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# Anesthetic activity of the essential oil of *Ocimum americanum* in *Rhamdia quelen* (Quoy & Gaimard, 1824) and its effects on stress parameters

Lenise de Lima Silva[1,2], Quelen Iane Garlet[1], Gessi Koakoski[1], Murilo Sander de Abreu[1], Carlos Augusto Mallmann[3], Bernardo Baldisserotto[4], Leonardo José Gil Barcellos[5] and Berta Maria Heinzmann[6]

The aim of this study was to evaluate the anesthetic activity of the essential oil (EO) of *Ocimum americanum* L. in silver catfish (*Rhamdia quelen*). In the first experiment, the depressor effects and chemical composition of the leaf EO (LEO) and inflorescence EO (IEO) were compared. Juveniles (n = 10) were placed in aquaria containing different concentrations of EO (25 - 500 mg $L^{-1}$) to determine the point at which anesthesia was induced and the length of the recovery period. In the following experiment, the effects of 300 and 500 mg $L^{-1}$ LEO exposure on stress parameters (plasma cortisol, glucose and sodium levels) after air exposure for 1 min were assayed. Fish (n = 10 per sampling time) were sampled immediately or transferred to anesthetic-free aquaria until sampling (15, 30, 60 or 240 min). LEO was composed mainly of β-linalool and 1,8-cineole in similar proportions, whereas IEO showed β-linalool as major compound. Anesthesia was obtained in silver catfish with 200-500 mg $L^{-1}$ between 4-8 min for LEO and 6-16 min for IEO. Lower EO concentrations did not reach anesthetic stage up to 30 min. LEO used as anesthetic prevented the cortisol increase and sodium loss induced by aerial exposure. Glucose levels were raised in catfish exposed to LEO compared to basal group (not air exposed) in almost all observation times. EO of *O. americanum* obtained from leaves was considered suitable to anesthetic procedures due to its fast induction and handling-induced stress prevention.

O objetivo deste trabalho foi avaliar a atividade anestésica do óleo essencial (EO) de *Ocimum americanum* L. em jundiás (*Rhamdia quelen*). No primeiro experimento, os efeitos depressores e a composição química dos óleos obtidos a partir das folhas (LEO) e das inflorescências (IEO) desta espécie vegetal foram comparados. Para isto, juvenis de jundiás (n = 10) foram transferidos para aquários contendo diferentes concentrações de EO (25 - 500 mg $L^{-1}$) a fim de determinar os tempos de indução aos estágios de anestesia e de recuperação. No experimento seguinte foram determinados os efeitos da exposição a 300 e 500 mg $L^{-1}$ de LEO em parâmetros de estresse (cortisol, glicose e níveis de sódio plasmáticos) após os animais serem expostos durante 1 min ao ar. Os peixes (n = 10 por tempo de amostragem) foram coletados imediatamente ou transferidos para aquários livres de anestésico até amostragem (15, 30, 60 ou 240 min). LEO demonstrou ser composto majoritariamente por β-linalol e 1,8-cineol em proporções similares, enquanto que IEO apresentou β-linalol como constituinte majoritário. O estágio de anestesia foi verificado em jundiás com 200-500 mg $L^{-1}$ entre 4-8 min para LEO e 6-16 min para IEO. Menores concentrações de ambos os EO não promoveram anesthesia até 30 min. O uso de LEO como anestésico preveniu o aumento de cortisol e a perda de sódio induzida pela exposição aérea. Já a glicemia encontrava-se aumentada em peixes expostos ao LEO em comparação ao grupo basal (não exposto ao ar) na maioria dos tempos de observação. Assim, EO de *O. americanum* obtido a partir das folhas foi considerado adequado para procedimentos anestésicos devido a sua rápida indução e capacidade de prevenir o estresse decorrente do manuseio.

**Keywords**: Anesthesia, Basil, Cortisol, Glucose, Sodium.

[1]Programa de Pós-Graduação em Farmacologia, Universidade Federal de Santa Maria - UFSM, Santa Maria, RS, Brazil. (LLS) ls.lenise@gmail.com, (QIG) quelenig@hotmail.com, (GK) gessikoakoski@yahoo.com.br, (MSA) murilosanderabreu@gmail.com
[2]Curso de Farmácia, Universidade Regional Integrada do Alto Uruguai e das Missões, Santiago, RS, Brazil.
[3]Departamento de Medicina Veterinária Preventiva, UFSM, Santa Maria, RS, Brazil. mallmann@lamic.ufsm.br
[4]Departamento de Fisiologia e Farmacologia, UFSM, Santa Maria, RS, Brazil. bbaldisserotto@hotmail.com
[5]Universidade de Passo Fundo, *Campus* Universitário do Bairro São José, Passo Fundo, RS, Brazil. lbarcellos@upf.br
[6]Departamento de Farmácia Industrial, Universidade Federal de Santa Maria, Avenida Roraima, 1000, Prédio 26, 97105-900 Santa Maria, RS, Brazil. berta.heinzmann@gmail.com (corresponding author)

## Introduction

Anesthetic and sedative drugs are used in aquaculture to improve fish welfare, minimize movement, handling trauma and pain, and attenuate the physiological response to stress (Neiffer & Stamper, 2009; Zahl *et al*., 2012). Some essential oil (EO) and their constituents have shown promising results in this context due their effectiveness, low incidence of side effects and ability to mitigate stress effects (Wagner *et al*., 2003; Cunha *et al*., 2010a, 2010b; Silva *et al*., 2012; Benovit *et al*., 2012; Gressler *et al*., 2014; Heldwein *et al*., 2014). Stress response in fish develops initially from the activation of neuroendocrine pathways, which promote the release of catecholamines and corticosteroids (cortisol). These hormones lead to physiological and behavioral changes that allow response/adaptation to the stressor, but lead to adverse whole-animal effects if extreme or sustained (Ellis *et al*., 2012; Zahl *et al*., 2012).

The plants of the *Ocimum* genus (Lamiaceae), collectively called basil, are considered good sources of EO used in food flavoring, oral products, fragrances and traditional medicines (Vieira & Simon, 2006). Recently, anesthetic and sedative effects of the EO of *Ocimum gratissimum* L. in fish and its potential application in aquaculture have been reported (Benovit *et al*., 2012; Silva *et al*., 2012). One important species of this genus due to its high EO content is *Ocimum americanum* L. (synonymy *O. canum* Sims), known as hairy basil (Silva *et al*., 2003; Chanwitheesuk *et al*., 2005). Preparations obtained from its aerial parts are widely used in folk medicine in the treatment of insomnia and anxiety (Hassane *et al*., 2011). However, until this moment, only the antimicrobial and antioxidant activities have been confirmed *in vitro* for its EO (Hassane *et al*., 2011; Nascimento *et al*., 2011; Selvi *et al*., 2015).

*Rhamdia quelen* is a nocturnal bagrid of the Heptapteridae commonly found in South American rivers. It has been considered a good alternative for fish production due to its fast growth rate in warmer months (Gomes *et al*., 2000; Barcellos *et al*., 2001, 2012). However, this species 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, 2010b), which makes the use of anesthetics and sedatives with attenuation of endocrine secretion advantageous for its production.

Thus, the aim of this study was to evaluate the anesthetic activity of the EO of *Ocimum americanum* L. in *R. quelen*. In the first moment, the depressor effects and chemical composition of the leaf EO (LEO) and inflorescence EO (IEO) were compared. Following, the effects of LEO exposure on stress parameters after handling were assayed.

## Material and Methods

**Plant material.** Aerial parts of *O. americanum* were collected in December 2011 in Encantado (RS, Brazil). Voucher specimen (no. SMDB 13163) identified by Dr. Sérgio Augusto de Loreto Bordignon was deposited in the Herbarium of the Department of Biology, UFSM.

**Essential oil extraction and analysis.** Leaves and inflorescences were separately hydrodistillated for 3 h in a Clevenger-type apparatus (Council of Europe (COE), 2007). Extractive yield was determined as % (w/w). EO samples were stored at -4ºC in amber glass bottles until analysis by gas chromatograph coupled to mass spectrometer (GC-MS) and biological tests.

GC-MS TIC analysis was performed using an Agilent-6890 gas chromatograph coupled with an Agilent 5973 mass selective detector using an HP5-MS column (5% phenyl, 95% methylsiloxane, 30 m x 0.25 mm i. d. x 0.25 μm) and EI-MS of 70 eV according to operational conditions described by Silva *et al*. (2012). The constituents were identified by comparison of the Kovats retention index and mass spectra with a mass spectral library (NIST, 2005) and with literature data (Adams, 2001).

**Animals.** Four month-old juvenile *R. quelen* were maintained in continuously aerated 250 L (experiment 1) and 95 L (experiment 2) tanks with controlled water parameters at loading density of 3.5 and 4.5 g $L^{-1}$, respectively. The dissolved oxygen levels and temperature were measured with an YSI oxygen meter (YSI Inc., Yellow Springs, OH, USA). The pH was determined with pH meters DMPH-2 (Digimed, São Paulo, SP, Brazil) and Solar SL110 (Solar, Florianópolis, SC, Brazil). Total ammonia levels were measured by the salicylate method (Verdouw *et al*., 1978). A semi-static system was used with independent tanks, where 50% of the water volume was changed daily. Fish were fed once a day with commercial feed (28% crude protein). Juveniles were fasted for 24 h prior to the experiments. The methodologies of the experiments were approved by the Ethical and Animal Welfare Committee of the Universidade Federal de Santa Maria (Process no. 46/2010).

**Experiment 1. Anesthesia induction and recovery.** Sedative and anesthetic effects of LEO and IEO were evaluated in *R. quelen* at concentrations of 25, 50, 100, 200, 300 and 500 mg $L^{-1}$. These concentrations were corrected by the density of the EO (LEO: 0.9273 g $mL^{-1}$; IEO: 0.8884 g $mL^{-1}$) and diluted in 95% ethanol (1:10) before bath administration. Control experiments were performed using aquaria containing ethanol at the highest concentration used to dilute the EO.

Juveniles of *R. quelen* (8.09 ± 0.22 g; 9.89 ± 0.11 cm) were transferred to 1 L aquaria containing the EO concentration to be tested. Ten fish were evaluated in each concentration, and each juvenile was used only once to the observation of deep sedation (S2), partial and total loss of equilibrium (S3a and S3b, respectively) and/or anesthesia (S4) (Schoettger & Julin, 1967). The animals remained in anesthetic bath until reaching S4 or for 30 min. The anesthesia was determined

by loss of reflex activity and no reaction to strong external stimuli. The anesthetic solution was changed for each animal. After the induction of anesthesia, each fish was measured, weighed, and transferred to an anesthetic-free aquarium to recover. The fish were considered to have recovered if their normal posture and behavior were restored. Following, the animals were transferred to 30 L tanks to evaluate possible side effects or mortality after 24 h of exposure.

**Experiment 2. Evaluation of stress parameters.** Fish (21.80 ± 1.00 g; 13.18 ± 0.19 cm) were subjected to one of the following treatments: water control, 300 or 500 mg $L^{-1}$ of LEO. Ten fish were used by treatment in each collection time (0, 15, 30, 60 or 240 min), and each animal was used only once. To prevent cumulative stress of the repeated captures, the fish used in each collection time were maintained in tanks of 96 L for one week before experiment without additional handling and simultaneously captured.

Experimental protocol consisted in fish capture with a hand net and their transference to continuously aerated aquaria containing only 1 L of water (water control) or the samples to be tested. The time between capture and release did not exceed 30 s. Juveniles stayed in the aquaria until reached stage 4 of anesthesia induction (Schoettger & Julin, 1967) with LEO, or for 8 min in case of the water control. This time was chosen for the controls because it corresponds to the highest induction time observed for the LEO concentrations in the experiment 1.

After these procedures, the fish were exposed to air for 1 min. Aerial exposure is a protocol previously described as able to induce stress in *R. quelen* (Cunha *et al*., 2010a, 2010b; Barcellos *et al*., 2006). Basal group was carried out with unmanipulated fish. Animals were sampled immediately or transferred to anesthetic-free aquaria containing 50 L of water until sampling. 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 min) in a microhematocrit centrifuge, and plasma was transferred to 1.5 mL eppendorf tubes and stored at −25°C until analysis. Cortisol was measured in unextracted plasma samples, using commercially available EIA kits (EIAgenTM Cortisol, Adaltis Italy S.p.A), previously validated for the species (Barcellos *et al*., 2006). Glucose was analyzed by a colorimetric test based on the oxidase/peroxidase reaction (Glicose – PP, Gold Analisa Diagnostica Ltda, Brasil). Plasma $Na^+$ concentrations were measured in appropriate diluted samples against four standard solutions of NaCl using flame photometry (Micronal B262). All measures were performed in duplicate.

**Statistical analysis.** Data are presented as median and interquartile range (Q1–Q3) or mean ± SEM. To verify the homogeneity of variances and normality, all data were submitted to Levene and Kolmogorov-Smirnov tests, respectively. Extractive yields and water parameters between experiments were compared using Student's t-test. The results of time of induction and recovery and stress parameters were analyzed by Scheirer-Ray-Hare extension of the Kruskal–Wallis followed by the Dunn test or two-way ANOVA and Tukey test. Minimum significance level was set at $P < 0.05$.

## Results

**Essential oil analysis.** Extractive yields of LEO (0.67 ± 0.02%) and IEO (0.62 ± 0.02%) did not differ statistically. Qualitative differences in the chemical composition of the EO obtained from different parts of the plant were detected to some minor compounds (0.02-0.51%). In relation to the major compounds, lower content of β-linalool was observed in LEO (20.18%) when compared to IEO (46.61%). LEO contained 1,8-cineole, eugenol and camphor in higher proportions than IEO (Table 1).

**Table 1.** Chemical composition of the essential oils of *Ocimum americanum* obtained from leaves (LEO) and inflorescences (IEO). Rt: Retention time; (%): Relative percentage; RI cal: calculated retention index; RI ref: reference retention index. [a] NIST (2005); [b] Adams (2001). Bold type indicates major components.

| Rt (min) | Constituent | LEO (%) | IEO (%) | RI cal | RI ref |
|---|---|---|---|---|---|
| 9.23 | tricyclene | 0.02 | 0.02 | 919.8 | 920[a] |
| 9.51 | α-thujene | 0.04 | 0.02 | 926.7 | 925[a] |
| 9.72 | α-pinene | 1.03 | 0.55 | 931.8 | 933[a] |
| 10.28 | camphene | 0.76 | 0.54 | 945.1 | 946[a] |
| 11.37 | sabinene | 0.88 | 0.25 | 971.6 | 975 [a,b] |
| 11.44 | β-pinene | 1.53 | 0.58 | 973.2 | 976[a] |
| 11.78 | 1-octen-3-ol | 0.06 | 0.03 | 981.5 | 980[a] |
| 12.19 | β-myrcene | 1.29 | 0.34 | 991.3 | 992[a] |
| 12.61 | α-phellandrene | 0.08 | 0.07 | 1001.5 | 1003[a,b] |
| 13.13 | α-terpinene | 0.12 | 0.08 | 1014.3 | 1017[a,b] |
| 13.46 | *o*-cymene | | 0.02 | 1022.5 | 1022[a] |
| **13.83** | **1,8-cineole** | **21.00** | **8.43** | **1031.4** | **1031[a]** |

| Rt (min) | Constituent | LEO (%) | IEO (%) | RI cal | RI ref |
|---|---|---|---|---|---|
| 14.13 | *Z*-β-ocimene | 0.03 | 0.03 | 1038.8 | 1038[b] |
| 14.31 | benzene acetaldehyde | 0.02 | 0.02 | 1043.1 | 1045[a] |
| 14.53 | *E*-β-ocimene | 0.29 | 0.39 | 1048.5 | 1050[a,b] |
| 14.88 | τ-terpinene | 0.26 | 0.16 | 1057.2 | 1056[a] |
| 15.22 | bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1α,2α,5α)- | 0.34 | 0.12 | 1065.5 | 1065[a] |
| 15.53 | 1-octanol | 0.17 | | 1073.1 | 1070[a] |
| 16.04 | fenchone | 1.41 | 3.59 | 1085.7 | 1087[a,b] |
| **16.84** | **β-linalool** | **20.18** | **46.61** | **1105.3** | **1101[a]** |
| 17.22 | exo-fenchol | | 0.11 | 1115.2 | 1115[a] |
| 17.46 | bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1α,2β,5α)- | 0.05 | 0.08 | 1121.2 | 1121[a] |
| 17.66 | α-campholenal | | 0.05 | 1126.3 | 1127[a] |
| **18.38** | **camphor** | **11.96** | **9.50** | **1144.8** | **1144[a]** |
| 18.45 | *Z*-β-terpineol | 0.09 | 0.08 | 1146.5 | 1144[a] |
| 19.13 | isoborneol | 0.08 | 0.11 | 1164.0 | 1162[a,b] |
| 19.21 | δ-terpineol | 0.49 | 0.20 | 1165.9 | 1166[b] |
| 19.59 | 1-terpinen-4-ol | 0.74 | 0.41 | 1175.7 | 1177[a] |
| 20.16 | α-terpineol | 3.12 | 1.79 | 1190.4 | 1190[a] |
| 20.35 | myrtenol | 0.06 | | 1195.2 | 1196[a] |
| 21.00 | acetic acid, octyl ester | 0.17 | | 1212.6 | 1210[a] |
| 21.24 | fenchyl acetate | | 0.07 | 1219.0 | 1223[a] |
| 22.57 | chavicol | 0.09 | | 1255.2 | 1250[b] |
| 23.67 | bornyl acetate | 0.21 | 0.28 | 1285.2 | 1284[a] |
| 25.11 | myrtenyl acetate | 0.10 | 0.03 | 1325.7 | 1327[a,b] |
| 25.49 | δ-elemene | | 0.04 | 1336.8 | 1338[b] |
| 25.91 | α-cubebene | | 0.05 | 1349.1 | 1351[b] |
| **26.38** | **eugenol** | **17.17** | **3.22** | **1362.5** | **1364[a]** |
| 26.84 | α-copaene | 0.24 | 0.21 | 1375.8 | 1376[a,b] |
| 27.14 | β-bourbonene | 0.28 | 0.45 | 1384.6 | 1384[a] |
| 27.40 | β-elemene | 0.60 | 2.08 | 1391.8 | 1391[a] |
| 28.30 | β-caryophyllene | 1.46 | 3.27 | 1418.9 | 1418[a] |
| 28.62 | β-copaene | 0.08 | 0.10 | 1428.5 | 1432[b] |
| 28.85 | α-bergamotene | 2.06 | | 1435.7 | 1436[a] |
| 28.94 | α-guaiene | 0.11 | 0.88 | 1438.3 | 1439[a] |
| 29.19 | *Z*-muurola-3,5-diene | 0.14 | 0.19 | 1445.9 | 1450[b] |
| 29.42 | α-caryophyllene | 0.46 | 1.18 | 1452.9 | 1452[a] |
| 29.54 | β-farnesene | 0.08 | | 1456.3 | 1456[a] |
| 29.74 | *Z*-muurola-4(14), 5-diene | | 0.35 | 1462.6 | 1467[b] |
| 30.35 | germacrene D | 3.49 | 4.76 | 1481.2 | 1480[a] |
| 30.51 | valencene | | 0.18 | 1485.8 | 1485[a] |
| 30.83 | τ-elemene | 0.53 | 0.96 | 1495.6 | 1492[a] |
| 31.10 | germacrene A | 0.51 | | 1504.2 | 1509[b] |
| 31.14 | δ-guaiene | | 1.72 | 1505.3 | 1505[a] |
| 31.39 | τ-cadinene | 0.97 | 1.06 | 1513.6 | 1513[a] |
| 31.68 | δ-cadinene | | 0.21 | 1523.0 | 1523[a,b] |
| 32.92 | *E*-nerolidol | | 0.12 | 1563.7 | 1564[a] |
| 33.53 | caryophyllene oxide | | 0.14 | 1583.8 | 1583[a] |
| 34.50 | 1,10-di-epi-cubenol | 0.49 | 0.47 | 1616.0 | 1619[b] |
| 35.29 | τ-cadinol | 3.36 | 3.09 | 1643.1 | 1642[a] |
| 35.55 | β-eudesmol | 0.12 | 0.26 | 1652.0 | 1651[a] |
| 35.67 | α-cadinol | 0.22 | 0.28 | 1656.0 | 1657[a] |
| Total identified (%) | | 99.16 | 99.83 | | |

**Water parameters.** Experiments 1 and 2 were performed at similar conditions of dissolved oxygen (7.31 ± 0.22 mg $L^{-1}$) and total ammonia levels (0.26 ± 0.16 mg $L^{-1}$). Water temperature (experiment 1: 18.53 ± 0.14 ºC; experiment 2: 24.57 ± 0.41 ºC) and pH (experiment 1: 6.33 ± 0.07; experiment 2: 7.00 ± 0.09) were statistically different between experiments.

**Experiment 1. Anesthesia induction and recovery.** Sedative and anesthetic effects were verified in fish exposed to both EO of *O. americanum*. Concentrations of 25-100 mg $L^{-1}$ were not able to induce anesthesia during 30 min of exposure, whereas both samples of EO at 200-500 mg $L^{-1}$ led to this depression level in all animals exposed (Table 2). A positive relationship was detected to stages 3b and 4 in both samples, where an increment of EO concentration caused a reduction in the time required for anesthesia induction. LEO (4-8 min) anesthetized (stage 4) *R. quelen* in significantly less time than IEO (6-16 min) at the same concentration. Ethanol alone did not produce any sedative and anesthetic effects.

The EO samples tested did not show differences in the recovery time. Fish exposed to 25 - 200 mg $L^{-1}$ of both EO recovered quickly (within 1-6 min) without significant differences between concentrations. Larger times of recovery (within 11-14 min) were observed to the highest concentrations tested (300 and 500 mg $L^{-1}$). No significant relationship was found between EO concentration and recovery time from anesthesia.

**Experiment 2. Evaluation of stress parameters.** In this experiment, anesthesia was reached with 300 and 500 mg $L^{-1}$ of LEO in 274.84 ± 5.79 s (4.5 min) and 197.26 ± 9.63 s (3 min), respectively. Lower cortisol levels were verified in fish exposed to 300 and 500 mg $L^{-1}$ of LEO in comparison to the control group up to 15 min after handling. Animals anesthetized previously with LEO did not change their cortisol concentrations during the evaluation time, and were able to maintain similar values to those of the basal group. The same did not occur with fish of the control group, which showed a rise of cortisol in 15 min followed by a decrease to levels below the basal group at 240 min (Fig. 1A).

Blood glucose levels increased in all experimental groups in relation to the basal group for almost all observation times. Glycemia only returned to baseline levels at 240 min for the control group and 300 mg $L^{-1}$ of LEO. There were no differences in the blood glucose contents between LEO and control groups, as well as during the evaluation time to animals of the control group or exposed to 500 mg $L^{-1}$ of LEO (Fig. 1B).

A significant decrease in plasma $Na^+$ levels occurred in the control group 30 min after handling compared to the basal group and previous observation times. At this time, fish anesthetized with LEO showed similar plasma $Na^+$ levels to the basal group. Differences in the ionic concentration were only verified between LEO concentrations immediately after handling (data statistic not shown) and during the observation time for fish exposed to 300 mg $L^{-1}$ LEO (Fig. 1C).

**Table 2.** Time required for induction and recovery from anesthesia using the EO of *Ocimum americanum* obtained from leaves (LEO) and inflorescences (IEO) in juvenile *R. quelen*. Stages are defined according to Schoettger & Julin (1967). Maximum observation time was 30 min. Time to reach each stage is given in seconds (s). n=10 for each concentration tested. Data are presented as the median and interquartile range (Q1–Q3). Different letters in the columns indicate significant difference between concentrations of the same sample, while * represents significant difference between samples at the same concentration based on Scheirer-Ray-Hare extension of the Kruskal–Wallis followed by the Dunn test ($P < 0.05$). In the equations, x= concentration of essential oil (mg/L); y = time to reach the stage of induction or recovery from anesthesia in seconds (s).

| Sample | Concentration (mg/L) | Induction time (s) | | | | Time to recovery (s) |
|---|---|---|---|---|---|---|
| | | Stage 2 | Stage 3a | Stage 3b | Stage 4 | |
| LEO | 25 | 392 (367-430)$^{a}$ | | | | 100 (43-110)$^{b}$ |
| | 50 | 146 (120-157)$^{ab}$ | 229 (195-266)$^{a}$ | | | 290 (230-344)$^{bc}$ |
| | 100 | 66 (56-82)$^{abc}$ | 88 (77-96)$^{ab}$ | 1120 (824.7-1529.2) | | 290 (265-332)$^{bc}$ |
| | 200 | 15 (9-19)$^{bcd}$ | 63 (46-68)$^{bc}$ | 349.5 (323-394) | 501.5 (472-584)* | 401 (322-448)$^{ac}$ |
| | 300 | 7 (6-11)$^{cd}$ | 22.5 (17-26)$^{cd}$ | 160.5 (116-194) | 362 (316-431)* | 730 (600-900)$^{a}$ |
| | 500 | 5 (4-5)$^{d}$ | 10 (9-17)$^{d}$ | 68 (63-90) | 240 (179-308)* | 813 (622-952)$^{a}$ |
| | Equation | - | - | $y = 45.061+7992.389 \exp(-0.5(\ln(x/9.089)/1.209)^2)$ ($r^2 = 0.999$) | $\ln y = 1.311+26.188/\ln(x)$ ($r^2 = 0.999$) | - |
| IEO | 25 | 157 (123-186)$^{a}$ | | | | 100 (58-116)$^{b}$ |
| | 50 | 117 (100-132)$^{ab}$ | 246 (194-282)$^{a}$ | | | 81 (61-123)$^{b}$ |
| | 100 | 77 (25-128)$^{abc}$ | 127 (91-142)$^{ab}$ | 773 (710-810) | | 392 (324-440)$^{ab}$ |
| | 200 | 49 (19-56)$^{bcd}$ | 70 (65-79)$^{b}$ | 448 (410-515) | 970 (696-1050) | 395 (325-502)$^{ab}$ |
| | 300 | 10.5 (8-19)$^{cd}$ | 41 (37-54)$^{bc}$ | 249 (230-270) | 487 (468-492) | 705 (619-1294)$^{a}$ |
| | 500 | 5 (4-10)$^{d}$ | 25 (24-30)$^{c}$ | 142 (110-160) | 413 (325-420) | 891 (679-989)$^{a}$ |
| | Equation | - | - | $y = 82.436 + 697.608 \exp(-0.5(\ln(x/84.545)/0.776)^2)$ ($r^2 = 0.999$) | $y = 2462.8 - 10.281x + 0.012x^2$ ($r^2 = 1$) | - |

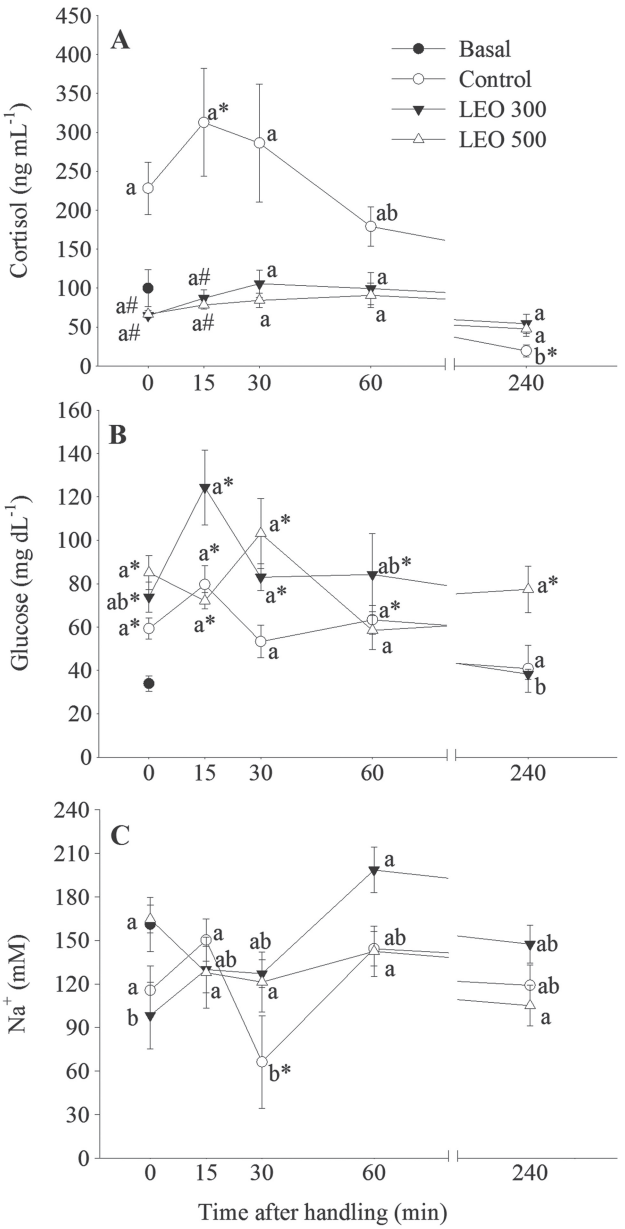


**Fig. 1.** Effects of the essential oil of *Ocimum americanum* (LEO) on cortisol (A), glucose (B) and $Na^+$ (C) levels of *R. quelen* after handling. Data are presented as the mean ± SEM. Lowercase letters indicate significant differences between times after handling within same experimental group, # represents statistical differences in comparison to water control at the same time after handling, and * corresponds to differences in relation to basal level. Scheirer-Ray-Hare extension of the Kruskal–Wallis test followed by the Dunn test or two-way ANOVA and Tukey test were used ($P < 0.05$).

## Discussion

Intermediary extractive yield was detected to EO samples of *O. americanum* in comparison to previous reports (Lawrence, 1989; Silva *et al*., 2003; Ngassoum *et al*., 2004; Vieira & Simon, 2006; Hassane *et al*., 2011; Nascimento *et al*., 2011). Their chemical compositions were also distinct from that reported for the same species growing in Brazil, where only *E*-methyl cinnamate and methylchavicol chemotypes were detected (Silva *et al*., 2003; Vieira & Simon, 2006; Nascimento *et al*., 2011). Furthermore, the presence of similar contents of 1,8-cineole and β-linalool in the LEO composition did not permit its classification in any other chemotype reported to this plant (Lawrence, 1989; Ngassoum *et al*., 2004; Hassane *et al*., 2011). Thus, the present study detected a distinct chemical profile for the EO samples of *O. americanum*.

The differences in the anesthetic effect of the EO samples of *O. americanum* can be related to their distinct chemical compositions. In this context, the high contents of β-linalool and eugenol in the LEO, the most active sample, must be highlighted. The results suggest that both compounds, which had their anesthetic effect in the same fish species recently reported (Cunha *et al*., 2010a; Heldwein *et al*., 2014), may act in synergistic form. Regarding the other constituents, only 1,8-cineole had its anesthetic activity evaluated, and did not show any depressor action up to 17 mg $L^{-1}$ for the same fish species (Heldwein, 2011).

In relation to induction time, other eugenol rich oils promoted fast anesthesia (about 4 min) at lower concentrations than LEO. For example, concentrations range of 100-150 mg $L^{-1}$ of EO of *O. gratissimum* are required to anesthetize Brazilian flounder (*Paralichthys orbignyanus*) at similar temperature (Benovit *et al*., 2012). On the other hand, β-linalool and the EO obtained from *Ocotea acutifolia*, *Lippia sidoides* or *Hyptis mutabilis* required higher induction times and/or concentrations to promote fast anesthesia in the same fish species at comparable water conditions (Heldwein *et al*., 2014; Silva *et al*., 2013a, 2013b). A similar concentration and anesthetic induction time from LEO was only described to EO of *Hesperozygis ringens* in *R. quelen* (Silva *et al*., 2013a). A possible explanation to the detected differences in induction time is the interaction between constituents of different samples and fish organism. According to Wagner & Ulrich-Merzenich (2009), the compounds of one extract or essential oil may act synergistically through change of pharmacokinetic or physicochemical parameters, *i. e.,* improved solubility, resorption rate and bioavailability.

A clear relationship between the concentration of EO of *O. americanum* and recovery time was not observed in *R. quelen*, which seems to be similar to that described to other types of EO (Cunha *et al*., 2010b; Silva *et al*., 2012, 2013a). However, fish exposed to 300 mg $L^{-1}$ of EO of *O. americanum* had an intermediary recovery time (about 12 min) when compared to those anesthetized at the same concentration of EO of *L. alba* (about 6 min) and EO of *O. gratissimum* (about 20 min) (Cunha *et al*., 2010b; Silva *et al*., 2012). These differences between samples may be resultant of distinct accumulation levels in the fish tissues or experimental conditions, and other studies should be performed to evaluate this question.

The experimental protocol used to induce a stress event was considered adequate despite the high cortisol levels detected in basal group. The cortisol peak in the control group occurred at the same time course and with similar magnitude to that described to juveniles of *R. quelen* after persecution with a pen net for 1 min (Barcellos *et al*., 2012). These results corroborate with those described by Barcellos *et al.* (2006), which verified that *R. quelen* exposed to a period of chronic stress did not impair its capacity to respond to further acute stressors.

The stress detected in basal group should not be related to deterioration in water quality or high loading density. Water physicochemical parameters were within the adequate range for *R. quelen* production (Baldisserotto & Gomes, 2010). Which excludes the possibility this environmental factor is responsible for the results. In relation to loading density, a value of 4.5 g $L^{-1}$ used in the experiment is almost eighty times lower than the described as stressful to this fish species (Carneiro *et al*., 2009). The most possible explanation to this pattern is the release of pre-synthesized pools of steroid hormones or their precursors. Hormone storage was suggested in studies with rainbow trout (*Oncorhynchus mykiss*), where cortisol was released immediately after netting procedures and 30 s of air exposure (Gerwick *et al*., 1999).

It is worth to note that the previous exposure to LEO was able to prevent the cortisol rise due to handling. A preventive effect was also verified in *R. quelen* anesthetized with EO of *L. alba* and eugenol after 1 and 4 h of the aerial exposure for 1 min (Cunha *et al*., 2010a, 2010b). Gressler *et al.* (2014) detected increases in the cortisol levels of *R. quelen* immediately after anesthesia with 150 mg $L^{-1}$ of MS-222 using a protocol without application of additional stressor. No similar pattern was verified to either concentrations of LEO after stress event, which can be considered a positive point to its use.

Secondary effects of stress can be evaluated from changes in glycemic and ionic levels (Ellis *et al*., 2012). *R. quelen* exposed to LEO showed hyperglycemia after handling, which was not accompanied by ionoregulatory changes. The maintenance of the plasma $Na^+$ levels in LEO groups corroborates its effect on cortisol levels. This hormone acts increasing $Na^+/K^+$-ATPase density in gill membranes, which favors the ion loss (Dang *et al*., 2000).

Effects on plasma glucose levels, but not on cortisol contents, were also detected in anesthesia of rainbow trout (*Oncorhynchus mykiss*) with clove oil, an eugenol rich sample (Wagner *et al*., 2003). According to the authors, this phenomenon occurs due to fish perception to anesthetic presence. It promotes cathecolamine release, which induces liver glycogenolysis and thus increases the plasma glucose levels.

The hyperglycemic effect detected to LEO seems to be transient mainly at the lowest concentration tested. Toni *et al.* (2014) also verified glucose increase and baseline levels return only with the lowest concentration of EO of *H. ringens* tested (150 μL $L^{-1}$) under the same experimental protocol. An opposite pattern was reported to linalool-rich EO of *L. alba* in the same study, since it did not induce glycemic changes after exposure (Toni *et al*., 2014).

In conclusion, the EO samples of *O. americanum* demonstrated sedative and anesthetic effects in *R. quelen* between 25-50 mg $L^{-1}$ and 200-500 mg $L^{-1}$, respectively. The compounds β-linalool and 1,8-cineole were detected in similar proportions in the LEO, together with great amounts of eugenol and camphor. LEO corresponded to the most active sample, which was considered suitable to the anesthetic procedures due to its fast induction and handling-induced stress prevention. However, other studies should be performed to evaluate its bioaccumulation in fish tissues aiming its approval as anesthetic prior to slaughter.

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