Effects of anesthesia with the essential oil of *Ocimum gratissimum* L. in parameters of fish stress

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ABSTRACT: The effects of anesthesia with the essential oil of *Ocimum gratissimum* (EOO) in parameters of stress after handling were investigated in silver catfish (*Rhamdia quelen*). EOO was obtained from the aerial parts by hydrodistillation. Juveniles were anesthetized with 70 or 300 mg L^{-1} EOO and submitted to air exposure for 1 minute. The fishes were sampled immediately or transferred to anesthetic-free aquaria until sampling. In the first experiment, juveniles had their blood collected at 0, 1, 4, and 8 h after handling to assay plasma cortisol and blood glucose levels. The unanesthetized animals were restrained manually for blood collection. In the second experiment, water samples of the recovery aquaria were collected to evaluate net ion fluxes at 0 - 4 h and 4 - 8 h. Water and ethanol controls were also performed under the same conditions. The results showed that the cortisol levels did not differ among the treatments. Hyperglycemia was verified in fish exposed to 70 and 300 mg L^{-1} EOO at 1 h and 4 h after handling. After 8 h, cortisol and glucose concentrations were lower or similar than those from immediately after handling for all treatments. EOO anesthesia prevented Na^+ efflux observed in the control groups in both flux periods. There were net Cl^- and K^+ effluxes at 0 - 4 h and influxes at 4 - 8 h after handling in most treatments, and these fluxes did not differ among the treatments. The results suggest that EOO did not impair stress recovery and did not act as an additional handling stressor in silver catfish.

Key words: clove basil, cortisol, glucose, net ion flux, silver catfish.

RESUMO: Efeitos da anestesia com o óleo essencial de *Ocimum gratissimum* L. em parâmetros de estresse de peixes. Os efeitos da anestesia com o óleo essencial de *Ocimum gratissimum* (EOO) em parâmetros de estresse após manuseio foram investigados em jundiás (*Rhamdia quelen*). EOO foi obtido a partir das partes aéreas por hidrodestilação. Os juvenis foram anestesiados com 70 ou 300 mg L^{-1} de EOO e expostos ao ar por 1 minuto. Os peixes foram amostrados imediatamente ou transferidos para aquários sem anestésico até amostragem. No primeiro experimento, os juvenis tiveram seu sangue coletado em 0, 1, 4, e 8 h após manuseio para avaliar os níveis de cortisol e glicemia. Os animais não anestesiados foram contidos manualmente para coleta sanguínea. No segundo experimento, amostras de água foram coletadas do aquário de recuperação dos animais para avaliação do fluxo iônico entre 0 - 4 h e 4 - 8 h. Grupos controles em água e etanol também foram realizados sobre as mesmas condições. Os resultados demonstraram que os níveis de cortisol não diferiram entre os tratamentos. Hiperglicemia foi detectada em peixes expostos a 70 e 300 mg L^{-1} de EOO em 1 h e 4 h após o manuseio. Após 8 h, os teores de cortisol e glicose foram menores ou similares aqueles imediatamente após o manuseio para todos os tratamentos. A anestesia com EOO preveniu o efluxo de Na^+ observado para os grupos controle em ambos os períodos avaliados. Ocorreram efluxos de Cl^- and K^+ entre 0 - 4 h e influxos entre 4 - 8 h após o manuseio para a

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maioria dos tratamentos, e estes eventos não diferiram entre os tratamentos. Os resultados sugerem que o EOO não prejudica a recuperação do animal frente ao evento estressor ou atua como estressor adicional ao manuseio em jundiás.

Palavras- chave: alfavaca, cortisol, glicose, fluxo iônico, jundiá.

INTRODUCTION

Anesthetics are often used to reduce fish movement and stress during fish farming procedures (Kiessling et al., 2009). Stress response corresponds to an animal's attempt to maintain its homeostatic state (Barton, 2002; Kiessling et al., 2009). It involves the activation of neuroendocrine pathways, which promote catecholamine and corticosteroid (cortisol) release. These hormones lead to secondary changes in metabolism, respiration, acid-base status, hydromineral balance, immune function, and cellular responses and over the long term these will negatively affect the whole animal performance (see reviews of Hontela, 1998; Barton, 2002).

However, not all anesthetics used in aquaculture have an effect on inhibit the stress cascade and some induce cortisol responses (Kiessling et al., 2009; Zahl et al., 2010). For silver catfish (*Rhamdia quelen*), the fish species used in this study, anesthesia with eugenol and the essential oil of *Lippia alba* prevented plasma cortisol increase in fish submitted to air exposure (Cunha et al., 2010a, b). This nocturnal species is one of the most widespread inhabitants of South American rivers, and has been considered a good alternative to fish production, as a result of its fast growth rate (Barcellos et al., 2001; Koakoski et al., 2012). It is susceptible to stress resulting from confinement and handling procedures, such as capture, tank transference, and air exposure (Barcellos et al., 2001, 2006; Cunha et al., 2010a, b).

The plant *Ocimum gratissimum* L. (Lamiaceae), commonly known as alfavaca or tree basil, is native to Africa, but it is cultivated and naturalized throughout Brazil. It has been used as a condiment and, in traditional medicine, as a sedative and in the treatment of stress, headache, among others (Albuquerque & Andrade, 1998; Di Stasi et al., 2002; Albuquerque et al., 2007). Recently, the essential oil of *O. gratissimum* (EOO) was also proposed as a new anesthetic for silver catfish juveniles (Silva et al., 2012; Benovit et al., 2012). Thus, the purpose of the present study was to evaluate the effects of such oil on the stress response induced by the handling of silver catfish.

MATERIALS AND METHODS

Fish

Juvenile silver catfish (15.68 ± 0.40 g, 12.40 ± 0.10 cm) were kept in continuously aerated tanks (130 L capacity in experiment 1 and 230 L capacity in experiment 2) for one week before the experiments in order to reduce the possible interferences of handling and environmental conditions on the parameters evaluated. A semi-static system was used and 50 % of the water volume was changed daily. The fish were fed once a day with commercially available food at 5 % of body weight (42 % crude protein, 3400 kcal kg^{-1} DE). The water parameters were daily monitored. Temperature and dissolved oxygen concentrations were measured with an YSI 550A oxygen meter, while pH values were measured with digital pH meters Solar SL110 (experiment 1) and DMPH-2 (experiment 2). Total ammonia nitrogen, alkalinity, and hardness were also measured through colorimetric tests.

Plant Material

The aerial parts of *O. gratissimum* L. were grown in Jardinópolis, São Paulo, Brazil. The plant material was collected in March 2007, dried for three days in a ventilated drying oven at 45 °C, and stored in dark closed packages until extraction. Voucher specimen (no. 1329) identified by Dr. Lin Chan Ming was deposited in the Biotechnology Department of the University of Ribeirão Preto.

Essential oil extraction

Essential oil was obtained from the aerial parts without flowers by means of hydrodistillation, using a Clevenger-type apparatus for 3 h (European Pharmacopoeia, 2007). It was stored at - 4 ºC in amber glass bottles until the experiments were initiated. The major compounds of EOO were eugenol (76.3 %) and β-bisabolene (18.3 %) (Silva et al., 2012).

General procedures

Juvenile silver catfish were subjected to one of the following treatments: water control, ethanol control, 70 mg L^{-1} or 300 mg L^{-1} EOO. These EOO concentrations were chosen because they induced the anesthesia of silver catfish within 1 - 6 minutes (Silva et al., 2012). Before dissolution in water, the

EOO concentrations were corrected by density (1.09 g mL^{-1}) and diluted in ethanol 95 % (1:10). Ethanol control corresponded to the same concentration used to dilute 300 mg L^{-1} EOO (about 2.48 mL). Eight fish were used by treatment and/or collection time, and each animal was used only once.

All the fish were captured with a hand net and transferred to continuously aerated aquaria containing only 1 L of water (water control) or added to the samples to be tested (ethanol or EOO). The time between capture and release did not exceed 30 seconds. Juveniles stayed in the aquarium until reaching stage 4 of anesthesia induction (Schoettger & Julin, 1967) with EOO or for 10 minutes in the case of the controls. This time was chosen for the controls because it corresponds to the highest induction time observed for EOO concentrations used in this study (Silva et al., 2012). Anesthesia was determined by loss of reflex activity and lack of reaction to strong external stimuli.

After the induction procedure, the fish were handled for biometric measurements, and exposed to air for 1 minute. Aerial exposure is a protocol previously described as able to induce stress in silver catfish (Barcellos et al., 2006; Cunha et al., 2010a, b). The animals were then transferred individually to anesthetic-free aquaria containing 450 mL of water or sampled immediately according to the protocol of experiments 1 and 2. Different fish were used for each experiment, and both experiments were conducted during daytime in low luminosity conditions. Methodologies of the experiments were approved by the Ethical and Animal Welfare Committee of the Federal University of Santa Maria (UFSM; Process no. 46/2010).

Experiment 1

Silver catfish from each treatment had their blood collected at 0, 1, 4, and 8 h after the procedures of anesthetic induction and handling. The unanesthetized animals were restrained manually for blood collection with damp cloth. The absence of anesthetic in this step is justified by the possible interference that other drug could promote in the results. Blood was collected (0.1 – 0.3 mL) using heparinized capillaries from caudal peduncles. Afterwards, the fish were euthanized by severing the spinal cord.

Capillaries were centrifuged (3000-g, 15 minutes) in a microhematocrit centrifuge, and plasma was transferred to 1.5 mL eppendorf tubes and stored at – 25 °C until cortisol measurement. Cortisol was measured in duplicates in the unextracted plasma samples by using commercially available EIA kits (EIAgenTM Cortisol, Adaltis Italy S.p.A). The specificity of the test was evaluated by comparing the parallelism between the standard curve and serial dilutions of the plasma samples in phosphate buffered saline (pH 7.4). The standard curve constructed with the human standards ran parallel to that obtained using serial dilutions of *R. quelen* plasma. In the linear regression test, high positive correlation (R^2 = 0.9818) was found between the curves. The inter- and intra-assay coefficients of variation ranged from 9 to 12 % and from 6 to 9 %, respectively. One sample of the total blood of each animal was used for glucose determination with a digital Accu-Check® Advantage II apparatus (Roche Diagnostics, Germany).

Experiment 2

Water samples (15 mL) were collected from recovery aquaria at 0, 4, and 8 h after handling, and stored under refrigeration until ion measurements were performed. Water for time 0 h was collected 10 minutes after fish transference. Such amount of time was allotted so that the animals could adapt to the new environment and, hence, water could be collected with minimal fish disturbance.

Chloride levels were determined according to Zall et al. (1956) and Na^+ and K^+ levels were measured by a flame spectrophotometer. Standard solutions for each ion were prepared in four different concentrations with analytical grade reagents dissolved in deionized water. Net ion fluxes were calculated according to Gonzalez et al. (1998):

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

where $[ion]_1$ and $[ion]_2$ represent ion concentrations (µmol) in water at the beginning and end of the flux period, respectively; V is the water volume in the recovery aquaria (in L), M stands for fish weight (kg), and t stands for duration of the flux period (h).

Statistical analysis

Data are shown as mean ± SEM. To verify the homogeneity of variances and normality, Levene and Kolmogorov-Smirnov tests were respectively applied to all the data. The results of the cortisol level were transformed into log form and analyzed by two-way ANOVA and the Tukey's test. Data on glucose level and net ion fluxes were analyzed by the Scheirer-Ray-Hare extension of the Kruskal–Wallis test and Dunn's test. Analysis was performed using software SigmaPlot ver. 11.0, and the minimum significance level was set at $p < 0.05$.

RESULTS

Experiments 1 and 2 were performed at a water temperature of 22.0 ± 0.27 °C and 16.2 ± 0.36 °C, and pH of 7.71 ± 0.09 and 7.56 ± 0.11, respectively. Dissolved oxygen concentrations were 7.92 ± 0.96 mg L^{-1}, and total ammonia was lower

than 0.6 mg N L^{-1}. Total hardness and alkalinity were 73.5 ± 4.5 mg $CaCO_3$ L^{-1} and 28.5 ± 1.5 mg $CaCO_3$ L^{-1}, respectively.

Silver catfish were anesthetized with 70 and 300 mg L^{-1} EOO in 531.03 ± 32.08 s and 116.74 ± 9.49 s, respectively. Anesthesia recovery occurred in less than 10 minutes for all the animals tested. No mortality was observed during the experiments.

Experiment 1

There were no significant differences in the cortisol levels of the fish anesthetized with 70 and 300 mg L^{-1} EOO in comparison to water and ethanol controls in all the evaluated times. Animals of ethanol control showed lower cortisol levels in 0 h than water control. For the experimental groups exposed to 70 mg L^{-1} EOO, ethanol and water controls, cortisol levels were also lower at 8 h than those detected at 0 h and 1 h. Only fish exposed to 300 mg L^{-1} EOO did not change their cortisol values statistically during the time after handling (Figure 1a).

Glucose peaks were observed 1 h after the procedure for all the experimental groups. The highest concentrations occurred in animals exposed to 300 mg L^{-1} EOO, followed by 70 mg L^{-1} EOO, ethanol and water control. Glucose rise at 1 h was followed by a reduction at 4 h and 8 h, except for fish exposed to 70 mg L^{-1} EOO at 4 h. At 8 h after handling fish from all treatments had lower glucose levels than those detected immediately after anesthesia (0 h) within each group (Figure 1b).

Juveniles exposed to 70 and 300 mg L^{-1} EOO had statistically higher glucose levels at 1 h and 4 h after handling than those of the water control. Fish from the ethanol group also showed increased glucose levels at 1 h when compared to water control fish, and this value did not differ from those obtained with 70 and 300 mg L^{-1} EOO. At 8 h after handling, juveniles anesthetized with 70 mg L^{-1} EOO still had higher glucose levels than the water control animals, while those treated with 300 mg L^{-1} EOO had lower values. Furthermore, significant lower glucose concentrations were detected in the fish treated with 300 mg L^{-1} EOO when compared to 70 mg L^{-1} EOO at 0 h, 4 h, and 8 h after handling (Figure 1b).

Experiment 2

Net Na^+ effluxes were observed in fish of water and ethanol controls in both evaluated periods, and this loss decreased significantly at 4 - 8 h after handling. However, net Na^+ influxes were observed in juveniles anesthetized with 70 and 300 mg L^{-1} EOO at 0 - 4 h and 4 - 8 h after handling (Figure 2a). Fish exposed to 300 mg L^{-1} EOO did not show differences for this ion between flux periods, while those treated with 70 mg L^{-1} EOO increased their net Na^+ influxes at 4 - 8 h.

There were net Cl^- and K^+ effluxes at 0 - 4 h and influxes at 4 - 8 h after handling in most treatments and these fluxes did not differ among the treatments within each period (Figures 2b and 2c). Exceptions to this pattern were observed in juveniles exposed to 70 mg L^{-1} EOO and ethanol control. Regardless of the period evaluated, the fish from the ethanol control showed net K^+ influx, while net Cl^- and K^+ losses occurred in the animals treated with 70 mg L^{-1} EOO. Statistical differences between 0 - 4 h and 4 - 8 h were observed for net Cl^- and K^+ fluxes in all treatments, except to 70 mg L^{-1} EOO in K^+ flux.

DISCUSSION

Higher induction times were observed for both EOO concentrations when compared to the previous report (Silva et al., 2012). The different induction times to anesthesia observed in both studies may have been influenced by biological factors such as age, sex, life stage, body weight, and physiological condition, as well as environmental factors such as water temperature, pH, and dissolved oxygen level (Zahl et al., 2012).

The increase in cortisol concentrations in plasma is recognized as the main hormonal response to stressors and is widely used as a stress response indicator (Barton & Iwama, 1991). In this study, no significant increases in cortisol levels could be detected at 1 h for all the experimental groups, which corresponds an opposite pattern to the one previously described for silver catfish after aerial exposure for 1 minute (Barcellos et al., 2006; Cunha et al., 2010a, b).

The hypotheses to explain the absence or loss of a cortisol response is the interrenal exhaustion, 'acclimatization' to stressor, environmental factors as deterioration in water quality, increase in the metabolic clearance rate of cortisol, and negative feedback of its release (Barcellos et al., 2006; Ellis et al., 2012). According to Barcellos et al. (2006), a period of chronic stress did not impair the capacity of the hypothalamic-pituitary-interrenal axis for this fish species to respond to further acute stressors. Thus, interrenal exhaustion cannot be deemed responsible for this lack of response. The acclimatization proposition also may not apply in this study because the animals were not submitted to the same stressor protocol during one week before the experiments. Water physicochemical parameters were within the adequate range for silver catfish production (Baldisserotto et al., 2010), which excludes the possibility that environmental factor are responsible for the results.

However, differences in the cortisol response

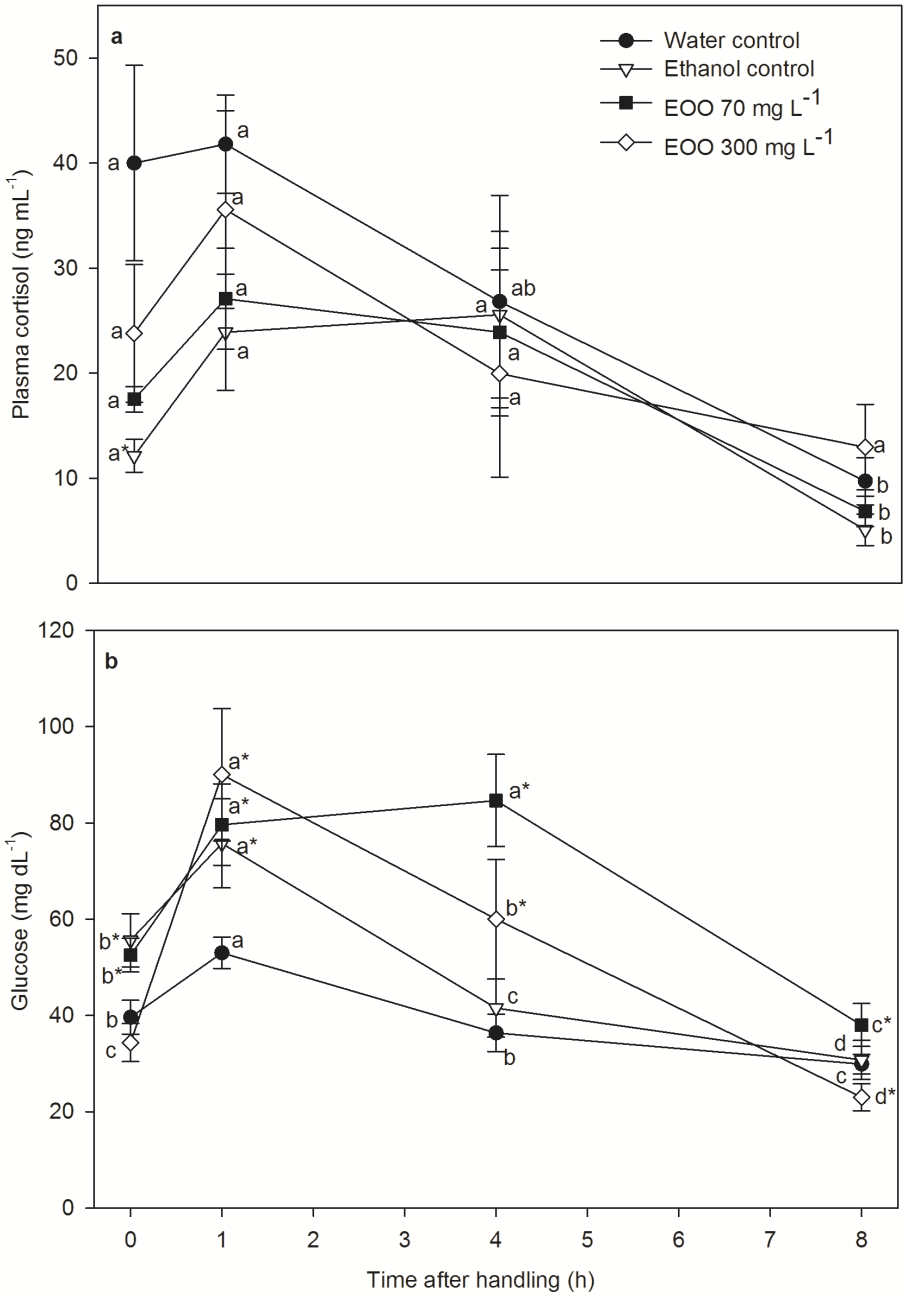

FIGURE 1. Effects of the essential oil of *Ocimum gratissimum* (EOO) on cortisol (a) and glucose (b) levels of silver catfish after handling. Data are expressed as the mean ± SEM (N = 8). Different letters indicate significant differences among the times after handling for each experimental group and * describes the significant differences among treatments and water control ($p < 0.05$).

as a result of fish characteristics cannot be discarded as a factor that can influence the results (Ellis et al., 2012; Koakoski et al., 2012). A recent report related the divergent time course of cortisol release in silver catfish of different ages. Fingerlings (60 days of age, approx. 2 g) and juveniles (180 days of age, approx. 10 g) attained peak cortisol concentrations within 5-30 minutes after active persecution with a pen net, whereas adults (360 days of age, approx. 440 g) experienced the correspondent peak 60 minutes after stressor exposure (Koakoski et al., 2012). Thus, the cortisol peak may not have been detected within 1 h, if the same release pattern occurred with the stressor-type used in this study.

Glucose peak was detected 1 h after handling for all experimental groups, but it had a lower magnitude than the one reported for this species after tank transference (Barcellos et al., 2001) or during the re-feeding period (Barcellos et al., 2010). Furthermore, the detected blood glucose

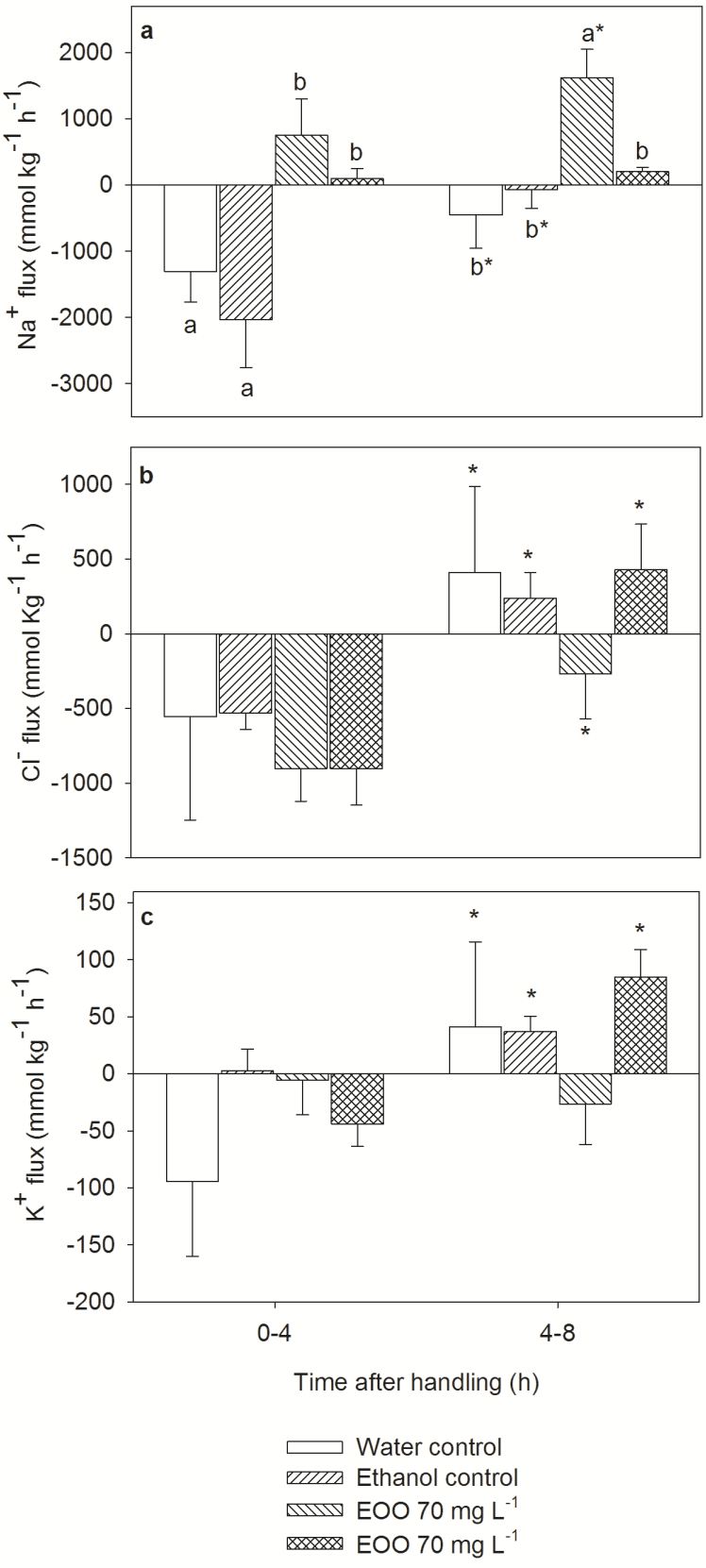

FIGURE 2. Net Na^+ (a), Cl^- (b) and K^+ (c) fluxes of silver catfish handling after the essential oil of *Ocimum gratissimum* (EOO) anesthesia. Data are expressed as the mean ± SEM (N = 8). Positive values indicate net influxes, and negative values indicate net effluxes. The different letters indicate the significant differences among treatments in the same flux period and * describes significant differences among the flux periods for the same treatment ($p < 0.05$).

concentrations were lower or similar than those described for silver catfish on the first day of fasting (Barcellos et al., 2010).

Glucose level is the most assessed indicator of secondary effects of stress, because the energy required for the fight or flight response in the face of an acute stressor agent is provided by hyperglycemia. A glucose rise is mediated initially by catecholamine release via liver glycogenolysis and is sustained by plasma cortisol increases if a stressful challenge persists (Maricchiolo & Genovese, 2011; Pankhurst, 2011).

The glucose rise along with the non-significant increase in cortisol levels verified in this study may have occurred due to the release of pre-synthesized pools of adrenergic and steroid hormones or their precursors. Hormone storage was suggested in studies with rainbow trout (*Oncorhynchus mykiss*), because it releases cortisol and adrenaline immediately after netting procedures and 30 s of air exposure (Gerwick et al., 1999). Furthermore, a hyperactivity period at the beginning of clove oil exposure, which also contains eugenol as the major compound, was described on Nile tilapia (*Oreochromis niloticus*) (Simões et al., 2011). As this pattern was also verified for the EOO tested (data not shown), pre-synthesized hormones could have been released during this time before the fish reached deep anesthesia, and may have produced the effects observed. This proposition corroborates the higher glucose levels detected in silver catfish anesthetized with 70 mg l^{-1} EOO, where the induction time to deep anesthesia was longer.

Stressor agents can also trigger changes in major blood ions (*e.g.,* Na^+, Cl^-, and K^+) and in their exchange between the internal and external fish medium (Dang et al., 2000; Barton, 2002; Pankhurst, 2011). Freshwater teleosts, as silver catfish, have hyperosmotic body fluids with respect to the surrounding water; thus, they show the same physiological mechanisms to compensate for the tendency towards water influx and to maintain adequate plasma ion levels (Hwang & Lee, 2007). These mechanisms are changed after a stressful event by the action of catecholamine and cortisol (Dang et al., 2000; Pankhurst, 2011). Catecholamine increases gill blood flow and lamellar recruitment to increase gas exchange (Sundin, 1999). This increase in the respiratory surface area to improve oxygen uptake would also increase ion loss by the gills. The stress of handling or confinement led to an increase of branchial ion efflux and high Na^+ loss in rainbow trout (McDonald et al., 1991) and net Na^+ efflux in silver catfish (Rosso et al., 2006), as observed in the present experiment with silver catfish in the treatments of water control and ethanol.

EOO anesthesia seems to prevent Na^+ efflux as verified in the control groups after handling. Other analyzed ion fluxes were not different among the experimental groups, only between different time periods after handling. Net Cl^- and K^+ fluxes had a similar range as those determined for silver catfish after tank transference (Rosso et al., 2006). Equivalent results were observed for tamoatas (*Hoplosternum littorale*) and pirarucus (*Arapaima gigas*) exposed to different Amazonian waters (Baldisserotto et al., 2008).

Regardless of EO exposition, the experimental groups showed the same pattern of Cl^- and K^+ flux after handling. As was the case with the results obtained in this study, a clear relationship between mechanisms regulating Cl^- and K^+ fluxes, and Na^+ fluxes was not observed in silver catfish by Rosso et al. (2006). Moreover, EOO anesthesia did not produce larger osmoregulatory changes in silver catfish until 8 h after handling, which characterizes a positive point for the use of this product as an anesthetic. Anesthetic procedure with eugenol in matrinxã (*Brycon amazonicus*) (Barbosa et al., 2007) and a clove powder solution in juvenile common carps (*Cyprinus carpio*) (Hoseini, 2011) also did not change plasma ion concentration after tank transference and crowding exposure, respectively.

Controversial results are reported in the literature about the stressor character of anesthetics and their influence on handling (Deriggi et al., 2006; Cunha et al., 2010a; Zahl et al., 2010). Anesthesia with eugenol, the major compound of EOO (Silva et al., 2012), did not reduce or promote any additional cortisol and plasma ions concentration responses in Nile tilapia (*Oreochromis niloticus*) after handling (Deriggi et al., 2006). In silver catfish, it prevented an increase of cortisol after handling and aerial exposure (Cunha et al., 2010a). Additionally, anesthesia with MS-222 and clove oil when associated with handling reduced the duration of stress response in blackspot sea bream (*Pagellus bogaraveo*) (Maricchiolo & Genovese, 2011).

Park et al. (2009) verified that in rock bream (*Oplegnathus fasciatus)* anesthetized with 100 mg l^{-1} of clove oil, plasma cortisol and glucose concentrations re-attained the levels detected before exposure only after two days. Silver catfish exposed to EOO before handling had a better recovery profile of these parameters, as lower or similar concentrations were detected at 8 h compared to 0 h. A similar pattern was verified in the cortisol levels in Atlantic salmon following bath administration with benzocaine, MS-222, and isoeugenol. After 360 minutes, all the fish exposed to such anesthetics had similar or lower concentrations than those detected at time zero (Kiessling et al., 2009).

In conclusion, exposure to EOO followed by handling enabled the promotion of a metabolic

response to stress in silver catfish, which was not accompanied by significant net ion fluxes and cortisol changes. The results suggest that EOO did not impair stress recovery of silver catfish and did not act as an additional handling stressor. However, more studies should be conducted to elucidate the pattern detected in fish exposed to EOO in the first hours after handling.

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REFERENCES

ALBUQUERQUE, U.P. et al. Medicinal plants of the caatinga (semi-arid) vegetation of NE Brazil: A quantitative approach. **Journal of Ethnopharmacology**, v.114, p.325-354, 2007.

ALBUQUERQUE, U.P.; ANDRADE, L.H.C. El género *Ocimum* L. (Lamiaceae) en el Nordeste del Brasil. **Anales Jardín Botánico del Madrid**, v.56, p.43-64, 1998.

BALDISSEROTTO, B. et al. 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**, v.34, p.405-412, 2008.

BALDISSEROTTO, B. et al. Jundiá (*Rhamdia* sp). In: BALDISSEROTTO, B.; GOMES, L.C. (Ed.) **Espécies nativas para piscicultura no Brasil.** Santa Maria: Editora da Universidade Federal de Santa Maria, 2010.

BARBOSA, L.G. et al. Metabolic responses of matrinxã to eugenol in anesthetic baths. **Acta Scientiarum: Biological Sciences**, v.29, p.255-260, 2007.

BARCELLOS, L.J.G. et al. Plasma levels of cortisol and glucose in response to capture and tank transference in *Rhamdia quelen* (Quoy & Gaimard), a South American catfish. **Aquaculture Research**, v.32, p.121-123, 2001.

BARCELLOS, L.J.G. et al. Previous chronic stress does not alter the cortisol response to an additional acute stressor in jundiá (*Rhamdia quelen*, Quoy & Gaimard) fingerlings. **Aquaculture**, v.253, p.317-321, 2006.

BARCELLOS, L.J.G. et al. The effects of fasting on cortisol, blood glucose and liver and muscle glycogen in adult jundiá *Rhamdia quelen*. **Aquaculture**, v.300, p.231-236, 2010.

BARTON, B.A.; IWAMA, G.K. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. **Annual Review of Fish Diseases**, v.1, p.3-26, 1991.

BARTON, B.A. Stress in Fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. **Integrative and Comparative Biology**, v.42, p.517-525, 2002.

BENOVIT, S.C. et al. Anesthesia and transport of Brazilian flounder, *Paralichthys orbignyanus*, with essential oils of *Aloysia gratissima* and *Ocimum gratissimum*. **Journal of the World Aquaculture Society**, v.43, p.896-900, 2012.

CUNHA, M.A. et al. Anesthesia of silver catfish with eugenol: time of induction, cortisol response and sensory analysis of fillet. **Ciência Rural**, v.40, p.2107-2114, 2010a.

CUNHA, M.A. et al. Essential oil of *Lippia alba*: a new anesthetic for silver catfish, *Rhamdia quelen*. **Aquaculture**, v.306, p.403- 406, 2010b.

DANG, Z. et al. Cortisol increases Na^+/K^+-ATPase density in plasma membranes of gill chloride cells in the freshwater tilapia *Oreochromis mossambicus*. **Journal of Experimental Biology**, v.203, p.2349-2355, 2000.

DERIGGI, F.G. et al. Stress responses to handling in Nile tilapia (*Oreochromis niloticus* Linnaeus): assessment of eugenol as an alternative anesthetic. **Acta Scientiarum: Biological Sciences**, v.28, p.269-274, 2006.

DI STASI, L.C. et al. Medicinal plants popularly used in the Brazilian Tropical Atlantic Forest. **Fitoterapia**, v.73, p.69-91, 2002.

ELLIS, T. et al. Cortisol and finfish welfare. **Fish Physiology and Biochemistry**, v.38, p.163-188, 2012.

European Pharmacopoeia. Strassbourg: European Directorate for the Quality of Medicines, 2007.

GERWICK, L. et al. Modulation of stress hormones in rainbow trout by means of anesthesia, sensory deprivation and receptor blockade. **Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology**, v.124, p.329-334, 1999.

GONZALEZ, R.J. et al. Effects of water pH and Ca^{+2} concentration on ion balance in fish of the Rio Negro, Amazon. **Physiological Zoology**, v.71, p.15-22, 1998.

HONTELA, A. Interrenal dysfunction in fish from contaminated sites: *in vivo* and *in vitro* assessment. **Environmental Toxicology and Chemistry**, v.17, p.44-48, 1998.

HOSEINI, S.M. Efficacy of clove powder solution on stress mitigation in juvenile common carps, *Cyprinus carpio* (Linnaeus). **Comparative Clinical Pathology**, v.20, p.359-362, 2011.

HWANG, P.P.; LEE, T.H. New insights into fish ion regulation and mitochondrion-rich cells. **Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology**, v.148, p.479-497, 2007.

KIESSLING, A. et al. Pharmacokinetics, plasma cortisol and effectiveness of benzocaine, MS-222 and isoeugenol measured in individual dorsal aorta-cannulated Atlantic salmon (*Salmo salar*) following bath administration. **Aquaculture**, v.286, p.301-308, 2009.

KOAKOSKI, G. et al. Divergent time course of cortisol response to stress in fish of different ages. **Physiology and Behavior**, v.106, p.129-132, 2012.

MARICCHIOLO, G.; GENOVESE, L. Some contributions

to knowledge of stress response in innovative species with particular focus on the use of the anaesthetics. **Open Marine Biology Journal**, v.5, p.24-33, 2011.

MCDONALD, D.G. et al. Gill design in freshwater fishes: interrelationships among gas exchange, ion regulation, and acid–base regulation. **Physiological Zoology**, v.64, p.103-123, 1991.

PANKHURST, N.W. The endocrinology of stress in fish: An environmental perspective. **General and Comparative Endocrinology**, v.170, p.265-275, 2011.

PARK, M.O. et al. Efficacy and physiological responses of rock bream, *Oplegnathus fasciatus* to anesthetization with clove oil. **Aquaculture**, v. 287, p.427-430, 2009.

ROSSO, F.L. et al. Ion fluxes in silver catfish (*Rhamdia quelen*) juveniles exposed to different dissolved oxygen levels. **Neotropical Ichthyology**, v.4, p.435-440, 2006.

SAKURAGUI M.M. et al. Gill chloride cell proliferation and respiratory responses to hypoxia of the neotropical erythrinid fish *Hoplias malabaricus*. **Journal of Comparative Physiology B**, v.173, p.309-317, 2003.

SCHOETTGER, R.; JULIN, M. Efficacy of MS-222 as an anesthetic on four salmonids. **Fish Control U.S. Department International**, v.13, p.1-15, 1967.

SILVA, L.L. et al. Essential oil of *Ocimum gratissimum* L.: anesthetic effect, mechanism of action and tolerance in silver catfish, *Rhamdia quelen*. **Aquaculture**, v.350-353, p.91-97, 2012.

SIMÕES, L.N. et al. Efficacy of clove oil as anesthetic in handling and transportation of Nile tilapia, *Oreochromis niloticus* (Actinopterygii: Cichlidae) juveniles. **Zoologia**, v.28, p.285-290, 2011.

SUNDIN, L. Hypoxia and blood flow control in fish gills. In: VAL, A.L.; ALMEIDA-VAL, V.M.F. (Ed.). **Biology of Tropical Fishes**. Manaus: INPA, 1999.

ZAHL, I.H. et al. Anaesthesia of farmed fish: implications for welfare. **Fish Physiology and Biochemistry**, v.38, p.201-218, 2012.

ZAHL, I.H. et al (2010) Anesthesia induces stress in Atlantic salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*) and Atlantic halibut (*Hippoglossus hippoglossus*). **Fish Physiology and Biochemistry**, v.36, p.719-30, 2010.