

## ESSENTIAL OIL OF *Aloysia triphylla* IS EFFECTIVE IN NILE TILAPIA TRANSPORT

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### ABSTRACT

The study evaluated the use of the essential oil of *Aloysia triphylla* (EOAT) as stress reducer in transporting Nile tilapia, *Oreochromis niloticus*. Juveniles were transported for 8 h in plastic bags by car with water containing 0 (control), 20 or 30  $\mu\text{L L}^{-1}$  EOAT (three replicates). We evaluated water quality, net ion fluxes ( $\text{Cl}^-$ ,  $\text{Na}^+$  and  $\text{K}^+$ ) and, plasma parameters (cortisol, glucose, lactate and paraoxonase) and ventilatory rate (VR) of Nile tilapia. There was no mortality in juveniles undergoing transport. Nile tilapia transported with 20 or 30  $\mu\text{L L}^{-1}$  EOAT presented lower pH than control group and with 30  $\mu\text{L L}^{-1}$  EOAT presented lower water conductivity, alkalinity and  $\text{Cl}^-$ ,  $\text{Na}^+$  and  $\text{K}^+$  net ion fluxes compared to other treatments. The EOAT did not alter plasma cortisol and paraoxonase levels, but reduced VR, and 30  $\mu\text{L L}^{-1}$  EOAT reduced plasma glucose levels compared to the control group after transport. The use of 20  $\mu\text{L L}^{-1}$  EOAT increased plasma lactate levels compared to other treatments. The use of 30  $\mu\text{L L}^{-1}$  EOAT for sedation in 8 h transport of Nile tilapia is recommended because it improved water quality and reduced ionic losses, plasma glucose levels and VR.

**Key words:** cortisol; glucose; *Oreochromis niloticus*; stress; water quality.

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### ÓLEO ESSENCIAL DE *Aloysia triphylla* É EFETIVO NO TRANSPORTE DE TILÁPIA DO NILO

### RESUMO

Este estudo avaliou o uso do óleo essencial de *Aloysia triphylla* (OEAT) como redutor de estresse no transporte de tilápia do Nilo, *Oreochromis niloticus*. Juvenis foram transportados em carro por 8 h em sacos plásticos contendo água com 0 (controle), 20 ou 30  $\mu\text{L L}^{-1}$  OEAT (em triplicata). Nós avaliamos qualidade da água, fluxos iônicos ( $\text{Cl}^-$ ,  $\text{Na}^+$  e  $\text{K}^+$ ), parâmetros plasmáticos (cortisol, glicose, lactato e paraoxonase) e taxa de ventilação (TV) de tilápia do Nilo. Nenhuma mortalidade ocorreu durante o transporte. Juvenis de tilápia do Nilo transportados com 20 ou 30  $\mu\text{L L}^{-1}$  OEAT apresentaram menor pH da água do que o grupo controle e com 30  $\mu\text{L L}^{-1}$  OEAT apresentaram menor condutividade, alcalinidade e fluxos de  $\text{Cl}^-$ ,  $\text{Na}^+$  e  $\text{K}^+$  da água comparado aos demais tratamentos. O OEAT não alterou níveis de cortisol e paraoxonase do plasma, mas reduziu TV, e 30  $\mu\text{L L}^{-1}$  OEAT reduziu os níveis de glicose plasmática comparada ao grupo controle depois do transporte. O uso de 20  $\mu\text{L L}^{-1}$  OEAT aumentou os níveis plasmáticos de lactato comparado aos demais tratamentos. O uso de 30  $\mu\text{L L}^{-1}$  OEAT para sedação em transporte de 8 h de tilápia do Nilo é recomendado por melhorar qualidade de água e reduzir perdas iônicas, níveis plasmáticos de glicose e TV.

**Palavras-chave:** cortisol; glicose; *Oreochromis niloticus*; estresse; qualidade da água.

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### INTRODUCTION

The production of fish depends on various factors, of which transportation is one of the most important. During transportation, water quality can change (e.g. decrease in oxygen levels, pH changes and increase in ammonia levels) and hence affect the well-being and survival of fish (COLT *et al.*, 2011; SENA *et al.*, 2016; BOSISIO *et al.*, 2017). The use of sedative substances in fish transport can reduce physical activity and prevent physical injury and reduce metabolism without total loss of equilibrium (BECKER *et al.*, 2012; ZEPPEFELD *et al.*, 2014; SENA *et al.*, 2016).

Fish react to stress with a primary neuroendocrine response, represented by a rapid release of catecholamines (adrenaline and noradrenaline) and by the activation of the hypothalamic–pituitary–interrenal (HPI) axis. The increase of these hormones may induce a wide range of secondary responses that can be observed through disturbance of metabolic processes (WENDELAAR-BONGA, 1997), like plasma lactate, glucose and cortisol levels and/or ion loss in freshwater fish. Evaluation of ionic flow, plasma chemistry and hormones, especially cortisol concentrations in plasma, is broadly used as stress response indicators in fish (BARTON and IWAMA, 1991), since such parameters may reflect alterations of physiological homeostasis, demonstrating its application as biomarkers in the evaluation of stress effects, as well as paraoxonase, an enzyme that acts to protect the fish against oxidative stress (SENA *et al.*, 2016). A sedative must have a stress-reducing capacity, blocking the HPI axis, and render the fish unable to respond to additional stressors (BOLASINA, 2006). In addition, sedation can contribute to the maintenance of water quality by reducing oxygen consumption and ammonia excretion and contribute to the fish culture.

Essential oils derived from different plant species have been used for sedation and transporting fish (BECKER *et al.*, 2012; LIMMA-NETTO *et al.*, 2016; HOHLENWERTGER *et al.*, 2017). The essential oil of *Aloysia triphylla* (L'Hér.) Britton (EOAT) contributes to maintaining water quality (PARODI *et al.* 2014) and improving physiological conditions of silver catfish (*Rhamdia quelen*) after transport (ZEPPEFELD *et al.*, 2014). EOAT has also proved to be efficient for sedation, anesthesia and stress reducer in Nile tilapia (TEIXEIRA *et al.*, 2017) and for transporting albino and grey strains of silver catfish (PARODI *et al.*, 2014) and post larvae of white shrimp (*Litopenaeus vannamei*) (PARODI *et al.*, 2012). Although EOAT has not been evaluated for the transport of Nile tilapia in a closed system such as a plastic bag, clove oil is not recommended due to increased osmoregulatory disorder and mortality (SIMÕES *et al.*, 2011), although it did not influence glucose levels (OLIVEIRA *et al.*, 2009). These data suggest the necessity for studies with essential oils which can be recommended for the transport of Nile tilapia species.

The Nile tilapia (*Oreochromis niloticus*) adjusts easily to different environments and is one of the most important species of freshwater fish for global aquaculture, primarily because of its known hardiness and good adjustment to captivity conditions (EL-SAYED, 2002). However, there are no studies about EOAT in Nile tilapia transport. Therefore, the purpose of this investigation was to verify the efficacy of EOAT for use during the transport of Nile tilapia. So, we performed experimental trials to compare the influence of EOAT on survival, water quality, net ion fluxes (Cl<sup>-</sup>, Na<sup>+</sup> and K<sup>+</sup>), biochemical determinations (plasma cortisol, glucose, lactate and paraoxonase) and ventilatory rate (VR) of Nile tilapia.

## METHODS

### Essential oil of *Aloysia triphylla*

Leaves of *A. triphylla* cultivated at Frederico Westphalen, RS, Brazil, were collected in winter 2013. A voucher specimen was registered in the herbarium of the Department of Biology of the Universidade Federal de Santa Maria (SMDB no. 11169). Oil extraction from fresh leaves was performed by hydrodistillation and was stored in amber glass bottles (-4°C). The chemical constituents were verified by TEIXEIRA *et al.* (2017), where geranial (28.97%) and neral were the main constituents (16.12%).

### Animals

Sex-reversed male Nile tilapia juveniles of Gift lineage (92.66 ± 28.76 g and 17.33 ± 1.66 cm) were purchased from Bebedouro Fish Farm, Petrolina, PE, Brazil. The fish were housed for 14 days in continuously aerated 300-L masonry tanks with a semi-static system. The fish were fed twice a day at a ratio of 5.0% body mass with a commercial diet (320 g kg<sup>-1</sup> crude protein; 3500 kcal digestible energy, Purina Nutripeixe SI). Individuals were fasted for a period of 24 h prior to the experiments.

We performed two experiments separately. Initially, we evaluated the effects of EOAT on ionic fluxes, water quality and biochemical responses in plasma of fish during transport (N=12 before transport + N=36 after transport for each treatment). Subsequently, we performed a second experiment to evaluate the effect of EOAT on ventilatory rate of fish (N=24). The experiments were approved by the Ethical Committee of the Biology Institute of the Universidade Federal da Bahia under registration no. 19-2014.

### Evaluation of stress during transport

The transport began at 8 a.m. from the Bebedouro Fish Farm, Petrolina, PE towards Universidade Federal da Bahia, Salvador, BA, Brazil (a distance of 534 km) and the Nile tilapia juveniles were transported in a car for 8 h, mean time of transport of juveniles in conditions of long distances. The fish were placed in 30-L plastic bags, closed with elastic straps, containing 10-L of water and the rest completed with oxygen. Fish were transported at a loading density of 12 fish per plastic bag (density of 9.26 g L<sup>-1</sup>). Fish were divided into three treatments (three replicates each): 0 (control), 20 and 30 µL L<sup>-1</sup> EOAT, both first diluted in ethanol (1:10). Previous studies demonstrated that ethanol does not induce sedation or anesthesia at concentrations up to 4500 µL L<sup>-1</sup> in Nile tilapia (HOHLENWERTGER *et al.*, 2016; TEIXEIRA *et al.*, 2017).

Another 12 fish were not subject to transport and designated 'before transport'. They remained in water free of EOAT throughout the trial. The concentrations of EOAT were chosen according to the range recommended for sedation of Nile tilapia by TEIXEIRA *et al.* (2017). Additionally, a pilot study was performed which showed that the fish reached only light sedation during 8 h of exposure at these concentrations. After transportation, fish were placed in 250-L tanks and mortality was assessed after 96 h.

## Water quality parameters and net ion fluxes

Physical and chemical parameters of water were measured before and after transportation (in triplicate). Dissolved oxygen and temperature were measured with an oxygen meter (Polterm POL 60), pH with a pH meter (Hanna Combo HI 98130), electrical conductivity with a portable meter (Phtek CD310) and alkalinity, hardness and nitrite were measured with a commercial kit (Alfatecnoquímica, Florianópolis, Brazil). Total ammonia was measured by a colorimetric method and reading in a spectrophotometer (photoLab® S12) and un-ionized ammonia levels were calculated according to COLT (2002). The levels of Na<sup>+</sup> and K<sup>+</sup> were determined by spectrophotometry (Espectro Blue, model FME) and the levels of Cl<sup>-</sup> were determined by ion chromatography (Dionex®, ICS 1000).

The net ion fluxes were calculated based on the following equation:  $J_{net} = V([ion]_1 - [ion]_2) \times (M \times t)^{-1}$ , where  $[ion]_1$  and  $[ion]_2$  are the ion concentrations in the water before and at the end of the experimental period, respectively,  $V$  is the water volume (L),  $M$  is the mass of the fish (kg) and  $t$  is the duration of the exposure (h) (BALDISSEROTTO *et al.*, 2008).

## Biochemical determinations

At the end of transportation, blood samples (1 mL) were collected from 12 fish per plastic bag with heparinized syringes, transferred to micro tubes (2 mL) and centrifuged at 3000 rpm for 15 min (at 6°C) to obtain the plasma. Another 12 fish were also used to evaluate plasma parameters before transport. The plasma samples were stored under constant refrigeration (-20 °C) and were sent to the Laboratory of Clinical Biochemistry of the Universidade Federal da Bahia to measure cortisol, glucose, lactate and paraoxonase levels.

A cortisol S kit was used for the determination of cortisol in the plasma aliquots in mini VIDAS® equipment, using the enzyme-linked fluorescent assay technique. The measurement values of the VIDAS® cortisol S kit ranged from 2 to 650 ng mL<sup>-1</sup>. The analytical detection limit was 2 ng mL<sup>-1</sup>. The observed values of total precision, dependent on serum concentration, ranged from 7.42 to 12.98% (coefficient of variance).

The plasma glucose levels were determined enzymatically by glucose oxidase/glucose peroxidase in BT 3000 apparatus (Wiener Lab, Rosario, Argentina). Lactate analysis was performed using the same equipment. The paraoxonase activity was determined by measuring *p*-nitrophenol reaction product according to the method described by SENTI *et al.* (2003), where paraoxonase activity = factor × Δ abs/min. The evaluation of paraoxonase activity has already been evaluated in previous studies with essential oil of *Lippia alba* in fish transport (HOHLENWERGER *et al.*, 2016; SENA *et al.*, 2016).

## Ventilatory rate

To test the possible sedative effect of EOAT, the second experiment about VR was quantified by visually counting 20 successive opercular/buccal movements, measuring the elapsed time with a chronometer (HOHLENWERGER *et al.*, 2017).

Eight fish per treatment (one individual for an 8-L aquarium) that were not submitted to transport were exposed to the same EOAT concentrations used in transport treatments. The evaluation times of VR were: 0, 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 h. After the VR experiment, fish were placed in 250-L tanks and mortality was assessed after 96 h and, each juvenile was used only once.

## Statistical analyses

All data are expressed as the mean ± SEM. All data were subjected to Levene's test to verify the homogeneity of the variances. The data normality was assessed by Shapiro-Wilk test. Because the data exhibited homogeneous variances, comparisons between different treatments were made using two-way ANOVA (time X EOAT concentration) for VR and one-way ANOVA for biochemical determinations, net ion fluxes and water quality parameters post-transport followed by Tukey post-hoc tests. We used t-test for comparison between the "before transport" and "control" groups. Significance was set at a critical level of 95% (P<0.05).

## RESULTS

### Survival and water quality parameters

No mortality or injury in the Nile tilapia juveniles was observed immediately after or 96 h after transport.

The dissolved oxygen and temperature after transport were significantly lower and higher, respectively, than before transport (P<0.05). After transport, pH was significantly lower with the use of 20 or 30 μL L<sup>-1</sup> EOAT compared with the control group (P<0.05). The electrical conductivity and alkalinity after transport was significantly higher than before transport and electrical conductivity and alkalinity of the fish transported with 30 μL L<sup>-1</sup> EOAT was significantly lower than control group or 20 μL L<sup>-1</sup> EOAT (P<0.05).

The total ammonia and un-ionized ammonia were significantly higher after transport than before transport (P<0.05). After transport, total ammonia was significantly higher in the 20 μL L<sup>-1</sup> EOAT group compared with the control group (P<0.05). Hardness and nitrite levels were not significantly affected by transport and treatments (Table 1).

### Net ion fluxes

The use of EOAT in the transport water led to a significant decrease in Cl<sup>-</sup> and Na<sup>+</sup> (20 and 30 μL L<sup>-1</sup> EOAT) and K<sup>+</sup> net fluxes (30 μL L<sup>-1</sup> EOAT) compared to the control group (P<0.05). Additionally, the net Cl<sup>-</sup> efflux was significantly lower in the 30 μL L<sup>-1</sup> EOAT group than in the 20 μL L<sup>-1</sup> EOAT group (P<0.05) (Figure 1).

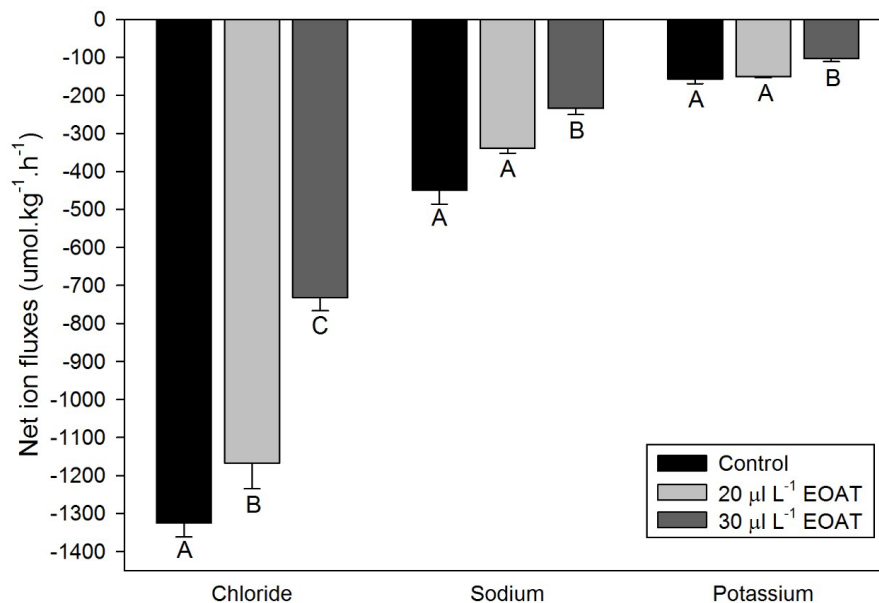
### Biochemical determinations

Nile tilapia juveniles of the control group had significantly higher plasma cortisol, glucose and, paraoxonase levels than fish not subjected to transport (P<0.05) (Figure 2A, B and C).

**Table 1.** Water parameters before and after transport (8 h) of Nile tilapia (n=3 plastic bags per treatment) with essential oil of *Aloysia triphylla* (EOAT) added to the water. Data are expressed as mean ± SEM.

Water parameter	Before transport	After transport		
		Control	EOAT 20 µL L <sup>-1</sup>	EOAT 30 µL L <sup>-1</sup>
Dissolved oxygen (mg L <sup>-1</sup> )	6.83±0.09*	3.63±0.17 <sup>a</sup>	3.70±0.11 <sup>a</sup>	3.33±0.28 <sup>a</sup>
Temperature (°C)	25.90±0.06*	30.33±0.17 <sup>a</sup>	30.07±0.03 <sup>a</sup>	30.67±0.13 <sup>a</sup>
pH	6.57±0.08	6.08±0.20 <sup>a</sup>	5.70±0.04 <sup>b</sup>	5.86±0.02 <sup>b</sup>
Electrical conductivity (mS cm <sup>-1</sup> )	0.74±0.10*	4.55±0.69 <sup>a</sup>	4.29±0.81 <sup>a</sup>	3.13±1.59 <sup>b</sup>
Alkalinity (mg CaCO <sub>3</sub> L <sup>-1</sup> )	36.67±3.33*	86.67±6.67 <sup>a</sup>	76.33±3.33 <sup>a</sup>	53.33±3.33 <sup>b</sup>
Hardness (mg CaCO <sub>3</sub> L <sup>-1</sup> )	40.00±0.00	40.00±0.00 <sup>a</sup>	40.00±0.00 <sup>a</sup>	40.00±0.00 <sup>a</sup>
Total ammonia (mg L <sup>-1</sup> N-NH <sub>3</sub> )	0.04±0.01*	0.91±0.09 <sup>b</sup>	1.45±0.08 <sup>a</sup>	1.06±0.16 <sup>ab</sup>
Un-ionized ammonia (µg L <sup>-1</sup> N-NH <sub>3</sub> )	0.09±0.01*	0.89±0.07 <sup>a</sup>	0.58±0.05 <sup>a</sup>	0.64±0.06 <sup>a</sup>
Nitrite (mg L <sup>-1</sup> N-NO <sub>2</sub> )	0.015±0.00	0.025±0.00 <sup>a</sup>	0.025±0.00 <sup>a</sup>	0.025±0.00 <sup>a</sup>

Different letters indicate significant difference between treatments post transport. Asterisk denote differences between the “before transport” and “control” groups (P<0.05).

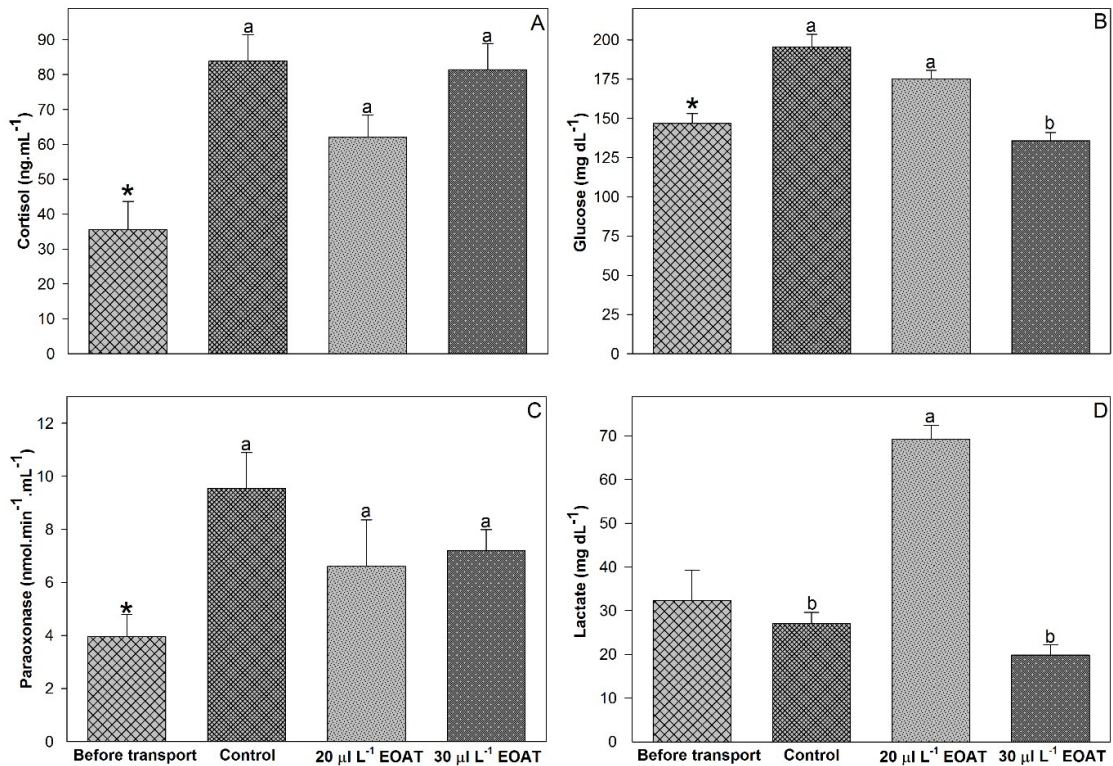


**Figure 1.** Effect of the essential oil of *Aloysia triphylla* (EOAT) added to the water on net ion fluxes (Cl<sup>-</sup>, Na<sup>+</sup> and K<sup>+</sup>) in Nile tilapia (n=3 plastic bags per treatment) transported for 8 h. Data are expressed as mean ± SEM. Different letters indicate significant difference between treatments (P<0.05).

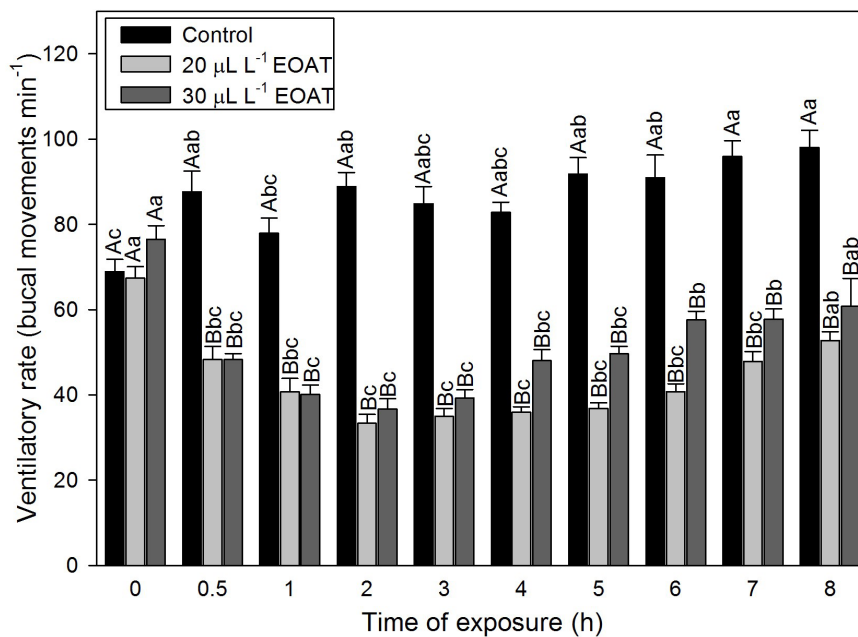
The plasma glucose levels of fish transported with 30 µL L<sup>-1</sup> EOAT were significantly lower than fish of the control group or transported with 20 µL L<sup>-1</sup> EOAT (P<0.05) (Figure 2B). The plasma paraoxonase levels were not significantly affected by transport or treatments (Figure 2C). Plasma lactate levels of fish transported with 20 µL L<sup>-1</sup> EOAT were significantly higher than fish of the control group or transported with 30 µL L<sup>-1</sup> EOAT (P<0.05) (Figure 2D).

### Ventilatory rate

After 0.5 h, fish sedated with EOAT (20 and 30 µL L<sup>-1</sup>) presented lower VR than those from the control group up to the end of the experiment (8 h) (P<0.05). The VR was significantly lower in the 20 and 30 µL L<sup>-1</sup> EOAT groups after 0.5 h (except at time 8 h) compared with time zero (P<0.05). In general, fish from the control group presented higher VR after 0.5 h compared to time zero (P<0.05) (Figure 3).



**Figure 2.** Effect of the essential oil of *Aloysia triphylla* (EOAT) added to the water on plasma cortisol (A), glucose (B), paraoxonase (C) and lactate (D) levels in Nile tilapia (n=12 before transport + 36 fish per treatment after transport) after 8 h transport. Data are expressed as mean  $\pm$  SEM. Different letters indicate significant difference between treatments (P<0.05). \*Indicate significant difference between before transport and control group (P<0.05).



**Figure 3.** Effect of the essential oil of *Aloysia triphylla* (EOAT) added to the water on ventilatory rate in Nile tilapia (n=8 per treatment). Data are expressed as mean  $\pm$  SEM. Different capital letters indicate significant differences between treatments at the same time. Different lowercase letters indicate significant differences between different times for the same treatment (P<0.05).

## DISCUSSION

### Water quality parameters

Dissolved oxygen in water levels are normally reduced during transport due to fish respiration (COLT *et al.*, 2011). The values found in this study were above the minimum dissolved oxygen level for Nile tilapia culture ( $2.9 \text{ mg L}^{-1}$  at  $32.7^\circ\text{C}$ ) (SODERBERG, 2006). The water temperature before transport was lower due to the environmental conditions of this experiment, with lower temperatures typical of early morning hours. Water temperatures throughout the experiment remained within the thermal comfort range for Nile tilapia ( $24\text{--}33^\circ\text{C}$ ) (BARAS *et al.*, 2000).

The electrical conductivity of water is closely related to the dissolved ions (WENDELAAR BONGA, 1997) and the increase in conductivity values after transport was possibly caused by ion net fluxes by the fish. The lower electrical conductivity values observed in the water of Nile tilapia transported with  $30 \mu\text{L L}^{-1}$  EOAT were probably related to the lower net ion net fluxes observed in this treatment. Water pH remained within tolerance values for Nile tilapia (EL-SHERIF and EL-FEKY, 2009). The highest pH value of the water in the control group was probably due primarily to un-ionized ammonia excretion (BALDISSEROTTO *et al.*, 2008).

Similar to our data, increases in alkalinity levels after transport were reported in previous studies with silver catfish (BECKER *et al.*, 2012; PARODI *et al.*, 2014). These authors reported that the increase in alkalinity and hardness was probably due to regurgitation of commercial food (which contained calcitic limestone) by the fish. Food debris in the transport plastic bags was not observed in the present study because there was a 24 h fasting period prior to transport, as recommended for Nile tilapia (OLIVEIRA *et al.*, 2009). The alkalinity increase in the control group after transport was probably due to the increase in the pH of the water and the higher efflux of  $\text{Na}^+$  and  $\text{K}^+$ , which were associated with the carbonates and bicarbonates present in the water (BOYD *et al.*, 1992). Moreover, the absence of regurgitation observed in the present study may explain the maintenance of levels of water hardness after 8 h of transport.

Total ammonia levels were higher after transport than before transport, as expected (BECKER *et al.*, 2012; PARODI *et al.*, 2014; ZEPPENFELD *et al.*, 2014). However, in the present study the lower pH in the 20 and  $30 \mu\text{L L}^{-1}$  EOAT treatments compared to the control group contributed to the lack of significant difference in un-ionized ammonia levels. Un-ionized ammonia levels remained well below lethal levels for Nile tilapia (above  $7400 \mu\text{g L}^{-1}$ ) (BENLI and KÖKSAL, 2005) suggesting that Nile tilapia could be transported for a longer period without mortality due to ammonia toxicity levels under the conditions used in this experiment.

### Net ion fluxes

The transport of Nile tilapia caused net ion ( $\text{Cl}^-$ ,  $\text{Na}^+$  and  $\text{K}^+$ ) net fluxes, as expected, because the stress of transport can cause an increased release of catecholamines in fish, with a resulting increase in blood flow and gill permeability. In freshwater fish this implies ion loss to the water, especially  $\text{Cl}^-$  and  $\text{Na}^+$  (URBINATI *et al.*, 2004).

Previous studies also found that EOAT ( $30\text{--}50 \mu\text{L L}^{-1}$ ) reduced net ion ( $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{K}^+$ ) losses in silver catfish transported for 5–6 h (PARODI *et al.*, 2014; ZEPPENFELD *et al.*, 2014). On the other hand, for the transport of Nile tilapia (6 to 24 h), clove oil ( $9$  and  $18 \text{ mg L}^{-1}$ ) is not recommended because it increases net ion fluxes and mortality (SIMÕES *et al.*, 2011). We hypothesized that the lower ion losses with the use of EOAT in Nile tilapia transport were due to lower catecholamine release during the transport process and, consequently, there was lower gill blood flow. However, further studies should be conducted for a more accurate determination of the role of EOAT on ionic uptake in fish.

### Biochemical determinations

A stressor can result in an endocrine response, such as a release of cortisol. Transport by car generates a change in the water flow of the plastic bags and exposes the fish to ambient temperature and insolation (SENA *et al.*, 2016). This is consistent with our findings to lower plasma cortisol levels before transport. Additionally, in this study, Nile tilapia transported with EOAT did not exhibit an increase in plasma cortisol levels compared with the control group. Similarly, HOHLENWERGER *et al.* (2017) found no change in plasma cortisol levels in Nile tilapia transported with essential oil of *L. alba* ( $10\text{--}20 \mu\text{L L}^{-1}$ ). Previous studies of fish transport have demonstrated that the addition of EOAT ( $30\text{--}50 \mu\text{L L}^{-1}$ ) had conflicting results in silver catfish: plasma cortisol levels did not change or decreased in the grey strain (PARODI *et al.*, 2014; ZEPPENFELD *et al.*, 2014), but increased in the albino strain compared to fish transported without EOAT (PARODI *et al.*, 2014).

Physiological stress responses include plasma hormones, metabolic processes and ionic balance (BARTON and IWAMA, 1991). The elevation of plasma glucose levels in fish after transport is due to the action of catecholamines and/or corticosteroids, which stimulate muscle and liver glycogenolysis (BARTON and IWAMA, 1991) as an adaptive response for supplying energy during transport and to maintain homeostasis (SENA *et al.*, 2016). Our results showed that  $30 \mu\text{L L}^{-1}$  EOAT was effective in reducing plasma glucose levels, reinforcing the hypothesis of lower catecholamine release. According to TAKAHASHI *et al.* (2006) plasma glucose can give better answers than cortisol in assessing the physiological condition due to stress caused by transport. In this study, EOAT presented sedative effect in Nile tilapia transport and reduced the energy demand.

Additionally, stress can result in a rapid increase in the plasma lactate levels, because lactate is a final product of anaerobic glycolysis for cellular energy supplies (URBINATI *et al.*, 2004). We found that  $30 \mu\text{L L}^{-1}$  EOAT, did not cause elevation of plasma lactate levels, which could indicate that sedation with EOAT at this concentration did not require the use of an anaerobic metabolic pathway as an important energy source during the transport (HOHLENWERGER *et al.*, 2016) as verified for Nile tilapias under handling ( $500 \mu\text{L L}^{-1}$ ) or transport ( $10$  or  $20 \mu\text{L L}^{-1}$ ) conditions with use of essential oil of *L. alba* (HOHLENWERGER *et al.*, 2017). Another hypothesis is that lactate has been produced and immediately consumed in the muscle (GLADDEN, 2000). In this sense, the lactate increase was already verified in Nile tilapia juveniles immediately after handling with  $300 \mu\text{L L}^{-1}$  EOAT, but not for 1 or 4 hours after

handling (TEIXEIRA *et al.*, 2017). So, further studies should be conducted for a clarification about the lactate response in the transport of Nile tilapia with the use of EOAT. Additionally, the increased plasma lactate levels observed in fish exposed to 20  $\mu\text{L L}^{-1}$  EOAT could be related to increased total ammonia levels seen in this treatment. According COPATTI *et al.* (2015), the increase in  $\text{NH}_3$  concentrations triggers the use of an anaerobic route with the use of lactate as substrate for gluconeogenesis.

Plasma paraoxonase is an important indicator of protection against oxidative stress, preventing damage in the immune response and changes in total plasma proteins (KARATAS and KOCAMAN, 2014). In this study, plasma paraoxonase and cortisol levels indicate that exposure to EOAT did not alter the protection mechanism for stress in Nile tilapia after 8 h of transport, unlike what occurred in the control group, where the activation of antioxidant defences may be an adaptive response which involves physiological adjustments caused by stress or transport. However, few studies have investigated paraoxonase in fish, and its relation to the use of anaesthetics or sedatives in fish should be further better investigated (SENA *et al.*, 2016).

### Ventilatory rate

VR is easily changed in cases of stressful stimuli (BARRETO and VOLPATO, 2004; BECKER *et al.*, 2012). According to BARRETO and VOLPATO (2004), in the absence of stress, the opercular movements of Nile tilapia vary between 96 and 109 per min. In the present study, except for time zero, the concentrations of 20 and 30  $\mu\text{L L}^{-1}$  EOAT reduced movements in relation to the control group over 8 h, and remained below 61 opercular movements per min.

In this sense, our data showed that EOAT reduced the VR of Nile tilapia after 0.5 h of exposure, however, as plasma cortisol levels were not affected by EOAT in the water of transport, probably this essential oil reduced catecholamine release and/or metabolism, which led to the lower VR observed (HOHLENWERGER *et al.*, 2017).

### CONCLUSIONS

The use of 30  $\mu\text{L L}^{-1}$  EOAT in Nile tilapia indicate that water quality was maintained, ionic balance improved since it reduced the loss of ions ( $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{K}^+$ ) and metabolic responses were prevented or not modified during transportation, and VR was reduced after 0.5 h and remained so over 8 h. So, 30  $\mu\text{L L}^{-1}$  EOAT is safe and effective as a sedative for Nile tilapia transport.

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### REFERENCES

- BALDISSEROTTO, B.; COPATTI, C.E.; GOMES, L.C.; CHAGAS, E.C.; BRINN, R.P.; ROUBACH, R. 2008 Net ion fluxes in the facultative air-breather *Hoplosternum littorale* (tamoata) and the obligate air-breather *Arapaima gigas* (pirarucu) exposed to different Amazonian waters. *Fish Physiology and Biochemistry*, 34(4): 405-412. PMID: 18958598. <http://dx.doi.org/10.1007/s10695-008-9200-y>.
- BARAS, E.; PRIGNON, C.; GOHOUNGO, G.; MEALARD, C. 2000 Phenotypic sex differentiation of blue tilapia under constant and fluctuating thermal regimens and its adaptive and evolutionary implications. *Journal of Fish Biology*, 57(1): 210-223. <http://dx.doi.org/10.1111/j.1095-8649.2000.tb00787.x>.
- BARRETO, R.E.; VOLPATO, G.L. 2004 Caution for using ventilatory frequency as an indicator of stress in fish. *Behavioural Processes*, 66(1): 43-51. PMID: 15062970. <http://dx.doi.org/10.1016/j.beproc.2004.01.001>.
- BARTON, B.A.; IWAMA, G.K. 1991 Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Review of Fish Diseases*, 1(1): 3-26. [http://dx.doi.org/10.1016/0959-8030\(91\)90019-G](http://dx.doi.org/10.1016/0959-8030(91)90019-G).
- BECKER, A.G.; PARODI, T.V.; HELDWEIN, C.G.; ZEPPEFELD, C.; HEINZMANN, B.M.; BALDISSEROTTO, B. 2012 Transportation of silver catfish, *Rhamdia quelen*, in water with eugenol and the essential oil of *Lippia alba*. *Fish Physiology and Biochemistry*, 38(3): 789-796. PMID: 21972065. <http://dx.doi.org/10.1007/s10695-011-9562-4>.
- BENLI, A.C.K.; KÖKSAL, G. 2005 The acute toxicity of ammonia on tilapia (*Oreochromis niloticus* L.) larvae and fingerlings. *Turkish Journal of Veterinary and Animal Sciences*, 29(2): 339-344.
- BOLASINA, S.N. 2006 Cortisol and hematological response in Brazilian codling, *Urophycis brasiliensis* (Pisces, Phycidae) subjected to anesthetic treatment. *Aquaculture International*, 14(6): 569-575. <http://dx.doi.org/10.1007/s10499-006-9055-0>.
- BOSISIO, F.; REZENDE, K.F.O.; BARBIERI, E. 2017 Alterations in the hematological parameters of Juvenile Nile Tilapia (*Oreochromis niloticus*) submitted to different salinities. *Pan-American Journal of Aquatic Sciences*, 12(2): 146-154.
- BOYD, C.E.; TUCKER, C.S.; SOMRIDHIVEJ, B. 1992 Alkalinity and hardness: critical but elusive concepts in aquaculture. *Journal of the World Aquaculture Society*, 47(1): 6-41. <http://dx.doi.org/10.1111/jwas.12241>.
- COLT, J. 2002 List of spreadsheets prepared as a complement. In: WEDEMEYER, G.A. (Ed.). *Fish hatchery management*. 2<sup>nd</sup> ed. Washington: Amer Fish Soc Pub. Available from: <www.fisheries.org/hatchery>. Accessed on: 26 June 2017.
- COLT, J.; MOMODA, T.; CHITWOOD, R.; FORNSHELL, G.; SCHRECK, C. 2011 Water quality in tilapia transport: from the farm to the retail store. *North American Journal of Aquaculture*, 73(4): 426-434. <http://dx.doi.org/10.1080/15222055.2011.629946>.
- COPATTI, C.E.; BOLNER, K.C.S.; ROSSO, F.L.; LORO, V.L.; BALDISSEROTTO, B. 2015 Tolerance of piava juveniles to different ammonia concentrations. *Semina: Ciências Agrárias*, 36(6): 3991-4002. <http://dx.doi.org/10.5433/1679-0359.2015v36n6p3991>.

- EL-SAYED, A.F.M. 2002 Effects of stocking density and feeding levels on growth and feed efficiency of Nile tilapia (*Oreochromis niloticus* L.) fry. *Aquaculture Research*, 33(8): 621-626. <http://dx.doi.org/10.1046/j.1365-2109.2002.00700.x>.
- EL-SHERIF, M.S.; EL-FEKY, A.M.I. 2009 Performance of Nile tilapia (*Oreochromis niloticus*) fingerlings. I. Effect of pH. *International Journal of Agriculture and Biology*, 11(3): 297-300.
- GLADDEN, L.B. 2000 Muscle as a consumer of lactate. *Medicine and Science in Sports and Exercise*, 32(4): 764-771. PMID:10776895. <http://dx.doi.org/10.1097/00005768-200004000-00008>.
- HOHLENWERGER, J.C.; BALDISSEROTTO, B.; COUTO, R.D.; HEINZMANN, B.M.; SILVA, D.T.; CARON, B.O.; SCHMIDT, D.; COPATTI, C.E. 2017 Essential oil of *Lippia alba* in the transport of Nile tilapia. *Ciência Rural*, 47(3): e20160040. <http://dx.doi.org/10.1590/0103-8478cr20160040>.
- HOHLENWERGER, J.C.; EDUARDO COPATTI, C.; CEDRAZ SENA, A.; DAVID COUTO, R.; BALDISSEROTTO, B.; HEINZMANN, B.M.; CARON, B.O.; SCHMIDT, D. 2016 Could the essential oil of *Lippia alba* provide a readily available and cost-effective anaesthetic for Nile tilapia (*Oreochromis niloticus*)? *Marine and Freshwater Behaviour and Physiology*, 49(2): 119-126. <http://dx.doi.org/10.1080/10236244.2015.1123869>.
- KARATAS, T.; KOCAMAN, E.M. 2014 Susceptibility to oxidative damage in wild and cultured brook trouts (*Salvelinus fontinalis* Mitchell, 1815). *International Journal of Fisheries and Aquatic Studies*, 2(1): 180-183.
- LIMMA-NETTO, J.D.; SENA, A.C.; COPATTI, C.E. 2016 Essential oils of *Ocimum basilicum* and *Cymbopogon flexuosus* in the sedation, anesthesia and recovery of tambacu (*Piaractus mesopotamicus* male x *Colossoma macropomum* female). *Boletim do Instituto de Pesca*, 42(3): 727-733. <http://dx.doi.org/10.20950/1678-2305.2016v42n3p727>.
- OLIVEIRA, J.R.; CARMO, J.L.; OLIVEIRA, K.K.C.; SOARES, M.C.F. 2009 Sodium chloride, benzocaine and clove oil in tilapia transport water. *Revista Brasileira de Zootecnia*, 38(7): 1163-1169. <http://dx.doi.org/10.1590/S1516-35982009000700001>.
- PARODI, T.V.; CUNHA, M.A.; BECKER, A.G.; ZEPPEFELD, C.C.; MARTINS, D.I.; KOAKOSKI, G.; BARCELLOS, L.G.; HEINZMANN, B.M.; BALDISSEROTTO, B. 2014 Anesthetic activity of the essential oil of *Aloysia triphylla* and effectiveness in reducing stress during transport of albino and gray strains of silver catfish, *Rhamdia quelen*. *Fish Physiology and Biochemistry*, 40(2): 323-334. PMID:23974669. <http://dx.doi.org/10.1007/s10695-013-9845-z>.
- PARODI, T.V.; CUNHA, M.A.; HELDWEIN, C.G.; SOUZA, D.M.; MARTINS, Á.C.; GARCIA, L.O.; WASIELESKY JUNIOR, W.; MONSERRAT, J.M.; SCHMIDT, D.; CARON, B.O.; HEINZMANN, B.; BALDISSEROTTO, B. 2012 The anesthetic efficacy of eugenol and the essential oils of *Lippia alba* and *Aloysia triphylla* in post-larvae and sub-adults of *Litopenaeus vannamei* (Crustacea, Penaeidae). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 155(3): 462-468. PMID:22198554.
- SENA, A.C.; TEIXEIRA, R.R.; FERREIRA, E.L.; HEINZMANN, B.M.; BALDISSEROTTO, B.; CARON, B.O.; SCHMIDT, D.; COUTO, R.D.; COPATTI, C.E. 2016 Essential oil from *Lippia alba* has anaesthetic activity and is effective in reducing handling and transport stress in tambacu (*Piaractus mesopotamicus* x *Colossoma macropomum*). *Aquaculture*, 465(1): 374-379. <http://dx.doi.org/10.1016/j.aquaculture.2016.09.033>.
- SENTI, M.; TOMAS, M.; FITO, M.; WEINBRENNER, T.; COVAS, M.I.; SALA, J.; MASIÁ, R.; MARRUGAT, J. 2003 Antioxidant paraoxonase 1 activity in the metabolic syndrome. *The Journal of Clinical Endocrinology and Metabolism*, 88(11): 5422-5426. PMID:14602783. <http://dx.doi.org/10.1210/jc.2003-030648>.
- SIMÕES, L.N.; LOMBARDI, D.C.; GOMIDE, A.T.M.; GOMES, L.C. 2011 Efficacy of clove oil as anesthetic in handling and transportation of Nile tilapia, *Oreochromis niloticus* (Actinopterygii: Cichlidae) juveniles. *Zoologia*, 28(3): 285-290. <http://dx.doi.org/10.1590/S1984-46702011000300001>.
- SODERBERG, R.W. 2006 Culture in flowing water. In: LIM, C.E.; WEBSTER, C.D. (Eds.). *Tilapia: biology, culture and nutrition*. London: Food Products Press. p. 289-307.
- TAKAHASHI, L.S.; ABREU, J.S.; BILLER, J.D.; URBINATI, E.C. 2006 Effect of post-transport environment on the stress indicators recovery of pacu juveniles, *Piaractus mesopotamicus*. *Acta Scientiarum Animal Science*, 28(4): 469-475.
- TEIXEIRA, R.R.; SOUZA, R.C.; SENA, A.C.; BALDISSEROTTO, B.; HEINZMANN, B.M.; COUTO, R.D.; COPATTI, C.E. 2017 Essential oil of *Aloysia triphylla* in Nile tilapia: anaesthesia, stress parameters and sensory evaluation of fillets. *Aquaculture Research*, 48(7): 3383-3392. <http://dx.doi.org/10.1111/are.13165>.
- URBINATI, E.C.; ABREU, J.S.; CAMARGO, A.C.S.; PARRA, M.A.L. 2004 Loading and transport stress of juvenile matrinxã (*Brycon cephalus*, Characidae) at various densities. *Aquaculture*, 229(1-4): 389-400. [http://dx.doi.org/10.1016/S0044-8486\(03\)00350-8](http://dx.doi.org/10.1016/S0044-8486(03)00350-8).
- WENDELAAR-BONGA, S.E.W. 1997 The stress response in fish. *Physiological Reviews*, 77(3): 591-625. PMID:9234959. <http://dx.doi.org/10.1152/physrev.1997.77.3.591>.
- ZEPPEFELD, C.C.; TONI, C.; BECKER, A.G.; MIRON, D.S.; PARODI, T.V.; HEINZMANN, B.M.; BARCELLOS, L.J.G.; KOAKOSKI, G.; ROSA, J.G.S.; LORO, V.L.; CUNHA, M.A.; BALDISSEROTTO, B. 2014 Physiological and biochemical responses of silver catfish, *Rhamdia quelen*, after transport in water with essential oil of *Aloysia triphylla* (L'Herit) Britton. *Aquaculture*, 418-419(1): 101-107. <http://dx.doi.org/10.1016/j.aquaculture.2013.10.013>.