

Commissioner:
Francien de Jonge
Wageningen UR Science Shop

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*Welfare of wild caught plaice (*Pleuronectes platessa*)*

An inventory how current practices in fisheries may affect welfare of plaice and possible indicators thereof



Project nr: 1311
Project title: An inventory how current practices in fisheries may affect welfare of fish and possibilities of improvement thereof

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5/2/2014

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Executive summary

During the last decades, the awareness about the welfare of fish in fisheries has been continuously increasing. Also in the Netherlands, the welfare of wild caught fish is becoming a relevant and important topic. However, compared with husbandry systems and farmed fish, the research on wild caught fish is lacking behind. To provide concrete in-depth knowledge, this study focused on one wild caught fish species. Since plaice (*Pleuronectes platessa*) is one of Europe's commercially most important species, plaice was chosen to be the species of focus for this study. The purpose of this report is to deliver a list of indicators for the welfare of plaice and to discuss the applicability of these indicators in practice. Subsequently, these indicators can be used as a measure for the welfare of wild caught plaice during the fishing process. The report is intended to provide the Wageningen UR Science Shop with background knowledge on factors that can affect the welfare of fish during the different stages of the fishing process (capturing, bringing on deck and killing) and indicators which can be used to assess the welfare of plaice. Interviews with experts and extensive literature research were conducted in order to collect the needed information. The evaluation of all the collected information showed that the welfare of plaice is likely to be impacted during the different stages of the fishing process and that several indicators can be used to assess the welfare of plaice. Physiological parameters are accurate to assess the welfare but are mostly not applicable on board of a fishing boat as a laboratory environment is needed to perform the measurements. Indicators that can be used in practice are mostly environmental based indicators, such as hauling speed and duration, the amount of fish and debris in the net and the handling of plaice. Applicable indicators that can be measured on plaice itself are limited to the amount of injuries, the condition of the slime layer and rigor mortis (the time it takes the animal to become stiff after dead). As rigor mortis is related to different physiological parameters, it can be a good indicator to assess the welfare. It is recommended that the effect of different stressors in the fishing process should be tested in a laboratory environment, so that the physiological response of plaice can be measured and linked to the environmental indicators, injuries and rigor mortis. To improve the welfare of wild caught plaice during the fishing process, it is important to investigate and engineer possible improvements of the methods used to catch, bring on deck and kill plaice. Examples hereof are the use of pumps to bring the fish on deck, stunning of the fish before killing them and more selective and less welfare impacting fishing gears. Furthermore, it should be investigated whether the results found on plaice can be extrapolated as well to other species. This because the ultimate goal is the creation of a welfare label for wild caught fish. Therefore, the role of stakeholders like supermarkets, NGOs, consumers and fisheries should also be investigated.

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Introduction

Animals are an important food source for humans and are therefore kept for production on a large scale. This might affect the welfare of these animals when they suffer due to the way in which they are kept and/or killed. The treatment and use of animals has long been a subject of intense debate in Western culture (Preece, 1999). This debate is dating back at least to ancient Greece (Sorabji, 1993) and has been involving deeply rooted cultural values (Fraser, 2001). During the last decades, the attention paid to animal welfare issues has increased, in the Netherlands as well as worldwide. Not only NGOs, but also the government, consumers and producers pay more attention to animal welfare. Welfare assessment programs and guidelines for animal welfare are well developed for husbandry systems. Extensive research has been done on the welfare of pigs (Mul *et al.*, 2010; Sainsbury, 1986; Grandin, 2003; Faucitano and Schaefer, 2008), cattle (Rushen *et al.*, 2007; Grandin, 1998; Philips, 2008; Von Keyserlingk *et al.*, 2009), and poultry (van Horne and Achterbosch, 2008; Prescott *et al.*, 2003; Kristensen and Wathes, 2000). Due to the increase in societal awareness about animal welfare, especially in Europe, Canada, Australia and New Zealand, attention has been drawn to fish welfare in aquaculture (van de Vis *et al.*, 2014) and since the last decade also to fish welfare in fisheries. However, when compared to husbandry systems, welfare assessment programs, guidelines and research are lacking behind for fish. Only little research is available on the welfare and guidelines for welfare in farmed fish (van de Vis *et al.*, 2012; Bovenkerk and Meijboom, 2013), while even less research addresses welfare in commercially caught wild fish (Mood, 2010). However, the fishing industry is a significant part of the economy in the Netherlands. It generates around €386 million per year and 450.000 tonnes of fish are caught every year (Government of the Netherlands, 2013). It is estimated that, with an average weight of 7 kg per fish, this corresponds to 0.064 billion fish and with an average weight of 0.3 kg per fish even to 1.5 billion fish captured each year (van de Vis, personal communication 2014). Thus one can imagine that fish welfare is a relevant and important topic in the Netherlands. The awareness towards of this topic is increasing since the last years and more research is being done on it (figure 1). Research indicates that, in contrast to the perception of people in the past, fish are likely to be able to feel and experience pain and fear (Braithwaite and Huntingford, 2004; Braithwaite *et al.*, 2013; Chandroo *et al.*, 2004). Therefore, it is important to investigate on the welfare of wild caught fish during the three different steps of the fishing process: capturing, bringing the fish on deck and killing.

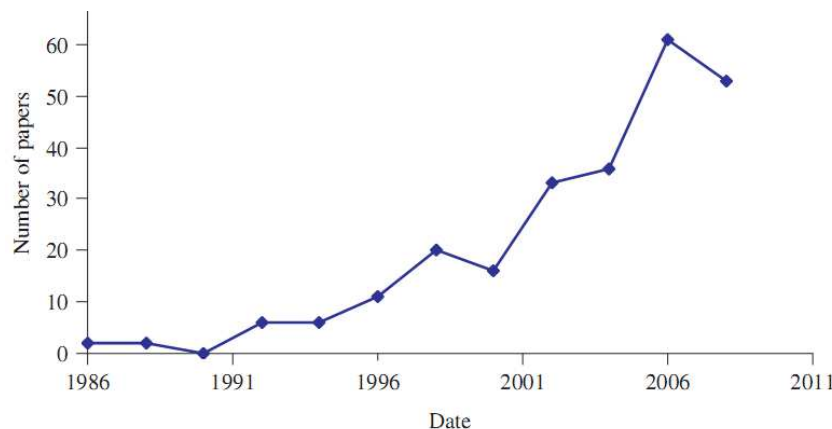


Figure 1. "The number of papers published with the keywords welfare and aquaculture in 3 years time bins, from a Web of Science search" (Huntingford and Kadri, 2009).

The Dutch Foundation for the Protection of Fish (Stichting Vissenbescherming) is concerned about the welfare of fish and encourages the development of new techniques to improve the welfare of fish in aquaculture and fisheries. The foundation wants to develop a fish welfare label for fisheries which takes fish welfare into account. Recent research has shown that 33% of the consumers prefers

products with a label, as labels are associated with better quality products and better conditions during production (De Hek *et al.*, 2012). In addition, the development of a fish-welfare label can create consumer awareness about the welfare of fish and stimulate the market for fish products with better welfare and thus more welfare concerning fishery practices. In order to develop such a label, knowledge is required about the factors that influence the welfare of the fish during the fishing process. To do that, the Dutch Foundation for the Protection of Fish requested a project at the Wageningen UR Science Shop. For this project, the factors which influence the welfare of fish during the fishing process are scientifically investigated. The project focuses on three captured fish species, namely plaice (*Pleuronectes platessa*), sole (*Solea solea*) and common dab (*Limanda limanda*). The overall goal of the project is to come up with indicators that can be used to assess the welfare of fish and that can be used to design a label. However, the main focus of this report will be on the commercially most important fish species in the Netherlands, namely plaice (Visbestanden in de Noordzee, 2013). The fact that only one species of focus is chosen allows to come up with concrete knowledge which is practical and can be implemented more easily. Nonetheless, the findings on plaice can be used to make assumptions about other fish species.

This project will deliver a list of indicators for the welfare of plaice and the applicability of these indicators in practice. These indicators can be used as a measure for the welfare of wild caught plaice during the three different steps of the fishing process.

“The main problem is that it is not clear how the welfare of plaice is affected in the current fishing practices during the capturing of the fish, bringing the fish on deck and killing of the fish. Research on this subject needs to be done in order to develop indicators of welfare that can be used to create a label.”

In order to solve this problem and to provide knowledge about how the welfare of plaice is affected during the capturing, bringing on deck and killing processes, the following research questions have been formulated and will be answered in this report:

Main research question:

What are operational indicators for the welfare of wild caught plaice during the three processes of fishing: capturing, bringing on deck and killing?

Sub research questions:

- How can fish welfare be assessed by using the allostasis concept?
- Which parameters are known from farmed and wild caught fish that indicate the welfare of fish?
- What are the life history characteristics of plaice?
- Which methods are used to capture, bring on deck and kill plaice?
- Which factors during the three processes of fishing (capturing, bringing on deck and killing) might affect the welfare of plaice, taking into account the life history characteristics?
- Which parameters indicating welfare known from literature can be used to assess the welfare of plaice?

Methods

This chapter about research methods gives an overview of the procedure used to address the research questions and for the data collection. These include literature reviews and interviews.

Literature review

Literature review involved extensive reading, analyzing, evaluating and summarizing of the available literature on a specific topic, in this case current practices in fisheries that may affect the welfare of plaice. Since there is still a knowledge gap in the area of wild caught plaice, articles were reviewed on farmed fish as a starting point. The knowledge obtained from the literature on farmed fish was extrapolated to wild caught plaice and represents an important source of information for this study. In this way for instance, information could be gathered on which types of indicators can be used to assess the welfare of fish. Furthermore, literature provided information on the life history of plaice, the concept of fish welfare and the fishing methods. The literature gave the background that was needed to perform interviews with experts that were needed to be able to answer the research questions about how the welfare of plaice might be affected and about the possible indicators for welfare and the applicability of these indicators.

Interviews

An interview is a method of data collection that involves asking a series of questions. Research interviews are seen as a specific kind of interaction in which the researcher and the interviewee produce data about how knowledge is categorized (Green and Thorogood, 2013). Depending on how the interview is structured, the researcher can either set the agenda in terms of the topics covered or allow the interviewees to develop their own accounts of the issues that are important to them. Two types of interviews were conducted in this study: face to face interviews and interviews via Skype. These are described in more detailed below. In total there were four interviews performed, two with experts on fish welfare and two with experts on fishery practices. In these interviews, an in depth approach was taken to gather valuable information and understanding of the topics instead of remaining at the surface. Therefore, the type of interview chosen for this study was a semi-structured interview, in which an additional set of questions form the basis of the interview, with room for follow-up questions and new ideas based on the information from the interviewee. As there is a limited amount of persons specialized in the area of fish welfare in the Netherlands, it was very valuable that the four people interviewed in this study were willing to cooperate.

Face to face interviews

Face to face interviews are also called in-person interviews and are probably the most popular and oldest form of a method for data collection. Face to face interviews can minimize non-responsiveness and maximize the quality of the data collected. It also makes it easier for the respondent to either clarify answers or ask for clarification about items on the questionnaire. The face to face interviews were arranged taking into account distance, cost, time and willingness of respondents to participate. The arrangement of the interview dates and times was done via email contact with the respondents. Two of the experts were interviewed via a face to face interview, Jacob Kramer (Director of Ekofish Group in Urk) and Ruud van den Bos (Researcher at the department of Organismal Animal Physiology, Radboud University Nijmegen). The questions for the interview were set up before the interview took place (as can be seen in appendix 1 and 2 respectively), but the interviews had an open and informal character with space for additional information and follow-up questions. In the report, the interviewees are cited by giving their name and the term: interview. (For example: (Kramer, interview)). A summary of the interviews can be found in the appendices (appendix 5 and 6).

Skype interviews

Skype interviews are interviews that are conducted via a video link using the computer program Skype™ (www.skype.com, 11-4-2014). The popularity of Skype interviews is increasing due to their cost effectiveness by reducing travelling time. Skype allows the interviewer and interviewees to talk in the same setting but effectively and at no cost to either party. The Skype interviews were prepared by first adding the respondent to the contact list of one of the interviewers. A voice recorder, Callnote Premium (www.kandasoft.com, 11-4-2014), was downloaded and installed to record the interviews so that no relevant information was missed. The equipment was tested on functionality by testing the sound, connection, camera and recording via a Skype call between two team members. In the same way as with the face to face interviews, the questions for the interview were set up before the interview took place (as can be seen in appendix 3 and 4 respectively), but the interviews had an open and informal character with space for additional information and follow-up questions. Two experts were interviewed via a Skype interview, Bob van Marlen (IMARES, expert on fishing practices) and Hans van de Vis (IMARES, expert on fish welfare in aquaculture and fisheries). In the report, the interviewees are cited in the same way as the face to face interviewees by giving their name and the term: interview. (For example: (Kramer, interview)). The transcripts of the Skype interviews can be found in the appendices (appendix 7 and 8 respectively).

Fish welfare

Concept of fish welfare

In order to assess how the welfare of plaice is affected during the process of capturing, bringing on deck and killing, it is important to address the concept of welfare in fish. Before the welfare of fish can be assessed, it is first important to investigate whether fish can experience pain, stress and fear, and thus whether their welfare can be compromised. An overview will be provided here on findings presented in the debate about the presence of feelings in fish.

The ability to experience emotions is an adaptive trait that allows animals to respond flexibly to their environment. Animals that avoid harmful experiences and look for positive experiences have a higher chance of survival compared to those that do not do this (Braithwaite *et al.*, 2013). The response of animals to aversive stimuli can be investigated by measuring their behaviour and physiology in response to a painful stimulus. The next step is to locate the neural areas that are associated to the experience of this stimulus and the response of the fish (Braithwaite *et al.*, 2013). The response of fish to aversive stimuli has been investigated by Sneddon *et al.* (2003) in rainbow trout for example. When the fish was injected with an acetic acid substance as a painful stimulus, it responded with both behaviour, for instance a longer time to resume feeding, and physiology, for instance an increase in opercular beat rate. This stimulus also showed the activation of nociceptors that are known to detect noxious stimuli in mammals (Sneddon *et al.*, 2003). However, it is argued that the presence of these nociceptors and its effect on the behaviour in fish is no proof that fish also experience pain (Rose, 2007). The implications for welfare do not only depend on the ability to adjust to pleasant and dangerous aspects of the environment, but also to the extent to which animals are consciously experiencing emotions. There is a difference in changes in behaviour as a consequence of neural reflexes or changes in behaviour of which the animal is aware (Braithwaite *et al.*, 2013). Different approaches are suggested to the importance of unconscious reflexes and conscious responses in the welfare of fish. According to Duncan (1996), to consider welfare of fish, they need to have cognition and be aware of their feelings. However, Rose (2007) suggests that also reflexive responses can be included in the welfare of fish, since a constant disturbance of homeostasis can cause disturbed behaviour and physiological regulation.

Responding consciously allows selective attention to stimuli (internal and external), anticipation or expectation and goal-directed behaviour, which allow animals to be more flexible in their response to stimuli (Chandross *et al.*, 2004). Several of these capacities have been found in fish (Braithwaite and Boulcott, 2007; Braithwaite *et al.*, 2013). Braithwaite and Boulcott (2007) argue that fish do not only respond with reflexes, because they learned to associate a light cue to an electric shock and consequently responded with avoidance behaviour towards the light cue. This learned avoidance is too complex to be just a reflex (Braithwaite and Boulcott, 2007). It has also been found that fish can determine their fighting behaviour based on what they observed from their opponent in the past, using not only the outcome (win or lose) but also the aggressiveness of the component during the observed fights (Chandross *et al.*, 2004). In their review, Braithwaite *et al.* (2013) present several other examples that show that the responses of fish to aversive stimuli are more than just reflexes and involve some cognitive processes: fish are able to solve the reversed reward task by choosing the smallest reward (fish choose between two rewards; they get the opposite reward than the one chosen), fish value their feeder differently when they have once experienced an electric shock when approaching the feeder and fish behave differently in response to pain depending on the social context. Following the reasoning of Braithwaite *et al.* (2013), the behaviours from these examples are likely to require both cognition and emotion in fish, following the analogy postulate to compare observations in fish to observations in humans and feelings associated with it. Furthermore, evidence was found that fish have similar brain structures to mammals involved in cognition and emotion (Broglio *et al.*, 2005). The hippocampus and the amygdala are areas in the mammalian brain that are

closely linked to cognition and emotions, which are involved in timing, spatial learning, memory and processing emotional valued information respectively (Braithwaite and Boulcott, 2007). Areas that are homologous to these areas have been identified in fish: the lateral pallial region of the forebrain has a similar function as the hippocampus and the medial pallial region of the forebrain is comparable in function to the amygdala (Broglia *et al.*, 2005). Damage to the lateral region shows an impaired ability to solve spatial tasks or associative learning based on a temporal relationship, while a damaged medial region leads to an impaired performance on avoidance in conditioning tasks (Broglia *et al.*, 2005).

According to several authors (Volpato *et al.*, 2007; Rose, 2007), these examples of fish behaving consciously in different tasks and situations, are still no proof that fish actually experience feelings. As Rose (2007) points out, the disturbance of homeostasis should be used as reasoning for animal welfare, but this is hard to use for the welfare of wild caught fish, since their welfare is only impacted by the fishery during the fishing process, capturing, bringing on deck and killing. In this short time frame, the disturbance of homeostasis is only of short duration and not a big concern for the welfare, whereas the potential experience of pain and suffering of the fish is an important issue for the welfare during the fishing process (Rose, 2007). According to Rose (2002), the absence of the cerebral cortex, which is responsible for the psychological experience of pain, in fish is reason to assume fish do not experience pain. However, it should be mentioned that following this reasoning, the experience of pain is also absent in very young children, elderly people with Alzheimer and all other animals except higher primates (van de Vis, personal communication 2014). Although it is not possible to prove the subjective experience of feelings in other beings, results from several studies on behaviour and neurological systems make the assumption of the experience of pain in several species of fish (rainbow trout, Atlantic cod, common goldfish and Atlantic salmon) likely (Sneddon, 2002; Nilsson *et al.*, 2002; Nordgreen, 2009).

Although the welfare of fish should be judged as objectively as possible, moral considerations do play a role as well. The public opinion is that the society is responsible for the welfare of the fish and that it should minimize any potential suffering (Ohl and van der Staay, 2012). As the results found from several studies suggest that fish are able to experience emotions and are capable of suffering (Braithwaite and Huntingford, 2004; Braithwaite and Boulcott, 2007; Braithwaite *et al.*, 2013; Chandroo *et al.*, 2004), a precautionary principle can be supported and a start can be made to investigate how fish welfare might be compromised and how this can be improved. However, it remains important to continue with investigating the more basic question on whether fish indeed experience pain, fear and stress.

Models to measure welfare

To measure the welfare of animals, a model is needed that can assist in giving indicators for welfare. The most commonly used model in husbandry systems is the model of the five freedoms: freedom from hunger and thirst, freedom from discomfort, freedom from pain, injury and disease, freedom to express normal behaviour and freedom from fear and distress (Botreau *et al.*, 2007). These five freedoms are used to formulate 4 criteria for a welfare assessment (good feeding, good housing, good health and appropriate behaviour), which are divided in twelve subcriteria (see Botreau *et al.*, 2007). These criteria include both the absence of negative aspects and the presence of positive aspects (Botreau *et al.*, 2007). The focus of this model based on the five freedoms is still on the negative aspects (Ohl and van der Staay, 2012). However, it is stated by Duncan (1996) that welfare is not only about negative emotions, but also about positive ones. The welfare of an individual cannot only be considered good when negative experiences are absent, also behaviours that satisfy needs and desires contribute to welfare by giving individuals a positive feeling (Ohl and van der Staay, 2012). Another remark on the five freedoms can be placed on the approach towards more ethical science, with less attention to the biology science (Korte *et al.*, 2007). Furthermore, the five

freedoms focus on the effect of the environment on the animal and do not pay much attention to the adaptive capacity of the animal to be able to interact with the environment (Ohl and van der Staay, 2012). Despite these critical notes on the five freedoms, it is a useful model for measuring welfare that is implemented most in husbandry systems.

A different model to measure the welfare of individuals is the allostasis concept, which was developed based on the following reasoning. When considering the definition of welfare proposed by Broom (1986), it states that 'the welfare of an individual is its state as regards its attempts to cope with its environment', indicating the importance of taking into account the ability of the individual to adapt to the environment. As Korte *et al.* (2007) argue, animals in their natural environment do not live in utopia, and challenges, like hunger and diseases, are realistic in their natural habitat. According to Broom (1986), the welfare of an individual can be considered poor when it has difficulties or is unable to cope with these challenges. This capacity of individuals to cope with environmental challenges depends on its history of environmental challenges and balance between negative and positive states (van den Bos, interview). Individuals that have experienced more environmental variation are better able to adjust to a challenge than those that have been kept in a stable environment (van den Bos, interview). When an individual is for example challenged by a pathogen, it is more likely to be able to cope with it when its immune system is built up by previous challenges than if the individual has never been into contact with any threats to its immune system. Furthermore, the ability to cope with environmental challenges depends on the internal balance between positive and negative experiences (van den Bos, interview). Individuals that have experienced too much negative situations and only little positive ones to balance the negative out, perceive a challenge as more stressful compared to those that have more positive experiences (van den Bos, interview). Based on this theory, the concept of allostasis is formulated, which uses the physiology of the animal to judge its welfare (Korte *et al.*, 2007). The allostasis concept entails that physiological parameters of an individual, such as cortisol, neurotransmitters and immune responses, can increase as a response to environmental changes. This increase can be adaptive, when the benefits of the appropriate response are higher than the costs of producing the responses, but damaging if the costs of the response outweigh the benefits (Korte *et al.*, 2005). In the latter case, known as allostatic load, the individual might not be able to cope with repeated challenges, or might fail to end the physiological response after the challenge is over, or it might not be able to produce an adequate response to the challenge (Korte *et al.*, 2005). Thus, in these situations it can be said that the individual is not able to cope and its welfare is impaired (Broom, 1986). The concept of allostasis can be visualised as an inverted U-shaped curve (figure 2). When the animal is not subjected to any environmental challenges, its welfare is impaired because it is not able to cope with even the smallest environmental challenges, as can be seen in the example of the immune system. However, when the animal is subjected to too big environmental challenges, its welfare is also impaired (Korte *et al.*, 2007). In between these extremes, there is a range in which the animal is able to adapt to the environmental challenges so that these challenges improve its welfare (Korte *et al.*, 2007). The consequence of under- or overstimulation to the animal, is a reduction in the range of environmental challenges to which the animal can adapt and thus under- or overstimulation are reached sooner (Korte *et al.*, 2007).

This research will reason mostly following the allostasis model. Although the five freedoms are mostly used, it seems less appropriate when discussing the welfare of wild caught plaice. In wild caught systems, the welfare is mostly affected on short term, and animals are not kept for longer periods of time as in husbandry systems and aquaculture. Consequently, most criteria (absence of prolonged hunger/thirst, comfort around resting, thermal comfort, absence of disease, expression of behaviours) used in the five freedom model are less applicable to this situation. The allostasis model fits this research better, since it includes measurable physiological parameters. The use of these physiological parameters also makes the assessment of welfare an objective matter.

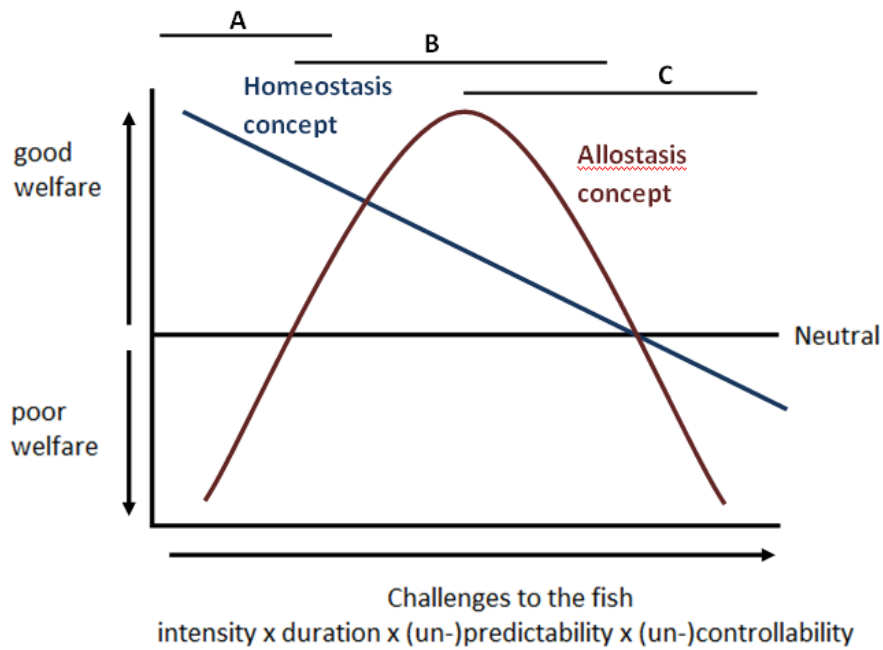


Figure 2. Diagram showing relationships between poor and good welfare and the environmental challenges experienced by the fish: linear model according to the homeostasis concept and hyperbolic model according to the allostatic concept. Challenge varies as a result of intensity, duration, predictability and controllability of stressors. At low environmental challenge hypostimulation may produce poor welfare conditions (distress). Certain degrees of environmental challenge (eustress) will improve welfare (hyperbola above the neutral welfare line). Experiments will be designed to expose fish to hypostimulation (A), to eustress (B) and to distress (C). Region B represents allostatic load, beneficial stress conditions that the fish can cope with; region C represents allostatic overload, stress conditions that will cause illness and death. Hypostimulation has not been studied in fish so far. Modified after: Korte *et al.*, 2007.

Indicators for fish welfare

To determine whether a fish is overtaxed or when the stress levels are considered to be too high, parameters need to be measured that can give an indication about the welfare of fish. There are many parameters which can be used to assess the welfare of fish. The possible indicators for fish welfare can be classified based on the following categories (Huntingford *et al.*, 2006):

- Behavioural indicators
- Physiological indicators
- Physical indicators
- Indicators of environmental quality

In this study, the focus will be on the physiological and physical indicators of fish welfare. This includes the response of the physical and chemical processes in the fish to stress and stimuli and how they impact on the welfare of the fish. Behavioural indicators include mostly behaviours in response to long term stressors, which can be very useful when assessing the welfare in farmed systems (Martins *et al.*, 2012). These indicators include foraging behaviour, ventilatory activity, aggression, individual and group swimming behaviour and stereotypic behaviours (Martins *et al.*, 2012). These behaviours are species specific and even depend on the coping style of the individual (Martins *et al.*, 2012). For this reason, it is difficult to extrapolate behavioural indicators to plaice, and since it is mostly used as indicator on the long term, it is not considered in this report as the focus lies on the fishing processes of capturing, bringing on deck and killing, which affects welfare more on short term. As for the indicators of the environmental quality, they are important for the welfare of the fish, because the welfare might be impacted by environmental variables as for instance the hauling speed. However, this kind of indicators differs a lot between farmed fish and wild caught fish and will be handled in much detail later in the report, while this chapter focuses on the welfare indicators that are known from literature and that might be extrapolated to wild caught plaice.

Physical parameters

Physical parameters concern injuries and deformities to various body parts (Noble *et al.*, 2012). Research on physical indicators of welfare in fish has mostly been done in farmed fish (Huntingford *et al.*, 2006; Noble *et al.*, 2012; Ashley, 2007). However, some aspects of aquacultural systems can be compared to aspects of fisheries for wild caught fish. Therefore, the knowledge on physical indicators of welfare in fish from farmed systems that can be extrapolated to wild caught fish will be investigated here. For this review, the focus will be on injuries, since these are most relevant when studying the impact on welfare of wild caught systems, which do not entail keeping fish for longer periods. Although there is still a debate about whether fish are sentient beings, there is some evidence that fish can feel pain (see Concept of fish welfare). Consequently, it is reasonable to assume that fish welfare will be impaired by injuries (Huntingford *et al.*, 2006). This is supported by the finding of Turnbull *et al.* (2005) that fish with a worse fin condition also have a higher plasma glucose and cortisol levels, suggesting that injuries on the fish are related to higher stress levels. High frequencies of injuries or slow recovery from injuries due to cortisol suppressing the immune system can be a sign of poor welfare (Huntingford *et al.*, 2006). As the slime layer and the scales of fish have a function in the osmoregulation of the fish, damage to these parts are expected to have a great impact on the fish (Ashley, 2007; van de Vis, interview). When the fish cannot maintain its osmolarity, its homeostasis is disturbed and this might cause stress in the fish (van de Vis, interview).

Besides the possible influence on the welfare, injuries also have a great importance from an economic perspective. Vagsholm and Djupvik (1998) report a price decrease of 2% in Atlantic salmon when the amount of injuries increases with 10%. It is likely that injuries have a similar effect on the

price of plaice. According to van de Vis (interview), the quality of plaice and, related to that, the price of plaice are affected by the appearance of the fish. Thus, more injuries are likely to reduce the price because of decreased appealing appearance. Moreover, injuries are also likely to reduce the shelf life of the meat. The slime layer and scales of fish function as a barrier to infection and in farmed fish, it has been found that injured fish are more susceptible to bacterial infections (Noble *et al.*, 2012). Although no research has been done on the effect of injuries on shelf life, it is likely that also shelf life is reduced by injuries through the same mechanism (van de Vis, interview).

Physiological parameters

Different physiological parameters have been proved to be possible indicators for the stress levels in fish. These indicators are mainly based on brain function, haematic parameters, tissue parameters and meat quality.

Brain function

Brain function can be an indicator for the sensibility and consciousness of fish. To assess the brain function, an Electrocardiogram (ECG) can be applied in a laboratory. But also responses to flashes of light directed towards the eyes (photic stimulation) and responses evoked by stimulating nerves (somatosensory) can be used to measure the consciousness of fish (Kestin *et al.*, 1991; Kestin, 1994; Robb *et al.*, 2000; van de Vis *et al.*, 2001, 2003; Kestin *et al.*, 2001; Lambooj *et al.*, 2002; Robb and Roth, 2003).

Haematic indicators

The term homeostasis refers to an open dynamic system that is kept in balance by internally regulated processes (Schulkin, 2004). In fish, like in all other vertebrates, this means that temperature, blood pH and solutes are kept in a range in which the normal physiological functions are maintained. Stress leads to an endocrine response (expressed by the brain) that causes changes of the previous parameters. The endocrine response as well as the caused changes in the blood can be measured and used as welfare indicators (Poli *et al.*, 2005).

Adrenaline

When stressed, fish first respond with the release of an increased amount of adrenaline (endocrine). Because it is difficult to determine and it is not staying in blood for a long time, adrenaline is not typically used as an indicator (Wendelaar Bonga, 1997).

Cortisol

As a consequence of stress, cortisol is being released from the interrenal tissue into the blood stream. Cortisol is a commonly used indicator for short- and long-term stress circumstances. It has been shown that multiple stress situations augment the cortisol response (Ortuño *et al.*, 2002). It is important to take into account that husbandry conditions, feeding, reproductive cycles and seasonal cycles can have an influence on the cortisol level, meaning that cortisol levels in the non-stressed state can vary (Pickering *et al.*, 1982; Pickering and Pottinger, 1985).

Plasma glucose

As a consequence of the endocrine response more energy is made available by accelerated plasma glucose production. Plasma glucose is an easily measurable indicator for stress. Nonetheless it has been stated that its release can be delayed (Barry *et al.*, 1993).

Non esterified fatty acids (NEFA)

Another mechanism that provides more energy is the mobilization of fat reserves as a consequence of the expression of cortisol. This leads to an increase in NEFA concentration in the blood. It has been shown that NEFA can also be used to determine the stress level in fish (Barton and Iwama, 1991).

Haematocrit value

The endocrine stress response causes higher muscular activity, as the fish is either more active or shows freezing behaviour because of a stressor. This leads to an elevation in heart-beat and a need of greater oxygen uptake. The latter causes a rise of the haematocrit value that can easily be measured. Nonetheless typical values need to be determined for each species to be able to use haematocrit as a stress indicator (Reddy and Leatherland, 1988).

Muscle Tissue

Muscle pH and lactic-acid concentration

Stress induces greater muscular activity which causes a higher energy demand. Because of that, anaerobic glycolysis is initiated which leads to an increase in plasma lactate that in most fish is stored in the muscle. The rise in lactate concentration leads to a decrease in muscle pH. (Erikson *et al.*, 1999, Oka *et al.*, 1990; Lowe *et al.*, 1993; Marx *et al.*, 1997; Robb and Warriss, 1997)

Muscle energy reserve

Phosphocreatine, ATP/ADP/AMP and glycogen are indicators for the amount of energy stored in the muscle and can be used as early stress indicators, as shown for eel (Morzel and van de Vis, 2003). Muscular activity as well as stress prior and during killing have a negative influence on the amount of available energy. Furthermore, early lack of energy leads to a faster onset of rigor mortis (Erikson, 1997; Pottinger, 2001; Tejada *et al.*, 2001).

Rigor mortis

Stress and the related consequences mentioned above can have a great influence on the biochemical processes after death (post mortem). As a result of increased muscular activity, high stress levels and higher endocrine response, the onset of rigor mortis can be affected (Poli *et al.*, 2012). Rigor mortis can be used as a good tissue indicator for welfare of plaice before killing. As Amlacher (1961) summarized, the cause of rigor mortis onset is the disappearance of ATP in the muscles. Where ATP normally binds with calcium ions in the fish muscle, when a fish is death this calcium is released and the softening effect of ATP disappears. With this information it can be argued that when fish is severely stressed before it is killed there is earlier rigor mortis onset because of the early lack of ATP (Poli *et al.*, 2012). Frequently, rigor mortis starts in the caudal muscles (Verma and No, n.d.), possibly because these muscles are most active in the death struggle and have therefore less ATP available. Rigor mortis onset can therefore be a good indicator to determine how stressed the fish was before it was killed. Lastly when there is delayed rigor mortis onset there is more time available to fillet the fish before the fish stiffens resulting in increased quality and size of the fillet and shelf life is increased (Jerret *et al.*, 1996, Robb and Kestin, 2002, Wilkinson *et al.*, 2007).

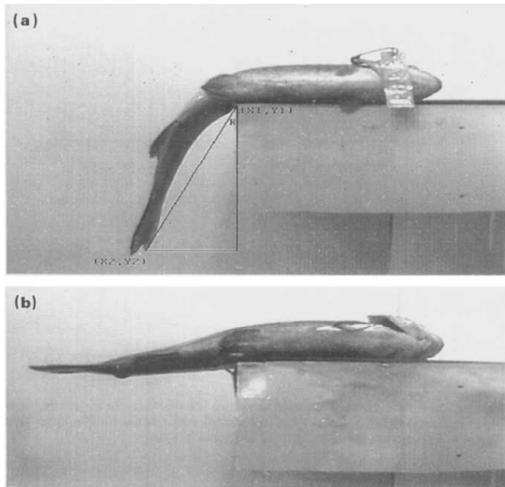


Figure 3. Rigor mortis (Azam *et al.*, 1990).

Meat quality

It has been shown that stress prior to killing and stress caused by killing can have an influence on the quality of the meat. For instance gaping and blood spotting of the meat are such indicators of stress. Also shelf life can be used as a indicator of stress. It is related to the time of rigor mortis, which can be used as a stress indicator as well. A shorter shelf life of the fillet might be an indicator of lower welfare in wild caught fish. Finally, increased stress levels showed to have an impact on the texture, taste, flavor and odors of cooked fish fillets too (Poli *et al.*, 2005).

Life history characteristics of plaice (*Pleuronectes platessa*)

Plaice is one of the flatfishes that are characterized by both of their eyes being on the right side of their body. The species can be recognized by the orange spots on the body. Furthermore, plaice can adjust its body pattern to match the environment for camouflage purpose (Kelman *et al.*, 2006). Plaice have no swimbladder (van Emmerik, 2007). They can reach a body length of maximum 100 cm, and they can get up to 50 years old (ICES, 2005). Plaice can be found over a large area following the coast of Europe, from north to south ranging from the White Sea and Iceland all the way to the western Mediterranean Sea (ICES, 2005). Plaice are benthic fish, living at the bottom of the sea. They predate mostly upon thin-shelled molluscs, crustaceans and polychaetes (ICES, 2005; FishWise, 2012).

Reproduction

The age of maturation is lower for males (2-3 years) than for females (4-5 years) (ICES). At the age of reproduction, males have reached a size of 18-26 cm, half of the males are reproductively active at a length of 22 cm. Females are 30-35 cm long at the age of reproduction and half of them are reproductively active at a length of 34 cm (van Emmerik, 2007). Depending on their size, females can lay around 60 000 to 100 000 eggs each year (ICES). Adult plaice are solitary animals (Barnes, 2014) and migrate around January to specific spawning areas to reproduce. During spawning time plaice rely entirely on energy reserves as capital breeders (Rijnsdorp, 1994). Spawning takes place when the water temperature is approximately 6 °C (van Emmerik, 2007; Freyhof, 2011). During the migration to spawning areas, and back to their feeding areas, plaice make use of selective tidal transport (Arnold and Metcalfe, 1995). They swim in mid-water to be carried downstream and move to the bottom when the tide turns. The tidal stream used to return to the feeding area is the opposite of the stream used to reach the spawning area (Arnold and Metcalfe, 1995). Plaice migrates solitary to the spawning areas where large groups of plaice are present (van Emmerik, 2007). The females lay the eggs in open water after which they are fertilized by the males (van Emmerik, 2007). When the eggs are laid, it takes three to four months for plaice offspring to develop from egg and larval stage to the final metamorphosis phase. Larvae of plaice and juvenile plaice can be found in shallow water close to the coast and in estuaries, while older, larger individuals gradually move to greater depths (ICES). At greater depths, more polychaetes and amphipods can be found, which are common species preyed upon by plaice (Gibson *et al.*, 2002). These species are larger prey and more profitable for larger plaice than the prey species found in more shallow water (Gibson *et al.*, 2002). This adaptive response also increases survival for larvae and juvenile plaice through reduction in predation risk, since they are most preyed upon. Being in shallow water has some direct and indirect benefits against predation. First, important predators like shrimps and shore crabs occur in deeper water, thus by settling at depths that may even be less than one meter, juvenile plaice minimize predation risk (Gibson *et al.*, 2002). Furthermore, plaice can escape predation by reaching their refuge size, at which they are much less vulnerable for predation. Juvenile plaice risk predation by shrimps until a size of 35 mm, when grown larger they are out of reach for shrimp (van der Veer and Bergman, 1987). For other predator species (grey gurnard, poor-cod, whiting and 0-group cod), it was found that plaice were not vulnerable for predation when a size of 45 mm was reached (Ellis and Gibson, 1995). Since temperatures are higher in less deep water, growth of plaice is stimulated here so that they sooner reach a size where predation risk is minimal (Gibson *et al.*, 2002). A research studying habitat quality and the distribution of plaice also shows a strong positive correlation between the amount of juvenile plaice and temperature (Lauria *et al.*, 2011). An additional benefit of the shallow water is a reduction in competition with dab. Plaice and dab have highly overlapping diets, and by settling on a more shallow habitat than dab, more food is available for plaice and thus plaice can achieve higher growth rates to reach their refuge size sooner (Gibson *et al.*, 2002). To reach the shallow water, plaice makes use of selective tidal transport by the same mechanism used by adults

during migration. Both larvae and juvenile plaice were found to use tides to move to more shallow water by travelling to mid-water during flood (Kuipers, 1973; Rijnsdorp *et al.*, 1985). Despite these adaptations, mortality of plaice is still very high in the egg and larval stage: more than 99% does not metamorphose into the juvenile stage (van Emmerik, 2007).

Habitat quality

Some studies have investigated the effects of habitat quality on the distribution of plaice (Gibson, 1994; Lauria *et al.*, 2011). Many factors of habitat quality are interrelated and which is most important is therefore difficult to determine (Gibson, 1994). As already mentioned before, predation risk is an important factor determining the distribution of mostly juvenile plaice. A clear difference in predation and mortality can be found between nursing areas. In the Wadden Sea, predation and mortality of larvae is relatively low. During summer, approximately 14 % of larval and juvenile plaice dies each month. Whereas the mortality in English nursing areas, where larvae settle in deeper water, can be up to 50% per month in summer (Bergman *et al.*, 1988). This is the result of the presence of much more predatory species in the deeper water in the English nursing areas, which are almost absent in the Wadden Sea (Bergman *et al.*, 1988). These results demonstrate the effects of depth on predation risk, and support the finding that juvenile plaice move towards shallow water to avoid predation (Gibson *et al.*, 2002). Water temperature and food availability are related to the water depth. These factors are not only important for juvenile plaice, but also for older plaice. Both have a strong effect on the growth rate of plaice, and therefore also on maturation. It was found that plaice occurring in more northern habitats matured later than plaice in the southern habitats, likely due to the lower temperature in the north (Bromley, 2000). Another important factor that determines habitat quality for plaice, is sediment structure (Gibson, 1994; Lauria *et al.*, 2011). Especially juvenile plaice prefer a fine sediment structure, older plaice are found on slightly coarser sediment structures (Lauria *et al.*, 2011). This preference is related to prey abundance and the possibility to bury themselves (Gibson, 1994). In finer sediment, plaice can bury themselves more easily and thus be more camouflaged for predators (Lauria *et al.*, 2011; Gibson and Robb, 1992). It is also suggested that flatfish having the possibility to bury themselves are more passive and in that way conserve energy by having lower metabolic, respiratory and heart rates. The latter was found in sole, a different flatfish species, when their activity in the presence of sand was compared to their activity in the absence of sand (Howell and Canario, 1987). Additionally, Gibson and Robb (1992) suggest that the burying of plaice is a mechanism to avoid drag forces from currents.

Environmental flexibility

As indicated before, plaice can be found at varying depths, with a positive correlation between size and depth. The depth range indicated in literature varies from maximal 100 m (Freyhof, 2011) to a maximum of 200 m (Barnes, 2014). In this range of depths, the temperature ranges from 2 to 15 degrees between seasons (FishWise, 2012). Temperatures above 28°C were found lethal for flatfish (Berghahn *et al.*, 1993). In hot summer months, juvenile flatfish in tidal pools can get overheated by the solar radiation and temperatures above 28°C, which causes damage to the skin and high mortality rates (Berghahn *et al.*, 1993). How sensitive plaice is to mortality due to temperature depends not only on the temperature, but also on the temperature difference and the speed at which the temperature changes (Berghahn *et al.*, 1993). Besides the temperature, also oxygen saturation is important for the survival of plaice. According to Scholz and Waller (1992), plaice need sufficient oxygen levels to survive. Plaice survive at a water oxygen saturation of approximately 30%. At a saturation of 20% half of the plaice die. When the oxygen saturation drops to 10%, plaice cannot survive anymore (Scholz and Waller, 1992).

Table 1. Life history characteristics of plaice

Life history characteristics	Reported for plaice	Reference
Maximum age	50 years	ICES, 2005
Maximum size	100 cm	ICES, 2005
Habitat	Demersal	Barnes, 2014
Feeding method	Predator/Scavenger	Barnes, 2014
Social structure	Solitary	Barnes, 2014
Age at maturity	2-3 (males), 4-6 (females)	ICES, 2005
Migratory	Seasonal (reproduction)	ICES, 2005
Reproductive season	Jan-Feb	Barnes, 2014
Reproductive strategy	Capital breeders	Rijnsdorp, 1994
Temperature range	2 – 15 °C	FishWise, 2012
Maximum depth	200 m	Barnes, 2014
Oxygen range	7.37 – 1.33 ml/l	OBIS, 2014

Methods for catching plaice

There are several methods used for catching fish. They range from using large nets to catch large groups of fish to fishing lines aimed to catch individual fish. For the catching of plaice in the Netherlands several methods are being used, namely: beam trawling, pulse trawling, SumWing, Hydrorig, Outrig, twinrig, flyshoot and gillnet (Visserij in cijfers, 2010). While beam trawling is still being used the most, adjustments are made to this method and new methods are developed. These new developments happen because of several reasons, being economical (to decrease fuel consumption) or ecological (less damage to the sea floor) (van Marlen, interview). In the next chapter the different methods that are being used in the Netherlands for catching plaice are explained.

Beam trawling

One of the most frequently used fishing methods is beam trawling. It is a very efficient method for fishing flatfish, especially for sole and plaice (van Beek *et al.*, 1990). The different trawling ships vary in width, from 4 to 12 meter, and can weigh up to 7.5 tons in air (de Groot and Lindeboom, 1994; Kaiser and Spencer, 1996). These ships have special designed tickler chains or a chain matrix that are situated in front of the net and are dragged over the sea bottom (Kaiser and Spencer, 1996). These chains scare up the fish and hereby increase the amount of targeted flatfish caught in the nets (Kaiser *et al.*, 1996). The concept of beam trawling is visualized in figure 4. Because the heavy fishing gear is dragged over the sea bottom, the bottom is ploughed and epifauna and infauna are damaged or dug out (Kaiser and Spencer, 1996). As specific parts of the North Sea can be trawled several times a year, this can have long lasting effects on the environment (North Sea Task Force, 1993; Kaiser and Spencer, 1996). Furthermore, there are excessive discards because trawling is not species and size specific. The heavy weight of the fishing gear and the resistance caused by the net and the tickler chains leads to a high fuel demand (Maarten *et al.*, 2012). There are several alternatives for the traditional beam trawling, as for example the SumWing, Hydrorig and Outrig. The SumWing can replace the beam in beam trawling. It has a wing profile which makes it more hydrodynamic. Besides it is filled with air, which makes it light in the water. Together this leads to 20% fuel saving compared with traditional beam trawling. This method can be used for all flatfish and has a comparable catch efficiency as the beam trawl, but it has less bycatch and the caught fish is of better quality (Productschap Vis, 2010^a; **Error! Hyperlink reference not valid.**, 7-4-2014). Hydrorig is another alternative for the traditional beam trawl. In this method, the beam is more hydrodynamic, which saves energy, and it generates a water flow which sucks the fish from the bottom. Because of this water flow, no tickler chains are necessary, which also saves energy and results in less bottom disturbance. The fish caught with this method is of good quality and there is also less bycatch compared to beam trawling, but the catching efficiency is lower than that of other methods (Productschap Vis, 2010^a; **Error! Hyperlink reference not valid.**, 7-4-2014). Outrig fishing is the last alternative for beam trawling that will be described here. In this method, both derricks of the ship (see figure 4) have a net attached, which is held open by trawl boards instead of by a beam. This is therefore also a variation on the otter trawl, which is used to catch roundfish. Light fishing gear is used and rubber lines are used instead of tickler chains to reduce energy costs (www.agrimatie.nl, 7-4-2014; www.vdbergfishing.nl, 7-4-2014).

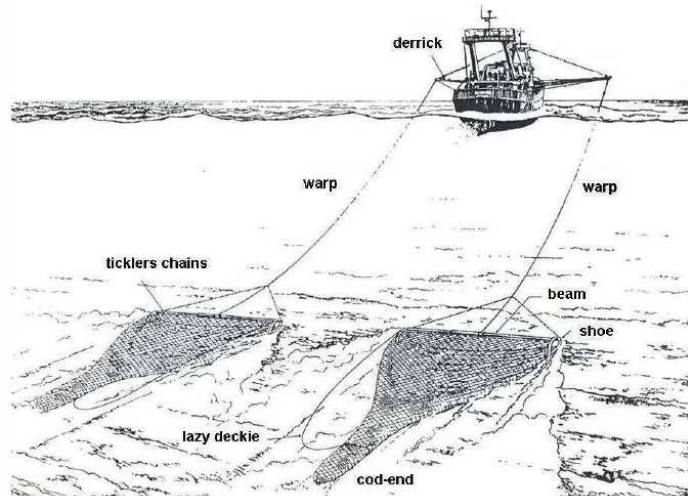


Figure 4. Beam trawling (de Boer and Vermeulen, 1976).

Pulse trawling

Pulse trawling, compared to beam trawling, uses electricity to startle the flatfish from the seabed instead of tickler chains. As an alternative for the tickler chains, the pulse trawl contains drag wires which conduct electric pulses from a pulse generator on board of the ship. These pulses are led from the generator to the net by an electrical feeding cable (figure 5). The drag wires generate a pulsating electric field, which startles the flatfish up from their position at the bottom and into the net. The response of the fish to this electric field is determined mainly by the strength of the electric field, the pulse frequency and amplitude and by the fish species and size of the fish (van Marlen *et al.*, 2014; Productschap Vis, 2010^a; www.visgroothandel.nl, 4-4-2014; Stewart, 1975).

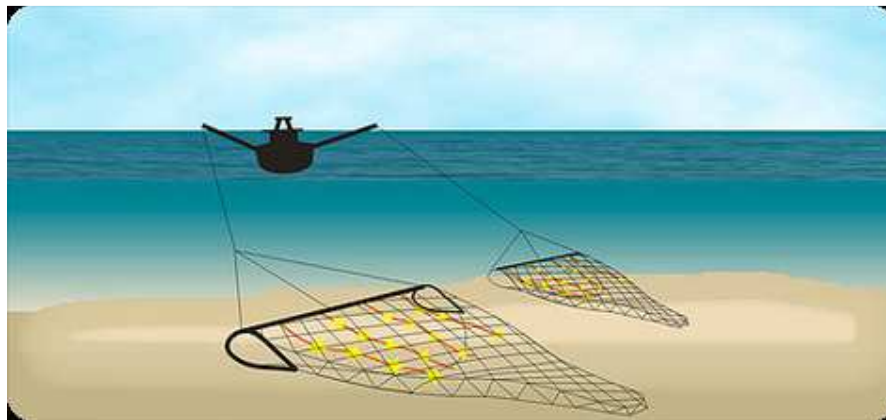


Figure 5. Pulse trawling (www.visgroothandel.nl, 4-4-2014).

Pulse trawling is more specific than beam trawling. While the catch efficiency (catch per unit area swept) does not differ significantly for pulse and beam trawling (van Marlen *et al.*, 2014). Furthermore, fewer undersized plaice is caught (figure 6) with the pulse trawl compared to the beam trawl. Also the catch of benthic invertebrates is reduced by 50% (van Marlen *et al.*, 1999, 2000). The probability to catch undersized plaice is lower in pulse trawling, because large fish are more strongly affected by the electric field than small fish and are therefore more likely to be caught with this method (van Marlen, interview; Stewart, 1975).

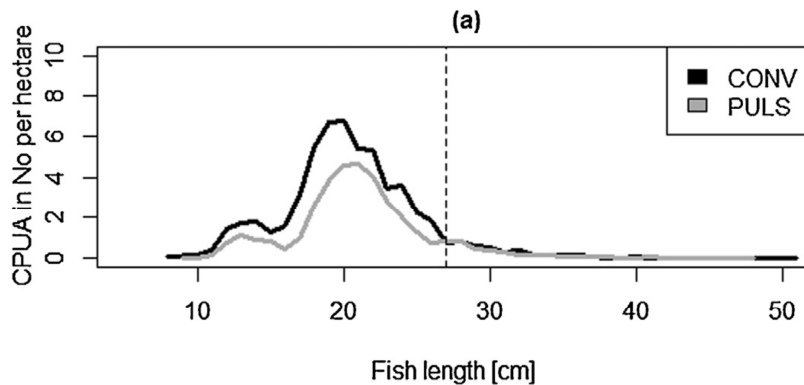


Figure 6. “Length distribution of plaice in numbers per hectare from hauls when landings and discards were both sampled. CONV = conventional ticklerchain beam trawl, PULS = pulse trawls (taken together), the dotted vertical line represents the Minimum Landing Size (MLS)” (van Marlen *et al.*, 2014).

The fishing gear used in pulse trawling has less contact with the sea bottom than the one used in beam trawling and is therefore less disturbing for the environment and less fuel consuming than beam trawling. Around 45% of energy savings is currently achieved when using pulse trawling compared with beam trawling. Besides this, also the quality of the caught fish is better, because the fish have less contact with fishing gear and material compared with beam trawling (Productschap Vis, 2010^a). An alternative form of pulse trawling is the Pulse Wing. This is a combination of pulse trawling and the previously described SumWing. This method is still in development, but the first indications are that the catch efficiency is comparable to that of the beam trawl and that this methods can lead to a 60% fuel saving compared to the traditional beam trawl (Productschap Vis, 2010^a).

Twinrig

Twinrig fishing uses two trawl nets that are connected to each other and are dragged behind the ship by three cables, one in the middle and two on both sides of the nets (figure 7). The two nets are connected with a weighting block, to pull the nets towards the bottom. On both sides of the nets are trawl boards to keep the net open. The trawl boards are connected to the net via the sweeps. The trawl boards and the centre weight are dragged over the bottom and the sweeps produce vibrations that startle the fish and make them swim to the middle of the net opening as they try to escape from the sweeps that pull the net. Due to exhaustion, the fish ends up in the net (Kramer, interview). Twinrig fishing is done at low speeds, to prevent the fish from escaping. This method can be used as an alternative for beam trawling to catch plaice. The fishing gear used in this method is relatively light compared with beam trawling and therefore this method uses less energy than beam trawling. Twinrig fishing is also a clean method, as the net does not scrape over the bottom, less stones and crustaceans are caught with this methods compared to beam trawling (Kramer, interview). (Productschap Vis, 2010^a; www.schmidtzeevis.nl, 7-4-2014).



Figure 7. Twinrig; a = trawl boards, b = cable to the centre weighting block, c = centre weighting block, d = sweeps (www.schmidtzeervis.nl, 7-4-2014).

Flyshoot

Flyshooting is a relatively clean and sustainable way of fishing (www.ekofishgroup.nl, 4-4-2014). At the start a seine rope is attached to a marking point (buoy) and subsequently the ship sails half a circle back to the starting point lowering the net halfway (Wieland *et al.*, 2009). When the ship reaches the starting point again the net is slowly pulled in as can be seen in figure 8. The seine ropes generate a dust cloud that startles the fish and in combination with the seine ropes forces the fish to keep swimming in front of the ropes towards the centre where the fish is finally caught in the net (www.ekofishgroup.nl, 4-4-2014). This means that the fish will only be in the net for the last 5 to 10 minutes of the haul, before the net is heaved in (Polet, n.d.). Because the vibrations created by the sweeps are too small to startle the fish, the dust cloud is needed to startle them. Therefore this method is only effective during daylight as the visibility of the dust cloud is crucial to startle the fish.

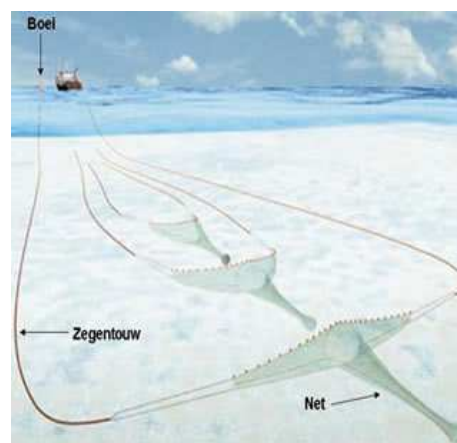


Figure 8. Flyshoot fishing method (www.ekofishgroup.nl, 4-4-2014).

Gillnet

Gillnet fishing is only used to a small extent to fish for plaice in the Netherlands. It is a passive fishing method in which the net is anchored at the sea bottom and kept to the bottom by a line with lead weights as can be seen in figure 9. The net is kept in vertical position by floats on the top of the net. Buoys are attached to the net as a mark for the fishermen. Gillnets are very selective, as their mesh size is specific for a certain size of fish. Small fish will easily swim through the meshes of the net, while fish that are too big are prevented from swimming through the net at all. Fish which match the size the net was designed for will attempt to swim through it but will get stuck in the net. When they try to swim backwards to escape from the net, the wires entangle the fish behind their gills. The fish will be kept in the gillnet until the fishermen come to pick the net up and thus the fish might be stuck in the net for a long time. Gillnets are usually placed in long rows next to each other (Productschap Vis, 2010^b; www.miseagrant.umich.edu, 8-4-2014; www.visserbond.nl, 8-4-2014).

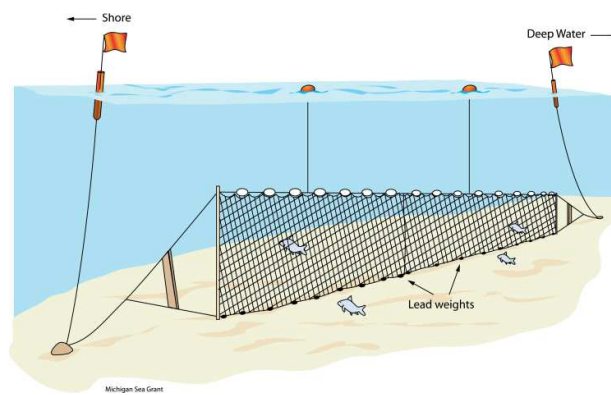


Figure 9. Gillnet fishing (www.miseagrant.umich.edu, 8-4-2014).

Bringing on deck and handling

The removal of fish from the water and the transportation to cages or containers can be a very stressful experience (Ashley, 2006). Nowadays the most commonly used method to transport the fish on board of the ship is by simply hauling the net in and open it on the ship (Huss, 1995). The fish are collected in tanks or containers waiting to be further processed. There are however some downsides to this method. Firstly, because of gravitational forces the fish that are located at the bottom of the net get crushed by other fish or receive injuries (Ashley, 2006; Conte, 2004). Secondly the time it takes for the fish to be removed from the water and moved into tanks is relatively long. In order to avoid these gravitational forces and limit the time spent outside the water a new method was designed in Norway. The fish in the net are pumped out of the net onto the ship (Isaksen and Midling, n.d.). It appears that moving the fish together with the water is the least invasive and stressful method for fish (Ashley, 2006; Conte, 2004). The handling of fish by fishers can also be a stressful situation which, if this handling is not done appropriately, can have serious consequences for the fishes. The handling can damage the mucus layer and fish scales (Conte, 2004). This coating protects the fish against diseases and helps with respiration, ionic and osmotic regulation (Shephard, 1994; Subramanian *et al.*, 2008) thus damage can reduce the level of protection. In order to minimize the impact of fish-handling on the fish wet hands or soft, wet gloves need to be worn during the handling (Conte, 2004; Theberghe and Parker, 2005). All the different handling processes need to be executed slowly and deliberate to avoid aversive reactions by the fish leading to increased activity and resulting exhaustion (Conte, 2004). Lastly temperature can play an important role by increasing stress levels in fish. Fish are stressed when there is a sudden change in temperature, as is happening when the fish is removed from the water, even when the temperature is in its tolerance range (Conte, 2004). Piper (1982) concluded that fish are especially vulnerable during periods of summer heat. Therefore it is recommended to avoid handling fish during high temperatures.

Killing Methods

There are several methods being used today in the Netherlands for the killing of plaice. In order for these methods to be considered humane these methods should induce unconsciousness immediately or induction should be without pain or fear (MAFF, 1995). According to Robb and Kestin (2002) the way of killing in farmed mammals and birds is to render them immediately unconscious by a physical blow to the head or an electrical current running through the head. However, the methods that are being used for the killing of plaice are not considered to be humane and may bring suffering to the plaice for long periods (Robb and Kestin, 2002). In the next chapter the methods used for the killing of plaice, namely asphyxiation, asphyxiation in ice slurry and evisceration (van de Vis, personal communication 2014) will be discussed. Also, electrical stunning, which can be an improvement of the killing methods with regard to fish welfare, will be discussed.

Asphyxiation

Asphyxiation is the most commonly used method for the killing of plaice and also of other fish. It is simply done by removing the fish from the water and expose it to air. However, the time that a fish remains alive outside the water is dependent on the fish species and the temperature (Kestin *et al.*, 1995; Robb and Kestin, 2002). Another factor that affects the time it takes for fish to die via this method is their resistance to anoxic conditions (Poli *et al.*, 2005). Because plaice lives in sometimes anoxic environments, it can survive on low oxygen concentration and therefore the time it takes for plaice to die via asphyxiation may take a long time. Research shows that this method is aversive to the fish since they attempt vigorously to escape and they exhibit a high stress responses (Robb and Kestin, 2002).

Asphyxiation in ice slurry

An alternative method for asphyxiation is asphyxiation in flake ice or ice slurry (Robb and Kestin, 2002; Poli *et al.*, 2005; Hastein, 2004). In this method plaice is moved directly in ice and the body temperature, metabolic rate and movements decrease fast (Poli *et al.*, 2005). In the end the fish will die because of anoxia and the temperature drop (Robb and Kestin, 2002). However, the oxygen requirements of the fish also decrease significantly when they are transferred into ice and therefore they will survive longer in the ice (Poli *et al.*, 2005). Research on the time it takes for plaice to die via this method is lacking, but from research on other fish species it is assumed that this can be for a long period (Kestin *et al.*, 1991; Poli *et al.*, 2005).

Evisceration

The second method being used to kill plaice is via evisceration. The intestines (viscera) and the heart are removed from the plaice and the plaice dies due to a combination of asphyxiation and exsanguination (Robb and Kestin, 2002). Exsanguination is performed, because blood within the fish body causes faster deterioration of the muscle after the fish has died and it causes undesirable marks in the flesh (Branson, 2008). According to the unpublished results by JW van de Vis (in Robb and Kestin, 2002) it takes around 40 minutes for plaice to die via the combination of asphyxiation and exsanguinations without ice slurry. For other animals exsanguination is preceded by electrical stunning which renders the animal insensible during the exsanguination period (Lines *et al.*, 2003). Research done by Robb *et al.* (2000) showed that unstunned fish show significant aversive reactions during exsanguinations, suggesting that the welfare of the fish is compromised. In order to make sure that fish welfare is not affected during exsanguination plaice needs to be permanently stunned and should not recover from the stunning (Branson, 2008). Lastly, Roth *et al.* (2007) conclude that when

using exsanguination as a killing method for fish, a rapid pH decline in the meat of the fish and earlier rigor mortis onset is caused, which affects food quality.

Electrical Stunning

The goal of electrical stunning is to make sure that information is not passed on to the brain or that the information is not processed in the brain by disrupting the sensory nerves (Kestin *et al.*, 1995; Lambooij *et al.*, 2004). The electricity can also result in paralysis of the nerves. This paralysis happens when there is frequent stimulation of the muscle fibres resulting in rapid contraction of these fibres which leads to paralysis (Branson, 2008). When a sufficient current is passed through the brains consciousness is lost immediately. This period may persist as long as the current is applied and a short period after shutting off of the current, depending on the application time (Robb *et al.*, 2002). Subsequently, a period of uncontrolled activity is induced, the so called clonic phase, which can last for 15-45 seconds. During the tonic and clonic phase the fish is insensible and not conscious of its environment (Lambooij *et al.*, 2014). These two phases are followed by a new phase in which the fish regains more consciousness and starts recovering again (Lambooij *et al.*, 2004; Robb *et al.*, 2002). During a “poor” stun the fish may be paralysed but not insensible and as soon as the current is removed the fish displays aversive and escape behaviour (Robb *et al.*, 2002; Branson, 2008). The stunning can occur inside or outside the water. When stunning is done in the water the most simple method is to apply an electrical current across the tank in which the fish are placed after being caught (Robb *et al.*, 2002). The fish are removed from the tank after the current is turned off but they could recover after they were being transferred back to water (Branson, 2008). Because the current induces muscle paralysis and not insensibility, the fish could consider the current as a stressful experience (Robb *et al.*, 2002). On the other hand, fish can be stunned outside the water on board of the ship. The fish will be delivered onto a conveyer belt at which electrodes are attached that will stun the fish. There are some important factors that need to be taken into account when applying electrical stunning. Firstly, the orientation of the fish is important, because the efficiency of the current is determined by the body parts that it is flowing through (only the head or through the whole body) (Lambooij *et al.*, 2002; Lines *et al.*, 2003). To stun the fish effectively, a sufficient current should be passed through the brain of the fish (van de Vis, personal communication 2014). However, at the Ekofish Group, a stunning machine is used where plaice goes through via a conveyer belt. In the machine, plaice is hit four times by a clapper which conducts an electrical current. It does not matter if the plaice is hit at the head or at the whole body, because the currents are conducted through the whole fish (Kramer, interview). The electrical stunning machine that is used by the Ekofish Group can be seen in figure 10. Secondly, different fish species need different electrical currents for stunning (Branson, 2008). To make sure that all the fish are stunned, the highest current that is needed should be used, provided that this does not lead to carcass damage (van de Vis, personal communication 2014). According to the report about the electrical stunning machine by Ekofish Group (2013) at least 98 Volt DC and 8.4 V_{rms} 100 Hz Ac is needed to stun plaice, provided that plaice is not lying on top of each other on the conveyer belt. Although electrical stunning can be a good method to improve the welfare of plaice during the killing process, there is still more research needed to complete the final details and to make an implementation on board possible.



Figure 10. The electrical stunning machine that is used by Ekofish Group.

How is plaice welfare affected during the processes of capturing, bringing on deck and killing?

The welfare of plaice can be affected during the different stages of the fishing process, namely catching, bringing on deck and processing of plaice. In the following chapter, it will be discussed how the welfare of plaice might be affected during these three stages.

Net material

It is suggested that the choice of the net material has a big impact on the amount of injuries caused to the fish (Noble *et al.*, 2012). Two nets with different knots (traditional and 'T90') were tested by Digre *et al.* (2010) to investigate the influence of these knots on injuries and fish quality. The 'T90' net was partly knotless, and partly used knots turned 90° from the traditional net. The result was a less narrow net with reduced flow and turbulence, which led to less injuries in haddock (Digre *et al.*, 2010). Similar results were found by Barthel *et al.* (2003), who compared net type to the number of injuries. They found that knotted nets caused more injuries on fish than rubber or knotless nets (Barthel *et al.*, 2003). The selectivity of the net can be increased by using a bigger mesh size. This leads to less bycatch of undersized fish, thereby improving their welfare. Furthermore, also the amount of bycatch of debris and other organisms could be reduced. As can be read in the part about the 'Amount of fish and stones caught in the net', this might increase the welfare of plaice (Kramer, interview).

Hauling duration

When plaice is caught in the net, it is dragged along with the net for the remaining duration of the haul. The time plaice spend in the net depends on when the fish is caught during the haul. It is assumed that when plaice is caught in the net just before the net is brought on deck (as is the case with flyshooting) the time that plaice spends in the net is minimal and that the welfare of plaice is not impacted as much as if it would be dragged along with the net for a longer period of time (van Marlen, interview). It is a stressful experience for plaice when it is caught in a net, thus spending a long time in the net may lead to even more stress (Mood, 2010; Borderios and Sánchez-Alonso, 2011). When hauling duration increases, also the amount of fish caught in the net increases. Several studies have found a relation between injuries and the amount of fish caught: when more fish is caught, it is subjected to more pressure and thus the amount of injuries is higher (Digre *et al.*, 2010). However, since there is a relation between the amount of fish in the net and the hauling duration, it is difficult to say which of these two factors is causing the impact (Digre *et al.*, 2010). The same relation is also found in other species, as for instance the mortality of sea snakes caught by trawlers also increases with both catch weight and hauling duration (Wassenberg *et al.*, 2001). Besides the amount of injuries (damaged mucus layer, removal of scales, deep cuts in dermal tissue), also the stress response, measured in cortisol levels, increased in red sea bream when the hauling duration increased (Chopin *et al.*, 1996). Based on farmed systems, one can reason that not only the hauling duration, but also the amount of fish in the net influences the welfare of fish. For example, in salmon it was found that increasing stocking density was related to a lower welfare score, calculated based on the number of injuries and the glucose and cortisol levels (Turnbull *et al.*, 2004).

Amount of fish and stones caught in the net

As mentioned in the previous paragraph, the amount of fish caught can influence the welfare of plaice. Additionally, large quantities of bycatch in the form of unwanted materials, debris and other organisms are caught, especially with bottom trawling (Jennings and Kaiser, 1998; Board, 2002; Mood, 2010). An example of this can be seen in figure 11. When plaice is caught in the net and

subjected to the water flow which causes pressure and collisions with the other material that passes through the net, its welfare can be affected. This exposure to other materials in the net may lead to injuries and damage to the slime layer (Braithwaite and McEvoy, 2005).



Figure 11. Stones in the net (www.wageningenur.nl, 22-4-2014).

Hauling speed of the net

The hauling speed differs between the different fishing methods. It is assumed that the welfare of plaice might be impacted when the hauling speed is too high for plaice to swim along with the net, as it is for instance the case in beam trawling. As long as the fish is able to swim in the net, the only stress factor is the limited amount of space. It is much more stressful for the fish when it is stuck in the net and not able to move or to do anything about its situation (van de Vis, interview). Therefore, if the hauling speed is higher than the swimming speed of plaice, the welfare of plaice is affected. Furthermore, higher hauling speed also increases the effect of stones and other material on plaice, which is also influencing the welfare (van Marlen, interview).

Bringing up the net

When the net is brought up, the fish inside it is suddenly gathered together with all the other material that is present inside the net. Due to speed of bringing up and the gravity that puts pressure on the fish and other materials inside the net when the net is taken outside the water, the fish might be heavily exposed to the pressure of other fish and other materials. This may damage the skin and mucus layer for instance (Ashley, 2007). The amount of fish in the net and the amount of debris influence the welfare of plaice during the bringing up of the net in the same way as indicated above. There is quite some variation in the stress response of different fish species during crowding: some species adapt well to the high densities of confinement while others show increased cortisol levels for a long time (Ashley, 2007). Plaice might be a species that shows a high stress response when confined in a net close to other fish, since it is a solitary animal and thus not used to these high densities. An alternative that has been used for some other species to bring the fish on deck is pumping the fish out of the net. This is supposed to be less stressful (Ashley, 2007). Grizzle *et al.* (1992) researched three different methods of bringing up the fish, namely lift net, turbine pump and vacuum pump. Grizzle *et al.* (1992) found less damages when the vacuum pump was used, but no improvements when the turbine pump was used. The amount of damages to the fish was found to depend on the pumping speed. On average more damages were found when pumping speed was

higher (Grizzle and Lovshin, 1994). Besides the process of bring up the net, also hauling up fish from a certain depth might have an impact on the welfare of fish. Often, pressure changes that occur during hauling up the net from deeper waters result in the inflation of organs in the fish (Davis, 2002). However, as plaice has no swim bladder, pressure changes are not an important issue, as the swim bladder is the most vulnerable organ to inflation (van de Vis, interview).

Handling of plaice

After plaice is caught and brought on deck, it is made ready for processing. There are several factors which can affect the welfare of plaice during this process. Firstly, the welfare might be affected when the net is emptied on deck or in a pipe or tank that leads to the conveyer belt. The roughness of this emptying, for instance dropping plaice from a height onto the deck or in a pipe or tank, can lead to increased stress levels in the plaice and to injuries. Next to this, sharp or protruding objects on the conveyer belt that is used for moving the plaice to the processing table can damage the fish and inflict injuries. These objects can for instance be stones that are caught along with the plaice. A third factor that might affect the welfare of plaice is the lack of water during the handling and processing. If plaice is not kept sufficiently wet with water on deck, it might have a negative impact on the welfare of plaice (van Marlen, interview). Subsequently, the handling of plaice by the fishermen may inflict damage to their mucus layer, which can result in aversive reactions, increasing the stress (Conte, 2004).

Stunning of plaice

Before plaice is killed, it might be stunned electrically to render the fish unconscious. Ekofish Group is working with this method, since the welfare of plaice is improved when it is unconscious during the killing process, avoiding suffering from pain during killing (Kramer, interview). However, in order to produce unconsciousness in plaice, an electrical current needs to be applied with a specific strength. If this current is too low, it might affect the welfare of plaice by not rendering the plaice unconscious, but by inflicting pain (Robb *et al.*, 2002). Furthermore, if the used current is not sufficient to render the fish unconscious, the fish may be paralysed, but not insensible. This means that the fish can still feel pain (Robb *et al.*, 2002; Branson, 2008). Besides, the unconsciousness of the plaice is not permanent after electrical stunning (van de Vis, interview). However, if plaice is stunned effectively and put on ice immediately afterwards, it does not get conscious again and it can be killed humanely (Kramer, interview).

Killing of plaice

The most obvious factor that influences the welfare of plaice is the killing method that is used. There are different methods to kill plaice: asphyxiation, asphyxiation in an ice slurry and evisceration (van de Vis, personal communication). When killed by asphyxiation, plaice is removed from the water and exposed to air. Because plaice cannot take up oxygen from the air, it dies as a consequence of exposure to air (Robb and Kestin, 2002). However, it takes quite long before plaice dies because of asphyxiation as plaice can survive without oxygen for longer time since it is used to lower oxygen concentrations because of its natural environment. Therefore, it can be regarded to be stressful for the plaice to be killed using this method (Poli *et al.*, 2005). Besides asphyxiation in air, plaice can also be killed by asphyxiation in an ice slurry. With this method, plaice is moved directly in ice. Because of the lower temperatures, the body temperature and metabolism of the plaice decreases, which eventually can lead to death due to anoxia (Poli *et al.*, 2005; Robb and Kestin, 2002). However, because of the lower temperature, the oxygen requirement of the plaice also decreases and that increases the time it takes for plaice to die (Poli *et al.*, 2005). From other species it is known that the time it takes to die in ice slurry can be quite long, for example up to 98 minutes for trout (Poli *et al.*,

2005). Considering that plaice lives in temperatures as low as 2°C (see Life history), it is likely that it will survive a long time in ice slurry. The last method that is commonly used to kill plaice is evisceration. This is often performed in combination with ice slurry. With this method, the organs are removed and plaice dies due to a combination of anoxia and exsanguination (Robb and Kestin, 2002). Research showed that the welfare of plaice is compromised during this method, because plaice is still conscious for around 40 minutes after its organs are removed (unpublished results van de Vis in Robb and Kestin, 2002). To avoid this, electrical stunning in combination with putting the fish into ice slurry might be used as is described above.

General factors and fish welfare

Besides the factors that influence the welfare of plaice during specific stages of the fishing process, there are also more general factors that influence the welfare of plaice. One of these factors is seasonality. The reproductive season of plaice is in January-February and during these months, the fish is in a worse condition, as it is sacrificing its muscle tissues (Rijnsdorp, 1994; van de Vis, interview). Because plaice is in a worse condition during this period, the impact on welfare by the fishing practices might be increased.

Furthermore, there are more general factors that influence plaice welfare. Exposure of plaice to higher temperatures can lead to additional stress. Warmer surrounding temperatures lead to increased body core temperature resulting in more physiological stress (Schreck *et al.*, 1997; Davis, 2002). As was mentioned in the section on the life history of plaice, this species lives in temperatures ranging from 2-15 degrees: higher temperatures can be considered stressful for plaice and temperatures above 28°C were even found to be lethal for plaice (Berghahn *et al.*, 1993). Exposure to sunlight is also a factor that has an influence on plaice welfare. Research indicated that it is more stressful for the fish, when it is exposed to bright sunlight (Woo *et al.*, 2011). Furthermore the removal of fish from the water is a very stressful event. Exposure to air for 3 minutes resulted in a 50-fold increase in plasma cortisol levels in gilthead sea bream, so it can be assumed that stress levels also increase in plaice (Arends *et al.*, 1999). The most important note here is that during the catching, plaice is taken from its natural environment and placed in a completely different environment. The combined effects of all factors of the change in environment can be considered very stressful for plaice (van de Vis, interview). A last important factor that needs to be taken into account is the time between the bringing on deck and the killing of plaice (Digre *et al.*, 2010). In order to reduce the impact on plaice welfare the time between these two processes should be minimized or the fish should at least be rendered unconscious as quickly as possible (Kramer, interview).

Discards

Although this report does not focus on the discards, the welfare of plaice and other organisms might be seriously impacted by the fishing and handling methods used. Therefore, it is important to take also the discards into account when discussing how the welfare of plaice is affected. The bycatch that is thrown overboard because it does not meet the requirements of the fishermen is a great problem with the fishing methods used to catch plaice. This bycatch is mostly discarded because of one of the following five reasons (Buisman *et al.*, 2011):

- Undersized fish. Undersized fish that are smaller than the minimum requirements are not allowed to be landed and thus the only way to avoid this is to discard the fish.
- Over quota. When quotas are already filled, all other fish needs to be discarded.
- High grading. Fish that are above the minimum requirements are put overboard, because they are still considered to be too small and larger fish are preferred.
- Less valuable species. When there is no profit to be made by landing these species or because of the small size of the market, landing can significantly decrease value prices.

- Non-commercial species. When there is no market to sell these species (especially benthos species).

The welfare of all discarded marine species is affected, however in this chapter the focus will be on discarded plaice. According to van Helmond and van Overzee (2010) in 2008 53% of all wild caught plaice was discarded again. As the mortality rate for certain fish species after discard is more than 90% (van Beek *et al.*, 1990), it is clear that the welfare of discarded plaice is also affected.

There are several factors that determine the survival of the discarded plaice. Firstly, hauling duration and fishing depth have an impact on the welfare as longer hauling duration and fishing at greater depths decrease survival chances (Davis, 2002). Secondly, delayed mortality (Davis and Ottmar, 2006) because of higher susceptibility to predation, physiological stress or disease (Davis, 2002) can occur. According to Davis (2002), there are several reasons for the high mortality rates in discarded fish. As was mentioned before, the slime layer and skin form the protection layer for fish, and damages to these areas make fish more vulnerable to diseases (Noble *et al.*, 2012). Higher mortality because of predation happens because fish are in a “catatonic state”, where the fish do not respond to stimuli. Furthermore, discarded fish experience more predation pressure as a result of disrupted schooling and because predators are more attracted because of visual, olfactory and mechanical cues from injured fish and the fishing gear. Another factor that leads to the high mortality rates of discards are the amount of sea birds that often accompany the fishing boats. Discards are a major food source for seabirds that are waiting for this easy food source in form of the discards (Kelleher, 2005). According to Camphuysen *et al.* (1995), the amount of discarded fish eaten by seabirds is greater than the amount of live-caught fish that is eaten by seabirds.

Of course the same factors that affect the welfare of plaice that is killed eventually on deck have an effect on the discarded fish as well. These factors can be for instance the handling, exposure to air, temperature and irradiation to which plaice is exposed before it is discarded.

In order to cope with the discard problem a discard ban will be put in place in 2016. After that, everything that is caught needs to be landed by law (Borges, 2013). The discard ban might lead to a reduction in discards and to more accurate catch data. However, in order to function properly, several measures should be taken. Firstly, success is dependent on the amount of surveillance and therefore an observation programme needs to be strictly implemented. Subsequently, Condie *et al.*, (2014) propose to implement gear restrictions and closure of specific areas (Uhlman *et al.*, 2012). Furthermore, a flexible management system to trade with quotas is needed. Lastly, allowable quota averages and economic landing incentives will reduce the need for discarding and will reduce the costs for landed fish.

Table 2. Indicators for good and bad welfare as described in the chapter 'Which indicators are applicable to indicate the welfare of wild caught plaice?'

✓ OR ✓	Net material	Hauling Duration	Amount of fish and debris in the net	Hauling speed of the net
Good welfare	Soft material and knotless nets.	Short hauling duration where plaice is in the net for a short time period.	No/small quantities of bycatch (unwanted materials, debris and other organisms).	Low hauling speed, plaice is still able to swim within the net and can still move.
Bad welfare	Hard material and knotted nets.	Long hauling duration where plaice is in the net for a long time period.	Large quantities of bycatch (unwanted materials, debris and other organisms) that can harm the plaice within the net.	High hauling speed, plaice is not able to swim within the net, restricted movement. Impact of other unwanted material on plaice is higher.

✓ OR ✓	Bringing up the net	Handling	Stunning
Good welfare	No/small quantities of bycatch in the net. Alternatives for conventional bringing on deck used (e.g. pump). Pressure changes are taking into account.	Gently emptying of the net on deck and conveyer belt. Sufficient water/wetting during handling and processing. Fishermen handle plaice responsible (with wet gloves, slowly).	When plaice is effectively stunned directly, rendered unconscious and does not get conscious again before it is dead.
Bad welfare	Large quantities of bycatch in the net that can harm plaice. Pressure and gravitational forces not taking into account.	Rough emptying of the net on deck and conveyer belt. Lack of water during handling and processing. Handling by fishermen damages the mucus layer and injures plaice.	There is no stunning or stunning does not render plaice unconscious but inflicts pain. Plaice gets conscious again before it is dead.

✓ OR ✓	Killing of plaice	General factors
Good welfare	Quick and effective killing methods used. Plaice is rendered unconscious during the killing process via stunning.	No fishing on plaice during the spawning season (Jan-Feb), no exposure to high temperatures/ irradiation levels or long exposure to air. Minimal time between bringing on deck and killing of plaice, or between bringing on deck and rendering plaice unconscious.
Bad welfare	It takes a long time before plaice is killed with the different killing methods and plaice is still conscious and sensible.	Fishing on plaice year round, exposure to higher temperatures, irradiation levels and exposure to air. Long time period between bringing on deck and killing while plaice is still conscious.

Which indicators are applicable to indicate the welfare of wild caught plaice?

The previous chapter described how the welfare of wild caught plaice might be affected during the different stages of the fishing process. In this chapter, the indicators that can give an indication on the welfare of plaice in these situations will be discussed. Since research is lacking on indicators for the welfare of wild caught fish, the proposed indicators are mostly based on assumptions. Almost no literature was found that can back up the proposed indicators, therefore, logical reasoning is used. The indicators that are not mentioned in this chapter but are mentioned in the chapter about the known indicators for fish welfare are simply not applicable on board of fishing ships (for instance blood sampling and brain activity), as these indicators can only be used under laboratory conditions.

Hauling duration

The duration of a haul can easily be monitored, however it is not that easy to determine what the maximal duration is that plaice is allowed to be in the net before it seriously impacts its welfare. In general one can expect that the shorter the hauling duration the better this is for the welfare. However, since it is very inefficient to continuously lower and then bring up the net again one cannot avoid that hauling has a certain duration. An indicator that can be used for the hauling duration is to determine the maximum time that hauling is allowed to take place. In order to set a maximum hauling time, plaice caught at different times need to be sampled to make an educated guess after which time welfare is impaired. In order to do so stress levels and survival rates need to be measured that can indicate plaice welfare. This should be done in an experimental setup, survival rates can be measured in an experimental setup on board of the fisher ships, but the stress levels have to be measured in laboratory experiments (van de Vis, interview).

Amount of fish and stones caught in the net

As the welfare of plaice is impacted by the amount of fish and stones, the amount of fish and stones in the net is an easy indicator for plaice welfare. It is very difficult to measure what is inside the net during the hauling, as you only know what is in the net when you bring it in (Kramer, interview). An easy method to monitor what is in the net could possibly be a camera attached to the net. In this way, one can monitor the amount of debris, stones, other material and other organisms within the net. If this amount is considered too much the ship needs to bring in the net and move to another location. This camera could also be used to determine what the best timing is to haul up the net, for instance when the net is considered too full or when welfare of plaice is affected (for instance dead or crushed fish in the back of the net). Furthermore, a device that can measure pressure changes within the net as a result of amount of fish in the net could be used to determine when the net is considered too full. This is an ambitious idea which needs a lot of engineering and testing before it could be implemented, since there are many forces acting upon the net. However, when all these factors are taken into account this could help to determine when the density and therefore the pressure on the individual fish is too high.

Speed of hauling of the net

The speed of hauling of the net is a difficult indicator. The speed of the fishing boat can be monitored, but the speed of hauling of the net is also linked to the speed of the water currents, which are not easily measurable and also not controllable. Therefore the hauling speed might not be applicable as an indicator. However, by placing a camera on the net, as mentioned for the amount of fish and stones caught in the net, one can also monitor if plaice is still able to swim inside the net, which could give an indication if the welfare of plaice is impacted or not. Furthermore, when plaice is

accumulating in the net and when the density at the back of the net increases (affecting the plaice in its welfare) because of too high hauling speed this can be noticed with the camera.

Seasonality

In order to map the spawning season of plaice the status of the gonads of the fish can be monitored when the fish is gutted. When all data of the different areas are collected, a general map could be made which fishermen can use to determine where they can and cannot fish for plaice. If the maturation of the gonads is too far, the fishing ship should move to another location to fish. Additionally, the condition of the fish should be monitored, since it can take a few months to recover after the spawning season (van de Vis, interview). However, as fishermen also want the best quality of fish that they can get, and seeing that plaice during the spawning season is in a worse condition, some already take this into account (Kramer, interview).

Handling of plaice

The most clear indicators for affected welfare of plaice are injuries and a damaged slime layer. However, it is difficult to distinguish whether this happened during handling or already when the plaice was in the net. The same holds for a second indicator, the drying out of plaice. It is difficult to pinpoint when plaice was not sufficiently wetted, as this can happen during the bringing on deck or during the processing of plaice. Only at the processing table one can see whether plaice has injuries, a damaged slime layer or is dried out. But one cannot say where in the process the injuring of the fish took place or which part of the process took too long. It would be ideal to measure these indicators continuously during the whole process, but this is not applicable on board of a ship because of the large amounts of plaice that is processed. However, these indicators can be used as general indicators to see if welfare of plaice is affected among the whole process. Two simple and easy controllable indicators that show if plaice welfare is affected are to see whether fishermen use wet gloves or their bare hands during the handling of the fish and if there is wetting of plaice during the whole process.

Stress related post mortem changes

There are several clear indicators that are measurable after the fish has died, which can be used to determine the welfare of plaice before being killed. When fish experience severe stress, it has negative effects on the physical properties of flesh quality (Poli *et al.*, 2005; Poli, 2009). According to Poli (2009) and Poli *et al.* (2005), indicators for quality changes are fish and fillet appearance, technological properties of the fish and fillet, freshness indicators and sensory qualities. Using fish and fillet appearance the amount of physical injuries, flesh gaping and colour can be used to indicate the welfare of the fish before death. Secondly, technological properties of plaice and fillet can indicate fish welfare. Like already mentioned, rigor mortis onset is a good indicator as are flesh texture (firmness, cohesiveness and elasticity), water holding capacity and fillet shrinkage. Thirdly, some indicators for freshness can indicate stress in plaice. These indicators are mostly spoilage indicators (for instance biogenic amines and lipid oxidation products) . And lastly, sensory qualities can indicate stress during the killing process. These sensory qualities are the appearance of the fish, eyes and gills and the colour, smell and condition of the flesh (Poli *et al.*, 2005; Poli, 2009).

Discussion

The results presented in this paper are the first steps towards the assessment of the welfare of wild caught plaice, and can ultimately be used as input for the development of a label to improve the welfare of wild caught fish. Since the welfare of wild caught fish has not been given much attention until recently, not much research has been done on this topic yet. Therefore, most knowledge from literature has been obtained from aquacultural systems and was extrapolated to wild caught plaice. This knowledge was applied to plaice mostly based on interviews. The results were kept as objective as possible by using literature sources where possible and by interviewing only experts. However, it is important to note that the choice of experts could affect the results. Within the time limit of the project, a limited number of experts were interviewed. It should be taken into account that a bias in the choice of experts might have influenced the results. The findings of this project will be discussed here following the different research topics.

When investigating how the fishing process affects the welfare of plaice, an unavoidable issue is whether increased stress levels and injuries matter for the welfare of wild caught fish. Although the debate on whether or not fish experience pain is still going on, evidence from behavioural and neurological research makes it likely that they do experience pain (Braithwaite *et al.*, 2013). Consequently, the assumption was made that fish are sentient beings, an assumption made in other researches as well (Huntingford *et al.*, 2006; Ashley, 2007). A number of parameters were investigated to be used as indicators for welfare. For the literature research, the focus was placed on physical and physiological indicators. Most behavioural indicators are species specific, resulting in different responses to stressors (Martins *et al.*, 2012). Moreover, behaviour can differ between individuals, as proactive copers can respond differently to stressors than reactive copers (Martins *et al.*, 2012). Furthermore, most behavioural indicators (foraging behaviour, stereotypic behaviour, aggression, swimming behaviour) apply mostly to aquacultural systems and to welfare on long term (Martins *et al.*, 2012), and are not relevant for the short term stressors induced during the fishing processes in wild caught fish. Consequently, behavioural indicators are not given much consideration in this report, while these may give a good indication of the welfare of the fish.

For the assessment of fish welfare, it is important to take into account the species characteristics. In previous research on several species kept in aquacultural systems, the species characteristics were taken into account and led to species specific conclusions on factors influencing the welfare of fish (van de Vis *et al.*, 2012). Thus, taking into account the life history of plaice can be considered crucial in making the proper analysis on the effects of fishing methods. However, research on plaice focuses mostly on its reproduction and survival of juveniles (Gibson *et al.*, 2002; Bergman, 1987; Ellis and Gibson, 1995; Kuipers, 1973; Rijnsdorp, 1985), and knowledge on other specific population characteristics is not known from literature. The response of a specific individual to the process of fishing, depends on its life stage as well as on its life history, determined by experiences of plaice in the past (van den Bos, interview). Although not much on the environmental challenges in later life stages is known in literature, populations are monitored by IMARES (van Marlen, personal communication). This information can be very valuable in the assessment of welfare, taking into account the life history of the population that is caught.

From the catching methods for plaice considered in this report, most attention is paid to the most commonly used method, beam trawling, and the alternatives to this method (pulse trawling, twinrig and flyshoot). Less attention is paid to the gillnet, since this method is not commonly used for plaice and does not seem to be an alternative where welfare of plaice could be improved (Mood, 2010). The long time (from several hours to even days) that fish can be entrapped in a gill net can be very stressful, as was shown for sea bream by the increase of stress levels when capture duration increased (Chopin *et al.*, 1996). Angling was not considered as a method for catching plaice in this

report. This method is used in recreational settings, but is not used to catch plaice in commercial fisheries (www.vissersbond.nl, 2-5-2014).

In the methods considered in this report, differences were found in selectivity and amount of bycatch. Bycatch that is thrown back into the sea has a high mortality rate and can have effects on the population structure, since it concerns mostly the smaller fish that were not intended to be caught. At the moment, fisheries are still allowed to discard their bycatch, but a ban on throwing bycatch back into the sea will be implemented in 2016 (Borges, 2013). As a consequence of this regulation, all bycatch is also counting for the quota of fisheries. This will increase the need for more selective methods of catching fish, so that less bycatch is brought on board. The Ekofish Group is a pioneer in this area, as it is already implementing small adjustments to the fishing gear to improve selectivity (Kramer, interview). Besides these improvements, Ekofish Group is also researching improvements on the methods of bringing plaice on board and killing.

The issue of bycatch is also related to the effects of catching fish on the remaining population of plaice. Over the years, it has already been noticed that the population dynamics of plaice have changed as a consequence of fisheries. Due to the big impact of fisheries on mortality of plaice compared to the natural mortality, evolutionary adaptations to fisheries can be expected (Grift *et al.*, 2003). The comparison of data on plaice over the years has shown that their growth rate significantly increases over the years, and both size and age at maturity are decreased as a consequence of the high fishing pressure (Grift *et al.*, 2003). Moreover, the increasing fishing pressure has led to a decrease in genetic variation in plaice and consequently, inbreeding has resulted in a higher incidence of genetic deficiencies (Hoarau *et al.*, 2005). Although this report has paid most attention to the effects of the fishing methods on the fish that are actually caught, it is important to realize that fisheries can also affect the welfare of the remaining population by changing their dynamics. An additional consequence could also be that the impaired welfare of the remaining population makes it less adapted to challenges, and thus might get overtaxed more easily when caught by fisheries.

When assessing the welfare of plaice during the processes of fishing, a choice has to be made to the level on which the welfare is assessed. Ideally, the welfare of each individual is assessed. However, due to practical limitations, it is more feasible to sample a part of the caught fish to assess the welfare. By assessing only a part of the catch, it is important to note that this results in an average level of welfare, and the extremes might not be considered.

One important point of discussion is the extrapolation on data found in other species to plaice. As mentioned before, the response to environmental challenges is species specific and even depends on the life history and life stage of the animal. For simplicity, the assumption was made that these data might also hold for plaice. Differences in responses have been reported, for example on cod and haddock, which showed a different amount of injuries as a consequence of the net material used (Digre *et al.*, 2010). On the physiological responses to stress, little is known in plaice. Considering that plaice is a different species than the species cultivated in aquaculture on which most physiological research has been done, and the different environment plaice is exposed to compared to aquacultural systems, it is very likely that plaice will also respond differently in terms of physiological parameters. Moreover, the physiological parameter that reflects the stress level of plaice most is not known. In this report, it was assumed that measurements on physiological parameters will be difficult on board, since a laboratory environment is necessary for these measurements. However, if the parameter that reflects stress best in plaice is known, the development of a device to measure this might be useful, since this could show the real stress level of plaice. Using environmental indicators such as hauling duration, speed and time until death, will only give an indication of the welfare of plaice. With these indicators, there can still be a big difference in welfare between individuals due to their life stage and life history. Even if experimental settings can show how plaice respond to different stressors, it cannot be assumed that the response will be similar in wild caught

plaice, because of the differences in life history. Therefore, a physiological indicator for welfare can have a lot of benefits.

Due to the abovementioned restrictions in this research, it is not yet possible to formulate concrete criteria that could be used for the implementation of a label. Ideally, criteria should include concrete numbers such as number of injuries, time until rigor mortis and maximum allowed amount of debris. The lack of data on how plaice responds to different stressors, and when physical or physiological parameters indicate that plaice is overtaxed makes it impossible to provide these concrete numbers in this research.

Conclusion

Since the last decades, the awareness about fish welfare in wild caught fisheries is increasing and it is becoming a relevant and important topic in the Netherlands. This study was conducted to see whether the welfare of plaice is impacted during the fishing process. The information and results that were found in this study by interviews and literature reviews seem to indicate that the welfare of plaice is threatened among the different stages of the fishing process, namely capturing, bringing on deck and killing.

To determine the welfare of plaice, the allostasis concept was used, because it considers the ability of an animal to cope with the environment. Thus it is both species specific, by including the species characteristics, and applicable to physiological parameters that indicate stress, which makes it an objective concept for animal welfare. Literature on farmed fish suggested that several indicators for fish welfare can be used, such as behavioural, physiological, physical and environmental quality indicators. However, this study focused on only the physiological and physical indicators for fish welfare known from literature since the other two types of indicators are only useful when one looks at the whole life span of plaice while this study only focuses on the last part of the fish its life, namely the catching, bringing on deck and killing of plaice. Physiological indicators that can be used to assess welfare of plaice are brain function, haematic indicators, adrenaline, cortisol, plasma glucose levels, haematocrit values, muscle pH, lactic-acid concentrations, muscle energy reserves and onset time of rigor mortis. Physical indicators that can be used to assess the welfare of plaice are mostly injuries and damages to the mucus slime layer. Additional environmental indicators were formulated for the welfare of plaice, after considering the factors of the fishing process that affect the welfare.

After combining the life history of plaice and the methods used for catching, bringing on deck, and killing of plaice, several factors that can affect the welfare of plaice during the whole process were indicated. Net material, hauling duration and speed, amount of fish and debris in the net, process of bringing on deck, handling of plaice and stunning and killing of plaice can all affect the welfare of plaice. Furthermore, several general factors can influence the welfare, such as higher temperature, irradiation and exposure to air influence plaice welfare.

Based on where welfare of plaice is affected during the process some applicable indicators are proposed for assessing the welfare of plaice. Again hauling duration and speed and the amount of fish and debris in the net can be used to assess plaice welfare. Furthermore, seasonality and handling of plaice can be used. Most physiological indicators are however only useful in a laboratory setting and not applicable on board of a fishing ship. Nonetheless, they can be useful to assess the welfare of plaice during the whole process when they can be linked to an indicator that can easily be measured, such as rigor mortis. Rigor mortis is a good indicator of stress (and hence fish welfare) where in fish that are stressed before killing the onset of rigor mortis is faster. Lastly, stress related post mortem changes can be used as applicable welfare indicators.

Recommendations

After taking all the different factors into account that may influence the welfare of plaice during the capturing process and identifying indicators that can be used to assess the welfare, some recommendations can be made to improve plaice welfare. These will be discussed in this chapter.

It has been shown that the methods that are being used nowadays for fishing plaice are likely to impact the fish its welfare during the catching, bringing on deck and killing. Improvements on these methods can be made, which can result in less impact on the welfare of plaice. A good replacement for the conventional bringing on deck of the net could be a pumping system where plaice is pumped out of the net directly on board of the fishing ship. In Norway such a pumping system is already used for salmon and with some adjustments this system might be used for plaice as well. The advantages of this system are that the fish spends no time out of the water and is not exposed to forces due to gravitation and crowding as it is when they are heaved out of the water inside the net. Because of these advantages, this method can be regarded as to be an improvement for the welfare compared to the conventional way of bringing up the net. Ekofish Group is a pioneer in the field of fish welfare and they already made some adjustments on their fishing gear that reduces the impact on the fish. Ekofish Group uses the twinrig method for fishing on plaice, however, by using different net materials and mesh sizes, the damage to plaice is decreased and the selectivity is increased. Besides this, some adjustments are made on the twinrig material. Instead of being dragged over the bottom, the trawl boards are floating just above the bottom while balls are attached to the sweeps so that these are dragged less over the seabed. Additionally, Ekofish Group designed a stunning machine which renders the fish unconscious before it is killed. This is a large step forward for fish welfare, as this minimizes the time that plaice is conscious while it is being killed or awaits for processing. Lastly, Ekofish Group is developing a gutting machine, so that more plaice can be processed in less time. Because of this machine, the time between bringing on deck and killing of plaice can be significantly decreased. More research is needed on all these adjustments and improvements, either already put into practice by Ekofish Group or mentioned in the report to see how they can improve the welfare of wild caught plaice. Thereby, also research is needed on the possibility of implementing these adjustments on a larger scale and on other innovative methods for catching, bringing on deck and killing of plaice. It is recommended to investigate these topics to be able to improve the welfare of wild caught plaice.

Subsequently, the knowledge that is acquired in this project on the welfare of wild caught plaice can be used as a starting point to examine the applicability to other fish species. However, much more research is needed to be able to extrapolate the findings in this report to other species. Additionally, the applicability of the indicators for assessing the welfare of plaice need to be researched to see how feasible these indicators are in reality. Therefore, it is recommended that the effect of the different stressors that have an effect on the welfare of plaice during the capturing process is tested in a laboratory environment. By linking the physiological response of plaice to the different environmental indicators, injuries and rigor mortis, it can be seen where welfare of plaice is mostly affected. In this experimental setting the recovery rates can be measured to see how long it takes for plaice to recover from the different stressors and to see when welfare is impaired.

Even though the discard of plaice is only briefly mentioned in this report, the welfare is also affected during this event. With more research on how to improve the survival rate of the discarded plaice the welfare of these fish can be improved.

With the increasing interest in fish welfare that is currently going on in society, it is important to actively involve the different stakeholders in the fishing industry as they play a major role in sustaining the welfare of plaice. These stakeholders are for instance the consumers, fishermen, NGOs

and the government. Since the ultimate goal of the Science Shop project is to explore the possibilities for creating a fish welfare label, it is recommended that research is done on the consumers perspective towards such a label. It is of vital importance for the success of such a label to explore if there is a market for it. And lastly, also the perspective of the fishermen needs to be taken into account. They are the ultimate implementers of measures for improving the welfare of plaice and resistance from them will make it impossible to apply these measures on a wide scale.

This report is only a small step towards revealing the total impact that fishing has on the welfare of fish, but hopefully it contributes to the overall goal of the Science Shop project of developing a fish welfare label by giving an overview of how the welfare of plaice is impacted in the current fishing practices, what possibilities there are to indicate the welfare of plaice, what possibilities there are for improvement and what information is missing to actually improve the welfare of plaice. The report can also be used as a framework that helps with setting strategic and research priorities for what should be done in the future. Knowledge gaps and research that is needed to fill these knowledge gaps should be identified in order to come up efficiently with feasible and theoretically validated indicators.

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Appendix 1

Questions for the interview with Jacob Kramer on 15-4-2014

(The interview was performed in Dutch)

Catch:

- Welke alternatieven voor boomkor gebruiken jullie bij Ekofish Group?
- Hoeveel schol vangen jullie per methode? Weet u of dit verschilt met de traditionele visserij methode?
- Waarom is de boomkor nog steeds de meest gebruikte methode om schol te vangen, gezien dat er andere methodes beschikbaar zijn?
- Wat is de toekomst voor pulskor? Zou het de boomkor kunnen vervangen?
- Wat is de toekomst voor twinrig? Zou het de boomkor kunnen vervangen?
- Wat is de toekomst voor flyshoot? Zou het de boomkor kunnen vervangen?
- Hoe lang is schol in het net? (per methode)
- Zijn er veel verwondingen en is er veel stress voor schol door overvolle netten? Hoe verschilt dit tussen de methodes? Hoeveel ander materiaal (stenen, krabben) wordt er mee gevangen per methode?
- Hoe groot is de schol gemiddeld die jullie vangen? Verschilt dit per methode?
- Welke methodes hebben volgens u het meeste effect op het welzijn van schol? Op welke manier?
- Wordt de kwaliteit van het vlees volgens u beïnvloed door de verschillende methodes?
- Zijn methodes die beter voor het welzijn van schol zijn ook meer duurzaam? (schade aan de omgeving, bijvangst)
- Wat zijn volgens u goede alternatieven om welzijn te verbeteren voor schol tijdens de vangst?

Bringing on deck

- Hoe wordt schol aan dek gebracht? (per methode)
 - Hoe wordt het welzijn van schol beïnvloed bij deze methodes van aan dek brengen? (Hoe snel, dichtheid)
- In Noorwegen wordt een nieuwe methode voor het aan dek brengen toegepast: pompen. Wat denkt u: zou dit een goed alternatief kunnen zijn voor de huidige methode om schol aan dek te brengen? Is dit toepasbaar voor schol? Omdat dit in Noorwegen soms al gedaan wordt, heeft u dit wel eens overwogen? Zo ja, waarom gebruikt u deze methode niet?
- Heeft u wel eens overwogen om capture based aquacultuur te gebruiken als methode om schol te vangen?
- Kunt u nog andere manieren bedenken om het proces van schol aan dek brengen te verbeteren wat betreft het welzijn?

Handling

- Hoe denkt u dat het handelen aan boord het welzijn van schol kan beïnvloeden? (laden op loopband, vastpakken, tijd tot dood)
- Hoeveel tijd zit er tussen het aan dek brengen en doden van de schol?

Killing

- Wat zijn de verschillende methodes die Ekofish Group gebruikt om schol te doden?
- Welke methode is volgens u het best, wat betreft het welzijn van schol? (Tijd tot bewusteloosheid van verschillende methodes?)

- Welke methode is volgens u het best, wat betreft de vlees kwaliteit? (gerelateerd aan stress respons maar niet perse aan welzijn)
- Welke methode geeft u de voorkeur aan gezien de kosten en toepasbaarheid?
- Kunt u uitleggen hoe elektrisch verdoven werkt?
 - Wordt schol aan boord verdoofd of in het water? Is het altijd hetzelfde?
 - Wordt schol individueel verdoofd of meerderen tegelijk?, alleen de hoofd of heel het lichaam?
 - Hoe lang wordt de elektrische schok toegediend en hoe sterk is deze schok?
 - Hoe effectief is de elektrische verdoving van schol? (ze kunnen weer bij bewustzijn komen)
 - Wat is het effect van deze methode op de kwaliteit van het vlees?
 - Waarom word schol niet gedood met een elektrische stroomstoot? In andere visensoorten gebeurt dit soms
- Wij weten uit de literatuur dat elektrisch verdoven en spiking meer humane methoden zijn vergeleken met de methoden die meest gebruikt worden, waarom wordt dit niet meer toegepast?

Welfare indicators:

- Is het mogelijk en toepasbaar om aan boord bloedmonsters te nemen van schol? (eventueel vervolg: wat wordt er dan gemeten?)
- Wat wordt er op dit moment al aan boord gemeten? (kan dit eventueel gebruikt worden voor indicatoren van het welzijn)

Overig:

- Vangen jullie schol het hele jaar door of alleen in bepaalde maanden? (De kwaliteit van schol is minder tijdens het paaiseizoen)

General questions:

- Wat is uw mening over het concept van vissenwelzijn/dat vissen pijn, angst en stress voelen?
- Denkt u dat het welzijn van schol negatief beïnvloed wordt door huidige visserijen? Op welke manier?
- Mogen wij in het verslag naar u refereren naar aanleiding van dit interview?

Appendix 2

Questions for the interview with Ruud van den Bos on 17-4-2014

(The interview was performed in Dutch)

Life history

- Uitleg allostasis/welzijn: volgens het allostasis concept is het welzijn van dieren goed binnen een bepaalde range van allerlei parameters. Zodra de omgeving zodanig is, dat deze parameters buiten deze range komen, kan worden aangenomen dat het welzijn verminderd is. Met dit concept wordt welzijn dus beoordeeld door te kijken naar het aanpassingsvermogen van dieren. Als de situatie buiten het aanpassingsvermogen valt van het dier, kan het dier hiervan stress, angst of pijn ervaren die het welzijn verminderen. Klopt dit met uw beeld van allostasis, waar missen we nog dingen, waar heeft u een ander idee over?
- Hoe zou u allostasis verbinden met het welzijn van vissen?
- In het paaiseizoen leeft schol op hun vet reserves. Zijn ze in dit seizoen daarom ook gevoeliger voor stress als ze gevangen worden? Zou het daarom ook verboden moeten worden om op schol te vissen in het paaiseizoen? Maakt dat hun buiten dit seizoen minder gevoelig voor stress omdat ze aan deze erbarmelijke omstandigheden gewend zijn?
- Predatiedruk is bij schol vooral hoog in het eerste levensjaar wanneer de schol nog klein is, daarna speelt dit een kleine rol. Dus normaal gesproken heeft volwassen schol weinig last van predatie. Hoe verwacht u dat de volwassen schol daarom het hele vangst proces ervaart? (twee ideeën: minder predatie --> minder stress omdat ze niet weten wat er gebeurt, of minder predatie dus minder aangepast om met stress van predatie om te gaan)
- Door de lage predatie druk en de leefstijl (op de bodem, ambush predator) kan worden gezegd dat schol een relatief passieve vis is. Verwacht u dat schol daarom meer gestrest is tijdens het vangen dan meer actieve vissen?
- Schol leeft normaal gesproken in temperaturen die verschillen tussen de 2 en 15 graden Celsius. Als ze vervolgens aan dek worden gehouden in hoge temperaturen, hoe beïnvloedt dit hun welzijn? En als ze aan dek worden gedood met ice-slurry, zou die temperatuur range daar ook effect op hebben? Zit hier ook nog een effect van seizoen bij, en kan het daarom aan te raden zijn om schol alleen met ice-slurry te doden in de zomer, maar niet in de winter?
- In de literatuur hebben we gevonden dat schol niet goed kan overleven bij een zuurstofverzadiging van 20% en 10% (50% en 100% gaat dood respectievelijk). Wij begrijpen het concept niet helemaal; andere bronnen noemen een range of 0.9 – 8.1 ml zuurstof per liter water. Betekent dit dat schol tegen relatief lage hoeveelheden zuurstof kan, en dat het lang kan overleven buiten het water? Gezien deze gegevens, wat denkt u van de methode van verstikking?
- Schol komt vooral voor op dieptes tussen de 10 en 50 meter. Als ze snel omhoog worden gehaald, worden ze blootgesteld aan een groot drukverschil; hoe zal dit hun welzijn beïnvloeden? Ze hebben bijvoorbeeld geen zwemblaas die kan knappen. Wat is het effect van de snelheid waarop ze naar boven worden gebracht hierbij?
- Deze grote dieptes houden ook in dat schol in een donkere omgeving leeft. Bij het omhooghalen van de vangst wordt schol blootgesteld aan grote hoeveelheden licht. Hoe zal dit hun welzijn beïnvloeden?
- Schol wordt in zijn habitat blootgesteld aan stromingen. Hebben stromingen een effect op het aanpassingsvermogen van schol en zijn welzijn tijdens de vangst?

- Zijn er nog meer factoren die u kan bedenken in het leefgebied van schol die effect kunnen hebben op de capaciteit van schol om zich aan te passen aan verschillende omstandigheden, en die daarom effect hebben op zijn welzijn tijdens de vangst?

Welfare indicators:

- Denkt u dat beschadigingen aan de slijmlaag en huidbeschadigingen het welzijn van schol beïnvloeden?
- Hoe reageert schol op stress wat betreft fysiologische parameters?
- Is het redelijk om aan te nemen dat het welzijn van schol beïnvloed wordt door een verandering in fysiologische parameters?
- Van andere vissoorten (zalm, kabeljauw) weten we dat metingen aan het bloed (pH, cortisol, glucose levels etc., gerelateerd aan stijfheid en houdbaarheid) gebruikt kunnen worden als stress indicatoren. In hoeverre kunnen we aannemen dat deze indicatoren ook voor schol gelden?

General questions:

- Wat is uw mening over het concept van vissenwelzijn en dat vissen pijn, angst en stress ervaren?
- Denkt u dat het welzijn van schol in gevaar komt in de huidige vispraktijken? Op welke manier?

Appendix 3

Questions for the interview with Bob van Marlen on 14-4-2014

Catch:

- What alternatives are available for beam trawling? Are they actively implemented?
- How much plaice is caught per method (beam trawl, pulse trawl, flyshoot, twinrig, gillnet)?
- Why is beam trawling still the most commonly used method for catching plaice seen as there are better methods available?
- What is the future for pulse trawling? Can it replace beam trawling?
- Could you explain something about the results you found in comparing pulse trawling and beam trawling, specifically for plaice welfare? (injuries, indicators)
- What is the future for twinrig? Can it replace beam trawling?
- What is the future for flyshoot? Can it replace beam trawling?
- How long is plaice in the net? (for each method)
- Are there a lot of injuries and stress to plaice due to overcrowding? How does this differ between the methods? (for each method)
- Which methods do you think affect welfare most? In what way?
- Are methods that are better for the welfare of plaice also more sustainable? (environmental damage, bycatch)
- What are good options to improve welfare of plaice during capture?

Bringing on deck:

- What are the different methods for bringing plaice on deck?
 - Is it related to the catching method?
 - What is the effect on the welfare of plaice for the different methods? (how fast brought on deck, how much overcrowding)
- In Norway a new method for bringing on deck is applied; pumping. What do you think: might this be a good alternative for current methods of bringing plaice on deck? (Is it applicable to plaice?)
- What do you think of using capture based aquaculture as method for catching plaice?
- Can you think of other options to improve the process of bringing on deck with regard to welfare of plaice?

Handling

- How do you think handling on deck can affect welfare of plaice? (transport on conveyer belt, fishermen holding plaice, time to killing)
- How much time is in between bringing on deck and killing?

Killing

- What are the different methods used to kill plaice?
- Which method is best according to you, considering the welfare of plaice? (duration to unconsciousness for different killing methods?)
- Which method is best according to you, considering the meat quality of plaice? (related to stress response, but not per se to welfare)
- Which method would you prefer considering costs and practical issues?
- Can you explain how electrical stunning works?
 - Is plaice stunned on board or in the water, always the same?
 - Is plaice stunned individually or several at the same time?
 - How long is the electrical shock applied?

- How effective is electrical stunning? (fish can get conscious again)
 - What is the impact of this method on the quality of the meat?
- We know from literature that electrical stunning and spiking is more humane compared to methods currently used for plaice, why is it not applied more?

Welfare indicators:

- Is it possible and feasible to take blood samples from plaice on board?

General questions:

- What is your opinion about the concept of fish welfare/fish feeling pain, fear stress?
- Do you think plaice welfare is compromised in current fishing practices? In what way?

Appendix 4

Questions for the interview with Hans van de Vis on 24-4-2014

Life history

- In the spawning season plaice lives on its fat reserves. Are they also more sensitive to stress in this season? Should it be prohibited to catch plaice in the spawning season? Does this make them less sensitive outside the spawning season because they are used to stress more from this spawning season?
- Predation is especially high in juvenile plaice. What strategy do juvenile plaice use to escape from predators? How about predation in adulthood; there was not much information on this issue; how is the predation pressure in adulthood? How does this influence its welfare during the catching process? Does the fact that there is little predation in adulthood influence the welfare of adults during catching (two ideas: less predation --> less stress because they do not know what is going to happen, or less predation --> less adapted to deal with stress from predation)
- Because of the low predation risk and the lifestyle (bottom-living, ambush predator), it can be concluded that plaice is a relatively passive fish. How do you expect that this influences the welfare of plaice during the process of catching?
- Plaice lives in areas with temperatures ranging from 2 to 15 °C. How does this influence their well-being when brought on board in high temperatures? If they are killed by use of ice-slurry, how is this affected by the temperature range, and what is the effect of the season? Could it be an option to use ice-slurry only in the summer and not in the winter?
- In literature it has been found that plaice cannot survive well when oxygen saturation is lower than 20% (half of all plaice dies) or below 10% (All plaice dies). In other sources an oxygen range of 0.9-8.1 ml/l water was mentioned. What does this mean? Is plaice well able to survive outside water? Given these numbers, how do you think asphyxiation affects welfare of plaice?
- At what depth is plaice usually caught?
- Plaice is mainly found in depths between 10 and 50 meters. If they are taken out of the water quick, they experience a great difference in pressure. How can this influence their welfare, seen as they are fish without a swimbladder? What is the effect of the speed at which they are taken out of the water?
- These great depths also entail that plaice lives in a dark habitat. When taken out of the water, plaice is exposed to much light. How will this influence their welfare?
- Plaice is exposed to currents in its habitat. Do these currents have an effect on the adaptive capacity of plaice and its welfare during catching and killing?
- Are there more factors you can think of in the habitat of plaice that can have an effect on the adaptive capacity of plaice and its welfare during catching and killing?

Fishing methods

- Which process do you think is most stressful for plaice: catching, bringing on deck or killing? So where is it most important to make changes?
- During the process of catching; what do you think stresses plaice more: keep swimming in front of the net to exhaustion or being in the net all the time and being crushed by everything that enters the net?
- Is there a maximum stress response, so that if this maximum is reached it doesn't matter what you do to the fish anymore?
- On basis of what do you judge which methods are affecting welfare more? Indicators measured on the fish or assumptions about how the methods probably affect the welfare?

Welfare indicators:

- Do you know what fraction of the discard dies from the stress of capture?
- Do you think damage to the slime layer and damage to the skin influences the welfare of plaice?
- How is plaice sold to consumers; do damages to the skin matter for quality because consumers can still see it?
- How does plaice respond to stress in terms of physiological parameters?
- How can osmoregulation change when plaice is damaged and its welfare is impaired. And can you measure this as indication of welfare?
- Is it reasonable to assume that the welfare of plaice is impaired by a change in physiological parameters?
- From other fish (salmon, cod) we know that blood measurements (pH, cortisol, glucose levels, etc., related to stiffness and shelf life) can be used as stress indicators. To which extent can we assume that these indicators also hold for plaice?
- We have come across blood parameters, tissue parameters, brain functioning and injuries. Which of these is best to indicate welfare; and which is applicable?