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EFFECT OF DIRECT CURRENT ELECTROANAESTHESIA ON NILE TILAPIA (*Oreochromis niloticus*)

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ABSTRACT

The present study was aimed to assess the effects of direct current (DC) on inducing the stage-IV anaesthesia in Nile tilapia (*Oreochromis niloticus*). Underwater DC of different magnitudes *viz.*, 30, 60, 90 and 200 V applied for 25 sec in all, except 200 V wherein exposure time varied from 15 to 30 sec. Notably, the exposure to 30, 60, and 90 V for 25 sec did not induce anaesthesia; while 200 V DC lead to successful anaesthesia. Opercular movements in fishes exposed to 200 V after 1 min of recovery was significantly higher than before exposure value. Opercular movements slowed down gradually; and after 15 min of recovery no significant difference in opercular movement was observed. Normal swimming activity and reactivity to tactile stimuli were restored after 10 min of recovery. The results suggest that DC at 200 V was effective in inducing anaesthesia in *O niloticus* with fast induction (15-30 sec) and recovery period (20-55 sec). This revealed that the electro-anaesthesia technique used in this study offer a cost-effective, safe and eco-friendly alternative of chemical anaesthetics.

Keywords: Anaesthesia, direct current, opercular movements, induction, recovery period

INTRODUCTION

Anaesthesia is a widely used for stress reduction in aquaculture activities like handling, tagging, transportation, etc. (Palic *et al*., 2006; Kim *et al*., 2017). Anaesthesia is also used to minimize the possibility of injury and trauma in fishes during the procedures which need a complete or partial sedation of fish (Ross and Ross, 1999). Numerous methods are practiced for fish anaesthesia, but several factors like cost, effectiveness, safety to fish, human and environmental impact, etc. have to be considered during selection of an anaesthetic (Pirhonen and Schreck, 2003). Reactions of fish in electric field depend on fish species, size, conductivity and temperature of the ambient water, duration of electric shock and orientation of fish in field.

Two techniques frequently used in anaesthesia are chemical anaesthesia (wherein chemical agents are used) and physical anaesthesia (wherein electricity, temperature or gas pressure are used (Vandergoot *et al*., 2011). Chemical anaesthesia can be achieved by using several agents including tricaine methane sulphonate (MS-222), benzocaine, quinaldine sulphate, methomidate and clove oil (Velisek *et al.*, 2011). Among these, MS-222 is the only FDA approved chemical with 21-day mandatory withdrawal period (Trushenski *et al*., 2012b). During this 21-day period fishes are held in post-treatment captivity for dissipation of tissue drug residues (Trushenski and Bowker, 2012). The most important parameter of an ideal anaesthetic is appropriate induction and rapid recovery of fish with minimum stress. Other parameters like cost, simplicity of use and safety to human, fish and environment are to be considered before the selection of any anaesthetic. The anaesthetic should not have any toxic effect on fish and handler. It should be biodegradable and have the property to get cleared from the tissue after exposure (Ackerman *et al*., 2005). The use of electricity has interesting advantages over chemical anaesthetics as it is cost-effective and free of chemical residue in fish body and environment, with fast recovery period (Ackerman *et al.*, 2005; Renault *et al*., 2011; Rous *et al*., 2015). Electricity has less effect on plasma and tissue-electrolyte changes in comparison to chemical anaesthetic, MS-222 (Jenning and Looney, 2011). The stages of anaesthesia depicted by Summerfelt and Smith (1990) have largely been referenced (Trushenski *et al*., 2012a,b; Rad *et al*,, 2016; Reid *et al.*, 2019). According to Summerfelt and Smith (1990) the total loss of muscle tone, slow but regular opercular rate and loss of spinal reflexes indicate the attainment of stage IV anaesthesia. Further, the stage V and VI anaesthesia is associated with low heart rate along with very low respiratory rate and cessation of respiratory movement. Extended maintenance at these stages may cause the death of fish due to respiratory acidosis as a result of diminution of blood oxygen (Ackerman *et al.,* 2005). In this study electric field was switched off immediately after attaining stage IV anaesthesia to minimize such hurtful effect on fish.

Oreochromis niloticus (Linnaeus, 1758) is found in various parts of Africa and widely distributed all over the world. This omnivorous hardy fish presently exists in several tropical and sub-tropical countries and the feral tilapia populations now exist in at-least 114 countries (Deines *et al*., 2016). Tilapia is the 2^{nd} most important farmed fish after carps (Fitzsimmons *et al.*, 2010). It is a popular food fish in India. Venturini *et al.* (2018) studied the efficacy of different frequencies for electronarcosis of *O. niloticus* as stunning method to ensure animal welfare and good meat quality. Many studies have documented the effect of electric shock on different fish species (Vandergoot *et al.*, 2011; Trushenski *et al.,* 2013; Baaberoo *et al.*, 2016; Durhack *et al*., 2020) but information is inadequate about the effectiveness of direct current anaesthesia on *O niloticus*. The present study was aimed to assess the effectiveness of underwater direct current employed at various volts on stage IV anaesthesia of *O. niloticus;* and to find out the induction and recovery period. The behavioural responses and opercular movements in electric field were also monitored.

MATERIALS AND METHODS

Experimental subjects and design

The experiments were conducted on Nile tilapia (*Oreochromis niloticus*) collected in March 2020 from a culture pond in Kolkata (India) with specifications: length: 151.88 ± 20.98 mm; weight: 59.65 ± 24.72 g and age: 2-3 months. They were housed in a 40 L PVC tank for a week and maintained in good health on a formulated feed diet (Optimum, Thailand). The tank water was oxygenated continuously through an air pump and temperature maintained between 28-30°C. The water conductivity was in the range of 167-175 $\mu S\ cm^{-1}$, pH 8.1-8.2, salinity 0.1 ppt (parts per thousand) and dissolved oxygen 7.1-7.5 $mg\ L^{-1}$. All the experiments were conducted in an indoor insulated glass tank of 120 x 28 x 30 cm size. To ensure a homogenous electric field, two aluminum plate electrodes (28 cm long, 27 cm wide and 2 mm thick) were placed at the two longest extremities of glass tank. Two electrodes were placed vertically at the bottom plane of tank and parallel to each other at the two longer extremities of glass tank.

O. niloticus was subjected to underwater direct current (DC) at 30, 60, 90 and 200 V. Exposure period was 25 sec in case of 30, 60 and 90 V. However, in case of 200 V, the exposure time varied from 15-30 sec as the electric field was applied until the attainment of stage IV anaesthesia. Induction period and recovery period was recorded for each fish (Table: 1). During experiments, one fish was randomly selected at a time, captured quickly (< 1 min) and transferred one at a time to the experimental tank, confined in the area between electrodes and allowed to remain for 5 min to become adopted to the new environment. A single fish was considered as experimental unit and for each applied voltage type, total 20 fishes were used (n = 20). Each fish was monitored for the time at which stage IV of sedation was achieved (Summerfelt and Smith, 1990). Stage IV of sedation is associated with the total loss of equilibrium, muscle tone and responsiveness to visual and tactile stimuli with reduced but steady opercular ventilation. After the loss of equilibrium, the fish was considered to reach the stage IV of sedation when there was no response to the manual tactile stimuli. Electricity was switched off immediately after attaining the stage IV anaesthesia. Then the fish was monitored for the time period taken for recovery of equilibrium and normal swimming. When normal swimming was regained, fish was transferred to the holding tank and monitored for survival for 48 h.

The reactions were recorded by means of a video camera (Nikon D5300) fixed at an overhead position of water tank. Opercular movements (beat min^{-1}) was noted by observing the movements before exposure to the electricity during stage IV anaesthesia, 1 min after regaining the equilibrium, and 10 min after recovery in case of 200 V exposure. In case of 30, 60 and 90 V exposure only before exposure and 15 min after exposure opercular movements were recorded.

Power supply and electrical parameters

A 0.5 KVA AC to DC inverter, capable of supplying 20 to 230 volts DC was used as a source of power supply during the experiments. The output voltage was stepped down by the introduction of three variable resistances (rheostat) of 500, 500 and 1000 Ω in series, between DC output terminals of the inverter and electrodes. This facilitates gradual rising of field intensity. The applied voltage to the electrode was measured by AVO meter (Universal Avo Meter, Model 8 MK II). The density of current in the field was measured with an electrically insulated probe of 1 cm^{-2} using a multimeter (Fluke 115). The copper plates of the probe were separated by a distance of 1 cm and capable for measurement in µA scale. The conductivity of water was checked by a conductivity meter (Bionics, BST-CT57) and also by an Ohmmeter in µ-Siemens and Ohm scale. A thermometer was used for recording the water temperature of experimental tank before the start of experiment.

Statistical analysis

Paired t test was used to compare the opercular movements during anaesthesia, 1 min after attaining equilibrium and 10 min after recovery with opercular movements before exposing to electric field.

RESULTS AND DISCUSSION

The fishes never tried to escape the experimental tank during exposure to the externally applied direct current in the range of 30, 60, 90 and 200 V. There was no reaction or behavioural change in fishes when exposed to 30 V for 25 sec. At 60 V fishes exhibited a jerk of body with extended fin. When exposed to 90 V fishes started to move violently between the electrodes but there was no loss of equilibrium after 25 sec exposure. All the fishes were anaesthesized when exposed to 200 V for 15-30 sec. During exposure to electric field, the fishes showed a clonic phase of epileptiform seizure with extended fin away from the body, closed mouth and slower opercular movement. Fishes lost their equilibrium, were upside down and there was no reaction to tactile stimuli (touched by a stick to the lateral line). This ensured that fish had reached stage IV of anaesthesia.

Opercular movements was significantly lower during anaesthesia ($P<0.05$). Opercular movements in all fishes (200 V exposure) after 1 min of recovery was significantly higher ($P<0.05$) than that of before exposure (Fig. 1). Opercular movements slowed down gradually and after 15 min of recovery there was no significant difference in opercular movements ($P>0.05$) with that of before exposure (Fig. 1). After 10 min of recovery, normal swimming activity and reactivity to tactile stimuli were regained completely. In case of 30 V (before, 98.20 ± 13.24; after 97.21 ± 12.32), 60 V (before 96.20 ± 13.24; after 97.21 ± 12.32), 90 V (before, 98.20 ± 13.24; after 100.21 ± 9.32) exposure there was no significant difference between the before exposure and 15 min after exposure opercular movement values. In all the applied voltage except 30 V fish appeared more pale and whiter in colour during the exposure period. They regain normal body color within 10 sec after turning off the electric field. There is no mortality instantaneously after the exposure to the electric field and not even within the 48 h follow up period.

Table 1: Induction and recovery period of fish at 200 V applied DC

Applied voltage	Total length of the fish (mm)	Exposure period (sec)	Induction period (sec)	Recovery (sec.)
30 V	113-195	25	-	-
60 V	113-198	25	-	-
90 V	113-198	25	-	-
200V	113-198	15-30	15-30	20-55

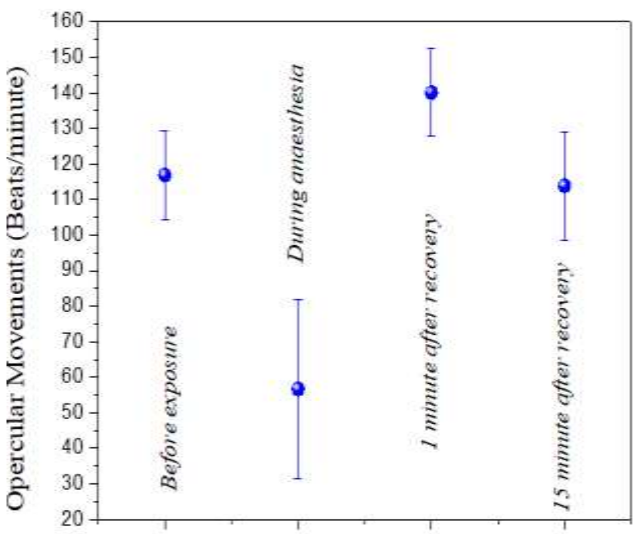

Fig. 1: Opercular beats min^{-1} of *O. niloticus* before and after exposure to 200 V electricity

During normal aqua-culture activities, anaesthesia is applied to reduce the unfavourable upshots of stress. Some activities like sorting, weighing, injections, tagging, blood sample collection, etc. require anaesthesia with faster induction and recovery times (Sattari *et al.*, 2009). The induction period is the time starting from the first exposure until the total loss of equilibrium with slow but regular ventilation (Jolley *et al*., 1972). The present study indicated that DC at 200 V was effective in inducing anaesthesia in Nile Tilapia with 15-30 sec of induction period. Different ranges of induction period have been reported for various species like 0.2 ± 0.1 min induction time and 3.8 ± 0.3 min recovery time in ship sturgeon *Acipenser nudiventris* (Baaberoo *et al*., 2016). *Rachycentron canadum* subjected to 100 V, 30 Hz pulsed direct current was reportedly sedated with 5 sec exposure with recovery time of 08 ± 0.1 min (Trushenski, 2012b), while in *Cyprinus carpio* the induction time of 37.73 ± 4.58 sec and recovery time of 4.40 ± 1.27 was recorded while exposed to direct current of 0.5 V.cm^{-1}. (Rad *et al.*, 2016). The present study indicates that the induction and recovery periods were similar to the previously observed ranges though in different species (Trushenski *et al.*, 2012a).

Removal of the fish while in narcosis or at the beginning of tetany from electric field either by netting or by switching off the current aids in faster recovery (Beaumont, 2016). In our experiments, the electric field was switched off immediately after the fish attained anaesthsia. This helps in faster recovery and prevents tetany. Unconsciousness during narcosis is may be due to the overstimulation of brain that brings either a epileptiform response or suppresses brain function which ultimately results in dysfunction of brain and makes it unable to respond to stimuli (Lambooij *et al.*, 2013). All the fishes in our study recovered within 55 sec; the fishes were ready to be released or to be used as food fish as there was no chemical residue. No visible reaction in *O niloticus* when subjected to 30 V DC probably indicates that the fishes were probably unable to perceive electric filed. The 25 sec exposure was not sufficient to induce anaesthesia in Nile Tilapia subjected to 30, 60 and 90 V. However, longer exposure to low voltage was reported to cause death of half of the total fish under study (Robb *et al*., 2002).

Respiratory activity of fish is the first physiological response to be affected after exposure to stress. The opercular movement has been used as an indicator of consciousness after electrical stunning especially for trout and salmon (Lines *et al.*, 2003). Opercular movements were significantly reduced or even ceased during anaesthesia. This may be due to the lactic acid accumulation due to rapid muscular contraction before attaining anaesthesia. Reduction of opercular movements by 50-70% in electric field has also been observed in *Oreochromis niloticus* by Robinson (1984). Significantly higher opercular rates after 1 min of attaining equilibrium indicates that probably the fish tries to overcome the oxygen deficiency faced during anaesthesia. *Piaractus mesopotamicus* when subjected to 100 V DC exhibited rapid opercular movements after 6 min of recovery and persisted until 10 min of recovery (Filho *et al.*, 2016). In our study after 15 min of recovery there was no significant difference in opercular movements. In accordance with Ross and Ross (2008) all the fishes are able to change their body colour to some extent as a response to stress by controlling the distribution of pigment in the chromatophores of skin both by nervous and pituitary hormonal means. Fading of body color of Nile Tilapia in all fishes when exposed to 60 V, 90 V and 200 V is may be due to the stress faced by the fish due to exposure to the externally applied electric field. Though there was no mortality within 48 h of post-exposure follow up period, it may be possible that fishes used in our study may have been susceptible to vertebral injuries or tissue damages (Snyder, 2003) which was not assessed in this study. However, no mortality immediately after the study or within 48 hours follow up period indicates that injuries if occurred were minor.

Conclusion: The results suggested that DC at 200 V was effective in inducing anaesthesia in *O. niloticus.* Fast induction and rapid recovery period indicated that the electroanaesthesia technique used in this study may offer a cost-effective, safe and ecofriendly alternative to chemical anaesthetics. Further studies on post-release behavioural response, physiology and neuro-physiologic response of electroanaesthesia on different fish species is recommended to improve the animal welfare.

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Conflict of interest: The authors do not have any conflict of interest to declare.

Ethical Statements: The experimental animals were taken care off well. For conducting experiments with the fishes or lower invertebrate any ethical clearance from local or national permitting authority is not required in India (according to “Breeding of and Experiments on Animals (Control & Supervision) Rules, 1998”, amended in 2001 & 2006, formulated by CPCSEA).

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