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RESEARCH PAPER

Oxidative and biochemical responses in *Brycon amazonicus* anesthetized and sedated with *Myrcia sylvatica* (G. Mey.) DC. and *Curcuma longa* L. essential oils

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Abstract

Objective To investigate the effects of rapid anesthesia and long-term sedation with the essential oils (EOs) of *Myrcia sylvatica* (EOMS) and *Curcuma longa* (EOCL) on biochemical and oxidative parameters in matrinxã.

Study design Prospective, randomized, laboratory experiment.

Animals A total of 72 matrinxã (*Brycon amazonicus*) adults weighing 404.8 ± 27.9 g were divided into eight groups of nine fish.

Methods Biochemical and oxidative effects were investigated in plasma and tissues of matrinxã subjected to rapid anesthesia (5 minutes) or long-term sedation (360 minutes, simulating the practice of transport) with EOMS (200 μL L^{-1} and 10 μL L^{-1}, respectively) and EOCL (500 μL L^{-1} and 40 μL L^{-1}, respectively).

Results Transport simulation without sedation or anesthesia increased lipid peroxidation levels in the gills and kidney of fish in the control group. Anesthesia and sedation with EOs decreased cortisol concentrations and increased lactate concentrations compared with controls. Lipid peroxidation was lower in the brain, gills, liver and kidney of sedated and anesthetized fish, than in the control group. Anesthesia with EOs increased the activity of superoxide dismutase and glutathione-S-transferase in the brain, and catalase in the liver and gills, compared with controls. Long-term sedation with EOs increased superoxide dismutase, glutathione peroxidase and glutathione reductase activities in the brain, catalase in the liver, glutathione peroxidase and glutathione reductase in the gills and superoxide dismutase in the kidney. In general, nonprotein thiols content and total reactive antioxidant potential of tissues were higher after anesthesia and sedation with EOs compared with the control group.

Conclusions and clinical relevance The concentrations of EOMS and EOCL used were effective at preventing a stress response and excess of reactive oxygen species formation. For these reasons, these substances may be recommended for use in the transportation of fish to improve survival and animal welfare.

Keywords animal welfare, antioxidant enzymes, fish, immersion anesthesia, natural anesthetic.

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Introduction

Brycon amazonicus (Characidae), popularly known as matrinxã, is a neotropical species of fish native of the Amazon, Orinoco, and Essequibo River basins (Lima 2003), which is highly valued in aquaculture and used in breeding sites for recreational and commercial fishing. However, common procedures in aquaculture and during research practices (netting, handling, loading, and transportation) are stressful to fish and can lead to economic losses in production or reduction in the sampling population. These procedures are often associated with the activation of the stress response, characterized by stimulation of the hypothalamic–pituitary–interrenal axis, with the release of corticosteroids and catecholamines. The activation of the endocrine cascade of stress may culminate in deleterious effects on fish, such as suppression of the immune system with reduced resistance to pathogens (Nardocci et al. 2014), a reduced ability to maintain homeostasis and resist to additional stressors (e.g., poor water quality and crowding) (Zahl et al. 2012), and inhibition of growth and reproductive failure (Barton 2002).

Variations in blood chemistry can occur immediately after the fish are disturbed by a stressful situation (Barton & Iwama 1991); therefore, analysis of blood and tissue parameters is a very important tool for monitoring the physiologic and health status of fish. Furthermore, there is an increase in oxygen demand under stress conditions. Abnormal oxidative reactions in the aerobic metabolic pathways can occur, resulting in the formation of excessive amounts of reactive oxygen species (ROS). The exposure of molecules, cells or tissues to excess levels of ROS is known as oxidative stress (Velisek et al. 2011).

Studies have demonstrated that anesthesia during aquatic biomonitoring or laboratory studies reduces pain in fish, as it triggers a calming state with a consequent loss of equilibrium and blockade of reflex action (Zahl et al. 2012). However, exposure to anesthetics may itself induce activation of the stress response in fish, with cortisol release (Small 2003; King et al. 2005; Zahl et al. 2010), due to depression of cardiovascular and respiratory function (Iversen et al. 2003) and other undesirable side effects.

Essential oils (EOs) have exerted potent sedative and anesthetic effects in fish in different experimental protocols (Cunha et al. 2010; Azambuja et al. 2011; Silva et al. 2012; Gressler et al. 2014; Parodi et al. 2014; Zeppenfeld et al. 2014). *Myrcia sylvatica* (G. Mey.) DC. (http://www.tropicos.org/Name/22101901, last accessed 15 August 2016; Myrtaceae) is a plant distributed in eastern South America that is used in popular medicine in the Amazon region (Zoghbi et al. 2003), despite the lack of studies confirming its pharmacological activities. *Curcuma longa* L. (http://www.tropicos.org/Name/34500029, last accessed 15 August 2016; Zingiberaceae), is well known in regions of America, Africa, and Asia for its medicinal effects (antioxidant, antiatherosclerotic and antidiabetic) and is a versatile spice for use in cookery (Priya et al. 2012).

In this context and based on results of a prior study (Saccol et al. 2016), the purpose of this study was to investigate the effects of rapid anesthesia and long-term sedation with the EOs of *Myrcia sylvatica* (EOMS) and *Curcuma longa* (EOCL) on physiological parameters of matrinxã. This experiment was performed in a laboratory-controlled setting to verify the sole effect of the EOs as anesthetics upon plasma biochemical parameters and oxidative response in tissues of matrinxã.

Materials and methods

Plant material

Plants were collected in an experimental area of the Universidade Federal do Oeste do Pará, classified and deposited in the herbarium of Empresa Brasileira de Pesquisa Agropecuária/Eastern Amazonia (Belém, PA, Brazil), under registration numbers IAN 184696 (*M. sylvatica*) and IAN 188089 (*C. longa*). Extraction and composition of EOs from fresh leaves was the same as found for Saccol et al. (2016), with β-selinene (9.96%), cadalene (9.36%), α-calacorene (9.17%) and Z-calamene (8.17%) as major constituents for EOMS, and β-phellandrene (31.48%), α-terpinolene (22.46%) and 1,8-cineole (15.21%) as major constituents for EOCL.

Fish and water quality

A total of 72 adult matrinxã, [mean ± standard deviation (SD)] 404.8 ± 27.9 g and 18.0 ± 1.2 cm, were captured from farm ponds in Station Production of Santa Rosa (Santarém, PA, Brazil) under authorization of Secretaria Estadual de Desenvolvimento Agropecuário e de Pesca (SEDAP, PA, Brazil). Fish were maintained for 1 week in continuously aerated 1000 L tanks (semistatic system) for acclimation, with dissolved oxygen (6.0 ± 0.4 mg L^{-1}), temperature (25.9 ± 0.6 °C) and pH (7.9 ± 0.3) monitored daily. Total ammonia (0.4 ± 0.1 mg L^{-1}) was

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determined by the direct nesslerization method and unionized ammonia (0.001 ± 0.0001 mg L^{-1}) was calculated (Colt & Tomasso 2002). Daily, 50% of the tank water volume was removed and renewed 1 hour after fish were fed a commercial diet for omnivorous fish (32% extruded crude protein). Food was withdrawn 24 hours before the experimental procedure. The experimental protocol was approved by the Committee on Ethics in Research of State University of Pará (no. 42/2012).

Experimental design

Fish were hand-transferred to the trial tanks (containing 10 L of water) and kept under the confined experimental conditions for a determined period. Three animals were placed in the same tank, simulating the loading density (120 g L^{-1}) that is used for the transport practices (Carneiro & Urbinati 2002). Two concentrations of each EO were used to induce rapid anesthesia and long-term sedation, respectively (Saccol et al. 2016). Sedation leads to decreased reactivity to external stimuli and partial loss of equilibrium and is the stage recommended for the transport of animals (Iwama et al. 1989). EOs were diluted first in ethanol (1:10) to enable better dissolution in water. The two highest concentrations of ethanol used for diluting the EOs were tested separately to verify the effects *per se* of ethanol.

A total of nine fish per treatment were tested (three replicates with three fish each) corresponding to: 1) anesthesia (animals were removed from the tank after 5 minutes of anesthetic bath): control group, free of anesthetic substances, simulation of immersion anesthesia; ethanol group, 4500 μL L^{-1}, vehicle; EOMS group, 200 μL L^{-1}, fish anesthetized; EOCL group 500 μL L^{-1}, fish anesthetized; and 2) sedation (animals were removed from the tank after 360 minutes, reproducing transport procedures): control group, free of anesthetic substances; ethanol group, 360 μL L^{-1}, vehicle; and EOMS group, 10 μL L^{-1}, fish sedated; EOCL group, 40 μL L^{-1}, fish sedated.

Constant aeration was used to minimize interference as dissolved oxygen declined, pH decreased and carbon dioxide accumulated. Measurements made during the experiments (27.3 ± 0.1 °C, pH 6.3 ± 0.1, dissolved oxygen 5.6 ± 0.2 mg L^{-1}, total ammonia 0.5 ± 0.1 mg L^{-1} and unionized ammonia 0.002 ± 0.0001 mg L^{-1}) confirmed that water parameters were kept similar to those of the acclimation tanks.

Sample collection and preparation

After each experimental period (5 or 360 minutes), a small nonlethal blood sample was immediately taken from the caudal peduncle using a heparinized sterile syringe (3 mL syringe and 25 gauge, 7 mm needle; Descarpack, SP, Brazil) from all fish in each treatment. Plasma was separated by centrifugation at 1100 ***g*** for 5 minutes and stored at −80 °C until biochemical analysis. Fish were euthanized by sectioning the spinal cord and the brain, liver, gills and kidney were carefully removed, frozen in liquid nitrogen and stored at −80 °C. Frozen tissue samples were weighed and homogenized as previously described (Saccol et al. 2016).

Biochemical analysis in plasma

Plasma cortisol concentration was measured in duplicate using a commercially available enzyme-linked immunosorbent assay kit (Diagnostics Biochem Canada Inc., Canada). Coefficients of variation inter- and intra-assay were 5.2 ± 0.5 and 4.1 ± 0.7%, respectively. Using commercial kits (Labtest, MG, Brazil), biochemical parameters analyzed in plasma were glucose, lactate, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, urea, creatinine, and uric acid concentrations and lactate dehydrogenase (LDH), alanine aminotransferase, aspartate aminotransferase, γ-glutamyl-transferase and alkaline phosphatase activities. Protein was measured according the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Metabolic analysis in liver

A portion of liver was used to measure the concentrations of glucose and glycogen according to Carr & Neff (1984) and lactate in agreement with Harrower & Brown (1972).

Oxidative stress biomarkers

Lipid peroxidation (LPO) was determined spectrophotometrically using two methods: 1) determination of lipid hydroperoxides (LOOH) for detection of the primary products of LPO by oxidation of Fe^{2+} in an acidic medium with xylenol orange dye, forming a Fe^{3+} complex (Södergren et al. 1998); and 2) using the thiobarbituric acid reactive substance (TBARS) assay, that evaluates LPO end products, such as malondialdehyde (Buege & Aust 1978).

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Tissue proteins were quantified as previously described for the plasma proteins. Total superoxide dismutase (SOD) activity was based on the inhibition rate of autocatalytic adrenochrome generation at 480 nm (Misra & Fridovich 1972). Catalase (CAT) activity was assessed by following the decrease in the 240 nm absorption of hydrogen peroxide (Boveris & Chance 1973). Glutathione peroxidase (GPx) activity was assayed following the rate of nicotinamide adenine dinucleotide phosphate oxidation at 340 nm by the coupled reaction with glutathione reductase (GR) (Flohé & Günzler 1984). GR activity was determined measuring nicotinamide adenine dinucleotide phosphate oxidation at 340 nm (Carlberg & Mannervik 1985). Glutathione S-transferase (GST) activity was measured based on the conjugation reaction with glutathione (GSH), using 1-chloro-2,4-dinitrobenzene as a substrate (Habig et al. 1974).

Nonprotein thiols (NPSH) represent an indirect measure of reduced GSH and were assayed at 412 nm after reacting with 5,5′-dithiobis-(2-nitrobenzoic acid) (Ellman 1959). Total reactive antioxidant potential (TRAP) was measured by a chemiluminiscence assay with 2,2′-azobis (2-aminodipropane) dihydrochloride as a source of free radicals. The standard curve was prepared with different Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) concentrations (1−4 μM). The comparison of the induction time after the addition of Trolox and the sample allowed calculation of TRAP as the equivalent of the Trolox concentration necessary to produce the same induction time (Evelson et al. 2001).

Statistical analyses

Data are presented as means ± standard error of the mean. Bartlett test was applied to assess normality and Levene test was used to verify homogeneity of variances. Because the data exhibited homogeneous variances, comparisons between different groups and times were made using two-way analysis of variance (ANOVA) and Tukey's test, separately for each EO. The statistical analyses were performed using the software Statistica 7.0 (StatSoft, Inc., OK, USA) and GraphPad Prism 6.01 (GraphPad Software, Inc., CA, USA), and differences were considered significant at $p < 0.05$.

Results

Lower concentrations of cortisol were measured in EOMS and EOCL than in the control and ethanol groups (Fig. 1a). Plasma glucose concentrations did not differ significantly among the groups at the same time point, but 360 minutes of sedation or simulation of sedation (control and ethanol) significantly increased glucose concentrations compared with anesthesia or simulation of anesthesia (Fig. 1b). Fish anesthetized with EOMS and EOCL showed higher concentrations of lactate than in the control group. After 360 minutes of sedation with EOs, lactate concentrations were higher than in the ethanol group (Fig. 1c).

The other biochemical indices in plasma of matrinxã were not affected by anesthesia and sedation with these oils (Table 1). Hepatic concentrations of glucose, glycogen and lactate did not differ among the experimental groups (Table 2).

LOOH and TBARS levels were lower in brain of matrinxã anesthetized and sedated with both EOs, compared with controls (Table 3). Activity of SOD was higher in fish anesthetized with EOCL and sedated with both EOs compared with the control group in their respective times. GPx activity was higher in matrinxã sedated with both EOs than in the control. Activity of GR was higher in fish sedated with EOCL compared with control. GST activity was higher in fish anesthetized with EOCL compared with control and ethanol groups. NPSH and TRAP were higher in fish anesthetized and sedated with both EOs, compared with controls.

In liver, LOOH were lower in matrinxã anesthetized with both EOs and sedated with EOMS, compared with controls (Table 4). Levels of TBARS were lower in fish anesthetized and sedated with both EOs compared with the control group at their respective times. CAT activity was higher in matrinxã anesthetized and sedated with EOs, compared with controls. NPSH content was higher in fish anesthetized with both EOs, and sedated with EOCL, than in the controls. TRAP was higher in matrinxã anesthetized with both EOs, and sedated with EOCL, compared with controls.

In gills, LOOH were lower in matrinxã anesthetized with EOCL than in the control group. TBARS were lower in fish sedated with both EOs compared with control (Table 5). TBARS levels were higher in gills of fish control 360 minutes (simulation of transport without sedation) than control 5 minutes. CAT activity was higher in fish anesthetized with EOCL compared with control. Activity of GPx was higher in fish sedated with EOMS and EOCL compared with control. GR activity was higher in matrinxã sedated with EOMS than in the control group. GST activity was lower in matrinxã sedated with EOCL compared with control. NPSH and TRAP were higher in fish

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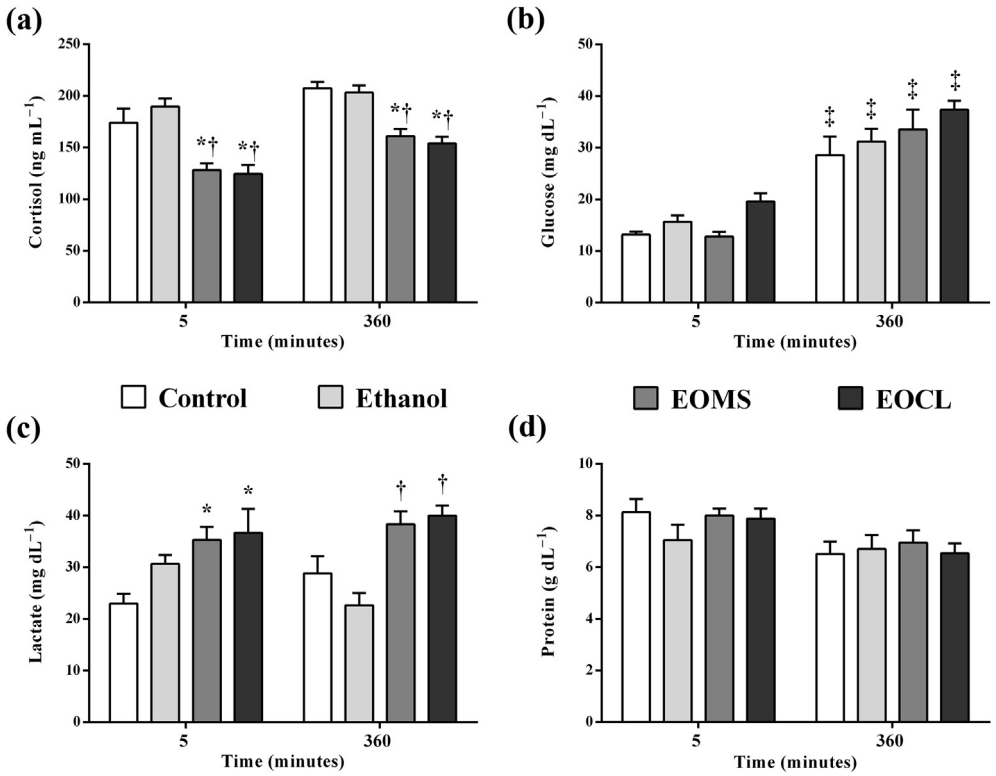

Figure 1 (a) Cortisol, (b) glucose, (c) lactate and (d) protein concentrations in plasma of matrinxã subjected to anesthesia (5 minutes) or sedation (360 minutes) with essential oils of *Myrcia sylvatica* (EOMS) and *Curcuma longa* (EOCL). Values are expressed as means ± standard error of the mean ($n = 9$). *Significantly different from control group at the same time point ($p < 0.05$). †Significantly different from the ethanol group at the same time point ($p < 0.05$). ‡Significantly different from time 5 minutes within the same treatment ($p < 0.05$).

anesthetized and sedated with both EOs, compared with controls.

In kidney, LOOH were lower in matrinxã sedated with both EOs compared with control. LOOH levels were higher in kidney of fish control 360 minutes (simulation of transport without sedation) than control 5 minutes (Table 6). TBARS were lower in fish anesthetized and sedated with both EOs, compared with controls. Activity of SOD was higher in fish sedated with EOCL than in the control. NPSH was higher in fish anesthetized with EOCL compared with control. TRAP was higher in fish anesthetized and sedated with EOCL, compared with controls (Table 6). Ethanol concentrations used in this study had no anesthetic effect and the oxidative parameters were not significantly altered.

Discussion

The anesthesia and long-term sedation with EOMS and EOCL reduced cortisol concentrations and did not alter the plasma glucose concentrations in matrinxã. Plasma cortisol concentrations were also lower in *Colossoma macropomum* exposed to EOCL (Saccol et al. 2016), *Rhamdia quelen* exposed to EO of *Aloysia triphylla* (Gressler et al. 2014) and *Salmo salar* L. (Iversen et al. 2003), *Ictalurus punctatus* (Small 2003) and *Centropristis striata* (King et al. 2005) exposed to clove oil. These results indicate that the EOs promote the suppression of cortisol synthesis by blocking or reducing the activity of the hypothalamic–pituitary–interrenal axis (Small 2003), attenuating the activation of this hormonal cascade associated with stress (Iversen et al. 2003). Moreover, these substances may affect enzymes related to the production of cortisol in interrenal cells, similar to the anesthetic etomidate, which interacts with the mitochondrial cytochrome P450-dependent enzymes that catalyze the synthesis of cortisol, as 11β-hydroxylase in mammals (Wagner et al. 1984).

Throughout the long period (360 minutes) of transport simulation, the fish exposed to sedative concentrations of EOs remained in a calm state.

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Table 1 Plasma biochemical indices in matrinxã subjected to anesthesia (5 minutes) and sedation (360 minutes) with essential oils of *Myrcia sylvatica* (EOMS) and *Curcuma longa* (EOCL)

Variable	5 minutes				360 minutes			
	Control	Ethanol	EOMS	EOCL	Control	Ethanol	EOMS	EOCL
CHO (mg dL^{-1})	324.9 ± 20.5	369.5 ± 28.0	283.1 ± 38.8	293.9 ± 44.1	300.7 ± 25.4	270.3 ± 23.6	311.1 ± 30.1	266.8 ± 30.9
LDL (mg dL^{-1})	76.5 ± 18.0	99.5 ± 12.7	79.2 ± 7.9	118.0 ± 18.2	100.3 ± 11.4	90.9 ± 20.6	113.7 ± 14.8	78.9 ± 16.6
HDL (mg dL^{-1})	143.0 ± 15.8	135.6 ± 13.6	148.1 ± 14.1	147.1 ± 10.3	160.0 ± 33.7	175.8 ± 31.5	130.4 ± 13.3	175.0 ± 21.6
TG (mg dL^{-1})	438.5 ± 62.1	364.6 ± 27.9	378.9 ± 43.1	404.3 ± 51.7	302.2 ± 17.6	394.3 ± 32.9	335.1 ± 39.7	282.1 ± 25.5
LDH (U L^{-1})	573.7 ± 85.9	635.5 ± 43.9	784.2 ± 64.8	588.5 ± 86.8	691.1 ± 23.4	549.7 ± 65.8	709.1 ± 71.1	653.3 ± 46.3
ALT (U L^{-1})	41.6 ± 4.1	35.6 ± 4.0	42.1 ± 4.5	38.2 ± 3.6	34.2 ± 4.8	39.5 ± 4.0	38.1 ± 2.4	34.8 ± 3.7
AST (U L^{-1})	31.6 ± 2.6	29.3 ± 2.3	37.4 ± 5.0	34.1 ± 2.7	40.0 ± 4.2	38.2 ± 2.8	40.5 ± 2.7	34.2 ± 4.0
GGT (U L^{-1})	70.3 ± 14.4	91.8 ± 4.1	94.0 ± 6.0	80.0 ± 8.4	65.0 ± 12.8	61.8 ± 9.3	90.5 ± 7.9	83.8 ± 11.1
ALP (U L^{-1})	68.8 ± 5.1	77.4 ± 8.9	54.7 ± 3.5	53.6 ± 10.1	56.4 ± 7.5	61.4 ± 8.8	58.7 ± 7.0	54.1 ± 2.6
Urea (mg dL^{-1})	23.6 ± 3.1	26.8 ± 4.4	24.2 ± 2.8	26.3 ± 1.9	22.6 ± 4.6	27.4 ± 2.7	22.2 ± 4.0	22.8 ± 2.5
CRE (mg dL^{-1})	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
URA (mg dL^{-1})	1.3 ± 0.0	1.3 ± 0.1	1.2 ± 0.0	1.2 ± 0.1	1.2 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	1.2 ± 0.0

Data are presented as the means ± standard error of the mean ($n = 9$). Means obtained showed no significant difference ($p > 0.05$). ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; CHO, total cholesterol; CRE, creatinine; GGT, γ-glutamyl-transferase; HDL, high-density lipoprotein cholesterol; LDH, lactate dehydrogenase; LDL, low-density lipoprotein cholesterol; TG, triglycerides; URA, uric acid.

Table 2 Effects of anesthesia (5 minutes) and sedation (360 minutes) with essential oils of *Myrcia sylvatica* (EOMS) and *Curcuma longa* (EOCL) on hepatic metabolism in matrinxã

Variable	5 minutes				360 minutes			
	Control	Ethanol	EOMS	EOCL	Control	Ethanol	EOMS	EOCL
Glucose (μg mg $tissue^{-1}$)	9.1 ± 0.3	8.8 ± 0.7	12.9 ± 1.2	9.7 ± 0.5	9.2 ± 0.6	11.9 ± 0.9	12.6 ± 1.1	12.0 ± 1.1
Glycogen (μg mg $tissue^{-1}$)	137.8 ± 24.2	127.5 ± 25.5	236.9 ± 41.2	223.6 ± 52.5	158.4 ± 20.7	148.7 ± 14.6	240.3 ± 47.1	234.4 ± 50.8
Lactate (μg mg $tissue^{-1}$)	0.5 ± 0.0	0.4 ± 0.0	0.5 ± 0.1	0.6 ± 0.0	0.5 ± 0.0	0.4 ± 0.1	0.5 ± 0.1	0.6 ± 0.1

Data presented as the means ± standard error of the mean ($n = 9$). Means obtained showed no significant difference ($p > 0.05$).

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Table 3 Effects of anesthesia (5 minutes) and sedation (360 minutes) with essential oils of *Myrcia sylvatica* (EOMS) and *Curcuma longa* (EOCL) on oxidative stress biomarkers in brain of matrinxã

Variables	5 minutes				360 minutes			
	Control	Ethanol	EOMS	EOCL	Control	Ethanol	EOMS	EOCL
PRO ($mg\ mL^{-1}$)	4.3 ± 0.3	4.4 ± 0.3	3.9 ± 0.2	4.2 ± 0.2	4.3 ± 0.2	4.3 ± 0.2	3.9 ± 0.1	4.0 ± 0.2
LOOH ($nmol\ g\ tissue^{-1}$)	128.3 ± 5.3	130.4 ± 5.7	103.0 ± 3.0*†	99.9 ± 3.4*†	133.2 ± 2.8	139.2 ± 5.0	96.0 ± 4.0*†	89.4 ± 4.7*†
TBARS ($nmol\ g\ tissue^{-1}$)	77.7 ± 2.4	77.5 ± 2.7	62.1 ± 2.3*†	58.3 ± 3.1*†	71.1 ± 3.7	70.9 ± 3.4	58.5 ± 3.1*†	45.7 ± 2.8*†
SOD ($U\ mg\ protein^{-1}$)	1.9 ± 0.1	1.9 ± 0.1	2.2 ± 0.1	2.8 ± 0.1*†	2.1 ± 0.1	2.1 ± 0.1	2.8 ± 0.2*†	3.0 ± 0.2*†
GPx ($nmol\ minute^{-1}\ mg\ protein^{-1}$)	16.2 ± 1.6	15.3 ± 1.7	21.5 ± 1.7	19.2 ± 3.1	12.1 ± 2.2	18.8 ± 3.6	23.0 ± 3.6*	26.4 ± 2.4*
GR ($nmol\ minute^{-1}\ mg\ protein^{-1}$)	4.9 ± 0.2	4.9 ± 0.2	6.8 ± 0.6	5.6 ± 0.6	4.5 ± 0.7	5.2 ± 0.9	6.7 ± 0.5	7.1 ± 0.7*
GST ($pmol\ minute^{-1}\ mg\ protein^{-1}$)	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	2.0 ± 0.1*†	1.6 ± 0.2	1.3 ± 0.2	1.7 ± 0.1	1.2 ± 0.2
NPSH ($\mu mol\ mg\ protein^{-1}$)	14.0 ± 0.8	14.4 ± 0.8	17.7 ± 0.3*†	18.8 ± 0.8*†	12.0 ± 0.5	14.6 ± 0.7	19.9 ± 0.7*†	19.9 ± 0.9*†
TRAP ($\mu mol\ mg\ protein^{-1}$)	2.7 ± 0.2	2.8 ± 0.2	4.7 ± 0.3*†	4.3 ± 0.4*†	3.0 ± 0.4	2.5 ± 0.1	4.9 ± 0.4*†	4.2 ± 0.2*†

Values are expressed as means ± standard error of the mean ($n = 9$). *Significantly different from control group at the same time point ($p < 0.05$). †Significantly different from the ethanol group at the same time point ($p < 0.05$). GPx, glutathione peroxidase; GR, glutathione reductase; GST, gluthatione S-transferase; LOOH, lipid hydroperoxides; NPSH, nonprotein thiols; PRO, protein; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substance; TRAP, total reactive antioxidant potential.

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Table 4 Effects of anesthesia (5 minutes) and sedation (360 minutes) with essential oils of *Myrcia sylvatica* (EOMS) and *Curcuma longa* (EOCL) on oxidative stress biomarkers in liver of matrinxã

Variable	5 minutes				360 minutes			
	Control	Ethanol	EOMS	EOCL	Control	Ethanol	EOMS	EOCL
PRO (mg mL^{-1})	10.2 ± 0.9	9.9 ± 1.0	9.8 ± 0.6	10.3 ± 1.2	10.7 ± 0.8	11.1 ± 0.6	11.7 ± 1.6	9.4 ± 0.5
LOOH (nmol g $tissue^{-1}$)	296.3 ± 17.1	290.5 ± 18.6	222.6 ± 11.5*†	213.1 ± 11.6*†	275.1 ± 16.9	288.4 ± 20.1	209.3 ± 11.7*†	214.5 ± 14.8†
TBARS (nmol g $tissue^{-1}$)	21.3 ± 1.2	21.8 ± 1.4	14.0 ± 0.8*†	11.7 ± 1.0*†	18.4 ± 1.4	19.1 ± 1.6	12.7 ± 0.8*†	11.4 ± 0.9*†
SOD (U mg $protein^{-1}$)	3.5 ± 0.3	3.4 ± 0.3	4.3 ± 0.2	4.5 ± 0.4	3.9 ± 0.4	4.2 ± 0.3	4.9 ± 0.1	4.9 ± 0.2
CAT (pmol mg $protein^{-1}$)	8.0 ± 0.5	7.8 ± 0.6	12.8 ± 1.4*†	11.6 ± 1.2*†	7.1 ± 0.7	7.1 ± 0.8	11.3 ± 1.1*†	12.3 ± 0.8*†
GPx (nmol $minute^{-1}$ mg $protein^{-1}$)	13.9 ± 1.3	14.7 ± 1.4	15.3 ± 1.5	15.7 ± 1.7	13.3 ± 2.1	12.4 ± 1.7	18.3 ± 2.8	19.3 ± 1.1
GR (nmol $minute^{-1}$ mg $protein^{-1}$)	2.1 ± 0.3	2.4 ± 0.3	3.8 ± 0.6	3.5 ± 0.5	2.9 ± 0.3	2.2 ± 0.3	4.1 ± 0.5†	4.0 ± 0.6†
GST (pmol $minute^{-1}$ mg $protein^{-1}$)	5.2 ± 0.5	5.2 ± 0.6	5.6 ± 0.4	5.5 ± 0.9	5.7 ± 0.4	5.7 ± 0.6	6.4 ± 0.8	6.0 ± 0.4
NPSH (μmol mg $protein^{-1}$)	5.0 ± 0.4	5.0 ± 0.4	6.5 ± 0.3*†	7.2 ± 0.5*†	6.0 ± 0.3	5.9 ± 0.4	6.8 ± 0.2	7.6 ± 0.3*†
TRAP (μmol mg $protein^{-1}$)	2.5 ± 0.2	2.4 ± 0.2	3.8 ± 0.2*†	4.6 ± 0.3*†	3.0 ± 0.2	2.6 ± 0.3	3.8 ± 0.3†	4.1 ± 0.3*†

Values are expressed as means ± standard error of the mean ($n = 9$). *Significantly different from control group at the same time point ($p < 0.05$). †Significantly different from the ethanol group at the same time point ($p < 0.05$). CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; GST, gluthatione S-transferase; LOOH, lipid hydroperoxides; NPSH, nonprotein thiols; PRO, protein; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substance; TRAP, total reactive antioxidant potential.

Table 5 Effects of anesthesia (5 minutes) and sedation (360 minutes) with essential oils of *Myrcia sylvatica* (EOMS) and *Curcuma longa* (EOCL) on oxidative stress biomarkers in gills of matrinxã

Variables	5 minutes				360 minutes			
	Control	Ethanol	EOMS	EOCL	Control	Ethanol	EOMS	EOCL
PRO (mg mL^{-1})	10.7 ± 0.3	10.5 ± 0.3	11.2 ± 0.5	11.7 ± 0.2	11.0 ± 0.4	10.8 ± 0.4	11.6 ± 0.3	11.4 ± 0.4
LOOH (nmol g $tissue^{-1}$)	139.1 ± 9.0	132.5 ± 7.6	98.6 ± 5.0	100.4 ± 6.2*	130.1 ± 8.2	140.4 ± 11.9	105.0 ± 5.2	102.6 ± 4.8†
TBARS (nmol g $tissue^{-1}$)	8.7 ± 1.0	8.0 ± 0.7	7.4 ± 0.6	7.5 ± 0.5	13.4 ± 0.8‡	12.7 ± 0.9	6.8 ± 0.3*†	7.2 ± 0.5*†
CAT (pmol mg $protein^{-1}$)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0*†	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
GPx (nmol $minute^{-1}$ mg $protein^{-1}$)	11.9 ± 1.0	12.3 ± 1.2	17.3 ± 0.9	15.8 ± 1.2	10.5 ± 1.4	12.1 ± 1.2	17.3 ± 1.6*	18.7 ± 1.3*†
GR (nmol $minute^{-1}$ mg $protein^{-1}$)	3.5 ± 0.6	3.7 ± 0.6	4.3 ± 0.4	4.1 ± 0.6	2.7 ± 0.4	3.1 ± 0.3	4.6 ± 0.5*	4.4 ± 0.4
GST (pmol $minute^{-1}$ mg $protein^{-1}$)	3.9 ± 0.2	3.7 ± 0.2	3.5 ± 0.4	2.7 ± 0.5	4.5 ± 0.3	4.5 ± 0.4	3.3 ± 0.3	3.2 ± 0.2*†
NPSH (μmol mg $protein^{-1}$)	4.8 ± 0.2	5.0 ± 0.1	5.6 ± 0.2*	5.7 ± 0.2*	4.4 ± 0.2	4.8 ± 0.3	5.6 ± 0.1*†	5.8 ± 0.2*†
TRAP (μmol mg $protein^{-1}$)	0.8 ± 0.0	0.7 ± 0.0	1.1 ± 0.1*†	1.1 ± 0.1*†	0.8 ± 0.1	0.9 ± 0.0	1.1 ± 0.1*†	1.1 ± 0.1*

Values are expressed as means ± standard error of the mean ($n = 9$). *Significantly different from control group at the same time point ($p < 0.05$). †Significantly different from the ethanol group at the same time point ($p < 0.05$). ‡Significantly different from time 5 minutes within the same treatment ($p < 0.05$). CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; GST, gluthatione S-transferase; LOOH, lipid hydroperoxides; NPSH, nonprotein thiols; PRO, protein; TBARS, thiobarbituric acid reactive substance; TRAP, total reactive antioxidant potential.

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Table 6 Effects of anesthesia (5 minutes) and sedation (360 minutes) with essential oils of *Myrcia sylvatica* (EOMS) and *Curcuma longa* (EOCL) on oxidative stress biomarkers in kidney of matrinxã

Variables	5 minutes				360 minutes			
	Control	Ethanol	EOMS	EOCL	Control	Ethanol	EOMS	EOCL
PRO (mg mL^{-1})	13.2 ± 0.5	12.9 ± 0.5	13.5 ± 0.5	12.5 ± 0.6	11.5 ± 0.5	12.1 ± 0.4	13.3 ± 0.6	12.1 ± 0.5
LOOH (nmol g $tissue^{-1}$)	9.2 ± 0.4	10.1 ± 0.7	7.7 ± 0.3	7.4 ± 0.4†	11.7 ± 0.6‡	10.9 ± 0.6	7.0 ± 0.4*†	7.3 ± 0.3*†
TBARS (nmol g $tissue^{-1}$)	63.6 ± 3.0	66.2 ± 3.4	45.2 ± 3.8*†	39.0 ± 2.9*†	68.0 ± 4.1	63.5 ± 4.6	46.9 ± 3.2*†	42.3 ± 4.4*†
SOD (U mg $protein^{-1}$)	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	0.6 ± 0.0	0.7 ± 0.1	0.7 ± 0.0	0.9 ± 0.1*
CAT (pmol mg $protein^{-1}$)	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.0	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
GPx (nmol $minute^{-1}$ mg $protein^{-1}$)	11.3 ± 0.8	11.2 ± 1.0	14.2 ± 0.7	15.0 ± 1.6	10.7 ± 1.4	11.4 ± 1.1	13.4 ± 1.4	15.6 ± 1.1
GR (nmol $minute^{-1}$ mg $protein^{-1}$)	2.6 ± 0.4	3.1 ± 0.5	3.1 ± 0.2	3.1 ± 0.4	2.9 ± 0.4	2.9 ± 0.4	3.3 ± 0.2	3.0 ± 0.4
GST (pmol $minute^{-1}$ mg $protein^{-1}$)	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.0	0.7 ± 0.0	0.8 ± 0.1	0.9 ± 0.1†
NPSH (μmol mg $protein^{-1}$)	3.8 ± 0.3	3.8 ± 0.3	4.4 ± 0.2	5.4 ± 0.3*†	3.8 ± 0.3	4.0 ± 0.4	4.7 ± 0.3	5.6 ± 0.3*†
TRAP (μmol mg $protein^{-1}$)	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.6 ± 0.1*†	0.4 ± 0.0	0.4 ± 0.1	0.5 ± 0.0	0.6 ± 0.0†

Values are expressed as means ± standard error of the mean ($n = 9$). *Significantly different from control group at the same time point ($p < 0.05$). †Significantly different from the ethanol group at the same time point ($p < 0.05$). ‡Significantly different from time 5 minutes within the same treatment ($p < 0.05$). CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; GST, gluthatione S-transferase; LOOH, lipid hydroperoxides; NPSH, nonprotein thiols; PRO, protein; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substance; TRAP, total reactive antioxidant potential.

However, this condition increased glucose concentrations compared with anesthetized fish, regardless of treatment, probably owing to variation in the release of catecholamines caused by simulation of transport. Additionally, anesthesia with EOs resulted in increased plasma lactate concentrations. The increase in lactate has been observed in *S. salar* (Iversen et al. 2003) and *R. quelen* (Toni et al. 2014) during the anesthesia process, which leads to decreased metabolism, requiring the use of an anaerobic pathway. According to McFarland (1959), the metabolic rate of sedated fish remains close to the basal metabolic rate, however, there is a reduction in oxygen consumption and release of degradation products such as ammonia and carbon dioxide. The findings of this study indicated that anesthesia and sedation with these EOs did not alter the hepatic metabolism.

The content of plasma proteins was also not changed by the treatments with EOs. Protein and amino acids metabolism can be affected by stress, since they can be used as an alternative source when carbohydrates and lipid reserves are exhausted (Hoseini et al. 2016). The other blood parameters analyzed are usually employed as general indicators of stress and the functioning liver and kidney of vertebrates. These parameters were not affected by the anesthesia and sedation with EOs, reinforcing the theory of the reduction in cortisol synthesis and demonstrating that the tested concentrations of EOs did not have toxic effects.

In addition to the modifications described, stress can interrupt the balance between oxidation and antioxidant systems by excess in ROS formation or depletion of antioxidants, characterizing the oxidative stress. Among the cellular components, unsaturated fatty acids with more than one double bond are particularly susceptible to the action of ROS. The chain reactions resulting in LPO disrupt the structure and function of biological membranes. Under physiological conditions, the production of ROS is controlled by the activity of antioxidant enzymes, such as SOD, CAT, GPx, GR, GST, and endogenous antioxidants as GSH (Halliwell & Gutteridge 1999).

The brain is considered the main target of oxidative stress due to a higher rate of oxygen utilization, abundant presence of polyunsaturated fatty acid, and relatively low levels of antioxidants and antioxidant enzymes (Maiti et al. 2006). However, the liver, the primary site of detoxification, is the most frequently studied organ in the analysis of oxidative imbalance. In addition to these two important organs, the

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present study also studied the gills because they are the first organ of contact and absorption of EOs, and the kidney that also plays an important role in detoxification.

LOOH and TBARS levels were higher in the kidney and gills, respectively, after the simulation of transport in the control group, with no added EOs. The study of Du et al. (2016) showed that during transport simulation, without addition of anesthetic substances, the levels of malondialdehyde increased in the liver of *Coilia nasus*, indicating that the rate and extent of LPO increased in response to stress. This study also showed that SOD and CAT activities were reduced 8 hours after transport without an addition of anesthetics (Du et al. 2016).

In general, anesthesia and long-term sedation with EOMS and EOCL resulted in lower levels of LPO in brain, liver, gills and kidney of matrinxã, representing lower ROS formation. These results were similar to those reported by Salbego et al. (2015), in which the methanolic extract of *Condalia buxifolia* added to transport water decreased the LPO in liver, kidney and muscle of *R. quelen*.

Anesthesia with EOs increased the activity of SOD and GST in the brain, and CAT in the liver and gills, compared with control. Long-term sedation (360 minutes) with EOs exacerbated the activity of these enzymes as there was an increase in SOD, GPx and GR activities in the brain, CAT in the liver, GPx and GR in the gills and SOD in the kidney. Addition of EO *Lippia alba* (10 μL L^{-1}) to the water in a 7 hours transport increased the SOD activity in liver and brain of *R. quelen* (Azambuja et al. 2011), whereas the addition of EO of *A. triphylla* (40 μL L^{-1}) increased the CAT activity in the liver and gills of the same species transported for 6 hours (Zeppenfeld et al. 2014).

However, GST activity in the gills was lower after sedation with EOCL, compared with the control group. The decrease of this enzyme occurred probably because the gills, as the first and most exposed organ in contact with EOs, can be depleted of this enzyme required for detoxification. This response was also found in the study of Gressler et al. (2015), where GST activity in the gills of *R. quelen* was lower after 12 hours of exposure to propofol (0.4 mg L^{-1}).

The nonenzymatic antioxidant GSH was quantitated indirectly by measurement of NPSH. NPSH content was higher in fish anesthetized and sedated with EOs. TRAP is a technique that measures the total capacity antioxidant of the tissue and can provide information regarding the system's capacity to withstand oxidative stress imbalances (Evelson et al. 2001). In this study, the highest value of TRAP in the tissue of fish anesthetized or sedated with EOs corresponds to the higher antioxidant capacity of the tissue, and can be attributed to the induction of the activity of antioxidant enzymes and nonenzymatic antioxidants. Furthermore, these results demonstrate that the EOs have antioxidant activity and probably acted as proton donors and regulators of gene expression that code the enzymatic and nonenzymatic antioxidants (Gülçin et al. 2012), repairing oxidative damage caused by increases in ROS formation during handling and simulation of transport.

In summary, anesthesia and sedation with EOs of *M. sylvatica* and *C. longa* reduced the synthesis or release of cortisol, thereby offering protection for the matrinxã from excess ROS formation. The reduction of metabolism by inhibitory effects of anesthesia on fish respiratory system altered the plasma lactate concentrations. These results suggest that the organism was induced to adjust to this condition, resulting in increased activity of antioxidant enzymes and GSH, to prevent oxidative damage when the oxygen concentration in tissues was restored to normal. Our results suggest that use of EOMS and EOCL may provide a protective effect to the fish and may be used in the transportation practice to improve survival and animal welfare.

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Authors' contributions

EMHS: study design, execution of experiments, analysis and interpretation of data, preparation of the manuscript. EPL: analysis and interpretation of data, preparation of the manuscript. CAB: analysis and interpretation of data, preparation of the manuscript. JS: study design, execution of experiments. LTG: analysis and interpretation of data, preparation of the manuscript. LVFS: study design, equipment supply, execution of experiments, preparation of the manuscript. RHVM: study design, equipment supply, execution of experiments, preparation of the

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manuscript. RBO: equipment supply, execution of experiments. SFL: analysis and interpretation of data, preparation of the manuscript. BB: study design, equipment supply, execution of experiments, analysis and interpretation of data, preparation of the manuscript. MAP: study design, equipment supply, execution of experiments, analysis and interpretation of data, preparation of the manuscript. All authors participated in critical review of the manuscript.

Conflict of interest statement

Authors declare no conflict of interest.

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