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Benzocaine and clove oil as anesthetics for pejerrey (*Odontesthes bonariensis*) fingerlings

[*Benzocaina e óleo de cravo como anestésicos para alevinos de peixe-rei* (Odontesthes bonariensis)]

J. *Morato-Fernandes*[1], R.A. *Tavares*[1]; C. B. *Rocha*[1], J.L.O.F. *Pouey*[2], S.R.N. *Piedras*[2*]

[1]Aluno de pós-graduação – FAEM – Universidade Federal de Pelotas, RS
[2]FAEM – Universidade Federal de Pelotas, RS

ABSTRACT

Pejerrey (*Odontesthes bonariensis*) is a native species from Rio Grande do Sul, Uruguay and Argentina where it is of great economic importance for artisanal fishing. One difficulty in laboratory research with pejerrey is related to its sensitivity, as it presents higher basal cortisol levels than other freshwater species. For this reason, the aim of this work was to evaluate the efficiency of benzocaine and clove oil as anesthetics for pejerrey fingerlings. Two experiments were done where fingerlings (57±7.8mm and 1.1±0.44g) were exposed to benzocaine with concentrations between 40mgL^{-1} and 120mgL^{-1} and to clove oil with concentrations between 12mgL^{-1} and 75mgL^{-1}. Survival, anesthesia induction time and recovery time for each pharmaceutics were evaluated. Both benzocaine and clove oil pharmaceutics showed efficiency as anesthetics for pejerrey fingerlings, with negative correlation between the dose of anesthetics and the anesthesia induction time. For benzocaine, the concentrations between 80mgL^{-1} and 100mgL^{-1} showed better results, as for clove oil the optimal concentrations were between 25mgL^{-1} and 50mgL^{-1}. On the other hand, the anesthesia recovery time did not present significant variation on the different concentrations of the tested products. The tested products are highly metabolizable by pejerrey.

Keywords: anesthesia, survival, induction, recovery, aquaculture

RESUMO

O peixe-rei (Odontesthes bonariensis) *é uma espécie nativa do Rio Grande do Sul, Uruguai e Argentina, onde tem grande importância econômica para a pesca artesanal. Uma dificuldade da pesquisa em laboratório com peixe-rei está relacionada à sua sensibilidade, pois apresenta níveis basais de cortisol mais elevados que outras espécies de água doce. Este trabalho avaliou a eficiência da benzocaína e do óleo de cravo como anestésicos para alevinos de peixe-rei. Foram realizados dois experimentos em que alevinos (57±7,08mm e 1,1±0,44g) foram expostos à concentração entre 40mg^{-1} e 120mgL^{-1} de benzocaína e entre 12mgL^{-1} e 75mgL^{-1} de óleo de cravo. Avaliaram-se a sobrevivência, o tempo de anestesia e o tempo de recuperação para cada um dos fármacos. Ambos os fármacos, benzocaína e óleo de cravo, mostraram eficiência para anestesiar peixe-rei, com correlação negativa entre a dose e o tempo de indução de anestesia. Para benzocaína, concentrações entre 80mgL^{-1} e 100mgL^{-1} mostraram melhor resultado, enquanto para óleo de cravo as melhores concentrações ficaram entre 25mgL^{-1} e 50mgL^{-1}. Por outro lado, o tempo de recuperação do estado de anestesia não apresentou variação significativa nas diferentes concentrações testadas. O peixe-rei tem elevada capacidade de metabolização dos produtos testados.*

Palavras-chave: anestesia, sobrevivência, indução, recuperação, aquicultura

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*Autor para correspondência (*corresponding author*)
E-mail: moratofernandes@hotmail.com

INTRODUCTION

The use of native species in aquaculture has a number of advantages, mainly because they do not present environmental constraints, are adaptable to local climate and there is already a consumer market. Of the many native species (traíra, jundiá, peixe-rei, lambari, pintado, viola, cascuda, grumatã, piava e cará) from Rio Grande do Sul with the potential for fish farming, the most studied are jundiá (*Rhamdia quelen*) and pejerrey (*Odontesthes bonariensis*) (Baldisserotto, 2009).

Pejerrey has been the subject of studies related to reproduction and bio-ecology, however there are few studies related to its handling techniques, due its sensitivity caused by higher basal cortisol levels than other freshwater species (Tsuzuki *et al*., 2001), which hinders laboratory research.

The handling of fish during the biometrics, classification, transport, artificial breeding and other procedures has increased stress levels that result in injuries and immunologic depletion that can lead to stunted growth, infections and even death. Moreira and Volpato (2004) claim that stress is a factor more responsible for the occurrence of disease and mortality in aquaculture, and in fish the recovery from a mnemonic stressful experience induces increased plasma levels of cortisol.

In contrast, Galhardo and Oliveira (2006) argue that stress situations in fish may be reversed by increasing fear response through the administration of analgesics. Moreover, the Federal Law No.11,794, 2008 that provides for the maintenance of an institutional national database of the Ethics Committees of Animal Use (CEUAs) (Brazil, 2008), has led to greater severity in conducting scientific research using animals, outlining handling standards, which in turn are not known for many species.

In this sense this study aims to evaluate two anesthetics, which allow the appropriate handling of *Odontesthes bonariensis fingerlings*.

MATERIALS AND METHODS

Ninety *Odontesthes bonariensis* fingerlings with average total length of 57±7.08mm and average weight 1.1±0.44g randomly selected from a population of 1,000 animals, stored separately in 1,000 liter polyethylene boxes were used. The entire population was subjected to fasting for 24 hours before the experiment.

The drugs were previously diluted in ethanol at a concentration of 10g $0.1mL^{-1}$ and then placed in a volumetric flask, which had its volume completed to 1.0 liter with distilled water, and this product with a final concentration of $10mgL^{-1}$ was the stock solution used to establish the desired concentrations.

Before the final exposure, preliminary tests were performed when concentrations were between 10 and $200mgL^{-1}$ of the products for subsequent establishment of the experimental concentrations.

Exposure to anesthetics was adapted from Ross and Ross (1999, 2008) and held in plastic containers with a capacity of two liters of water. Two experiments were conducted with a completely randomized design, where the experimental unit was an individual. In the first experiment five treatments with benzocaine at concentrations of 40, 60, 80, 100 and $120mgL^{-1}$, with ten replicates per treatment were tested. In the second experiment four treatments with clove oil at concentrations of 12, 25, 50, $75mgL^{-1}$, also with ten replicates per treatment were tested.

For each anesthetic concentration, ten individual animals were exposed and the time that each animal reached the third stage of anesthesia (total loss of equilibrium) proposed by Ross and Ross (2008) was evaluated. After reaching the desired stage of anesthesia, fish were removed from the anesthetic solution, measured in length and weight and then kept in a 20 liter tank, containing 18 liters of water with constant aeration to determine the recovery time.

The recovery time of the anesthesia state was measured from the removal of the fingerling from anesthetic until its full recovery, including the time that it remained out of the water for the biometric implementation. The two times were not measured separately. The animals were considered recovered when they resumed swimming movement and balance, as described for Stage four by Ross and Ross (2008). The fish were kept in aquaria to recover for 96 hours to assess mortality.

The physical and chemical environmental conditions in anesthesia and recovery tanks were monitored. Temperature and dissolved oxygen were measured by oxymeter YSI-F-150, pH and alkalinity with pot Alfakit by titration (Apha, 1998). The time to reach the stage of anesthesia with the different products and doses tested were analyzed by regression. The anesthesia recovery time data were subjected to variance analysis and showed no significant difference. Analyses were performed by the Statistic 5.0® statistical package.

RESULTS AND DISCUSSION

The environmental condition during the tests showed no significant differences between treatments. Temperatures of 23.4±0.94°C, dissolved oxygen 6.3±0.98mgL^{-1}, pH 7.4±0.17 and alkalinity of 55±7.5mgL^{-1} $CaCO_3$, are suitable parameters for the pejerrey (Piedras *et al*., 2009).

Preliminary tests showed that benzocaine concentrations above 120mgL^{-1} caused deep anesthesia without recovery of animals, and consequent death. These conditions correspond to anesthesia state six according to Storkopf (1993). Moreover, concentrations of benzocaine lower than 40mgL^{-1} did not cause satisfactory anesthesia effects. In this case, the fish stayed in a pre-induction state (Ross and Ross, 2008). The same occurred with a clove oil concentration of 10mgL^{-1}. On the other hand, clove oil concentrations higher than 75mgL^{-1} were not tested due to formation of froth, a dilution decrease and increase in water cloudiness.

The effectiveness of benzocaine as an anesthetic for pejerrey fingerlings showed a negative time dependence correlation in induction of anesthesia in relation to concentration (Figure 1).

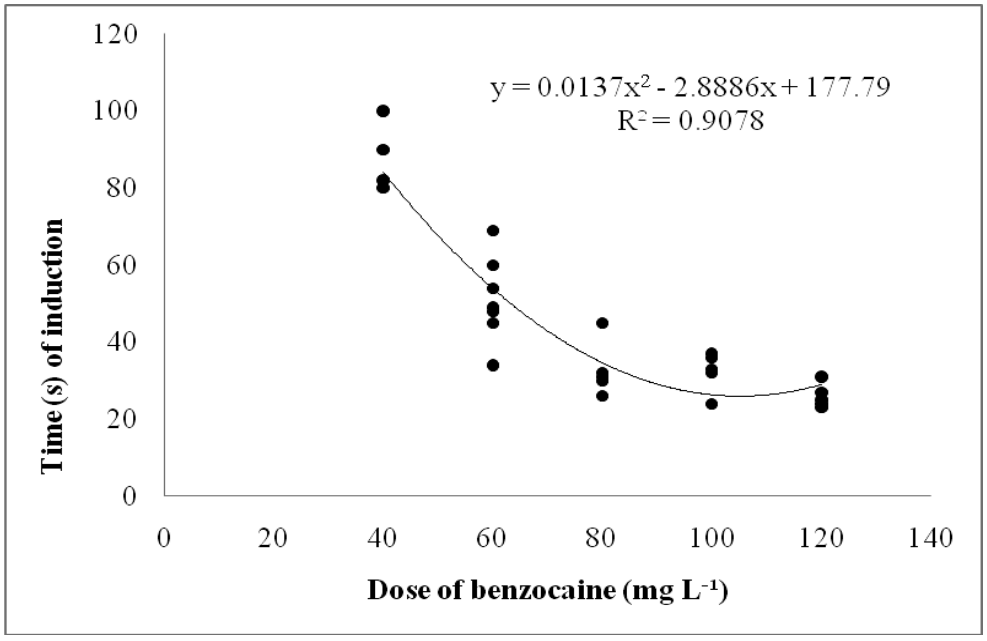

Figure 1. Relation between the dose of benzocaine and anesthesia induction time for pejerrey fingerlings (*Odontesthes bonariensis*).

The benzocaine dosage that provided the desired anesthetic effect for 30 seconds, as considered satisfactory by Roubach and Gomes (2001) and Iwama *et al*. (2004) was 87.35mgL^{-1} (Figure 1). These results are lower than 100mgL^{-1} reported by Delbon (2006) for tilapia (*Oreochromis niloticus*), and 125mg L^{-1} for lambari (*Astyanax altiparanae*) (Gimbo *et al*., 2008). Moreover, Okamoto *et al*. (2009) established the dosing between 50 and 75mgL^{-1} of benzocaine for pampo (*Trachinotus marginatus*) to reach the third stage of anesthesia in 30 seconds. Although

the values reported for several species are approximate, differences in the concentration of benzocaine, yet small, show the specificity of action of the drug, as suggested by Graham and Iwama (1990) and Iwama *et al*. (1994).

The concentration of 120mgL^{-1} of benzocaine resulted in a mortality of 20% of animals 96 hours after exposure, suggesting that the lethal concentration of this anesthetic for pejerrey is close to this value, this result being similar to 125mgL^{-1}, recorded by Gimbo *et al*. (2008) for lambari (*Astyanax altiparanae*). Antunes *et al*. (2008) argue that the boundaries between the optimal concentration and the lethal concentration of the anesthetic for a given species are very close.

The correlation between the anesthesia induction time and the benzocaine concentration confirms the hypothesis that higher concentrations of anesthetic result in shorter time to reach the desired state of anesthesia. Although this fact is mathematically proven, doses should be considered within physiological limits of this species, because high doses, although not lethal, can lead to metabolic disorders and deleterious effects such as vision and olfactory damage (Barbosa *et al*., 2007, Okamoto *et al*., 2009).

Clove oil as an anesthetic for pejerrey fingerlings showed a negative correlation between the product concentration and anesthesia induction time that is represented in Figure 2.

Considering the ideal anesthesia induction time of 30 seconds, the concentration of clove oil resulted in 31.74mgL^{-1} (Figure 2). This result shows the efficiency of clove oil as an anesthetic for pejerrey fingerlings, confirming the records of 40mgL^{-1} obtained by Keene *et al*. (1998) for rainbow trout (*Oncorhynchus mykiss*), 100mgL^{-1}, recorded by Simões *et al*. (2010) for tilapia and 50mgL^{-1} for pampo (*Trachinotus marginatus*) (Okamoto *et al*., 2009). There was no mortality on the doses of clove oil tested.

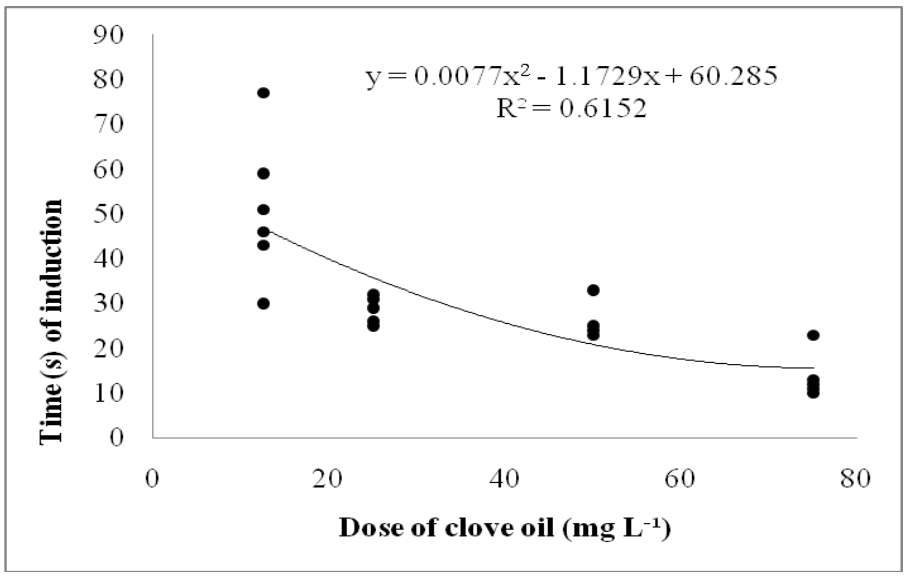

Figure 2. Relation between the dose of clove oil and anesthesia induction time for pejerrey fingerlings (*Odontesthes bonariensis*).

There is no dependence between dose and recovery time, which had been registered by Gimbo *et al*. (2008) for lambari (*Astyanax altiparanae*) exposed to concentrations of 50 to 125mgL^{-1} of benzocaine. This recovery time is lower than 4 to 7 minutes found by Pereira-Da-Silva *et al*. (2009) for lambari (*Astyanax* sp.) and 2 to 8 minutes for juvenile pampo (*Trachinotus marginatus*) found by Okamoto *et al* (2009).

Although the physiological mechanisms of fish recovery anesthesia are not sufficiently clear, Park *et al*. (2008) argue that the shorter the induction of anesthesia, the greater the recovery

time. This rule does not apply to pejerrey because regardless of the concentration and anesthesia induction time, recovery time showed no significant difference. In the case of pejerrey, that can be explained by the fact that its blood cortisol levels are higher (Tsuzuki *et al*., 2001), which results in a higher gill ventilation and blood oxygenation, therefore these higher cortisol levels shown by pejerrey are not entirely negative.

Lima *et al*. (2006) discussed stress in fish and said that in certain situations high cortisol levels taken can be beneficial, which can be applied to the pejerrey regarding recovery time from the anesthesia state. On the other hand, Barbosa *et al*. (2007) studying the physiological effects of clove oil in matrinxã (*Brycon amazonicus*) reported that clove oil does not reduce the levels of lactate, which is the product of anaerobic glycolysis. The low blood oxygenation caused by the drug does not stop the cellular energetic activity, but reduces it, since anaerobic glycolysis produces less ATP. In pejerrey fingerlings, the recovery time of the anesthesia state was not related to the anesthetic concentration used, because as soon as the blood oxygenation was restored, the metabolic activities returned to the physiological status of the species.

Recovery time for animals exposed to concentrations between 40 and 120mgL^{-1} of benzocaine showed no significant difference ranging between 38.5 and 48.5, average 40.5±11.5 seconds (Table 1).

Table 1. Recovery time from the anesthesia state and mortality of pejerrey fingerlings (*Odontesthes bonariensis*) submitted to benzocaine and clove oil.

Benzocaine					
Concentration (mg L^{-1})	40	60	80	100	120
Recovery time (s)	38.5±14.4	48.5±9.1	47.2±9.6	46,8±10.7	48.3±11.3
Mortality (%)	0	0	0	0	20
Clove Oil					
Concentration (mg L^{-1})	12	25	50	75	
Recovery time (s)	115±16.5	151.0±36	131.5±40.5	132±50.7	
Mortality (%)	0	0	0	0	

Similar to what occurred with benzocaine, the animals exposed to clove oil at concentrations between 12 and 75mgL^{-1} presented no significant differences in recovery time from the anesthesia state. The average recovery time was 132.03±37.8 seconds, ranging from 115 to 151 seconds.

CONCLUSIONS

The benzocaine and clove oil are effective as anesthetics for pejerrey fingerlings, providing suitable conditions for the handling of animals in research activities. The recovery of the anesthesia state is satisfactory without the occurrence of mortality, if concentrations between 80 and 100mgL^{-1} of benzocaine and between 25 e 50mgL^{-1} of clove oil are used. In the continuing quest for knowledge of this species, further studies are needed to evaluate the use of anesthetics in adults. Also, it is important to evaluate side effects that may produce stress, causing physiological, behavioral, reproductive changes in performance, which are still unknown.

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