18	0.062	17.5	54.23	0.042	19.2	81.78	0.078	
17.76	61.03	0.072	18.08	66.23	0.060	18.6	69.60	0.092	Mean
0.76	8.1	0.011	1.47	17.92	0.010	1.30	16.46	0.010	Standard Deviation

CHAPTER IV.

DISCUSSION

The physiological responses of fish to changing environmental osmotic stress are most easily measured by following osmotic or ionic changes in the blood. Osmotic concentration of fish blood is maintained relatively constant over a wide range of external salinities. There are comparatively small differences between the blood concentrations of fish endemic to fresh water and those endemic to sea water. Strictly stenohaline fish usually maintain a constant blood concentration of about 300 mOsm/Kg in fresh water and about 400 mOsm/Kg in sea water (Parry 1966). Almost all marine fish have body fluids that are more dilute than sea water, and all fresh water fish have body fluids more concentrated than fresh water.

Kelp greenling were hyperosmotic at a salinity of 7.5 ppt, but at the higher salinities tested became hyposmotic, able to maintain the plasma osmolarity at about 360 mOsm/Kg in seawater. *Tilapia vendalli* were found to be hyperosmotic in salinities below 10 ppt and maintained their plasma osmolarity below 360 mOsm/Kg (Whitfield et al 1976).

The experimental results indicated that Kelp greenling required about 48 hours for stabilization of plasma osmolarity when transferred from sea water

(30 ppt) to a series of 4 salinities ranging from 7.5 to 45 ppt. In contrast, steelhead trout (*Salmo gairdneri*) transferred to sea water required between 80 to 170 hours for the blood concentration to stabilize (Houston 1959). The relatively rapid adaptation of Kelp greenling to changing environmental salinities clearly has survival value. Winter salinities are low and variable in Yaquina Bay, where the fish were caught (Frolander et al 1973).

The plasma osmolarity of Kelp greenling changed from 262 mOsm/Kg at 5 ppt to 455 mOsm/Kg at 45 ppt, a change of 74%. Similarly the starry flounder's (*Platichthys stellatus*) serum osmolarity changed from 306 mOsm/Kg in fresh water to 459 mOsm/Kg at 46 ppt (Hickman 1959). In contrast the adult salmon (*Salmo salar*) showed a change of only 5% in plasma osmolarity between fresh and sea water. In fresh water the blood osmolarity was 328 mOsm/Kg and it changed to 344 mOsm/Kg in sea water (Parry 1961). The Kelp greenling and the starry flounder, moving between the sea and estuaries, showed greater osmotic fluctuations in the blood, compared to the migratory salmonids. This suggests that Kelp greenling have less control of their internal environment and greater tolerance to internal fluctuations.

The increase observed in plasma osmolarity of Kelp greenling with the increase of salinity from 7.5 to 30 ppt was accompanied by a slight increase

in haematocrit from 28 to 30 ppt. Kashiwagi and Sato (1968) reported that the haematocrit values for young (18-month-old) *Oncorhynchus keta* were 36.7% and 47.1% repectively in fresh and salt water and suggested that the fish adapted to salt water with difficulty and that the blood became concentrated through loss of water. Older fish (25-month-old) adapted readily and their haematocrit did not change between the two media.

The moisture content of Kelp greenling was only reduced from 73.9% to 73.1% when the salinity was changed from 7.5 to 30 ppt. Parry (1961) found a reduction from 78% to 68% in water content of muscle fibers in Atlantic salmon *(Salmo salar)* migrating from fresh to sea water. A similar decrease from 79% to 75% in water content of the muscle tissue of brown trout *(Salmo trutta)* was reported by Gordon (1959) while Kinne (1960) found no significant differences in the percentage body water content in *Cyprindon macularius* living in 15 or 35 ppt or fresh water and concluded that the fish was an excellent regulator because it was able to maintain normal body water content over a wide range of salinities. Arunachalam et al (1979) studied the effects of different salinities (0, 2, 4, 6 and 10 ppt) on body composition of the fresh water catfish *(Mystus vittatus)* and found that water content of the fish decreased with increase of salinity,

while maximum fat (42.25%) was exhibited by fish reared in 10 ppt salinity. They reported also that the ash content increased from 21.7 to 25.6% with increase of salinity from 0 to 10 ppt. A small increase in ash values of Kelp greenling was also observed with increase of salinity in the present study.

My results indicated that Kelp greenling do not tolerate salinities greater than 45 ppt. At 50 ppt all fish died after 72 hours of exposure. On the other extreme the fish tolerated a salinity of 5 ppt with no mortality for 8 days, but did not survive in fresh water. With respect to their regulating capacities, Kelp greenling were referred to as "euryhaline osmo-regulators" by Kinne (1964), because of their ability to regulate in water of fluctuating reduced or increased salinities, but require more salt than is available in pure fresh water to survive.

In general, acclimation to low salinities tends to shift the lower lethal limit downward, and acclimation to higher salinities tends to shift the upper limit upward (Kinne 1964). Acclimation of Kelp greenling to low salinity of 7.5 ppt for more than 2 months had no effect on its tolerance to fresh water.

Interestingly, large Kelp greenling (150 g) showed more resistance to abrupt transfer to fresh water than the smaller ones (50 g). The large fish survived for at least 10 hours after they were transferred

to fresh water before they all died, but the smaller ones survived only for less than 5 hours. Nordlie et al (1982) reported that juvenile striped mullet *(Mugil cephalus)* ranging in size from 2.0 to 3.9 cm in standard length were not able to tolerate instantaneous transfer from the brackish water in which they were captured to fresh water, while fish of larger size (4.0 to 6.9 cm standard length) were tolerant. They also reported that at high environmental salinities, the osmotic regulatory capabilities improved with growth in size. The starry flounder *(Platichthys stellatus)* showed a size-dependency in the development of osmotic regulatory capability also (Hickman 1959).

The experimental results indicated that Kelp greenling are both hypotonic and hypertonic regulators, possessing compensating mechanisms for internal osmotic regulation for quick and drastic alteration in the salinity of the external environment. The experimental changes in the salinity of the external environment were also accompanied by significant changes in the standard metabolic activity of the fish. Kelp greenling at 7.5 and 30 ppt consumed more oxygen than those at 15 ppt. The metabolic cost of maintaining water balance and ion exchange probably accounts for the increased oxygen consumption at high and low salinities. The magnitude and not the direction of the osmotic gradient is of importance in the energy requirements

for osmoregulation. The osmolarity of water at 15 ppt was closest to the plasma osmolarity of Kelp greenling, thus energy spent in osmoregulation of fish at 15 ppt was relatively low when compared to fish at higher or lower salinities. Similarly, rainbow trout *(Salmo gairdneri)* had the lowest rate of oxygen consumption at salinities isosmotic with their blood (Rao 1968). Farmer and Beamish (1969) measured oxygen consumption of *Tilapia nilotica* for various swimming speeds at a series of salinities ranging from 0 to 30 ppt. They found that oxygen consumption values at 11.6 ppt were lowest and those at 30 ppt highest and presumed energy required for osmoregulation was least in the absence of an osmotic gradient (11.6 ppt) and greatest when the osmotic gradient was highest (30 ppt). Approximately 29% of the total oxygen consumption was required for osmoregulation at 30 ppt according to their estimates. Oxygen consumption of Kelp greenling was approximately 35% less on the average at 15 ppt than it was at 30 ppt. Results from this study support their findings: as the osmotic gradient decreased between plasma of the fish and the external environment, oxygen consumption decreased. Hickman (1959) estimated standard oxygen consumption of the starry flounder *(Platichthys stellatus)* and concluded that in high salinity a large proportion of the animal's minimum energy requirements must be devoted to maintaining homoiosmoticity.

He stated that the different metabolic rates at 0 and 25 ppt were related to the metabolic demands for transportation of electrolytes and water in the principal organs of exchange; the gills, kidney and gut.

Generally, oxygen consumption rate of euryhaline fish is lowest in an iso-osmotic dilution of sea water, which presumably is a reflection of minimal osmoregulatory work that exists in a condition of minimal osmotic gradient between the environment and body fluids (Rao 1968, 1971; Farmer and Beamish 1969; Frame 1973). However, many investigators have found that salinity has either no effect (Muir and Niimi 1972; Courtois 1976) or opposite effect (Hickman 1959; Job 1969a, b) on oxygen consumption rate of euryhaline fish.

The assessment of salinity effects on oxygen consumption is complicated by the fact that the oxygen content of water depends on its salinity; the higher the salinity the lower the oxygen content. As oxygen uptake of fishes is, to a large extent, governed by the concentration of oxygen in the water, it is often difficult to determine the responses due strictly to salinity.

Phaloheimo and Dickie (1966) speculated an increase in metabolic rate to maintain osmotic equilbrium took place at the expense of energy for growth.

My study supports this hypothesis, since Kelp greenling grew best at 15 ppt as a result of the low costs of osmoregulation. The growth of Atlantic menhaden *(Brevoorita tyrannas)* was found to be faster in low salinity (5-10 ppt) when compared to high salinity (23-34 ppt) (Hettler 1974). Atlantic menhaden was found to be isosmotic with sea water of 12 ppt (Hettler et al 1973).

Canagaratnam (1959) studied growth of two series of coho fry *(Oncorhynchus kitsutch)*. In the first series, a group of fish was maintained at each of 3 salinities (0, 6, and 12 ppt); in the second series, groups of the same size were followed at 0 and 18 ppt. He found that the percentage increase in body weight was highest in the 12 ppt salinity in the first series and at 18 ppt in the second.

Salinity affected the growth and food conversion of Kelp greenling. DeSilva and Perera (1976) reported similar findings when they studied the effects of different levels of salinity (30, 20, 10 and less than 1 ppt) on the growth, food intake and food conversion efficiency of young grey mullet *(Mugil cephalus)*. The maximum growth was found to occur at 20 ppt. The percentage conversion efficiency of fish fed on excess diet at 10 ppt was the highest and when a constant ration was given the percentage conversion efficiency was found to decrease with increasing salinity.

Kinne (1964) reported that changes in total osmolarity of the surrounding water not only affect the growth rate, but also the efficiency of metabolic processes. In different salinities a given amount of food may be converted by fish into different amounts of body substance and energy available for biological processes.

The responses of fish to salinity are often the net result of a number of factors; these include not only the osmolarity and salt content of the water but also associated variables such as oxygen content, specific gravity of the water and its ionic composition.

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