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Faculty of Biosciences, Fisheries and Economics
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Live-storage of wild mature cod (*Gadus morhua* L.) without feed supply. Effects on biological and quality properties

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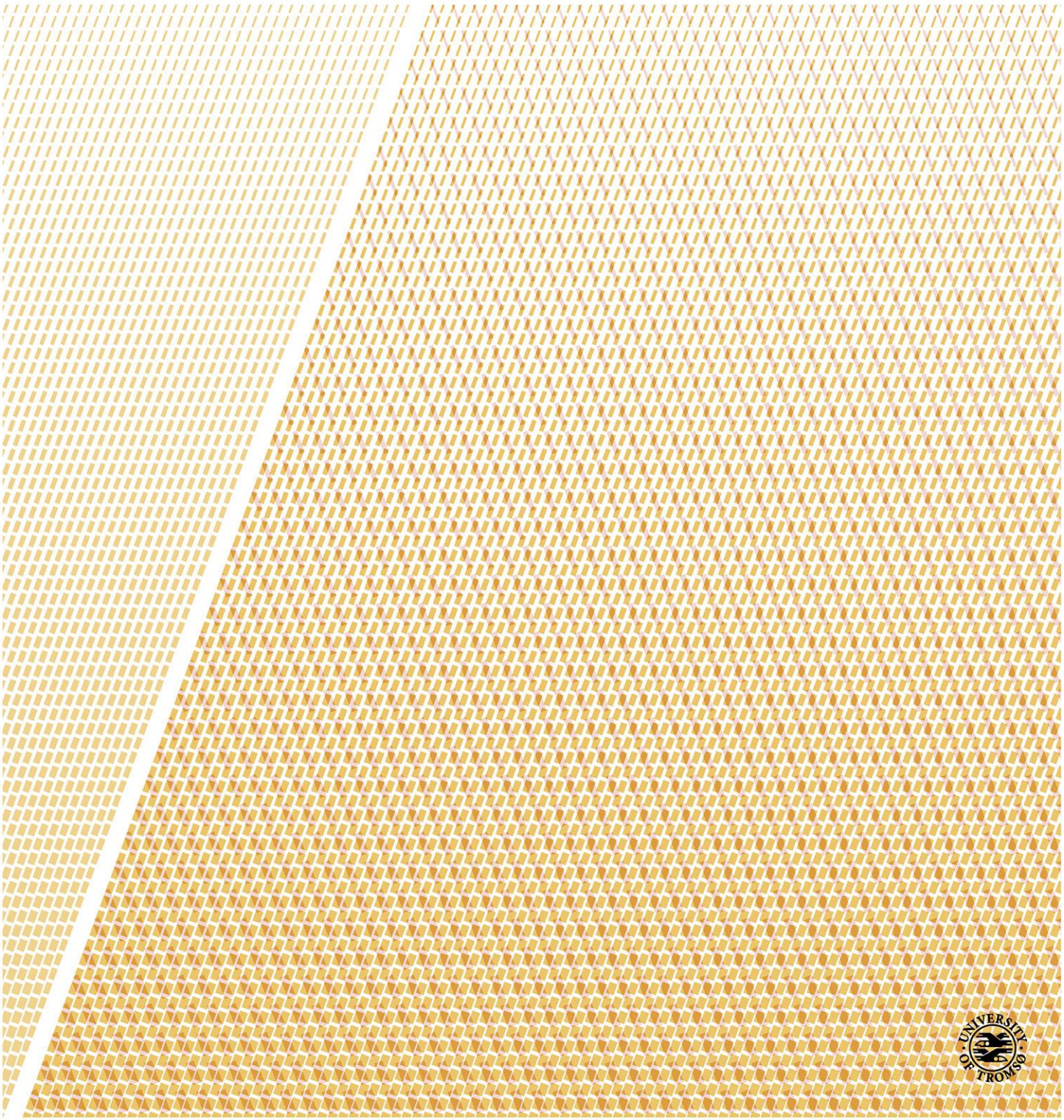
Tatiana N. Ageeva

A dissertation for the degree of Philosophiae Doctor – June 2018



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The Norwegian College of Fishery Science

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The Arctic University of Norway

and

Nofima, Tromsø

Tromsø

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Tatiana N. Ageeva Tromsø, June 2018

English summary

The overall aim of this work was to obtain better understanding of how live-storage without feeding affects wild mature Atlantic cod (*Gadus morhua* L.) and quality of products obtained from such cod. Atlantic cod were live-stored without feeding for 82 days. First, gender-specific weight changes in both whole and gutted fish, as well as certain organs were studied (Paper I). Next, the effects of feed deprivation on development of *rigor mortis*, as well as aspects of muscle properties were investigated (Paper II). Finally, changes in quality of fillet products, both due to feed deprivation and different times of processing were studied (Paper III).

The results showed that the cod were spawning during the first 54 days in captivity, leading to a great loss of total weight. The main changes in gutted weight were detected in the post-spawning period. Females were affected more severely by feed deprivation than males. This sexual dimorphism became more evident towards the termination of the trial, when females had lost more total and gutted weights, liver mass and had a significantly lower muscle protein concentration than males.

Feed deprivation for 23 days reduced *pre rigor* time from 29 to 17 h while further feed deprivation did not reduced this time significantly. If the fish are slaughtered under non-stressful conditions, the *pre rigor* time in feed-deprived cod can still be long enough to process the fish prior to the onset of *rigor mortis*.

Feed deprivation resulted in just a small reduction in contraction of *pre rigor* made products. The tail products, especially made *pre rigor*, contracted more and had higher drip loss than loins and whole fillets. The sensory profile of fillets changed during the feed deprivation. Most fillets had an unpleasantly soft texture, atypical white colour and less fresh sea odour at day 82.

The results in this thesis suggest that the tolerable period of live-storage without feeding of wild mature cod is approximately 54 days.

Norsk sammendrag

Hovedmålet med dette arbeidet var å få bedre forståelse for hvordan levendelagring uten fôring påvirker vill kjønnsmoden Atlantisk torsk (*Gadus morhua* L.) samt kvalitet på produkter laget av slik torsk. Atlantisk torsk ble lagret levende uten fôring i 82 dager. Først ble kjønnsbaserte endringer i fiskens total og sløyd vekt, samt gonader og lever bestemt (Artikkel I). Deretter ble effektene av sultetid på utvikling av *rigor mortis*, samt flere kvalitetsaspekter av muskel undersøkt (Artikkel II). Til slutt, ble kvalitetsendringer i forskjellige filetprodukter vurdert opp mot ulike sultetider og fileteringstidspunkt *post mortem* (Paper III).

Resultatene viste at torsken i fangenskap gytte i løpet av de første 54 dagene, noe som reduserte sterkt fiskens totalvekt. De største endringene i sløyd vekt ble detektert i perioden etter gyting. Hunnene ble mer påvirket av mangel på fôr enn hannene. Denne kjønnsbaserte dimorfismen ble mer tydelig mot avslutningen av forsøket, da hunnene hadde tapt mer total og sløyd vekt, samt levervekt og hadde en signifikant lavere proteinkonsentrasjon i muskel enn hannene.

En sulteperiode på 23 dager reduserte *pre rigor* tid fra 29 til 17 timer, mens ytterligere tid uten fôring reduserte ikke denne tiden signifikant. Dersom fisken slaktes under skånsomme forhold, kan *pre rigor* tid for sultet torsk fortsatt være lang nok til å prosessere fisken før inntredelse av *rigor mortis* (dødsstivhet).

Uten fôring ble sammentrekningen av *pre rigor* produserte filet produkter bare redusert i mindre grad. Sporstykker (tails), spesielt laget *pre rigor*, krympet mer og hadde høyere drypptap enn både tykkfiskstykker (loins) og hele fileter. I tillegg, ble den sensoriske profilen til fileter endret med lengre sultetid. De fleste filetene hadde en bløt (geléaktig) tekstur, atypisk hvit farge og mindre fersk sjølukt på dag 82.

Resultatene i denne doktorgraden tydet på at den tolerable perioden for levendelagring av vill kjønnsmoden torsk uten fôring er cirka 54 dager, utover det syntes negative kvalitetsendringer å bli betydelige.

Краткое содержание

Основной целью этой диссертации было исследование влияния промежуточного хранения в садках без корма на биологическое состояние дикой, живой, половозрелой Атлантической трески (Gadus morhua L.), а также на качество полученных из неё продуктов. Атлантическую треску поместили в садки для хранения без корма на 82 дня. В течении этого периода было произведено 4 выборки рыбы: в начале эксперимента и, последовательно, каждую четвертую неделю. Сначала были изучены отличительные особенности самок и самцов трески в их адаптации к условиям голода. Наблюдение основывалось на изменениях в массе целой и потрошеной рыбы, а также в массе гонад (половых желез) и печени каждого индивидиума (Научная статья I). Затем было изучено влияние голодания на развитие посмертного окоченения, а также на другие свойства мышечной ткани рыбы (Научная статья II). В заключение были исследованы изменения качества продуктов (целого филе, спинки филе – loin и хвостовой части филе - tail) в зависимости от продолжительности периода голодания и времени переработки рыбы (Научная статья III).

Результаты показали, что треска, хранящаяся в садках, нерестилась в течение первых 54 дней эксперимента, что значительно уменьшило массу целой рыбы до её отправления в производство. Главные изменения в массе потрошеной рыбы произошли после нереста. Голодание повлияло на самок в значительно большей мере, чем на самцов. Дифференциация между самками и самцами трески стала более очевидной к концу эксперимента, т. к. у самок была значительно большая потеря в массе целой и потрошеной рыбы, в том числе в массе печени. Кроме того, концентрация белка в мышечной ткани самок была намного меньше, чем в мышечной ткани самцов.

Голодание в течении 23 дней сократило время наступления посмертного окоченения с 29 до 17 часов, в то время как продолжение голодания до 82 дней существенного влияния не оказало. Следовательно, если такую треску усыпляют в нестрессовых для неё условиях, то время перед началом посмертного окоченения еще может быть достаточно долгим для переработки рыбы до посмертного уплотнения мышечной ткани.

Голодание сократило степень контракции (сжатия) охлаждённых продуктов, произведенных до посмертного окоченения, но только в незначительной степени. За время хранения во льду, контракция и потеря массы хвостовой части филе (tail) были значительно выше, чем у филе и спинки филе (loin). Особенно, это было выражено в продуктах, произведённых до посмертного окоченения. Кроме того, вкусовое качество филе тоже изменилось после длительного периода голодания. После 82 дней голодания большинство филе приобрели желеобразную, мягкую консистенцию, атипичный белый цвет и имели менее выраженный запах свежей рыбы.

На основании изложенных данных можно предположить, что допустимый период хранения без обеспечения дикой, половозрелой трески кормом составляет приблизительно 54 дня.

List of publications

The thesis is based on the following articles:

Paper I.

Ageeva, T. N., Jobling, M., Olsen, R. L., and Esaiassen, M. (2017). Gender-specific response of mature Atlantic cod (*Gadus morhua* L.) to feed deprivation. *Fisheries Research*. 188: 95-99.

Paper II.

Ageeva, T. N., Olsen, R. L., Joensen, S., & Esaiassen, M. (2018). Effects of Long-Term Feed Deprivation on the Development of Rigor Mortis and Aspects of Muscle Quality in Live-Stored Mature Atlantic Cod (Gadus Morhua L.). *Journal of Aquatic Food Product Technology*, *27*(4), 477-485.

Paper III.

Ageeva, T. N., Olsen, R. L., Joensen, S., & Esaiassen, M. (2018). Quality aspects of fillet, loin and tail products made from live-stored feed-deprived Atlantic cod (Gadus morhua L.) at different times *post mortem*. LWT – Food Science and Technology, revised and submitted.

1 Introduction

Norway has a unique location and a long coastline, and for centuries Norwegians have used the sea in many ways. However, cod fisheries have always had a special place in the hearts of the people (Kolle et al., 2017). As the Norwegian parson poet, Petter Dass wrote, the cod was extremely important as trade goods for the people already in the 17th century (Jorgenson & Holmboe, 2015). Nowadays, cod is still important as a commercial fish species in Norway.

> Well, now I must come to the king of the fish,
> The aim and the object of northerners' wish,
> The cod, called the "skrei" in Norwegian.
>
> It hangs on the rocks, and it fills all the stores;
> Praise God that this fish comes each year to our shores;
> It feeds both the wives and the husbands.
>
> O cod, you are truly our livelihood high;
> You bring us from Bergen the much needed rye
> And feed Norland's fishermen amply.
>
> Petter Dass
> «The Trumpet of Nordland»

1.1 Atlantic cod (Northeast Arctic cod)

There are several stocks of Atlantic cod (*Gadus morhua* L.), each having different geographical distributions, life-histories and migration patterns. The cod stocks are located in Northern America, Greenland, Island, Faroe Islands, Irish Sea, North Sea, Barents Sea, Skagerrak and Baltic Sea as well as several local coastal cod stocks. (Bakketeig, Hauge, & Cecilie, 2017; Bratland, Krishnan, & Sundnes, 1976). In Northern Norway, there are two main groups of cod, coastal cod and ocean cod. The latter one, northeast Arctic cod, is also called Arcto-Norwegian cod (ICES, 2005; Kolle et al., 2017; Nordeide, Johansen, Jørgensen, Karlsen, & Moum, 2011). Coastal cod include two main groups, sedentary "fjord cod" and more mobile "bank cod", but both groups live in the Norwegian coastal waters all their lives (Institute of Marine Research, 2009).

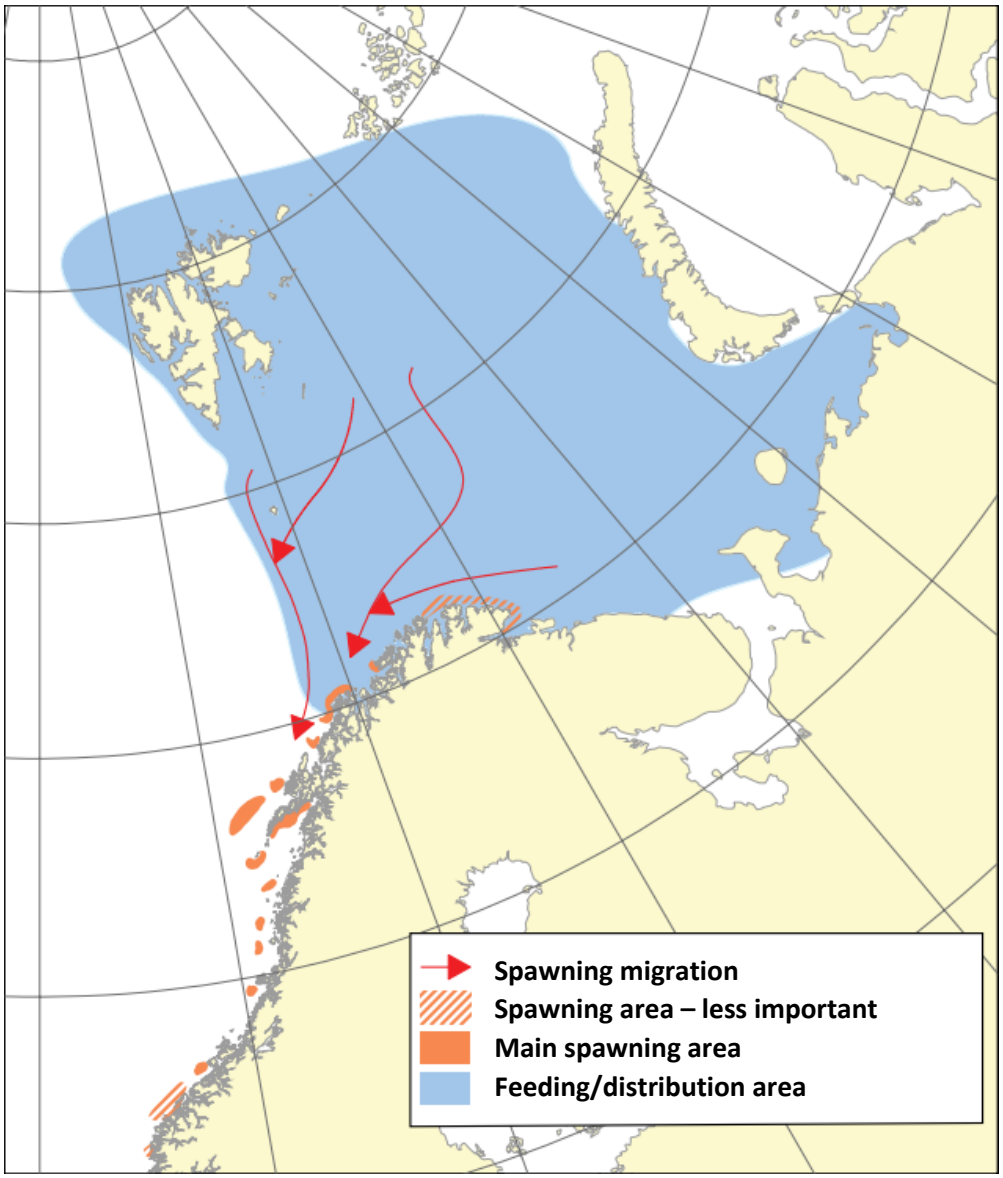

Figure 1. The distribution and migrating pattern of northeast Arctic cod. Retrieved and modified 15.02.2018 from Bakketeig, I. E., Hauge, M., & Cecilie, K. (2017).

Northeast Arctic cod is the largest cod stock in the world, and the stock is managed by Norway and Russia in cooperation (Bakketeig et al., 2017). These cod inhabit the Barents Sea for most of the year, but migrate towards the Norwegian coast for spawning or feeding. The first spawning migration, mainly to Lofoten and Vesterålen, occurs at the age of 6-8 years, when the cod reach maturity (Fig. 1). Such cod are called *skrei*, from Old Norse *skrida* – to move (Kolle et al., 2017).

The skrei spawn from the end of January to April/May, forming the foundation for Lofoten fisheries, because in contrast to large trawlers, the small coastal vessels cannot cover large distances and have to wait until the fish come to spawn near the coast. The young and immature cod initially follow the skrei, but instead of migrating to the Lofoten area, the immature cod head towards the Finnmark coast to feed on spawning capelin. Such cod are often called "capelin cod" and provide a basis for so called "capelin cod fishery" or the "spring cod fishery" which is also an important Norwegian coastal fishery (Akse & Midling, 1997; Kolle et al., 2017).

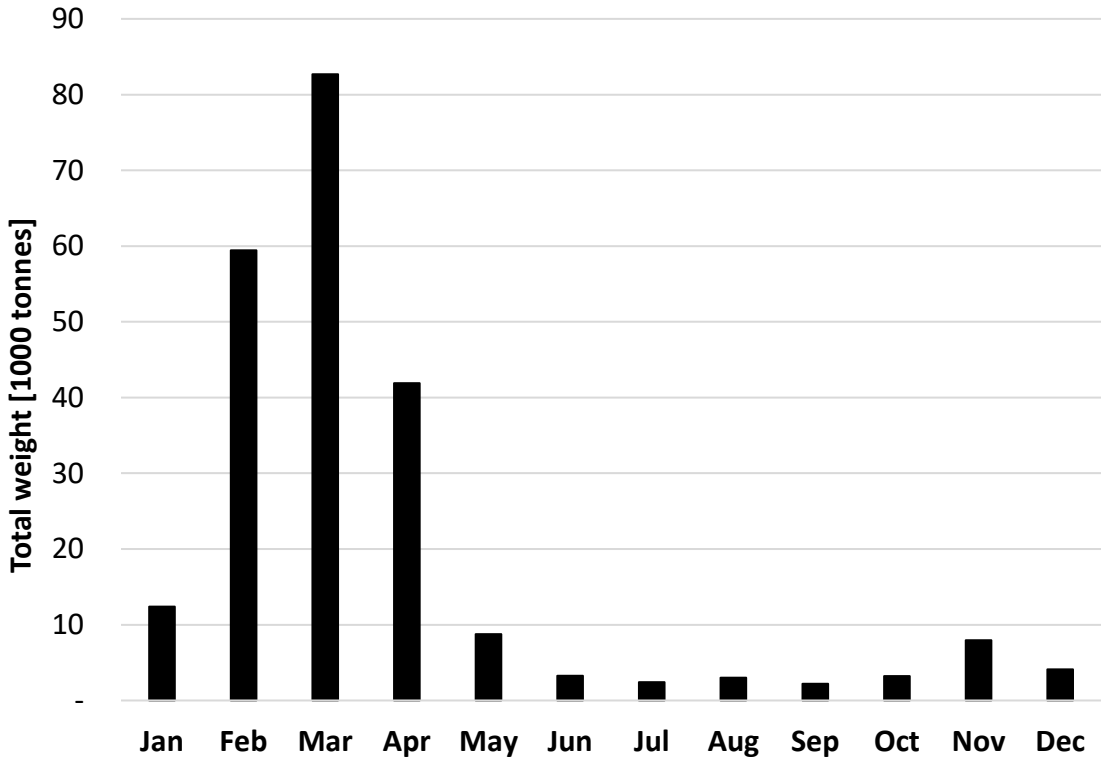

Figure 2. The amount of Atlantic cod (Total weight, 1000 tonnes) delivered by coastal fleet each month during 2016. Figure: Øystein Hermansen, Nofima, Tromsø.

The migration patterns of northeast Arctic cod results that the most cod are landed during the first four months of each year (Fig. 2). This seasonality, as well as weather-dependent unpredictability in landings of cod, lead to a shortage of fresh raw materials and discontinuities in production, which in turn lead to uncertainty in deliveries to the markets (Hermansen & Dreyer, 2010). Such instability makes it difficult to plan production and to make long-term

contracts for both fishing vessels and fish processing facilities. In addition, correct handling of raw materials and maintaining high product quality are much more difficult when large quantities are delivered in a short period of time. However, by keeping cod alive after capture, these challenges can be met and the market season of fresh cod can be extended. Furthermore, live-storage of wild cod costs less compared to the 2-3 years needed to produce farmed cod of market size, and makes it possible to control the quality of fish from capture to consumption, as well as resulting in by-products of high quality (Sogn-Grundvåg, Egeness, Hermansen, & Larsen, 2012).

1.2 Live-storage of wild cod

The term "Live-stored cod" means wild cod that have been caught alive, transported to shore and kept alive in sea cages. The idea of storing of wild-caught Atlantic cod (*Gadus morhua* L.) alive is not new. In fact, one of the first live-stored cod were delivered in the 1880s to Grimsby, England, by the Norwegian ship-owner Sigvart Waage (Hovland, 1980; Midling, Aas, Isaksen, Pettersen, & Jørgensen, 1998). Waage used a boat, equipped with a 21-foot long fish well, which was perforated on both sides to allow for seawater exchange. The cod were caught in the North Sea, kept alive in the well during transport to England, and delivered to the fish market in Grimsby. Such fish was in high demand and obtained a very high price. For instance, one live cod was sold for 3.29 NOK while the traditional salted fish obtained 9.42 NOK per tonne (Hovland, 1980).

The first application of live-storage of cod on an industrial scale in Norway was in early 1900s when fish traps were used in cod fisheries from Skagerrak to Helgeland, Northern Norway (Hermansen, Sogn-Grundvåg, & Dreyer, 2018). Live-stored fish were transported to Trondheim, Bergen and Oslo, where the fish were slaughtered and sold. However, due to high mortality of cod, reduced market prices and higher efficiency in the traditional fisheries, live-storage of cod became less profitable compared to traditional cod fisheries. Thus, live-storage

activity decreased in the mid-1950s. In 1980s, Norwegian cod fisheries adapted seine technology and started to store the cod in conic sea cages, as are used in salmon aquaculture. This revived live-storage and was used mostly in Myre, Vesterålen, and Alta, Finnmark (Hermanson et al. 2018, personal communication Sæther, 2018). Still, there were challenges like high mortality. After changing to storage of the wild cod recovery in flat-bottom transportation tanks and sea cages, mortality has been strongly reduced (Midling et al., 1998). Nowadays, live-storage of cod might have bright economic prospects (Fig. 3).

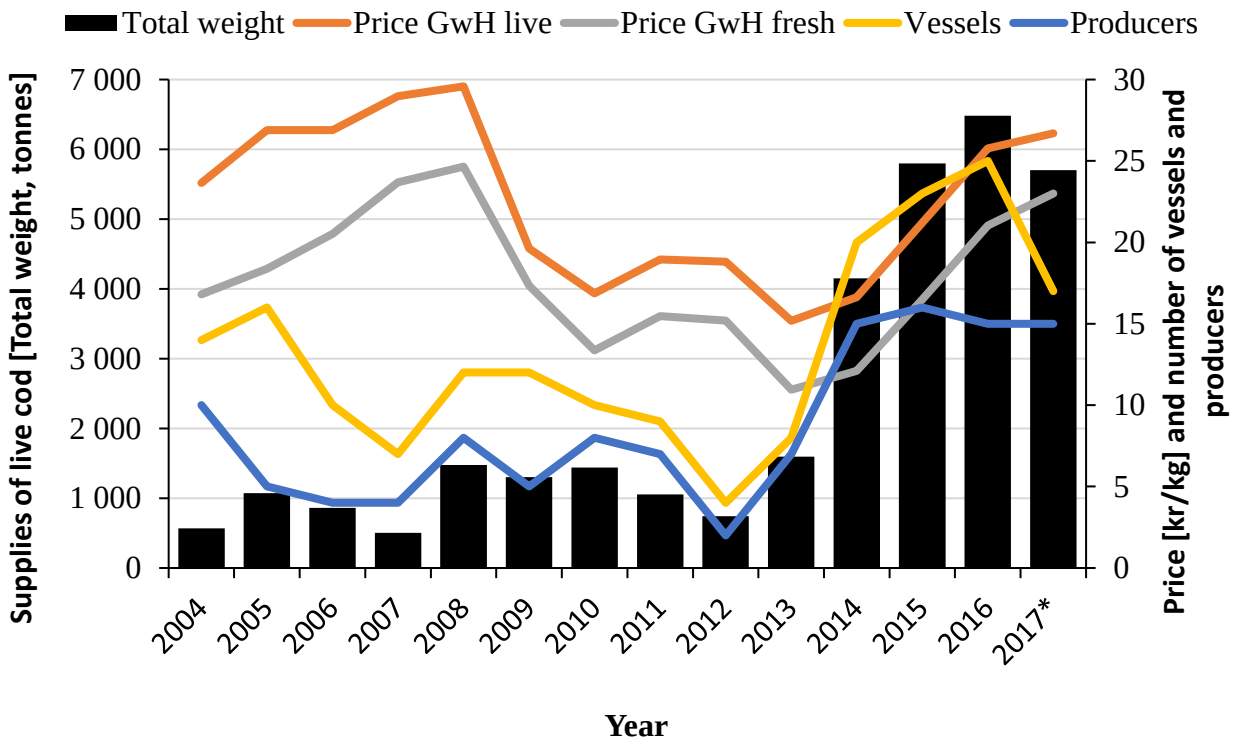

Figure 3. Development of live-storage of Atlantic cod in Norway from 2004 to 2017. Total weight – tonnes of live cod landed each year; Price GwH live – average price for gutted without head cod delivered as live; Price GwH fresh – average price for gutted without head of fresh cod slaughtered at sea; Vessels – number of vessels which delivered live cod; Producers – number of producers /sites where the live fish were delivered and stored until processing; 2017* - preliminary results.
From Charles Aas, The Norwegian Fishermen's Sales Organization, Tromsø, 2018

Live-storage provides the possibility to control the processing of fish and to improve the quality of raw material, which is of the outmost importance for premium final products (Akse, Tobiassen, Joensen, Midling, & Aas, 2005; Karlsen, Krag, Albertsen, & Frandsen, 2015; Pedrosa-Menabrito & Regenstein, 1990). For instance, it has been shown that given time, fish

can recover from the stress caused during harvesting. Live Atlantic cod need just hours to reduce the fillet discoloration caused by capture stress (Olsen, Tobiassen, Akse, Evensen, & Midling, 2013; Svalheim, Karlsson-Drangsholt, Olsen, Johnsen, & Aas-Hansen, 2017) and up to 5-6 weeks to remove the more severe blood residues caused by swim bladder rupture (Midling, Koren, Humborstad, & Sæther, 2012; Sæther et al., 2016). Furthermore, live-storage gives the opportunity to control the nutritional status of fish. It is known that feeding of live-stored cod captured during spring, will contribute to their weight gain (Sæther et al., 2016). On the other hand, short-term starvation of both "capelin cod" (cod that had been feeding on capelin) and farmed Atlantic cod may improve fillet texture and processing quality (Akse & Midling, 1997; Akse, Kristiansen, Tobiassen, Dahl, & Eilertsen, 2008; Olsson, Gundersen, & Esaiassen, 2006). Often, well-fed cod has an unusual soft muscle texture and gives a low product yield. However, there is little knowledge about how the long-term live-storage of wild Atlantic cod can affect the biological and quality properties of the fillets.

According to the current Norwegian regulations (FOR-2004-12-22-1878, 2004) wild-caught fish can be kept alive in sea cages for up to 12 weeks, with the initial 4 weeks without feeding. After 12 weeks of live-storage, the cod are considered "farmed" fish, and managed by legislations for the capture-based aquaculture (FOR-2014-12-15-1831, 2014). The industry wishes to extend the period without feed supply (Sæther et al., 2016), mainly because the wild cod hardly accept formulated dry feed, and thus expensive feed such as whole capelin, herring or mackerel has to be used (Dreyer, Nøstvold, Midling, & Hermansen, 2008; Sæther, 2009; Sæther & Bogevik, 2017). In addition, the fatty acids in the fatty pelagic species may undergo peroxidation during frozen storage prior to being fed to the cod. This may affect the growth and development of fish negatively (Sargent, Tocher, & Bell, 2002). Thus, it is of interest to examine how long time the cod can be stored alive in absence of feed without negatively affecting the fish welfare or product quality.

Last, but not least, it is common to locate the sea cages with cod relatively close to fish processing facilities. This makes it possible to plan the time of production, improve the slaughter procedures and process the fish before the onset of *rigor mortis*. Processing the fish *pre rigor* facilitates that fish products may enter the market with a higher degree of freshness compared to traditional fisheries where the cod have to be stored until resolution of *rigor* before processing (Tobiassen et al., 2006). Consequently, it is interesting to study how the long-term feed deprivation of live-stored wild cod will affect the development of *rigor mortis* and the quality of final products made at different time spans after slaughter.

1.3 Aims of the study

The work has been part of the project CATCH: Market-oriented and sustainable value chains for cod products based on live-storage, funded by the Research Council of Norway (No. 233751/E50). The main objective of the project was to obtain the maximal sustainable value of wild Atlantic cod based on live-storage, and the research scope includes the whole value chain from the capture of fish in the sea to the distribution of cod products to consumers.

The overall aim of this work was to obtain better understanding of how live-storage without feeding affects mature Atlantic cod and quality of products obtained from such cod. Consequently, the main objectives were:

- Investigate gender-specific responses to feed deprivation during the spawning and post-spawning periods (Paper I).
- Investigate effects of feed deprivation on the onset and development of *rigor mortis* and muscle quality (Paper II).
- Study the quality of fresh products (fillet, loin and tail) made from feed-deprived cod at different times *post mortem*. (Paper III).

2 General background

2.1 Fish quality

The term *quality* can be defined in several ways since it is an abstract concept, which depends on a variety of factors including type, reason a product is being produced, and on a person's impressions of a product (Botta, 1995). Several factors can define the quality of a fish or a fish product (Fig. 4), and the quality is affected both by natural conditions like fish species, feeding, maturation, spawning, and by fish handling and processing such as catching methods, immediate post-harvest handling, slaughter procedures, processing, packaging methods and storage temperature (Bremner, 2000; Love, 1988; Strasburg, Xiong, & Chiang, 2008). Freshness is one of the most important attribute for the quality of fish products, and can be even more important than visual attributes (Heide & Olsen, 2017; Olafsdóttir et al., 1997).

Figure 4. Overview of factors determining the quality of a fish or a fish product. After Olafsdóttir et al., 1997.

However, sensory properties of fish products like odour, texture, colour, etc. are also essential (Sveinsdóttir et al., 2009). Texture can be extremely important both to the processors and to the consumers. For instance, soft texture of farmed salmon can cause up to 40% loss in value (Michie, 2001). Liquid loss from muscle is also economically important since it is equivalent to a weight loss. In addition, the accumulated liquid in the product package is unattractive to consumers and contains water-soluble nutrients (vitamins, minerals, amino acids, etc.) lost from the muscle (Foegeding, Lanier, & Hultin, 1996; Kristoffersen, Vang, Larsen, & Olsen, 2007). As previously mentioned, the quality of fish muscle may change depending on several factors, and in particular, intake/absence of feed and time of processing can both strongly affect the fish quality. Fortunately, these factors can be managed when practising live-storage of fish. In the following, the changes in fish muscle due to feed deprivation and time of filleting *post mortem* are presented.

2.2 Feed deprivation

Feed deprivation/starvation refers to the biological condition when the animals are willing to eat but unable to do so due to extrinsic limitation on food resources (McCue, 2010). Feed deprivation is distinct from fasting, when the animals choose to abstain food even if it is available. In nature, starvation can be caused by several reasons, such as acute weather conditions, seasonal cycles, etc. All wild fish face food absence and are well adapted to prolonged periods of fasting, which may occur, for example during winter months, spawning migration, pre-spawning and spawning phases. Food limitation forces fish to use its own endogenous energy stores to survive (Bar & Volkoff, 2012). Different fish species, and even individuals of the same species, can respond differently to feed deprivation. However, as described by Bar and Volkoff (2012), all animals, including ectotherms like fish, undergo several phases of starvation that are defined by the type of nutrients that are being mobilized.

Transition between these phases of deprivation may be indirectly identified by changes in hormones, enzyme activities, blood metabolites or body mass.

Atlantic cod is a lean fish species and stores lipids mainly in the liver (Huss, 1995; Love, 1988), where the lipid content can rise up to 70% (Black and Love, 1986). In cod muscle, the fat content is approximately 0.1-0.9%, the proportion of water vary between 78% and 83%, while the protein content ranges from 15% to 19% and ash content is about 1.16% (Huss, 1995; Strasburg et al., 2008). The majority of a cod fillet consists of white muscle whereas the dark (red) muscle is located on the fillet's surface, right under the skin, along the lateral line on each side of the body as shown in Figure 5 (Foegeding et al., 1996; Love, 1988). The proportion of dark to white muscle increases toward the tail area.

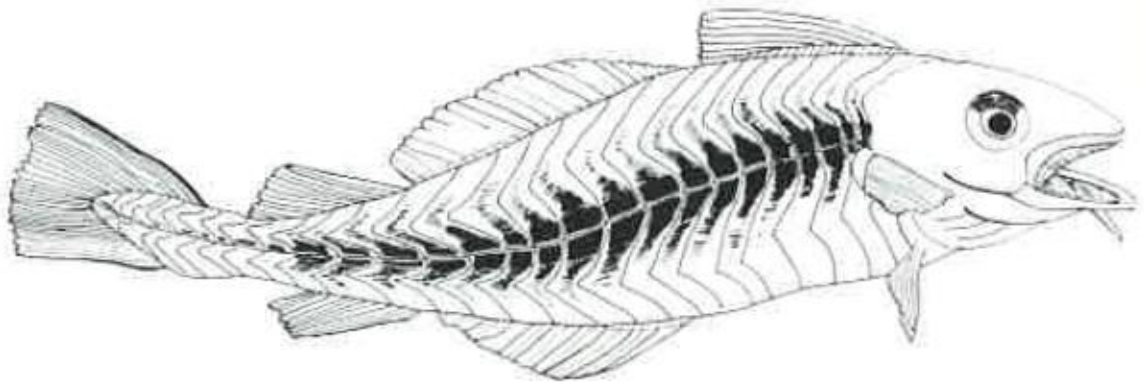

Figure 5. Cod without skin to show the proportion of dark muscle. Love, 1988.

It has been observed that the composition of white muscle in fish is more uniform and independent of its location while the dark muscle composition may vary as a function of its location (Foegeding et al., 1996). Specifically, dark muscle in the anterior part of the fish may contain more lipids while in the posterior section has more water and protein. Dark muscle contains also more glycogen, mitochondria and myoglobin. As opposed to white muscle, which is more based on anaerobic metabolism, dark muscle is more dependent on the tricarboxylic acid cycle and oxidative metabolism and is used for continuous and long-term swimming. Such

differences in composition, metabolism and purpose of the two muscles lead to the different muscle responses to starvation.

For instance, Martinez et al. (2003) reported that white muscle in Atlantic cod were affected more by starvation than dark muscle during starvation for 16 weeks. Feed deprivation reduced the activity of glycolytic and mitochondrial enzymes both in white and dark muscle, but the changes were greater in white muscle, and the activity of glycolytic enzymes in white muscle were reduced more than mitochondrial enzymes. In addition, starvation reduced the differences in metabolic capacities of muscles from different areas of fillet, and this homogeneity was more pronounced in white than red muscles. The greater degradation of white muscle than of dark muscle during starvation for 12 weeks was also detected in saithe (*Pollachius virens*) by Beardall and Johnston (1983).

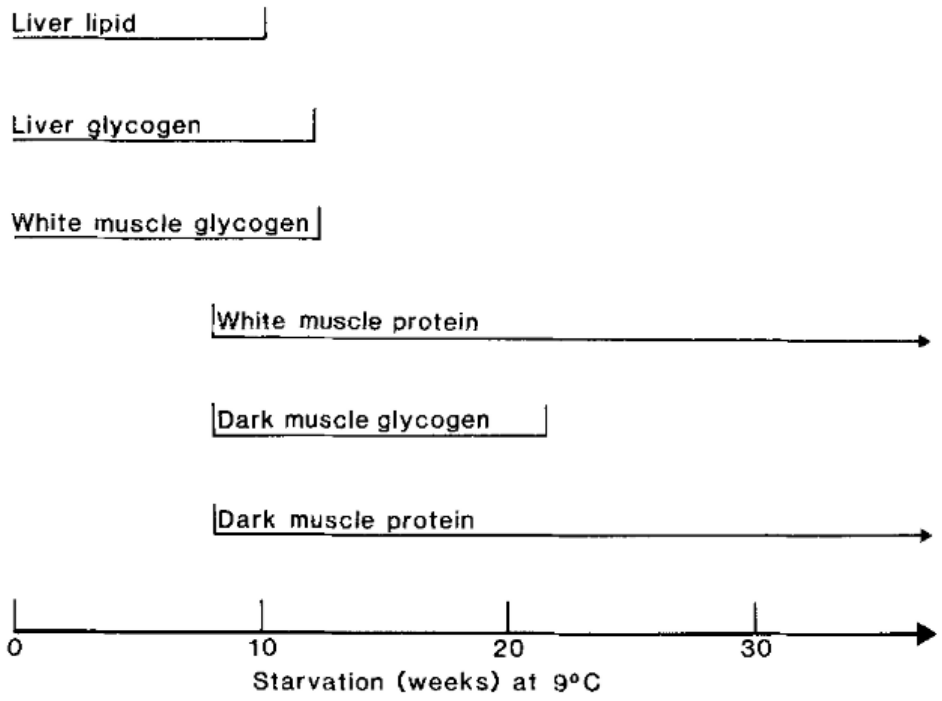

Figure 6. Overview of systematic mobilisation of the main energy reserves in cod starved at 9°C. Time values are approximate. After Black and Love (1986).

The sequential utilization of nutrients in cod during starvation were studied more thoroughly by Black and Love (1986). They concluded that early in starvation, the cod simultaneously used liver lipids, liver glycogen and glycogen in white muscle, and of these, the liver lipids became depleted first (Fig. 6). Next, the proteins in red and white muscles as well as glycogen in red muscle started to decrease. However, the use of glycogen in red muscle ended first, resulting in that muscle proteins subsequently became the major energy resource.

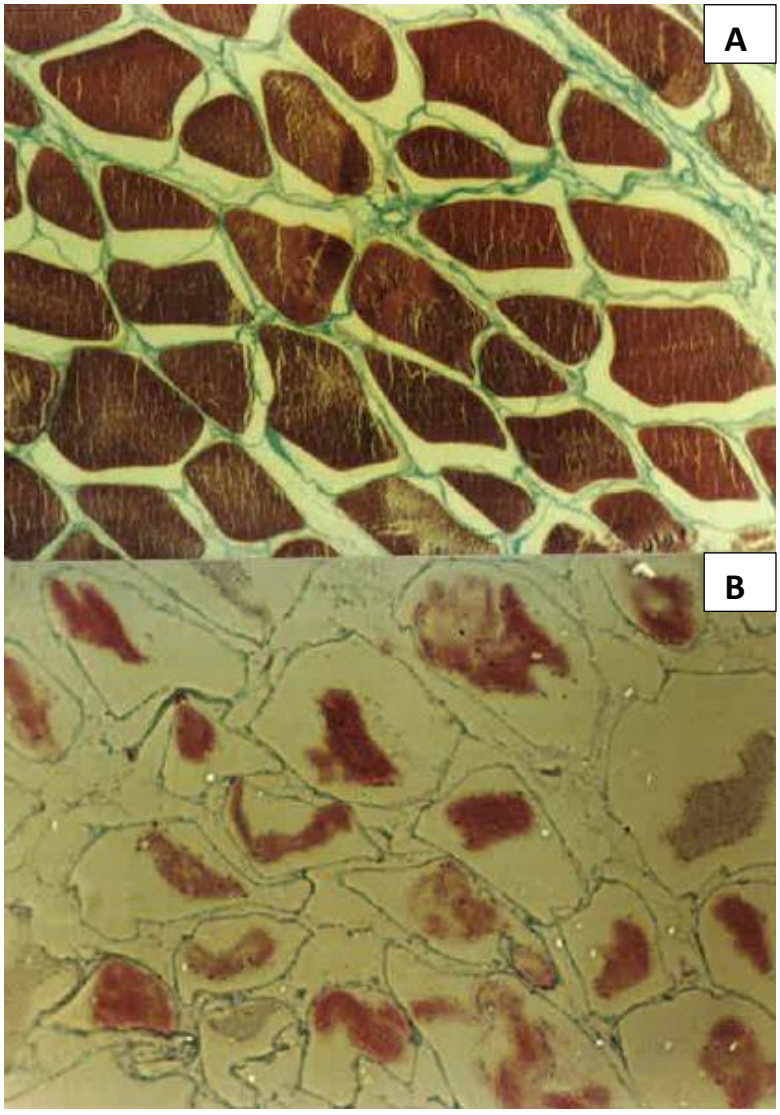

Figure 7. Cross-sections of the white muscle of feed-deprived cod. A - moderate starvation, water content 83.3%, B – extreme severe starvation, water content 95.3%. After Love, 1988.

As the content of muscle protein decreases, the water content in the muscle increases (Lavéty & Love, 1972; Love, 1988). Love (1988) wrote that water content in muscle of severe

feed-deprived cod can rise up to 95%. Figure 7 illustrates the histology of the white muscle of cod feed-deprived to moderate (A) and to severe (B) degrees.

The red/brown spaces of each cell (representing contractile proteins) almost disappeared in the long-term feed-deprived cod, and the cells were filled with fluid (Fig. 7). In addition, the extracellular space increased and contained even more fluid. In contrast to muscle proteins, the connective tissue appeared to be unchanged. In fact, the previous studies have reported that concomitant with changes in protein and water contents in muscle during starvation, myocommata became stronger and thicker (Lavéty & Love, 1972; Love, Yamaguchi, Creac'h, & Lavéty, 1976). This was linked to an increased proportion of collagen to total muscle protein, with additional formation of collagen and higher amount of intermolecular crosslinks in collagen of starved cod compared to that in fed cod (Foegeding et al., 1996).

Furthermore, if starvation coincides with maturation and spawning, the depletion of fish muscle can be more severe (Idler & Bitners, 1960; Karlsen, Holm, & Kjesbu, 1995; Takama, Love, & Smith, 1985). Actually, in salmonids, the starvation may inhibit sexual maturation (Rowe & Thorpe, 1990; Thorpe, Talbot, Miles, & Keay, 1990). In contrast, the feed-deprived farmed cod may still mature and spawn despite the reduced growth, liver size and fecundity, with female cod being affected more severely than males (Karlsen et al., 1995).

The quality of fillets from starved and fed cod differs. Starved fish show less fillet gaping, probably caused by strengthen connective tissue and higher ultimate pH. Viewed from the processing perspective that can be positive, but such fillets have reduced nutritional quality. Fillets from starved cod have a high water content and increased amount of extracellular fluid, which contains more sodium than intracellular fluid, giving nearly twice as much sodium as fillet of fed cod (Love, 1988). In addition, according to Love (1988) starved cod contains more glycine, proline, hydroxiproline, sphingomyelin, and cholesterol and less phosphatidyl choline and phospholipid than fed cod. Finally, fillets obtained from starved fish may have wetter

surfaces, an unpleasantly soft (sloppy) texture, and are more prone to bacterial spoilage than fed cod due to near-neutral muscle pH.

2.3 *Pre rigor* processing

By *pre rigor* processing of fish means that the fish are processed/filleted before the onset of *rigor mortis*. Time before the onset of death stiffness (*pre rigor* time) may vary from less than one hour to more than a day, and depends on a number of factors such as fish species, *ante mortem* nutritional status, degree of exhaustion before slaughter, fish size, *post mortem* handling and temperature (Strasburg et al., 2008; Stroud, 1969). In other words, the onset of *rigor mortis* depends on the amount of energy remaining in the muscle after slaughter, and occurs when the energy is almost depleted. After death, when there is no longer oxygen supply, the muscle still keeps trying to maintain cellular homeostasis. This requires energy, which in the body is supplied by ATP (adenosine triphosphate). There are some resources of ATP that can be used by *post mortem* muscle (Strasburg et al., 2008). CrP (creatine phosphate) can donate its phosphate to ADP (adenosine diphosphate), thus, regenerating ATP:

$$\mathrm{CrP + MgADP \xrightarrow{\text{Creatine kinase}} MgATP + Cr}$$

ATP can also be produced very early *post mortem* from ADP by the sarcoplasmic enzyme adenylate kinase:

$$\mathrm{2ADP \xrightarrow{\text{Adenylate kinase}} ATP + AMP\ (adenosine\ monophosphate)}$$

In live fish, these ATP sources are used if there is a high-energy demand in live muscle, such as during sprinting, and are often depleted if the fish struggles prior to death.

The main source of ATP in white muscle is glycogen, which is used for ATP production both before death (through aerobic metabolism) and *post mortem* (through anaerobic metabolism) (Campbell & Farrel, 2008; Strasburg et al., 2008). After death, the synthesis and hydrolysis of ATP occur concomitant with anaerobic production of lactic acid until the reserves

of glycogen are depleted. Hydrolysis of ATP (reaction steps 1 and 2) results in accumulation of hydrogen ions (H^+) that initiate the fall in muscle pH. The accumulation of H^+ correlates also with accumulation of lactic acid. However, it is the formation of H^+ during the hydrolysis of ATP that mainly causes the drop in pH *post mortem* (Foegeding et al., 1996; Robergs, Ghiasvand, & Parker, 2004).

$$ATP + H_2O \rightarrow ADP + P_i + H^+ \qquad (1)$$

$$ADP + H_2O \rightarrow AMP + P_i + H^+ \qquad (2)$$

When almost all the ATP are depleted, the process of *rigor mortis* begins (Figure 8). In Atlantic cod, the ATP content at the onset of *rigor mortis* has been reported to be 1.25 µmol/g muscle (Cappeln & Jessen, 2002; Fraser, Dingle, Hines, Nowlan, & Dyer, 1967).

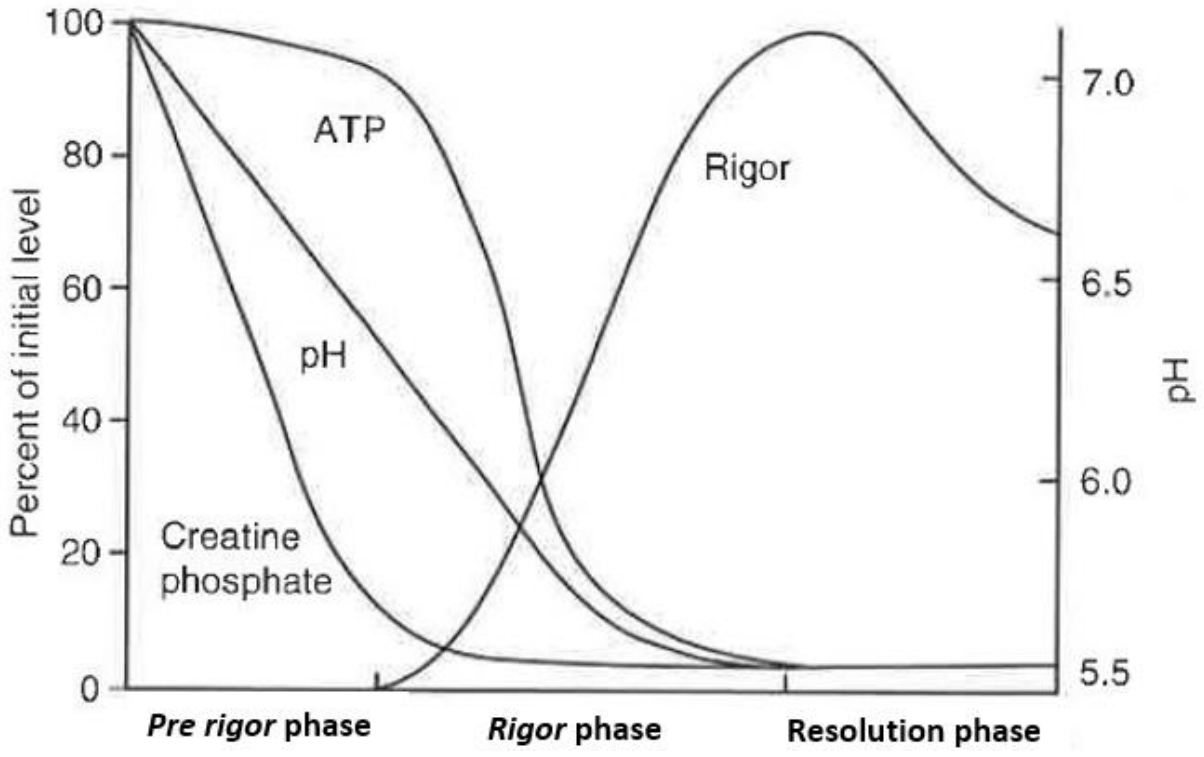

Figure 8. Overview of *post mortem* changes in pH, in concentrations of ATP and creatine phosphate, and rigor development as a function of time. After Strasburg, G., Xiong, Y. L., & Chiang, W. (2008).

The biochemical changes in the muscle lead to the changes in physical properties of *post mortem* muscle. Immediately after slaughter, the fish is elastic and has naturally firm texture (Figure 9). Eventually, the lack of ATP results in the accumulation of sarcoplasmic Ca^{2+} in

muscle and the formation of the actomyosin complex, also called the "*rigor* complex" (Strasburg et al., 2008). In other words, the muscles start to contract and pull against each other, making the fish stiffen and harden (Love, 1988; Stroud, 1969). If the fish struggles before death, the initial muscle pH is low and the time elapsed before the fish stiffens is strongly decreased (Kristoffersen, Tobiassen, Steinsund, & Olsen, 2006; Misimi, Erikson, Digre, Skavhaug, & Mathiassen, 2008; Roth et al., 2012). The fish can be in *rigor mortis* from one hour to several days, then, during resolution of *rigor mortis*, the muscles relax, and the fish becomes flexible again (Figure 9).

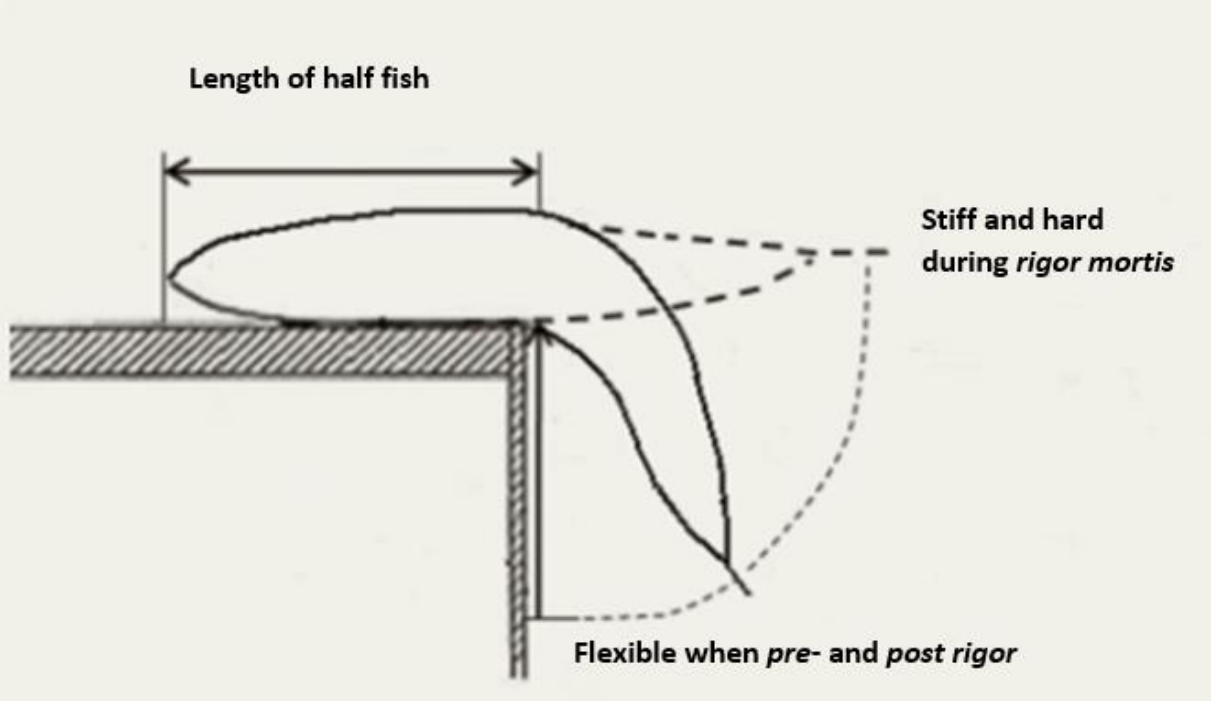

Figure 9. The fish in *pre-*, in- and *post rigor* condition. Retrieved and modified 18.03.2018 from imr.no, Report on Ocean Research 2011.

However, the texture of *post rigor* processed fish differs from the texture of *pre rigor* produced fish, by being softer and deforming more easily from pressure applied by a fingertip, often resulting in a more or less permanent depression in the muscle. This muscle softening occurs due to the activity of endogenous proteolytic enzymes that partially degrade the structure of the muscle, such as myofiber-myofiber attachment, structure of connective tissue and

myofiber-myocommata attachment, without breaking the actomyosin complex (Strasburg et al., 2008; Taylor, Fjaera, & Skjervold, 2002).

Fresh cod harvested in a traditional way, are usually in- or entering *rigor* when delivered to the processors. The processing of death-stiff fish is undesirable, since mechanical handling of such fish often leads to misalignment in both gutting and filleting machines, resulting in reduced fillet yield and severe fillet gaping (Love, 1988; Midling, 2011; Stroud, 1969). Thus, the processors commonly await a few days before production, losing the precious days from an already short shelf life of fresh cod products. Previously, *pre rigor* processing is known from the on-board production of frozen fish products on the larger trawler (Stroud, 1969; Tobiassen et al., 2006). Today, *pre rigor* filet production may be carried out in on-shore facilities as can be done with live-stored cod slaughtered under non-stressful conditions, similar to what often is done with farmed salmon. This creates the opportunity to produce very fresh fillets products and high quality by-products from the gutting process. It should be also noted that transport of fillets or fillet products will probably be less energy demanding in comparison to gutted head-on or gutted head-off fish.

The products made while the fish is in *pre rigor* phase differ from fillets made when the fish is in- or after *rigor mortis*. *Pre rigor* made products have less gaping, firmer texture, higher lightness and may arrive in the market 2-4 days earlier, giving a longer marketing time than the traditional *post mortem* products (Kristoffersen, Tobiassen, Esaiassen, et al., 2006; Kristoffersen et al., 2007; Mørkøre, Hansen, & Rørvik, 2006; Rosnes et al., 2003; Tobiassen et al., 2006). On the other hand, it has been revealed that *pre rigor* filleting may result in lower liquid-holding capacity, strong contraction and high drip loss of fillets during storage (Jørpeland, Imsland, Stien, Bleie, & Roth, 2015; Kristoffersen et al., 2007; Mørkøre et al., 2006; Ofstad et al., 1996; Sørensen, Brataas, Nyvold, & Lauritzen, 1997). Strong fillet contraction leads to changes in shape of the fillet, making the fillet thicker and wider (Misimi et al., 2008;

Skjervold et al., 2001). These issues are still not solved completely, but there are studies reporting several methods to reduce the fillet contraction or drip loss. For instance, vacuum packaging of *pre rigor* made salmon fillets can physically limit the contraction, but this may negatively affect the colour of the fillets (Veiseth-Kent et al., 2010). It has also been shown that *pre rigor* produced skin-on loins contracted less compared to skin-off loins (Kristoffersen, Kristiansen, Eilertsen, & Olsen, 2009). Another study has shown that by immersing the *pre rigor* cod fillets in salt solution, the drip loss during storage may be reduced but the extent of fillet contraction may increase (Larsen, Olsen, Kristoffersen, & Elvevoll, 2008). If possible, these challenges should be solved and there is a need for further investigations.

3 Experimental design

Atlantic cod were feed-deprived for 82 days, and effects on biological parameters of live fish as well as quality of the fillet products were studied. First, gender-specific weight changes in both whole and gutted fish, as well as in certain organs were studied, and the results are presented in Paper I. Next, the effect of feed deprivation on the onset and development of *rigor mortis*, as well as aspects of fillet quality were addressed, and the results are presented in Paper II. Finally, changes in quality of fillet products, both due to feed deprivation and different times of processing were studied (Paper III). The study was carried out in compliance with Norwegian legislations and approved by veterinary authorities (Code number: 7327).

The experimental design is shown in Figure 10. Atlantic cod were caught on 18 March near Andenes, Norway, and transferred to a sea cage at Bjarkøya, Norway. At Bjarkøya, two days after capture, 25 fish were sampled, weighted and measured (more detailed description in Paper I). Of those, ten fish were used for the assessment of development of both *rigor mortis* and muscle pH, as well as for analysis of muscle protein concentration, water content and water-holding capacity (Papers I and II). Another ten fish were filleted and skinned 24 h *post mortem*. Products: whole fillets, loins and tails, were made, and then stored in ice until the sensory evaluation at Nofima, Tromsø (Paper III). All products were used to study the changes in length and weight during ice storage. In addition, the fillets were used for measurements of muscle pH, water content and sensory evaluation. Seven days after capture, the remaining cod (~ 10 tonnes) at Bjarkøy were transported to the Aquaculture Research station at Skulgambukt, Tromsø. The next day, 400 individuals were transferred to the onshore facility at Kraknes, Tromsø. The day after, the fish were tagged and distributed equally between two indoor tanks (n=200 each). The fish were then live-stored without feeding for the remaining 73 days. Subsequently, 60 fish were sampled on days 26, 54 and 82 after capture. These were weighed, measured and filleted at different times *post mortem* and used for different analyses (Figure

10). In addition, three days before each of those samplings, ten fish were sampled for measurements of developments of both *rigor mortis* and muscle pH as well as protein- and water contents in muscle, and water-holding capacity (Paper II).

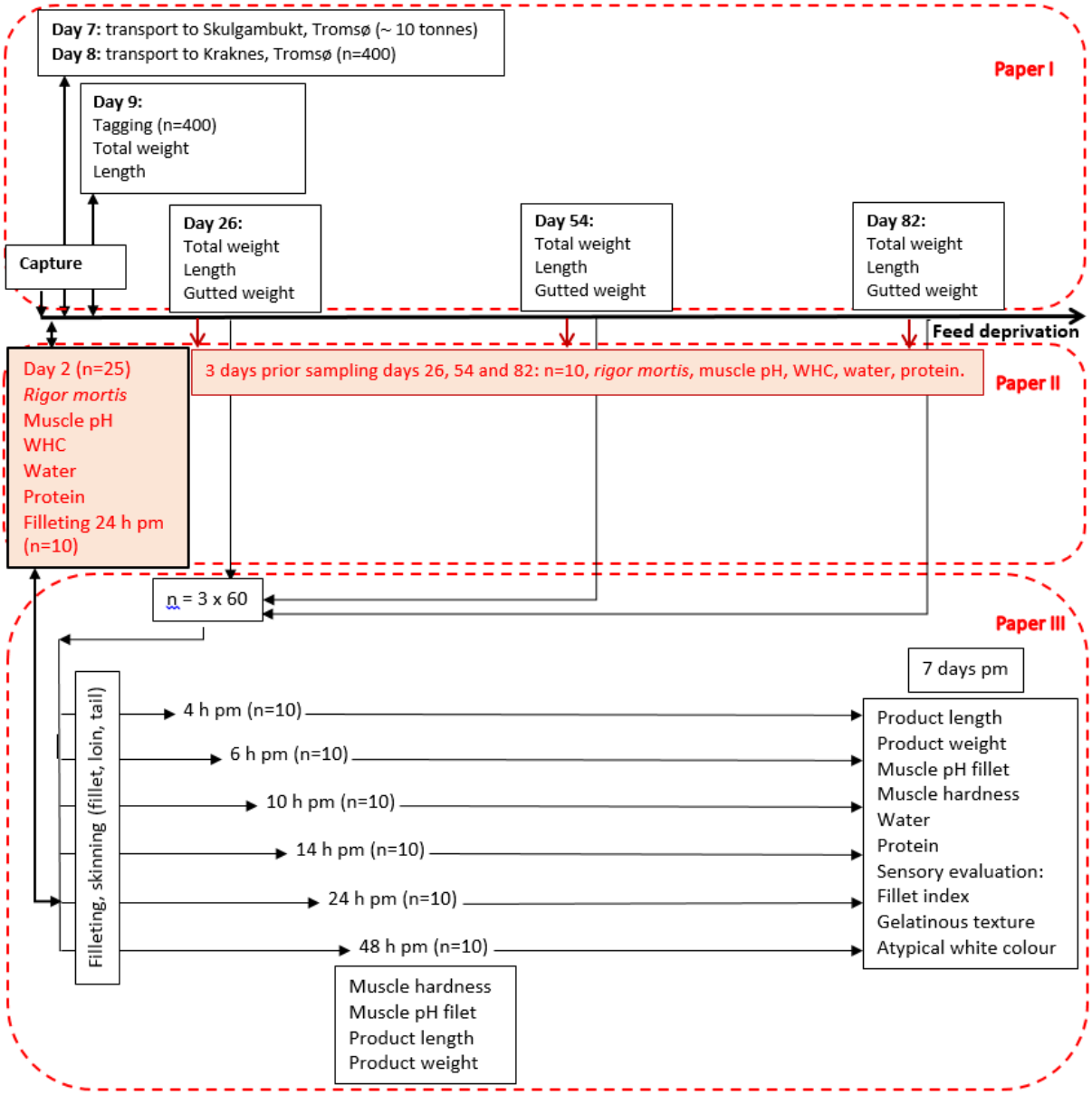

Figure 10. The experimental design and overview of papers, based on different steps of the trial. pm = *post mortem.*

4 Main results and general discussion

According to Norwegian regulations (FOR-2004-12-22-1878, 2004), wild fish can be kept in sea cages for up to 12 weeks, with the initial four weeks in absence of feed. Live-stored wild cod unfortunately do not accept formulated dry feed well (Sæther & Bogevik, 2017). Consequently, there is a need to study both the biological response of wild, feed-deprived cod and the quality changes of final products that occur due to feed deprivation.

4.1 Weight loss during feed deprivation

First, the effects of feed deprivation upon weight loss in mature Atlantic cod were studied. The cod (skrei) were caught in spring 2015 in their spawning area (Paper I). To simulate the situation, occurring during live-storage of skrei in fish industry, initially, the data from this study were not gender-differentiated.

Skrei are usually of large size and sexually mature Atlantic cod, which also was the case for the cod used in the present study (Table 1). The fish had high GSI (Gonadosomatic index) and were in good condition based on HSI (Hepatosomatic index). The size of liver in cod is related to feed intake, and thus, HSI can be used as an indicator of the nutritional status of wild Atlantic cod (Black & Love, 1986; Hemre, Karlsen, Lehmann, Holm, & Lie, 1993; Jobling, 1988). According to Jobling (1988), for wild cod, the HSI values within the range of 2-6% should be considered as "normal" and the range of 8-9% as "abnormal".

Table 1. Biological data (not gender-differentiated) of the experimental Atlantic cod (n=25) slaughtered 2 days after capture.

Biological parameters	
Length (cm)	90.6 ± 8.9
Body weight (g)	6820 ± 2105
HSI[a]	5.0 ± 1.9
GSI[b]	13.2 ± 4.8

(Mean ± STDEV of mean)
[a] HSI (Hepatosomatic index) = (liver weight / body weight) x 100
[b] GSI (Gonadosomatic index) = (gonad weight / body weight) x 100

Large values of GSI and HSI can have great economic consequences for the producers, as fish body weight and muscle quality can be reduced already before slaughter due to spawning and prolonged feed deprivation during live-storage, as it was also detected during our study. Figure 11 shows the total and gutted weights, as well as weights of liver and gonads of the cod measured during trial. The fish, kept in indoor tanks, were spawning despite the absence of feed. The depletion of gonads, the reduction of total weight (~18%), liver size (~22%) and minor reduction of gutted weight (~5%) during the first 54 days reflected the spawning. The main changes in the gutted weight of cod occurred during the post-spawning period, i.e. the last 28 days of the trial. After 82 days of feed deprivation, the total weight of fish decreased by about 29%, gutted weight by around 15% and liver size by 55%.

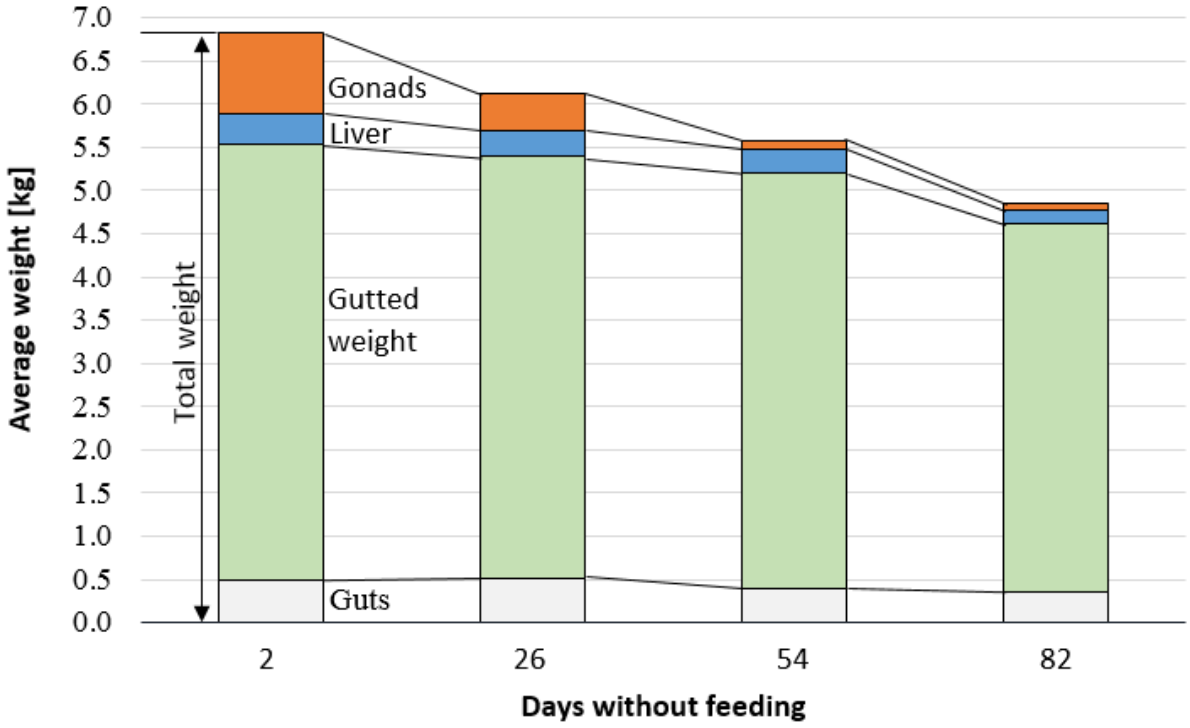

Figure 11. The total and gutted weight, as well as weights of gonads, liver and the remaining viscera (guts) of Atlantic cod sampled on days 2 (n=25), 26 (n=60), 54 (n=60) and 82 (n=60) after capture.

The changes in gutted weight concurred with the significant reduction of protein concentration and increase in water content in cod muscle during the post-spawning period (Figure 12). In other words, despite the absence of feed, skrei may spawn and, thus, lose both the high quality

by-products (roe and liver) and body weight during storage in sea cages before the processing. If the fish are still kept without feeding in the post-spawning period, the nutritional quality of the fillets will also be reduced due to the spawned-out and feed-deprived cod starting to utilize the muscle proteins as a main energy source to survive. This also contributes to the reduced fillet yield.

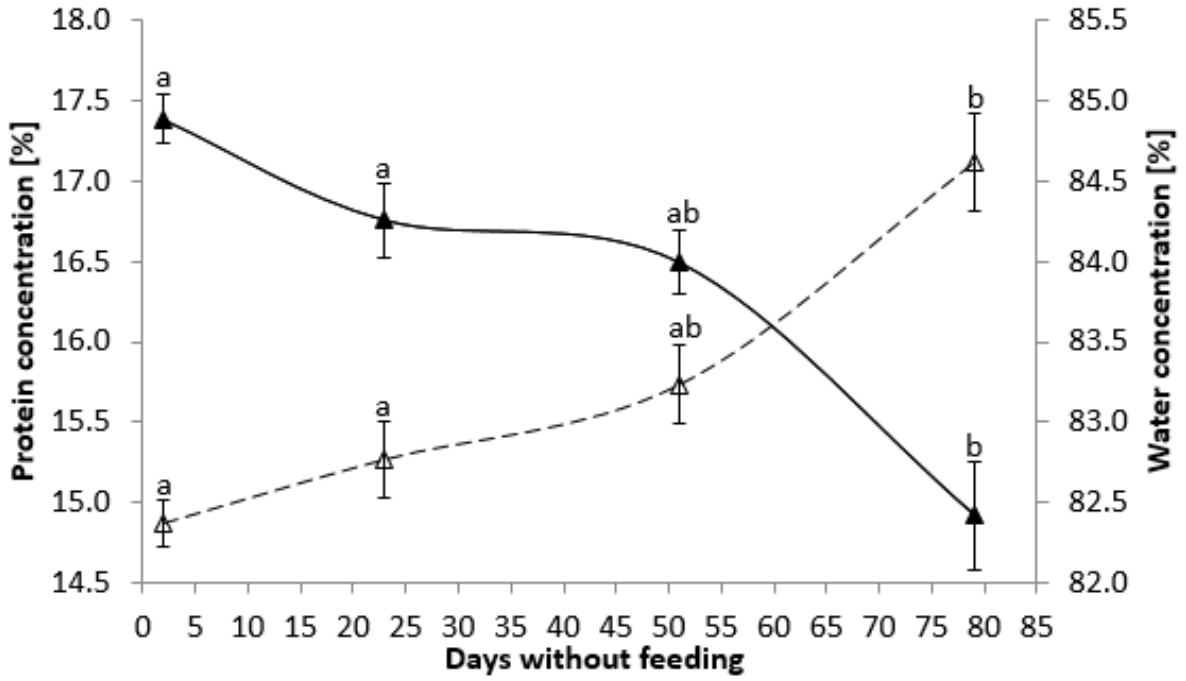

Figure 12 Changes in concentrations of protein (▲) and water (Δ) in muscle of live-stored cod sampled on days 2, 23, 51 and 79 after capture (each day: n=10). The data were analysed using ANOVA, and the different letters indicate significant changes in protein- and water concentrations ($p < 0.05$) during feed deprivation.

4.2 Sexual dimorphism during feed deprivation

It has been reported that female and male cod can respond differently to feed deprivation (Hagen & Solberg, 2010; Karlsen et al., 1995), thus, the data in this study were also gender-differentiated, and the results are presented in Paper I. In addition, the data were standardised to a fish with body length 90 cm. That was done due to the wide range of fish sizes detected in

each sampling. The average body length (90 cm) of females and males in each sampling was used for calculations, as the fish length did not change during live-storage.

At the start of the trial, the standardised females had slightly lower total weight than males of the same length (Figure 13). However, females had somewhat higher weights of gonads and liver, and thus significantly lower gutted weight than males. During the trial, both sexes decreased in total weight but at the end of the trial, the 90 cm females weighted significantly less than males of the same length.

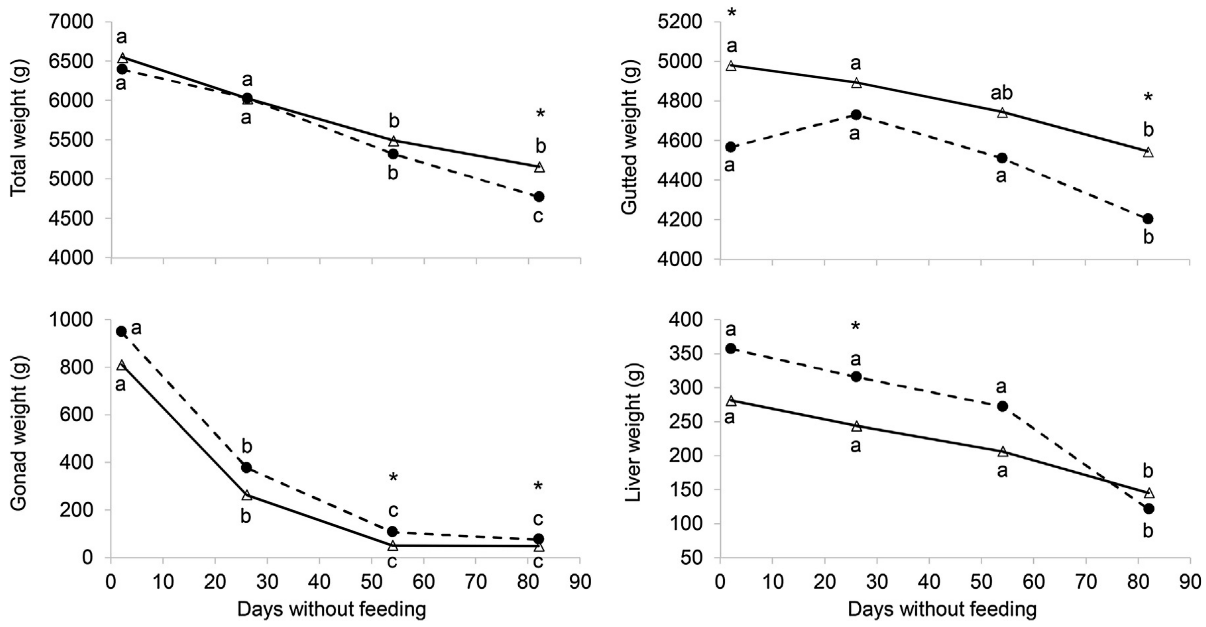

Figure 13. Changes in total and gutted weights, the weights of gonads and liver in standardized to 90 cm length female (●) and male (Δ) Atlantic cod during 82 days of feed deprivation. Lower case letters indicate significant differences ($p < 0.05$) between sample days within sexes, and asterisks (* = $p < 0.05$) show the significant differences between sexes on a given sampling date.

The major decrease in total weight in both sexes occurred during the first 54 days of the experiment, when the fish were spawning. It seems that males were more active during this period, as they depleted more gonads (93.7%) and liver (26.5%) than females of the same length (88.6% and 24.1%, respectively). Despite that, males tolerated better the absence of feed in post-spawning period, as the total loss of liver registered at the end of the trial in 90 cm males

(48.2%) was much lower than in 90 cm females (66%). In addition, the gutted weights of both sexes decreased with prolonged feed deprivation. However, this reduction did not affect significantly the concentration of muscle protein in males for up to 82 days but muscle protein content in females was significantly reduced at the end of the study. The protein concentration in muscle of females was significantly lower than in males after feed deprivation for both 54 and 82 days (Figure 14). The sexual dimorphism became more evident at the end of the trial, when the females had significantly lower total weight, gutted weight as well as had depleted more liver and muscle protein than males (Figures 13 and 14). Hagen and Solberg (2010) determined the muscle protein concentrations in farmed, male and female Atlantic cod during 11 weeks of feed deprivation and found that females had significantly lower muscle protein concentration than males during the whole period of starvation. The cod used in their study were smaller and not sexually mature.

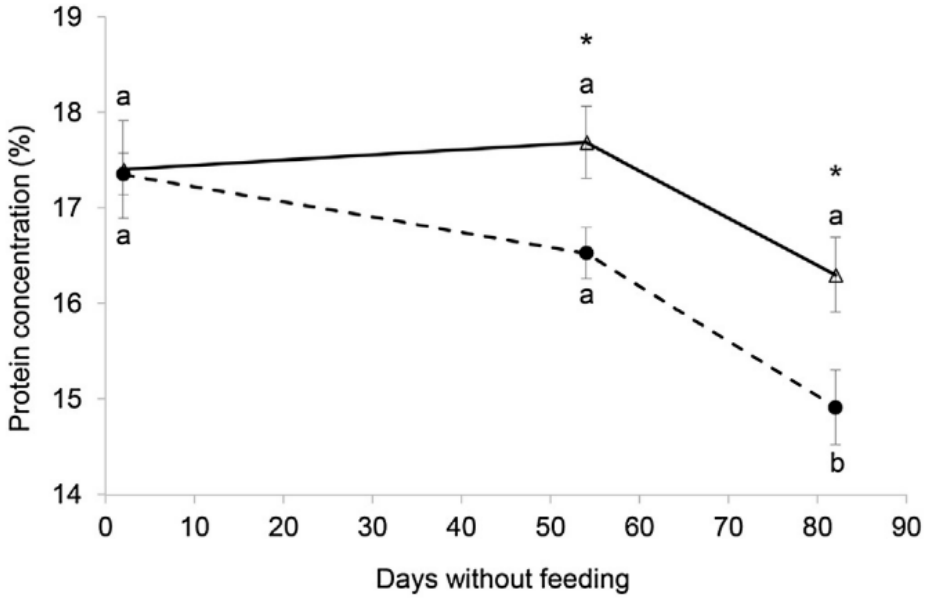

Figure 14. Changes in concentration of muscle protein in female (●) and male (Δ) Atlantic cod after feed deprivation for 2 (♀=5, ♂=5), 54 (♀=10, ♂=10) and 82 (♀=10, ♂=10) days after capture. Lower case letters indicate significant differences ($p < 0.05$) between sample days within sexes, and asterisks (* = $p < 0.05$) show the significant differences between sexes on a given sampling date.

There are different findings about cod's need for feed intake during spawning. Krumsick and Rose (2012) found that Atlantic cod from the Newfoundland-Labrador area do feed during the spawning period. Similar results were reported for mature cod spawning near Lofoten (Michalsen, Johannesen, & Bogstad, 2008). In both studies, the data were gender-differentiated and the results reported were contradictory regarding genders. Krumsick and Rose (2012) declared that females consumed less feed than males while Michalsen et al. (2008) stated the opposite. In the present work, the stomachs of all cod sampled 2 days after capture were empty and our findings support the statement made by Fordham and Trippel (1999). They registered that both sexes supressed feeding at the start of the spawning season and fed more towards the end of spawning, with males consuming less than females.

It is clear that mature female and male Atlantic cod have different metabolic needs and therefore respond differently to feed deprivation. Sexual dimorphism may have technological consequences since the lower protein concentration in female cod after long-term feed deprivation must result in a higher water content in the muscle. This may be of interest in further investigations.

4.3 The tolerable period of feed deprivation

Based on the changes in gutted weight and concentration of muscle proteins, the acceptable feed deprivation period for mature Atlantic cod during spawning and post-spawning period is approximately 54 days (Paper I). However, this statement might not be applied to all cod captured throughout the year as the tolerance for feed deprivation of Atlantic cod may vary depending on the original nutritional condition and size of energy resources of each individual.

Atlantic cod show seasonal changes in pattern of feeding, growth, metabolism and energy storage throughout the year (Jens-Eric Eliassen & Ola Vahl, 1982; J-E Eliassen & O Vahl, 1982; Fordham & Trippel, 1999; Love, 1988; Schwalme & Chouinard, 1999; Solberg & Willumsen, 2008). Other scientists studied the response to feed deprivation in wild, live-stored

Atlantic cod caught at different times of the year. The young immature fish, such as "capelin cod", response differently than spawning cod to feed deprivation. Akse and Midling (1997) carried out two experiments where "capelin cod" were live-stored without feeding for 44 days (1990) and for 73 days (1991) after capture. In the former, the main changes in muscle protein and water contents occurred after 28 days of feed deprivation while in the latter after 54 days. The different response to feed deprivation of capelin cod in the experiments were probably linked to the nutritional condition of fish at the start of the trials as it was mentioned that the cod from 1990 were of atypical poor quality in contrast to the fish studied the year after.

In the study to Sæther et al. (2016), the effects of feed deprivation in spawning cod from Lofoten area were studied. They found that the total weight in mature cod was reduced by ~20% after 48 days. In our study, the mature cod were used and the detected body weight loss was ~18% after 54 days. Both results are higher than loss of body weight (10%) detected by Akse et al. (1997) after 73 days of feed deprivation of immature cod studied in 1991 as well as reduction of body weight (~15%) reported in the same report by Sæther et al. (2016) of "capelin cod" after 56 days of feed deprivation. This difference is clearly due to the loss of gonads as the mature fish were spawning. Furthermore, the time of main body weight loss in mature cod may also differ between batches depending on their time of spawning. Sæther et al. (2016) reported that some cod individuals lost up to 20% of body weight already after 7 days of live-storage without feeding. This is due to Atlantic cod is being a multiple batch spawner (Kjesbu, 1989; Rakitin, Ferguson, & Trippel, 2001) and individual fish can be in different phases of spawning at the time of sampling.

4.4 Development of *rigor mortis* in feed-deprived mature Atlantic cod

Long-term feed deprivation changes the protein and water contents in the muscle (Paper I) and reduces the amounts of muscle glycogen in fish (Black & Love, 1986; Mørkøre, Tahirovic, & Einen, 2008). Muscle glycogen serves as the main energy source for the anaerobic glycolysis

that occurs in *post mortem* muscle (Strasburg et al., 2008). The amount of *post mortem* energy reserves in the muscle affects the time before ATP is depleted and thereby the onset and development of *rigor mortis*. The pattern of *rigor mortis* is important for the quality of fillet. Thus, it was of interest to study any changes in time before the onset and development of *rigor mortis* as well as changes in muscle properties in the feed-deprived cod (Paper II).

The results showed that development of *rigor mortis* in the least feed-deprived cod differed much from that observed in the fish starved for longer periods (Figure 15, Table 2). Specifically, cod sampled 2 days after capture had almost twice as long *pre rigor* time (t_{CF100g}), moderately strong maximum *rigor* (CF_{max}), lowest rate of rigor resolution (r_{res}) and moderate level of muscle hardness (CF_{term}) after *rigor* resolution (Table 2). Lowest r_{res} indicates that there were small changes in muscle hardness occurred after maximum *rigor mortis* and that it took more time for *rigor* resolution (see equation for r_{res} under Table 3). Feed deprivation reduced the time elapsing before the onset of *rigor mortis*. However, to our surprise, only feed deprivation of Atlantic cod for 23 days strongly reduced *pre rigor* time while further feed deprivation did not decrease this time significantly (Table 2). The *pre rigor* time was halved after 23 days of feed deprivation while further feed deprivation resulted in only one hour shorter *pre rigor* time every fourth week.

This reduction pattern indicates that even spawned-out and feed-deprived cod do not deplete the energy reserves completely and may still have a relatively long *pre rigor* time, making *pre rigor* processing possible. However, it should be taken into consideration that if the fish struggles before slaughter, much of energy reserves will be depleted and the muscle will obtain low pH earlier, resulting in the strongly reduced time prior *onset* of *rigor mortis* (Aursand, Erikson, & Veliyulin, 2010; Bjørnevik & Solbakken, 2010; Digre et al., 2011; Erikson, Digre, & Misimi, 2011; Kristoffersen, Tobiassen, Steinsund, et al., 2006; Misimi et al., 2008; Roth et al., 2012).

Table 2. *Pre rigor* time (t_{CF100g} [h]) and *rigor mortis* development at each sampling day assessed on individual basis as maximal compression force (CF_{max} [g]), time elapsed before maximal compression force (t CF_{max} [h]), rate of *rigor mortis* resolution (r_{res} [g h-1]) and compression force determined 5 days *post mortem* (CF_{term} [g]).

Sampling days	t_{CF100g}	t_{CFmax}	CF_{max}	r_{res}	CF_{term}
2	28.7 ± 2.1[a]	53.4 ± 1.9[a]	455.2 ± 39.7	4.4 ± 0.3	223.0 ± 22.8[a]
23	17.1 ± 1.7[b]	49.2 ± 2.8[ab]	543.6 ± 57.1	5.4 ± 1.2	238.8 ± 31.1[a]
51	16.0 ± 1.3[b]	45.7 ± 3.9[ab]	499.0 ± 74.2	8.5 ± 1.9	181.4 ± 31.1[ab]
79	14.9 ± 1.9[b]	40.4 ± 3.5[b]	397.0 ± 45.7	6.7 ± 2.2	123.6 ± 13.7[b]
Significant difference between days	$F(3,36) = 13.350$	$F(3,36) = 3.110$	$F(3,36) = 1.261$	$F(3,36) = 1.217$	$F(3,36) = 4.003$
	$p = 0.000$	$p = 0.038$	$p = 0.302$	$p = 0.318$	$p = 0.015$

(Mean ± SE of mean).
$r_{res} = (CF_{max} - CF_{\pm 50g})/(t_{CF\pm 50g} - t_{CFmax})$.
CF constant ($CF_{\pm 50g}$ [g]) is the compression force when individual change was less than ±50 g.
$t_{CF\pm 50g}$ = time elapsed before CF constant.
Different lowercase letters indicate significant differences ($p < 0.05$) between sampling days.

The effects of combination of feed deprivation and stress on *rigor* development have been studied for farmed Atlantic salmon. According to Mørkøre et al. (2008), stressed starved salmon have half as long *pre rigor* period compared to non-stressed starved salmon, but it has also been registered that stressed fed salmon have even shorter *pre rigor* time compared to stressed starved salmon. In contrast, there are findings indicating that farmed feed-deprived cod are more prone to stress than fed cod, based on changes in blood and plasma chemistry, but the impact of stress on *rigor mortis* in such cod was not studied (Olsen et al., 2008). Thus, it may be of interest to investigate the development of *rigor mortis* related to different stress levels in live-stored Atlantic cod exposed to different periods of feed deprivation.

Furthermore, it seems that long-term feed deprivation may also reduce the strength of maximum *rigor mortis* and level of muscle hardness, in other words texture, after *rigor* resolution (Table 2). In this study, the most feed-deprived cod had the shortest *pre rigor* time, lowest maximum *rigor* and the softest muscle of all cod in the trial. This is probably related to the sequential utilization of muscle nutrients, increased proteolytic activities, and also to increased water content in muscle.

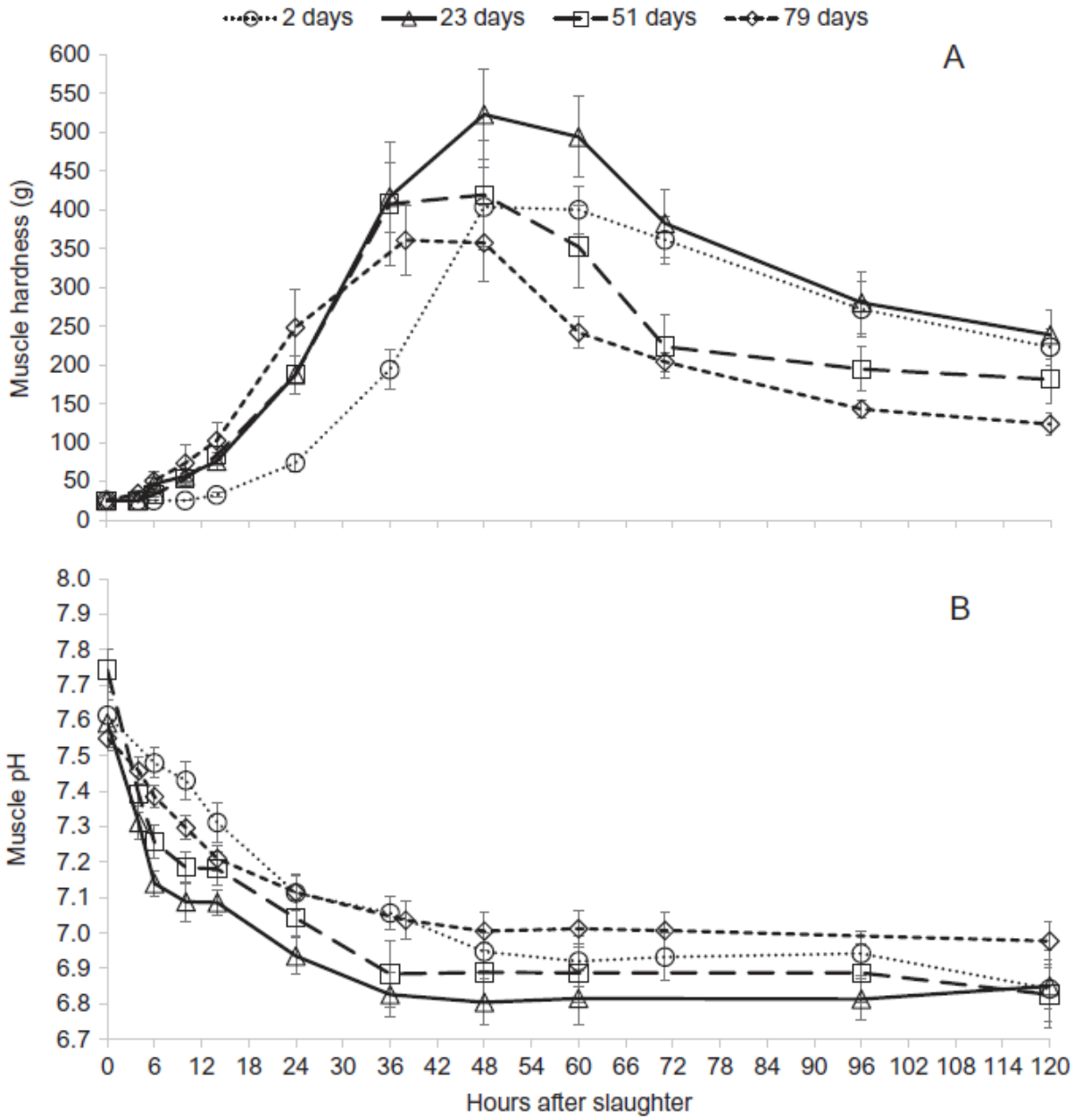

Figure 15. Development of *rigor mortis* in Atlantic cod feed-deprived for 2, 23, 51 and 79 days. The changes in muscle hardness (A) and muscle pH (B) were detected at time points 4, 6, 10, 14, 24, 36, 48, 60, 72, 90 and 120 h *post mortem* while the fish were stored in boxes with ice. Each point is represented as MEAN ± SE of mean (n=10).

4.5 Effects of feed deprivation and time of processing on product quality

The period of feed deprivation prior to slaughter and time of processing are the factors that can strongly affect the cod in biological, biochemical and physical ways (Papers I and II), and the quality of raw material has a strong impact on the quality of end product (Akse et al., 2005; Borderías & Sánchez-Alonso, 2011; Kiessling et al., 2007). Most producers process the cod by using filleting machines, followed by manually trimming of the fillets. There are different standards of trimming, from removing just blood spots to removing pin bones and cutting the fillet in different sections. Often, such fillets sections like loins (Figure 16) and tails (Figure 17) are being made and then commercialized for different prices.

Figure 16. Loin.

Figure 17. Tail.

To our knowledge, most of the scientific findings about quality changes in fish muscle are made based on the data obtained from whole fillet. Thus, it was of interest to investigate the quality of different fillet products, such as fillets, loins and tails, made from feed-deprived cod at different times *post mortem* (Paper III). The investigated aspects were product contraction, drip loss, muscle hardness, water content, and sensory attributes like texture, colour and odour.

In the trial, all *pre rigor* products (made during the first 14 h after slaughter) contracted more than products made after onset of *rigor* (24 h and 48 h *post mortem*), independent of period of feed deprivation (Figure 20 A, C and E). The earlier after slaughter the products were made, the higher product contraction was registered. For instance, fillets obtained from cod feed-deprived for 82 days and made 4 h *post mortem* contracted by ~22% while fillets made 48 h *post mortem* only by ~0.9% (Figure18).

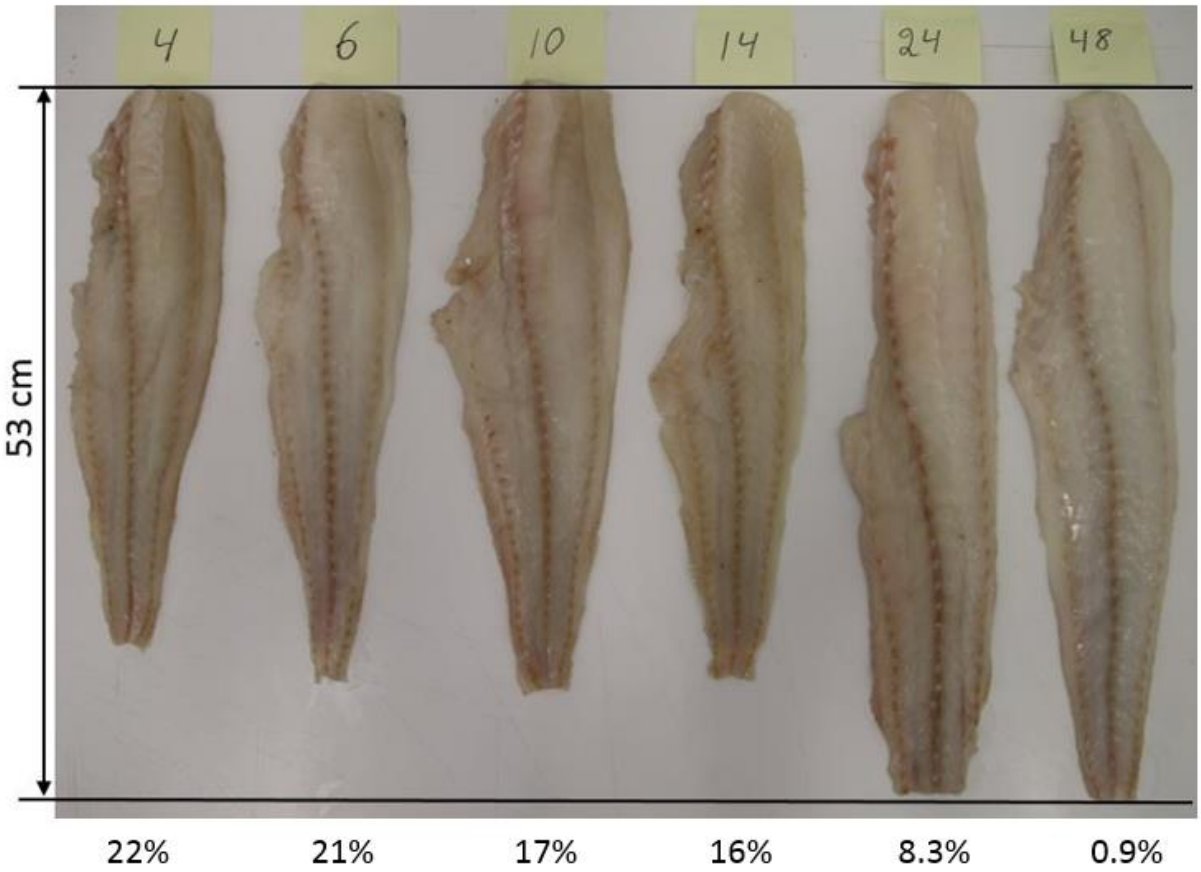

Figure 18. Fillets made at 4, 6, 10, 14, 24 and 48 h *post mortem* from Atlantic cod feed-deprived for 82 days. The fillets shown were initially 53 cm long. The percentages given under each fillet correspond to averaged contraction in fillet length for a group (n=10) ice stored from the time of filleting to day 7 *post mortem*.

There is a correlation between fillet contraction and development of *rigor mortis* (Figure 19). In the Figure 19, it is shown that despite the fact that fillets (shown in the figure) were made from cod feed-deprived for three days more than the cod used for *rigor* measurements, the differences in length of fillets made during different stages of *rigor* development are clear. When fish are filleted *pre rigor*, the muscle are more prone to contraction since there is still much energy to use and, at the same time, all mechanical support from the vertebrae and skin has been removed. The greater contraction in *pre rigor* fillets than in *post rigor* fillets is a known phenomenon and has been reported in several studies (Jørpeland et al., 2015; Kristoffersen et al., 2007; Misimi et al., 2008; Mørkøre et al., 2008).

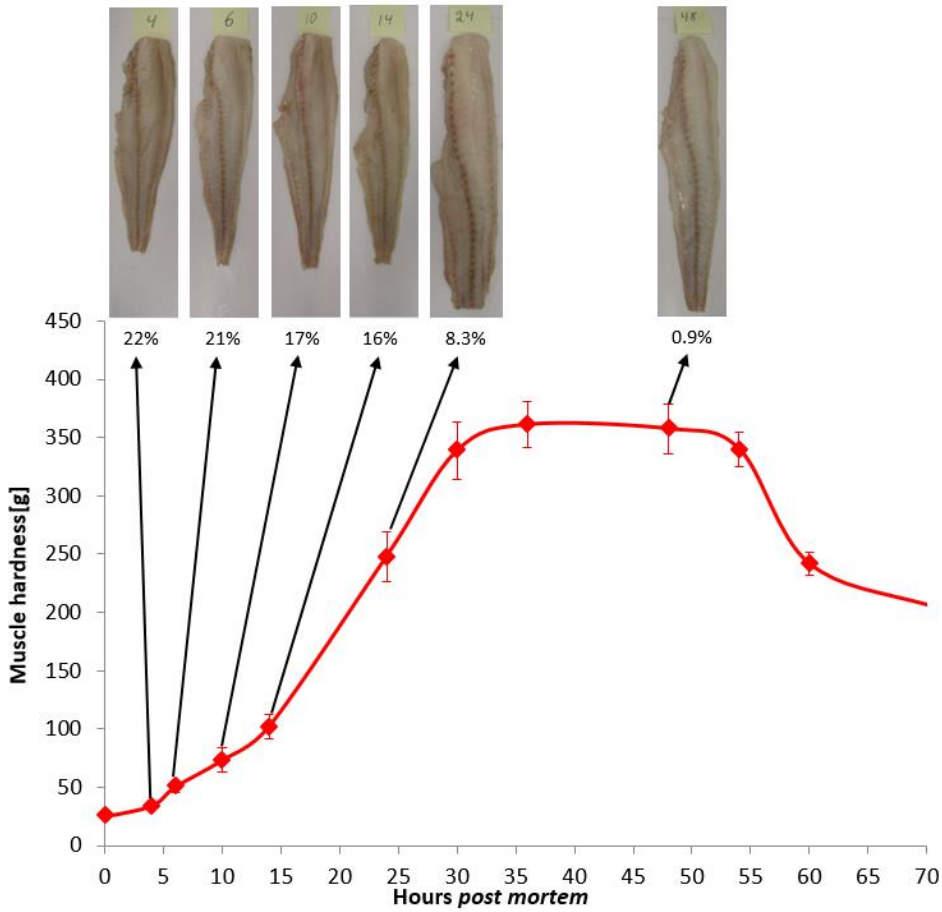

Figure 19. Fillets made during different stages of *rigor mortis* development, assessed as changes in muscle hardness. Muscle hardness was determined by measuring compression force (g) at 4, 6, 10, 14, 24, 30, 36, 48, 54, 60 h *post mortem* in Atlantic cod live-stored without feeding for 79 days. The fillets were made at 4, 6, 10, 14, 24 and 48 h *post mortem* from Atlantic cod feed-deprived for 82 days after capture and stored in ice from the time of filleting to day 7 *post mortem*. Initially, these fillets were 53 cm long (n=10).

Further, the contraction in all products tended to be reduced towards the end of feed deprivation (Figure 20 A, C and E). This is consistent with the results presented in Paper II where the most feed-deprived cod had the shortest *pre rigor* time and the lowest muscle hardness during maximum *rigor*. However, viewed from the producers' perspective these minor differences do not justify prolonged feed deprivation as the contraction of *pre rigor* products could still be higher than 20% (Figure 18).

The pattern of drip loss was not similarly clear as *rigor* contraction and the changes in drip loss of all products were not significantly dependent on the time of filleting (Figure 20 B, D and F). At the same time, *pre rigor* fillets had slightly lower water content than *post rigor*

fillets (Paper III). These differences became more evident after 54 d of feed deprivation and remained until the termination of the experiment, when the most feed-deprived cod had also highest water content in the muscle. There are contradictory statements about variations in drip loss and water content in fillets during storage. Kristoffersen, Tobiassen, Esaiassen, et al. (2006) and Jørpeland et al. (2015) reported increased drip loss and decreased water content in *pre rigor* fillets from farmed cod after ice storage for 6 and 12 days, respectively. No significant differences in water content between *pre* and *post rigor* processed fillets were found by Esaiassen, Dahl, Eilertsen, Gundersen, and Sivertsvik (2008). Akse et al. (2008) reported about almost equal drip loss in *pre* and *post rigor* loins after feed deprivation of Atlantic cod for four weeks. The possible explanation to non-clear changes in water content in fillets used in our trial could be the way the samples were taken, as the group of fillets (n=10) from each time of filleting were divided in two, and five samples were homogenised together before the water analysis (Paper III).

The changes in length and drip loss of tails differed from that found for fillets and loins but the latter ones did not differ significantly from each other (Figure 20). It has been noticed that tails, especially made *pre rigor* after 26 days of feed deprivation, were more susceptible to shortening than loins (Figure 20 A, C and E). This may be explained by higher surface to volume ratio, and higher proportion of dark muscle in tails than in loins. Dark muscle contain more mitochondria, lipid and glycogen than white muscle (Buttkus, 1963; Cappeln & Jessen, 2002; Foegeding et al., 1996), and thus, the *rigor* development in tails can differ from that in loins. For instance, Buttkus (1963) has shown that contraction in length can be three times higher in red than in white muscle of lingcod (*Ophiodon elongatus*). In a study with rats it has also been found that red muscle goes into *rigor mortis* faster than white muscle (Kobayashi et al., 2000).

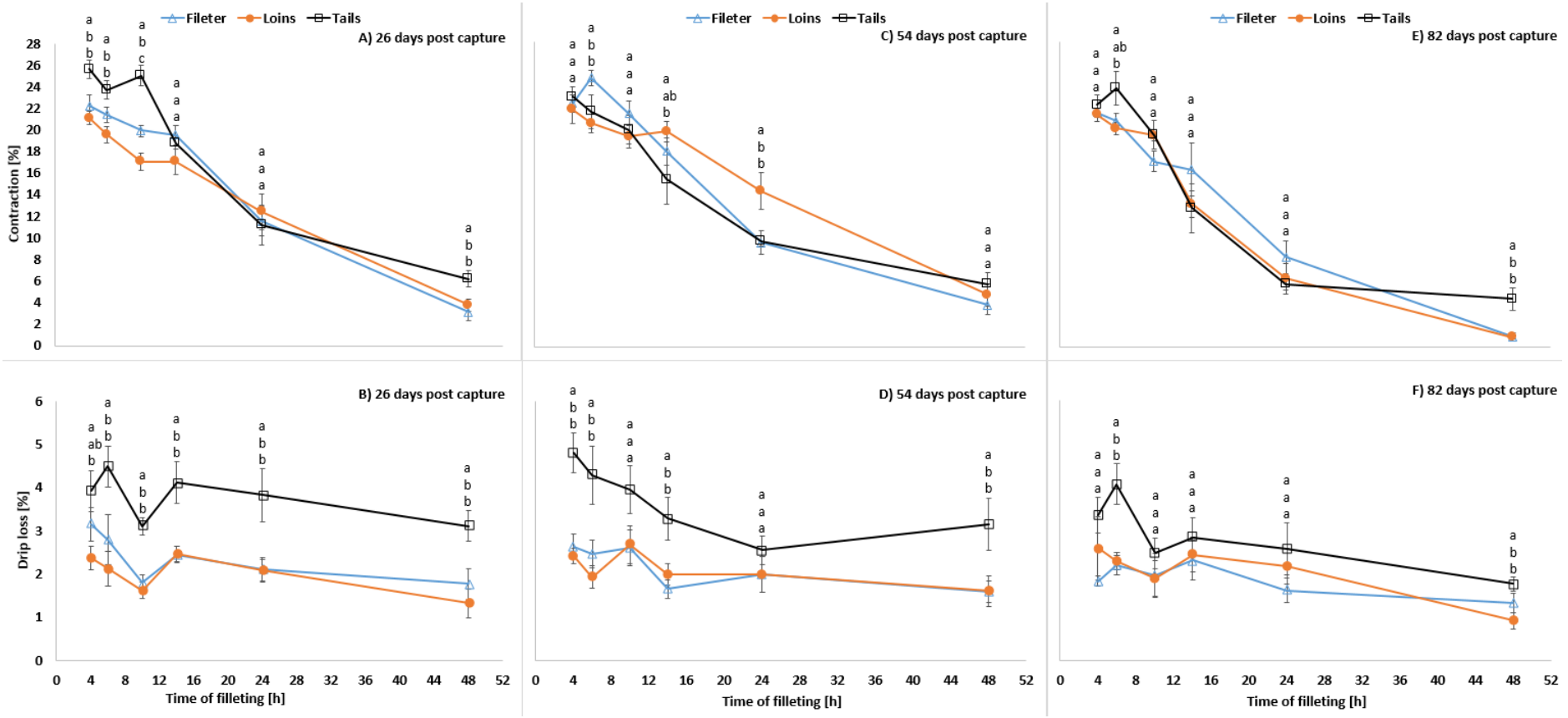

Figure 20. Contraction and drip loss of fillets, loins and tails were determined 7 days *post mortem* of cod feed-deprived for 26 days, 54 days and 82 days after capture. Filleting was carried out at 4, 6, 10, 14 , 24 and 48 h post mortem. The small letters indicate significant differences between fillet-, loin- and tail products made at the same time of filleting but on different samplings.

The drip loss during ice storage was higher in tails than in loins, independently of how long the fish were feed-deprived before slaughter or when the fish were processed after slaughter (Figure 20 B, D and F). The geometry of the tails with greater surface to volume ratio makes the tail products more prone to loss of muscle water. The fact that tails were more prone to contraction than loins may also contribute. Muscle shrinkage, occurring during *rigor* contraction, may lead to increased drip loss during the first days after *pre rigor* filleting (Kristoffersen et al., 2007), as it may press muscle water from the intramyofibrillar spaces into extramyofibrillar areas, where it is more easily lost as drip loss (Bertram, Purslow, & Andersen, 2002; Huff-Lonergan & Lonergan, 2005; Offer & Trinick, 1983). It is also possible that tail section of the fillet have higher water content than other fillet areas, as it has been reported for farmed salmon by Aursand et al. (2010). To prevent or reduce drip loss during storage, leaving the skin on products could be considered. Kristoffersen et al. (2009) reported that *pre rigor* produced loins with skin-on had lower drip loss than *pre rigor* made skin-off loins of cod after ice storage for 14 days.

Furthermore, it was detected that long-term feed deprivation reduced drip loss in tails but there were no changes in drip loss in loins despite the higher water content in muscle towards the termination of the trial (Figure 20 B, D and F). The possible explanation could be related to the reduced energy reserves in red muscle, or higher muscle pH and higher water holding capacity, or stronger myocommata after prolonged feed deprivation. These ideas could be tested in further investigations.

Whether the time of filleting or feed deprivation may change the sensory aspects of tail product may also be interesting to investigate. According to Love (1988), the red muscle has more flavour intensity than white muscle but it is also more prone to rancidity during storage. In this trial, only fillets were evaluated by a sensory panel of three experts. The evaluation was carried out to determine how sensory properties of the fillets were affected by feed deprivation

and time of filleting *post mortem*. Fillet index method was used (Esaiassen et al., 2008) in addition to determining gelatinous texture of fillet and colour (Paper III). The latter two were evaluated because of previous findings about starving cod may develop gelatinous (sloppy) texture and an atypical white colour (Love, 1988; Sæther et al., 2016).

The results showed that the fillet texture was affected by time of processing (Figure 21).

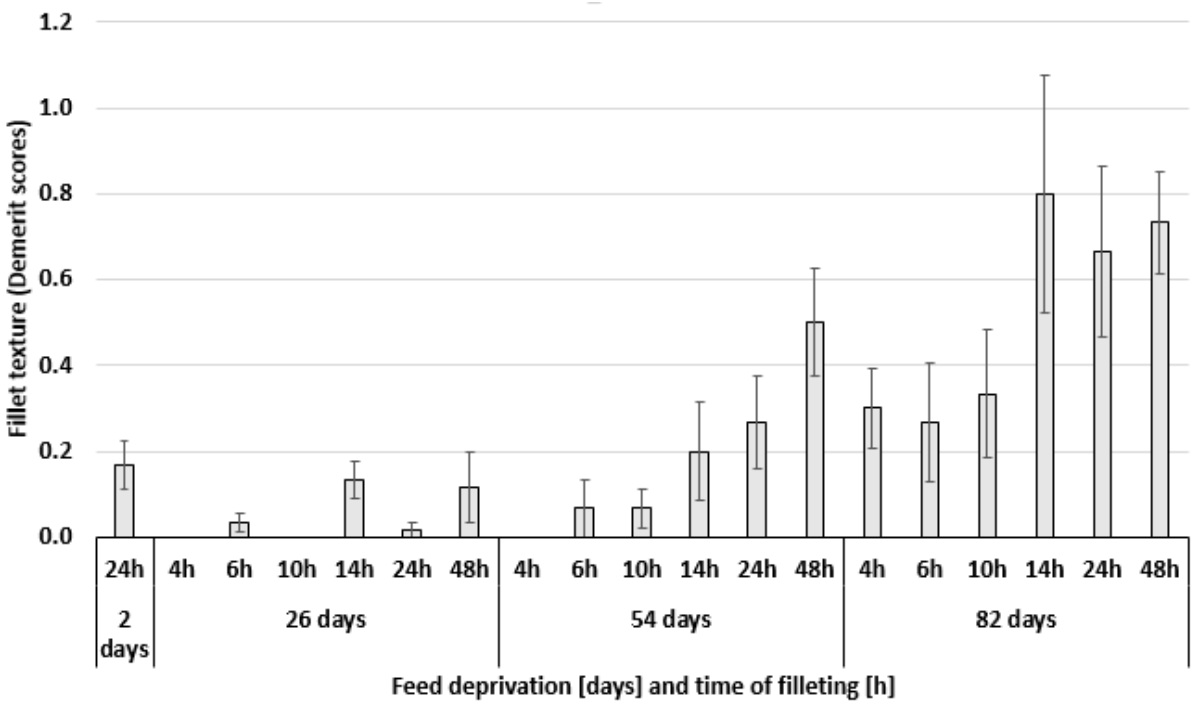

Figure 21. Fillet texture was assessed by a sensory panel of three experts on sampling days 2, 26, 54 and 82 after capture in fillet groups (n=10) processed at 4, 6, 10, 14, 24 and 48 h *post mortem* and stored in ice until day 7 after slaughter. The fillet texture was evaluated by using fillet index method and the given demerit scores were 0: naturally firm, 1: little soft, 2: moderate soft and 3: severe soft texture.

Early processing (during the first 10 h after slaughter) provided fillets with firmer texture. This was also confirmed by measuring the of muscle hardness after ice storage, on day 7 post mortem (Paper III). At the same time, the fillets made from the most feed-deprived cod (82 days) had more neutral odour, brighter colour and softer texture independently of time of processing. This could be related to higher water content and higher proteolytic activity in the muscle of long-term feed-deprived cod. The amount of fillets having gelatinous texture and atypical white

colour increased with prolonged feed deprivation. For instance, only 1 of 60 fillets had gelatinous texture and atypical white colour after 26 days of feed deprivation. In contrast, 34 of 60 fillets were affected by feed deprivation for 82 days, 21 of affected fillets had both gelatinous texture and atypical white colour and the reminder fillets had just one of such two defects.

5 Conclusions

In this thesis it has been shown that prolonged feed deprivation of wild mature Atlantic cod during live-storage affected negatively both the biological condition of fish, the development of *rigor mortis*, and finally, the quality of fresh products made from such fish. In addition, the quality of final products changed depending on the time of processing of the fish.

Despite the absence of feed, the mature cod were spawning during live-storage, resulting in a great loss of total weight before processing. Female cod responded differently to feed deprivation than males. Specifically, females have a greater loss of total and gutted weight, reduction of liver and muscle proteins, but the reduction of gonads weight was significantly higher in males.

Feed deprivation made *pre rigor* time shorter. However, feed deprivation for 23 days reduced *pre rigor* time from 29 to 17 h while further feed deprivation did not reduced this time significantly. If the fish is slaughtered under non-stressful conditions, the *pre rigor* time in feed-deprived cod can still be long enough to process the fish prior to the onset of *rigor mortis*.

Feed deprivation of mature cod also slightly reduced the strength of maximum *rigor* and product contraction during ice storage. A higher drip loss in tails was detected independently of the period of feed deprivation and time of filleting.

Feed deprivation reduced gutted weight, and thus, fillet yield as well as nutritional quality of cod muscle, as the cod feed-deprived for more than 51-54 days had a reduced protein concentration and a higher water content in muscle. In addition, the sensory profile of fillets was also changed with length of feed deprivation, as most fillets obtained after feed deprivation of fish for 82 days had unpleasantly soft texture, atypical white colour and less fresh sea odour. The results in this thesis suggest that tolerable period of live-storage without feeding of wild mature cod is approximately 54 days.

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Paper I

Paper II

Paper III