The effect of continuous light at low temperatures on growth in Atlantic salmon reared in commercial size sea pens

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Abstract

The aim of this study was to investigate the effect of continuous light of different duration, applied from late autumn to spring in the second year of the production cycle, on the production performance of Atlantic salmon in Northern Norway. The underlying hypothesis is that the introduction of continuous light (LL) superimposed on the natural light before December (the preferred continuous light regime in Northern Norway) could enhance growth and inhibit maturation in the subsequent year. To test this, two large, commercial scale experiments were performed [Experiment 1 in 2014 at 69.47°N, 18.26°E, and Experiment 2 in 2015 at 69.80°N, 19.42°E] where salmon of initial size of 1-1.5 kg were subjected to LL at different time points during the period between 11 November to 13 December, and reared under LL until 31 March the following year. In Experiment the water temperature at 6 m depth ranged between 6.7 °C in November to 3.6 °C in March and in experiment 2 the water temperature at 6 m depth ranged between 8.3 °C in November to 3.6 °C in March and 6.8 °C in May 2016. Before and after the period with LL, all fish were reared under natural light. Growth was improved by 13-20 % in the early exposed groups (15 Nov and 11 Nov) compared to the late exposed groups (13 Dec.). No maturation was seen in the experimental groups at slaughter (Exp. 1: July – September 2015, Exp. 2: May 2016). Vertebra deformities did not differ between the early and late exposed groups suggesting that continuous light promotes growth at lower temperatures, while supporting normal vertebra development. Only minor differences in flesh texture (measured as differences in Cathepsin L+B activity) were found in both experiments. It is concluded that a considerable growth benefit may be achieved by exposing Atlantic salmon to continuous light from early November in their first year in seawater, i.e. one month earlier than presently used by the salmon farming industry in Northern Norway.

1. Introduction

To better utilize available area for an increasing Atlantic salmon, *Salmo salar* L., production, the industry has expanded at high latitudes in Northern Norway, north of the Arctic Circle (66.6°N). In southern Norway slaughtering may start in early summer after about 12-14 months in sea water due to good winter growth, while in Northern Norway, growth rate is lower and production time is 2-3 months longer in order to regain lost winter growth (Roth et al., 2005). These sub-optimal production conditions are particularly related to short day-length and low temperatures during the winter. For Atlantic salmon, Handeland, et al. (2008) suggested an optimum temperature for growth of 12.8 °C for 70–150 g and 14.0 °C for 150–300 g post-smolts, whereas ambient temperatures in Northern Norway decline from approx. 6°C in October to 3°C in March and an average of 5°C during this period.

The growth enhancing effect of continuous light (LL) has been reported for Atlantic salmon in both freshwater (Stefansson et al., 1991) and sea water (Kråkenes et al., 1991; Handeland et al., 2003); however, these studies have been performed at near optimal temperatures for Atlantic salmon (Handeland et al., 2008). For the early post smolt stage (size range 96-300 g), Døskeland et al. (2016) investigated the interactive effects of low temperatures (4.3, 6.5 or 9.3°C) and photoperiods (continuous light, LL or simulated natural photoperiod (69ºN), LDN) on growth and found significant interactive effect between temperature and photoperiod, as post-smolts exposed to low temperature and continuous light regime (4LL) had a significantly higher growth (30 % gain in overall SGR) than the LDN group corresponding to the effect of approx. 1.2 °C temperature increase. Similar interaction between temperature and photoperiod was reported for other aquaculture species. In turbot, *Scophthalmus maximus*, the interactive effects of temperature and photoperiod can cause a downward shift in the optimum temperature for growth when the photoperiod is altered

(Imsland and Jonassen, 2001). The growth-promoting effect of continuous light has been shown to be inversely related to temperature for turbot (Imsland et al., 1995), and Atlantic halibut, *Hippoglossus hippoglossus* (Norberg et al., 2001). It is, therefore, of interest to identify to what extent light can compensate for the growth disadvantages associated with rearing at low temperatures (Handeland et al., 2008) during the seawater phase of Atlantic salmon farming. At present little is known about possible interactions between temperature and photoperiod at the post-smolt stage of Atlantic salmon in seawater. Imsland et al. (2014) investigated the long term effect of continuous light and constant temperature and their interaction on growth physiology in Atlantic salmon pre- and post-smolts. They reported a growth-enhancement in fresh water of continuous light corresponding to a 4.5 °C increase in water temperature. Imsland et al. (2014) also found that proportion of mature males was higher at 12.7°C (66%) compared to 8.3°C (11%); however, those findings have not been validated under full scale rearing conditions. Continuous light is commonly used from December to March in the current production regime of Atlantic salmon in Norway to promote growth without triggering maturation (Oppedal et al., 1997, 2006).

In Atlantic salmon the later stages of sexual maturation involve a redistribution of the somatic resources and the development of nuptial colouration responsible for the low commercial value of mature fish (Leclercq et al., 2010), altered feeding activity (Kadri et al., 1997) and increased pathogen susceptibility (Currie and Woo, 2007). The suppression of pre-harvest sexual maturation is therefore a priority in the salmon on-growing industry (Leclercq et al., 2010). This is achieved by photoperiod manipulation of the stock in the form of continuous artificial light (LL) applied between the winter and summer solstice during the second year at sea. This 4-6-month period LL-regime is recognized as the most efficient by providing a key environmental signal that phase-advances the so-called ‘spring decision window’ such that a reduced proportion of the stock meets the developmental/energetic

thresholds required to proceed through maturation (Taranger et al., 1998 Oppedal et al., 2006). Current knowledge on the photoperiodic control of puberty in Atlantic salmon suggests that terminating LL-exposure before the summer solstice could be equally efficient at suppressing sexual maturation (Leclercq et al., 2010), whereas knowledge about the effect of onset time point of LL are currently lacking. Such knowledge may be an important tool to increase winter growth and reduce the production time of Atlantic salmon in sea cages in Northern Norway.

Development of vertebra deformities is a slow process that manifests itself months after the actual induction (Grini et al., 2011; Fjelldal et al., 2012a), and can be modulated by temperature (Grini et al., 2011). Further, Fjelldal et al. (2005) found that vertebrae in the trunk and tail regions displayed a differential growth rate in response to photoperiod in Atlantic salmon post-smolts reared in sea cages at ambient temperature in Southern Norway. Also, continuous light was shown to promote bone resorption in postsmolts (Fjelldal et al., 2012b). However, there is limited knowledge on the impact of different production regimes in sea cages, i.e. duration of continuous light during winter, on the development of vertebra deformities up to harvest size.

In fish, flesh texture is shown to be influenced by a number of different factors, such as light regime (Hemre et al., 2004; Hagen and Johnsen, 2016), temperature (Roth et al., 2005), feeding (Einen et al., 1999), slaughter and filleting method (Kiessling et al., 2004; Kristoffersen et al., 2007). It has also been reported that cathepsin B, D and L activities have an impact on specific structural proteins correlating to texture (Godiksen et al., 2009; Bahuaud et al., 2010). Textural changes can be related to somatic muscle growth and following protein turnover are an important factor that is affected by the intracellular enzyme activity, in particular cathepsins and calpains (Lysenko et al., 2015). High protease activity is

related to decomposition of muscle proteins post mortem (Delbarre-Ladrat et al., 2006), which in turn, would probably influence the drip loss (loss of fluid during storing and thawing).

The aim of this study was to investigate the effect of continuous light of different duration applied from autumn to spring in the production cycle on Atlantic salmon on growth, maturation and flesh quality, and thus, test the hypothesis that the introduction of additional light before December (the preferred continuous light regime in Northern Norway) would enhance growth and delay maturation in the subsequent year.

2. Materials and methods

Two large scale experiments were performed at a commercial Atlantic salmon sea farm [Experiment 1 at 69.47°N, 18.26°E and Experiment 2 at 69.80°N, 19.41°E, Lerøy Aurora, Troms county, Norway]. The salmon used in the study were S1 smolts produced at the commercial smolt hatchery of Lerøy Aurora (location Laksefjord, Finnmark, Norway) moved to sea cages in April 2014 (Experiment 1) and May 2015 (Experiment 2). The fish were from the Aqua Gen strain and were vaccinated with Pentium Forte Plus (Novartis Aqua, Oslo, Norway). The fish were held under natural light (NL) conditions until the start of each experiment. Both experiments were performed during winter, but Experiment 2 lasted two month longer. The initial fish size differed in the two experiments (see below).

2.1 Experimental design

2.1.1 Experiment 1

On 11 November 2014, six cages (160 m circumference, 37688 m^3 volume) holding one sea-winter (1-SW) Atlantic salmon (n=124086 ± 11898 fish pen^{-1}) with a mean (± standard error of mean, SEM) live body-weight (BW) of 1190 ± 106 g were exposed to LL using four 360 W BlueLED lights per pen (AKVA group ASA, Tromsø, Norway). Three photoperiod treatments were tested in duplicate where additional light was introduced on three different dates i.e. 11 November (LL-11 Nov), 24 November (LL-24 Nov) and 13 Dec (LL-13 Dec). The LL-13 Dec is the current production regime used by the commercial partner. Water temperature at 6 m depth ranged between 6.7 °C in November to 3.6 °C in March. All six cages were returned to NL on 31 March the following year (2015) and all fish

slaughtered from July to September 2015. The fish were fed a commercial dry diet according the manufacturer recommendations (Ewos, Bergen, Norway).

2.1.2 Experiment 2

On 15 November 2015, four cages (160 m circumference, 37688 m^3 volume) holding one sea-winter (1-SW) Atlantic salmon (n=20742 ± 2033 fish pen^{-1}) with a mean (±SEM) live body-weight of 1536 ± 166 g were exposed to LL using four 360 W BlueLED light per pen (AKVA group ASA, Tromsø, Norway). Two photoperiodic treatments were tested in duplicate where additional light was introduced on two different dates i.e. 15 November (LL-15 Nov) and 13 Dec (LL-13 Dec). Water temperature at 6 m depth ranged between 8.3 °C in November to 3.6 °C in March to 6.8 °C in May 2016. The cages were returned to NL on 31 March and reared on NL until slaughtered in May 2016. The fish were fed a commercial diet according the manufacturer recommendations (Biomar, Myre, Norway).

2.2 Growth

Growth measurements in the present study are based on counting and bulk weighing of all fish in all sea pens at the start and termination of each experiment. In between, biomass was controlled using an electronic data base system from FishTalk® (AkvaGroup, Norway) for stock control and further documentation of the production. From the numbers stocked, fish lost during production due to mortalities were deducted. Monitoring of biomass development (growth) was based on daily recording of the fed amount and subsequent conversion into biomass using an expected feed conversion ratio. Mean weights were indirectly derived as total biomass divided by the number of fish in the unit. Supplemental biomass estimation was

done using automated systems and used to adjust the FCR and subsequent mean weight estimations. These automated systems comprise stereo-camera systems or frame systems for single fish biomass estimation in situ, both being based on optical readout and specific weight algorithms (Beddow and Ross, 2005; Aunsmo et al., 2013). These daily estimates were compiled into monthly estimates of biomass in each sea pen. Specific growth rate (SGR) was calculated according to Houde and Schekter (1981):

$$SGR = (e^{g} - 1)\ 100$$

where g is the instantaneous growth coefficient; expressed as $(\ln(W_2)-\ln(W_1))\ (t_2-t_1)^{-1}$ and W_2 and W_1 are weights on days t_2 and t_1, respectively.

2.3 Feeding and feed conversion ratio

For both studies, the feeding regime was based on automatic feeding using commercial automated feeding systems (Akvasmart CCS feeding system, AKVA group ASA, Tromsø, Norway). Daily feed-delivery to each cage was registered, and changes in appetite noted together with a continuous evaluation of the usage-characteristic of the feed. For each duplicate treatment, mean weekly feed consumption and standard deviation (SD) was calculated and those data compiled into monthly average. Feed conversion ratio (FCR) was calculated as:

$$FCR = C / (B_2 - B_1)$$

where C is feed consumption in the sea pen during the period and B_2 and B_1 are biomass in tank (g) at days t_2 and t_1, respectively. Daily feeding rate (F) was calculated from $F=100\ C / \overline{W}$ where $\overline{W}$ is the mean daily fish weight over the experimental period.

2.4 Radiography and vertebra morphometry

At the terminal sampling in Experiment 1, 20 fish per treatment had their vertebral columns carefully dissected for lateral radiographs, and evaluated for vertebra deformities (Witten et al., 2009). The vertebral columns were radiographed (Porta 100 HF; Eickemeyer Medizintechnik für Tierärzte KG, Tuttlingen, Germany) onto a 35 × 43 cm image plate in a rigid cassette (Dürr Medical, Bietigheim-Bissingen, Germany) with 40 kV and 10 mAs with a distance of 70 cm. The image plate was scanned (CR 35 VET; Dürr Medical) and the resulting image converted into a TIFF file (Vet-Exam Plus Software, version 4.14.0).

2.5 Cathepsin B and L activities

Samples for cathepsin activity were taken from November 2015 to January 2016 from 6 fish from each sea pen (N = 12 from each experimental group) in Experiment 2. Analyses were prepared, using a modified method described by Bahuaud et al. (2010). Muscle samples were taken from the dorsal part of the Norwegian Quality Cut (NQC) from six fish in LL-15 Nov and LL-13 Dec on 16 November, 17 December and 14 January, immediately frozen at −80 °C prior to further analyses. Cathepsin B + L, cathepsin B and cathepsin L total activities were measured on muscle homogenates, prepared by homogenising 100 mg of muscle tissue in 300 µl of extraction buffer (100 mM Na-acetate in 0.2% Triton X-100, pH 5.5) in Precellys tubes CK 28 (2 ml), and homoganized using Ultra Turrax (IKA, USA) at 12500 rpm. Obtained homogenates were centrifuged at 16,016×g (4 °C, 30 min) and the supernatants were used to measure enzyme activities.

Cathepsin B + L and cathepsin B activities were measured fluorometrically, according to a modified method described by Kirschke et al. (1983). The release of the fluorogenic reagent 7-amido-4-methylcoumarin was determined by fluorescence measurements

(excitation and emission wavelengths were 360 and 460 nm, respectively). As substrates, Z-l-phenylalanine-l-arginine-7-amido-4-methylcoumarin (Z-Phe-Arg-AMC) was used for cathepsin B + L activity, whereas Z-l-arginine-l-arginine-7-amido-4-methylcoumarin (Z-Arg-Arg-AMC) was used for cathepsin B activity. To estimate cathepsin L activity, the activity of cathepsin B was subtracted from cathepsin B + L activity (Bahuaud et al., 2009). All samples were analysed in triplicate, and the mean was calculated. The activity was expressed in $\mu U\ g^{-1}$ muscle where 1 U was defined as 1 μmol product produced per minute at 40°C.

2.6 Statistical methods

To assess normality of distributions a Kolmogorov-Smirnov test (Zar, 1984) was used and homogeneity of variances was tested using Levene's F test (Brown and Forsythe, 1974). Possible differences in mean weights, specific growth rates, feed conversion ratio, feed intake and cathepsin activity among treatments were tested using a two way nested Model III ANOVA, where the replicates (random) were nested within continuous light treatment groups (fixed). Significant ANOVA were followed by a Student-Newman-Keuls multiple comparison test (Zar, 1984) to identify differences among treatments. Data on mortality was tested with a χ^2 test with the LL-13 Dec group in both experiments as expected value. A significance level (α) of 0.05 was used if not stated otherwise.

3. Results

3.1 Growth, maturation and mortality

Mortality was higher (χ^2 = 4.1, $P < 0.05$) for the smaller salmon in Experiment 1 (overall mean 4.7 %) compared to the larger salmon in Experiment 2 (overall mean 2.0 %). There were no systematic differences in mortality related to duration of continuous light in either experiment. In Experiment 1, mortality was 4.6, 5.8 and 2.9% for the LL-11 Nov, LL-24 Nov and LL-13 Dec groups, respectively, whereas it was 1.9 and 2.1% for the LL-15 Nov and LL-13 Dec groups in Experiment 2. Mean weight varied between experimental groups in both experiments (Figs. 1-2). In Experiment 1, the mean weight of the LL-11 Nov group was significantly higher (Student-Newman-Keuls (SNK) test, $P < 0.05$, Fig. 1) compared to the LL-13 Dec group in February and March, and the final weight was 20% higher in the LL-11 Nov group. In Experiment 2, the final weight in June varied between the two experimental groups (SNK test, $P < 0.05$, Fig. 2) and was 13% higher in the LL-15 Nov group compared to the LL-13 Dec group. Growth rate differed between the experimental groups in both experiments (Table 1). In Experiment 1, the overall growth was 20% higher (SNK test, $P < 0.05$, Table 1) for the LL-11 Nov group compared to the LL-13 Dec group. Similar significant growth differences were seen between the LL-15 Nov and LL-13 Dec groups in Experiment 2 (SNK test, $P < 0.05$, Table 1).

During the primary processing at slaughter, maturity status was evaluated in all fish in all experimental groups based on external examination. All examined fish were classified as immature.

3.2 Feed intake and feed conversion efficiency

Both daily feeding rate and feed conversion ratio (FCR) differed between the experimental groups (Table 1). In Experiment 1 LL-11 Nov had significantly better FCR compared to the other two groups (SNK test, $P < 0.05$) and in Experiment 2 the LL-15 Nov had significantly higher daily feeding rate compared to the LL-13 group (Table 1).

3.3 Cathepsin activity

Cathepsin L+B activity differed between the two experimental groups in Experiment 2 (two way nested ANOVA, $P < 0.05$, Fig. 3) as the LL-15 Nov group had higher cathepsin activity in January. No other differences in cathepsin activity were observed.

3.3 Vertebra deformities

No differences in vertebra deformities were found between the experimental groups in Experiment 1. In analysed groups 30 % of the sampled fish had vertebra deformities. In general, these were mild and consisted of aggravations of 2 to 4 fused vertebrae (Fig. 4A-C). Only one fish had a severe vertebra deformity with 9 deformed vertebrae (Fig. 4D).

4. Discussion

Despite the fact that growth was 13-20 % higher for the early November groups (LL-11 Nov in Exp. 1, and LL-15 Nov in Exp. 2) compared to the commercial reference group in both experiments (LL-13 Dec), there are surprisingly few studies specifically evaluating the post-smolt stage in sea water relevant for direct comparison. A 13-20 % increase in SGR at the sea temperatures in the present experiments (average, 5.2 °C) corresponds to approx. 1.5 °C increase in water temperature (Handeland et al., 2003). Furthermore, Imsland et al. (2014) reported a growth enhancing effect of continuous light for Atlantic salmon in fresh water corresponding to a 4.5 °C increase in temperature in an experiment investigating both smolt and post-smolt at 8.3 and 12.7°C. Due to the low temperatures in the present experiments , maturation was not expected (Hutchings and Jones, 1998) and was not observed at slaughter. Accordingly, we can conclude that the positive growth effect is associated with photoperiod alone, with no interaction of early maturation. The optimal temperature for growth and FCE for Atlantic salmon in this size range is approximately 11-14°C (Bromage et al. 2001; Handeland et al. 2003, 2008) so all groups were reared far below their optimal rearing temperature. The precise mechanism of photoperiod action on growth is not entirely understood (Stefansson et al., 2007); however, it is clear Atlantic salmon differs from several other species in that light plays a key role for ontogenetic shifts (Boeuf and Le Bail, 1999). The findings of Døskeland et al. (2016) suggest that the magnitude of the effect of continuous light on growth be inversely related with temperature which results in significant interaction between temperature and photoperiod. Further support for this is found in studies on juvenile turbot (Imsland et al., 1995) and Atlantic halibut (Jonassen et al., 2000) demonstrating that the growth promoting effect of continuous light can be stronger at low temperature compared to near optimum temperature. Clarke et al. (1978) suggested that the rate-controlling effect of

temperature might be the reason for the short duration of the growth-enhancing effect of long photoperiod at higher temperature in sockeye, *Oncorhynchus nerka*, and coho, *O. kisutch*, salmon.

Higher feed intake in the LL-15 Nov group in Experiment 2 could be linked to better conditions for feeding due to the superimposed light in this group from November compared to December. Although minor in magnitude, feed conversion ratio was significantly better in the LL-11 Nov group in Experiment 2. This may reflect the positive effect of LL on FCR similar to that seen in Døskeland et al. (2016) where FCR was lower at 4°C-LL compared to 4°C- LDN.

No maturation was seen in any of the groups at slaughter. The study was performed under commercial conditions with low ambient temperatures. Previous studies have shown that the switch from short to long days is the key photoperiodic signal regulating Atlantic salmon maturation. An arrest of sexual development is indeed observed within 6 weeks of LL-exposure in fish remaining immature (Taranger et al., 1998, 1999). This photo-inhibition would have occurred before late March in all regimes tested here such that timing of LL termination had no effect on maturation rates at harvest. The study of Vikingstad et al. (2015) demonstrated that final sexual maturation and spawning in large (6-7 kg) Atlantic salmon is strongly influenced by temperature, with elevated temperatures (14-16 °C) having a deleterious effect on these processes. In contrast, rearing the females at cold (decreasing from 7 to 3°C) amplified and advanced the profiles of all three endocrine steroids investigated compared with the ambient group (decreasing from 11 to 5°C), and increased the survival rates to the eyed egg stage. However, the fish in Vikingstad et al. (2015) was reared at NL in contrast to continuous light in the present study. As no maturation was seen in the present study this demonstrates that continuous light from November to March is a very potent mechanism to inhibit maturation.

Activity of proteases, such as cathepsins, is widely described in the literature to be an important contributor to protein degradation and muscle softening (Bahuaud et al., 2009; Lerfall et al., 2015). In present study, increased cathepsin activity was seen in the LL-15 Nov group in January 2016 corresponding with higher growth in this group. Previously increased cathpesin activity has been associated with pre-mortem stress-related factors (Bahuaud et al., 2010). Lerfall et al. (2015) found a significant relation between cathepsin L and the drip loss from fillets during storage. The higher cathapsin activity seen in the LL-15 Nov, is in line with newer studies by Hagen and Johnsen (2016) showing that an exposure to continuous light increases the activity of cathepsin L+B, and can be seen as an indication of higher somatic muscular growth. Since cathepsins are involved in fast muscle protein breakdown and turnover (Hagen et al., 2008) and may reflect softening of the muscle tissue. The present findings indicate that slaughter of salmon should be avoided in the period of continuous light superimposed on the natural light.

No differences in vertebra deformities were found in the samples analysed from Experiment 1. A total of 30 % of the fish analysed had one or more deformed vertebra. In earlier studies adult Atlantic salmon have shown prevalence of deformities ranging from 12 to 92% (Fjelldal et al., 2007, 2009; Korsøen et al., 2009; Grini et al., 2011; Taylor et al., 2013), and between 33 and 50% in wild fish (Fraser et al., 2014; Sambraus et al., 2014). Development of vertebra deformities is a slow process that manifests itself months after the actual induction (Grini et al., 2011; Fjelldal et al., 2012a), and the vertebra fusions (Witten et al., 2006) observed herein are most probably the result of changes prior to the onset of the experiments in this study (Witten et al., 2006; Fjelldal et al., 2007). The fish used in the present study were reared at near optimal temperatures (12-14 °C) during the late parr and early smolt stage. It is known that vertebra deformities can be modulated by temperature (Grini et al., 2011) as post-smolts at 16°C developed vertebra deformities, while post-smolts

at 10°C did not. Since the deformity prevalence in the present study were within that reported for wild salmon, the present situation must be considered as normal.

5. Conclusion

It is concluded that a considerable growth benefit may be achieved by exposing post-smolt Atlantic salmon to continuous light from early November i.e. one month earlier than presently used by the salmon farming industry in Northern Norway.

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Table 1. Specific growth rate (SGR, % day -1), daily feeding rate (F%) and feed conversion ratio (FCR) of Atlantic salmon reared at different periods of continuous light. Values are given as mean (SEM). Significant differences between treatment groups are indicated with superscripted letters (Student–Newman–Keuls test, $P < 0.05$).

Treatment group	SGR	F (%)	FCR
Experiment 1 ($N = 20$)			
LL-11 Nov	0.49 (0.03)^{a}	0.44 (0.02)	1.09 (0.001)^{b}
LL-24 Nov	0.42 (0.02)^{b}	0.45 (0.03)	1.12 (0.006)^{a}
LL-13 Dec	0.39 (0.02)^{b}	0.44 (0.03)	1.13 (0.007)^{a}
Experiment 2 ($N = 28$)			
LL-15 Nov	0.39 (0.04)^{a}	0.43 (0.04)^{a}	1.06 (0.03)
LL-13 Dec	0.31 (0.03)^{b}	0.33 (0.05)^{b}	1.07 (0.04)

Figure legends

Fig. 1. Mean weight (g) of Atlantic salmon reared at three different periods of continuous light (LL) from 11 November (LL-11 Nov), 24 November (LL-24 Nov) and from 13 December (LL-13 Dec). LL treatment was terminated on 31 March in all groups. Prior to onset all groups were reared under natural light. Vertical whiskers indicate standard error of mean (SEM). Letters indicate significant difference between treatments on sampling date (Student–Newman–Keuls test, $P < 0.05$).

Fig. 2. Mean weight (g) of Atlantic salmon reared at two different periods of continuous light from 15 November (LL-15 Nov) and from 13 December (LL-13 Dec). LL treatment was terminated on 31 March in both groups. Vertical whiskers indicate standard error of mean (SEM). Letters indicate significant difference between treatments on sampling date (Student–Newman–Keuls test, $P < 0.05$).

Fig. 3. Cathepsin L+B activity in Atlantic salmon reared at two different periods of continuous light from 15 November (LL-15 Nov) and from 13 December (LL-13 Dec). Vertical whiskers indicate standard error of mean (SEM). Letters indicate significant difference between treatments on sampling date (Student–Newman–Keuls test, $P < 0.05$).

Fig. 4. Lateral radiographs of different vertebra deformities seen in the sampled fish from Experiment 1. (A) An individual with vertebra fusion in vertebrae nos. 18 and 19. (B) An individual with vertebra fusion in the two most caudal vertebrae (white and black arrowheads). The urostyl is indicated by a black astrix. (C) An individual with vertebra fusion in vertebrae nos. 34 and 35, and 37 and 38. (D) An individual with 9 deformed vertebrae; one-

sided compression in vertebra no. 2 (most cranial vertebra on the radiograph), fusion in vertebrae nos. 3 to 6, and 7 to 10.

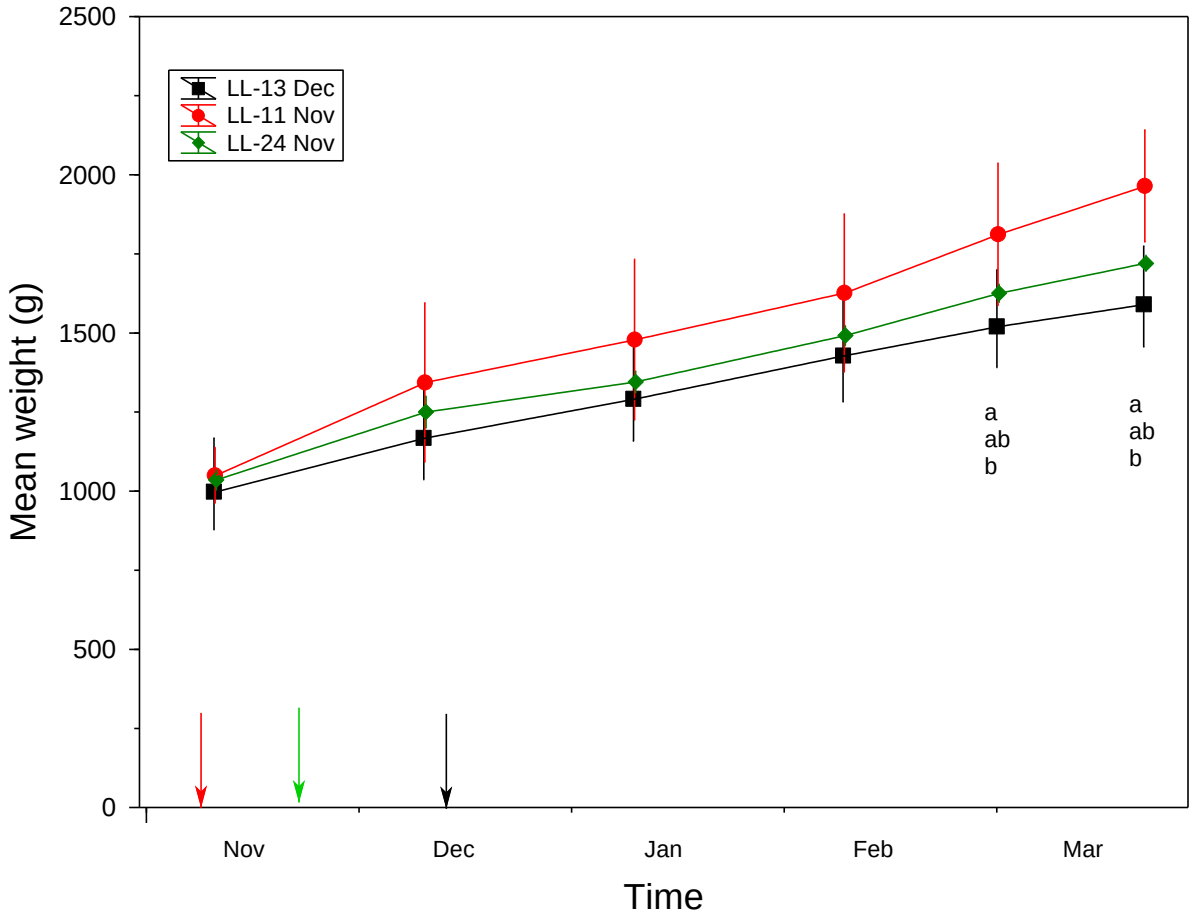

Fig. 1. Imsland et al.

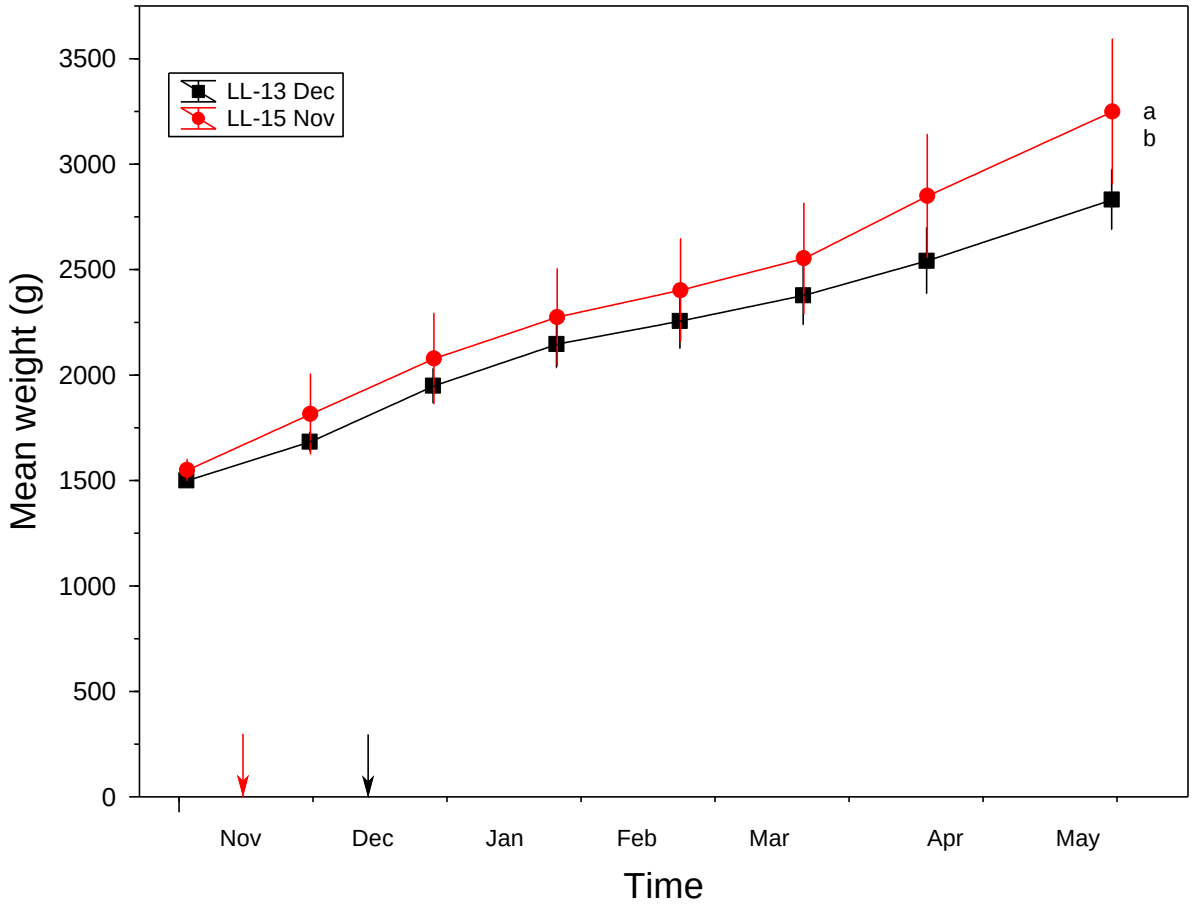

Fig. 2. Imsland et al.

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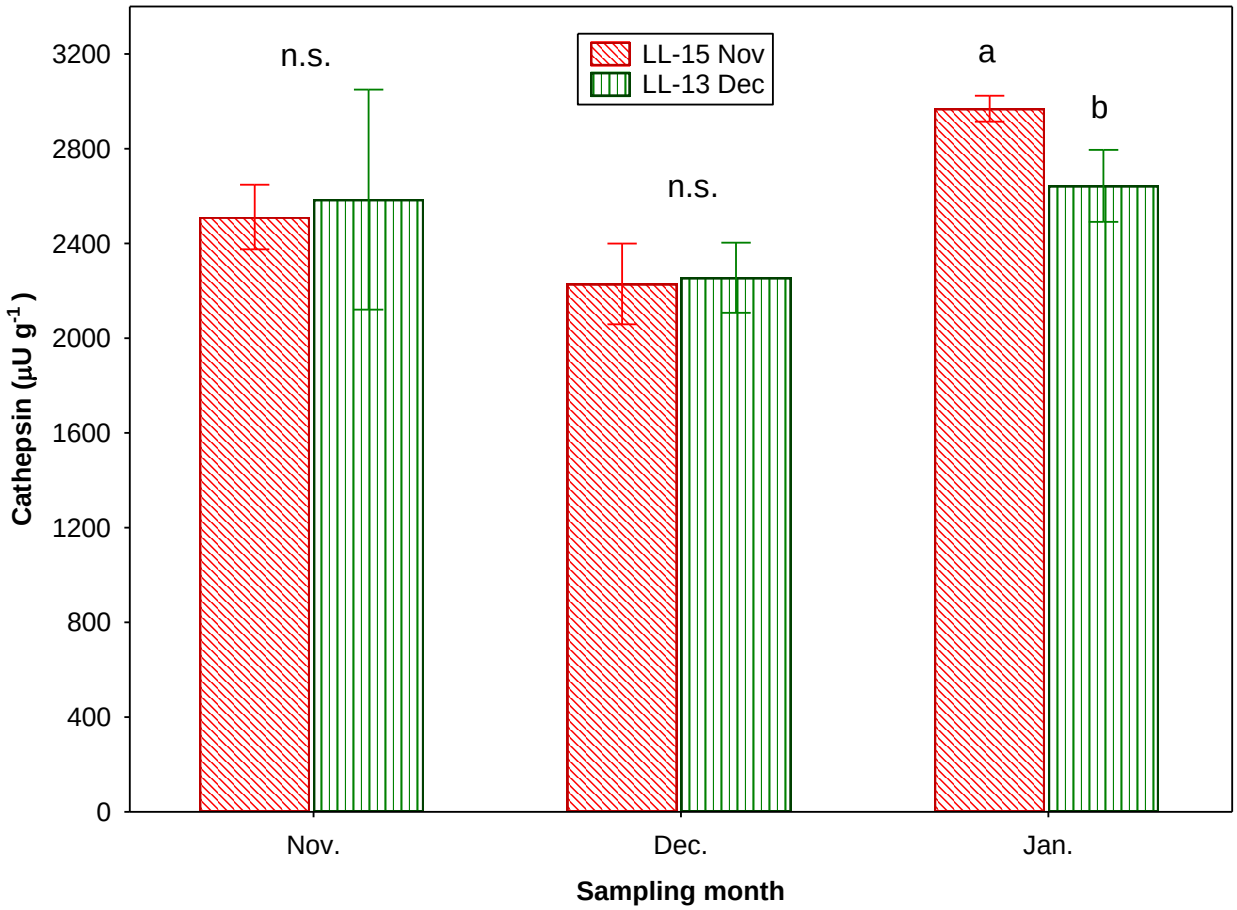

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4 Fig. 3. Imsland et al.

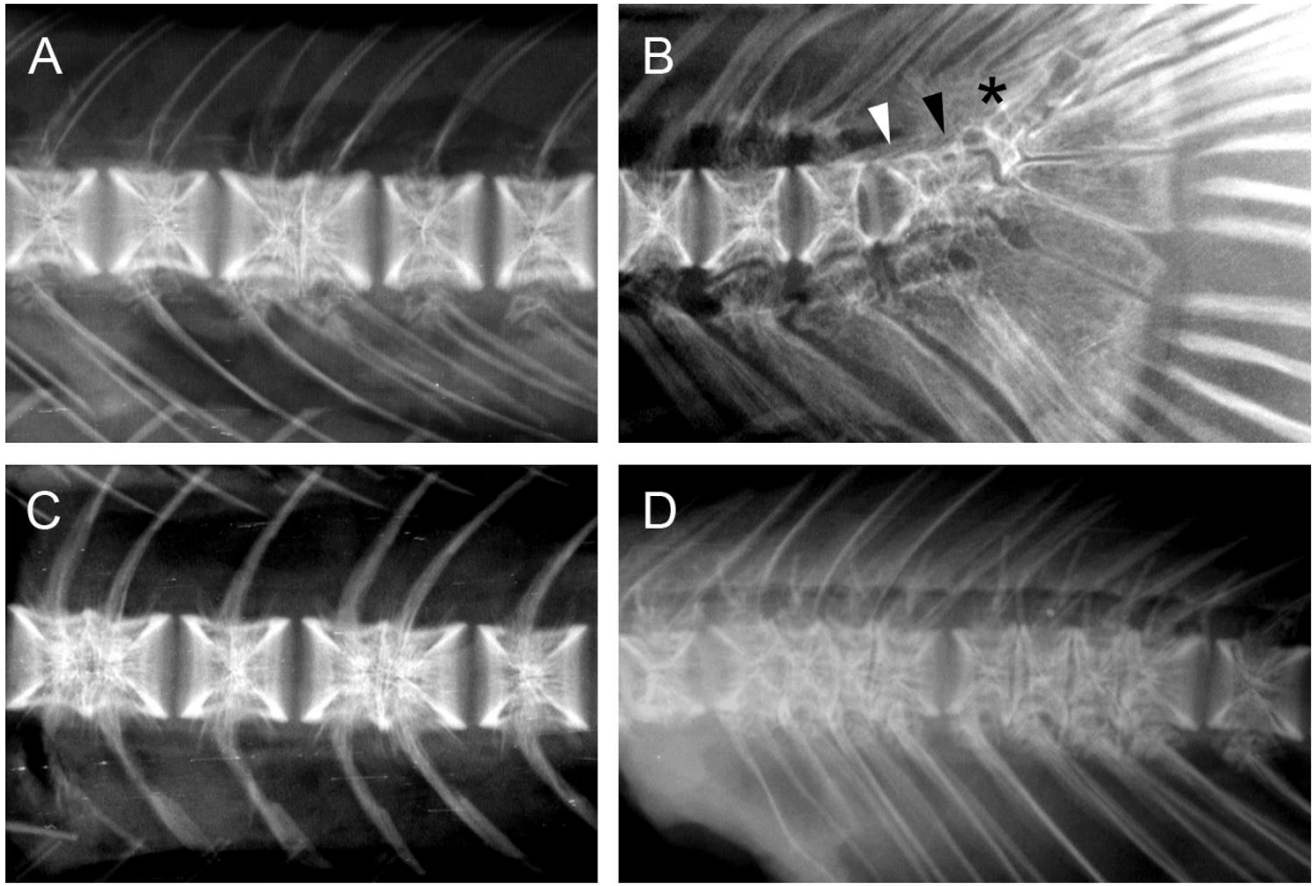

Fig. 4. Imsland et al.