

# NEURAL, PHYSIOLOGICAL AND BEHAVIOURAL OBSERVATIONS AFTER HEAD-ONLY ELECTRICAL AND CAPTIVE NEEDLE STUNNING OF AFRICAN CATFISH (*Clarias gariepinus*).

E. Lambooi<sup>1</sup>, J.W. van de Vis<sup>2</sup>, R.J. Kloosterboer<sup>2</sup>, M.A. Gerritzen<sup>1</sup>

<sup>1</sup>Institute for Animal Science and Health (ID-Lelystad), P.O. Box 65, 8200 AB Lelystad, The Netherlands

<sup>2</sup>Netherlands Institute for Fisheries Research (RIVO), P.O. Box 68, 1970 AB IJmuiden, The Netherlands

## Background

The suborder of *Clariidae* is found in Africa and Asia. African catfish (*Clarias gariepinus* is member of the suborder and can be farmed at high stocking densities. The fish is adapted to its habitat under wet and dry circumstances, as it is an air-breathing fish. The major characteristics of the fish are: the presence of two branchial chambers for air-breathing, a tight and smooth skin without scales, long barbels in the mouth region and an opening of the gills that is wide and has a deep indentation with a firm operculum. The branchial chambers are derived from and located above the gills in the dorsal part of the skull. In each branchial chamber there are anterior and posterior respiratory trees. In the Poland, Hungary and the Netherlands African catfish are farmed as food fish. It is known that slaughter can adversely influence both product quality and welfare of slaughter animals. The concept of animal welfare has gained acceptance for mammals, however, fish welfare is a relatively new concept. Based on similarities in basic structure of neurones and neuronal biochemistry to that of mammals, and similarities in stress responses and behaviour to that of higher vertebrates it is likely that the welfare of fish can be affected, especially at slaughter (Kestin, 1994). It is therefore recommended to render fish unconscious and insensible instantaneously prior to killing (Verheijen and Flight, 1997; Van de Vis et al, submitted). An assessment of current slaughter methods used for African catfish revealed that the fish are not stunned without avoidable stress prior to killing (Lambooi et al, unpublished results). Stunning of 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 during 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 without a detrimental effect on the welfare of the animal and the meat quality of the carcass.

## Objective

The objective of the study was to assess pre-slaughter stunning methods of African catfish. Two methods were studied: head-only electrical stunning and the use a captive needle pistol.

## Methods

### Fish

Heads (n= 5) of African catfish were obtained and anatomically dissected to determine the position of the electrodes for measurement of the EEG (electro-encephalogram) and position of the captive needle pistol. Sixty-seven African catfish were used for stunning. Seven days before the experiment the required number of catfish were fasted and after that delivered to the laboratory. The catfish were kept in a tank containing tap water at 24 °C. After the experiment the fish were weighed and dissected to determine the sex. The experiment was performed with 6 fish per day.

### Registration of EEG and behaviour of restrained African catfish

Prior to stunning the fish was equipped with EEG electrodes. In order to facilitate the implantation of the electrodes the fish were restrained. The restrainer was developed especially for African catfish.

Prior to implantation of the electrodes the fish were locally anaesthetised using Xylocaine® 10% spray (lidocaine 100 mg/ml; Astra Pharmaceutica BV, Zoetermeer, Netherlands). For the implantation 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 to the left of the sagittal suture and 4 cm caudal to the imaginary line between the eyes. The earth electrode for the EEG was placed subcutaneously caudal to the dorsal fin. The EEG was recorded during 1 minute before and during 2 minutes immediately after stunning and during 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 recordings were later analysed for changes in the waveforms, frequency and suppression. The behaviour of the animals was monitored for the occurrence of tonic, clonic cramps and exhaustion.

### Stunning

**Head-only as a first stunning method.** A number of individually restrained 38 fish were subjected to head-only electrical stunning by applying 150 to 350V (50 Hz a.c.) for 1 s. 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. In order to assess the effect of electrical stunning on the behaviour of freely moving catfish, 8 animals were stunned head-only for 1 s. On basis of the results from the previous experiment the required voltage for this experiment was established. Subsequently, the animals were placed immediately in fresh water.

**Captive needle as second stunning method.** A number of 21 electrically stunned animals that recovered were stunned for a second time and killed by insertion of air under pressure in the brains during 1.5 s, by using the captive needle pistol as described by Lambooi et al., (1999). 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, which pressed the air in 3 directions radial 120°, where one direction was caudally towards the spinal cord.

**Captive needle as first stunning method.** For a sound assessment of captive needle stunning 21 fishes were stunned for the first time with this technique. In order to assess the effect of captive needle stunning on the behaviour of freely moving catfish, 8 animals were stunned and placed in fresh water.

## Preliminary results and discussion

### Head-only electrical stunning

The weight of the fishes (21 males and 17 females) was between 900 and 2100 g. When head-only stunning was applied at 150, 200, 270 and 320 V with 4 catfish the characteristics of a general epileptiform insult were not observed, which indicates that the animals were still conscious. On the EEG an epileptiform 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. During these phases an animal is considered to be unconscious and insensible (Lopes da Silva, 1983). In the next trial another 34 catfish were stunned at  $362 \pm 32$  V,  $629 \pm 180$  mA for  $1.2 \pm 0$  s, respectively. It appeared that the characteristics of a general epileptiform insult were observed on the EEG. The tonic, clonic phase and exhaustion phase were  $8 \pm 3$ ,  $12 \pm 7$  and  $7 \pm 5$  s on the EEG, respectively. For 11 fish a distinct exhaustion phase was not clear. The total duration of the insult was  $23 \pm 8$  s, which is rather short. A similar duration was also observed in broilers and ostriches (Lambooij et al, 1999).

The durations of the tonic, clonic and exhaustion phases in the free swimming fish were  $11 \pm 8$ ,  $20 \pm 5$  and  $23 \pm 20$  s. All fish recovered after stunning which implies that head-only stunning should be used in combination with a killing method, e.g. exsanguination. Taking into account existing knowledge on bleeding times of other species (Blackmore & Delany, 1988), it is likely that a combination of head-only stunning and exsanguination should not be introduced in practice for welfare reasons.

### Captive needle stunning

The weight of the fish (23 males and 19 females) was between 900 and 2000 g. Due to positioning the pistol and the shot artefacts were observed on the EEG for a few seconds. Subsequently, the EEG revealed theta and delta waves (4–8 Hz and < 4 Hz, respectively) and spikes tending to no brain activity in 12 fish after  $10.5 \pm 9.2$  s and in 11 animals after  $16.2 \pm 17.4$  s, after the application of captive needle stunning as first and second method, respectively. The stunned fish showed some slow muscle cramps for  $2.1 \pm 3.3$  s and  $6.3 \pm 9.8$  s after the first and second time of stunning, respectively. No responses to pain stimuli on the EEG and in behaviour were observed. Similarities in basic structure of neurones and neuronal biochemistry (Kestin, 1994; Verheijen and Flight, 1997) supports the assumption that catfish may be unconscious and insensible as gauged by analogy with similar EEG changes in man and other vertebrates (Lopes da Silva, 1983). Also for broilers, ostriches and eel the presence of theta and delta waves tending to no brain activity on the EEG and the absence of responses to pain stimuli on the EEG and in behaviour were observed after captive needle stunning. These results indicated that the latter animals lost consciousness immediately and irreversibly (Lambooij et al, 1999; 1999; 2002).

Observation of free catfish revealed that the animals showed some uncoordinated swimming movements after stunning. It is known that the movements of catfish are also co-ordinated by the spinal cord (Spierts, 1999). Since co-ordination was lost, this suggests that the spinal cord was at least partly damaged.

### Conclusion

The results showed that captive needle stunning is effective. For prevention of movements after stunning, which may hinder further processing, it is recommended to chill the animals immediately after stunning.

It may be concluded from our results that African catfish were effectively stunned with a current of  $629 \pm 180$  mA and  $362 \pm 32$  V for  $23 \pm 8$  s. The animals, however, recovered. Taking into account the short duration of the loss of consciousness it cannot be recommended to use head-only stunning followed immediately by exsanguination in industry.

### Literature

- Blackmore, D.K. and Delany, M.W., 1988. Slaughter of stock. Publ. No. 118. Vet. Con. Ed. Massey University. Palmerston North, New Zealand.
- Kestin, S.C., 1994. Pain and stress in fish. Royal Society for the Prevention of Cruelty to Animals
- Kooi, K.A., Tucker, R.P. Marshal, R.R., 1978. Fundamentals of electro encephalography. Second ed. Harper and Row, New York.
- Lambooij, E., Pieterse, C., Potgieter, C.M., Snyman, J.D. & Nortjé, G.L., 1999. Meat Sci. 52, 339-345.
- Lambooij, E., Pieterse, C., Hillebrand, S.J.W. and Dijksterhuis, 1999. Poultry Sci. 78, 600-607.
- Lambooij, E., van de Vis, J.W., Kloosterboer, R.J. and Pieterse, C., 2002. Aquaculture in press.
- Lambooij, E., van de Vis, J.W., Kloosterboer, R.J. and Pieterse, C., 2002. Aquaculture Res. in press.
- Lopes da Silva, H.F., 1983. In: Stunning of animals for slaughter (G. Eikelenboom ed.) pp 3-12, The Hague: Martinus Nijhoff.
- Spierts, I.L.Y., 1999. Swimming and muscle structure in fish. Thesis, Wageningen
- Van de Vis, J.W., Kestin, S.C., Robb, D.F.H., Oehlenschläger, J., Lambooij, E., Münkner, W., Kuhlmann, H., Kloosterboer, R.J., Tejada, M., Huidobro, A., Otterå, H., Roth, B., Sørensen, N.K., Akse, L., Byrne, H. and Nesvadba, P. Submitted to Aquaculture Research
- Verheijen, F.J. and Flight, W.F.G., 1997. Aquaculture Research, 28, 361-366.