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EFFECT OF DIRECT AND PULSED DIRECT CURRENT ON BEHAVIORAL RESPONSE OF NILE TILAPIA (*Oreochromis niloticus*)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Authors NC, KPB and TKD designed the study, and planned the experiments. Authors NC and KPB performed the experiments and managed the analyses of the study. Author NC managed the literature searches and wrote the first draft of the manuscript. Authors TKD and KPB critically commented and corrected the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

We have investigated the detailed behavioral responses of *Oreochromis niloticus* exposed to direct current (DC) and 3 low frequencies of pulsed DC (PDC) (1 Hz, 3 Hz, 6 Hz). The study was aimed to find out the threshold current densities ($\mu A/mm^2$) for the perception of electric field, galvanotaxis (involuntary movement towards the electrode) and narcosis (loss of equilibrium, unconsciousness). Effects on opercular movements before and after electrical exposure was extensively studied. The threshold values for all three responses were found to be higher in DC in comparison with PDC. Among three types of PDC (1, 3 and 6 Hz) applied in the experiments, the threshold values for all the three reactions (perception, taxis and narcosis) were lowest in PDC 6 Hz and highest in PDC 1 Hz followed by continuous DC and PDC 3 Hz. Anodal galvanotaxis was most effectively shown in 90% test individuals in both DC sharp rise and PDC 3 Hz. However, PDC 3 Hz is preferred over DC due to low current requirement. In case of DC, opercular movements were regained immediately after the removal of current, whereas fishes regained opercular movements within 20seconds- 1 minute after exposure in PDC. Though recovery after exposure was delayed upto 1 minute in PDC, it can be ignored as there was no mortality in the present study. So PDC 3 Hz may be a more sustainable option over DC sharp rise due to lower power consumption.

Keywords: Galvanotaxis; narcosis; *Oreochromis niloticus*; behavioral response**;** threshold.

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1. INTRODUCTION

Catching fish with electricity is a common technique for population survey to determine density, abundance, and composition of fish population. The definite sequences of reactions with an increase in field strength have been observed by many investigators [1-3]. But the process involves dynamic and complex mix of physics, physiology and behavior which remain poorly understood still now [4]. Reaction of fishes to an electric field can change strikingly depending on the intensity of electric field, the duration of electrical stimulus and the morphology of fish body [5].

Electric fishing depends on the creation of electric potential gradients between one or more cathodes and one anode in the water. The effect of potential gradient depends on the orientation of fish in the field, fish species and size, water conductivity, temperature, the intensity and type of current [6]. An electric field affects the fish more strongly when the potential difference between their longest body extremities (head to tail) is higher [7]. In order to achieve this, the fish is required to be placed exactly along the lines of current conduction, i.e. perpendicular to the equipotential lines. On the other hand when the fish place itself along the equipotential lines, the potential difference is reduced to minimum and the fish hardly perceive any electrical stimulus. For similar orientation, larger fish intercepts greater potential difference than the smaller fish [8]. Different behavioral responses are either due to electrostimulation of both the central nervous system (CNS) and autonomous nervous system (ANS) and the direct response of muscles of the fish or basically due to the electrically induced epilepsy [9-11].

Direct current (DC), alternating current (AC), pulsed direct (PDC) or pulsed alternating current (PAC) was used in different studies depending on the conductivity, temperature of the water and the fish species to be sampled. Only the DC and PDC induce involuntary movement of the fish towards the electrode (galvanotaxis or electrotaxis) and this behavior is exploited for catching fishes [12]. When AC is used, fish do not swim towards an electrode rather they take a transverse position to tap off a minimum voltage (oscillotaxis) [7]. DC and PDC are preferred over AC because of less detrimental effect on fish [13]. Low power consumption is the main advantage of using pulsed currents. DC and low frequency PDC were found to be effective enough for population estimation by Pajos and Weise. [14]. Recently, electric barriers have been used to restrict the movement of the invasive species in water bodies in different countries [15].

Oreochromis niloticus (Nile tilapia) is an African native fish found mostly in Egypt, central Africa and in Gambia. They are distributed in Nile river, other African rivers, lakes and reservoirs. At present this omnivorous, hardy fish exists in a large parts of tropical and sub- tropical countries and feral Tilapia populations right now presents in 114 countries [16]. Tilapia has become the second most important farmed fish after the carps [17]. Barham et al. studied the effect of electro narcosis on *Oreochromis mossambicus* and denoted that narcosis time increases with body length [18]. Robinson [19] reported brief opercular flaring in *Oreochromis niloticus* at any exposed voltages [19]. Jonathan et al. 2012 [20] studied behavioral and physiological responses of Mozambique Tilapia *(Oreochromis mossambicus*) to an electric stimulus applied to tailfin and suggested that fish perceived electric shock as painful stimuli. Venturini et al. [21] investigated the effectiveness of different frequencies for electronarcosis of *Oreochromis niloticus* as stunning method to ensure animal welfare and good meat quality [21]. However, data is lacking on the detailed behavioral responses of *Oreochromis niloticus* in DC and low frequency PDC electric field.

In this regard, the present study was undertaken to determine detailed behavioral reactions of *Oreochromis niloticus* (Nile tilapia) in homogeneous DC field (DC gradual rise and DC sharp rise) and PDC field with three frequencies namely 1 Hz, 3 Hz, and 6 Hz, respectively. Additionally, the study was aimed to find out the threshold values (current density at which the reaction initiates) of current intensities observed for three major reactions. These reactions are as follows: the first reaction (perception of the electric field) where the fish perceives the electric field with jerk of body, extended fins; the second reaction or galvanotaxis (movement toward electrode) and the third reaction where narcosis (immobilization) of the fish is monitored. Moreover, the aim of the present study was to observe physiological responses of *Oreochromis niloticus* in DC and PDC field. For this purpose, effects on opercular movements before and after electrical exposure was extensively studied.

2. MATERIALS AND METHODS

2.1 Experimental Subjects and Design

Experiments were conducted with Nile Tilapia (*Oreochromis niloticus*) collected from culture pond (Size: 112-198 mm; Weight: 25.6gm-106.2 gm). 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 -30°C. The conductivity of the water varies in the range of 167-175 µS cm^{-1}, pH between 8.1-8.2, Salinity 0.1 ppt, dissolved oxygen 7.1-7.5 mg/L.

All experiments were conducted in an indoor insulated glass tank of dimension of 120 cm, 28 cm, 30 cm (L×W×D) respectively. 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 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.

Oreochromis niloticus were subjected to five different types of underwater direct current (DC) and pulsed direct current (PDC) namely i) DC with Gradual rise ii) DC with sharp rise at the peak iii) PDC 1 Hz iv) PDC 3 Hz v) PDC 6 HZ. For each current type, total 25 (n=25) fishes were used. 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, the density of current was increased by raising the voltage gradient slowly, while in sharp rise, the voltage was raised to a pre-set value. The density of current in the homogeneous field was taken as the criteria for exhibiting the reaction at three phases (first reaction, second reaction and third reaction); which was measured as threshold values, required for initiating those reactions. The density of current was measured by a probe (1 cm^2 separated at a distance of 1 cm from each other).

Live fishes were held in a glass tank for a week and maintained in good health on a diet of formulated feed. 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 minutes to become adjusted to the new environmental condition. After treatment, electricity was switched off and fish was observed for 20 minutes. After 20 minutes fish was removed from the experimental tank and total length and girth of the body was measured. Then the fish was returned to a separate well aerated recovery tank and monitored for 48 hours for survival.

2.2 Power Supply and Electrical Parameters

A 0.5 KVA AC to DC inverter, capable of supplying 40 to 230 volt DC was used as a source of power supply during the experiments. An electronic pulser of low frequency (1 Hz to 6 Hz) was connected in the circuit between the inverter input and AC supply to obtain pulsed direct current (PDC) which was transmitted to the electrodes.

The output Voltage (DC) was further stepped down by the introduction of three variable resistances (rheostat) of 500,500 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 density of current in the field was measured with an electrically insulated probe of 1 square cm using a multimeter (Fluke 115). The copper plates of the probe were separated by a distance of 1 cm and are capable for measurement in µA scale. 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 Behavioral Assessment

The fish was subjected to electric current in water by gradually raising the potential difference (voltage) between the electrodes till the fish perceives the current flow (first reaction). This was followed by either involuntary forced swimming at a higher speed to either of the electrodes when they were parallel to the lines of current conduction or anodic curvature when perpendicular to the field lines at a further rise of current density (second reaction or galvanotaxis). With the further increase in the intensity of the applied electric field, the fish was unable to move, lost consciousness and equilibrium, body relaxed, is upside down (third reaction or narcosis). The reactions were recorded by means of a video camera fixed at an overhead position of the water tank (Nikon D5300).

The body voltage was calculated by observing the orientation of the fish body with relation to the lines of current conduction for each reaction stated above and measuring the voltage gradient of the experimental field between the electrodes along the equipotential lines.

Normal breathing rate of the fishes were monitored by counting opercular movements (number/minute).

The physiological stress of the exposed fish was checked by the time taken for undergoing narcosis and the recovery time from narcosis after switching off the current flow. The rate of opercular movement before the current exposure and after the recovery from narcosis was compared. Paired T-test was done to assess any significant change in respiratory rates before and after exposure. The difference of potential between head and tail (Body voltage) for the first reaction, galvanotaxis and narcosis were calculated from the required voltage input, length of the fish, orientation of the fish in the field and distance of the fish from the anode by using a MATLAB program. It is to be noted that, in the present study, any physiological response was evaluated, however physiological changes was only observed, but not evaluated or quantified.

3. RESULTS AND DISCUSSION

3.1 Results

Current densities (µA/mm²) to bring out first, second and third reactions and respective body voltages (V) are depicted in Table 1 and Table 2 respectively.

During gradual rise of the applied DC electric field, all the fishes perceived the presence of electric field with irritation of body accompanied with jerks of anterior portion during first reaction. Forced movement towards the negative electrode was noticed in almost all the fishes during second reaction. Except in one case out of 25, all the fishes have undergone narcosis in the midfield and lay on its side within 2- 4 minutes of exposure.

In sharp DC field, 90% fishes moved to the anode and 10% to the cathode during the second reaction. All the fishes were narcotized and laid on their sides near the anode within 2-3 minutes of exposure.

Fishes when treated in pulsed direct current (PDC) of 1Hz, at a current density of 0.46 – 1.75 µA/mm², 46% fishes glided to +ve electrode and exhibited circular movement near the anode. Rest 54% of them maneuvered to orient either perpendicular or 45^0 angle to the lines of the current conduction. Only 1 fish exhibited anodic curvature when its body was perpendicular to the lines of current flow. Notably, 77% of the fishes were narcotized within 5-6 minutes of electric exposure. They lay on their sides near positive electrode and recovered immediately after switching of the current. Remaining 23% though not narcotized, lost their swimming equilibrium as long as the current continued to flow.

When exposed to PDC 3 Hz, during second reaction, 90% fishes moved to anode and 10% to the cathode. All the fishes under investigation lost their consciousness and lay on their sides near the anode as long as the current densities flowing in the tank lies within the range of third reaction (within 3-4 minutes of exposure).

In PDC (6 Hz), 75% fishes showed cathodic taxis, while 12.5% of fishes remained in the midfield, head pointing to anode and another 12.5% fishes swam at the water surface to tap off the minimum body voltage. At higher current densities during third reaction, 75% of exposed fishes have undergone narcosis near negative electrode and 12.5% each near positive electrode and in the midfield during the effective period of current flow within 4-5 minutes of exposure.

Table 1. Threshold current densities (µA/mm²) for first reaction, galvanotaxis and narcosis in different current types

Current type	Threshold current density (µA/mm²) for first reaction	Threshold current density (µA/mm²) for galvanotaxis	Threshold current density (µA/mm²) for narcosis	Total length range(mm)
DC Gradual	0.25-0.90	0.70-2.30	0.75-9.50	113-195
DC Sharp	0.25-0.65	0.75-7.50	5.00-11.00	112-198
PDC 1 Hz	0.11-0.85	0.46-1.75	0.50-8.50	116-198
PDC 3 Hz	0.20-0.85	0.30-1.50	1.15-6.00	115-198
PDC 6 Hz	0.25-0.80	0.55-1.25	1.10-3.00	113-196

Table 2. Body voltage (V) for first reaction, galvanotaxis and narcosis in different current types

Current type	Body voltage for first reaction (V)	Body voltage for galvanotaxis (V)	Body voltage for narcosis (V)	Total length range(mm)
DC Gradual	2.03-5.82	4.48-8.72	5.65-9.52	113-195
DC Sharp	1.31-3.49	2.73-13.65	5.56-23.51	112-198
PDC 1 Hz	0.88-3.87	2.35-7.15	5.21-17.65	116-198
PDC 3 Hz	0.49-3.41	1.56-4.14	2.04-6.96	115-198
PDC 6 Hz	1.35-3.77	3.85-8.62	3.65-14.78	113-196

All fishes exposed to PDC exhibited muscle contractions at each pulse. During the experiments in all current types 100% individuals experienced body discoloration but they regained normal color within 10-30 seconds of switching off the current source.

Opercular movement (beats/min) was reduced significantly after recovery from narcosis in case of DC gradual rise [P=0.039], PDC 3Hz [P=0.026] and PDC 1Hz [P=0.014]. However, difference was not significant in DC sharp rise [P=0.052] and PDC 6Hz [P=0.056] (Fig. 1). In all current types, opercular movements were remarkably reduced during narcosis. In addition, all the fishes regained opercular movements and equilibrium within 20 seconds-1 minute after exposure in PDC and within 1-20 seconds after the current was switched off for the case of applied DC field. 100% fishes in all current type experienced increase in opercular movements during the recovery period (for the initial 2-3 minutes after exposure) which subsequently decreases at a slower rate and within 5 minutes after exposure, all fishes were breathing normally and regained swimming activity. We recorded opercular beat rates after 10 minutes of recovery. Any case of mortality was not found upto 48 hours after exposure.

3.2 Discussion

In general, the responses or reactions of fish in electric field are mostly same though the threshold levels and intensity of those responses may vary [11]. In our experiments, *O. niloticus* when exposed to DC and low frequency PDC (1 Hz,3Hz and 6 Hz), their behavior were more or less similar in all the current types. Immediately after exposures, fishes showed two different responses. At lower current densities, fishes swam restlessly and were trying to escape the field. At higher current densities, fishes showed strong involuntary movement towards electrode which ultimately results in loss of equilibrium (narcosis) within 2-5 minutes after exposure. In three cases, it was accompanied with release of egg or sperm.

The threshold values for all three responses were found to be higher in DC in comparison with PDC. Wherein, the threshold values also vary between the two applied DC current and between PDC frequencies. Vincent (1971) reported that PDC induces DC like responses at comparatively lower field intensity than DC [22]. Because of its higher threshold values, DC is considered as the least damaging current type for fishes [23].

We have envisaged that the fishes perceived the electric field at lower current densities in sharp DC in comparison to the DC with gradual rise. However, for the subsequent second and third reaction, the threshold values of current densities exhibit an opposite trend as evident in its recorded threshold values. For continuous DC, the threshold values for second reaction and third reaction were significantly lower

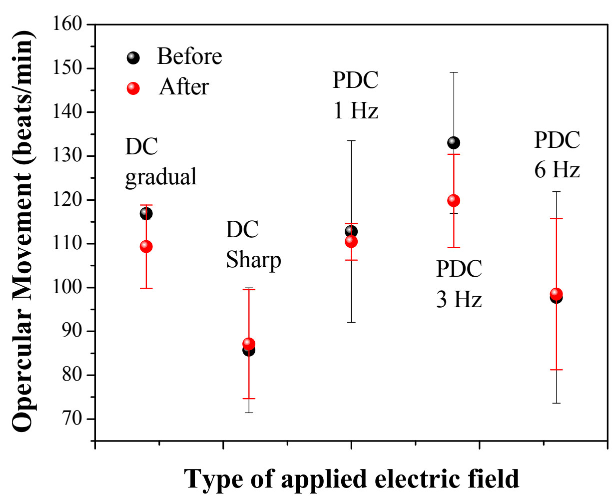

Fig. 1. Average Opercular Beats (per minute) of *O. niloticus* before and after exposure at different current types [reduction of opercular movement was significant in DC gradual rise (P=0.039), PDC 1 Hz (P=0.014) and PDC 3 Hz (P=0.026) as evident from the obtained "P" values from paired "T" test]

than the threshold values for DC with sharp rise. In case of DC gradual rise, continuously rising current flow causes a series of stimuli below the threshold level for nerve and muscular response over a shorter period. Cumulative effect of these stimuli may cause to earlier onset of taxis and narcosis with lower threshold values [24-26].

The threshold value of current densities for perception of electric field by *O. niloticus* was highest in continuous DC and was marginally lower in PDC. For the second reaction, the range of threshold values were found to be higher in DC gradual rise than PDC 1 Hz, followed by PDC 3 Hz and PDC 6 Hz. Similar trends have been noticed during third reaction as well. Here, the threshold values required for narcosis and immobilization was higher in DC gradual rise in comparison to PDC 1 Hz, and progressively lowers in PDC 3 Hz and PDC 6 Hz. Among three (3) PDC applied in the experiments, the threshold values for all the three reaction (perception, taxis and narcosis) was lowest in PDC 6 Hz and highest in PDC 1 Hz. Earlier study also reported decreasing threshold values of voltage gradient for anodic taxis and narcosis with the increasing frequencies of PDC for both *Gila elegans* and *Gila cypha* [27].Similarly Meismer [28] recorded higher threshold values of twitch, taxis and narcosis for 15 Hz PDC in comparison with 60 Hz PDC, even the values of 15 Hz were comparable to the thresholds of DC of respective responses [28]. In this study similar trends were observed for threshold values of current densities. Threshold value of narcosis was found to be higher in DC than PDC in different fish species [29]. As DC has far greater attractive effect than PDC but is less efficient as stimulator, fish do not narcotize so easily [30]. This requires greater threshold values for narcosis in DC. It is perceived that, since the fish skin act like the two conducting plates of the capacitor, the easiness with which the applied electric field can be transmitted into the fish depends on the change in the applied voltage potential around the fish [23]. For instance, the observed threshold values for a fish to react is lower in case where the applied voltage is switched on and off simultaneously in comparison to the gradually rising DC voltage applied around the fish. This leads to the fact that, in our experiment the lowest threshold value for perception of electric field is observed in DC sharp rise, followed by PDC and DC gradual rise. However, for the second and third reaction, the trend changes.

During the experiments with marine fishes Stewart [30] concluded that PDC acted directly on fish muscles in parallel with the nervous system of the fish [30]. This results in reaction of muscle at each applied pulse. Here in our experiments, we have also observed contraction of fish muscle at each pulse in all the three frequencies of PDC. Responses exhibited by fish in an electric field are results of direct stimulation of not only the central nervous system but also the autonomic nervous system which indeed controls involuntary reactions and muscles [9,25]. Muscular bends of body towards the anode may be a result of overstimulation of efferent nerves and nerve endings of muscles [31]. This muscular bends habitually leads to the movement towards anode or Galvanotaxois. Lamarque [31] concluded that anodic taxis caused by PDC is markedly different from that of DC [31]. DC generally directs the fish toward the anode whereas, PDC causes an involuntary movement towards the anode at each circuit closure [24]. Intensity of anodic taxis in PDC field depends on the wave shape, frequency, and duty cycles used. We have observed desirable anodic galvanotaxis during second reaction in 90% cases when the animals were treated in sharp DC and PDC 3 Hz. PDC 6 Hz failed to induce any anodic galvanotaxis in *O. niloticus* in this study. Earlier studies with brown trout also revealed no significant taxis until frequency of PDC reduced from 60 Hz to 15 Hz but intensity of taxis increased significantly when the frequency reduced to 1-2 Hz [24]. Halsband et al. reported that there are specific pulse frequencies for which number of temperate fish species respond favorably leading to galvanotaxis [8]. It was argued that, when the applied PDC frequencies match the swimming rhythm of a particular fish species, galvanotactic reaction occurs successfully.

While comparing the effect on behavioral reactions of two types of DC on the fishes, we have observed that for gradual rise, fishes under investigations did not move to the anode rather moved either to or near about the cathode. The fishes were narcotized subsequently near the cathode. However, in case of sharp rise of peak voltage, majority of fishes exhibited anodic taxis including narcosis near the anode. The effect of DC field was observed on *Tilapia mossambica* where the orientation of the fish in the electric field was found to be an important factor for the behavioral reactions [32]. Cathodic galvanotaxis in DC with gradual rise and PDC 6 Hz in this study corroborates the results documented by earlier study [32]. If one takes into account the power requirement, the PDC 3 Hz is favorable than DC sharp rise as it require less power, whereas the effective duration of current flow is longer in DC.

Removal of the fish under narcosis or at the beginning of tetany from the electric field either by netting or by switching off the current aids in faster recovery [23]. In our experiments, current was switched off immediately after the fish displayed narcosis. This helps in faster recovery and prevents tetany.100% fishes experienced narcosis only in DC sharp rise, PDC 3 Hz and PDC 6Hz; wherein in case of DC sharp and PDC 3 Hz narcosis was induced near anode region.

Reduction of ventilation rate upto 50-70%, even cessation in some cases during narcosis and low ventilation rate after recovery was also reported by Robinson [19] in *Oreochromis niloticus* exposed to AC current [19]. The present study revealed that, fishes exposed to DC fields regained opercular movements immediately after the removal of current but recovery delayed upto 20seconds- 1 min in PDC. This corroborates well to the literature for gold fish in DC [29]. Chemielewski et al., noted that brown trout requires 1 to 2 min recovery time for reestablishment of equilibrium and normal respiratory movements [33]. It was found that the ventilation recovery time lies in the range of 19 s to 3 min after removal of the current when exposed to PDC [34]. Rapid muscular contractions during taxis may cause lactic acid build up and oxygen debt [26]. When the fish is drawn nearer to anode with further increase of field intensity it becomes impossible for the fish to breathe; this leads to lower respiratory rates during narcosis. Reduced respiration in all cases during narcosis and increased opercular movements during recovery from narcosis was also reported earlier [35]. Increased rate of respiration during recovery may help the fish to overcome the oxygen debt faced by the tissues during narcosis. In the present study, all fishes regained swimming activity after 5 minutes after switching off the current.

Afterwards, the effect on body color of *Oreochromis niloticus* has been investigated due to the application of different type of the electric field. Body color was found to fade during electrical exposures in all cases of the applied electric field. This is due to the pigment contraction owing to the tension perceived in the fish during the electric exposure [7]. Notably, all the fishes regained normal pigmentation after the stoppage of the current.

4. CONCLUSION

For population survey or catching fish with electricity, anodic taxis is the most important behavior for catching efficiency; more taxis towards anode ensures greater catchability. In this study, DC sharp rise and PDC 3 Hz induced anodic taxis in 90% cases. Therefore, DC sharp rise and PDC 3 Hz may exhibit better catching potential than the other current types used in this study. Though recovery after exposure was delayed upto 1 minute in PDC, it can be ignored as there was no mortality in the present study. So PDC 3 Hz may be a more sustainable option over DC sharp rise due to lower power consumption. However, we recommend that the use of lowest threshold of current density may be advantageous to minimize the occurrence of unforeseen injuries and physiological changes. However, detailed physiological and biochemical studies may be carried out to monitor whether any severe internal damage is caused to *Oreochromis niloticus* due to electrical exposure. In addition, different frequencies of PDC may be included in such studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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