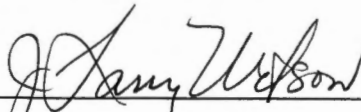


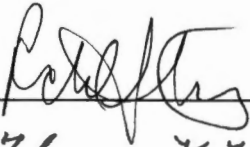
To the Graduate Council:

I am submitting herewith a thesis written by Jingjuan Gong entitled "Effects of Dietary Sodium Chloride on Chloride Cell Development and Growth of Rainbow Trout". I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Wildlife and Fisheries Science.



Dr. J. Larry Wilson, Major Professor

We have read this thesis
And recommended its acceptance:



Thomas E. Hill

Accepted for the Council:



Associate Vice Chancellor and
Dean of The Graduate School

**Effects of Dietary Sodium Chloride on Chloride Cell Development and
Growth of Rainbow Trout**

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Jingjuan Gong

August 1999

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Thesis
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DEDICATION

This thesis is dedicated to my parents

Mr. Mingfeng Gong

and

Mrs. Xiaoling Luo

Who have given me invaluable educational opportunities.

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ABSTRACT

The effects of increased dietary salt on growth and morphology and numbers of chloride cells of rainbow trout (*Oncorhynchus mykiss*) were investigated using three isonitrogenous and isocaloric diets containing 1.5, 4.5 and 10.5% sodium chloride, respectively. The basal trout diet contained approximately 0.7 - 0.8% sodium chloride; there was no nutrient dilution in any of the diets caused by salt addition.

The added salt did not affect growth rate and food conversion efficiency of the fish. The numbers of chloride cells in the interlamellar region decreased significantly as the salt level in the diet increased, while the numbers of chloride cells at the base of the lamella increased only slightly with the increase of salt level. The total chloride cell numbers, including cells in the interlamellar region and at the base of the lamella, increased somewhat as the salt level in the diet increased. Fish fed on 4.5% and 10.5% of salt had significantly higher numbers of chloride cells than those of fish fed on the control diet after six weeks of feeding. The size of chloride cells at the base of the lamella did not differ among different treatment groups. Total numbers of chloride cells in fish fed 4.5 and 10.5% NaCl were significantly higher than the control groups; however, there were no statistical differences among the treatment diet groups with regard to chloride cell numbers. It was concluded that the dietary salt did not significantly affect either the fish growth or chloride cell size of yearling rainbow trout.

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CHAPTER I

INTRODUCTION

The early development of fish culture in the United States centered primarily on the propagation of trout and salmon (Davis 1965). More recently, in the southeastern United States, three main species of freshwater fish are cultured: channel catfish (*Ictalurus punctatus*), crawfish (*Procambarus* spp.), and rainbow trout (*Oncorhynchus mykiss*). Striped bass (*Morone saxatilis*) and tilapia (*Tilapia* spp.) are also cultured in this area of the country. The trout industry in the South has developed mostly in the southern Appalachian area, and depends primarily on diverted springs for water supplies (Hinshaw 1992).

The growth of trout production in Tennessee has been part of a regional expansion in the southeastern Appalachians, currently being led by North Carolina. The trout producers of Tennessee are mainly located in Middle, Upper East, and Lower East Tennessee, and on the Cumberland Plateau; no trout production is feasible in West Tennessee. Trout producers utilize surface runoff from mountain streams or cold-water springs, a natural resource of relative abundance in Tennessee (Gockowski and Keller 1988).

Commercial trout production depends on several factors: water flow, volume, temperature, oxygen content, and pH level (Gockowski and Keller 1988). The production capacity of a water source is limited by the low levels of discharge during the fall months. The average temperature of the springs utilized by Tennessee trout producers ranges from 54-59 F. At these temperatures, trout can be raised intensively and in relatively high densities. The

saturation level of dissolved oxygen at 56 F is approximately 10.5 mg/L which allows higher stocking rates than water at higher temperatures and lower dissolved oxygen saturation levels (Leitritz and Lewis 1976). Based on a survey by Gockowski and Keller (1988), the average pH of commercial trout springs in Tennessee was a slightly alkaline 7.1 with the majority (80%) of springs having a pH of 7.0 or above and the remainder between 6.0-6.9; the range of pH values measured in the survey was 6.0 to 7.5.

The acidification of fresh water has become a serious environmental problem in some areas of America (Fromm 1980, Adams et al. 1985). Trout farms in western North Carolina supplied with very soft water have suffered increased mortality rates associated with pulses of low pH water (as low as 4.5) following precipitation (Hinshaw, unpublished data).

Fish maintain osmotic and acid/base equilibrium via a sophisticated branchial mechanism (McDonald 1983). The chloride cell of fish branchial epithelium has been reported as the site of active ion exchange (Avella et al. 1987). Sodium ions are exchanged for H^+ or NH_4^+ , and Cl^- is exchanged for HCO_3^- (McDonald 1983). Increasing the salinity of the ambient water is protective in low pH environments by reducing the ionic gradient, and by providing Na^+ ions to exchange for H^+ (NH_4^+) across the gill membrane (Cameron and Iwama 1989). Manipulating the internal electrolyte levels may be partially protective in low pH conditions (Hinshaw, unpublished data).

The numbers and size of chloride cells have been reported to increase in response to either sea water or soft water (Laurent and Hebibi 1989, Kultz et al. 1992). After adaptation to sea water, fish increase the number and size of chloride cells to secrete the excess salt in response to increased external NaCl. Fish adapted to soft water respond to the ion poor medium by producing more chloride cells to enhance ionic uptake from the

water (Perry and Laurent 1989). Salman and Eddy (1987) concluded that chloride cell numbers increased with the level of dietary NaCl to excrete the excessive internal NaCl.

The objective of this study was to investigate the relationship of chloride cell numbers and sizes in response to an increase in dietary NaCl, and the effect of the dietary NaCl on the growth of rainbow trout. In this study, it was hypothesized that: (1) rainbow trout fed higher levels of NaCl would have more and larger chloride cells than rainbow trout fed a basal level of NaCl, and (2) the dietary NaCl would not be detrimental to the growth of rainbow trout. If dietary NaCl does increase the proliferation of chloride cells and enhance osmoregulatory function, it could help mitigate environmental stresses on rainbow trout which are exposed to the low pH environment by altering their internal electrolyte milieu.

CHAPTER II

LITERATURE REVIEW

Salt Feeding and Effect on Growth of Fish

The feeding of salt-enriched diets has been mainly used for improving the survival of freshwater fish being transferred to seawater (Zaugg and McLain 1969, Basulto 1976, Zaugg et al. 1983). A numbers of studies have noted that fish fed supplemental salt have adapted to full strength sea water with fewer mortalities than those fish receiving a normal diet. The mechanism of this process can be briefly described as follows: Fish living in a marine environment must be able to excrete large quantities of inorganic ions in order to maintain a suitable electrolyte balance by using a highly efficient sodium pump, i.e., Na^+/K^+ - stimulated ATPase activities (Zaugg and McLain 1969). The dietary sodium chloride produces an increase in Na^+/K^+ -ATPase; therefore, salt feeding increases the seawater adaptability of freshwater fish.

Researchers have also found that supplemental dietary salts influence growth, osmoregulation, gill morphology, and ionic fluxes as well as kidney function of fish (Laurent and Hebibi 1989). However, the effects of salt feeding on growth were not consistent. Zaugg and McLain (1969) found that growth and food conversion efficiency were reduced in young coho salmon fed diets containing from 1.5 to 12% of sodium chloride. Salman and Eddy (1990) indicated that rainbow trout fed on salt diets with NaCl at 1.3, 4.5, 9.2, and 11.6% showed lower food conversion efficiency and growth rate due to nutrient dilution. In the similar study, Salman and Eddy (1988) found that food conversion efficiency of fish fed the

isonitrogenous diets was not affected by the added salt. Ogino and Kamizono (1975) noted that the optimal content of the salt mixture in the diet was proven to be 4-5% for young rainbow trout; furthermore, the diet deficient in the salt-mixture resulted in retarded growth, high mortality, anemia, and malformation of the head.

The conflicting results noted in these studies may have been due to differences in diet preparation. In some studies, dietary protein was removed as the level of NaCl increased in the diets; this subsequently caused nutrient dilution.

Chloride Cells

The maintenance of the osmotic, ionic balance is an important function of the fish gill. Numerous studies have indicated that fish regulate their osmotic and ionic balance either by the uptake of ions from the environment or by the excretion of excess endogenous ions via the gills (Evans 1980). This process is always accompanied by morphological changes in the gill. One of the most often studied and physiologically significant changes involves variations in the number and /or size of the chloride cells.

Keys and Willmer (1932) found the chloride secreting activity of a specific kind of cell in teleost gills. These large, ovoid cells in the gills of these fish resembled the oxyntic cells of the mammalian stomach; their function was the secretion of chloride and were therefore termed “chloride-secreting cells”. Later, these cells were called chloride cells (Copeland 1948). In marine fish, the branchial and opercular chloride cells are the sites of active NaCl efflux (Karnaky et al. 1977, Foskett and Scheffey 1982). In freshwater fish, chloride cells play an important role in maintaining a balanced internal ionic environment by absorbing Na^+ and Cl^- (Laurent and Hebibi 1989, Laurent and Perry 1990). Chloride

cells are characterized typically by the presence of large, well-developed mitochondria, so the chloride cell is also referred to as a mitochondria-rich cell.

Teleosts, the bony fishes, possess four gill arches on each side of the body. Projecting from each of these gill arches are hundreds of gill filaments with each filament having numerous branching lamellae. The filament and lamella are also termed the primary lamella (PL) and secondary lamella (SL) in the older literature (Laurent and Dunel 1980). Chloride cells are largely confined to the trailing edge of the filament epithelium and in particular are concentrated in the interlamellar regions and at the junctions between the filament and lamella.

Changes in branchial chloride cell numbers and sizes under challenging environments are well documented (Laurent and Dunel 1980, Krous et al. 1982, Laurent and Hebibi 1989, Perry and Laurent 1989). The proliferation of branchial chloride cells occurs on both the filament and lamellar epithelia in response to environmental conditions such as ion-poor (soft) water; this causes an increase in the ion-transporting capacity of the gill (Bindon et al. 1994). Na^+/K^+ -ATPase activity, chloride cell number, and cell size increased after seawater adaptation of fish (Kultz et al. 1992). Laurent and Hebibi (1989) found that in fish adapted to Strasbourg tap water, few chloride cells were present on the lamella. After adaptation to sea water, chloride cells were present at the base of the lamella (trailing edge only) and the numbers of chloride cells on filament epithelium increased significantly. Adaptation to ion-poor water causes chloride cells to proliferate on both the filament and lamellar epithelia. They indicated that there was no difference in morphology or size between filament and lamellar chloride cells, except that they were oriented horizontally in the lamellar epithelium and vertically in the filament

epithelium. In the same study, the mean linear dimensions and surface area of chloride cell section profiles indicated that these cells decreased in size when fish were adapted to sea water. Salman and Eddy (1987) fed rainbow trout dietary NaCl up to a level of 12% and found a positive linear relationship between the increased level of salt in the diet and both chloride cells numbers and gill Na^+/K^+ -ATPase activity.

CHAPTER III

METHODOLOGY

Fish Rearing

Three hundred and sixty yearling rainbow trout were obtained from the Tennessee Wildlife Resources Agency (TWRA) hatchery at Buffalo Springs. The average weight of each fish was 115 grams and the average length was 21.9 centimeters. A flow-through rearing system (Figure 1) was housed in the University of Tennessee Institute of Agriculture (UTIA) Cherokee Animal Facility. The system included three rectangular fiberglass troughs, each measuring 3.05 x 0.62 x 0.45 meters, and had a continuous flow of dechlorinated municipal water at a flow rate of 38 L/min passing through them. Each trough was divided into four individual rearing compartments with each section measuring approximately 0.61 x 0.62 x 0.45 meters. Each compartment contained approximately 170 liters of water and held 30 trout.

Fish were acclimated to trough conditions for two months prior to the initiation of the feeding trials. During the acclimation period, all fish were fed commercial trout pellet. After acclimation, fish were fed experimental diets containing additional NaCl levels of 1.5, 4.5, and 10.5 %; the control group was maintained on a control test diet containing approximately 0.7-0.8% sodium chloride.

The feeding rate for all treatments ranged from 1.3 to 1.4% of body weight. These rates were based on recommended amounts of dry feed for trout for different size groups at different temperatures (Piper et al. 1982). The specified amount of feed was provided twice

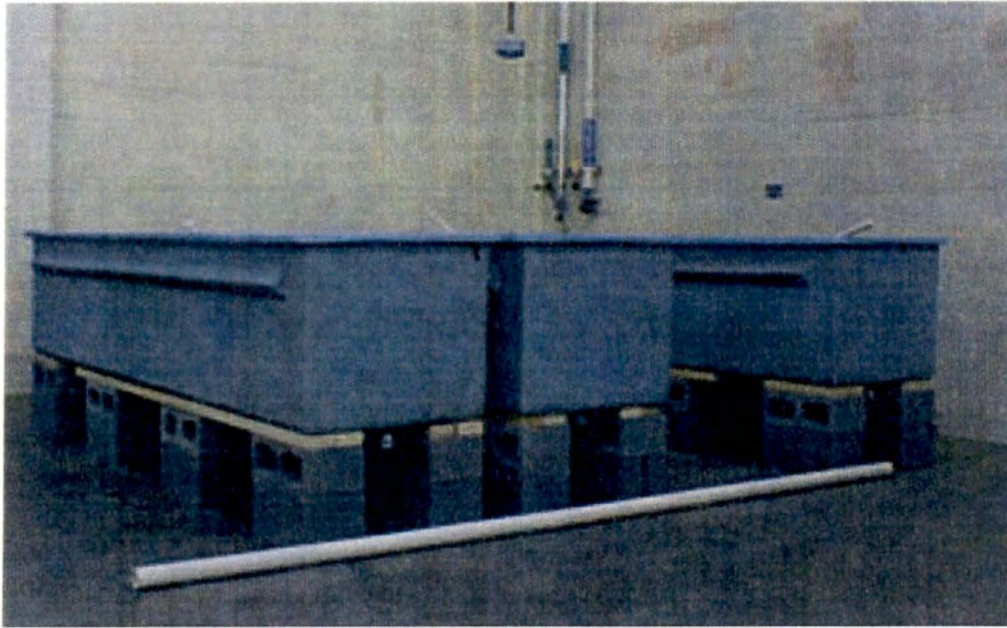


Figure 1. Flow-through rearing system housed in the University of Tennessee Institute of Agriculture (UTIA) Cherokee Animal Facility.

daily, in the morning and evening, six days a week. Water quality was checked daily; water temperature was maintained at 58 F and the dissolved oxygen was kept at 12 mg/l.

Experimental Diets

Experimental diets were formulated by Purina Mills Inc. and mixed and pelleted at the Purina TestDiet® facility (Richmond, IN). Diets were identical with respect to basic ingredients and were identically prepared except for added levels of sodium chloride, i.e., 1.5, 4.5, and 10.5%. The basal diet contained approximately 0.7-0.8% sodium chloride (M.Griffin, Purina Mills Inc., personal communication). Diets were isonitrogenous and isocaloric, thereby avoiding nutrient dilution caused by the removal of protein as the level of NaCl increased.

Experimental Design

A randomized block design with four treatments and three replicate troughs (blocks) was used in this experiment (Table 1). The treatments were the basal control diet and three test diets supplemented with different levels of sodium chloride, i.e., 1.5, 4.5, and 10.5%. Each of the four treatments was randomly assigned to one of the four compartments within each trough.

Table 1. Summary of experimental feeding trials with salt-enriched (NaCl) diets

Treatment group (% added NaCl)	1.5	4.5	10.5	Control
No. of fish/treatment	90	90	90	90
Mean wt. of fish (g)	114.4	117.7	113.2	114.7
Duration (weeks)	6	6	6	6

Experimental Parameters

Determination of growth rate and feed conversion

Growth data (length and weight) were collected every two weeks from the onset of the experiment; fifteen fish were sampled from each treatment and individually weighed (grams) and measured in total length (centimeters). The growth rate and feed conversion values were calculated using the following formulas:

a. $\text{Growth rate (g/week)} = (W_f - W_i) / N,$

where W_f is the final weight of the fish, W_i is the initial weight of the fish, and N is the number of weeks of feeding

b. $\text{Feed conversion} = \text{dry weight feed (g)} / \text{wet weight gain (g)}$

Determination of chloride cell number and size

Chloride cell data were collected from treated fish at two-week intervals during the study. At the onset of the experiment, three fish per treatment were used for data collection of chloride cell size and number. After the salt feeding began, six fish per treatment were used. Fish were euthanized with an overdose (100 mg/L) of MS-222 and the fish head was immediately immersed into a 10% neutral buffered formalin (BNF) solution. After several minutes, all fish were beheaded with all gill arches intact and fixed in the BNF for 48 hours. The central portion of the second left gill arch from each fish was used as the standard reference point for comparisons. Each piece of the arch contained several pairs of filaments. After fixation, gill tissue samples were dehydrated using alcohol, cleaned by xylene, infiltrated with paraffin and embedded in paraffin wax, and were sectioned on a A/O A20 rotary microtome. The 5.0 μm -thick sections were stained with a hematoxylin and eosin (H&E) stain (Trombetti et al. 1996). The stained sections were observed at 400X magnification with a Fisher Scientific Compound light microscope.

Chloride cells are large round-shaped cells located mainly at the base of the lamella on the trailing edge of the filament or in the interlamellar regions (Figure 2). The first 25 pairs of lamellae were always examined for chloride cells and five filaments were examined for each fish. The chloride cells at the base of the lamella and in the interlamellar region were counted separately. The total numbers of chloride cells for each fish was expressed as the sum of the numbers of chloride cells in the interlamellar region and at the base of the lamella.



Figure 2. A stained cross-section (200X) through the gill filament of a yearling rainbow trout showing: (a) chloride cell at the base of lamella, (b) filament, (c) lamella, (d) chloride cell in the interlamellar region, (e) interlamellar region

Chloride cells at the base of the lamella (Figure 3) were measured with a light microscope fitted with an objectmicrometer; the calibration value for 400X magnification was 2.5 μ m. Ten cells from each of six fish per treatment group were measured (in diameter) to the nearest 0.01 μ m. The longest possible distance between the two poles of the round-shaped chloride cell was measured as the cell diameter.

Statistical Analyses

Statistical analyses were performed using SAS (SAS 1997). Analysis of variance was used to test for treatment and week (time) differences. The Least Significant

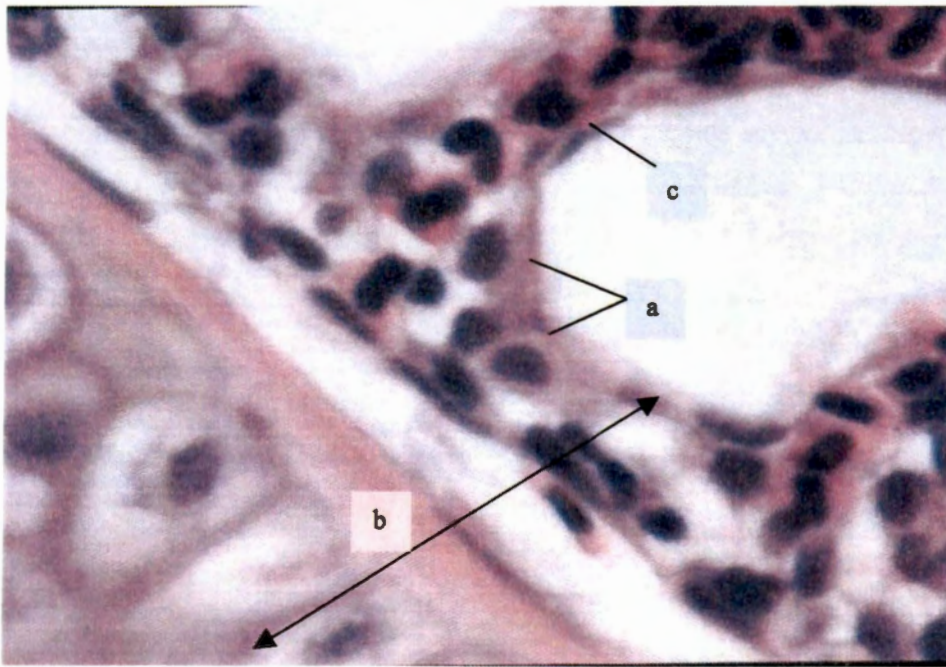


Figure 3. Magnified section (600X) of gill filament of rainbow trout showing (a) chloride cell at base of lamella, (b) filament, (c) lamella

Difference test (Sokal and Rohlf 1981) was used to compare means. All values were considered significant at the $P < 0.05$ level.

CHAPTER IV

RESULTS

Growth Rate

The effects of different levels of dietary NaCl on somatic growth rate are presented in Table 2. Mean somatic growth rates among the four treatments did not differ significantly ($F = 0.51$; $P > 0.05$). Fish fed at the 10.5% NaCl level had the highest mean somatic growth rate (9.94 ± 1.70). The effects of different levels of dietary NaCl and time period on the somatic growth rates are presented in Figure 4.

Table 2. Mean somatic growth rates of rainbow trout fed four levels of dietary NaCl (control and 1.5, 4.5, and 10.5%) during the 6-week study. All values reported as the mean $\pm$ SE. Within a column, values without a letter in common were significantly different.

% Added NaCl	Time (weeks)	Somatic growth rate (g/ week)	Mean somatic growth rate(g/ week)
1.5	0-2	$11.65 \pm 3.86abc$	$8.12 \pm 1.70a$
	2-4	$8.78 \pm 3.86abc$	
	4-6	$3.93 \pm 3.86bcd$	
4.5	0-2	$7.57 \pm 3.86abc$	$8.12 \pm 1.70a$
	2-4	$9.99 \pm 3.86abc$	
	4-6	$6.81 \pm 3.86abc$	
10.5	0-2	$13.01 \pm 3.86abc$	$9.94 \pm 1.70a$
	2-4	$1.97 \pm 3.86cd$	
	4-6	$14.83 \pm 3.86a$	
Control	0-2	$14.38 \pm 3.86ab$	$7.01 \pm 1.70a$
	2-4	$11.05 \pm 3.86abc$	
	4-6	$-4.39 \pm 3.86d$	

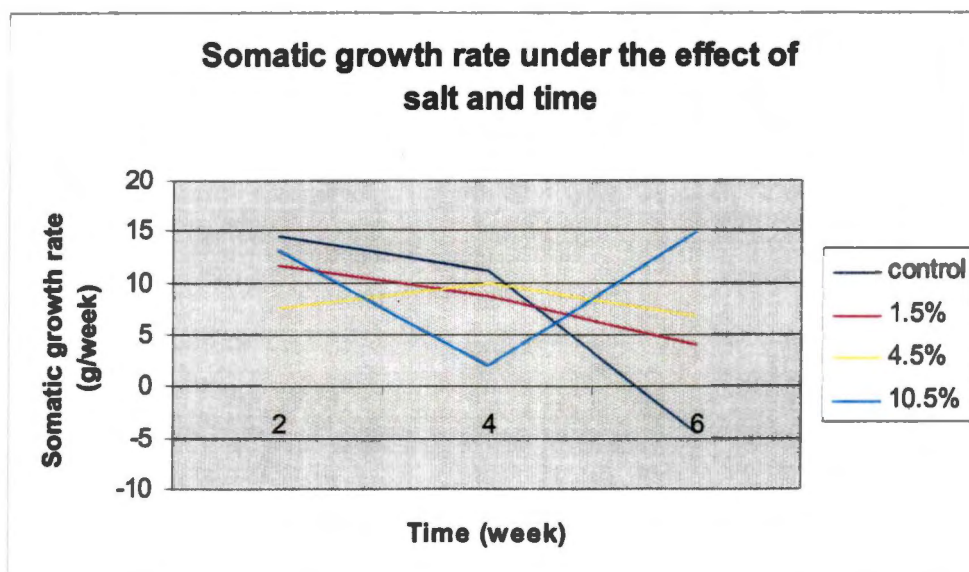


Figure 4. Somatic growth rate of yearling rainbow trout fed diets containing four salt levels (control and 1.5, 4.5, and 10.5% NaCl) during the 6-week study.

Food Conversion

The effects of four different levels of dietary salt on the food conversion are presented in Table 3. The highest food conversion efficiency was found in fish fed the 4.5% level of dietary salt; however, there were no significant differences among all the treatment diets.

Number and Size of Chloride Cells

Chloride cells had the typical shape, size, and location as described in the literature for branchial chloride cells (Laurent and Dunel 1980; Zadunaisky 1984). The number of chloride cells in the interlamellar region decreased significantly with an increase of salt level in the diet among experimental groups while the control group had the lowest number of chloride cells (Table 4). Fish fed at the 1.5% level of NaCl had the highest

Table 3. Mean food conversion efficiency of rainbow trout fed four levels of dietary salt (control and 1.5, 4.5, 10.5% NaCl) for six weeks. Food conversion rates are reported as the mean $\pm$ SE. Within a column, values without a letter in common were significantly different.

Diet (% added NaCl)	Food Conversion
1.5	1.17 $\pm$ 0.14a
4.5	1.32 $\pm$ 0.14a
10.5	1.26 $\pm$ 0.14a
Control	1.13 $\pm$ 0.14a

Table 4. Mean numbers and diameter of chloride cells from rainbow trout fed four levels of dietary salt (control and 1.5, 4.5, 10.5% NaCl) for six weeks. The total number of chloride cells is the sum of numbers in the interlamellar region and at the base of the lamella. Values are reported as the mean $\pm$ SE. Within a column, values without a letter in common were significantly different.

NaCl Level (% added)	1.5	4.5	10.5	Control
No. of chloride cells				
Interlamellar	20.66 $\pm$ 1.16a	18.53 $\pm$ 1.16ab	16.91 $\pm$ 1.16bc	14.41 $\pm$ 1.16c
Base of lamella	48.15 $\pm$ 1.68b	52.20 $\pm$ 1.68ab	53.88 $\pm$ 1.68a	49.39 $\pm$ 0.68ab
Total	68.81 $\pm$ 2.01ab	70.73 $\pm$ 2.01a	70.78 $\pm$ 2.01a	63.80 $\pm$ 2.01b
Diameter (μ m)				
	10.26 $\pm$ 0.17a	10.40 $\pm$ 0.16a	10.37 $\pm$ 0.16a	9.93 $\pm$ 0.17a

number of chloride cells (20.66 ± 1.16). The number of chloride cells at the base of the lamella increased with an increase in salt level among the experimental groups, but the differences were not statistically significant. When the numbers of chloride cells in the interlamellar region and at the base of the lamella were added together, the total number of chloride cells increased as the level of salt in the diet increased (Figure 5). The numbers of chloride cells of fish fed at the 4.5 and 10.5% levels of dietary salt were significantly higher than the control group. The highest number of chloride cells was 70.78 ± 2.01 and was found in the highest salt-level diet (10.5% NaCl). However, there were no statistically significant differences among the three treatment levels of salt feeding (1.5, 4.5, and 10.5%).

The mean diameters of chloride cells were not significantly different among the treatment groups but were different across the time period. The size of chloride cells increased by 50% and then decreased gradually after two and four weeks of salt feeding. However, since the control group displayed the same pattern of changes over time, these changes were not in response to the dietary salt.

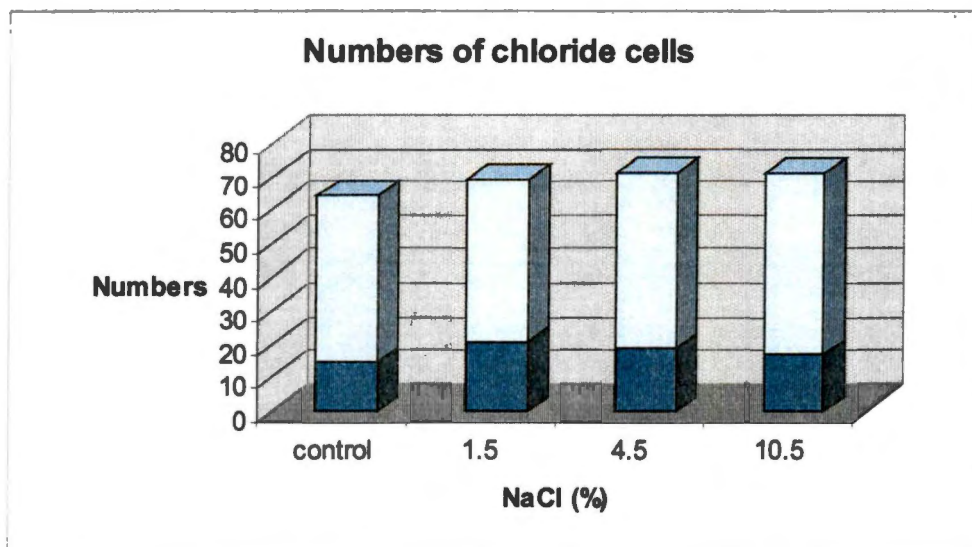


Figure 5. Numbers of chloride cells from rainbow trout fed four different levels of dietary salt (control and 1.5, 4.5, 10.5% NaCl) during the 6-week study. The lower bar represents chloride cell numbers in the interlamellar region and the upper bar represents chloride cell numbers at the base of the lamella. The whole bar represents the total numbers of chloride cells.

CHAPTER V

DISCUSSION

Effect of Dietary Salt on Growth of Rainbow Trout

Although there are contradictory results concerning the effects of dietary salt on growth and feeding activities of fish (Zaugg and McLain 1969, Shaw et al. 1975, Basulto 1976, MacLeod 1978, Wilson et al. 1985), the general consensus in the literature is that dietary NaCl inversely affects growth rate when the level of supplemental salt interferes with the balance of other essential dietary components such as protein (Salman and Eddy 1988). Therefore, the conflicting results noted in those previous studies may have been due to differences in diet preparation. In preparing the diet for the present study, the dietary protein was not removed as the level of NaCl increased as in some previous investigations. Thus, the isonitrogenous/isocaloric diets used in this study prevented nutritional dilution effects caused by dietary supplementation with salt.

The results of this study indicated that supplemental dietary NaCl, up to the level of 10.5%, had no significant effects on the growth rate and food conversion efficiency of yearling rainbow trout when isonitrogenous/isocaloric diets were used. These results agreed with several previous findings. Salman and Eddy (1988) reported that dietary NaCl up to the level of 11.6% did not affect the growth of rainbow trout using isonitrogenous/isocaloric diets; the dietary NaCl was found to be fully absorbed by rainbow trout (Salman and Eddy 1987). No differences were observed in growth rates of channel catfish fed experimental diets containing various levels of sodium chloride up to

2% (Murray and Andrews 1979). Musselman et al. (1995) found that mean growth rates did not differ for juvenile bluegill fed diets containing 0, 2, and 4% dietary NaCl. MacLeod (1978) fed rainbow trout commercial diets containing 8.5% of added sodium chloride with no significant effect on growth. Shaw et al. (1975) fed diets containing up to 12% sodium chloride to Atlantic salmon reared in both fresh water and sea water with no apparent effect.

Some researchers have also reported that moderate levels of salt mixture (approximately 4%) have a beneficial effect on growth. Ogino and Kamizono (1975) suggested such an effect to promote growth of carp and trout. Dietary NaCl levels of 1-4% are commonly used in salmon diets and such supplements seem to be useful in trout food as well (Tacon and De Silva 1983). In this study, increasing the level of NaCl in the diet appeared to have no significant effect on somatic growth of rainbow trout.

Effect of Dietary Salt on Chloride Cells

According to Salman and Eddy (1987), most of the dietary salt absorbed into the blood is excreted via the gills, with the indication that chloride cells and Na^+/K^+ -ATPase are involved in this process. They found that increasing dietary salt resulted in an increase in chloride cell numbers, an increase in chloride cells as a proportion of gill cells, and an increase in branchial Na^+/K^+ -ATPase activity. Numbers of chloride cells of fish fed at 9 and 12% salt in the diet were significantly higher than in the control group. In the present study, the total numbers of chloride cells in rainbow trout (i.e., by summing the numbers of chloride cells in both the interlamellar region and at the base of the lamella) increased with an increase in salt levels. However, there were different responses

in the numbers of chloride cells in the interlamellar region and at the base of the lamella with the increasing salt level. For the interlamellar region, the numbers of chloride cells decreased significantly with the increasing level of salt while the numbers of chloride cells at the base of the lamella increased (but not significantly) with the increasing level of salt. However, the increase in total chloride cell numbers is consistent with Salman and Eddy's (1987) results. The total numbers of chloride cells of fish fed at 4.5% and 10.5% levels of NaCl were significantly higher than those of fish fed on the control diet while there were no significant differences among the three treatment levels of NaCl (1.5, 4.5, and 10.5%).

Increased numbers of chloride cells in freshwater fish have been noticed in response to external environments such as: seawater (Laurent and Dunel 1980), lowered external NaCl concentration (Perry and Laurent 1989), or the water of very low ionic content or high nitrite level (Krous et al. 1982). The number of chloride cells of rainbow trout adapted to sea water is larger than in fresh water in order to extrude the excess ions (Laurent and Hebibi 1989). An important adaptational response of rainbow trout to low environmental NaCl is enlargement of branchial epithelial chloride cells which, in turn, enhances the NaCl-transporting capacity of the gill as a result of the proliferation of Na^+ and Cl^- transport sites (Perry and Laurent 1989). Krous et al. (1982) exposed rainbow trout to water with a nitrite concentration of 175 mg/L and reported that nitrite may be transported across gill epithelia by means of the branchial anion exchange mechanism attributed to lamellar chloride cells in fresh water. A fish in soft water (ion-poor water) is in an "ion depleted" medium and responds by producing more chloride cells, or cells resembling chloride cells, presumably to enhance ionic uptake from the water (Salman

and Eddy 1987, Perry and Laurent 1989). Salman and Eddy (1987) found that, similar to the response to seawater transfer, rainbow trout fed the high level of salt showed an increase in numbers of chloride cells. These suggestions together with the present results indicate that chloride cells are involved in salt transport in freshwater fish, not only in ionic absorption, but also in ionic secretion. The increase of chloride cell numbers in freshwater fish fed on salt enriched diet is the response to the increasing dietary salt and the need for excretion of excess internal NaCl.

Numerous studies have indicated the change of chloride cell size of both freshwater and marine fish in response to different environments. In freshwater fish, the chloride cell is the site of Na^+ and Cl^- ion uptake. Several studies (Laurent and Hebibi 1989; Perry and Laurent 1989,1993) have established a positive correlation between Na^+ and Cl^- influxes and the fractional apical surface area of chloride cells on the filament. Physiological or environmental circumstances that promote internal electrolyte loss are associated with increased density of chloride cells as well as with the size of individual chloride cells. Laurent and Hebibi (1989) found that chloride cells of rainbow trout increased in size when the fish were adapted to ion-poor water.

Chloride cells of marine fish have been reported to be larger than chloride cells of freshwater fish in several non-salmonid species (Shirai and Utida 1970). Langdon (1984) found that saltwater adaptation induced proliferation and enlargement of the chloride cells of Atlantic salmon. His study indicated that the increase in mean chloride cell size in fish transferred to salt water was evident after 14 days but not after 24 hours; therefore, this presumably reflected the developing functions of these cells in salt water rather than an acute osmotic swelling effect.

However, no literature was found to show that chloride cells change size in response to oral NaCl. In the present study, there were no significant differences in cell sizes among the different treatment groups.

CHAPTER VI

SUMMARY

In the present study, the effects of dietary sodium chloride on growth rate, food conversion efficiency, and chloride cell number and size were studied. Rainbow trout were fed at different levels of dietary sodium chloride (1.5, 4.5, and 10.5% NaCl and control) in a flow-through rearing system.

The major findings from this study are:

1. There were no significant differences ($P>0.05$) in somatic growth rates of fish fed three different levels of dietary salt and the control diet.
2. Food conversion efficiency did not differ significantly ($P>0.05$) among the four treatment groups.
3. The numbers of chloride cells in the interlamellar region decreased significantly ($P<0.05$) with the increase of salt level.
4. The number of chloride cells at the base of the lamella increased slightly ($P>0.05$) with the increase of salt level in the diet.
5. The total numbers of chloride cells (including both numbers of chloride cells in the interlamellar region and at the base of the lamella) increased as the level of salt in diet increased but no significant differences were found among three treatment levels of NaCl (1.5, 4.5, and 10.5%). The numbers of chloride cells fed on 4.5% and 10.5% of dietary salt were significantly higher than chloride cell

numbers of fish fed on the control diet. However, there was no significant difference between fish fed on 1.5% NaCl and the control diet.

6. The mean diameters of chloride cells were not significantly different among the four different treatment groups.

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