Aquaculture 596 (2025) 741825

Contents lists available at ScienceDirect

Aquaculture

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

Assessment of brain function during stunning and killing of channel catfish (*Ictalurus punctatus*)

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ARTICLE INFO

Keywords:
Brain activity
VERs
Fish welfare
Slaughter
Aquaculture

ABSTRACT

Measurement of brain activity is a reliable method to determine loss of consciousness during the slaughter of terrestrial farm animals. In fish, the ability to obtain an electroencephalogram (EEG), even in unrestrained individuals, has enabled the assessment and optimization of stunning and killing methods in aquaculture. This study evaluated the effect of percussive and in-water electrical stunning in channel catfish (*Ictalurus punctatus*) using the loss of visual evoked responses (VERs) as indicator of loss of consciousness. Our results show that percussive stunning with a fish bonker effectively caused permanent disruption of normal brain function when applied correctly. However, there is a risk of mis-stuns, with 20 % of the catfish showing temporarily or permanently responsiveness after stunning (*i.e.* presence of VERs). Exposure to an electric field strength of 13 V_{rms} cm^{-1} and a current density of 1.3 A_{rms} dm^{-2} for 1 s caused immediate but, in contrast to successful percussive stunning, transient loss of VERs. Extending the exposure time to 10 s using the same electrical parameters did not affect recovery based on VERs, nor did increasing the field strength to 24 V_{rms} cm^{-1} and a current density to 2.1 A_{rms} dm^{-2} using a 10 s exposure time. The recovery time was also unaffected by post-stun placement of fish in air or water. However, a 10 s shock with an electric field of 24 V_{rms} cm^{-1} and a current density of 2.1 A_{rms} dm^{-2} immediately followed by decapitation, prevented recovery in 70 % of the catfish with the remaining 30 % experiencing temporary recovery (*i.e.* presence of VERs) for a few minutes. Our findings show that different stunning methods offer distinct advantages and limitations. Modifying the slaughter protocol in commercial catfish production, considering these methods, could significantly enhance the welfare of channel catfish during slaughter.

1. Introduction

The welfare of farmed fish has gained increased attention over the last decades, as research on fish cognition and their responses to noxious stimuli has enhanced our understanding of humane fish handling (Braithwaite et al., 2013; Huntingford and Kadri, 2014). A critical situation where fish welfare is at risk is during slaughter (Brijs et al., 2020; Retter et al., 2018; Van De Vis et al., 2003). A slaughter scheme that does not compromise animal welfare is often referred to as humane slaughter. To be considered humane the animal should be rendered insensible before killing and remain so until death, without experiencing avoidable fear, anxiety, pain, suffering or distress (EFSA, 2004). Addressing humane slaughter issues in aquaculture proactively allows for sustainable improvements and prepares the industry for potential new or revised regulations and private standards. This proactive approach has led to a re-evaluation of many existing slaughter schemes for fish to ensure they meet high standards (Jung-Schroers et al., 2020; Retter et al., 2018). However, all stunning and killing schemes at slaughter must be optimized and adapted to species-specific requirements, which is a significant challenge given the large number of farmed fish species (Van De Vis et al., 2003). Additionally, most research has historically focused on economically important fish species, such as Atlantic Salmon (*Salmo salar*), farmed in the European Union where slaughter legislations is relatively strict. Nonetheless, there is a need for further research on slaughter practices to align with welfare regulations at the time of killing, even for European salmonid production. Moreover, evaluation of slaughter methods is lacking for the majority of farmed fish species worldwide (Gräns and Bowman, 2019). Fortunately, the aquaculture

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https://doi.org/10.1016/j.aquaculture.2024.741825
Received 27 June 2024; Received in revised form 9 October 2024; Accepted 28 October 2024
Available online 29 October 2024

industry is increasingly willing anticipate and comply with forthcoming animal welfare legislation, providing an opportunity to investigate farmed fish species where optimized slaughter processes remains largely unexplored. One such example is the channel catfish (*Ictalurus punctatus*) processing industry in the United States. The slaughter process for this species typically consists of electrical stunning in air to facilitate safe handling by humans, followed by killing *via* decapitation. In a previous study conducted in collaboration with the industry, we aimed to identify optimal stunning procedures for channel catfish using electroencephalogram (EEG) recordings and provide suggestions for improvements to the existing slaughter methods (Hjelmstedt et al., 2024). We found that in-air electro-stunning can induce immediate but transient loss of consciousness, necessitating a minimal time from stunning to decapitation to prevent recovery. Therefore, it is crucial to further investigate alternative stunning and killing methods for the catfish processing industry. However, practical limitations and economic considerations must be addressed when evaluating and introducing alternative methods to an existing processing infrastructure, ensuring they fit the specific needs of each industry.

Physical destruction of the brain is a common method to immediately stun and kill animals. In fish, this is mainly achieved through spiking (also known as pithing or iki-jime) or percussive stunning. Spiking involves physically disrupting the brain using an awl or a knife. When preformed correctly it quickly kills the fish. However, due to the small brains of most farmed fish species and the welfare hazard associated with failed attempts, this method is rarely used in large-scale industry processing (Poli et al., 2005). Percussive stunning involves administering a blow to the fish's head with a tool like fish priest or fish bonker. A correctly applied force cause physical trauma and brain haemorrhaging, leading to permanent unconsciousness in various fish species (Brijs et al., 2020; Hjelmstedt et al., 2022; Lambooij et al., 2007; Retter et al., 2018; Robb et al., 2000). However, performing manual percussion on an alert fish is challenging and poses significant welfare risks due to potential miss-hits. There it is also a workplace safety concern since operators may inadvertently strike their hands if the fish are struggle during stunning. Additionally, percussive stunning often involves restraining the fish and exposing them to air, both of which are stressful for most fish species (Lines and Spence, 2012).

An alternative method is electro-stunning in water, which efficiently induces immediate unconsciousness in several fish species (Brijs et al., 2020; Hjelmstedt et al., 2022; Lambooij et al., 2013; Robb and Roth, 2003). This method involves passing a current between electrodes submerged in water, creating an electric field around the fish placed between them. The effectiveness of the stun dependent on factors such as voltage, current, frequency, duration, and electrode position (Lambooij et al., 2008a; Robb et al., 2002; Robb and Roth, 2003; Rucinque et al., 2021). However, electro-stunning (whether in water and in air) often results in only temporary unconsciousness. Therefore, a suitable method for killing or using a combination of stunning methods may be necessary to prevent recovery during bleeding. For instance, electro-stunning of African sharptooth catfish (*Clarias gariepinus*) followed by percussive stunning, immersion in ice-slurry or decapitation, has been shown to prolong unconsciousness sufficiently for death by gill cut without the fish regaining consciousness (Brijs et al., 2020; Lambooij et al., 2006a, 2006b). Similar results, where placing stunned fish in ice water prevented recovery following electrical stunning, have been observed in Nile tilapia (*Oreochromis niloticus*) (Lambooij et al., 2008b). Collectively, previous studies on stunning of fish have demonstrated species-specific tolerance to electrical stunning and suitability of different methods of brain destruction based on fish morphology. This is crucial given the wide variety of species farmed in commercial aquaculture and the need for species-specific slaughter protocols.

The primary challenge hindering large-scale evaluations of stun efficiency in aquaculture today is the difficulty in obtaining reliable data on unconsciousness in fish (Poli et al., 2005; van de Vis et al., 2014). This stems from the belief only neurophysiological investigations, *i.e.* EEG measurements, provides robust and accurate insights into on loss of consciousness (Bowman et al., 2020; Kestin et al., 1991; Retter et al., 2018). Visual indicators of unconsciousness, such as loss of movement, ventilation or the vestibulo-ocular reflex, may not reliably indicate unconsciousness if neurophysiological indicators of consciousness are still present (Bowman et al., 2020; Kestin et al., 1991; Retter et al., 2018).

Fortunately, EEG methods for measuring neurophysiological indicators of consciousness in fish are available and can serve as a mean of verification. For example, exposing a conscious fish to a flashing light will evoke a visual evoked response (VER) on the EEG. Absence of VERs suggests a degree of brain dysfunction inconsistent with consciousness or awareness (Daly et al., 1987; Gregory and Wotton, 1986; Kestin et al., 1991). The absence or presence of VERs effectively gauge and estimate loss of consciousness post-stunning (Bowman et al., 2019; Kestin et al., 1991; Robb et al., 2000). Additionally, EEG measurements can detect the presence of an epileptic-like insult (also referred to as a *grand mal* or a tonic-clonic seizure) induced by current passage through the brain. This process involves rapid neuron depolarization followed by a period of minimal brain activity (*i.e.* an isoelectric or quiet phase), a phenomenon commonly observed in mammals (Terlouw et al., 2016). Presence of an epileptic-like insult can be monitored on an EEG as a period with high-amplitude brain activity and has been used to confirm immediate loss of consciousness in several fish species (Hjelmstedt et al., 2022; Lambooij et al., 2006a, 2006b; Lambooij et al., 2007). During both the epileptic-like insult and the subsequent isoelectric phase the animal is considered unconscious and in a state where it can be killed without experiencing pain or suffering (Blumenfeld, 2012). However, recent studies on fish subjected to electro-stunning have revealed that the isoelectric phase is not always present and fish can recover consciousness much quicker following a epileptic-like insult compared to mammals (Brijs et al., 2020; Hjelmstedt et al., 2022; Retter et al., 2018). Nevertheless, EEG remains a crucial tool for verifying the electrical parameters necessary to achive immediate unconsciousness following a brief exposure to electricity (Hjelmstedt et al., 2022; Lambooij et al., 2007).

The aim of this study was to assess the potential of in-water electro-stunning and percussive stunning in enhancing the welfare of channel catfish during slaughter. EEG measurements were employed to determine the electrical parameters required to induce immediate unconsciousness characterized by an epileptic-like. Additionally, we utilized visual evoked response (VERs) to assess and estimate loss or recovery of consciousness post-stunning. Factors influencing the efficacy of electro-stunning, such as extended exposure time, increased current density, electric field strength, and recovery conditions (in water or air), were also investigated. Finally, the study examined the impact of decapitation following electro-stunning.

2. Materials and methods

2.1. Animals and housing

Channel catfish ($n = 60$, mean ± standard error of the mean (SEM) mass: 803 ± 30 g (range: 445–1415 g), mixed sexes) were kept in 6.2 m^3 indoor tanks (ø = 2.3 m, depth = 1.5 m, fish density of approximately 8 kg/m^3) supplied with well water that was aerated with air stones and kept at a water temperature of ~24 °C at the animal facility at Mississippi State University. The fish were fed twice a week and fasted for a minimum of two days prior to experimentation. The experimental protocols were in accordance with an approved institutional animal care and use committee protocol (#22–234).

2.2. Data acquisition and signal filtering

Individual fish EEGs were recorded using two intracranial needle electrodes positioned on each side of the brain as active electrodes (25 mm 18G hypodermic needles, Becton Dickinson & CO, New Jersey, US) and a smaller reference needle electrode (MLA1213 Needle electrodes for FE136, ADInstruments, Oxford, United Kingdom) implanted in the proximal dorsal muscle tissue. The electrodes were connected to a digitally controlled relay-box that was used to disconnect the electrodes from the hardware during the electric discharge. The relay-box enabled the possibility to acquire EEG-data immediately after (< 1 s) the electrical stun. For this, an animal bio amplifier (FE136; ADInstruments) with a sensitivity range of 2 ± mV and a band-pass filter (0.1–50 Hz) was used to optimize the EEG signals. The EEG-signal was recorded at a sampling rate of 1 kHz using a PowerLab data acquisition instrument (ML 870, 8/30, ADInstruments) and monitored and analyzed with the LabChart Pro software (version 8.1.22, ADInstruments). A custom-built LED strobe light, programmed to deliver 10:490 ms light:dark light flashes (*i.e.* 2 flashes s^{-1}), was used to induce the VER in the fish. The strobe light also enabled 450 ms epochs of the EEG, where the epochs were triggered and time-locked by a signal from a small solar panel (Velleman SOL1N, Gavere, Belgium) that was connected to the data acquisition instrument. Analysis of presence or absence of VERs was done using the beta wave frequency (12–32 Hz) separated from the rest of the EEG with a band pass filter in the software. To detect the VERs, the Scope View module of the software used the average of 120 non-overlapping consecutive epochs that displayed 50 ms before and 400 ms after the flash. All epochs where the beta wave amplitude exceeded 10 μV were automatically excluded to reduce influence of muscle activity from activity bursts. Voltage and current measurements during stunning were obtained using a current clamp (Hantek CC-650, Qingdao Hantek Electronic Co., Ltd., Qingdao, China) and a voltage probe (Micsig DP10013, Shenzhen Micsig Technology Co., Ltd., Guangdong, China) connected to a PC oscilloscope (PicoScope 5204, Pico Technology, Cambridgeshire, UK) and monitored using the PicoScope 7 software (Pico Technology).

2.3. Assessment of EEGs

Presence of an epileptic-like insult was used to determine settings capable to induce immediate loss of consciousness following a 1 s electrical shock. The insult was characterized by a rapid increase in EEG amplitude of the fish compared to the EEG prior to stunning (Fig. 1a). The insult was deemed over once the EEG amplitude had returned to "normal", *i.e.* similar to pre-stunning amplitude. The duration of unconsciousness was determined as the time from the end of electrical shock to reappearance of the VERs. For the period immediately after electrical shock, recovery of VER was determined by manual averaging of consecutive epochs to obtain the best approximation of recovery time. Ventilation post stunning was determined from the unfiltered (0.1–50 Hz) EEG signals which were characterized as rhythmic waves caused by muscle activity from opercular movements.

2.4. Set up for electrical stunning

All catfish used for the investigations of electrical shocks were individually caught with a dip net and anesthetized by exposure to MS222 (ethyl-3-aminobenzoate methane sulphonic acid) at a concentration of 150 mg l^{-1} buffered with 400 mg l^{-1} $NaHCO_3$ prior to experimentation. This was done to prevent stress and ease handling and to prevent distress from noxious stimuli from implantation of needle-

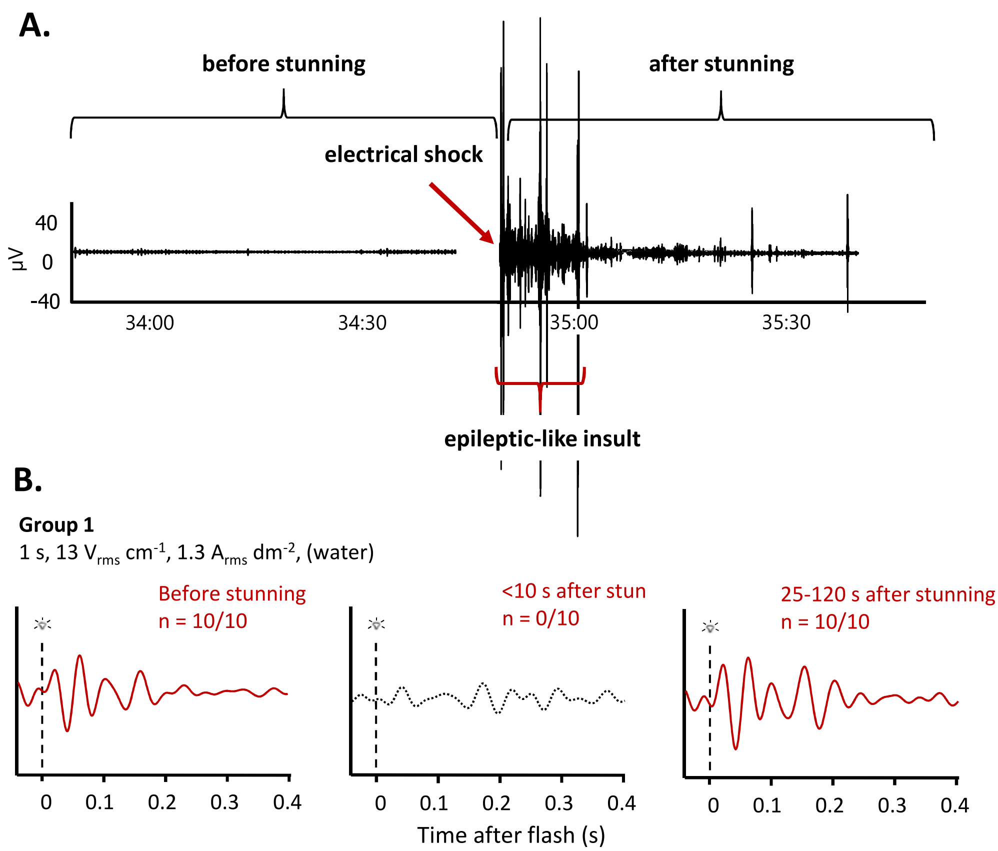

Fig. 1. Epileptic-like insult and visually evoked responses (VERs) in channel catfish before, during, and after and 1 s electrical shock of 13 V_{rms} cm^{-1} and 1.3 A_{rms} dm^{-2} from representative individuals. (A).
Representative trace of the beta waves (12–32 Hz) in the EEG-signal. Before the shock, the EEG amplitude is stable in the conscious fish. Immediately after the shock, a dramatic increase in EEG amplitude, indicative of an epileptic-like insult, lasts approximately 15 s. No isoelectric phase is observed following the insult. The EEG signal is missing during the stun exposure (<1 s) as it was disconnected. (B) (B) VERs observed before the shock confirmed that the catfish was awake and that EEG electrodes were correctly positioned. After the 1 s shock, VERs were transiently absent in all fish but recovered within 2 min.

electrodes. Once the fish were deemed to be anesthetized (belly up, no movements or eye roll reflex), they were gently held with only the upper part of the head exposed to air, and electrodes were inserted on each side of the brain. The fish were then quickly placed a plastic experimental tank (dimensions 12 × 55 cm) and fully submerged with a water level of 12 cm, allowing free movement. The tank was gravity fed with fresh and aerated 23.6 (22.7–25.2) °C water with a conductivity of 928 (918–935) μS cm^{-1} at a water exchange rate of approximately 2 l min^{-1}. The LED strobe-light was placed over the tank approximately 20 cm above water level. The fish were kept in the tank until displaying righting behaviour, characterized by return of body movements and ventilation (opercular movements) and clear VERs observed using computer software (LabChart). After clear return of VERs for a few minutes, fish were exposed to an electric field after which EEG was recorded for 30 min to determine recovery of the VER. Electric fields were created in the experimental tank using side-to-side stainless steel electrodes (11.5:52.5 cm l:w, area = 6.04 dm^2) with a plate separation of 11.5 cm. The electrodes were connected to an in-house built electro-stunner, consisting of a transformer delivering 50 Hz with adjustable voltage (0–150 V AC or 0–240 V AC) and a built-in timer with a 1 ms resolution to program stunning duration. Current density of electrodes (A_{rms} dm^{-2}) and electric field strength (V_{rms} cm^{-1}) were calculated as follows:

$$Current\ density\ \left(A_{rms}\ dm^{-2}\right) = \frac{Current\ (A_{rms})}{Electrode\ area\ (dm^2)}$$

$$Electric\ field\ \left(V_{rms}cm^{-1}\right) = \frac{Voltage\ (V_{rms})}{Electrode\ seperation\ (cm)}$$

2.5. Treatments

The 60 catfish were divided into 6 treatment groups with 10 fish per group. The various stunning protocols for each treatment group are summarized in Table 1.

Treatment group 1 was used to determine if a 1 s exposure to a field strength of 13 V_{rms} cm^{-1} and a current density of 1.3 A_{rms} dm^{-2} (which was the maximum capacity using the transformer used to deliver 150 V AC) will cause an immediate loss of consciousness (*i.e.* induce an epileptic-like insult). This electrical parameters are slightly lower compared for settings used for stunning of African sharptooth catfish (Brijs et al., 2020; Lambooij et al., 2006a, 2006b) but markedly higher than the electrical parameters required to induce epileptic-like seizures in rainbow trout (Hjelmstedt et al., 2022).

Treatment group 2 was used to determine if an increased shock duration (*i.e.* from 1 s to 10 s) will prolong the period of insensibility (*i.e.* loss of VERs) when the fish are exposed to a field strength of 13 V_{rms} cm^{-1} and a current density of 1.3 A_{rms} dm^{-2} (delivered using 150 V AC).

Treatment group 3 was used to determine if increasing the field strength of 24 V_{rms} cm^{-1} and a current density of 2.1 A_{rms} dm^{-2} (which was the maximum capacity using the transformer delivering 275 V AC) will prolong the period of insensibility when the fish are exposed for 10 s.

Treatment group 4 was used to determine if the period of unconsciousness differed between fish recovering in water compared to fish recovering in air. Therefore, these fish were, following a 10 s exposure to field strength of 24 V_{rms} cm^{-1} and a current density of 2.1 A_{rms} dm^{-2}, lifted out of the stunning tank and placed on a moist plastic tray in air.

Treatment group 5 was used to determine if recovery could be prevented from quick bleeding that occurs following decapitation. To do so these fish were, following a 10 s exposure to a field strength of 24 V_{rms} cm^{-1} and a current density of 2.1 A_{rms} dm^{-2}, lifted out of the stunning tank, decapitated, and placed on a moist plastic tray in air. An electric knife was used for decapitation to mimic the circular saw blades normally used to decapitate this species at commercial processing plants (Hjelmstedt et al., 2024) It took <10 s from the end of stunning to a complete severance of the head, which was placed on a moist plastic tray in air for EEG-recording.

Treatment group 6 was used to determine the efficacy of percussive stunning. Fish were individually netted from the holding tank, quickly transferred onto a moist cutting board, and administered two percussive blows to the head over the brain *via* a commercially available aluminum bat fish bonker (Offshore Angler™ Aluminum Fish Bat, BPS direct, L.L.C., Springfield, Missouri, US). For these fish it was not possible to obtain EEG measurements prior to percussive stunning as EEG-electrode position on the head made a correct application of the blow impossible. Instead, EEG-electrodes were implanted immediately after the stun, a process that took <30 s, after which the fish was moved to the water-filled recovery tank and EEG was measured for 30 min to monitor for recovery of VERs and ventilation.

Table 1
Summary of the treatment groups investigated in the this study (n = 10). Mass is presented as mean (min-max) and the water flowing through the tank was kept at a temperature of ~24 °C with a conductivity of ~930 μS cm^{-1}.

Group	Stunning	Duration	Post-stunning	Mass
1	13 V_{rms} cm^{-1} / 1.3 A_{rms} dm^{-2}	1 s	Water	868 (475–1190) g
2	13 V_{rms} cm^{-1} / 1.3 A_{rms} dm^{-2}	10 s	Water	761 (515–1145) g
3	24 V_{rms} cm^{-1} / 2.1 A_{rms} dm^{-2}	10 s	Water	786 (465–1080) g
4	24 V_{rms} cm^{-1} / 2.1 A_{rms} dm^{-2}	10 s	Air	778 (470–1345) g
5	24 V_{rms} cm^{-1} / 2.1 A_{rms} dm^{-2}	10 s	Air + decapitation	748 (575–880) g
6	Mechanical percussion	–	Water	882 (535–1415) g

2.6. Statistical analyses

Statistical analyses were conducted using SPSS Statistics 29 (IBM Corp., Armonk, NY, USA). All data were assessed to ensure that they met the assumptions of the statistical model outlined below. Kaplan-Meier tests were used to test if the various treatments affected the time it took for catfish to resume VERs or ventilation independently. Time until recovery of VERs and ventilation was treated as the dependent variable, with the six treatment groups included as factors. "Recovery" was defined as the status, and fish that had not recovered by the end of the 30-min (1800 s) observation period were censored (*i.e.* the subject is removes from the denominator and no longer considered to be at risk of recovering). Pairwise comparisons among treatments were evaluated using the Log rank test statistic, where all time points are weighted equally. Treatment groups with 100 % success in the investigated variable were excluded from analysis. Throughout the text, p-values from statistical analyses are reported, with values <0.05 considered statistically significant. Unless otherwise specified, results are presented as mean (min-max).

3. Results

VERs were clearly present in all fish prior to electro-stunning. However, VER amplitude from epoching of the EEG signal varied among individuals which is likely explained by slight variations in electrode positions in relation to vicinity of the brain. All electrically shocked catfish became rigid with pectoral fins locked in an outwards position, but this effect was less pronounced for the group that was shocked for 1 s using a field strength of 13 V_{rms} cm^{-1} and a current density of 1.3 A_{rms} dm^{-2}. Although rigidness of the body decreased during recovery, voluntary movement was greatly depressed and the spines remained in the locked position. Some individuals displayed sluggish movements when touched during recovery but spontaneous activity was rarely observed.

All fish electrically shocked for 1 s with a field strength of 13 V_{rms}

cm^{-1} and a current density of 1.3 A_{rms} dm^{-2} showed a period of increased brain activity (an epileptic-like insult) indicating loss of consciousness from stunning. The magnitude of the increase in amplitude was similar to a previous study on rainbow trout of an approximately 20-fold increase in amplitude during the insult compared to pre-stunning levels (Hjelmstedt et al., 2022). Therefore, it was deemed that this setting was sufficient to cause immediate loss of consciousness (Fig. 1a). The duration of the insult, *i.e.* the time it took for the EEG to return to pre-stun amplitude, was 31 s (7–67 s) and none of the fish displayed an isoelectric period following the insult. VER returned 82 s (25–120 s) after the electrical shock which corresponds to 3–60 s after the end of the insult (Fig. 1b & 2a).

Increasing the duration of the electrical shock to 10 s with a field strength of 13 V_{rms} cm^{-1} and a current density of 1.3 A_{rms} dm^{-2} did not significantly affect the time until recovery for VERs ($p = 0.72$) or ventilation ($p = 0.98$). However, in this group variance in responses were high, with one individual's VERs observed immediately after cessation of the shock compared to another individual where the VERs were not regained for over 16 min (Fig. 2a & 3a). Further, increasing intensity of the 10 s electrical shock to a field strength of 24 V_{rms} cm^{-1} and a current density of 2.1 A_{rms} dm^{-2} did not significantly affect the recovery of either VERs ($p = 0.78$) or ventilation ($p = 0.61$). Greater shock intensity resulted in high variation of recovery time, which ranged from 10 to 170 s in 9 out of 10 individuals, with one individual not recovering until after 420 s (Fig. 2a & 3b). Using the same stun setting but immediately removing the fish from the water following the shock, prevented recovery of VER in one individual (Fig. 2 & Fig. 3c). Yet, no significant effect was found when the fish were left in air compared to water on the recovery of VERs ($p = 0.18$) or ventilation ($p = 0.14$).

Interestingly, when the 10 s shock with an electric field of 24 V_{rms} cm^{-1} and a current density of 2.1 A_{rms} dm^{-2} was immediately followed by decapitation, recovery of VER was prevented in 70 % and ventilation in 40 % of the fish (Fig. 2 & Fig. 3d). For the three individuals that recovered the VER (after 15, 30 and 70 s respectively), the response disappeared after a few minutes following decapitation and the EEG signal became flat after 3 to 8 min. Also, the regained ventilatory movements were transient and lost after 3.5 to 20 min. Consequently, the addition of decapitation following the electrical shock both increased the proportion of fish that did not recover and significantly increased the time until recovery of both VERs ($p = 0.003$, $p = 0.018$) and ventilation ($p < 0.001$, $p = 0.015$) when compared to fish left to recover in water or air, respectively.

Percussive stunning caused immobilization and loss of ventilation in all catfish and also caused permanent loss of VERs in 8 out of 10 individuals (Fig. 2a & 4). The amplitude of the EEG following a successful percussive stun was very low compared to an EEG from a conscious fish, and also low compared to fish following electrical stunning. One individual displayed VERs around 8 min post-stunning that again disappeared, and another individual was clearly not sufficiently stunned as it had VERs during the whole recovery, albeit without ventilation or other movements. Taken together, the proportion of fish that did not recover VERs was similar to the group that was decapitated (*i.e.* 80 and 70 % respectively). The time until recovery of VERs following percussive stunning was significantly longer compared to electrically stunned fish left to recover in water ($p < 0.001$) or in air ($p < 0.001$) but not significantly different from the recovery time of decapitated fish ($p = 0.51$).

Altogether, four fish started ventilating without recovering VERs. In contrast, both fish that regained VER from percussive stunning did not show any opercular movements for the entire 30 min recovery period.

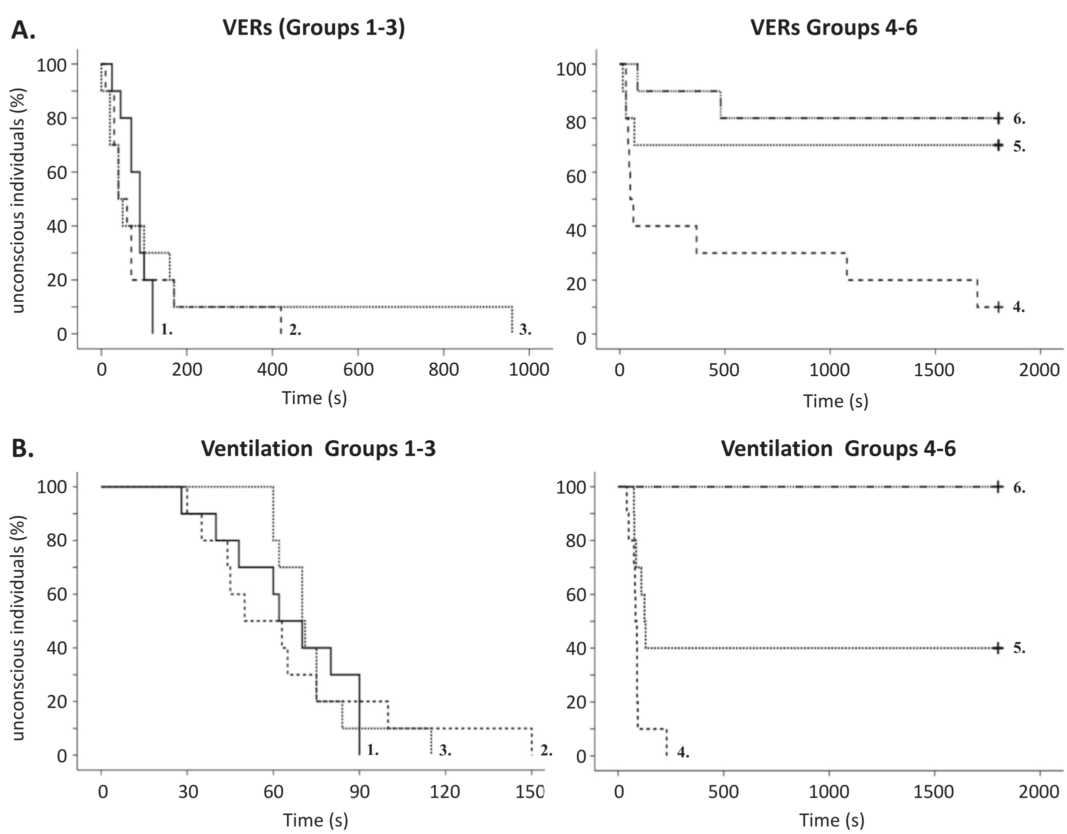

Fig. 2. Kaplan Meier curves of the recovery of VERs (A) and ventilation (B) in channel catfish following different stunning methods. Group 1 was shocked with a field strength of 13 V_{rms} cm^{-1} and a current density of 1.3 A_{rms} dm^{-2} for 1 s. Group 2 was exposed to the same electrical parameters for 10 s. Groups 3–5 were all shocked with 24 V_{rms} cm^{-1} and 2.1 A_{rms} dm^{-2} for 10 s, then left to recover in water (group 3), in air (group 4), or in air after decapitation (group 5). Group 6 underwent percussive stunning. Each step in the curves represents the recovery of an individual. A steeper slope indicates a faster recovery, while flatter slopes show slower recovery. Plateaus represents periods without recoveries, highlighting the variability within treatment groups.

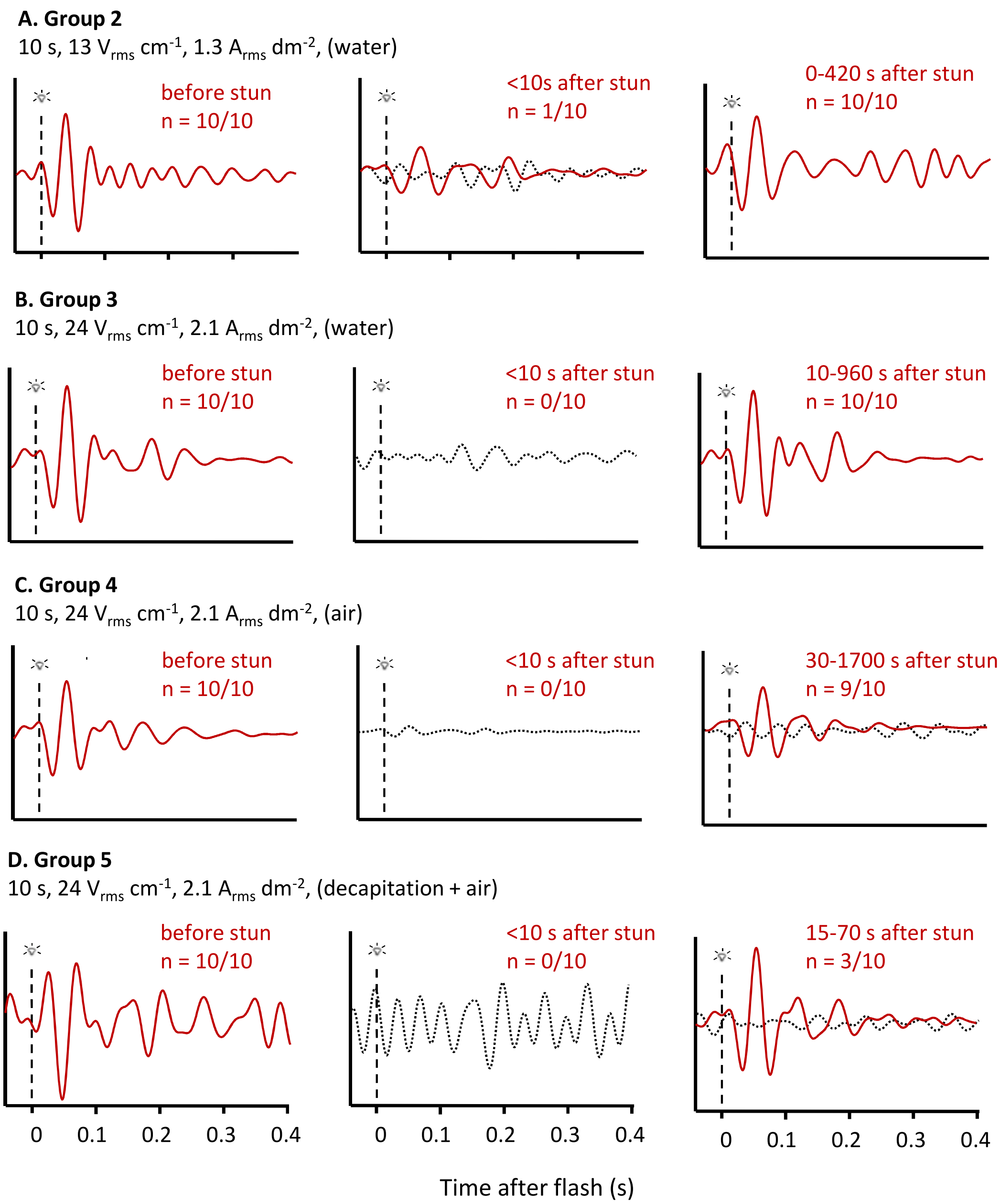

Fig. 3. Proportion of channel catfish with visually evoked responses (VERs, red lines) before (left panels) and immediately (<10 s) following a 10 s electrical shock (middle panels), and during a 1800 s recovery period (right panels). The treatments groups were either exposed to two different strengths of electrical shocks (A & B) or recovering in either water (B), air (C) or air following decapitation (D) after being shocked with 24 Vrms cm^{-1} and 2.1 Arms dm^{-2} for 10 s. The dashed lines are from fish where no VERs could be detected. No units are presented for the y-axis as signal strengths varied slightly depending on electrode position and fish size among trials (columns) making the scale on the y-axis, measured in μV, not directly comparable due to within-individual differences. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Overall, out of the 42 individuals that recovered both VER and ventilation, 45 % recovered VER first, 48 % recovered ventilation first and both recovered simultaneously in 7 % (Fig. 4). The difference in recovery time between these indicators was generally <1 min but in three instances VERs were preceded by ventilation for >10 min (not included in Fig. 5).

4. Discussion

As concern for fish welfare grows, more aquaculture facilities are adopting slaughter schemes that involves stunning before killing. The objective of pre-slaughter stunning is to immediately render the fish unconscious ensuring consciousness does not return before death (EFSA, 2004; OIE, 2019). Pre-slaughter stunning also facilitates fish handling, improving the effectiveness of the killing procedure and enhancing workplace safety (Retter et al., 2018). However, many current stunning methods used for fish lack evidence-based validation regarding their ability to render fish unconscious (Gräns and Bowman, 2019). Obtaining reliable data of unconsciousness in fish remains challenging, primarily due to the necessity for neurophysiological EEG investigations to validate stun effectiveness (Poli et al., 2005; van de Vis et al., 2014). Conducting such investigations in fish is inherently complex (Bowman et al., 2020; Kestin et al., 1991; Retter et al., 2018).

In our study, we integrated EEG and ventilation measurements in channel catfish, show that both physical destruction of the brain and in-water electro-stunning followed immediately by decapitation

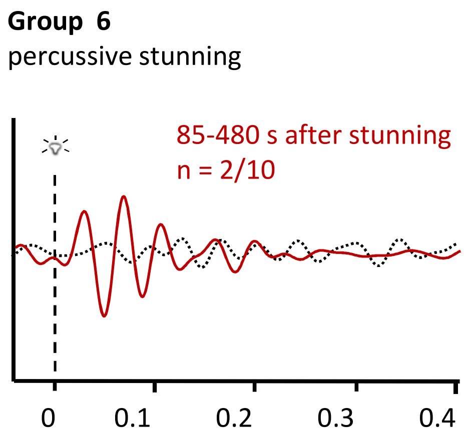

Fig. 4. Proportion of channel catfish recovering visually evoked responses (VERs) during a 1800 s recovery period following percussive stunning. The dashed line is from one of the fish where no VERs could be detected. No units are presented for the y-axis as signal strengths varied slightly depending on electrode position and fish size among trials (columns) making the scale on the y-axis, measure in μV, not directly comparable due to within-individual differences.

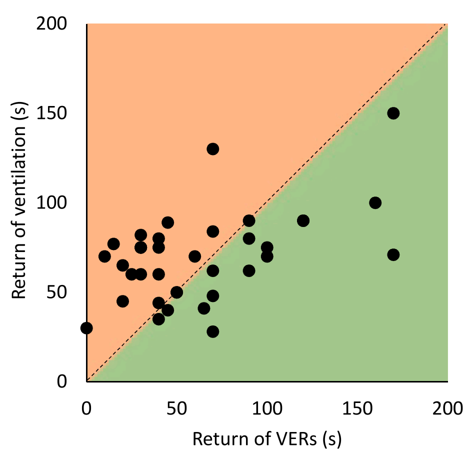

Fig. 5. Time to recovery of ventilation and visually evoked responses (VERs) of channel catfish following electrical stunning. Dots in the upper orange area represent individuals where VERs returned before ventilation indicating that they may have recovered consciousness without any visual signs. Dots on the lower green area represent individuals where ventilation returned before VERs. A few ($n = 3$) outliers have been removed from the figure 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.)

significantly enhanced welfare during slaughter. In addition, our results underscore the need to optimize these methods for achieve immediate and sustained unconsciousness in aquaculture practices.

Consistent with previous research our findings demonstrate that percussive stunning can induce immediate and permanent unconsciousness in channel catfish, aligning with studies on other fish species (Hjelmstedt et al., 2022; Roth et al., 2007). However, two fish in our study regained VERs, resulting in an 80 % success rate. Instances of miss-hits during manually percussive stunning have been documented in previous studies on different fish species (Brijs et al., 2020; Lambooij et al., 2010; Lambooij et al., 2007) likely due to the challenges of accurately handling and targeting a struggling fish and generating sufficient force with each blow. In a prior investigation, we analyzed the impulse (N) and kinetic energy (J) generated by a percussive blow using a fish priest. Our results revealed significant variability in these metrics across blows, despite efforts to apply consistent force and accuracy (Brijs et al., 2020). Overall, our results show that percussive stunning can efficiently induce immediate and permanent unconsciousness in channel catfish. However, to safeguard fish welfare and working safety, future studies should focus on minimizing stress and struggling behaviours during fish handling before percussive stunning. Additionally, research should explore methods to design tools and protocols that minimize variability in the force generated during percussive stunning. Thereby optimizing the procedure and improve its success rate in rendering fish unconscious effectively.

Electro-stunning in water can also successfully induce immediate loss of consciousness in channel catfish, as evident by the presence of an epileptic-like insult following a 1 s electrical shock (13 V_{rms} cm^{-1}, 1.3 A_{rms} dm^{-2}). Comparable EEG patterns have been observed in numerous other fish species, although the intensity of the shock needed to induce such an insult varies substantially among species (Robb and Roth, 2003; Rucinque et al., 2018). The electrical field strength and current density used in this study are similar to those previously applied for the related sharptooth catfish but notably higher than the 2.8 V_{rms} cm^{-1}, 0.22 A_{rms} dm^{-2} required to induce an epileptic-like seizure in the more sensitive rainbow trout (*Oncorhynchus mykiss*) (Brijs et al., 2020; Hjelmstedt et al., 2022; van de Vis et al., 2014). The duration of the seizures observed in our study aligns with reported times in other fishes, and no isoelectric period was observed on the EEG for any of the channel catfish shocked for 1 s (Hjelmstedt et al., 2022; Lambooij et al., 2008a; Lambooij et al., 2008b; Lambooij et al., 2007). Consistent with findings in various fish species, the seizure's effect was transient, with all catfish recovering (based on presence of VERs) within a minute after the 1 s electrical shock (Brijs et al., 2020; Hjelmstedt et al., 2022; Robb and Roth, 2003; Rucinque et al., 2018).

In contrast to earlier studies on other fish species (Lines et al., 2003; Robb et al., 2002; Roth et al., 2004), prolonging the period of unconsciousness in channel catfish following an electrical shock was not achieved by increasing either the duration or strength of the shock. When the duration of the electrical shock was extended from 1 to 10 s (13 V_{rms} cm^{-1}, 1.3 A_{rms} dm^{-2}), one individual recovered VERs immediately after the 10 s shock ended, suggesting potential recovery during the electrical exposure. Similarly, increasing the strength of the electrical shock to 24V_{rms} cm^{-1} (2.1 A_{rms} dm^{-2}) for 10 s, did not improve stunning effectiveness, as 80 % of the catfish recovered VERs within 3 min, with one individual recovering after only 10 s.

Our results show that in this species, recovery of ventilatory movements following electro-stunning in water is not a reliable indicator of consciousness. A high proportion of individuals regained VERs before exhibiting ventilation, indicating potential earlier recovery of consciousness without visible signs. Similarly, many individuals recovered ventilatory movements before regaining VERs. These results are consistent with earlier studies across various fish species, which also showed limited correlation between neurophysiological indicators of consciousness and observable signs (Bowman et al., 2020; EFSA, 2004; Retter et al., 2018).

The resilience of channel catfish to electrical shocks is remarkable. For instance, the electric field employed here was nearly eight times higher than that recently suggested to induce unconsciousness until death in juvenile Atlantic salmon (Bouwsema et al., 2022). From a product quality standpoint, this electrical resilience poses potential challenge, as high electric fields, current densities, low frequencies, and prolonged exposures are all known to cause injuries, such as vertebrae fractures and petechial haemorrhage in muscle tissue (Lines et al., 2003; Robb, 2001). Injuries, that can impede automated processing or diminish market value of the final products. In summary, our findings suggest that electro-stunning alone may have limitations for inducing long-term unconsciousness in channel catfish.

One potential solution to address the electrical resilience in fish species is to incorporate post-stunning treatment or a second stunning method into the slaughtering process to ensure irreversible unconsciousness (Brijs et al., 2020). Given that severe oxygen deprivation can by itself be life-threatening, we initially explored whether preventing recovery by allowing catfish to recover in air instead of water following electrical shock would be effective. However, recovering in air did not significantly alter the recovery time in this species. Another combined stunning and killing method that has proven to be affective for several other species involves immediately following electro-stunning with throat cutting and immersion in ice water. This method has been demonstrated effective in previous experimental studies involving African sharptooth catfish, yellowtail kingfish (*Seriola lalandi*), Nile tilapia, turbot (*Scopthalmus maximus*) and common sole (*Solea solea*) (Brijs et al., 2020; Daskalova et al., 2015; Lambooij et al., 2008a; Llonch et al., 2012). However, it is important to note that this combined approach is currently impractical for much of the catfish industry, as they are process the fish before chilling and the onset of rigor mortis (*i.e.* stiffening of the corpse caused by chemical changes in the muscles) (Hjelmstedt et al., 2024).

Another proposed alternative is electro-stunning followed by decapitation, which have been effective in preventing recovery in African sharptooth catfish (Lambooij et al., 2006a, 2006b). Similarly, for channel catfish the success rate of the stunning procedure increased significantly if the electrical shock ($24V_{rms}$ cm^{-1} 2.1 A_{rms} dm^{-2}, delivered for 10 s) was immediately followed by decapitation, with only the 3 out of 10 fish regained signs of consciousness, all of which were lost within a few minutes. Since decapitation is already a standard practice at many catfish processing plants, this finding suggests that relatively simple modifications to existing equipment could greatly improve the welfare of channel catfish during slaughter. One potential solution to explore further is the integration of an additional electro-stunner into the beheading machine, delivering a brief second electrical shock. If the fish can be rendered unconscious immediately before decapitation, the likelihood of recovery may be significantly reduced. However, this remains highly speculative and further investigation is needed to determine whether sequential electrical stunning can enhance fish welfare in channel catfish production. It is important to note that the physical response of channel catfish to in-water electro-stunning is very similar to the more commonly used electro-stunning with rod-electrodes in air, characterized by body stiffening and the pectoral fins locking in an outwards position. This characteristic is a crucial for the proper function of many existing beheading machines (Hjelmstedt et al., 2024).

5. Conclusion

Our study, using EEG measurements in channel catfish confirms the importance of neurophysiological indicators in assessing stunning methods. We caution against relying solely on behavioural cues, which can be misleading, particularly for methods that may induce paralysis or immobilization. Channel catfish exhibit resilience and resistance to both electrical and percussive stunning methods; however, a well-applied percussive blow or electrical shock can swiftly induce insensibility. These findings emphasize the necessity to consider the entire process - including pre- and post-stun handling and the chosen killing method - when evaluating the humaneness of stunning methods. Futhermore, our research underscores the importance of critically analyzing and integrating EEG data and empirical optimization to ensure reliable and humane stunning in aquaculture settings. Optimization efforts can yield significant improvements in slaughter practices with minimal modifications to existing designs, particularly for channel catfish. The sequential application of electrical shock followed by prompt decapitation presents a promising strategy to reduce the risk of fish regaining sensibility before death. In summary, our study identifies opportunities to enhance welfare standards in aquaculture and fisheries, demonstrating how simple adjustments can lead to substantial improvements in fish welfare during slaughter.

CRediT authorship contribution statement

Per Hjelmstedt: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Filip To:** Writing – review & editing, Methodology, Investigation. **Peter J. Allen:** Writing – review & editing, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Albin Gräns:** Writing – review & editing, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Data curation, 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 are grateful to the staff at the South Farm Aquaculture Facility at Mississippi State University for technical and practical support during the experimental trials. The project was initiated by the Center for Responsible Seafood and the study was performed in 2023. The project was 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).

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