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Original Article

On the investigation of electroanaesthesia in *Labeo rohita* under DC and PDC electric field

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Abstract
This study investigates electroanaesthesia under Direct Current (DC) and three low frequency Pulsed Direct current (PDC) of 1 Hz, 3 Hz and 6 Hz in *Labeo rohita*. All the current types in this study were effective in anaesthetizing *L. rohita* with fast induction and rapid recovery period in all fishes. The threshold value of voltage gradient (V cm^{-1}) for inducing anaesthesia was higher in DC than PDCs. Opercular movements were significantly lower during anaesthesia, significantly higher one minute after recovery in all current types and reduced significantly in DC, PDC 1 Hz and PDC 3 Hz after 10 min of recovery from anaesthesia. Blood glucose level returned to the near control values after 8 hours of exposure in all current types in this study. DC sharp rise and PDC 3Hz may be preferred for inducing anaesthesia considering the recommended criteria of anaesthetic induction and recovery time. Result of this study indicates that electroanaesthesia technique with fast induction and rapid recovery may proffer a cost effective, safe and ecofriendly alternative for aquaculture activities in *L. rohita*.

Keywords: Anesthesia; electroanaesthesia; induction; *Labeo rohita*; recovery

1 | INTRODUCTION

Anaesthesia is an important technique used to reduce stress in various aquaculture activities including sampling, manage fisheries, tagging, collection of species specific data, collection of egg / milt for artificial spawning (Palić *et al.* 2006; Ross and Ross 2008; Velisek *et al.* 2005). Fishes are generally stressed by handling and transport which ultimately results in immune-suppression, injury or even mortality. It is indispensable to anaesthetize fish prior to performing even a simple task as struggling of fishes during capture and handling also have an effect on the physiology and behavior (Ross and Ross 2008). In addition to stress reduction, anaesthetics are used in aquaculture field during transportation to avoid injury and to reduce dissolved oxygen consumption (Coyle *et al.* 2004).

The most important parameter of an ideal anaesthetic is appropriate induction and rapid recovery with minimum stress on the fish. Besides that, other parameters like cost, simplicity of use and safety to human, fish and the environment must be considered before selection of the anaesthetic (Pirhonen and Schreck 2003; Ackerman *et al.* 2005; Ross and Ross 2008). The anaesthetic should not have any toxic effect on the fish and the handler. It should be biodegradable and should have the property to get clear from the tissue after exposure (Ackerman *et al.* 2005). The effect of an anaesthetic depends on numerous factors including the concentration of chemical, ambient water temperature, fish species and size (Soto and Burhanuddin 1995; Ross and Ross 2008). Several chemical agents have been used as anaesthetic for fish such as tricaine methanesulfonate (MS 222), clove oil (Eugenol), carbon di-oxide (CO_2), quinaldine, benzocaine-hydrochloride, and carbonic acid (Ackerman *et al.* 2005; Palić *et al.* 2006; Velisek *et al.* 2005). Among the all, MS-222 is the most commonly used and the only US Food and Drug administration approved anaesthetic agent for the food fish. However, there is a recommended and mandatory withdrawal period for 21 days for MS-222 where the exposed fish must be held in captivity for removal of the drug residues from tissues before human consumption or releasing them back to nature (Trushenski and Bower 2012). These chemical agents are used to minimize the harmful outcomes of stress responses (Summerfelt and Lynwood 1990). However, there were some evidences of unwanted and traumatic post exposure upshots of chemical anaesthetic agents. Arnolds *et al.* (2002) reported that MS-222 alters the brain current required to extract an action potential of supramedullary / dorsal neurons of cunner (*Tautogolabrus adspersus*). According to Palić *et al.* (2006) MS-222 and eugenol cannot avert the cortisol induced stress response of the fish and MS-222 is not able to prevent the degranulation of neutrophil primary granules in fish. On the other hand, CO_2 is not an approved fish anesthetic and may not be used effectively for all fishes. Quinaldine, clove oil, benzocaine-hydrochloride may only be used for research purpose (Trushenski and Bower 2012).

Due to the limitations of chemical anaesthetics fishery professionals are presently exploring cost effective alternative. In the recent past electroanaesthesia has attained interest of fishery professionals as a potential alternative (Ackerman *et al.* 2005; Renault *et al.* 2011; Rous *et al.* 2015). Electroanaesthesia is reported to have very less effects on plasma and tissue electrolytes in contrast with MS-222 (Jenning and Looney 1998). Electrofishing has been used as popular survey techniques for decades (Kynard and Lonsdale 1975). Generally alternative current (AC), direct current (DC) and different frequencies of pulsed AC and DC can be used for electrofishing (Beaumont 2016). This technique has been later modified particularly for immobilizing fish for transport, handling, surgery and other reasons (Jennings and Looney 1998; Zydlewski et al. 2008; Trushenski and Bower 2012). The response of fish to electric field depends on intensity of the field, duration of exposure, water temperature, conductivity, fish size and species.

Ross and Ross (2008) used the phrases “electroanaesthesia” or “electronarcosis” for the effects of AC electric field and “galvanonarcosis” for the effects of the DC and pulsed DC electric field. However the terms ‘anaesthesia’, ‘electrosedation’, and ‘immobilization’ are used interchangeably for fishes (Trushenski and Bower 2012). The terms ‘narcosis’ and ‘anaesthesia’ have been considered synonymous (Reid *et al.* 2019) and the term ‘anaesthesia’ is used throughout this article.

Electroimmobilization may have several beneficial effects in comparison to chemical sedatives. Electricity offers quicker recovery time in comparison with chemical anaesthetics with lower post anaesthetic effects on fish biochemistry and physiology (Gosset and Rivers 2004). It is cost effective, eradicates the problem of withdrawal periods, chemical residues and ecological pollution (Sattari *et al.* 2009). Moreover the use of electrosedation as a harvest method in the food processing industry is increasing as a substitute to chemicals for preparation of the fish for euthanization (Zydlewski *et al.* 2008). Alternatively shortcomings of electrosedation includes possibility of injury due to prolonged muscle tetany (Zydlewski *et al.* 2008; Vandergoot *et al.* 2011), elevated glucose levels though the impacts are reasonably nominal (Trushenski and Bower 2012).

Indian major carp *Labeo rohita* (Hamilton-Buchanan, 1822) belongs to the family Cyprinidae is commonly known as ‘Rohu' is one of most popular food fishes in India and adjacent countries. It is a naturally found in the riverine ecosystems primarily in North and central India and the rivers of Pakistan, Bangladesh and Myanmar (FAO 2009). It is the most important fish species of the three major carps used in polyculture (FAO 2009). As *L. rohita* is a popular aquaculture species in India and the region, results and observations of this study may be helpful for desirable handling in future.

Electroanaesthesia has been found to be an effective method in several fishes like Atlantic herring, hybrid striped bass, largemouth bass (Nordgreen *et al.* 2008; Trushenski *et al.* 2012a, 2012b). Electrosedation has been reported as a quick and proficient form of anaesthesia in *Cyprinus carpio* and *Carassius auratus* (Kim *et al.* 2017). Though anaesthetics effect of different concentration MS-222 and iso-eugenol derivate (AQUI-S) on *L. rohita* has been reported (Farid *et al.* 2008; Hussen and Sharma 2016; Devi and Kamilya 2019) but there is no documentation of efficacy of electroanaesthesia in case of *L. rohita*.

The main objective of this study was to find out threshold electric field in the form of voltage gradient (V cm^{-1}) at which anaesthesia was induced (gradual rise and sharp rise) and low frequency PDC (1 Hz, 3 Hz and 6 Hz). Post sedation blood glucose levels at different intervals and changes of opercular movements were evaluated along with the observation of behavioral changes in *L. rohita*.

2 | METHODOLOGY

2.1 Experimental design

Experiments were conducted with *L. rohita* collected from local aquaculture pond. They were housed in a 40 L PVC tank. The animals were fed daily with commercial fish pellets (Optimum, Thailand). The water was oxygenated continuously by placing air pump and the temperature was maintained between 28°C and 31°C. The conductivity and pH varied from 165 – 177 µS cm^{-1} and 7.9 – 8.1 respectively. Salinity was maintained at 0.1 ppt whereas dissolved oxygen (DO) was 7.8 ± 0.5 mg L^{-1}.

All experiments were conducted in an indoor insulated glass tank of dimension of 120 cm, 28 cm and 30 cm (length × width × depth) respectively. To ensure a homogenous electric field, two aluminum plate electrodes (28cm long, 27 cm wide and 2 mm thick) were placed at the two extremities of the glass tank. Two electrodes were placed vertically at the bottom plane of the tank and parallel to each other at the two longer extremities of the glass tank.

Labeo rohita (130 – 270 mm) were subjected to five different types of underwater DC and PDC, namely (i) DC with gradual rise of current intensity, (ii) DC with sharp rise at the peak, (iii) PDC 1 Hz, (iv) PDC 3 Hz, and (v) PDC 6 Hz. For each current type 25 fish individuals were used and size of fish was more or less same across the groups. A single fish was used per treatment in all experiments. The difference in the two types of DC lies in the fact that for gradual rise, voltage gradient was increased gradually up to the attainment of anaesthesia. In case of DC sharp rise fish was exposed to 20 V, 35 V, 45 V, 60 V, 85 V, 170 V sequentially for 8 seconds in each case until the attainment of anaesthesia.

Threshold electric field in the form of voltage gradient were recorded for exhibiting the reaction at three phases (first reaction or initial perception of the electric field, second reaction or taxis towards the electrode and anaesthesia); which was measured as threshold values, required for initiating those reactions. Each fish individual was observed to determine the time at which Stage IV of anaesthesia (Summerfelt and Smith 1990) was achieved. Stage IV of anaesthesia is associated with total loss of equilibrium, muscle tone and responsiveness to visual and tactile stimuli with reduced but steady opercular ventilation. After loss of equilibrium fish was considered to reach the Stage IV of anaesthesia when there was no response to the manual tactile stimuli (touch by a stick to the lateral line). Electricity was switched off immediately after attaining the Stage IV anaesthesia.

Before exposure to the external electric field, length and opercular movement was recorded for each fish individual. For control, 10 individuals (n = 10) were removed from the tank and placed in water containing 2% lidocaine hydrochloride solution at 40 mg L^{-1} for 10 minutes and blood sample was collected from caudal vasculature into a 1 ml heparinized syringe within 5 minutes of anaesthesia. These fishes were never used in the electric exposure. After electric field exposure blood samples were collected in a similar manner of control at regular interval in a group of 5 / 4 fish individuals (1 h, 2 h, 4 h, 6 h, 8 h). Immediately after blood collection blood glucose was determined with the glucose meter ACCU CHEK Active (Roche Diabetes care GmbH, Germany) (Bartonkova *et al.* 2016).

2.2 Power supply and electrical parameters

A 0.5 KVA AC to DC inverter, capable of supplying 20 to 230 volt DC was used as a source of power supply during the experiments. A customized electronic pulse generator for generating low frequency (1 Hz to 6 Hz) with duty cycle of 20 milisecond was connected in the circuit between the inverter input and AC supply to obtain PDC which was transmitted to the electrodes.

The output voltage (DC) was further stepped down by the introduction of three variable resistances (rheostat) of 500 Ω, 500 Ω and 1000 Ω in series, between the DC output terminals of the inverter and the electrodes. This facilitates gradual rising of the field intensity. The applied voltage to the electrode was measured by AVO meter. The conductivity of water was checked by a conductivity meter and also by an Ohmmeter in µ-Siemens and Ohm scale. Thermometer was used for recording the temperature of water of experimental tank before starting the experiments.

2.3 Data analysis

Induction and recovery times were analyzed by the one way Analysis of Variance (ANOVA) to detect significant differences among different current types. Paired *t*-test was used to compare the opercular movements during anaesthesia, one minute after attaining equilibrium and 10 minutes after recovery with opercular movements recorded before electric field exposure.

3 | RESULTS

Regardless of the different current types, all fishes were successfully anaesthetized within 25 – 126 seconds of exposure. Threshold voltage gradient (V cm^{-1}) was comparatively higher in PDC than DC (Table 1). All the fishes recovered swimming activities within 372 seconds in DC gradual rise, within 30 seconds in DC sharp rise and within 336, 270 and 325 seconds for PDC 1Hz, 3 Hz and 6 Hz respectively (Table 1).

Immediately after the exposure to electric field fishes displayed escape behavior with extended fins, tried to jump out of the experimental tank. With increase in field density fishes exhibited forced swimming towards the anode; with further increase in field density (Table 1) fishes lost their equilibrium and finally anaesthetized. In case of pulsed direct current muscle contraction was found at every pulse. One fish individual died in PDC 3 Hz and one in PDC 6 Hz immediately after exposure but there was no mortality during the 7 day follow-up period.

Opercular movements remain more or less similar in all current types. There was no significant change between the opercular movements before and 15 minutes after exposures (DC gradual, p = 0.99; DC Sharp, p = 0.53; PDC 1 Hz, p = 0.77; PDC 3 Hz, p = 0.93; PDC 6 Hz, p = 0.56). However, opercular movements reduced significantly during anaesthesia in all current types (DC gradual, p = 0.012; DC Sharp, p = 0.025; PDC 1 Hz, p = 0.027; PDC 3 Hz, p = 0.03; PDC 6 Hz, p = 0.041). Opercular movements increased significantly during recovery in all current types (Dc Gradual, p = 0.03; DC Sharp, p = 0.05; PDC 1 Hz, p = 0.01; PDC 3 Hz, p = 0.01; PDC 6 Hz, p = 0.045) (Figure 1).

TABLE 1 Threshold electric field intensity (mean ± SD) for perception of electric field, galvanotaxis and anaesthesia and induction and recovery time (seconds) (mean ± SD) in *Labeo rohita* in different type of electric fields.

Current type	Length (mm)	Threshold electric field intensity (V cm^{-1})			Time (second)	
		Perception	Galvanotaxis	Anaesthesia	Induction	Recovery
DC gradual	147.52 ± 19.81	0.10 ± 0.03	0.28 ± 0.11	0.45 ± 0.09	78 ± 10	164 ± 84
DC sharp	146.76 ± 24.92	0.21 ± 0.05	0.47 ± 0.09	0.76 ± 0.19	37 ± 16	19 ± 5
PDC 1 Hz	147.32 ± 19.64	0.16 ± 0.05	0.35 ±0.20	0.65 ± 0.25	82 ± 11	207 ±75
PDC 3 Hz	149.28 ± 28.97	0.12 ± 0.05	0.31± 0.05	0.46 ± 0.07	86 ± 13	137 ± 73
PDC 6 Hz	152.68 ± 22	0.13 ± 0.02	0.26 ± 0.07	0.44 ± 0.07	86 ± 12	93 ± 78

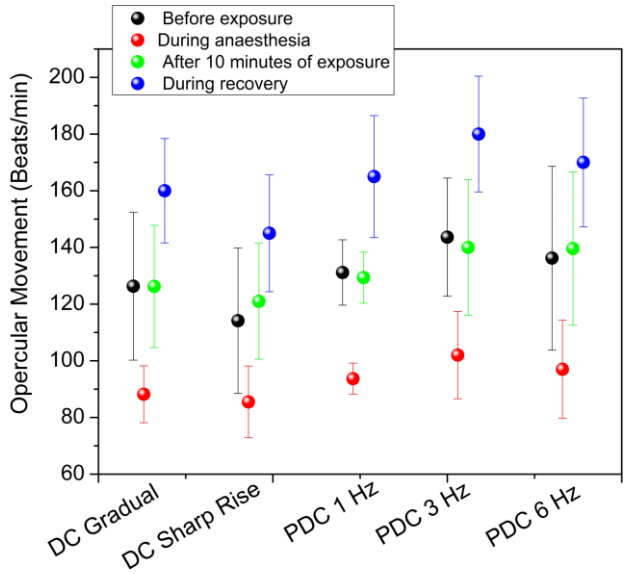

FIGURE 1 Opercular movements in *Labeo rohita* before exposure, during anaesthesia, during recovery (1 minute after recovery) and after 10 minutes of exposure at different current types.

There was no significant difference in blood glucose concentrations among the 5 current types at all samplings. But blood glucose levels were significantly higher in comparison to the control group after 2 h, 4 h and 6 h of exposure in all current types (all p < 0.05). However, there was no significant difference after 8 h post exposure (Figure 2).

4 | DISCUSSION

During normal aquaculture activities, anaesthesia is applied to reduce stress in fish. Some activities like sorting, weighing, injections, tagging, blood sample collection etc. require anaesthesia with fast 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 respiration (Jolley *et al.* 1972). According to Marking and Meyer (1985) the preferable induction and recovery period would be within 3 minutes and 5 minutes respectively. In this study, all fishes were immobilized within 25 – 126 seconds of induction period. Although minor numeric differences were observed for induction period among the current types in this experiments, but they were not statistically significant except in DC sharp rise. Recovery period of DC sharp rise and PDC 3 Hz in all the fishes investigated during this study were within the recommended period of less than 5 minutes suggested by Marking and Meyer (1985). The significantly lower recovery period for DC sharp rise may be due to the lower induction period.

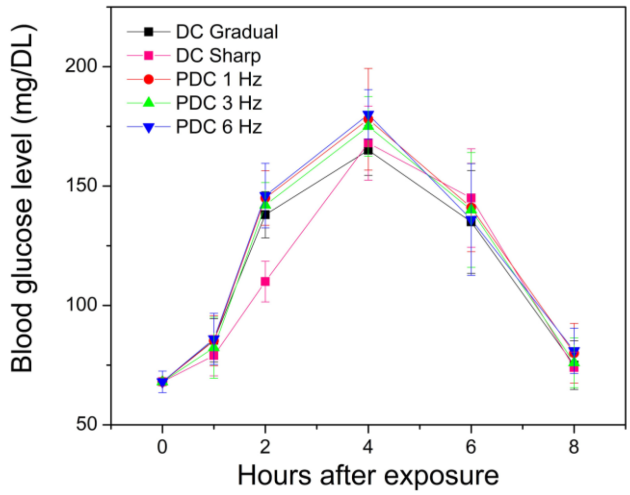

FIGURE 2 Blood glucose levels in *Labeo rohita* at 0 h (control), 1 h, 2 h, 4 h, 6 h and 8 h after exposure at different current types.

In the present study, the use of electric field was competent in inducing anaesthesia in *L. rohita* in all the fishes exposed to electric field in all the current types documented in this study. The threshold values of voltage gradient that induced anaesthesia was lowest in DC gradual rise followed by PDC and highest in DC sharp rise. In DC gradual rise continuously flowing electricity creates a series of stimuli below the threshold level for nerve response and their cumulative effect may cause the onset of narcosis at a lower threshold value (Haskell *et al.* 1954; Wydoski 1980; Emery 1984). Wherein the highest threshold values of field density can be explained by the fact that for DC, higher gradients required due to the lower effect per unit volt as DC is less efficient as stimulator in comparison with PDC (Beaumont 2016). For PDC, the threshold values were observed to decrease with increasing pulse frequencies. Similar inverse relation between the pulse frequencies and voltage gradient was reported by Taube (1992) and Meismer (1999) for rainbow trout, by Muth and Ruppert (1997) for juvenile bonytail and humpback chub. Difference of the threshold values in different PDC frequencies can be explained by the fact that the brain impedance can be changed by the frequency. As a result the current reaching the brain is changed which alters the response of the neurons (Finlay *et al.* 1978). Immediate post exposure mortality was detected in one fish in PDC 3 Hz and in one fish in PDC 6 Hz; no mortality was observed in other current types. Any internal injuries in fishes were not evaluated in this study. However, the absence of any delayed mortality or behavioral changes within a week after the exposure advocates that internal injuries if occurred were minor.

Significantly reduced opercular rates in all of the current types during anaesthesia may be explained by the lactic acid build up due to rapid muscular contraction before attaining narcosis. Reduction of Opercular movements by 50 – 70% in electric field was also observed in *Oreochromis niloticus* (Robinson 1984). Significantly increased opercular rate during recovery may help the fish to overcome the oxygen debt faced by the tissues during narcosis.

Previous research works with chemical anaesthetics and electrosedation have reported that fish undergo the generalized stress response after sedation (Wagner *et al.* 2002; Davis and Griffin 2004; Sattari *et al.* 2009; Trushenski *et al.* 2012a, 2012b, 2012c). Fish body starts the release of stress hormones like cortisol and catecholamines as primary response. Immediately after the perception of stress or the changed state of body by the central nervous system, the chromaffin cells releases stored catecholamines. Wherein after perception of the stressor interrenal cells release cortisol after stimulation by adrenocorticotropic hormone as part of the hypothalamic-pituitary-interrennal axis (Iwama 1998). Released stress hormones alter the blood and tissue chemistry as secondary response like an increase in plasma glucose (Barton and Dwyer 1997; Begg and Pankhurst 2004). These stress hormones mainly cortisol promote glucose production in fish through glycogenolysis and gluconeogenesis (Iwama 1998) and even halts peripheral glucose uptake (Wedemeyer *et al.* 1990) which ultimately released to the blood circulation. This entire pathway helps the fish to cope with high energy demand of stress exposure. Hyperactivity during induction, excessive muscle contraction during taxis and reduced ventilation during narcosis leads to more accumulation of lactate within tissues (Trushenski *et al.* 2012a). This lactate accumulation was observed to resolve within 6 h of exposure as it is used for resynthesis of glycogen (Trushenski and Bower 2012; Trushenski *et al.* 2012b). However blood glucose levels remain higher for relatively longer period may be due to the preferential tissue uptake. This is probably the reason of significant higher blood glucose values after 6 h of exposure in our study. However, the blood glucose level returned to near control values after 8 h of exposure.

Electroanaesthesia provides feasible sedation with a zero withdrawal effect without the restraints of chemical anaesthesia (Rous *et al.* 2015). The MS-222, the predominantly used chemical anaesthetic agent requires a 21-day withdrawal period post exposure in food fishes in USA to avoid any accidental ingestion (Summerfelt and Smith 1990). There is not yet sufficient evidence that most of the available chemical sedatives like AQUI-S, benzocaine are safe for using in food fish; not even universally approved (Trushenski *et al.* 2013). All the fishes in our study recovered within 372 seconds; the fishes were ready to be released or to be used as food fish as there was no chemical residue. Our results indicate that electroanaesthesia is effective in all the current types used in this study. We did not found any significant difference in induction and recovery times between DC gradual rise, PDC 1 Hz, PDC 3 Hz and PDC 6 Hz. However, recovery time was significantly lower in DC Sharp rise than the other current types. This is may be due to the lower induction period in DC Sharp rise. No significant difference in other current types makes it difficult to recommend preeminence of one current type over another. However, the recovery period in DC Sharp rise and PDC 3 Hz in all fishes were within 5 minutes which is recommended by Marking and Meyer (1985).

5 | CONCLUSIONS

Electroanaesthesia technique with fast induction and rapid recovery may proffer a cost effective, safe and eco-friendly alternative for aquaculture activities in *L. rohita*. The results of this study indicate that electroanaesthesia can be used for tagging, fin clipping and other noninvasive procedures particularly where immediate release of the fish is required. Although all the current types in this study induced anaesthesia successfully in all the fishes under treatment, DC sharp rise and PDC 3 Hz may be preferred considering the recommended criteria of anaesthetic induction (within 3 min) and recovery time (within 5 min). The changes in blood glucose level observed in this study were consistent with the generalized secondary stress response in fishes. The threshold field intensities were investigated to minimize the prevalence of unanticipated injuries or physiological and behavioral changes. Future studies on neurophysiologic response of electrosedation on different species and evaluation of post release behavior, physiology, survival rate is recommended to improve animal welfare.

ETHICAL STATEMENTS

The experimental animals were taken care off well. For conducting experiments with 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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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

NC research design, laboratory experiments, analysis of the study, first draft and final draft of the manuscript; **AD** laboratory experiment; **SKG** research design and supervision; **TKD** research design, supervision, and review of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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