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Mitochondrial enzyme and Na^+,K^+-ATPase activity, and ion regulation during parr-smolt transformation of Atlantic salmon (*Salmo salar*)

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Abstract

Atlantic salmon (*Salmo salar*) exposed to either simulated natural photoperiod (SNP) or continuous light (L24) were used to examine developmental changes in the presence and absence, respectively, of the parr-smolt transformation. Plasma osmolarity and ion concentrations were unaffected by photoperiod treatment. Gill Na^+,K^+-ATPase specific activity increased 150% between February and June in SNP fish and was low and unchanged in L24 fish. Kidney Na^+,K^+-ATPase specific activity varied within similar, narrow limits in both groups. Citrate synthase of liver, gill and kidney, expressed as specific activity or activity/g total body weight (relative activity), increased 25–60% between March and June in SNP fish. With the exception of kidney relative activity, citrate synthase activity declined to initial (March) levels by August. Liver, gill and kidney cytochrome c oxidase activity of the SNP group underwent similar though less marked changes. Liver, gill and kidney citrate synthase and cytochrome c oxidase activities of the L24 group remained relatively constant between March and August, and where significant differences occurred, they were lower than those of the SNP group. These results indicate that respiratory capacities of the liver, gill and kidney increase in smolts concurrent with preparatory osmoregulatory changes, and subsequently decline. The findings are consistent with a hypothesized transient increase in catabolic activity during the parr-smolt transformation that may be due to the metabolic demands of differentiation.

Introduction

Coincident with their migration from fresh water to seawater, Atlantic salmon (*Salmo salar*) and other anadromous salmonids undergo a variety of changes, collectively known as the parr-smolt transformation, that are adaptive for life in a marine environment. Among these is a photoperiod-cued increase in salinity tolerance that results from differentiation of osmoregulatory organs in fresh water, prior to and in preparation for seawater entry (Hoar 1976; McCormick and Saunders 1987). Increased gill Na^+,K^+-ATPase activity occurs in spring in several salmonid species (Zaugg and McLain 1970; Saunders and Henderson 1978). In coho salmon (*Oncorhynchus kisutch*) there are increases in the number of gill chloride cells (Richman *et al.* 1987) and net intestinal fluid absorption (Collie and Bern 1982). Seasonal changes in urine flow of Atlantic salmon have also been observed

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(Holmes and Stainer 1966; Eddy and Talbot 1985).

In addition to the large number of physiological changes which occur during the parr-smolt transformation, there are several lines of evidence indicating that the transformation is accompanied by increased metabolic rate. These include decreases in muscle and liver lipid content (reviewed in McCormick and Saunders 1987), increased liver glycogenolysis and lipolysis (Sheridan *et al.* 1985) and increased liver mitochondrial enzyme activity (Blake *et al.* 1984). It is unclear, however, whether these metabolic changes are permanent or transient, and to what extent they also occur in osmoregulatory organs (*e.g.* gill and kidney). It has been suggested that increased metabolism during the parr-smolt transformation may be partly due to the catabolic demands of differentiation (Sheridan *et al.* 1985; McCormick and Saunders 1987).

In the present study we investigated whether changes in metabolic capacity which occur in the liver also occur in the gill and kidney, whether these changes are permanent or transient, and finally whether seasonal and/or size-related changes in metabolic capacity and osmoregulation could be distinguished from those which are due directly to the parr-smolt transformation. We have previously demonstrated that exposure to continuous light early in development inhibits osmoregulatory changes (increases in salinity tolerance and gill Na^+,K^+-ATPase activity) that normally occur in spring, but has little or no effect on growth rate in spring (Saunders *et al.* 1985; McCormick *et al.* 1987). Atlantic salmon exposed to continuous light, therefore, provide a useful control for examining metabolic and osmoregulatory changes in the presence and absence of the parr-smolt transformation.

Materials and methods

Rearing and sampling

Fertilized Atlantic salmon eggs were transported to St. Andrews Biological Station, N.B., Canada, just after hatching in April and reared in 1 m^2 Swedish style tanks supplied with fresh water at 12 $l \cdot min^{-1}$. All fish were initially exposed to simulated natural photoperiod which provided a seasonal change in daylength (McCormick *et al.* 1987). Overhead lighting to all tanks was by standard fluorescent bulbs which provided light intensities at the water surface of 430–540 lx. On September 15 fish were separated into four tanks of approximately 100 fish each; two tanks were exposed to continuous (24h/d) light (designated the L24 group) for the remainder of the study, while the other two tanks were exposed to simulated natural photoperiod (designated the SNP group). Water temperature fluctuated seasonally (6–18°C) during early rearing (April through December) and then was kept constant (6–8°C) during the period of physiological sampling (January through August). The fish were maintained in fresh water throughout the experiment. Salmon were fed a commercial dry pellet at maximum ration (adjusted to body size and temperature) at half hour intervals during daylight hours corresponding with the SNP group.

On February 20, gill tissue from 8 fish in each group were sampled for Na^+,K^+-ATPase activity determination in order to verify that the time-course of development was proceeding as in previous years (McCormick *et al.* 1987). On March 21, April 17, May 7, June 11 and August 1, 8 fish from each group were sampled. Animals were starved overnight and sampled between 0900 and 1700 Atlantic standard time. After being stunned by a blow to the head, fish were measured for length and weight, and blood was collected from the caudal blood vessels with a heparinized syringe immediately before tissue removal.

The first two pairs of gill arches on each side were removed, the bony tissue (ceratobranchials) trimmed away, and each pair was blotted dry of external fluid and weighed to the nearest mg. The kidney and liver were removed, blotted dry and weighed. The posterior third of the kidney was severed logitudinally. One of these portions of the kidney and one of the two pairs of gill arches were used for mitochondrial enzyme analysis, while the remaining kidney and gill tissue was used for Na^+,K^+-ATPase determination. The liver was minced into 3 to 5 mm^3 pieces and several of these were chosen at random for mitochondrial enzyme determination. Epaxial white muscle just anterior to the dor-

sal fin was weighed and dried to a constant weight at 80°C. Blood was centrifuged at 4,000 × g for 5 min and plasma was removed and frozen at −20°C. Plasma cations were measured by atomic absorption spectrophotometry, [Cl^-] by Buchler-Cotlove chloridometry and osmolarity by vapor pressure osmometry.

Enzyme assays

Citrate synthase and cytochrome c oxidase activities were measured within 1h of tissue removal. Tissues for Na^+,K^+-ATPase measurement were stored in SEI buffer containing 0.3 M sucrose, 0.02 M ethylene diamine tetraacetic acid and 0.1 M imidazole (pH 7.3), at −80°C for up to 4 weeks. All enzyme assays were performed at 20 (± 0.1)°C.

Na^+,K^+-ATPase (E.C. 3.6.1.3) was measured by a modification of the method of Zaugg (1982). Tissues (0.05–0.12 g wet weight) were homogenized in 0.8 ml SEI buffer with 0.1% sodium deoxycholate in a hand-operated conical glass homogenizer (0.13 mm clearance) and then centrifuged for 5 min at 3,000 × g. Na^+,K^+-ATPase activity of the supernatant was determined by measuring the production of inorganic phosphate following incubation for 10 min in 114 mM NaCl, 17 mM $MgCl_2$, 55 mM KCl, 85 mM imidazole and 4 mM Na_2ATP at pH 7.3, in the presence or absence of 0.5 mM ouabain. All substrates were experimentally determined to be at saturating concentrations. Inorganic phosphate was measured by the method of Heinonen and Lahti (1981) and protein by the method of Miller (1959). All assays were performed in duplicate and expressed as μmoles P_i · mg $protein^{-1}$ · h^{-1}. Intra- and inter-assay coefficients of variation for the Na^+,K^+-ATPase assay, including homogenization, were 3% and 12%, respectively.

For determinations of citrate synthase (CS; E.C. 4.1.3.7) and cytochrome c oxidase (CCO; E.C. 1.9.3.1) activity, tissues were placed in 20 volumes 25 mM Tris, 1 mM EDTA at pH 8.1, and homogenized for two-8 second bursts with a Polytron (set at 70% maximum) and two 8-second bursts with an ultrasonic tissue disrupter (set at 60% maximum) while on ice. Homogenization and sonication for longer periods (> 10 s) decreased mitochondrial enzyme activity. Prior to routine measurement, CS and CCO activities were measured at several buffer concentrations (15, 30, 50 and 80 mM) and pH (6.8 to 8.4 at 0.3-unit intervals) using several preparations of gill, kidney and intestine, and the concentration and pH that yielded the greatest activity were used thereafter. All substrates were experimentally determined to be at saturating concentrations. CS activity was determined by measuring the linear rate of release of sulfhydryl-coenzyme A with Ellman's reagent in 50 mM Tris, 0.5 mM oxaloacetate and 0.15 mM acetyl CoA (pH 8.1) at 412 nm in a recording spectrophotometer (Alp *et al.* 1976). CCO activity was determined by measuring the linear rate of oxidation of 0.057 mM ferrocytochrome c in 50 mM phosphate buffer (pH 7.1) at 550 nm in a recording spectrophotometer (Blake *et al.* 1984). All assays were performed in duplicate. Intraassay coefficient of variation for both the CS and CCO assays, including homogenization, was 6%.

To compare organ mitochondrial enzyme activity among fish with differing organ weights, changes in wet weight specific mitochondrial enzyme activity were normalized for changes in relative organ weight and termed the relative enzyme activity. The latter was calculated by multiplying the organ wet weight activity (μmoles product · g organ wet $weight^{-1}$ · h^{-1}) by the relative organ weight (g organ wet weight/g body weight) and was expressed as μmoles product produced by the organ/g total body weight/h. This method permits direct comparison of organ enzyme activity in instances, such as the present, in which relative organ weight differs with time or between experimental groups. Specific activity was also calculated and expressed as μmoles product · mg $protein^{-1}$ · h^{-1}.

Calculations and statistics

Condition factor was calculated as (weight/$length^3$) · 100. The first two pairs of gill arches were determined to be 59.7 ± 1.0% of total gill weight (n = 10, 50–100g fish); therefore, a factor of 1.67 was used to calculate total gill weight.

Table 1. Length, weight, condition factor (100 · [weight/length^3]) and muscle moisture content (%) of Atlantic salmon exposed to stimulated natural photoperiod (SNP) or continuous light (L24).

		March 21	April 17	May 7	June 11	August 1
Length (cm)	SNP	16.2 ±0.4	15.8 ±0.2	16.3 ±0.2	18.6 ±0.5	20.0±0.8
	L24	16.5 ±0.3	16.3 ±0.4	16.7 ±0.2	17.8 ±0.8	19.2±0.8
Weight (g)	SNP	46.1 ±2.7	42.2 ±1.6	46.6 ±1.9	66.0 ±4.3	87.3±9.1
	L24	51.3 ±2.3	49.5 ±2.7	54.7 ±1.4	63.1 ±8.1	76.0±8.4
Condition factor	SNP	1.08±0.02	1.07±0.01	1.06±0.01*	1.01±0.02*	1.05±0.02
	L24	1.15±0.03	1.14±0.03	1.18±0.02	1.09±0.02	1.05±0.02
Muscle moisture content	SNP	75.6 ±0.3	75.4 ±0.4	75.1 ±0.2*	75.7 ±0.4*	75.5 ±0.2
	L24	74.7 ±0.4	74.4 ±0.3	73.5 ±0.2	74.4 ±0.5	75.5 ±0.4

Values are mean ± SEM of 6–8 fish in each group at each time interval. An asterisk signifies a significant difference between the SNP and L24 groups at a given time interval ($p < 0.05$, Student-Newman-Keuls test, preceded by two-way ANOVA, $p < 0.05$).

Determination of the statistical significance of photoperiod treatment (SNP versus L24) and date of sampling (changes over time) were determined by two-way ANOVA ($p < 0.05$). If photoperiod effects were significant, SNP and L24 groups were compared at each time interval using one-way ANOVA followed by Student-Newman-Keuls procedure ($p < 0.05$). If changes over time were significant, sampling dates within each group were compared using one-way ANOVA followed by Student-Newman-Keuls procedure ($p < 0.05$).

Results

Increase in length and weight occurred in both the SNP and L24 groups between May and August (Table 1, $p < 0.001$), but there was no significant difference in length and weight between the two groups ($p > 0.1$, two-way ANOVA). Condition factor of the SNP group decreased slightly (7%; $p = 0.02$) between March and June. Condition factor of the L24 group was significantly higher than the SNP group in May and June, but declined to the same level as the SNP group by August (Table 1). Muscle moisture content of the SNP group did not significantly change between March and August ($p > 0.1$). Muscle moisture content of the L24 group decreased in May and June and was significantly lower than the SNP group at these times.

Plasma osmolarity, $[Na^+]$ and $[Cl^-]$ of SNP and L24 fish decreased slightly (5%) but significantly ($p < 0.02$) between March and April, and remained constant thereafter (Fig. 1). There was no significant difference in plasma osmolarity, $[Na^+]$, $[Cl^-]$ and $[K^+]$ between the SNP and L24 groups at any time ($p > 0.08$), whereas plasma $[Mg^{++}]$ did differ between the two groups ($p < 0.001$). Plasma $[Mg^{++}]$ of SNP fish was 10–18% lower than that of L24 fish from March to June, but was not significantly different in August.

Photoperiod treatment had a marked effect on gill Na^+,K^+-ATPase activity which increased from 6.4 to 15.0 μmoles $P_i \cdot$ mg protein$^{-1} \cdot h^{-1}$ between February and June in SNP fish ($p = 0.02$; Fig. 2). In contrast, gill Na^+,K^+-ATPase activity of L24 fish remained between 6.8 and 8.5 μmoles $P_i \cdot$ mg protein$^{-1} \cdot h^{-1}$ throughout the study ($p > 0.1$). Kidney Na^+,K^+-ATPase activity remained relatively constant between March and August (between 25 and 29 μmoles $P_i \cdot$ mg protein$^{-1} \cdot h^{-1}$) and was not significantly affected by photoperiod treatment ($p > 0.1$).

Relative liver and gill weight (organ weight as a percent of total body weight) of all fish decreased with increasing size ($r = -0.43$ and -0.42, respectively, $p < 0.001$). There was no significant difference in relative liver weight of SNP and L24 fish ($p > 0.1$), whereas relative gill weight was significantly larger in SNP fish in April, May and June (Table 2). Relative kidney weight was not correlated with changes in size ($p > 0.1$), but was significantly larger in SNP fish in June and August.

Citrate synthase (CS) activity of liver, gill and

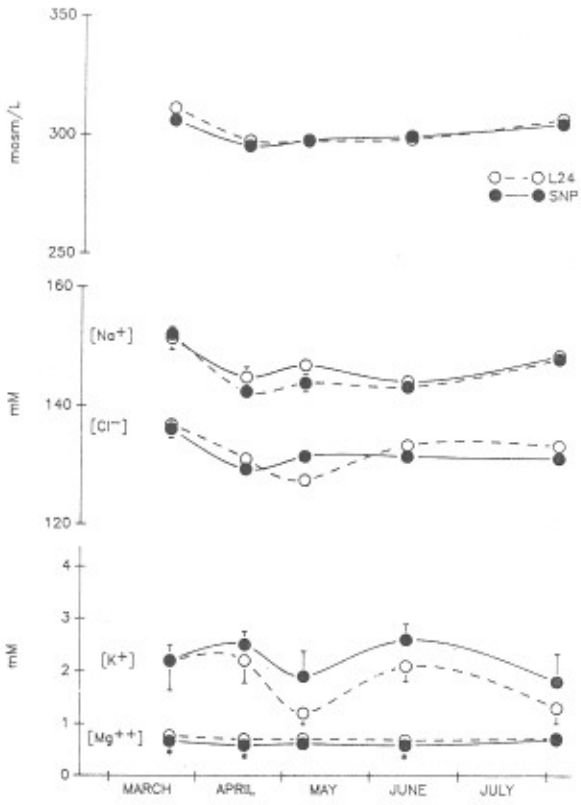

Fig. 1. Plasma osmolarity, $[Na^+]$, $[Cl^-]$, $[K^+]$ and $[Mg^{++}]$ of Atlantic salmon exposed to simulated natural photoperiod (SNP) or continuous light (L24). Values are mean ± SEM of 6–8 fish per group per sampling date. SEM's not shown are less than the value overlaid by the symbol of the mean. Intervals marked with an asterisk are significantly different from the L24 group ($p < 0.05$, two-way ANOVA followed by Student-Newman-Keuls test).

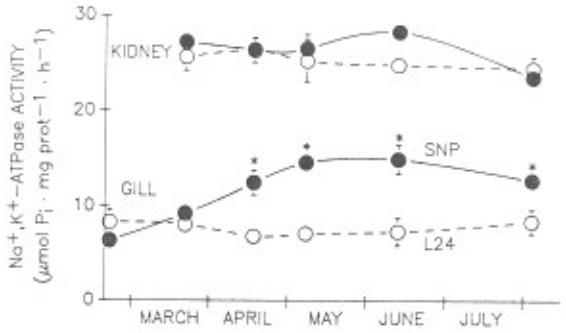

Fig. 2. Na^+,K^+-ATPase specific activity in gill and kidney of Atlantic salmon exposed to simulated natural photoperiod (SNP) or continuous light (L24). Values are mean ± SEM of 6–8 fish per group per sampling date. SEM's not shown are less than the value overlaid by the symbol of the mean. Intervals marked with an asterisk are significantly different from the L24 group ($p < 0.05$, two-way ANOVA followed by Student-Newman-Keuls test).

kidney, expressed as either specific activity (μmoles · mg protein^{-1} · h^{-1}; Table 2) or relative activity (μmoles · g body weight^{-1} · h^{-1}; Fig. 3), increased significantly between March and June in SNP fish ($p < 0.03$). CS specific activity in liver increased by 25% between March and June, followed by a decline of 20% between June and August. Relative CS activity of liver increased 35% between March and April, followed by a gradual decline through August. The L24 fish had significantly lower liver CS specific activity than the SNP group in March, April and May, though levels in L24 fish increased gradually and significantly ($p < 0.001$) from March to August. When expressed as relative activity, however, liver CS of L24 fish was unchanged over time ($p > 0.1$; Fig. 3).

Specific and relative CS activity of gill tissue of SNP fish increased by 25% and 40% ($p < 0.001$ and $p = 0.02$), respectively, between March and May, followed by a subsequent decline to initial levels by August (Table 1 and Fig. 3). Although there were significant changes in gill CS specific activity of L24 fish ($p = 0.001$), the magnitude of these fluctuations was relatively small (less than 20%) and the activity was always lower than that of SNP fish, significantly so in March, May and August. Gill CS relative activity of L24 fish was stable over time ($p > 0.1$) and was significantly lower than that of SNP fish in April through June.

CS specific activity in kidney tissue from SNP fish remained constant from March to May, increased significantly (25%; $p < 0.001$) in June and subsequently declined to initial levels in August (Table 2). Relative kidney CS activity of SNP fish rose by over 60% ($p = 0.002$) between March and June and remained elevated in August (Fig. 3). Specific and relative kidney CS activities of L24 fish were initially lower than SNP fish; relative activity of L24 fish did not change significantly between March and August ($p > 0.1$), whereas specific activity increased slightly but significantly ($p = 0.04$).

Changes in cytochrome c oxidase activities (CCO; Table 3) of liver, gill and kidney were, for the most part, similar to those in CS activity. Within-group

Table 2. Citrate synthase specific activity (CSSA) and relative organ weight of Atlantic salmon exposed to stimulated natural photoperiod (SNP) or continuous light (L24).

		March 21	April 17	May 7	June 11	August 1
CSSA (μmol·mg prot^{-1}·h^{-1})						
Liver	SNP	1.01 ± 0.04*	1.14 ± 0.05*	1.23 ± 0.07*	1.27 ± 0.04	1.08 ± 0.05
	L24	0.84 ± 0.05	0.96 ± 0.04	1.04 ± 0.03	1.16 ± 0.06	1.16 ± 0.05
Gill	SNP	4.02 ± 0.25*	4.64 ± 0.23	5.20 ± 0.19*	4.63 ± 0.21	3.85 ± 0.16*
	L24	3.38 ± 0.04	4.20 ± 0.17	3.90 ± 0.21	4.54 ± 0.37	3.14 ± 0.16
Kidney	SNP	5.15 ± 0.26	4.98 ± 0.21	5.16 ± 0.16	6.29 ± 0.13*	4.97 ± 0.16
	L24	4.16 ± 0.13	4.20 ± 0.17	5.22 ± 0.27	5.18 ± 0.29	4.66 ± 0.32
Relative organ weight (% body weight)						
Liver	SNP	1.30 ± 0.06	1.78 ± 0.17	1.45 ± 0.14	1.10 ± 0.04	1.14 ± 0.03
	L24	1.43 ± 0.04	1.66 ± 0.15	1.49 ± 0.10	1.09 ± 0.09	1.01 ± 0.03
Gill	SNP	0.74 ± 0.05	0.85 ± 0.02*	0.74 ± 0.03*	0.85 ± 0.12*	0.71 ± 0.09
	L24	0.79 ± 0.02	0.70 ± 0.02	0.64 ± 0.02	0.73 ± 0.01	0.65 ± 0.06
Kidney	SNP	0.72 ± 0.03	0.88 ± 0.04	0.77 ± 0.04	0.77 ± 0.03*	0.77 ± 0.02*
	L24	0.68 ± 0.03	0.74 ± 0.05	0.64 ± 0.04	0.64 ± 0.03	0.64 ± 0.03

Values are mean ± SEM of 6–8 fish in each group at each time interval. An asterisk signifies a significant difference between the SNP and L24 groups at a given time interval ($p < 0.05$, Student-Newman-Keuls test, preceded by two-way ANOVA, $p < 0.05$).

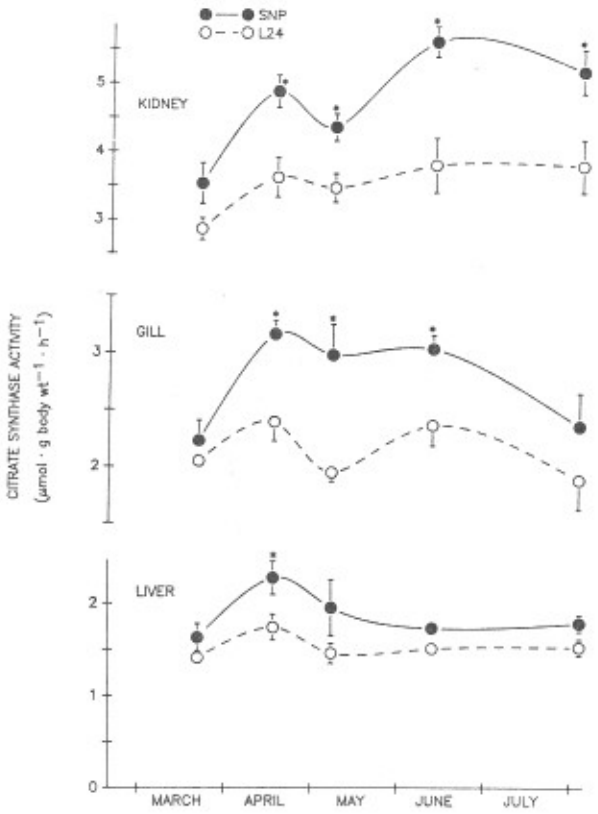

Fig. 3. Citrate synthase relative activity in liver, gill and kidney of Atlantic salmon exposed to simulated natural photoperiod (SNP) or continuous light (L24). Values are mean ± SEM of 6–8 fish per group per sampling date. SEM's not shown are less than the value overlaid by the symbol of the mean. Intervals marked with an asterisk are significantly different from the L24 group ($p < 0.05$, two-way ANOVA followed by Student-Newman-Keuls test).

variations in CCO activity were about twice those seen in CS activity, and the differences between SNP and L24 fish were of a lesser magnitude and were less often of statistical significance than those seen for CS activity. Liver and gill CCO specific activities did not differ significantly between the two photoperiod treatments ($p > 0.1$). Relative liver CCO activity of SNP fish was greater than that of L24 fish in August, whereas relative gill CCO activity was greater in both April and May. In spite of 15%–30% increases between March and June, liver and gill CCO relative and specific activity did not change significantly over time in SNP fish ($p > 0.1$). In L24 fish, significant changes occurred over time in CCO relative activity of gill ($p = 0.004$), but not of liver ($p = 0.09$).

Specific and relative CCO activity of kidney tissue from SNP fish increased by 60% and 100%, respectively, between March and June ($p < 0.001$). These levels declined slightly in August, but remained higher than levels seen initially in March. Specific and relative CCO activity of L24 fish also increased significantly ($p < 0.01$) with time, but to a lesser extent than the SNP fish. Specific CCO activity of SNP fish was significantly greater than that of L24 fish in June, whereas relative CCO activity was greater in SNP fish at every time point except March.

Discussion

Gill Na^+,K^+-ATPase (the sodium pump) plays a

Table 3. Changes in cytochrome c oxidase specific (CCOSA) and relative activity (CCORA) of Atlantic salmon exposed to stimulated natural photoperiod (SNP) or continuous light (L24).

		March 21	April 17	May 7	June 11	August 1
CCOSA (μmol·mg prot^{-1}·h^{-1})						
Liver	SNP	5.4 ± 0.4	5.1 ± 0.3	5.2 ± 0.5	5.9 ± 0.5	5.5 ± 0.5
	L24	4.1 ± 0.3	5.0 ± 0.3	4.6 ± 0.4	6.0 ± 0.5	5.2 ± 0.3
Gill	SNP	7.7 ± 0.4	8.5 ± 0.4	8.5 ± 0.4	8.7 ± 0.6	8.0 ± 0.6
	L24	7.1 ± 0.4	8.1 ± 0.4	7.1 ± 0.3	10.1 ± 0.3	6.8 ± 0.2
Kidney	SNP	10.3 ± 0.8	10.5 ± 0.4	11.0 ± 0.4	16.1 ± 0.8*	12.0 ± 0.5
	L24	8.9 ± 0.8	10.0 ± 0.5	11.0 ± 0.6	13.7 ± 0.7	11.3 ± 0.6
CCORA (μmol · g body wt^{-1} · h^{-1})						
Liver	SNP	8.6 ± 0.2	10.4 ± 1.2	8.0 ± 1.0	8.0 ± 0.7	8.9 ± 0.5*
	L24	7.0 ± 0.7	9.0 ± 1.0	6.2 ± 0.4	8.0 ± 0.8	6.9 ± 0.8
Gill	SNP	4.3 ± 0.4	5.8 ± 0.4*	4.8 ± 0.4*	5.7 ± 0.4	5.0 ± 0.8
	L24	4.3 ± 0.2	4.6 ± 0.3	3.6 ± 0.1	5.3 ± 0.3	4.1 ± 0.4
Kidney	SNP	6.9 ± 0.3	10.2 ± 0.4*	9.3 ± 0.3*	14.3 ± 0.7*	12.3 ± 0.6*
	L24	6.0 ± 0.6	8.1 ± 0.6	7.2 ± 0.3	10.0 ± 1.0	9.1 ± 0.8

Values are mean ± SEM of 6–8 fish in each group at each time interval. An asterisk signifies a significant difference between the SNP and L24 groups at a given time interval ($p < 0.05$, Student-Newman-Keuls test, preceded by two-way ANOVA, $p < 0.05$).

central role in current models of the salt-secreting function of the gills (Silva *et al.* 1977; Evans 1984). The strong correlation between gill Na^+, K^+-ATPase activity and salinity tolerance in Atlantic salmon (Langdon and Thorpe 1985; McCormick *et al.* 1987) indicates that gill Na^+,K^+-ATPase activity is both a good indicator and a functional part of the developmental changes that occur during the parr-smolt transformation. The results of the present study confirm previous findings (Saunders *et al.* 1985; McCormick *et al.* 1987) and those of the accompanying paper (McCormick *et al.* 1988), that exposure to continuous light in autumn will inhibit the increases in salinity tolerance, hypoosmoregulatory ability and gill Na^+,K^+-ATPase activity that normally occur in spring. The relatively long period during which gill Na^+,K^+-ATPase activity remained elevated in the present study may have resulted from the relatively low rearing temperatures used in this experiment. Johnston and Saunders (1981) found that exposure of Atlantic salmon to elevated temperatures in late winter and early spring will cause an earlier decline in gill Na^+,K^+-ATPase activity.

The large differences in osmoregulatory physiology between SNP and L24 fish justify the use of the terms smolt and non-smolt, respectively, for these groups, with the understanding that other aspects of the transformation (e.g. behavior) may not be as dramatically affected by photoperiod. Although parr can possess elevated gill Na^+,K^+-ATPase activity in spring (Langdon and Thorpe 1985; McCormick *et al.* 1987), the increase is smaller in magnitude than that seen in smolts. Furthermore, parr, like L24 fish in the present study, do not increase salinity tolerance or hypoosmoregulatory ability in spring, whereas SNP fish do (Saunders *et al.* 1985; McCormick *et al.* 1989).

The greater specific Na^+,K^+-ATPase activity of the kidney relative to the gill (Fig. 2) is indicative of the large active ion transport capacity of the kidney. Freshwater teleosts produce a copious and dilute urine to counteract the passive influx of water (Hickman and Trump 1969). In the present study there was neither an increase with time in SNP fish nor a significant difference between SNP and L24 fish that would constitute evidence for a developmental change in kidney Na^+,K^+-ATPase activity in Atlantic salmon during the parr-smolt transformation. McCartney (1976) reported spring

increases in kidney Na^+,K^+-ATPase specific activity in Atlantic salmon. These changes occurred in the absence of changes in gill Na^+,K^+-ATPase activity (or other indicators of the parr-smolt transformation), and it is therefore difficult to determine whether the changes in kidney Na^+, K^+-ATPase activity reported by McCartney (1976) were the result of developmental change.

Baggerman (1960) hypothesized that changes in osmotic balance may occur during the parr-smolt transformation and act to initiate downstream migration. Although a number of studies have found changes in plasma ions coincident with the parr-smolt transformation, a similar number have been unable to find such changes (see review by Folmar and Dickhoff 1980). The absence of differences in plasma osmolarity, $[Na^+]$, $[Cl^-]$ and $[K^+]$ between SNP and L24 groups (smolt and non-smolt, respectively) suggests that disturbances in extracellular osmotic balance are not a necessary consequence of the parr-smolt transformation. It has been suggested that a releasing factor or stimulus (such as increased temperature or flow rate) is also required to cause osmotic perturbation which could in turn initiate migratory behavior (Baggerman 1960; McCormick and Saunders 1987).

Although there was no difference in regulation of the major plasma ions between SNP and L24 fish, plasma $[Mg^{++}]$ of SNP fish was 10–18% lower than that of the L24 group from March to June (Fig. 1). In August, plasma $[Mg^{++}]$ of SNP fish increased to that seen in the L24 fish. While having no impact on total osmotic balance, these differences may be of physiological significance, perhaps reflecting a difference in kidney function between smolts and non-smolts in fresh water. The low variation in plasma $[Mg^{++}]$ within each group suggests that $[Mg^{++}]$ is tightly regulated in these fish.

The relative organ weight of the gill and kidney of smolts was larger than that of non-smolts at several time points, even though fish in the two groups were of equal size throughout the study. Although it seems likely that these differences are a consequence of the parr-smolt transformation, it is difficult to conclude this with certainty because of the fluctuations which occurred in the SNP group. These fluctuations may reflect a complex interaction between size- and development-related changes in relative organ weight.

To compare organ mitochondrial enzyme activity between smolts and non-smolts, changes in wet weight specific mitochondrial enzyme activity were normalized for changes in relative organ weight, and termed the relative enzyme activity (expressed as μmoles product produced by the organ/g total body weight/h). This calculation permits direct comparison of organ enzyme activity in instances, such as the present, in which relative organ weight differs with time or between experimental groups. The utility of this calculation is most apparent in the case of liver citrate synthase specific activity of L24 fish which increases progressively with time (and size); when corrected for changes in relative liver weight, the citrate synthase activity of L24 fish is seen to remain constant through time (Fig. 3). Goolish and Adelman (1987) recently reported a significant correlation between relative liver weight and CCO activity in largemouth bass (*Micropterus salmoides*).

It was deemed inappropriate to normalize Na^+, K^+-ATPase activity to relative organ weight because the assay included a partial purification procedure (as opposed to the whole homogenates used in mitochondrial enzyme assays). In the case of the gill such a calculation would increase the already significant differences in Na^+,K^+-ATPase activity between smolts and non-smolts. The slightly larger relative kidney weight of SNP fish would result in slightly greater kidney Na^+,K^+-ATPase relative activity compared to that of L24 fish.

Measurement of mitochondrial enzyme activity has been widely used as an indication of differences in respiratory capacity in tissues and cell types (Sidell 1983; Goolish and Adelman 1987). Citrate synthase and cytochrome c oxidase activities have been most useful in this regard because they are generally believed to be rate-limiting and, therefore, most accurately reflect respiratory capacity *in vivo* (Erecinska and Wilson 1982; Mommsen 1984). The activities of these enzymes are directly related to differences in oxygen consumption between tissue types, and to changes in metabolic capacity and oxygen consumption of a tissue following environmental change, such as thermal adaptation in tele-

osts (Sidell 1983). In the present study citrate synthase and cytochrome c oxidase activities responded in a similar manner to photoperiod treatment, although CCO activity displayed greater variation among individuals and apparent fluctuations over time. Such variation may have obscured statistically significant differences between the SNP and L24 groups which were found in gill and liver CS specific activity but not in CCO specific activity. However, we cannot rule out the possibility that the small differences of the two enzymes in response to photoperiod treatment may reflect real differences in their regulation in response to developmental change.

Between March and May, mitochondrial enzyme activity of liver, gill and kidney of SNP fish increased between 20% and 100%, concurrent with increases in gill Na^+,K^+-ATPase activity. Although there were some fluctuations in the L24 group, liver, gill and kidney mitochondrial enzyme activity of this group remained relatively constant over time, and where significant differences occurred, were lower than those of SNP fish. Liver and gill mitochondrial enzyme activity had returned to initial (March) levels by August, whereas kidney relative activity remained elevated. These results indicate that an increase in respiratory capacity of liver, gill and kidney occurs during the parr-smolt transformation of Atlantic salmon.

Increased succinate dehydrogenase activity of gill tissue has been observed in spring in Atlantic salmon (Chernitsky and Shterman 1981; Langdon and Thorpe 1985), and has been correlated with increases in gill Na^+,K^+-ATPase activity and salinity tolerance. In the present study, gill Na^+,K^+-ATPase and the activity of rate-limiting mitochondrial enzymes increased concurrently. Mitochondrial enzyme activity decreased by August whereas gill Na^+,K^+-ATPase activity remained elevated, a result that is difficult to explain solely through changes in the number of chloride cells which contain higher Na^+,K^+-ATPase and mitochondrial enzyme activities than other gill cell types (Sargent *et al.* 1975; Langdon and Thorpe 1984). As discussed above, this may have been partly due to the low rearing temperatures used in the present study. McCormick *et al.* (1989) found that adaptation of Atlantic salmon to 30 ppt seawater did not result in increased gill CS or CCO activity relative to fish in 0 ppt, despite a 5-fold increase in gill Na^+,K^+-ATPase activity. From the present study we cannot determine whether the observed increase in respiratory capacity is due to an increase in the number of chloride cells or to a general increase in gill metabolism. Such a distinction will require enumeration of cell types and their specific biochemical characteristics during the parr-smolt transformation.

Seasonal changes in kidney mitochondrial enzyme activity of salmonids have not been previously reported. Increases of up to 100% occurred in CS and CCO activity of smolts between March and June. Such large increases in respiratory capacity of this tissue suggest an increased metabolic rate that may be due to increased water and ion transport or to the metabolic demands of differentiation that may occur during this period, or to both. Holmes and Stainer (1966) found that urine flow and water excretory rates of steelhead (*Salmo gairdneri*) smolts is less than that of early and late smolts, and that these differences were entirely due to decreases in glomerular filtration rate. In contrast, Eddy and Talbot (1985) recorded increases in urine production of Atlantic salmon during the parr-smolt transformation. These conflicting results make it difficult to conclude whether and to what extent kidney function is altered during the parr-smolt transformation. The large changes in respiratory capacity of the kidney found in the present study suggest that changes in the function of this organ may occur during transformation.

It is difficult to distinguish whether increased respiratory capacity of osmoregulatory organs is due to increases in the metabolic demands of ion transport or to metabolic demands of differentiation which may occur during the parr-smolt transformation. Metabolic changes in the liver are easier to interpret. Results of the present study agree with those of Blake *et al.* (1984), which demonstrated 50% greater liver cytochrome c oxidase and succinate dehydrogenase activities in smolts relative to parr. Sheridan *et al.* (1985) found increased lipolytic and decreased lipogenic activity in coho salmon livers during the parr-smolt transformation which results in the depletion of lipids in the liver

and whole body. In the present study we have found that increases in liver respiratory capacity are reversible. These findings indicate that there is a transient catabolic increase that occurs during the parr-smolt transformation, which may be due in part to the energetic demands of differentiation.

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