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Aquaculture 273 (2007) 337–344
Aquaculture
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# Differential hormonal responses of Atlantic salmon parr and smolt to increased daylength: A possible developmental basis for smolting

Stephen D. McCormick [a,*], J. Mark Shrimpton [b], Shunsuke Moriyama [c], Björn Thrandur Björnsson [d]

[a] *USGS, Conte Anadromous Fish Research Center, Turners Falls, MA, USA*
[b] *Biology Program, University of Northern British Columbia, Prince George, B.C. Canada*
[c] *Laboratory of Molecular Endocrinology, School of Fisheries, Kitasato University, Sanriku, Iwate, Japan*
[d] *Fish Endocrinology Laboratory, Department of Zoology/Zoophysiology, Göteborg University, Sweden*

**Abstract**

In order to elucidate the developmental basis for smolting, Atlantic salmon, *Salmo salar*, parr (<11.5 cm) and smolts (>12.5 cm) were exposed to natural daylength (LDN) and increased daylength (LD16:8) starting in late February and gill $Na^+$,$K^+$–ATPase activity and circulating hormone levels monitored from January to May. Gill $Na^+$,$K^+$–ATPase activity remained low and constant in both groups of parr. In smolts, gill $Na^+$,$K^+$–ATPase began increasing in late February in both photoperiods, but was significantly higher in the LD16:8 group from March through April. Smolts exposed to LD16:8 had dramatically elevated plasma GH within one week of increased daylength that remained high through April, whereas plasma GH of LDN smolts increased steadily beginning in late February and peaking in late April. Plasma GH levels of parr remained low in spring and did not respond to increased daylength. Plasma insulin-like growth factor I (IGF-I) levels were substantially higher in smolts than parr in January. Plasma IGF-I levels of parr increased steadily from January to May, but there was no influence of increased daylength. In smolts, plasma IGF-I of LD16:8 fish initially decreased in early March then increased in late March and April, whereas plasma IGF-I of LDN smolts increased steadily to peak levels in early April. Plasma cortisol was low in parr throughout spring and did not differ between photoperiod treatments. Plasma cortisol of LD16:8 smolts increased in early March and remained elevated through April, whereas in LDN smolts plasma cortisol did not increase until early April and peaked in late April. Plasma thyroid hormones were generally higher in smolts than in parr, but there was no clear effect of increased daylength in parr or smolts. The greater capacity of the GH/IGF-I and cortisol axes to respond to increased daylength may be a critical factor underlying smolt development.


## 1. Introduction

The parr–smolt transformation is an endocrine-driven developmental event that is preparatory for downstream migration and ocean entry. In Atlantic salmon (*Salmo salar*) this is a size-related process in which fish that have achieved a minimum size (e.g. 12 cm in winter) will become smolts the following spring (McCormick et al., 1998). A variety of physiological, morphological and behavioral changes occur in smolts, and these changes do not occur in parr, even though they may be the same age and are reared (in nature or in the laboratory) under the same environmental conditions. One of the most important of these changes to occur during smolting is

* Corresponding author. Tel./fax: +1 413 863 9810.
*E-mail address:* mccormick@umext.umass.edu (S.D. McCormick).

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doi:10.1016/j.aquaculture.2007.10.015

the development of salinity tolerance which is adaptive for rapid seawater entry. Increased salinity tolerance is brought about by changes in the major osmoregulatory organs (gill, gut and kidney) and include upregulation of ion transporters such as gill $Na^+$,$K^+$–ATPase activity. Changes in osmoregulatory physiology of smolts are regulated by growth hormone (GH), insulin-like growth factor I (IGF-I) and cortisol (Hoar 1988). Thyroid hormones probably play an indirect role in osmoregulatory physiology and are important in controlling smolt morphology and behavior.

The underlying mechanism(s) that control the size-related development of smolts are unknown. It seems likely that they involve neuroendocrine changes in the brain and/or hypothalamus (McCormick et al., 1998). Prior work has demonstrated that photoperiod is the primary environmental factor that controls smolt development in spring, and that this control is through stimulation of plasma GH, IGF-I and cortisol (Bjornsson, 1997). We hypothesized that fish that are large enough to become smolts may have a differential endocrine response to photoperiod that might be the underlying basis for the parr–smolt transformation. To test this hypothesis, we exposed small (<11 cm in winter) and large (>12.5 cm) juvenile Atlantic salmon to normal seasonally increasing daylength (LDN) and artificially increased daylength (LD16:8 in February) and monitored their physiological and endocrine responses.

## 2. Materials and methods

Juvenile Atlantic salmon were obtained from the White River National Fish Hatchery (Bethel, VT, USA) and brought to the Conte Anadromous Fish Research Center (Turners Falls, MA, USA) in autumn. Rearing and experimental conditions are similar to those reported in McCormick et al. (1995). On December 15, fish were separated by size into putative parr (<11 cm fork length) and putative smolts (>12.5 cm fork length) and placed in isolated photoperiod rooms containing two 1-m diameter tanks supplied with ambient river water at a flow rate of 4 L $min^{-1}$ and supplemental aeration. Each tank contained approximately 100 fish. The fish were fed to satiation (Zeigler Bros., Gardners, PA, USA) using automatic feeders. Initially all groups were maintained on a simulated natural photoperiod (LDN) with seasonal changes in daylength. Lighting was supplied by overhead fluorescent lights (500 lx at the water surface) and the LDN photoperiod was adjusted twice a week. From December through January, fish were maintained on ambient temperatures (1–6 °C). On February 3, temperatures in all tanks were gradually increased and maintained at 9–10 °C throughout the remainder of the experiment. On February 15, half of the fish in each group of parr and smolt were exposed to long days (LD16:8; 16 h daylight) while the other half remained on LDN. A plumbing malfunction led to loss of fish in the smolt LD16:8 group after April 4.

Feed was withheld for 24 h prior to sampling which was carried out from 1000–1100 h Eastern Standard Time on January 25, February 13 and 22, March 3 and 21, April 4 and 24, and May 16. Fish were anesthetized (100 mg $L^{-1}$ MS-222 neutralized to pH 7.0) and fork length to the nearest mm and weight to the nearest 0.1 g were recorded. Blood was drawn from the caudal vein into a 1 ml ammonium heparinized syringe and spun at 8000 g for 5 min at 4 °C. Plasma was aliquoted and stored at −80 °C. Four to six gill filaments were severed above the septum, placed in 100 µl of ice-cold SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3) and frozen at −80 °C within 30 min.

$Na^+$,$K^+$–ATPase activity was determined with a kinetic assay run in 96-well microplates at 25 °C and read at a wavelength of 340 nm for 10 min as described in McCormick (1993). Gill tissue was homogenized in 125 µl of SEID (SEI buffer and 0.1% deoxycholic acid) and centrifuged at 5000 ×g for 30 s. Ten µl samples were run in two sets of duplicates; one set containing assay mixture and the other assay mixture and 0.5 mM ouabain. The resulting ouabain-sensitive ATPase activity is expressed as µmoles ADP/mg protein/h. Protein concentrations are determined using BCA (bicinchoninic acid) Protein Assay (Pierce, Rockford, Il, USA). Both assays were run on a THERMOmax microplate reader using SOFTmax software (Molecular Devices, Menlo Park, CA, USA).

Plasma cortisol levels were measured by a validated direct competitive enzyme immunoassay as outlined in Carey and McCormick (1998). Sensitivity as defined by the dose–response curve was 1 to 400 ng $ml^{-1}$. The lower detection limit was 0.3 ng $ml^{-1}$. Using a pooled plasma sample, the average intra-assay variation was 5.5% ($n$=10) and the average inter-assay variation was 8.8% ($n$=10). Plasma growth hormone levels were measured by a radioimmunoassay validated for Atlantic salmon (Björnsson et al., 1994). Typical measuring range was 0.1 to 50 ng $ml^{-1}$ with $ED_{50}$=2.2 ng $ml^{-1}$ and an average intra-assay and inter-assay variation of 5.4% ($n$=9) and 3.9% ($n$=9), respectively. Plasma IGF-I was measured by homologous radioimmunoassay as described by Moriyama et al. (1994). Sensitivity as defined by the dose–response curve was 1 to 250 ng $ml^{-1}$. The lower detection limit was 0.20 ng $ml^{-1}$. Using a pooled plasma sample, the average intra-assay variation was 7% ($n$=5) and the average inter-assay variation was 6.5% ($n$=5). Thyroxine ($T_4$) and 3,5,3′-triiodo-L-thyronine ($T_3$) concentrations were measured by a direct radioimmunoassay described by Dickhoff et al. (1978) and modified by McCormick et al. (1995). Sensitivity as defined by the dose–response curve was 1 to 64 ng $ml^{-1}$ for thyroxine and 0.5–16 ng $ml^{-1}$ for triiodothyronine. Intra- and inter-assay coefficients of variation for these assays were 4.3–11% and 3.2–5%, respectively.

For each parameter a three-way analysis of variance (ANOVA) was used to determine the significance of photoperiod treatment, parr vs smolt and changes over time (January–April when all groups were represented). If photoperiod treatment was significant ($p$<0.05) or there was a significant interaction with photoperiod and parr vs smolt, the difference

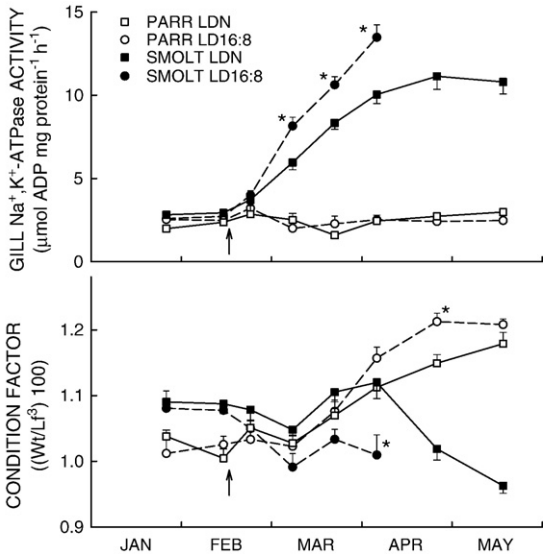


Fig. 1. Gill $Na^+,K^+$–ATPase activity (Φmol ADP/mg protein/h) and condition factor ((weight/length$^3$)100) in Atlantic salmon parr and smolt subjected to normal and increased daylength. Increased daylength in the LD16:8 groups began on February 15 (arrowhead). Values are mean±standard error ($n$=10–12). Asterisk indicates significant difference between the LD16:8 and LDN treatments within each of the parr and smolt groups ($p$<0.05, Student–Neuman–Keuls test).

between photoperiod treatments at each time point for parr and smolt was tested using Student–Neuman–Keuls test ($p$<0.05). Correlation and regression analyses were performed on mean values from each group, treatment and time point when all groups were represented (January–April). All statistics were run using the Statistica (StatSoft Inc., Tulsa, OK) software package.

## 3. Results

Gill $Na^+,K^+$–ATPase activity remained low throughout the experiment for both LDN and LD 16:8 parr (mean values < 3 μmol ADP/mg protein/h; Fig. 1). Gill $Na^+,K^+$–ATPase activity was initially low in each of the smolt groups. In March gill $Na^+,K^+$–ATPase activity increased in both smolt groups, but this increase was greater in the LD16:8 than LDN group; activity was significantly higher in the LD16:8 beginning March 3 (16 days after initiation of increased daylength) and continued through April. There was a significant effect of photoperiod ($p$<0.0001), parr vs smolt ($p$<0.0001) and time ($p$<0.0001) on gill $Na^+,K^+$–ATPase activity (3-way ANOVA).

Condition factor was initially low (1.00–1.04) in parr, then increased in both photoperiod treatments in late March (Fig. 1). Condition factor of the parr LD16:8 group increased to a significantly higher level than the parr LDN group in late April. Condition factors of smolts were initially higher than parr (1.08–1.10). In the smolt LD16:8 group condition factor decreased in early March and was significantly lower than the smolt LDN group in early April. Condition factor of the smolt LDN group decreased in late April. There was a significant effect of photoperiod ($p$=0.002) and time ($p$<0.001), but not parr vs smolt ($p$<0.10) on condition factor (3-way ANOVA), and a significant interaction between parr vs smolt and time ($p$<0.0001).

Plasma GH was initially low (mean January values <2 ng ml$^{-1}$) in all groups (Fig. 2). Both parr groups increased to 5–6 ng ml$^{-1}$ in early March and subsequently declined; there was no apparent affect of photoperiod treatment on plasma GH in parr. In contrast, 7 days after the initiation of increased daylength plasma GH of the smolt LD16:8 group increased to 8 ng ml$^{-1}$, and after 16 days was 15 ng ml$^{-1}$. Plasma GH in the smolt LDN group increased gradually beginning in March and reached peak values of 8 ng ml$^{-1}$ in late April. There was a significant effect of photoperiod ($p$<0.0001), parr vs smolt ($p$=0.0001) and time ($p$=0.0006) on plasma GH (3-way ANOVA).

Plasma IGF-I levels in January and February were more than 2-fold higher in smolts than in parr (Fig. 2). Plasma IGF-I of both

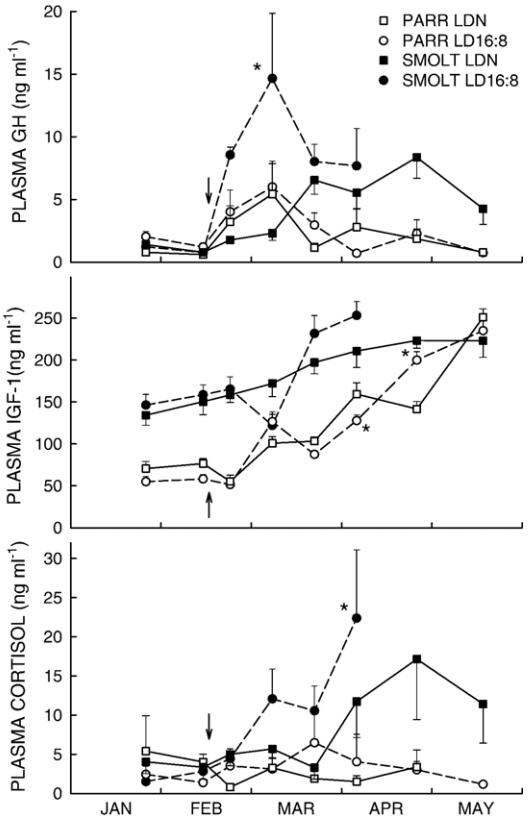


Fig. 2. Plasma growth hormone, insulin-like growth factor I and cortisol (ng ml$^{-1}$) in Atlantic salmon parr and smolt subjected to normal and increased daylength. Increased daylength in the LD16:8 groups began on February 15 (arrowhead). Values are mean±standard error ($n$=10–12). Asterisk indicates significant difference between the LD16:8 and LDN treatments within each of the parr and smolt groups ($p$<0.05, Student–Neuman–Keuls test).

parr groups increased steadily between January and May and there was no substantial difference between the two groups. Plasma IGF-I of the smolt LDN group increased steadily through the spring. There was a decrease in plasma IGF-I in the smolt LD16:8 group in early March followed by an increase in late March, but there was no significant difference between the smolt LDN and LD16:8 groups. There was a significant effect of parr vs smolt ($p<0.0001$) and time ($p<0.0001$) but not photoperiod ($p=0.93$) on plasma IGF-I (3-way ANOVA).

Plasma cortisol was initially low in the both parr groups (mean January values <2 ng ml$^{-1}$) and remained relatively low throughout the study (mean values <7 ng ml$^{-1}$; Fig. 2). There was no apparent effect of photoperiod treatment on plasma cortisol of parr. Plasma cortisol was slightly higher in the smolt groups relative to parr in January (mean values 4–6 ng ml$^{-1}$). Plasma cortisol increased in the smolt LD16:8 group 16 days after initiation of increased daylength (12 ng ml$^{-1}$) and remained elevated in April (23 ng ml$^{-1}$). Plasma cortisol of the smolt LDN group remained relatively constant from January through March then increased in early April and remained elevated through May (11–16 ng ml$^{-1}$). There was a significant effect of parr vs smolt ($p=0.003$) and time ($p=0.024$) but not photoperiod on plasma cortisol (3-way ANOVA). There was a significant interaction between time and photoperiod ($p<0.0001$).

Plasma $T_4$ was generally lower in parr than smolt throughout the study (Fig. 3). Plasma $T_4$ was highly variable over time in smolts during most of the study, though a general pattern of high levels at the end of January, February, March and April was present in both photoperiod treatments. A similar pattern appeared to exist in parr, but it was not as clearly discernible. No clear photoperiod effect on plasma $T_4$ was evident in either parr or smolt. There was a significant effect of parr vs smolt ($p<0.0001$) and time ($p<0.0001$), but not photoperiod ($p=0.052$) on plasma $T_4$ (3-way ANOVA).

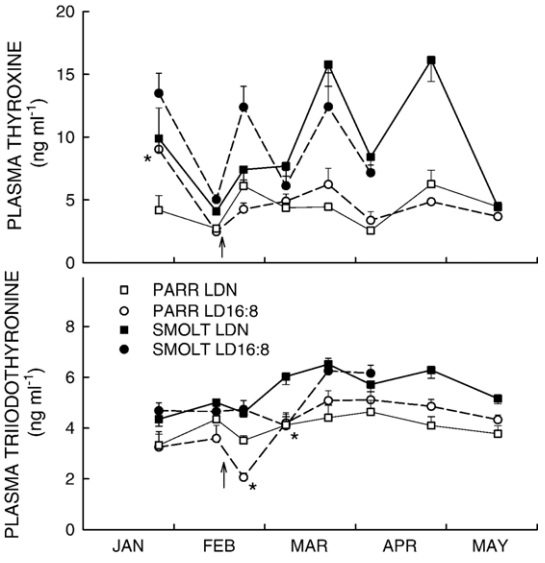


Fig. 3. Plasma thyroxine (ng ml$^{-1}$) and triiodothyronine (ng ml$^{-1}$) in Atlantic salmon parr and smolt subjected to normal and increased daylength. Increased daylength in the LD16:8 groups began on February 15 (arrowhead). Values are mean±standard error ($n=10$–12). Asterisk indicates significant difference between the LD16:8 and LDN treatments within each of the parr and smolt groups ($p<0.05$, Student–Neuman–Keuls test).

Plasma $T_3$ levels were initially low in parr in January (3–4 ng ml$^{-1}$) and increased slightly through the course of the study. Plasma $T_3$ in smolts increased gradually from 4–5 ng ml$^{-1}$ in January to peak values of 6 ng ml$^{-1}$ in late March. There was no clear photoperiod effect in either parr or smolt. There was a significant effect of parr vs smolt ($p<0.0001$) and time ($p<0.0001$) but not photoperiod ($p=0.12$) on plasma $T_3$ (3-way ANOVA).

There was a positive correlation between gill $Na^+,K^+$–ATPase activity and all of the plasma hormones: GH $r^2=0.42$, $p<0.00001$; IGF-I $r^2=0.36$, $p=0.0002$; cortisol $r^2=0.77$, $p<0.0001$; $T_4$ $r^2=0.21$, $p=0.007$; $T_3$ $r^2=0.43$, $p<0.0001$. In a stepwise regression model of gill $Na^+,K^+$–ATPase activity only cortisol and IGF-I were included as significant ($r^2=0.88$). There was a significant positive correlation between condition factor and plasma GH ($r^2=0.21$, $p=0.012$) and cortisol ($r^2=0.23$, $p<0.0084$). In a stepwise regression model of condition factor only cortisol and IGF-I were included as significant ($r^2=0.54$).

## 4. Discussion

The present results indicate a differential endocrine response to photoperiod between Atlantic salmon parr and smolt. We observed that increased daylength caused a significant elevation in plasma growth hormone and cortisol levels in smolts, but not in parr. As these hormones trigger many of the physiological changes that occur during smolting (Hoar 1988), it is likely that there is a causal relationship between differentiated endocrine response seen in the present study, and the concomitant changes in gill $Na^+,K^+$–ATPase activity, condition factor and other physiological and behavioral parameters that occur in smolts, but not in parr.

Photoperiod has been established as a major controlling factor in smolt development (Saunders and Henderson 1970), partly through a "light-pituitary-axis" which involves the activation of GH-producing somatotrophs (Komourdjian et al., 1976). In Atlantic salmon, plasma GH levels increase during normal smolting (Björnsson et al., 1989, 1995; Prunet et al., 1989; Stefansson et al., 1991; McCormick et al., 1995), increase rapidly in response to artificially increased daylength in Atlantic salmon (Björnsson et al., 1989; McCormick et al., 1995, 2000, 2002), and are correlated with increased gill $Na^+,K^+$–ATPase and the development of salinity tolerance (McCormick et al., 1995, 2000). The present results are consistent with these previous findings, with plasma GH levels increasing

within one week of increased daylength, followed by increased gill $Na^+$,$K^+$–ATPase activity.

In previous studies, Atlantic salmon parr that are large enough to become smolts in the coming spring respond to increased daylength with elevated GH levels as early as October (Björnsson et al., 1995) and February (McCormick et al., 1995). The parr tested in the present study are not on track to smoltify during the coming spring, but during the following year. Shrimpton et al. (2000) observed that one-year old parr had very low levels of plasma GH in spring compared to one-year old smolts, and during the following spring the now two-year old parr developed into smolts with large spring increases in plasma GH. The present study extends these observations by determining that the lack of increased plasma GH in parr is due to a reduced photoperiodic response of parr. The differential GH-response to photoperiod in parr and smolt indicates that a developmental process takes place in Atlantic salmon in which the "light-growth hormone" axis matures or develops (i.e. is prepared to respond to increased daylength) in smolts prior to the final stages of smolting in spring. The mechanisms behind such activation are unknown, but could possibly involve the light-melatonin axis, which in birds and mammals is known to be able to modulate the regulation of GH secretion at the hypothalamic level (Zeman et al., 1999) and in salmonid fish can modulate GH secretion *in vitro* (Falcon et al., 2003). Parr and smolt could differ in their seasonal melatonin pattern, or, alternatively, the GH axis of smolts could be more sensitive than parr to photoperiod-controlled melatonin patterns.

Although there was no photoperiod-related increase in plasma GH in parr, GH levels increased significantly in both parr groups in late February and early March. This increase may be related to the increased temperature (1–10 °C) in early February. Increased temperature (8 vs 16 °C) increased plasma GH levels in rainbow trout (*Oncorhynchus mykiss*), independent of food intake (Gabillard et al., 2003), and higher levels of plasma GH are found in Atlantic salmon juveniles acclimated to 10 °C compared with fish acclimated to low ambient winter river temperatures (1–3 °C) (McCormick et al., 2000). These lower temperatures can also limit the ability of photoperiod to increase plasma GH and advance smolt development (McCormick et al., 2000).

The much-used term "GH-IGF-I axis" implies a functional relationship between these two hormones. Indeed, GH is a strong stimulator of hepatic IGF-I secretion in salmonid fish (Moriyama 1995; Moriyama et al., 1994), and during Atlantic salmon smoltification, significant positive correlation between GH and IGF-I levels has been noted (McCormick et al., 2000). Thus, it is notable that in the present study, there is a clear dissociation between the GH and IGF-I plasma profiles over time. This is in line with a growing number of studies which indicate that IGF-I levels in fish must be strongly influenced by other regulatory mechanisms than by circulating GH levels (cf. Reinecke et al., 2005). The most obvious dissociation is related to nutritional status and growth, which is positively correlated with IGF-I levels, but less, or negatively correlated with GH levels (Perez-Sanchez et al., 1994; Duan, 1998; Beckman et al., 2004). The photoperiod-induced increase in plasma GH levels in smolts in the present study was not accompanied by a similar increase in IGF-I levels. This dissociation of plasma GH and IGF-I levels is consistent with several other studies. Photoperiod-induced smoltification in yearling and underyearling Atlantic salmon includes a large increase in GH levels without parallel changes in IGF-I levels (McCormick et al., 2002; B.Th. Björnsson and T. Hansen, unpublished data). There are further physiological situations, such as during exposure to estrogenic compounds, where plasma GH and IGF-I levels in Atlantic salmon do not move in parallel (McCormick et al., 2005).

In addition to the differential effect of photoperiod on circulating GH and cortisol, parr and smolt differed in circulating IGF-I levels, which were substantially higher in smolts through most of the winter and early spring. These elevated levels of plasma IGF-I were apparently independent of photoperiod, as they occurred in early winter and were not advanced by artificially increased daylength in early February. As plasma GH did not differ in parr and smolt in early winter, one possible explanation is that GH receptors in the liver are more numerous in smolt than in parr. Fryer and Bern (1979) reported lower levels of tilapia GH binding in liver of coho salmon (*Oncorhynchus kisutch*) smolts compared with parr, though the date of sampling is not reported. Gray et al. (1992) found that liver GH receptors increased progressively in smolts from winter through spring. To our knowledge there is no comparison of GH receptors of salmon parr and smolt in winter or early spring. Pierce et al. (2005) have recently reported that insulin, cortisol and glucagon can all decrease the effectiveness of GH in stimulating the IGF-I release from the liver. Plasma cortisol did not differ between parr and smolt in winter and early spring, though it is possible that glucagon or insulin is elevated in parr. The elevated plasma IGF-I in smolts observed in this study is likely to play a role in smolt development in spring, so determining the source of these differences will be

important in understanding underlying mechanisms of smolt development.

There was a relatively strong correspondence between plasma cortisol and GH levels, especially in smolts. One week after initiation of LD16:8 in February, plasma GH increased dramatically in smolts, and this was followed two weeks later by increased plasma cortisol. Similarly, when plasma GH increased in LDN smolts in late March, plasma cortisol increased in early April. These results suggest that increased plasma GH may play a role in later increases in plasma cortisol. Young (1988) has shown that in coho salmon (*O. kisutch*), GH sensitizes the interrenal, making cortisol production more responsive to ACTH. It should be noted, however, that the increase in GH that occurred in parr in March, apparently unrelated to photoperiod, did not result in increased cortisol. Although it is possible that GH does not sensitize the interrenal in parr, a more likely explanation is that that ACTH is not increasing in parr in March, as cortisol remained low in parr throughout winter and spring. To our knowledge there are no studies examining changes in ACTH during the parr or smolt development.

Plasma cortisol did not differ between parr and smolt in winter. Cortisol levels remained low throughout the spring in parr, whereas cortisol increased with photoperiod in smolts. The low circulating cortisol levels throughout the spring in parr relative to smolt are consistent with earlier findings (Shrimpton and McCormick, 1998; Shrimpton et al., 2000). These authors also found that the number of receptors for cortisol increased in spring, but there was little difference between parr and smolt in receptor abundance or affinity. Consequently, the sensitivity of the gill to cortisol is not likely to differ between parr and smolt throughout the spring in the present study. The differences observed in plasma cortisol, therefore, may contribute to the photoperiod response found for smolts, and the lack of physiological smolt development in parr.

Two major patterns were observed in circulating thyroid hormones in the present study. Plasma $T_4$ and $T_3$ levels in parr remained stable throughout the spring and were always lower than those of smolts. Along with the higher levels of GH, IGF-I and cortisol, the higher levels of thyroid hormones in smolts confirm the view that smolts are 'pan hyper-endocrine' relative to parr (Hoar, 1988). Although plasma $T_4$ levels varied throughout winter and spring, there was no strong spring increase or 'thyroxine surge' such as that often reported for Pacific salmon. We have previously found that plasma $T_4$ increases dramatically (surge-like) in Atlantic salmon smolts coincident with downstream migration in both hatchery and wild fish (McCormick et al., 2003). The environmental and neuroendocrine regulation of this thyroxine surge is still uncertain, and thyroid stimulating hormone (TSH) has not been measured during salmonid smolting. Although the release of TSH in fish, like mammals, may be primarily under inhibitory control, Larsen et al. (1998) have shown that corticotrophin-releasing hormone (CRH) can cause the *in vitro* release of TSH from the pituitary of coho salmon, and that thyrotropin releasing hormone (TRH) is generally ineffective. Although both the thyroid and interrenal axes may both be regulated to some degree by CRH, the difference in cortisol and $T_4$ patterns in smolts makes it clear that other regulatory mechanisms are also involved.

The second major pattern in thyroid hormones seen in the present study is the approximately monthly increase and subsequent decrease in plasma $T_4$, a pattern which is not present in plasma $T_3$. The changes in plasma $T_4$ appear to be largely independent of photoperiod, and no strong seasonal change is observed. The monthly increases in plasma $T_4$ suggests a possible lunar rhythm, which have been observed for thyroid hormones in other smolting salmon (Grau et al., 1981). It was suggested that these rhythms may be important for synchronization of migration in salmon smolts that might be adaptive for prey acquisition or predator avoidance. Recently, DeVries et al. (2004) found that Pacific salmon smolts have a lunar rhythm in the timing of migration into estuaries. Unfortunately, these studies did not measure circulating thyroid hormones. During the present study, the new moon occurred on February 17, March 18, April 16 and May 16 and there is a rough correspondence between these dates and the highest levels of plasma $T_4$. As the present study was carried out in light-tight rooms, lunar-related light is not responsible for the observed cycle. It should be noted that we have not observed such a rhythm in previous studies on Atlantic salmon smolts under similar conditions (McCormick et al., 2000), though it is possible that the two to three week sampling regime was insufficient to detect such a pattern, and that finding this a pattern in the present study was fortuitous. Although there is circumstantial evidence for lunar rhythms of thyroid hormones in Atlantic salmon, the results to date have been variable (see Boeuf and Prunet, 1985) and there have been no systematic studies on lunar patterns in this species.

Our results indicate that there is a differential effect of photoperiod on the circulating levels of growth hormone and cortisol in parr and smolt. We also observed higher levels of plasma IGF-I in smolts in

early winter. These differences may be the critical developmental differences that distinguish parr and smolt. It will therefore be of interest to examine the neuroendocrine basis of parr–smolt differences in the 'light-endocrine' axes as a means of establishing the developmental events controlling smolt development.

## Acknowledgements

We thank Judy Carey for analysis of plasma cortisol, Barbro Egnér for analysis of plasma GH and IGF-I levels, and Mike O'Dea for analyzing gill Na,K–ATPase activity and preparation of figures. This study was funded by USGS and the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning.

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