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Benzocaine and eugenol as anesthetics for *Brycon hilarii*

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ABSTRACT. Anesthetic products are frequently employed during fish handling practices; however, the correct doses of the various chemicals for different species are still unknown. This study determined the ideal concentrations of benzocaine and eugenol as anesthetics used in *Brycon hilarii* juveniles. The fish were acquired from a commercial fish farm located in western Paraná state, Brazil, totaling 104 juveniles, with average body weight and length of 50.04 ± 20.80 g and 16.30 ± 12.32 cm respectively. The study was carried out at the Aquiculture Laboratory from the Aquaculture Management Study Group - GEMAq at the West Parana State University. Five benzocaine concentrations (50.0, 100.0, 150.0, 200.0 and 250.0 mg L^{-1}) and seven eugenol concentrations (50.0, 100.0, 150.0, 200.0, 250.0, 300.0 and 350 mg L^{-1}) were evaluated in order to assess the induction time to anesthesia. The fish were transferred to anesthetic-free tanks to recover, and the time needed to return to normal activity was recorded. The best results were observed with the benzocaine dose of 100 mg L^{-1} and the eugenol dose between 100 and 150 mg L^{-1}.

Keywords: anesthesia, light sedation, handling, native fish, fish farming, lethargy.

Benzocaína e eugenol como anestésicos para piraputanga (*Brycon hilarii*)

RESUMO. A utilização de produtos anestésicos durante práticas de manejo é frequentemente empregada, porém doses corretas de diferentes fármacos e para espécies distintas ainda estão em fases de pesquisa. O objetivo do estudo foi determinar a melhor concentração de benzocaína e eugenol para juvenis de piraputanga (*B. hilarii*). Foram utilizados 104 juvenis de piraputanga com peso médio de 50,04 ± 20,80 g e comprimento total médio de 16,30 ± 12,32 cm adquiridos em uma piscicultura comercial localizada na região Oeste do Estado do Paraná. O trabalho foi conduzido no Laboratório de Aquicultura do Grupo de Estudos de Manejo na Aquicultura - GEMAq da Universidade Estadual do Oeste do Paraná. Os animais foram submetidos a cinco concentrações de benzocaína (50,0; 100,0; 150,0; 200,0 e 250,0 mg L^{-1}) e sete concentrações de eugenol (50,0; 100,0; 150,0; 200,0; 250,0; 300,0 e 350 mg L^{-1}), para a aferição dos tempos referentes à letargia. Para a recuperação, os animais foram mantidos em aquários livre do anestésico e observado o tempo em que retornaram às atividades normais. A melhor dose de benzocaína verificada foi de 100 mg L^{-1}, enquanto a melhor dose de eugenol foi entre 100 e 150 mg L^{-1}.

Palavras-chave: anestesia, sedação leve, manejo, peixe nativo, piscicultura, letargia.

Introduction

Fish farming has experienced uncontrolled growth in Brazil and worldwide and the commercial potential of species is frequently discovered. Therefore, an improved understanding on the behaviors of different species in cultures is important to establish effective handling techniques reducing losses during fish rearing period.

One of these newly farmed species in Brazil is known as piraputanga, belonging to the genus *Brycon* and Briconinae family. This family comprises around 40 species (LIMA, 2003), reported in few studies in the literature. This species is endemic to Paraná and Paraguay river basins and belongs to the

group of the highly Neotropical migratory fish (HOWES, 1982). It is pursued by both local small fishing communities, for sport fishing due to its aggressive behavior (GANECO; NAKAGHI, 2003), and it is also considered the main species of tourist interest in the area of Bonito, Mato Grosso do Sul State, Brazil (SANCHES; GALETTI JR., 2007).

Anesthetics are used in animals to lower their metabolism during physiological interventions and some procedures of cultures, making them less susceptible to ailments caused by inadequate handling techniques.

Benzocaine and eugenol are among the most important chemicals used for fish sedation in Brazil due to their availability, safety, cost, and efficacy.

Benzocaine has been widely studied as a fish anesthetic to prevent stress during transportation due to its effects on the metabolism (INOUE et al., 2010). According to Gomes et al. (2001) and Oliveira et al. (2009), it is the most widely used anesthetic in Brazil.

Eugenol has been proposed as a low-cost alternative of strong, safe, and non-toxic anesthetic for humans (ROUBACH et al., 2005). It is a natural compound, obtained from distillation of leaves and flowers of the clove tree. Several studies evaluated its use to reduce the animal hypermotility, reducing thus the fish stress during handling (CUNHA et al., 2010; INOUE et al., 2011; VIDAL et al., 2008).

Thus, the present study aimed to determine the best dosage of benzocaine and eugenol for *Brycon hilarii* juveniles as for anesthesia induction, the time spent to reach lethargy for each dosage, and the time required to recover the normal behavior.

Material and methods

The study was carried out at the Aquiculture Laboratory from the Aquaculture Management Study Group - GEMAq at the State University of Western Paraná - Unioeste, Toledo Campus, State of Paraná, Brazil.

Fish were acquired from a commercial fish farm located in the western of Paraná State, Brazil, totaling 104 juveniles, with mean body weight and length of 50.04 ± 20.80 g and 16.30 ± 12.32 cm, respectively.

In laboratory, fish were acclimated during the first 30 minutes and maintained in four 250-liter circular fiberglass tanks with constant aeration and water renewal for 72 hours; the water temperature was kept constant at 25ºC through an electronic heating system. The animals were fed to satiety twice a day, with commercially extruded feed containing 40% crude protein.

Following the acclimatization period, two 30-liter glass tanks were filled with water; one with 10 liters including the respective dose of anesthetic and other with only clean water and constant aeration for recovery of the individuals.

The experimental design consisted of 12 treatments, corresponding to five concentrations of benzocaine (50, 100, 150, 200, and 250 mg L^{-1}), seven of eugenol (99% purity grade) (50, 100, 150, 200, 250, 300, and 350 mg L^{-1}), and a control group subjected to the same handling process without sedation. For each treatment, eight fish were randomly selected (n = 8) and individually exposed to each concentration. The water in the experimental tanks was replaced at the end of each concentration test.

The times required to display the patterns of behavior described in the Table 1 were monitored using a digital chronometer.

Table 1. Behavioral characteristics in fish according to the different stages of anesthesia.

Stage	Behavioral characteristic
I	Visibly slow or erratic opercular movement.
II	Partial loss of balance and difficulty to maintain the normal swimming position when not in movement.
III	Total loss of balance and inability to recover from the vertical swimming position ('belly-up').
IV	Lack of reaction to any stimulus.
Recovered	Recovery to the normal swimming position and ability to swim

Source: Woody et al. (2002).

The absence of reaction to any stimulus was verified by touching the side of the body of the specimen with a glass rod. The animals were removed from the induction tank when achieved the stage IV (Table 1), dried in paper towels, measured and weighed in order to simulate the handling process, and subsequently placed in the recovery tank.

After recovery, fish were transferred to the 250-liter tanks, fed, and monitored to mortality for 96 hours as recommended by Vidal et al. (2006).

Stock solutions of benzocaine and eugenol were prepared in ethyl alcohol (92.8º) at the

concentration of 100 mg mL^{-1}.

A regression analysis of the data was performed comparing the mean values observed through the analysis of variance (ANOVA) at 5% significance level, using the statistical program Sistema de Analises Estatísticas e Genéticas - SAEG 7.1 (SAEG, 1997).

Results and discussion

Benzocaine and eugenol showed significant differences in the performance as anesthetics ($p < 0.05$) for the respective doses applied. The dosage of 50 mg L^{-1} benzocaine produced the longest induction time and was statistically different ($p < 0.05$) from the other doses. The concentrations 50, 100, and 150 mg L^{-1} did not show any recovery times significantly different, except the dosage of 250 mg L^{-1}, which although taking less time to induce the anesthesia in the animals, led to mortality (Table 2).

Table 2. Induction time to anesthesia and length of time to recovery in *Brycon hilarii* juveniles subjected to different doses of benzocaine.

Concentration (mg L^{-1})	Anesthesia (seconds)	CV (%)	Recovery (seconds)	CV (%)
50	112.50	21.19	207.00	39.84
100	81.50	21.94	189.50	46.15
150	66.00	47.26	198.71	44.17
200	50.85	30.49	303.00	34.49
250**	31.88	19.58	253.83	39.10

Anesthesia = linear effect $\hat{y} = 129.11 - 0.3923x$; $r^2 = 0.96$. Recovery = linear effect $\hat{y} = 166.96 - 0.4966x$; $r^2 = 0.40$. **Caused mortality in two animals. C.V. (%) = coefficient of variation.

An inverse proportional effect (Figure 1) was observed with the use of benzocaine, between the increase in concentration and the decrease in the induction time to anesthesia. The same pattern was not observed for the recovery time, which was independent from the doses.

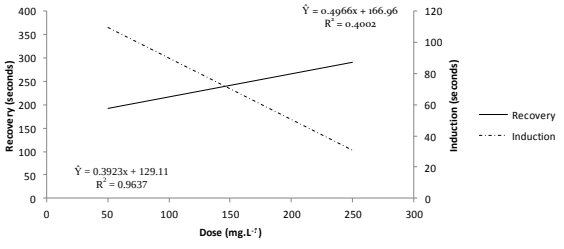

Figure 1. Induction and recovery of piraputanga juveniles subjected to anesthesia with benzocaine.

The experiments with eugenol showed that the higher the concentration tested the shorter the time necessary to induce coma. The same pattern was not observed for recovery, regardless of the concentration applied.

Significant differences ($p < 0.05$) were observed between the concentrations of eugenol, for both the anesthetic effect and the time spent to return to normal activities (Table 3). The 50 mg L^{-1} dose was associated with the time longer than 300 seconds to reach sedation, whereas the fish subjected to the highest dose (350 mg L^{-1}) reached the stage of sensory absence to any stimulus within only 29 seconds.

Table 3. Induction time to anesthesia and length of time to recovery in *Brycon hilarii* juveniles subjected to different doses of eugenol.

Concentration (mg L^{-1})	Anesthesia (seconds)	CV (%)	Recovery (seconds)	CV (%)
50.00	306.38	20.43	342.25	20.79
100.00	122.63	18.78	317.38	22.10
150.00	95.88b	23.41	348.88	16.22
200.00*	65.23	30.60	468.13	16.37
250.00*	39.25	24.42	336.13	23.53
300.00***	30.88	12.15	443.25	19.20
350.00**	29.00	24.51	835.63	25.76

Anesthesia = quadratic effect $\hat{y} = 397.16 - 2.7085x + 0.0049x^2$; $r^2 = 0.92$. Recovery = quadratic effect $\hat{y} = 476.29 - 2.508x + 0.0093x^2$; $r^2 = 0.75$. *Caused mortality in one animal. **Caused mortality in two animals. ***Caused mortality in three animals. C.V.(%) = coefficient of variation.

A quadratic effect (Figure 2) was observed for induced anesthesia through the regression analysis, and by derivation of the equation, a value of 276.38 mg L^{-1} was obtained as the best concentration. Nevertheless, although concentrations higher than 200 mg L^{-1} have shown the shortest values of time, they resulted in fish mortality due to toxicity, in addition to the increased recovery times of fish.

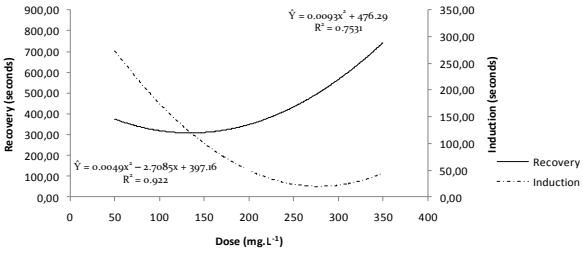

Figure 2. Induction and recovery of piraputanga juveniles subjected to anesthesia with eugenol.

Through the regression analysis, an equation with quadratic effect was obtained for *Brycon hilarii* juveniles subjected to benzocaine. The best dosage of benzocaine obtained by derivation of this equation, for this species, was 100 mg L^{-1} (Figure 1). No death was observed with the use of this concentration, and the normal mobility was recovered after 189 seconds.

According to Okamura et al. (2010), the last stage of anesthesia represents the threshold between the reversible anesthesia and the medullary collapse which leads the animal to death. This is also observed when the animals experience the most intense effect of the drug, showing high levels of anesthetization. In the present study, the animals reached this condition under all tested concentrations. However, because they have remained under the anesthetic effect after reaching that stage for only a short time, they were able to recover the normal patterns of movement except when subjected to the concentration of 250 mg L^{-1} benzocaine, from which two fish did not recover.

Gimbo et al. (2008), by using benzocaine as anesthetic for *Astyanax altiparanae*, observed that 100 mg L^{-1} leads to loss of reaction to handling after approximately one minute, and three minutes were required for the recovery. Similar results were also observed for the present study with *B. hilarii* juveniles, according to the ranges of time recommended by Marking and Meyer (1985), in which the animal sedation should not exceed three minutes and the recovery time should occur within five minutes.

In a study with matrinxã, a species of the same genus of *B. hilarii*, Inoue et al. (2002) observed that the concentration of 60 mg L^{-1} benzocaine was enough to sedate the animals for approximately one minute. This length of time is shorter than observed in the present study; fish were sedated after one minute only when subjected to concentrations above 150 mg L^{-1}.

The lengths of time observed in the present study are shorter than reported by Okamoto et al. (2009) with *Trachinotus marginatus* juveniles, both for induced anesthesia and recovery, in all concentrations used. This reinforces the differences in the species-specific behavior observed herein. Antunes et al. (2008) highlighted that a 1-gram increase in the body weight corresponds to a 0.158 mg L^{-1} rise in the dosage of benzocaine hydrochloride for common carp (*Cyprinus carpio*). This possibly occurs with all fish species - an increase in the body weight requires a larger dose to induce sedation, corroborating with the idea that the smaller the fish, the larger the area of chemical absorption.

Bastos-Ramos et al. (1998) reported that benzocaine satisfactorily meets the requirements for surgical procedures in Antarctic fish and for fish species of temperate climate in general. These authors observed that, with a dose of 100 mg L^{-1}, the animals required from 6 to 8 minutes to reach the stage of sedation and remained sedated for approximately 15 to 20 minutes; in addition, this dose also allowed for, if necessary, 2 more applications on the same fish.

Hegedus and Herb (2005) described that benzocaine at high concentrations may lead to methemoglobinemia, preventing iron in the hemoglobin to transport oxygen to the tissues. This was probably the cause of death of two fish subjected to the 250 mg L^{-1} dose.

The recovery time recorded for the animals sedated with eugenol were also significantly different for the different doses used. This recovery time was considered as the time required for the fish to regain their normal functions, including functions of escaping and reacting to disturbances imposed. The exposure to concentrations of 300 mg L^{-1} or higher, led to recovery times exceeding 400 seconds and consequently, to death. A quadratic effect was evidenced from the regression analysis as well as by derivation of the equation; the ideal dosage of 134.40 mg L^{-1} was then obtained (Figure 2).

Although eugenol is still considered as an alternative anesthetic to induce lethargy in fish, it has also been reported in studies performed in different animals. This demonstrates the facility to obtain the product and the efficacy of its use, related to the time necessary for the animals to undergo the anesthesia procedure and to regain their behavioral activities, such as, for instance, of escaping.

For matrinxã juveniles, Vidal et al. (2007a) concluded that the ideal doses of eugenol are within the range of 50-100 mg L^{-1}, providing less times than those for the present study. This difference may reflect the difference in fish size between piraputanga (50.04 ± 20.80 g) and matrinxã (3.31 ± 0.57 g) juveniles. In addition, *B. hilarii* juveniles can be anesthetized with 100 mg L^{-1} eugenol instead of 150 mg L^{-1} because the recovery time is around five minutes, which is recommended for the normal resumption of the normal activity in fish, depending on the objective of the sedation. According to Vidal et al. (2007b), there is no influence of the live weight in matrinxã juveniles on the induction or recovery time for the anesthetic effect of eugenol, which places the anesthetic concentration as the main factor in animal sedation.

Inoue et al. (2003) reported shorter anesthetic induction and recovery times when compared with our results. Those authors observed doses

between 40 and 60 mg L^{-1} as ideal concentrations for clove oil in matrinxã juveniles. These same doses, applied in *B. hilarii* juveniles, resulted in longer induction and recovery times in the present study.

Moreira et al. (2010) considered the eugenol at 120 mg L^{-1} as ideal for adults of Nile tilapia in studies regarding biometry because it reduces the opercular beat rate. This dose is in accordance with the present study, where the anesthesia induction and recovery times are within tolerable limits.

The use of anesthetics is extremely important to enable handling practices, when administered in correct dosages. It reduces the animal hypermotility and, consequently reduces the stress and lowers the risks of death and infections by opportunistic pathogens.

Conclusion

The ideal dose for induction of anesthesia and recovery to normal behavior in *Brycon hilarii* juveniles was 100 mg L^{-1} for benzocaine and within the range of 100-150 mg L^{-1} for eugenol. The time required for the animals to be completely sedated ranged from 31 to 112 seconds for the highest and lowest concentration of benzocaine, and from 29 to 306 seconds for the highest and lowest concentration of eugenol applied to the water. For the animal recovery, the elapsed time ranged from 207 to 303 seconds when under the effect of benzocaine and from 317 to 835 seconds when under the effect of eugenol.

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