Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Ocimum gratissimum essential oil in the transport water of *Brycon hilarii*: implications at water quality, blood parameters and residues in tissue and plasma

Óleo essencial de *Ocimum gratissimum* na água de transporte de juvenis de *Brycon hilarii*: implicações na qualidade da água, parâmetros sanguíneos e resíduos em tecido e plasma

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Abstract

Transporting live fish is a common practice in fish farming, and is certainly one of the main problems that affect fish homeostasis. In this scenario, the use of natural additives has shown promise in improving fish resistance to adverse situations. This study aimed to assess the impact of *Ocimum gratissimum* L. essential oil (OGEO) on water quality, hematological parameters, and residue levels in the plasma, fillet, and liver of juvenile piraputanga (*Brycon hilarii)* during a two-hour transportation period. The fish were divided into plastic bags (4 L) and exposed to three different OGEO concentrations (10, 20, and 30 mg L^{-1}), while a control group received no OGEO (three repetitions each). After the two-hour transportation, blood samples were collected, as well as portions of the fillet and liver for quantifying essential oil compounds, which were also measured in the plasma. Oxygen levels remained high throughout the transportation period, in all groups, while the pH decreased. Hemoglobin, MCHC, and MCH increased in fish exposed to OGEO concentrations of 20 and 30 mg L^{-1}, compared to the control group. However, lymphocyte counts and the concentrations of essential oil compounds in plasma, fillet, and liver increased with higher OGEO concentrations. The use of 10 mg L^{-1} OGEO in the two-hour transport water is promising to ensure the survival and well-being of *Brycon hilarii* juveniles (weighing 16 g), showing to be safe and effective. The residual concentration of eugenol the major compound of OGEO in the fillet remains below the maximum limit of the recommended daily intake.

Keywords: anesthetic, piraputanga, residual compound, water quality.

Resumo

O transporte de peixes vivos é uma prática comum na piscicultura, e é certamente um dos principais problemas que afetam a homeostase dos peixes e neste cenário o uso de aditivos naturais tem-se mostrado promissor para melhorar a resistência dos peixes frente a situações adversas. Este estudo teve como objetivo avaliar o impacto do óleo essencial de *Ocimum gratissimum* L. (OEOG) na qualidade de água, perfil hematológico e níveis de resíduos no plasma, filé e fígado de juvenis de piraputanga (*Brycon hilarii*) durante um período de transporte de duas horas. Os peixes foram divididos em sacos plásticos (4 L) e expostos a três concentrações diferentes de OE OG (10, 20 e 30 mg L^{-1}) enquanto um grupo não recebeu OEOG. Cada grupo com três repetições. Após o transporte de duas horas, foram coletadas amostras de sangue, bem como de filé e do fígado para a quantificação dos compostos do óleo essencial, que também foram mensurados no plasma. Os níveis de oxigênio permaneceram elevados durante todo o período do transporte, em todos os grupos, enquanto o pH diminuiu. A hemoglobina, CHCM e HCM aumentaram nos peixes dos grupos EOOG 20 e 30 mg L^{-1}, em comparação ao grupo controle. No entanto, a contagem dos linfócitos e as concentrações decompostos no plasma, filé e fígado aumentaram com a concentração mais elevada de OEOG. O uso de 10 mg L-1 de OGEO na água de transporte de duas horas é promissor para garantir a sobrevivência e o bem-estar dos juvenis de *Brycon hilarii* (pesando 16 g), mostrando-se seguro e eficaz. A concentração residual de eugenol, composto majoritário do OGEO no filé, permanece abaixo do limite máximo da ingestão diária recomendada.

Palavras-chave: anestésico, piraputanga, composto residual, qualidade da água.

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Received: November 11, 2023 – Accepted: February 27, 2024

1. Introduction

In recent years, the production and trade of live fish have experienced significant growth in Brazil and worldwide, primarily driven by intensive production methods (Valenti et al., 2021). Consequently, management practices such as capture, biometrics, classification, and transport have become increasingly frequent and necessary (Becker et al., 2016). However, these management practices can induce stress in fish (Barton, 2002).

The transport of live fish is a major procedural impediment in the fish farming production chain and many factors must be considered, such as fasting period (Kubitza, 1997), oxygenation (Ferreira et al., 2022), load density (Espinoza-Ramos et al., 2022), and time of transport (Sampaio and Freire, 2016; Paranhos et al., 2023). In this sense, live fish transport involves a combination of these stressful practices, along with factors like crowding and potential deterioration of water quality, which can negatively impact productivity (Esmaeili, 2021; Tavares-Dias and Martins, 2017; Valladão et al., 2018). It is known that induced stress (caused by transport) can affect the survival rates of animals, through delayed mortality (Schreck et al., 1989; Iversen et al., 1998). Therefore, evaluating the recovery time after transporting live fish is extremely important to ensure adequate and safe handling of fish.

Stress in fish is defined as a condition in which homeostasis is disturbed or influenced by an adverse stimulus (Balasch and Tort, 2019). Stress responses can alter the distribution of necessary resources, such as blood flow, release of energy stores, in addition to compromising the immune system, allowing the invasion of pathogens and the incidence of diseases that generate losses in production (Green and Haukenes, 2015; Schreck and Tort, 2016). Given its great importance in the physiological cascade of stress, glucose concentration is one of the main indicators of stress measured in fish (Vijayan et al., 1990; Van Der Vyver et al., 2013). Many researchers have demonstrated that poorly planned management (biometrics and fish transport) were responsible for increasing cortisol levels, activating glycogenolysis and gluconeogenesis with increases in plasma glucose values, as reviewed by Aydin and Barbas (2020) and Souza et al. (2019).

Therefore, to mitigate the physiological effects of stress and promote animal welfare during transport, various compounds have been investigated for their potential benefits. These compounds act on multiple physiological mechanisms, including sedation, mucus integrity and protection, and disease prevention (Vanderzwalmen et al., 2019). Examples of such compounds include salt (Baldisserotto et al., 2007; Tacchi et al., 2015), probiotics (Carvalho et al., 2009; Sutthi and Doan, 2020), plant extracts (Mattos et al., 2023) and even synthetic anesthetics (Topic Popovic et al., 2012; Bolasina et al., 2017).

Another area of growing research focuses on the use of plant-derived anesthetics, such as essential oils, which have demonstrated sedative effects, reducing fish metabolism e consequently maintaining water quality and animal survival, when used at appropriate concentrations (Ross and Ross, 2008; Becker et al., 2017; Aydin and Barbas, 2020; Boaventura et al., 2021). Essential oils are complex, volatile, natural compounds consisting of hydrocarbons and alcohols, possessing distinct aromas, and obtained from various plant sources (Edris, 2007; Bakkali et al., 2008; Swamy et al., 2016). Their efficacy during fish transportation has been confirmed by several studies reporting sedative effects, (Benovit et al., 2012; Barbas et al., 2020), reduced ammonia excretion and oxygen consumption, improved immune status (Zeppenfeld et al., 2014; Salbego et al., 2017; Boaventura et al., 2021) and extended shelf life of fish meat due to its antimicrobial and antioxidant activity (Moosavi-Nasab et al., 2019). Among the essential oils investigated for fish transport, *Ocimum gratissimum* stands out.

Ocimum gratissimum L., commonly known as African basil or clove basil, is a globally distributed plant. It is used as a culinary spice and in traditional medicine for its sedative properties and therapeutic applications (Akara et al., 2021). The main compounds found in *Ocimum gratissimum* L. essential oil (OGEO) are eugenol (43.3%) and 1,8-cineole (28.2%) (Chagas et al., 2021), both known for their antimicrobial properties (Zhang et al., 2013; Boijink et al., 2016) and anesthetic/sedative effects in fish (Silva et al., 2012b, 2015; Adewale et al., 2017). Previous studies have investigated the effects of OGEO on the transport of matrinxã (*Brycon cephalus*) (Inoue et al., 2003), tilapia (*Oreochromis niloticus*) (Ferreira et al., 2021), and pacamã (*Lophiosilurus alexandri*) (Boaventura et al., 2021). However, there is a lack of information in the literature regarding the use of anesthetics added to fish transport water and on residues of theses compounds in plasma, liver and fillet. Something important to be investigated, as it is essential these anesthetic does not leave residues in the meat.

Fish species belonging to the genus *Brycon*, such as *Brycon orbignyanus*, *Brycon amazonicus*, and *Brycon hilarii*, are highly regarded in fisheries and animal production due to their excellent growth performance (Zaniboni Filho et al., 2006; Antunes et al., 2010). Specifically, *B. hilarii* is appreciated for its excellent meat quality.

Therefore, this study aimed to evaluate the efficacy of OGEO in the transport water of *B. hilarii* juveniles, focusing on hematological and biochemical parameters, water quality, and tissue and plasma residue levels.

2. Material and methods

2.1. Animals and experimental conditions

The present study followed the guidelines for experimental procedures in animal research by Animal Use Ethics Committee (CEUA) of the State University do Mato Grosso do Sul – UEMS, Aquidauana, MS, Brazil (Protocol nº 013/2021).

Piraputanga (*Brycon hilarii*) juveniles were acquired from a commercial fish farm and acclimated for a period of 30 days in an 8 m^{-3} hapa net cages installed in a pond with continuous water flow at the fish farming unit of UEMS, located in Aquidauana - MS, Brazil. During the acclimation period, the average water quality parameters were monitored as follows: dissolved oxygen, 5.21 mg L^{-1}; temperature, 29.36 °C (measured using Alfakit AT 160); and pH, 6.48 (measured with a pH meter, Quimis QA338). The fish were fed twice daily with a commercial feed

containing 360 g kg^{-1} crude protein, 80 g kg^{-1} ether extract, 150 g kg^{-1} mineral matter, and 600 mg kg^{-1} vitamin C, as specified by the manufacturer. Prior to the transport experiment, all fish were fasted for a period of 24 h.

2.2. Ocimum gratissimum essential oil production and dissolution in water

The OGEO was obtained by hydrodistillation of fresh leaves of *Ocimum gratissimum* at the medicinal plant unit of Embrapa Western Amazon in Manaus - AM, Brazil.

To achieve complete dissolution in water, *Ocimum gratissimum* essential oil (OGEO) was diluted in ethyl alcohol (95%). The dilution ratio used for all concentrations of OGEO studied was 1:10 (v/v). Figure 1 illustrates the chemical composition of OGEO.

2.3. Experimental design

A total of 108 juvenile piraputanga fish (16.54 ± 1.65 g and 11.56 ± 0.42 cm) were randomly assigned to four treatment groups, with three replications each (n=9 per group). The experimental units consisted of 12 plastic bags (59.5 × 80.5 cm) with a total volume of 10 L, containing 4 L of water and 2/3 pure oxygen. The OGEO stock solution was diluted in ethyl alcohol 95% P.A (1:10) and tested at concentrations of 10 mg L^{-1} (group 1), 20 mg L^{-1} (group 2), and 30 mg L^{-1} (group 3), along with a control group (group 4) where only ethyl alcohol was added.

The plastic bags were sealed with an elastic band and placed on the body of a vehicle for a two-hour transport period (Ventura et al., 2020). After transport, the bags were opened, and water quality parameters were measured. Four fish from each bag (n=12 per treatment) were sampled for blood and tissue collection. The remaining fish from each treatment were relocated to four circular tanks with a useful volume of 100 L, equipped with continuous water flow and aeration, for assessment of survival rate and return to feeding for up to five days post-transport.

2.4. Blood collection and analysis

Blood samples were collected by caudal puncture using needles and syringes dipped in EDTA (3%). Blood glucose levels were quantified using individual aliquots of blood added to a glucose meter (Accu Chek - Active Roche), followed by reading. Hematocrit (Ht) was determined using the microhematocrit method described by Goldenfarb et al. (1971). Hemoglobin (Hb) levels were measured by spectrometry using the cyanmethemoglobin method described by Collier (1944), with a Labtest kit and a spectrophotometer set at 540 nm. The number of erythrocytes (Er) was counted in a Neubauer chamber after diluting the blood in formalin citrate solution (1:200). From these data, the following hematimetric indices were calculated: mean corpuscular volume (MCV, fL) = hematocrit / erythrocytes × 10; mean corpuscular hemoglobin concentration (MCHC) = hemoglobin / hematocrit × 100; and mean corpuscular hemoglobin (MCH) = hemoglobin / erythrocytes × 10, as proposed by Ranzani-Paiva et al. (2013).

Blood smears were prepared and stained with May Grünwald-Giemsa-Wright according to Tavares-Dias and Moraes (2004). Total leukocyte count, total thrombocyte count, and leukocyte differentiation were performed using an optical microscope with an oil immersion objective (100x). Plasma was obtained by centrifuging the blood (10 min) and used for the analysis of residual OGEO compounds.

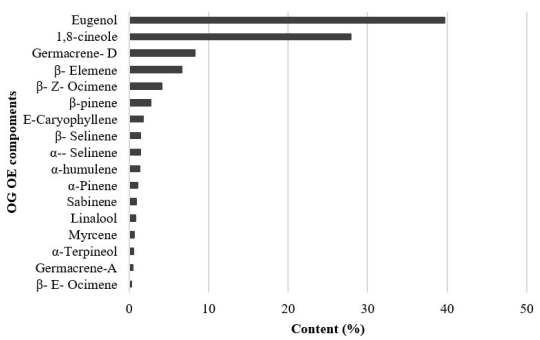

Figure 1. Chemical composition (%) of *Ocimum gratissimum* L. essential oil (OGEO) determined by gas chromatography-mass spectrometry (GC-MS).

2.5. Analyses of residual OGEO compounds in plasma, liver, and fillet

Fish from the blood collection were euthanized by deepening hypothermia in isothermal boxes with ice and rapid brain concussion. Liver and fillet samples were collected, labeled, and frozen at -20 °C. The analysis residual OGEO compounds ware carried out collaboration with the Instrumental Analysis Laboratory (CERNA) of the State University of Mato Grosso do Sul in Dourados - MS, Brazil.

Two grams of each tissue from each fish were weighed, and 10 mL of chromatographic grade hexane was added. The samples were homogenized and stirred in an ultrasonic tank (L-100-Schuster) for 30 min. Plasma samples (500 µL) were extracted with 2 mL of hexane (chromatographic grade) in an ultrasonic chamber for 5 min.

The hexane fraction was filtered, and the residue was extracted again three times consecutively using the same sample. The hexane fractions were combined and evaporated in a fume hood. The residue was then redissolved in 200 µL of hexane for analysis using gas chromatography coupled to mass spectrometry (GC-MS-2010 Ultra, Shimadzu, Kyoto, Japan).

To construct the analytical curve, concentrations ranging from 0.1 to 100 µg L^{-1} for plasma analysis and 0.1 to 100 µg kg^{-1} for liver and fillet analysis were prepared using 1,8-cineole, β-Z-ocimene, eugenol, β-elemene, and germacrene D. The limits of detection and quantification were determined using the signal-to-noise ratio approach.

Gas chromatography coupled to mass spectrometry was used for the analyses under the following conditions: helium gas (99.99% purity and flow rate of 1.0 mL min^{-1}), 1 µL injection volume in splitless mode. The oven temperature was programmed from 50 °C to 280 °C at a rate of 3 °C per minute, using a DB-5 column (30 m length × 0.25 mm internal diameter, 0.25 µm thickness).

Compound identification was performed by calculating the retention index using linear alkanes standard (C_7-C_{40}, Sigma Aldrich, purity ≥ 98%) and comparing the data and mass spectra of the samples with references (Adams, 2007) as well as databases National Institute of Standards and Technology (NIST Chemistry Webook).

Plasma and tissue analyses were performed using the same equipment and analysis conditions. The EO sample was prepared at a concentration of 1000 µg mL^{-1} in hexane (HPLC grade) and further diluted to a concentration of 100 µg mL^{-1} for analysis. Tissue samples (fillet and liver) were thawed in a temperature-controlled environment (22 °C).

2.6. Water quality

Water quality parameters including temperature, oxygen levels (measured using an oximeter, Alfakit AT160), pH (measured using a digital device, Quimis QA338), and total ammonia ($NH4^+$) (Labcon Test) were measured before and after transport.

2.7. Statistical analysis

The data were assessed for normality (Shapiro-Wilk test) and homoscedasticity of variances (Bartlett test). Water quality parameters were analyzed using ANOVA, followed by Tukey's post-test ($P < 0.05$), except for ammonia, which was subjected to the Kruskal-Wallis test and Dunn's test ($P < 0.05$). Blood parameters and residual compounds data were analyzed using ANOVA and linear regression ($P < 0.05$). All statistical analyses were performed using R statistical program version 3.4.3 (ExpDes.pt package).

3. Results

3.1. Blood parameters

The concentrations of *Ocimum gratissimum* essential oil (OGEO) used in the transport water of *Brycon hilarii* juveniles were found to be effective and safe, with minimal impact on the hematological and biochemical parameters of the fish.

The concentration of *Ocimum gratissimum* L. essential oil (OGEO) had a quadratic effect on the parameters of hemoglobin, mean corpuscular hemoglobin concentration (MCHC) e mean corpuscular hemoglobin (MCH), with values increasing at OGEO concentrations of 20 and 30 mg L^{-1} compared to the control group (Figure 2). Hematocrit, erythrocytes, mean corpuscular volume (MCV), and glucose levels were not significantly affected by transport or the OGEO treatments ($P > 0.05$).

The lymphocyte count showed a significant linear increase, which was attributed to the increasing OGEO concentrations in the transport water. However, in neutrophils, the linear regression equation did not indicate an increasing trend, as the highest values were observed in fish transported with a concentration of 10 mg L^{-1}, followed by the group of fish that did not receive OGEO in the water. Monocytes, thrombocytes, and leukocytes were not significantly affected by transport or the OGEO treatments (Table 1).

3.2. Residual compounds

The presence of residual OGEO compounds in the plasma, fillet, and liver at the end of transport did not

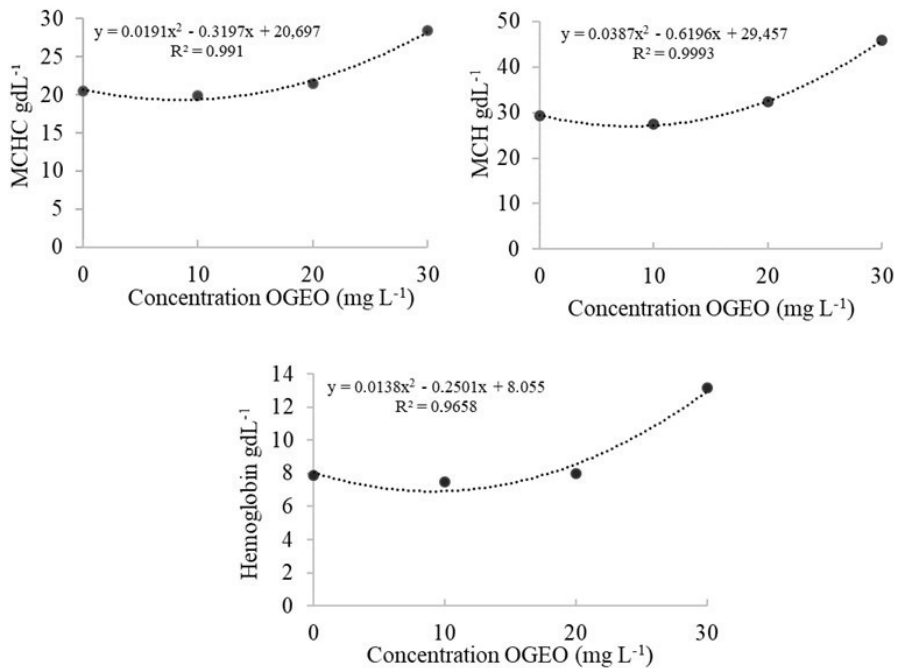

Figure 2. Mean (+SD) hemoglobin and hematimetric indices (MCHC and MCH) of juvenile Brycon hilarii after two hours of transport in plastic bags with different concentrations of *Ocimum gratissimum* essential oil (OGEO) in water.

Table 1. Means (+SD) differential and total leukocyte counts of juvenile *Brycon hilarii* after two hours of transport in plastic bags with different concentrations of *Ocimum gratissimum* essential oil (OGEO) in water.

Parameters	Concentrations of OGEO (mg L^{-1})				*P*-value	Regression model	R^2	CV (%)
	0	10	20	30				
Lymphocytes (x10 $^3\mu L^{-1}$)	61.16 ± 6.56	62.94 ± 9.60	81.74 ± 31.62	116.62 ± 0.52	0.038	Y= 53.565 + 1.674x	0.85	24.14
Neutrophils (x10 $^3\mu L^{-1}$)	44.85 ±16.26	55.53 ± 5.34	23.23 ±13.95	21.50 ± 3.24	0.040	Y= 51.635 -1.023x	0.62	37.67
Monocytes (x10 $^3\mu L^{-1}$)	27.63 ± 6.90	22.67 ± 10.80	7.60 ± 1.95	11.41 ±1.94	0.088	ns	-	54.19
Thrombocytes (x10 $^3\mu L^{-1}$)	44.68 ±3.49	33.34 ±8.13	36.58± 9.82	44.96 ±11.06	0.640	ns	-	33.14
Leukocytes (x10 $^3\mu L^{-1}$)	38.55 ±10.98	24.91 ± 5.06	11.16 ± 1.13	19.48 ± 12.13	0.072	ns	-	46.69
Erythrocytes (x10 $^3\mu L^{-1}$)	2.76 ± 0.53	2.76 ± 0.32	2.55 ± 0.79	2.89 ± 0.12	0.860	ns		18.33

cause any toxic effects. Residues of eugenol, 1,8-cineole, germacrene D, β-elemene, and B-Z-ocimene were detected in the plasma, fillet, and liver of juvenile *B. hilarii*. Linear regression analysis ($P < 0.05$) indicated that the levels of residual compounds increased with higher concentrations of *Ocimum gratissimum* essential oil (OGEO) in the transport water. No residues of the analyzed compounds were found in the control treatment (Table 2).

Eugenol, is the main constituent of OGEO, and exhibited the highest residual values in the plasma, fillet, and liver of *B. hilarii* across all concentrations used in the transport water.

3.3. Water quality and mortality

Table 3 displays the water quality parameters and survival rates before and after transport. No mortalities were recorded during transport, but within the five-day post-transport observation period, three deaths occurred in the control group, while one death each occurred in the 10, 20, and 30 mg L^{-1} OGEO groups. Nonetheless, there was no significant difference ($P > 0.05$) in mortality rates between the treatments.

The concentration of dissolved oxygen remained high in the control group and in the group receiving 20 mg L^{-1}, 12.31 mg L^{-1} and 12.08 mg L^{-1} of OGEO, respectively, after transport. Regarding temperature, no significant differences were observed between the groups. However, the temperature was higher (30 °C), and the values were significantly ($P < 0.05$) elevated in all treated groups compared to the temperature observed before transport. The pH decreased in all groups compared to the pre-transport values ($P < 0.05$). Ammonia levels did not differ significantly between the groups ($P < 0.05$).

4. Discussion

The concentrations of *Ocimum gratissimum* essential oil (OGEO) used in the transport water of *Brycon hilarii* juveniles were found to be effective and safe, with minimal impact on the hematological and biochemical parameters of the fish. The presence of residual OGEO compounds in the plasma, fillet, and liver at the end of transport did not cause any toxic effects.

The use of anesthetics before and during fish transport is a common practice in fish farming as it helps reduce fish metabolism and oxygen consumption, resulting in lower production of CO_2 and ammonia (Harmon, 2009; Sampaio and Freire, 2016). The increase in temperature after transport was expected, as the air temperature during transport was 32 °C, and the packages were not insulated to prevent temperature fluctuations. Higher temperatures can lead to increased oxygen consumption and ammonia concentration in the water.

pH plays a role in regulating the toxicity of metabolites such as ammonia and maintaining the balance between CO_2 and HCO_3^- by releasing H+ ions when the pH decreases (Kubitza, 1998). The decrease in pH observed in the transport water was likely due to the accumulation of CO_2 generated by the natural respiration of the fish, causing acidification (Boyd and Tucker, 2012; Kamalam et al., 2017), which prevented an increase in ammonia toxicity. The biggest problem encountered in transporting fish in plastic bags is the accumulation of carbon dioxide and ammonia (caused by physiological processes such as respiration and excretion), which can generate stress in fish (Carneiro et al., 2009; Sampaio and Freire, 2016).

Dissolved oxygen concentration is a critical parameter for the transport system. Dissolved oxygen levels remained high after transporting *B. hilarii*, which can be attributed to the use of pure oxygen in transport bags, a common practice among fish producers (Inoue et al., 2005; Sampaio and Freire, 2016). Vehicle movements and the pressure on the transport bags can also increase dissolved oxygen levels (Zeppenfeld et al., 2014; Mazandarani et al., 2017). The water fluctuations observed during transport were within acceptable levels, as the *B. hilarii* juveniles arrived with 100% survival.

Studies have shown that OGEO can act as an efficient natural anesthetic (Silva et al., 2015; Ribeiro et al., 2016; Aydin and Barbas, 2020; Silva et al., 2020), anthelmintic (Boijink et al., 2016), immunomodulator, and growth

Table 2. Residual compounds in plasma fillet and liver (mean ± SD) after a two-hour transport of *Brycon hilarii* juveniles in plastic bags containing water with different concentrations of *Ocimum gratissimum* essential oil (OGEO) in water.

Compounds OGEO	Concentrations of OGEO (mg L^{-1})				*P* - value	Regression model	R^2	CV (%)
	0	10	20	30				
Plasma								
Eugenol (µg kg^{-1})	0.00 ± 0.00	9.62 ± 0.05	20.18 ± 0.21	29.60 ± 0.02	< 0.001	Y = - 0.0518+ 0.9936x	0.99	0.07
1,8-Cineole (µg kg^{-1})	0.00 ± 0.00	5.01 ± 0.03	11.03 ± 0.14	16.08 ± 0.01	< 0.001	Y = - 0.4256 + 0.1311x	0.93	0.49
Germacrene-D (µg kg^{-1})	0.00 ± 0.00	1.48 ± 0.03	2.75 ± 0.01	4.97 ± 0.05	< 0.001	Y = - 0.1282 + 0.1616x	0.99	1.14
β-Elemene (µg kg^{-1})	0.00 ± 0.00	1.10 ± 0.03	2.23 ± 0.02	3.37 ± 0.06	< 0.001	Y = - 0.0113 + 0.1123x	0.99	2.10
B- Z-ocimene (µg kg^{-1})	0.00 ± 0.00	0.77 ± 0.01	1.42 ± 0.02	2.12 ± 0.03	< 0.001	Y = 0.0259 + 0.700x	0.99	1.64
Muscle								
Eugenol (µg kg^{-1})	0.00 ± 0.00	12.17 ± 0.01	24.03 ± 0.05	36.32 ± 0.12	< 0.001	Y = 0.0082 + 1.2083x	0.99	0.07
1,8-Cineole (µg kg^{-1})	0.00 ± 0.00	7.07 ± 0.01	14.04 ± 0.02	21.04 ± 0.02	< 0.001	Y= 0.0262 + 0.7009x	0.99	0.49
Germacrene-D (µg kg^{-1})	0.00 ± 0.00	2.15 ± 0.02	4.30 ± 0.02	6.53 ± 0.03	< 0.001	Y= - 0.0169 +0.2178x	0.99	1.14
β-Elemene (µg kg^{-1})	0.00 ± 0.00	1.87 ± 0.01	3.10 ± 0.04	4.58 ± 0.02	< 0.001	Y= 0.1430 + 0.1497x	0.99	2.10
β-Z-Ocimene (µg kg^{-1})	0.00 ± 0.00	0.87 ± 0.01	1.58 ± 0.06	2.35 ± 0.01	< 0.001	Y= 0.1913 + 0.0492x	0.91	2.47
Liver								
Eugenol (µg kg^{-1})	0.00 ± 0.00	8.04 ± 0.06	17.09 ± 0.10	26.36 ± 0.03	< 0.001	Y = -0.3477 + 0.8813x	0.99	0.46
1,8-Cineole (µg kg^{-1})	0.00 ± 0.00	7.75 ± 2.97	10.24 ± 0.18	15.04 ± 0.02	< 0.001	Y = 1.1183 + 0.4760x	0.95	18.04
Germacrene-D (µg kg^{-1})	0.00 ± 0.00	1.50 ± 0.02	2.66 ± 0.01	4.59 ± 0.03	< 0.001	Y = - 0.0500 + 0.1492x	0.99	0.76
β-Elemene (µg kg^{-1})	0.00 ± 0.00	0.95 ± 0.00	1.95 ± 0.02	3.50 ± 0.03	< 0.001	Y = - 0.1262 + 0.1151x	0.98	1.20
β-Z-Ocimene (µg kg^{-1})	0.00 ± 0.00	0.86 ± 0.00	1.56 ± 0.01	2.35 ± 0.01	< 0.001	Y = 0.0316 + 0.0774x	0.99	0.68

Table 3. Water quality parameters and survival before and after (mean ± SD) a two-hour transport of *Brycon hilarii* in plastic bags containing different concentrations of *Ocimum gratissimum* L. essential oil (OGEO) in water.

Parameter	Before transport	After transport Concentrations of OGEO (mg L^{-1})			
		0	10	20	30
Dissolved oxygen (mg L^{-1})	5.13^{b}	12.31 ± 3.25^{a}	8.98 ± 1.14^{ab}	12.08 ± 3.68^{a}	7.63 ± 1.88^{ab}
Temperature (°C)	26.40^{b}	30.53 ± 1.18^{a}	30.93 ± 0.31^{a}	30.40 ± 0.90^{a}	30.06 ± 0.69^{a}
pH	6.69^{a}	6.13 ± 0.01^{b}	6.14 ± 0.00^{b}	6.31 ± 0.01^{ab}	6.26 ± 0.01^{b}
Total ammonia	0.00^{a}	0.012 ± 0.006^{a}	0.011 ± 0.00^{a}	0.010 ± 0.007^{a}	0.012 ± 0.003^{a}
Survival (%)	100^{a}	88.98^{a}	96.30^{a}	96.30^{a}	96.30^{a}

Notes below = Different letters in the rows indicate significant differences between groups after transport. The ammonia was submitted to the Kruskal-Wallis test followed by the Dunn's test *P* (<005).

promoter when added to the diet of *O. niloticus* (Brum et al., 2017). In addition to its use as a safe alternative for short-term transport (Benovit et al., 2012), as it does not affect hematological parameters, there is a lack of studies evaluating the clearance period of OGEO in fish.

The use of OGEO during transport did not prevent hyperglycemia in *B. hilarii* juveniles, which is consistent with observations in *Pseudoplatystoma reticulatum* anesthetized with OGEO (Silva et al., 2020). Fish release corticosteroids and catecholamines in stressful situations, activating glycogenolysis and gluconeogenesis, resulting in increased glucose levels as an adaptive response to provide energy during transport and help maintain homeostasis (Barton and Iwama, 1991; Pankhurst, 2011; Sena et al., 2016). Although OGEO has a sedative effect, there was no reduction in energy demand during the transport conducted in this study. The disturbance in the water inside the plastic bags caused by transport via car, along with exposure to ambient temperature, may have contributed to the observed lack of reduction in glucose levels in this

study. The catecholamines released also regulate cardiac and respiratory functions, by promoting increased blood flow, resulting in an increase in red cells and a greater affinity of hemoglobin for oxygen. This process optimizes tissue oxygenation, ensuring adequate cardiorespiratory function (Reid, 2011; Wendelaar Bonga, 2011).

Hemoglobin, along with erythrocytes and red cells, is responsible for oxygen transport in the blood (Maekawa and Kato, 2015), and its concentration can increase in response to stress and hypoxia to enhance oxygen transport and supply energy (Wojtaszek et al., 2002; Souza and Bonilla-Rodriguez, 2007). The OGEO concentration of 30 mg L^{-1} in the transport water significantly increased the hemoglobin concentration in the fish, leading to greater oxygen expenditure. Chagas et al. (2012) also observed an increase in hemoglobin concentration in tambaqui fed diets containing β-glucan after a 3-h transport.

The hematimetric indices MCHC and MCH exhibited increased values at the highest concentration of OGEO. These indices are useful in monitoring pathologies and stress and reflect the physiological state of the animal (Silva et al., 2012a). The increase in hemoglobin concentration is associated with the observed changes in these indices. No significant difference was found in the erythrocyte count, consistent with the findings of Abreu et al. (2008) during a 4-h transport of *B. amazonicus*. Overall, the hematological values of juvenile *B. hilarii* observed in this study align with the established hematological profiles for the genus Brycon (Dal'Bó et al., 2015). Stressors can, in certain situations, cause harmful deformation in the morphology of red blood cells, while the number of erythrocytes remains constant (Esmaeili, 2021).

The fish that received OGEO concentrations of 20 and 30 mg L^{-1} in the transport water exhibited a reduction in the number of neutrophils, monocytes, and leukocytes compared to the fish that did not receive OGEO. This decrease in immune cell count can be attributed to the higher OGEO concentrations and suggests a potential weakening of the immune system (Vosyliené, 1999) due to exposure to OGEO. Lymphocytosis was observed as the concentrations of OGEO in the transport water increased.

Lymphocytes are the predominant cells in most fish species and play a crucial role in antigen recognition (Tavares-Dias et al., 1999; Ranzani-Paiva, 2007), consistent with the findings of this study. They contribute significantly to immune function, and an increase in their numbers may indicate immune stimulation (Clauss et al., 2008; Montanha and Pimpão, 2012). Similar findings of increased lymphocyte numbers were reported in *Piaractus mesopotamicus* anesthetized with *Ocimum basilicum* essential oil at concentrations up to 350 mg L^{-1} (Ventura et al., 2021). Neutrophils play a vital role in the microbicidal activity of the respiratory burst, converting molecular oxygen into oxygen compounds and metabolites (Plyzcz et al., 1989). Stressors can lead to an increase in the number of neutrophils, as observed in this study with fish transported at 10 mg L^{-1} of OGEO. Glucocorticoid hormones, supposedly, can influence the redistribution of lymphocytes from the blood to other tissues and promote the release of neutrophils from the leukopoietic organs into the blood (Dhabhar et al., 1996; Grzelak et al., 2017)

Studying the residues of essential oil compounds in animals is important for determining their various applications. The residual compounds analyzed in the transport water were absorbed by the plasma, fillet, and liver of juvenile *B. hilarii*. The concentrations of each compound found were proportional to the percentage composition of OGEO and the concentrations applied in the transport water. This observation can be explained by the rapid absorption, exposure time, and low water solubility of OGEO, as also observed in the fillet of *O. niloticus* anesthetized with clove oil and benzocaine (Pereira et al., 2015). Although anesthetic residues are not harmful to human health according to Stone and Tostin (1999), they can affect the taste of fish meat, emphasizing the need for a purification period to reduce compound concentrations (Botrel et al., 2017).

Eugenol, the major compound found in plasma, fillet, and liver, is consistent with the composition of OGEO. Essential oils are known to be complex, containing up to 60 compounds in varying concentrations, with two to three major compounds that often determine their biological properties (Bakkali et al., 2008). Eugenol is a well-known natural compound used in aquaculture due to its sedative and anesthetic effects on fish. It is readily available in the market at a low cost (Inoue et al., 2003) and possesses various beneficial properties such as analgesic, antiseptic, anti-inflammatory, antibacterial, and antifungal actions (Kamatou et al., 2012; Singh et al., 2018). After anesthesia, eugenol residues are eliminated from the fish organism within 24 h (Delbon and Ranzani-Paiva, 2012). According to the FAO (2007), the limit of eugenol intake in muscle tissue is 2.5 mg kg^{-1}, which is higher than the recorded value of 26.32 µg kg^{-1} in this study.

Cineole, also known as eucalyptol, is a terpene compound found in several essential oils, primarily in eucalyptus oil (*Eucalyptus globulus* Labill) (Dhakad et al., 2018). It exhibits anti-inflammatory, antioxidant (Ryu et al., 2014; Caceres et al., 2017), and insecticidal properties (Kiran and Prakash, 2015). Mazandarani and Hoseini (2016) reported that 1,8-cineole induced deep anesthesia in *Cyprinus carpio* at concentrations ranging from 300 to 800 mgL^{-1}, while concentrations from 200 to 800 µL L^{-1} induced deep anesthesia in trout (*Oncorhynchus mykiss*) (Mirghaed et al., 2018). Nevertheless, these studies did not report on residues in fish. According to Bullangpoti et al. (2018), 1,8-cineole inhibited acetylcholinesterase and exhibited moderate toxicity to guppy (*Poecilia reticulata*) after 24 h of exposure, with the LC50 concentrations for females and males being 3997 and 1701 mg L^{-1}, respectively.

The health benefits of essential oils are closely related to their chemical constituents. While the main compounds often determine the biological properties of the oils, the synergistic effect of secondary compounds also plays a significant role (Rattan, 2010; Raut and Karuppayil, 2014; Zhou et al., 2019). Considering the low residual concentrations of cineole found in the analyzed tissues, together with eugenol, this study demonstrates the promising use of OGEO in the transport water of juvenile *B. hilarii* to ensure the well-being of the fish.

5. Conclusion

The use of 10 mg L^{-1} OGEO in the two-hour transport water is promising to ensure the survival and well-being of *Brycon hilarii* juveniles (weighing 16 g), showing to be safe and effective. The residual concentration of eugenol the major compound of OGEO in the fillet remains below the maximum limit of the recommended daily intake.

Acknowledgements

The authors acknowledge Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES for the doctoral scholarship granted to Oliveira, F.C. and for the postdoctoral fellowship granted Ferreira, A. L. (nº 88887.691567/2022-00 and 88887.808250/2023-00). We also appreciate the Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul – FUNDECT for supporting the research project (Process: 71/049.087/2021).

References

ABREU, J.S., SANABRIA-OCHOA, A.I., GONÇALVES, F.D. and URBINATI, E.C., 2008. Stress responses of juvenile matrinxã (*Brycon amazonicus*) after transport in a closed system under different loading densities. *Ciência Rural*, vol. 38, no. 5, pp. 1413-1417. http://dx.doi.org/10.1590/S0103-84782008000500034.

ADAMS, R.P., 2007. *Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy*. 4th ed. Illinois: Allured Publishing Corporation.

ADEWALE, A.Y., ADESHINA, I. and YUSUF, O.Y., 2017. Anaesthetic effect of *Ocimum gratissimum* extract on *Oreochromis niloticus* juveniles. *European Journal of Experimental Biology*, vol. 7, pp. 2-7.

AKARA, E.U., EMMANUEL,EO., UDE, V.C., UCHE-IKONNE, C., EKE, G. and UGBOGU, A.E., 2021. *Ocimum gratissimum* leaf extract ameliorates phenylhydrazine-induced anaemia and toxicity in Wistar rats. *Drug Metabolism and Personalized Therapy*, vol. 36, no. 4, pp. 311-320. http://dx.doi.org/10.1515/dmpt-2020-0185. PMid:34821128.

ANTUNES, R.S.P., GOMES, V.N., PRIOLI, S.M.A.P., PRIOLI, R.A., JÚLIO JÚNIOR, J.H.F., PRIOLI, L.M., AGOSTINHO, C.S. and PRIOLI, A.J., 2010. Molecular characterization and phylogenetic relationships among species of the genus *Brycon* (Characiformes: Characidae) from four hydrographic basins in Brazil. *Genetics and Molecular Research*, vol. 9, no. 2, pp. 674-684. http://dx.doi.org/10.4238/vol9-2gmr759. PMid:20449799.

AYDIN, B. and BARBAS, L.A.L., 2020. Sedative and anesthetic properties of essential oils and their active compounds in fish: a review. *Aquaculture*, vol. 520, pp. 734999. http://dx.doi.org/10.1016/j.aquaculture.2020.734999.

BAKKALI, F., AVERBECK, S., AVERBECK, D. and IDAOMAR, M., 2008. Biological effects of essential oils - *A review*. *Food and Chemical Toxicology*, vol. 46, no. 2, pp. 446-475. http://dx.doi.org/10.1016/j.fct.2007.09.106. PMid:17996351.

BALASCH, J.C. and TORT, L., 2019. Netting the stress responses in fish. *Frontiers in Endocrinology*, vol. 10, no. 62, pp. 62. PMid:30809193.

BALDISSEROTTO, B., ROMERO, J.M.M. and KAPOOR, B.G., 2007. *Fish osmoregulation*. Enfield: Science Publishers, 527 p.

BARBAS, L.A.L., ARAÚJO, E.R.L., TORRES, M.F., MALTEZ, L.C., GARCIA, L.O., HEINZMANN, B.M. and SAMPAIO, L.A., 2020. Stress relieving potential of two plant-based sedatives in the transport of juvenile tambaqui *Colossoma macropomum*. *Aquaculture*, vol. 520, pp. 734681. http://dx.doi.org/10.1016/j.aquaculture.2019.734681.

BARTON, B.A. and IWAMA, G.K., 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Review of Fish Diseases*, vol. 1, pp. 3-26. http://dx.doi.org/10.1016/0959-8030(91)90019-G.

BARTON, B.A., 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology*, vol. 42, no. 3, pp. 517-525. http://doi.org/10.1093/icb/42.3.517. PMid:21708747.

BECKER, A.G., PARODI, T.V., ZEPPENFELD, C.C., SALBEGO, J., CUNHA, M.A., HELDWEIN, C.G., LORO, V.L., HEINZMANN, B.M. and BALDISSEROTTO, B., 2016. Pre-sedation and transport of *Rhamdia quelen* in water containing essential oil of *Lippia alba*: metabolic and physiological responses. *Fish Physiology and Biochemistry*, vol. 42, no. 1, pp. 73-81. http://dx.doi.org/10.1007/s10695-015-0118-x. PMid:26297516.

BECKER, A.G., LUZ, R.K., MATTIOLI, C.C., NAKAYAMA, C.L., SILVA, W.S., LEME, F.O.P., MENDES, H.C.P.M., HEINZMANN, B.M. and BALDISSEROTTO, B., 2017. Can the essential oil of *Aloysia triphylla* have anesthetic effect and improve the physiological parameters of the carnivorous freshwater catfish *Lophiosilurus alexandri* after transport? *Aquaculture*, vol. 481, pp. 184-190. http://dx.doi.org/10.1016/j.aquaculture.2017.09.007.

BENOVIT, S.C., GRESSLER, L.T. and SILVA, L.L., 2012. Anesthesia and transport of Brazilian flounder *Paralichthys orbignyanus* with essential oils of *Aloysia gratissima* and *Ocimum gratissimum*. *Journal of the World Aquaculture Society*, vol. 43, no. 6, pp. 896-900. http://dx.doi.org/10.1111/j.1749-7345.2012.00604.x.

BOAVENTURA, T.P., SOUZA, C.F., FERREIRA, A.L., FAVERO, G.C., BALDISSERA, M.D., HEINZMANN, B.M., BALDISSEROTTO, B. and LUZ, R.K., 2021. The use of *Ocimum gratissimum* L essential oil during the transport of *Lophiosilurus alexandri:* water quality hematology blood biochemistry and oxidative stress. *Aquaculture*, vol. 531, pp. 735964. http://dx.doi.org/10.1016/j.aquaculture.2020.735964.

BOIJINK, C.L., QUEIROZ, C.A., CHAGAS, E.C., CHAVES, F.C.M. and INOUE, L.A.K.A., 2016. Anesthetic and anthelminthic effects of clove basil (*Ocimum gratissimum*) essential oil for tambaqui (*Colossoma macropomum*). *Aquaculture*, vol. 457, pp. 24-28. http://dx.doi.org/10.1016/j.aquaculture.2016.02.010.

BOLASINA, S.N., AZEVEDO, A. and PETRY, A.C., 2017. Comparative efficacy of benzocaine tricaine methanesulfonate and eugenol as anesthetic agents in the guppy *Poecilia vivipara*. *Aquaculture Reports*, vol. 6, pp. 56-60. http://dx.doi.org/10.1016/j.aqrep.2017.04.002.

BOTREL, B.M., ABREU, D.C., SACZK, A.A., BAZANA, M.J., COELHO, S.M.E., ROSA, P.V., MAGRIOTIS, Z.M. and DE LIMA, R.M., 2017. Residual determination of anesthetic menthol in fishes by SDME/GC-MS. *Food Chemistry*, vol. 229, pp. 674-679. http://dx.doi.org/10.1016/j.foodchem.2017.02.087. PMid:28372230.

BOYD, C.E. and TUCKER, C.S., 2012. *Pond aquaculture water quality management*. New York: Springer Science and Business Media.

BRUM, A., PEREIRA, S.A., OWATARI, M.S., CHAGAS, E.C., CHAVES, F.C.M., MOURIÑO, J.L.P. and MARTINS, M.L., 2017. Effect of dietary essential oils of clove basil and ginger on Nile tilapia (*Oreochromis niloticus*) following challenge with *Streptococcus agalactiae*. *Aquaculture*, vol. 468, pp. 235-243. http://dx.doi.org/10.1016/j.aquaculture.2016.10.020.

BULLANGPOTI, V., MUJCHARIYAKUL, W., LAKSANAVILAT, N. and JUNHIRUN, P., 2018. Acute toxicity of essential oil compounds

(thymol and 18-cineole) to insectivorous guppy *Poecilia reticulata* Peters 1859. *Agriculture and Natural Resources*, vol. 52, pp. 190-194.

CACERES, A.I., LIU, B., JABBA, S.V., ACHANTA, S., MORRIS, J.B. and JORDT, S.E., 2017. Transient receptor potential cation channel subfamily M member 8 channels mediate the anti-inflammatory effects of eucalyptol. *British Journal of Pharmacology*, vol. 174, no. 9, pp. 867-879. http://dx.doi.org/10.1111/bph.13760. PMid:28240768.

CARNEIRO, P.C.F., KAISELER, P.H.S., SWAROFSKY, E.A.C. and BALDISSEROTTO, B., 2009. Transport of jundiá *Rhamdia quelen* juveniles at different loading densities: water quality and blood parameters. *Neotropical Ichthyology*, vol. 7, no. 2, pp. 283-288. http://dx.doi.org/10.1590/S1679-62252009000200021.

CARVALHO, E.S., GOMES, L.C., BRANDÃO, F.R., CRESCÊNCIO, R., CHAGAS, E.C. and ANSELMO, A.A.S., 2009. Uso do probiótico Efinol®L durante o transporte de tambaqui. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, vol. 61, no. 6, pp. 1322-1327. http://dx.doi.org/10.1590/S0102-09352009000600011.

CHAGAS, E.C., ARAÚJO, L.D., BOIJINK, C.D.L., INOUE, L.A.K.A., GOMES, L.D.C. and MORAES, F.R., 2012. Respostas de tambaquis ao estresse por transporte após alimentação com dietas suplementadas com β-glucano. *Biotemas*, vol. 25, no. 4, pp. 221-227. http://dx.doi.org/10.5007/2175-7925.2012v25n4p221.

CHAGAS, E.C., BRANDÃO, F.R., FARIAS, C.F.S., MONTEIRO, P.C., BATISTA, E.S., SOUZA, D.C.M., CHAVES, F.C.M. and INOUE, L.A.K.A., 2021. Physiological changes in juvenile tambaquis (*Colossoma macropomum*) transported using essential oil of *Ocimum gratissimum. Agrarian*, vol. 14, pp. 102-111. http://dx.doi.org/10.30612/agrarian.v14i51.12279.

CLAUSS, T.M., DOVE, A.D.M. and ARNOLD, J.E., 2008. Hematologic disorders of fish. *Veterinary Clinics of North America: Exotic Animal Practice*, vol. 11, no. 3, pp. 445-462, v. http://dx.doi.org/10.1016/j.cvex.2008.03.007. PMid:18675728.

COLLIER, H.B., 1944. Standardization of blood haemoglobin determinations. *Canadian Medical Association Journal*, vol. 50, no. 6, pp. 550-552. PMid:20323122.

DAL'BÓ, G.A., SAMPAIO, F.G., LOSEKANN, M.E., QUEIROZ, J.F., LUIZ, A.J.B., WOLF, V.H.G., GONÇALVES, V.T. and CARRA, M.L., 2015. Hematological and morphometric blood value of four cultured species of economically important tropical foodfish. *Neotropical Ichthyology*, vol. 13, no. 2, pp. 439-446. http://dx.doi.org/10.1590/1982-0224-20140115.

DELBON, M.C. and RANZANI-PAIVA, M.J.T., 2012. Eugenol em juvenis de tilápia do Nilo: concentrações e administrações sucessivas. *Boletim do Instituto de Pesca*, vol. 38, pp. 43-52.

DHABHAR, F.S., MILLER, A.H., MCEWEN, B.S. and SPENCER, R.L., 1996. Stress-induced changes in blood leukocyte distribution-role of adrenal steroid hormones. *Journal of Immunology*, vol. 157, no. 4, pp. 1638-1644. http://dx.doi.org/10.4049/jimmunol.157.4.1638. PMid:8759750.

DHAKAD, A.K., PANDEY, V.V., BEG, S., RAWAT, J.M. and SINGH, A., 2018. Biological, medicinal and toxicological significance of *Eucalyptus* leaf essential oil: a review. *Journal of the Science of Food and Agriculture*, vol. 98, no. 3, pp. 833-848. http://doi.org/10.1002/jsfa.8600. PMid:28758221.

EDRIS, A.E., 2007. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. *Phytotherapy Research*, vol. 21, no. 4, pp. 308-323. http://dx.doi.org/10.1002/ptr.2072. PMid:17199238.

ESMAEILI, M., 2021. Blood performance: a new formula for fish growth and health. *Biology*, vol. 10, no. 12, pp. 1236. http://dx.doi.org/10.3390/biology10121236. PMid:34943151.

ESPINOZA-RAMOS, L.A., PEPE-VICTORIANO, R., HUANACUNI, J.I. and NANDE, M., 2022. Effect of transportation time and stocking density on seawater quality and survival of *Anisotremus scapularis* (Perciformes: haemulidae). *Journal of the World Aquaculture Society*, vol. 53, no. 5, pp. 1042-1050. http://dx.doi.org/10.1111/jwas.12865.

FERREIRA, A.L., FAVERO, G.C., BOAVENTURA, T.P., FREITAS SOUZA, C., FERREIRA, N.S., DESCOVI, S.N., BALDISSEROTTO, B., HEINZMANN, B.M. and LUZ, R.K., 2021. Essential oil of *Ocimum gratissimum* (Linnaeus 1753): efficacy for anesthesia and transport of *Oreochromis niloticus. Fish Physiology and Biochemistry*, vol. 47, no. 1, pp. 135-152. http://dx.doi.org/10.1007/s10695-020-00900-x. PMid:33196935.

FERREIRA, A.L., DOS SANTOS, F.A.C., SOUZA, A.D.S., FAVERO, G.C., BALDISSEROTTO, B., PINHEIRO, C.G., HEINZMANN, B. and LUZ, R.K., 2022. Efficacy of *Hesperozygis ringens* essential oil as an anesthetic and for sedation of juvenile tambaqui (*Colossoma macropomum*) during simulated transport. *Aquaculture International*, vol. 30, no. 3, pp. 1549-1561. http://dx.doi.org/10.1007/s10499-022-00868-w.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS – FAO, 2007. *Fish Stats Plus: universal software for fishery statistical time series: version 2.32*. Rome: FAO.

GOLDENFARB, P.B., BOWYER, F.P., HALL, E. and BROSIOUS, E., 1971. Reproducibility in the hematology laboratory: the microhematocrit determinations. *American Journal of Clinical Pathology*, vol. 56, no. 1, pp. 35-39. http://dx.doi.org/10.1093/ajcp/56.1.35. PMid:5556212.

GREEN, C. and HAUKENES, A., 2015. *The role of stress in fish disease.* Stoneville: Southern Regional Aquaculture Center, pp. 1-3. SRAC Publication, no. 474.

GRZELAK, A.K., DAVIS, D.J., CARAKER, S.M., CRIM, M.J., SPITSBERGEN, J.M. and WIEDMEYER, C.E., 2017. Stress leukogram induced by acute and chronic stress in zebrafish (*Danio rerio*). *Comparative Medicine*, vol. 67, no. 3, pp. 263-269. PMid:28662755.

HARMON, T.S., 2009. Methods for reducing stressors and maintaining water quality associated with live fish transport in tanks: a review of the basics. *Reviews in Aquaculture*, vol. 1, no. 1, pp. 58-66. http://dx.doi.org/10.1111/j.1753-5131.2008.01003.x.

INOUE, L.A.K.A., SANTOS NETO, C. and MORAES, G., 2003. Clove oil as anaesthetic for juveniles of matrinxã *Brycon cephalus* (Gunther 1869). *Ciência Rural*, vol. 33, no. 5, pp. 943-947. http://dx.doi.org/10.1590/S0103-84782003000500023.

INOUE, L.A.K.A., AFONSO, L.O.B., IWAMA, G.K. and MORAES, G., 2005. Effects of clove oil on the stress response of matrinxã (*Brycon cephalus*) subjected to transport. *Acta Amazonica*, vol. 35, no. 2, pp. 289-295. http://dx.doi.org/10.1590/S0044-59672005000200018.

IVERSEN, M., FINSTAD, B. and NILSSEN, K.J., 1998. Recovery from loading and transport stress in Atlantic salmon (*Salmo salar* L.) smolts. *Aquaculture*, vol. 168, no. 1-4, pp. 387-394. http://dx.doi.org/10.1016/S0044-8486(98)00364-0.

KAMALAM, B.S., PATIYAL, R.S., RAJESH, M., MIR, J.I. and SINGH, A.K., 2017. Prolonged transport of rainbow trout fingerlings in plastic bags: optimization of hauling conditions based on survival and water chemistry. *Aquaculture*, vol. 480, pp. 103-107. http://dx.doi.org/10.1016/j.aquaculture.2017.08.012.

KAMATOU, G.P., VERMAAK, I. and VILJOEN, A.M., 2012. Eugenol-from the remote maluku islands to the international market place: a review of a remarkable and versatile molecule. *Molecules*, vol. 17, no. 6, pp. 6953-6981. http://dx.doi.org/10.3390/molecules17066953. PMid:22728369.

KIRAN, S. and PRAKASH, B., 2015. Toxicity and biochemical efficacy of chemically characterized *Rosmarinus officinalis* essencial oil against *Sitophilus oryzae* and *Oryzaephilus surinamensis*. *Industrial Crops and Products*, vol. 74, no. 15, pp. 817-823.

KUBITZA, F., 1997. Transporte de peixes vivos. *Revista Panorama da Aquicultura*, vol. 7, pp. 20-26.

KUBITZA, F., 1998. Qualidade da água na produção de peixes: parte II. *Revista Panorama da Aquicultura*, vol. 8, no. 46, pp. 35-41.

MAEKAWA, S. and KATO, T., 2015. Diverse of erythropoiesis responding to hypoxia and low environmental temperature in vertebrates. *BioMed Research International*, vol. 2015, pp. 747052. http://dx.doi.org/10.1155/2015/747052. PMid:26557695.

MATTOS, D.C., MANHÃES, J.V.A., CARDOSO, L.D., ARIDE, P.H.R., LAVANDER, H.D., OLIVEIRA, A.T., RADAEL, M.C., AZEVEDO, R.V. and VIDAL JUNIOR, M.V., 2023. Influence of garlic extract on larval performance and survival of juvenile angelfish *Pterophyllum scalare* during transport. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 83, e244480. http://doi.org/10.1590/1519-6984.244480. PMid:34259780.

MAZANDARANI, M. and HOSEINI, S.M., 2016. Menthol and 18-cineole as new anaesthetics in common carp *Cyprinus carpio* (Linnaeus 1758). *Aquaculture Research*, vol. 48, no. 6, pp. 3041-3051. http://dx.doi.org/10.1111/are.13136.

MAZANDARANI, M., HOSEINI, S.M. and DEHGHANI GHOMSHANI, M., 2017. Effects of linalool on physiological responses of *Cyprinus carpio* (Linnaeus 1758) and water physico-chemical parameters during transportation. *Aquaculture Research*, vol. 48, no. 12, pp. 5775-5781. http://dx.doi.org/10.1111/are.13400.

MIRGHAED, A.T., GHELICHPOUR, M., ZARGARI, A. and YOUSEFI, M., 2018. Anaesthetic efficacy and biochemical effects of 18-cineole in rainbow trout (*Oncorhynchus mykiss* Walbaum 1792). *Aquaculture Research*, vol. 49, no. 6, pp. 2156-2165. http://dx.doi.org/10.1111/are.13671.

MONTANHA, F.P. and PIMPÃO, C.T., 2012. Efeitos toxicológicos de piretróides (cipermetrina e deltametrina) em peixes: revisão. *Revista Científica Eletrônica de Medicina Veterinária*, vol. 18, pp. 1-58.

MOOSAVI-NASAB, M., MIRZAPOUR-KOUHDASHT, A. and OLIYAEI, N., 2019. *Application of essential oils for shelf-life extension of seafood products*. Rijeka: Intech Open, pp. 1-13.

PANKHURST, N.W., 2011. The endocrinology of stress in fish: an environmental perspective. *General and Comparative Endocrinology*, vol. 170, no. 2, pp. 265-275. http://dx.doi.org/10.1016/j.ygcen.2010.07.017. PMid:20688064.

PARANHOS, C.O., NEVES, L.C., SILVA, W.S. and LUZ, R.K., 2023. Transport of killifish *Hypsolebias flagellatus*: effects of salt use and previous feeding in association with transport time. *Journal of Applied Aquaculture*, vol. 35, no. 1, pp. 100-111. http://dx.doi.org/10.1080/10454438.2021.1943102.

PEREIRA, R.A., SACZK, A.A., OKAMURA, D., CARDOSO, M.G., COSTA, L.S., SANTIAGO, W.D., SOUZA, T.M., LIMA, R.M.F. and ROSA, P.V., 2015. Quantification of residual clove oil benzocaine and tricaine in fish fillets using SPE and UPLC-DAD. *Journal of Advances in Chemistry*, vol. 10, no. 5, pp. 2661-2668. http://dx.doi.org/10.24297/jac.v10i5.6967.

PLYZCZ, B., FLORY, C.M., GALVAN, I. and BAYNE, C.J., 1989. Leucocytes of rainbow trout (*Oncorhynchus myliss*) pronephros: cell types producing superoxide anion. *Developmental and Comparative Immunology*, vol. 13, no. 3, pp. 217-224. http://dx.doi.org/10.1016/0145-305X(89)90002-5. PMid:2551747.

RANZANI-PAIVA, M.J.T., 2007. Hematologia como ferramenta para avaliação da saúde de peixes. In: *Anais do 2º Simpósio de Nutrição e Saúde de Peixes*, 2007, Botucatu. Botucatu: Universidade Estadual Paulista, 74 p.

RANZANI-PAIVA, M.J.T., PÁDUA, S.B. and TAVARES-DIAS, M., 2013. *Métodos para análise hematológica em peixes*. Maringá: Eduem, 135 p. no. 1. http://dx.doi.org/10.7476/9788576286530.

RATTAN, R.S., 2010. Mechanism of action of insecticidal secondaty metabolites of plant origin. *Crop Protection*, vol. 29, no. 9, pp. 913-920. http://dx.doi.org/10.1016/j.cropro.2010.05.008.

RAUT, J.S. and KARUPPAYIL, S.M., 2014. A status review on the medicinal properties of essential oils. *Industrial Crops and Products*, vol. 62, pp. 250-264. http://dx.doi.org/10.1016/j.indcrop.2014.05.055.

REID, S.G., 2011. Catecholamines. In: A. P. FARREL, J. J. CECH, J. G. RICHARDS and E.D. STEVENS, eds. *Encyclopedia of fish phisiology: from genome to enviromment*. London: Academic Press, pp. 1541-1552.

RIBEIRO, A.S., BATISTA, E.S., DAIRIKI, J.K., CHAVES, F.C.M. and INOUE, L.A.K.A., 2016. Propriedades do óleo essencial de *Ocimum gratissimum* como anestésico para juvenis de matrinxã. *Acta Scientiarum. Animal Sciences*, vol. 38, no. 1, pp. 1-7. http://dx.doi.org/10.4025/actascianimsci.v38i1.28787.

ROSS, L.G. and ROSS, B. 2008. *Anaesthetic and sedative techniques for aquatic animals*. 3rd ed. Oxford: Blackwell Science, 240 p. http://dx.doi.org/10.1002/9781444302264.

RYU, S., PARK, H., SEOL, G.H. and CHOI, I.Y., 2014. 18-Cineole ameliorates oxygen-glucose deprivation/reoxygenation-induced ischaemic injury by reducing oxidative stress in rat cortical neuron/glia. *The Journal of Pharmacy and Pharmacology*, vol. 66, no. 12, pp. 1818-1826. http://dx.doi.org/10.1111/jphp.12295. PMid:25088014.

SALBEGO, J., TONI, C., BECKER, A.G., ZEPPENFELD, C.C., MENEZES, C.C., LORO, V.L., HEINZMANN, B.M. and BALDISSEROTTO, B., 2017. Biochemical parameters of silver catfish (*Rhamdia quelen*) after transport with eugenol or essential oil of *Lippia alba* added to the water. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 77, no. 4, pp. 696-702. http://dx.doi.org/10.1590/1519-6984.16515. PMid:28492807.

SAMPAIO, F.D.F. and FREIRE, C.A., 2016. An overview of stress physiology of fish transport: changes in water quality as a function of transport duration. *Fish and Fisheries*, vol. 17, no. 4, pp. 1055-1072. http://dx.doi.org/10.1111/faf.12158.

SCHRECK, C.B., SOLAZZI, M.F., JOHNSON, S.L. and NICKELSON, T.E., 1989. Transportation stress affects performance of coho salmon, *Oncorhynchus kisutch*. *Aquaculture*, vol. 82, no. 1-4, pp. 15-20. http://dx.doi.org/10.1016/0044-8486(89)90391-8.

SCHRECK, C.B. and TORT, L., 2016. The concept of stress in fish. In: C.B. SCHRECK, L. TORT, A.P. FARRELL and C.J. BRAUNER, eds. *Biology of stress in fish.* Amsterdam: Academic Press, pp. 1-34. Fish Physiology. http://dx.doi.org/10.1016/B978-0-12-802728-8.00001-1.

SENA, A.C., TEIXEIRA, R.R., FERREIRA, E.L., HEINZMANN, B.M., BALDISSEROTTO, B., CARON, B.O., SCHMIDT, D., COUTO, R.D. and COPATTI, C.E., 2016. Essential oil from *Lippia alba* has anaesthetic activity and is effective in reducing handling and transport stress in tambacu (*Piaractus mesopotamicus* x *Colossoma macropomum*). *Aquaculture*, vol. 465, no. 1, pp. 374-379. http://dx.doi.org/10.1016/j.aquaculture.2016.09.033.

SILVA, A.S.E., LIMA, J.T.A.X. and BLANCO, B.S., 2012a. Hematologia em peixes. *Revista Centauro*, vol. 3, pp. 24-32.

SILVA, L.L., PARODI, T.V., RECKZIEGEL, P., GARCIA, V.O., BÜRGER, M.E., BALDISSEROTTO, B. and HEINZMANN, B.M., 2012b. Essential oil of *Ocimum gratissimum* L: ansthetic effects mechanism of action and tolerance in silver catfish *Rhamdia quelen*.

Aquaculture, vol. 350, pp. 91-97. http://dx.doi.org/10.1016/j.aquaculture.2012.04.012.

SILVA, L.L., GARLET, Q.I., KOAKOSKI, G., OLIVEIRA, T.A., BARCELLOS, L.J.G., BALDISSEROTTO, B., PEREIRA, A.M.S. and HEINZMANN, B.M., 2015. Effects of anesthesia with the essential oil of *Ocimum gratissimum* L in parameters of fish stress. *Revista Brasileira de Plantas Medicinais*, vol. 17, no. 2, pp. 215-223. http://dx.doi.org/10.1590/1983-084X/13_034.

SILVA, L.A., MARTINS, M.A., SANTO, F.E., OLIVEIRA, F.C., CHAVES, F.C.M., CHAGAS, E.C., MARTINS, M.L. and CAMPOS, C.M., 2020. Essential oils of *Ocimum gratissimum* and *Zingiber officinale* as anesthetics for the South American catfish *Pseudoplatystoma reticulatum*. *Aquaculture*, vol. 528, pp. 735595. http://dx.doi.org/10.1016/j.aquaculture.2020.735595.

SINGH, S., LAL, R.K., MAURYA, R. and CHANOTIYA, C.S., 2018. Genetic diversity and chemotype selection in genus *Ocimum*. *Journal of Applied Research on Medicinal and Aromatic Plants*, vol. 9, pp. 19-25. http://dx.doi.org/10.1016/j.jarmap.2017.11.004.

SOUZA, P.C. and BONILLA-RODRIGUEZ, G.O., 2007. Fish hemoglobins. *Brazilian Journal of Medical and Biological Research*, vol. 40, no. 6, pp. 769-778. http://dx.doi.org/10.1590/S0100-879X2007000600004. PMid:17581674.

SOUZA, C.F., BALDISSERA, M.D., BALDISSEROTTO, B., HEINZMANN, B.M., MARTOS-SITCHA, J.A. and MANCERA, J.M., 2019. Essential oils as stress-reducing agents for fish aquaculture: a review. *Frontiers in Physiology*, vol. 10, pp. 785. http://dx.doi.org/10.3389/fphys.2019.00785. PMid:31281264.

STONE, D. and TOSTIN, N. 1999. Clove bud oil a big yawn for silver perch: fish. *NSW Maganize Spring*, vol. 19, pp. 30-34.

SUTTHI, N. and DOAN, H.V., 2020. *Saccharomyces crevices* and *Bacillus* spp effectively enhance health tolerance of Nile tilapia under transportation stress. *Aquaculture*, vol. 528, pp. 735527. http://dx.doi.org/10.1016/j.aquaculture.2020.735527.

SWAMY, M.K., AKHTAR, M.S. and SINNIAH, U.R., 2016. Antimicrobial properties of plant essential oils against human pathogens and their mode of action: an updated review. *Evidence-Based Complementary and Alternative Medicine*, vol. 2016, pp. 3012462. http://dx.doi.org/10.1155/2016/3012462.

TACCHI, L., LOWREY, L., MUSHARRAFIEH, R., CROSSEY, K., LARRAGOITE, E.T. and SALINAS, I., 2015. Effects of transportation stress and addition of salt to transport water on the skin mucosal homeostasis of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, vol. 435, pp. 120-127. http://dx.doi.org/10.1016/j.aquaculture.2014.09.027. PMid:25705060.

TAVARES-DIAS, M., SANDRIM, E.F.S. and CAMPO-FILHO, E., 1999. Características hematológicas de tambaqui (*Colossoma macropomum*) Cuvier (osteichthyes Characidae) em sistema de monocultivo intensivo II Leucócitos. *Revista Brasileira de Zoologia*, vol. 16, no. 1, pp. 175-184. http://dx.doi.org/10.1590/S0101-81751999000100012.

TAVARES-DIAS, M. and MORAES, F.R., 2004. *Hematologia de peixes teleósteos*. Ribeirão Preto: Eletrônica e Arte Final, 144 p.

TAVARES-DIAS, M. and MARTINS, M.L., 2017. An overall estimation of losses caused by diseases in the Brazilian fish farms. *Journal of Parasitic Diseases*, vol. 41, no. 4, pp. 913-918. http://dx.doi.org/10.1007/s12639-017-0938-y. PMid:29114119.

TOPIC POPOVIC, N., STRUNJAK-PEROVIC, I., COZ-RAKOVAC, R., BARISIC, J., JADAN, M., PERSIN BERAKOVIC, A. and SAUERBORN KLOBUCAR, R., 2012. Tricaine methane-sulfonate (MS-222) application in fish anaesthesia. *Journal of Applied Ichthyology*, vol. 28, no. 4, pp. 553-564. http://dx.doi.org/10.1111/j.1439-0426.2012.01950.x.

VALENTI, W.C., BARROS, H.P., VALENTI, P.M., BUENO, G.W. and CAVALLI, R.O., 2021. Aquaculture in Brazil: past present and future. *Aquaculture Reports*, vol. 19, pp. 100611. http://dx.doi.org/10.1016/j.aqrep.2021.100611.

VALLADÃO, G.M.R., GALLANI, S.U. and PILARSKI, F., 2018. South American fish for continental aquaculture. *Reviews in Aquaculture*, vol. 10, no. 2, pp. 351-369. http://dx.doi.org/10.1111/raq.12164.

VAN DER VYVER, J.S.F., KAISER, H., POTTS, W.M. and JAMES, N., 2013. Using blood plasma cortisol concentration and fish behaviour to determine temperature avoidance in the estuarine-dependent fish species *Rhabdosargus holubi* (Steindachner, 1881) (Sparidae). *Journal of Applied Ichthyology*, vol. 29, no. 6, pp. 1275-1278. http://dx.doi.org/10.1111/jai.12268.

VANDERZWALMEN, M., EATON, L., MULLEN, C., HENRIQUEZ, F., CAREY, P., SNELLGROVE, D. and SLOMAN, K.A., 2019. The use of feed and water additives for live fish transport. *Reviews in Aquaculture*, vol. 11, no. 1, pp. 263-278. http://dx.doi.org/10.1111/raq.12239.

VENTURA, A.S., JERÔNIMO, G.T., OLIVEIRA, S.N., GABRIEL, A.M.A., CARDOSO, C.A.L., TEODORO, G.C., CORRÊA FILHO, R.A.C. and POVH, J.A., 2020. Natural anesthetics in the transport of Nile tilapia: hematological and biochemical responses and residual concentration in the fillet. *Aquaculture*, vol. 526, pp. 735365. http://dx.doi.org/10.1016/j.aquaculture.2020.735365.

VENTURA, A.S., ARAÚJO, G.A.M.D., GANDRA, J.R., NOIA, I.Z., POVH, J.A. and JERÔNIMO, G.T., 2021. Thermal dynamics and physiological implications in pacu *Piaractus mesopotamicus* anaesthetised with *Ocimum basilicum* essential oil. *International Aquatic Research.*, vol. 13, pp. 261-270.

VIJAYAN, M.M., BALLANTYNE, J.S. and LEATHERLAND, J.F., 1990. High stocking density alters the energy metabolism of brook charr, Salvelinus fontinalis. *Aquaculture*, vol. 88, no. 3-4, pp. 371-381. http://dx.doi.org/10.1016/0044-8486(90)90162-G.

VOSYLIENÉ, M.Z., 1999. The effects of heavy metals on haematological indices of fish (Survey). *Acta Zoologica Lituanica*, vol. 9, no. 2, pp. 76-82. http://dx.doi.org/10.1080/13921657.1999.10512290.

WENDELAAR BONGA, S.E., 2011. Hormone response to stress. In: A. P. FARREL, J. J. CECH, J. G. RICHARDS and E.D. STEVENS, eds. Enc*yclopedia of fish phisiology: from genome to enviromment*. London: Academic Press, pp. 1515-1523.

WOJTASZEK, J., DZIEWULSKA-SZWAJKOWSKA, D., ŁOZIŃSKA-GABSKA, M., ADAMOWICZ, A. and DUGAJ, A., 2002. Hematological effects of high dose of cortisol on the carp (*Cyprinus carpio* L): cortisol effect on the carp blood. *General and Comparative Endocrinology*, vol. 125, no. 2, pp. 176-183. http://dx.doi.org/10.1006/gcen.2001.7725. PMid:11884063.

ZANIBONI FILHO, E., TATAJE, D.R. and WEINGARTNER, M., 2006. Potencialidad del género *Brycon* en la piscicultura brasileñ. *Revista Colombiana de Ciencias Pecuarias*, vol. 19, no. 2, pp. 233-240.

ZEPPENFELD, C.C., TONI, C., BECKER, A.G., MIRON, D.S., PARODI, T.V., HEINZMANN, B.M., BARCELLOS, L.J.G., KOAKOSKI, G., ROSA, J.G.S., LORO, V.L., CUNHA, M.A. and BALDISSEROTTO, B., 2014. Physiological and biochemical responses of silver catfish *Rhamdia quelen* after transport in water with essential oil of *Aloysia triphylla* (L'Herit). *Aquaculture*, vol. 418-419, pp. 101-107. http://dx.doi.org/10.1016/j.aquaculture.2013.10.013.

ZHANG, L.J., XUE, Y., ZHANG, C.R. and HU, S.H., 2013. Antibacterial and anti-inflammatory effects of eucalyptol. *Chinese Journal of Veterinary Drug*, vol. 47, no. 3, pp. 21-24.

ZHOU, L., ZHANG, Z., WEI, M., XIE, Y., HE, S., SHI, H. and LIN, Z., 2019. Evaluation of the antifungal activity of individual and combined monoterpenes against *Rhizopus stolonifer* and *Absidia coerulea*. *Environmental Science and Pollution Research International*, vol. 26, no. 8, pp. 7804-7809. http://dx.doi.org/10.1007/s11356-019-04278-z. PMid:30675711.