Aquaculture 364–365 (2012) 143–149

Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Assessing effectiveness of electrical stunning and chilling in ice water of farmed yellowtail kingfish, common sole and pike-perch[☆]

P. Llonch [b,*], E. Lambooij [a], H.G.M. Reimert [a], J.W. van de Vis [c]

[a] Livestock Research, Wageningen UR, P.O. Box 65, 8200 AB Lelystad, The Netherlands
[b] IRTA, Animal Welfare Subprogram, Finca Camps i Armet s/n. Monells, 17121, Girona, Spain
[c] IMARES, Wageningen Aquaculture, Wageningen UR, P.O. Box 77, 4400 AB Yerseke, The Netherlands

ARTICLE INFO

Article history:
Received 26 September 2011
Received in revised form 15 June 2012
Accepted 7 August 2012
Available online 17 August 2012

Keywords:
Stunning
Fish
EEG
Yellowtail
Common sole
Pike-perch

ABSTRACT

Animals should be rendered unconscious before slaughter in order to avoid suffering or pain. The objective of this study was to evaluate an electrical stunning after dewatering to induce instantaneous unconsciousness and insensibility in yellowtail kingfish (*Seriola lalandi*), common sole (*Solea solea*) and pike-perch (*Stizostedion lucioperca*). To kill without recovery, the current was applied for 5 s, followed by chilling in ice water for 10 min. Loss of consciousness and sensibility were assessed by neural (EEG), physiological (ECG) and behavioural parameters. An epileptiform insult was observed in all yellowtail kingfish (n = 27), common sole (n = 25) and pike-perch (n = 25) after administering a current of 0.54 ± 0.12 A_{rms} (124 V dc and 11 V_{rms} ac; 100 Hz), 0.65 ± 0.23 A_{rms} (98 V dc and 8.4 V_{rms} ac; 100 Hz) and 0.75 ± 0.24 A_{rms} (144 V dc and 13 V_{rms} ac; 100 Hz) during 1 s through the head of individual fish, respectively.
When yellowtail kingfish (n = 11) was submitted to a 5 s electrical stun followed by chilling in ice water, this resulted in passing 0.72 ± 0.13 A_{rms} for 5 s and no recovery during chilling. In the case of common sole (n = 10) and pike-perch (n = 12), passing 1.4 ± 0.64 and 0.75 ± 0.24 A_{rms} during 5 s followed by chilling in ice water for 10 min resulted in an irrecoverable stun in 9 out 10 fish and 11 out 12 fish, respectively. We conclude that for yellowtail kingfish the investigated combination of electrical stunning and chilling is suitable for humane slaughter, whereas for common sole and pike-perch this procedure needs to be optimised.

1. Introduction

The European aquaculture production sector, which is dominated by few fish species, has expanded extensively over the last decade. To solve the glutting of the markets with a few species and the drop in prices, the aquaculture chain in Europe also focuses on diversification of species (Dosdat et al., 2006).

The classification scheme of Le-Francois et al. (2002) was used to evaluate the potential culture of different fish species. This revealed that yellowtail kingfish (*Seriola lalandi*), common sole (*Solea solea*) and pike-perch (*Stizostedion lucioperca*) have potential for aquaculture in recirculation aquaculture systems (RAS) (Kals et al., 2005).

Yellowtail kingfish is a pelagic marine species found globally in temperate and sub-tropical coastal waters. We performed trials with yellowtail kingfish in RAS at IMARES and the outcome confirmed the high potential of the yellowtail kingfish culture in Dutch aquaculture (W. Abbink pers. com.). Common sole (*S. solea*) is an interesting species for marine aquaculture in Europe, as it is a high valued species with a large market (Schram et al., 2006). Recent research has demonstrated the feasibility of cultivating intensively pike-perch, which is a highly valued fish in Europe (Dil, 2008).

Besides diversification of species, sustainability of production is another important driver for the aquaculture sector in Europe (Dosdat et al., 2006). Within the concept of sustainability, the maintenance of an optimal animal welfare is an important issue. In the view of NGOs and an accruing number of companies in the aquaculture sector, especially at slaughter, welfare of fish can be impaired.

Currently, asphyxia on ice or chilling on ice slurry is commonly used for killing of conscious fish. It is known that asphyxia on ice or chilling on ice slurry does not result in immediate loss of consciousness and it is likely that application of this methods stresses fish (EFSA, 2009).

For the protection of the welfare of fish at the moment of slaughter, it is essential to apply appropriate stunning methods prior to killing, as reviewed by Van de Vis et al. (2003). Stunning is a process that renders an animal unconscious without avoidable stress prior to killing/slaughter, which should not recover until death occurs (Council Regulation (EC) No, 1099/2009).

Various studies show that electronarcosis, which is defined as unconsciousness induced by passing an electric current through the

☆ Wageningen Aquaculture is a consortium of IMARES (Institute for Marine Resources & Ecosystem Studies) and AFI (Aquaculture and Fisheries Group, Wageningen University), both part of Wageningen University & Research Centre (WUR).

* Corresponding author. Tel.: +34 972630052; fax: +34 972630373.
E-mail address: pol.llonch@irta.cat (P. Llonch).

0044-8486/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aquaculture.2012.08.015

brain, is a suitable method to stun fish within 1 s (EFSA, 2009). When fish are exposed long enough to the electricity, they can be subsequently killed by means of additional methods like transferring the stunned fish into ice water (Sattari et al., 2010).

Electronarcosis is currently applied in practice for stunning Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) (EFSA, 2009), European eel (*Anguilla anguilla*) (Anonymous, 2011), African catfish and a hybrid of African catfish and Vundu catfish (*Heterobranchus longifilis*) (Anonymous, 2011). Electrical stunning does not require individual handling of fish and the animals can be rendered unconscious in batches or continually. Electrical stunning can be performed in water or after dewatering. The latter method has an advantage with respect to safety, as it was established that in low current densities (0.1 A/dm^2) in seawater, hydrogen production could occur (Bennet, 1980). For electrical stunning of turbot in seawater, a current density of at least 2.5 A/dm^2 is needed to provoke immediate loss of consciousness (Digre, 2011). Another advantage of electrical stunning after dewatering is that, as stated for Atlantic salmon, a very low incidence of injuries occurred, which is contrary to the current technologies used for water electrical stunning (Roth et al., 2009).

To determine whether an electrical current of sufficient strength for an instantaneous stun is applied, it is necessary to assess the brain activity by means of electroencephalography (EEG) (Lambooij et al., 2010). For electrical stunning a general epileptiform insult should be induced in fish instantaneously, which is indicative of unconsciousness and insensibility. The epileptiform insult is characterised by rapid and extreme depolarisation of the neuron membrane potential, and the resulting changes in overall brain activity differ between animals (Kooi et al., 1978).

Several types of commercial electrical stunning equipment, both in water and dry stunners, are available. However, they have not already been evaluated for yellowtail kingfish, common sole and pike-perch, using EEG and electrocardiography (ECG) (analysis of heart function) recordings.

Therefore, our objective was to assess electro-stunning after dewatering followed by chilling in ice water by neural (EEG), physiological (ECG) and behavioural parameters in yellowtail kingfish, common sole and pike-perch.

2. Materials and methods

2.1. Animals

2.1.1. Yellowtail (S. lalandi)

At the laboratory of IMARES 43 yellowtail kingfish of 2100 ± 320 g (mean ± SD) were fasted for 48 h prior to the start of the experiments. Throughout the entire experiment, animals were kept in a circular tank of 5000 l for 25 °C and 30‰ salinity seawater. A photoperiod of 16:8 h light:dark was applied. The oxygen supply and the aeration of each tank provided oxygen to the fish and promoted removal of carbon dioxide from the water.

2.1.2. Common sole (S. solea)

At IMARES 41 common soles of 287 ± 57 g (mean ± SD) were fasted for 48 h and placed in 5 glass tanks (50 × 65 × 45 cm) with aerated seawater of 22 °C and 30‰ salinity. Each tank housed 10 animals. The soles were exposed to photoperiod of 16:8 h light:dark.

2.1.3. Pike-perch (S. lucioperca)

Forty one pike-perches of 682 ± 124 g (mean ± SD) were fasted for 48 h at a commercial farm prior to purchase. Upon arrival at IMARES, the fish were placed in a circular tank of 5000 l with aerated 22 °C freshwater.

2.2. EEG and ECG registration

Each fish was randomly netted from the holding tank and transported to the laboratory room. Four fish of each species were dissected to determine the position of the electrodes for measuring the EEG and the ECG. To facilitate the implantation of the electrodes, each individual fish was restrained. The method for restraining depended on the fish species' anatomy. The pisciform species (yellowtail kingfish and pike-perch) were restrained, as described by Sattari et al. (2010). For the common sole, a flatfish species, a restrainer was used that consisted of a plastic rectangular plate (25 × 80 cm). At the end of the plate an equilateral triangle of 8 cm was cut out in the middle. On each side of this triangular shape holes were drilled to facilitate restraining an individual sole by using cable ties. A duct tape around the fish body was used to restrain the fish on the plastic plate.

Prior to stunning, each fish was equipped with EEG and ECG electrodes, as described by Lambooij et al. (2003). To implant the electrodes, the fish was locally anaesthetised using Xylocaine® 10% spray (Lidocaine 100 mg/ml; Astra Pharmaceutica BV, Zoetermeer, The Netherlands). 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 each species, into the surface of the cortex. The ECG electrodes (the same composition as the EEG electrodes) were placed subcutaneously, two caudal of the implantation of both the left and right pectoral fins. The earth electrode for both the EEG and ECG was placed subcutaneously, caudal to the dorsal fin. The EEG and ECG were recorded during 30 s before the stunning and until 5 min after. The EEG and ECG data were recorded 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. Behavioural responses registered on the EEG after the administered noxious stimuli (using needle scratches applied to the dorsal skin) were monitored at the start of each recording period. Afterwards, the EEG and ECG recordings were analysed for changes in the waveforms. The animals were monitored visually for the occurrence of behavioural responses to the administered stimuli.

2.3. Electro-stunning after dewatering

For each species, the fish were divided into 2 groups. The first group was subjected to electric stunning for 1 s to determine the conditions (voltage and amperage) necessary for an instantaneous stun. Fish of the second group were exposed for 5 s to electricity to establish whether the application of a killing method could prevent recovery of the stunned animal. We assessed 10 to 15 fish a day. After the completion of the experiments, fish that recovered were killed by percussion.

For electrical stunning, an experimental unit, similar to the larger Stansas 01 (Digre et al., 2010), was used. Our unit of 150 cm length, 30 cm height and 40 cm width was equipped with 18 rows of above-suspended flexible positive electrodes and with 4 cm separation in-between. Each row consisted on 14 electrodes of 2.4 cm and 12 cm length. The distance between these electrodes and a steel plate, which was the negative electrode, was 1.2 cm. The experimental unit was designed by IMARES and built by SeaSide A/S, Stranda, Norway. The experimental unit was connected to a source of power that generated a combination of a direct (dc) and sinusoidal (ac) 100 Hz electrical current (see Fig. 1 for the waveform), which was built by IMARES.

In order to measure the voltage and amperage used for stunning, an oscilloscope was used. A laptop computer (Panasonic, Secaucus, New Jersey, USA) with PicoScope 2000 software (Pico Technology, Cambridgeshire, UK) served as oscilloscope. A Fluke i30 S AC/DC