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Seasonal differences in plasma cortisol and gill corticosteroid receptors in upper and lower mode juvenile Atlantic salmon

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Abstract

Circulating plasma cortisol and gill corticosteroid receptors (CR) have been observed to change seasonally in conjunction with smolting in Atlantic salmon. To differentiate whether these changes are seasonal or ontogenic, juvenile Atlantic salmon parr were separated by size into upper (UM) and lower mode (LM) in September. At monthly intervals, the fish were sampled for plasma cortisol, gill Na^+K^+ATPase activity and CR abundance (B_{max}) and dissociation constant (k_D). UM were significantly larger than LM, and showed the silver appearance characteristic of smolts in April and May. Gill Na^+K^+ATPase activity of UM fish increased 6-fold during the spring; LM fish increased 1.5-fold. Plasma cortisol levels increased significantly (10-fold) in UM fish in May, but not in LM fish. Gill CR B_{max} increased 5-fold over the duration of the study in both groups. CR k_D was lowest in October and highest in May; a 1.8- and 2-fold increase in LM and UM, respectively. There were no significant differences in gill CR B_{max} and k_D between the two groups during the study, except in May, when k_D was significantly greater and B_{max} lower in UM than LM. Peak levels of gill Na^+K^+ATPase activity occur coincident with an increase in plasma cortisol concentration. Seasonal increases in CR B_{max} and k_D are similar in UM and LM fish and occur independent of smolting in juvenile Atlantic salmon. In UM fish, plasma cortisol increases in spring are concurrent with increased smolt characteristics.

Keywords: Atlantic salmon; Parr; Smolt; Gill corticosteroid receptors; Gill Na^+K^+ATPase activity; Plasma cortisol

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1. Introduction

A variety of physiological and morphological changes occur during the parr–smolt transformation. Notable among these is an increase in gill Na^+K^+ATPase activity, which is correlated with the development of saltwater tolerance (McCormick and Saunders, 1987). This enzyme has been shown to be regulated by cortisol in vitro (McCormick and Bern, 1989) and in vivo (Bisbal and Specker, 1991). The mechanism of cortisol action in the gills is likely mediated by high affinity, intracellular corticosteroid receptors (CR) which have been found in the gills of salmonids (Chakraborti et al., 1987; Maule and Schreck, 1990). Plasma cortisol and gill CR concentration have been observed to change seasonally in conjunction with smolting in coho salmon (*Oncorhynchus kisutch*) (Shrimpton et al., 1994; Shrimpton, 1996) and hybrid steelhead-rainbow trout (*O. mykiss*) (McLeese et al., 1994). The physiological importance of these changes remains to be established.

Bimodal growth distributions are common in hatchery and laboratory-reared fish (Thorpe, 1977) and have also been documented in wild populations (Heggenes and Metcalfe, 1991) of Atlantic salmon (*Salmo salar*). In hatchery fish the larger, faster growing upper mode (UM) will smolt in their first spring as 1 + juveniles, whereas the smaller, slower growing lower mode (LM) require an additional year of growth before smolting as 2 + juveniles. To date most of the laboratory studies examining physiological changes associated with smolting in Atlantic salmon have focused on UM fish. It is not known, however, if the LM fish in their first spring respond to seasonal changes in environment with physiological changes similar to those observed in UM individuals. In the present study, seasonal changes in plasma cortisol concentration, gill CR concentration (B_{max}) and affinity (k_D) and gill Na^+K^+ATPase activity in juvenile Atlantic salmon were examined in UM and LM in order to determine if these changes are specific to smolts or also occur in parr.

2. Materials and methods

2.1. Fish and rearing conditions

Juvenile Atlantic salmon were transported from the White River National Fish Hatchery in Bethel, VT, to the Conte Anadromous Fish Research Center in Turner Falls MA on June 27, 1994. Fish were reared in dechlorinated city water from June to September. On September 12, 1994 fish were sorted into potential UM (102 fish > 11 cm) and LM (166 fish < 9 cm) groups, and each housed in two identical 1 m tanks. Water was maintained at approximately 16°C until October 14, when the tanks were switched to Connecticut River water to follow a naturally changing temperature regime (Fig. 1). From the date of sorting in September until the sampling date in May, no mortality was observed in UM or LM groups. In the first 2 weeks of June, however, most of the fish in the LM group died leaving insufficient numbers of fish to sample in June.

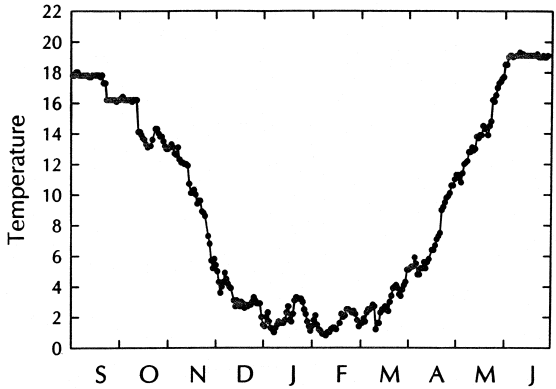

Fig. 1. Seasonal change in water temperature of the experimental tanks. Tanks were fed by Connecticut River water at 4 l min^{-1}.

In April, two apparent smolts from the LM group and one parr from the UM group were removed from the study based on size, CF, appearance and Na^+K^+ATPase activity. Modal groups are not clearly distinct and recruitment of LM fish into the UM has been shown to occur throughout the autumn if the fish are greater than 10 cm and water temperature exceeds 10°C (Kristinsson et al., 1985). The fish removed from the LM sample were greater than 15 cm in length and 30 g in weight, and may have taken advantage of growth opportunities that existed in the fall after grading to enter the UM (Fig. 1).

Fish were sampled at monthly intervals from October 1994 to June 1995, except for January when a storm resulted in turbid water and prevented normal sampling. Eight UM and eight LM fish were rapidly removed from their respective tanks and transferred to a bucket containing 200 mg l^{-1} tricaine methane sulphonate (neutralized and buffered with sodium bicarbonate, pH 7.0). Once the fish were anesthetized, length (L) and weight (W) were measured. Blood was collected in heparinized capillary tubes after the caudal peduncle was severed (LM) or in heparinized syringes from the caudal vasculature (UM). Collection of blood was complete within 5 min of first disturbing the fish to ensure that a stress associated rise in cortisol did not occur (Sumpter et al., 1986). Blood was stored on ice for less than 30 min, centrifuged at $3000 \times g$ for 5 min and plasma removed and frozen at -80°C. A gill biopsy (approximately six to eight primary gill filaments) was taken and placed in 100 ml of SEI (150 mM sucrose, 10 mM Na_2EDTA, 50 mM imidazole, pH 7.3) on ice for determining Na^+K^+ATPase activity. Samples were frozen at -80°C within 30 min. The rest of the gill tissue was removed and placed in 2 ml of TEMS (10 mM Tris–HCl, 1 mM Na_2EDTA, 12 mM monothioglycerol, 20 mM sodium molybdate, 10% v/v glycerol, pH 7.4) and frozen immediately at -80°C for later analysis of corticosteroid receptor concentration and affinity.

2.2. Determination of plasma cortisol

Plasma cortisol was quantified using a competitive solid-phase microtitre enzyme immunoassay (EIA) following a protocol similar to that of Munro and Stabenfeldt

(1984) as outlined by Carey and McCormick (1998). Rabbit anti-cortisol antibodies (Cat F3-314, lot 345-10-22-80, Endocrine Science Products, Calabasas Hills, CA) were coated to microtitre plates. Cortisol–horseradish peroxidase conjugate (Gift from Coralee Munro, University of California, Davis, CA) was used as the label. Colour development was with 3,3′,5,5′-tetramethylbenzidine (TMB) containing 0.01% hydrogen peroxide. The reaction was terminated with 0.5 M HCl and absorbance read at 450 nm.

2.3. Analysis of gill Na^+K^+ ATPase activity

Gill Na^+K^+ATPase activity was measured according to the microassay protocol of McCormick (1993). Gill filaments were homogenized in SEI buffer containing 0.1% sodium deoxycholate. Following centrifugation ($3000 \times g$ for 0.5 min) to remove large debris, Na^+K^+ATPase activity was determined linking ATP hydrolysis to the oxidation of nicotinamide adenine dinucleotide (NADH), measured at 340 nm for 10 min at 25°C, in the presence and absence of 0.5 mM ouabain. Protein content in the gill homogenate was measured using a bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL, USA). Specific activities were expressed as μmol ADP mg^{-1} of protein h^{-1}.

2.4. Corticosteroid receptor analysis

Corticosteroid receptors were measured using the method of Maule and Schreck (1990) as modified by Shrimpton and Randall (1994). All procedures were carried out with samples on ice. Cartilage was removed from the gills, and then the gills were homogenized using an IKA-Ultra Turrax TP 18/10S1 homogenizer for two 10-s bursts. Homogenates were centrifuged in a Beckman GPKR knee-well centrifuge at $3000 \times g$ for 15 min. The supernatant was removed and placed on ice. The pellet was resuspended with 0.5 ml of TEMS containing 50 mg ml^{-1} bacitracin, 20 mg ml^{-1} benzidine, 0.5 mg ml^{-1} aprotinin, 10 mg ml^{-1} *o*-phenanthroline to wash more CR from the pellet and recentrifuged at $3000 \times g$. The supernatants were combined and centrifuged at $48{,}000 \times g$ for 2 h in a Beckman J2-21M centrifuge with a JA-21 rotor. The supernatant was removed and mixed with 1.0 ml of TEMS containing 10% (w/v) activated charcoal and 1.0% (w/v) dextran and incubated for 10 min to remove endogenous steroids. To separate the charcoal from the liquid, the samples were centrifuged at $3000 \times g$ for 15 min. The final supernatant was used to quantify cortisol binding. Protein content of this fraction was assayed with Bradford reagent (Bradford, 1976).

Cortisol binding receptor studies were conducted with [^{3}H]triamcinolone acetonide (TA; 1,4-pregnadien-9α-fluoro-11β,16α,-17α,21-tetrol-3,20-dione-16,17 acetonide) with a specific activity of 43.8 Ci $mmol^{-1}$ (Dupont-NEN). TA was used as we have found that in binding and competition studies on duplicate gill homogenates, TA and cortisol bound to the same number of receptors, except TA had a higher affinity. To determine the number of high affinity cortisol receptors, 100 ml of the final supernatant was incubated in aliquots with 100 ml of buffer containing [^{3}H]TA with or without a 500-fold excess of cold TA. Final concentration of [^{3}H]TA ranged from 0.1 to 6 nM. Duplicate aliquots were incubated when enough tissue could be obtained. Single aliquots

were run on fish less than approximately 18 g. The tubes were vortexed and incubated for 2 h. After incubation, 0.5 ml of TEMS containing 2.5% (w/v) activated charcoal and 0.25% (w/v) dextran was added and vortexed. After 10 min, the charcoal containing unbound ligand was separated from bound ligand by centrifuging at $3000 \times g$ for 15 min in a Beckman GPKR refrigerated centrifuge. Supernatant (0.5 ml) was added to 3 ml of Scintisafe Econo 2 (Fisher Scientific) aqueous counting scintillant. Samples were counted on a Beckman LS 6000IC liquid scintillation counter. Specific binding was determined by subtracting non-specific bound from the total bound.

Considerable controversy has existed over the subcellular location of CR. In mammals, recent immunocytochemical studies indicate that unoccupied CR are located in the nucleus (Brink et al., 1992; Pekki et al., 1992). The subcellular distribution of CR in fish has not been investigated. Using the protocol outlined above, gill CR are found in the cytosolic fraction possibly due to redistribution from the nucleus during tissue processing (Welshons and Jordan, 1987). We have been unable to quantify CR in the nuclear fraction, as has also been reported by Pottinger et al. (1994). The CR concentration measured, therefore, is comprised of the unbound 'cytosolic' receptor population. The equilibrium dissociation constant (k_D) and the concentration of corticosteroid receptor sites (B_{max}) were calculated according to Scatchard (1949). B_{max} was divided by the homogenate protein concentration, and CR concentration was expressed as fmol mg^{-1} of protein. To estimate whether cooperativity between CR and ligand existed, the Hill Coefficient was calculated according to Sandor et al. (1984).

2.5. Statistical analysis

Condition factor (CF) was calculated as $CF = 100(W \cdot L^{-3})$, where W is weight in gram and L is fork length in centimeter.

A Two-way analysis of variance (ANOVA) was used to determine the effects of modal group and changes over time on the measured parameters. If significant differences existed between UM and LM groups, UM and LM at each time point were compared by *t*-test. If the Two-way ANOVA revealed significant changes over time, a One-way ANOVA followed by a Neuman–Keuls test was used to find significant differences among time points within each modal group. Statistical significance was taken at a level of $P = 0.05$. All values are expressed as means $\pm$ 1 SE.

3. Results

UM fish were significantly heavier ($P < 0.001$) and longer ($P < 0.001$) than LM fish at all sampling intervals, and both groups increased in size during the study (weight, $P < 0.001$; length, $P < 0.001$; Fig. 2). Condition factor (CF) changed significantly over the duration of the study ($P < 0.001$) and between modal groups ($P < 0.05$) and an interaction effect existed ($P < 0.001$). CF declined significantly in winter in LM fish from October to February, but increased again in the spring. In UM fish, there was an initial decline in CF between October and November, but from November to April CF

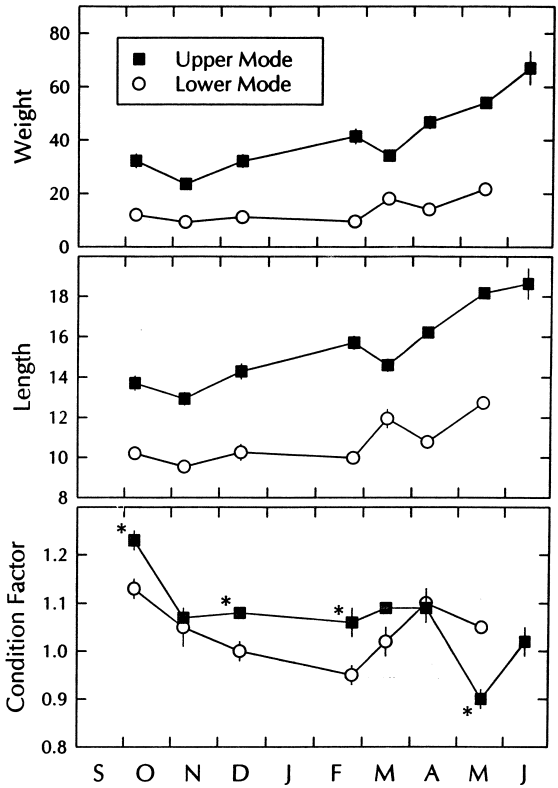

Fig. 2. Weight (g), length (cm) and condition factor (100 g cm^{-3}) of juvenile Atlantic salmon sampled from October 1994 to June 1995. * indicates CF values for the UM are significantly different from the LM for the same sampling interval. Values are mean $\pm$ 1 SEM. Error bars are not shown when they are smaller than the symbol.

remained constant. CF of UM fish dropped significantly from 1.09 ± 0.03 in April to 0.88 ± 0.02 in May, then rebounded to 1.02 ± 0.03 in June. CF was significantly lower in LM than UM throughout the winter. In May, CF was significantly higher in LM than UM.

In April UM fish developed silver coloration and dark fin margins characteristic of smolts, which persisted until the end of the study in mid-June. LM fish did not loose parr marks at any time during the study.

Representative binding curves, Scatchard plots and Hill plots are shown in Fig. 3 for UM fish sampled in October and April. Analysis indicated saturable binding as the specific binding plateaued. A single class of receptors was indicated by the linear Scatchard analysis. There was no indication of cooperative binding as the Hill plot was linear and the coefficient equivalent to one. In October, B_{max}, k_D, and Hill coefficient were 33.5 ± 2.9 fmol mg^{-1} of protein, 0.44 ± 0.03 nM, and 1.01 ± 0.02, respectively. In April, B_{max}, k_D, and Hill coefficient were 166.3 ± 12.6 fmol mg^{-1} of protein, $0.88 + 0.08$ nM, and 1.02 ± 0.01, respectively.

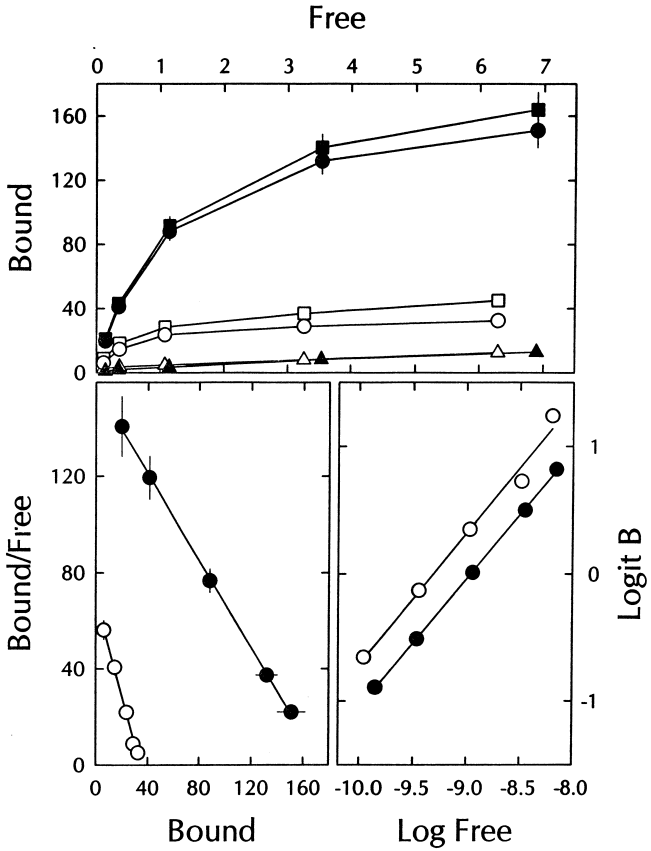

Fig. 3. Representative binding plots (upper), Scatchard plots (left) and Hill plots (right) for October (open symbols) and April (closed symbols) upper mode (UM) fish. (■) Indicates total binding, (▲) indicates non specific binding, and (●) indicates specific binding. Values are mean ± 1 SEM. Units are fmol mg^{-1} of protein for bound and nM for free. Error bars are not shown when they are smaller than the symbol.

Seasonal changes in CR B_{max} are shown in Fig. 4. There was a significant difference over time ($P < 0.001$), no significant difference between modal groups ($P = 0.91$), but there was an interaction effect ($P < 0.001$). CR B_{max} increased 5-fold in both UM and LM fish, peaking in April; B_{max} was 166.3 ± 12.6 for UM and 123.5 ± 14.0 for LM. Although B_{max} was 30% greater in UM than LM in April, the values did not differ significantly ($P = 0.079$). After the peak in April, B_{max} declined significantly by May in both groups; dropping by 73% and 40% in UM and LM fish, respectively. Comparison between the two modal groups for each sample interval indicated that UM fish values of B_{max} were significantly lower than LM for the May sample ($P < 0.05$).

Seasonal changes in CR k_D are shown in Fig. 4. Significant differences occurred over time ($P < 0.001$) and between groups ($P < 0.001$), but no interaction effect was observed ($P = 0.22$). k_D was lowest in the fall and increased over the course of the study for both UM and LM. By April, k_D had increased 2-fold for UM and 1.8-fold for

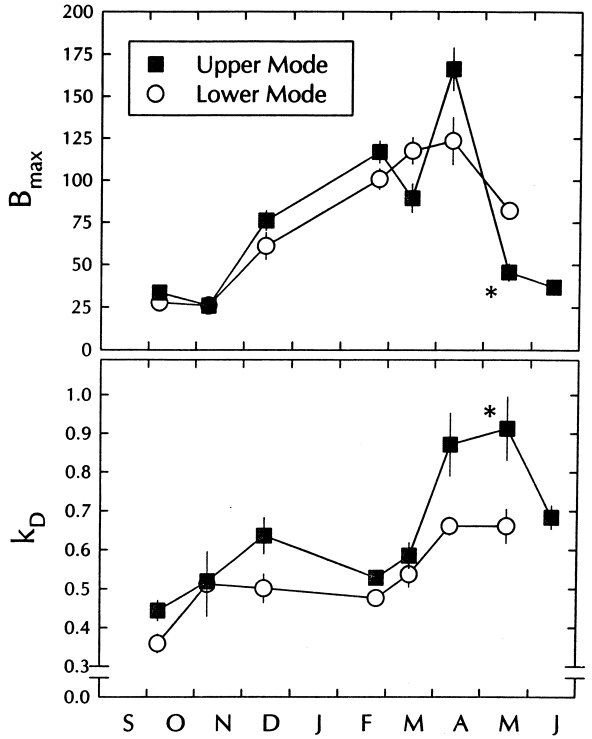

Fig. 4. Seasonal changes in corticosteroid receptor concentration (B_{max}; fmol mg^{-1} of protein) and dissociation constant (k_D; nM) for juvenile Atlantic salmon sampled from October 1994 to June 1995. * indicates values for the UM are significantly different from the LM for the same sampling interval. Values are mean $\pm$ 1 SEM. Error bars are not shown when they are smaller than the symbol.

LM. Values of k_D did not differ between the two groups except for May when UM were significantly greater than LM. In June k_D decreased significantly in the UM group.

The Hill coefficient did not differ significantly over time or between the two modes and was not significantly different from one.

Fig. 5 shows the change in gill Na^+K^+ATPase activity observed over the course of the study. Two-way ANOVA indicated that there was a significant effect due to sample date ($P < 0.001$), modal group ($P < 0.001$) and an interaction effect ($P < 0.001$). Na^+K^+ATPase activity increased 6-fold in UM fish and 1.5-fold in LM fish over the duration of the study. In UM and LM fish, the increase in gill Na^+K^+ATPase activity from November to March was gradual and similar for both groups. This gradual increase in gill Na^+K^+ATPase activity in LM fish continued between March and May, with highest activities of 4.1 ± 0.2 μmol ADP mg^{-1} of protein h^{-1} in May. UM fish, however, showed a 4-fold increase in gill Na^+K^+ATPase activity during the same period; peak levels were 12.4 ± 0.8 μmol ADP mg^{-1} of protein h^{-1} in May. Following the peak, gill Na^+K^+ATPase activity in UM fish declined significantly to 2.4 ± 0.3 μmol ADP mg^{-1} of protein h^{-1} by mid-June.

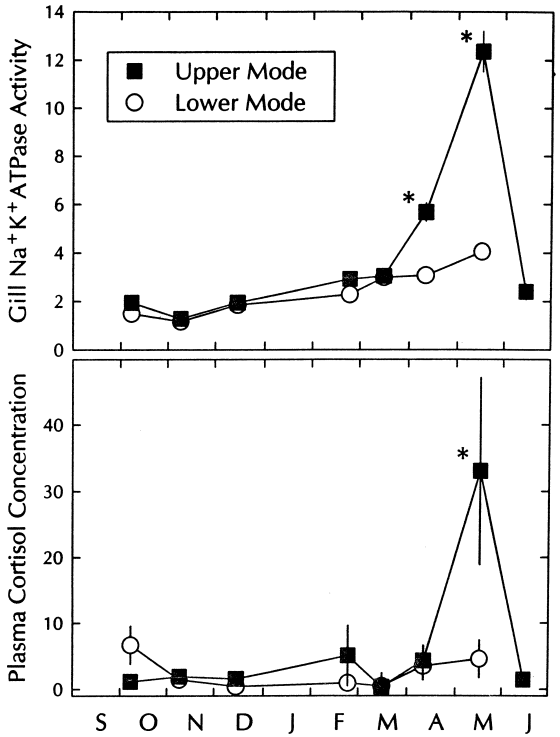

Fig. 5. Gill $Na^+ K^+$ATPase activity (μmol ADP mg^{-1} of protein h^{-1}) and mean plasma cortisol concentration (ng ml^{-1}) in juvenile Atlantic salmon sampled from October 1994 to June 1995. * indicates values for the UM are significantly different from the LM for the same sampling interval. Values are mean ± 1 SEM. Error bars are not shown when they are smaller than the symbol.

Plasma cortisol showed little seasonal variation in either group until May when a 10-fold increase was observed in UM fish (Fig. 5). A similar rise in cortisol concentration during the spring was not seen in the LM fish. Two-way ANOVA indicated a significant effect of time ($P < 0.001$), modal group ($P < 0.003$) and an interaction ($P < 0.001$). The May peak in the UM fish was the only point that differed significantly.

4. Discussion

Atlantic salmon show a bimodal growth distribution developing in the autumn after hatch; UM fish smolt the following spring and LM fish reside in fresh water an additional year before smolting (Thorpe, 1977). In the present study, fish that were initially designated as LM were significantly smaller than UM throughout the year (Fig. 2). From January to March the water temperature averaged less than 2°C, and growth was low in both groups. CF measurements, however, suggest that the pattern of growth

over the winter months differed between UM and LM groups. After an initial drop in November, CF of UM fish was unchanged until May. In contrast, CF of LM fish declined through the winter. This loss of CF may have been associated with reduced food intake due to lower appetite as suggested by Metcalfe et al. (1988). Decreases in CF in spring occur in most smolting salmonids (McCormick and Saunders, 1987; Hoar, 1988). A decline in CF was not observed in the UM fish until May, when a significant drop was observed; CF rebounded by June. Over the same interval of time, LM fish that were not smolting exhibited an increase in CF. The decline in CF in May, therefore, is clearly associated with the parr–smolt transformation.

4.1. Seasonal changes in corticosteroid receptors

CR concentration has been observed to change with developmental stage and seasonally in several vertebrates (Gendreau et al., 1987; Lange and Hanke, 1988). In teleosts, gill B_{max} changes have been observed in conjunction with smolting in coho salmon (Shrimpton et al., 1994; Shrimpton, 1996) and hybrid rainbow-steelhead trout, but not in steelhead trout (McLeese et al., 1994). Endocrine factors are known to regulate CR abundance. For example, GH increases gill CR B_{max} in coho salmon (Shrimpton et al., 1995). A gradual increase in GH may stimulate the seasonal increase in CR B_{max} in both UM and LM fish. The 30% higher CR B_{max} in UM fish than LM fish in April, though not significantly different, suggests that GH (or tissue sensitivity to GH) may have been greater in UM fish. Examination of seasonal changes in GH in parr and smolt would be of interest as large differences were reported in wild parr and smolts by McCormick and Björnsson (1994).

Cortisol also affects receptor concentration and has been shown to downregulate CR B_{max} in coho salmon gill (Maule and Schreck, 1991; Shrimpton and Randall, 1994) and rainbow trout liver (Pottinger et al., 1994). Gill CR B_{max} declined significantly in both UM and LM from April to May. In UM fish the decline in B_{max} may in part be due to downregulation of CR by cortisol. As plasma cortisol in LM fish did not increase, however, cortisol cannot be the only factor causing decreases in B_{max}.

The similarity in seasonal changes in CR between UM and LM fish suggests that gill cortisol responsiveness is the same in both groups. McCormick et al. (1991) examined gill responsiveness to cortisol in vitro in Atlantic salmon smolts and parr during the spring. Smolts with initially high levels of gill Na^+K^+ATPase activity were unresponsive to cortisol, whereas parr with initially low levels of gill Na^+K^+ATPase activity responded to cortisol in vitro. When gill Na^+K^+ATPase levels peaked in May in the present study, plasma cortisol, gill CR B_{max} and k_D differed significantly between UM and LM. Although, the experiment by McCormick et al. (1991) was conducted at the end of March, these fish were reared at elevated temperature and it is possible that the smolts were at or near their peak Na^+K^+ATPase levels for the spring, and B_{max} and affinity may have already decreased.

Gill CR affinity decreases have been observed seasonally in coho salmon (Shrimpton et al., 1994; Shrimpton, 1996), but not in steelhead trout (McLeese et al., 1994). Cortisol treatment has also been shown to decrease CR affinity in coho salmon gill (Maule and

Schreck, 1991; Shrimpton and Randall, 1994) and rainbow trout liver (Pottinger et al., 1994). Our findings, however, do not indicate that cortisol is driving the seasonal changes in k_D. Changes in k_D were observed in UM fish before plasma cortisol concentration was observed to increase. Despite the large increase in plasma cortisol in May in UM fish, values of k_D did not differ between April and May. The absence of any change in circulating cortisol levels in LM fish also suggests that cortisol is not driving the seasonal changes in k_D in Atlantic salmon in the present study. Other endocrine factors may contribute to seasonal changes in k_D. Exogenous administration of GH to Atlantic salmon parr has been shown to significantly increase k_D (Shrimpton and McCormick, unpublished data). If plasma GH levels were greater in UM than LM, GH may have contributed to the changes in k_D in the UM group which were significantly greater than those in LM.

The mechanism for affinity changes in CR is unclear. Our data suggest that it is unlikely that a new receptor population with a different affinity is present as Scatchard analysis resulted in linear plots and Hill coefficients were approximately one for all binding analyses performed (Fig. 3). It is possible that cellular factors associated with CR may affect affinity following transcription. In mammals, heat shock proteins (HSP) are associated with CR regulation (Pratt, 1993). Some forms of HSP have been shown to change seasonally (T.M. Bradley, University of Rhode Island, personal communication). Whether these changes can affect CR affinity in fish tissues is not clear, but this has been suggested for mammalian receptors (Vamvakopoulos, 1993). Alternatively, fatty acids have also been shown to affect CR affinity (Lee and Struve, 1992), and are altered by thyroxine, GH, cortisol and during the parr–smolt transformation (Sheridan, 1994).

4.2. Seasonal changes in gill Na^+K^+ATPase activity

Gill Na^+K^+ATPase activity shows seasonal variation in both LM and UM (Fig. 5). The rise in gill Na^+K^+ATPase activity in LM fish indicate that parr undergo seasonal changes in osmoregulatory physiology, but the extent of the change is much greater in smolts. Duston (1994) suggested that a physiological gradation in seasonal changes exists between parr and smolts in spring, and that large LM fish partially develop smolt characteristics. These observations are not limited to gill Na^+K^+ATPase activity. Elevations in plasma thyroid hormones during the spring have been observed in coho salmon, but the degree of change is dependent on age and size (Dickhoff et al., 1982). Our observations on changes in gill CR B_{max} and k_D in both UM and LM also indicate that seasonal changes occur in parr and smolts, but of a diminished magnitude in parr (Fig. 4).

From November to March there was a gradual increase in gill Na^+K^+ATPase activity in UM and LM fish with no increase in plasma cortisol concentration. Cortisol, however, may still influence this increase in enzyme activity as CR concentration increases during this period. An increase in CR B_{max} is likely to enhance gill sensitivity to cortisol. In mammalian cell cultures, a direct relationship between CR B_{max} and cellular response is equivocal. CR concentration has been correlated with a physiological response in mouse thymoma-derived cells (Danielsen and Stallcup, 1984), but not in

human leukocytes from leukemia patients (Homo et al., 1980). In fish, Shrimpton et al. (1994) found that wild coho salmon with higher gill CR B_{max} and plasma cortisol levels, exhibited significantly greater increases in gill Na^+K^+ATPase activity and seawater tolerance when compared to their hatchery-reared counterparts. Recently, we have found that a direct relationship between gill CR B_{max} and in vitro responsiveness of gill Na^+K^+ATPase activity to cortisol exists in rainbow trout (Shrimpton and McCormick, unpublished data).

In April, gill Na^+K^+ATPase activity in UM fish doubled, whereas little change was seen in the LM fish. This increase in enzyme activity in UM fish occurred prior to an increase in plasma cortisol. Gill CR B_{max} was 30% higher in UM than LM fish and this difference though not significant may have played a role in increased gill Na^+K^+ATPase activity. These observations indicate that other hormones are involved in stimulating gill Na^+K^+ATPase activity. Seasonal increases in growth hormone (GH) are correlated with increased gill Na^+K^+ATPase activity in Atlantic salmon (Boeuf et al., 1990; McCormick et al., 1995). Injection of Atlantic salmon with GH increased gill Na^+K^+ATPase activity (Boeuf et al., 1990; McCormick, 1996). GH, therefore, may be responsible for the April increase in Na^+K^+ATPase activity.

The peak in gill Na^+K^+ATPase activity in UM fish occurred in May when plasma cortisol levels had risen significantly. Given the temporal correlation between the peak in plasma cortisol levels and gill Na^+K^+ATPase activity (Fig. 5), it seems likely that cortisol is stimulating enzyme activity in UM fish. Throughout the study CR B_{max} increased and was greatest in April, the month prior to the cortisol increase in UM fish. The sensitivity of the gill to cortisol, therefore, was highest prior to the increase in plasma cortisol in May, enhancing the responsiveness of gill Na^+K^+ATPase to cortisol. Due to the high circulating levels of plasma cortisol in UM fish, however, we do not know what the total receptor concentration (unbound and bound) was for May. The rise in plasma cortisol and enhanced gill sensitivity to cortisol both contributed to the significant increase in gill Na^+K^+ATPase activity. GH may also enhance the effect of cortisol by a synergistic increase in Na^+K^+ATPase activity (Madsen, 1990; McCormick, 1996).

The specific activity of Na^+K^+ATPase in UM declined significantly between May and June; falling from a high of 12.3 ± 0.8 μmol ADP mg^{-1} of protein h^{-1} in May to 2.4 ± 0.3 μmol ADP mg^{-1} of protein h^{-1} in June. Loss of smolt characteristics occurs in salmon that remain in fresh water beyond the normal date of entry into seawater. The rate of the decline in gill Na^+K^+ATPase activity and saltwater tolerance is dependent on temperature (Duston et al., 1991; McCormick et al., 1997), and it seems likely that the high temperature of the water from May to June is responsible for the rapid decline in gill Na^+K^+ATPase activity. Low gill CR levels in late spring may play a role in these precipitous declines.

This study has shown that significant changes in CR concentration and affinity occur seasonally in UM and LM juvenile Atlantic salmon. Thus, changes in gill CR are more strongly regulated by season than ontogenic changes. The seasonal increase in CR concentration will result in a greater responsiveness of gill tissue to cortisol for both UM and LM fish. An increase in plasma cortisol, however, was only seen in the UM group, which provided a stimulus for the large increase in gill Na^+K^+ATPase activity.

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