STUDY ON THE ELECTROANESTHESIA OF SOME SPECIMENS OF *CYPRINUS CARPIO*

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Abstract

The study on electroanesthesia is part of a larger project that refers to research on the genetic determinism of the growth process in some breeds of Cyprinus Carpio species. One of the problems encountered was the handling of fish specimens in optimal conditions, without inducing stress, without causing injuries and without introducing chemicals, with a sedative or narcotic effect, into the aquatic environment. This was necessary to collect blood samples, tissue and semiological material to obtain results as relevant and compliant with those of the living environment. Among the anesthesia methods without chemicals (the method of electroanesthesia, the method of hypothermia and the method with CO_2), the method of electroanesthesia was used due to its immediate efficiency and easy recovery of fish material. Thus, the present study revealed that the most efficient current to be applied to obtain the desired effect of electroanesthesia was the alternating current rectified monoalternation.

Key words: electroanesthesia, fish, manipulation, monoalternation

INTRODUCTION

Anesthesia is an important procedure used to immobilize fish specimens in order to reduce the physical damage and the stress during aquaculture practices (transport, sorting, reproduction and vaccination), stock assessment (specimen marking and collection) and experimental procedures (implantation of the telemetry transmitter; Summerfelt and Smith 1990).

When the anesthesia is applied, it is manifested by the loss of senses, installing depression in the central and peripheral nervous system (Iwama and Ackerman 1994). Specimens subjected to this procedure suffer a series of physiological and behavioral changes in response to the anesthetic exposure. In the first phase, the specimens calm down, then they successively lose their mobility, balance, consciousness and final reflection reaction (Summerfelt and Smith 1990).

The progression of these changes has been classified into various stages of anesthesia by many researchers (McFarland 1959; Bell 1987; Yoshikawa et al. 1988; Iwama et al. 1989), but the six stages of anesthesia described by Summerfelt and Smith (1990) are widely mentioned: stage 0 of anesthesia refers to normal behavior, while stage 6 refers to death. Stage 4 of anesthesia, characterized by total loss of balance and lack of handling reactions, is usually the necessary step for aquaculture procedures (Summerfelt and Smith 1990).

The level of stress in fish is increased by the intensification of aquaculture practices. Transport, handling, sorting, weighing, increasing density and degrading water quality are stressors in aquaculture practices that show physiological responses to stress from fish specimens. These stressors cause changes in plasma concentrations of cortisol, glucose, lactase, plasma chloride, sodium and lymphocytes in fish (Ackerman et al., 2005).

The few anesthetics available, registered for use in fish farming and the tendency to give up chemicals, have stimulated a renewed interest in research into methods of anesthesia and the search for non-chemical means of anesthetizing fish (Ackerman et al., 2005).

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Following studies on the effects of anesthetics on aquaculture organisms, some authors have stated that these effects are optimized using electricity, CO_2 and substances such as clove oil as viable alternatives to chemical anesthesia. They also established that to be useful, an anesthetic should induce anesthesia in less than 3 minutes and recovery should take place within 5 minutes of removing the anesthetic medium (Ackerman et al., 2005).

Table 1 Stages of anesthesia and post-anesthetic recovery (source: Ackerman et al., 2005)

Anesthesia stage	Description
I	Loss of balance
II	Loss of raw body movements, but with the presence of continuous operculum movements
III	Loss of operculum movements
Recovery stage	
I	The body is immobilized, but the operculum movements are just beginning
II	Regular operculum movements and raw body movements begin
III	Restoration of balance and preanesthetic appearance

MATERIAL AND METHOD

The following experimental set-up (Fig. 8) was used to study the effects of electronarcosis and electroanesthesia on some specimens of Cyprinus carpio:

1. Glass aquarium (L = 80cm, l = 50 cm and H = 50 cm) in which a volume of 100 l of water and 2 copper electrodes was introduced (Fig. 1).

2. PPW-8011 TWINTEX programmable direct current laboratory source with the following characteristics: output voltage: 0 ... 80V DC, output current: 0 ... 11A, maximum power: 880W, current stabilization: ≤0.2% + 5mA, voltage stabilization: ≤0.1% + 5mV (Fig. 2).

Fig. 1 Glass aquarium (original photo)

Fig.2 Programmable DC Power Source (original photo)

3. Hewlett Packard 54600B digital oscilloscope with the following characteristics: digital type, number of channels: 2, frequency band: 100MHz (Fig. 3)

4. Adjustable laboratory autotransformer with the following characteristics: input voltage: 220V = 240V AC, output voltage 0-300V AC (Fig. 4)

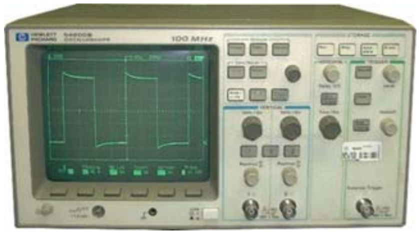

Fig. 3 Hewlett Packard 54600B digital oscilloscope

Fig. 4 Adjustable laboratory autotransformer

5. NIMEX NI2100 Digital Multimeter Meter in ammeter configuration (Fig. 5)
6. Rectifier bridge KBP 1010 10 Amps (Fig. 6)
7. Rectifier diode 10 Amps NTE5812HC (Fig. 7)

Electronarcosis is a common method for juvenile and adult fish harvesting in aquaculture management (Cowx & Lamarque, 1990; Reynolds, 1996).

Three types of electric current were used to immobilize fish: alternating current (AC), direct current (DC) and pulsating forms of AC and DC. Direct current can cause anodotaxis (displacement to the anodic pole), electronarcosis and electrotetany (tetanic muscle contractions). The purpose of electroanesthesia is to induce electronarcosis and to avoid severe muscle tetany that can lead to spinal cord injuries.

Fig. 5 NIMEX NI2100 Digital Multimeter

Fig. 6 KBP 1010 rectifier bridge

Fig. 7 Rectifier diode NTE5812HC

Fig. 8 Experimental stand (original photo)

The response of fish to electricity depends on the intensity of the electric field and the duration of the shock. Other factors such as water conductivity, temperature, fish size and species may also affect the effectiveness of electroanesthesia.

Electroshock was found to induce an immediate increase in plasma corticosteroids and lactase concentrations in rainbow trout, with persistent increases in plasma glucose and corticosteroids for at least 6 hours after capture and cardiovascular changes, including changes in rhythm (Bisson 1976).

Alternating current has been widely used in the past (Madden and Houston, 1976; Ross and Ross, 1984); however, it is now known to be the most harmful form of current for fish (Lamarque, 1990; Walker et al., 1994) and electroanesthesia is performed with DC or pulsed DC.

Pulsed DC is the most common waveform used in aquaculture today, and a setting of 60 V and 50 Hz has been used successfully for electroanesthesia (Walker et al., 1994; Redman et al., 1998).

The main advantages of electroanesthesia over chemical anesthetics include the speed of induction times, the recovery and immediate release of treated fish, without the need for a recovery period (Ackerman, 2005).

The biological material used in the present study was represented by 2 carp specimens, one male and one female, 35 and 38 cm long and 1000 g each. The male is of the carp breed with scales distributed in frame and the female is of the carp breed with scales, both 18 months old.

The aim of the experiment was to establish, with precision, the type and level of tension (stabilized continuous, bialternant rectified voltage, monoalternated rectified voltage) at which the specimens enter the state of anesthesia to collect blood samples, tissue and semiologic material samples.

Studies have shown that if too low a voltage and current is applied, fish, when they feel the electric current, begin to jump uncontrollably. This is a high risk of injuries due to bumps on the walls of the aquarium. Sudden discharges of adrenaline, caused by these jumps, affect the hematological parameters that are monitored.

An important electrical parameter is the type of DC voltage used, some authors recommending DC voltage, others the continuous voltage over which a train of pulses overlapped.

It is known that the power density (*Pw*), the amount of power applied to water, was calculated as:

$Pw = C_w(V/D)^2$, where *C*w = is the conductivity of the water (μS/cm), *V* = the voltage, and *D* = the distance between the electrodes (cm); (Kolz 1989).

METHOD

The first set of tests was performed with direct current voltage from the laboratory source PPW-8011 TWINTEX, connected to the electrodes in the aquarium according to Fig. 9.

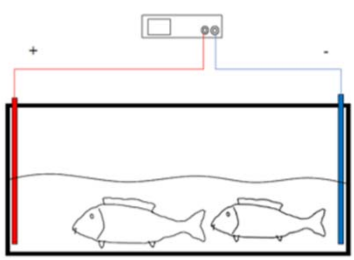

Fig. 9 DC diagram

Fig. 10 Application of DC

Fig. 11 The effect of DC

The second set of tests was performed with electrodes powered by the KBP 1010 rectifier bridge which generates bi-alternating rectified current (Fig. 13). The rectifier bridge is supplied with alternating voltage which is adjusted to the desired level by the laboratory autotransformer. The signal is visualized on the oscilloscope. The oscilloscope, also has the role of measuring the input and the output signal. For measuring the current applied on the electrodes, the NIMEX ampermeter was inserted in serial in the circuit (Fig. 12)

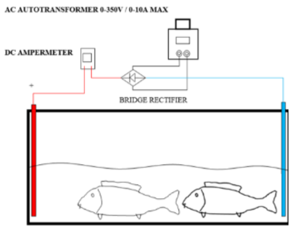

Fig. 12 Scheme of bialternant rectified AC

Fig. 13 Application of bialternant rectified AC

Fig. 14 Effect of bialternant rectified AC

The last set of tests was performed with mono-alternating rectified AC through NTE5812HC rectifier diode. The rectifier diode is powered by the laboratory autotransformer which provides an adjustable voltage, and the current applied to the electrodes is monitored using the NIMEX ammeter. The Hewlett Packard oscilloscope was used to visualize the values of the input and output voltages on the rectifier diode. The connections to the electrodes are represented in Fig. 15 and the oscillogram of mono-alternating rectified AC current is captured on Fig. 16.

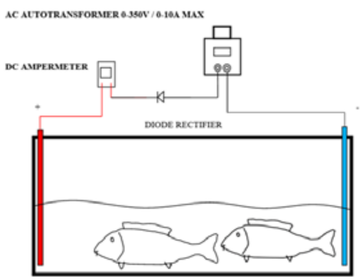

Fig. 15 Scheme of monoalternant rectified AC

Fig. 16 Mono-alternating rectified AC on oscilloscope

The tests on the regulated voltage levels were performed respecting the established interval of 6 hours. This interval is necessary for the specimens to fully recover from the application of electroanesthesia (Bisson, 1976).

RESULTS AND DISCUSSIONS

Compared to the chemical methods of anesthesia, electronarcosis and electroanesthesia are much more effective and easier to apply. They are also suitable for harvesting fish material directly from the water without causing stress and injury.

Following the application of the three types of current, direct current, bialternating rectified AC current and monoalternating rectified AC current, to induce electroanesthesia, without affecting the blood parameters and without stressing the cyprinid specimens, the following were found:

- electroanesthesia times depend on the supply voltage of the electrodes;
- the low blood pressure level causes a violent reaction of the specimens subjected to anesthesia, a reaction materialized by jumping;
- in stabilized direct current, (Fig. 10) electronarcosis and electroanesthesia are the most difficult to install, and the recovery times are longer (Fig. 11). Regardless of the value of the voltage, there are always violent jumps when the electric current appears in the electrodes;
- in bi-alternating rectified AC current rectified (Fig. 13), this manifestation by violent jumps appears at a low voltage level, but at the voltage level according to it disappears. The specimens reach the stage of electroanesthesia more difficult, but immediately enter electronarcosis (Fig. 14);

- in mono-alternating rectified AC curent, the effect of electronarcosis is observed immediately: there are no jumps and it has been established to be the most suitable technology for electroanesthesia. After the tissue and blood samples were taken, the specimens recovered the fastest.

CONCLUSIONS

Most advantages were observed when applied mono-alternating rectified AC current. First of all, the specimens showed the state of anesthesia throughout the sampling (5 minutes), the muscles did not show tetany, making the sampling procedure very easy.

The recovery time of the specimens was the shortest following the application of the mono-alternating rectified AC current, the recovery starting with the moment of introducing the specimens into the aquatic environment, after collecting the samples and reaching stage III of recovery (tab. 1) in 20-30 seconds.

Regarding the voltage level used, it was lower than the method of applying stabilized direct current or bi-alternating rectified AC current, and the cost of the equipment for obtaining the mono-alternating rectified AC current is the lowest.

These observations led to the conclusion that the most effective method of electroanesthesia was the one in which mono-alternating rectified AC current was applied to the electrodes.

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