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THE ARCTIC UNIVERSITY OF NORWAY

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Blood coagulation time in response to simulated trawling and recovery time of wild Atlantic Cod (*Gadus morhua* L.)

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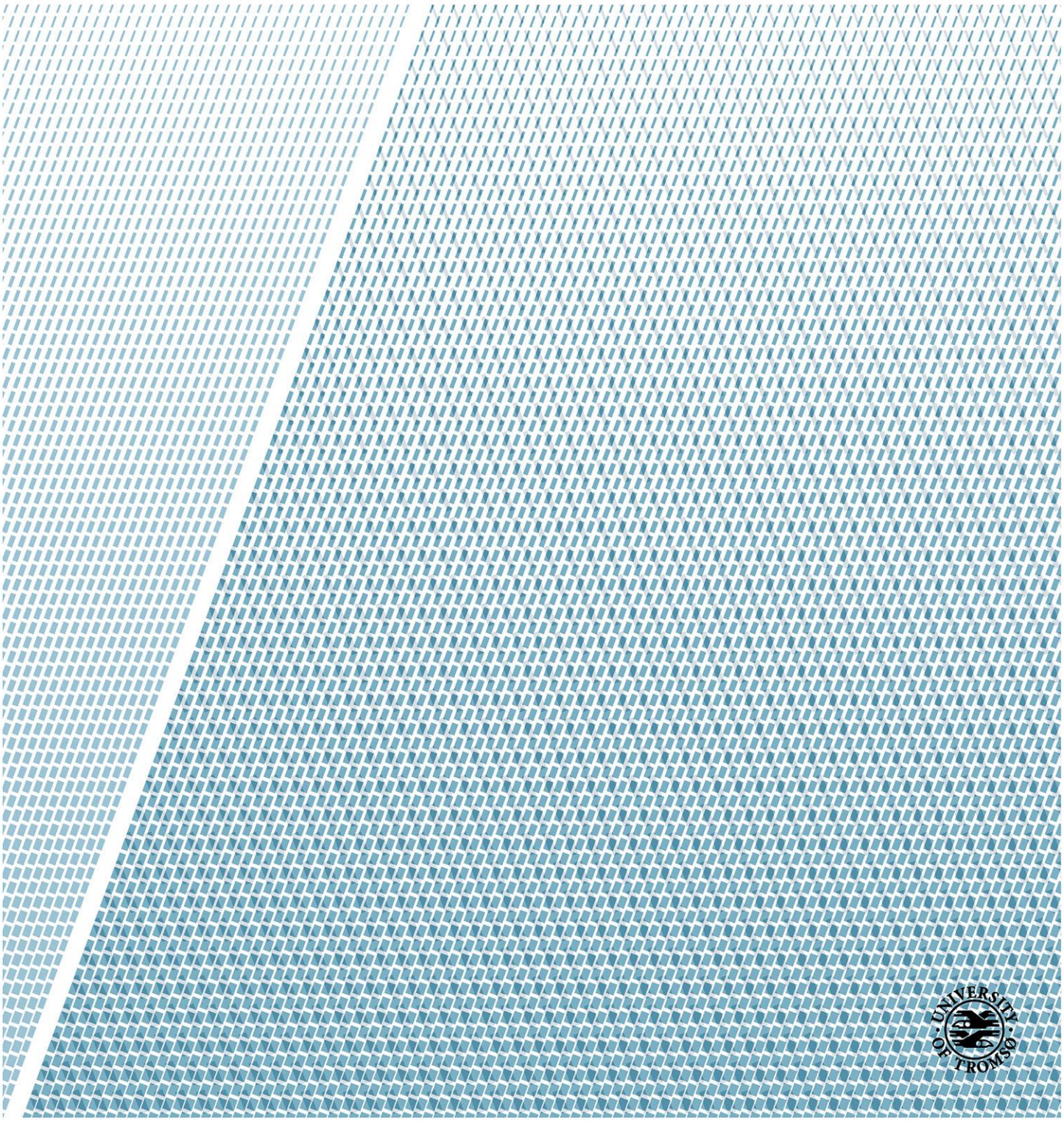
Master thesis in International fisheries management
May 2015



ACKNOWLEDGEMENTS

This thesis is a summit of two beautiful and challenging years at the University of Tromsø and the Norwegian College of Fishery Science. There are many people who made those years both exciting and instructive. All of them positively contributed to the whole educational process and to this thesis especially, and so deserve big thanks.

First and foremost I would like to thank to my supervisors Stefano Peruzzi and Anders Karlsson for many advices and for their patient guidance through the whole process of writing.

One big thanks go to Ragnhild A. Svalheim, Helge K. Johnsen, Stein H. Olsen and to all members of project Crisp who participated in research and created both educational and friendly atmosphere.

Big thanks go to people from Tromsø aquaculture research station who contributed with their effort and hard work to smooth proceedings of research. Special thanks is aimed to personal of seacages who with their constructive ideas and discussions enriched this work, and many times created relaxed, stress-free atmosphere necessary for successful proceeding from the first day to the last one.

Thanks to all classmates in International Fisheries Management for fellow “suffering” during the whole study period and to all personal responsible for program, from professors to administration for their incredible effort and patience.

In the end I would like to thank to my parents and sister for all the support and good advice not only during my study life. Huge thanks to all my family and my friends both in the south and in the north.

Tromsø, 12 may 2015

Jakub Tichy

PREFACE

This study is part of the CRISP (Centre for Research-based Innovation in Sustainable fish capture and Pre-processing technology) project funded by the Norwegian Research Council and managed by Institute for Marine Research. CRISP is working on development of advanced technical solutions for exploiting marine resources in a sustainable manner. The project CRISP and rationales are outlined in Appendix 1. The University of Tromsø (Department of Arctic and Marine Biology/Faculty of Biosciences, Fisheries and Economics) together with NOFIMA are carrying out the fifth work package – V. Quality improvement. One part of quality assessment deals with blood coagulation time as a reaction to exhaustive exercise, packing in experimental cod-end followed by variable recovery time. Blood coagulation time may be important in determining quality of the final product and research done on blood coagulation time during project CRISP is elaborated in this Master thesis. While scientific literature is used to provide updated background knowledge on the field study, data obtained during the experimental field are presented, analyzed and discussed.

SUMMARY

Wild Atlantic cod (*Gadus morhua* L.) was used to assess possible effects of different treatments on blood coagulation time, fillet redness and possible correlationship between blood coagulation time and fillet redness by simulating fish capturing and handling activities. Assessment of blood coagulation was conducted in February 2015 at the Tromsø aquaculture research station in Kårvika, Ringvasøya, under natural weather conditions. The fillet redness assessment was conducted during the same period at facilities of Nofima, Tromsø.

During blood coagulation time assessment fish were tested for different treatments simulating commercial fishing operations. Three different treatments were tested – exhaustive exercise represented by swimming in experimental swimming tunnel, packing in experimental cod-end for two different time periods, recovery in recovery containers for three different time periods. Combination of treatments divided tested fish into eight groups in order to be able to assess the influence of each treatment separately.

One group of fish did not undergone any treatment and served as control for 'rested fish'. A second group underwent only experimental swimming and represented the swimming control group.

All other fish after swimming were packed in experimental cod-end. Half of the fish was packed for 1 hour, and half for period of 3 hours. Within packing groups were fish divided into three groups depending on the duration of the recovery period. Recovery periods were 0, 3 and 6 hours. Described division allowed assessment of different activities separately. All the research was done during a period of three weeks within February 2015.

Coagulation time showed no significant difference between controls groups and treated groups. The only significant difference in blood coagulation time was observed within 3 hours packing between groups with no recovery and three hours recovery. Surprisingly, the group with three hours recovery had shorter coagulation time than the group without recovery. The fillet redness assessment showed two important findings. The packing in experimental cod-end had negative effect on fish quality. The redness of fillet was higher in groups with packing and no recovery compared to control groups in all but one cases. On the contrary, a positive effect on fillet status of 6 hour recovery period was proven. The fillet redness of the groups with 6 hours packing was not different from control groups with one exception which was lying on the borderline for statistical significance. Regression assessment between blood coagulation time and fillet redness showed no correlation in any part of the fillet and residual blood in veins.

Content

1. INTRODUCTION

1.1 Atlantic cod

Atlantic cod (*Gadus morhua* L.) is widely spread in the northern Atlantic Ocean into the Arctic Sea (see Figure 1). Fish can be found along eastern and western coast of North America, along the coast of Greenland, waters around Iceland, the North Sea and the Barents Sea (FAO, 2015).

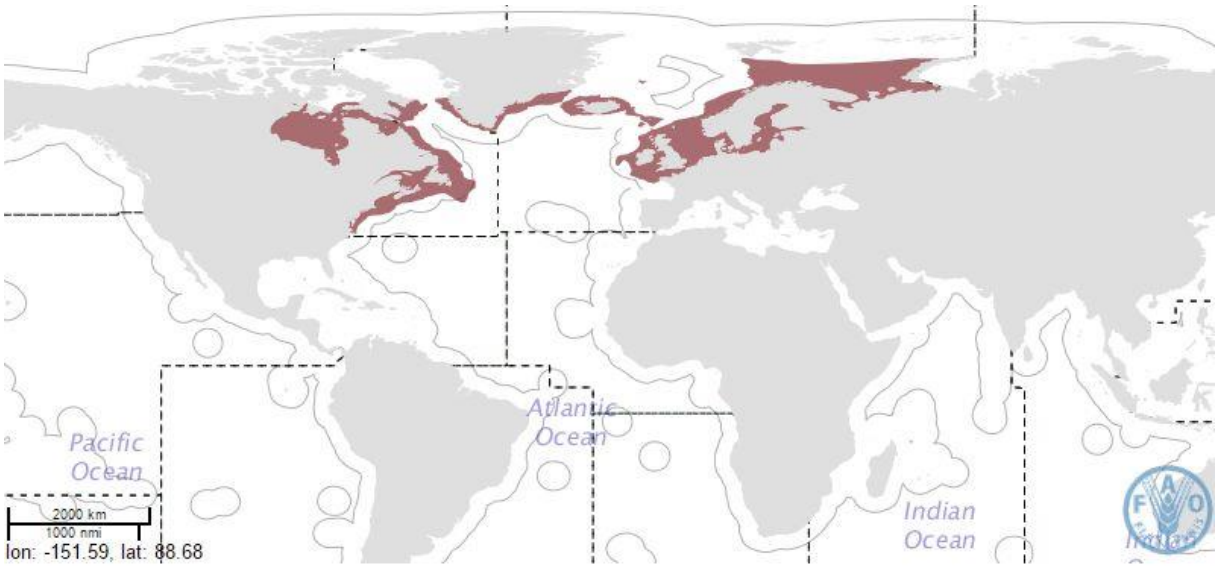


Figure 1: Geographical distribution of Atlantic Cod (FAO, 2015).

The Atlantic cod is generally considered as a demersal fish and is distributed in a variety of habitats ranging from shoreline down to continental shelf. Cod can become pelagic under certain hydrography conditions, when feeding or spawning (FAO, 2015). Cod can tolerate wide range of environmental conditions and can be found in water with various ranges of salinity and temperature. Therefore the presence of cod is determined rather by prey distribution than by environmental conditions (FAO, 2015). Atlantic cod is an omnivorous species. Nauplii of *Calanus finmarchius* is the main food sources for larvae and post-larvae in Norwegian waters, while juveniles mainly fed on invertebrates, and sub adult and adult cod prey on both invertebrates and fish, including young cod (FAO, 2015).

The North-East Arctic (NEA) stock of Atlantic cod is the most important for the Norwegian cod fishery. The stock is distributed widely along the Norwegian coast and in the oceanic regions of Barents Sea (Blanchard *et al.*, 2005). Distribution of (NEA) cod is widest during summer-autumn feeding season when cod feed close to polar front on capelin and shrimp.

Spawning of cod in Norwegian waters normally occurs in winter/spring on well-known spawning sites (ICES, 2005), and lasts until the end of April/early May. The date of 50% spawning during the period 1976-1986 varied between 29 March and 5 April (Ellertsen *et al.*, 1989). Spawning is occurring in the same sites every year and most of the eggs are spawned along a limited part of coastline in Lofoten and Vesterålen (Blanchard *et al.*, 2005).

Apart from NEA cod there is also another Atlantic cod stock in the Northeast Atlantic. Norwegian coastal cod (NCC) is one of high importance. NCC is found along the coast of Norway and is characteristic for fjord areas, with dominant fishing areas along the coast from Varangerfjord to Lofoten (ICES, 2012). NCC is fished throughout the whole year with peak in the first half of the year. The quantities of NCC are quite low with exception for fjords in eastern Finnmark and recreational fisheries takes important piece of total landings. Fishing gear dominantly used for catching NCC is gillnet (50%), Danish sein (20%), long line/hand line (20%) and less than 5% for bottom trawl (ICES 2012). NCC was introduced on the national red list of threatened species in 2006 as a 'near threatened' stock (Kålås et al. 2006). For the years 2004 – 2011 was no catch suggested by ICES. In 2010 was recovery plan finally suggested by Norway and adopted in same year.

1.2 Norwegian cod fishery

Fisheries on Atlantic cod can be characterized as traditional in Norway. Cod have represented one of the main sources of protein for civilisation localized in area of modern Norway through its whole history. Even today cod fisheries represent a significant part of the society particularly in Northern Norway. According to Statistics Norway, Norwegian vessels delivered 2,3 million tonnes of fish, crustaceans and molluscs which together represented almost NOK 14,2 billion in 2014. Out of this portion cod largely dominated the fishery landings both in terms of weight and value (Statistics Norway, 2015).

In Norway there are two management plans for the Atlantic cod stocks. One is managing NEA cod in Barents Sea and one is for North Sea cod. The North Sea stock is managed together with EU and according to International Council for the Exploration of the Sea (ICES) the stock has a reduced reproductive capability and recruitment is low (ICES, 2013). The quota was set to 26 475 tons in 2013.

The NEA cod stock is the largest in the world and is managed together with Russia. The stock is in good condition, something that is reflected in increase of quotas which were set to 1 million tons in 2013 – representing an increase of 30% compared to 2012. In 2014 quotas for

Barents Sea were set to 993 000 tons (Institute of Marine Research, 2013). The cod fishery is conducted year-around with peaks in the first half of the year. Fisheries on NEA cod follow the migration pattern of the stock (see Figure 2) which in winter and spring migrates to the southern Barents Sea in coastal areas to spawn. During autumn, the fish migrate northwards to their main feeding areas along the polar front where they feed on capelin, which is abundant in the areas in the autumn period.

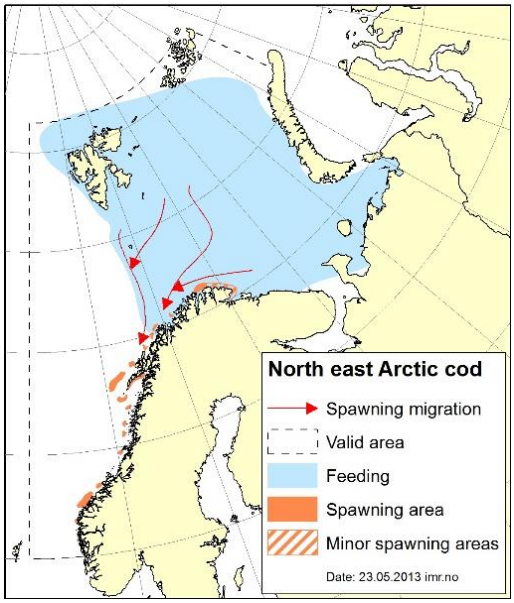


Figure 2: Seasonal spawning migration patterns, spawning, feeding and nursery areas for North east Arctic cod. (ICES, 2005).

Gear used in Norwegian fishery rather varies. In typical year the catch of Norwegian fleet is made up of about 30% from bottom trawl, 30% from gill net, 15% from longline, 15% from Danish seine and 10% from hand line (The Norwegian Ministry of Trade, Industry and Fisheries, 2013). The reason behind using appointed types of fishing gear is fact that cod is demersal specie and thus is usually taken from seabed.

The abundance of fish in North-East arctic stock and the record size of quota proposed by ICES in 2012 for the 2013 season led to dramatic price decrease for cod already in summer 2012. Price level was moving on the same level as in 1970 and the decrease continued through winter 2013 and in the end was index 40% lower in spring 2013 compare to price for cod at it's top in 2008 (Henriksen, 2013). The price decrease for cod product is creating pressure on fleet to increase effectivity of processes. It seems that there are two possible ways to obtain higher effectivity – reducing costs, or make the markets pay higher price for products. The first factor, cost reduction, is significantly dependent on fuel price which is difficult to influence by industry. But higher price levels for products could be obtained by

different methods. As presented in Figure 3, most of the landings are delivered during the first four months (NOFIMA, 2011). Consequence is then market overloading with lower product prices than in later periods when there is shortage of product and price for cod is therefore higher. Keeping cod alive until the shortage periods and feeding fish to obtain bigger size is then one of the ways how to obtain higher price for products. Another way is to obtain better quality by using advanced fishing techniques, more effective bleeding and improved slaughtering techniques.

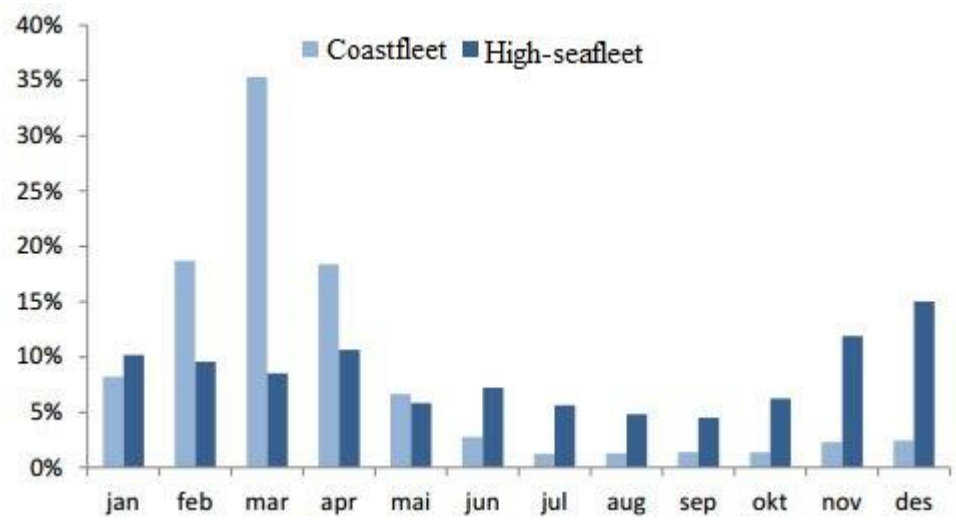


Figure 3: Structure of landings of Norwegian fleet of cod by month over period of 1 year. Averaged for period 2000-2010 (NOFIMA, 2011)

1.3 Quality improvement – development of capture and handling practices to optimize quality and thus value of captured fish

White fleshed-fish species caught by trawling can have poor quality and are often worth less than fish caught by other types of fishing gear. Fishing gear can affect flesh quality since gear used during different fishing methods is usually coming into contact with fish. Fishing gears have multiple impacts on flesh quality and can result in quality degradation of final products (Botta *et al.*, 1987; Esaiassen *et al.*, 2004; Margeirsson *et al.*, 2006; Digre *et al.*, 2010). Insufficient bleeding usually causes bruises and discoloration. Those quality defects are typical for bottom trawling and Danish seine operations. The bruises can be linked to pressure in the trawl and tough handling of the fish before it is bleed (Digre *et al.*, 2010; Rotabakk *et al.*, 2011). In general, the sensory quality depends on a multitude of factors such as seasonal variations, capture methods and processing procedures on board vessels. In large scale trawling operations, bleeding and gutting fish represent challenging tasks. Fish taken by different methods in large hauls are stored in bins before bleeding and gutting which can lead

to bruises and muscle discoloration due to insufficient bleeding and pressure on the fish (Olsen *et al.*, 2013). After exhaustive swimming, blood flow to the white muscle rises (Farell *et al.*, 2001) in order to regenerate the intracellular energy status of the tissue. The blood flow gradually decreases to normal levels over time as the tissue nears its pre swimming state. Therefore is expected that restitution after exhaustive exercise, represented by fishing operations, can lead to improved flesh colour and thus quality (Olsen *et al.*, 2013).

New practices and regulations in management system of fisheries are needed as new technology and processes open possibilities of managing fisheries in a more sustainable way. The CRISP (Centre for Research-based Innovation in Sustainable fish capture and Pre-processing technology) project funded by the Norwegian Research council (2011-2018) is working on the development of advanced technical solutions for exploiting marine resources in such a way. The main outcome of CRISP is expected to be sustainable trawl and purse-sein technology followed by optimal capture practices in order to optimize quality and thus value of captured fish (see Appendices). In particular, the objective of one of the project work-packages (Workpackage V. Quality improvement) is to increase product value through improved product quality. This will be achieved through the implementation of procedures and technology for minimal stress during capture, optimized live storage of fish on-board, and automated individual slaughtering and bleeding of recovered fish for processing. Such practices are already in use in capture-based aquaculture and slaughtering of farmed fish, and technology transfer from aquaculture to fisheries is therefore a key aspect of this work. This implementation will not only offer increased value-adding through improved product quality, but will also address concerns regarding fish welfare and result in improved ethical standards of the industry.

1.3.1 Blood coagulation and flesh quality improvement

All multicellular animals require effective hemostatic mechanism in order to prevent significant loss of fluid and cells by vascular or other general injuries (Doolittle 1993, Rowley *et al.*, 1997, Aird 2003). The hemostatic mechanism is a process which causes bleeding to stop. That means blood is kept within a damaged blood vessel and not released into the surroundings. Aquatic vertebrates, particularly fish, have delicate gills where blood is in proximity to the surrounding environment. Therefore, fish would easily bleed to death if the appropriate hemostatic mechanism was missing. The cells involved in hemostasis in fish are the thrombocytes. The number of thrombocytes varies between and within fish species. Those variations are attributed to number of factors both biotic (age, maturity), abiotic (water

temperature, pH) and in particular to stress (Tavares-Dias *et al.*, 2008). Rapid decline of whole blood clotting time occurs when the number of thrombocytes increases in a fish (Casilas and Smith 1977). Prothrombin, thrombin, fibrinogen, tissue factor and plasminogen are documented factors involved in the coagulation process (Wang *et al.*, 1989, Doolitle 1993).

Coagulation is a complex process which is divided into two phases. Coagulation begins within few seconds after a vessel is damaged. In the damaged site a thrombocyte plug is formed, as the thrombocytes adheres to the collagen fibers of the blood vessels using a specific receptor (Tavares-Dias and Oliveira 2009). Afterwards, a secondary hemostatic process takes place. In this phase, several plasma components designated from coagulation factors form the fibrin, which reinforces the thrombocyte plug. The secondary hemostasis is divided into intrinsic, extrinsic and common pathways. Completion of intrinsic and extrinsic pathways is a necessary condition for reaching a common pathway. As was already mentioned, the final outcome of common pathway during secondary hemostasis is fibrin. In the teleost fish, intrinsic, extrinsic and common pathway factors of the blood coagulation have been demonstrated in biochemical studies (Doolitle and Surgenor 1962; Smit and Schoonbee 1988).

Factors such as stress, K-vitamin, exposure to chemical products, blood thrombocytes, vascular abnormalities and liver damage can influence the coagulation mechanism. Stress is the coagulation factor imposed by fishing and handling activities and so can be controlled in some degree. Activation of hemostatic mechanisms following period of stress has been noted resulting in decline of the whole blood clotting time corresponding with increase in number of thrombocytes (Wedemeyer *et al.*, 1976, Casilas and Smith 1977). It indicates that decrease in clotting time is possibly due to increase in number of thrombocytes, which in turn are induced by an increase of catecholamines and cortisol, released during stress (Tavares-Dias and Oliveira 2009). Clotting time can then be used as a stress indicator.

The capture of wild fish imposes various degrees of handling stress for a significantly long period of time. Consequently, a number of factors influencing exsanguination may result into insufficient bleeding during slaughtering processes and degraded product quality. Stress caused by exhaustive exercise induces an increase in blood volume in the muscle and slows down the exchange of blood between parts of the vascular system. As a result only the blood present in the gills, or in the vessels close to them, is purged when the gills are cut (Hoar and Randall, 1970). Stress also affects the coagulation rate of the blood, as the rate of coagulation increases as stress increases (Ruis and Bayne, 1997). After the death of the fish, the blood in

the veins starts to coagulate. Both temperature and pre-slaughter stress is known to influence the blood viscosity and its ability to clot (Ruis and Bayne, 1997; Olsen *et al.*, 2006). The blood that is forced into the muscle before death will therefore not be removed during bleeding. Increase in volume of blood in muscle, as consequence of exhaustive exercise, may be solved by providing fish time to recover, as physical activity is reduced in the phase of recovery. Physical activity is causing need for more blood in muscles and therefore can live storage and restitution before bleeding improve bleeding and flesh colour (Olsen *et al.*, 2013).

1.4 Research strategies, aims and research questions

The thesis is executed by reviewing scientific literature and statistical information about the state of Norwegian cod fishery and Atlantic cod. On review of existing knowledge about blood coagulation system of fish with special focus on its effects on flesh quality special importance is assigned as it is the main topic of whole thesis. Aim of the thesis is to investigate the possible links between blood coagulation time and flesh quality, measured as fillet redness, after exhaustive exercise and packing, followed or not by recovery. Effect of different treatments on blood coagulation time is assessed separately for every treatment. To be able to achieve this aim, the following questions have to be answered:

1. *Are there any differences between fish groups (divided by swimming, packing and recovery) in blood coagulation time?*
2. *Which of the mentioned variables (swimming, packing, recovery) are most affecting coagulation time?*
3. *Is there a difference in fillet redness between different groups according to sensory panel evaluation?*
4. *Is there a relationship between the different treatments and fillet redness?*
5. *What is the optimal practice for capturing and handling Atlantic cod according to blood coagulation research executed in this work in order to avoid or limit redness of flesh?*

2. MATERIALS AND METHODS

2.1 Fish and research facilities

Project was carried out at the Tromsø Aquaculture Research Station (Havbruksstasjonen i Tromsø) – HiT; 69°N. Two facilities of HiT were involved during the project. In first phase the fish were kept in sea cages of Skulgambukt for a period of four months. Afterwards they were moved to the land facility of Kårvika.

The fish used in this study was Atlantic cod originated from wild populations of NEA from the Barents Sea. Fish were caught in Finnmark county in Fruholmen area (71°06'N; 23°58'E) in May 2014. Afterwards the fish were moved to sea cages facilities of HiT. The number of fish moved to sea cages was approximately 4000 and composed of different sizes (1.5 – 6 kg) and age. Fish was placed into a sea cage of size 660 m^3. Fish was fed three times per week with 40 kg of Barents Sea capelin (*Mallotus villosus*), its natural prey in the area. Fish was placed in sea cage under the above mentioned regime from the beginning of June to early September. Afterwards approximately 400 fish were moved to the land-based facility by using a boat equipped for transporting live fish. None of the fish died under transportation. Fish were then equally divided into two acclimatization tanks (10 m^3**)** provided with flowing water current of temperature corresponding with natural seawater temperatures. Fish were fed once weekly with 20 kg of frozen capelin per tank. Both tanks were covered with black netting and plastic cover in order to avoid impact of any stress factors from the surroundings.

After euthanization all fish were marked with a number on a paper stapled on the fish and registered in the system for processing data.

Crucial accessory used in the experiment was a fully functional, large-size fish swimming tunnel designed by CRISP for controlled experimental studies with live fish. The swimming tunnel is of size 6x 0.9 x0.9 meter with 1.4 m^3 fish chamber (see Figure 4).

The tunnel is equipped with suitable instrumentation for physical monitoring and control of water speed, flow conditions, cameras, oxygen probes and instruments for fish telemetry. The maximum speed of current in tunnel is 1.2 m/s. The more detailed description of experimental swimming system is provided in Appendix 2.

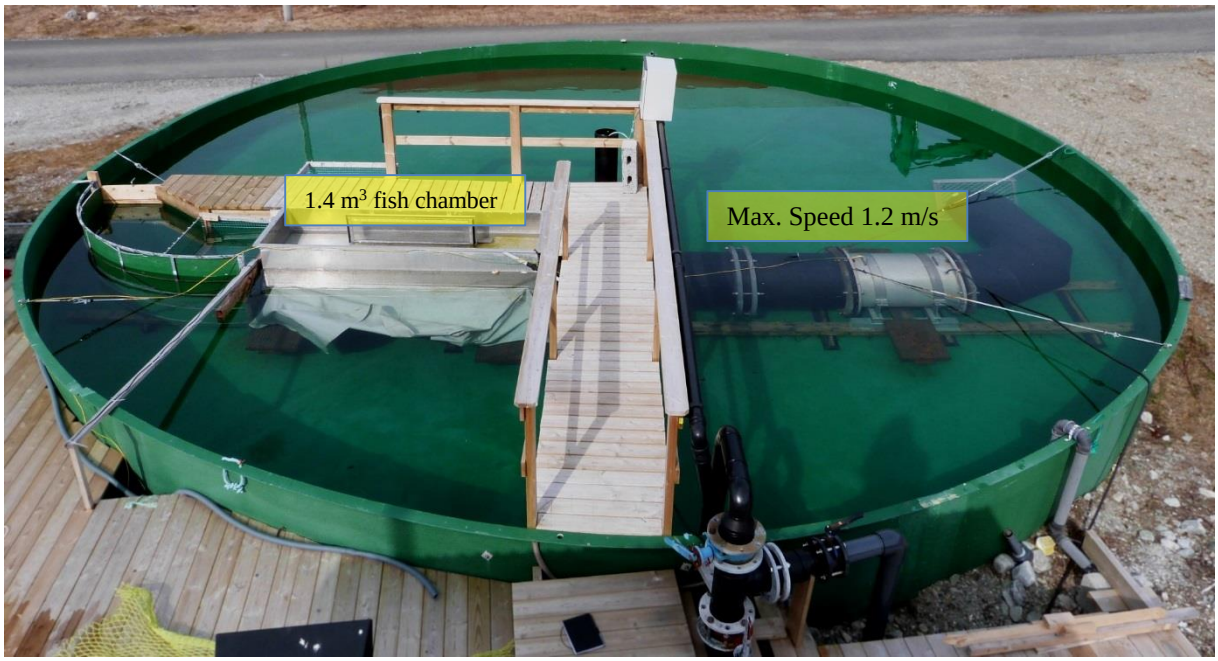


Figure 4: Swimming tunnel for experimental studies of live fish in Tromsø aquaculture research station (Photo by R. A. Svalheim, 2014)

2.2 General experimental setup and fish handling

In the experiment, there were examined three factors which were expected to affect blood coagulation time – swimming, packing in experimental cod-end and recovery. In order to be able to compare different factors and their impact on blood coagulation time the following groups of fish subjected to different conditions were investigated:

1. Control group (CTRL) – taken directly from tank (21 fish)
2. Control swimming group (CTRLS) – taken immediately after swimming (49 fish)
3. Group P1R0: Swimming and packing for 1 hour, 0 hours of recuperation (21 fish)
4. Group P1R3: Swimming and packing for 1 hour, 3 hours of recuperation (21 fish)
5. Group P1R6: Swimming and packing for 1 hour, 6 hours of recuperation (21 fish)
6. Group P3R0: Swimming and packing for 3 hours, 0 hours of recuperation (21 fish)
7. Group P3R3: Swimming and packing for 3 hours, 3 hours of recuperation (21 fish)
8. Group P3R6: Swimming and packing for 3 hours, 6 hours of recuperation (21 fish)

During the experiment the total of 196 fish were randomly taken from both tanks in 1:1 ratio. As the experiment involved different treatments (swimming, packing, recuperation), it was necessary to triplicate experiment in order to obtain reliable data. The total duration of the experiment was of three weeks.

A control group (CTRL) was taken directly from the acclimatization tank in order to obtain basal values for untreated fish. The control swimming group (CTRLS) was sampled directly

after an exhaustive exercise. Exhaustive exercise was simulated by forced swimming in the experimental tunnel. Current speed in the swimming tunnel started at 0.15 m/s and increased to 1.25 m/s over period of 20 minutes. Speed increase in tunnel was considered continuous for the fish since the steps (1200 steps = 1 sec/step) were too small and short to have any effect on fish. Packing was done by use of experimental cod-end and fish was packed right after exhaustive exercise. During experiment were used 2 time periods for packing – 1 hour (P1) and 3 hours (P3). Recovery followed after packing in experimental cod-end and ended fish treatments. During recovery fish were placed in a recovery container placed in the water tank of the swimming tunnel. For this purpose, three time periods of recovery were used – no recovery (R0), 3 hours recovery (R3) and 6 hours recovery (R6).

Experiments were conducted on Sunday, Tuesday and Thursday. Days of experiments were selected as follows: Sunday was chosen as day for control group (CTRL) sampling in order to minimize stress impact from daily operations at the research station and obtained basal value for 'rested fish'. On Tuesday were sampled swimming control group (CTRLS) and groups with packing time of 1 hour (P1R0, P1R3, P1R6). On Thursday were sampled control swimming group (CTRLS) and swimming groups with packing time of 3 hours (P3R0, P3R3, P3R6). There was a single rotation of Tuesday and Thursday during one week because of other planned activities at the research station that could have affected the results.

Fish in the two acclimatization tanks were fed weekly on Wednesday providing fish sufficient time to digest feed without affecting samples. All experimental fish were killed by sharp single blow on the head before being sampled.

2.3 Detailed experimental set up

The experimental week began with taking samples from the control group followed by the relocation of 28 fish (4 groups of n=7 fish) from the acclimatization tanks into the swimming chamber of the experimental swimming tunnel. Fish were taken randomly from tanks in the number of 14 individuals from each tank. Next sampling day was Tuesday therefore providing the fish with approximately 39 hours acclimatization period. After collecting all samples and measurements were new fish moved from tanks into swimming chamber following the same key as used on Sunday. This fish was than sampled on Thursday which provided them with approximately same time for acclimatization (39 hours).

Detailed experimental set up of exercise is presented in Figure 5.

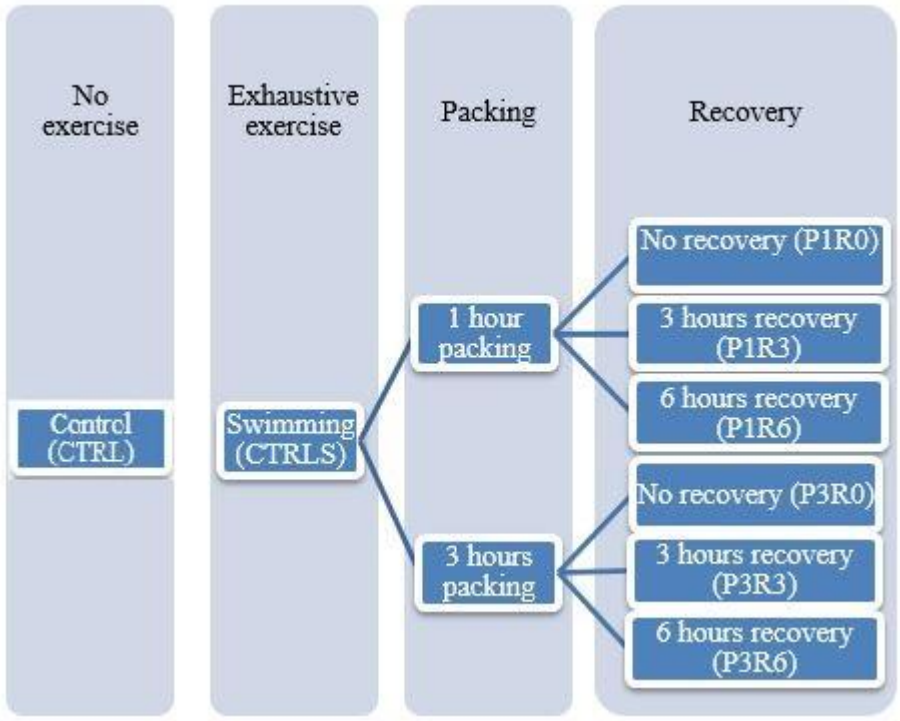


Figure 5: Experimental set up. Exhaustive swimming/non swimming, followed by retention in an experimental cod end for 1 or 3 hours and subsequent recovery in cages for 0, 3 or 6 hours divided tested fish into following groups: CTRL – control group; CTRLS – control swimming group; P1R0 – swimming, 1 hour packing, no recovery, P1R3 – swimming, 1 hour packing, 3 hours recovery; P1R6 – swimming, 1 hour packing, 6 hours recovery; P3R0 – swimming, 3 hours packing, no recovery; P3R3 – swimming, 3 hours packing, 3 hours recovery; P3R6 – swimming, 3 hours packing, 6 hours recovery.

2.4 Blood sampling

Blood was drawn from the caudal vein of fish. From each individual two samples were taken by using two different Vacutainer (BD Vacutainer®, Becton Dickinson, Franklin lakes, New Jersey, USA) systems. A first blood sample (7 ml) was taken using a Vacutainer without anticoagulant. This sample was used for blood coagulation assessment. A second sample (4 ml) was taken using a Vacutainer with anticoagulation treatment (Lithium Heparin) and was used for further blood analysis not included in the present work. Each Vacutainer was labelled and further processed using tag number of fish.

2.5 Blood coagulation assessment

The blood sample was transferred from the Vacutainer without anticoagulant by using a pipette (Finnpipette F1, 100 – 1000 µl) into four Thrombotest tubes (Trombotestrør PS 14x80mm, 7 ml (Art.nr: HEGR100), HEGER A.S, Rjukan Norge) in the amount of 1 ml per tube. For each individual were then performed four independent blood coagulation time measurements in order to obtain consistent results. All TT tubes were placed in a rack as

presented in Figure 6. The rack was placed in a water bath with a temperature equivalent to the one used in the swimming tunnel. Water temperature was constantly measured for the whole duration of experiment by use of a Thermotester (Hanna instruments®, Thermotester HI 93503, ®, (-50, +150), Canada).

Fish 1	Fish 2		Fish 3		Fish 4		Fish 5		Fish 6		Fish 7
Fish 1	Fish 2		Fish 3		Fish 4		Fish 5		Fish 6		Fish 7
Fish 1	Fish 2		Fish 3		Fish 4		Fish 5		Fish 6		Fish 7
Fish 1	Fish 2		Fish 3		Fish 4		Fish 5		Fish 6		Fish 7

Figure 6: Scheme of blood coagulation assessment. Red positions represent test tubes with 1 ml of blood from different individuals.

Setup of blood coagulation assessment in real condition is presented in Figure 7.

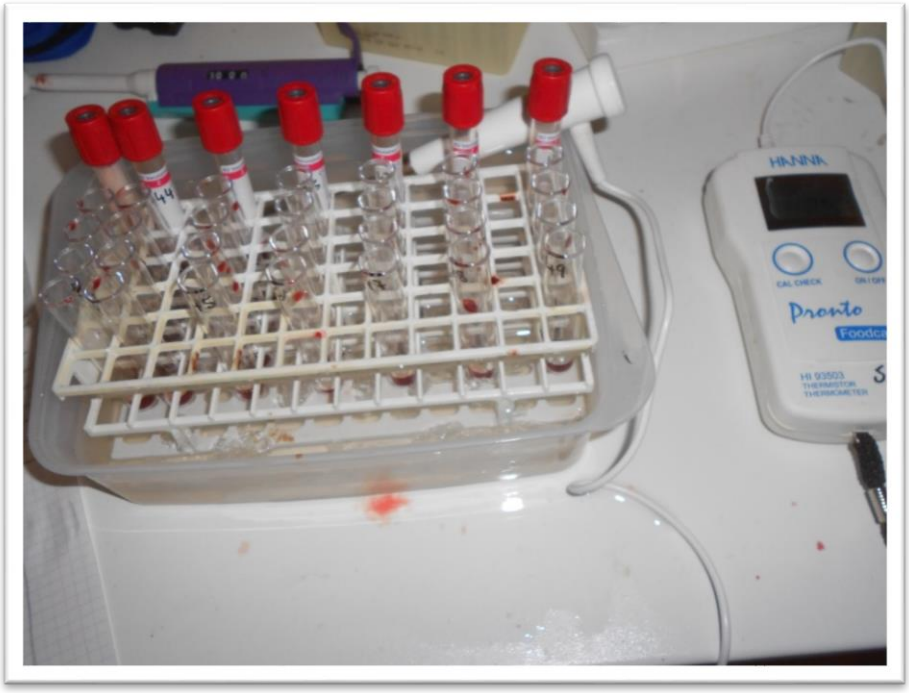


Figure 7: Setup of blood coagulation assessment (Photo by J. Tichy, 2015).

Since the method used for blood coagulation assessment was based on subjective evaluation, all the work and assessment was performed by the same person for the whole period of the study. In order to obtain reliable results, it was necessary to avoid contamination of samples

with sea water. Timing of blood coagulation test started when the operator received the blood samples, immediately after taking blood from fish. Samples were evaluated constantly and notes were made when the first blood clots were visible on the wall of TT tube under an angle of approximately 45° (see Figure 8).

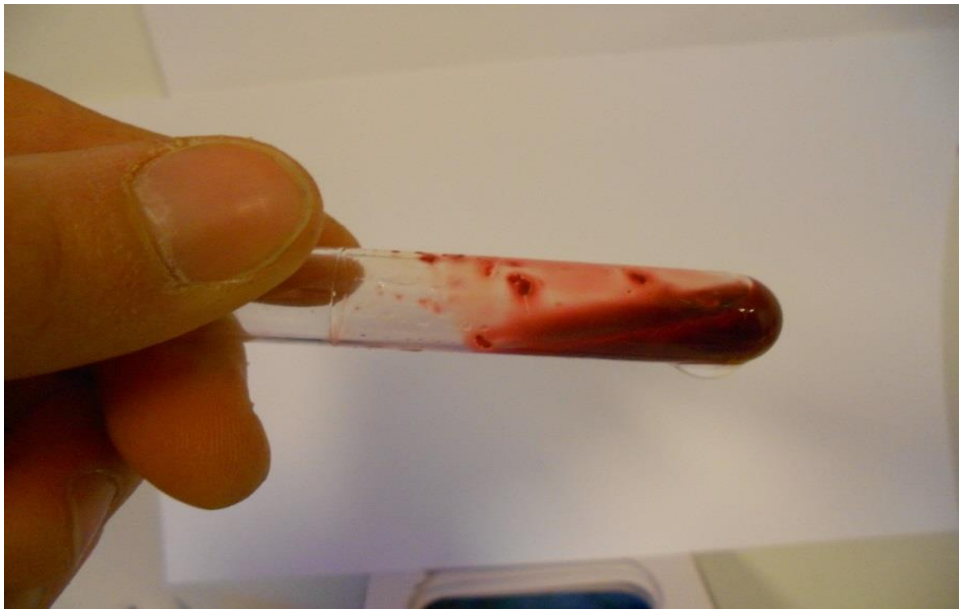


Figure 8: Beginning of blood coagulation. There are visible blood clots hanging on the TT tube (Photo by J. Tichy, 2015).

Sample was considered as fully coagulated when the blood clots formed visible formations and blood created a more or less single mass on the bottom of TT tube holding on the wall even when the tube was being under a horizontal position (see Figure 9).

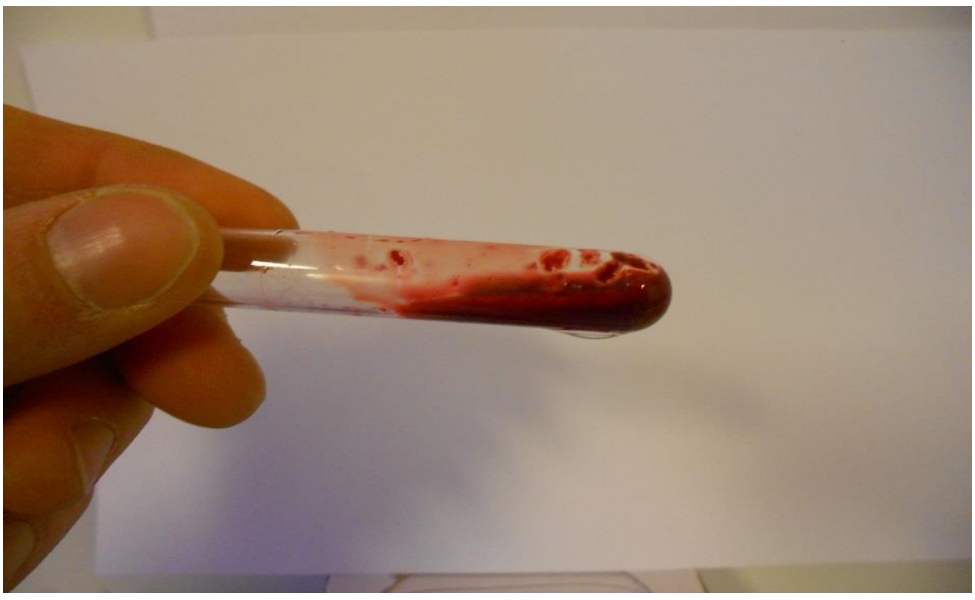


Figure 9: Final coagulation of sample (Photo by J. Tichy, 2015).

In this phase of coagulation process the final time was recorded.

2.6 Post mortem measurements

Fish were then bled by cutting the Bulbus arteriosus and both the *vena cardinalis* and *communis*, and placed in a tank with running sea water for 30 minutes. After bleeding, fish were measured for body length and weight. Liver, gonads and intestine were also weighted. Hepatosomatic index, gonadosomatic index and condition factor were calculated (see below). Gutted fish was placed in the box filled with ice and ready for following fillet redness measurements (see chapter 2.8). The fish were placed in polystyrene boxes with their body cavity facing downwards during ice-storage (ice put in the box and boxes placed in a refrigerator) in order to avoid gravity effect on the blood content in right or left fillet.

2.7 Calculation formulas

The hepatosomatic index (HIS) is defined as ratio of liver weight to body weight.

$$\boldsymbol{HSI = \frac{Liver\ weight\ (g)}{Fish\ weight\ (g)} x\ 100}$$

The gonadosomatic index (GSI) is the ratio of gonad weight to body weight..

$$\boldsymbol{GSI = \frac{Gonad\ weight\ (g)}{Fish\ weight\ (g)} x\ 100}$$

The condition factor (K) is calculated from relation between weight and length of fish.

$$\boldsymbol{K = \frac{Fish\ weight\ (g)}{Fish\ length\ (cm)^3}}$$

2.8 Evaluation of fillet redness

For the purpose of fillet quality assessment by a sensory panel (see below) fish were processed 72 hours post-mortem. During evaluation was skin on but the black abdominal lining was removed. Fillet was then placed on white surface and three parts were evaluated: the belly, the loin and the caudal part of the fillet. Sensory panel consisted of three trained persons who rated each fillet on a scale of 0 to 3. For white fillet it was assigned a score of 0 and for red was given a score of 3. On the same principle was evaluated also residual blood in the flesh with 3 being blood filled and 0 meaning no residual blood. Based on the principle described above, the overall lowest score represented filet of highest quality. An example of fillet redness as performed by sensory evaluation is presented in Figure 10.

Figure 10: Sensory evaluation of fillet redness. Fillet 1 was given score 3 and fillet 2 was given score 0 (Photo by R. A. Svalheim, 2015).

2.9 Statistics

The data were analyzed with R, version 3.0.2 (R core team, 2013), utilizing the supplementary R packages xlsx (Dragulescu, 2013), lsmeans (Lenth, 2013) and nparcomp (Konietschke, 2012). All data response variables were evaluated for normality and variance homogeneity using quantile and density plots and Shapiro Wilkins normality test. In response variables where uniform variance and normality could be assumed (before or after log-transformation), ANOVA with type III sum of squares (F-test) was used to determine if packing and/or recovery time affected the results. Depending on the response variable, the cofactors sex, body length, body weight, condition factor, hepatosomatic index, gonadosomatic index and water temperature were included in the tested models. The cofactor effects were always small if present and are not reported in the thesis. Sum of squares corrected means (LSmeans) were then constructed for each packing and recovery time combination as well as swam and unswam control (corrected for effect of cofactor). Post-hoc t-tests with Tukey adjustment for multiple comparisons were then performed between the LSmeans.

Flesh redness (belly, loin and caudal) and vessel blood score are categorical variables and were analyzed for effect of packing and recovery time using the nparcomp package (Konietschke, 2012) in R.

All data are presented as LSmeans ± their standard errors (se). Results were considered statistically significant when P-values < 0.05.

3. RESULTS

Results of the thesis are divided into three parts: – blood coagulation, regression between blood coagulation and fillet redness, fillet redness and effect of different treatments on fillet redness.

3.1 Blood coagulation time

Based on results of F-test was determined that packing, recovery and packing with recovery combined affect blood coagulation time significantly. Out of the potential cofactors sex, body length, body weight, K, HSI, GSI and water temperature, only body length and sex significantly affected blood coagulation time. These were included in the model as cofactors. In the Figure 11 are results of blood coagulation assessment presented.

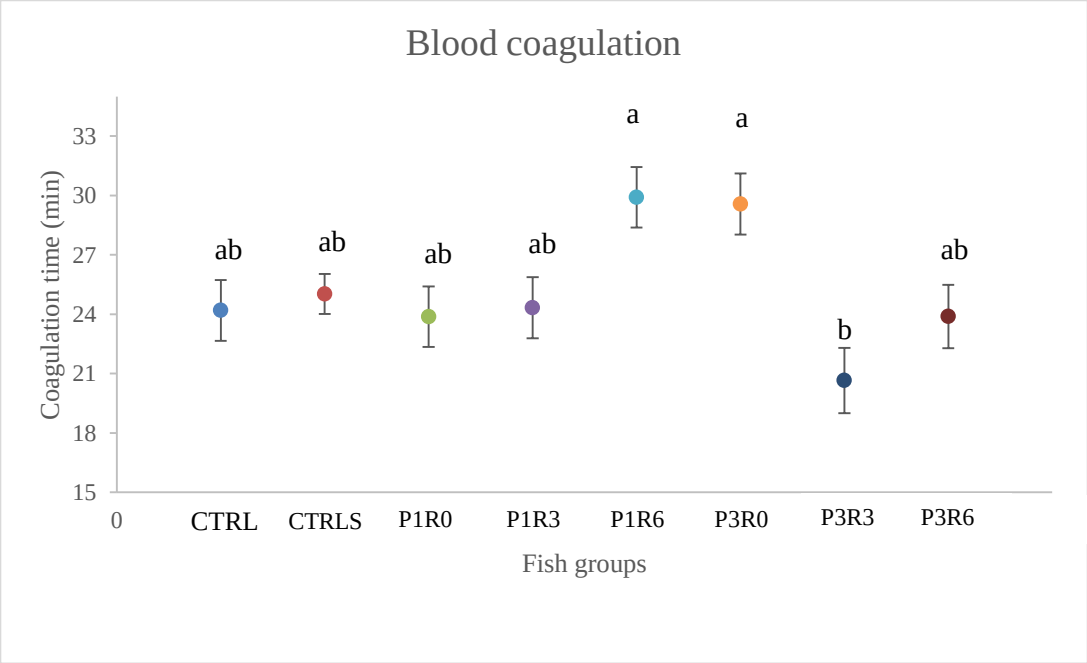


Figure 11: Average (LSmean ± SE) blood coagulation time in Atlantic cod in different treatment groups. Different letters (a, b) represent a significant difference (P < 0.05) between treatment groups. Exhaustive swimming/non swimming, followed by retention in an experimental cod end for 1 or 3 hours and subsequent recovery in cages for 0, 3 or 6 hours divided tested fish into following groups: CTRL – control group; CTRLS – control swimming group; P1R0 – swimming, 1 hour packing, no recovery, P1R3 – swimming, 1 hour packing, 3 hours recovery; P1R6 – swimming, 1 hour packing, 6 hours recovery; P3R0 – swimming, 3 hours packing, no recovery; P3R3 – swimming, 3 hours packing, 3 hours recovery; P3R6 – swimming, 3 hours packing, 6 hours recovery.

The coagulation time was 24-25 min for cod in the CTRL, CTRLS, P1R0, P1R3 and P3R6 groups and these groups were not different from each other or any of the remaining groups. The only meaningful difference in coagulation time occurred between the no recovery (P3R0; 29±1.5 min) and 3 hours of recovery (P3R3; 20.6±1.6 min) groups after three hours of

packing in the cod end. Group P1R6 had a coagulation time of 29.9±1.5 min and was different from group P3R3. This difference is not considered meaningful since neither packing nor recovery time are identical, thus effect of different treatment cannot be compared. There is no difference between group P1R6 and any other group it should be compared to (CTRL, CTRLS, P1R0, P1R3, P3R6).

3.2 Fillet redness and effect of different treatment on fillet redness

Results of fillet redness are presented in 4 parts in order to follow assessment of sensory panel.

3.2.1 Belly redness

The fillet redness in the belly part with scores of 0.1-0.4 for cod in the CTRL and CTRLS groups were not different from each other. Both control groups were not different from groups with 6 hours recovery time and 1 hour packing (P1R6; 0.995±0.15) and 3 hours packing (P3R6; 0.781±0.15). The swimming control group was not different from 3 hours packing with 3 hours recovery group (P3R3; 1.19±0.15). Both control groups were different from groups P1R0, P1R3 and P3R0. The group with 3 hours recovery and 6 hours packing P3R6 was different from group with 3 hours packing and no recovery (P3R0; 1.26±0.13).

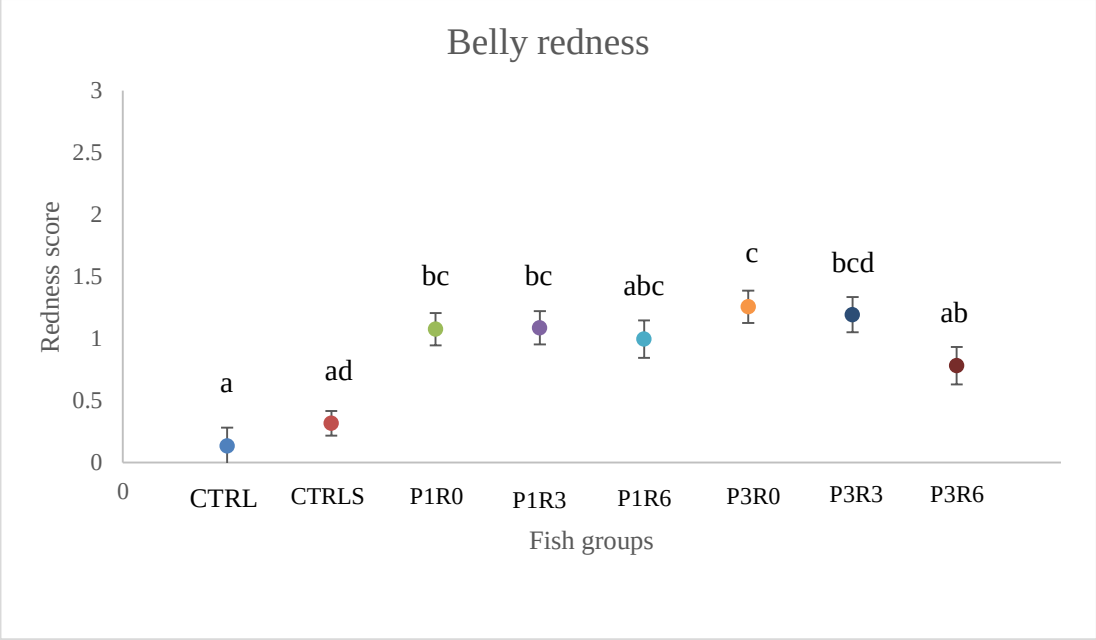


Figure 12: Average (LSmean ± SE) blood coagulation time in Atlantic cod in different treatment groups. Different letters (a, b, c, d) represent a significant difference (P < 0.05) between treatment groups. Exhaustive swimming/non swimming, followed by retention in an experimental cod end for 1 or 3 hours and subsequent recovery in cages for 0, 3 or 6 hours divided tested fish into following groups: CTRL – control group; CTRLS – control swimming group; P1R0 – swimming, 1 hour packing, no recovery, P1R3 – swimming, 1 hour packing, 3 hours recovery; P1R6 – swimming, 1 hour packing, 6 hours recovery; P3R0 – swimming, 3 hours packing, no recovery; P3R3 – swimming, 3 hours packing, 3 hours recovery; P3R6 – swimming, 3 hours packing, 6 hours recovery.

3.2.2 Loin redness

The redness score in the loin part was 0.1-0.12 in both control groups and those groups were not different from each other. Both control groups were not different from groups with 6 hours recovery period and 1 hour packing (P1R6; 0.476±0.12) and (P3R6; 0.368±0.13). Control group CTRL was not different from group with 1 hour packing and no recovery (P1R0; 0.476±0.12). Both control groups were different from groups P1R3, P3R0 and P3R3.

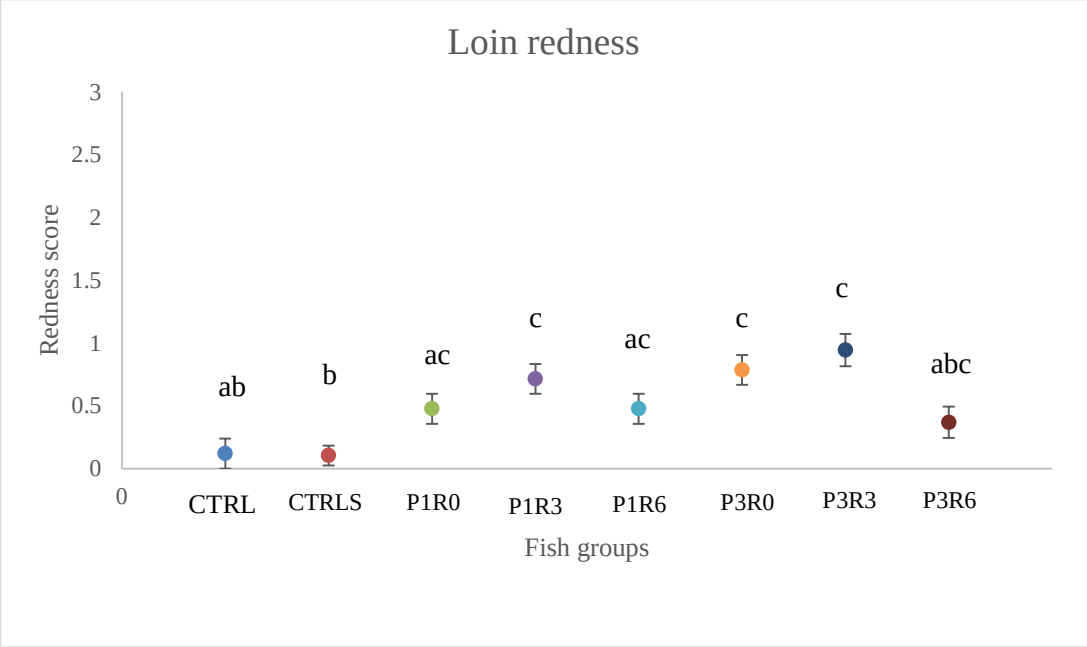


Figure 13: Average (LSmean ± SE) blood coagulation time in Atlantic cod in different treatment groups. Different letters (a, b, c) represent a significant difference (P < 0.05) between treatment groups. Exhaustive swimming/non swimming, followed by retention in an experimental cod end for 1 or 3 hours and subsequent recovery in cages for 0, 3 or 6 hours divided tested fish into following groups: CTRL – control group; CTRLS – control swimming group; P1R0 – swimming, 1 hour packing, no recovery, P1R3 – swimming, 1 hour packing, 3 hours recovery; P1R6 – swimming, 1 hour packing, 6 hours recovery; P3R0 – swimming, 3 hours packing, no recovery; P3R3 – swimming, 3 hours packing, 3 hours recovery; P3R6 – swimming, 3 hours packing, 6 hours recovery.

3.2.3 Caudal redness

The redness score in the caudal part was 0.35-0.41 for control groups. Control groups were not different from each other. Both control groups were not different from 1 hour packing with 6 hours recovery (P1R6; 0.762±0.12) and 3 hours packing with 6 hours recovery (P3R6; 0.632±0.13). Control group CTRL was not different from group with 1 hour packing and 3 hours recovery (P1R3; 0.929±0.13). Both control groups were different from groups P1R0, P3R0 and P3R3. The group with 3 hours packing and 6 hours recovery was different from other two groups with 3 hours packing and no recovery (P3R0; 1.190±0.13) and 3 hours recovery (P3R3; 1.128±0.14).

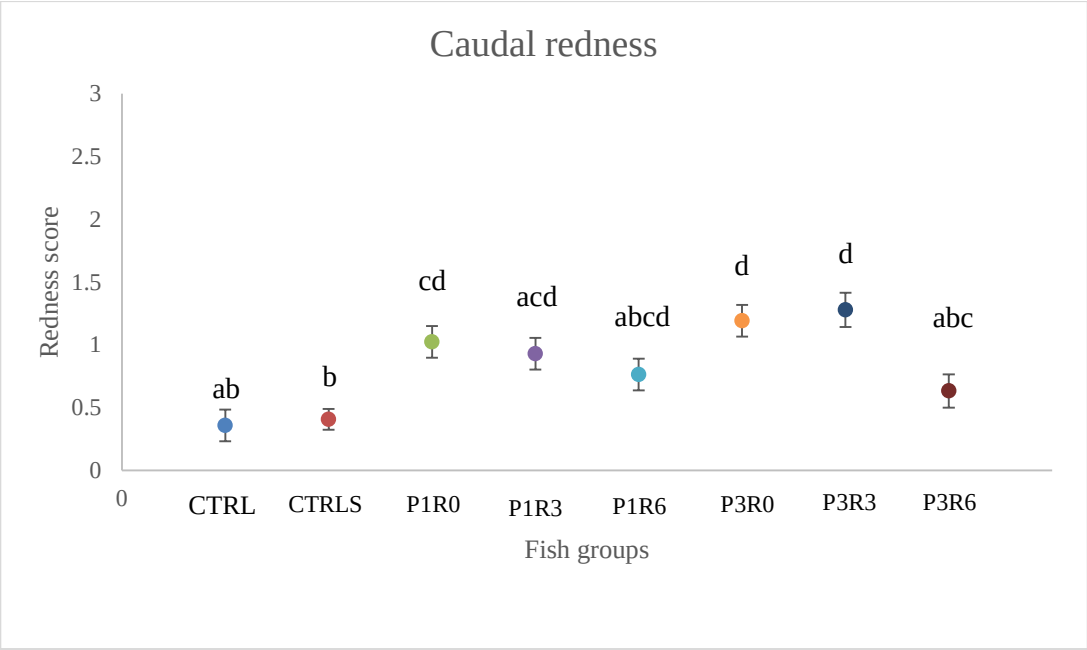


Figure 14: Average (LSmean ± SE) blood coagulation time in Atlantic cod in different treatment groups. Different letters (a, b, c, d) represent a significant difference (P < 0.05) between treatment groups. Exhaustive swimming/non swimming, followed by retention in an experimental cod end for 1 or 3 hours and subsequent recovery in cages for 0, 3 or 6 hours divided tested fish into following groups: CTRL – control group; CTRLS – control swimming group; P1R0 – swimming, 1 hour packing, no recovery, P1R3 – swimming, 1 hour packing, 3 hours recovery; P1R6 – swimming, 1 hour packing, 6 hours recovery; P3R0 – swimming, 3 hours packing, no recovery; P3R3 – swimming, 3 hours packing, 3 hours recovery; P3R6 – swimming, 3 hours packing, 6 hours recovery.

3.2.4 Residual blood in vessels

The score for the residual blood in vessels was not different between control groups CTRL and CTRLS. Control group CTRL was different from groups with no recovery and with 1 hour packing (P1R0; 1.13±0.14) and 3 hours packing (P3R0; 0.970±0.14). Otherwise was control group not different with any other of the treatment groups P1R3, P1R6, P3R3 and P3R6. Control swimming group CTRLS was not different from any of the groups.

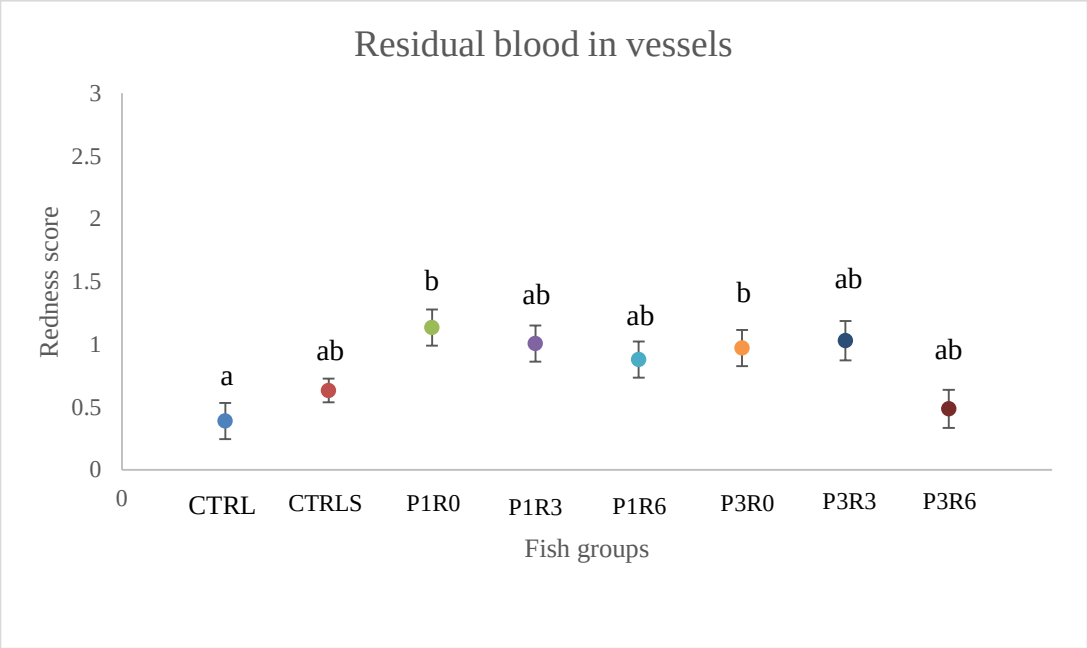


Figure 15: Average (LSmean ± SE) blood coagulation time in Atlantic cod in different treatment groups. Different letters (a, b, c, d) represent a significant difference (P < 0.05) between treatment groups. Exhaustive swimming/non swimming, followed by retention in an experimental cod end for 1 or 3 hours and subsequent recovery in cages for 0, 3 or 6 hours divided tested fish into following groups: CTRL – control group; CTRLS – control swimming group; P1R0 – swimming, 1 hour packing, no recovery, P1R3 – swimming, 1 hour packing, 3 hours recovery; P1R6 – swimming, 1 hour packing, 6 hours recovery; P3R0 – swimming, 3 hours packing, no recovery; P3R3 – swimming, 3 hours packing, 3 hours recovery; P3R6 – swimming, 3 hours packing, 6 hours recovery.

3.3 Regression between blood coagulation and fillet redness

In the blood coagulation and fillet redness regression assessment was found out that there is no significant correlation between blood coagulation time and redness of different parts of fillet. Close to the confidence level of 0.95 was only found the caudal part with P = 0.054. Also testing of residual blood in vessels showed no significant correlation with blood coagulation time. The results are presented in Table 1.

Table 1: Regression between blood coagulation and fillet redness/blood in vessels. All the groups are assessed together as there was no effect of coagulation time on fillet redness/blood in vessels of separate groups.

Fillet part	P-value
Belly	0.9211
Loin	0.1843
Caudal	0.05369
Residual blood in vessels	0.5205

4. DISCUSSION

Blood coagulation time was not significantly different between treatment groups (P1R0, P1R3, P1R6, P3R0, P3R3, P3R6) and control groups (CTRL, CTRLS). It was expected that increased level of stress due to exhaustive exercise followed by packing in experimental cod-end will reduce blood coagulation time. Another study on effects of acute stress on blood clotting of rainbow trout found that blood from fish exposed to four different levels of stress differed consistently with respect to clotting times (Marko and Christopher, 1997). In their study, blood from stressed fish always clotted, and did so more rapidly after higher levels of stress. On the contrary, blood from resting fish either clotted very slowly or even failed to clot. Different fish species could be the reason for the mismatch between the findings in the two studies, but more likely the different stress factors and treatments are the main reasons for the different results.

There was a significant difference between two groups within three hours packing time; no period of recovery (P3R0; 29±1.5 min) and three hours recovery (P3R3; 20.6±1.6 min). Similar packing time may indicate that the difference in coagulation time is due to different recovery period and thus recovery causes decrease in coagulation time. The group with same packing time and six hours recovery (P3R6; 23.9±1.6 min) resulted in seemingly longer coagulation time although not significantly different. Coagulation time results of group P3R0 were affected by six individuals sampled on the same day whose coagulation times were considerably longer than those of fish from the same group sampled on different days. The groups with one hour packing time followed by three hours recovery (P1R3; 24.3±1.5 min) and six hours recovery (P1R6; 29.9±1.5) showed the tendency of increased coagulation time with increasing recovery period even though was not considered statistically significant by test.

The mechanism of blood in fish is a complicated system that can be influenced by many factors such as vitamin K-deficiency, liver damage, exposure to chemical products, vascular abnormalities, thrombocyte disorders, coagulation factor deficiencies and stress (Tavares-Dias and Oliveira, 2009). Any of those factors could have affected blood coagulation time during the present study. Such factors are not investigated in this thesis and thus are not going to be discussed further.

The fillet redness assessment showed that control (CTRL) and control swimming (CTRLS) groups had the lowest score in all fillet parts assessed and thus the best quality. Treatment groups not different from control group were then considered of high quality.

The general pattern for the redness development was similar in all parts of fillet. The redness in both packing groups without recovery (P1R0, P3R0) was significantly higher than both control groups in all parts of fillet with one exception. The redness development of the rest of the groups was rather uniformed. The redness of groups with 3 hours recovery increased in most of the fillet parts compared to control groups with two exceptions. The redness in 6 hours recovery groups then returned to both control groups levels with one exception. Nevertheless, the redness in 6 hours recovery groups was in most cases not different from other recovery groups.

The redness in the belly part of fillet was similar to the general pattern described above. The only exception was the group with 3 hours packing and 3 hours recovery (P3R3) which was not different from the control swimming group (CTRLS). Result of the redness test between those groups was on the borderline of statistical difference for the trait described.

Described pattern for fillet redness applies also in loin part of the fillet. The only exception was the group with 1 hour packing and no recovery (P1R0). This was surprisingly not different from the control group (CTRL). Assuming packing to be a stress factor, the fillet redness of the group with only packing and no recovery was expected to be different from the control group.

The caudal redness follows general pattern with the exception of the group with 1 hour packing and 3 hours recovery (P1R3) which was not different from control group (borderline probability value). In the 3 hours packing groups, the caudal redness of the group with 6 hours recovery (P3R6) was similar to the control groups but significantly different from other recovery groups (P3R0, P3R3) indicating a positive effect of recovery on this trait.

The results of quality assessment of residual blood in vessels showed the same trend with no difference between all of the treatment groups and the control swimming group (CTRLS). The only difference was observed between control group and groups with no recovery (P1R0, P3R0).

The fillet redness assessment presented negative effect of packing as the fillet redness in the groups with only packing and no recovery was always significantly different from control swimming group (CTRLS). Packing is likely the treatment responsible for the discoloration of fillet in the studied fish. On the contrary, with one exception in the loin part of the fillet (P1R6), all the groups with 6 hours recovery had the same level of redness as control swimming group (CTRLS) indicating positive effect of 6 hours recovery period. It is probable, that fish can redistribute discoloration from muscle during recovery period.

In the fillet redness assessment, the packing time and the recovery period were expected to be the most important factors. The packing in cod-end may cause discoloration of flesh, while recovery may allow fish to redistribute discoloration from fillet. As reported previously by Olsen *et al.* (2013), live storage and slaughtering procedures have an impact on discoloration of fillet and thus quality in Atlantic cod. It was also reported that fillets from fish slaughtered immediately after hauling were considerably lighter compared to cod slaughtered after three hours recovery. This may be due to ability of fish to redistribute the blood flow, glucose and lactate between organs and muscles under physical stress in order to prioritize recovery over digestion (Wood, 1991; Farrell *et al.,* 2001; Soldatov, 2006). After six hours recovery period was most of discoloration removed from the muscle probably due to the reduced need of blood supply to the muscle as fish is prioritizing digestion over recovery with reduced stress (Olsen *et al.,* 2013).

In the blood coagulation time and fillet redness assessment no regression was found in any parts of fillet. This finding indicates that blood coagulation time is not affecting significantly fillet redness and thus quality of the product. The presence of blood in anaerobic white muscle seems to be factor with higher importance on fillet's discoloration then coagulation time itself.

The findings of thesis confirmed results from previous research (Olsen *et al.,* 2013) that six hours live storage contributes to redistribution of blood from muscle back to the other organs and reducing discoloration of the muscle. After exposing fish to exhaustive exercise during fishing activities and thus increasing blood volume in muscle, providing fish with prolonged period for recovery may contribute to improved fillet quality as the presence of blood in musculature may be the main factor affecting fillet colour.

As reported by Stien *et al.* (2005), both stress and storage temperature affect the final quality of pre-rigor filleted cod. The change of colour is more observable with increasing levels of stress and temperature. These authors based his assessment on pre-rigor filleted fish and claimed that pre-rigor filleted fillets of unstressed cod stored at 4°C kept their original colour compared to the fillets of fish with increased levels of stress and temperature.

Poli *et al.* (2005) listed general trends for pre-slaughter and slaughter practices as they have important effect on the flesh quality in fish. Emphasis is given on minimizing stress as factor affecting quality of product. Less stressful practices are described by these authors as methods for inducing less intense physical exercise and thus minimize the stress response.

Optimal capturing and handling practice in order to obtain high quality products is killing and bleeding fish as soon as possible after capture. With the volumes currently handled by

Norwegian fleet and the technology used on-board vessels, the adaptation of such a practice might be far from reality, especially so with large hauls. Therefore keeping fish alive after taking it on-board the vessel is crucial in order to achieve sufficient bleeding. Keeping fish alive after exhaustive exercise and packing in cod-end may help fish to recover from fishing and handling activities, resulting in sufficient bleeding and thus limiting the redness of flesh resulting in higher quality products.

Nevertheless, the results of the present study are limited by location, period, fish size and species, and further research considering physiological factors affecting fillet quality is needed. More research on fish tolerance to processes connected to capture, transport and live storage of wild captured Atlantic cod is also needed.

5. CONCLUSION

Three assessments were done in this project in order to answer all the scientific questions defined in the aim of thesis. The blood coagulation time assessment highligthted differences between some of the fish groups divided by swimming, packing and recovery though none of the fish groups was different from either control or control swimming groups. The only meaningful difference was between group P3R0 and P3R3. The groups have the same factor packing, but different recovery period indicating difference in blood coagulation time caused by different recovery period. The results of blood coagulation present then recovery time as factor affecting blood coagulation time the most.

The assessment of fillet redness revealed differences in fillet redness between different treatment groups. In general increased fillet redness in groups with packing followed by 0 or 3 hours recovery compared to control groups. In the groups with 6 hours recovery redness returned to controls but was in most cases not different from other recovery groups. The relationship between different treatments and fillet redness based on the redness assessment is than increased redness as consequence of packing in cod-end and decreased redness, back to control levels, when fish are recovering for 6 hours.

In order to find the optimal practice for capturing and handling wild Atlantic cod according to blood coagulation assessment, the regression test showed no correlation between blood coagulation time and the fillet redness in any of the fillet part, nor residual blood in vessels. The blood coagulation time is then not relevant for fillet redness and optimal practice may not consider the blood coagulation time as significant factor. Nevertheless, the results of the fillet

redness assessment indicate that the optimal practice for capturing and handling Atlantic cod is to provide fish with sufficient recovery time of 6 hours instead of minimizing packing time in cod end.

References

Aird, W.C. 2003. Hemostasis and irreducible complexity. Journal of Thrombosis and Haemostasis, 1, 227-230.

Blanchard, J. L., Heffernan, O. A., and Fox, C. J. 2005. North Sea (ICES Divisions IVaec and VIId). In Spawning and Life History Information for North Atlantic Cod Stocks. ICES Cooperative Research Report, 274, 154 pp.

Botta, J.R., Kennedy, K.M., and Squires, B.E. 1987. Effect of method of catching and time of season on the composition of Atlantic cod (*Gadus morhua)*. Journal of Food Science. 52, 922–924.

Cassilas, E, and Smith LS. 1977. Effect of stress on blood coagulation and haematology in rainbow trout (*Salmo gairdneri).* Journal of Fish Biology, 10, 481-491.

Digre, H., Hansen, U.J., and Erikson, U., 2010. Effect of trawling with traditional and 'T90' trawl codends on fish size and on different quality parameters of cod *(Gadus morhua)* and haddock (*Melanogrammus aeglefinus)*. Fisheries Science. 76, 549–559.

Doolittle, R.F., and Surgenor, D.M. 1962. Blood coagulation in fish. American Journal of Physiology, 203, 964-970.

Doolittle, R.F. 1993. The evolution of vertebrate blood coagulation: A case of yin and yang. Thrombosis and Haemostasis, 70, 24-28 pp.

Dragulescu A.A. 2013. xlsx: Read, write, format Excel 2007 and Excel 97/2000/XP/2003 files. R package version 0.5.5. http://CRAN.R-project.org/package=xlsx

Ellertsen, B., Fossum, P., Solemdal, P., and Sundby, S. 1989. Relations between temperature and survival of eggs and first feeding larvae of Northeast Arctic cod *(Gadus morhua L.*). Rapports et Procès-verbaux des Réunions du Conseil International pour l'Exploration de la Mer, 191, 209–219 pp.

Esaiassen, M., Nilsen, H., Joensen, S., Skjerdal, T., Carlehog, M., Eilertsen, G., Gundersen, B., and Elvevoll, E. 2004. Effects of catching methods on quality changes during storage of cod *(Gadus morhua)*. LWT – Food Science and Technology. 37, 643–648.

Farrell, A.P., Thorarensen, H., Axelsson, M., Crocker, C.E., Gamperl, A.K., and Cech, J.J. 2001. Gut blood flow in fish during exercise and severe hypercapnia. Comparative biochemistry and physiology A-molecular and integrative physiology 128, 551–563.

Henriksen E; Lønnsom foredling av hvitfisk i Norge – hva skal til ? – Oppsummering av foredragsserie holdt for LO, supplert med relevant litteraturen, Rapport 44/2013, Utgitt oktober 2013; NOFIMA

Hoar, W.S., and Randall, D.J., Fish Physiology, Vol. 4, , Academic Press, Orlando (FL), 133-168 .

ICES. 2005. Spawning and life history information for North Atlantic cod stocks. ICES Cooperative Research Report, No. 274, 152 pp.

ICES. 2012. Report of the Arctic Fisheries Working Group 2012 (AFWG). 20-26 April 2012, ICES Headquarters, Copenhagen. ICES CM 2012/ACOM., 05, 670 pp.

ICES. 2013. The future for Cod recovery in the North Sea. (NSRAC). July 2013, Demersal working group meeting.

Jacobsen, C., Luten, J.B., Bekaert, K., Sæbø, A., and Oehlenschlager, J. 2006, Seafood Research from Fish to Dish – Quality, Safety and Processing of Wild and Farmed Fish. Wageningen Academic Publishers, Wageningen, Netherland, 265–274 pp.

Konietschke F 2012 nparcomp: Perform multiple comparisons and compute simultaneous confidence intervals for the nonparametric relative contrast effects. R package version 2.0. http://CRAN.R-project.org/package=nparcomp

Kålås, J.A., Viken, Å. and Bakken, T. 2006. Norwegian Red List 2006. Artsdatabanken, Norway, 416 pp.

Lenth RV, 2013 lsmeans: Least-squares means. R package version 1.10-4. http://CRAN.R-project.org/package=lsmeans

Margeirsson, S., Nielsen, A.A., Jonsson, G.R., and Arason, S. 2006. Effect of catch location, season and quality defects on value of Icelandic cod (*Gadus morhua*) products. In: Jacobsen, C., Luten, J.B., Bekaert, K., Sæbø, A., and Oehlenschlager, J. 2006. Seafood Research from Fish to Dish – Quality, Safety and Processing of Wild and Farmed Fish. Wageningen Academic Publishers, Wageningen, Netherland, 265–274 pp.

NOFIMA, 2011. Landingsmønsteret I torskefiskeriene. Et tilsvar på Fiskeri-og kystedepartments spørsmål. Tromsø, Oktober 2011.

Olsen, S.H., Sorensen, N.K., Stormo, S.K., and Elvevoll, E.O. 2006. Effect of slaughter methods on blood spotting and residual blood in fillets of Atlantic salmon (*Salmo salar*). Aquaculture 258, 462–469.

Olsen S.H., Tobiassen T., Akse L., Evensen T, H., and Midling., K, Ø, 2013; Capture induced stress and live storage of Atlantic cod *(Gadus morhua)* caught by trawl: Consequences for the flesh quality. Fisheries Research 147, 446-453.

Poli, B.M., Parisi, G., Scappini, F., and Zampacavallo, G. 2005. Fish welfare and quality as affected by pre-slaughter and slaughter management. Aquaculture International 13, 29-49.

Randall, D.J. 1970. The circulatory system. In: Fish Physiology, Vol. 4, W.S. Hoar, D.J. Randall (eds.), Academic Press, Orlando (FL), 133-168.

Rotabakk, B.T., Skipnes, D., Akse, L., and Birkeland, S. 2011. Quality assessment of Atlantic cod *(Gadus morhua)* caught by longlining and trawling at the same time and location. Fisheries Research. 112, 44–51.

Rowley, A.F., Hill, D.J., Ray, C.E. and Munro, R. 1997. Haemostasis in fish. An evolutionary perspective. Thrombosis and Haemostasis, 77, 227-233.

R core team (2013) R: A language and environment for statistical computing, version 3.0.2, R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Ruis, M. A.W. and Christopher J. Bayne. 1997. Effects of acute stress on blood clotting and yeast killing phagocytes of rainbow trout, Journal of aquatic animal health, 9:3, 190-195.

Smit, G.L., and Schoonbee, H.J. 1988. Blood coagulation factors in fresh water fish *Oreochromis mossambicus.* Journal of Fish Biology, *32*, 673-677.

Soldatov, A.A., 2006. Organ blood flow and vessels of microcirculatory bed in fish. Journal of Evolutionary Biochemistry and Physiology. 42, 243–252.

Stien, L.H., Hirmas, E., Bjørnevik, M., Karlsen, Ø., Nortvedt, R., Rørå, A.M.B., Sunde, J., and Kiessling, A. 2005. The effects of stress and storage temperature on the colour and texture of pre-rigor filleted farmed cod (*Gadus morhua* L.). Aquaculture research, 36, 1197-1206.

Tavares-Dias, M., Affonso, E.G., Oliveira, S.O., Marcon, J.L., and Egami, M.I. 2008. Comparative study on haematological parameters of farmed matrinxa, *Brycon amazonicus* Spix and Agassiz, 1829 (Characidae: Bryconinae) with others Bryconinae species. Acta Amazonica, 38, 799-806.

Tavares-Dias, M., and Oliveira, S.R. 2009. A review of the blood coagulation system of fish. Brazilian Journal of Biosciences. 2, 205-224.

Wang, Y.Z., Patterson, J.E., Gray, J.E., Yu, C., Cottrell, B.A., Shimizu, A., Graham, D., Riley, M and Doolitle, R.F. 1989. Complete sequence of the lamprey fibrinogen α chain. Biochemistry, 28, 9801-9806.

Wedemeyer, G.A., Meyer, F.P., and Smith, L. 1976. Environmental stress and fish disease. Neptune City: T.F.H. Publications. 192 pp.

Wood, C.M. 1991. Acid–base and ion balance, metabolism, and their interactions, after exhaustive exercise in fish. J. Exp. Biol. 160, 285–308.

Internet sources:

http://www.fao.org/fishery/species/2218/en

http://www.fisheries.no/ecosystems-and-stocks/marine_stocks/fish_stocks/cod/#.VRvjW_mUffd

International innovation (2013), CRISP – A novel approach to sustainable fish capture: http://www.scantrol.com/wp-content/uploads/2013/03/2013-CRISP-brosjyre-Improving-commercial-trawling.pdf

APPENDIX 1

Project CRISP

CRISP is a constructive research and development mechanism which is aiming to develop the advanced technical solutions needed to exploit marine resources in a sustainable manner in the future. The Centre aims to develop technical solutions that bring the highest fish quality to consumers using smart technologies consisting of proper instruments and modern fishing gears. Since this centre started its research activities on April 1st 2011, the consortium has involved four research partners (Institute of Marine Research, Nofima AS, University of Bergen and University of Tromsø), four industry partners (Kongsberg Maritime AS, Simrad, Scantrol AS, Egersund Group and Nergård Havfiske AS) and two sponsors (Norges Råfisklag and Norges Sildesalgslag). The main outcome of CRISP is expected to be sustainable trawl and purse-sein technology followed by optimal capture and handling practices in order to optimize quality and thus value of captured fish. New practices and regulations in management system of fisheries will be needed as new technology and processes open possibilities of managing fisheries in a more sustainable way. For example with obtaining advanced technology for reducing by-catch could be this kind of technology forced as compulsory for the whole fleet by changes in laws and regulations.

The vision of CRISP is to enhance the position of Norwegian fisheries-related companies as leading suppliers of equipment and seafood products through the development of sustainable trawl and purse seine technology. The research activity of the Centre is organised in six scientific work packages, which represent objectives:

I. To develop and implement instrumentation to identify species and sizes prior to the catching process,
II. To develop and implement instrumentation for commercial fishing to monitor fish behaviour and gear performance during fishing operation,
III. To develop methods and instrumentation to actively release unwanted by-catch unharmed during trawl and purse seine fishing,
IV. To develop new trawl designs that minimize the environmental impact on bottom habitats and reduce air pollution,
V. To develop capture and handling practices to optimize quality and thus value of captured fish,

VI. To analyse and document the economic benefits for the fishing industry resulting from implementation of the new technologies developed by the project.

Fishing is a complex activity involving everything from finding the fish to bringing quality products to the consumer. In this long and complicated production line, economy for the operators and acceptable environmental impact while fishing are key factors (International innovation, 2013). Most of the Norwegian fleet is relatively advanced in terms of the technology available to the crew and their working conditions. However, in areas such as fuel efficiency, seabed impact and the number of unwanted fish being caught, there is still much work to do. The multidisciplinary approach taken by CRISP reflects the complexity of fishing activities. It is hoped that better technologies and practices can be developed and introduced to the global fishing industries. Development of such practices and technologies would have a massive impact on the whole industry and on its governance.

APPENDIX 2

Experimental swimming tunnel system

Drawing of the experimental system used in Kårvika showing the detail of the swimming tank where fish were challenged (1) and the whole propeller unit (2).

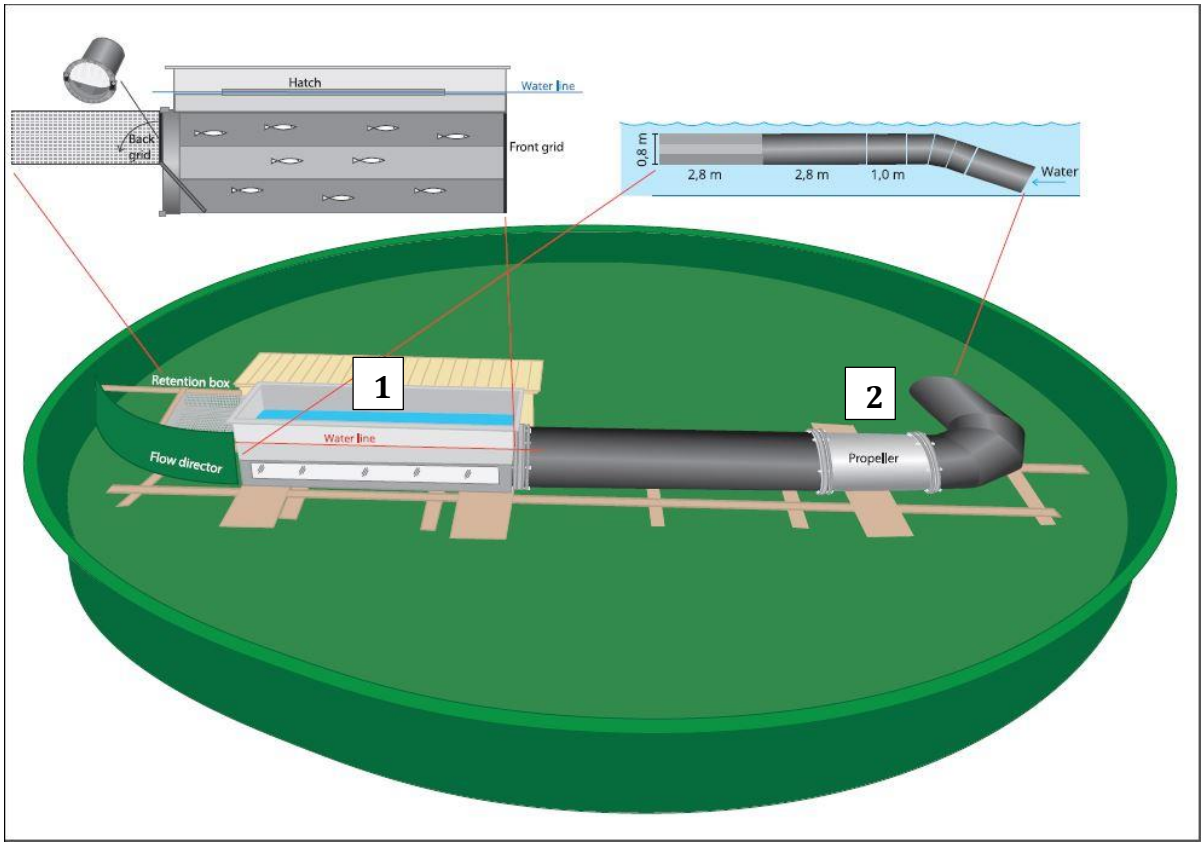


Figure 1: Drawing of experimental swimming tunnel system. Kårvika, HiT (Frøydis Strand, AMB)