PARR-SMOLT TRANSFORMATION IN NONANADROMOUS ATLANTIC SALMON SALMO SALAR AND EFFECTS OF SEXUAL MATURATION ON THE PARR-SMOLT TRANSFORMATION IN MALE ANADROMOUS ATLANTIC SALMON

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PARR-SMOLT TRANSFORMATION IN NONANADROMOUS ATLANTIC SALMON *Salmo salar* AND EFFECTS OF SEXUAL MATURATION ON THE PARR-SMOLT TRANSFORMATION IN MALE ANADROMOUS ATLANTIC SALMON

BY

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ABSTRACT

The parr-smolt transformation was compared in male and female anadromous (SRxSR), nonanadromous (LLxLL) and hybrid (SRxLL) Atlantic salmon, *Salmo salar*. Seasonal patterns in total lipid content, moisture content, condition factor, silvering, and branchial Na^+-K^+ ATPase activity indicate that smoltification occurred in immature SRxSR females while previously mature SRxSR males did not smoltify. It was also apparent that, in general, smoltification was not completed in either sex of the SRxLL or LLxLL salmon. Histological examination of gill tissue revealed that chloride cells appeared to decline in number over the summer in fish retained in freshwater, whereas seawater acclimated fish, at this time, had numerous well developed chloride cells.

Wild anadromous smolts of the Exploits River stock had high branchial Na^+-K^+ ATPase activity and adapted to seawater without marked elevation of plasma sodium or chloride ion concentration. Mortality was high in LLxLL salmon during the course of exposure to seawater and the group exhibited elevated plasma ion concentrations. Induction of branchial Na^+-K^+ ATPase activity did not appear to lower plasma ion concentrations in LLxLL salmon exposed to seawater. It appears that genetic differences in smolting patterns occur in mature male salmon from anadromous populations. As well, differences appear to exist in adaptability to seawater among stocks of nonanadromous Atlantic salmon. Differences in smoltification patterns in anadromous and nonanadromous salmon are discussed in terms of the evolution of the different life history patterns observed in the two forms.

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Chapter 1
INTRODUCTION

In the sea, salmonid fishes encounter an environment very different from the freshwater rivers and lakes in which they were hatched. Migrants are therefore forced to adopt a new lifestyle more appropriate to the conditions encountered in the marine environment, and to make the transition over a short period of time. To permit successful adaptation these fishes undergo a preparatory set of physiological and behavioral changes collectively termed the parr-smolt transformation.

Essentially, the changes that occur during the parr-smolt transformation permit bottom-dwelling, territorial fish living in an hypoosmotic medium to adopt a pelagic, non-territorial lifestyle in the hyperosmotic-seawater medium. The various changes have been the subject of many studies and are well documented. Perhaps the most obvious of these is body silvering that occurs during the spring due to deposition of purine crystals (chiefly guanine and hypoxanthine) just under the scales and deep in the dermis adjacent to the underlying muscle (Markert and Vanstone, 1966; Johnston and Eales, 1967, 1968, 1970). Such purine accumulation effectively masks the vertical parr marks that are characteristic of pre-smolt salmonids residing in fresh water. During the parr-smolt metamorphosis melanization occurs along the fin margins, especially the caudal and pectorals (Saunders and Henderson, 1978). A third morphological alteration observed during smoltification is a decrease in condition factor associated with a reduction in total lipid content and changes in the relative amounts of constituent lipids (Lovern, 1934; Hoar, 1939; Woo *et al.*, 1978; Sheridan *et al.*, 1983).

The adaptive significance of integumentary purine deposition is not well known, however Hoar (1976) suggests that water conservation is involved. As the marine environment is "osmotically dry", this phenomenon may be a means by which migrating fish can dispose of nitrogenous waste products while keeping urinary water loss at a low level. As well, the silver smolt is more appropriately coloured for a pelagic lifestyle than are the colourful parr. Likewise, the changes observed in fat metabolism are not fully understood. It is generally assumed that the changes associated with the parr-smolt transformation are energy demanding and that the reduction in total lipid levels is caused by mobilization of depot reserves to fuel these changes. Smoltification occurs during the spring when growth and activity rates are high, so it may be that food intake is not sufficient to meet the increased energy demand imposed by smoltification. The removal of depot fat is a selective process, resulting in higher relative amounts of unsaturated fats. Hoar (1939) suggests that this may be adaptive to the cool temperatures encountered at sea. As well, the lipids associated with cell membranes may be altered during the parr-smolt transformation such that their permeabilities are suited to the hypertonic marine environment.

Prior to seaward migration salmonids are hyperosmoregulators, that is the osmolarity of their internal fluids is higher than that of the surrounding medium. In the sea salmonids are hypoosmoregulators and maintain their internal osmolarity below that of the seawater medium. Thus during the seaward migration the fish moves from an environment where active salt uptake and water excretion are necessary, to one where salts must be excreted and water conserved. While in seawater dehydration is prevented by ingestion of seawater and decreasing urinary output (Smith, 1930). Excess ions that accumulate from drinking, food intake, and diffusion must be eliminated. Keys and Willmer (1932) described special cells (chloride cells) in the gill epithelium and suggested they are the sites for ion excretion. Indirect evidence from several studies has supported this view, however it was not until recently that direct evidence has been found (Foskett and Scheffey, 1982). An increase in chloride cell number has been

reported on transfer of various euryhaline fish from freshwater to saltwater including eels, *Anguilla spp.* (Jozuka, 1966; Utida *et al.*, 1971; Thomson and Sargent, 1977), pupfish, *Cyprinodon variegatus* (Karnaky *et al.*, 1976), and salmonids (Morrison, 1979; Burton and Idler, 1984; Langdon and Thorpe, 1984). Copeland (1948) described apical "excretory vesicles" that showed a strong positive reaction for chloride in the chloride cells of *Fundulus heteroclitus* adapted to saltwater and freshwater adapted fish that were salt loaded.

Associated with the increase in chloride cell numbers during seawater adaptation is an elevation in branchial Na^+-K^+ ATPase activity. This enzyme occurs principally in the chloride cells (Utida *et al.*, 1971; Kamiya, 1972; Sargent *et al.*, 1975; Langdon and Thorpe, 1984) and is believed to function in monovalent ion regulation (Epstein *et al.*, 1971; Boeuf *et al.*, 1978; Saunders *et al.*, 1983). Branchial Na^+-K^+ ATPase activity increases during the late winter and spring in smolting salmonids and reaches a high level at the time of seaward migration (McCartney, 1976; Saunders and Henderson, 1978; Johnston, 1983). Activity remains high in smolts that acclimate to seawater, or declines in smolts retained in freshwater (Zaugg and McLain, 1970; Johnston, 1983; Johnston *et al.*, 1983; Langdon and Thorpe, 1984).

Behavioral changes associated with the parr-smolt transformation are also adaptive for life at sea. Pre-smolt salmonids aggressively defend feeding territories and maintain instream position, while migrating smolts are less aggressive, abandon territories, and often form schools that proceed downstream to the sea. Comprehensive accounts of the parr-smolt transformation are offered by Hoar (1976), Folmar and Dickhoff (1980), and Wedemeyer et al. (1980).

Adult runs of Atlantic salmon, *Salmo salar* in Newfoundland are comprised predominantly of female fish (Davis and Farwell, 1975; Chadwick *et al.*, 1978). This finding can be traced back to an imbalanced sex ratio in the smolt run in many rivers. Dalley (1978) reported that females constitute 81-92 percent of the smolts migrating from several Newfoundland rivers. Similarly Davis and Farwell

(1975) report that 77 percent of Exploits River smolts are female, while Chadwick *et al.* (1978) found that female salmon comprise 79 percent of smolts migrating from Western Arm Brook in northern Newfoundland. The small proportion of males in these smolt runs is believed to be related to the incidence of so called precocious sexual maturation in pre-smolt male parr. Dalley *et al.* (1983) found a correlation between the incidence of sexual maturity and imbalanced sex ratios in smolt runs in Newfoundland rivers. The preponderance of females appears to result from high mortality among mature male parr. High mortality has been documented in mature male Baltic salmon parr (Mitans, 1973) and in chinook salmon, (*Oncorhynchus tshawytsha*) parr (Gebharts, 1960).

The bulk of literature concerning Atlantic salmon deals with anadromous fish. There are however, many populations of nonanadromous salmon in North America and Europe (Dahl, 1928; Power, 1958; Havey and Warner, 1970). These salmon are known by such local names as Sebago salmon, ouananiche, landlocked salmon, blege, or smablank. In Newfoundland, nonanadromous salmon are widespread and exhibit geographical variation in characteristics such as growth rate, age at maturity, fecundity, maximum size, and lifespan (Andrews, 1966; Leggett and Power, 1969; Lee, 1971; Bruce, 1976; Barbour *et al.*, 1979; Barbour and Garside, 1983). Despite different life history characteristics, Wilder (1947) could find no consistent morphometric differences between anadromous and nonanadromous Atlantic salmon and concluded that classification of the two as subspecies is unwarranted. The most obvious difference between the two forms is the degree of migratory activity that occurs during the smolt stage. The anadromous form moves downstream and enters the sea for some period of time while the nonanadromous forms remain in fresh water throughout the life cycle. It is generally assumed that nonanadromous salmon arose from anadromous stocks that recolonized rivers after the most recent glaciation, and became "landlocked" by impassible obstacles created by isostatic rebound (Power, 1958). If this theory is correct, the ancestors of nonanadromous salmon presumably underwent the same metabolic changes associated with smoltification that are seen in present day

anadromous salmon. The present study was undertaken to determine if smoltification occurs in cultured fish from a population of nonanadromous Atlantic salmon from insular Newfoundland. In addition, the question of whether sexual maturation in anadromous male salmon parr affects subsequent smoltification is addressed.

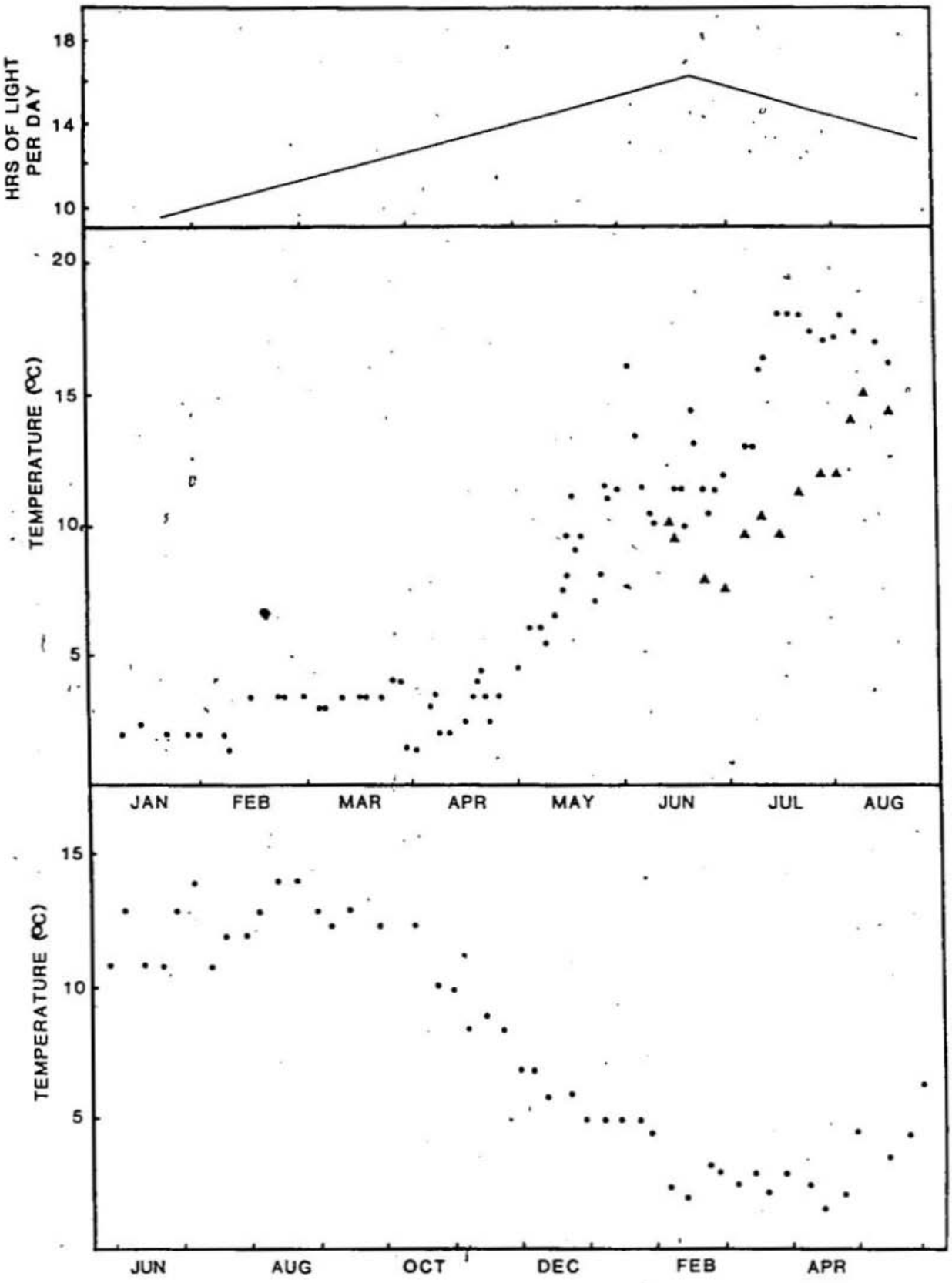
Chapter 2
METHODS AND MATERIALS

2.1. Fish Source and Sampling Procedures

Anadromous Atlantic salmon (SRxSR) were hatched from eggs obtained in November 1980 from the Exploits River stock (Newfoundland) and were tank reared at the Marine Sciences Research Laboratory (MSRL), Memorial University. Nonanadromous salmon (LLxLL) of the Five Mile Pond East stock (Avalon Peninsula, Newfoundland) and a hybrid group (SRxLL, anadromous dam and nonanadromous sire) were similarly reared. All salmon were cultured in $1m^2$ center draining fiberglass tanks under ambient temperature (Figure 2-1) and simulated natural photoperiod regimes. Fish were fed by hand three times daily to satiation with moist pellets formulated from capelin meal (48.8%), capelin (35%), middlings (10%), capelin oil (5%), and vitamin mix (1.2%). Sexually mature males with running milt were fin clipped (adipose) for future identification. All male salmon and female LLxLL salmon that were used as experimental animals had matured the previous autumn (eggs had been stripped from ripe females). Female SRxSR salmon were immature, while both mature and immature female salmon were present among the SRxLL females. Salmon from the latter group were sampled randomly with no attempt made to select fish on the basis of reproductive status.

At 3-week intervals beginning in January 1984, 5 male and 5 female salmon were drawn from each group for assessment of characteristics associated with smoltification. Fish were killed with a sharp blow to the head, weighed and measured, and a blood sample was collected in lithium-heparinized tubes from the

Figure 2-1. Seasonal photoperiod (top panel) and water temperatures at MSRL. Middle panel shows freshwater temperatures (dots) and seawater temperatures (triangles) during the seawater acclimation experiment in 1984. Bottom panel shows seawater temperatures during the growth experiment between June 1983 and June 1984.


HRS OF LIGHT PER DAY
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14
10
TEMPERATURE (°C)
20
15
10
5
JAN
FEB
MAR
APR
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AUG
TEMPERATURE (°C)
15
10
5
JUN
AUG
OCT
DEC
FEB
APR

severed caudal peduncle. Whole blood was centrifuged and the resulting plasma stored at -80°C. The gill apparatus was removed and a portion of the first arch fixed in Bouin's solution for histological examination. Filaments from the left arches were removed for determination of Na^+-K^+ ATPase activity. The carcass was opened mid-ventrally and dried to constant weight at 80°C in preparation for moisture and lipid content determinations.

2.2. Silvering and Condition Factor

Periodically over the winter and spring, all salmon were anaesthetized in tertiary-amyl alcohol (2 percent), weighed and measured, and the degree of body silvering assessed according to the procedure of Johnston and Eales (1967). Each fish was classified as either parr (parr marks distinct with little or no silvering), silvery parr (parr marks somewhat obscured but still visible), or smolt (parr marks totally obscured or just visible and black margin on caudal fin).

Condition factor (K) was determined from length/weight data according to Hoar (1939):

$$K = (W/L^3) \times 100$$

where L denotes fork length in centimeters and W denotes weight in grams.

2.3. Gill Na^+-K^+ ATPase Activity

Determination of gill Na^+-K^+ ATPase activity (ouabain sensitive) was done according to the method described by Johnston and Saunders (1981). After removal the gill apparatus was washed in cold 250mM sucrose, 5mM ethylenediaminetetra-acetic acid (EDTA). Filaments were blotted dry, excised and homogenized in cold sucrose/EDTA solution (36 mg tissue/ml) using a motor driven teflon pestle. The resulting homogenate was quickly frozen in an alcohol/dry ice bath and then stored at -80°C until enzyme activity was determined (usually within 2 days). For the enzyme activity assay a 0.2 ml

aliquot of tissue homogenate was added to each of three tubes containing 0.1 ml 1000mM NaCl/200mM KCl solution and 0.5 ml 200mM Tris buffer (pH 7.6), and to each of three tubes containing 0.1 ml 1000mM NaCl/200mM KCl/2mM ouabain solution and 0.5 ml 200mM Tris buffer (pH 7.6). The reaction was started with the addition of 0.2 ml 30mM ATP (disodium salt)/25mM MgCl solution, and stopped exactly 10 minutes later with the addition of 4 ml cold 1 percent ammonium molybdate/40 mg/ml ferrous sulfate solution prepared in 1.15 N H_2SO_4. The reaction was run at 37°C. The reaction mixture was then centrifuged (2500 rpm) for 6 minutes, and the optical absorbance of the blue supernatant was read at a wavelength of 700 nm. A K_2HPO_4 solution was used as a phosphate standard.

Protein determination was carried out using a modification of the Lowry technique (Hartree, 1972), using bovine serum albumin as a standard. Na^+-K^+ ATPase activity was measured by subtracting the activity in the reaction mixture containing ouabain from the activity in the reaction mixture not containing ouabain, and is expressed as micromoles inorganic phosphate (Pi) liberated per mg protein per hour(μmoles Pi/mg protein per hr).

2.4. Moisture and Lipid Content

After drying to a constant weight, the carcass was reweighed and ground to a fine consistency in a 50 ml Waring blender. Moisture is expressed as percent fresh body weight. Lipid content was determined on a 2 g sample of dried tissue according to the method of Hara and Radin (1978). Tissue was extracted for approximately 4 hours in 15 ml solvent (3:2 hexane/ isopropanol mixture) and filtered through a Whatman GF/A glass microfibre filter under vacuum. Tissue and glassware were rinsed with fresh solvent until the final volume was 36 ml (1 g tissue to 18 ml solvent). Non-lipid materials were salted out from the solvent solution by vigorously mixing with 15 ml warm 6.7 percent sodium sulfate in a separatory funnel. After allowing the mixture to separate into 2 phases, the lower aqueous phase was discarded and the upper hexane phase was transfered to a

preweighed petri plate and allowed to evaporate to dryness in an air stream. The resulting lipids were dried to a constant weight at 80°C and the plates reweighed. Lipid content is expressed as percent dry body weight.

2.5. Chloride Cells

Fixed gill tissue was embedded in parafin, sectioned transversely (5 μm thickness), and stained with hematoxylin and eosin. Chloride cells were identified as large acidophilic cells occuring principally in the basal region of the secondary lamellae. Due to difficulty in standardizing counts, only qualitative observations were made of chloride cell number. The length of five representative chloride cells (from basal to apical surface) was measured in each fish using a calibrated occular micrometer. Only cells sectioned through the nucleus were measured.

2.6. Seawater Acclimation

On 6 June 1984, 200 wild Atlantic salmon smolts were transported from the Exploits River counting facility (Bishop's Falls) to the MSRL. These were held in three $1m^2$, center-draining tanks identical to those used for rearing purposes. A group of 150, 2 year old nonanadromous cultured salmon was similarly distributed among three tanks. Two tanks from each group received water from a common header tank supplied with fresh and salt water such that the desired salinity could be achieved by adjusting the flow rates appropriately. The remaining two tanks were supplied with fresh water only. After one week the salinity was gradually increased until full strength seawater was achieved (approximately 31 ppt) in 2.5 weeks. During the acclimation period and until the middle of August, salmon were sampled for examination of gill chloride cells, gill Na^+-K^+ ATPase activity, and plasma ion concentrations. Sampling techniques and sample sizes were the same as those outlined above. Plasma sodium was assayed with a Scientific Instruments flame photometer using a 20 μl sample. Chloride concentration was determined using a Corning chloridometer.

2.7. Growth Rates in Seawater

In June 1983, a small number of cultured salmon from the three groups (3 LLxLL, 6 SRxLL, 9 SRxSR) was acclimated to seawater and cultured in a circular tank (1 meter depth, 1.5 meter diameter). At intervals through the summer and autumn the salmon were anaesthetized with tertiary-amyl alcohol and weighed and measured. Fish were fed three times daily to satiation.

2.8. Statistical Analysis

Statistical manipulations were made using the MINITAB package (Ryan et al., 1982). Within group comparisons (between sexes) were made using T-Tests, and comparisons between groups were made using one-way analysis of variance. Significant differences detected by analysis of variance were isolated using Scheffe's multiple contrast test (Zar, 1974). Where heterogeneity of variance was detected (Bartlett's Test), the Mann-Whitney or Kruskal-Wallis test was applied where appropriate. An acceptance level of not less than 95 percent ($P<0.05$) was considered significant.

Chapter 3
RESULTS

3.1. Silvering and Condition Factor

Figure 3-1 presents the percentages of parr, silvery parr, and smolts in both sexes from each group of cultured salmon in freshwater between January and May, 1984. Many males and females in each group exhibited some degree of silvering at all sampling dates. In general however, silvering was more apparent in females than males and there was an overall trend toward increased silvering with time. In the January and March samples no salmon showed complete development of smolt colouration (parr marks completely obscured by silver pigment and black fin margins). In April a few fish in each group (mostly females) had fully developed smolt colouration, while most were partially silvered. In May both sexes in all groups contained fully silvered salmon with black fin margins. A very small number of LLxLL males were fully silvered while approximately 20 percent of SRxLL and SRxSR males and LLxLL females were so coloured. About 50 percent of SRxLL females and 80 percent of SRxSR females were fully silvered by May. Tables 3-1 to 3-4 provide a summary of the numbers of parr, silvery parr, and smolts, as well as the mean fork length of each group over the experimental period. Figure 3-2 illustrates the degree of silvering in representative specimens in each group in mid June.

Figure 3-3 presents mean condition factor for male and female salmon in each group on each sampling date. A significant difference was found in mean condition factor between male and female LLxLL salmon in January ($t=7.32$, $df=136.1$, $P<0.001$) and March ($t=3.66$, $df=116.0$, $P<0.001$). Females in this

Figure 3-1. Seasonal percentages of cultured, freshwater male and female parr (solid bars), silvery parr (open bars), and smolts (stippled bars),in LLxLL, SRxLL and SRxSR salmon.

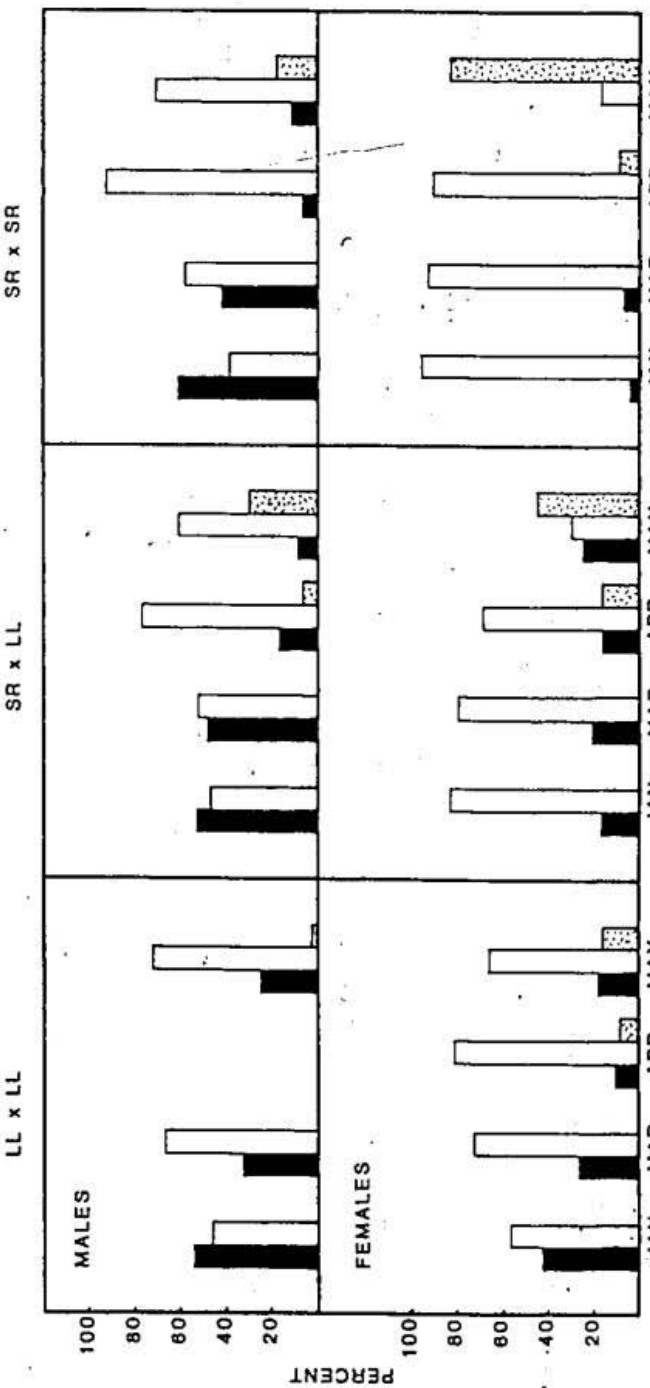

LL x LL
SR x LL
SR x SR
MALES
FEMALES
PERCENT
100
80
60
40
20
JAN
MAR
APR
MAY

Figure 3-2. Representative specimens of cultured, freshwater LLxLL (upper photo), SRxLL (middle photo), and SRxSR (lower photo) salmon showing body and fin colour in mid June. The upper two fish in each photograph are mature males while the lower two are females. The bottom salmon in the lower photograph is a wild anadromous smolt from the Exploits River.

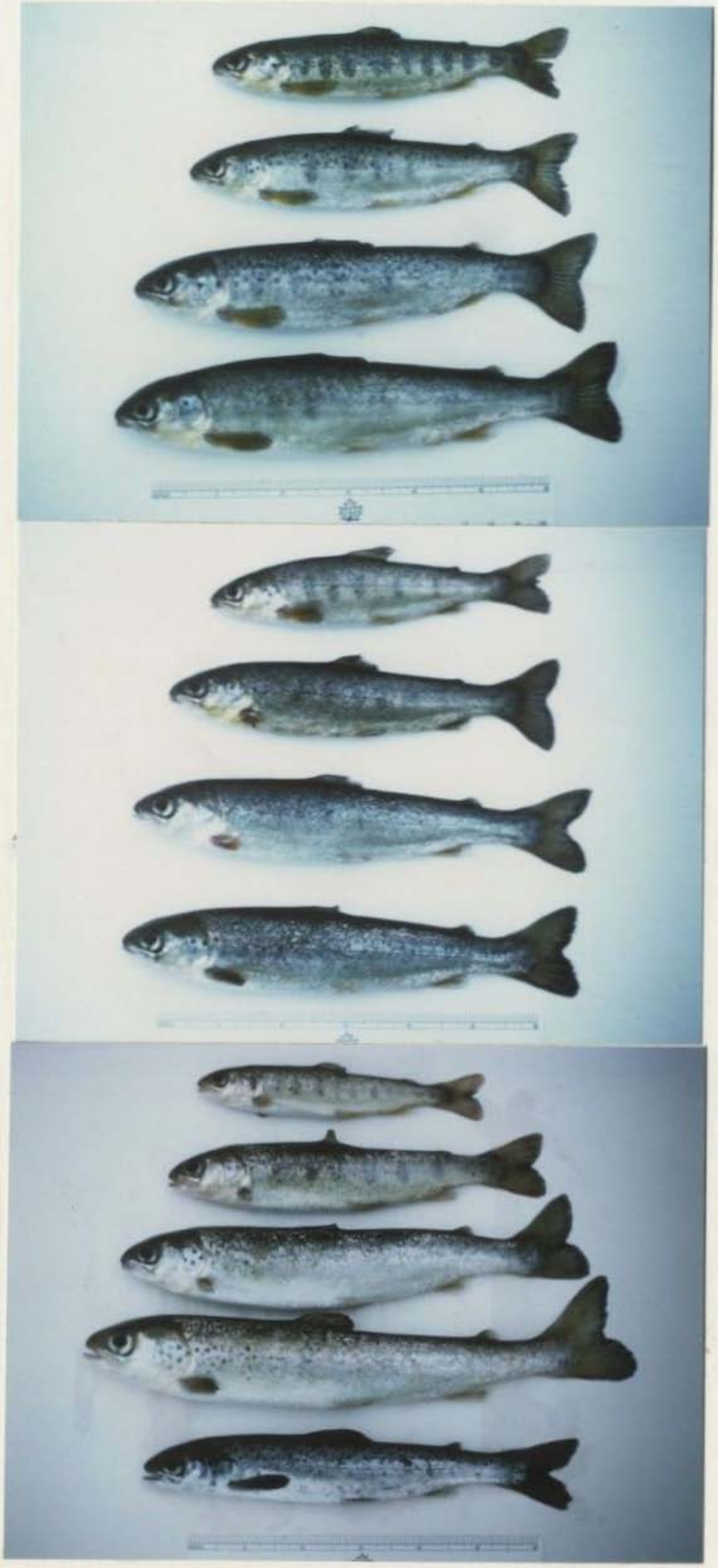


TABLE 3.1: Mean fork length (FL in cm) and number (N) of cultured, freshwater male and female parr, silvery parr and smolts in each group on January 21. Standard errors appear in parentheses.

STOCK	SEX		PARR	SILVERY PARR	SMOLT
LLXLL	M	FL	13.8 (0.27)	14.7 (0.26)	-
		N	36	30	-
	F	FL	14.1 (0.26)	16.1 (0.34)	-
		N	32	44	-
SRxLL	M	FL	13.6 (0.16)	15.6 (0.30)	-
		N	41	36	-
	F	FL	13.5 (0.68)	16.5 (0.22)	-
		N	12	59	-
SRxSR	M	FL	15.1 (0.19)	15.5 (0.64)	-
		N	24	16	-
	F	FL	16.6 (0.80)	18.3 (0.30)	-
		N	2	49	-

TABLE 3.2: Mean fork length (FL in cm) and number (N) of cultured, freshwater male and female parr, silvery parr and smolts in each group on March 11. Standard errors appear in parentheses.

STOCK	SEX		PARR	SILVERY PARR	SMOLT
LLxLL	M	FL	13.3 (0.34)	14.8 (0.25)	-
		N	18	36	-
	F	FL	13.7 (0.25)	15.9 (0.33)	-
		N	17	47	-
SRxLL	M	FL	13.5 (0.19)	15.7 (0.29)	-
		N	31	34	-
	F	FL	12.6 (0.40)	16.7 (0.23)	-
		N	13	50	-
SRxSR	M	FL	14.4 (0.32)	16.2 (0.58)	-
		N	13	18	-
	F	FL	15.2 (1.45)	18.9 (0.33)	-
		N	3	38	-

TABLE 3.3: Mean fork length (FL in cm) and number (N) of cultured, freshwater male and female parr, silvery parr and smolts in each group on April 22. Standard errors appear in parentheses.

STOCK	SEX		PARR	SILVERY PARR	SMOLT
LLxLL	M	FL	12.5 (0.36)	14.5 (0.24)	16.5 (0.26)
		N	11	43	5
	F	FL	13.7 (0.38)	15.5 (0.31)	18.5 (0.59)
		N	7	47	5
SRxLL	M	FL	13.1 (0.20)	15.1 (0.25)	17.3 (0.46)
		N	9	47	4
	F	FL	12.5 (0.46)	16.5 (0.35)	17.3 (0.47)
		N	9	40	8
SRxSR	M	FL	13.2 (1.15)	15.8 (0.44)	-
		N	2	25	-
	F	FL	-	18.7 (0.40)	20.0 (1.30)
		N	-	31	3

TABLE 3.4: Mean fork length (FL in cm) and number(N) of cultured, freshwater male and female parr, silvery parr, and smolts in each group on May 31. Standard errors appear in parentheses.

STOCK	SEX		PARR	SILVERY PARR	SMOLT
LLxLL	M	FL	12.4 (0.33)	15.0 (0.36)	16.8
		N	9	26	1
	F	FL	13.8 (0.35)	15.5 (0.41)	17.8 (0.57)
		N	8	29	7
SRxLL	M	FL	12.7 (0.39)	14.6 (0.28)	17.1 (0.49)
		N	4	27	13
	F	FL	12.1 (0.23)	15.3 (0.62)	17.3 (0.31)
		N	10	13	17
SRxSR	M	FL	14.7 (0.71)	15.2 (0.34)	21.0 (1.82)
		N	5	9	3
	F	FL	-	16.2 (0.96)	19.3 (0.40)
		N	-	4	20

group had a mean condition factor of 0.94 in January, the lowest value observed in any group throughout the study. By late May however, the mean condition factor for this group had increased to 1.10, and was not significantly different from the mean value for males of this group. A significant increase in mean condition factor was observed in female ($t=4.65$ $df=92.0$ $P<0.001$) and male ($t=3.50$ $df=60.6$ $P<0.001$) LLxLL salmon between April and May.

No significant difference was noted between male and female SRxLL salmon at any time, nor was any seasonal change observed. Mean condition for both sexes remained above unity on all sampling dates.

There was a significant difference in mean condition factor observed between male and female SRxSR salmon in January ($t=3.43$ $df=86.2$ $P<0.001$). This difference was not evident in March or April but reappeared by late May ($t=3.31$ $df=34.8$ $P<0.01$). Between March and May a significant decrease in mean condition factor occurred in female SRxSR salmon ($t=3.78$ $df=49.3$ $P<0.001$) while no such change was observed in males.

Mean condition factor of female LLxLL salmon was significantly lower than female SRxLL and female SRxSR salmon in January ($F=41.87$ $P<0.01$). Similarly male SRxSR salmon had a higher mean condition factor in January than SRxLL and LLxLL males ($F=21.44$ $P<0.01$). In May LLxLL females were found to have higher mean condition factor than SRxLL and SRxSR females ($F=4.51$ $P<0.05$). At this time mean condition factor in SRxLL males was lower than in LLxLL ($F=6.72$ $P<0.01$) and in SRxSR ($F=6.72$ $P<0.05$) male salmon. Table 3-5 presents the mean condition factor, fork length, and number of animals of each sex in the three groups on each sampling date.

A sample of 8 wild anadromous smolts collected at Bishop's Falls on June 7 had a mean condition factor of 0.93. This value was significantly lower than the mean value in SRxSR females in May ($t=9.22$, $df=23.0$, $P<0.001$).

Figure 3-3. Seasonal mean condition factor (± SE) for cultured, freshwater male and female LLxLL, SRxLL, and SRxSR salmon. In most cases standard errors are too small to be shown.

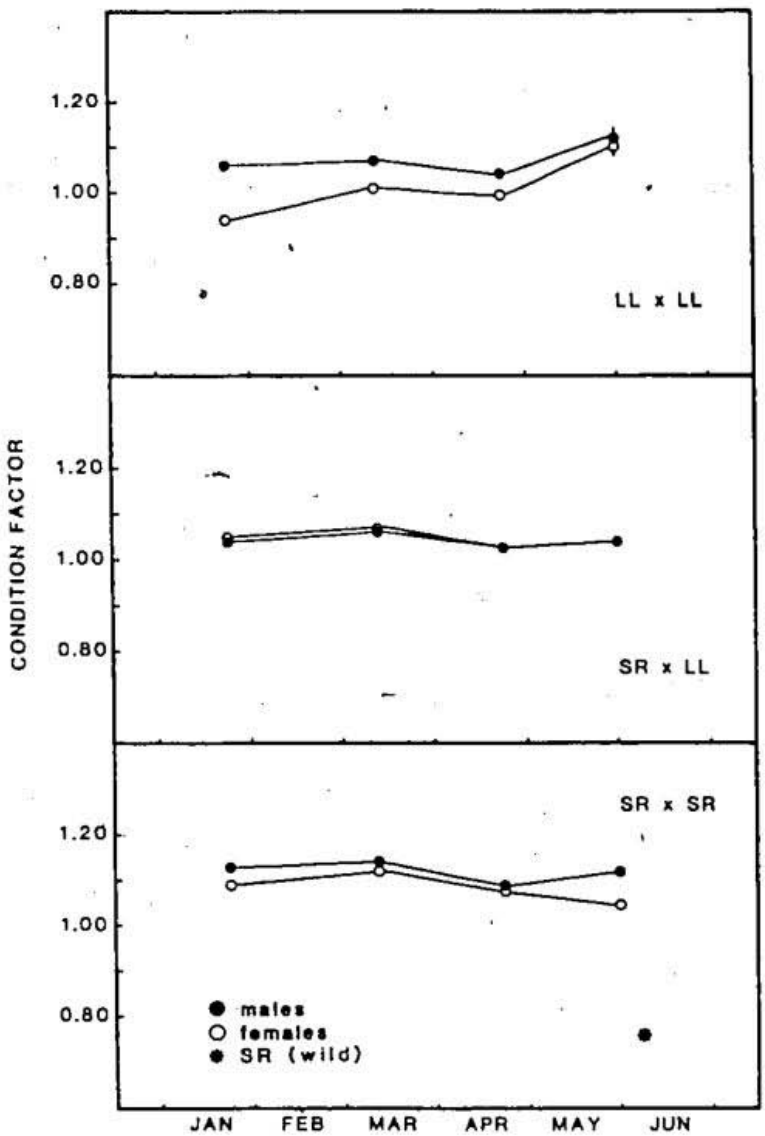

CONDITION FACTOR
1.20
1.00
0.80
LL x LL
SR x LL
SR x SR
males
females
SR (wild)
JAN
FEB
MAR
APR
MAY
JUN

TABLE 3.5: Mean condition factor (CF), fork length (FL in cm), and number (N) of cultured, freshwater male and female salmon in each group over experimental period. Standard errors appear in parentheses.

DATE	STOCK	SEX	CF	FL	N
JAN 21	LLxLL	M	1.06 (0.010)	14.2 (0.20)	65
		F	0.94 (0.013)	15.2 (0.25)	76
	SRxLL	M	1.04 (0.008)	14.5 (0.20)	77
		F	1.05 (0.012)	16.0 (0.25)	71
	SRxSR	M	1.13 (0.009)	15.2 (0.28)	40
		F	1.09 (0.008)	18.2 (0.30)	51
MAR 11	LLxLL	M	1.07 (0.012)	14.3 (0.22)	54
		F	1.01 (0.014)	15.3 (0.28)	64
	SRxLL	M	1.06 (0.011)	14.6 (0.22)	65
		F	1.07 (0.014)	15.9 (0.29)	63
	SRxSR	M	1.12 (0.011)	15.5 (0.40)	31
		F	1.12 (0.012)	18.7 (0.35)	41
APR 22	LLxLL	M	1.04 (0.011)	14.3 (0.23)	59
		F	0.99 (0.015)	15.5 (0.29)	59
	SRxLL	M	1.03 (1.011)	15.0 (0.23)	60
		F	1.03 (0.014)	16.0 (0.33)	57
	SRxSR	M	1.09 (0.013)	15.6 (0.43)	27
		F	1.08 (0.014)	18.8 (0.38)	34
MAY 31	LLxLL	M	1.12 (0.019)	14.4 (0.34)	36
		F	1.10 (0.017)	15.3 (0.34)	44
	SRxLL	M	1.04 (0.016)	15.1 (0.31)	44
		F	1.04 (0.014)	15.4 (0.41)	40
	SRxSR	M	1.12 (0.018)	16.1 (0.68)	17
		F	1.05 (0.015)	18.8 (0.43)	24

3.2. Total Lipid Content

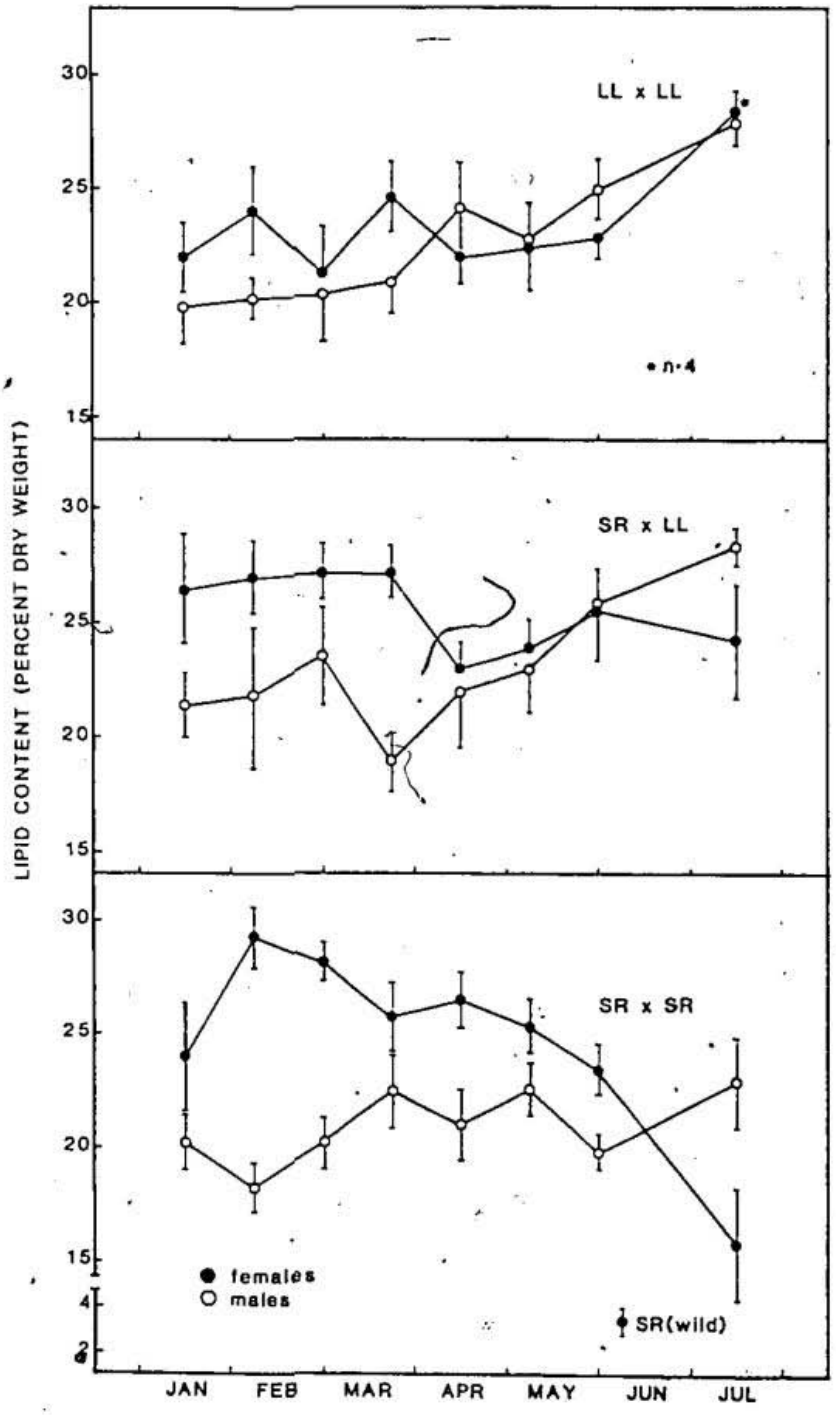
Seasonal total lipid content for male and female salmon from the three groups of cultured salmon, between January and July 1984, is presented in Figure 3-4. There was no significant difference observed between the sexes at any time in LLxLL salmon, however a seasonal increase in total lipid levels occurred in males ($t=4.13$ $df=6.5$ $P<0.01$) and females ($t=3.75$ $df=6.3$ $P<0.01$) between the initial and final samples.

Lipid dynamics during the late winter and spring were similar in LLxLL and SRxLL salmon. Total lipid levels in males of the latter group increased steadily from late March until July ($t=6.61$ $df=7.0$ $P<0.001$). The increase in females was slight and insignificant. Female SRxLL salmon had higher values than males on March 24 ($t=5.34$ $df=7.9$ $P<0.001$) while differences at other sampling dates were insignificant. Although condition factors in male and female SRxLL salmon were very similar throughout the experiment the total lipid content in males was generally lower than in females. This is probably due to the large gonadal mass present in the mature males. Immature female SRxLL and SRxSR fish had much more fat along the gut and mesentaries than was observed in mature males.

In the SRxSR group, females had higher total lipid levels than males on all dates with the exception of July 17. A rather sharp, although statistically insignificant increase was noted in the females between January 15 and February 7. From the latter date until the end of the study a steady decline in total lipid levels occurred ($t=4.62$, $df=6.3$, $P<0.005$) with a rather sharp decline occurring between May 27 and July 17 ($t=2.48$, $df=7.1$, $P<0.05$). Male SRxSR salmon maintained essentially constant lipid levels throughout the experiment with no springtime decrease.

Analysis of variance detected no significant difference in total lipid among females from the three groups in January or February. Similarly no difference was observed in males at these times. On July 17 female LLxLL fish had greater

Figure 3-4. Seasonal total lipid content (±SE) in cultured, freshwater male and female LLxLL, SRxLL and SRxSR salmon.


LIPID CONTENT (PERCENT DRY WEIGHT)
LL x LL
SR x LL
SR x SR
• n=4
females
males
SR(wild)
JAN
FEB
MAR
APR
MAY
JUN
JUL

lipid levels than SRxSR females ($F=7.12$, $P<0.05$), and SRxLL males had higher levels than SRxSR males ($F=4.86$, $P<0.05$).

Wild anadromous smolts were found to have extremely low lipid levels with a mean of only 3.6 percent. This level is significantly lower ($t=5.55$, $df=4.2$, $P<0.01$) than the lowest observed among any of the cultured salmon (16.8 percent in SRxSR females on July 17).

3.3. Moisture Content

An inverse relationship was observed between lipid and moisture levels in all groups of cultured salmon between January and July 1984. A significant decrease in moisture occurred in male ($t=5.35$, $df=6.9$, $P<0.005$) and female ($t=10.65$, $df=5.6$, $P<0.001$) LLxLL salmon between January 15 and July 17 (Figure 3-5). Moisture levels fell slowly until the end of May when the rate of decline increased. At no time was a significant difference between males and females observed.

Moisture content was not different between male and female SRxLL except on March 27 ($t=2.90$, $df=6.9$, $P<0.05$). There was no seasonal change seen in females, a finding consistent with the constant total lipid levels noted above. Male SRxLL fish showed a significant drop in moisture between March 27 and July 17 ($t=6.39$, $df=7.8$, $P<0.001$) that parallelled increased total lipid levels occurring at the same time.

Moisture content was significantly greater in male than female SRxSR salmon on February 7 ($t=6.37$, $df=7.8$, $P<0.001$), March 1 ($t=4.55$, $df=6.6$, $P<0.005$), and on April 16 ($t=3.35$, $df=7.2$, $P<0.005$). From May 6 until the termination of the study, moisture content in females increased ($t=2.63$, $df=8.0$, $P<0.05$). No significant change was observed in male SRxSR salmon over the entire study period.

In January it was determined that the mean moisture content of female SRxSR salmon was lower than that of LLxLL females ($F=9.85$, $P<0.01$) but not

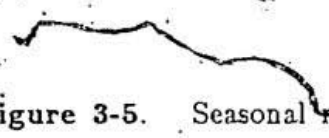
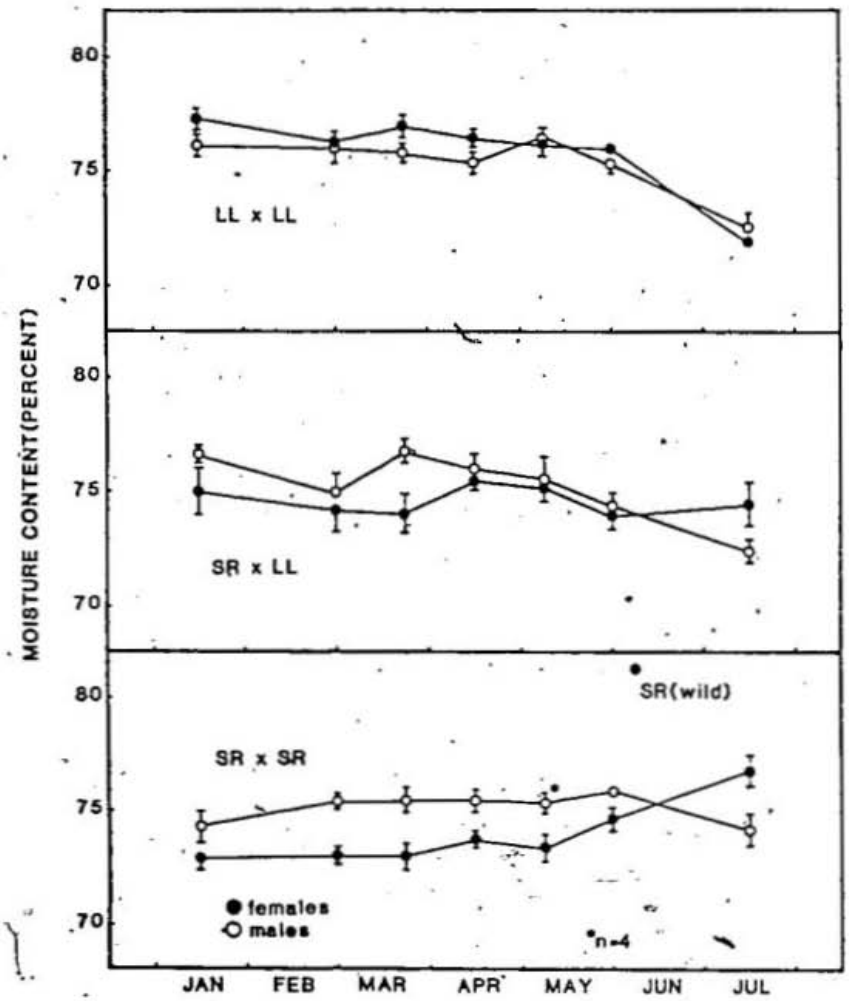


Figure 3-5. Seasonal moisture content (±SE) for cultured, freshwater male and female LLxLL, SRxLL and SRxSR salmon.


MOISTURE CONTENT(PERCENT)
80
75
70
LL x LL
SR x LL
SR(wild)
SR x SR
females
males
n=4
JAN
FEB
MAR
APR
MAY
JUN
JUL

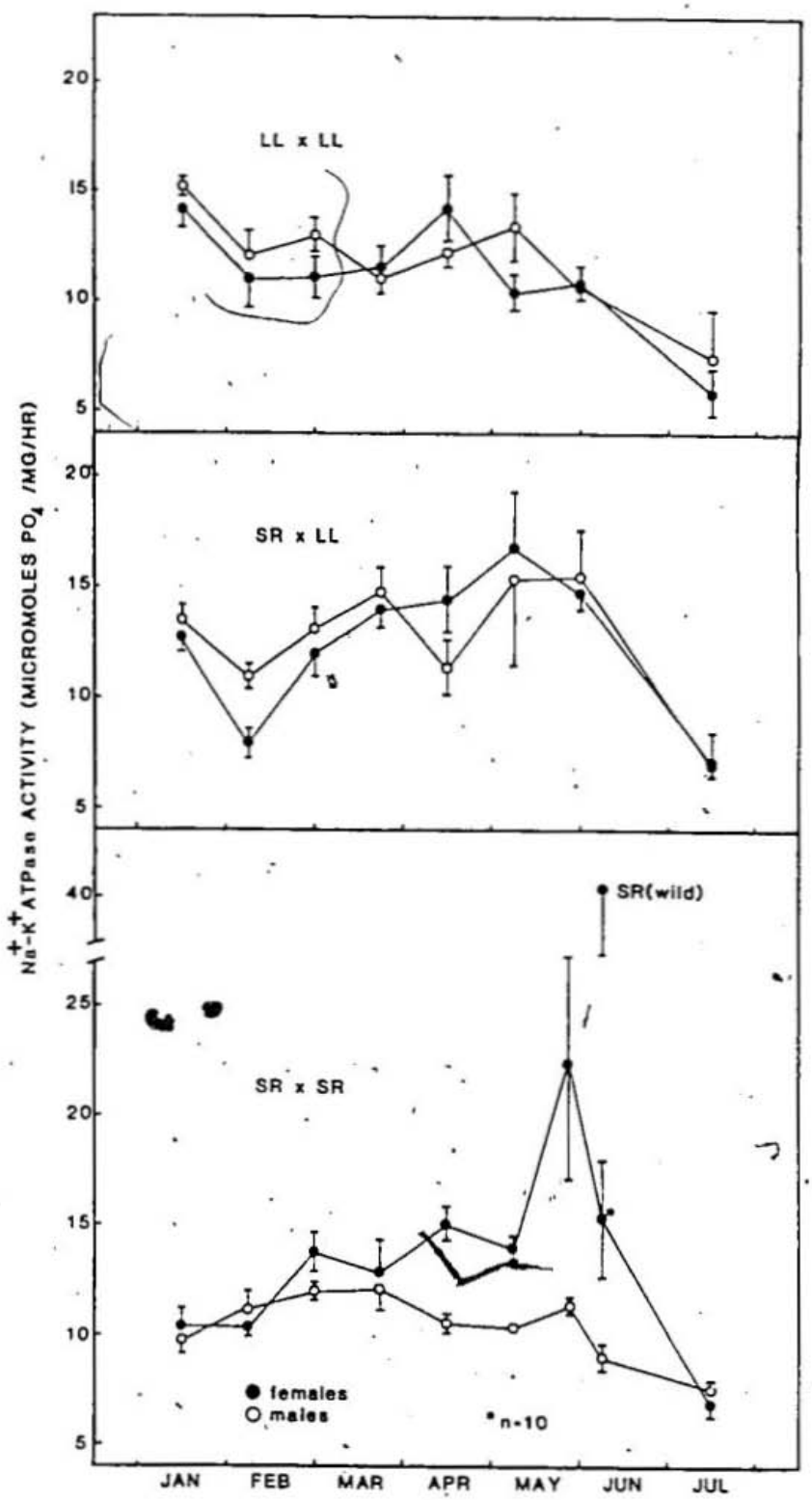
different from SRxLL females. By the end of the experiment the situation was reversed and LLxLL females had significantly lower moisture content than SRxSR females ($F=11.15$, $P<0.01$). Again SRxLL females had intermediate values not significantly different from SRxSR or SRxLL females. SRxSR males had lower moisture content than LLxLL males ($F=7.75$, $P<0.05$) and SRxLL males ($F=7.75$, $P<0.01$) on January 15. On July 17 no difference was noted among males in the three groups.

3.4. Na^+-K^+ ATPase Activity

Seasonal branchial Na^+-K^+ ATPase activity in male and female salmon in the three groups of cultured salmon in freshwater is shown in Figure 3-6. There was no significant difference in enzyme activity between male and female LLxLL fish at any point. A decline occurred between January 15 and February 7 in LLxLL males ($t=2.99$, $df=4.9$, $P<0.05$). From February 7 until May 27 the activity in LLxLL males did not change significantly. A small, insignificant drop in activity occurred between May 27 and July 17. Female LLxLL salmon followed a similar seasonal pattern with a decrease between January 15 and February 7 (insignificant) followed by a period of similar values. A small peak in activity occurred on April 16, however the activity was not significantly higher than activities observed on prior or subsequent dates. Branchial Na^+-K^+ ATPase activity was significantly higher on January 15 than on July 17 ($t=5.42$, $df=7.9$, $P<0.001$).

No significant difference was observed in branchial Na^+-K^+ ATPase activity between male and female SRxLL salmon except on February 7 when the activity in males was higher ($t=3.92$, $df=8.0$, $P<0.01$). There was no obvious seasonal pattern in enzyme activity in SRxLL males between January 15 and May 27. The highest level occurred on the latter date and was followed by a decrease in activity on July 17 ($t=3.76$, $df=4.3$, $P<0.05$). Na^+-K^+ ATPase activity decreased in female SRxLL salmon between January 15 and February 7 ($t=4.83$, $df=7.7$, $P<0.001$), and then increased steadily until peak activity occurred on

Figure 3-6. Seasonal branchial Na^+-K^+ ATPase activity ($\pm$SE) in cultured, freshwater male and female LLxLL, SRxLL and SRxSR salmon.


LL x LL
SR x LL
SR x SR
SR(wild)
Na+-K+ ATPase ACTIVITY (MICROMOLES PO4 /MG/HR)
20
15
10
5
40
25
● females
○ males
*n-10
JAN
FEB
MAR
APR
MAY
JUN
JUL

May 6. The increase between February 7 and May 6 was significant ($t=3.44$, $df=4.5$, $P<0.05$). Between May 6 and July 17 enzyme activity decreased significantly ($t=3.53$, $df=6.4$, $P<0.05$).

Na^+-K^+ ATPase activity was not significantly different between male and female SRxSR salmon on January 15, February 7, March 1, or March 24. Activity was higher in females on April 16 ($t=4.48$, $df=6.4$, $P<0.005$), May 6 ($t=7.13$, $df=6.6$, $P<0.01$), and June 8 ($t=3.67$, $df=10.6$, $P<0.01$). A small but significant increase in activity occurred in male SRxSR salmon between January 15 and March 1 ($t=2.65$, $df=5.7$, $P<0.05$), followed by decreasing values until May 6. Between May 27 and July 17 a significant decrease occurred ($t=6.95$, $df=7.7$, $P<0.001$).

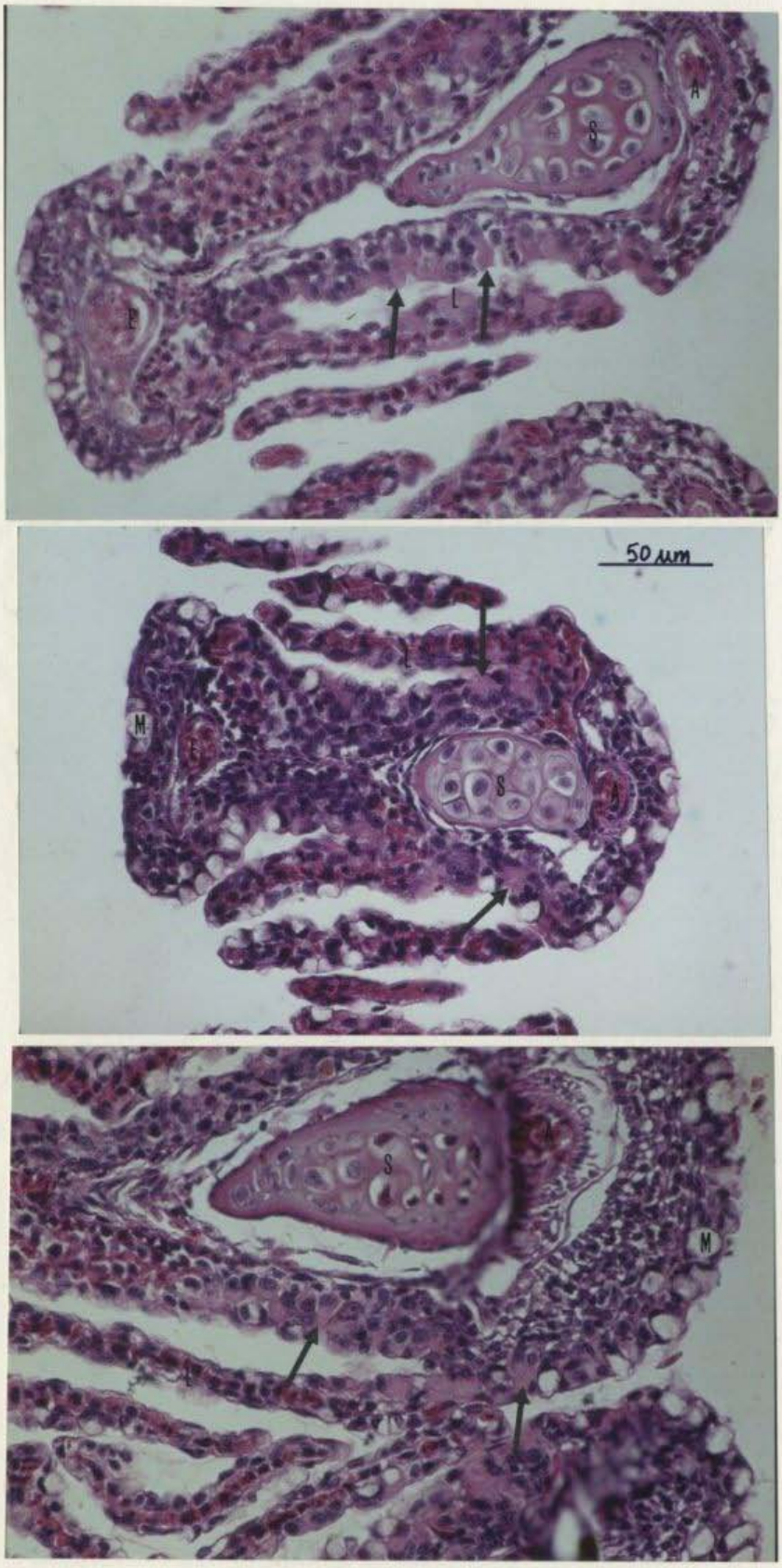
Analysis of variance revealed that Na^+-K^+ ATPase activity was higher on January 15 in LLxLL females than in SRxSR females ($F=4.74$, $P<0.05$). Male SRxSR salmon had lower activity than male LLxLL and SRxLL salmon on this date ($F=19.66$, $P<0.01$). Heterogeneity of variance was detected (Bartlett's Test) between male and female SRxSR salmon on May 27. Statistical comparisons involving females from this group were therefore made using the Mann Whitney t Test or the Kruskal-Wallis Test. The latter detected higher enzyme activity in SRxSR and SRxLL females than in LLxLL females ($H=6.977$, $P<0.05$) on May 27. No difference was noted between males on this date. On July 17 no significant difference was observed between groups in males or females.

3.5. Chloride Cells

Chloride cells were evident in most gill sections from male and female fish in the three groups cultured in freshwater on January 15 (Figure 3-7) and May 27. By July 17 chloride cells became difficult to locate in many cases.

In general mean chloride cell length was not different between males and females. Significant differences were noted in LLxLL salmon on January 15 when

Figure 3-7. Representative gill sections from cultured, freshwater LLxLL (top), SRxLL (middle) and SRxSR (bottom) salmon as sampled on January 15. Chloride cells are indicated with arrows. Abbreviations: A, afferent filamental artery; E, efferent filamental artery; L, secondary lamella; M, mucus cell; S, supporting element.


A
S
L
E
50 um
M
E
L
S
A
S
M
E
L

cells were longer in females ($t=3.01$, $df=7.6$, $P<0.05$) and in SRxLL salmon on July 17 when cells were longer in males ($t=4.08$, $df=7.3$, $P<0.01$). Table 3-6 presents mean chloride cell length in male and female salmon in the three groups on January 15, May 27, and July 17. Chloride cell lengths for wild Exploits River smolts are shown in Table 3-7. In no group did an increase in chloride cell length occur at the time of smoltification. A significant decline in mean chloride cell length occurred between January 15 and July 17 in all groups except in SRxLL males.

3.6. Seawater Acclimation: Gill Na^+-K^+ ATPase Activity

There was considerable mortality (42 percent) among the wild smolts (transported on 6th June, 1984) during the experiment because of travelling stress, infection (probably vibriosis), and refusal of some animals to eat the prepared pellets that were offered. Many salmon suffered from considerable scale loss during transport resulting in decreased disease resistance. However, no fish was observed to suffer from obvious osmotic stress during and after the acclimation period, and only healthy-appearing fish were used as experimental animals.

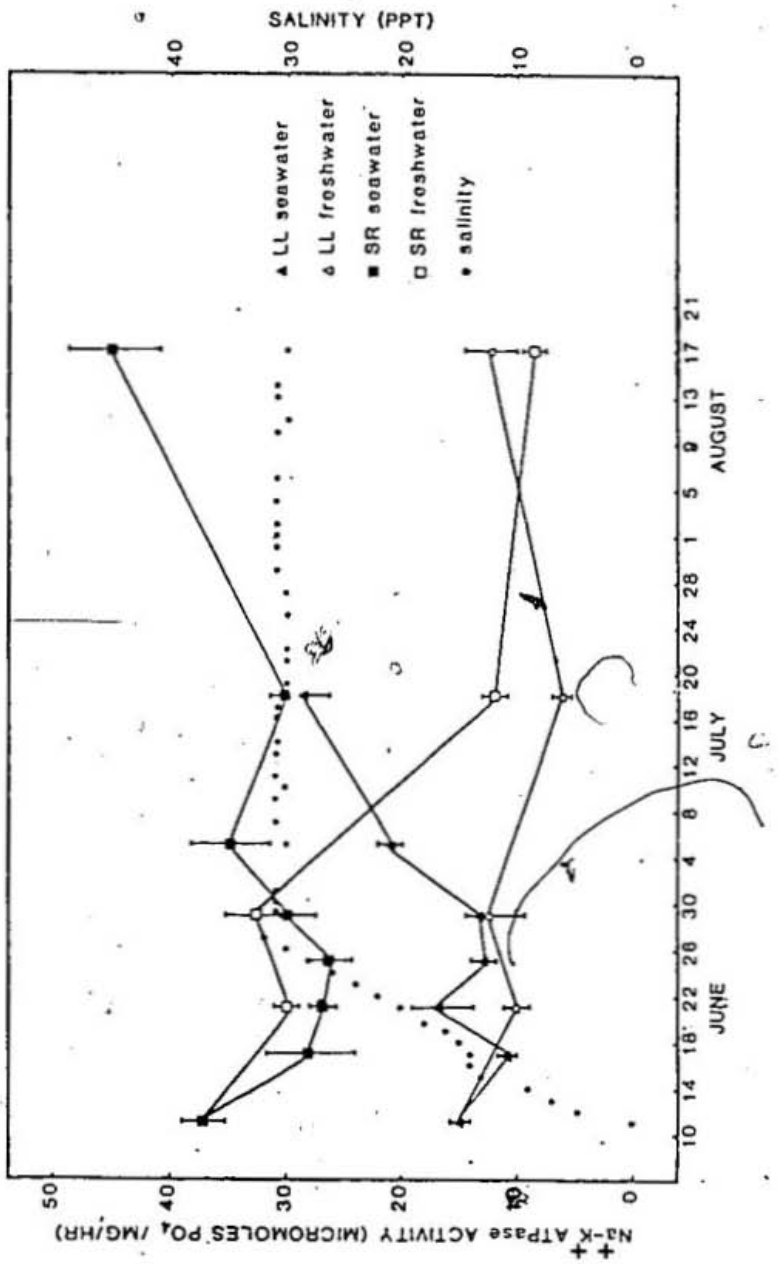
Figure 3-8 presents branchial Na^+-K^+ ATPase activity values for wild Exploits River smolts and cultured LLxLL salmon in freshwater and elevated salinity. Initial enzyme activity in salmon unexposed to increasing salinity was higher in the wild smolts ($t=11.21$, $df=4.9$, $P<0.001$). Mean enzyme activity in wild smolts retained in fresh water fell from an initial level of 37.4 μmoles Pi/mg protein per hr to 8.5 μmoles Pi/mg protein per hr by the end of the experiment ($t=11.97$, $df=4.7$, $P<0.001$). Wild smolts exposed to increasing salinity experienced a temporary reduction in Na^+-K^+ ATPase activity until the salinity reached approximately 25 ppt, and thereafter activity increased to initial levels about 7 days after full strength seawater was achieved (about 30 ppt).

LLxLL salmon retained in fresh water showed little change in Na^+-K^+ ATPase activity over the course of the experiment. On August 17 activity values

TABLE 3.6: Mean chloride cell length (μm) in cultured, freshwater LLxLL, SRxLL and SRxSR salmon on Jan. 15, May 27 and July 17. Each entry is a mean of 5 fish; 5 cells were measured in each fish. Standard errors appear in parentheses.

	Sex	January 15	May 27	July 17
LLxLL	M	14.4 (0.28)	13.8 (0.47)	11.9 (0.19)
	F	15.8 (0.35)	14.2 (0.46)	12.1 (0.38)
SRxLL	M	14.4 (0.55)	13.0 (0.54)	13.8 (0.45)
	F	14.4 (0.24)	12.8 (0.44)	11.6 (0.33)
SRxSR	M	14.7 (0.19)	14.5 (0.47)	11.7 (0.39)
	F	14.5 (0.45)	13.8 (0.94)	11.8 (0.21)

Figure 3-8. Branchial Na^+-K^+ ATPase activity (±SE) in cultured LLxLL and wild SR salmon in fresh water and during exposure to increasing salinity.


SALINITY (PPT)
40
30
20
10
0
▲ LL seawater
△ LL freshwater
■ SR seawater
□ SR freshwater
• salinity
10 14 18 22 26 30 4 8 12 16 20 24 28 1 5 9 13 17 21
JUNE
JULY
AUGUST
50
40
30
20
10
0
Na-K ATPase ACTIVITY (MICROMOLES PO4 /MG/HR)

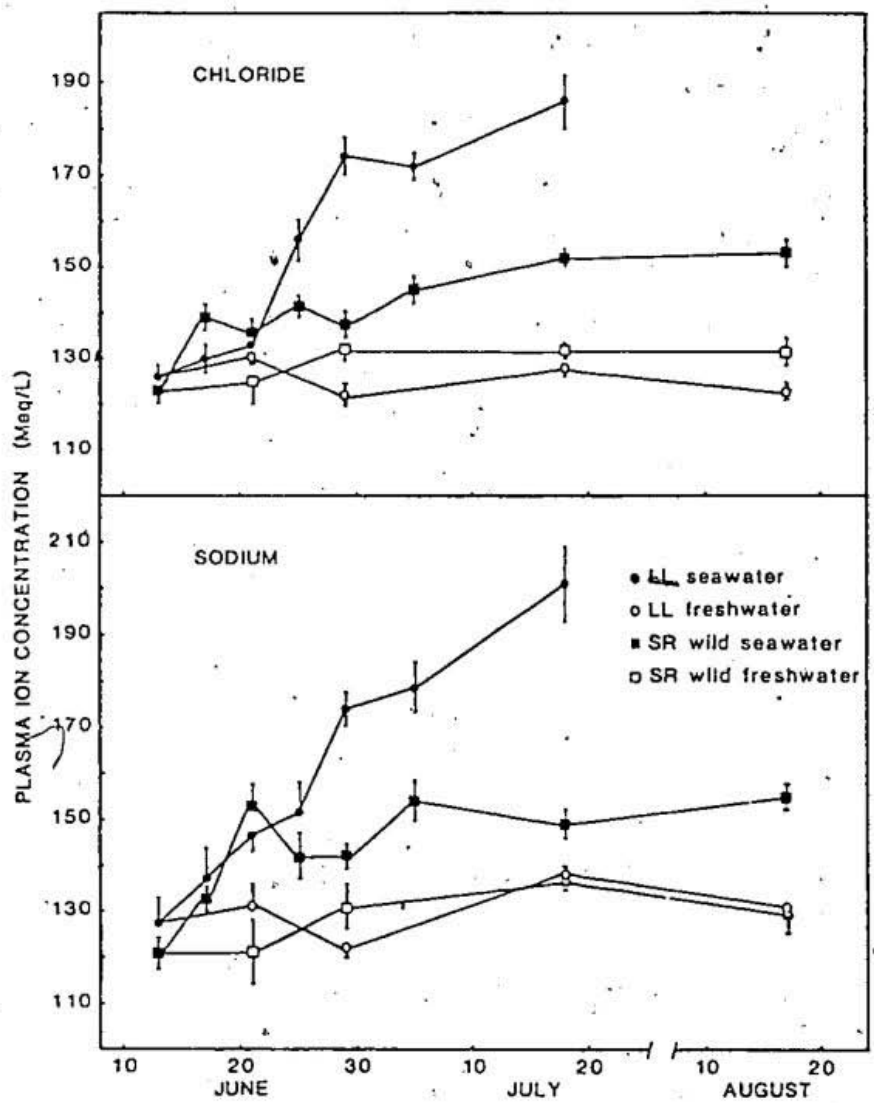
were not significantly different from the wild smolts retained in fresh water. LLxLL salmon exposed to seawater had essentially unchanged activity until full strength seawater was achieved; mean values then began to increase. Activity increased from a mean of 13.3 μmoles Pi/mg protein per hr on June 29 to 28.5 μmoles Pi/mg protein per hr on July 18 ($t=6.93$, $df=4.4$, $P<0.005$). On the latter date the mean Na^+-K^+ ATPase activity in LLxLL salmon exposed to seawater was not significantly different from that of wild smolts similarly exposed.

3.7. Seawater Acclimation: Plasma Ion Concentration

Plasma sodium and chloride concentrations in LLxLL salmon increased immediately as salinity increased and continued until the final sample was taken on July 18 (Figure 3-9). On July 27 all LLxLL fish exposed to seawater had either been sampled or had died from osmotic stress (with the exception of a single fish which successfully adapted). Highly significant increases in both sodium ($t=6.33$, $df=8.0$, $P<0.001$) and chloride ($t=8.87$, $df=4.3$, $P<0.001$) occurred between the initial (June 13) and final (July 18) samples in LLxLL salmon exposed to seawater.

Wild smolts exposed to seawater also experienced increased plasma sodium and chloride levels. Mean plasma chloride increased from an initial concentration of 122.6 Meq/L to 145.2 Meq/L by July 5 ($t=6.00$, $df=7.8$, $P<0.001$), while mean sodium concentration increased from 121.0 Meq/L to 154.0 Meq/L over the same interval ($t=5.41$, $df=6.9$, $P<0.005$). Plasma ion concentrations remained stable from July 5 through the remainder of the experiment in this group. Plasma sodium and chloride concentrations were greater in seawater exposed LLxLL salmon than in wild smolts on June 29 and on all subsequent dates ($P<0.001$). There was little fluctuation in plasma ion levels in either group retained in freshwater.

Figure 3-9. Plasma Na^+ and Cl^- concentrations ($\pm$SE) in wild anadromous and cultured LLxLL salmon while in fresh water and during exposure to increasing salinity.


CHLORIDE
SODIUM
PLASMA ION CONCENTRATION (Meq/L)
190
170
150
130
110
210
190
170
150
130
110
LL seawater
LL freshwater
SR wild seawater
SR wild freshwater
10
20
30
10
20
10
20
JUNE
JULY
AUGUST

3.8. Growth in Seawater

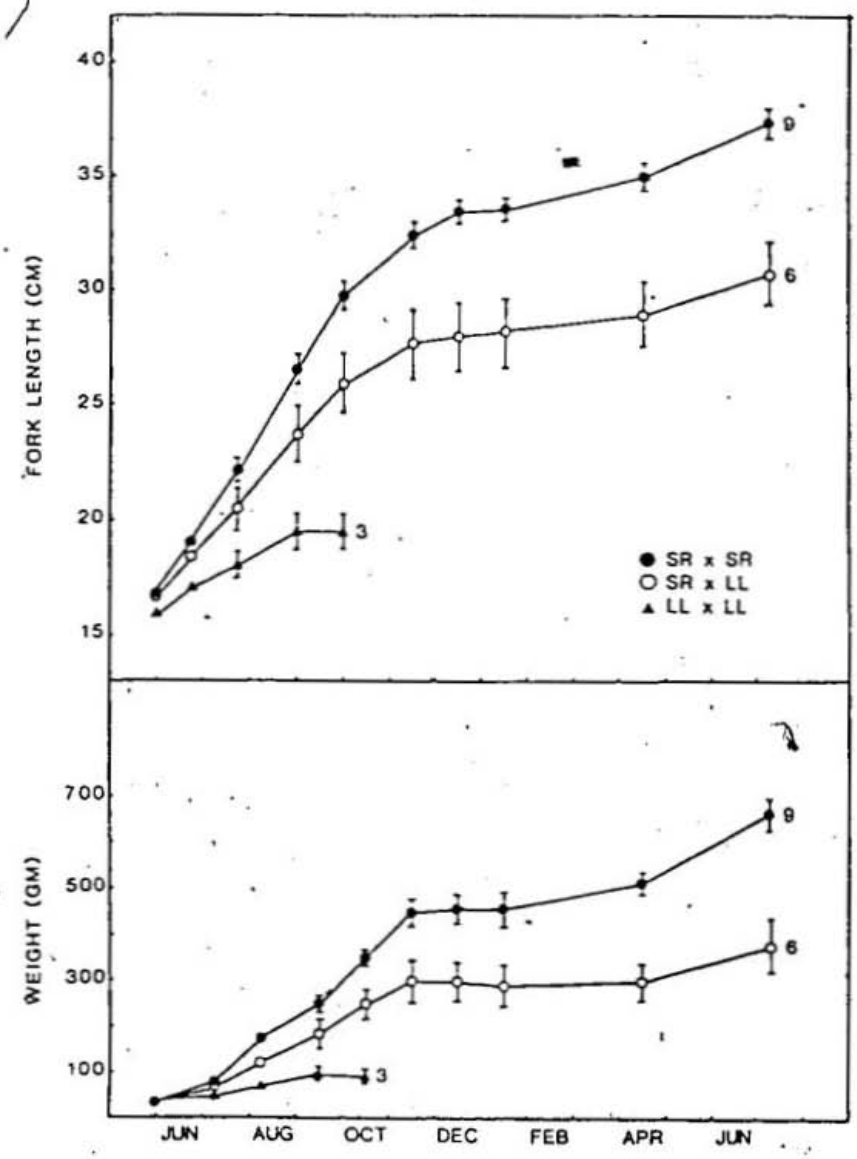
Growth performance for LLxLL, SRxLL, and SRxSR salmon adapted to full strength seawater between June 1983 and June 1984 is illustrated in Figure 3-10. Mean fork length was initially greatest in SRxSR fish and smallest in LLxLL fish, but the size differences were not significant. By July 7 the SRxSR salmon were longer than LLxLL salmon ($F=4.30$, $P<0.05$) but not significantly longer than the SRxLL fish. On September 15, SRxLL salmon had become longer than LLxLL salmon ($F=14.23$; $P<0.05$). A slight drop in mean weight occurred in LLxLL fish between September 15 and October 15 and two of the three salmon in this group died during the next month. The remaining fish died in January. SRxSR salmon were significantly longer than SRxLL salmon on November 17 ($t=3.02$, $df=6.6$, $P<0.05$). Figure 3-11 shows the relative sizes of one representative specimen from each group in December, after 7 months in seawater. While in seawater, all LLxLL salmon and 3 SRxLL salmon matured (all were female).

3.9. Seawater Acclimation: Chloride Cells

Micrographs of representative gill sections from cultured LLxLL and wild salmon smolts sampled prior to seawater exposure (June 13, 1984) are shown in Figure 3-12. Chloride cells appeared to decline in number over the summer in fish retained in freshwater and were difficult to locate on August 17 (Figure 3-13). Seawater acclimated wild anadromous salmon and the single remaining seawater adapted LLxLL salmon had numerous well developed chloride cells on this date (Figure 3-14).

On June 13 the mean chloride cell length was greater in wild smolts than in cultured LLxLL salmon, but not significantly so. A gradual decrease in chloride cell length occurred over the summer in fish retained in freshwater. Fish acclimated to seawater developed elongated chloride cells (Table 3-7). By July 18, mean chloride cell length was greater in seawater-acclimated wild smolts than in those smolts retained in freshwater ($t=11.72$, $df=6.2$, $P<0.001$). In cultured

Figure 3-10. Fork length and weight (±SE) of LLxLL, SRxLL and SRxSR salmon during 1983-84 growth experiment in seawater. Sample sizes are indicated to the right of each curve.


FORK LENGTH (CM)
40
35
30
25
20
15
9
6
3
SR x SR
SR x LL
LL x LL
WEIGHT (GM)
700
500
300
100
9
6
3
JUN
AUG
OCT
DEC
FEB
APR
JUN

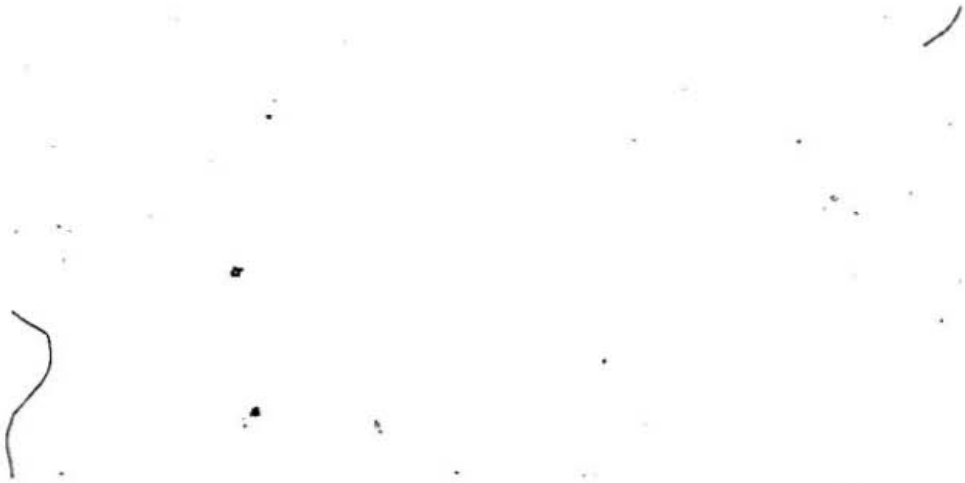
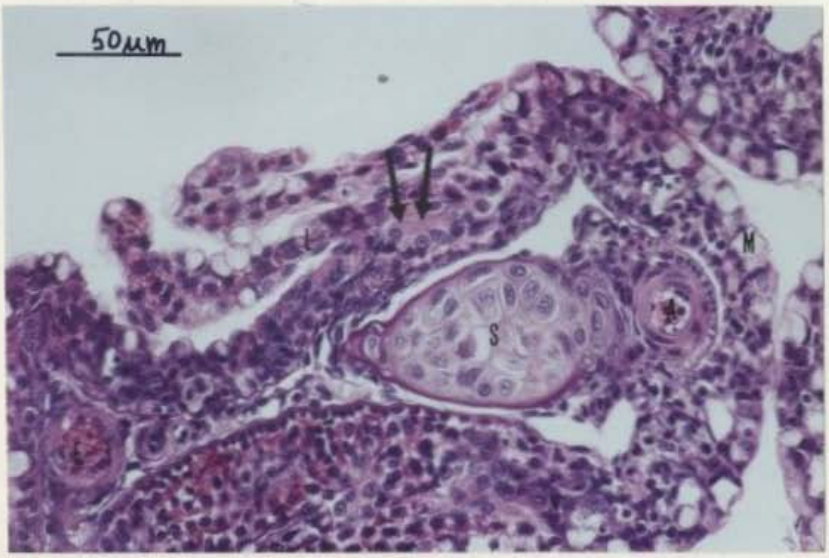


Figure 3-11. Representative specimens of LLxLL (right), SRxLL (lower left) and SRxSR (upper left) salmon in late December 1983, after 7 months growth in seawater.

Figure 3-12. Representative gill sections from cultured LLxLL (top) and wild anadromous salmon (bottom) sampled on June 13, 1984 prior to seawater acclimation. Chloride cells are indicated with arrows. Abbreviations: A, afferent filamental artery; E, efferent filamental artery; L, secondary lamella; M, mucus cell; S, supporting element.


50μm
M
S

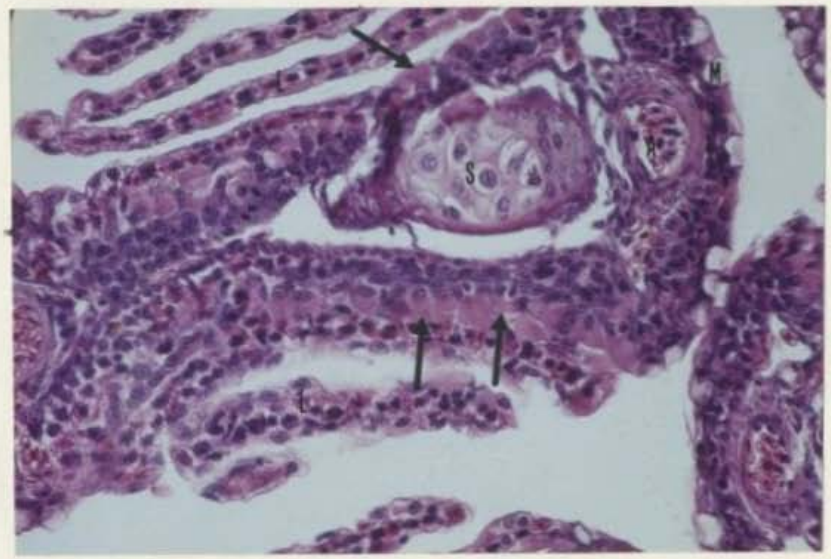
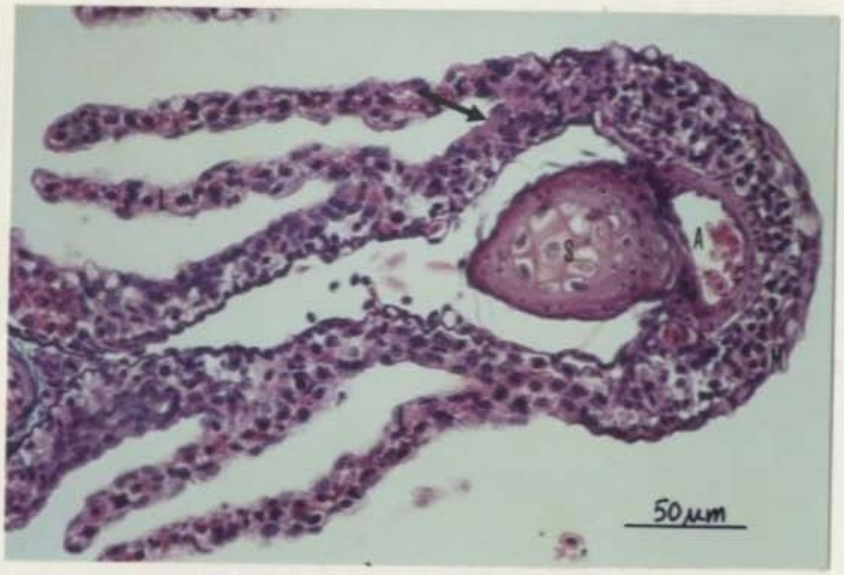

M
S

Figure 3-13. Representative gill sections from cultured LLxLL (top) and wild anadromous salmon (bottom) retained in fresh water until August 17 1984. Chloride cells are indicated with arrows. Abbreviations: A, afferent filamental artery; E, efferent filamental artery; L, secondary lamella; M, mucus cell; S, supporting element.


S
A
M
50µm

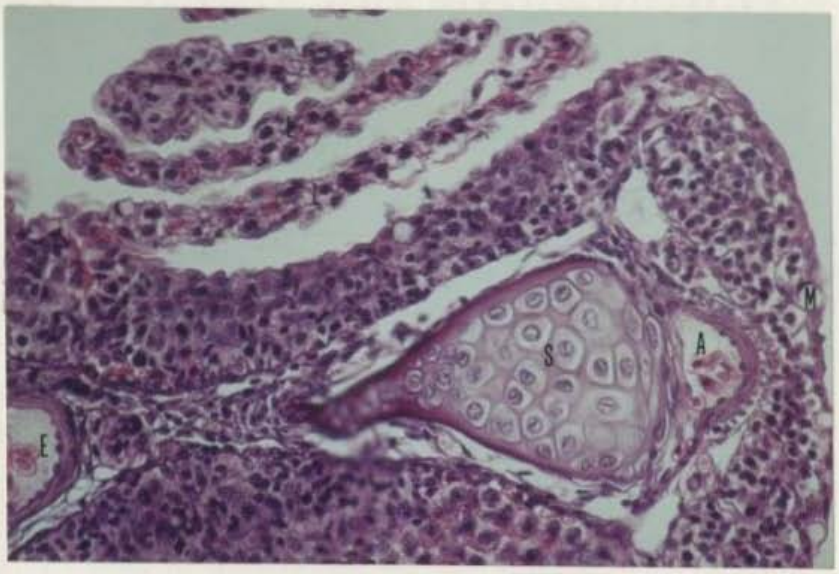
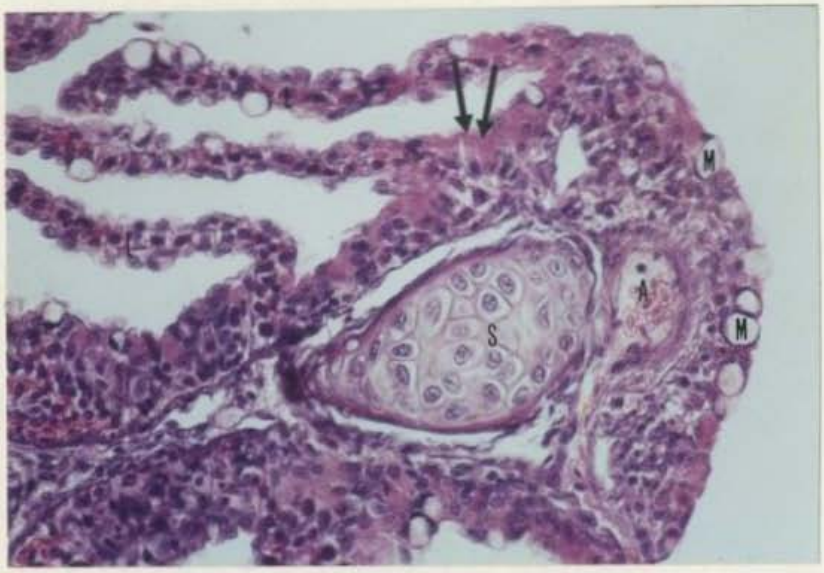
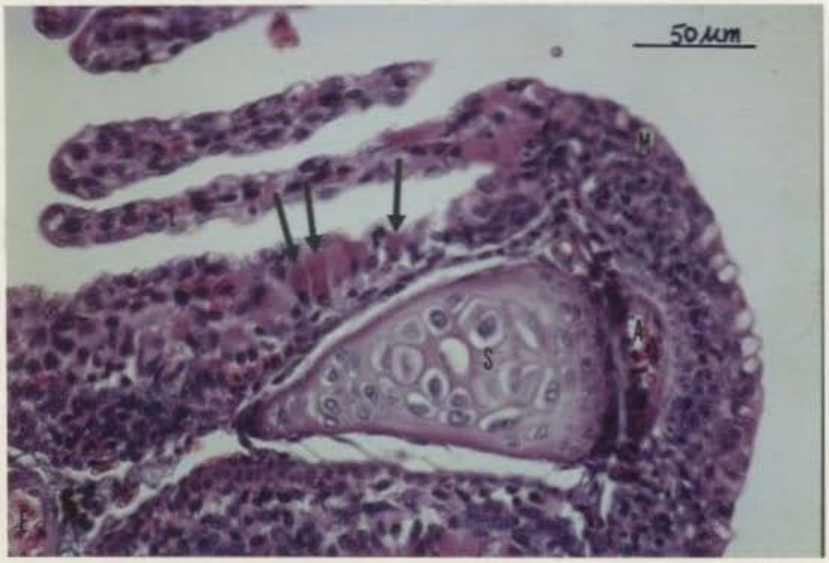

E
S
A
M

Figure 3-14. Representative gill sections from cultured LLxLL (top) and wild anadromous (bottom) salmon acclimated to seawater and sampled on August 17 1984. Chloride cells are indicated with arrows. Abbreviations: A, afferent filamental artery; E, efferent filamental artery; L, secondary lamella; M, mucus cell; S, supporting element.


M
S
A
M


50 µm
M
S
A

LLxLL salmon, mean chloride cell length was greater in seawater-exposed fish by June 29 ($t=4.36$, $df=5.9$, $P<0.001$). On August 17 the mean chloride cell length in seawater-acclimated fish was almost twice as great as in fish retained in freshwater. Chloride cells in seawater-acclimated salmon were not only much larger than in freshwater fish, but were more eosinophilic and deeply stained.

TABLE 3.7: Mean chloride cell length (μm) in wild anadromous smolts and cultured LLxLL salmon exposed to seawater (SW) and retained in freshwater (FW) in 1984. Except where indicated each point is a mean of 5 fish; 5 cells were measured in each fish. Standard errors appear in parentheses.

		June 13	June 20	July 18	August 17
Wild	FW	15.3 (0.72)	17.5 (0.57)	11.6 (0.22)	10.5 (0.34)
	SW		17.8 (0.60)	16.9 (0.40)	21.9 (0.84)*
LLxLL	FW	13.9 (0.32)	12.9 (0.18)	12.5 (0.46)	10.7 (0.27)
	SW		14.6 (0.35)	18.9 (0.43)	18.8**

*n=3
**n=1

Chapter 4
DISCUSSION

Seasonal changes in the various indices of smoltification followed the patterns typically associated with the parr-smolt transformation more closely in SRxSR females than SRxSR males or in either sex of the other groups. SRxSR females were the only group to experience a decrease in total lipid content and condition factor accompanied by increased moisture levels during the spring months. Similarly this was the only group in which a distinct peak in branchial Na^+-K^+ ATPase activity was observed. There were peaks in enzyme activity in male and female SRxLL salmon in early May and June respectively, but the mean values did not approach those observed in SRxSR females. The seasonal pattern of Na^+-K^+ ATPase activity in LLxLL salmon did not resemble that expected for fish preparing to enter a highly saline medium. Activity peaked in April and early May in females and males respectively, but mean values in both sexes were less than 15 μmoles Pi/mg protein per hr. Johnston (1983) suggests that a value of 20 μmoles Pi/mg protein per hr as the minimum enzyme activity to permit normal seawater survival.

The nonanadromous salmon (LLxLL) became quite silvery by late May, however they did not attain the degree of silvering observed in the anadromous (SRxSR) salmon. The hybrids (SRxLL) attained an intermediate degree of silvering. Silvering has been reported in several other populations of nonanadromous salmon (Dahl, 1928; Wilder, 1947; Havey and Warner, 1970; Barbour *et al.*, 1979) so its occurance in the present study is not surprising. Many stocks of nonanadromous salmon undergo a pelagic phase (including the LLxLL

salmon used in this study) in lakes or ponds analogous to the marine phase that occurs in anadromous stocks. It is probable that development of a cryptic silvery colouration is adaptive to salmon of both forms.

The seasonal patterns of lipid and moisture content and condition factor in LLxLL and SRxLL salmon and SRxSR males did not resemble those of smoltifying fish. While lipid content in SRxSR females was falling (from April until the termination of the study) both sexes of SRxLL and LLxLL salmon were experiencing increasing levels. These changes were accompanied by falling moisture content (and increasing condition factor in LLxLL salmon). Lipid content, moisture and condition factor did not change appreciably in male SRxSR salmon at any time. Barbour (1979) and Barbour and Garside (1983) made a similar study of the parr-smolt transformation in cultured nonanadromous salmon from Chamcook Lake, New Brunswick. They also found that lipid/moisture dynamics did not follow patterns typical of smolting fish. The apparent absence of smoltification in the sexually mature SRxSR males does not agree with the findings of Saunders *et al.* (1982), who reported that smoltification patterns in hatchery reared mature male parr were similar to those in immature parr. They found that salinity tolerance, gill Na^+-K^+ ATPase levels, survival, growth, and thyroid hormone levels were not significantly lower in the mature parr.

Gill histology did not prove to be a reliable indicator of smolt status. Chloride cells were plentiful in the gill epithelium of cultured, freshwater salmon in the winter and spring, but became much smaller and fewer in number in July and August. Similarly there were many chloride cells present in wild smolts caught during their seaward migration, and a reduction during the summer in fish retained in fresh water. Seawater acclimation caused an increase in size of chloride cells which were, based on qualitative criteria, also more numerous than in fish retained in freshwater. Hypertrophy of chloride cells after seawater adaptation has been reported previously in eels, *Anguilla japonica* (Shirai and Utida, 1970) and in Atlantic salmon (Langdon and Thorpe, 1984). Based upon

qualitative histological observation, the increased Na^+-K^+ ATPase activity in SRxSR females on May 27 was not associated with an increase in chloride cell number. Barbour (1979) observed no increase in chloride cell number or size associated with the parr-smolt transformation in anadromous and nonanadromous salmon exposed to manipulated photoperiods and water temperatures. Langdon and Thorpe (1984) report similar chloride cell numbers in Atlantic salmon parr and smolts, the latter having approximately 7 times as much branchial Na^+-K^+ ATPase activity. Thomson and Sargent (1977) demonstrated that increased levels of Na^+-K^+ ATPase activity in silver eels, *Anguilla anguilla* adapting to seawater resulted principally from increased enzyme concentration rather than from increased chloride cell number. However, irrespective of possible change in Na^+-K^+ ATPase activity, Burton and Idler (1984) observed an increase in the number of chloride cells in anadromous Atlantic salmon smolts after adaptation to seawater. Also in a small percentage of landlocked salmon which successfully adapted to seawater there was a greatly increased number of gill chloride cells. Langdon and Thorpe (1984) also claim that saltwater adaptation of *S. salar* induced proliferation and enlargement of chloride cells as well as stimulation of Na^+-K^+ ATPase activity. The relationships between chloride cells and salinity in cultured and wild LLxLL and SRxSR salmon appear to be complex and warrant more extensive study.

Despite observations that indicate the cultured SRxSR females smoltified, the degree of change that occurred in the various criteria of smoltification was of lesser magnitude than that reported in wild fish. There was a trend toward decreased condition factor in cultured SRxSR females, but at no time did mean values fall below unity. Hoar (1939) studied the weight/length relationship in wild Atlantic salmon and reported that mean condition factor in migrating smolts fell to less than 0.80. The low condition factor noted in the present study in wild Exploits River smolts (0.93) agrees with the findings of Hoar. Johnston and Saunders (1981) found that condition factor in cultured yearling Atlantic salmon tended to increase over the winter and spring while total lipid levels remained

constant. On the other hand, Farmer *et al.* (1978) found that lipid levels in cultured Atlantic salmon smolts fell to less than half the initial value between late February and mid July while moisture increased from 71.8 to 76.3 percent over the same period. Although a seasonal drop in lipid level was seen in cultured SRxSR females, the lowest values attained were much greater than those found in wild smolts. Cultured SRxSR females had a mean total lipid level of 16.8 percent compared to 3.6 percent in the wild smolts. Consistent with the low lipid value in wild smolts was the high moisture content found in these fish. In the same vane, cultured SRxSR females did not attain Na^+-K^+ ATPase activities comparable to those in wild smolts. The highest mean value recorded for cultured salmon in the present study was 22.3 μmoles Pi/mg protein per hr on May 27 compared to 40.5 μmoles Pi/mg protein per hr in wild smolts on June 6. Johnston (1983) found higher peak Na^+-K^+ ATPase activity levels in hatchery salmon reared outdoors (30 μmoles Pi/mg protein per hr) than in salmon reared indoors (20-25 μmoles Pi/mg protein per hr) (Johnston and Saunders, 1981).

It is apparent that caution is warranted when comparisons are made between studies concerned with proximate analysis and other indices of smoltification. Obviously culture conditions including ration size, temperature and photoperiod regimes, and fish stock will influence directly the values obtained in such studies. Comparison of seasonal trends in such analyses therefore may be more useful in assessing smolt status than the absolute values obtained.

The seawater acclimation experiment demonstrated a clear difference in osmoregulatory ability between the cultured LLxLL salmon and the wild anadromous smolts. The LLxLL fish were unable to cope with high salinity and their plasma ion concentrations increased throughout the experiment. In the present study an increase of approximately 23 percent occured in plasma Na^+ and Cl^- concentration in wild fish after acclimation to seawater, while plasma sodium in the LLxLL salmon increased by about 53 percent over freshwater levels. Parry (1961) found that the plasma ionic concentration increased by approximately 12 percent in wild smolts acclimating to seawater.

Few studies have been conducted on salinity tolerance in nonanadromous Atlantic salmon, and the few that have been done do not report identical findings. Evropeytseva (1963) compared salinity tolerance in four stocks of salmon smolts: anadromous salmon of Barents Sea and White Sea origin, Baltic salmon, and freshwater salmon of Lake Lagoda stock. It was determined that survival time in highly saline water (56 ppt) was 40 percent longer in the anadromous salmon than in the lake salmon. Baltic salmon performed only slightly better than the lake salmon, an unsurprising observation since the salinity in the Baltic Sea is only about 5 ppt. In another study dealing with European nonanadromous salmon, Koch (1983) examined plasma sodium regulation in a hatchery stock originating from Lake Vanern, Sweden. These fish experienced a slight increase in plasma sodium during the first 24 hours after direct transfer to seawater (30 ppt), followed by a reduction over the next 12 days to levels slightly above those noted in freshwater. Leduc (1972) reported that the behaviour of plasma Cl^- in small ouananiche parr from the Lake Victor stock (Quebec) was similar to that observed in anadromous parr. Burton and Idler (1984) performed salinity tolerance tests on fish from the same stock of nonanadromous salmon that was used in the present study. Only about 11 percent survived after 42 days in full strength seawater. Barbour and Garside (1983) compared salinity tolerance in Chamcook Lake nonanadromous salmon with anadromous smolts. At 30 ppt the anadromous salmon displayed significantly higher survival time while at 40 ppt both stocks survived for less than one day.

Adaptation to hyperosmotic media in salmonids involves two phases. In experiments in which rainbow trout, *Salmo gairdneri* were transfered to seawater, an initial adjustive phase has been observed wherein plasma and body ion levels are elevated and osmotic water loss occurs. The adjustive phase is followed by a regulative phase in which ions are actively excreted, urine becomes concentrated and reduced in volume, and plasma ion levels return to near freshwater values (Bath and Eddy, 1979; Houston, 1959). Reports do not agree about the duration of the adjustive phase. Houston (1959) reported an adjustive period of 70-180

hours while Bath and Eddy (1979) reported a duration of only 8 hours for the same species. Parry (1960) found that Atlantic salmon smolts, transfered to seawater from freshwater, remain for only an hour in the adjustive phase. Houston (1960) reported a duration of approximately 60 hours for Atlantic salmon. The results from the present study are not directly comparable with those cited above because a slow acclimation was involved, however chloride levels remained stable after approximately 100 hours exposure to increasing salinity.

The continued high branchial Na^+-K^+ ATPase activity in seawater acclimated wild anadromous smolts agrees with patterns observed in coho salmon, *Oncorhynchus kisutch* (Folmar and Dickhoff, 1981) and rainbow trout (Johnston *et al.*, 1983). Such high levels of enzyme activity are necessary to maintain acceptable internal ion concentrations. Reduction in Na^+-K^+ ATPase activity in salmon held in freshwater after the parr-smolt transformation has been reported previously (Zaugg and McLain, 1970, Zaugg et al., 1972; Johnston, 1983) and is part of the desmoltification process observed by Malikova (1959) and Evropeytseva (1963). Evropeytseva (1963) found that when Atlantic salmon smolts were retained in freshwater beyond the time of normal seaward migration the degree of silvering decreased, lipid levels increased to pre-smolt values, salinity tolerance decreased, and signs of reduced thyroid activity occured. This process represents a readaptation of the smolt to a freshwater habitat. The period of reduced enzyme activity in this study corresponds with seasonal temperature elevation. Temperatures in excess of 15°C have been shown to inhibit gill Na^+-K^+ ATPase activity in migrating coho salmon and rainbow trout (Zaugg *et al.*,1972; Adams *et al.*, 1973; Zaugg and McLain, 1976). Comparison of Na^+-K^+ ATPase activity in *S. salar* under freshwater and seawater conditions requires further rigorously controlled experimentation to assess the relative importance of temperature and salinity.

There was no preparatory elevation in branchial Na^+-K^+ ATPase activity

observed in the LLxLL salmon in freshwater although an apparent induction of enzyme activity occurred in animals exposed to seawater. By July 18 the mean Na^+-K^+ ATPase activity in the seawater exposed LLxLL salmon was only slightly less than that in the wild anadromous smolts acclimated to seawater. This induction in enzyme activity however, was not sufficient to reduce plasma ion levels, so it appears that the inability of LLxLL fish to hypoosmoregulate is due to some other cause. Hypoosmoregulation has been shown to involve more than just an increase in branchial Na^+-K^+ ATPase activity. Holmes and Stainer (1966) determined that during the parr-smolt transformation in rainbow trout, urine volume decreases while ionic concentration increases. Farmer *et al.* (1978) demonstrated that the urine of seawater (31 ppt) acclimated Atlantic salmon is more than six times as concentrated as urine produced by salmon acclimated to a salinity of 0.1 ppt. Collie and Bern (1982) reported an increase in intestinal fluid transport in coho salmon during the parr-smolt transformation. The ocean is a dehydrating medium wherein osmotic loss of body water is counteracted by drinking seawater. Increasing intestinal water transport facilitates replacement of water lost through osmotic action. The failure in hydromineral regulation observed in the LLxLL salmon could be associated with either or both of these seawater adaptation mechanisms.

Growth rate is an excellent indicator of adaptation to seawater in salmonids. If good growth is achieved then one can assume that the fish are well adapted for such a medium (i.e. that smoltification has occured). Poor growth and endocrine dysfunction have been reported by Clarke and Nagahama (1977) in underyearling coho salmon transferred to seawater prior to smoltification. The growth rates observed among the three groups in this study clearly indicate that the SRxSR salmon were the best adapted to the marine environment. It is probable that growth in the SRxLL and LLxLL salmon was retarded at least in part due to the initiation of sexual maturation. Since none of the SRxSR group matured, they were able to direct more energy toward somatic growth than could the maturing SRxLL and LLxLL salmon. In the latter two groups energy that could otherwise

be devoted to somatic growth was used in gonadal development. It is also possible that the SRxSR salmon were more efficient hypoosmoregulators and therefore expended less energy to maintain homeostasis than the other groups. If this is true then still more energy was diverted away from somatic growth in those salmon that matured.

It would appear that growth in seawater was influenced by at least two factors. Immature SRxLL fish were larger than mature fish from the same group. However the SRxLL salmon that matured were still larger than the LLxLL salmon on Oct. 15 (all of which matured). This indicates that some genetic factor(s) quite separate from those that determine the the timing of the onset of gonad maturation, and associated with growth rate, are received in the SRxLL salmon from the anadromous parent.

Atlantic salmon do not commonly migrate to sea as smolts and then mature the following autumn. Typically, the seaward migration in anadromous salmon is at least one year in duration. Evropeytseva (1959) therefore concluded that the processes of smoltification and maturation are biologically incompatible and cannot occur during the same year. Such a view implies that a smolt is not simply a fish capable of tolerating seawater, but is also a fish that is about to undertake an extended migration and thus will not spawn for at least one year. If this is true then the SRxLL and LLxLL salmon in this study that matured in seawater were not true smolts; rather they would be more accurately described as nonanadromous salmon capable of acclimation to seawater.

The observation that some nonanadromous salmon in the studies cited above were capable of seawater adaptation is of interest with respect to the evolution of these forms. Such findings support the contention of Behnke (1972) that nonanadromous Atlantic salmon have arisen since the last glaciation from anadromous ancestral stocks rather than from nonanadromous ancestors surviving the glaciation in lake refugia. Such an evolutionary history implies that nonanadromous stocks in each drainage basin arose independently from discrete

anadromous stocks. It is not unreasonable to suggest that individuals in present day nonanadromous stocks that are capable of osmoregulation in seawater have simply retained features characteristic of their anadromous ancestors. Those salmon from nonanadromous stocks that are not able to acclimate to seawater are indicative of a recent tendency away from anadromy. The parr-smolt transformation is widely assumed to be an energy intensive process, and therefore represents an inefficient utilization of resources when retained in stocks that do not normally go to sea. The suppression of the parr-smolt transformation would therefore be adaptive and selected for in these forms. The absence of morphometric differences between anadromous and nonanadromous salmon (Wilder, 1947) also supports a recent origin of the latter form. Ryman (1983) found only slight genetic differences between anadromous and nonanadromous forms of Atlantic salmon, brown trout, and rainbow trout, and similarly concluded that such forms are of recent origin.

The work of Ryman (1983) however does not preclude the existence of genetic differences between the two forms. Since the salmon in the present study were cultured under similar conditions, the differences noted in the patterns of smoltification presumably represent genetic differences. Sutterlin and MacLean (1984) found genetic differences in oocyte recruitment pattern in the same anadromous and nonanadromous stocks that were investigated in the present study. It appears therefore that there are important genetic differences between anadromous and nonanadromous Atlantic salmon. These differences however are behavioral (i.e. different migratory patterns) and physiological in nature and are not expressed morphologically.

The observation that smoltification was reduced in cultured SRxSR males is of interest with respect to reports of imbalanced sex ratios among naturally produced smolts, and may partially explain the preponderance of females among such groups. Gibson (1983) has shown that sexual maturation in male parr can result in a delay in the onset of smoltification because of reduced growth. Under

natural conditions, extended river residence probably reduces the proportion of male smolts because of increased predation, and the mature males that do smoltify and migrate are probably older than immature smolts. It could be argued therefore that the differences in the various criteria of smoltification observed in the SRxSR salmon occured because the mature males were too young to smoltify. It could also be argued that these differences occured because the immature females were larger than the mature males since the parr-smolt transformation is size dependent (Elson, 1957; Parry, 1960; Conte and Wagner, 1965; Ewing et al., 1980). However there was no significant difference in the mean length of the cultured SRxSR males compared to a sample of wild anadromous smolts captured while migrating to sea. Clearly the cultured males had attained the critical size necessary to permit smoltification and migration in wild salmon of this stock.

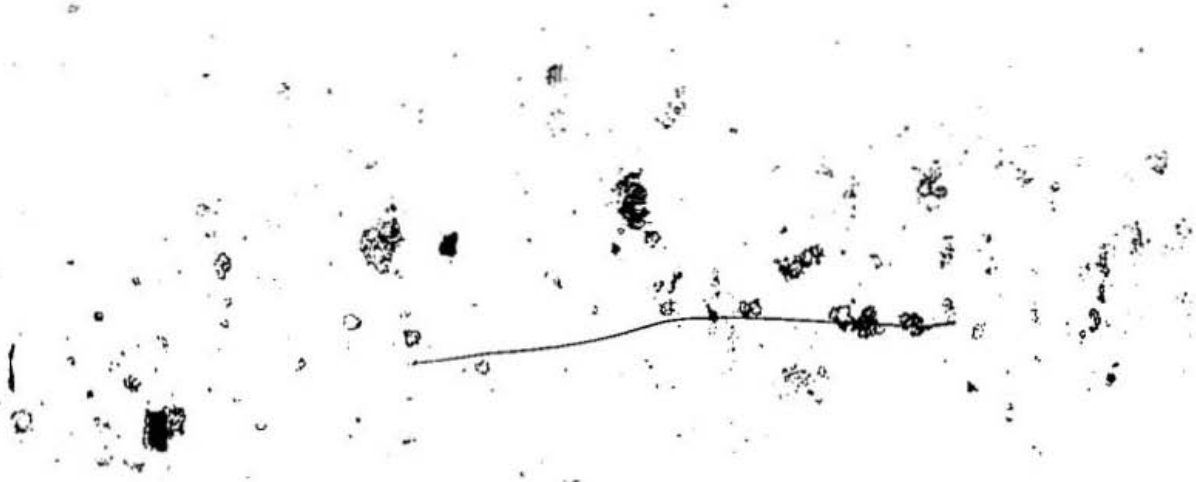
Circumstantial evidence (low proportion of males in smolt runs and low proportion of mature stream resident males older than the age of smoltification) led Dalley *et al.* (1983) to conclude that in Newfoundland rivers, only a small proportion of mature male salmon of smolt size actually smoltifies and migrates to sea, and that there is high mortality in the remaining fish. In the Matamek River (Quebec), Gibson, (1983) found that a proportion of mature male salmon parr does not appear to become anadromous, but rather remains in the river permanently. Such parr become considerably larger than the smolts that migrate to sea. It is quite possible however that precocious maturation in male parr does not have such effects on subsequent smoltification in all populations of Atlantic salmon. Saunders *et al.* (1982) found that the parr-smolt transformation was not compromised in cultured mature male parr from New Brunswick. Similarly, seaward migration of mature males has been reported in European stocks (Mitans, 1973; Thorpe and Morgan, 1980). While different rearing conditions may account for the disagreement between studies, it is possible that genetic differences are involved. Further investigation would seem warranted.

Jones (1959) expresses the view that large anadromous males are the primary spawners in Atlantic salmon, and that sexually mature male parr therefore represent a form of "biological insurance". Osterdahl (1969) stated the opposite view, that male parr are the primary spawners and that large anadromous males function as "biological insurance" (and are necessary for colonization of rivers without salmon). If salmonids originated in freshwater (Tchernavin, 1939; Hoar, 1976) then the latter position appears plausible since reproduction without prior seaward migration would represent the original life history pattern. There is nothing to suggest that maturation in male parr is a new phenomenon, hence it appears that anadromy and the precocious maturation in nonanadromous male parr represent alternative reproductive strategies. Thus, recent studies have applied the theory of games to salmonid life history patterns (Gross, 1984; Myers in prep.). Such treatment is appropriate in situations where competition occurs between alternative reproductive strategies, and where frequency dependent mechanisms operate such that an evolutionarily stable combination of strategies is maintained. Gross (1984) offered a theory for coho salmon in which frequency dependent agonistic behavior among males on the spawning ground ensures the fitness of both strategies. Myers (in prep.) suggests an alternative hypothesis leading to a mixed evolutionarily stable strategy wherein a high incidence of precocious male maturation increases the fitness of large anadromous males because of their ability to spawn with many females.

An assumption inherent in any biological application of game theory is that the alternative reproductive strategies have a genetic basis. It may be argued that environmental factors determine if males mature as parr or smoltify and migrate to sea. Several studies have shown that rapidly growing parr are more apt to mature precociously than slowly growing parr (Leyzerovich, 1973; Simpson and Thorpe, 1976; Saunders et al., 1982; Gibson, 1983), and that growth rate is influenced by environmental factors (density, food availability, temperature etc.). Gibson (1983) noted that growth rate of salmon parr in the Matamek River has increased due to reduced density between 1967 and 1976. This has resulted in an

increased incidence of precocious male maturation that is reflected by a decrease in the male:female ratio in recent smolt runs. Such population shifts would presumably result in fewer large anadromous males on the spawning ground relative to mature parr. If frequency dependent reproductive success (fitness) is operating then the few large anadromous males that do spawn will achieve greater success than the mature parr and therefore anadromy will be favoured. Future monitoring of anadromous sex ratios in this river may provide evidence for or against the operation of frequency dependent reproductive success in Atlantic salmon populations.

In conclusion, this study indicates that, apart from the attainment of silvery colouration, the nonanadromous Atlantic salmon that inhabit Five Mile Pond East, Newfoundland, do not undergo a parr-smolt transformation comparable to that observed in anadromous salmon from the Exploits River, and in general cannot acclimate to seawater. As well, cultured mature male parr of Exploits River stock did not smoltify, while immature females of the same stock did show evidence of smoltification. This finding provides direct support for the contention of Dalley *et al.* (1983) that mature male parr in some Newfoundland populations are not likely to smoltify and migrate to sea.



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