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Vertebrae of the trunk and tail display different growth rates in response to photoperiod in Atlantic salmon, *Salmo salar* L., post-smolts

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Abstract

This study shows that exposure to continuous light induces regional differences in growth in the vertebral column of Atlantic salmon under-yearling post-smolts reared in seawater cages. Exposure to continuous light during the period from January to the summer solstice (June) generally produced significantly larger fish than those reared under natural light. Until April, however, the fish exposed to continuous light grew more slowly, as reflected in the vertebral column by decreased growth in the truncal vertebrae, while the growth of the tail vertebrae was similar in the two groups. From April to June the fish exposed to continuous light displayed the fastest growth, with a higher growth rate in the vertebrae of the trunk than among fish reared under natural light. The mineral rate and mechanical strength of the vertebrae at the summer solstice was significantly lower in fish exposed to continuous light. Our results suggest that the growth of different regions of the vertebral column of salmon may be regulated partly independently, and that mineralisation is also affected during fast growth.

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Keywords: Atlantic salmon; *Salmo salar*; Vertebral column; Vertebra; Growth; Photoperiod; Mineralisation; Mechanical properties

1. Introduction

Seasonal changes in photoperiod are probably the most important environmental cue that synchronizes

smoltification, somatic growth and sexual maturation in salmonids (Hoar, 1988; Boeuf and Falcon, 2001; Bromage et al., 2001). Information about the photoperiod is conveyed by the pineal organ via its diurnal pattern of secretion of melatonin. Unlike most teleosts, which possess a self-sustained oscillator that drives the melatonin secretion rhythm, melatonin secretion in salmonids is regulated by the photoperiod

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and by the direction of change in the light–dark cycle (Ekström and Meissl, 1997). Melatonin also modulates pituitary activity and directly affects the secretion of both growth hormone and prolactin (Falcón et al., 2003). When cultured Atlantic salmon, after transfer to seawater, are exposed to continuous light, their rate of somatic growth increases, and the proportion of fish that mature sexually decreases relative to those reared under natural light (Saunders and Harmon, 1988; Kråkenes et al., 1991; Hansen et al., 1992). When continuous light is restricted to the period from January to the summer solstice, the growth rate is lower in the period from January to March–April, and higher from March–April to summer solstice, than in salmon under natural light (Oppedal et al., 1997, 1999; Nordgarden et al., 2003a). Continuous light thus influence the seasonal growth pattern, and may also be used as a model for the study of somatic processes related to fast growth. In salmon, the use of this model has been restricted to growth studies on the axial muscle cellularity (Johnston et al., 2003), quality (Nordgarden et al., 2003b) and composition (Nordgarden et al., 2003c). How the growth rate affects other tissues, such as the vertebral column, has yet to be elucidated.

The vertebral column of teleosts consists of a series of amphicelous vertebrae that have specific characteristics. Based on characters such as the presence of ribs, neural and haemal arches and the dimensional proportions of the vertebral body, the vertebral column may be divided into regions, using various criteria (Ford, 1937; Ramzu and Meunier, 1999; Morin-Kensicki et al., 2002). The vertebral column of the Atlantic salmon may be divided into four regions: cranial trunk, caudal trunk, tail and tail fin (Kacem et al., 1998). Studies of mammals have revealed that the regionalization of the vertebral column is achieved in part by specific patterns of *Hox* gene expression (van der Hoeven et al., 1996; Prince et al., 1998; Ahn and Gibson, 1999). In teleosts there also seems to be a correlation between *Hox* gene expression and some specific vertebral regional features (Morin-Kensicki et al., 2002).

A solid comprehension of regional functionality in the vertebral column of teleosts, and how the different regions respond to growth stimuli, for instance, is lacking. The objectives of the present study were therefore to use continuous light to alter growth

rates, and to study the effect of different growth rates on vertebral growth and mineralization in the different regions of the vertebral column of Atlantic salmon.

2. Materials and methods

2.1. Experimental design

Atlantic salmon (*Salmo salar* L.) under-yearling post-smolts ($n=2264$) with an average weight of 75 g were transferred to a seawater cage at the Institute of Marine Research, Matre, Norway (61°N) 2 months before the start of the experiment. One week after transfer to seawater, 924 fish were tagged with Trovan® transponders ID 101 (BTS Scandinavia AB, Sweden), and their adipose fins removed.

The experiment was designed with one experimental and one control group, each with three replicates in separate cages, and the experiment lasted from mid January until the summer solstice (June 21). At the start of the experiment, the fish were randomly allocated to six cages (5 × 5 × 7 m), so that each cage contained 154 tagged and 220 untagged fish. Three cages of the experimental group were exposed to continuous light, while the three cages of the control group received natural light.

For continuous illumination, one asymmetric metal halide lamp per cage was employed (EUROFLOOD, Siemens, Trondheim, Norway; Metal halide lamp: Osram Powerstar HQI-TS 150W/NDL UVS). The lamps were mounted on the side of the cages, 2 m above the water surface, yielding an illuminance of 105 ± 7 lux (lx) at a depth of 1 m. A light-tight barrier separated the illuminated cages from the others. The fish were reared at ambient temperature, which increased from 7.9 °C in January to 10.5 °C in June (measured at a depth of 5 m). The salinity at 5 m was stable at approximately 31‰.

The fish were fed Bio-optimal® dry feed (BioMar Ltd, Trondheim, Norway), to excess, using a computer-operated feeding system (ARE, Storebø, Norway). Three pellet sizes (3 mm, 4 mm and 6 mm) were used as the fish grew throughout the experiment. To control sea lice infestation, the fish were given SLICE® at a dose of 0.5% of biomass per day (Schering-Plough AS, Farum, Denmark) for 1 week in the middle of the experimental period.

2.2. Sampling, radiography and vertebral morphometry

In all the tagged fish ($n=462$ per group), fork length (FL=the length from the snout to the indentation of the tail fin) and body mass were recorded on January 13th, February 3rd, March 25th, April 29th and June 19th. In addition, on January 13th, April 29th and June 19th, 30 untagged fish per group (10 per cage) were measured for fork length and body mass and lateral radiographs were taken for analysis of vertebrae morphology. The condition factor (CF) was calculated according to the formula: $CF=(M_b/L^3) \times 100$. For measurements of mineral rate, the vertebral columns were dissected from 36 radiographed fish from June 19th, i.e. 18 (6 per cage) from each of the two groups. For measurement of mechanical properties of the vertebrae, five radiographed fish of equal length (NL 33.6 cm \pm 0.30 S.D. and LL 33.5 cm \pm 0.34 S.D.) from each group were sampled at the termination of the experiment. Radiographs were made with a portable X-ray apparatus (HI-Ray 100, Eickenmeyer Medizintechnik für Tierärzte e.K., Tuttlingen, Germany), the images were digitalized by scanning, and the length and dorso-ventral diameter (hereafter denoted diameter) of each vertebral body were measured by employing image analysing software (Image-Pro Plus, version 4.0). Malformed vertebrae were not included in the analysis, since the incidence of malformed vertebrae was low in both groups, 0.6% in the NL group and 0.7% in the LL group. At sampling the fish were anaesthetized by immersion in seawater containing metomidate hydrochloride (7 mg/l) (Wild-life Pharmaceuticals, CO, USA).

2.3. Estimation of vertebral growth

In the 30 untagged fish from each group, sampled in January, April and June, the sum of vertebral body lengths, for each fish, was highly correlated with the fork length. This correlation was used to estimate vertebral body lengths for the total populations in both groups. This was done according to the following procedure:

In order to obtain standard vertebral body length (SVBL), the vertebral body length (VBL) of each

vertebra (V1→V58) was divided by the sum of the vertebral body lengths of each fish.

$$SVBL_{Vn} = VBL_{Vn} / \sum_{V1}^{V58} VBL$$

n =vertebra number.

The mean standard vertebral body length (MSVBL) was calculated for each vertebra and cage at each sampling point.

$$MSVBL_{Vn} = 1/10 \sum_{i=1}^{10} SVBL_{Vn}$$

i =fish number at each sampling point (10 fish per cage), n =vertebra number.

The equation expressing the correlation between the sum of vertebral body length (ΣVBL) and the fork length (FL) was: $y = -1.1647x + 0.7051$, $r^2 = 0.99$. By employing this equation and the mean fork length MFL of the tagged fish ($n=154$ per cage), the mean cage-wise ΣVBL of the tagged fish was estimated ($E\Sigma VBL$) for each cage at each sampling point.

$$E \Sigma VBL = -1.1647MFL + 0.7051.$$

Estimates of the mean vertebral body length (EMVBL), for each vertebra in the tagged fish in each cage, were made by multiplying the mean standard vertebral body length (MSVBL) with the estimated sum of vertebral body length ($E\Sigma VBL$).

Estimates of specific growth rates (SGR, % per day) of the length of the vertebral bodies were based on the estimated mean vertebral body length for each cage ($n=3$ per group), and were calculated according to the formula: $SGR = (e^q - 1) \times 100$ (Ricker, 1958; Houde and Scheckter, 1981), where $q = [\ln(EMVBL_2) \div \ln(EMVBL_1)] / (t_2 \div t_1)$.

The vertebral column was divided into four main regions based on Kacem et al. (1998): region 1 (cranial trunk) comprise V1→V8; region 2 (caudal trunk) comprise V9→V30; region 3 (tail) comprise V31→V49 and region 4 (tail fin) comprise V50→V58. Most of the vertebrae of regions 1 and 2 are rib-bearing and situated above the abdomen. Region 3 is characterized by fused haemal arches, while region 4 forms the operative base of the tail fin.

2.4. Mechanical testing of the vertebrae

Before load-deformation measurements were made, each vertebra (V1→V58) was dissected free, and the neural and haemal arches were cut near their attachments to the vertebral body. Each vertebra was subjected to a compressive load along the cranial–caudal axis, employing a TA-XT2 Texture Analyzer (Stable Micro Systems, Haslemere, UK) with a progressing piston (6 mm/min). The resulting load-deformation data were continuously recorded, and the stiffness and yield load were calculated for each vertebra according to Fjelldal et al. (2004).

2.5. Mineral rate

Individual vertebrae were dissected, and for each fish, the vertebrae within the same region of the vertebral column were pooled. The vertebrae were defatted in hexane, dried overnight at 90 °C, and analysed according to Kacem et al. (2000).

2.6. Statistics

Differences between groups in fish growth (length, weight and condition factor) and vertebral body morphology (vertebral body length and the ratio between the vertebral body length and the vertebral body dorso-ventral diameter) were tested using a 2-way mixed model ANOVA with cages as random factor nested in groups, followed by a Newman–Keuls test; $P < 0.05$ was considered significant. The statistical analyses of vertebral morphology were based on mean values within different regions of the vertebral column. Group differences in estimated vertebral growth rate within the trunk and tail regions were tested using a 2-way ANOVA with group and vertebral number as categorical predictors. Group differences in the biomechanical properties of the vertebral bodies throughout the vertebral column were tested using a 2-way ANOVA with group and vertebral number as categorical predictors. Group differences in the mineral rate of the vertebral bodies were tested using a 2-way ANOVA with group and vertebral region as categorical predictors, followed by a Newman–Keuls test. $P < 0.05$ was regarded as statistically significant.

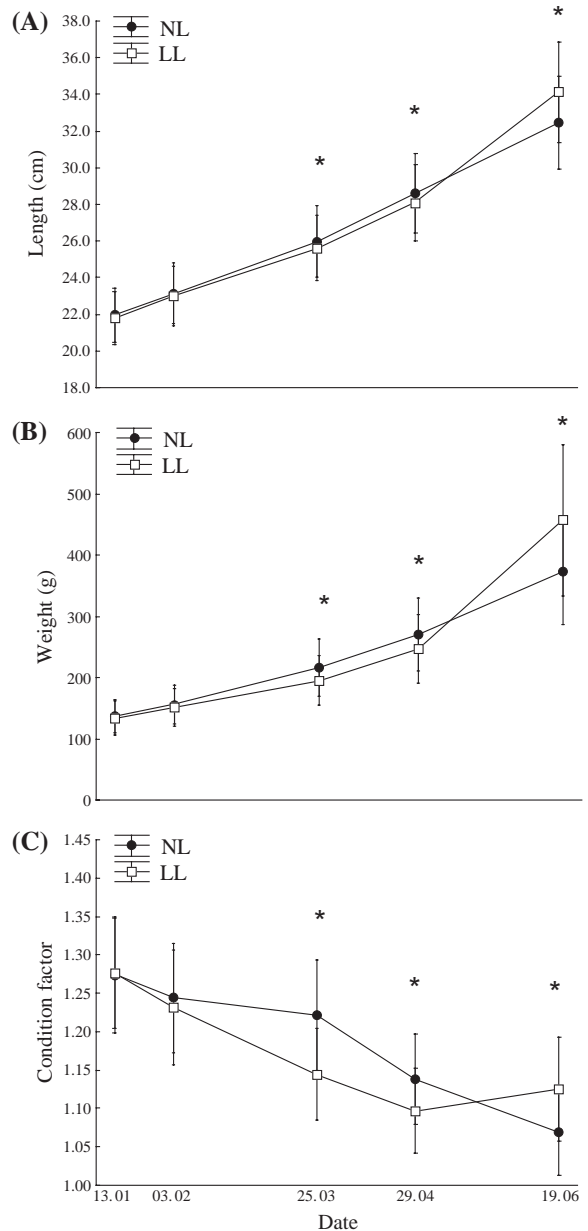


Fig. 1. Fish growth (mean values \pm S.D.). (A, B, C) Changes in (A) length, (B) weight and (C) condition factor in fish reared under natural light (NL) ($n=460$) and continuous light (LL) ($n=460$). *Significant differences between groups within a sampling.

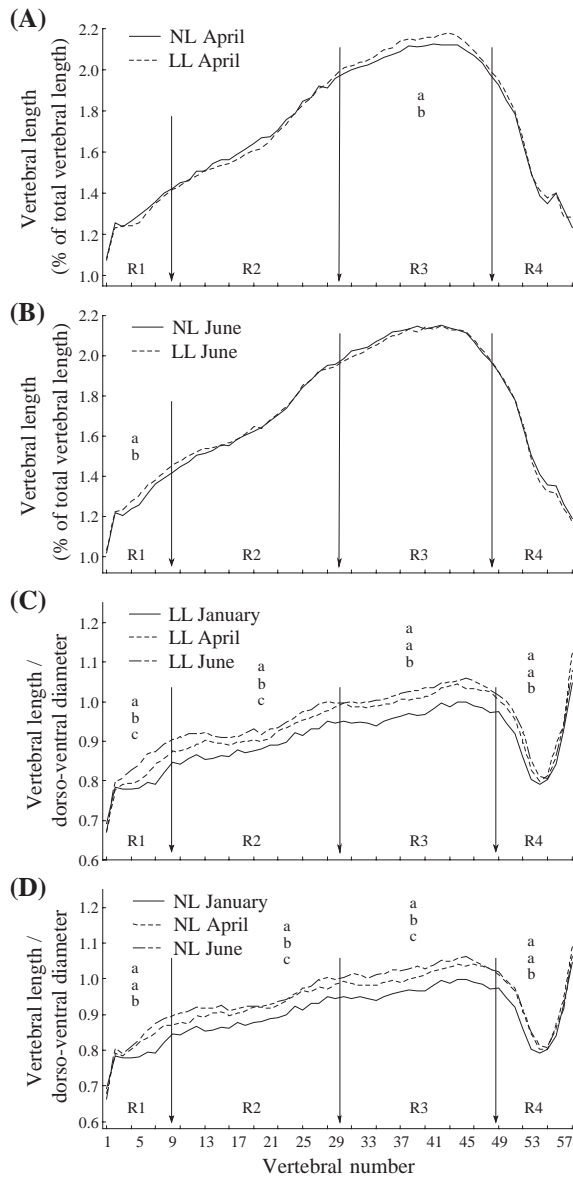


Fig. 2. Vertebral body (V) morphology (mean values) in different regions of the vertebral column: R1 (cranial trunk, V1→V8), R2 (caudal trunk, V9→V30), R3 (tail, V31→V49) and R4 (tail fin, V50→V58). (A, B) Measured vertebral body length (% of the sum of vertebral body lengths) in fish reared under natural light (NL) ($n=30$) and continuous light (LL) ($n=30$) in (A) April and (B) June. (C, D) Vertebral body proportions (measured vertebral body length/measured dorso-ventral diameter) ($n=30$ per group) in January, April and June under (C) continuous light and (D) natural light. Different lower case letters indicate significant differences between groups within a vertebral region.

3. Results

3.1. Somatic growth

In the period from February to April the group exposed to continuous light grew significantly more slowly in both weight and length than those in natural light (Fig. 1A, B), and the condition factor declined in both groups, indicating a relatively higher rate of growth in length than in weight, especially in the group exposed to continuous light (Fig. 1C). Between April and the summer solstice the group exposed to continuous light grew significantly faster in both length and weight (Fig. 1A, B), although most in

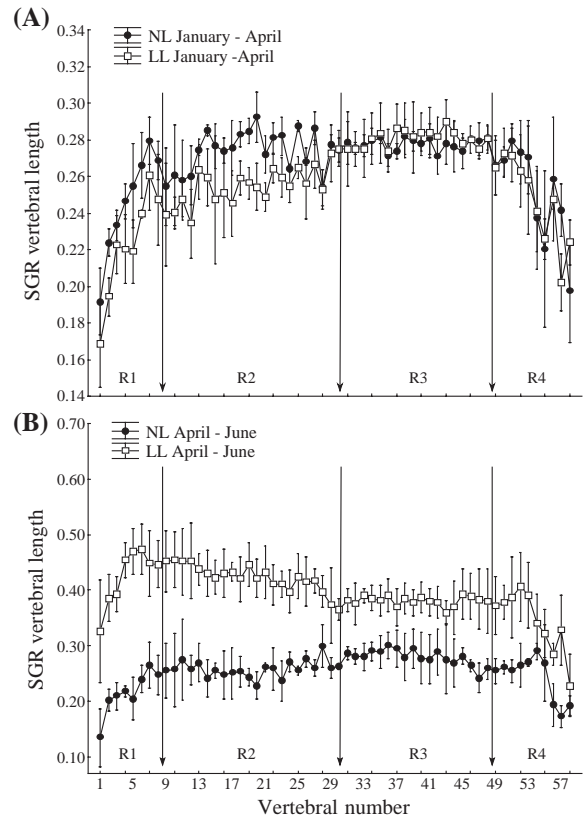


Fig. 3. Estimated specific growth rates (SGR, mean values \pm S.D.) along the cranio-caudal axis of the vertebral bodies (V) in fish reared under natural light (NL) and continuous light (LL). The different regions of the vertebral column are indicated: R1 (cranial trunk, V1→V8), R2 (caudal trunk, V9→V30), R3 (tail, V31→V49) and R4 (tail fin, V50→V58). (A) In the period from January to April, and (B) in the period from April to June.

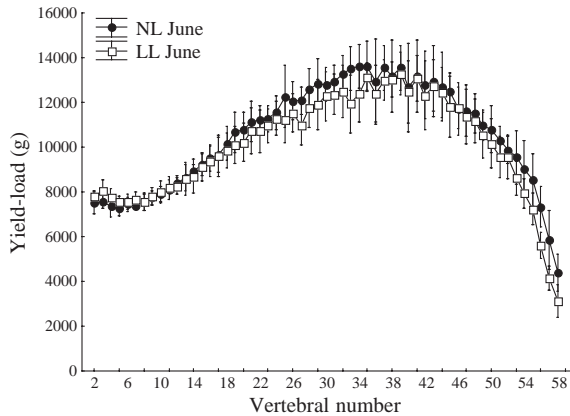


Fig. 4. Yield-load (mean values \pm S.D.) measured at the end of the experiment in fish reared under natural light (NL) ($n=5$) and continuous light (LL) ($n=5$), in different regions of the vertebral column: R1 (cranial trunk, V1→V8), R2 (caudal trunk, V9→V30), R3 (tail, V31→V49) and R4 (tail fin, V50→V58).

weight, resulting in an increase in condition factor (Fig. 1C).

3.2. Growth rate of the vertebral column

The distribution of vertebral body average lengths along the vertebral column was significantly different between the groups both in April and June (Fig. 2A, B). In April the vertebrae of region 3 accounted for a significantly higher proportion of the total vertebral length in the group exposed to continuous light than in those reared in natural light (Fig. 2A), whereas in June vertebrae of region 1 made up a significant higher portion of the total vertebral length (Fig. 2B). The dimensional proportions of the vertebral bodies changed significantly with time within both groups, with a growth in length relatively higher than that of the diameter. In both groups the increase in the ratio between vertebral length and diameter was significant in both periods: from January to April and from April to June (Fig. 2C, D). This may reflect that the cone angle of the amphicoel decreased, and eventually became less than 45° .

The estimated SGR of vertebral body length was significantly lower in the group exposed to continuous light from January to April (Fig. 3A), while from April to June the estimated SGR of vertebral body length was significantly higher in this group (Fig. 3B). The group exposed to continuous light displayed a

significantly lower SGR of vertebral body length in the trunk regions (pooled R1 and R2) in the period from January to April, whereas the SGRs in the tail regions (pooled R3 and R4) of the two groups were equal (Fig. 3A). In the period from April to June the group exposed to continuous light displayed a significantly higher SGR in vertebral body length in all regions. The difference in growth rate was most pronounced in the trunk regions (Fig. 3B).

3.3. Biomechanics of the vertebrae

In June, the vertebrae from the group exposed to continuous light had a significantly lower overall

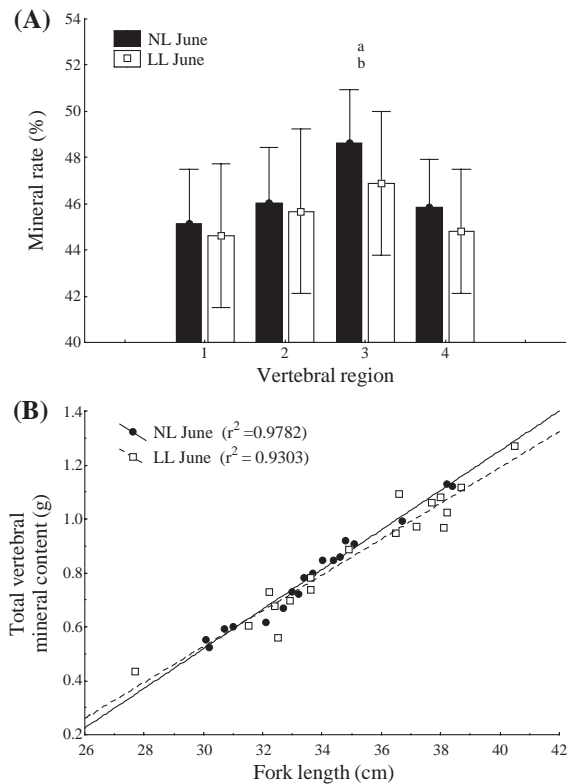


Fig. 5. Mineral analysis (mean values \pm S.D.) of vertebral bodies from fish sampled in June, reared under natural light (NL) ($n=18$, 6 per cage) and continuous light (LL) ($n=18$, 6 per cage). The different regions of the vertebral column are indicated: 1 (cranial trunk, V1→V8), 2 (caudal trunk, V9→V30), 3 (tail, V31→V49) and 4 (tail fin, V50→V58). (A) Mineral rate and (B) the correlation between fork length and total vertebral mineral content. Different lower case letters indicate significant differences between groups within a vertebral region.

yield-load (pooled R1–R4) than the vertebrae from the group reared under the natural photoperiod (Fig. 4). However, there were no differences in stiffness (data not shown).

3.4. Mineral content of the vertebrae

At the end of the experiment, the group exposed to continuous light had a significantly lower mineral rate in region 3 than those exposed to natural light (Fig. 5A). A high correlation between fork length and total vertebral mineral content was found in both groups in June (Fig. 5B).

4. Discussion

The growth pattern observed in the present experiment is in accordance with previous findings. Reduced growth and appetite in the first period after an abrupt change to continuous light, followed by a subsequent increase in growth and appetite, have been reported in seawater-reared Atlantic salmon (Saunders and Harmon, 1988; Kråkenes et al., 1991; Hansen et al., 1992; Oppedal et al., 1997; Nordgarden et al., 2003a).

Our results show that the reduced growth in length, during the first period after change to continuous light, is due to decreased growth in the abdominal part of the vertebral column, which suggests that different mechanisms regulate growth in the vertebrae of the trunk and tail. How these regional differences are acquired is unknown, but they may originate through different gene expressions that modulate cell metabolism, response to growth-regulating hormones or, for instance, through differences in mechanical stimulation during swimming.

In the Atlantic salmon the transition between the typical trunk and tail vertebrae occurs gradually, over five to six vertebrae. Due to these transitional vertebrae, the exact division between the trunk and tail is defined in different ways dependent on the primacy of vertebral characters, such as the morphology of the neural and haemal arches or the presence of ribs. In our study, regionalization has been based on the criteria described by Kacem et al. (1998).

The trunk and tail have different embryonic origins and are patterned during the blastophore stage, togeth-

er with other main components of the body axis, such as the head (Gilbert and Saxen, 1993; Kiecher and Niehrs, 2001). The trunk, together with the head, originate from the Spemann organizer, while the tail originates from the combined effects of the tail organizer that forms the caudal somites and the Spemann organizer, which contributes the notochord and ventral neural tube (Agathon et al., 2003). Furthermore, in zebrafish embryos, *Hox* genes are expressed only in the regions that originate solely from the Spemann organizer, where they are expressed in different combinations in numeric order in a specified segment and may participate in determining the regional identity of the vertebrae (Kimmel et al., 1995; van der Hoeven et al., 1996; Prince et al., 1998; Ahn and Gibson, 1999; Morin-Kensicki et al., 2002; Agathon et al., 2003). How the vertebrae of the tail and tail fin are patterned and acquire their regional identity remains to be elucidated. These distinct developmental differences between the trunk and tail may lay down different routes for the regulation of growth later in life, as our study has demonstrated.

The rapid growth in the group exposed to continuous light from April to June may be an effect of altered activity in the pineal gland, and the observed reduction in mechanical strength and mineral content of the vertebrae may also be an effect of altered bone tissue metabolism, as has been observed after pinealectomy (Fjellidal et al., 2004). Through its secretion of melatonin, the pineal gland has a wide range of effects on the growth and development of bone (for review, see Cardinali et al., 2003). The anabolic effects of melatonin on skeletal growth may either be through direct action on osteoblasts and osteoclasts, or indirectly, through modulation of growth hormone (GH) secretion by the pituitary (Falcón et al., 2003). Direct effects of melatonin on bone tissue have been demonstrated in a number of studies. In mammalian bone tissue cultures, for example, melatonin induced osteoblast proliferation, stimulated type I collagen synthesis (Nakade et al., 1999) and enhanced osteoid mineralisation (Roth et al., 1999). An increase in bone mass has also been observed in mice after administration of melatonin (Koyama et al., 2002). On the other hand, in scales from sexually mature goldfish, melatonin inhibited both osteoblast and osteoclast activity (Suzuki and Hattori, 2002). The indirect effects of melatonin on vertebral bone may be

facilitated through receptors for GH and insulin-like growth factor I (IGF-I), both of which are expressed in vertebrae of the Atlantic salmon (Wargelius et al., 2005). In rainbow trout, melatonin has complex dose-dependent modulatory effects on GH secretion, probably through the actions of multiple melatonin receptor systems on somatotrophic cells, and administration of melatonin at doses close to nighttime levels induces GH release (Falcón et al., 2003). In Atlantic salmon, continuous light (Porter et al., 1999; Bromage et al., 2001) and pinealectomy (Porter et al., 1996; Mayer, 2000) abolish the natural nighttime increase in plasma melatonin. On the basis of our current knowledge, however, a rationale capable of connecting melatonin secretion, GH release and their combined effects on skeletal growth is difficult to construct.

Under natural light conditions the fish cease to swim and are evenly distributed throughout the water column at night, while fish exposed to continuous light maintain a constant swimming speed in circular polarised schools, congregating in the warmer upper water (Oppedal et al., 2001). The increased exercise that is induced by exposure to continuous light may augment anabolic processes, for instance via endocrine mechanisms, and result in increased appetite, feed-conversion efficiency and improved growth performance (for review, see Davison, 1997). In Atlantic salmon an increase in exercise is associated with greater mechanical stimulation, especially in the tail region of the vertebral column, and whether this induces regional differences in vertebral body growth is still unclear.

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