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Original article

Stress-reducing and anesthetic effects of the essential oils of *Aloysia triphylla* and *Lippia alba* on *Serrasalmus eigenmanni* (Characiformes: Serrasalmidae)

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Anesthetic effect of *Aloysia triphylla* and *Lippia alba* essential oils (EOs) in the Amazonian fish *Serrasalmus eigenmanni* was evaluated. The fish were placed in aquaria containing *A. triphylla* or *L. alba* EOs (25 to 200 μL L^{-1}). Then, fish were transferred to aquaria containing EO-free water to evaluate their recovery time. In another experiment, fish were transferred to aquaria containing *A. triphylla* or *L. alba* EOs (3 to 10 μL L^{-1}) and swimming behavior was analyzed for up to 240 min of exposure. Water samples were collected at 0 and 240 min and blood samples were collected at 240 min. Tested concentrations induced all stages of anesthesia, except 25 μL L^{-1} *A. triphylla* EO and 50 μL L^{-1} *L. alba* EO, which only induced sedation. Prolonged exposure to both EOs reduced swimming time compared to the control at all evaluated times. The fish exposed to 3 μL L^{-1} *A. triphylla* EO showed a lower net K^+ efflux compared to ethanol-exposed fish; in those exposed to 5 μL L^{-1}, ammonia excretion was reduced. The blood parameters did not show significant differences between treatments. In conclusion, both EOs can be used as anesthetics and sedatives for transport of *S. eigenmanni*.

Keywords: Amazonian blackwaters, Anesthesia, Ion fluxes, Piranha, Stress.

Avaliou-se o efeito anestésico dos óleos essenciais de *Aloysia triphylla* e de *Lippia alba* no peixe amazônico *Serrasalmus eigenmanni*. Os peixes foram colocados em aquários contendo OEs de *A. triphylla* ou *L. alba* (25 a 200 μL L^{-1}). Após, foram transferidos para aquários com água sem anestésicos para avaliar o tempo de recuperação. Em outro experimento, peixes foram transferidos para aquários contendo OEs de *A. triphylla* ou *L. alba* (3 a 10 μL L^{-1}) e o comportamento natatório foi analisado até 240 min de exposição. Foram coletadas amostras de água em 0 e 240 min e de sangue em 240 min. As concentrações testadas induziram todos estágios de anestesia, exceto 25 μL L^{-1} OE de *A. triphylla* e 50 μL L^{-1} OE de *L. alba*, que causaram somente sedação. Exposição prolongada a ambos OEs reduziu o tempo de natação comparado ao controle. Peixes expostos a 3 μL L^{-1} OE de *A. triphylla* apresentaram menor efluxo de K^+ comparado aos expostos ao etanol e nos expostos a 5 μL L^{-1} a excreção de amônia reduziu. Parâmetros sanguíneos não diferiram entre tratamentos. Conclui-se que ambos OEs podem ser utilizados como anestésicos e no transporte de *S. eigenmanni*.

Palavras-chave: Águas negras amazônicas, Anestesia, Estresse, Fluxo iônico, Piranha.

Introduction

Stress can cause fish homeostasis loss, and it can be lethal if not reversed. Exposure to stressors is unavoidable in aquaculture because fish are routinely submitted to practices such as handling, transportation, and high stocking density, among others (Sampaio, Freire, 2016). Synthetic anesthetic substances have been used to prevent stress in fish (Small, 2003; Weber *et al*., 2009; Pramod *et al*., 2010). However, several of these substances can cause a loss of mucus, an increase of serum cortisol levels, immunosuppression, acidosis, tissue hypoxia (Sneddon, 2012; Zahl *et al*.,

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2012), and be aversive (Readman *et al.*, 2013). Tricaine methanesulfonate (MS-222), the only anesthetic approved by the Food and Drug Administration for use in fish destined for consumption, induces hepatic lipoperoxidation in silver catfish *Rhamdia quelen* (Gressler *et al*., 2014). Due to these adverse effects, there has been an increase in research involving natural products such as essential oils (EOs), their isolated compounds and other types of extracts from plants to replace synthetic anesthetics (Mazandarani, Hoseini, 2017; Hoseini *et al.*, 2018; Yousefi *et al*., 2018). Essential oils are permitted in organic aquaculture by the Food and Agriculture Organization of the United Nations (FAO) (Bansemir *et al*., 2006) and also by Brazilian legislation (MAPA, 2011).

Studies have shown the efficacy of anesthesia and/or the prevention of stress damage using EOs extracted from plants, such as: *Ocimum gratissimum* (Silva *et al*., 2012; Boijink *et al*., 2016), *Hesperozygis ringens* (Silva *et al*., 2013), *Nectandra megapotamica* (Tondolo *et al*., 2013), *Hyptis mutabilis* and *Ocotea acutifolia* (Silva *et al*., 2013). However, some EOs may have adverse effects. For example, the EO of *Aloysia gratissima* provoked involuntary muscle contractions during anesthesia induction and around 30% mortality up to 24 h later, besides not being safe for transport of the Brazilian flounder *Paralichthys orbignyanus* (Benovit *et al*., 2012). The EOs of *L. alba* and *A. triphylla* showed anesthetic efficacy and a stress-reducing effect in *Centropomus parallelus* (Parodi *et al*., 2016), *R. quelen* (Cunha *et al*., 2010; Parodi *et al*., 2014; Becker *et al*., 2016) and *Hyppocampus reidi* (Cunha *et al*., 2011), and did not induce aversiveness in fish (Bandeira Junior *et al*., 2018). Nevertheless, anesthesia with *L. alba* EO increased plasma cortisol of juvenile meagre, *Argyrosomus regius* (Cárdenas *et al.*, 2016), indicating that the effect of a given EO may be species-specific. Almeida *et al*. (2018) demonstrated the anesthetic and sedative efficacy of *A. triphylla* and *L. alba* EOs in *Serrasalmus rhombeus* (Linnaeus, 1766), an Amazonian fish living in the blackwaters of the Negro River, but did not evaluate whether these EOs prevent stress damage, such as ionoregulatory and blood disturbances, when applied in blackwaters.

The Amazonian blackwaters have a high concentration of humic and fulvic acids from dissolved organic matter decomposition, resulting in an acidic pH close to 5.0-6.0 (Küchler *et al*., 2000; Mortatti, Probst, 2003; Matsuo, Val, 2007) and with an average annual temperature of 30.6 °C (Fonseca *et al*., 1982). Given that the pH and temperature of the water can affect anesthetic induction and recovery times (Gomes *et al*., 2011), the aim of this study was to evaluate whether *A. triphylla* and *L. alba* EOs can be used as anesthetics and transport stress-reducing agents for *Serrasalmus eigenmanni* Norman, 1929, a species that can be found in the Amazonian blackwaters (Jégu, 2003; Dórea *et al.*, 2004).

Material and Methods

Essential oil extraction and analysis. The plants *A. triphylla* and *L. alba* were cultivated at the campus of the Universidade Federal de Santa Maria, in the city of Frederico Westphalen, southern Brazil. A voucher specimen (SMDB No. 11169) was deposited in the herbarium of the Biology Department. The EO extraction and analysis was made as described previously by Almeida *et al*. (2018). The main compounds of the EO of *A. triphylla* are geranial (24.3%), limonene (21.7%) and cis-carveol (*Z*-carveol) (18.5%) and the main compounds of the EO of *L. alba* are linalool (66.3%) and eucalyptol (10.6%) (Almeida *et al*., 2018).

Animals. Specimens of *S. eigenmanni* (171.01 ± 6.38 g; 18.26 ± 0.39 cm) (voucher number: INPA-ICT 53105) were collected during an expedition to Anavilhanas Islands of the Negro River, 110 km upstream from Manaus (2°23'41"S, 60°55'14"W). Fish were maintained in tanks supplied with flow through water, pumped directly from Negro River (30 °C, pH 5.1, dissolved oxygen levels 5-6 mg L^{-1}) and continuously aerated for a few hours before testing.

Experiment I: Anesthesia induction and recovery in *S. eigenmanni* exposed to *A. triphylla* and *L. alba* EOs. The fish were transferred individually to aquaria containing 5 L (30 ± 0.81 °C, pH 5.1 ± 0.09) of water with the *L. alba* or *A. triphylla* EOs in the 25 - 200 μL L^{-1} concentration range, first diluted in ethanol at a proportion of 1:10. These concentrations were chosen based on a previous study with these EOs in *S. rhombeus* (Almeida *et al.*, 2018). The time for anesthesia induction was evaluated according to Small (2003): stage I corresponds to sedation, when the reactivity to external stimuli is decreased; stage II involves the partial loss of equilibrium and erratic swimming, and stage III involves the total loss of equilibrium and the cessation of locomotion. In recovery, the fish returns to regular swimming and regular reactions to external stimuli. Eight fish were used for each tested concentration and each fish was only tested once. The maximum observation time was 15 min; in the review by Hoseini *et al.* (2018), this period of time was enough in almost all experiments with fish to determine the time to induce deep anesthesia using essential oils. Control experiments were performed using aquaria containing only water and aquaria containing water and ethanol at a concentration equivalent to the highest dilution (1800 μL L^{-1}). After the induction of anesthesia, fish were transferred to a tank containing only water to evaluate their recovery time. The animals were considered recovered when they showed regular swimming and regular reaction to external stimuli (*i.e*., when the peduncle caudal fin was pressed with a glass rod).

Experiment II: Prolonged exposure of *S. eigenmanni* to the *A. triphylla* and *L. alba* EOs. The fish were placed into tanks containing 15 L of water and *A. triphylla* (3 and 5 μL L^{-1}) or *L. alba* (5 and 10 μL L^{-1}) EOs, first diluted in ethanol at a proportion of 1:10, and kept for 4 hours. Control experiments were performed using aquaria containing only water and aquaria containing water and ethanol at a concentration equivalent to the highest dilution (90 μL L^{-1}).

These concentrations were chosen based on the study of Almeida *et al*. (2018) with a related species, *S. rhombeus*. Three fish per aquaria were used for each concentration (three replicates for each concentration).

Behavioral analyses. The fish were filmed for 30 s for the analysis of the total swimming time and equilibrium (partial or total loss of equilibrium, or normal) at 0, 5, 30, 60, 120, 180 and 240 min of exposure.

Water sampling and analyses. Water samples were collected at 0, 5, 30, 60, 120, 180 and 240 min of exposure. The total ammonia levels were checked according to Eaton *et al*. (2005). The levels of Na^+ and K^+ were determined with a flame photometer (Analyzer) and the chloride levels were determined using the colorimetric method described by Zall *et al*. (1956). The net ion fluxes and ammonia excretion were calculated according to Val *et al*. (1998): Jnet (ammonia excretion) = V ($[ion]_1$-$[ion]_2$) · $(M.t)^{-1}$, where $[ion]_1$ and $[ion]_2$ are the ion or ammonia concentrations in the water in which the fish were kept at the beginning and at the end of the experimental period, respectively; V is the volume of the water (in L); M is the fish mass (in kg); and t is the duration of the exposure (in h).

Blood sampling and analyses. After 240 min of exposure to the EOs, fish were collected with a hand net, and held tightly with a wet cloth. Blood samples were collected through the caudal vein using heparinized syringes (n=9 per concentration). The blood was centrifuged for 5 min at 665.1 *g* and the obtained plasma was frozen in liquid nitrogen. All samples were kept in a freezer at -80 °C until further analysis.

Plasma concentrations of cortisol (DBC Diagnostics Biochem Canada, ON, Canada) and total thyroxine (T_4; Symbiosys-Alka Tecnologia®, SP, Brazil) were quantified in triplicate using the enzyme-linked immunosorbent assay (ELISA) method in a microplate reader (Molecular Devices, CA, USA). Lactate was determined by reacting the lactate dehydrogenase enzyme (LDH Kit-Sigma Diagnostics, St. Louis, MO, USA), according to Gutmann, Wahlefeld (1974). Total lipids concentration was determined by the colorimetric spectrophotometry method, according to Frings *et al*. (1972), using cod liver oil (Sigma Diagnostics, St. Louis, MO, USA) as the standard; the reaction was measured at 540 nm. Total protein was determined by the colorimetric method described by Lowry *et al*. (1951), using bovine serum albumin (Sigma Diagnostics, St. Louis, MO, USA) as the standard; the reaction was measured at 625 nm. Glucose was determined by the enzymatic colorimetric method based on the oxidase/peroxidase reaction (Glucose LiquiColor Test-InVitro®, Fr).

Statistical analyses. All data were expressed as mean ± standard error of the mean (SEM). The homogeneity of variances among treatments was tested using Levene's test. Anesthetic induction and recovery times and net ion flux comparisons between treatments were assessed using a parametric one-way ANOVA and Tukey test. When data violated the premises of ANOVA, the non-parametric Kruskal-Wallis test was used for the comparisons of mean ranks. The comparisons between the different treatments and swimming time were assessed using the non-parametric Scheirer-Ray-Hare extension of the Kruskal-Wallis test, followed by the *post-hoc* Nemenyi test. Analyses was performed using the Statistica 7.0 software (Stat Soft. Inc.). The minimum significance level was set at $P < 0.05$.

Results

The concentration of 25 µL L^{-1} *A. triphylla* EO and 50 µL L^{-1} *L. alba* EO only induced a sedative effect within 15 min; however, all other EO concentrations induced all stages of anesthesia. Fish exposed to 100 and 150 µL L^{-1} *A. triphylla* EO reached deep anesthesia (stage III) significantly faster than at lower concentrations, and the concentration of 100 µL L^{-1} *A. triphylla* EO showed a lower recovery time (Fig. 1a). In the case of *L. alba* EO, fish reached stage III with 100 and 200 µL L^{-1}; the recovery time did not differ between these concentrations (Fig. 1b). Exposure to 1800 µL L^{-1} ethanol alone did not produce any anesthetic effect.

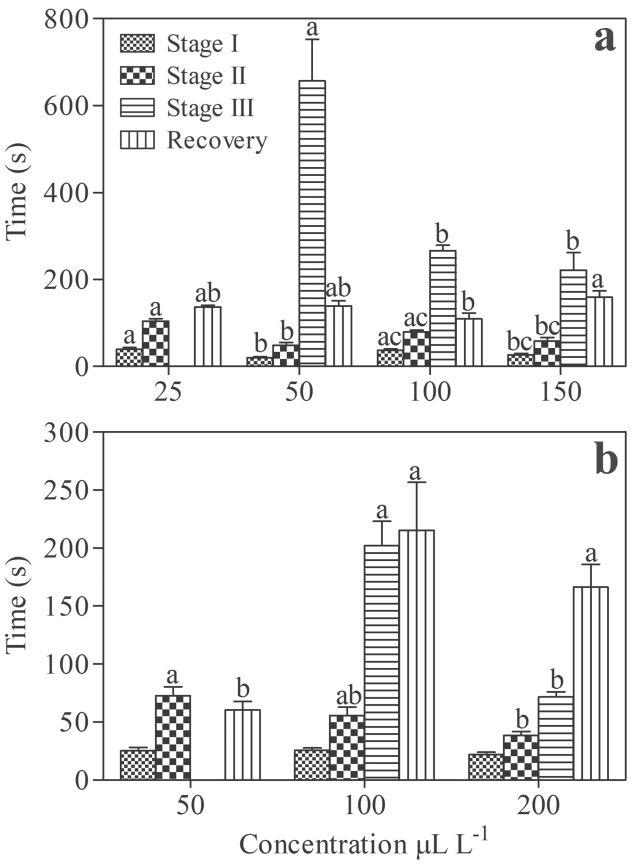

Fig. 1. Time required for induction and recovery of anesthesia in *Serrasalmus eigenmanni*. **a**. *Aloysia triphylla* essential oil. **b**. *Lippia alba* essential oil. Different letters indicate significant differences at each stage between treatments based on one-way ANOVA and Tukey tests ($P < 0.05$).

In the second experiment, fish exposed to 3 and 5 µL L^{-1} *A. triphylla* EO and 5 and 10 µL L^{-1} *L. alba* EO presented lower swimming times than control fish over all analyzed times. Out of all the fish exposed to 10 µL L^{-1} *L. alba* EO, 22.22% showed a loss of equilibrium after 1 h of exposure. The fish did not show a loss of equilibrium in the other tested concentrations of both EOs. Ethanol-exposed fish showed lower swimming times compared to control fish in the first 5 min and in the last 60 min of exposure (Fig. 2).

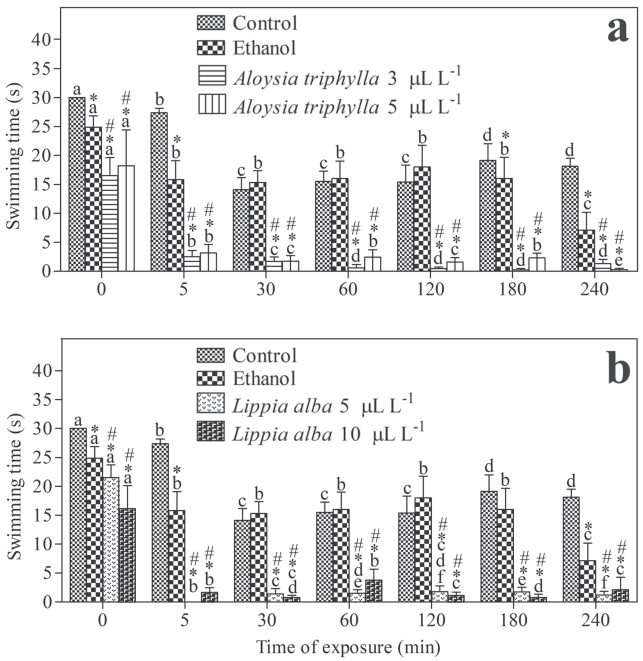

Fig. 2. Swimming time of *Serrasalmus eigenmanni* exposed to **a.** *A. triphylla* essential oil. **b.** *L. alba* essential oil. Different letters indicate significant differences at each exposure time between treatments based on the non-parametric Scheirer-Ray-Hare extension of the Kruskal-Wallis test, followed by a *post-hoc* Nemenyi test. * indicates a significant difference from control fish. # indicates a significant difference from ethanol-exposed fish.

There was no significant difference in net ion (Na^+, K^+ and Cl^-) fluxes, except at 3 µL L^{-1} *A. triphylla* EO, in which the K^+ efflux was lower compared to ethanol-exposed fish (Fig. 3).

Ammonia excretion did not differ between treatments, except at 5 µL L^{-1} *A. triphylla* EO, in which ammonia excretion was lower than ethanol-exposed and control fish (Fig. 4).

The plasma levels of cortisol, total T4, glucose, lactate and lipids showed no significant differences across all treatments. Protein levels decreased in fish exposed to 5 µL L^{-1} *L. alba* EO compared to control and ethanol-exposed fish (Tab. 1).

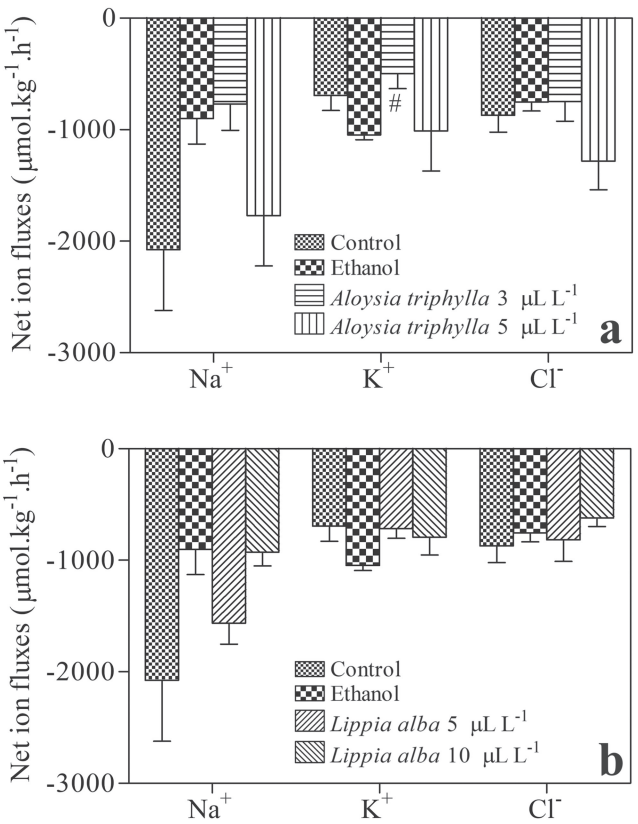

Fig. 3. Net ion fluxes of *Serrasalmus eigenmanni* exposed to essential oils for 4 hours. **a.** *A. triphylla* essential oil. **b.** *L. alba* essential oil. # indicates a significant difference from ethanol-exposed fish, based on one-way ANOVA and Tukey tests ($P < 0.05$).

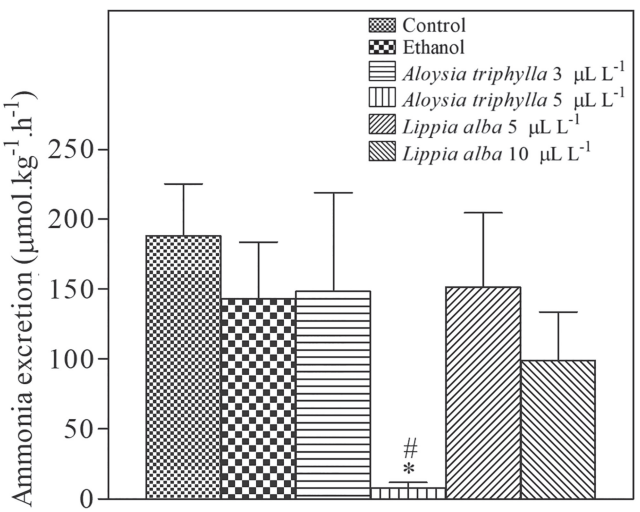

Fig. 4. Ammonia excretion of *Serrasalmus eigenmanni* exposed to essential oils for 4 hours. **a.** *A. triphylla* essential oil. **b.** *L. alba* essential oil. * indicates a significant difference from control fish and # indicates a significant difference from ethanol-exposed fish, based on one-way ANOVA and Tukey tests ($P < 0.05$).

Tab. 1. Plasma levels of cortisol (μg dL^{-1}), total T4 (μg dL^{-1}), glucose (mg dL^{-1}), lactate (mg dL^{-1}), protein (mg mL^{-1}) and lipids (mg mL^{-1}) in *Serrasalmus eigenmanni* exposed to different concentrations of *Aloysia triphylla* and *Lippia alba* essential oils. * indicates a significant difference from control fish and # indicates a significant difference from ethanol-exposed fish, based on one-way ANOVA and Tukey tests ($P < 0.05$).

Treatment	Cortisol	Total T4	Glucose	Lactate	Protein	Lipids
Control	83.21 ± 16.06	15.55 ± 5.31	79.79 ± 8.98	21.10 ± 2.83	70.15 ± 2.40	11.65 ± 0.79
Ethanol	38.40 ± 5.07	17.64 ± 5.69	82.92 ± 7.02	63.34 ± 19.67	69.08 ± 6.57	11.28 ± 1.51
Lippia alba 5 μL L^{-1}	81.60 ± 6.48	12.55 ± 1.75	55.10 ± 4.92	35.78 ± 20.80	40.59 ± 4.46*#	11.03 ± 1.66
Lippia alba 10 μL L^{-1}	70.00 ± 9.90	20.90 ± 1.54	82.33± 5.76	31.07 ± 10.95	60.8 ± 10.19	11.80 ± 0.47
Aloysia triphylla 3 μL L^{-1}	47.22 ± 9.34	4.43 ± 2.70	64.32 ± 7.81	43.70 ± 22.62	67.57 ± 7.50	9.26 ± 0

Discussion

Fish are exposed to several stressors during their life cycle, both in the natural environment and on fish farms. Anesthetic substances are used to prevent or reduce stress damage in cultured fish. According to Ross (2008), the maximum acceptable time to induce deep anesthesia is 600 s. The present study showed that both EOs induced deep anesthesia in *S. eigenmanni* in less than 600 s; therefore, they can be used as anesthetics in this species. There are no studies of the anesthetic efficacy of geranial, limonene, and cis-carveol (*Z*-carveol) in fish, the main compounds of *A. triphylla* EO, but other EOs that anesthetize fish also contain these compounds in different amounts (Hoseini *et al.*, 2018; Lopes *et al.*, 2018). The anesthetic efficacy of linalool and eucalyptol (syn 1,8-cineole), the main compounds of *L. alba* EO, was determined for *R. quelen* (Heldwein *et al*., 2014) and *Oncorhynchus mykiss* (Mirghaed *et al*., 2018), respectively; the anesthetic efficacy of both compounds was also determined for *Cyprinus carpio* (Mazandarani *et al*., 2017; Khumpirapang *et al*., 2018). The anesthetic effects of these EOs are due to the association of these main compounds (and maybe other minor compounds) and result from additive and/or synergistic activities.

The lower concentrations of *A. triphylla* and *L. alba* EOs, 25 μL L^{-1} and 50 μL L^{-1}, respectively, induced only the sedation stage in which fish presented a low reactivity to external stimuli and a partial loss of equilibrium. The more efficient concentration for rapid deep anesthesia in *S. eigenmanni* was 100 μL L^{-1} *A. triphylla* EO, with an induction time of 266 s and a recovery time of 110 s; increasing the concentration to 150 μL L^{-1} did not reduce the time to reach deep anesthesia. The recommended concentration for deep anesthesia in both the albino and gray strains of *R. quelen* (2.6-3.0 g, 24 ºC, pH 7.0) and in *C. parallelus* (2.7 g, 30 ppt seawater, 23 ºC) is 200 μL L^{-1} with the same EO (Parodi *et al*. 2014, 2016), while for *S. rhombeus* (110.9 g, 30 ºC, pH 5.0), it is 150 μL L^{-1} (Almeida *et al.*, 2018); these species need a concentration higher than that required for the deep anesthesia of *S. eigenmanni*. In the case of *L. alba* EO, the best concentration to induce deep anesthesia in *S. eigenmanni* was also 100 μL L^{-1}, with induction and recovery times of 236 s and 242 s, respectively. *Serrasalmus eigenmanni* needs a lower *L. alba* EO concentration for the same anesthesia induction time as *S. rhombeus* (110.9 g, 30 ºC, pH 5.0; 200 μL L^{-1}; Almeida *et al.* (2018)), Nile tilapia *Oreochromis niloticus* (4.2, 25 ºC, pH 7.5; approximately 450 μL L^{-1}; Hohlenwerger *et al*. (2016)) and silver catfish *R. quelen* (6.5 g, 21 ºC, pH 6.5-7.0; approximately 450 μL L^{-1}; Cunha *et al*. (2010)), but a similar concentration as tambacu, the hybrid of *Piaractus mesopotamicus* × *Colossoma macropomum* (72.8 g, 26 ºC, pH 7.5; 200 μL L^{-1}; Sena *et al.* (2016)). As these studies with the EOs of *A. triphylla* and *L. alba* were performed with the same chemotypes, *i.e*., similar compositions, differences in the best concentrations to induce deep anesthesia are probably related to the different temperatures, pH, fish size, and/or species used. These water parameters can change the efficacy of the EOs (Hoseini *et al.*, 2018).

In the second experiment, low concentrations were tested to verify the viability of the use of these EOs for the transport of *S. eigenmanni*. The lower swimming activity of the fish when exposed to the EOs is an indication that these products can be recommended for the transport of Amazon fish at high temperatures. For fish transport, it is recommended that animals remain in the deep sedation stage, in which there is a loss of responsiveness to external stimuli, but without the loss of equilibrium and supposedly with lower metabolic activity (Summerfelt, Smith, 1990; Pirhonen, Schreck, 2003). The equilibrium loss of 22.22% of fish after 1 h of exposure suggests that the concentration of 10 μL L^{-1} of *L. alba* EO cannot be used for fish transport at high temperatures, because according to Cooke *et al*. (2014), in this stage, the fish may die of suffocation.

In stressful situations, the organism responds with the sympathetic activation of the hypothalamic-hypophysis-interrenal (HHI) axis, which triggers a change in the blood hormone levels, mainly catecholamines secreted by the chromaffin cells and the release of cortisol by the interrenal tissue (Mazeaud *et al*., 1977; Barton, Iwama, 1991; Barton, 2002). Consequently, metabolic changes, such as the increase of glucose, lactate, and tyrosine (T4) and a decrease in protein levels, as well as ionoregulatory disorders (Barton, 2002), may occur.

The addition of EOs of *A. triphylla* and *L. alba* to the transport water of silver catfish *R. quelen* showed that these EOs minimized stress transport effects through the reduction of cortisol release, ammonia excretion and ion loss (Becker

et al., 2012, 2013; Parodi *et al*., 2014; Zeppenfeld *et al*., 2014). The addition of 20 μL L^{-1} of *A. triphylla* EO in the transport water of *C. paralellus* decreased cortisol plasma levels and ammonia excretion (Parodi *et al*., 2016). The catching of *S. eigenmanni* specimens from the maintenance tanks and their transfer to the experimental aquaria led to a net ion loss to the water in the first few hours after handling. Similar results were found in a previous study with the same species (Baldisserotto *et al.*, 2008a) and other Amazonian fish in blackwater (Baldisserotto *et al.*, 2008b). The metabolic parameters, ammonia excretion, and ion fluxes of *S. eigenmanni* were not affected by exposure to EOs, except at 3 and 5 μL L^{-1} of *A. triphylla* EO, in which there was a reduction of K^+ efflux (compared to ethanol-exposed fish) and ammonia excretion, respectively. The decrease of ammonia excretion may be related to metabolism reduction, in accordance with the lower swimming activity in *S. eigenmanni* induced by this EO. Lower ammonia excretion was also observed in silver catfish (Zeppenfeld *et al.*, 2014) and *C. paralellus* transported with this EO (Parodi *et al*., 2016). On the other hand, Mazandarani *et al*. (2016) attributed the lower ammonia excretion, associated with higher serum urea levels in common carp *Cyprinus carpio* transported with linalool (the main constituent of the *L. alba* EO), as a sign of impaired gill function. Additional studies analyzing plasma ammonia and urea levels in fish transported with *A. triphylla* and *L. alba* EOs will provide more insight into this phenomenon.

Regarding the active substances of the studied EOs, literature reports demonstrated central depressor effects for geranial, limonene, and linalool in *in vivo* and *ex vivo* rodent models (Vale *et al*., 1999, 2002; Sousa *et al*., 2015), and also for linalool in fish (Heldwein *et al*., 2014). In addition, carveol itself did not induce anesthesia, but enhanced the anesthetic effects of propofol in mice (Lin *et al.*, 2006). As such, the available information indicates that the observed effects may result from the interaction of the different EO components.

In conclusion, both EOs showed anesthetic effects in *S. eigenmanni*. The recommended concentrations are 25 and 50 μL L^{-1} for sedation and 100 μL L^{-1} for deep anesthesia. A 5 μL L^{-1} concentration of both EOs is recommended for the transport of *S. eigenmanni* lasting up to 4 h; at this concentration, fish decreased ammonia excretion with the *A. triphylla* EO and maintained swimming equilibrium with both EOs.

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