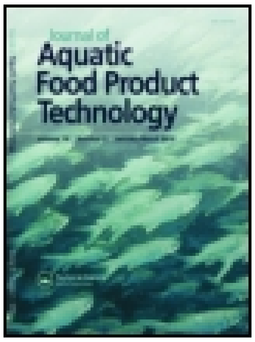

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# Establishing a Method for Electrical Immobilization of Whitefish on Board Fishing Vessels

U. Erikson, L. Grimsmo, and H. Digre

Seafood Technology, SINTEF Ocean, Trondheim, Norway

**ABSTRACT**

A downscaled version of an electrical dry stunner used in aquaculture has been adapted for use on fishing vessels to immobilize the catch (cod, haddock and saithe) shortly after capture to facilitate immediate and easy catch handling, and to minimize downgrading due to poor bleed-out. Possible effects of electrical stimulation of white muscle (blood lactate, initial pH and twitch ability) were determined. Eye roll, respiration, equilibrium, swim ability and noxious stimuli were assessed after stunning and fillets were examined with respect to color shade, possible blood spots and discolorations. Suitable voltages, exposure times to electricity, and rows of electrodes were 40 and 70 V, 4 to 6 s, and 3 to 5 rows of electrodes, respectively. Cod and haddock can be immobilized without skeletal damages and occurrence of blood spots and discolorations. In contrast, 11% of the saithe had fractured spine usually accompanied by a single large blood spot.

**KEYWORDS**

Cod; saithe; haddock; electrical immobilization; on board processing

## Introduction

Whitefish like Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), and saithe (*Pollachius virens*) are routinely bled after capture on board fishing vessels. The main reason for this is to avoid downgrading due to presence of blood and discoloration since consumers prefer white fillets without color-related flaws. To minimize discoloration, it is well established that the most important factor is to bleed the catch before the blood starts to coagulate. In practice, this means that the catch should be bled immediately or within 30 min (Olsen et al. 2014) to 1 h post capture (Botta et al. 1986). On large vessels, like trawlers and Danish seiners, however, there are certain factors that may impede adequate bleeding of the whole catch. Shortly after capture, the catch is transferred to a so-called dry bin, which is typically a steel tank without water located below the shelter deck. Fish are then consecutively processed from the dry bin (bled, gutted, washed, chilled or frozen, and packed). Fishermen tend to wait until the fish become calm or moribund before processing starts, since the catch will then be easier and safer to handle during bleeding and gutting operations. Furthermore, when large quantities of fish are caught, processing can take several hours. At some point, most fish in the dry bin will be dead by the time they are processed. Considering fish welfare, extended storage in dry bin will result in slow death due to asphyxia, as well as distress caused by the weight of surrounding fish. These factors can be minimized if fish processing can start shortly after the catch is taken aboard. Delayed processing can lead to reduced product quality due to inferior fillet color characteristics (Erikson et al. 2019). A remedy to minimize discolorations can be to transfer the catch to on-board holding tanks with good water quality from which live fish are consecutively processed (Digre et al. 2017; Olsen et al. 2013). To facilitate immediate start of processing, as well as safe and easy handling of fish from either dry bin or live storage tank, the introduction of an automated stunning method could be a possible solution.

**CONTACT** U. Erikson ulf.erikson@sintef.no Seafood Technology, SINTEF Ocean, Brattørgata 17C, Trondheim 7010 (P.B. 4762 Sluppen), Norway.

For usage in the aquaculture industry, an electrical dry stunner (STANSAS #1, SeaSide AS, Stranda, Norway) was developed to render Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) instantly unconscious before exsanguination. Suitable electrical conditions for humane stunning, valid for salmonids, have been described by Lambooij et al. (2010). The slaughter process starts as salmon from well boats or waiting cages are pumped to a processing plant where the water is drained off just before the fish enter the electrical stunner. Depending on the capacity of the plant, salmonids are distributed into several slots (e.g. three) where the fish, carried on a stainless-steel conveyor belt, pass several rows (e.g. six) of hinged electrodes (steel flaps, e.g. four in each slot). It is required by welfare regulations in aquaculture in Norway that fish must be rendered unconscious instantly as they touch the first row of electrodes. The conveyor belt acts as counter electrode. The remaining rows of electrodes are necessary to increase exposure time to electricity to ensure the fish remain unconscious until they die due to loss of blood after the subsequent bleeding step.

A smaller version of the stunner with only one slot with 10 rows of steel electrodes was used to test the method as a possible stunning method for farmed Atlantic cod (Digre et al. 2010a). An average of 41 V pulsed direct current (pDC), where the alternating current (AC) component of the signal had frequency of 100 Hz, supplied 0.2 A to individual cod for 18 to 27 s. Exposure to electricity resulted in partial depletion of muscle ATP stores, shortening time to rigor onset. However, flesh quality was not affected. The method was considered promising for stunning of farmed cod. To comply with welfare regulations for stunning and killing of cod in aquaculture, however, it was necessary to establish electrical conditions for rendering cod instantly unconscious and prevent early recovery during the exsanguination step. For the same type of stunner, it was later established that rested farmed cod can be instantly (0.5 s) stunned at 107 V pDC with a current of 0.5 + 0.2 $A_{rms}$. Furthermore, the fish needed to be exposed to electricity for 15 s to avoid recovery during exsanguination. No fillet discolorations or spinal fractures were observed under these conditions (Erikson et al. 2012). Regarding fillet quality and rigor onset after stunning of cod at 107 V pDC, similar results were achieved as mentioned by Digre et al. (2010a).

Since farmed cod can be successfully stunned by the STANSAS #1 stunner, it was suggested that the equipment should be tested on board fishing vessels to facilitate immediate processing of whitefish as described above. However, due to limited space on board most fishing vessels, it was necessary to devise a shorter version of the stunner, with one slot only. Since fish welfare regulations for ethical stunning of farmed fish do not yet apply to wild-caught fish, stunning at 107 V and an exposure time of 15 s were not strictly necessary to adhere to at sea.

Our main goal was to immobilize whitefish effectively to facilitate rapid catch handling to minimize the frequency of fillet discolorations. We hypothesized that adequate immobilization could be achieved by establishing a suitable combination of “low” voltages ($\ll$ 107 V) and lowest possible number of rows with electrodes (short exposure time to electricity). Since it is well known that different fish species respond differently upon exposure to electricity, the major whitefish species in the Norwegian fisheries (cod, saithe and haddock) were included in the present study.

## Materials and methods

### Capture conditions and fish

The study was carried out between 9 and 14 November 2012 on board the research vessel R/V Helmer Hanssen (63.8 m length overall; 4080 horse power). The fish were caught off the coast of Tromsø in northern Norway (69–71°C N/17-25°CE) with two identical two-panel Euronete trawls built entirely of 155 mm nominal mesh size polyethylene netting with 453 meshes of circumference. The trawl had a 36.5 m headline and 19.2 m fishing line. Fish from eight hauls were used, where the towing time was 1.5 h, except for 1.0 h in one case. In all cases, catches were <400 kg, and the fishing depth varied between 130 and 335 m. Wind and sea temperature ranged from 2 to 14 $ms^{-1}$ and 7.0 to 8.4°C, respectively. Three whitefish species were caught; data for the selected experimental fish are shown in Table 1.

## Sampling of fish

### Dry bin

Groups of saithe (S_DB) and cod (C_DB) (Table 2) were sampled from the dry bin (DB) between 30 and 60 min post capture. The fish were killed by a sharp blow to the head before the throat was cut and blood was collected for analysis of lactate. Then, they were put in a tank containing seawater for 30 min bleed-out. Subsequently, initial pH in white muscle, muscle twitches, and body temperatures were determined. Round weight and total length were also recorded along with gender as well as weights of liver and gonads. Then, the fish were filleted, briefly washed, and gently dried with paper towels before they were subjectively examined for possible blood spots, discolorations, whole fillet color, as well as skeletal damages. In terms of processing on board, these fish represented typical current commercial practices (processing directly from dry bin).

**Table 1.** Description of whitefish used in the present study (mean values ± SEM).

| Species | n | Total length (cm) | Round weight (kg) | CF[(1)] (-) | GSI[(2)] (%) | HSI[(3)] (%) | Females/Males (%) |
|---|---|---|---|---|---|---|---|
| Saithe | 141* | 67 ± 1 | 2.9 ± 0.1 | 0.9 ± 0.0 | 1.5 ± 0.2 | 6.9 ± 0.3 | 52/48 |
| Cod | 114* | 71 ± 1 | 3.5 ± 0.2 | 0.9 ± 0.0 | 1.1 ± 0.1 | 6.6 ± 0.3 | 46/54 |
| Haddock | 46 | 52 ± 1 | 1.5 ± 0.0 | 1.0 ± 0.0 | n.a. | n.a. | n.a. |

[(1)] Fulton's condition factor; [(2)] Gonadosomatic index: GSI = (gonad weight/body weight) x 100%; [(3)] Hepatosomatic index: HSI = (liver weight/body weight) x 100%; *For length, weight, CF, GSI, HIS and gender: n = 54 and 59 for saithe and cod, respectively; n.a. = not analyzed.

**Table 2.** Definition of experimental groups and test conditions (applied voltage, exposure time to electricity, and number of stunner rows equipped with electrodes) for electrical immobilization of saithe, cod, and haddock on board a trawler. Fish from dry bin (DB) and live storage tank only (LS) were not subjected to electrical exposure. Groups exposed to electricity were always taken directly from the live storage tank.

| Group | n | pDC voltage (V) | Exposure time (S) | # Rows (R) |
|---|---|---|---|---|
| *Saithe (S)* | | | | |
| S_DB* | 20 | - | - | - |
| S_LS* | 14 | - | - | - |
| S_20V_6S_5 R | 10 | 20 | 6 | 5 |
| S_40V_6S_5 R* | 20 | 40 | 6 | 5 |
| S_40V_4S_5 R | 11 | 40 | 4 | 5 |
| S_40V_4S_3 R | 28 | 40 | 4 | 3 |
| S_70V_6S_5 R | 23 | 70 | 6 | 5 |
| S_70V_4S_3 R | 10 | 70 | 4 | 5 |
| S_70V_5S_3 R | 20 | 70 | 5 | 3 |
| *Cod (C)* | | | | |
| C_DB* | 20 | - | - | - |
| C_LS* | 19 | - | - | - |
| C_40V_6S_5 R | 5 | 40 | 6 | 5 |
| C_40V_4S_5 R | 11 | 40 | 4 | 5 |
| C_70V_6S_5 R* | 28 | 70 | 6 | 5 |
| C_70V_4S_5 R | 16 | 70 | 4 | 5 |
| C_70V_5S_3 R | 15 | 70 | 5 | 3 |
| *Haddock (H)* | | | | |
| H_40V_4S_5 R | 11 | 40 | 4 | 5 |
| H_70V_4S_5 R | 18 | 70 | 4 | 5 |
| H_70V_5S_3 R | 17 | 70 | 5 | 3 |

**Assessments of handling stress were carried out on these groups of fish*

### Electrical immobilization and recovery

From pre-tests with the stunner, we knew that it is more challenging to stun viable fish (less stressed) than severely stressed and moribund fish from a dry bin. Hence, we decided to collect fish subjected to live storage on board for the experiments with electrical immobilization.

Immediately after the trawl gear was hauled onto deck, live fish were randomly collected from the codend and quickly transferred batchwise in baskets for temporary storage on deck in two 1035-L tanks with running seawater. To enable rapid transfer (few seconds) from tank to the stunner located below deck, the fish were subsequently moved to two other 1000-L live storage tanks (LS) with running seawater (flow-through principle). Cod were contained in one tank, whereas another tank contained saithe or haddock. Throughout live storage, dissolved oxygen (DO) levels and water temperatures varied between 58 and 95% saturation and 7.4 and 9.0°C, respectively.

All groups of fish subjected to exposure to electricity were processed similarly to fish from the dry bin, except that they were killed after behavioral observations for 10 min post-stunning. A 200-L observation tank filled with seawater (8.3°C, 86% DO saturation) was used.

A smaller version of the STANSAS #1 stunner, as used in the aquaculture industry, was constructed by SeaSide AS (Stranda, Norway) and adapted to whitefish processing lines on trawlers and seiners. The single-slot stunner had five rows of electrodes where each row had six stainless-steel electrodes (each 5 cm in width). The stainless-steel conveyor belt acted as negatively charged counter electrode as the fish were transported through the stunner. The control box, where the electrical pulses were generated, was similar to the full-size version of the stunner used in aquaculture. In the present feasibility study, the stunner was not installed in the vessel's processing line, but rather, in the research vessel's laboratory.

A priori, we decided to test combinations of different voltages (20, 40 and 70 V pDC), 3 or 5 rows of electrodes, as well as three exposure times to electricity (4, 5, and 6 s) by adjusting conveyor belt speed (between 19 and 25 cm $s^{-1}$). One or two fish at a time were quickly netted from the live-holding tank and placed head-first on the conveyor belt within 10 s. Fish behavior and applied voltage across the electrodes and conveyor belt were determined. As shown in Table 2, DB and LS groups of saithe and cod, as well as saithe exposed to 40 V, and cod exposed to 70 V, were selected for determination of stress levels (blood lactate, white muscle pH, and twitch ability) pre and post stunning. This was done to check whether differences in remaining energy content of the white muscle (degradation of glycogen and ATP, already partly degraded by capture stress) played a role regarding immobilization efficiency or tendency to cause skeletal damage by, for example, excessive muscle twitches upon electrical exposure. After 10 min in the observation tank, the fish were killed and bled as described above.

## Analytical methods

### Blood lactate, initial white muscle pH, and body temperature

Blood lactate was used as an indicator of anaerobic metabolism. Immediately after the fish had been killed by a blow to the head and the throat had been cut, a lactate test strip was briefly soaked in blood. The strip was inserted into a Lactate Scout+ meter (EKF Diagnostics GmbH, Magdeburg, Germany), and the lactate concentration was read directly on the display in mmol $l^{-1}$. The measuring range of the instrument is 0.5–25 mmol $l^{-1}$. While the method has been found useful for field studies (Brown et al. 2008), where the main purpose is to compare experimental groups of fish, the method generally seems to underestimate lactate concentrations compared with traditional analytical methods (Stoot et al. 2014). Subsequently, the initial pH of dorsal white muscle was measured after an incision had been made through the skin using a scalpel. A shielded glass electrode (WTW SenTix 41, WTW, Weilheim, Germany) connected to a portable pH meter (model WTW 315i) was used. The body temperature was measured in deep muscle through the incision made for measuring pH in white muscle. A Testo 110 thermometer (Testo AG, Lenzkich, Germany) was used.

### Muscle twitches

The ability of the white muscle to contract immediately after stunning and killing was determined using a Twitch Tester Quality Assessment Tool (AQUI-S Ltd., Lower Hutt, New Zealand). The instrument generates an electrical pulse (9 V DC for 0.6 s). A few (1–3) measurements were performed on one side of each fish. For each measurement, the electrodes were in contact with the fish for about 1–2 s. The following scale was used:

- ***3*** *– Tail twitch (electrodes placed along the entire lateral line, behind the head, and near the caudal fin)*
- ***2*** *– Weak tail twitch (electrodes placed as above)*
- ***1*** *– Minor muscle contractions in (small) restricted areas of the fish surface (electrodes placed a few cm apart)*
- ***0*** *– No contractions whatsoever.*

### Fish behavior during and after exposure to electricity

Visual assessments of the behavior of saithe, cod, and haddock during exposure to electricity and possible recovery during 10 min in the observation tank were carried out. After exposure to electricity, the following parameters were recorded:

*Vestibulo-ocular reflex (VOR)*, or “eye roll,” was assessed where VOR = 0 means no “eye roll,” which indicates that a fish is unconscious or dead. VOR = 1 means “eye roll” is present; that is, the fish is conscious (Kestin et al. 2002).

*Swimming behavior*: score 0 – none; score 1 – intermittent/erratic; score 2 – weak; score 3 – normal

*Respiration/opercular motion*: score 0 – none; score 1 – slow; score 2 – normal

*Noxious stimuli*: Needle scratches were applied to the dorsal skin; score 0 – no response; score 1 – fish responding

*Equilibrium*: score 0 – belly up; score 1 – fish lying sideways on bottom; score 2 – normal (upright position).

### Fillet blood spots, discoloration, and skeletal damage

Just after bleed-out and sampling (see above), the fish were filleted. The fillets were briefly washed and gently dried with paper towels before they were examined by two trained panelists. The color shade of whole fillets was assessed as: score 0 – normal; score 1 – pink; score 2 – reddish. Since it is known that exposure to electricity may cause skeletal damage and hemorrhages, spines and fillets were examined for possible fractures and associated blood spots, respectively.

### Statistical analysis

Only fish from the same haul (saithe: Haul 6 and cod: Haul 7) were compared statistically in terms of stress. Blood lactate and initial muscle pH data were tested for normality and homogeneity of variance using the Shapiro-Wilk and Levene Median tests, respectively. Where either normality or homogeneity of variance tests failed, the Mann–Whitney Rank Sum test was used (saithe and cod lactate as well as saithe pH in muscle). Since muscle pH data for cod passed both Shapiro-Wilk and Levene Median tests, fish groups were compared using a t-test. Fish groups were considered different when $P < 0.05$.

## Results

### Electrical immobilization

Variation in voltages, stunner-set point versus measured values on the stunner, during exposure to electricity is shown in Table 3. For simplicity, stunner set point values are always referred to herein.

**Table 3.** Relationship between stunner course voltage setpoints and actual voltage ranges as measured during immobilization of whitefish.

| Stunner voltage set point (V pDC) | Actual voltage* (V pDC) |
|---|---|
| 20 | 19–20 |
| 40 | 38–40 |
| 70 | 67–70 |

*Voltage as measured across hinged electrodes (+) vs conveyor belt (-).

Generally, all species of whitefish were calm during the 5 to 10 s transfer from live storage tank to stunner. Immediately following exposure to electricity (contact with first row of electrodes), ventilation ceased. For some fish, regardless of species, exposure to electricity immediately resulted in extended opercula or pectoral fins; whereas in other cases, the whole fish body rapidly became stiff. Other fish showed merely vague reactions or hardly any reactions at all, as they passed through the stunner. Furthermore, no strong muscle twitches were observed as fish passed the stunner.

## Handling stress

Blood lactate concentrations and initial pH in white muscle in fish from DB and LS, saithe and cod exposed to 40 and 70 V, respectively, are shown in Table 4. In all cases, the levels of lactate were high. In case of cod from Haul 7, lactate was significantly lower in fish subjected to live storage followed by stunning than those from dry bin.

In terms of white muscle pH, it was evident that DB and LS groups of saithe experienced significant perimortem anaerobic muscle activity, since the values were low at about pH 7.1. On the other hand, saithe exposed to 40 V displayed significantly higher pH values at pH 7.30, as determined after 8 to 9 h of live storage before stunning. Likewise, cod from DB exhibited severe acidosis (pH 6.99) compared with fish stored live for 12 to 13 h before stunning at 70 V (pH 7.53).

The ability of white muscle of saithe and cod to twitch after sampling from dry bin, live storage tanks, as well as after live storage followed by electrical exposure and storage in observation tank is shown in Figure 1. Direct comparisons between treatments are not straightforward since experimental fish were caught from different hauls and because sampling times on board were different (twitch ability also decreases during the early postmortem phase). Nevertheless, the data indicates, as can be expected, that several fish from DB were in a poor physiological condition, possibly moribund or dead, since no twitches were observed for some fish. Furthermore, post-capture recovery during live storage for about 10 h followed by exposure to 40 or 70 V did not seem to cause excessive depletion of white muscle energy reserves.

**Table 4.** Blood lactate and initial pH in white muscle of fish from dry bin (DB, without water) and live storage tank (LS, with circulating seawater), as well as after electrical immobilization where both fish species were taken from the LS tank just before exposure to electricity. Fish body temperatures varied between 8.7 and 9.7°C.

| Group | Haul | Blood lactate (mmol $l^{-1}$) | Initial pH in muscle | Storage time (min)* |
|---|---|---|---|---|
| *Saithe* | | | | |
| S_DB | 6 | 6.7 ± 0.6 (1.2)[a] | 7.05 ± 0.03 (0.05)[a] | 30–40 |
| S_LS | 8 & 9 | 10.3 ± 0.5 (1.0) | 7.06 ± 0.05 (0.11) | 50–140 |
| S_40V_6S_5 R | 6 | 8.3 ± 1.0 (2.2)[a] | 7.30 ± 0.07 (0.14)[b] | 487–543 |
| *Cod* | | | | |
| C_DB | 7 | 8.2 ± 0.4 (0.9)[a] | 6.99 ± 0.03 (0.06)[a] | 60–90 |
| C_LS | 8 & 9 | 8.9 ± 0.9 (1.8) | 7.24 ± 0.05 (0.11) | 15–215 |
| C_70V_6S_5 R | 7 | 5.6 ± 0.6 (1.4)[b] | 7.53 ± 0.05 (0.10)[b] | 704–764 |

Mean values ± SEM (95% C.I. of mean; n = 20); Only fish from the same haul were compared statistically where different letters, a or b, mean significant difference ($p < 0.05$); *post capture.

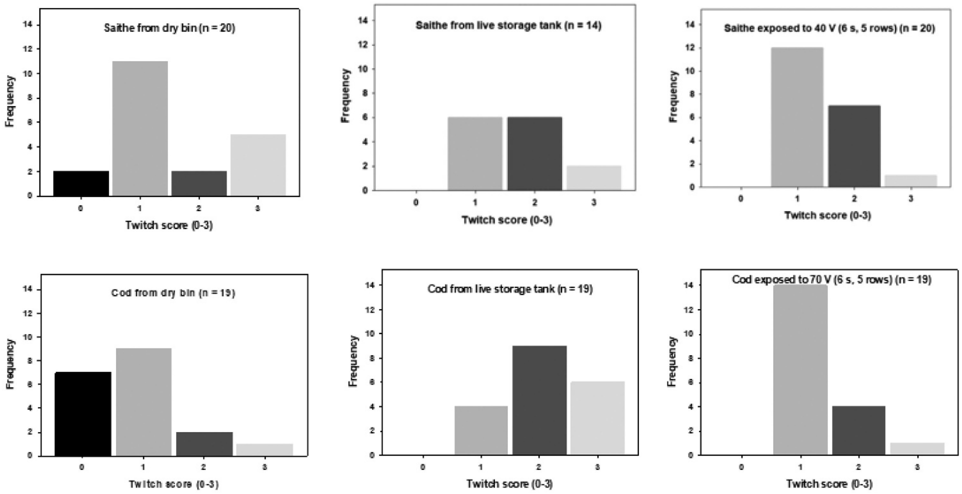

**Figure 1.** White muscle twitches of saithe and cod from (1) dry bin: after 30 to 40 min (saithe, Haul 6, upper left) and after 60 to 90 min (cod, Haul 7, lower left); (2) live storage tank: after 50 to 140 min (saithe, Hauls 8 & 9, upper centre) and after 15 to 215 min (cod, Hauls 8 & 9, lower centre); (3) stunner (including live storage before stunning and 10 min in observation tank after stunning): after 487 to 543 min (saithe, Haul 6, upper right) and after 704 to 764 min (cod, Haul 7, lower right). All points in time refer to post capture.

## *Behavior after exposure to electricity*

Immediately after the fish had passed the stunner, they were transferred to the observation tank to monitor behavior for the next 10 min (Table 5). Since VOR were observed in several fish within all groups, except from C_40V_6S_5 R group with n = 5 only, none of the voltages (20, 40, or 70 V) applied for 4–6 s or by using 3 or 5 rows of electrodes were able to render all fish within a group unconscious for 10 min. Otherwise, no clear patterns regarding VOR *vs* electrical parameters were clearly evident.

**Table 5.** Visual assessment of saithe, cod, and haddock during 10 min in seawater after exposure to electricity. The table shows the number of fish assigned to each category.

| | | VOR (0–1) | Swimming behaviour (0–3) | | | | Respiration (0–2) | | | Noxious stimuli (0–1) | Equilibrium (0–2) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | n | *1* | *0* | *1* | *2* | *3* | *0* | *1* | *2* | *1* | *0* | *1* | *2* |
| | | | | | | *Saithe* | | | | | | | |
| S_20V_6S_5 R | 10 | 7 | 5 | 2 | 1 | 2 | 3 | 1 | 6 | 5 | 2 | 3 | 5 |
| S_40V_6S_5 R | 20 | 10 | 19 | 0 | 1 | 0 | 12 | 5 | 3 | 10 | 0 | 19 | 1 |
| S_40V_4S_5 R | 11 | 2 | 11 | 0 | 0 | 0 | 10 | 1 | 0 | 1 | 8 | 3 | 0 |
| S_40V_4S_3 R | 20 | 3 | 20 | 0 | 0 | 0 | 16 | 3 | 1 | 4 | 1 | 19 | 0 |
| S_70V_6S_5 R | 23 | 1 | 23 | 0 | 0 | 0 | 22 | 1 | 0 | 2 | 5 | 18 | 0 |
| S_70V_4S_3 R | 10 | 3 | 10 | 0 | 0 | 0 | 7 | 2 | 1 | 2 | 2 | 8 | 0 |
| S_70V_5S_3 R | 20 | 2 | 19 | 1 | 0 | 0 | 18 | 1 | 1 | 1 | 20 | 0 | 0 |
| | | | | | | *Cod* | | | | | | | |
| C_40V_6S_5 R | 5 | 0 | 5 | 0 | 0 | 0 | 5 | 0 | 0 | 1 | 0 | 5 | 0 |
| C_40V_4S_5 R | 11 | 4 | 10 | 1 | 0 | 0 | 9 | 1 | 1 | 0 | 10 | 1 | 0 |
| C_70V_6S_5 R | 28 | 11 | 28 | 0 | 0 | 0 | 18 | 8 | 2 | 6 | 4 | 23 | 1 |
| C_70 V_4S_5 R | 16 | 7 | 16 | 0 | 0 | 0 | 7 | 8 | 1 | 3 | 10 | 4 | 2 |
| C_70V_5S_3 R | 15 | 9 | 14 | 0 | 1 | 0 | 6 | 5 | 4 | 5 | 6 | 8 | 1 |
| | | | | | | *Haddock* | | | | | | | |
| H_40V_4S_5 R | 11 | 3 | 10 | 1 | 0 | 0 | 6 | 3 | 2 | 4 | 4 | 6 | 1 |
| H_70V_4S_5 R | 18 | 1 | 18 | 0 | 0 | 0 | 16 | 2 | 0 | 2 | 7 | 10 | 1 |
| H_70V_5S_3 R | 17 | 3 | 16 | 0 | 1 | 0 | 14 | 2 | 1 | 1 | 5 | 11 | 1 |

**VOR** (score 1 -"eye roll" as evaluated after 10 min post stunning); **Swimming behavior**: score 0 – none, score 1 – intermittent/erratic, score 2 – weak, score 3 – normal; **Respiration/opercular motion**: score 0 – none, score 1 – slow, score 2 – normal; **Noxious stimuli**: score 1 – fish responding; **Equilibrium**: score 0 – belly up, score 1 – fish lying sideways on bottom; score 2 – normal.

For most fish, regardless of species, swimming activity was significantly hampered after exposure to electricity at 40 and 70 V. Whether exposure times between 4 and 6 s or 3 and 5 rows of electrodes were used did not seem to matter. Half of the haddock exposed to 20 V exhibited erratic to normal swimming behavior. When saithe is considered, except for exposure to 20 V, respiration ceased following exposure to electricity in case of most fish in the experimental groups. The same was basically true for haddock. Cod, on the other hand, seemed to be less inclined to lose respiration by comparison, perhaps with exception of the C_70V_6S_5 R group. With two exceptions, S_20V_6S_5 R and S_40V_6S_5 R groups, most fish in the various groups did not respond to noxious stimuli. Merely a few fish, for some of the treatments, did not lose equilibrium following electrical exposure. Typically, many fish in the various groups were either lying sideways on bottom or floating belly up after exposure to electricity. As with the other behavioral assessments, exposure to 20 V was less effective to immobilize saithe.

### *Fillet discoloration and skeletal damage*

In general, the occurrence of small blood spots was modest for all three species, apparently without any correlation to electrical exposure (data not shown). In the case of saithe and cod from dry bin, and all treatments of cod and haddock according to Table 1, no spinal fractures were observed. In contrast, spinal fractures were observed in five out of seven experimental groups for saithe, as shown in Table 6. In total, electrical exposure resulted in spinal fractures in 11% of the saithe. In cases where the spine was broken, only a single fracture per fish was invariably identified. The fracture point was approximately located below the 2nd dorsal fin. Incidences within experimental groups varied between 0% and 30% of the fish. The fractures were identified by successive gentle bending of small sections of the spine. Loss of flexibility in the examined section was caused by vertebra fracture. In most cases, the fracture was clearly accompanied by a single, large blood spot covering both fillet sides. Apparently, there was no unambiguous correlation between voltage (20, 40, or 70 V), exposure time (4, 5, or 6 s), or number of rows with electrodes (3 or 5) and inclination to spinal fractures.

The color shades of cod and haddock fillets were close to normal white appearance (score 0) and on the average considerably lower than all pink appearance (score 1). For saithe fillets, the situation was largely the same, although with values slightly more towards a pink shade. The fillets were subsequently salted to visually enhance the appearance of possible residual blood. The situation remained largely the same after re-examination, with no significant differences between treatments (data not shown). The results suggest that electrical exposure did not alter fillet color shade.

## Discussion

### *Handling stress*

In terms of blood lactate (Table 4), it was evident that all groups of fish must be defined as considerably stressed, since their mean values ranged from 5.6 to 10.3 mmol $l^{-1}$. The lower detection limit of our

**Table 6.** Number of spinal fractures for different experimental groups of saithe. Saithe from dry bin (DB) and live storage (LS) were not exposed to electricity.

| Group | No. of fish with broken spine/No. of fish in group |
|---|---|
| S_DB | 0/20 |
| S_LS | 0/14 |
| S_20V_6S_5 R | 3/10 |
| S_40V_6S_5 R | 5/20 |
| S_40V_4S_5 R | 0/11 |
| S_40V_4S_3 R | 1/28 |
| S_70V_6S_5 R | 1/23 |
| S_70V_4S_3 R | 3/10 |
| S_70V_5S_3 R* | 0/20 |

*Eight fish were judged as possibly dead before exposure to electricity.

lactate meter is 0.5 mmol $l^{-1}$, a value which is also typical of unstressed cod (Brown et al. 2008). Just after capture of cod by trawl, mean lactate levels of around 3.4 mmol $l^{-1}$ have been reported (Digre et al. 2017; Olsen et al. 2013). The mean lactate levels of saithe from DB and after stunning at 40 V groups were not significantly different in spite of the fact that the latter group of fish could have recovered during 8 to 9 h of live storage. This may indicate a slow clearance rate of lactate in blood. On the other hand, the levels of lactate in cod blood after stunning at 70 V were in fact significantly lower (5.6 mmol $l^{-1}$) than in cod from DB (8.2 mmol $l^{-1}$). This might be explained by a longer recovery period for cod (12 to 13 h) than for saithe or by differences between species. Nevertheless, we do not have clear evidence that electrical exposure affected lactate levels, but it should be mentioned that stunning of farmed cod in air at 107 $V_{rms}$ did not affect mean lactate values after exposure to electricity for 0.5 s and 15 s (Erikson et al. 2012).

Regarding acidity in white muscle, the S_DB, S_LS and C_DB groups had mean initial pH values of pH 7.05/7.06 (saithe) and pH 6.99 (cod), indicating that the cod were severely stressed since comparable values of rested (anaesthetized) and severely stressed cod have been reported as pH 7.54 to 7.64 and pH 7.09, respectively (Erikson et al. 2011). We are not aware of controlled experiments where the magnitude of a given stress response of rested saithe is assessed in terms of white muscle activity (PCr/ATP and glycogen degradation to IMP and lactate/$H^+$, respectively). It, nevertheless, seems that the stress response of saithe to capture was of similar magnitude to that of cod.

Initial pH in white muscle of cod caught by trawlers typically varies between pH 7.2 and 7.3 (Digre et al. 2010b), pH 7.01 (Olsen et al. 2013) and pH 7.11 (Digre et al. 2017). Stunning of farmed cod at 107 $V_{rms}$ for 0.5 (pH 7.06 to 7.10) and 15 s (pH 7.00) did not significantly affect initial pH in muscle (Erikson et al. 2012). Moreover, the significantly higher pH values of both S_40V_6S_5 R (8 to 9 h recovery) and C_70V_6S_5 R (12 to 13 h recovery) groups can be explained by recovery before stunning as well as that stunning per se did not in turn deplete pH back to post-capture levels. The C_LS fish also indicated signs of recovery.

When the levels of blood lactate versus initial pH in muscle of S_40V_6S_5 R, C_LS, and C_70V_6S_5 R groups are compared individually (Table 4), the overall picture does not necessarily seem to make sense at first sight since the lactate values indicated severe stress whereas the pH values indicated anything from partial recovery (pH 7.24 to 7.30) to rested fish (pH 7.54). Common for these groups of fish was that they were kept in the live storage tank with circulating seawater. This means that under favorable conditions, the fish could recover from capture stress. Beamish (1968) reported that blood lactate continues to increase for up to about 1 h after strenuous exercise and returned to baseline levels, dependent on conditions, up to about 24 h post exercise. In white muscle, post-exercise movements of lactate and protons into the extracellular compartment appear to be dissociated processes (Wood 1991), where the elimination of lactate from muscle cells during recovery is a slow process (Neumann et al. 1983). Recovery of white muscle pH is a considerably faster process compared with that of plasma lactate (Booth et al. 1995). Hence, our blood lactate and white muscle pH values shown in Table 4 probably reflect that the relevant groups of fish were at different stages of recovery during storage in the LS tank, while the DB groups basically reflected capture stress and possibly some struggling in air before they became quiescent.

As could be expected, fish without twitch ability were observed in the dry bin only (Figure 1). Also, it is evident that exposure to electricity at 40 and 70 V for 6 s did not lead to dramatic depletion of the energy stores in white muscle. This suggests that by using the stunner for immobilization of the catch, under the current conditions, will not likely hamper processing on board by early onset of rigor mortis. Otherwise, it is not possible to draw firm conclusions regarding the distribution of twitch scores between 1 and 3 for the various groups of fish. Mean twitch scores of cod just after capture by trawl has been reported as 2.1 (Digre et al. 2017) whereas for rested, farmed cod in cage and after carbon dioxide stunning, the values were significantly different at 3.0 and 2.6, respectively (Erikson et al. 2011). Furthermore, when cod were stunned in air at 107 $V_{rms}$ for 0.5 and 15 s, the twitch values were not significantly different at 2.9 and 2.7, respectively (Erikson et al. 2012).

Generally, it should be mentioned that the catch sizes in this study were modest. Considerably larger catch sizes and rougher fishing conditions can prevail during commercial fishing. This will likely affect the condition of the fish. Compared with the fish used in the present experiment, such fish may be more stressed and would then require longer recovery times.

### Electrical immobilization

Since our goal was to immobilize fish to facilitate immediate catch processing and easy handling of individual fish, our results indicated that a voltage of 20 V was too low to achieve that whereas both 40 and 70 V can be applied to provide acceptable immobilization (Table 5). Exposure times between 4 and 6 s, as well as using three or five rows of electrodes, seemed to be adequate for the purpose. Thus, a compact stunner with only 3–5 rows of electrodes with accompanying short exposure time can be recommended. In practice, this would imply that fishermen have at least 10 min to process (exsanguinate) either saithe, cod, or haddock on the processing line at any one time before the fish (might) start to recover significantly. Since another goal was to minimize the applied voltage, it is evident that 40 V is sufficient for immobilization on commercial vessels.

Assessment of behavior (Table 5) basically showed that exposure to voltages up to 70 V and 6 s did not render all fish unconscious. If welfare is considered, fish would have to be stunned instantly as required in the aquaculture industry. To achieve a larger portion of fish without VOR, stunner voltage and/or exposure time need to be increased. As already mentioned, consistent stunning and prevention of early recovery of rested cod in aquaculture can be achieved by using 107 V for 15 s (Erikson et al. 2012). By using a small experimental version of the same stunner, although with only one row of electrodes, where the fish were restrained on a wooden strip for EEG and ECG measurements and then brought into contact with the electrodes, Lambooij et al. (2012) reported that on board a trawler, cod and haddock could be immediately stunned (0.7 s) at 52 $V_{rms}$ at a combined AC and DC (pDC) of 0.34 and 0.36 $A_{rms}$. Recovery was prevented by exposure for at least 3 s in combination with a throat cut. This suggests that a voltage of 70 V and exposure time of 4 to 6 s, as in our case, could be sufficient to achieve humane stunning of whitefish. This was clearly not the case after 10 min post stunning (Table 5), since 4 to 60% of the whitefish were conscious (VOR = 1) after removal from the observation tank. We cannot rule out, however, that these fish could in fact have been instantly stunned before recovery in the observation tank. If so, a voltage of 70 V followed by cutting the throat just after stunning can be the solution to upgrade the system to perform humane stunning on board vessels. It should be mentioned that animal welfare in the fisheries sector is currently gaining more attention (Veldhuizen et al. 2018), so improving the performance of the stunner would be a timely thing to do.

### Blood spots and skeletal damages

In contrast to cod and haddock, only saithe had occasional skeletal damages due to exposure to electricity. No such damages were observed in DB and LS groups for saithe (Table 6). Based on visual assessment of saithe behavior during immobilization, we did not observe distinct differences from cod and haddock regarding, for example, excessive white muscle twitches. Since saithe is sensitive to electricity (Roth et al. 2004) it seems that the spine of this species is more fragile than that of cod and haddock. From our current set of electrical variables, it is not possible to elucidate whether certain combinations of voltages and exposure times were better than others to minimize the problem. Regardless of treatment, 13 out of 122 saithe (11%) exposed to electricity exhibited spine fractures, which in most cases were accompanied by a single, large blood spot. This is probably not acceptable since such fillets may be downgraded. As an attempt to solve these issues with saithe, a study was subsequently conducted where the effects of pre-stunning stress, applied voltage (40 to 100 V), as well as stunner configuration were assessed. None of the mentioned factors fully solved the problem with fractured spines. However, it was found that the frequency of skeletal damage can be minimized by using the same stunner configuration as was used in the present study and by exposing stressed fish to electricity rather than rested fish (Erikson et al. 2016). When saithe is considered, it therefore seems to be a disadvantage, for a certain fraction of the catch (Table 6), to use live storage tanks since the fish eventually can recover from capture stress (Table 4) before immobilization. In such cases, fish will be more susceptible to spinal fractures.

### Commercial usage of the electrical stunner

The stunner described herein has been quite successfully adapted on many fishing vessels. For the time being, approximately 20 trawlers and 30 Danish seiners, liners, or other vessels are using the stunner to immobilize their catches. According to anecdotal feedback from fishermen, other advantages of using the system are improved SHE conditions (safer and easier catch handling and bleeding of individual fish) as well as higher fish processing speeds.

## Conclusions

Suitable voltages to immobilize whitefish to facilitate processing just after capture and easy catch handling were 40 or 70 V pDC, whereas 20 V pDC was considered too low. To minimize the size of the stunner commonly used in aquaculture, 3 or 5 rows of electrodes and exposure to electricity for 4 to 6 s were sufficient to achieve efficient immobilization. Cod and haddock can be immobilized using the present method without skeletal damage and occurrence of blood spots. In contrast, 11% of the saithe ended up with fractured spine, usually accompanied by a single, large blood spot in the fillets. Further research is needed to minimize the occurrence of skeletal damage in saithe. Exposure to electricity under the present conditions did not seem to excessively activate the glycolytic pathway in whitefish.

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