Physiology during smoltification in Atlantic salmon: effect of melatonin implants

S. O. Handeland · A. K. Imsland · B. Th. Björnsson · S. O. Stefansson · M. Porter

Abstract Melatonin implants were used to override natural melatonin rhythm in groups of juvenile Atlantic salmon, *Salmo salar*, raised at simulated natural photoperiod (SNP) and constant light (LL) from mid-March until end of August. The experiment contained also both sham control (with non-melatonin implants) and control (no implants). No differences were found in the experimental variables between these two control groups. Growth and food intake were negatively affected by melatonin implantation. Overall, higher GH levels were observed in the SNP melatonin-implanted group, whereas no differences in GH levels were seen between the SNP control, LL control, or the LL melatonin-implanted groups. Highest food intake was seen in the LL control group. No differences in food intake were recorded between the LL melatonin-implanted and SNP control groups. Gill Na^+, K^+, ATPase (NKA) activity was influenced by time as well as the interaction between photoperiod and time. No differences in gill NKA activity or plasma chloride levels following transfer to seawater were seen between the groups with melatonin implants and their controls. Based on the present results, it seems apparent that melatonin does play a role in regulating food intake and growth in Atlantic salmon smolts.

Keywords Melatonin implant · Growth · Food intake · Growth hormone · Atlantic salmon

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Introduction

Smoltification in Atlantic salmon involves complex changes in behavior, morphology, and physiology that pre-adapt the young salmon for life in seawater. Although the physiological mechanisms by which the changes in day length influence the development of hypo-osmoregulatory ability in salmonids are unclear, stimulation through the increase in circulating levels of GH, cortisol, and/or thyroid hormones seems likely (Björnsson 1997; Ebbesson et al. 2008). In smolts kept on natural photoperiod, circulating growth hormone (GH) levels generally increase from January to May

concurrent with an increase in branchial ATPase activity (Stefansson et al. 2007). GH is known to increase food intake and growth (Björnsson 1997) and to stimulate the development of hypo-osmoregulatory ability in smolts (Björnsson et al. 1995, 2002). Considering the effect of increasing day length on circulating GH levels and development of hypo-osmoregulatory ability, little work have been done to investigate the mechanisms behind the translation of photo information in salmon smolts. In salmonids, the melatonin profile has been shown to reflect the prevailing light/dark cycle and has been suggested to be the internal measuring device of day and night and seasonal changes (Bromage et al. 2001; Porter et al. 2001; Aarseth et al. 2010).

Pinealectomy appears to delay the onset of smoltification, whereas the combination of pinealectomy and melatonin implants increased survival rates in sea in March (Porter et al. 1998). In the same study, Porter et al. (op cit.) reported increased growth in Atlantic salmon parr (inserted when fish were 3–7 g) treated with melatonin implants. Whether this was due to increased food intake and/or increased food conversion remains unknown. Increased number of chloride cells and NKA activity in steelhead trout (*Oncorhynchus mykiss*) treated with melatonin implants has been seen (Rourke 1994), suggesting that this hormone play a role during smoltification.

The present study was undertaken to provide more information on the effect of melatonin on circulating GH levels, NKA activity, food intake, and growth in Atlantic salmon during smolting and after transfer to seawater. This was achieved by overriding the natural melatonin rhythm in two experimental groups of juvenile Atlantic salmon, raised at simulated natural photoperiod (SNP) and constant light (LL), by the use of melatonin implants from mid-March until end of August. Two control groups were established; one at SNP and one at LL. All groups were transferred from freshwater to seawater in mid-May when the control fish showed normal morphological signs of smolting.

Materials and methods

Fish stock and rearing conditions

On 11 and 12 March 2002, 600 potential S1 Atlantic salmon smolts from the Lakeland stock (mean weight 60.2 ± 1.7 g) were transferred from the hatchery at Howietoun fish farm to Buckieburn Freshwater Research Facility, Stirling University (latitude 56°20′N). The juvenile salmon were distributed equally ($n = 50$ in each tank) in twelve 1 m^2 with covered fiberglass tanks rearing volume of 440 l supplied with river water at a rate of 15 l/min and at ambient seasonal temperatures (∼3 °C in March and ∼8 °C in May). Oxygen saturation in the outlet water was kept above 80 % throughout the study. Light was supplied by 100 W fluorescent daylight tubes mounted under the tank lid and positioned 0.45 m above the water surface, providing an intensity of approximately 120 lux at the bottom of the tank. On 25 March, a sub-population of 20 fish from each tank was individually tagged (Avid pit-tags, total 160 fish). Melatonin implants (2.5 × 1 mm), containing 18 mg of melatonin ('Regulin'; Schering Agrochemicals, Alexandria, Australia), were used to elevate plasma melatonin levels in the smolts maintained in 4 tanks with 4 tanks left intact as controls. In the remaining 4 tanks (two tanks on SNP and two on LL), fish were given sham implants. A detailed description of the surgical procedure used to implant the fish is given by Porter et al. (1998) and Mazurais et al. (2000). It has been established by the use of sham implants that the surgical procedure itself has minimal effect on the physiological response of the fish (Porter et al. 1998; Mazurais et al. 2000).

Experimental design

On 25 March, the photoperiod in 4 tanks was changed from simulated natural photoperiod (SNP) to constant illumination (LL) in a 2 × 2 factorial design including two photoperiods (SNP and LL) and two treatment groups (controls and melatonin-implanted groups), each treatment with 2 replicate tanks. By mid–May, all fish showed normal morphological signs of smolting, that is, dark fin margins, absence of parr marks, and loose silvery scales. Hence, on 22 May, all individually tagged fishes were carefully transported by car to Macrihanish Research Facility (latitude 55°20 N) and thereafter transferred to the experimental tanks with flow-through seawater until 15 August. All groups were reared in identical tanks and under the same photoperiods and light source as used during the freshwater period. The day length (hours of light (L):hours of darkness (D)) in the SNP group was around L12:D12 in late March, rising to L16:8D during June and then decreasing to L14:D10 in

mid-August. Temperature and oxygen content was recorded daily. During the seawater rearing period, temperature increased from approximately 8 to 16 °C. Water flow was kept at 15 1/min, maintaining oxygen saturation in the water above 80 %. In both freshwater and seawater, all tanks were checked daily and dead fish were removed immediately.

Sampling

Blood plasma and gill tissue were collected from each group in freshwater on 25 March, 18 April, and 21 May and in seawater on 18 June and 15 August ($n = 12$). In addition, to evaluate hypo-osmoregulatory ability during the early seawater period, blood plasma from fish in seawater was collected on 23 May, 28 May, and 6 June. Blood was always collected in heparinized syringes from the caudal vessels and centrifuged for 10 min. at 4 °C and 4,000 rpm. The supernatant was then divided in three equal parts and kept at −70 °C until analyses. Blood samples collected on 23 May, 28 May, and 6 June were analyzed for plasma chloride levels using a Radiometer CMT 10 chloride titrator. Plasma growth hormone levels were assessed in a homologous salmon GH-RIA validated for Atlantic salmon (Björnsson et al. 1994). Gill tissue from the second gill arch was collected for determination of NKA activity. The gill tissue was immediately immersed in ice-cold SEI buffer, frozen at −70 °C, and analyzed according to the procedure of McCormick (1993). Finally, the fish was X-rayed to assess individual food intake. After sampling, all fishes were frozen individually.

Fork length (to nearest 0.1 cm) and weights (to nearest 0.1 g) of individually tagged smolts were determined on 25 March, 18 April, and 21 May, 18 June and 15 August to allow specific growth rate and condition factor to be calculated. Growth rate for individual fish between two dates was calculated as specific growth rate (SGR):

$$SGR = (\ln W_2 - \ln W_1) \times 100/(T_2 - T_1)$$

where W_1 and W_2 are weights at days T_1 and T_2. Fultons condition factor (CF) was calculated from the formula:

$$CF = 100 \times W \times L^{-3}$$

Food and feeding

During the experimental period, all groups were fed a dry diet (EWOS micro, 2 and 3 mm, stored at 5 °C prior to use) according to temperature and fish size, from automatic feeders during daylight between 09:00 A.M and 17:00 P.M. Two days before each sampling, the ordinary diet was replaced with an identical diet containing X-ray dense ballotoni glass beads (Talbot and Higgins 1983). EWOS Innovation (Dirdal, Norway), using the same methods, nutrient composition, color, particles size and texture as the normal commercial diet, prepared this diet. Together with X-radiography, this feed allowed the individual rates of food consumption in fish to be measured. At pre-arranged intervals (see sampling procedures), a sub-population in each tank was euthanized, and immediate death assured by hard blow to the head, and X-rayed (GeR XT-100 X-ray machine, AGFA Structurix DX7, Talbot and Higgins 1983). Following the development of the X-ray plates, the number of marker particles present in the stomach was counted and the amount feed consumed calculated. This calculation was achieved using a standard curve prepared by X-raying known weights of the marked food and assessing the number of ballotini glass beads. A standard curve was prepared by using this information to make a linear regression of the relationship between the weight of the marked feed and number of ballotini particles, see below:

$$\begin{aligned} &\text{Feed intake}(\text{g feed} \times \text{g/fish}) \\ &= 0.05 \times (\text{ballotini glass beads}) \\ &\quad + 0.05, (n = 12, \ r^2 = 0.97) \end{aligned}$$

Statistical analysis

All statistical analyses were performed with Statistica^{TM} 11.0. A Kolmogorov–Smirnov test (Zar 1996) was used to assess for normality of distributions. The homogeneity of variances was tested using the Levene's F test (Zar 1996). Three-way nested ANOVA, where replicates are nested within photoperiods and treatments groups, was used to test mean values among groups and was followed by a Student–Newman–Keuls test in the case of significant ANOVAs.

Individual growth trajectories were analyzed using a growth curve analysis model (GCM, Chambers and

Miller 1995) which is an extension of the multivariate repeated measurements analysis of variance (MANOVA) model. The model equation of the GCM had the form:

$$\mathbf{Y}(n \times p) = \mathbf{X}(n \times q)\mathbf{B}(q \times p) + \mathbf{E}(n \times p)$$

where $\mathbf{Y}$ $(n \times p)$ are the growth at age vectors, $\mathbf{y} = (y_1, y_2, \ldots, y_p)$ for each p (age) measurements on n individual fish; $\mathbf{X}$ $(n \times q)$ is the design matrix or the set of extraneous variables measured for each individual, that is, $q = \text{age}_p + \text{photoperiod}_i$ ($i = \text{SNP}$, LL) $+ \text{treatment}_j$ ($j = \text{control, melatonin implant}$); $\mathbf{B}$ $(q \times p)$ is the matrix of parameters estimated by the model; $\mathbf{E}$ $(n \times p)$ is the matrix of deviations for each individual from the expected value of $\mathbf{Y} = \mathbf{XB}$.

A linear regression was used to test for differences over time between experimental groups. A significance level (α) of 0.05 was used if not stated otherwise.

As statistical analysis revealed no differences in growth, food intake GH, and hypo-osmoregulatory ability between sham groups and their controls, the sham-operated groups are excluded in all figures.

Results

Growth and condition

Individual growth trajectories were affected by photoperiod both in freshwater (MANOVA $_{(\text{photoperiod})}$, Wilk's $\Lambda_{2,150} = 0.69$, $P < 0.001$) and seawater ((MANOVA $_{(\text{photoperiod})}$, Wilk's $\Lambda_{2,140} = 0.91$, $P < 0.05$). In freshwater, no growth differences were seen between the melatonin-implanted groups and their controls (Table 1, $P > 0.15$). A significant increase in mean weight was observed in the LL groups between 25 March and 21 May (linear regression, Fig. 1, $P < 0.001$). In contrast, no changes in size were recorded in the SNP groups during the same period.

During the seawater period, highest growth (Student–Newman–Keuls (SNK) test, $P < 0.05$, Table 1) was observed in the LL control group. Furthermore, higher growth was seen during the first month in seawater in the control groups compared to their respective melatonin-implanted groups (SNK test, $P < 0.05$). On 15 August, mean weight was higher in the LL control compared to the LL melatonin group (SNK test, $P < 0.05$, Fig. 1), which in turn was higher than those of the SNP control ($P < 0.05$) and the SNP melatonin groups ($P < 0.05$).

A significant decrease in condition factor (CF) was recorded in the SNP control (linear regression, $P < 0.05$) during the freshwater period, whereas no changes were seen in the SNP melatonin-implanted group ($P < 0.05$, Fig. 2). In contrast, a significant increase in CF was observed in all the LL groups during the same period ($P < 0.05$). Following transfer to seawater, a transient decrease in CF was recorded in all groups. After 3 months in seawater, CF was higher in fish at LL compared with those at SNP ($P < 0.05$), whereas no differences were observed between the melatonin-implanted groups and their controls.

Food intake

Food intake was influenced by photoperiod (three-way nested ANOVA, $P < 0.001$) and melatonin implantation ($P < 0.001$, Fig. 3). Overall, the highest food intake was observed in the LL control (5.7 g × g fish^{-1}), significantly higher than in the LL melatonin (4.8 g × g fish^{-1}, SNK test, $P < 0.05$) and SNP control (4.7 g × g fish^{-1}, SNK test, $P < 0.05$). No differences in food intake were observed between the LL melatonin and the SNP control group, whereas both groups showed a food intake significantly higher than the SNP melatonin group (3.1 g × g fish^{-1}, SNK test, $P < 0.001$). A significant increase in food intake was observed in both the SNP control and LL control groups between 25 March and 21 May (linear regression, $P < 0.001$). In contrast, no changes in food intake were recorded in melatonin-implanted groups (SNP and LL) between 25 March and 18 April, whereas an increase was evident between 18 April and 21 May ($P < 0.01$). Following transfer to seawater, a decrease in food intake was observed in all groups ($P < 0.01$). On 18 June and 15 August, higher food intake was observed in the LL control compared to the SNP melatonin-implanted group ($P < 0.01$). No differences were seen between the SNP control and LL melatonin-implanted and the other groups. No further changes in food intake were recorded during the remainder of the study.

Hypo-osmoregulatory ability

Gill NKA activity was influenced photoperiod (three-way nested ANOVA, $P < 0.001$, Fig. 4). No differences were observed between the melatonin-implanted groups and their controls. In the LL groups, a
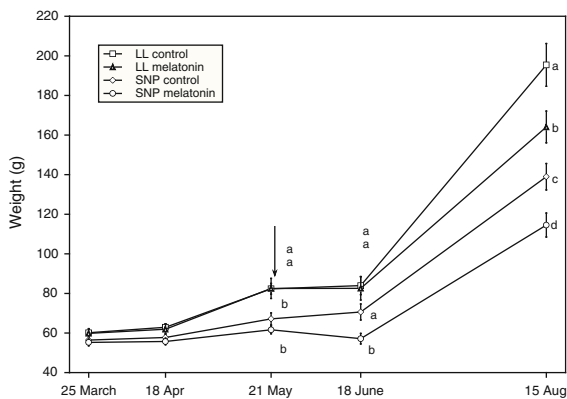
Table 1 Specific growth rates for weight (SGR % day^{-1}) in treated (melatonin implanted) and untreated (control) juvenile Atlantic salmon at two different photoperiods (SNP and LL) in freshwater (25 March–21 May) and after transfer to seawater (22 May–15 Aug)

Group	Freshwater 25 March–21 May	Seawater 22 May–15 August
LL control	0.28[a]	1.04[a]
LL melatonin	0.27[a]	0.87[b]
SNP control	0.15[b]	0.78[b]
SNP melatonin	0.09[b]	0.70[c]

Different letters indicate significant differences among groups (Student–Newman–Keuls test, $P < 0.05$)

Fig. 2 Condition factor in treated (melatonin implanted) and untreated (control) juvenile Atlantic salmon at two different photoperiod (SNP and LL) in freshwater and after transfer to seawater. See Fig. 1 for further details

Fig. 1 Growth in treated (melatonin implanted) and untreated (control) juvenile Atlantic salmon at two different photoperiod (SNP and LL) in freshwater and after transfer to seawater. *Different letters* (Student–Newman–Keuls test, $P < 0.05$) indicate significant differences among groups. The *arrow* indicates timing of seawater transfer. Values are given as mean ± SE ($n = 12$)

Fig. 3 Food intake in treated (melatonin implanted) and untreated (control) juvenile Atlantic salmon at two different photoperiod (SNP and LL) in freshwater and after transfer to seawater. See Fig. 1 for further details

significant increase (linear regression, $P < 0.001$) in gill ATPase activity was observed between 25 March and 21 May, reaching peak levels at approximately 8–10 µmol ADP mg protein^{-1} h^{-1}. No significant differences in ATPase activity were seen in the SNP groups between 25 March and 18 April. However, activity increased in these groups between 18 April and 21 May, reaching peak levels of approximately 10–12 µmol ADP mg protein^{-1} h^{-1}. Following transfer to seawater, gill NKA activity decreased to levels between 5 and 7 µmol ADP mg protein^{-1} h^{-1} in all groups ($P < 0.05$). No differences in enzyme activity were seen among groups for the rest of this study.

No significant differences in plasma chloride levels were observed among groups following transfer to
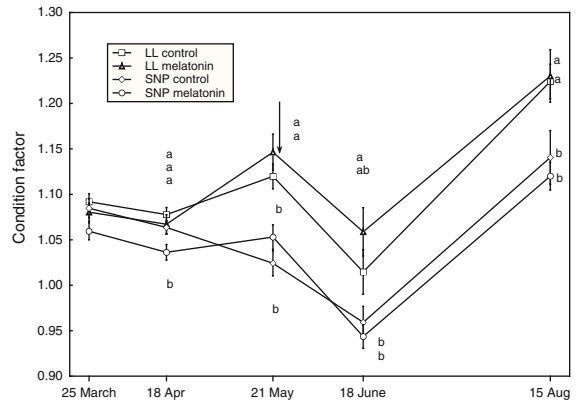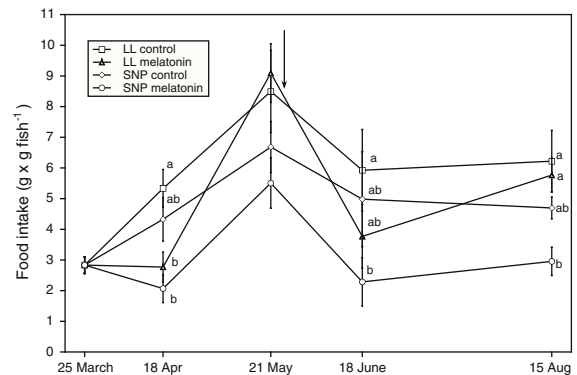
seawater. Plasma chloride levels increased transiently in all groups to peak values at approximately 150–155 mmol l^{-1} during the first 24 h and were followed by a decrease and stabilization at approximately 145 mmol l^{-1} after 14 days in seawater.

Plasma growth hormone levels

Growth hormone (GH) levels were significantly affected by melatonin implants (three-way nested ANOVA, $P < 0.001$) and the interaction between melatonin implants and photoperiod ($P < 0.01$, Fig. 5). No significant differences in plasma GH levels were observed among groups during the
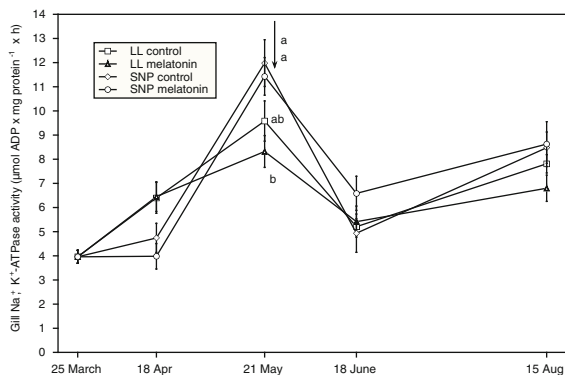
Fig. 4 Gill Na^+, K^+, ATPase activity in treated (melatonin implanted) and untreated (control) juvenile Atlantic salmon at two different photoperiod (SNP and LL) in freshwater and after transfer to seawater. See Fig. 1 for further details

Fig. 5 Plasma GH levels following seawater transfer in treated (melatonin implanted) and untreated (control) juvenile Atlantic salmon at two different photoperiods (SNP and LL). See Fig. 1 for further details

freshwater period. Between 25 March and 21 May, a significant increase in plasma GH levels was observed in the control groups (linear regression, $P < 0.05$). In the melatonin-implanted groups, a significant rise in plasma GH was recorded between 25 March and 18 April (linear regression, $P < 0.05$), followed by a decrease in GH in only the LL melatonin-implanted group between 18 April and 21 May ($P < 0.05$).

A transient increase in plasma GH was recorded in all groups following transfer to seawater (linear regression, $P < 0.05$). The greatest increase was seen in the SNP melatonin-implanted group, with GH levels significantly higher than the SNP control on 18 June and 15 August. ($P < 0.05$). No differences in circulating GH levels were seen in the SNP melatonin-implanted group between 18 June and 15 August. In contrast, a significant decrease in GH was evident in all other groups ($P < 0.05$), reaching GH levels significantly below the SNP melatonin-implanted group on 15 August ($P < 0.05$).

Discussion

In the present study, growth and food intake was negatively affected by melatonin implantation. The results of the present study contrast the results of Porter et al. (1998) who reported higher growth in Atlantic salmon parr treated with melatonin implants. In the study of Porter et al. (1998), all groups were implanted from early July at high temperatures compared to mid-March and low temperatures in the present study. The size at
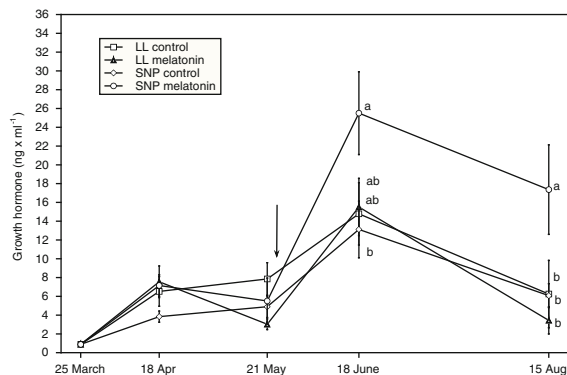
implantation was 3–7 g in the study of Porter et al. (1998) compared to 60 g in present study. Bromage et al. (1995) and Sanchez-Vazquez et al. (2000) proposed that the effect of pinealectomy was dependent on photoperiod, temperature, and also timing of the operation within the developmental cycle. Therefore, it is possible that the reported discrepancy in growth is related to the different experimental protocols as the two groups were implanted at different temperatures, different times of the year, and different stages of smolt development.

In teleost fishes, photoreceptor cells within the pineal gland produce melatonin with synthesis increasing during the hours of darkness (Randall et al. 1995; Porter et al. 1998; Falcón et al. 2010). Plasma melatonin profiles reflect the prevailing day/night cycle and have been suggested to play a role in the interpretation of photoperiod signal and in the regulation of endocrine activity in salmonids (Bromage et al. 1995, 2001; Porter et al. 1998; Aarseth et al. 2010; Falcón et al. 2010). This suggests that a constant high level of plasma melatonin may be interpreted as a prolonged period of darkness, which in juvenile salmon is known to influence negatively on growth (Skilbrei et al. 1997). However, in the present study, no effects of melatonin implants on circulating GH levels and growth were recorded during the freshwater period, whereas during the seawater period the groups with melatonin implants displayed lower growth concomitant with significant higher GH levels. It appears likely that when GH levels increase rapidly, as they do in salmonids in response to increased day length in freshwater (Björnsson, 1997) and seen here during the seawater phase, an initial GH-induced

stimulation of growth results in a positive correlation, as seen in the early part of the seawater phase of the present study. However, it is likely that subsequently, a new equilibrium is reached between the secretion rate of GH, GH-receptor densities, and GH turn-over rate, leading to a situation where low GH plasma levels are associated with rapid growth (Nordgarden et al. 2006) and seen for the SNP groups in the present study. Earlier findings have showed that melatonin implants increased weight in Atlantic salmon parr (Porter et al. 1998) but reduced body weight and growth rate in trout (Taylor et al. 2005). In the study of Taylor et al. (2005) where growth was negatively affected by high melatonin levels in implanted fish, plasma IGF-I levels were not significantly different between controls and implants, suggesting that melatonin does not act directly on the GH-IGF axis to control growth, as lower IGF-I levels are generally associated with poorer growth (Imsland et al. 2007, 2008). Falcón et al. (2010) reviewed current knowledge of the interrelationship between melatonin, growth and feeding and suggested that the observed discrepancies (see above) might reflect a seasonal regulation of feeding and growth. This seems plausible given the differences in the melatonin, GH, and growth response seen in the present study and the study of Taylor et al. (2005) (both conducted during spring and summer) on one hand and the study of Porter et al. (1998) (conducted in winter) on the other.

Following seawater exposure, circulating GH levels increased transiently in all groups. This endocrine response appears to be a consistent feature when salmonids are transferred to seawater (see Björnsson 1997). Furthermore, higher GH levels, decreased food intake, and low overall growth were all recorded in the melatonin-implanted SNP group compared to the SNP control group, thus resembling the situation observed in stunted Atlantic salmon smolts (Björnsson et al. 1988). These results may indicate that long-term melatonin treatment can be interpreted as a short day signal, further suggesting a link between melatonin, GH, and growth in salmon smolts. According to Hernandez-Rauda et al. (2000), increasing melatonin levels from daytime to night-time has been associated with a decrease in the content of dopamine in hypothalamus and pituitary. Dopamine has been shown to influence on GH secretion in rainbow trout (*Oncorhynchus mykiss*, Augustsson et al. 2000). In addition, a high degree of common localization of

2–125 iodo melatonin-binding sites and immunoreactive neurons for growth-hormone-releasing factor has been reported in the brain of Atlantic salmon (Ekström and Vanacek 1992). Furthermore, studies of rainbow trout have revealed the presence of melatonin receptors Mel1a and Mel1b in peripheral tissues, with both receptors expressed in retina, heart, muscle, and intestine (Mazurais et al. 1999, 2000). These findings may suggest a mechanism by which circulating levels of melatonin could bind directly to its receptors in, for example, intestine and muscle, thereby influencing nutrient uptake and growth. Falcón et al. (2003) investigated whether melatonin acts directly on the pituitary to control GH and prolactin (PRL) secretion in rainbow trout. They showed that 2-[125I]-iodomelatonin, a melatonin analog, binds selectively to membrane preparations and tissue sections from trout pituitaries. They also reported that melatonin modulates GH and PRL secretion as GH release was reduced in the presence of picomolar concentrations of melatonin. At higher concentrations, the effects of melatonin became stimulatory. The authors concluded that the complexity of the observed responses suggests several types of melatonin receptors might be involved.

Recently, melatonin administration has been suggested to have anorexigenic effect in fish. Piccinetti et al. (2010) investigated the effect of two different melatonin doses (100 nM and 1 μM) administered for 10 days, via water, to zebrafish, *Danio rerio*, adults was evaluated at both physiological and molecular level, and the effect of melatonin was considered in relation to the most prominent systems involved in appetite regulation. The results obtained indicate that melatonin significantly reduces food intake and the reduction is in agreement with the changes observed at molecular level. A significant increase in genes codifying for molecules involved in feeding inhibition, such as leptin and MC4R, and a significant reduction in the major orexigenic signals including ghrelin, NPY, and CB1 were found. This is in line with the present findings where growth and food intake were negatively affected by melatonin implantation. In contrast, Aarseth et al. (2010) found higher melatonin levels in implanted Arctic charr (*Salvelinus alpinus*) (average mid-day plasma melatonin levels 1,106 ± 147 pg/ml) compared to those of fish with natural plasma melatonin levels (vehicle implanted and untreated fish with average mid-day plasma

melatonin levels of 94 ± 13 and 58 ± 6 pg/ml, respectively). However the high melatonin levels in implanted fish did not affect feed intake, body mass or body length, or the timing of the seasonal growth rhythm in the Arctic charr. This issue clearly warrants further investigations.

The data presented here may provide additional support to the results of Handeland et al. (2003) who reported a photoperiod-induced change in feeding pattern in wild and selected salmon smolts. Following transfer to seawater, no differences in food intake and growth were recorded between the LL melatonin-implanted and the SNP control groups. In fishes, photoperiod information can enter through both the eyes and the pineal window (Guthrie and Muntz 1993; Ekström and Meissl 1997). Besides photoreceptor cells and supporting cells, the pineal gland also includes nerve cells with projections that terminate in pre-tectum and dorsal thalamus (Guthrie and Muntz 1993; Ekström and Meissl 1997). In addition, both melatonin-binding sites and the nerve cell projections show a common localization, further indicating an overlap of optic stimulation in the same area (Ekström and Vanacek 1992). Taken together, this may suggest that photoperiod input through the eye and/or pineal nerves in the LL group may have masked the physiological effect of melatonin implants. Future studies are needed to examine these possibilities.

In Atlantic salmon, changes in plasma melatonin profiles reflect changes in day length and are assumed to provide the fish with exact seasonal information (Porter et al. 1999, 2001; Bromage et al. 2001, Falcón et al. 2010). Hence, by using melatonin implants, to override the natural melatonin rhythm from 25 March onward, we expected to see a disruption or altered timing of the normal smolt development in the same groups. However, independent of photoperiod, no differences in gill Na +, K +, ATPase activity, condition factor and seawater tolerance were observed between the implanted groups and their respective controls. There are at least two ways to explain this as Bromage et al. (1995) suggested that the alternating periods of high and low melatonin concentrations might be necessary to transmit photoperiod information in fish. By using melatonin implants to mask this rhythm, the endogenous rhythm could start to run free and thus further affect the completion of the smolting process. Alternatively, the effect of melatonin implants could potentially have caused a down-

regulation of the melatonin receptors in the brain as suggested by Porter et al. (1998).

The higher overall growth found in the LL groups in freshwater is in agreement with previous studies on juvenile Atlantic salmon (Stefansson et al. 2008) and can further be explained by a higher food intake in this group compared to smolts raised at SNP. The present results correspond to the results of Handeland et al. (2003), who reported enhanced growth and increased food intake in smolting salmon reared at constant light compared to simulated natural photoperiod. Similar results have been found in studies with turbot (*Scophthalmus maximus*, Imsland et al. 1997), halibut (*Hippoglossus hippoglossus*, Imsland et al. 2009), and cod (*Gadus morhua*, Imsland et al. 2012), although the underlying mechanism causing the positive effect of continuous light on growth and food intake remains unknown.

In conclusion, the present study showed that growth and food intake were negatively affected by long-term melatonin implantation. No differences in food intake were recorded between the LL melatonin-implanted and SNP groups, suggesting a compensatory mechanism of photoperiod on the effect of melatonin implantation.

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