A comparative study of diploid versus triploid Atlantic salmon (*Salmo salar* L.). The effects of rearing temperatures (5, 10 and 15 °C) on raw material characteristics and storage quality.

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Abstract

Several major market operators argue that the current level of knowledge about quality is too scant to justify a switch to a large-scale production of triploid salmon. The aim of the present study was, therefore, to elucidate how rearing conditions (5, 10 and 15 °C) affect the flesh quality of triploid Atlantic salmon (*Salmo salar* L., 1.6 ± 0.3 kg). As a reference, diploid salmon kept under equal conditions and with equal genetics were used. The main design discriminant was the holding temperature; increased temperature gave increased blood lactate, rigor index (I_r), drip loss (DL), content of astaxanthin and intensity of redness, but reduced muscle pH, cathepsin activity and fillet lightness. Salmon kept at 10 °C grew the fastest. It is concluded that ploidy gave less variation than temperature. Triploids were characterized by lower blood haematocrit (Hct) and I_r, higher DL and collagenase activity, and on average, paler and less yellowish fillets.

Keywords: Triploid Atlantic salmon; rearing temperature; *rigor mortis*; quality.

1. Introduction

Triploid salmon are sterile (O'Flynn, McGeachy, Friars, Benfey, & Bailey, 1997), so no genetic material is transferred from triploid farmed salmon to wild fish. Several conservation and management organizations, including the North Atlantic Salmon Conservation Organization (NASCO), and the Food and Agricultural Organization (FAO) (Taranger & Albretsen, 2014), support triploid production to protect wild species. Triploids used in aquaculture are brown trout in the UK, rainbow trout in the USA and now a few Atlantic salmon production concessions in Norway. The production in Norway emerged due to the announcement of the 45 "green production concessions" (FOR-2013-06-24-754). To qualify for the concessions, farmers were strictly advised to reduce the risk for escapees to breed into wild salmon populations.

Triploid salmon are easily produced. After the sperm has entered the egg, the second set of genetic material produced during the second mitotic division exits the egg through a polar body, leaving the egg with the normal 2n genetic setup. However, eggs subjected to shock by pressure or temperature will not produce the polar body, giving an egg with 2n + 1n chromosomes, *i.e.* the triploid genetic setup (Benfey, 1999). Norwegian law does not define triploid salmon as a genetically modified organism (GMO) due to the production method (§ LOV-1993-04-02-38). For the same reason, triploid salmon production does not need a separate breeding program (Taylor, Preston, Guy, & Migaud, 2011).

Earlier studies show that triploids often have poor performance, and higher mortalities and deformities when compared to diploids (O'Flynn, McGeachy, Friars, Benfey, & Bailey, 1997). These problems seem partly related to altered nutritional requirements. Phosphorus levels in feeds must increase to reduce the skeletal deformations of triploid salmon to the same level as for diploids (Fjelldal, Hansen, Breck, Ørnsrud, Lock, Waagbø, et al., 2012).

On the other hand, triploid salmon grows faster than the diploids in the freshwater phase (Cotter, O'Donovan, O'Maoileidigh, Rogan, Roche, & Wilkins, 2000), thus contributing to a shortened production cycle. Better performance for triploids compared to diploids at low temperatures (e.g. 6 °C) make triploids interesting for cold-water aquaculture. At 15 and 18 °C, triploids are struggling compared to diploids (Hansen, 2012). Triploids are more sensitive to hypoxia than diploids, particular at higher temperatures where larger fish~~es~~ are most sensitive (Hansen, 2012; Hansen, Olsen, Stien, Oppedal, Torgersen, Breck, et al., 2015). Diploid and triploid salmon, reared at 10 °C with low oxygen levels, have the same aerobic capacity (Stillwell & Benfey, 1997).

There has been little investigation of the effects of ploidy on the quality characteristics of Atlantic salmon. The fraction of the superior quality of triploids at slaughter is reduced compared to that of diploids (Cotter, O'Donovan, O'Maoileidigh, Rogan, Roche, & Wilkins, 2000; Fraser, Hansen, Skjæraasen, Mayer, Sambraus, & Fjelldal, 2013; Taylor, Preston, Guy, & Migaud, 2011). Triploid salmon muscle is softer, with increased gaping, and is darker (lower L^*-value) and more reddish (higher a^*-value) compared to that of diploids (Bjørnevik, Espe, Beattie, Nortvedt, & Kiessling, 2004). As far as we know, that is the only literature comparing the quality of diploid and triploid salmon. Softer fillets and increased gaping are related to the muscle cellularity, i.e. larger cell size (Johnston, Alderson, Sandham, Dingwall, Mitchell, Selkirk, et al., 2000). Triploid fish have fewer but larger muscle fibres (Johnston, Strugnell, McCracken, & Johnstone, 1999). Fish texture is generally affected by season, connective tissue, pH post mortem, fish size, muscle fibre size, etc. It is presently unclear if the differences between diploid and triploid flesh are related to genetic variations, variations in the muscle fibre density, or are caused by seasonal changes (Bjørnevik, Espe, Beattie, Nortvedt, & Kiessling, 2004; Choubert, Blanc, & Vallée, 1997).

Several major market operators argue that the current level of knowledge about quality is too scant to justify a switch to a large-scale production of triploid salmon. The aim of the present study was, therefore, to elucidate how rearing conditions affect the flesh quality of triploid salmon. As a reference, diploid salmon kept under equal conditions and with equal genetics were used.

2. Material and methods

2.1. Fish material and experimental design

The Atlantic salmon eggs used were from the Aquagen strain (Aqua Gen AS, Trondheim, Norway) and were produced at the company`s farm in Hemne, Norway (Strike date: 18-19.10.2012).

After fertilization, eggs from the first batch were incubated directly, whereas eggs from the second batch were subjected for approximately 6 minutes to a hydrostatic pressure of 65,500 kPa (TRC-APV, Aqua Pressure Vessel, TRC Hydraulics Inc., Dieppe, Canada), 35 minutes after fertilization at 8 ˚C, to induce triploidy. The eggs were then incubated at 5.8 ˚C and transported to the Institute of Marine Research (IMR), Matre, Norway on the 20th of December 2012. The feeding commenced on the 5th of March 2013. Following smoltification, both groups (diploid and triploid smolts less than a year old) were transferred to an IMR sea-pen system (seawater, mass salinity 34 g/kg) in Smørdalen (Masfjord, Norway). The fish were reared under natural light conditions until the 23rd of June 2014, when 180 fish with an average weight of 1 kg for both groups were hauled and transported (sea vessel Salma) to the experimental facilities at IMR, Matre. The fish were evenly distributed into six 3m tanks (9m^3) with three tanks for each ploidy. The temperature was then adjusted to 5, 10 and 15 ˚C over 30 days and thereafter held constant over 27-29 days until the fish were slaughtered. The fish were fed a commercial salmon feed (Skretting Spirit 600, pellet size: 7mm, protein: 40-

43%, fat: 30-33%, pigment: 50 mg/kg) using automatic feeders (ARVO-TEC T Drum 2000, Arvotec, Huutokoski, Finland) controlled by a computer operated system (Normatic AS, Nordfjordeid, Norway). In addition, the farmer controlled the feed intake. The natural light rhythm was simulated according to season. After four days of starvation, 162 farmed Atlantic salmon (50% diploid and 50% triploid, average weight of 1.6 ± 0.3 kg) were slaughtered between the 19th and 21st of August 2014. The fish were killed one by one by a sharp blow to the head (approximately 3 min between each fish).

On the first day, 60 salmon (10 from each group) were sampled to assess *rigor mortis* development. The first five salmon from each group (n = 30) were sampled for a blood analysis of the lactate, haematocrit (Hct) and plasma levels of sodium (Na^+), potassium (K^+) and chlorine (Cl^-). All the fish were analysed for muscle pH, temperature at death, length and whole body weight. The fish were then stored (not bled) on ice in a refrigerated room (5-6 °C) before they were assessed for *rigor mortis* development (Bito, 1983) and muscle pH over 60 hours.

The day after (August 20th), twelve fish from each group (n = 72) were sampled for fillet shrinkage, drip loss (DL), water holding capacity (WHC), dry matter (DM), colour and texture. The muscle pH, death temperature, length (fork length), and whole body weight were measured before the fish were transferred to the bleeding tank (ice water), gutted and hand filleted pre-rigor. The fillets were then packaged individually in aluminium foil and stored at 5-6 °C for 15 days.

On the last sampling day (August 21st), five fish~~es~~ from each group (n = 30) were sampled for chemical analysis. The muscle pH, death temperature, length and whole body weight were measured before the fish were transferred to the bleeding tank (ice water). Thereafter, the salmon were gutted and hand filleted pre-rigor. The content of astaxanthin, and the cathepsin and collagenase activity of the raw material was determined by analysing muscle tissue from

147 the Norwegian Quality Cut (NQC). On day 0, the NQC from the right fillet, was split into
148 two, vacuum packed separately and frozen at -80 °C. The left fillets were wrapped in
149 aluminium foil and stored at 5-6 °C. After 15 days of storage, the left NQC underwent the
150 same procedure as the right. Then, half the samples were sent to the Norwegian University of
151 Science and Technology (NTNU, Trondheim, Norway) for an analysis of astaxanthin content.
152 The other half were sent to Nofima AS (Stavanger, Norway) for analyses of cathepsin and
153 collagenase activity.

154 *2.2. Muscle pH and temperature*

155 The muscle pH and temperature were measured immediately after death in the anterior dorsal
156 muscle close to the gills by using a Mettler Toledo SevenGo pro^{TM} pH-meter (Mettler Toledo
157 International Inc., USA) connected to an Inlab puncture electrode. The muscle pH and
158 temperature were also measured during storage (see experimental design).

159 *2.3. Blood parameters and rigor mortis development*

160 Blood samples were immediately extracted from the caudal vein using lithium heparinised
161 syringes (n = 30). The blood lactate was measured immediately using a Lactate Pro 2 analyzer
162 (Arkray Factory Inc., Japan). The plasma was prepared by centrifugation (9500 g, 1 min, 6
163 °C, Eppendorf, 5415R, Hamburg, Germany), frozen in liquid nitrogen, and further stored at -
164 80 °C until analysed. The plasma levels of Na^+, K^+ and Cl^- were then analysed on a 9180
165 Electrolyte Analyzer (Roche Diagnostics GmbH, Germany). Hct was obtained using
166 heparinized micro capillary tubes and a Compur M1100 Hct centrifuge.

167 The development of *rigor mortis* was measured by Cuttingers Method (tail drop) (Bito, 1983).
168 The rigor index (I_r) was calculated by the formula $I_r = [(L_o-L_t)/L_o] \times 100$, where L represents
169 the vertical drop (cm) of the tail when half of the fish fork length is placed on the edge of a

table as a function of time. The tail drop at the beginning of the experiment is L_o, while L_t represents measurements throughout the experiment (t = 0-60 hours with interval of 4 hours).

2.4. Shrinkage, DL, WHC, DM, colour and texture

Fillet shrinkage was calculated after a method by Stien, Suontama, and Kiessling (2006). The right fillets were photographed with an SLR camera (Canon EOS 1000D, Canon Inc.) on days 0, 5, 10 and 15, respectively. A ruler was used as a sentinel. The exact area of each fillet was calculated from the representative pictures using Matlab® (MathWorks Inc., Natick, MA, USA).

The DL from the right and left fillets was calculated as the difference in fillet weight between day 0 and days 5, 10 and 15, respectively. An average of the left and right fillets was used for statistical analyses. Before each weighting, the fillets were dried gently with a piece of paper.

$$\text{DL} = \frac{m_0 - m_x}{m_0} \times 100\,\%, \text{ where}$$

m_0: fillet weight at day 0

m_x: fillet weight at day X, X being 5, 10 or 15

The WHC was measured in the dorsal muscle anterior to the dorsal fin of all the left fillets after Skipnes, Ostby, and Hendrickx (2007). The WHC was measured in duplicates at each sampling (5, 10 and 15 days post mortem) on a defined sample (diameter 31 mm, high 6 mm, approximately 5 g). The DM was estimated gravimetrically after drying at 105 °C for 24 hours (ISO 6496 1983).

The surface colour (CIE 1994) was measured by a MiniScan XE, HunterLab Inc., where L^* describes the lightness (L^* = 100 = white, and L^* = 0 = black) of the sample, a^* the intensity in red ($a^* > 0$) and b^* the intensity in yellow ($b^* > 0$). The colour measurements were

192 performed at five defined areas in the dorsal muscle anterior to the dorsal fin of four fillets at
193 day 0 and repeated on the respective fillets 15 days post mortem. An average of the five
194 measurements of each fillet was used for data analyses.

195 Instrumental textural analyses were performed in the dorsal part of the NQC using a Texture
196 Analyzer TA-XT2 (SMS Ltd., Surrey, England) equipped with a 30 kg load cell. A flat-ended
197 cylinder probe (10 mm diameter, type P/1SP) was used. The force-time graph was recorded
198 by a computer equipped with the Texture Exponent software for windows (version 6.1.7.0,
199 SMS), which was also used for the data analyses. The analyses were performed in duplicates
200 (average values were used for data analyses) of four randomly chosen left fillets from each
201 group, 5 and 15 days post mortem. The resistance force (N) was recorded with a constant
202 speed of 5 mm/sec, and the force required to press the cylinder down to 80% of the fillet
203 thickness was used to describe the firmness.

204 *2.5. Chemical composition*

205 The total carotenoid content in the raw fish was determined by analysing muscle tissue from
206 the NQC of five individual fish from each group. Carotenoids were extracted by the method
207 of Bligh and Dyer (1959). The carotenoid content was analysed by high performance liquid
208 chromatography (HPLC), using an Agilent1100 liquid chromatograph (Agilent Technologies,
209 Paolo Alto, CA, USA connected to an Agilent photodiode array UV-VIS detector) after a
210 method by Vecchi, Glinz, Meduna, and Schiedt (1987) using a Lichrosorb SI60-5, 125 × 4.0
211 mm, 5 µm, Hichrom, Reading, UK, HPLC column. The astaxanthin was quantified by a
212 response factor (RF) prepared from a standard of known concentration. The standard was
213 prepared from crystalline all-*E*-astaxanthin (AcrosOrganics, 328612500), and the exact
214 astaxanthin concentration was measured using a spectrophotometer (UV-1700, Shimadzu)
215 using a molar absorptivity of E1%, 1 cm= 2100 (acetone, λmax = 472 nm) (Aas, Bjerkeng,
216 Hatlen & Storebakken, 1997).

The activity of the cathepsin B+L and collagenase was measured as described by Sovik and Rustad (2006). The substrates used were Z-Phe-Arg-AMC (VWR, 102996-428, 14.8 µM/L, 100µl) for the cathepsin B+L, and Suc-Gly-Pro-Leu-Gly-Pro-AMC (VWR, 72698-36-3, 14.8 µM/L, 100µL) for the collagenase (Kojima, Kinoshita, Kato, Nagatsu, Takada, & Sakakibara, 1979). The increases in emission and excitation were measured using a spectrophotometer (Synergy 2, BioTek Instruments, USA) at 460 and 360 nm, respectively. Activity is expressed as the increase in fluorescence, and given in arbitrary units (U) based on the mean of three measurements.

2.6. Statistics

The data were analysed by a general linear model (GLM) with the ploidy, holding temperature and storage time as fixed factors. A multivariate GLM with *L*, a* and b** as multiple Y were used to analyse fillet appearance. Pearson`s correlation coefficient (r) was used to calculate the linearity dependence between the variables X and Y. To compare different groups, one-way ANOVA and Duncan`s comparison test was used. All statistical analyses were performed using an IBM Statistical Package for the Social Sciences statistics software (release 23, IBM corporation, USA). The alpha level was set to 5% ($P < 0.05$). All results are given as an average ± standard deviation (SD), unless otherwise stated.

3. Results and Discussion

All the fish~~es~~ examined in the present study were of the Aquagen strain (Aqua Gen AS, Trondheim, Norway) but differed in ploidy, and in holding temperature throughout the last period (27-29 days) of their life cycle. The feeding and rearing strategies were, on the other hand, equal. Hence, the observed differences in the growth, physicochemical and autolytic processes were most likely caused by differences in ploidy and/or holding temperature.

241 *3.1. Biometrics, death temperature and pH*

242 Several studies have shown that the rearing temperature affects the growth rate of Atlantic
243 salmon (Austreng, Storebakken, & Åsgård, 1987; Hevrøy, Hunskår, de Gelder, Shimizu,
244 Waagbø, Breck, et al., 2013). In a controlled experiment reported by Hevrøy, et al. (2013),
245 diploid salmon were fed (45 days) at 13, 15, 17 and 19 °C, respectively. The most efficient
246 growth was achieved at 13 °C. Furthermore, salmon reared at 15 and 17 °C grew efficiently
247 for the first two weeks but then exhibited reduced feed intake and growth over the last part of
248 the study. Austreng, Storebakken, and Åsgård (1987) reported, however, an increased growth
249 rate as a consequence of increased water temperature (examined between 2-14 °C). These
250 findings, together with those presented by Hevrøy et al. (2013), indicated that the best rearing
251 temperature, or the "comfort zone" for Atlantic salmon, should be somewhere around 10-14
252 °C. When the water temperature falls below the "comfort zone", the fish starts to consume
253 less feed due to decreased appetite (Austreng, Storebakken, & Åsgård, 1987). In the present
254 study, significant effects of the holding temperature on the whole body weight (GLM, $P <$
255 0.001), fork length (GLM, $P = 0.001$) and condition factor (CF, GLM, $P < 0.001$) were found
256 (Table 1). Salmon kept at 10 °C grew significantly faster compared to salmon kept at 5 and 15
257 °C, respectively. There were no effects of ploidy on either of these parameters (GLM, $P >$
258 0.12 - 0.65). Significant differences in fish weight observed in the present study only after 27-
259 29 days at adjusted temperatures (5, 10, or 15 °C), show the importance of temperature
260 control during salmon growth. Friars, McMillan, Quinton, O'Flynn, McGeachy, and Benfey
261 (2001) found better growth and higher CF, together with higher variance of growth within and
262 between families, in triploid compared to that of diploid salmon. In the present study, no such
263 effects were seen. That may be due to the relatively short time at a stable temperature. The
264 larger cell size of triploids does, however, not induce any growth advantages (Benfey, 1999).
265 In the present study, normal growth and feed intake were observed for all fish at the

respective water temperatures (5, 10, or 15 °C). It is therefore likely that our data reflects fish reared under the respective conditions. The average death temperature of the fish~~es~~ from each group reflected the water temperature of the respective tanks, whereas the muscle pH at the point of death decreased as a function of increased temperature (Table 1).

3.2. Blood parameters and rigor mortis development

Blood analyses of the fish were performed to investigate the fish resilience against handling stress (Lerfall, Roth, Skare, Henriksen, Betten, Dziatkowiak-Stefaniak, et al., 2015). In the present study the blood parameters (Hct, K^+, Cl^- and lactate), but not Na^+ (GLM, $P > 0.59$), were significantly affected by the experimental design (GLM, Table 2). The main discriminant was the holding temperature where fish kept at 5 °C were found to be more resilient compared to fish stored at higher temperatures. The blood lactate increased as a function of time from the first to the last fish for salmon kept at 15 °C. This was not observed for salmon kept at lower temperatures. Na^+ was about 159 mM regardless of ploidy and treatment. Both the K^+ and Cl^- levels were lowest in the groups maintained at 10 °C, and increasing at 5 and 15 °C. The Hct level was lowest in the triploid salmon reared at 5 °C and was affected by ploidy (GLM, $P = 0.021$) and holding temperature (GLM, $P = 0.002$).

The development of *rigor mortis* was clearly affected by the experimental design (GLM, $P < 0.001$), where an increased holding temperature showed a stepwise acceleration of *rigor mortis* (GLM, $P < 0.001$, Fig. 1, Table 2). The highest average I_r was measured in salmon kept at 15 °C (97.6 ± 3.2%), whereas the lowest I_r was observed in salmon kept at 5 and 10 °C (87.4 ± 7.9% and 88.9 ± 6.2%, respectively). Temperature influences *rigor mortis* in bony fish (Arimoto, Gang, & Matsushita, 1991; Bito, 1983). High rearing temperatures might act as a stress factor, which can have a large impact on the onset of *rigor mortis*. In the present study, the blood lactate was higher, and the H^+ (pH) lower in salmon kept at 15 °C. This was most likely caused by a higher metabolism and faster depletion of stored energy, followed by

a faster onset and stronger maximum I_r, as compared to those of the salmon kept at 5 and 10 °C.

The effects of ploidy in the development of *rigor mortis* is, however, unclear. The I_r was found to be affected by ploidy where higher I_r was observed for diploid compared to triploid salmon (on average 93.5 ± 7.5% and 89.1 ± 6.9%, respectively, GLM, $P = 0.004$). An important difference between diploid and triploid salmon is the cell size, which may influence the processes inside the cell and the *rigor mortis* development. The cross-bridge complex between actin and myosin (Currie & Wolfe, 1979) causes the rigidity of *rigor mortis*. These bindings cannot be regenerated post rigor because of a lack of adenosine triphosphate (ATP) to transport Ca^{2+}. *Rigor mortis* must be the breakage of cellular membranes, destruction of the osmotic potential and proteolysis (Hultin, 1984; Tsuchiya, Kita, & Seki, 1992), in addition to the cross-bridges between actin and myosin. Slinde, Roth, Balevik, Suontama, Stien, and Kiessling (2003) suggested that the rigor process is caused by water movements from inter- to intracellular space in the muscle (not actomyosin contraction). Ando, Yoshimoto, Inabu, Nakagawa, and Makinodan (1995) showed increased proteolytic activities caused by ruptures in the cell membranes during the early stages of the rigor process. In larger cells, as in triploids, the equalization of the osmotic pressure may be slower and thereby cause a slower onset of *rigor mortis*. No differences in the onset of *rigor mortis* were observed here between diploid and triploid salmon. However, the time before maximum rigor (hour) correlated significantly to the maximum I_r ($r = -0.45$, $P < 0.001$) and muscle pH ($r = 0.39$, $P = 0.002$), and the contents of lactate ($r = -0.61$, $P < 0.001$), Hct ($r = -0.41$, $P = 0.026$) and K^+ ($r = 0.39$, $P = 0.038$).

315 *3.3. Shrinkage, DL, WHC and DM*

316 The amount of fillet shrinkage assessed after 5, 10 and 15 days of storage was significantly
317 affected by the experimental design (GLM, $P < 0.001$, Table 3) where significant effects of
318 the holding temperature (GLM, $P < 0.001$) and storage time (GLM, $P < 0.001$) were
319 observed. Ploidy had no effect on fillet shrinkage ($P > 0.30$, GLM). The highest shrinkage
320 was observed in salmon kept at 5 and 10 °C, and the lowest in those originally kept at 15 °C
321 (average of diploid and triploid salmon, 4.7 ± 2.3% (5 °C) and 5.5 ± 2.8% (10 °C) versus 2.9
322 ± 2.5% (15 °C) GLM, main effects of holding temperature, $P < 0.001$). During storage, fillets
323 reshaped, which resulted in less shrinkage measured at the end of the storage period (15 days).
324 When fillets shrink, an increased squeezing of the cells occurs, which may lead to increased
325 cell damage and consequently higher DL. In the present study a weak but significant
326 correlation was observed between the fillet DL and fillet shrinkage during storage ($r = 0.32$, P
327 < 0.001).

328 The DL was significantly affected by the experimental design (GLM, $P < 0.001$, Table 3)
329 where significant effects of the ploidy (GLM, $P = 0.001$), holding temperature (GLM, $P <$
330 0.001) and storage time (GLM, $P < 0.001$) were observed. In general, the DL in triploids was
331 higher throughout the 15 days of storage as compared to that in diploids (5.5 ± 0.7% versus
332 5.0 ± 1.2%, respectively). The largest differences in DL were observed between diploid and
333 triploid salmon kept at the medium temperature (10 °C). This is probably linked to the larger
334 cell size (caused by the extra set of chromosomes) and consequent higher amounts of cytosol
335 in triploid cells (Benfey, 1999). Moreover, the DL was found to increase in a stepwise manner
336 with increased temperature.

337 Both the WHC and DM were significantly affected by the experimental design (GLM, $P <$
338 0.001, Table 3), where significant effects of the holding temperature (GLM, $P = 0.026$ and $<$
339 0.001, respectively) and storage time (GLM, $P < 0.001$) were observed. Ploidy did not affect

the fillet WHC or DM (GLM, $P > 0.50$ and > 0.57, respectively). After 5 days of storage the highest WHC was observed in the diploid salmon kept at 15 °C, whereas no significant differences were observed after 10 and 15 days of storage. In general, both the WHC and DM were negatively correlated to the DL (r = -0.40, $P = 0.001$ and r = -0.28, $P = 0.018$), which resulted in a slight increase of the WHC and DM of the fillets during storage.

3.4. Colour, texture and chemical composition

The fillet appearance (CIE 1994) was affected by the ploidy, holding temperature and storage time (Multivariate GLM, $P < 0.001$, Table 4). Salmon kept at 5 °C was paler (higher L^*-value) and less reddish (lower a^*-value) as compared to salmon kept at 10 and 15 °C. The L^*-value was found to decrease stepwise with an increased holding temperature. A comparable intensity of redness as observed in salmon kept at 5 °C was also observed in those kept at 10 and 15 °C. The yellowness (b^*) was not affected by holding temperature (GLM, $P > 0.23$). Ploidy affected fillet lightness (L^*, GLM, $P = 0.016$) and yellowness (b^*, GLM, $P = 0.003$), where triploid salmon on average were paler (higher L^*-values) and less yellowish (lower b^*-values) as compared to diploids. Fillet redness (a^*) was not affected by the ploidy (GLM, $P > 0.23$). Ploidy has been previously found to affect the flesh colour in rainbow trout (Choubert, Blanc, & Vallée, 1997) and Bjørnevik, Espe, Beattie, Nortvedt, and Kiessling (2004) reported a darker and a more reddish colour of triploid salmon. No effect of ploidy on colour at the time of slaughter was observed here. After 15 days of storage, however, triploids kept at 10 °C had paler fillets (higher L^*) and increased yellowness (higher b^*) compared to the diploids. On average, however, triploids were significantly paler and less yellowish. This effect is, however, probably too small to be recognized by the human eye. The intensity of redness (a^*-value) increased stepwise with an increased holding temperature independent of ploidy. A medium correlation (r = 0.41) between the contents of astaxanthin and fish weight indicated an increased redness/astaxanthin content with an increased feed intake. The content

of astaxanthin was affected by the holding temperature (GLM, $P < 0.001$), but not by ploidy (GLM, $P > 0.67$) or storage time (GLM, $P > 0.97$, Table 5). The lowest concentration of astaxanthin was found in salmon kept at 5 °C with a stepwise increase as a function of increased water temperature. Moreover, a significant correlation was observed between astaxanthin and fish weight ($r = 0.41$, $P = 0.026$). In addition, the distribution of astaxanthin 13Z-isomers increased slightly as a function of increased holding temperature. Choubert and Blanc (1989) reported triploid rainbow trout to have better muscle pigmentation (canthaxanthin) compared to sexually maturing female diploids. Better muscle pigmentation of triploids was not observed in the present study, presumably due to the immaturity of the diploid salmon used. The increased content of the astaxanthin 13*Z*-isomer with an increased holding temperature might be a result of disturbances in the metabolic pathways of astaxanthin induced by increased metabolism and consequently increased formation of reactive oxygen species (ROS). Z-isomers are known to have better antioxidant properties than all-*E* astaxanthin (Liu & Osawa, 2007).

Triploid salmon flesh has been reported to be softer compared to that of diploids. This has been related to fewer small muscle fibres and a 23% larger mean cross-sectional fibre in triploids (Bjørnevik, Espe, Beattie, Nortvedt, & Kiessling, 2004). An inverse relationship between the average fibre diameter and flesh firmness is indicated (Hurling, Rodell, & Hunt, 1996). In the present study, however, fillet firmness was significantly affected by the experimental design (GLM, $P = 0.037$, Table 4), with holding temperature as the only significant factor (GLM, $P = 0.015$). There were no effects of ploidy or storage time (GLM, $P > 0.48$ and > 0.99, respectively). The softest fillets were observed in diploid salmon kept at 10 °C, whereas diploid salmon kept at 15 °C were the firmest. Fillet firmness and CF were, moreover, uncorrelated ($r = -0.34$, $P > 0.11$).

389 Different families of Atlantic salmon have different cathepsin activities (Bahuaud, Gaarder,
390 Veiseth-Kent, & Thomassen, 2010), where high activity is related to a soft flesh texture
391 (Bahuaud, Mørkøre, Østbye, Veiseth-Kent, Thomassen, & Ofstad, 2010). The cathepsin
392 activities are related to pre slaughtering stress (Bahuaud, Mørkøre, Østbye, Veiseth-Kent,
393 Thomassen, & Ofstad, 2010; Lerfall et al., 2015). In the present study, the cathepsin activities
394 were related to the holding temperature but not to ploidy. As far as we know, there have been
395 no studies of collagenase activities in triploid salmon. During the chilled storage of fish, a
396 progressive *post mortem* breakdown of the fine collagenous fibrils that anchor the muscle
397 fibres to the myocommata occurs (Ando, Yoshimoto, Inabu, Nakagawa, & Makinodan, 1995).
398 Due to the higher collagenase activity in triploids, a softer texture should be expected.
399 However, this was observed only for the fish kept at 15 °C. The mechanism of post mortem
400 flesh softening is complex and further research is needed to understand all the underlying
401 mechanisms. Cathepsin and collagenase activities were significantly affected by the
402 experimental design (GLM, $P < 0.001$ and $= 0.003$, respectively, Table 5). The highest level
403 of collagenase activity was found in the triploid salmon (GLM, $P < 0.001$), whereas the
404 cathepsin activity was not affected by ploidy ($P > 0.42$). The opposite effect was observed for
405 holding temperature. The holding temperature affected cathepsin activity (GLM, $P < 0.001$)
406 but not collagenase activity (GLM, $P > 0.34$). Moreover, the cathepsin activity increased and
407 the collagenase activity decreased as an effect of storage time (GLM, $P < 0.001$ and $= 0.001$,
408 respectively). No significant correlation between the activity of cathepsin and collagenase was
409 observed ($r = -0.12$, $P = 0.49$).

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411

412

4. Conclusion

It is concluded that the main discriminant was the holding temperature; increased temperature gave increased blood lactate, I_r, DL, content of astaxanthin and intensity of redness, but reduced muscle pH, cathepsin activity and fillet lightness. Ploidy contributed less to the variation than the temperature did. However, triploid salmon showed lower blood Hct and I_r, higher DL, fat content and collagenase activity, and on average paler and less yellowish fillets than diploids. Moreover, it is concluded that an increase in storage time gave an increased DL, contents of DM, yellowness and cathepsin activity, but decreased the collagenase activity of the salmon flesh.

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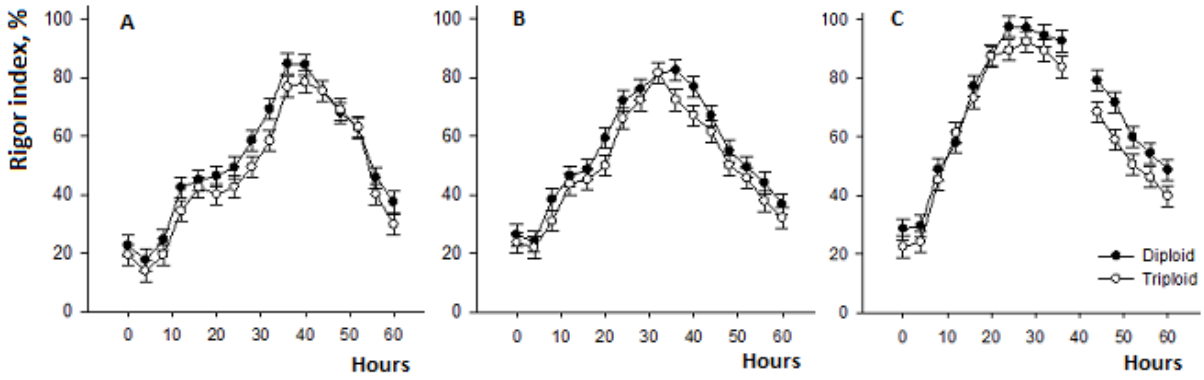
534

Figure caption

Fig. 1. Rigor index (I_r) (Bito, 1983) of Atlantic salmon kept at different water temperatures. The rigor index was measured with a time interval of 4 hours from 0-60 hours post mortem. **A:** 5 °C; **B:** 10 °C and **C:** 15 °C. Results are shown as average ± SD. Bars indicate one SD.



554
555 **Fig. 1.**

Table 1

Average biometrics, death temperature and pH of diploid and triploid Atlantic salmon kept at 5, 10 and 15 °C

	5 °C		10 °C		15 °C		*GLM* [b]			
	Diploid	**Triploid**	**Diploid**	**Triploid**	**Diploid**	**Triploid**	P_M	P_P	P_T	$P_{P\times T}$
Whole weight, kg [a]	1.4±0.4[b]	1.4±0.3[b]	1.7±0.3[a]	1.7±0.3[a]	1.5±0.3[b]	1.5±0.3[b]	*<0.001*	*0.65*	*<0.001*	*0.98*
Fork length, cm [a]	50.7±3.6[b]	51.7±2.6[ab]	53.3±2.9[a]	53.4±3.3[a]	52.0±3.3[ab]	53.2±2.5[a]	*0.005*	*0.12*	*0.001*	*0.64*
Condition factor [a]	1.0±0.1[c]	1.0±0.1[c]	1.1±0.1[ab]	1.1±0.2[a]	1.1±0.1[bc]	1.0±0.1[c]	*<0.001*	*0.28*	*<0.001*	*0.22*
Death temp., °C [a]	6.0±0.2[d]	6.0±0.2[d]	11.2±0.1[c]	11.1±0.1[c]	15.8±0.1[a]	15.7±0.2[b]	*<0.001*	*0.011*	*<0.001*	*0.36*
Muscle pH [a]	7.3±0.1[ab]	7.3±0.1[a]	7.2±0.2[bc]	7.2±0.2[bc]	7.2±0.2[c]	7.2±0.2[c]	*<0.001*	*0.23*	*<0.001*	*0.97*

[a] Average values of 27 individuals per group, in total 162 individuals.

[b] General Linear Model (GLM) analyses of variance, where P_M, P_P, P_T, and $P_{P\times T}$ are the significance levels for the effects of the model, ploidy, holding temperature and the interaction between ploidy and holding temperature, respectively. Different superscripts ([abcd]) within each row indicate significant differences ($P < 0.05$) by a one-way ANOVA and Duncan's comparison test.

Table 2
Blood parameters (Hct, Na^+, K^+, Cl^- and lactate) at point of death and maximum rigor contraction (index and hour) of diploid and triploid Atlantic salmon kept at 5, 10 and 15 °C

	5 °C		10 °C		15 °C		*GLM* [c]			
	Diploid	**Triploid**	**Diploid**	**Triploid**	**Diploid**	**Triploid**	P_M	P_P	P_T	$P_{P\times T}$
Blood parameters:										
Hct, % [a]	31.2±2.6^{a}	25.8±4.5^{b}	36.2±3.4^{a}	33.4±3.1^{a}	32.6±3.4^{a}	31.2±4.0^{a}	*0.004*	*0.021*	*0.002*	*0.46*
Na^+, mmol l^{-1} [a]	158.8±1.6	157.6±3.6	156.8±2.4	159.6±2.3	159.0±1.4	159.0±4.0	*0.59*	*0.60*	*0.76*	*0.25*
K^+, mmol l^{-1} [a]	6.4±0.9^{a}	6.6±1.7^{a}	3.1±0.5^{c}	3.9±0.7^{bc}	5.3±0.8^{ab}	5.2±1.2^{ab}	*<0.001*	*0.48*	*<0.001*	*0.68*
Cl^-, mmol l^- [a]	136.6±3.1^{ab}	137.0±1.9^{a}	133.2±1.6^{b}	136.2±2.7^{ab}	138.4±1.3^{a}	140.0±4.4^{a}	*0.016*	*0.099*	*0.004*	*0.55*
Lactate, mmol l^{-1} [a]	0.9±0.3^{b}	1.1±0.7^{b}	1.7±0.8^{ab}	2.1±1.4^{ab}	2.5±1.0^{a}	2.6±1.1^{a}	*0.031*	*0.52*	*0.004*	*0.94*
Rigor mortis										
Max. index, % [b]	91.5±8.2^{bc}	83.4±5.2^{d}	89.7±7.2^{c}	88.1±5.3^{cd}	99.3±1.4^{a}	95.8±3.5^{ab}	*<0.001*	*0.004*	*<0.001*	*0.178*
Max. time, h [b]	38.8±4.2^{a}	39.6±4.4^{a}	32.0±5.7^{b}	31.6±5.8^{b}	25.6±7.4^{c}	22.8±5.7^{c}	*<0.001*	*0.58*	*<0.001*	*0.59*

[a] Average values of five individuals per group, in total 30 individuals.
[b] Average values of 10 individuals per group, in total 60 individuals.
[c] General Linear Model (GLM) analyses of variance, where P_M, P_P, P_T, and $P_{P\times T}$ are the significance levels for the effects of the model, ploidy, holding temperature and the interaction between ploidy and holding temperature, respectively. Different superscripts (^{abcd}) within each row indicate significant differences ($P < 0.05$) by a one-way ANOVA and Duncan's comparison test.

Table 3

Average drip loss (DL), shrinkage, water holding capacity (WHC) and dry matter (DM) of diploid and triploid Atlantic salmon kept at 5, 10 and 15 °C

		5 °C		10 °C		15 °C					
	Day	**Diploid**	**Triploid**	**Diploid**	**Triploid**	**Diploid**	**Triploid**	P_M	P_P	P_T	P_S
*DL, %**	*5*	1.7±0.3^{ab}	1.8±0.3^{a}	1.4±0.5^{bc}	1.8±0.2^{a}	1.4±0.4^{c}	1.5±0.4^{bc}				
	10	2.8±0.5^{c}	2.9±0.4^{bc}	2.5±0.8^{c}	3.1±0.4^{bc}	3.4±0.7^{ab}	3.8±0.9^{a}				
	15	4.4±0.8^{c}	4.4±0.7^{c}	4.6±0.9^{c}	5.6±0.7^{b}	6.2±1.0^{ab}	6.4±1.1^{a}				
	GLM [c]							*<0.001*	*0.001*	*<0.001*	*<0.001*
Shrinkage, % [a]	*5*	6.6±1.9^{ab}	6.4±2.3^{ab}	5.8±2.1^{b}	8.0±3.4^{a}	4.9±1.7^{bc}	3.3±2.4^{c}				
	10	4.3±1.9^{ab}	3.2±2.2^{bc}	4.2±1.4^{b}	5.8±2.5^{a}	3.8±2.2^{bc}	2.3±1.8^{c}				
	15	4.8±1.7^{ab}	3.1±1.9^{b}	3.9±2.0^{ab}	5.4±3.4^{a}	2.8±2.^{4b}	0.5±2.3^{c}				
	GLM [c]							*<0.001*	*0.30*	*<0.001*	*<0.001*
WHC, % [b]	*5*	87.2±2.2^{b}	86.9±2.6^{b}	86.9±2.6^{b}	87.3±2.7^{b}	92.0±1.5^{a}	89.8±2.5^{ab}				
	10	89.8±4.5	90.6±3.3	91.5±3.4	90.5±4.0	94.2±3.2	91.6±3.3				
	15	92.4±1.6	95.0±1.6	91.0±2.1	94.7±2.3	91.8±3.4	94.5±3.1				
	GLM [c]							*<0.001*	*0.50*	*0.026*	*<0.001*
DM, % [b]	*5*	28.9±1.9	27.8±0.9	30.2±0.8	30.6±1.9	29.4±1.2	29.4±1.5				
	10	27.1±0.7^{c}	29.3±1.0^{abc}	30.0±1.7^{ab}	31.6±2.5^{a}	27.3±1.6^{c}	28.7±0.9^{bc}				
	15	29.5±2.5^{bc}	27.5±1.3^{c}	36.8±2.8^{a}	35.2±1.6^{a}	30.2±2.5^{bc}	31.3±1.6^{b}				
	GLM [c]							*<0.001*	*0.57*	*<0.001*	*<0.001*

[a] Average values of 12 individual fillets per group, in total 72 individuals.

[b] Average values of four individual fillets per group per day, in total 72 individuals.

[c] General Linear Model (GLM) analyses of variance, where P_M, P_P, P_T, and P_S are the significance levels for the effects of the model, ploidy, holding temperature and storage time, respectively. Different superscripts (^{abc}) within each row indicate significant variation ($P < 0.05$) between groups by a one-way ANOVA and Duncan's comparison test.

Table 4

Colorimetric parameters (Hunter Lab-values) and fillet firmness (force at 80% compression, N) of diploid and triploid Atlantic salmon kept at 5, 10 and 15 °C

Parameter [a]	Day	5 °C		10 °C		15 °C					
		Diploid	Triploid	Diploid	Triploid	Diploid	Triploid	P_M	P_P	P_T	P_S
L	*0*	53.5±1.6^{a}	53.2±0.7^{a}	51.3±1.6^{b}	51.8±0.7^{ab}	50.7±1.3^{b}	51.3±1.4^{b}				
	15	52.5±1.3^{b}	53.2±1.4^{ab}	51.6±1.1^{bc}	54.1±0.6^{a}	50.7±1.1c	51.7±0.4^{bc}				
	GLM [b]							*<0.001*	*0.016*	*<0.001*	*0.31*
a	*0*	19.1±3.2^{c}	20.0±1.2^{bc}	22.7±1.3^{a}	22.0±1.7^{ab}	22.6±1.5^{a}	22.3±1.0^{ab}				
	15	20.8±2.7^{c}	20.4±1.1^{c}	22.7±1.6^{abc}	20.3±1.5^{c}	23.3±1.2^{a}	23.1±0.5^{ab}				
	GLM [b]							*0.004*	*0.26*	*<0.001*	*0.54*
b	*0*	23.3±2.3	23.2±0.7	25.0±1.1	23.5±1.0	24.4±1.0	23.6±1.1				
	15	23.0±2.3^{a}	22.0±0.6^{a}	22.8±1.3^{a}	20.0±1.3^{b}	23.3±0.7^{a}	22.6±0.3^{a}				
	GLM [b]							*0.001*	*0.003*	*0.23*	*<0.001*
Fillet firmness	*5*	12.8±2.0^{bc}	14.6±2.6^{ab}	10.7±1.6^{c}	13.7±1.7^{bc}	17.7±2.3^{a}	14.3±1.6^{b}				
80% (N)	*15*	14.3±2.8	15.0±2.8	12.7±3.0	13.4±2.1	14.2±2.0	14.1±1.3				
	GLM [b]							*0.037*	*0.48*	*0.015*	*0.99*

[a] Average values of 4-5 individuals per group per day.

[b] General Linear Model (GLM) analyses of variance, where P_M, P_P, P_T, and P_S are the significance levels for the effects of the model, ploidy, holding temperature and storage time, respectively. Different superscripts (^{abc}) within each row indicate significant variation ($P < 0.05$) between groups by a one-way ANOVA and Duncan's comparison test.

Table 5

Contents of astaxanthin, distribution of astaxanthin isomers, and cathepsin and collagenase activity of diploid and triploid Atlantic salmon kept at 5, 10 and 15 °C

		5 °C		10 °C		15 °C					
	Day	**Diploid**	**Triploid**	**Diploid**	**Triploid**	**Diploid**	**Triploid**	P_M	P_P	P_T	P_S
Astaxanthin mg kg^{-1} [a]	*0*	3.0±0.4^{b}	3.0±0.2^{b}	3.4±0.4^{b}	3.2±0.5^{b}	4.3±0.6^{a}	4.3±0.5^{a}				
	15	3.1±0.5^{bc}	2.7±0.7^{c}	3.2±0.3^{bc}	3.5±0.0^{b}	4.4±0.4^{a}	4.3±0.5^{a}				
	GLM [c]							*<0.001*	*0.67*	*<0.001*	*0.97*
All-E astaxanthin, % [a]	*0*	93.5±0.9^{ab}	93.9±1.0^{a}	92.5±0.8^{bc}	93.0±0.8^{abc}	90.0±0.4^{d}	92.4±0.8^{c}				
	15	93.6±0.7^{a}	92.4±1.9^{ab}	91.7±0.7^{b}	90.9±0.1^{b}	91.5±0.6^{b}	91.8±0.5^{b}				
	GLM [c]							*0.021*	*0.59*	*0.12*	*0.072*
9-Z astaxanthin, % [a]	*0*	1.1±0.2	1.1±0.2	1.1±0.3	0.8±0.2	1.0±0.0	1.0±0.2				
	15	1.2±0.3^{a}	1.2±0.3^{a}	1.0±0.1^{ab}	1.0±0.1^{ab}	1.0±0.1^{ab}	0.9±0.1^{b}				
	GLM [c]							*0.087*	*0.66*	*0.013*	*0.17*
13-Z astaxanthin, % [a]	*0*	5.4±0.8^{c}	5.1±0.8^{c}	6.4±0.6^{b}	6.2±0.6^{b}	9.0±0.4^{a}	6.7±0.8^{b}				
	15	5.2±0.6^{c}	6.4±1.6^{bc}	7.2±0.8^{ab}	8.1±0.1^{a}	7.5±0.6^{ab}	7.2±0.5^{ab}				
	GLM [c]							*0.004*	*0.59*	*0.014*	*0.065*
Cathepsin mU g^{-1} [b]	*0*	1.1±0.1	2.0±0.4	2.1±1.2	1.9±0.2	0.8±0.3	0.9±0.2				
	15	2.9±0.3^{a}	2.7±0.3^{a}	2.4±0.3^{ab}	2.4±0.1^{ab}	1.9±0.5^{b}	2.1±0.1^{ab}				
	GLM [c]							*<0.001*	*0.42*	*<0.001*	*<0.001*
Collagenase mU g^{-1} [b]	*0*	2.0±0.3^{bc}	2.8±0.1^{a}	2.1±0.4^{bc}	2.8±0.4^{a}	1.9±0.3^{c}	2.5±0.2^{abc}				
	15	1.5±0.4	2.0±1.0	1.3±0.2	2.4±0.3	1.2±0.3	1.9±0.9				
	GLM [c]							*0.003*	*<0.001*	*0.34*	*0.001*

[a] Average values of 10 individuals per group, in total 60 individuals.

[b] Average of three individuals per group, in total 18 individuals. For all parameters the same fillet was analysed both at day 0 and 15.

[c] General Linear Model (GLM) analyses of variance, where P_M, P_P, P_T, and P_S are the significance levels for the effects of the model, ploidy, holding temperature and storage time, respectively. Different superscripts (^{abcd}) within each row indicate significant variation ($P < 0.05$) between groups by a one-way ANOVA and Duncan's comparison test.