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Effects of on-board storage and electrical stunning of wild cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) on brain and heart activity

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ABSTRACT

Cod and haddock captured with commercial trawling gear were taken immediately after landing on deck to on-board storage in dry bins for measuring brain and heart activity, and behaviour. Other groups were first stored in holding tanks and then electrically stunned with a prototype “dry stunner”. For stunning 52 V_{rms} was applied on individual fish for 1 s. As a result, the cod and haddock received an electrical current of 0.34 ± 0.09 and 0.36 ± 0.12 A_{rms}, respectively. Electrical activity in the brain and heart was measured before and after electrical stunning. The fish remained conscious for at least 2 h after landing and during on-board storage as indicated by the electrical activity measured in brain and heart. Behavioural responsiveness to administered stimuli was absent in both species. After electrical stunning, both species showed a general epileptiform insult which was characterised by a tonic phase followed by a clonic phase and terminating with an exhaustion phase.

Since the fish remained conscious after landing and storage, electrical stunning and subsequent killing with a throat cut, may provide an option for improving fish welfare on-board commercial fishing vessels. In particular, we recommend to stun and kill wild cod and haddock as soon as possible after landing on deck using a dry stunner applying 52 V_{rms} (coupled AC/DC current) for more than 3 s.

1. Introduction

Atlantic cod (*Gadus morhua*) is a well-known demersal food fish. Haddock (*Melanogrammus aeglefinus*) is a marine fish also distributed on both sides of the North Atlantic. On average about 220–1100 million Atlantic cod and 160–310 million haddock are wild-caught in commercial fisheries across the world each year (FAO, 2009). There is a need for more efficient and automated solutions for initial handling of wild-caught sea fish on-board vessels, gentle transfer of fish from sea to deck, rapid controlled bleeding and gutting, and controlled rinsing. There is also a demand for the development of technologies for automated online documentation of quality assurance. Preliminary experiments with on-board electro-stunning of fish at trawling boats indicated that this treatment could allow more rapid gutting and rinsing of fish. This could increase fish quality while at the same time safeguarding the health and security environment (HSE) of the fishermen.

Other developments in Europe stimulate an increasing awareness within the fishing industry about the need to improve the sustainability of the fisheries and its environment. However, until now little consideration has been given to the welfare of the fish that are caught. There is increasing evidence that fish may perceive pain and fear, and have the capacity to suffer. Literature indicates that fish can detect harmful stimuli, respond to nociceptive stimuli, are able to conceptualise pain (Braithwaite and Huntingford, 2004, Chandro et al., 2004; Sneddon, 2002, 2003; Roques et al., 2010) and have explicit memory (Nilsson et al., 2008). Therefore, it is reasonable to hypothesise that fish indeed have a capacity to feel pain. To date, fish welfare studies have been concentrated on aquaculture and recreational angling, and there is a lack of knowledge of fish welfare in commercial fisheries. It is anticipated that fish welfare during commercial fishing practices will become an issue of great importance in the future (Kaiser and Huntingford, 2009). This paper addresses an important aspect of fish welfare in commercial fisheries, namely the pain associated with the slaughter process.

Throughout the world electrical stunning is used to reduce pain during slaughter in farmed animals such as cattle, sheep, pigs, poultry and fish. Electrical stunning is based on the induction of a general epileptiform insult (grand mal or seizure-like state) caused by an electrical current going through the brain (Lambooij, 2004). The epileptiform insult is characterised by a rapid and extreme depolarisation of membrane potentials throughout the brain, and there is heterogeneity of findings (Kooi et al., 1978). The brain thus stimulated is unable to respond to stimuli and consensus exists

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that a vertebrate animal is unconscious and thus cannot experience pain during a generalised epileptiform insult (Lopes da Silva, 1984). Stunning is typically performed to induce unconsciousness and insensibility to pain stimuli lasting for a sufficient duration of time to secure that the animals do not recover during exsanguination (which causes the animal to die). Unconsciousness and insensibility should be induced promptly to reduce adverse animal welfare effects but also to minimise potentially detrimental effects of these procedures on meat quality (Blackmore and Delany, 1988).

EEG and ECG registrations are widely used to record brain and heart activity under different circumstances to determine states of consciousness and brain and heart disorders in both humans and animals. The electrical activity of the EEG can be classified as delta (<4 Hz), theta (4–7 Hz), alpha (8–13 Hz) and beta (>13 Hz) waves. Conscious animals typically show alpha and beta waves in the EEG (Kooi et al., 1978). Electrical head to body stunning may induce cardiac fibrillation with extreme depolarisations of the membrane potentials or cessation of heart beat or a stop, extra systolae or irregular heart rate as reported by Dubin (1999).

The aim of this research was to measure consciousness and survival using measurements of brain and heart activity (EEG and ECG), and behaviour after landing on board and during storage of cod (*G. morhua*) and haddock (*M. aeglefinus*). Moreover, a method of electrical stunning (so-called "dry stunning") was tested on board during which dewatered fish received a current between a row of positive electrodes and a plate that was negatively charged.

2. Material and methods

2.1. Fish

Atlantic cod and haddock were obtained for the experiments after landing on deck of the research vessel and stern trawler R/V "*Jan Mayen*". After landing on deck 5 cod and 5 haddock fish were dissected to determine the positions of electro-encephalogram (EEG) and electrocardiogram (ECG) electrodes. For measurement of survival times cod ($n = 60$) and haddock ($n = 40$) were selected after landing on deck and put into dry storage bins before evisceration. Another 36 cod and 37 haddock fish were used for the stunning experiments after landing on board and placed in live holding tanks continuously supplied with sufficient amounts of flowing seawater. They were held in the tanks for a maximum of 24 h and removed from the tank for the stunning experiment.

2.2. Placement of electrodes and registration of EEG and ECG

Each fish was equipped with electrodes for EEG and ECG recordings as described previously (Lambooij et al., 2010). In order to facilitate implantation of the electrodes, and to keep the instrumented fish in a stable and straight position during electrical stunning, the fish were restrained on a wooden strip using plastic cable ties. A data recording module (DI 720) was used equipped with a WinDaq Waveform browser (Dataq Instruments, Akron, OH, USA). Two channels on the DI 720 were used with a 250 Hz sample frequency for each channel. The EEG and ECG recordings were analysed for patterns and changes of waveforms, frequency and suppression after stunning.

2.3. Survival after landing and storage

Three hauls of Atlantic cod and 2 hauls of haddock were used for measurement of persistence of brain and heart activity after landing and slaughter. During each test the first 5 fish were removed from the net immediately after hauling, i.e. within 0.5 h. The following group of 5 fish were taken from the dry storage bins immediately before evisceration, i.e. 1, 1.5 and 2 h after hauling. In total 60 cod and 40 haddock were examined. Each fish was weighted and examined for its ability to maintain equilibrium, breathing, vestibulo-ocular reflex (VOR or 'Eye roll'), response to a gentle scratch along the lateral line, squeezing the tail and response to electrical stimulation (9 V for 1–2 s) along the entire lateral line, using the Twitch Tester Quality Assessment Tool. This examination was performed according to the protocol published by Kestin et al. (2002). After these measures each fish was equipped with EEG and ECG electrodes to record brain and heart function for approximately 1 min. The Atlantic cod in this examination weighed 2.6 ± 1.2 kg (mean $\pm$ S.D.), and had a fork length of 62 ± 9 cm. Haddock weighed 0.7 ± 0.5 kg and had a fork length of 37 ± 8 cm.

2.4. Electro stunning

2.4.1. Equipment

A small portable unit resembling the larger STANSAS #1 commercial dry stunner from SeaSide AS (Stranda, Norway) was used. Whereas the commercial version has 10–12 rows of 4 parallel electrodes per slot, the experimental unit used here had 3 rows of 4 electrodes (one slot). Since our main goal was to achieve immediate stunning at the first point of contact between the fish's head and an electrode, a larger model was not required. The power source used to generate a voltage across the electrodes was the same as used in a previous study (Lambooij et al., 2010). The row of electrodes was positively charged and the plate beneath the rows was negatively charged. The current administered was a noisy AC signal with a DC offset, where the DC component dominated and the AC voltage signals were not fully sinusoidal with a root mean square (RMS) frequency of 100 Hz. A laptop computer Panasonic (Secaucus, NJ, USA) with PicoScope 2000 software (Pico Technology, Cambridgeshire, UK) and a Pico scope 4224 AD converter served as oscilloscope to determine the waveform of the electrical stunning current. A Fluke i30 S AC/DC current clamp (Fluke Inc., Everett, WA, USA) was connected to the AD converter, which was connected to the laptop. Root mean square values for voltage and current were measured with a 20 MHz FLUKE 123^{TM} industrial scope metre (Fluke Inc., Everett, WA, USA) together with a FLUKE 80i-110s AC/DC current probe (Fluke Inc., Everett, WA, USA) and Fluke View Scope Metre using Software for Windows SW90W (B. V. Tilburg, The Netherlands). Additional voltage measurements were performed using a NI PXI-4071 Digital Multimeter (National Instruments Corporation, Austin, TX, USA) with special software developed in Lab VIEW.

2.4.2. Electrical stunning

In order to establish electrical settings required to stun fish instantly and maintain unconsciousness, one fish each was stunned at 105, 79 and 52 V_{rms} for 1 s. On our third attempt, the voltage was set at 52 V_{rms} for 1 s which was chosen to establish whether or not this voltage provided sufficient current to provoke an instantaneous stun. The remaining fish were subjected to the latter conditions. We verified that at 52 V_{rms}, the waveform consisted of a direct current of 48 V and an alternating current of 4 V_{rms} (100 Hz). Stunning and recovery tests were performed on individual cod ($n = 27$) and haddock ($n = 27$) netted from the holding tank over a period of 4 days. After electrode placement, the fish, which were attached to the wooden stick, were positioned to assure direct contact between the head and one of the electrodes on the first row. The metal frame (corresponding to the metal conveyor belt of the commercial the stunning unit) acted as the negative electrode. When adequate contact was established, the current was turned on. The fish immediately responded with a strong twitch. EEGs and ECGs were recorded from 30 s before stunning to up to 5 min after stunning and recovery. Noxious stimuli (i.e. needle scratches applied to the dorsal skin) were administered at 0.5, 1, 2, 3, 4 and 5 min