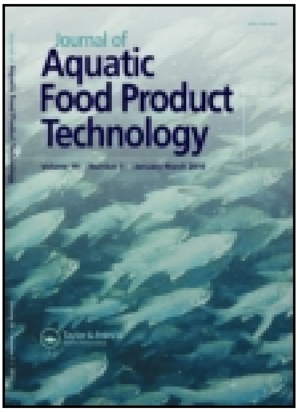

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Evaluation of Electrical Stunning of Atlantic Cod (*Gadus morhua*) and Turbot (*Psetta maxima*) in Seawater

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The aim of this study was to assess electrical stunning of Atlantic cod and turbot in seawater to develop a protocol for the process of stunning and killing. An induced general epileptiform insult (unconscious) had a duration of 40 ± 27 s (n =14) in cod (2.6 ± 0.5 kg) and 34 ± 18 s (n = 19) in turbot (520 ± 65 g). Seven cod and 3 turbot displayed a physical reaction, and 11 turbot registered an electroencephalogram (EEG) response to pain stimuli administered 30 s post-stun. The heart rate was 32 ± 6 beats/min in cod and 25 ± 7 beats/min in turbot prior to stunning. Post-stunning, the electrocardiogram (ECG) revealed fibrillation and reduced activity post-stun. EEG, ECG recordings, and behavioral observations indicate that when a bipolar square wave current was applied with a frequency of 133 Hz and 43% duty cycle side to side (turbot) and at 170 Hz and 33% duty cycle (cod) head to tail, both species were stunned in seawater at current densities of 3.2 A/dm^2 and 2.5 A/dm^2, respectively. For turbot, a 5 s exposure to electricity followed by chilling in ice water for 15 min is sufficient to prevent recovery. For cod, a killing method needs to be established.

Keywords: electrical stunning, Atlantic cod, turbot, fish welfare, brain activity, heart rate

INTRODUCTION

Consumer awareness in Europe is not limited to food safety, taste, and wholesomeness of farmed fish products. Environmental and animal welfare, especially at slaughter, are also important topics to consumers. Various studies show that welfare at slaughter can be improved by sparing an animal

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avoidable stress by applying effective stunning methods (Robb et al., 2000; Lambooij et al., 2006, 2010; van de Vis et al., 2003). Stunning is a process that renders an animal unconscious without avoidable stress prior to killing/slaughter. A stunned animal should not recover before death occurs (Anonymous, 1993).

The most commonly used method for killing turbot is chilling in seawater (−1.5 to −0.8°C) for 30–60 min (Roth et al., 2009b). Live chilling is also applied as a procedure for killing farmed Atlantic cod. According to Roth et al. (2009b), live chilling of turbot is a highly questionable practice in terms of animal welfare. Claireaux et al. (1995) reported that Atlantic cod became stressed when exposed to a sudden drop in temperature.

Contrary to turbot and Atlantic cod, stunning of Atlantic salmon (Roth et al., 2007, 2009a) is a common commercial practice. Two stunning methods (percussion or electro-stunning) are used for these species. When sufficient percussive force is used, Atlantic salmon immediately loses consciousness. However, consequences of this method were hemorrhages in the brain cavity, broken upper or lower jaws, and eye bursts (Lambooij et al., 2010). Studies on electrical stunning of various farmed fish species has revealed that carcass damage can be minimized or avoided (van de Vis et al., 2003; Roth et al., 2009a). The most common electro-stunning method for livestock uses a frequency of 50 Hz alternating current (AC) with sinusoidal waveform.

When a square wave pulsed AC is applied for electrical stunning of Nile tilapia (*Oreochromis niloticus*) instead of a 50 Hz sinusoidal, the overall current density can be reduced from 1 to 0.6 A/dm^2. The overall field strength that is needed for the current density can be reduced 26%, compared to the 50 Hz waveform (Lambooij et al., 2008a). Hence, the use of a square wave pulsed AC makes it possible to save electrical energy for stunning of fish with less effects on carcass muscles (Gregory et al., 1995).

There are two approaches of electrical stunning applicable for use in practice. The fish species can be either stunned in water or after withdrawal from the water. Stunning in water involves exposing the fish to an electrical current administered via water between plates in a tank (Figure 1). For stunning after dewatering, the fish is placed in a device that consists of rows of steel flaps as positive electrodes and a conveyer belt or steel plate as negative electrodes (Lambooij et al., 2010). In principle, electrical stunning in water reduces stress in the fish, whereas applying stunning after dewatering the fish may result in exposing the fish to air longer. No commercial stunning equipment is presently available for farmed turbot in seawater. Recent studies have revealed that development of electrical stunning equipment should take account of electroencephalogram (EEG) recordings to ensure the correct specifications for an instantaneous and effective stun. However, caution is advised when using the vestibulo-ocular reflex (VOR) as indicator of loss of consciousness in electrically stunned Atlantic salmon. Some Atlantic salmon have been seen to recover from an electrical stun, based on EEG recordings, yet the VOR can still be absent (Lambooij et al., 2010).

The current status regarding stunning of farmed turbot and cod warrants research to establish specifications for electrical stunning. In Table 1, an overview is given of established conditions that have to be met to induce an immediate loss of consciousness and sensibility in fish by applying electricity. The data in this Table clearly show that these conditions cannot be predicted for other species using published data. In the present study, we evaluated electro-stunning by assessment of behavioral, neural, and physiological parameters. Atlantic cod (*Gadus morhua*) and turbot (*Psetta maxima*) were electro-stunned in seawater. We selected a bipolar square wave current for stunning, as it appeared for Nile tilapia (Lambooij et al., 2008a) that energy can saved. This study is a first step to develop a human protocol for slaughter of these species in practice.

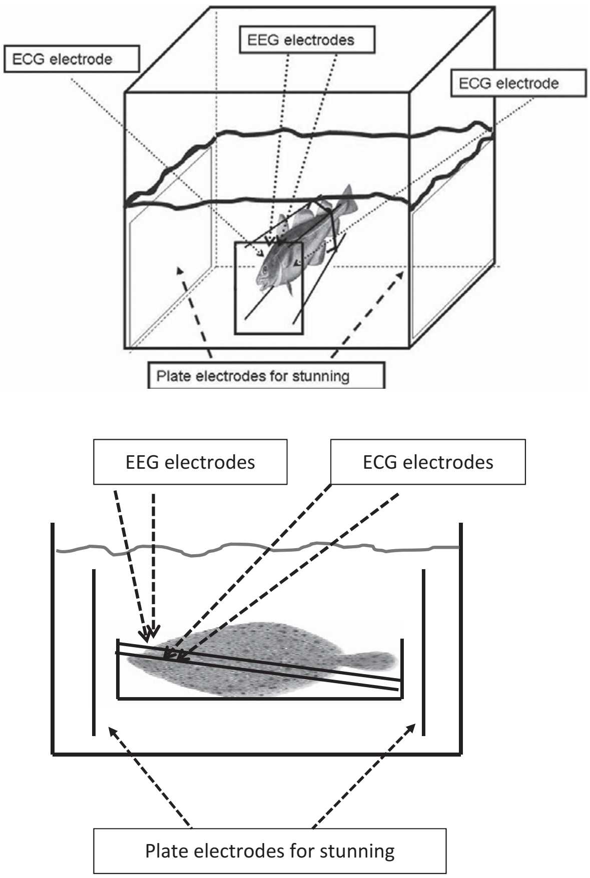

FIGURE 1 Schematic drawing of a fish in a restrainer for electro-stunning in a tank with seawater. The body was then restrained between four PVC pipes with plastic tie-ribs. Individual cod were electro-stunned side to side and turbot head to tail.

MATERIALS AND METHODS

Fish

Farmed Atlantic cod, mean weight (± *SD*) 2.6 ± 0.5 kg and fork length 56 ± 3 cm, $n = 33$, 50% female) were obtained from a fish farm located on the northwest coast of Norway. The fish were lifted from the cage and transferred to two tubs into which seawater was pumped continuously. A small boat transported the tubs ashore in 5 min. The fish were immediately transferred by net into three transport tanks on a truck used for commercial transport of live fish. The tanks contained seawater that was constantly oxygenated (8°C) during transportation (about 3 h) to the laboratory at SINTEF in Trondheim. Transport density of the fish was approximately 49 kg m^{-3}. Upon arrival, the fish were transferred to holding tanks (4,000 L) and left to recover from transport stress for 8 days at a density of 34 kg m^{-3} (more detailed information is given in Digre et al., 2010). The experiment was performed with 7–12 fish day^{-1} and completed within 4 days.

Farmed turbot were obtained from a Dutch fish farm and delivered to the laboratory of IMARES. The fish were kept without feed in a 1,000 L tank containing oxygenated seawater at 17°C.

TABLE 1
Stunning parameters for different seawater and freshwater species, and the effects on the electrical activity in the brains (general and heart rate)

Species	*Weight (kg)*	*Field strength (V/cm)*	*Current (A/dm²)*	*Wave form*	*Conductivity (mS/cm)*	*Duration gen. ep. insult (s)*	*Heart rate prior to stunning (beats/min)*	*Duration of fibril-lation (s)*	*Heart rate 2 min post-stun (Beats/min)*	*Heart rate 5 min post-stun (Beats/min)*	*Reference*
Sea bass	0.25	0.70	3.4	Bipolar square wave 133 Hz, 43% duty cycle	53	23 ± 11	28 ± 6	5 ± 5	37 ± 11	39 ± 15	Lambooij et al., 2008b
Sea bass	0.25	1.0	4.3	Sinusoidal 50 Hz	53	48 ± 34	61 ± 18	8 ± 6			Lambooij et al., 2008b
Fresh- water											
Eel	0.8	13	0.64	Sinusoidal 50 Hz	0.51	46 ± 28	26 ± 10	36 ± 17	30 ± 11	28 ± 12	Lambooij et al., 2002
African catfish	1.9	18	1.6	Sinusoidal 50 Hz	0.51	28 ± 8	77 ± 20	11 ± 7	86 ± 34	69 ± 24	Lambooij et al., 2004
Carp	1.3	7.1	0.14	Sinusoidal 50 Hz	0.20	–	–	–	–	–	Lambooij et al., 2007
Nile tilapia	0.7	13	1.0	Sinusoidal 50 Hz	0.70	26 ± 10	27 ± 9	9 ± 4	37 ± 14	32 ± 8	Lambooij et al., 2008a
Nile tilapia	0.7	9.2	0.60	Bipolar square wave 133 Hz, 43% duty cycle	0.69	51 ± 37	28 ± 8	16 ± 7	32 ± 10	32 ± 10	Lambooij et al., 2008a

Experiments were performed over a period of 2 weeks using 10 animals per day. Upon completion of the experiments, the turbot were weighed; mean (± *SD*) 520 ± 65 g.

Experimental Design

A device was designed and built at IMARES to produce a pulsed square wave with AC. Power was provided by a series of Marathon L12V80 12V 80A batteries (GNB Industrial Power, Exide Technologies AS, Oslo, Norway).

Individual cod were stunned with a peak voltage of 89 V (33% duty cycle, 170 Hz) applied for 1 s across plate electrodes (25 cm × 80 cm) placed 57 cm apart. Each individual fish was placed perpendicular to the electric field in a container (Figure 1).

Individual turbot were stunned with a peak voltage of 55 V (43% duty cycle, 133 Hz) applied across electrode plates (10 cm × 33 cm) 53 cm apart in a Plexiglas tank. They were stunned side to side in a container (Figure 1).

Twenty-nine cod and 22 turbot were used to determine the overall current density required to induce a general epileptiform insult. EEG and electrocardiogram (ECG) recordings were used to assess loss of consciousness. The EEG electrodes became disconnected in four cod and three turbots and were not included in the results.

Both fish species were allowed to recover (indicated by a steady EEG) and stunned again for 5 s. For cod, we chose this approach to assess whether or not electricity could kill the fish. A study with yellowtail king fish and pike-perch revealed that only by using electricity can a fish be killed (unpublished data).

It is known that turbot is a sturdy fish that can survive for a long period in air (Roth et al., 2009b). Therefore, for turbot we tested whether a 5 s stun followed by chilling in ice water for 15 min could result in an immediate and permanent loss of consciousness and sensibility. Upon completion of the experiments, the fish were killed by a manually applied blow to the head.

Registration of EEG and ECG

Prior to stunning, each individual fish was restrained in order to apply the EEG and ECG electrodes. Implantation of EEG and ECG electrodes has been described previously (Lambooij et al., 2004). During passage of the electrical current through seawater, the cod were restrained by using a 16 cm × 16 cm wooden plate fitted with four PVC pipes (30 cm long, inner diameter: 1.5 cm). The pipes were mounted at the corners of the wooden plate. A plastic clamp in the middle of the plate restrained the fish by clasping the lower jaw. Each cod was restrained between the four PVC pipes using plastic tie-ribs. Flexibility of the pipes accommodated for variation in size of fish. For turbot, a PVC restrainer was used, and each individual fish was placed on a grill. A plastic clamp in the middle of the plate restrained the mouth of the fish by clasping the lower jaw. Subsequently, plastic pipes, which were adjustable parallel to the grill, were used to restrain the body between grill and pipes.

EEG and ECG recordings began 30 s prior to electro-stunning and were terminated either 5 (turbot) or 15 (A. cod) min after electro-stunning. EEG and ECG traces were recorded using a DI 720 data recording module with a WinDaq Waveform browser (Dataq Instruments, Akron, OH, USA). Behavioral and EEG responses to pain stimuli (needle scratches applied to the dorsal skin) were monitored at 0.5, 2, 5, 10, and 15 min after stunning.

EEG and ECG recordings were analyzed for changes in waveforms, frequency, and levels of suppression. The behavior of the animals was monitored for the occurrence of:

- tonic spasms: severe tension of all muscles;
- clonic spasms: uncontrolled severe muscle contractions;

- exhaustion: muscle flaccidity;
- recovery: return to consciousness indicated by righting to normal position and normal swimming behavior.

The experiments were approved beforehand by an ethical board for experiments involving animals.

Statistical Analysis

Each fish represents an experimental unit with a probability (p) that the fish is rendered unconscious during a general epileptiform insult. For *n* fish, which are treated independently, the number (x), unconscious, is binomially distributed with total (*n*) and probability. A confidence interval can be calculated for probability based on a relation between the binomial and beta distribution. The number of effective stuns follows a binomial distribution. A 95% confidence limit on the probability of an effective stun can be estimated by means of a well-known relationship with the beta distribution (Johnson and Kotz, 1969).

RESULTS

Cod

EEG recordings in cod stunned in seawater (conductivity: 48 mS/cm) with a side to side applied current for 1 s. At a current density of 2.5 ± 0.2 A/dm^2, immediate loss of consciousness and sensibility was provoked in cod. On average, the overall field strength in the tank was $0.89 + 0.01$ V/cm. A distinct tonic, clonic phase followed by a phase of muscle flaccidity was observed in behavior. On the EEG, a tonic/clonic phase of 19 ± 23 s was followed by exhaustion lasting 31 ± 15 s in 18 fish, while 7 fish showed minimal brain activity and did not recover. The general epileptiform insult found in the remaining fish lasted 40 ± 27 s ($n = 14$). Cod that displayed responses in behavior and in the EEG to pain stimuli administered are presented in Table 2. The heart rate prior to stunning was 32 ± 6 beats/min. After stunning, the ECG revealed fibrillation for 27 ± 30 s in all fish. The mean ($\pm$ *SD*) rate was 23 ± 15, 18 ± 12, 13 ± 9, 13 ± 8, and 11 ± 7 beats/min after 30 s, 2, 5, 10, and 15 min, respectively. In four fish, the heart began to flutter, and in one fish, the heartbeat stopped.

Within a confidence limit of 95%, taking into account the number of cod with a reliable EEG ($n = 25$), the minimum probability of an effective stun for all cod is 0.89, when a current density of 2.5 A/dm^2 is applied between the plate electrodes in water with a conductivity of 48 mS/cm.

TABLE 2
Number of fish with positive response to a noxious stimulus after electrical stunning for 1 s in a tank with seawater

	N	*0.5 min*	*2 min*	*5 min*	*10 min*	*15 min*
Atlantic	25					
cod		7	13	24	20	20
physical EEG		0	6	7	9	9
Turbot	17					
physical		3	0	5		
EEG		11	13	15		16

Attempts were made to kill cod that had recovered from the first stun with a second electrical stun of 5 s. The EEG data showed that eight fish recovered, and these were killed by percussion. The remaining 17 cod did not respond to pain stimuli in the EEG. For the 8 cod that recovered, the heart rate was approximately 20 beats/min after stunning, and in the other 17 animals, the heart fluttered uncontrollably.

Turbot

Exposure of turbot for 1 s to a current density of 3.2 ± 0.3 A/dm^2 head to tail in seawater of 48 mS/cm resulted in immediate loss of consciousness and sensibility. This current density was obtained by applying average overall field strength of 0.68 ± 0.03 V/cm. The turbots showed tonic/clonic phases followed by a phase of muscle flaccidity. Derived from the EEG, a tonic/clonic phase of 17 ± 10 s was followed by a phase of exhaustion lasting 17 ± 19 s. The total duration of the general epileptiform insult was 34 ± 18 s ($n = 19$). In Table 2, data on recovery from the 1 s stun are shown. The heartrate prior to stunning was 25 ± 7 beats/min. After stunning, the ECG revealed fibrillation for 18 ± 12 s in all fish. The mean ($\pm$ *SD*) rate was 30 ± 16, 25 ± 5, and 22 ± 6 beats/min at 0.5, 2, and 5 min after stunning, respectively. One fish did not recover, and the heart stopped within 2 min post-stun.

Within a confidence limit of 95%, taking into account the number of animals with a reliable EEG ($n = 19$), the minimum probability for an effective stun of turbot is 0.82, when a current of 3.2 A/dm^2 was administered between the plates. After the second stun of 5 s followed by chilling in ice water for 15 min, all the turbot showed minimal (EEG) brain activity but did not display EEG or behavioral responses to pain stimuli. The heart stopped directly after stunning within 2 min, except in one fish which displayed an irregular heartbeat 5 min post-stun.

DISCUSSION

Stunning is applied to animals to induce unconsciousness and insensibility to pain stimuli of sufficient duration to ensure that an animal does not recover during exsanguination. Unconsciousness and insensibility should be induced promptly to optimize animal welfare and also to minimize possible detrimental effects of exposure to electricity on meat quality (Blackmore and Delany, 1988). Electro-stunning of farmed animals such as cattle, sheep, pigs, poultry, and fishes is common practice worldwide in slaughterhouses and is accruing popularity in aquaculture. Electro-stunning involves passing an electric current through the skull of an animal. The stunning method is based on the induction of a general epileptiform insult (grand mal or seizure-like state). The epileptic process is characterized by rapid and extreme depolarization of the membrane potential. The brain is in a stimulated condition and unable to respond to stimuli (Lambooij, 2004). Behavioral and clinical signs of recovery are not sufficient for the assessment of electro-narcosis. Therefore, the use of EEG recordings alongside responses to stimuli (visual evoked response and somato-sensory evoked responses) to assess unconsciousness and insensitivity are recommended (Wageneder and Schuy, 1967).

Exposure to an electrical current to provoke immediate loss of consciousness in Atlantic cod and turbot was also studied in Atlantic salmon, eel, and gilthead sea bream (van de Vis et al., 2003). Our study showed that a bipolar square wave with an alternating current and a frequency of 133 Hz delivered with a 43% duty cycle for turbot, as well as bipolar square wave of 170 Hz with a 33% duty cycle for cod can be applied to provoke immediate loss of consciousness and sensibility in the chosen fish species. Similar to turbot and cod, a bipolar square wave was also applied for effective electrical stunning of Nile tilapia loss of consciousness and sensibility was provoked immediately in *N. tilapia* (Lambooij et al., 2008a).

Contrary to our expectations, the current density used for stunning of cod was 14% lower than calculated, considering the configuration of electrodes, conductivity of the seawater, and field strength applied. It is possible that the restrainer used reduced the overall conductivity. The restrainer used for turbot was specially designed for flatfish species; the current density was the same as calculated.

Electricity has been used in various studies to stun farmed fish species. It is known that the specifications for electrical stunning are not only dependent on fish species, but are also partly determined by waveform of the current applied (Lambooij et al., 2008a). A summary of data on electrical stunning, based on EEG and ECG recordings, is presented in Table 2. Comparison of these results shows that consciousness and sensibility were lost for longer periods when pulsed square waves were applied, compared to sinusoidal waves of 50 Hz (on average 51 and 26 s, respectively). A prolonged loss of consciousness facilitates application of a killing method, and therefore the use of a bipolar square wave is recommended for electrical stunning. In addition, the use of pulsed, square wave alternating currents can also be recommended for application in practice as power can be saved with respect to a 50 Hz alternating current (Lambooij et al., 2008a).

Clearly, differences in current density and water conductivity as well as anatomical and physiological differences of the fish influence to the current density needed for an immediate stun. Lines and Kestin (2004) also suggested that any observed differences in the electric fields required to stun fish of different sizes are not simply a consequence of the physical volume of the fish. In addition, they revealed that a fish stunned side to side required a 35% stronger electric field, compared to head-to-tail stunning.

The European Food Safety Authority (EFSA, 2009) established that a hazard in the process of stunning and killing is recovery of the fish using the method presented in this study. A similar phenomenon was observed in Atlantic salmon where recovery occurred during exsanguination. For implementation in practical operations, possible options to prevent recovery in turbot and cod are prolonging exposure to the electric current for at least 5 s followed by chilling in ice water, and then percussion or decapitation to kill the unconscious and insensible fish (Lambooij et al., 2010; Sattari et al., 2010).

Our study on electrical stunning of cod and turbot in seawater is a first step that is needed to put this technology into practice. We foresee that more experimental work is required to establish whether the specifications for stunning of cod on average 2.6 kg and turbot of 0.52 kg are also valid for fish in other weight ranges. Moreover, a method for killing of stunned cod needs to be established to prevent recovery. Prior to testing electrical stunning of these species in a commercial setting, a dosing system needs to be designed and built. A dosing system is needed to ensure that the process of stunning is performed at a slaughter rate that is demanded by a company. Welfare aspects of a dosing system are also of interest, as a fish should be placed in a stunner without any avoidable stress; for example, the animal should not be exposed to electricity when it is not in a stunner yet. We foresee that the design of a dosing system is species specific.

CONCLUSIONS

When a bipolar square wave current (133 Hz) 43% duty cycle and (170 Hz) 33% duty cycle were applied for turbot head to tail and cod side to side, respectively, current densities required to stun turbot and cod in seawater were 3.2 and 2.5 A/dm^2, respectively. For turbot, a 5 s exposure to electricity followed by chilling in ice water for 15 min is sufficient to prevent recovery of the stunned animals. For cod, a killing method to prevent recovery of the stunned species needs to be studied. A combination of electro-stunning and a killing procedure may be applicable, with species-specific modifications to a variety of fish species. Our study is the first step, and therefore further research prior to implementation in practice is necessary.

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