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Eugenol and tricaine methanesulfonate as anhestesics for the pearl cichlid

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ABSTRACT. Anesthesia reduces the handling process duration and prevent fish injuries. The anesthetic effect and ideal concentrations of eugenol and tricaine methanesulfonate (MS-222) were tested for pearl cichlid (*Geophagus brasiliensis*) juveniles with an average weight of 4.4 g in water at 24ºC. The criterion for determining the optimal dose considered an induction time of one minute. Experiment 1 tested the concentrations of 25, 75, 150 and 300 mg L^{-1} of eugenol. The best results were obtained at doses of 150 and 300 mg L^{-1}. Experiment 2 aimed to establish a more accurate result by testing the concentrations of 180, 210, 240 and 270 mg L^{-1}, and led to an estimation of 217 mg L^{-1} of eugenol to induce anesthesia in one minute. Experiment 3 evaluated 200, 300, 400, 500 and 600 mg L^{-1} of tricaine, of which the concentration of 294 mg L^{-1} was estimated to induce anesthesia in one minute. No significant differences were observed for recovery times when using either of the anesthetics. No mortality was observed within 24 hours after the experiments for any concentration of the anesthetics. The present study recommends 217 mg L^{-1} of eugenol or 394 mg L^{-1} of tricaine for anesthesia of the pearl cichlid.

Keywords: *Geophagus brasiliensis*; biometrics; clove oil; native species; MS-222.

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Introduction

The species *Geophagus brasiliensis* (Quoy & Gaimard, 1824), popularly known as pearl cichlid, is found in freshwater habitats throughout South America, from the Amazon River basin to Uruguay (Baumgartner et al., 2012). This species prefers lentic environments such as lakes, floodplains, coastal lagoons, as well as streams and rivers with natural shelter and slow currents, such as backwaters or banks with abundant vegetation (Amaral Júnior, Netto, Garcia, & Mello, 2011, Baumgartner et al., 2012).

The pearl cichlid is a popular ornamental species in the USA, Taiwan and Australia because of its morphology and coloration and has been shown to establish wild populations in the importing countries (Beatty, Morgan, Keleher, Allen, & Sarre, 2013). This species also has high commercial importance in Brazil as a popular sport fish and is targeted for artisanal fishing in various reservoirs and lagoons (Porcher, Poester, Lopes, Schonhofen, & Silvano, 2010). Nevertheless, aquaculture of the pearl cichlid in artificial conditions has only been carried out for research purposes (Beatty et al., 2013).

The commercial production of fish for the ornamental and human consumption markets requires management and manipulation of individuals for biometrics, transportation, or induced reproduction (Hoseini, Taheri Mirghaed, & Yousefi, 2018). These practices can lead to excessive stress and may cause a loss of production and mortality, therefore, it is necessary to use anesthetics to avoid inadequate conditions during management (Priborsky & Velisek, 2018).

Anesthetics reduce physiological stress of fish by restricting metabolic activity, movement and oxygen consumption (Kubitza, 1998). Furthermore, anesthesia reduces the handling process duration and prevent fish injuries. Many chemical products are currently used for anesthesia of fish, of which tricaine methanesulfonate (MS-222) and eugenol (Velisek, Stara, Li, Silovska, & Turek, 2011) are among the more popular anesthetics.

Tricaine is used worldwide for anesthesia of fish and is approved for use with animals destined for human consumption (Carter, Woodley, & Brown, 2011). This anesthetic is derived from benzocaine and has shown

promising results with several fish species (Hinostroza & Martínez, 2013; Chambel et al., 2015). However, tricaine remains accumulated in fish tissue for long periods after dosing, requiring a grace period of at least 21 days for safe consumption (Kubitza, 1998; Hinostroza & Martínez, 2013). Furthermore, tricaine causes a sharp reduction in water pH due to its high solubility and acidity, of which the acidity is corrected with the addition of basic compounds such as sodium bicarbonate (Hinostroza & Martínez, 2013).

Eugenol is a natural compound extracted from the clove tree and is commonly traded. This anesthetic is considered safe for the handler and the environment, and it is beneficial for aquaculture activities because it reduces stress in teleost fishes thus, it is a widely used anesthetic in researches and field (Priborsky & Velisek, 2018). Eugenol is also advantageous because it has no influence on the pH of water and it is eliminated from the fish bloodstream within 48 hours after dosing (Kubitza, 1998; Woody, Nelson, & Ramstad, 2002).

The objective of the present study was to evaluate the anesthetic effect and the optimal concentrations of eugenol and tricaine methanesulfonate (MS-222) for the pearl cichlid based on the criterion of rapid anesthesia, with an induction time of 1 minute and a recovery of less than 5 minutes.

Material and methods

Specimens of the pearl cichlid were captured using artisanal traps in an artificial lake located in Laguna, Santa Catarina state, Brazil (28° 28' 13.9" S; 48° 46' 41.9" W). The fish were then transported to the Aquaculture Laboratory (LAQ) of the Santa Catarina State University (UDESC – Laguna campus) where they were screened to obtain individuals of similar size. The fish were kept for 24 hours in a recirculation system composed of three 250 L polypropylene tanks, mechanical and biological filter and one sump (250 L), where the submerged pump was placed.

Two assays were carried out with eugenol (under different concentrations) and one with tricaine (MS-222). Individual specimens were exposed to different experimental solutions of the anesthetics and were then measured and weighed to simulate the handling of animals for biometrics during commercial activities. After measurements, the fish were transferred to another aquarium of 10 liters and with constant aeration (OD > 6.00 mg L^{-1}) for recovery. Each specimen was subjected to only one exposure to the anesthetics. The water in this aquarium was replaced after each treatment to avoid contamination from wastes, using clean water with similar conditions to which the fish were adapted. Temperature and pH of the water remained constant during the experiments at 24.0 ± 1.0 °C and 7.2 ± 0.2, respectively.

The present study used the criteria of total anesthesia and recovery as described in Woody et al. (2002): total anesthesia was characterized by complete loss of balance and reaction to stimuli, and recovery was characterized by the return of balance and normal swimming. A maximum time of 15 minutes was set for anesthesia. The fish that did not lose balance within this period, it was removed and placed in the recirculation system without the recovery period. The animals were monitored for mortality over 24 hours.

Experiment 1 – Eugenol (25, 75, 150, 300 mg L^{-1})

Eugenol was diluted to a concentration of 1 mL per 10 mL of 95% ethanol as the stock solution. Seven animals were used per treatment, totaling 28 specimens with a mean weight of 4.30 ± 1.22 g and length of 6.63 ± 0.61 cm. The anesthetic was diluted to concentrations of 25, 75, 150 and 300 mg L^{-1}.

Experiment 2 – Eugenol (180, 210, 240, 270 mg L^{-1})

Based on the results of experiment 1, the present study sought to establish a more accurate concentration between 150 and 300 mg L^{-1} for an anesthetic induction time of 1 minute. The concentrations of 180, 210, 240 and 270 mg L^{-1} were tested. Seven fish with a mean weight of 4.59 ± 1.41 g and length of 6.53 ± 0.75 cm were used for each concentration (N = 28).

Experiment 3 – Tricaine (200, 300, 400, 500 and 600 mg L^{-1})

Tricaine methanesulfonate (MS-222) was diluted in deionized water for a stock solution of 2 g L^{-1}. Sodium hydroxide (NaOH, 39.9971 g mol^{-1}) was added to neutralize the pH of the solution as tricaine has high solubility and acidity in water (Huang et al., 2010). This solution was then diluted according to the established treatments. Due to the lack of studies on the use of tricaine as an anesthetic for the pearl cichlid and the high resistance of this species to anesthesia shown in experiments 1 and 2 of the present study, this assay consisted of treatments with high doses of tricaine (from 200 mg L^{-1}) when compared to studies with other fish species

(Hinostroza & Martínez, 2013; Braz, Silva, Tesser, Sampaio, & Rodrigues, 2017). Five treatments were used in this assay and the concentrations of tricaine tested were 200, 300, 400, 500 and 600 mg L^{-1}. Each treatment used seven fish with a mean weight of 4.26 ± 0.75 g and length of 6.49 ± 0.43 cm.

Statistical analysis

Anesthesia induction and recovery times were compared using an analysis of variance (ANOVA) with the significance level set at 5%. When significant differences were found, treatments were compared using the Tukey test. All analyses were performed using the statistics software program R Statistics® (R Core Team, 2018).

Results and discussion

In experiment 1, the fish showed no anesthetic effects after 15 minutes with 25 mg L^{-1} of eugenol. The other concentrations (75, 150 and 300 mg L^{-1}) showed differences in induction time, of which 300 mg L^{-1} of eugenol had the shortest induction time (Table 1, Figure 1A).

Nevertheless, the present study analyzed eugenol concentrations between 180 and 270 mg L^{-1} to obtain a more accurate dose with anesthetic effects in less than one minute. Induction time varied according to the concentration of eugenol, of which the highest levels (240 and 270 mg L^{-1}) presented the shortest induction times (Table 1, Figure 1B).

The regression model established in experiment 2 is represented by the equation $y = 91801.[x]^{-1,363}$, (Figure 1B) where y is the time required for induction and [x] is the concentration of eugenol. The regression showed that a concentration of 217 mg L^{-1} of eugenol will induce anesthesia in one minute for the pearl cichlid. Vidal et al. (2008) evaluated doses between 50 and 250 mg L^{-1} of eugenol for tilapia (initial weight of 5.34 g; temperature 25°C; pH 7.5) and recommended the concentration of 75 mg L^{-1} since this dose induced anesthesia in 82 seconds and showed a total recovery in 143 seconds, which were within the set induction and recovery time criteria of less than 3 and 5 minutes, respectively. However, Vidal et al. (2008) showed a concentration of 107 mg L^{-1} as estimated from the regression model for an induction time of one minute, which is lower than the concentration obtained in the present study (217 mg L^{-1}).

Table 1. Induction and recovery times of anesthesia with different concentrations (mg L^{-1}) of eugenol for the pearl cichlid (*Geophagus brasiliensis*).

Concentrations (mg L^{-1})	25	75	150	300
IND (s)	NA	340.27 ± 148.21 a	121.17 ± 45.40 b	50.58 ± 18.97 c
REC (s)	NA	60.60 ± 20.99 a	102.98 ± 38.08 a	97.00 ± 40.86 a
Concentrations (mg L^{-1})	180	210	240	270
IND (s)	73.50 ± 11.98 a	69.62 ± 20.11 ab	50.68 ± 12.37 bc	43.96 ± 7.02 c
REC (s)	103.86 ± 16.85 a	108.82 ± 33.03 a	70.88 ± 25.73 a	119.42 ± 50.84 a

Mean ± standard deviation. Values followed by different letters in the same row indicate significant difference according to the Tukey test set at a probability at 5%. IND, Time of anesthetic induction. REC, Time of recovery. NA, not anesthatized after 15 minutos of exposure to the anesthetic.

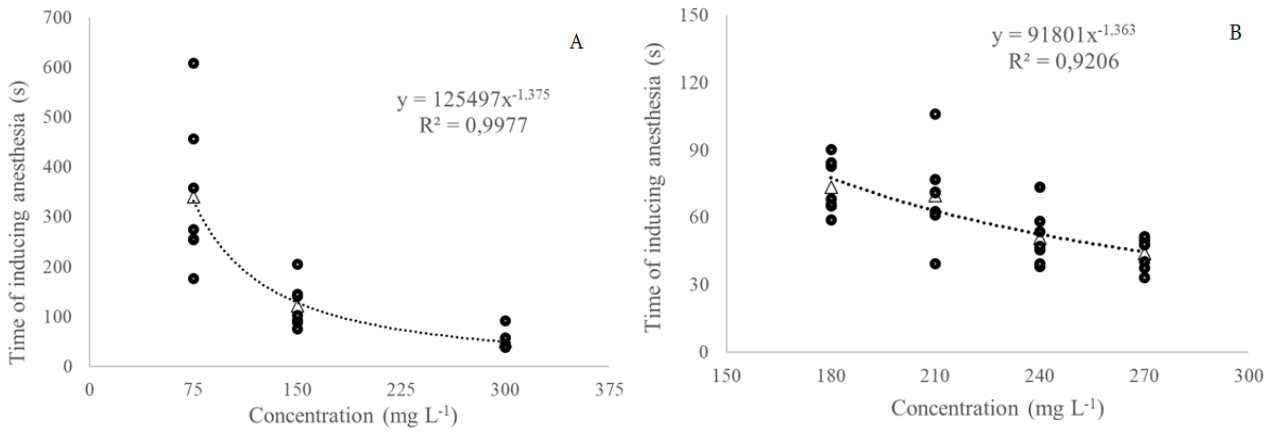

Figure 1. Time of inducing anesthesia with eugenol for the pearl cichlid (*Geophagus brasiliensis*) with concentrations, A: 75, 150 and 300 mg L^{-1}; B: 180, 210, 240 and 270 mg L^{-1}. Circles represent the time of anesthesia for each individual specimen and triangles represent the means of each concentration.

Rocha, Stech, Pinheiro, Pagani, and Sukekava, (2015) evaluated doses between 50 and 200 mg L^{-1} for pearl cichlids with a mean weight of approximately 17.5 g and in water with a temperature of 22.5°C, and observed

that fish were anesthetized almost immediately with 50 mg L^{-1} of eugenol. The previous study also reported an induction time of 110 s with 150 mg L^{-1} of eugenol, similar to that of the present study (121.17 s). Both studies with the pearl cichlid were also similar when considering that no anesthetic effects were observed for fish within 1 minute with concentrations of up to 200 mg L^{-1} of eugenol.

Vidal et al. (2007) used eugenol as an anesthetic for matrinxã (*Brycon cephalus*) juveniles (3.31 ± 0.57 g) and showed that 50 mg L^{-1} was effective to induce anesthesia for this species in less than 3 minutes. The same study suggested that 98 mg L^{-1} of eugenol induces anesthesia in 1 minute based on the calculated regression model. Other regression models estimated that a concentration of 24 mg L^{-1} of eugenol is sufficient to induce anesthesia for pacamã (*Lophiosilurus alexandri*) juveniles (7.44 ± 0.44 g) at one minute (Ribeiro et al., 2013), whereas anesthesia of the snook (*Centropomus undecimalis*) (7.63 ± 0.15 g) at one minute was estimated to require 117 mg L^{-1} of this compound (Bernardes Júnior, Nakagome, Mello, Garcia, & Amaral Júnior, 2013). These results show that the pearl cichlid is resistant to eugenol when compared to other species (Table 2).

Table 2. Studies with the anesthetics of eugenol and tricaine in fish.

	Species	CT (mg L^{-1})	Weight (g)	Temp. (ºC)	pH	CR (mg L^{-1})	C1M (mg L^{-1})	Reference
Eugenol	*Geophagus brasiliensis*	50-200	17.47	22.5	6.0	50-80	-	(Rocha et al., 2015)
	Oreochromis niloticus	50-200	5.34	25	7.5	75	107	(Vidal et al., 2008)
	Brycon cephalus	50-200	3.31	25	NI	50	98	(Vidal et al., 2007)
	Lophiosilurus alexandri	20-120	7.44	28.1	6.4	120	24	(Ribeiro et al., 2013)
	Mugil Liza	50-110	6.9	23.8	7.7	70	-	(Braz et al., 2017)
	Centropomus undecimalis	25-150	7.63	25.8	8.14	50	117	(Bernardes Júnior et al., 2013)
	Oreochromis niloticus	40-120	47,73	23,1	7,8	100	127	(Delbon & Paiva, 2012)
Tricaine MS-222	*Arapaima gigas*	25-100	730	28-30	5.5-6.0	100	100	(Hinostroza & Martínez, 2013)
	Rhamdia quelen (albino)	50-300	21.3	21	7.4	300	308	(Gressler, Parodi, Riffel, Costa, & Baldisserotto, 2012)
	Mugil Liza	100-175	6.9	23.8	7.8	150	-	(Braz et al., 2017)
	Hippocampus kuda	25-150	11.1	28.5	7.9	125	166	(Pawar et al., 2010)

CT, Concentrations tested (mg L^{-1}). Temp, Temperature. CR, Concentration recommend by the authors. C1M, Concentration estimated by the mathematic model to induce anesthesia in 1 minute.

In the present study, the assays with the eugenol showed that increasing concentrations influenced anesthetic induction times, but no significant differences were shown for recovery times ($p > 0.05$). Vidal et al. (2007) also observed that increasing concentrations of eugenol had little influence on the recovery time in anesthetized matrinxã juveniles. For sea bass, recovery time after anesthesia with eugenol showed no consistent correlation with increasing doses (Bernardes Júnior et al., 2013).

The present study evaluated concentrations of 200 to 600 mg L^{-1} of tricaine (MS-222) and found that the shortest induction times ($p < 0.05$) were obtained with concentrations of 400, 500 and 600 mg L^{-1} (Table 3, Figure 2). When considering the criteria used for the eugenol in the present study, the regression model ($y = 75460[x]^{-1.194}$) estimated that 396 mg L^{-1} of tricaine induces anesthesia in one minute for the pearl cichlid. No significant differences were shown between all concentrations of tricaine regarding the recovery time, but full recovery was obtained in less than 5 minutes for each concentration.

Table 3. Induction and recovery times of anesthesia with the concentrations of 200, 300, 400, 500 and 600 mg L^{-1} of tricaine for the pearl cichlid (*Geophagus brasiliensis*).

Time	200	300	400	500	600
IND (s)	147.72 ± 24.73 a	71.70 ± 26.19 b	58.42 ± 12.69 bc	47.97 ± 10.43 bc	36.91 ± 4.30 c
REC (s)	42.48 ± 17.14 a	59.93 ± 28.29 a	84.26 ± 48.89 a	82.27 ± 34.78 a	64.50 ± 25.53 a

Mean ± standard deviation. Values followed by different letters in the same row indicate significant difference according to the Tukey test set at a probability at 5%. IND, Time of anesthetic induction. REC, Time of recovery.

Gressler et al.(2012) evaluated tricaine as an anesthetic for two strains of jundia (*Rhamdia quelen*) with doses of up to 300 mg L^{-1} and showed that the concentrations were inversely proportional to the time to induce anesthesia, of which the highest dose tested (300 mg L^{-1}) were able to anesthetize jundia in less than 3

minutes. For albino jundia (21 g) of the previous study, the regression model estimated that 308 mg L^{-1} of tricaine is required to induce anesthesia in one minute while the regression in the present study estimated that 396 mg L^{-1} is necessary for the pearl cichlid. Pawar et al. (2010) recommended 125 mg L^{-1} of tricaine for anesthesia with the *Hippocampus kuda* and estimated that a concentration of 166 mg L^{-1} was adequate to induce anesthesia within 1 minute. The concentration of tricaine recommended to induce anesthesia in mullets in less than 3 minutes is 150 mg L^{-1} (Braz et al., 2017) and in *Alosa pseudoharengus* between 75 to 100 mg L^{-1} (Berlinsky, Watson, Dimaggio, & Breton, 2016).

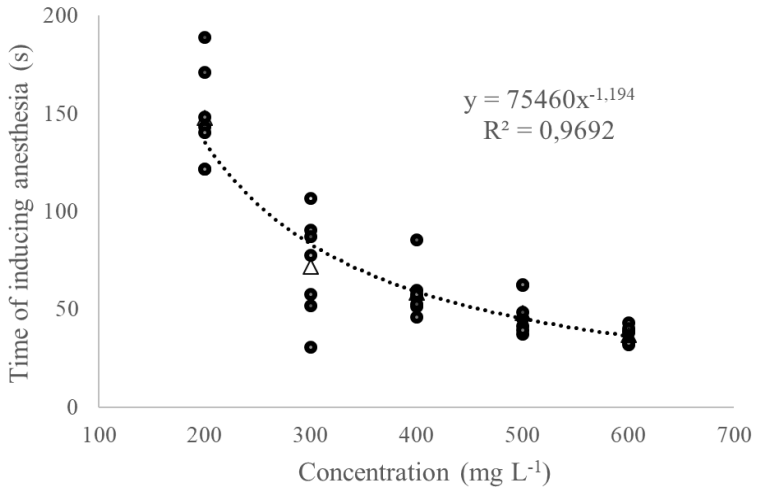

Figure 2. Time of inducing anesthesia with tricaine for the pearl cichlid (*Geophagus brasiliensis*) with concentrations of 200, 300, 400, 500 and 600 mg L^{-1}. Circles represent the time of anesthesia for each individual specimen and triangles represent the means of each concentration.

The variation between studies regarding the recommended dose concentrations of both anesthetics is probably due to differences between species (body shape and gill area) and individual characteristics of each fish such as size, nutritional status and stress (Chambel et al., 2015). Environmental factors such as water temperature, pH, salinity, oxygen and minerals may influence the time to induce anesthesia as well (Sneddon, 2012; Hoseini, Rajabiesterabadi, & Tarkhani, 2015).

Regression curves obtained for all assays in the present study had a pattern similar to those established by other authors for eugenol (Vidal et al., 2007; Bernardes Júnior et al., 2013; Ribeiro et al., 2013) and for tricaine (Pawar et al., 2010), indicating a marked decrease in induction time between lower concentrations and a tendency toward stability as doses increase. Furthermore, the standard deviation was inversely proportional to the doses tested, of which the lowest values of standard deviation were shown with the highest dosages. Thus, higher concentrations of anesthetics are advantageous when considering a greater homogeneity and precision regarding the time for handling anesthetized fish.

No mortalities were observed within 24 hours after the assays despite the exposure of fish to high concentrations of the anesthetics. The same result was observed in Rocha et al (2015), which used eugenol with the same species as the present study. However, post-experiment mortalities have been reported when using tricaine (up to 250 mg L^{-1}) to induce anesthesia of zebra fish, guppy and discus (Chambel et al., 2015).

Results of the present study show that the pearl cichlid is highly resistant to anesthesia and requires higher doses of anesthetics when compared to those reported in the literature for other freshwater fish species. In addition to promoting a faster induction, the criteria of one minute for inducing anesthesia as adopted in the present study optimizes the time used for management that is common in commercial fish farms and has greater precision regarding the time to induce anesthesia as indicated by the lower standard deviation.

Conclusion

The present study recommends 217 mg L^{-1} of eugenol or 394 mg L^{-1} of tricaine for rapid anesthesia (in 1 minute) of the pearl cichlid (*Geographus brasiliensis*) when exposure is carried out with a water temperature and pH of 24 ± 1°C and 7.2 ± 0.2, respectively.

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