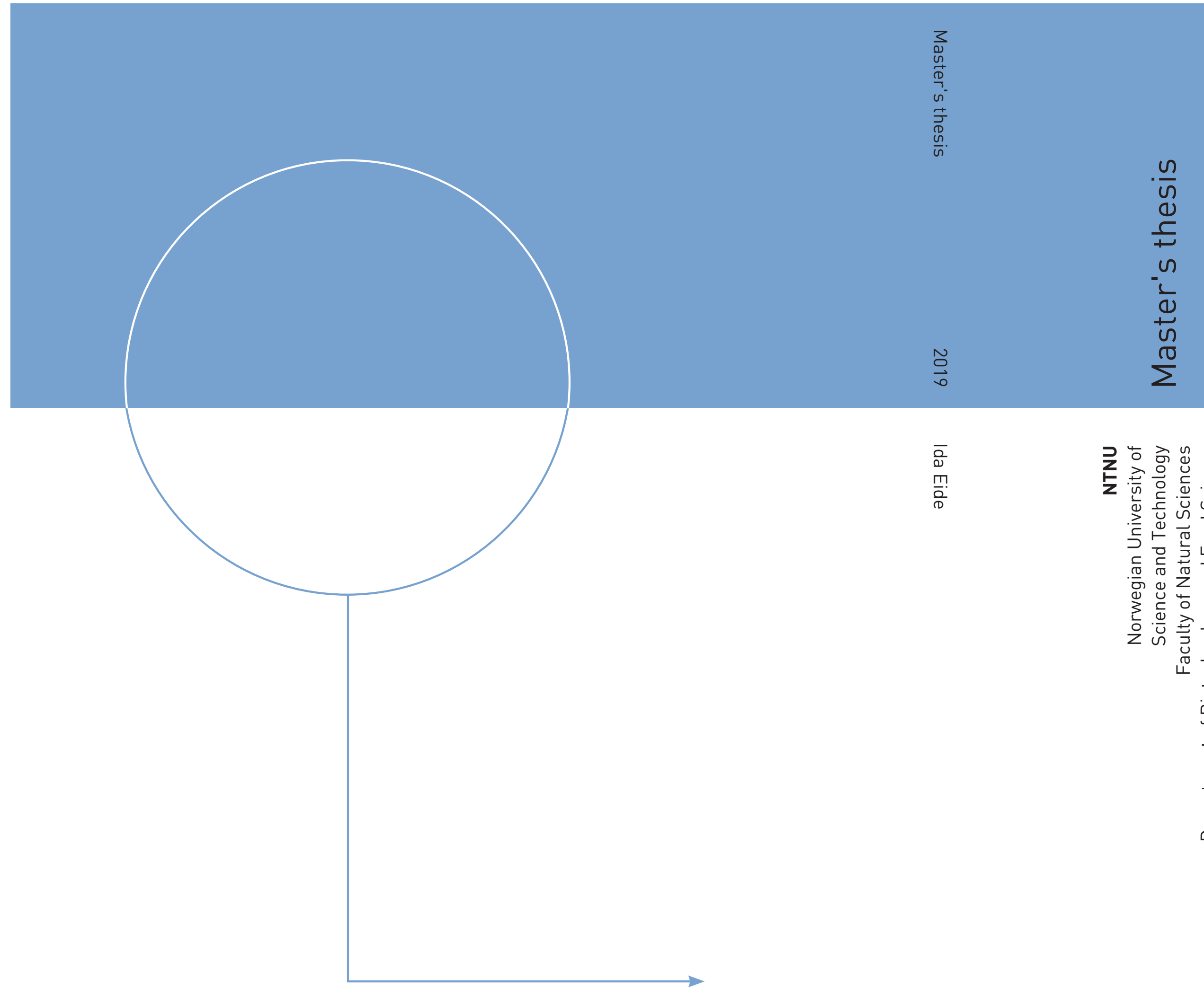

Ida Eide

The effect of pre-mortem crowding on fillet quality of live caught Atlantic mackerel (*Scomber scombrus L.*)

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Abstract

This study examines the impact of pre-mortem crowding stress and its subsequent effect on fillet quality of live caught Atlantic mackerel (*Scomber scombrus L*). The study entailed two different experiments conducted on; 1) 32 mackerel caught from two different land-based tanks (3x3x1m) and 2) 20 mackerel caught from an experimental net pen (5x5x5m inside a 12x12x12m stock pen). In both experiments the first group of mackerel was caught without any treatment and acted as a control, with a second group stressed using a reduction in the netting volume. Blood parameters (lactate and glucose), muscle pH and *rigor mortis* development were measured to state the level of stress induced to each fish. To evaluate fillet quality; drip loss (DL), water holding capacity (WHC), textural properties, gaping, fillet color, degradation of ATP and activity of cathepsin B+L (only in experiment 2) were measured two and/or seven days post mortem. In experiment 1 frozen storage was added as a factor in addition to ice storage. However, in experiment 2 only fresh fillets were evaluated.

In the present study, fillet DL and WHC were found to be unaffected by pre-mortem crowding stress, whereas a significant effect was observed on fillet gaping and texture. The stressed mackerel were found to be softer and had higher gaping score than the controls. Moreover, fillet firmness was found to have a weak correlation to gaping ($r=-0.304$, $p=0.042$). Regarding fillet color, only small effects of stress were observed. The a* value was the only parameter showing differences between groups with a slightly greener muscle (lower negative a*) for non-stressed mackerel in comparison to stressed mackerel. There were no effects of stress regarding degradation of ATP (K- or H-value) or activity of cathepsin B+L. Freeze-thawing was moreover assumed to cover up differences in the effects of stress.

It is concluded that pre-mortem crowding stress did not reduce the WHC or increase the muscle DL in Atlantic mackerel. However, there were effects on fillet texture and gaping, with results indicating stressed Atlantic mackerel as having a higher gaping score and reduced firmness. The stressed mackerel were significantly less green in comparison to the control group, however no effect of stress on ATP degradation was found. Furthermore, it is concluded that the flesh quality of mackerel sampled from the full-scaled experiment in net pen followed the same pattern observed of fish kept in land-based tanks. However, the effect of reducing netting volume was observed less on stress parameters of fish harvested from the net pen in comparison to fish from smaller land-based tanks.

Sammendrag

I denne masteroppgaven ble påvirkningen av pre-mortem trenging og effekten av det medførende stresset har på filetkvaliteten til atlantisk makrell (*Scomber scombrus L.*) undersøkt. To ulike forsøk ble gjennomført; 1) 32 makrell hentet fra landbaserte tanker på 3x3x1 m, og 2) 20 makrell hentet fra en eksperimentell merd (5x5x5m nett innenfor en 12x12x12 m merd) i sjø ble slaktet med et slag i hodet. I begge forsøkene fungerte én gruppe makrell som kontroll, mens de resterende ble stresset ved å redusere volumet på nettet før fangst. Blodparametere (laktat og glukose), samt utvikling av muskel pH og *rigor mortis* ble målt for å bekrefte effekten av stresset som ble påført ved trenging. Til å vurdere filetkvaliteten ble vannbindingskapasitet (WHC), drypp tap (DL), teksturegenskaper, filetspalting, farge på filet, nedbrytning av ATP og aktivitet av katepsin B+L (kun i forsøk 2) analysert to og/eller sju dager etter slakting. I det første forsøket ble fryselagring inkludert som en faktor i tillegg til islagring, mens det i andre forsøk kun ble evaluert ferske, islagrede fileter.

I denne studien ble DL og WHC funnet til å være upåvirket av trenging, mens det ble observert en effekt av stress på tekstur og filetspalting. Atlantisk makrell fra de stressede gruppene ble funnet til å være mindre fast, og hadde i tillegg høyere grad av filetspalting sammenlignet med kontrollene. Filetfasthet var dessuten funnet til å ha en liten, negativ korrelasjon til filetspalting ($r=-0.304$, $p=0.042$). Med tanke på filetfarge, var det kun enkelte effekter av tilført stress i det eksperimentelle designet som ble observert. a*-verdien var eneste fargeparameter som viste forskjell mellom kontroll og stresset fisk (lavere, negativ verdi), noe som betyr en litt grønnere muskel i stresset makrell sammenlignet med kontroll. Det var ingen effekt av stress på verken degradering av ATP (K- og H-verdi) eller i aktivitet av katepsin B+L. Fryse-tining ble tolket som en kamuflerende faktor, da forskjeller i effekt av stress nærmest forsvant ved analysering av filetene etter tining.

Det konkluderes med at påført stress før slakt ved hjelp av trenging ikke påvirker vannbindingskapasiteten (WHC) eller øker drypp tapet (DL) i atlantisk makrell. Det var derimot forskjeller i tekstur og filetspalting, hvor stresset makrell ble mindre fast og hadde høyere grad av filetspalting. Stresset fisk hadde en signifikant mindre grønn filetfarge enn kontrollfisk, men det ble ikke funnet effekt av stress på ATP-nedbrytning. Videre ble det konkludert med at filetkvaliteten til makrellen fra full-skala eksperimentet i merd (forsøk 2) viste samme mønster som makrellen holdt i landbaserte tanker (forsøk 1). Effekten av å redusere nettingvolumet ble derimot funnet til å være lavere på stressparametere i makrellen som ble høstet fra eksperimentell merd sammenlignet med makrellen fra små tanker.

Preface

This thesis marks the completion of a master´s degree in Food and technology (FTMAMAT) at the Department of Biotechnology and Food Science (IBM) at the Norwegian University of Science and Technology (NTNU). It was performed in collaboration with the Institute of Marine Researh (IMR), Nofima and SINTEF Ocean as part of the project *Catch control in purse seine fishing for pelagic species* financed by FHF.

In order to make this thesis happen, I would like to thank my supervisor Jørgen Lerfall, for professional help and guidance throughout the whole process and to external co-supervisor Bjørn Roth for inspiration and advices. I would also like to thank the research group at The Institute of Marine Harvest (IMR), Bergen and Nofima, Stavanger, for all help with my experimental design and analyses. A special thanks to PhD candidate Neil Anders for helping me with laboratory duties, and generally giving me a lot of help and guidance.

Lastly, I want to thank my friends and family for help and moral support. I could not have finished this without you.

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Ida Eide

Abbreviations

ADP: Adenosine-5´-diphosphate

AMP: Adenosine-5´-monophosphate

ATP: Adenosine-5´-triphosphate

DL: Drip loss

HPLC: High-performance liquid chromatography

Hct: Hematocrit

Hx: Hypoxanthine

IMP: Inosine-5´-monophosphate

IMR: The Institute of Marine Research

Ino: Inosine

NEA: North East Atlantic

NTNU: The Norwegian University of Science and Technology

WHC: Water holding capacity

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1. Introduction

Mackerel (*Scomber scombrus L.*) supports a productive and valuable fishery in Norway, with a reported total catch of 280 855 tons in 2018 that represents a sales value of NOK 3,6 billion (Norges Sildesalgslag, 2019). Mackerel is consumed worldwide and is a good source of nutrition that strengthens the population´s health. In fact, mackerel is a brilliant source of omega-3 and is known for lowering blood pressure, strengthen the skeleton, help against depression, promote weight loss and to be favorable for the cardiovascular and cognitive health (Cardoso et al., 2016; Swanson et al., 2012). As well as the nutritional content, the quality of the mackerel is highly significant for the industry/consumer. The majority of the marked require good quality of the fish, referring to fresh color, minimal gaping and firm texture (Irish Sea Fisheries Board, n.d.). The importance of skippers having adequate control over the catching process is therefore evident for the fisheries to avoid catching fish of poor quality that gives a less profitable production and weaker reputation of the fish.

During purse seining, it is important to control the entire catching process from fishing exploration until the catch is delivered. The main reason for this is to get the best possible financial benefit from the quotas that have been allocated. At the same time, it is important to take care of the initial quality of the raw material, the safety on board and to comply with the fisheries regulations. During capture and slaughter fish are exposed to different stress factors, such as crowding, hypoxia and quick changes in temperature (Sone et al., 2019). Understanding how mackerel responds to stress would make it more desirable to promote welfare friendly fishing practices that maximize survival potential if the catch is to be slipped and meat quality if the catch is to be retained. This would potentially contribute towards a more sustainable and profitable Norwegian fishing industry.

The present MSc thesis is part of a bigger project named *Catch control in purse seine fishing for pelagic species* financed by Fiskeri- og havbruksnæringens forskningsfond (FHF). The main goal of the project was to improve catch control of Atlantic mackerel by developing instruments and analytical methods that provide a better basis for making decisions during the catching process. (FHF, n.d.). Over time, this may give the fleet better control over gear and catch, and potentially lead to increased catching values and higher profitability.

Several studies have shown that pre-slaughter stress accelerates flesh softening, increases gaping, affects flesh color and induce increased drip loss (DL) of fish fillets (Erikson and Misimi, 2008; Kiessling et al., 2004; Lerfall et al., 2015; Robb et al., 2000). A sub-goal in this project, and the aim of this thesis, was therefore to investigate the influence of pre-mortem crowding stress and how it affects the fillet quality of ice stored fresh Atlantic mackerel. In order to investigate this, the following research questions were formed:

- Does pre-mortem crowding stress induce increased drip loss, reduced water holding capacity and flesh softening in Atlantic mackerel?
- Does pre-mortem crowding stress affect flesh color of Atlantic mackerel?
- Does pre-mortem crowding stress affect the degradation of ATP in Atlantic mackerel?

2. Theoretical background

2.1 Atlantic mackerel *(Scomber scombrus L.)*

As one of the most extensive and distributed migratory fish species in the North Atlantic (Jansen and Gislason, 2013), mackerel is a fast-swimming shoal fish that can migrate near the surface over long distances. It forms large schools and migrate to southern deep waters in winter for higher temperatures but move closer to the continental shelf edge during the spring season. They get fed by several species of zooplankton (Langøy et al., 2006) and pelagic larval, and their lower boundary of preferred sea temperature is 8 °C (Sette, 1952). After summer the mackerel starts eating less and lives near the seabed off the southwest coast of Norway (Jansen, T. et al., 2012). In Norwegian waters it is found three different populations of mackerel. One stock spawns centrally in the North Sea and Skagerrak from May to July, a western stock spawns west of Ireland and the British Isles from March to July, and a southern stock spawns outside Spain and Portugal from February to May (ICES, 1999; Sylvia Frantzen et al., 2010). The western and southern stocks migrate to the Norwegian sea and the North Sea after spawning, and blends with the North Sea component (Havforskningsinstituttet, 2018; Uriarte, A. et al., 2001). It is impossible to separate the catches from the different spawning stocks, thus the mackerel is managed as one stock; North East Atlantic (NEA) mackerel (Havforskningsinstituttet, 2018).

With its blue green iridescent back and silvery white belly, the mackerel has a distinctive appearance. On the dorsal part of the fish there is 20 to 30 black curved lines across a narrow black line that runs below on both sides. With these characteristics of appearance, the school of mackerel communicates with other schools of fish about their movement (Denton and Rowe, 1998). The average length of an adult mackerel is about 30 cm, but they can be up to 60 cm and 3.4 kg (Luna, Susan M., n.d.). Mackerel is a brilliant source of proteins, selenium, vitamins A, D and B12, and together with the high content of EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) fatty acids (Moujahed, N. and Guizani, SEO., 2015), it is favorable for human health. Whereas the protein content remains relatively constant around 18-20%, the total fat content can vary a lot throughout the seasons. The amount of omega-3 fatty acids will also vary as the relationship between total lipid content and the composition of fatty acids is not static (Soriguer et al., 1997). As a consequence, the water content also varies, as fat and water have an inverse relationship (Hardy and Keay, 2007; Rehbein and Oehlenschläger, 2009). The mackerel is observed on its leanest in February and richest on fat in September and October, which corresponds to when most purse sein fishing in Norway takes place. The total fat percentage varies from approximately 18-31% (Pelagia AS, n.d.) and the premium catch period is between September and November, when it's most nutritious. On the other hand, the high fat content makes the fish easily perishable and mackerel is considered a very delicate species.

2.2 Common catching methods

The methods used for catching and slaughtering of fish can affect the skin and scales, and also have an impact on the fillet quality. In fact, most methods of catching provides some sort of physiological or physical stress on the fish. The methods used for catching Atlantic mackerel is usually trawls or purse seines, where Norwegian fisheries primarily uses purse seines (Havforskningsinstituttet, 2018).

In mid-water trawling a large bag-shaped fishing net (figure 1) is pulled along the sea by one or two boats to catch schools of fish (Gregory and Grandin, 1998). The net has a wide opening in front, which then narrows to a closed end where the fish are trapped. Wings extending from the opening increases the area swept by the net and allows the fish to enter the net. Thereafter, otter boards keep the wings outwards, as well as opposing floats and weights to keep the front open. Even though a mid-water trawl does not pull the netting gear along the bottom, it can sometimes cause damage to sensitive seabed habitats and organisms if the water is not to deep. (NOAA Fisheries, 2018).

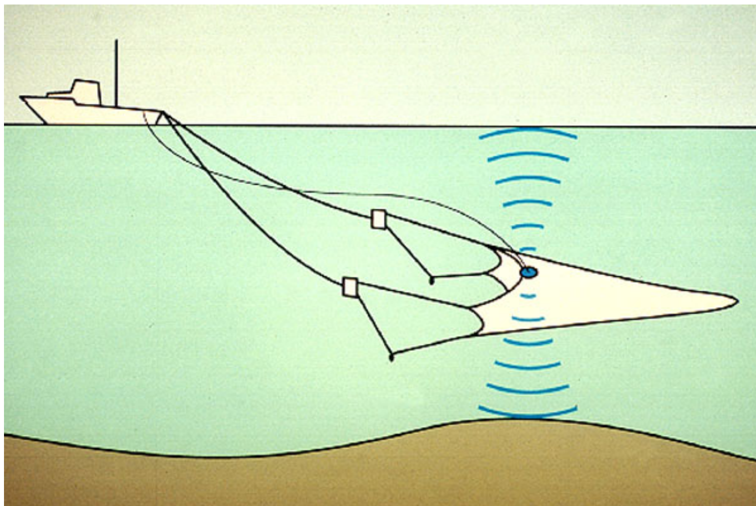

Figure 1. Illustration of how trawling works (FAO Fisheries and Aquaculture Department, 2018).

Purse seining is a common method in NEA fisheries. It is performed to surround the pelagic schools near the surface by shooting a deep net, with bunt first, that floats at the surface (=float line) (figure 2) (Breen et al., 2012; NOAA Fisheries, 2019). The vessel then follows a certain direction to encircle the school (Breen et al., 2012). On the bottom of the net there is weights to ensure that the purse line sinks fast while a wire gets the net closed under the shoal. The purse net is thereafter hauled aboard which leads to a rapidly reduced total volume enclosed by the net. As the reduced volume reaches a particular point, it will prevent the mackerel´s movement, and the fish will be acting as individuals rather than a shoal (Lockwood et al., 1983). Finally, the fish is pumped from the net to the vessel (Seafood Scotland, 2019). In most cases the purse nets do not affect the seabed and its habitats, but the small amount of bycatch can have a negative impact on the environment due to purse seining.

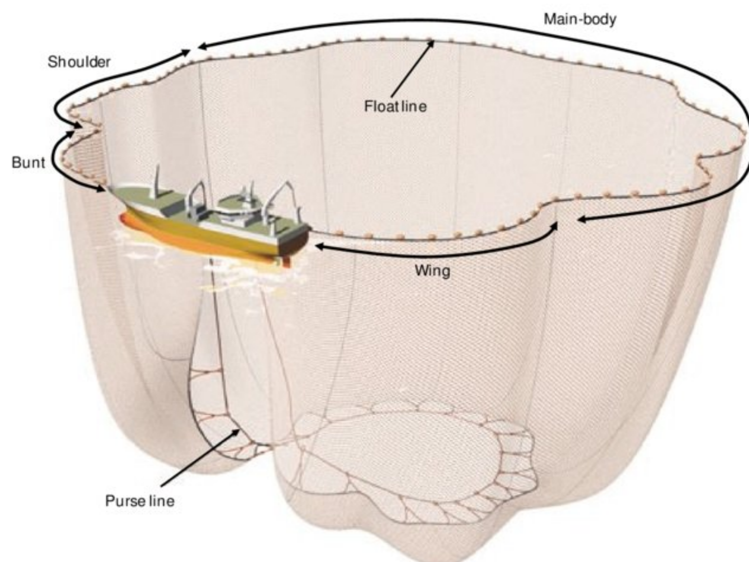

Figure 2. Illustration of a purse seine with accompanying explanations (Breen et al., 2012)

Both of these catching methods includes factors that may induce stress to the mackerel and hence decrease the fillet quality post mortem. In the present experiments, the crowding stress were simulated in purse seines.

2.3 Stress and stress responses

Stress can be defined and interpreted in many different ways. For instance, it can be defined as a physical or chemical factor that cause bodily reactions contributing to disease and death (Rottmann et al., 1992), or as the response of the cell or organism to any demand placed on it such that it causes an extension of a physiological state beyond the normal resting state (Iwama, 1998). The response to stress is considered an adaptive mechanism that makes the fish able to deal with stressors in order to maintain its homeostatic state (Barton, 2002). Damaging stress occurs when the organism´s homeostasis is disturbed by an overly severe stressor or the actions of a single or several, long-lasting stressors or stimuli (Gutzeit, 2001). Furthermore, the stress responses may involve physiological responses (Rottmann et al., 1992) which may have implications for fillet quality.

Previous studies have shown that small pelagic schooling fish, such as mackerel and sardines, are highly vulnerable to gear-inflicted injuries (Marçalo et al., 2006; Misund and Beltestad, 2000). Compared to cod and plaice, mackerel are a lot more sensitive to physical stress (Pawson and Lockwood, 1980), and even moderate handling can lead to high mortality. Catching of fish by for instance purse nets includes several activities that causes stress, such as crowding and pumping. All of these activities could cause physiological reactions in the fish and change the levels of biochemical substances (Huse and Vold, 2010).

Crowding stress was simulated in order to determine likely physiological stress response to crowding stress in mackerel purse seine fishing and formed the basis for the assessment in the present MSc thesis. These stress responses are grouped in primary, secondary and tertiary stress responses (Barton, 2002). Primary responses follows the activation of brain centers which leads to the release of catecholamines and

corticosteroids (Wendelaar Bonga, 1997), while the secondary responses are effects of these and refer to metabolic and cellular changes as well as changes in immune function and hematological features. Finally, the tertiary responses is alteration in physical performance and behavior (Barton, 2002).

One of the primary stress responses is the release of the hormones cortisol and adrenalin (Erikson et al., 1997). Acute primary responses to stress are an immediate massive release of adrenaline and noradrenaline into the blood followed by a gradual adrenocorticotropic hormone (ACTH) release. ACTH triggers release of cortisol from the renal tissue into the blood. (Poli et al., 2005) With a lack of data on mackerel, it was found that levels of plasma cortisol in unstressed salmonid fish are less than 30-40 ng/mL, but should ideally be <5 ng/mL (Pickering and Pottinger, 1989). On the other hand, variations of cortisol in resting fish may be due to other factors than stress. For example, cortisol levels can increase ten times during smoltification (Barton et al., 1985).

Pre-mortem stress accelerate the degradation of adenosine-5´-triphosphate (ATP), and results in an earlier, faster and stronger rigor contraction (Sigholt et al., 1997). ATP is a nucleoside triphosphate in muscle tissue of vertebrates, which transports chemical energy within cells to different intracellular spaces and to enzymes so that they can carry out their metabolic processes. Most of this energy is allocated for muscle cells to do mechanical work and to synthesize metabolic intermediates (Hong et al., 2015). Therefore, living organisms try to maintain the amount of ATP within each cell. ATP degrades in a series of biochemical reactions during post mortem storage, and as the muscle ATP decreases, no energy is there to separate the linkages of actin and myosin, thus the state of *rigor mortis* is a fact (Hong et al., 2015; Pate and Brokaw, 1980). The degree of onset and resolution of rigor varies between species and is affected by temperature, handling, size and physical condition of the fish (Huss, 1995). Furthermore, a fast and strong *rigor mortis* can lead to a decrease in the fillet quality of the fish, resulting in gaping, soft muscle texture, color changes, larger drip loss (DL) and decreased shelf life (Lowe et al., 1993; Sigholt et al., 1997; Stroud, 2001)

The ATP is degraded (figure 3) by endogenous enzymes causing the formation of adenosine-5′-diphosphate (ADP), adenosine-5′-monophosphate (AMP), inosine-5′-monophosphate (IMP), inosine (Ino) and hypoxanthine (Hx), which further degrades to xanthine (X) and uric acid (UA) by the developing spoilage microflora (Huss, 1995; Rehbein and Oehlenschläger, 2009). The value X and UA is not included in the present project.

Figure 3. Illustration of post mortem degradation of ATP to Hx, and further to UA, in fish muscle.

The breakdown of ATP leads to an increase in ADP, which is the substrate for the adenylate kinase reaction that produces 1 mol of ATP and 1 mol of AMP from 2 moles of ADP. The level of AMP increases therefore consequently and deaminates to IMP, which

further accumulates in the fish muscle post mortem (Massa et al., 2005). The degradation steps from ATP to IMP goes very fast, whereas the following degradation of IMP is slow (Rehbein and Oehlenschläger, 2009). IMP is therefore the main nucleotide present and contributes to the pleasant fresh flavor of the fish muscle, and is additionally associated with the umami taste (Hong et al., 2015). The accumulation of Hx in fish is apparently responsible for the loss of good flavor and developing a bitter taste. Hx forms either by the autolytic breakdown of nucleotides or by bacteria, such as *Pseudomonas spp*., *S. putrefaciens*, and *P. phosphoreum* (Huss, 1995). However, all this considered, the degradation patterns of ATP catabolites are species-dependent. The compounds in degradation of ATP increases as the temperature rises above the freezing point of fish muscle from temperate waters (Hong et al., 2015). Nevertheless, between −0.8°C and −5°C, the degradation of ATP continues at a higher speed than in room temperature because the intracellular water is only partially frozen which again concentrates all the soluble components (e.g. enzymes) in the water phase (Van den Thillart et al., 1990). On that account, rapid freezing is necessary to pass through this range of temperatures as quickly as possible to optimize the quality of the frozen fish muscle.

Glucose is an important substance giving energy to all the cells. In fact, some fish tissues are dependent on glucose for energy metabolism. As a result of stress and a decrease in ATP, the increase of cortisol stimulate the glycogen stores and thereby increases the glucose levels in the circulating blood (Schreck et al., 2016). The amount of stored glycogen in the living tissue depends on the nutritional status of the fish and the amount of stress applied (Huss, 1995). Glycogen breaks down to pyruvate during anaerobic glycolysis and the pyruvate is further reduced to lactic acid when oxygen is limited (Hong et al., 2015).The fish starts using anaerobic energy when there is not sufficient with oxygen (Erikson et al., 1997). In parallel with the acidification caused by the increase in lactic acid, rigor in fish muscle develops. As the heart beat increases during stress, and the fish needs a higher oxygen uptake, an increase in the number of moving erythrocytes happens and thus a higher hematocrit (Hct) value (Poli et al., 2005).

As the lactic acid increases in the fish muscle, the post mortem pH decreases which has an effect on the physical properties of the muscle. As the pH drops, the net surface charge of the muscle proteins is reduced, causing them to partially denature and lose some of their water holding capacity (WHC). Muscle pH is normally 6.5 to 7.3 immediately post mortem, but the ultimate pH can go as low as between 6.0 and 6.5 (Sen, 2005; Sone et al., 2019). When the resolution of rigor starts the pH will increase gradually due to protein degradation (Sayas-Barberá et al., 2010; Sen, 2005). Loss of water has a detrimental effect on the texture of fish muscle, and it has been shown by Love (1975) that there is an inverse relationship between muscle toughness and pH, unacceptable levels of toughness occurring at lower pH levels. Intrinsic pH between species is very variable, and together with the variability in effects of biological conditions and harvesting procedures, and between fish within a batch, it prevents the pH of being a successful measure of spoilage (Rehbein and Oehlenschläger, 2009).

2.4 Quality parameters

In fatty fish like mackerel and herring, most of the lipid reserves are found in the fish muscle (Hall, 2012). The high level of polyunsaturated fatty acids is known to make the mackerel fillet more susceptible to oxidation, resulting in off flavors, color and texture changes and a loss of nutrients (Standal et al., 2018). However, this depends on the season and the composition of fat and water. Worth mentioning is that stress is known to affect the susceptibility to infectious diseases (Snieszko, 1974). A consequence of this is shorter shelf life and a more rapid development of unwanted sensory properties (Barton & Iwama, 1991).

The phenomenon of gaping happens when the connective tissue fails to hold the muscle fragments together, making the fillet non-attractive and difficult to process (Hagen and Johnsen, 2016; Robb et al., 2000). Several factors cause gaping, such as change in muscle pH (as a result of pre-mortem handling stress), slow freezing, seasonal variations and mechanical damage (Jacobsen et al., 2017). Also increased time in frozen storage has been shown to result in slightly more gaping (Fletcher et al., 1997). Furthermore, gaping is often accompanied by tissue softening, and may be due to weaker tissue strength and change in structure. However, the mechanisms behind gaping is far from fully understood (Ofstad et al., 2006).

Several studies have reported that pre-mortem stress has a negative impact on fillet color and texture (Robb et al., 2000; Roth et al., 2006; Sigholt et al., 1997). The most influencing factor on texture is the muscle pH (Hall, 2012). As a consequence, softening of the fish fillet due to slaughter stress have been observed in different species, such as salmon and chub mackerel (Roth et al., 2002; Sato et al., 2002). Intense muscle activity before slaughter may also affect the fillet color (Robb et al., 2000).

Water is the major component in muscle foods, and the interactions between water and macromolecules defines the water holding capacity (WHC) (Offer & Knight, 1988). The WHC is considered a very important quality attribute in muscle food, and the distribution of water is believed to be affected by the physical and biochemical changes in the muscle, which occur during for example *rigor mortis* (Aursand et al., 2010). Short-term stress pre-mortem can accelerate the post mortem metabolism in the muscle, associated with anaerobic glycolysis, followed by a rapid decrease in pH (Warner, 2017). A drop in pH initiates protein denaturation, and DLs can be greater than in non-stressed muscle that has a slower rate of pH decline. DL is inversely related to WHC, and occurs when the WHC decreases, which results in loss of protein-containing fluids (Kristoffersen et al., 2007). It is also known that there is a connection between the pre slaughter energy status of salmon and cod and the DL (Kristoffersen et al., 2006; Lerfall et al., 2015).

Cathepsins are proteases usually found in intracellular lysosomes, which is believed to be responsible for protein breakdown at places with injury. Therefore, cathepsins are mostly inactive in living tissue but become released into the extracellular fluids upon physical damage or freezing and thawing of post mortem muscle (Huss, 1995). Previous studies consider that release of cathepsins D and L are one of the main reasons of autolytic degradation and post mortem tissue softening (Aoki and Ueno, 1997; Huss, 1995).

K-value is a freshness indicator defined as the percentage of the amount of Ino and Hx to the total amount of ATP-related compounds (Saito et al., 1959).

$$K = \frac{Ino + Hx}{ATP + ADP + AMP + IMP + Ino + Hx} \times 100$$

The acceptable K-values differ between species, but a general limit for consumption is about 80% for example white fish and salmon (Erikson et al., 1997; Hattula et al., 1993). Furthermore, a more rapid increase in K-value has been reported in stressed fish compared to non-stressed (Lowe et al., 1993)

On the other side, K is not considered reliable as a freshness index for all marine finfish. As ATP breaks down to IMP rapidly after death and remains very low for some species, other formulas that do not require measurements of ATP, ADP and AMP, have been formed to evaluate fish freshness. Hong et al. (2015) reviewed the importance of ATP-related compounds for the freshness for several fish species, and for mackerel it is proposed to maybe use other freshness indicators (Hong et al., 2015), for example, the H-value (Luong and Male, 1992):

$$H = \frac{Hx}{IMP + Ino + Hx} \times 100\%$$

There is increasing interest in the combined technology of freezing and chilling (O'Leary et al., 2000). Freeze-thawing is an important method for preservation and give logistics benefits, and for pre-packed fish fillets it is replacing the traditional ice counter in supermarkets more and more. The term freeze-thawing means freezing and then frozen storage, followed by thawing and storage in chilled temperatures (Fagan et al., 2003). It is, however, reported that this process of freezing and thawing accelerates quality loss of the fish muscle, and also hydrolysis and oxidation of lipids (Fagan et al., 2003). In the present study freeze-thawing was not analyzed as a main factor, however it was considered if freeze-thawing emphasized the effect of pre-mortem crowding stress or not.

3. Materials and Methods

Regarding this MSc thesis, two experiments were carried out at Institute of Marine Research (IMR), Austevoll, Norway in the period of January-February 2019. The purpose of the first experiment was to see how catch-related stressors affect chemo-physical quality of live stored mackerel in land-based tanks, and thereafter look at the quality of fresh and frozen mackerel during ice storage. The second experiment was carried out by using experimental fish live stored in net pen, as it was feared that the pelagic mackerel would not adapt well to the tanks and therefore their stress response may have been very different for wild fish. Experiment 2 was also carried out to further increase the sample size.

3.1 Raw material and experimental design

The raw material used in these experiments was Atlantic mackerel. The mackerel used in experiment one and two respectively was slaughtered on 16th of January and 28th of February at IMR, Austevoll.

3.1.1 Experiment 1

In mid-January a total of 32 fish from two different tanks (3x3x1 meter) were collected, where approximate half of the school in each tank were caught with a dip net as gently as possible to serve as a control. The remaining mackerel were trapped and crowded by reducing the volume in the netting for approximate 15 min, and further slaughtered immediately post stressor (figure 4 and 5). All fishes were tagged with an individual ID and killed manually by a blow in the head.

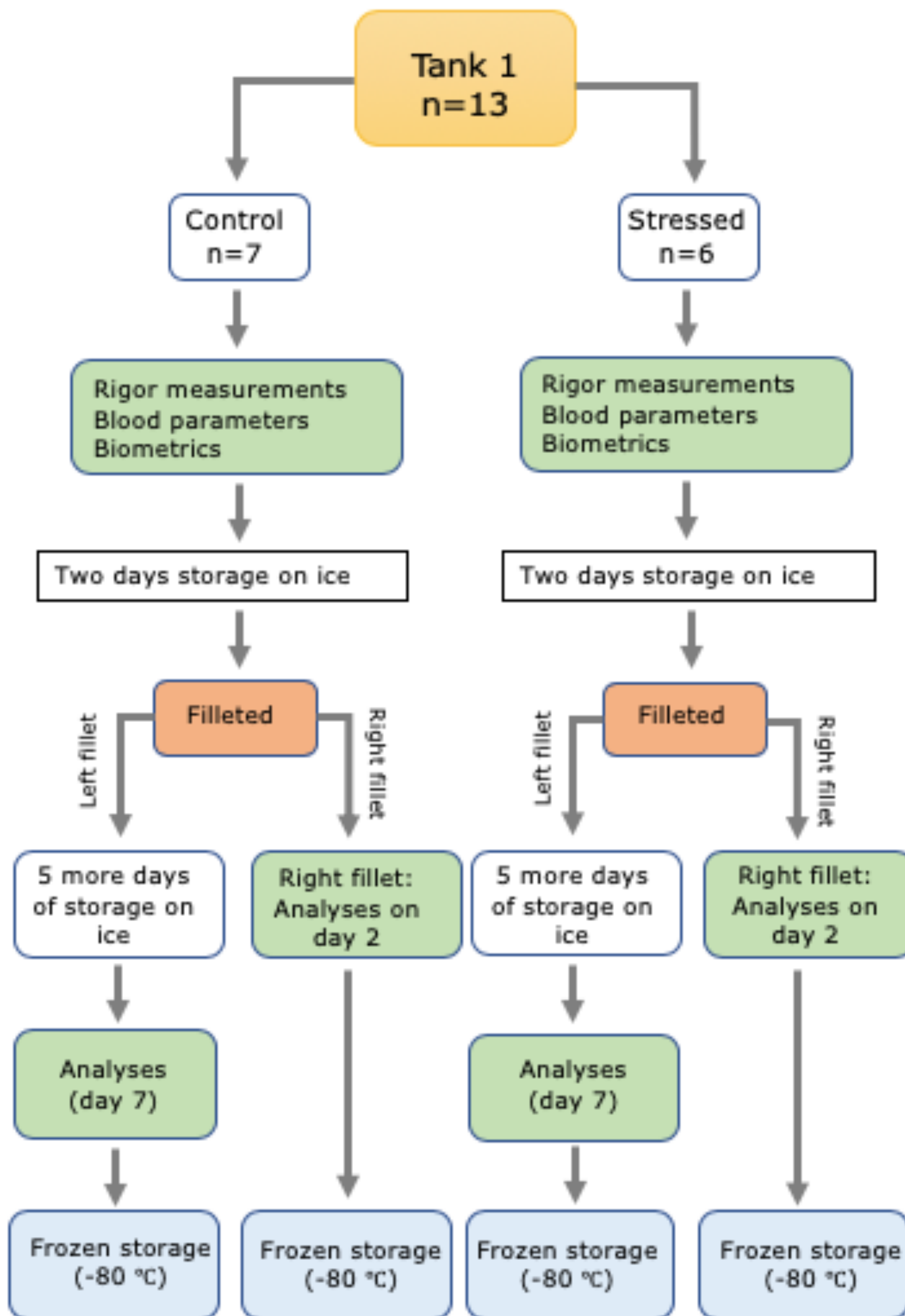

Figure 4. Distribution of mackerel collected from tank 1 (experiment 1), including the following measurements and storage conditions for control and stressed group.

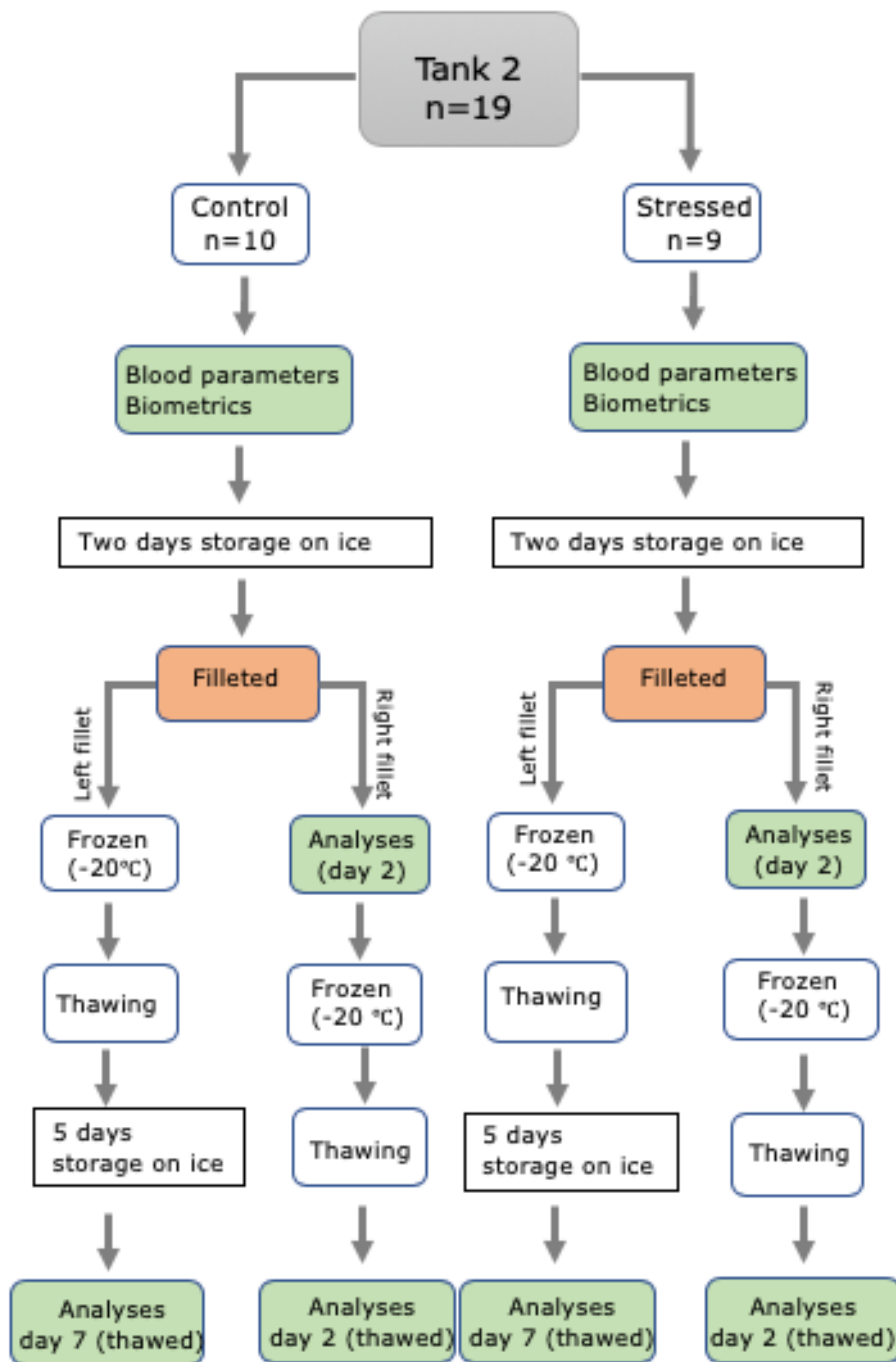

Figure 5. Distribution of mackerel collected from tank 2 (experiment 1), including the following measurements and storage conditions for control and stressed group. After all analyses were completed, the fillets got frozen in – 80℃.

Right after slaughter blood and tissue samples were collected from each fish. The blood was drawn from the caudal vein and measured immediately for whole blood glucose and lactate. Afterwards, the whole blood was stored chilled and centrifuged later on for analyzing hematocrit values. In addition, plasma was sent to an extern laboratory (IMR, Matre Research station) for analyzing several blood plasma parameters, such as cortisol, ALAT, ASAT, phosphate and triglycerides. Although, these parameters did not form part of the present MSc thesis. Weight and fork length were also measured subsequently with muscle pH and rigor index measurements. The development of *rigor mortis* and muscle pH were only measured of fish from tank 1 (experiment 1) 3, 6, 12, 18, 24 and 43 hours post mortem. Muscle samples were taken from the anterior/central dorsal area (figure 6) and directly frozen on liquid nitrogen and transported on ice to NTNU – The Norwegian University of Science and Technology, Trondheim.

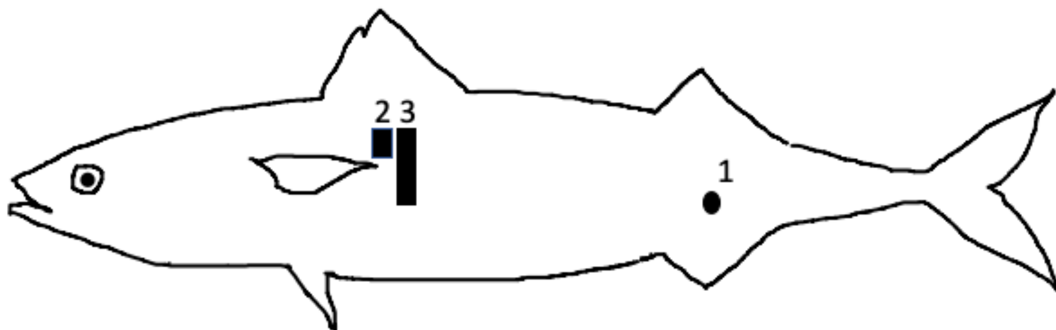

Figure 6. Illustration of where blood was drawn (1) and muscle tissue for ATP analysis (2 and 3) cut out.

Two days post slaughtering, mackerel from experiment 1 was filleted and the viscera examined. The liver and gonads were moreover weighed, and the sex distribution was evaluated. Gaping and distinct injuries were thereafter noted before color measurements were performed by imaging with a DigiEye full system (VeriVide Ltd., Leicester, UK). The left fillets from the experimental fish kept in tank 1 was stored fresh on ice in 4 ℃ for further measurement on day seven, while the right fillets were used to measure texture before the rest of the muscle was frozen at -80℃ for further analysis. Both fillets of the fish from tank 2 were frozen in -20 ℃ after gutting and thawed later on before any further measurements to compare fresh and frozen mackerel fillets.

3.1.2 Experiment 2

Late February, the last experiment of 20 fish living in a net pen were completed. Half of the school was collected as gently as possible by hooking and killed manually by a blow to the head. The other half was crowded by decreasing the purse net volume significantly for approximate 15 mins. The mackerel was further trapped with a dip net, and then slaughtered by a blow in the head. Blood and muscle samples were taken in same procedure as in experiment one. All fish from this experiment were gutted in Austevoll and furthermore stored fresh on ice and transported to NTNU, Trondheim.

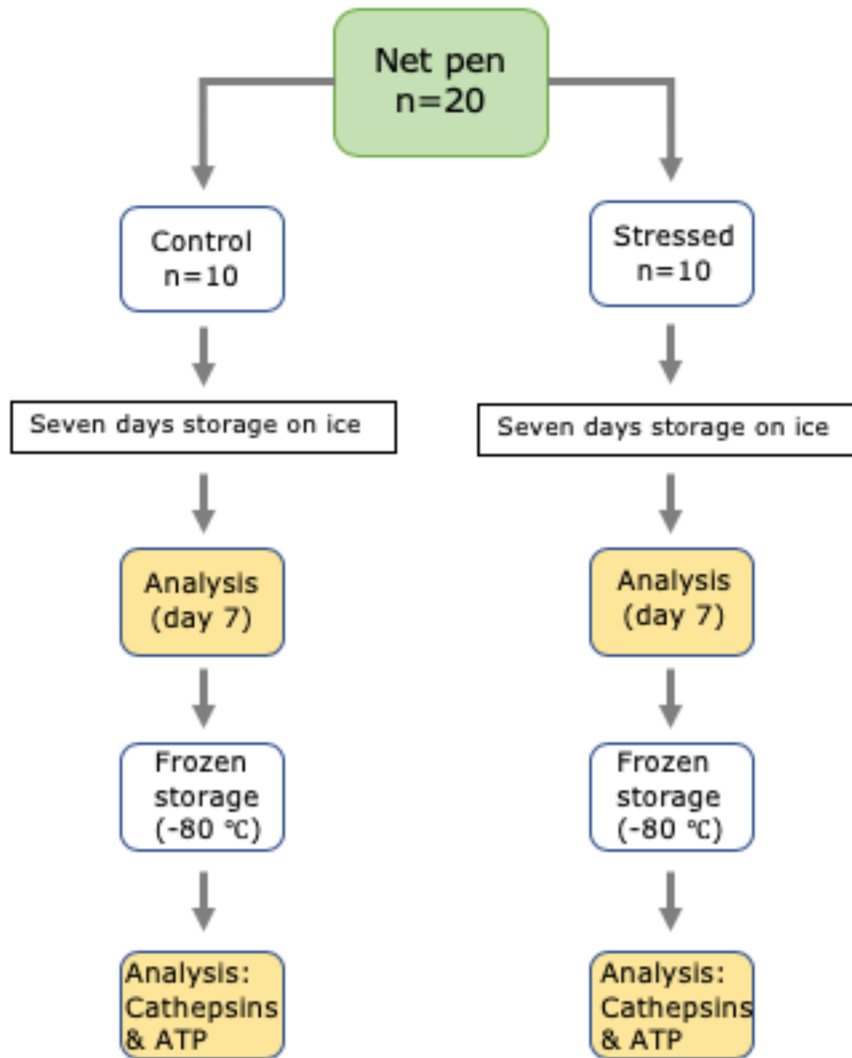

Figure 7. Design of experiment two with mackerel collected from net pen.

The fish from experiment 2 (figure 7) were caught from a net pen to serve like a full-scaled experiment, and to increase the sample size. The same parameters as in experiment 1 were analyzed (table 1), but also the activity level of cathepsin B+L. However, development of rigor and muscle pH was not included in experiment 2.

Table 1. Overview of the parameters, and when they got analyzed, in experiment 1 and 2.

Experiment 1	**Day**	**Experiment 2**	**Day**
Glucose, mmol/l	0	Glucose, mmol/l	0
Lactate, mmol/l	0	Lactate, mmol/l	0
Hematocrit, %	0	Hematocrit, %	0
Condition factor (K)	0	Condition factor (K)	0
Rigor development[2,]	0	Muscle pH	0
Muscle pH[3]	0	DL, %	7
WHC, %	2, 7	Texture	7
DL, %[4]	2, 7	Gaping	7
Texture	2, 7	Color	7
Gaping	2, 7	ATP metabolites	0, 7
Color	2, 7	Cathepsins, μU/g	7
ATP metabolites	2, 7		

[1]Day = x day(s) post mortem
[2]Rigor development was only measured from fish in tank 1.
[3]including development of pH measured over several hours (up to 43 h).
[4]Measured both fresh and frozen on day 2 post mortem.

3.2 Stress parameters and quality measurements

3.2.1 Blood analysis

Blood samples were taken from the caudal vein (figure 6) using EDTA (Ethylenediaminetetraacetic acid) as an anticoagulant immediately after death. The lactate level was measured by a Lactate Pro 2 analyzer (Arkray Factory Inc., Japan) and the glucose level by a glucose test meter (Contour Next ONE, Ascensia Diabetes Care Holdings AG, Switzerland). Hematocrit (Hct) values was further measured by filling capillary tubes and centrifuge them using a micro hematocrit centrifuge (DSC-100MH-3, Digisystem Laboratory Instruments INC., Taiwan) in room temperature for 5 minutes.

3.2.2 Condition factor

Fulton´s condition factor (K) is a formula used to calculate the relationship between the weight and length of an individual fish, with the intention of describing the physical condition of it (Nash et al., 2006). The formula is:

$$K = \frac{W}{L^3} \times 100\%$$

where W= wet weight of whole body in grams and L= fork length in centimeters.

3.2.3 Rigor index and muscle pH

Rigor index (I_r) was measured at 0, 3, 6, 12, 24 and 43 hours post mortem using Cutting's tail drop method (Bito et al., 1983). The rigor index measurements were calculated by the formula:

$$I_r = \frac{L_0 - L_t}{L_0} \times 100\%$$

where L_0 indicates half of the fork length, and L_t equals the value at selected time intervals after death. A completely relaxed fish with a hanging tale has rigor index 0,

however, if it is totally rigid lying in a horizontal line with the table, the rigor index is at its maximum.

Muscle pH was measured in white muscle immediately post mortem and after every rigor measurement, spesifically in the loin fillet immediately posterior to the pectorial fin. The instrument used was a Mettler Toledo SevenGo pro™ pH-meter (Mettler Toledo Inc., USA).

3.2.4 Drip loss (DL) and water holding capacity (WHC)

WHC of the white fish muscle was measured after seven days of fresh storage, with an exception of the freeze-thawed fillets that were measured right after thawing (day 2) as well. Cylindrical muscle samples were cut in duplicates from the upper back loin(diameter 28mm, approximately 5 g), and was weighed and placed into metal carriers (Part No. 4750, Hettich Lab Technology, Germany) and centrifuged (Rotina 420 R, Hettich Lab Technology, Germany) for 15 min at 4 °C, using a free swing rotor at 530 xg. WHC was calculated from the following formula (Skipnes et al., 2007):

$$WHC = \frac{W_0 - \Delta W}{W_0} \times 100\%$$

Where,

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

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

V_0 = the water content of the muscle.

D_0 = dry matter of the muscle.

ΔV_0 = the weight of the liquid separated from the sample during centrifugation.

The dry mass (D_0), and hence the water content (V_0), was determined by drying the samples in duplicates for 20-22 hours at 105 ℃ (ISO, 1999) and calculated using the formula:

$$D_0 = \frac{x1}{x2} \times 100\%$$

Drip loss (DL) was calculated as the difference between the fillet weights at the selected sampling days using the following formula mentioned by (Bjørnevik and Solbakken, 2010):

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

where m_0 indicates the fillet weight post mortem, and m_x the weight at the selected sampling day. In this present experiment, m_0 equals measurements on day two, because that was the day the mackerel got filleted. In both the WHC and the dry mass the averages of duplicates was evaluated for further data analysis.

3.2.5 Texture

Texture analyzes were performed using a Texture analyzer TA-XT2 (SMS Ltc., Surrey, England) with a flat-ended cylinder probe (20 mm diameter, type P/1SP), equipped with a 25 kg load cell. The force–time graph was recorded by a computer equipped with the Texture Exponent light software for windows (version 4.13, SMS). The max force (N) and the total force (Ns) detected by force at 80% compression of the height was reported. The measurement was taking place in the middle of the fillet, across the spinal cord, and the average of two replicates beside each other was evaluated during data analysis.

3.2.6 Gaping

The degree of muscle gaping (table 2) was visually assessed in both fresh and freeze-thawed fillets on a scale from 0 to 5 based on the number and length of the slits in the fillet surface (Andersen et al., 1994).

Table 2. Scale of gaping score.

0	No gaping
1	<5 small slits
2	<10 small slits or <2 large slits
3	>10 slits or a few large slits
4	Many large slits
5	Extreme gaping

3.2.7 Color

Color measurements of the fillet surface were performed by digital photo imaging (DigiEye full system, VeriVide Ltd., Leicester, UK) and analyzed with DigiPix software (VeriVide Ltd., Leicester, UK). It was taken one image of the mid back loin and one with the spine included at day two and seven. The fillets were photographed in a standardized light-box with daylight (6400 K) using a calibrated digital camera (Nikon D80, 35 mm lens, Nikon Corp., Japan). Afterwards, the color was quantified, where L* describes the lightness of the product from 0 to 100 (100 = white, 0 = black) , a* says something about the intensity of color on the red–green axis (a* > 0 = red, a* < 0 = green) and b* the intensity of color on the yellow–blue axis (b* > 0 = yellow, b* < 0 = blue). Furthermore, chroma (C*) represents the color saturation and hue (H*) the color angle between a* and b*, where H* = 0° for reddish hue and H* = 90° for yellowish hue.

3.2.8 ATP metabolites

Tissue samples was cut out immediately post mortem and immediately immersed in liquid nitrogen to avoid further degradation of ATP metabolites prior to extraction. The muscle samples needed to be extracted before analyzing, and duplicates of approximately 0.6 g frozen muscle sample kept on ice was homogenized with 5.0 ml perchloric acid ($HClO_4$, 0.42 M) for 2 min by a disperser (Ultra Turrax T25, IKA®-Werke GmbH & Co. KG, Germany, 13 000 rpm). Thereafter, 1 ml of potassium hydroxide (KOH, 1 M) was added, and finally the samples were centrifuged (Rotina 420 R, Hettich Lab Technology, Germany) in 4°C for 10 minutes using an angle rotor at 4800rpm. Afterwards, the supernatant was transferred to a 10 ml tube and stored in the freezer (-80 °C) until further analyzes.

Before analyzing, the samples were aspirated into syringes (1 ml) and pressed through filters (Chromacol 30 mm, nylon, 0,45 µm) and further placed in 1,5 ml tubes fitted for the High-performance liquid chromatography (HPLC). Analysis of the degradation products of ATP is based on the HPLC methodology of Sellevold et al. (1986). The system for the HPLC analysis was an Agilent 1290 connected to an infinity Diode Array Detector (Agilent Technologies) with a Poroshell 120 column (EC-C18 3,0 x 100 mm, pore size 2,7 µm with a Poroshell 120 pre-column, 3,0 x 5 mm, Agilent Technologies). The mobile phase that was used was made out of 1:1 dihydrogen potassiumphosphate (KH_2PO_4, 0,215M) and tetrabutylammonium hydrogensulfate buffer (0,023 M) in 3,5 % acetonitrile (C_2H_3N) dissolved in water.

Standard solutions used was ATP (adenosine-5′-triphosphate disodium salt trihydrate, Roche diagnostics, cas: 51963-61-2), ADP (adenosine-5′-diphosphate sodium salt, Sigma-Aldrich, cas: 20398-34-9), AMP (adenosine-5′-monophosphate sodium salt, Sigma- Aldrich, cas: 149022-20-8), IMP (inosine-5′- monophosphate disodium salt hydrate, Sigma-Aldrich, cas: 352195-40-5), In (inosine, Sigma-Aldrich cas: 58-63-9), Hx (hypoxathin, Sigma-Aldrich, cas: 68-94-0). The metabolites were detected at 210 nm (ATP and ADP) and 260 nm (AMP, IMP, In and Hx).

3.2.9 Cathepsin B + L activities

Fish samples from experiment 2 stored fresh for seven days were used to analyze the level of cathepsins. A pre-trial was performed for an optimization of the procedure, and a pH of 6.0 on the phosphate buffer and an incubation temperature of 50 ℃ showed the most stable results.

Duplicates of approximate 1 g of partly defrosted white fish muscle was cut out and homogenized by a disperser (Ultra Turrax T25, IKA®-Werke GmbH & Co. KG, Germany, 13 000 rpm) in 40 seconds together with 5 ml of phosphate buffer (3.38 mM Na_2HPO_4, 15 mM NaH_2PO_4, 0.25 M sucrose, 1mM EDTA, 100 mM NaCl). Thereafter the samples were centrifuged (Avanti JXN-26, Beckman Coulter, U.S.) for 20 minutes at 4 °C and 11 200 rpm, and the supernatant transferred to 2 ml eppendorf tubes. 15 µl sample was added to 135 µl activation buffer (340 mM CH_3COONa, 60 mM CH_3COOH, 4 mM EDTA, 0.1% Brij 35, 0.8 M Dithiothreitol ($C_4H_{10}O_2S_2$), and then mixed and placed in an incubator (50 ℃, 10 min). Thereafter 100 µl 12.5 µM substrate solution (10 mM Z-Phe-Arg-Nmec/AMC in 1 ml Dimethyl sulfoxide) was added and further incubated in 50 degrees for 10 minutes. Finally, 1 ml of stop buffer (100 mM NaOH, 30 mM CH_3COONa, 70 mM CH_3COOH, 100mM $CH_2ClCOOH$) was added.

250µl of prepared sample was added to a microtiter plate together with a standard dilution series (10 mM 7-amino-4-methyl coumarin) and three blank samples. Thereafter, samples got analyzed by a fluorometer (Synergy 2, BioTek Instruments Inc., USA) with excitation and emission wavelengths 355nm and 460nm respectively. Accordingly, the results were recorded by Gene5 software program and finally assessed in Microsoft excel.

3.3 Statistics

For the statistical analysis the IBM SPSS Statistics 25 for macOS (IBM Corporation, Armonk, New York, USA) was used. To test the significance of any differences between the groups of controls and stressed mackerel a univariate general linear model (GLM) was used. For colorimetric parameters (Lab*) specifically, the multivariate GLM was

performed. The impact of storage was analyzed by sampled t-tests or Wilcoxon´s signed-rank test when no normality of the data was a fact. Pearson's correlation coefficient, r, was used to analyze if variables were related. The analyses of quality measurements were based on averages of duplicates, and all results are given as mean ± SD. All significant differences were defined as $p<0.05$

4. Results

The results of the present MSc thesis were based on two experiments, where a total of 32 fish (38.3 ± 2.2 cm and 527 ± 113 g) was caught and analyzed in experiment 1 and 20 fish (38.0 ± 1.9 cm and 595 ± 88 g) in experiment 2. The mackerel in experiment 2 (1.05 ± 0.14) had a significantly higher (p=0.001) K-factor than tank 1 (0.94 ± 0.09) and tank 2 (0.92 ± 0.10) (experiment 1). Significant differences between control group and stressed group of mackerel was examined, as well as in storage time. Comparison between fresh and frozen storage was taken in to considerations, and additionally correlations between parameters were analyzed.

4.1 Experiment 1

The averages and the associated standard deviations of blood parameters of the raw material of Atlantic mackerel was obtained together with the significance levels (P) between control group and stressed group (table 3). In order to prevent tank 1 and 2 having had an effect on the result of the raw material, differences between tanks were analyzed. Finally, the muscle pH and *rigor mortis* were measured as starting points for subsequent pH (figure 8) and rigor development (figure 9) lasting over a period of 43 hours.

4.1.1 Blood parameters and muscle pH

There was no significance (P=0.113) in glucose levels between control group (3.7 ± 0.7 mmol/l) and stressed group (4.2 ± 0.8 mmol/l), although the control averages were somewhat lower than the stressed ones (table 3). Between tanks there was no significant difference (p=0.203), which means the replicates in the tanks did not affect the results.

Table 3. Descriptive statistics of raw material in experiment 1.

	Control	**Stressed**	**P**[1]
Glucose, mmol/l	3.7 ± 0.7	4.2 ± 0.8	0.113
Lactate, mmol/l	1.6 ± 0.8	16.8 ± 3.2	<0.01
Hematocrit, %	42.9 ± 5.8	49.2 ± 12.1	0.085
Rigor index, %	35 ± 4	58 ± 20	<0.01
Muscle pH	7.4 ± 0.1	6.6 ± 0.4	<0.01

[1]Significance level p<0.05, GLM Univariate.

Lactate levels were significantly higher (p<0.01) in stressed fish than in controls. The replicates in the tanks did not affect the results as there was no significant difference in lactate between the tanks (p=0.869).

Measurements showed no significant difference (p=0.064) in Hct-values between control and stressed group, although the numerical means were somewhat a little lower for control fish than the stressed ones. There was no significant difference in hematocrit between tanks (p=0.067).

4.1.2 Muscle pH and rigor index development

The muscle pH and rigor index were measured at equal times, with the purpose to be followed throughout a 43-hour period. The results are illustrated to visualize how the muscle pH changes throughout the hours (figure 8) and when the mackerel was in a state of *rigor mortis* (figure 9).

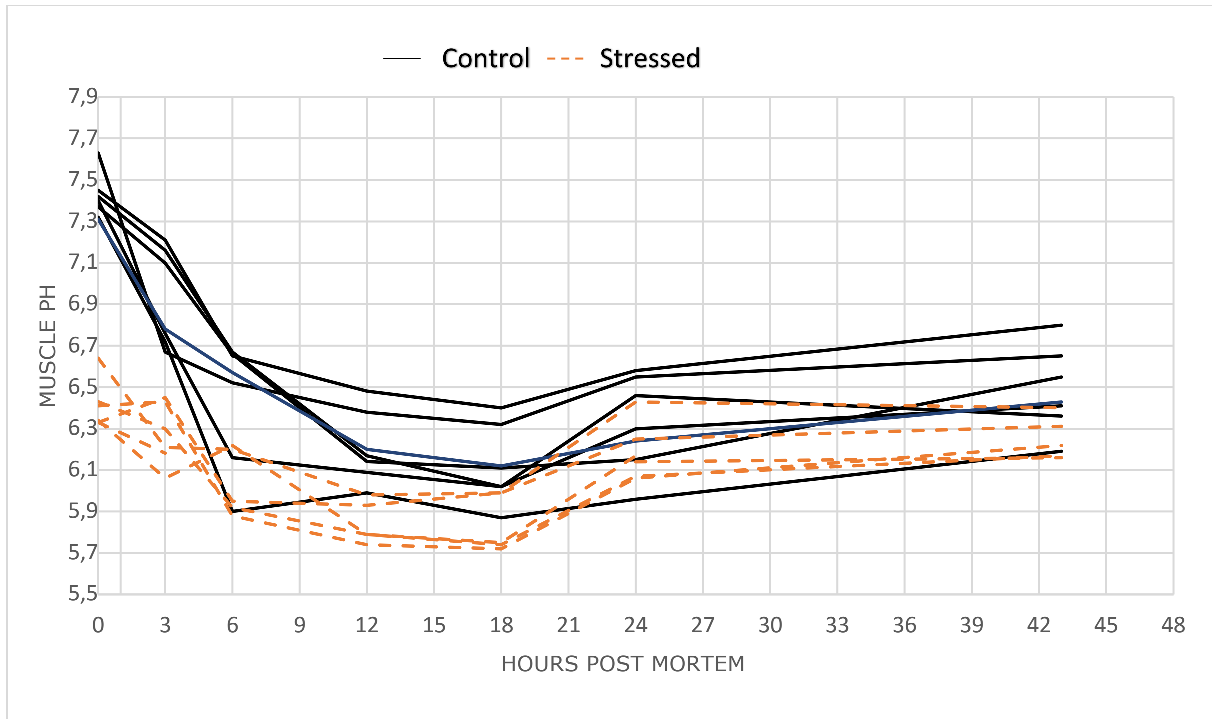

Figure 8. Development of muscle pH of control and stressed mackerel measured 0, 3, 6, 12, 18, 24 and 43 hours post mortem.

The muscle pH (table 3) was significantly lower ($p<0.01$) in stressed mackerel than in the control group immediately post mortem, and the results showed no significant difference ($p=0.474$) between replicates. The muscle pH was significantly lower ($p<0,05$) in stressed mackerel than in the control fish at all measurements (figure 8), except the one after 24 hours ($p=0,235$).

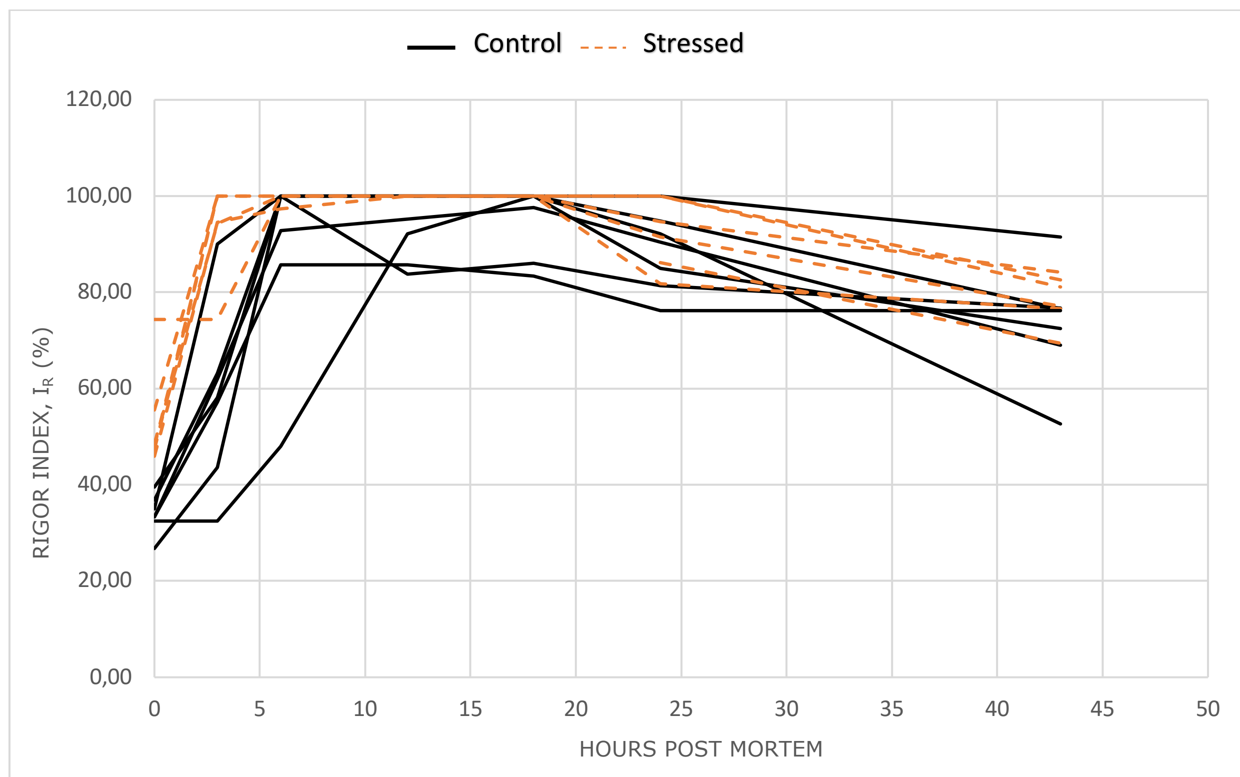

Figure 9. Development of rigor mortis of control and stressed mackerel measured 0, 3, 6, 12, 18, 24 and 43 hours post mortem.

The calculated rigor index (table 3) was significantly higher ($p<0.01$) for stressed mackerel (58 ± 20 %) than for the control fish (35 ± 4 %) immediately post slaughter. There was moreover a significantly lower ($p<0.01$) rigor index for the control fish compared to the stressed fish three hours post mortem (figure 9). The remaining measurements did not show any significant differences ($p>0.05$) between controls and stressed fish.

The rigor index did correlate with the muscle pH immediately post mortem (r=-0.811, p= 0.001), and after 3 hours (r=-0.723, p=0,005).

4.1.3 Drip loss (DL) and water holding capacity (WHC)

From two until seven days post mortem the DL of fresh fillets was measured, but no significant difference (p=0.314) between control and stressed fish was found (table 4). Nor were there any significant difference (p=0.226) in WHC between control fish and stressed fish seven days post mortem.

Table 4. DL and WHC of fresh fillets stored in seven days, and the significance (p) of induced stress.

	Day	**Control**	**Stressed**	**p[1]**
DL, %	7	3.0 ± 1.1	2.4 ± 1.0	0,314
WHC, %	7	87.5 ± 3.1	85.6 ± 2.1	0,226

[1] Significance level $p<0.05$, GLM Univariate.

All fillets of fish from tank 2 (n=19) were frozen two days post mortem, and then thawed again. Fishes analyzed on the same day as thawing were named *day 2*, while *day 7* represents thawed fish stored on ice for five more days.

Table 5. DL and WHC of freeze-thawed fillets measured after thawing (day 2) and five days later (day 7), and the significance (p) of induced stress.

	Day	Control	Stressed	p[1]
DL, %	2	3.2 ± 1.7	3.1 ± 1.1	0.810
	7	8.9 ± 3.8	9.4 ± 3.3	0.743
WHC, %	2	78.6 ± 4.1	80.3 ± 4.9	0.438
	7	82.2 ± 4.5	80.7 ± 3.8	0.522

[1] Significance level $p<0.05$, GLM Univariate.

No significant difference ($p=0.810$) was found in DL between control fish and stressed fish right after thawing (=day 2) (table 5). The total DL of these fishes measured five days later (=day 7) did not show any significant difference ($p=0.743$) between the control and the stressed fish. Although, there was a significant higher ($p<0.01$) DL on day 7 compared to day 2.

There was no significant difference ($p=0.438$) in WHC between control and stressed fish right after thawing. No significant difference ($p=0.522$) was either found in WHC between control and stressed fish five days after thawing, and no significant difference ($p=0.320$) in WHC between the two measurements (day 2 and 7) for fish that was freeze-thawed before analyzing.

4.1.4 Texture

The maximum resistance force (N) at 80% compression of the fillet indicated the firmness of the fillet, while the total force (Ns) reflected the total amount of pressure that was applied during the whole test.

Table 6. Firmness (N) and total force (Ns) applied in texture analysis of fresh fillets, and the significance of induced stress.

	Day	Control	Stressed	P[1]
Firmness (N)	2	70.5 ± 7.2	59.6 ± 13.3	0.180
	7	64.9 ± 9.3	54.4 ± 10.5	0.013
Total force (Ns)	2	302.5 ± 74.0	259.9 ± 90.7	0.163
	7	251.1 ± 69.1	216.8 ± 61.0	0.285

[1] Significance level $p<0.05$, GLM Univariate.

No significant differences ($p>0.05$) were observed in either firmness or total force between control and stressed fish two days post mortem (table 6). However, there was a significant higher firmness in control fish compared to stressed seven days post mortem ($p=0.013$). There was a significant higher ($p<0.01$) firmness on day two compared to day seven for fresh stored fillets. There was moreover used a significant higher ($p<0.01$) total force on day two compared to fillets stored in seven days post mortem.

Table 7. Firmness (N) and total force (Ns) applied in texture analysis of freeze-thawed fillets and the significance (p) of induced stress.

	Day	Control	Stressed	p[1]
Firmness (N)	2	56.5 ± 9.8	55.6 ± 6.2	0.824
	7	59.0 ± 10.9	51.9 ± 5.1	0.094
Total force (Ns)	2	238.8 ± 61.3	211.2 ± 23.9	0.224
	7	214.1 ± 59.2	203.1 ± 15.5	0.597

[1] Significance level $p<0.05$, GLM Univariate.

There were no significant differences ($p>0.05$) in either firmness or total force between freeze-thawed control and stressed fish (table 7). No significant difference ($p=0.757$) in firmness between day two and day seven was either found, but there was a significant higher ($p<0.01$) total force value measured on day two compared to day seven.

The main effect of stress for fresh and freeze-thawed fillets measured on day two and seven was further analyzed, and control fish showed an overall significantly higher ($p=0.007$) firmness than stressed fish.

4.1.5 Gaping

No significant difference ($p=0.159$) in gaping (table 8) between control and stressed fish was observed after two days of fresh storage. Nor any significant difference ($p=0.058$) in gaping score between the control fish and the stressed fish after seven days of storage. Although, the control fish had a slightly lower mean value.

In the freeze-thawed mackerel, there was no significant difference (p=0,608) in gaping between control and stressed fish after thawing, nor in control fish and stressed fish stored five days after thawing ($p=0.477$).

Table 8. Gaping score for fillets stored fresh and frozen for 2 and 7 days, and the significance (p) of induced stress.

	Day	Control	Stressed	p[1]
Gaping, fresh	2	0,9 ± 0.9	1.7 ± 1.0	0.159
	7	1.0 ± 0.8	2.2 ± 1.2	0.058
Gaping, freeze-thawed	2	2.2 ± 1.3	1.9 ± 1.3	0.608
	7	2.8 ± 1.2	3.2 ± 1.3	0.477

[1] Significance level $p<0.05$, GLM Univariate

Significant lower gaping scores was moreover found on day two compared to day seven for both fresh ($p=0.046$) and frozen mackerel fillets ($p<0.01$).

The main effect of stress was analyzed, and for fresh and freeze-thawed fillets the control fish was given a significantly higher ($p=0.007$) gaping score than stressed fish. Furthermore, gaping scores correlated slightly with total force ($r=-0.304$, $r=0.042$).

4.1.6 Color measurements

The flesh color was analyzed both in back loin (table 9) and in the center of the fillet with spinal cord included (table 10).

Table 9. Colorimetric results (mean ± SD) of back loin of fresh and freeze-thawed mackerel fillets, and the significance (p) of induced stress.

		Fresh (tank 1)			**Freeze-thawed (tank 2)**		
	Day	Control	Stressed	p	Control	Stressed	P[1]
L*	2	52.1 ± 2.5	50.8 ± 3.0	0.406	51.9 ± 1.5	53.1 ± 2.3	0.190
	7	51.4 ± 2.3	51.1 ± 2.1	0.798	54.1 ± 1.5	54.7 ± 1.6	0.358
a*	2	-5.3 ± 0.8	-1.9 ± 2.4	**0.005**	-3.8 ± 1.7	-2.9 ± 2.6	0.408
	7	-4.6 ± 1.3	-1.4 ± 2.1	**0.007**	-3.2 ± 1.1	-3.1 ± 1.3	0.862
b*	2	6.5 ± 3.1	9.2 ± 5.6	0.297	8.9 ± 2.8	6.5 ± 5.3	0.232
	7	8.3 ± 3.4	9.2 ± 5.0	0.708	3.5 ± 2.7	2.5 ± 2.8	0.413
C*	2	8.7 ± 2.0	10.5 ± 3.5	0.277	10.0 ± 1.9	8.3 ± 3.0	0.228
	7	9.8 ±2.4	9.9 ± 3.8	0.943	5.3 ± 1.5	4.6 ± 1.6	0.370
H*	2	132.0 ± 17.4	114.9 ± 41.7	0.339	115.7 ± 17.1	129.8 ± 37.1	0.295
	7	122.3 ± 17.1	109.0 ± 30.7	0.343	142.2 ± 36.2	149.1 ± 33.7	0.670

[1] Significance level p<0.05, GLM Multivariate and Univariate.

The L* and b* values were not significantly (p>0.05) different between the control group and the stressed group in the back loin of the mackerel (table 9). Although, the a* value was significantly lower (p<0.01) in the control fish compared to the stressed fish that was stored fresh. There was significantly lower (p=0.010) a* value and higher (p=0.008) H* value for measurements on day two compared to day seven, but no significant differences (p>0.05) in L*, b* and C* values between storage days.
As a response to stress, the rise of lactate correlates with the a* value (r=0.822, p=0,001). Additionally, the decrease in muscle pH correlates with the a* value (r=-0.749, p=0,003).

No significant differences (p>0.05) was found in any of the colorimetric parameters between control and stressed freeze-thawed fish. However, significant differences (p<0.01) was found between storage of two and seven days for every parameter, except the a* value (p=0.415). On the other hand, the a* value correlated with the hematocrit (r=0,504, p=0,028).

Table 10. Colorimetric results (mean ± SD) of fresh and freeze-thawed mackerel fillets with spine included, and the significance (p) of induced stress.

	Fresh				**Freeze-thawed**		
	Day	Control	Stressed	p	Control	Stressed	p[1]
L*	2	51.2 ± 1.8	51.4 ± 2.1	0.918	53.3 ± 0.9	54.0 ± 1.8	0.316
	7	51.8 ± 1.9	52.5 ± 1.1	0.480	54.5 ± 0.9	54.9 ± 1.3	0.447
a*	2	-3.1 ± 1.1	-1.4 ± 1.7	0.059	-2.5 ± 1.1	-2.0 ± 1.8	0.539
	7	-2.4 ± 1.1	-1.0 ± 0.9	**0.033**	-2.1 ± 0.9	-1.9 ± 0.9	0.674
b*	2	6.1 ± 3.4	6.0 ± 4.6	0.976	4.3 ± 2.4	2.6 ± 4.5	0.301
	7	6.2 ± 4.0	4.9 ± 3.5	0.536	1.4 ± 2.0	0.3 ± 2.8	0.344
C*	2	7.3 ± 2.4	7.2 ± 2.6	0.961	5.3 ± 1.6	4.8 ± 3.0	0.639
	7	7.3 ± 2.9	5.5 ± 2.5	0.278	3.2 ± 0.8	3.3 ± 0.9	0.775
H*	2	123.4 ± 25.0	116.8 ± 47.4	0.758	128.0 ± 31.5	151.5 ± 47.7	0.218
	7	121.5±31.8	113.1 ± 41.2	0.685	154.1 ± 41.7	177.2 ± 54.2	0.308

[1] Significance level p<0.05. GLM Multivariate and Univariate.

The L* and b* value was not significantly (p>0.05) different between the control group and the stressed group in the fillet with spine included (table 10). Although, the a* value was significantly lower (p=0.033) in the control fish compared to the stressed fish when stored fresh in seven days. In view of C* and H* values there were no significant differences (p>0.05) between control fish and stressed fish for mackerel stored fresh for two and seven days. The L*, a* and C* values were significantly different between two and seven days, but the b* and H* values were not significantly different (p>0.05) between storage days for fresh fillets. The increase in lactate in stressed fish correlated with the a* value (r=0,640, p=0,025), as well as the decrease in muscle pH (r=-0,639, 0,025).

There were no significant differences (p>0.05) in Lab* values, nor in C* and H*, when comparing control fish and stressed fish that was freeze-thawed and stored in further five days (=day 7). When comparing the colorimetric values measured the same day as thawing (day 2) and five days after (day 7), there were significant differences (p<0.05) in L*, b*, C* and H*. a* (p>0.05) did not differ from day 2 to day 7. Furthermore, the hematocrit values in the freeze-thawed fillets correlated with the a* value (r=0,513, p=0,025).

4.1.7 ATP, K-value and H-value

No ATP was found in any sample. Therefore, there are no analyses on ATP levels in post mortem fish muscle in the present results. The detected metabolites were used in the formulas of K- and H-value to indicate the mackerel´s freshness after two and seven days of storage, either fresh or freeze-thawed.

Table 11. K-value for mackerel in both tank 1 and 2, measured at given sampling days, and the significance (p) of induced stress.

Tank 1				Tank 2		
Day	**Control**	**Stressed**	**p**	**Control**	**Stressed**	**p[3]**
2	50.8 ± 1.3	46.6 ± 4.2	0.028	52.1 ± 0.8	51.8 ± 1.0	0.556
7	93.5 ± 3.4	89.8 ± 3.4	0.042	94.6 ± 1.8[1]	95.1 ± 1.1[1]	0.461

[1]At day 7 (tank 2) mackerel have been freeze-thawed
[2]Values denominated in percent
[3] Significance level $p<0.05$, GLM Univariate.

In tank 1 the control fish and stressed fish had significant different K-values ($p<0.01$) after two and seven days. The K-value (table 11) were also significantly higher ($p<0.01$) after seven days of storage. Although, no differences ($p>0.05$) was found in tank 2.

Table 12. H-value for mackerel in tank 1 and 2 at given sampling days.

Tank 1				Tank 2		
Day	**Control**	**Stressed**	**p**	**Control**	**Stressed**	**p**
2	1.9 ± 0.5	2.1 ± 0.4	0.352	2.8 ± 1.0	2.4 ± 0.5	0.219
7	4.3 ± 0.9	3.9 ± 0.6	0.411	6.9 ± 3.8[1]	7.0 ± 2.3[1]	0.963

[1]At day 7 (tank 2) mackerel have been freeze-thawed
[2]Values denominated in percent

There was no significant difference ($p>0.05$) in H-value (table 12) between control and stressed fish at any sampling day. However, there were calculated a significant higher ($p<0.01$) H-value on day seven compared to day 2.

4.2 Experiment 2

In experiment 2 mackerel were caught from a net pen to see if the results from the tanks were transferable to cages at sea, but also to expand the sample size. This would potentially simulate the crowding stress by purse seine fishing better and become a more full-scaled experiment. The blood analyzes and biometrics were carried out at HI, Austevoll immediately post mortem, while additional quality measurements were executed seven days post mortem at NTNU, Trondheim. The fish were stored on ice in 4 ℃.

4.2.1 Blood parameters

The blood parameters (table 13) were analyzed out of whole blood immediately post mortem.

Table 13. Blood parameters measured immediately post mortem, and the significance (p) of induced stress..

	Control	Stressed	p[1]
Glucose, mmol/l	3.5 ± 0.5	4.7 ± 0.8	0.001
Lactate, mmol/l	0.6 ± 0.1	11.3 ± 4.9	<0.01
Hematocrit, %	42.3 ± 6.3	44.1 ± 6.5	0.537

[1] Significance level $p<0.05$, GLM Univariate.

Both the glucose levels and lactate levels were significantly lower ($p<0.001$) in control fish compared to the stressed (figure 10). The hematocrit values were not significant different ($p=0.537$) between control fish and stressed fish.

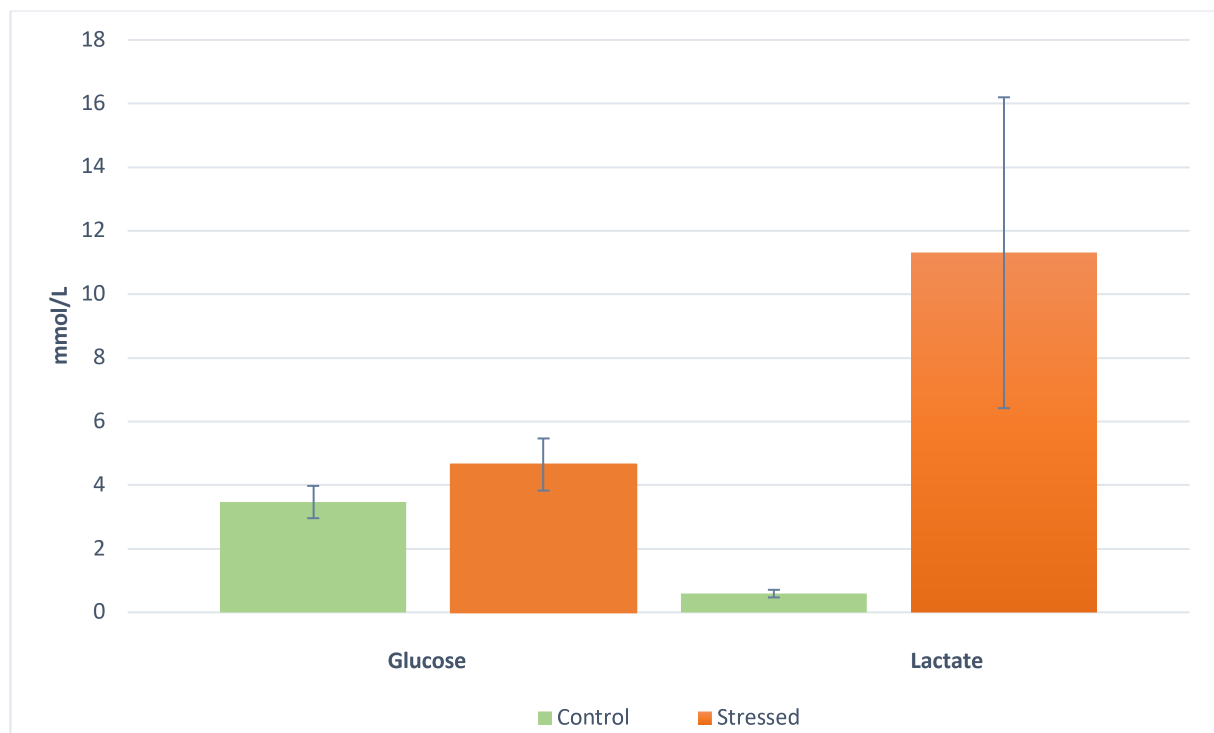

Figure 10. Comparison of control and stressed fish considering glucose and lactate values with standard deviations.

4.2.2 Muscle pH, texture and gaping

There was significantly higher ($p<0.01$) muscle pH in control fish (7.4 ± 0.1) compared to stressed fish (6.7 ± 0.3) immediately post mortem (figure 11).

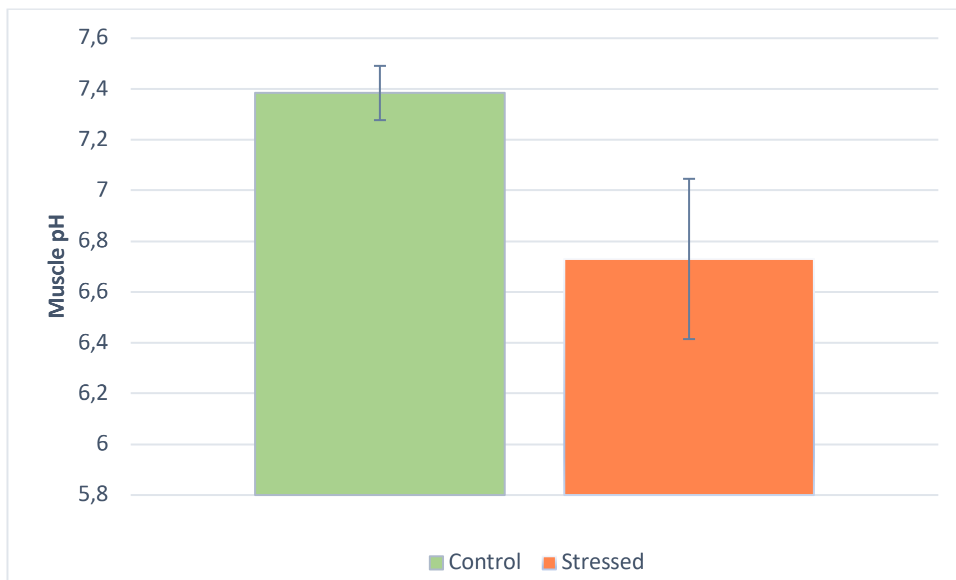

Figure 11. Means of muscle pH of control and stressed group of mackerel in net pen.

Table 14. Averages of muscle pH, texture parameters and gaping score of mackerel stored fresh for seven days, and the significance (p) of induced stress.

	Control	**Stressed**	**p[1]**
Muscle pH	7.38 ± 0.11	6.73 ± 0.32	<0.01
Firmness (N)	61.7 ± 12.6	65.5 ± 12.0	0.497
Total force (Ns)	250.6 ± 48.4	288.1 ± 65.8	0.164
Gaping	2.2 ± 1.14	2.5 ± 1.3	0.584

[1] Significance level $p<0.05$, GLM Univariate.

There was no significance between the control fish and the stressed fish ($p=0.497$, $p=0.164$) in firmness or in the total force used during the analysis (table 14).

In gaping score (table 14) there was no significant difference ($p=0.584$) between control fish and stressed fish after seven days storage on ice in 4 ℃.

4.2.3 Color

None of the color variables showed significant difference ($p>0.05$) between control and stressed fish when analyzing back loin (table 13), as well as the fillet including spine (table 14), seven days post mortem.

Table 15. Colorimetric parameters analyzed of fillets back loin seven days post mortem. and the significance (p) of induced stress.

	Control	Stressed	p[1]
L*	52.85 ± 2.18	53.82 ± 2.16	0.329
a*	-4.41 ± 1.05	-3.64 ± 1.33	0.168
b*	6.18 ± 3.39	4.04 ± 4.44	0.242
C	7.94 ± 2.56	6.22 ± 3.37	0.213
H	130.75 ± 20.28	142.75 ± 29.43	0.303

[1] Significance level p<0.05, GLM Multivariate and Univariate.

The L* value of the analyzed back loin was, however, slightly correlated with the glucose level ($r=0.463$, $p= 0.040$), and the a* value showed a moderate negative correlation with muscle pH ($r=-0.459$, $p=0.042$).

Table 16. Averages of colorimetric parameters analyzed of fillets with spine included seven days post mortem, and the significance (p) of induced stress.

	Control	Stressed	p[1]
L*	52.70 ±1.83	53.12 ± 1.43	0.572
a*	-2.64 ± 1.15	-2.31 ± 1.24	0.551
b*	5.22 ± 3.72	3.90 ± 3.92	0.447
C	6.32 ± 2.96	5.15 ± 3.20	0.405
h	127.80 ± 29.06	134.23 ± 30.16	0.633

[1] Significance level p<0.05, GLM Multivariate and Univariate.

4.2.4 ATP, K-value and H-value

As in experiment 1, no ATP was found in either of the samples.

Table 17. Calculated K- and H-value of mackerel analyzed in frozen condition, 0 and 7 days post mortem, and the significance (p) of induced stress.

	Day	Control	Stressed	p[1]
K-value, %	0	80.4 ± 15.8	82.2 ±24.7	0.842
	7	89.4 ± 1.5	90.2 ± 2.9	0.480
H-value, %	0	0.1 ± 0.1	0.3 ± 0.6	0.295
	7	3.7 ± 1.2	4.2 ± 2.1	0.557

[1] Significance level p<0.05, GLM Multivariate and Univariate.

No significant differences between controls and stressed mackerel was found regarding K- and H-value measured at start and seven days post mortem. There was no significant difference ($p=0.469$) in K-value from start to day seven, however, the H-value was significantly lower ($p<0.01$) immediately post slaughter than after seven days of fresh storage.

4.2.5 Cathepsin B + L activities

No significant difference ($p=0.148$) in total activity level of cathepsin B and L (figure 12) between control fish (2276 ± 827 µU/g) and stressed fish (3080 ± 1465µU/g) after seven days storage on ice in 4 ℃. Nevertheless, numerical difference was observed between means, where controls tended to have a lower activity than stressed fish.

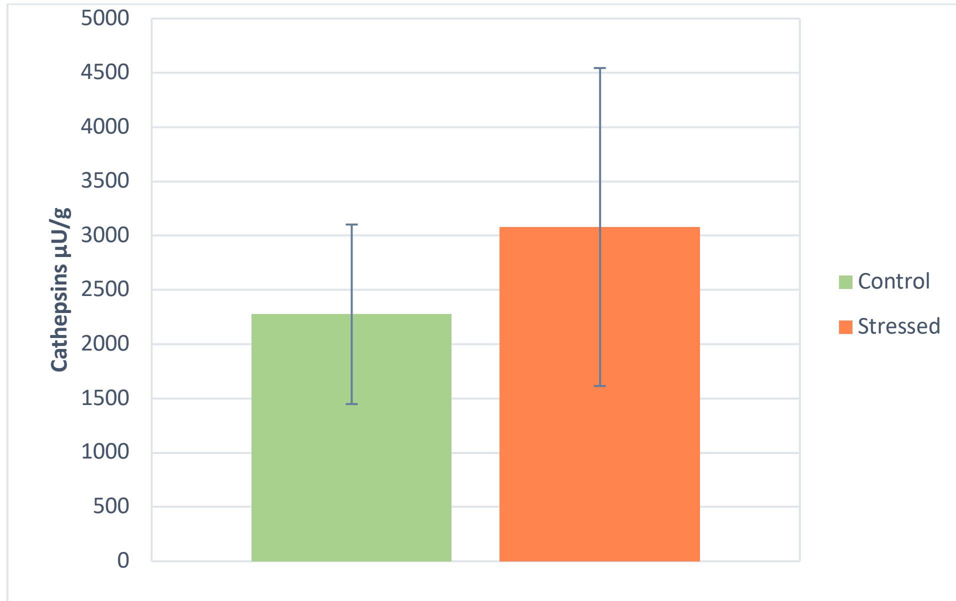

Figure 12. Mean values of cathepsin B and L for control and stressed mackerel seven days post mortem.

5. Discussion

The aim of this present thesis was to investigate the effect of crowding stress, and furthermore how stress responses affect the fillet quality of fresh and freeze-thawed Atlantic mackerel. Biometrics and blood data were analyzed immediately post mortem, while the whole fillets of the mackerel were examined two days later and further taken quality measurements of. Thereafter, data analyses of these measurements were evaluated and discussed up against the literature, whether the stress had an effect on the fillet quality or not.

5.1 Experiment 1

The present results from experiment 1 stated that the blood lactate levels increased strongly in the stressed mackerel immediately post mortem, and was moreover confirmed with the significant lower muscle pH, which is well known to decrease very quickly post mortem (Watabe et al., 1989). The blood glucose level was lower in control fish, but not significantly different from the stressed fish. The lack of glucose elevation in stressed fish, has moreover been observed by Einarsdóttir and Nilssen (1996) as well (Einarsdóttir and Nilssen, 1996). Anyway, these blood data results are consistent with the literature on effects of stress in fish (Barton and Iwama, 1991; Wendelaar Bonga, 1997) The hematocrit, however, did not differ between control and stressed fish, but the stressed fish showed a tendency ($p=0.085$) to have a higher Hct value. An explanation of why there was no significance can be that some of the mackerel was more stressed previous to crowding, as a result of the tanks in experiment 1 not being suitable for mackerel. That could mean the group of fish in each tank was more exposed to individual differences in oxygen tension (pO_2), which is in conjunction with the Hct (Lücker et al., 2017).

In experiment 1 *rigor mortis* development was examined, and the result stated that stressed fish goes faster into rigor. This is consistent in other studies that have investigated the effects of crowding or handling stress for other fish species (Roth et al., 2012; Skjervold et al., 2001). In the present study maximum rigor tension occurred very fast, and all fish that was crowded before slaughter was reported in rigor within 3 hours. This may indicate that Atlantic mackerel is a species that gets easily affected by slaughter and handling stress. Also, the fast rigor development was correlating negatively ($r=-0.811$) with the rapid decrease in muscle pH, which was significantly lower for stressed mackerel throughout the period of 43 hours post mortem. Sigholt et al. (1997) made a hypothesize that a strong rigor mortis together with a rapid decline in post mortem pH could increase the extent of protein denaturation, enhance the availability of substrates to proteolytic enzymes and finally induce a faster softening of the muscle (Sigholt et al., 1997).

The mackerel in the present study did not differ in DL or in WHC, neither between controls and stressed fish or in storage time. Similar results were obtained by Lerfall et al. (2015) on Atlantic salmon, however, differences were found as a result of storage time. In the present study, freeze-thawed mackerel did not show any significant differences in DL or WHC, however there was a higher DL of fish stored in five days after thawing. It is reasonable to think that the freezing and thawing steps affected the control fish almost equal to the stressed, and in that way eliminated the stress as a factor of quality degradation. Besides, Nott et al. (1999) did a study on freeze-thawing of fresh and individually quick frozen (IQF) cod and mackerel, and found out that the main reason

of damage to the fish quality was the process of freezing and thawing, and not the difference in raw material (Nott et al., 1999).

Regarding the texture analysis, the results of the fresh fillets stated that the firmness and total force applied during the test was higher for control fish than stressed fish. That means the stressed fish got softer post mortem. However, the results were not significant and individual differences needs to be taken in to consideration. The firmness was moreover significantly higher on day 2 compared to day 7, which means that the fillet became less firm the longer storage. On the other side, there was no differences in either firmness or total force in freeze-thawed fillets, only between storage days 2 and 7. A reason for that could be that the freezing and thawing process itself, combined with inter-species differences, was affecting the fillets so much that the differences applied by pre slaughter stress became insignificant. Also, mackerel varies a lot in fat and water content influenced by e.g. sexes, age and feeding activity (Chidambaram et al., 1952). Irrespective of this, the main effect of pre-mortem stress was analyzed and stated that the control fish was significantly firmer than the stressed fish (p=0.007). Lower firmness in stressed fish is in convenience with the lower muscle pH and rigor development. In addition, (Bahuaud et al., 2010) reported on Atlantic salmon that the rapid decrease in muscle pH may lead to increased cathepsin activity, protein denaturation and therefore induce a faster softening of the muscle. Looking at the result of cathepsins (figure 12) in experiment 2, this is in convenience with the present study as well.

The controls of fresh stored mackerel had a slightly lower gaping score than the stressed, but the difference was not significant. Freeze-thawed fillets did not show any significant differences in gaping. However, both fresh and freeze-thawed fish tended to have averages higher on day 7 compared to day 2. Increased gaping during fresh ice storage is in accordance with the literature (Eskin and Shahidi, 2013), but as the average values for freeze-thawed mackerel was slightly higher than fresh, it indicates that the freezing induced the gaping. There was moreover significant higher gaping in stressed than in control fish when analyzing the main effect of stress for fresh and freeze-thawed mackerel. (Espe et al., 2004) stated that "the softer the fish, the higher the gaping score" and that is adequate with the present texture results. Besides, total force correlated slightly with gaping of fresh fillets (r=-0.304, p=0.042), which means fillets with higher firmness had lower gaping score and vice versa.

The back loin of fresh stored mackerel showed a significantly lower a* value in control fish, which means that the fillet got a greener color than the stressed. Also, the fresh fillets had a lower a* value and a higher H* value on day 2 compared to seven days post mortem, which indicates that the mackerel got less green with a redder hue during storage. This is not in convenience with the study (Robb et al., 2000) did on rainbow trout. Both the rise in lactate and decrease in muscle pH correlated with the a* value and strengthens the results of a* being significantly different between control and stressed mackerel. Hct was also moderate correlated to the a* value (r=0.504, p=0.028). For freeze-thawed back loin of mackerel, none of the colorimetric parameters differed when comparing control against stressed fish. Although, they all differed from day 2 to day 7, except the a* value.

Color measurements were also taken with the spine included, and these results showed the same tendency as back loin, with significant difference in a* value on day 7 for fresh fillets. The freeze-thawed fillets had no difference in colorimetric parameters between the

control and stressed group, but significant differences in L*, a* and C* between storage days, which was different from measurements of the back loin. The pictures that was taken of the spine included involved a lot more interference and might have disturbed the results and explain the different results from the back loin. Since all of the parameters except the a* value differed from day 2 to day 7 for the freeze-thawed mackerel, in both back loin and with the spine included, is was reasonable to believe that the freezing and thawing steps had a large effect on flesh color.

None of the analyzed samples of mackerel contained any ATP, but that was expected. ATP degrades very fast to IMP (Tarr, 1966) and it was therefore not optimal to transport the samples for so many hours. In addition, the samples could have been exposed to temperature fluctuation. Sigholt et al. (1997) reported that a very little rise in storage temperature had a distinct negative effect on fillet quality of Atlantic salmon, as an increase in K-value appeared. Depletion of ATP is known to correlate with the onset of rigor (Eskin et al., 1971), and that confirms why there was no ATP in the present results, as almost all mackerel was in rigor within 5 hours.

A quicker increase in K-value has been reported in stressed fish (Lowe et al., 1993), but that was not in convenience with the present results. After processing the present results and reading literature, it looked like the H-value was more reliable than the K-value. As the level of ATP, ADP and AMP decreases quickly post mortem, and the K-value has its dependence on e.g. species and handling condition (Olafsdóttir et al., 1997), Hong et al. (2015) have reviewed and presented other freshness indicators. For several species, including mackerel, a substantial accumulation of inosine has been observed during ATP depletion. As a result of that, the K-value would obtain high values and is expected to not be a reliable indicator of taste and freshness (Luong et al., 1992). Saito et al. (1959) stated that a K-value higher than approximate 70% indicates that the fish is not edible. The mackerel in the present experiment reached a K-value over 50% after two days of storage and seems to be consistent with this theory. It is therefore conceivable that the H-value is more targeted and adapted to Atlantic mackerel as Luong et al. (1992) stated that H-value would be much lower than K-value for inosine producing species. There was no significant difference in H-value between control and stressed fish, but there was a higher value on day 7 compared to day 2. A study on sea bass of (Özogul et al., 2010) confirms that H-value correlated well with storage time.

5.2 Experiment 2

A second experiment was executed to perform more as a full-scaled experiment, and to better simulate the crowding stress mackerel get applied during purse seine fishing. The results were also used to increase the sample size, and to compare the results from experiment 1.

The blood data results corresponded with the ones in experiment one, however in the second experiment glucose was significantly affected by the pre-mortem stress as well. This may indicate that the control fish was more relaxed and that the welfare in the net pen was better than in land-based tanks. In addition, this supports the glucose results from experiment 1, which was not significant but higher in stressed than in control fish. The post mortem muscle pH in stressed mackerel was more or less the same in both experiments, 6.6 ± 0.4 and 6.7 ± 0.3 respectively.

The firmness and total force applied did not differ between control and stressed fish. In a matter of fact, the present texture results showed large standard deviations. The mean gaping score had big standard deviations as well. These results may indicate large individual differences.

None of the colorimetric parameters L*, a*, b*, C* and h* were significant different between control and stressed mackerel, either in back loin or in flesh with spine included. However, the L* value of the back loin correlated moderately with the glucose ($r=0.463$, $p=0.040$), as well as the a* value correlated negatively with muscle pH ($r=-0.459$, $p=0.042$). That indicates that stressed Atlantic mackerel with higher glucose and lower muscle pH, was more translucent and had a less green flesh color than non-stressed mackerel. That is in convenience with the significant lower a* value in control fish from experiment 1.

There was no significant difference in K-value, as the standard deviations was large. However, the H-value was significantly lower immediately post mortem than after seven days of fresh storage. As mentioned before, this also confirms the theory about H-value being a more fitting freshness indicator than the K-value. In experiment 2 the H-value was vaguely lower on day 7 compared to the results from experiment 1 and may further indicate that the mackerel thrived better in net pen than in land-based tank.

In the second experiment the level of cathepsins was analyzed and showed that the control fish had a tendency to contain less of the enzyme than the stressed fish seven days post mortem. Although, the result was not significant in this present study, but furthermore in convenience with the results on a study of farmed cod (Hultmann et al., 2012). Cathepsin L and B are well described to be important contributors to protein degradation and flesh softening (Bahuaud et al., 2008; Huss, 1995; Yamashita and Konagaya, 1991), and the higher average in stressed fish fits with the texture results in experiment 1, as the stressed fish had a softer texture.

5.3 A comparison of experiment 1 and 2

Most of the results in experiment 1 showed the same tendency in experiment 2, however the average lactate level was higher in experiment 1 than 2. That verifies the assumption about the land-based tanks being an additional stress factor to the mackerel. The condition factor in experiment 1 and 2 did not differ from each other and suggests that the mackerel got the same feeding conditions. However, the mackerel caught in experiment 2 had a significantly higher K-factor, which may indicate that the mackerel thrived better and had better feeding conditions in net pen. The land-based tank was small in volume, and it seemed like the mackerel in general was more relaxed in the net pen than in the tanks. Also, the number of fishes in tanks were very low, and opposite of what mackerel is used to in large schools. This can give account to why the mackerel was more clearly significantly affected by the induce of pre slaughtering stress. However, it has to be taken in to consideration that the number of samples were lower in experiment 2, and that with more fish samples the significantly differences might have appeared in experiment 2 as well. On the other hand, the stress induced in the second experiment corresponded more to purse seine fishing, and the results from land-based tanks might have been extreme.

6. Conclusion

Pre-mortem crowding stress did not reduce the water holding capacity or increase the muscle drip loss. However, there was a main effect of stress on texture and gaping, where stressed Atlantic mackerel became less firm, and firmer fillets tended to have lower gaping scores. Pre-mortem crowding stress had an effect on flesh color, with the stressed mackerel having a significantly less green color than the control group. There was not found any effects of stress on ATP degradation.

Furthermore, it is concluded that the flesh quality of fish sampled from the full-scaled experiment (experiment 2) followed the same patterns as observed of fish caught from land-based tanks. However, the effect of reducing netting volume was found less on stress induced parameters of fish harvested form the net pen in comparison to mackerel from smaller land-based tanks.

7. Future perspectives

It would have been advantageous to repeat the experiment in the net pen with a greater sample size because of big individual differences in the selection of mackerel, and with that gives insecurity in the results of the actual effects of stress. In addition, it would be sufficient to repeat a trial during summer/early fall when the mackerel is more robust with a higher K-factor.

The experiments in the present MSc only simulated the crowding stress applied in purse seine fishing, and a future experiment could perhaps include analyses of actual pre-mortem crowding stress in the commercial fisheries.

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