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Increased daylength stimulates plasma growth hormone and gill Na^+, K^+-ATPase in Atlantic salmon (*Salmo salar*)

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Abstract Atlantic salmon juveniles reared at constant temperature (9–10 °C) were exposed to four photoperiod treatments and sampled every 2 weeks from January through May. Fish reared under normal photoperiod exhibited eight- and three fold increases in plasma growth hormone and gill Na^+, K^+-ATPase activity, respectively, between January and April. Fish exposed to abrupt increases in daylength (LD 15:9) in February or March responded with earlier increases in plasma growth hormone and gill Na^+, K^+-ATPase activity, and earlier decreases in condition factor relative to fish in the normal photoperiod group. Fish maintained under short daylength (LD 9:15) from January to May exhibited delayed and muted increases in plasma growth hormone and gill Na^+, K^+-ATPase activity. Plasma thyroxine exhibited a 2.5-fold increase from February to late March in the normal photoperiod group, was generally lower in the LD 9:15 group, but exhibited no obvious response to abrupt increases in daylength. There was an increase in plasma 3,5,3′-triiodo-L-thyronine with time in all groups (43–80%) but no significant response to photoperiod. Plasma levels of somatostatin-25 were highest in the LD 9:15 group, but there was no detectable response to increased daylength in any of the photoperiod treatments. The results indicate that plasma growth hormone is responsive to increased daylength and may be causally related to subsequent increases in gill Na^+, K^+-ATPase.

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Abbreviations *ANOVA* two-way analysis of variance · *BCA* bicinchoninic acid · *BSA* Bovine serum albumin · *EDTA* ethylene diamine tetraacetic acid · *ELISA* enzyme-linked immunosorbent assay · *EST* eastern standard time · *GH* growth hormone · *GLU* Glucagen · *IgG* Immunoglobulin G · *INS* Insulin · *LDN* simulated natural photoperiod · *RIA* radio immuno assay · *SEI* Sucrose EDTA imidazole · *SS*-25 somatostatin-25 · *SW* sea water · T_3 3,5,3′triiodo-L-thyronine · T_4 thyroxine

Introduction

Many seasonal cycles of animal behavior and physiology can be greatly influenced by changes in daylength (Farner 1985). Reproductive cycles, including associated migratory behaviors, have been most widely examined in this regard. However, not all vertebrate migrations are for the immediate purpose of reproduction. Juvenile anadromous fishes such as Atlantic salmon undergo seasonal changes in migratory behavior and physiology that result in movement from streams to oceans. In this developmental phenomenon, known as the parr-smolt transformation or smolting, only juveniles of the appropriate size-related developmental stage undergo the physiological and behavioral changes that are adaptive for downstream migration and an oceanic existence (McCormick and Saunders 1987; Hoar 1988).

The development of salinity tolerance is an important part of the preparatory adaptations that comprise the parr-smolt transformation. Biochemical and physiological changes in all osmoregulatory

organs (gill, gut, and kidney) occur during smolting, although the gill has been most widely studied in this regard (McCormick and Saunders 1987). Increased numbers of gill chloride cells and Na^+, K^+-ATPase activity accompany and are causally related to increased salinity tolerance during smolting. Increased gill Na^+, K^+-ATPase activity is also associated with migratory behavior during smolting (Zaugg et al. 1985; Ewing et al. 1994; McCormick and Björnsson 1994), though the precise temporal relationship between the two has not been established, and they are unlikely to have identical mechanisms of control.

Several hormones have been implicated in regulating increased gill Na^+, K^+-ATPase activity, chloride cell development and salinity tolerance during the parr-smolt transformation. Exogenous GH and cortisol can increase all of these parameters in several salmonid species (McCormick 1994), and both of these hormones increase during smolting (Young et al. 1989; Björnsson et al. 1989b). Numerous studies have found that thyroid hormones increase during smolting (Dickhoff and Sullivan 1987), and recent evidence suggests that these are involved in mediating the action of GH in SW acclimation (Leloup and Lebel 1993). Although SS-25 has not been implicated in osmoregulatory physiology of salmonids, it does play a role in lipid mobilization which is substantially increased during the parr-smolt transformation (Eilertson and Sheridan 1993).

Previous studies have strongly implicated increased daylength as a major environmental factor in regulating the parr-smolt transformation (Saunders and Henderson 1970; Komourdjian et al. 1976; Clarke et al. 1978; Duston and Saunders 1990). Information on the photoperiodic regulation of hormones in fish is limited (McCormick et al. 1987; Bjornsson et al. 1989a, 1994), and the present study was undertaken to examine the effect of increased daylength on endocrine factors involved in the regulation of the parr-smolt transformation. Specifically, we examined whether plasma GH, thyroid hormones and SS-25 change in concert with physiological changes whose timing has been altered by increased daylength or by the absence of increased daylength.

Materials and methods

Juvenile Atlantic salmon (*Salmo salar*) were obtained from the White River National Fish Hatchery (Bethel, Vt., USA) and brought to the Anadromous Fish Research Center (Turners Falls, Mass., USA). They were randomly divided into four photoperiod rooms each with two 1-m diameter tanks. Each tank contained 80–85 fish. Water was maintained at a constant temperature of 9–10 °C and a flow rate of 4 l·min^{-1}. The fish were fed to satiation twice daily (Zeigler Bros., Gardners, Pa., USA). The control group was exposed to LDN (Fig. 1). Two groups initially kept on LDN were then subjected to sudden increases in daylength (LD 15:9) on February 11 and March 11. The fourth group was kept on a short daylength (LD 9:15) from December 30. Lighting was supplied by overhead fluorescent lights (500 lx at the water surface) and LDN was adjusted twice a week.

Feed was withheld for 24 h prior to sampling which occurred from 1000 to 1100 hours EST. Blood and gill samples were taken every 2 weeks from January 21 through May 11 ($n = 10$ per treatment). Fish were anesthetized (100 mg·l^{-1} MS-222 neutralized to pH 7.0 with 2 *N* NaOH) and fork length to the nearest millimeter 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 ice-cold SEI buffer (150 mmol·l^{-1} sucrose, 10 mmol·l^{-1} EDTA, 50 mmol·l^{-1} imidazole, pH 7.3) and frozen at −80 °C within 30 min.

Na^+, K^+-ATPase activity was determined using the microassay method of McCormick (1993). The gill tissue was homogenized in 125 µl SEID (SEI buffer and 0.1% deoxycholic acid) and centrifuged at 5000 g for 30 s. Two pairs of duplicate 10-µl samples of the supernatant were run in the presence and absence of 0.5 mmol·l^{-1} ouabain in 96-well microplates at 25 °C and read at a wavelength of 340 nm for 10 min. The ouabain-sensitive Na^+, K^+-ATPase activity is expressed as µmoles ADP·mg protein^{-1}·h^{-1}. Protein concentration was determined using a BCA Protein Assay (Pierce, Rockford, Ill., USA). Both assays are run on a THERMOmax microplate reader using SOFTmax software (Molecular Devices, Menlo Park, Calif., USA).

Thyroxine (T_4) and 3,5,3'-triiodo-L-thyronine (T_3) concentrations were measured by a direct radioimmunoassay (Dickoff et al. 1978) modified by McCormick and Naiman (1984). L-[^{125}I]-Thyroxine (1250 µCi·µg^{-1}), and L-3,5,3'- [^{125}I] -triiodothyronine (1200 µCi·µg^{-1}) were purchased from DuPont NEN Research Products (Boston, Mass., USA). T_4 and T_3 antisera were purchased from Endocrine Sciences Products (Calabasas Hills, Calif., USA). A stock solution of T_4 or T_3 was made in ethyl alcohol and standards were diluted into a Ringers' solution with 0.05% BSA. Using a 5 µl standard and plasma sample size, the detection limit was as ng·ml^{-1}. Intra- and interassay coefficients of variation for these assays were 4.3–11% and 3.2–5%, respectively.

Plasma GH levels were measured in duplicate 50-µl samples using a specific double-antibody salmon GH radioimmunoassay developed by Bolton et al. (1986) and modified by Björnsson et al. (1994). Recombinant chum salmon GH (Kyowa Hakko Kogyo, Tokyo) was used for iodination and assay standards. The primary antibody (HU-85) was raised in rabbit against chum salmon GH, and the secondary antibody used was goat anti-rabbit IgG (R-0881; Sigma, Mo., USA). The ED_{50} of the RIA was 2.22 ng·ml^{-1} and intra- and interassay coefficients of variation were 5 and 4%, respectively.

SS-25 was measured by a novel ELISA adopted from the non-competitive procedure outlined by Engvall (1980). ELISAs were performed using coho salmon SS-25 as standard and antiserum against coho salmon SS-25. Plates were read at 450 nm using a BioRad (Model 3550) Microplate Reader (Richmond, Calif., USA). Antiserum specificity under ELISA conditions was previously examined by evaluating cross-reactivity with several peptides. The antiserum does not significantly cross-react with mammalian (m) SS-28, mINS, or mGLU; however, there is a 10% cross-reactivity with mSS-14. The lowest detectable level was 20 pg per well using 75 µl standard or sample volume. Inter- and intra-assay coefficients of variation were 12 and 8%, respectively.

Plasma glucose was measured by the *o*-toluidine colorimetric method (Hüvarinen and Nikkila 1962).

Two-way analysis of variance (ANOVA) was used to determine the significance of photoperiod treatment and changes over time. If photoperiod treatment was significant, one-way ANOVA was used to test differences at a single time point and Dunnett's test used to compare artificial photoperiods to the LDN group. Similarly, if two-way ANOVA determined significant changes over time, one-way ANOVA was used to determine the significance of changes over time in each photoperiod treatment. Results of statistical analysis are primarily presented in figure legends.

Results

Condition factor is a relative ratio of weight to length which normally decreases during the parr-smolt transformation. In the present study, condition factor of fish under LDN was constant from January to mid-March and then continually decreased to the end of the study (mid-May; Fig. 1). Within 3 weeks of increased daylength in the LD 15:9 (FEB) group condition factor began to decrease and was significantly lower than the LDN group in March and April. Condition factor of the LD 15:9 (MAR) group also began to decline within 3 weeks of increased daylength and was consistently lower than the LDN group though not significantly different. During the period when condition factor was declining in the LDN group, fish under constant short daylength (LD 9:15) had significantly higher condition factor. Although there was no net change in condition factor in this group over the course of the experiment, there was a rise in late March followed by a decrease from April to mid-May.

Gill Na^+, K^+-ATPase activity remained constant (2 μmol ADP · mg protein^{-1} · h^{-1}) in all groups from January to early March (Fig. 2). A three fold increase occurred in the LDN group in the next 6 weeks. This increase was more abrupt and peaked at least 2 weeks earlier in the LD 15:9 (FEB) group. The LD 15:9 (MAR) group had a similar pattern of increase but was not significantly different from the LDN group. Gill Na^+, K^+-ATPase activity in the LD 9:15 group began increasing at the same time as the LDN group but increased at a slower rate and exhibited only a two fold increase by the end of the experiment (mid-May).

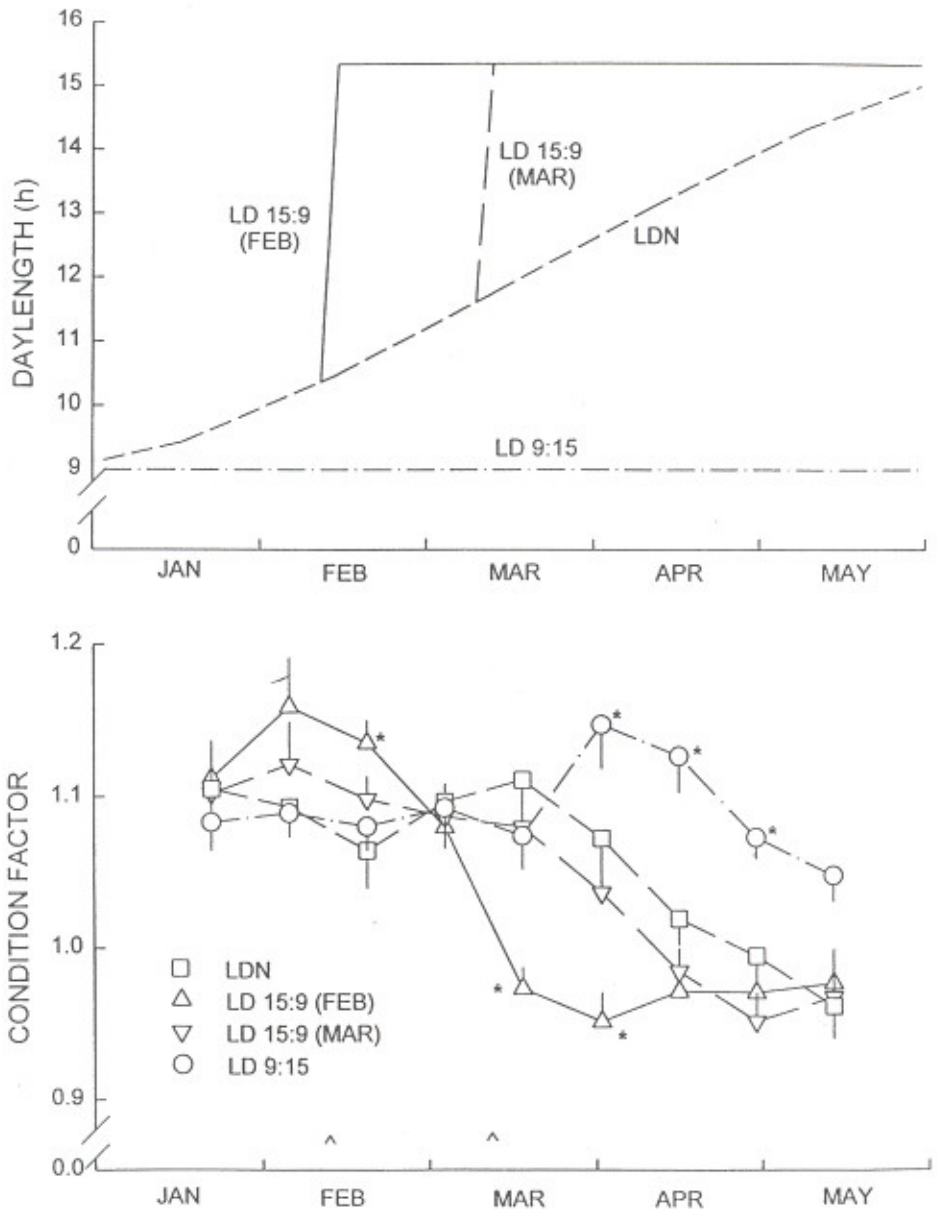

Fig. 1 *Upper*: change in daylength in four photoperiod treatments. For LD 15:9 (*FEB*) and (*MAR*) groups abrupt increases in daylength occurred on February 11 and March 11 (*arrowheads*). Prior to January fish were reared on ambient (seasonal) water temperatures and natural photoperiod; during photoperiod treatments fish were maintained at 9–10 °C. *Lower*: condition factor $\left(\frac{\text{weight}}{(\text{length})^3} \cdot 100\right)$ in juvenile Atlantic salmon subjected to photoperiod treatments. Values are mean ± standard error ($n = 10$). *Arrowheads* indicate time of increased daylength in LD 15:9 (*FEB*) and (*MAR*) groups. *Asterisk* indicates a significant difference from the LDN group sampled at the same time (one-way ANOVA followed by Newman-Keuls test). There was a significant effect of time ($P < 0.0001$) and photoperiod treatment ($P = 0.001$) and a significant interaction ($P < 0.0001$). There was a significant change in condition factor with time in all photoperiod treatments ($P < 0.002$)

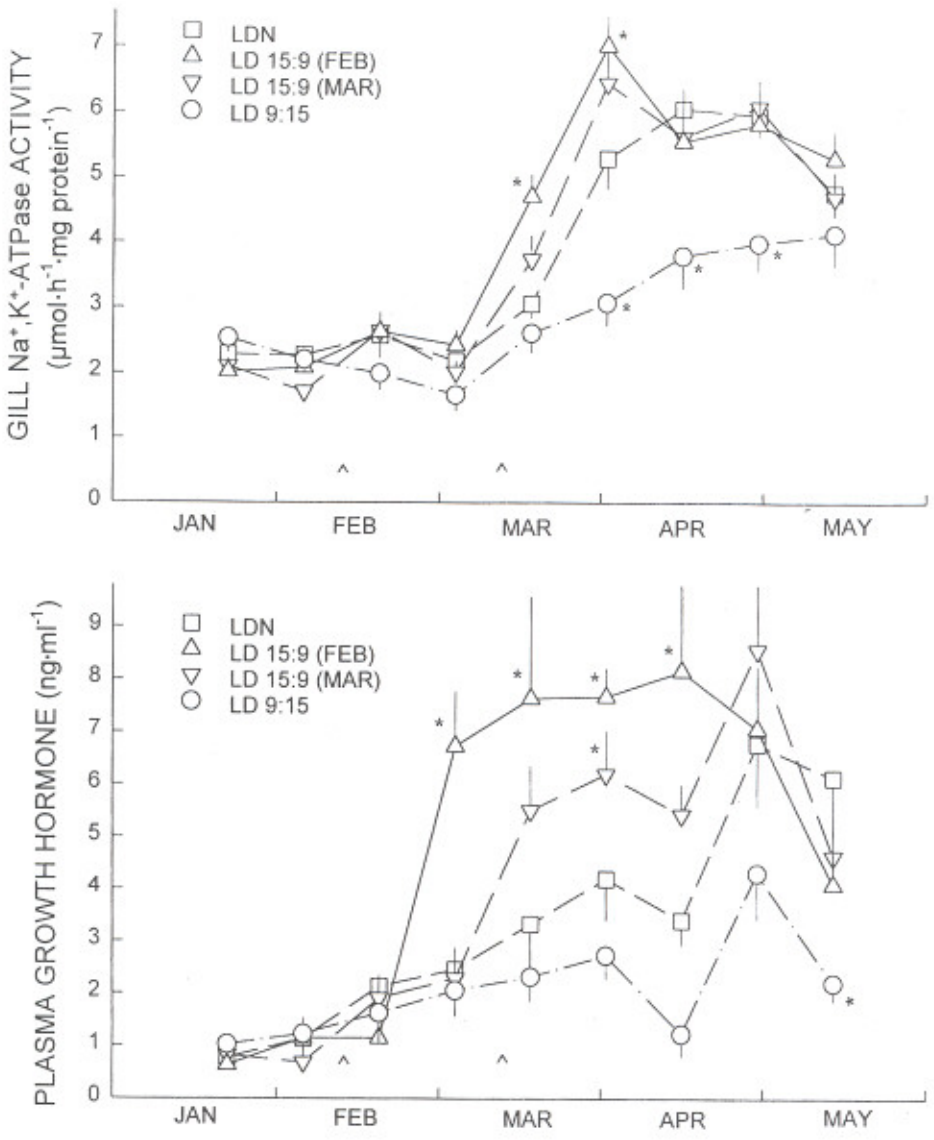

Fig. 2 Gill Na^+, K^+-ATPase activity (*upper*) and plasma growth hormone (*lower*) in juvenile Atlantic salmon subjected to photoperiod treatments (see Fig. 1). Values are mean ± standard error ($n = 10$). *Arrowheads* indicate time to increased daylength in LD 15:9 (*FEB*) and (*MAR*) groups. *Asterisk* indicates a significant difference from the LDN group sampled at the same time (one-way ANOVA followed by Newman-Keuls test). For gill Na^+, K^+-ATPase activity there was a significant effect of time ($P < 0.0001$) and photoperiod treatment ($P < 0.0001$) and a significant interaction ($P < 0.0001$). There was a significant change in gill Na^+, K^+-ATPase activity with time in all photoperiod treatments ($P < 0.0001$). For plasma GH there was a significant effect of time ($P < 0.0001$) and photoperiod treatment ($P = 0.003$) and no interaction ($P = 0.3$). There was a significant change in plasma GH with time in all photoperiod treatments ($P < 0.0001$)

Plasma GH in the LDN group increased continually from January (0.8 ng · ml^{-1}) to late April (6.8 ng · ml^{-1}), an eight fold increase (Fig. 2). One week following an abrupt increase in daylength in the LD 15:9 (FEB) group there was no change in plasma GH (1.1 ng · ml^{-1}), but 3 weeks later there was a dramatic increase to 6.7 ng · ml^{-1} which remained elevated for 8 weeks. One week after the abrupt increase in daylength in the LD 15:9 (MAR) group there was a three fold increase in plasma GH which also remained elevated. As in the LDN group, plasma GH in the LD 9:15 group increased steadily from January to late April but did not increase to the same extent (a four fold increase over this period). Values remained lower than the LDN group from March through May, but were significantly lower only in mid-May.

Plasma T_4 exhibited a 2.5-fold increase from February (4.3 ng · ml^{-1}) to late March (10.9 ng · ml^{-1}) in the LDN group (Fig. 3). Plasma T_4 also significantly increased in both the LD 15:9 (FEB) and (MAR) groups but there was no clear relationship between the time of abrupt increases in daylength and increased hormone levels. In the LD 9:15 group plasma T_4 exhibited no significant change over time and was significantly lower than the LDN group during the peak of plasma T_4.

Plasma T_3 in the LDN group increased steadily from January (2.5 ng · ml^{-1}) to early April (4.5 ng · ml^{-1}) then fell slightly thereafter (Fig. 3). Plasma T_3 significantly increased with time in all groups (43–80%) but there was no significant difference among the photoperiod treatments.

Plasma levels of SS-25 in the LDN group remained relatively constant at 0.8 ng · ml^{-1} from January to mid-March; in early April the levels increased to approximately 1.2 ng · ml^{-1} where they remained until the end of the study (Fig. 4). A similar pattern with increases in early April occurred in the LD 15:9 (FEB) and (MAR) groups. Plasma SS-25 was low in the LD 9:15 group in January (0.9 ng · ml^{-1}), increased to 1.4 ng · ml^{-1} in mid-February and remained elevated.

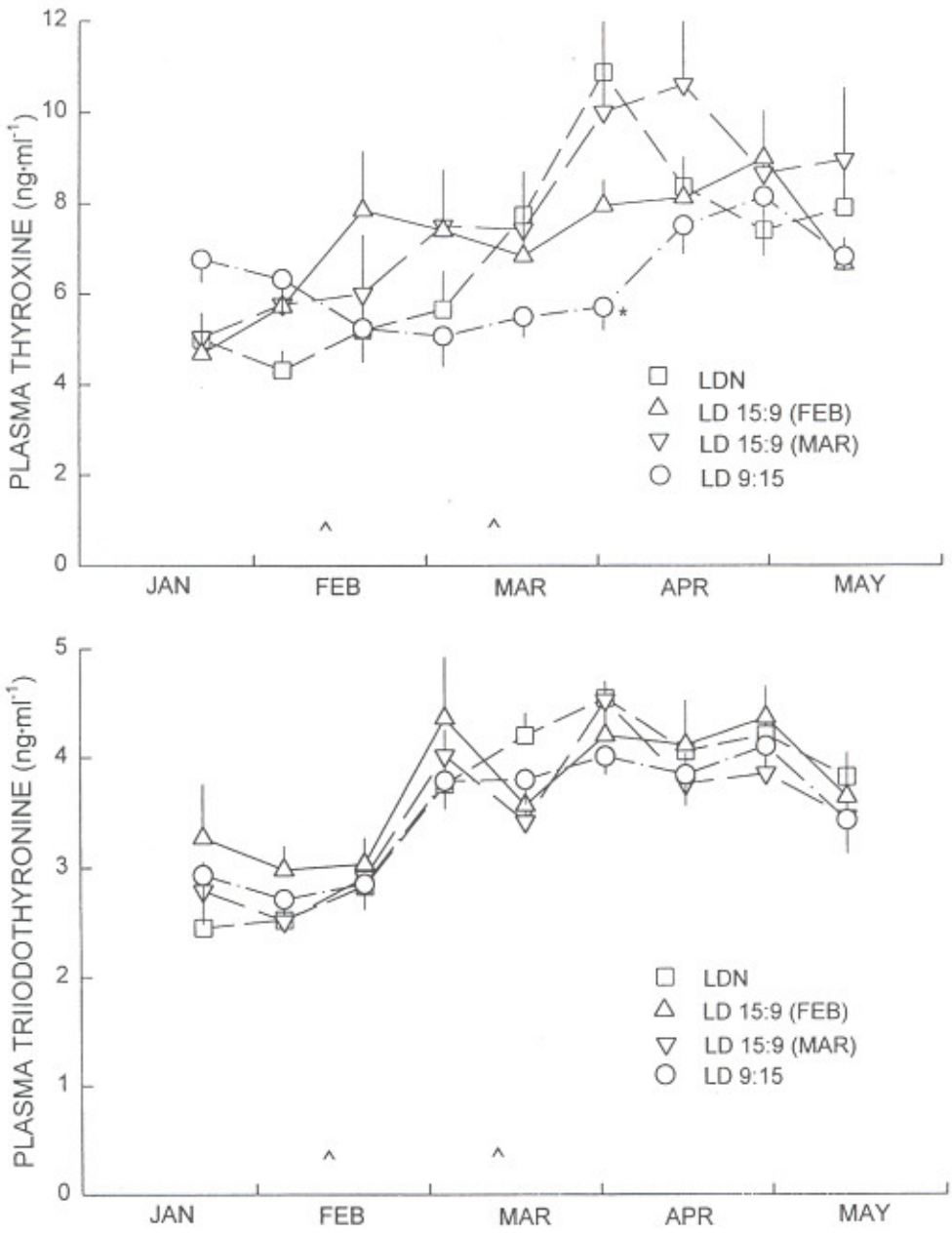

Fig. 3 Plasma thyroxine (T_4; *upper*) and plasma 3, 5, 3'-triiodo-L-thyronine (T_3; *lower*) in juvenile Atlantic salmon subjected to photoperiod treatments (see Fig. 1). Values are mean ± standard error ($n = 10$). *Arrowheads* indicate time of increased daylength in LD 15:9 (*FEB*) and (*MAR*) groups. *Asterisk* indicates a significant difference from the LDN group sampled at the same time (one-way ANOVA followed by Newman-Keuls test). For plasma T_4 there was a significant effect of time ($P < 0.0001$) and photoperiod treatment ($P = 0.016$) and a significant interaction ($P = 0.045$). There was a significant change in plasma T_4 with time in all photoperiod treatments ($P < 0.05$). For plasma T_3 there was a significant effect of time ($P < 0.0001$) but no effect of photoperiod treatment ($P = 0.2$) and no interaction ($P = 0.9$) There was a significant change in plasma T_3 with time in all photoperiod treatments ($P < 0.05$)

Changes over time in this group, however, were not statistically significant.

Plasma glucose was 3.5–4.5 mmol·l^{-1} in all groups in mid-January, and rose steadily throughout the experiment to 5.5–7.0 mmol·l^{-1} by mid-May (Fig. 4). Although there were significant differences among the groups, there was no pattern of change that would indicate a response to abrupt increases in daylength or short days.

There was a strong correlation between plasma GH and condition factor ($r^2 = 0.66$) which was increased if GH was correlated with condition factor of the subsequent sampling period (2 weeks later, $r^2 = 0.78$, Fig. 5). Plasma GH and gill Na^+, K^+-ATPase activity were also strongly correlated ($r^2 = 0.65$), and this correlation was also increased if a 2-week lag was included ($r^2 = 0.72$, Fig. 5). Correlations of condition factor and gill Na^+, K^+-ATPase activity with other endocrine changes were weaker ($r^2 = 0.18$–0.58).

Discussion

Gill Na^+, K^+-ATPase activity is strongly correlated with the development of salinity tolerance of Atlantic salmon in spring and has been widely used as an indicator of smolt development. In the present study gill Na^+, K^+-ATPase activity under normal photoperiod remained constant from January to February,

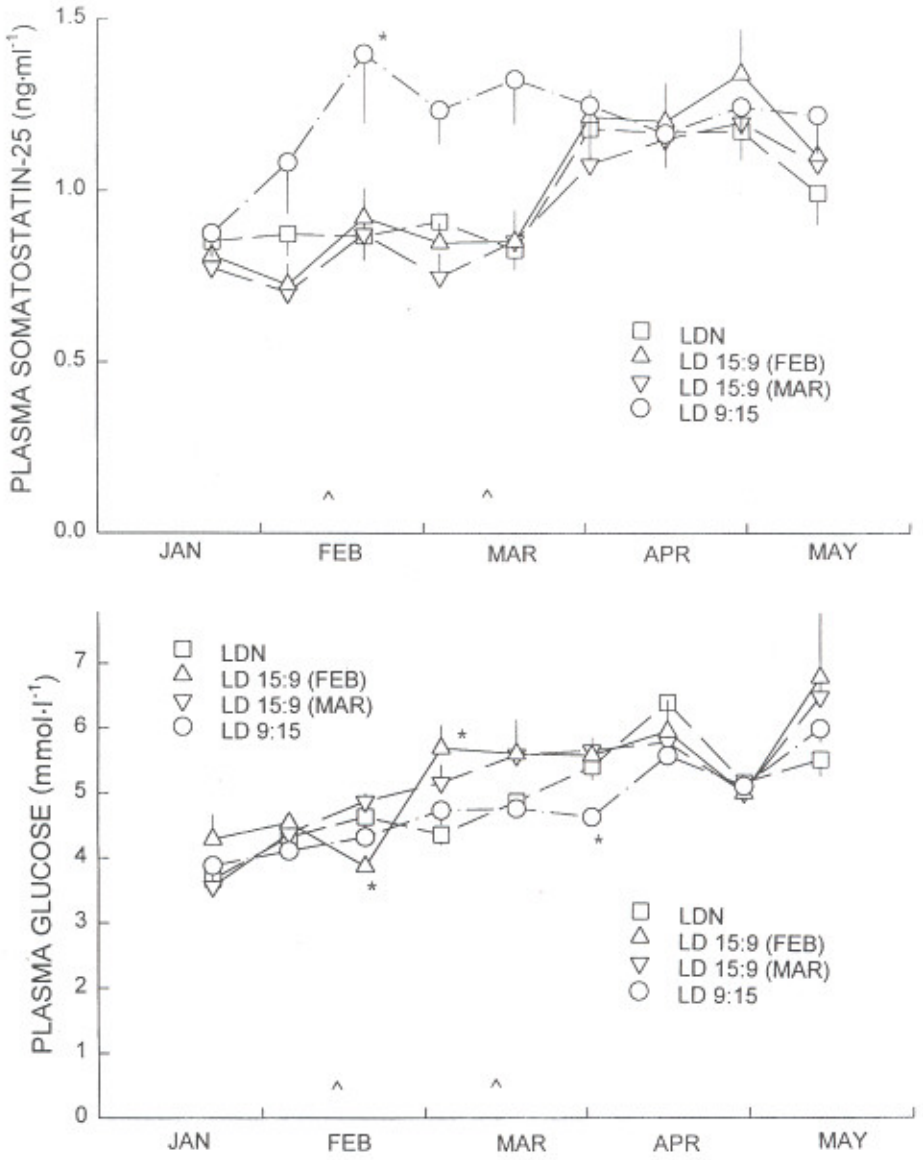

Fig. 4 Plasma somatostatin-25 (*upper*) and plasma glucose (*lower*) in juvenile Atlantic salmon subjected to photoperiod treatments (see Fig. 1). Values are mean ± standard error ($n = 10$). *Arrowheads* indicate time of increased daylength in LD 15:9 (*FEB*) and (*MAR*) groups. *Asterisk* indicates a significant difference from the LDN group sampled at the same time (one-way ANOVA followed by Newman-Keuls test). For plasma somatostatin-25 there was a significant effect of time ($P < 0.0001$) and photoperiod treatment ($P = 0.0001$) and no significant interaction ($P = 0.2$). There was a significant change in plasma somatostatin-25 with time in all photoperiod treatments ($P < 0.0005$) except the LD 9:15 group. For plasma glucose there was a significant effect of time ($P < 0.0001$) and photoperiod treatment ($P = 0.0005$) and no interaction ($P = 0.07$). There was a significant change in plasma glucose with time in all photoperiod treatments ($P < 0.01$)

then increased three fold in the next 6 weeks. Abrupt exposure to 16 h daylength in February resulted in earlier and more rapid increases in gill Na^+, K^+-ATPase activity and condition factor relative to controls. It should be noted, however, that there was no response for 3 weeks following the onset of increased daylength in the 15:9 FEB group, nor was there a significant difference between the two advanced photoperiod groups. These results suggest a limitation in the ability of photoperiod to advance increases in gill Na^+, K^+-ATPase activity. Other researchers have found that a minimum period of 6–8 weeks on short daylength is necessary for a subsequent response of salmonids to long days (Thorarensen and Clarke 1989; Clarke et al. 1989; Björnsson et al. 1989a; Okumoto et al. 1989). This would not appear to be a limitation of a photoperiod effect in the present study as the fish experienced short days (less than 10 h light) for more than 8 weeks. Using an experimental design similar to that of the present study, Duston and Saunders (1990) report an 8-week advance in maximum salinity tolerance of Atlantic salmon when abrupt increases in daylength occurred in early January and February, compared to the 2- to 4-week advance in gill Na^+, K^+-ATPase activity in the present study. This greater advance may relate in part to the later onset of maximum salinity tolerance under normal photoperiod (mid-May) relative to the peak in gill Na^+, K^+-ATPase activity (mid-April) in our study. As in the present study, several authors have found that physiological response to photoperiod does not occur in the first 2–3 weeks following increased daylength (Thorarensen and Clarke 1989; Okumoto et al. 1989; Duston and Saunders 1990). A possible explanation for this time course of response relates to the endocrine mechanism controlling these physiological changes and their response to photoperiod.

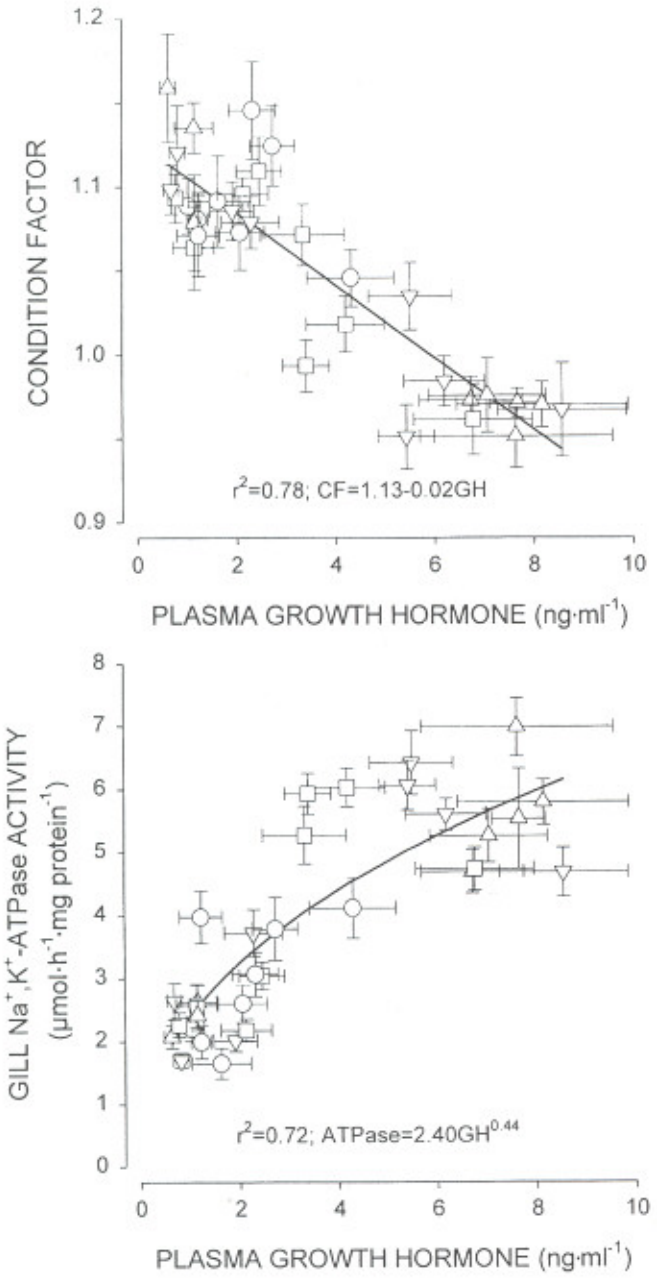

Fig. 5 Correlation of plasma growth hormone with condition factor (*upper*) and gill Na^+, K^+-ATPase activity (*lower*) in juvenile Atlantic salmon subjected to photoperiod treatments (see Fig. 4 for symbol definitions). Values for condition factor and gill Na^+, K^+-ATPase activity are 2 weeks later than the corresponding growth hormone values. Data are expressed as mean ± standard error (n = 10)

Plasma GH showed the most direct response to increased daylength. Whereas plasma GH rose steadily throughout the study in the normal photoperiod group, abrupt increases in daylength resulted in large increases in plasma GH within 1–3 weeks which remained elevated throughout the study. Although previous studies have implicated photoperiod in seasonal increases in plasma GH in smolting salmonids (Björnsson et al. 1989a; Okumoto et al. 1989), such rapid, large and sustained increases in response to increased daylength have not previously been reported for any teleost. Although each of the LD 15:9 groups responded with similar magnitude of increases in plasma GH, interestingly the timing of onset of increases differed. In the LD 15:9 FEB group there was no increase 1 week after increased daylength, whereas in the LD 15:9 MAR group there was a significant response within 1 week. This result suggests a greater sensitivity of the 'light-hypothalamo-pituitary' complex to increased daylength in March compared to February. Such an increase in sensitivity may itself be cued by prior increases in daylength. It would be interesting to see whether GH secretion by isolated pituitaries of juvenile smolting salmonids changes seasonally and how the response to hypothalamic agents implicated in regulating GH differs developmentally.

Several lines of evidence have implicated GH in preparatory adaptations to SW (smolting) and the process of SW acclimation [review: Sakamoto et al. (1993)]. Komourdjian et al. (1976) found that the histological 'activity' of GH-secreting cells increased in Atlantic salmon smolts following increased daylength and were contemporaneous with increased salinity tolerance. Subsequent investigations have found that altered photoperiod can result in increased circulating levels of GH in several smolting salmonids (cited above). Several studies have also shown that exogenous GH can increase gill Na^+, K^+-ATPase activity, chloride cell numbers and salinity tolerance (Sakamoto et al. 1993). To date, no direct action of GH on gill tissue has been demonstrated (McCormick et al. 1991a), although GH receptors are present in gill tissue (Aguenaou et al. 1989).

It has recently been shown that GH can increase gill cortisol receptors (Shrimpton et al. 1995) in coho salmon; cortisol has been shown to increase gill Na^+, K^+-ATPase activity and chloride cell numbers in vivo and in vitro (Bisbal and Specker 1991; Madsen, 1990; McCormick and Bern 1989; McCormick 1990). GH may also act by increasing circulating and local production of IGF-I (Sakamoto and Hirano 1993; Duan et al. 1993) which has been shown to increase salinity tolerance in vivo (McCormick et al. 1991b) and gill Na^+, K^+-ATPase activity in vitro (Madsen and Bern 1993). Exogenous GH can have effects on salinity tolerance within 48 h (Bolton et al. 1987; McCormick et al. 1991b), whereas effects on gill Na^+, K^+-ATPase activity generally require a week or more of treatment (Sakamoto et al. 1993). This time-course of action of GH on gill Na^+, K^+-ATPase activity is consistent with the present study in which increases in GH after abrupt increases in daylength were followed 1–3 weeks later by increased gill Na^+, K^+-ATPase activity.

Exposure of Atlantic salmon to short days (LD 9:15) from January onward resulted in damped and delayed increases in plasma GH and gill Na^+, K^+-ATPase activity (Fig. 2). As no 'signal' for increased daylength or seasonal temperature change occurred in this group, an endogenous cycle of endocrine activity and smolt physiology may exist in Atlantic salmon. Establishment of such an endogenous cycle will require long-term experiments to follow smolt-related changes over more than one annual cycle. Such experiments have been carried out on smolt-related growth dynamics: Eriksson and Lundqvist (1982) found that growth rate, condition factor and smolt appearance under constant conditions (LD 12:12, 11 °C) conformed to an 'annual' cycle with a period of 10 months.

Decreased condition factor during smolting and the marked influence of photoperiod on this parameter are consistent with previous studies on several salmonids (McCormick and Saunders, 1987; Hoar 1988). Changes in condition factor during smolting are probably related to changes in lipid and carbohydrate metabolism, specifically increased lipolysis accompanied by decreased lipogenesis and increased glycogenolysis accompanied by decreased glycogen synthesis (Sheridan 1989). Hormones such as GH, T_4, cortisol and prolactin modulate much of the changes in lipid metabolism that occur during smolting (Sheridan 1986); the effect of GH on lipolysis has been shown to result from direct effects on target cells (O'Connor et al. 1993). The strong correlation between GH and condition factor observed in the present study suggests that photoperiod-induced alterations in GH may underlie the changes in condition factor.

A 'peak' in plasma thyroid hormones in spring is typical of most smolting salmonids (Dickhoff and Sullivan 1987). Although plasma T_4 and T_3 increased significantly in the LDN groups, there was no clear response to increased daylength. Several other studies have shown a lack of correspondence between manipulations in the timing of smolting and changes in thyroid hormones. Exposure of Atlantic salmon to continuous light from first feeding prevented normal spring increases in gill Na^+, K^+-ATPase activity and salinity tolerance but did not prevent three fold increases in plasma T_4 (McCormick et al. 1987). Photoperiod manipulations which altered the proportion of masu salmon that became smolts as well as the timing of smolting also failed to alter plasma levels of T_4 and T_3 (Okumoto et al. 1989). These results indicate that circulating thyroid hormones are not responsive to photoperiod during the parr-smolt transformation. That is not to say, however, that thyroid hormones have no role in smolting. Studies of the role of thyroid hormones in teleosts suggest that an intact thyroid is necessary for normal growth and development (Leatherland 1982), although the mechanism (s) of action for thyroid hormones are unclear. Of even greater relevance to smolting are recent findings that inhibition of T_4 to T_3 conversion by iopanoic acid decreases the salinity tolerance of brown trout *Salmo trutta* (Lebel and Leloup 1992). Similar treatment also blocks the ability of exogenous GH to increase salinity tolerance (Leloup and Lebel 1993), indicating that an intact thyroid system may also be necessary for photoperiodic stimulation of smolting.

SS-25 is an N-terminally extended form of SS uniquely found within the salmonid pancreas and purportedly derived from a separate gene from that which encodes the invariant SS-14 common to all vertebrates studied thus far (Plisetskaya et al. 1986). Although SS-25 was higher in the LD 9:15 group compared to other groups early in the study, there was no obvious response to increased daylength. Levels of SS-25 were generally higher in smolts than pre-smolts (in January), consistent with previous observations in chinook salmon (Cowley et al. 1994). Such seasonal alterations in SS-25 may be involved in metabolic changes that occur during smolting. SS-25 may have indirect effects on nutrient flux by inhibiting insulin release and direct actions through stimulation of lipolysis and glycogenolysis in target tissues (Eilertson et al. 1991; Eilertson and Sheridan 1993). Although no strong correlation between SS-25 and condition factor ($r^2 = 0.18$) was observed, SS-25 may be related to observed increases in plasma glucose and other metabolic changes that occur during smolting.

This and previous studies support a role for photoperiod in controlling the timing of the parr-smolt transformation. Photoperiod manipulations of smolting will be useful in aquaculture, enhancement or restoration programs under circumstances when early SW releases of salmon are beneficial. The present results strongly implicate GH in the regulation of smolting under normal conditions and when increased daylength is used to alter the timing of smolting. Since smolting is regulated by a number of endocrine systems, further investigation is necessary to determine the importance of interaction of GH with other hormones. The mechanism by which photoperiod affects GH (and other hormones) is also unclear. Although the pineal has been implicated in regulating seasonal and photoperiodically controlled rhythms in teleosts, there is little information on the possible direct or indirect regulation of GH by the pineal (Gern and Karn 1983). The parr-smolt transformation is an interesting and accessible system for further examination of these questions.

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