



Effects of Onboard Processing on Groundfish Quality

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**Faculty of Industrial Engineering, Mechanical
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Dissertation submitted in partial fulfillment of a
Philosophiae Doctor degree in Industrial Engineering

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Abstract

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The scope of this study is processing onboard fresh fish trawlers, focusing on bleeding and cooling processes and the effects on product quality and shelf-life. In recent years there has been a shift from less valuable frozen products to higher value fresh fish export of fillets and loins from Iceland. This development in the fresh fish fillets markets was made possible by technological improvements in the value chain in the last decade. However, the primary onboard processing has been a weak link in terms of research studies in the value chain of groundfish. This research gap in the value chain and the renewal of the Icelandic fleet led to the current study, in which the objective is to evaluate primary processing onboard fresh fish trawlers focusing on the bleeding and cooling processes. The study evaluation is aimed at the effects of new onboard processes, to provide design criteria for further technical innovation and provide measurement results that producers can use as references regarding shelf-life and quality indicators. This is done with onboard research on time and temperature control, variable bleeding conditions and methods, different cooling and superchilling methods, and on superchilled storage with focus on the effects on shelf-life and product quality.

The results of the study show that the waiting time in the reception hold is the main deciding factor regarding fillet colour. As for the method of bleeding and gutting fish, the two-step process of bleeding and gutting showed only marginally better results in terms of fillet colour, compared to direct gutting. Results of bleeding media conditions show the effects of time, temperature, water flow and recirculation on fillet quality, and indicate that pump flow recirculation has more effect on bleeding efficiency than water replacement. Comparison of newly developed onboard primary processing systems to older ones confirms improvements in terms of uniformity of both bleeding and cooling. The study results show that it is not advisable to store whole ungutted cod in onboard RSW for more than 24 hours. The onboard superchilling of whole gutted fish reduces some spoilage factors but superchilled storage after fillet processing was found to be more important for extended shelf-life. Simulation of a superchilled value chain from catch to market indicates that the freshness period and shelf-life can be extended by up to four days, compared to traditional ice cooling of whole fish and storage of fillets at 1 °C. The study outcomes can provide technical developers with premise and requirements for further technical solutions needed to deliver fish of the highest possible quality and with a maximum shelf-life to secondary processing.

Útdráttur

Áhrif vinnslu um borð í ferskfisktogurum á gæði bolfisks

Viðfangsefni þessa verkefnis er vinnsla um borð í ferskfisktogurum, með áherslu á að greina mismunandi aðferðafræði við meðhöndlun, blæðingu og kælingu bolfisks við vinnslu um borð og áhrif þessara þátta á gæði og geymsluþol afurða. Undanfarin ár hefur á Íslandi orðið aukning í vinnslu og útflutningi á ferskum bolfiskafurðum, flökum og hnakkastykkjum, á kostnað verðminni frosinna afurða. Rannsóknir innan virðiskeðju bolfisks hafa stutt við þessa þróun síðastliðinn áratug, en tækniþróun vinnslu um borð í skipum hefur setið á hakanum og verið veikur hlekkur samanborið við rannsóknir og þróun landvinnslu og flutningaferla. Þörf fyrir endurnýjun flotans, ásamt nauðsyn þess að þróa nýjar lausnir fyrir vinnslu á millidekkjum ferskfisktogara, var kveikjan að þessu verkefni. Markmið verkefnisins eru að greina og leggja mat á meðhöndlun og vinnslu um borð í ferskfisktogurum, með áherslu á mismunandi aðferðir við blæðingu og kælingu. Stefnt er að því að skilgreina bestu aðferðafræði við meðhöndlun fersks bolfisks gegnum vinnslu um borð og að leggja mat á áhrif nýrra lausna í togurum. Markmið þeirrar greiningar er að geta skilgreint hönnunarforsendur fyrir frekari tækniþróun og lagt fram niðurstöður mælinga sem framleiðendur geti notað sem viðmið um geymsluþol og gæði. Í því felst að rannsaka og greina áhrif mismunandi aðferðafræði við meðhöndlun, blæðingu, kælingu og ofurkælingu fisks við vinnslu um borð á gæði og geymsluþol afurða.

Niðurstöður verkefnisins sýna að biðtími í móttöku er stærsti áhrifaþátturinn á lit ferskra flaka, og einnig að verklag þar sem blóðgun og slæging er framkvæmd í tveimur aðgerðum skilar lítillaga betri lit, samanborið við hefðbundna blóðgun og slægingu í einu handtaki. Niðurstöður blæðingartilrauna sýna hvaða tími og hitastig gefa ljósan flakalit, og einnig áhrif endurnýjunar og hringrásunar sjávar í kerjum. Þær benda til að hringrásun blæðingarvökva hafi meiri áhrif á virka blóðtæmingu fisks en endurnýjun á sjó í kerjum. Mat á nýjum vinnslukerfum sýnir árangur þeirra í samanburði við eldri kerfi, þ.e. meiri einsleitni afurða eftir bæði blæðingu og kælingu. Niðurstöður verkefnisins sýna að það er ekki ráðlegt að geyma heilan óslægðan þorsk lengur en 24 klukkustundir í RSW-sjókælingu um borð. Ofurkæling á heilum slægðum fiski um borð skilaði hægari skemmdarferlum, en ofurkæld geymsla á flökum eftir vinnslu hafði meiri áhrif á geymsluþol en ofurkæling á heilum slægðum fiski. Hermun á ofurkældri virðiskeðju frá veiðum til markaðar gefur til kynna að ferskleiki og geymsluþol bolfisks geti aukist um allt að fjóra daga, samanborið við hefðbundna ískælingu á heilum fiski og geymslu flaka við 1 °C. Niðurstöður verkefnisins geta nýst framleiðendum sem hönnunarforsendur fyrir tæknilausnir sem taka mið af því að hámarka gæði og geymsluþol þess hráefnis sem ferskfisktogarar skila í land til áframhaldandi vinnslu.

Preface

The origin of this study was a research project initiated by HB Grandi (now BRIM), one of the largest fisheries companies in Iceland. In 2013, they involved Matis in research work to define the ideal methodology for saithe (*Pollachius virens*) through the primary onboard processing without being fixed on specific technical solutions but rather to focus on the optimal raw material handling and improvement solutions. The research provided results on physical properties of raw material and the effects of different bleeding technologies on saithe fillet quality. The research output then provided technical developers with premise and requirements for the technical solutions needed to deliver quality raw material. The involvement of the PhD candidate in the project provided an opportunity for the methodology to develop and implement solutions based on in-depth research on raw material properties and handling through primary processing. The project then led to this PhD study which relates to developments in the Icelandic fishing industry, where in recent years there has been a shift from frozen to fresh fish processing, resulting in all the major fisheries companies renewing their fleet. The research field has been on the processing decks as they develop from the old to the new trawlers. This development in the fresh fish markets was made possible by technological developments in the value chain in the last decade. There has been research done on models for decision making, land-based processing, temperature control in transport chains and at the market end of the value chain. However, the primary onboard processing has been a weak link in terms of research studies in the value chain of groundfish. This research gap and need for the fleet renewal therefore led to the current study.

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List of Papers

This thesis is based on the work contained in the following six papers:

Paper I:	Eliasson, S., Arason, S., Margeirsson, B., Bergsson, A.B., Palsson, O.P. (2019). The effects of superchilling on shelf-life and quality indicators of whole Atlantic cod and fillets. <i>LWT – Food Science and Technology</i> , 100 (1), pp.426-434. DOI: 10.1016/j.lwt.2018.10.062.
Paper II:	Eliasson, S., Arason, S., Margeirsson, B., Palsson, O.P. (2020). Onboard Evaluation of Variable Water Flow and Recirculation Effects on Bleeding of Atlantic Cod (<i>Gadus morhua</i>). <i>Foods</i> , 9(11), 1519, pp.1-14. DOI: 10.3390/foods9111519.
Paper III:	Eliasson, S., Ragnarsson, S.O., Arason, S., Margeirsson, B., Palsson, O.P. (2021). Onboard pre-chilling of ungutted and gutted Atlantic cod in different cooling media -Temperature measurements and analytical modelling-. <i>International Journal of Refrigeration</i> , 132, pp.72-81. DOI: 10.1016/j.ijrefrig.2021.08.027.
Paper IV:	Eliasson, S., Arason, S., Margeirsson, B., Bergsson, A.B., Palsson, O.P. (2019). Effects of on-board bleeding methods and superchilling on quality of cod and saithe. In the proceedings of <i>25th IIR International Congress of Refrigeration</i> , ICR 2019, 24-30 th August, pp.3248-3255. International Institute of Refrigeration. DOI: 10.18462/iir.icr.2019.1639.
Paper V:	Eliasson, S., Arason, S., Margeirsson, B., Palsson, O.P. Effects of onboard refrigerated seawater (RSW) storage of whole ungutted cod on quality parameters of fillets. <i>Submitted for publication</i> .
Paper VI:	Eliasson, S., Arason, S., Margeirsson, B., Palsson, O.P. Effects of different bleeding methods and storage time on the colour of cod fillets. <i>Submitted for publication</i> .

Contribution to the study and papers

The PhD candidate is the main author of all the study papers and wrote the papers in collaboration with co-authors. The PhD candidate carried out the onboard experiments, investigation, formal analysis and data curation, validation, visualisation and the original draft preparation for the papers. The main exceptions to this are; a) the work of Sigurdur Orn Ragnarsson, in Paper III, who built the thermal model to simulate the cooling as a part of his MSc thesis work and provided the simulation results based on the PhD candidate's measurements, and b) the sensory evaluation and chemical measurements done by Matis staff and Hildur I. Sveinsdottir as a part of her MSc study.

The PhD committee and the PhD candidate collaborated on the study conceptualisation, methodology, funding acquisition, project administration, statistical analysis, and on writing, reviewing and editing the papers. The PhD committee supervised the PhD candidate during the study period.

Glossary

Nomenclature:

Symbols	Description [unit]
A	overall surface area of fish [m ²]
a	parameter for surface area formulae [-]
b	parameter for surface area formulae [-]
b_i	parameter for regression model [-]
ε	regression model error/residual
Bi	Biot number [-]
c_p	specific heat [J·kg ⁻¹ ·K ⁻¹]
D	fraction of volume and surface area [m]
h	convective heat transfer coefficient [W·m ⁻² ·K ⁻¹]
k	thermal conductivity [W·m ⁻¹ ·K ⁻¹]
m	mass/weight [kg]
n	number of samples [-]
\dot{Q}	heat flow rate [W]
T_f	initial freezing point [°C]
T_s	surface temperature of fish [°C]
T_∞	ambient temperature [°C]
V	volume [m ³]

Abbreviations	Description
ANOVA	analysis of variance
CSW	chilled seawater
DG	direct gutting
FFA	free fatty acids
FOB	free on board
GDA	generic descriptive analysis
GC	gill-cut
Hb	hemoglobin
HI	heme iron
ISK	icelandic krona currency
PCA	principal component analysis
PFR	pump flow recirculation
PL	phospholipids
RQ	research question
RSW	refrigerated seawater
SC	superchilled
SD	standard deviation
SEM	standard error of mean
TC	traditional ice cooling
TMA	trimethyleamine
TC	throat cut
TVB-N	total volatile base nitrogen
TVC	total viable psychrotrophic counts
WHC	water holding capacity
WRR	water replacement ratio

Terms explained in the following terminology refer to their use in this dissertation and apply to fish as a food substance.

Term	Meaning	Reference
Cooling	Removal of heat, resulting in a lower temperature and/or phase change.	https://eurovent.eu/?q=articles/new-definitions-five-terms-used-in-industry-gen-101500
Chilling	Cooling of fish without freezing it.	https://eurovent.eu/?q=articles/new-definitions-five-terms-used-in-industry-gen-101500
Superchilling	Reducing the temperature of fish to between the initial freezing point of the fish and 1-2 °C lower.	https://www.efsa.europa.eu/en/efsajournal/pub/6378
Freezing	Solidification phase change of a liquid or the liquid content of a substance, usually due to cooling.	https://eurovent.eu/?q=articles/new-definitions-five-terms-used-in-industry-gen-101500
Groundfish	The term groundfish is used in this thesis for the two bottom fish species included in the research, Atlantic cod (<i>Gadus morhua</i>) and saithe (<i>Pollachius virens</i>). Groundfish, also known as demersal fish, is defined as a bottom fish and the term can refer to many species, the most familiar in the North Atlantic seas are cod, haddock, saithe/pollock, halibut, and related species.	https://atlanticgroundfishcouncil.ca/understanding-the-fishery/
Chilled seawater (CSW)	The term chilled seawater refers to seawater that is cooled by adding ice to the seawater to generate the cooling medium.	https://www.fao.org/3/x5944e/x5944e01.htm#Introduction
Refrigerated seawater (RSW)	The term refrigerated seawater refers to seawater cooled by mechanical refrigeration.	https://www.fao.org/3/x5944e/x5944e01.htm#Introduction

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The present work was carried out in collaboration with the University of Iceland, Matís and partners in the Icelandic fishing industry. The study was conducted as part of the research projects “Redesign of demersal wet-fish trawler processing decks” and “Fast-cooling for fast-fishing boats”, both supported by the Icelandic Technology Development Fund (grants number 142667-0611 and 164866-0611) and the AVS R&D Fund of the Ministry of Fisheries and Agriculture in Iceland (grants number R 15 068-14 and R 19 024-17).

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1 Introduction

Due to an increasing demand for high quality fresh fish in recent years, there has been a shift by Icelandic fish producers from less valuable frozen products to high-end fresh products. Figure 1 shows the development of fresh export from Iceland in the last decade with Atlantic cod (*Gadus morhua*) products the most valuable from groundfish processing. The increased fillet and loin production is the main contributor to the fresh fish export value. With better handling in the last decade, there has been a change in the groundfish production option with a higher proportion of the raw material fit for more valuable products. This change has been supported by various technological developments along the fresh fish value chain with improvements in onboard processing and raw material handling resulting in added value of the products. Temperature control improvements, resulting in extended shelf-life, have increased fresh fish export and automation has helped to improve yield, product quality and the working environment. The new systems onboard fresh fish trawlers are based on time controlled bleeding and superchilling technology intended to chill the fish below its initial freezing point and then store it at the same temperature using no ice. The goals of the improvements are to provide more uniform raw material of high quality for further processing and to support more sustainable and economic fishing and processing. Along with extended shelf-life, raw material for high-end products, like fresh fish loins, needs to be well drained of blood and uniform in colour and quality. Considering the current status of the fresh whole fish export, there are opportunities to further increase the export value if the raw material fulfils the standards for fresh loin and fillet production.

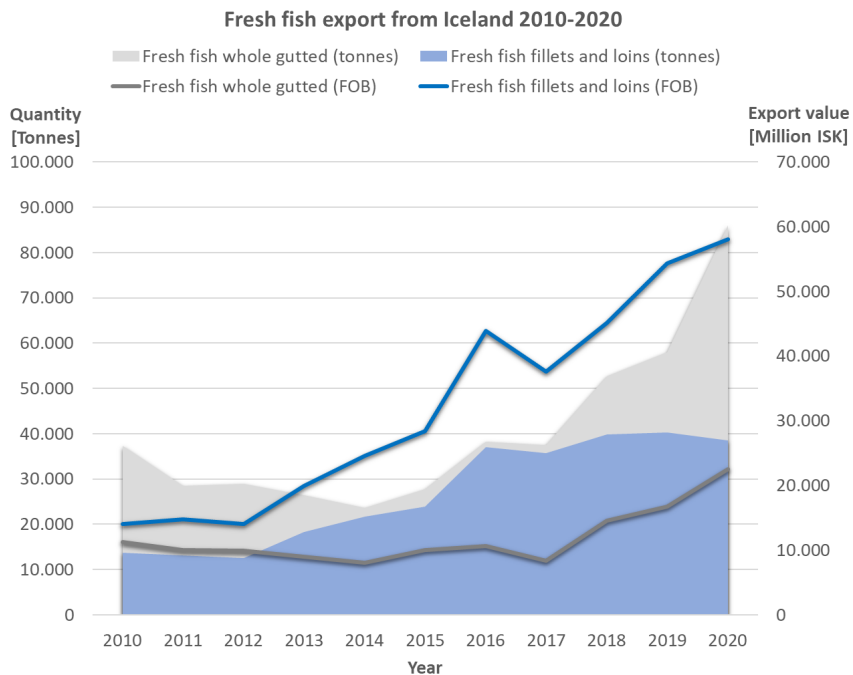


Figure 1: Fresh fish export from Iceland, 2010-2020 (Statistics Iceland, 2021). FOB: Free on board, ISK: Icelandic krona currency.

The origin of this study was a research project initiated by the industry back in 2013 when the three largest groundfish producers in Iceland were planning to build several new wet-fish trawlers in the coming years. This was due to an aging fleet and the shift from frozen to fresh fish products, which required redesign of the trawlers processing decks. This development of the fresh fish market has been supported by technological developments in the value chain in the last decade, but the primary onboard processing has been a weak link in terms of research studies. Figure 2 shows the onboard processing research focus of the current study within the fresh fish value chain. Focused research in other links of the value chain include studies on decision making (processing forecast, fisheries management, catch location, seasonal yield and quality parameters), secondary on-shore processing (improved yield and automation, physical and chemical properties of raw material), temperature control (chilling and cold chain transport) and marketing (distribution, traceability and sustainability studies). These studies have led to vast improvements and technical developments. The onboard processing research gap in the value chain, and the pending renewal of the Icelandic fleet, led to the current study where the main objective was to define the ideal methodology for groundfish handling through primary processing, with the goal to deliver fish of the highest possible quality and with a maximum shelf-life.

Accessibility issues regarding the research environment onboard fishing trawlers is one of the reasons for a lack of in-depth research and limited research focus on the primary onboard processing. Among the challenges of the onboard research environment are time consuming experiments and sampling and the many variables that influence the research results; seasonal variability for both fish and environment, trawling mix, handling prior to onboard processing, limited work space and the variable operating and/or processing time. After the bleeding process, there are challenges regarding the onboard cooling process where the sensitive superchilling method is carried out on mixed fish sizes under more demanding conditions and with more variability compared to land-based systems.

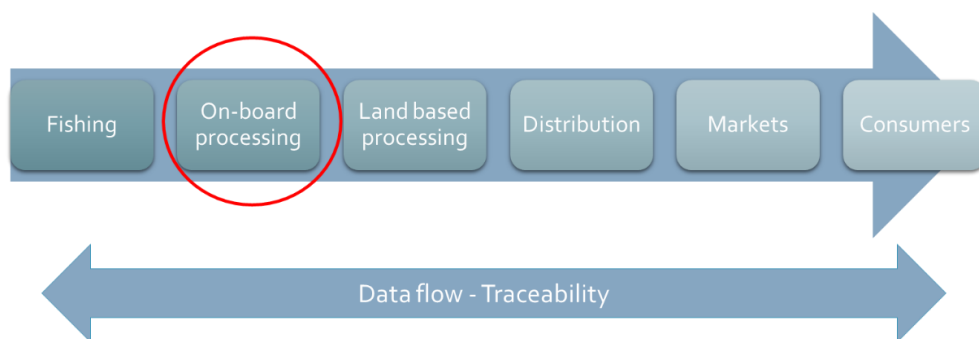


Figure 2: Groundfish value chain and study focus.

Figure 3 shows the study design and the scope of six papers, on which the study is based, in relation to the onboard processing. The papers include research on the bleeding process, i.e. on the methods of cut and gutting (**Papers IV and VI**), followed by evaluation of variable conditions during the bleeding (**Papers II and IV**). The chilling profiles of the new systems are compared to the systems being replaced (**Paper IV**) and measured for both ungutted and gutted fish in different cooling media (**Paper III**). Onboard storage of whole ungutted cod in refrigerated seawater (RSW) is also investigated (**Paper V**). Finally, the effects of onboard bleeding methods and superchilling on shelf-life and quality are evaluated through the value chain (**Papers I and IV**).

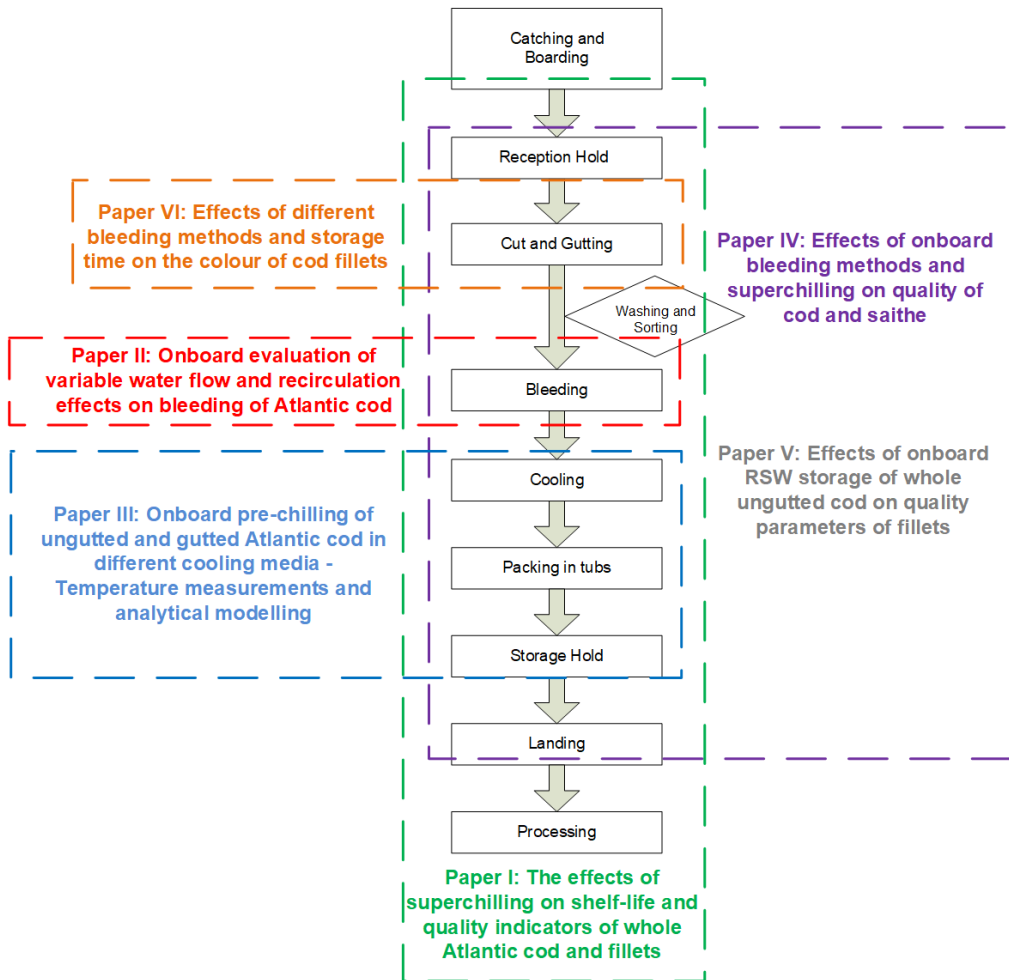


Figure 3: Study design. Papers linked to onboard processing flow chart.

1.1 Research questions and dissertation structure

The research focus of this study is on the onboard processing, a part of the fresh fish value chain where technical development in the last decades has been far less than in land-based processing. Land-based processing has seen a dramatic increase in automation and quality control and the aim of the industry is to implement that methodology in the onboard processing. A part of this development is also to improve data gathering in this sector, where data flow from fishing and onboard processing further along the value chain is often insufficient. This study is important for the evaluation of the impact of new industrial technology in the groundfish value chain. The innovation of processing onboard is aimed towards improving automation, sustainability and process control and the benefits of the new solutions need to be analysed for verification and for further technological development.

Also, the results can set a benchmark for fresh fish producers in Iceland regarding shelf-life and quality indicators for fresh fish products.

The main objectives of the study are to:

- evaluate new onboard processes from catch to storage, focusing on the bleeding and cooling processes.
- provide technical developers with design criteria for innovation in the onboard bleeding and cooling process.
- produce measurement results that can be used by fresh fish producers as a reference regarding shelf-life and quality indicators.

To meet these objectives the following research questions (**RQs**) are defined for the study:

RQ: *What are the effects of onboard processing on groundfish quality?*

This is the main research question and the study focuses specifically on the bleeding process in **RQ1** and the cooling process in **RQ2**.

RQ1: *How do different bleeding process methods affect the quality of groundfish?*

RQ1.1: *What are the effects of reception hold waiting time prior to bleeding on the fillet colour of cod and saithe?*

RQ1.2: *How do different methods of cut and gutting affect the bleeding process of cod and saithe?*

RQ1.3: *How do time and temperature variables in onboard bleeding systems affect the bleeding efficiency of cod and saithe?*

RQ1.4: *What are the recommended design parameters for water flow and recirculation in onboard bleeding systems?*

RQ2: *How do different cooling methods affect cooling profiles and the shelf-life of cod?*

RQ2.1: *What are the effects of different cooling methods on gutted and ungutted whole fish?*

RQ2.2: *How do industrial onboard systems compare to controlled experimental cooling profiles?*

RQ2.3: *What are the benefits of onboard superchilling of cod compared to traditional ice cooling?*

RQ2.4: *How does superchilling affect whole fish and fillets in terms of shelf-life?*

The links between each research question and the study papers are shown in Table 1. **Papers II, IV and VI** cover the bleeding process and **Papers I, III and V** cover the cooling process research. **Papers I, IV and V** focus on the onboard process effects from catch to storage.

Table 1: Research questions (RQs) linked to Papers I-VI.

	PAPER I	PAPER II	PAPER III	PAPER IV	PAPER V	PAPER VI
RQ1.1				X		
RQ1.2				X		X
RQ1.3				X		
RQ1.4		X				
RQ2.1			X		X	
RQ2.2			X	X		
RQ2.3	X		X		X	
RQ2.4	X					

This dissertation presents a summary of research studies performed onboard trawlers aimed to evaluate the process performance in terms of product quality and shelf-life. It is based on three published research papers, one conference paper and two submitted research papers. The papers are linked to research questions (Table 1) and the onboard processing value chain (Figure 3).

The thesis is divided into two main parts. The first part is this dissertation which consists of five main sections (chapters). In the first section, the context of the research is introduced along with objectives and research questions. The second section charts the scientific background and previous research relevant to this thesis. Section 3 explains the methodology applied and describes the data used. The fourth section presents a summary of the main results and discussions in relation to the theoretical framework and existing literature set forth in Section 2. The fifth section presents the conclusions of the study and an evaluation of the scientific contribution of the research, an evaluation of the study's contribution to the industry and, finally, future recommendations. The second part of the thesis consists of the six papers on which the thesis is based.

2 Background

The scientific background of the study and relevant literature are presented in this section, which is structured in relation to the research topics and questions. Figure 4 shows a flow chart of the onboard processing research topics in relation to the RQs with the relevant background literature review presented in Sections 2.1 Bleeding process, 2.2 Cooling process, and 2.3 Storage.

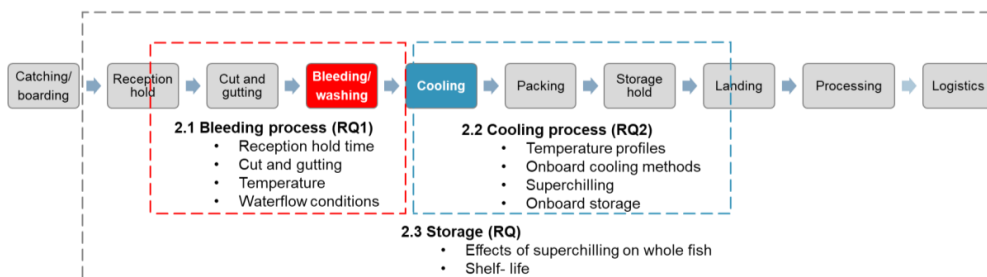


Figure 4: Overview of research topics relating to onboard trawler processing.

The increasing global demand has led fish producers in Iceland to focus on high-quality fresh fish products. Improvements in raw material handling, processing, temperature control and packaging have extended shelf-life and promoted an increase in fresh fish processing and export (Olafsdottir *et al.*, 2006, Lauzon *et al.*, 2010, Margeirsson *et al.*, 2010, Margeirsson *et al.*, 2012a,b,c). High-quality fresh products, like loins and fillets from cod (*Gadus morhua*) and saithe (*Pollachius virens*), require the raw material to be chilled, well drained of blood and uniform in colour. Low temperature slows microbial growth in the fish muscle and effective removal of blood also contributes to extended product shelf-life (Huss, 1995, Olafsdottir *et al.*, 2006). Superchilling of whole fish studied by Thorvaldsson *et al.* (2010), Bjarnason (2012) and Olafsdottir *et al.* (2012) has, however, not included a comparison of chilling processes for gutted and ungutted fish. Industrial superchilling of salmon fillets was studied by Kaale (2014) and Kaale *et al.* (2014). Their study considered quality indicators but did not include the chilling of whole fish. Tolstorebrov *et al.* (2019a,b) have studied the chilling of whole salmon using industrial systems, with the focus on the limitations of the cooling systems rather than fish quality.

The processing decks and fishing gear development have also aimed to avoid unnecessarily harsh treatment of the raw material which can cause various defects such as gaping and bruising. The volume in each haul should therefore be limited to reasonable amounts, reception hold designed to minimise strain to the fish, conveyor belts designed so that fish is not subjected to unnecessary drops and the drops to the tubs minimised. Icelandic wet-fish trawlers typically catch 20-50 tons of groundfish per day in fishing trips lasting from three to six days. Prior to being taken aboard a trawler, the fish has been battling against the trawl net, up the ramp and into the reception hold. Olsen *et al.* (2013) showed the negative effects of large haul sizes and long duration on the fish quality. The fish show a stronger reaction towards air exposure in terms of an increase in residual blood and decrease in blood clotting time when stressed by crowding (Svalheim *et al.*, 2019). This can be the case in the trawl

and the reception hold, and the study suggests quick euthanising after capture and minimising air exposure time prior to slaughter. A large part of the groundfish catch of small boats comes ashore ungutted, as this is the usual procedure when a sea trip takes less than one day. In Iceland, however, most of the catch of larger boats is gutted at sea. Groundfish are therefore often chilled and stored ungutted onboard smaller boats and the principal difference between cooling ungutted and gutted cod investigated in this study has not been widely studied. The method that is commonly used for pelagic fish in Iceland, chilling whole fish in onboard RSW tanks, has not been used for groundfish to any extent. RSW is preferred to ice as a cooling and storage medium in certain fisheries due to advantages such as speed of cooling and bulk chilling, reduced pressure on the fish, lower holding temperature, quicker handling of large quantity catches and less processing labour (Graham *et al.*, 1992). Roach *et al.* (1961) studied RSW storage for various species of fish, including ungutted groundfish. The interest of Icelandic producers in this method is motivated by their desire to lower fishing costs by bringing the gutting process ashore and thus provide the opportunity to utilise more rest raw materials from the fish.

2.1 Bleeding process

Bleeding and gutting are essential to rid the fish of contamination bacteria which reduce the quality of the raw material. These bacteria start to grow at accelerated rates post rigor mortis after death, especially where there is blood and inside the viscera. To slow the deterioration process of the fish muscles during storage, and to get rid of the bacteria and parasites, the viscera must be removed. The viscera contain enzymes, bacteria, microorganisms and parasites which are not desirable in contact with the muscles. The fish blood contains iron which can act as an oxidation agent when exposed to oxygen and can therefore have a severe impact on the quality of the fish fillets, most noticeably in colour and taste. Bleeding is generally the first process the fish goes through after catching and is aimed to drain most of the blood from the muscle (Borderías & Sanchez, 2010). Blood makes up 1.5-7% of a fish's total body weight, depending on the species, with about 20% of it localised in the muscular tissue (Huss, 1995). Insufficient bleeding can affect the shelf-life of products through product taste, odour, visual appearance and residual blood which can also promote lipid oxidation, provide nourishment for bacteria and cause increased enzymatic activity (Richards & Hultin, 2002). Research has shown that the bleeding process of the fish is an important factor for improving the colour of the flesh (Huss, 1995). Various research has shown the many influence factors on the bleeding process; **live storage** (Olsen *et al.*, 2013, Olsen *et al.*, 2014, Digre *et al.*, 2017), **fish stress** (Misimi *et al.*, 2008, Poli *et al.*, 2005, Olsen *et al.*, 2013, Olsen *et al.*, 2014, Digre *et al.*, 2017, Tobiassen *et al.*, 2016, Tobiassen *et al.*, 2018, Svalheim *et al.*, 2020), **fishing gear** (Rotabakk *et al.*, 2011, Grundvåg *et al.*, 2020), **blood coagulation** (Bantle *et al.*, 2015a, Tobiassen *et al.*, 2016), **bleeding media conditions** (Tobiassen *et al.*, 2018), **bleeding time** (Roth *et al.*, 2009, Erikson *et al.*, 2010, Olsen *et al.*, 2014, Tobiassen *et al.*, 2018). Other influencing factors include the **cooling of live fish** (Tolstorebrov, 2014, Skjervold *et al.*, 2001) and use of an **electronic stunner** before bleeding (Tobiassen *et al.*, 2018, Svalheim *et al.*, 2019, Erikson *et al.*, 2021).

The most important aspects of the procedure of bleeding were noted in the studies of Botta *et al.* (1986) and Olsen *et al.* (2014). They indicated that the time factor was more important than the method itself. Olsen *et al.* (2014) noted that, to ensure the best exsanguination of the blood from the muscles, the fish needs to start bleeding within 30 minutes after arrival

on the processing deck. In another study, Roth *et al.* (2009) observed that the washing of the fish for a minimum of 12 minutes, with a constant supply of clean seawater streaming into a washing bin/tub, could improve the exsanguination of the blood from the muscles. Karlsdottir *et al.* (2014) researched the effect of different bleeding methods and temperature of the bleeding medium on the quality and storage life of cod and saithe products. Their main results indicated that washing the cod in slurry ice rather than unchilled seawater resulted in better product quality. However, saithe showed the opposite reaction to chilled seawater compared to the cod, as saithe showed better end-quality bled in unchilled seawater. There are debates as to the best methods of cutting and bleeding fish. The three main methods are a gill-cut, cutting of the throat and direct gutting. Cutting the isthmus and gutting in a one-step procedure, where the isthmus is cut without touching the backbone and the fish then immediately gutted, is the most common practice in fresh fish processing. Olsen *et al.* (2014) and Karlsdottir *et al.* (2014) had better results in terms of exsanguination with two-step bleeding/gutting (throat cut, bleeding for 30 minutes, gutting and washing) compared to the commercial method of direct gutting (a one-step procedure as described by Botta *et al.*, 1986). A study by Digre *et al.* (2011a) indicated that the drainage of blood from a stressed cod was worse than for unstressed cod.

Lipid oxidation is one of the major problems associated with seafood during processing and storage as it affects fish quality and shelf-life (Ackman, 1980, Tavares *et al.*, 2021). As stated by Erickson (2002), typical oxidative response by muscle foods is variable, depending on processing treatment, and freezing generally inhibits the oxidative response. Research by Karlsdottir *et al.* (2014) and Olley & Lovern (1960) has shown that when phospholipids (PL) are degraded due to enzymatic activity the amount of free fatty acids (FFA) increases. Measurements of the amount of FFA and PL can therefore be a good indicator of quality when working with lean fish. Several variables can have impact on the amount of enzymatic activity in fish, including freezing rate, storage temperature, storage time, temperature fluctuations during storage and bleeding efficiency (Karlsdottir *et al.*, 2014, Olley & Lovern, 1960). Intact lipids, FFA and oxidised lipids can interact with proteins and result in quality deterioration of lean fish species. Results by Burggaard & Jorgensen (2010) and Dang *et al.* (2018) show how changes in lipids during frozen storage of fish can lead to quality deterioration, especially at temperatures around -20 °C to -10 °C. Temperature variations during transportation and short-term freezer storage commonly cause fish temperature to reach above -20 °C, resulting in negative changes in quality (Hyldig *et al.*, 2012). Blood components in the fish muscle have been proved to contribute to lipid oxidation of fish products during processing and storage. Also, the iron ions released from heme proteins are thought to promote lipid oxidation (Richards & Hultin, 2002). Heme proteins, including hemoglobin (Hb) and heme iron (HI), play an important role in lipid oxidation as pro-oxidants (Richards *et al.*, 1998). Phospholipids account for almost half of the total lipids in the blood of fish (Lizenko *et al.*, 2008) and it has been suggested that they contribute to lipid oxidation of fish muscle since phospholipids are known to be highly susceptible towards oxidation (Pamplona, 2008). Karlsdottir *et al.* (2014) concluded that if myoglobin and hemoglobin proteins remain in the white muscle tissue, the effect of an improper exsanguination can cause increased rancidity of the product and shorten the shelf-life.

2.2 Cooling process

The well-known method of cooling and storing the catch in ice brings the fish temperature slowly and surely to the ice melting point, at 0 °C. Superchilling means reducing the fish temperature to below the melting point of ice, which extends storage life. The method involves the partial freezing of water in food (e.g. cod muscle). The food is commonly cooled 1-2 °C below the initial freezing point (T_f) which leads to 5-30% of the water inside the product forming ice crystals (Aune, 2003, Kaale *et al.*, 2011). In this study, this is referred to as “superchilling”, however, various other terms are used to describe the method of superchilling food. It is also known as partial freezing (Duun & Rustad, 2007), deep chilling (Wu *et al.*, 2014) or, a more recent industry term, “sub-chilling” (Skaginn 3X, 2018). Figure 5 shows how the fraction of ice in fish changes during superchilling, where the first ice crystals are formed between 0 and -2 °C depending on the fish species and water content. According to Rahman *et al.* (2009), the initial freezing point for whitefish with water content around 80% is close to -1 °C (Margeirsson *et al.*, 2012b, modelled it at -0.92 °C for cod) and ice crystal formation increases rapidly at slightly lower temperatures. The chilling of fish and different cooling techniques have been widely studied, by e.g. Margeirsson & Arason (2008), Thorvaldsson *et al.* (2010), Kaale (2014), Bantle *et al.* (2015b), Laguerre *et al.* (2018), Tolstorebrov *et al.* (2019a,b). The advantages of superchilling fresh fish and the beneficial effects on quality are further covered in reviews by Magnussen *et al.* (2008), Kaale *et al.* (2011), and in EFSA BIOHAZ Panel (2021).

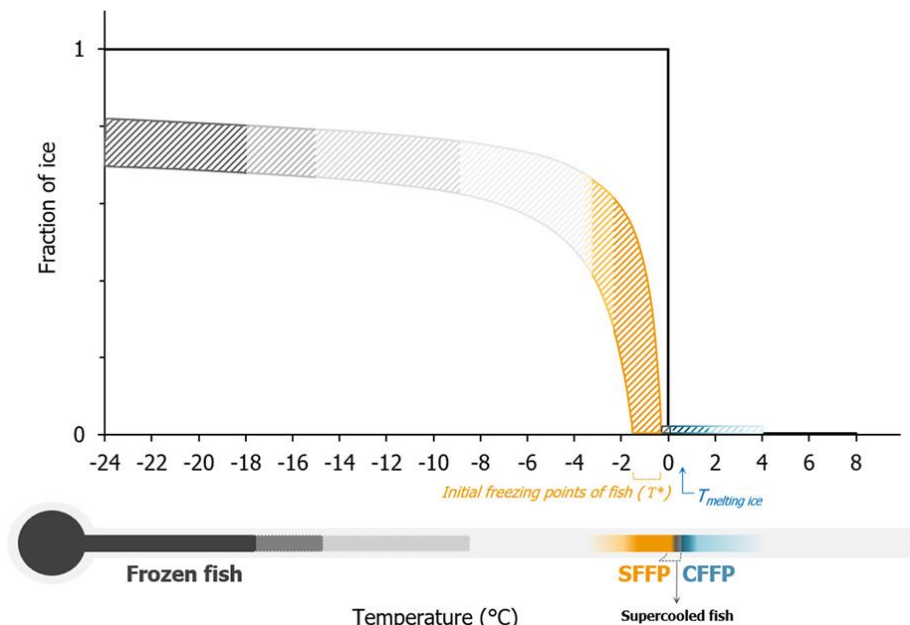


Figure 5: Conceptual representation of the fraction of frozen water in fish during superchilling. CFFP denotes conventional fresh fish cooled with ice and SFFP the superchilled fresh fish in the orange patterned area, below the initial freezing point of the fish. (Figure from EFSA BIOHAZ Panel, 2021).

Onboard cooling systems use various cooling media such as chilled seawater (CSW), ice slurry, refrigerated seawater (RSW) or refrigerated brine (Ruiz, 2012). Recent developments in onboard cooling of groundfish include superchilling the fish on the processing deck rapidly after catching and storing it without ice in the hold at -1 °C. Instead of a large part

of the cooling process taking place with iced fish in the storage hold, the new processes are designed to finish the temperature reduction process on the processing deck and store the fish with no ice, or a limited amount of ice, at a temperature close to the initial freezing point (T_f) of the fish, i.e. close to $-1\text{ }^{\circ}\text{C}$ for Atlantic cod (Stevik & Claussen, 2011).

Based on experience and theoretical calculations, approximate chilling times may be estimated. However, implementing superchilling in industrial processing of wild catch requires dynamic temperature control during the chilling process. As pointed out by Bantle *et al.* (2016), there are challenges in industrial superchilling where predictions on the state of superchilling, in terms of ice crystal formation, are complex to pinpoint. Avoiding excessive surface freezing and limiting ice crystal growth is a challenge in these conditions regarding structural damage and negative texture changes to the fish flesh and increased drip loss (Bahuaud *et al.*, 2008, Magnussen *et al.*, 2008, Kaale *et al.*, 2011). The onboard cooling process design, regardless of the chilling method or cooling medium, needs to handle energy removal proportional to the trawlers fishing capacity and to optimise the cooling process and temperature control of the system, and variable size and flow of fish to the processing must also be considered.

2.3 Storage

Fish spoilage is mainly caused by autolytic changes, enzymatic degradation (Boziaris, 2013, FAO, 2005), lipid oxidation (Hultin, 1994) and microbial spoilage (Boziaris, 2013, Church, 1998, Gram & Huss, 2000). Maintaining low temperatures during catching, transportation and storage of fish is key for preservation. Increased knowledge and improvements in packaging design and temperature control have extended shelf-life and supported an increase in fresh fish processing and export (Olafsdottir *et al.*, 2006, Lauzon *et al.*, 2010, Margeirsson *et al.*, 2012a,b,c, Paquette *et al.*, 2017, Tavares *et al.*, 2021). There are also positive effects in less gaping in the fish muscle with lower temperatures. Temperature is a key factor influencing the onset of the rigor mortis stage and rigor mortis can be delayed by cooling the fish to around $0\text{ }^{\circ}\text{C}$. If that is done, the contractions of the muscles will be less extreme when the fish goes through rigor mortis, resulting in less gaping in the flesh (Huss, 1995).

For most lean whitefish species caught in temperate waters, such as cod and haddock, the shelf-life is generally up to 15 days given traditional cooling, storage at $0\text{ }^{\circ}\text{C}$ and proper bleeding and gutting (Martinsdottir *et al.*, 2001, Tavares *et al.*, 2021). Haugland (2002) and Haugland *et al.* (2005) showed the positive effects of pre-chilling and superchilling iced whole, gutted cod on both bacterial growth and product temperature after filleting. According to Magnussen *et al.* (2008), the method of superchilling can extend the shelf-life of fresh cod by 2-3 days, compared to traditionally iced cod with a shelf-life of 13-14 days (Lauzon *et al.*, 2010). This is in accordance with Digre *et al.* (2011b) whose results showed lower microbiological activity and better quality for superchilled cod at Day 14, compared to cod stored in ice. Studies by Lauzon *et al.* (2010) and Olafsdottir *et al.* (2006) also indicated that lowering the temperature from $0\text{-}0.5\text{ }^{\circ}\text{C}$ to $-1.5\text{ }^{\circ}\text{C}$ prolonged the shelf-life of fresh fish by 2-3 days. Olafsdottir *et al.* (2006) concluded that controlling and keeping the correct temperature during storage is an important factor in preserving the quality of the fish, since a high product temperature can influence the growth of bacteria. If superchilling can be maintained through temperature control during transport, it has proved to have a positive effect on product quality (Aune, 2003, Mai *et al.*, 2011). It also has potential for positive

environmental impacts such as abiotic depletion, global warming potential and marine aquatic ecotoxicity (Hoang *et al.*, 2016). Using simple modified atmosphere packaging (MAP) can further prolong the shelf-life of cod loins by 4-5 days. A study by Wang *et al.* (2008) even showed a shelf-life of fresh cod loins from nine days for ice-chilled storage and up to 16-17 days in superchilled storage. Combining MAP with chilling and superchilling methods increased the shelf-life to 14 and 21 days, respectively. A stable storage temperature is essential for prolonged superchilled storage of products to minimise the risk of recrystallisation and formation of larger ice crystals in the fish muscle.

3 Materials and methods

This section gives an overview of the study methods and the necessary prerequisites for performing the research presented in the thesis. The experimental setup, measurement and modelling procedures for each experiment are described in more details in contributing **Papers I-VI**. The experiments in the study were mostly done onboard wet-fish trawlers, first on the older ones which have now been replaced, and then on newer ones as they were developed during the study period. Time control of bleeding and cooling systems was done with a screw conveyor/Archimedes screw. The first experiments were done with pilot units that were installed alongside older equipment (Figure 6) and then with fully developed industry equipment (Figure 7).



Figure 6: Experimental screw conveyor (pilot unit).

Figure 7: Onboard industry screw conveyor.

The onboard measurements took place during several fishing trips, as shown in Table 2. An overview of analytical methods used in the study is presented in the following sections (3.1, 3.2, 3.3) and, for each experiment, the methods are explained in more detail in **Papers I-VI**.

Table 2: Fishing trips and experiment focus.

Fishing trip	Time	Experiments	Measurements	Paper
Trawler Ottó	February 2013	- Bleeding - Cooling profiles	- Colour evaluation - Temperature	IV
Trawler Ottó	March 2013	- Bleeding - Cooling profiles	- Colour evaluation - Temperature	IV
Trawler Helga M.	March 2014	- Bleeding - Cooling profiles	- Temperature	III IV
Trawler Kaldbakur	May 2015	- RSW cooling - Storage	- Water and WHC - Microbial analysis - TVB-N, TVC, H ₂ S	V
Trawler Málmei	March 2016	- Cooling profiles - Storage - Shelf-life - Bleeding conditions	- Temperature - TVB-N, TVC, H ₂ S - Sensory evaluation - FFA, PL, HI	I II III
Trawler Björg	September/ October 2018	- Cooling profiles	- Temperature - Microbial analysis - Colour evaluation	III VI

3.1 Bleeding process

Experiments that were done on the bleeding process in this study included measurements to assess the influence of reception hold waiting time, temperature of the bleeding medium, method of cut and gutting, different bleeding times, and the effects of Pump Flow Recirculation (PFR) and Water Replacement Ratio (WRR) on the quality of fresh fish products. Among conditions in industrial bleed-out tanks that influence the bleeding efficiency are the WRR, which is the ratio of bleeding medium renewal (seawater in onboard systems), and the PFR, which impacts the flow turbulence in the tanks. The influence of both the WRR and PFR on the bleeding process of cod has, according to the author's best knowledge, not been studied before. The methods used to evaluate the effects on the fish quality in this study include chemical analysis (FFA, PL, HI) and colour analysis (visual evaluation and CIE L*a*b*). In **Paper II** a multivariable linear regression model shown in Equation (1) was constructed in order to evaluate the effects of PFR and WRR on the results:

$$\text{Dependent variables (FFA, PL, HI)} = b_0 + b_1 PFR + b_2 WRR + \varepsilon \quad (1)$$

The objectives of the regression model were, on one hand, to evaluate the relation between the dependent variables, FFA, PL, and HI and, on the other hand, the effects of the independent variables, PFR and WRR (b_i represents the model parameters and ε its residuals). The experiment setup and methods to evaluate the bleeding process are described in more detail in **Papers II, IV and VI**.

Chemical analysis

Methods for evaluation of quality were chemical analysis of free fatty acids (FFA) and phospholipids (PL) and heme iron (HI). Lipid hydrolysis was evaluated through the quantification of FFA. The method used for determination of FFA content was the method from Lowry & Tinsley (1976), with modification by Bernardez *et al.* (2005). To evaluate lipid oxidation susceptibility of the different muscle tissues according to their anatomical origin, a quantification of heme iron in the muscle was performed. The analysis was performed using the method by Gomez-Basauri & Regenstein (1992) as described in **Paper II**. Studies have identified heme iron and other similar compounds as highly pro-oxidative (Gomez-Basauri & Regenstein, 1992, Richards & Hultin, 2002, Undeland *et al.*, 2004). Therefore, an increased amount of heme iron was assumed to relate to an increase in the oxidation susceptibility of the different muscle types.

Colour analysis

Evaluation of the bleeding process, looking at fillet colour, was done with sensory evaluation by a colour scale, Minolta meter CIE L*a*b* measurements and digital image processing. Visual fillet colour was evaluated by trained experts. Figure 8 shows the scale for fillet colour evaluation, developed by Icelandic Ltd (2012) for cod and adjusted for saithe in this study (**Paper IV**).



Figure 8: Fillet colour chart scale for cod, from Icelandic Ltd (2012). Figure from Paper IV.

Colour changes can often predict changes occurring in the raw material, e.g. an increase in yellow colour of fillets has been linked to increased lipid oxidation (Hamre *et al.*, 2003). The colour of fillets in the L*a*b* scale was determined with a Minolta Chroma Meter CR-300 (Minolta, Osaka, Japan) using the CIE L*a*b* system. The instrument recorded the L*-value, indicating lightness on the scale from black to white, 0 to 100 respectively, the a*-value, ranging from (+) red to (-) green, and the b*-value, ranging from (+) yellow to (-) blue (shown in Figure 10, Hutchings, 1999). When used on whole fillets, the colour was measured above the lateral line in five positions, from the loin to the tail end. In this case, the measurements were performed to evaluate changes in colour throughout storage.



Figure 9: Minolta measurement locations. Figure from Paper VI.

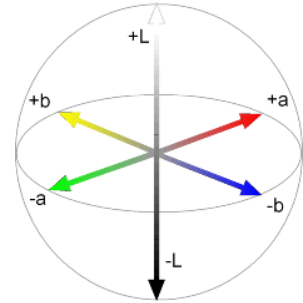


Figure 10: Values derived by using the CIE L*a*b* system (Hutchings, 1999).

Average L* (lightness), a* (redness), and b* (yellowness) values of the muscle surfaces were calculated using the LensEye software. In addition, whiteness (W*) of the samples was calculated with Equation (2), as described by Park (1994):

$$W^* (\%) = 100 - [(100 - L)^2 + a^2 + b^2]^{1/2} \quad (2)$$

In **Paper VI** the total colour difference between groups relative to the reference group was calculated by Equation (3):

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (3)$$

In terms of colour differences perceivable to the human eye, universally valid ΔE^* values can be used as follows: 0–1: observer does not notice the difference; 1–2: only an

experienced observer can notice the difference; 2–3.5: inexperienced observer also notices the difference; 3.5–5: a clear difference in colour is noticed; >5: observer notices two different colours (Mokrzycki & Tatol, 2011). When calculating results, whole fillets were given average values, as well as the loins and tail end individually.

Image analysis of CIE L*a*b* in **Paper VI** was done as in Jiang *et al.* (2020) using the IMAGE COLOR SUMMARIZER RGB, HSV, LCH & L*a*b* image colour statistics and clustering program (Circos—Circular Genome Visualization/Martin Krzywinski). Images of a 4x4 cm² area in the loin, belly flap and tail end sections (n=20 fillets for each group) were analysed, as shown in Figure 11.

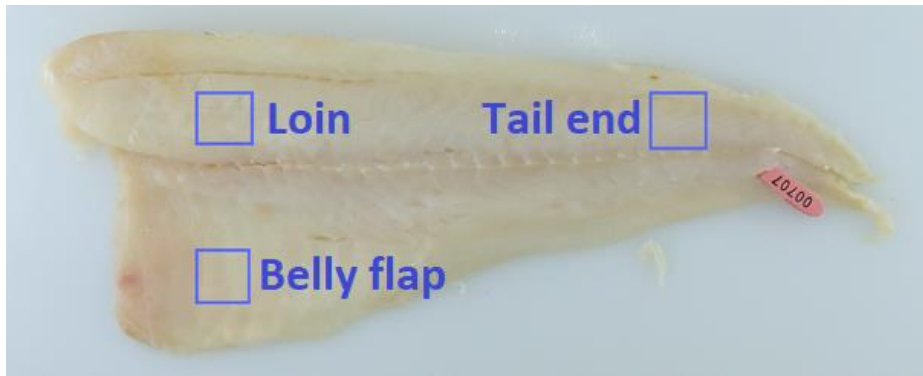


Figure 11: Image analysis areas of fillet. Figure from Paper VI.

3.2 Cooling process

One of the novelties of this study was to evaluate the temporal difference between chilling ungutted and gutted cod and to evaluate the performance of industrial chilling processes by comparing them to results obtained from controlled experiments. Experiments of chilling cod were carried out in both controlled environment and industrial processing onboard wet-fish trawlers, using different chilling methods. The measurements of industrial chilling systems were conducted to evaluate the challenges for industrial implementation of superchilling. The experimental results of the study were used to build a model for chilling gutted cod with variable cooling medium temperatures and to estimate the convective heat transfer coefficient for different industrial systems. The experiment setups and methods are described in detail in **Papers I, III, IV** and **V**.

The onboard cooling systems measured in this study were all screw/spiral conveyor types, i.e. an Archimedes screw transferring fish through the cooling tanks. The ratio of cooling medium to fish was around 3:1 for all systems. The following is a description of the different trawler cooling equipment:

- Trawler A: Ice slurry/CSW: The equipment used was a screw conveyor (Skaginn 3X, Akranes, Iceland, 2018) with one-step slurry ice chilling at -1.2 to -1.0 °C.
- Trawler B: Refrigerated brine, two-step chilling: The onboard superchilling method was in two chilling steps; the first step cooling in seawater at -0.8 °C (3% salt

concentration) and the second step cooling in brine at -2.8 °C (6% salt concentration). The equipment used was a screw conveyor (Skaginn 3X, Akranes, Iceland, 2018).

- Trawler C: Refrigerated seawater, one-step chilling: The equipment used was a screw conveyor manufactured by Stranda (2019) and adapted by Slippurinn, Akureyri, a one-step RSW system with seawater at -1 °C.

The weights of the fish are shown in Table 3; small (2-3 kg), medium (4-5 kg) and large cod (6-9 kg) defined in three size groups.

Table 3: Fish size groups for cooling experiments and modelling. Table from Paper III.

		Gutted weight [kg]	Ungutted weight [kg]	Location and time of catch
Experiment 1, controlled environment	Size group I, small cod	2.5	2.5	Southwest (SW) of Iceland in April
	Size group II, medium cod	4.1	5.1	
	Size group III, large cod	9.1	9.3	
Experiment 2, onboard systems	Size group I, II, III	2, 4, 6		Trawler A: Southwest (SW) of Iceland in March
				Trawler B: Northwest (NW) of Iceland in March
				Trawler C: Northeast (NE) of Iceland in September
Model	Size group I, II, III	2, 4, 6		

As the exact same fish was measured in both chilled brine and flake ice, applying the same uniform initial temperature in the experiments, the difference between fish in different cooling media can be directly compared. The weights of gutted and ungutted fish however, were selected as close as possible from the size range available for this experiment.

Temperature measurements

Temperature loggers were used to measure the temperature inside the fish, in the cooling media/ice, in insulated boxes during real transport, and in the packaging and transport simulations. Temperature measurements were mainly done with the temperature loggers iButton and Tidbit. Red marks on the cod in Figure 12 show where they were inserted in the fish when measuring core temperature or tail end temperature. Outside surface and ambient temperatures were either measured with the iButton loggers or Tidbit v2 temperature loggers from Onset Computer Corporation (Bourne, MA, USA). All temperature loggers were factory calibrated and recalibrated by the author in a thick mixture of fresh, crushed ice and water at 0 °C.

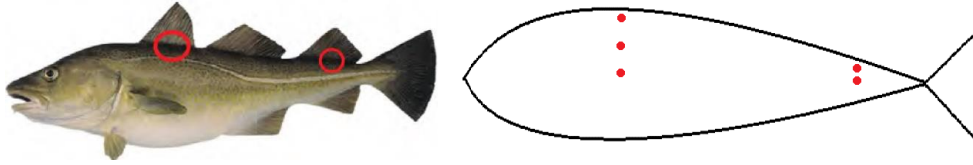


Figure 12: Temperature logger placement in fish (red circles denote the insertion points), to the left. A representation of the measuring points of fish in the model (red points), to the right. Figure from Paper III.

Different types of temperature data loggers were used in the experiments. For the ambient temperature, Tidbit v2 loggers from OnSet Computer Corporation (Bourne, MA, USA) were used. The accuracy of the data loggers is ± 0.2 °C for temperatures between 0 °C and 5 °C. Variable depth temperature measurements were done with a Testo 176 T4 data logger (Testo Inc., Lenzkirch, Germany) with TC type K and T thermocouples probes (accuracy ± 0.3 °C and resolution 0.1 °C). Maxim Integrated's iButton data loggers were used to measure the fish core temperature. The measurement resolution of these loggers is 0.0625 °C and the error margin for temperatures between -15 °C and 65 °C, as specified by the manufacturer, is no more than 0.5 °C. However, van Marken Lichtenbelt *et al.* (2006) reported that, during a test for error margins, these loggers measured temperatures with a reading error of no more than 0.09 °C.

Modelling of cooling profiles

The model for simulating chilling was built using Simulink and Matlab and includes three measurement points within the loin section of the fish and two measurements within the tail section, shown in Figure 12. A first order differential equation is combined for each point and they are interlinked in a 2nd and 3rd order equation, for the tail and loin respectively. The ambient temperature is set to interact with the surface of the fish through convection, and heat transfer inside the fish is through conduction. The model is described in more detail in **Paper III**. The initial temperature of the whole fish is set to an initial value, and the surface temperature of the fish is the same temperature as the outermost block. At the surface of the fish, the heat flow is calculated based on the temperature difference between the outermost layer and the surroundings, and a constant coefficient of heat transfer. The results of the model were compared to temperature measurements to estimate the convective heat transfer coefficient for fish in controlled experiments and trawler systems. The heat flow from the surface of the fish to the ambient (refrigerated water or ice) is represented by Equation (4):

$$\dot{Q} = h \cdot A \cdot (T_s - T_\infty) \quad (4)$$

where \dot{Q} represents the heat transfer per unit time, h is the convective heat transfer coefficient, A the surface area of the fish, T_s the surface temperature and T_∞ the ambient temperature.

The overall surface area of the fish was estimated by considering the mass (m) of the fish and then using formulated quantities based on work by O'Shea *et al.* (2006) in Equation (5):

$$A = a \cdot m^b \quad (5)$$

where a and b are constants defined by the fish species. Each cross section, represented by a temperature logger placement point shown in Figure 12, was then defined with a reference area surface based on the total surface area, A, found by Equation (5).

3.3 Storage

Analytical methods for evaluation of shelf-life and quality indicators include chemical measurements (TVB-N), microbial analysis (TVC and H₂S-producing bacteria) and sensory evaluation using Generic Descriptive Analysis and the Torry scale. The experiment setups and methods are described in detail in **Papers I, III and V**.

Chemical and microbial analysis

Quality difference was evaluated by microbial analysis and total volatile base nitrogen (TVB-N). The method of Malle & Tao (1987) was used for TVB-N measurements, measured by steam distillation and titration, after extracting the fish muscle with 7.5% aqueous trichoroacetic solution. The distilled TVB-N was collected in boric acid solution and titrated with sulfuric acid solution.

Total viable psychrotrophic counts (TVC, 15 °C, 4–5 days) were evaluated by spreadplating aliquots onto modified Long & Hammer's medium; counts of H₂S-producing bacteria and presumptive pseudomonads were evaluated on spreadplated Iron Agar (15 °C, 4–5 days) and modified Cephaloridine-Fucidin-Cetrimide medium (22 °C, 3 days), respectively (as described by Magnusson *et al.*, 2009).

Water content and water holding capacity

Measurements of water holding capacity (WHC), water content and salt were performed on mid-section parts. Pieces were minced and used for chemical analysis. The water content of the samples was measured by determining the difference in weight of the minced muscle samples following drying for 4 hours at 102-104 °C (ISO, 1999). The muscle sample was taken from the fish mid-section. The WHC was determined with approximately 2 g minced white epaxial muscle, taken at the fillet mid-section, and was subjected to low-speed centrifugation as described by Eide *et al.* (1982), except that a centrifugation force of 210 g was used. The WHC was expressed as the percentage of retained weight after centrifugation (1350 rpm, 5 min) at 4 °C, divided by the initial sample weight and multiplied by 100.

Sensory analysis

Sensory analysis was performed to determine shelf-life and whether, and/or how, the treatments performed affected the sensory attributes evaluated. The Generic Descriptive Analysis (GDA) as described by Lawless & Heymann (2010) in **Paper I** and the Torry freshness score sheet (Shewan *et al.*, 1953) were used to assess cooked samples. For the freshness evaluation of cooked fillets, the Torry scheme is used to assess the fish flavour and odour on a scale from 10, very fresh, to 3, very spoiled (Martinsdottir *et al.*, 2001).

Visual assessment of quality parameters such as gaping, blood, bile spots etc. in **Paper V**, was done with a 4x4 cm grid on the fillets as described by Margeirsson *et al.* (2007). Two trained persons with experience in sensory and quality evaluation of fish products did the visual assessments on the same pool of samples.

4 Summary of results and discussion

The main results obtained in the study are presented and discussed in this section. This includes six individual studies presented in **Papers I-VI** on onboard processing time and temperature control, variable bleeding conditions, superchilling methods and superchilled storage, with the main focus on the effects on shelf-life and product quality. Figure 13 shows a flowchart of the onboard processing in relation to the main research topics focusing on bleeding, cooling and storage, and the effects of these processes on the quality and shelf-life of groundfish.

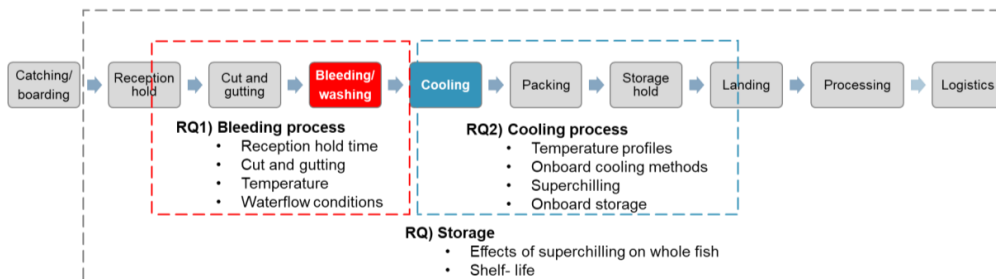


Figure 13: Focus of research questions relating to onboard fresh fish processing - overview.

The main research question **RQ** in this dissertation is:

RQ: “What are the effects of onboard processing on groundfish quality?”

To approach this main question the study presents, through research **Papers I-VI**, topic related questions on the bleeding process (**RQ1**) and the cooling process (**RQ2**) as shown in Figure 13. These topic questions are further investigated with relevant research questions (**RQ1.1-1.4** and **RQ2.1-2.4**).

Papers II, IV and **VI** all contribute to the research questions on bleeding process methods and their effects on quality (**RQ1**). **Papers IV** and **VI** contribute directly to research questions **RQ1.1** and **RQ1.2**, **Paper IV** to research question **RQ1.3** and **Paper II** to research question **RQ1.4**, as discussed in Section 4.1. Bleeding process.

Papers I, III, IV and **V** all contribute to research questions on cooling methods and their effects on chilling profiles and shelf-life (**RQ2**). **Papers III, IV** and **V** contribute directly to research questions **RQ2.1** and **RQ2.2**, as discussed in Section 4.2 Cooling process. **Papers I, III** and **V** contribute directly to research questions **RQ2.3** and **RQ2.4**, as discussed in Section 4.3. Storage.

4.1 Bleeding process

Papers II, IV and VI contribute to the research questions on the bleeding process (**RQ1.1, RQ1.2, RQ1.3 and RQ1.4**). The results of the bleeding experiments indicate that the fish should be bled within 30 minutes after catch to maintain quality in terms of fillet colour. The bleeding process should exceed 12 minutes and be carried out in unchilled seawater, ensuring movement and refresh rate of the bleeding medium. Evaluation of the older uncontrolled systems showed that they are unreliable in terms of both bleeding time and cooling. As for the method of bleeding and gutting saithe, the two-step process of bleeding and gutting showed slightly better results in terms of fillet colour compared to direct gutting. The two-step method is suitable for automation of the bleeding process onboard trawlers to shorten the waiting time in the reception hold, which was found to be the main deciding factor influencing fillet colour.

In this section, the main factors that were found to influence the bleeding process are presented in relation to the relevant RQs and the results discussed, linking the papers from which results and conclusions are drawn directly to the relevant research questions. The five factors mainly discussed are; Bleeding process methods, Reception hold waiting time, Method of cut and gutting, Bleeding process design variables, and Water flow and recirculation.

Research question **RQ1** focuses on the bleeding process methods:

RQ1: “How do different bleeding process methods affect the quality of groundfish?”

The main conclusion from experiments presented in **Paper IV** on the bleeding process methods is that the reception hold waiting time is most crucial for maintaining lighter fillet colour. This concurs with the results of Botta *et al.* (1986) and Olsen *et al.* (2014). The bleeding time and the method of cutting were also found to influence fillet colour to a lesser extent (**Papers IV and VI**). The results presented in **Papers II and IV** also indicate that different conditions of the bleeding medium (temperature, time, PFR and WRR) should be adjusted to optimise the bleeding process for cod.

Research question **RQ1.1** focuses on the reception hold waiting time:

RQ1.1: “What are the effects of reception hold waiting time prior to bleeding on the fillet colour of cod and saithe?”

Results presented in **Paper IV** show that the waiting time in the reception hold is a deciding factor regarding fillet colour (Figure 14a,b). A higher number represents a lighter fillet colour. The graph on the left is for saithe and on the right for cod, both show darker fillet colours as the fish is kept longer in the reception hold.

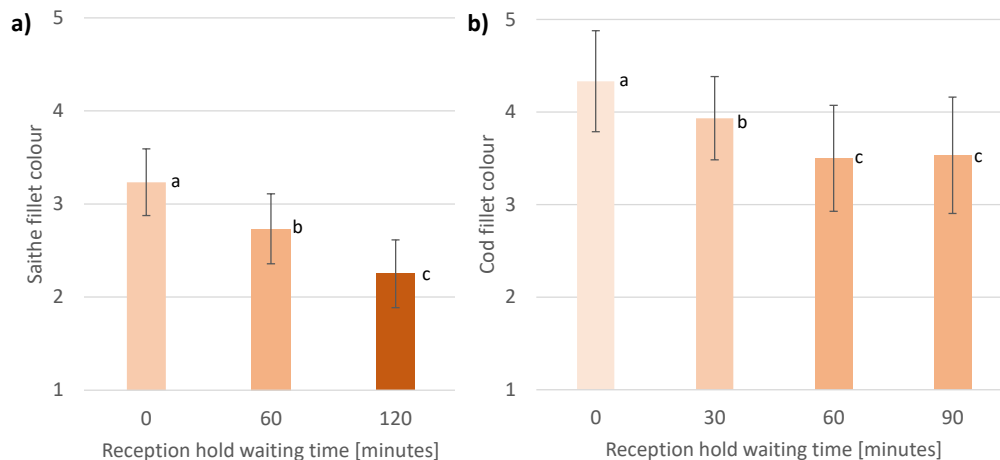


Figure 14: Colour of a) saithe and b) cod fillets for different waiting times in a trawler reception hold (n=30 fillets). Significant difference between groups is represented by different letters a, b, c ($p < 0.05$). Error bars show standard deviation from mean values ($\bar{\mu} \pm \sigma$). Figure from Paper IV.

The results in Figure 14a) show a significantly lighter fillet colour for saithe bled straight from the reception hold after catch compared to saithe that was bled 60 minutes and 120 minutes later. Results for different waiting times for cod in Figure 14b) show a significant difference for cod bled straight after catch compared to cod bled after 30 and 60 minutes. There was no significant difference found in fillet colour between cod that waited for 60 and 90 minutes. The results indicate that, to maintain a lighter fillet colour, the fish must be bled within 30 minutes from catch. This is in accordance with results by Botta *et al.* (1986) and Olsen *et al.* (2014) who both concluded that the time from catch to bleeding was a deciding factor influencing exsanguination of cod. The fisheries companies involved in this study have, during the study period, increasingly set a reference limit on the acceptable size of hauls, aiming to reduce the waiting time in the reception hold, underlining the importance of fillet quality. Reduced haul size also has positive effects in terms of the physical pressure on the catch and crowding stress as shown by Rotabakk *et al.* (2011), Olsen *et al.* (2013) and Svalheim *et al.* (2019).

The results of the current study therefore indicate that, to minimise the negative effects of the reception hold waiting time prior to bleeding on fillet colour, the fish must be bled within 30 minutes after catch.

Research question **RQ1.2** focuses on the method of cut and gutting:

RQ1.2: “How do different methods of cut and gutting affect the bleeding process of cod and saithe?”

For investigation on the method of bleeding and gutting fish in **Papers IV** and **VI**, the two-step process of bleeding and gutting showed marginally better results in terms of fillet colour, compared to direct gutting. Figure 15 shows three groups of saithe; the first is direct gutting in a controlled system, then a two-step bleed and gut method, also in a controlled first-in first-out system, and the last is direct gutting in an older uncontrolled system. Blue columns show fillet colour at Day 4 from catch and red columns at Day 9, after simulation of a 5 day sea transport to European markets. The difference is clearer looking at the fillets after the transport simulation.

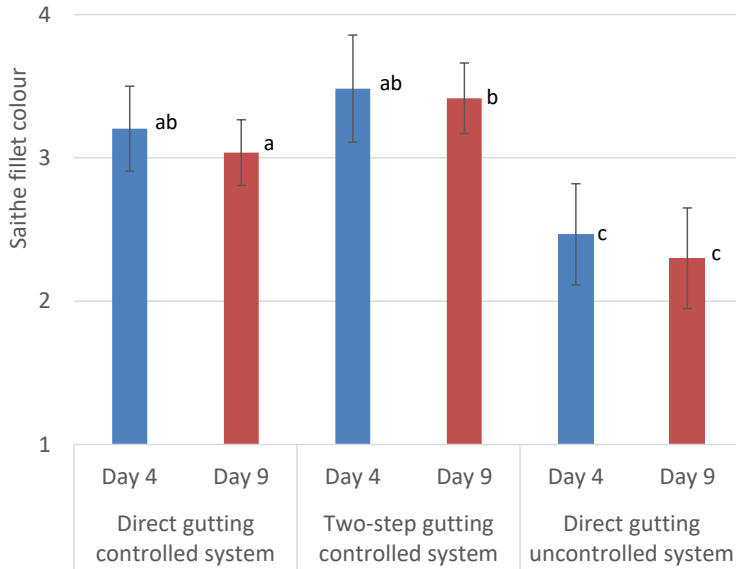


Figure 15: Fillet colour of saithe for different gutting methods and onboard processing systems. Significant difference between groups is represented by letters a, b, c ($p < 0.05$). Error bars show standard deviation from mean values ($\bar{\mu} \pm \sigma$). Figure from Paper IV.

The purpose of **Paper VI** is to evaluate the effects of different bleeding methods and storage of Atlantic cod on the product quality. This is done with an experiment comparing the efficiency of different onboard bleeding methods for three groups; direct gutting (DG), gill-cut (GC) and throat cut (TC). The evaluation of fillets was done with visual colour assessment, a Minolta meter and using digital image analysis of different fillet locations. The study results, shown in Table 4, did not show a clear difference between the cutting method groups DG, GC and TC. The difference found was a minor difference in the redness (a^* -value) of the belly flap and tail end for group DG. The colour evaluation, however, showed a significant difference in fillet colour affected by the six days storage time and different location on the fillet.

Table 4: Fillet colour of cod for different cutting methods. Minolta Average $L^*a^*b^*$ (n=20 fillets). Direct gutting (DG), gill-cut (GC) and throat cut (TC). Table from Paper VI.

	Day 6			Day 12		
	DG	GC	TC	DG	GC	TC
L^* (lightness)	69.2±0.5	69.0±0.4	69.1±0.4	74.6±0.4	74.5±0.4	75.3±0.4
a^* (redness)	1.5±0.2	1.2±0.1	1.2±0.1	1.8±0.1	1.8±0.1	1.5±0.1
b^* (yellowness)	7.3±0.2	7.0±0.2	7.2±0.1	9.8±0.3	9.3±0.2	9.4±0.2
W^* (whiteness)	68.3±0.5	68.2±0.3	68.3±0.3	72.7±0.4	72.8±0.3	73.5±0.4

Table 5 shows the results of the image analysis for the loin, belly flap and tail end sections. There is no significant difference between the cutting method groups apart from the a^* -value of the belly flap and tail end for group DG. Compared to the Minolta values, the whiteness (W^*) of the fillets is of similar value for Day 6 and considerably lower for Day 12. Olsen *et*

al. (2014) evaluated whiteness of loin and belly for cod with different bleeding methods and found lower whiteness in the loin area. The total colour differences show ΔE^* values of 2.5 to 4.2 between Days 6 and 12, indicating a marginal to clearly noticeable difference in the colour depending on storage time. The ΔE^* values are between 0.3 and 0.9 for the loin groups on Day 6, indicating no difference. On Day 12, the ΔE^* values indicate a marginal difference between groups GC and TC, and DG.

Table 5: Fillet colour of cod for different cutting methods. Image analysis by CIE L*a*b* colour scale. Direct gutting (DG), gill-cut (GC) and throat cut (TC). Table from Paper VI.

Fillet part	CIE LAB colour	Day 6			Day 12		
		DG	GC	TC	DG	GC	TC
Loin	L* (lightness)	68.6±0.6	68.5±0.4	69.3±0.5	70.5±0.7	72.0±0.4	71.2±0.4
	a* (redness)	-0.3±0.7	-0.1±0.3	-0.6±0.4	-0.2±0.6	-0.3±0.3	-0.5±0.2
	b* (yellowness)	8.1±0.4	8.2±0.3	8.5±0.4	10.4±0.4	10.6±0.3	9.3±0.3
	W* (whiteness)	67.6±0.6	67.5±0.4	68.1±0.5	68.7±0.7	70.1±0.5	69.7±0.5
Belly flap	L* (lightness)	64.8±1.3	66.3±0.7	65.1±0.9	64.7±0.5	68.2±0.4	65.7±0.7
	a* (redness)	4.6±0.6	2.3±0.5	3.3±0.7	5.0±0.6	2.9±0.6	3.6±0.6
	b* (yellowness)	8.7±0.4	9.8±0.3	10.4±0.7	11.1±0.7	12.0±0.4	11.1±0.4
	W* (whiteness)	63.5±0.3	64.8±0.4	63.4±0.5	62.7±0.6	65.9±0.4	63.8±0.5
Tail end	L* (lightness)	63.1±0.6	63.8±0.6	63.3±0.8	61.8±0.3	62.5±0.4	62.9±0.3
	a* (redness)	1.8±0.3	0.7±0.3	0.9±0.2	3.2±0.3	1.1±0.3	1.6±0.3
	b* (yellowness)	7.7±0.3	8.0±0.3	7.5±0.3	9.8±0.3	10.9±0.3	9.5±0.2
	W* (whiteness)	62.3±0.4	60.4±0.6	62.5±0.6	60.4±0.3	61.0±0.4	61.7±0.4

The difference found was a minor difference in the redness (a*-value) of the belly flap and tail end for group DG. The reason for only a minor difference in this study could be that, when bleeding time exceeds 20 minutes and the fish is bled within 30 minutes after catch, the difference between cutting methods becomes negligible. The colour evaluation, however, showed a significant difference in fillet colour affected by the six days storage time compared to 12 days storage time and different locations on the fillet. According to the digital assessment of the Minolta meter and image analysis, the fillets show a higher lightness value and higher yellow after six days of cold storage. The belly flap and tail end also show higher redness in contrast to the loin.

In terms of fillet colour, the results of the current study show only marginally better results for a two-step bleeding method, compared to traditional direct gutting which is more common due to the simpler onboard processing flow design.

Research question **RQ1.3** focuses on the bleeding process design variables:

RQ1.3: “How do time and temperature variables in onboard bleeding systems affect the bleeding efficiency of cod and saithe?”

Paper IV shows an evaluation of different bleeding times for saithe; 5, 12, 18 and 30 minutes in Figure 16. Blue columns show fillets at Day 4 and red columns at Day 9, after a 5 day sea transport simulation. Comparison of the fillet colour after filleting (4 days after catch) did

not show significant difference but after further storage of the fillets (9 days from catch) there was a significant difference ($p < 0.05$). Groups bled for 5 and 12 minutes showed a darker colour compared to a lighter colour for groups bled 18 and 30 minutes. The results after 9 days from catch indicate that the bleeding time for saithe should exceed 12 minutes and that the effects of insufficient bleeding are not necessarily apparent immediately after filleting. The fillet storage between Days 4 and 9 was simulating sea transport of fresh fish at $-1\text{ }^{\circ}\text{C}$, so Day 9 from catch is approximately the time of delivery to buyers in Europe. Significant results between the groups are visible only after storage simulation, indicating that the critical bleeding time is between 12 and 18 minutes, and that quality assessment should consider that evaluation immediately after processing does not always show the negative effect of shorter bleeding time. Therefore, the fillets may look different to the end user.

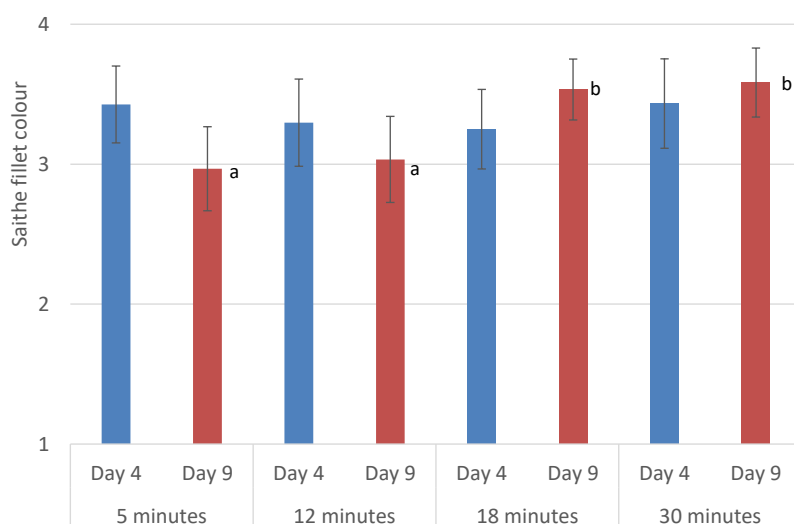


Figure 16: Fillet colour of saithe after 5, 12, 18 and 30 minutes bleeding in seawater. Blue columns represent fillet colour after fillet processing (Day 4 from catch) and red columns fillet colour after transport (Day 9 from catch). Error bars show standard deviation from mean values ($\bar{\mu} \pm \sigma$). Figure from Paper IV.

Paper IV also contributes to address the temperature of the bleeding medium. Figure 17 shows cod fillets bled for 20 minutes at three different temperatures; $-1\text{ }^{\circ}\text{C}$, $2\text{ }^{\circ}\text{C}$ and $6\text{ }^{\circ}\text{C}$. Fillets bled in unchilled seawater ($6\text{ }^{\circ}\text{C}$) show the best results in terms of uniform colour. Blood is more apparent in the fillets when bled at lower temperatures, especially in the belly flap area. Karlsdottir *et al.* (2014) showed the same results for saithe, however, for cod they had better exsanguination at lower temperatures.



Figure 17: Cod fillets bled in seawater for 20 minutes at three different temperatures; -1 °C, 2 °C and 6 °C. Photo taken after filleting, four days after catch. Figure from Paper IV.

In terms of time and temperature, the results of the current study support that the bleeding process should be carried out in unchilled seawater and that the time in the bleeding tank should exceed 12 minutes.

Research question **RQ1.4** focuses on water flow and recirculation during the bleeding process:

RQ1.4: “*What are the recommended design parameters for water flow and recirculation in onboard bleeding systems?*”

In **Paper II**, a time- and flow-controlled process was used to create variable bleeding conditions for whole gutted cod onboard a wet-fish trawler. The two examined design variables influencing the bleeding process were the pump flow recirculation (PFR) and the water replacement ratio (WRR). They were studied in five different combinations (groups) and the effects of the different bleeding conditions were evaluated by free fatty acids (FFAs), phospholipids (PLs) and total heme iron (HI) during freezer storage for up to four months. The results of the measurements of FFA content in Figure 18 and PL content in Figure 19 show that prolonged frozen storage affects the amount of both FFAs and PLs negatively (i.e., the FFA content increases with longer storage time, while the PL content is reduced). The values of FFAs (Figure 18) in the fresh cod samples after seven days show a significant difference between Group B and Group C and no significant difference for other groups. After one month of frozen storage, FFA was higher in all groups and there was a linear trend between the three groups bled in the screw conveyor tanks (A, B, and C). Significantly higher FFA content was measured in Group E, with no PFR, compared to the other groups after one month of frozen storage. All groups except E showed a rise in FFA content between Months 1 and 4. The FFA trend for Groups A, B, and C was similar between one and four months, while the difference was relatively smaller for Groups D and E.

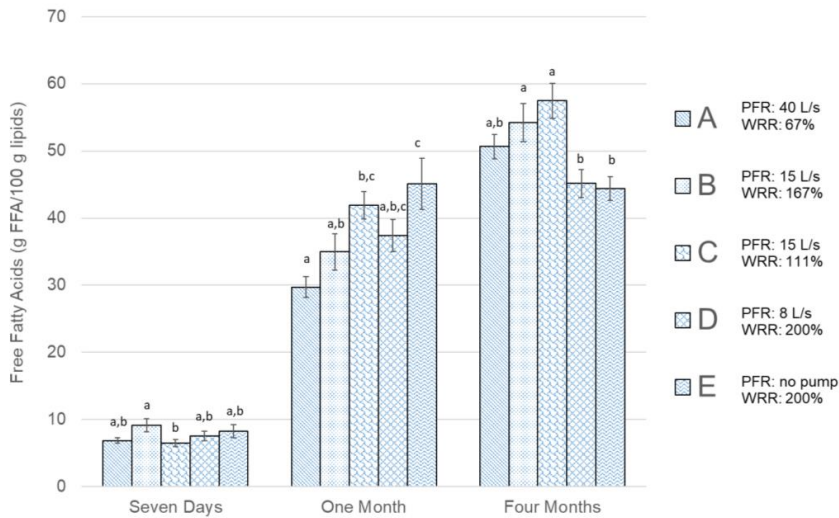


Figure 18: Free Fatty Acids [g FFA/100g lipids] results for all groups. The error bars show standard error of the mean values, n=6. Different letters a, b and c within storage times represent significant difference between experimental groups. PFR: Pump Flow Recirculation and WRR: Water Replacement Ratio. Figure from Paper II.

The PL content results in Figure 19 show that Group A had significantly the highest PL content in fresh cod after seven days of storage. After freezing, and as the storage time became longer, there was a decline in PL content for all groups, except Group E, which showed lower PL values after one month of freezer storage compared to four months. Therefore, after one month, Group E showed significantly different results compared to the other groups, showing both the highest FFA content and the lowest PL content. After four months of frozen storage, there was no significant difference in PL content between the groups.

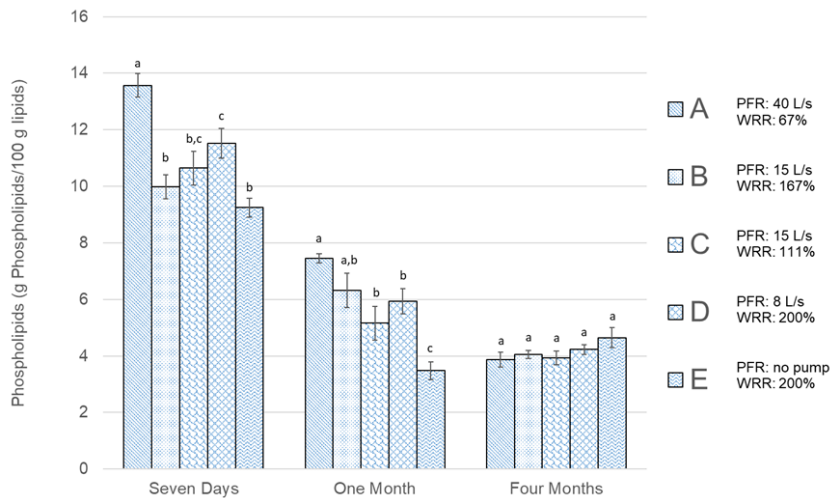


Figure 19: Phospholipids [g PL/100g lipids] results for all groups. The error bars show standard error of the mean values, n=6. Different letters a, b and c within storage times represent significant difference between experimental groups. PFR: Pump Flow Recirculation and WRR: Water Replacement Ratio. Figure from Paper II.

The reason for evaluating the bleeding process using FFA, PL, and HI content is because of their relation to the enzyme activity in the muscle at different storage temperatures (Karlsdottir *et al.*, 2014). The results of this study are mostly directly comparable to the findings of Minh *et al.* (2013, 2018) and Karlsdottir *et al.* (2014), who also applied similar methods to investigate the bleeding of cod and other lean fish. The results, showing FFA and PL development during simulated frozen storage in this study, support the contribution of blood components in the fish to lipid oxidation (Richards & Hultin, 2002). The trend of FFA formation in Figure 18 is like what Karlsdottir *et al.* (2014) and Dang *et al.* (2018) have seen during frozen storage of cod with different bleeding processes, however, the FFA values in this study are relatively higher. A decrease in the PL content of lean fish, like cod, during frozen storage is the main factor driving the accumulation of FFA, so a lower rise in FFA formation for Groups D and E can indicate less enzyme activity (and slower PL formation) in fillets from those groups, therefore indicating more effective blood removal. The PL content results in Figure 19 show a reverse pattern compared to the FFA content, as they decreased as storage time passed, likely due to enzyme activity. After seven days, the highest significant PL value was for Group A, which had the highest PFR and the lowest WRR. The second highest significant PL content after seven days was in Group D, with relatively low PFR but high WRR. This indicates that, for fresh cod, positive effects of both high PFR and WRR can be seen in the corresponding PL content. The results for PL content and the regression model indicate that the enzyme activity in the fish muscle is lower in cases where PFR exerts greater influence on the bleeding process than WRR. The effects of successful blood removal also seem to be most noticeable after one month of freezer storage, rather than in fresh cod after seven days or after four months of simulated frozen food-chain storage.

The study results therefore indicate that, with the bleeding medium to fish ratio of around 3:1 and enough WRR (over 100% replacement in 20 min), the PFR becomes the limiting design parameter regarding efficient blood removal and should be at least 10% of the tank volume per minute to ensure enough recirculation and flow of water in the bleed-out tanks.

4.2 Cooling process

In this section, the main factors that were found to influence the cooling process are presented in relation to the relevant RQs and the results discussed, linking the papers from which results and conclusions are drawn directly to the relevant research questions. The four factors mainly discussed are; Cooling methods and profiles, Cooling of gutted and ungutted cod, RSW storage of ungutted whole cod, and Evaluation of onboard systems.

Paper III contributes to research questions on the cooling process (**RQ2.1, RQ 2.2, RQ 2.3**), specifically the effects of different cooling media and comparison of cooling systems and chilling profiles, as discussed in this section. The main results show that the cooling time is 30-50% shorter with RSW compared to flake ice chilling and that the cooling time difference between gutted and ungutted fish is smaller with flake ice chilling than with RSW. Also, the comparison of the model results to onboard measurements indicates that the thermal conduction of the fish can be limiting to the energy transfer rather than the heat transfer coefficient. **Papers I and V** contribute further to research questions **RQ 2.3** and **RQ 2.4** in terms of onboard cooling storage of whole fish and the effects on shelf-life indicators.

Research question **RQ2** focuses on the cooling methods and profiles:

RQ2: “How do different cooling methods affect cooling profiles and the shelf-life of cod?”

Paper III addresses how different cooling methods affect the chilling profiles, while **Paper I** evaluates the difference in shelf-life between onboard ice cooling and superchilling methods. One of the aims of the study is to evaluate chilling methods of whole cod in controlled first-in first-out superchilling systems with different approaches, and compare them to the more traditional and well known method of flake ice chilling, studied e.g. by Thorvaldsson *et al.* (2010) and Laguerre *et al.* (2018). This is done in **Papers III** and **IV**.

Research question **RQ2.1** focuses on the cooling of gutted and ungutted cod:

RQ2.1: “What are the effects of different cooling methods on gutted and ungutted whole fish?”

A part of **Paper III** is dedicated to answering **RQ2.1**. The temperature graphs in Figure 20 and Figure 21 show results from controlled experiments. The chilling time from 8 °C to 0 °C in flake ice and chilled brine is presented for different size categories of ungutted and gutted fish. Figure 20a) shows the chilling profiles for the cod loin in flake ice and Figure 20b) for the same fish in chilled brine. The loin in chilled brine reaches 0 °C in approximately half the time compared to flake ice chilling. With flake ice there is limited difference in chilling time between ungutted and gutted fish, and less difference for larger fish in flake ice than for smaller fish. In chilled brine, however, the difference between ungutted and gutted fish is apparent, especially for medium sized cod. Figure 20a) shows shorter chilling times for ungutted fish in the smallest size category compared to the gutted (the gutted fish, however, was larger than the ungutted fish before gutting).

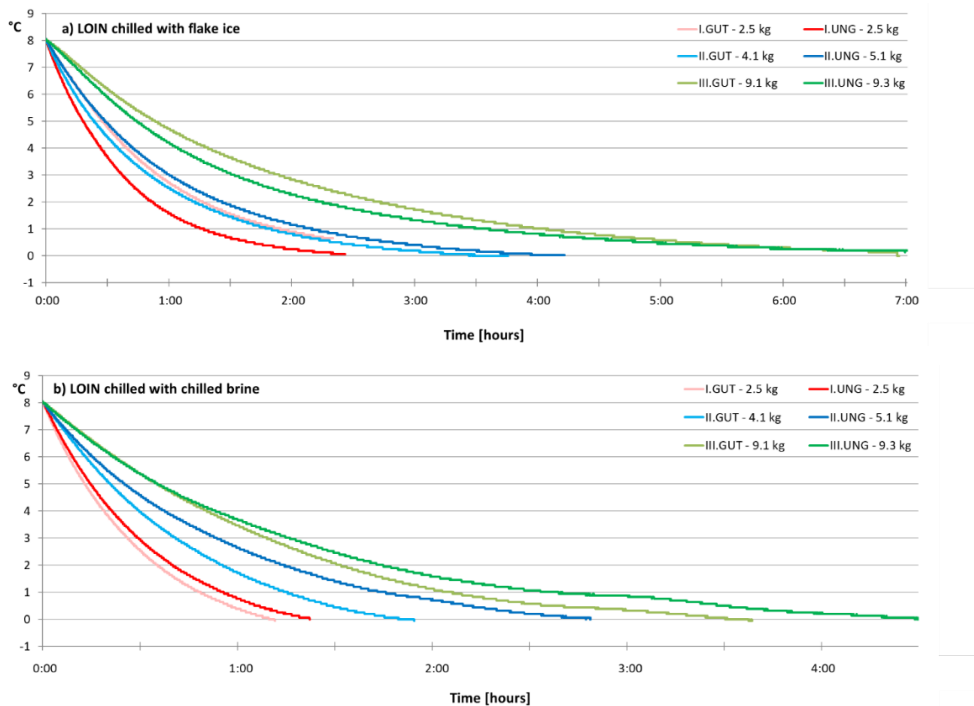


Figure 20: a) Core temperature of cod loin chilled with flake ice (at 0 °C) and, b) Core temperature of cod loin chilled with chilled brine (at -1 °C). Figure from Paper III.

Figure 21 shows temperature profiles of the cod tail during the same experiment. The tail reaches 0 °C between 3 and 5 times faster in chilled brine compared to flake ice chilling. Also, there is less noticeable difference between whole ungutted and gutted fish for the cod tail, compared to chilling times for the loin. This clearly shows that the gutting process does not affect the thermal resistance between the core and the surface of the tail as compared to the loin.

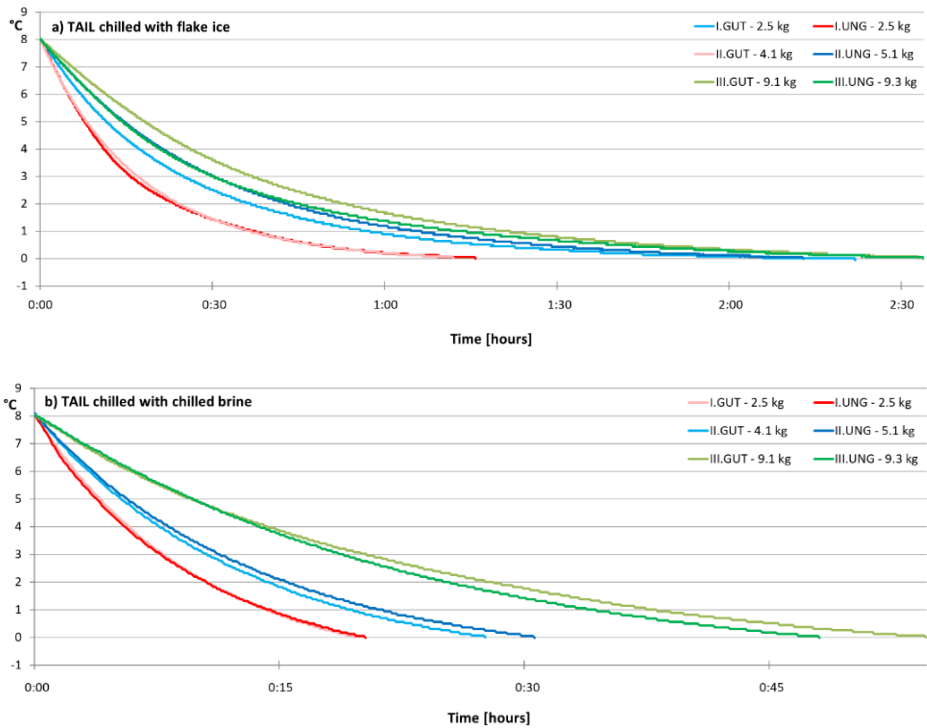


Figure 21: a) Temperature of cod tail chilled with flake ice (at 0 °C) and, b) Temperature of cod tail chilled with chilled brine (at -1 °C). Figure from Paper III.

Comparing chilling times of whole cod in Figure 20 to previous controlled experiments (Margeirsson & Arason, 2008, Thorvaldsson *et al.*, 2010, Bjarnason, 2012, Olafsdottir *et al.*, 2012), the chilling of cod, saithe and haddock in different cooling media shows mostly similar cooling profiles for similar sized fish. The main difference between chilling gutted cod with flake ice and chilled brine in this study is that the contact area of the brine and fish is larger than with ice as the brine rather flows inside the fish gut area. As expected, compared to flake ice chilling, the difference in chilling rate for ungutted vs. gutted fish is greater in chilled brine, where the cooling medium reaches a larger heat exchange area of the fish.

The chilling of whole ungutted fish is relevant in smaller boats where onboard facilities for gutting are not available or when the aim is to RSW chill whole ungutted fish and gut after landing (studied by Roach *et al.*, 1961). This was investigated in **Paper V** in an onboard RSW storage experiment for whole ungutted cod. The temperature inside and around the fish in the RSW tank is shown in Figure 22. The core temperature of the measured fish is between -0.7 to -0.9 °C during the RSW storage time and the ambient temperature (cooling medium or RSW temperature) of the fish is between -1 and -2°C during the fishing trip. The

temperature mapping during the RSW storage shows a stable core temperature of the fish through the onboard storage. The change in the temperature of the cooling medium is most likely due to a change in location of the measured fish within the RSW tub, as the temperature loggers were placed inside the fish loin and outside the fish. The storage conditions are similar to the experiments by Roach *et al.* (1961) and Longard *et al.* (1971) for onboard RSW storage of cod, and the results therefore comparable.

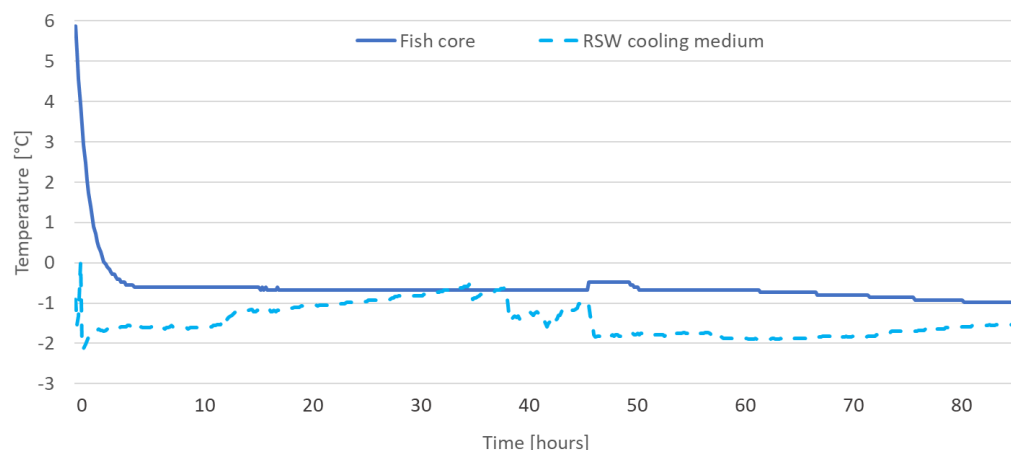


Figure 22: Temperature profiles of a fish (solid line) and the cooling medium (dotted line) during the fishing trip. Figure from Paper V.

Quality parameters evaluated after fillet processing, in the study described in **Paper V**, are shown in Table 6. The assessment of quality parameters after filleting shows negligible effects of RSW storage for 24 hours compared to direct gutted, bled and stored in ice (DC-ICE) but the groups that were kept longer, for 60 and 84 hours, in the RSW tub indicated significant increase in fillet gaping and yellow bile spots. The salt content (g/100g) was measured for all groups and showed a significantly ($p < 0.05$) lower content of 0.3% for groups DC-ICE and GC-RSW24h, compared to 0.4% for groups GC-RSW60h and GC-RSW84h. This indicates that longer RSW storage results in some salt absorption. Nematodes were counted in both the loin and the belly flap to evaluate whether prolonged storage of the whole fish with the gut and liver would result in nematodes moving into the flesh. No nematodes were found in the fish loins and, as seen in Table 6, the nematodes found in the belly flap are not clearly related to longer RSW storage, although group GC-RSW84h did show a higher count.

Table 6: Visual assessment defects in fillets after processing, 4 days after catch. Defects per fillet (n=10 per group), Mean \pm SEM; NS = no significant difference between groups ($p < 0.05$); Different superscript letters within each defect assessment denote a significant difference ($p < 0.05$) between the groups. Table from Paper V.

	DG-ICE	GC-RSW24h	GC-RSW60h	GC-RSW84h
Gaping	0.4 \pm 0.2 ^a	0.6 \pm 0.2 ^a	1.2 \pm 0.2 ^b	1.2 \pm 0.3 ^b
Bile spots	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.4 \pm 0.1 ^b	0.8 \pm 0.2 ^b
Nematodes in belly flap	0.0 \pm 0.0 ^a	0.6 \pm 0.3 ^{ab}	0.1 \pm 0.1 ^a	1.2 \pm 0.3 ^b
Blood spots ^{NS}	0.2 \pm 0.1	0.4 \pm 0.2	0.2 \pm 0.1	0.2 \pm 0.1

The study results show a significantly shorter cooling time for whole fish using RSW, compared to flake ice chilling. The cooling time difference between gutted and ungutted fish, however, is less with flake ice chilling than with RSW. The results of prolonged onboard RSW storage of whole ungutted fish show negative effects on quality parameters, compared to storage in ice.

Research question **RQ2.2** focuses on the evaluation of onboard systems:

RQ2.2: “How do industrial onboard systems compare to controlled experimental cooling profiles?”

Measurements in **Paper IV** show an example of the weakness of the older processing systems, showing how much difference there is in individual fish treatment. Figure 23 shows time and temperature profiles for the first and last fish to be processed in a saithe haul. The dotted lines represent the ambient temperature around the fish in the system and the solid lines the flesh temperature in the core of the fish. The first fish (blue line) is gutted after 5 minutes from catch and only stays in the bleeding tank for 4 minutes, after which it tumbles in the cooling tanks for over 60 minutes and therefore gets enough cooling time. The last fish (red line) to be gutted gets 15 minutes in the bleeding tank but only stays in the cooling tanks for a few minutes before being transferred down to the storage hold. The temperature profiles show the inconsistency of an uncontrolled system, with variable bleeding time and the final temperature of the first fish at 2 °C while the last fish is packed at 7 °C in the storage hold.

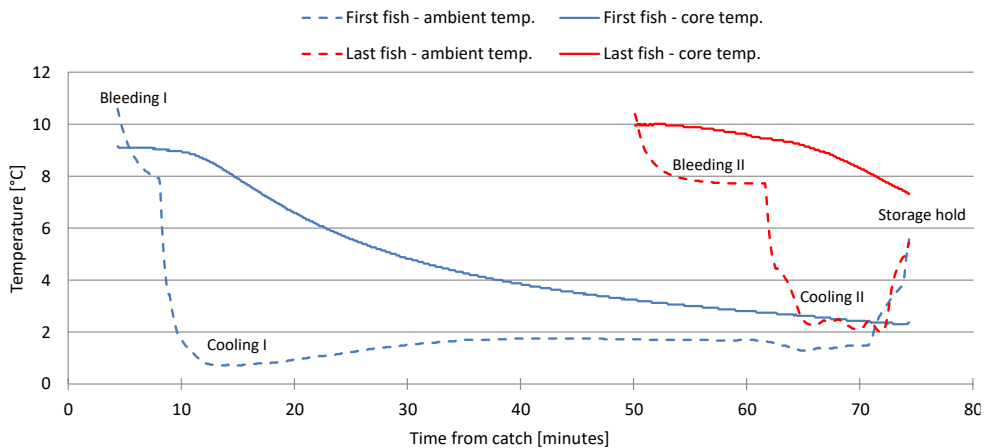


Figure 23: Temperature profiles for the first and last fish (3 kg) in an uncontrolled bleeding/cooling system. Figure from Paper IV.

Comparison of newly developed onboard systems to older ones confirms improvements in terms of both uniform bleeding and chilling. Figure 24 shows a fish temperature profile in a first-in first-out superchill system (the darker line) compared to traditional flake ice cooling as a reference. In the superchill system, the core temperature of the fish can reach close to -1 °C in about 1 hour after which it can be packed in a tub and stored in the storage hold at -1 °C ambient temperature without any ice. After landing, the superchilled fish is kept in a storage at -1 °C before fillet processing.

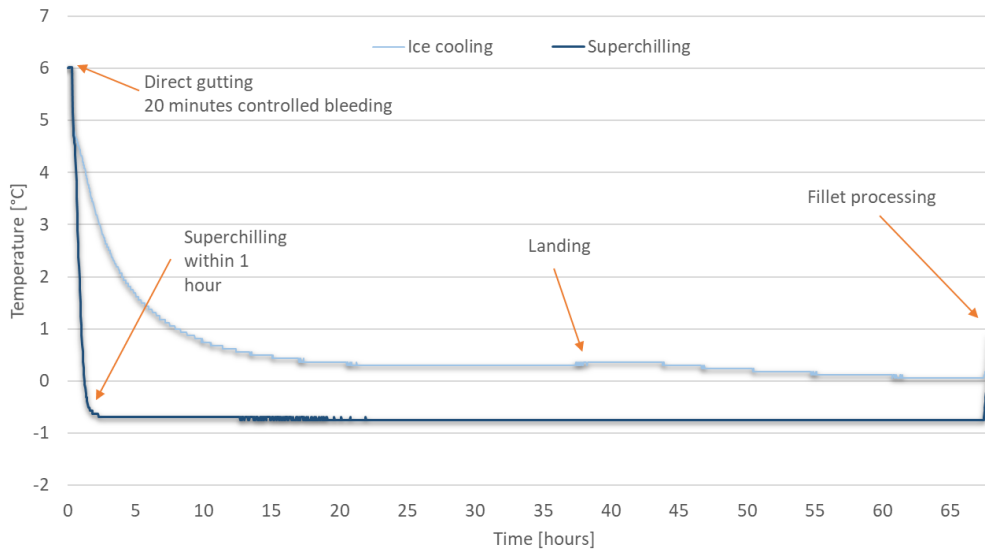


Figure 24: Controlled superchilling system compared to traditional flake ice cooling. Core temperature from catch to fillet processing for 3 kg fish. Figure from Paper IV.

The cooling process is further investigated in **Paper III** with onboard measurements on different wet-fish trawlers (Trawler A: slurry ice/CSW, Trawler B: RSW and refrigerated brine, two-step chilling, Trawler C: RSW, one-step chilling) to answer **RQ2.2**. Figure 25 shows temperature profiles measured for gutted cod onboard trawlers with different chilling systems. Trawlers A and C have steady chilling temperatures while Trawler B has a separate chill tank where the cooling medium drops to $-3.5\text{ }^{\circ}\text{C}$. The fish measured were weighed and sorted in three size categories, 2, 4 and 6 kg ($n=8$, average of eight fish for each size group). The chilling times here are similar to results of controlled experiments, but a more rapid chilling is seen in the controlled experiments at $-1\text{ }^{\circ}\text{C}$ chilled brine.

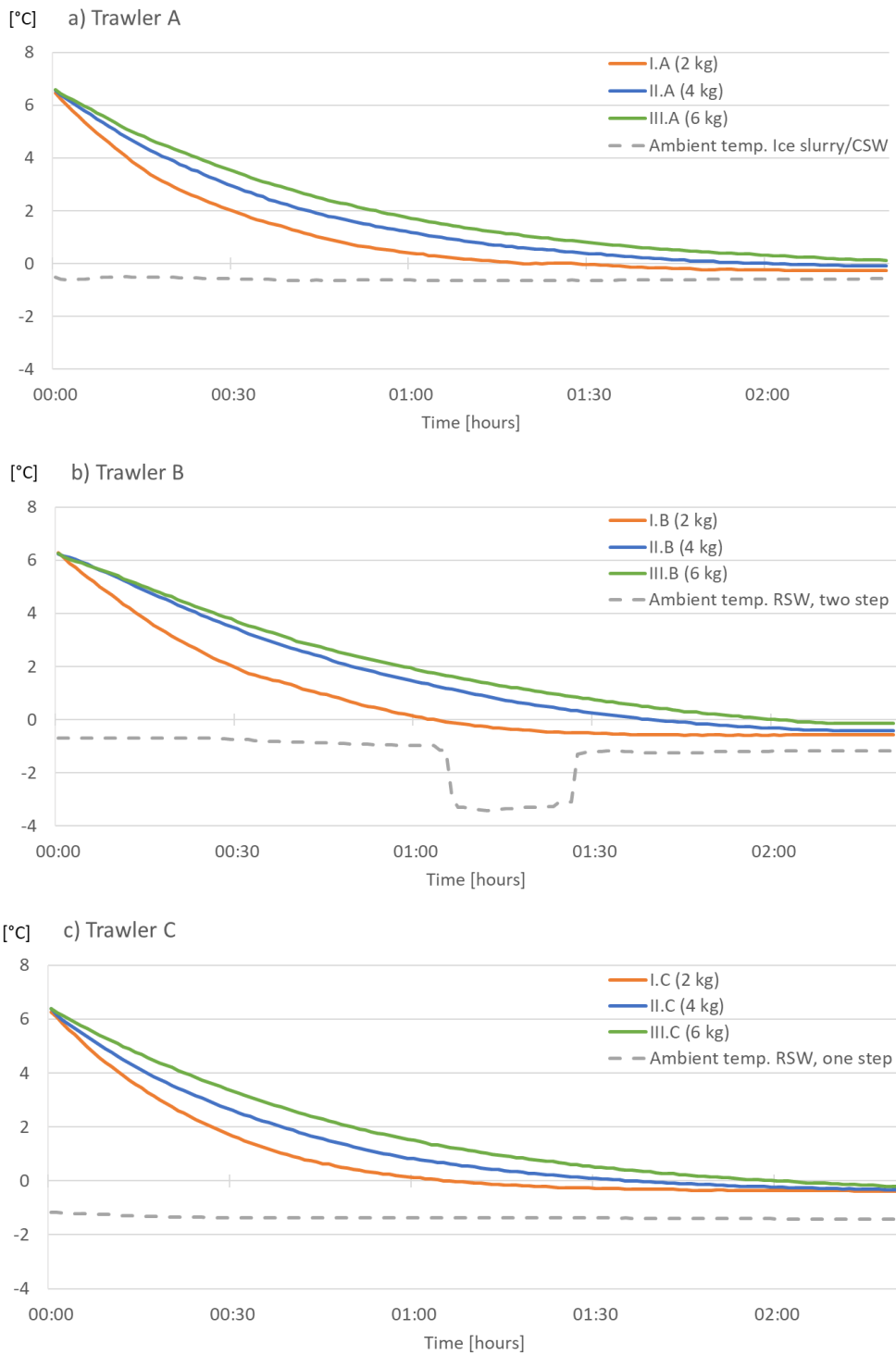


Figure 25: Chilling profiles for three size categories of gutted cod (loin in 2, 4 and 6 kg fish) for a) Trawler A: Ice slurry/CSW, b) Trawler B: RSW, two-step and c) Trawler C: RSW, one-step. Figure from Paper III.

Results of industrial onboard measurements show that the systems using RSW chilling methods are more effective than a slurry ice/CSW system. Compared to the results of the controlled experiment, the industrial systems show similar results, suggesting that the onboard cooling systems are effective in terms of heat transfer and cooling medium to fish ratio.

A part of **Paper III** is using these onboard temperature measurements to generate a model to estimate chilling time and the convective heat transfer coefficient (h) between 2, 4 and 6 kg fish and the cooling medium. This definition of chilling time refers to the time it takes to lower the initial fish temperature to 0 °C, regardless of the ambient temperature used. The results of the modelled chilling profiles for different convective heat transfer coefficients are compared to the results of the onboard experiments. The model uses data from onboard measurements, shown in Figure 25 for each trawler, to predict the cooling profile for the fish size groups. The estimation of the fish surface area was calculated with Equation (5), in relation to the fish mass.

Figure 26 shows the comparison of modelled chilling profiles for 4 kg gutted fish and measured profiles for different values of h . The cooling medium temperatures for each trawler are the same as were measured in the onboard systems, shown in Figure 25. The examples shown are results from Simulink modelling of loin for 4 kg gutted cod fish size. The temperature profiles are shown for different convective heat transfer coefficients ($h = 100, 300$ and $700 \text{ W}\cdot\text{m}^{-2}\cdot\text{K}^{-1}$). For h -values higher than $300 \text{ W}\cdot\text{m}^{-2}\cdot\text{K}^{-1}$, the difference in chilling time simulations becomes negligible for the model. A Biot number calculated using $h = 300 \text{ W}\cdot\text{m}^{-2}\cdot\text{K}^{-1}$, $k = 0.43 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$ and volume/surface area of the small fish size gives $\text{Bi} = 11.6$. For the larger fish sizes, the Biot number will increase. As the Biot number is considerably larger than 1, the conclusion is that the convective heat transfer is more efficient than the thermal conduction, i.e. the surface of the fish will exchange heat much more quickly than the rest of the body.

For 4 kg gutted cod, the model is closer to the final temperature for Trawler A but fits the measured chill curve best for Trawler B. The measurements of Trawler A and C, however, show a little more effective chilling during the first hour, compared to the model. The efficiency of the onboard cooling systems is supported by the results of the model, where industrial systems fit chilling profiles with h -values ranging from 300 to $700 \text{ W}\cdot\text{m}^{-2}\cdot\text{K}^{-1}$. The model shows negligible differences for h -values higher than $700 \text{ W}\cdot\text{m}^{-2}\cdot\text{K}^{-1}$ as the thermal conductivity (k), (estimated k -value for cod is $0.43 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$), becomes a limiting factor in the model, which is consistent with the results of Bantle *et al.* (2015b). With conventional methods for water chilling, the convective heat transfer coefficient is considered intensive at $h \approx 350 \text{ W}\cdot\text{m}^{-2}\cdot\text{K}^{-1}$ and can, with a high degree of turbulence of the refrigeration medium, be up to $h = 720\text{-}760 \text{ W}\cdot\text{m}^{-2}\cdot\text{K}^{-1}$ (Fikiin, 1992). Estimations of ice slurry systems have shown h around $750 \text{ W}\cdot\text{m}^{-2}\cdot\text{K}^{-1}$ (Fikiin *et al.*, 2005). The heat transfer coefficient for flake ice is estimated between 15 and $35 \text{ W}\cdot\text{m}^{-2}\cdot\text{K}^{-1}$ (Jain *et al.*, 2005, Tolstorebrov *et al.*, 2019a,b).

4 kg gutted cod

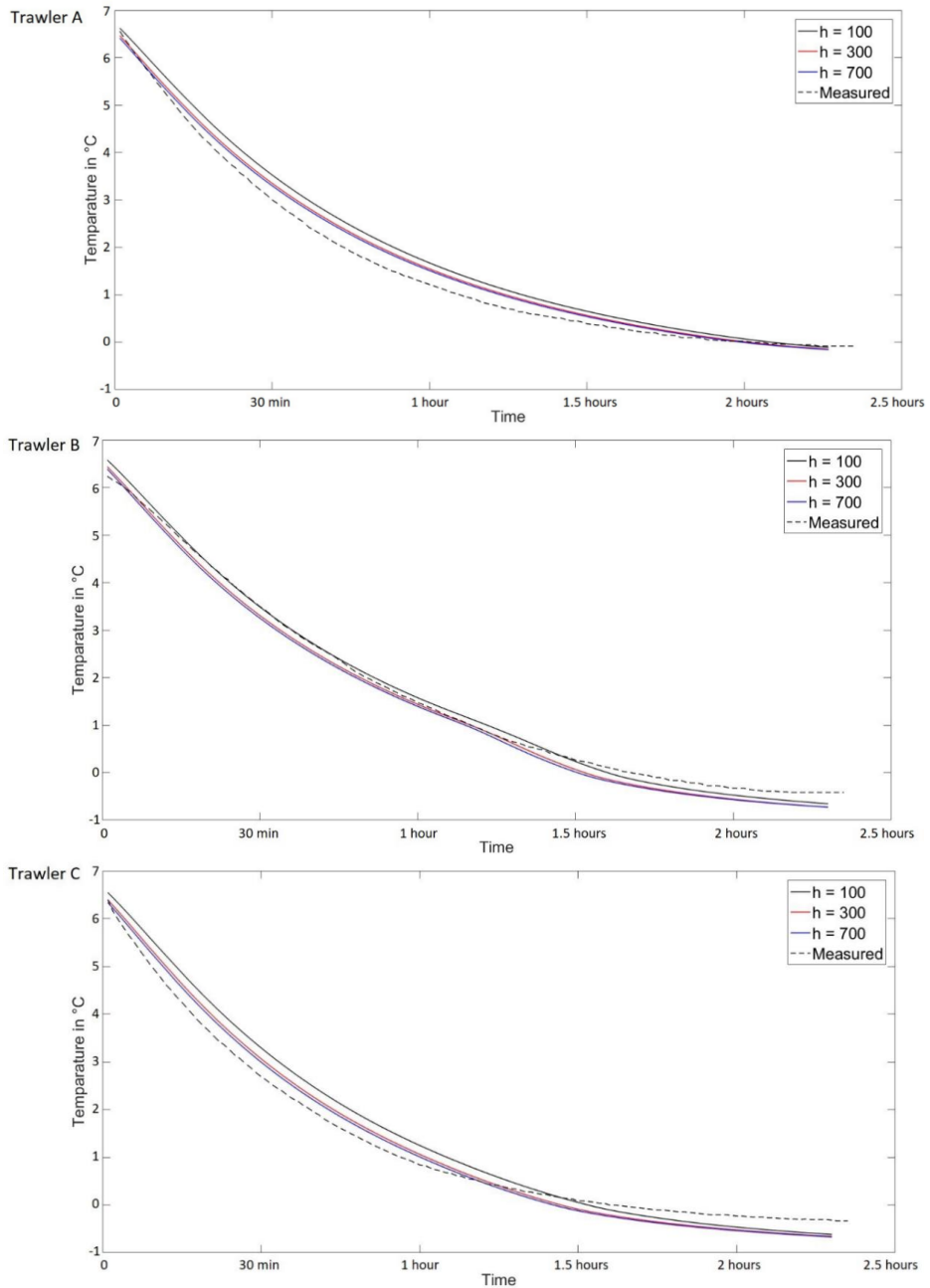


Figure 26: Comparison of chilling profiles for loin measurement and model for 4 kg gutted cod, Trawlers A, B and C. Figure from Paper III.

Table 7 shows chilling times generated from simulations and for measurements, i.e. the chilling time of gutted cod loin in minutes, from 6 °C to 0 °C. Also shown in the table are average cooling rates [°C/min] for the temperature interval from 6 °C to 0 °C.

Table 7: Cooling time (in minutes) of gutted cod (loin) from 6 °C to 0 °C. Average cooling rate is shown in brackets [°C/min]. Table from Paper III.

		Size group I (2.5 kg)	Size group II (4.1 kg)	Size group III (9.1 kg)
	Cooling method	Cooling time [ave. cooling rate °C/min.]	Cooling time [ave. cooling rate °C/min.]	Cooling time [ave. cooling rate °C/min.]
Experiment 1, controlled environment	Flake ice (0 °C)	120 min. [0.05]	205 min. [0.03]	390 min. [0.02]
	Chilled brine (-1 °C)	70 min. [0.09]	105 min. [0.06]	185 min. [0.03]
Experiment 2, industrial systems	Trawler A	80 min. [0.08]	120 min. [0.05]	-
	Trawler B	65 min. [0.09]	105 min. [0.06]	-
	Trawler C	70 min. [0.09]	110 min. [0.05]	-
Model	h=700	65 min. [0.09]	100 min. [0.06]	145 min. [0.04]

A study by Tolstorebrov *et al.* (2019b) on industrial RSW systems for salmon showed that optimal effectiveness was achieved when the density of fish in the spiral tanks was decreased to below 33% (fish to seawater ratio) and with RSW flow close to 200 m³·h⁻¹. The results of this study suggest that the fish density and RSW flow rate of the industrial systems are not limiting factors as their chilling profiles are close to the controlled experiments and the model results. The systems are, however, close to their limitations in terms of lowering the temperature of the cooling medium if they are to avoid the risk of freezing the tail end of the fish (or the surface, or belly flap, of gutted fish). This is one of the challenges of industrial superchilling systems, as pointed out by Bantle *et al.* (2016), in lowering the cooling medium temperature to below T_f and thus risking partial freezing. This study also concurs with Bantle *et al.* (2016) and Tolstorebrov *et al.* (2019a) on a common industry challenge that large fish sizes are difficult to chill effectively within reasonable onboard processing time intervals. With more groundfish trawlers moving from traditional use of ice to superchilling the fish, RSW and (super)chilled storage without ice, the new systems have become more efficient in chilling time and temperature control, labour factor, and in some ways energy efficiency. However, they have become more fragile in terms of maintaining the chill chain and the risk of excessive partial freezing of the fish. An example of the importance of maintaining a superchilled chill chain for cod is shown in **Paper I** where most benefits of onboard superchilling are lost when superchilling is not maintained throughout processing and transportation.

4.3 Storage

Papers I, III and V contribute to research questions on the cooling process in terms of storage (**RQ2.3** and **RQ2.4**), specifically the effects of superchilling on fish quality and shelf-life as discussed in this section. The results in **Paper I** show that onboard superchilling of whole fish reduces some spoilage factors but does not considerably extend shelf-life. However, the simulation of a superchilled value chain to the market indicates that the freshness period and shelf-life can be extended by up to four days, compared to traditional ice cooling and +1 °C storage. Also, results from **Paper V** show that onboard RSW storage of whole ungutted cod for more than 24 hours is not recommended.

In this section, the main factors that were found to influence storage and shelf-life are presented in relation to the relevant RQs and the results discussed, linking the papers from

which the results and conclusions are drawn directly to the relevant research questions. The three factors mainly discussed are; Onboard superchilling of whole gutted fish, Onboard storage of whole ungutted fish, and Superchilling of fillets/portions.

Research question **RQ2.3** focuses on the onboard superchilling process:

RQ2.3: “What are the benefits of onboard superchilling of cod compared to traditional ice cooling?”

Temperature reduction is evaluated in **Paper III**, which aim is to evaluate the chilling methods for whole cod in controlled first-in first-out superchilling systems with different approaches and compare them to the more traditional and well known method of flake ice chilling, studied e.g. by Thorvaldsson *et al.* (2010) and Laguerre *et al.* (2018). **Papers I** and **III** address **RQ2.3**, in terms of the evaluation of onboard superchilling on fish quality and shelf-life.

The main objective of **Paper I** is to evaluate the effects of superchilling wild cod on product quality and shelf-life compared to traditionally iced cod, both regarding onboard cooling and storage of whole gutted fish, and chilled or superchilled storage of fillets throughout shelf-life of the product. Samples from the temperature profiles shown in Figure 24 are evaluated at different storage temperatures simulating the whole value chain in **Paper I**. The results for different storage temperatures at +1 °C and -1 °C show (Figure 27) that the main deciding factor in the fresh fish chill chain is the temperature of the fillets after processing. However, the superchilling of whole gutted fish onboard results in less bacterial growth and slows down some spoilage factors.

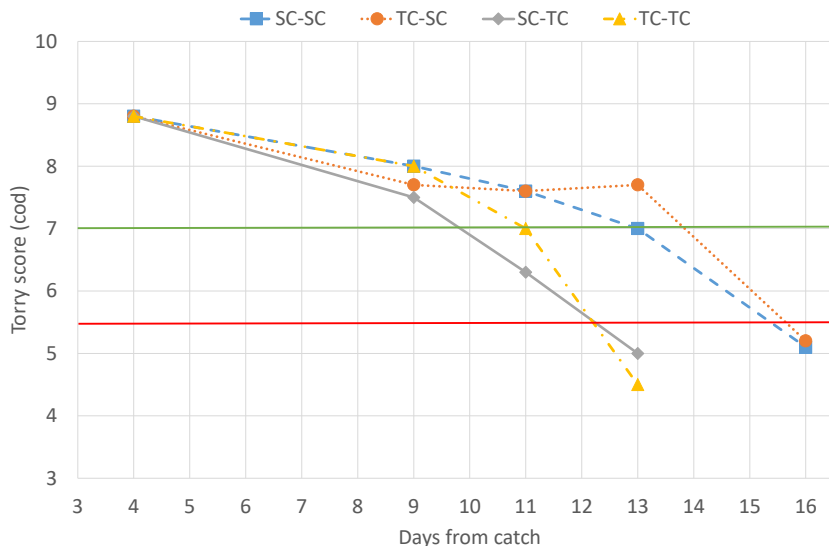


Figure 27: Mean Torry freshness scores for cooked cod samples on Days 4, 9, 11, 13 from catch. Groups are labelled by different onboard chilling methods (SC-superchilled, TC-traditional ice cooling) and different fillet storage temperatures (SC stored at -1 °C and TC stored at +1 °C). The green line indicates loss of freshness (Torry score = 7) and the red line indicates end of shelf-life (Torry score = 5.5). Figure from Paper IV (adapted from Paper I).

The results of **Paper V** show negative effects of prolonged onboard RSW storage of whole ungutted cod on some quality parameters of cod fillets. Figure 28 shows the microbial

growth for all groups measured on Days 4, 7 and 11 from catch. The TVC count and H₂S producing bacteria increases with time between Days 4, 7 and 11 for all groups. There is also a notable trend of increasing bacterial growth with longer RSW storage.

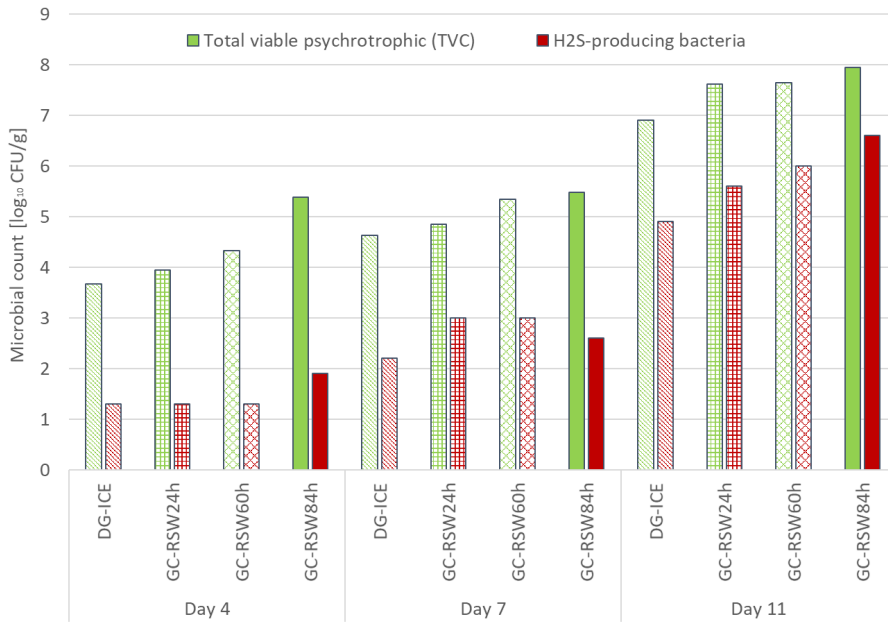


Figure 28: Development of microbial count for all groups on Days 4, 7 and 11 from catch. DG-ICE: Direct gutting, bled, iced in tub. GC-RSW24h: Gill-cut, bled and stored in RSW for 24 hours. GC-RSW60h: Gill-cut, bled and stored in RSW for 60 hours. GC-RSW84h: Gill-cut, bled and stored in RSW for 84 hours. Figure from Paper V.

The microbial measurements show a clear trend in bacterial growth related to longer RSW storage, both for TVC and H₂S bacteria (reference consumption limit of 6 log₁₀ for TVC). In a study by Reynisson *et al.* (2010), whole gutted haddock was stored in liquid ice and plate ice for 8 days. The results showed that the microbial growth was delayed at early storage independent of the cooling method, however, while the liquid ice provided faster initial cooling it created unfavorable conditions under extended storage (where active spoiler *P. Phophereum* became dominant). Digre *et al.* (2011b) found a significantly lower difference of 1.3 log₁₀ for TVC and 0.6 log₁₀ for H₂S bacteria for cod stored in ice-slurry compared to flake ice. However, neither of these studies were storing whole ungutted cod like the current study and were therefore dealing with less gut bacteria during storage. Olsson *et al.* (2007) measured the bacterial count CFU and H₂S bacteria ratio in wild cod after different storage times for fillets stored in ice. Their results were 3-4 log₁₀ after 3 days, 7-8 log₁₀ after 7 days and 9 log₁₀ after 14 days, which are comparable or higher values than the microbial values shown in Figure 28.

Compared to the study by Watson (1996) for gutted cod stored on ice, the current study shows lower microbial values for group DG-ICE. Watson (1996) measured TVC 5-6 log₁₀ after 6 days on ice and 7 log₁₀ on Day 12. For gutted cod stored in RSW by Watson (1996), similar to the conditions of the current study, the TVC values were 3-4 log₁₀ after 5 days in RSW and 4-5 log₁₀ after 12 days. These values are considerably lower than the current study values, likely due to the contamination of the guts in the ungutted storage period.

The results of spoilage bacteria and TVB-N in Figure 28 and Figure 29 are, for Days 4 and 7, similar to quality and shelf-life estimations by Lauzon *et al.* (2010) for cod processed 3 days from catch and stored at 2 °C. The bacteria and TVB-N results in the current study are higher on Day 11, likely due to the higher storage temperature of 4 °C. Olafsdottir *et al.* (2006) measured the shelf-life of ice cooled cod stored at an average temperature of 2 °C and found a shelf-life limit on day 11 when the TVB-N content was 34 (mg N/100g), the TVC 8 log₁₀ and the H₂S bacteria 6.6 log₁₀. The reference group in this study, DG-ICE, is lower in both TVB-N and spoilage bacteria, compared to the Olafsdottir *et al.* (2006) experiment, indicating reasonable quality changes and shelf-life considering the handling and storage temperature. The trend between groups seen in the bacterial growth was also noticeable in the TVB-N formation, shown in Figure 29, with insignificant difference between groups after Days 4 and 7. A significant difference between groups is found only on Day 11, in higher TVB-N content for groups GC-RSW60h and GC-RSW84h on Day 11 compared to groups DG-ICE and GC-RSW24h. This indicates that the rise in TVB-N occurs between 24 and 60 hours in RSW storage. The results of TVB-N development in the current study concurs to TMA-N development during storage of cod stored in crushed ice and RSW at 0 °C in the study by Roach *et al.* (1961). The development remains similar for the first week of storage but, after that, the RSW 60h and 84h groups show significantly higher values compared to DG-ICE cod and the RSW 24h group, showing slower trimethylamine (TMA) development. In a study by Longard *et al.* (1971), RSW storage of gutted and whole ungutted cod showed slower development of TMA during the first 7 days and more rapid formation after that. Another experiment in the same study, where the fish was kept in fluctuating RSW temperature (-1 °C to +1 °C), showed an exponential growth for whole ungutted cod compared to a linear TMA development in the gutted group.

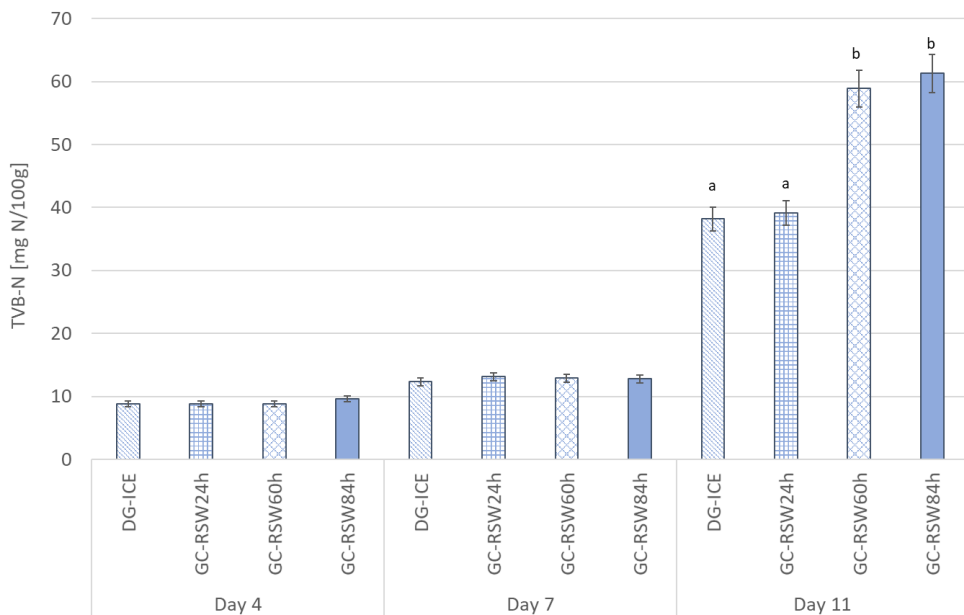


Figure 29: TVB-N values (mean ± SEM, n=3) on Days 4, 7 and 11 for all groups. Different superscript letters within each group and day denote a significant difference of p<0.05. Figure from Paper V.

The effects of RSW storage in **Paper V** are noticeable in water content and WHC, most likely affected by bacterial growth and the higher salt content in groups GC-RSW60h and

GC-RSW84h. Olsson *et al.* (2007) concluded that the relation of percentage of liquid loss (same definition as WHC) of cod muscle to bacterial growth was only broadly related and indirect, likely to be temporal and not causal.

The current study results therefore indicate that, compared to traditional ice cooling, superchilled storage after fillet processing is the main deciding factor regarding shelf-life, although the onboard superchilling process for whole ungutted fish can delay some spoilage factors. In terms of onboard storage of whole fish, however, the results show that onboard RSW storage of whole ungutted fish is not advisable for more than 24 hours, prior to gutting.

Research question **RQ2.4** focuses on the effects of superchilling on shelf-life:

RQ2.4: “How does superchilling affect whole fish and fillets in terms of shelf-life?”

The effects of superchilling on shelf-life is further investigated in **Paper I** where H₂S-producing bacteria appears to grow more rapidly in the fish that was iced whole compared to the samples that were superchilled onboard (Figure 30). Total viable counts in fillets are lower under superchilled storage conditions. The TVB-N level of fish iced onboard and stored chilled rose to above the consumption limit on Day 11 after catch, while the other samples were below (Figure 31). TVB-N levels where fillets were stored superchilled were still under the limit 16 days after catch, sensory evaluation however rejected both groups at that point. According to the sensory evaluation, the effects of superchilled storage of fillets had a much greater impact on quality changes than the onboard cooling method, resulting in a 2-4 day extension of the freshness period and 3 days longer shelf-life. The effects of superchilling whole fish onboard were less noticeable, however, the method slowed the growth of H₂S-producing bacteria and spoilage attributes.

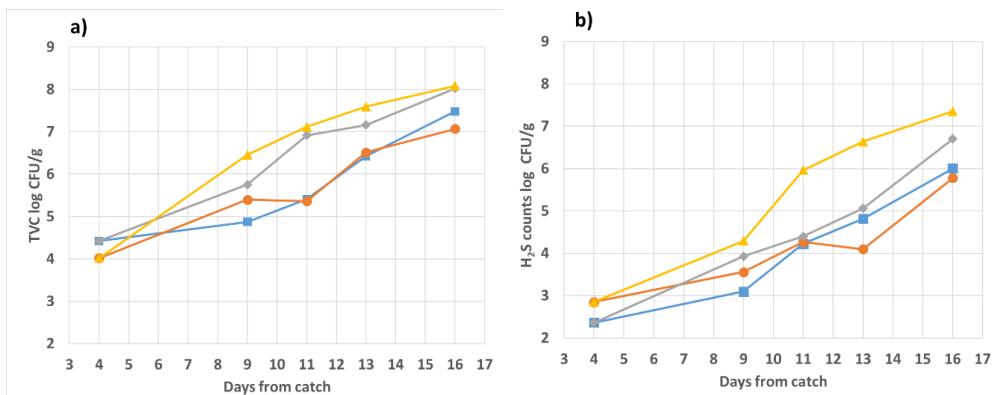


Figure 30: Bacteria counts for groups on Days 4, 9, 11, 13 and 16 from catch. Group A (blue ■), B (orange ●), C (yellow ▲), D (grey ◆). Each point represents an average of two independent measurements (n=2). (a) Development of the total viable psychrotrophic counts (TVC) for different cooling and storing conditions. (b) Development of the H₂S-producing bacteria count for different cooling and storage conditions. Figure from Paper I.

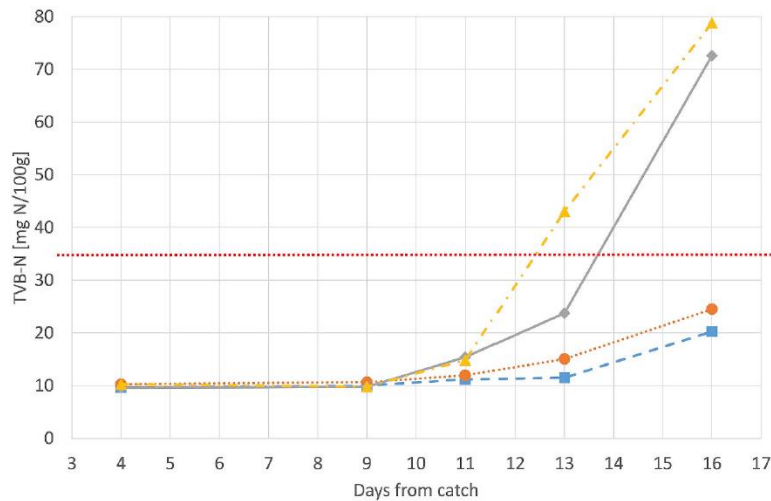


Figure 31: TVB-N values for groups on Days 4, 9, 11, 13 and 16 from catch. Group A (blue ■), B (orange ●), C (yellow ▲), D (grey ◆). The dotted red line shows the consumption limit for cod by EU regulation (2005). Each point represents an average of two independent measurements (n=2). Figure from Paper I.

Temperature monitoring showed that the industrial superchilling method under consideration onboard the trawler manages to bring the core temperature of fish close to -1°C within 1 hour and the ice-less storage hold was relatively stable, showing minimal ambient temperature fluctuations. The superchilling temperature profiles were like the ones Olafsdottir *et al.* (2012) produced, resulting in a two to three day shelf-life extension of superchilled as compared to non-superchilled whole cod. In that study, however, the two groups for whole cod only showed different Torry scores after 14 days from catch. Superchilling whole gutted fish onboard did not show improved product quality or lengthen the freshness period or the shelf-life of the cod, as suggested by Olafsdottir *et al.* (2012). A relatively short time from catch to processing plant, three days, could be a factor that minimises the benefits of the onboard whole fish superchilling, however, three days is a common time from catch to processing plant for Icelandic fresh fish trawlers. The timing of rigor mortis, which is delayed at lower temperatures, could influence the shelf-life and gapping, as Aune *et al.* (2014) showed, with less fillet contraction when filleting at lower temperatures. This could be a deciding factor in slower bacterial growth for the superchilled whole fish if the rigor mortis period were longer.

Compared to ice cooling, the study results do not show a significant extension of shelf-life for superchilling whole ungutted fish onboard. The onboard superchilling of whole fish was, however, found to slow the growth of H_2S -producing bacteria and the formation of TVB-N.

4.4 Overview of key findings of Papers

An overview of the six papers on which this dissertation is based, and their contribution to the research questions (RQs), is given in Table 8. The research that was conducted includes studies on time and temperature control, variable bleeding conditions, superchilling methods and superchilled storage, with the focus on the effects of these factors on shelf-life and product quality.

Table 8: Overview of research papers - key findings and contribution to RQs.

Paper I: The effects of superchilling on shelf-life and quality indicators of whole Atlantic cod and fillets.	
Study aim	Key findings
To evaluate the effects of superchilling cod on product quality and shelf-life compared to traditionally iced cod, regarding both onboard cooling and storage of whole gutted fish and chilled or superchilled storage of fillets throughout the product shelf-life.	<ul style="list-style-type: none"> • Superchilling whole fish onboard did not notably extend shelf-life compared to ice cooling. • Onboard superchilling of whole fish was found to slow the growth of H₂S-producing bacteria and formation of TVB-N. • Superchilled storage after fillet processing was found to be the main deciding factor regarding shelf-life.
Contributes to RQ2.3 and RQ2.4 .	
Paper II: Onboard Evaluation of Variable Water Flow and Recirculation Effects on Bleeding of Atlantic Cod (<i>Gadus morhua</i>).	
Study aim	Key findings
To explore the effects of WRR and PFR of the bleeding medium on the bleeding efficiency of Atlantic cod and the resulting product quality by evaluating FFA, PL and HI content.	<ul style="list-style-type: none"> • The pump flow recirculation had more effect on reducing lipase activity than the water replacement. • Effects of successful bleeding were most noticeable after one month of frozen storage. • After four months simulated frozen food-chain storage, the lipase activity had evened out the effect of bleeding methods on fat derivatives.
Contributes to RQ1.4 .	
Paper III: Onboard pre-chilling of ungutted and gutted Atlantic cod in different cooling media - Temperature measurements and analytical modelling -	
Study aim	Key findings
To evaluate chilling methods of whole cod in controlled onboard superchilling systems with different approaches and compare them to the more traditional and well-known method of flake ice chilling.	<ul style="list-style-type: none"> • Chilling time difference between gutted and ungutted fish was less with flake ice chilling than with RSW. • Chilling time was found to be 30-50% shorter with RSW compared to flake ice chilling. • Results from onboard systems were comparable to controlled experiments. • Comparison of the model results with onboard measurements indicate that the thermal conduction within the fish is limiting to the energy transfer, rather than the convective heat transfer coefficient.
Contributes to RQ2.1 , RQ2.2 and RQ2.3 .	

Paper IV: Effects of on-board bleeding methods and superchilling on quality of cod and saithe.	
Study aim	Key findings
To evaluate the onboard processing in terms of product quality for fresh cod and saithe. This mainly includes studies on time and temperature control and different bleeding conditions and methods.	<ul style="list-style-type: none"> • The waiting time in the reception hold was found to be the main deciding factor influencing fillet colour. • Fish should be bled within 30 minutes after catch to maintain quality in terms of fillet colour. • The bleeding process should exceed 12 minutes and be carried out in unchilled seawater. • Older uncontrolled systems are unreliable in terms of both bleeding and cooling. • The two-step process of bleeding and gutting showed better results in terms of fillet colour, compared to direct gutting.
Contributes to RQ1.1, RQ1.2, RQ1.3 and RQ2.2 .	
Paper V: Effects of onboard refrigerated seawater (RSW) storage of whole ungutted cod on quality parameters of fillets.	
Study aim	Key findings
To evaluate the effects of onboard RSW storage of ungutted cod on the quality parameters of fillets.	<ul style="list-style-type: none"> • Negative effects of prolonged onboard RSW storage, compared to storage in ice, of whole ungutted cod on quality parameters; gaping, bile spots, spoilage bacteria, and TVB-N. • Not recommended to store whole ungutted cod in onboard RSW for more than 24 hours.
Contributes to RQ2.1 and RQ2.3 .	
Paper VI: Effects of different bleeding methods and storage on cod fillet colour.	
Study aim	Key findings
To evaluate the effects of different bleeding methods and storage on cod fillet colour.	<ul style="list-style-type: none"> • Results did not show a clear difference between the cutting method groups; direct gutting (DG), gill-cut (GC) and throat cut (TC) • Colour evaluation showed a significant difference in fillet colour affected by the six days storage time and different locations on the fillet.
Contributes to RQ1.2 .	

All six papers contribute to answering the dissertation's main research question:

RQ: *What are the effects of onboard processing on groundfish quality?*

The overall conclusions of the study are presented in Section 5 Conclusions, aiming to answer the main research question with the principal focus on evaluating new onboard processes from catch to storage.

5 Conclusions

This study was initiated to contribute to filling a research gap in the onboard value chain of groundfish. The goal was to evaluate new onboard processes, focusing on bleeding and cooling, and provide measurement results to be used for defining design criteria or as a benchmark for fresh fish producers regarding shelf-life and quality indicators. The overall conclusions of the study are that the new onboard processes, from catch to post processing storage, are more reliable than the older systems in terms of bleeding and cooling efficiency and that they have a positive impact on raw material quality and shelf-life. The results contribute to deciding which design parameters should be adjusted during the bleeding process to achieve more effective bleeding of the fish. The results on the cooling process show that the temperature profiles of the industrial onboard systems measured are comparable to the ones obtained from controlled experiments, indicating that fish bulk density and flow rates are not limiting or restricting factors for the industrial systems examined.

The onboard superchilling of whole gutted fish, compared to traditional ice cooling, does not considerably extend shelf-life but was found to reduce the growth of H₂S-producing bacteria and TVB-N. The storage and/or transport methods for fresh fish products after the processing of fillets clearly have a greater impact on the quality of fish products than the specific method of onboard cooling. Superchilled storage after fillet processing is the main deciding factor regarding shelf-life. Simulation of a superchilled value chain to the market indicates that the freshness period and shelf-life can be extended by up to four days, compared to traditional ice cooling and +1 °C storage. This means that, with the best superchilling conditions, the shelf-life of fresh fish products can be extended by a time period corresponding to the sea freight transport time from Iceland to Europe, making the more economic sea freight a more viable option compared to transport by airplane.

The novelty of the research lies in studying the effects of onboard superchilling on the groundfish value chain, including the onboard superchilling of whole fish after gutting, while other studies have focused on superchilling later in the chain. Examining the influence of the WRR and PFR on the bleeding process of cod is novel to this study and other research involving cooling of whole fish has not included a comparison of chilling processes for gutted and ungutted fish.

The main research question put forth in the study is:

RQ: *What are the effects of onboard processing on groundfish quality?*

The main conclusions of the study in relation to the **RQ** are:

- Innovation in the onboard groundfish processing has resulted in more uniform processing and quality of fresh products.
- Effective processing from the reception hold is key to the bleeding process outcome.
- Bleeding process improvements can be achieved by adjusting design variables in the bleeding tanks.

- The positive effects of successful bleeding of the fish can become more noticeable later in the value chain, rather than immediately after filleting.
- The onboard cooling systems investigated are effective in terms of heat transfer and cooling medium to fish ratio.
- In terms of shelf-life, superchilling of fillets and post processing superchilled storage is more important than the superchilling of whole fish onboard.

5.1 Scientific contribution

The scientific contribution of the Ph.D. study includes an evaluation of the impact of new industrial technology in the first stages of the groundfish value chain in Iceland. The innovation of trawler processing is aimed at improving automation, sustainability and process control, and the benefits of the new solutions need to be analysed for verification and for further technological developments in next-generation fresh fish trawlers. The following results of the study contribute, as guidelines for producers and technical developers, towards achieving the ideal handling of groundfish through primary onboard processing. Regarding the bleeding process, groundfish should be bled within 30 minutes after catch to maintain best quality. The two-step method of bleeding and gutting is suitable for automation of the bleeding process onboard trawlers to shorten the waiting time in the reception hold, which was found to be the main deciding factor influencing fillet colour. The bleeding process following direct gutting should exceed 12 minutes and be carried out in unchilled seawater ensuring sufficient movement and refresh rate of the bleeding medium. Onboard superchilling of whole fish reduces some spoilage factors but does not considerably extend shelf-life compared to ice cooling. However, superchilled storage after fillet processing is the main deciding factor regarding shelf-life. Simulation of a superchilled value chain from catch to market indicates that the freshness period and shelf-life can be extended by up to four days, compared to traditional ice cooling of whole fish and storage of fillets at 1 °C. These results have been considered when redesigning the new processing decks for Icelandic trawlers in the last few years and have contributed to the delivery of higher quality raw material to the secondary processing. The results of the study can set a benchmark for fresh fish producers regarding shelf-life and quality indicators of the fish.

For the scientific contribution of the study, there are also novel results on the comparison of chilling gutted and ungutted fish, as well as on the effects of superchilling on whole fish and the effects of water flow conditions on bleeding. The results also validate the effects of time in the reception hold, the method of cut and gutting, and the chilling time of fish for different cooling media.

5.2 Evaluation of the research

In this section, the reliability and validity of the research findings are discussed. Evaluation of *reliability* in research deals with the consistency of the study results and their accuracy. Consistency is generally achieved when the results can be reproduced using a similar methodology and performed under similar conditions. The evaluation of the research's *validity* considers the generalisability of the study and to which extent the research is credible in measuring to the aims of the study (Golafshani, 2003).

When analysing the reliability of this study there are environmental and natural variances that have obvious effects on the reproduction of the results. The study relies mostly on individual experiments that involve the catching of wild fish in the ocean around Iceland. The variability is therefore considerable in terms of seasonal and natural changeability. Many limitations in the experimental setup were encountered when choosing time periods and hauls where the fish is in a similar natural condition (e.g. with regard to the spawning period). The timing and method of taking fish samples from the trawl and reception hold was, however, carried out in the same way in all experiments.

In terms of validity, the indicators for evaluating fish quality are not generalised and the specific indicators and methods chosen to evaluate the bleeding process in the study can be questioned. The indicators were chosen in consultation with the industry partners, focusing on specific products. The methods are therefore limited in comparison to studies using other indicators, for example studies that measure residual blood or iron content of the fish muscle. Some of the measurements used rely partly on human factors, like the expert panel for the sensory evaluation. As for the validity of the cooling profile measurements, they rely on a more generalised indicator, i.e. temperature. In terms of reliability, there is the accuracy and placement of the loggers to consider as well as the precision of the experimental setup environment.

5.3 Recommendations for the industry

The study originated around the redesign of processing decks in trawlers built by the Icelandic fishing industry. The study results can be an important input for adjusting the design variables of bleeding and cooling systems and can improve onboard processes. The benefits of controlled onboard processing systems are apparent in more uniform fish quality and the reduced risk of overloading the systems, which generally results in less effective bleeding and cooling. The new system designs also result in gentler handling of the catch as there are fewer drops and less physical load to the catch, especially when the fish is packed into the tubs on the processing deck rather than down in the storage hold. The reduction or elimination of ice usage, as a result of the superchilling and superchilled storage, results in several benefits including less time and work when loading the ship, less work packing the fish onboard and reduced risk of accidents that occur in the storage hold when packing and icing the catch.

Bleeding

The first practical guideline is that groundfish should be bled within 30 minutes after catch to maintain quality in terms of fillet colour and that the waiting time in the reception hold is the main deciding factor influencing fillet colour. As for the method of bleeding and gutting saithe, the two-step process of bleeding and gutting showed slightly better results in terms of fillet colour, compared to direct gutting. Saithe and cod must be bled within 30 minutes from catch to maintain a light fillet colour. During the study period, the fisheries companies involved have considered the study results for beneficial changes and, for example, adjusted their limit on the size of hauls, partly in order to minimise the load to the catch but mainly to reduce the waiting time in the reception hold. With manual gutting, there are slightly better results when bleeding the fish before gutting compared to direct gutting in one step. The two-step procedure, however, requires an additional bleeding tank or a buffer storage tank

on the already packed processing deck. The automation of the bleeding process would facilitate this option and speed up the overall processing time.

The bleeding process is mainly influenced by time, temperature, water flow conditions and the method of cut and gutting. The bleeding process for cod (*Gradus morhua*) should exceed 12 minutes and be carried out in unchilled seawater ensuring movement and refresh rate of the bleeding medium. Regarding the bleeding medium temperature, there are better results when bleeding the fish above or close to the current sea temperature than in chilled seawater. The design criteria are that the bleeding time should reach 15 minutes, but that is dependent on the water flow and circulation in the bleeding medium. Pump flow recirculation, or active movement of the fish in the bleeding medium, can be a more deciding factor than the rate of water replacement. The effects of successful bleeding are most noticeable after 1 month of frozen storage but lipids in the fish even out after 4 months of frozen storage regardless of bleeding conditions. Producers of loins are often freezing other parts of the fillet for products in which successful blood removal would become more noticeable after freezer storage, rather than in fresh cod, further supporting the importance of a sufficient bleeding process.

Cooling

As expected, applying time control to both the bleeding and cooling processes are apparent improvements from older systems. The study results show how the temperature profiles of the industrial onboard systems measured are mostly comparable to the ones obtained from controlled experiments. The results indicate that fish bulk density and flow rate are not limiting or restricting factors for the industrial systems examined. The cooling systems will, however, be sensitive to overload with higher fish to cooling medium ratios, and also to the risk of excessive partial freezing of the fish when they are operating on cooling medium temperatures lower than the initial freezing point of the fish. When wet-fish trawlers can effectively chill the fish to below 0 °C on the processing deck, the storage hold can be run at -1 °C and ice can be greatly reduced or eliminated from the onboard chill chain. As the superchilling method has only recently been applied in general industrial usage onboard groundfish trawlers, there is a lack of regulations regarding definitions and monitoring of the application. There is an Icelandic government regulation, no.745/2016, regarding superchilling of fish, which allows for 0.6% reduction from the catch weight at landing due to estimated drip loss. Therefore, due to lack of other regulation and definitions, when superchilled fish is transported and stored at chilled temperature later in the value chain, the consumers are unlikely to become aware of the prior superchilling.

Storage

Superchilling whole gutted fish onboard can reduce spoilage and slow the growth of bacteria and TVB-N, however, the systems that use temperatures below -1 °C risk freezing the fish, especially the tail and belly flaps. To optimise the cooling process and minimise the risk of freezing, the onboard systems should consider size grading fish for more uniform chilling. Also, if the cooling systems stop for unexpected reasons, the fish can be kept in tanks that keep the cooling media temperature down at -1 °C but at lower temperatures there is the risk of freezing fish being stored due to system stop. The onboard storage of fish is generally a relatively short time period in the value chain of fresh fish and superchilled storage after fillet processing proves to be a deciding factor regarding shelf-life. The benefits of a carefully time controlled and superchilled transport chain are considerable in terms of shelf-life, however the producers will have to evaluate the benefits in terms of economic factors

and risks associated with interruptions in the temperature chain. Practical problems in the superchilled value chain of fish are the potential temperature fluctuations during processing, storage and transportation.

5.4 Future research

The focus of this study is the onboard processing, specifically the processes of bleeding, cooling and storage, and the results of the study have led to recommendations for improving these processes. To give a wholistic view and thus enable further development, further research should build on the results of this study to focus on improving fish handling in the prior value chain steps, improving fishing gear and methods, and on possible reception hold improvements.

Due to complications in the working process, when bleeding and gutting in a two-step process, there is an uncertainty for producers on whether to carry out the onboard bleeding and gutting procedure in one step or whether it is better to bleed first and gut later. The reason that more producers are considering bleeding the whole catch first and gut later is that, in that way, the crew is able to bleed the catch more quickly. There is a need for technological solutions onboard trawlers that could help the crew to bleed and gut the whole catch faster than can be done today, especially on larger vessels. Such solutions could ensure higher and more uniform quality as the catch is processed sooner and this would also make the onboard work much easier. The next expected steps are the development of bleeding robots to speed up the onboard processing time, as described by Toldnes *et al.* (2014).

For further research on the cooling process there is work to be done developing more accurate models for heat transfer and the quality criteria. With the development of more complex 2D and 3D heat transfer models, there are interesting options to develop a relationship between product temperature and quality criteria (FFA, PL, HI). One way to do this would be by predicting the physical reaction rates of quality deterioration in relation to the fish temperature dependence. With this work, the heat transfer model could be linked directly to a quality model, enabling prediction of the product temperature and the resulting quality changes.

In Iceland, the focus should be on future research aiming to increase the proportion of the groundfish catch processed to higher value products, i.e. fresh loins and fillets. While the research focus has mainly been on Atlantic cod, improvements in onboard bleeding and cooling processes provide more uniform and higher quality fish and therefore opportunities for an increase in fresh products from other groundfish, such as saithe, haddock and redfish. For the Icelandic fresh fish trawlers, further research is needed to develop solutions to minimise capture-induced stress and for live storage of fish in the reception hold. This has been studied by Olsen *et al.* (2013), Digre *et al.* (2017) and Erikson *et al.* (2019) and should be studied further for the specific Icelandic processing setup. Also, for further research focus related to live fish handling, one of the companies participating in this project has during the project's duration upgraded a trawler for onboard live storage of fish. The concept is that the fish can be brought alive to land-based storage tanks close to the processing to provide flexibility for more strategic processing, taking into account selective raw material condition and current market demand. The fish quality and shelf-life will benefit from the better temperature-controlled chill chain but further studies on specific fish quality factors, such as

sensory acceptance and nutritional value retention, are necessary. Also, future comparison studies on energy, environmental issues and economic factors are needed for further evaluation. The current study provides an important contribution to these future research topics.

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Contributing Papers

Paper I:	Eliasson, S., Arason, S., Margeirsson, B., Bergsson, A.B., Palsson, O.P. (2019). The effects of superchilling on shelf-life and quality indicators of whole Atlantic cod and fillets. <i>LWT – Food Science and Technology</i> , 100 (1), pp.426-434. DOI: 10.1016/j.lwt.2018.10.062.
Paper II:	Eliasson, S., Arason, S., Margeirsson, B., Palsson, O.P. (2020). Onboard Evaluation of Variable Water Flow and Recirculation Effects on Bleeding of Atlantic Cod (<i>Gadus morhua</i>). <i>Foods</i> , 9(11), 1519, pp.1-14. DOI: 10.3390/foods9111519.
Paper III:	Eliasson, S., Ragnarsson, S.O., Arason, S., Margeirsson, B., Palsson, O.P. (2021). Onboard pre-chilling of ungutted and gutted Atlantic cod in different cooling media -Temperature measurements and analytical modelling-. <i>International Journal of Refrigeration</i> , 132, pp.72-81. DOI: 10.1016/j.ijrefrig.2021.08.027.
Paper IV:	Eliasson, S., Arason, S., Margeirsson, B., Bergsson, A.B., Palsson, O.P. (2019). Effects of on-board bleeding methods and superchilling on quality of cod and saithe. In the proceedings of <i>25th IIR International Congress of Refrigeration</i> , ICR 2019, 24-30 th August, pp.3248-3255. International Institute of Refrigeration. DOI: 10.18462/iir.icr.2019.1639.
Paper V:	Eliasson, S., Arason, S., Margeirsson, B., Palsson, O.P. Effects of onboard refrigerated seawater (RSW) storage of whole ungutted cod on quality parameters of fillets. <i>Submitted for publication</i> .
Paper VI:	Eliasson, S., Arason, S., Margeirsson, B., Palsson, O.P. Effects of different bleeding methods and storage time on the colour of cod fillets. <i>Submitted for publication</i> .

Paper I



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The effects of superchilling on shelf-life and quality indicators of whole Atlantic cod and fillets

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ABSTRACT

A newly developed industrial process was used to achieve superchilling of whole gutted cod on-board a fresh-fish trawler. It was compared to iced cod and after fillet processing, storage conditions at $-1\text{ }^{\circ}\text{C}$ and $+1\text{ }^{\circ}\text{C}$ were used to differentiate the groups. Quality difference was evaluated by microbial analysis, total volatile base nitrogen (TVB-N) and sensory evaluation. H_2S -producing bacteria appeared to grow more rapidly in the fish that was iced whole compared to the samples that were superchilled on-board. Total viable counts in fillets were lower under superchilled storage conditions. The TVB-N level of fish iced on-board and stored chilled rose above the consumption limit on day 11 after catch, while the other samples were below. TVB-N levels where fillets were stored superchilled were still under the limit 16 days after catch, sensory evaluation however rejected both groups at that point. According to the sensory evaluation the effects of superchilled storage of fillets had a much greater impact on quality changes than the on-board cooling method, resulting in a 2–4 day extension of freshness period and 3 days longer shelf-life. The effects of on-board whole fish superchilling was less noticeable, however the method slowed the growth of H_2S -producing bacteria and spoilage attributes.

1. Introduction

Superchilling is a method involving partial freezing of water in food (e.g. cod muscle), in which commonly the food is cooled $1\text{--}2\text{ }^{\circ}\text{C}$ below the initial freezing point (T_f) resulting in 5–30% of water inside the product forming ice crystals (Aune, 2003; Kaale, Eikevik, Rustad, & Kolsaker, 2011). This study refers to this as “superchilling”, however the method of superchilling food is also known as partial freezing (Duun & Rustad, 2007), deep chilling (Wu et al., 2014) or a more recent industry term “sub-chilling” (Skaginn 3X, 2017). According to Magnussen, Haugland, Torstveit Hemmingsen, Johansen, and Nordtvedt (2008), the method can extend shelf life of fresh cod by 2–3 days, compared to traditionally iced cod with shelf-life of 9–13 days (Lauzon et al., 2010). This corresponds with previous studies on superchilled cod products which have shown extended shelf life of 3 days for fresh fillets (Martinsdottir, Lauzon, & Tryggvadottir, 2005) and similar time for shrimp (Ando, Nakamura, Harada, & Yamane, 2004). For salmon the extended shelf life can be considerably longer, as shown by

Duun (2008). Haugland et al. (2002, 2005) showed positive effects of pre-chilling and superchilling of iced whole, gutted cod on both bacterial growth and product temperature after filleting. During transport superchilling has also showed to have a positive effect on product temperature control (Aune, 2003; Mai et al., 2011) and on environmental impacts, such as abiotic depletion, global warming potential and marine aquatic ecotoxicity (Hoang, Brown, Indergard, Leducq, & Alvarez, 2016). Due to an increasing demand for high quality fresh fish, fish producers globally have been focusing on high value products and in the last decade there have been vast improvements in raw material handling. In recent years there has been a shift by Icelandic fish producers from less valuable frozen products to high end fresh products, made possible by technological developments along the value chain. Increased knowledge and improvements in packaging design and temperature control have extended shelf-life and supported an increase in fresh fish processing and export (Olafsdottir, Lauzon, Martinsdottir, Oehenschlauger, & Kristbergsson, 2006; Lauzon et al., 2010; Margeirsson et al., 2012a; b; c; Paquette, 2017; Laguerre, 2018).

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Table 1
Definition of experimental groups A, B, C and D. Storage temperature of whole fish and fillets is shown in parenthesis.

Group	On-board storage of whole fish	Storage of fillets
A	Superchilled (-1°C)	Superchilled (-1°C)
B	Traditionally iced (0°C)	Superchilled (-1°C)
C	Superchilled (-1°C)	Chilled (1°C)
D	Traditionally iced (0°C)	Chilled (1°C)

Automation in the fishing industry has generally improved yield, product quality and working environment. The present experiment was conducted at one of Iceland's major seafood processor (FISK Seafood) that has played a key role in this development, investing in new high technology equipment for both ships and land based processing. A part of this initiative included installing a newly developed system including superchilling technology for whole gutted fish on-board a fresh-fish trawler. The goal of the development was to provide uniform and high quality raw material but also to reduce physical work and provide more economic fish handling and processing. The novelty of the current research is to study the effects superchilling cod on the whole value chain, including the on-board superchilling after gutting, while other studies have focused on superchilling later in the chain. Based on experience and theoretical calculations, approximate chilling times may be estimated. However, as pointed out by Bantel et al. (2016), there are challenges in industrial superchilling and measurements of temperature and time-dependent ice fraction have to be carried out under realistic industrial conditions. In industrial fishing the fish varies in size, shape and composition making predictions on the state of superchilling (in terms of ice crystal formation) complex to pinpoint. Avoiding excessive surface freezing and limiting ice crystal growth is a challenge in these conditions regarding structural damage and negative texture changes to the fish flesh and increased drip loss (Bahuaud et al., 2008; Kaale et al., 2011; Magnussen et al., 2008). Olafsdottir et al. (2012) studied superchilling of whole gutted cod, however the fish was only superchilled after landing and received chilling in slurry ice on-board. In the current study the experiment groups received different cooling immediately after on-board gutting. Also the whole process of on-board superchilling followed by superchilled fillet storage has not been studied before, according to the authors' best knowledge. The objective of this research was to evaluate the effects of superchilling wild cod on product quality and shelf-life compared to traditionally iced cod, both regarding on-board cooling and storage of whole gutted fish and chilled or

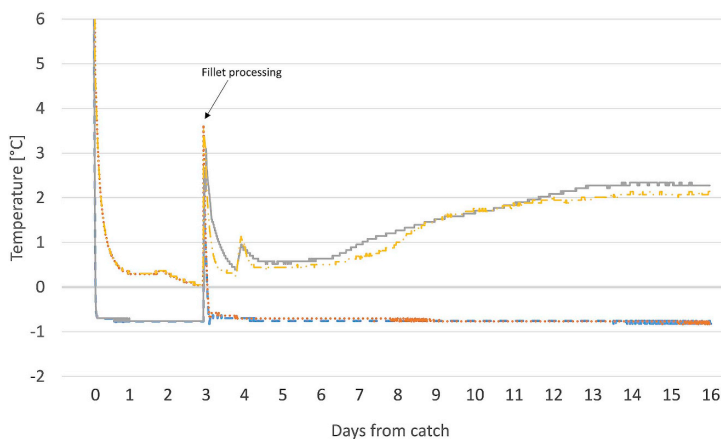


Fig. 1. Temperature profiles for all groups through the experiment period. Whole fish from catch to fillet processing and then fillet storage. Fish temperature of groups is represented by; A (blue dashed line), B (orange dotted line), C (grey whole line), D (yellow dashed/dotted line). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Measurement scheme of cod fillets.

Days from catch	Sensory evaluation	TVB-N	Bacteria TVC & H ₂ S
Day 3 (8th March)		x	x
Day 4 (9th March)	x	x	x
Day 9 (14th March)	x	x	x
Day 11 (16th March)	x	x	x
Day 13 (18th March)	x	x	x
Day 16 (21st March)	x	x	x

superchilled storage of fillets throughout the product shelf-life.

2. Materials and methods

The on-board superchilling method was in two steps; first step cooling in seawater -0.8°C (3% salt concentration), second step cooling in brine -2.8°C (6% salt concentration). The equipment used was a screw conveyor (Skaginn 3X, 2017), however different producers offer various equipment and ways of achieving industrial superchilling (Stevik & Claussen, 2011). Generally, cod is superchilled to around -1°C in modern industrial applications (Stevik & Claussen, 2011; Valtysdottir, Margeirsson, Arason, Lauzon, & Martinsdottir, 2010). The on-board superchilling system manages to bring the core temperature of the fish to around -1°C and the current study aims to evaluate the gained shelf-life of both the on-board chilling as well as further storage under superchilled conditions.

2.1. Experimental design

The experiment was done during a fishing trip where cod from the same haul (in order to limit variation) was split into a total of four groups; two different on-board cooling and storage methods and two different storage temperatures after filleting and skinning. On-board cooling methods included traditional ice cooling and storage (at $0-1^{\circ}\text{C}$) and the other superchilling and storage on-board without ice (-1.0 to -0.7°C). The four groups compared in the study are shown in Table 1.

Cod for the experiment was caught by the fresh-fish trawler Málmei SK-1 on the 5th of March 2016, North West of Iceland in sea temperature of $5-6^{\circ}\text{C}$. The cod came from a haul of size 9.4 tons with a hauling time of 25 min and 3.2 kg average fish weight. After gutting all samples by hand, they were placed under time controlled bleeding for 20 min in a screw conveyor (manufactured by Skaginn (Akranes, Iceland) in 2016). The temperature of the seawater used for bleeding

Table 3
Sensory attributes used for cooked cod and their descriptions. Adopted from Lawless and Heymann (2010).

Sensory attribute	Short name	Scale anchors	Description of attribute
<i>Odour</i>			
sweet	o-sweet	none much	Sweet odour
shellfish, algae	o-shellfish	none much	Shellfish, characteristic fresh odour
vanilla/warm milk	o-vanilla	none much	Vanilla, sweet heated milk
boiled potatoes	o-potatoes	none much	Reminds of whole warm boiled potatoes
dishcloth	o-cloth	none much	Reminds of dishcloth (damp cloth to clean kitchen table, eft for 36 h)
TMA	o-TMA	none much	TMA odour, reminds of dried salted fish, amine
spoilage sour	o-sour	none much	Sour odour, sour milk, spoilage sour, acetic acid
sulphur	o-sulphur	none much	Sulphur, matchstick, boiled cabbage
<i>Appearance</i>			
colour	a-colour	light dark	Sample surface. Light; white colour, Dark, yellowish, brownish, grey
heterogenous	a-hetero	homogenous heterogenous	Sample surface. Hererogenous, discoloured, stains
white precipitation	a-prec	none much	White precipitation on the sample surface and in liquid
flakiness	a-flakes	none much	The fish portion slides into flakes when pressed with the fork
<i>Flavour</i>			
salt	f-salt	none much	Salty taste
metallic	f-metallic	none much	Characteristic metallic flavour of fresh cod
sweet	f-sweet	none much	Characteristic sweet flavour of very fresh (boiled cod)
bitter	f-bitter	none much	Bitter flavour
spoilage sour	f-sour	none much	Sour taste, spoilage sour
TMA	f-TMA	none much	TMA flavour, reminds of dried salted fish, amine
putrid	f-putrid	none much	Strength of putrid flavour
<i>Texture</i>			
soft	t-soft	firm soft	Evaluate how firm or soft the fish is in the first bite
juicy	t-juicy	dry juicy	Dry, draws juice from the mouth. Juicy, releases juice while chewing
tender	t-tender	tough tender	Evaluate after chewing several times
mushy	t-mushy	none much	Mushy texture
rubbery	t-rubbery	none much	Rubbery texture, springy

was 6 °C. After the bleeding process groups B and D were iced in 460 L insulated tubs (closed with lids, without drain plugs to facilitate draining) with flake ice and stored on the processing deck throughout the fishing trip. Groups A and C were superchilled after bleeding with chilled seawater in a screw conveyor with a two stage cooling process; first –0.8 °C for 20 min and then –2.8 °C for 8 min. The samples were then stored in 460 L insulated tubs with no ice stored in the ship hold at –1 °C (tubs without lids and without drain plugs). Fish in all groups were filleted and packed 3 days after slaughtering, on March 8th at FISK Seafood in Saudarkrokur, Iceland. To avoid replicate samples each group contained 10 skinless cod fillets from 10 fish. The fish in groups A and C were processed at –0.8 °C after which the surface temperature of the fillets rose to 2 °C. Fish in groups B and D were processed at 0 °C and the surface temperature of fillets measured 3 °C after filleting and skinning (see temperature profiles in Fig. 1). Fillets in groups A and B were packed with a 250 g ice pack on top of and with an absorbent pad below fillets in 5 × 10-kg expanded polystyrene (EPS) boxes without drain holes (Tempra, Hafnarfjordur, Iceland). The boxes were kept open for 4 h in a freezer storage until transportation then closed and stored at –1 °C (at Matis in Reykjavik, Iceland) throughout the storage. Transportation of the samples from processing plant (FISK Seafood) to research facilities (Matis) was 290 km by a refrigerated truck. The fillets in groups C and D were also packed in 5 × 10-kg EPS boxes and stored at 0–2 °C (at Matis) throughout the storage. Four to five fillets were placed in each box. Ambient conditions during storage were controlled in controllable air climate chambers (Celsius, Reykjavik, Iceland).

2.2. Temperature monitoring

Temperature loggers used were iButton (Micro-T DS1922L) from Maxim Integrated Products distributed by NexSens Technology (Dayton, OH, USA). The logger has an accuracy of ± 0.5 °C and a resolution of 0.0625 °C and an operating range of –40 to 85 °C. The diameter is 17 mm and the thickness is 6 mm. All temperature loggers were calibrated in ice/water mixture.

Two fish in each group were monitored from catch to processing plant (eight fish in total). For each fish one temperature logger was placed inside the flesh (center of the loin) and one outside the fish to monitor the ambient temperature throughout cooling and storage. After filleting and skinning two temperature loggers were stored within boxes for each group to monitor product temperature (surface temperature of fillets in the center of the box) and two loggers for the ambient temperature for each group (16 loggers total).

2.3. Chemical and microbial analyses

Table 2 shows the timing and type of measurements done on the cod fillets. Total volatile base nitrogen content (TVB-N) and bacteria measurements (TVC and H₂S-producing) were done on the same fillet (measurements in triplicate). The method of Malle and Tao (1987) was used for TVB-N measurements, measured by steam distillation and titration, after extracting the fish muscle with 7.5% aqueous trichloroacetic (TCA) solution. The distilled TVB-N was collected in boric acid solution and titrated with sulfuric acid solution.

For microbial analysis fillets were aseptically minced, assessing 2 pooled fillets for each sample. Minced flesh (25 g) was mixed with 225 mL of cooled Maximum Recovery Diluent (MRD, Oxoid, UK) in a stomacher for 1 min. Successive 10-fold dilutions were done as required. Total viable psychrotrophic counts (TVC, 15 °C, 4–5 days) were evaluated by spreadplating aliquots onto modified Long & Hammer's medium; counts of H₂S-producing bacteria and presumptive pseudomonads were evaluated on spread-plated Iron Agar (15 °C, 4–5 days) and modified Cephaloridine-Fucidin-Cetrimide (CFC) medium (22 °C, 3 days), respectively (as described by Magnusson et al., 2009).

2.4. Sensory evaluation

Cod fillets from the four groups, eight fillets from each group, were evaluated with sensory evaluation during a twelve day period, the first evaluation four days from catch. Cod fillets were evaluated on day four,

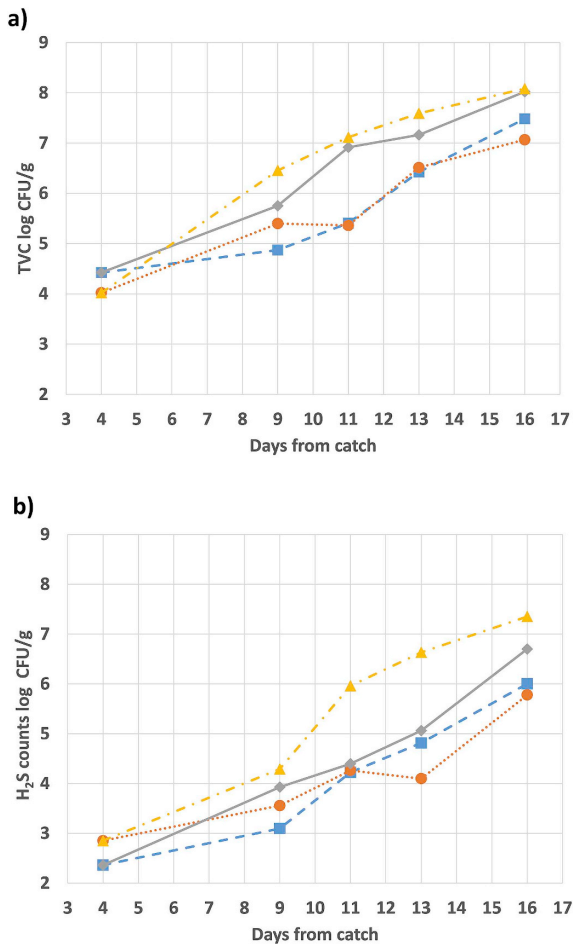


Fig. 2. Bacteria measurements for groups on days 4, 9, 11, 13 and 16 from catch. Group A (blue ■, dashed line), B (orange ●, dotted line), C (grey ◆, whole line), D (yellow ▲, dashed/dotted line). Each point represents an average of two independent measurements ($n = 2$). (a) Development of the total viable psychrotrophic counts (TVC) for different cooling and storing conditions. (b) Development of the H₂S-producing bacteria count for different cooling and storage conditions. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

nine, eleven, thirteen and sixteen from catch. The main purpose of the evaluation was to study the effect of superchilling immediately after catch and superchilled storage, compared to traditional chilling and storage temperature, on quality deterioration and shelf life (see experimental groups in Table 1). The Generic Descriptive Analysis (GDA) as described by Lawless and Heymann (2010) and the Torry freshness score sheet (Shewan, Macintosh, Tucker, & Ehrenberg, 1953) were used to assess cooked samples. Freshness evaluation of cooked fillets using the Torry scheme implies the assessment of the fish flavour and odour (a scale from 10, very fresh, to 3, very spoiled, from Martinsdottir et al., 2001). Quality deterioration is first characterised by the initial loss of the fresh fish flavour (sweet, seaweedy) which is followed by the development of a neutral odour/flavour (i.e. the end of freshness period, Torry score = 7), leading to the detection of off-odours/flavours. End of shelf life is usually determined when sensory attributes related to

spoilage such as sour, pungent, TMA odour and/or flavour become evident (below Torry score = 5.5).

Evaluation of the GDA method describing 24 attributes relating to odour, appearance, flavour and texture (see Table 3). The fillets from groups A and C were stored under the same conditions until day 4 from catch and were then split in two storage temperatures. The same was done for groups B and D. Two groups were evaluated on day four, superchilled (A) and traditionally chilled (C). Only groups A and B were evaluated on day 16 since groups C and D were then spoiled. Ten panellists participated in the Torry sensory evaluation and six to eight sensory panellists participated in each GDA sensory session. All panellists had been trained according to international standards (EN ISO 8586; 2014); including detection and recognition of tastes and odours, use of scales and in the development, and use of descriptors. The members of the panel were experienced in using the GDA method and Torry freshness score sheet for cod. The intensity of each attribute for a given sample was evaluated using a 15 cm unstructured line scale which in analysis was transformed to numbers from 0 to 100. All attributes were defined and described by the sensory panel during earlier projects. Portions weighing about 40 g were cut from the cod loins and placed in aluminium boxes coded with three-digit random numbers. The samples were cooked for 6 min in a pre-warmed oven (Convotherm Elektrogeräte GmbH, Eglfing, Germany) at 95–100 °C with air circulation and steam, and then served warm to the panel. Each panellist evaluated duplicates of each test group in a random order (four samples per session). A computerised system (FIZZ, Version 2.0, 1994–2000, Biosystèmes) was used for data recording.

2.5. Statistical analyses

The sensory evaluation program Panelcheck V1.3.2 (Nofima, Tromsø, Norway) was used to assess panel performance. The programs NCSS 2000 (NCSS, Utah, USA), and Microsoft Excel 2007 were used for statistical analysis of the results. Analysis of variance (ANOVA, General linear model method) and Duncan's test were used to perform multiple comparisons on GDA data. Data from each sampling day were analysed as a separate dataset. Difference between the panellists' use of the scale was corrected for. One Way ANOVA was used for statistical analysis of sensory data, the significance level was set at 5% ($p < 0.05$). Principal Component Analysis (PCA) on significant means level corrected values of sensory attributes and samples was performed, using full cross validation (with XLSTAT version 2018.5) as described by Magnusson et al. (2009).

3. Results

Temperature profiles from catch through the storage for the all four groups can be seen in Fig. 1. Since the on-board handling was the same for groups A and C the temperature profiles are almost identical (same for groups B and D). After filleting, skinning and packing the temperature profiles are similar but easier to distinguish.

Fig. 2 (a) shows TVC for the groups. Groups C and D, stored under chilled conditions (1 °C) show higher values compared to groups A and B, stored at superchilled conditions. For H₂S-producing bacteria, shown in Fig. 2 (b), there was a similar increasing bacterial development as the storage time passes, however here group D separates from the other groups with higher bacterial values.

Fig. 3 shows TVB-N measurements for all groups for the same measurements points. The dotted line in Fig. 3 represents the consumption limit of 35 as defined by EU regulation (no 2074/2005). Until day 11 the TVB-N values are close for all the groups. On day 13 group D rises above the limit and on day 16 both C and D are both above the consumption limit while A and B measure around TVB-N 20 [mg N/100 g].

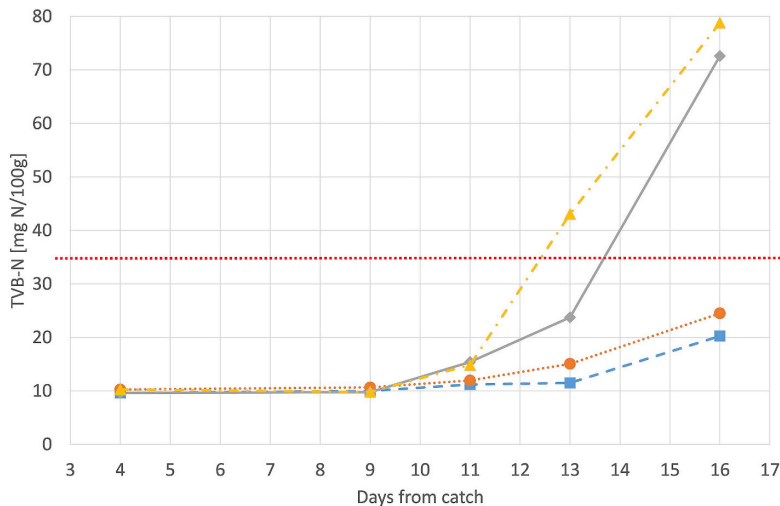


Fig. 3. TVB-N measurements for groups on days 4, 9, 11, 13 and 16 from catch. Group A (blue ■, dashed line), B (orange ●, dotted line), C (grey ◆, whole line), D (yellow ▲, dashed/dotted line). The horizontal red line (square dotted) shows the consumption limit for cod at 35 by EU regulation. Each point represents an average of two independent measurements ($n = 2$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Fig. 4 shows the mean values for GDA sensory attributes for cooked cod according to Table 3. The figure shows eight sensory attributes that showed statistical difference (ms (marginal significance, $p = 0.05$ – 0.10); * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$)). No differences were seen between GDA values of groups on day four from catch (Fig. 4). Only minor differences were seen on day nine where group C had a small trace of TMA not seen in other groups (Fig. 4 (a)). On day 11 group C had a trace of spoilage sour odour that was not seen in other groups (Fig. 4 (c)). A sour flavour was also starting to develop in group C. Big differences were seen between groups on day 13 from catch in odour, appearance and flavour but no differences were seen in texture. Groups A and B had a sweeter odour, more vanilla odour, less dishcloth odour and less sour odour than groups C and D. Groups A and B also had much more metallic flavour than groups C and D. Group D had more bitter flavour than groups A and B and more TMA odour than other groups. Groups A and B were also lighter in colour, and less discoloured than groups C and D. These results indicated that groups C and D were spoiled and were therefore not analysed further. On day 16 from catch only minor differences were seen between groups A and B but group B had a slightly more sour odour than group A. The PCA analysis in Fig. 5 (a) and Fig. 5 (b) shows an overview of how the significant sensory attributes described the samples, explaining altogether 81.13% of the sensory variation between the samples in the first (F1) and second (F2) principal components. Fig. 5 (a) shows sensory attributes in relation to the principal components, the first principal component clearly showing storage time with high intensities of fresh cod to the far right (sweet, shellfish) and variables describing spoilage to the far left (TMA, sour). Fig. 5 (b) shows how the sample groups change with the storage time along the first principal component (F1).

From interpretation of the Torry scale (Shewan et al., 1953) the cod has lost its freshness below a mean score of seven. A score of five indicates that the cod has developed spoilage characteristics and therefore the limit for shelf life was set at mean score of 5.5 (Martinsdottir et al., 2001). The Torry results showed no significant differences between experimental groups on storage days four and nine (Table 4). On day 11 group C received a significantly lower score than groups A and B and a slightly lower than group D. On day 13 groups A and B received much higher mean scores than groups C and D. No difference was seen between groups A and B on day 16. From the Torry data it can be concluded that group A had a freshness period of 13 days and group B 14 days (Fig. 6). Both groups A and B had a shelf life of slightly more

than 15 days. Freshness in group C was lost around day 10 and in group D after day 11. Both groups were spoiled around day 12.

4. Discussion

Temperature monitoring showed that the industrial superchilling method under consideration on-board the trawler manages to bring the core temperature of fish close to -1 °C within 1 h and the ice-less storage hold was relatively stable, showing minimal ambient temperature fluctuations. The superchilling temperature profiles were similar to the ones Olafsdottir et al. (2012) produced, resulting in a two to three days shelf-life extension of superchilled as compared to non-superchilled whole cod. However in that study the two groups for whole cod only showed different Torry scores after day 14 from catch.

According to sensory results the superchilled fillet groups A and B had a considerably longer freshness period (two to four days) and shelf life (two to three days) compared to the non-superchilled fillet groups C and D. Differences between groups A and B on one hand and between C and D on the other were less. Groups A and C, that were superchilled on-board, had a shorter freshness period compared to B and D, that were chilled in ice on-board. The spoilage was mostly described as spoilage sour odour, dishcloth odour, TMA flavour and bitter flavour. No consistent differences were seen in texture between groups. The difference between results for groups A and B on one hand and between C and D on the other hand show that storage temperature was a deciding factor regarding shelf life of fillets, as shown before by Olafsdottir et al. (2006), Bahuauud et al. (2008) and Dunn (2008). The PCA in Fig. 5(a) and (b) shows that at the beginning of storage the groups are very similar, described with fresh cod characteristics. However at the end of the storage period the difference between groups C and D on day 13 indicated that the on-board superchilling is resulting in reduced spoilage characteristics with group D showing more TMA flavour and sour odour.

Superchilling whole gutted fish on-board did not show improved product quality or lengthen the freshness period or the shelf life of the cod as suggested by Olafsdottir et al. (2012). A relatively short time from catch to processing plant, three days, could be a factor that minimizes the benefits of the on-board whole fish superchilling, however three days is a common time from catch to processing plant for Icelandic fresh fish trawlers. The timing of rigor mortis, which is delayed at lower temperatures, could influence the shelf life and gaping,

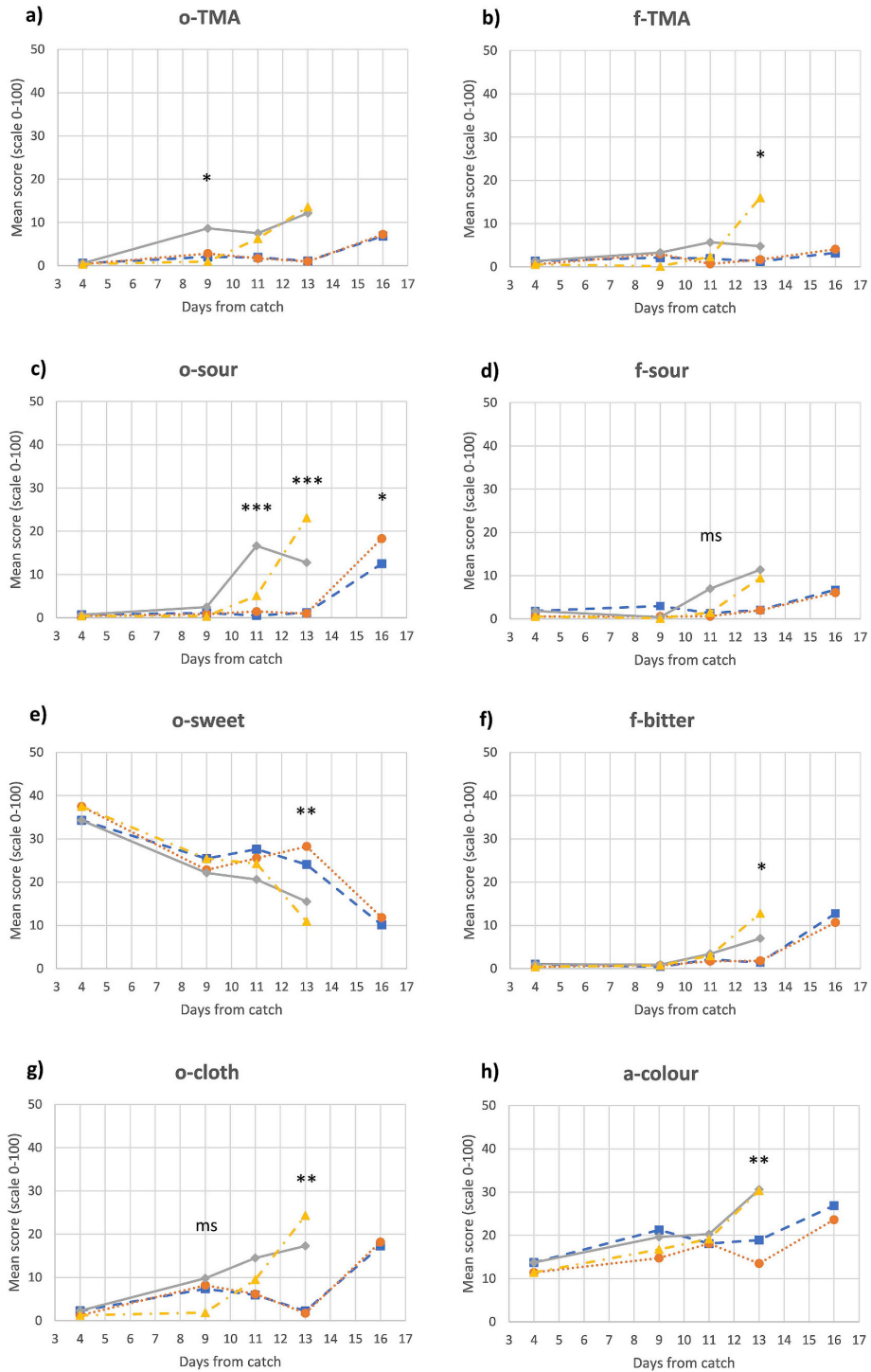


Fig. 4. (a)–(h) Mean values for GDA sensory attributes for cooked cod on days 4, 9, 11, 13 from catch. Different marks on the graphs represent a significant difference between the relevant groups. (o-odour, a-appearance, f-flavour, t-texture.). ms (marginal significance, $p = 0.05\text{--}0.10$); * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$). Group A (blue ■, dashed line), B (orange ●, dotted line), C (grey ◆, whole line), D (yellow ▲, dashed/dotted line). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

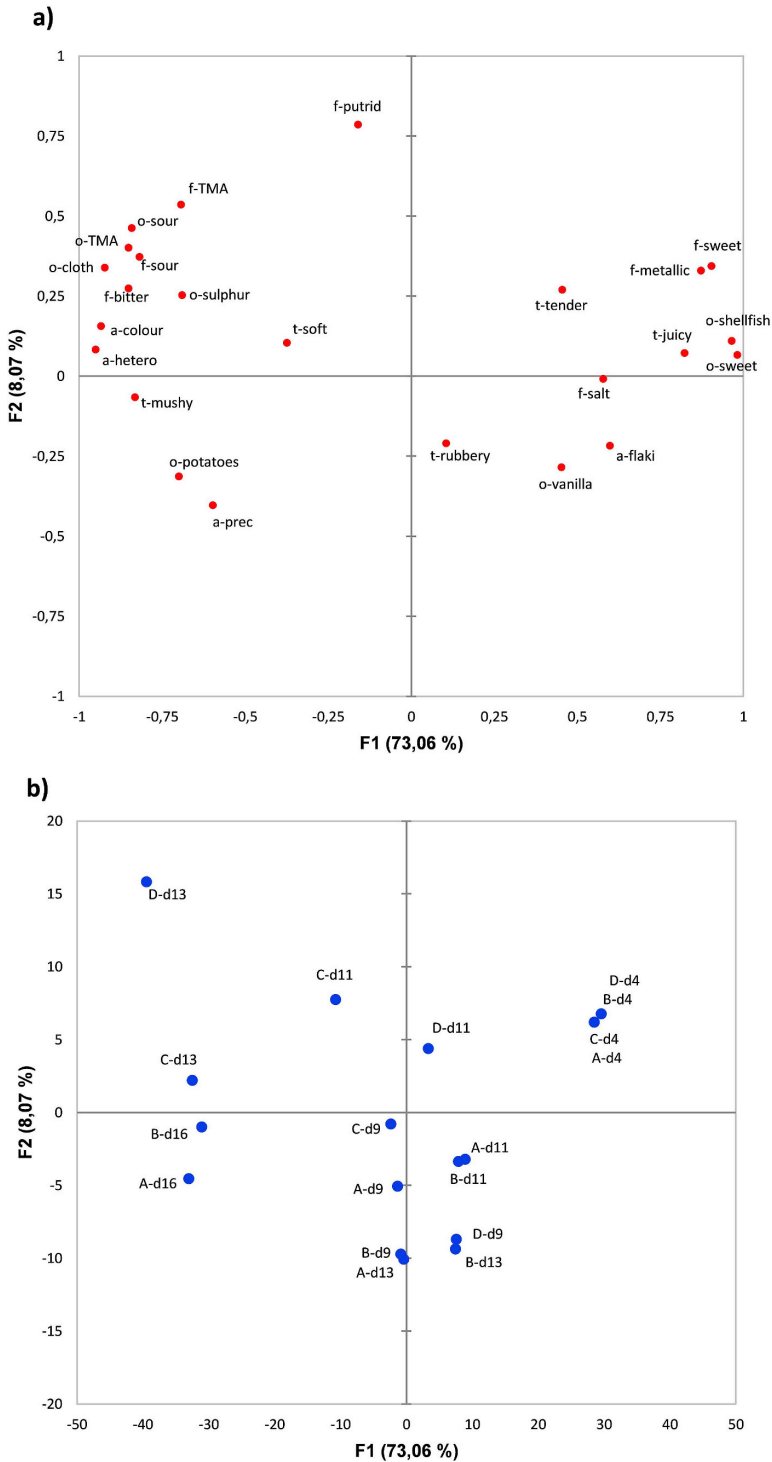


Fig. 5. (a) PCA of sensory attribute variables for the first (F1) and second (F2) principal components. (b) PCA showing sample group observations for the first (F1) and second (F2) principal components. Capital letters of observation labels refer to groups and numbers to days from catch.

Table 4

Mean Torry scores for groups A, B, C and D on sampling days. Different letters within a row indicate a significant difference between relevant groups. Standard deviations are shown in parenthesis. Ten panellists (n = 10) participated in each Torry sensory evaluation session.

Day	A	B	C	D	p-value
4	8.8 (0.75)	8.8 (0.54)	8.8 (0.75)	8.8 (0.54)	0.901
9	8.0 (1.18)	7.7 (0.98)	7.5 (1.85)	8.0 (1.09)	0.599
11	7.6 a (1.06)	7.6 a (0.76)	6.3 b (1.66)	7.0 (1.77)	0.009
13	7.0 a (1.57)	7.7 a (1.21)	5.0 b (1.42)	4.5 b (1.42)	0.000
16	5.1 (1.26)	5.2 (1.46)			0.703

as Aune, Olsen, Akse, Ytterstad, and Esaiassen (2014) showed with less fillet contraction when filleting at lower temperatures. This could be a deciding factor in slower bacterial growth for the superchilled whole fish if the rigor mortis period was longer. Microbacterial development for both TVC and H₂S-producing measured highest in group D, which was kept at the highest temperature throughout the study period. The populations of TVC are in accordance with Ahmed, Akand, Islam, Mamun, and Bari (2015) where iced fish (Ruhi: *Labeo rohita*) was 6.5 log CFU/g compared to 6.5 for group D, both after nine days of storage. A factor that could influence the storage conditions in this study is that while storing the fish in melting ice, the fish was still being washed through the storage period and some residual blood was still leaking from the tubs. In the iceless storage at -1 °C however there was no extended washing of the fish.

Groups A and B, which were stored superchilled after filleting and skinning, showed lower bacterial counts compared to fillets stored at +1 °C. This is in accordance to results by Olafsdottir et al. (2006) and Duun and Rustad (2007), showing delayed growth rate of all bacterial groups under superchilled conditions. The difference between on-board storage of whole gutted fish did not show an extension in shelf-life results but the microbial measurements indicate that bacterial growth may be delayed by superchilling whole fish. While the TVC measured on day four were more than three times higher (11,000 vs. 34,000, Fig. 2 (a)) for the fish superchilled on-board the H₂S-producing bacteria were lower on day four for the same groups (230 vs. 960, Fig. 2 (b)). The reasons for higher initial TVC values for the superchilled fish could be microbial growth in the brine throughout the fishing trip. The comparison of initial values (on day four) for TVC and H₂S-producing bacteria indicated that the on-board superchilling slows the growth of H₂S-producing bacteria. This is further supported by TVB-N

measurements as muscle proteins are decomposed by H₂S-producing bacteria that results in increased TVB-N levels. This was seen when comparing the results of H₂S-producing bacteria and TVB-N measurements for groups C (superchilling on-board) and D (ice cooling on-board). TVB-N for group D rises above the consumption limit on day 13 when group C is still below. On day 16 however groups C and D are both above the limit while groups A and B with fillets stored superchilled, are below the consumption limit.

5. Conclusions

The results from the sensory evaluation indicate that the superchilling process has a greater effect on shelf life after the cod was filleted than before. However the relatively short time from catch to filleting could be a factor in this study that devaluates the effects of the on-board superchilling. From this it can be concluded that the storage temperature after filleting and skinning had much more effect on the shelf life and quality of the fish than the chilling method on-board. Superchilling whole gutted fish on-board did not show improved product quality or lengthen the freshness period or the shelf life of the cod. In fact looking at storage of fillets at 1 °C the Torry scores from day 11 rate the traditionally ice cooled fish (group D) higher than the superchilled (group C). Furthermore the freshness period for both groups that were traditionally iced on-board (B and D) was 1–2 days longer compared to the groups superchilled on-board (A and C). The results from H₂S-producing bacteria, TVB-N and GDA sensory analysis however indicate that the superchilling of whole fish on-board slows down the microbial activity and the decomposition of muscle proteins in the cod.

Acknowledgements

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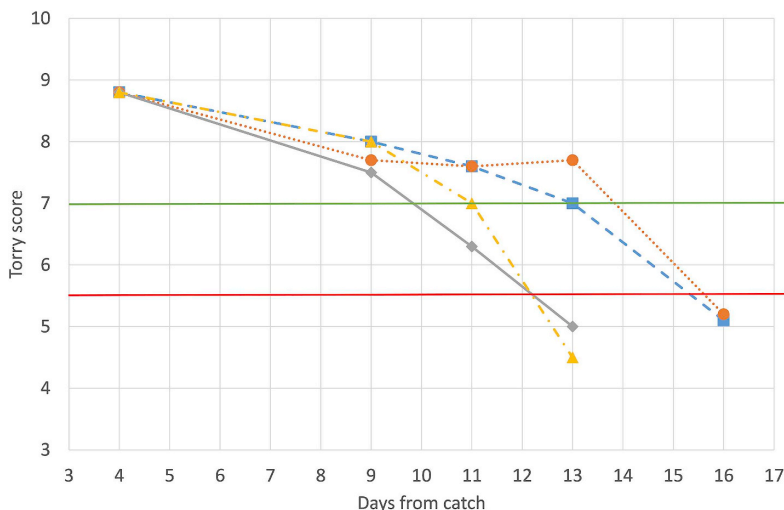


Fig. 6. Mean Torry freshness scores for cooked cod samples on days 4, 9, 11, 13 from catch. Group A (blue ■, dashed line), B (orange ●, dotted line), C (grey ◆, solid line), D (yellow ▲, dashed/dotted line). The green line indicates loss of freshness (Torry score = 7) and the red line indicates end of shelf life (Torry score = 5.5). Ten panellists (n = 10) participated in each Torry sensory evaluation session. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



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Paper II

Article

Onboard Evaluation of Variable Water Flow and Recirculation Effects on Bleeding of Atlantic Cod (*Gadus morhua*)

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Abstract: The aim of the study was to explore the effects of different design variables in the onboard bleeding process of cod on bleeding efficiency and the resulting product quality. A time- and flow-controlled process was used to create variable bleeding conditions for whole gutted cod onboard a wet-fish trawler. Two main design variables influencing the bleeding process are the pump flow recirculation (PFR) and the water replacement ratio (WRR); they were studied in five different combinations (groups). The effects of different bleeding conditions were evaluated by measurements of free fatty acids (FFAs), phospholipids (PLs), and total heme iron (HI) content during freezer storage for up to four months. The results for PL content and the regression model indicate that the enzyme activity in the fish muscle is lower in cases where PFR exerts greater influence in the bleeding process than WRR. The effects of successful blood removal also seem to be most noticeable after one month of freezer storage, rather than in fresh cod after seven days or after four months of simulated frozen food-chain storage. The study indicates that, with the bleeding medium to fish ratio of around 3:1 and enough WRR (over 100% replacement in 20 min), the PFR becomes the limiting design parameter regarding efficient blood removal and should be at least 10% of the tank volume per minute to ensure enough recirculation and flow of water in the bleed-out tanks.

Keywords: Atlantic cod; bleeding; water flow; recirculation; fatty acids; lipids; heme iron

1. Introduction

An increasing demand for high-quality fresh fish has led fish producers globally to focus on and improve raw material handling and processing. Improvements in temperature control and packaging have extended the shelf-life of fish and promoted an increase in fresh fish processing and export [1–4]. High-end products, like fresh cod loins, also require the raw material to be well drained of blood and uniform in color. The present experiment was conducted onboard a wet-fish trawler with newly developed time- and temperature-controlled bleeding and superchilling equipment.

Bleeding is generally the first process the fish goes through after catching and is aimed to drain most of the blood from the muscle [5]. Depending on the species, blood makes up about 1.5–7% of a fish's total body weight with around 20% of it localized in the muscular tissue [6]. Insufficient bleeding can affect the shelf-life of products through product taste, odor, visual appearance, and residual blood, which can also promote lipid oxidation, provide nourishment for bacteria, and cause increased enzymatic activity [7].

Botta et al. [8] demonstrated in their research that the bleeding process of the fish is an important factor for improving the color of the flesh. Cutting the isthmus and gutting in a one-step procedure, where the isthmus is cut without touching the backbone and then the fish is immediately gutted, is the most common practice in fresh fish processing. Studies [8–10] have indicated that the time-factor was a more important aspect of the procedure of the bleeding process than the cutting method itself. Others have studied the influence of stress [11–13], time [9,10,14], and temperature [10,15,16] on the bleeding process. Karlsdottir et al. [16] concluded that if myoglobin and hemoglobin proteins remain in the white muscle tissue, the effect of an improper exsanguination can cause increased rancidity of the product and shorten the shelf-life.

Lipid oxidation is one of the major problems associated with seafood during processing and storage [17]. Typical oxidative response by muscle foods is variable, depending on processing treatment, and freezing generally inhibits the oxidative response [18]. Research has shown that when phospholipids (PLs) are degraded due to enzymatic activity, the amount of free fatty acids (FFAs) increases [16,19]. Measurements of the amount of free fatty acids (FFAs) and phospholipids (PLs) can therefore be a good indicator of quality when working with lean fish. Several variables can impact the amount of enzymatic activity in fish, including freezing rate, storage temperature, storage time, temperature fluctuations during storage, and bleeding efficiency [16,19]. Intact lipids, FFAs, and oxidized lipids can interact with proteins and result in quality deterioration of lean fish species. Results by Burggaard and Jorgensen [20] and Dang et al. [21] show how changes in lipids during frozen storage of fish can lead to quality deterioration, especially at temperatures around $-20\text{ }^{\circ}\text{C}$ to $-10\text{ }^{\circ}\text{C}$. Temperature variations during transportation and short-term freezer storage commonly cause fish temperatures to reach above $-20\text{ }^{\circ}\text{C}$, resulting in negative changes in quality [21,22].

Blood components in the fish muscle have been proven to contribute to the lipid oxidation of fish products during processing and storage. Also, the iron ions released from heme proteins are thought to promote lipid oxidation [7]. Heme proteins, including hemoglobin (Hb) and heme iron (HI), play an important role in lipid oxidation as pro-oxidants [23]. Phospholipids account for almost half of the total lipids in the blood of fish [24], and it has been suggested that they contribute to lipid oxidation of fish muscle, since phospholipids are generally known to be highly susceptible towards oxidation [25].

Conditions in industrial bleed-out tanks that influence the bleeding efficiency are mainly the water replacement ratio (WRR), which is the ratio of bleeding medium replaced (seawater in onboard systems), and the pump flow recirculation (PFR), which impacts the flow turbulence in the tanks. The influence of the WRR and PFR on the bleeding process of cod has, according to the authors' best knowledge, not been studied further. The aim of this research is to explore the effects of WRR and PFR of the bleeding medium on the bleeding efficiency (exsanguination) of Atlantic cod (*Gadus morhua*) and the resulting product quality by evaluating FFA, PL, and HI content.

2. Materials and Methods

The experiments took place during a fishing trip where cod from the same haul was bled for 20 min in five different conditions (groups) but otherwise handled in the same way. The cod was then processed at a fish processing plant in Saudarkrokur, Iceland, four days after the catch, and the fillets were kept frozen and measurements were made at Matis (Icelandic Food and Biotech R&D) in Reykjavik, Iceland. The measurements were done after three storage times: on fresh cod seven days from catch, then frozen after 1 month and 4 months. As the general industry standard is variable, the samples were stored at a relatively high freezer storage temperature (-12 to $-18\text{ }^{\circ}\text{C}$) during the last period of frozen storage to presumably result in a more rapid quality decline (PLs and FFAs), simulating imperfect and realistic temperature control in transport and long-term freezer storage [21].

2.1. Experimental Design

The cod for the experiment was caught by the wet-fish trawler Malmey SK-1 (no. 1833) on 4 March 2016, north west of Iceland in a sea temperature of $5\text{--}6\text{ }^{\circ}\text{C}$. The cod came from a haul of

5.3 tons with a tow-time of 90 min—the haul size was within the company guidelines of around 7 tons and the tow-time was below average for the fishing trip. After gutting all samples alive (within 30 min after hauling), cutting the isthmus and gutting in a one-step procedure, they were placed under different bleeding conditions with regard to recirculation and flow rate but otherwise handled and stored the same way. The temperature of the seawater in the bleed-out tanks was 5 °C. Groups A, B, and C were bled for 20 min in time-controlled bleed-out tanks equipped with a screw conveyor (manufactured by Skaginn 3X, Akranes, Iceland [26], see Figure 1A,B). The screw conveyor is basically an Archimedes screw that transfers the gutted fish through the bleed-out tanks in chamber batches. The volume capacity of all the bleed-out tanks is about 7 m³, and each of the ten slots of the bleed-out tank system holds about 150 kg of cod at a time.

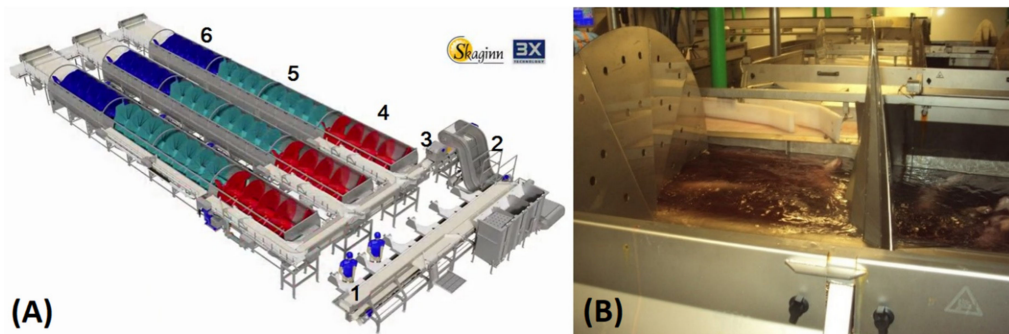


Figure 1. (A) Three-dimensional model of screw conveyor used for controlled bleeding and superchilling. Figure labels: No.1: Gutting; No.2: Washing; No.3: Grading; No.4: Bleeding; No.5: Chilling; No.6: Superchilling [23]. (B) Photo of screw conveyor used for controlled bleeding.

The power of the recirculating pump and recirculation rate in the tank were varied between the groups; the different settings between the bleeding systems are shown in Table 1. The table shows the conditions for each group during the bleeding procedure. The cod in Groups D and E were bled for 20 min in separate 460-L tubs, and the water was replaced in one batch after 10 min during the bleeding procedure. Each tub contained around 100 kg of cod and 200 kg of seawater. In one of the tubs (Group D), a pump was installed to recirculate the seawater, while another (Group E) had no pump (still seawater).

Table 1. Experiment groups settings.

Group	Bleeding Water to Fish Ratio	Pump Flow Recirculation (PFR) (Pump Power)	Water Replacement Frequency	Water Replacement Method	Water Replacement Ratio (WRR) * (Replacement Time)
A	3:1	40 L/s (8 kW)	235 L/min	25 s injection intervals	67% (30 min)
B	3:1	15 L/s (3 kW)	585 L/min	15 s injection intervals	167% (12 min)
C	3:1	15 L/s (3 kW)	390 L/min	10 s injection intervals	111% (18 min)
D	2:1	8 L/s (1.5 kW)	20 L/min	batch replacement	200% (10 min)
E	2:1	No pump	20 L/min	batch replacement	200% (10 min)

*WRR: Ratio of seawater replaced in the bleeding tank/tub during 20 min.

After the bleeding process, the samples (whole gutted fish) from each group were superchilled with chilled seawater/saltwater in a screw conveyor with a two-stage cooling process: first at −0.8 °C for 20 min and then at −2.8 °C for 8 min. The seawater in the chilling process also has the effect of surface cleaning and washing the fish after the bleeding. The samples were then packed in 460-L insulated tubs with no ice and stored in the ship hold at −1 °C for 40 h (tubs without lids and without

drain plugs). Then 20 fish from each group were headed, filleted, and skinned and then manually trimmed. The fillets were then transported in 5-kg expanded polystyrene (EPS) boxes (manufactured by Temptra, Hafnarfjordur, Iceland) at 0 °C. Upon arrival to the laboratory, the fillets were kept at 0 °C storage for two days before being analyzed (on day seven from catch). For each sampling point, measurements were performed on three fillets from each group, and duplicate samples were measured from each fillet ($n = 6$). The rest of the fillets were frozen down to -24 °C in a blast freezer (20T 2/1 POS/NEG REMO, Ilsa Spa, San Fior, Italy). They were stored at -24 °C for four weeks prior to analysis, the one-month sampling point, where three fillets from each group were measured. Subsequently, the remaining samples were transported directly (no thawing) to a -12 °C storage for six weeks followed by -18 °C for eight weeks before analysis. These variations of common storage temperature at -18 °C and high storage temperature at -12 °C were selected to simulate the rapid quality deterioration during an uncontrolled transport and storage in the value chain of frozen fillets. The frozen samples were measured after simulated food-chain freezer storage (six weeks at -12 °C followed by eight weeks at -18 °C).

2.2. Chemical Analyses

Fillet samples were taken from a freezer storage at -24 °C and thawed in air at 0 °C for about 48 h on a metal frame, covered with a plastic sheet to prevent drying. Mid-section fillet parts were used for measurements of fat, FFAs, and PLs, but unbound and bound iron, water content, and salt were measured in all parts of the fillet samples. Total fat content in the muscle was measured using extraction of total lipids [27]. This extraction was then used to estimate the amount of FFAs and the proportion of PLs in the fat measured. The method used for determination of FFA content was the method from Lowry and Tinsley [28] with modification by Bernardez et al. [29]. The procedure of the modified method was to use duplicate samples in two screw cap glass tubes with the removed solvent from the lipid extraction [27]. Then 3 mL of cyclohexane and 1 mL of cupric acetate-pyridine reagent were added, with contact time >30 s. The mixture was vortexed for 40 s and centrifuged at $2000\times g$ for 10 min at 4 °C (Heraeus Biofuge Stratos, Thermo Scientific, Bremen, Germany). The upper layer of the sample was read at 710 nm (Amersham Pharmacia Biotech, Ultrospec 3000 pro, Buckinghamshire, United Kingdom). Quantification was based on a calibration curve constructed from oleic acid standards and the FFA content was calculated by the following equation [29] (oleic acid in Equation (1) stands for μmol of oleic acid and 282.46 is its molecular weight):

$$\text{FFA : free fatty acid content (\%)} = \frac{\text{oleic acid} \times 282.46 \text{ M} \times 1 * 10^{-6}}{\text{g lipid in the sample}} \times 100 \quad (1)$$

The method used for the estimation of PL content was the colorimetric method based on the formation of a complex between phospholipids and ammonium ferrothiocyanate [30]. Duplicates were made of each extract from the lipid extraction. First, 2 mL of chloroform was added to a 15-mL plastic tube with a screw cap. Next, 10 μL of the lipid extract was then added to the tube as well as 1 mL of thiocyanate reagent. The mixture was vortexed for 1 min and centrifuged at 2000 rpm for 5 min at 4 °C. (Beckman Coulter Inc., TJ-25 Centrifuge, Indianapolis, IN, USA). The lower layer was read at 480 nm and compared with known amounts of a standard phospholipid solution.

The HI (heme iron) content was determined according to the method described by Gomez-Basauri and Regenstein [31] with the main reagent being 40 mM phosphate buffer at pH 6.8 (disodium hydrogen phosphate). A 2-g grounded sample was weighed into a 50-mL centrifuge tube and 20 mL of cold 40 mM phosphate buffer was added. The content was homogenized at 13,500 rpm for 10 sec and then centrifuged at $3000\times g$ for 30 min at 4 °C (Beckman Coulter Inc., TJ-25 Centrifuge, Indianapolis, IN, USA). The supernatant was filtered using Whatman No. 1 filter paper and then the filtrate read at 525 nm (Amersham Pharmacia Biotech, Ultrospec 3000 pro, Buckinghamshire, United Kingdom). Myoglobin content was calculated from the millimolar extinction coefficient 7.6 and a molecular

weight of 16.110. The HI content was calculated based on myoglobin, which contains 0.35% iron, and expressed as mg/100 g sample.

2.3. Statistical Analyses

SigmaStat v. 3.5 for Windows (Systat Software, Inc., San Jose, CA, USA) and Microsoft Excel 2016 (Microsoft Inc. Redmond, WA, USA) were used for statistical analysis. One-way analysis of variance (ANOVA) and Duncan's post hoc test were applied for evaluating significance between groups. During analysis, a difference at the level of $p < 0.05$ was considered statistically significant. In order to evaluate the results for the five different groups, a multivariable linear regression model shown in Equations (2)–(4) was constructed [32]:

$$FFA = b_0 + b_1PFR + b_2 WRR + \varepsilon \quad (2)$$

$$PL = b_0 + b_1PFR + b_2 WRR + \varepsilon \quad (3)$$

$$HI = b_0 + b_1PFR + b_2 WRR + \varepsilon \quad (4)$$

where FFA, PL, and HI are dependent variables, and PFR and WRR are independent variables, b_i represents the model parameters and ε is the error or residuals (with mean 0 and variance σ^2).

The objectives of the regression model are to evaluate the relation between the dependent variables, FFA, PL, and HI, on one hand and the effects of the independent variables, PFR and WRR, on the other hand. The parameters in Equations (2)–(4) are estimated in SigmaStat based on the total available observations.

3. Results

The results of the measurements of FFA content in Figure 2 and PL content in Figure 3 show that prolonged frozen storage affects the amount of both FFAs and PLs negatively (i.e., the FFA content increases with longer storage time, while the PL content is reduced). The values in Figures 2–6 are presented as mean values \pm standard error of the mean. The values of FFAs (Figure 2) in the fresh cod samples after seven days show a significant difference between Group B and Group C and no significant difference for other groups. After one month of frozen storage, Figure 2 shows that FFA was higher in all groups and there was a linear trend between the three groups bled in the screw conveyor tanks (A, B, and C). Significantly higher FFA content was measured in Group E compared to the other groups after one month of frozen storage. All groups except E showed a rise in FFA content between months one and four. The FFA trend for Groups A, B, and C was similar between one and four months, while the difference was relatively smaller for Group D and E. The PL content results in Figure 3 show that Group A had significantly the highest PL content in fresh cod after seven days of storage. After freezing, and as the storage time became longer, there was a decline in PL content for all groups, except Group E, which showed lower PL values after one month of freezer storage compared to four months. Therefore, after one month, Group E showed significantly different results compared to the other groups, showing both the highest FFA content and the lowest PL content. After four months of frozen storage, there was no significant difference in PL content between the groups.

The variables PFR and WRR (parameters shown in Table 1) can influence the difference between groups in more than one way. For Group A, both variables are set with different parameters from the other groups. As PFR is comparable between Groups B and C, and WRR is comparable between Groups D and E, the same FFA and PL content results are shown together in Figures 4 and 5. The FFA and PL content of Groups B and C, for which the PFR is the same but the is WRR different, are shown and compared in Figure 4. The greatest difference between the groups was after one month of freezer storage; however, the difference there was determined to be insignificant ($p > 0.05$). Looking at a comparison between Groups D and E in Figure 5, where the WRR is the same but the PFR is different, a significant difference in FFA content was not observed between the groups. The PL values were,

however, significantly different between the groups, both after seven days and one month of frozen storage, showing higher PL content for Group D. However, after four months of storage, there was no significant difference between Groups D and E.

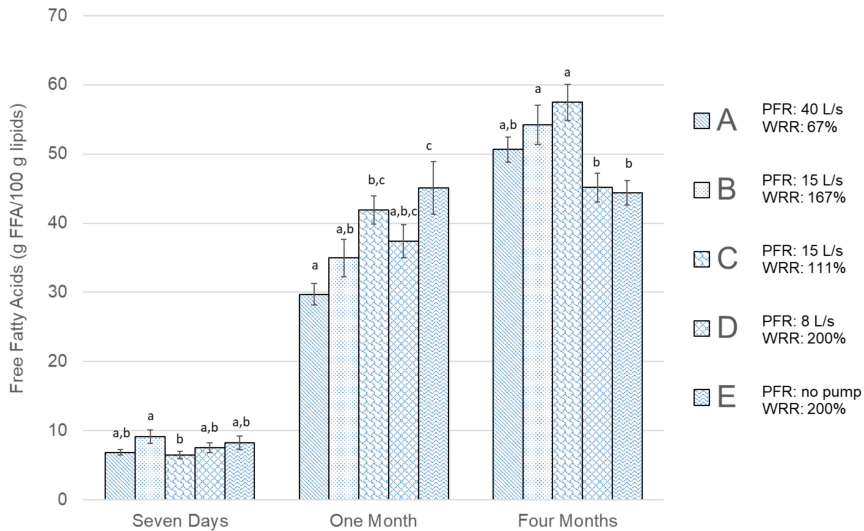


Figure 2. Free fatty acids (g FFA/100 g lipids) results for all groups shown in Table 1. The error bars show the standard error of the mean values ($\bar{\mu} \pm \sigma_{\bar{\mu}}$), $n = 6$. Different letters (a, b, and c) within storage times represent significant differences between experimental groups ($p < 0.05$).

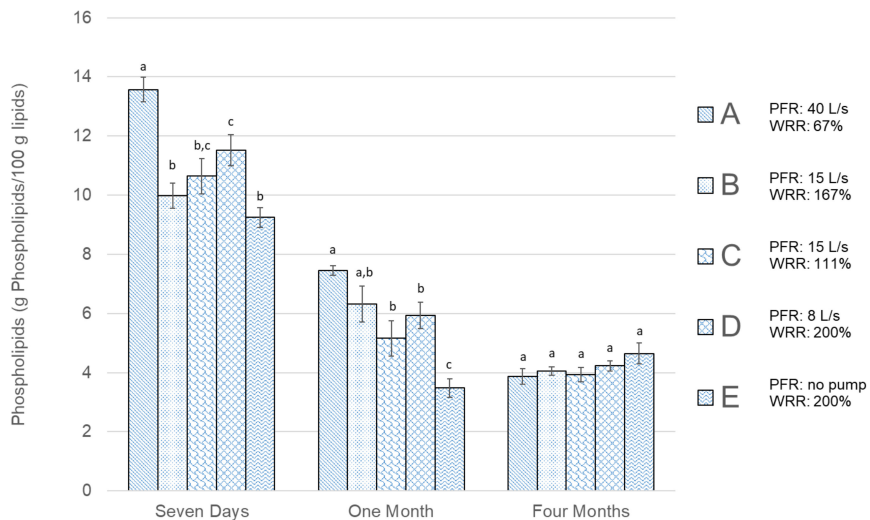


Figure 3. Phospholipids (g PL/100 g lipids) results for all groups shown in Table 1. The error bars show the standard error of the mean values ($\bar{\mu} \pm \sigma_{\bar{\mu}}$), $n = 6$. Different letters (a, b, and c) within storage times represent significant differences between experimental groups ($p < 0.05$).

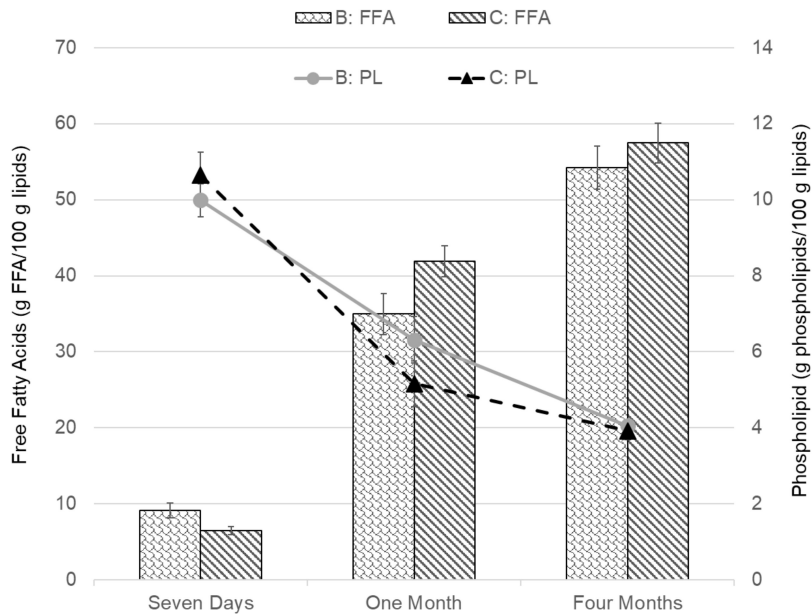


Figure 4. Comparison of Free Fatty Acids (FFA) and Phospholipids (PL) results for Groups B and C. The error bars show the standard error of the mean values ($\bar{\mu} \pm \sigma_{\bar{\mu}}$).

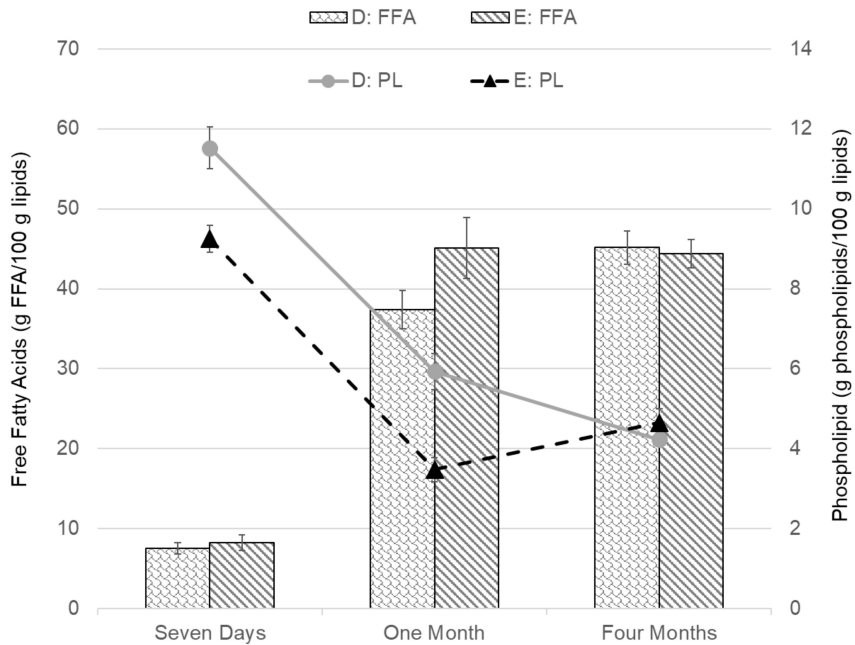


Figure 5. Comparison of Free Fatty Acids (FFA) and Phospholipids (PL) results for Groups D and E. The error bars show the standard error of the mean values ($\bar{\mu} \pm \sigma_{\bar{\mu}}$).

Figure 6 shows the measurement results of HI content for samples of unfrozen fillets seven days from catch and fillets stored frozen from one month at $-24\text{ }^{\circ}\text{C}$ up to four months in a freezer storage ranging from $-12\text{ }^{\circ}\text{C}$ to $-18\text{ }^{\circ}\text{C}$. After seven days, the highest HI values in fresh cod were in Group

A, which were significantly different from other groups, which did not show a significant difference between them. After one month of freezer storage, the trend was the same, with significantly higher HI in Group A compared to the other groups. After four months, the difference in HI values was negligible between groups, except for Group D, which showed significantly lower HI content compared to the other groups.

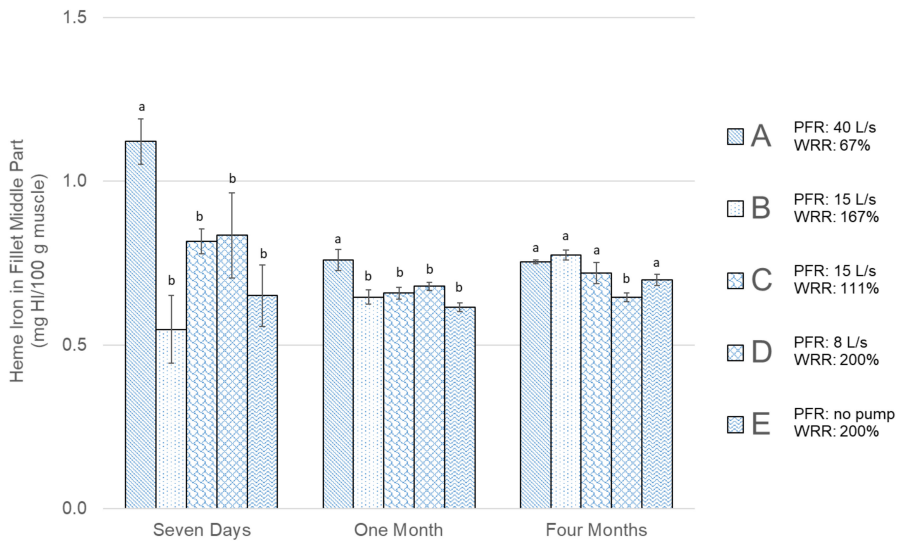


Figure 6. Heme Iron (g HI/100g muscle) results for all groups shown in Table 1. The error bars show the standard error of the mean values ($\bar{\mu} \pm \sigma_{\bar{\mu}}$), $n = 6$. Different letters (a and b) within storage times represent significant differences between experimental groups ($p < 0.05$).

The results of the regression model are shown in Table 2. For each of the dependent variables (FFA, PL, and HI), the model parameters (b_0 , b_1 , and b_2) and their significance are shown. The overall model significance, Prob(F), and R^2 values are also listed for dependent variables. The model results show that the model parameters are mostly significant after one month of freezer storage for variables FFA and PL, where all parameters except one are significant to $p < 0.05$. For the variable HI and the seven-day and four-month time periods for all dependent variables, fewer significant parameters were found.

Table 2. The regression model estimates. Significant parameter values (with $p < 0.05$) are shown in bold.

Dependent Variables	Parameter/Period	7 Days	1 Month	4 Months
Free Fatty Acids (FFAs)—Equation (2)	b_1	0.06	-0.75	-0.31
	(p -value)	(0.36)	(1×10^{-3})	(0.15)
	b_2	2.50	-11.47	-13.04
	(p -value)	(0.13)	(0.03)	(0.02)
	b_0	3.03	66.60	74.67
	(p -value)	(0.36)	9×10^{-7}	(4×10^{-7})
	R^2	0.12	0.45	0.24
	Prob(F)	0.17	(3×10^{-4})	0.02
Phospholipids (PLs)—Equation (3)	b_1	0.14	0.16	-0.01
	(p -value)	(3×10^{-3})	(3×10^{-4})	(0.70)
	b_2	1.18	2.17	0.25
	(p -value)	(0.29)	(0.04)	(0.62)
	b_0	7.08	-0.07	3.90
	(p -value)	(4×10^{-3})	(0.97)	(8×10^{-4})
	R^2	0.53	0.54	0.14
	Prob(F)	4×10^{-5}	3×10^{-5}	0.14
Heme Iron (HI)—Equation (4)	b_1	0.01	0.002	-0.002
	(p -value)	(0.05)	(0.40)	(0.60)
	b_2	0.07	-0.06	-0.13
	(p -value)	(0.69)	(0.33)	(0.16)
	b_0	0.53	0.71	0.92
	(p -value)	(0.13)	(5×10^{-6})	(4×10^{-5})
	R^2	0.38	0.40	0.17
	Prob(F)	2×10^{-3}	1×10^{-3}	0.08

4. Discussion

The design of this study was industry based, as it was performed onboard a trawler during fishing, and is consequently difficult to repeat in full detail due to variability. This makes the study unique but also presents the challenge of isolating the specific variables of interest, PFR and WRR. Studies show that the residual blood of bled fish can be substantial [6,7,13,14]. The bleeding process in this study was designed to provide optimal bleeding conditions with regard to other factors previously studied [5,33–35]. The fish were also bled alive within 30 min, as suggested by Olsen et al. [9], and a washing time of 20 min was ensured [10,14]. While the subject of the bleeding of cod has been studied with regard to optimum bleeding time and bleeding methods [5,9,10,16], few have attempted to evaluate the effect of PFR and WRR, which are important design parameters. This study aimed to evaluate the effects of these variables at three different storage times based on the FFA, PL, and HI contents of cod fillets. The storage times studied were chosen to represent the effects of variable bleeding conditions on fresh cod (iced at 0 °C) seven days after catch, then on the freezing quality (quick frozen and stored at -24 °C), and, lastly, on a simulated frozen storage food-chain (between -12 and -18 °C). The simulated storage temperature used can be compared to industrial frozen storage studied by Dang et al. [21], where similar patterns for FFA and PL content in catfish were observed during the frozen storage period. Most studies on the bleeding of cod use residual blood measurements or some form of color evaluation [9,13,35] to assess the resulting effects. In this study, the reason for evaluating the bleeding using FFA, PL, and HI content is because of their relation to the enzyme activity in the muscle at different storage temperatures [16,36]. The results of this study are mostly directly comparable with the findings of Minh et al. [37,38] and Karlsdottir et al. [16], who also applied

similar methods to investigate the bleeding of cod and other lean fish. Other studies using FFA, PL, and HI content as measurement references to bleeding efficiency and storage apply to other fish species (e.g., cobia, seabass, tilapia, and catfish [21,38–40]).

The results showing FFA and PL development during simulated frozen storage in this study support the contribution of blood components in the fish to lipid oxidation [7]. The trend of FFA formation is similar to what others [16,21] have seen during frozen storage of cod with different bleeding processes, however, the FFA values in this study were relatively higher. The strong connection linking HI to susceptibility of lipid oxidation in the fish muscle [31] was less noticeable in the current study. The measured FFA content was generally low in fresh cod after seven days and similar to values for fresh cod that others have measured [37]. Some patterns were observed, indicating that during frozen storage the FFA content increased at different rates within the fish muscle depending on the group. The results shown in Figure 2 indicated that for groups bled in a screw conveyor, FFA was formed at a slower rate in Group A, which had the largest PFR but lowest WRR. However, after four months of simulated frozen food-chain storage, Groups D and E (which bled out in 460-L tubs with higher WRR) showed significantly lower values of FFA content compared to Groups B and C. This indicates the positive effects of higher values for both PFR and WRR. The FFA results in Figure 2 also show that through the storage period there was relatively less increment in FFA in Groups D and E that were bled in tubs, where PFR was low and WRR was high, compared to groups bled in a screw conveyor with lower WRR.

As suggested by Hardy et al. [41], a decrease in the PL content of lean fish like cod during frozen storage is the main factor driving the accumulation of FFA. A lower rise in FFA formation for Groups D and E indicate less enzyme activity (and slower PL formation) in fillets from those groups, indicating more effective blood removal. The results of total HI content (Figure 6) in the samples showed a significantly higher value for Group A for fresh cod after seven days and also after one month of freezer storage. After four months, there was relatively little difference between groups, with only Group D resulting in significantly lower HI content. The results of HI after seven days and one month, however, indicate that there were some negative effects relating to low WRR.

The PL content results in Figure 3 show a reverse pattern compared to the FFA content, as they decreased as storage time passed, due to enzyme activity [42]. After seven days, the highest significant PL value was for Group A, which had the highest PFR and the lowest WRR. The second highest significant PL content after seven days was in Group D, with relatively low PFR but high WRR. This indicates that for fresh cod, positive effects of both high PFR and WRR can be seen in the corresponding PL content. The lowest PL value average was for Groups E, with the lowest PFR and the highest WRR, however, it was not significantly lower than Groups B and C. The quantity of PL measured in fresh cod was relatively low, compared to the results of Minh et al. [37], although the sampling methods and handling were similar. After one month of freezer storage, these Groups, A and E, still showed the lowest and highest average in PL values among the five groups, with Group E significantly lowest. This indicates that blood removal was more effective in Group A, as PL values support that there was less enzyme activity compared to the other groups. However, after one month freezer storage, Group E showed both the significantly highest FFA and the lowest PL content, indicating negative effects of no pump/PFR even though the WRR was set relatively high. After four months of freezer storage, the PL values for all groups seemed to even out at around 4%, with no significant difference between groups. The FFA content for the corresponding period indicates that the increase in FFA originated from another source than decomposing PLs [21,42].

Comparing specifically Groups B and C (in Figure 4), where the WRR was variable but the PFR was constant, there was not much difference noticeable between the groups, indicating that the change in WRR did not affect the blood removal. For these specific parameters, it can therefore be concluded that WRR over 100% in 20 min (111% for group C) was sufficient. The comparison between Groups D and E (in Figure 5), where the PFR was variable and the WRR was constant, showed a more significant difference, especially for the PL content after seven days and one month of storage. The results of

that comparison support that there was more residue blood in Group E, which was bled at a high WRR but which had no pump/PFR. This indicates that with these specific parameters, the PFR had a greater effect on the bleeding efficiency and storage quality of the fillets than the WRR. This is further supported by looking at Group A, which had the highest PFR and lowest WRR, resulting in the highest PL content after seven days and slower formation of FFAs compared to Groups B and C, which bled out in the screw conveyor. Bleeding conditions affected the HI content measured in fresh cod after seven days but evened out to around 0.6–0.7 mg HI/100g after freezer storage (Figure 6). This is in accordance with Minh and Phan [38], where total HI content was initially varied for different bleeding conditions for cobia fish and evened out after 24 weeks of freezer storage. The total HI content in this study was at the same level as the ice water bled cobia studied by Minh and Phan [38] but higher compared to HI in other studies on seabass [39] and tilapia [40], likely due to the different fish species.

The regression analysis shows a significant overall model for six models out of nine, the R^2 values, however, are relatively low (highest at 0.54), indicating that while the WRR and PFR are correlated with the FFA, PL, and HI content, there are other factors explaining some of the variability. The results of the regression model indicate that the variables PFR and WRR have the most significant effects on FFA and PL after one month of freezer storage. For HI content, fewer parameters were found to be significantly different, which is consistent with the results of the ANOVA test. The model showed that more significant parameters were related to the PFR than the WRR. The results of the model also indicate that after four months of freezer storage with temperature fluctuations, there was no difference between the groups in PL results, as there was no significant parameter for PL content. The HI results in Figure 6 indicate a difference between groups for unfrozen cod seven days from catch, as residual blood could act as an oxygen donor effecting PL. After seven days of refrigerated storage, there could also be some effects of residue blood that have yet to emerge in FFA and PL end-point results.

5. Conclusions

In this study, the influence of variable pump flow in a bleed-out tank and the rate of seawater recirculation rate during the bleeding process on the changes in FFA, PL, and total HI content in cod muscle was evaluated. Measurements were done on different storage times: on fresh cod seven days after catch, after one month of frozen storage at $-24\text{ }^{\circ}\text{C}$, and, lastly, after four months of simulated frozen food-chain storage (-12 to $-18\text{ }^{\circ}\text{C}$). The results for PL content and the regression model indicate that the enzyme activity in the fish muscle was lower in cases where PFR exerted more influence in the bleeding process than WRR. The effects of successful blood removal also seem to be most noticeable after one month of freezer storage, rather than in fresh cod after seven days or after four months of simulated frozen food-chain storage. The temperature fluctuations during frozen storage for four months resulted in PL values evening out at around 4%, while FFA values were still rising, causing more risk of rancidity in the cod. FFA measurements after four months showed better results for cod bled in tubs compared to the groups bled in a screw conveyor, indicating that higher WRR could have some long-term positive effects. Changes in HI content were less noticeable, as the regression analysis parameters for PFR and WRR were not significant for HI content.

In terms of design parameters for the industry, the study indicates that with the bleeding water to fish ratio of 3:1 and enough WRR (over 100% in 20 min), the PFR becomes the limiting design parameter regarding efficient blood removal and should be at least 10% of the tank volume per minute to ensure enough recirculation and flow of water in the bleed-out tanks.

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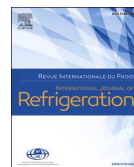
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Paper III



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Onboard pre-chilling of ungutted and gutted Atlantic cod in different cooling media -Temperature measurements and analytical modelling-

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ABSTRACT

Cooling experiments using flake ice and chilled brine/seawater were carried out on ungutted and gutted cod. Core temperatures of the fish loins and tails were measured during the cooling process. The study aims were to investigate different approaches to chilling gutted and ungutted whole fish and compare controlled brine chilling with traditional flake ice chilling. Experiments were conducted in controlled environment using fish in three weight categories, around 2.5, 4 and 9 kg. The experimental results were used as design parameters for newly developed onboard industrial chilling processes using different approaches to superchill whole gutted cod (slurry ice and refrigerated seawater/brine). Industrial chilling processes were evaluated by mapping temperature profiles onboard three wet-fish trawlers using different chilling systems and methods. Temperature measurements were then used to generate a model to estimate chilling time and the convective heat transfer coefficient between 2, 4 and 6 kg fish and cooling medium. The chilling time of equal weight fish from +8°C to 0°C proved to be 30-50% shorter with refrigerated brine chilling compared to flake ice chilling. The difference between chilling gutted and ungutted cod was apparent, as expected, due to a greater surface area between gutted fish and the cooling medium. The chilling time difference between gutted and ungutted fish was less with flake ice chilling than with refrigerated brine chilling. Comparison of the model results with onboard measurements indicate that the thermal conductivity of the fish is limiting to the energy transfer and efficiency of the onboard chilling systems rather than the heat transfer coefficient.

1. Introduction

The development of fish processing onboard wet-fish trawlers has been ongoing in recent years. A part of this development has been improvements in cooling and storage processes onboard wet-fish trawlers. Icelandic wet-fish trawlers typically catch 20-50 tons of groundfish per day in fishing trips lasting from three to six days. The well-known method of chilling and storing the catch in ice brings the fish temperature slowly and surely to the ice melting point, at 0°C. Superchilling however means reducing the fish temperature uniformly below melting point of ice resulting in extended storage life. Recent developments of onboard cooling include superchilling the fish on the processing deck rapidly after catch and storing it without ice in the hold at -1°C. These

onboard systems use various cooling media such as chilled seawater (CSW), ice slurry or, refrigerated seawater (RSW) or refrigerated brine (Ruiz, 2012). In this study the definition of RSW refers to cooling with the use of mechanical refrigeration as part of the cooling tank system while CSW refers to cooling with adding ice or ice slurry to the cooling tank seawater (Graham *et al.*, 1992). Instead of a large part of the cooling process taking place with iced fish in the storage hold, the new processes are designed to finish the temperature reduction process on the processing deck and store the fish with no ice, or a limited amount of ice, at a temperature close to the fish initial freezing point (T_f), i.e. close to -1°C for Atlantic cod (Stevik and Claussen, 2011). According to Rahman *et al.* (2009), the initial freezing point for whitefish with water content around 80% is close to -1°C (Margeirsson *et al.* (2012) modelled it at -0.92°C for cod) and ice crystal formation increases rapidly at slightly

Abbreviations: CSW, Chilled seawater; RSW, Refrigerated seawater.

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Nomenclature

Symbols	Meaning
A	Overall surface area of the fish [m ²]
a	Parameter for surface area formulae [-]
b	Parameter for surface area formulae [-]
Bi	Biot number
c _p	Specific heat [J·kg ⁻¹ ·K ⁻¹]
D	Fraction of volume and surface area [m]
h	Heat transfer coefficient [W·m ⁻² ·K ⁻¹]
k	Thermal conductivity [W·m ⁻¹ ·K ⁻¹]
m	Mass/Weight [kg]
n	Number of samples [-]
Q̇	Heat flow [W]
T _f	Initial freezing point [°C]
T _s	Surface temperature of fish [°C]
T _∞	Ambient temperature [°C]
V	Volume [m ³]

lower temperatures.

The chilling of fish and different cooling techniques have been widely studied, by e.g. Margeirsson and Arason (2008), Margeirsson et al. (2010), Thorvaldsson et al. (2010), Kaale (2014), Bantle et al. (2015), Tolstobrev et al. (2019a,b). Reviews by Magnussen et al. (2008) and Kaale et al. (2011) cover the advantages of superchilling fresh fish and the beneficial effects on quality. Based on experience and theoretical calculations, approximate chilling times may be estimated. However, implementing superchilling in industrial processing of wild catch requires dynamic temperature control during the chilling process. As pointed out by Bantle et al. (2016), there are challenges in industrial superchilling where predictions on the state of superchilling, in terms of ice crystal formation, are complex to pinpoint. Avoiding excessive surface freezing and limiting ice crystal growth is a challenge in these conditions regarding structural damage and negative texture changes to the fish flesh and increased drip loss (Bahuaud et al., 2008; Magnussen et al., 2008; Kaale et al., 2011). The onboard chilling process design, regardless of the chilling method or cooling medium, needs to handle energy removal proportional to the trawlers fishing capacity. To optimize the chilling process and temperature control of the system, variable size and quantity/flow of fish must be considered.

It is common that a large part of the groundfish catch of small boats comes ashore ungutted when the sea trip takes less than one day, while most of the catch of larger boats is gutted at sea, at least in Iceland. Therefore, groundfish are often chilled and stored ungutted onboard smaller boats and, according to the authors' best knowledge, the principal difference between cooling ungutted vs. gutted cod has not been studied. The main reason for gutting groundfish is to prevent autolytic spoilage and to some extent spoilage by removing the main source of bacteria (Borderias and Sanchez, 2011). In terms of onboard processing of groundfish the advantages of skipping immediate gutting is that the catch can be effectively bled within 30 minutes resulting in better exsanguination and lower microbial content (Olsen et al., 2014). The method that is now used for pelagic fish, chilling in onboard RSW tanks, was studied by Roach et al. (1961) for various species of fish, including ungutted groundfish. The interest of Icelandic producers in this method lies in lowering fishing costs by bringing the gutting process ashore and providing the opportunity to utilize more rest raw materials from the fish. Other research involving superchilling of whole fish (Thorvaldsson et al., 2010; Bjarnason, 2012; Olafsdottir et al., 2012) has not included a comparison of chilling processes for gutted and ungutted fish. Kaale and Eikevik (2016) studied industrial superchilling of fillets with regards to quality indicators but did not include the chilling of whole fish.

The aim of the study is to evaluate chilling methods of whole cod in

controlled first-in first-out superchilling systems with different approaches and compare them to the more traditional and well-known method of flake ice chilling, studied e.g. by Thorvaldsson et al. (2010) and Laguerre et al. (2018). Experiments of chilling cod were carried out in both controlled environment and industrial processing onboard wet-fish trawlers using different chilling methods. The novelty of this study is to evaluate the temporal difference of chilling ungutted and gutted cod with controlled experiments and to evaluate the performance of industrial onboard chilling processes. The measurements of industrial chilling systems were conducted to evaluate the challenges for industrial implementation of superchilling as, pointed out by Bantle et al. (2016), the precision of the onboard chilling process becomes increasingly important since the new systems do not rely on ice as a cooling medium in the ship hold. Therefore, any thermal energy transferred in to the ship hold can disrupt the storage temperature. The experimental results of the study were used to build a model for chilling gutted cod with variable cooling medium temperatures and to estimate the convective heat transfer coefficient for different industrial systems.

2. Materials and methods

The temperature measurements in this study were composed of two main parts. During the first part, temperature measurements were carried out in a controlled environment, chilling ungutted and gutted cod with flake ice and in a refrigerated brine system. In the second part of the study, different industrial superchilling processes were evaluated by measuring temperature profiles onboard three wet-fish trawlers; Trawler A (slurry ice/CSW), Trawler B (RSW and refrigerated brine, two-step chilling), Trawler C (RSW, one-step chilling). During the measurements in this study, both the temperature inside the fish and the ambient temperature surrounding the fish were logged. In the current study, the term "ambient" refers to the temperature of the fish environment, i.e. the cooling medium, ice or liquid surrounding the fish.

3. Experiment design

The cod used in the controlled environment experiment was caught by a wet-fish trawler southwest (SW) of Iceland in April, and the fish were chilled both in chilled brine and flake ice for comparison. As the exact same fish were measured in both chilled brine and flake ice, applying the same uniform initial temperature in the experiments, the difference between fish in different cooling media can be directly compared. The weight of gutted and ungutted fish however, selected as close as possible from the size range available for this experiment. In the onboard experiment, six fish in each size group were measured. Cod used for onboard measurements was caught at different locations, as shown in Table 1. The weight of the fish is also shown in Table 1, small (2.5 kg), medium (4-5 kg) and large cod (6-9 kg) defined in three size groups. Average water content measured in fish loin was 82.7% and in the fish tail 83.2% (average of three samples, n=3).

The chilling of fish in a controlled environment was done in a 660 litre insulated tub equipped with a circulating pump (1.5 kW) as shown in Fig. 1. In order to measure the temperature gradient in the tub, temperature data loggers were located at three different depths in the brine solution. This way, the temperature of the liquid at the bottom of the brine solution could be compared to the temperatures closer to the surface.

The fish was then prepared for measurements by placing temperature data loggers inside the core of both the loins and near the tail, by the back fin (insertion points are shown in Fig. 2). When measuring core temperature of loin and tail the temperature loggers were inserted as close to the fish spine as possible.

When placing the loggers in the tail, care was taken so that minimal disruption was inflicted on the muscle structure. The temperature of the chilled brine was kept just below -1°C, and the salt concentration of the brine at 3.5%. Fish chilled with ice were well covered with flake ice and

Table 1
Fish size groups, weights and catch locations.

		Gutted weight [kg]	Ungutted weight [kg]	Location and time of catch
Experiment 1, controlled environment	Size group I, small cod	2.5	2.5	Southwest (SW) of Iceland in April
	Size group II, medium cod	4.1	5.1	
	Size group III, large cod	9.1	9.3	
	Size group I, II, III	2, 4, 6		
Experiment 2, onboard systems	Size group I, II, III	2, 4, 6		Trawler A: Southwest (SW) of Iceland in March Trawler B: Northwest (NW) of Iceland in March Trawler C: Northeast (NE) of Iceland in September
Model	Size group I, II, III	2, 4, 6		



Fig. 1. Setup of chilling experiment in controlled environment.

stored in insulated boxes, made from expanded polystyrene, during the chilling period.

The onboard cooling systems measured in this study were all screw/spiral conveyor types, i.e. an Archimedes screw transferring fish through the cooling tanks. The ratio of cooling medium to fish was around 3:1 for all systems. The following is a description of the different trawler cooling equipment:

- **Trawler A, Ice slurry/CSW:** The equipment used was a screw conveyor (Skaginn et al., 2018) with one-step slurry ice chilling at -1.0 to -1.2 °C.
- **Trawler B, Refrigerated brine, two-stepchilling:** The onboard super-chilling method was in two chilling steps; the first step cooling in

seawater at -0.8 °C (3% salt concentration) and the second step cooling in brine at -2.8 °C (6% salt concentration). The equipment used was a screw conveyor (Skaginn 3X, Akranes, Iceland, 2018).

- **Trawler C, Refrigerated seawater, one-stepchilling:** The equipment used was a screw conveyor manufactured by Stranda (2019) and adapted by Slippurinn, Akureyri, a one-step RSW system with seawater at -1 °C.

3.1. Temperature measurement devices

Different types of temperature data loggers were used in the experiments. For the ambient temperature, Tidbit v2 loggers from OnSet Computer Corporation (Bourne, MA, USA) were used. The accuracy of the data loggers is ± 0.2 °C for temperatures between 0 °C and 5 °C. Variable depth temperature measurements were done with a Testo 176 T4 data logger (Testo Inc., Lenzkirch, Germany) with TC type K thermocouples probes (accuracy ± 0.3 °C and resolution 0.1 °C). The loggers used to measure the fish core temperature were Maxim Integrated's iButton data loggers. The measurement resolution of these loggers is 0.0625 °C and the error margin for temperatures between -15 °C and 65 °C specified by the manufacturer is no more than 0.5 °C. However, van Marken Lichtenbelt et al. (2006) reported that during a test for error margins, these loggers measured temperatures with a reading error of no more than 0.09 °C.

3.2. Heat transfer model

The model for simulating chilling was built using Simulink and includes three measurement points within the loin section of the fish and two measurements within the tail section, shown in Fig. 2. A first order differential equation is combined for each point and they are interlinked in a 2nd and 3rd order equation, for the tail and loin respectively. The ambient temperature is set to interact with the surface of the fish through convection and heat transfer inside the fish is through conduction. Each section of the model relies on parameters that are estimated through optimization (Matlab script running the iterations). When running the script, the initial simulation of the model used pre-determined initial values from Johnston et al. (1994) for temperature dependent thermal properties of white fish. Thermal properties of the fish were kept constant for temperatures above 5 °C in the model. For fish temperatures between 0 °C and 5 °C, square interpolation was used to get fitted values based on temperature. However, for temperatures lower than 0 °C, cubic interpolation was used to increase estimation precision for thermal properties of the fish during phase change. The cubic interpolation uses a third-degree polynomial to derivate between grid points (The Math Works, Inc., 2020). As the model is not simulating the partial freezing of the fish, the cooling was modelled in such a way that the physical properties of the muscle hit an initial freezing point threshold at $T = -0.9$ °C. This was done by creating a large thermal resistance, defining the apparent specific heat (c_p) of the muscle at 300 $\text{kJ kg}^{-1}\text{K}^{-1}$ when the temperature reached the initial freezing point threshold of -0.9 °C. As Pham (2006) states, due to inaccurate results, the apparent specific heat method is not recommended for modelling the freezing process, however in this study the fish core temperature is not

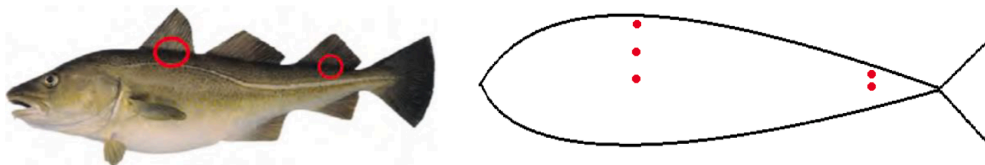


Fig. 2. To the left, temperature logger placement in fish (red circles denote the insertion points) and to the right, a representation of the measuring points of fish in the model (red points).

being chilled below T_f , although the ambient temperature can be lower. To simulate supercooling, the specific heat above freezing is assumed to continue to apply below T_f until the coldest node reaches nucleation temperature, at which point all the nodes are assumed to freeze instantaneously (Pham, 2006). To fully model the freezing and thawing of the muscle as well as the cooling, further background work is needed as a wide variety of protein changes take place during freezing and thawing, affecting the thermal properties of the muscle by less water-holding capacity (Kaale, 2014; Kaale and Eikevik, 2016; Kaale et al., 2011).

For each section representing either a thermal mass, convection or conduction in the model layers, the following parameters were estimated: mass, apparent specific heat, thermal conductivity, thickness and surface area. The position of the loin and tail sections were decided to be the location of the front and back dorsal fin (see Fig. 2 and 3). These positions were used to place loggers in experiments, insertion locations in the fish measured and used in the fish model. Factors related to the total weight of the cod (m), for both loin and tail sections, were estimated empirically with experiments and comparison of the modelled temperature response and the controlled experiment chilling profiles measured for gutted cod. The factors were estimated in an iterative way to obtain cooling curves that gave the best fit to the measurements. The overall surface area of the fish was estimated by considering the mass of the fish and then using formulated quantities based on work from O’Shea et al. (2006) in Eq. (1):

$$A = a \cdot m^b \tag{1}$$

where the constants $a = 11.2$ and $b = 0.65$ are constants defined by the fish species. Each cross section, represented by a temperature logger placement point shown in Fig. 2, was then defined with a reference area based on the total surface area found by Eq. (1). These parameters were defined as in Fig. 3, where A denotes the total surface area. For each section of the fish, in addition to the surface area, a specific reference thickness was determined for each weight category simulated, shown in Fig. 3.

In the model a specific time is defined, determining how long each simulation runs. To build the initial model, these time intervals were set as in the controlled experiment measurements. The initial temperature of the whole fish is set to an initial value, and the surface temperature of the fish is the same temperature as the outermost block. At the surface of the fish the heat flow is calculated based on the temperature difference

between the outermost layer and the surroundings and a constant coefficient of heat transfer. The results of the model were compared to temperature measurements to estimate the convective heat transfer coefficient for fish in controlled experiments and trawler systems. To estimate the convective heat transfer coefficient during experiments Testo thermocouples (Testo Inc., Lenzkirch, Germany) were installed at different depths under the fish skin (approximately 25 mm, 10 mm and 3 mm) to compare with iButton core temperature measurements. The convective heat transfer coefficient is estimated, similar to what Bantle et al. (2015) and Tolstorebrov et al. (2019a,b) did for RSW chilling of salmon. The heat flow from the surface of the fish to the ambient (refrigerated water or ice) is represented by Eq. (2):

$$\dot{Q} = h \cdot A \cdot (T_s - T_\infty) \tag{2}$$

where \dot{Q} represents the heat transfer per unit time, h is the convective heat transfer coefficient, A the surface area of the fish, T_s the surface temperature and T_∞ the ambient temperature.

The Biot number defines the ratio of the heat transfer resistance inside the fish and at the surface:

$$Bi = \frac{h}{k} \cdot D \tag{3}$$

where h is the convective heat transfer coefficient, k is the conductivity of the fish, D is the fraction of volume (V) and surface area (A) of the fish (long cylinder approximation).

4. Results

In this chapter the results from controlled and onboard experiments are presented. The definition of chilling time refers to the time it takes to lower the initial fish temperature to 0°C, regardless of the ambient temperature used. Finally, the model result chilling profiles for different convective heat transfer coefficients are compared to the results of the onboard experiments. The model uses data from onboard measurements for the cooling medium temperature for each trawler to predict the cooling profile for the fish size groups.

4.1. Chilling profiles, controlled experiments

The temperature graphs in Fig. 4 and Fig. 5 show results from

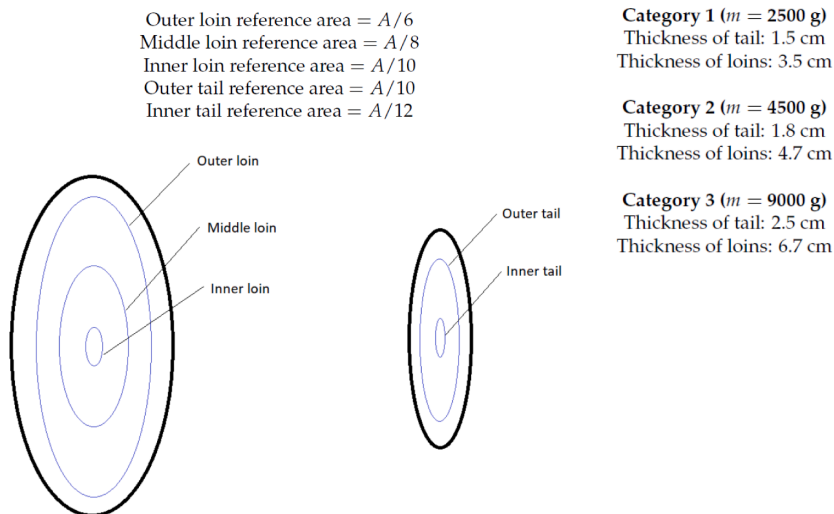


Fig. 3. Cross sectional areas of loin and tail sections for three size categories of the fish model. Reference area refers to the surface of a specific cross section.

controlled experiments. The chilling time from 8°C to 0°C in flake ice and chilled brine is presented for different size categories of ungutted and gutted fish. Fig. 4 a) shows the chilling profiles for the cod loin in flake ice and in Fig. 4 b) for the same fish in chilled brine. The loin in chilled brine reaches 0°C in approximately half the time compared to flake ice chilling. With flake ice there is limited difference in chilling time between ungutted and gutted fish, and little difference for larger fish in flake ice than for smaller fish. In chilled brine, however, the difference between ungutted and gutted fish is apparent, especially for medium sized cod. Fig. 4 a) shows shorter chilling time for ungutted fish in the smallest size category compared to the gutted, however the gutted fish was larger than the ungutted before gutting.

Fig. 5 shows temperature profiles of the cod tail during the same experiment. The tail reaches 0°C between 3 and 5 times faster in chilled brine compared to flake ice chilling. Also, there is less noticeable difference between whole ungutted and gutted fish for the cod tail, compared to chilling times for the loin. This clearly shows that the gutting process doesn't affect the thermal resistance between the temperature measuring point at the core and the surface of the tail as compared to the loin.

4.2. Chilling profiles, onboard experiments

Fig. 6 shows temperature profiles measured for gutted cod on board trawlers with different chilling systems. Trawlers A and C have steady chilling temperatures while Trawler B has a separate chill tank where the cooling medium drops to -3.5°C. The fish measured were weighed and sorted in three size categories, 2, 4 and 6 kg ($n=8$, average of eight fish for each size group). The temperature is shown from around 6°C,

measured in the loin of the fish, and is comparable to temperatures in the controlled measurements for gutted fish in Fig. 4 b). The chilling times are mostly similar but more rapid in the controlled experiments at -1°C chilled brine.

4.3. Model validation

Fig. 7 shows the comparison of modelled chilling profiles and measured profiles for different values of the convective heat transfer coefficient (h). The cooling medium temperatures for each trawler are the same as were measured in the onboard systems, shown in Fig. 6. The example shown are results from Simulink modelling of loin for 4 kg gutted cod fish size. The temperature profiles are shown for different convective heat transfer coefficients ($h = 100, 300$ and $700 \text{ W m}^{-2} \text{ K}^{-1}$). For h values higher than $300 \text{ W m}^{-2} \text{ K}^{-1}$ the difference in chilling time simulations becomes negligible for the model.

A Biot number calculated with Eq. 3 using $h = 300 \text{ W m}^{-2} \text{ K}^{-1}$, $k = 0.43 \text{ W m}^{-1} \text{ K}^{-1}$ and volume/surface area of the small fish size gives $Bi = 11.6$. For the larger fish sizes the Biot number will increase. As the Biot number is considerably larger than 1 it concludes that the convective heat transfer is more efficient than the thermal conductivity, i.e. the surface of the fish will exchange heat much more quickly than the rest of the body.

For 4 kg gutted cod the model is closer to the final temperature for Trawler A but fits the measured chill curve best for Trawler B. The measurements of Trawler A and C however show a little more effective chilling during the first hour compared to the model.

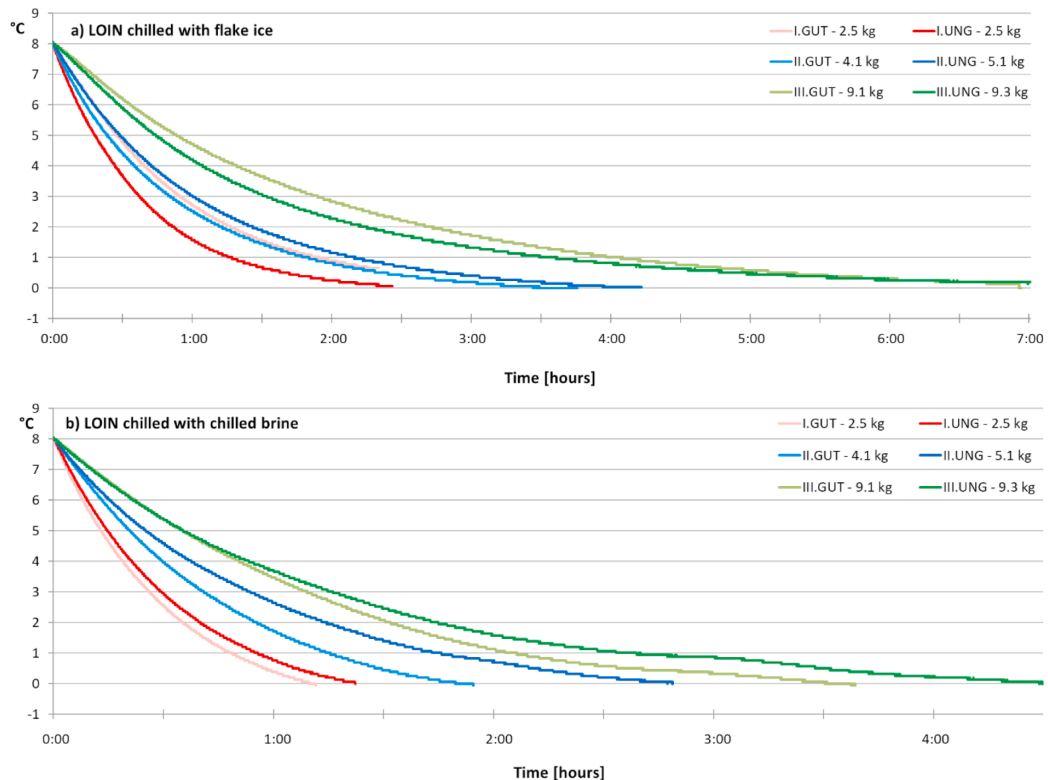


Fig. 4. Experimental measurements of controlled conditions for a) Core temperature of cod loin chilled with flake ice (at 0°C) and, b) Core temperature of cod loin chilled with chilled brine (at -1°C). Core temperature is measured close to the fish spine.

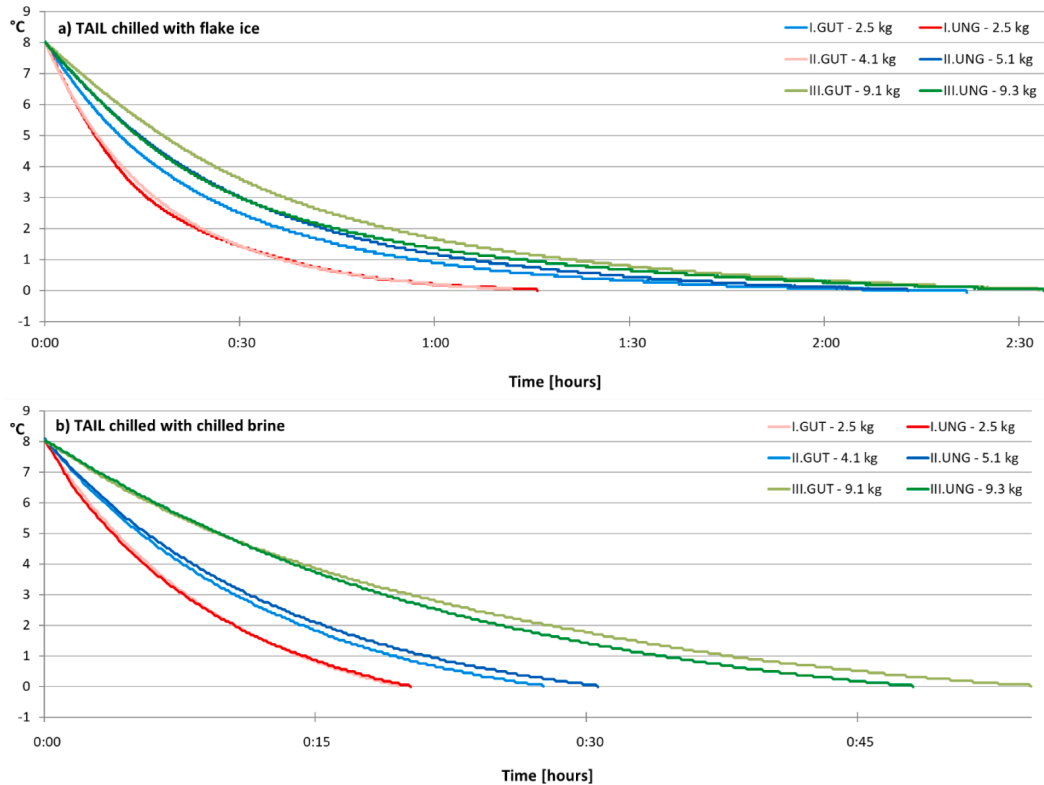


Fig. 5. Experimental measurements of controlled conditions for a) Temperature of cod tail chilled with flake ice (at 0°C) and, b) Temperature of cod tail chilled with chilled brine (at -1°C). Core temperature is measured close to the fish spine.

4.4. Chilling time summary

Table 2 shows chilling times generated from simulations and for measurements, i.e. the chilling time of gutted cod loin in minutes, from 6°C to 0°C. Also shown in the table are average cooling rates [°C/minute] for the temperature interval from 6°C to 0°C.

5. Discussion

While the chilling of fish generally has been widely studied, the focus of this study was set to support the development of industrial super-chilling of groundfish, with more specific estimations of chilling times for common groundfish size categories, considering ambient temperatures below the fish initial freezing point (T_f), and gutted vs. ungutted fish. Comparing chilling times of whole cod in Fig. 4 with previous controlled experiments (Margeirsson and Arason (2008), Thorvaldsson et al. (2010), Bjarnason (2012), Olafsdottir et al. (2012)), chilling cod, saithe and haddock in different cooling media shows mostly similar but in some cases more rapid chilling rates. The main difference between chilling gutted cod with flake ice and chilled brine in this study is that the contact area of the brine and fish is larger than with ice as the brine rather flows inside the fish gut area. As expected, compared to flake ice chilling, the difference in chilling rate for ungutted vs. gutted fish was greater in chilled brine, where the cooling medium reaches a larger heat exchange area of the fish. The chilling of whole ungutted fish is relevant in smaller boats where onboard facilities for gutting are not available or when the aim is to RSW chill whole fish and gut after landing (as studied by Roach et al., 1961).

Results of industrial onboard measurements showed that the systems

using RSW chilling methods were more effective than a slurry ice/CSW system. Compared to the results of the controlled experiment, the industrial systems were similar, suggesting that the systems are effective in terms of heat transfer and cooling medium to fish ratio. This is further supported by the results of the model, where industrial systems fit chilling profiles with h -values ranging from 300 to 700 $W \cdot m^{-2} \cdot K^{-1}$. The model shows negligible differences for h -values higher than 700, as the thermal conductivity (k -value for cod 0.43 $W \cdot m^{-1} \cdot K^{-1}$) becomes a limiting factor in the model, which is consistent with the results of Bantle et al. (2015). With conventional methods for water chilling, the surface heat transfer coefficient is considered intensive at $h \approx 350 W \cdot m^{-2} \cdot K^{-1}$ and can, with a high degree of turbulence of the refrigeration medium, be up to $h = 720\text{--}760 W \cdot m^{-2} \cdot K^{-1}$ (Fikiin, 1992). Estimation of ice slurry systems have shown h around 750 $W \cdot m^{-2} \cdot K^{-1}$ (Fikiin et al. 2005). The heat transfer coefficient for ice is estimated between 15 and 35 $W \cdot m^{-2} \cdot K^{-1}$ (Jain, 2005, Tolstorebrov et al. (2019a,b)).

Tolstorebrov et al. (2019a) modelled RSW systems for salmon and concluded the convective heat transfer coefficient for laboratory chilling tanks to be close to 300 $W \cdot m^{-2} \cdot K^{-1}$ and for large-scale industrial chilling units between 112 and 139 $W \cdot m^{-2} \cdot K^{-1}$, due to the density of fish in the systems. For comparison of this study results for cod with a fattier fish lower water content and thermal conductivity will have to be accounted for, Rahman et al. (2009) report k -value 0.47 $W \cdot m^{-1} \cdot K^{-1}$ for salmon with 72.5 % water content. These factors would generally result in decreased heat transfer. A study of Tolstorebrov et al. (2019b) on industrial RSW systems for salmon showed that optimal effectiveness was achieved when the density of fish in the spiral tanks was decreased to below 33% (fish to sea water ratio) and with RSW flow close to 200 $m^3 \cdot h^{-1}$. The results of this study suggest that the fish density and RSW

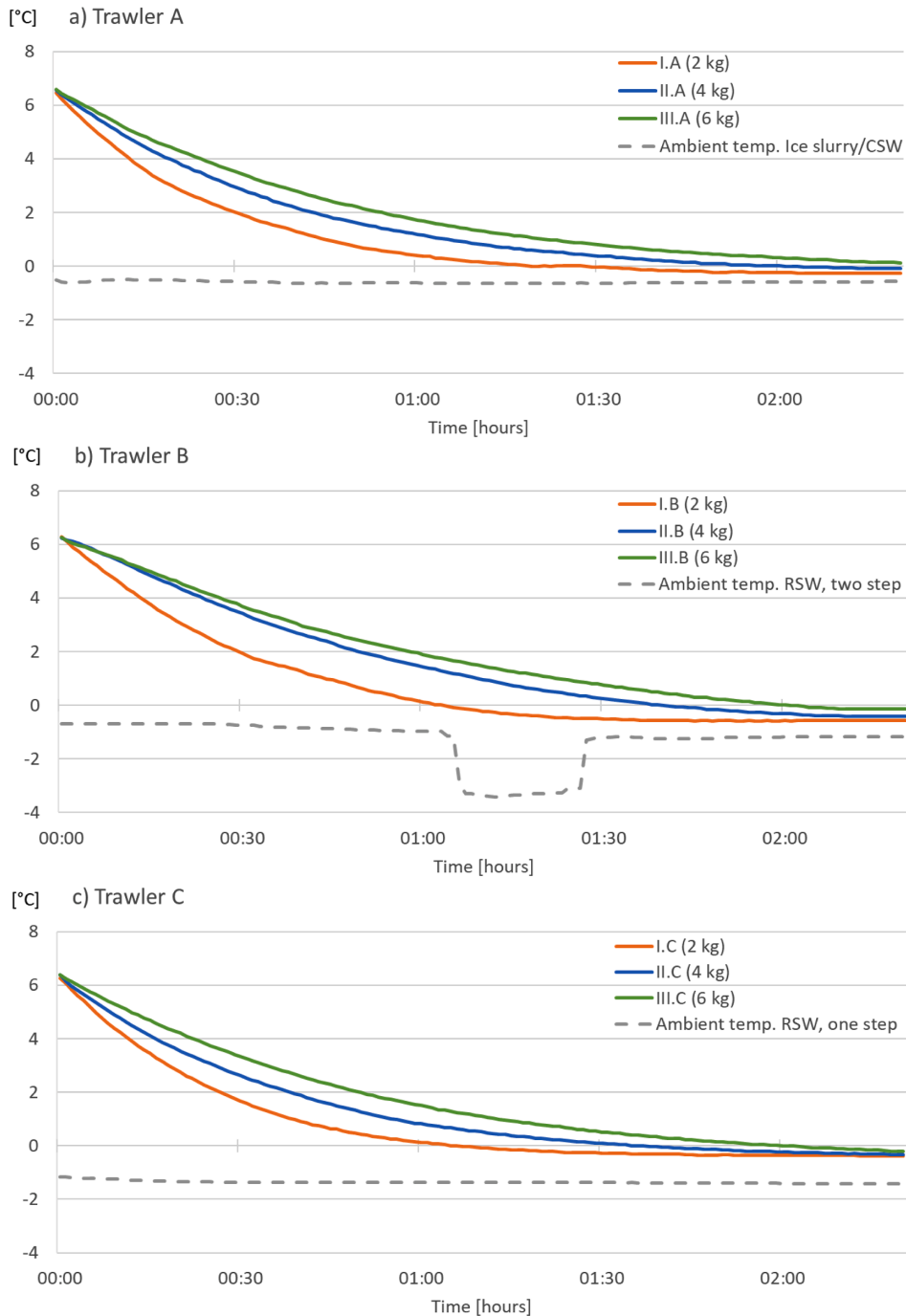


Fig. 6. Chilling profiles for three size categories of gutted cod (LOIN in 2, 4 and 6 kg fish) for a) Trawler A: Ice slurry/CSW, b) Trawler B: RSW, two step and c) Trawler C: RSW, one step.

flow rate of the industrial systems are not limiting factors as their chilling profiles are close to the controlled experiments and the model results. The systems are, however, close to their limitations in terms of lowering the temperature of the cooling medium if they are to avoid the

risk of freezing the tail end of the fish (or the surface or belly flap of gutted fish). This is one of the challenges of industrial superchilling systems, as pointed out by [Bantle et al. \(2016\)](#), in lowering the cooling medium temperature below T_f and thus risking partial freezing. This

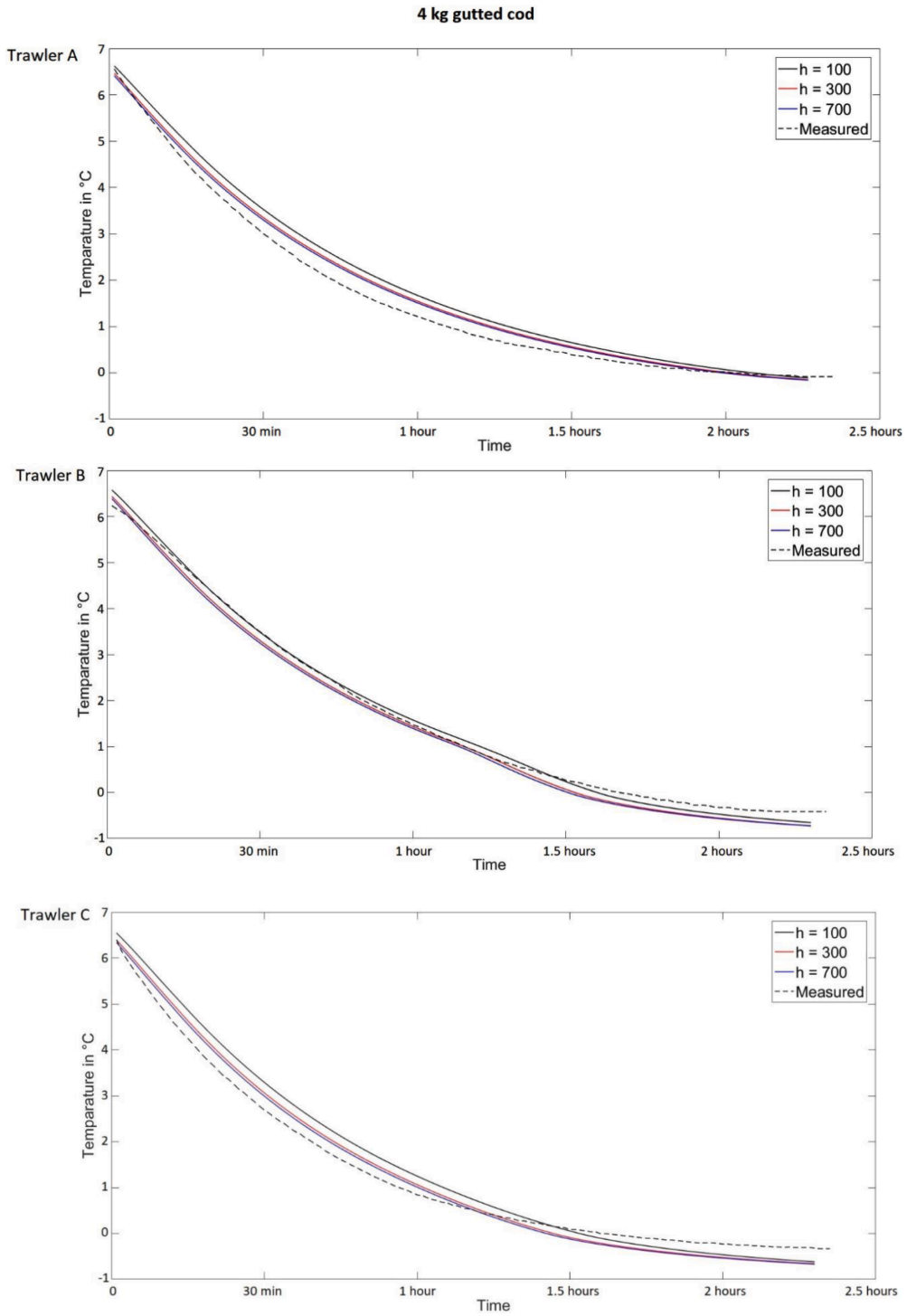


Fig. 7. Comparison of chilling profiles for loin measurement and model for 4 kg gutted cod, Trawlers A, B and C.

Table 2
Chilling of gutted cod (loin) from 6°C to 0°C, time in minutes (min).

		Size group I (2.5 kg)	Size group II (4.1 kg)	Size group III (9.1 kg)
	Cooling method	Cooling time [cooling rate°C/ min.]	Cooling time [cooling rate°C/ min.]	Cooling time [cooling rate°C/ min.]
Experiment 1, controlled environment	Flake ice (°C)	120 min. [0.05]	205 min. [0.03]	390 min. [0.02]
	Chilled brine (-1°C)	70 min. [0.09]	105 min. [0.06]	185 min. [0.03]
Experiment 2, industrial systems	Trawler A	80 min. [0.08]	120 min. [0.05]	-
	Trawler B	65 min. [0.09]	105 min. [0.06]	-
	Trawler C	70 min. [0.09]	110 min. [0.05]	-
Model	h=700	65 min. [0.09]	100 min. [0.06]	145 min. [0.04]

study also concurs with Bantle *et al.* (2016) and Tolstorebrov *et al.* (2019a) on a common industry challenge that large fish sizes are difficult to chill effectively within reasonable onboard processing time intervals.

With more groundfish trawlers moving from traditional use of ice to superchilling the fish, RSW and chilled storage without ice, the new systems have become more efficient in chilling time and temperature control, labour factor, and in some ways energy efficiency. However, they have become more fragile in terms of maintaining the chill chain and the risk of excessive partial freezing of the fish. An example of the importance of maintaining a superchilled chill chain for cod was shown by Eliasson *et al.* (2019), where most benefits of onboard superchilling are lost when superchilling is not maintained throughout processing and transportation.

6. Conclusions

The aims of this study were to investigate different approaches to chilling whole fish and compare controlled superchilling with traditional flake ice chilling. Comparison of chilling time of equal size fish from +8°C to 0°C for flake ice and chilled brine, which are cooling media at different temperature 0°C and -1°C, results in 30–50% shorter time for refrigerated brine chilling. The difference between brine chilling gutted and ungutted cod was apparent, as expected, due to a greater surface area between gutted fish and the cooling medium. The difference in chilling time between gutted and ungutted fish was less with flake ice chilling than with refrigerated brine chilling. The results of this study show that the temperature profiles of the industrial onboard systems measured are comparable to the ones obtained from controlled experiments, indicating that fish bulk density and flow rate are not limiting or restricting factors for the industrial systems examined. These systems will however be sensitive to overload with higher fish to cooling medium ratios. Also, systems that run cooling medium temperatures lower than the initial freezing point of the fish, like Trawler B (refrigerated brine, two step cooling), will risk excessive partial freezing of the fish. Comparison of the model results with onboard measurements indicate that the thermal conductivity of the fish is limiting to the energy transfer rather than the heat transfer coefficient.

When wet-fish trawlers can effectively chill the fish below 0°C on the processing deck, the storage hold can be run at -1°C and ice can be greatly reduced or eliminated from the onboard chill chain. The fish quality and shelf-life will benefit from the lower temperature chill chain but further studies on specific fish quality factors such as sensory acceptance and nutritional value retention should be done. Also, future comparison studies on energy, environmental and economic factors are

needed for further evaluation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Paper IV

REFRIGERATION SCIENCE AND TECHNOLOGY
PROCEEDINGS



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RENDUS

Effects of on-board bleeding methods and superchilling on quality of cod and saithe

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ABSTRACT

An overview of studies for technological developments in the first stages of the value chain of groundfish in Iceland is introduced. It presents the results of recent research performed on board fresh fish trawlers and is aimed to evaluate the process performance in terms of product quality and shelf-life for fresh cod and saithe. This includes studies on time and temperature control, different bleeding conditions, superchilling methods and storage. The main objectives of the research are to provide design parameters to the processing deck redesign and to evaluate the impact of new on-board processes from catch to post-processing storage. The results of the studies indicate that storage methods have a greater impact on fish quality than the specific method of cooling. Also, the results indicate which parameters should be adjusted during the bleeding process to achieve the most effective drainage of blood from the fish.

Keywords: Superchilling, Bleeding, Fresh-fish, Colour, Shelf-Life.

1. INTRODUCTION

This study is related to developments in the Icelandic fishing industry where a large proportion of the fish processing companies have switched from frozen to fresh products and many main companies have or are starting to build new vessels. The aim of this research is to provide some design parameters and prerequisites to the redesign process of fresh fish trawlers processing. The goal of the development is to produce uniform and high-quality raw material for fish processing but also to provide better working conditions and more economic fishing and processing. High quality fresh products, like loins and fillets from cod (*Gadus morhua*) and saithe (*Pollachius virens*), require the raw material to be chilled, well drained of blood and uniform in colour. Low temperature slows microbial growth in the fish muscle and effective removal of blood also contributes to extended product shelf life (Huss, 1995; Olafsdottir et al., 2006). The research focus in this study is on on-board processing, a part of the fresh fish value chain where technical developments in the last decades has been less than in land-based processing. Land-based processing has seen a dramatic increase in automation and quality control and the aim is to implement that methodology in the on-board processing. A part of this development is also to improve data gathering in this sector, where data flow from fishing and on-board processing further along the value chain is often insufficient. This study is important to evaluate the impact of new industrial technology in the groundfish value chain. The innovation of processing on board is aimed to improve automation, sustainability and process control and the benefits of the new solutions need to be analysed for verification and for further technological development. Also, the results can set a benchmark for fresh fish producers in Iceland regarding shelf-life and quality indicators of fresh fish products. Prior to being taken aboard a trawler the fish has been battling against the trawl net, up the ramp and into the reception hold. Olsen et al. (2013) showed the negative effects of large haul sizes and long haul duration on the fish quality. They also had better results in terms of exsanguination with two-step bleeding/gutting (throat cut, bleeding for 30 minutes, gutting and washing) compared the commercial method of direct gutting (one-step procedure as described by Botta et al., 1986). Exsanguination of fish can be influenced by many factors. Insufficient bleeding can affect the shelf life of products through product taste, odour,

visual appearance and residual blood can also promote lipid hydration, provide good nourishment for bacteria and cause increased enzymatic activity (Richard and Hutlin, 2002). In a study by Digre et al. (2011a), the drainage of blood from a stressed cod was indicated to be worse than for unstressed cod. Botta et al. (1986) demonstrated in his research that the bleeding of the fish is an important factor for improving the colour of the flesh. Studies by Botta et al. (1986) and Olsen et al. (2014) implied that the most important factor is to process the fish as soon as possible at the bleeding stage. Olsen et al. (2014) indicated that bleeding and exsanguination should take place within 30 minutes after the catch has been hauled on board. Karlsdottir et al. (2014) researched the effect of different bleeding methods and temperature of the bleeding medium on the quality and storage life of cod and saithe products. Their main results indicated that washing the cod in slurry ice rather than unchilled seawater resulted in better product quality. However saithe showed the opposite reaction to chilled seawater compared to the cod, as saithe showed better end-quality bled in non-chilled seawater. There are debates as to the best methods of cutting or bleeding fish. The main three methods are a gill cut, cutting of the throat and direct gutting. The most important aspect of the procedure of bleeding, was noted in the studies of Botta et al. (1986) and Olsen (2014). They indicated that the time-factor was more important than the method itself. In the study by Olsen (2014), it was noted that the fish needed to start bleeding within 30 minutes after arrival to the processing deck, to produce the best exsanguination of the blood from the muscles. Another study by Roth (2009) observed that the washing of the fish for minimum of 12 minutes in a constant supply of clean seawater streaming into a washing bin/tub, could result in improving the exsanguination of the blood from the muscles. Superchilling is a method involving partial freezing of water in food (e.g. cod muscle), in which commonly the food is cooled 1-2 °C below the initial freezing point (T_f) resulting in 5-30% of water inside the product forming ice crystals (Aune, 2003; Kaale et al., 2011). For whitefish the initial freezing point is close to -1 °C (Margeirsson et al. (2012) modelled it -0.92 °C for cod) and ice crystal formation increases rapidly at lower temperatures. This study refers to this as "superchilling", however the method of superchilling food is also known as partial freezing (Duun and Rustad, 2007), deep chilling (Wu et al., 2014) or a more recent industry term "sub-chilling" (Skaginn 3X, 2018). According to Magnussen et al. (2008), the method can extend shelf life of fresh cod by 2-3 days, compared to traditionally iced cod with shelf-life of 9-13 days (Lauzon et al., 2010). This is in accordance with Digre et al. (2011b) whose results showed lower microbiological activity and better quality for superchilled cod at day 14 compared to cod stored in ice. According to Olafsdottir et al. (2006) it was concluded that controlling and keeping the correct temperature during storage is an important factor in preserving the quality of the fish, since a high product temperature can influence the growth of bacteria. Studies by Lauzon et al. (2010) and Olafsdottir et al. (2006) indicated that lowering the temperature from 0-0.5 °C to -1.5 °C prolonged shelf-life of fresh fish. As the temperature is one of the key factors in the onset of rigor mortis stage by cooling down the fish to around 0 °C, the onset of rigor mortis can be delayed and thus the contractions of the muscles will be less powerful and extreme when the fish goes through rigor mortis at higher temperatures, resulting in gaping in the flesh (Huss, 1995). A study by Eliasson et al. (2019) showed that while shelf-life effects of on-board superchilling of whole gutted fish are minimal compared to ice cooling it can reduce the growth of spoilage bacteria. The bleeding and cooling processes in the new ships are time and temperature controlled, first in-first out systems. There has been developed equipment for automatic transport of the fish tubs down to the cargo hold, which helps to standardize the quality of the catch. Also, there is no ice in the tubs that presses into the fish, which is stored at around -1 °C. Next expected steps are developments of bleeding robots to speed up the on-board processing time, as described by Toldnes et al. (2014).

2. METHODS

The experiments were performed on-board four fresh fish trawlers fishing saithe and cod around Iceland during the period 2014-2017. Fig.1 shows a flow chart of the on-board processing research topics; I) Bleeding, II) Chilling and III) Storage/shelf-life. The bleeding process includes methods of cut and gutting with regard to possible automation, temperature of the bleeding medium and waterflow conditions for effective bleeding. The newer trawlers studied are equipped with different methods of superchilling aimed to bring the core temperature of the fish down to -1 °C before on-board storage. Some of the systems are using chilled seawater (around -1.5 °C) and other use brine with temperature down to -4 °C. The chilling process involves whole gutted fish and the effects of the chilling method and on-board storage on shelf-life and quality were evaluated. For experimental design, comparison samples were always taken from the same haul in order to limit variation. Haul

sizes of samples ranged from 3 to 6 tonnes with hauling times 80 to 160 minutes. The samples were generally taken at the start of processing so they represent the best possible quality from the haul. During experiment for waiting time in the reception hold, samples were gutted after different waiting time (0, 60 and 120 minutes for saithe and 0, 30, 60 and 90 minutes for cod) and put in the same bleeding and chilling process before being iced in tubs. Samples bled at different temperatures were bled for 20 minutes in medium at different temperatures and then put through the same superchilling process before being packed without ice in a superchilled storage at -1°C . Saithe samples with different bleeding times were bled for 5, 12, 18 and 30 minutes before being packed with ice in tubs. When evaluating different gutting methods samples were bled and chilled in a controlled system, a first-in first-out screw conveyor (SUB-CHILLING™ – Onboard, 2018). They were either directly gutted from the reception hold and into bleeding tanks or a two-step bleeding/gutting process as described by Botta et al. (1986), where the fish is bled by cutting the ventral aorta, bled for 15 minutes in seawater and then gutted, washed and chilled. Another sample from this group was directly gutted into an older uncontrolled trawler system for comparison (the uncontrolled system was made of three separate tanks with elevator conveyor belts transporting the catch through).

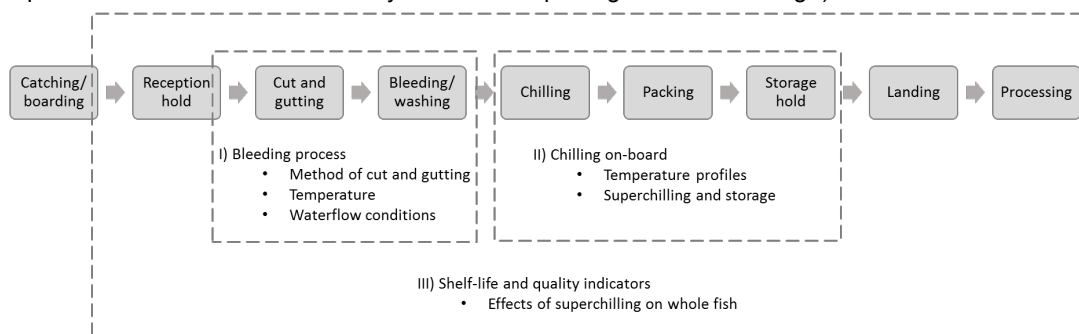


Figure 1: Overview of research topics relating to on-board trawler processing

Temperature monitoring was done with loggers placed both inside the flesh (centre of the loin for core temperature of the whole gutted fish) and one outside the fish to monitor the ambient temperature throughout bleeding, cooling and storage. Temperature logging and shelf-life evaluation were performed as described in Eliasson et al. (2019). Temperature loggers used were iButton (Micro-T DS1922L) from Maxim Integrated Products distributed by NexSens Technology (Dayton, OH, USA). Sensory evaluation and fillet quality was evaluated by standards as described by Martinsdottir et al. (2001). Fillets colour was evaluated by trained experts, Fig.2 shows the scale for fillet colour evaluation, developed by Icelandic Ltd (2012) for cod and also adjusted for saithe in this study. One Way ANOVA was used for statistical analysis of fillet colour measurements (with XLSTAT version 2018.5), the significance level was set at 5% ($p < 0.05$).

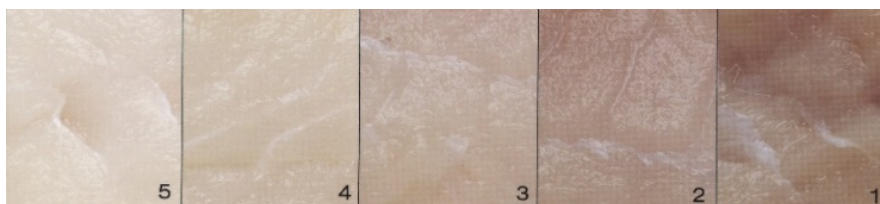


Figure 2: Fillets colour chart scale for cod, from Icelandic Ltd (2012)

3. RESULTS AND DISCUSSION

Fig.3 shows results of experiments where different waiting time in the trawler reception hold is evaluated from fillet colour after processing. The results in Fig.3a) for saithe show a significant reduction in fillet colour comparing saithe bled straight from the reception hold after catch to saithe that was bled 60 minutes and 120 minutes later. Results for different waiting time for cod show a significant difference for cod bled straight after catch and again after 30 and 60 minutes, Fig.3b). There was no significant difference found in fillet colour between cod that waited for 60 and 90 minutes. The results indicate that the fish must be bled within 30 minutes from catch to maintain a light fillet colour. This is in accordance with results by Botta et al. (1986) and Olsen et al. (2014)

which both concluded that the time from catch to bleeding was a deciding factor influencing exsanguination of cod. The fisheries companies involved in this study have now set a reference limit on the acceptable size of hauls, to minimize the load to the catch but mainly to reduce the waiting time in the reception hold, underlining the importance of fillet quality.

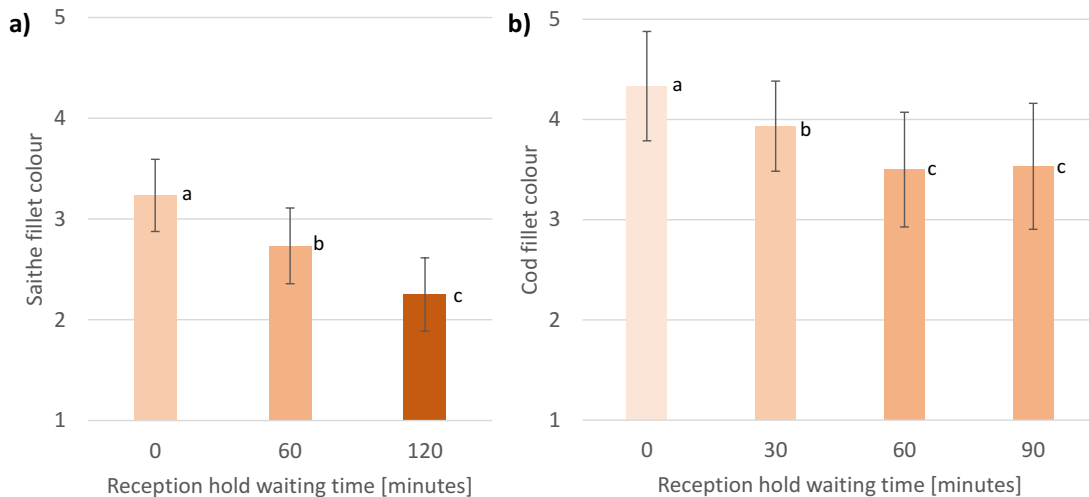


Figure 3: Colour of a) saithe and b) cod fillets for different waiting times in a trawler reception hold (n=30 fillets). Significant difference between groups is represented by different letters a, b, c (p<0.05). Error bars show standard deviation from mean values ($\bar{\mu} \pm \sigma$)

Fig.4 shows cod fillets bled for 20 minutes at three different temperatures; -1 °C, 2 °C and 6 °C. Fillets bled in unchilled seawater (6 °C) showed the best results in terms of uniform colour. When bled at lower temperatures blood was more apparent in the fillets, especially in the belly flap area. Karlsdottir et al. (2014) showed the same results for saithe, however for cod they had better exsanguination at lower temperatures.



Figure 4: Cod fillets bled in seawater for 20 minutes at three different temperatures; -1 °C, 2 °C and 6 °C. Photo taken after filleting, four days after catching

Fig.5 shows fillets colour for saithe after 5, 12, 18 and 30 minutes bleeding process before tub packing. Comparing the fillets colour after filleting (4 days after catch) did not show significant difference but after further storage of fillets (9 days from catch) there were significant difference (p<0.05). Groups bled 5 and 12 minutes showed darker colour compared to lighter colour for groups bled 18 and 30 minutes. The results after 9 days from catch indicate that the bleeding time for saithe should exceed 12 minutes and that the effects of insufficient bleeding are not necessarily apparent immediately after filleting. The fillet storage between day 4 and 9 was simulating sea transport of fresh fish at -1 °C so day 9 from catch is approximately the timing of delivery to buyers in Europe.

The results for 9-day fillets support that bleeding time should exceed 12 minutes, for system design however that is depended on the waterflow and circulation in the bleeding medium. Results of on-board bleeding systems have also shown that circulation or active movement of the fish in bleeding medium is a more deciding factor than the rate of water replacement.

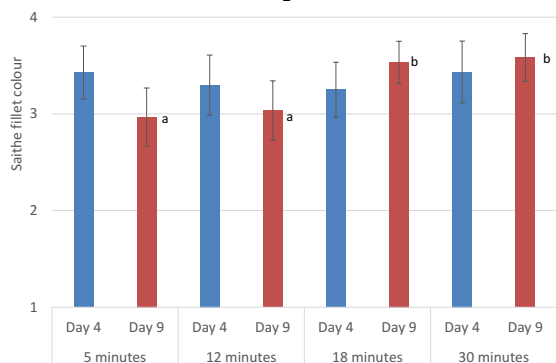


Figure 5: Saithe fillet colour after 5, 12, 18 and 30 minutes bleeding in seawater. Blue columns represent fillet colour after fillet processing (Day 4 from catch) and red columns fillet colour on day 9 from catch. Error bars show standard deviation from mean values ($\bar{\mu} \pm \sigma$)

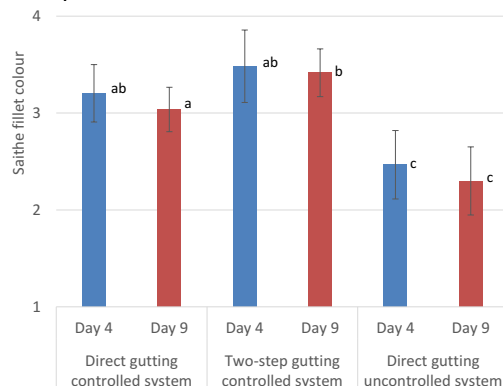


Figure 6: Fillet colour of saithe for different gutting methods and on-board processing systems. Significant difference between groups is represented by letters a, b, c ($p < 0.05$). Error bars show standard deviation from mean values ($\bar{\mu} \pm \sigma$)

Results of an experiment comparing direct gutting of saithe to bleeding (15 minutes) and gutting in a two-step procedure are shown in Fig.6. The groups were time controlled first-in first-out systems and were also compared to a group using an uncontrolled trawler system (as described in methods). Similar to results by Olsen et al. (2014) the method of bleeding and gutting in a two-step procedure resulted in slightly better colour, significantly apparent after simulated storage, 9 days from catch. The difference of using a controlled first-in first-out system however was more decisive factor than the method of gutting. Temperature profiles of the uncontrolled system are shown in Fig.7.

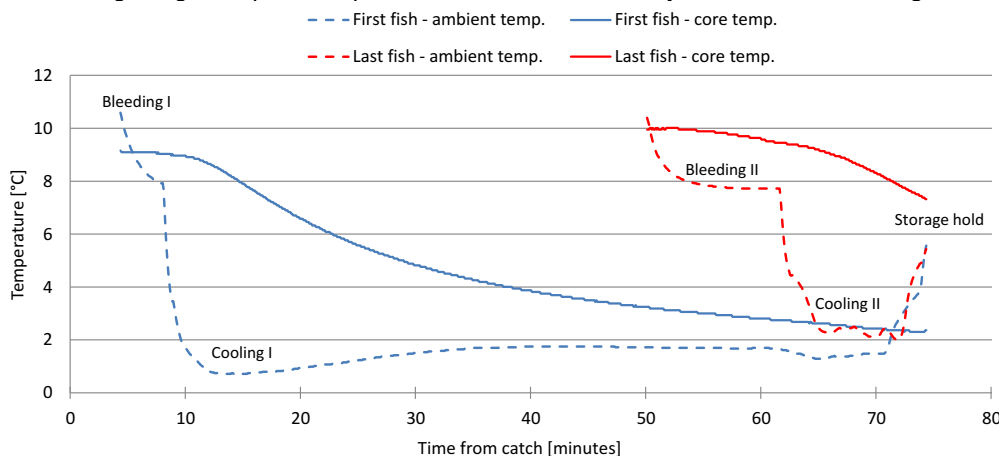


Figure 7: Temperature profiles for the first and last fish (3 kg) in an uncontrolled bleeding/cooling system

The example shows profiles of measured fish, the first and last fish to be processed in a saithe haul. The dotted lines represent the ambient temperature around the fish in the system and the solid line the flesh temperature in the core of the fish. The first fish (blue line) is gutted after 5 minutes from catch and only stays in the bleeding tank for 4 minutes, after which it tumbles in the cooling tanks for over 60 minutes and therefore gets sufficient cooling time. The last fish (red line) to be gutted gets 15 minutes in the bleeding tank but only stays in the cooling tanks for a few minutes before being transferred down to the storage hold. The initial temperature of the fish was 9 °C but the last fish is at 10 °C into the system because of the waiting time in the reception hold. The temperature profiles

show the inconsistency of an uncontrolled system with variable bleeding time and the final temperature of the first fish at 2 °C while the last fish is packed at 7 °C in the storage hold. Fig.8 shows fish temperature profile in a first-in first-out superchill system compared to traditional flake ice cooling as a reference. In the superchill system the core temperature of the fish can reach -1 °C within 1 hour, after which it is packed in a tub and stored in the storage hold at -1 °C ambient temperature without any ice. After landing the superchilled fish is kept in a storage at -1 °C before fillet processing.

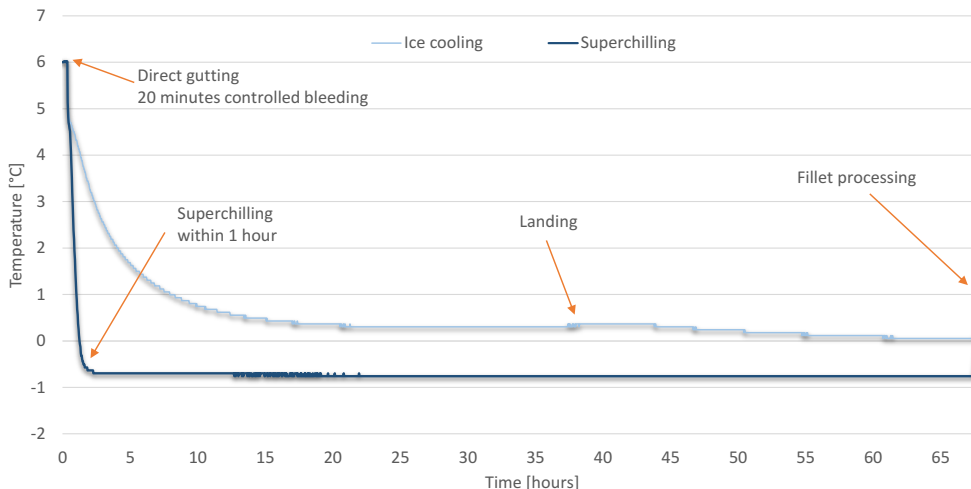


Figure 8: Controlled super-chilling system compared to traditional flake ice cooling. 3 kg fish core temperature from catch to fillet processing

Samples from the temperature profiles shown in Fig.8 were evaluated at different storage temperature simulating the whole value chain in at study by Eliasson et al. (2019). The results for different storage temperatures at +1 °C and -1 °C showed (Fig.9) that the main deciding factor in the fresh fish chill chain is the temperature of the fillets after processing. However, the superchilling of whole gutted fish on-board results in less bacterial growth and slows down some spoilage factors.

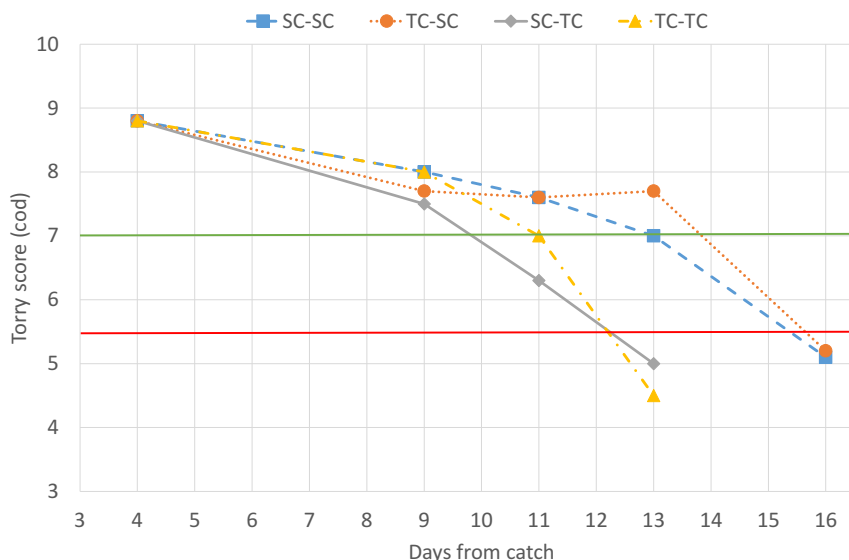


Figure 9: Mean Torry freshness scores for cooked cod samples on days 4, 9, 11, 13 from catch. Groups are labelled by different on-board chilling methods (SC-super-chilled, TC-traditional ice cooling) and different fillet storage temperature (SC stored at -1 °C and TC stored at +1 °C). The green line indicates loss of freshness (Torry score = 7) and the red line indicates end of shelf life (Torry score = 5.5). Figure adapted from Eliasson et al. (2019)

4. CONCLUSIONS

The results of the bleeding experiments indicate that the fish should be bled within 30 minutes after catch to maintain quality in terms of fillet colour. The bleeding process should exceed 12 minutes and be carried out in un-chilled seawater ensuring movement and refresh rate of the bleeding medium. Evaluation of the older uncontrolled systems showed that they are unreliable both in terms of bleeding and cooling. As for the method of bleeding and gutting saithe, the two-step process of bleeding and gutting showed slightly better results in terms of fillet colour, compared to direct gutting. The two-step method is suitable for automation of the bleeding process on-board trawlers to shorten the waiting time in the reception hold, which was found to be the main deciding factor influencing fillet colour. The on-board super-chilling of whole fish reduces some spoilage factors but did not considerably extend shelf-life. However, the simulation of a super-chilled value chain to the market indicates that the freshness period and shelf-life can be extended by up to four days, compared to traditional ice cooling and +1 °C storage.

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Paper V

Effects of onboard refrigerated seawater (RSW) storage of whole ungutted cod on quality parameters of fillets

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Abstract: The aim of this investigation was to evaluate the effects of onboard refrigerated seawater (RSW) storage of ungutted cod on the quality parameters of fillets. The traditionally processed reference group was directly gutted, bled and stored in ice, the other three groups were gill-cut, bled and stored in an onboard RSW tub for 24, 60, and 84 hours respectively, then gutted and stored in ice until processing. The results showed a difference between groups in spoilage bacteria (TVC; H₂S), total volatile basic nitrogen content (TVB-N), water content and water holding capacity (WHC), gaping, bile spots and salt content. Negative effects of prolonged RSW storage were obvious after 60 and 84 hours of storage, while the group stored in RSW for 24 hours was close to the quality parameters of the reference group. It is, therefore not recommended to store whole ungutted cod in onboard RSW for more than 24 hours.

Keywords: Atlantic cod, refrigerated seawater (RSW) storage, whole ungutted cod, water holding capacity, spoilage bacteria, total volatile basic nitrogen content (TVB-N).

Highlights:

- Onboard RSW storage of whole ungutted cod was studied and compared to iced cod
- Prolonged onboard storage in RSW resulted in decreased quality
- RSW storage for 60 hours and more led to a significant growth of spoilage bacteria and TVB-N
- RSW storage for over 60 hours was a deciding factor for quality parameters
- Difference in quality between the 24 hours RSW group and iced cod was negligible

1. Introduction

Icelandic fish producers are increasingly producing more fresh fish products that require high quality raw material. Generally, processing and temperature control improvements have supported an increase in the export of fresh fish fillets, with extended shelf-life, improved yield and more stable product quality (Olafsdottir *et al.*, 2006; Margeirsson, 2008; Margeirsson *et al.*, 2010; Lauzon *et al.*, 2010). The current study topic is onboard RSW storage of whole ungutted groundfish for different gutting procedures, to investigate the viability of storing ungutted fish onboard and gutting it later in land-based processing facilities. The gill-cut can be a simpler and more effective onboard processing method compared to the direct gutting. The experiment was designed to evaluate the effects of RSW storage of whole gill-cut cod on fillet quality, compared to direct gutting.

RSW is preferred to ice as a cooling and storage medium in certain fisheries due to various advantages such as speed of cooling and bulk chilling, reduced pressure on the fish, lower holding temperature, quicker handling of large quantity catches and less processing labour (Graham *et al.*, 1992). These systems can also result in extended storage times for the catch, as is the case for pelagic fishing. The use of RSW systems for onboard cooling and storage of pelagic fish is common and widely used. In Iceland, improved methods for chilling and storing pelagic species onboard purse seiners were developed in the last decade, resulting in more valuable products (Thorvaldsson *et al.*, 2011). For groundfish caught by trawl, however, the procedure is different. Prior to boarding, the fish has often been battling against the trawl net, up the ramp and into the reception hold where it is kept until bleeding. The reception hold waiting time has a negative effect on the fish quality (Olsen *et al.*, 2014; Eliasson *et al.*, 2019) so speeding up the processing from the reception will increase the overall quality of the catch. The benefits of a more rapid bleeding procedure (gill-cut instead of direct gutting) are therefore mainly to increase the processing productivity, resulting in a shorter reception hold waiting time. By cooling the fish below 0 °C, the onset of rigor mortis can be delayed and thus the contractions of the muscles will be less powerful and extreme than when the fish goes through rigor mortis at higher temperatures, resulting in gaping in the flesh (Huss, 1995). The common disadvantages of RSW include excessive uptake of salt, uptake of water by species with a low-fat content, loss of protein, and problems with anaerobic spoilage bacteria (Graham *et al.*, 1992). In most Northern European fisheries, the gutting of lean species like cod is considered compulsory, based on the assumption of reduced quality if they are not gutted. In the case of cod, it has been shown that the omission of gutting can cause a considerable quality loss and a reduction in storage life of five to six days. After two days from catch, discoloration of the belly area is often visible (Huss, 1995).

The onboard RSW storage of ungutted fish also gives the option of delivering the raw material with well chilled guts to the land-based processing for more economic processing and increased utilisation since the guts, apart from liver and roes, are generally thrown overboard. The industry's interests in these procedures for groundfish are therefore related to simpler onboard handling of fresh fish, shorter fishing trips and the increasing focus of Icelandic fish producers on delivering higher quality raw material to produce fresh fish products. The method of RSW cooling ungutted whole fish onboard is most common for pelagic fish in Icelandic fisheries. For pelagic fish this requires no prior processing or bleeding before being stored in large onboard RSW tanks. Prior studies on refrigerated sea water (RSW) systems as a means of storing Atlantic cod in onboard tanks are few and outdated. Onboard RSW storage of groundfish prior to processing has been carried out chiefly on the Pacific coast with salmon and halibut, where it has met with considerable success (Roach *et al.*, 1961). Only laboratory

scale work has been reported on the Atlantic coast for holding cod in RSW onboard trawlers (MacCallum and Chan, 1961). Watson (1996, 1997) found that holding mackerel in RSW treated with sodium hypochlorite reduced the rate of bacterial spoilage by over 50%, however, little improvement was seen in the quality of whole gutted cod with the same treatment.

The aim of this investigation is to evaluate the effects of onboard RSW storage of whole ungutted cod on the end-products' quality parameters. The experiments were done onboard a large wetfish trawler during a fishing trip where cod in the reference group was direct gutted, bled and iced (**DG-ICE**) and compared to cod from the same haul that was gill-cut, bled and stored ungutted for different time periods in an RSW tub onboard. The cod from the RSW tub was gutted after 24, 60, and 84 hours respectively (groups **GC-RSW24h**, **GC-RSW60h**, and **GC-RSW84h**) and iced in tubs. The samples were processed at a fisheries company and the fillets transported to and measured at Matis in Reykjavik.

2. Materials and methods

This section describes the experimental setup of the study and the measurement methods. The study included experiments with four trial groups; one reference group, direct gutted, bled and ice cooled and three groups, gill-cut, bled and stored whole ungutted in RSW for different time periods.

2.1. Experimental setup

Wild Atlantic cod was caught by trawl on May 29th in the Northwest of Iceland (tow time 45 minutes). The average fish size was 4.29 kg, haul size 7,145 kg (6,825 kg cod and 320 kg saithe). The fish (n=90) was gill-cut (one side gill-cut, **GC**), bled for 25 minutes in 5-6 °C seawater and then stored in a 1000L tub connected to RSW and a circulating pump (Figure 1). The temperature in the RSW tank was kept at an average of -1.4 °C through the sea trip storage period, Figure 3 shows the temperature profile. The traditionally processed comparison group **DG-ICE** was direct gutted at the same time, iced in a 460L tub and kept in the trawler storage hold. Groups **GC-RSW24h**, **GC-RSW60h** and **GC-RSW84h** were stored ungutted for 24, 60 and 84 hours respectively in the RSW tub before being gutted and iced in tubs in the storage hold. After landing, all groups (n=30 fish for each group) were processed, filleted and evaluated. Day 0 is defined as the time of catch. The first measurements were conducted after processing on Day 4 after catch. The fillets were then packed in expanded polystyrene (EPS) boxes and kept in a 2 ± 2 °C cold storage at Matis until measurements continued on Day 7 and Day 11. Visual quality assessment of fillets for gaping, blood and bile spots (see Figure 2) was carried out after fillet processing on Day 4 from catch. Water content, WHC and bacterial measurements were done on Days 4, 7 and 11.

Description of experiment groups:

- **DG-ICE**: Direct-gutted (DG), bled and iced in 460 L tub.
- **GC-RSW24h**: Gill-cut (GC), bled and stored in RSW tank for 24 hours.
- **GC-RSW60h**: Gill-cut (GC), bled and stored in RSW tank for 60 hours.
- **GC-RSW84h**: Gill-cut (GC), bled and stored in RSW tank for 84 hours.



Figure 1: 1000L tub for onboard RSW trial.



Figure 2: Bile spot on fillet belly flap (yellow discoloration).

2.2. Chemical and microbial analysis

Total volatile basic nitrogen content (TVB-N) and spoilage bacteria measurements, total viable psychotropic counts (TVC) and measurements of H₂S-producing bacteria, were done by assessing two pooled fillets for each sample (measurements in triplicate). The method of Malle & Tao (1987) was used for TVB-N measurements, measured by steam distillation and titration, after extracting the fish muscle with a 7.5% aqueous trichoroacetic (TCA) solution. The distilled TVB-N was collected in a boric acid solution and titrated with a sulfuric acid solution.

For microbial analysis, fillets were aseptically minced, assessing 2 pooled fillets for each sample. Minced flesh (25 g) was mixed with 225 mL of cooled Maximum Recovery Diluent (MRD, Oxoid, UK) in a stomacher for 1 min. Successive 10-fold dilutions were done as required. Total viable psychotropic counts (TVC, 15 °C, 4 to 5 days) were evaluated by spread-plating aliquots onto modified Long & Hammer's medium; counts of H₂S-producing bacteria and presumptive pseudomonads were evaluated on spread-plated Iron Agar (15 °C, 4 to 5 days) and modified Cephaloridine-Fucidin-Cetrimide (CFC) medium (22 °C, 3 days), respectively, as described by Magnusson *et al.* (2009).

2.3. Water content and water holding capacity

Measurements of water holding capacity, water content and salt were performed on mid-section parts. Pieces were minced and used for chemical analysis. The water content of the samples was measured by determining the difference in weight of minced muscle samples, following drying for 4 hours at 102-104 °C (ISO, 1999). The muscle samples were taken from the fish mid-section. The water holding capacity (WHC) was determined with 2 g of minced white epaxial muscle, measured at about the same location as the water content. The muscle was subjected to low-speed centrifugation as described by Eide *et al.* (1982), except that a centrifugation force of 210 g was used. The WHC was expressed as the percentage of retained weight after centrifugation (1350 rpm, 5 min) at 4 °C, divided by the initial sample weight and multiplied by 100.

2.4. Visual quality analysis

Two trained persons with experience in sensory and quality evaluation of fish products did the visual assessments on the same pool of samples. Visual assessment of the quality parameters gaping, blood and bile spots was carried out with a 4x4 cm grid on the fillets, as described by Margeirsson *et al.* (2007). The measurement method counts a defect within the grid area as one unit on the fillet. The defects were then presented as the average of a 10-fillet assessment. Nematodes were counted as the total number of nematodes in each loin or belly flap.

2.5. Statistical analysis

Statistical analysis of data was performed using Microsoft Excel 2016 (Microsoft Inc. Redmond, Wash, USA) and IBM SPSS Statistics v. 26 (International Business Machines, Armonk, New York, USA). Values are presented as standard error of mean (SEM). One-way analysis of variation (ANOVA) and Duncan's post hoc test were applied on all samples for each group/day and the significance level was set $p < 0.05$ for all statistical analyses. A t-test was then used to determine the significance of differences between means of two groups.

3. Results and discussion

The study results are presented and discussed in this section. The temperature inside the fish loin, along with the ambient temperature of the fish in the RSW tank, is shown in Figure 3. The core temperature of the measured fish is between -0.9 and -0.7 °C during the RSW storage time and the ambient temperature (cooling medium or RSW temperature) of the fish is between -2 and -1 °C during the fishing trip. The temperature mapping during the RSW storage shows a stable core temperature of the fish through the onboard storage. The change in the cooling medium temperature is most likely due to a changed location of the measured fish within the RSW tub, as the temperature loggers were placed both inside the fish loin and outside the fish. The storage conditions are similar to the studies by Roach *et al.* (1961) and Longard *et al.* (1971) for onboard RSW storage of cod, and therefore comparable.

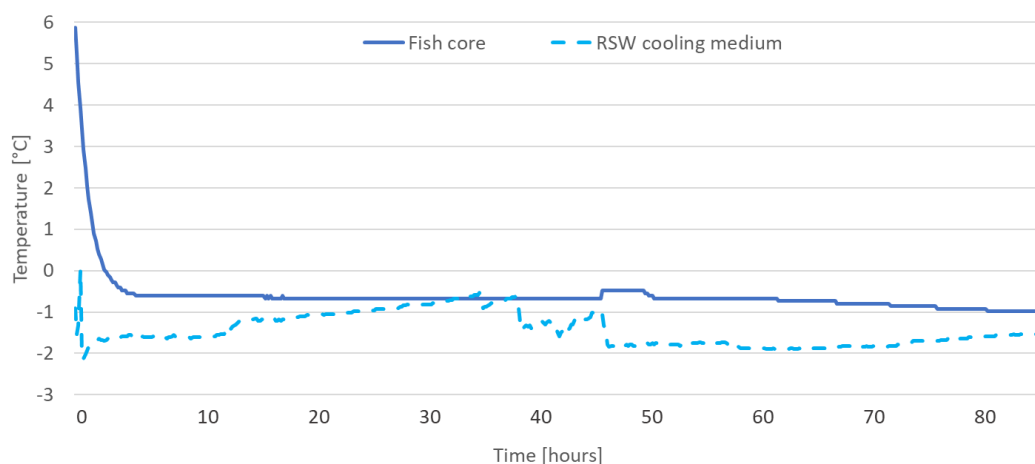


Figure 3: Temperature profiles of a fish loin (solid line) and the RSW cooling medium (dotted line) during the fishing trip

Quality parameters evaluated after fillet processing are shown in Table 1. The assessment of quality parameters after filleting showed negligible effects of RSW storage for 24 hours compared to **DC-ICE** but the groups that were kept longer, for 60 and 84 hours, in the RSW tub showed a significant increase in fillet gaping and yellow bile spots. The salt content (g/100g) was measured for all groups and showed a significantly ($p < 0.05$) lower content of 0.3% for groups **DC-ICE** and **GC-RSW24h** compared to 0.4% for groups **GC-RSW60h** and **GC-RSW84h**. This indicates that longer RSW storage results in some salt absorption. Nematodes were counted in both the loin and the belly flap to evaluate whether prolonged storage of the whole fish, with the gut and liver, would result in nematodes moving into the flesh. No nematodes were found in the fish loins and, as seen in Table 1, the nematodes found in the belly flap are not clearly related to longer RSW storage, although group **GC-RSW84h** did show a higher count.

Table 1: Visual assessment defects in fillets after processing, 4 days after catch. Defects per fillet ($n=10$ per group), Mean \pm SEM; NS = no significant difference between groups ($p < 0.05$); Different superscript letters within each defect assessment denote a significant difference ($p < 0.05$) between the groups.

	DG-ICE	GC-RSW24h	GC-RSW60h	GC-RSW84h
Gaping	0.4 \pm 0.2 ^a	0.6 \pm 0.2 ^a	1.2 \pm 0.2 ^b	1.2 \pm 0.3 ^b
Bile spots	0.0 \pm 0.0 ^a	0.0 \pm 0.0 ^a	0.4 \pm 0.1 ^b	0.8 \pm 0.2 ^b
Nematodes in belly flap	0.0 \pm 0.0 ^a	0.6 \pm 0.3 ^{ab}	0.1 \pm 0.1 ^a	1.2 \pm 0.3 ^b
Blood spots ^{NS}	0.2 \pm 0.1	0.4 \pm 0.2	0.2 \pm 0.1	0.2 \pm 0.1

Figure 4 shows the microbial growth for all groups measured on Days 4, 7 and 11 from catch. The TVC count and H₂S producing bacteria increases with time between Days 4, 7 and 11 for all groups. The microbial measurements show a clear trend in bacterial growth related to longer RSW storage, both for TVC and H₂S bacteria (reference consumption limit of 6 log₁₀ for TVC).

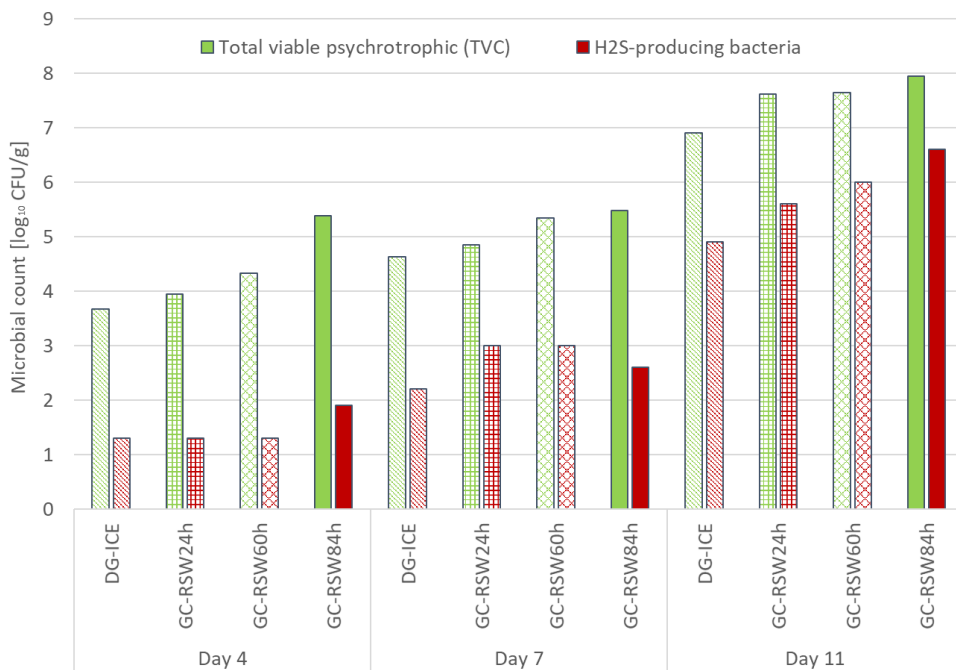


Figure 4: Development of microbial count for all groups on Days 4, 7 and 11 from catch. DG-ICE: Direct gutted, bled, iced in tub. GC-RSW24h: Gill-cut, bled and stored in RSW for 24 hours. GC-RSW60h: Gill-cut, bled and stored in RSW for 60 hours. GC-RSW84h: Gill-cut, bled and stored in RSW for 84 hours.

In a study by Reynisson *et al.* (2010), whole gutted haddock was stored in liquid ice and plate ice respectively, for 8 days. The results showed that the microbial growth was delayed at early storage for both cooling methods, however, while the liquid ice provided faster initial cooling it created unfavorable conditions under extended storage (where active spoiler *P. Phophereum* became dominant). Digre *et al.* (2011a) found a significantly lower difference of 1.3 log₁₀ for TVC and 0.6 log₁₀ for H₂S bacteria for gutted cod stored in ice-slurry compared to flake ice. However, neither of these studies were storing whole ungutted cod like in the current study and were therefore dealing with less gut bacteria during storage. Olsson *et al.* (2007) measured the bacterial count CFU (Colony-Forming Unit) and H₂S bacteria ratio in wild ungutted cod after different storage times for ice stored fillets. The CFU results by Olsson *et al.* (2007) were 3-4 log₁₀ after 3 days, 7-8 log₁₀ after 7 days, and 9 log₁₀ after 14 days, which are comparable or higher values than the microbial values shown in Figure 4.

Comparison to the study by Watson (1996) for gutted cod stored on ice shows lower microbial values in the current study, as Watson (1996) measured TVC 5-6 log₁₀ after 6 days on ice and 7 log₁₀ on Day 12. For gutted cod stored in RSW, similar to the conditions of the current study, the TVC values measured by Watson (1996) were 3-4 log₁₀ after 5 days in RSW and 4-5 log₁₀ after 12 days. These are considerably lower than the values in the current study, most likely due to the contamination of the guts in the ungutted storage period.

The results of the spoilage bacteria count and TVB-N content in Figure 4 and Figure 5 are, for Days 4 and 7, similar to quality and shelf-life estimations by Lauzon *et al.* (2010) for cod processed 3 days from catch and stored at 2°C. The bacteria and TVB-N results in the current study are higher on Day 11, most likely due to the higher storage temperature of 4 °C. Olafsdottir *et al.* (2006) measured the shelf-life of ice cooled cod stored at an average temperature of 2 °C and found a shelf-life limit on Day 11 when the TVB-N content was 34 (mg N/100g), the TVC 8 log₁₀ and the H₂S bacteria count 6.6 log₁₀. The reference group in this study, **DG-ICE**, is lower in both TVB-N and spoilage bacteria, compared to the Olafsdottir *et al.* (2006) experiment, indicating reasonable quality changes and shelf-life considering the handling and storage temperature of ice cooled gutted cod. The trend between groups seen in the bacterial growth was also noticeable in the TVB-N formation, shown in Figure 5, with insignificant difference between groups after Days 4 and 7. A significant difference between groups is found only on Day 11, in higher TVB-N content for groups **GC-RSW60h** and **GC-RSW84h** on Day 11 compared to groups **DG-ICE** and **GC-RSW24h**. This indicates that the rise in TVB-N occurs between 24 and 60 hours in RSW storage. The results of TVB-N development measured in the current study concurs to trimethylamine (TMA) development during storage of cod stored in crushed ice and RSW at 0° C in the study by Roach *et al.* (1961). The development remains similar for the first week of storage but after that the RSW 60h and 84h groups showed significantly higher compared to **DG-ICE** cod and the RSW 24h group, showing slower TMA development. In a study by Longard *et al.* (1971), RSW storage of gutted and ungutted whole cod showed slower development of TMA during the first 7 days and more rapid formation after that. Another experiment in the same study, where the fish was kept in fluctuating RSW temperature (-1 °C to +1 °C), showed an exponential growth for whole ungutted cod compared to a linear TMA development in the gutted group.

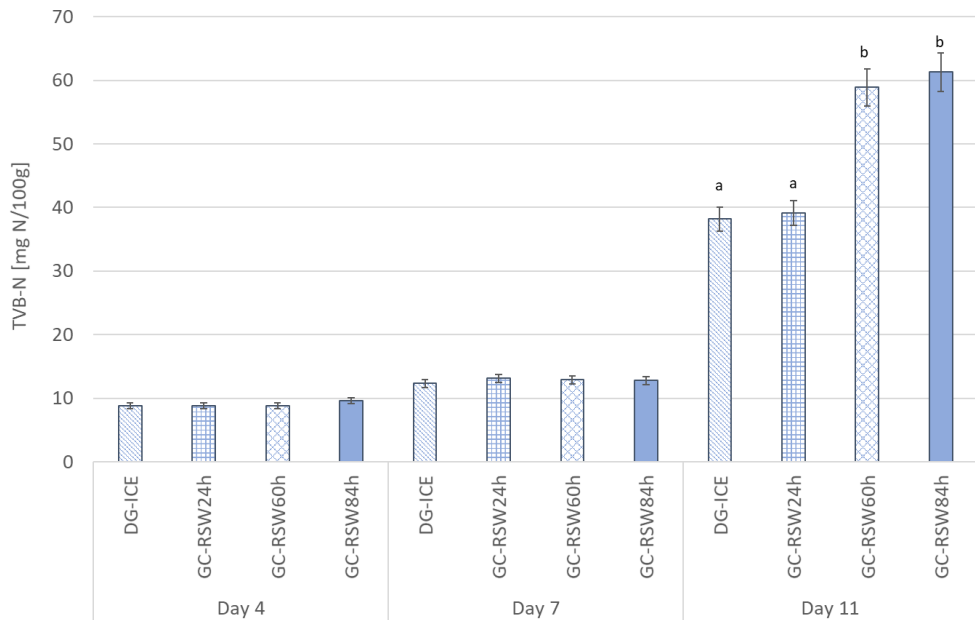


Figure 5: TVB-N measurements (mean \pm SEM, n=3) for all groups on Days 4, 7 and 11 from catch. Different superscript letters within each group and day denote a significant difference ($p < 0.05$).

Figure 6 shows the water content and WHC for all groups on Days 4, 7 and 11. On Day 4 there is no difference between group **DG-ICE** and **GC-RSW24h** but the water content is lower in group **GC-RSW60h** and the WHC lower in groups **GC-RSW60h** and **GC-RSW84h**, indicating that the longer RSW storage is affecting the samples. On Day 7, there no difference in the water content but the WHC is significantly higher in groups **GC-RSW24h** and **GC-RSW84h**. On Day 11, there is no difference in the groups' water content but the effects of RSW storage is noticeable in the WHC as all RSW groups show significantly higher values compared to **DG-ICE**. A reason for this could be the higher salt content and the microbial count causing higher WHC.

Olsson *et al.* (2007) concluded that the relation of percentage of liquid loss (same definition as WHC) of cod muscle to bacterial growth was only broad and indirect, that the relation was likely to be temporal and not causal. The results of WHC in the study by Olsson *et al.* (2007) showed WHC 83.2 - 90.2% after 2-3 days, WHC 75.5 - 90.2% after 7-8 days and WHC 72.9 - 89.9% 14-17 days. This concurs with the WHC of cod in group **DG-ICE**, as WHC is lower with longer storage time. The current study results for groups kept in RSW, however, show an opposite trend with WHC rising with increased storage time in RSW. This is most likely due to higher salt content in the cod muscle of groups **GC-RSW60h** and **GC-RSW84h**. The results of the current study were also compared to the study of Digre *et al.* (2011b) which showed WHC of 82.7-85.6% and water content of 78.8-80.3% for fresh farmed direct gutted cod (anesthetized pre and post rigor), similar values as in the current study.

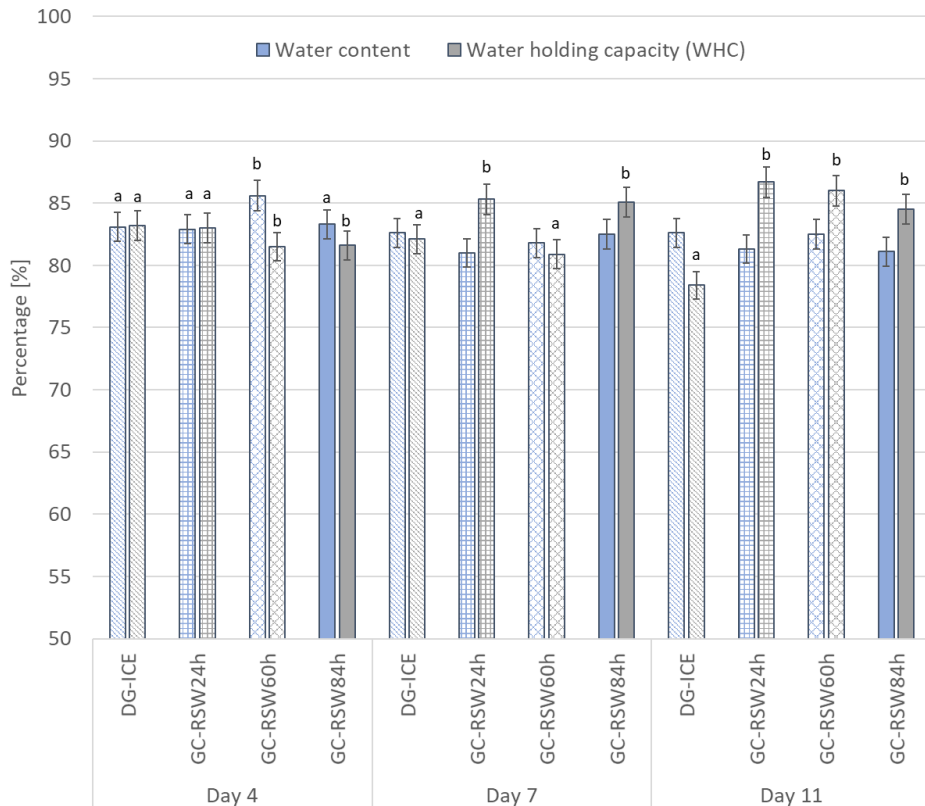


Figure 6: RSW experiment water and WHC (mean \pm SEM, n=3) for all groups on Days 4, 7, and 11 from catch. Different superscript letters within each group and day denote a significant difference ($p < 0.05$).

4. Conclusions

The results of this investigation show negative effects of prolonged onboard RSW storage of whole ungutted cod on some of the quality parameters of cod fillets. The groups stored in RSW for 60 and 84 hours showed significantly higher gaping, bile spots and salt content compared to the reference group and the 24 hours RSW storage group. The spoilage bacteria measurements also resulted in higher values with prolonged RSW storage, for both TVC and H_2S bacteria. The TVB-N content was not significantly different between the groups on Days 4 and 7. On Day 11, however, the 60 and 84 hours RSW storage groups were higher in TVB-N. The effects of RSW storage were noticeable in water content and WHC, most likely affected by bacterial growth and the higher salt content in groups **GC-RSW60h** and **GC-RSW84h**. The negative effects of prolonged RSW storage of ungutted cod on fillet quality could be clearly seen after 60 and 84 hours of RSW storage, whereas the difference in quality parameters between the RSW group stored for 24 hours and the DG-ICE group was almost negligible. Therefore, the results indicate that it is not advisable to store whole ungutted cod in onboard RSW storage for more than 24 hours.

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Paper VI

Effects of different bleeding methods and storage time on the colour of cod fillets

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Abstract:

The purpose of the study is to evaluate the effects of different bleeding methods and storage time of Atlantic cod on the fillet colour. An experiment was conducted comparing the efficiency of different onboard bleeding methods for three different cut and gutting procedures; direct gutting (DG), gill-cut (GC) and throat cut (TC). The evaluation of fillets was done with visual colour assessment, a Minolta meter on different fillet locations, and using digital image analysis for three different areas on the fillet (loin, belly flap, and tail end). The study results did not show a clear difference between the cutting method groups. The variation found was a minor difference in the redness (a* value) of the belly flap and tail end for group DG. The colour evaluation, however, showed a significant difference in fillet colour affected by the time of cold storage, compared on Day 6 and Day 12 from catch. Also, a significant difference was found in colour depending on location on the fillet. With only minor differences in fillet colour between cutting methods, the results support either DG and a simpler processing-deck setup, or GC or TC for two-step bleeding and faster processing from the reception hold.

Keywords: Atlantic cod, bleeding, cutting methods, fillet colour, whiteness.

Highlights:

- Different bleeding methods for Atlantic cod were evaluated
- Only marginal difference was found between bleeding methods
- A cold storage time of 6 days affected the fillet colour
- Difference in colour was found to depend on the fillet area location

1. Introduction

In recent years there has been a shift by Icelandic fish producers from less valuable products to high-end fresh fish portions and fillets, which export has been made possible by a decade of technological developments in the value chain. The major seafood processors in Iceland have played a key role in this development, investing in new high technology equipment for both ships and land-based processing. A part of this initiative included installing a newly developed system based on time-controlled bleeding and superchilling technology onboard large wetfish trawlers, on which this investigation was conducted. Along with extended shelf-life, high-end products, like fresh fish loins from cod, require the raw material to be well drained of blood and uniform in colour. The total blood volume of fish depends on the species and ranges from 1.5 to 7% (Huss, 1995, Itazawa *et al.*, 1983) of the body weight. For Atlantic cod, the volume is close to 3% (Ronald *et al.*, 1964), with only 20% of the blood volume generally localised in muscular tissues, while the rest is localised in internal organs (Huss, 1995).

The production of fresh cod products requires the fillets to be white in colour, which is best achieved with immediate bleeding and good exsanguination of the fish (Olsen *et al.*, 2014, Eliasson *et al.*, 2019). This most important aspect of the procedure of bleeding was noted in the studies of Botta *et al.* (1986) and Olsen *et al.* (2014). They indicated that the time-factor was more important than the method itself. In the study by Olsen *et al.* (2014), it was noted that the fish needed to start bleeding within 30 minutes after arrival to the factory deck to produce the best exsanguination of the blood from the muscles. Studies by Eliasson *et al.* (2019, 2020) observed that the washing of cod for a minimum of 12 minutes, to ensure enough recirculation and flow of water in the bleed-out tanks, improves the exsanguination of the blood from the muscles. In the study by Digre *et al.* (2011), the drainage of blood from a stressed cod was indicated to be worse than for unstressed cod. When the fish are transported from the bunker through the processing line, they can be subjected to poor handling along the processing line. Digre *et al.* (2011) also saw that the fillet colour of farmed cod changed significantly when assessed immediately after slaughter, as compared to after 7 days of ice storage. However, after the storage time, effects of stress on fillet colour was not discernible. Digre *et al.* (2010) found that, after 8 days of ice storage, cod showed redder and less yellow colour due to stress prior to electrical stunning. Bjernevik and Solbakken (2010) studied the difference between stressed vs unstressed cod and did not find a significant difference in colour after 8 days of ice storage.

Common bleeding procedures for groundfish are throat cut with direct gutting (DG), throat cut (TC) where the throat (ventral aorta) or the arteries in the neck (dorsal aorta) are cut and gill-cut (GC), cutting the gill arches. DG is a one-step procedure where the throat is cut without touching the backbone and the fish is then directly gutted. These methods are used to facilitate death and drain most of the blood from the fish muscle (Botta *et al.*, 1986, Warriss & Wilkins, 1987, Robb *et al.*, 2000, Borderias & Sanchez-Alonso, 2011). It has been argued that the most efficient exsanguination is obtained only if the fish is alive during bleeding (Botta *et al.*, 1986, Huss, 1995). However, muscle activity during bleeding has also been reported to be of less importance to obtain a good exsanguination (Robb *et al.*, 2000; Roth *et al.*, 2009). DG is a method that removes the heart, while with gill-cut and throat cut the heart is present during the bleed-out process. A fish heart is constructed for a single circulation (a two-chambered structure) and studies have indicated that a beating heart is not necessary for effective exsanguination during slaughter (Olsen, 2011). The immediate bleeding of live fish after capture has been shown to be of more importance than the bleeding method (Valdimarsson *et al.*, 1984, Botta *et al.*, 1986, Roth *et al.*, 2009). The one-step method of direct gutting (DG) is the most common practice in fresh fish fisheries in Iceland. Several researchers have questioned the necessity of handling the fish in a two-step procedure involving a special bleeding period (Botta *et*

al., 1986, Huss & Asenjo, 1977, Valdimarsson *et al.*, 1984). Huss & Asenjo (1977) found the best bleeding if a deep throat cut including the dorsal aorta was applied, but this was not confirmed in the work of Botta *et al.* (1986). Botta *et al.* (1986) recommended including a bleeding period (two-step procedure) when live fish were handled (fishing with pound net, trap, seine, longline or jigging), while Valdimarsson *et al.* (1984) found that the quality of dead cod (4 hours after being brought onboard) was slightly improved using the two-step procedure. However, the effect of bleeding should also be considered against the advantages of having a more effective handling procedure resulting in rapid processing, bleeding and chilling of the catch.

The study topic is onboard bleeding methods with focus on delivering better raw material to secondary processing. The study experiments cover the effects of three different bleeding methods on cod fillet colour; direct gutting (DG), gill-cut (GC) and throat cut (TC). The interest in these different bleeding procedures is related to the onboard handling of fresh fish and the increasing focus of Icelandic fish producers on delivering better raw material to produce fresh fish products. The experiment was done onboard a fresh fish trawler during a fishing trip where cod from the same haul was bled with three different cutting methods, superchilled and stored onboard at -1 °C without ice. The cod was then processed in a land-based processing plant in Akureyri and the fillets transported and measured at Matis in Reykjavik. The aim of this investigation is to evaluate the efficiency of different bleeding methods (DG, GC, TC) and storage time of Atlantic cod and the effects on fillet colour.

2. Materials and methods

This section describes the experimental setup of the study experiments and the measurement methods.

2.1. Experimental setup

The study included experiments with different cutting methods.

Experiment groups:

DG - Direct gutting: DG -> bled -> washed -> chilled -> stored.

GC - Gill-cut: GC -> bled -> gutted -> washed -> chilled -> stored.

TC - Throat cut: TC -> bled -> gutted -> washed -> chilled -> stored.

Figure 1 shows the onboard processing deck during the experiment sampling.



Figure 1: Onboard bleeding experiment

Wild Atlantic cod was caught in a bottom trawl by a wetfish trawler on September 29th, 2018. The onboard equipment was developed and installed by Slippurinn in Akureyri (Stranda) and includes an improved gutting line, batch bleeding tanks and first-in first-out cooling equipment. Three groups were taken from the same 7,700 kg haul where the towing time was 180 minutes and the average \pm standard deviation cod weight from the haul was 3.8 ± 0.3 kg. The time from catch to bleeding was kept at a minimum as the fish ($n = 30$ fish for each group) was bled live directly from the reception hold using different cutting methods; DG, GC and TC. The groups were bled for 25 minutes in seawater at a temperature of 6 °C and then groups GC and TC were gutted. After that, the groups were chilled in the same superchilling process. The catch was stored in 460 L tubs in a storage hold that was kept at -1 °C and, untraditionally, no ice was added to the tubs. The fish was processed at Utgerðarfélag Akureyrar four days post-catch. The fish was de-headed, filleted and skinned in Baader machines and then manually trimmed. The sample fillets were transported fresh to Matis in Reykjavík where colour evaluation and measurements were conducted. Day 0 is defined as the time of catching. Landing and processing took place on Day 3 from catch. The Minolta colour assessment and image analysis proceeded on Day 6 and Day 12 from catch. The fillets were kept fresh in EPS boxes in a 2 ± 2 °C cold storage between measurements.

2.2. Colour analysis

Visual evaluation of the colour of the fillets was done with a scale for fillet colour evaluation developed by Icelandic Ltd (2012) for cod, (scale of 5 (lightest) to 1 (darkest), see Figure 2). Two trained persons with experience in sensory and quality evaluation of fish products did the visual assessments on the same pool of samples ($n=20$ for each group).



Figure 2: Fillets colour chart scale for cod, from Icelandic Ltd (2012)

The colour of the samples (cod fillets) was determined with a Minolta Chroma Meter CR-300 (Minolta, Osaka, Japan) using the CIE $L^*a^*b^*$ system. The instrument recorded the L^* value, indicating lightness on the scale from black to white, 0 to 100 respectively, the a^* value, ranging from (+) red to (-) green, and the b^* value, ranging from (+) yellow to (-) blue (see Figure 4, Hutchings, 1999). The meter was calibrated on a white colour pad from the meter manufacturer. The colour was measured above the lateral line in seven positions, as shown in Figure 3, from the loin to the tail of 20 raw fillets for each group. In addition, whiteness of the samples was calculated as described by Park (1994):

$$\text{Whiteness (\%)} = 100 - [(100 - L)^2 + a^2 + b^2]^{1/2} \quad (1)$$

Total colour difference between groups relative to the DG group was calculated by equation 2:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (2)$$

In terms of color differences perceivable to the human eye, universally valid ΔE^* values can be used as follows: 0–1: observer does not notice the difference; 1–2: only an experienced observer can notice

the difference; 2–3.5: unexperienced observer also notices the difference; 3.5–5: a clear difference in color is noticed; >5: observer notices two different colors (Mokrzycki & Tatol, 2011).

Twenty fillets from each group were photographed inside a white plastic box in a semi-closed environment. White LED lighting panels were placed inside the box on either side of the fillets, 10 cm above the fillets and tilted towards them. The camera used was a Canon G7X Mark II (Canon Inc., Tokyo, Japan).



Figure 3: Minolta measurements locations

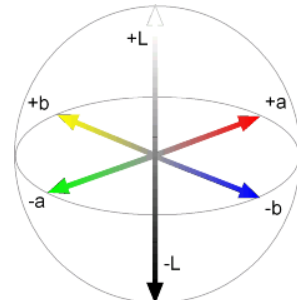


Figure 4: Description of the meaning of values gotten through using the CIE L*a*b* system (Hutchings, 1999)

Image analysis of CIE L*a*b* was done as in Jiang *et al.* (2020) using the IMAGE COLOR SUMMARIZER RGB, HSV, LCH & L*a*b* image color statistics and clustering program (Circos—Circular Genome Visualization/Martin Krzywinski). Images of 4x4 cm² area section in the loin, belly flap and tail end sections (n=20 fillets for each group) were analysed, as shown in Figure 5.

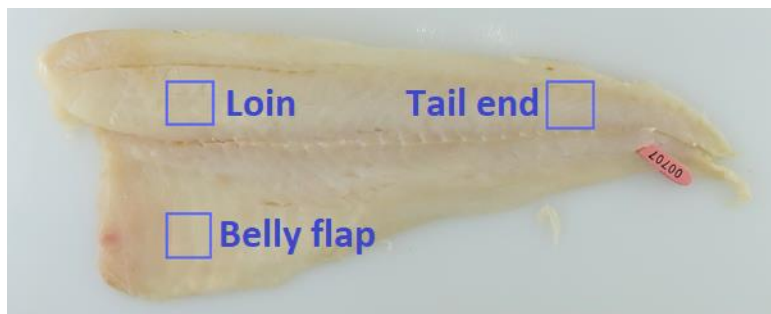


Figure 5: Image analysis sections of fillets, 4x4 cm² area

2.3. Statistical analysis

Statistical analysis of data was performed using Microsoft Excel 2016 (Microsoft Inc. Redmond, Wash, USA) and IBM SPSS Statistics v. 26 (International Business Machines, Armonk, New York, USA). Values are presented as mean ± standard error of mean (SEM). A t-test was used to determine the significance of differences between means of two groups.

3. Results and discussion

The results of the investigation are presented and discussed in this section. The visual evaluation of fillet colour shown in Figure 6 (evaluated by the Icelandic Ltd (2012) scale in Figure 2) showed a significantly higher fillet colour score on Day 6 compared to Day 12 (fillet colour score scale from 5 (lightest) to 1 (darkest)). The evaluation, however, did not show a significant difference between groups compared on the same day.

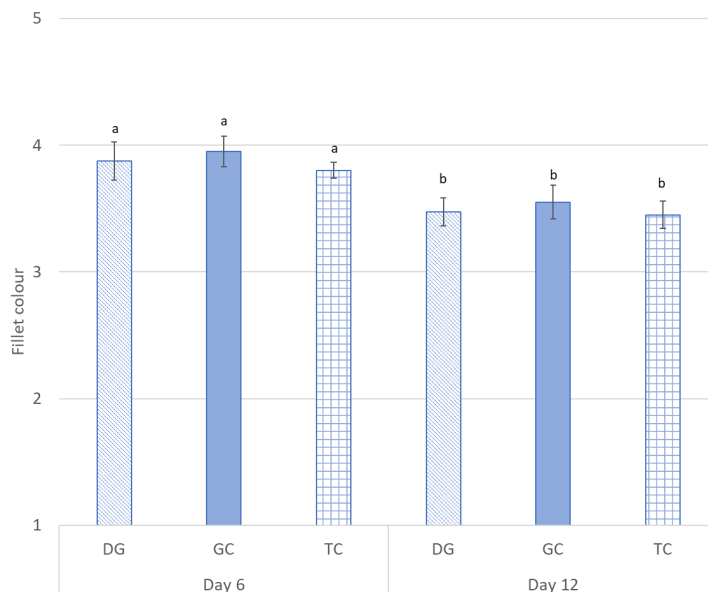


Figure 6: Average of visual fillet colour score (measured by Icelandic Ltd (2012) scale). Values are mean \pm SEM. Different superscript letters denote a significant difference ($p < 0.05$).

Table 1 shows the results of Minolta CIE $L^*a^*b^*$ measurements where average L^* , a^* and b^* values are shown for each group. The whiteness is calculated by equation 1. The average values on each day are similar between the groups. Olsen *et al.* (2014) found significantly more discoloration in fillets subjected to direct gutting, compared to fish that was throat or gill-cut and bled for 30 minutes prior to gutting. The difference between Days 6 and 12, however, is clearer, with higher values of L^* , b^* and a^* values. The fillets are therefore showing a more yellow colour with longer storage and show a lighter colour, but they show more redness. Comparing this to the visual assessment shown in Figure 6, the results indicate that the visual assessment weights the red colour of the fillets more significantly than the yellow colour changes. Total colour differences show ΔE^* between 0.3 and 0.9 for groups measured within the same day, indicating no noticeable difference. However, comparison between Day 6 and 12 indicates a clear difference in colour, with ΔE^* values above 5 (5.9-6.6).

Table 1: Fillet colour of cod for different cutting methods. Minolta Average $L^*a^*b^*$ ($n=20$ fillets).

	Day 6			Day 12		
	DG	GC	TC	DG	GC	TC
L^* (lightness)	69.2 \pm 0.5	69.0 \pm 0.4	69.1 \pm 0.4	74.6 \pm 0.4	74.5 \pm 0.4	75.3 \pm 0.4
a^* (redness)	1.5 \pm 0.2	1.2 \pm 0.1	1.2 \pm 0.1	1.8 \pm 0.1	1.8 \pm 0.1	1.5 \pm 0.1
b^* (yellowness)	7.3 \pm 0.2	7.0 \pm 0.2	7.2 \pm 0.1	9.8 \pm 0.3	9.3 \pm 0.2	9.4 \pm 0.2
W (whiteness)	68.3 \pm 0.5	68.2 \pm 0.3	68.3 \pm 0.3	72.7 \pm 0.4	72.8 \pm 0.3	73.5 \pm 0.4

Different fillet locations in Figure 7 show difference in values for L*, a* and b*, depending on the fillet measurement location according to Figure 3 (location points M1 (loin end) to M7 (tail end), see Figure 3). The L* and b* values are significantly higher at each end of the fillet, i.e. the loin and the tail end compared to the fillet mid-section. The value of the redness (a*), however, is higher in the fillet tail end. The results in Table 1 do not show a clear difference in average values between groups based on different cutting methods. However, in accordance with the study of Digre *et al.* (2011) on farmed cod, the colour values change during storage. Digre *et al.* (2011) saw the values of whiteness (W), L* and b* rise (L* from 59-67 to 74-78, b* from 17-19 to 20-22) and values of a* (redness) drop (from 5-11 to -2 to +2). The effects of gill-cut (GC) vs direct gutting (DG) in the Digre *et al.* (2011) study showed changes, with GC whiter and less red (showing higher L* and b* values, and lower a* values), compared to the direct gutting method.

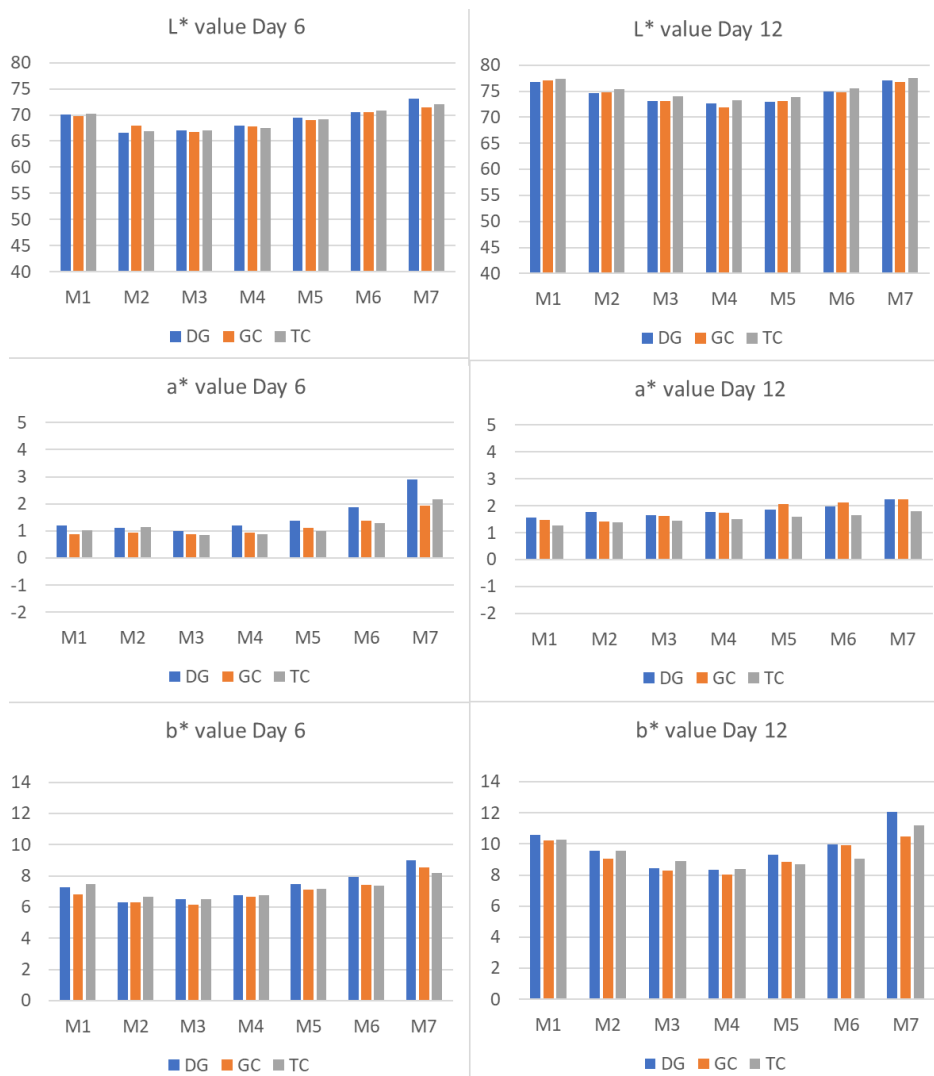


Figure 7: L*a*b* in different fillet locations (see measurement locations in Figure 3)

Another comparison to the study by Digre *et al.* (2017) showed the loin colour of cod stored for different time periods before slaughter. The results showed L* values of the loin 84-86, a* values of 2-

5, and b* values of 2.5-3.0, with the filleting and colour measurement proceeding one day after catching. In the study by Digre *et al.* (2010), after 8 days of ice storage, cod showed L* values 81.8-85.0, a* values of -3.2 to 3.7, and b* values of 17.7-20.5. Bjernevik & Solbakken (2010) studied the difference between stressed vs unstressed cod and did not find significant differences in CIE L*a*b* values after 8 days of ice storage. Measured on Days 0 and 8 with a Minolta meter, the values they measured were L* 43.7-48.4, a* 3.6 to -4.7, and b* -0.8 to 1.4. The L* value was significantly higher, and the a* and b* values were significantly lower on Day 8 compared with Day 0. In Erikson *et al.* (2019), colour analysis of fresh cod showed L* values of 86.6-88.2, a* values of 2.5 to 6.0, and b* values of 1.7 to 2.4. The L*a*b* values in these studies are in some cases similar but in other cases different from the colour analysis in the current study. However, the difference between groups and storage time, and their effects on the fillet colour, can be evaluated.

Table 2 shows the results of the image analysis for the loin, belly flap and tail end sections shown in Figure 5. There is no significant difference between the cutting method groups apart from the a* value of the belly flap and tail end for group DG. This concurs with the a* value results on Day 6 for tail end locations M6 and M7, in Figure 7. Compared to the Minolta values, the whiteness of the fillets shows similar values for Day 6 and considerably lower values for Day 12. Olsen *et al.* (2014) evaluated the whiteness of loin and belly for cod with different bleeding methods and found, in contrast to the results of this study, lower whiteness in the loin area. Total colour differences by equation 2 show a change in ΔE^* values of 2.5 to 4.2 between Days 6 and 12, indicating a marginal to clearly noticeable difference in the colour, depending on storage time. The ΔE^* values are between 0.3 and 0.9 for the loin groups within Day 6, indicating no difference. On Day 12, the ΔE^* value of loin in groups GC and TC is 1.5 higher than the DG, indicating a marginal difference in colour. The same trend is noticeable in the belly flap colour on Day 6, with noticeable difference (ΔE^* of 2.2 and 2.9) comparing groups GC and TC to the DG group. On Day 12, the belly flap colour for the GC group shows a clear difference from the DG (ΔE^* value of 4.2) and a noticeable difference from TC (ΔE^* value of 2.7). A similar trend is seen for the tail end as for the belly flap, the a* value of group DG indicates more redness in colour compared to groups GC and TC. The ΔE^* values also indicate a more uniform colour difference in the loin between the groups than in the belly flap and the tail end.

Table 2: Fillet colour of cod for different cutting methods. Image analysis by CIE L*a*b* colour scale (values are mean \pm SEM)

Fillet part	CIE LAB colour	Day 6			Day 12		
		DG	GC	TC	DG	GC	TC
Loin	L* (lightness)	68.6 \pm 0.6	68.5 \pm 0.4	69.3 \pm 0.5	70.5 \pm 0.7	72.0 \pm 0.4	71.2 \pm 0.4
	a* (redness)	-0.3 \pm 0.7	-0.1 \pm 0.3	-0.6 \pm 0.4	-0.2 \pm 0.6	-0.3 \pm 0.3	-0.5 \pm 0.2
	b* (yellowness)	8.1 \pm 0.4	8.2 \pm 0.3	8.5 \pm 0.4	10.4 \pm 0.4	10.6 \pm 0.3	9.3 \pm 0.3
	W (whiteness)	67.6 \pm 0.6	67.5 \pm 0.4	68.1 \pm 0.5	68.7 \pm 0.7	70.1 \pm 0.5	69.7 \pm 0.5
Belly flap	L* (lightness)	64.8 \pm 1.3	66.3 \pm 0.7	65.1 \pm 0.9	64.7 \pm 0.5	68.2 \pm 0.4	65.7 \pm 0.7
	a* (redness)	4.6 \pm 0.6	2.3 \pm 0.5	3.3 \pm 0.7	5.0 \pm 0.6	2.9 \pm 0.6	3.6 \pm 0.6
	b* (yellowness)	8.7 \pm 0.4	9.8 \pm 0.3	10.4 \pm 0.7	11.1 \pm 0.7	12.0 \pm 0.4	11.1 \pm 0.4
	W (whiteness)	63.5 \pm 0.3	64.8 \pm 0.4	63.4 \pm 0.5	62.7 \pm 0.6	65.9 \pm 0.4	63.8 \pm 0.5
Tail end	L* (lightness)	63.1 \pm 0.6	63.8 \pm 0.6	63.3 \pm 0.8	61.8 \pm 0.3	62.5 \pm 0.4	62.9 \pm 0.3
	a* (redness)	1.8 \pm 0.3	0.7 \pm 0.3	0.9 \pm 0.2	3.2 \pm 0.3	1.1 \pm 0.3	1.6 \pm 0.3
	b* (yellowness)	7.7 \pm 0.3	8.0 \pm 0.3	7.5 \pm 0.3	9.8 \pm 0.3	10.9 \pm 0.3	9.5 \pm 0.2
	W (whiteness)	62.3 \pm 0.4	60.4 \pm 0.6	62.5 \pm 0.6	60.4 \pm 0.3	61.0 \pm 0.4	61.7 \pm 0.4

Figures 8-13 show examples of the same fillet from each group on Day 6 and Day 12, when they were evaluated.



Figure 8: Group DG on Day 6 from catch - Direct gutting



Figure 9: Group DG on Day 12 from catch - Direct gutting



Figure 10: Group GC on Day 6 from catch - Gill-cut



Figure 11: Group GC on Day 12 from catch - Gill-cut



Figure 12: Group TC on Day 6 from catch - Throat cut



Figure 13: Group TC on Day 12 from catch - Throat cut

4. Conclusions

The study results did not show a clear difference in overall fillet colour between the cutting methods; direct gutting (DG), gill-cut (GC) and throat cut (TC). The difference found was a minor difference in the redness (a^* value) of the belly flap and tail end for group DG. The reason for only a minor difference in this study could be that, when bleeding time exceeds 20 minutes and the fish is bled within 30 minutes after catch, the difference between cutting methods becomes negligible. The colour of the loin was found to be similar for all groups evaluated on the same day. The colour evaluation, however, showed a significant difference in fillet colour affected by the six days storage time compared to 12 days storage time and different locations on the fillet. According to the digital assessment of the Minolta meter and image analysis, the fillets became lighter in colour, more yellow and redder after six days of cold storage. The visual assessment colour scale likely tends to evaluate the red colour of the fillets more significantly than yellow colour changes, or changes in lightness. An obvious colour difference was seen between loin, tail end and belly flap. The loin is lighter, less red and less yellow while the belly flap and tail end are darker and with redness. The difference in yellowness however between these fillet locations was only marginal. With only minor differences between cutting methods, the results support either DG and a simpler processing-deck setup, or GC or TC for two-step bleeding and faster processing from the reception hold.

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