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Menthol as a natural anesthetic for guppy

Mentol como anestésico natural para guppy

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ABSTRACT

The objective of this study was to evaluate different menthol concentrations for the anesthesia of adult females, adult males, and juvenile guppy (*Poecilia reticulata*). To this end, 30 adult females (0.379 ± 0.108 g), 30 adult males (0.220 ± 0.049 g), and 30 juveniles (0.033 ± 0.016 g) were used. The animals were individually exposed to five concentrations of menthol (50, 100, 150, 200, and 250 mg L^{-1}), six fish per concentration. The induction times, anesthetic recovery, and mortality up to 96 h after the experiment were evaluated. The concentration of 50 mg L^{-1} did not induce anesthesia in any of the studied groups. The concentrations of 100 and 250 mg L^{-1} resulted in 100% mortality in the adults (male and female) and juveniles, respectively. The concentrations from 150 to 250 mg L^{-1} and from 150 to 200 mg L^{-1} of menthol were efficient and safe for use as anesthesia for guppy adults (male and female) and juveniles, respectively.

KEYWORDS: anesthetic, induction, ornamental fish, *Poecilia reticulata*, recovery.

RESUMO

O objetivo deste estudo foi avaliar diferentes concentrações de mentol para anestesia de fêmeas adultas, machos adultos e juvenis de guppy (*Poecilia reticulata*). Para isto, foram utilizadas 30 fêmeas adultas (0,379 ± 0,108 g), 30 machos adultos (0,220 ± 0,049 g) e 30 juvenis (0,033 ± 0,016 g). Os animais foram expostos, individualmente, a cinco concentrações de mentol (50, 100, 150, 200 e 250 mg L^{-1}), seis peixes por concentração, sendo avaliados os tempos de indução e recuperação anestésica e a mortalidade até 96 h após a realização do experimento. A concentração de 50 mg L^{-1} não induziu a anestesia em nenhum dos grupos avaliados. As concentrações de 100 e 250 mg L^{-1} ocasionaram a mortalidade de 100% dos adultos (machos e fêmeas) e juvenis, respectivamente. As concentrações de 150 a 250 mg L^{-1} e 150 a 200 mg L^{-1} de mentol demonstram eficácia e segurança para anestesia de adultos (machos e fêmeas) e juvenis de guppy, respectivamente.

PALAVRAS-CHAVE: anestésico, indução, peixe ornamental, *Poecilia reticulata*, recuperação.

In ornamental fish farming, practices such as batch count, biometry, sorting by size, and transport trigger physiological stress responses in fish (STEVENS et al. 2017). The anesthesia during this management is an alternative to mitigate harmful effects from stress (PEREIRA-DA-SILVA et al. 2016, BALAMURUGAN et al. 2016). Among the anesthetics used, natural substances (extracted from plants), such as menthol, have emerged as an alternative for fish sedation opposite synthetic anesthetics often used, such as benzocaine and MS-222 (tricaine methanesulfonate) because of their easy acquisition, low cost, and low risk to handler and fish (GONÇALVES et al. 2008, PEDRAZZANI & OSTRENSKY NETO 2016, ROMANELI et al. 2018).

The efficacy of anesthetic substances varies between species (CHAMBEL et al. 2015) and between individuals of the same species due to the biological characteristics (lineage, size, sex) of each organism (TEIXEIRA et al. 2011, PARODI et al. 2014, CUNHA et al. 2015). Thus, studies on the assessment of concentrations and appropriate safety margins of each anesthetic for each species considering their biological differences are essential.

Given the importance of this information for a proper management, recent years have shown an increase in studies with anesthesia of ornamental fish. Promising results were reported with natural anesthetic for zebrafish (*Danio rerio*) (DOLEŽELOVÁ et al. 2011, WONG et al. 2014), guppy (*Poecilia reticulata*) (DOLEŽELOVÁ et al. 2011, CUNHA et al. 2015, BOLASINA et al. 2017), platy (*Xiphophorus maculatus*) (HOSHIBA et al. 2015), clown anemonefish (*Amphiprion ocellaris*) (OSTRENSKY et al. 2016, PEDRAZZANI & OSTRENSKY NETO 2016), freshwater angelfish (*Pterophyllum scalare*) (ROMANELI et al. 2018), and siamese fighting fish (*Betta splendens*) (PATTANASIRI et al. 2016). However, studies that evaluate the differences between individuals of the same species during anesthesia are scarce. Thus, the objective of this study was to evaluate different concentrations of menthol in the anesthesia of adult females, adult males, and juvenile guppy (*Poecilia reticulata*).

The experiment was conducted at the Laboratório de Aquariofilia of the Universidade Federal do Pampa (UNIPAMPA), Campus Uruguaiana, RS, Brazil. The experiment consisted of 30 adult females (0.379 ± 0.108 g), 30 adult males (0.220 ± 0.049 g), and 30 juveniles (0.033 ± 0.016 g) of guppy (*Poecilia reticulata*). After preliminary tests, different responses to anesthesia induction were observed between females and males. Thus, animals were separated into three categories. The specimens that had not yet presented apparent sexual dimorphism were considered juveniles and, therefore, evaluated as a separate group. The animals were separated in tanks (100-liter water capacity) according to their respective categories (female, male, and juvenile) and acclimated during 15 days under same experimental conditions. The fish were fed daily with flaked commercial feed (Alcon Basic, SC, Brazil) for ornamental fish, at 9:00 and 17:00, *ad libitum*. Tank siphonage was performed daily to remove the feces.

For anesthetic induction, 5-liter tanks were used, containing 2.5 liter of water. The menthol (Vetec Química Fina Ltda.) was diluted in ethanol (99.8%) at 1:10. Preliminary tests confirmed that the alcohol concentrations used for diluting the menthol caused no anesthetic effects on fish. A maximum exposure time (30 minutes) to the concentrations of anesthetic was also determined. For each treatment, six females, six males, and six juveniles were randomly selected and submitted, one by one, to the concentrations of 50, 100, 150, 200, and 250 mg L^{-1} of menthol, totaling 18 fish per treatment. After the exposure, the animals were removed from the anesthetic induction tanks and introduced into a recovery tank containing seven liters of water, with constant aeration, and without anesthetic, to assess fish recovery time. The anesthesia and recovery were divided into three stages, of which specific characteristics can be seen in Table 1.

Table 1. Behavioral characteristics of fish in three stages of anesthesia and recovery.

Stage	Induction	Recovery
1	Reduced swimming movement, reaction to external stimuli and normal balance	Mild recovery of opercular movement and swimming movements
2	Loss of muscle movement and balance, reduction of opercular movement and responses to external stimuli	Balance recovery and mild reaction to external stimuli
3	Total loss of reflexes to external stimuli and opercular movement almost absent	Normal movement and swimming balance

Adapted from: OKAMURA et al. (2010).

The time required for the animals to reach stages of induction and recovery from the anesthesia was monitored using a digital chronometer. Loss of response to external stimuli was verified by touching the fish with a glass rod. After the reestablishment of animals, they were placed in tanks containing 20 L of water and maintained with constant aeration and daily food for 96 hours after the experiment to monitor mortality.

During the experimental period, the physical and chemical parameters of the water were monitored using a portable multi-parameter (HI 9829). The recorded values (mean ± standard deviation) of pH 8.33 ± 0.06, dissolved oxygen 6.80 ± 0.48 mg L^{-1}, and temperature 28.02 ± 0.87 ºC were within the standards considered suitable to fish farming (BOYD & TUCKER 1998).

The experimental design was completely randomized. The data were submitted to analysis of variance and, when there were significant differences, the Tukey test (5%) was applied (SAS 9.0). The data are presented as mean ± standard error (SE).

According OKAMURA et al. (2010), the third stage of anesthesia is the limit of reversible anesthesia and the phase in which animals remain in the highest effect of the drug, with a high desensitization degree, which eventually leads to their immobilization. This immobility is considered a favorable feature for fish handling, as it can avoid any injury from occurring due to usual agitation of the specimens when exposed to manipulation. In this study, a concentration of 50 mg L^{-1} of menthol was unable to induce the third stage of anesthesia in guppy (female, male, and juvenile) within 30 minutes of exposure, reaching only the first stage, in which swimming movement slowed (Tables 2, 3, and 4).

Table 2. Induction and recovery time (in minutes) of adult females of guppy exposed to different concentrations of menthol.

Menthol (mg L^{-1})	Induction time			Recovery time		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
50	0.96±0.23a	*	*	*	*	*
100	0.60±0.07ab	2.52±0.47a	10.12±2.43a	2.28±0.62a	3.38±0.65a	6.00±1.18a
150	0.55±0.03ab	1.03±0.08b	2.35±0.55b	0.75±0.18b	1.42±0.18b	2.67±0.32b
200	0.60±0.07ab	1.00±0.05b	1.70±0.17b	0.75±0.12b	1.78±0.20b	2.92±0.27b
250	0.48±0.02b	0.85±0.03b	1.43±0.17b	1.00±0.23ab	1.70±0.13b	2.97±0.32b

*Stage not reached in 30 minutes. Mean values ± standard error. Means followed by same letter in the same column do not differ at 5% significance by Tukey test.

Table 3. Induction and recovery time (in minutes) of adult males of guppy exposed to different concentrations of menthol.

Menthol (mg L^{-1})	Induction time			Recovery time		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
50	1.20±0.21a	*	*	*	*	*
100	0.62±0.05b	1.33±0.08a	11.62±2.57a	1.75±0.40a	2.83±0.52a	6.80±1.25a
150	0.63±0.02b	0.85±0.05b	1.30±0.17b	0.78±0.22a	2.00±0.67a	3.35±0.63b
200	0.57±0.05b	0.85±0.03b	1.45±0.15b	1.08±0.18a	2.22±0.38a	3.52±0.42b
250	0.55±0.03b	0.92±0.10b	1.53±0.23b	0.97±0.17a	1.50±0.05a	3.48±0.50b

*Stage not reached in 30 minutes. Mean values ± standard error. Means followed by same letter in the same column do not differ at 5% significance by Tukey test.

Table 4. Induction and recovery time (in minutes) of juveniles of guppy exposed to different concentrations of menthol.

Menthol (mg L^{-1})	Induction time			Recovery time		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
50	0.95±0.18a	*	*	*	*	*
100	0.50±0.07ab	2.50±0.45a	4.60±0.67a	2.00±0.48a	2.92±0.57a	5.40±0.42a
150	0.67±0.15ab	1.20±0.17b	2.22±0.28b	2.03±0.38a	4.63±0.58a	6.58±0.57a
200	0.47±0.03ab	0.83±0.13b	1.57±0.18b	2.63±0.55a	4.47±0.78a	6.42±0.93a
250	0.37±0.02b	0.68±0.08b	1.52±0.15b	2.80±0.63a	4.83±0.33a	7.15±0.47a

*Stage not reached in 30 minutes. Mean values ± standard error. Means followed by same letter in the same column do not differ at 5% significance by Tukey test.

GONÇALVES et al. (2008) found similar results when testing menthol concentrations of 50, 100, 150, and 200 mg L^{-1} as a possible replacement for benzocaine in the anesthesia induction of pacu (*Piaractus mesopotamicus*), in which only the concentration of 50 mg L^{-1} was ineffective in inducing anesthesia. By contrast, in lambaris (*Astyanax altiparanae*) anesthetized with menthol concentrations from 50 to 300 mg L^{-1}, the concentration of 50 mg L^{-1} was effective, inducing anesthesia in a short time (1 minute) and attenuating animal response to stress (PEREIRA-DA-SILVA et al. 2016). These studies demonstrate variations in responses for different individuals and the need for specific studies for each species.

Many authors observed a reduction in the time required for fish anesthesia when increasing the concentration of anesthetic (PÁDUA et al. 2010, ROMANELI et al. 2018). These results were similar to those found with guppy (Tables 2, 3, and 4). According to KEENE et al. (1998), recommended characteristics of an anesthetic are their fast action (approximately 3 minutes), ease of use, and low risk to animals. In this study, the concentrations that allowed the fish to reach the third stage of anesthesia within the time recommended by the authors, with no mortality, were from 150 mg L^{-1} to 250 mg L^{-1} for adults (female and male) and 150 to 200 mg L^{-1} for juveniles. For the three groups, the concentration of 100 mg L^{-1} had the longest induction time, differing ($p<0.05$) from the higher concentrations, and causing 100% mortality in adults (male and female). One possible cause for this toxicity in guppy females and males may be the long exposure time to the anesthetic, which exceeded 10 minutes for both sexes to reach the final stage of anesthesia. For juveniles, the concentration of 250 mg L^{-1} caused 100% mortality, which leads us to emphasize the importance of biological characteristics to induce anesthesia. More studies are necessary to evaluate the harmful effects of these concentrations of anesthesia in different groups of guppies.

When anesthetizing juvenile robalo peva (*Centropomus parallelus*) with menthol, SOUZA et al. (2012) determined the concentration of 150 mg L^{-1} as the ideal minimum since it has a faster induction time when compared to lower concentrations (50 and 100 mg L^{-1}), and does not cause animal mortality. For freshwater angelfish (*Pterophyllum scalare*), menthol concentrations from 150 to 250 mg L^{-1} resulted in induction times inferior to 3 min (ROMANELI et al. 2018), which corroborates with the results in guppy.

In this study there was a significant difference ($p<0.05$) in recovery times for adults (males and females), in which the concentration that provided the longest anesthetic induction time (100 mg L^{-1}) also caused the longest recovery time. For juveniles, there was no significant difference ($p>0.05$) in recovery period. In general, at all menthol concentrations, animals (females, males, and juveniles) recovered in less than 10 minutes within the interval considered appropriate by ROSS & ROSS (2008) for anesthetic recovery.

Studies observing the security levels of menthol use as an anesthetic have found promising results. FAÇANHA & GOMES (2005) observed no mortality when exposing Tambaqui to within 30 minutes at the ideal concentration of 150 mg L^{-1}. SIMÕES & GOMES (2009) tested twice the optimal concentration (500 mg L^{-1}) for juvenile tilapia (*Oreochromis niloticus*) and reported no deaths. PEREIRA-DA-SILVA et al. (2016) observed no mortality of lambari fingerlings when using the appropriate concentration for the species, 50 mg L^{-1}, even exceeding three minutes of considered limit. Thus, the results of these studies make it clear the potential use of menthol as an anesthetic for fish farming and demonstrate that it is safe and effective for fish anesthesia when used at optimal concentrations for each species.

Menthol proves to be a safe and effective anesthetic for guppy. The menthol concentrations recommended for guppy anesthesia induction are from 150 to 250 mg L^{-1} for adults (female and male) and from 150 to 200 mg L^{-1} for juveniles.

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