Flesh quality of Atlantic salmon smolts reared at different temperatures and photoperiods

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Running head: Flesh quality in salmon

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Abstract
Possible interactive effects of temperature and photoperiod on flesh quality in Atlantic salmon post-smolts were studied. Juvenile (initial mean weight 96.0 g ± 3.1 SEM) Atlantic salmon were reared at six different combinations of temperatures (4.3, 6.5 or 9.3°C) and photoperiods (continuous light or simulated natural photoperiod). At termination of the trial the fish were slaughtered and flesh samples taken to investigate quality and textural properties in the different experimental groups. Final weight in the six experimental groups varied between 174 and 345 g. Softer texture was seen in the fast growing groups. Photoperiod has only minor effect on flesh quality and textural properties whereas temperature had significant impact on most of the measured variables. Although positive for growth, higher temperatures might be less favourable in relation to softer muscle tissue.
**INTRODUCTION**

Historically the Atlantic salmon, *Salmo salar*, industry was primarily located in the western and central parts of Norway. To better utilize available area for an increasing production, more activity has been localized at high latitudes in Northern Norway above the Arctic Circle. Fish farming in high latitude areas may give shorter growth seasons and longer production cycles (Koskela, Pirhonen & Jobling, 1997). In southern Norway slaughtering may start in early summer due to good winter growth, while this is less profitable in the north where production time is longer in order to regain lost winter growth (Roth et al. 2005). These sub-optimal production conditions are particularly related to photoperiod and temperature. For Atlantic salmon Handeland, Imsland & Stefansson (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. 9°C in October to 3°C in March (Imsland et al., 2018).

Salmon filet is the main end product in Norwegian fish farming, but growth as such is not enough if quality is compromised. Texture quality is important for consumer acceptability of Atlantic salmon and insufficient firmness causes downgrading in the processing industry (Michie, 2001, Torgersen et al., 2014). Flesh quality is a complex set of characters involving factors such as texture, chemical composition, color and fat content (Fauconneau, Alami-Durante, Lorache, Marcel & Vallot, 1995). Firmness in relation to fiber size and distribution is a major factor influencing acceptability of raw fish products and is therefore important for characteristics like hardness of fish flesh (Veland & Torrissen 1999). In teleost fish, muscle growth is characterized by its high plasticity, and may be altered by a wide range of environmental and endogenous signals (Larsen, Imsland, Lohne, Pittman & Foss, 2011; Espe et al., 2004; Torgersen et al., 2014). The influence of temperature on muscle texture hardness has been studied in Atlantic salmon and is known to decrease during summer months (Espe et
al. 2004; Roth et al. 2005). The impact of temperature and light on these mechanisms depends
on the affected life stages, as reviewed by Rowlerson and Veggetti (2001). The effect of season
may overshadow endogenous rhythms and affect quality (Roth et al., 2005). Johnston et al.
(2003) studied Atlantic salmon during their first sea winter and found significantly higher
numbers of fast muscle fibers and a shift in the distribution of fiber diameter in groups reared
at continuous light compared with groups reared at natural daylight at the same temperature,
while no effect on hypertrophy was found. These authors added that an effect of continuous
light on muscle fiber recruitment was obtained only during a discrete seasonal window of
decreasing day length, and that these effects may be enhanced or inhibited by changing the
timing of light treatment. It is therefore interesting to consider how muscle hardness as an
expression of fillet quality, is affected by different light regimes at sub-optimal temperatures.

The aim of this study was to study the combined effect of two photoperiod regimes,
continuous light (LL) and simulated natural photoperiod (LDN, Tromsø) at low temperatures
(4.6 and 9°C) on flesh quality and textural properties in Atlantic salmon smolts.
2 | MATERIALS AND METHODS

2.1 | Experimental fish and conditions

On 15 October 2013 a total of 1140 juvenile salmon (initial mean weight 90.0 g ± 3.1 SEM) arrived at Bergen High Technology Centre (BHTC), Bergen, Norway, where the experiment was carried out in the period from 16 October 2013 to 17 March 2014. At arrival at BHTC the salmon (95 fish in each tank) were distributed among twelve 1 m² (400 l) and transferred to 32 ppt during 16-23 October. The fish were fed using a commercial formulated feed (Smolt 30, Ewos AS, Florø, Norway, 3-4 mm). Feed was delivered by automatic screw feeders (Arvo-Tec Oy, Finland) during daytime. These were calibrated and tested at regular intervals during the experiment. Amount of feed was adjusted according to biomass development, temperature and visual inspection in order to feed approx. 10% in surplus. The surplus feeding was done to counteract development of any form of feeding hierarchy in the tanks. Feed was only administrated during daytime in the LDN group.

To study individual growth a subgroup (mean weight ±SEM, 86.2 g ± 3.1) within each tank (N = 20, Total Ntagged = 240) were on 16 October 2013, anaesthetized (metacain, 0.03 g l⁻¹) and individually tagged using Carlin tags (McAllister, McAllister, Simon & Werner, 1992). Temperature was gradually (four days) lowered to the three experimental temperatures on an average (± SEM) of 9.3 (±0.1), 6.5 (±0.2) and 4.3 (±0.2)°C. All temperature groups were reared in replicate groups at either continuous light (LL) or simulated natural photoperiod (LDN) for Tromsø (N 69° 40'). The experimental groups are abbreviated hereafter as: 4LDN, 4LL, 6LDN, 6LL, 9LDN and 9LL. For more information about the background of the fish and experimental groups see Døskeland et al. (2016).

The experimental fish were anaesthetised and individually weighed (0.1 g) on the following dates: 16 October, 27 November, 8 January, 18 February and 17 March.
2.2 | Fish quality measurements

At termination of the trial on 17 March 2014 all fish were starved for one day and killed with a blow to the head. Immediately after slaughter, the fish were exsanguinated by a gill cut, placed into ice water for 30-40 min, gutted, filleted and stored on ice in a cooled storage room. The fillets were used for chemical analysis 6 days post mortem as some time is expected to elapse prior to consumption of the product so investigation of quality aspects shortly after slaughtering may not reflect changes seen in the final product from a consumptive perspective. The chemical analysis included flesh gaping, muscle pH, water content of fillet, water holding capacity (WHC), and texture properties (hardness and breaking force).

The left fillet of the sampled fish was divided into two parts. One part of the fillet was weighed and dried at 105°C from 16 to 24 h (NMKL 123,1991), for estimating the dry content of the muscle and hence the water content (WC) of the muscle. The other part was weighed and centrifuged (Sorvall® RC5Cplus, Thermo Fisher Scientific Inc, USA) for 15 minutes at 4°C using 1500 rpm with a SLA 1500™ rotor. Water holding capacity (WHC) was calculated from the following formula (Skipnes, Ostby & Hendrickx, 2007).

\[
\text{WHC} = \frac{W_0 - \Delta W}{W_0} \times 100
\]

Where:

\[
W_0 = \frac{V_0}{(V_0 + D_0)} \times 100
\]

\[
\Delta W = \frac{\Delta V_0}{(V_0 + D_0)} \times 100
\]

\[V_0: \text{ is the water content of the muscle}\]

\[D_0: \text{ Dry matter of the muscle}\]

\[\Delta V_0: \text{ The weight of the liquid separated from the sample during centrifugation}\]

Muscle pH was measured, by a Mettler Toledo Seven Go pro™ with an Inlab 489 pH probe (Mettler Toledo INC, USA).
2.3 | Texture analysis

Information on hardness, breaking strength and profile were obtained using a Texture Analyzer (TA-XT®-plus Texture Analyzer, Stable Micro Systems, Surrey, UK) with a load cell of 25 kg. A flat-ended cylinder (12.5 mm) was used as test probe. Seven days after collection the puncture test was assessed in two locations on the Norwegian quality cut (NQC, NS 1975) directly on the fillets (skin on) transverse to the muscle fiber orientation. The probe was programmed to penetrate 80 % into the initial fillet height and max forces were recorded in addition to forces at 20, 40 and 60 % compression (Roth et al., 2008, 2010). The speed of the probe was set to 1 mms⁻¹. The breaking force was defined as the force required to penetrate the cylinder through the fillet surface and hardness (N) as the highest force recorded during the first compression cycle (Bourne, 1977).

2.4 | Growth

Specific growth rate (SGR) was calculated as:

\[ SGR = \left( e^g - 1 \right) \times 100 \]

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

2.5 | Statistics

A two-way factorial ANOVA (Zar, 1996) was applied to analyse possible effects of different temperature and photoperiod groups. Analysis of covariance (ANCOVA, Zar, 1996) was used to test for possible effect of temperature and photoperiod and flesh quality, textural hardness and breaking force with final weight and individual growth rates (SGR) as covariates. Student-Newman-Keuls multiple comparison test (Zar, 1996) was used to identify differences among
treatments. A linear regression was used to test the relationship between fillet texture hardness and individual growth rates.
3 | RESULTS

3.1 | Growth

The overall mortality was 0.9%. No difference in mortality was found between experimental groups. There were significant differences in mean weight between temperature treatments with the 9 °C groups having the highest mean weight from week 27 November (two-way ANOVA, $p < 0.001$, Fig 1). Specific growth rate differed between the two photoperiod groups at 4°C during the whole experimental period (SNK test, $p < 0.05$). The 4LL group was significantly larger ($p < 0.05$) compared to the 4LDN group from January onwards (Fig. 1) and displayed 30% higher overall growth rates, whereas no growth enhancing effect of LL was seen at 6 and 9°C. As a result, an overall interaction (two-way ANOVA, $p < 0.001$) effect of photoperiod and temperature on growth rate was found.

3.2 | Flesh quality and texture

No effect of photoperiod on gaping, muscle pH, water content or water holding capacity (WHC) was found (ANCOVA, $P > 0.2$, Table 1). Mean gaping was low in the 9LL group (0.1), but the high within variation within this group and the other experimental groups did possibly prevent any findings of between group effect. Muscle pH was related to size and temperature (ANCOVA, $p < 0.01$, Table 1), but did not vary systematically between the experimental groups. The textural properties of the salmon fillets were softer in the groups displaying higher growth (SNK post hoc test, $p < 0.01$, Table 2). Hardness decreased with increasing temperature, size and growth (Table 2), whereas no effect of photoperiod was found. Accordingly, there was an overall significant linear relationship between fillet hardness and individual growth (linear regression, $p < 0.001$, $R^2 = 0.38$, Fig. 2).
Results on textural properties in the present study, measured as breaking and hardness, suggest that changes in quality was effected by growth properties and temperature, but photoperiod played only a minor role. Previous studies on Atlantic halibut (*Hippoglossus hippoglossus*, Haugen et al., 2006) show the shear forces of the muscles increases in periods with low growth (Hagen, Solber, Sirnes & Johnston, 2007). This could help to explain the overall relationship between textural hardness and somatic growth rate seen in the present study (Fig. 2). Flesh quality of fish is also influenced by season (Espe et al., 2004; Hagen et al., 2007) and is therefore an obvious and relevant parameter in commercial aquaculture. The analysis of fillet quality gave indications of reduced filet hardness with increasing growth rate in accordance with Johnston (1999) and Rasmussen (2001). In line with present findings Mørkøre & Rørvik (2001) investigated product quality of farmed Atlantic salmon for hardness, and found highest values during the winter period. The two photoperiods tested here had only a minor effect on textural properties. This is similar to the findings of Imsland et al. (2009) on Atlantic halibut where photoperiod regimes only have a minor effect on flesh-quality, but a seasonal effect was seen with a tendency towards lower hardness in summer time compared to winter.

Although the results of filet quality measurement (fillet hardness) were based on a relatively simple experiment set-up at one point at the end of the experiment, the results show that the quality in terms of hardness is lower for 9°C group (red symbols to the right in Fig. 2). This could be due to the rapid growth phases for the medium and high temperature groups related to muscle tissue becoming looser to allow growth (Johnston, 1999). In fish, flesh texture is shown to be influenced by a number of different factors, such as light regime (Hemre et al., 2004; Hagen & Johnsen, 2016), temperature (Roth et al., 2005), feeding (Einen et al., 1999), slaughter and filleting method (Kiessling et al., 2004; Kristoffersen et al., 2007) and season (Espe et al., 2004; Imsland et al., 2017). Fast growth has been found to promote softness of salmon fillets
(Mørkøre & Rørvik, 2001), but there is limited knowledge on underlying causes of the correlation between fast growth and softness (Moreno et al., 2016). According to Swatland (1990), the connective tissue (endomysium) associated with individual muscle fibres cannot keep up with rapid muscle fibre growth and as a result is less developed and immature. A recent study by Torgersen et al. (2014) revealed myofibre–myofibre detachments and disappearance of the endomysium in soft salmon muscles, coinciding with deterioration of important connective tissue constituents, such as collagen type I (Col I). Further, 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). In a recent study in a commercial salmon farm in Northern Norway Imsland et al. (2017) investigated 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. Growth was improved by 13-20 % in the early exposed groups (15 Nov. and 11 Nov.) compared to the late exposed groups (13 Dec.), and this was accompanied by minor differences in flesh texture (measured as differences in Cathepsin L+B activity) where increased cathepsin activity was seen groups with corresponding higher growth. 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). The higher cathapsin activity seen in the faster growing group in the study of Imsland et al. (2017) is in line with newer studies by Hagen & 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 findings of Imsland et al. (2017) indicate that harvesting Atlantic salmon during periods of high growth can negative effect on flesh quality in the form of softer muscle tissue. Although the fish in the current study were not of harvesting size a similar relationship between fast growth and tissue softness was found as seen in the study of Imsland et al. (2017).

A detailed analysis of part of the growth data presented here was given by Døskeland et al. (2016). An interactive effect of photoperiod and temperature on somatic growth was found as the fish exposed to low temperature and continuous light regime (4LL) had a significantly higher growth (30 % gain in overall SGR) than the 4LDN group, corresponding to the effect of approx. 1.2°C temperature increase. Further, both daily feeding rate and feed conversion efficiency (FCE) increased with increasing temperature. Feed conversion efficiency (FCE) was significantly higher for the 4LL group compared to the 4LDN group, whereas no differences were seen within the other two temperatures groups. Interactive effect of temperature and photoperiod with increased effect of continuous light at low temperature has previously been reported for juvenile turbot (Imsland, Folkvord & Stefansson, 1995) and Atlantic halibut (Jonassen, Imsland, Kadowaki & Stefansson, 2000) demonstrating that the growth promoting effect of continuous light can be stronger at low temperature compared to near optimum temperature.

5 | CONCLUSION

We conclude that quality in salmon muscle is dependent on growth, where temperature has the major impact, whereas photoperiod only has minor effect on flesh quality and textural properties. The present findings indicate that slaughter of salmon should be avoided in periods of high growth.
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Figure legends

**FIGURE 1** Mean weight (g) of juvenile Atlantic salmon reared at three temperatures (4.3, 6.5 and 9.3°C) and two light regimes (LL = continuous light, LDN = simulated natural photoperiod for Tromsø, Norway). Broken line = LDN, solid line = LL. Blue line = 4.3°C and circle symbol, green line = 6.5°C and square symbol and red line = 9.3°C and diamond symbol. Vertical whiskers indicate standard error of mean (SEM). Letters indicate significant difference between treatments on sampling date (Student–Newman–Keuls test, *p* < 0.05). Asterisk, * denotes significant interaction (two-way ANOVA *p* < 0.05) between photoperiod and temperature.

**FIGURE 2** Texture hardness of PIT tagged juvenile Atlantic salmon reared at two different photoperiods (LDN = simulated natural photoperiod for Tromsø and LL = continuous light) at three temperatures (4, 6 and 9°C). The three temperature groups and two light regimes are separated by color and box symbol. Open symbol = LDN. closed symbol = LL. Blue = 4°C and circle symbol, green = 6°C and square symbol and red = 9°C and diamond symbol.