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# **Seasonal variation in fillet quality of Atlantic salmon (*Salmo salar*)**

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Master of science in Aquaculture  
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Master`s Thesis in Aquaculture  
(30 Credits)

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## **Abstract**

The Aquaculture industry are providing fresh seafood all year around, and the demand for safe and nutritious, quality food is increasing. Atlantic salmon are released into sea cages where they are reared until slaughter. During the sea water phase, Atlantic salmon are exposed to changes in the sea temperature and day length that are caused by season. The seasonal differences are therefore of importance to farmers as seasonally challenges in Atlantic salmon will occur when reared at sea.

In this experiment there was sent out surveys to Atlantic salmon farms reared in sea cages in Chile, Tasmania and Norway, where Norway was geographically divided in South, Central and North. There were seasonal variations regarding the fillet fat content, gaping and colour. Autumn was a problematic season for fillet colour and gaping, while autumn and summer showed a higher problem reading to fat content than winter and spring. There was not indicated any seasonal variation for melanin spots, which were high throughout the year.

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# 1.0 Introduction

Norway is the second biggest exporter of seafood followed by China, while for salmon production, Norway is the largest producer followed by Chile (FAO, 2016b). The world’s population is rapidly expanding and 50 percent more food will be required in 2050 to sustain the current quality of life (Diana et al., 2013). In 2013 - 2015, the global fish production was 56 percent from captured fisheries, while 44 percent from aquaculture (fig.1.1). However, the fish production has been estimated to be higher for aquaculture than wild catch by 2025. The same estimation has been done for human consumption, where there is predicted that more fish from aquaculture will be consumed than wild catch in the future (FAO, 2016b).

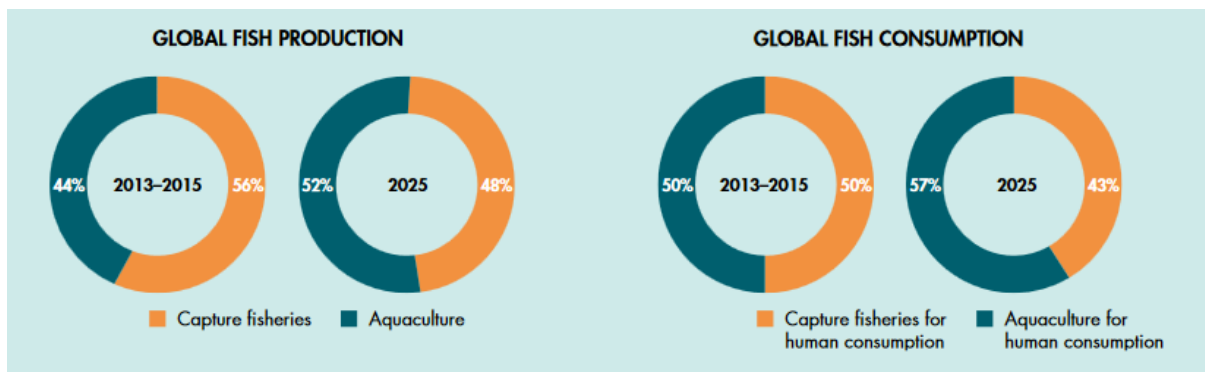
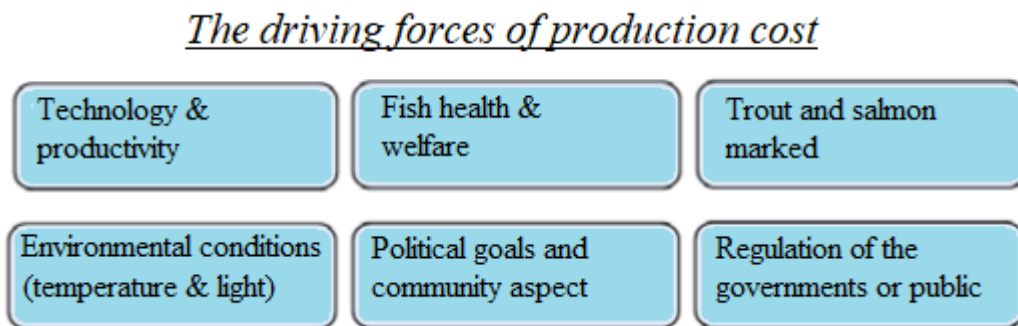


Figure 1.1 The global production and consumption of fish in 2013 – 2015 and the prediction of global fish production and consumption in 2025. Retrieved from OECD and FAO.

There are different driving forces affecting the production cost of Atlantic salmon (*Salmo salar*), where the production cost of salmon has increased in recent years. Environmental conditions (light and sea temperature) have been mentioned as one of the driving forces that affects the production cost, where the sea temperature and daylength is changed according to season (fig. 1.2) (Iversen & Hermansen, 2016). The sea temperature changes throughout the year and has an impact on fish growth. Lower sea temperature limits growth, while higher sea temperatures increase the growth rate (Austreng, Storebakken, & Åsgård, 1987).

Photoperiod and light intensity is another environmental parameter that affects the growth rate (Rørvik et al., 2018), reproduction (Taranger et al., 2010) and smoltification process (Saunders & Henderson, 1970). The growth and reproduction cycle can influence the quality of Atlantic salmon (Hansen, Stefansson, & Taranger, 1992; Rørvik et al., 2018; Taranger et al., 2010). Furthermore, fish health and welfare have been listed as another major reason for

the increased production cost in the aquaculture industry. If the environmental conditions are sub optimal, the fish's health and well-being can affect the flesh quality. It is thus clear that season is important for the fillet quality of Atlantic salmon (Iversen & Hermansen, 2016).



*Figure 1.2. The driving forces affecting the production cost of the salmonid aquaculture industry. Environmental condition is here listed as one of the major driving forces (Iversen & Hermansen, 2016).*

There are four seasons in Tasmania, Chile and Norway; winter, spring, summer and autumn. The environmental conditions vary considerably between Chile, Tasmania and along the Norwegian coast, while sea temperature and photoperiod show large seasonal variations (Skaugen & Tveito, 2004). Salmon is slaughtered and supplied to the market throughout the year. It is therefore important for the industry to know about the temporal variations in the product quality that are associated with seasons (Towers, 2010).

#### The objective of the study:

There has not yet been completed any research of farmed Atlantic salmon comparing the seasonal variation in fillet quality in Norway, Chile and Tasmania. The aim of this study was therefore to compare the quality issues of farmed Atlantic salmon obtained from sea cages in Chile, Norway and Tasmania. The quality factors targeted in the Atlantic salmon fillet were fat content, melanin spots, colour and gaping. The hypothesis is thus;

- There are seasonal variations amongst the quality issues; fillet fat content, colour, melanin spots and gaping when comparing the seasons in Chile, Tasmania and Norway.

## **2.0 Literature review**

The literature review is divided in three parts. Part A will give an overview of the Atlantic salmon marked in Chile, Norway and Tasmania. After this the different climate and environmental factors in these countries will be described. Part B gives an overview of the growth pattern of Atlantic salmon, while Part C gives a knowledge status of the fillet quality issues; fat content, colour, melanin spots and gaping.

### **Literature review – Part A**

Chile, Tasmania and Norway are located on three different continents, and have great differences in day length and sea temperature. Day length and sea temperature are controlled by season, which can greatly affect the quality of the Atlantic salmon fillet.

### **2.1 The Atlantic salmon marked in Chile, Norway, Tasmania**

#### *2.1.1 The Chilean salmon production*

The Chilean aquaculture industry started in the middle of 1980 (Iversen & Hermansen, 2016) where the salmonid species dominate the Chilean aquaculture sector, both in harvest volume and export value (FAO, 2017a).

The industry had production growth and decreased production cost until 2007/2008, but in 2008-2009 Chile experienced a substantial production fall due to a disease outbreak of infectious salmon anemia (ISA) that resulted in a reduction in the Atlantic salmon production from almost 400.000 tons to 122.000 tons. The situation came under control, and in 2014 Chile produced approximately 600.000 tonnes of Atlantic salmon. In 2015 – 2016, the production dropped due to algae growth and resulted in an increased amount of mortality (fig.2.1) (Iversen & Hermansen, 2016).



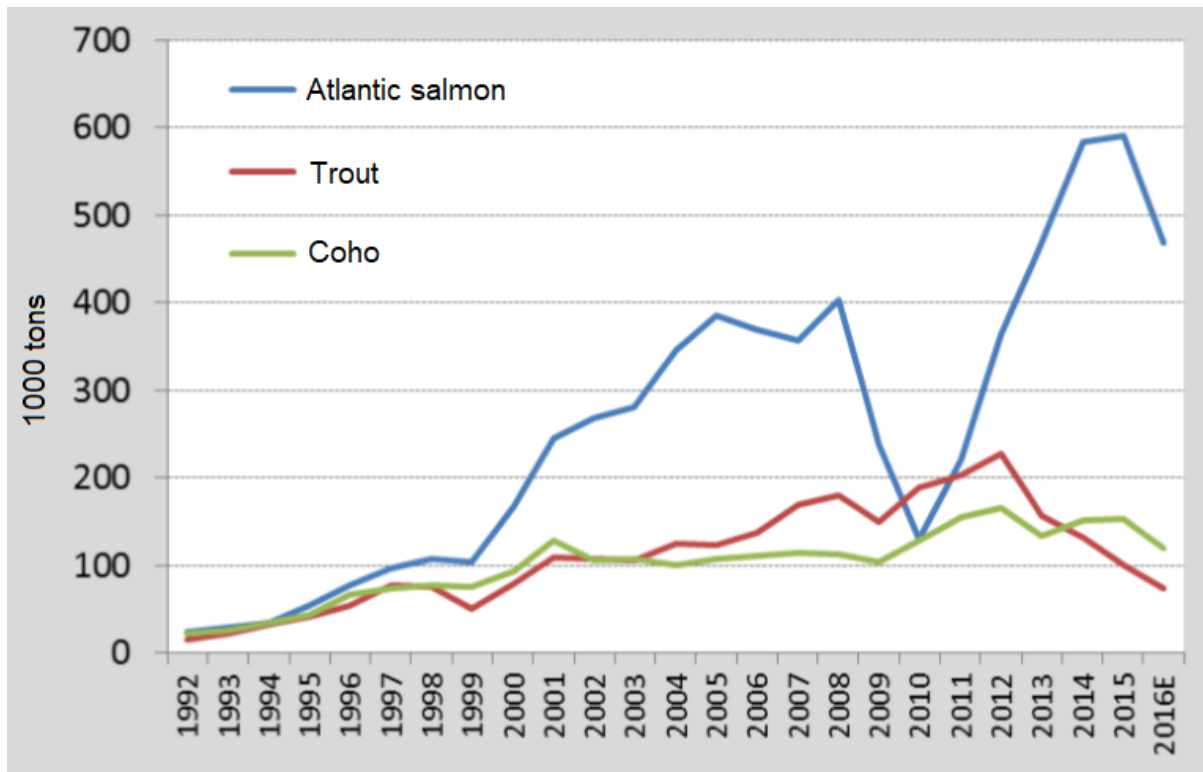


Figure 2.1. The production of Atlantic salmon, trout and Coho salmon in Chile from 1992 to 2016 (Iversen & Hermansen, 2016).

### Marked and export

Chile export salmon and trout mainly to Japan, USA, Brazil and Russia (fig.2.2), where Japan buy most of the trout. Fish is exported to USA by plain and is therefore expensive. Almost 70 percent of the Chilean salmon is processed before export, where most of the fillets are sold as frozen fillets. The cost of transporting the salmon is a challenge for Chile, as the cost for airfare is higher from Chile to Asia compared to Norway to Asia. Furthermore, the Chilean salmon marked have had trouble obtaining demand due to a series of disease outbreaks and algae problems (Elizondo-Patrone, Hernández, Yannicelli, Olsen, & Molina, 2015; Mardones, Martinez-Lopez, Valdes-Donoso, Carpenter, & Perez, 2014). The customers of the Chilean salmon marked have therefore turned to elsewhere for their supply of seafood (FAO, 2016a; Hovland, 2017). Another challenge is the quality of the Chilean salmon, where salmon produced in Chile is more often declassified from being superior than the Norwegian salmon (Iversen & Hermansen, 2016).

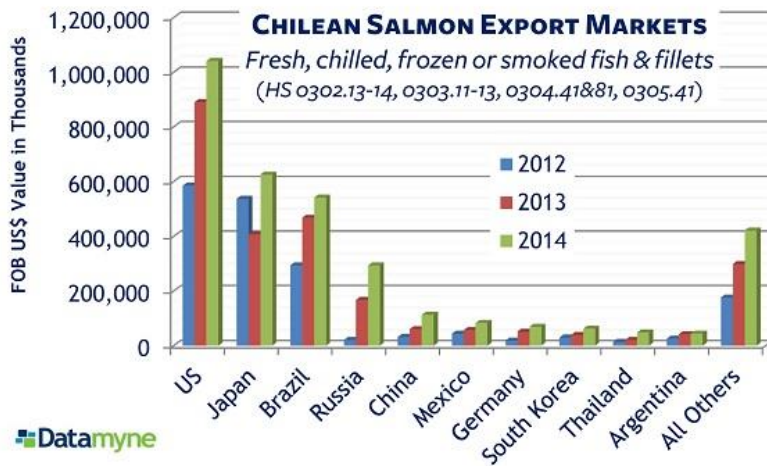


Figure 2.2. The Chilean salmon export market from 2012 to 2014 (Iversen & Hermansen, 2016).

## Problems

The Chilean aquaculture sector have been expected to meet several internal and external problems, where the aquaculture sector have to show willingness to grow and develop under sustainable conditions (FAO, 2017a). The regulations of the aquaculture sector in Chile have been poor resulting in multiple problems for Chilean farms, where the country's high allowance of biomass in cages and the massive use of antibiotics have been brought to attention (Tipping, 2017). Besides several disease outbreaks, Chile have experienced harmful algae blooms (HAB) (Anderson & Rensel, 2017) that occurred twice in 2016, one in Los Lagos where 40 000 tonnes of salmon got killed. This equalled 12 percent of Chile's annual production (abcNews, 2016).

### *2.1.2 The Norwegian salmon production*

The Norwegian salmon aquaculture industry started in 1970 and in 2017 the production accounted for more than 80 percent of the Norwegian aquaculture. According to The Food and Agriculture Organization of the United Nations (FAO), Norway is the largest producers of Atlantic salmon in the world (FAO, 2017b).

The production of Atlantic salmon in Norway increased exponentially from 1980 and stabilized at around 1.2 million tons in 2015 (fig. 2.3).

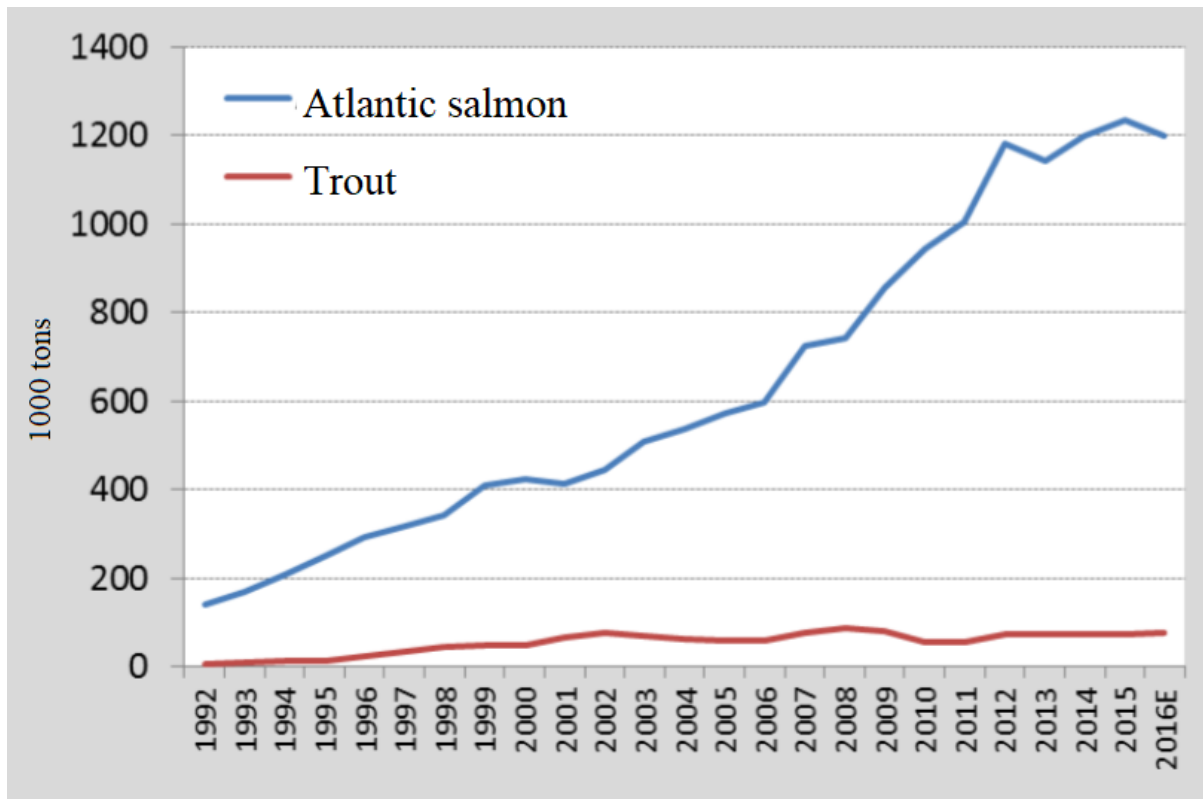


Figure 2.3. Aquaculture production of Atlantic salmon, rainbow trout and other species in Norway from the period 1980 – 2016 (Iversen & Hermansen, 2016)

### Marked and export

For the Norwegian Aquaculture industry the EU is the most important marked (FAO, 2017b). Most of the Atlantic salmon is exported to Polen, France, Denmark and the USA (fig. 2.4) and in 2017, Norway exported 736.000 tons of Atlantic salmon to the European Union (EU) at a cost off 45.7 billion NOK. Both 2016 and 2017 was measured as record years for the export of farmed Atlantic salmon. (Aandahl, 2018).

The Norwegian krone has remained weak against the Euro and the US dollar. This has resulted in Atlantic salmon being cheaper than before to import from Norway (FAO, 2016a; Hovland, 2017). Seafood analyst Aandahl in Norway`s sjømatråd states that the main reason why 2016 and 2017 were extraordinary years were because of the stable EU market. In 2017, Norway exported 49 000 tons of Atlantic salmon valued 4.4 billion to the USA. That is an increase of 25 percent Atlantic salmon in export volume compared to the export volume in 2016. In addition, there was an increase of Atlantic salmon to Asia in 2017, where the largest import country was Japan (Aandahl, 2018).

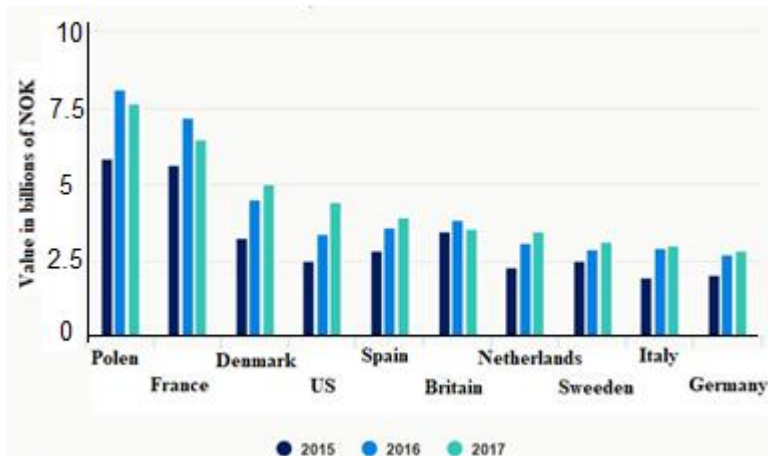


Figure 2.4. The 10 biggest export markets for the Norwegian salmon industry in 2015, 2016 and 2017 (Iversen & Hermansen, 2016).

## Problems

Sea lice have been a huge problem facing the Norwegian fish farming industry. In addition to sea lice, the development of viral diseases like pancreatic disease (PD) and infectious salmon anemi (ISA) have increased significantly. There have also been a substantial increase of the bacterial disease yersiniosis in Central of Norway (Hjeltnes, Jensen, Bornø, Haukaas, & Walde, 2018).

### *2.1.3 The Tasmanian salmon production*

Salmon farming in Tasmania started in 1984 with importation of eggs from Canada (Governments, 2016). Tasmania represents approximately 60 percent of the nation's total aquaculture production (fig. 2.5) (Wynn, Terrill, & Cameron, 2017), and are accountable for 98 percent of the Australian salmonid production. Farmed salmon is therefore more or less restricted to Tasmania (Hatfield, 2017).

Between 2005/2006 to 2015/2016, the salmon production in Tasmania increased by 35 344 tonnes to 56 319 tonnes. The increased growth resulted in farmed salmon to be Australia's most valuable fisheries product in 2015/2016, worth AUD \$718 million and production volume being 63 138 tonnes (Hatfield, 2017). Tasmania is expanding their aquaculture industry (Hatfield, 2017) and FAO has predicted that the Australian aquaculture will exceed the wild fish production by 2018 (Governments, 2016).

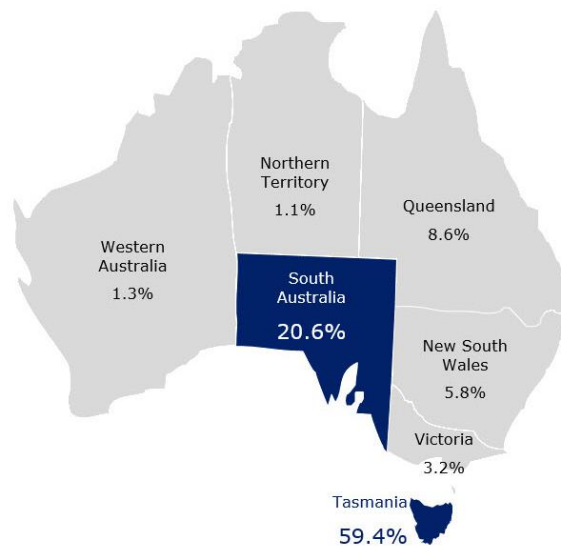


Figure 2.5. The share of Australia's aquaculture production, where Tasmania and South Australia are the two largest producing areas (Iversen & Hermansen, 2016).

### Marked and export

The most important market is spread throughout Asia (TAS, 2007). In 2015 – 2016 over half of all salmonids were exported to the Chinese market. The Chinese market became the largest market for exporting salmonids in 2015 – 2016 (Hatfield, 2017).

### Problems

Favourable sea temperature and isolation from other wild and farmed Atlantic salmon decreased the major infectious disease problems. However, the Tasmanian salmon industry have recently met some problems related to high sea temperatures and thereby resulting in lower oxygen levels (Davis, 1975; Whiting, 2017). Intensive fish farming in Macquarie Harbour experienced in 2016 hot summers with low oxygen levels. As a result, Huon Aquaculture experienced a loss of AUD \$1.3 million the first half of 2016 financial year (Bolger, 2016). In addition, FAO predict that lack of suitable sites will most likely limit the expansion of the industry (Jones, 2004).

## 2.2 Climate & environmental factors

Chile and Tasmania are located in the southern hemisphere, while Norway is located in the northern hemisphere (fig. 2.6). Norway has therefore opposite seasons to Chile and Tasmania, where the countries seasons are summer, autumn, winter and spring (Skaugen & Tveito, 2004).

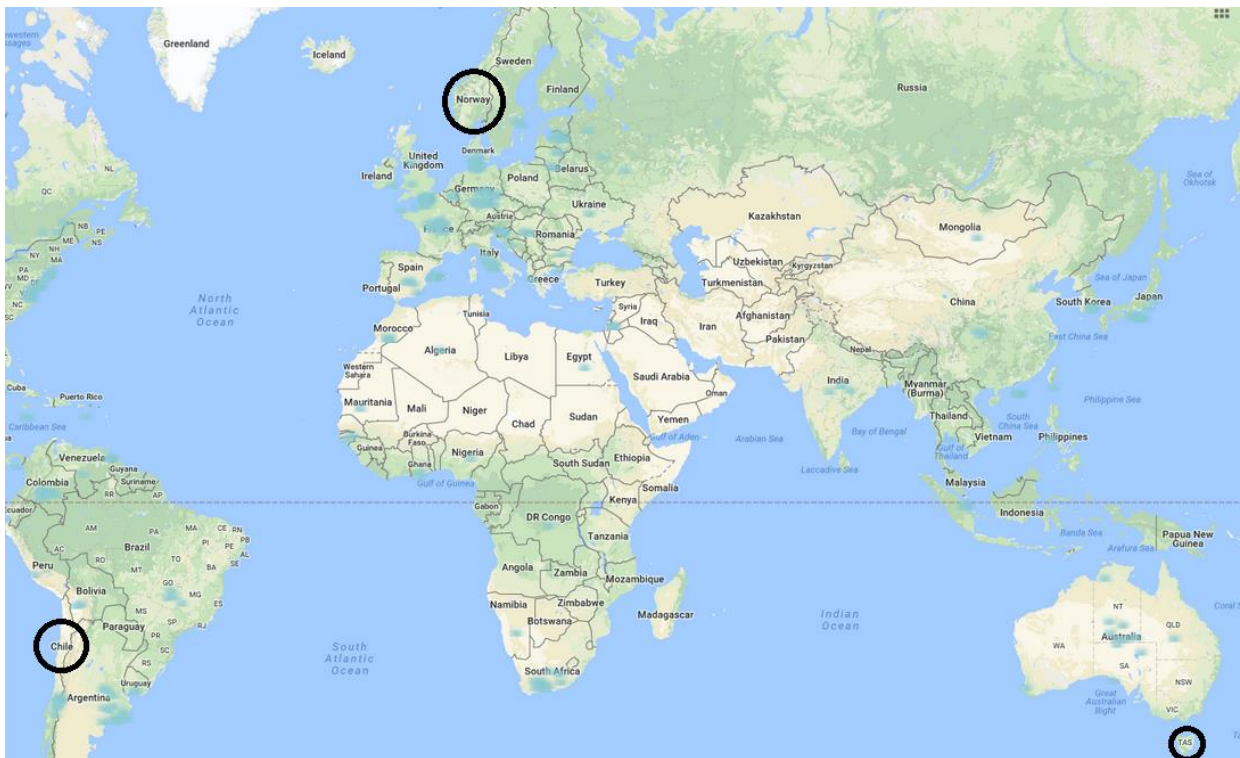


Figure 2.6. World map, where Chile, Norway and Tasmania are localized by black circles. Equator is indicated by a dotted line and divide the northern hemisphere and the southern hemisphere. Retrieved from World Map – Google.

Atlantic salmon are farmed in net cages in the fjords and sea (sec. 2.3.1), where water flows freely into the cages, exposing salmon for biophysical factors (European, 2017). The environmental conditions vary considerably along the Chilean-, Norwegian- and Tasmanian-coast, with sea temperature and day length showing great seasonal changes within a fish farm. Therefore, the two major environmental factors, sea temperature and day length will be highlighted in the following sections.

### 2.2.1 Sea temperature

Atlantic salmon can be farmed in very different environments, where they show best appetite around their sea temperature optimum; 13 degrees Celsius. During a prolonged thermal exposure on 19 degrees Celsius, salmon reduced its feed intake and had a reduced growth (Hevrøy et al., 2013).

In the summer months, Chile seem to have the highest sea temperature, followed by South of Norway and Tasmania. In the winter months, Tasmania and Chile have similar sea temperatures, while Norway holds a much lower sea temperature. Norway's geographic location results in large seasonal variations in sea temperature, where the sea temperature is lower for all the seasons in North of Norway (fig. 2.7).

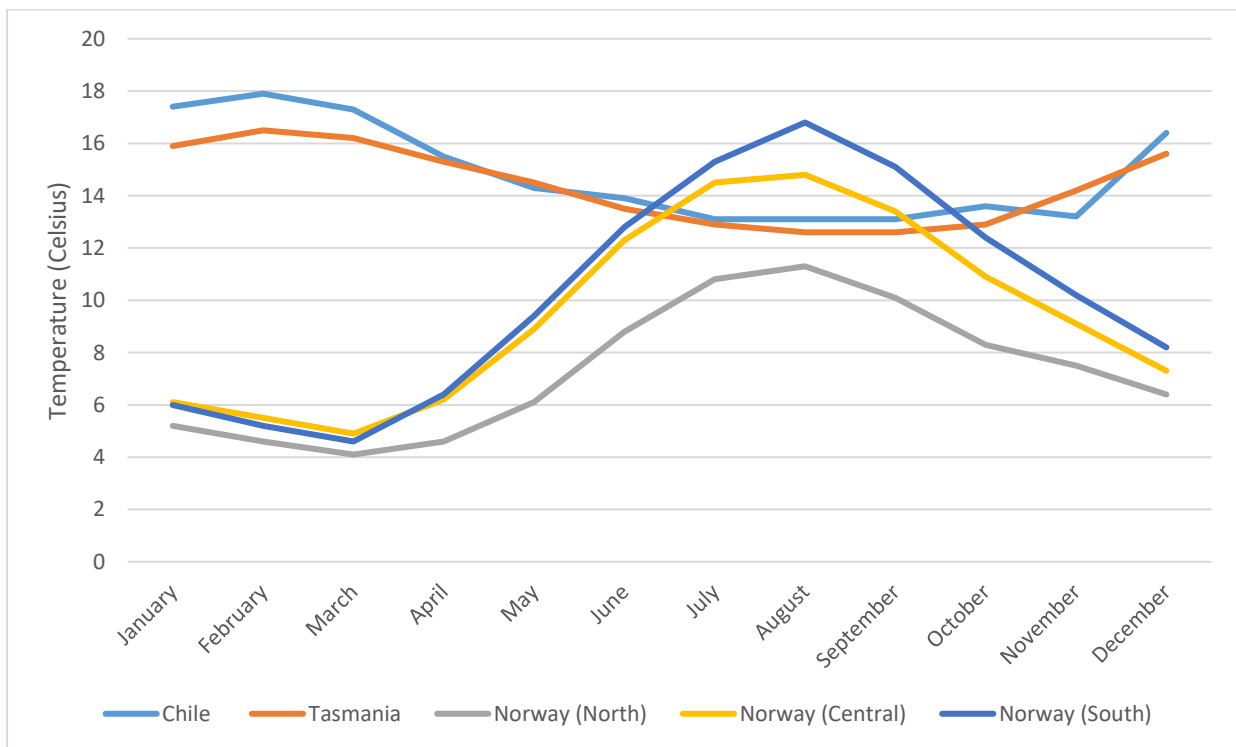


Figure 2.7. Average sea temperature in Vina del Mar (Outside the coast of Santiago, Chile), Hobart (Tasmania), Tromsø (Norway, North), Trondheim (Norway, Central) and Stavanger (Norway, South). Data retrieved from <https://www.seatemperature.org>

### 2.3.2 Daylight

Photoperiod is used in the aquaculture industry to stimulate growth by regulating the appetite (Smith, Metcalfe, Huntingford, & Kadri, 1993), delay the sexual maturation process and to control the smoltification process (Hansen et al., 1992; Kråkenes, Hansen, Stefansson, & Taranger, 1991; Saunders & Henderson, 1970). When smolt is released into the sea cages, the natural light will affect the salmon depending on the season and day length.

In the Summer, the day length is longer for Norway and shorter for Tasmania and Chile. In the Winter, the day length is shorter in Norway and longer in Tasmania and Chile (fig. 2.8). Norway's geographic location results in large seasonal variations in the number of daylight hours. In North of Norway there are minimal sunlight to none at winter from late November to late January, while approximately 20 – 24 hours sunlight from May to late July. South and Central of Norway show a similar tendency, but less extreme pattern as North of Norway. The day length is shorter the higher North you get in the Winter months, and longer the higher North you get in the Summer months. Tasmania have a bit longer daylength in Summer and a bit shorter in the Winter months compared to Chile. However, the day length in Chile and Tasmania show a similar tendency.

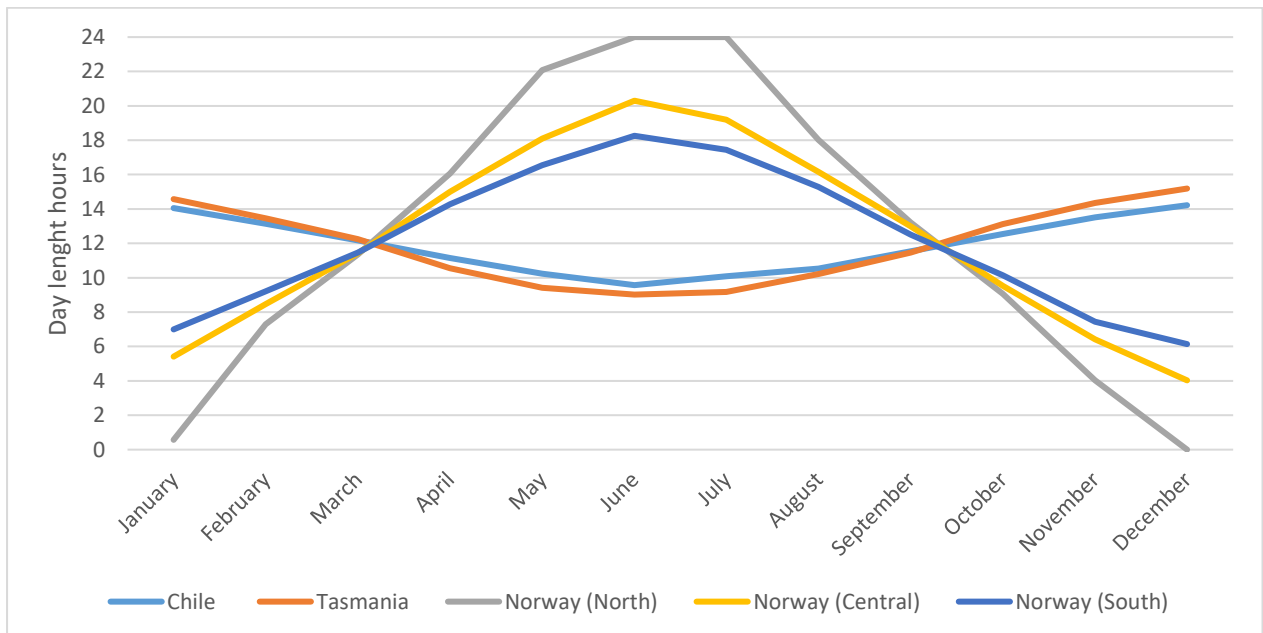


Figure 2.8. Day length regime in Santiago, (Chile), Hobart (Tasmania), Tromsø (Norway, North), Trondheim (Norway, Central) and Stavanger (Norway, South). All data is from 2017, the 15th in each month and retrieved from <https://www.timeanddate.com/sun/>



## Literature review – Part B

This chapter will give an overview of the growth pattern of salmon and look into the reproduction cycle of the salmonids. Furthermore, the life cycle of salmonids will be briefly described.

The biological principals will be the same where you are in the world. However, the differences in sea temperature and day length between seasons will affect the growth pattern of salmon.

### **2.3 Growth pattern of Atlantic salmon**

#### *2.3.1 Life cycle*

The brood stock of Atlantic salmon is stripped, and eggs are mixed with milk. When the eggs are fertilized they cannot be moved after 24 hours and will be shipped after they have reached the eye - age stage. After hatching, they will grow to become smolts and later be transferred to sea cages in the ongrowing farms. In the sea cages, they will grow until they are ready for slaughter (fig. 2.9) (Towers, 2010).

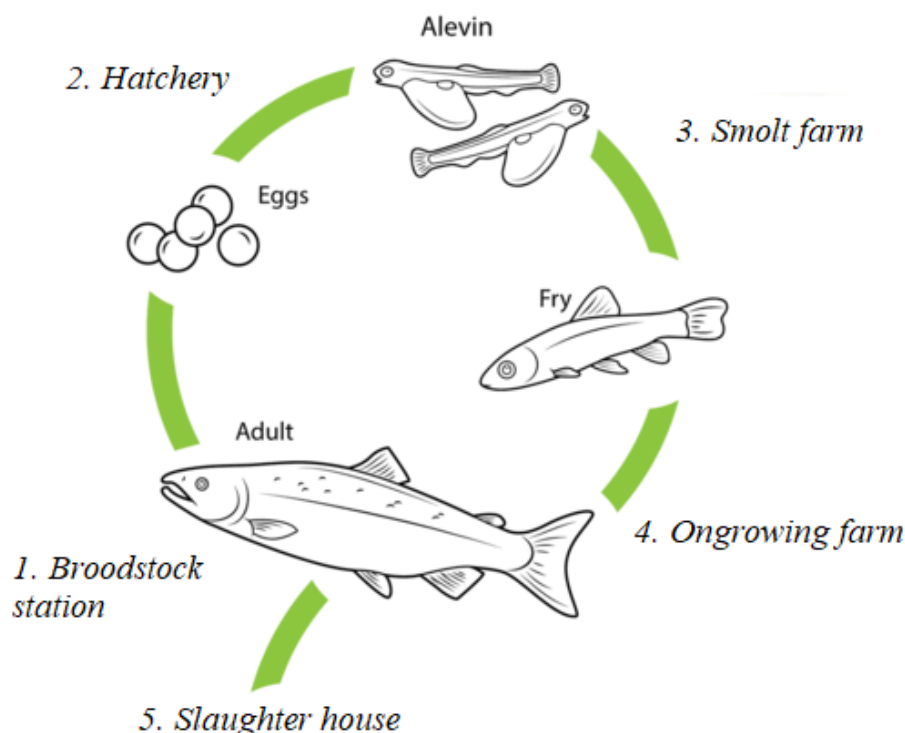


Figure 2.9. The life cycle of Atlantic salmon. The different stations in the aquaculture industry are labelled with numbers; broodstock station (1), hatchery (2), smolt farm (3), ongrowing farm (sea cages) (4) and slaughter house (5). Salmonids that are close to the breeding goals will be chosen to be a part of the broodstock (broodstock station) (Artsashina, 2015).

### 2.3.2 The reproduction cycle

The goal of an organism is to survive and reproduce, so their genes can be passed on to the next generation. The reproduction cycle is therefore highly relevant for the growth pattern of Atlantic salmon. Reproduction in animals is an energy demanding process and requires energy in the form of fat or glycogen (Ofteidal & Gittleman, 1989), where the accumulation of fat/glycogen is controlled by the day length (Huber & Bengtson, 1999; Ortavant et al., 1988). It is therefore the energy and day length that decide if the salmon can sexually mature and thereby reproduce (Alne, Oehme, Thomassen, Terjesen, & Rørvik, 2011). When smolt is transferred to sea, they will be affected by the day length. From Summer to Autumn, the day length gets shorter and salmon will respond to this by accumulating fat. The accumulation of fat in autumn will decide if salmon can sexually mature in spring (fig. 2.10) (Alne et al., 2011). The fat accumulation during autumn is depending on the fish feed and how fat or lean the fish is in the start of autumn, where a lean salmon will accumulate more fat and grow faster (Rørvik et al., 2018).

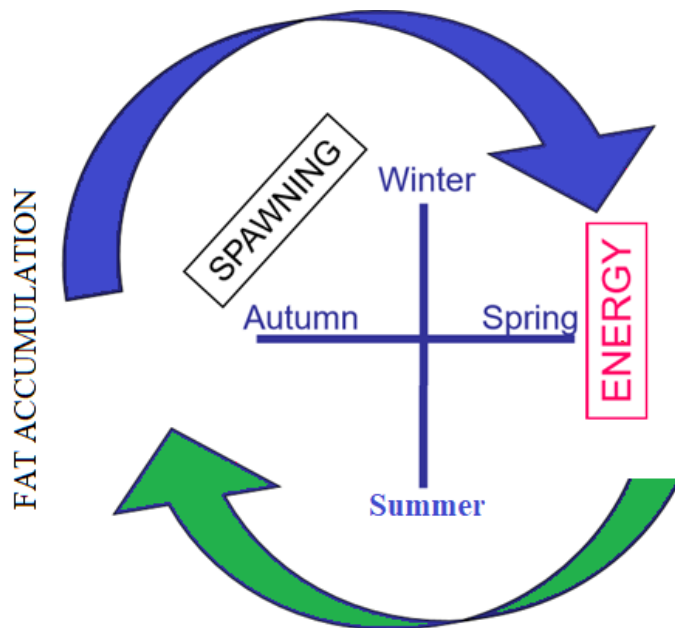


Figure 2.10. The reproduction cycle of Atlantic salmon. Retrieved from Professor Kjell-Arne Rørvik (Nofima).

On the other hand, if the salmon does not meet the need for energy required in spring, sexual maturation will be postponed. Males need less fat (energy) than the female salmon to mature

and therefore more males are observed to mature in the on-growing farms than females (Alne, Thomassen, Sigholt, Berge, & Rørvik, 2009).

Sexual maturation will reduce the flesh colouration where energy is moved from the fillet to the gonads and flesh (Kadri, Mitchell, Metcalfe, Huntingford, & Thorpe, 1996). There is a decrease in fat content and protein content, and an increase of water in the salmon fillet (Aksnes, Gjerde, & Roald, 1986).

In Norway, salmon is normally released into the sea in the autumn or spring as 0+ smolt or 1+ smolt, where the day length and sea temperature will affect the salmonids growth pattern and thereby their life cycle (Fiskeridirektoratet, 2015). Growth rate and feed utilisation have shown to vary significantly with season, where the changes seem to be highest for 1 + smolt. However, after weight difference correction, there have not been found any correlations between the production efficiency or product quality and the smolt age (0+ or 1+) when transferred to seawater (Mørkøre & Rørvik, 2001).

## Literature review – Part C

This chapter gives an overview of the fillet quality of farmed Atlantic salmon, targeting the quality issues; fat content, colour, melanin spots and gaping. The first section will give an introduction to quality and quality measurements. The next section will give an overview of each quality issue and describe the variation of each quality issue within fillets and variation between season.

Seasonal changes in live Atlantic salmon will be emphasized. Slaughter, handling and storage is also crucial for quality, but will not be discussed as these problems will be the same regardless of the season. Insufficient fillet quality resembles a severe economic loss for companies independent on farming region. If the consumers receive sub optimal quality, the company might lose customers to other companies (sec. 2.1).

### **2.4 Quality**

#### *2.4.1 What is quality?*

According to FAO, quality refers to the degree of spoilage, freshness and the appearance of the fish, and can also involve the safety aspects; free from bacteria, parasites and chemicals (FAO, 1995). In general fillet quality is divided into five main factors; hygienic, sensory, technological, ethical and nutritional quality (Thomassen et al., 2007).

Melanin spots, pale and irregular colour, high fat content, soft texture or gaping are known causes of quality downgrading (Koteng, 1992; Robb, Kestin, Warriss, & Nute, 2002). When the fish is alive, the feed intake, diseases and environmental factors will affect the fillet (Thomassen et al., 2007).

#### *2.4.2 Quality measurements*

Quality traits vary within the same fillet. Hence, quality characteristics should be analysed on a standard area of the fillet. The Norwegian Quality Cut (NQC/Norwegian Standard NS9402) is a cutlet that used for analysing the colour and fat content. NQC refers to a cross-section, where the first cut is behind the dorsal fin and the second cut is done in front of the anal fin (fig. 2.11) (Daczowska-Kozon & Pan, 2011). There are no standard methods for assessing gaping, but the dorsal fillet part is mostly used for texture measurements (Mørkøre, 2008).

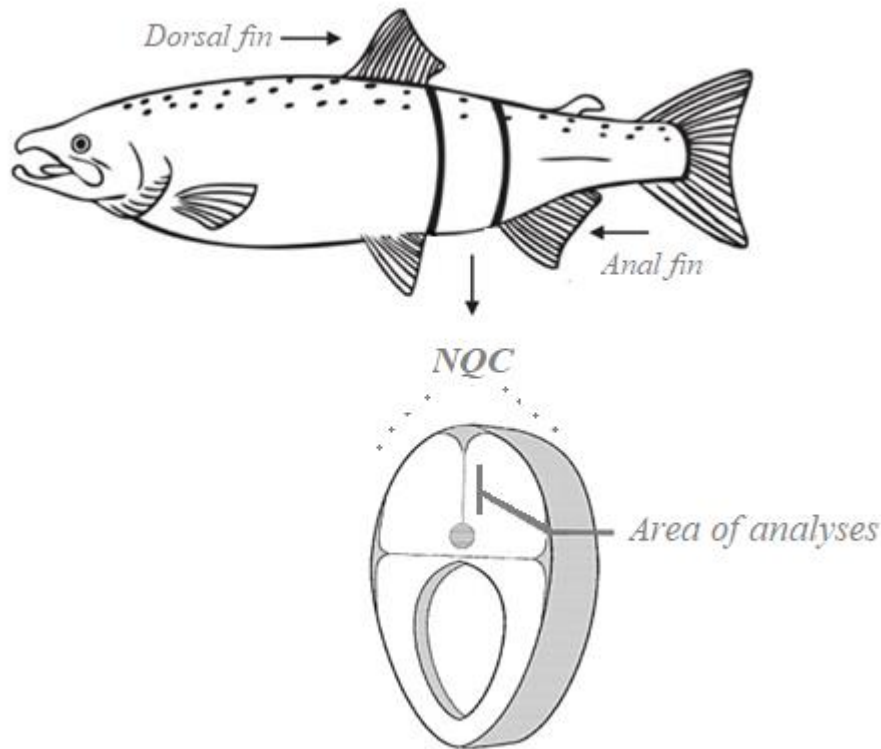


Figure 2.11. The Norwegian Quality Cut (NQC) and the area of analyses. Retrieved from Fjord-Lab AS and (Espe et al., 2004).

Fish quality can be measured indirectly (non-destructive way) or directly (destructive way) by sensory analysis or chemical analysis. Non-destructive testing is when the fillet is being inspected, tested and evaluated without being destroyed. Non – destructive analyses are generally faster in comparison with destructive tests, which are done on a limited number of samples to lower the cost (ASNT, 2017).

A sensory analysis is when your own sensory system is used to evaluate the fish appearance, odour, colour and texture. Sensory methods are considered as most for assessing freshness and quality in the fish (Olafsdottir et al., 2004). However, the human sensory system may not be as accurate as an instrument. Image analyses are also used for fish quality analyses. An example is Photofish that gives information about the fillet concerning fat percentage, the condition factor, the colour based on the SalmonFan, (fig. 2.16) and the astaxanthin content based on images (Kraugerud, 2015). Measurements can also be done direct by the use of a chemical analysis, where astaxanthin or fat are extracted from the fillet (Lambertsen & Braekkan, 1971)

## 2.5 Fat content

Salmon is an oily fish and can accumulate high amounts of fat (Stead & Laird, 2001). The importance of a balanced diet for fish has long been known (Lee, Roehm, Yu, & Sinnhuber, 1967; Owen, Adron, Middleton, & Cowey, 1975). In 1975, juvenile rainbow trout (*Oncorhynchus mykiss*) were fed diets rich on linoleic acid (LA, n-6) which gave reduced survival (Owen et al., 1975), while including a diet with n-3 polyunsaturated fatty acids (PUFA) gave an increased survival (Lee et al., 1967). The awareness of n-3 for human health have grown during the past years (Calder & Yaqoob, 2009).

LA is converted to arachidonic acid (AA, n-6) and  $\alpha$  – linolenic acid (ALA, n-3) is converted to docosahexaenoic acid (DHA, n-3) and eicosapentaenoic acid (EPA, n-3) in a series of steps involving desaturation and elongation reactions (fig. 2.12). The fatty acids, AA, EPA and DHA are converted to different local signal hormones called eicosanoids and docosanoids.

The eicosanoids that arises from EPA and DHA maintain normal physiological functions and homeostasis in cells. Furthermore, AA give rise to pro – inflammatory eicosanoids.

Eicosanoids from the n-3 and n-6 family have therefore in some cases opposite biological effects on biological functions (Saini & Keum, 2018). Both, n-3 and n-6 PUFA compete for the same enzymes for desaturation and elongation. It is therefore a constant competition between the n-3 and n-6 pathway (Contreras & Rapoport, 2002). Atlantic salmon can convert ALA to EPA and DHA, but the conversion is not very efficient. Therefore, their essential FA requirements are not met by ALA alone, and must be provided with some dietary EPA and DHA in order to obtain good growth and health (Ruyter, Røsjø, Einen, & Thomassen, 2000). The nutritional requirement for n-3 PUFA for fast-growing Atlantic salmon during grow out in the sea cages is not well documented (Rosenlund, Torstensen, Stubhaug, Usman, & Sissener, 2016). Atlantic salmon fed 10 g/kg levels of EPA + DHA was previously regarded as sufficient. However, this level was too low to maintain fish health under demanding environmental conditions in sea cages (Bou, Berge, Baeverfjord, Sigholt, Østbye, et al., 2017). Requirement for optimal DHA and EPA levels have therefore suggested to be 2.7 percent (Rosenlund et al., 2016). Furthermore, feed with 2 g/kg EPA + DHA levels showed changes in the fatty acid composition in the cell membrane. There was found an increased level of n-6 in the phospholipid membrane and a decreased levels of EPA and DHA (Bou, Berge, Baeverfjord, Sigholt, Østbye, et al., 2017).

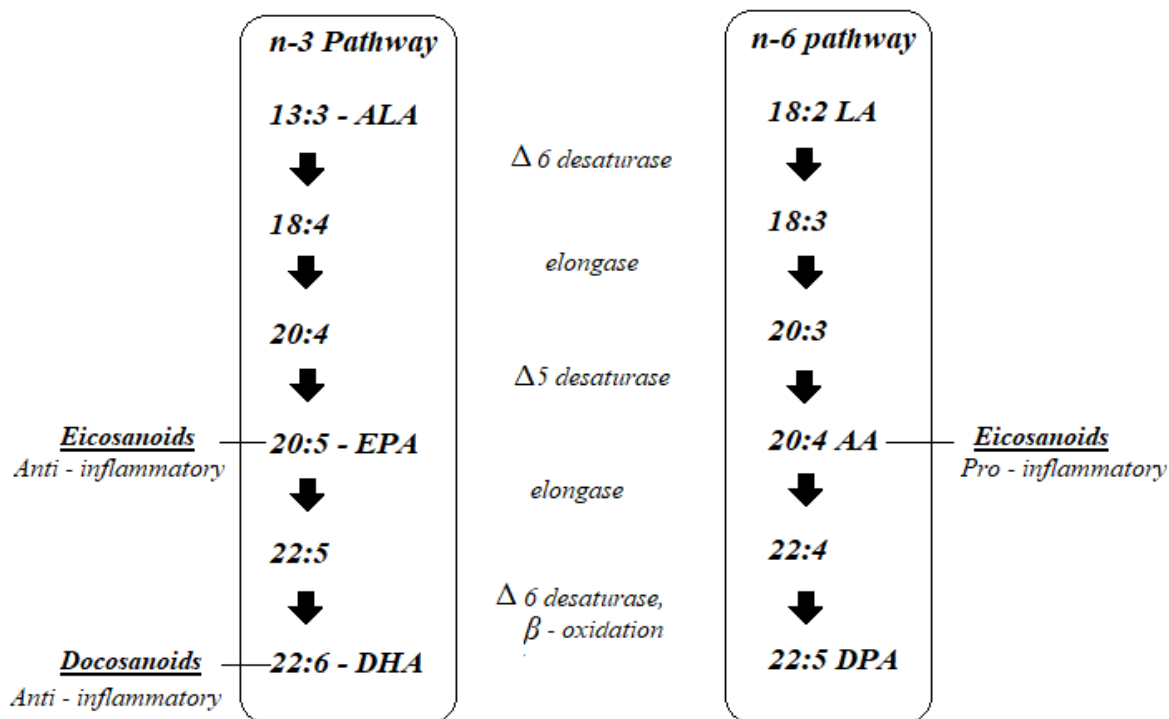


Figure 2.12. Pathway of n-3 and n-6, where double bonds are added (desaturation, using the enzymes  $\Delta 5$  and  $\Delta 6$ -desaturase) and where the chain length is increased by two carbon atoms (elongation, using *elongase* enzymes) and finally an abbreviation of the carbon chain by beta oxidation (Lau, Cohen, Ward, & Ma, 2013).

Diets for farmed salmon has changed from marine-based with high levels of EPA and DHA to a feed that is currently based on 70 percent plant-based feed with low levels of EPA and DHA (Bou, Berge, Baeverfjord, Sigholt, Ostbye, et al., 2017). Limited availability of marine oils has resulted in the use of vegetable oils in fish feed (Thomassen et al., 2007), which have shown to be rich in n-6 fatty acids and can result in increased inflammations (Chapkin et al., 2007; Saini & Keum, 2018). Even though EPA and DHA have decreased during the years (Sprague, Dick, & Tocher, 2016), replacing 50 percent of the fish oil with vegetable oils had no significant effects on growth, survival, body traits or fillet quality of salmon (Rosenlund, Obach, Sandberg, Standal, & Tveit, 2001). Another study showed that a rich diet in soya oil resulted in increased fat accumulation in the intestine and especially increased the pro-inflammatory, n-6 fatty acids at low sea temperature (B. Ruyter, Moya-Falcón, Rosenlund, & Vegusdal, 2006).

Salmonids ability to accumulate high amounts of fat in their muscles can cause challenges in processing industry, because salmon with very high fat content may exude oil from cut surfaces when smoked and give an oily mouthfeel after heat treatment (Robb et al., 2002; Stead & Laird, 2001). High fat leakage have been observed in smoked salmon containing 20

percent or more fat in the fillet (Mørkøre et al., 2001). Late autumn have specially a high appearance of oil exude in the flesh and variation in lipid content is high between individuals (Bell et al., 1998), even between the individuals that are reared in the same sea cage (Rørå et al., 1998).

### 2.5.1 Fat distribution

The fat content is of importance of the fillet as water and fat represent approximately 80 percent of the muscle weight in an immature salmon (Haard, 1992). According to Stead and Laird, the overall fat content of the fillet should be around 8 percent to 12 percent (Stead & Laird, 2001). However, farmed Atlantic salmon market size has a lipid content between 6 percent and 22 percent, with an average of 15/16 percent measured in the NQC (fig.2.11) (Rørå et al., 1998; Sigurgisladottir, Torrissen, Lie, Thomassen, & Hafsteinsson, 1997).

The distribution of the fat content in the fillet is visualized in figure 2.13. The fat percentage increases in the salmon fillet when moving horizontally from the posterior end (tail) towards the anterior end (head) of the salmon, and when moving vertically from the dorsal side towards the ventral side. The belly flap, which is located on the ventral side has the highest fat percentage (Einen, Waagan, & Thomassen, 1998). The belly tends to accumulate fat because of the higher concentration of adipocytes found in this region (Aursand, Bleivik, Rainuzzo, Jörgensen, & Mohr, 1994).

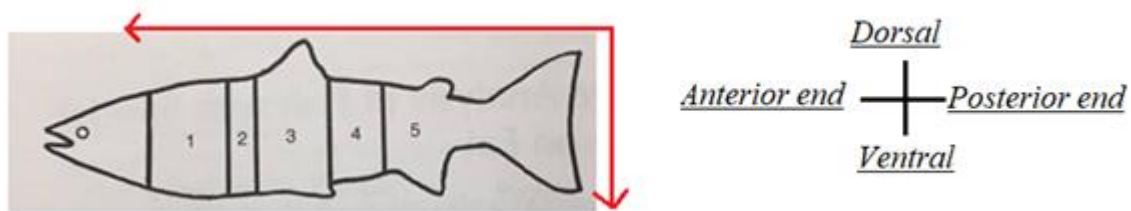


Figure 2.13. The fat distribution in Atlantic salmon. Fat content increases from the dorsal to the ventral side, and fat content increases from the tail to the head (red arrows). Received from (Einen et al., 1998) and Nutreco ARC, unpubl. Data.

### 2.5.2 Seasonal variation

A research project completed in Norway concluded that there was an increased retention of fat in the muscle of salmonids during autumn, and a lower fat content in the muscle of salmonids during winter (fig. 2.14) (Mørkøre & Rørvik, 2001). According to section 2.3.2, the day length affects the fat accumulation, which will have a higher percentage of fat



retention<sup>1</sup> as daylength decreases (fig. 2.8). A newly study showed that fat was mostly stored intra muscular during the autumn, while in spring the fat was mostly stored in and around the organs (visceral) than intra muscular (Weihe et al., In press).

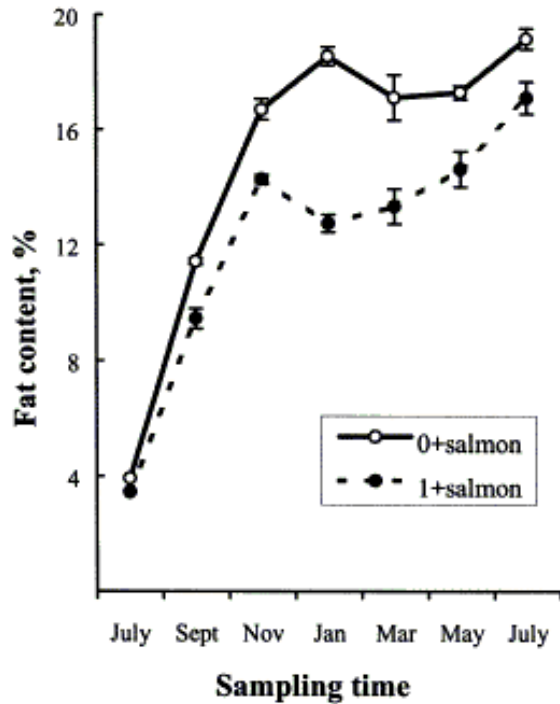


Figure. 2.14. Seasonal variation of the fat content in 0 + and 1 + smolt given in percentage (Mørkøre & Rørvik, 2001).

Another experiment done in Averøy in Norway showed that growth, feeding rate and feed utilization was low during the first spring in sea for both Atlantic salmon released to the sea cages in the autumn (0+ smolt) and the spring (1+ smolt). The fat retention however increased from summer to autumn for both groups (Alne et al., 2011).

<sup>1</sup> Percentage of fat retained/accumulated in the fish

## 2.6 Flesh colour

Astaxanthin is an antioxidant and carotenoid, that are found in the flesh of wild salmon (fig. 2.15). It is the most common pigment used in intensive fish farming for flesh colouration of Atlantic salmon (Torissen, Hardy, & Shearer, 1989). Astaxanthin accumulates in the flesh of salmonids as they grow, where the process stops when they become sexually mature (Bjerkeng, Storebakken, & Liaaen-Jensen, 1992). Carotenoids are usually biosynthesised from photosynthesising organisms and certain bacteria and fungus (Thomassen et al., 2007). Salmon are unable to biosynthesize astaxanthin (Alfnes, Guttormsen, Steine, & Kolstad, 2006), and astaxanthin is therefore added to salmon feeds to give the flesh a pink – red colour (Quevedo, Aguilera, & Pedreschi, 2010).

Carotenoid feeding is approximately 15 percent to 20 percent of the total feed cost (Torrissen, Christiansen, Struksnæs, & Estermann, 1995), and even though it is expensive it is important for the fish itself, both for reproduction (Thomassen et al., 2007) and the immune system, where it prevents free radicals to react with proteins, DNA and other tissue (Ambati, Siew, Ravi, & Aswathanarayana, 2014; Anuradha, 2018). Astaxanthin is also important for the market, where an insufficient colouration will be rejected (Torissen et al., 1989). In addition a feed without astaxanthin has showed to have a negative effect on growth (Christiansen, Glette, Lie, Torrissen, & Waagbø, 1995). It has therefore been recommended that all fish feed should contain at least 10 mg/kg astaxanthin (Torrissen et al., 1995).

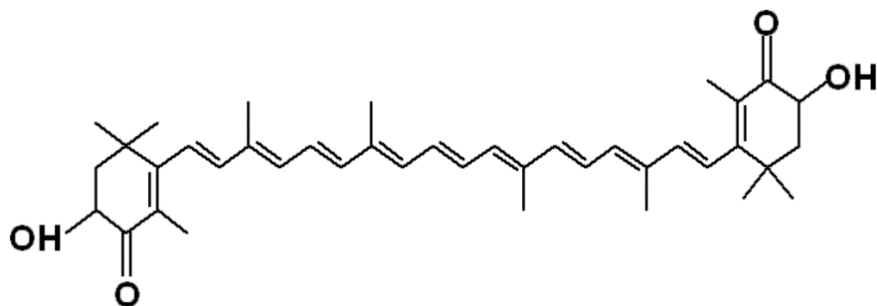


Figure 2.15. The structure of astaxanthin. Retrieved from (Ambati et al., 2014).

Carotenoids are absorbed into the body like lipids and transported via the lymphatic system to the liver (Page & Davies, 2006), where high fat content in the feed correlates positive to the absorption of carotenoids (Bjerkeng et al., 1997; Rosenlund et al., 2016). The muscle retention of astaxanthin in Atlantic salmon is usually less than 10 percent. One reason for this is that the digestibility of astaxanthin is poor and typically less than 50 percent (Bjerkeng & Berge, 2000). Furthermore, the absorption, utilization and pigmentation of the flesh in salmon

have been reported to differ between types of carotenoids. For example, have the astaxanthin in rainbow trout (*Oncorhynchus mykiss*) shown to be 1.4 more efficient than the carotenoid canthaxanthin for muscle pigmentation (Torrissen, 1986), and canthaxanthin is reported to give a red pigmentation with a more brown tone colour compared to astaxanthin (Torrissen, 1996). The deposition of carotenoids are dependent on carotenoid mix and can be influenced by several factors; the digestibility of the carotenoid (Foss, Storebakken, Austreng, & Jensen, 1987), the absorption from the intestine (Hardy, Torrissen, & Scott, 1990), transport in the blood by lipoproteins (Choubert, Milicua, & Gomez, 1994), the metabolism of the pigment (Choubert et al., 1994) and the affinity to the muscle fibre (Henmi, Hata, & Hata, 1990). These processes relay on each other, which means that limitation in one of these processes mentioned above will influence the deposition of the carotenoid and therefore the flesh colour.

The flesh pigments in the salmonids can be measured by the DSM SalmoFan scale (20 – 34) (Skrede, Risvik, Huber, Enersen, & Blumein, 1990). The SalmoFan is developed on the basis of salmonid flesh pigments with astaxanthin to e.g. check if the product is ready for marked (fig. 2.16) (Quevedo et al., 2010). The normal colour range are between 25 – 27 for farmed Atlantic salmon sold in Norway (Alfnes et al., 2006).



Figure 2.16. SalmoFan™ measuring the fillet colour of Atlantic salmon. Retrieved from DSM.

### 2.6.1 Colour distribution

The colour distribution of Atlantic salmon fillets are most intense in the posterior end (tail) and on the dorsal side. The colour decreases horizontally towards the anterior end (head) and

vertically towards the ventral side (fig. 2.17) (March & Macmillan, 1996). The colour distribution and fat distribution are therefore opposite of each other (fig. 2.12 and fig. 2.17).



Figure 2.17. The variation in colour within a salmon fillet. Flesh pigmentation increasing towards the posterior end and dorsal side (red arrows). Retrieved from <http://packaging-materials.xtraplast.com/packaging-materials/rollstock-film/skin>

### 2.6.2 Seasonal variation

The flesh pigmentation of Atlantic salmon is affected by the season (Mørkøre & Rørvik, 2001; Torrissen et al., 1995), where astaxanthin concentrations in the fillet generally increases over time with the size of the fish (Torrissen, 1986). An increased growth rate and intestinal rate in autumn was found problematic, as it resulted in a poorer pigmentation of the flesh of Atlantic salmon (Rørvik et al., 2010).

An experiment completed by Mørkøre and Rørvik in Møre og Romsdal county (Ekkilsøy) in Central of Norway (sec. 3.1 for geographical location) measured the seasonally pigmentation in 0 + and 1 + smolt. According to figure 2.18, the flesh pigmentation was low in autumn and increased in winter. The fillet colour had a drop in spring and started increasing again in summer (Mørkøre & Rørvik, 2001).

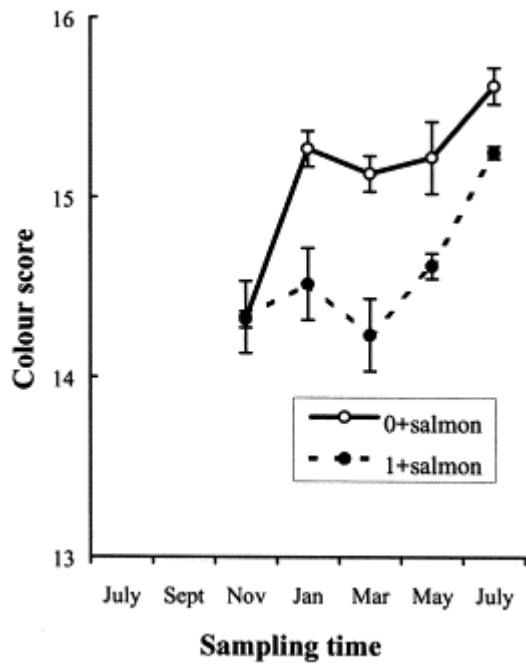


Figure 2.18. Seasonal changes in fillet colour in 0+ and 1+ smolt during November – July (Roche colour card scores). Retrieved from (Mørkøre & Rørvik, 2001).

## 2.7 Melanin spots

Melanin spots are dark pigments in the fish fillet that are produced by melanocytes (Hearing & Tsukamoto, 1991), which are controlled by melanocyte- stimulating hormone and melatonin (fig. 2.19). There are three basic types of melanin; eumelanin, pheomelanin, and neuromelanin, where eumelanin is the only one found in teleosts and produce primary brown or black spots (Agius & Roberts, 2003).

The major quality issue in the Atlantic salmon industry are the occurrence of dark spots in fillets (Berg, Yurtseva, Hansen, Lajus, & Fjellidal, 2012). It is especially the melanin deposition in the fillet that gives a quality problem. Melanin is not dangerous to eat but is not appetizing for customers and causes quality downgrading (Koteng, 1992).

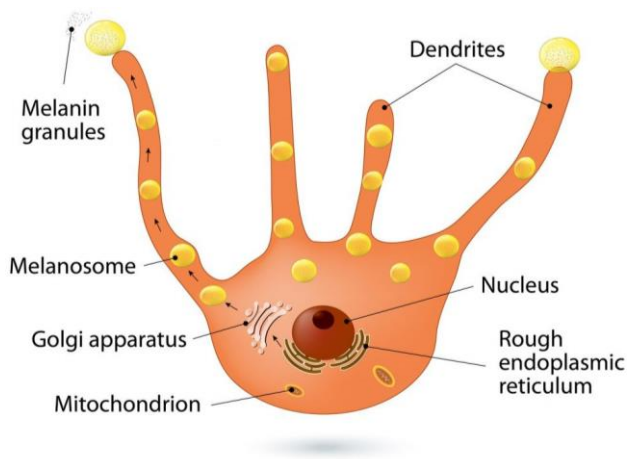


Figure 2.19. Melanocyte producing melanin granules. Retrieved from <https://www.shutterstock.com/image-vector/melanocyte-melanin-producing-cells-pigment-responsible-281633990>

A major function of melanin is to inhibit bacterial, fungal and other parasitic infections of the dermis and epidermis (Mackintosh, 2001), where melanin spots often have been found in a wide range of species in relation to injury or infection (Somerset, Krossøy, Biering, & Frost, 2005). Melanin therefore works as a strong antioxidant and help the fish repair itself. Studies have shown that induced pancreas disease (PD) stimulates melanin pigmentation in Atlantic salmon (Lerfall et al., 2012), and collected data from the industry likewise showed that PD resulted in higher frequency of melanin spots after PD outbreak in the fillet (fig. 2.20) (Mørkøre, 2012).

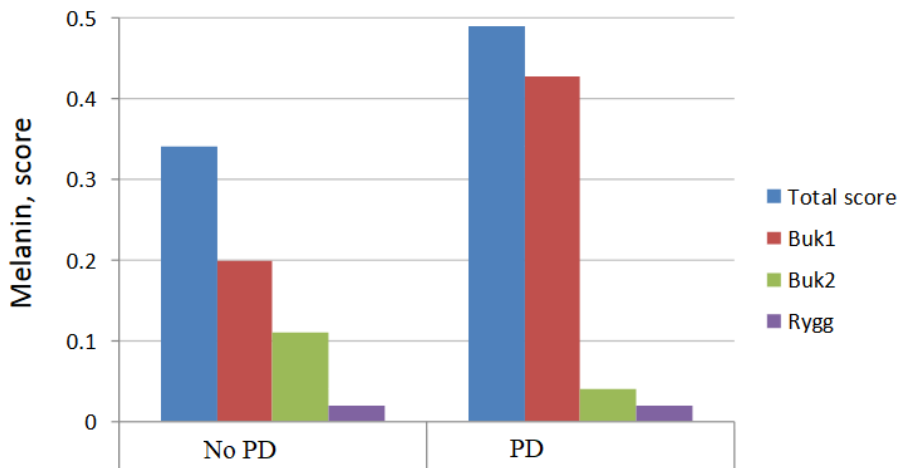


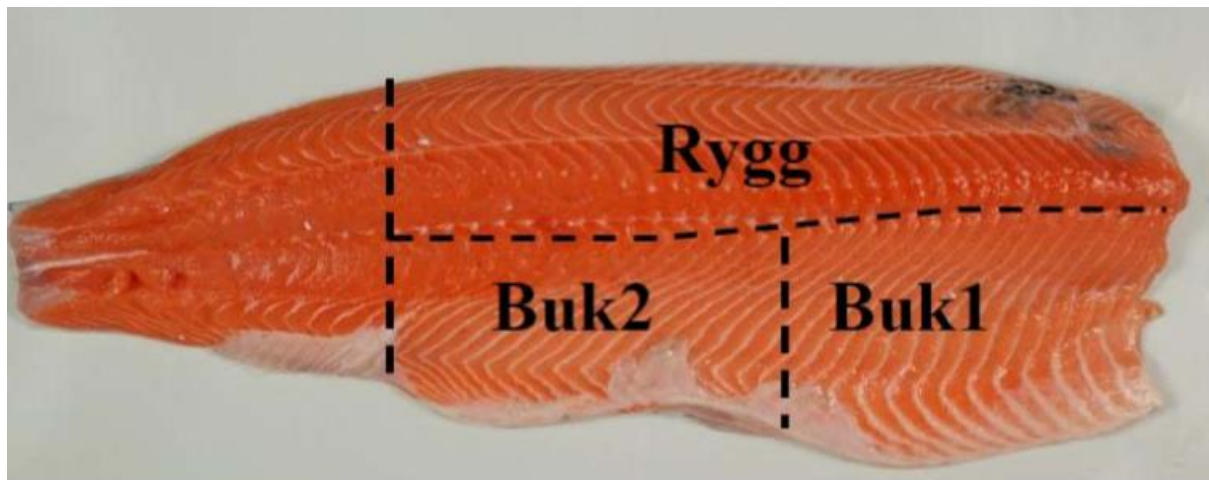
Figure 2.20. Average melanin score in different parts of the fillets of salmon after a PD outbreak farmed in sea cages in Central of Norway. See figure 2.21 for classification scale (Buk1, Buk2, Rygg)(Mørkøre, 2012).

Antioxidants are types of molecules that are capable to terminate the process of chain reaction during oxidation, where free radicals "steal" electrons and cause damage that can result in rancidity of fats, destruction of vitamins and pigments (Tacon, 1987).

Selenium is a micronutrient and is essential for both humans and animals. Selenium is a component of the enzyme glutathione peroxidase and removes hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and lipid peroxidases from cells (Gatlin & Wilson, 1984). There is a balance between antioxidants (vitamin C, vitamin E, selenium) and melanin, where supplementing antioxidants in fish feed decreased the size and presence of melanin spots in the fillet of Atlantic salmon (Wang, 2016). Furthermore, more melanin spots have been observed in salmon fed low levels of DHA and EPA (Mørkøre, 2017).

### 2.7.1 Distribution of melanin

Prevalence of melanin spots increases with fish size. Most melanin spots are located in the anterior fillet part below the lateral line (Buk 1) (fig. 2.24), where the occurrence of melanin spots on the dorsal side (Rygg) have shown to be the lowest (fig. 2.21) (Mørkøre, 2012).



<i>Score</i>	<i>Melanin in fillet</i>
0	No discolouration
1	Gray shadow
2	Spot less than 3 cm
3	Spot 3 cm - 6 cm
4	Spot greater than 6 cm

Figure 2.21. The classification scale for the distribution of melanin spot in the fillet of Atlantic salmon.

Retrieved from (Mørkøre, 2012).

### 2.7.2 Seasonal variation

Melanin spots have shown to vary geographically and within farms located on the same geographic area. The spots have different sizes, but most of them have a diameter around 3 centimetre (Mørkøre, Dessen, Limenez, & Rørvik, 2016). In 2011, data were collected from 283 different fish farms (350.000 Atlantic salmon) in order to study the prevalence and geographical variations of melanin spots. The highest occurrence of melanin spots was observed in South of Norway with 22 percent and lowest in the North with 12 percent (fig. 2.22). Even though the sea temperature was different in these regions it was not a major cause for the different results (fig. 23) (Mørkøre, 2012). Another study completed in-vitro showed that temperature above 20 degrees increased melanin production (Larsen et al., 2013).



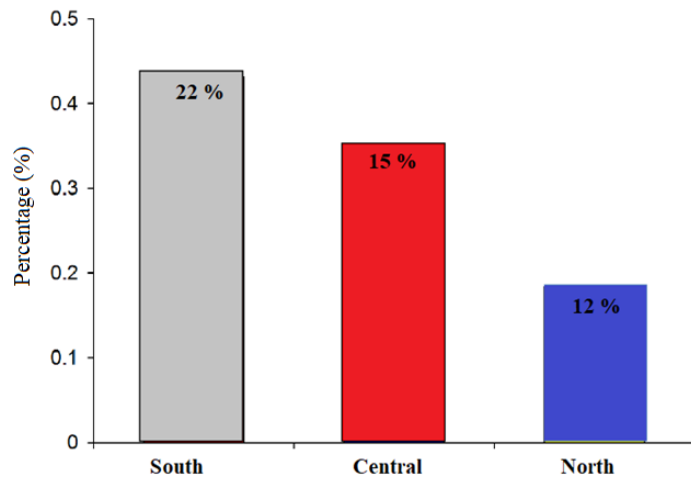


Figure 2.22. The average total score of melanin in South, Central and North of Norway (Mørkøre, 2012).

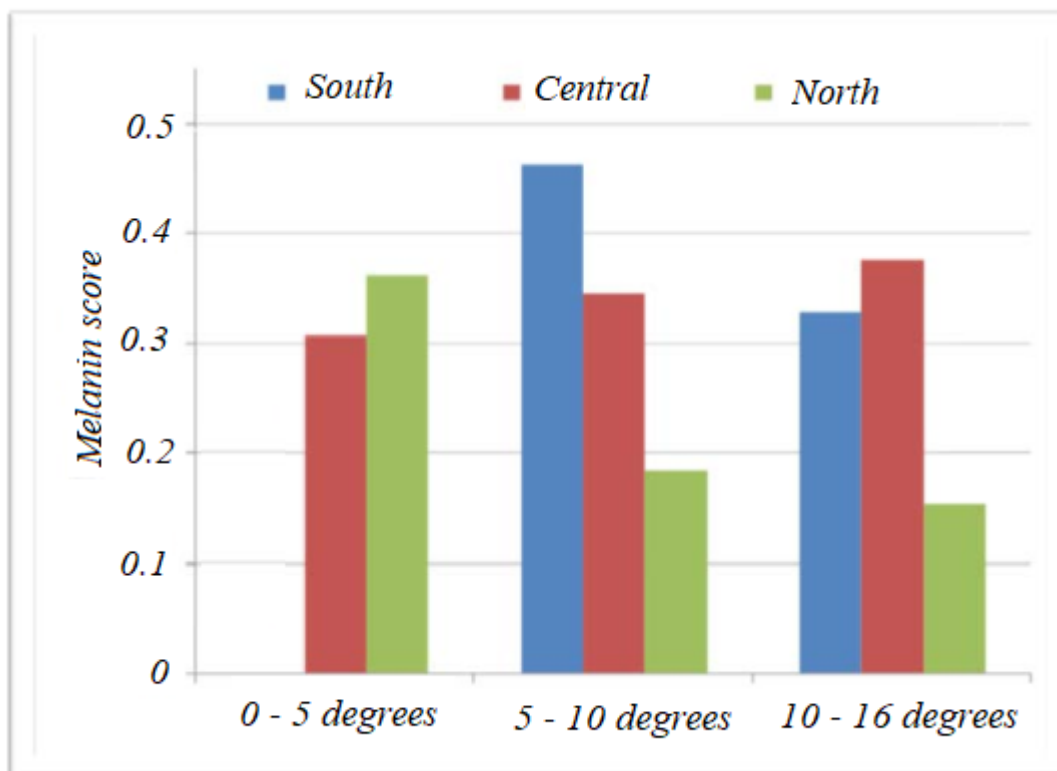


Figure 2.23. Average melanin score of the different sea temperatures in South, Central and North of Norway. No data of 0 – 5 degrees in South of Norway (Mørkøre, 2012).

Mørkøre (2012) found that smolt transferred to sea in autumn had a lower total score of melanin spots than smolt transferred to sea in spring. However, the distribution of the melanin spots varied significantly (fig. 2.24). Showing higher occurrence of melanin spots in “Buk1” in spring, while higher occurrence of melanin spots in “Buk2” and “Rygg” in

autumn. Seasonal variation of melanin spots showed likewise result with an increase of melanin spots in spring (fig. 2.25).

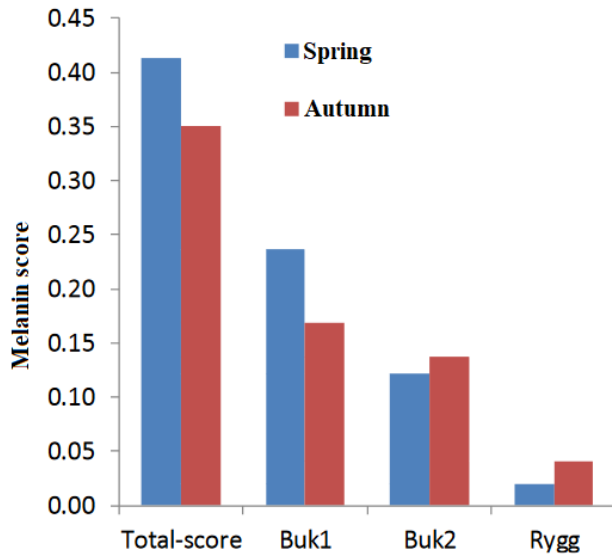


Figure 2.24. Average melanin score of salmon transferred to seawater in Spring or Autumn (Mørkøre, 2012).

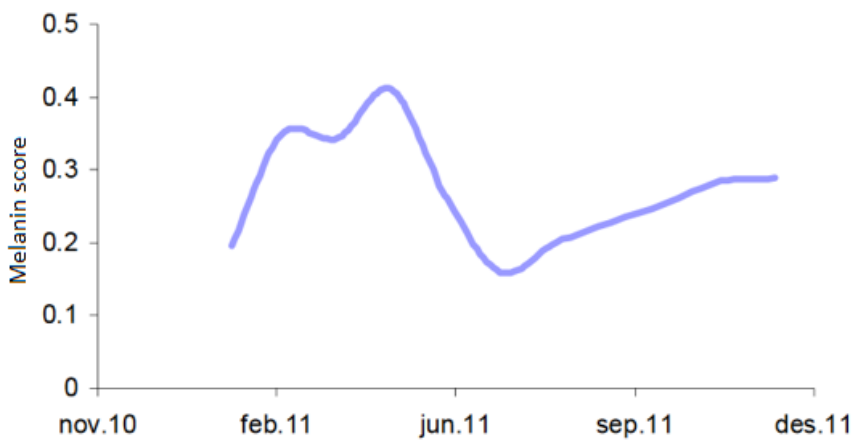


Figure 2.25. Average seasonal variation of melanin score in Norway from January 2011 to December 2011 (Mørkøre, 2012).

## 2.8 Flesh texture and gaping

Gaping is a phenomenon where there occur holes in the fish fillet due to breakage of the connective tissues between the muscle segments. Soft texture can have more gaping than firm texture (Espe et al., 2004; Mørkøre & Rørvik, 2001). In sea cages, environmental conditions, feed and the health of the fish are linked to soft texture and gaping (Mørkøre, 2008).

Firm and juicy flesh is preferred by the consumer, where it is the amount of moisture and intra muscular fat that determine the juiciness of the flesh (Ofstad et al., 1996). In addition, soft texture and gaping is to be avoided as it makes further processing hard, affects the appearance and thereby increases the quality downgrading (Koteng, 1992).

The flesh of the salmon consist of many muscle fibres that are bound together by connective tissues (fig.2.26) (Hyldig & Nielsen, 2001). The muscle fibres consist of muscle proteins while the connective tissue consists of collagen, matrix and fat. Decomposition of the proteins in the muscle fibres results in a softer texture, while changes in the connective tissue will affect the elasticity strength in the connective tissue and result in a softer texture and increased gaping. Salmon with more connective tissue will therefore give a firmer texture (Mørkøre, 2008).

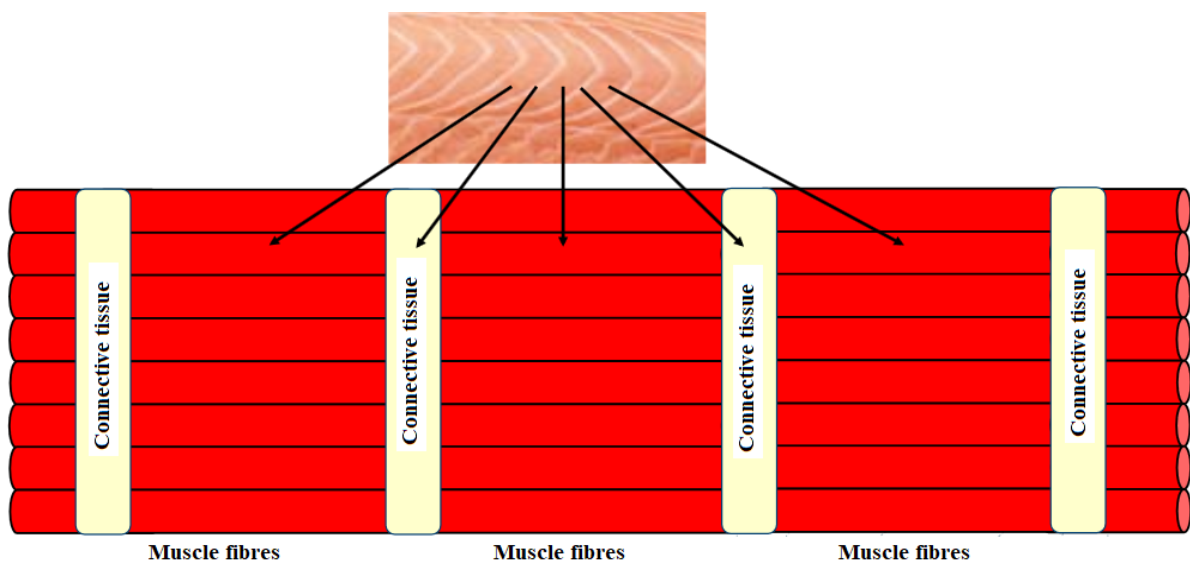


Figure 2.26. Schematic illustration of the composition of a salmon fillet with layers of muscle fibres and connective tissues. The muscle fibres contract, while the connective tissue is elastic and works as a glue and keeps the muscle fibres together (Veiseth, 2009).

Salmon grow by increasing the thickness of the muscle (hypertrophy) and by increasing the amounts of muscle fibres (hypoplasia). Salmon with more and thinner muscle fibres give a firmer texture than a salmon with fewer and larger muscle fibres (Mørkøre, 2008). Smaller

fish gaped more than larger fish as the connective tissue was stronger in larger fish (Borderías & Sánchez-Alonso, 2011).

Hydroxylysyl pyridinoline (PYD) are cross-linkages which are important for the flesh quality where it helps stabilizing the collagen in fillet. An increase amount of cross – linkages are therefore positively correlated with fillet firmness in fresh and smoked Atlantic salmon (Li et al., 2005). Both the macronutrients and micronutrients are important for optimal production of connective tissue. Fillets with an average fat percentage of 19 – 20 percent gradually evolved softer texture (Mørkøre, 2008). Furthermore, little copper (Cu) in filets showed high gaping (Mørkøre & Austreng, 2004).

Salmon with a soft texture was found to often have deviant cell structure. Stressful situations due to sub optimal environmental conditions can cause changes in the cell structure. In addition, melanin spots have showed to disturb the cell formation in the connective tissue, muscle and fat (Mørkøre, 2008).

### 2.8.1 Distribution of soft and firm texture

The texture firmness increases from the anterior end (head) to the posterior end (tail) (fig. 2.27). A study of 80 salmon from the same sea cage revealed that the texture behind the dorsal fin had better texture firmness than the area in front (Mørkøre, 2008).

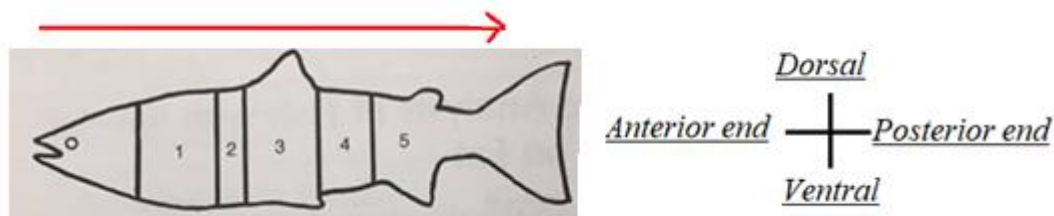


Figure 2.27. The distribution of texture firmness increases from the anterior end to the posterior end in general (red arrow). Received from (Einen et al., 1998) and Nutreco ARC, unpubl. Data.

### 2.8.2 Seasonal variation

Mørkøre and Rørvik (2001) showed significant higher incidence of gaping in spring and summer for 0+ salmon and 1+ salmon reared in sea cages (fig. 2.28). Specific growth rate (SGR) was negatively correlated with firmness of 0+ salmon and 1+ salmon indicating that fast growth might result in a softer texture. Two other experiment likewise showed that harvesting immediately after a high growth period resulted in a softer salmon fillet (Einen, Mørkøre, Rørå, & Thomassen, 1999; Folkestad, Rørvik, Kolstad, & Mørkøre, 2008). Einen et

al. (1999) found higher contents of glycogen, a lower pH and a lower content of connective tissue in the fillet. Rørvik et al. (2018) found an increased growth rate in autumn (sec. 2.3). Furthermore, Scottish salmon fillets are found to gape more in June – July (Lavety, Afolabi, & Love, 1988).

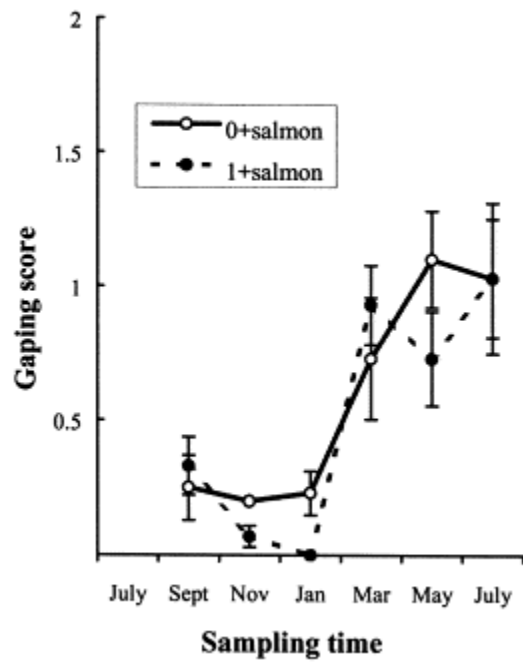


Figure 2.28. Seasonal variation in gaping score, measured by Anderson method (total range: 0 – 5), where 0 is no gaping and 5 indicates extreme gaping (Mørkøre & Rørvik, 2001).

### 3.0 Materials and methods

This thesis is composed of A) a literature-based theory part and B) a survey.

A) The collection of scholarly sources was a continuously job that went on through the entire master thesis. The scholarly sources were collected by using mostly Google Scholar and publications on Nofima`s webpage.

B) Involved a two-step workplan (fig. 3.1).

Step 1. Collection of contact details of salmon farming companies in Chile, Norway and Tasmania, where Norway was divided into the Northern part of Norway (North), Central part of Norway (Central) and southern part of Norway (South).

Step 2. Creation of a survey. The survey presented information regarding what months Chile, Norway (North, Central, South) and Tasmania experienced problems related to Atlantic salmon fillet quality. The survey was sent to on-growing farms.

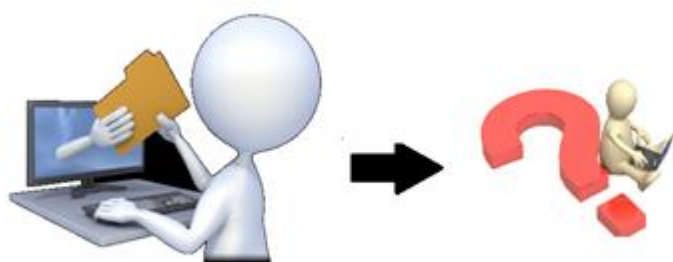


Figure 3.1. Illustration of the workplan. Locating on-growing farms in Chile, Tasmania and Norway. Retrieved from <https://www.spondylitten.no/kartlegger-fysioterapitilbudet-i-norge/> and <http://www.spydeberg.kommune.no/ending-i-byggereglene-fra-nyttaar.5831723-194641.html>

#### 3.1 Collection of contact details

Contact information was found by online research. Exploring company webpages, the use of social network sites (LinkedIn and Facebook) and attending aquaculture conferences (Håp I Havet) and events (YoungFish) were strategies used to connect with companies (fig. 3.2).

Companies received the survey mostly through email.



Figure 3.2. Contacting companies in Chile, Tasmania and Norway through social media and networking. Retrieved from Facebook, LinkedIn and fotolia.

### 3.2 Survey

A survey was made based on the collection of data from companies in Chile (6), South (4), Central (4), North (7) and Tasmania (3). In total 24 companies participated during the period October 2017 – February 2018, when the surveys were sent. Figure 3.3 illustrates how Norway was divided into three geographical areas. North included Finnmark, Troms and Nordland (blue), Central included Trøndelag and Møre og Romsdal (yellow), and South included Sogn og Fjordene, Hordaland, Rogaland and Vest-Agder county (green).

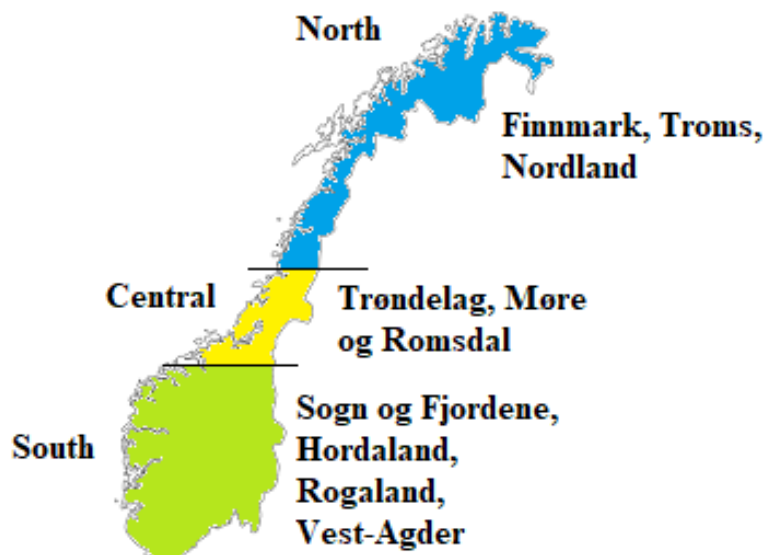


Figure 3.3 The geographical division of Norway. Blue illustrates North of Norway, yellow illustrates Central of Norway and green illustrates South of Norway. County are written to the right of the areas they include. Retrieved from <http://fargelegg.com/norgeskart-tegning-til-fargelegging/>

The survey was designed to be quick and was estimated to take two minutes to complete. The survey was composed of four questions with a comment box on the bottom of the survey. They four survey questions were closed<sup>2</sup>, while the comment box was a written question that gave additional information regarding the quality issues; fillet fat content, colour, melanin spots and gaping. The survey contained a brief introduction about the data sampling usage, contact details of person accountable for the data sampling and when the date of the survey was completed (Appendix A).

### 3.2.1 Layout of survey

The first two questions involved around the participants, where they could tick off the right answer (Appendix A). Hence, the collection of information such as company localization and the occupation of participants were given (fig. 3.4).

1. In which country is your company located?

Australia (Tasmania)

Chile

Norway; what part       North      Central      South

2. What is your position in the company?

Managing director

Financial manager or sales and marketed manager

Production manager

Quality manager

Veterinarian

IT-consultant

Apprentice

Other

Figure 3.4 Layout of question 1 and 2 in the survey.

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<sup>2</sup> No written questions



The third question was designed as a table where participants could choose multiple answers. The table showed the months in a two-month interval in the column. The fillet quality issues; fat content, colour, melanin spots and texture (gaping) were listed in one row each.

Participants were asked when these quality issues were problematic. Problematic being when consumers gave complaints back to the company regarding the fillet.

The fourth question was regarding the sea temperature. Participants were asked to fill in the sea temperature when they experienced most problem with each of the quality issues.

Temperatures ranged from 0 degrees Celsius to 25 degrees Celsius, but there was also an option to choose “lower” or “higher”.

3. Cross of the section where there are occurring problems resulting in bad fillet quality due to any of the factors (melanin spots, fat content, colour, texture; gaping) listed below. Multiple answers are possible.

	Jan - Feb	Mar - Apr	May - Jun	Jul - Aug	Sep - Oct	Nov - Dec
Melanin spots	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Fat content	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Colour	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Texture; gaping	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

4. What is the measured sea temperature when there are problems due to any of the factors listed below:

Melanin spots      Choose an item.

Fat content          Choose an item.

Colour                Choose an item.

Texture; gaping      Choose an item.

Figure 3.5 Layout of question 3 and 4 in the survey.

### 3.2.2 Confidentiality

The survey was anonymous, and this information was stated clearly in the e-mails and in the introduction of the survey. Anonymous meant that the information given by the company could not be linked to them in any way (StatisticsSolutions, 2013).

### 3.2.3 Description of months and seasons

The months in the different countries did not respond to the same seasons. There were four seasons in Chile, Tasmania and Norway; winter, spring, summer, autumn. Therefore, four different descriptions were made. The summer and winter season represented four months each, while spring and autumn represented two months each (table 3.1).

Table 3.1. Description of the different seasons in relation to months in Chile, Tasmania, Norway.

Months	Chile	Tasmania	Norway Central	Norway North	Norway South
November – February	Summer	Summer	Winter	Winter	Winter
Mars – April	Autumn	Autumn	Spring	Spring	Spring
May – August	Winter	Winter	Summer	Summer	Summer
September – October	Spring	Spring	Autumn	Autumn	Autumn

The calculation of months to season were completed separately for each country. The calculation of one specific month in Norway, would be opposite for Chile and Tasmania. According to table 3.1, November – February are summer in Chile and Tasmania. Mars – April are autumn in Chile and Tasmania and spring in Norway. May – August are winter and Chile and Tasmania and summer in Norway. September – October are spring in Chile and Tasmania and autumn in Norway. The calculations of months to seasons were therefore as followed (Appendix B, table 8.3);

$$Nov - Feb = \frac{Complaints\ Jan - Feb + Complaints\ Nov - Dec}{2 * n\ participants} \quad \text{Equation 3.1}$$

$$Mars - April = \frac{Complaints\ Mars - April}{n\ participants} \quad \text{Equation 3.2}$$

$$May - Aug = \frac{Complaints\ May - June + Complaints\ July - Aug}{2 * n\ participants} \quad \text{Equation 3.3}$$

$$Sept - Oct = \frac{Complaints\ Sept - Oct}{n\ participants} \quad \text{Equation 3.4}$$

Where  $n$  was the number of participants from Chile, Tasmania and Norway.

The seasons were used to describe the results of the survey in section 4.0 and discuss them in section 5.0.

### 3.3 Excel calculations and figures

This section is divided in two lower sections, where survey question one and two is presented in the first section (sec. 3.3.1) and survey question three is presented in the second section (Sec. 3.3.2).

- Section 3.3.1: Calculations of the total number of participants, response rate and occupations.
- Section 3.3.2. Customer complaints received from Chile, Tasmania and Norway was pooled and listed in table 8.1 (Appendix B). From this table, two new tables were created:
  - One pivot table the overall quality issues and country complaints (Appendix B, table 8.2).
  - One table showing seasons in relation to quality issue complaints (Appendix B, table 8.3).

#### 3.3.1 Participants information

The country and the number of survey participants and response rate in Chile, Tasmania and Norway was presented in a table (table 4.1). Response rate was calculated in percentage using formula:

$$\text{Response rate (\%)} = \left( \frac{\text{Number of } X}{\text{Total amount of } X} \right) 100 \% \quad \text{Equation 3.5}$$

Where  $X$  was companies.

The different occupation was presented in a table and converted to percentage. The results were then shown in a pie chart (fig. 4.1)

Similar statements from the survey comment box were pooled and calculated in percentage.

### 3.3.2 *Quality issues*

#### Overall and compared by country

A pivot table (Appendix B, table 8.2) was made for the creation of a pie chart (fig.4.2) and a clustered column graph (fig. 4.3).

The pie chart was calculated into percentage and showed the average quality issue for fillet fat content, colour, melanin spots and gaping, excluding seasons and countries.

The clustered column graph presented the quality issue in Chile, Tasmania and Norway, excluding the seasons. For the calculation of the column graph (fig. 4.3). The calculation of the percentage of the quality issues was completed separately for each country. All the data was calculated into average percentage of customer complaints using equation 3.6:

$$\begin{aligned} & \text{Average customer complaints (\%)} \\ & = \frac{(x \text{ participants complaints})}{(n \text{ participants} * 6 \text{ complaints})} * 100 \% \end{aligned} \qquad \text{Equation 3.6}$$

Where  $x$  is the overall number of complaints from participants for Chile, Tasmania and Norway (Appendix B, table 8.1), and  $n$  is the number of participants from each of these countries. In the equation, participants were multiplied with 6 complaints as question three were presented as two and two months together. Therefore, the highest complaints for each quality issue being 6.

#### Seasonal variations

Table 8.3 (Appendix B) showed seasons in relation to fillet quality issues; fat content, colour, melanin spots and gaping. The months were calculated and rearranged into season by equation 3.1 – 3.4 (sec. 3.2.3). Four box-plots for each quality issue and season were made. Boxplot shows the spread of the data and is good to use for comparison of different data sets. The boxplot gives five values; median, the lower quartiles (Q1), the upper quartile (Q3), the maximum and minimum value (fig. 3.5).

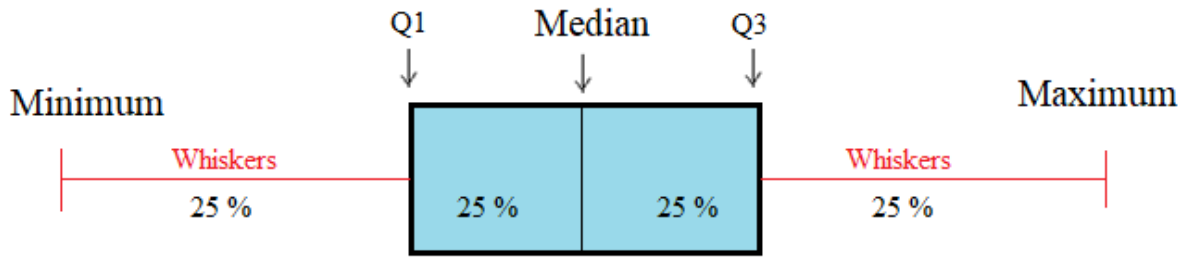


Figure 3.6. The layout of a boxplot, showing the lower quartile (Q1), upper quartile (Q3), median, minimum and maximum value.

The spread of the data is shown by the whiskers and can be divided in four sections. Each section representing where 25 percent of the data is localized. An extension of one of the sections indicates that there is a wider range in the values of the data set. Data that are more condensed, indicates a lower spread in the data set (closer together).

Table was used in statistical analysis, where there was made five column graphs from the results received from the Duncan test (sec. 3.4). Standard error (ER), average complaints for each quality issue and significant and non-significant season were here shown.

### 3.4 Statistical analysis

There was performed a one-way ANOVA F-test in the statistical analysis software (SAS) (Appendix C) using the frequency of quality complaints from each season calculated by the equation 3.1 – 3.4 (Appendix B, table 8.3). This was to find out whether there was an overall seasonal effect on the quality issues. A Duncan – test was performed to see which seasons that differed from the others (Bewick, Cheek, & Ball, 2004). For the fat content complaints there was run another ANOVA F-test in SAS comparing autumn and summer with winter and spring.

There are two types of error that can be committed when testing a hypothesis. Type 1 error is when a hypothesis is rejected even though it is true, while type 2 error is when a hypothesis is accepted even though it is false. The hypothesis stated that there were seasonal variations amongst the quality issues; fillet fat content, colour, melanin spots and gaping ( $H_1$ ). The null hypothesis ( $H_0$ ) stated therefore that it was no seasonal variations amongst the quality issues; fillet fat content, colour, melanin spots and gaping.

Duncan test was especially protective against error 2 but had a greater risk of making type 1 errors and reject  $H_0$  even though it was true (fig. 3.7) (Bewick et al., 2004).

		Reality	
		$H_0$ Is True	$H_1$ Is True
Conclusion	Do Not Reject $H_0$	Correct Conclusion	Type II Error
	Reject $H_0$	Type I Error	Correct Conclusion

Figure 3.7. Type 1 and type 2 error in statistical analysis. Retrieved from <https://mat117.wisconsin.edu/3-type-i-and-type-ii-errors/>

The Standard error (S.E.) represents the standard deviation of the mean within a dataset. This serves as a measure of variation for random variables ( 3.7).

$$SE = \frac{SD}{\sqrt{n}}$$

Formula 3.7

The higher the denominator or sample size (n), the smaller the S.E. because the statistic will approach the “true value”. The sample size will in this experiment be  $n = 5$  as there were 5 locations: Chile, Tasmania and South-, Central- and North of Norway. Meaning that the variable in would be the Standard deviation or the numerator (SD). Therefore, the higher the SD, the higher the spread of the data from the average value and therefore less accuracy.

## 4.0 Results

### 4.1 Participants information

There was a total of 24 participants, which corresponded to 53 percent response rate. Highest response rate was from Tasmania, followed by North of Norway, Chile, South of Norway and lowest response rate in Central part of Norway relative to the surveys that were sent to the companies (table. 4.1).

Table 4.1. The number of participants and the response rate given in percentage in Chile, Tasmania and Norway.

Country	No. of participants	Response rate (%)
Tasmania	3	100
Chile	6	46
Norway South	4	44
Norway Central	4	40
Norway North	7	70
<b>Total</b>	<b>24</b>	<b>53</b>

The survey was mostly answered by quality managers, followed by veterinarians and other occupations in the company, where “others” often was clicked in addition to quality managers and veterinarians (fig. 4.1).

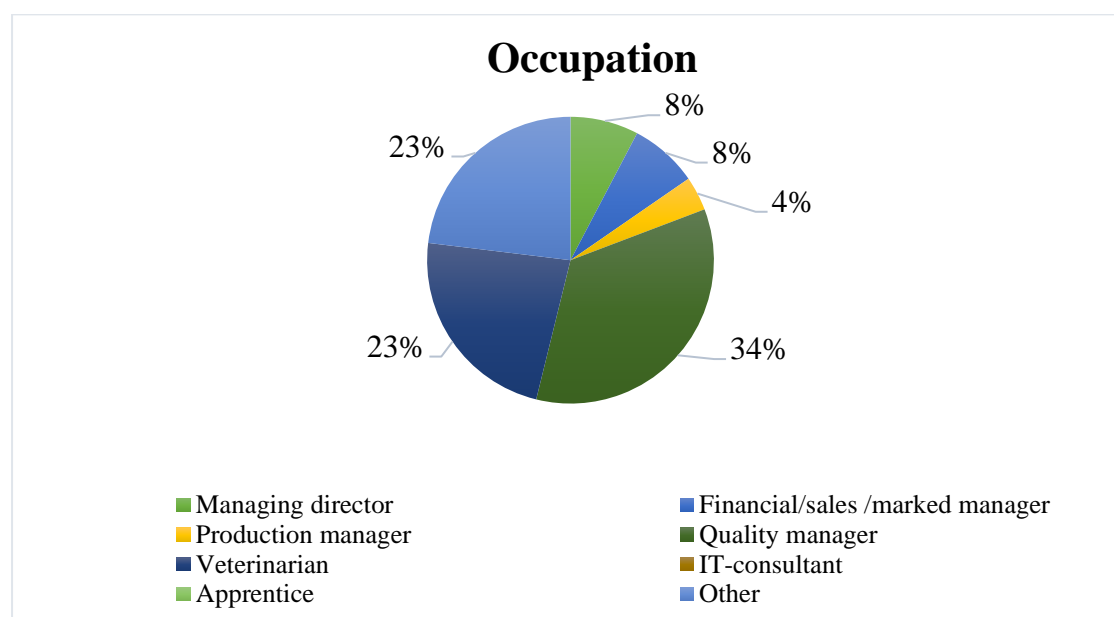


Figure 4.1. The occupations of the survey participants given in percentage of the total response.

Comments from participants:

- 50 percent of the participants mentioned that sea temperature wasn't a seasonal quality issue for fillet fat content, colour, melanin spots and gaping.
- 17 percent of the participants mentioned that poor fillet colour was a result of sexual maturation.
- 14 percent of the participant mentioned that melanin spots were a problem that occurred sporadic throughout the year.
- 4 percent of the participants mentioned that there was found a solution in the feed that could reduce melanin spots by 90 percent.



## 4.2 Quality issues – overall and compared by country

Melanin spots was the largest quality issue in Chile, Tasmania and Norway, followed by gaping, colour and fat content (fig. 4.2). The yearly occurrence of customer complaints related to melanin spots was highest in Central Norway, while customer complaints concerning gaping was most problematic in Chile. Colour however was most problematic in South of Norway and fat content was most problematic in North of Norway and fat content was most problematic in North of Norway (fig. 4.3).

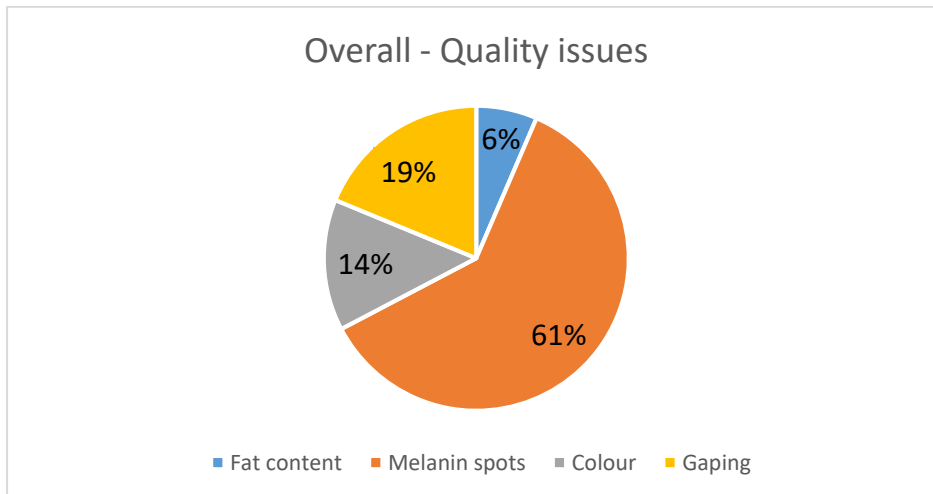


Figure 4.2. Pie chart comparing the overall quality issues in Chile, Tasmania and Norway given in average percentage of customer complaints.

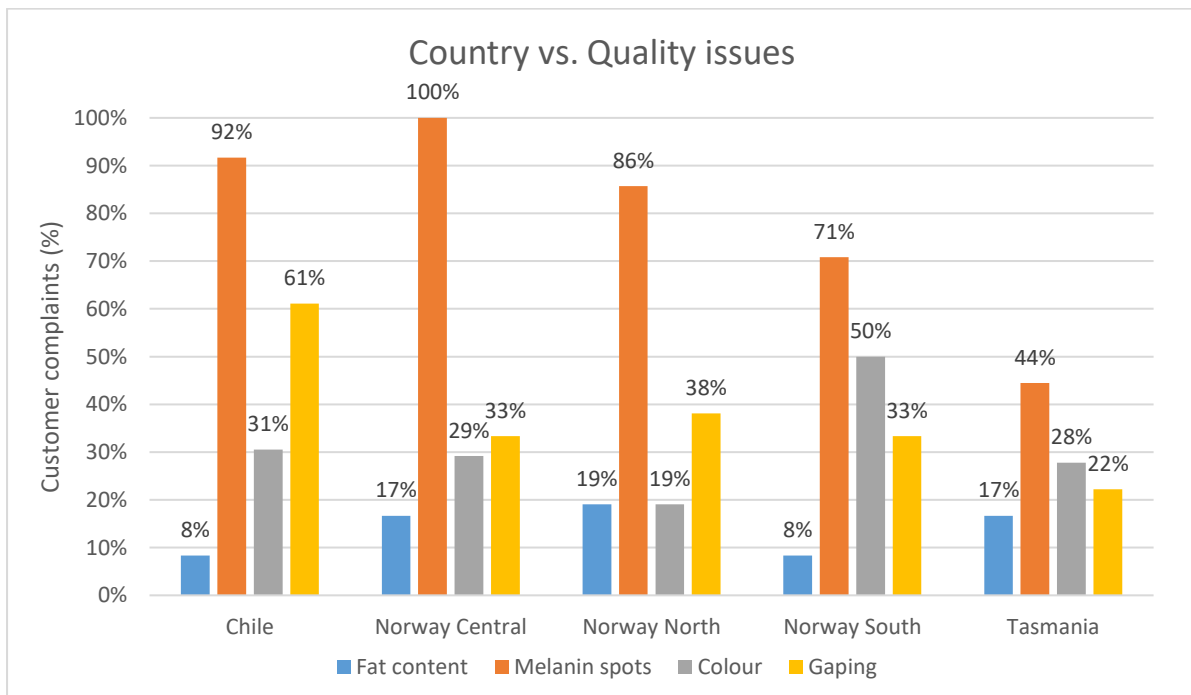


Figure 4.3. Average percentage of customer complaints of the quality issues fat, melanin, colour and gaping in Chile, Norway and Tasmania.

### 4.3 Seasonal variations

A one - way ANOVA F – test was performed to check if there were any overall seasonal significance effects regarding fat content, colour, melanin spots and gaping (table 4.2).

Table 4.2. Statistical data from one-way ANOVA analysis of seasonal effects regarding the fillet quality issues; fat content, colour, melanin spots and gaping. *P* value and *R* – square is noted.

Quality Issues	<i>P</i> value	<i>R</i> -square
<b>Fat</b>		
ANOVA	0.29	0.20
ANOVA (autumn, summer vs. winter, spring)	0.05	0.20
<b>Colour</b>		
ANOVA	0.0005	0.66
<b>Melanin</b>		
ANOVA	0.85	0.05
<b>Gaping</b>		
ANOVA	0.08	0.34

#### 4.3.1 Fat content

For the complaints regarding fat content the *R-square* and *p* value were calculated to be 0.20 and 0.29, respectively. The *p* value was higher than 0.05, and it was therefore no overall significant effect of seasons regarding the fat content complaints.

For the second ANOVA test, the *R-square* and *p* value were calculated to be 0.20 and 0.05, respectively (table 4.2). There was a seasonal difference between summer, autumn and winter, spring (fig. 4.5), where the average values for summer and autumn were significantly higher than the average value of winter and spring (fig. 4.4).

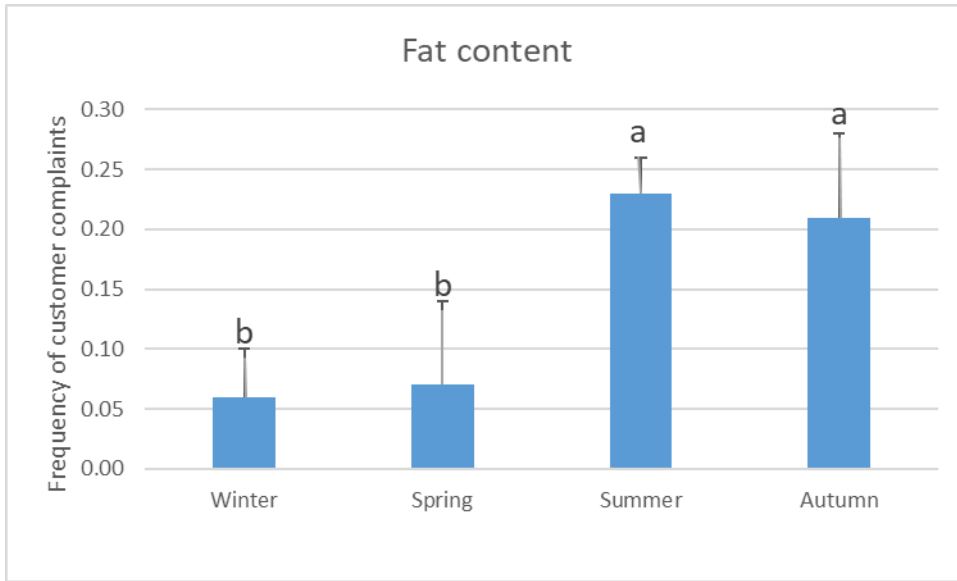


Figure 4.4. Average fat content complaints in the fillet of Atlantic salmon according to season, where the y- axis shows the frequency of customer complaints. The error bars show the standard error (S.E.), while significance differences amongst the seasons are indicated with different letters.

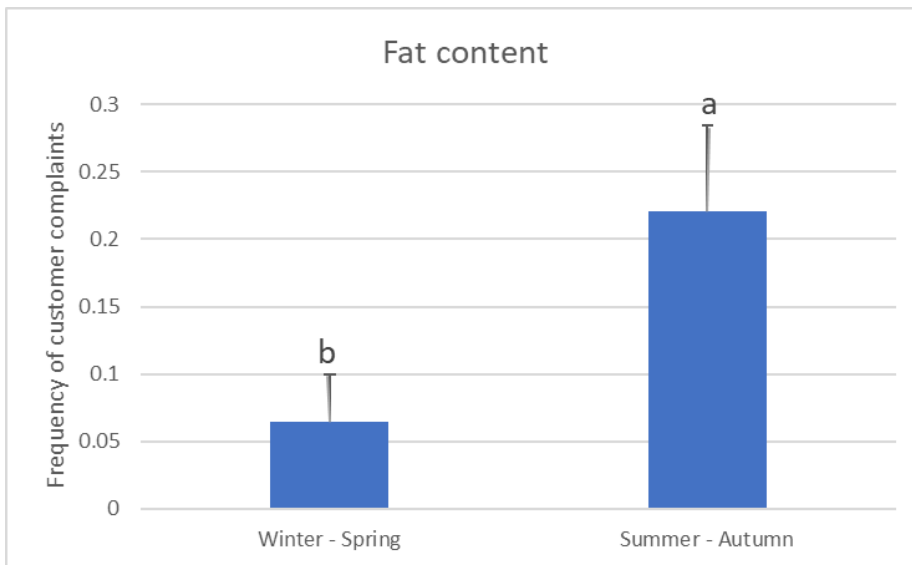


Figure 4.5. Average fat content complaints in the fillet of Atlantic salmon according to season, where the y- axis shows the frequency of customer complaints. The error bars show the standard error (S.E.).

The variation among the respondents concerning fat within season is shown in figure 4.5. There was a numerically high variation amongst the respondents in autumn (total range 0 percent – 57 percent) compared to the other seasons (fig. 4.6).

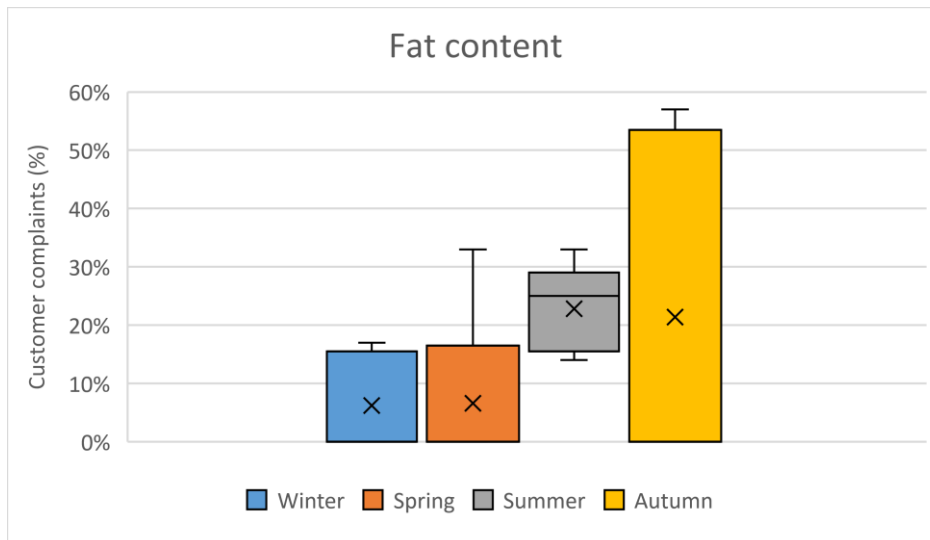


Figure 4.6. Occurrence of customer complaints of the fat content in the fillet of Atlantic salmon according to seasons. The whiskers represent the spread of the data from the minimum to the maximum value. The upper and lower quartiles are represented in the figures as the top and the bottom of the box. The median is indicated by a line close to  $x$ , which represents the average value of the dataset. If median is not shown, it is because it is a part of the lower quartile.

#### 4.3.2 Fillet colour

For the complaints regarding colour, the *R-square* and *p value* were calculated to be 0.66 and 0.0005, respectively. The *p value* was lower than 0.05, and it was therefore an overall seasonal significance regarding to the colour complaints (table 4.2). From the Duncan – test, autumn was found to be significantly different from the other seasons that were similar. Spring and autumn had a somewhat higher S.E. than summer and winter (figure 4.7).

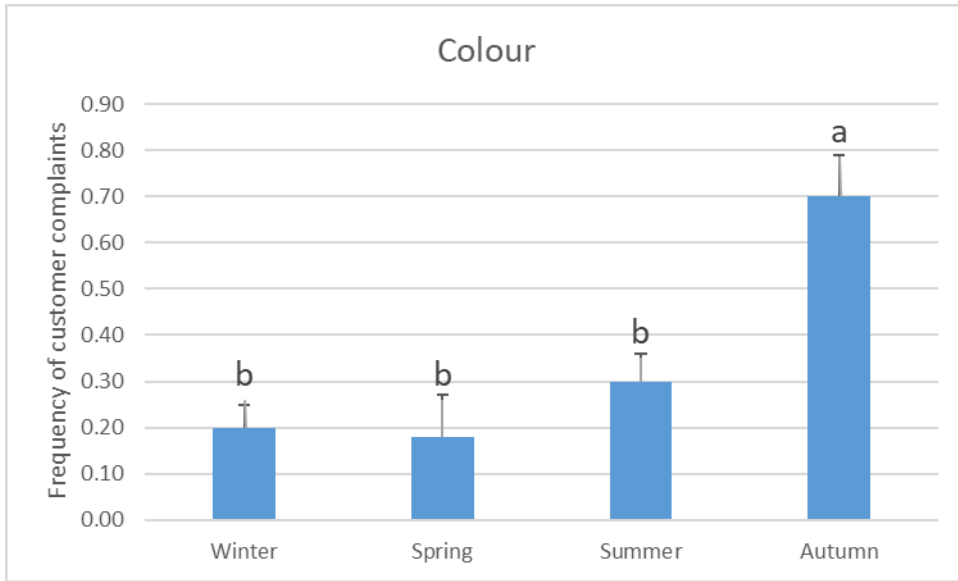


Figure 4.7. Average colour complaints in the fillet of Atlantic salmon according to season, where the y-axis shows the frequency of customer complaints. The error bars show the standard error (S.E.).

There was no overlap amongst the customer complaints received in autumn (total range 50 percent – 100 percent) compared to the other season (0 percent – 50 percent) (fig. 4.8).

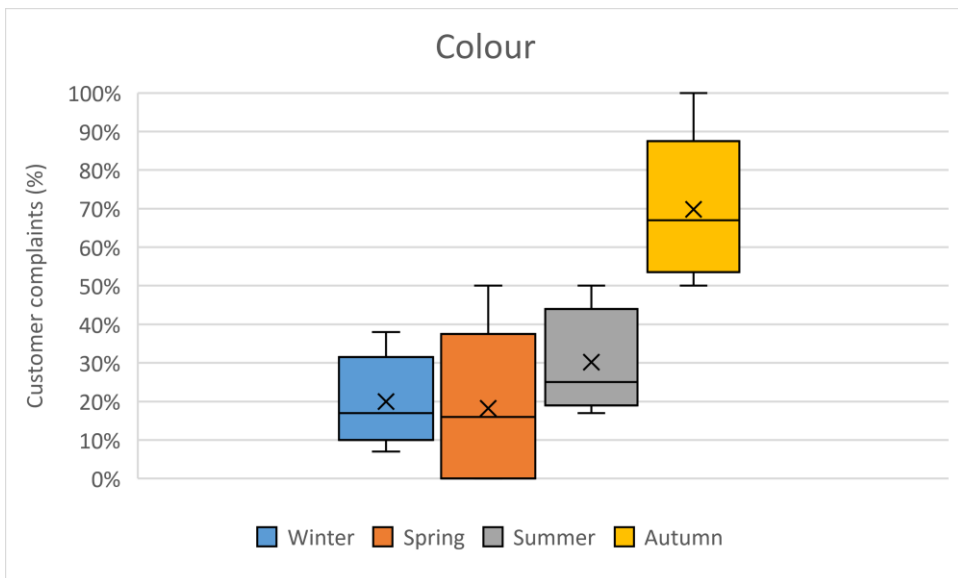


Figure 4.8. Occurrence of customer complaints of the fillet colour in Atlantic salmon according to seasons. See text in figure 4.6 for more information.

### 4.3.3 Melanin spots

For the complaints regarding melanin spots the *R-square* and *p value* was calculated to be 0.05 and 0.85, respectively. The *p value* was higher than 0.05, and it was therefore no overall significant effect of seasons regarding to the melanin spots complaints. (table. 4.2).

The average value for complaints regarding melanin was numerically high for all the seasons, where winter and spring showed a somewhat higher S.E. than summer and autumn (fig. 4.9).

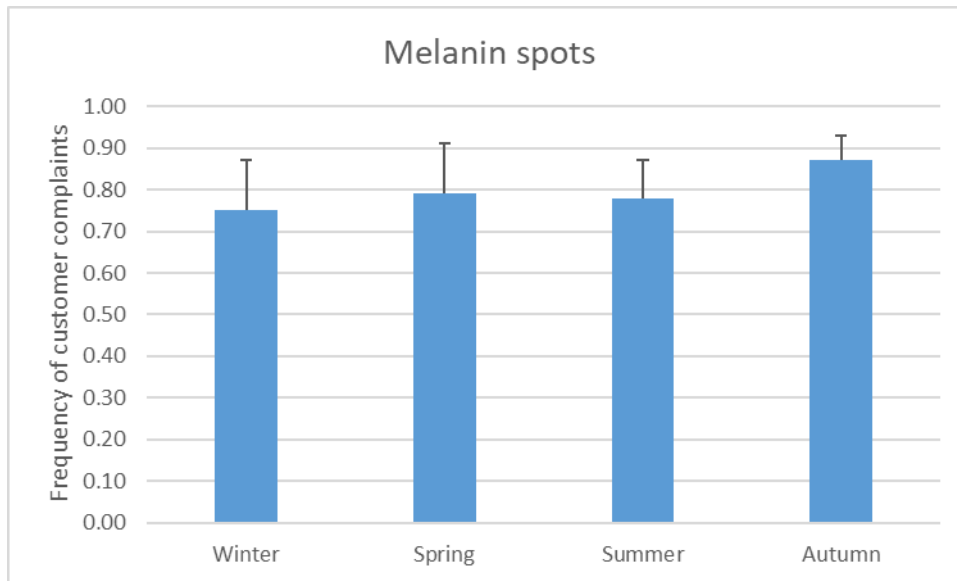


Figure 4.9. Average melanin spots complaints in the fillet of Atlantic salmon according to season, where the y-axis shows the frequency of customer complaints. The error bars show the standard error (S.E.).

All the seasons showed high percentage of customer complaints. The data amongst the respondents was more condensed in autumn (total range 67 percent – 100 percent) compared to the other seasons (total range 33 percent – 100 percent). However, all the seasons overlapped each other. Therefore, it was no indication of seasonal customer complaints regarding melanin spots (fig. 4.10).

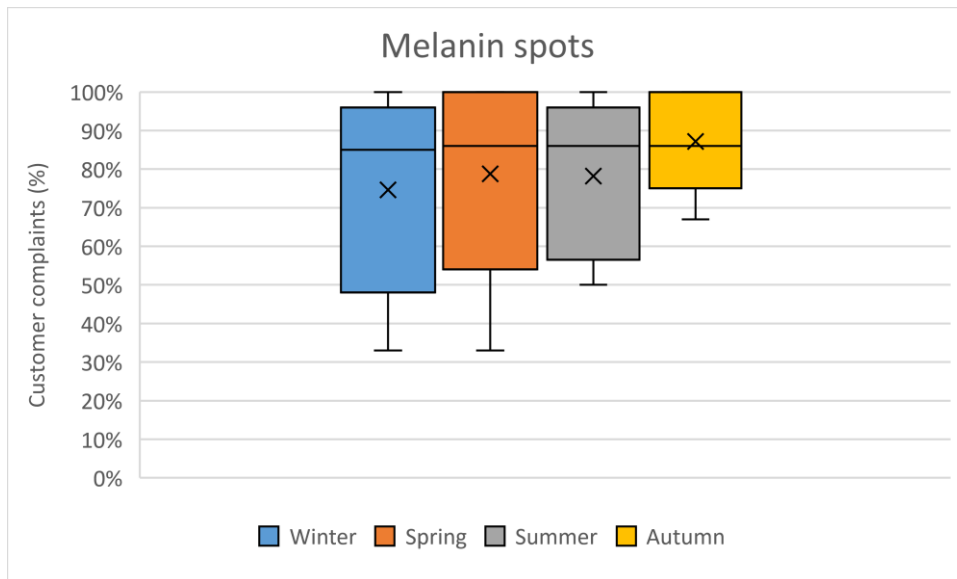


Figure 4.10. Occurrence of customer complaints of melanin spots in the fillet of Atlantic salmon according to seasons. See text in figure 4.6 for more information.

#### 4.3.4 Gaping

For the complaints regarding gaping the *R-square* and *p value* were calculated to be 0.34 and 0.08, respectively. A *p value* of 0.08 indicates an overall seasonal trend, but not an overall significant difference (table. 4.2). However, there was revealed a significant difference within the seasons from the Duncan test (fig. 4.11).

Comparisons of complaints on gaping amongst seasons, showed a significantly higher frequency of complaints in autumn compared with summer, spring and winter. The S.E. was numerically highest for summer and lowest for spring (fig. 4.11).

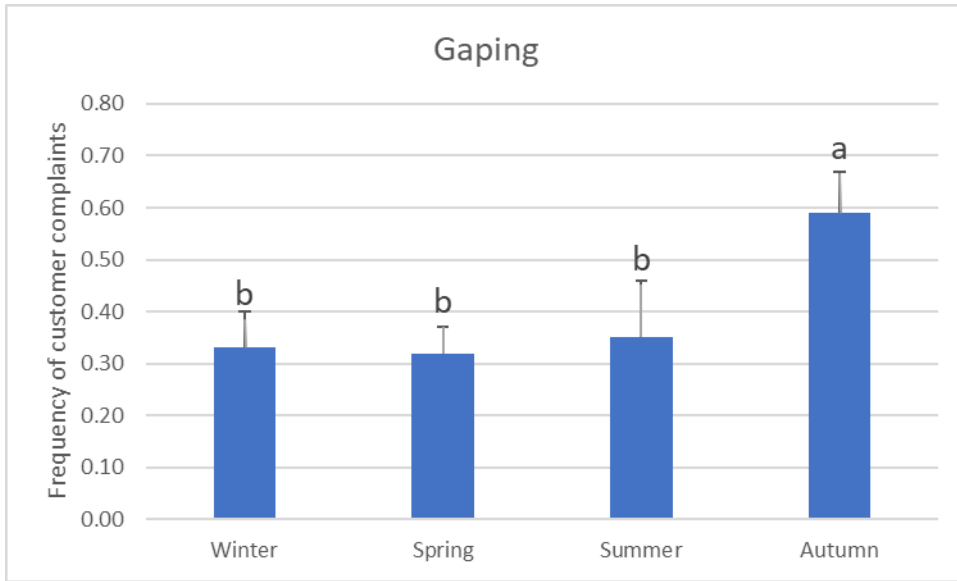


Figure 4.11. Average gaping complaints in the fillet of Atlantic salmon according to season, where the y-axis shows the frequency of customer complaints. The error bars show the standard error (S.E.), while significance differences amongst season are indicated with different letters.

There was a numerically higher variation amongst the respondents in summer (total range 17 percent – 75 percent), followed by autumn (total range 33 percent – 75 percent), winter (total range 17 percent – 50 percent) and spring (total range 17 percent – 75 percent), where there was an overlap between the range of customer complaints between the seasons (fig. 4.12).

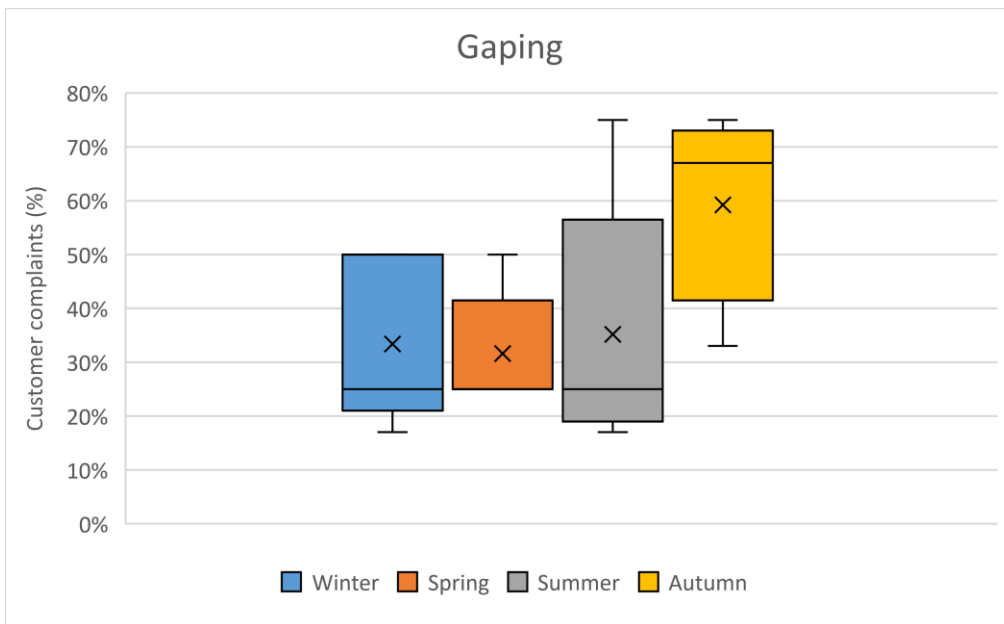


Figure. 4.12. Occurrence of customer complaints of the gaping in the fillet of Atlantic salmon according to seasons. See text in figure 4.6 for more information.



## **5.0 Discussion**

### **5.1 Participants information**

The response rate was 53 percent and a total of 24 participants (table 4.1). The highest response rate in Tasmania correlates to the few salmon farming (three in total). In contrast, there are 98 and 76 salmon farming companies in Norway and Chile, respectively (Ibieta, Tapia, Venegas, Hausdorf, & Takle, 2011; Marineharvest, 2016). The relatively high response rate in North of Norway can be explained by direct contact with the industry by participating at an Aquaculture conferences in North of Norway (Tromsø). Face-to-face communication in earlier studies has revealed to be an information rich medium and a good way to get in contact with people (sec. 3.1) (Doherty et al., 1997; Short, Williams, & Christie, 1976). The lower response rate found in Chile compared to Tasmania and North of Norway might have been because of a language barrier, where Tasmania and Norway could receive the survey in their mother tongue.

There were mostly quality managers and veterinarians that answered the survey, often with the help from other people in the company with different occupations (fig.4.1). Meaning that two participants often were cooperating on answering the survey. The data from the survey are therefore considered credible.

### **5.2 Quality issues – overall and compared by country**

Melanin spots was the numerically biggest quality issues, where problems regarding gaping, colour and fat content occurred in a smaller extent (fig. 4.2).

#### *5.2.1 Fat content*

The reason for fat content being a numerically higher problem in North of Norway compared to Chile, Tasmania, South and Central of Norway might be because the day length in North of Norway show a higher variation between the seasons (fig. 2.8), where declining day length in autumn has shown to increase the growth and accumulation of fat (Rørvik et al., 2018).

#### *5.2.2 Fillet colour*

The reason for fillet colour being a numerically higher problem in South of Norway compared to Central and North of Norway might be because the average summer sea temperature of South of Norway is higher than the sea temperature of Central and North of

Norway according to figure 2.7. High sea temperature increases oxidative stress, were an increased metabolic rate will result in more free radicals to be produced. The reason for a poor fillet colour could therefore be that astaxanthin is metabolised to prevent the free radicals to react with the cell membrane and DNA (Ambati et al., 2014; Anuradha, 2018; Nakano et al., 2014). However, if sea temperature was the only reason for poor fillet colour we would expect to see a numerically higher occurrence of problems regarding to colour in Chile as the sea temperature is higher. In addition, we would also have expected to see similarities in Tasmania regarding the colour as the sea temperature here are similar to the sea temperature in South of Norway. Companies from Chile and Tasmania argued that sexual maturation was the reason for bad flesh colour and did therefore not “tick” of problems related to colour in question 3 in the survey (Appendix A). The complaints regarding colour in Chile and Tasmania should therefore probably have been higher for Tasmania and Chile.

### *5.2.3 Melanin spots*

Norway and Chile had numerically higher problem regarding to melanin spots than Tasmania (fig. 4.3). Atlantic salmon is not a native species in Tasmania (Governments, 2016), and is therefore isolated from other wild and farmed salmon that can carry diseases. This might result in a decreased problem of the major infectious disease in salmon, which might be a possible explanation for Tasmania having numerically lower problems related to melanin spots as melanin seem to occur synchronously with disease outbreaks (Bjørngen et al., 2015; Lerfall et al., 2012; Mackintosh, 2001).

### *5.2.3 Gaping*

Gaping was the second overall largest quality issue (fig. 4.2) and showed a numerically higher problem in Chile (fig.4.3). Temperature can result in serious damage in poikilothermic organisms and cause metabolic stress in the body (Nakano et al., 2014). When sea temperature reach up to 19 degrees Celsius, the feed intake and growth is reduced which is a sign that salmon doesn't thrive in the environment (Hevrøy et al., 2013). This causes the fish to stress and might therefore increase the gaping in the fillet (Suzuki, 1981). Chile reach an average sea temperature of 18 degrees Celsius in the end of summer and are also containing a higher overall sea temperature compared to Norway and Tasmania (fig. 2.7).

### 5.3 Seasonal variations

There were seasonal quality issues in the salmon fillets, where autumn was the most problematic season for fillet colour and gaping, while summer and autumn was the most problematic season for fat content (fig. 5.1). There was no seasonal effect on melanin spots which seemed to be a numerically high problem throughout the year (table 4.2).

According to half of the participants, sea temperature wasn't a direct issue related to seasonal fillet quality and the sea temperature was therefore inconsistent when comparing the season to the quality issues; fillet fat content, colour, melanin spots and gaping (fig. 5.1)

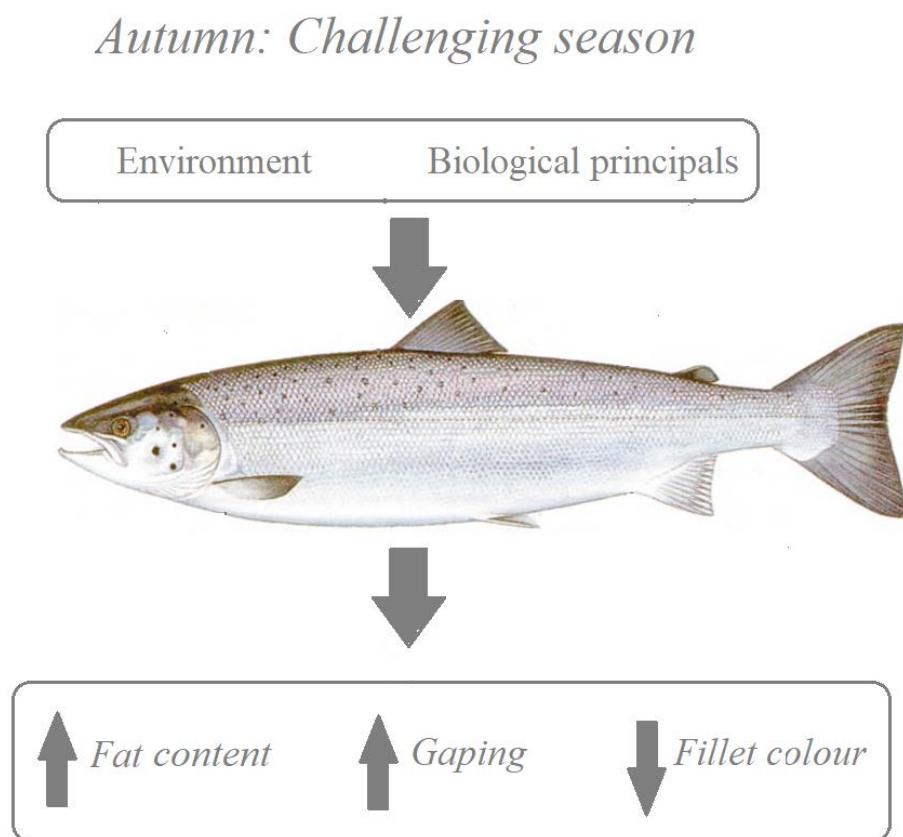


Figure 5.1. Atlantic salmon reared in sea cages in autumn. Illustrating the affect environment and biological principals has on the quality issues: fillet fat content, gaping and colour. Retrieved from <https://wdfw.wa.gov/fishing/salmon/atlantic.html>

#### 5.3.1 Fat content

Fat content was problematic in summer and autumn, where the seasonal variations were found to explain 20 percent of the problems with excessive fat accumulation (table 4.2). Declining day length in autumn increases the accumulation of fat in the fillet (Mørkøre & Rørvik, 2001; Rørvik et al., 2018). The reason for fat accumulation in autumn are most likely

linked to the sexual mature process (Alne et al., 2011), but the fat accumulation can also be linked to the evolutionary need of energy in winter as the availability of food can be low during winter for wild salmon.

Autumn and spring had higher complaints regarding the fat content, where the average value was close for autumn and summer. According to table 3.1, August is the last summer month for Norway, while February is the last summer months for Chile and Tasmania. In figure 2.8, daylength starts declining in February for Chile and Tasmania, while a decline in day length is first seen in August for Norway. Therefore, when summer is pooled with autumn we get the last summer month (August and February) where a decline in day length is noticeable. Therefore, this might be the reason for why autumn and summer becomes significantly different from winter and spring.

Tasmania, Chile and Norway are in different parts of the world on three different continents, where the day length and sea temperature vary considerably between and within the countries (fig. 2.7 and fig. 2.8). According to figure 2.7, the summer sea temperature in North of Norway doesn't reach the winter sea temperature in Chile and Tasmania. It is therefore understandable that seasons in one location can be shorter than the same season found in another location, or vice versa. The same can be said for seasons within Norway too, where Central and South of Norway show less variation than North as it geographically stretches far South and far North. Meteorologists in Norway argued that the first day of summer was when the average temperature was above 10 degrees. If this meteorological definition is used, some of the places in North of Norway will not have summer (Pedersen & Mamen, 2012). There might therefore be a big difference on the length of the season between countries, where the summer in North of Norway might be shorter than anticipated. Fat content complaints from North of Norway that are "clicket" of in question 3 in the survey in August (Appendix A) might therefore actually be from autumn and not summer (table 3.1).

North of Norway had the numerically highest problem with fat compared to Chile, Tasmania, South and Central of Norway (fig. 4.3). Low sea temperatures in North of Norway causes slower growth (Austreng et al., 1987) and results in a leaner fish when entering autumn. Salmon will therefore have a higher accumulation of fat and an increased growth in autumn (Rørvik et al., 2018). In recent studies, rapid growth was found to result in more gaping (Einen et al., 1999; Folkestad et al., 2008). Therefore, it would be expected to see higher problems related to gaping in North of Norway (sec. 8.3.4).

### 8.3.2 Fillet colour

Fillet colour was revealed to be highly significant in autumn (fig. 4.7), where seasonal variations could explain 66 percent of the problems with pale colour (table 4.2). Astaxanthin is poorly digested and absorbed in the intestine, where the muscle retention is less than 10 percent (Bjerkeng & Berge, 2000). The reason for a reduced fillet colour in the autumn can be associated with an increased growth rate that will result in an increased intestinal rate, which will result in a poorer absorption of astaxanthin over the intestine (Rørvik et al., 2010). When the intestinal rate increases, astaxanthin have less time in the intestine and therefore less time to be digested and absorbed.

### 8.3.3 Melanin spots

There was no overall seasonal effect on melanin spots, where melanin spots seemed to be a problem throughout the year (fig 4.9 and fig. 4.10). The data could only explain 5 percent of the problems concerning melanin spots to be because of season (table 4.2). Meaning that 95 percent of the occurrence of melanin spots complaints could not be explained by season. There is a balance between antioxidants in the fish, where a supplementation of antioxidants decreased the size and presence of melanin spots (Wang, 2016). Decreased levels of astaxanthin might therefore increase the occurrence of melanin spots. There was a higher problem related to fillet colour in autumn (fig. 4.7) and the complaints regarding melanin spots have a numerically higher average value and a numerically lower S.E. in autumn (fig. 4.9), where the data amongst the respondents was more condensed in autumn compared to the other seasons for melanin spots (fig. 4.10). Therefore, there is a slightly similar tendency between problems regarding melanin spots and fillet colour in autumn, where a poorer fillet colour and a slightly numerically higher problem related to melanin. Christiansen et al. (1995) showed a decreased growth of the Atlantic salmon fed without astaxanthin. However, there was no effects regarding melanin spots.

One of the participant claimed that an ingredient in the fish feed reduced the melanin spots by 90 percent. The ingredient of this feed was confidential; However, there are most likely differences in the fish feed given to the salmon in Chile, Tasmania and Norway which can affect all the quality issues. Many factors influence the occurrence of melanin spots.

Supplementing of antioxidants and increase the levels of DHA and EPA can be mentioned as one of the factors (Mørkøre, 2017). There (Contreras & Rapoport, 2002). The growth rate increases in autumn and large amounts of fat are stored (Rørvik et al., 2018). The n-3 and n-6

pathways use the same enzymes, and it is therefore crucial with a balanced diet between n-3 and n-6 (Lee et al., 1967; Owen et al., 1975), where salmon fed low amounts of DHA and EPA (2 g/kg EPA and DHA) had increased levels of n-6 in their cell membrane (Bou, Berge, Baeverfjord, Sigholt, Østbye, et al., 2017), and diets rich on linoleic acid (n-6) showed reduced survival (Owen et al., 1975).

#### 8.3.4 *Gaping*

Gaping showed a seasonal trend, and even though the seasonal variations could explain 34 percent of the problems with gapping, no overall seasonal effect was found (table 4.2). However, within the seasons, autumn was significantly different from the other seasons (4.11). Therefore, gapping was expected to be a bigger problem in autumn than the other seasons. Fast growth have been linked to a softer texture (Einen et al., 1999; Folkestad et al., 2008; Mørkøre & Rørvik, 2001), where a softer texture is found to correlate with more gapping (Espe et al., 2004). The problems with gapping in autumn is therefore probably caused by the increased growth rate (Rørvik et al., 2018), where most of the fat is stored intramuscular as suggested by Weihe et al. (Inpress) due to documentation of increased slaughter yield in the autumn, compared to the spring.

Figure 4.11 and 4.12 show a slightly lower average gapping in the spring with low S.E. and little spread in the data set. The reason for a numerically lower customer complaints in spring are unknown.

The reason for more gapping in autumn might be because the production of muscle fibres and connective tissue doesn't keep up with the fast growth when large amounts of fat is stored in the fillet (Weihe et al., In press). Restricted feeding has seen to give a firmer texture, where the fillet with fast growth and softer texture had a lower content of connective tissue, lower pH and are probably connected with the texture of salmon somehow. Furthermore, starvation prior to slaughter time might be a way to decrease gapping (Einen et al., 1998).

## 6.0 Conclusion

Fillet fat content, colour and gaping showed seasonal variations. A higher problem related to gaping and colour was found in the autumn. Fat content was a higher problem in summer and autumn than winter and spring. Problem with excessive fat accumulation, gaping and pale colour seem to occur synchronously and are linked to the sea temperature, day length and the biological growth cycle (biological principals) of Atlantic salmon.

Chile, Tasmania and Norway are located in three different continents. There are large variations in sea temperature and day length between the countries and within the localities North, Central and South of Norway. However, seasonal quality issues seem to occur in farmed Atlantic salmon regardless of where the fish is reared in the world. The interaction between sea temperature, day length and growth pattern of Atlantic salmon are affecting the quality issues. However, based on existing information there are several knowledge gaps and further knowledge is required to provide information about why autumn is a problematic season.

### Further recommendations:

#### *Experiments*

- For further studies, there are needed an increased knowledge of the underlying mechanisms that causes seasonal quality downgrading to minimize the problem regarding gaping, fat content and colour in the fillet of Atlantic salmon in autumn.
- Further studies are needed to understand why melanin spots are continuously high throughout the year.

#### *Survey*

- For further studies, more personal contact would be recommended and calling rather than emails to increase the response rate.

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## 8.0 Appendix

### 8.1 Appendix A

#### Survey - Season and quality issues in Atlantic salmon fillets

This questionnaire is a part of a master thesis at the University of life sciences (NMBU) in Ås, Norway. The aim of the questionnaire is to study the different quality issues perceived of Atlantic salmon in relation to season variation.

All information will be anonymous and will be used in a statistical analyse. The questionnaire will take approximately 2 minutes. If you have any questions regarding the questionnaire, please do not hesitate to contact Monica Nordberg.

Email: [Monicanordberg93@gmail.com](mailto:Monicanordberg93@gmail.com)

Tlf: 46421893

Your help is much appreciated! Thank you for taking the time to answer this survey.

Please insert today's date: Click or tap to enter a date.

1. In which country is your company located?

Australia (Tasmania)

Chile

Norway; what part

North

Central

South

2. What is your position in the company?

Managing director

Financial manager or sales and marketing manager

Production manager

Quality manager

Veterinarian

IT-consultant

Apprentice

Other

3. Cross of the section where there are occurring problems resulting in bad fillet quality due to any of the factors (melanin spots, fat content, colour, texture; gaping) listed below. Multiple answers are possible.

	Jan - Feb	Mar - Apr	May - Jun	Jul - Aug	Sep - Oct	Nov - Dec
Melanin spots	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Fat content	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Colour	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Texture; gaping	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

4. What is the measured sea temperature when there are problems due to any of the factors listed below:

Melanin spots      Choose an item.

Fat content      Choose an item.

Colour      Choose an item.

Texture; gaping      Choose an item.

Comments:

Click or tap here to enter text.

## 8.2 Appendix B

### Tables from survey – Quality issues (Question 3)

Table 8.1 Overall customer complaints (blue) for fat, colour, melanin and gaping (pink) for every second month in Chile, Tasmania, Norway Central, Norway North and Norway South.

Sum of Fat content					
Months	Chile	Tasmania	Norway Central	Norway North	Norway South
Jan-Feb	1	1	0	0	0
Mar-Apr	0	0	0	0	0
May-Jun	0	0	1	1	1
Jul-Aug	1	0	1	1	1
Sep-Oct	0	1	2	4	0
Nov-Dec	1	1	0	2	0
Grand Total	3	3	4	8	2
Sum of Colour					
Months	Chile	Tasmania	Norway Central	Norway North	Norway South
Jan-Feb	2	1	0	0	1
Mar-Apr	4	3	1	0	2
May-Jun	2	1	1	0	2
Jul-Aug	1	0	2	3	2
Sep-Oct	1	0	2	4	3
Nov-Dec	1	0	1	1	2
Grand Total	11	5	7	8	12
Sum of Melanin					
Months	Chile	Tasmania	Norway Central	Norway North	Norway South
Jan-Feb	6	2	4	6	3
Mar-Apr	5	2	4	6	3
May-Jun	5	1	4	6	3
Jul-Aug	6	1	4	6	2
Sep-Oct	6	1	4	6	4
Nov-Dec	5	1	4	6	2
Grand Total	33	8	24	36	17
Sum of Gaping					
Months	Chile	Tasmania	Norway Central	Norway North	Norway South
Jan-Feb	5	0	1	2	1
Mar-Apr	4	1	1	1	1
May-Jun	3	1	1	1	1
Jul-Aug	3	0	1	2	2
Sep-Oct	3	1	3	5	2
Nov-Dec	4	1	1	5	1
Grand Total	22	4	8	16	8

Table 8.2. Data from pivot table country vs quality issue

Country	Sum of Fat content	Sum of Melanin	Sum of Colour	Sum of Gaping	Overall score
Chile	3	33	11	22	69
Norway C	4	24	7	8	43
Norway N	8	36	8	16	68
Norway S	2	17	12	8	39
Tasmania	3	8	5	4	20
Overall score	20	188	43	58	309

Table 8.3 Data calculated to seasons. Table used in SAS for statistical analysis (see appendix C)

	Season	Region	Fat	Colour	Melanin	Gaping
	Winter	Chile	0.17	0.25	0.92	0.50
	Winter	Tasmania	0.00	0.17	0.33	0.17
	Winter	NorwayN	0.14	0.07	0.85	0.50
	Winter	NorwayC	0.00	0.13	1.00	0.25
	Winter	NorwayS	0.00	0.38	0.63	0.25
	Spring	Chile	0.00	0.16	1.00	0.50
	Spring	Tasmania	0.33	0.00	0.33	0.33
	Spring	NorwayN	0.00	0.00	0.86	0.25
	Spring	NorwayC	0.00	0.25	1.00	0.25
	Spring	NorwayS	0.00	0.50	0.75	0.25
	Summer	Chile	0.17	0.25	0.92	0.75
	Summer	Tasmania	0.33	0.17	0.50	0.17
	Summer	NorwayN	0.14	0.21	0.86	0.21
	Summer	NorwayC	0.25	0.38	1.00	0.25
	Summer	NorwayS	0.25	0.50	0.63	0.38
	Autumn	Chile	0.00	0.67	0.83	0.67
	Autumn	Tasmania	0.00	1.00	0.67	0.33
	Autumn	NorwayN	0.50	0.57	0.86	0.71
	Autumn	NorwayC	0.57	0.50	1.00	0.75
	Autumn	NorwayS	0.00	0.75	1.00	0.50

## 8.3 Appendix C

### 8.3.1 Fat content - Statistical analysis software (SAS)

#### The SAS System

#### The GLM Procedure

Dependent Variable: Fat

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.12377500	0.04125833	1.36	0.2901
Error	16	0.48480000	0.03030000		
Corrected Total	19	0.60857500			

R-Square	Coeff Var	Root MSE	Fat Mean
0.203385	122.1537	0.174069	0.142500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Season	3	0.12377500	0.04125833	1.36	0.2901

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Season	3	0.12377500	0.04125833	1.36	0.2901

Parameter	Estimate		Standard Error	t Value	Pr >  t
Intercept	0.0620000000	B	0.07784600	0.80	0.4374
Season Autumn	0.1520000000	B	0.11009087	1.38	0.1864
Season Spring	0.0040000000	B	0.11009087	0.04	0.9715
Season Summer	0.1660000000	B	0.11009087	1.51	0.1511
Season Winter	0.0000000000	B	.	.	.

**Note:** The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.



The SAS System

The GLM Procedure

Duncan's Multiple Range Test for Fat

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	16
Error Mean Square	0.0303

Number of Means	2	3	4
Critical Range	.2334	.2447	.2518

Means with the same letter are not significantly different.			
Duncan Grouping	Mean	N	Season
A	0.2280	5	Summer
A			
A	0.2140	5	Autumn
A			
A	0.0660	5	Spring
A			
A	0.0620	5	Winter

Second ANOVA F – test and Duncan test regarding fat content is listed below (winter, spring vs. summer, autumn).

The SAS System

The GLM Procedure

Dependent Variable: Fat

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.12324500	0.12324500	4.57	0.0465
Error	18	0.48533000	0.02696278		
Corrected Total	19	0.60857500			

R-Square	Coeff Var	Root MSE	Fat Mean
0.202514	115.2305	0.164203	0.142500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Halvar	1	0.12324500	0.12324500	4.57	0.0465

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Halvar	1	0.12324500	0.12324500	4.57	0.0465

Duncan's Multiple Range Test for Fat

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

**Alpha** 0.05

**Error Degrees of Freedom** 18

**Error Mean Square** 0.026963

**Number of Means** 2

**Critical Range** .1543

**Means with the same letter are  
not significantly different.**

<b>Duncan Grouping</b>	<b>Mean</b>	<b>N</b>	<b>Halvar</b>
A	0.22100	10	SommerHo
B	0.06400	10	WinterSp

### 8.3.2 Colour - Statistical analysis software (SAS)

#### The SAS System

#### The GLM Procedure

Dependent Variable: Colour

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.87025500	0.29008500	10.22	0.0005
Error	16	0.45424000	0.02839000		
Corrected Total	19	1.32449500			

R-Square	Coeff Var	Root MSE	Colour Mean
0.657047	48.76797	0.168493	0.345500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Season	3	0.87025500	0.29008500	10.22	0.0005

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Season	3	0.87025500	0.29008500	10.22	0.0005

Parameter	Estimate		Standard Error	t Value	Pr >  t
Intercept	0.2000000000	B	0.07535250	2.65	0.0173
Season Autumn	0.4980000000	B	0.10656453	4.67	0.0003
Season Spring	-.0180000000	B	0.10656453	-0.17	0.8680
Season Summer	0.1020000000	B	0.10656453	0.96	0.3527
Season Winter	0.0000000000	B	.	.	.

**Note:** The XX matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

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## The SAS System

### The GLM Procedure

#### Duncan's Multiple Range Test for Colour

**Note:** This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	16
Error Mean Square	0.02839

Number of Means	2	3	4
Critical Range	.2259	.2369	.2438

Means with the same letter are not significantly different.			
Duncan Grouping	Mean	N	Season
A	0.6980	5	Autumn
B	0.3020	5	Summer
B			
B	0.2000	5	Winter
B			
B	0.1820	5	Spring

### 8.3.3 Melanin spots - Statistical analysis software (SAS)

#### The SAS System

#### The GLM Procedure

Dependent Variable: Melanin

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.04266000	0.01422000	0.27	0.8475
Error	16	0.84916000	0.05307250		
Corrected Total	19	0.89182000			

R-Square	Coeff Var	Root MSE	Melanin Mean
0.047835	28.90523	0.230375	0.797000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Season	3	0.04266000	0.01422000	0.27	0.8475

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Season	3	0.04266000	0.01422000	0.27	0.8475

Parameter	Estimate		Standard Error	t Value	Pr >  t
Intercept	0.7460000000	B	0.10302670	7.24	<.0001
Season Autumn	0.1260000000	B	0.14570175	0.86	0.3999
Season Spring	0.0420000000	B	0.14570175	0.29	0.7768
Season Summer	0.0360000000	B	0.14570175	0.25	0.8080
Season Winter	0.0000000000	B	.	.	.

**Note:** The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

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## The SAS System

### The GLM Procedure

#### Duncan's Multiple Range Test for Melanin

**Note:** This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	16
Error Mean Square	0.053073

Number of Means	2	3	4
Critical Range	.3089	.3239	.3333

Means with the same letter are not significantly different.			
Duncan Grouping	Mean	N	Season
A	0.8720	5	Autumn
A			
A	0.7880	5	Spring
A			
A	0.7820	5	Summer
A			
A	0.7460	5	Winter

### 8.3.4 Gaping - Statistical analysis software (SAS)

#### The SAS System

#### The GLM Procedure

Dependent Variable: Gaping

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.25285500	0.08428500	2.76	0.0760
Error	16	0.48820000	0.03051250		
Corrected Total	19	0.74105500			

R-Square	Coeff Var	Root MSE	Gaping Mean
0.341209	43.83395	0.174678	0.398500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Season	3	0.25285500	0.08428500	2.76	0.0760

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Season	3	0.25285500	0.08428500	2.76	0.0760

Parameter	Estimate		Standard Error	t Value	Pr >  t
Intercept	0.3340000000	B	0.07811850	4.28	0.0006
Season Autumn	0.2580000000	B	0.11047624	2.34	0.0329
Season Spring	-.0180000000	B	0.11047624	-0.16	0.8726
Season Summer	0.0180000000	B	0.11047624	0.16	0.8726
Season Winter	0.0000000000	B	.	.	.

**Note:** The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

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## The SAS System

### The GLM Procedure

#### Duncan's Multiple Range Test for Gaping

**Note:** This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	16
Error Mean Square	0.030513

Number of Means	2	3	4
Critical Range	.2342	.2456	.2527

Means with the same letter are not significantly different.			
Duncan Grouping	Mean	N	Season
A	0.5920	5	Autumn
B	0.3520	5	Summer
B			
B	0.3340	5	Winter
B			
B	0.3160	5	Spring





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