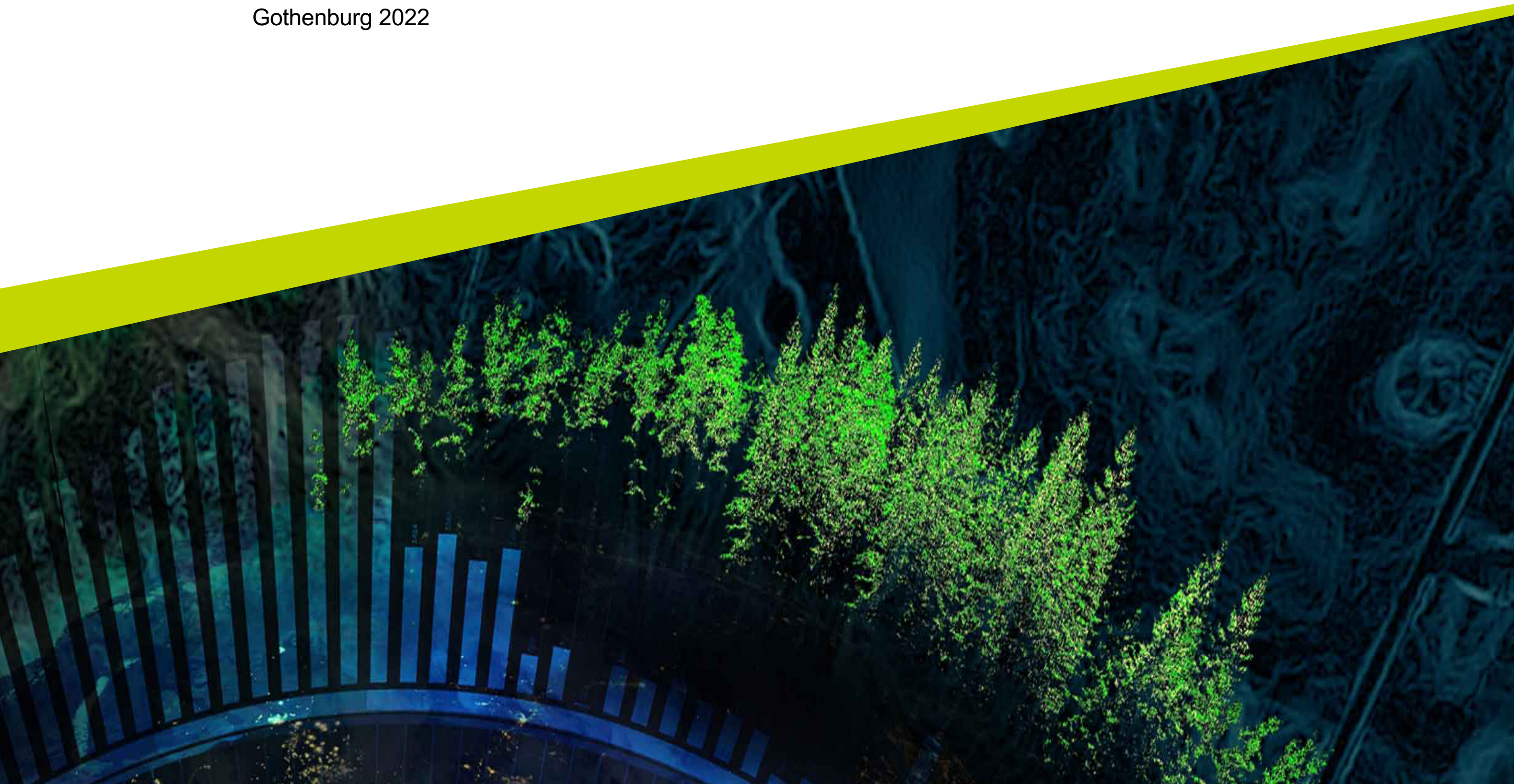
Assessment of Fish Welfare in Trawl Caught Atlantic Herring

Mafalda de Leitão Tomás

Independent project • 60 credits
Swedish University of Agricultural Sciences, SLU
Department of Aquatic Resources
NJ faculty
Master program in Biology
Gothenburg 2022



Assessment of fish welfare in trawl caught Atlantic Herring

Mafalda de Leitão Tomás

Supervisor:	**Albin Gräns, Swedish University of Agricultural Sciences, Animal Environment and Health**
Assistant supervisor:	Jane Behrens, Technical University of Denmark, DTU AQUA
Examiner:	Erik Petersson, Swedish University of Agricultural Sciences, Aquatic Resources

Credits:	60 hp
Level:	Advanced level, A2E
Course title:	**Independent project in biology**
Course code:	**Ex0900**
Programme/education:	**Master program in Biology**
Course coordinating dept:	**Department of Aquatic Sciences and Assessment**
Place of publication:	**Gothenburg**
Year of publication:	**2022**
Copyright:	All featured images are used with permission from the copyright owner.

Keywords:	Welfare, Pelagic Fisheries, *Clupea harengus,* Stress

Swedish University of Agricultural Sciences
Department of Aquatic Resources
NJ Faculty

Abstract

With the gain of scientific evidence concerning nociception in fish, the awareness about fish's welfare has been increasing not only for authorities but also for consumers. However, there is still a long way to go concerning how fish are being caught and slaughtered. In terms of welfare, fisheries are without any doubt a source of both stress and pain.

Trawling has proved to be the most stressful fishing method; nevertheless, little has been done to try to minimise or better avoid such stress. This study's goal was to assess the welfare of pelagic fisheries using Atlantic herring (*Clupea harengus*) as a model. This study stands out from others because it follows the fish from the minute it is pumped until they are dead. Two research trips were made, one onboard a commercial trawler and the other on a vessel that uses a hook in line as its fishing gear. Each captured individual went through a series of tests. First, consciousness assessments and morphological injuries and then blood sampling to get the physiological indicators of stress and x-ray to check for internal damage.

Overall, individuals captured by a commercial trawler present higher mortality and extreme stress levels when compared with the ones caught by hook in line. However, the morphological assessment showed that all the individuals caught during the first research trip appeared to be fine apart from the scale loss. Therefore, based on the results of this study, it is possible to see that the welfare of fish is highly compromised during pelagic fisheries. However, it is hard to pinpoint the exact cause of death. Most likely, the individuals died due to a combination of factors, where osmotic imbalance due to the impairment of the skin and the overcrowding at the cod end are believed to be the most significant ones.

Keywords: Welfare, Pelagic Fisheries, *Clupea harengus,* Stress

Table of contents

List of tables

List of figures

Abbreviations

DTI	Danish Technological Institute
DTU	Technical University of Denmark
FAO	Food and Agriculture Organization
GAS	General Adaptation Syndrome
HPI	Hypothalamic-Pituitary-Interrenal
RAMP	Reflex Action Mortality Predictors
ROA	Rhythmic Opercular Activity
RTS	Response to Tactile Stimuli
RSW	Refrigerated Seawater System
SLU	Swedish University of Agricultural Sciences
VOR	Vestibular Ocular Response

1. Introduction

Humans have been interacting with fishes in various ways, many of which present serious welfare hazards to the fish (Huntingford *et al.*, 2006). Fishes constitute a significant source of protein for the human population. Nevertheless, wild populations are incapable of coping with the high demand for fish which has led to many fish stocks being severely overfished. To meet the high demand, approximately half of the fish consumed by humans today are farmed, and aquaculture is expected to increase even further in the upcoming years. Approximately 0.80 to 2.5 trillion fish are killed in commercial fisheries and 50 to 200 billion are slaughtered in fish farms (Mood, 2010; FishCount, 2020; FAO, 2020).Both contexts carry significant ethical and welfare matters, the reason why to this time there has been an increase interest in fish welfare. One of the matters would be how stressful different forms of humans-fish interactions are and if there is, any possible way to minimize the stress inflicted on the fish. The limited knowledge on fisheries welfare when compared with farmed individuals makes one wonder and try to dig deeper. Pelagic fisheries are widely spread and responsible for approximately 30% of the annual catches (Stephenson and Smedbol, 2019). They have been known to inflict high levels of stress. This information combined with those high percentages makes it an area of great concern and importance for fisheries welfare.

1.1 Stress Response in Fish

The term 'stress' tends to be used with no agreement on its definition. It first started as a response to any harmful stimuli (Selye, 1950a), however, after reconsideration, a distinction was made, and two new concepts appeared: 'stressor' and 'stress response'. A stressor is a stimulus that risks perturbation of homeostasis, while the stress response is how the individual deals with such stressor to recover or protect homeostasis (Chrousos, 2009). According to Cannon, (1932) the term homeostasis was first defined as an internal physiological equilibrium. Later, Sapolsky, (2004) changed the concept of homeostasis to allostasis. The new and more relaxed notion suggests "stability through change", where throughout distinct life stages, the internal balance adjusts itself to distinct necessities. Undoubtedly, stress is related to welfare, and Broom, (2007) stated that flawed welfare starts when mechanisms fail to adapt the individual to the surrounding environment. When considering the capture process in pelagic fisheries there's a clear disturbance of the individuals' necessities that will inflict stress on fishes. It is then crucial to minimize the stressful procedure to ensure fish's welfare.

1.1.1 Three Stress Responses

Selye (1950b) was the first to distinguish three different responses: the primary, secondary and tertiary response, together also known as the General Adaptation Syndrome (GAS). The principle behind these stages in fish is identical to other vertebrates (Barton, 2002; Iwama, Afonso and Vijayan, 2006). The primary response works as an alarm call involving the activation of the hypothalamus-sympathetic-chromaffin cell axis, responsible for catecholamines (noradrenaline and adrenaline) production, and the hypothalamic-pituitary-interrenal tissue (HPI) axis, responsible for corticosteroids (cortisol) production (Nelson and Chabot, 2011). The second response is the resistance stage, where there are behavioural and physiological adaptations to the stressful circumstance. Several metabolic pathways are triggered, causing shifts in respiration, osmoregulation, immune and cellular response, and blood chemistry (Barton, 2002; Iwama, Afonso and Vijayan, 2006). Finally, the tertiary response happens when the individual cannot maintain homeostasis. Here the individuals' resistance is highly compromised, leading to immune suppression and high mortality (Broom and Johnson, 1993). Both primary and secondary responses are adaptive, allowing the individual to gather all the energy to defend itself against the stressor, while the tertiary response leads to damaging effects, such as diseases, changes in growth, variations in reproduction, also compromises the survival of the individual (Iwama, Afonso and Vijayan, 2006; Weber Iii, 2011).

1.1.2 Assessing Stress in Fish

Although the tertiary response is the most aversive to animal welfare, efforts are usually made to prevent these aversive effects from occurring. Preventing stress from becoming distressed and poor welfare. Therefore, it is quite common to mainly focus on some of the primary and secondary stress responses when assessing animal welfare (Sopinka *et al.*, 2016). For the primary response, there is cortisol. Cortisol is the main corticosteroid hormone in fish, a stress hormone that is released into the individual's bloodstream (Donaldson and Fagerlund, 1968) and has been used as a primary stress response measure due to its unquestionable link to fish welfare (North *et al.*, 2006; Varsamos *et al.*, 2006). Plasma cortisol concentration increases rather drastically during and following stressful situations. Cortisol is one of the most commonly used indicators to estimate stress in fish. In most investigated fishes the levels of unstressed fish are low (< 5 ng L^{-1}) and progress by 10 to 100 folds during stress (Iwama, Afonso and Vijayan, 2006). Plasma levels of cortisol start to increase within minutes and normally reach a peak after 2 to 24 hours depending on the situation and the type of stressor (Mommsen, Vijayan and Moon, 1999). Stress can be quantified with the direct relation between stress intensity and cortisol concentration. Higher concentrations indicate stressful perturbations (Davis, 2006).

As for the secondary response, there are metabolic changes such as increases in lactate and glucose, decreases in glycogen found in the tissue and variations in ion levels (Barton, 2002; Iwama, Afonso and Vijayan, 2006; Cook *et al.*, 2011; Weber Iii, 2011). Lactate is a product of glycolysis that occurs when there is not enough oxygen for aerobic metabolism, for example during intense periods of physical response or in waters with low oxygen levels (Milligan and Girard, 1993; Barton, 2002).Like cortisol, lactate gradually increases after a stressful period and after the peak presents a long recovery time (Hatløy, 2015).Another easily measured stress indicator in fish is changes in plasma osmolality. Osmolality in saltwater fish varies from 300 to 350 mOsm per kg H2O, while for freshwater fish it varies from 230 to 280 mOsm per kg H2O (Freire and Prodocimo, 2007). To function correctly, any fish in saltwater needs to maintain an internal osmolality of about one-third of seawater (Soengas et al., 2018).There is a constant loss of water to the surrounding via osmosis, a passive water exchange process mainly done through the gills to maintain such balance. Fish, being osmoregulators, need to invest a lot of their metabolic energy, maintaining the equilibrium of their medium. Therefore, osmolality has been considered one of the primary parameters to estimate the effects of stress on homeostasis. Furthermore, as the effects on plasma osmolality cortisol and lactate are all closely related, any variation in osmolality - quantifies the dissolved particles existing in a liquid (Evans, 2009), and ion concentration (*e.g.,* chloride, calcium, potassium, magnesium) can be used to quantify the secondary response in fish, especially saltwater (Veiseth et al., 2006).

1.1.3 Stress Management in Fish

Historically there has been a large difference in how stress has been managed in aquaculture compared to fisheries. Many aquaculture practices have been developed to minimize stress and safeguard fish welfare as they are all related to aversive and costly tertiary responses to stress. Consequently, in aquaculture it is generally accepted that it is possible to improve fish welfare while still increasing both products' economic benefits (FAO, 2021). However, the situation is somewhat contrasting for commercial fisheries where humans only affect fish welfare during the fish's final stage, where tertiary stress responses will, if at all, only have moderate effects on the economy of the operation. In addition, the lack of profitable, welfare-aware fishing methods makes it harder to have any legal requirements to safeguard fish's welfare (Jennings *et al.*, 2016). However, with growing evidence of fish being sentience beings with the capacity to feel pain and the increasing consumer awareness regarding the importance of fish welfare, the ethical considerations have now been extended in order to minimise stress and pain in fish also when caught by commercial fisheries.

1.2 Pain in Fish

Whether or not fish are capable of feeling pain has been a long-lived debate (Huntingford *et al.*, 2006; Braithwaite, 2010; Sneddon, 2019). Pain is defined as an unpleasant emotional and receptive event associated with harmful or possibly harmful tissue damage (IASP, 2021). While pain assessments in humans can be done via personal communication, there is a need to rely on both physiological and behavioural responses to pain in animals (Rose *et al.*, 2014; Sneddon *et al.*, 2014, 2018). Even though there is this continuing discussion (Sneddon *et al.*, 2018) convincing evidence indicates that fish feel pain in a similar way as other vertebrates, including humans (Sneddon, 2015; Woodruff, 2018).

In the early 2000s, with the discovery of nociceptors (i.e., nerve endings that recognise harmful stimuli that result in tissue damage) in rainbow trout (Sneddon, Braithwaite and Gentle, 2003), the debatable topic of whether fish feel pain took a turn. Like stress pain started to be associated with welfare. The question was no longer whether fishes could detect painful stimuli but rather how they respond to them and if they cause any unpleasant emotions for fish (Rose *et al.*, 2014). When assessing pain, the observed behavioural responses are divided into two primary parts 1) the simple reflex, in which the individual moves away from the unpleasant sensation and 2) the understanding of the situation, where the individual recognizes the painful sensation and shows an adaptive behaviour, avoiding it. As Broom (2001) mentioned it is not worth it to feel the painful stimuli without learning to stay away from it. Following the discovery of nociceptors in fish there have been significant efforts to better understand fish pain and how it can be minimized or better, avoided. This topic is now step by step entering commercial fisheries a place where trillions of animals are affected. Now, there will be room for improvements that might bring not only ethical but also economic benefits (Savina et al., 2016).

1.3 How's Fish Being Caught and Killed by Humans?

One important issue of concern with wild fish in relation to welfare is how they are being caught and killed. The majority of fish are captured by commercial vessels, which is a multi-billion-dollar industry with major socio-economic effects both locally and on a global scale (Raby *et al.*, 2014; Pauly and Zeller, 2016). However, there are always implications in how fish welfare is handled depending on their value to humans as both employment and food sources. FAO (2020), in their *State of World Fisheries and Aquaculture* evaluated that more than 85% of wild-caught fish went for human consumption while the remaining percentage were used for non-food uses such as fish oil or fishmeal. Additionally, it was estimated that in 2018 more than 38 million people were employed in the fisheries sector and a lot more engaged in the trade, processing, and retail sectors, thus stressing the socio-economic importance of fisheries.

Fisheries were industrialized when Englishmen started working with steam trawlers in the nineteen hundreds. After World War II, the power of the English fleet grew bigger with diesel motors, radar, and acoustic finders (Cushing, 1987; Jennings *et al.*, 2001). These improvements allowed higher catches to respond to the fish demand and precision while locating the fleet. Improved fleets also meant new scientific discoveries. The new technology allowed for the study of population and mathematical modelling, altering the concept of fisheries science and giving humans a better understanding of not only the fish but also their environment (Finley, 2016). Today a wide variety of fishing gears are being used depending on what species are targeted and the fishing environment (Cashion *et al.*, 2018). Fishing gears are generally divided into two main categories depending on the mechanism: the passive gears where the fishes are attracted to the gear (*e.g.*, simple handlines, nets and traps), and active gears when the gear pursuits the target species- such as mechanically operated trawls and large purse seines (Nédélec and Prado, 1990; Jennings *et al.*, 2001). From a welfare perspective, it is important to understand both the internal and external damage that different gears might have on the fish (Noble et al., 2020). Trawls are responsible for 30% of the annual catches (Stephenson and Smedbol, 2019), making it one of the most important fishing gears for humans but also one of the most critical for the fish, with high levels of morphological impairment and mortality but also for their habitat.

1.3.1 Trawling

Trawling has been known to trigger a series of behavioural responses of the targeted fish (Gregory, 1998; Benoît, Hurlbut and Chassé, 2010; Winger, Eayrs and Glass, 2010). Furthermore, if the gear is towed on or close to the seabed it can disturb not only the resident species but also the sediment. The ecological damages may *e.g.,* include destruction of habitat complexity, resuspension of sediments, bycatch, and changes in the food web (Dayton *et al.*, 1995; Jennings and Kaiser, 1998; Kaiser and de Groot, 2000). However, even with all the discussion of the negative effects related to trawling the welfare of the caught fish is often forgotten.

Trawling can be done midwaters (pelagic) or at the bottom (demersal). The trawl itself is a funnel-shaped net with a wide opening that narrows down to the cod end, which is a meshed bag where the fish gets trapped (Sistiaga *et al.*, 2015; Joseph, Shipley and Siskey, 2019). Pelagic trawls are normally larger than bottom trawls. They are designed to target species that inhabit the mid-water column and sometimes also surface species such as herring, mackerel, pilchard, and blue grenadier (Joseph, Shipley and Siskey, 2019). Even though this type of trawling does not impact the seabed, bycatch of non-target species may in some cases be problematic (Davies *et al.*, 2009; Crespo and Dunn, 2017).

Regular towing speeds go from 1 to 7 knots, depending on both the fishing location and the target species. Such pursuing speeds are likely to exceed the swimming speed of the target species, getting the fish fatigated or exhausted when entering the cod end (He, 1991; Breen and Pedersen, 2012). The amount of time that the fish keeps on swimming depends on several factors. Not only size and length influence the swimming (Suuronen, Lehtonen and Jounela, 2005) but also individuals' physiological condition when entering the net (Winger, Eayrs and Glass, 2010).

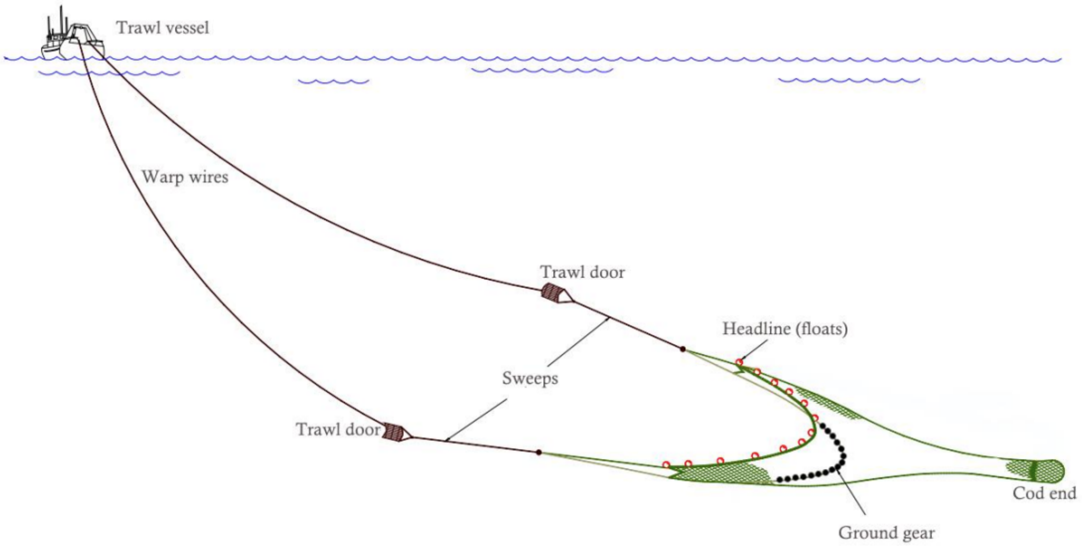


Figure 1: Illustrative representation of a bottom trawl (Svalheim, 2018).

1.3.2 Post Capture

From an animal welfare perspective, a quick and painless loss of consciousness after being caught is a criterion for human killing. Nevertheless, during larges catches, this technique can be challenging. Hence, fish are often left air-exposed on deck, leading to asphyxiation, before exsanguination, making them easier to handle (van de Vis et al., 2003).Asphyxiation is described as an extended time of suffocation prior to death, and likely involves long periods of stress. This time depends on several aspects, such as temperatures and the resistance of the targeted species (Poli *et al.*, 2005). Ice slurries besides being a preservation method are also a common way of killing, where after capture, the fish goes directly to tanks filled with cold water or ice slurry. In this case, there is a decrease in fish body temperature that will slow down the individual's metabolism and decrease oxygen consumption leading to extended periods until death (Robb and Kestin SC, 2002; Poli *et al.*, 2005).

During the 90s refrigerated seawater systems (RSW) were gradually introduced into commercial vessels, ensuring the preservation of big catches with a rapid cooling process to temperatures that allow storage onboard without compromising fish quality (Bell, 1984). Keeping the captured individuals' alive increases shelf life and allows for better control when slaughtering. Even with some debate, it is believed to be a method with space for improvement in fish welfare. Experimental studies showed that refrigerated systems are less stressful than the common method of air asphyxiation with lower plasma cortisol and lactate. Additionally, the quick reduction of body temperature improved product quality and shelf life (Robb and Kestin SC, 2002; van de Vis *et al.*, 2003; Poli *et al.*, 2005; Erikson, Hultmann and Erik Steen, 2006). Throughout the years, the system has been optimized. The system goes as follows: pumps recirculate seawater through tanks and the cooling system. The seawater is cooled down before being evenly distributed to the tanks. The cold water goes upwards into both the tank and the fish, allowing them to float while still cooling it. Suggestively, RSW with both oxygen and carbon dioxide is a less stressful method than the traditionally used ice slurries and carbon dioxide tanks. Although this might work in Norwegian salmon farms, there's still a long way to go for commercial fisheries, most likely due to their limitations (Erikson, Hultmann and Erik Steen, 2006). In fisheries, it's impossible to get the precise weight that farms get and there's always variation between species. High reformation of the fleet would be needed, which would be costly and not all vessels have the capacity for both oxygen and carbon dioxide distribution.

1.4 Aims of the Study

This present study aimed to assess the effects of trawling on the welfare of fish, using wild Atlantic herring caught in Danish pelagic fisheries as a model setup. Herring are found both in North Pacific (*Clupea pallasii Val.)* and North Atlantic (*Clupea harengus L.*), where each species can be further divided depending on spawning periods, their migratory behaviour and geographical dispersal (Hay *et al.*, 2001; Stephenson *et al.*, 2001; Power M. J. *et al.*, 2007). Herring are captured both for human consumption and to produce fish meal and oil for animal feeds. For centuries, Atlantic herring has been an essential source of nutrition and economic income in countries surrounding the North Atlantic (Alder *et al.*, 2008; Pikitch *et al.*, 2014; í Kongsstovu *et al.*, 2022). According to NOAA Fisheries (2022), in 2020, more than 9 million kilograms of Atlantic Herring were captured, indicating how important this species is. Being a species that is both, caught in great numbers for feed-production and a symbol of celebration by human consumers, Atlantic herring was chosen as model species for this work.

Consciousness assessments, physiological and physical state were studied to see their effect on fish welfare. Each individual followed the reflex action mortality predictors (RAMP), such as response to tactile stimuli (RTS), rhythmic opercular activity (ROA), equilibrium and vestibular ocular response (VOR). In addition, any physical damage was recorded and after blood collection four physiological indicators were studied: cortisol, lactate, osmolality and chloride concentration.

An assortment of fishing gear is used in capture fisheries, such as traps, handlines, purse seines, and trawls (Gabriel *et al.*, 2005). Each gear has its own impact on fish welfare. Therefore, a study on the effect of the capture fisheries on fish welfare should consider the various gear types. The idea behind the second aim was to compare the stress and welfare of herring caught by a commercial fish trawler with herring caught at low depts using hook and line. The choice was made by acknowledging the fact that trawling is the most traumatic capture method while hook and line has far less effects (Veldhuizen *et al.*, 2018).

The third aim of this study was to assess how and when the fish loses their consciousness: if they lose it before going through the pump, after the pump or once they reach the RSW tanks. Suppose they were conscious once reaching the tanks what makes them nonresponsive. Overall, it was hypothesised that trawling has a negative effect on the fish welfare when comparing to other methods and that trawling presents less levels of consciousness and higher stress levels when compared to hook and line. In addition, trawled individuals would present worse shape with high external and internal damage.

2. Material and Methods

The study was divided into two research trips. One onboard of a pelagic fisheries boat to collect data from a commercial large-scale fishing boat and the other one onboard of a hook and line fishing boat to see if there are any differences between the two methods in the individual's welfare.

2.1 Description of the Two Different Fishing Methods Investigated

In the first part of the study, wild herring were caught by trawl from a Danish flagged boat (Beinur) from September 13th until September 21st at 145–160 m depths. First, shoals of herring were located with the help of sonar systems. This modern pelagic fishing vessel uses multiple sonar systems to locate and catch the fish. Once a shoal was located, the net was hauled out from the ship's stern and dragged through the open water column so that it never reaches the seabed. The net is funnel-shaped with a wide opening that narrows down to the cod-end, which is a bag with 50 mm mesh where the fish finally gets trapped (Joseph, Shipley and Siskey, 2019). When the trawl was filled, after 2 to 5 h, the net was hauled back upon the deck leaving only the cod-end in the water. Next, the cod-end was temporarily lifted out of the water so that it could be connected via a large hose to a fish-pump. The fish was then pumped, with a 24 inches pump with a capacity of 25-30 tons per minute, from the cod-end into a separator, a resistant aluminium divider with steel grilles that allows the water separation from the pumped fish to the RSW tanks and examine for bycatch. After the separator, the fish continued down into one out of eight refrigerated seawater (RSW) tanks, where pumps recirculate the water through the containers and the cooling system. To guarantee high quality, the fish pump on the particular fishing vessel was maintained at around 20-25 tons per minute. Throughout the trip, general information (*e.g.,* trawl size, mesh size) was complemented with information specific for each haul (*e.g.,* trawling speed, fishing depth, haul duration and catch size, see Table 1).

In the second part of the study wild herring were caught by hook and line from a Danish boat (Havstrygeren) in Øresund (Helsingør, Denmark) on November 23rd, 2021, at 15–22 m depths. Anglers lined up along the boat where the reels were attached to a rod. The reels are used to deploy and retrieve the line to resemble a free-moving bait and typically are fitted with a 'brake' system where there's a resistance in the reel once the fish takes the line. Once the herring were winded upon to the boat, they were quickly de-hooked and euthanized by a sharp blow to the head using a fish priest.

2.1.1 Fish Sampling After Capture with a Commercial fish Trawl

Initially, a protocol was designed to collect information about the trawled fish and investigate if different sampling sites would impact the assessments of consciousness, the health and physical injuries, and the data obtained after blood sampling. To do so we collected and sampled fish at three different sites on the vessel. In the first haul, individuals were randomly collected directly after the pump at the separator (n=20), followed by the collection of random individuals (n=20) in one of the eight RSW tanks. Finally, random individuals (n=20) were collected at the separator but put into buckets. After collection, each individual went through a sampling protocol, including a condition assessment followed by blood sampling. Individuals were then measured, photographed and frozen for further x-ray (see descriptions of the specific methods below). During the first haul of the trip, it became clear that the vast majority of fish we sampled were either moribund or dead. In addition, the blood samples were largely haemolysed upon plasma preparation preventing us from conducting any further investigations of the composition of the plasma. Because of this, the protocol for the blood sampling was altered for the two upcoming hauls.

In the second haul, again, fish were sampled from either the separator (n=20) or the RSW tank (n=20). However, instead of a complete random selection, we started the sampling at the separator by selecting out individuals (n=10) that looked alive. Again, most fish sampled were moribund or dead. However, now the first ten fish sampled stood out, as the blood from these fish could be separated into plasma and a pellet of erythrocytes making further analyses of the composition of the blood possible.

For the third haul, we consequently decided to separate the fish from which we took blood samples and the fish used for condition assessments. The reason for this was that we did not want to bias the condition assessments by selecting lively looking fish and at the same time, we needed plasma that was not polluted by ruptured erythrocytes for the subsequent analysis. Hence, we now sampled fish that looked alive (n=40) at the separator for blood while at the same time randomly subsequently selected a different subset of fish (n=90), also at the separator, for assessments of consciousness and the health and physical injuries. Once the blood sampling was done, each fish was measured and documented (*i.e.,* photographed and tagged). For additional information, all fish sampled for blood were individually bagged, tagged and frozen at -20˚C for later assessments of internal damage (see details below).

Table 1: General information, such as date, haul duration, fishing depth and catch size, that was gathered from the trip onboard the commercial trawl Beinur. All the information is divided by hauls.

Haul Number	Date	Duration of the Haul	Depth	Catch Size
1	18 Sept.	2 Hours	145-160 m	200 ton
2	18 Sept.	5 Hours	145-160 m	350 ton
3	19 Sept.	3 Hours	145-160 m	280 ton

2.1.2 Fish Sampling After Capture with Hook and Line

Here, the fish were divided into two experimental groups. The first group of fish caught (n=20) were used for the assessments of consciousness, killed and sampled for blood as quickly as possible. The fish from this group represents a control, as the method used here is considered the mildest and quickest way to capture a wild herring. Following euthanasia, all fish were sampled for blood, measured and photographed. To assess the haemolysis problem observed on the commercial fishing vessel, a second group of individuals (n=20) was captured and randomly divided into two buckets filled with 12L of no aerated seawater containing tricaine methanesulphonate (MS-222, 150mg/L), the primary anaesthesia agent for fish (Topic Popovic *et al.*, 2012), buffered with $NaHCO_3$ (300 mg/L). From the buckets fish were then sampled at six different time intervals (*i.e.* 15, 30, 45, 60, 120 and 180 min). At each time, three random fish were pulled out of the buckets and sampled for blood, measured and photographed.

2.1.3 Assessments of Consciousness and Morphological Indicators of Welfare

For assessments of consciousness and morphological indicators welfare, fish were individually placed into a 20 L assessment bucket filled with aerated seawater. Here, the fish were evaluated by applying reflex action mortality predictors

(RAMP): (1) equilibrium, seeing if the fish could maintain their righting reflex when placed upside down in the assessment bucket; (2) vestibular ocular response (VOR), rotating the fish around, along the axis, and observing if the eyes rotate to maintain orientation, *i.e.,* following the horizon; (3) rhythmic opercular activity (ROA), observing the opercula for any rhythmic movement; (4) and response to tactile stimuli (RTS), pinching the caudal peduncle area and see if the fish responded to the stimuli (Uhlmann *et al.*, 2016). The absence of responses, meaning no reflexes, scored 0, while positive responses scored a 1 (Table 2).

Immediately after the assessments of consciousness, seven different morphological indicators were scored to evaluate fish welfare: opercular damage, fins condition, eye damage, scales loss, skin lesion and effects of pressure such as bulging eyes and bloated swim bladder (Table 3) (Veldhuizen et al., 2018; Noble et al., 2020).

Table 2: Visual assessments of the reflex action mortality predictors, used to determine herring's state of consciousness, and their respective scores and descriptions (Noble et al., 2020).

Parameter	**Score**	**Description**
Equilibrium	1	Fish rights itself and retains upright orientation
	0	Fish shows no equilibrium
Vestibular Ocular Response (VOR)	1	Eye tracks angler or level plane
	0	Fish shows no Vestibular Ocular Response
Rhythmic Opercular Activity (ROA)	1	There's opercula movement
	0	Fish shows no Rhythmic Opercular Activity
Response to Tactile Stimuli (RTS)	1	Swimming as an escape response
	0	Fish shows no reaction to Tactile Stimuli

2.1.4 Blood Sampling

Blood samples were taken from the caudal vein immediately after fish collection. In the first two hauls on the commercial fish trawler, the samples were taken after performing assessments of consciousness and seeing the morphological indicators. In the last haul and for fish caught with hook and line the sample was taken immediately, after the fish were removed from the separator. In all fish 0.5-1 ml of blood was taken with each sample using a BD Microlance ™ 3 23G needle with a

1 ml heparinised syringes. Samples were then centrifuged at 6000 min^{-1}/2000 ×g with VWR MiniStar for 10 minutes. Plasma samples were frozen at -80°C until further analyses.

Table 3: Indicators of external injuries possibly caused by the gear with their respective scores and description. Scoring goes from zero evidence (0) to extreme injuries (1). The morphological indicators are based on the welfare indicators for farmed rainbow trout by Nobel et al. 2020.

Parameter	Score	Description
Opercular Damage	1	Both operculum absent, exposed gills
	0	No opercular damage
Fins Condition	1	Little fin remaining
	0	No fin damage or most of the fin remaining
Eye Damage	1	Large haemorrhages and ruptured eye
	0	No eye damage
Scale Loss	1	Whole fish has lots of scale loss areas Large areas of scale loss
	0	No scale loss
Skin Lesion	1	Significant bleeding, wounds, muscle exposed
	0	No skin lesion
Pressure Injuries	1	Some minor injuries in parts of the fish
	0	No injuries

2.2 Plasma Analysis: Physiological Indicators of Stress

Lactate concentration was measured using Sigma's colourimetric test (ref. MAKO64; St. Louis, MO, USA) following the manufacturer's instructions. Subsamples of plasma were diluted with Milli-Q water instead of Lactate Assay Buffer to get the readings within the standard curve range. A threshold of 2.2 abs was created, allowing further calculations. The first batch followed a dilution of 1:60 (10 µL of plasma for 590 µL of Milli-Q water), while the second was a 1:180 dilution (10 µL of plasma for 1170 µL of Milli-Q water). Plasma cortisol concentration was estimated using an Enzyme-Linked ImmunoSorbent Assay

(ELISA) commercial kit (ref. 042710; Neogen Europe, Ayrshire, Scotland, UK). For the cortisol concentration, plasma subsamples were diluted (1:25, 10 µL of plasma for 240 µL of diluted extraction buffer).

Total plasma osmolality was measured using a vapor pressure osmometer (Vapor Model 5600, South Logan, UT, USA). Calibration premeasurement was guaranteed using the three Opti-Mole ampule osmolality standards (OA-100, OA-029, and OA-010) (ref: oa-100; oa-029; oa-010, South Logan, UT, USA). Duplicates were run for each sample. Plasma chloride was estimated using a chloride analyser (Sherwood M926, Cambridge, UK). Precession was checked running duplicates of the first five samples, since all the values were perfectly matched, the rest of the samples were measured only once. As some samples had less than the reading volume (20 µL), the plasma analysis was done with 10 µL, and all the values were doubled to get the right concentration.

Not all the fish tested were sampled for cortisol (79), lactate (78), and osmolality (61). Samples that suffered haemolysis presented values above detection, precluding calculations and ended up coagulating, increasing the contamination level of the chloride analyser.

2.2.1 Lactate and Cortisol Calculations

Since both cortisol and lactate absorbance values are displayed as Optical density (OD), some calculations were done to obtain the concentration values.

Starting with the D-Lactate Colorimetric Assay Kit, duplicates from the 0 (blank) D-Lactate Standard were averaged and subtracted from all the readings. This was done because background values can show significance, so it is necessary to correct them. The corrected absorbance values were then used to plot the standard curve, the mean of the corrected duplicates (OD570) against the standard concentration (µM).

The trendline equation based on the standard curve was determined as a linear regression (y=mx+b) and used to calculate D-Lactate concentration.

$$\text{Lactate Concentration} = \frac{Sa \times D}{1000}$$

Where:
Sa = The amount of lactic acid in the wells obtained from the standard curve (µM)
D = Dilution factor
1000 was used to transform the µM to mM

As for the cortisol ELISA kit, the idea is the same. Again, the background was corrected by subtracting the blank (0,032), so the background absorbance was driven by the reagent from all the wells, including those from the non-specific binding (Standards). The duplicates from the standards were averaged and divided by the maximum binding (B0), the maximum amount of the tracer that the antibody can bind without a free analyte. Afterwards, the data was linearized following a log transformation. The equation of such transformation is shown below. The new values were used to plot the standard curve, the logit of the bond divided by the maximum binding (B/B0) against the log of the standard concentrations. The sample concentration was determined from the standard curve and then multiplied by the dilution factor, that in this case was 25.

$$logit\left(\frac{B}{B0}\right) = \ln\left[\frac{B}{B0}/(1-\frac{B}{B0})\right]$$

Where:
B = The bond, absorbance of the sample
B0 = Maximum Binding

A new standard curve was set up each time the assay was run for both cortisol and lactate to minimize any external interference.

2.3 Scale Loss Percentage

Photographs from each individual were taken right after the blood sampling using a photo box with a digital camera placed from above at a distance of roughly 30 cm. Individuals were positioned horizontally, caudal fin facing the left side and with their respective barcode (Figure 2). The shots were later analysed using ImageJ (National Institutes of Health, Bethesda, MD, USA), an image processing software developed by Wayne Rasband (Abràmoff, 2004). Because of lively fish selection, the second and third haul were not taken into consideration for this part of the study.

Scales areas were calculated using the Fiji polygon selection tool, a distribution of ImageJ that focuses on biological-image analysis (Schindelin *et al.*, 2012). Each individual from both first haul and control group was surrounded, and the total area was calculated using the measurements found in the *menubar*, a tool that makes measurements under the manually specified area of interest. The same procedure was followed for areas with visible scales that were then subtracted from the total area. These values were converted from pixel to cm^2 using the barcode as scale, 4.8 cm and then expressed as percentage.

Figure 2: Picture of a herring caught during the hook and line trip. A) Total area of scales obtained with ImageJ running both the polygon and measure tool. B) The First area of loss brought with ImageJ using the polygon and measure tool. The white bar represents the scale, 4,8 cm.

2.4 Internal Damage - Algoritme to Image Analysis

The main goal was to develop algorithms to detect any spine damage in the Atlantic herring. With the use of machine learning, it would be possible to train networks in order to identify the factors that one is interested in. The software used was Pytorch, an "Open-Source Deep Learning Framework" developed by Facebook's AI Research lab (Paszke *et al.*, 2019). Pytorch was chosen based on its flexibility within frameworks, allowing to speed the strategy between prototyping and deployment.

X-ray examinations with customized folios adjusted to the fish's size were performed on the 120 individuals collected from the commercial trawler to check for any internal damage. With an image plate scanner, this process allowed the team to look for traumas such as fractures and other scale deformities in the vertebrate and skull. All the images were pre-processed, removing both the background and surroundings, allowing only the fish in the field of view. Unfortunately, the image volume was limited, where there were only 121 DICOM images making it impossible to train the algorithm, but the internal damage was still quantified. This part of the work was led by *DBN* Dennis Brandborg Nielsen, and *DSC* Dorte Lene Schrøder-Petersen from the Danish Technological Institute (DTI) and performed in collaboration with the university hospital for companion animals in Copenhagen.

2.5 Statistical Analyses

Statistical analysis was performed using the statistical software R, version 4.1.2 (http://www.R-project.org), with the packages ggpubr, dplyr, rcompanion and ggstatsplot. Scale loss, and both lactate and cortisol concentration were calculated in Excel, version 16.59. All response variables, such as cortisol (ng/mL), lactate (Mm), osmolality (Mmol/Kg), chloride (Mmok/L), size (cm) and area (cm^3), were assessed for equal variance and normality for each treatment group using density plots and Shapiro Wilkins normality test (Shapiro and Wilk, 1965). The method was chosen based on its effectiveness and powerfulness for small samples, ≤ 50 (Bowers, 2019).The null hypothesis assumes that the data is normally distributed. If $P > 0.05$, the null hypothesis is accepted (Kim, 2013; Bowers, 2019).

To see if the haul duration influenced stress levels, linear models were used to compare cortisol, lactate, osmolality, and chloride with the haul number, operating the lm' function. In addition, a linear analysis was done to assess if the individual's length among different hauls had any influence on all the previously mentioned variables. Since the linear models showed no significance, no multiple comparisons using a post hoc Tuckey Honest Significant Differences Test (Tukey HSD) were followed (Quinn and Keough, 2002).

A Kruskal Wallis rank-sum test (Kruskal and Wallis, 1952) was done to see if there were any differences between the two different fishing methods on the stress levels. The choice of the test was based on the fact that both gears had extreme values that even after transformations (log transformation, square root transformation and 1/x transformation) would not meet the assumptions of the linear models. Ideally, stress levels would vary depending on the fishing method, so cortisol, lactate, chloride, and lactate were used as response variables while haul number was used as an explanatory variable. The Kruskal-Wallis test proves that at least two treatment groups are different, however, it does not provide multiple comparisons to know which ones. The non-parametric test was then followed by a Wilcoxon rank-sum test also known as a Mann–Whitney U test (Mann H.B. and Whitney D.R., 1947). The individual length was used as a covariant, but since it did not show any significance it was excluded from further analysis. Scale loss percentage would also vary depending on whether it's a passive or an active fishing method, which is why a Kruskal-Wallis rank-sum test was followed.

While trying to investigate possible explanations for the haemolysis, there was a visible trend in the Helsingør trip with one of the response variables. Consequently, a linear regression was used to study the potential significance between time and lactate concentration. In addition, some individuals had plasma cortisol levels below the detection limit (0.4 mM). Therefore, those observations were substituted with the highest value possible between the detection range.

3. Results

Understanding how stressful and painful a fishing method is, presents a significant impact on the fisheries industry, both financially and ethically. In addition, it brings space for improvements from a welfare point of view. In this study, the main idea was to look at morphological metrics, physical injuries, assessments of consciousness, and physiological indicators of stress to quantify the levels of stress inflicted by the fishing gear and assess how responsive the individuals are after being trawled. When compiling all the information, ideally, it will be possible to pinpoint the exact cause of death and how painful the process is for Atlantic herring, which will help to improve the way fish are being slaughtered during pelagic fisheries.

3.1 Assessments of Consciousness

The reflex action mortality predictors included equilibrium, response to tactile stimuli (RST), vestibular ocular response (VOR) and rhythmic opercular activity (ROA). During the commercial trawl, in the first haul, only 12% of the fish showed any type of response. While in the third haul, the number of responsive individuals was even less, with just 10% reacting to any of the predictors. Contrasting to these high mortality levels, all the individuals from the hook in line were conscious during the capture (Figure 3). During the second haul, only alive individuals were targeted, therefore, the results from such haul were not analysed, as this comparison would be biased by the sampling method.

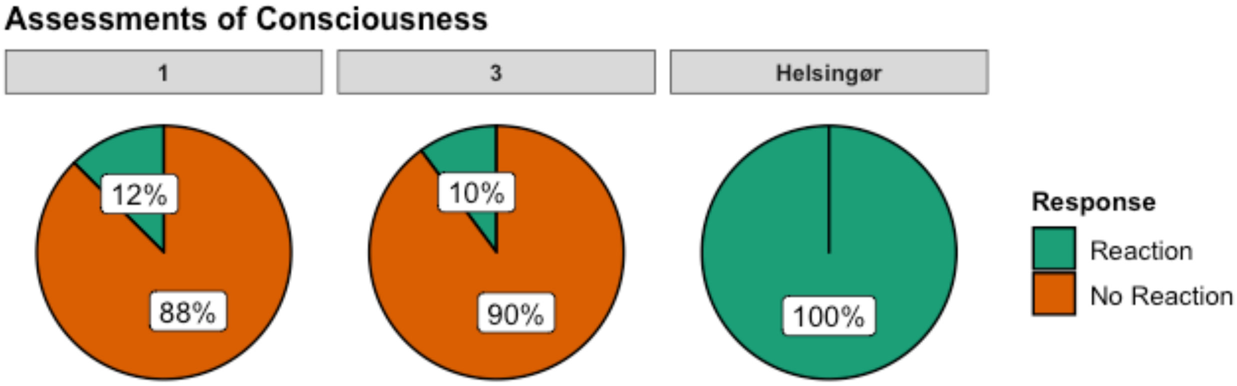


Figure 3: Percentage of lively fish after being caught with a pelagic trawler (1 and 3) and with hook in line (Helsingør). Calculations were made using the scores of the three reflex action mortality predictors: VOR, RST, ROA, where one positive indicator was enough to be considered conscious.

3.2 Morphological Metrics and Physical Injuries

As for the morphological indicators, only four of them are shown (Figure 4), opercula damage, eye damage, scale loss and skin condition. The other three, fin condition, bulging eyes and bloated swim bladder, scored 0 for all the individuals. In general, all the individuals captured during the commercial trawl were in perfect condition, showing almost no signs of damage, except for the scale loss, where most of the individuals presented no scales or lost an extensive area of scales. Individuals from the commercial trawl presented an average total body length of 28.00 cm ± 1.06. This value is significantly higher ($p < 0.001$) than the average length of 25.70 cm ± 2.66 recorded for the individuals from the hook and line.

Scale loss was evident throughout the study. Individuals caught in the first haul lost an average of 98.00% ± 4.30 of their scales, while those from the hook and line, the control group, lost an average of 62.52% ± 24.66. Even with such percentages coming from the control group, significant differences were observed between both methods ($p< 0.001$, Figure 5).

The internal damage analysis only showed a group of no harm, where none of the individuals presented any sign of trauma like fractures or any other deformities in the vertebrate and skull (Figure 6). It was impossible to develop the algorithm due to the lack of injured fish.

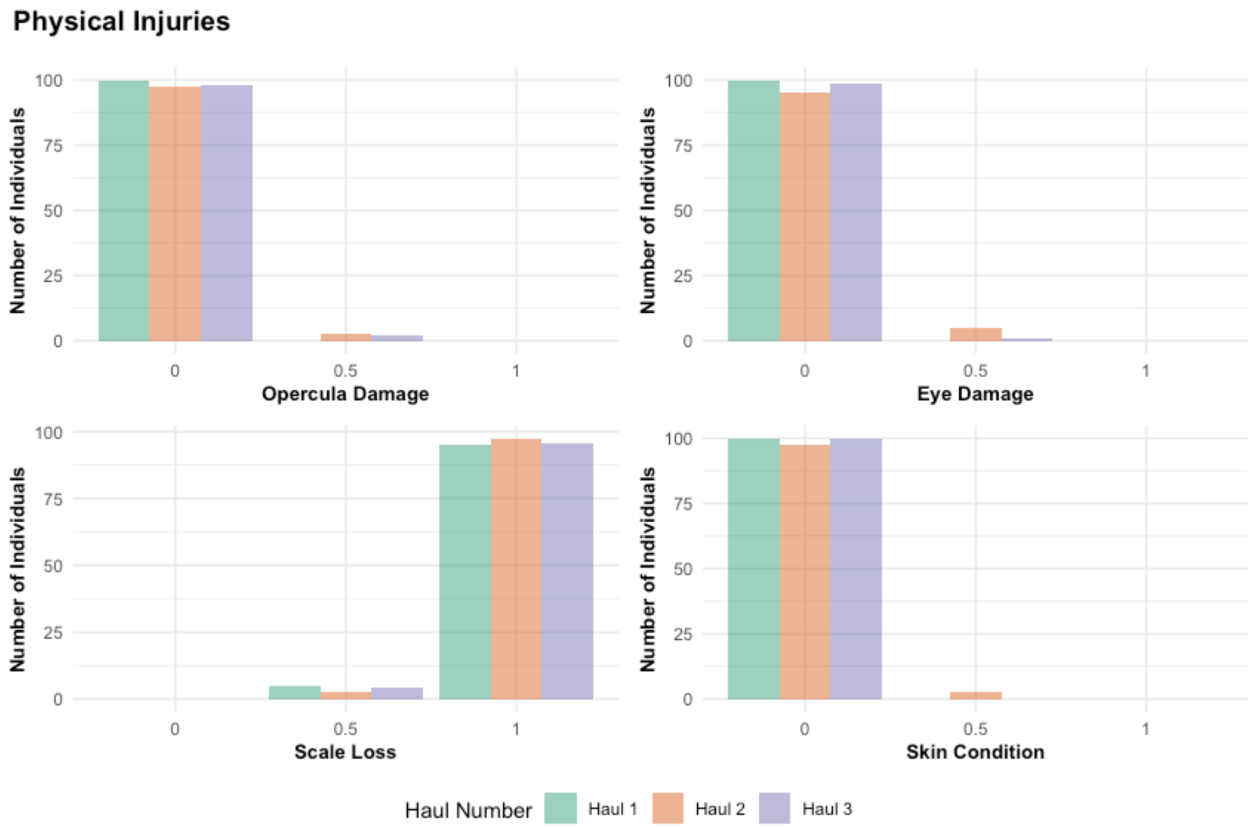


Figure 4: Relative values of four indicators of external injurie: Opercula damage, Eye damage, Scale loss and Skin condition for the three hauls onboard of the commercial trawler. Scoring 0: no evidence of damage, 0.5: minor evidence of damage, 1: clear evidence.

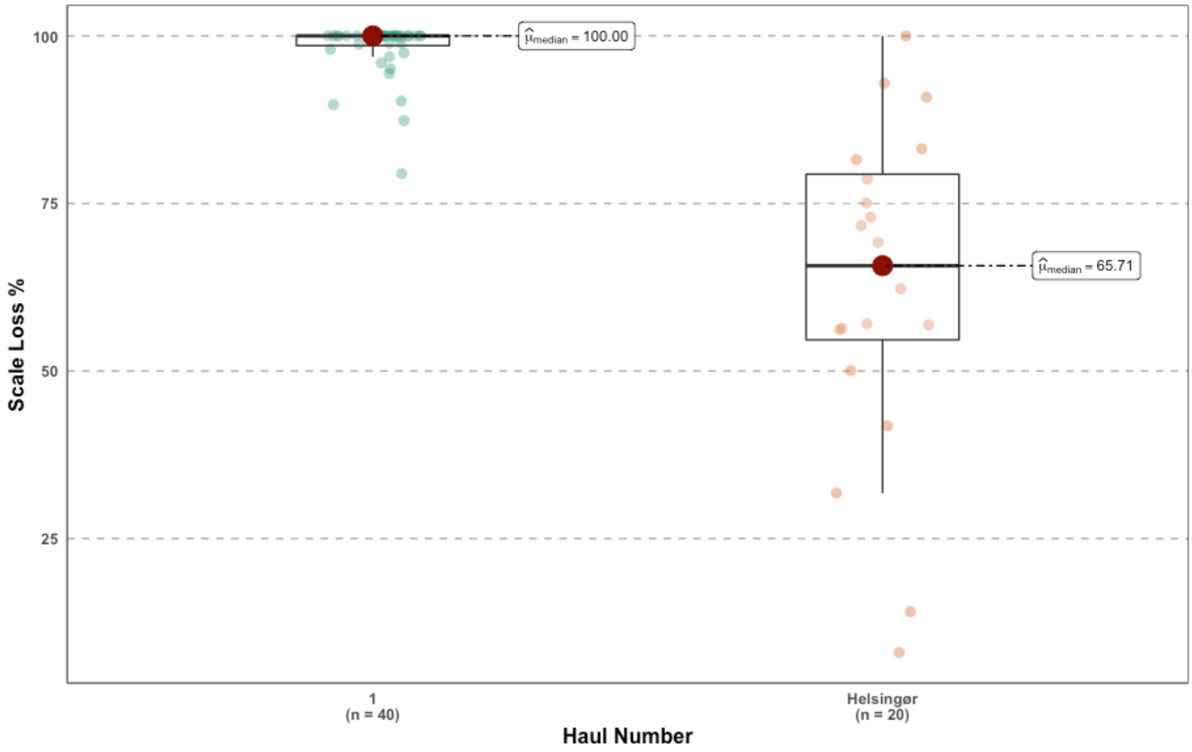


Figure 5: Differences in scale loss percentage between the fish caught in first haul from the commercial trawler and those caught with hook in line in the Helsingør trip. Each point represents one individual. Medians represented as the dark red circle. Upper edge and lower edge denotate third and first quartile respectively. Beginning/End of the vertical line represents the maximum/minimum observation bellow/above the upper/lower

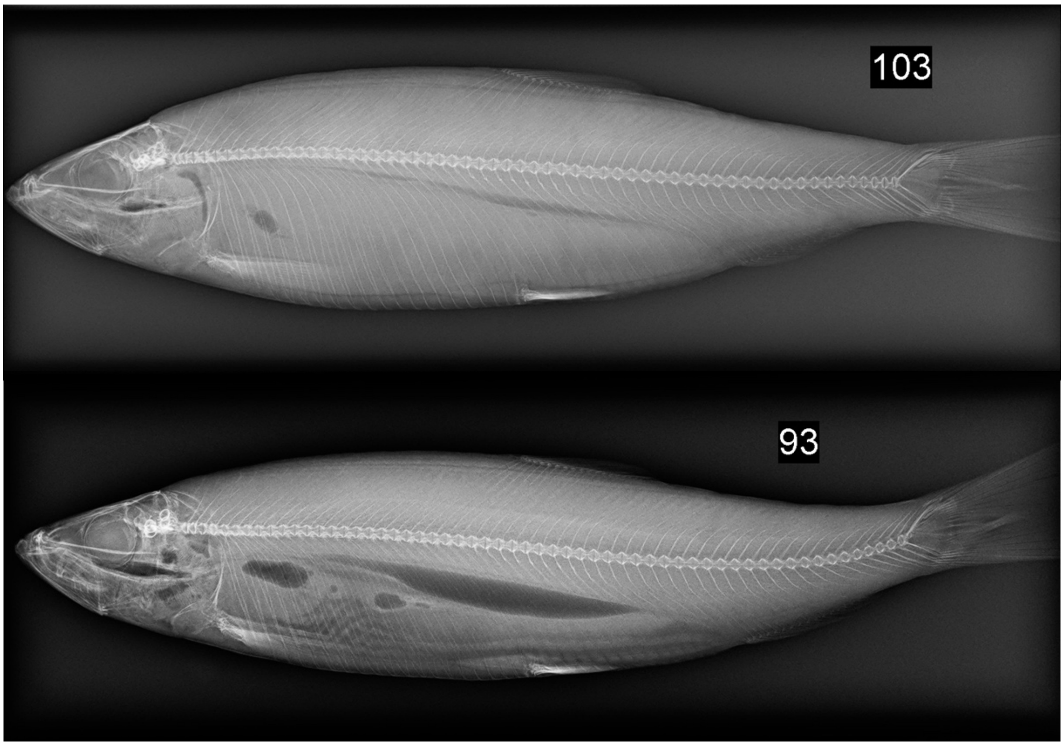


Figure 6: Scanned image of the lateral X-ray examination on fish number 103 and 93 (third haul) caught in the commercial trawler.

3.3 Physiological Indicators of Stress

The physiological indicators of stress considered in this work were plasma cortisol, plasma lactate, plasma osmolality and chloride concentration. There were no significant differences between the second and the third haul from the commercial trawl for all the parameters (osmolality, plasma lactate, plasma cortisol and chloride) but a substantial difference ($p < 0.001$) was found when comparing the hauls from the commercial trawl with the hook and line (Figure 6). In the first haul, almost all the plasma samples were completely haemolysed. Due to that, all samples obtained from haul one and any haemolysed samples from the second and third haul were disregarded from the analysis.

Although both hauls presented significant differences when compared to the hook in line, there was no trend between haul duration and concentration of the stress indicators. Trawled fish often exhibited values close to the ones obtained from the hook in line, while others showed values of extremely stressed individuals. This riff made getting a proper test between haul duration and the response parameters quite challenging.

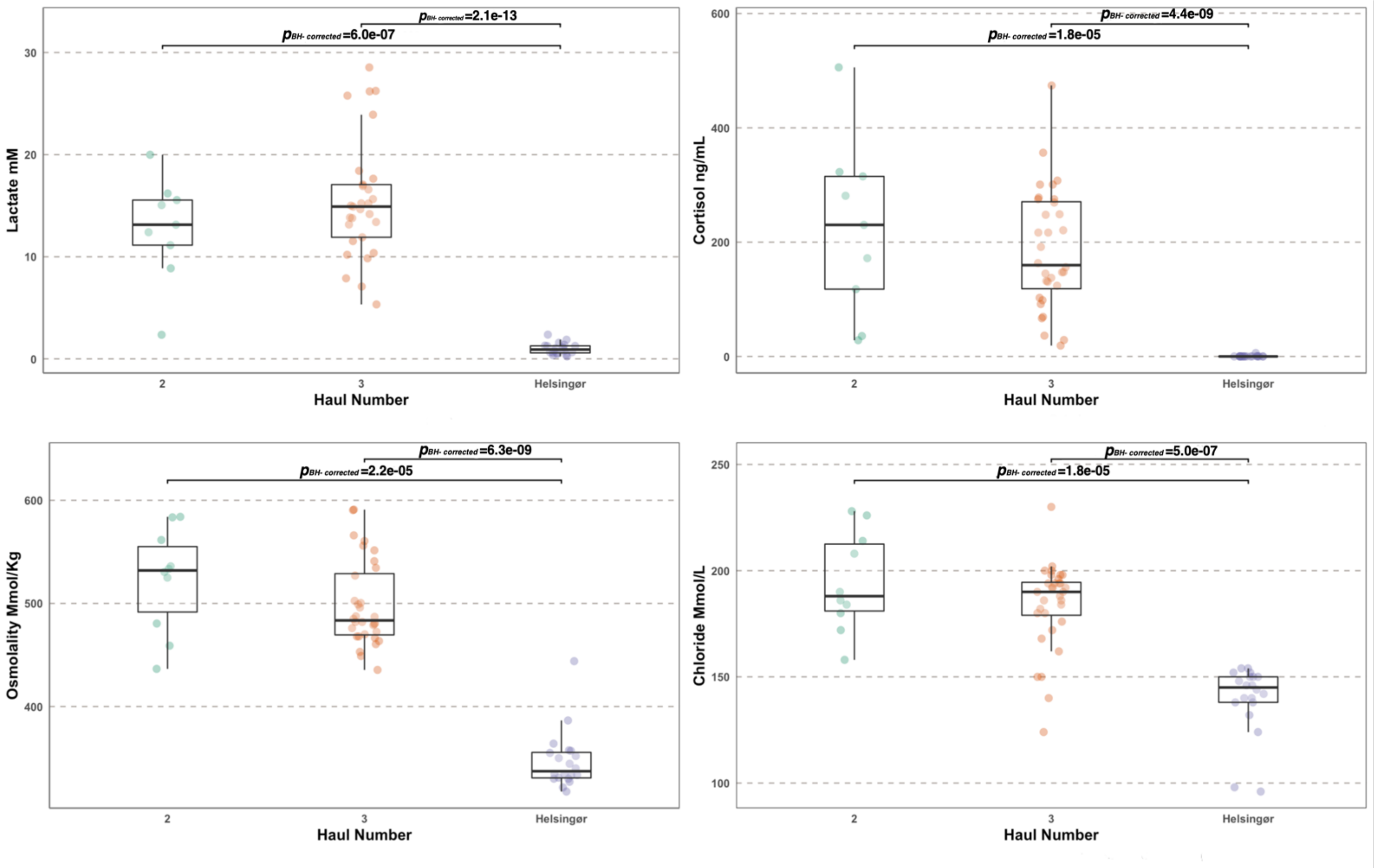


Figure 7: Differences in the physiological stress indicators (Lactate, Cortisol, Osmolality and Chloride) between the commercial trawler and the hook and line. Each circle represents one individual. The bold black line gives the average value in the boxplot. Upper edge and lower edge denotate third and first quartile respectively. Statistical significance p<000.1 when comparing hauls with Helsingør. Beginning/ End of the vertical line represents the maximum/minimum observation bellow/above the upper/lower fence

During the first research trip onboard a commercial trawler, many of the blood samples were haemolysed. Because of the lack of information on such a matter, the idea was to understand why it happened, what effect that had on the fish, and how it could be avoided. During the Helsingør trip, while studying a passive gear method, hook and line, some individuals were left in buckets to see if time has any influence on haemolysis. In the first bucket the individuals were sampled in three different time intervals – 15, 30 and 60 minutes, while in the second bucket individuals were sampled in four different time intervals – 45, 60, 120 and 180 minutes. Although no answers were obtained while analysing the data, it was possible to see a clear trend with plasma lactate and time (Figure 8). Time presented a significant effect on lactate concentrations ($P< 0.001$), contrasting with the rest of the stress indicators, plasma cortisol, osmolality and chloride (data not shown).

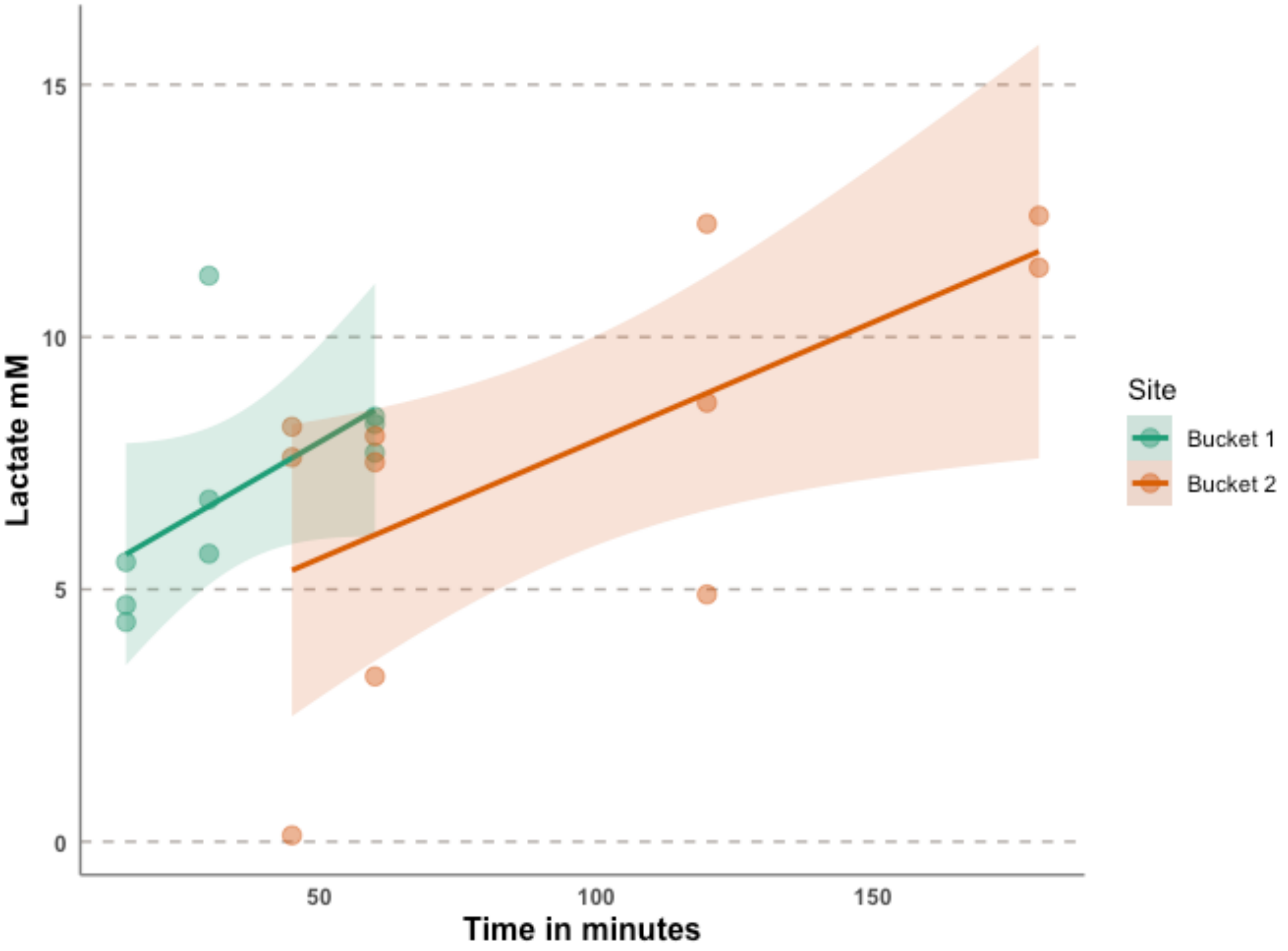


Figure 8: Relationship between time and lactate concentration on individuals caught with hook and line from the first bucket (green dots and line) and the second bucket (orange dots and line).

4. Discussion

Concerns about fish welfare in general and in commercial fisheries, in particular, have arisen along with new evidence suggesting that fish are sentient beings. However, there is still little information about fish welfare and how it is affected by human activities such as commercial fishing. Here we aimed to assess the effects of trawling on the welfare of Atlantic herring caught and slaughtered in pelagic fisheries. In this present study the pelagic fishery using a trawl proved to have major effects on fish welfare with significant levels of scale loss, high-stress levels and few survivors. A number of different risk factors, including water temperature, oxygen deprivation, extensive periods of swimming, fishing practices and seasonality (*e.g.,* Parker et al., 2003; Broadhurst, Suuronen and Hulme, 2006), are known to influence the wellbeing and mortality of fish caught by commercial fisheries and therefore may have affected the result of the present study are discussed below.

Assessing fish consciousness and mortality in open sea is challenging. Here we used a modified protocol of reflex action mortality predictors (RAMP) to assess fish consciousness and mortality (Davis, 2010; Raby *et al.*, 2012). Our results show that there is an overwhelming risk of fish dying when caught using a commercial trawl (>85% mortalities). This result was in great contrast with fish being caught hook and line where 100% ranked as conscious and lively. These results are in line with previous studies that showed that active fishing gear such as trawls and seines are related to high levels of mortality (*e.g.,* Smith and Scharf, 2011; Olsen et al., 2012; Depestele et al., 2014) when compared to passive fishing gear such as traps and hook in line (*e.g.,* Davis, Olla and Schreck, 2001; Benoît, Hurlbut and Chassé, 2010; Rudershausen, Buckel and Hightower, 2014) However, our results, specifically the absence of morphological abnormalities except for scale loss, show that there also must be other explanatory factors for the high mortality associated with trawling. This result is in contrast with other studies that report fin and skin injuries caused by either contact with other individuals (Davis and Ottmar, 2006; Suuronen and Erickson, 2010) or contact with the net (Gregory, 1998; Davis and Ottmar, 2006).

What we did find was an extremely high degree of scale loss following trawling (~98.00%). Some degree of scale loss during trawling was expected as it is quite common to see scales spread over the sea (Olsen *et al.*, 2012). Yet, the magnitude of the scale losses observed was somewhat surprising. Most previous studies on scale loss and how it affects fish have been conducted in controlled environments, probably due to the difficulty of isolating one specific effect at sea. It has been suggested that the high mortalities observed in Atlantic Salmon following manual descaling related to damage to the skin (dermis) barrier originated osmoregulatory drawbacks, with a clear increase of plasma ions that is related to a decrease of osmoregulation (Zydlewski, Zydlewski and Danner, 2010).Although most believe that the scales are the most outermost layer of the fish this is not true. There's a thin epidermis, covering the scales, that's responsible for mucus production. So, when removing scales that first layer is also removed (Zydlewski, Zydlewski and Danner, 2010).Our results showed as well a relationship between scale loss and compromised osmoregulatory function as the individuals with high percentage of scale loss also showed a high concentration of chloride and high osmolality plasma. However, it has also been suggested that scale loss is not the only possible cause of death. Both Smith (1993) and Olsen et al., (2012) have gotten an increase in plasma cortisol and lactate suggesting that scale loss was responsible for activating the secondary stress response, which might be lethal. Here, individuals with higher scale loss were also more stressed as evident from their higher levels of plasma cortisol and lactate. A phenomenon that has previously been found in de-scaling studies of Atlantic salmon and Atlantic herring (Gadomski, Mesa and Olson', 1994; Zydlewski, Zydlewski and Danner, 2010; Olsen et al., 2012). Furthermore, our results show that the scales of Atlantic herring are extremely sensitive as even individuals caught with hook and line lost a relatively high percentage of their scales (>60%). However, this comparison needs to be carefully interpreted as the vast majority of this scale loss happened when dehooking the fish and while sampling blood, meaning that only part of this is directly related to the fishing method itself. In future studies this could try to be avoided by photographing the individuals before the proceeding with the blood sampling and the measurements.

Another potential stressor and welfare hazard during commercial fishing is crowding. Usually, the air exposure is what is associated with high levels of stress and pain (Gingerich *et al.*, 2007) however, intense crowding with high densities in the trawls has been lifted as a possible explanation for high mortalities often seen when fishing with trawl (Suuronen, Lehtonen and Jounela, 2005; Depestele *et al.*, 2014). High densities can cause compaction of individuals that incapacitate fish to breathe, eventually causing asphyxiation (Gregory, 1998). Naturally, there are both interspecific and intraspecific differences in how a fish responds to crowding, yet the most common reaction is an increase in both primary and secondary stress responses (Marçalo *et al.*, 2006; Tenningen, Vold and Olsen, 2012). Anders, Roth and Breen (2021), when studying the response of Atlantic mackerel to crowding saw that there was a rapid response to high densities with an increase in the physiological parameters of stress (lactate, cortisol, osmolality and ion concentration) when comparing with their control group.

Therefore, it comes as no surprise that longer trawling times will aggravate these stress responses. Although it has not before been shown with Atlantic herring, Lockwood, Pawson and Eaton, (1983) showed that mackerel mortality due to high densities in a purse seine increased rather drastically with increased fishing times. Our significant densities and the same stress levels independently of the haul duration make density in the net the most crucial cause. Fish swim exhaustively, weakening its energy resources which is related to an increase in both plasma cortisol and blood lactate. Theoretically, with hauls going from 2 to 5 hours and catching 200 to 350 tons prolonged oxygen depletion will happen and will for sure lead to extended periods of anaerobic metabolism. However, due to the similarities in the concentration of plasma cortisol and lactate it is unlikely that haul duration is the most important factor in this case. Both crowding density and duration vary depending on the size of the trawl and the catch's size (Tenningen et al., 2019).According to Lerfall et al. (2015), generally, 200kg/m^3 to 250 kg/m^3 densities are pumped onboard the vessel. In this particular study, we are dealing with a massive commercial vessel with a gross tonnage of 2600 that can accommodate more than 250 kg/m^3 in the cod end. Since Tenningen, Vold and Olsen (2012), stated that crowding and its indirect conditions could lead to 30-50% of mortality before any pumping, with these high densities the mortality encountered comes as no surprise.

It has been suggested that, in theory, the last fish entering the trawl will also be the last fish pumped out of the trawl and onto the ship. If so, one would expect to find an increasing ratio of lively fish as the pump empties the trawl. Our observations were not in accordance with this as we detected a few unconscious fish widely dispersed with the dead ones. The same was observed in recent studies (Olsen *et al.*, 2013; Digre *et al.*, 2016; Svalheim *et al.*, 2020). When looking for the effects of exhaustive swimming in Atlantic Cod they found that the individuals tend to get mixed up in the nets depending on their swimming capacity and recuperation time after long periods of swimming. Consequently, it is possible that the outliers present in the physiological indicators, especially in the second haul, might be from fish that got caught late in the net. Levels of cortisol and lactate need to be different from a fish that was caught right at the beginning of the fishing than from a fish that was caught 5 seconds before the end.

Throughout the study, there was an obvious problem with haemolysed plasma samples complicating reliable readings and making us lose essential data from the first haul in the commercial trawler. It is still unclear if the fish died due to the haemolysed blood or if that was a direct consequence of death Olsen et al. (2012), faced the same problem when studying the effects of scale loss in Atlantic herring, but no answers were given on why it was happening. A trial was done during the second research trip to see if time affected the plasma sampling and if the same problem would be faced. None of the samples was haemolysed, even the ones above 2 hours which makes us think that haemolyse was not a consequence of death. It also allowed us to see a positive trend in lactate levels throughout the time, like what was found in other articles (Davis, 2005; Olsen *et al.*, 2013; Karlsson-Drangsholt *et al.*, 2018; Svalheim *et al.*, 2020). However, nothing was found when looking for this same trend with plasma cortisol. Cortisol concentration varied throughout time. Nevertheless, it cannot be ruled out that each bucket was filled with ten individuals and the overlap of individuals together with the lack of aeration may have created respiratory distress that is highly related to increasing levels of plasma lactate (Davis, 2005; Olsen et al., 2013).

Based on our results, it is still impossible to pinpoint the leading cause of death in pelagic trawlers. As of now, there is no primary cause, and the high mortality is likely to be a combination of several factors. Several individuals lost a considerable number of scales (~98.00%), indicating that such loss might have affected mortality. In addition, the potential shortage of oxygen available, both when crowded in the trawl and due to the exhaustive swimming led to high blood lactate levels during trawling, which might have also given rise to such high mortality percentage. Given the difficulty of isolating all the factors in only one responsible factor, a combination of laboratory experiments and fieldwork would be beneficial. Furthermore, it would be advantageous to study the relation between mortality and fish characteristics. Recent studies have proven that smaller fish are more likely to die during trawling when compared to larger ones (Marçalo *et al.*, 2010; Olsen *et al.*, 2012; Tenningen, Vold and Olsen, 2012; Depestele *et al.*, 2014) and that different populations show different resistance not only to stress but also to gear type (Davis, 2007; Broadhurst, Millar and Brand, 2009). Moreover, further studies on different seasons, different salinities, different depths, additional hauls duration and density within the net would also lead to important and comparable findings. Regardless, the fish were in very poor shape indicating that the way they are being fished might not be the best from a welfare point of view and that improvements need to be made. Even though all individuals looked okay to the naked eye, we cannot forget that most of them were dead, and after laboratory work, it was possible to see that they were in distress when alive.

There is evidence that fish can feel pain, and such evidence is as good as the one we have for mammals. So, we are now in a place to minimise any adverse conditions for fish and create measures for better welfare. But unfortunately, with our results, the high concentration of physiological stress indicators together with mortality percentage and the poor fish shape, it is clear that there is still a long way to go. The way fisheries are being done needs to be improved, fish are being killed in a way that would not be accepted if we were talking about any other animal. Besides the obvious mechanical stress involved, our results showed that there is evident stress that could be minimised for better welfare. Nevertheless, it is a complex subject. One can appeal for improved nets, shortened cod-end densities, shorter times, and a different way of pulling the net. Still, one also needs to understand how vital these commercial fisheries are to humans and how costly these improvements will be. It is now an ethical decision more than anything else.

References

Abràmoff, M.D. (2004) “Image Processing with ImageJ,” *Biophotonics international*, 11(7), pp. 36–42.

Alder, J. *et al.* (2008) “Forage fish: From ecosystems to markets,” *Annual Review of Environment and Resources*, 33, pp. 153–166. doi:10.1146/annurev.environ.33.020807.143204.

Anders, N., Roth, B. and Breen, M. (2021) “Physiological response and survival of Atlantic mackerel exposed to simulated purse seine crowding and release,” *Conservation Physiology*, 9(1). doi:10.1093/conphys/coab076.

Barton, B.A. (2002) “Stress in Fishes: A Diversity of Responses with Particular Reference to Changes in Circulating Corticosteroids,” *Integrative and Comparative Biology*, 42, pp. 517–525. Available at: https://academic.oup.com/icb/article/42/3/517/723932.

Bell, G.R. (1984) *An outline of anesthetics and anesthesia for salmonids, a guide for fishculturists in British Columbia. Canadian Technological Report of Fisheries andAquatic Sciences*. Nanaimo, BC.

Benoît, H.P., Hurlbut, T. and Chassé, J. (2010) “Assessing the factors influencing discard mortality of demersal fishes using a semi-quantitative indicator of survival potential,” *Fisheries Research*, 106(3), pp. 436–447. doi:10.1016/j.fishres.2010.09.018.

Bowers, D. (2019) *Medical Statistics from Scratch : An Introduction for Health Professionals*. 2nd edn. Edited by d. Bowers. Chichester, West Sussex: John Wiley & Sons.

Braithwaite, V. (2010) *Do Fish Feel Pain?* 1st edn. Oxford: Oxford University Press.

Breen, M. and Pedersen, A.O. (2012) “A review of possible mitigation measures for reducing mortality caused by slipping from purse-seine fisheries,” *ICES Journal of Marine Science* [Preprint]. Available at: https://www.researchgate.net/publication/266200767.

Broadhurst, M.K., Millar, R.B. and Brand, C.P. (2009) “Mitigating discard mortality from dusky flathead platycephalus fuscus gillnets,” *Diseases of Aquatic Organisms*, 85(2), pp. 157–166. doi:10.3354/dao02063.

Broadhurst, M.K., Suuronen, P. and Hulme, A. (2006) “Estimating collateral mortality from towed fishing gear,” *Fish and Fisheries* , 10, pp. 180–218.

Broom, D. (2007) “Cognitive Ability and Sentience: Which aquatic animals should be protected,” *Diseases of Aquatic Organisms*, 75, pp. 99–108. Available at: www.int-res.com.

Broom, D. and Johnson, K. (1993) *Animal Welfare Stress and Animal Welfare*. 2nd edn. London: Chapman & Hall. Available at: http://www.springer.com/series/5675.

Broom, D.M. (2001) *The evolution of pain*, *Flemish Veterinary Journal*.

Cannon, W.B. (1932) *The wisdom of the body*. New York: W.W. Norton & Company, Inc,.

Cashion, T. *et al.* (2018) “Reconstructing global marine fishing gear use: Catches and landed values by gear type and sector,” *Fisheries Research*, 206, pp. 57–64. doi:10.1016/j.fishres.2018.04.010.

Chrousos, G.P. (2009) “Stress and disorders of the stress system,” *Nature Reviews Endocrinology*, 5(7), pp. 374–381. doi:10.1038/nrendo.2009.106.

Cook, K. v. *et al.* (2011) “Fitness and behavioral correlates of pre-stress and stress-induced plasma cortisol titers in pink salmon (Oncorhynchus gorbuscha) upon arrival at spawning grounds,” *Hormones and Behavior*, 60(5), pp. 489–497. doi:10.1016/j.yhbeh.2011.07.017.

Crespo, G.O. and Dunn, D.C. (2017) “A review of the impacts of fisheries on open-ocean ecosystems,” *ICES Journal of Marine Science*, 74(9), pp. 2283–2297. doi:10.1093/icesjms/fsx084.

Cushing, D.H. (1987) “Population biology and the management of fisheries,” *Trends in Ecology & Evolution*, 2(5), pp. 138–139.

Davies, R.W.D. *et al.* (2009) “Defining and estimating global marine fisheries bycatch,” *Marine Policy*, 33(4), pp. 661–672. doi:10.1016/j.marpol.2009.01.003.

Davis, K.B. (2006) “Management of Physiological Stress in Finfish Aquaculture,” *North American Journal of Aquaculture*, 68(2), pp. 116–121. doi:10.1577/a05-007.1.

Davis, M.W. (2005) “Behaviour impairment in captured and released sablefish: ecological consequences and possible substitute measures for delayed discard mortality,” *Journal of Fish Biology*, 66, pp. 254–265. doi:10.1111/j.1095-8649.2004.00602.x.

Davis, M.W. (2007) “Simulated fishing experiments for predicting delayed mortality rates using reflex impairment in restrained fish,” *ICES Journal of Marine Science*, 64(8), pp. 1535–1542. Available at: https://academic.oup.com/icesjms/article/64/8/1535/612639.

Davis, M.W. (2010) “Fish stress and mortality can be predicted using reflex impairment,” *Fish and Fisheries*, 11(1), pp. 1–11. doi:10.1111/j.1467-2979.2009.00331.x.

Davis, M.W., Olla, B.L. and Schreck, C.B. (2001) “Stress induced by hooking, net towing, elevated sea water temperature and air in sablefish: Lack of concordance between mortality and physiological measures of stress," *Journal of Fish Biology*, 58(1), pp. 1–15. doi:10.1006/jfbi.2000.1399.

Davis, M.W. and Ottmar, M.L. (2006) "Wounding and reflex impairment may be predictors for mortality in discarded or escaped fish," *Fisheries Research*, 82(1–3), pp. 1–6. doi:10.1016/j.fishres.2006.09.004.

Dayton, P.K. *et al.* (1995) "Environmental effects of marine fishing," *Aquatic Conservation: Marine and Freshwater Ecosystems*, 5(3), pp. 205–232. doi:10.1002/aqc.3270050305.

Depestele, J. *et al.* (2014) "Short-term survival of discarded target fish and non-target invertebrate species in the 'eurocutter' beam trawl fishery of the southern North Sea," *Fisheries Research*, 154, pp. 82–92. doi:10.1016/j.fishres.2014.01.018.

Digre, H. *et al.* (2016) "Pumping of mackerel (Scomber scombrus) onboard purse seiners, the effect on mortality, catch damage and fillet quality," *Fisheries Research*, 176, pp. 65–75. doi:10.1016/j.fishres.2015.12.011.

Donaldson, E.M. and Fagerlund, U.H.M. (1968) "Changes in the Cortisol Dynamics of Sockeye Salmon (Oncorhynchus nerka) Resulting from Sexual Maturation," *General and Comparative Endocrinology*, 11(3), pp. 552–561.

Erikson, U., Hultmann, L. and Erik Steen, J. (2006) "Live chilling of Atlantic salmon (Salmo salar) combined with mild carbon dioxide anaesthesia: I. Establishing a method for large-scale processing of farmed fish," *Aquaculture*, 252(2–4), pp. 183–198. doi:10.1016/j.aquaculture.2005.05.013.

Evans, D.H. (2009) *Osmotic and Ionic Regulation: Cells and Animals*. 3rd edn. Edited by D.H. Evans. Boca Raton: CRC Press.

FAO (2021) *The State of Food Security and Nutrition in the World 2021*, *The State of Food Security and Nutrition in the World 2021*. ROME: FAO, IFAD, UNICEF, WFP and WHO. doi:10.4060/cb4474en.

Finley, C. (2016) "The Industrialization of Commercial Fishing, 1930–2016," in *Oxford Research Encyclopedia of Environmental Science*. Oxford University Press. doi:10.1093/acrefore/9780199389414.013.31.

FishCount (2020) *Fishcount estimates of numbers of individuals killed in (FAO) reported fishery production*. Available at: http://fishcount.org.uk/studydatascreens/2016/fishcount_estimates_list.php (Accessed: May 5, 2022).

Freire, C.A. and Prodocimo, V. (2007) "Special Challenges to Teleost Fish Osmoregulation in Environmentally Extreme or Unstable Habitats," in Baldisserotto, B., Mancera Romero, J.M., and Kapoor B.G. (eds) *Fish Osmorregulation*. 1st edn. Enfield: Science Publishers, pp. 249–276.

Gabriel, Otto. *et al.* (2005) *Fish catching methods of the world*. 4th edn. Oxford: Blackwell Pub.

Gadomski, D.M., Mesa, M.G. and Olson', T.M. (1994) "Vulnerability to predation and physiological stress responses of experimentally descaled juvenile chinook salmon, Oncorhynchus tsha wytscha," *Environmental Biology of Fishes*, 39, pp. 191–199.

Gingerich, A.J. *et al.* (2007) "Evaluation of the interactive effects of air exposure duration and water temperature on the condition and survival of angled and released fish," *Fisheries Research*, 86(2–3), pp. 169–178. doi:10.1016/j.fishres.2007.06.002.

Gregory, N.G. (1998) *Animal Welfare and Meat Science*. Edited by Gregory N.G. Oxon: CABI Publishing.

Hatløy, T. (2015) *Effekten av akutt allostatisk belastning på hypothalamus-hypofyse-interrenal aksen, og dets betydning på dyrevelferden hos diploid og triploid atlantisk laksesmolt (Salmo salar L.)*.

Hay, D.E. *et al.* (2001) *Herring: Expectations for a New Millennium Taking Stock: An Inventory and Review of World Herring Stocks in 2000*.

He, P. (1991) "Swimming endurance of the Atlantic cod, Gadus morhua L., at low temperatures," *Fisheries Research*, 12, pp. 65–73.

Huntingford, F.A. *et al.* (2006) "Current issues in fish welfare," *Journal of Fish Biology*, 68, pp. 332–372. doi:10.1111/j.1095-8649.2005.01046.x.

í Kongsstovu, S. *et al.* (2022) "Atlantic herring (Clupea harengus) population structure in the Northeast Atlantic Ocean," *Fisheries Research*, 249. doi:10.1016/j.fishres.2022.106231.

International Association for the Study of Pain (IASP) (2021) *Pain Terms and Definitions* . Available at: https://www.iasp-pain.org/resources/terminology/#pain (Accessed: December 28, 2021).

Iwama, G.K., Afonso, L.O.B. and Vijayan, M.M. (2006) "Stress in Fish," in Evans, D.H. and Claiborne, J.B. (eds) *Physiology of Fishes*. 3rd edn. Boca Raton, Flo.: CRC press, pp. 320–342. doi:10.1080/17451000.2016.1169299.

Jennings, S. *et al.* (2001) *Marine Fisheries Ecology*. 1st edn. New Jersey: Blackwell Science. Available at: www.cefas.co.uk.

Jennings, S. *et al.* (2016) "Aquatic food security: insights into challenges and solutions from an analysis of interactions between fisheries, aquaculture, food safety, human health, fish and human welfare, economy and environment," *Fish and Fisheries*, 17(4), pp. 893–938. doi:10.1111/faf.12152.

Jennings, S. and Kaiser, M.J. (1998) "The Effects of Fishing on Marine Ecosystems," *Advances in Marine Biology*, 34, pp. 201–352.

Joseph, J., Shipley, O.N. and Siskey, M.R. (2019) "Open ocean fisheries for large pelagic species," in Cochran, J.K., Bokuniewicz, H.J., and Yager, P.L. (eds) *Encyclopedia of Ocean Sciences*. 3rd edn. Stony Brook, NY,: Academic Press, pp. 470–479. doi:10.1016/B978-0-12-409548-9.11375-2.

Kaiser, M.J. and de Groot, S.J. (2000) *Effects of fishing on non-target species and habitats: biological, conservation and socio-economic issues*. 2nd edn, *Aquatic*

Conservation: Marine and Freshwater Ecosystems. 2nd edn. Oxford: Blackwell Science. doi:10.1002/aqc.492.

Karlsson-Drangsholt, A. *et al.* (2018) "Recovery from exhaustive swimming and its effect on fillet quality in haddock (Melanogrammus aeglefinus)," *Fisheries Research*, 197, pp. 96–104. doi:10.1016/j.fishres.2017.09.006.

Kim, H.-Y. (2013) "Statistical notes for clinical researchers: assessing normal distribution (2) using skewness and kurtosis," *Restorative Dentistry & Endodontics*, 38(1), p. 52. doi:10.5395/rde.2013.38.1.52.

Kruskal, William.H. and Wallis, W.Allen. (1952) "Use of Ranks in One-Criterion Variance Analysis," *Journal of the American Statistical Association*, 47(260), pp. 583–621.

Lerfall, J. *et al.* (2015) "Pre-mortem stress and the subsequent effect on flesh quality of pre-rigor filleted Atlantic salmon (Salmo salar L.) during ice storage," *Food Chemistry*, 175, pp. 157–165. doi:10.1016/j.foodchem.2014.11.111.

Lockwood, S.J., Pawson, M.G. and Eaton, D.R. (1983) "The effects of crowding on mackerel (Scomber scombeus L.)- Physical condition and mortality," *Fisheries Research*, 2, pp. 126–147.

Mann H.B. and Whitney D.R. (1947) "On a test of whether one of two random variables is stochastically larger than the other," *Annals of Mathematical Statistics*, 18, pp. 50–60.

Marçalo, A. *et al.* (2006) "Sardine (Sardina pilchardus) stress reactions to purse seine fishing," *Marine Biology*, 149(6), pp. 1509–1518. doi:10.1007/s00227-006-0277-5.

Marçalo, Ana *et al.* (2010) "Fishing simulation experiments for predicting the effects of purse-seine capture on sardine (Sardina pilchardus)," *ICES Journal od Marine Science* , 67(2), pp. 334–344. Available at: https://academic.oup.com/icesjms/article/67/2/334/694741.

Milligan, C.L. and Girard, S.S. (1993) "Lactate Metabolism in Rainbow Trout," *J. exp. Biol*, 180, pp. 175–193.

Mommsen, T.P., Vijayan, M.M. and Moon, T.W. (1999) "Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation," *Reviews in Fish Biology and Fisheries*, 9, pp. 211–268.

Mood, A. (2010) *Worse Things Happen at Sea: The Welfare of Wild-Caught Fish*.

Nédélec, C. and Prado, J. (1990) "Definition and classification of fishing gear categories." Rome: FAO.

Nelson JA and Chabot D (2011) "General Energy Metabolism," *Encyclopedia of Fish Physiology: From Genome to Environment,*. e. d. Edited by Farrell A.P. London: Academic Press. doi:10.1016/B978-0-1237-4553-8.00148-9.

NOAA Fisheries (2022) *Atlantic Herring*. Available at: https://www.fisheries.noaa.gov/species/atlantic-herring (Accessed: May 6, 2022).

Noble, C. *et al.* (2020) *Welfare Indicators for farmed rainbow trout: tools for assessing fish welfare*. Available at: https://nofima.no/fishwell/trout/.

North, B.P. *et al.* (2006) "The impact of stocking density on the welfare of rainbow trout (Oncorhynchus mykiss)," *Aquaculture*, 255(1–4), pp. 466–479. doi:10.1016/j.aquaculture.2006.01.004.

Olsen, R.E. *et al.* (2012) "Physiological response and mortality caused by scale loss in Atlantic herring," *Fisheries Research*, 129–130, pp. 21–27. doi:10.1016/j.fishres.2012.06.007.

Olsen, S.H. *et al.* (2013) "Capture induced stress and live storage of Atlantic cod (Gadus morhua) caught by trawl: Consequences for the flesh quality," *Fisheries Research*, 147, pp. 446–453. doi:10.1016/j.fishres.2013.03.009.

ood and Agriculture Organization of the United Nations (FAO) (2020) *The State of World Fisheries and Aquaculture 2020*, *INFORM*. American Oil Chemists Society. doi:10.4060/ca9229en.

Parker, S.J. *et al.* (2003) "Discard Mortality of Trawl-Caught Lingcod in Relation to Tow Duration and Time on Deck," *North American Journal of Fisheries Management*, 23(2), pp. 530–542. doi:10.1577/1548-8675(2003)023<0530:dmotcl>2.0.co;2.

Paszke, A. *et al.* (2019) "PyTorch: An Imperative Style, High-Performance Deep Learning Library."

Pauly, D. and Zeller, D. (2016) "Catch reconstructions reveal that global marine fisheries catches are higher than reported and declining," *Nature Communications*, 7, pp. 1–9. doi:10.1038/ncomms10244.

Pikitch, E.K. *et al.* (2014) "The global contribution of forage fish to marine fisheries and ecosystems," *Fish and Fisheries*, 15(1), pp. 43–64. doi:10.1111/faf.12004.

Poli, B.M. *et al.* (2005) "Fish welfare and quality as affected by pre-slaughter and slaughter management," *Aquaculture International*, 13, pp. 29–49.

Power M. J. *et al.* (2007) *Evaluation of 4VWX herring*.

Quinn, G.P. and Keough, M.J. (2002) *Experimental Design and Data Analysis for Biologists*. Cambridge: Cambridge University Press.

Raby, G.D. *et al.* (2012) "Validation of reflex indicators for measuring vitality and predicting the delayed mortality of wild coho salmon bycatch released from fishing gears," *Journal of Applied Ecology*, 49(1), pp. 90–98. doi:10.1111/j.1365-2664.2011.02073.x.

Raby, G.D. *et al.* (2014) "The understudied and underappreciated role of predation in the mortality of fish released from fishing gears," *Fish and Fisheries*, 15(3), pp. 489–505. doi:10.1111/faf.12033.

Robb, D.H.F. and Kestin SC (2002) "'Methods Used to Kill Fish: Field Observations and Literature Reviewed,'" *Animal Welfare*, 11, pp. 269–282.

Rose, J.D. *et al.* (2014) “Can fish really feel pain?,” *Fish and Fisheries*, 15(1), pp. 97–133. doi:10.1111/faf.12010.

Rudershausen, P.J., Buckel, J.A. and Hightower, J.E. (2014) “Estimating reef fish discard mortality using surface and bottom tagging: Effects of hook injury and barotrauma,” *Canadian Journal of Fisheries and Aquatic Sciences*, 71(4), pp. 514–520. doi:10.1139/cjfas-2013-0337.

Sapolsky, RM. (2004) *Why Zebras Don’t Get Ulcers*. 3rd edn. New York: New York: Henry Holt and Company.

Savina, E. *et al.* (2016) “Testing the effect of soak time on catch damage in a coastal gillnetter and the consequences on processed fish quality,” *Food Control*, 70, pp. 310–317. doi:10.1016/j.foodcont.2016.05.044.

Schindelin, J. *et al.* (2012) “Fiji: An open-source platform for biological-image analysis,” *Nature Methods*, 9(7), pp. 676–682. doi:10.1038/nmeth.2019.

Selye, H. (1950a) “Stress and the general adaptation syndrome,” *British medical journal*, 1(4667), pp. 1383–1392.

Selye, H. (1950b) *The Physiology and Pathology of Exposures to Stress*. Montreal: Acta Medica Publ.

Shapiro, S.S. and Wilk, M.B. (1965) “An Analysis of Variance Test for Normality (Complete Samples),” *Biometrika*, 52, pp. 591–611.

Sistiaga, M. *et al.* (2015) “Effect of lifting the sweeps on bottom trawling catch efficiency: A study based on the Northeast arctic cod (Gadus morhua) trawl fishery,” *Fisheries Research*, 167, pp. 164–173. doi:10.1016/j.fishres.2015.01.015.

Smith, L.S. (1993) “Trying to Explain Scale Loss Mortality: A Continuing Puzzle,” *Reviews in Fisheries Science*, 1(4), pp. 337–355. doi:10.1080/10641269309388549.

Smith, W.E. and Scharf, F.S. (2011) “Postrelease survival of sublegal southern flounder captured in a commercial gill-net fishery,” *North American Journal of Fisheries Management*, 31(3), pp. 445–454. doi:10.1080/02755947.2011.590116.

Sneddon, L.U. *et al.* (2014) “Defining and assessing animal pain,” *Animal Behaviour*, 97, pp. 201–212. doi:10.1016/j.anbehav.2014.09.007.

Sneddon, L.U. (2015) “Pain in aquatic animals,” *Journal of Experimental Biology*. Company of Biologists Ltd, pp. 967–976. doi:10.1242/jeb.088823.

Sneddon, L.U. *et al.* (2018) *Fish sentience denial: Muddying the waters*, *Animal Sentience*. Available at: www.liv.ac.uk/integrative-.

Sneddon, L.U. (2019) “Evolution of nociception and pain: Evidence from fish models,” *Philosophical Transactions of the Royal Society B: Biological Sciences*, 374(1785). doi:10.1098/rstb.2019.0290.

Sneddon, L.U., Braithwaite, V.A. and Gentle, M.J. (2003) “Do fishes have nociceptors? Evidence for the evolution of a vertebrate sensory system,” *Proceedings of the Royal Society B: Biological Sciences*, 270(1520), pp. 1115–1121. doi:10.1098/rspb.2003.2349.

Soengas, J.L. *et al.* (2018) “Oxygen Uptake and Osmotic Balance of Atlantic Salmon in Relation to Exercise and Salinity Acclimation Salmon Swimming in Different Salinities,” 5. doi:10.3389/fmars.2018.00368.

Sopinka, N.M. *et al.* (2016) “Stress Indicators in Fish,” in Schreck, C.B., Tort, L., and Farrell, A.P.B.C.J. (eds) *Fish Physiology*. Academic Press, pp. 405–462. doi:10.1016/B978-0-12-802728-8.00011-4.

Stephenson, R.L. *et al.* (2001) “Herring Stock Structure, Stock Discreteness, and Biodiversity,” in Funk, F. et al. (eds) *Herring : expectations for a new millennium : proceedings of the symposium Herring 2000, expectations for a new millennium*. 1st edn. Anchorage, Alaska, USA: University of Alaska Sea Grant College Program, pp. 559–573.

Stephenson, R.L. and Smedbol, R.K. (2019) “Small Pelagic Species Fisheries,” *Encyclopedia of Ocean Sciences*, pp. 503–509. doi:10.1016/B978-0-12-409548-9.11491-5.

Suuronen, P. and Erickson, D.L. (2010) “Mortality of Animals that Escape Fishing Gears or Are Discarded after Capture: Approaches to Reduce Mortality,” in He, P. (ed.) *Behavior of Marine Fishes*. Ames: Wiley-Blackwell, pp. 265–293.

Suuronen, P., Lehtonen, E. and Jounela, P. (2005) “Escape mortality of trawl caught Baltic cod (Gadus morhua) - The effect of water temperature, fish size and codend catch,” *Fisheries Research*, 71(2), pp. 151–163. doi:10.1016/j.fishres.2004.08.022.

Svalheim, R.A. *et al.* (2020) “Simulated trawling: Exhaustive swimming followed by extreme crowding as contributing reasons to variable fillet quality in trawl-caught Atlantic cod (Gadus morhua),” *PLoS ONE*, 15(6 June). doi:10.1371/journal.pone.0234059.

Tenningen, M. *et al.* (2019) *Fangstkontroll og fiskevelferd i ringnotfiske etter sild*.

Tenningen, M., Vold, A. and Olsen, R.E. (2012) “The response of herring to high crowding densities in purse-seines: Survival and stress reaction,” *ICES Journal of Marine Science*, 69(8), pp. 1523–1531. doi:10.1093/icesjms/fss114.

Topic Popovic, N. *et al.* (2012) “Tricaine methane-sulfonate (MS-222) application in fish anaesthesia,” *Journal of Applied Ichthyology*, 28(4), pp. 553–564. doi:10.1111/j.1439-0426.2012.01950.x.

Uhlmann, S.S. *et al.* (2016) “Injury, reflex impairment, and survival of beam-trawled flatfish,” *ICES Journal of Marine Science*, 73(4), pp. 1244–1254. doi:10.1093/icesjms/fsv252.

Varsamos, S. *et al.* (2006) “Husbandry stress during early life stages affects the stress response and health status of juvenile sea bass, Dicentrarchus labrax,” *Fish and Shellfish Immunology*, 20(1), pp. 83–96. doi:10.1016/j.fsi.2005.04.005.

Veiseth, E. *et al.* (2006) “Accelerated recovery of Atlantic salmon (Salmo salar) from effects of crowding by swimming,” *Comparative Biochemistry and*

Physiology - B Biochemistry and Molecular Biology, 144(3), pp. 351–358. doi:10.1016/j.cbpb.2006.03.009.

Veldhuizen, L.J.L. *et al.* (2018) "Fish welfare in capture fisheries: A review of injuries and mortality," *Fisheries Research*, 204, pp. 41–48. doi:10.1016/j.fishres.2018.02.001.

van de Vis, H. *et al.* (2003) *Is humane slaughter of fish possible for industry?*, *Aquaculture Research*.

Weber Iii, E.S. (2011) "Fish analgesia: Pain, stress, fear aversion, or nociception?," *Veterinary Clinics of North America - Exotic Animal Practice*, 14(1), pp. 21–32. doi:10.1016/j.cvex.2010.09.002.

Winger, P.D., Eayrs, S. and Glass, C.W. (2010) "Fish Behavior near Bottom Trawls," in He, P. (ed.) *Behavior of Marine Fishes: Capture Processes and Conservation Challenges*. Wiley-Blackwell, pp. 65–103. doi:10.1002/9780813810966.ch4.

Woodruff, M.L. (2018) "Pain in fish: Evidence from peripheral nociceptors to pallial processing," *Animal Sentience*, 3(21). doi:10.51291/2377-7478.1321.

Zydlewski, J., Zydlewski, G. and Danner, G.R. (2010) "Descaling Injury Impairs the Osmoregulatory Ability of Atlantic Salmon Smolts Entering Seawater," *Transactions of the American Fisheries Society*, 139(1), pp. 129–136. doi:10.1577/t09-054.1.

Popular science summary

With scientific proof that fish experience stress and pain the same way as mammals, there has been an increasing interest in fish wellbeing. Humans are constantly interacting with fish, and directly or indirectly, some of the ways they interact impact fish's wellbeing. The most detrimental one is, without a doubt, commercial fisheries. There's a wide variety of fishing methods, and all of them depend on the captured species and their environment.

This research project aims to assess fish welfare in pelagic fisheries, an active commercial fishing method and how fish are being killed. Two research trips were made to obtain this information: One onboard a pelagic trawler, a boat designed to target fish in the middle of the water column, and the other is a smaller vessel that caught fish with hook and line. First, it was necessary to quantify how many of the sampled individuals were reactive to human touch and then check for any physical damage. Additionally, blood was taken to quantify the stress levels using specific physiological indicators and X-rays were done to check for internal damage.

The results indicate that pelagic trawls are extremely stressful and painful compared to hook and line. Although most fish showed no physical or internal damage, a significant percentage was dead coming to the trawler. In addition, there was a major scale loss in trawled individuals, but such was not found when capturing with hook and line. Looking at the stress indicators, it is clear that trawled fish presented extreme values indicating that they are submitted to more stressful conditions than the hook and line.

There's a combination of factors that lead to high levels of stress and mortality. Fish are dragged in a packed net for hours, and they clash with each other and the gear making them lose their scales. Although it is still not possible to tell the exact cause of death, one can tell those fisheries are not precisely the synonym for fish wellbeing. Further studies are needed, but there is room to make fisheries a more ethical and sustainable practice.

Acknowledgements

First and foremost, I'd like to thank my supervisor Albin Gräns for his time, patience and constant insight. I am incredibly grateful as well for Jane Behrens, my second supervisor, whose discussion and advice contribute a lot to my thesis. I owe a great depth to you both for letting me "pick a bit of your brain". A huge thanks to all the people involved in the project "Humane slaughter in pelagic fishery", I'm inspired and grateful for all our work

I also need to thank the friends who made Sweden feel like home, both in Uppsala and Gothenburg. You guys are pretty sweet. A special appreciation to the Vasagatan group who handled all my breakdowns: Laura Gyllix, Laura Lechner, Léna Reverte, Matilda Floresjö and Vera Roberts, who supported me through this process whether through discussing my work even when not understanding half of the words, dinners filled with laughter or just their company. I spent a blissful time with you girls. But unfortunately, the universe does not have enough cake to express my appreciation. Everyone should have you in their lives!

An appreciative hug filled with *saudade* to all the friends back in Portugal, I can't wait to see you again. It would be impossible to name you all, but your support was not unnoticed. Thank you for filling my life with magic and being patient even when I disappear for months not answering any calls. A massive thanks to Sofia Raimundo, whose voice on our calls always sounds like home. You are the best! Much love to the brilliant Margarida Gomes for a friendship of a lifetime filled with outlandish support and encouragement. Thank you for being there! You a beautiful human being.

Finally, I need to thank my family for their love and constant support. My parents for allowing me to pursue my dreams, for everything really. There are not enough words in this world to show my gratitude and love. Million thanks to my aunt for the friendship, generosity and excellent advice. Love and appreciation to my sister Diana, who always makes me feel extra clever; I lean on you more than you know.

Publishing and archiving

Approved students' theses at SLU are published electronically. As a student, you have the copyright to your own work and need to approve the electronic publishing. If you check the box for **YES**, the full text (pdf file) and metadata will be visible and searchable online. If you check the box for **NO**, only the metadata and the abstract will be visible and searchable online. Nevertheless, when the document is uploaded it will still be archived as a digital file. If you are more than one author, the checked box will be applied to all authors. Read about SLU's publishing agreement here:

- https://www.slu.se/en/subweb/library/publish-and-analyse/register-and-publish/agreement-for-publishing/.

☒ YES, I/we hereby give permission to publish the present thesis in accordance with the SLU agreement regarding the transfer of the right to publish a work.

☐ NO, I/we do not give permission to publish the present work. The work will still be archived and its metadata and abstract will be visible and searchable.