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Ontwikkelen van een welzijnsvriendelijke slachtmethode voor de Afrikaanse meerval (*Clarias gariepinus*)

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Samenvatting

Het onderzoek was gericht op een het ontwikkelen van een bedwelmingsmethode/ bedwelmingsmethodes voor gekweekte Afrikaanse meerval op laboratoriumschaal, waarbij onnodige stress kan worden vermeden en de vleeskwaliteit op zijn minst gelijkwaardig is aan die van de huidige industriële methoden, het levend onderkoelen. In samenwerking met verwerkers van de meervallen werd nagegaan in hoeverre de ontwikkelde laboratoriummethode/methodes geschikt is/zijn voor opschaling.
De huidige industriële methode, het onderkoelen van Afrikaanse meerval die nog bij bewustzijn is, leidt tot vermijdbare stress. Tijdens het onderkoelen konden er in de meervallen die bij bewustzijn waren, gedurende 4,7 minuten spierkrampen voorkomen en was er sprake van een extreem hoge hartslag (tussen 294 en 311 slagen per minuut). Deze onnodige stress kan worden vermeden door individuele meerval onmiddellijk en permanent te bedwelmen door lucht onder druk in de hersenen te injecteren met een naaldschietmasker, hetgeen bleek uit registratie van de hersen-, hartfilms en observatie van gedrag. Door toepassing van het naaldschietmasker wordt met een druk van 8 bar een naald in de hersenen ingeschoten, die onder een druk van 3 bar gedurende 1,5 s lucht in de hersenen injecteert.
Tijdens overleg met verwerkers van Afrikaanse meerval werd duidelijk dat hun voorkeur uitging naar elektrisch bedwelmen in combinatie met ontkoppen of 15 min onderkoelen in een trommel met scherfijs, omdat zij vonden dat beide procedures in principe geschikt waren voor verdere opschaling. Daarom zijn deze methodes verder onderzocht in het laboratorium en bij een bedrijf. Uit het meten van hersen-, hartfilms en het doen gedragsobservaties bleek dat met beide methodes de bewusteloosheid en gevoelloosheid onmiddellijk kon worden opgewekt zonder dat de vissen weer bijkwamen.
De condities voor het bedwelmen met stroom waren de volgende. De bedwelmingstank bestond uit een tank van plexiglas die was gevuld met leidingwater (500 µS geleidbaarheid). De plaatelektrode op de bodem en de andere net onder het wateroppervlak hadden ieder een oppervlak van 648 cm2. De afstand tussen de plaatelektrodes bedroeg 16 cm. De Afrikaanse meerval werd individueel in het water bedwelmd door gedurende 5 s een stroomdichtheid van 1,5 A/dm^2, 50 Hz a.c. te gebruiken. Het spanningsverschil tussen de plaatelektrodes was gemiddeld 301,9 $\pm$ 3,8 V. De vis werd vervolgens zo snel mogelijk uit het water gehaald, machinaal ontkopt of in het ijs geplaatst. Voor het ontkoppen en fileren werd de daartoe beschikbare apparatuur in het bedrijf gebruikt
Uit de verkregen resultaten blijkt dat de vleeskwaliteit die verkregen was door stroom in combinatie met ontkoppen of afkoelen toe te passen, gelijkwaardig was aan die van de industriële methode (onderkoelen gevolgd door strippen). Het fileerrendement en vochtverlies tijdens opslag van de filets werd niet beïnvloed door de gebruikte methode om de vissen te bedwelmen.
Tijdens de uitvoering van de experimenten werd duidelijk dat na toepassen van elektrisch bedwelmen in combinatie met ontkoppen de nog aanwezige slijmlaag op de ontkopte dieren op bezwaren stuitte bij de bedrijven. Om deze werd de voorkeur gegeven aan elektrisch bedwelmen in combinatie met onderkoelen. Het is hierbij essentieel dat de elektrisch bedwelmde vissen na 15 min onderkoelen in scherfijs of ijswater meteen worden verwerkt.

Executive summary

HEAD-ONLY ELECTRICAL STUNNING AND BLEEDING OF AFRICAN CATFISH (*Clarias gariepinus*); ASSESSMENT OF LOSS OF CONSCIOUNNESS

The overall objective was to evaluate the suitability of electrical stunning prior to bleeding of farmed African catfish as an alternative for live chilling in combination with gutting. The EEG (electro-encephalogram), and ECG (electro-cardiogram) recordings, observation of behaviour and responses to noxious stimuli were used to assess unconsciousness, insensibility and cardiac function in African catfish (1571 ± 362 g, 32 males and 26 females).

In the first experiment the minimum electrical current needed to induce a general epileptiform insult by head-only stunning was assessed. The individual catfish were fixed in a specially designed restrainer. The applied voltage of 150, 200 or 250, 300 or 350 V, 50 Hz, AC was delivered via scissor-model stunning tongs for approximately 1 s. A general epileptiform insult was observed in 31 catfish for which a successful EEG recording was obtained, using 362 ± 32 V, 629 ± 180 mA for 1.2 s. The duration of the tonic, the clonic and the exhaustion phase were 8 ± 3, 12 ± 7 and 7 ± 5 s on the EEG, respectively, where a distinct exhaustion was not clear in 11 fish. The total duration of the insult was 23 ± 8 s. After the insult the fish recovered. The heart rate was 63 ± 29 beats/minute prior to stunning. After stunning the ECG revealed extra systolae and was irregular. By using an average current of 629 ± 180 mA (at approx. 360 V, 50 Hz AC) at least 91% of the catfish are effectively stunned within a confidence level of 95%.

In the second experiment the behaviour of 10 individual catfish, which were able to move freely in water was observed following head-only stunning (370 V). The durations of the tonic, clonic and exhaustion phases in free swimming fishes were 11 ± 8, 20 ± 5 and 23 ± 20 s. All fishes recovered.

In the third experiment a first group of 7 catfish was head-only stunned followed by gill-cutting to kill them as a second procedure, i.e. after recovery from head-only stunning. No brain activity on the EEG occurred 12 ± 5 s after stunning. However, 2 fish showed responses to noxious stimuli after 2 and 5 min. A second group of 7 catfish was only gill-cut. They responded to noxious stimuli for at least 15 min. The blood loss was 1.2% and 1.0% of live weight for the first and second group, respectively.

It may be concluded from our results that African catfishes are effectively stunned for 23 ± 8 s with a current of 629 ± 180 mA for 1.2 s after which they recover. Since evoked responses may remain for at least 5 min after stunning and gill-cutting, it is recommended to optimise the stunning and killing procedure.

STUNNING OF FARMED AFRICAN CATFISH (*Clarias gariepinus*) BY USING CAPTIVE NEEDLE PISTOL; ASSESMENT OF WELFARE ASPECTS.

The objective of the study was to assess whether high-pressure injection of air in the brain of African catfish (*Clarias gariepinus*) could render the animal unconscious and insensible immediately and permanently. In the study, 48 African catfish with a live weight of 900 to 1900 g were restrained and equipped with EEG and ECG electrodes and then stunned. The catfish were stunned mechanically using a captive needle pistol. The pressure to shoot the needle was 8 bar and to inject the air at 3 bar for 1.5 s. Behaviour was observed during and after stunning.
The appearance of theta, delta waves and spikes, which precede a stoppage in brain activity as measured on the EEG, were used as indices for the measurement of immediate induction of unconsciousness and insensibility In 23 of 42 fish, an iso-electric line was observed after an

average of 13.4 s, while in the remaining fish the theta, delta waves and spikes remained on the EEG during the recording period. In all cases the ECG showed an irregular heart rate with fibrillation and extra systolae. Moreover, the configuration showed ischaemia.
Prior to captive needle stunning, free-swimming fish (n=7) explored the tank for an average of 21 ± 12 s before they layed down on the bottom. After stunning they showed clonic uncoordinated swimming movements. The movements stopped after an average of 38 ± 50 s. In another group (n=7) that was stunned and subsequently placed in ice water, clonic cramps were observed in two out of seven animals.
When taking into account the number of animals with a reliable EEG (n=42) and using 95% confidence intervals, it was concluded that at least 93% of the catfish were effectively stunned using a correctly positioned captive needle pistol. Furthermore, it is recommended to immobilize the stunned fish by chilling, as the post-stun clonic cramps may hinder gutting and filleting.

STUNNING OF AFRICAN CATFISH (*Clarias gariepinus*) BY CHILLING IN ICE WATER: ASSESSMENT OF LOSS OF CONSCIOUSNESS AND SENSIBILITY

The overall objective of the study was to evaluate an industrial slaughter method of African catfish, which consisted of chilling in ice water to immobilize and deslime followed by evisceration.
In the first experiment 28 individual catfish with an average live weight of 1738 ± 441 g and a body temperature of 22.0 ± 2.1°C were restrained and equipped with EEG and ECG electrodes. A temperature sensor was inserted in the body. Then they were placed one by one in ice water of 0.1 ± 0.5°C for 30 min to assess live chilling by measurement of brain and heart function. Indices for the induction of unconsciousness and insensibility were the appearance of theta and delta waves, no response to administered pain stimuli and low brain activity, which appeared after a median of 5 min, 12.5 min and 15 min, respectively. These appearances were observed at a body temperature of 17.3 ± 2.3°C, 13.7 ± 2.6°C and 13.3 ± 2.7°C, (n=22), respectively.
When taking into account the number of animals with a reliable EEG (n=22) and using 95% confidence intervals, it was concluded that at least 90% of the catfish were unconscious and insensible at a decrease in body temperature of approximately 8.7°C.
Live chilling of African catfish resulted in an extremely high heart rate (tachycardia). Values between 294 ± 47 and 311 ± 038 beats/minute (n=13) were measured. A vigorous response in behaviour of restrained catfish was observed in 9 fish when placed in ice water and during live chilling 10 fishes displayed vigorous movements.
In the second experiment 10 individual unrestrained African catfish with a live weight of 2422 ± 484 g were placed in ice water of - 0.1 ± 0.1°C. The observation of the catfish, which could move freely, revealed three phases. The animals were 1) swimming or moving in the ice water, 2) showing clonic muscle cramps, whilst being conscious, or 3) motionless. In the control experiment 7 individual catfish with a live weight of 1765 ± 178 g were placed in fresh water of 22.8 ± 1.3°C. They explored the tank for on average 21 ± 12 s before they lay down on the bottom of the tank.
The obtained results show that the catfish transferred to ice water became unconscious and insensible after a median of 12.5 min when the body temperature was decreased by approximately 8.7°C. They may become stressed by live chilling, as muscle cramps and a tachycardia during consciousness were observed.

FEASIBILITY OF ELECTRICAL STUNNING FOLLOWED BY DECAPITATION OR CHILLING OF AFRICAN CATFISH (*Clarias gariepinus*); ASSESSMENT OF BEHAVIOURAL AND NEURAL PARAMETERS AND PRODUCT QUALITY

The objective of the study was to assess effects of electrical stunning of farmed African catfish (*Clarias gariepinus*) followed by decapitation or chilling on neural and, behavioural responses. To assess the possibility of scaling up one or both experimental methods flesh quality was compared to live chilling under practical conditions.
After electrical stunning in combination with decapitation the fishes showed spikes alternated with theta and delta waves on the EEG, which was followed by minimal brain activity after 20 ± 10 s. The same characteristics were seen after electrical stunning in combination with chilling. Minimal brain activity occurred after 22 ± 11 s.

Within a confidence level of 95%, the percentage of African catfishes that was effectively stunned after administration applying of an electrical current of 1.5 A/dm2, 300 V (50 Hz a.c.) followed by decapitation or chilling is at least between 91% and 100%.
Analysis of weight, yield and drip moisture loss showed no specific differences between the groups. The overall values of the pH in the different groups showed a significant ($p<0.05$) decrease during the 48 hours post mortem. Afterwards there is a slight increase. No difference in brightness, redness and yellowness was observed between the different groups.
It is concluded that cat fish can be stunned effectively using electrical stunning in a water tank which is followed by decapitation or chilling in ice. Dutch industry prefers to combine electrical stunning with chilling in flake ice in a rotating tumbler, as the outer slime layer is then removed, which facilitates further processing.

1. Inleiding

In juni 2001 is tussen Ministerie van LNV, het Nederlands Instituut voor Visserijonderzoek (RIVO) en de Divisie Voeding van de Animal Sciences Group, Wageningen UR contact geweest over het bedwelmen van Afrikaanse meerval op bedrijfsschaal en de beleidsvragen van het Ministerie van LNV.
Het Ministerie van LNV maakte duidelijk dat het wenselijk is om een praktisch toepasbare methode voor het bedwelmen van Afrikaanse meerval *(Clarias gariepinus*) te ontwikkelen. Bij deze ontwikkeling zal gebruik worden gemaakt van de resultaten die zijn verkregen in het kader van het onderzoek “Ontwikkeling protocol met criteria voor beoordeling van bedwelmingsprocessen bij vissen” (Lambooij et al., 2001). In het kader van dit onderzoek zijn criteria vastgesteld voor het toetsen van bedwelmings-/dodingsmethoden voor de paling.

Doel
Op basis van deze criteria zal tijdens het voorgestelde onderzoek een optimale bedwelmingsmethode voor de Afrikaanse meerval worden geselecteerd. Het uitgangspunt voor deze selectie is dat voorafgaand aan het slachten bij een vis de bewusteloosheid en gevoelloosheid dient te worden opgewekt tot het dier dood is, zonder dat er sprake is van vermijdbare stress en er geen ongewenste neveneffecten optreden zoals beschadigingen aan of in het dier. Vervolgens wordt de geselecteerde methode verder onderzocht. Hierbij wordt nagegaan of de methode kan worden geautomatiseerd en of de methode veilig toepasbaar is. Uiteraard dient de methode aan wettelijke bepalingen te voldoen. In het kader van het voorgestelde onderzoek wordt geen apparaat gebouwd.

Het is wenselijk dat verwerkers van Afrikaanse meerval betrokken zijn bij de uitvoering van het onderzoek. Experimenten zullen in aanwezigheid van medewerkers van bedrijven en indien mogelijk ook bij bedrijven worden uitgevoerd. Tijdens het laatste overleg van 29 maart 2002 zijn er nadere afspraken gemaakt over de te verrichten werkzaamheden. Het ministerie van LNV gaf aan dat het onderzoek naar effecten van bedwelmen op de *post mortem* fysiologie geen prioriteit heeft.

2. Achtergrondinformatie

Sinds de laatste tien jaar wordt voedsel gezien als een totaalconcept en hiervan maken aspecten als milieu en dierenwelzijn ook deel van uit. Supermarkten gaan daarom in toenemende mate eisen stellen aan de wijze waarop de productieomstandigheden en het welzijn van gehouden vissen worden afgestemd (Anon, 2001; Cooke et al., 2001; Waitrose, 2003). In het kader van deze ontwikkeling is van het belang dat gehouden vissen eerst bewusteloos worden gemaakt voordat ze worden gedood. Door vissen eerst bewusteloos te maken worden ongewenste effecten op het welzijn voorkomen bij het slachten of doden.
In het belang van het welzijn van het dier zijn algemene uitgangspunten in de Europese Regelgeving (Council Directive 93/119/EC, 1993) beschreven die bij het toetsen van methoden worden gehanteerd. In de praktijk moet het bedwelmen, slachten en doden van dieren gefokt of gehouden voor de productie van vlees, huid, bont of andere producten voldoen aan het volgende criterium in de Europese regelgeving (Council Directive 93/119/EC, 1993): “Bij het verplaatsen, onderbrengen, fixeren, bedwelmen, slachten en doden moet ervoor worden gezorgd dat de dieren elke vermijdbare opwinding of pijn of elk vermijdbaar lijden wordt bespaard”.
De Gezondsheids- en Welzijnswet voor Dieren (Ministerie van LNV, 1992) is gebaseerd op de Europese Regelgeving**,** maar geldt niet voor vissen. De uitgangspunten zijn ook bruikbaar om bedwelmings- en dodingsmethoden van vissen te toetsen. Tijdens een inventariserende studie naar het doden van vissen in de praktijk zijn de uitgangspunten gebruikt (Van de Vis en Kestin, 1996).
Bedwelmen is gedefinieerd als: “Iedere methode die, bij toepassing op een dier, dit dier onmiddellijk brengt in een staat van bewusteloosheid die aanhoudt totdat de dood is ingetreden”. Onder doden verstaat men: “Iedere methode die resulteert in de dood van het dier”. Het slachten is omschreven als “het doden van een dier door verbloeding” (Council Directive nº 93/119, 1993). Slachten is ook gedefinieerd als doden met het oog op het te verkrijgen vlees. Binnen het bestek van dit rapport wordt de laatstgenoemde definitie van slachten gebruikt.

3. Resultaten en discussie

3.1. Head only electrical stunning and bleeding of African catfish (*Clarias gariepinus*); assessment of loss of consciousness

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Introduction

The present status of pre-slaughter and slaughter methods used at fish processors is leading to an increasing concern of governments, animal protection associations and consumers in Europe. The reason for the concern is that it has become clear that most industrial slaughter methods do not provoke unconsciousness in fish without avoidable stress prior to killing. These methods may, therefore, affect welfare of fish (Robb and Kestin, 2002; Van de Vis et al., 2003). The concept of animal welfare has gained acceptance for mammals. However, fish welfare is a relatively new concept. There is some evidence that the term pain is applicable to fish too, since results of anatomical, physiological and behavioural studies were very similar to those of studies performed on birds and mammals (Overmier & Hollis, 1990; Kestin, 1994; Verheijen & Flight, 1997; Wiepkema, 1997; Clarke & Squire, 1998; Nieuwenhuys et al., 1998). Contrary to these reported studies Rose (2002) stated that it is implausible that fishes can experience pain or emotions. Nevertheless, he (Rose, 2002) also reported “they display robust, non-conscious, neuro-endocrine, and physiological stress responses to noxious stimuli. Thus avoidance of potentially injurious stress responses is an important issue in considerations about the welfare of fishes.” In line with the reported studies and the changing public opinions, detrimental effects on welfare of fish at slaughter should be avoided. To achieve this, fish should be stunned (i.e. rendered unconscious) until death without avoidable stress, pain or discomfort and effects on product quality. Stunning should also result in sufficient immobility to facilitate the initiation of exsanguinations (Blackmore and Delany, 1988).
The current pre-slaughter process used in the Netherlands for African catfish (*Clarias gariepinus*) consists of chilling to immobilise them prior to evisceration (Robb and Kestin, 2002). It is unlikely that live chilling provokes unconsciousness in African catfish immediately or slowly and without avoidable stress, as it has been reported for Atlantic salmon (Skjervold et al., 2001), rainbow trout (Sneddon, 2002), eel (Lambooij et al., 2002) and gilthead seabream (Van de Vis et al., 2003) that this is stressful. An alternative method to provoke immediate loss of consciousness is electrical stunning, as its application induced an immediate loss of consciousness in Atlantic salmon, eel and gilthead seabream (Van de Vis et al., 2003).
Methods to assess stunning of fish have been optimised recently (Kestin et al., 2002; Van de Vis et al., 2003). The studies revealed that a sound assessment consists of registration of the EEG (electro encephalogram) and ECG (electro cardiogram) in fish in combination with the observation of behaviour.
Brain and heart activity can be measured by recording the electrical potentials (EEG and ECG, respectively). Recording of an EEG is necessary to determine whether an electric current has been sufficient to induce a general epileptiform insult indicating unconsciousness and insensibility (Wageneder & Schuy, 1967; Hoenderken, 1978). The absence of a somato-sensory evoked response (induced by e.g. a noxious stimulus) indicates a profound form of brain failure and provides an unequivocal diagnosis of insensibility following stunning (Gregory & Wotton, 1990). However, it should be noted that the presence of an evoked response implies that the afferent pathways to the higher brain centers are intact, but not necessarily whether the animal is aware of the stimulus.
During electrical stunning, the heart is affected, which is recordable on an ECG. The authors considered that the observation of behaviour alone is not sufficient for assessment of electro narcosis. If sufficient current is administered through the head of an animal a general epileptiform insult (all brain parts are stimulated) will occur. The epileptic process is characterised by rapid and extreme depolarisation of the membrane potential and there is heterogeneity of findings (Kooi et al, 1978). As measured on the EEG such an insult consists of relatively small waves increasing in

amplitude in the tonic phase, and decreasing in frequency in the clonic phase to result ultimately in a period of strong depression of electrical activity (Lambooy, 1982; Lambooy & Spanjaard, 1982). A human being is unconscious during the three phases of a general epileptiform insult. Moreover, the brain is in a stimulated condition and unable to respond to stimuli. By analogy, a mammal is supposed to be also unconscious and insensible (Lopes da Silva, 1983). The objectives of this study were therefore to determine the minimal current which should be passed through individual farmed African catfish to provoke an immediate loss of consciousness and to assess exsanguination with and without prior stunning of the fish to establish the time needed before unconsciousness is induced.

Materials and methods

Fish

A number of heads of African catfish were obtained and dissected to determine the position of the electrodes for measurement of the EEG.
Fifty-eight African catfish were used and obtained from a commercial farm. Three days before the experiment the animals were fasted and after that delivered at the laboratory. The catfish were placed in a tank containing aerated filtered tap water of 24 oC. The experiment was performed with approximately 10 animals per day. During the experiment the fishes were placed one by one in a special developed restrainer for registration of EEG and ECG or placed in the water to monitor the behaviour of freely moving fish. After the experiment the fish was weighed and dissected for gender.

Registration of EEG, ECG and behaviour of restrained African catfishes

Prior to stunning each individual fish was equipped with EEG electrodes. In order to facilitate the implantation of the electrodes the fish was restrained. The restrainer consisted of a platform (3 cm diameter) and a metal pallet to fix the head of the fish. Both of these were adjustable, as required by the size of the fish. The distance between the platform and a U-shaped steel grill (20 cm long) was also adjustable. The platform, pallet and U-shaped grill were mounted on a steel plate.
An individual African catfish was restrained by placing the lower jaw on the platform, inserting the metallic pallet in the mouth and subsequently the pallet was pressed down on the platform. A substantial part of the abdomen and most of the tail were fixed in place by the U-shaped grill. The various sizes of fish were accommodated by using plastic tiebacks, which held the fish in place in the grill.
Prior to implantation of the EEG and ECG electrodes the skin was locally anaesthetised using Xylocaine? 10% spray (lidocaine 100 mg/ml; Astra Pharmaceutica BV, Zoetermeer, Netherlands). Subsequently, holes were drilled in the skull. The silver spiked EEG electrodes (6 mm long and 1.5 mm in diameter) were positioned in the holes: one electrode 1 cm to the right and one electrode to the left of the sagittal suture and 4 cm caudal the imaginary line between the eyes. The earth electrode for the EEG and ECG was placed subcutaneous caudal to the dorsal fin. The steel ECG electrodes (35 mm long and 1 mm in diameter) were placed subcutaneous one caudal to the implantation of the left pectoral fin and one 2 cm caudal of the first electrode.
The EEG and ECG were recorded for 1 minute before and for 2 minutes immediately after stunning and for 30 s at 5 and 10 min after stunning. The recorder used was a DI-151RS serial port data-recording module with a WinDaq Waveform browser (Dataq Instruments, Akron, Ohio, USA). During the stunning itself, the EEG and ECG recordings were interrupted and the applied voltage and current were recorded using a Mingograf 34 (Elema Schönander, Stockholm, Sweden) recorder.
Responses to noxious stimuli (i.e. needle scratches applied to the skin of the tail) in the behaviour as well as on the EEG were assessed after 30 s, 2, 5 and 10 min after stunning. The EEG recordings were analysed for changes in the waveforms, frequency and suppression. The behaviour of the animals was monitored on video and assessed for the occurrence of tonic, clonic cramps, exhaustion and recovery.

Head-only electrical stunning

Determination of minimal current

Forty-one fishes were subjected to head-only electrical stunning for approximately 1s, by applying voltages ranging from 150 to 350V (50 Hz a.c.). The current was delivered by using scissor-model stunning tongs with steel electrodes. Each electrode consisted of a cylinder with a base diameter of 7.0 mm and a height of 5.5 mm equipped with a rim of 1.5 mm long spikes. The electrodes were placed on each side of the head between the eye and opening of the gill. The power supply (Stork RMS, Lichtenvoorde, Netherlands) delivered constant voltages at 150, 200, 250, 300 and 350 V (50 Hz a.c.). In addition, 4 catfish were stunned by applying 600 V (50 Hz a.c.) for approximately 1 s. to determine whether the period of loss of consciousness could be increased.
All but 7 catfishes were killed at the end of the experiment by a modified captive needle pistol (Lambooij et al., 1999). The 7 fish were allowed to recover as judged from the EEG and used in a second procedure to assess head-only stunning in combination with bleeding.

Behaviour of freely moving African catfish

The behaviour of 10 African catfish, which were able to move freely in fresh water, was observed after the application of head-only electrical stunning. Prior to the stun each individual fish was placed in a V-shaped device and restrained. The catfish was stunned with 350 V, as described previously. Immediately after stunning the individual fish was placed in a tank (100 x 50 x 60 cm) containing aerated tap water of 24oC. The behaviour of each animal was recorded on video and assessed afterwards for the occurrence of tonic, clonic cramps, exhaustion and recovery. When the experiment was finished the fish was killed with the modified captive needle pistol.

Exsanguination

Head-only stunning followed by gill-cutting

The 7 African catfish, which had been allowed to recover from head-only stunning for 20 minutes were used in this experiment. The fish were head-only stunned with 350 V (n=4) or 600 V (n=3) for 1 s. As soon as possible they were bled by gill-cutting using a pair of sharp scissors. The blood was collected and weighed after the experiment.

Gill-cutting

Another group of 7 catfish was restrained one by one, implanted with EEG and ECG electrodes and subsequently bled by gill-cutting. The registration of the EEG, EEG and observation of behaviour was the same as described in the previous sections.

Ethics

A Dutch governmental ethical committee approved the experiments beforehand.

Statistical analyses

Within a confidence limit of 95% taking into account the number of animals with a reliable EEG, the probability of an effective stun was calculated (Johnson & Kotz, 1969).

Results

Fish

The live weight of the African catfish was on average 1572 ± 362 g. It was observed that 32 were male and 26 female.

Head-only electrical stunning

Determination of minimal current

Head-only stunning by application of 150, 200, 250 and 300 V was performed to determine the minimal current for an instantaneous stun. For each voltage 1 catfish was used. The characteristics of a general epileptiform insult were not observed in these 4 catfish. Subsequently 37 African catfish were stunned with on average 362 ± 32 V, 629 ± 180 mA during 1.2 ± 0 s. The electrodes were disconnected in 6 out of 37 fish in this stunning procedure. The successful ones showed the characteristics of a general epileptiform insult on the EEG (Figure 1). The tonic, the clonic phase and the exhaustion phase were 8 ± 3, 12 ± 7 and 7 ± 5 s on the EEG, respectively. A distinct exhaustion phase was not clear for 11 fish. The total duration of the insult was 23 ± 8 s. Observation of behaviour of the restrained catfish revealed only clonic cramps of the tail. The duration was on average 18 ± 6 s. Using the number of reliable EEGs obtained (n=31) and a confidence limit of 95%, the chance of effectively stunning African catfish was determined to be between 0.91 and 1.00 when a current of 629 mA (50 Hz AC) is applied to the head.
The heart rate was 63 ± 14 beats/minute prior to stunning. After stunning the ECG revealed fibrillation (Figure 1) for 18 ± 9 s. The heart rates measured at 0.5, 2, 5, and 10 minutes after stunning were 70 ± 19, 71 ± 19, 66 ± 16 and 66 ± 16 beats/minute, respectively. The ECG showed extra systolae in 10 fishes and was irregular in 13 fish after stunning.
The current flow was 1235 ± 360 mA for 1.2 s in the 4 fish stunned with 600 V. The duration of the epileptiform insult was 58 ± 22 s and the brain activity was depressed afterwards. The ECG showed extra systolae and an irregular heart rate.

Behaviour of freely moving African catfish

The 10 catfish were placed immediately in water after electrical stunning with 673 ± 184 mA (~ 370 V) for 1.2 s. Three phases were distinguished: tonic and clonic cramps in a horizontal area followed by tonic and clonic cramps in a horizontal and vertical area and subsequently an exhaustion phase was observed. The recovery was defined as swimming smoothly forward or drooping the body in the water. The duration of the 3 successive phases were 11 ± 8 s, 20 ± 5 s and 23 ± 20 s, respectively. The total duration of the observed general epileptiform insult was 51 ± 20 s.

Exsanguination

Head-only stunning followed by gill-cutting

After recovery from head-only stunning for 20 min the group of 7 catfish were head-only stunned followed by gill-cutting to kill them. The applied current was on average 651 ± 272 (n=4) and 1327 ± 232 (n=3) mA for 1.2 s using a voltage of 350 and 600 V, respectively. The cut was performed as soon as possible. Following the occurrence of a general epileptiform insult no brain activity occurred after 12 ± 5 s. However, 2 fish, stunned with 350 V, responded to noxious stimuli, one after 5 min and one after 2 and 5 min. The heart rate was 60 ± 11 beats/min before the cut and 0.5, 2, 5 and 10 min after that 65 ± 29, 60 ± 17, 57 ± 21 and 50 ± 26 beats/min, respectively. The pattern was ischaemic in 5 out of 7 fishes and irregular in all of them. The blood loss was 17 ± 2 g, which was 1.2% of the live weight.

Gill-cutting

The EEG pattern became depressed approximately 2 to 5 min after the cut. Nevertheless, the responses to noxious stimuli could be recorded for at least 15 min after gill-cutting in all fish. After this period they were killed by captive needle pistol. The heart rate was before and 0.5, 2, 5, 10 and 15 min after the cut 82 ± 11, 84 ±23, 98 ± 24, 90 ± 12, 98 ± 30 and 80 ± 8 beats/min, respectively. The heart rate was irregular. The blood loss was on average 10 ± 2 g, which was 1.0% of the live weight.

Discussion

Interest in sustainable farming of fish has emerged due to the problems associated with intensive livestock production, the increasing imbalance between amounts of fish caught (limited by EU quota) on one hand and an increasing consumer demand for a variety of high quality fish on the other hand. Based on the experience with fish farming in the recent past a number of potential problems can be indicated. From a consumer perspective these are the perceived problems with residues from, for instance, antibiotics and animal welfare aspects associated with housing systems and slaughter. From a producer's point of view, the disease susceptibility of farmed fish forms a serious threat to sustainable farming. Animal welfare affects acceptance by consumers in two ways. The first one concerns the emotional aspects of farming, stunning (or lack thereof), and slaughter. The second one is that the farming and slaughter practices may have a major impact on the sensory quality of fish. A slaughter method is considered to be humane when unconsciousness is induced immediately or without avoidable stress, pain, and discomfort prior to killing and lasts until death.
If sufficient current is administered through the head of an animal a general epileptiform insult (all brain parts are stimulated) will occur. As measured on the EEG such an insult consists of relatively small waves increasing in amplitude in the tonic phase, and decreasing in frequency in the clonic phase to result ultimately in a period of strong depression of electrical activity (Lambooy, 1982; Lambooy & Spanjaard, 1982). The same characteristics were observed in African catfish, which indicates that the fish was unconscious during the general epileptiform insult. A minimum current, which depends on the impedance of the body, is necessary for the occurrence of such an insult. In head-only stunning studies of fish, the minimum current has been determined as 500 mA for rainbow trout (Kestin et al, 1995) and 545 mA for eel (Lambooij et al, 2002). Within a confidence level of 95% at least 91% of the African catfishes are effectively stunned by applying 629 mA (at approx. 370 V, 50 Hz AC).
Observation of behaviour of sheep after stunning revealed extension of the muscles and tonic spasms succeeded by clonic spasms eventually followed by exhaustion (Lambooy, 1982). In mammals the extensors are stronger than the flexors, which caused the extension. Two phases were distinguished in eel: limited tonic and clonic cramps combined with much backward swimming were followed by heavy clonic cramps combined with uncoordinated movements such as jumping out of the water (Lambooij et al, 2002), whereas in African catfish cramps in a horizontal and vertical area were observed. The most common type of swimming consists of traveling waves of bending with increasing amplitude towards the tail as found in the majority of fish. For carp it has been reported that white muscle tissue has a high contraction velocity and produce a high power output, however, only for a short period of time (10 to 60 s). It uses glycogen, which is broken down anaerobically. The red muscle fibres contract 3 times slower, have anaerobic metabolism using lipids, and are practically infatiguable (Spierts, 1999). The observed behaviour during a general epileptiform insult is for 51 ± 20 s. This suggests that most muscles involved have white muscle fibres, which become fatigued during the process. After recovery smooth swimming is observed, for which red muscle tissue may be used.
Several studies in which neurotransmitters have been measured and combined with pharmacological experiments suggest that the general epileptiform insult induced in mammals by an electrical stun is dependent on the release of vasopressin, oxytocin, glutamate, aspartate and GABA (gamma amino-4-butyric acid) (Lambooy et al, 1985; Cook et al, 1995, 1996). Combining head-only stunning with exsanguination has a synergistic effect on the release of glutamate and aspartate, which increases the duration of unconsciousness. Sticking following a stun should be carried out as quickly as possible when using head-only stunning, as it takes time, depending on the species before brain responsiveness is lost as a result of sticking (Cook et al, 1996). The combination of head-only stunning and gill-cutting affected the possibility of recovery and the EEG pattern, which revealed loss of brain activity after 12 ± 5 s. Two fish, however, respond to a noxious stimulus. A positive response implies an intact afferent pathway to the higher brain centers, where a negative response provides an unequivocal diagnosis of insensibility following stunning (Gregory & Wotton, 1990). Gill-cutting without a previous head-only stun did not result in a delete of evoked responses for at least 15 min. The long period may be due to the limited blood loss of approximately 1%. The loss of blood is affected mostly by the method of sticking, muscle contraction and gravity (Wariss & Wilkins, 1987), which were not optimal during our experiments with African catfish. It is assumed that the blood volume for rainbow trout (*Oncorhynchus mykiss*)

is 5% of the body weight, where 66% of it is located in the white muscle. The slow exchange of blood between parts of the vascular system may also reduce the loss of the blood due to gill-cutting. In addition it is known that adrenalin and nor-adrenalin dilate the vessels and decrease the resistance to flow through the gills (Hoar & Randall, 1970). Due to the handling these hormones may have been increased in African catfish. It is therefore possible that due to dilatation of the vessels only blood, which is present in the gills or in the vessels close to them, is obtained by gill-cutting.

Conclusions

It can be concluded from our results that using a confidence limit of 95%, the chance of effectively stunning African catfishes was determined to be between 0.91 and 1.00 when a current of 629 mA (~370V, 50 Hz AC) is applied to the head. All catfishes recovered after the stunning process. Head-only stunning followed by gill-cutting resulted for two out seven fishes in responses to noxious stimuli on the EEG up to 5 min, which implies that afferent pathways to higher centers of the brain are still in tact. In previous studies with electrical stunning of farmed eel we showed that this can be avoided and therefore optimisation of electrical stunning and killing for humane slaughter of African catfish is the subject of a future study.

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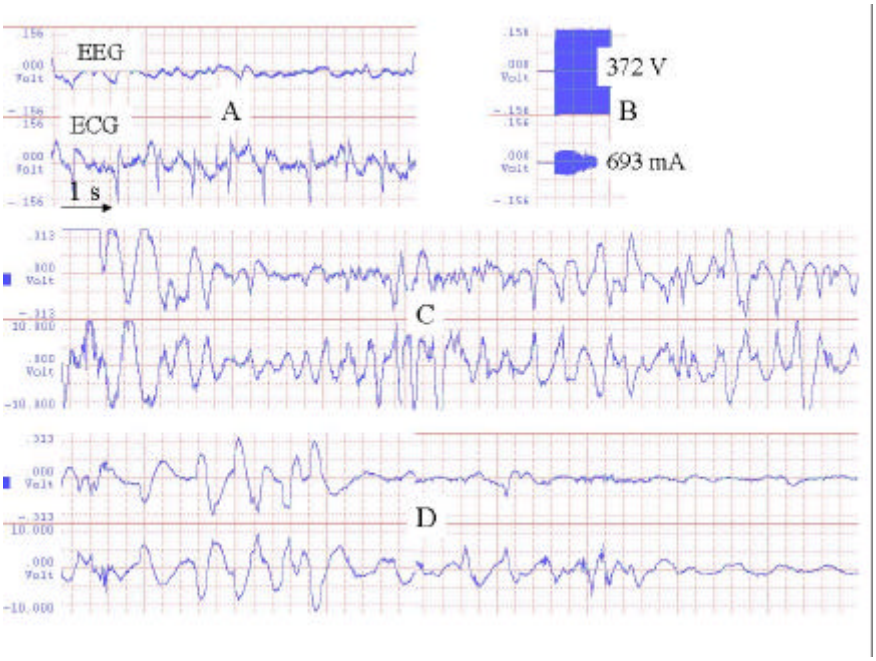

Figure 1 A EEG and ECG traces before stunning
B Voltage and current applied
C Tonic/clonic phase on the EEG and heart fibrillation on the ECG
D Clonic phase followed by exhaustion on the EEG and fibrillation followed by recovery on the ECG

3.2 Stunning of farmed African catfish (*Clarias gariepinus*) by using captive needle pistol; assessment of welfare aspects

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Introduction

Fish belonging to the suborder of Clariidae are found in Africa and Asia. African catfish (*Clarias gariepinus*), which can be farmed at high stocking densities, is a member of this group. This fish is well adapted to the wet and dry extremes of its habitat. Its skin is tight and smooth without visible scales. The gills are wide and have a deep indentation with a strong operculum. The jaw extremity terminates in a special skin fold, by which the fish can be attached when processing, the so-called "catfish grip". The telencephalon of the central nervous system is bulged in a tractus olfactorius, which ends in the bulbus olfactorius. The mesencephalon with the tectum opticum, which contains sensible and motoric elements, is located caudal to the diencephalon (Nieuwenhuys et al, 1998).
Harvest is the sequence of events in aquaculture whereby a living animal is processed into an edible product. Improper processes can adversely impact both product quality and animal welfare. The concept of animal welfare, which is generally accepted for mammals, is a relatively new concept for fish. It is likely that conventional harvest adversely affects the welfare of fish particularly when the similarities in basic structure of neurones and neuronal biochemistry to that of mammals, and the similarities in stress responses and behaviour to that of higher vertebrates are considered. (Overmier and Hollis, 1990; Kestin, 1994; Chervova, 1997; Wiepkema, 1997). Contrary to these studies, Rose (2002) stated that it is implausible that fish can experience pain or emotions. Nevertheless, he also reported "They display robust, non-conscious, neuro-endocrine, and physiological stress responses to noxious stimuli. Thus avoidance of potentially injurious stress responses is an important issue in considerations about the welfare of fish." Fish, including African catfish, should therefore be rendered unconscious and insensible instantaneously and permanently prior to slaughter as are warm-blooded slaughter animals.
According to the Council Directive for the protection of animals at slaughter (1993) the animals have to be rendered unconscious until death without avoidable pain, suffering, stress or discomfort. In the case of fish, however, no methods are prescribed and only general provisions apply. Prescribed methods for stunning of warm-blooded animals can be grouped into mechanical, electrical and chemical methods.
Research on development and optimisation of these methods for stunning of warm-blooded animals has been carried out since the beginning of the 20th century. It was observed that concussion stunning was not satisfactory, from a humanitarian point of view, and the need for improved stunning methods became obvious. Stunning by captive bolt was introduced at the end of the 19th century (Breidert, 1902), electrical stunning at the end of the 1920's (Raschke, 1928) and CO_2-gas stunning in the 1950's (Wernberg, 1957).
Contrary to the research with warm-blooded animals, development and optimisation of stunning methods for fish has started only recently. In an overview (Robb and Kestin, 2002) evidence suggests that in most cases methods applied for stunning are stressful. For African catfish, live chilling is applied prior to gutting. The authors suggest that live chilling is stressful and therefore alternative methods should be investigated. Lambooij et al. (2002) showed that high-pressure injection of air in the brain, using a captive needle pistol, resulted in an immediate and permanent loss of consciousness in eel. The conclusion for eel was based on registration of electrical activity in the brains (EEG) and heart (ECG) combined with behavioural observations.
The objective of this study was therefore to evaluate high-pressure injection of air in the brains as a pre-slaughter stunning method for African catfish. The combination with post stun chilling was also investigated.

Materials and methods

Fish

Heads of African catfish (n=5) were dissected to determine the position of the electrodes for measurement of the EEG and position of the captive needle pistol (Figure 1).
Seven days before the experiment sixty-three African catfishes were fasted and after that delivered to the laboratory. The catfishes were kept in a tank containing aerated tap water at 24°C. The experiment was performed with approximately 10 animals per day. After the experiment the fishes were weighed and dissected to determine the sex.
The average weight of the African catfish was 1083 ± 125 g (n=21), and 1688 ± 264 g (n=21) for captive needle stunning as first and second stunning method, respectively. There were 8 males and 13 females in the first stunning and 15 males and 6 females in the second one.
The average weight of fish, which were allowed to move freely, was 1793 ± 168 g. The group consisted of 4 males and 3 females. The group with animals, which were placed in ice water after stunning had an average weight of 1704 ± 273 g. This one consisted of 4 males and 3 females.

Registration of EEG and ECG and behaviour of restrained African catfishes

Prior to stunning each individual fish was equipped with EEG electrodes. In order to facilitate the implantation of the electrodes the fish was restrained. The restrainer consisted of a steel plate on which a platform, a pallet and an U-shaped grill were mounted. The platform (3 cm diameter) and a metal pallet were used to fix the head of the fish. Both of these were adjustable, as required by the size of the fish. The distance between the platform and the U-shaped grill (20 cm long) was also adjustable. An individual African catfish was restrained by placing the lower jaw on the platform, inserting the metallic pallet in the mouth and subsequently the pallet was pressed down on the platform. A substantial part of the abdomen and most of the tail were fixed in place by a U-shaped grill. The various sizes of fish were accommodated by using plastic tiebacks, which held the fish in place in the grill.
Prior to implantation of the electrodes each fish was locally anaesthetised using Xylocaine? 10% spray (lidocaine 100 mg/ml; Astra Pharmaceutica BV, Zoetermeer, Netherlands). For implantation of EEG electrodes two holes were drilled in the skull. The silver spiked EEG electrodes (6 mm long and 1.5 mm in diameter) were placed in the holes: one electrode 1 cm to the right and one electrode 1 cm to the left of the sagittal suture and 4 cm caudal of the imaginary line between the eyes. The earth electrode for the EEG was placed subcutaneous caudal of the dorsal fin. The steel ECG electrodes (35 mm long and 1 mm in diameter) were placed subcutaneous one caudal of the implantation of the left pectoral fin and one 2 cm caudal of the first electrode. The earth electrode for both the EEG and ECG was placed subcutaneous caudal of the dorsal fin. The EEG and ECG were recorded from 1 minute before until 2 minutes immediately after stunning and for 30 s after 5 and 10 min after stunning. The recorder used was a DI-151RS serial port data-recording module with a WinDaq Waveform browser (Dataq Instruments, Akron, Ohio, USA). Responses to pain stimuli (i.e. needle scratches applied to the skin of the tail) in the behaviour as well as on the EEG were monitored after 30 s, 2, 5 and 10 min after stunning. The EEG and ECG recordings were analysed for changes in the waveforms, frequency and suppression afterwards. The behaviour of the animals was monitored for the occurrence of tonic, clonic cramps, exhaustion and recovery.

Captive needle stunning

The captive needle pistol was adapted to the African catfish with regard to the length and shape of the needle. Only one cone shaped needle of 16 mm was used, by which pressed air in 3 directions radial 120°C was injected into the brains. One direction was directed caudally to the spinal cord (Figure 1).
First stunning method: For 22 fish captive needle stunning was applied as first stunning method. The fish were stunned mechanically by captive needle pistol using a shooting pressure of 8 bar and an air injection of 3 bar during 1.5 s, similar to the method described by Lambooij et al., (2002) for eel.
Second stunning method: Another group of 26 fishes as second stunning method had been subjected to head-only electrical stunning for 1s, 350V (50 Hz AC) as part of another series of experiments, prior to the captive needle stunning. The fish were left for approximately 20 minutes

to establish whether the animals could recover. The recovery was established on basis of the EEG traces.

Registration of behaviour of free-swimming African catfishes

Seven fish were placed in a tank with water (24°C) to swim freely for 10 min. Subsequently they were stunned using the captive needle pistol and after that placed in the water. Another group of 7 fish were placed in ice water after captive needle stunning.
The behaviour of the animals was monitored on video and analysed for:
- Swimming: The fish swims around in the water mostly along the wall and may explore its environment.
- Lying on the bottom: the fish breathes but shows no other movements. It lies on its abdominal side on the bottom.
- Collisions with the walls of the tank: The fish bombs its nose to a wall or corner.
- Motionless in the water: The body of the fish is slightly bent, resulting in an elevated head. An U-shape may be present.

Ethics

The experiments were approved beforehand by a governmental ethical committee.

Statistical analyses

The obtained data were analysed to determine the probability for an effective stun at 95 % lower confidence limit. A 95% confidence limit on the probability for an effective stun can be obtained by means of a well-known relationship with the beta distribution (Johnson & Kotz, 1969).

Results

Registration of EEG and behaviour of restrained African catfish

The electrodes became disconnected in 1 and 5 fish respectively in the two stunning procedures used. This was possibly a result of positioning the captive needle pistol, shooting of the needle in the skull, the injection of the air or a combination thereof. Due to positioning of the pistol and the subsequent air injection, artefacts were seen briefly on the EEG. The artefacts were followed by theta and delta waves (4–8 Hz and <4 Hz, respectively) and spikes, which decreased to nihil (i.e. no brain activity) in 12 fishes after 11 ± 9 s and in 11 fishes 16 ± 17 s respectively, for the two stun methods used. In the other animals, theta and delta waves and spikes continued during the recording period. An example of an EEG is presented in Figure 2. The fish had slow muscle cramps for 2 ± 3 s and 6 ± 10 s after application of the respective stunning methods. No responses to pain stimuli on the EEG and in the behaviour were observed.
Within a confidence limit of 95 % and taking into account the number of animals with a reliable EEG (n=42), the chance on an effective stun for African catfish with a live weight of 900 to 1900 g lies between 0.93 and 1.00.

Registration of ECG of restrained African catfish

The heart rate before stunning was 71 ± 19 and 58 ± 16 beats/minute for the first and second stun groups, respectively. The ECG showed fibrillation immediately after stunning in 9 fish in the first and in 3 fish in the second stunning method used. All other fish showed an irregular heart rate with multi-focal extra systolae. The configuration was ischaemic (ST-deviation and sometimes inversion of the T-top; Figure 2). The heart rates measured at 0.5, 2, 5 and 10 minutes after stunning in the first procedure were 60 ±22, 68 ± 25, 62 ± 27 and 53 ± 30 beats / minute and 51 ± 17, 53 ± 12, 49 ± 15 and 48 ± 13 respectively, following stunning in the second procedure. An example of such a record is presented in Figure 2.

Registration of behaviour of free swimming African catfish

Prior to stunning, the fish were swimming normally and explored their environment. The fish sometimes touched the wall of the tank and raised their heads out of the water in an attempt to escape. They laid down on the bottom after 21 ± 12 s. The water temperature was 22.8 ± 1.3°C.

After captive needle stunning, the fish were unable to swim normally, sometimes bending in a U-shape or colliding with the walls of the tank. Movements stopped after 38 ± 50 s and the fish remained motionless in the water. Severe but temporary convulsions occurred, however, when they were grasped by hand. They could afterwards be handled without a visible response.
When placed one by one in ice water after stunning, movements were not observed for 5 of the 7 animals tested. The other 2 had clonic cramps for 3 and 63 s .

Discussion

Captive bolt stunning applied to the head has previously been investigated with the aid of EEG in sheep and calves (Freeseman 1975; Gross, 1976; Lambooij, 1982). Conclusions were that captive bolt stunning almost certainly eliminates perception of pain based on major changes on the EEG (occurrence of delta and theta waves, < 4 Hz and 4 – 8 Hz, respectively). Percussive stunning may also induce immediate unconsciousness and insensibility. In contrast, a study with Atlantic salmon revealed that brain function ceased 0.3 min after application of the blow (Robb et al., 2000). Similarities in basic structure of neurones and neuronal biochemistry (Overmier and Hollis, 1990; Kestin, 1994; Verheijen and Flight, 1997) supports the assumption that catfish can be rendered unconscious and insensible based on analogy with EEG changes in humans and other vertebrates (Lopes da Silva, 1983). Major changes on the EEG combined with a lack of response to pain stimuli led to the conclusion that broilers, ostriches and eels could be rendered unconscious immediately following stunning with the captive needle pistol (Lambooij et al, 1999a; Lambooij et al, 1999b; Lambooij et al, 2002). In this study, the African catfish tested showed the same phenomena after stunning. However, brain activity remained in several fish during the recording period of 10 min but these showed no response to pain stimuli.
It is likely that the compressed air administered through the captive needle pistol results in damage of the brains by laceration of higher brain regions, leading to unconsciousness and insensibility. The flow in the caudal direction causes laceration of the upper spinal cord, which may prevent post stun convulsions (Lambooij et al, 2002). Contrary to warm-blooded animals, the spinal cord in African catfish controls a major part of the coordinated movements. A decapitated African catfish can make swimming movements under special stimuli (Spierts, 1999). This could explain why some of the catfish stunned in our experiment were able to make swimming movements when placed in water. The swimming movements were, however, uncoordinated and they could not remain in equilibrium. In addition, the animals responded to manual handling with temporary but severe convulsions. Severe convulsions, previously observed in some eels, might be related to a sub-optimal positioning of the pistol consequently inflicting less damage to the upper spinal cord. Muscle contractions may appear in eels as well as in broilers following captive needle stunning (Lambooij et al, 1999a; Lambooij et al 2002). However, no post-stun convulsions were observed in Atlantic salmon after percussive stunning (Robb et al., 2000). Stunning by captive needle pistol did not by itself provoke sufficient immobility to facilitate slaughter of the fish. At lower body temperatures, however, muscle activity is significantly reduced making it possible to gut or bleed. Therefore, chilling the fish between stunning and slaughter is recommended. .

Conclusion

We conclude that within a confidence level of 95%, at least 93% of African catfish are effectively stunned by a correctly positioned captive needle pistol using a shooting pressure of 8 bar and an air injection of 3 bar for 1.5 seconds. After stunning, fish retain some ability to make swimming movements. Chilling the animals after captive bold needle stunning facilitates bleeding or gutting through immobilisation of the muscles.

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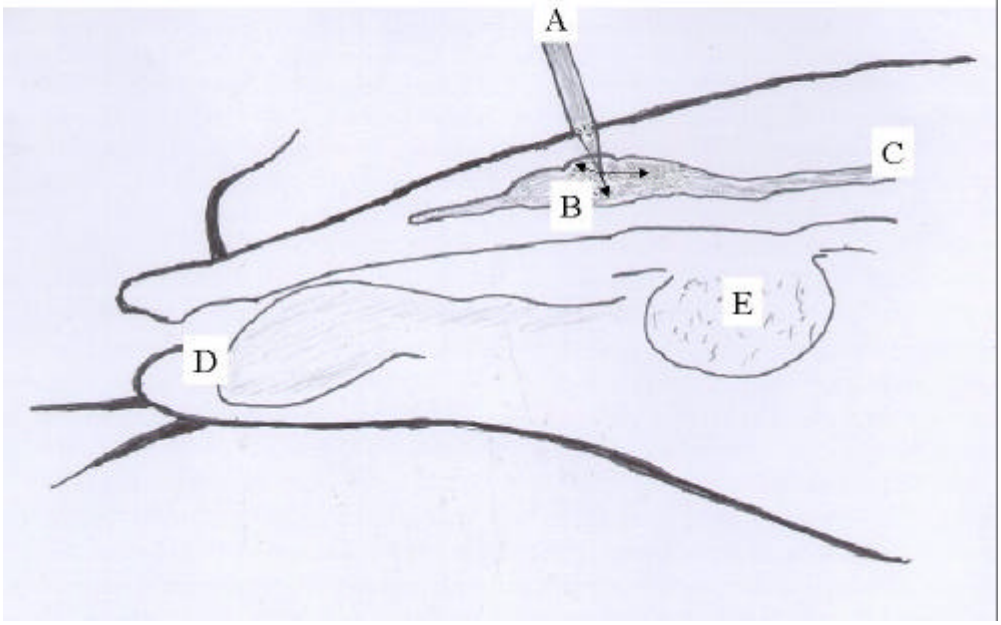

Figure 1 Schematic medial suture of the head of an African catfish for determination of the position of a captive needle pistol. A) captive needle, B) central nerve system, C) spinal cord, D) skin bag, E) branchial chamber.

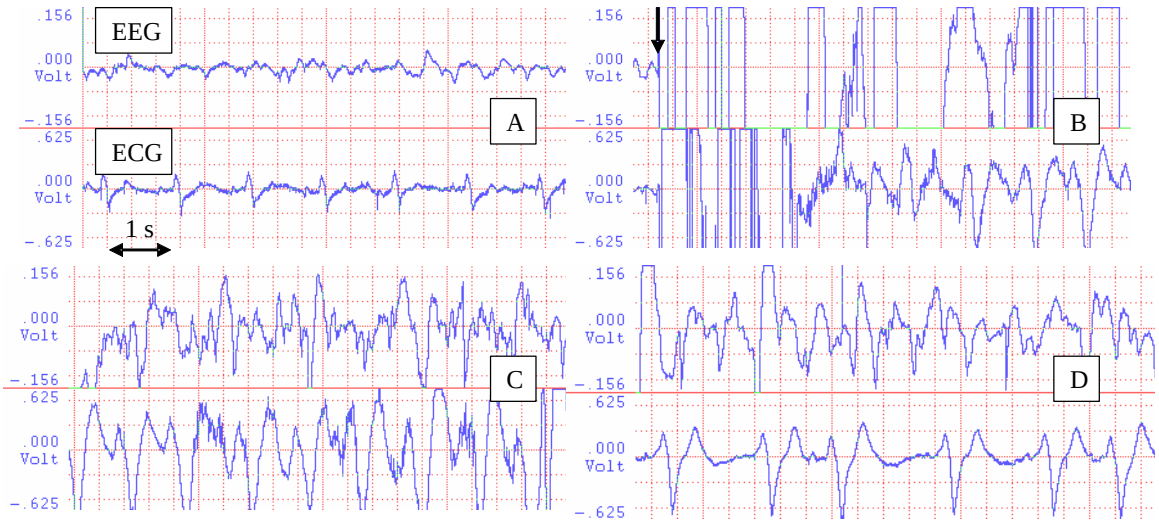

Figure 2: Example of an EEG and ECG before and after stunning by using the captive needle pistol.

A	EEG and ECG (normal heart beat) trace before the shot (= ▼)
B	After the shot artefacts lasted for 5 s.
C	The artefacts were followed by theta and delta waves and spikes on the EEG. After the shot fluttering of the heart on the ECG.
D	Some brain and heart activity remain on the EEG and the ECG until 10 min after stunning.

3.3 Stunning of African catfish (*Clarias gariepinus*) by chilling in ice water: assessment of loss of consciousness and sensibility

Submitted for publication in *Aquaculture*

Introduction

For the economic viability and sustainability of husbandry of animals, farming fish is an alternative and addition to red and white meat production. Interest in sustainable farming of fish has emerged due to the problems associated with intensive livestock production, the increasing imbalance between amounts of fish caught (limited by EU quota) on one hand and an increasing consumer demand for a variety of high quality fish on the other hand.
Nowadays quality of fish also comprises ethical aspects during production. Especially during pre-slaughter welfare of fish can be compromised. For various farmed fish species in the Netherlands and other countries death in air, live chilling, carbon dioxide narcosis and a manual applied blow on the head are used as pre-slaughter methods in industry. Lambooij et al., 2002, Robb and Kestin, 2002; Van de Vis et al., 2003 established that these pre-slaughter methods can be stressful. According to the EU Council Directive 93/119/EC (1993) on the protection of animals at the time of slaughter or killing, slaughter animals must be restrained in an appropriate manner to spare them any avoidable pain, suffering, agitation, injury or contusions. Permitted methods for stunning are: 1) captive bolt pistol, 2) concussion, 3) electro narcosis and 4) exposure to carbon dioxide.
In contrast to warm-blooded slaughter animals, there are no specific requirements for fish. However, there is evidence that the term pain is applicable to fish too, since results of anatomical, physiological and behavioural studies were very similar to those of studies performed on birds and mammals (Mathews and Wickelgren, 1978; Neuman, 1991; Kestin 1994; Sneddon, 2002, 2003; Sneddon and Gentle, 2002). Contrary to the conclusions of these authors, Rose (2002) concluded that fish are not capable of pain perception, as the necessary brain structure, a neocortex, is not present in fish. However, there is evidence that fish are capable of nociception, which is detection of a noxious stimulus and the reflex response to this, and that pain related behaviours are not simply reflexes (Sneddon, 2002, 2003; Sneddon and Gentle, 2002).
The present status of pre-slaughter and slaughter methods used at fish processors is leading to an increasing concern of government, animal protection associations and consumers. It is well known that stress may have profound effects on the physiological status of the fish. The term stress is used when the control systems are overtaxed (e.g. the heart rate) and there is likely to be a reduction of biological fitness (Broom and Johnson, 1993). Short-term stress may occur at crowding, transport, grading, restraining (i.e. restricting the movements to facilitate the performance of the next step), stunning and killing. Stunning of slaughter animals is in the first place applied to induce a state of unconsciousness and insensibility of sufficient duration to ensure that the animal does not recover while bleeding to death (exsanguination). Secondly, stunning should produce sufficient immobility to facilitate the initiation of exsanguination (Blackmore and Delany, 1988). It is generally stated that unconsciousness and insensibility should be induced as soon as possible and without a detrimental effect on the welfare of the animal and the meat quality of the carcass.
The current pre-slaughter process used in the Netherlands for African catfish consists of live chilling to immobilise them prior to evisceration (Robb and Kestin, 2002). Assessment of live chilling of eel revealed that this method is stressful as vigorous activity of the animals and an irregular heart rate were observed. Responses to pain stimuli disappeared at a body temperature of approximately 8.0 ˚C, which occurred after 12 ± 5 min, which suggests that consciousness is lost (Lambooij et al., 2003). It is unlikely that live chilling provokes unconsciousness in African catfish immediately or slowly and without avoidable stress, as it has been reported for carp (Arends et al., 1998), eel (Lambooij et al., 2003) and gilt-head seabream (Van de Vis et al., 2003) that this is stressful.
The objective of the study was the evaluation of behavioural, neural and physiological responses during live chilling of African cat fish (*Clarias gariepinus*).

Materials and methods

Fish

Three days before the experiment 45 African catfishes were fasted and after that delivered to the laboratory. The catfishes were kept in a tank containing aerated tap water at 24 °C. The experiment was performed with approximately 10 animals per day. After the experiment the fishes were weighed and dissected to determine the sex.
The average weight of the 28 catfish, which were restrained one by one and equipped with EEG and ECG electrodes for neural and physiological assessment of live chilling was 1738 ± 441 g. There were 15 males and 13 females. The 10 fishes, which were allowed to move freely in ice water had an average weight of 2422 ± 484 g and they consisted of 4 males and 6 females. In the control group the average weight of the 7 fishes, which were allowed to move freely in fresh water, was 1765 ± 178 g and they consisted of 4 males and 3 females.

Registration of EEG and ECG and body temperature of restrained African catfish

Prior to stunning each individual fish was equipped with EEG electrodes, as described by Lambooij et al. (2003). In order to facilitate the implantation of the electrodes the fish was restrained. The restrainer consisted of a steel plate on which a platform, a pallet and an U-shaped grill were mounted. The platform (3 cm diameter) and a metal pallet were used to fix the head of the fish. Both of these were adjustable, as required by the size of the fish. The distance between the platform and the U-shaped grill (20 cm long) was also adjustable. An individual African catfish was restrained by placing the lower jaw on the platform, inserting the metallic pallet in the mouth and subsequently the pallet was pressed down on the platform. A substantial part of the abdomen and most of the tail were fixed in place by a U-shaped grill. The various sizes of fish were accommodated by using plastic tie-ribs, which held the fish in place in the grill.
Prior to implantation of all electrodes each fish was locally anaesthetised using Xylocaine® 10% spray (lidocaine 100 mg/ml; Astra Pharmaceutica BV, Zoetermeer, Netherlands). For implantation of EEG electrodes two holes were drilled in the skull. The silver spiked EEG electrodes (6 mm long and 1.5 mm in diameter) were placed in the holes: one electrode 1 cm to the right and one electrode 1 cm to the left of the sagittal suture and 4 cm caudal of the imaginary line between the eyes. The steel ECG electrodes (35 mm long and 1 mm in diameter) were placed subcutaneously one caudal of the implantation of the left pectoral fin and one 2 cm caudal of the first electrode. The earth electrode for both the EEG and ECG was placed subcutaneously caudal of the dorsal fin. The EEG and ECG were recorded from 30 s before until 2 minutes immediately after placement in the ice water and for 30 s after 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5 and 30 min. The recorder used was a DI 720 data-recording module with a WinDaq Waveform browser (Dataq Instruments, Akron, Ohio, USA). Responses to pain stimuli (i.e. needle scratches applied to the skin of the tail) in the behaviour as well as on the EEG were monitored at the start of each recording period. The EEG and ECG recordings were analysed for changes in the waveforms, frequency and suppression afterwards. The behaviour of the animals was monitored for the occurrence of tonic and clonic cramps.
An incision of 3 mm was made at the height of the anus along the lateral line and a copper-constantan thermocouple was inserted 1.5 cm deep medially. A thermocouple was also placed in the ice water. Both thermocouples were connected to a Chessel 4001 recorder (Chessel Ltd, Sussex, UK). The temperature was measured at the mentioned recording times.

Registration of behaviour of unrestrained African catfish

A group of 10 fish were placed individually in ice water and allowed to swim freely for 10 min. Another group of 7 fish were placed individually in a tank with fresh water of 24 °C and allowed to swim freely for 10 min.
The behaviour of the animals was monitored on video and analysed for:

- Swimming: The fish swims around in the water and may explore its environment.
- Lying on the bottom: the fish breathes but shows no other movements. It lies on its abdominal side on the bottom.
- Motionless in the water: The body of the fish is slightly bent, resulting in an elevated head.

Ethics

The experiments were approved beforehand by a governmental ethical committee.

Statistical analyses

The obtained data were analysed to determine the probability for an effective stun at 95 % lower confidence limit. A 95% confidence limit on the probability for an effective stun can be obtained by means of a well-known relationship with the beta distribution (Johnson and Kotz, 1969).

Results

Body temperature and registration of EEG and ECG of restrained African catfish

The initial temperature of the ice water was on average 0.1 ± 0.5°C. When the restrained catfish were placed in this ice water, their body temperature was 22.0 ± 2.1°C (n=22). After 30 min the average body temperature was decreased to 8.2 ± 1.6°C in 30 min. The decrease is presented as a function of time in Fig. 1.
As the electrodes were disconnected in 6 catfish, 22 EEG and ECG recordings were obtained. Prior to placement in ice water, alpha and beta waves (8 – 13 Hz and > 13 Hz, respectively) were recorded on the EEG. After a median time of 5 min these traces changed to theta and delta waves (4 – 8 Hz and < 4 Hz, respectively) and the median body temperature was lowered from 22 ± 2.1 to 17.3 ± 2.3°C (n=22). Low brain activity was observed after a median time of 15 min at a body temperature of 13.3 ± 2.7°C (n=22). Responses to pain stimuli disappeared at a body temperature of 13.7 ± 2.6°C (n=22), which occurred after a median time of 12.5 min. It was observed that the shortest period after which the responses to pain stimuli had disappeared, was 5 min whereas the longest one was 20 min.
When taking into account the number of animals with a reliable EEG (*n*=22) and using 95% confidence intervals, it can be concluded that at least 90% of the catfish is unconscious and insensible at a decrease in body temperature of approximately 8.7°C .
The heart rate before placing in ice water was 295 ± 38 beats/minute, varied during cooling between 294 ± 47 and 311 ± 38, and was after 30 min 300 ± 33 (n=13). With regard to this extremely high heart rate, which was recorded prior to placement in the ice water, it should be noted that the restrainer was not at ambient temperature. The restrainer had been taken out of the ice water to restrain a next fish in our experiments. The heart showed a tachycardia (Fig 1 and 2).
When placed in ice water 9 restrained catfish responded immediately by displaying vigorous muscle activity. During live chilling behavioural responses i.e. vigorous muscle activity to the applied pain stimuli were observed in 10 fish.

Observation of behaviour of unrestrained African catfish

Ten individual catfish were placed in ice water of on average – 0.1 ± 0.1 °C. The observation of the unrestrained catfish revealed three phases. Firstly, the catfish swam or moved in the ice water for 13 ± 4 s. Secondly, the catfish showed clonic cramps for 284 ± 208 s and finally they were motionless. Movements were lost after 300 ± 208 s.
In the control experiment the 7 unrestrained fishes were placed in water of 22.8 ± 1.3 °C. The animals displayed normal behaviour. They were swimming and explored their environment. The fish sometimes touched the wall of the tank and went to the surface of the water to breath air. They laid down on the bottom after 21 ± 12 s.

Discussion

Aquaculture is growing in importance throughout the European Community. Up to now, most research has been directed at increasing production with little emphasis on welfare. As the industry matures the quality of fish products becomes more important. In addition, concern for animal welfare is increasing, with welfare at slaughter being especially important. In bird and mammal slaughtering industries it has been found that improvements in animal welfare can lead to an optimised meat quality. For farmed fish species such as Atlantic salmon (Byrne, 2002)), trout,

(Byrne, 2002) eel (Morzel and Van de Vis, 2003), turbot (Morzel et al., 2003) it is shown that reduced-stress at the process of slaughter has a positive influence on the flesh quality. Freshness, which is a major product quality attribute in the opinion of consumers, can be improved when due to the reduction of stress the muscle activity of the fishes is reduced during the slaughter process (Morzel and Van de Vis, 2003; Byrne, 2002).
Hypothermia is not considered acceptable for euthanasia of fish, because it prolongs the period of consciousness and does not reduce the ability to feel pain (Close et al., 1997). The Farmed Animal Welfare Council (1996) reported that cooling of live trout on ice should be prohibited. Exposure of carp (*Cyprinus carpio*) to a rapid drop in temperature of 9˚C resulted in a time-dependent cortisol response and induced a differential expression of both the POMC and mRNAs. Plasma cortisol levels increased up to 6 times the control level 20 min after the start of the experiment, and remained high until the end of the temperature shock (Arends et al., 1998). It is likely that eels, which are transferred from water at 18˚C to ice water are stressed, as specific behaviour and an irregular heart rate were observed. Brain activity and responses to pain stimuli disappeared at a body temperature of 8.0 ± 2.1˚C, which occurred after approximately 12 min (Lambooij et al, 2002). In the present study with African catfish, low brain activity and loss of responses to pain stimuli was observed after approximately 15 min at a body temperature of 13.3 ± 2.7˚C (Fig 1). In both eel and catfish this physiological point was reached after a decrease in body temperature of 9 to 10°C. Low brain activity and no response to pain stimuli both on the EEG and in behaviour, supports the assumption that the catfish were unconscious and insensible, as gauged by analogy with similar EEG changes in man (Lopes da Silva, 1983) and similarities in basic structure of neurones and neuronal biochemistry (Overmier and Hollis, 1990; Kestin, 1994; Verheijen and Flight, 1997; Clarke and Squire, 1998). It was also observed that freely moving catfish became motionless after 5 min in ice water, whereas in restrained cat fish theta and delta waves appeared on the EEG after the same period. This relationship pointed to an onset of loss of consciousness after 5 min in ice water.
Heart rate is often measured as a response to stressors. It may either increase (tachycardia) or decrease (bradycardia). Changes in heart rate can be caused by physical as well as psychological factors, and they are difficult to distinguish (De Jong, 2000). A tachycardia (approximately 300 beats/min) occurred when the catfish was placed in the cold restrainer and remained after placing the fish and restrainer in ice water. It is likely that the high heart rate of the animals prior to live chilling is caused by placing the fish in a cold restrainer, as the restrainer was not heated up to room temperature prior to use for the next fish. In a previous study (Lambooij et al., 2003) we established that the heart rate in restrained catfish at room temperature was 71 $\pm$ 19 beats/min. During the course if live chilling it appeared that the heart rate remained extremely high.
The tachycardia observed can be classified as a sinoatrial tachycardia, because this rhythm is originated from the sinoatrial node and may be caused by excitement (Dubin, 1999).
When eels were placed in ice water, an exponential decrease was observed in heart rate and in body temperature (Lambooij et al, 2002). The African catfish may be more sensitive to a cold environment than species adapted to a cold climate. After live chilling recovery is possible when the fish is kept at ambient temperature (Robb and Kestin, 2002). Recovery can be stressful, because the slime layer has been removed from the skin.
The anatomy, physiology and behaviour of fish suggest that it is likely that they can perceive pain. It suggests that the concepts of animal welfare can be applied legitimately to farmed fish (Chandroo et al, 2004; Sneddon 2003). Contrary to the research with warm-blooded animals, development and optimisation of stunning methods for fish has started only recently. In an overview (Robb and Kestin, 2002) showed evidence that in most cases, methods applied for stunning are stressful. The authors suggest that live chilling is stressful and therefore alternative methods should be investigated (Van de Vis et al, 2003).

Conclusion

It may be concluded that African catfish that are transferred from water at 22 C to ice water are unconscious and insensible after a median of 12.5 min. They were excited during the full conscious period of 5 min, as muscle cramps and a sinoatrial tachycardia (extreme increased heart rate) were observed. Live chilling does not kill the catfish, which makes recovery possible prior to killing.

Acknowledgements

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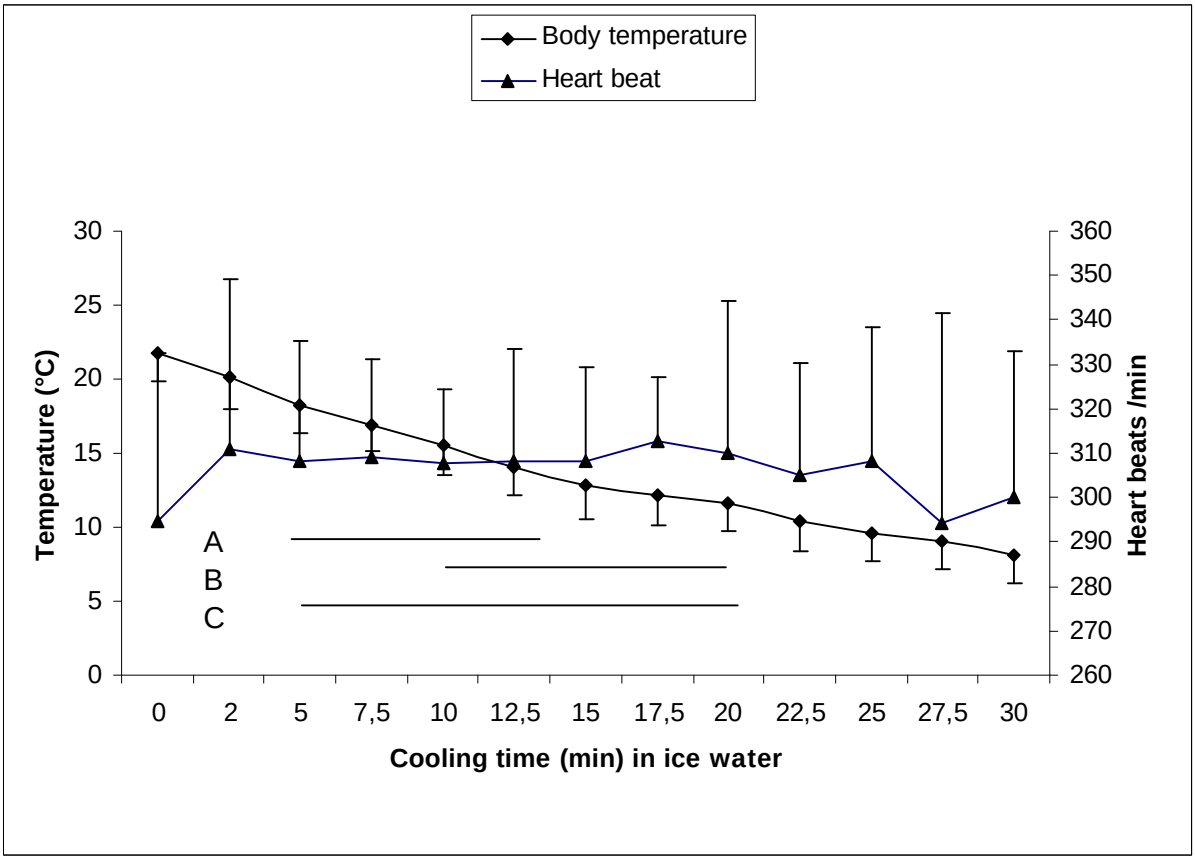

Figure 1: Live chilling of African catfish: effect on body temperature (°C) and heart rate (beats/min). The average body weight was 1738 ± 441 g and the temperature of the ice water 0.1 ± 0.5 °C.
A= occurrence of theta and delta waves; B=low brain activity on the EEG; C= loss of pain response.

Prior to placement in the ice water the fish was fixed, using a cold restrainer.

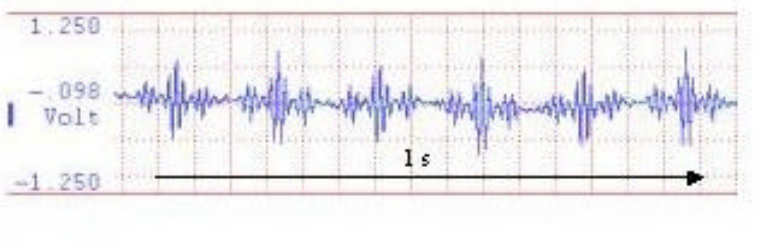

Figure 2: Tachycardia in an African cat fish caused by live chilling

3.4 Feasibility of electrical stunning followed by decapitation or chilling of African catfish (*Clarias gariepinus*); assessment of behavioural and neural parameters and product quality

Submitted for publication in *Aquaculture Research*

Introduction

In order to meet the European consumers' and retailers' increasing demand for high quality fish it is necessary to increase the production by aquaculture of emerging fish species. In addition, maximising the quality of emerging fish, including ethical quality, species is necessary to fulfil their demands. Quality of fish can be affected by various factors, such as feed and welfare of these animals in the entire chain of production. It is obvious that these factors influence consumers' concern and acceptability. Animal welfare influences acceptance by consumers, retailers and animals protectionists in two ways (FAWC 1996; Cooke, 2001, Waitrose, 2003). The first one concerns the emotional aspects of farming, stunning (or lack thereof), and slaughter, as perceived them. The second one for consumers and retailers is that the farming and slaughter practices may have a major impact on sensory quality of fish.

Various studies have shown that stress at slaughter can affect product quality of farmed fish. Reduced stress at slaughter by application of effective stunning methods prior to killing has been shown to have a positive influence on freshness (Byrne, 2002; Morzel et al., 2003; Morzel and Van de Vis, 2003) and flesh quality (Azam et al., 1989; Berg et al., 1997; Byrne, 2002; Sigurgisladóttir, 2001; Roth et al., 2002; Morzel and Van de Vis, 2003). Most of the current slaughter methods of farmed fish, for instance placing the animals in seawater saturated with carbon dioxide, in ice or ice water followed by gutting or bleeding, can be classified as slow and stressful. These methods do not cause immediate loss of consciousness.

Various studies show that fish can be stunned immediately without recovery until death occurs by applying an electrical current of sufficient strength for 1 second to induce unconsciousness immediately (Robb and Roth, 2002; Lambooij et al., 2004). Another recommended method to provoke loss of consciousness immediately and permanently is the use of a captive needle pistol. In this case air is injected in the skull under high pressure (Lambooij et al, 2002a, 2003). At present effective stunning methods are not applied at processors of farmed eel and African catfish in the Netherlands. (Robb and Kestin, 2002; Van de Vis et al., 2003). Experiments performed in collaboration with industry demonstrated that stress at slaughter of eels can be avoided by applying a peak stun followed by maintenance stun in combination with removal of oxygen from the water (Lambooij et al., 2002b). Comparison of effects by applying this experimental method to the current Dutch industrial method, i.e. de-sliming of live eels in a salt bath followed by gutting, revealed that the experimental improved quality of the fish flesh (Morzel and Van de Vis., 2003). It is foreseen that the application of electricity is suitable for stunning batches of other fish species (Van de Vis et al., 2003). Contrary to eel, for African catfish an effective laboratory scale method, consisting of applying electricity in combination with a killing method, has not been developed yet. Results obtained in a study performed by us suggest that electrical stunning in combination with bleeding can be optimised to induce loss of consciousness immediately and permanently in African catfish (Lambooij et al., 2004).

The objective of the study was therefore evaluation of electrical stunning of African catfish (*Clarias gariepinus*) in combination with bleeding or chilling with respect to neural and behavioural parameters. To assess the possibility of scaling up both experimental methods, flesh quality was compared to a batch, which was live chilled under practical conditions.

Materials and methods

Laboratory experiment

Fish

Seven days before the experiment 37 African catfishes were fasted for 3 days and after that delivered to the laboratory. The catfishes were kept in a tank containing aerated tap water at 24ºC. The experiment was performed with approximately 10 animals per day. After the experiment the fishes were weighed and dissected to determine the sex.
The average weight of the 15 catfish (6 males and 9 females), which were restrained one by one and equipped with EEG electrodes for assessment unconsciousness after electrical stunning and decapitation was 1536 ± 290 g.
The 12 catfish (4 males and 8 females), that were restrained and equipped with EEG electrodes for assessment of unconsciousness after electrical stunning and chilling in ice water weight 1622 ± 233 g.
A batch of 10 fishes (4 males and 6 females), which were allowed to move freely and stunned electrically tank and subsequently chilled in ice water, was used for observation of behaviour. The average live weight was 1640 ± 244 g.

Registration of EEG of restrained African catfish

Prior to stunning each individual fish was equipped with EEG electrodes as described by Lambooij et al. 2003. In order to facilitate the implantation of the electrodes the fish was restrained. The restrainer consisted of a square wooden plate (16 x 16 cm) equipped with four 56 cm long PVC pipes with an inner diameter of 1.5 cm. The pipes were mounted at the corners of the wooden plate. In the middle of the plate a plastic clamp was attached which was placed in the mouth of the catfish to clasp the lower jaw of the animal. The body of the catfish was fixed between the four PVC pipes by using plastic tie-ribs. The flexibility of the pipes enabled to accommodate for different sized fishes.
Prior to implantation of all electrodes each fish was locally anaesthetised using Xylocaine? 10% spray (lidocaine 100 mg/ml; Astra Pharmaceutica BV, Zoetermeer, Netherlands). For implantation of EEG electrodes two holes were drilled in the skull. The silver spiked EEG electrodes (6 mm long and 1.5 mm in diameter) were placed in the holes: one electrode 1 cm to the right and one electrode 1 cm to the left of the sagittal suture and 4 cm caudal of the imaginary line between the eyes. The earth electrode for the EEG was placed subcutaneously caudal of the dorsal fin and after decapitation replaced in the skin at the back site of the head.
The EEG was recorded 30 s before, for 30 s immediately after stunning and at 5, 7.5, 10, 12.5, 15 min after application of the method. The recorder used was a DI-720 data recording module with a WinDaq Waveform browser (Dataq Instruments, Akron, Ohio, USA). Responses to pain stimuli (i.e. needle scratches applied to the skin of the tail) in the behaviour as well as on the EEG were monitored at the start of each recording period. The EEG recordings were analysed for changes in the waveforms, frequency and suppression afterwards. The behaviour of the animals was monitored for the occurrence of tonic and clonic cramps.
Each individual catfish was electrically stunned in a Plexiglas box filled with (size 50 x 20 x 70 cm) of tap water (500 ?S conductivity). The bottom and top plate electrodes measured an area of 648 cm^2 each at 16 cm distance. For the prevention of a short-circuit, the top plate electrode was placed on the wooden square of the restrainer of (16 x 16 cm). Opposite of the wooden square a PVC pipe was squeezed between the walls of the tank to separate the ends of the plate electrodes.
The power supply (Stork RMS, Lichtenvoorde, Netherlands) delivered a constant voltage of 300 V (50 Hz a.c.). Voltage and current were measured with a FLUKE multimeter (tongs ammeter; B. V. Tilburg, The Netherlands). Immediately after stunning the catfish was removed from the tank and decapitated or placed in ice water for a period of 15 min. The time that elapsed between switching of the current and decapitation or chilling in ice water, was recorded. Prior to placement in the ice water an incision of 3 mm was made at the height of the anus along the lateral line and a copper-constantan thermocouple was inserted 1.5 cm deep medially. A thermocouple was also placed in the ice water. Both thermocouples were connected to a Chessel 4001 recorder

(Chessel Ltd, Sussex, UK). The temperature was measured at the mentioned recording times for the EEGs.

Registration of behaviour of unrestrained African catfish

A group of 10 fish were individually electrically stunned for 5 s and after that placed in ice water. Each animal could move freely and was observed for 15 min, using a video camera. The recorded pictures were analysed for occurrence of tonic, clonic cramps, exhaustion and recovery.

Experiments at a commercial processor of African catfish

Fish

The fish was fasted for 3 days (at a farmer and after that delivered at the fish processor's 0 to 2 days before slaughter. Slaughter was carried out batch-wise. The 450 kg fish were chilled for at least 15-20 min in tumbler filled with 225 kg flake ice to immobilise and de-slime the animals. Afterwards they were eviscerated, decapitated, de-skinned and filleted. The fillets were wrapped in plastic foil and placed in polystyrene boxes with flake ice (5 kg fillets with approx. 2 kg flake ice). It was decided to assess whether a batch of 20 fishes stunned by applying electricity and subsequently chilling for 15 min in flake ice could recover by placing the 20 animals for 75 min in fresh water of 20°C. This was assessed by observation of behaviour, as described by Kestin et al. (2002).
Two experiments were performed at a commercial fish slaughter, viz. 1 electrical stunning in combination with decapitation; 2 electrical stunning in combination with chilling for 15 min in a stainless steel tumbler filled with approx 15 kg flake ice. In both experiments the industrial slaughter method served as control. In both experiments 30 live and conscious catfish were stunned individually as described in the laboratory experiment with 300 V for 5 s (water 500 µS). In the first experiment they were decapitated and after this the animal was subjected to the same primary processing steps as fish that had been stunned by applying the industrial method. We traced each electrically stunning fish during decapitation, gutting and filleting and its fillets were collected. In the second experiment the electrically stunned fishes were chilled and deslimed in ice in a stainless steel tumbler for 15 min. Subsequently the fish were subjected to the regular processing steps and traced by us during primary processing in order to collect the fillets The number of males, females and average live weight of the whole fish were recorded.
During both experiments 30 control fish were randomly selected after live chilling, weighed, subjected to the regular processing steps and traced. At the end of the slaughter line the fillets of each traced fish were collected.
In both experiments the same person filleted all carcasses.

Flesh quality

The whole fishes were weighed before slaughter and the filets after slaughter to determine the yield. Of each fish the right fillet was used for measurement of pH values, temperature and colour of the muscle tissue at 30 min, 1, 2, 5, 7, 9 and 12 days, *post mortem*. In experiment 1 pH values were not measured 1 day *post mortem* due to problems with the equipment. Drip loss was determined as percentage weight loss after 2, 5 and 7 days storage at 4°C according to Lundström and Malmfors (1985). Analysis of pH was performed directly in the tissue of each right-hand side fillet on the visceral side, using a spear electrode PH62 (WTW, Weilheim, Germany). The other 30 left-hand side fillets were used for determination of drip loss during storage. For each fish, three measurements on one fillet were carried out. pH was measured rostral, mid-axial and caudal. Colour of fillets was monitored using a Chroma-meter CR-200. Due to a defect in the CR-200 apparatus after day 1 we decided to continue the measurements with the CR 300 (Minolta, Osaka, Japan). The major differences between the CR-200 and the CR-300 are the size of the opening of the light-projection tube (1 cm for the CR-200 vs. 3 cm for the CR-300). Measurements were taken in triplicate on the visceral and skin side of the right-hand side of the 30 fillets, which were also used for analysis of pH. Colour was monitored just behind the area of the ribs in the live animal (mid-axial area). The measurements were carried out mid-axial on the dorsal, lateral and ventral sides. Chromatic values are given in the CIE-L*a*b* system.

Ethics

A governmental experimental committee approved the experiments beforehand.

Statistical analyses

The obtained data were analysed to determine the probability for an effective stun at 95 % lower confidence limit. A 95% confidence limit on the probability for an effective stun can be obtained by means of a well-known relationship with the beta distribution (Johnson and Kotz, 1969).
The data of the meat quality parameters were analysed using ANOVA and Tukey.

Results

Laboratory experiment

Decapitation after electrical stunning of restrained African catfishes

Subsequently 15 African catfishes were stunned with on average 269 ± 4 V, 1.5 A/dm^2 during 5 ± 0 s. The catfish were decapitated in 50 ± 9 s. The electrodes were disconnected in 2 fishes in this stunning procedure. The successful ones showed spikes alternated with theta and delta waves on the EEG, which was followed by minimal brain activity after 20 ± 10 s. Observation of behaviour of the restrained catfishes revealed only clonic cramps of the eyes. The head of the fish did not respond on pain stimuli, as no movements of the eyes, gill covers, mouth or barbels were observed. Using the number of reliable EEGs obtained (n=15) and a confidence limit of 95%, the chance of effectively stunning and killing African catfishes was determined to be between 0.91 and 1.00 with 300 V (1,5 A/m^2 50 Hz a.c.) applied for 5 s in water of 500 µS followed by decapitation.

Chilling in ice water after electrical stunning of restrained and unrestrained African catfishes

The restrained African catfishes were stunned with on average 299 ± 3 V, 9.6 ± 0.6 A (current density was on average 1.5 A/dm^2) during 5 ± 0 s (n=13). The catfish were placed in ice water 34 ± 13 s after stunning. The electrodes were disconnected in 2 fishes in this stunning procedure. The successful ones showed spikes alternated with theta and delta waves on the EEG, which was followed by minimal brain activity after 22 ± 11 s. The temperature of the body was 24 ±1^{o}C before stunning and 23 ±1 ^{o}C, 20 ± 2^{o}C and 18 ± 2 ^{o}C at 5, 10 and 15 min after stunning, respectively. The fishes did not respond on pain stimuli. Using the number of reliable EEGs obtained (n=15) and a confidence limit of 95%, the chance of effectively stunning and killing African catfishes was determined to be between 0.91 and 1.00 when 300 V (1.5 A/m^2, 50 Hz a.c.) is applied in water of 500 µS conductivity, followed by live chilling.
The 10 unrestrained African catfishes were stunned with on average 300 ± 2 V, (current density 1.5 A/dm^2) during 5 ± 0 s. The catfish were placed in ice water 14 ± 4 s after stunning. The temperature of the body was 17 ± 3^{o}C at 15 min after stunning. They all showed some clonic muscle cramps during chilling. The fishes did not recover during chilling.

Experiments at a commercial processor of African catfish

Fish

Fishes stunned by applying electricity and subsequently chilling for 15 min in flake ice, which were placed for 75 min in fresh water of 20°C were classed as *dead*, which suggests that the animals did not recover. During gutting of the 20 fish, however, one animal, which was classed as *dead* during observation of behaviour, responded to removal of its intestines.

Flesh quality

The results of the weight, yield and drip loss are presented in Table 1. Significant differences ($p<0.05$) were found between experiments, methods, males and females. Since the differences are divided over all groups, it cannot be attributed to a method.
The pH and colour measurements are presented in the Figures 1 and 2. The overall values of the pH in the different groups showed a significant ($p<0.05$) decrease during the 48 hours *post*

mortem. Afterwards there is a slight increase. For experiment 1 the average mid-axial pH value, of the fish that was stunned by electricity and decapitation was significantly lower at the day of slaughter than the average value measured for the live chilled batch. The a values decreased and the b values increased during storage of all batches, which implies that the colour of the flesh changed to a more reddish and yellowish colour, respectively. For the live chilled batch in experiment 1 the a values measured *post mortem* on 2 up to day 7 and those on day 5 up to 9 on the visceral and skin side, respectively were significantly ($p<0.05$) higher than for the batch stunned by electricity followed by decapitation.
No significant difference in redness between fillets of male and females was observed.

Discussion

Results from observation of behaviour and displayed responses to stimuli as procedure to assess electrical stunning is do not provide a definite answer about loss of consciousness. No firm conclusions can be drawn from observation of behaviour and responses, as an animal may be paralysed or responses may be caused by electrical stunning itself (Roos & Koopman, 1940). Thus, it is difficult to consider this behaviour and these responses of indicators of loss of consciousness. These drawbacks can be avoided by measuring the electrical activity of the brain by an EEG (Müller, 1968). However, it is difficult to estimate the relation between electrical brain activity and presence or absence of consciousness. From medical research a lot is known about the relation between electrical activity of the brain and perception of pain or the state of consciousness (Davidson & Davidson, 1980; Lopez da Silva, 1983). A combination of measurement of an EEG, and observation of behaviour and evoked responses in behaviour and on the recorded EEG, such as was carried out in this experiment, can provide sufficient scientific information concerning the consciousness of the animal and the effectiveness of the stunning method.
The African catfish were easily placed in the restrainer to position the EEG electrodes and placed in the tank with water. After electrical stunning the fish was taken out of the water tank and taken out of the restrainer for decapitation on a table to bleed it or for placement in the ice water or flake ice to achieve an irrecoverable loss of consciousness. It was considered that both methods could be used under commercial conditions. During chilling in ice, using the stainless steel tumbler, the fish was de-slimed and cleaned within approximately 15 min, which meets the demands for further processing. These demands were established during two workshops for all Dutch processors of African catfish in our laboratory, as well as in discussions with staff and manager the industrial processor of the fish species.
Psychological or physical short-term stress before slaughter may lead to excessive glycogenolysis in the muscles. The formed lactate will lower the pH relatively fast. As a consequence, meat or flesh can become pale and soft, exuding much of the tissue's water. This explanation must not be generalised, as the physiological response to stressors from the environment is partly influenced by the genotype of the animal (Nicol & Scott, 1990). In well-fed rested animals, meat pH falls to approximately 6.0-6.5 45 minutes post mortem and ultimate to 5.5 to 5.8 after slaughter (Hillebrand, 1993; Klont et al, 1993;). Colour and water binding capacity are determined by protein denaturation, caused by a rapid acidification after death (Tarrant, 1989). The rate of acidification after death is controlled by the degree of hormonal and contractile stimulation of muscle immediately before and during slaughter, whereas muscle temperature at death and rate of cooling is also important (Warriss, 1987; Tarrant, 1989; Monin & Ouali, 1992).
The occurrence of dark, firm and dry (DFD) meat is more readily attributable to effects of long-term stress, which may occur at the transport or husbandry and this type of stress is less variable amongst genetic lines. It occurs when the animals are fatigued. In this case glycogen energy store is exhausted at slaughter, resulting in no acidification, an increase in rigor mortis value and dark coloured meat (Tarrant 1989). However, in our study the fishes showed a relatively high pH but no dark coloured flesh.
The expected reduction of stress, which can be reflected into less acidification of muscle tissue, by applying electrical stunning in combination with decapitation or chilling may have been masked by stressful steps that preceded stunning. The steps, which are performed prior to stunning consists of fasting of the fishes, crowding, transport, lairage and subsequently transport to the slaughter facilities in a company.

No differences in, brightness, redness and yellowness was observed between electrical stunning in combination with decapitation or chilling followed by gutting on one hand and live chilling followed by gutting on the other hand could be detected (see figure 2). For other fish species, however, it has been reported that applying electricity resulted in a darker flesh colour. This has been previously observed in channel catfish (Boggess et al., 1973) and eel (Morzel & Van de Vis., 2003). In addition, for eel it was reported that effective stunning resulted in an increased redness of red muscle tissue on the skin side of the fillet, compared to applying the traditional salt bath to de-slime conscious eel (Morzel & Van de Vis, 2003). The observation that reduction of stress by effective stunning did not influence brightness, redness or yellowness suggest that the reduction may have been masked by more stressful steps that precede stunning. Contrary to results reported by Wedekind (1991), fillets obtained from males were not significantly redder than those from females.

Conclusion

The results of this study show that of the application of an electrical current of 1.5 A/dm2, 300V (50 Hz) to individual African catfish in a tank in combination with decapitation with de-bleeding or chilling in ice water is an effective procedure for stunning of catfish before slaughter. The flesh quality is not affected by this stunning method, compared to the commercial procedure. During our study it became clear that processors of African catfish prefer to stun the species by applying electricity in combination with chilling in flake ice in tumbler prior to gutting, because the fish is de-slimed and cleaned, which facilitates further processing.

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Table 1: Live chilling, electrical stunning + decapitation and electrical stunning + chilling of African catfish: yield and drip loss during storage of fillets.

	Experiment 1				Experiment 2			
	El. stunning + decapitation		Chilling in ice		El. stunning + chilling in ice		Chilling in ice	
	Male n=21	Female n=9	Male n=18	Female n=12	Male n=11	Female n=19	Male n=12	Female n=18
Weight whole fish (g)	1252±331	1169±104	1257±344	1181±408	1960±572	1929±658	1925±646	1968±417
fillet (g)	598±85	531±54	614±76	591±91	874±288	774±279	859±306	839±205
yield (%)	45.4±1.1 [a]	45.4±1.1 [a]	46.9±2.1 [b]	46.8±1.6 [b]	44.3±5.5 [a]	40.3±4.3 [b]	44.3±2.5 [a]	42.4±2.3 [ab]
Drip loss								
day 2 (%)	0.8±0.2	0.8±0.2	0.8±0.2	0.8±0.2	*	*	*	*
day 5 (%)	1.2±0.3	1.1±0.2	1.3±0.3	1.2±0.2	2.3±0.8	2.4±0.7	1.8±0.4	2.3±0.9
day 7 (%)	1.3±0.3	1.2±0.2	1.5±0.4	1.4±0.3	4.2±1.0	4.1±0.9	3.8±0.9	3.8±0.9

[*] Due to a temporarily defect in the measuring equipment the drip loss is measured from day 2
Means with a different superscript differ significantly ($p<0.05$)

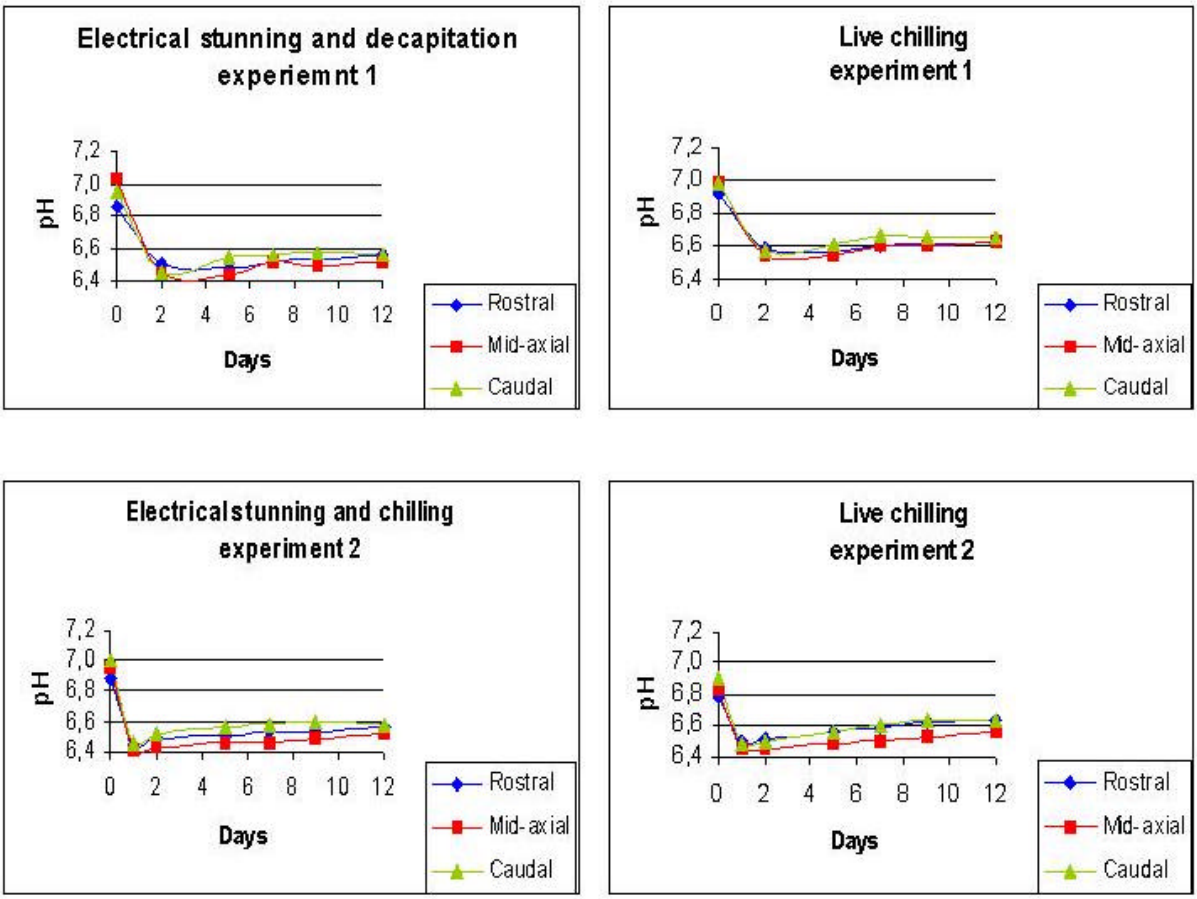

Figure 1: Electrical stunning followed by decapitation or chilling compared to live chilling: course of pH values during storage

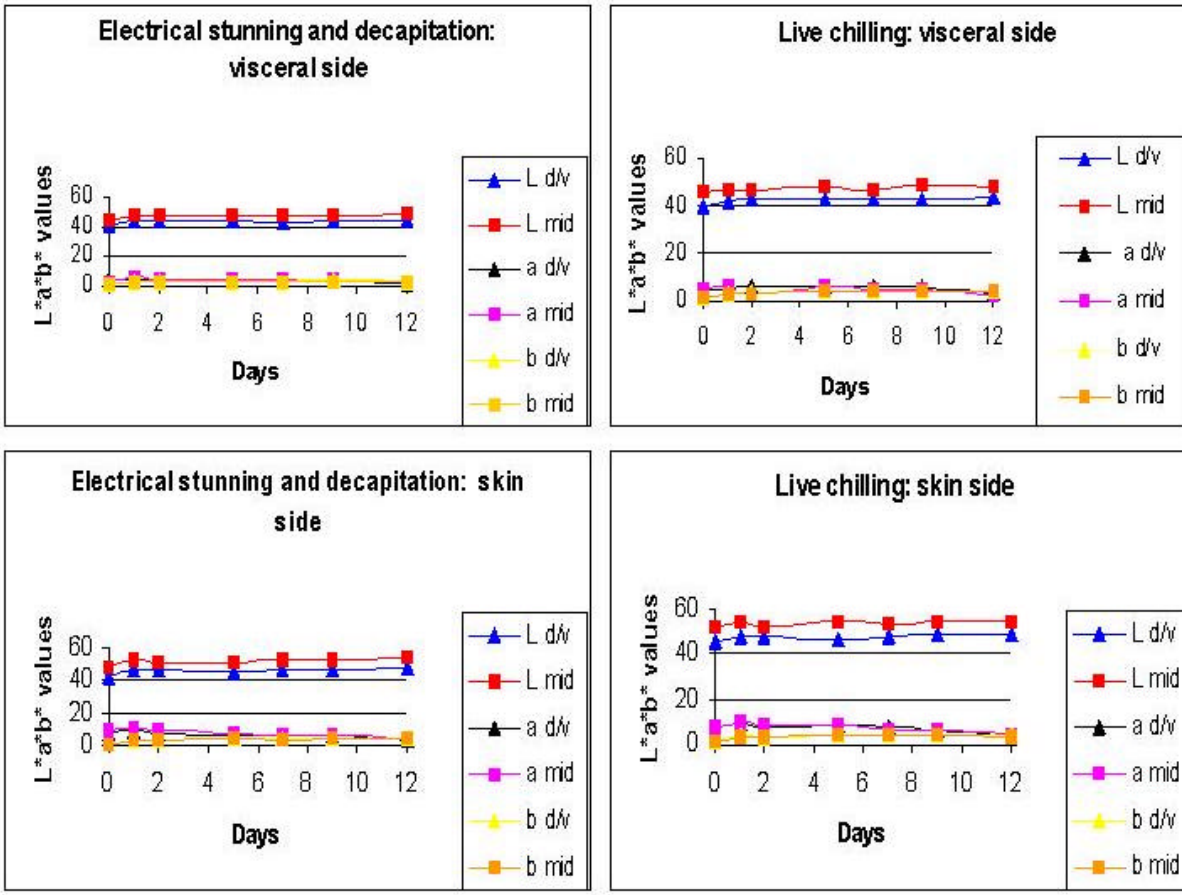

Figure2a: Electrical stunning followed by decapitation compared to live chilling: course of CIE L*a*b* on visceral and skin sides of fillets during storage in experiment 1.

m= mid-axial, d= dorsal, v= ventral, d/v= average of values measured dorsal and ventral

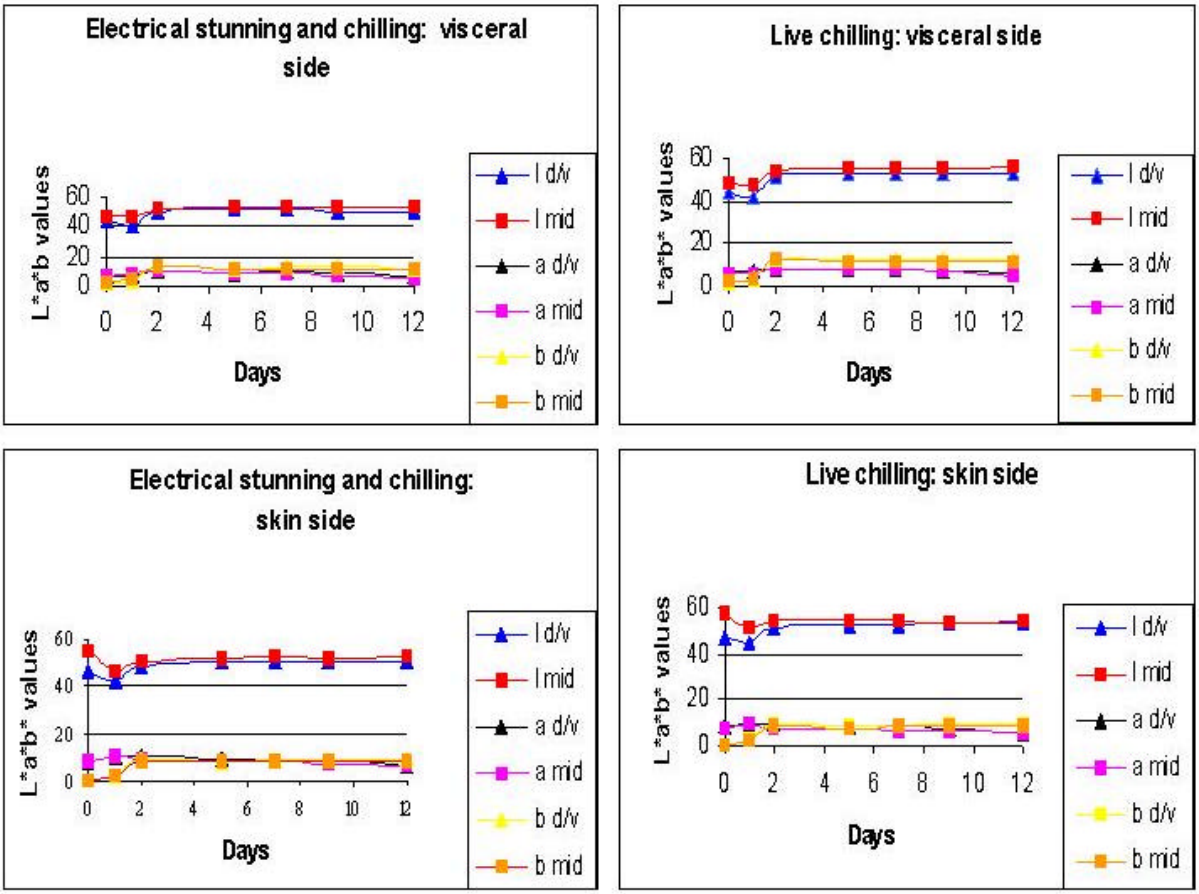

Figure 2b: Electrical stunning followed by chilling compared to live chilling: course of CIE L*a*b* on visceral and skin sides of fillets during storage in experiment 2

m= mid-axial, d= dorsal, v= ventral, d/v= average of values measured dorsal and ventral

4. Conclusies

Kop-kop elektrisch bedwelmen en verbloeden van Afrikaanse meerval (*Clarias gariepinus*): toetsen van verlies van bewustzijn

Het direct verbloeden van de meervallen die bij bewustzijn zijn, door de kieuwbogen door te snijden is onderzocht om na te gaan hoe lang het kan duren voordat de bewusteloosheid en gevoelloosheid is ingetreden. Met het oog op de haalbaarheid van bedwelmen in combinatie met verbloeden voor meervallen, is het noodzakelijk is om vast te stellen hoelang het duurt voordat een meerval bedwelmd is door het dier direct op deze wijze te verbloeden.
Vijftien minuten na het doorsnijden van de kieuwbogen waren de meerval nog niet bedwelmd omdat de dieren reageerden op toegediende prikkels. Het experiment werd gestopt door het dier te bedwelmen met een naaldschietmasker. Blijkbaar is verbloeden door het doorsnijden van de kieuwbogen niet geschikt om snel bewusteloosheid en gevoelloosheid op te wekken.
Afrikaanse meerval met een gemiddeld levend gewicht van 1571 g kan door 629 mA, 50 Hz a.c. gedurende 1 s door de kop te voeren onmiddellijk worden bedwelmd. Om deze stroomsterkte te bereiken moet gemiddeld 362 V spanning op de kop van een individuele vis worden gezet. Uit de resultaten blijkt dat deze condities niet voldoende zijn om een permanente bewusteloosheid en gevoelloosheid op te wekken.
Kop-kop elektrische bedwelming in combinatie met het doorsnijden van de kieuwbogen is ook getest om na te gaan of hiermee kan worden vermeden dat de meervallen weer bijkomen. De metingen lieten zien dat het verbloeden van de bewusteloze meervallen door het doorsnijden van de kieuwbogen niet leidt tot een permanente bedwelming. Twee van de zeven dieren kwamen bij, één na twee en de andere na 5 minuten. Blijkbaar voldoet deze wijze van verbloeden van bewusteloze meervallen niet.

Bedwelmen van Afrikaanse meerval (*Clarias gariepinus*) met een naaldschietmasker: toetsen van welzijnsaspecten

Uit de experimenten blijkt dat Afrikaanse meervallen met gemiddeld levend gewicht van 900 tot 1900 g onmiddellijk en permanent kunnen worden bedwelmd met het naaldschietmasker dat met een druk van 8 bar een naald in de hersenen schiet die onmiddellijk lucht onder een druk van 3 bar gedurende 1,5 s injecteert.
Observatie van het gedrag liet zien dat de bedwelmde meervallen soms spierbewegingen vertoonden. Dit kan vermeden of gestopt worden door de bedwelmde dieren te onderkoelen in ijs of ijswater.

Bedwelmen van Afrikaanse meerval (*Clarias gariepinus*) door onderkoelen in ijswater: toetsen van verlies van bewustzijn en gevoeligheid

Wanneer Afikaanse meerval met een levend gewicht van gemiddeld van 1738 g, die waren voorzien van EEG en ECG elektrodes, in ijswater van 0°C werd geplaatst, duurde het ca. 12,5 minuten voordat de bewusteloosheid en gevoelloosheid was ingetreden. Tijdens de gehele periode van bewustzijn was de meerval gestresst, hetgeen bleekt uit een extreem hoge hartslag en spierkrampen tijdens het onderkoelen. De spierkrampen in vrijzwemmende meervallen met een gemiddeld gewicht van 2422 g die bij bewustzijn waren, duurden gemiddeld 4,7 minuten.

Haalbaarheid van elektrisch bedwelmen in combinatie met ontkoppen of onderkoelen voor Afrikaanse meerval (*Clarias gariepinus*): beoordeling van gedragsparameters, neurologische parameters en de productkwaliteit

Elektrisch bedwelmen van Afrikaanse meervallen in water met een levend gewicht van gemiddeld 1536 g gevolgd door ontkoppen als methode van verbloeden of door onderkoelen gedurende 15 minuten in een roterende trommel met scherfijs (verhouding w/w vis: ijs= 1: 0,5) zijn effectieve methoden om de dieren onmiddellijk en permanent te bedwelmen. De condities voor het elektrisch bedwelmen waren voor individuele vissen gebruik van eens stroomdichtheid van 1,5 A/dm2, 50 Hz a.c gedurende 5 s.
Metingen aan de vleeskwaliteit lieten zien dat deze voor beide procedures vergelijkbaar was met die van de huidige industriële methode, namelijk het onderkoelen van meervallen die nog bij bewustzijn zijn.
Tijdens de twee workshops, die bij het Nederlands Instituut voor Visserijonderzoek waren gehouden en twee slachtproeven bij een meervalverwerker werd duidelijk dat de voorkeur van de bedrijven, die de meervallen verwerken, uitgaat naar de combinatie van elektrisch bedwelmen en onderkoelen omdat hiermee de vissen worden ontslijmd voordat verdere verwerking plaastvindt. Bovendien is deze procedure na opschaling waarschijnlijk in te passen in de bestaande proceslijn bij de verwerkers, hetgeen ook voor het gebruik van stroom in combinatie met ontkoppen het geval is.

5. Aanbevelingen

Op basis van de verkregen resultaten met het elektrisch bedwelmen in combinatie met onderkoelen is duidelijk geworden dat het mogelijk en wenselijk is om deze procedure op te schalen voor gebruik in de praktijk.
Het is essentieel dat de elektrisch bedwelmde vissen na onderkoeling meteen worden verwerkt, om te vermijden dat de vissen mogelijk bijkomen als gevolg van opwarming.

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