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Effects of an advanced temperature cycle on smolt development and endocrinology indicate that temperature is not a zeitgeber for smolting in Atlantic salmon

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Summary

Atlantic salmon (*Salmo salar*) juveniles were reared under simulated conditions of normal photoperiod (LDN) or short days (LD 9:15) and ambient temperature (AMB: normal temperature increases in April) or an advanced temperature cycle (ADV: temperature increases in February). Under both photoperiod conditions, the timing of increased and peak levels of gill Na^+,K^+-ATPase activity were not altered by temperature, although the rate of increase was initially greater under ADV. ADV/LD 9:15 resulted in peak gill Na^+,K^+-ATPase activity that was half of that seen under normal photoperiod and temperature conditions. Plasma growth hormone (GH) levels increased threefold in late March under ADV/LDN, but not under ADV/LD 9:15, indicating that there is a photoperiod-dependent effect of temperature on levels of this hormone. Plasma insulin-like growth factor I (IGF-I) increased in spring in all groups, with increases occurring significantly earlier in the ADV/LDN group. In each photoperiod condition, the advanced temperature cycle resulted in large decreases in plasma thyroxine (T_4) levels in March, which subsequently recovered, whereas plasma 3,5,3′-triiodo-L-thyronine (T_3) levels were not substantially affected by either photoperiod or temperature. There was no consistent pattern of change in plasma cortisol levels. The results do not provide support for the role of temperature as a zeitgeber, but do indicate that temperature has a role in the timing of smolting by affecting the rate of development and interacting with the photoperiod.

Key words: temperature, photoperiod, smolting, Atlantic salmon, *Salmo salar*, zeitgeber, gill Na^+,K^+-ATPase, growth hormone, insulin-like growth factor I, thyroid hormone.

Introduction

As part of their anadromous life history, juvenile salmon abandon the freshwater habitat and migrate downstream as smolts. The preparatory adaptations for seawater entry that constitute the parr–smolt transformation normally occur in spring, coincident with increased daylength and temperature. Advanced photoperiod regimes can advance the timing of the physiological changes that occur during smolting, and it is generally accepted that photoperiod is the dominant environmental cue for this process (Hoar, 1988; Duston and Saunders, 1990). The role of temperature in controlling smolt development is more enigmatic (McCormick et al., 1996). Studies using differing constant temperatures during smolt rearing have found that increased temperature results in earlier smolt development in Atlantic salmon and steelhead trout (although at temperatures of 15°C and higher, smolt development of the latter is inhibited) (Adams et al., 1973; Johnston and Saunders, 1981; Solbakken et al., 1994). More recent work with Atlantic salmon confirms this effect of temperature and indicates a strong relationship between the cumulative temperature experience of fish (degree days) and smolt development (Sigholt et al., 1998), indicating that temperature is acting to control the rate of development. Staurnes et al. (1994) found that Atlantic salmon reared under a constant long-day photoperiod and seasonal spring increases in temperature underwent normal smolt development, and suggested that '*an increase in water temperature may induce development of preparatory seawater tolerance*'. These results indicate that temperature may act primarily by controlling the rate of smolt development, or may have a more direct role by acting as a zeitgeber (acting to cue circannual rhythms), independent of photoperiod.

The physiological changes that comprise smolting are under

the control of the neuroendocrine system. Growth hormone (GH) and cortisol interact to increase salinity tolerance and the underlying physiological changes such as gill Na^+,K^+-ATPase activity (McCormick, 1995). Thyroid hormones may interact with these hormones to control osmoregulatory changes, but have a more direct role in morphological changes (such as silvering) and the development of behavioral changes that occur during smolting (Hutchison and Iwata, 1998). Although levels of cortisol and thyroid hormones can be influenced by photoperiod, growth hormone is the most responsive to changes in daylength and is strongly correlated with the capacity of photoperiod to alter the timing of smolting (Bjornsson, 1997). The effect of temperature on endocrine changes during smolting has received less attention. Recent work indicates that low temperature can limit the capacity of photoperiod to advance smolting and that this is controlled by changes in circulating hormones. However, it is unclear whether other aspects of the impact of temperature on smolting, particularly its potential as a zeitgeber, are subject to endocrine control.

The present study was undertaken to determine the impact of temperature on smolt development and the underlying endocrine control mechanisms. A particular aim was to determine whether temperature alone could act as a zeitgeber for smolting. Thus, in addition to keeping fish on a normal photoperiod cycle, fish were also kept on a short-day photoperiod regime to determine if temperature can affect smolting in the absence of any photoperiod cues. Further, under each photoperiod regime, fish were exposed to either an ambient temperature regime (low temperature in winter, increasing in spring), or an advanced temperature regime (same pattern as ambient, but advanced by six weeks). Changes in gill Na^+,K^+-ATPase activity were used to monitor physiological smolt development, and plasma GH, IGF-I, cortisol and thyroid hormones were measured in order to examine the underlying endocrine signaling pathways.

Materials and methods

Rearing and sampling of fish

Juvenile Atlantic salmon (*Salmo salar* L.) were obtained from the White River National Fish Hatchery (Bethel, VT, USA) and brought to the Anadromous Fish Research Center (Turners Falls, MA, USA) in the autumn. Rearing and experimental conditions were similar to those reported by McCormick et al. (1995). 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^{-1}, with supplemental aeration. Each tank contained approximately 80 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 increases in daylength. Lighting was supplied by overhead fluorescent lights (500 lux at the water surface) and the LDN photoperiod was adjusted twice a week. On January 12, two groups remained on short days (LD 9:15; 9 h daylight) while the remaining two groups continued on a simulated natural photoperiod (LDN). The fish at this time had a fork length of 14–16.5 cm and body mass of 29–50 g. On February 14, 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 six weeks.

Food was withheld for 24 h prior to sampling, which occurred from 10.00–11.00 h Eastern Standard Time. Blood and gill samples were taken approximately every 2 weeks from January 5 through May 19 (N=10 per treatment). Fish were anesthetized with MS-222 (100 mg l^{-1}, neutralized to pH 7.0), and fork length to the nearest mm and mass to the nearest 0.1 g were recorded. Blood was drawn from the caudal vein into a 1 ml ammonium heparinized syringe and centrifuged at 5000 ***g*** for 5 min at 4°C. Plasma was divided into portions and stored at –80°C. 4–6 gill filaments were severed above the septum, placed in 100 µl of 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.

Measurement of gill Na^+,K^+-ATPase activity

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 (McCormick, 1993). Gill tissue was homogenized in 125 µl of SEID (SEI buffer containing 0.1 % deoxycholic acid) and centrifuged at 5000 ***g*** for 30 s. 10 µl samples were run in two sets of duplicates, one set containing assay mixture and the other assay mixture plus 0.5 mmol l^{-1} ouabain. The resulting ouabain-sensitive ATPase activity measurement is expressed as µmol ADP mg^{-1} protein h^{-1}. Protein concentrations were determined using bicinchoninic acid (BCA) Protein Assay (Pierce, Rockford, Il, USA). Both assays were run on a THERMOmax microplate reader using SOFTmax software (Molecular Devices, Menlo Park, CA, USA).

Hormone immunoassays

Plasma cortisol levels were measured by a validated direct competitive enzyme immunoassay as outlined by Carey and McCormick (1998). The typical measuring range was 1–400 ng ml^{-1}, with a lower detection limit of 0.3 ng ml^{-1}. Using a pooled plasma sample, the mean intra-assay variation was 5.5% (N=10) and the mean inter-assay variation was 8.8% (N=10). Plasma growth hormone levels were measured by a radioimmunoassay validated for Atlantic salmon (Björnsson et al., 1988). The typical measuring range was 0.1–50 ng ml^{-1} with mean intra-assay and inter-assay variations of 5.4% (N=9) and 3.9% (N=9), respectively. Plasma IGF-I concentration was measured by homologous radioimmunoassay, as described by Moriyama et al. (1994). The typical measuring range was 1–250 ng ml^{-1}, with a lower detection limit of 0.20 ng ml^{-1}. Using a pooled plasma sample, the mean intra-assay variation was 7% (N=5) and the mean 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

(Dickhoff et al., 1978). The typical measuring range was 1–64 ng ml^{-1} for thyroxine and 0.5–16 ng ml^{-1} for triiodothyronine. Intra-and interassay coefficients of variation for these assays were 4.3–11% and 3.2–5%, respectively.

Calculations and statistics

A non-parametric three-way analysis of variance (ANOVA) on ranks was used to determine the significance of photoperiod, temperature and changes over time. When significant treatment effects were established (P<0.05), differences among treatments at each time point were tested using the non-parametric Kruskal–Wallis test. Only sampling points after initiation of experimental treatments were used in statistical analyses (thus, the January sampling point is presented graphically, but not included in statistical analyses).

Results

All fish were greater than 14 cm fork length at the beginning of the study (Fig. 1). The fork length of fish in the two advanced temperature groups was greater than in the ambient temperature groups beginning in late March. For both temperature treatments, fish in the LDN groups were larger than LD 9:15 fish in late April and early May.

In the AMB/LDN group condition factor remained relatively stable from February through March and then decreased at a constant rate thereafter (Fig. 1). A stable winter condition factor (mass×length^{-3})×100 was also seen in the ADV/LDN group, but in late April this group showed a large and rapid decrease in condition factor. Decreased condition factor was not seen in either of the LD 9:15 groups, and the ADV/LD 9:15 group showed an increase in condition factor in mid-April.

Under ambient temperature and normal daylength (AMB/LDN), gill Na^+,K^+-ATPase activity increased from 2.4 μmol ADP mg^{-1} protein h^{-1} in early February to peak values of 12.4 μmol ADP mg^{-1} protein h^{-1} in late April (Fig. 2A). The increase was relatively constant from February to early April when the temperature was low, but accelerated from early to late April, coincident with increasing temperature. In the ADV/LDN group, gill Na^+,K^+-ATPase activity rose more quickly than in the AMB/LDN group, resulting in significant differences between the two groups in late March and early April, but the timing and absolute values of the peak levels were the same. In the AMB/LD 9:15 group, gill Na^+,K^+-ATPase activity increased at a similar rate to the AMB/LDN group in winter, but rose more slowly than that group from late March onward, reaching only 9.3 μmol ADP mg^{-1} protein h^{-1} by the end of the study in mid-May. Gill Na^+,K^+-ATPase activity of the ADV/LD 9:15 group rose at a rate similar to that of the ADV/LDN group from February to March, but reached peak levels of 7.1 μmol ADP mg^{-1} protein h^{-1} in late March and subsequently declined throughout the rest of the study.

Plasma GH in the AMB/LDN group increased steadily from 1.0 ng ml^{-1} in February to 1.9 ng ml^{-1} in early April, and then underwent a rapid increase to peak levels of 8.7 ng ml^{-1} in late April, coincident with increasing temperature (Fig. 2B). In the ADV/LDN group, plasma GH concentration was low (0.6–1.8 ng ml^{-1}) from February to mid-March, then rose steeply in mid March to 6.2 ng ml^{-1} several weeks after the increase in temperature, and remained high with peak levels of

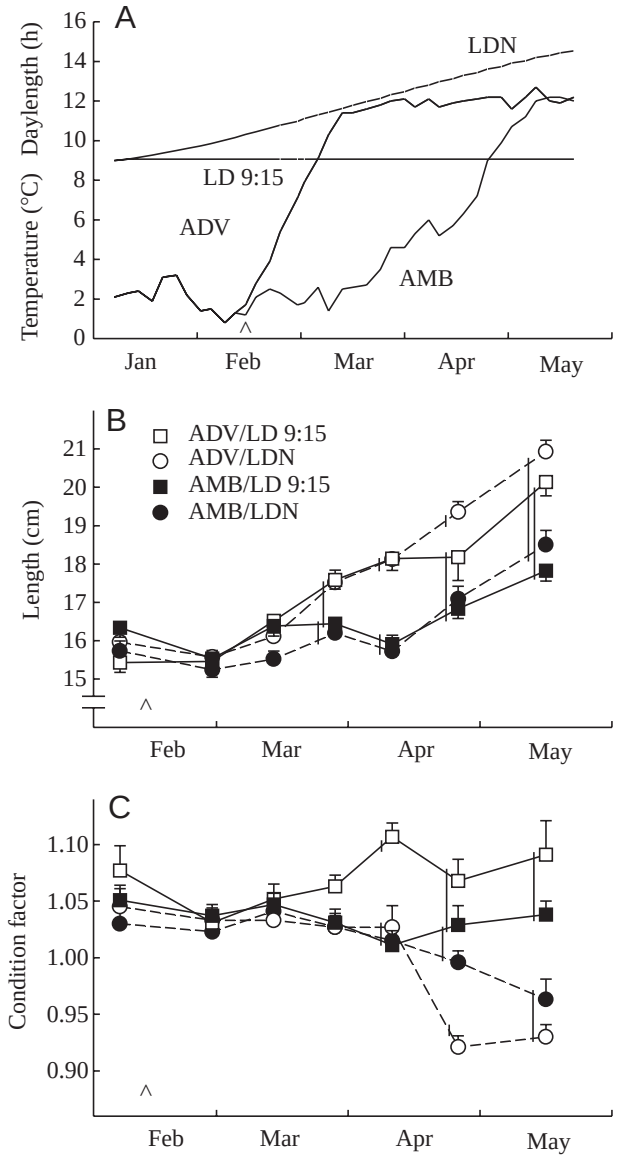

Fig. 1. (A) Seasonal change in temperature and daylength of the four experimental groups. Temperature increases in the advanced temperature group (ADV) began on February 14 (arrowhead). (B) Length (cm) and (C) condition factor (mass×length^{-3})×100 in juvenile Atlantic salmon subjected to different photoperiod and temperature treatments. Values are means ± S.E.M. (N=12). There were significant effects of photoperiod, temperature and time on condition factor, and significant effects of temperature and time on length (P<0.05, three-way ANOVA). Vertical lines unconnected to other lines indicate a significant difference from other groups at that time; points without vertical lines are not significantly different from one another (P>0.05, Kruskal–Wallis test).

8.9 ng ml^{-1} in late April. In the AMB/LD 9:15 group, plasma GH remained relatively constant from February to the end of March (0.8–1.0 ng ml^{-1}), increased slightly through April and then increased steeply to peak values of 6.9 ng ml^{-1} in mid-May. Plasma GH concentration of the ADV/LD 9:15 group rose at a rate similar to that of the ADV/LDN group from February to mid-March, but did not show a steep rise in response to temperature, and peak levels in this group were only 3.5 ng ml^{-1} in late April.

Plasma IGF-I levels of the AMB/LDN group were low (88 ng ml^{-1}) in February and remained relatively constant until mid-April, after which they increased steadily to a peak level

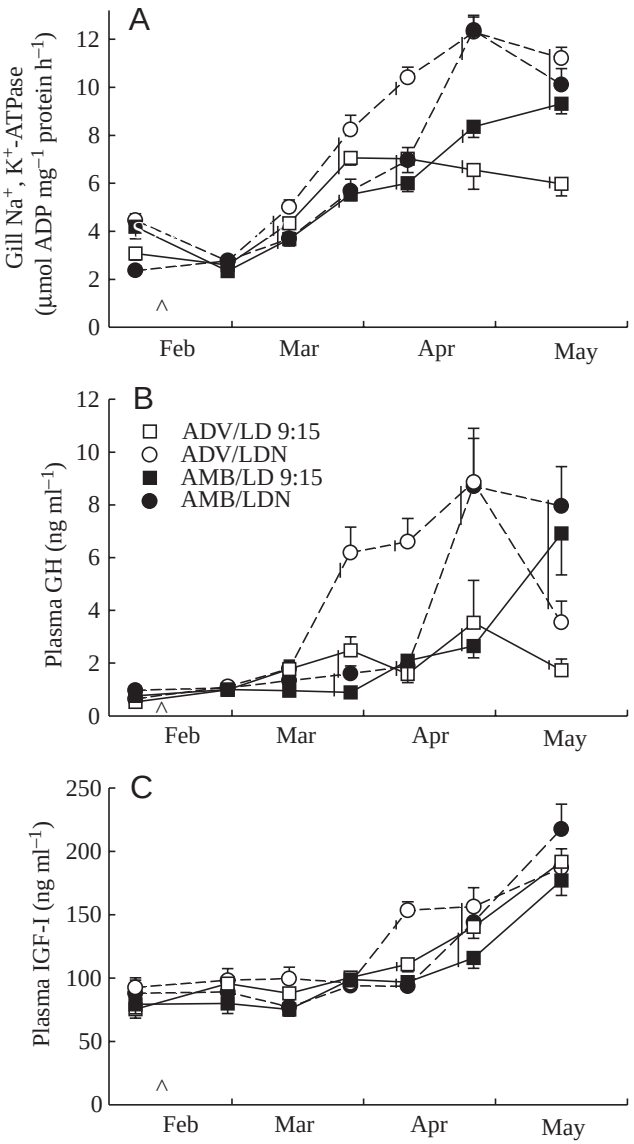

Fig. 2. Gill Na^+,K^+-ATPase activity (μmol ADP mg^{-1} protein h^{-1}) (A), plasma growth hormone levels (GH, ng ml^{-1}; B) and insulin-like growth factor I levels (IGF-I, ng ml^{-1}; C) in juvenile Atlantic salmon subjected to different temperature and photoperiod treatments (see Fig. 1). Values are means ± S.E.M. (N=12). Temperature increases in the advanced temperature group began on February 14 (arrowhead). There were significant effects of photoperiod, temperature and time on gill Na^+,K^+-ATPase activity and plasma IGF-I levels, and significant effects of photoperiod and time on plasma GH levels (P<0.05, three-way ANOVA). Vertical lines unconnected to other lines indicate a significant difference from other groups at that time point; time points with no vertical lines are not significantly different from one another (P=0.05, Kruskal–Wallis test).

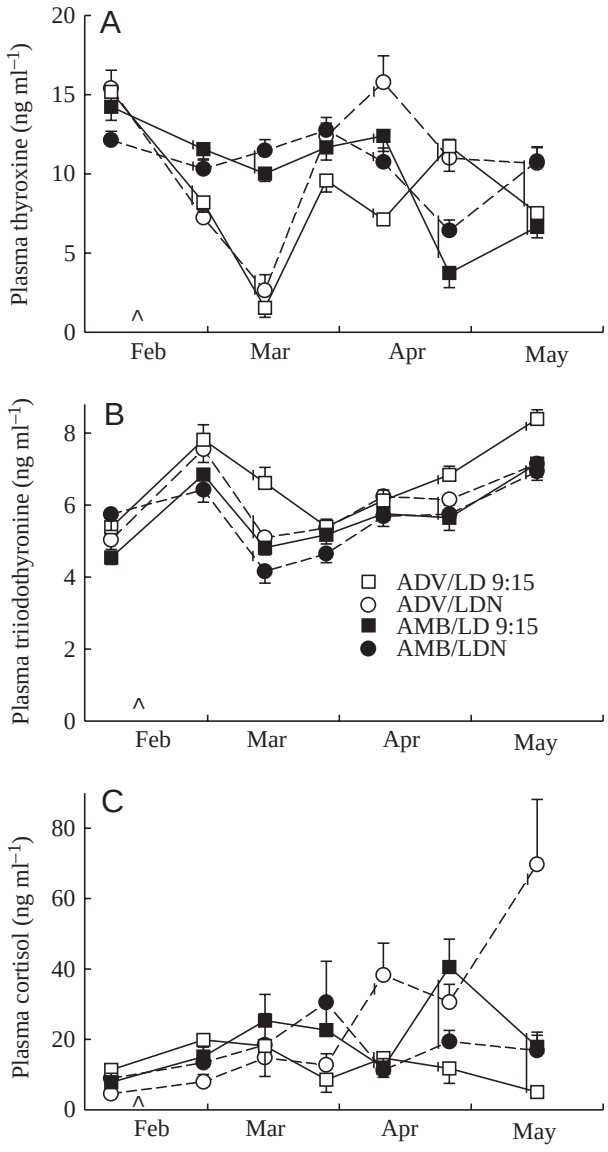

Fig. 3. Plasma thyroxine (T_4, ng ml^{-1}; A), triiodothyronine (T_3, ng ml^{-1}; B) and cortisol (ng ml^{-1}; C) levels in juvenile Atlantic salmon subjected to different temperature and photoperiod treatments (see Fig. 1). Values are means ± S.E.M. (N=12). Temperature increases in the advanced temperature group began on February 14 (arrowhead). There were significant effects of photoperiod, temperature and time on plasma T_4 and T_3 levels (P<0.05, three-way ANOVA). There was a significant effect of time on plasma cortisol and a significant interaction between time, photoperiod and temperature (P<0.05, three-way ANOVA). Vertical lines unconnected to other lines indicate a significant difference from other groups at that time; points without vertical lines are not significantly different from one another (P=0.05, Kruskal–Wallis test).

of 218 ng ml^{-1} in mid-May (Fig. 2C). A similar pattern and magnitude of increase was seen in the ADV/LDN group, except that the increase began 2 weeks earlier. Both of the LD 9:15 groups were similar to the AMB/LDN groups and only differed in mid-April.

Plasma thyroxine (T_4) levels of the AMB/LDN group remained relatively stable at 10.3–12.8 ng ml^{-1} from February to late March and then declined to 6.4 ng ml^{-1} in late April, coincident with increasing temperature (Fig. 3). A similar pattern in plasma T_4 concentration was seen in the AMB/LD 9:15 group. By contrast, both of the advanced temperature groups underwent a precipitous decline in plasma T_4 levels after the temperature was increased in these groups in early February. Following this decrease, both temperature-advanced groups had increased plasma T_4 levels in late March, with the LDN group having substantially higher levels (15.8 ng ml^{-1}) in mid-April than the LD 9:15 group (7.1 ng ml^{-1}).

Plasma triiodothyronine (T_3) levels of the AMB/LDN group were 5.8 ng ml^{-1} in February, dropping to 4.2 ng ml^{-1} in mid-March and then rising slowly but steadily to peak levels of 6.9 ng ml^{-1} in mid-May (Fig. 3). A similar pattern was seen in all of the other groups, the only consistent difference being significantly higher levels of plasma T_3 in the ADV/LD 9:15 in early March, late April and May compared to all other groups.

Plasma cortisol levels of the AMB/LDN group were low in February (8.9 ng ml^{-1}) and rose steadily to peak values of 30.5 ng ml^{-1} in late March, declining to intermediate levels (11.3–19.4 ng ml^{-1}) for the remainder of the study (Fig. 3). In the ADV/LDN group, the plasma cortisol levels rose steadily from February to late March, then rose sharply in early April (38.3 ng ml^{-1}) and again in mid-May (69.7 ng ml^{-1}). In the AMB/LD 9:15 group, the plasma cortisol level was 7.9 ng ml^{-1} in February, rose through mid-March, declined in early April and rose again in late April to peak values of 40.6 ng ml^{-1}. A similar pattern in plasma cortisol levels was seen in the ADV/LD 9:15 group, except that a secondary increase was not seen and values were significantly lower in late April.

Discussion

Two major findings from the present study support the concept that temperature is acting primarily by controlling the rate of development and not acting as an independent zeitgeber. First, under normal daylength conditions, an advanced temperature-increase regime resulted in a more rapid rate of increase in gill Na^+,K^+-ATPase activity, but the date at which increases began and peak levels occurred was the same for both temperature regimes. Second, smolting remained incomplete under a regime of short days and an advanced temperature-increase regime (as judged by half-normal peak gill Na^+,K^+-ATPase activity and no decrease in condition factor), indicating that temperature increase alone cannot act to advance smolting in the absence of photoperiod cues. These results indicate that temperature is acting primarily on the rate of development, but is not acting independently of photoperiod as a zeitgeber. Previous studies on Atlantic and Pacific salmon show that the date at which peak levels of gill Na^+,K^+-ATPase activity or salinity tolerance occur is advanced by higher temperatures, especially if constant elevated temperatures are used (see Introduction). This may relate to the higher degree days experienced by fish under constant elevated temperatures (McCormick et al., 1996; Sigholt et al., 1998). In the present study, gill Na^+,K^+-ATPase activity was significantly elevated from late March to mid-April in the ADV/LDN group compared with all other groups. The strong correlation between gill Na^+,K^+-ATPase activity and salinity tolerance, demonstrated in previous studies (McCormick, 1996), indicates that salinity tolerance will be increasing throughout this period. Such an increase in salinity tolerance will have survival advantages for fish that experience high spring temperatures (such as salmon in more southerly latitudes and hatchery-reared smolts) and enter seawater early. This effect of temperature may have negative impacts on salmon survival if it results in both earlier development and loss of salinity tolerance (the latter also being driven by temperature; McCormick et al., 1996) prior to ocean entry.

The present study agrees with most previous studies that have found an interaction between photoperiod and temperature in the control of smolt development (Zaugg, 1981; Solbakken et al., 1994; Sigholt et al., 1998). In the present study, the most rapid increase in gill Na^+,K^+-ATPase activity occurred under normal (increasing) daylength and an increasing temperature regime, as has been observed in several previous studies (Johnston and Saunders, 1981; Sigholt et al., 1998). This more rapid increase suggests that there may be stages in the photoperiod-regulated circannual rhythm that are more sensitive to temperature. The possible mechanism for such a response may lie in an altered temperature response of the endocrine system at different times of the circannual cycle.

Several lines of evidence indicate that an increased level of plasma growth hormone is causal to the osmoregulatory changes that occur during smolting (Bjornsson 1997; McCormick et al., 1998). Previous studies have demonstrated that photoperiod-induced alterations in the timing of smolt development are strongly correlated with changes in circulating levels of growth hormone. The effect of temperature may also be mediated in part by changes in plasma growth hormone levels. Exposure of Atlantic salmon to a temperature increase from 6 to 12°C resulted in a fivefold increase in plasma growth hormone followed by an advance in development of seawater tolerance (Björnsson et al., 1989). McCormick et al. (2000) found that fish kept at a low winter temperature (<3°C) had lower plasma GH levels and slower smolt development than fish kept at an elevated temperature, and that there was a strong correlation between plasma GH levels and gill Na^+,K^+-ATPase activity. There was a similar strong correspondence between changes in plasma GH levels and the timing of smolt development in the present study, both as a function of temperature and of photoperiod. In the LDN group, an ADV temperature cycle resulted in earlier increases in plasma GH levels and gill Na^+,K^+-ATPase activity. In the AMB temperature group, a similar increase in both parameters

occurred in late April, corresponding with increased temperatures. In the LD 9:15 group, an increase in temperature resulted in only slight increases in plasma GH level. This strongly suggests that increased daylength is necessary for the large increases in GH levels normally seen during smolting, and increased temperature by itself will have only moderate effects on plasma GH levels. Together, these data suggest that plasma growth hormone plays a large role in mediating the effect of temperature on smolt development, although the mechanism(s) by which this occurs in Atlantic salmon remains to be elucidated.

An increase in plasma IGF-I (Agustsson et al., 2001) and hepatic IGF-I mRNA levels occurs during spring in smolting salmonids (Duan, 1998), and a significant positive correlation between plasma GH levels and IGF-I levels has been found during smoltification of Atlantic salmon (McCormick et al., 2000). Such correlation is in agreement with the general view that plasma IGF-I in vertebrates is mainly of hepatic origin, and that the IGF-I secretion from the liver is under direct control by GH. However, a closer inspection of the available data (present study; McCormick et al., 2000; Agustsson et al., 2001) reveals that such a causal relationship between GH and IGF-I levels must be modulated by other factors. Thus, in a number of instances, large increases or decreases in plasma GH levels are not accompanied by similar changes in IGF-I levels. An example from the present study is the ADV/LDN group, in which GH levels increase rapidly in March and decrease in May, without similar changes occurring in IGF-I. The available plasma IGF-I profiles during Atlantic salmon smoltification (present study, McCormick et al., 2000; Agustsson et al., 2001) rather give the general impression of a gradual increase during spring, irrespective of photoperiod and temperature regimes, or plasma GH levels. This suggests an endogenous circannual component to IGF-I secretion, but the mechanism by which plasma IGF-I levels can rise without an increase in plasma GH levels (see, for example, mid-March plasma values; Agustsson et al., 2001) remains unclear. It could either involve changed GH-receptor densities or activity of other IGF-I secretagogues.

Relatively little is known of the environmental factors that affect circulating levels of IGF-I in salmon or in fish in general. Higher plasma IGF-I levels in response to increased rearing temperatures have been found in chinook *Oncorhynchus tshawytscha* and coho salmon *O. kisutch* (Beckman et al., 1998; Larsen et al., 2001). Higher plasma IGF-I levels due to elevated temperature were also found in the present study, though this was limited to one sampling point in mid-April. Although the ADV temperature regime caused a significant increase in plasma IGF-I levels in both photoperiod groups at this time, the increase was greater in the LDN group, suggesting an interaction between temperature and photoperiod in controlling circulating IGF-I levels. There was a consistent increase in plasma IGF-I levels in all groups throughout the study, irrespective of photoperiod and temperature treatment. This suggests that some aspects of the regulation of plasma IGF-I level may be under an endogenous circannual rhythm. There was relatively little impact of photoperiod treatment in the present study, which contrasts with previous work in which increased daylength at an elevated temperature resulted in significant increases in plasma IGF-I levels that lasted for almost 2 months (McCormick et al., 2000). This affect was only seen under an elevated temperature regime, and under ambient temperature conditions only moderate increases in plasma IGF-I levels were observed, similar to the present study. Based on these limited studies, smolt-related increases in plasma IGF-I can be expected to be substantially advanced only when both temperature and photoperiod are advanced.

The clearest effect of environmental manipulation on thyroid hormones in the present study was a marked decrease in plasma thyroxine concentration that occurred coincidentally with increased temperature in both temperature regimes (decreases in late February and late April for the ADV and AMB groups, respectively). This was a transient increase lasting for 4 weeks in ADV groups and 2 weeks in AMB. Overall, photoperiod had relatively little impact on plasma thyroxine and triiodothyronine levels, in agreement with previous work in which it was concluded that, although photoperiod had some influence on plasma thyroxine levels (and limited effects on plasma triiodothyronine levels), plasma thyroxine concentration was substantially higher in Atlantic salmon smolts reared under cool (ambient) temperatures than at 10°C (McCormick et al., 2000). These results are somewhat at odds with the widely observed plasma thyroxine ‘surge’ during smolting, at a time when water temperatures are normally increasing. This may be due to rearing conditions and/or stock differences, as increases in plasma thyroxine levels may also be absent in some hatchery-reared smolts, although they increase substantially after these fish are released into the wild (S. D. McCormick, unpublished results). Such post-release increases in plasma thyroxine concentration may be due to a number of environmental and biotic changes experienced by the fish, including flow, turbidity, water quality, food availability and the act of downstream migration (Iwata, 1995; Specker et al., 2000). These are environmental signals that do not occur under highly controlled laboratory settings like the present study.

Plasma cortisol levels increased during the spring, but the magnitude and timing of the changes differed greatly among the groups. The ADV/LDN group had significantly greater plasma cortisol levels than other groups in early April and May, and the increase in April was synchronous with elevated gill Na^+,K^+-ATPase activity. There was also a significant increase in cortisol in the AMB/LD 9:15 group in late April that may have driven the rise in gill Na^+,K^+-ATPase activity. However, little increase in plasma cortisol levels was seen in the AMB/LDN group despite significant increases in gill Na^+,K^+-ATPase activity. Although a seasonal increase in cortisol concentration has been described as one of the endocrine factors that stimulate smolting (Hoar, 1988), the available experimental evidence is often equivocal. A temporal relationship between plasma cortisol levels and gill Na^+,K^+-ATPase activity has been seen previously in this stock of

Atlantic salmon (Shrimpton and McCormick, 1998). Increases in plasma cortisol levels were seen in response to increased daylength for fish in warm (10°C) water and cold (<3°C) water (McCormick et al., 2000). At 10°C, elevated cortisol levels were coincident with an increase in gill Na^+,K^+-ATPase activity, but this was not seen at the low temperature. In a different study conducted on the same stock of Atlantic salmon over 2 years, a seasonal increase in cortisol concentration was only seen in one year, not in both (Shrimpton et al., 2000). The lack of a strong correspondence between circulating cortisol levels and physiological changes during smolting, however, does not preclude an important role for cortisol in these changes. The gill cortisol receptor number increase during spring (Shrimpton and McCormick, 1998) and responds to temperature and photoperiod manipulations (J. M. Shrimpton and S. D. McCormick, unpublished data) potentially altering tissue responsiveness to cortisol without a significant change in circulating cortisol levels. This is supported by Patiño et al. (1985), who found increased cortisol turnover during smolting that was coincident with increased gill Na^+,K^+-ATPase activity.

The present results indicate that temperature can influence smolt development, most likely by affecting the rate of response to an endogenous circannual rhythm cued by changing photoperiod, and that increased temperature alone probably does not act as a zeitgeber. Plasma thyroxine concentration decreased following increased temperatures, whereas plasma IGF-I, T_3 and cortisol levels are only moderately affected by the altered pattern of temperature increases used in this study. In contrast, changes in plasma growth hormone levels precede and are strongly correlated with the physiological changes that resulted from the manipulations of both temperature and photoperiod. The present results therefore provide further evidence that growth hormone in the major endocrine mediator of the environmental effects on the timing of smolt development. There is likely to be an adaptive value involved in the evolution of photoperiod as the main zeitgeber and temperature controlling the rate of development, if it allows for the correct timing of physiological and behavioral development with seasonal environmental changes such as prey abundance in the ocean that are beneficial to survival.

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