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Atlantic salmon smolts are more responsive to an acute handling and confinement stress than parr

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Abstract

Atlantic salmon parr and smolts reared under a natural temperature and photoperiod regime were subjected to an acute handling and confinement stress in early May. Smolts had a mean plasma cortisol concentration of 10 ng/ml before stress and 242 ng/ml 3 h after initiation of stress which returned to pre-stress levels within 8 h. Parr had a plasma cortisol concentration of 4 ng/ml prior to stress which increased to 11 ng/ml 3 h after initiation of stress and returned to pre-stress levels within 8 h. Plasma glucose was significantly higher in parr and smolts 3 h after initiation of stress; in parr, plasma glucose returned to pre-stress levels within 8 h, but not until 48 h in smolts. Plasma chloride concentration in smolts decreased from 139 to 124 mM 3 h after initiation of stress but returned to pre-stress levels within 24 h; plasma chloride in parr was not altered by stress. Plasma thyroxine of parr and smolts peaked at 3 h after initiation of stress and returned to pre-stress levels within 8 h, but smolts had 72% higher levels at 3 h. Pre-smolts (February) and smolts (May) reared under constant temperature (8–10°C) were also subjected to a handling and confinement stress. Although peak levels of plasma cortisol 3 h after initiation of stress were twice as high in smolts, other physiological and endocrine responses were not substantially different between pre-smolts and smolts. The results demonstrate that Atlantic salmon smolts are more responsive to stress than parr and that developmental differences are more important than seasonal changes.

Keywords: Atlantic salmon; Smolt; Parr; Stress

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1. Introduction

The parr–smolt transformation consists of a series of complex morphological and physiological changes that prepare anadromous salmonids for entry into seawater. These changes include silvering of the skin, darkening of the fin margins, use of metabolic reserves, increased growth, oxygen consumption and saltwater tolerance (Hoar, 1988). Increased salinity tolerance during smolting is due at least in part to increased Na^+, K^+-ATPase activity in the gill epithelium and intestinal mucosa (McCormick and Saunders, 1987). In Atlantic salmon (*Salmo salar*) smolting normally occurs in the spring, is under photoperiod and temperature control, and is stimulated by several endocrine changes (Saunders and Hendersen, 1970; see Hoar, 1988 for review).

Plasma cortisol levels are known to rise in the spring during the parr–smolt transformation (Specker, 1982; Barton et al., 1985; Young, 1986). The rise in cortisol that accompanies smolting is responsible for some of the changes in lipid metabolism during this phase (Sheridan, 1994). Cortisol has been shown to increase hyperosmoregulatory abilities by acting directly to increase gill Na^+, K^+-ATPase activity (McCormick and Bern, 1989; Bisbal and Specker, 1991) and cause differentiation of chloride cells (Foskett et al., 1983; McCormick, 1990). Changes in the response of osmoregulatory tissues to cortisol over the course of the parr–smolt transformation indicate developmental differences in tissue responsiveness (McCormick et al., 1991; Veillette et al., 1995).

Exposure to environmental stressors also stimulates the hypothalamic–pituitary–interrenal axis resulting in elevated plasma cortisol levels (Wedemeyer et al., 1990; Barton and Iwama, 1991). Cortisol is only one of several hormones that increase following stress (Pickering, 1981). Catecholamines cause an initial rise in plasma glucose by glycogenolysis, whereas cortisol is involved in the secondary metabolic effect of stress by maintaining hyperglycemia through protein catabolism and gluconeogenesis (Hanke et al., 1986; Thomas, 1990; van der Boon et al., 1991; Soengas et al., 1992). A prolonged elevation of cortisol due to stress has been considered harmful due to increased susceptibility to disease and suppression of reproductive processes (Pickering, 1993). However, an increased sensitivity of the interrenal axis may also signal an increased adaptability (Thorpe et al., 1987) by mobilizing energy, stimulating respiratory capacity, and helping to maintain ionic balance (Pickering, 1993).

Only a few studies have addressed developmental differences in the stress response of fish. Larval rainbow trout (*Oncorhynchus mykiss*) show increased cortisol levels after acute stress at 6 weeks of age (2 weeks after hatching) indicating that the hypothalamic–pituitary–interrenal axis becomes functional at this stage (Barry et al., 1995). Pottinger et al. (1995) found that sexually-mature male rainbow trout have a reduced responsiveness to stress during spawning. Young (1986) examined interrenal responsiveness to adrenocorticotropic hormone (ACTH) in vivo in coho salmon (*Oncorhynchus kisutch*) from the parr through the post-smolt stages, and found at maximum sensitivity to ACTH occurred coincident with the peak of the parr–smolt transformation. Barton et al. (1985) found that coho salmon smolts have higher plasma cortisol in response to handling stress than do parr. To our knowledge this is the only study to examine smolts-related physiological responses to acute stress.

The present study was undertaken to determine whether an increased stress response occurs during smolting of Atlantic salmon and whether such a change is more dependent on developmental stage (parr vs. smolt) than on a progressive seasonal change (smolt in February vs. May). The primary stress response, indicated by a rise in plasma cortisol, and three secondary stress responses; changes in plasma glucose, lactate and selected ions, were decided in order to characterize physiological changes after stress. We also examined thyroid hormones, which are known to be involved in the parr–smolt transformation; the response of thyroid hormones to stress has not been fully characterized (Leatherland, 1982). The limited number of studies to date indicate that thyroid hormones increase following acute stress (Reddy et al., 1995) but decrease during chronic stress (Leatherland and Cho, 1985).

2. Materials and methods

2.1. Fish and rearing conditions

Progeny of sea-run Atlantic salmon returning to the Connecticut River, USA were spawned in October at the Cronin National Salmon Station, Sunderland, MA, USA. Early rearing of juvenile fish occurred at the White River National Fish Hatchery, Bethel, VT, USA. Fish were transferred to the Anadromous Fish Research Center (Turners Falls, MA, USA) in the autumn of 1992. Thereafter, parr and smolt used in Experiment 1 were held in separate 1.5-m diameter tanks under natural photoperiod (LDN; light:dark normal) and ambient water temperature which changed seasonally (minimum 1.1°C in January, maximum 16.6°C in May) with a flow rate of 4 l min^{-1}. Pre-smolts and smolts used in Experiment 2 were held in 1-m diameter tanks, 75 fish per tank, under LDN, and water was maintained at 8.7–10.1°C with a flow rate of 4 l min^{-1}. Natural photoperiod was maintained by overhead, sunlight-spectrum fluorescent lights (500 lux at the water surface) and the photoperiod was adjusted twice a week. Fish were fed to satiation twice daily with commercial feed (Zeigler Bros., Gardeners, PA, USA).

2.2. Experimental design

Experiment 1 compared responses of parr and smolts and Experiment 2 compared responses of pre-smolts and smolts using the same test protocol. Parr were defined as fish that are too small to undergo the parr–smolt transformation (< 12 cm fork length in May). Pre-smolts were defined as fish that are large enough to smolt in spring (> 15 cm in February) but have not completed smolt-related physiological changes. Smolts are the same cohort as pre-smolt, but had completed smolt-related physiological changes (> 16 cm in May).

In Experiment 1, parr and smolts were subjected to acute handling and confinement stress on May 10. This allowed us to compare fish of different developmental stages at

the same season. In Experiment 2, pre-smolts (stressed on February 3) were compared to smolts (stressed on May 5). This allowed us to compare seasonal changes during smolt development.

2.3. Sampling protocol

Fish were captured and held in a net out of water for 30 s and then crowded at an approximate density of 100 g/l in a plastic mesh cage in their original tank for 3 h. This protocol was chosen to simulate transport of Atlantic salmon smolts from White River National Fish Hatchery in Bethel, VT. Initiation of the stressor began between 8:00–8:30 a.m. EST. Immediately before the stressor was applied, fish from the test tank were sampled and designated as the control group at time 0. The control fish sampled at time 8 h came from a tank that had not been previously disturbed. This tank was sampled again at 48 h. The experimental fish were sampled as they were released from the confinement stress (time = 3 h) and again at 8, 24, and 48 h. Fish were not fed during or after the stress event. Fish were anesthetized in 200 mg/l tricaine methanesulfonate (MS-222, pH 7.0) and fork length and weight were measured. Blood was drawn from the caudal vasculature into a 1-ml ammonium-heparinized syringe. All blood removal was accomplished on a group of fish ($n = 8$) in less than 10 min to eliminate any possible sampling stress effect. Blood was centrifuged at $3000 \times g$ for 5 min at 4°C, and plasma was aliquoted and stored at −80°C. Four to six gill filaments were severed above the septum, placed in 100 μl ice-cold SEI buffer (150 mmol l^{-1} sucrose, 10 mmol l^{-1} EDTA, 50 mmol l^{-1} imidazole, pH 7.3) and frozen at −80°C within 30 min.

2.4. Na^+, K^+-ATPase

Gill Na^+, K^+-ATPase activity was determined using the microassay method of McCormick (1993). Gill tissue was homogenized in 125 μl of SEID (SEI buffer with 0.1% deoxycholic acid) and then centrifuged at $3000 \times g$ for 30 s. Duplicate 10-μl homogenate samples were added to 200 μl assay mixture with and without 0.5 mM ouabain in 96-well microtitre plates at 25°C and measured at 340 nm for 10 min with intermittent mixing. Ouabain-sensitive ATPase activity was detected by enzymatic coupling of ATP dephosphorylation to NADH oxidation and expressed as μmol ADP mg $protein^{-1}$ h^{-1}. Protein concentration was determined using a Bicinchoninic Acid (BCA) Protein Assay (Pierce, Rockford, IL, USA). Both assays were run on a THERMOmax microplate reader using SOFTmax software (Molecular Devices, Menlo Park, CA, USA).

2.5. Plasma analysis

Plasma glucose was measured by enzymatic coupling with hexokinase and glucose-6-phosphate dehydrogenase (Stein, 1963). Plasma lactate was measured by reduction of nicotinamide adenine dinucleotide with lactate dehydrogenase (Marbach and Weil, 1967). Plasma osmolality was measured with a vapor pressure osmometer (Wescor

5500, Logan, UT, USA). Plasma sodium, calcium, and potassium were measured using ion-selective electrodes (AVL 984-S, Roswell, GA, USA). Plasma chloride was measured on a digital chloridometer (Model 442-5000, Labconco, Kansas City, MO, USA).

2.6. Thyroid hormones radioimmunoassay

Plasma concentrations of thyroxine (T4), and 3,5,3′-triiodo-L-thyronine (T3) were measured by a direct radioimmunoassay (Dickhoff et al., 1978) as modified by McCormick and Naiman (1984). L-[^{125}I]-Thyroxine (1250 μCi μg^{-1}), and L-3,5,3′-[^{125}I]-triiodo-L-thyronine (1200 μCi μg^{-1}) were the labled ligands used in the RIA. T4 and T3 antisera were purchased from Endocrine Sciences Products (Calabasas Hills, CA, USA). A stock solution of T4 or T3 was made in ethyl alcohol and standards were diluted in Ringers' solution with 0.05% BSA. Using a 5-μl standard and plasma sample size, the detection limit was 1 ng ml^{-1}. Intra- and interassay coefficients of variation for the T4 and T3 assays were 4.3–11% and 3.2–5%, respectively.

2.7. Cortisol enzyme immunoassay

Plasma cortisol was measured by a fully validated direct enzyme immunoassay (EIA). Corning Easy Wash microtitre plates (Corning, Corning, NY, USA) were coated with rabbit anti-cortisol antibody (cat # F3-314, lot # 345-10-22-80, Endocrine Sciences Products). Using a final dilution of 1:16,000 in coating buffer (0.05 M carbonate–bicarbonate, pH 9.6), 150 μl were added to each well. Plates were incubated overnight at 4°C and then washed with a 0.15 M NaCl and 0.05% Tween 20 solution in a microplate washer. Plates were pounded dry by inverting them and snapping briskly against a towel. A blocking solution of EIA buffer (0.1 M phosphate, 0.15 M NaCl, pH 7.0, with 0.1% BSA) was added before the addition of 2.5 μl of standard or sample along with 100 μl of cortisol–horseradish peroxidase conjugate (horseradish peroxidase linked to cortisol in the 3-position by a carboxymethyloxime bridge, a gift from Coralee Munro, University of California, Davis, CA, USA). Plates were incubated overnight at 25°C, washed as before and 200 μl of 3,3′,5,5′-tetramethylbenzidine containing 0.01% hydrogen peroxide (TMB, Kirkegaard and Perry, Gaithersburg, MD, USA) was added to each well. Each plate was incubated at 25°C with shaking in a THERMOmax plate reader (Molecular Devices) until the desired optical density was reached, usually 5–7 min. Then 0.5 M HCl, 50 μl/well, was added to stop the color reaction and an endpoint reading was taken at 450 nm. Analysis of standards and samples was done on a 4-parameter logistic curve fit (SOFTmax 2.01, Molecular Devices).

A stock solution of cortisol was made in ethyl alcohol and standards were diluted in Ringers' after comparison with charcoal stripped plasma standards. Sensitivity, as defined by the dose–response curve, was measurable from 1 ng/ml to 400 ng/ml. The lower detection limit was 0.30 ng/ml. There was a strong correlation of cortisol values obtained by EIA and RIA ($r = 0.986$, $n = 36$). Three different plasma samples gave displacement curves that were parallel to the standard curve. Testosterone and estradiol showed negligible (less than 1%) cross-reactivity. Cortisone had 1.6%, 7.7%, and 4.2%

cross-reactivity at 10 ng/ml, 100 ng/ml, and 400 ng/ml, respectively. Heat denaturation and ethanol extraction of plasma samples gave mean calculated values of 98% and 87% ($n = 9$), respectively, compared to unextracted plasma. Using a pooled plasma

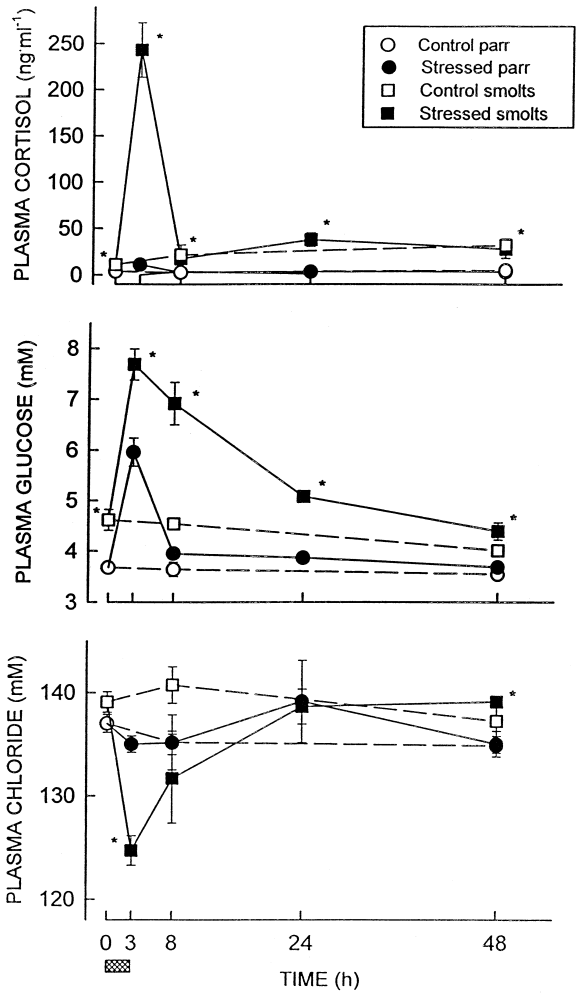

Fig. 1. Plasma cortisol (ng/ml) upper, plasma glucose (mM) middle and plasma chloride (mM) lower, in juvenile Atlantic salmon parr and smolt subjected to an acute handling followed by 3 h of crowding stress on May 10 ($n = 8$ at each time point). Two-way analysis of variance (ANOVA) determined that for plasma cortisol and glucose there was a significant difference between parr and smolts ($P < 0.0001$), a significant effect of stress ($P < 0.0001$), and a significant interaction ($P < 0.05$). Two-way ANOVA for plasma chloride determined that there were no significant differences between parr and smolt ($P > 0.05$), there was a significant effect of stress ($P < 0.0001$), and a significant interaction ($P < 0.05$). Values are means $\pm$ standard error. The hatched box below *X*-axis corresponds to the length of the crowding. Asterisk ($*$) indicates significant differences between parr and smolts at each time point.

sample, the average intra-assay variation was 5.5% ($n = 10$) and the average inter-assay variation was 8.8% ($n = 10$).

2.8. Statistical analysis

Condition factor (CF) was calculated $100 \times (\text{weight}/(\text{length}^3))$. All statistical analyses were performed on ranked values (non-parametric analysis). Two-way analysis of variance (ANOVA) was used to determine whether there were significant changes over time (0, 3, 8, 24 and 48 h) and between stressed groups (parr vs. smolts; pre-smolts vs. smolts). Interaction effects are only reported if they were significant ($P < 0.05$). If the stressed groups were significantly different, a one-way ANOVA was used to test the differences between stressed groups at each time point. Similarly, if a two-way ANOVA detected differences over time, a one-way ANOVA was used to test significant differences over time within each group. Student–Newman–Keuls Multiple Comparison test (SNK test) was used in cases where a one-way ANOVA showed significant differences over time within a group. The probability for establishing statistical significance was $P < 0.05$.

3. Results

3.1. Experiment 1: parr vs. smolt

Parr and smolts maintained on natural photoperiod and ambient temperatures were sampled on May 10. Parr had fork lengths of 9.6–12.1 cm, weights of 9.3–18.0 g, and a

Table 1
Plasma thyroxine (T4) and plasma triiodothyronine (T3) in Atlantic salmon parr and smolts subjected to acute handling followed by 3 h of crowding stress on May 10 ($n = 8$ at each time point)

Time (h)	0	3	8	24	48
Plasma T4 (ng ml^{-1})					
Parr (control)	2.0 ± 0.37	–	1.3 ± 0.13	–	1.7 ± 0.18
Parr (stress)	2.0 ± 0.37	8.6 ± 1.33[a]	2.7 ± 0.42	1.6 ± 0.20	2.7 ± 0.52
Smolt (control)	3.0 ± 0.55	–	2.3 ± 0.30	–	3.2 ± 0.55
Smolt (stress)	3.0 ± 0.55	14.9 ± 0.97 *[a]	4.1 ± 0.37 *	3.9 ± 0.45 *	4.9 ± 0.51 *[a]
Plasma T3 (ng ml^{-1})					
Parr (control)	2.2 ± 0.21	–	1.7 ± 0.08	–	1.6 ± 0.10
Parr (stress)	2.2 ± 0.21	2.5 ± 0.20	1.9 ± 0.13	2.1 ± 0.14	2.1 ± 0.11
Smolt (control)	3.1 ± 0.23	–	3.5 ± 0.10	–	3.2 ± 0.11
Smolt (stress)	3.1 ± 0.23	4.1 ± 0.24 *[a]	3.0 ± 0.10 *	3.4 ± 0.11 *[a]	2.9 ± 0.09 *

For each of the parameters, a two-way ANOVA determined that the groups were different from each other, that there was a stress effect, and there were no significant interactions ($P = 0.05$).
Values are means ± standard error.
* Indicates significant differences between parr and smolt at each time point.
[a] Indicates significant differences from initial (pre-stress) levels within a group (Student–Newman–Keuls test, $P < 0.05$).

mean condition factor of 1.03 ± 0.01. Smolts had fork lengths of 16.4–20.6 cm, weights of 38–82 g, and mean condition factor of 0.90 ± 0.01. Parr had an initial plasma cortisol of 4 ng ml^{-1}, which increased to 11 ng ml^{-1} at 3 h after initiation of stress and returned

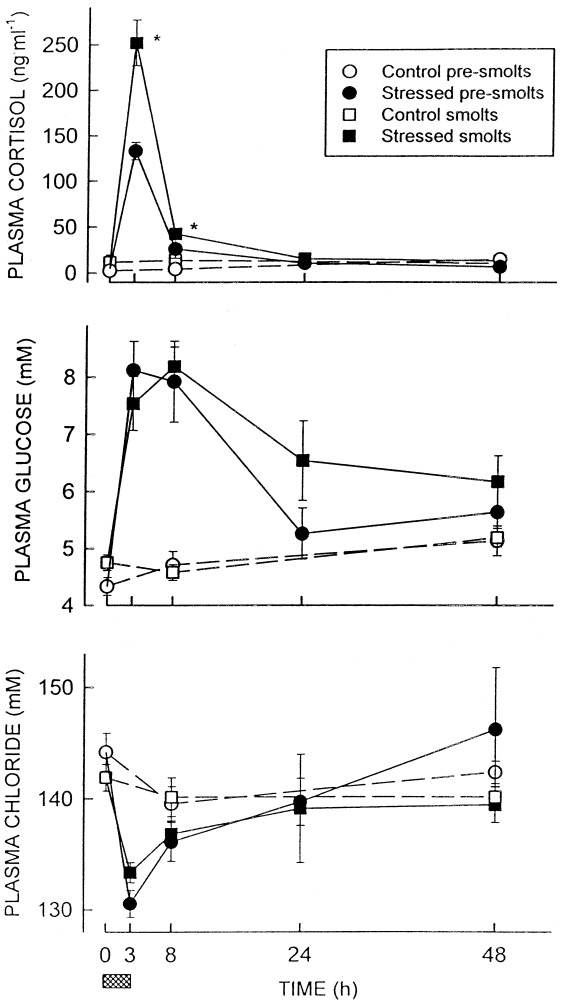

Fig. 2. Plasma cortisol (ng/ml) upper, plasma glucose (mM) middle and plasma chloride (mM) lower, in juvenile Atlantic salmon pre-smolts and smolts subjected to an acute handling followed by 3 h of crowding stress on February 3 (pre-smolts) or May 5 (smolts) ($n = 8$ at each time point). Two-way ANOVA for plasma cortisol and glucose determined that there was a significant difference between pre-smolts and smolts ($P < 0.05$), a significant effect of stress ($P < 0.0001$), but no significant interaction ($P > 0.05$). Two-way ANOVA for plasma chloride determined that there were no significant differences between pre-smolts and smolts ($P > 0.05$), there was a significant effect of stress ($P < 0.0001$), and that there was no interaction ($P > 0.05$). Values are means ± standard error. The hatched box below X-axis corresponds to the length of the crowding. Asterisk (*) indicates significant differences between pre-smolts and smolts at each time point.

to initial levels by 8 h; levels remained low for the remainder of the experiment. Smolts had an initial plasma cortisol concentration of 10 ng ml^{-1}, which rose sharply to 243 ng ml^{-1} at 3 h and declined to 18 ng ml^{-1} at 8 h (Fig. 1). Plasma glucose was significantly higher in both parr and smolts by 3 h. Plasma glucose levels in parr were initially 3.7 mM, rose to 5.9 mM at 3 h and decreased to 3.9 mM by 8 h. Plasma glucose concentrations in smolts were initially 4.6 mM, rose to 7.7 mM at 8 h, but did not return to initial levels until 48 h. Plasma chloride concentrations of parr were unchanged over the course of the experiment while plasma chloride in smolts decreased from 139 mM to 124 mM at 3 h and returned to initial levels by 24 h.

Plasma T4 peaked at 3 h in both parr and smolts (Table 1). In parr, the plasma T4 level was initially at 2.0 ng ml^{-1} and rose to 8.6 ng ml^{-1} within 3 h, but fell back to initial levels by 8 h. In smolts, plasma T4 was initially 3.0 ng ml^{-1} (not significantly different from parr), then rose to 14.9 ng ml^{-1} at 3 h, which was 72% higher than parr levels, and remained significantly higher than parr throughout the experiment. At all time points plasma T3 was higher in smolts than in parr. Plasma T3 in smolts increased 32% 3 h after initiation of stress whereas T3 levels in parr after stress did not differ from initial levels.

Control parr (unstressed) at 48 h had gill Na^+, K^+-ATPase activity of 4.1 ± 0.4 μmol ADP mg $protein^{-1}$ h^{-1} and stressed parr had gill Na^+, K^+-ATPase activity of 4.4 ± 0.4 μmol ADP mg $protein^{-1}$ h^{-1}. Control smolts (unstressed) had gill Na^+, K^+-ATPase activity of 11.4 ± 0.7 μmol ADP mg $protein^{-1}$ h^{-1} and stressed smolts had gill Na^+, K^+-ATPase activity of 11.9 ± 0.6 μmol ADP mg $protein^{-1}$ h^{-1}.

Table 2
Plasma thyroxine (T4), and plasma triiodothyronine (T3) of Atlantic salmon pre-smolts and smolts subjected to acute handling followed by 3 h of crowding stress on February 3 (pre-smolts) or May 5 (smolts) ($n = 8$ at each time point)

Time (h)	0	3	8	24	48
Plasma T4 (ng ml^{-1})					
Pre-smolt (control)	6.0 ± 0.60	–	8.9 ± 0.70	–	9.0 ± 1.39
Pre-smolt (stress)	6.0 ± 0.60	11.1 ± 1.48[a]	8.5 ± 1.15	5.8 ± 0.57	5.8 ± 0.38
Smolt (control)	9.9 ± 1.65 *	–	5.4 ± 0.80	–	7.5 ± 0.66
Smolt (stress)	9.9 ± 1.65 *	10.0 ± 1.33	8.5 ± 0.96	9.4 ± 1.47 *	8.5 ± 0.58 *
Plasma T3 (ng ml^{-1})					
Pre-smolt (control)	2.5 ± 0.19	–	2.5 ± 0.21	–	2.2 ± 0.24
Pre-smolt (stress)	2.5 ± 0.19	2.9 ± 0.35	2.7 ± 0.34	1.7 ± 0.13	1.8 ± 0.11
Smolt (control)	3.1 ± 0.33	–	2.5 ± 0.24	–	2.6 ± 0.22
Smolt (stress)	3.1 ± 0.33	3.5 ± 0.18	3.0 ± 0.24	2.5 ± 0.2 *	2.6 ± 0.29 *

For each of these parameters, a two-way ANOVA determined that pre-smolts and smolts were different from each other, that there was a stress effect, and there were no significant interactions.
Values are means ± standard error.
* Indicates significant differences between pre-smolts and smolts at each time point.
[a] Indicates significant differences from initial (pre-stress) levels within a group (Student–Newman–Keuls test, $P < 0.05$).

3.2. Experiment 2: pre-smolts vs. smolts

Pre-smolts and smolts were subjected to stressors on February 3 and May 5, respectively. Pre-smolts had fork lengths of 15.3–20.5 cm, weights of 37–92 g, and a mean condition factor of 1.04 ± 0.01. Smolts had fork lengths of 18.4–23.5 cm, weights of 58–122 g, and a mean condition factor of 0.90 ± 0.01. Plasma cortisol of pre-smolts and smolts (Fig. 2) reached their maximum levels at 3 h; in pre-smolts plasma cortisol rose from 2 ng ml^{-1} at 0 h to 133 ng ml^{-1} at 3 h, returning to initial levels by 24 h, while smolts had initial cortisol levels of 11 ng ml^{-1}, which then peaked at 252 ng ml^{-1} at 3 h, and declined back to initial levels by 24 h. Plasma cortisol concentrations of

Table 3
Plasma ions and lactate of Atlantic salmon pre-smolts and smolts subjected to acute handling followed by 3 h of crowding stress on February 3 (pre-smolts) or May 5 (smolts) ($n = 8$ at each time point)

Time (h)	0	3	8	24	48
Plasma [Na^+] (mM)					
Pre-smolt (control)	156 ± 1.1	–	153 ± 1.5	–	155 ± 1.0
Pre-smolt (stress)	156 ± 1.1	149 ± 1.2[a]	150 ± 1.0[a]	152 ± 1.3[a]	153 ± 0.7[a]
Smolt (control)	157 ± 0.5	–	159 ± 2.8	–	160 ± 1.1
Smolt (stress)	157 ± 0.5	154 ± 0.8[*]	152 ± 0.8[a]	156 ± 1.0[*]	157 ± 0.6[*]
Plasma [Ca^{++}] (mM)					
Pre-smolt (control)	1.54 ± 0.02	–	1.53 ± 0.04	–	1.53 ± 0.02
Pre-smolt (stress)	1.54 ± 0.02	1.53 ± 0.04	1.52 ± 0.03	1.46 ± 0.03	1.57 ± 0.08
Smolt (control)	1.49 ± 0.02	–	1.51 ± 0.03	–	1.62 ± 0.02
Smolt (stress)	1.49 ± 0.02	1.43 ± 0.01[*]	1.43 ± 0.02[*]	1.47 ± 0.02	1.58 ± 0.02[a]
Plasma [K^+] (mM)					
Pre-smolt (control)	2.6 ± 0.10	–	2.7 ± 0.16	–	2.1 ± 0.12
Pre-smolt (stress)	2.6 ± 0.10	2.2 ± 0.09[a]	1.9 ± 0.15[a]	2.7 ± 0.09	1.4 ± 0.04[a]
Smolt (control)	3.1 ± 0.12	–	2.8 ± 0.13	–	0.9 ± 0.06
Smolt (stress)	3.1 ± 0.12	1.7 ± 0.16[*a]	2.2 ± 0.16[a]	2.7 ± 0.07[a]	0.7 ± 0.05[*a]
Plasma lactate (mM)					
Pre-smolt (control)	3.4 ± 0.31	–	2.7 ± 0.18	–	2.9 ± 0.14
Pre-smolt (stress)	3.4 ± 0.31	3.0 ± 0.19	2.8 ± 0.22	2.6 ± 0.14	2.6 ± 0.11
Smolt (control)	3.0 ± 0.22	–	2.8 ± 0.17	–	3.0 ± 0.14
Smolt (stress)	3.0 ± 0.22	3.5 ± 0.41	2.6 ± 0.26	2.7 ± 0.23	2.3 ± 0.20

A two-way ANOVA determined that there were significant differences in plasma [Na^+] and [Ca^{++}]between pre-smolts and smolts and there was a stress effect ($P < 0.05$). There was no significant interaction of plasma [Na^+] ($P > 0.05$), but there was a significant interaction of plasma [Ca^{++}] ($P < 0.05$).
Plasma [K^+] was not significantly different between pre-smolts and smolts but there was a stress effect. There was a significant interaction ($P < 0.05$). Plasma lactate was not significantly different between pre-smolts and smolts but there was a stress effect ($P < 0.05$).
Values are means ± standard error.
[*] Indicates significant differences between pre-smolts and smolts at each time point.
[a] Indicates significant differences from initial (pre-stress) levels within a group (Student–Newman–Keuls test, $P < 0.05$).

pre-smolts and smolts were significantly different at 3 and 8 h. Plasma glucose levels in pre-smolts and smolts almost doubled in both groups (Fig. 2), but were not statistically different between the two groups. Plasma chloride concentrations in pre-smolts decreased from 144 mM at 0 h to 130 mM at 3 h, recovering to initial levels by 24 h, while in smolts chloride concentrations decreased from 142 mM at 0 h to 133 mM at 3 h, returning to initial levels by 24 h (Fig. 2). Plasma chloride concentrations were not significantly different between pre-smolts and smolts.

Initially, plasma T4 levels were significantly higher in smolts than in pre-smolts (Table 2). In pre-smolts plasma T4 rose from 6 ng ml^{-1} at 0 h to 11 ng ml^{-1} at 3 h and returned to initial levels by 24 h ($P < 0.05$, one-way ANOVA), while plasma T4 in smolts did not significantly change over the course of the experiment ($P = 0.85$, one-way ANOVA). Plasma T3 levels after stress were not significantly different from initial levels in either pre-smolts or smolts ($P > 0.05$, SNK test).

In Experiment 2 because the fish were large enough to draw more blood, more features could be investigated. Plasma sodium concentration of pre-smolts dropped to their lowest levels at 3 h and remained significantly below initial levels throughout the experiment (Table 3). Plasma sodium concentrations in smolts fell to their lowest level at 8 h but all other time points were not significantly different from initial levels. Plasma calcium of pre-smolts did not vary over the course of the experiment. Plasma calcium in smolts was significantly different at 48 h but the magnitude of change was small. Plasma potassium of pre-smolts and smolts dropped from their initial levels at 3 h, began to recover at 24 h and then fell to their lowest concentrations at 48 h; however, similar changes occurred in the control (unstressed) groups. Plasma lactate in pre- smolts did not significantly differ over the course of the experiment. Plasma lactate in smolts initially rose slightly after 3 h and then fell below initial levels over the remainder of the experiment.

Control pre-smolts (unstressed) at 48 h had gill Na^+, K^+-ATPase activity of 3.6 ± 0.4 μmol ADP mg $protein^{-1}$ h^{-1} and stressed pre-smolts had gill Na^+, K^+-ATPase activity of 4.1 ± 0.5 μmol ADP mg $protein^{-1}$ h^{-1}. Control smolts (unstressed) had gill Na^+, K^+-ATPase activity of 9.5 ± 0.8 μmol ADP mg $protein^{-1}$ h^{-1} and stressed smolts had gill Na^+, K^+-ATPase activity of 9.1 ± 0.5 μmol ADP mg $protein^{-1}$ h^{-1}

4. Discussion

In this study, Atlantic salmon smolts were clearly distinguishable from parr by their silvery appearance, dark fin margins, low condition factor, and higher gill Na^+, K^+-ATPase activity. Plasma cortisol levels in stressed Atlantic salmon were directly related to their developmental stage. Smolts had resting plasma cortisol levels that were three times that of parr or pre-smolts and a stress response that was 25 times greater than parr and twice that of pre-smolts. These results indicate an increased responsiveness of the interrenal tissue to handling stress during smolting. Young (1986) demonstrated increased responsiveness of the interrenal to ACTH during smolting, but it would also

be of interest to determine if plasma ACTH (or other factors affecting plasma cortisol levels) are higher following stress in smolts compared with parr. In the present study, pre-smolts had resting cortisol levels comparable to parr but exhibited a greater stress response than parr. This difference suggests that the increased interrenal responsiveness to ACTH characteristic of smolts begins to develop by February in Atlantic salmon reared at 10°C. This is interesting in light of the fact that other aspects of smolt development, such as gill Na^+, K^+-ATPase, did not differ between parr and pre-smolts.

Barton et al. (1985) studied the effect of handling stress on coho salmon and found increased plasma cortisol levels during the parr-smolt transformation. Virtanen and Forsman (1987) found that plasma cortisol levels in response to enforced swimming in freshwater were greater in wild smolts than in parr. They also found that smolts had low fat stores and their liver glycogen was depleted during the swimming test. Corticosteroids in fish presumably mobilize energy reserves, which may contribute to preventing exhaustion (Specker, 1982). The increased response of the interrenal to stress may be important for sustaining short-term increases in energy mobilization immediately after a stress event. This may have, however, negative consequences for available energy during smolt migration over the long term. Schreck (1982) suggests that stress may reduce energy available for routine activities and decrease the performance capacity of smolts.

While release of corticosteroids from the interrenal is considered to be a primary physiological response to stress, changes in plasma glucose are part of a secondary metabolic response to stress (Mazeaud et al., 1977; Woodward and Strange, 1987; Wedemeyer et al., 1990). Acute rises in plasma glucose are probably mediated by catecholamines whereas sustained plasma glucose levels are mediated by cortisol through stimulation of protein catabolism and gluconeogenesis (Thomas, 1990). Vijayan and Leatherland (1992) found that increases in plasma glucose 24 h after stress could be abolished using the corticosteroid antagonist RU486, providing direct evidence for the role of cortisol in sustained increases in plasma glucose. While their experimental evidence points to a direct effect of cortisol on glucose levels during stress they also state that cortisol's role may be permissive in hyperglycemia through its capacity to increase adrenergic receptors (Reid et al., 1992). In the present study, increased plasma glucose due to stress was apparent in all experimental groups, but the magnitude and duration differed. Smolts and pre-smolts had higher plasma glucose levels, which remained elevated longer after stress than parr. A temporal correspondence of the peaks in plasma cortisol and glucose was evident at 3 h but plasma glucose levels remained elevated over a longer period of time, suggesting that cortisol's influence goes beyond the relatively brief period that it is elevated by acute stress.

Plasma ion concentrations of chloride and sodium were measured to monitor ionoregulatory disturbance following acute stress. The magnitude of post-stress change in plasma ions appeared to be related to developmental stage; pre-smolts and smolts had similar decreases in plasma chloride and sodium levels, whereas plasma chloride levels of parr did not change in response to stress. The rise in catecholamines following stress causes increases in intralamellar blood pressure and gill permeability that result in increased respiratory capacity. This change is presumably adaptive in a 'flight or fight' response (Perry and McDonald, 1993). There are, however, negative consequences to

these changes, including increased ion efflux and lower plasma sodium and chloride. Fontaine et al. (1963) found higher plasma epinephrine in Atlantic salmon smolts than in parr, but circulating catecholamines in parr and smolts have not been measured previously after an acute stress.

While stress in smolts caused an initial decline in plasma chloride and sodium levels, plasma ion levels generally recovered to initial levels by 24 h. Postlethwaite and McDonald (1995) found a similar response in rainbow trout held in freshwater following exercise and stress. They found initial ion loss was due to ion efflux, but recovery by 24 h was due to both increased ion influx and reduced ion efflux. Cortisol injections in rainbow trout over 10 days increased whole body influxes of Na^+ and Cl^- but acute (3 h) arterial infusion of cortisol did not affect ion influx (Laurent and Perry, 1990). While the rapid activation of ion uptake is largely unknown, Postlethwaite and McDonald (1995) provided evidence that growth hormone is involved in increased ion uptake and postulated that prolactin and somatolactin may also be involved in regulating responses of gill ion transport and permeability during exercise and stress.

Alterations in ion regulation during smolting may be involved in the development of downstream migratory behavior. Specifically, Thorpe (1984) theorized that ''abandonment of such adaptations ... to maintain hydromineral balance in a hypotonic medium ... results in downstream emigration.'' There are, however, contradictory findings regarding changes in plasma ions during smolting (McCormick and Saunders, 1987). In the present study there were initially no differences in plasma chloride between parr and smolts, but substantially lower plasma chloride existed after stress in smolts but not parr. These findings suggest that rather than being a necessary consequence of smolt development, ion losses are due to a combination of smolt development and presence of an external stressor. Increases in flow, temperature or siltation may be perceived as stressful by smolts in their natural environment, resulting in decreases in plasma ions that act to stimulate downstream movement. This may be one of several 'signals' required for downstream movement of smolts. More research is needed to determine the possible connection between stress-related physiological changes and the migratory behavior of smolts. Changes in plasma potassium levels after stress were highly variable in pre-smolts and smolts and their corresponding controls. Plasma potassium dropped at the end of the confinement stress, recovered to initial levels at 24 h, and then declined again by half in pre-smolts and over 4-fold in smolts. However, plasma potassium levels of control fish had also dropped by 48 h, indicating that some of the observed changes may not be due to stress. Barton et al. (1986) found an increase of plasma potassium in juvenile chinook salmon *(Oncorhynchus tshawytscha)* after multiple acute stressors. McDonald and Milligan (1992) reported that plasma potassium rises in teleosts after exercise strenuous enough to result in intracellular acidosis, which causes an outward leak of potassium from cells. Our handling and confinement stressors resulted in a significant but small and transient rise of plasma lactate levels in smolts. Plasma lactate levels would have been expected to rise in Atlantic salmon during exercise or hypoxia (McDonald and Milligan, 1992), but the confinement used in our study may have actually restricted exercise and exhaustion.

Alternatively, plasma lactate may have increased after the acute stress but recovered within 3 h, similar to the finding of Waring et al. (1992). Plasma calcium of pre-smolts

did not respond to stress and plasma calcium of smolts was slightly elevated after stress. The relatively minor changes in plasma calcium in the present study are consistent with previous work showing tight regulation of plasma calcium in fresh water (and seawater) that is generally not affected by stress (McDonald and Milligan, 1992).

Thyroid hormones are involved in growth, guanine deposition leading to a silvery appearance, change in forms of hemoglobin and changes in intermediary metabolism that occur during the parr–smolt transformation (Dickhoff and Sullivan, 1987). Plasma T4 levels rise dramatically during smolting while T3 levels generally remain stable (Hoar, 1988). A differential response of T4 was observed in the present experiments. In Experiment 1, parr had a 4-fold increase in plasma T4 levels in response to stress while smolts had a 5-fold increase with each returning to initial levels by 8 h. The response of fish in Experiment 2 was less dramatic; in pre-smolts plasma T4 levels doubled 3 h after initiation of stress and returned to pre-stress levels by 24 h, whereas plasma T4 of smolts did not change. Pre-smolts and smolts used in Experiment 2 were maintained on constant temperature (10°C), whereas parr and smolt in Experiment 1 were under increasing ambient temperature (1.1–16.6°C). Thyroid activity in fishes parallels ambient temperature (Dickhoff and Sullivan, 1987) and if the natural rise in temperature is prevented during smolting the usual increase in thyroid function is not seen (Grau et al., 1982). The constant temperature in Experiment 2 may explain why the T4 response of pre-smolts and smolts was much less pronounced than for parr and smolts in Experiment 1.

As discussed earlier, serum cortisol levels in fish rise in response to environmental stressors and also during the parr–smolt transformation. Redding et al. (1984) state that a cortisol surge is needed to stimulate gill Na^+, K^+-ATPase at the time of seawater acclimation. The ability of smolts to mount a heightened stress response may be important for survival at the time of seawater entry. The results of these experiments point to the importance of developmental stage in the stress response. Evaluation of stress responsiveness may be helpful in determining optimal release times for migratory salmonids. Under natural conditions a heightened stress response may be beneficial to migrating salmon smolts to mobilize energy reserves, avoid swimming exhaustion, and possibly increase ionoregulatory abilities. Hatchery-reared salmon smolts, however, may experience the deleterious effects of acute and chronic stress due to culture conditions, netting and transportation (Maule et al., 1987). The importance of developmental differences in the stress response is underscored by high mortalities of smolts, but no mortality of parr, following some instances of long distance transportation (Wendt and Saunders, 1973). Rearing and transport conditions should account for the heightened stress response of Atlantic salmon smolts to minimize these negative effects.

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