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Electrical stunning and killing of Lumpfish with commercial waterbath and dry stunners

Final report

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Report

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Electrical stunning and killing of lumpfish with commercial waterbath and dry stunners

Tittel:
Bedøving og avliving av rognkjeks med kommersielle enheter for elektrisk tørr- og vannbedøving.

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Se avsnitt 1.1

Table of contents

1 Abstract

Lumpfish, ranging in size from 146-4200 g, were placed in an electric dry stunning unit head first and exposed to 50 Hz, 240 V AC for 0.5 or 1 s and thereafter 4.5 or 9 s of electricity, thus 5 and 10 s respectively. In seawater, lumpfish were exposed to an electric field equivalent of 4.6 V_{rms}/cm, 100 pps square AC, with 15% duty cycle for 1 s and thereafter 9 s. This to verify whether the animal is rendered unconscious prior to killing by a throat cut, immersion into cold brine (-14 to -18 °C) or hypoxic water supersaturated with CO2. Behaviour, heart (ECG) and brain (EEG) activity were monitored until the animal was classed as dead. Post the treatment, the internal temperature of the animal was measured by loggers placed into the brain and heart cavity. The electric potential across the brain was also measured. A preliminary evaluation of the EEG and ECG registrations in the individual fish showed that loss of consciousness may occur within 0.5 seconds stun as the fish displays epileptic insult, but unconsciousness could not be verified with EEG. A one-second stun did, however, result in verified unconsciousness both for dry and waterbath stunning. A prolonged electric exposure in combination with a cold brine resulted in permanent loss of consciousness until death, whereas a throat cut or exposure to water supersaturated with CO_2 was insufficient and resulted in some fish recovering. Measurements of the electric potential difference across the brain showed that the electric field is dependent on the size of the fish for dry stunning, but not in water. Unconsciousness was reached as low as 2.8 V/cm V_{pp} across the brain, but with an average of 45 and 14 V_{pp} for dry and waterbath stunning, respectively. We conclude that electrical stunning in combination with cold brine is effective for humane stunning and killing of lumpfish.

1.1 Sammendrag

Rognkjeks, i varierende størrelse fra 146-4200 g, ble plassert i en elektrisk tørr bedøvingsenhet med hodet først og eksponert for 50 Hz, 240 V AC i 0,5 eller 1 s og deretter 4,5 eller 9 s elektrisitet, henholdsvis 5 og 10 s. I sjøvann ble rognkjeks eksponert for et elektrisk felt tilsvarende 4,6 Vrms/cm, 100 pps AC, med 15% driftssyklus i 1 s og deretter 9 s. Dette for å verifisere om dyret blir bevisstløst før avliving ved strupekutt, plassering i saltlake (-14 til -18 °C) eller hypo-oksisk vann overmettet med CO2. Atferd, hjerte (EKG) og hjerne (EEG) aktivitet ble overvåket til dyret ble klassifisert som dødt. Etter behandlingen ble dyrets indre temperatur målt av loggere plassert i hjernen og hjertehulen. Det elektriske potensialet over hjernen ble også målt. En foreløpig vurdering av EEG- og EKG-registreringene hos den enkelte fisk viste at bevissthetstap kan oppstå innen 0,5 sekunder da fisken får epileptiske anfall, men støy som følge a epileptiske anfall kombinert med rask oppvåkning gjorde at dette ikke verifiseres med EEG. Men med 1 sekund eksponeringstid resulterte imidlertid i verifisert bevisstløshet med EEG for både for tørr- og vannbedøvere. En langvarig elektrisk eksponering i kombinasjon med en kald saltlake resulterte i permanent tap av bevissthet frem til død, mens et halskutt eller eksponering for vann overmettet med CO2 var utilstrekkelig og resulterte i at noen fisk kom seg. Målinger av den elektriske potensialforskjellen over hjernen viste at det elektriske feltet er avhengig av størrelsen på fisken for tørrbedøving, men ikke i vann. Bevisstløshet ble nådd så lavt som 2,8 V/cm Vpp over hele hjernen, men med et gjennomsnitt på 45 og 14 Vpp for henholdsvis tørr- og vannbadbedøvelse. Vi konkluderer med at elektrisk bedøving i kombinasjon med kald saltlake er effektivt for human bedøving og avliving av rognkjeks.

2 Introduction

For production of Atlantic salmon (*Salmo salar*), cleaner fish such as the lumpfish (*Cyclopterus lumpus*) are commonly used for managing the sea lice infestations within a salmon culture. This fish must also, like most aquaculture species, be killed and slaughtered in a humane way, according to act of conduct by EFSA and Norwegian rules and regulation (Anon 2006, EFSA 2004, EFSA 2009$_{a,b}$). In general, cleaner fish will maintain its purpose until salmon are slaughtered, where cleaner fish must undergo the same procedures as salmon and often follow same path as salmon. Previous studies on cleaner fish do show that stunners used for salmonids (105 V, AC+DC; Lambooij et al. 2010), works well on larger wrasse sp., but not for smaller cleaner fish that do not touch the electrodes (Foss et al. 2017). For lumpfish the situation is far worse as applying 105 V AC+DC does not stun the animal at all (Foss et al. 2017) and should be sorted out and undergo separate stunning and killing processes. There are in principle 3 ways of stunning and killing of farmed fish. Either percussive, electric stunning in combination with exsanguination or an overdose of anaesthetics (Lines and Spence 2012). Naturally, fish exposed to anaesthetics cannot be introduced into the circular food chain, and thus create waste rather than feed or food. As an alternative to anaesthetics, electrical stunning in combination with immediate grinding is commonly used for killing fish for ensilage purposes, which also accounts for lumpfish (Roth and Foss 2021).

In order to render lumpfish unconscious within 0.5 s with electricity, 220 V, 50 Hz AC is required for dry stunners and 322 V/m in water resulting in epileptic seizure and loss of behavioural reflexes for a brief period of time (Foss et al. 2017). The challenge is however to induce a prolonged period of unconsciousness, as a 5 second stun duration with 220V resulted in a rather fast recovery (Foss et al. 2017). Studies investigating commercial waterbath and dry stunning of lumpfish with prolonged periods of time or alternating voltages show a complex picture for stunning and killing of this animal as 150 V, 50 Hz AC in dry and 225 V/m in water was insufficient for stunning lumpfish nor kill (Foss et al. 2021). Increasing the current duration to 10 s in combination with a cold shock could prevent the animal from recovering, resulting in death (Foss et al. 2021). Although the presence of an epileptic insult and loss of basic reflexes after a short electrical insult is a good indicator for loss of consciousness, the lack of behavioural responses are often a bad indicator while the animal is regaining consciousness (Kestin et al. 2002; Hjelmstedt et al. 2022). Therefore, the best strategy for stunning lumpfish should be to stun them with electricity and mince them shorty after stunning, which has been the current practice at slaughterhouses (Roth and Foss 2021).

In recent years there has been a shift in how one should stun and kill animals also for feed and side stream purposes. An ultimate intervention would be slaughter, which can be needed for legal, health, welfare and sustainability reasons. However, when killing at a farm is required, stunning methods that are applied in a slaughterhouse are not always feasible or practicable. This also goes for cleaner fish, where the animals are often sorted out at the farm and the industry is striving to avoid using not only anaesthetics, but also mincing the animals in order to use these animals not only for feed, but also food. Alternative methods for humane killing therefore required. Newer studies by van de Vis et al. (2023) show that salmon can stunned and killed are humanely exposing the animals directly to 240 V, 50 Hz AC for 5 to 10 s before placing them into a freezing brine or water supersaturated with CO_2. This allows the industry to stun and kill salmonids on-site without the use of anaesthetics or grinding, increasing the sustainability. The big question is whether the same principles apply for lumpfish.

The aim of this study was therefore to investigate whether lumpfish could be stunned unconscious in air or in water in accordance with regulations, and remain unconscious until killed by throat cut, cold brine immersion or exposure to hypoxic water supersaturated with CO_2. The unconscious conditions applied for stunning and killing will be verified by registration of brain activity (EEG) in combination with registration of heart activity (ECG). A secondary objective is to determine if the conditions for stunning can be modelled by registering the electrical potential difference across the brain as a function of stunning method, species and size.

3 Materials and methods

Experiments on dry stunning was carried out in June and September 2023 where a total of 69 lumpfish with mean (SD) size of 1309 (756.2) g (ranging from 164 to 4200 g) were selected from different production tanks at the Akvaplan-niva research station at Kvaløya, Norway. For waterbath stunning the experiments were carried out in September 2023 using 47 lumpfish with a mean (SD) size of 653 (468,8) g (ranging from 280 to 2326 g). All fish were held under seawater conditions at 6-8 °C and fed daily. Before the experiment one fish at the time was captured from the tank with a dipnet and transported in a transfer tank to the experimental facilities. Oxygen was at all times above 80% saturation. Prior to neurophysiological measurements (EEG and ECG), each fish was then fixed in a net and kept in a holding tank with water of appropriate temperature, salinity, and oxygen concentration. Then, with the fish in the water, the EEG and ECG electrodes are invasively applied under local anaesthesia, using injectable 2% lidocaine (Ross and Ross, 2008). This procedure does not require a period of recovery prior to registration of an EEG and ECG in fish.

3.1 Stunning and killing

In a laboratory setting, the specifications for immediate stunning for dry stunning for lumpfish were set to 0.5 (June) and 1 second (September), without recovery until death were established, using registration of the electrical activity in the brains (EEG) and heart (ECG). We assessed whether a transfer of the stunned fish to a cold brine of -14 to -18 °C or seawater of 8 °C supersaturated with carbon dioxide (< pH 5.2) prevented recovery, thus killing the fish.

To assess whether immediate unconsciousness in lumpfish could be achieved, individual fish were exposed to 240 V_{rms} 50 Hz AC for 0.5 to 1 s in a dry stunner (Stansas, Optimar, Norway). According to the study of van de Vis et al. (2023) on Atlantic salmon, the stun duration was set to 5 and 10 seconds to prevent recovery prior to the application of one of the two killing methods. After a 10 second stun in total, each individual lumpfish was placed in the cold brine or water supersaturated with CO_2 and followed until death was ensured by an isoflat line with EEG.

For stunning in water a commercial module from Askvik Akva, Hjelmeland, Norway. Conditions for stunning was in accordance with the findings of Foss et al. (2021), where lumpfish were placed in a tank containing seawater with titanium electrodes 30 cm apart placed in parallel to the fish, whereas the fish is in a 90° to the electric field. The electric output was pulsed 720V_{pp} pulsed AC at ≈48Hz with ≈1.6ms pulse length, thus a ≈15.5% duty cycle. The electric V_{rms} across the electrodes, calculated from the machines output values, was 4.645 V/cm. As previously described the first stun was set to 1 s in order to assess unconsciousness and then set to 9 s prior to exsanguinating the fish with a throat cut or placing it into brine.

3.2 EEG and ECG registrations

EEG and ECG registrations were carried out in individual lumpfish. The same procedure was repeated for each fish. No fish were re-used.

Immediately after the electrodes were placed, a baseline for both the EEG and ECG was measuredin fish held in a small tank of water. Then, each fixed fish was placed between the stunning electrodes and the current was applied head to tail. In case head to tail appeared not to result in an immediate loss of consciousness, head-first stunning was applied. Based on changes in the EEG and ECG, we determined if unconsciousness was indeed induced within 0.5 second. EEG and ECG registrations continued for at least 15 min after a maintenance stun followed by the application of one the two killing methods.

3.3 Voltage, temperature measurements

For measuring the potential difference across the brain. The EEG electrodes were placed in a fixed position 10 mm from centre to centre. Connected to the electrodes, in parallel to EEG equipment was an oscilloscope (PXI multimeters). During recording, the peak to peak electric potential difference (V_{pp}) was recorded within 0.5 sec. For temperature logging in brine, fish classed as dead were re-thawed to ambient temperature (from brine studies) or directly after CO2 experiments placed into the brine having temperature loggers placed into the neuro and heart cavity until passing freezing temperature for the carcass (below -3 °C).

3.3.1 Ethics

The experiment was approved by the Norwegian Food Safety Authority in accordance with application id FOTS 23/74319.

4 Results and Discussion

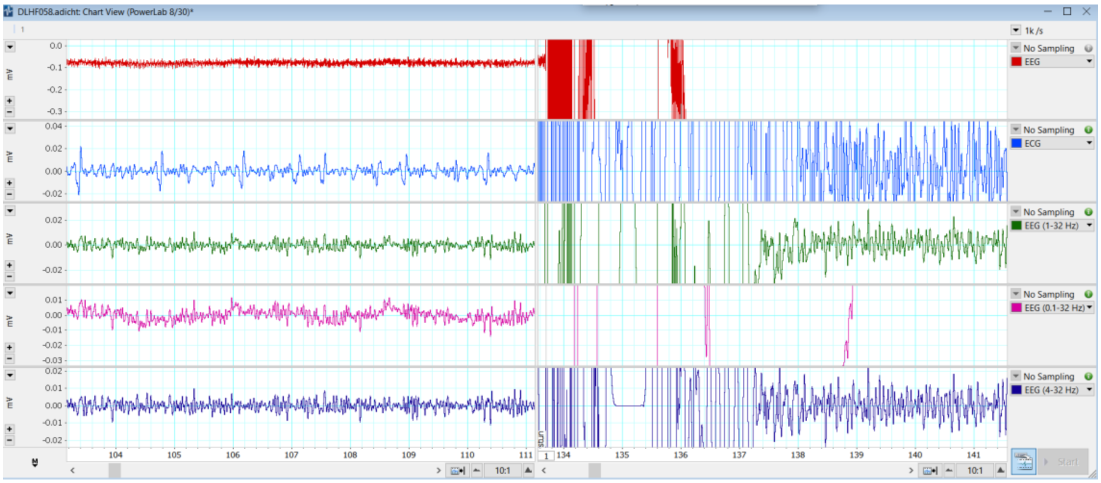
4.1 Dry electric stunning- immediate loss of consciousness

A preliminary evaluation of the EEG and ECG registrations in individual lumpfish shows that loss of consciousness can be induced within 0.5 second by applying 240V 50 Hz AC head-first exposure after dewatering (Figure 1A). Stunning the fish tail to head by applying 240V 50 Hz did not result an immediate loss of consciousness, thus head-first stunning was applied for all further experiments.

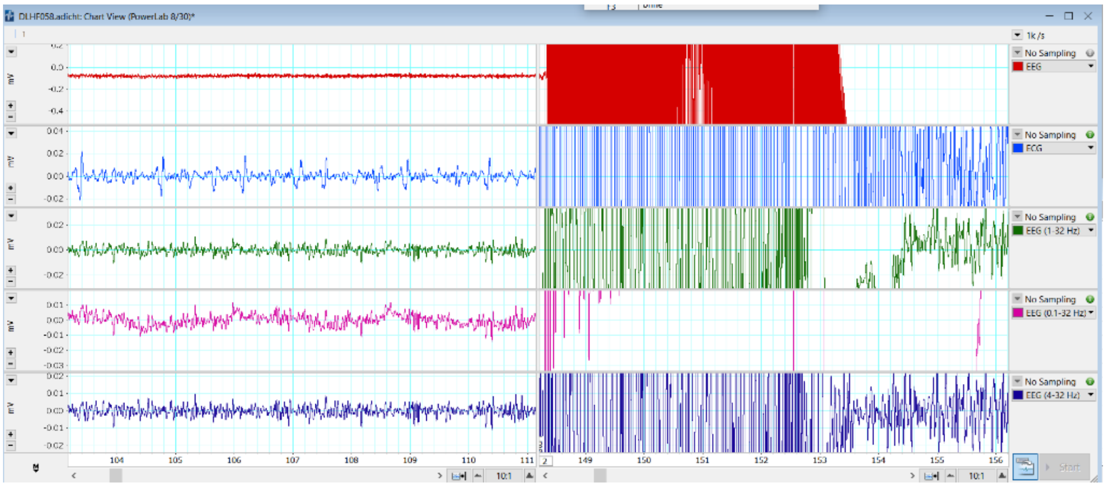
However, a 0.5 second application head-first was not feasible for all lumpfish used in our experiment, as a proper contact between the fish and the positive electrode was hindered by movement(s) of the individual fish. The movement(s) were probably caused by muscle contractions that resulted from the exposure to the electrical current. Obviously, these muscle contractions are unavoidable. We, therefore, recommend that the criterion for an immediate loss of consciousness in lumpfish should be that this achieved by exposing the fish head-first for one second to 220 V 50 Hz AC.

4.2 Dry electric stunning followed by immersion in a cold brine

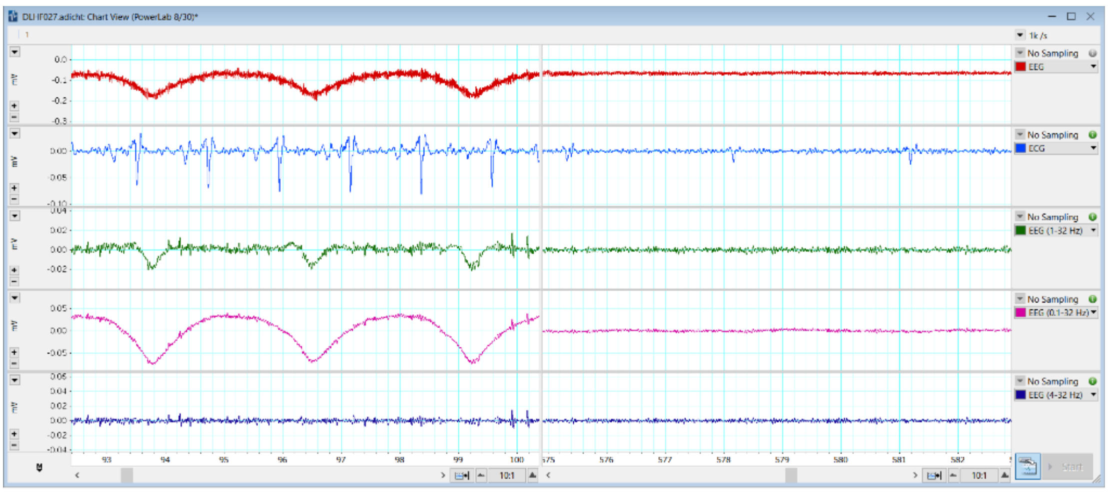
Loss of consciousness can be prolonged without recovery until death when a 5 second electric stun 240 V 50 Hz AC is followed by immersion of the unconscious lumpfish in a cold brine of –14-18 ºC (Figure 1C). When the 5 stun (Figure 1 B) is followed by immersion in the cold brine, recovery is prevented, and death occurs in the unconscious fish due to freezing of the brain.



1A: EEG and ECG in a conscious lumpfish (**left side only**) and the induced general epileptiform insult (unconscious fish) and fibrillation of the heart (**right side only**) after a 0.5 second stun 240 V 50 Hz ac



1B: EEG and ECG of a conscious lumpfish (**left side only**) and the induced general epileptiform insult (unconscious fish) and fibrillation of the heart (**right side only**) after a 5 second stun 240 V 50 Hz ac



1C: EEG and ECG in a conscious lumpfish (**left side**) and after a 5 stun 240 V 50 Hz ac followed by a 410 second exposure to the cold brine (**right side**).

Figure 1 Registration of EEG and ECG in lumpfish prior to dry electric stunning, thereafter and during immersion in the cold brine. In all these figures the red line represents the raw EEG data, the light blue line ECG data and the green line the EEG data (bandpass filter 1-32 Hz). The purple line represents filtered EEG data (bandpass filter 0.1-32 Hz) and the dark blue line shows the EEG data (bandpass filter 4-32 Hz).

4.3 Dry electric stunning followed by immersion in seawater saturated with carbon dioxide

A 5 second stun 240 V 50 Hz AC head-first followed by immersion in seawater saturated with carbon dioxide did most likely not result in complete loss of consciousness without recovery. Figure 2 shows that after 420 s exposure to seawater saturated with carbon dioxide, recovery may occur. Hence, we recommend using the cold brine as killing method for stunned lumpfish.

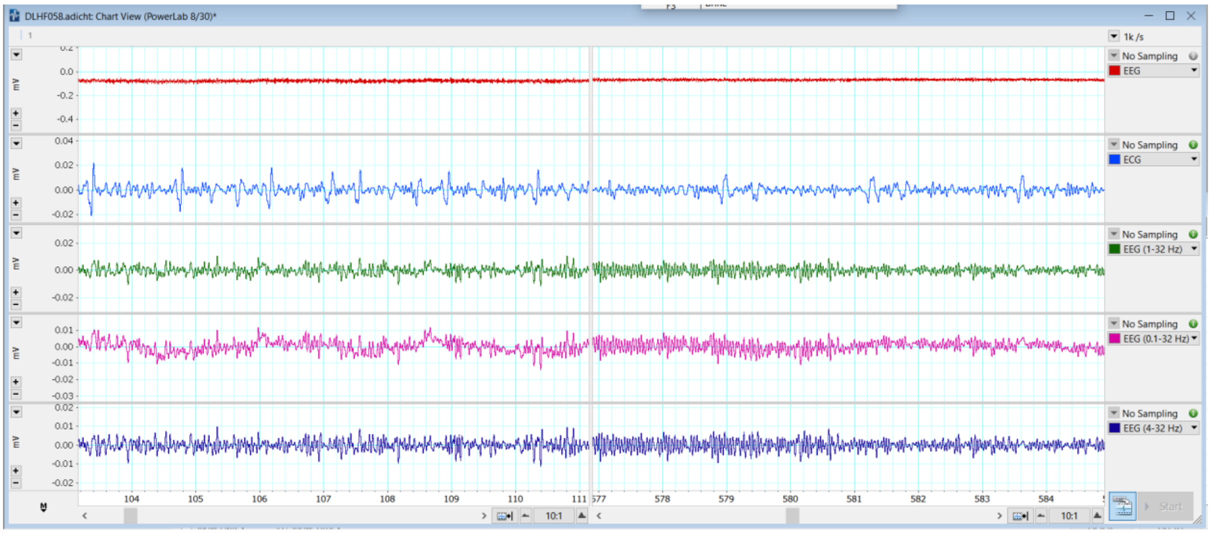


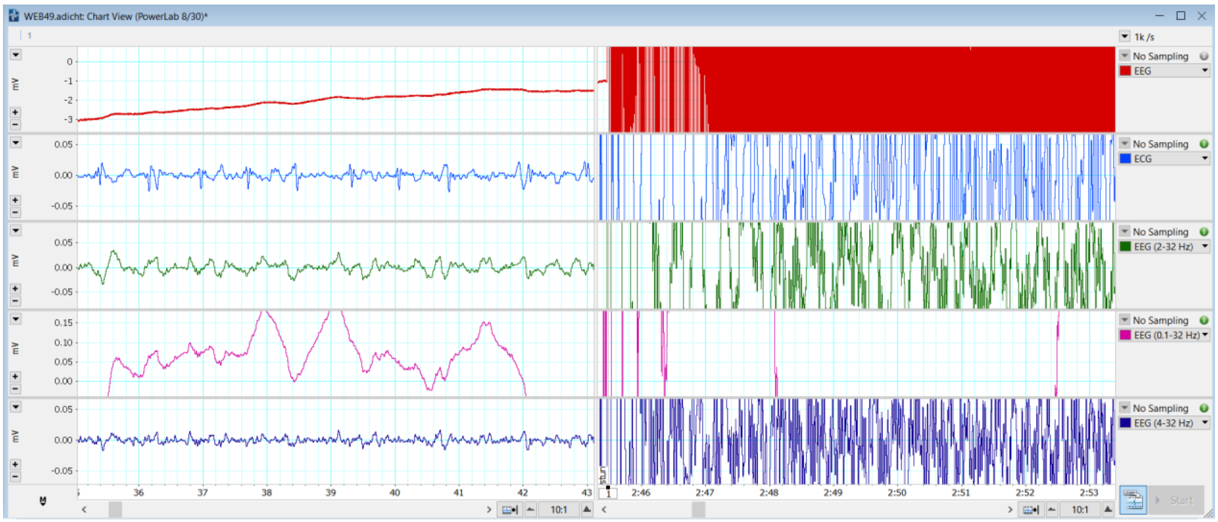
*Figure 2: EEG and ECG in a conscious lumpfish (**left side**) and after a 5 second stun 240 V 50 Hz ac followed by a 420 exposure to the cold brine (**right side**)*

4.4 Electric stunning in seawater - immediate loss of consciousness

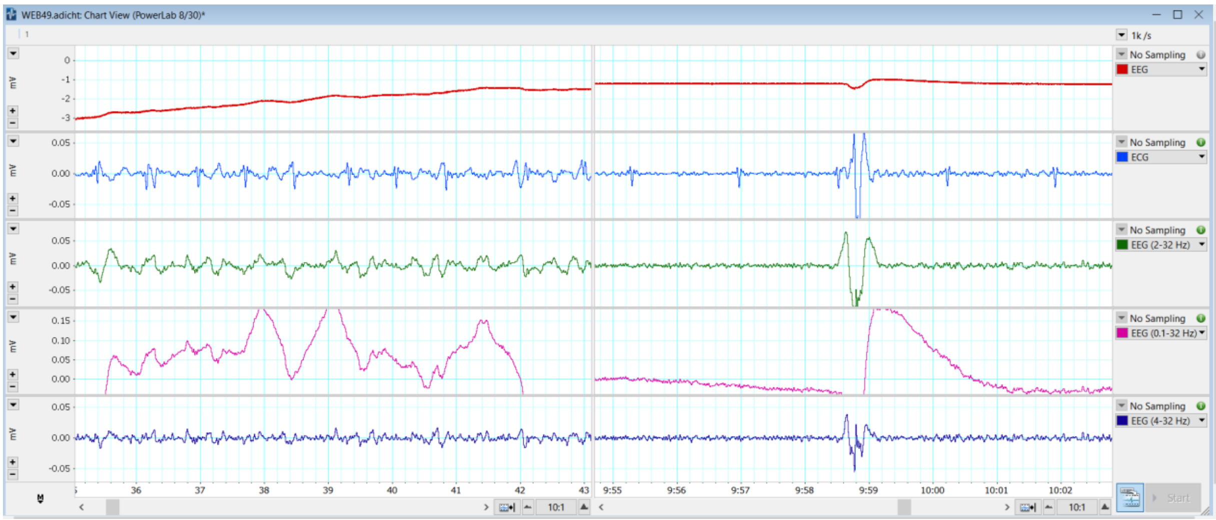
After a one second stun in seawater, the changes in the EEG and ECG patterns in lumpfish where similar to those observed after dry stunning (Figure 1). A field strength of 4.6 V_{rms}/cm had to be applied to pass sufficient electric current through an individual fish in seawater to induce an immediate loss of consciousness. Achieving a loss of consciousness in lumpfish by applying a 0.5 stun in seawater was not considered feasible by us, as this approach leads to extremely large increase in power source output. This increase leads to sharp and undesirable increase in the purchase costs of equipment for electric stunning of lumpfish in seawater.

4.5 Electric stunning in seawater followed by a throat cut

A 10 second stun (field strength of 4.6 V_{rms}/cm) followed by a cutting of the ventral aorta (i.e. a throat cut) did not result in loss of consciousness without recovery. Figure 3 shows that 7 min after the application of the throat cut, recovery may occur. We, therefore, recommend using the cold brine as killing method for lumpfish after stunning with electricity in seawater.



3A EEG and ECG of a conscious lumpfish (**left side only**) and the induced general epileptiform insult (unconscious fish) and fibrillation of the heart (**right side only**) after a 10 second stun (field strength of 4.6 V_{rms}/cm)



3B The **left-hand side** shows the EEG and ECG recordings in a conscious fish. The **right-hand side** shows the EEG and ECG recordings 7 min after the application of a 10 s stun (field strength of 4.6 V_{rms}/cm) and throat cut.

Figure 3 Registration of EEG and ECG in lumpfish prior to electric stunning in seawater, thereafter and during immersion in the cold brine. In all these figures the red line represents the raw EEG data, the light blue line ECG data and the green line the EEG data (bandpass filter 2-32 Hz). The purple line represents filtered EEG data (bandpass filter 0.1-32 Hz) and the dark blue line shows the EEG data (bandpass filter 4-32 Hz).

To summarise, the tests in a laboratory setting show that stunning and killing of lumpfish is in principle feasible when dry electric stunning or electric stunning in water is followed by immersion in a cold brine. We recommend performing tests in a commercial setting prior to implementing the recommended methods, as a limit of the time interval between fish leaving the stunner and the application of the killing method has not yet been established. Experience with other fish species show that this is essential to avoid recovery prior to the application of a killing method.

4.6 Electric potential difference across the brain

For stunning lumpfish in dry stunner with 240 V 50 Hz the average (SD) electric potential difference across the brain was 45 (23.7) V_{pp}/cm. As shown in Figure 4 the electric potential difference was significantly dependent of size (P<0.0001; R=0.44, n=67, linear regression) providing following equation:

$$V_{pp} = e^{(4.0746-0.000289*x)}$$

These results correspond with previous results on salmon (van de Vis et al. 2023), where the electric potential difference was a function of size, but unlike salmon, the variation in potential difference amongst lumpfish was very high variating from 17-181 V/cm. Reason seems to be contact resistance in the skin and good contact to the electrodes. Like previous studies on lumpfish (Foss et al. 2017) the lack of good contact and resistance causing the skin to burn and the electric potential can rapid increase or decrease or time. This effect becomes more obvious in waterbath stunning, where the lack of contact resistance resulted in a homogeneous electric field during the appliance period with a rather low variation (P<0.0001, F-test). As shown in Figure 4 the electric potential difference across the brain remained independent on size of the fish (P>0.18, Linear regression) while the fish was immersed in seawater with an average (SD) V_{pp} equal of 14 (6.9) V/cm. Since the fish remained in free water, the variation in reading in waterbath stunning could also be caused by small changes in the fishes position or angle to the electric field.

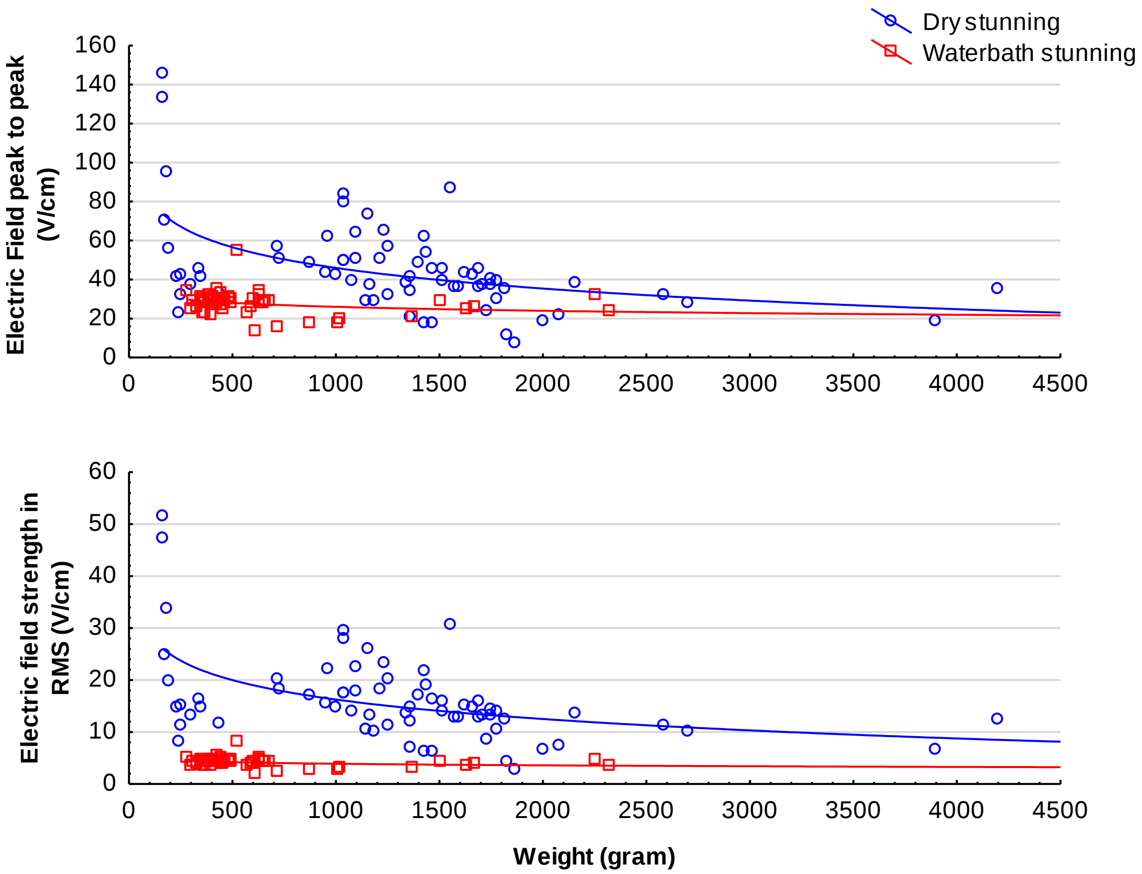


Figure 4 Potential difference across the brain as a function of size of lumpfish stunned in air or water.

4.7 Conclusion

The preliminary conclusion is that stunning Lumpfish with electricity in both dry or seawater will render the animal unconsciousness within 1 s. Increasing the current duration to 10 s and immersing unconscious fish into cold brine will result in permanent insensibility. The electric potential difference across the brain is negatively correlated with the size of the fish in dry stunners meaning it becomes more efficient thus in smaller animals. For waterbath stunning size is of minor influence.

4.8 Main findings

Exposing lumpfish 240 V_{rms} dry or 4.6 V_{rms}/cm in seawater will render lumpfish unconscious within 1 second.

Although behavior indicates unconsciousness within 0.5, this could not be verified by EEG due to lack of proper contact and noise.

Potential difference across the brain required to render the fish unconscious potential difference across the brain had the lowest average of 14 V_{pp}/cm.

After exposing the fish for 5 and 10 s of electricity it can be efficiently killed using cold brine or mince

Attempts to kill the lumpfish humanly with a throat cut or placing into CO_2 saturated water failed.

4.9 Acknowledgement

This study was funded by FHF, Elektrisk bedøving av rognkjeks av Fisk, project no. 901813. Also thanks to staff at Akvaplan for good support and maintaining our fish in a good way. Also thanks to Optimar and Askvik Aqua for technical support.

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