EFFICACY OF EUGENOL AS AN ANESTHETIC IN JUVENILE TAMBAQUI, *Colossoma macropomum*

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Anesthetics are important in fish culture for reducing handling stress and mortality. A number of chemicals are used for fish anesthesia, but conventional anesthetics such as tricaine methane sulphonate (MS-222), quinaldine, and 2-phenoxiethanol are toxic to species and expensive.

The most widely available anesthetic in Brazil is benzocaine. Results obtained by Gomes et al. (2001) with tambaqui juvenile suggest that benzocaine meets most of the criteria established for an ideal fish anesthetic. As subsequent phase, alternative anesthetics for tambaqui are necessary, as such eugenol, which is at

the moment one of the most studied alternative fish anesthetic for several species. In a similar manner to benzocaine, eugenol has several characteristics, which qualify it as a safe anesthetic: low cost, efficacy, good margin of safety for fish and is not toxic to humans at the concentrations used. Therefore, the main goal of the present study is to verify the efficiency and to establish a protocol using eugenol as a fish anesthetic for tambaqui juveniles.

Eugenol was dissolved in alcohol at a rate of 10 mL/20 mL (1:2) to make a stock solution. Aliquots of the stock solution (1 mL of eugenol equals to 3 mL from the stock solution) were used to achieve the desired concentrations into the water prior to the addition of the fish. Ten fish were individually exposed to dosages of 0.10, 0.15, 0.20, 0.25, 0.30 and 0.40 ml/L eugenol stock solution for a period of ten minutes at 28 ^{o}C. Behavioral responses were noted, then fish were removed from the anesthetic solution and placed in 60-L aquarium containing 40 L of aerated fresh rearing water for recovery. Immediately following recovery, blood was drawn from the caudal vein with a heparinized syringe from five fish at each concentration of eugenol and glucose was measured. Control value for glucose level was obtained from fish exposed to the highest alcohol concentration (0.8 mL/L) in the water.

Stages of anesthesia were based on criteria of Stoskopf (1993): I/1 - slight loss of reactivity to visual and tactile stimuli; II/1 - loss of equilibrium; II/2 - total loss of equilibrium; III/1 - reduced opercular movement, III/2 - Minimal opercular movement; Recovery - recovery of equilibrium and swimming actively.

Fish exposed to eugenol passed sequentially through the various stages of anesthesia (Table 1). To induce fish with a total loss of equilibrium a dosage of 0.1 was adequate. However, at this dosage, a relatively long time (3.7 min) was needed to induce the minimal opercular movement. To induce fish in a surgical anesthetic state (with minimal opercular movement) a more adequate dosage is 0.25. Recovery time was significantly similar for dosages for up to 0.30 and significantly superior at a dosage of 0.40. Eugenol did not have a significant effect on fish plasma glucose values.

In the present study, eugenol dosage of 0.25 induced all stages of anesthesia in tambaqui within the desired time according to Ross and Ross (1999). The dosage range is also similar to doses used with other anesthetics like MS-222 (Ross and Ross 1999).

Table 1. Behavioral events of tambaqui juveniles exposed to various concentrations of eugenol.

Conc. mg/L	Behavioral event (min.)					
	Loss of reactivity to stimuli	Loss of equilibrium	Total loss of equilibrium	Reduced opercular movement	Minimal opercular movement	Recovery of equilibrium
0.10	0.32±0.11[a]	0.72±0.37[a]	1.93±0.47[a]	2.81±0.57[a]	3.71±0.67[a]	5.83±2.37[a]
0.15	0.38±0.09[a]	1.03±0.28[a]	1.84±0.66[a]	2.67±0.82[a]	3.43± 0.95[a]	6.58±3.53[a]
0.20	0.32±0.11[a]	0.77±0.38[a]	1.45±0.46[a]	2.26±0.39[a]	2.53±0.39[b]	6.82±3.73[a]
0.25	0.35±0.09[a]	0.83±0.46[a]	1.87±0.44[a]	1.32±0.18[b]	2.30±0.50[b]	8.17±2.86[a]
0.30	0.39±0.05[a]	0.66±0.26[a]	1.90±0.51[a]	1.32±0.14[b]	2.28±0.47[b]	9.25±3.81[a]
0.40	0.30±0.08[a]	0.52±0.29[a]	1.41±0.44[a]	0.92±0.42[b]	1.74±0.53[b]	20.59±9.27[b]

Values are means (± SD) of 10 fish for each concentration. Means in each column followed by different letters are significantly different at $P < 0.05$ by Tukey's test. Conc. = Concentration.

Recovery time with eugenol took longer than the ones observed with benzocaine and with values above the ideal time span considered by Ross and Ross (1999), although is not limiting for the use of this anesthetic.

Stress response have been reported with different anesthetics drugs, such as MS-222 and quinaldine (Davies et al. 1982; Ross and Ross 1999) and normally are associated with exposure to high anesthetic dosages or a prolonged exposure. Such result was not observed with eugenol, where blood plasma glucose was similar ($P>0.05$) for all dosages. Mean (± SD) plasma glucose values of five fish for each concentration were between 77.40±7.16 mg/dL for the 0.20 mL/L dosage, 92.60±24.40 mg/dL for the highest dosage (0.40 mL/L), and a value of 71.60±15.82 mg/dL for the control.

Recent research showed the possibilities of using alternative drugs as fish anesthetics, such as AQUI-S and some plants extracts (Mbgenka and Ejiofor 1999), but in Brazil the use of eugenol is most feasible, since this anesthetic showed as a good alternative to benzocaine with tambaqui juveniles.

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