Aquaculture 592 (2024) 741200

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Use of electroencephalogram (EEG) to optimize stunning efficiency and animal welfare in commercial catfish production

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ARTICLE INFO

Keywords:
Brain activity
Slaughter
Fish welfare
Electric stunning
Ictalurus punctatus

ABSTRACT

The welfare of farmed fish has gained increasing attention during recent decades, and as technological advances have facilitated measurements of brain activity in fish, the slaughter process has been highlighted as an area for assessment and potential improvement. Here, we used electroencephalograms (EEG) to assess brain activity in channel catfish (*Ictalurus punctatus*), and used commercial slaughter practices to guide optimization of stunning conditions in a laboratory setting. Following in-air electro-stunning at processing plants, individual fish responses to the shock varied based on EEG and corresponding ventilation measurements prior to physical euthanasia. Results from laboratory experiments showed stunning efficacy is dependent on shock duration and the location where electrodes contact fish. Electrodes contacting the head for 1 s using 50 Hz 132 AC V_{RMS}, with a current >380 mA_{RMS}, caused immediate loss of consciousness lasting 10–40 s. When the exposure period was prolonged to 6 s, recovery time was significantly longer, ranging from 45 to 240 s (mean 125 s). If the electrodes contacted the body instead of the head, shock delivered for 6 s resulted in a shorter recovery time of 0–100 s (mean 48 s). These findings highlight that shock duration and electrode position are important when stunning channel catfish and presumably other fishes, and indicate the time from stunning to killing should be kept as short as possible.

1. Introduction

It is estimated that 51–167 billion farmed fish were slaughtered for human consumption in 2019 (fishcount.org.uk, website visited 10/112023). Channel catfish (*Ictalurus punctatus*) is an economically important species produced mainly in the United States (US) and China with an annual global production of approximately 380,000 t in 2017 (FAO, 2019). The US catfish industry is predominantly located in southeastern US, where catfish are normally reared in land-locked, earthen ponds and harvested at a weight of approximately 1000 g. During harvest, catfish are corralled in large seine nets before being loaded into cooled water tanks and transported by truck to processing plants which are customized for slaughter of catfish. Upon arrival at the processor, catfish are weighed and then conveyed through a multi-stage electro-stunner where an electric current passes through the fish when the body touches hanging electrodes before killing by decapitation (Fig. 1A-D). When a high enough electrical current/amount of energy is delivered to the catfish, it becomes immobilized and rigid which makes all subsequent handling safer. The electrical exposure also causes their pectoral fins and spines to erect into a locked position, perpendicular to the body, which is used for hanging the fish onto the shackling mechanism of the carousel of an automated de-heading machine prior to fileting. The slaughter process ensures worker safety (*e.g.* by preventing or reducing the risk of catfish fins and spines inducing tissue damage and bacterial infection, such as the 'Fish Handler's Disease'; Fry et al., 2019) and processing efficiency while meeting the current US regulations for animal welfare. Notably, scientific technological advances have continued to provide a more refined understanding of cognitive abilities in fish and their ability to respond to noxious external stimuli which has led to an increased awareness from the industry, legislators, and consumers regarding the wellbeing of farmed fish (Braithwaite et al., 2013; Sneddon and Roques, 2023). In light of this, the US catfish industry desires to continue to adapt and improve practices to meet or exceed current and future animal welfare regulations.

Assessments of fish welfare are challenged by our limited understanding of cause and effect of the different rearing, handling, and

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https://doi.org/10.1016/j.aquaculture.2024.741200
Received 28 February 2024; Received in revised form 1 June 2024; Accepted 7 June 2024
Available online 10 June 2024

slaughter protocols used in fish aquaculture. There are also species-specific variations in factors that may affect the wellbeing of fish, and the whole production cycle should be adapted to the specific needs and requirements of each farm species (Ashley, 2007). Recent technical advances in the collection of physiological data and novel understanding of behavior have improved welfare assessments in aquaculture, but the reliability and application of such methods needs further studying and is limited to a few species (Barreto et al., 2021). Furthermore, it has been suggested that electro-stunning can be used, not only to facilitate handling and work safety, but also to cause unconsciousness (*i.e.* stunning) in farm animals, including fish, during the slaughter process (Lines and Spence, 2012). From an animal welfare perspective this is highly desired as it will spare the animal of any experience associated with the killing process. However, it is well-known that the tolerance to electrical exposure is highly variable between different fish species and the efficacy of different electro-stunning devices to effectively stun the fish have to date only been evaluated for a handful of species and never for in-air electro-stunning of channel catfish. One major reason for this is the technical difficulties related to assessing consciousness in fish. For reliable evaluations of stunning effects, the normally used visual verification of consciousness (*e.g.* equilibrium, ventilation, movement, vestibulo-ocular reflex) must be paired with neurological investigations (*e.g.* using electroencephalograms, EEGs) of consciousness (Bowman et al., 2020; EFSA, Welfare, A, et al., 2018; Hjelmstedt et al., 2022; Kestin et al., 1991; Robb et al., 2000; Van De Vis et al., 2003). This is because visual verification of consciousness can be lost long before the neurological investigations show evidence of severe brain failure. For animal welfare, this means that the animal risks being falsely determined as insensible (Bowman et al., 2020; Hjelmstedt et al., 2022; Retter et al., 2018). Although measurement of EEG is technically challenging and not practically applicable for large-scale, on-site evaluation of stunning success, it provides a useful tool for developing novel stunning methods and equipment, as well as for optimizing protocols that can subsequently scaled up for commercial application.

One conservative and reliable assessment of unconsciousness is the absence of visually evoked responses (VER, *i.e.* the ability of the brain to react to an external visual stimuli) within an EEG reading. This is indicative of profound brain failure during which the abolition of VERs has been previously confirmed as an objective and unequivocal indicator of brain dysfunction and hence, loss of sensibility, in fish species such as Atlantic salmon, *Salmo salar* (Robb and Roth, 2003; Robb et al., 2000a), rainbow trout, *Oncorhynchus mykiss* (Bowman et al., 2019,

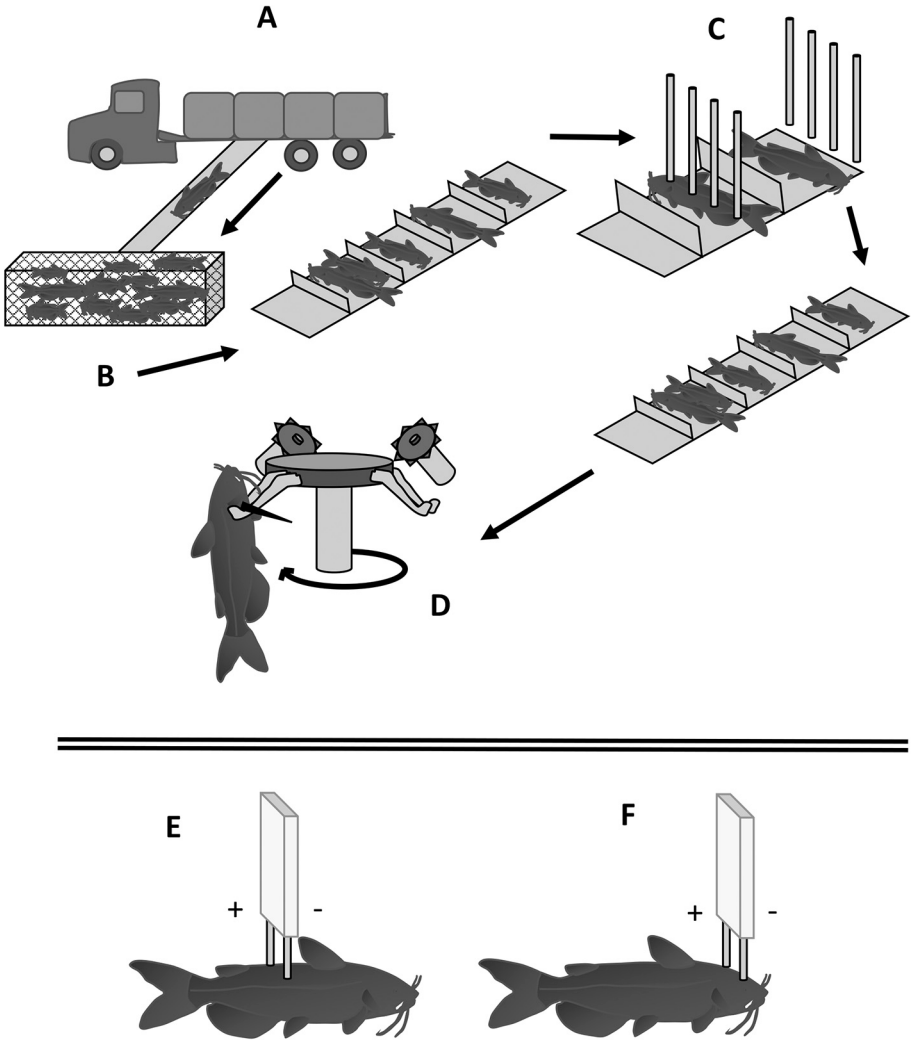

Fig. 1. Schematic image summarizing electro stunning of channel catfish (*Ictalurus punctatus*) at the processor (A-D) and in the lab study (*E*-F). At a processor fish normally arrive after transportation by trucks from fish ponds in the area (A). During transport the fish are kept at high density (50:50 fish:aerated water) in 1 m^3 tanks. At a processor, fish are batch weighed (*e.g.*, draining of water in a large scale) (B). Next, the fish are transported on a conveyor belt, while being sprayed with water, through an electrical stunner (C). When a fish touches the hanging steel electrodes, a current is delivered through their body which leaves them immobilized. After stunning, fish are transported to an automatic decapitation machine that kills the fish by removing the head *via* three circular saw blades (D). In the laboratory study, the electrodes of the electrical stunner were pressed either against the body (E) or the head (F) of the catfish.

2020; Kestin et al., 1991), common carp, *Cyprinus carpio* (Retter et al., 2018), goldfish, *Carassius auratus* (Quick and Laming, 1990) and European eel, *Anguilla anguilla* (Lambooij et al., 2002). Normally VERs are induced using high intensity flashing of light, which is harmless to the animal and can be delivered repetitively over a long time. In this way measurements of VERs can be used to validate that the animal does not regain consciousness before death following a stunning and killing procedure. This is especially important when working with ectothermic animals like fish as their brain can stay functional long after its blood supply has been disrupted due to their lower metabolic activity and energy consumption (Holleben et al., 2010; Morzel et al., 2003; Robb et al., 2000; van de Pol et al., 2017).

The overarching aim of the study was to, in collaboration with the catfish processing industry, evaluate stunning procedures and optimize stunning efficiency using EEG to identify areas where fish welfare improvements can be made during the slaughter of channel catfish. To do so, we evaluated the slaughter protocol of two commercial catfish processing plants by evaluating roles of two electro-stunners for inducing unconsciousness. Importantly, the on-site investigation was complemented with a laboratory investigation where we determined how the duration of the electrical exposure and placement of the electrodes delivering the current modulate the effectiveness of the stun. Investigations were done using recordings of VERs and ventilation to determine the onset and duration of brain failure following electrical exposure.

2. Materials and methods

2.1. Animals and housing

Channel catfish of mixed sexes were sampled following electro-stunning at two commercial catfish processing plants. Stunning and processing were preceded by transport (~ 0.5–2.5 h) from regional catfish farms *via* hauling trucks, with fish held in cooled and oxygenated water tanks. Upon arrival at processing plants, tanks were batch-weighed on a scale, and transferred on a conveyor belt into the stunner station (Fig. 1A-D). Afterwards, fish were randomly sampled for EEG measurements.

For the laboratory trials, channel catfish of mixed sexes were housed at the South Farm Aquaculture Facility at Mississippi State University in circular, indoor tanks (2.3-m diameter; 1.5-m deep; 6.2 m^3), supplied with well water using a flow-through system, at a temperature of ~24 °C and aerated with air stones and a fish density of approximately 8 kg/m^3. The fish were fed *ad libitum* twice weekly, with food withheld for 48 h

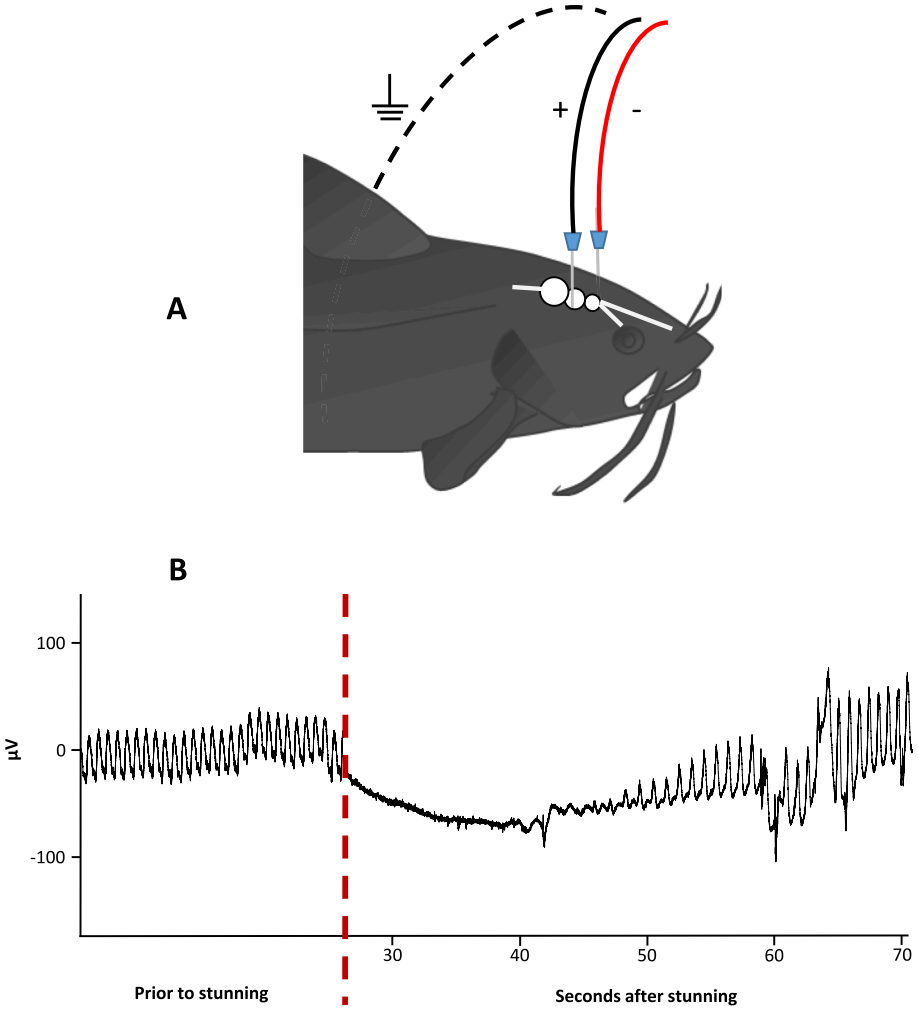

Fig. 2. EEG measurements in channel catfish (*Ictalurus punctatus*) was done using two intracranial electrodes implanted in the proximity of the optic lobe on each side of the brain and a third smaller reference electrode needle electrode positioned into the proximal dorsal muscle tissue (A). Ventilation could be determined both by observing channel catfish for opercular movements and directly from the unfiltered EEG-signal. B shows an example of the electrical signal characteristics from muscle activity observed as rhythmic waves when opercular movements were present in an awake catfish prior to stunning (left of the red hatched line). Ventilation is inhibited for approximately 50 s following exposure to electricity, whereafter opercular movements return and rapidly increase in strength (right of the red hatched line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

prior to experimentation. Values are presented as mean ± s.e.m. and range (min – max). All procedures followed an approved institutional animal care and use committee protocol (#22–234).

2.2. Data acquisition and signal filtering

To determine the status of the catfish, EEG signals were continuously recorded using two intracranially implanted active electrodes (25 mm 18G hypodermic needles, Becton Dickinson & CO, New Jersey, US) positioned on each side of the brain and a third smaller reference electrode positioned into the proximal dorsal muscle tissue (Fig. 2A-B). The shielded wires of the electrodes were connected to a custom-made relay box, used to disconnect the electrodes from the instrument when the electricity was administered to the fish. The instrument used was an animal bio-amplifier (FE136; ADInstruments). The bio-amplifier was set with a sensitivity range (±2 mV) and a band-pass filter with a cutoff frequency of 0.1 and 50 Hz to optimize the EEG signals. To induce VERs on the EEG, a custom-built LED strobe light delivering ~3 ms light flashes at 2 Hz (2% duty cycle) was used. Signals from the bio-amplifier and a custom-made light detector (made from a solar panel [Velleman SOL1N, Gavere, Belgium]) were recorded using a data acquisition instrument (Power Lab, ML 870, 8/30, ADInstruments) at a sampling rate of 1 kHz (Bowman et al., 2019; Brijs et al., 2020; Hjelmstedt et al., 2022). Data collected were subsequently analyzed using LabChart Pro software (version 7.3.2, ADInstruments).

When analyzing EEG recordings in LabChart Pro software, a band-pass filter was used to separate the beta wave frequency (12–32 Hz) from the rest of the EEG, which has been shown to provide reliable readings of VER activity in rainbow trout and African sharptooth catfish (*Clarias gariepinus*) (Bowman et al., 2019, 2020; Brijs et al., 2020; Hjelmstedt et al., 2022). VERs were detected using the Scope View module in the software, which was set to display time windows starting 50 ms before, and ending 400 ms after, each strobe-light flash (total time window of 450 ms). To reduce the effects of noise caused by strong muscular movements, all time windows where the amplitude of the beta wave exceeded 10 μV were automatically excluded from the analyses. The Scope View module was then used to average 120 consecutive, non-overlapping time windows into a single 450 ms time window representative of the VERs for 60 s of recording (see (Hjelmstedt et al., 2022) for detailed description). In addition, ventilation was determined by observing the opercular movements of fish and from a clearly visible rhythmic wave pattern on the unfiltered EEG-signal (Fig. 2C). For measurements of the current and voltage delivered during each stun in the lab, an oscilloscope (PicoScope 5204, Pico Technology, Cambridgshire, UK) with a current clamp (Hantek CC-650, Qingdao Hantek Electronic Co., Ltd., Qingdao, China) and a voltage differential probe (Micsig DP10013, Shenzhen Micsig Technology Co., Ltd., Guangdong, China) were used. The signal traces of the oscilloscope were stored using the PicoScope 7 software. Average values of the alternating current/voltage waveforms are reported as root mean square (RMS). RMS current/voltage of the alternating current/voltage represents the direct current/voltage that dissipates the same amount of power as the average power dissipated by the alternating current/voltage. For sinusoidal oscillations waveform, as the one used here, the RMS value equals peak value divided by the square root of 2.

2.3. Stunning protocol

2.3.1. Sampling at processing plants

Channel catfish were randomly collected and assessed for signs of brain failure following electrical exposure at two commercial processing plants. At both plants all fish were stunned by receiving an electric shock when passing through a stunning station. The stunning station consisted of hanging rod electrodes pointing down toward the conveyor belt. The electrodes were arranged in an interleaving manner between cathode and anode, with stunning stations at one processing plant consisting of two sets of electrodes, while the other consisted of three sets of electrodes. The fish were stunned prior to being transferred onto another segment of conveyor belt that led to an automated decapitator. The rod electrodes were energized with 127 V_{RMS}AC at a maximum current limit of 5 A. Following stunning, a total of 32 catfish were randomly selected, implanted with EEG-electrodes, and placed in a moist, opaque, plastic cooler for further assessments. The cooler was covered in black plastic bags to keep the animals in darkness during measurements to avoid light stimulus other than the flashing light used to induce VERs. Inside the cooler, EEG was recorded for 15 min or until VERs had clearly recovered whereafter they were euthanized with a sharp blow to the head and measured for weight and length. A total of 14 (mean mass 926 g (570–1470 g)) and 18 (mean mass 817 g (472–1266 g)) catfish were assessed for EEG measurements at the first and second processing plant, respectively.

2.3.2. Laboratory study

All fish used in laboratory trials were individually caught from the holding tank with a dip net and transferred to an opaque plastic cooler containing 15-L of anesthetic (150 mg l^{-1} MS222; ethyl-3-aminobenzoate methane sulphonic acid) buffered with 400 mg l^{-1} $NaHCO_3$. Once anaesthetized, electrodes were quickly implanted and the fish was subsequently placed in a flow-through experimental tank (volume = ~ 8.1 L) where fresh, aerated ~24 °C water was gravity fed at a rate of ~2 L min^{-1}. Once the fish had recovered from anaesthesia and was conscious (*i.e.* had regained equilibrium and was actively moving), it was transferred to a moist plastic cooler for subsequent stunning. A custom-made electro-stunner designed to mimic the equipment used at the processing plants was used to test the effect of stunning duration and electrode position (Fig. 1E-F). The stunner was powered by a 50 Hz, 240 VAC transformer and the duration of stunning was controlled with a built-in timer with a resolution of 1 mS. The electrodes used to stun the fish were made using two stainless steel hollow rods (ø = 10 mm) with a 5 cm separation attached to a non-conductive handle. In order to investigate whether unconsciousness was induced immediately, 16 catfish were given an electric shock for 1 s by pressing the electrodes firmly on the head of the fish with one electrode on each side of the brain (Table 1). For this group, EEG was monitored until VERs and ventilation were clearly recovered. After this, and to investigate the effect of stun duration on the recovery time, another 10 catfish were given an electric shock to the head but this time for 6 s and monitored until the VER and ventilation were clearly recovered (Table 1). Lastly, to investigate the effect of the positioning of the stunner electrodes on the recovery time, another 10 individuals were given an electric shock for 6 s by applying the electrodes to the middle part of the body and monitored until VERs and ventilation were clearly recovered (Table 1).

2.4. Statistical analyses

All statistical analyses were performed using IBM SPSS 27.0.1 (IBM Corp., Armonk, New York, USA). All data were tested for homogeneity

Table 1
Stunning electrode position, exposure time, current, voltage and mass of channel catfish (*Ictalurus punctatus*) for each experimental group in the laboratory trials. For all the groups (n = 10–16 fish/group), an alternating current (AC) was delivered at 50 Hz.

Electrode position	Exposure time	Current	Voltage	Mass
	s	mA_{RMS}	V_{RMS}	g
Head	1	510 (210–890)	134	822 (535–1195)
Head	6	420 (270–680)	134	866 (555–1315)
Body	6	540 (350–880)	134	876 (480–1230)

and normality. Data from the processing plants are largely descriptive.

For the laboratory groups, an independent samples *t*-test was used to evaluate the effect of electric shock exposure duration when stunning electrodes are applied to the head. Time to recovery of VER and ventilation was compared between catfish electro-shocked for 1 s ($n = 16$) or 6 s ($n = 10$). An additional unequal variance t-test was used to explore the effect of electrode position by comparing time to recovery of VERs and ventilation between fish that were exposed to an electric shock for 6 s to the head compared to the fish shocked over the body. A regression analysis was performed to test whether current had an impact on time to recovery of VERs for the catfish that were exposed to 1 s of stunning in the laboratory trials. All results are reported as mean and range (min – max) and statistical significance was accepted at $p < 0.05$.

3. Results

3.1. Processing plants

The duration of the electric shock was approximately 6 s for individual fish that passed through the electro-stunners at the processing plants. When leaving the electro-stunner, the activity level of most catfish at both processing plants was greatly reduced. The behavior of different individuals following the electrical exposure varied from motionless and apparently euthanized to undulatory movements and some responsiveness to touch. The investigation of the EEGs revealed that VERs were already present when the measurements began in 7 of 14 and 7 of 18 fish from each processing plant, respectively (Fig. 3). However, it took >1 min to move the fish from the conveyor belt and implant the EEG-electrodes, and, consequently, neurological assessment from the first minute immediately following the electric shock was practically not possible. From the remaining fish, 3 of 14 and 4 of 18 individuals, respectively, did not recover VERs within 15 min following stunning. In the fish that recovered, presence of VERs could be seen between 0 and 480 s and 0–780 s after EEG measurements began for the two processing plants, respectively, with the majority recovering within 2 min.

Time to recovery of ventilation ranged from 0 to 210 s and 0–525 s at the two processing plants respectively, with 7 individuals from each facility already ventilating when EEG measurements started. One fish that had VERs present lost the response after 320 s. This individual was also the only one of the recovered fish that did not recover ventilation. Another catfish recovered VERs but not ventilation while one more individual recovered VER as late as 13 min after beginning of EEG recording. Ventilation was present without the fish regaining VER in one instance.

3.2. Laboratory trials

In the laboratory, three different electrical exposures were investigated (*i.e.* 1 s to the head, 6 s to the head and 6 s to the body). A few individuals had to be excluded from the analyses as powerful spasms in these fish interrupted the period of exposure. Thus, the analyses include only data from fish exposed to a full and continuous 1 ($n = 16$) or 6 s ($n = 10$) period of electrical exposure. Most fish became rigid and immobilized following the shock except for two individuals exposed for 1 s over the head and one individual stunned for 6 s over the body that was clearly not stunned at all. The current varied between 213 and 890 mA among fish stunned for 1 s using 132–134 V_{RMS}, 50 Hz AC. All fish receiving a current >380 mA when exposed to the head for 1 s lost VER for at least 10 s (Fig. 4).

VERs and ventilation were absent immediately for 88% (*i.e.* 14/16) of the fish exposed for 1 s to the head (Fig. 5). In two individuals that did not lose VERs or ventilation (*i.e.* "failed"), notable behavioural reactions were displayed following the shock. When exposed to the head for 1 s, the time to recovery of VERs and ventilation were relatively short ranging from 0 to 40 s (18 ± 3 s) and 0–65 s (29 ± 5 s) respectively.

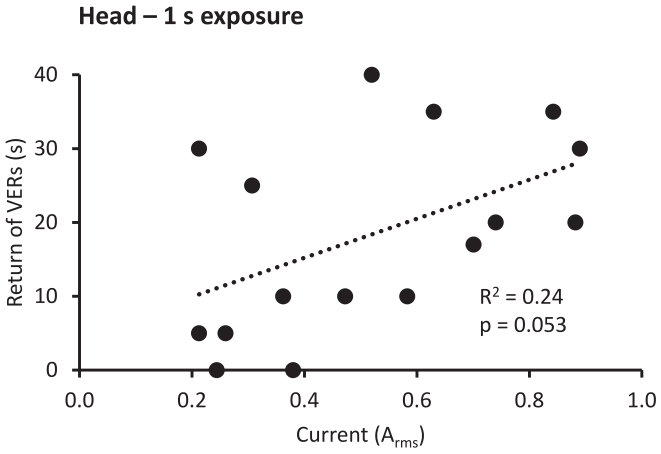

Fig. 4. The relationship between 1 s currents delivered to the head of channel catfish (*Ictalurus punctatus*) and return of VERs. Worth noting is that all fish who received currents >380 mA_{RMS} to the head were immediately stunned for at least 10 s.

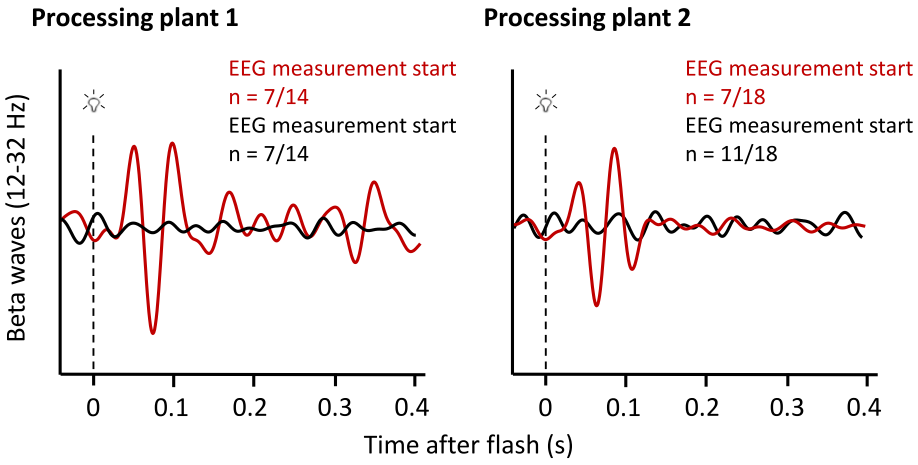

Fig. 3. Presence (red) and absence (black) of visually evoked responses (VERs) in the beta waves of channel catfish (*Ictalurus punctatus*) after passing through the electrical stunner. When the EEG measurements were started 50% and 39% of the fish were responding to the flashing light at the two processing plants respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

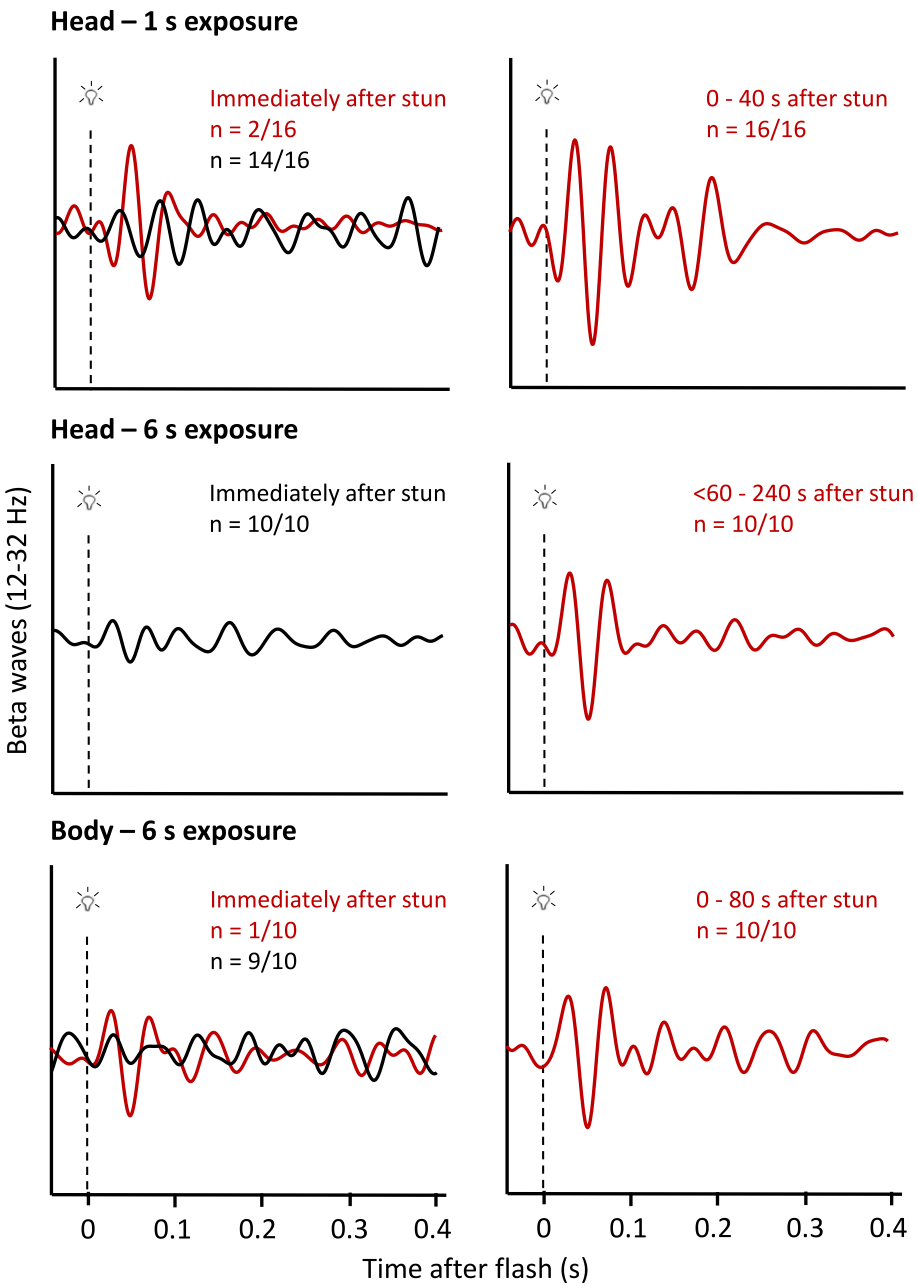

Fig. 5. VERs in channel catfish (*Ictalurus punctatus*) following electrical exposure to 134 V_{RMS} AC at 50 Hz. Left panels show indices of absence (black) or presence (red) of VER immediately after the electric shock was delivered, and right panels display the range of time it took for all fish to recover VERs after the electric shock. Exposure to a 1 s shock to the head resulted in loss of VERs in 88% of the fish and within 40 s all fish had regained VERs (**top**). With a 6 s delivery to the head, VERs were lost in 100% of the fish and the effective period was significantly prolonged (**middle**). If the 6 s exposure was instead delivered to the body of the fish, VERs were lost in 90% of the fish and within 80 s all fish had regained VERs (**bottom**). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Increasing the duration of the exposure to 6 s resulted in an absence of VERs and ventilation in all individuals for 45–240 s (126 ± 15 s) and 80–220 s (132 ± 15 s), respectively (Fig. 5), which was significantly longer compared to a 1 s stun delivered over the head for both VERs ($t_{9,837} = -6861$, $p < 0.001$) and ventilation ($t_{13,917} = -5826$, $p < 0.001$). When the 6 s electrical exposure instead was delivered to the body, 90% (*i.e.* 9/10) of the fish lost VERs after 40–80 s (48 ± 7 s) and ventilation after 5–100 s (53 ± 12 s) and 1/10 had both VER and ventilation after the shock (Fig. 5). However, the post-stunning signal was noisy for three individuals, and it is possible VERs returned earlier. Delivering the electrical shock to the body for 6 s resulted in significantly shorter recovery times for both VERs and ventilation compared to that of fish exposed to the head for 6 s ($t_{12,466} = 4629$, $p < 0.001$ and $t_{16,803} = 4145$, $p < 0.001$ respectively). Also the individual in the 6 s body group that did not lose VER reacted by powerful movements/escape attempts and was deemed as "failed".

Data on recovery times of VERs and ventilation show that 59% of the individuals recovered VER first, 30% recovered ventilation first while both indicators recovered at the same time in 11%. Difference in recovery time between VERs and ventilation was often <1 min but could be substantially longer (*i.e.* > 10 min) in some cases, particularly when ventilation recovered before VERs (Fig. 6).

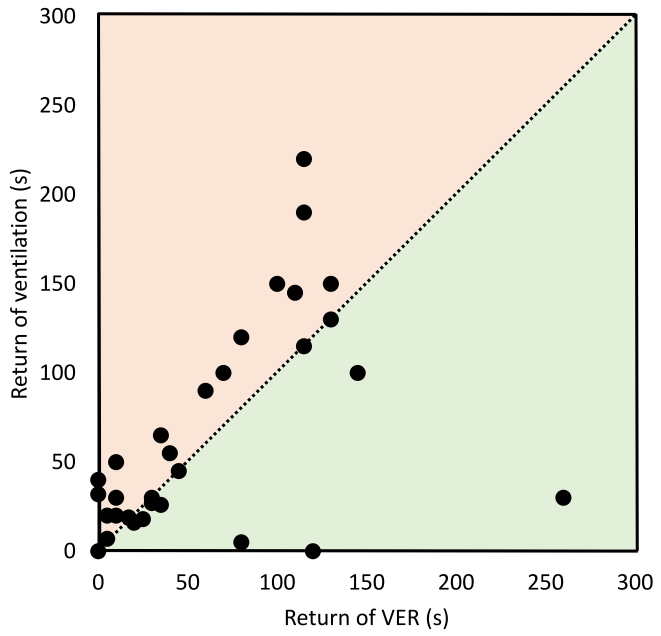

Fig. 6. Recovery of VERs and ventilation in channel catfish (*Ictalurus punctatus*) following electrical exposure to 134 V_{RMS} AC at 50 Hz. The black dashed line is a 1:1 line and has been included to demonstrate the welfare implications of using ventilation as an indicators of sensibility. Each dot in the green shaded area represents an individual where ventilation appeared before VERs which is acceptable from a welfare perspective. Contrariwise, each dot in the red shaded area represents an individual where VERs returned prior to ventilation, highlighting the risk of misjudging insensibility in fish from ventilation alone. The figure exclude individuals (n = 13) where timing of recovery could not be determined exactly (*i.e.* when VER was already present when EEG started) and individuals that only had recovery of either VER or ventilation (n = 3). Three outliers have been removed to improve visualization. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

This is the first neurophysiological investigation of the effect of electro-stunning at time of slaughter for channel catfish. Our findings show that channel catfish can be immediately stunned if a strong enough current is delivered to their head. Furthermore, channel catfish is a comparably robust fish species against exposure to electricity and can regain sensibility shortly after the circuit is broken. The findings presented and discussed here will aid in the development and optimization of stunning equipment and slaughter protocol for commercial processing plants in the catfish industry. This will benefit the industry, as they can better align with future concerns about the welfare of farmed channel catfish at time of slaughter with changing regulatory and consumer preferences. Historically, the catfish processing industry has been developed primarily to ensure work safety and product quality when handling large quantities of fish. Through exposure to electricity, voluntary movements is inhibited in the fish, which facilitate handling prior to killing during slaughter at the processor (Silva et al., 2001). In fact, a commonly used decapitation machine is designed to take advantage of the physical reaction of channel catfish when exposed to electricity. When shocked, channel catfish will lock their pectoral fins perpendicular to the body, allowing processing plant personnel to manually position fish vertically, in a machine where catfish are quickly killed (< 1 s) and prepared for subsequent processing by three circular saw blades while large fish were manually decapitated using a band saw.

The issue of fish recovering from electro-stunning, observed in the present study, is a phenomenon reported in various fish species (Gräns et al., 2016; Hjelmstedt et al., 2022; Kestin et al., 1995; Lambooij et al., 2007; Lambooij et al., 2002; Retter et al., 2018; Robb and Roth, 2003). For some species (*e.g.* many fish in the family Salmonidae), the high electrical strength and low frequencies needed to cause permanent loss of sensibility (*i.e.* electrocution) have also been reported to cause tissue and spinal damage, lowering the quality and value of the product (Robb et al., 2002; Roth et al., 2004). Consequently, electrical settings may need to be restricted in order to maintain high product quality. For other species, including fish in the order Siluriformes (*i.e.* catfishes), short recovery periods following electro-stunning are more likely a consequence that these species evolved an innate high resistance to electricity (Brijs et al., 2020; Lambooij et al., 2006). Therefore, for channel catfish processing, considerations for the use of increased electrical currents must be balanced with higher electricity costs and, more importantly, considerations for worker safety.

In our laboratory trials, channel catfish lost VERs for approximately 1–4 min when exposed to 134 V_{RMS} AC at 50 Hz with a current flow to the head of around 0.5 A $_{RMS}$ for 6 s. Such a short period of guaranteed insensibility highlight the necessity to keep the stun-to-decapitation period as short as possible in the commercial catfish processing industry. In addition, an optimal period of insensibility should not only last long enough for the killing procedure to be performed, but it should also be long enough to avoid recovery during exsanguination (Brijs et al., 2020). Notably, several studies have shown that fish can stay sensible for several min following gill or throat cut and even after decapitation (Lambooij et al., 2004; Morzel et al., 2003; Robb et al., 2000; Verheijen and Flight, 1997). It should be noted that VERs were not always continuously present during recovery, but VERs fluctuated between being present and absent in approximately half of the individuals, similar to previous observations on electro-shocked rainbow trout (Brijs et al., in press). However, until further research is able to conclusively determine whether or not this phenomena corresponds to a state of sensibility, the fluctuating presence of VERs must represent the possibility that these individuals are not insensible for transient periods of time during recovery as the brain to some extent can respond to the activation of primary sensory pathways.

Consequently, in order to minimize the risk of recovery before killing or during exsanguination when slaughtering and processing channel catfish, time between electro-stunning and decapitation could be optimized or electro-stunning could be combined with a second treatment preventing recovery (or both). Suggested solutions that can be used to prevent fishes from recovering sensibility following electro-stunning are *e.g.* a two-stage electro-stun (Lines and Kestin, 2005), electro-stunning followed by chilling in ice slurry (Brijs et al., 2020; Lambooij et al., 2006; Lambooij et al., 2007; Llonch et al., 2012) or electro-stunning followed by decapitation (Lambooij et al., 2006).

The laboratory trials showed significant effects of both exposure time (6 *versus* 1 s) and electrode position (head-only *versus* body-only) on the time it takes for the catfish to recover VERs and ventilation. Interestingly, at the processing plants the effect of electro-stunning was more variable, as the majority of catfish regained VERs and ventilation within a few minutes; ranging from some individuals displaying VERs at the onset of measurements to others remaining unresponsive for >15 min.

A plausible explanation for the more variable outcome observed at the processing plants is that when running the electro-stunner at full scale, the current each individual receives, the duration of exposure and the point of delivery can vary much more compared to a controlled laboratory trial. Because many fish were stunned simultaneously at the processing plants, the electrode position on the fish during stunning varied with some individuals entering the stunner head first while others entered tail first or sideways. Occasionally, fish also laid on top of each other when passing though the stunner. Moreover, in our laboratory studies, we observed instances of both mis-stuns and immediate recoveries when fish were exposed for shorter durations or when the shock was not delivered to the head of the fish. These results are not surprising as similar relationships between stun efficiency, electrical exposure period, and stun-electrode positioning have also been shown in other

species such as Atlantic cod (*Gadus morhua*) (Erikson et al., 2012). In addition, it was obvious during the laboratory experiments that the strong spasms sometimes induced immediately when current was passed thought the fish can cause a disruption of the delivered current. It is possible that increasing the number of rows of hanging electrodes could mitigate the potential issue of spasms causing a premature disconnection of the circuit.

In the laboratory trials all successful stuns (*i.e.* when VERs were lost) resulted in rigidness of the fish and pectoral fins being locked in an outward position which often lasted even when the fish was recovered based on EEG measurements. In these stunned catfish, ventilation, which is often used as a visual indicator of sensibility (Anders et al., 2019; Jung-Schroers et al., 2020; Rucinque et al., 2018), started approximately 1–2 min after the exposure period and corresponded roughly to the recovery of VERs. However, occasions of both VERs being present without obvious signs of ventilation and *vice versa* were observed in the laboratory trial and it is thus possible that gill movements can be observed in channel catfish that have been rendered unconscious from an electric shock as this indicates that some brain stem function remain despite the animal being able to respond to external stimuli (Kestin et al., 1991). Similar findings with ventilation often, but not always, being associated with the presence of VERs, have been reported also for rainbow trout and common carp (*Cyprinus carpio*) (Hjelmstedt et al., 2022; Retter et al., 2018). Overall, our findings suggest further improvements can be made to enhance effectiveness of protocols used for electrical stunning of channel catfish. Plausible improvements that could increase the likelihood of successful stuns and the time it takes until the fish recover include: enhanced control of the direction in which the fish enters the electro-stunner, a more even distribution of fish and more rows of stun-electrodes.

It is worth noting the electrical current passing though the fish varied greatly (*i.e.* 210–890 mA_{RMS}) among individuals despite constant voltage (132 V_{RMS} AC, 50 Hz) indicating difference in impedance among individuals. Similar patterns have been reported for African sharptooth catfish when exposed to 1.2 s head-only electrical stuns and for Atlantic cod during 0.5 s exposure (Erikson et al., 2012; Lambooij et al., 2004) and we have observed similar patterns also for rainbow trout (Brijs et al., in press). Although the relationship between 1 s currents delivered to the head and return of VERs in our regression analysis did not quite reach the threshold for significance ($p = 0.053$) it should be noted that all three individuals that did not lose VERs in the laboratory trials were exposed to currents <380 mA_{RMS}. Whether fish exposed to the lower end of the range immediately lost their sensibility in the group exposed to the head for 6 s is unknown and not within the scope of this study but warrants further investigations if longer stun exposures are to be recommended in the future.

5. Conclusion

In the present study we used neurophysiological investigation to identify areas where electro-stunning and fish welfare improvements can be made in the slaughter procedures for channel catfish. In our laboratory trials we show that channel catfish can be immediately rendered insensible if exposed to 132 V_{RMS} AC at 50 Hz for 1 s with a current flow to the head of around 0.5 A_{rms}. However, if the current flow is not strong enough or if it is delivered to the body of the catfish, immediate insensibility is not guaranteed. In our laboratory trials we also show that even following exposure to an electric current to the head for 6 s, channel catfish regain sensibility after <1 to 4 min. Also of interest, catfishes may differ from salmonids in resilience and responses to electrical stunning, with an example that typical ventilatory movements do not always link directly to VERs. These findings most likely explain the more variable outcome, in stun efficiency, observed at the processing plants where the electrical exposure will be more inconsistent compared to a controlled laboratory environment. Although the slaughter process at catfish processing facilities meets current regulatory criteria, the use of EEG measurements in this study has helped to identify where improvements can be made to enhance the effectiveness of electrical stunning of channel catfish. Plausible improvements include: minimizing the time between stunning and decapitation, combining the electro-stunning with a second treatment that prevents recovery, enhanced control of the direction in which individual fish enter the electro-stunner, a more even distribution of fish into the stunning-machine, and more rows of stun-electrodes. Taken together our findings will aid the catfish industry to safeguard the welfare of channel catfish at time of slaughter.

CRediT authorship contribution statement

Per Hjelmstedt: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Filip To:** Writing – review & editing, Resources, Investigation, Conceptualization. **Albin Gräns:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Peter Allen:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgement

The authors would like to thank the staff and managers at the two processing plants for their hospitality and for allowing and aiding us to perform the on-site studies. We are also grateful for the valuable help from the staff at the South Farm Aquaculture Facility at Mississippi State University for technical and practical support during the laboratory trials. The project was initiated by The Center for Responsible Seafood and funded by Open Philantropy, with additional support provided by the Mississippi Agricultural and Forestry Experiment Station and the US Department of Agriculture Agricultural Research Service (grant: 58-6066-5-042) and the Swedish research council for sustainable development FORMAS (grant: 2021-02303). Experiments were performed in 2023.

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