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# Efficacy of eugenol and the methanolic extract of *Condalia buxifolia* during the transport of the silver catfish *Rhamdia quelen*

Alexssandro Geferson Becker[1], Mauro Alves da Cunha[1], Luciano de Oliveira Garcia[1], Carla Cristina Zeppenfeld[1], Thaylise Vey Parodi[1], Graciela Maldaner[2], Ademir Farias Morel[2] and Bernardo Baldisserotto[1]

This study evaluated extracts of *Condalia buxifolia* as anesthetics for the silver catfish *Rhamdia quelen*. The effectiveness of eugenol and of the methanolic extract (ME) of *C. buxifolia* during the transport of this species was also assessed. Fish of two different weights (1.50±0.02 g and 165.70±22.50 g) were transferred to aquaria containing water with the *C. buxifolia* ME or with fractions obtained from the ME, such as the n-hexane, dichloromethane, ethyl acetate, n-butane and aqueous fractions, at concentrations from 0-300 μL $L^{-1}$. The *C. buxifolia* ME in the 0.5-120 μL $L^{-1}$ range caused only light sedation, and the fractions did not have an effect on the fish. In the second experiment, another group of fish was transported for 12 h in 15 plastic bags. The fish were divided into five groups: control, 1 or 2.5 μL $L^{-1}$ eugenol and 25 or 50 μL $L^{-1}$ *C. buxifolia* ME. The non-ionized ammonia levels were lower at the end of transport in the groups with the compounds than in that with water alone. Moreover, both compounds decreased the $Na^+$, $Cl^-$, and $K^+$ net effluxes; therefore, their addition to the water during transport is advisable because they reduce fish mortality and ion loss.

Este estudo investigou extratos de *Condalia buxifolia* como anestésico para jundiá *Rhamdia quelen*, e também a eficiência do eugenol e do extrato metanólico (EM) de *C. buxifolia* para utilização durante o transporte dessa espécie. Peixes de dois diferentes pesos (1,50±0,02 g e 165,70±22,50 g) foram transferidos para aquários contendo água com o EM de *C. buxifolia* ou frações obtidas a partir do EM (n-hexano, acetato de diclorometano, etil n- butano e aquoso, em concentrações na faixa de 0 - 300 μL $L^{-1}$. O EM de *C. buxifolia* em concentrações na faixa de 0,5 - 120 μL $L^{-1}$ causou somente uma sedação leve e as frações não tiveram efeito. No segundo experimento outro grupo de peixes foi transportado por 12 h em 15 sacos plásticos divididos em cinco tratamentos: controle, 1 ou 2,5 μL $L^{-1}$ de eugenol e 25 ou 50 μL $L^{-1}$ de EM de *C. buxifolia*. Os níveis de amônia não-ionizada foram menores nos tratamentos com ambos compostos em relação à água (controle). Além disso, ambos compostos diminuíram os efluxos líquidos de $Na^+$, $Cl^-$ e $K^+$ e, portanto, sua adição na água de transporte é aconselhável, pois reduzem a mortalidade e a perda de íons dos peixes.

**Key words:** Anesthesia, Fish transport, Heptapteridae, Ion fluxes, Sedative.

## Introduction

In the pursuit of new fish sedatives or anesthetics, researchers have searched for compounds that are easily acquired and of low cost to fish farmers. Moreover, these compounds should not present a risk to the health of the fish or the farmers, and the compounds should have little or no withdrawal period (Gilderhus & Marking, 1987). It is also important that a new substance does not induce physiological and/or biochemical changes in the fish (Soto & Burhanuddin, 1995; Anderson *et al.*, 1997; Iversen *et al.*, 2003; Façanha & Gomes, 2005; Cunha *et al.*, 2010a).

Plant extracts or essential oils appear to be viable alternatives as anesthetics for fish because of the high costs and difficulties of obtaining chemical products for this purpose (Façanha & Gomes, 2005). Eugenol [(2-methoxy-4-(2-propenyl) phenol], which is the major component in clove oil (70-90% weight)], or clove oil has been used as an anesthetic in several studies with native Brazilian fish (Inoue *et al.*, 2003, 2005; Roubach *et al.*, 2005; Vidal *et al.*, 2006, 2008; Barbosa *et al.*, 2007; Gonçalves *et al.*, 2008; Cunha *et al.*, 2010b). Moreover, this anesthetic is listed in the FDA category of materials "generally regarded as safe" (Ross & Ross, 2008). Because of their efficacy, low price, lack of

[1]Departamento de Fisiologia e Farmacologia, [2]Departamento de Química, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil. bbaldisserotto@hotmail.com (BB)

withdrawal period and lack of negative effects on fish feeding, eugenol, clove oil and iso-eugenol have been considered "modish anesthetics" of choice in the aquaculture industry (Harper, 2003). However, eugenol impairs the flavor of the silver catfish, *Rhamdia quelen*, fillet; therefore, its use is not recommended immediately prior to slaughter (Cunha *et al.*, 2010b).

The tree *Condalia buxifolia* Reissek (Rhamnaceae) is found primarily in South America, including in Brazil, Uruguay, and Argentina (Bastos, 1989), and a study of its chemistry identified several peptide alkaloids in its root bark (Morel *et al.*, 2002). These peptide alkaloids possess a variety of biological activities, including sedation (El-Seedi *et al.*, 2007).

The transport of live fish is problematic in aquaculture. The success of transporting fish depends on many factors, including the duration of transport, water parameters, the size, density and physical condition of the fish and the duration of the depuration period before fish transport (Berka, 1986; Golombieski *et al.*, 2003; Carneiro *et al.*, 2009; Becker *et al.*, 2012). The most used system of juvenile transport in Brazil is the closed system that uses plastic bags. The limitations of this system are the supply of oxygen and the build-up of ammonia and carbon dioxide produced during transport (Gomes *et al.*, 1999; Golombieski *et al.*, 2003; Gomes *et al.*, 2006a, b; Carneiro *et al.*, 2009; Becker *et al.*, 2012).

The use of anesthetics during fish transportation has been proposed to reduce stress responses (Guo *et al.*, 1995; Inoue *et al.*, 2005; Azambuja *et al.*, 2011; Cunha *et al.*, 2011; Becker *et al.*, 2012). Monitoring physiological parameters, such as plasma ions, cortisol and glucose levels and ventilatory frequency, during transport can provide valuable data for the establishment of adequate management practices (Barton *et al.*, 2003; Sulikowski *et al.*, 2005; Carneiro *et al.*, 2009; Becker *et al.*, 2012).

Studies conducted on the transport of *R. quelen* have evaluated different times, densities, temperatures and salt concentration in the water (Gomes *et al.*, 1999; Golombieski *et al.*, 2003; Carneiro *et al.*, 2009). Recently, a study on the effectiveness of eugenol (1.5 or 3.0 iL $L^{-1}$) and of an essential oil of *Lippia alba* was performed that evaluated several blood and water parameters, survival and ionoregulatory balance (Becker *et al.*, 2012).

The objective of this study was to evaluate the extracts of *C. buxifolia* as *R. quelen* anesthetics and to evaluate the time to induction and recovery from anesthesia. Moreover, this study investigated the effectiveness of eugenol and of the *C. buxifolia* methanolic extract (ME) for use during the transport of *R. quelen* using the following indicators: water parameters, mortality and ionoregulatory balance.

## Material and Methods

### Plant material

The freeze-dried bark of *C. buxifolia* (2.2 kg) was extracted with methanol (MeOH) in a Soxhlet extractor. The solvent was evaporated under reduced pressure to obtain 430 g of a dark viscous residue (methanolic crude extract - ME). A portion of the ME (100 g) was dissolved in water (500 mL) and successively extracted with n-hexane (3 x 0.5 L), dichloromethane (3 x 0.5 L) and ethyl acetate (3 x 0.5 L), yielding the following fractions: n-hexane (10 g), dichloromethane (7 g), ethyl acetate (5 g), n-butane (20.5 g) and aqueous (55.5 g). Identification of the botanical material was performed by comparison with existing samples in the herbarium of the Departamento de Biologia-UFSM (SMDB3296).

### Animals

Specimens of *R. quelen* were purchased from a fish farm and transported to the Laboratory of Fish Physiology at the Universidade Federal de Santa Maria, where they were maintained for two weeks in continuously aerated 250 L tanks in a semi-static system (temperature 21 ± 1°C, pH 6.8 ± 0.5, dissolved oxygen 6.5 ± 0.8 mg $L^{-1}$). The fish were fasted for 24 h prior to the experiments. Two experiments were performed. Experiment I evaluated the anesthesia induction times in *R. quelen* exposed to extracts of *C. buxifolia*, and experiment II evaluated several physiological responses after the transport of *R. quelen* with eugenol and with the *C. buxifolia* ME added to the water during transport.

### Experiment I: Anesthesia induction in *R. quelen* exposed to extracts of *C. buxifolia*

After the adaptation period to laboratory conditions, the fish (n = 260) of two different weights (mean ± SEM: 1.50±0.02 g and 165.7±22.5 g) were transferred to aquaria containing 1 L of water with one fish in each aquarium and with the *C. buxifolia* ME in the following concentrations: 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 10, 30, 50, 80, 120, and 300 µL $L^{-1}$ first diluted in ethanol at a proportion of 1:10. A previous analysis demonstrated that ethanol at the tested concentrations did not induce sedation or anesthesia in *R. quelen* (Cunha *et al.*, 2010a). The time for anesthesia induction was evaluated according to Schoettger & Julin (1967) (Table 1). The maximum observation time was 30 min. The same procedure was used to test the other fractions of this extract, including the n-hexane, dichloromethane, ethyl acetate, n-butane and aqueous extracts. After these analyses, the fish were exposed to the *C. buxifolia* ME at 1.0-50 µL $L^{-1}$ in continuously aerated 40 L tanks to determine whether 6 h of sedation would be anesthetic. This concentration range was chosen because sedation time increased at concentrations greater than 50 µL $L^{-1}$ *C. buxifolia* ME (see results). After induction, the fish were transferred to anesthetic-free aquaria to eliminate any residue of the *C. buxifolia* extracts. The recovery time was not measured because sedation with the *C. buxifolia* ME was very light (see results) and the determination of full recovery would be imprecise. Twenty juveniles were used for each tested concentration, and each fish was used only once. The water parameters were the same as in the acclimation period.

**Table 1.** Stages of anesthesia in fish (from Schoettger & Julin, 1967).

| Stage | Description | Behavioral response |
|---|---|---|
| 1 | Light sedation | Partial loss of reaction to external stimuli |
| 2 | Deep sedation | Partial loss of equilibrium, no reaction to external stimuli |
| 3a | Total loss of equilibrium | Fish usually turn over but retain swimming ability |
| 3b | Total loss of equilibrium | Swimming ability stops but responds to pressure on the caudal peduncle |
| 4 | Anesthesia | Loss of reflex activity, no reaction to strong external stimuli |
| 5 | Medullary collapse (death) | Respiratory movement ceases (death) |

**Experiment II: Transport**

Another group of *R. quelen* (n = 225, mean ± SEM: 12.01±1.73 g, 10.27±1.85 cm) was captured using a cage net inside an earth pond at a fish farm near Santa Maria city, Southern Brazil. The fish did not go through a depuration period because this procedure, although recommended (Amend *et al.*, 1982), is not followed by the majority of fish producers in southern Brazil (Golombieski *et al.*, 2003). The fish were transported at a density of 186.7 g $L^{-1}$ for 12 h in 15 plastic bags (32 x 60 cm) with 1.5 L of water and 3 L of pure oxygen. The fish were divided into five treatment groups with three replicates each. These treatments were as follows: control, 1.0 or 2.5 µL $L^{-1}$ eugenol (Odontofarma™, Porto Alegre, Brazil) (equivalent to 1.0 or 2.5 mg $L^{-1}$, respectively, because the density of this anesthetic is approximately 1.06) and 25 or 50 µL $L^{-1}$ *C. buxifolia* ME (both first diluted in ethanol 1:10). The transport time was defined as the maximum transport time utilized by the producers from Rio Grande do Sul State (Brazil), which is 12 h. The selected eugenol concentrations were similar to those recommended by Becker *et al.* (2012) for the transport of the same species. The loading density used in this study was higher than the maximum recommended for silver catfish (168 g $L^{-1}$) (Golombieski *et al.*, 2003) to expose the fish to a very stressful situation and to determine the efficacy of the substances used.

The water parameters were measured before and after transport. The dissolved oxygen (DO) and temperature were measured with an YSI oxygen meter (Model Y5512; YSI Inc., Yellow Springs, OH, USA). The pH was verified with a DMPH-2 pH meter (Digimed, São Paulo, SP, Brazil). Nesslerization verified the total ammonia nitrogen (TAN) levels according to the method of Eaton *et al.* (2005). Non-ionized ammonia ($NH_3$) levels were calculated according to the method of Colt (2002). Water hardness was analyzed using the EDTA titrimetric method. Alkalinity was determined according to the method of Boyd & Tucker (1992). Carbon dioxide ($CO_2$) was calculated using the method of Wurts & Durborow (1992).

Water samples (5 mL) were collected before and after transport. Chloride levels were determined according to Zall *et al.* (1956), and $Na^+$ and $K^+$ levels were determined with a B262 flame spectrophotometer (Micronal, São Paulo, Brazil). Standard solutions were made with analytical-grade reagents dissolved in deionized water, and the standard curves of each ion to be tested were constructed for five different concentrations. The net ion fluxes (Jnet) were calculated according to the method of Gonzalez *et al.* (1998) as follows:

$$Jnet = V([ion]_1 - [ion]_2) \times (M \times t)^{-1},$$

where $[ion]_1$ and $[ion]_2$ are the ion concentrations in the transport water at the beginning and end of the transport period, respectively, V is the water volume (L), M is the mass of the fish (kg) and t is the duration of the transport (h).

**Statistical analyses**

All data are expressed as the mean ± SEM. The homogeneity of the variances between treatments was tested with Levene's test. The data exhibited homogeneous variances; therefore, comparisons among the different treatments and times were performed using a one-way ANOVA and Tukey's test. The analysis was performed using the Statistica ver. 7.0 software (Stat Soft. Inc., wwwstatsoft.com), and the minimum significance level was set at $P<0.05$. The relation between the time to reach the stage of sedation and the concentration of the *C. buxifolia* ME was calculated with the Sigma Plot 11.0 software ($P<0.05$).

## Results

**Experiment I: Anesthesia induction in *R. quelen* exposed to *C. buxifolia* extracts**

*R. quelen* exposed to the n-hexane, dichloromethane, ethyl acetate, n-butane and aqueous extracts of *C. buxifolia* did not present any evidence of sedative or anesthetic effects during the 30 min evaluation period. The *C. buxifolia* ME at concentrations from 0.5-120 µL $L^{-1}$ caused only light sedation (stage 1) in the *R. quelen* of both tested weights. Higher concentrations did not alter the silver catfish behavior within the 30 min evaluation period, and no difference was observed in the response to this extract between the two weight groups. In fish exposed to concentrations of the *C. buxifolia* ME greater than 10 µL $L^{-1}$, it was observed that the time to induce sedation increased as the concentration increased (Fig. 1). *R. quelen* exposed to 1.0-50.0 µL $L^{-1}$ *C. buxifolia* ME for 6 h maintained a uniform depth of sedation, *i.e.*, they remained in stage 1.

**Experiment II. Transport**

After transport, the highest mortality was observed in the control group followed by the 1 µL $L^{-1}$ eugenol and 25 µL $L^{-1}$ *C. buxifolia* ME treatment groups. Conversely, the

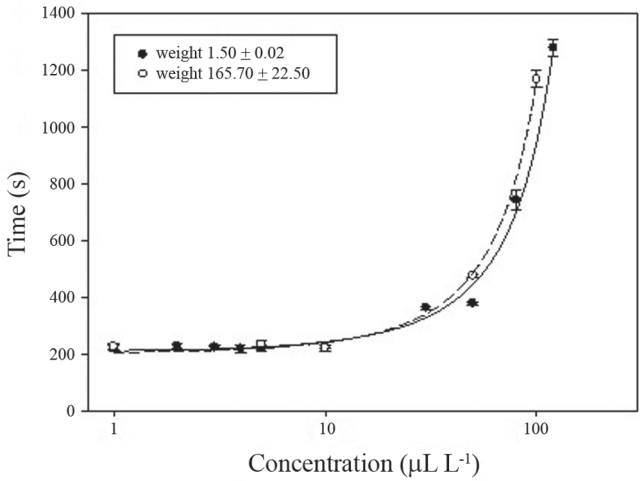


**Fig. 1.** Time to reach the light sedation stage in *Rhamdia quelen* juveniles of two different weight classes exposed to the methanolic extract of *Condalia buxifolia*. The following equations were fitted to the data: For fish weighing 1.50 ± 0.02 g; $y = 209.629\ e^{0.015x}$; $r^2 = 0.996$. For fish weighing 165.7 ± 22.5 g; $y = 2039.020\ e^{0.017x}$; $r^2 = 0.999$. Where x = the concentration of the methanolic extract of *C. buxifolia* (μL $L^{-1}$) and y = time for sedation(s).

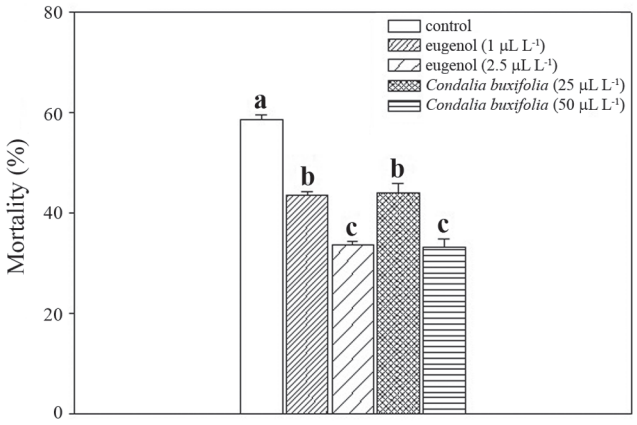


**Fig. 2.** Mortality after the transport of *Rhamdia quelen* in plastic bags with eugenol and with the methanolic extract of *Condalia buxifolia* added to the water. The values are the means ± SEM. The different letters indicate significant differences between the treatments ($P<0.05$).

lowest mortality was observed in the 2.5 μL $L^{-1}$ eugenol and 50 μL $L^{-1}$ *C. buxifolia* ME treatment groups (Fig. 2).

The 1 μL $L^{-1}$ eugenol and 25 μL $L^{-1}$ *C. buxifolia* ME treatments exhibited the highest DO levels in the water after transport. Additionally, the lowest $CO_2$ and TAN levels were found in the water of the control group. The total alkalinity, water hardness levels and water temperature did not exhibit any significant differences among the treatments at the end of transport. Additionally, the pH and $NH_3$ levels were significantly higher in the control group than in the other groups (Table 2).

The net $Na^+$, $Cl^-$, and $K^+$ effluxes were significantly higher in the fish from the control treatment group than in the fish from the other treatment groups. Moreover, the lowest net $Na^+$, $Cl^-$, and $K^+$ effluxes were found for the 1 μL $L^{-1}$ eugenol and 25 μL $L^{-1}$ *C. buxifolia* ME treatment groups (Fig. 3).

## Discussion

### Experiment I. Anesthesia induction in *R. quelen* exposed to *C. buxifolia* ME

The *R. quelen* specimens were lightly sedated with the *C. buxifolia* ME, and there was no induction of anesthesia even after six hours. The recovery time of the *C. buxifolia* ME could not be precisely verified because the sedation was too light. Consequently, the use of this extract as a sedative rather than as an anesthetic is suggested. The best concentration range of the *C. buxifolia* ME appears to be 0.5-10 μL $L^{-1}$ because higher concentrations increased the time of sedation for both *R. quelen* weight classes. Additionally, this extract is very safe because even a concentration 30-fold higher than the maximum concentration recommended did not cause mortality.

Because the fractions of the *C. buxifolia* ME did not present any sedative or anesthetic effects when tested separately, the effect of ME is most likely not due to a specific compound, which would have been separated in at least one of the fractions, but rather to the synergism of its compounds. There are no studies regarding the effects of these fractions of the *C. buxifolia* ME or even the synergism of compounds on anesthetizing fish, but the same principle can be found in some isolated components from the essential oil of two species of *Ocimum* that exhibited either low or no insecticidal activity and became potently toxic when blended together (Bekele & Hassanali, 2001). Another interesting effect is that the time to reach the slight sedation stage increases in *R. quelen* exposed to higher concentrations of the *C. buxifolia* ME. Again, there are no similar results regarding fish anesthetics, but some interactions between plant compounds have revealed a clear concentration-dependent interaction (Goñi *et al.*, 2009). It is possible that a concentration-dependent interaction occurs with the compounds of the *C. buxifolia* ME regarding its sedative effect in *R. quelen*. The lower efficacy of this ME at higher concentrations may be due to the increased concentration of minor compound(s) that antagonize the sedative effect. At lower concentrations, this compound would not effectively decrease the sedative effect.

### Experiment II: Transport

In this study, there was significantly higher mortality in the control group than in the other treatment groups at the end of the transport period. Therefore, the anesthetics added

**Table 2.** Water parameters before and after the transport (12 h) of *Rhamdia quelen* in plastic bags with eugenol and with the methanolic extract of *Condalia buxifolia* added to the water. The values are the means ± SEM. The asterisks indicate significant differences compared to the values before transport (P<0.05). The different letters in the rows indicate significant differences between the treatments after transport (P<0.05). The dissolved oxygen and carbon dioxide levels are expressed as mg $L^{-1}$, and the total ammonia nitrogen and non-ionized ammonia are expressed as mg N $L^{-1}$. The alkalinity and water hardness are expressed as mg $CaCO_3$ $L^{-1}$.

| Water parameter | Before transport | After transport (treatments) | | | | |
|---|---|---|---|---|---|---|
| | | control | eugenol (1 µL L-1) | eugenol (2.5 µL L-1) | *C. buxifolia* (25 µL L-1) | *C. buxifolia* (50 µL L-1) |
| Dissolved oxygen | 5.60±0.06 | 1.46±0.06*d | 2.22±0.09*a | 1.73±0.04*c | 2.22±0.04*a | 1.97±0.04*b |
| Carbon dioxide | 4.86±0.11 | 51.04±1.33*c | 85.07±1.12*a | 78.48±1.27*b | 79.04±1.77*b | 76.60±2.13*b |
| Alkalinity | 24.7±0.5 | 43.0±0.5*a | 45.2±1.3*a | 41.7±0.8*a | 42.0±0.5*a | 40.7±2.7*a |
| Water hardness | 21.6±0.5 | 26.5±0.5*a | 28.0±0.5*a | 28.0±0.5*a | 26.5±0.5*a | 26.5±0.5*a |
| pH | 6.98±0.09 | 6.21±0.05*a | 6.04±0.04*b | 6.05±0.04*b | 6.03±0.05*b | 6.03±0.06*b |
| Temperature | 23.1±0.2 | 28.1±0.3*a | 28.1±0.2*a | 28.1±0.2*a | 28.1±0.2*a | 28.1±0.3*a |
| Total ammonia nitrogen | 0.10±0.02 | 5.25±0.12*c | 6.12±0.12*a | 5.73±0.09*b | 5.58±0.09*b | 5.66±0.10*b |
| Non-ionized ammonia | 0.0005 | 0.0060*a | 0.0047*b | 0.0045*b | 0.0042*b | 0.0043*b |

to the transport water reduced the mortality of *R. quelen*. The transport of largemouth black bass (*Micropterus salmoides*) with MS-222 (Carmichael *et al*., 1984) and of Indian major carp fry (*Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala*) (Singh *et al*., 2004) and guppies (*Poecilia reticulata*) (Teo *et al.*, 1989) with 2-phenoxyethanol also decreased mortality after transport.

The lethal concentrations (96 h) of TAN and $NH_3$ for *R. quelen* under normoxic conditions (total hardness: 20 mg $CaCO_3$ $L^{-1}$; 25°C) are 7.73 and 0.44 mg $L^{-1}$, respectively, at pH 6.0 (Miron *et al.*, 2008). In this study, the total ammonia and $NH_3$ levels were much lower at the end of transport than the lethal values, but the DO levels were very low and near the lethal concentration for *R. quelen* (Braun *et al.*, 2006). Additionally, exposure to high waterborne $NH_3$ (0.1 mg $L^{-1}$) and low DO (3.5 mg $L^{-1}$) levels for 6 and 24 h caused ionoregulatory changes in this species (Becker *et al.*, 2009). Therefore, the low DO levels and high $NH_3$ levels found in the control treatment group may explain the increase in mortality for this group.

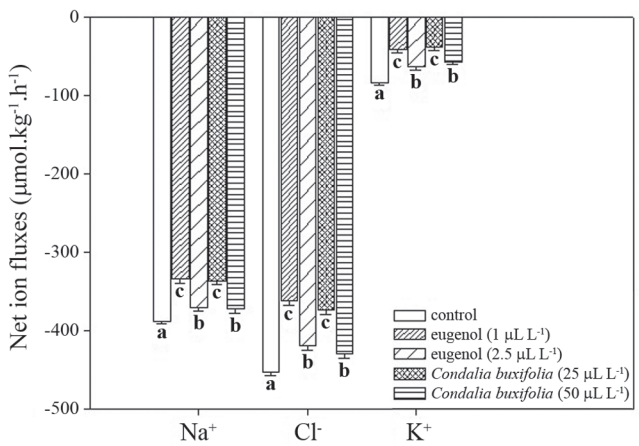


**Fig. 3.** The net ion ($Na^+$, $Cl^-$ and $K^+$) fluxes measured for the transport of *Rhamdia quelen* in plastic bags with eugenol and with the methanolic extract of *Condalia buxifolia* added to the water. The values are the means ± SEM. The different letters indicate significant differences between the treatments for the same ion (P<0.05).

*Rhamdia quelen* exposed to concentrations of eugenol between 20 and 70 µl $L^{-1}$ could reach stage 2 anesthesia, *i.e.*, deep sedation, which is observed as a partial loss of equilibrium and lack of reaction to external stimuli, within a few minutes (Cunha *et al.*, 2010b). Conversely, *R. quelen* exposed to concentrations of the *C. buxifolia* ME between 1.0 and 50 µL $L^{-1}$ for 6 h maintained a uniform sedation in that they remained in stage 1. During the transport of the fish, the anesthetic concentrations must induce stage 2 anesthesia at most. Carmichael *et al.* (1984) reported increased survival and reduced stress parameters, such as decreased plasma glucose and corticosteroids and increased plasma $Cl^-$ and osmolality, during the transport of the largemouth black bass *Micropterus salmoides* in water with MS-222. Moreover, the use of benzocaine-hydrochloride (25 mg $L^{-1}$) on the Mozambique tilapia *Oreochromis mossambicus* reduced oxygen consumption by approximately $^1/_3$ and decreased both ammonia and $CO_2$ excretion (Ferreira *et al.*, 1984). Additionally, the Indian carp fry *C. catla*, *L. rohita*, and *C. mrigala* exposed to 2-phenoxyethanol (0.09 mg $L^{-1}$) exhibited decreased $NH_3$ excretion (Singh *et al.*, 2004). Park *et al.* (2009) suggested that lidocaine hydrochloride at concentrations of 5, 10 or 20 mg $L^{-1}$ decreased the metabolic activity of the flounder *Pleuronectes americanus* (= *Pseudopleuronectes americanus*) compared to that of the control group after 5 h of transport time because this substance reduced ammonia excretion (approximately 27.4 to 30.5%) and oxygen consumption (approximately 82.7 to 86%).

The increase in the $CO_2$ levels observed in all treatment groups at the end of *R. quelen* transport was most likely

responsible for the decrease in the pH of the water, which was similar to that observed by Golombieski *et al.* (2003) and Becker *et al.* (2012). Regardless of the treatment, the alkalinity levels increased after transport, most likely due to regurgitated food because the fish did not undergo a depuration period and the commercial food provided to the fish contained calcitic limestone ($CaCO_3$). Similar results were found by Golombieski *et al.* (2003) and Becker *et al.* (2012).

Transportation and handling operations are stressful situations that can increase ion loss in freshwater fish by increasing gill blood flow and paracellular permeability (Cech Jr. *et al.*, 1991). In the present study, eugenol and the *C. buxifolia* ME in the transport water reduced ion loss in *R. quelen*. These results were similar to those of the same species transported with 1.5 or 3.0 μL $L^{-1}$ eugenol and 10 or 20 μL $L^{-1}$ *L. alba* essential oil added to the transport water for 4 h (Becker *et al.*, 2012). Additionally, other studies have reported that the anesthetics used for fish transport reduced agitation and fish stress (Guo *et al.*, 1995; Singh *et al.*, 2004; Park *et al.*, 2009). Therefore, eugenol and the *C. buxifolia* ME may have sedated the *R. quelen* during transport and reduced ion loss.

In conclusion, the best concentration range for the *C. buxifolia* ME is 0.5-10 μL $L^{-1}$ because higher concentration levels increase the time of sedation. Moreover, the addition of eugenol and of the *C. buxifolia* ME to the transport water at the concentrations tested is advisable because they reduce fish mortality and ion loss.

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