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Influence of salinity on the energetics of gill and kidney of Atlantic salmon (*Salmo salar*)

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Abstract

The effect of seawater acclimation and adaptation to various salinities on the energetics of gill and kidney of Atlantic salmon (*Salmo salar*) was examined. Smolts and non-smolts previously reared in fresh water were exposed to a rapid increase in salinity to 30 ppt. Plasma osmolarity, [Na^+], [Cl^-], [K^+] and [Mg^{++}] increased in both groups but were significantly lower in smolts than non-smolts. Gill Na^+,K^+-ATPase specific activity, initially higher in smolts, increased in both groups after 18 days in seawater. Kidney Na^+,K^+-ATPase specific activity was not affected by salinity in either group. Gill and kidney citrate synthase specific activity was not affected by seawater exposure in smolts but decreased in non-smolts. In a second experiment, Atlantic salmon smolts reared in fresh water were acclimated to 0, 10 or 30 ppt seawater for 2 months at a temperature of 13–14°C. Gill Na^+,K^+-ATPase was positively correlated with salinity, displaying 2.5- and 5-fold higher specific activity at 10 and 30 ppt, respectively, than at 0 ppt. Kidney Na^+,K^+-ATPase specific activity was not significantly affected by environmental salinity. Citrate synthase and cytochrome c oxidase specific activities in gill were slightly (6–13%) lower at 10 ppt than at 0 and 30 ppt, whereas kidney activities were lowest at 30 ppt. Oxygen consumption of isolated gill filaments was significantly higher when incubated in isosmotic saline and at 30 ppt than at 0 ppt, but was not affected by the prior acclimation salinity. The results indicate that although high salinity induces increased gill Na^+,K^+-ATPase activity, it does not induce substantial increases in metabolic capacity of gill or kidney.

Introduction

Euryhaline and diadromous teleosts have the capacity to maintain nearly constant osmotic and ionic balance under conditions of widely differing salinity. Whereas both active ion transport and passive mechanisms are necessary for this osmoregulatory capacity, the energetic demands of these processes remain unclear (Evans 1984). Theoretical calculations of the steady-state energy requirements of ion transport for salmonids yield values of 1% of resting metabolic rate in fresh water and 0.5% in seawater (Eddy 1982). These calculations are based on the energy necessary to overcome electrical gradients for observed net fluxes of sodium and chloride, and yield similar conclusions as those of Potts

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et al. (1973) for flounder and Eddy (1975) for goldfish.

In sharp contrast with theoretical calculations, measurements of whole animal oxygen consumption yield much higher estimates of the cost of osmoregulation. Rao (1968) reported that oxygen consumption of rainbow trout (*Salmo gairdneri*) at rest increased 20% and 27% in fresh water and seawater, respectively, over that in isosmotic salinity. Similarly, Farmer and Beamish (1969) found that oxygen consumption of inactive tilapia (*Oreochromis nilotica*) was 19% and 29% higher at 0 and 30 ppt, respectively, than at isosmotic salinity. Febry and Lutz (1987) reported similar changes in oxygen consumption due to salinity in a tilapia hybrid (*Oreochromis nilotica* × *O. hornorum*).

In the present study we attempt to resolve this discrepancy between the calculated and observed energetic demands of osmoregulation by examining energetic changes in osmoregulatory organs of Atlantic salmon (*Salmo salar*). The activity of the energy-demanding sodium pump (Na^+,K^+-ATPase) and two rate-limiting mitochondrial enzymes, citrate synthase (Krebs cycle) and cytochrome c oxidase (respiratory chain), were measured in the gill and kidney of Atlantic salmon following exposure to seawater. The activities of these enzymes were also measured in the gill and kidney following long-term acclimation to hypoosmotic (0 ppt), isosmotic (10 ppt) and hyperosmotic (30 ppt) salinities. In addition, oxygen consumption of isolated gill filaments was measured for each environmental salinity in several different incubation media.

Materials and methods

Experiment I

Atlantic salmon fry were transported to St. Andrews Biological Station, N.B. Canada, immediately after hatching in April and reared as previously described (McCormick *et al.* 1988). All fish were initially exposed to simulated natural photoperiod which provided a seasonal change in daylength. On September 15 one group of fish was exposed to continuous light (24h/d) and held under this regime for the remainder of the study (L24; non-smolts), while the other group remained on simulated natural photoperiod (SNP; smolts). The former treatment has previously been shown to inhibit increases in salinity tolerance and gill Na^+,K^+-ATPase activity that normally occur during the parr-smolt transformation (Saunders *et al.* 1985; McCormick *et al.* 1987, 1988). Water temperature fluctuated seasonally (6–18°C) during early rearing (April through December) and was then kept constant (6–8°C) from January through June (including the time of seawater exposure).

On May 13 the SNP and L24 tanks were partially drained and the incoming water switched from fresh water to seawater. Within 2 hours each tank reached the salinity of the incoming seawater (30 ± 1 ppt). Five fish from each group were sampled just prior to and after 6 hours, 1, 2, 4, 8, and 18 days following the change from fresh water to seawater. Fish were fasted overnight and during the morning prior to sampling. 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 prior to removal of gill, kidney and muscle as described below.

Experiment II

Post-smolt Atlantic salmon (1.5 years old) that had been reared in fresh water at St. Andrews Biological Station were randomly separated into 3, 1-m diameter tanks supplied with non-recirculating water. Two of these were gradually acclimated to either 10 or 30 (± 1) ppt, over a two-week period. Ten ppt seawater was achieved by mixing pre-heated or precooled 30 ppt and 0 ppt in a 2:1 ratio in an insulated header tank. Salinity and temperature of rearing tanks were checked daily. Water was maintained at 13 (± 0.2)°C from May 1 to July 20 and 14 (± 0.2)°C from July 20 to August 23. Dissolved oxygen was 8.3–9.3 mg O_2/L and was approximately 5 and 10% lower at 10 and 30 ppt, respectively, than at 0 ppt. This difference is less than the inherent oxygen solubility differences due to increased salt

concentration. With this exception, all other rearing conditions were identical among the groups. Fresh water (0 ppt) contained 2.3 mM Na^+, 3.0 mM Cl^-, 0.3 mM K^+, 0.5 mM Mg^{+2}, 3.0 mM Ca^{+2} and 4.0 mM SO_4^{-2}. Round tanks with an average current velocity of 10–15 cm/s caused fish to maintain their position by swimming at a speed of approximately 0.5–1.0 body lengths/s. Fish were exposed to simulated natural photoperiod and fed commercial (Ewos) salmon pellets (1.5% body weight/d) at half-hour intervals during daylight hours using automatic feeders.

After at least a two-month acclimation, fish were sampled for tissue enzymes (July 10–15, 83–139 g wet weight) and gill oxygen consumption (August 19–23, 122–201 g wet weight). Animals were starved overnight and sampled between 0900 and 1700 Atlantic standard time. Fish were stunned by a blow to the head, blood was collected from the caudal blood vessels with a heparinized syringe followed immediately by tissue removal.

Gill, kidney and epaxial white muscle were sampled as described in McCormick *et al.* (1988). Gill oxygen consumption and mitochondrial enzyme activities were measured within 20 min and 1h, respectively, of removal of the fish from water. 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. 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 were performed as previously described (McCormick *et al.* 1988). Since salinity had no significant effect on relative gill and kidney weight, enzyme activities are expressed as specific activity (μmoles product · mg protein^{-1} · h^{-1}).

Gill oxygen consumption

Groups of 8 to 14 primary gill filaments were severed from the first or second gill arch and placed in ice-cold Cortland's medium (124 mM NaCl, 5.1 mM KCl, 3.0 mM NaH_2PO_4, 11.9 mM $NaHCO_3$, 1.9 mM $MgSO_4$, 1.3 mM $CaCl_2$ and 5.6 mM glucose, pH 7.3). Only filaments whose tissue damage was solely at the proximal (severed) end were used. Filaments were rinsed in the appropriate incubation medium and suspended from the gill septum in a 1-mL chamber containing 0 ppt, 30 ppt or Cortland's isosmotic saline. Cortland's saline was used to provide an incubation medium that was similar to fish plasma and would therefore minimize the difference in osmotic and ionic concentrations across the gill epithelium. Experiments with and without glucose demonstrated that its inclusion had no effect on gill oxygen consumption ($n = 6$, $p > 0.5$, one-way ANOVA). Two separate preparations in each incubation medium were made for each fish. Temperature was maintained at 14 (±0.1)°C and oxygen uptake was monitored with a Clark-type electrode (0.2 nmoles/min sensitivity). Stirring rates were optimized to dissipate O_2 gradients without damaging tissue. Only linear tracings between 85 and 100% of O_2 saturation in fresh water were used (this required slight super-saturation in Cortland's saline and 30 ppt). Following incubation, filaments were removed, blotted dry, weighed and dried to a constant weight at 80°C. The inter-assay coefficient of variation in Cortland's medium was 18% ($n = 4$).

The statistical significance of photoperiod treatment (SNP versus L24) and seawater exposure 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 Student-Newman-Keuls procedure ($p < 0.05$). If seawater exposure was significant, the significance of changes over time within each group was tested using one-way ANOVA ($p < 0.05$). One-way ANOVA followed by Student-Newman-Keuls procedure ($p < 0.05$) was used to test the statistical significance of adaptation to 0, 10 and 30 ppt seawater. Two-way ANOVA was used to test the statistical significance of environmental salinity and incubation media on gill oxygen consumption.

Results

Experiment 1

Following exposure to seawater, plasma osmolarity, $[Na^+]$, $[Cl^-]$ and $[K^+]$ increased in both SNP fish (smolts) and L24 fish (non-smolts) ($p < 0.05$, one- and two-way ANOVA). In smolts, plasma osmolarity, $[Na^+]$ and $[Cl^-]$ increased after 6h in seawater, peaked between 1 and 2 days (after a 7–8% increase over initial freshwater levels), and remained stable at this slightly elevated level through 18 days. A different pattern of ion regulation was seen in non-smolts. Plasma osmolarity, $[Na^+]$ and $[Cl^-]$ increased within 6h, peaked after 4 days in seawater (after a 18–23% increase), and declined slightly from these peak levels after 18 days. Between 1 and 18 days in seawater, plasma osmolarity of non-smolts was significantly higher than smolts at all time points, and plasma $[Na^+]$ and $[Cl^-]$ were significantly higher at several time points (Fig. 1).

Plasma $[K^+]$ increased in smolts and non-smolts after 6h and peaked between 2 and 4 days (60% and 100% increases, respectively, $p < 0.02$; Fig. 1). After 18 days in seawater, plasma $[K^+]$ returned to initial levels in smolts but remained elevated in non-smolts. Plasma $[K^+]$ was significantly higher in non-smolts relative to smolts after 2 days in seawater (Fig. 1). Despite a 25% increase in mean levels within the first few days, plasma $[Mg^{++}]$ did not change significantly in smolts following seawater exposure ($p = 0.051$). Plasma $[Mg^{++}]$ increased by 100% ($p < 0.001$) in non-smolts after 1 day in seawater and remained elevated thereafter. Plasma $[Mg^{++}]$ of smolts was significantly lower than that of non-smolts after 1, 2, 8 and 18 days in seawater. Muscle moisture content of smolts decreased 2% ($p = 0.006$, one-way ANOVA; Table 1) after 6h of seawater exposure but returned to initial levels at the end of 1 day. Muscle moisture content of non-smolts, initially lower than smolts, declined 2% ($p < 0.001$, one-way ANOVA) after 2 days, and returned to initial levels after 18 days.

Gill Na^+,K^+-ATPase specific activity was 140% greater in smolts than in non-smolts prior to seawater exposure (Fig. 2). Following seawater exposure there was an apparent decline in gill Na^+,K^+-ATPase specific activity in both groups (between 1 and 4 days in seawater) that was statistically significant only in non-smolts ($p = 0.05$, Student-Newman-Keuls test). After 18 days in seawater, there

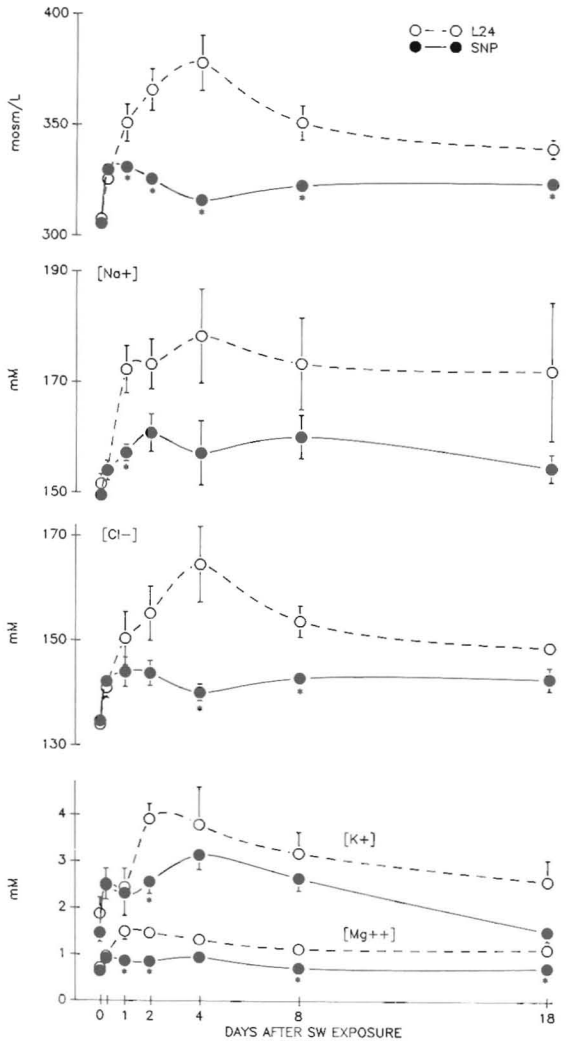


Fig. 1. Plasma osmolarity, $[Na^+]$, $[Cl^-]$, $[K^+]$ and $[Mg^{++}]$ of Atlantic salmon exposed to simulated natural photoperiod (smolts) or continuous light (non-smolts) and subsequently exposed to seawater. Values are mean ± SEM of 5 fish per group at each sampling date. Standard errors not shown are less than the value taken up by the symbol of their respective mean. An asterisk signifies a significant difference between non-smolts and smolts at a given time interval ($p < 0.05$, two-way ANOVA followed by Student-Newman-Keuls test).

Table 1. Length, weight, condition factor (100 × [weight/length^3]) and muscle moisture content (%) of Atlantic salmon exposed to simulated natural photoperiod (SNP; smolts) or continuous light (L24; non-smolts) and subsequently exposed to seawater.

		Time after seawater exposure						
		0	6 h	1 d	2 d	4 d	8 d	18 d
Length (cm)	SNP	16.2 ±0.5	17.1 ±0.5	17.4 ±0.6	17.7 ±0.6	17.0±0.6	18.4 ±0.6	18.6 ±0.5*
	L24	17.6 ±0.4	16.9 ±0.5	17.3 ±0.4	16.9 ±0.2	17.1±0.5	17.4 ±1.4	16.4 ±0.7
Weight (g)	SNP	42.7 ±4.1*	50.0 ±3.5	54.4 ±4.7	56.4 ±6.0	49.4±4.8	64.0 ±5.9	66.6 ±5.4*
	L24	65.9 ±3.5	55.2 ±5.1	55.9 ±4.2	52.1 ±1.7	54.8±5.2	57.9 ±5.8	48.0 ±4.6
Condition factor	SNP	0.99±0.03*	1.00±0.02*	1.02±0.02*	1.00±0.02*	0.98±0.01*	1.01±0.01	0.98±0.01
	L24	1.21±0.02	1.13±0.03	1.07±0.01	1.09±0.01	1.09±0.01	1.08±0.01	1.08±0.03
Muscle moisture content	SNP	75.5 ±0.1*	73.8 ±0.4	75.4 ±0.5	75.0 ±0.4*	75.1 ±0.3*	75.7 ±0.6*	76.4 ±0.4*
	L24	73.9 ±0.3	74.6 ±0.5	72.5 ±0.5	71.9 ±0.4	72.2 ±0.5	72.7 ±0.8	75.3 ±0.2

Values are mean ± SEM of 5 fish/group at each time interval. An asterisk signifies a significant difference between the SNP and L24 fish at a given time interval (two-way ANOVA followed by Student-Newman-Keuls test, $p = 0.05$).

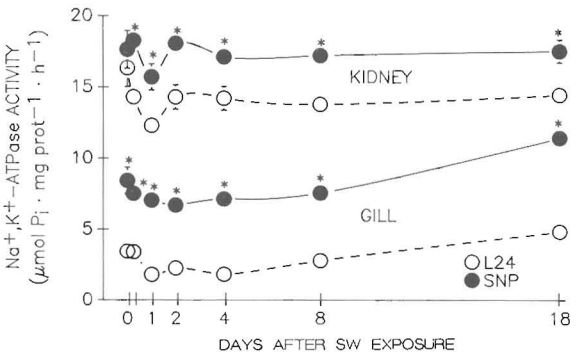
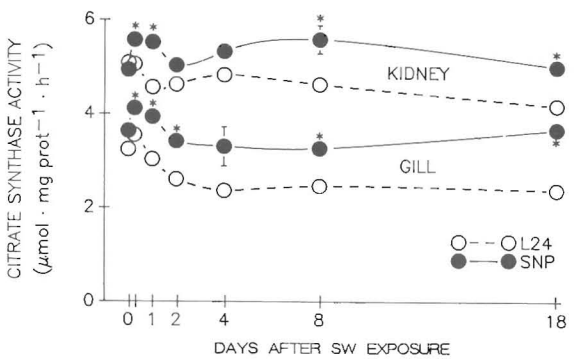


Fig. 2. Gill and kidney Na^+,K^+-ATPase specific activities of Atlantic salmon exposed to simulated natural photoperiod (smolts) or continuous light (non-smolts) and subsequently exposed to seawater. Values are mean ± SEM of 5 fish per group at each sampling date. Standard errors not shown are less than the value taken up by the symbol of their respective mean. An asterisk signifies a significant difference between non-smolts and smolts at a given time interval ($p < 0.05$, two-way ANOVA followed by Student-Newman-Keuls test).



were 30–40% increases in gill Na^+,K^+-ATPase specific activity over initial levels in both groups ($p < 0.05$, Student-Newman-Keuls test).

Kidney Na^+,K^+-ATPase specific activities in smolts and non-smolts were similar prior to seawater exposure (Fig. 2). At every sampling following seawater exposure kidney Na^+,K^+-ATPase specific activity of smolts was greater than that of non-smolts ($p < 0.05$, Student-Newman-Keuls test), due to an apparent decline in kidney Na^+, K^+-ATPase activity of non-smolts. The biological significance of this difference is unclear, however, since separate comparison of the effect of seawater exposure on smolts and non-smolts failed to detect a significant effect ($p = 0.34$ and 0.061, respectively, one-way ANOVA).

Gill and kidney citrate synthase specific activities of smolts were not significantly affected by seawater exposure ($p = 0.07$ and 0.07, respectively, one-way ANOVA; Fig. 3). Gill and kidney citrate

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Fig. 3. Gill and kidney citrate synthase specific activities of Atlantic salmon exposed to simulated natural photoperiod (smolts) or continuous light (non-smolts) and subsequently exposed to seawater. Values are mean ± SEM of 5 fish per group at each sampling date. Standard errors not shown are less than the value taken up by the symbol of their respective mean. An asterisk signifies a significant difference between non-smolts and smolts at a given time interval ($p < 0.05$, two-way ANOVA followed by Student-Newman-Keuls test).

synthase specific activities of non-smolts, however, declined by 33% ($p < 0.001$) and 20% ($p = 0.006$), respectively, within 4–6 days of seawater exposure and remained at these low levels through 18 days. Because of the significant decline in non-smolts, gill and kidney citrate synthase specific activities of non-smolts was less than that of smolts at most time points following seawater exposure (Fig. 3).

Experiment II

There was no significant difference in plasma osmolarity, sodium, chloride, potassium, magnesium, muscle moisture content or relative gill and kidney weights among Atlantic salmon maintained at 0, 10 or 30 ppt for two months (Table 2). The absence of differences among the groups, and the low variability (< 2%) in the major plasma ions, osmolarity and muscle moisture content suggest that a two month acclimation period was sufficient to allow a new equilibrium in osmotic and ionic regulation to be reached following a change in environmental salinity.

Gill Na^+,K^+-ATPase activity was 2.5- and 5-fold higher at 10 and 30 ppt, respectively, than at 0 ppt (Fig. 4). Kidney Na^+,K^+-ATPase activity was not significantly affected by salinity. The highest Na^+,K^+-ATPase activity, irrespective of salinity, was found in the kidney.

Patterns of change in citrate synthase and cytochrome c oxidase activity as a function of environmental salinity were similar, though the level of statistical significance was greater in the case of citrate synthase (Fig. 4). Gill citrate synthase activity was significantly lower in Atlantic salmon adapted to 10 ppt than to 30 ppt (11% lower), whereas the activity at 0 ppt was intermediate between the two (Fig. 4). Kidney citrate synthase activity decreased with increasing salinity, and was significantly lower at 30ppt than at 0 ppt. The kidney displayed the highest activities of both citrate synthase and cytochrome c oxidase, irrespective of salinity.

Although significant differences in citrate synthase and cytochrome c oxidase activites were detected in osmoregulatory organs, changes in the mean activity levels of these enzymes due to salinity

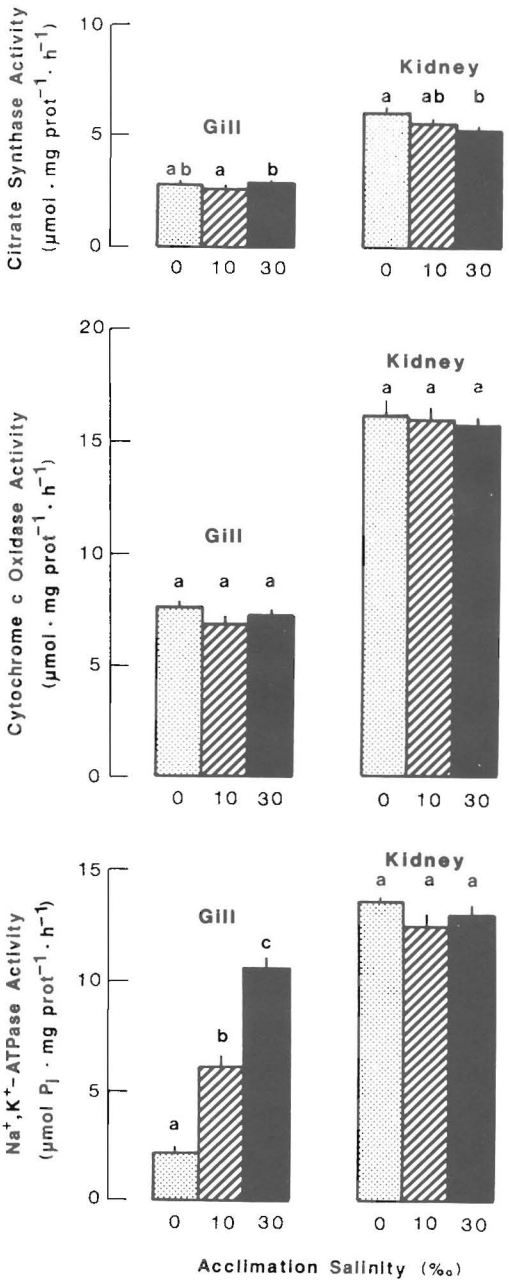

Fig. 4. Na^+,K^+-ATPase, citrate synthase and cytochrome c oxidase specific activities of gill and kidney of Atlantic salmon following a 2-month acclimation in 0, 10 or 30 ppt seawater. The same letter above histograms for each tissue indicate that activities at a given salinity are not significantly different from one another (e.g. for gill Na^+,K^+-ATPase specific activity 0, 10 and 30 ppt were all significantly different from one another, whereas with kidney Na^+,K^+-ATPase there was no significant effect of salinity; $p = 0.05$, Student-Newman-Keuls procedure). Values are mean ± SEM of eight fish at each salinity.

Table 2. Plasma osmolarity (mosm/l) and ion concentrations (mM), muscle moisture content (%) and relative organ weight (% of total body weight) of Atlantic salmon adapted to 0, 10 and 30 ppt seawater.

	Plasma					Muscle moisture content	Relative organ weight	
Salinity	Osm	$[Na^+]$	$[Cl^-]$	$[K^+]$	$[Mg^{++}]$		Gill	Kidney
0 ppt (13 mOsm)	302 ± 1	160 ± 1	137 ± 1	1.98 ± 0.51	0.79 ± 0.03	76.6 ± 0.2	0.523 ± 0.023	0.565 ± 0.032
10 ppt (319 mOsm)	304 ± 3	163 ± 2	139 ± 1	2.44 ± 0.49	0.79 ± 0.02	77.5 ± 0.3	0.553 ± 0.043	0.591 ± 0.032
30 ppt (956 mOsm)	308 ± 2	164 ± 2	139 ± 1	2.37 ± 0.61	0.82 ± 0.03	77.1 ± 0.2	0.581 ± 0.029	0.637 ± 0.024
ANOVA: p =	0.14	0.17	0.44	0.81	0.71	0.07	0.48	0.13

Values are the mean (± SEM) of 8 fish/group.

were in all cases less than 20%. Such changes are small relative to the 500% increase in gill Na^+, K^+-ATPase activity between 0 and 30 ppt. These changes are equivalent if enzyme activities are expressed on a wet weight rather than per mg protein basis.

Because of the large increase in energy-demanding gill Na^+,K^+-ATPase activity without concomitant increases in gill metabolic capacity, we deemed it of interest to examine the effect of changes in salinity on gill oxygen consumption. Gill oxygen consumption in all incubation media was linear throughout the period of measurement (up to 10 minutes), and there was no edema or cell shrinkage observed in any medium when isolated gill tissue was examined under a light microscope immediately after incubation. These results indicate that exposure of isolated gill tissue to anisosmotic media over this time period did not result in cellular or metabolic damage.

Oxygen consumption of isolated gill filaments was most strongly influenced by the incubation media; incubation in isosmotic Cortland's medium or 30 ppt seawater resulted in 15–30% greater oxygen consumption than incubation in 0 ppt; this increase occurred irrespective of the acclimation salinity (Table 3). The overall effect of prior acclimation to different environmental salinities was not significant ($p = 0.08$, two-way ANOVA). As with changes in citrate synthase and cytochrome c oxidase activities, changes in mean gill oxygen consumption due to environmental salinity were relatively small (30% or less).

Table 3. Oxygen consumption (nmoles O · mg dry wt^{-1} · min^{-1}) of isolated gill filaments of Atlantic salmon that were previously acclimated for 3 months in 0, 10 or 30 ppt seawater.

	Acclimation salinity		
Incubation media	0 ppt	10 ppt	30 ppt
0 ppt	3.81 ± 0.17	3.79 ± 0.20	3.70 ± 0.15
Cortland's saline	4.80 ± 0.17	4.23 ± 0.21	3.99 ± 0.17
30 ppt	4.69 ± 0.25	4.34 ± 0.29	4.44 ± 0.15

Values are the mean (± SEM). For each of 7 fish per salinity, six filament preparations were incubated in duplicate in 0 ppt, Cortland's saline or 30 ppt. The overall effect of incubation media was significant ($p < 0.01$), whereas the overall effect of prior acclimation salinity was not ($p = 0.08$, two-way ANOVA).

Discussion

The present study corroborates previous findings that the hypoosmoregulatory ability of Atlantic salmon smolts is greater than that of non-smolts (see reviews by Hoar 1988; McCormick and Saunders 1987), and demonstrates that these differences are not due to size, but to physiological differences resulting from photoperiod-cued developmental changes. Greater gill Na^+,K^+-ATPase specific activity prior to seawater exposure is associated with greater salinity tolerance (McCormick *et al.* 1987) and hypoosmoregulatory ability (present study). In spite of their low initial gill Na^+,K^+-ATPase activity, non-smolts possessed some ability to regulate plasma ions following exposure to seawater, and had increased gill Na^+,K^+-ATPase activity after

18 days of seawater exposure. Langdon and Thorpe (1984) found that parr responded to seawater exposure with increased gill Na^+,K^+-ATPase activity but with abnormal hypertrophy and hyperplasia of chloride cells. These results suggest that, as in the other euryhaline teleosts, at least some of the mechanisms for hypoosmoregulation in non-smolt Atlantic salmon can be induced by exposure to seawater.

There were no significant changes in gill or kidney citrate synthase specific activities in smolts in the first 18 days following exposure to seawater (Fig. 3). In contrast, both gill and kidney citrate synthase specific activities decreased in non-smolts. These changes were not due to a change in protein content of the tissue as the results are unchanged if expressed on a wet weight basis. The explanation for this decrease is not immediately obvious as the demand for maintenance of metabolic capacity for active ion transport would appear to be as great or greater for non-smolts as for smolts. It is possible that an increased degradation rate of mitochondrial enzymes, mitochondria or mitochondria-rich cells may occur in non-smolts as a result of the greater osmotic and ionic perturbations following seawater exposure. Chretien and Pisam (1986) found that both renewal and loss of chloride cells in *Poecilia reticulata* are greater following transfer from fresh water to 50‰ seawater.

Most euryhaline and diadromous teleosts possess higher gill Na^+,K^+-ATPase activity following seawater adaptation (Epstein *et al.* 1967; Kirschner 1980). This enzyme is generally believed to be responsible for generating ionic and electrical gradients necessary for increased net ion efflux across the gills in seawater (Towle 1981). The five-fold increase in gill Na^+,K^+-ATPase activity following seawater adaptation of Atlantic salmon observed in the present study is similar in magnitude (generally two- to five-fold) to that observed in other teleosts (Kirschner 1980). Increases in gill Na^+, K^+-ATPase activity following seawater adaptation are primarily located in the mitochondria-rich chloride cells (Sargent *et al.* 1975; Langdon and Thorpe 1984) which are responsible for active chloride secretion (Foskett and Scheffey 1982). When Atlantic salmon were acclimated to isosmotic salinity (10 ppt), gill Na^+,K^+-ATPase activity increased 2.5-fold over that in fresh water. This is of particular interest since oxygen consumption of several teleosts is minimal at isosmotic salinity (Rao 1968; Farmer and Beamish 1969). Since net fluxes of Na^+ and Cl^- are presumably minimal at 10 ppt, the physiological importance of increased gill Na^+,K^+-ATPase activity is unclear. Such an increase may be due to a direct response to increased salinity, perhaps via corticosteroid-induced synthesis of this enzyme (Butler and Carmichael 1972; Forrest *et al.* 1973; McCormick and Bern 1989). In spite of elevated gill Na^+, K^+-ATPase activity of smolts, seawater exposure resulted in further increases in the activity of this enzyme in a time course similar to that seen in non-smolts (Fig. 2), suggesting that each of these groups may be responding to the same stimuli despite their different hypoosmoregulatory abilities.

Changes in gill Na^+,K^+-ATPase activity in response to salinities between 8 and 12 ppt, relative to 0 ppt, are species-dependent. Gill Na^+,K^+-ATPase activity increases in the orange chromid (*Etroplus maculatus*), rainbow trout and Atlantic salmon (Jurss *et al.* 1984; Dange 1985; present study), remains constant in Japanese eel (*Anguilla japonica*) and tilapia (*Oreochromis mossambicus*) (Utida *et al.* 1971; Dange 1985) and declines in American eel (*Anguilla rostrata*; Butler and Carmichael 1972). Gill Na^+,K^+-ATPase activity of killifish (*Fundulus heteroclitus*) and pupfish (*Cyprinodon salinus*) at 15–16 ppt is lower than at either 0 or 30 ppt (Towle *et al.* 1977; Stuenkel and Hillyard 1980). This diversity among teleosts suggests that there are several tactics for utilizing or regulating gill Na^+,K^+-ATPase at isosmotic salinity. Whether or not the pump activity *in situ* at isosmotic salinity is similar to that of gill homogenates, and how this activity relates to ion fluxes at isosmotic salinity are matters for future investigation.

Following seawater adaptation, the teleost kidney produces smaller volumes of a more concentrated urine than is produced in fresh water (see review by Evans 1979). Kidney Na^+,K^+-ATPase activity increases two-fold following freshwater adaptation of the marine euryhaline teleosts *Crenimugil labrosus* and *Dicentrarchus labrax* (Lasserre

1978), but does not change in freshwater or seawater adapted American eel, rainbow trout or Australian bass *Macquaria novemaculeata* (Jampol and Epstein 1970; Jurss *et al.* 1985; Langdon 1987). In the present study, adaptation of Atlantic salmon to 0, 10 or 30 ppt had no influence on kidney Na^+, K^+-ATPase activity.

Measurement of mitochondrial enzyme activity has been widely used as an indication of differences in respiratory capacity in tissues and cell types (Sidell 1983; McCormick *et al.* 1988). In the present study citrate synthase and cytochrome c oxidase activities of the gill of Atlantic salmon were lowest at 10 ppt, whereas kidney activity was lowest at 30 ppt, although these differences were small in magnitude. To a limited degree these changes match the presumed functional changes in the transport activity of these organs: reduced active ion transport at isosmotic salinity in the gill and reduced glomerular filtration rates with increasing salinity in the kidney. It is important to note, however, that for each of these tissues, changes in CS and CCO activity are not correlated with changes in Na^+,K^+-ATPase activity. This is particulary apparent in the gill where Na^+,K^+-ATPase activity increased 150% and 400% in Atlantic salmon adapted to 10 and 30 ppt, whereas gill citrate synthase and cytochrome c oxidase activities in 10 and 30 ppt were not significantly different from fish in 0 ppt.

The findings of relatively small changes in respiratory capacity of the gill following salinity change reported here are supported by several other studies. Conte (1969) found that gill succinate-dependent cytochrome c reduction was unaltered following seawater adaptation of coho salmon (*Oncorhynchus kisutch*). Jurss *et al.* (1984) found that enzymes of intermediary metabolism in gill homogenates of rainbow trout were also unaffected by seawater adaptation. Langdon and Thorpe (1984), however, found a 100% increase in succinic dehydrogenase (SDH) activity in Atlantic salmon smolts adapted to seawater for 28 days. Using a cytochemical method, Sargent *et al.* (1975) found that SDH in dispersed gill tissue of eels (*Anguilla anguilla*) increased 2.5-fold following exposure to seawater. The difference between these results and those of the present study may reflect a difference in regulation of rate-limiting and non-rate-limiting mitochondrial enzymes such as SDH. In addition to its role in the citric acid cycle, SDH is important in anaerobic fermentations and metabolism of isoleucine, valine and methionine to pyruvate for gluconeogenesis (Hochachka and Somero 1984), which may contribute to its differential regulation following salinity change. Nonetheless, in *Gillichthys mirabilis* gill SDH activity increased by only 25% following an increase in salinity from 0 to 60 ppt (Doneen 1981). Epstein *et al.* (1967) found that gill SDH activity decreased by 45% following adaptation of killifish from freshwater to seawater.

Mandel (1986) has demonstrated that ouabain-inhibitable respiration of proximal kidney tubules of the rabbit kidney is 30% of the maximum respiratory capacity and 50% of resting respiratory rate. Up to a three-fold increase in Na^+,K^+-ATPase dependent active ion transport of the rabbit proximal tubules could occur without increasing maximum respiratory capacity. It would appear that only large increases in active ion transport would require increased mitochondrial enzyme activity (though to maintain rate-limiting enzymes below V_{max}, an increase would probably be required before maximum respiratory capacity is reached). The two- to five-fold increase in gill Na^+,K^+-ATPase activity and the large increase in ion flux rates that accompany adaptation of teleosts to seawater (Evans 1984) appear to present the possibility of a large increase in active ion transport; the absence of any significant change in mitochondrial enzyme activity is, therefore, paradoxical. It is possible that gill respiratory capacity is maintained in excess, irrespective of salinity. Alternatively, the increased energetic demands imposed by the sodium pump in seawater may simply be a relatively small component of the energy demands of the entire gill tissue. It is therefore of considerable interest to examine rate-limiting enzyme activity in chloride cells (in fresh water and seawater) relative to other cell types of the gill; to date only SDH activity has been measured (Sargent *et al.* 1975; Langdon and Thorpe 1984).

The level of *in vitro* oxygen consumption of gill tissue observed in the present study is similar to that reported for gills of other teleosts (Stagg and Shutt-

leworth 1982; Itazawa and Oikawa 1983). The use of isolated gill filaments has the advantage of circumventing stress that occurs during measurement of whole animal oxygen consumption, and the cellular degradation and edema that may occur in perfused gill preparations (Perry *et al.* 1984). It is limited, however, by the inability to provide separate mucosal and serosal media that might precisely duplicate *in vivo* conditions for ion transport. Increases in salinity of the incubation media resulted in a maximum increase of 30% in gill oxygen consumption. Prior acclimation salinity had no statistically significant effect and resulted in a maximum difference of 20%. These results indicate that the metabolic capacity of the gill is changed only slightly (a maximum of 20–30%) due to environmental salinity. Such a conclusion is in agreement with the small or undetectable changes in rate-limiting mitochondrial enzyme activity of salmonids in response to salinity (Conte 1969; present study) and by other studies of gill oxygen consumption in teleosts. Leray *et al.* (1981) found that oxygen consumption of the saline-perfused head of rainbow trout was not different in fresh water or seawater. Ouabain-inhibited and total oxygen consumption of gill slices of flounder were not affected by prior adaptation to fresh water or seawater (Stagg and Shuttleworth 1982).

Based on a whole-animal resting metabolic rate of 50 mg O_2/kg/h (Rao 1968; McCormick, unpublished data), weight-specific oxygen consumption of the gill is five times that of the whole animal. Since the gill constitutes less than 1% of total body weight, changes in gill metabolic rate of up to 20% would alter whole-animal metabolic rate by only 1%. By estimating the contribution of osmoregulatory organs to whole animal oxygen consumption, it is possible to calculate the increase in respiratory rate of these tissues necessary to account for changes in whole-animal oxygen consumption due to environmental salinity. The gill, kidney and entire gut account for approximately 14% of whole animal metabolic rate of the carp (*Cyprinus carpio*; Itazawa and Oikawa 1983). (This is probably an overestimate of the contribution of osmoregulatory organs to metabolism since it includes gut musculature which is unlikely to be involved in osmoregulation). Oxygen consumption of these osmoregulatory organs would have to increase 150% to account for a 20% change in whole-animal metabolic rate as observed by Rao (1968) and Farmer and Beamish (1969). The results of the present study indicate that energetic changes in osmoregulatory organs of Atlantic salmon are not of sufficient magnitude to bring about such large changes in the metabolic rate of the whole animal.

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