Available online at www.sciencedirect.com
SCIENCE DIRECT®
Aquaculture
Aquaculture 222 (2003) 83–99
www.elsevier.com/locate/aqua-online

Environmental and endocrine control of gill corticosteroid receptor number and affinity in Atlantic salmon (*Salmo salar*) during smolting

J. Mark Shrimpton*, Stephen D. McCormick

Conte Anadromous Fish Research Center, USGS, Biological Resources Division, US Geological Survey, Turners Falls, MA, USA
Department of Biology, University of Massachusetts, Amherst, MA, USA

Received 31 October 2002; accepted 15 December 2002

Abstract

During smolting, cortisol acts on the gill through intracellular corticosteroid receptors (CR). Regulation of CR concentration (B_{max}) and dissociation constant (k_d) by environmental factors, however, has not been investigated. We subjected juvenile Atlantic salmon (*Salmo salar*) to changes in photoperiod and temperature to determine the effect on gill CR B_{max} and k_d. Cortisol, growth hormone (GH), insulin-like growth factor 1 (IGF-1), thyroxine (T_4), and triiodothyronine (T_3) were measured to determine endocrine factors that correlated with changes in CR B_{max} and k_d. Gill Na^+,K^+-ATPase activity was measured as an indicator of smolting. Control fish were maintained under ambient Connecticut River water temperatures and natural photoperiod (LDN). In the first experiment, fish were also reared at elevated temperature (constant 10 °C), or long day photoperiod (LD 16:8; 16 h light), or a combination of these two treatments. In the second experiment, fish were subjected to an advanced river temperature regime, or short day photoperiod (LD 9:15; 9 h light), or a combination of these two treatments. Seasonal changes in CR B_{max} were found to be significantly affected by temperature, but not photoperiod. A decline in CR B_{max} occurred when mean daily temperature increase exceeded 1.5 °C per week, preceding the increase in gill Na^+,K^+-ATPase activity. CR B_{max} was found to be correlated positively with T_4 and negatively with IGF-1. Gill CR k_d changed significantly over the spring,

* Corresponding author. Present Address: Biology Program, University of Northern British Columbia, 3333 University Way, Prince George, BC, Canada V2N 4Z9. Tel.: +1-250-960-5991; fax: +1-250-960-5539.
E-mail address: shrimptm@unbc.ca (J.M. Shrimpton).

0044-8486/03/$ - see front matter
doi:10.1016/S0044-8486(03)00104-2

but manipulation of temperature and photoperiod had little effect. CR k_d was found to be positively correlated with gill Na^+,K^+-ATPase activity, plasma GH, cortisol, IGF-1 and T_4. Temperature appears to influence seasonal changes in CR B_{max} observed, whereas endocrine factors appear to be more closely related to seasonal changes in CR k_d.

Keywords: Smolting; Corticosteroid receptors (CR); B_{max}

1. Introduction

Smolting is a preparatory physiological adaptation in which salmon develop tolerance to increased salinity just prior to migration to the sea. This physiological transformation is stimulated by the increase in day length that occurs during the spring (McCormick and Saunders, 1987). Altering the natural photoperiod cycle has been shown to shift the timing of smolting, evidence that photoperiod acts as a zeitgeber for smolting (Clarke et al., 1978). Temperature has also been found to affect smolting. Seasonal increase in water temperature concurrent with an increase in photoperiod is a stronger stimulus to smolting than each alone (Muir et al., 1994). The evidence for temperature directly stimulating smolting is equivocal in the absence of a photoperiod stimulus (see McCormick et al., 1987; Staurnes et al., 1994). Low temperature, however, has been shown to limit the effect of photoperiod on smolting when Atlantic salmon were subjected to long day length (16 h light) in mid-February (McCormick et al., 2000).

The endocrine system is the primary signaling pathway between the external stimuli and the seasonal physiological response. Sensitivity of cells and tissues to a given hormone is dependent on stability of the hormone receptor complex, affinity of the ligand for the receptor, and the number of receptors specific for the hormone in question (Clark and Peck, 1977). Changes in receptor concentration and affinity have been observed during development indicating that sensitivity of tissues changes with life stage. For example, during metamorphosis in amphibians, the increase in circulating thyroxine levels is associated with increased liver expression of the thyroid hormone receptor gene (Yaoita and Brown, 1990) and higher concentrations of the liver thyroid hormone receptor protein (Eliceiri and Brown, 1994). Unlike metamorphosis where a single endocrine pathway is dominant, smolting involves a number of interacting endocrine systems. Cortisol, growth hormone (GH), insulin-like growth factor 1 (IGF-1), and thyroid hormones increase during the spring in response to seasonal changes in photoperiod and temperature and stimulate smolting (Hoar, 1988). For one of these hormones, cortisol, we have observed changes in corticosteroid receptor (CR) concentration (B_{max}) and dissociation constant (k_d) when fish smolt during the spring (Shrimpton and McCormick, 1998a; Shrimpton et al., 2000). Given the importance of photoperiod and temperature on the parr–smolt transformation, we varied these variables to determine their influence on gill CR of Atlantic salmon during smolting.

2. Materials and methods

2.1. Experiment 1—increased temperature and long-day photoperiod

Juvenile Atlantic salmon were obtained as parr from the White River National Fish Hatchery (Bethel VT, USA) and brought to the Conte Anadromous Fish Research Center in mid-October. Atlantic salmon show bimodal growth distribution and by November, there is a clear difference in size between the upper mode fish which smolt the following spring and the lower mode fish that will smolt 1 year later. The fish were graded, and upper mode fish were randomly divided into four isolated photoperiod rooms containing two 1-m diameter tanks supplied with ambient river water at a flow rate of 4 l/min and supplemental aeration. Lighting was supplied by overhead fluorescent lights (500 lx at the water surface), and photoperiod adjusted twice a week. Each tank contained approximately 80 fish. The fish were fed to satiation (Zeigler, Gardners, PA) with automatic feeders.

Initially, all groups were maintained on a natural photoperiod (LDN) and water was maintained at ambient temperature in all tanks. On 7 January, two of the groups were supplemented with heated water to maintain a temperature of 9–10 °C. On 8 February, one group in each of the temperature regimens was subjected to an abrupt increase in day length to 16 h (LD 16:8).

2.2. Experiment 2—advanced temperature and short-day photoperiod

Juvenile Atlantic salmon were obtained and treated as described above. Initially, all groups were maintained on a simulated natural photoperiod and water was maintained at ambient temperature in all tanks. On 12 January, two groups remained on short days (LD 9:15; 9 h daylight) while the remaining two groups continued on a light dark natural photoperiod (LDN). On 14 February, one group in each of the photoperiod treatments was supplemented with heated water to mimic the natural temperature increase that occurs during the spring, but advanced by approximately 1 month.

2.3. Fish and sampling procedures

At approximately 2-week intervals throughout the spring, six fish were rapidly netted from a tank 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, fork length (L) and body weight (W) were measured. Blood was collected in heparinized syringes from the caudal vasculature. Collection of blood was completed 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, plasma removed and frozen on dry ice. A gill biopsy (approximately six to eight primary gill filaments) was taken and placed in 100 μl 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 on dry ice within 30 min. The remaining 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 on dry ice for later analysis of corticosteroid receptor concentration and affinity. All samples were stored at −80 °C until analyses. After the first tank from each room was sampled, six fish from the second tank were sampled as described above. Only the first three fish sampled from each tank, however, were included in this data set due to the length of time needed to remove all the tissue for corticosteroid receptor samples. There was no significant difference between tanks for any of the parameters measured. Samples from both tanks were pooled for analysis and sample size (n) equals six for each parameter reported in this paper.

2.4. 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 × g for 0.5 min) to remove large debris, Na^+,K^+-ATPase activity was determined by 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}.

2.5. Determination of plasma hormone levels

Plasma GH levels were measured in duplicate samples using a specific double-antibody salmon GH radioimmunoassay outlined by Björnsson et al. (1994). Plasma IGF-1 levels were measured by a homologous radioimmunoassay as outlined by Moriyama et al. (1994). Plasma cortisol levels were quantified using a competitive solid-phase microtitre enzyme immunoassay (EIA) following the protocol of Carey and McCormick (1998).

2.6. Corticosteroid receptor analysis

Corticosteroid receptors were measured on the cytosol fraction of gill tissue using the method of Maule and Schreck (1991) as modified by Shrimpton and McCormick (1998b). Binding 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). Gill cytosol (100 μl) was incubated in aliquots with 100 μl of buffer containing [^{3}H]TA with or without a 500-fold excess of cold TA for 2 h on ice. Final concentration of [^{3}H]TA in each assay were 0.1, 0.3, 1, 3, and 6 nM. After incubation, unbound steroids were removed by incubation for 10 min with 0.5 ml of TEMS containing 2.5% (w/v) activated charcoal and 0.25% (w/v) dextran and then centrifuged at 3000 × g for 15 min. Supernatant (0.5 ml) was added to 3 ml of aqueous counting scintillant and radioactivity counted. Specific binding was determined by subtracting nonspecific bound from the total bound.

The origin of corticosteroid receptors in the gills may be cytosolic or nuclear, but are referred to as cytosolic as they are found in the cytosol fraction following tissue processing (Welshons and Jordon, 1987). The CR concentration measured is comprised of the unbound 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} protein. To estimate cooperativity between CR and ligand, the Hill coefficient was calculated according to Sandor et al. (1984).

Affinities of CR for cortisol measured in this study are similar to those found for the glucocorticoid receptor (GR) in vertebrates, and of lower affinity than for the mineralocorticoid receptor (MR) (Diaz et al., 1998). Competition experiments with other steroids indicated that the two synthetic glucocorticoids TA and dexamethasone showed the highest affinity for the ligand (Shrimpton and McCormick, 1999), also a characteristic of GR (Ducouret et al., 1995), but distinct from the MR homologue recently found in rainbow trout (Colombe et al., 2000). A true mineralocorticoid function of this receptor in fish has yet to be determined. Receptors measured in this study are characteristic of GR, but we refer to the receptors as CR to avoid any confusion concerning their physiological action.

2.7. Calculations and statistical analysis

For seasonal changes in gill CR B_{max}, K_d, and gill Na^+,K^+-ATPase activity, a three-way analysis of variance (ANOVA) was used to determine whether time of sampling, temperature regime or photoperiod regime had a significant effect on these variables. When factors were found to be statistically significant, Tukey's test was used to determine differences between the treatments and time interval. Statistical significance was taken at a level of $P < 0.05$. All values are expressed as mean $\pm$ 1 S.E.

To examine the role that temperature plays on seasonal changes in gill B_{max} and k_d, changes in these parameters were plotted as a function of the accumulated thermal units (ATU). ATU is calculated as the additive daily temperature in degrees Celsius experienced since 1 January. To determine a relationship between the decline in CR B_{max} and temperature, we calculated the daily change in temperature between the sample date when the decline was observed and the previous sample intervals (2 weeks). The rate of change in temperature was then expressed as temperature change per week (ΔT, °C/week). The difference in CR B_{max} between the two sample dates (ΔB_{max}) was compared to ΔT by linear regression analysis for each of the different temperature regimes. Photoperiod was not included in the analysis.

Linear regression analysis was conducted on data from all individual animals for both B_{max} and k_d as a function of all endocrine parameters. Correlation analysis was also conducted on gill CR and gill Na^+,K^+-ATPase activity. To examine the relative explanatory power of endocrine parameters on gill B_{max} and k_d, a best subsets regression analysis was used. Endocrine parameters (cortisol, GH, IGF-1, triiodothyronine (T_3), and thyroxine (T_4)) were logarithmically transformed for these analyses.

3. Results

3.1. Experiment 1

Gill CR B_{max} increased steadily from January to April in all groups (Fig. 1). Highest levels were reached in all groups at the end of March, and subsequently declined. Three-way ANOVA indicated that there was a significant effect of time ($P<0.001$) and temperature ($P<0.005$), but not photoperiod ($P=0.576$). There was also an interaction

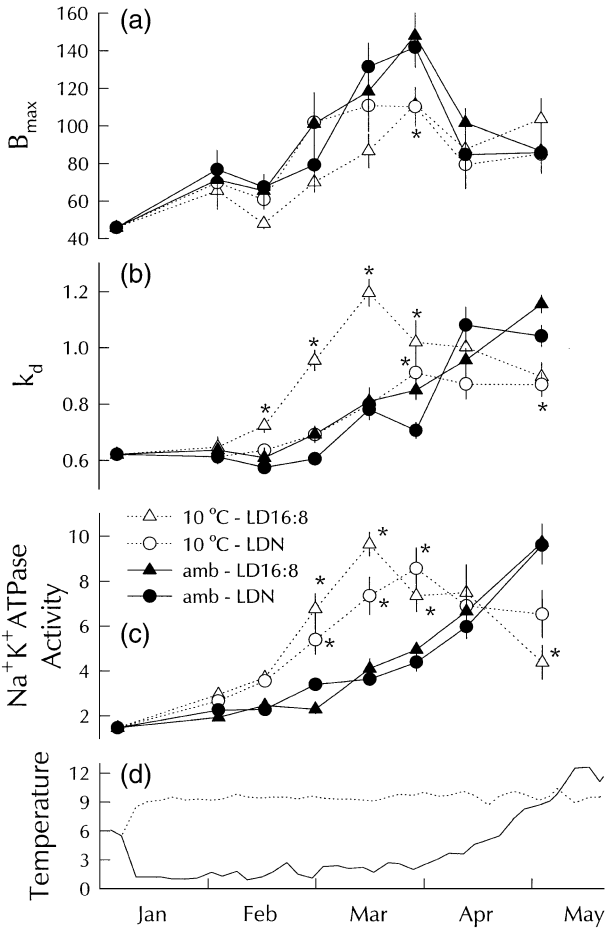

Fig. 1. Seasonal changes in gill corticosteroid receptor (a) concentration (B_{max}; fmol mg^{-1} of protein), (b) dissociation constant (k_d; nM), (c) gill Na^+,K^+-ATPase activity (μmol ADP mg^{-1} of protein h^{-1}) for juvenile Atlantic salmon, and (d) water temperature for Experiment 1. 10 °C are groups reared at constant 10 °C temperature, and amb is the natural water temperature of the Connecticut River. LDN is a simulated natural photoperiod and LD 16:8 groups were subjected to 16 h light and 8 h dark photoperiod regime from 8 February to the end of the experiment. * Indicates value is significantly different from the amb–LDN for the same sampling interval. Values are mean ± 1 S.E.M.

effect between time and temperature ($P<0.05$). The 10 °C temperature groups tended to be lower than the ambient temperature groups. Tukey's test found that B_{max} was significantly lower (40%) in the 10 °C groups than the ambient groups for the sample point at the end of March.

Gill CR k_d was significantly affected by time ($P<0.001$), photoperiod ($P<0.001$), temperature ($P<0.005$), and there was an interaction between the three factors ($P<0.001$). CR k_d stayed fairly constant until mid-March in three of the groups, but increased steadily after this point. The 10 °C–LD 16:8 group was the exception and showed an earlier rise (1 week after increased day length) (Fig. 1). Tukey's test on all pairwise comparisons indicated that k_d in the 10 °C–LD 16:8 group was significantly greater than the amb–LDN group at all time points between mid-February and the end of March. There was also a significant difference between the two-photoperiod groups held at 10 °C for the first two March samples. Following the maximum value of 1.20 ± 0.05 nM for the 10 °C–LD 16:8 group, k_d declined 25% by the end of the experiment. The 10 °C–LDN group showed a smaller increase during the spring than the LD 16:8 group at the same temperature and was also significantly greater than the amb–LDN at the end of March and then declined slightly. The ambient temperature groups continued to increase throughout the study. By the last sample point in mid-May, the amb–LDN group was significantly greater than the 10 °C–LDN group.

Gill Na^+,K^+-ATPase activity increased during the spring. Three-way ANOVA revealed that there was a significant effect of time ($P<0.001$), and temperature ($P<0.001$), but not photoperiod ($P=0.575$). There was also a significant interaction between time and temperature ($P<0.001$). For fish held in 10 °C water, gill Na^+,K^+-ATPase increased steadily from January to mid-March; however, the increase was advanced in the LD 16:8 group, but the activities did not differ significantly between the two LD 16:8 groups at any time (Fig. 1). Increases in gill Na^+,K^+-ATPase activity were slower in the ambient temperature groups and differed significantly from the 10 °C groups throughout March.

3.2. Experiment 2

Gill CR B_{max} showed significant changes over the spring (Fig. 2). CR B_{max} increased from January until April in the ambient temperature groups, whereas in the advanced temperature groups CR B_{max} increased slightly until late February and subsequently declined. There was a significant effect of time ($P<0.001$), and temperature ($P<0.001$), but not photoperiod ($P=0.418$) on CR B_{max}. There was also an interaction effect between time and temperature ($P<0.001$). Pairwise comparisons indicated that B_{max} was significantly lower in the advanced temperature groups compared to ambient temperature groups during March and early April.

There was a significant increase in k_d for all groups over the spring, and a significant drop in k_d between the last two sample times (Fig. 2). Seasonal changes in gill CR k_d were less than those seen in the first experiment. ANOVA indicated a significant effect of time ($P<0.001$), but not photoperiod ($P=0.121$) or temperature ($P=0.311$).

Gill Na^+,K^+-ATPase activity increased during the spring. Three-way ANOVA revealed that there was a significant effect of time ($P<0.001$), and temperature ($P<0.001$), and

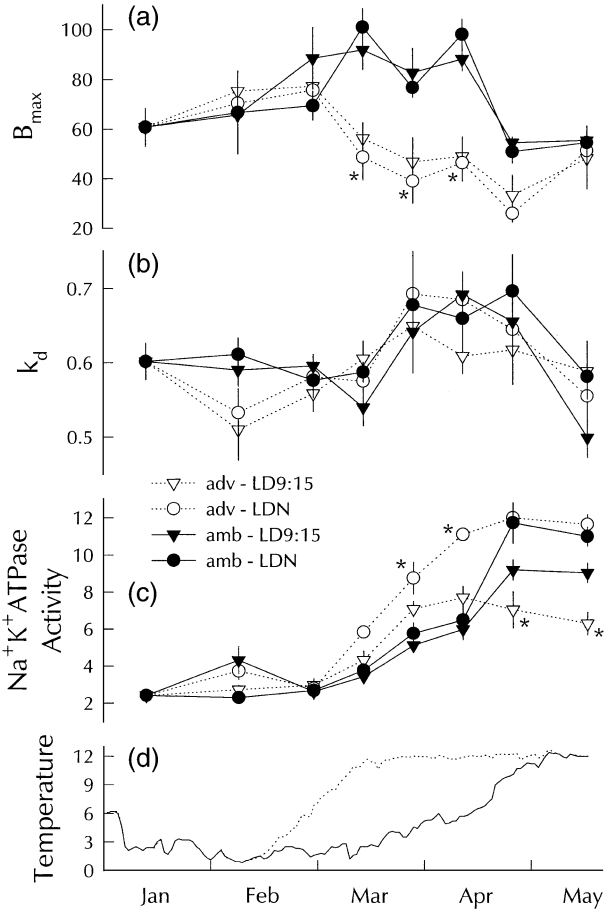

Fig. 2. Seasonal changes in gill corticosteroid receptor (a) concentration (B_{max}; fmol mg^{-1} of protein), (b) dissociation constant (k_d; nM), (c) gill Na^+,K^+-ATPase activity (μmol ADP mg^{-1} of protein h^{-1}) for juvenile Atlantic salmon, and (d) water temperature for Experiment 2. adv are groups reared at a temperature regime that was advanced over ambient temperature increases by approximately 6 weeks, and amb is the natural water temperature of the Connecticut River. LDN is a simulated natural photoperiod and LD 9:15 groups were subjected to 9 h light and 15 h dark photoperiod regime from 14 February to the end of the experiment. * Indicates value is significantly different from the amb−LDN for the same sampling interval. Values are mean ± 1 S.E.M.

photoperiod ($P<0.001$). There was also a significant interaction between time, temperature, and photoperiod ($P<0.001$). In the advanced temperature LDN group, gill Na^+,K^+-ATPase increased steadily after temperature began to increase but diverged over the spring; activities differed significantly between the two advanced temperature groups in late April and May (Fig. 2). The increase in Na^+,K^+-ATPase activity was more gradual in the ambient temperature groups, but highest gill Na^+,K^+-ATPase levels were seen concurrent with the adv−LDN group. There was no significant difference between the two-photoperiod treatments at ambient temperature.

3.3. Environmental effects on gill CR

Photoperiod treatments did not appear to influence gill CR B_{max} (Figs. 1 and 2). Changes in photoperiod also had little effect on gill CR k_d, except for the 10 °C group in Experiment 1; transition from LDN to LD 16:8 on 8 February advanced the increase in k_d.

Temperature, however, did have an affect on gill CR B_{max}. This was most noticeable in Experiment 2 when the two temperature regimes are compared. The maximum B_{max} and decline in B_{max} values were seen earlier in the season in the advanced temperature groups. This trend was not seen in Experiment 1. Maximum gill CR B_{max} values were reached at approximately 170 ATU for all the temperature groups, except for constant 10 °C (Table 1). For the seasonally increasing temperature regimes, this corresponded to a difference in time of approximately 1 month. Absolute temperature did not appear to affect the maximum value of B_{max} as it varied from 2 to 6.9 °C among the different groups. Maximal k_d did not appear to be related to the temperature, accumulated thermal units, or date (Table 1).

For the groups that were reared on water that changed seasonally, there was a marked decrease in CR B_{max} that occurred after the peak. The interval between the highest B_{max} and the decline differed for the different experimental treatments; it ranged from 2 to 6 weeks. The decrease in CR B_{max} is temperature dependent as B_{max} remained near the seasonal maximum value until the temperature began to increase in the ambient temperature and advanced temperature groups (Figs. 1 and 2). The rate of increase in temperature over the 2-week interval prior to the decline in B_{max} was calculated to be greater than 1.5 °C/week. There was less of a decline in the constant 10 °C groups, but this is also reflected in a smaller increase in CR B_{max}. A summary of the ΔB_{max} as a function of environmental factors in given in Table 2. An examination of the data for each of the temperature groups indicated that there was a significant relationship between ΔB_{max} and ΔT for three of the temperature regimes, but not the constant temperature group. For Experiment 1 ambient and constant temperature groups, $R^2 = 0.463$, $P = 0.007$, and $R^2 = 0.036$, $P = 0.52$, respectively. For Experiment 2 ambient and advanced temperature groups, $R^2 = 0.738$, $P = 0.0003$, and $R^2 = 0.408$, $P = 0.025$, respectively.

Table 1
The maximum gill CR k_d and B_{max} for each of the treatment groups for Experiments 1 and 2

	B_{max}	Maximum B_{max}			k_d	Maximum k_d		
		ATU	Day	°C		ATU	Day	°C
amb–LDN #1	142 ± 11	178	88	2	1.08 ± 0.06	230	102	3.6
amb–LD 16:8	148 ± 13	178	88	2	1.16 ± 0.03	357	122	9.1
amb–LDN #2	101 ± 8	167	73	2.5	0.70 ± 0.05	400	115	10.1
amb–LD 9:15	92 ± 8	167	73	2.5	0.69 ± 0.07	216	87	4.6
adv–LDN	76 ± 5	170	59	6.9	0.69 ± 0.03	629	101	11.9
adv–LD 9:15	77 ± 8	170	59	6.9	0.65 ± 0.06	463	87	12
10 °C–LDN	111 ± 14	674	75	9.1	0.91 ± 0.04	801	88	9.7
10 °C–LD 16:8	111 ± 8	801	88	9.7	1.20 ± 0.05	674	75	9.1

The accumulated thermal units (ATU, °C × day), number of days since 1 January, and the temperature (°C) when maximal values for k_d and B_{max} were reached are listed.

Table 2
Temperature change over the 2-week interval prior to the decline in B_{max} for each of the study groups

	Mean ΔB_{max}	Environmental conditions			
		ΔT	Average T	ATU	Day
Ambient #1	− 51.6	1.81 ± 0.18	2.1 ± 0.1	227	102
Ambient #2	− 40.4	2.03 ± 0.13	2.3 ± 0.1	400	115
Advanced	− 24.0	2.53 ± 0.05	2.9 ± 0.2	300	73
10 °C	− 27.2	0.05 ± 0.06	8.9 ± 0.1	928	102

The temperature change (ΔT) is expressed as the degree temperature change per week (°C/week) and was calculated over the interval of 2 weeks prior to the decline in B_{max}. Average temperature is calculated as the average from 1 January to the maximum value of B_{max} in °C for each of the treatment groups. The accumulated thermal units (ATU, °C × day) were calculated from 1 January. Day is the number of days since 1 January.

3.4. Gill CR correlations with endocrine factors and gill Na^+,K^+-ATPase activity

Correlations for gill CR B_{max} with individual plasma hormone concentrations are shown in Table 3. Correlations with B_{max} were strong for plasma T_4 and plasma IGF-1, weak for plasma GH and plasma cortisol, and not significant for plasma T_3. A best subsets regression model of hormones on gill B_{max} found that plasma GH, IGF-1, and T_4 were significant parameters ($P < 0.0001$, $R^2 = 0.119$); cortisol and T_3 were excluded from the analysis during the stepwise regression. Coefficients for GH (11.6) and T_4 (20.6) were positive, but the coefficient for IGF-1 (− 71.8) was negative.

Correlations for gill CR k_d and plasma hormone concentrations are shown in Table 3. Strong correlations existed for k_d and all hormones measured except for T_3 which was not correlated with k_d. The best fit for a single hormone on k_d was for GH; mean gill CR k_d is plotted against mean plasma GH (Fig. 3; $R^2 = 0.48$). A best subsets regression model of hormones on gill k_d found that plasma cortisol, GH, and T_4 had a significant effect on the analysis ($P < 0.0001$, $R^2 = 0.214$); IGF-1 and T_3 were excluded from the analysis during the stepwise regression. Coefficients for all hormones were positive; 0.153, 0.079, and 0.059 for GH, T_4, and cortisol, respectively.

Table 3
Results of correlation analysis for CR B_{max} and k_d when regressed against measured plasma levels of cortisol, growth hormone (GH), insulin-like growth factor 1 (IGF-1), thyroxine (T_4), and triiodothyronine (T_3)

	Hormone	Coefficient	P
B_{max}	Cortisol	− 4.44	0.079
	GH	6.49	0.068
	IGF-1	− 39.14	< 0.001
	T_4	21.85	< 0.001
	T_3	− 3.95	0.808
k_d	Cortisol	0.072	< 0.0001
	GH	0.164	< 0.0001
	IGF-1	0.192	0.001
	T_4	0.072	0.048
	T_3	− 0.066	0.463

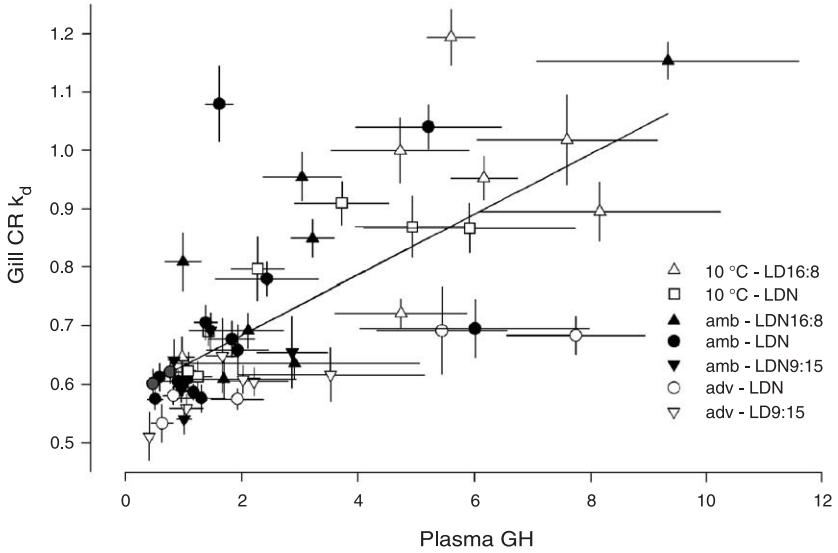

Fig. 3. Regression of plasma growth hormone with gill CR k_d. Values are means ($n=6$) for each time point from each of the photoperiod and temperature treatments. Groups as described in Figs. 1 and 2.

Gill CR B_{max} was not correlated with gill Na^+,K^+-ATPase activity. Regression analysis, however, was significant for gill CR k_d and gill Na^+,K^+-ATPase activity and is plotted in Fig. 4 ($R^2=0.33$).

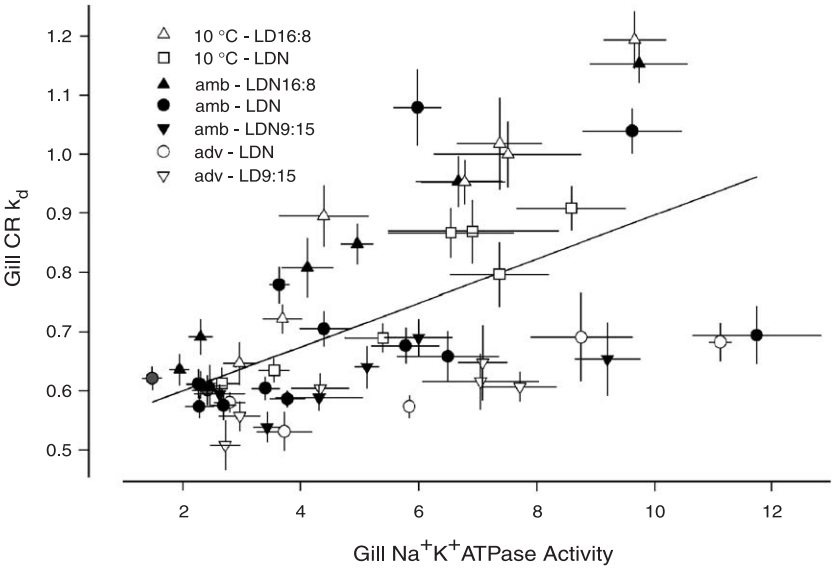

Fig. 4. Regression of gill Na^+,K^+-ATPase activity with gill CR k_d. Values are means ($n=6$) for each time point from each of the photoperiod and temperature treatments. Groups as described in Figs. 1 and 2.

4. Discussion

Changes in the corticosteroid receptor have been seen during development in fish and are thought to be important to the ontogeny of hormone action. During early embryonic development in tilapia, (*Oreochromis mossambicus*), the highest concentration of cortisol receptor mRNA was found just after fertilization (Tagawa et al., 1997). Gill CR concentration (B_{max}) and dissociation constant (k_d) have been observed to change seasonally in Atlantic salmon (Shrimpton and McCormick, 1998a; Shrimpton et al., 2000). The density of GR immunoreactive neurons was greater in olfactory regions of the brain in sexually mature sockeye salmon (*Oncorhynchus nerka*) compared to immature fish (Carruth et al., 2000). These observations indicate that CR dynamics change seasonally with developmental stage in fish and increase when cortisol is functionally important. The present study sought to characterize environmental and endocrine factors that regulate gill CR in Atlantic salmon during the parr–smolt transformation.

A seasonal increase in plasma cortisol is one of the endocrine factors that stimulate smolting in juvenile salmon (Hoar, 1988). The mechanism of cortisol action in the gills is mediated by the CR (Clark and Peck, 1977). A direct relationship between tissue sensitivity to cortisol and CR concentration, and to a lesser extent affinity, has recently been shown by Shrimpton and McCormick (1999). For all study groups in Experiments 1 and 2 (except 10 °C), maximal values of B_{max} were reached earlier in the year than gill Na^+,K^+-ATPase activity. The increase in B_{max} prior to the peak in smolting will increase sensitivity of the gill to cortisol, before the springtime increase in cortisol. The increase in B_{max}, therefore, appears to be preparatory for the seasonal increase in cortisol that plays a role in stimulating smolting. Changes in gill CR k_d occurred synchronously with changes in gill Na^+,K^+-ATPase activity, and appear to reflect physiological changes associated with smolting.

4.1. Environmental regulation of CR

Photoperiod is the primary stimulus for smolting (McCormick et al., 1987). The lack of a change in CR B_{max} following photoperiod manipulation, therefore, is surprising. It appears that the seasonal changes in B_{max} are independent of photoperiod. Temperature does have an effect on CR B_{max} (Tables 1 and 2). Temperature affects CR B_{max} in two ways; first, the seasonal increase is independent of a change in temperature but correlated with ATUs, and second, the decrease in B_{max} is dependent on the seasonal increase in temperature.

The maximum values of B_{max} corresponded to approximately 170 ATU for the ambient and advanced temperature groups, but were not a function of date. An examination of other studies that have shown changes in CR B_{max} over the spring in Atlantic salmon indicates that ATU is also related to maximum B_{max}. A calculation of maximum B_{max} in the study by Shrimpton and McCormick (1998a) found that the maximum was reached at 247 ATU. This is greater than the ATU calculated in the present study; however, the sampling interval was monthly. Two weeks earlier, the mid-point between the two sampling intervals would correspond to 184 ATU and a value similar to that calculated in the present study. Average temperature was 2.5 °C from January to May, and the temperature was 5.6 °C when the maximum was reached. The relationship between ATU

and maximum B_{max} did not hold for another study where water temperature during the winter months was warmer (>8 °C during January and February) (Shrimpton et al., 2000). It appears, therefore, that when water temperatures are below 3 °C, B_{max} is closely associated with ATU. The strong correlation between maximum B_{max} and ATU is suggestive that the rate of development is dependent on temperature not date, as the timing of the highest values of B_{max} differed by approximately 1 month (Table 1).

The decline in B_{max} was found to be a function of temperature change for groups where temperature was not held constant. As the rate of increase in temperature exceeded 1.5 °C/week during the 2-week interval before the sample, there was a marked decrease in B_{max} (Table 2). Regression analysis of ΔB_{max} on ΔT also indicate that the seasonal increase in temperature drives the decline in B_{max}. A similar relationship was calculated for the data published by Shrimpton and McCormick (1998a). In the 10 °C constant temperature group, there was less seasonal change in CR B_{max}; values in the ambient temperature groups were significantly greater than the 10 °C groups in early April, the maximum values for B_{max} (Fig. 1). Correspondingly, there was a smaller decrease in B_{max} following the peak. In the study by Shrimpton et al. (2000), there was a change in temperature over the year, but the temperature regime did not follow a profile characteristic of natural systems. Temperature was warmer in the winter (mean greater than 7 °C in both years) and decreased during the spring. The effect of this thermal regime is not clear, but the seasonal decreases in CR B_{max} were certainly protracted.

Although photoperiod is the main environmental factor to stimulate smolting, increases in water temperature can affect the timing of smolting. As assessed by peak gill Na^+,K^+-ATPase activity, smolting occurs several weeks earlier when rearing temperature is increased (McCormick et al., 1997). Smolting has also been linked to changes in temperature as the seasonal increase in gill Na^+,K^+-ATPase activity is more pronounced when temperature increases in conjunction with photoperiod (Muir et al., 1994). Other smolt related characteristics such as time of migration have also been found to be strongly associated with a temperature threshold (Jonsson and Ruud-Haansen, 1985). The increases in B_{max} were not affected by absolute temperature changes or a threshold in temperature, but the seasonal increase in temperature is associated with the decline in B_{max} at the end of the spring. The elevation of temperature to 10 °C at the start of Experiment 1, however, had no effect on B_{max}. Whether the increase in temperature in the first experiment occurred too early in development for B_{max} to be affected is not clear.

Seasonal changes in CR k_d were also observed in the present study consistent with findings of other studies on Atlantic salmon (Shrimpton and McCormick, 1998a; Shrimpton et al., 2000) and coho salmon (Shrimpton et al., 1994), but not in steelhead or rainbow trout (McLeese et al., 1994). Changes in CR k_d were correlated with gill Na^+,K^+-ATPase activity, unlike B_{max} (Fig. 4). A strong correlation between these two variables has been seen in previous studies. Shrimpton and McCormick (1998a) found that highest k_d values were coincident with maximum gill Na^+,K^+-ATPase activity. Strong seasonal correlation was also seen in the study by Shrimpton et al. (2000). In coho salmon, a similar relationship was also found for fish captured in the wild, but not for hatchery fish reared at a constant temperature (Shrimpton et al., 1994). The seasonal increase in k_d, therefore, appear to be a function of physiological changes associated with smolting that also alter k_d.

Advances in photoperiod (Experiment 1) increased gill CR k_d, but not at low temperature (Fig. 1). The increase in k_d correlated well with an increase in gill Na^+,K^+-ATPase activity and in turn plasma GH (see McCormick et al., 2000). It is possible that the changes in k_d that respond to photoperiod are a function of endocrine changes associated with smolting. The short day treatments in Experiment 2 did not affect k_d (Fig. 2).

4.2. Endocrine control of CR

In the present study, there were a number of correlations between endocrine parameters and B_{max}. Individual regression of hormone data showed that there was a significant relationship between CR B_{max} and T_4. This was not seen with T_3, which would appear contrary to the findings of Shrimpton and McCormick (1999) who showed that exogenous T_3 significantly increased CR B_{max}. This may be due to species differences or due to the activity of these two hormones. Seasonal increases in T_4 is a normal feature of smolting, yet there appears to be little increase or change in T_3 (Hoar, 1988). The correlation of gill CR B_{max} with T_4 and not T_3, therefore, may reflect deiodination of T_4 in the gill tissue itself.

There was a weak, but positive correlation between GH and B_{max}. Given the evidence from injection studies (Shrimpton et al., 1995; Shrimpton and McCormick, 1998b), it is surprising that the relationship between B_{max} and GH is not stronger. GH has been shown to interact with cortisol to increase saltwater tolerance (Madsen, 1990). A number of mechanisms have been proposed to account for this finding. GH may have a direct action on the gill through gill GH receptors (Gray et al., 1990), GH may act to increase gill response to cortisol through an increase in CR B_{max} (Shrimpton et al., 1995), or GH cause release of IGF-1 which may have a direct effect (Sakamoto et al., 1993). The hypoosmoregulatory action of IGF-1 on salmonids has been shown by McCormick et al. (1995) and an additive effect with cortisol has been shown by McCormick (1996). If the GH effect is through IGF-1, then we would expect that a strong correlation should exist between CR B_{max} and IGF-1. Indeed a strong relationship exists between CR B_{max} and IGF-1, but the correlation is negative. The reason for this apparent discrepancy is unclear particularly given the findings of McCormick (1996).

Interestingly, plasma cortisol was not significantly correlated with CR B_{max}. A strong negative correlation between plasma cortisol and CR B_{max} would be expected and has been demonstrated in several previous studies (Maule and Schreck, 1991; Pottinger et al., 1994; Shrimpton and Randall, 1994). The results of these studies, however, may not contradict the present findings as they were not conducted during the spring when the fish were smolting. During the spring, the seasonal increases in plasma T_4 and GH may function to limit the decline in CR B_{max} when cortisol also increases. Indeed, the multi-hormonal control of the gill cortisol receptor may explain why correlations with individual hormones are weak or contradictory.

In the present study, plasma cortisol, GH, IGF-1 and T_4 were positively correlated with gill CR k_d. There was no relationship with T_3. Injection experiments with cortisol have shown an increase in CR k_d in the gills of coho salmon (Maule and Schreck, 1991; Shrimpton and Randall, 1994) and rainbow trout liver (Pottinger et al., 1994). GH injections increase k_d

in Atlantic salmon (Shrimpton and McCormick, 1998a,b), but GH and T_3 were not found to affect CR k_d in rainbow trout (Shrimpton and McCormick, 1999). Whether the correlation between IGF-1 and T_4 are causal is not known. Further experimentation is required to determine a regulatory role for these two hormones on gill CR k_d.

Under natural temperature regimes CR B_{max} increased prior to elevations in gill Na^+,K^+-ATPase activity, while changes in k_d correlated with the seasonal increases in gill Na^+,K^+-ATPase activity. The change in B_{max} may be preparatory, as the gill will be more responsive to cortisol as part of the endocrine system driving higher activities of gill Na^+,K^+-ATPase and seawater tolerance. Higher temperatures will result in earlier development of salinity tolerance (McCormick et al., 1997). The present study, however, indicates that advances in the seasonal increase in water temperature will lead to a decrease in CR B_{max} with little change in CR k_d, reducing gill sensitivity to cortisol earlier in the year. Higher temperatures also result in more rapid losses of salinity tolerance and decreases in gill Na^+,K^+-ATPase activity (McCormick et al., 1997). The earlier decline in CR B_{max} in fish reared under temperature regimes that are advanced or warmer may be a mechanism for the shortened smolt window in fish reared under seasonally advanced or warmer water temperatures.

Acknowledgements

We thank the staff of the White River National Fish Hatchery, US Fish and Wildlife Service for providing and maintaining the fish used throughout the study. We also thank Judy Carey, Jill Leonard, Michael O'Dea and Joseph Zydlewski for their assistance in sampling the fish. We express our gratitude to Judy Carey for cortisol analyses, Dr. Thrandur Björnsson and Gunilla Eriksson for growth hormone analyses, and Dr. Shunsuke Moriyama for IGF-1 analyses. We also thank Dr. Carl Schreck and an anonymous reviewer for the helpful comments, which improved the manuscript.

References

Björnsson, B.Th., Taranger, G.L., Hansen, T., Stefansson, S.O., Haux, C., 1994. The interrelation between photoperiod, growth hormone, and sexual maturation of adult Atlantic salmon (*Salmo salar*). Gen. Comp. Endocrinol. 93, 70–81.

Carey, J.B., McCormick, S.D., 1998. Atlantic salmon smolts are more responsive to handling and confinement stress than parr. Aquaculture 168, 237–253.

Carruth, L.L., Jones, R.E., Norris, D.O., 2000. Cell density and intracellular translocation of glucocorticoid receptor–immunoreactive neurons in the kokanee salmon (*Oncorhynchus nerka kennerlyi*) brain, with an emphasis on the olfactory system. Gen. Comp. Endocrinol. 117, 66–76.

Clark, J.H., Peck, E.J., 1977. Steroid hormone receptors: basic principles and measurement. In: O'Malley, B.W., Birnbaumer, L. (Eds.), Receptors and Hormone Action. Academic Press, New York, pp. 383–410.

Clarke, W.C., Shelburn, J.E., Brett, J.R., 1978. Growth and adaptation to seawater in underyearling sockeye (*Oncorhynchus nerka*) and coho (*Oncorhynchus kisutch*) salmon subjected to regimes of constant or changing temperature and daylength. Can. J. Zool. 56, 2413–2421.

Colombe, L., Fostier, A., Bury, N., Pakdel, F., Guiguen, Y., 2000. A mineralocorticoid-like receptor in the rainbow trout, *Oncorhynchus mykiss*: cloning and characterization of its steroid binding domain. Steroids 65, 319–328.

Diaz, R., Brown, R.W., Seckl, J.R., 1998. Distinct ontogeny of glucocorticoid and mineralocorticoid receptor and 11β-hycroxysteroid dehydrogenase types I and II mRNAs in the fetal rat brain suggest a complex control of glucocorticoid actions. J. Neurosci. 18, 2570–2580.

Ducouret, B., Tujague, M., Ashraf, J., Mouchel, N., Servel, N., Valotaire, Y., Thompson, E.B., 1995. Cloning of a teleost fish glucocorticoid receptor shows that it contains a deoxyribonucleic acid-binding domain different from that of mammals. Endocrinology 136, 3774–3783.

Eliceiri, B.P., Brown, D.D., 1994. Quantitation of endogenous thyroid hormone receptors α and β during embryogenesis and metamorphosis in *Xenopus laevis*. J. Biol. Chem. 269, 24459–24465.

Gray, E.S., Young, G., Bern, H.A., 1990. Radioreceptor assay for growth hormone in coho salmon (*Oncorhynchus kisutch*) and its application to the study of stunting. J. Exp. Zool. 156, 190–296.

Hoar, W.S., 1988. The physiology of smolting salmonids. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology, vol. XIB. Academic Press, New York, pp. 275–344.

Jonsson, B., Ruud-Haansen, J., 1985. Water temperature as the primary influence on timing of seaward migration of Atlantic salmon (*Salmo salar*) smolts. Can. J. Fish. Aquat. Sci. 42, 592–596.

Madsen, S.S., 1990. Enhanced hypoosmoregulatory response to growth hormone after cortisol treatment in immature rainbow trout, *Salmo gairdneri*. Fish Physiol. Biochem. 8, 271–279.

Maule, A.G., Schreck, C.B., 1991. Stress and cortisol treatment changed affinity and number of glucocorticoid receptors in leukocytes and gill of coho salmon. Gen. Comp. Endocrinol. 84, 83–93.

McCormick, S.D., 1993. Methods for nonlethal gill biopsy and measurements of Na^+,K^+-ATPase activity. Can. J. Fish. Aquat. Sci. 50, 656–658.

McCormick, S.D., 1996. Effects of growth hormone and insulin-like growth factor I on salinity tolerance and gill Na^+,K^+-ATPase in Atlantic salmon (*Salmo salar*): interaction with cortisol. Gen. Comp. Endocrinol. 101, 3–11.

McCormick, S.D., Saunders, R.L., 1987. Preparatory physiological adaptations for marine life of salmonids: osmoregulation, growth, and metabolism. Am. Fish. Soc. Symp. 1, 211–229.

McCormick, S.D., Saunders, R.L., Henderson, E.B., Harmon, P.R., 1987. Photoperiod control of parr–smolt transformation in Atlantic salmon (*Salmo salar*): changes in salinity tolerance, gill Na^+,K^+-ATPase activity, and plasma thyroid hormones. Can. J. Fish. Aquat. Sci. 44, 1462–1468.

McCormick, S.D., Björnsson, B.Th., Sheridan, M., Eilertson, C., Carey, J.B., O'Dea, M., 1995. Increased daylength stimulates plasma growth hormone and gill Na^+,K^+-ATPase in Atlantic salmon (*Salmo salar*). J. Comp. Physiol., B 165, 245–254.

McCormick, S.D., Shrimpton, J.M., Zydlewski, J.D., 1997. Temperature effects on osmoregulatory physiology of juvenile anadromous fish. In: Wood, C.M., McDonald, D.G. (Eds.), Global Warming: Implications for Freshwater and Marine Fish. Society for Experimental Biology Seminar Series, vol. 61. Cambridge Univ. Press, Cambridge, UK, pp. 279–301.

McCormick, S.D., Moriyama, S., Björnsson, B.Th., 2000. Low temperature limits the photoperiod response: endocrinology of smolting in Atlantic salmon. Am. J. Physiol. 278, R1352–R1361.

McLeese, J.M., Johnsson, J., Huntley, F.M., Clarke, W.C., Weisbart, M., 1994. Seasonal changes in osmoregulation, cortisol, and cortisol receptor activity in the gills of parr/smolt of steelhead trout and steelhead-rainbow trout hybrids, *Oncorhynchus mykiss*. Gen. Comp. Endocrinol. 93, 103–113.

Moriyama, S., Swansen, P., Nishii, M., Takahashi, A., Kawauchi, H., Dickhoff, W.W., Plisetskaya, E.M., 1994. Development of a homologous radioimmunoassay for coho salmon insulin-like growth factor-I. Gen. Comp. Endocrinol. 96, 149–161.

Muir, W.D., Zaugg, W.S., Giorgi, A.E., McCutcheon, S., 1994. Accelerating smolt development and downstream movement in yearling chinook salmon with advanced photoperiod and increased temperature. Aquaculture 123, 387–399.

Pottinger, T.G., Knudsen, F.R., Wilson, J., 1994. Stress-induced changes in the affinity and abundance of cytosolic cortisol-binding sites in the liver of rainbow trout, *Oncorhynchus mykiss* (Walbaum), are not accompanied by changes in measurable nuclear binding. Fish Physiol. Biochem. 12, 499–511.

Sakamoto, T., McCormick, S.D., Hirano, T., 1993. Osmoregulatory actions of growth hormone and its mode of action in salmonids: a review. Fish Physiol. Biochem. 11, 155–164.

Sandor, T., DiBattista, J.A., Mehdi, A.Z., 1984. Glucocorticoid receptors in the gill tissue of fish. Gen. Comp. Endocrinol. 53, 353–364.

Scatchard, G., 1949. The attraction of protein for small molecules and ions. Ann. N.Y. Acad. Sci. 51, 661–672.

Shrimpton, J.M., McCormick, S.D., 1998a. Seasonal differences in plasma cortisol and gill corticosteroid receptors in upper and lower mode juvenile Atlantic salmon. Aquaculture 168, 205–219.

Shrimpton, J.M., McCormick, S.D., 1998b. Regulation of gill cytosolic corticosteroid receptors in juvenile Atlantic salmon: interaction effects of growth hormone with prolactin and triiodothyronine. Gen. Comp. Endocrinol. 112, 262–274.

Shrimpton, J.M., McCormick, S.D., 1999. Responsiveness of gill Na^+,K^+-ATPase to cortisol is related to gill corticosteroid receptor concentration in juvenile rainbow trout. J. Exp. Biol. 202, 987–995.

Shrimpton, J.M., Randall, D.J., 1994. Downregulation of corticosteroid receptors in the gills of coho salmon due to stress and cortisol treatment. Am. J. Physiol. 267, R432–R438.

Shrimpton, J.M., Bernier, N.J., Randall, D.J., 1994. Changes in cortisol dynamics in wild and hatchery-reared juvenile coho salmon (*Oncorhynchus kisutch*) during smoltification. Can. J. Fish. Aquat. Sci. 51, 2179–2187.

Shrimpton, J.M., Devlin, R.H., McLean, E., Byatt, J.C., Donaldson, E.M., Randall, D.J., 1995. Increases in gill cytosolic corticosteroid receptor abundance and saltwater tolerance in juvenile coho salmon (*Oncorhynchus kisutch*) treated with growth hormone and placental lactogen. Gen. Comp. Endocrinol. 98, 1–15.

Shrimpton, J.M., Björnsson, B.Th., McCormick, S.D., 2000. Can Atlantic salmon smolt twice? Endocrine and biochemical changes during smolting. Can. J. Fish. Aquat. Sci. 57, 1969–1976.

Staurnes, M., Sigholt, T., Gulseth, O.A., 1994. Effects of seasonal changes in water temperature on the parr–smolt transformation of Atlantic salmon and anadromous Arctic char. Trans. Am. Fish. Soc. 123, 408–415.

Sumpter, J.P., Dye, H.M., Benfey, T.J., 1986. The effect of stress on plasma ACTH, α-MSH, and cortisol in salmonid fishes. Gen. Comp. Endocrinol. 62, 377–385.

Tagawa, M., Hagiwara, H., Takemura, A., Hirose, S., Hirano, T., 1997. Partial cloning of the hormone-binding domain of the cortisol receptor in tilapia, *Oreochromis mossambicus*, and changes in the mRNA level during embryonic development. Gen. Comp. Endocrinol. 108, 132–140.

Welshons, W.V., Jordon, V.C., 1987. Heterogeneity of nuclear steroid hormone receptors with an emphasis on unfilled receptor sites. In: Clark, C.R. (Ed.), Steroid Hormones: Their Intracellular Localization. Ellis Horwood, Chichester, pp. 128–154.

Yaoita, Y., Brown, D.D., 1990. A correlation of thyroid hormone receptor gene expression with amphibian metamorphosis. Genes Dev. 4, 1917–1924.