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INDUCTION AND RECOVERYTIMES OF ANESTHESIA IN CYPRINIDS USING VARIOUS DOSES OF CLOVE OIL (*EUGENIA CARYOPHYLLATA*)

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Abstract

Anesthetics are widely used in fish farming. Their purpose is to reduce the mobility and stress of fishes due to manipulation. In the present study we tested the efficiency of clove oil (Eugenia caryophyllata) in different doses [4.70 mL anesthetic/50 liter of water (0.094 mL/Lwater); 2.36 mL anesthetic/50 liter of water (0.0472 mL/Lwater); 1.82 mL anesthetic/50 liter of water (0.0364 mL/Lwater)] in carp (Cyprinus carpio) and in Prussian carp (Carassius gibelio) with different body mass. Anesthesia induction times were monitored. At each of these stages the respiratory rate was monitored by recording the opercular movements. The results show a direct correlation between the fish size and the induction times of anesthesia. Regardless of species, larger and heavier specimens require longer periods for anesthesia. Instead, recovery times in larger-body specimens were shorter. Different doses have influenced the duration of anesthesia induction and recovery times, the most effective dose being 4.70 mL/50 liter of water followed by 2.36 mL/50 liter of water. The dose of 1.82 mL/50 liter of water did not induce anesthesia.

Key words: *efficiency, fish anesthesia, natural anesthetics, opercular movements.*

INTRODUCTION

Anesthetics are synthetic or natural medicinal products used for the temporary loss of sensations. In terms of mechanisms of action, they are classified under general, regional and local anesthesia. General anesthetics lead to total immobility, amnesia, sleep, analgesia, unconsciousness, and a reduced autonomic response to harmful stimuli.

Regional and local anesthetics interrupts neuronal conduction by inhibiting the influx of sodium ions, thus blocking painful sensations in the regions where they are administered. In fishery practice, anesthetics are widely used (Zahl et al., 2012), when the fish must be immobilized for a longer period of time. In general, fish are anesthetized when invasive surgical procedures or biological sampling (blood samples, different types of tissues) are required. Anesthetics are also used in situations which require fish handling during transport, sorting, artificial breeding, or administration of vaccines. Different types of anesthetics and methods of anesthesia are used for the purpose of stunning the fishes (electronarkosis, hypotermia, etc.), eliminating stress-causing situations (Robb and Kestin, 2002; Zydlewski et al., 2008; Wilson et al., 2009).

Administration of anesthetics can be done in several ways: by immersing fish in short-term baths containing anesthetics or by subcutaneous or intramuscular injection. The anesthetics used are found in a wide range, both natural (Rezende et al., 2017; Hoseini et al., 2018) and synthetic anesthetics. These generally present a period of persistence in the fish body, especially at the musculature level, which is why after administration, a period of quarantine it is necessary before the fish are destined for human consumption and in accordance with EU regulations (Nicolae et al., 2018; Totoiu et al., 2018). The anesthetics most commonly used in fish are: MS-222 (tricaine methanesulfonate) (Küçük, 2018), Benzocaine (Fabiani et al., 2013), Propiscin (Kazuń and Siwicki, 2012),Quinaldine (Sneddon, 2012), 2-Phenoxyethanol (Varkey and Sajeevan, 2014),

Metomidate, Clove Oil (Palić et al., 2006), Aqui-S^{TM} (Javahery and Moradlu, 2012) and carbon dioxide (Bernier and Randall, 1998).
Anesthetics efficiency is dependent on several factors as the size of the doses administered or the size of fish, water temperature and species (Skår et al., 2017). Although so far many studies have been conducted on the use of anesthetics in aquaculture, there are still many unknown data on their efficiency. Induction of anesthesia should be done in accordance with the purpose of research or the manipulation of fish in such a manner that the correct dose it's used (Ferreira et al., 2018). Any error can lead to the induction of a state of stress to the fish, with all the following consequences or in the worst case scenario, the death of the fish. Clove oil is a dark brown liquid obtained by the distillation of strains, leaves and flower buds of *Eugenia caryophyllata*, with the active component which is eugenol [2-Methoxy-4-(prop-2-en-1-yl)phenol] in a proportion of 85-95%, along isoeugenol [2-Methoxy-4-(prop-1-en-1-yl)phenol] andmethyleugenol [1,2-Dimethoxy-4-(prop-2-en-1-yl)benzene]. It has a wide range of uses: as an antioxidant (Ghadermazi et al., 2017), antimycotic (Estrada-Cano et al., 2017), antibacterial (Xu et al., 2016) and as an anesthetic. Since the clove oil has a certain amount of persistence time in the organism of which is subject to the anesthesia (Zhao et al., 2017), its use is prohibited in fish that are used for human consumption or in fish who are going to be released into the natural environment (Gueretz et al., 2017).

MATERIALS AND METHODS

The dose efficiency (0.094 mL/L water; 0.0472 mL/L water; 0.0236 mL/L water) of clove oil was tested on 2 groups of carp (*Cyprinus carpio*) and 2 groups of Prussian carp (*Carassius gibelio*) with different body size and mass (Table 1).

Table 1. Body weight average values of the experimental groups and dispersion indices

Clove Oil 0.094 mL/Lwater				
Experimental Group	Abr.	$X \pm s_x$	V%	s
Common carp	Group 1.1	150.8 ± 1.374	9.11	13.737
Common carp	Group 1.2	442.4 ± 14.413	32.58	144.133
Prussian carp	Group 1.3	100.6 ± 1.679	16.69	16.787
Prussian carp	Group 1.4	240.8 ± 10.12	42.03	101.199
Clove Oil 0.0472 mL/Lwater				
Experimental Group	Abr.	$X \pm s_x$	V%	s
Common carp	Group 2.1	153.2 ± 1.108	7.23	11.077
Common carp	Group 2.2	441.4 ± 14.882	33.71	148.818
Prussian carp	Group 2.3	98.6 ± 1.601	16.24	16.009
Prussian carp	Group 2.4	241.6 ± 9.911	41.02	99.11
Clove Oil 0.0236 mL/Lwater				
Experimental Group	Abr.	$X \pm s_x$	V%	s
Common carp	Group 3.1	157.6 ± 1.292	8.19	12.915
Common carp	Group 3.2	428.6 ± 5.42	12.65	54.201
Prussian carp	Group 3.3	98.4 ± 1.264	12.85	12.641
Prussian carp	Group 3.4	229 ± 4.205	18.36	42.048

The experiment was carried out in the Aquaculture Laboratory of UASVM Cluj-Napoca. Fish anesthesia was done by successively immersing the specimens in a clove oil solution at a water temperature of 21 °C in 50L basins. Throughout the experiment, the dissolved oxygen level was maintained at 8 mg O_2/L of water. Since the clove oil is hardly miscible in water, having a density of 1.040 – 1.067 g/cm^3 (Nowak et al., 2012), it has previously been mixed into a separate container by vigorous stirring with a smaller amount of water. Afterwards, it was discharged and homogenized in the experimental pools. Following the induction of total anesthesia, the fish were transferred to fresh water pools which

had the same water temperature. Prior to anesthesia (24 hours), the feeding of fishes was stopped. The times for induction and recovery of anesthesia (MI – mild imbalance; LD – lateral decubitus; TA – total anesthesia; FSR – first signs of recovery; TR – total recovery; OR – opercular rate) were monitored by filming the experiment with a Nikon Coolpix P540 digital camera. Based on the filming, anesthesia and recovery times and phases were then chronometer and the database for the analysis of the results regarding the relationship between body mass and induction and recovery times (seconds) from anesthesia was established for the two different species.

RESULTS AND DISCUSSIONS

In Figures 1, 2 and 3 the anesthesia induction times for the experimental groups, respectively the opercular rate (respiratory movements) in each phase of anesthesia are represented. In each figure the average values and the standard error for each phase are presented.

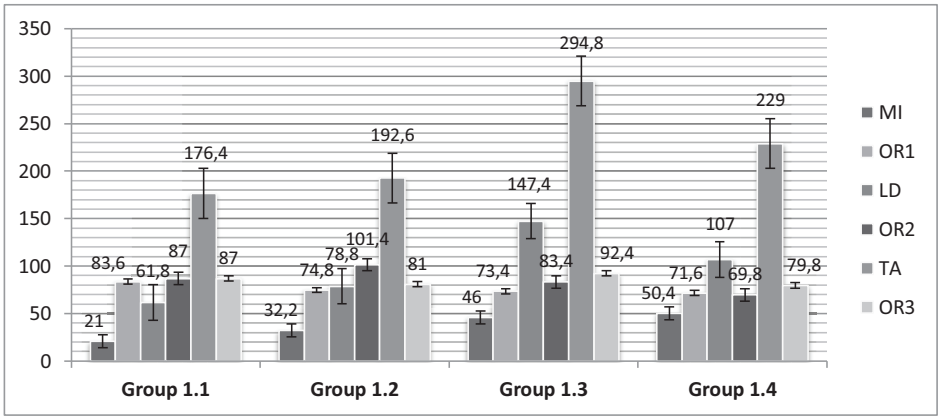

Figure 1. Induction times (seconds) of anesthesia and the rate of operculum movements to the four groups (average values of time and standard error) (Clove Oil doses – 0.094 mL/L water)

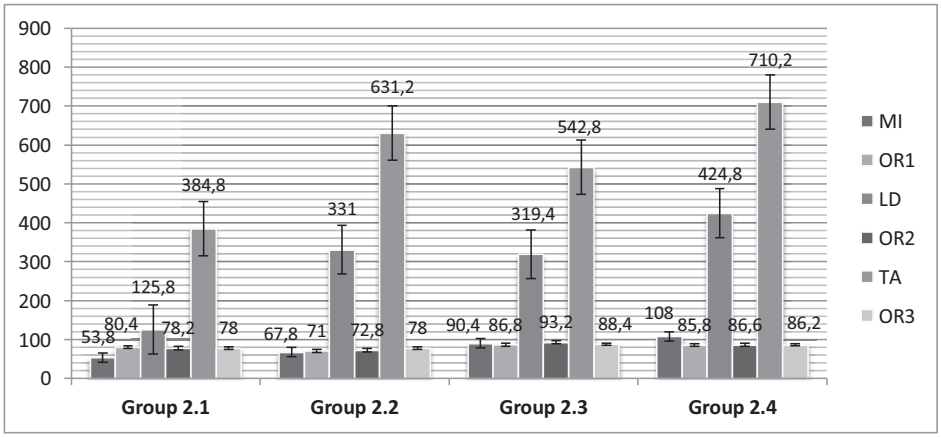

Figure 2. Induction times (seconds) of anesthesia and the rate of operculum movements to the four groups (average values of time and standard error) (Clove Oil doses – 0.0472 mL/L water)

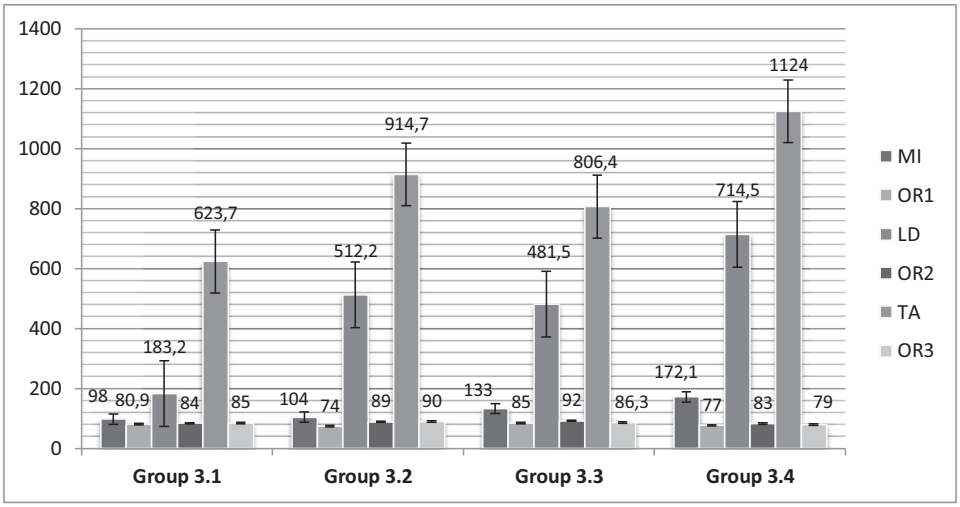

Figure 3Induction times (seconds) of anesthesia and the rate of operculum movements to the four groups (average values of time and standard error) (Clove Oil doses – 0.0236 mL/L water)

From graphical representation, it can be observed that anesthesia induction times increase as anesthetic doses are lower, as found in other studies (Hseu et al., 1998).Thus, in the case of carp with lower body mass (group 1.1, 2.1, 3.1) total anesthesia (TA) is induced at 176.4 (1.1) 384.8 sec (2.1) to 623.7 sec (3.1). It can be seen therefore, that with halving the dose of clove oil, the total times of induction of anesthesia doubles. The same situation was observed for the other phases of anesthesia (mild imbalance MI and lateral decubitus LD). The first signs of imbalance were observed at 21 sec (1.1), 53.8 sec (2.1) and 98 sec (3.1), and the lateral decubitus phase it was observed at 61.8 sec (1.1), 125.8 sec (2.1) and 183.2 sec (3.1).Similar results were obtained in the other experimental groups, and it seems to be a general rule that with the increase in anesthetic dose, the induction times of anesthesia decrease. By reference to the species and size of the specimens, it was also observed that in the case of the smaller specimens (group 1.1, 2.1, 3.1 and 1.3, 2.3, 3.3), anesthesia is induced more rapidly as compared with larger specimens (group 1.2, 2.2, 3.2 and 1.4, 2.4, 3.4).However, an atypical situation was observed in experimental group 1.3 (small Prussian carp specimens with a body weight of 100.6 ± 1.679 g) where the induction times of anesthesia were higher compared to the large Prussian carp specimens (group 1.4) with a body mass of 240.8 ± 10.12 g. Thus, in group 1.3, the induction phases of lateral decubitus and total anesthesia were longer (LD = 147.4 sec; TA = 294.8 sec) compared to group 1.4 (LD = 107 sec, TA = 229 sec). This situation was observed only at the clove oil concentration of 0.094 mL/L water. In the other two concentrations of anesthetic (0.0472 mL/L water, 0.0236 mL/L water), induction time of anesthesia were greater in specimens with higher body mass.

The opercular rate (OP1, OP2, OP3), corresponding to the respiratory rate, regardless of the anesthesia induction phase and the experimental group, was more pronounced than the species limits (carp and Prussian carp). In the experimental group 1.1, the opercular rate corresponding to the first phase of anesthesia (OP1) presented 83.6 movements / minute. At the lateral decubitus phase, the opercular rate (OP2) was 87.0 movements / minute, the same rate being maintained for total anesthesia (OP3 = 87.0 movements / minute). In the experimental group 1.2 and the 0.094 mL clove oil/L water concentration, the opercular rate also ranged within normal limits, but an acceleration was observed in the lateral decubitus phase, which returned to lower values at the time of total anesthesia installation (OP1 = 74.8 movements / minute, OP2 = 101.4 movements / minute, OP3 = 81.0 movements / minute).In other studies on the

induction of anesthesia in fish (Al-Hamdani et al., 2010) were obtained lower values of respiratory rate (34.2 - 43.3 movements / minute). It seems that clove oil induces hypoxia conditions (Stecyk and Farrel, 2002), with similar respiratory and opercular rates being obtained (7.8±1.7 – 10.7±1.8 / min^{-1}). Similar values were obtained for opercular rate at the use of lower doses of anesthetic (Group 2.1 = 78.0 – 80.4movements/min; Group 2.2 = 71.0 – 78.0 movements/min.; Group 2.3 = 86.8 – 93.2 movements/min.; Group 2.4 = 85.8 – 86.6 movements/min.; Group 3.1 = 80.9 – 85.0 movements/min.; Group 3.2 = 74.0 – 90.0 movements/min.; Group 3.3 = 85.0 – 92.0 movements/min.; Group 3.4 = 77.0 – 83.0 movements/min.).

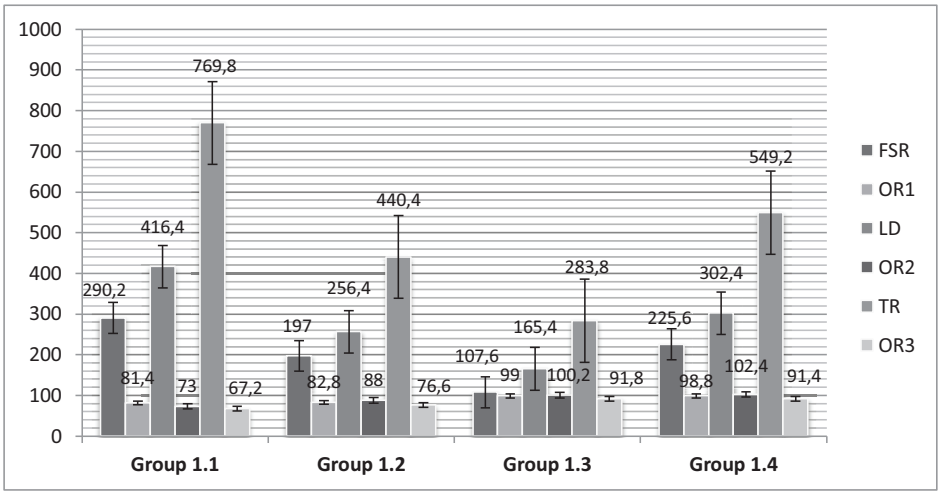

Figure 4. Recovery times (seconds) of anesthesia and the rate of operculum movements to the four groups (average values of time and standard error) (Clove Oil doses – 0.094 mL/L water)

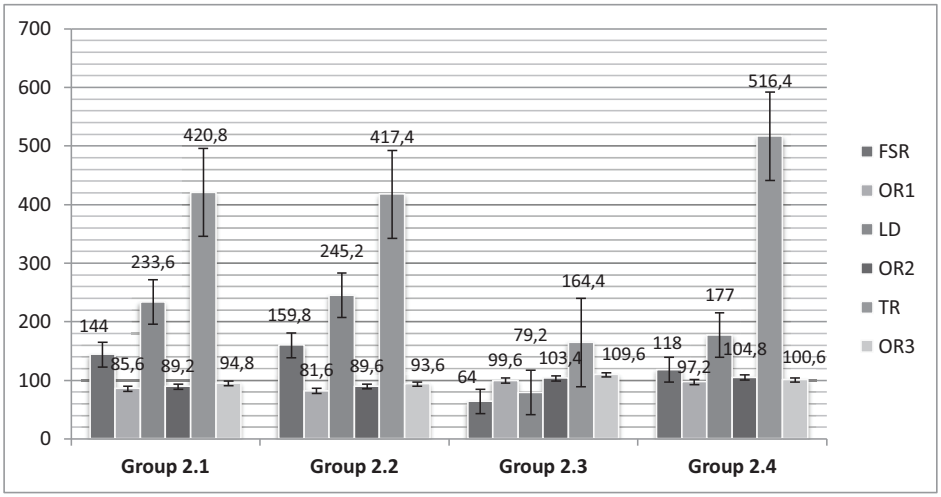

Figure 5. Recovery times (seconds) of anesthesia and the rate of operculum movements to the four groups (average values of time and standard error) (Clove Oil doses – 0.0472 mL/L water)

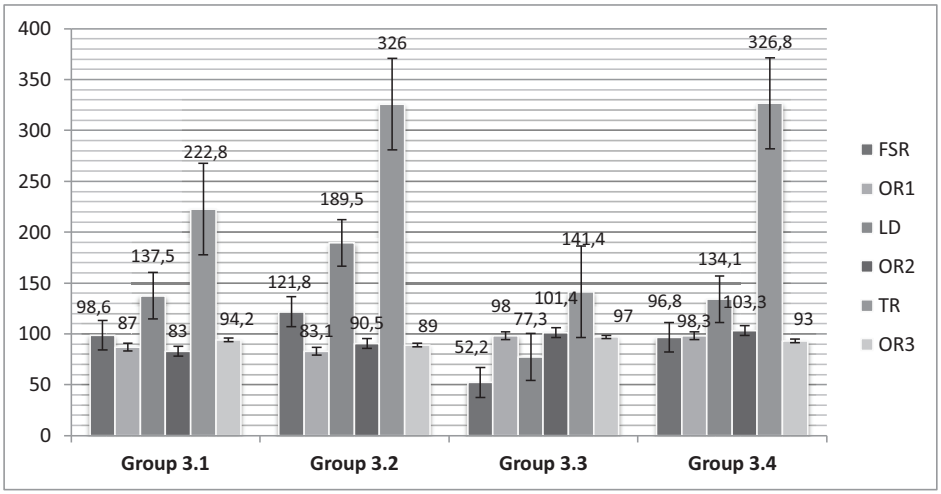

Figure 6. Recovery times (seconds) of anesthesia and the rate of operculum movements to the four groups (average values of time and standard error) (Clove Oil doses – 0.0236 mL/L water)

Recovery from anesthesia times is shown in Figures 4, 5 and 6. As a general rule, it can be seen that the longer they are, the concentration of anesthetic is higher. Thus, for groups 1.1, 1.2, 1.3 and 1.4 (Clove Oil doses = 0.094 mL/Lwater), total recovery times were: Group 1.1 TR = 769.8 sec; Group 1.2 TR = 440.4 sec.; Group 1.3 TR = 283.8 sec.; Group 1.4 TR = 549.2 sec. In the case of carp, total recovery times were longer than in the Prussian carp. In experimental group 1.3 (Prussian carp BW = 100.6 ± 1.679 g), the recovery times are approximately equal to the induction anesthesia times (TA = 294.8 seconds vs. TR = 283.8 seconds). For group 2.1, 2.2, 2.3, 2.4 (Clove Oil doses = 0.0472 mL/Lwater), the recovery times were: Group 2.1 TR = 420.8 sec.; Group 2.2 TR = 417.4 sec.; Group 2.3 TR = 164.4; Group 2.4 TR = 516.4 sec. Similarly, it can be seen that in group 2.3 (Prussian carp BW = 98.6 ± 1.601 g), the average recovery times were the shortest. For experimental group 3.1, 3.2, 3.3, and 3.4 (Clove Oil doses = 0.0236 mL/Lwater), total recovery times were as follows: Group 3.1 TR = 222.8 sec.; Group 3.2 TR = 326.0 sec; Group 3.3 TR = 141.4 sec.; Group 3.4 TR = 326.8 sec.

Operculum movements during recovery from anesthesia were within normal range, but varied according to the doses of anesthetics used and species. Thus, in carps, anesthetized at doses of 0.094 mL/L water (Group 1.1 BW = 150.8 ± 1.374 g), the opercular rate at the time of the first signs of recovery (FSR) was 81.4 movements / min, and for group 1.2 (BW = 442.4 ± 14.413 g) this was 82.8 movements / min. For both groups of carps, at the time of total recovery, the respiratory rate was lower (Group 1.1 TR-OR3 = 67.2 movements / min vs. Group 1.2 TR-OR3 = 76.6 movements / min.).In Prussian carps, anesthetized at doses of 0.094 mL/Lwater, the opercular movements showed at the time of the first signs of recovery the following frequencies: Group 1.3 (BW = 100.6 ± 1.679 g) FSR-OR1 = 99.0 movements/min., Group 1.4 (BW = 240.8 ± 10.12 g) FSR-OR1 = 98.8 movements / min. When total recovery installed, the opercular rates were 91.8 movements / min.(Group 1.3), respectively 91.4 movements / min. (Group 1.4).

In Group 2.1, 2.2, 2.3, 2.4 anesthetized with 0.0472 mL/L water Clove oil, an increase in the opercular rate was observed from the start of recovery to the time of total recovery. Thus, for group 2.1 (carp BW = 153.2 ± 1.108 g), the frequency of the opercular rate at the time of the first signs of recovery (FSR) was 85.6 movements / min, so that at the moment of total recovery (TR), the frequency would reach 94.8 movements / min. For group 2.2 (carps BW = 441.4 ± 14.882 g) OR1-FSR = 81.6 movements / min. vs. OR3-TR = 93.6 movements / min. Similar situations were also observed for

Prussian carps in group 2.3 (BW = 98.6 ± 1.601 g) OR1-FSR = 99.6 movements / min vs. OR3-TR = 109.6 movements / min, respectively group 2.4 (BW = 241.6 ± 9.911 g) OR1-FSR = 97.2 movements/min. vs. OR3-TR = 100.6 movements/min.

The increase of the respiratory frequency rate at the dose of 0.0472 mL/L Water Clove Oil is the physiological response of fish subjected to a long time under the influence of anesthetics.

For the group 3.1, 3.2, 3.3, 3.4, the frequency of the opercular movements did not show very large differences from the start of recovery (FSR) to the total recovery (TR), thereby indicating the inefficiency of a small dose of anesthetic.

CONCLUSIONS

Anesthetics administered to fish when they are manipulated or suffer invasive interventions, have a beneficial effect by reducing stress. However, the correct dosing of anesthetics is extremely important, and must be effective both in duration and effect.

The results of our study demonstrate the efficacy of the 0.094 mL/Lwater Clove Oil dose, both in terms of anesthesia induction and recovery times and respiratory rate.

The dose of 0.0472 mL / Water Clove Oil is efficient but induces a state of stress to fish due to long induction of anesthesia and recovery times. We do not recommend using the 0.0236 mL / Water Clove Oil dose due to the extremely long anesthesia induction times.

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