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Effect of ploidy and temperature on quality attributes of diploid versus triploid Atlantic salmon (*Salmo salar* L.), and the effect of locality (north-south) and season (spring-autumn) for quality attributes under commercial production.

Even Flønes Skare

ACKNOWLEDGEMENTS

This work was conducted as part of education at Norwegian University of Life Science (NMBU), Department of Chemistry, Biotechnology and Food Science. This thesis lasted from January 2014 to May 2015, where the task has been part of two projects.

The first project has been part of a larger project, "Reduced drip loss from salmon fillet", funded by the Norwegian Research Council and Marine Harvest ASA. The project was carried out in collaboration with Nofima (Stavanger) and Sør-Trøndelag University College (HiST), Trondheim.

The second project, "Quality differences between diploid and triploid salmon", has been going on at Institute of Marine Research (IMR) station at Matre in Masfjorden municipality (Hordaland). There has been a close collaboration with fellow student Pål Rune Hasli in this project. We have divided the tasks between us, and the introduction and illustrations in the present thesis are performed in close cooperation, beyond this we have separate thesis. This project had not been feasible without researcher Rolf Erik Olsen v / IMR research station at Matre, now researcher at Norwegian University of Science and Technology (NTNU). The presented work has been funded by Researchers from several institutions; researcher Rolf Erik Olsen (IMR), Professor Bjørg Egelandsdal (NMBU), senior researcher Bjorn Roth (Nofima) and Associate Professor Jørgen Lerfall (HiST).

In all, this thesis was a collaboration between NMBU (Ås), HiST (Trondheim), IMR (Matre) and Nofima (Stavanger). Outsourced analyses was contraction analysis which was conducted by researcher Lars Helge Stien v / IMR's research station in Austevoll, and protein analysis of diploid/triploid salmon which was performed by researcher Line Bach Christensen v / Nofima, Stavanger. Colour and fat analyses were performed at HiST. There are dozens to thank for help and guidance in dealing with my thesis. First, I want to thank my main supervisors Professor Erik Slinde and Associate Professor Jørgen Lerfall. The same applies Professor Bjørg Egelandsdal, researcher Rolf Erik Olsen, senior researcher Bjorn Roth and researcher Bjorn Tore Rotabakk. Thanks to Lene Ruud Lima (NMBU) and all employees at IMR research station at Matre, a special thanks to Stian Morken, Grethe Thorsheim, Ivar Helge Matre, Britt Sværen Daae, Karen Anita Kvestad and base commander Øivind Torslett. Finally, thanks to my fellow student; Pål Rune Hasli.

Ås, May 2015 Even Flønes Skare

ABSTRACT

Fillet quality of farmed Atlantic salmon (*Salmo salar* L.) is affected by a broad range of *pre-* and *post-mortem* factors. The "green A licenses" in North-Norway has led increased focus on triploidisation of Atlantic salmon (again) in the Norwegian aquaculture. Based on that, this thesis focused mainly on the effect of ploidy and temperature on quality of raw salmon fillets. In addition, the effect of locality (north-south) and season (spring-autumn) on quality during commercial production of diploid Atlantic salmon was studied. Both *pre-* and *post mortem* factors are investigated, and among *post mortem* factors temperature, drip loss, water holding capacity (WHC), fillet firmness, rigor development and chemical composition are important.

To study the effect of locality (north-south) and season (spring-autumn) on fillet quality, salmon were divided into four groups. Salmon were sampled from the waiting cage or well boat by Marine Harvest facilitys at Hjelmeland and Herøy, both in May and November 2014 (average full weight 5.42 ± 0.99 kg). This resulted in a sample design with four groups based on locality (north-south) and season (spring-autumn). To study the effect of ploidy and temperature on fillet quality, salmon (average full weight 1.55 ± 0.34 kg) were picked from the Institute of Marine Research experimental indoor facilities (Matre). This resulted in a sample design with six groups based on their ploidy (diploid and triploid) and growth temperature (5, 10 and 15°C).

In the first project, "The effect of locality (north-south) and season (spring-autumn) on quality", it is concluded that locality (north-south) and season (spring-autumn) did affect the drip loss of commercial farmed diploid salmon. Moreover, it is concluded that locality (north-south) had a clear main effect on fillet firmness.

In the second project, "Quality differences between diploid and triploid salmon", it is concluded that triploid salmon has higher collagenase and fat content compared to diploids. Moreover, it is concluded that growth temperature affect rigor mortis, but the effects of ploidy is however not clear. The drip loss in triploid salmon were found to be nominally higher compared with diploids, and growth temperature were found to increase the drip loss from the fillets. The quality of triploid salmon seemed however to be remarkably similar to diploids when the fish had the same background.

In accordance with the results in this thesis, the presented study demonstrate significant variation in biometrical traits and quality attributes between locality (north-south), season (spring-autumn), ploidy (diploid-triploid) and growth temperatures (5, 10 and 15°C).

SAMMENDRAG

Filet kvalitet hos oppdrettet atlantisk laks (*Salmo salar* L.) blir påvirket av ulike faktorer *pre* og *post mortem*. De "grønne A konsesjonene" i Nord-Norge har ført til økt fokus på triploidisering av atlantisk laks (igjen) i norsk akvakultur. På bakgrunn av det, ble hovedfokuset i masteroppgaven rettet mot å studere effekten av ploiditet (diploid-triploid) og temperatur (5, 10 og 15°C). på kvalitet hos rå laksefileter. I tillegg ble effekten av lokalitet (nord-sør) og sesong (vår-høst) på kvalitetsegenskaper under kommersiell produksjon i diploid atlantisk laks studert. Det er sett på *pre* og *post mortem* faktorer som temperatur, væskeslipp, vannbinding, filet fasthet, rigor utvikling og kjemisk sammensetning.

For å studere effekten av lokalitet (nord-sør) og sesong (vår-høst) på filetkvalitet, ble laks delt inn i fire grupper. Laks ble hentet fra brønnbåt og ventemerd ved Marine Harvests anlegg på henholdsvis Hjelmeland og Herøy, både i mai og november 2014 (gjennomsnitt helvekt 5.42 ± 0.99 kg). Dette resulterte i et prøvedesign med fire grupper basert på lokalitet (nord-sør) og sesong (vår-høst). For å studere effekten av ploiditet og temperatur på filetkvalitet, ble det i august 2014 hentet ut laks (gjennomsnitt helvekt 1.55 ± 0.34 kg) fra Havforskningsinstituttet sitt innendørs forsøksanlegg (Matre). Uttaket resulterte i et prøvedesign med seks grupper basert på deres ploiditet (diploid og triploid) og veksttemperatur (5, 10 og 15°C).

I det første prosjektet, "Effekten av lokalitet (nord-sør) og sesong (vår-høst) på filetkvalitet", er det konkludert at lokalitet (nord-sør) og sesong (vår-høst) påvirket drypptapet i kommersiell oppdrettet diploid laks. Videre, er det konkludert at lokalitet (nord-sør) hadde en klar hovedeffekt på filetfasthet.

I det andre prosjektet, "Kvalitetsforskjeller mellom diploid og trploid laks", er det konkludert at triploid laks har høyere collagenase og fettinnhold sammenlignet med diploid. Videre er det konkludert med at veksttemperatur påvirker *rigor mortis*, men effekten av ploiditet er ennå ikke helt klar. Drypptap i triploid laks ble funnet å være nominell høyere sammenlignet med diploid, og veksttemperatur økte drypptapet i filetene. Kvaliteten i triploid laks ser uansett til å være lik diploid med samme bakgrunn.

I samsvar med resultatene i denne avhandlingen, viser studien variasjon i biometri egenskaper og kvalitetsegenskaper mellom lokalitet (nord-sør), sesong (vår-høst), ploiditet (diploid-triploid) og vekst temperatur (5, 10 og 15°C).

ABBREVIATIONS

Abbreviations	Full name
*a**	Reddish when *a** > 0
ATP	Adenosine tree phosphate
*b**	Yellowish when *b** > 0
DL	Drip loss
DM	Dry matter
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
FAO	Food and Agriculture Organisation
GMO	Genetic Modified Organism
HiST	Sør-Trøndelag University College
HPLC	High performance liquid chromatography
ICES	International Council of the Exploration of the Sea
IMR	Institute of Marine Research
IPN	Infectious pancreatic necrosis
*L**	Lightness / translucence (0-100; 0 = black, 100 = diffuse white)
NASCO	North Atlantic Salmon Conservation Organization
NINA	Norwegian Institute of Nature Reasearch
NMBU	Norwegian University of Life Science
NTNU	Norwegian University of Science and Technology
NQC	Norwegian Quality Cut
pH	Pondus Hydrogenii
SD	Standard Derivation
WHC	Water Holding Capacity

CONTENTS

1. INTRODUCTION

1.1 Background

1.1.1 History of Norwegian aquaculture, a breeding adventure

Commercial production of farmed Atlantic salmon (*Salmo salar* L.) has been a success story for Norway. Enthusiasts behind the pioneer time of Norwegian aquaculture in the 50ties and 60tiest did not reach all the way up with full-scale aquaculture. However, they put unproven guidelines for the production systems that were of great help when the salmon adventure escalated (Hovland 2014 p.57-58).

Mowi AS, a firm in Bergen, was the first to put a substantial volume of salmon smolts in cages in the sea. This happened in the spring of 1969 at Sotra outside of Bergen (Hordaland) (Gjedrem et al. 1991; Hovland 2014 p.77). One year later, the Grøntvedt brothers Sivert and Ove from Hitra (Sør-Trøndelag), managed to put 20 000 smolts in the sea (Gjedrem et al. 1991). Their octagonal floating cages filled with salmon was an immediate success, and two years later the first profitable salmon farming was a fact (Hovland 2014 p.79).

Professor Harald Skjervold (1917-1995) worked with breeding at the Institute of Animal Breeding at Agricultural College at Ås (Akershus) and together with Professor Trygve Gjedrem (1929-) which joined the research-team in 1971 they created a breeding program for anadromous fish, from the same ideology as the livestock (Gjedrem et al. 1991). At Institute of Marine Research (IMR) in Matre (Masfjorden, Hordaland), Gunnar Nævdal (1936-) and Dag Møller (1931-) also wanted to conduct breeding research. A partnership was at one point considered, but collapsed when they disagreed about the distribution of species. As a result, two independent research stations for fish farming were born (Hovland 2014 p.111-114).

The pioneers Skjervold and Gjedrem started in 1971 selection trials with Atlantic salmon at Sunndalsøra and Averøy. The experiments and the premises both became owned and operated by Akvaforsk with Skjervold as leader (Gjedrem et al. 1991). Later, the same year, the genetic research on Matre also started (Hovland 2014 p.115) and the breeding on anadromous fish for commercial use had started.

Skjervold and Gjedrem collected wild Atlantic salmon during four years from 41 different rivers in Norway. The rivers were selected by assumptions that the river gave them great wild salmon. Salmon origin and geographical base were not taken into account when the genetic

breeding crossing. After 1.5 years in onshore tanks and two years in sea cages, salmon were selected by size, and then the genetic progress were started (Gjedrem et al. 1991).

"The salmon were selected by size." pers.comm Professor Trygve Gjedrem.

It took several generations of salmon to wean them from cannibalism, acquire a life in small tanks and cages, and at least learn them to eat pellets (pers.comm Trygve Gjedrem 2014).

The breeding program is today based on the collection of salmon from the 1970s, which is today owned by AquaGen AS. AquaGen AS is a part of the EW Group, which has the jurisdiction of the breeding stock from Skjervold and Gjedrem. According to the company AquaGen AS, there has been a tremendous genetic progress from startup to today. Breeding work has resulted in a sharp reduction in production time, improved survival, higher age maturation, improved feed utilization, and slaughter quality. Other factors that have had an impact on farming are production equipment, operating procedures, separate vaccine program experienced over time, and research-based knowledge. Today, the 11th generation of farmed salmon lives in the cages along the Norwegian coast.

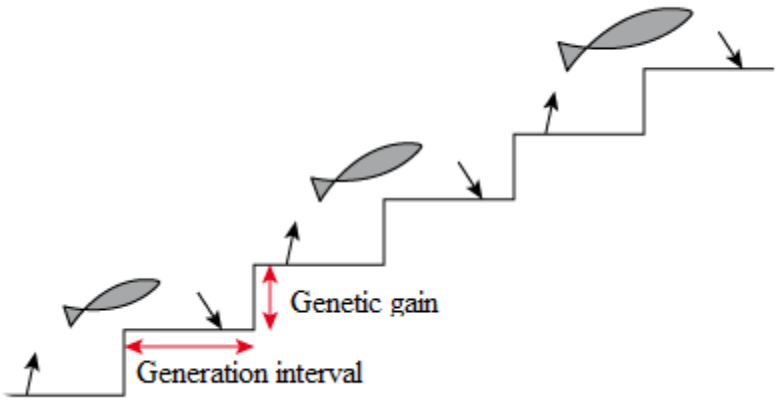


Figure 1.1 The figure shows a breeding stair. A instep is a generation interval (4 years), and the scene is the progress generated by selection. The height of the step is partly dependent on the genetic level. From the 1970s to 2000s, the genetic gain contributed to: a reduction in production time in freshwater (roe - smolt) from 16 to 8 months and seawater (smolts - harvest size) from 24 to 12 months, higher age maturation, improve feed utilization (less feed per pound of meat produced), higher survival against viral disease infectious pancreatic necrosis (IPN) and improved fillet quality (fat and colour). It is well documented that at least 40% of the overall increase in productivity within salmon attributable genetic progress through breeding (AquaGen 2015). *www.aquagen.no* – last access 19.02.2015

Aquaculture production of Atlantic salmon is today the most important marine industry in Norway and salmon is the species along the Norwegian coast with decidedly largest population with a share of almost 94% of the total. In 2013, the Norwegian aquaculture in total exported salmon for 40 480 million, which corresponds to the harvest volume of 1 168 324 tons of salmon (Statistisk sentralbyrå 2013). To put the amount of salmon in perspective, exports of salmon provides 37 million meals around the world every single day (Norges Sjømatråd 2014).

1.1.2 Triploid salmon a candidate for commercial farming?

The interest around triploid salmon have intermittently come up as a topic, most recently now by the awarding of the green concessions in the Norwegian aquaculture. The use of sterile, triploid salmon for aquaculture has two benefits, namely, the prevention of gene flow from aquaculture escapees to wild stocks and the control of sexual maturation in aquaculture (O'Flynn et al. 1997). The use of triploids for aquaculture originated with the industry's need to prevent sexual maturation of production fish before they reach market size, because maturing salmon, being chronically stressed, have reduced flesh quality and are more susceptible to disease (Mazeaud et al. 1977).

The current interest in triploids is based on the perceived need for "genetic containment" of those fish that inevitably escape from aquaculture facilities, and is therefore driven by forces outside the industry (Benfey 2001). If fish farmers are to be readily encouraged to use triploid Atlantic salmon (*Salmo salar* L.), then their performance must equal or be superior to diploid fish under commercial production (O'Flynn et al. 1997).

Today, we see commercial companies preparing for a triploid salmon industry. In 2012, AquaGen introduced product series for triploid salmon, under the name "AquaGen® Atlantic GREEN STERILE" (AquaGen 2014), and BioMar AS was the first feed company with a 1.generation product line for triploid salmon (BioMar 2014).

Triploid salmon are not considered to be a genetically modified organism (GMO). According to the Norwegian Gene Technology Act §4, GMOs are defined as: *"Microorganisms, plants and animals whose genetic composition is modified by means of gene or cell technology"* (Klima- og miljødepartementet 1993). Genetic engineering is techniques that involve genetic isolation, characterization, modification and inserted into living cells or viruses. Cell technology, techniques for the production of living cells with new combinations of genetic

material by the fusion of two or more cells (Klima- og miljødepartementet 1993). Triploid salmon are in accordance with Norwegian law not considered genetically modified based on the production method.

Norwegian Research Council (Nævdal 2003 p.44) wanted in 2003 a collective answer about including triploid should be consumer accepted before practical use in breeding and research (Nævdal 2003 p.45). Today, there are two main reasons to produce triploid salmon. First, we can sell salmon eggs / fry to the world market without "giving away" the genetic material. The second reason, as mentioned earlier, triploid salmon cannot be sexually mature, do not produce functional sex cells (Hansen 2012).

1.1.3 Politics and salmon escape

In 1973 the Norwegian Parliament approved a temporary law on aquaculture based on public licenses (Hovland 2014 p.118) whereas eight years later (in 1981) a permanent farming law was proposed by the Fishing Minister Thor Listau (1938-2014) in the Willoch government. This law is the background for today`s management of fish farming licenses (Hovland 2014 p.163). The allocation of new licenses announced in 2013 (Regulation 2013-06-24 No. 754) was named: "The green concessions" and it has been emphasized that the licenses should be environmentally good for the industry.

"§ 1 Purpose: Regulations will contribute to facilitat a sustainable and competitive aquaculture industry that can contribute to activity and value creation along the coast, and encourage the realization of new technological solutions or operational ways that help promote reducing environmental challenges with escaped farmed fish and the spread of lice." (Fiskeridepartementet 2013)

The distribution of the 45 green concessions are set by the government and should be distributed as followed: A) 20 licenses through Troms and Finnmark for a fixed price. B) 15 licenses without region-specific criteria with a closed bidding, and C) 10 licenses without region-specific criteria for a fixed price. The total allowable biomass differs both within- and across the groups (A-C) (Fiskeridepartementet 2013). Conditional use of sterile salmon is required in group A, which consider 7 licenses in Troms and 5 licenses in Finnmark. The assignment of the licenses has not jet started because several companies believe that the professional groups assessment are not adequately documented (Fiskeridirektoratet 2014a). Moreover several aquaculture companies believe that triploid salmon in full scale are not

enough explored and therefore sued the state. 22th December 2014 the complaints were rejected by Industry- and Fisheries Ministry by Fisheries Minister Elisabeth Aspaker (Nærings- og fiskeridepartementet 2014) under Erna Solberg government. Interest Organizations that work with the Wild existence; North Atlantic Salmon Conservation Organization (NASCO), Food and Agricultural Organization (FAO) and International Council for the Exploration of the Sea (ICES) has made it clear that the genetic contamination from escaped farmed salmon should be solved by using triploid farmed salmon (Taranger & Albretsen 2014 p.152).

It is no secret that escape of salmon occurs from fish farms. In 2013, it was reported 198 181 escaped salmon, and it was considered to be a minimum number (Fiskeridirektoratet 2014b). Escape of salmon from fish farms are not desirable either from the concessionaire, farmers or others who are involved. No one wants to lose money. It is important to emphasize that it is not illegal per definition that salmon escapes. The owner of the farm has a duty under the Operating Regulations for Aquaculture §37 to prevent and limit the escape (Fiskeridepartementet 2008). In Norway, we have large and solid environmental organizations that will report escapes, where some escapes ends with juicy fines, while others are not (vilvitevillaks.no 2012).

Research shows that escaped farmed salmon can spawn freely and leave offspring in nature (Glover et al. 2013). To what extent farmed salmon have crossed into wild salmon germplasm is still unknown (Taranger & Albretsen 2014 p.76). In autumn, several rivers along the Norwegian coast have been monitored over a period from 2006-2012 to count escaped salmon. Average year percent proportion of escaped salmon was estimated to be 8.0 to 10.7% (Taranger & Albretsen 2014 p.87). Trend measurements have been criticized for low statistical basis (Skilbrei et al. 2011), and which strains the measurements actually measure against (Brekke 2012).

The Directorate has commissioned Norwegian Institute for Nature Research (NINA) for Nature Management to investigate error trek to both farmed and wild salmon. The project was based on more than 20 years brand surveys from the river Imsa in Sandnes municipality in Rogaland County. The results of the surveys showed that 15.4% of farmed salmon wrong wandered (deferred from 1981 to 1999) as compared to 5.8% of the wild salmon (emigrated 1976-1999) (Jonsson 2001).

Until now, no escapes from cages with triploid salmon are reported. Research has shown that triploid male salmon shows spawn behavior towards diploid female salmon in tanks (Fjelldal et al. 2014). This is probably because the mitotic division in the testes was not disturbed enough (Thorgaard 1983).

1.2 Salmon quality

Quality is a complex concept, which originates from the Latin word *qualitas*, meaning property. Defining quality is difficult, because everyone has a subjective perception of what characteristics the fish should have for the product to please us. The traditional requirement is that the fish must be fresh, but the term also concern about how the fish looks, how it is packaged, texture, taste, and smell. Although the term quality seems self-explanatory in everyday usage, there are in practice many different views of what it means and how it should be achieved. According to the standard ISO 8402:1994 quality is defined as; "*the totality of features and characteristics of a product or service that bear on its ability to satisfy stated or implied needs*" (Standardization 1994). Garvin (1984) distinguishes between four overall approaches to defining quality.

- *Product quality:* an inherent characteristic of the product determined by the presence or absence of measurable product attributes.
- *Manufacturing quality:* a product which conforms to specified requirements.
- *User perceived quality:* the combination of product attributes which provide the greatest satisfaction to a specified user.
- *Economic quality:* a product which provides performance at an acceptable price, or conformance to requirements at an acceptable cost. (Lengnick-Hall 1996)

Fish products depend on several attributes of food quality, such as safety, nutrition, flavor, texture, colour, appearance and the suitability of the raw material for processing and preservation. The term quality depends on regional preferences, consumer attitudes and methods of preservation and consumption. When it comes to quality preferences of salmon fillets, it depends on regional preferences, consumer attitudes, different markets with different criteria, methods of preservation, and consumption (Einen et al. 1998; Folkestad et al. 2008; Haard 1992; Love 1988; Torrissen et al. 1995).

1.2.1 Drip loss

The muscle's ability to retain water is an important quality factor affecting dryness, chewing resistance, and consistency of the fillet. The fillet water content is moreover affected by muscle structure, duration and strength of the rigor process, and seasonal variation. Additionally, freezing procedures will affect the muscle water content. Freezing of salmon before entering *rigor mortis*, will lead to an extra powerful muscle contraction (Bito 1983; Ma & Yamanaka 1991; Ma et al. 1992). This can lead to muscle fibers ruptures, fillet gaping and increased water loss (Cappeln & Jessen 2001). If the fish are frozen pre-rigor, thaw rigor might occur with a possible increase in drip loss, which has a negative impact on quality (Elvevoll et al. 1996).

The main drip loss of raw salmon is water (Ofstad et al. 1995), but some loss of lipids, proteins, and carotenoids occur naturally in small amounts. Factors such as starvation (Morkore et al. 2008) and stress prior to slaughtering (Lerfall et al. 2015; Roth et al. 2006) affects the drip loss of raw salmon. Most of the water in the fish muscle is held in either the myofibrils, between myofibrils and between myofibrils and the cell membrane (sarcolemma), between muscle cells and between muscle bundles (groups of muscle cells) (Huff-Lonergan & Lonergan 2005).

The water holding capacity (WHC) is defined as *"the product's ability to retain water when it is exposed to a coalescing force"* (Lynum & Rustad 2005; Olsson et al. 2003). Many factors affect the water binding capacity in fish muscle such as heat-induced structural changes, sarcomere length, osmotic pressure, pH, ionic strength, state of *rigor mortis* (Ofstad et al. 1995), capillary size, charges on protein network type ions, temperature, equilibrium between protein and water, and the presence of low molecular weight substances (Albarracín et al. 2011). Water binding in fresh fish is a key issue when it comes to juiciness and flavor, and thawing of fish results in increased drip loss. Some obvious quality parameters for costumers are; flaking (whether the fish falls apart on the plate), texture, tenderness and juiciness, and all these parameters are related to the WHC (Skipnes et al. 2007).

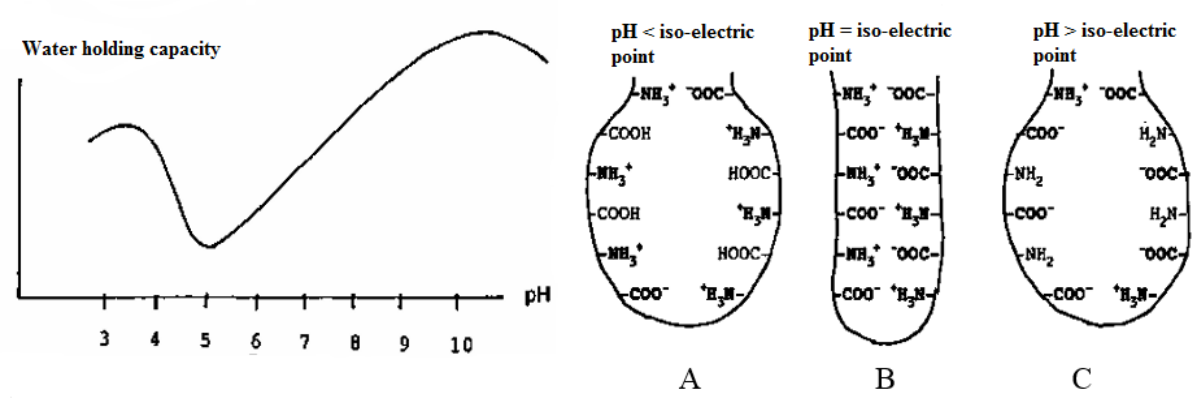


Figure 1. 2 The left figure show the isoelectric point of the muscle proteins in fish, and is between pH 4.5-5.5. Water molecules bind to vacant ions (±), A + C and muscle proteins swell. When there is a balance between the charges of the ions they will bind to each other. At the isoelectric point B is not available ions obtainable and proteins will naturally remain unswollen in this phase (Lynum & Rustad 2005 p.116).

In muscle tissue we distinguish between free water and bound water. The free water is kept in the muscle tissue with capillary forces, for example in cavities between myofilaments and myofibrils. Bound water is bound to proteins by hydrogen bonds, and affected by the surface charges and polarity of the proteins. In minced muscle tissue is also a large proportion of water located in the network of myofibrillar proteins (Strasburg et al. 2008).

1.2.2 Fillet texture

The texture of fish can be defined by its dryness, hardness, and juiciness, that is typically tested in the industry by the 'finger method' (Lie 2001). Texture is considered as one of the most important quality characteristics of salmon muscle, were soft flesh leads to reduced acceptability by the consumers (Koteng 1992). Fillet quality is very complex because it can be influenced by many factors and is affected throughout the entire value chain. The terms gaping and texture are used interchangeably. Fillet quality can be linked to breeding phase (Gjedrem 1997), genetics and growth (Gjøen & Bentsen 1997; Thodesen et al. 2001), feed composition (Aksnes 1995; Einen et al. 1999), feeding regime (Noble et al. 2007), seasonal variations (Mørkøre & Rørvik 2001), handling and slaughtering process (Erikson et al. 1999; Kiessling et al. 2004; Mørkøre et al. 2008), muscle fibre density (Hatae et al. 1984; Johnston et al. 2000), ice chilling and temperature during frozen storage (Espe et al. 2004; Hultmann & Rustad 2004).

The salmon muscle consists mainly of water, fat and proteins. The salmon consists of two kinds of muscle, a dark aerobic muscle used for continuous swimming and a white muscle with anaerobic metabolism (Lynum & Rustad 2005 p.54). If we look at the nutritional composition between the muscle types, dark muscle consists of more lipids than the white muscle (Aursand et al. 1994). The white muscle predominates in salmon, while the dark muscle is located as a stripe just under the skin of the body running beneath the lateral line. Water content and fat has negative correlation with an average content of around 80 % (Lynum & Rustad 2005 p.54).

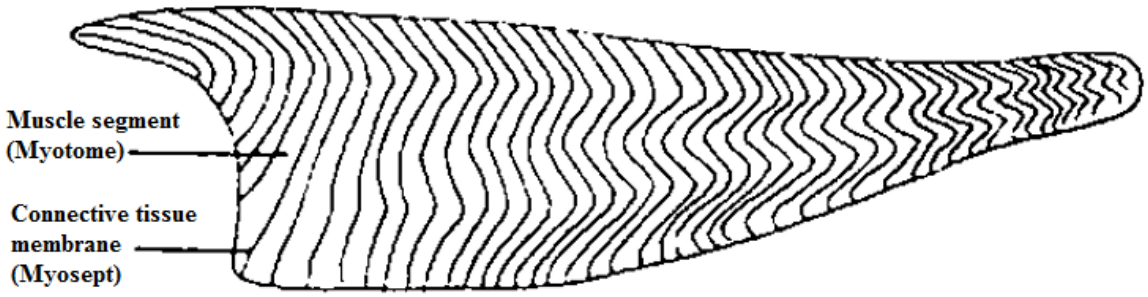


Figure 1. 3 Muscles of fish consists of muscle segments and connective tissue membranes where the segments forming a vertically W (Lynum 1999 p.28).

The salmon muscle is layered instead of bundled to the skeleton as in the other vertebrates. Each segment of the muscle contains of horizontal muscle fibers (myotom) and are separated from each other by a sheet of connective tissue (myosept) (Kryvi & Totland 1997; Lynum & Rustad 2005). In triploid salmon it is discovered an abnormal cell size of muscle fibers and altered development of muscle fibers as myotoms and myofibrils (Johnston et al. 1999). The myotoms consist of myofibrils located horizontally, ranging from myosepta to myosepta. Muscle contraction between actin and myosin is well known. Myofibrils consist of actin and myosin, built up as long parallel bundles of protein strands, where actin thread is stuck in a transverse slice called Z-line. The myosin threads is contracted when pulled together. The length between two Z lines is called sarcomas length and operates as the functional unit (Lynum & Rustad 2005).

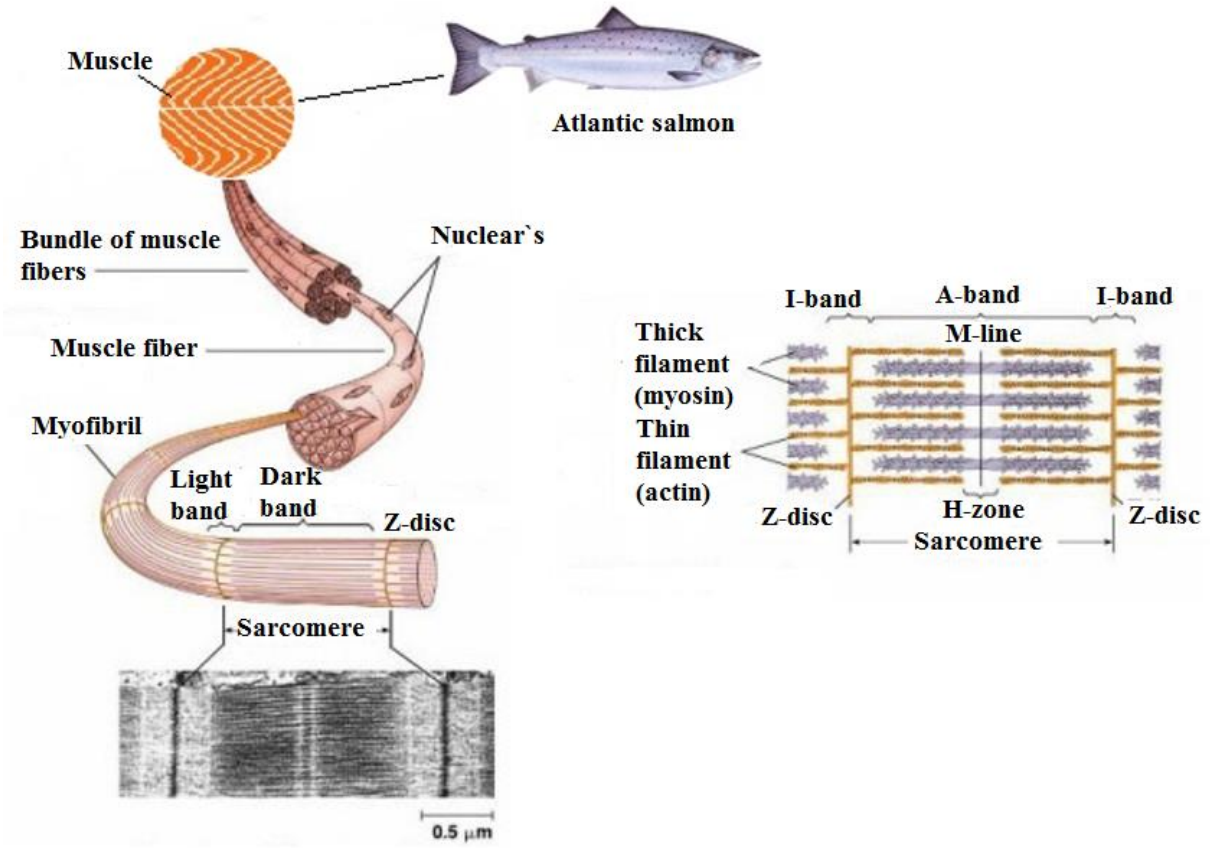


Figure 1. 4 Formed crosslinks between the muscle proteins actin and myosin provides a muscle in locked position when the adenosine tree phosphate (ATP) value is low (<5%). The reaction is not reversible. To the right, you can see a muscle fiber with Z-disc, the M-line and sarcomas lengths. (Retrieved from: www.sakshieducation.com - last access 22.02.2015)

Fish show a significant capacity throughout the year to change muscle composition. In salmon growing season will protein utilization be worse than outside season (Einen & Roem 1997). This leads to changes in fat and water composition while the protein level is stable (Shearer et al. 1994). Ploidy does not affect the chemical composition (Bjørnevik et al. 2004).

When the fish muscle grows, the individual muscle fibers grow in size or new muscle fibers are formed. In winter, formation of new fibers are highest, because the fish growth are small at this time. The ratio of small and large fibers is highest in autumn and winter, and then fall into the spring, before start of the new growing season. Fish with higher proportion of small fibers have less gaping than fish with large fibers. Quality is affected when the relationship between connective tissue protein and muscle protein changed (Bjørnevik et al. 2004; Einen & Thomassen 1998; Espe et al. 2004; Lavety et al. 1988; Mørkøre & Rørvik 2001).

Fish-muscle proteins

Proteins are complex polymers composed of up to 21 different α-L-amino acids (Damodaran 1996). They are bounded together by amide linkages. The function of proteins is determined by the three-dimensional structure, as determined by amino acid sequence. Structure of proteins are arranged in four levels: the primary structure describes all covalent bonds which bind together the amino acids in the polypeptide chain, and comprise the sequence of amino acids. Secondary structure is organizing specific parts of the polypeptide chain in either α-helix, β- sheet or β-folds. Tertiary structure is the three-dimensional structure of the folded protein. Quaternary structure describes how the different polypeptide chains is arranged relative to each other (Damodaran 1996; Lynum & Rustad 2005).

The fish proteins are grouped into three parts based on their solubility characteristics (Haard 1992). These are salt soluble myofibril proteins (65-80%, contractile network; actin, myosin, tropomyosin, troponin), water-solouble sarcoplasmic proteins (18-20%, mainly enzymes; globulin, myogen, myoalbumin), and insoluble stroma proteins (3-5%, connective tissue; collagen, reticulum, elastin) (Hall & Ahmad 1997). Textural and water-holding properties of the fish are considerably dependent on the distribution between groups of protein. Endogenous proteases, which are able to hydrolyze different proteins in the muscle, are important early in the deterioration process during iced storage of raw fish (Cepeda et al. 1990) .

Ante mortem and *post mortem* reactions, *rigor mortis*

Post-mortem is a generic term for changes that occur after death. After death, enzymatic degradation of structural proteins starts, called autolysis. Autolytic changes leads to protease degradation of proteins, connective tissue and the formation of peroxides due to lipid hydrolysis (Delbarre-Ladrat et al. 2006).

After death the blood circulation in animals' ceases, but the catabolic processes of the muscle cells continue, as long as energy is available. Oxygen transport to cells stops and causes anaerobic conditions, followed by biochemical reactions such as accumulation of lactic acid, leading to pH fall, and reduction of ATP. The muscle cells indicates *rigor mortis* function when the ATP level reach a minimum (ATP < 5%). Actin and myosin form cross-bridge complexes, and these complexes are used to explain the rigidity or stiffness characterizing of

these bridges (Currie & Wolfe 1979). Since actomyosin bridges are irreversible, the *rigor mortis* can not be explained by resolution of the bridges, but the breakage of cellular membranes and destruction of the osmotic potential (Bendall 1951). There have been some theories about how *rigor mortis* resolution actually takes place, especially widely discussed is the breakdown of muscle structure using proteolytic activity in dead tissue (Hultin 1984; Khan 1977; Tsuchiya et al. 1992), and osmotic changes in cells (Balevik 2004; Slinde et al. 2003).

We distinguish between three types of death: brain-, organ- and muscle death. Death rigidity (*rigor mortis*) is a physical change that occurs in the muscles of all vertebrates *post mortem* (Bendall 1973). Muscle death occurs because individual blood circulation stops, and the transport of oxygen to the mitochondria cease. This again leads to anaerobic metabolism and production of lactate (lactic acid). Immediately after death a structural change in the muscles starts, combined with increased osmotic pressure and the lack of available ATP contributes to the development of rigor. Death rigidity will make the fish hard and inflexible, the extent to which this happens depends on fish type, glycogen storage, stress before death and temperature (Haard 1992). Rigor is a phenomenon where all features in the process probably not yet has been discovered, neither the *rigor mortis* or what triggers the process (Lynum & Rustad 2005 p.83; Østvik 1991 p.19-21).

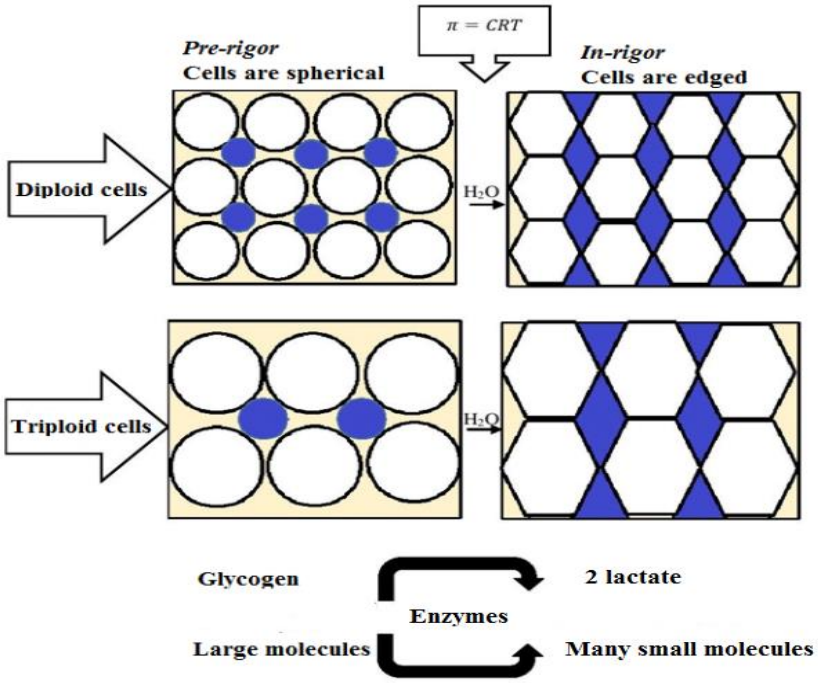


Figure 1. 5 Osmotic change in muscle cells in diploid and triploid salmon. The figure are based on literature reported by Balevik (2004) and Slinde et al. (2003). (Figure: own)

1.2.3 Colour

The flesh colour of salmon is one of the main characteristics that influence consumer perception of quality (Francis 1995). The most commonly used carotenoid for muscle pigmentation of salmon is synthetically natural identic astaxanhtine (3,3`-dihydroxy-β,β-carotene-4,4`dione) (Bjerkeng 2000; Christiansen et al. 1995; Skrede & Storebakken 1986), that gives the muscle its reddish colour. Together with synthetic astaxanthin some natural astaxanthin are produced by the yeast *Xanthophyllomyces dendrorhous* (previously *Phaffia rhodozyma*) and the bacteria *Paracoccus carotinifaciens* (Panaferd-AX) (Lerfall 2011). Carotenoids occur in different stero isomers (*cis* and *trans*) and optical isomers (*R* and *S*) where the geometrical all-*trans* isomer is dominant in the salmon muscle (~90 %) (Bjerkeng, Bjørn et al. 1997; Henmi et al. 1987).

In the seawater phase, normal range of astaxanthin in commercial salmon feed is between 20-50 mg kg^{-1}. There are many parameters that affect the colour of salmon flesh, among others; genetic background (Torrissen & Naevdal 1988), composition and amounts of carotenoids in the feed (Bjerkeng 2000), seasonal variations (Mørkøre & Rørvik 2001), starvation (Einen et al. 1998; Morkore et al. 2008), stress prior to slaughtering (Erikson & Misimi 2008; Lerfall et al. 2015), slaughtering procedures (Kiessling et al. 2004; Roth et al. 2010), ice chilling and temperature during frozen storage (Espe et al. 2004), muscle fibre density (Johnston et al. 2000), and the health status of the fish (Bjerkeng 2004).

The human eye becomes saturated leading to inaccurate perception of colour when the astaxanthin concentration in salmon muscle exceed 6-8 ppm (Foss et al. 1984).

1.2.4 Autolysis and sensoric perception

For the costumer, sensory quality is the key factor that shapes the overall impression of a fish product. Fishken (1990) defines sensory quality as: *"Sensory quality is that complex set of sensory characteristics, including appearance, aroma taste and texture, that is maximally acceptable to a specific audience of consumers, those who are regular users of the product category, or those who, by some clear definition, comprise the target market"* (York & Sereda 1994).

In the first storage time of fresh iced fish, autolytic processes like glycolysis and depletion of ATP occurs. Glycolysis leads to accumulation of lactic acid and pH decreases. Proteolysis increases the content of free amino acids and other chemical changes. These autolytic

reactions affect consistency because the fish runs through rigor mortis. Meanwhile, the fish loses its "fresh taste and aroma" (Lynum & Rustad 2005 p.86).

When autolytic reactions start in the muscle tissue, it affects the fillet freshness. pH is controlled by lactate dehydrogenase, and stops at pH 6.2 in fish (Lynum & Rustad 2005 p.94). Water loss can also be explained by microbiological contamination (Delbarre-Ladrat et al. 2006), whereas microorganisms are not important for changes in muscle texture (Hultmann & Rustad 2004).

Both cathepsin and collagenase play important roles in the quality deterioration of fish (Hernández-Herrero et al. 2003; Kolodziejska & Sikorski 1996). Cathepsins are lysosomal proteases that are involved in fish *post-mortem* muscle softening (Bahuaud, D et al. 2010; Chéret et al. 2007). Several investigators shows that enzyme-catalysed degradation of collagen and other extracellular matrix components is related to quality deterioration of seafood products (Ando et al. 1992; Sikorski et al. 1984). Collagenases are generally defined as enzymes that are capable of degrading the polypeptide backbone of native collagen under conditions that do not denature the protein (Sovik & Rustad 2006).

1.3 Salmon genetics

1.3.1 Salmon genome evolution

The ancestor of all salmonids underwent a whole genome duplication ~80-100 million years ago (Allendorf & Thorgaard 1984). Today, most of the Atlantic salmon genome have returned to a functional diploid state (i.e. bivalent pairing in meiosis) with 29 chromosomes. However, 60-70 % of the genes are still dublicated although only ~10-15 % of the salmon genome still behaves as a tetraploid genome (pers.comm Simen Rød Sandve; Danzmann et al. 2008; Fuerst 1972).

Fossil (Behnke 1992) and molecular data (Devlin 1993) suggest that the genus *Salmo* (which includes the Atlantic salmon and Brown trout) and *Oncorhynchus* (Rainbow trout) diverged early in the Miocene epoch, just prior to or at the onset of the cooling of the Arctic Ocean. Miocene is the first geologic epoch of the Neogene period 23-2.6 million years ago (Waples et al. 2008). A key feature of the Salmo and the Oncorhyncus lineages is that they contain species that has evolved the ability to be anadromous and there is evidence supporting that the ancestral genome duplication could have enabled evolution of anadromy (Lorgen et al. 2015).

1.3.2 Production of triploid salmon

Genetic substance in all living organisms is deoxyribonucleic acid (DNA), which consists of filamentous molecules that are built together into a double helix. The thread-shaped molecules are composed of bases; Adenine (A), Cytosine (C), guanine (G), Thymine (T), where C + G and A + T form base pairs (Harvey & Ferrier 2011 p.395-413). The genome (the entire genome) are structured in linear molecules called chromosomes (Harvey & Ferrier 2011 p.464-470). Number of chromosomes in a cell may vary from species to species. This is called ploidy (Table 1.1).

Table 1. 1 The table shows a list of some ploidy in the nature and glossarys, number of copies of each chromosome and som example of a species/cell or other living organisms.

Ploidy	**Number of copies of each chromosome**	**Example**
Hapolid	1	Sex cells
Diploid	2	Mammalian cells
Triploid	3	Various fruit
Tetraploid	4	Potatoes, cotton
Pentaploid	5	Woods
Heksaploid	6	Bread wheat
Octaploid	8	Strawberries
Polyploid	Common name (2 or more copies of each chromosome)	
Autotriploid	Diploid and haploid procreation (Zygoten, 3 copies)	
Autotetraploid	Doubler own (even) chromosomes (2x2 copies)	
Pseudotetraploid	"Illegitimate", "false" tetraploid fish there chromosome number varies on the individual level.	

When a triploid salmon is produced eggs are first collected. At this stage the egg cells are in fact still diploid (Figure 1.8). This is a result of normally occurring lag in the timing of the last stage of meiosis, when female gametes go though reduction of their chromosome content. Post fertilization salmon eggs are in fact naturally occurring triploid cells. What happens next is what differentiates between production of a normal diploid and a triploid salmon. During production of diploid salmon the triploid fertilized egg get rid of the third genome component, resulting in a diploid developing embryo. In triploid production, triploid fertilized eggs are subjected to pressure treatment that stops the egg from returning to a diploid state (Benfey, Tillmann J. 1999; Tave 1993). The method is performed with a constant pressure of 655 bar from where the fertilized salmon eggs between 300-350 minutes degrees (Hansen 2012 p.41). Further changes in procedures developed for diploid salmon is the disinfecting and lower incubation temperature for triploid eggs (Fraser et al. 2015) to reduce the deformations in

spinal and heart in triploid salmon (Sambraus et al. 2014). The triploid cell nuclei, contain by definition of 50 % more DNA than the diploid cell nuclei, which results in an increased of the nuclear volume in triploids to accommodate this extra genetic material (Benfey, Tillmann J 1999). In addition triploids differ from diploids in that they are more heterozygous (Allendorf & Leary 1984; Leary et al. 1985), they have larger but fewer cells in a variety of tissues, and their gonadal development is disrupted to some extent (Benfey, Tillmann J 1999).

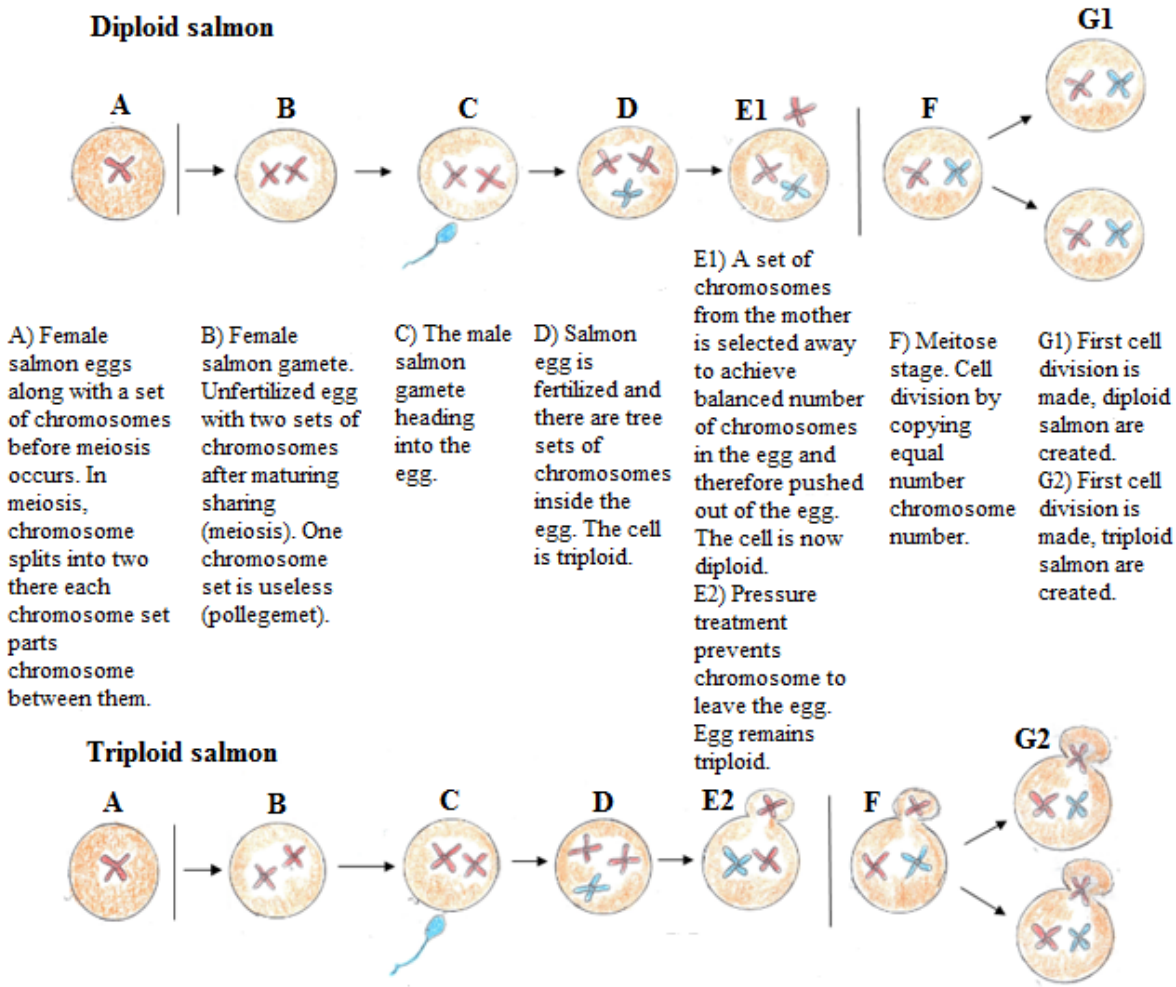


Figure 1. 6 The figure explains the process from the first maturing sharing in female salmon eggs (meiosis) until the first chromosome duplication (mitosis) and cell division in triploid salmon. The female chromosome set that does not connect with the male chromosome set (pollegmet) tried thrown out of the egg before meitose to maintain a balanced number of sets of chromosomes. Once the egg pressure treated unable "pollegmet" to get out of the cell before meitose turn and thus remains within the cell. This creates more chromosome within the cell and larger cells (Hansen 2012 p.10). (Figure: own)

1.3.4 General differences and quality aspects of triploidity

A significant correlation between production results of diploid and triploid salmon suggested that there is no overall differences between the production cycle, and it is therefore not necessary with a separate breeding program for triploid fish (Taylor et al. 2011).

Early research on triploid salmon has showed some challenges; clearly higher mortality in triploid than diploid salmon (O'Flynn et al. 1997), poorer growth in triploid than diploid salmon (Galbreath & Thorgaard 1997), and higher rates of skeletal deformations in jaw and back (O'Flynn et al. 1997; Sadler et al. 2001).

Recent trials with triploid fish with lessons from previous years, has provided research reports showing that there are no significant differences in growth between the groups in total, but several reports show that triploid fish grow faster in the freshwater phase compared with diploids (Bonnet et al. 1999; Cotter et al. 2000; Oppedal et al. 2003; Tave 1993). Test experiments with increased phosphorus levels in feed for triploid salmon in the transition to seawater, has given skeletal deformations at the same level as for diploid salmon (Fjelldal et al. 2012).

In the study by Hansen (2012) triploid salmon (300-400 gr) have shown to make it better or equally good as diploids in water temperatures up to 12°C, but worse at 15°C and 18°C. Large fish are more sensitive to high temperatures and hypoxia (Hansen 2012 p.32). Hypoxia and temperature sensitivity analyses shows major differences between triploid and diploid salmon at 19°C and low oxygen levels (Hansen et al. 2015), although diploid and triploid fish seems to have the same aerobic capacity (Stillwell & Benfey 1997). Triploid fish showing disapproval of swimming in warm water, which is well documented by several researchers (Altimiras et al. 2002; Hyndman et al. 2003; Ojolick et al. 1995).

Until now, the diploid salmon have shown to give significantly higher proportion of superior quality as compared to triploid salmon at slaughter (Fraser et al. 2013; Taylor et al. 2013) in two of three studies (Cotter et al. 2002). It is however discovered some differences in quality between diploid and triploid salmon when it comes to gaping and softness (Bjørnevik et al. 2004). This can be related to muscle cellularity (Johnston et al. 2000). Diploid had one third fewer fibers than triploid salmon (Johnston et al. 1999). When it comes to colour, researchers are unsure whether there are differences or only genetic variations, variation in muscle density or if the differences are caused by the attempts made at different seasons (Bjørnevik et al. 2004; Choubert et al. 1997; Johnston et al. 2000).

1.4 Locality, life cycle and season

1.4.1 Environment, currents and temperature

No one knows exactly how the anticipated climate change will affect salmon, neither in rivers or in cages. The effect of likely future climate changes related to fish are studied by several disciplines (Finstad et al. 2010; Framstad et al. 2006; Friedland et al. 2000). Since the Gulf Stream carries temperate water along the Norwegian coast, we have relatively high seawater temperatures in winter, where minimum temperatures are not below four degrees Celsius. Skagerrak coast and north of Finnmark County have however lower winter temperatures in the sea due to week influence of the warm currents. Salmon have a high oxygen requirement and therefore does not cope well at high water temperatures. It is normally to assume that water temperature above 18 to 20 ˚C is unfavorable for the fishes (Gjedrem 1975; Hansen et al. 2015).

Many factors influence salmon growth, like density, the number of fish in the sea cage, the feeding pattern and type of feed. The variation in the grow period however mainly occurred as an effect of changes in sea temperature, sea current, waves, disease outbreaks and daylight hours (Lorentzen 2008). There are large seasonal variations in the number of daylight hours, varies along the coastline and due to season as an effect of Norway's geographic location and its high latitude. In the northern part of Norway (above the Arctic Circle), there are 24 hours of daylight (midnight sun) from late May to late July, while the rest of the country experiences approximately 20 h of daylight at the same periode. From late November to late January, there are no daylight hours (polar nights) in the northern part of the country, while the daylight hours are very short in south. The salmon industry uses additional artificial light during the winter and spring however to compensate for the lack of natural light (Oppedal et al. 2001). The use of artificial light in fish cages has reduced the proportion of fish that undergo sexual maturation and enhanced the growth of Atlantic salmon (Oppedal et al. 2003).

Fish are highly reliant on temperature (Boeuf & Le Bail 1999), and the variation in sea temperature is considered to be the most important factor that influences salmon growth. Efficient salmon growth was previously believed to be best promoted at water temperatures between 13–17°C (Wallace 1993). Recent studies however shown that growth is better achieved at colder temperatures (Hevrøy et al. 2013). When the food supply is not limited, the specific growth rate increases with increasing sea temperature, while at any sea temperature, the specific growth rate decreases with increasing body weight (Talbot 1993). This observation indicates that any feeding regime will increase the feed conversion ratio (i.e., the

feed quantity per kilogram of growth) and that little variation will be observed in feeding patterns after controlling for climatic and environmental variables (Asche & Bjorndal 2011).

It is well known that fish is affected by the temperature fluctuations. Temperature affects salmon in many contexts throughout the lifecycle; growth, health, variations in sea temperature, after slaughter, and when presented to the customer. Temperature is an important factor in determining the rate of many reactions in salmon, and the reaction rate increases with temperature. A temperature rise of 1ºC could increase the speed of the reaction by 10 % (Pedersen 2014).

The classic Arrhenius model is commonly used to describe rate–temperature relations in food and biological systems. It is particularly suitable for systems over a broad range of temperatures without a major change of mechanisms that determine kinetic rate constants. However, there are situations where changes in system parameters are only noticeable above certain threshold temperatures (Kong et al. 2007). Arrhenius equation (equation 1) shows the relationship between the rate constant *k* in a reaction and absolute temperature (Helbæk & Kjelstrup 2006 p.570). Formulated in another way, Arrhenius Law describe the temperature dependence of the specific reaction rate constant in chemical reactions.

$$\ln k = \ln A - \frac{E}{RT} \qquad \text{(equation 1)}$$

where, *k* is the rate constant, *T* is absolute temperature, *G* is the activation energy of the reaction, *R* is the gas constant, and *A* is a constant (Arrhenius/frequency factor).

The Arrhenius plot is a graphical representation of the logarithm of the rate constant (ln k) against the inverse of the absolute temperature (1 / T). The slope can thus be used to determine the activation energy (G) in any reaction (Helbæk & Kjelstrup 2006).

1.4.2 The life cycle of salmon

The life cycle of salmon consists of eight different natural life stages.

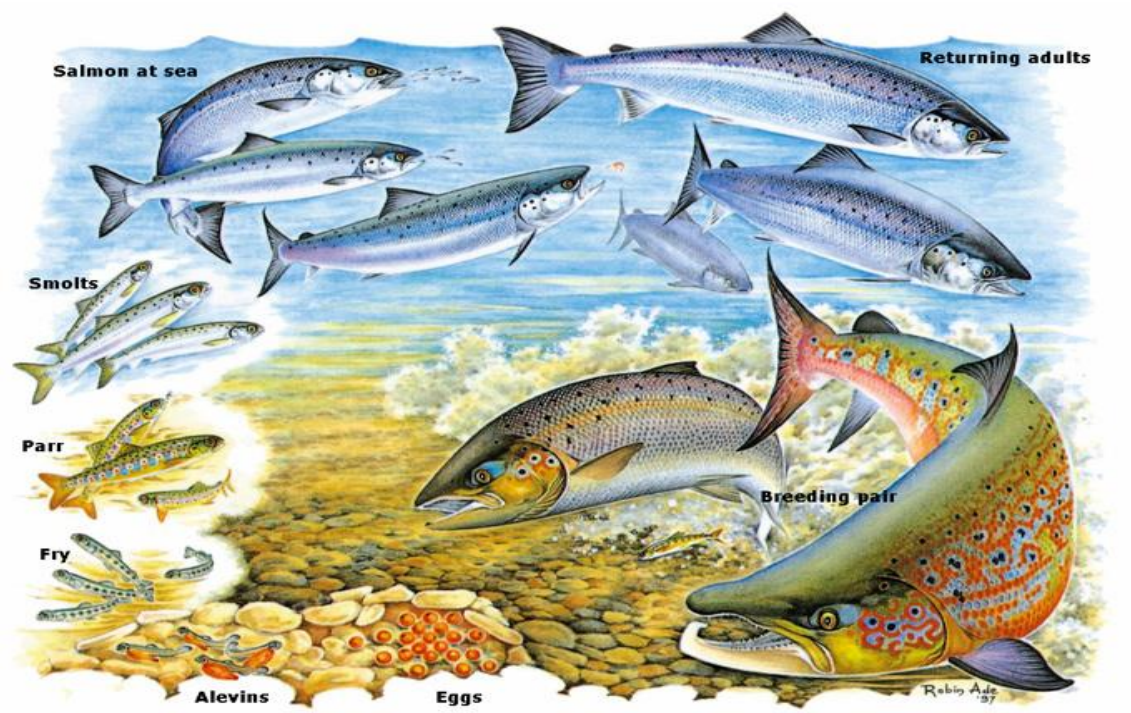


Figure 1. 7 Salmon cycle of nature goes from egg stage in the gravel where it is all winter to the fertilized eggs is 350-400 degree days. Yolk fry, parr and smolts is very critical eras in wild salmon life where competition in the rivers to eat and not be eaten is elementary. After 1-6 years traveling smolts from birth river and out into the Atlantic. Many salmon never come back to the birth river, probably the strongest returns after grazing journey at 106 400 000 km² Atlantic Ocean. Those who come back spawns in autumn and wanders again back out to sea. Retrieved from: http://www.nasco.int - last access 20.11.2014.

Every summer and early autumn, salmon swims from the sea and into freshwater rivers to spawn were only the strongest salmon becomes spawning salmon. The fertilized eggs hatch in the spring when the eggs are around 350-400 degree days old. Freshwater stage of egg, yolk sac fry, fry, parr and smolts can take from 1-6 years depending on the environment, temperature, food availability and competition in habitat. The salmon parr swim when the time comes onto the estuary where it becomes silvery smolts and smoltification can occur. Then, wild salmon wander from their maternity river and out to sea. Salmon that become sexually mature without walking into the sea, is called dwarf males and forms the basis for increased genetic variability in rivers.

In farming, it is natural to divide life stages into three different "stations"; brood stock facility, hatchery and sea sites. Broodstock are carefully selected out where eggs and milk are mixed together and added to incubation.

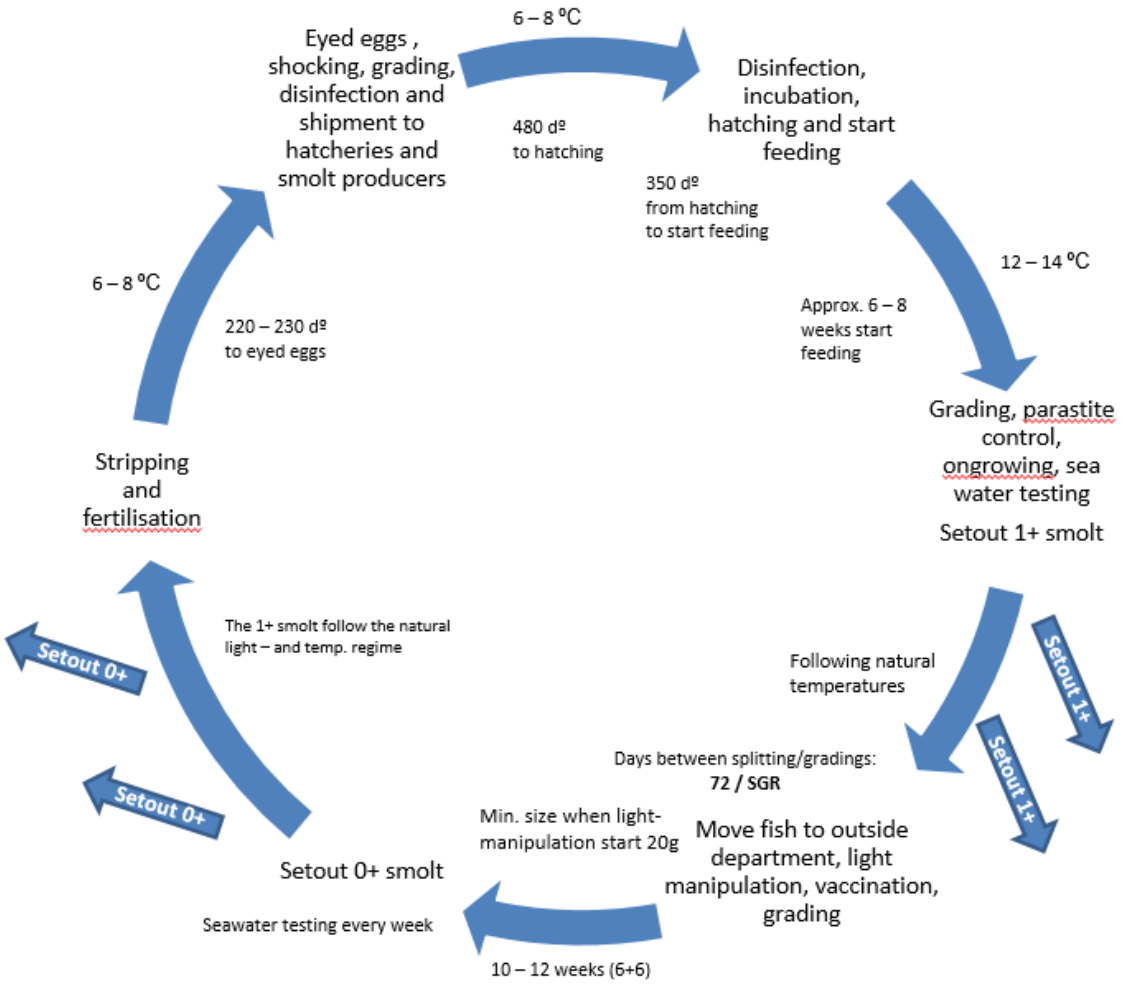


Figure 1. 8 The figure show the production circle in farming. Farmed salmon growth cycle can be prepared as a wheel where the three different stations is represented. Broodstock facilities: Ironing, incubation, shocking and grading. Hatchery: hatching, grading, start feeding, vaccination, light manipulation, testing the chloride stocking 0+ & 1+. Sea sites: Growth in saltwater, light manipulation, seasonal variation and starvation before slaughter. (Retrieved from: Lecture in the course AQP 211, NMBU, spring 2014)

After 230 days of degrees, the eggs get eyes, and between 350-400 degree days, they are changed to yolk sac fryes. After this stage, the fry "float up" and the feeding can begin. The hallmark of salmon (called parr in this stage) has clear black marks in the skin, but these disappear during smoltification. When the salmon becomes 20 grams large, light manipulation starts, and when they reach 40 grams, they will be vaccinated. Smolt is characterized as adolescence in the fish, where it undergoes physiological, morphological and behavioral changes. The chloride developed by enzyme activity, and when the cells reach on appropriate developmental level, the fish can control salt regulation in the blood itself. In this way at that point, the salmon can wander from their maternity river or hatcheries and out to the sea. Naturally, this takes 2-4 years of the life. With light, we can manipulate the salmon so the

salmon smoltifies after only one year. This is called 0+ smolts. Farmers may also use 1+ smolts, which takes a year and a half to produce. Sexual maturation can also be controlled with light. In salmon farming the salmon seawater period starts between 12 to 24 months, depending on which size they want to produce and seawater temperature.

1.4.3 Seasonal changes

Several research reports show that season affects salmon during the year. Atlantic salmon may be classified among the fatty fish species, where the proximate composition of slaughter size salmon flesh is water (60-65%), followed by protein (18-20%) and lipids (16-19%) (Einen et al. 1998; Mørkøre & Rørvik 2001; Shearer et al. 1994). In general, the content of lipids varies individually and between seasons (Mørkøre & Rørvik 2001; Shearer et al. 1994; Shearer 1994) and is strongly related to the content of water and vice versa (Katikou et al. 2001; Quinton et al. 2005; Shearer 1994). The protein content in healthy slaughter sized Atlantic salmon is relatively stable (Shearer et al. 1994).

Wild fish have quality differences related to season, because of changes in maturation, growth rate and fat content (Lavety et al. 1988; Love 1975). Also farmed salmon is known to change with season. The process of maturation predominants (Aksnes et al. 1986), and seasonal change in gaping score (Lavety et al. 1988) and texture hardness (Bjørnevik et al. 2004; Espe et al. 2004; Mørkøre & Rørvik 2001) has been reported.

Product quality includes a variety of aspects, both biological and non-biological parameters. Several studies have shown biological variations in commercial farmed Atlantic salmon, were they see effects of genetic variations in feed intake, growth and feed utilization, meat quality, disease resistance and age at sexual maturation (Kolstad et al. 2004; Powell et al. 2008; Thodesen et al. 1999; Vieira et al. 2007; Wild et al. 1994). Other factors that is important and influence the biological variation are; seasonal variations (Mørkøre & Rørvik 2001; Shearer et al. 1994; Shearer 1994), geographic and local environments such as temperature, light, water quality and local current conditions (Austreng et al. 1987; Boeuf & Le Bail 1999; Brett 1979; Poli 2009; Saunders & Harmon 1988; Shearer 1994; Smith et al. 1993). Regarding geographical variations in Norway, there is a north-south gradient in light and water temperature. Effects of feed on biological variations in commercial farming are relatively small in comparison to the similarity of feed managements amongst the farmers (Einen & Roem 1997). Furthermore, fish nutrition has an impact on several parameters directly

influencing the quality, such as colour and appearance, smell and taste, texture, nutritional quality, shelf life, and level of contaminants (Lie 2001). The quality of the fish varies throughout the year, affecting consistency (firmness / softness), colour, and fat content (Mørkøre & Rørvik 2001).

The quality of the fish may also be influenced by pre-, ante- and *post mortem* conditions, including handling before slaughtering, slaughtering methods, and storage conditions (Concollato et al. 2014). The quality of the end-product is also affected by the *rigor mortis* process (Lerfall et al. 2015). Duration and strength of this process will again be affected by factors such as the nature, size, method of capture, handling of fish (stress), temperature, and how fish condition was before it was slaughtered (Ando et al. 1991; Arimoto et al. 1991; Kumano & Seki 1993; Sigholt et al. 1997; Thomas et al. 1999; Tsuchiya et al. 1992).

Anyway, season is an important factor on the quality of farmed salmon due to increase in body size with season (Veland & Torrissen 1999), growth rate variations at different seasons (Mørkøre & Rørvik 2001), and relations to fibre size and densities (Bjørnevik et al. 2004; Johnston et al. 2002).

2. AIM

Title: Effect of ploidy and temperature on quality attributes of diploid versus triploid Atlantic salmon (*Salmo salar* L.), and the effect of locality (north-south) and season (spring-autumn) for quality attributes under commercial production.

Issue / description of objectives:

Objective 1: Understand the effect of locality (north-south) and season (spring-autumn) on quality attributes of diploid pre-rigor filleted Atlantic salmon *(Salmo salar* L.*)* in commercial production. The main objective will be studied through three subsidiary objectives:

1. Determine the effect of locality (north-south) and season (spring-autumn) on drip loss and water holding capacity (WHC).
2. Determine the effect of locality (north-south) and season (spring-autumn) on fillet firmness.
3. Determine the effect of locality (north-south) and season (spring-autumn) on chemical composition.

Objective 2: Understanding the effect of ploidy status (diploid-triploid) and growth temperature (5, 10 and 15°C) on quality attributes of Atlantic salmon (*Salmo salar* L.). The main objective will be studied through four subsidiary objectives:

1. Investigate the importance of temperature of diploid and triploid salmon growth.
2. Investigate the effect of ploidy status (diploid versus triploid) in quality attributes at different temperatures (5, 10 and 15°C).
3. Investigate *rigor mortis* development of diploid and triploid salmon at different temperatures (5, 10 and 15°C).
4. Investigate the consequences and aspects around production of triploid salmon.

3. MATERIAL

3.1 Locality and season (Marine Harvest)

3.1.1 Fish material and experimental design

In May and November 2014 a total of 110 farmed Atlantic salmon with mean ± SD weight equal of 5.42 ± 0.99 kg, were sampled during commercial slaughtering from two slaughterhouses, one in Rogaland (Hjelmeland) and one in Nordland (Herøy), Norway. The sampling procedure resulted in a full factorial design with locality and growth season as variables. The experimental design resulted in four groups hereby named 1. Rogaland, May) = *South-spring* (salmon farmed at 59°41`N 5°86`E); 2. Rogaland, November = *South-autumn* (salmon farmed at 60°18`N 4°59`E); 3. Nordland, May = *North-spring* (salmon farmed at 66°63`N 13°29); and 4. Nordland, November = *North-autumn* (salmon farmed at 64°94`N 11°76`E).

At the sampling place, fish were picked manually from the waiting cage (Herøy) or the well boat (Hjelmeland). All fish were manually killed by a sharp blow to the head. Then lactate, muscle pH and temperature were measured before the fish were transferred to the bleeding tank filled with ice water (0 ˚C). All salmon were thereafter gutted and manually filleted pre-rigor. Before weighing, all fillets were dried gently with a piece of paper. Fillet weight of both the right and left fillet were measured, then all fillets were individually packed in aluminum foil, and thereafter placed in plastic bag in fish boxes on ice and transported to Sør-Trøndelag University College (HiST), Trondheim, Norway. The fish were there stored in a refrigerated room (3-4°C) on ice for 20 days.

On day 6, 11 and 20, muscle pH, temperature and fillet weight were measured. Analyses of drip loss, texture and water holding capacity (WHC) were performed on eight randomly handpicked left fillets at day 6, 11 and 20 respectively. Before weighing, all fillets were dried gently with a piece of paper. At sampling day 6, five samples of each selection (*South-spring, South-autumn, North-spring and North-autumn*) were collected, packed and frozen (-80°C) until later analyses of total fat, protein and astaxanthin.

Table 3.1 Fish material; weight, condition factor, temperature, muscle pH and lactate in the different groups sampled for analyses. Results are shown as average ± SD.

	Hjelmeland spring	**Herøy spring**	**Hjelmeland autumn**	**Herøy autumn**	***P-value***
Whole weight, kg	4.87±0.68^{b}	6.34±1.07^{a}	5.26±0.98^{b}	5.16±0.58^{b}	*<0.001*
Gutted weight, kg	4.37±0.60^{b}	5.60±0.95^{a}	4.47±0.81^{b}	4.59±0.52^{b}	*<0.001*
Condition factor	1.27±0.11^{b}	1.24±0.13^{b}	1.41±0.14^{a}	1.27±0.11^{b}	*<0.001*
Death temperature	11.85±0.28^{a}	8.90±0.12^{b}	11.81±0.38^{a}	8.23±0.22^{c}	*<0.001*
pH	7.16±0.14^{c}	7.21±0.12^{c}	7.55±0.10^{a}	7.35±0.17^{b}	*<0.001*
Lactate, mmol l^{-1}	2.34±1.30^{b}	2.85±1.75^{ab}	1.81±1.60^{b}	3.61±1.60^{a}	*<0.001*

Average values of 25 individuals per group, in total 100 individuals.
Different lower case letters in the same row indicate significant variation (P<0.05) by one-way ANOVA.

3.1.2 Fish genetics and growth

The fish used in this study were all from the company Marine Harvest ASA. Fish genetics and growth from Hjelmeland autumn is missing. The genetic background differs between the groups. Hjelmeland spring and Herøy autumn used Mowi strain, and Herøy spring used AquaGen strain. 0+ smolt, seems to be used at all localityies. The fish in this experiment were fed feeds from several feed companies; Biomar, Skretting and Marine Harvest. The fish were moreover starved differently before they were transported by the well-boat from the rearing cage to the slaughterhouse. Since the experiment were performed on commercial farmed salmon, it was difficult to organize equal genetic- and farming conditions for all experiment. See Appendix 1-3 for specific information about the fish history in the different localities (Hjelmeland spring, Herøy spring, Herøy autumn).

Figure 3. 1 Map showing sea localities and slaughterhouses where the experiment were performed in Norway (Hjelmeland and Herøy) (Figure: own).

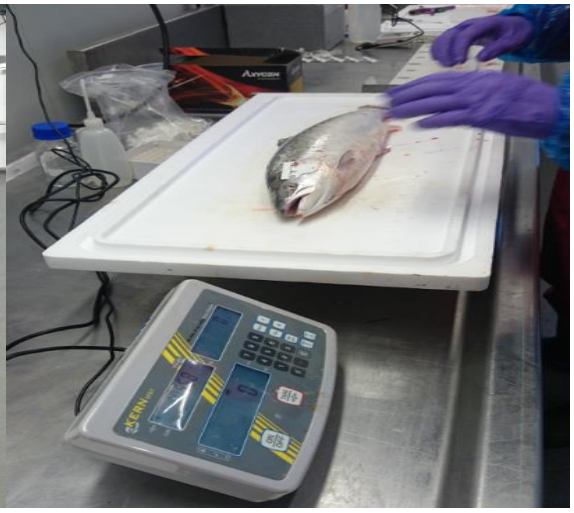
3.2 Quality diploid versus triploid Atlantic salmon (Institute of Marine Research)

3.2.1 Fish material and experimental design

In August 2014 a total of 162 farmed Atlantic salmon (50 % diploid and 50 % triploid) with mean ± SD weight equal of 1.55 ± 0.34 kg, were sampled at a research station at the West coast of Norway, Institute of Marine Research, Matre. The sampling procedure resulted in a full factorial design with six groups of salmon with different ploid and seawater temperature: 1. Diploid 5°C (hereby named D-5); 2. Triploid 5°C (hereby named T-5); 3. Diploid 10°C

(hereby named D-10); 4. Triploid 10°C (hereby named T-10); 5. Diploid 15°C (hereby named D-15) and 6. Triploid 15°C (hereby named T-15). All fish were sampled by taking out one by one fish which were killed immediately by a sharp blow to the head.

Picture 1: Fish tanks (Photo: own).



Picture 2: Weight of the fish (Photo: own).

Due to the complexity of the experimental design, the trial was divided into three separate selections. The first experiment was set up to analyse the rigor development (n=10 of each group, in total 60 salmon). The second experiment was set up to follow drip loss, shrinkage, WHC, colour and texture during 15 days refrigerated storage (5-6 ^{o}C, n=12 of each group, in total 72 salmon). In the third experiment, 30 salmon (5 of each group) were used to determine changes in contents of astaxanthin, lipid and protein, and protein denaturation during 15 days refrigerated storage (5-6 ^{o}C).

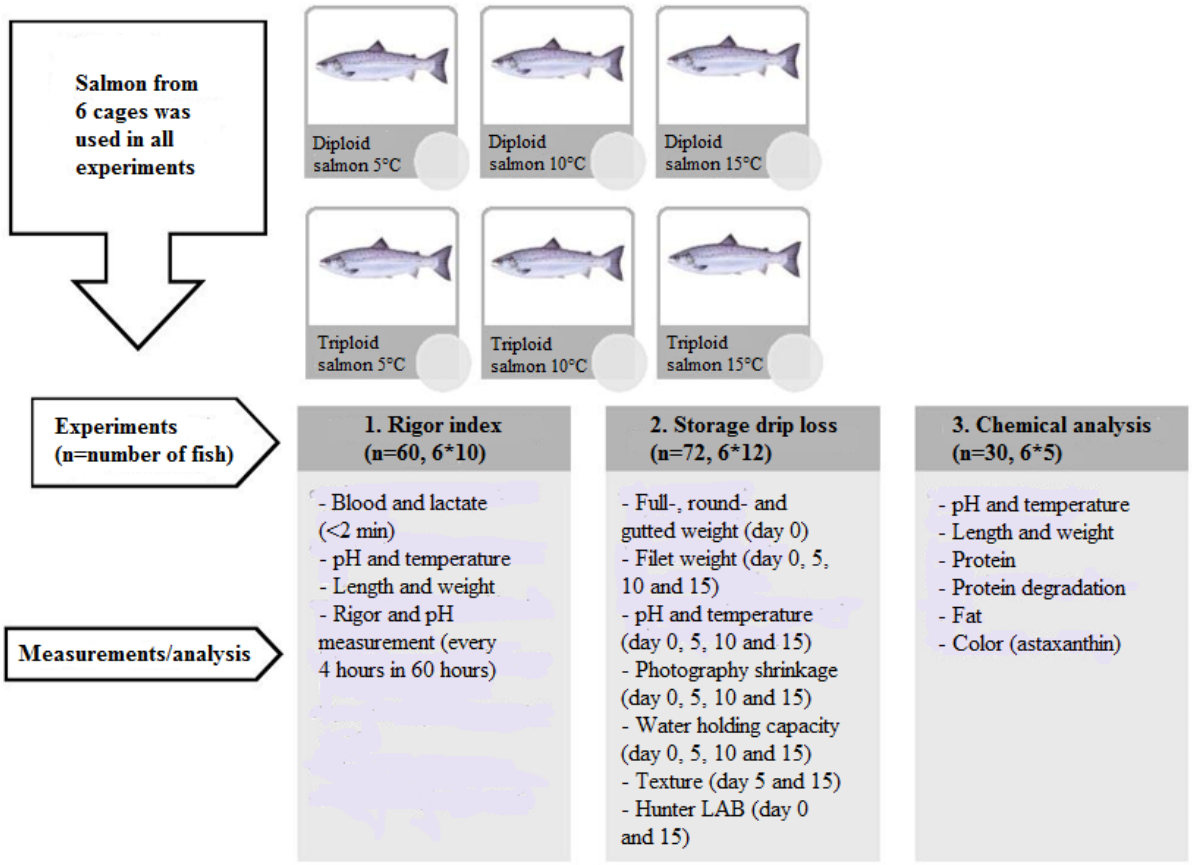


Figure 3. 2 Experimental design. The figure shows the flow chart for all quality analyses, chemical analyses and rigor index analysis for this task. The fish had two different ploidy (diploid and triploid) from six different vessels with three different temperature regimes. Fish from all the vessels was used in all experiments. It was performed different analyses at different times based on what was feasible. Fish were taken out carefully, to minimize the risk of affecting the experiment.

In the first experiment, rigor index (Cuttingers method (tail drop) (Bito 1983)) and muscle pH was measured on 60 fish (10 fish*6 groups, n=60) with a time intervall of four hours from 0-60 hours *post mortem*. Before rigor index measurements, blood and lactate were measured on the five first salmon from each group. Muscle pH, death temperature, length and whole weight were measured before the fish were packed in expended polystyren boxes, and stored on ice for 15 days in a refrigerated room.

In the second experiment, twelve fish from each group (n=72) were filleted for determining drip loss, WHC, colour and texture. Muscle pH, death temperature, length, whole and round weight were measured before the fish were transferred to the bleeding tank (ice water). Thereafter, the salmon were bled, gutted (gutted weight listed) and hand filleted pre-rigor, and after filleting the fillets were packed individually in aluminum foil and stored at a table in a refrigerated room (5-6°C) for 15 days. Before packaging, all right fillets were photographed

(Canon EOS 1000D, SLR camera, Canon Inc.), and four left fillets from each group were measured for surface colour (Hunter LAB). On day 5, 10 and 15, all right fillets were photographed and muscle pH, temperature and fillet weight were measured. Measurements of the drip loss and water holding capacity (WHC) were performed on four randomly handpicked left fillets from each group at day 5, 10 and 15, respectively. Before each weighing, the fillets were dried gently with a piece of paper. Measurements of texture were performed on four random choosen left fillets from each group at day 5 and 15, and colour (MiniScan XE, HunterLab Inc.) were measured on the same fillets at day 0 and 15.

In the third experiment, chemical composition of the raw material was determined in the fillets by analysing muscle tissue from the Norwegian Quality Cut (NQC) of five individuals from each group (n=30). On day 0, NQC from the right fillet, was split into two, vacuum packaged separately and frozen at -80°C. The left fillets were thereafter wrapped in aluminum foil and stored refrigerated for 15 days, before the left NQC underwent the same procedure as the right NQC (at day 15). Then, half of the fish samples were sent to HIST in Trondheim for analysis of fat and astaxanthin content, and the other half of the samples to Nofima Stavanger for total protein and protein degradation analyses.

3.2.2 Fish genetics and growth

Fish used in this study are from the brood stock of AquaGen AS produced at the company's farm in Hemne municipality, Sør-Trøndelag county.

Table 3. 2 The table shows egg production data from Aqua Gen AS.

Egg production data		
Type of salmon:	Diploid	Triploid
Batch number:	71	78
Strike date:	18.10.2012	19.10.2012
Origin fish stock:	Aqua Gen	Aqua Gen
Average temperature:	5,8°C	5,8°C
Delivery date:	20.12.2012	20.12.2012

Start feeding started 5th March 2013 and vaccination was carried out 19th August 2013 for both groups. Both groups have smolts from the 30th September 2013 rared in separate cages at the IMR sea site Smørdalen in Masfjorden municipality to the average fish weight of 1 kg. Monday 23th June 2014 around 360 fish (average weight of 1 kg) was transported by the sea

vessel Salma to Matre, a trip of approximately 10 minutes. The fish were thereafter re-dispersed in six $9m^3$ vessels inside the environmental hall where they was farmed for 57-59 days. The water temperature was slowly changed during a periode of 30 days, where they reached experimental temperature of 5°C, 10°C and 15°C at 24th July 2014. The temperature was thereafter stable until slaughter (27-29 days). Natural light rhythm according to season was used, and fish were fed with Skretting Spirit 600, 7mm large pellets (Protein; 40-43% fat; 30-33% pigment; 20,30,40,50mg). The feeding was controlled by the responsible farmer. All fish were starved 4 days before each selection. All the fish were slaughtered between 19-21/8-2014.

Table 3. 3 Fish material; weight, condition factor, temperature, muscle pH and lactate in the different groups sampled for analyses. Results are shown as average ± SD.

	5°C		**10°C**		**15°C**		***P-value***
	Diploid	**Triploid**	**Diploid**	**Triploid**	**Diploid**	**Triploid**	
Whole weight, kg [(1)]	1.37±0.35^{b}	1.40±0.25^{b}	1.71±0.33^{a}	1.74±0.34^{a}	1.52±0.32^{ab}	1.53±0.28^{ab}	*<0.001*
Gutted weight (HOG), kg [(2)]	1.16±0.33^{b}	1.20±0.16^{b}	1.47±0.37^{ab}	1.56±0.28^{a}	1.28±0.30^{ab}	1.40±0.23^{ab}	*0.007*
Condition factor [(1)]	1.03±0.08^{bc}	1.00±0.06^{c}	1.11±0.08^{ab}	1.13±0.23^{a}	1.06±0.07^{abc}	1.01±0.08^{c}	*<0.001*
Death temperature [(1)]	6.03±0.17^{c}	6.01±0.19^{c}	11.18±0.14^{b}	11.11±0.13^{b}	15.79±0,12^{c}	15,69±0,20^{c}	*<0.001*
Muscle pH [(1)]	7.31±0.11^{ab}	7.34±0.12^{a}	7.22±0.15^{abc}	7.24±0.21^{abc}	7.16±0.19^{c}	7.20±0.16^{bc}	*<0.001*
Lactate, mmol l^{-1} [(3)]	0.88±0.29^{a}	1.06±0.70^{a}	1.74±0.80^{a}	2.14±1.41^{a}	2.52±1.04^{a}	2.62±1.08^{a}	*0.031*

[(1)] Average values of 26-27 individuals per group, in total 161 individuals.
[(2)] Average values of 12 individuals per group, in total 72 individuals.
[(3)] Average values of 5 individuals per group, in total 30 individuals.
Different lower case letters in the same row indicate significant variation (P<0.05) by one-way ANOVA.

4. METHODS

4.1 Locality and season (Marine Harvest)

4.1.1 Lactate

Blood was collected from the tail section right after death, and lactate was measured by using a Lactate Pro 2 analyzer together with Lactate Pro 2 test strips (Arkray Factory Inc, Japan).

4.1.2 Muscle pH and temperature

Muscle pH and temperature was measured right after death in the anterior dorsal muscle close to the gills by a Mettler Toledo SevenGo proTM pH-meter (Mettler Toledo International Inc, USA) connected to an Inlab puncture electrode. During storage, muscle pH and temperature was measured at each sampling day (6, 11 and 20 days *post mortem*). See figure 4.1.

4.1.3 Drip loss and water holding capacity

Drip loss (DL) from the fillets was calculated as the difference in fillet weight between day 0 and day X of both the left and right fillets. An average of the left and right fillets was used for statistical analyses.

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

m_0: fillet weight at day 0

m_x: fillet weight at day x

Water holding capacity (WHC) of all left fillets were measured in the dorsal muscle after a method described by Skipnes, Østby, Hendrickx (2007). WHC was measured in duplicates at each sampling day (6, 11 and 20 days *post mortem*) on a defined area of the fillet (diameter 31mm, high 6 mm, approximately 5 g), see figure 4.1. The dry matter (DM) was estimated gravimetrically after drying at 100°C for 24 hours (ISO 1983).

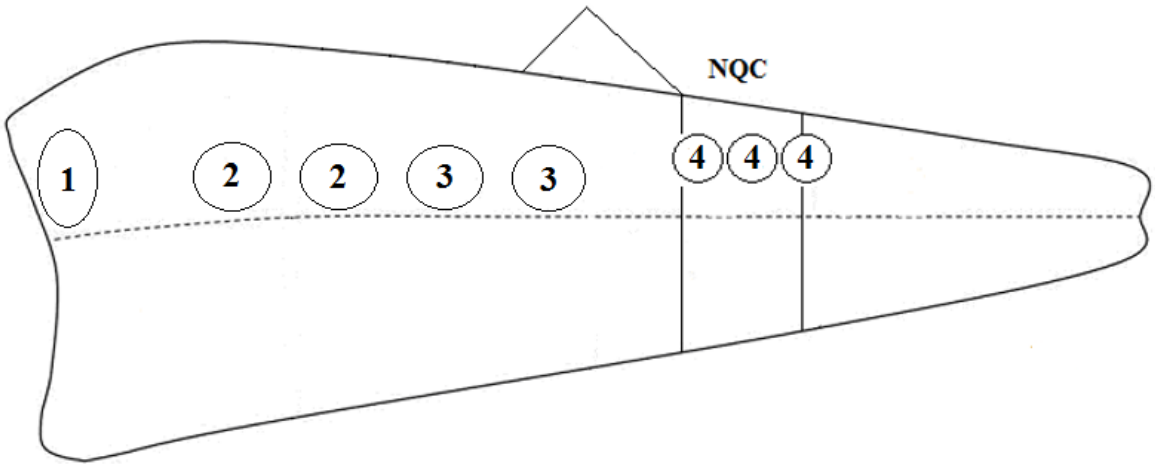


Figure 4. 1 Schematic illustration showing the areas upon the left fillet from witch analyses were conducted. 1: Muscle pH and temperature during 20 days storage, 2: Water holding capacity (WHC), 3: Fat, protein and astaxanthin analyses, 4: Instrumental texture analyses.

4.1.4 Fillet firmness

Instrumental textural analyses were performed using a Texture Analyser TA-XT2 (SMS Ltd., Surrey, England) equipped with a 25 kg load cell. A flat-ended cylinder probe (20 mm diameter, type P/1SP) was used. The force-time graph was recorded by a computer equipped with the Texture Exponent light software for windows (version 5.1.1.0), which was also used for data analyses. Analyses were performed in triplicates (average values were used for statistical analyses) of each fillet 6, 11 and 20 days *post mortem* (figure 4.1). The resistance force (N) was recorded with a constant speed of 5 mm sec-1, and the force required to press the cylinder down to 80 % of fillet thickness was used to describe fillet firmness.

4.1.5 Chemical composition

Chemical composition of the raw fish material was determined in the fillets (figure 4.1) by analysing muscle tissue (day 6) from the NQC of five individuals from each group. Total fat and carotenoids were extracted and total fat calculated by the method of Bligh and Dyer (Bligh 1959) with slight modifications. Nitrogen content was measured on a Tecator Kjeltec system (Model 2020 Digestor and 1026 Distilling unit, Tecator, Höganäs, Sweden) (NCFA 2003). Protein content was calculated from nitrogen measurements using the formula: % protein = % nitrogen*6.25. Astaxanthin were analysed by high performance liquid chromatography (HPLC) using a Agilent1100 liquid chromatograph (Agilent Technologies, Paolo Alto, CA, USA connected to an Agilent photodiode array UV-VIS detector) after a

method by Vecchi, Glinz, Meduna, Schiedt (Vecchi et al. 1987) using a Lichrosorb SI60-5, 125*4.0 mm, 5 um, Hichrom, Reading, UK, HPLC column. Before use, the column was washed with ortophosphoric acid (0.1% in CH_3OH).

4.2 Quality diploid versus triploid Atlantic salmon (Institute of Marine Research)

4.2.1 Muscle pH and temperature

Muscle pH and temperature was measured right after death in the anterior dorsal muscle close to the gills by a Mettler Toledo SevenGo proTM pH-meter (Mettler Toledo International Inc, USA) connected to an Inlab puncture electrode. During storage, muscle pH and temperature was measured at each sampling day (5, 10 and 15 days *post mortem*). See figure 4.1.

4.2.2 Experiment 1 – Lactate and rigor mortis development

Blood lactate was measured by using a Lactate Pro 2 analyzer together with Lactate Pro 2 test strips (Arkray Factory Inc, Japan).

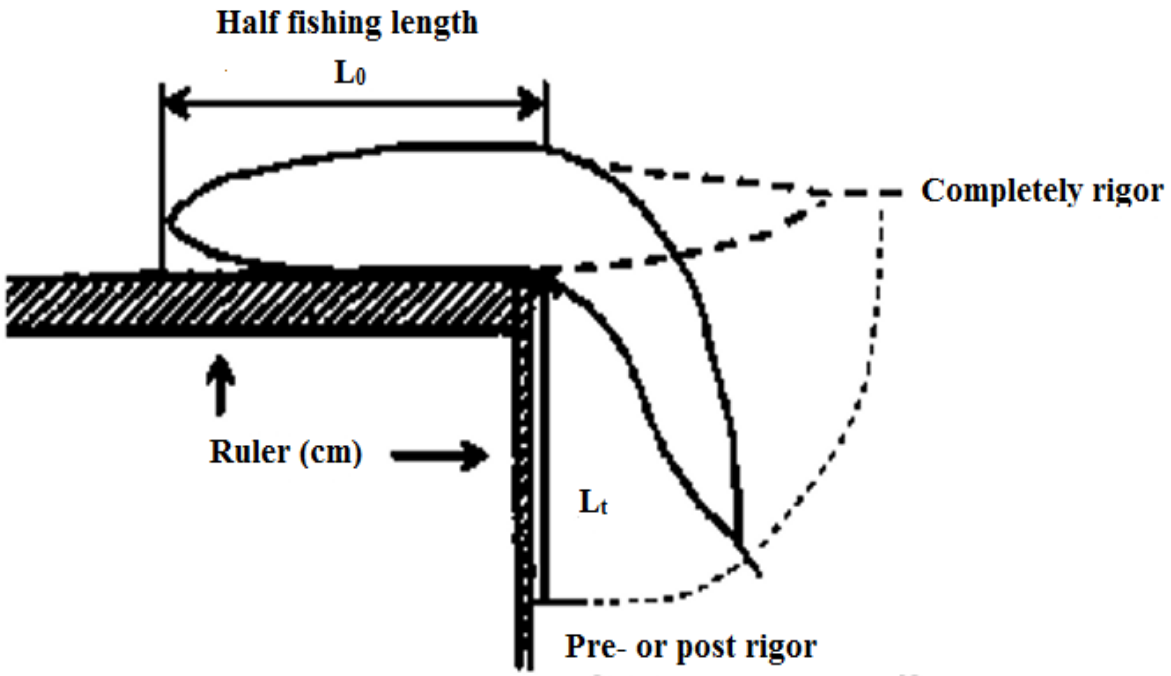


Figure 4. 2 Measurement of rigor index: $I_R = ((L_0 - L_t) \times 100$. L_0 indicates half of the body length of the fish. L_t represents the distance from the caudal fin to the horizontal line of the table, measured at intervals during storage. A hanging relaxed fish has rigor index 0, and when it is completely stiff, and lying horizontal with the table, the rigor index is at its maximum. (Sundet et al. 2011)

Rigor mortis was measured by using Cuttingers Method (tail drop, figure 4.2) (Bito et al. 1983). The rigor index (I_r) was calculated by following formula $I_r = [L_o - L_t)/L_o] \times 100$. *L* represents the vertical drop (cm of the tail, when half of the fish fork length is placed on the edge of a table. L_o is the tail drop at the beginning of the experiment, while L_t represents measurements throughout the experiment.

4.2.3 Experiment 2 – Contraction, DL, WHC, DM, colour and texture

Fillet contraction was calculated after a method by Stien, Suontama and Kiessling (Stien et al. 2006). Right fillets were photographed with a SLR camera (Canon EOS 1000D, Canon Inc.) at day 0, 5, 10 and 15. A ruler was used as sentinel at each shooting. The area of the fish fillets were calculated in the computer program Matlab.

Drip loss (DL) from the fillets was calculated as the difference in fillet weight between day 0 and day X of both the left and right fillets. An average of the left and right fillets was used for statistical analyses.

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

m_0: fillet weight at day 0

m_x: fillet weight at day x

WHC of all left fillets was measured in the dorsal muscle (figure 4.3) after a method described by Skipnes, Østby and Hendrickx (Skipnes et al. 2007). WHC was measured in duplicates at each sampling day (5, 10 and 15 days *post mortem*) on a defined area of the fillet (diameter 31mm, high 6 mm, approximately 5 g). The dry matter was estimated gravimetrically after drying at 105°C for 24 hours (ISO 1983).

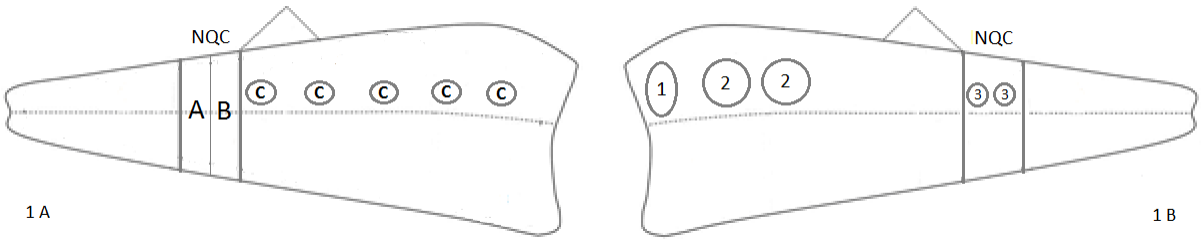


Figure 4. 3 1A) Schematic illustration showing the areas upon the right fillet from witch analyses were conducted. A: Astaxanthin and fat at day 0 and 15, B: Protein and protein degredation, C: area for spectrophotometrycally analysis of colour (MiniScan XE, HunterLab Inc. 1B) Schematic illustration showing the areas upon the left fillet from witch analyses were conducted. 1: Muscle temperature and pH, 2: Water holding capacity (WHC), 3: Instrumental texture analyses.

Surface colour was measured by a MiniScan XE, HunterLab Inc were *L** describes the lightness of the sample, *a** intensity in red (*a**>0) and *b** intensity in yellow (*b**>0). Colour measurements were performed at a defined area (Figure 4.3) of four fillets at day 0 and respectively at day 15 *post mortem*. *L**, *a**, *b** is presented as an average of five parallels of each fillet.

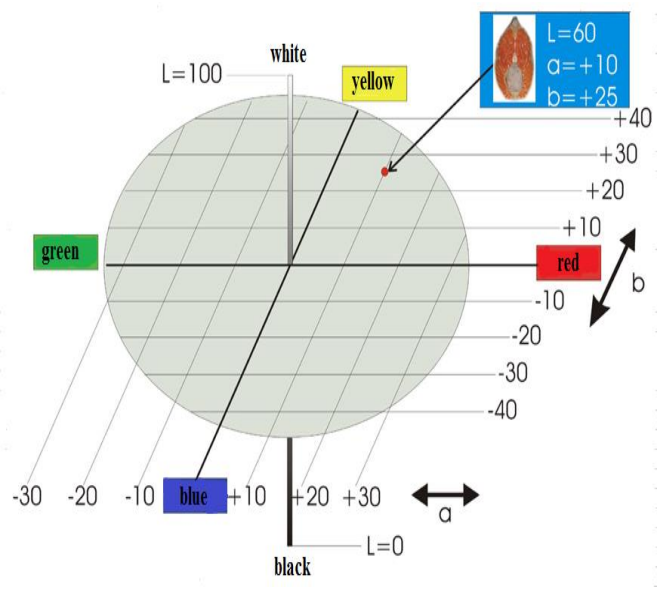


Figure 4. 4 Colour hue. The *L** variable represents lightness (*L** = 0 for black, *L** = 100 for white), the *a** scale represents the intensity in red and the *b** scale represents the intensity in yellow. (Photo: http://www.premiercolorscan.com/products/spectrophotometer/hunterlab/mini_scan.htm)

Figure 4. 5 Texture Analyser (Photo: own)

Instrumental textural analyses were performed using a Texture Analyser TA-XT2 (SMS Ltd., Surrey, England) equipped with a 30 kg load cell. A flat-ended cylinder probe (10 mm

diameter, type P/1SP) was used. The force-time graph was recorded by a computer equipped with the Stable Micro Systems Exponent software for windows (version 6.1.7.0) which was also used for data analyses. Analyses were performed in duplicates (average values were used in data analysis) of each fillet 5 and 15 days *post mortem*. The resistance force (N) was recorded with a constant speed of 5 mm sec-1, and the force required to press the cylinder down to 80 % of fillet thickness was used to describe fillet firmness.

4.2.4 Experiment 3 – Chemical composition

Chemical composition of the raw fish material was determined by analysing muscle tissue from the NQC (Figure 4.3) of five individuals from each group. Fat and carotenoids were extracted and total fat calculated by the method of Bligh and Dyer (Bligh 1959) with slight modifications. Astaxanthin were analysed by HPLC using a Agilent1100 liquid chromatograph (Agilent Technologies, Paolo Alto, CA, USA connected to an Agilent photodiode array UV-VIS detector) after a method by Vecchi, Glinz, Meduna, Schiedt (Vecchi et al. 1987) using a Lichrosorb SI60-5, 125*4.0 mm, 5 um, Hichrom, Reading, UK, HPLC column. Before use, the column was modified with ortophosphoric acid (0.1% in CH_3OH).

Crude extracts were prepared as described in Sovik & Rustad (2006) with modifications (Sovik & Rustad 2006). Frozen samples (1-2 g) were cut in small pieces, mixed with 5 ml distilled water in centrifuge tubes and homogenized with an Ultra Turrax (small; 18.000 rpm) for 20 sec, stirred for 10 min and centrifuged at 10.400×g (8300 rpm in Sorwall) for 20 min at 4°C. Supernatants were decanted and stored at -80°C for further analysis.

Protein concentration in the crude extracts was determined by DC™ Protein Assay, Bio-Rad, (Lowry et al. 1951) in triplicate, with bovine serum albumin (BSA) as standard (0,008 g BSA in 4 ml distilled water (Stock = 2,000 mg/ml)). 5 µl of the sample was pipetted out and into a microtiter plate, were 25 µl of reagent A and 200 µl of reagent B were added into each well. The plate was mixed for 5 sec in the multiplate reader (Synergy 2, BioTek Instruments, USA), therafter incubated for 15 min at room temperature. Absorbance was read at 750 nm.

Enzyme measurements were performed as described in Sovik & Rustad (2006) with modifications. Activity of cathepsin B+L and collagenase were measured against synthetic substrates, were crude enzyme (10µl) extracts were added to small glass tubes and substrate Z-Phe-Arg-4-methylcoumaryl-7-amide (14.8 µM/L, 100µl for cathepsin B+L); Suc-Gly-Pro-

Leu-Gly-Pro-4-methylcoumaryl-7-amide (14.8 µM/L for collagenase) (Kojima et al. 1979)) was added and thoroughly mixed. A substrate (6.25 mmol/l) in dimethyl sulfoxide (1 ml, DMSO) was further diluted to (14,8 µmol/L (500*; 20 ul in 8.5 ml) in 0.3 mol/l) phosphate-citrate buffer + dithiothreitol (4 mmol/l, DTT) and EDTA (2 mmol/l, pH 7) and Bis-Tris buffer (0.1 mol/l) with $CaCl_2$ (0.05 mol/l + pH 7) for the analyses of cathepsins and collagenase, respectively. The reaction mixture was incubated in a water bath for 10 min at 40°C, before the reaction were stopped by adding 1 ml of 1% SDS in 0.05 mol/L Bis-Tris (pH 7).

Blank samples was added a stopbuffer (1% SDS in 0.05 mol/L Bis-Tris, (pH 7)) before the preincubation. Appropriate dilutions of standards (6) (in stopbuffer) were used. Increases in emission and excitation was measured using a spectrofotometer (Synergy 2, BioTek Instruments, USA) at 460 and 360 nm, respectively, in black multiplates (250 µl). Activities are expressed as the increase in fluorescence and given in arbitrary units (U) based on the mean of three measurements.

4.3 Statistics

Microsoft Exel (2013) was used to process raw data from the experiments. Data were analysed by one-way ANOVA, general linear model (GLM), regression (R) and correlation (Pearson`s correlation coefficient, r) analyses using version Minitab 17. To compare different groups, Tukey`s pairwise comparison test were used. The alpha level was set to 5 % ($P<0.05$). All results are given as mean ± SD.

5. RESULTS

5.1 Locality and season (Marine Harvest)

5.1.1 Main effects on drip loss

Figure 5.1 shows the variation in drip loss between all fillets sampled for analyses during 20 days ice storage. Season (P=0.043, GLM) and locality (P=0.013, GLM) affect the drip loss significantly during 20 days ice storage (Figure 5.2).

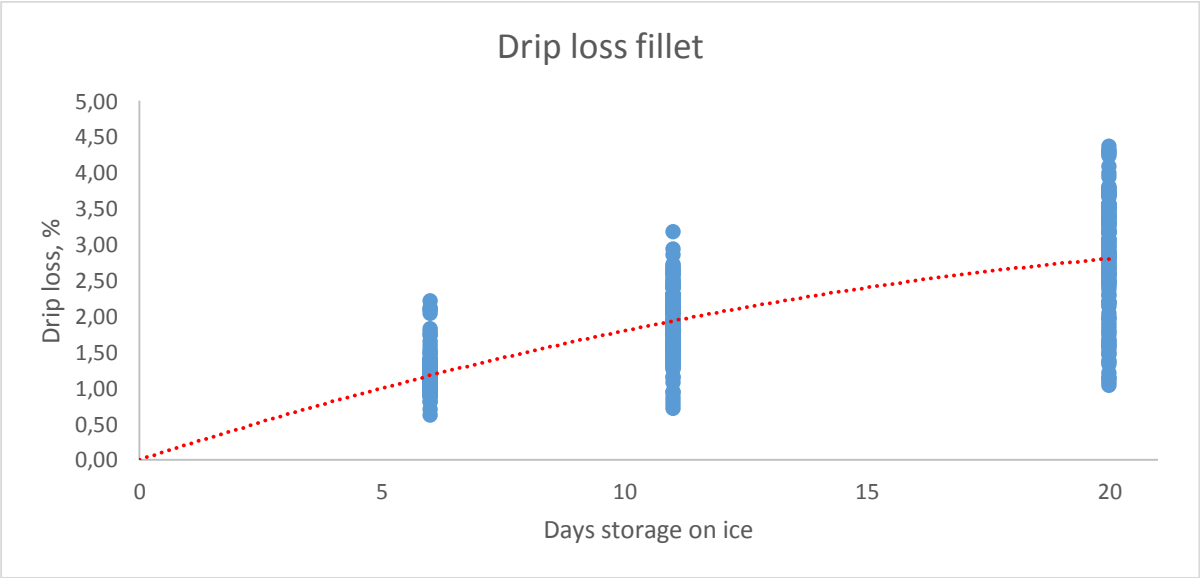


Figure 5. 1 Drip loss during 20 days ice storage of salmon fillets (average of right + left fillets) sampled for analyses (n=25 fish per group, in total 100 fish). Overall, at each sampling day there was differences in drip loss between season (P=0.043, GLM), locality (P=0.013, GLM) and days (P<0.001, GLM).

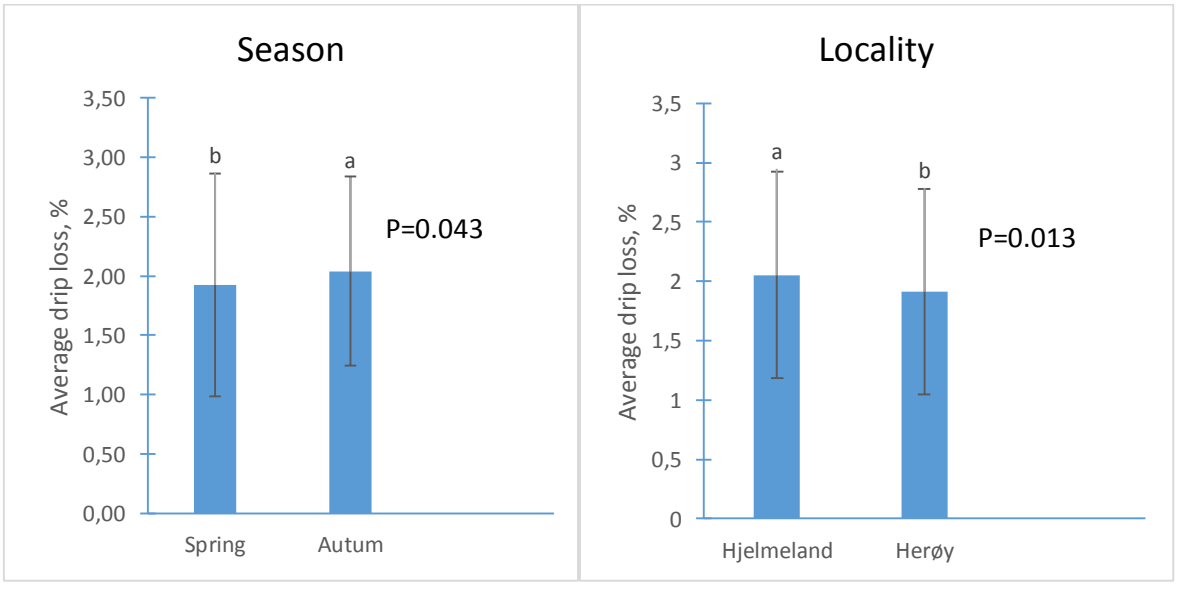


Figure 5. 2 Main effects of drip loss (season: spring/autumn, locality: Hjelmeland/Herøy) after 20 days ice storage of salmon fillets sampled for analyses (n=50 fish per column, in total 100 fish). Different lower case letters indicate significant variation (P<0.05) by general linear model (GLM). Bars indicate one SD.

5.1.2 Drip loss and WHC

Table 5.1 shows a significant effect of storage time on the drip loss from the salmon fillet (P<0.001). At day 6, significantly (P<0.001) lowest drip loss were observed of salmon sampled in spring independent of locality. At day 11 however, the variation between the individuals increased in all groups (higher SD). Significantly highest drip loss was found for salmon sampled at Hjelmeland in autumn. This group was moreover followed nomerically by Hjelmeland spring (2.0), Herøy autumn (1.9) and Herøy spring (1.7). At end of the storage period (day 20) the differences in drip loss between the groups were insignificant (P=0.902).

There were no significant differences in WHC between groups on day 6 (P=0.099) and 20 (P=0.119). A significant main effect of locality (north-south) was however found (P=0.004, GLM). Moreover, no effect of season (P=0.475, GLM) was observed on the WHC was.

Table 5. 1 Average drip loss and WHC (mean±SD) in pre rigor filleted salmon measured after 6, 11 and 20 days on ice between groups.

	Day	**Hjelmeland spring**	**Herøy spring**	**Hjelmeland autumn**	**Herøy autumn**	***P-value***
Drip loss, % (1)	*6*	1.2±0.2^{b}	1.1±0.2^{b}	1.4±0.4^{a}	1.2±0.1^{ab}	*<0.001*
	11	2.0±0.5^{ab}	1.7±0.6^{b}	2.1±0.5^{a}	1.9±0.3^{ab}	*0.003*
	20	2.8±1.0	2.8±0.9	2.9±0.8	2.8±0.6	*0.902*
WHC, % (2)	*6*	96.0±1.0	93.4±3.1	94.1±2.4	94.6±1.1	*0.099*
	11	95.3±0.6^{a}	92.7±1.7^{b}	95.2±1.5^{a}	94.4±1.3^{ab}	*<0.001*
	20	94.7±1.4	95.2±0.6	94.6±1.4	92.8±3.7	*0.119*

(1) Average values of 24 individuals per group and day, in total 96 individuals.

(2) Average values of 8 individuals per group and day, in total 24 individuals.

Different lower case letters indicate significant variation (P<0.05) by one-way ANOVA.

5.1.3 Fillet firmness

Fillet firmness (N) was significantly affected by locality (P<0.001, GLM), and not by season (P=0.360, GLM) (Figure 5.3).

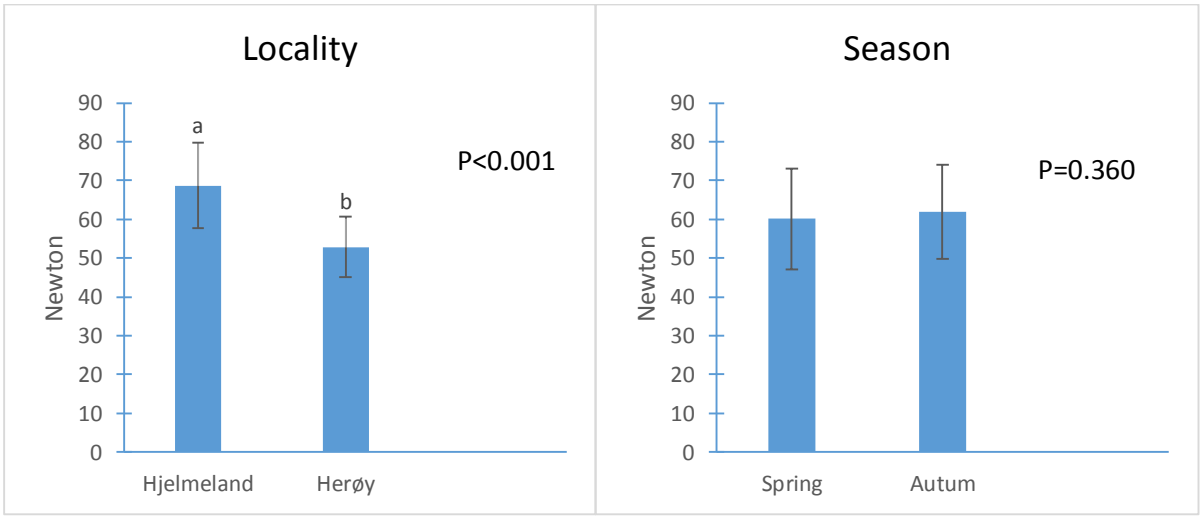


Figure 5. 3 Firmness (mean±SD) of pre rigor filleted salmon determined instrumentally as the force (N) at 80 % compression of the fillet height. Bars indicate one SD and different lower case letters indicate significant variation (P<0.05) by general linear model ANOVA (n=48 fillets per locality, in total 96 fillets).

Fillet firmness (N) was significantly different between the locality both at day 6, 11 and 20 (Table 5.2). Significantly lowest firmness (N) was observed in fillets from the locality Herøy, both at spring and autumn.

Table 5. 2 Firmness of pre rigor filleted salmon determined instrumentally after 6, 11 and 20 days on ice as the force (N) at 80% compression of the fillet height. Results are shown as average per group ± SD.

Day	**Hjelmeland spring**	**Herøy spring**	**Hjelmeland autumn**	**Herøy autumn**	***P-value***
6	75.8±10.8^{a}	52.2±10.6^{b}	71.4±13.3^{a}	54.0±6.9^{b}	*<0.001*
11	61.1±10.3^{ab}	54.7±7.9^{b}	70.2±8.0^{a}	53.6±8.3^{b}	*0.002*
20	63.3±9.2^{ab}	49.5±8.1^{c}	69.9±10.0^{a}	53.0±6.0^{bc}	*<0.001*

Different superscripts (^{abc}) in the same row indicate significant variation (P<0.05) between groups by one-way ANOVA (n=8 fillets per sampling day at each locality, in total 96 fillets).

5.1.4 Chemical composition

No significant differences were found between the groups in chemical composition, except for contents of lutein (Table 5.3). Significantly higher contents of lutein was found in salmon

sampled at Herøy (autumn) compared to the other groups. Water content was significantly affected by locality (P=0.050, GLM), and not by season (P=0.157, GLM).

Table 5. 3 Chemical composition (water, protein and fat) and contents of carotenoids (astaxanthin and lutein) in the different groups sampled for analyses. Results are shown as average ± SD.

	Hjelmeland spring	**Herøy spring**	**Hjelmeland autumn**	**Herøy autumn**	***P-value***
Water content, % [(1)]	66.3±2.7	65.3±2.4	65.6±1.4	64.8±1.9	*0.116*
Protein content, %[(2)]	21.5±1.5	21.4±0.9	22.9±1.1	22.4±0.8	*0.157*
Fat content, % [(2)]	11.8±4.9	13.6±3.8	11.6±1.1	11.2±1.6	*0.648*
Astaxanthin, mg/kg [(2)]	5.82±1.73	5.26±0.81	6.21±1.76	5.79±1.46	*0.796*
Lutein mg/kg [(2)]	0.19±0.09[b]	0.09±0.02[b]	0.16±0.06[b]	0.34±0.11[a]	*<0.001*
Total carotenoid mg/kg [(2)]	6.00±1.82	5.35±0.82	6.37±1.81	6.13±1.57	*0.765*

[(1)] Average values of 24 individuals per group, in total 96 individuals.
[(2)] Average values of 5 individuals per group, in total 20 individuals.
Different lower case letters indicate significant variation (P<0.05) by one-way ANOVA.

5.2 Quality diploid versus triploid Atlantic salmon (Institute of Marine Research)

The six groups of salmon sampled for analyses (5°C diploid, 5°C triploid, 10°C diploid, 10°C triploid, 15°C diploid, 15°C triploid) were slaughtered at Institute of Marine Research (Matre) with equal fish history. They differs in whole weight (P<0.001), gutted weight (P=0.007) and condition factor (P<0.001) (Chapter 3, table 3.3). Between diploid and triploid salmon no differences in whole body weight was observed (P=0.649, GLM). There were however, significant differences in whole body weight between salmon farmed at different temperatures (P<0.001, GLM), were 10°C differs from 5°C and 15°C (Tukey pairwise comparison).

5.2.1 Rigor index

The results of rigor development are shown in figure 5.4, 5.5 and table 5.4, were the results are presented in three different ways. Figure 5.4 clearly shows the rigor development picture of all six groups. The speed of the rigor development was clearly affected by temperature. Based on the rigor index (Figure 5.5), diploid salmon went faster into rigor as compared to triploids at all temperatures. After 24 hours, a significant difference between temperatures (P<0.001, GLM) and ploidy (P=0.013, GLM) was found.

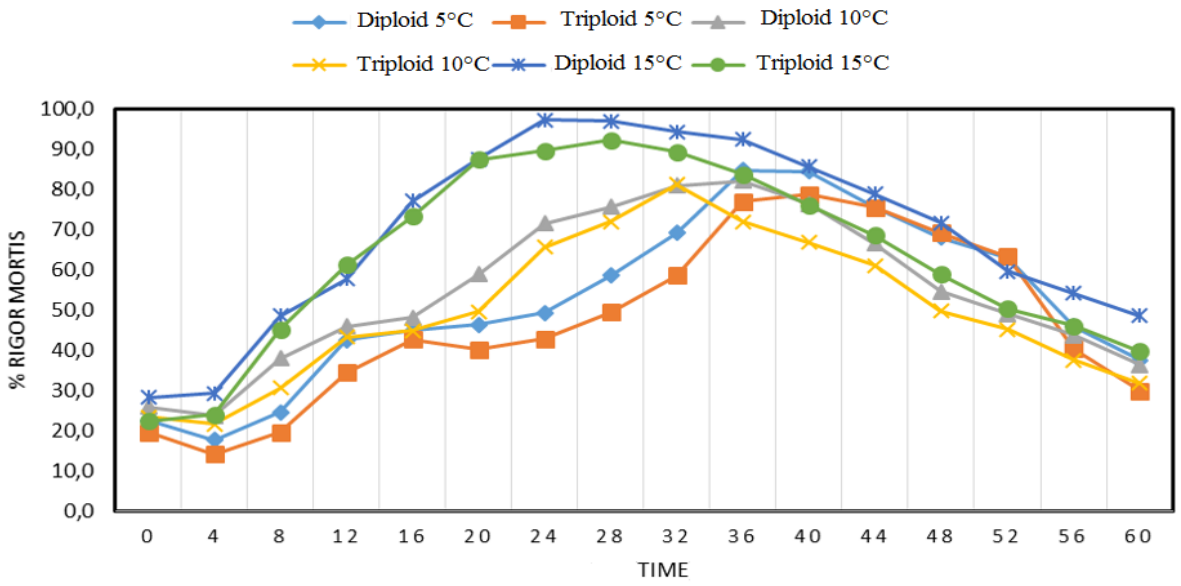


Figure 5. 4 Rigor index course in the different groups measured with a time interval of 4 hours from 0-60 hours. Presented values are an average of 10 individuals per group, in total 60 individuals.

Table 5.4 shows the rigor index values of the different groups. The rigor index were significantly different between groups throughout rigor development (P<0.001), except 44 hours *post mortem* (P=0.066).

Table 5. 4 Rigor index in the different groups measured with a time intervall of 4 hours from 0-60 hours. Results are shown as average ± SD.

Time	Diploid 5°C	Triploid 5°C	Diploid 10°C	Triploid 10°C	Diploid 15°C	Triploid 15°C	*P-value*
0	23 ± 3^{bc}	20 ± 3^{c}	26 ± 3^{ab}	23 ± 5^{bc}	28 ± 3^{a}	22 ± 3^{bc}	*<0.001*
4	18 ± 3^{cd}	14 ± 3^{d}	24 ± 3^{ab}	22 ± 6^{bc}	29 ± 6^{a}	24 ± 5^{ab}	*<0.001*
8	25 ± 8^{cd}	20 ± 5^{d}	38 ± 9^{abc}	31 ± 14^{bcd}	49 ± 10^{a}	45 ± 22^{ab}	*<0.001*
12	43 ± 10^{bc}	35 ± 9^{c}	46 ± 6^{abc}	43 ± 12^{bc}	58 ± 16^{ab}	61 ± 18^{a}	*<0.001*
16	45 ± 6^{b}	43 ± 8^{b}	48 ± 6^{b}	45 ± 14^{b}	77 ± 11^{a}	73 ± 17^{a}	*<0.001*
20	46 ± 7^{bc}	40 ± 10^{c}	59 ± 12^{b}	50 ± 15^{bc}	88 ± 11^{a}	87 ± 10^{a}	*<0.001*
24	49 ± 8^{a}	43 ± 12^{a}	72 ± 13^{b}	66 ± 15^{b}	97 ± 3^{a}	90 ± 8^{a}	*<0.001*
28	59 ± 14^{cd}	50 ± 10^{d}	76 ± 16^{b}	72 ± 13^{bc}	97 ± 3^{a}	92 ± 5^{a}	*<0.001*
32	69 ± 16^{bc}	59 ± 12^{c}	81 ± 5^{ab}	81 ± 8^{ab}	94 ± 7^{a}	89 ± 8^{a}	*<0.001*
36	85 ± 16^{ab}	77 ± 10^{b}	82 ± 8^{ab}	72 ± 12^{b}	92 ± 9^{a}	84 ± 11^{ab}	*0.004*
40	84 ± 8^{a}	79 ± 5^{ab}	76 ± 12^{ab}	67 ± 19^{b}	85 ± 11^{a}	76 ± 11^{ab}	*0.022*
44	76±10	76±8	67±15	61±23	79±14	69±12	*0.066*
48	68 ± 18^{ab}	69 ± 11^{ab}	55 ± 15^{ab}	50 ± 19^{b}	72 ± 14^{a}	59 ± 15^{ab}	*0.013*
52	63 ± 11^{a}	63 ± 13^{a}	49 ± 13^{a}	45 ± 18^{a}	60 ± 13^{a}	50 ± 13^{a}	*0.014*
56	46 ± 13^{ab}	40 ± 8^{ab}	44 ± 13^{ab}	38 ± 11^{b}	54 ± 9^{a}	46 ± 12^{ab}	*0.031*
60	38 ± 12^{ab}	30 ± 5^{b}	36 ± 8^{b}	32 ± 9^{b}	49 ± 11^{a}	40 ± 7^{ab}	*<0.001*

Presented values are an average of 10 individuals per group, in total 60 individuals.
Different superscripts (^{abc}) in the same row indicate significant variation (P<0.05) between groups by one-way ANOVA.

Figure 5.5 shows the *rigor mortis* progress and pH at each temperature of diploid and triploid salmon.

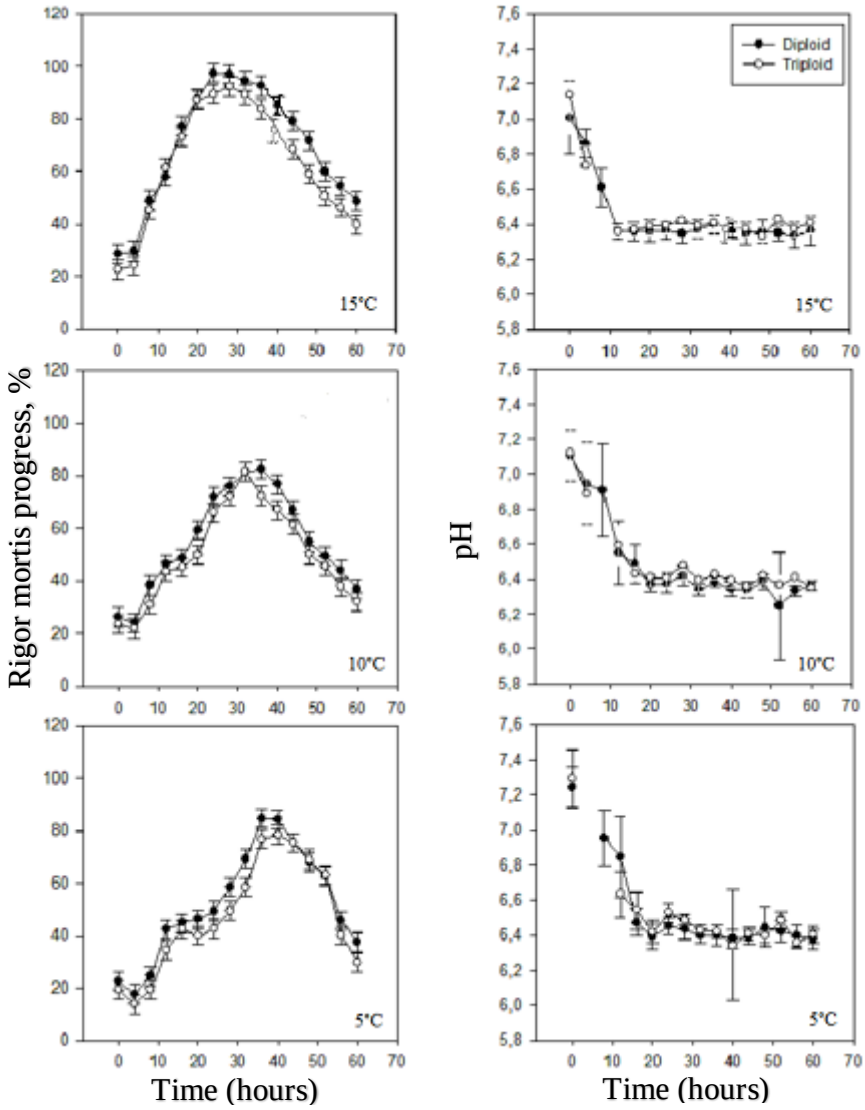


Figure 5. 5 Rigor index and pH at different temperatures sampled with a time interval of 4 hours from 0-60 hours. Results are shown as average ± SD. Bars indicate one SD.

5.2.2 Drip loss

The drip loss of the salmon fillets during 15 days refrigerated storage is presented in table 5.6. In the presented study, triploid salmon shows a nominal higher drip loss then diploids during 15 days refrigerated storage. Overall, there were significant main differences in drip loss between ploidy (P=0.003, GLM), temperature (P<0.001, GLM) and days (P<0.001, GLM) (Figure 5.7). A significant effect of temperature was however observed on the average drip

loss during 15 days refrigerated storage (P=0.010), were 15°C differed from 5°C and 10°C (Tukey pairwise comparison).

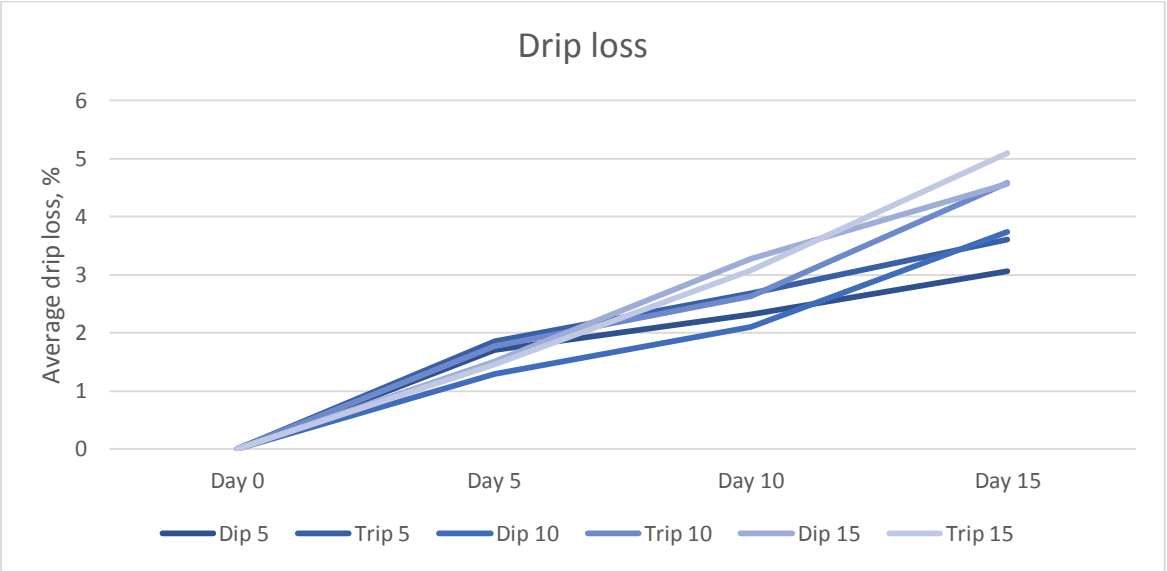


Figure 5. 6 Average drip loss (right + left fillet) during 15 days refrigerated storage (5-6°C) of salmon fillets sampled for analyses (n=12 fish per sampling day, in total 72 fish). Overall, significant differences between ploidy (P=0.003, GLM), temperature (P<0.001, GLM) and days (P<0.001, GLM) were observed.

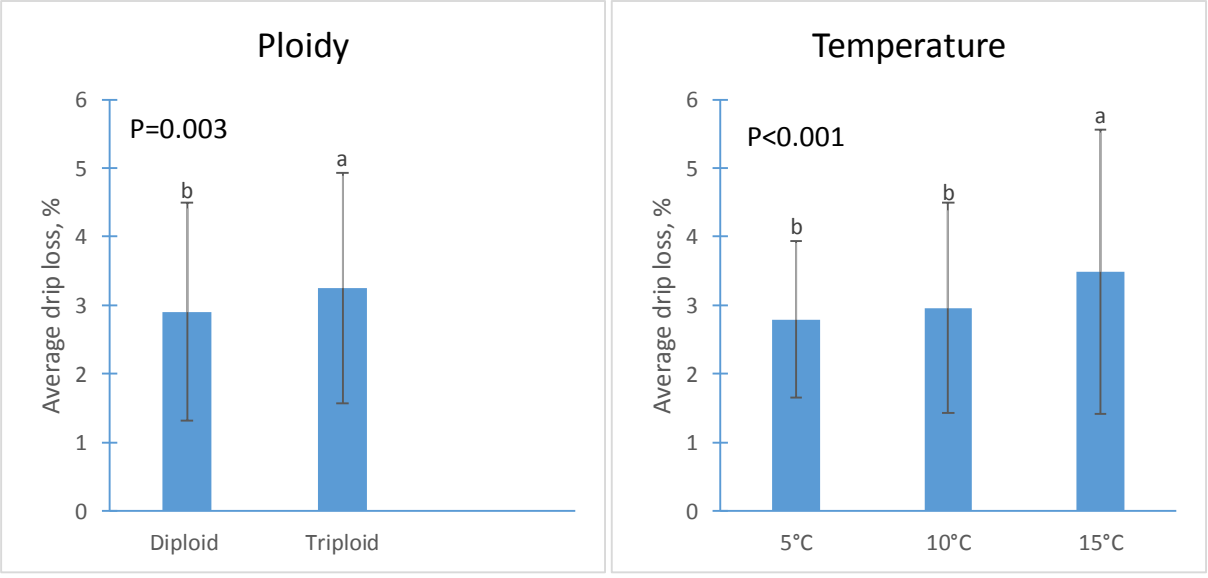


Figure 5. 7 Main effects of drip loss (ploidy: diploid/triploid, temperature: 5, 10 and 15°C) after 15 days refrigerated storage (5-6°C) of salmon fillets (right + left fillet) sampled for analyses (n=36 fish per column (diploid/triploid) and n=24 fish per column (5, 10 and 15°C), in total 72 fish). Different lower case letters indicate significant variation (P<0.05) by general linear model ANOVA. Bars indicate one SD.

5.2.3 Drip loss, contraction, water holding capacity and dry matter

The six groups of salmon sampled for analyses differed in total drip loss during 15 days dry storage (P=0.019), contraction (P<0.001) and dry matter (P<0.001) (Table 5.5). Only at growth temperature of 10 degrees, a significant difference in drip loss between diploid and triploid salmon fillets (P=0.039) was found. Moreover, a significant but week correlation was observed between measured drip loss and contraction (r=0.321, P<0.001). The WHC of fillets did not differ between the groups (P=0.302).

Table 5. 5 Average drip loss, contraction, WHC and dry matter in the different groups sampled for analyses. Results are shown as average ± SD.

	Diploid 5°C	**Triploid 5°C**	**Diploid 10°C**	**Triploid 10°C**	**Diploid 15°C**	**Triploid 15°C**	***P-value***
DL, %	2.7±1.2^{ab}	2.9±1.08^{ab}	2.6±1.4^{b}	3.3±1.6^{ab}	3.4±2.0^{ab}	3.6±2.2^{a}	*0.019*
Contraction, %	5.2±2.0^{ab}	4.3±2.6^{b}	4.6±2.0^{b}	6.4±3.3^{a}	3.8±2.2^{b}	2.0±2.5^{c}	*<0.001*
WHC, %	89.8±3.5	90.8±4.2	89.8±3.3	90.8±4.2	92.7±2.8	92.0±3.4	*0.302*
DM, %	28.5±2.0^{b}	28.2±1.3^{b}	32.3±3.8^{a}	32.5±2.8^{a}	29.0±1.8^{b}	29.8±1.7^{ab}	*<0.001*

Average values of 12 individuals per group, in total 72 individuals. Different superscripts (^{abc}) in the same row indicate significant variation (P<0.05) between groups by one-way ANOVA.

5.2.4 Hunter LAB

A significant effect were found between groups on fillet translucence (*L**) and redness (*a**) measured 0 and 15 days *post mortem* (Table 5.6). Overall, no main effects of ploidy on translucence (*L**) (P=0.263, GLM), redness (*a**) (P=0.908, GLM) or yellowness (P=0.487, GLM) were observed. No main effects were either found on temperature (*L**, P=0.742, GLM); *a**, P=0.194, GLM) or *b**, P=0.120, GLM). Thus, neither ploidy or temperature has an impact on colour. Moreover, there was found a correlation between redness (*a**) and contraction (r= -0.269, P=0.050).

Table 5. 6 Surface colour (Hunter LAB) of the pre-rigor fillets in the different groups on day 0 and 15 in the same fillet. Results are shown as average ± SD.

	Day	Diploid 5°C	Triploid 5°C	Diploid 10°C	Triploid 10°C	Diploid 15°C	Triploid 15°C	*P-value* [1)]
L	*0*	53.5±1.6^{a}	53.2±0.7^{ab}	51.3±1.6^{ab}	51.8±0.7^{ab,x}	50.7±1.3^{b}	51.3±1.4^{ab}	*0.010*
	15	52.5±1.3^{abc}	53.2±1.4^{ab}	51.6±1.1^{bc}	54.1±0.6^{a,y}	50.7±1.1^{c}	51.7±0.4^{bc}	*0.003*
	P-value[2)]	*0.336*	*0.984*	*0.757*	*<0.001*	*0.986*	*0.610*	
*a**	*0*	19.1±3.2^{b}	20.0±1.2^{ab}	22.7±1.3^{a}	22.0±1.7^{ab}	22.6±1.5^{ab}	22.3±1.0^{ab}	*0.016*
	15	20.8±2.7^{a}	20.4±1.1^{a}	22.7±1.6^{a}	20.3±1.5^{a}	23.3±1.2^{a}	23.1±0.5^{a}	*0.029*
	P-value[2)]	*0.443*	*0.678*	*0.975*	*0.157*	*0.432*	*0.238*	
*b**	*0*	23.3±2.3	23.2±0.7^{x}	25.0±1.1^{x}	23.5±1.0^{x}	24.4±1.0	23.6±1.1	*0.264*
	15	23.0±2.3^{a}	22.0±0.6^{ab,y}	22.8±1.3^{ab,y}	20.0±1.3^{b,y}	23.3±0.7^{a}	22.6±0.3^{ab}	*0.017*
	P-value[2)]	*0.856*	*0.032*	*0.030*	*0.002*	*0.125*	*0.125*	

Presented values are average of 4-5 individuals per group. Different superscripts (^{abc}) in the same row indicate significant variation (P<0.05) between groups[1)] and (^{xy}) days[2)] were tested by one-way ANOVA and Tukey`s pairwise comparison test.

5.2.5 Fillet firmness

There was a significant difference between groups on day 5 when it came to fillet firmness (P=0.004) (Table 5.7). On the other hand, no differences between groups were observed at day 15 (P=0.822) or between days (P=0.057-0.887). No main effects of ploidy was found on fillet firmness (P=0.534, GLM), but a main effect of temperature (P=0.018, GLM) was observed. Moreover, no significant correlations between force (N) and whole body weight (r= -0.204, P=0.263) were detected.

Table 5. 7 Firmness of pre rigor filleted salmon determined instrumentally at day 5 and 15 as the force (N) at 80% compression of the fillet height. Results are shown as average ± SD.

Day	Diploid 5°C	Triploid 5°C	Diploid 10°C	Triploid 10°C	Diploid 15°C	Triploid 15°C	*P-value* [1)]
5	12.8±2.0^{b}	14.6±2.6^{ab}	10.7±1.6^{b}	13.7±1.7^{ab}	17.7±2.3^{a}	14.3±1.6^{ab}	*0.004*
15	14.3±2.8^{a}	15.0±2.8^{a}	12.7±3.0^{a}	13.4±2.1^{a}	14.2±2.0^{a}	14.1±1.3^{a}	*0.822*
P-value [2)]	*0.396*	*0.848*	*0.268*	*0.887*	*0.057*	*0.840*	

Presented values are average of 4 individuals per group and day. Sampling at day 5 and 15 is different fillet. Significant variation (P<0.05) between the different groups[1)] and days[2)] were tested by one-way ANOVA and Tukey`s pairwise comparison test. There was no significant differences between days.

5.2.6 Chemical composition

There was a significant difference between groups when it came to total carotenoid (P<0.001) and astaxanthin content (P<0.001) (Table 5.8), were 15°C differed from 5°C and 10°C (Tukey pairwise comparison). No differences in protein (P=0.075) and fat (P=0.062) between groups was found. A significant main effect of temperature was however observed on contents of total carotenoids (P<0.001, GLM), astaxanthin (P<0.001, GLM), lutein (P<0.001, GLM), all-trans astaxanthin (P<0.001, GLM), 9-cis astaxanthin (P<0.004, GLM) and 13-cis astaxanthin (P<0.001, GLM). No main effects of ploidy was found on contents of total carotenoids (P=0.850, GLM), astaxanthin (P=0.812, GLM), lutein (P<0.001, GLM), all-trans astaxanthin (P=0.248, GLM), 9-cis astaxanthin (P=0.543, GLM) and 13-cis astaxanthin (P=0.256, GLM).

Table 5. 8 Chemical composition of salmon from the different groups sampled for analyses. Results are shown as average ± SD.

	Diploid 5°C	**Triploid 5°C**	**Diploid 10°C**	**Triploid 10°C**	**Diploid 15°C**	**Triploid 15°C**	***P-value***
Protein content, %[(1)]	14.0±5.2	14.0±3.2	11.7±1.6	11.6±1.3	16.3±4.1	15.4±0.9	*0.075*
Fat content, % [(2)]	2.9±1.5	5.0±2.5	3.4±0.9	5.1±3.1	3.7±1.2	6.8±2.4	*0.062*
Tot Carotenoid, mg/kg[(3)]	3.12±0.48^{b}	3.10±0.20^{b}	3.43±0.30^{b}	3.35±0.43^{b}	4.46±0.51^{a}	4.48±0.47^{a}	*<0.001*
Astaxanthin, mg/kg[(3)]	3.04±0.47^{b}	3.02±0.20^{b}	3.35±0.30^{b}	3.26±0.42^{b}	4.34±0.49^{a}	4.35±0.46^{a}	*<0.001*
All trans, % [(3)]	93.57±0.75^{a}	93.21±1.54^{ab}	92.25±0.82^{b}	92.14±1.15^{abc}	90.76±0.91^{c}	92.10±0.67^{b}	*<0.001*
9-cis, % [(3)]	1.16±0.22^{a}	1.14±0.23^{a}	1.00±0.16^{a}	0.94±0.29^{a}	0.97±0.05^{a}	0.95±0.13^{a}	*0.045*
13-cis, % [(3)]	5.28±0.67^{d}	5.65±1.34^{cd}	6.76±0.77^{bc}	6.92±0.96^{bc}	8.27±0.91^{a}	6.95±0.68^{b}	*<0.001*
Lutein, mg/kg[(3)]	0.08±0.02^{c}	0.07±0.01^{c}	0.09±0.01^{c}	0.10±0.02^{bc}	0.12±0.03^{ab}	0.13±0.03^{a}	*<0.001*

(1) Average values of 6 individuals per group, in total 36 individuals. Sampled day 0 and 15.
(2) Average values of 5 individuals per group, in total 30 individuals. Sampled day 0.
(3) Average values of 10 individuals per group, in total 60 individuals. Sampled day 0 and 15.
Different lower case letters indicate significant variation (P<0.05) by one-way ANOVA.

Analyses of cathepsins showed a significant main effects of days stored (P<0.001, GLM) and temperatures (P<0.001, GLM), whereas no effect of ploidy (P=0.446, GLM) was found (Table 5.9). The cathepsin level was found to increase during storage. Moreover, a significant effect of temperature was observed (P=0.011), were 15°C differed from 5°C and 10°C (Tukey pairwise comparison). Lowest level of cathepsins were found in salmon farmed at 15°C.

Contents of collagenase (Figure 5.8) were found to be significant different between diploid and triploid salmon (P<0.001) (Tukey pairwise comparison), where triploid salmon have the

highest level. There was a significant main effect of days stored on contents of collagenase (P<0.001, GLM). The level of collagenase were moreover found to decrease during storage (Table 5.9). No main effects of temperature on contents of collagenase (P=0.571, GLM) was found. No significant correlation between contents of cathepsin and collagenase were observed (r= -0.119, P=0.488).

Table 5. 9 Contents of cathepsin and collagenase in salmon fillets measured at day 0 and 15 in the different groups sampled for analyses. Results are shown as average ± SD.

Parameter	**Day**	**Diploid 5°C**	**Triploid 5°C**	**Diploid 10°C**	**Triploid 10°C**	**Diploid 15°C**	**Triploid 15°C**	***P-value*** [(1)]
Cathepsin (mU/g fish)	*0*	1.13±0.06^{y}	2.02±0.44	2.21±1.40	1.92±0.11^{y}	0.78±0.30^{y}	0.92±0.21^{y}	*0.052*
	15	2.88±0.28^{a,x}	2.72±0.25^{a}	2.44±0.32^{ab}	2.43±0.10^{ab,x}	1.89±0.46^{b,x}	2.12±0.09^{ab,x}	*0.009*
	P-value[2)]	*<0.001*	*0.071*	*0.791*	*0.004*	*0.025*	*<0.001*	
Collagenase (mU/g fish)	*0*	2.02±0.31^{abc}	2.75±0.13^{a}	1.99±0.36^{bc,x}	2.69±0.35^{ab}	1.85±0.26^{c,x}	2.47±0.19^{abc}	*0.006*
	15	1.53±0.43	2.04±0.96	1.26±0.24^{y}	2.45±0.25	1.16±0.28^{y}	1.92±0.85	*0.121*
	P-value[2)]	*0.185*	*0.274*	*0.042*	*0.386*	*0.033*	*0.340*	

Presented values are average of 3 individuals per group. The same fillet was used both day 0 and 15 is the same fillet. Significant variation (P<0.05) between the different groups[1)] and days[2)] were tested by one-way ANOVA and Tukey`s pairwise comparison test.

Comparing fat contents of diploid (3,3%) and triploid (5,7%) salmon stored 15 days (Figure 5.8), diploid salmon was found to be significantly leaner than triploids (P=0.002, GLM).

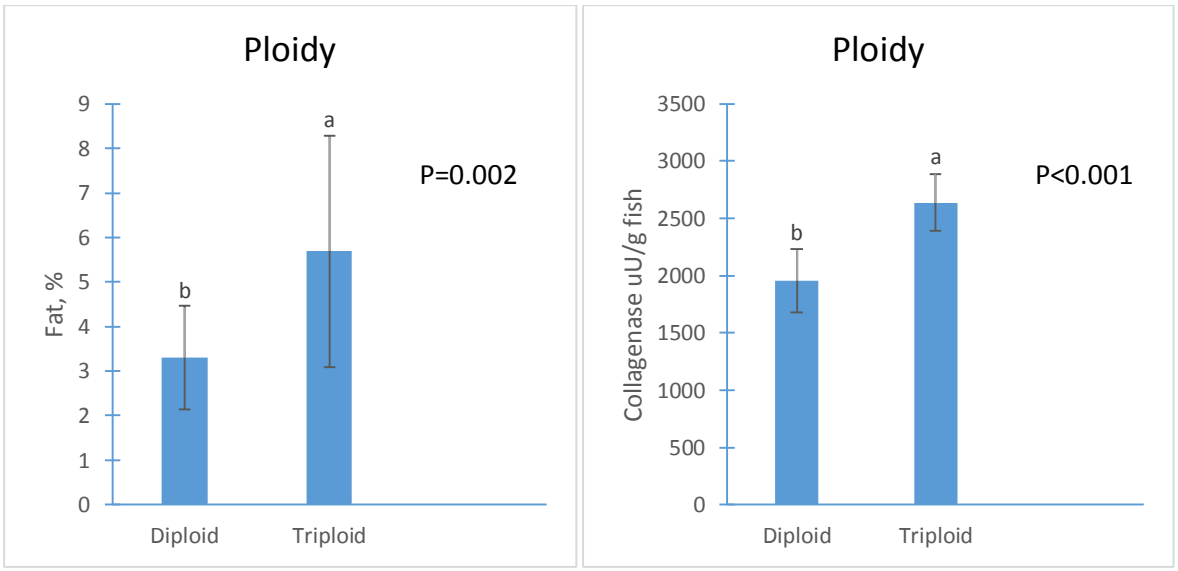


Figure 5. 8 Main effects of ploidy (diploid/triploid) on fat and collagenase content of salmon fillets (average of day 0 and 15 days refrigerated storage (5-6°C)). Fat: n=30 fish per column, in total 60 fish, collagenase: n=18 fish per column, in total 36 fish. Different lower case letters indicate significant variation (P<0.05) by general linear model (GLM). Bars indicate one SD.

5.2.7 Arrhenius plot

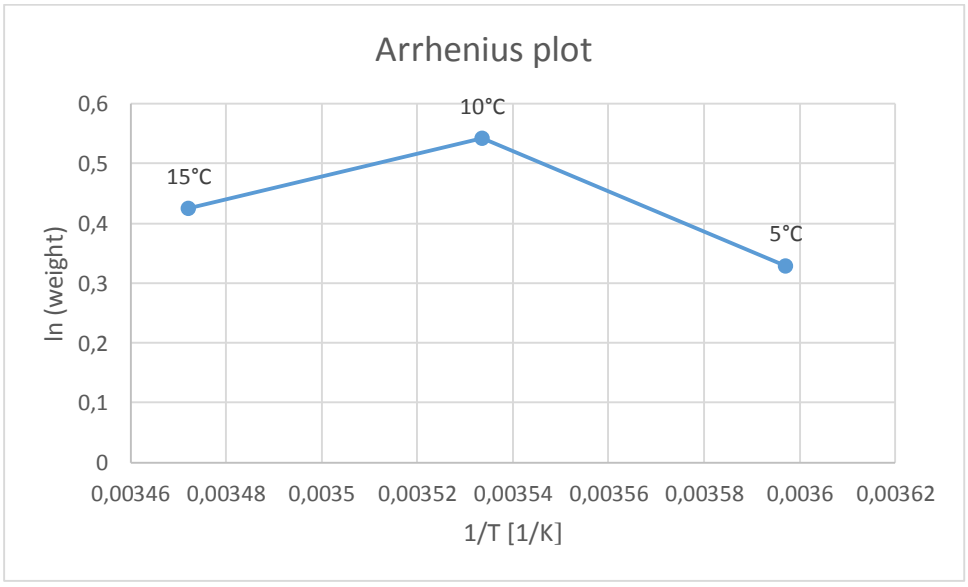


Figure 5. 9 Arrhenius plot. Ln whole weight against 1/temperature. 5°C (ln (1,39)), 10°C (ln (1,72)), 15°C (ln (1,53)).

Temperature is an important factor in determining the rate of many reactions in salmon. We expected the growth rate to increase linear with the temperature. Results of the arrhenius plot (figure 5.9) shows however that salmon stops to grow at a specific temperature.

6. DISCUSSION

6.1 Locality and season (Marine Harvest)

The division of groups in this study was based on different locality (north-south) and season (spring-autumn). All the fish were slaughtered within a short period in the end of May (spring) and November (autumn) 2014. It is natural to belive that there are general differences between salmon in the North and South along the Norwegian coast. This is due to different conditions in terms of light and temperature. In the North, the summer is short and hectic with continuous daylight while winter is long and hard. This imposes other requirements compared to salmon in the south, where summer is long, water temperature higher and winter is milder.

The fish material being compared in this experiment (Chapter 3, table 3.1), were significant different between the groups. Anyway, no appreciable differences when it comes to weight and stress parameters were observed between the groups. The death temperature of the fish sampled in spring and autumn of each locality was equal. On the other hand, the fish background and feed used was different. There are many independent factors which have affected the results in this experiment.

6.1.1 Drip loss and WHC

It is well documented that quality parameters of salmon fillets show seasonal variation (Lavety et al. 1988; Mørkøre & Austreng 2004; Roth et al. 2005). In the present study, locality and season has a significant main effect on the drip loss during 20 days ice storage (Figure 5.2). Significantly highest drip loss was found for salmon sampled in autumn. The season results are in line with Mørkøre et al. (2010). They reported that the drip loss increased significantly in October (during 13 days of storage inside the packages). On the other hand, earlier research shows that the drip loss of raw salmon fillets is affected both by factors such as starvation (Mørkøre et al. 2008) and stress prior to slaughtering (Roth et al. 2006). Comparing initial pH (Figure 3.1) and drip loss (Table 5.1), there seems to be no relationship between those factors. In the present study, a significant effect of drip loss in relation to storage time was observed (Table 5.1), which was in line with earlier findings (Lerfall et al. 2015; Mørkøre et al. 2010). Furthermore, drip loss in relation to storage time coincides with *post mortem* muscle degradation (Ofstad et al. 1995; Ofstad et al. 1996), which corresponds with the results reported in this study.

A significant main effect of locality wheras no effect of season on the WHC of salmon fillets was observed. pH close to the isoelectric point is known to reduce the WHC (Huff-Lonergan & Lonergan 2005). In the present study, a significantly lower WHC at locality Herøy (spring day 11) was not explainable by observed muscle pH at point of death. Furthermore, muscle pH throughout *rigor mortis* should have been followed, to investigate effects of muscle pH on the WHC. It has been suggested that increased WHC during ageing of meat is due to reduced water content, described as the "leaking out" effect (Van Moeseke & De Smet 1999). The presented results were however in line with Hultmann & Rustad (2002), who reported WHC of salmon muscle to not be affected by storage time (analysed 4, 7, 9 and 11 days *post mortem*). Moreover, another study had recently reported that the WHC of pork meat first decreased and then increased during the subsequent storage (Kristensen & Purslow 2001), which is in line with observation of the WHC in fillets from Herøy spring.

6.1.2 Fillet firmness

In the present study, no main effect of growth season on fillet firmness was observed (Figure 5.3). Literature about seasonal effects on quality are conflicting, first of all, texture is very complex. Previously research on salmon, shows however that softness to vary throughout season for fish originating as 0+ and 1+ smolts, and could be correlated to growth rates (Mørkøre & Rørvik 2001). Similar to softness, gaping can also be related to season (Lavety et al. 1988), and Mørkøre & Rørvik (2001) found that the degree of fillet gaping was highest during spring and summer. Comparing stress parameters (pH and lactate) (Table 3.1) and fillet firmness (Table 5.2), no significant relationships between those factors were found in the present study. However, in a study by Lerfall et al. (2015) fillet firmness were found to be affected by pre-slaughtering stress induced. Texture is regarded as a sensory attribute (Bourne 2002). It is therefore essential that instrumental techniques that measure the textural quality of fish fillets can predict a human`s sensory assessment.

In the present study, fillet firmness (N) was however significantly affected by locality (Figure 5.3). Significantly lowest firmness was observed in fish from the locality Herøy (north), both spring and autumn. According to the results, locality Hjelmeland (south) had firmer fillets. Reasons for this can be several. It is however natural to discuss the protein content first. Comparing protein content (Table 5.3) and fillet firmness (Table 5.2), no significant relationship between those factors was observed. Previous studies shows however that fillet quality can be linked to breeding phase (Gjedrem 1997), genetics and growth (Gjøen &

Bentsen 1997; Thodesen et al. 2001; Veland & Torrissen 1999), feed composition (Aksnes 1995; Einen et al. 1999), feeding regime (Noble et al. 2007), environmental factors (Johnston & Børresen 2008), seasonal variations (Mørkøre & Rørvik 2001), health status (Larsson et al. 2012), stress (Sigholt et al. 1997), handling and slaughtering process (Erikson et al. 1999; Kiessling et al. 2004; Mørkøre et al. 2008), muscle fibre density (Hatae et al. 1984; Johnston et al. 2000), ice chilling and temperature during frozen storage (Espe et al. 2004; Hultmann & Rustad 2004).

In a report by Mørkøre (2008) which describe the knowledge status of salmon texture, soft flesh can be attributed to bad breeding and genetics. Family is also an important factor, but it is also a great variation in texture within families (Bahuaud, D. et al. 2010). Secondly, soft salmon has a connection with the processes inside the cell, particularly in mitochondria, carnitine (correlation with firm texture) and the glycolysis. Thirdly, soft fish have cracks in the cells (glycogen accumulation in the cells), where missing proteins are fixed between muscle fibers, give connective tissue with a stretched structure. Soft fish is found to have higher activity of muscle degrading enzymes (Hultmann & Rustad 2004). Generally, we need to know more about interactions to increase the understanding of the complexity behind fillet texture.

In aquaculture, sea cages are exposed too high water currents in the farming locality. Today, each farming locality is divided into different levels according to the weather and sea currents. It would be interesting to see if there was a correlation between firm texture and increased sea currents. Castro et al. (2011) reported that interval aerobic training improved growth and increased robustness of Atlantic salmon, manifested by better disease resistance. These findings, can possibly explain observed differences of fillet firmness seen in this experiment. Texture is very complex, but the locality appears to be a determining factor for the fillet firmness.

Within the industry, there is a general concern that the fast growth rates made by the modern production techniques, result in an increased downgrading due to soft flesh and gaping. The results in the presented study, did not show any negative effect on the fillet firmness in autumn. Folkestad et al. (2008) showed a correlation between growth rate in the last two months before slaughter (march-may) and fillet firmness (tensile strength) of raw salmon (round weight 4 kg). On the other side, Johnston et al. (2007) concluded that there was no evidence that fast growing fish had a materially higher incidence of soft flesh and gaping than slow growing fish. Several customers who purchase large quantities of fish prefers however,

to, buy fish from the north in winter and south in summer, which they mean gives them the best quality.

6.1.3 Chemical composition

In the present study, the water content was significantly affected by locality (north-south). No clear differences in chemical composition were however observed between the experimental groups (Table 5.3). Fat contents presented in the present study are in contrast to Aksnes et al. (1986), Mørkøre & Rørvik (2001) and Roth et al. (2005) who all reported a higher fat accumulation during autumn. Since localities in this experiment do not have the same background (fish history) and differs in feed suppliers, it was difficult to identify the main causes. There were however, both endogenous and exogenous factors that may have affected the proximate composition of the salmon (Shearer 1994). Protein content is determined solely by fish size (endogenous controlled) whereas fat content is effected both by endogenous and exogenous factors.

6.1.4 Future aspects

To get an overall picture of differences in salmon quality between localities, the fish has to be equal in fish history and feeding strategy. Seasonality throughout the entire production time is another aspect which should have been investigated. Quality of salmon is complex, and includes a number of factors, thus putting extra demands on the researcher accuracy and experimental design is important. Further research is needed to understand the correlation between fillet quality and mechanisms around the locality/environment, growth, feed and starving. The fish muscle lose water, which is important economically since fish is sold by weight.

6.2 Quality diploid versus triploid (Institute of Marine Research)

One of the main challenges for the aquaculture industry in Norway is salmon escapes. Today, some companies in Norway, have started to produce triploid salmon for commercial use. The use of sterile, triploid salmon for aquaculture has been considered as a possible strategy to reduce genetic interactions between escaped farmed and wild salmon. However, for triploids to be accepted in aquaculture they must perform as well as diploids. Today, triploid salmon is a "hot topic" in the Norwegian aquaculture in many ways.

Production development has been tremendous, and the pursuit of the perfect farmed salmon has been going on for a long time. It is a paradox however that the discussions around triploid salmon primarily is about escapes and not which ploidy who has the best quality.

There are many questions related to triploid salmon, for example; dangerous for nature, animal welfare aspects, good enough for commercial production, finance, quality, save the wild salmon, consumer accepted, good enough documented, and finally, is sterile salmon the solution of the future? Anyway, this thesis documented the quality differences between diploid and triploid salmon in seawater. The division of groups in this study were based on different ploidy (diploid-triploid) and growth temperature (5, 10 and 15°C). Before slaughtering, the fish had one month with acclimatization to the specific temperatures, then one month at a constant temperature, before all the fish were slaughtered in August (19-21/8) 2014.

6.2.1 Temperature and growth

The six groups of salmon sampled for analyses (D-5, T-5, D-10, T-10, D-15, T-15) with equal fish history, did differ in whole weight, gutted weight and condition factor (Table 3.3). Differences in biometrics seemed however not to have any major effects on the flesh quality. Numerous studies on growth rates of triploid fish have been published. In the present study, no main effects were however found between diploid and triploid salmon on whole fish weight. These results agrees with a previous work by Hansen (2012) who found triploid salmon (300-400g) to make it better or equally good as compared to diploids in water temperatures up to 12°C. Moreover, Galbreath & Thorgaard (1997) reported poorer growth rate in triploid salmon than diploids. However, Friars et al. (2001) indicated higher within and between family variances in growth in triploid salmon than diploids. On the other hand, other fish species like ayu (*Plecoglosus altivelis*), showed in a study by Aliah et al. (1990) that

triploids were more sensitive to environment stimuli and were also less active than diploids in seeking food. This is a positive factor for growth. In contrast, Hussain et al. (1995) found no significant differences in growth between diploid and triploid tilapia (*Oreochromis niloticus* L.). Anyway, increased cell size does not appear to confer any growth advantages to triploids (Benfey, Tillmann J 1999).

Significant differences in whole weight between temperatures were however found, where 10°C differs from 5°C and 15°C. Moreover, optimal controlled sea temperature throughout the year will therefore results in increased financial gain to the producers. In a controlled experiment reported by Hevrøy et al. (2013), diploid salmon were fed (45 days) at 13°C, 15°C, 17°C and 19°C, respectively. The experiment showed that the most efficient growth was achieved in water temperature of 13°C (Hevrøy et al. 2013). Furthermore, salmon reared at 15°C and 17°C grew efficiently the first two weeks but exhibited reduced feed intake and growth in the last part of the studiey period. These findings together, indicated that the best temperature interval, or the comfort zone for the salmon, should be somewhere around 10-13°C. Additional research is however necessary to determine the optimal temperature. Moreover, when the water temperature is below this range, the fish consume less feed because the appetite depends on sea temperature (Austreng et al. 1987). A significant difference in fish weight only after 30 days at different temperature, stressing the importance of temperature for salmon growth. A temperature rise of 1ºC could increase the speed of the reaction by 10 % (Pedersen 2014), and the Arrhenius equation may also helping to describe the correlation between temperature and growth in the salmon. In this study a linear Arrhenius plot (Figure 5.9) was expected, but not observed (stable between 10 and 15°C). However, a few reports done by Davison (1997) and Jobling et al. (1993) showed that moderate aerobic training stimulates fish growth, especially in salmonid species at water speeds between 1 and 2 body lengths s^{-1}. Anyway, this is however not the reason for differences in growth observed in this experiment, where all salmon were reared at exactly the same environment conditions.

6.2.2 Rigor index

In the present study, the reaction speed of *rigor mortis* were clearly affected by temperature. Temperature influence the rigor in bony fish (Arimoto et al. 1991; Bito 1983; Sigholt et al. 1997), which is in line with our findings. After 24 hours, significant differences between growth temperatures was found (Figure 5.5). The reaction rate increased with increased

temperature. The results of Cuttingers method in this study shows that the development into *rigor mortis* is temperature dependent.

Based on the rigor index (Figure 5.5), diploid salmon went slightly faster into rigor compared to triploids at all temperatures. After 24 hours, a significant difference between ploidy was found. The effects of ploidy in *rigor mortis* is however not clear. A week and long rigor development is a benefit for salmon slaughterhouses. Stiff salmon leads to problems during gutting and damages due to in-rigor filleting. Furthermore, ploidy seems to affect the rigor input and rigor hardness, where triploid fish had a generally lower rigor index value as compared to diploids at all experimental temperatures. The cell size is an important difference between diploid and triploid salmon (Small & Benfey 1987) which seemes to influence the processes inside the cell and the development of *rigor mortis.* There is a general agreement that the rigidity of *rigor mortis* is caused by the cross-bridge complex between actin and myosin (Currie & Wolfe 1979), and that these bindings can not be regenerated because of lack of ATP (Bendall 1951). Since actomyosin bridges are irreversible, the *rigor mortis* can not be explained by resolution of the bridges, but the breakage of cellular membranes, destruction of the osmotic potential and proteolysis (Bendall 1951). There have been some pronunciation theories of rigor mortis resolution. Widely discussed is the breakdown of muscle structure using proteolytic activity in dead tissue (Hultin 1984; Khan 1977; Tsuchiya et al. 1992), and osmotic changes in cells (Balevik 2004).

On the other hand, Slinde et al. (2003) suggest that the increase in hardness observed during rigor process is the result of water movements from inter- to intracellular space in the muscle (not actomyosin contraction). This is powered by an increase in intracellular osmolality (concentration of molecules), mainly due to the formation of lactic acid and protons from the breakdown of glycogen. This hypothesis is based on an observed change in fibre conformity to a more inflated shape (the cell border changes from a wavy to a more stretched appearance) and on the fact that shear force (resistance to cutting) decreases at the same time as hardness increases (Skjervold et al. 2001), indicating that protein structure is weakened rather than strengthened during the rigor process (Slinde et al. 2003). However, a report done by Ando et al. (1992) shows that cell membrane repture early in the rigor process due to proteolytic activity. In larger cell, it will take longer time to equalize the osmotic pressure and to fill the cell with liquids. This may explain the slower and softer *rigor mortis* observed in triploid salmon (Figure 5.6 or 5.7). The metabolic state at point of death through starvation (level of glycogen) and muscle activity (stress) can accelerate the onset of *rigor mortis*. Lactate, pH

and temperature are all factors that affect the development of *rigor mortis*. A combination of these factors and high sea temperatures will increase the rate of diffusion of water into muscular cells. This may explains why slaughterhouses sometimes in summer experience stiff salmon less than two hours *post mortem*.

6.2.3 Quality attributes

Until today, effects of ploidy on quality traits have been received less attention, and is not well documented. In the present study, both ploidy, temperature and days stored showed significant effects on the drip loss during 15 days refrigerated storage. A significant effect of temperature was moreover observed on the average drip loss during 15 days refrigerated storage, were 15°C differed from 5°C and 10°C. Temperature was the factor which had the greatest, positive affect upon drip loss. Higher growth temperature increase the drip loss. The results show however, a nominal higher drip loss in triploid salmon compared to diploids. It is likely to belive that this tendency of higher drip loss of triploid salmon is related to larger cells (Benfey, Tillmann J 1999). As far as I know, no one has investigate the drip loss in triploid salmon. It is therefore difficult to compare observed results with previous research. Increased cell volum is however interesting to discuss since larger cells may be related to increased drip loss. In the present study, a significant correlation between contraction and drip loss was observed. Looking at the results however, a tendency towards less contraction with increased temperature was observed. Moreover, the results shows a tendency towards more drip loss with increased growth temperature.

In the present study, no relationship between fillets colour (L^*, a^* and b^* values) and ploidy was found. Ploidy is earlier reported to affect the flesh colour in rainbow trout (Choubert et al. 1997), and Bjørnevik et al. (2004) reported a darker (higher L^* value) and a more reddish colour (a^* value) of triploid salmon. The flesh colour reported in this study, did not support earlier studies by (Bjørnevik et al. 2004; Choubert et al. 1997). By looking at the colour intensity, a trend caused by temperature can be observed. Fish farmed at 5°C were lightest (highest L^* value), while fish reared at 15°C were darker. The same tendency were found according to redness (a^* value), where fish reared at 5°C were less reddish as compared to those reared at 15°C. Furthermore, this correlated with the contents of astaxanthin and total carotenoids, which is in line with other studies (No & Storebakken 1991; Ytrestøyl et al. 2005).

No significant difference in fillet firmness between ploidy was observed. This was not in agreement with earlier studies by Bjørnevik et al. (2004) which reported triploids to display more gaping and softer fillets. Triploid salmon have less small muscle fibres and 23 % larger mean cross-sectional fibres area than diploids (Bjørnevik et al. 2004). There is also found indications on an inverse relationship between average fibre diameter and flesh firmness (Hurling et al. 1996). Anyway, a higher lipid content has been reported to give softer texture (Dunajski 1980; Hatae et al. 1990; Hurling et al. 1996), although no relationship between lipid content and fillet firmness was confirmed in this experiment.

In the presented experiment, a significant main effect of ploidy was observed on fat content (Figure 5.8). Regardless of temperature, the fat deposition was higher in triploid than diploid salmon. Those findings supported research done by Thorgaard & Gall (1979), who observed large fat deposits around the internal organs of triploid rainbow trout (*Salmo gairdneri*). The relationship between fat content and body weight of salmon is reported to be linear (Mørkøre & Rørvik 2001). The lipid content of the feed is moreover important for pigment deposition in the salmon muscle (Bjerkeng, B et al. 1997; Bjerkeng et al. 1999). It is well known that astaxanthin is bound to actomyosin in the salmon fillet (Henmi et al. 1987; Henmi et al. 1989). Early research have shown that triploid trout have better muscle pigment accumulation than diploid trout (Choubert & Blanc 1989). This was not in line with observed results in this study. On the other hand, high fat content in salmon fillets is unfavorable (>18%), leading to negative consumer responses (Gjedrem 1997).

The level of cathepsin increased during storage. Moreover it was a clear difference in collagenase between diploid and triploid salmon (Figure 5.8). As far as I know, no one has investigate the collagenase in triploid salmon. It is therefore difficult to compare observed results with previous research. On the other hand, it has been suggested that a muscle consisting of smaller fibres may have higher contents of collagen. During chilled storage of fish a progressive *post mortem* breakdown of fine collagenous fibrils that anchor the muscle fibres to the myocomata occur (Ando et al. 1995; Montero & Mackie 1992; Sato et al. 1991). Considering triploid salmon to have a larger mean cross-sectional muscle fiber areas, it seems to be a link between higher collagenase and areas of cross-sectional muscle fibers in triploid salmon. In practice, this means that the triploid muscle will decompose faster, which affects the quality and durability of the salmon fillet.

6.2.4 Future aspects, consequences of commercial production of triploid salmon

Triploid salmon is debated, with ethical issues, consumer skepticism, growth and food quality. In a discussion about the use of triploid salmon in salmon farming, it is important to consider the ethical and moral aspects of human management. The production of triploid salmon is not defined as a product of human GMO in accordance with Norwegian law (Klima- og miljødepartementet 1993), but it is controversial, and food supervision, animal welfare and environmental organizations seems to be negative to triploid salmon production. Today, animal welfare is important both for producers of animals and the consumers. An report by the Norwegian Research Council (Nævdal 2003 p.44) weighted the consumer skepticism about including triploids. They pointed out that it should be consumer accepted before practical use in both breeding and research continues.

Another question raised is related to the environmental impacts on the escape of triploid salmon. Salmon stop growing when it reach sexual maturity. Will triploid salmon continue to grow and create unforeseen effects in the rivers? Today, the quality traits in triploid salmon is not well documented, and have received less attention. Can triploid salmon have a different nutrient content?

Economically, salmon has been a huge success. In 2014, Norway produced over 1,2 million tons of salmon. During the last 40 years, the salmon has gone from being a small business to become Norway`s largest livestock. Today, new establishments of salmon production in northern Norway increases. Low seawater temperature slow down the fish growth, which means increased feed costs.

Today, a large Norwegian research project will clarify whether aquaculture companies can harvest similar amonts triploid salmon as diploid salmon. Norwegian aquaculture companies are participating in the largest commercial testing of sterile salmon ever. In spring 2015, the first triploid salmons are going to be slaughtered. Anyway, experiments shows that triploid salmon can make it equal good and better than diploid salmon under optimal conditions. Which direction the Norwegian aquaculture choose, will be affected by the coming years.

7. CONCLUSION

The present study demonstrated significant variation in biometrics and quality attributes between locality (north-south), season (spring-autumn), ploidy (diploid-triploid) and temperatures (5, 10 and 15°C).

Based on the results obtained in the first project, "The effect of locality (north-south) and season (spring-autumn) on quality", it is concluded that locality (north-south) and season (spring-autumn) did affect the drip loss of commercial farmed diploid salmon. Moreover, it is concluded that locality (north-south) had a clear main effect on fillet firmness, whereas no effects of season (spring-autumn) were observed. A significant main effect of locality on the WHC and water content in fillets was moreover found.

Based on the results obtained in the second project, "Quality differences between diploid and triploid salmon", it is concluded that triploid salmon has higher collagenase and fat content compared to diploids. No significant main effect of ploidy on whole body weight was observed. A significant effect of temperature was however observed, were 10°C differed from 5°C and 15°C. Moreover, it is concluded that growth temperature affect rigor mortis, but the effects of ploidy is however not clear.

The drip loss in triploid salmon were found to be nominally higher compared with diploids. A significant effect of growth temperature was however observed on the drip loss, were 15°C differed from 5°C and 10°C. No main effects of ploidy was found on fillet firmness, but a main effect of temperature was observed.

Overall, no effects of ploidy or temperature on translucence (L^*), redness (a^*) and yellowness (b^*) was found. A significant main effect of temperature was however observed on contents of total carotenoids, astaxanthin, lutein, all-trans astaxanthin, 9-cis astaxanthin and 13-cis astaxanthin. On the other hand, no effects of ploidy was found on contents of total carotenoids, astaxanthin, lutein, all-trans astaxanthin, 9-cis astaxanthin and 13-cis astaxanthin.

In the present study, the triploid salmon seemed however to be remarkably similar to diploids when the fish had the same background. Finally, the consequences and aspects around commercial production of triploid salmon are still not well documented, which may lead to more research about the presented topic.

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APPENDIX 1: Fish history Hjelmeland (spring)

License No.	11964
Cage No.	BBL - 0008
Smolt supplier	BXB-Fister
Type of smolt	Q3
Year class	2012
Strain	Mowi

◄ Q1 Q2 = Spring, Q3 Q4 = Autumn

Harvest

Harvest period	
Average weight (g)	4848,49
Last day of feeding	2014-05-15

Fat and Colour Analysis

Date of last analysis	2014-04-23
Colour, LA ROCHE	0,0
Colour, SALMOFAN	26,3
Colour mg/Kg	6,7
%Fat	17,8

Feed

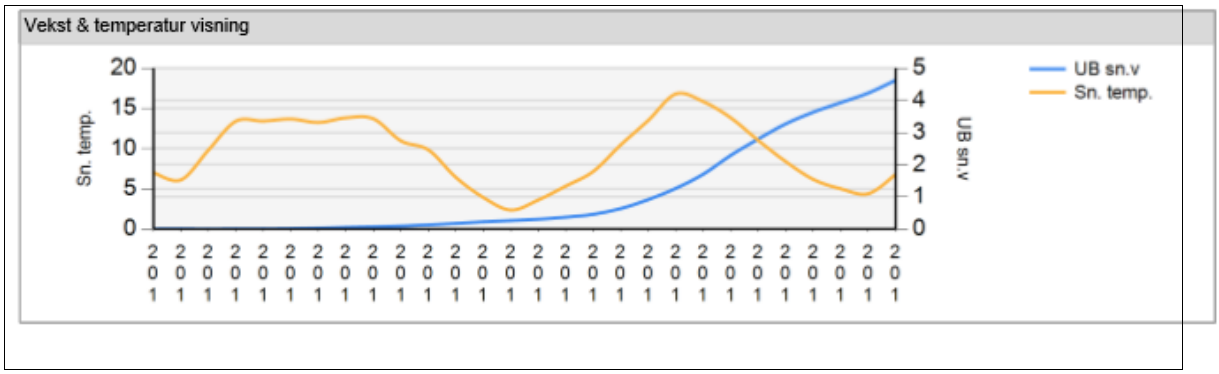
Feed supplier	Skretting
Type of feed	Optiline Premium 2500 - 50A 9mm
Type of pigment	ASTA
Amount of Pigment (mg/Kg)	30 - 70 mg/kg
% Fat	30 - 40 %
% Protein	40 - 45 %

Vaccinations

Vaccine	Vaccination Date
Norvax Compact PD	13.09.2012
Pentium Forte Plus	26.09.2012

Treatments

Treatment	Start of Treatment	Released from quarantine
Finquel 100% (M)	2014-04-08	2014-04-30
Alpha Max 10 mg / ml (M)	2014-02-17	2014-02-17
Salmosan (M)	2014-01-10	2014-01-12
Slice 10 mg/kg (M)	2013-03-22	2013-05-22
Releeze vet. 0,6g/kg (M)	2012-12-29	2013-01-13



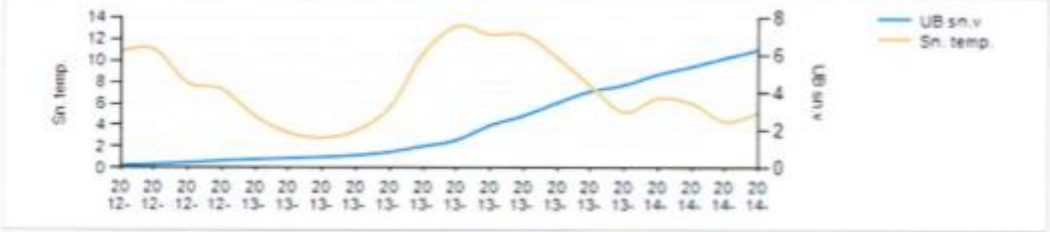
APPENDIX 2: Fish history Herøy (spring)

Produktinformasjon	
Merd nr	BIJ - 0001
Fiskegruppe	NSAGI112AM60-
Årgang	2012
Art	Atlantic Salmon
Opphav	AquaGen
Lokalitetsinformasjon	
Lokalitetsnavn	BIJ-Jektvika II
Kons-nr:	
Lokalitetsnr.	10885
Vannveg (Fjord)	
Stamfiskinformasjon	
Stamfiskanlegg	ST-Stamfisk
Stamfisklisens	
Strykedato	
Leveringsdato	

Settefiskanleggsinformasjon	
Settefiskanlegg	BXY-Haugvik
Konsesjon	
Klekkedato	2011-12-03
Smoltinformasjon	
Settefiskanlegg	BXX-Glomfjord
Smolt-lisens	
Utsett-vekt sjø	96,3
Overført til sjø	2012-09-12
Brønnbåt	Romaster
Vaksine 1 Type	Alphaject Micro 6
Vaksine 1 Dato	2012-07-19

Produksjon	
Antall	36 218
Sn.v (g)	6 523
Biomasse	236 250
Tilvekst (kg)	523 354
Øk FF	1,24
Bio FF	1,18
Siste fôringsdato	2014-05-14
Temperatur	
Min	1,10
Snitt	7,57
Maks	16,20
Tetthet	
Min	0,52
Snitt	10,46
Maks	23,62

Vekst & temperatur visning



Fôr opplysning (smolt)

Fôrtype	Leverandør	Første dato i bruk	Asta	Vekt	% av total
Total					

Fôr opplysning (lokalitet)

Fôrtype	Leverandør	Første dato i bruk	Asta	Vekt	% av total
SPIRIT SUPREME 75 50A 3,0mm	Skretting	2012-09-12	50,0	5 893	0,909%
CPK 75 Q SS40	Biomar	2012-10-28	0,0	9 821	1,514%
CPK 200 Q	Biomar	2012-12-01	0,0	15 534	2,395%
CPK 200 SS 20 Q	Biomar	2013-01-31	20,0	8 534	1,316%
CPK 500 20 A	Biomar	2013-03-16	20,0	8 638	1,332%
CPK 500 40A	Biomar	2013-04-01	40,0	2 450	0,378%
CPK 500 40 A Q	Biomar	2013-04-29	40,0	55 418	8,546%
Energy X 500Q 40A 7mm	Biomar	2013-07-10	40,0	12 553	1,936%
Energy 1000 40A 10mm	Biomar	2013-07-26	40,0	97 563	15,045%
Energy X 1000 40A 10mm	Biomar	2013-08-21	40,0	28 730	4,43%
Energy 2500 40A 10mm	Biomar	2013-09-23	40,0	158 634	24,462%
Energy 1000Q 40A 10mm	Biomar	2013-11-03	40,0	6 096	0,94%
Energy X 2500 40A 10mm	Biomar	2013-11-27	40,0	53 283	8,216%
Energy X 2500 20A 10mm	Biomar	2013-12-20	20,0	78 963	12,176%
Focus Winter 2500 20mg 9mm	Biomar	2013-12-31	20,0	33 393	5,149%
Focus Winter 2500 20A 10mm	Biomar	2014-01-20	20,0	5 040	0,777%
Energy 2500 20A 10mm	Biomar	2014-02-11	20,0	2 793	0,431%
Focus lice 2000 ss40	Biomar	2014-03-29	40,0	24 744	3,816%
Focus Lice 2500 40A 9mm	Biomar	2014-04-14	40,0	38 298	5,906%
Focus Winter 2500 40 mg	Biomar	2014-04-27	40,0	2 117	0,326%
Total			34,3	648 496	

Kvalitetsinformasjon

APPENDIX 3: Fish history Herøy (autumn)

Product certificate

HarvestId 123845

Product Information

Fish group:	NSMGI412AM615NStb131	Species:	Atlantic Salmon
Generation:	2013	Egg strain:	Mowi
Transfer period	Q3 : Jul-Sep	Smolt type	0+

Marine production site

Farm name:	Torgerhaugen	Cage no:	0004
Site code:	BRK	Max Density:	25 kg/m3
Location no:	30177		

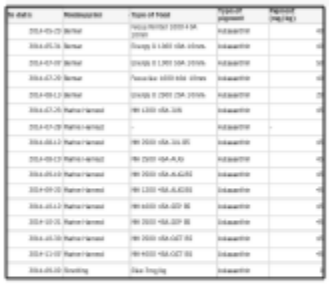
History of production

Egg supplier:	ST Stamfisk	Waiting cage:	
Smolt supplier:	Haugvik, Glomfjord	Kill date:	2014-11-14
Hatching date:	2012-07-01, 2012-07-04	Packing date:	2014-11-14
S.water entrance:	2013-07-29	Packing station	Herøy
Last feeding date		Stunning method	Mechanical Stun/Bleed
Sea temperature	8		

Fat and colour - product

Date of analysis:	2014-10-28	Fat (%):	18,87
Color (Salmofan):	25,80	Condition fact.::	1,25
Pigment (mg/kg):	5,82		

Feed last 6 months

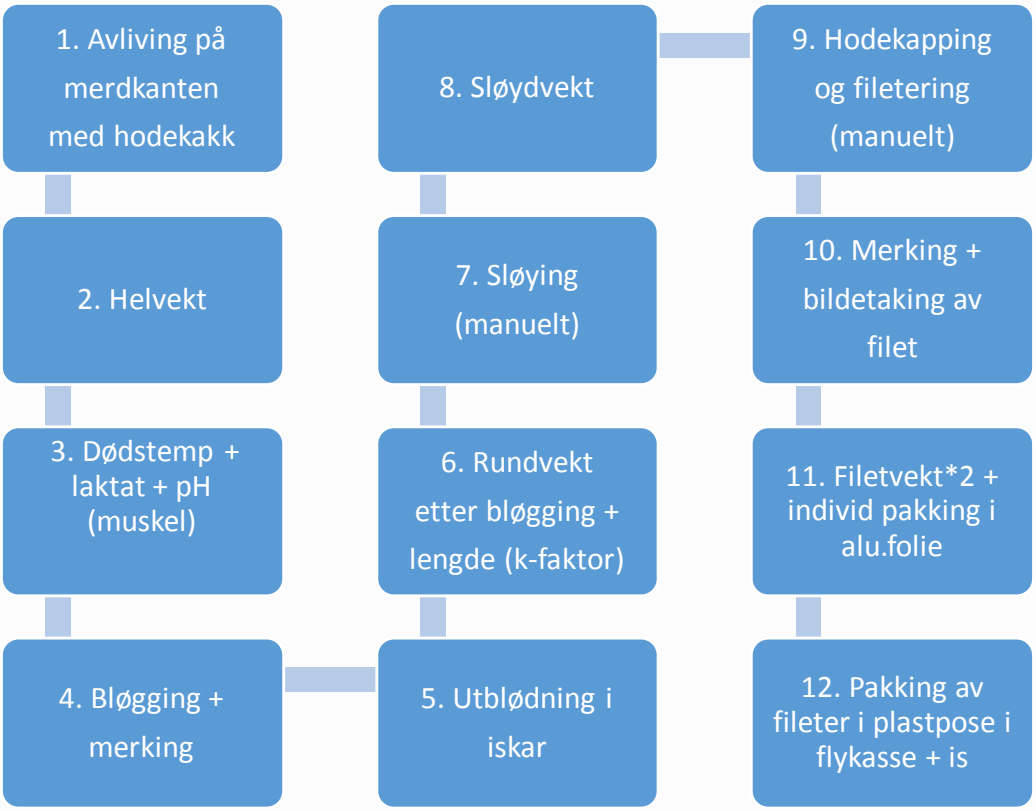


Last treatments

Product	Last day of treatment	Withdrawal time	Time from last treatmen to harvest	Avg temp last treatment to harvest	First allowed harvest date
Finquel 100%	2014-10-13	21 days	32 days	9	2014-11-03
Hydrogen Perox...	2014-06-14	0 none	153 days	10	2014-06-14
Slice 7 mg/kg 1...	2014-09-02	175 daydegrees	717 ddg	10	2014-09-20

APPENDIX 4: Locality and season experimental setups

Flowchart tasks sampling at slaughter facility Hjelmeland and Herøy in (May and November) 2014.



APPENDIX 5: Kronikk til Norsk Fiskeoppdrett juni 2015

Hvor mye gener tror du det er i laks?

Uten å fornærme hverken forskere innen genetikk eller biologer generelt, må vi kunne si at vi faktisk ikke vet helt sikkert hvor mange gener den atlantiske laksen har. Et dypdykk inn i evolusjonen til den atlantiske laksen gjennom to biologisk rettede masteroppgaver på Norges – miljø og biovitenskapelige universitet (NMBU) har vi sett nærmere på laksens evolusjonære historie. Oppgavenes formål var å se på energimetabolisme, samt kvalitetsforskjeller mellom diploid og triploid laks basert på de vedtatte «grønne» A konsesjonene i Troms og Finnmark, hvor spørsmålet om økt antall kromosomer kan ha en betydning. Vi skal derfor prøve å gi svar på spørsmål som; Hva er triploid laks, hvordan blir den triploid, hvorfor ønsker norske oppdrettsselskaper å triploidisere og problemer knyttet til dette.

Nå tenker du sikkert at dette er enda en søvnig kronikk skrevet av to hovedfagsstudenter som har levd inne i sin egen boble så alt for lenge. Kanskje har du aldeles rett i det, men hvis du altså er en av de ytterst få som har alle svarene på det genetiske dilemmaet norsk akvakultur har viklet seg inn i, kan du droppe å lese her. For oss andre kan en kort historie om nesten alt av de ørsmå endringene evolusjonen og det genetiske mangfoldet i laksen vi nå vet, være en interessant refleksjon, nå som «de grønne» konsesjonene har blitt en realitet.

Vi ble første gang introdusert for begrepet triploid atlantisk laks høsten 2013 da vi fant felles hovedveileder til avsluttende masteroppgaver ved Norges miljø- og biovitenskapelige Universitet (NMBU), Institutt for kjemi, bioteknologi og matvitenskap. Triploidisering av atlantisk laks er blitt «hot» igjen på bakgrunn av de såkalte «grønne A konsesjonene» i Nord-Norge. I 12 av 20 konsesjoner baseres det på bruk av såkalt steril laks. Med god hjelp fra fagfolk utenfor universitetet ble det formet to masteroppgaver som skulle undersøke kvalitetsforskjeller og energimetabolisme hos diploid og triploid atlantisk laks fra samme avlsstamme.

Hva er triploid atlantisk laks (*Salmo salar* L.)?

For hva er egentlig en diploid atlantisk laks og mer spesielt; hva er en triploid atlantisk laks? Ingen av oss hadde verken lest oss opp på laks, fisket villaks i elv, eller hatt interesser i oppdrettsnæringen. Og det eneste vi hadde hørt om kromosomer fra før, var mennesker med

feil kromosomoppsetning får downs syndrom, en triploidisering på kromosomarm 21. Har triploid laks samme nedsatt funksjonsevne eller gir hel kromosomsettøkning andre ulemper/fordeler? Vi ble nysgjerrige.

Vi tok fatt på arbeidsoppgavene i den tro at vi nå gjør jobben, får resultatene og leverer oppgavene. Slik skulle det overhode ikke bli takket være energiske veiledere som stilte to nye spørsmål, da vi spurte om svaret på et. Før vi i det hele tatt hadde fått noen som helst resultater tok nysgjerrigheten overhånd, noe som ledet oss helt ned til evolusjon av slekten *Salmo* i epoken Miocen for cirka 20 millioner år siden.

Polyploiditet er hos dyr svært sjeldent, men noen spesielle arter har skapt seg denne fordelen. Dette gjelder f.eks. øgler, gullfisk, frosker og ikke minst flere arter i laksefamilien. Blant blomsterplanter og planter som gir oss mat er derimot polyploiditet svært utbredt. Mange velkjente ville og dyrkede planter er faktisk polyploide. Kanskje løvetannens over hundre kromosomsett har betydning for dens gode tilpasningsevne? Den er overalt, og på sitt beste akkurat nå. I grunnskolen lærer man at mennesker er diploide individer med 46 kromosomer (2*23 kromosomer), altså 2 kopier av hvert kromosom (unntatt kjønnsceller som er haploide). Så enkelt er det ikke for den atlantiske laksen. Den enkleste og oftest brukte forklaringen er at diploid laks (vanlig oppdrettslaks) har to kopier av hvert kromosom (2*29=58 kromosomer) og triploid laks har tre kopier av hvert kromosom. Det at atlantisk laks i dag regnes som diploid er en forenklet virkelighet, noe som vi skal utdype senere.

Tabell 1 gir en oversikt over ploiditeter og ordforklaringer som vil kunne hjelpe deg å forstå det du nå skal få lese. Kromosomene er genmaterialet til hver enkelt celle i en organisme. Antall kromosomoppsetninger i en celle kan variere fra art til art. Dette kalles ploiditet.

Ploiditet	Antall kromosomoppsetning	Eksempel
Hapolid	n=1	Kjønnsceller
Diploid	n=2	Pattedyrceller
Triploid	n=3	Diverse frukt
Tetraploid	n=4	Potet, bomull
Pentaploid	n=5	Tresorter
Heksaploid	n=6	Brødhvete
Octaploid	n=8	Jordbær
Decaploid	n=10	Acipenser (Stør familien)
Dodecaploid	n=12	Noen typer frosker
Polyploid	n=>2	-
Autotriploid	Diploid og haploid formering (Zygoten n=3)	-
Autotetraploid	Dobler egne (like) kromosomer n=2x2	-
Pseudotetraploid	"Uekte", "falsk" tetraploid fisk der kromosomantallet varierer på individnivå.	

Laksens evolusjon

Stamfaren til de laksefiskene som i dag lever både i merd og i elv var tilsynelatende tetraploide (4 kopier av hvert kromsom). For mellom 65-95 millioner år siden doblet fisk i *Salmonidae* familien sine to kromosomsett og ble autotetraploid. Antageligvis skjedde dette fra naturens side for at arten skulle klare å overleve. Ved å doble like kromosom øker sjansen for at kromosomene som fisken behøver for å tilpasse seg uttrykkes og kombinasjonsmulighetene naturlig nok blir mangedoblet. Dagens atlantiske laks anslåes å være 0-15% tetraploid («feilduplisert»).

Som et resultat av dette har laks flere kromosomarmer og dobbel så mye deoksyribonukleinsyre (DNA) enn andre nært beslektede familier. Dette gjør *Salmonidae* slekten mer evolusjonært fleksibel enn andre arter, og derfor kan kromosomantallet i Atlantisk laks også variere uavhengig av antall kromosom kopieringer. I dag refereres det til 29 kromosom i hvert kromosomsett for den diploide nordeuropeiske laksen, altså 58 kromosom totalt. Andre kilder opererer med at den diploide atlantisk laksen i dag har mellom 54-60 kromosom. Antagelig har triploid atlantisk laks 81-90 kromosomer i cellen. Hvor mange av «de ekstra» kromosomene som er i funksjon i den triploide laksen vet vi enda ikke. Triploid laks inneholder i tillegg til økt kromosomoppsetning også 1,3-1,7 ganger mer DNA i cellekjernen kalt nukleært DNA. Stor spredning i mengde nukleært DNA tyder på at den pseudotetraploide variasjonen også den dag i dag må være stor.

Laksen har et komplisert arvemateriale. Deler av genomet i laks er fortsatt tetraploid, hvor det i dag skjer en konstant rediploidisering for å komme ned igjen til diploid status. Om vi får rene diploide individ i neste generasjon eller om 300 nye generasjoner vet vi ingenting om. Det at laks har det desidert største genomet og med en ekstra genomduplisering bak seg, byr det på mange utfordringer når genetikerne skal kartlegge arvemateriale.

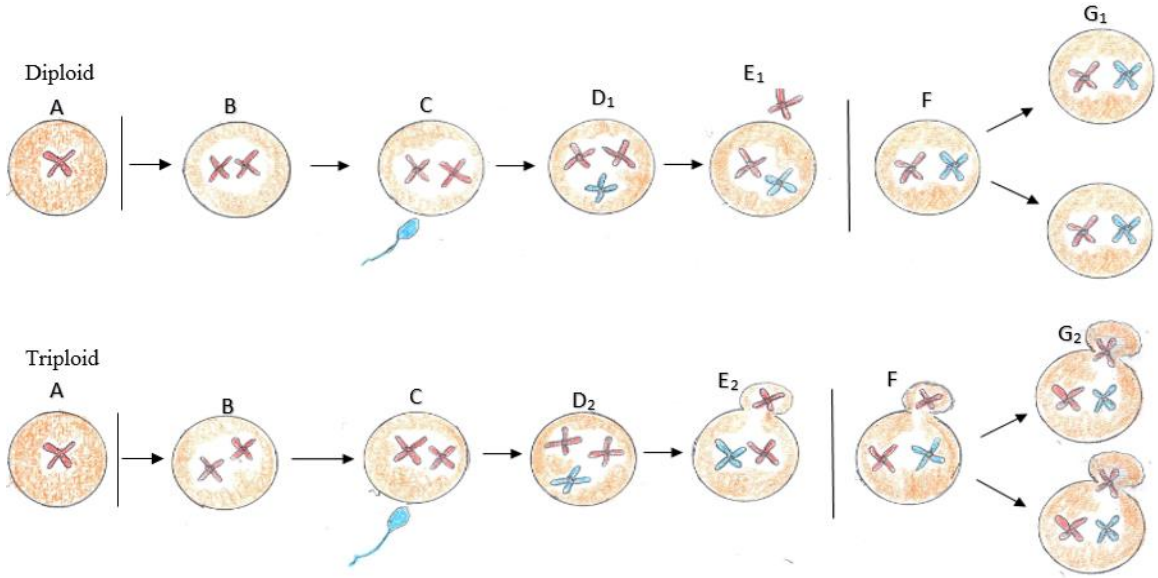
Det vi vet helt sikkert er at dagens oppdrettslaks er 11.generasjon mot den ville atlantiske laksens som er rundt 250 000 generasjoner. Hvor mye har vi mennesker faktisk klart å røre rundt med genene til laksen? Uten å ta noe som helst parti i den evigvarende krangelen mellom interessene fra oppdrett og interessene fra villaks, så er det merkelig at naturens egne genetiske antenner ikke er mere diskutert, og ikke minst satt i et evolusjonært perspektiv.

Hvordan blir laks triploid?

Nå som vi har lagt bak oss den evolusjonære delen for å kunne forstå forskjellen mellom diploid og triploid laks er det på tide å forklare hvordan 1 haploid celle + 1 haploid celle blir til et individ på 3 kromosomsett basert på trykkbehandling. Hvordan naturen klarte dette for millioner av år siden kan vi veldig lite om. Antagelig er naturens «lek» med kromosomer mer utbredt enn vi kan forestille oss, selv i dag. Nylig har laksens genom blitt sekvensert, som gjør selektering av egenskaper enda mer spesifikt og vil åpne nye dører i avlsarbeidet.

Vi mennesker har gjennom prøving og feiling funnet vår måte å triploidisere. I fisk har vi hatt en metode som er beskrevet så langt tilbake som år 1943. Med dagens teknologi blir det brukt konstant trykkbehandling for å oppnå triploide individ. Dette skjer ved 655 bar trykk fra de befruktede lakseeggene er 300-350 minuttgrader gamle.

Figur 1 forklarer prosessen fra første modningsdeling hos hunnlaksens egg (meiosen), frem til første kromosomkopiering (mitose) og celledeling hos diploid og triploid laks. Figuren er forenklet med «X og X» i egget som symboliserer kromosomsett, og baserer seg på andre tegninger og fagrelaterte artikler. Figuren er beskrevet i detalj til tross for at dette ikke er helt forstått enda.



A) Hunnlaksens egg sammen med et kromosomsett før meiosen inntreffer. I meiosen også kalt reduksjonsdeling deler kromosomet seg til to kromosom i hunnlaksens egg der hvert kromosomsett deler antall kromosom mellom seg (f.eks 58/2=29).

B) Hunnlaksens kjønnscelle. Ubefruktet egg med to kromosomsett etter modningsdeling (meiose) Det ene kromosomsett blir ikke en del av det nye individet etter befruktning. Denne hendelsen har betydning for genetisk variasjon i populasjonen fra mor-siden.
C) Hannlaksens kjønnscelle på vei inn i egget. Egget blir befruktet.
D_{1+2}) Lakseegget er befruktet og det er tre kromosomsett inne i egget. Det er tre kromosomsett inne i cellen for øyeblikket.
E_1) Et kromosomsett fra mor blir selektert bort for å oppnå balansert antall kromosomer i egget og av den grunn blir dyttet ut. Cellen blir diploid. E_2) Et kromosomsett fra mor blir selektert bort for å oppnå balansert antall kromosom i egget og blir av den grunn forsøkt dyttet ut av egget. Trykkbehandlingen hindrer det ene morkromosomet å forlate egget. Cellen forblir triploid.
F) Meitose stadiet. Celledeling med kopiering av likt antall kromosomantall.
G_1) Første celledeling er foretatt. En diploid laks er skapt. G_2) Første celledeling er foretatt. En triploid laks er skapt og cellen forblir cirka 30% større enn en diploid celle.

Hvorfor er det ønskelig for norske oppdrettsselskaper å triploidisere laks?
Det er to svar på akkurat det spørsmålet. For det første er det viktig for norsk akvakultur at genmaterialet til et godt innarbeidet avlsprogram ikke blir solgt som fertiliserte egg med kjøpers mulighet for videre avlsfremgang (selv om vi allerede har solgt arvegullet fra Ås til Erich Wesjohann Group GmbH (EW Group) i Tyskland).

Det andre svaret og det mest diskuterte spørsmålet i dagens oppdrettsnæring; rømt oppdrettsfisk sin mulighet til å krysse seg inn i villaksstammer. På den andre siden må vi være klar over at vi også kan få problemer med triploide individer på rømmen i norske farvann.

Problemer knyttet til produksjon av triploid fisk
Bruk av triploid fisk har vært diskutert flere ganger tidligere i norsk fiskeoppdrett, men pga. utfordringer av ulik karakter har det ikke vært aktuelt å innføre dette i Norge, selv om andre land i verden produserer triploid fisk. Takket være godt forskningsarbeid fra blant annet forskere fra Havforskningsinstituttet, har det resultert i ny kunnskap som gjør at det nå blir kommersiell oppdrett på denne «nye» arten. *(Flere tar til ordet for at oppdrettet triploid laks må karakteriseres som egen art).*

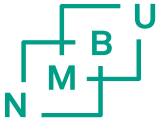
Uavhengig av dette har vi flere ubesvarte spørsmål til benyttelse av triploid laks knyttet opp mot; velferd, vaksinasjon, sykdom, adferd og forbrukeraspekt. Det retoriske spørsmålet alle kritikere alltid stiller: Vet vi nok om triploid laks og er det godt nok dokumentert? Ofte blir alle svar skyldig.

Uavhengig av dette ha vi satt spørsmåltegn om vi kjenner hele det genetiske aspektet triploid laks fører med seg. Atlantisk laks er en evolusjonært «sleip» fisk. Den evolusjonære tidslinjen til laksefamilien kan umulig være skapt på rene tilfeldigheter. Den har nok tilpasset seg for å kunne overleve. Satt i perspektiv er gjennomsnittlig levealder på en art på vår blå planet, 4 millioner år.

En konsekvens av økt mengde kromosom kan være at vi får større kvalitetsmessig spredning (genetisk variasjon) på den totale biomassen i norsk laks, der det faktisk kan være små forskjeller i avlslinjene. Forsøk viser at triploid laks kan gjøre det like bra og bedre enn diploid laks under optimale forhold. Hvilken retning norsk havbruk velger vil tiden vise, hvor flere instanser skal ha et ord med i laget. Uansett, økonomien får normalt det siste ordet.

Vi lever i herrens år 2015, og vi vet ikke helt sikkert hvor mange gener den atlantiske laksen faktisk har.

Skrevet av Even og Pål Rune



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