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Effectiveness of tail-first dry electrical stunning, followed by immersion in ice water as a slaughter (killing) procedure for turbot (*Scophthalmus maximus*) and common sole (*Solea solea*)

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ABSTRACT

To protect the welfare of fish at slaughter, these animals should be rendered unconscious and insensible prior to killing. Furthermore, the state of unconsciousness must be long enough to allow killing without recovery. The objective of this study was to determine the stunner settings for effective tail-first dry electrical stunning of turbot (*Scophthalmus maximus*) and common sole (*Solea solea*). The fish were separated in two batches (B1 and B2). The turbot and sole in B1 were subjected to a short tail-first stun lasting for 1 s and after 1 min of recovery to a second, longer (20 s) stun. The fish in B2 were exposed to a single long (20 s) stun, which was tail-first in sole, but head-first in turbot. The short stun was applied to verify that the loss of consciousness was instant (i.e. within 1 s), whereas the long stun (followed by immersion in ice water) was performed with the aim of showing that it is feasible to kill the fish without recovery. Loss of consciousness and sensibility were assessed using electrophysiological (EEG and ECG) and behavioural parameters.

After administering a current of 2.39 ± 0.91 A_{rms} by applying 125.5 ± 0.6 V_{rms} (100 Hz) in turbot and 1.22 ± 0.68 A_{rms} by applying 152.4 ± 0.5 V_{rms} in sole for 1 s, 25 out of 26 turbot and 9 out of 10 sole in B1 exhibited EEG patterns showing that the fish were rendered unconscious instantly.

The long tail-first exposure of turbot in B1 to 3.88 ± 1.26 A_{rms} for 1 s, followed by 1.44 ± 0.41 A_{rms} for 19 s, followed by immersion in ice water, led to an irrecoverable stun in 21 out of 22 fish, whereas the long head-first stunning of turbot in B2 (n = 13) resulted in passing 1.27 ± 0.40 A_{rms} for 1 s and 0.65 ± 0.21 A_{rms} for 19 s through the fish and no recovery during chilling. After the long, tail-first exposure of sole in B1 (n = 9) and B2 (n = 22) to 1.18 ± 0.49 A_{rms} for 1 s + 0.35 ± 0.22 A_{rms} for 19 s, and 1.20 ± 0.59 A_{rms} for 1 s + 0.36 ± 0.15 A_{rms} for 19 s, respectively, none of the fish regained consciousness during the chilling.

We conclude that the tail-first electrical stunning, followed by immersion in ice water can be developed into an effective stunning and killing method for turbot and sole.

Statement of relevance

The paper expedites stunning of turbot and sole in practice.

1. Introduction

Over the past years an effective method for electrical stunning of farmed fish after dewatering has been developed for various species (Roth et al., 2009; Lambooij et al., 2010; Erikson et al., 2012; Llonch et al., 2012).

For stunning and killing of farmed fish no specific requirements have been formulated in the current EU legislation. Thus, only general provisions apply to fish, i.e. avoiding pain and minimizing distress and suffering (Council Regulation, EC No 1099/2009, 2009).

In humans the capacity to suffer depends on the proper functioning of specific regions of the pre-frontal cortex. Given the apparent absence of a pre-frontal cortex in fish, it might be argued that fish do not have a capacity for mental awareness, e.g. to perceive pain and fear. The issue whether fish may have experiences that relate to a negative affective state such as suffering is still debated in the scientific literature (Rose, 2002; Rose et al., 2014), as reviewed by Braitwaite and Ebbesson (2014). However, recent research (as reviewed by Braithwaite et al. (2013)) shows that teleost fish have a capacity for mental awareness,

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as in teleost fish species relevant functional areas in the telencephalon have been found. Hence, studies indicate that it is possible that teleost fish perceive pain and fear when they are not stunned before killing or slaughter.

Existing stunning/killing procedures for turbot (*Scophthalmus maximus*) comprise live chilling, exsanguination and asphyxia on ice. EFSA (2009a) concluded that the application of these methods leads to prolonged periods of consciousness during which stress responses occur and, therefore, the development of alternative methods, such as electrical or percussive stunning was recommended. For common sole (*Solea solea*) asphyxia on ice or chilling on ice slurry is commonly used for killing. Hence, the application of the current methods to kill turbot and sole may be stressful for these fish.

To protect the welfare of turbot and sole at the moment of slaughter, it is essential to apply appropriate stunning methods prior to killing. Various studies show that electrical stunning, may be appropriate for rendering an animal unconscious and insensible within 1 s by passing an electric current of sufficient strength through the brain (EFSA, 2009a).

Electrical stunning causes a condition, resembling a generalized epileptic seizure (general epileptiform insult) (Lambooij et al., 2008). This condition is characterised by a rapid and extreme depolarisation of membrane potentials throughout the brain (Kooi et al., 1978), resulting in immediate loss of consciousness and inability of the brain to respond to stimuli (Lopes da Silva, 1984). Behaviourally, the general epileptiform insult, which is induced by passing an electrical current through the fish, is manifested by tonic cramps alternated by clonic ones followed by an exhaustion phase (Lambooij et al., 2002).

Although there is evidence that loss of consciousness can be induced immediately, after exposure to electricity (Lambooij et al., 2010), this stunning method also poses certain welfare challenges with respect to orientation of the fish (EFSA, 2009b) and recovery post stunning (Lambooij et al., 2010; Llonch et al., 2012). In practice fish may enter commercial electrical dry-stunners in a random position, meaning that fish entering tail-first could be exposed to electricity before the brain is subjected to the electric field a few seconds later (EFSA, 2009b). To date no EEG recordings have verified whether tail-first stunning is effective in fish, i.e. whether it can induce immediate loss of consciousness and sensibility. Furthermore, to prevent recovery from an electrical stun a killing method has to be applied. Killing methods that are currently used for farmed fish are for instance bleeding by gill-cutting, tail or throat cutting or killing by chilling in ice or ice water. Previous experiments have shown that electrical stunning, followed by chilling in ice or ice water is suitable for humane slaughter of some fish species, such as African catfish (*Clarias gariepinus*), Nile tilapia (*Oreochromis niloticus*) and yellowtail kingfish (*Seriola lalandi*) (Lambooij et al., 2006, 2008; Llonch et al., 2012). In this context, it must be noted that fish species may differ in their sensibility and/or resistance to the applied electrical stunning and killing procedure. Previous studies with EEG on turbot (unpublished data) and sole (Llonch et al., 2012) showed that a 5 s head-first exposure to 98 V DC and 8.4 Vrms AC (100 Hz) after dewatering, followed by immersion in ice water was not sufficient, as 6–10% of the fish did recover 2–3 min post stunning. Hence, there is a need to establish the appropriate electrical current parameters for both fish species.

The effectiveness of the stunning/killing method, i.e. the duration of the unconscious state, is highly dependent on both current duration and current magnitude (Robb et al., 2002; Roth et al., 2003). When a current with a sufficient magnitude is applied, the fish can be rendered unconscious instantly (i.e. within 1 s). However, previous studies have shown that such a brief exposure to the current is usually followed by regaining of consciousness within a short period of time (Lines et al., 2003; Lambooij et al., 2008). Therefore, a longer (more than 1 s) current application is needed to provide a prolonged period of unconsciousness that allows subsequent killing of the fish without recovery (Erikson et al., 2012). In line with this, a two-stage stunning procedure, involving a short exposure to a higher current immediately followed by a longer exposure to a lower current, has evidenced to be efficient in terms of maintaining the state of unconsciousness (Lines and Kestin, 2005).

The main objective of our study was to determine the stunner settings for effective electrical dry stunning of turbot and sole when the fish enter the stunner tail-first instead of head-first and are subsequently immersed in ice water. Under this main objective the following specific research questions were subsumed: 1. Can unconsciousness be induced immediately (i.e. within 1 s) in turbot and sole? 2. Can unconsciousness be maintained for a sufficient period of time (for a period of at least 5 min) to facilitate the application of a killing method without recovery? 3. How are behavioural measures of nociception, gill movements and tap responses (i.e. response to vibration) (cor-)related to each other and to brain and heart activity? 4. What are the main differences between the two species of flatfish (turbot and sole)?

Given the phylogenetic relatedness of *S. solea* and *Solea senegalensis*, which are considered very close sister lineages in most reconstructions (as reviewed by Imsland et al. (2004)), we decided that *S. solea* could also serve as model species for *S. senegalensis* in this study.

2. Materials and methods

2.1. Animals

For this study in total 85 animals (47 turbot and 38 sole) were used. The turbot were purchased from a commercial fish farm and transferred to the research lab of IMARES at IJmuiden where the experiment was conducted. The sole were obtained from the IMARES aquaculture facility in IJmuiden.

The turbot were kept in a recirculation system in a tank ($0.6 \times 2.5 \times 2.5$ m) containing aerated seawater of 17 °C. The sole were kept separate from the turbot in a similar tank at 22 °C. Mean ($\pm$ SD) body weights were 416 ± 39 g for turbot ($n = 32$) and 244 ± 66 g for sole ($n = 33$).

2.2. EEG and ECG recordings

Prior to stunning, each fish was equipped with EEG and ECG electrodes, as described by Lambooij et al. (2003, 2010). To implant the electrodes, the turbot and the sole were fixated on a plastic rectangular plate (25×80 cm) using cable ties and/or duct tape depending on the species (sole were much more responsive than turbot; the latter could even be instrumented without additional fixation). For stunning, at the end of the fixation plate an equilateral triangle of 8 cm was cut out in the middle leaving the head/tail free. For tail-first stunning of sole a strip of aluminium foil was applied to the plastic rectangular plate. The strip of foil made also contact with the negatively charged bottom plate of the experimental STANSAS dry-stunner (see next section). Each sole was placed on top of the strip of aluminium foil and, subsequently, fixated. Thus, the presence of this strip avoided an insulation of each sole from the negatively charged bottom plate of the experimental STANSAS stunner.

The EEG electrodes (20 mm long and 1.5 mm diameter; 55% silver, 21% copper and 24% zinc) were placed percutaneously in the skull, taking into account the position of the brain in these species, into the surface of the cortex. Two ECG electrodes (the same composition as the EEG electrodes) were placed subcutaneously, ventrally and dorsally of the upper pectoral fin. The earth electrode for both the EEG and ECG was placed subcutaneously near the tail.

The EEG and ECG data were recorded during about 30 s before the stunning and until 5 min after stunning, using a DI-720 data recording module with a WinDaq Waveform browser (Dataq Instruments, Akron, Ohio, USA). Two channels on the DI-720 data-recording module were used, with a 250 Hz sample frequency for each channel.