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Low temperature limits photoperiod control of smolting in Atlantic salmon through endocrine mechanisms

STEPHEN D. McCORMICK,[1] SHUNSUKE MORIYAMA,[2] AND B. THRANDUR BJÖRNSSON[3]
[1]*Conte Anadromous Fish Research Center, Biological Resources Division, US Geological Survey, Turners Falls, Massachusetts 01376;* [2]*Laboratory of Molecular Endocrinology, School of Fisheries, Kitasato University, Sanriku, Iwate, Japan; and* [3]*Fish Endocrinology Laboratory, Department of Zoology, Göteborg University, Göteborg, Sweden*

McCormick, Stephen D., Shunsuke Moriyama, and B. Thrandur Björnsson. Low temperature limits photoperiod control of smolting in Atlantic salmon through endocrine mechanisms. *Am J Physiol Regulatory Integrative Comp Physiol* 278: R1352–R1361, 2000.—We have examined the interaction of photoperiod and temperature in regulating the parr-smolt transformation and its endocrine control. Atlantic salmon juveniles were reared at a constant temperature of 10°C or ambient temperature (2°C from January to April followed by seasonal increase) under simulated natural day length. At 10°C, an increase in day length [16 h of light and 8 h of darkness (LD 16:8)] in February accelerated increases in gill Na^+-K^+-ATPase activity, whereas fish at ambient temperature did not respond to increased day length. Increases in gill Na^+-K^+-ATPase activity under both photoperiods occurred later at ambient temperature than at 10°C. Plasma growth hormone (GH), insulin-like growth factor, and thyroxine increased within 7 days of increased day length at 10°C and remained elevated for 5–9 wk; the same photoperiod treatment at 2°C resulted in much smaller increases of shorter duration. Plasma cortisol increased transiently 3 and 5 wk after LD 16:8 at 10°C and ambient temperature, respectively. Plasma thyroxine was consistently higher at ambient temperature than at 10°C. Plasma triiodothyronine was initially higher at 10°C than at ambient temperature, and there was no response to LD 16:8 under either temperature regimen. There was a strong correlation between gill Na^+-K^+-ATPase activity and plasma GH; correlations were weaker with other hormones. The results provide evidence that low temperature limits the physiological response to increased day length and that GH, insulin-like growth factor I, cortisol, and thyroid hormones mediate the environmental control of the parr-smolt transformation.

growth hormone; insulin-like growth factor I; cortisol; thyroxine; osmoregulation; sodium-potassium-adenosinetriphosphatase; fish; anadromous; rhythm; development

PHOTOPERIODIC REGULATION of seasonal changes in physiology is a well-known phenomenon in vertebrates and is generally thought to be the result of entrainment of an endogenous circannual rhythm by the annual photocycle (17). Circadian rhythms exhibit temperature compensation, such that the free-running period is largely independent of temperature within the physiological range (47). In contrast, the potential influence of temperature on circannual rhythms and their entrainment by photoperiod is not well characterized. This interaction is especially interesting for ectothermic vertebrates, which can experience a wide range of body temperatures (up to 40°C) during the year. In these vertebrates, temperature may limit the rate of physiological changes (including signaling pathways) and has the potential to act as a cue or zeitgeber for seasonal changes.

The parr-smolt transformation (smolting) is a preparatory physiological adaptation that occurs in spring in Atlantic and Pacific salmon (18). Changes in salinity tolerance, visual pigments, buoyancy, metabolism, morphology, and behavior precede and are adaptive for downstream migration and seawater entry. Osmoregulation has been the most widely studied physiological change during smolting. Increased gill Na^+-K^+-ATPase activity and differentiation of chloride cells result in greater salt secretory capacity and increased salinity tolerance of smolts (34). Morphological changes during smolting include silvering, darkened fin margins, and decreased condition factor (weight-to-length ratio). Many of the physiological and morphological changes that comprise smolting are known to be responsive to photoperiod, advanced by increased day length and/or delayed by short days (51). Temperature has also been shown to affect the timing of smolting, although to a more limited degree than photoperiod (36).

The endocrine system is the primary signaling pathway between external zeitgebers, internal rhythms, and seasonal physiological responses (17). In contrast to metamorphic events that rely heavily on a single stimulatory endocrine pathway, smolting involves a number of interacting endocrine systems. Growth hormone (GH), insulin-like growth factor I (IGF-I), cortisol, and thyroid hormones increase during smolting and stimulate various physiological changes that occur during smolting, whereas prolactin is generally inhibitory (11, 18). In most salmonids, plasma GH levels increase in spring as a result of increased day length (3). Thyroid hormones can also be affected by changes in photoperiod, although the responses are not always consistent and temperature effects are largely unexamined (20). Although seasonal changes in plasma cortisol have been demonstrated (49), there is only limited

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evidence for an effect of photoperiod on circulating cortisol (6, 19). Homologous assays for teleost IGF-I have only recently been established, and the response of IGF-I to environmental change in teleosts in general and smolts in particular has not been previously examined. Use of exogenous hormone treatments has demonstrated that GH, IGF-I, and cortisol interact to upregulate gill Na^+-K^+-ATPase activity and salinity tolerance (29). Thyroid hormones may also be involved in the control of osmoregulation and other physiological changes during smolting (18).

The present study was undertaken to examine the interaction of photoperiod and temperature on the hormones that regulate smolting and the physiological changes that comprise smolting. Specifically, we examined the ability of increased day length to alter the timing of smolting at 10°C and 2°C (ambient temperature). Changes in gill Na^+-K^+-ATPase activity and condition factor were used to follow smolt development, and the effect of environmental manipulation on circulating levels of GH, IGF-I, cortisol, and thyroid hormones was examined.

METHODS

Rearing and sampling of fish. Atlantic salmon (*Salmo salar*) were obtained as parr from the White River National Fish Hatchery (Bethel, VT) and brought to the Conte Anadromous Fish Research Center on 29 October, 8 mo after hatching. Rearing and experimental conditions are similar to those previously reported (31). Fish were randomly divided into four, isolated photoperiod rooms containing two 1-m-diameter tanks supplied with ambient river water at a flow rate of 4 l/min and supplemental aeration. Each tank contained ~80 fish. The fish were fed to satiation (Zeigler, Gardners, PA) by use of automatic feeders. Water was maintained at ambient temperature in all tanks until 7 January, when two of the groups were supplemented with heated water to maintain a temperature of 9–10°C (Fig. 1). Fish at this time were 15.2–18.6 cm long and weighed 34.3–72.2 g. Initially, all groups were maintained on a simulated natural photoperiod [light-dark natural (LDN)] with seasonal increases in day length matching normal daylight hours. On 8 February, one group in each of the temperature regimens was subjected to an abrupt increase in day length to 16 h [16 hours of light and 8 hours of darkness (LD 16:8)]. Lighting was supplied by overhead fluorescent lights (500 lx at the water surface), and the LDN photoperiod was adjusted twice a week.

Feed was withheld for 24 h before sampling, which occurred from 1000 to 1100 Eastern Standard Time. Blood and gill samples were taken approximately every 2 wk from 5 January through 19 May (n = 10/treatment). Fish were anesthetized (100 mg/l MS-222 neutralized to pH 7.0), and fork length to the nearest millimeter and weight to the nearest 0.1 g were recorded. Blood was drawn from the caudal blood vessels into a 1-ml ammonium heparinized syringe and centrifuged at 8,000 *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 buffer containing (in mM) 150 sucrose, 10 EDTA, and 50 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 (28). Gill tissue was homogenized in 125 µl of buffer containing 150 mM sucrose, 10 mM EDTA, 50 mM imidazole (pH 7.3), and 0.1% deoxycholic acid and centrifuged at 5,000 *g* for 30 s. Ten-microliter samples were run in two sets of duplicates: one set contained assay mixture and the other assay mixture and 0.5 mM ouabain. The resulting ouabain-sensitive ATPase activity measurement was expressed as micromoles of ADP per milligram of protein per hour. Protein concentration was determined using bicinchoninic acid protein assay (Pierce, Rockford, IL). Both assays are run on a THERMOmax microplate reader with use of SOFTmax software (Molecular Devices, Menlo Park, CA).

Hormone assays. Plasma cortisol levels were measured by a validated direct competitive enzyme immunoassay (8). The lower detection limit was 0.30 ng/ml. With use of a pooled plasma sample, the average intra- and interassay variations were 5.5% (n = 10) and 8.8% (n = 10), respectively. Plasma GH levels were measured by an RIA validated for Atlantic salmon (5). The lower detection limit was 0.1 ng/ml with average intra- and interassay variations of 5.4% (n = 9) and 3.9% (n = 9), respectively. Plasma IGF-I was measured by homologous RIA (37). The lower detection limit was 0.20 ng/ml, with average intra- and interassay variations of 7% (n = 5) and 6.5% (n = 5), respectively. Thyroxine (T_4) and 3,5,3′-triiodo-L-thyronine (T_3) concentrations were measured by a direct RIA (32). The lower detection limits were 0.5 ng/ml (T_4) and 0.2 ng/ml (T_3). Intra- and interassay coefficients of variation for these assays were 4.3–11% and 3.2–5%, respectively.

Calculations and statistics. Condition factor was calculated as follows: [weight/(length^3)] $*$ 100. A nonparametric three-way ANOVA on ranks was used to determine the significance of photoperiod, temperature, and changes over time and their interaction. Nonparametric analysis was used because not all parameters were normally distributed. If photoperiod or temperature treatments were significant ($P < 0.001$), differences among treatments at each time point were tested using the nonparametric Kruskal-Wallis test. First- and second-order polynomial regressions were calculated for all combinations of physiological variables. With one exception (plasma IGF-I vs. GH), r^2 values for all significant regressions were greater for second- than for first-order polynomials; therefore, only second-order polynomial regressions are reported. To test the effect of treatment on these regression analyses, a covariate ANOVA with "treatment" as a class variable (covariable) was conducted using SAS, general linear model procedure. To examine the relative explanatory power of endocrine parameters on gill Na^+-K^+-ATPase activity and condition factor, a best subsets regression analysis was used ($P < 0.05$). Endocrine parameters were logarithmically transformed for best subset regression analysis. Plasma T_3-to-T_4 ratio was not included in this analysis to prevent multiple colinearity with T_4 and T_3. Only sampling points after initiation of experimental treatments were used in statistical analyses. Thus the January sampling point is presented graphically but is not included in statistical analyses.

RESULTS

Condition factor of the 10°C-LDN group remained high until late March and declined steadily thereafter. A significant decrease in condition factor occurred within 1 wk of increased day length in the advanced photoperiod group (LD 16:8 in February) at 10°C and declined steadily until April (Fig. 1). Condition factor was significantly lower for the 10°C-LD 16:8 group than for the 10°C-LDN group from mid-February to mid-April. Condition factor of both photoperiod groups was lower in February at ambient temperature than at 10°C, remained at this level until late March, and

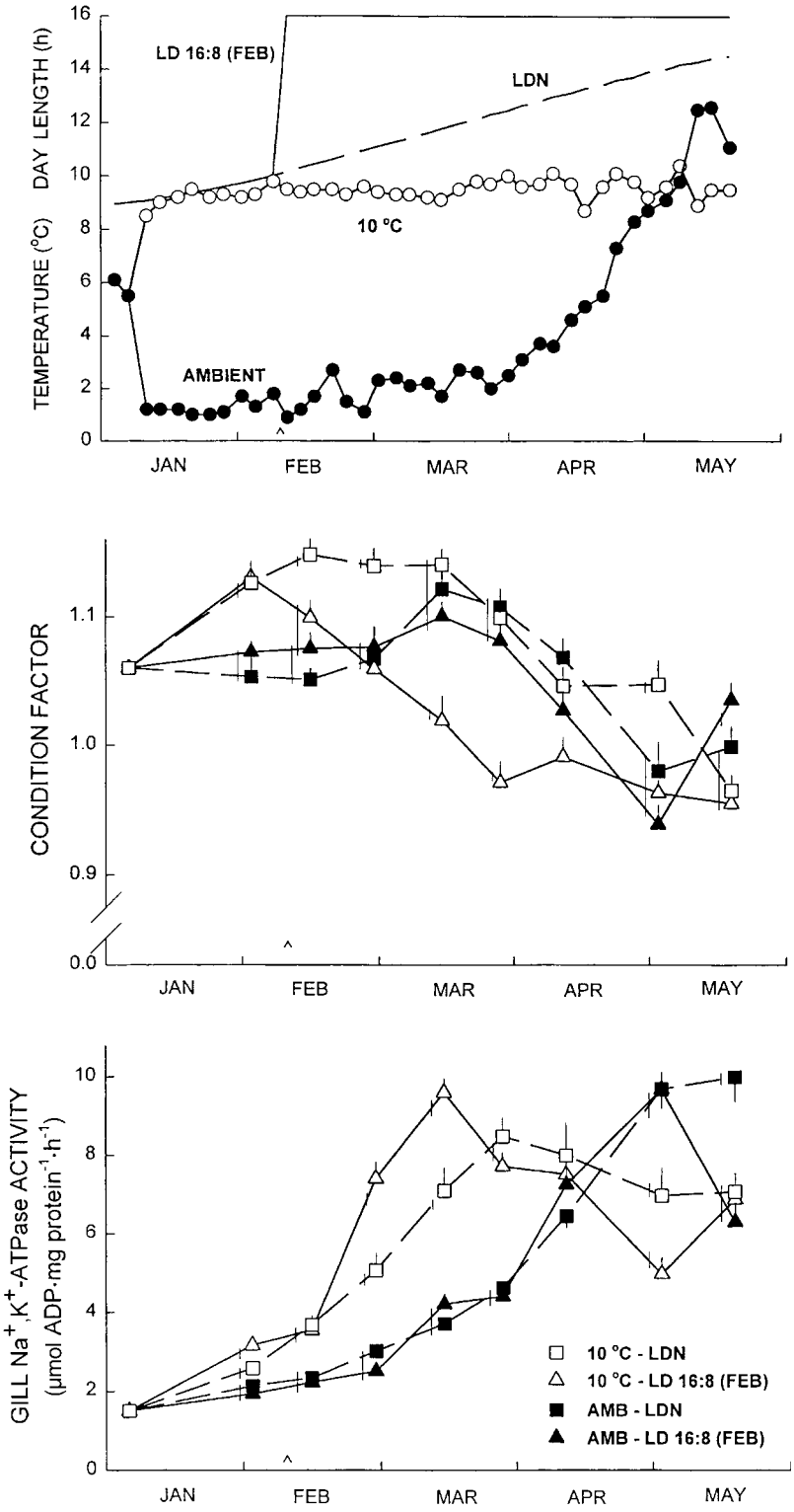

Fig. 1. *Top*: seasonal change in day length and temperature of 4 experimental groups. Increased day length [16 h of light and 8 h of darkness (LD 16:8)] occurred on 8 February 8 (caret). Condition factor [[weight/(length^3)] × 100; *middle*] and gill Na^+-K^+-ATPase activity (*bottom*) in juvenile Atlantic salmon subjected to photoperiod and temperature treatments are shown. LDN, natural day length; AMB, ambient temperature. Values are means ± SE (n = 12). 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).

declined steadily thereafter. There were no significant differences between the two photoperiod treatments at ambient temperature until the last sampling in late May. Condition factor was significantly influenced by time, photoperiod, and interaction between temperature and photoperiod (3-way ANOVA, $P < 0.001$).

Gill Na^+-K^+-ATPase activity increased steadily from January until late March in the 10°C-LDN group (Fig. 1). In the 10°C-LD 16:8 group, gill Na^+-K^+-ATPase activity rose abruptly 3 wk after increased day length and remained significantly higher than in the 10°C-LDN group throughout March. The Na^+-K^+-ATPase activity increased more slowly in both ambient temperature groups than in the 10°C groups and reached peak levels in early May. There was no significant difference between the two photoperiod treatments at ambient

temperature until late May, when there was a decrease in the LD 16:8 group. Gill Na^+-K^+-ATPase activity was significantly influenced by time, temperature, and temperature-photoperiod interaction ($P < 0.02$).

Plasma GH levels of the 10°C-LDN group were low (1–2 ng/ml) from January through late February and then increased steadily from mid-March to peak levels of 9.3 ng/ml in late May (Fig. 2). GH levels in the 10°C-LD 16:8 group increased abruptly after 1 wk of increased day length and remained significantly higher than in the LDN group for 7 wk (mid-February to late March). Increased day length in the ambient tempera-

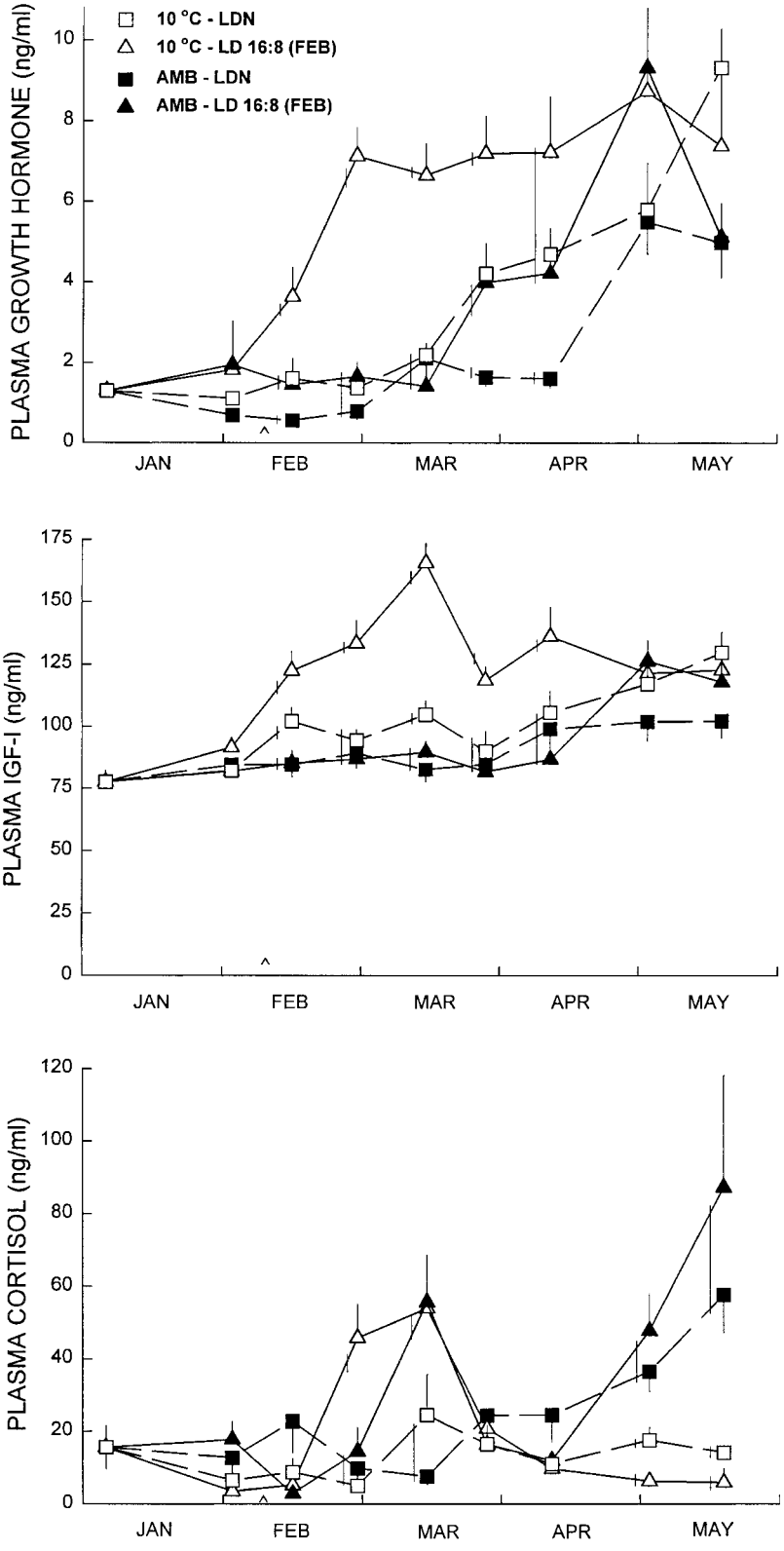

Fig. 2. Plasma growth hormone, insulin-like growth factor I (IGF-I), and cortisol in juvenile Atlantic salmon subjected to photoperiod and temperature treatments (see Fig. 1). Values are means ± SE ($n = 12$). Increased day length in LD 16:8 groups occurred on 8 February (caret). Vertical lines unconnected 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).

ture-LD 16:8 group had a more modest effect on plasma GH. Plasma GH levels did not increase above 2 ng/ml until late March. In late March and early April, GH levels were significantly higher in the ambient temperature-LD 16:8 group than in the ambient temperature-LDN group. In general, ambient (low) temperatures resulted in later increases in plasma GH, although peak levels were similar. Plasma GH was significantly influenced by time, temperature, photoperiod, and an interaction among the three ($P < 0.001$).

Plasma IGF-I levels increased modestly but steadily (from 75 to 125 ng/ml) from January to May in the 10°C-LDN group (Fig. 2). IGF-I levels of the 10°C-LD 16:8 group increased abruptly after 1 wk of increased day length and remained significantly higher than in the 10°C-LDN group for 9 wk (mid-February to late mid-April). Increased day length in the ambient temperature-LD 16:8 group did not significantly alter plasma IGF-I relative to the ambient temperature-LDN group. Plasma IGF-I was significantly influenced by time, temperature, photoperiod, and temperature-photoperiod interaction ($P < 0.001$).

Plasma cortisol levels of the 10°C-LDN group were low in February (<10 ng/ml) and then increased in March and remained relatively stable through May (mean 12–25 ng/ml; Fig. 2). Increased day length resulted in significant increases in plasma cortisol after 3 wk, and these values were significantly higher than in the 10°C-LDN group from late February to mid-March. There was also a significant effect of increased day length in the ambient temperature-LD 16:8 group, but this was of shorter duration than in the 10°C group (significant at 7 wk after increased day length). Both ambient temperature groups had increased plasma cortisol concurrent with increased temperature beginning in late April. Plasma cortisol was significantly influenced by time, temperature, and interaction among time, temperature, and photoperiod ($P < 0.001$).

Plasma T_4 levels of the 10°C-LDN group remained relatively stable throughout the study (mean 5–8 ng/ml; Fig. 3). Plasma T_4 levels were significantly higher after 1 wk of increased day length in the 10°C-LD 16:8 group and were significantly higher than in the 10°C-LDN group for 5 wk (mid-February to mid-March). The effect of increased day length at ambient temperature on plasma T_4 levels was not detectable until mid-May. Plasma T_4 levels in the ambient temperature groups were higher than in the 10°C groups in February and increased approximately twofold through the spring. Plasma T_4 was significantly influenced by time, temperature, photoperiod, and temperature-photoperiod interaction ($P < 0.01$).

Plasma T_3 levels of both 10°C groups were low in January, increased in early February, and decreased progressively thereafter (Fig. 3). Although plasma T_3 levels were significantly higher in the 10°C-LD 16:8 group in late February, this was due to a decrease in the 10°C-LDN group rather than a change in the 10°C-LD 16:8 group. Similarly, increased day length had little effect at ambient temperature, with only a slight difference between the LD 16:8 and LDN groups in early April. Plasma T_3 levels were generally higher at 10°C than at ambient temperatures, particularly in February. Plasma T_3 was significantly influenced by time, temperature, photoperiod, and interaction among time, temperature, and photoperiod ($P < 0.01$).

The ratio of T_3 to T_4 in plasma of the 10°C-LDN group was low in January, increased in early February, decreased in late February, and then leveled off for the remainder of the study (Fig. 3). A similar pattern was observed in the 10°C-LD 16:8 group, but T_3:T_4 ratio was significantly lower in this group from mid-February to mid-March. The T_3:T_4 ratio was significantly lower in the ambient temperature groups than in the 10°C groups at all time points. There was no significant difference in T_3:T_4 ratio between the photoperiod treatments at ambient temperatures at any time. Plasma T_3:T_4 ratio was significantly influenced by time and temperature ($P < 0.001$).

There were significant positive correlations between gill Na^+-K^+-ATPase activity and plasma GH ($r^2 = 0.63$; Fig. 4), IGF-I ($r^2 = 0.42$), and cortisol ($r^2 = 0.31$), but not with plasma T_4, T_3, or T_3:T_4 ratio. A best subsets regression model of hormones on gill Na^+-K^+-ATPase activity included plasma GH and cortisol ($r^2 = 0.71$), but not IGF-I, T_4, or T_3.

There were significant negative correlations between condition factor and plasma GH ($r^2 = 0.67$), IGF-I ($r^2 = 0.35$), and T_3 ($r^2 = 0.20$), but not with plasma cortisol, T_4, or T_3:T_4 ratio. A best subsets regression model of hormones on condition factor included plasma GH and T_3 ($r^2 = 0.78$), but not IGF-I, cortisol, or T_4.

Plasma GH was positively correlated with IGF-I ($r^2 = 0.62$; Fig. 4), but not with any other endocrine parameter. Plasma cortisol was positively correlated with plasma T_4 ($r^2 = 0.24$) and negatively correlated with plasma T_3:T_4 ratio ($r^2 = 0.28$). Plasma T_4 was negatively correlated with plasma T_3 ($r^2 = 0.16$). There were no other significant correlations among the endocrine parameters.

DISCUSSION

In the present study, smolting of Atlantic salmon as measured by increased gill Na^+-K^+-ATPase and decreased condition factor was advanced by increased day length when fish were held at elevated temperatures (10°C) through the winter, but not when fish were held at cooler, ambient temperatures of 2°C. These findings indicate that low temperature prevents artificial increases in day length from advancing the parr-smolt transformation in Atlantic salmon. At elevated winter temperatures (10°C), increased day length resulted in higher levels of plasma GH, IGF-I, T_4, and cortisol, whereas plasma T_3 levels were not affected. At ambient winter temperatures (2°C), increased day length resulted in increases in plasma GH, IGF-I, T_4, and cortisol that are much smaller, are of shorter duration, and occur later than at 10°C. Our results indicate that low temperature limits the ability of photoperiod to advance the physiological aspects of smolting and that this limitation occurs through a delayed and dampened

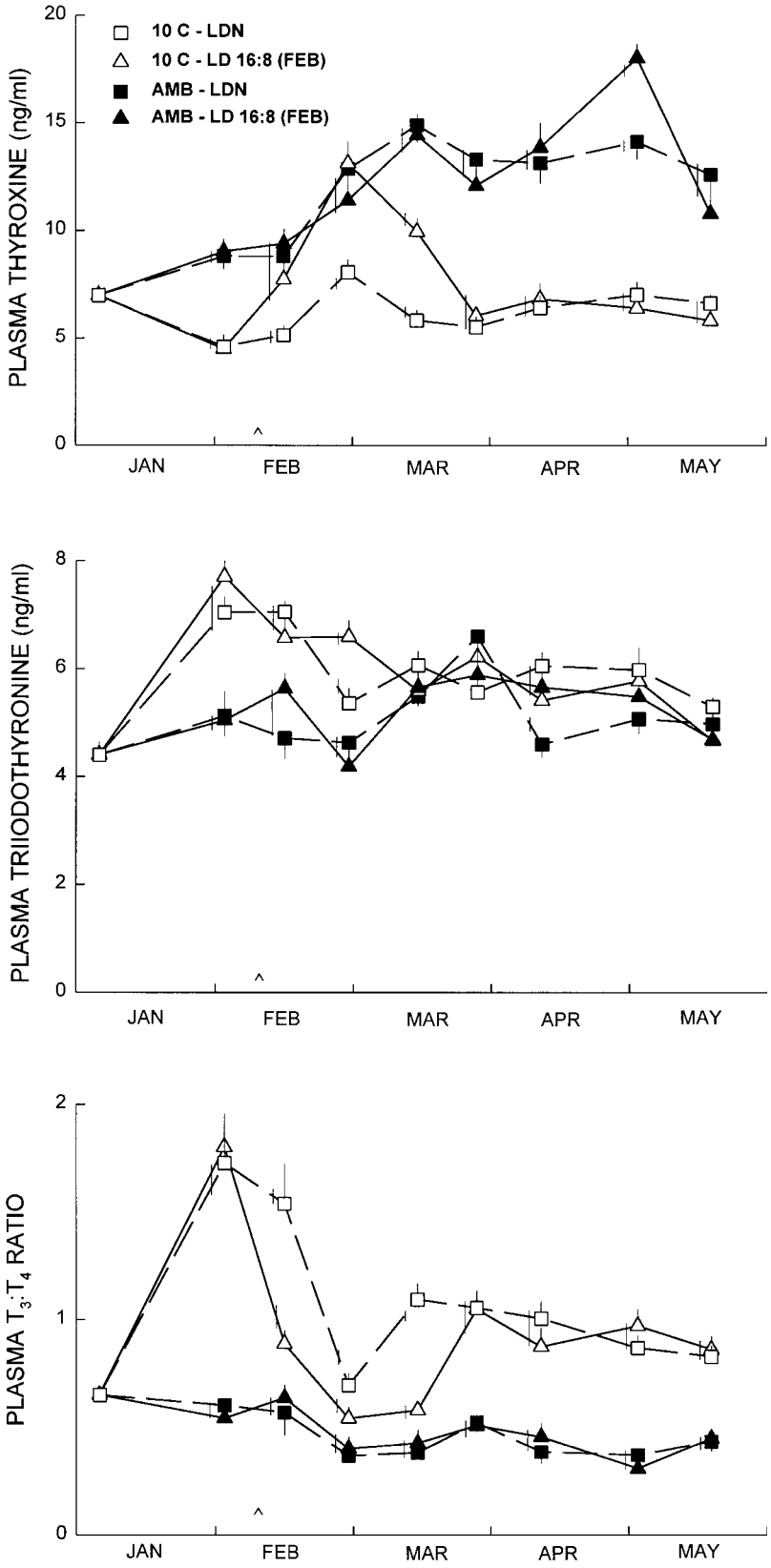

Fig. 3. Plasma thyroxine (T_4), triiodothyronine (T_3), and T_3:T_4 ratio in juvenile Atlantic salmon subjected to photoperiod and temperature treatments (see Fig. 1). Values are means ± SE (n = 12). Increased day length in LD 16:8 groups occurred on 8 February 8 (caret). Vertical lines unconnected to 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).

response of the endocrine system to increased day length at low temperature.

Previous work has demonstrated a strong effect of day length on smolt development in Atlantic and Pacific salmon (4, 9, 15, 35, 43, 44). Photoperiod exerts a similar effect on teleostean circannual rhythms, such as reproduction (7, 40). Exposure to short days for 6 wk is an apparent requirement for increased day length to

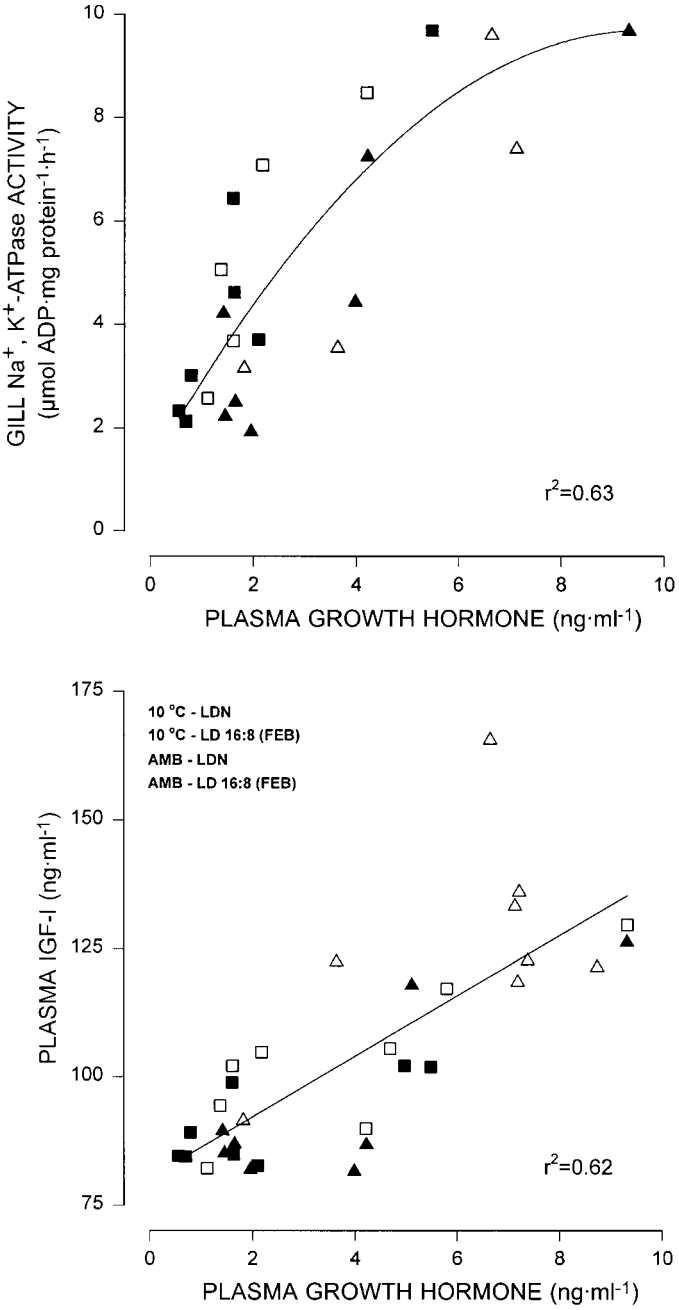

Fig. 4. Regression of plasma growth hormone with gill Na^+-K^+-ATPase activity and IGF-I. Values are means (n = 12) for each time point from each of the photoperiod and temperature treatments. Analysis of covariance with treatment as a covariable indicated that treatment was not significant ($P > 0.1$) but increased r^2 to 0.75 and 0.69 for gill Na^+-K^+-ATPase activity and IGF-I, respectively. Symbols as in Figs. 1–3.

advance smolting (48). Increases in salinity tolerance and gill Na^+-K^+-ATPase under a short day photoperiod provide limited evidence for a circannual rhythm of smolting (15, 31). All previous studies that have examined the influence of photoperiod on smolting have used elevated temperatures (>7°C), higher than those normally seen in temperate North America in winter. To our knowledge, the present study is the first to demonstrate a limiting effect of low temperature on the ability of photoperiod to alter a seasonal physiological response.

There are several possible mechanisms through which temperature may interact with and limit the endocrine response to increased day length. Temperature influences the rate at which physiological changes, such as enzyme synthesis, occur in response to environmental and endocrine signals (22). However, the strong relationship between circulating hormone levels, particularly GH, and physiological response in all treatments suggests that this mechanism is operating at the level of the pituitary or above, and not at the physiological target organs. One possible mechanism is a direct metabolic (Q_{10}) effect of temperature on hormone synthesis and release. In this case, the photoperiodic stimulation received by endocrine organs would be the same, but the capacity to respond is lower at low temperatures. This possible direct effect of temperature seems be most applicable to GH, where plasma levels increased in both temperature groups after day length increased (with a more substantial increase at 10°C) and increased under ambient conditions when temperature increased in late April. Other endocrine systems, however, did not respond to temperature in the same manner. Enzyme systems involved in hormone degradation will have lower activity at low temperature, and binding of hormones to their receptors may also be affected.

It is also possible that pineal and circannual time keeping are affected by temperature. Several authors have suggested that the pineal transduces photic and thermal information (1, 16, 50). Although temperature compensation occurs in mammalian circadian rhythms, this compensation is not complete, and temperature may have significant effects on phase changes (41). Incomplete temperature compensation in a circannual rhythm could explain the present results. Several teleosts have demonstrated circadian oscillation of the pineal melatonin cycle, but the rainbow trout, a close relative of Atlantic salmon, has no circadian melatonin cycle and responds directly to the light-dark cycle (16). Temperature has a direct effect on the trout pineal: higher temperatures increase the amplitude of the melatonin cycle and increase the sensitivity of the pineal to light. If the daily melatonin rhythms are involved in transducing information to the hypothalamic-pituitary cycle, this influence of temperature on the pineal could explain the present results. However, in contrast to the situation in mammals, the importance of melatonin rhythms in controlling circannual rhythms in fish is not well established (27). The intensity and duration of hypothalamic signals may also be affected by temperature, although to our knowledge this has not been investigated in fish.

Analysis of previous studies on Atlantic salmon reared at different temperatures indicates that a change in rearing temperature from 2 to 10°C can advance smolting by up to 4 wk (36). These findings are consistent with the present study in which the peak of gill Na^+-K^+-ATPase activity was delayed by 4 wk in fish in LDN and ambient temperature compared with fish at

LDN and 10°C. Atlantic salmon reared under continuous light and three increasing temperature regimens do not develop normal smolting in spring (46), indicating that temperature alone is not a sufficient cue for smolting. It is possible that the effect of temperature within a given photoperiod also worked through a temperature-photoperiod interaction, such that the stimulatory effect of increased day length in both photoperiod treatments was lower at low temperature. If this were the case, one would expect the ambient temperature groups to have the same endocrine profiles until temperatures began to increase, after which the LD 16:8 group would have higher levels. This pattern was not seen in any of the hormones examined in this study. Alternatively, temperature can have a more direct effect on circulating hormones, similar to the Q_{10} effect discussed above. This is seen to some degree in all the hormones measured in this study, inasmuch as plasma GH, IGF-I, cortisol, and T_4 were generally lower in each photoperiod at cooler temperatures. Furthermore, plasma GH, IGF-I, and especially cortisol increased in the ambient temperature groups coincident with increasing temperature in late April and May. These results suggest that temperature may act on the GH-IGF-I and interrenal axes directly and through a temperature-photoperiod interaction. These effects differ slightly but significantly for each hormone. Combinations of photoperiod and temperature will therefore have a complex effect on endocrine patterns and the timing of smolt development.

Decreased condition factor is associated with the increased metabolic rate, lipid utilization, and growth rate that accompany smolting (18). Condition factor had decreased within 1 wk of increased day length at 10°C, indicating a rapid response of growth and metabolism to photoperiod changes at elevated temperatures. In contrast, increases in gill Na^+-K^+-ATPase activity were not seen in the 1st wk after increased day length at 10°C but were seen after 3 wk. This period of response is consistent with the known time course of action of hormones on gill Na^+-K^+-ATPase, generally requiring 7–14 days for significant increases to occur (29). Na^+-K^+-ATPase mRNA in gills of brown trout can increase within 48 h of salinity transfer or hormone treatment (25). Salinity tolerance also has a relatively rapid response to exogenous GH and IGF-I, increasing within 2 days of treatment (33). The long response time necessary for increased gill Na^+-K^+-ATPase activity may be due to the proliferation and differentiation of chloride (salt secretory) cells, increased synthesis of enzyme subunits, and their transport to the basolateral membrane that comprise this physiological response (26, 53).

The present study provides evidence for the central importance of GH in smolt development. Of the endocrine factors examined, plasma GH had the earliest and most robust response to increased day length at 10°C and the most severely dampened response at low temperature. Plasma GH also had the greatest correlation with condition factor and gill Na^+-K^+-ATPase activity. Although smolting is clearly a multihormonal process, GH may be the primary endocrine mediator of environmental information for smolt development (3, 11) and may coordinate and facilitate the action of other hormones involved in smolting. As in other vertebrates, GH is in teleost fish the major secretagogue for liver and locally produced IGF-I (13), which has been shown to carry out at least some of the osmoregulatory actions of GH (33). There is a clear interaction between GH and cortisol in stimulating salinity tolerance, gill chloride cells, and gill Na^+-K^+-ATPase activity (24, 30). ACTH-induced cortisol secretion in salmon can be potentiated by GH in vivo and in vitro (52). GH may also promote responsiveness to cortisol by increasing the number of gill cortisol receptors (45). There are also clear links between the osmoregulatory actions of GH and thyroid hormones in salmonids (21), although the nature of this interaction is unclear.

High spring levels of plasma IGF-I and hepatic IGF-I mRNA have been found in Atlantic and coho salmon smolts (2, 14, 23, 42). In the present study, plasma IGF-I increased in all groups between March and May. Increased day length in the 10°C group resulted in substantial and sustained increases in plasma IGF-I, providing the first evidence that photoperiod regulates circulating levels of IGF-I in fish. Temperature had a less pronounced effect on plasma IGF-I. There were only occasional differences between the 10°C and ambient temperature groups under LDN and only a slight increase in plasma IGF-I when temperature rose in the ambient temperature group in May. Plasma IGF-I of smolting chinook salmon is also higher when fish are reared at warmer temperatures (2). We have shown a strong correlation between plasma GH and IGF-I in Atlantic salmon, supporting the importance of GH as the major releasing factor for circulating IGF-I. This correspondence between circulating IGF-I and GH is not perfect, inasmuch as large increases in plasma GH late in the study resulted in only slight changes in IGF-I. In addition, temperature seemed to be more important in regulating GH than IGF-I. It seems likely that other endocrine factors are involved in regulating plasma IGF-I. Recent studies have shown that, as in mammals, insulin can affect IGF-I production and release (37, 39).

Plasma T_4 levels increased after increased day length at 10°C, but not at 2°C. In contrast, plasma T_3 was not significantly affected by photoperiod treatment. Although the number of studies is low, there has not been a consistent effect of photoperiod in regulating thyroid hormones in salmonids (19, 35, 38). GH has been shown to increase T_4:T_3 conversion in some teleosts (10), but plasma T_3 was decreasing when GH was at high levels in all the groups, and there was no correlation between plasma GH and T_3:T_4 ratio. Given that a plasma T_4 "surge" is characteristic of Pacific and Atlantic salmon smolts (12, 31, 35), the absence of changes in plasma T_4 in the 10°C-LDN group is somewhat surprising. Temperature, rather than photoperiod, seemed to be more important in regulating thyroid hormones in the present study. Plasma T_3:T_4 ratio was lower in fish reared at ambient temperature, suggesting that

deiodinase activity may be lower at cold temperatures. However, plasma T_3:T_4 ratio was higher in the ambient temperature group throughout the study, even when temperature was similar in the two groups in May. Although temperature had a clear effect on thyroid hormones, the relationship is complex and not direct.

Changes in plasma GH, IGF-I, cortisol, and thyroid hormones observed in this study are consistent with their interactive effects in mediating environmentally induced changes in smolt development. The ultimate biological role of the environmental cues governing the parr-smolt transformation is to time completion of smolting to coincide with river and ocean conditions that are optimal for smolt survival. These optimal conditions are not only related to season, so that the fish enter the ocean in early summer when near-coast food supplies have increased, but are also related to temperature. In the river, increased temperatures often signify increased water flows, which in turn make downstream migration faster and diminish predation risk in rivers and estuaries. Food availability also usually increases with increased river temperatures. Thus our results which show that low temperature limits the capacity of photoperiod to advance smolting by delaying changes in the endocrine system are consistent with the biological advantages for the organism to delay smolting and migration during unusually cold springs or in higher latitudes.

Perspectives

Many poikilothermic animals use photoperiod to time seasonal events such as migration and reproduction. In salmon and other species, the interaction of temperature and photoperiod may be adaptive for the optimal timing of seasonal responses. In other animals, however, such as those that must respond before increased spring temperatures, an interaction of temperature and photoperiod may not be adaptive. It will thus be of interest to determine whether temperature modulation of the photoperiod response is a common feature of "cold-blooded" vertebrates or whether some animals possess compensatory mechanisms for the influence of temperature on the response to changing day length.

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Address for reprint requests and other correspondence: S. D. McCormick, Conte Anadromous Fish Research Center, Biological Resources Division, USGS, PO Box 796, Turners Falls, MA 01376.

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