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Head-only electrical stunning and bleeding of African catfish (Clarias gariepinus): assessment of loss of consciousness

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Abstract

The objective was to evaluate the welfare implications of electrical stunning prior to gill-cutting of farmed African catfish as an alternative to live chilling in combination with gutting. Electroencephalogram (EEG) and electrocardiogram (ECG) recordings, in combination with observation of behaviour and responses to noxious stimuli, were used to assess brain and cardiac function in African catfish (body-weight 1571 ± 362 g [mean ± standard deviation], 32 males and 26 females). In the first experiment, the minimum electrical current required to induce a general epileptiform insult by head-only stunning was determined. The individual catfish were fixed in a specially designed restrainer, and applied voltages of 150 V, 200 V, 250 V, 300 V or 350 V (50 Hz AC) were delivered via scissor-model stunning tongs for approximately 1 s. A general epileptiform insult was observed in 31 fish, for which a successful EEG recording was obtained using 362 ± 32 V, 629 ± 180 mA for 1.2 s. The durations of the tonic, the clonic and the exhaustion phases were 8 ± 3 s, 12 ± 7 s and 7 ± 5 s as measured by EEG, respectively; a distinct exhaustion phase 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 min^{-1} prior to stunning. After stunning, the ECG revealed extrasystole and was irregular. By using an average current of 629 ± 180 mA (at approximately 360 V, 50 Hz AC), at least 91% of fish are effectively stunned with 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 fish were 11 ± 8 s, 20 ± 5 s and 23 ± 20 s, respectively. All fish recovered. In the third experiment, a group of seven catfish was head-only stunned followed by gill-cutting to kill them as a second procedure (ie after recovery from head-only stunning). No brain activity was seen on the EEG 12 ± 5 s after stunning. However, two fish showed responses to noxious stimuli after 2 min and 5 min. A second group of seven catfish was gill-cut only. 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 catfish 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, we recommended that the stunning and killing procedure should be optimised.

Keywords: *animal welfare, catfish,* Clarias gariepinus*, killing, slaughter, stunning*

Introduction

The present status of pre-slaughter and slaughter methods used by fish processors is causing increasing concern among governmental agencies, animal protection associations and consumers in Europe. The reason for this concern is that it has become clear that most industrial slaughter methods do not induce unconsciousness in fish without avoidable stress prior to killing. These methods may, therefore, affect the welfare of fish (Robb & 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 also, as the results of anatomical, physiological and behavioural studies of fish are very similar to the results of studies of 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 fish can experience pain or emotions. Nevertheless, he (Rose 2002) also reported that "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 changing public opinions, detrimental effects on welfare of fish at slaughter should be avoided. To achieve this, fish should be stunned (ie rendered unconscious) until death ensues without avoidable stress, pain or discomfort or effects on product quality. Stunning

should also result in sufficient immobility to facilitate the initiation of exsanguination (Blackmore & Delaney 1988).

The current pre-slaughter process used in the Netherlands for African catfish (*Clarias gariepinus*) comprises chilling to immobilise the fish prior to evisceration (Robb & Kestin 2002). It is unlikely that live chilling induces unconsciousness in African catfish immediately and without avoidable stress, as it has been reported to be stressful 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). An alternative method is electrical stunning, which results in immediate loss of consciousness in Atlantic salmon, eel and gilthead seabream (Van de Vis *et al* 2003). Methods to assess stunning of fish have recently been optimised (Kestin *et al* 2002; Van de Vis *et al* 2003). These studies reveal that reliable assessment involves the recording of brain activity with an electroencephalogram (EEG) and of heart activity with an electrocardiogram (ECG), in combination with the observation of behaviour.

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 somatosensory evoked response (induced by, for example, 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 centres are intact, but does not necessarily imply that the animal is aware of the stimulus.

The authors considered that the observation of behaviour alone is not sufficient for assessment of electrically induced unconsciousness. If sufficient current is administered through the head of an animal, a general epileptiform insult (during which all parts of the brain are stimulated) will occur. The epileptiform insult is characterised by rapid and extreme depolarisation of the neuronal membrane potential, and the resulting changes in overall brain activity differ between subjects (Kooi *et al* 1978). As measured on the EEG, such an insult consists of relatively low-amplitude waves increasing in amplitude during the tonic phase, and decreasing in frequency during the clonic phase to result ultimately in a period of strong depression of electrical activity (the 'exhaustion phase') (Lambooy 1982; Lambooy & Spanjaard 1982). Humans are unconscious during the three phases (tonic, clonic and exhaustion phases) of a general epileptiform insult. Moreover, the brain is in a stimulated condition for the duration of the three phases and unable to respond to stimuli. By analogy, it is supposed that other mammals also are unconscious and insensible for the duration of the epileptiform insult (Lopes da Silva 1983). On the basis of similarities in the basic structure of neurones and neuronal biochemistry (Kestin 1994; Verheijen & Flight 1997), it can be argued that this analogy is valid for fish. The objectives of this study were to determine in individual farmed African catfish the minimum current that is required to provoke immediate loss of consciousness, and to assess exsanguination with and without prior stunning of the fish to establish the time period before unconsciousness is induced.

Materials and methods

Fish

A number of heads of African catfish were dissected in order to determine the position of the electrodes for measurement of the EEG. Fifty-eight African catfish were then obtained from a commercial farm. The fish were fasted for three days before the experiment (for commercial reasons, eg to empty the guts and improve flavours) and then delivered to the laboratory, where they were placed in a tank containing aerated filtered tap water at 24°C. The experiment was performed with approximately 10 fish per day. During the experiment the fish were placed individually into a specially developed restrainer for recording of EEG and ECG, or placed in the water for monitoring of their behaviour when moving freely. After the experiment the fish were weighed and dissected to ascertain their gender.

Recording of EEG, ECG and behaviour of restrained African catfish

Prior to stunning, each individual fish was equipped with EEG electrodes. In order to facilitate the implantation of the electrodes the fish were 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 variable 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. Each individual African catfish was restrained by placing the lower jaw on the platform, inserting the metallic pallet into the mouth and then pressing the pallet 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 use of plastic tie-backs 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^{-1}; Astra Pharmaceutica BV, Zoetermeer, The Netherlands). Subsequently, holes were drilled in the skull, and silver EEG electrodes (6 mm long, 1.5 mm diameter) were positioned with one electrode 1 cm to the right and one electrode 1 cm to the left of the sagittal suture, 4 cm caudal to an imaginary line between the eyes. The earth electrode for the EEG and ECG was placed subcutaneously caudal to the dorsal fin. The steel ECG electrodes (35 mm long, 1 mm diameter) were placed subcutaneously, the first electrode caudal to the implantation of the left pectoral fin and the second electrode 2 cm caudal to the first.

The EEG and ECG were recorded for 1 min before and for 2 min immediately after stunning, and for 30 s at 5 min and 10 min after stunning, using 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

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interrupted and the applied voltage and current were recorded using a Mingograf 34 (Elema Schönander, Stockholm, Sweden) recorder.

Responses to noxious stimuli (ie needle scratches applied to the skin of the tail), as demonstrated by changes in the behaviour of the fish as well as by the EEG, were assessed at 30 s, 2 min, 5 min and 10 min after stunning. The EEG recordings were analysed for changes in the waveforms, frequency and depression of neuronal activity. The behaviour of the fish was monitored on video and assessed for the occurrence of tonic and clonic muscle spasms, exhaustion and recovery.

Head-only electrical stunning

Determination of minimum current

Forty-one fish were subjected to head-only electrical stunning for approximately 1 s, by application of voltages ranging from 150 V to 350 V (50 Hz AC). The current was delivered 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 the opening of the gill. The power supply (Stork RMS, Lichtenvoorde, The Netherlands) delivered constant voltages of 150 V, 200 V, 250 V, 300 V and 350 V (50 Hz AC). In addition, four fish were stunned by applying 600 V (50 Hz AC) for approximately 1 s to determine whether the period of loss of consciousness could be increased.

All but seven catfish were killed at the end of the experiment using a modified captive needle pistol (Lambooij *et al* 1999). The seven remaining fish were allowed to recover, as judged by return to normal activity on the EEG, and were 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 able to move freely in fresh water was observed after head-only electrical stunning. Prior to the stun, each individual fish was placed in a V-shaped device and restrained. The catfish were stunned with 350 V, as described previously. Immediately after stunning each fish was placed in a tank (100 cm × 50 cm × 60 cm) containing aerated tap water at 24°C. Behaviour was recorded on video and assessed afterwards for the occurrence of tonic and clonic muscle spasms, exhaustion and recovery. Following the experiment, the fish were killed using a modified captive needle pistol.

Exsanguination

Head-only stunning followed by gill-cutting

The seven African catfish that had been allowed to recover for 20 min following head-only stunning 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 seven catfish was restrained one by one, implanted with EEG and ECG electrodes and subsequently bled by gill-cutting. Recording of the EEG and ECG and observation of behaviour were as described in the previous sections.

Ethics

The experiments were approved beforehand by a Dutch governmental ethical committee.

Statistical analyses

With a confidence limit of 95%, taking into account the number of fish from which a reliable EEG had been obtained, 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 (mean ± standard deviation). It was observed that 32 were male and 26 female.

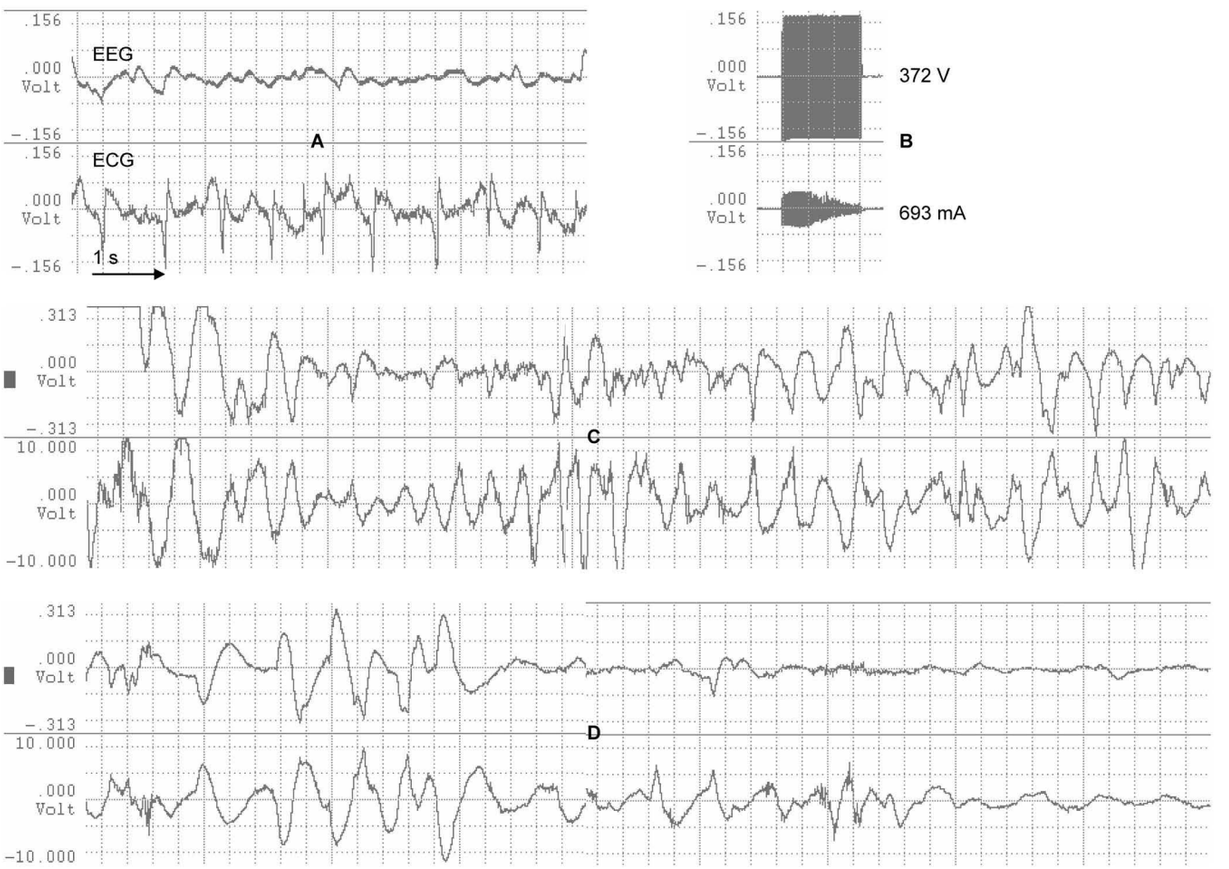
Head-only electrical stunning

Determination of minimum current

Head-only stunning by application of 150 V, 200 V, 250 V, 300 V and 350 V was performed to determine the minimum current for an instantaneous stun. The minimum current depends on the impedance of the body, which is related to the physics of the fish and cannot be changed. For each voltage, one catfish was used. The characteristics of a general epileptiform insult were not observed in the first four fish, demonstrating that the voltage required was at least 350 V. Subsequently, 37 African catfish were stunned with 362 ± 32 V (measured voltage, mean ± standard deviation), 629 ± 180 mA for a duration of 1.2 ± 0 s. The electrodes became disconnected in six out of 37 fish during this stunning procedure, but the successful ones showed the characteristics of a general epileptiform insult on the EEG (Figure 1). The tonic, clonic and exhaustion phases on the EEG were of durations 8 ± 3 s, 12 ± 7 s and 7 ± 5 s, 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 muscle spasms of the tail, the duration of which was, on average, 18 ± 6 s. Using the number of reliable EEGs obtained (n = 31) and a confidence limit of 95%, the probability 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 min^{-1} prior to stunning. After stunning, the ECG revealed fibrillation (Figure 1) for 18 ± 9 s. The heart rates measured at 0.5 min, 2 min, 5 min, and 10 min after stunning were 70 ± 19, 71 ± 19, 66 ± 16 and 66 ± 16 beats min^{-1}, respectively. The ECG showed extrasystole in 10 fish and was irregular in 13 fish after stunning.

Figure 1



The effect of stunning on the EEG and ECG. (**A**) Normal 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.

The current flow was 1235 ± 360 mA for 1.2 s in the four fish stunned with 600 V. The duration of the epileptiform insult was 58 ± 22 s and brain activity was depressed afterwards. The ECG showed extrasystole and an irregular heart rate.

Behaviour of freely moving African catfish

Ten catfish were placed in water immediately after electrical stunning with 673 ± 184 mA (~370 V) for 1.2 s. Analysis of the video tape allowed three phases to be distinguished: tonic and clonic muscle spasms in the horizontal plane, followed by tonic and clonic spasms in the horizontal and vertical planes, and subsequently an exhaustion phase. Recovery was defined as swimming smoothly forward or hanging down/drooping the body in the water. The durations of the three 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 for 20 min from head-only stunning, seven catfish were head-only stunned followed by gill-cutting to kill them. The applied currents were 651 ± 272 mA (n = 4) and 1327 ± 232 mA (n = 3) for 1.2 s using voltages of 350 V and 600 V, respectively. The cut was performed as soon as possible after the stun. Following the occurrence of a general epileptiform insult, no brain activity occurred after 12 ± 5 s. However, two 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^{-1} before the cut, and 0.5 min, 2 min, 5 min and 10 min following the cut it was 65 ± 29, 60 ± 17, 57 ± 21 and 50 ± 26 beats min^{-1}, respectively. The pattern was ischaemic (the ECG showed ST-devaluation and T-top inversion) in five out of seven fish and was 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 2–5 min after the cut. Nevertheless, responses to noxious stimuli could be recorded for at least 15 min after gill-cutting in all fish. After this period they were killed using a captive needle pistol. The heart rate before and 0.5 min, 2 min, 5 min, 10 min and 15 min after the cut was 82 ± 11, 84 ± 23,

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98 ± 24, 90 ± 12, 98 ± 30 and 80 ± 8 beats min^{-1}, respectively. The heart rate was irregular. The blood loss was 10 ± 2 g, which was 1.0% of the live weight.

Discussion

There is growing interest in sustainable farming of fish because of the problems associated with intensive livestock production and the increasing imbalance between the amounts of fish caught (limited by EU quota) and the rise in consumer demand for a variety of high-quality fish. On the basis of recent experience with fish farming, a number of potential problems can be indicated. From a consumer perspective, these are the perceived problems with residues from treatments such as 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 considerations affect acceptance of the product by consumers in two ways. The first concerns the emotional aspects of farming, stunning (or lack thereof), and slaughter. The second is the major impact that farming and slaughter practices may have 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 is maintained until death.

If sufficient current is administered through the head of an animal, a general epileptiform insult (during which all parts of the brain are stimulated) will occur. The brain activity pattern of such an insult has been well characterised in mammals using EEG (Lambooy 1982; Lambooy & Spanjaard 1982), and the same pattern was observed in African catfish, which indicates that the fish were unconscious during the general epileptiform insult. A minimum current, the level of 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). With a confidence level of 95%, at least 91% of African catfish are effectively stunned by applying 629 mA (at approximately 370 V, 50 Hz AC).

Observation of the behaviour of sheep after stunning reveals 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, causing the extension. Two phases were distinguished in eel: limited tonic and clonic spasms combined with much backward swimming, followed by heavy clonic spasms combined with uncoordinated movements such as jumping out of the water (Lambooij *et al* 2002). In African catfish, on the other hand, spasms in the horizontal and vertical plane were observed. The most common type of swimming found in the majority of fish consists of travelling waves of muscle contraction which increase in amplitude towards the tail. For carp, it has been reported (Spierts 1999) that white muscle fibres have a high contraction velocity and produce a high power output, but only for a short period of time (10–60 s). They use glycogen, which is broken down anaerobically. The red muscle fibres contract three times more slowly, metabolise lipids anaerobically, and are practically indefatigable (Spierts 1999). The observed behaviour during a general epileptiform insult has a duration of 51 ± 20 s. This suggests that most of the 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.

Neural and pharmacological studies 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 (γ-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 (ie cutting of the major blood vessels) following a stun should be carried out as quickly as possible when head-only stunning is being used, in order to avoid recovery after the stun (the time before brain responsiveness is lost as a result of sticking depends on the species; 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, continued to respond to a noxious stimulus. A positive response implies an intact afferent pathway to the higher brain centres, whereas a negative response provides unequivocal evidence of insensibility following stunning (Gregory & Wotton 1990). Evoked responses continued to be measurable for at least 15 min after gill-cutting without prior head-only stunning. The length of this period may be attributable to the limited blood loss of approximately 1% only. Blood loss is affected mainly by method of sticking, muscle contraction and gravity (Warriss & Wilkins 1987), which were not optimal during our experiments with African catfish. In rainbow trout (*Oncorhynchus mykiss*), it is assumed that the blood comprises 5% of the bodyweight, and that 66% of the blood is located in the white muscle (Hoar & Randall 1970). Slow exchange of blood between parts of the vascular system may also reduce the loss of blood resulting from gill-cutting. In addition, it is known that adrenaline and noradrenaline dilate the vessels and decrease the resistance to flow through the gills (Hoar & Randall 1970). Levels of these hormones may have been increased in these African catfish as a result of handling. It is therefore possible that, because of dilation of the vessels, only blood which was present in the gills or in the vessels close to them was obtained by gill-cutting.

Conclusions

It can be concluded from our results that, with a confidence limit of 95%, the chance of effectively stunning African catfish is between 0.91 and 1.00 when a current of 629 mA (~370 V, 50 Hz AC) is applied to the head. All catfish recovered after the stunning process.

For two out of seven fish, head-only stunning followed by gill-cutting resulted in continuation of response to noxious

stimuli for up to 5 min, as shown on an EEG, implying that afferent pathways to higher centres of the brain were still intact. In previous studies with electrical stunning of farmed eel, we have shown that this can be avoided (Lambooy *et al* 2002); therefore, optimisation of electrical stunning and killing for humane slaughter of African catfish is the subject of a future study.

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References

Blackmore DK and Delaney MW 1988 Slaughter of stock. Publication No 118. Veterinary Continuing Education. Massey University: Palmerston North, New Zealand

Clark RE and Squire LR 1998 Classical conditioning and brain systems: the role of awareness. *Science 280*: 77-81

Cook CJ, Devine CE, Gilbert KV, Smith DD and Maasland SA 1995 The effect of electrical head-only stun duration on electroencephalographic-measured seizure and brain amino acid neurotransmitter release. *Meat Science 40*: 137-147

Cook CJ, Maasland SA, Devine CE, Gilbert KV and Blackmore DK 1996 Changes in the release of amino acid neurotransmitters in the brains of calves and sheep after head-only electrical stunning and throat cutting. *Research in Veterinary Science 60*: 225-261

Gregory NG and Wotton SB 1990 Effect of stunning on spontaneous physical activity and evoked activity in the brain. *British Poultry Science 31*: 215-220

Hoar WS and Randall DJ 1970 *Fish Physiology. Volume IV: The Nervous System, Circulation and Respiration* pp 133-168. Academic Press: New York, USA

Hoenderken R 1978 *Elektrische bedwelming van slachtvarkens*. PhD thesis, State University of Utrecht, The Netherlands [Title translation: Electrical stunning of slaughter pigs]

Johnson NL and Kotz S 1969 *Discrete Distributions*. John Wiley: New York, USA

Kestin SC 1994 *Pain and Stress in Fish*. Royal Society for the Prevention of Cruelty to Animals: Horsham, West Sussex, UK

Kestin SC, Vis JW van de and Robb DHF 2002 Protocol for assessing brain function in fish and the effectiveness of method used to stun and kill them. *Veterinary Record 150*: 302-307

Kestin SC, Wotton SB and Adams S 1995 The effect of CO_2, concussion or electrical stunning of rainbow trout on fish welfare. In: Scheire K, Apselagh L and Jonkeere H (eds) *Quality in Aquaculture: Proceedings of the International Conference in Aquaculture Europe 1995, Trondheim, Norway* pp 380-381

Kooi KA, Tucker RP and Marshal RR 1978 *Fundamentals of Electroencephalography, Edn 2*. Harper & Row: New York, USA

Lambooy E 1982 Electrical stunning of sheep. *Meat Science 6*: 123-135

Lambooy E and Spanjaard WJ 1982 Electrical stunning of veal calves. *Meat Science 6*: 15-25

Lambooy E, Mens WBJ and Wimerma Greidanus TjB van 1985 Vasopressin and oxytocin in plasma of veal calves, sheep and pigs after high voltage electrical stunning. *Meat Science 14*: 127-138

Lambooij E, Pieterse C, Potgieter CM, Snyman JD and Nortjé GL 1999 Some neural and behavioural aspects of electrical and mechanical stunning in ostriches. *Meat Science 52*: 339-345

Lambooij E, Vis JW van de, Kloosterboer RJ and Pieterse C 2002 Evaluation of head-only and head to tail electrical stunning of farmed eels (*Anguilla anguilla, L*) for development of a humane slaughter method. *Aquaculture Research 33*: 323-331

Lopes da Silva HF 1983 The assessment of consciousness: general principles and practical aspects. In: Eikelenboom G (ed) *Stunning of Animals for Slaughter* pp 3-12. Martinus Nijhoff: The Hague, The Netherlands

Nieuwenhuys R, ten Donkelaar HJ and Nicholson C 1998 *The Central Nervous System of Vertebrates. Volume 2: Holosteans and Teleosts* pp 759-913. Springer Verlag: Heidelberg, Germany

Overmier JB and Hollis KL 1990 Fish in the think tank: learning, memory and integrated behaviour. In: Kesner RP and Olson DS (eds) *Neurobiology of Comparative Cognition* pp 205-236. Lawrence Erlbaum: Hillsdale, NJ, USA

Robb DFH and Kestin SC 2002 Methods used to kill fish: field observations and literature reviewed. *Animal Welfare 11*: 269-282

Rose JD 2002 The neurobehavioral nature of fishes and the question of awareness and pain. *Reviews in Fisheries Science 10*: 1-38

Skjervold PO, Fjæra SV, østby PB and Einen O 2001 Live-chilling and crowding stress before slaughter of Atlantic salmon (*Salmo salar*). *Aquaculture 192*: 265-280

Sneddon LU 2002 Anatomical and electrophysiological analysis of the trigeminus nerve in a teleost fish, *Oncorhynchus mykiss*. *Neuroscience Letters 319*: 167-171

Spierts ILY 1999 *Swimming and muscle structure in fish*. PhD thesis, Wageningen University, The Netherlands

Van de Vis JW, Kestin SC, Robb DFH, Oehlenschläger J, Lambooij E, Münkner W, Kuhlmann H, Kloosterboer RJ, Tejada M, Huidobro A, Otterå H, Roth B, Sørensen NK, Akse L, Byrne H and Nesvadba P 2003 Is humane slaughter of fish possible for industry? *Aquaculture Research 34*: 211-220

Verheijen FJ and Flight WFG 1997 Decapitation and brining: experimental tests show that after these commercial methods for slaughtering eel *Anguilla anguilla* (L.), death is not instantaneous. *Aquaculture Research 28*: 361-366

Wageneder FM and Schuy St 1967 Electro-therapeutic sleep and electro-aneasthesia. *Proceedings of the First International Symposium of the Excerpta Medica Foundation, Graz, Austria, 1966*. Excerpta Medica Foundation: Amsterdam, The Netherlands

Warriss PD and Wilkins LJ 1987 Exsanguination of meat animals. In: Carter HE and Carter VR (eds) *Pre-Slaughter Stunning of Food Animals* pp 150-158. Royal Society for the Prevention of Cruelty to Animals: Horsham, Sussex, UK

Wiepkema PR 1997 The emotional vertebrate. In: Dol M, Kasanmoentalib S, Lijmbach S, Rivas E and van den Bos R (eds) *Animal Consciousness and Animal Ethics* pp 93-102. Van Gorcum & Comp BV: Assen, The Netherlands