UiT THE ARCTIC UNIVERSITY OF NORWAY

Faculty of Bioscience, Fisheries and Economics
The Norwegian College of Fishery science

Stress responses influencing fillet quality of trawled Atlantic cod and haddock

Ragnhild Aven Svalheim

A dissertation for the degree of Philosophiae Doctor – November 2018

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‘If we knew what is was we were doing, it would not be called research, would it?’

Albert Einstein

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I. Acknowledgement

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II. Thesis abstract

During trawling, fish become stressed through exercise, interactions with fishing gear and crowding in the cod-end. As catches are dropped on deck and exposed to air, light and temperature differences, fish are exposed to cumulative stress as well as potential bruising and death by asphyxiation. There is increasing evidence suggesting that pre-mortem stress is of great importance to muscle quality of fish, and this may explain why the catches from trawl fisheries have variable quality. Yet, little is known about how individual steps of the trawling process affect the muscle quality of the fish.

In this thesis, an experimental swim tunnel and cod-end was used as a model to investigate how stress during various stages of trawl capture affects fillet quality in terms of residual blood, time and hardness of post-mortem muscle stiffness and muscle colour of cod and haddock. In addition, the effect of stress on the importance of timing of euthanasia was also addressed.

The first stage of trawl capture that was chosen to study was the herding of fish in front of the trawl mouth. Two experiments were conducted to address this issue; the first involved exhaustive swimming of cod and the second focused on critical swimming speed of haddock. These studies showed that exhaustive swimming causes a moderate stress response, recovery takes longer than 6 hours and that exercise has a short-lasting effect on muscle texture, with little or no effect on muscle colouration. It was concluded that other stages of trawl capture have a higher impact on fillet quality.

The third study for this thesis aimed to investigate how extreme crowding for 1 or 3 hours in the cod-end, following exhaustive swimming, would affect the physiology and muscle quality of cod. Findings from this study showed that crowding caused a severe stress response and that fish probably suffered from hypoxia due to a significantly reduced ability to move their opercula. In addition, fillet quality was significantly reduced due to increased amount of residual blood in the muscles. Moreover, the detrimental effects of crowding are not fully reversed after 6 hours of recuperation.

In the last investigation, the final stage of trawl capture process, i.e. the effect of air exposure on deck, was studied. Fish were stressed by mild crowding and then exposed to air for 15 or 30 minutes, or directly euthanised by terminal blow to the head and then left in air for 0, 15 or 30 minutes before exsanguination. We found that stress/crowding triggered a stronger response to the air exposure by faster increase in residual blood in the muscles, resulting in lower fillet quality. However, direct euthanasia stopped blood flow to the muscle and quality was significantly improved.

Together these four studies show that there is a strong connection between the type of stress inflicted on the fish during capture and the quality of the fish product (fillets). Measures that may secure top quality fish from trawlers, include reducing crowding time in the cod-end and implementing direct euthanasia or live recuperation for more than 6 hours.

III. List of papers

Paper I

Recovery from exhaustive swimming and its effect on fillet quality in haddock (*Melanogrammus aeglefinus*)

Anders Karlsson-Drangsholt, Ragnhild Aven Svalheim, Øyvind Aas-Hansen, Stein-Harris Olsen, Kjell Midling, Michael Breen, Endre Grimsbø, Helge Kreutzer Johnsen

Fisheries Research 197, (2018), 96-104.

Paper II

Effects of exhaustive swimming and subsequent recuperation on flesh quality in Atlantic cod (*Gadus morhua*)

Ragnhild Svalheim, Anders Karlsson, Stein-Harris, Helge K. Johnsen, Øyvind Aas-Hansen,

Fisheries Research 193 (2017) 158-163.

Paper III

Simulated trawling: Exhaustive swimming followed by extreme crowding as contributing reasons to variable fillet quality in trawl-caught Atlantic cod (*Gadus morhua*)

Ragnhild Aven Svalheim, Øyvind Aas-Hansen, Karsten Heia, Anders Drangsholt-Karlsson, Stein Harris Olsen, Helge Kreutzer Johnsen

PLoS One: In review

Paper IV

Differential response to air exposure in crowded and uncrowded Atlantic cod (*Gadus morhua*): Consequences for fillet quality

Ragnhild Aven Svalheim, Erik Burgerhout, Karsten Heia, Sjurdur Joensen, Stein-Harris Olsen, Heidi Nilsen, Torbjørn Tobiassen

Food Bioscience: In press

Introduction

Ethical and economical aspects of quality improvements in white fish fisheries

Fish as food represents an important resource for human nutrition and health and is also appreciated for cultural and gastronomic reasons. Globally, regionally, nationally and locally, the use and importance of aquatic foods vary greatly. Production of wild captured seafood has more or less stabilized the last two-three decades (Fig 1), with most fish stocks assessed by FAO to be fully, but sustainably exploited (FAO 2018). However, the global demand for aquatic food sources are expected to increase faster than population growth, due to an increasing proportion of middle-class people with greater spending power who typically consume more animal protein than people with lower income. Therefore, it becomes more important to ensure that harvested fish are utilised in such a way that the proportion of the fish suitable for human consumption is maximized. That is, by reducing waste that is caused by quality impairment activities.

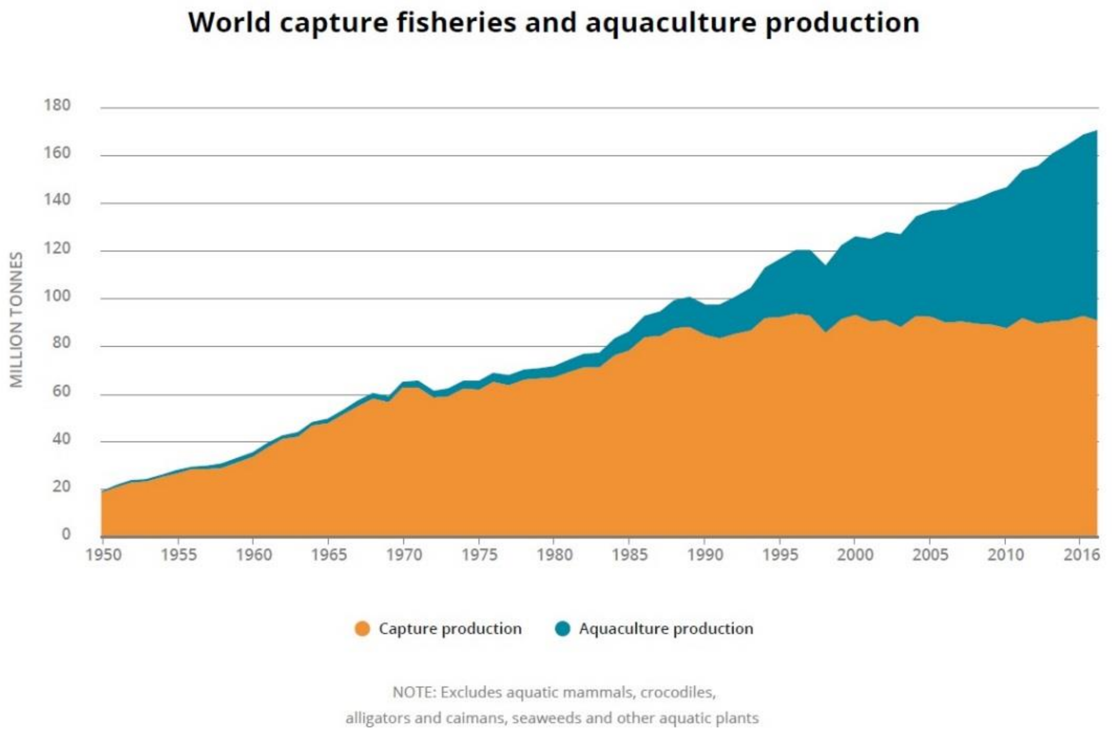

Figure 1. Trends in world capture fisheries and aquaculture production the last ~70 years. Aquaculture production is both food fish (~80 tons) and aquatic plants (~30 tons).

In Norway, governmental authorities, sales organizations, industry and researchers often emphasize the need for quality improvement of white fish. Nevertheless, the quality of landed fish from the coastal fisheries shows a negative trend (Akse et al. 2014, Akse et al. 2004). This is especially true for fish caught by gillnet and Danish sein, whereas fish caught by line are stable, and overall, have good quality. Furthermore, the difference in quality of fish caught by different gears is often not reflected in the ex-vessel price of fresh fish, where the trend is that vessels delivering the largest quanta per time unit get the highest price (The Norwegian Fishermen's Sales Organization, 2018). However, this may lead to a direct financial loss for the fish processors. A study comparing high and low quality cod and haddock showed that poor quality fish could lead to a potential value loss of 13% in the filleting industry, which corresponds to about NOK 100 million (EURO 11 million) based on the export value in 2013 (Svorken et al. 2015).

It is important to keep in mind that the gadoid fishery is diverse, extending from small coastal vessels utilizing hand-baited long-lines, gillnets, and jig machines with daily deliveries of fresh catches to local fish plants, to massive ocean going bottom trawlers and auto liners that process and freeze the catch at sea. The frozen fish is usually sold at auctions, which presents the opportunity to evaluate product quality and raise complaints when quality standards are not met. This creates a better, but not perfect, correlation between quality and price and emphasizes the fact that quality does matter. For example, fish caught by trawls and Danish seine are known to yield more variable quality than fish caught by auto line. In an ongoing study of prices for frozen cod and haddock covering a period of nine years (2009-2017), it is found that for Atlantic cod, fish caught by autoline gain 9.5% and 16.1% higher prices than trawl and Danish seine, respectively, controlled for the influence of fish size and season (Sogn-Grundvåg, unpublished data). For haddock, autoline get 22.5% higher price compared with Danish seine. Trawl, considered to be one of the most catch efficient fishing gears, lands the largest quantum of cod and haddock in Norway (Fig 2), and hence improvement to the quality of trawl-caught cod and haddock may therefore have a great ethical and financial impact on the fishery industry.

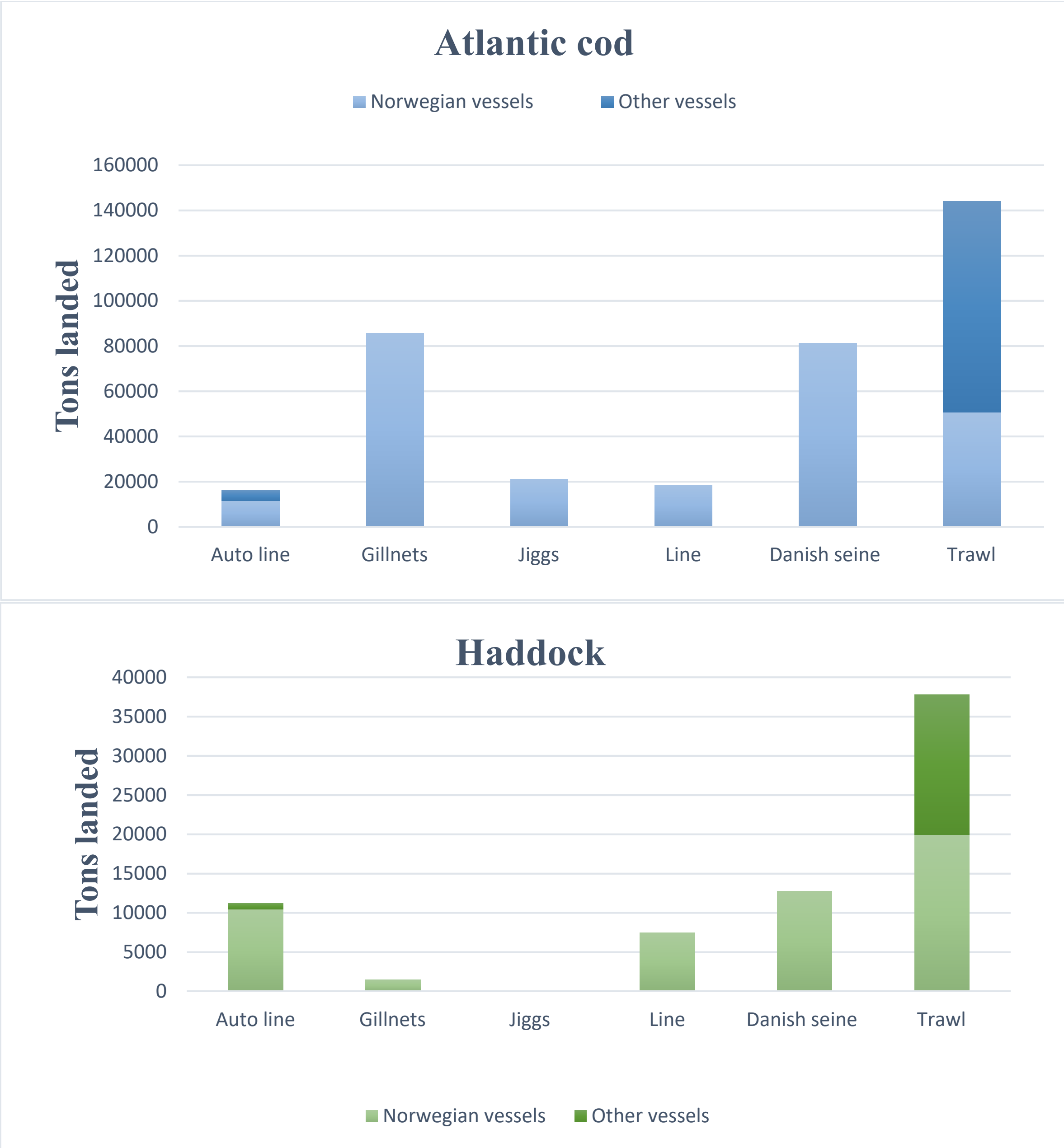

Figure 2. Metric tons landed haddock (a) and cod (b) by fishing gear. Period 01.01.2018 - 01. 10. 2018. Source: The Norwegian Fishermen's Sales Organization, 2018.

Fillet quality

There are many factors which determine the quality of a fish or a fish product (Fig 3). Fillet quality is a complex set of characters involving intrinsic factors such as chemical composition, texture, fat content and colour which in turn are influenced by extrinsic factors such as feeding regime, diet composition and pre- or post-slaughter handling procedures. The term 'quality' frequently refers to the visual appearance and freshness or degree of spoilage the fish has undergone. These features often have bearing on food safety in terms of harmful bacteria, viruses, parasites or chemicals, that can create an off odour and bad taste, soft texture or altered muscle colour. Many quality traits of a fish product also depend on other biotic and abiotic factors such as fish species, season (Botta et al. 1987b), gender (Ageeva et al. 2017), and type of food eaten (Ageeva et al. 2018).

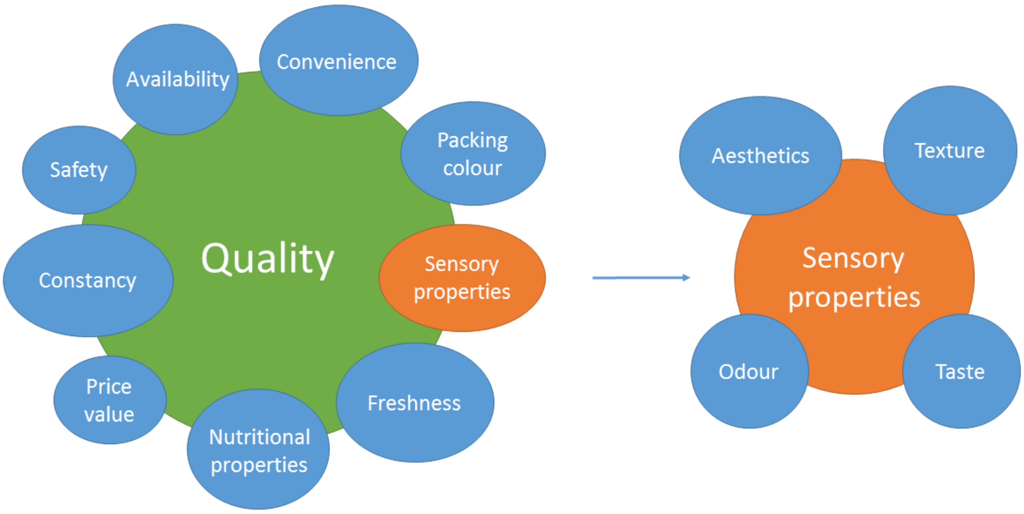

Figure 3. Overview over multiple factors influencing the quality of fish products. Modified from Olafsdóttir et al. (1997).

Texture of fish muscle is a highly important quality parameter, as softness may cause downgrading in the processing industry (Michie 2001). The texture of a fish muscle is influenced by inherent characteristics such as amount and composition of connective tissue and muscle fibres density, which in turn undergo substantial seasonal variations (Botta et al. 1987b). For the fish, white muscle with its high protein content, constitutes an important energy resource, and seasonal events like spawning, periods with starvation and prolonged stress may reduce protein content of the muscle (Ageeva et al. 2017, Ladrat 2000, Ageeva et al. 2018). These changes can alter contractile properties and metabolic characteristics of the muscles, and may ultimately influence the flesh quality (Delbarre-Ladrat et al. 2006). Post-mortem proteolysis in fishes is not considered beneficial to flesh quality, as fish meat generally does not need to be tenderized. Rather, the protease mediated muscle tissue degradation contributes to softening of the meat, increased drip loss, and increased gaping (Bahuaud et al. 2010, Mørkøre et al. 2008, Ofstad et al. 1996, Roth et al. 2006, Sigholt et al. 1997, Thomas et al. 1999).

The colour of fish muscle is another important quality parameter. When potential consumers evaluate fillets, they expect white fish to be white and may therefore reject pinkish or dark fillets. Residual blood in the fillets is the main factor responsible for colour change in white fish (Olsen et al. 2008). The haem pigments in red blood cells contain iron molecules, which bind oxygen and makes the blood cells appear red. Hence, residual blood in the muscles makes the fillet appear pink or reddish. In addition, large amounts of haemoglobin may accelerate lipid oxidation, causing an unpleasant odour (Maqsood and Benjakul 2011, Richards and Hultin 2002, Terayama and Yamanaka 2000). Therefore, exsanguination of fish by cutting the throat or gills is decreed by Norwegian legislation (FOR-2013-06-28-844).

The type of fishing gear can also greatly influence the quality of the fish product (Botta et al. 1987a, Digre et al. 2010, Esaiassen et al. 2004, Huse et al. 2000, Larsen and Rindahl 2008, Rotabakk et al. 2011). Some quality defects are directly related to the gear, such as gaffing damages from longline (Larsen and Rindahl 2008) and bruising and net marks from trawls (Digre et al. 2010). Other quality issues may arise as an effect of the stress inflicted on to the fish by the capture process itself and are not necessarily notable until the fish is processed into fillets. These issues involve increased amount of residual blood in fillets and faster onset of *rigor mortis*, followed by textural changes such as gaping and dry flesh (Stien et al. 2005, Hultmann et al. 2016, Aursand et al. 2010, Digre et al. 2017, Olsen et al. 2008). These effects are most likely related to altered physiological characteristics of the fish, due to pre-mortem stress.

Trawls

The use of trawl to catch fish triggers a complex sequence of behavioural responses by the captured fish. A large part of the knowledge on behavioural patterns of trawl-caught fish is based on studies from the 1960s, using underwater observations of fish during trawling (Glass and Wardle 1989, Beamish 1969, Reviewd in Winger et al. 2010).

The trawl itself is a cone shaped net made from two, four or more panels, which is towed by one or two boats. The net is wide at the opening and then narrows to a bag called the cod-end, where the fish become trapped (Fig 4). The net opening is held open by beams, otter boards (doors) or distance between two towing vessels (pair trawling).

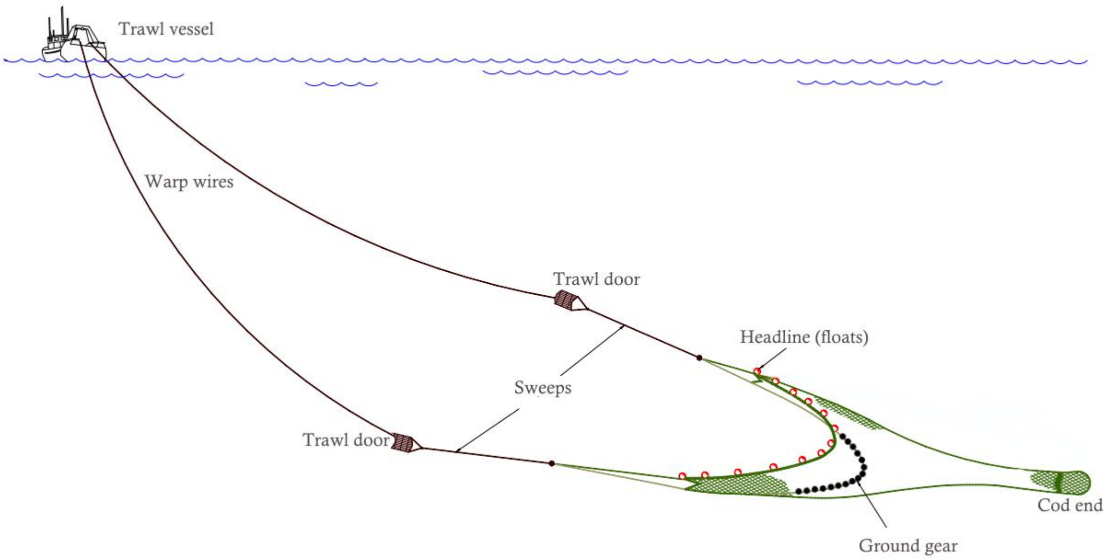

Figure 4. Schematic overview of a bottom trawl. Modified from and image by Institute of Marine research, Norway.

Otter trawl is the most commonly used type in Norway. The boards rest on the sea bottom and creates large mud clouds when they are dragged along the sea floor. These clouds are important for the catch efficiency, as they mask the visual appearance of the netting and footgear, making the fish swim towards the net instead of escaping to the side (Sistiaga et al. 2015). When these components become visible to the fish, the fish tend to alter course, turn around and if towing speed allows it, start swimming in the direction of the tow in the net opening. This behaviour occurs when light intensities are high enough for the fish to see the movement of the approaching net and is most likely an optomotor reflex (Winger et al. 2010). Normal towing speeds vary from 1-7 knots depending on target species. Common towing speeds for cod and haddock is 2-5 knots (1 to 2.5 m s^{-1}). These towing speeds exceed the sustained swimming speed of the target species (Breen et al. 2004, He 1991), suggesting that the fish may be exhausted or fatigued as they enter the cod-end.

Fish swimming in the net opening

Before ending up in the cod-end, fish engage in numerous behaviours that all involve swimming. These behaviours include reacting to and trying to avoid an approaching trawl, fleeing from the net opening and avoiding the gear or actively trying to escape once inside (Suuronen et al. 2005, Suuronen et al. 1996). In general, the swimming performance has been classified into three distinct categories: sustained, prolonged and burst swimming. Sustained swimming speed are speeds which can be maintained for more than 200 minutes, whereas prolonged activity, as classified by Beamish (1978), can be maintained for 20 seconds – 200 minutes. Speeds which cannot be maintained for more than 20 seconds are classified as burst swimming (Beamish 1978). The endurance of cod is highly sensitive to changes in towing speed, with higher towing speeds reducing how long time the cod can swim in the trawl opening (Winger et al. 2010). By choosing a towing speed higher than the sustained swimming speed of the target species, the fish in front of the net opening will eventually drift back into the net. However, the extent to which fish are able to maintain their position in front of a trawl opening, is highly species specific and also depend the on physical (i.e. size and length) (Suuronen et al. 2005) and physiological conditions of the fish when presented with the trawl opening (Winger et al. 2010).

Video footage from a trawl opening shows that fish engage in a burst and glide behaviour before drifting into the net. This behaviour represents an intermediate mode between prolonged and burst swimming and has a predicted energy savings of about 50% (Weihs 1974). It is however, not an endless swimming mode as fish eventually terminate this swimming behaviour. Most likely, fish cease swimming due to a combination of metabolic exhaustion and accumulation of anaerobic waste products in combination with a behavioural decision of the fish to stop swimming (Tudorache et al. 2013). For example, Breen et al. (2004) found that exhausted haddock where in fact not exhausted, but were seemingly ‘unwilling’ to continue swimming under laboratory conditions. It has since been clearly shown that ‘exhaustion’ and ‘fatigue’ are not interchangeable descriptions and that ‘exhaustion’ relates to a condition in which the energy stores of the fish are fully depleted, whereas ‘fatigue’ is a behavioural decision of the fish that may occur before the fish has depleted its energy stores (Farrell 2007).

Crowding in the cod-end

As fish are captured they accumulate in the cod-end and eventually become the catch, and the same time, crowding pressure increases. The intensity of the crowding situation vary with the amount of fish entering and exiting the cod-end and the water flow in the cod-end. The water flow in the cod end depends on the type of twine used, mesh-type and amount of fish (Winger et al. 2010). There are few studies on the extent and exact measurements of crowding pressure inside a cod-end. However, large catch sizes (15–30 metric tons) and visual observation strongly indicate that the degree of crowding pressure is sever (Fig 5). The most dramatic crowding probably occurs during the haul back when lifting of the cod-end reduces ambient pressure, causing the swim bladder, and hence the whole fish, to expand (Tytler and Blaxter

1973, Taylor et al. 2010, Ferter et al. 2015). Swim bladder expansion occurs in cod and haddock because they have a physoclist (i.e. closed) type of swim bladder. Fish with this type of swim bladder can only adjust the gas inside by actively secreting gas from the blood to the swim bladder via the gas gland (*rete mirable*) and reabsorb gas by controlled passive diffusion over a highly vascularised area, called the oval. Both the secretion into the bladder and reabsorption of gas from the swim bladder are slow processes which take several hours to complete (Midling et al. 2012).

Figure 5. Recently captured gadoids crowded in the cod end on deck of the fishing vessel. Photo by Jesse Brinkhof.

Furthermore, because the Northeast Atlantic cod tend to be found in high abundance and in dense aggregation, massive catches can be obtained during short towing times (10-20 minutes). This has led to a practice among Norwegian trawlers, called ‘buffer towing’. This practice involves deploying the trawl immediately after the catch is on board. In the case when the desired amount of fish is caught prior to completion of processing of the catch from the previous haul, the trawl is simply lifted off the seabed and towed at low speed (~1-2 knots). This tactic is employed in order to ensure a continuous supply of fish for processing, but has negative effects on quality and will lengthen the time the fish are crowded in the cod-end (Brinkhof et al. 2018).

Post-capture air exposure

Processing of live fish to food necessarily involves a moment of slaughter. The most commonly employed slaughtering technique of wild fish involves cutting the throat followed by exsanguination. However, large catches and vigorous fish can make bleeding challenging. It is therefore common practice on many fishing vessels that the fish are exposed to air prior to exsanguination, as fish then become moribund and easier to handle. For this reason, bleeding of the fish is often performed after a period of air exposure, which lead to asphyxiation (Van De Vis et al. 2003).

Asphyxia is characterized by a prolonged period with suffocation before death. The time it takes for fish to die from asphyxiation before bleeding depends on the hypoxia resistance of the species and temperature (Poli et al. 2005). Furthermore, previous recommendations states that the fish should be bled within 30 minutes of slaughter in order to ensure proper exsanguination (Olsen et al. 2014). However, cod and haddock show brain activities up to 2 hours after being taken on board and kept in dry tanks (Lambooij et al. 2012). Hence, air exposure cannot be considered a slaughter method for these fish species, but is rather an additional stressor in the capturing process which can potentially lead to reduced quality and shorter shelf life of the fish product (Lambooij et al. 2012). In addition, the practice with air exposure is considered unacceptable in terms of animal welfare (Van De Vis et al. 2003).

Live storage of fish on board fishing vessels

Storing live fish after catch may improve the quality of the captured fish. In Norway, live cod captured by demersal seine are placed into capture based aquaculture to supply markets with fish throughout the year, and thus increase the value of the catches (Ottolenghi et al. 2004, Midling et al. 2012). During the last decade, a similar procedure has been investigated for short-term storage of trawl-captured cod and haddock (Olsen et al. 2013, Lambooij et al. 2012, Digre et al. 2017). Short-term storage involves keeping fish in water-filled tanks until the crew is prepared for slaughter. The main advantages of this practice are improved fish welfare and potentially higher product quality. Keeping fish alive for as long as possible may increase shelf-life of the product, as less time will pass from slaughter to market. Furthermore, because time from slaughter to bleeding is important for proper exsanguination, live storage could be a practical approach allowing better control of the timing of slaughter. Furthermore, the study by

Olsen et al., (2013) showed that muscle quality of cod and haddock improved after 6 hours of live storage, although Digre et al., (2017) found that quality only improved marginally with recuperation in water with variable oxygen saturation (46 - 117% dissolved oxygen).

Fish musculature and cardiovascular system

White and red muscles

The fish skeletal muscles are organized in segments (myotomes) shaped like a sideways W and arranged in series so that they stack like cones (Fig 6). The myotomes are separated by connective tissue (the *myoseptum*) and are easily visible in heat treated fish, as high temperatures breaks down the connective tissue allowing separation of the muscle blocks. Fish swim with two types of muscle fibres, the metabolically aerobic red muscles and the metabolically anaerobic white muscles. The red muscles typically represent maximum 10% of the total muscle mass and is used for slow to moderate, sustained swimming. The white muscles constitutes about 90% of the total muscle mass (and sometimes over 50% of the total body mass) and is used for brief burst and high speed swimming, such as predator-pray chase (Nelson 2011).

The red muscles are located directly under the skin, parallel to the length of the fish. They get their colour from a high myoglobin content, which functions as an internal oxygen transport system. The red muscles are made up primarily from slow-twitched oxidative fibres generating their ATP by mitochondrial oxidative phosphorylation, which produces 36 ATP for each glucose equivalent. This process require fuel in forms of substrates (lipids, carbohydrates or protein) and oxygen as terminal electron acceptor. Oxygen and substrates are provided via rich blood supply (Wang and Richards 2011).

White muscles are composed of fast glycolytic fibres that produce most power at high contraction frequencies and which rely almost exclusively on intracellular fuel stores to generate ATP anaerobically via substrate-level phosphorylation (predominantly glycolysis). Glycolysis produces two ATP in the conversion of glucose to pyruvate and then lactate. White muscles are poorly vascularised and contain no or little myoglobin. Although oxidation of glucose in the mitochondria yields more ATP then fermentation (30-36 vs 2 ATP), there are at least two physiological conditions where anaerobic pathways are preferred over aerobic, both of which occurs when mitochondria meets their limit to generate ATP.

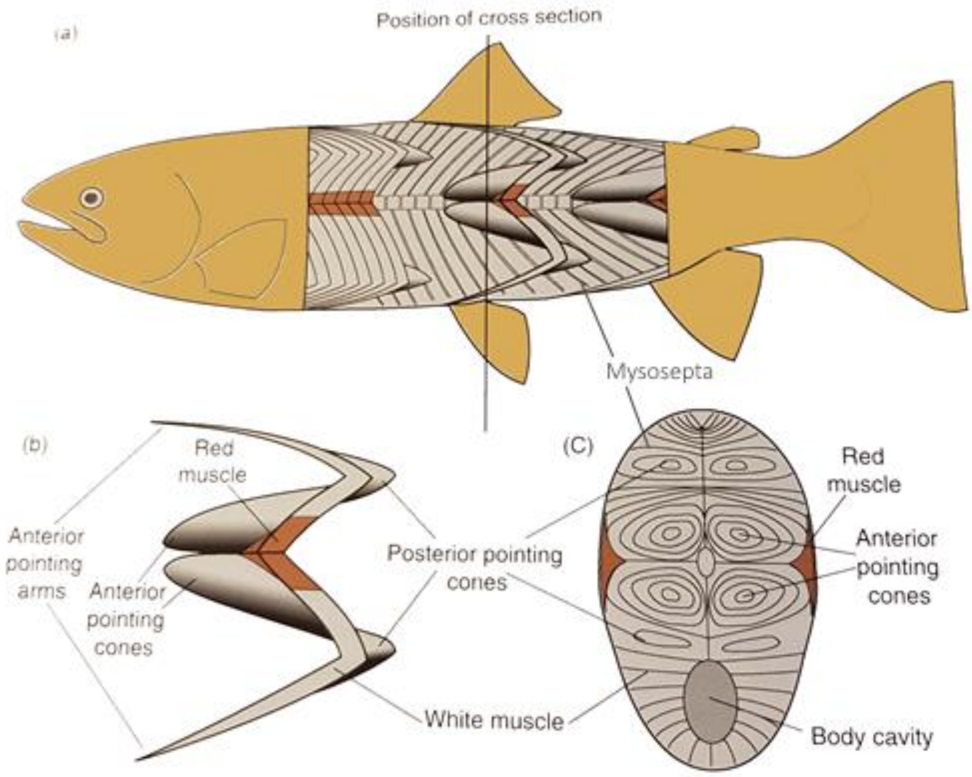

Figure 6. Myotomal muscle anatomy. (a) Lateral view of typical teleost. One myotome of the body axis is shown in situ with adjacent tissue removed to illustrate its three-dimensional structure. (b) Component part of the myotome. (c) Body cross section revealing muscle fibre types and myosepta. Modified from Nelson (2011).

The first involves lack of oxygen, for example due to exposure to a hypoxic environment. The second situation is where there is the requirement for a high generation rate of ATP in order to support intensive muscle contractions, for example during periods of intensive exercise. This is because ATP can be produced about twice as fast by anaerobic metabolism compared to aerobic metabolism (Wang and Richards 2011). Anaerobic metabolism leads to a rise in lactate levels, which can more than double in situations that cause the body to shift from aerobic metabolism to anaerobic metabolism. Therefore, lactate is frequently used as a stress indicator in fish. The rate of production and disposal of lactate is dependent on the stressor itself. For example, exercise may cause a more rapid formation and disposal of lactate than hypoxia does (Weber et al. 2016). This can be explained by differences in blood flow and in metabolic rate of the tissues that metabolize lactate.

Muscle contraction and development of *rigor mortis*

Although the anatomical structure of fish muscles is different from those of mammals, the process of muscle contraction by the striated muscles is the same. Production of force from shortening of the skeletal muscles is caused by myosin cross-bridge cycling, which involves a sequence of molecular events that underlie the sliding filament theory (Huxley and Hanson

1954, Huxley and Niedergerke 1954). In short, the myosin (thick) filaments of muscle fibres slide past the actin (thin) filaments during muscle contraction, while the two groups of filaments remain at relatively constant length. This process requires ATP energy and the binding and release of Ca^{2+} from troponin C, which is a protein component of thick myosin filaments. Crossbridge cycling can continue as long as there are sufficient amounts of ATP and Ca^{2+} in the cytoplasm

In the first hours to days following slaughter, the texture of fish muscle is particularly influenced by the process of *rigor mortis*. After death, fish cease respiration and aerobic production of ATP is no longer possible. However, the tissue will continue to produce ATP via anaerobic glycolysis until the glycogen stores are depleted. Following glycogen depletion, the ATP concentration declines and the body enters *rigor mortis* because there is no ATP available to break the crossbridges. Additionally, Ca^{2+} enters the intracellular fluids after death, due to the deterioration of the sarcoplasmic reticulum. Ca^{2+} allow the myosin heads to bind to the active sites of actin proteins and the muscle is unable to relax until further enzyme activity degrades the complex. Rigor completion has been achieved when cross-bridge affinity and tension are at their maximum. During rigor, nearly 100% of all possible binding sites form cross-bridges, as opposed to about 20% during normal muscle contraction. Muscle tension will decrease as a result of proteolytic degradation.

The process of *rigor mortis* has consequences for the processing of the fish as traditionally captured fish are often delivered in rigor. Mechanical handling of such fish by gutting or filleting machines can cause severe quality defects such as gaping and reduced fillet yield (Love 1988, Stroud 1969). It is therefore common practice to halt production until rigor is completed. This evidently has consequences for freshness and shelf life of the fish product as rigor may last for several days. Prolonging the time before onset of rigor opens up for pre-rigor filleting and production of exceptionally fresh fish that can have longer marketing time and may serve additional benefits in terms of less weight and thus energy for transport (fillet vs. whole fish). With that said, there are some issues related to pre-rigor filleting such as lower water holding capacity and increased drip loss, strong fillet contraction and fillet shrinkage (Kristoffersen et al. 2006, Kristoffersen et al. 2007).

Circulation

The fish circulatory system consists of two major vascular beds, the respiratory gill and the systemic circulation. The heart of the fish is situated in the *pericardium*, which is a membranous sac. Within the *pericardium*, there are four distinct chambers that constitute the fish heart: the *Sinus venous*, the atrium, the ventricle, and an outflow tract (*Bulbus arteriosus*) (Farrell and Pieperhoff 2011). The heart pumps deoxygenated blood through a short arterial network into the gills via *Aorta ventralis*. After passing through the capillary network of the gills, oxygenated blood is collected into *A. dorsalis* and distributed to the peripheral tissue via the systemic vessels. The venous system returns deoxygenated blood from the peripheral tissues to the heart (Olson 2011a).

White and red muscles are supplied with oxygenated blood by the segmental arteries. These arteries branch off *A. dorsalis* at each vertebra, usually in alternating myosepta. There are three groups of segmental arteries: dorsal, lateral and ventral (Fig 7). Dorsal and lateral arteries travel in the septa between the myotomes and branches leave to enter myotomes and perfuse the capillaries of muscle fibres. Circulation of the peripheral tissue is regulated by vessel diameter, which affects the resistance and hence the rate of blood flow through the tissue. The vessel diameter in turn is regulated by layers of smooth muscle cells wrapped around the arteries. Large arteries can be heavily innervated, but the degree of innervation becomes progressively less in smaller vessels (Olson 2011a).

The resistance is under the influence of two control systems; the remote system and the local system. The remote system includes the autonomic part (both the sympathetic and parasympathetic division) of the central nervous system and circulating catecholamines (CA; noradrenalin and adrenalin) released primarily from the chromaffin cells in the head kidney. Both components operate throughout the entire circulatory system and may affect blood flow through the heart (cardiac output), the gills as well as the arteries and some veins. In general, increased sympathetic activity will lead to increased blood flow and redistribution of blood from the intestinal tract to oxygen consuming tissues, including the red swimming muscles. This system is usually activated both during exercise and during hypoxia.

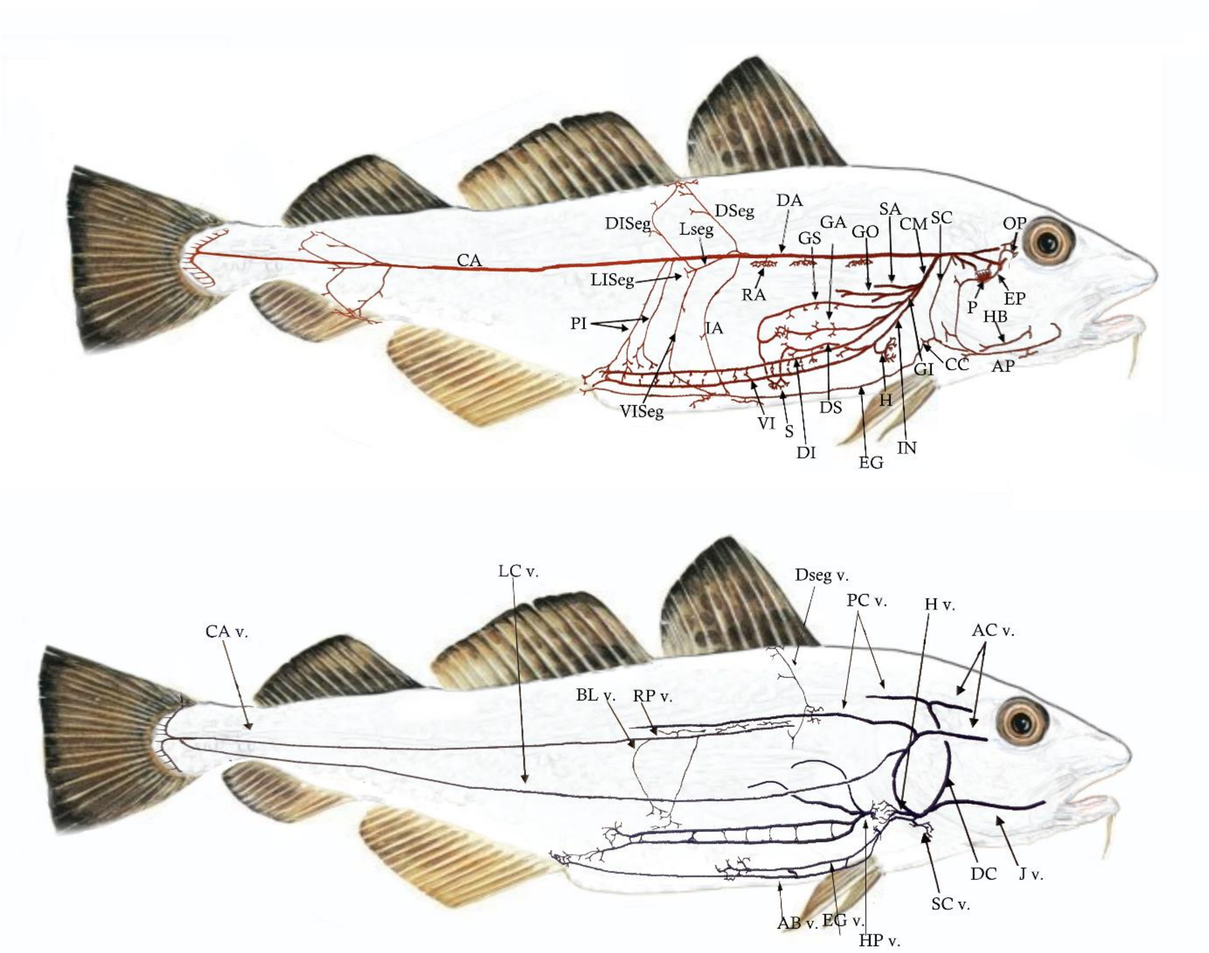

Figure 7. Major systemic arteries (top) and veins (bottom) in fish. AB v., abdominal vein; AC v., anterior cardinal vein; AP, afferent pseudobranch; CA, caudal artery; CA v., caudal vein (central vein); CM, celiacomesenteric artery; DA, dorsal aorta; DC, ductus cuvieri; DI, dorsal intestinal artery; DISeg, dorsal intersegmental; DS, duodenosplenic artery; Dseg v., dorsal segmental vein; EG v., epigastric vein; EG, epigastric artery; EP, efferent pseudbranch artery; GA, gastric artery; GI, gastrointestinal artery; GS, astrosplenic artery; H, hepatic artery; H v., hepatic vein, HB, hypobranchial artery; HP v., hepatic portal vein; IA, intercostal artery; IN, intestinal artery; J v., jugular vein; LC v., lateral cutaneous vein; LISeg, lateral intersegmental; Lseg, lateral segmental,; OP, ophthalmic artery; P, pseudobranch; PC v., posterior cardinal vein; RA, renal artery; RP v., renal portal vein; S, spleen; SA, swim bladder artery; SC, subclavian artery; SC v., subclavian vein; VI, ventral intestinal artery; VISeg, ventral intersegmental artery. Modified from Olson (2011a).

The local system, on the other hand, regulate blood flow locally in the peripheral tissue in response to production of metabolic waste products, which acts directly on smooth muscle cells in precapillary sphincters, causing vasodilation and increased blood flow in the tissue. This mechanism is referred to as local hyperemia and is important to ensure adequate oxygen supply locally in response to increased metabolism (e.g. muscle activity). Some of the putative vasodilatory agents include, but are not restricted to, H^+, CO_2, ATP, ADP, AMP and nitric oxide (NO) (Satchell 1991).

During sustained swimming, blood flow to the skeletal muscle of fish can increase four-fold due to local regulatory mechanisms, and blood is directed to the red muscles at the expense of the white muscles (Satchell 1991). At higher swimming speeds, blood flow to the gastrointestinal tract is reduced due to increased vascular resistance triggered by sympathetic activity (Axelsson and Fritsche 1991). The redistribution of blood from the gastrointestinal tract to the red muscles ensures that the heart can maintain supply of oxygen to the working muscles. Temperature and hypoxia will also affect vascular resistance in fish through local mechanisms. For example, a decrease in temperature will lower metabolism, thereby increasing resistance. Furthermore, stimulation of vascular smooth muscle cells by circulating catecholamines may be blocked locally by NO, which is synthesized by the endothelium and the perivascular nerves and is a potent dilator of fish blood vessels (Olson 2011b).

The stress response in fish

Capture and transport are acknowledged causes of acute stress in fish (Sampaio and Freire 2016). All stages of trawl capture, including exhaustive swimming during the initial stage of trawling, crowding in the cod-end during trawling and air exposure after the fish are landed on deck, have the potential to induce stress in fish. Hence, each stage of the trawling operation may be considered a stressor, which can potentially affect the physiology of the fish and eventually also the quality of the flesh.

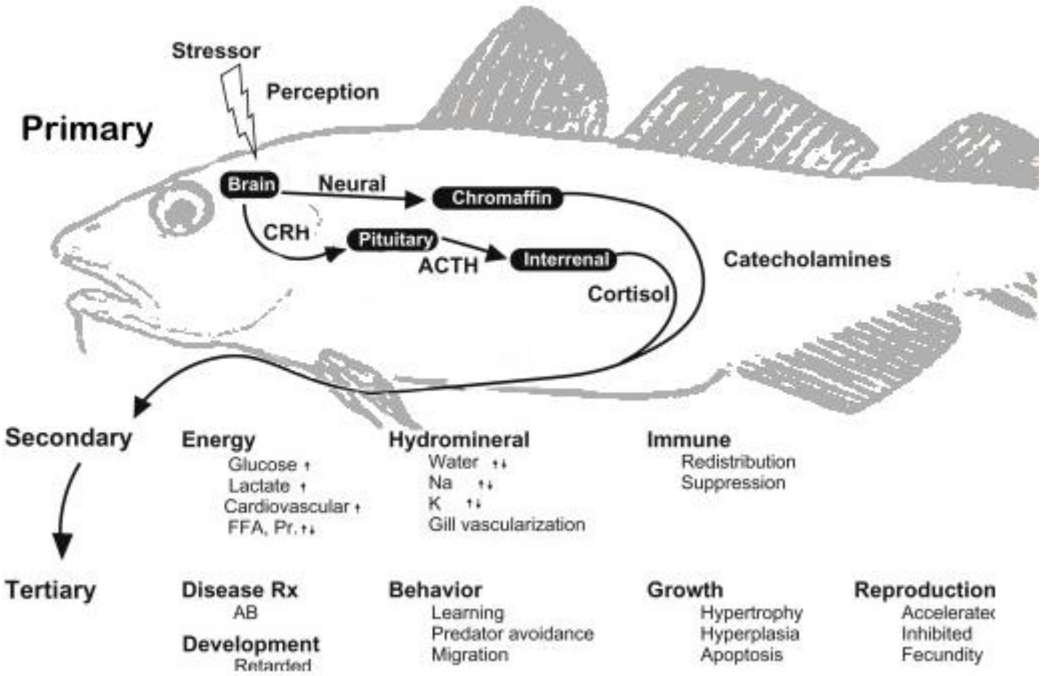

Figure 8. Overview over the primary, secondary and tertiary stress responses. CRH, Corticotropin releasing hormone, ACTH; Ardenocorticotropic hormone. Modified from Schreck and Tort (2016).

The term ‘stress’ is often a loosely used expression without consensus on its definition. Originally, stress was defined as the non-specific response of the body to any noxious stimuli (Selye 1950a). Later, this concept was revised and a distinction was made between a ‘stressor’ and a ‘stress response’. The stressor is the stimulus that jeopardize homeostasis and the stress response is how the organism copes with the stressor to regain or defend homeostasis (Chrousos 2009). The concept of homeostasis was first used to describe the physiological inner equilibrium (Cannon 1932) in terms of maintaining e.g. blood pressure, fluid volume, pH, salt concentrations etc. However, as nearly all activities of an organism relate directly or indirectly to the defence of homeostasis, the definition of stress as a threat to homeostasis seem illogical. For example, feeding will increase blood sugar and the mere action of waking up will cause a rise in level of the ‘stress hormone’ cortisol, but neither of these actions are considered stressors. Activities such as feeding and waking up are more or less predictable actions, and a more recent suggestion is that physiological stress is either the absence of an anticipatory response or a reduced recovery of the neuroendocrine reaction (Koolhaas et al. 2011).

Selye (1950b) made the distinction between primary, secondary and tertiary responses, collectively known as the General Adaptation Syndrome (GAS). The concept of GAS describes the overall stress response as a cascade of responses consisting of a primary response (alarm stage) which includes neural and endocrine responses, a secondary response (resistance stage) which covers changes in metabolic, respiratory, osmoregulatory, haematological and immunological responses, and a tertiary response in which the animal can no longer maintain homeostasis (Fig 8). The primary and secondary responses are considered adaptive, enabling the organism to mobilize sufficient energy to cope with the stressor, while the tertiary response is considered maladaptive leading to long-term detrimental effects. Behaviourally, the stress response triggers the organism to either move away from the stressor or to stay and fight the stressor, known as the ‘fight-or-flight’ reaction. Energy is attained via a set of catabolic reactions that brake down organic compounds such as carbohydrates, fats or protein in order to generate adenosine triphosphate (ATP) (Nelson 2011). ATP is a universal metabolic carrier of chemical bond potential energy and can be produced aerobically by mitochondrial oxidative phosphorylation and anaerobically via anaerobic glycolysis. To match the energy demand associated with stress, physiological mechanisms must be activated to ensure the availability of sufficient energy substrates. This is accomplished by a two-way communication between the central nervous system (CNS), the cardiovascular system, the immune system and other systems via neural and endocrine mechanisms that are responsible for increasing concentrations of circulating glucose and lipids.

Primary stress response

Briefly, the CNS perceives the stressor and CAs are secreted from chromaffin cells, which are located in the walls of the posterior cardinal vein with the highest concentration of cells found in the rostral region of the vein in the head kidney (Reid et al. 1998). Circulating CAs rises rapidly and act on the heart to increase both heart rate and stroke volume, as well as on blood vessels to alter resistance and blood flow. In the gills, CAs enhance O_2 uptake. Furthermore, CAs increases the blood oxygen transport capacity and causes the spleen to contract, thereby releasing more erythrocytes into the blood stream. This together with erythrocyte swelling increases the haematocrit in stressed fish (Reid and Perry 2003). CAs also have a direct effect on the swimming performance (Moon 2011), although the exact underlying mechanism for how CAs are involved in muscle contractions is unclear. In the liver, CAs stimulate gluconeogenesis and glycogenolysis, while in adipose tissue they stimulate lipolysis. Gluconeogenesis is the metabolic pathway responsible for glucose production from non-carbohydrate sources such as amino acids, whereas glycogenolysis is the production of glucose from glycogen.

Cortisol has a broad range of functions in fish and important target tissues are gills, intestine and liver. These organs reflect some of its major purposes, namely regulation of hydromineral balance and energy metabolism. In addition, cortisol has immune suppressive effects. Furthermore, cortisol may stimulate a proteolytic function in white muscle cells, and possibly also in the liver, which can fuel gluconeogenesis (Moon 2011). Cortisol is produced in the head kidney, more specifically in interrenal cells. The production and secretion cortisol is regulated via the brain-pituitary-interrenal axis (Fig 8). During acute stress, the concentration of plasma cortisol tends to increase rapidly, within a minute to an hour, followed by a gradual decrease to pre-stress levels within a day. Cortisol is perhaps the most commonly used indicator for stress in fish because basal levels are low (<5 ngL-1) for most fish species and usually increases by 10-100 folds during stressful situations, depending on the stressor and the species (Sopinka et al. 2016). Some of the critiques on using only cortisol as a stress indicator comes from the fact that circulating glucocorticoids respond rapidly (i.e. often within 3–5 min) to capture and handling (Romero and Reed 2005), and so it is often difficult to obtain baseline levels in wild animals.

Secondary stress response

The secondary stress response in fish involves metabolic changes such as increase in circulating glucose and lactate and decrease in tissue glycogen (Barton 2002), as well as osmoregulatory disturbances by altering levels of plasma chloride, plasma sodium and water balance. These changes are caused by the release of hormones during the primary stress response.

Glucocorticoids (primarily cortisol) also increases circulating levels of glucose by stimulating gluconeogenesis and glycogenolysis (Moon 2011). Glucose is an important oxidative substrate to many cells and tissues in fishes. In fishes, glucose is most likely subordinate to lipids and protein and blood glucose levels varies tremendously between fish species and even within species. The variations depend on life stage, temperature, feeding regimes etc., and baseline values of glucose can therefore be difficult to interpret. The role of glucose increases with stressful situations, where energy requirements are high and urgent, because glucose have the potential to create energy in the form of ATP quickly and in the absence of oxygen. Therefore, glucose can be useful for assessing the acute stress response to specific stressors (Sopinka et al. 2016).

There are also studies reporting that both cortisol and CAs may affect glycogenesis in fish skeletal muscle (Pagnotta and Milligan 1991, Girard et al. 1992, Milligan and Girard 1993). Glycogenesis is the process of glycogen synthesis, and production of glycogen from lactate is one is the main end-points of lactate in fish. The white muscles of rainbow trout can retain as much as 80-85% of the lactate produced during exercise (Milligan and Girard 1993) and this retention of lactate is stimulated by catecholamines (Wardle 1978). Lactate is an important metabolite in fish that serves as an oxidative fuel, a glycolytic end-product, a gluconeogenic precursor, and an intracellular signalling molecule, and can also be a useful stress indicator because the rate of appearance is faster than the rate of disposal during situations where ATP supply is limited, such as during stress (Sopinka et al. 2016).

Acute stress also has a pronounced effect on cardiovascular function and tends to cause an increase in heart rate and stroke volume. In addition CAs lead to a rise in blood haematocrit by causing erythrocytes to swell and increasing the number of red blood cells and reducing blood clotting time due to higher levels of circulating thrombocytes (Tavares-Dias et al. 2009). The circulating CAs also stimulate branchial blood flow and oxygen diffusing capacity and increased oxygen transport capacity of the blood. Furthermore, vascular resistance of the systemic blood vessels are affected by high levels of circulating CAs (Wendelaar Bonga 1997).

The primary and secondary stress responses are highly adaptive in fish, in terms of mobilizing and distributing energy, thus preparing the fish for fight or flight. However, it may influence the quality of final fish product because it changes the chemistry of the muscle tissue and may influence the efficiency of bleeding fish (Jørpeland et al. 2015, Olsen et al. 2013, Olsen et al. 2008, Digre et al. 2017).

Aims of the study

During trawl capture, fish are exposed to a number of stressors which may reduce the quality of the final product. These stressors involve swimming to exhaustion, crowding in the cod end, severe barotrauma, and lack of controlled killing and bleeding. Identifying or singling out the most important factors is very challenging on board trawlers, due to weather conditions, differences in haul size and duration. Therefore, the overall aim of this thesis is to isolate and experimentally test how exhaustive swimming, exhaustive swimming followed by crowding and traditional slaughtering techniques (asphyxiation) effects the physiology and the fillet quality of haddock and Atlantic cod, and to see how short-term recuperation affect these parameters.

General results and discussion

Trawlers land the largest quantum of cod and haddock in Norway. Understanding why quality from trawl-caught fish varies will have great ethical and financial impact on the fishery industry, as it sets the base for development of technologies to prevent or reverse impairment of fish quality.

Swimming

Capture of cod and haddock by trawl involves an initial phase where the fish swim in the net opening for some time before capture. The duration of this phase is dependent on towing speed, water temperature and condition of the fish (Winger et al. 2010). At some stage, the fish usually change its swimming behaviour from sustained to burst-and-glide swimming, which is an indication of a switch from the use of aerobic red muscles to anaerobic white muscles. During swimming, blood flow to the red muscles may increase four-fold (Satchell 1991). In Atlantic cod, exercise is reported to induce a decrease in the total vascular resistance and redistribution of blood from the intestinal tract to the other parts of the systemic vasculature (Axelsson and Fritsche 1991). This can be explained by hyperaemia in the working muscles, due to release of metabolites, which leads to increased blood flow to the muscles. We therefore hypothesized that an increase in blood flow to the working muscles could lead to deposition of residual blood and hence an increase in fillet redness after exhaustive exercise. Furthermore, Olsen et al., (2013) observed an increase in fillet redness after 3 hours of recuperation from trawl capture. Blood flow to both white and red muscles have been documented to increase after muscular activity (Neumann et al. 1983), and therefore we speculated whether short-term recuperation would reduce residual blood in fillets. We addressed these issues by using a large-scale swim tunnel to physically exhaust haddock (Paper I) and Atlantic cod (Paper II and III), followed by recuperation for 0, 3 or 6 hours (paper I) or 0, 2, 4, 6 or 10 hours (paper II).

When comparing the swimming experiments of haddock (paper I) and cod (paper II) to studies done on commercial trawlers (Digre et al. 2010, Olsen et al. 2013) it was clear that the changes we found in muscle pH, blood lactate and fillet redness were less pronounced than had been observed previously. This indicates that the exhaustive swimming procedure we used should probably be considered a moderate stressor. Yet, the observed swimming behaviour in the experimental set-up with kick and glide just before the fish ceased swimming, were similar to that observed in the net opening of a trawl (Beamish 1969).

We further concluded that exhausted swimming is not the main cause of reduced quality in terms of fillet colour. However, exhaustive swimming did have a significant effect on time and strength of post-mortem muscle stiffness. We found the time it took for haddock to reach maximum muscle stiffness was significantly reduced (paper I) and that swum cod had a higher maximum muscle stiffness during *rigor mortis* than rested cod (paper II). This indicates that, although swimming is not a severe stressor, it reduces the white muscle stores of glycogen, thereby causing a faster and stronger onset of *rigor mortis* (Stroud 1969). This effect was reversed sometime between 0 and 3 hours of recuperation for haddock (paper I) and 0 and 2 hours for cod (paper II), indicating that muscle energy stores are beginning to recover. However, a full recovery may take up to 12 hours (Kieffer 2000), as indicated by elevated blood glucose levels throughout the whole recovery period (6 hours for haddock, paper I) and (10 hours for cod, paper II).

Although exercise-induced hyperaemia may result in increased blood flow to the working muscles, we did not detect any increase in fillet redness for either haddock (paper I) or cod (paper II and III). It seems therefore that the amount of blood entering the working muscles during exercise is removed at the same rate as it appears. We concluded that exhaustive exercise probably has an effect on the textural quality of the fillets of both cod and haddock, but no effect on visual residual blood in the muscle. Hence, swimming in front of the net is not the major source of the quality impairment frequently seen in trawl-captured cod and haddock.

Crowding

In paper I and II, we concluded that the stress associated with exhaustive swimming had a low overall impact on fillet quality. Several studies have reported a relationship between the duration of trawl hauls and the frequency of fillet quality defects, and literature suggests that crowding may trigger a severe stress response in fish (Bahuaud et al. 2010, Lerfall et al. 2015, Montero et al. 1999, Ortuno et al. 2001, Pickering and Stewart 1984, Skjervold et al. 2001, Tort et al. 1996, Wedemeyer 1976). There is, however, little information on the timing of the stress response associated with the ‘crowding’ stage of the trawling operation, and we therefore addressed this issue by swimming commercially sized cod to exhaustion followed by extreme crowding (736 ± 50 kg m^3) of the fish for 0, 1 or 3 hours in an experimental cod-end. The fish were then allowed to recuperate for 0, 3 or 6 hours in a net pen prior to slaughter, in order to assess if any potential impairment of fillet quality could be reversed within a reasonable timeframe (Olsen et al. 2013).

We found that exhaustive swimming combined with crowding was associated with a marked metabolic stress response, as indicated by high levels of plasma cortisol, blood lactate and blood haematocrit levels, as well as a reduction of fillet quality in terms of increased visual redness and a drop in muscle pH. The severity of the metabolic stress response, as judged from the metabolic markers, was comparable to that reported for lengthy (>5 hrs) and large (>20 tons) trawl hauls (Olsen et al. 2013) . Furthermore, the evaluation of fillet redness presented in paper III indicated that the fillets of our fish were assessed as having even more red colouration than fillets of commercially caught fish (Fig 9).

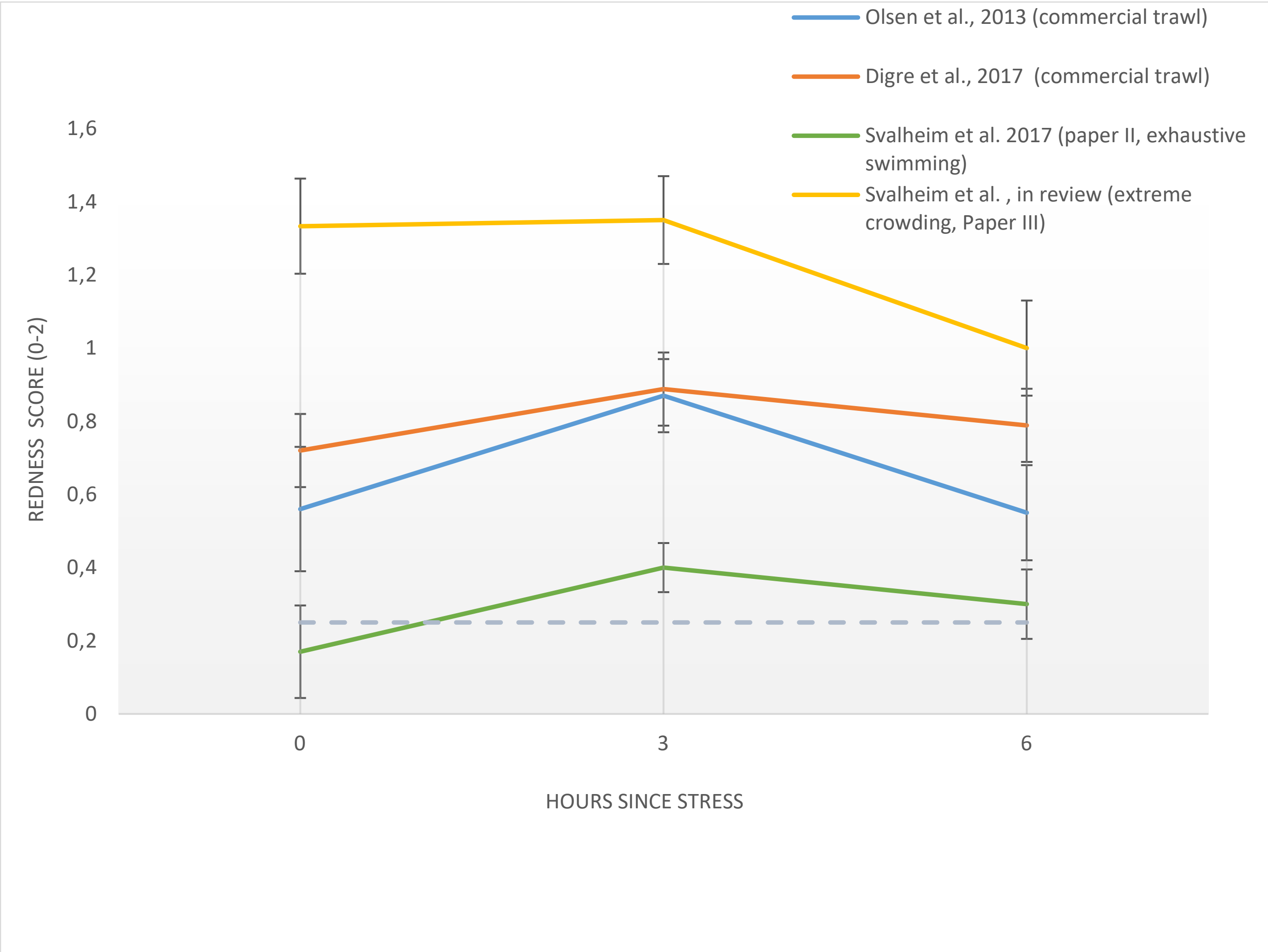

Figure 9. Comparison of five different experiments with sensory evaluation of fillet redness. All numbers have been transformed to the same scale (0-2), 0 being perfectly white, 2 being very red and blood filled. Stress in this setting is defined as either exhaustive exercise, exhaustive exercise followed by crowding or commercial trawl capture.

However, this result must be interpreted with caution, as the sensory evaluation of redness may be better suited for evaluating relative differences within the same experiment, rather than making comparisons across experiments.

Short-term recuperation only had positive effects on quality in terms of time to maximum muscle stiffness (paper I), the level of maximum muscle stiffness (paper II) in the process of *rigor mortis* and increase in muscle pH (paper III), but did not have a large impact on quality in terms of fillet redness (paper III). Studies conducted on board commercial trawlers, found that fillet redness tended to increase during the first 3 hours of recuperation after capture and then decreased to at-capture levels after 6 hours (Olsen et al. 2013, Digre et al. 2017) (Fig 9). Notably, no such increase was found in experimental setting (paper III), where redness remained unchanged throughout the recuperation period. Based on these findings, recuperation for 6 hours in our experiments had little effect on quality in terms of fillet redness.

Air exposure

On board trawlers, the final phase of the trawling operation include hauling the catch from the water and on-board the fishing vessel, where the catch is usually stored dry in bins until further processing or exsanguination. During this stage, the fish is normally exposed to air for some time prior to exsanguination, so they become asphyxiated and easier to handle in terms of bleeding (Van De Vis et al., 2003). In paper IV we investigated the effects of asphyxiation on stress parameters (muscle and blood pH, lactate and glucose) and fillet quality in terms of amount of haemoglobin in muscle of cod.

As clearly stated in paper III, and previously shown in studies on board commercial trawlers, the trawling operation represents a strong overall stressor that affects the fillet quality of cod. We therefore hypothesized that the accumulation of stress throughout the trawl capture, from swimming to crowding to air exposure, is the major cause of variable quality of trawl captured gadoids. The last paper of this thesis (paper IV) investigated how pre-mortem stress by mild crowding followed by air exposure for 0, 15 or 30 minutes effected metabolic stress parameters, blood coagulation time and muscle haemoglobin concentrations (as a measure of residual blood in the fillet).

As in paper III, paper IV also showed that stress from crowding resulted in increased levels of muscle haemoglobin in the fillets. Furthermore, we found that air exposure had an additional negative effect on fillet quality, which was stronger when the fish were crowded prior to air exposure. However, by euthanising the fish before air exposure, the accumulation of residual blood was delayed. Interestingly, crowding for four hours did not cause a significant increase in lactate or pH. Nevertheless, we did find significantly higher concentrations of haemoglobin in muscles of crowded individuals, indicating that ‘mild’ crowding may already affect the quality of the fish in terms of residual blood in the fillet.

Previous recommendation states that fish should be bled within 30 minutes of slaughter. However, the study asserting that recommendation is done on unstressed fish. In our study, we found that the effect of crowding on haemoglobin concentration in the muscles masked the effect of bleeding fish. This suggests that the practice of leaving fish in air may be detrimental to the fillet quality and should be avoided.

Why is crowding important for fillet redness?

It was surprising to us that mild to moderate stress from exercise (paper I and II) did not cause any increase in muscles redness, whereas stress from mild crowding (paper IV) did. It appears as if the amount residual blood in fillets is dependent on whether stress is induced from exercise or crowding. It should be noted that the evaluation of residual blood in these three papers is not the same; sensory evaluation in paper I and II versus VIS/NIR spectroscopy in paper and IV. In paper III, we tested the correlation between spectroscopy and sensory evaluation of fillet redness and found a significant positive correlation between fillet redness and muscle haemoglobin (Fig 10). Furthermore, both the spectroscopic and the sensory evaluation in paper III indicated that swimming did not cause an increase in redness, whereas crowding did. It is therefore worth speculating on why crowding appears to have the highest impact on fillet redness, even when it is done in such a way that it does not induce a severe stress response.

It is tempting to assume that the fillet redness is directly linked to the degree of stress. In paper III we found a positive correlation between cortisol and fillet redness. However, a correlation is not the same as causality and, in paper II the increase in cortisol was not linked to the increase in redness. Furthermore, metabolic stress parameters such as glucose, lactate and pH measured in crowded fish in paper IV indicated that the fish were only mildly stressed, yet haemoglobin concentration in the muscles was significantly increased.

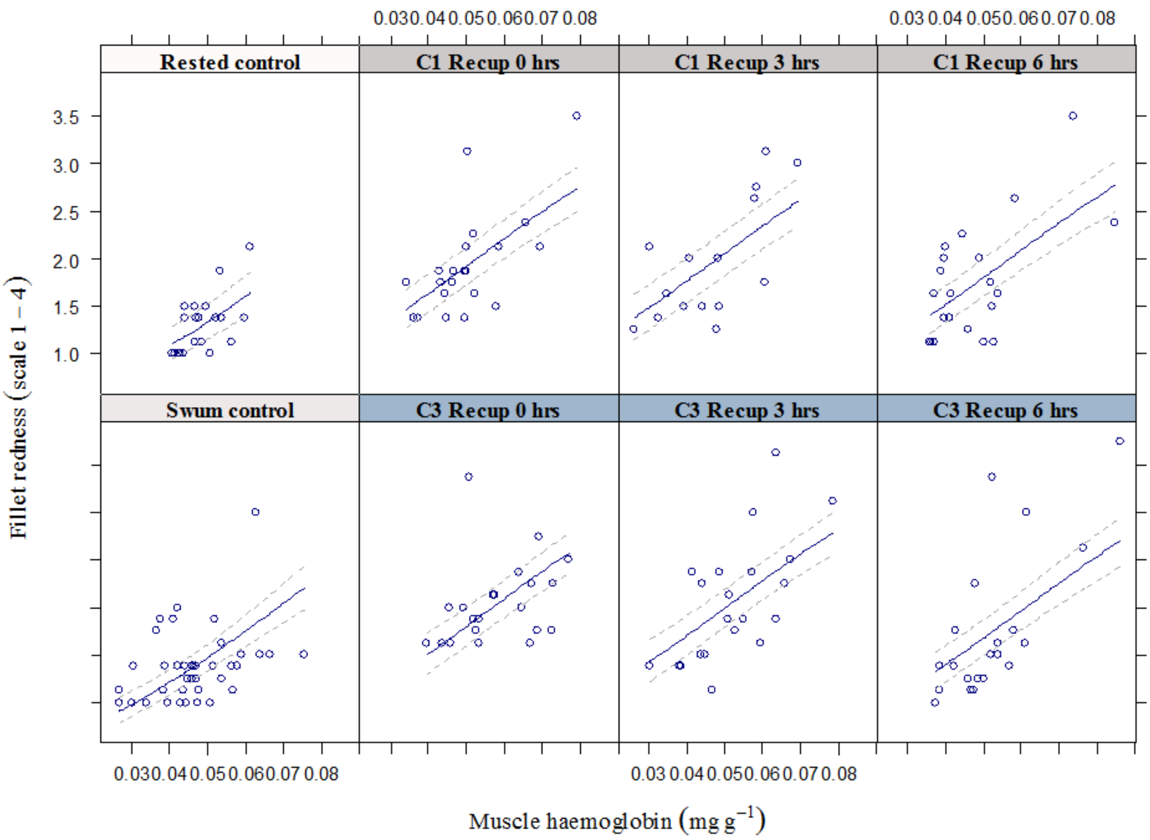

Figure 10. Correlation between filet redness and muscle haemoglobin. Continuous lines are trend lines estimated from the GLM and dotted lines indicate 95% confidence intervals (Paper III).

Previous studies have shown that blood is redistributed to white muscles during swimming (Neumann et al. 1983). However, as discussed in paper I and II, no increase in visual redness was found in fillets of cod or haddock during exercise, whereas crowded fish (paper III and paper IV) were evaluated as having a greater red colouration than swum or rested fish. It has been suggested that fillet redness is due to increased blood flow to the muscles to flush out waste products and protect against acidosis (Olsen et al. 2013). But, this does not explain why there is a difference between exhaustive exercise and crowding in terms of redness. It is interesting to speculate on some possible contributing reasons for increased fillet redness and why it occurs in crowded, but not exercising fish.

As previously mentioned, hyperaemia is the process by which the body adjusts blood flow to meet the metabolic needs of its different tissues (Satchell 1991). Functional, or active, hyperaemia leading to increased blood flow, is mediated by a rise in vasodilatory agents during periods of increased cellular metabolism. A possible explanation for the difference in residual blood content of white muscle in exercised, compared to exercised and crowded fish, is that accumulation of residual blood in the crowded fish is caused by insufficient emptying/return of venous blood from the segmental veins, due to impaired movement of the swimming muscles. The venous blood from the swimming muscles of fish is passed to the central veins via the segmental veins and depends on the alternating movement of the lateral muscles, which squeezes the blood out of the segmental veins and into the central veins when the ipsilateral muscles contract. The segmental veins, in turn, are guarded by ostial valves (Fig 11), which prevents backflow of blood when the ipsilateral muscles relax. Hence, return of venous blood from the swimming muscles is dependent on the continuous, alternating sideways muscular movement of the body of the fish. This mechanism is similar to the muscular pump of the lower limb in humans (Ludbrook 1966), which also depend on muscle contraction to facilitate the return of venous blood to the heart. It is possible therefore that in situations where the alternating contraction/relaxation of the swimming muscles is blocked, e.g. when the fish is tightly packed in the cod end (paper III), that the return of the venous blood from the swimming muscles is impaired, resulting in accumulation of blood in the muscles. This may also be the case during recuperation in the crowding experiment (paper III), where the fish laid still on the bottom of the cage during the first few hours of hours of recuperation (unpublished observations). Interestingly, the fish in this experiment began to swim slowly sometime between 3 and 6 hours of recuperation, corresponding to the time when fillet redness began to decrease.

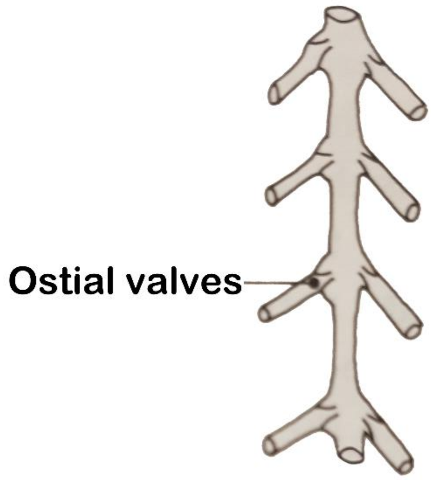

Figure 11. Location of the ostial valve on a large longitudinal (central) vein.

Conclusions

Together these four studies show that there is a connection between type of stressor affecting the fish during capture and the quality of the fish product (fillets) in terms of colour. Crowding and air-exposure cause an increase in fillet redness and concentration of haemoglobin in the muscle that was not seen for exercised fish. It is suggested that the accumulation of residual blood in the white muscles of crowded fish may be the result of insufficient emptying of segmental veins due to the static condition of the muscles during crowding. Measures that may secure top quality fish from trawlers, include reducing crowding in the cod-end and implementing direct slaughter. Recuperation may have beneficial effects on fillet quality by reducing air exposure time and gaining control over slaughter time. However, in terms of fillet colour, recuperation must be longer than 6 hours.

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Paper I

Paper II

Paper III

Paper IV