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ANIMAL PRODUCTION

Ginger essential oil as a sedative for yellowtail tetra transport: effects on water quality, gill histopathology, and fillet residues

Gabriel da Silva Barros[1] Andre Lima Ferreira[2*] Welison Aparecido Bispo Caitano[3] Leandro Borges Cavalcanti[3] Alysson Martins Wanderley[1,3] Lidiane Cecília Pereira[6] Carlos Eurico Fernandes[6] Francisco Célio Maia Chaves[5] Edsandra Campos Chagas[5] Claudia Andrea Lima Cardoso[4] Reíssa Alves Vilela[3] Robson Pereira de Paula[1] André Luiz Julien Ferraz[1,3] Cristiane Meldau de Campos[1,3,6] Deliane Cristina Costa[1,3]

[1]Programa de Pós-graduação em Zootecnia, Produção Animal no Cerrado e Pantanal, Centro em Excelência Animal do Cerrado e Pantanal (CECA), Setor de Piscicultura, Universidade Estadual de Mato Grosso do Sul (UEMS), Aquidauana, MS, Brasil.
[2]Grupo de Pesquisa em Vida Aquática e Produção Sustentável (AQUAVITA), Centro de Excelência em Bioinsumos (CEBIO), Instituto Federal de Educação, Ciência e Tecnologia Goiano (IF Goiano), 73840-000, Campos Belos, GO, Brasil. E-mail: andre.lima@ifgoiano.edu.br. [*]Corresponding author.
[3]Programa Institucional de Bolsas de Iniciação Científica (PROPPI), Graduação em Zootecnia, Universidade Estadual de Mato Grosso do Sul (UEMS), Aquidauana, MS, Brasil.
[4]Programa de Pós-graduação em Recursos Naturais, Universidade Estadual de Mato Grosso do Sul (UEMS), Dourados, MS, Brasil.
[5]Embrapa Amazônia Ocidental, Manaus, AM, Brasil.
[6]Programa de Pós-graduação em Ciência Animal, Laboratório de Patologia Experimental, INBIO, Universidade Federal de Mato Grosso do Sul (UFMS), Campo Grande, MS, Brasil.

ABSTRACT: The current study investigated the use of different concentrations of the essential oil of *Zingiber officinale* (EOZO) in the transport water of *Astyanax lacustris* by evaluating the behavioral responses of the fish, water quality, gill histopathology and residues in the fillet after transport. Four hundred juvenile *A. lacustris* (1.99 ± 0.72 g) were randomly distributed in 20 plastic bags, 20 fish each, with 4 L of water and 2/3 of pure oxygen. Five treatments were applied: control and four concentrations of EOZO (5, 10, 15 and 20 µL L^{-1}), with four replicates each in a completely randomized design. The bags were sealed and shaken periodically during a simulated 2-h transport. Water quality variables were measured before and after transport, while fish survival was recorded, and gill and muscle samples were collected, at different times after transport. Gill histopathology was analyzed to detect tissue alterations and muscle (fillet) residues were quantified. The use of low concentrations of EOZO during transport of *A. lacustris* was effective at sedating the fish, ensuring survival. The histopathological changes observed in the gills of fish transported with 5 and 10 µL L^{-1} of EOZO were reversible. Furthermore, EOZO preserved water quality, with an observed reduction in ammonia levels for animals transported with 10 µL L^{-1}. Although small histopathological changes were caused to the gills, EOZO residues in fillets were minimal and disappeared quickly (in less than 12 h). Therefore, the use of 10 µL L^{-1} of EOZO in the transport water of *A. lacustris* is recommended.
Key words: *Astyanax lacustris*, depletion of bioactive compounds, gill lesions, total ammonia, *Zingiber officinale.*

Óleo essencial de gengibre como sedativo para o transporte de tetra-amarelo: efeitos na qualidade da água, histopatologia branquial e resíduos de filés

RESUMO: O presente estudo investigou o uso de diferentes concentrações do óleo essencial de *Zingiber officinale* (EOZO) na água de transporte de *Astyanax lacustris* avaliando as respostas comportamentais dos peixes, qualidade da água, histopatologia branquial e resíduos no filé após o transporte. Quatrocentos juvenis de *A. lacustris* (1,99 ± 0,72 g) foram distribuídos aleatoriamente em 20 sacos plásticos, de 20 peixes cada, com 4 L de água e 2/3 de oxigênio puro. Foram aplicados cinco tratamentos: controle e quatro concentrações de EOZO (5, 10, 15 e 20 µL L^{-1}), com quatro repetições cada, em delineamento inteiramente casualizado. Os sacos foram selados e agitados periodicamente durante um transporte simulado de 2 horas. Variáveis de qualidade da água foram medidas antes e depois do transporte, enquanto a sobrevivência dos peixes foi registrada e amostras de brânquias e músculos foram coletadas, em diferentes momentos após o transporte. A histopatologia branquial foi analisada para detectar alterações teciduais e resíduos musculares (filé) foram quantificados. O uso de baixas concentrações de EOZO durante o transporte de *A. lacustris* foi eficaz na sedação dos peixes, garantindo a sobrevivência. As alterações histopatológicas observadas nas brânquias dos peixes transportados com 5 e 10 µL L^{-1} de EOZO foram reversíveis. Além disso, o EOZO preservou a qualidade da água, com redução observada nos níveis de amônia para animais transportados com 10 µL L^{-1}. Embora pequenas alterações histopatológicas tenham sido causadas nas brânquias, os resíduos de EOZO nos filés foram mínimos e desapareceram rapidamente (em menos de 12 horas). Portanto, recomenda-se a utilização de 10 µL L^{-1} de EOZO na água de transporte de *A. lacustris.*
Palavras-chave: amônia total, *Astyanax lacustris*, depleção de compostos bioativos, lesões branquiais, *Zingiber officinale.*

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INTRODUCTION

The transport of live fish is a crucial step in aquaculture, as it directly impacts the survival and quality of the species sold and is considered one of the most stressful handling procedures for the animals (SAMPAIO & FREIRE, 2016; LUZ & FAVERO, 2024). According to LUZ & FAVERO (2024), during transport, fish may suffer from overcrowding, which can lead to hypoxia, sudden temperature fluctuations, and the accumulation of toxic metabolites, compromising water quality, fish welfare, and survival. Therefore, implementing strategies to minimize stress during transport is essential to ensure sustainability in aquaculture, with particular emphasis on the use of essential oils, either through dietary supplementation (FERREIRA et al., 2017; VANDERZWALMEN et al., 2019) or by adding them to the transport water (FERREIRA et al., 2024; LUZ & FAVERO, 2024).

Essential oils, volatile metabolites from aromatic plants, have gained prominence in aquaculture due to their phytochemical properties and potential to reduce stress in fish during transport (AYDIN & BARBAS, 2020). Several studies have reported promising results with different oils, such as *Hesperozygis ringens* (TONI et al., 2015; FERREIRA et al., 2022), *Ocimum gratissimum* (BOAVENTURA et al., 2021; FERREIRA et al., 2024; VENTURA et al., 2024), *Lippia alba* (CUNHA et al., 2010), *Lippiasidoides*, and *Mentha piperita* (BRANDÃO et al., 2022a), reinforcing their potential as natural alternatives to synthetic anesthetics. In general, synthetic anesthetics such as tricaine methanesulfonate (MS-222) and benzocaine act quickly and have a short duration but can cause physiological disturbances (cardiovascular and respiratory depression, corneal lesions, excessive mucus production, delayed recovery), as well as tissue damage, compromising animal welfare. Moreover, they can lead to residue accumulation in fillets, raising concerns about food safety and environmental impact (PURBOSARI et al., 2019; AYDIN & BARBAS, 2020). In contrast, essential oils usually require lower concentrations to achieve the same anesthetic effect, present fewer adverse effects, and are considered safer for both fish and consumers due to their natural origin and biodegradability (CUNHA et al., 2010; OLIVEIRA et al., 2024; VENTURA et al., 2024). However, their chemical composition can vary depending on the species, plant part used, and extraction method, potentially affecting their efficacy and standardization (SOUZA et al., 2019).

Among these natural agents, the essential oil of ginger, *Zingiber officinale*, stands out for its multifaceted properties, including immunomodulatory, anti-stress, antimicrobial, antioxidant (FAZELAN et al., 2020; ALMEIDA et al., 2021; DAWOOD et al., 2022) and anesthetic (SILVA et al., 2020; SILVA et al., 2023) effects. Recently, MOREIRA et al. (2024) reported that the use of low concentrations of the essential oil of *Z. officinale* (EOZO) in the transport water of pacu (*Piaractus mesopotamicus*) was able to reduce the effects of physiological stress and mortality and preserve the water quality for juveniles transported in plastic bags. Given the increasing demand for natural anesthetics that ensure greater safety and sustainability in fish handling, evaluating the effects of EOZO on yellow tail tetra (*Astyanax lacustris*) can provide valuable insights for aquaculture.

In addition to the immediate effects on fish survival and welfare during transport, it is essential to assess long-term impacts, such as histopathological changes in tissues, as anesthetic absorption and metabolism occur primarily in the gills and liver (EVANS et al., 2005; WANG et al., 2021). This aspect is particularly relevant when assessing anesthetic compounds (BRANDÃO et al., 2021; LIU et al., 2022). SILVA et al. (2023) reported that EOZO concentrations above 300 $\mu L\ L^{-1}$, used to induce deep anesthesia in *A.lacustris*, can cause irreversible gill damage. Gills play a central role in respiration, osmoregulation, and excretion of nitrogenous wastes, among other functions, and their integrity is fundamental to the health and survival of fish (EVANS et al., 2005). Therefore, histopathological analyses of gills can provide detailed information on tissue injuries and the physiological response to stress, which are essential for understanding the impacts of EOZO on fish anesthesia, as recently reported by SILVA et al. (2023). Also relevant is the potential residual effect of the compounds in EOZO on fish fillets (MOREIRA et al., 2024), which may have implications for both food safety and market acceptance.

Astyanax lacustris, popularly known in Brazil as lambari-do-rabo-amarelo, is a small, omnivorous fish that is widely distributed in South America. It stands out as an important species in Neotropical aquaculture, with Brazilian annual production exceeding 1000 tons due to its popularity as live bait and appetizer (VALENTI et al., 2021; FONSECA et al., 2022). Its reproductive characteristics, such as high fecundity and rapid maturation, combined with easy handling (SILVA et al., 2022), reinforce its potential for aquaculture.

In this context, the search for natural anesthetic alternatives aims to enhance efficiency, sustainability, and possible animal welfare during routine handling procedures of commercial relevance.

Therefore, the present study investigated the effects of different concentrations of EOZO in the transport water of *A. lacustris*, assessing water quality parameters, gill histopathology, and the presence of residual compounds in the fillet post-transport.

MATERIALS AND METHODS

Cultivation, preparation, extraction, and chemical profiling of EOZO

Specimens of *Z. officinale* were grown at Embrapa Amazônia Ocidental (Manaus, Brazil). After harvest, the rhizomes were air-dried and stored (MONTEIRO et al., 2021). EOZO was extracted from the rhizomes by hydrodistillation using a Clevenger apparatus (2 h) and stored in amber bottles at - 4 °C. Stock solutions were prepared using ethanol (99.5%) in a ratio of 1:10 (V/V) at all concentrations to homogenize the EOZO in water, as also adopted by MOREIRA et al. (2024). The volume of ethanol equivalent to the highest concentration of EOZO was used for the control group (0 µL L^{-1} EOZO), i.e., 200 µL Ethanol L^{-1}.

The chemical profile of EOZO was analyzed by gas chromatography-mass spectrometry (GC-MS) using a Shimadzu GCMS2010 Plus (Shimadzu Corporation, Japan) at the Laboratório de Análise Instrumental do Centro de Estudos em Recursos Naturais, UEMS (Dourados, Mato Grosso do Sul, Brazil), which identified zingiberene (38.31%), β-sesquiphellandrene (22.14%) and β-bisabolene (14.01%) as the major constituents, together with smaller amounts of other compounds (Table 1).

Fish and environmental acclimatization

Specimens of *A.lacustris* were acclimated for two weeks in cages placed within an earthen pond, maintained under continuous water flow and aeration, with a daily water renewal rate of 185%. The water had a temperature of 26.97 ± 0.08 °C, pH of 7.97 ± 0.09, dissolved oxygen of 4.17 ± 0.28 mg L^{-1}(measured with a Hanna HI98194 multiparameter probe), and total ammonia of 0.01 ± 0.004 mg L^{-1}, determined using a portable photocolorimeter (Checker® HC HI715 Hanna). The fish were fed a commercial diet (42% crude protein, Supra - AQUAline, Brazil) twice a day until satiety. All animals were fasted for 24 h prior to the experiment.

Experimental design

A total of 400 *A. lacustris* (1.99 ± 0.72 g and 5.80 ± 0.66 cm) were transferred from their acclimation tanks to plastic bags. The water used to fill the plastic bags before transport had a temperature of 27.37 ± 0.07 °C, pH of 7.17 ± 0.08, dissolved oxygen of 3.47 ± 0.10 mg L^{-1} and total ammonia of 0.01 ± 0.008 mg L^{-1}. Twenty fish were distributed in each of 20 plastic bags (70 x 100 cm), with a capacity of 12 L, of which 4 L were water and the remainder supplemented with pure oxygen (Figure 1), the same loading density used by FERREIRA et al. (2017). Five treatments, adapted from MOREIRA et al. (2024), were applied, with four replicates each in a completely randomized design, namely: fish transported in water free of EOZO (Control - 200 µL Ethanol L^{-1}); fish transported in water + 5 µL L^{-1} of EOZO; fish transported in water +

Table 1 - Chemical profiling of the essential oil of *Zingiber officinale* (EOZO) determined through gas chromatography-mass spectrometry (GC-MS).

Compounds	Content (%)
Zingiberene	38.31
β-Sesquiphellandrene	22.14
β-Bisabolene	14.01
1,8 Cineole	9.12
Germacrene D	2.13
Farnesene	2.11
Camphene	1.62
(E,E) α-Farnesene	1.21
β-Elemene	0.89
Camphor	0.73
β-Phellandrene	0.71
Terpinolene	0.69
δ-Terpinene	0.67
Limonene	0.64
β-Pinene	0.57
β-Caryophyllene	0.37
Sabinene	0.25
α-Copaene	0.24
Bergametene	0.23
α-Pinene	0.23
α-Humulene	0.23
(Z) - β- Farnesene	0.22
β-Selinene	0.13
Calamenene	0.14
γ-Elemene	0.13
δ-Elemene	0.12
α-Cubebene	0.12
Santalene	0.12
α-Gurjumene	0.11
Allaromadendrene	0.11

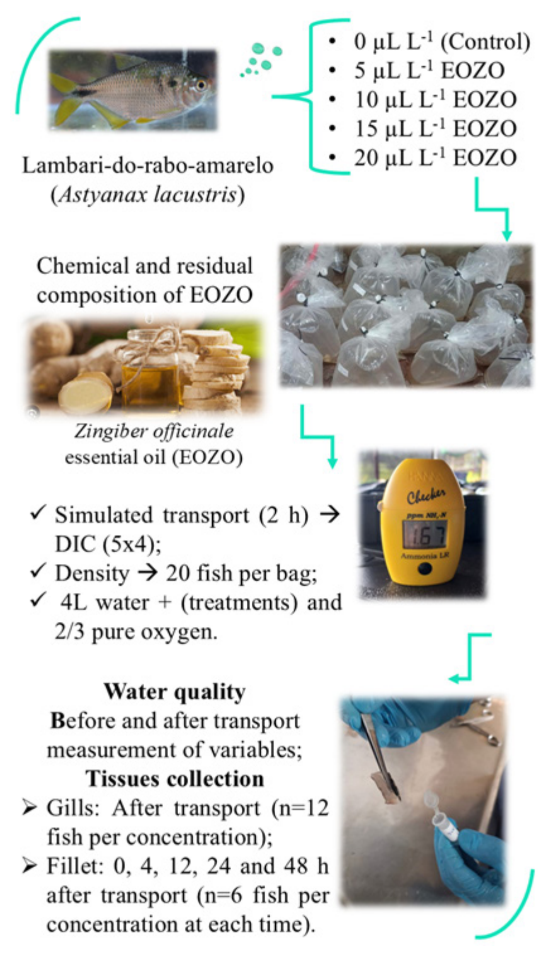

Figure 1 - Schematic diagram of the experimental design used in the study.

10 μL L^{-1} of EOZO; fish transported in water + 15 μL L^{-1} of EOZO; and fish transported in water + 20 μL L^{-1} of EOZO.

The plastic bags were sealed with elastic material at 8:00 am. Transport simulation involved randomly relocating the bags after being identified and shaken every 30 min, which consisted of shaking the bags for approximately 10 seconds to mimic the disturbance naturally occurring during live fish transport, as suggested by FERREIRA et al. (2022). A 2 h transport period was adopted, in accordance with previous studies (VENTURA et al., 2020; OLIVEIRA et al., 2024). Water quality variables [total ammonia (mg L^{-1}), temperature (ºC), pH and dissolved oxygen (mg L^{-1})] were measured before and after transport. The plastic bags were opened immediately after the end of the simulated transport and the water quality variables were measured and the survival of the animals determined.

After the initial stage (time 0: immediately after transport), six specimens of *A. lacustris* from each treatment were euthanized by immersion in ice and sectioning of the cervical spinal cord, as also adopted by FERREIRA et al. (2024). Samples of gill arches were collected and kept in Bouin's solution for 24 h, after which the solution was replaced with 70% alcohol for later preparation of histological slides. Muscle tissue samples (fillet) from the same fish were also collected for future residue analyses.

At the end of the test, the remaining fish from each evaluated concentration were kept in five previously identified 100-liter tanks, with continuous water renewal and supplemental aeration. Subsequently, muscle samples were collected from six fish per concentration at 4, 12, 24, and 48 h after transport.

After the last tissue collection (48 h), the remaining fish were fed twice a day until apparent satiety and observed for 30 min to evaluate food intake and survival for 96 h, as suggested by FERREIRA et al. (2022).

Tissue collection

Samples of branchial arches were collected, identified, prepared in Bouin's solution for 24 h and fixed in ethanol (70%) for subsequent preparation of histological slides. Carcass samples (1 g of muscle or fillet) were removed and immediately frozen at -20 °C for future analyses of residual compounds.

Gill histopathology analyses

Histopathological analyses of the gills were performed in the Laboratório de Patologia Experimental, of the Instituto de Biociências da Universidade Federal de Mato Grosso do Sul, located in Campo Grande, Mato Grosso do Sul, Brazil. Gill fragments from the fish transported with EOZO and of the control group were fixed in 10% buffered formalin solution for 24 h and then transferred to a 70% alcohol solution until they were histologically processed for Paraplast® (Sigma-Aldrich) inclusion. Histological sections (3 μm) stained by Hematoxylin and Eosin (HE) were analyzed using bright field microscopy(Optican 500R, OPTCAM 14.3 digital camera Lopet14003)(CARSON & HLADIK, 2009). The Degree of Tissue Change (DTC) was estimated according to the method reported by POLEKSIC & MITROVIC-TUTUNDZIC (1994) and BERNET et al. (1999). Briefly, DTC was based on the standard reaction (*a*) traits and their importance scores (*w*). Table 2 presents the histological changes considered and their respective degrees of importance. The following formula was used to estimate DTC:DTC = ∑alt (*a* × *w*), where *a*represents the distribution of damage (0, absent; 1, minor; 2, moderate; and 3, marked occurrence), "w" represents the degree of reversibility of the damage (w1, easily reversible; w2, moderated alterations with probable reversion after exposure; and w3, irreversible alterations), and the sum ofalterations (∑alt).

Chemical profiling of EOZO and its constituents in fillet

The chemical composition of EOZO was examined through GC-MS, followed by analysis of fillet samples (1 g). For chemical profiling, the EOZO was first diluted in hexane to a concentration of 100 µg mL^{-1}. For muscle analysis, 1 g of the sample was mixed with 5 mL of hexane (chromatographic grade), then shaken and homogenized in an ultrasonic tank for 30 minutes. The hexane fraction was filtered, and the extraction was repeated five times using the same sample. The collected hexane fractions were dried and subsequently redissolved in 100 µL of hexane for GC-MS analysis. The samples were analyzed in method described by MOREIRA et al. (2024). An injection volume of 1 µL was used, with a split ratio of 1:20 for EOZO and a splitless mode for muscle samples. The identification of EOZO components involved calculating the retention index using a C_7-C_{40} linear alkane standard (Sigma Aldrich, ≥ 98% purity) and comparing the data with mass spectra and retention indices from ADAMS (2007) and Willey and NIST databases.

Fillet metabolite levels were evaluated by constructing analytical curves using zingiberene and β-sesquiphellandrene at concentrations of 50, 25, 10, 5, 0.5 µg kg^{-1}. Detection and quantification limits were determined at various concentrations based on the signal-to-noise ratio. Detection and quantification limits were 0.15 µg kg^{-1} and 0.5 µg kg^{-1}, respectively for two compounds.

Statistics

Data were assessed for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test. Water quality results obtained immediately after transport were analyzed by ANOVA followed by Tukey's post-test ($P < 0.05$), while Dunnett's post-test ($P < 0.05$) was used for comparison with baseline results (before transport). A non-parametric ANOVA was applied to the gill histopathology results to compare DTC among the treatments, with pairwise comparisons by the Mann-Whitney U test ($P < 0.05$). Furthermore, the frequency of the degree of reversibility of lesions (w1, w2, and w3) was described for each experimental group. Data on residual compounds were analyzed using descriptive statistics. All analyses were performed using Infostat and R software.

Table 2 - Degree of Tissue Change (DTC)* according to stages of lesions observed in the gills.

Histological changes	Score
Hypertrophy of lamellar epithelium	1
Hyperplasia of lamellar epithelium	1
Fusion of lamelar epithelium	1
Mucous cell proliferation	1
Edema of epitelial cell	1
Shortening of secondary lamella	1
Lamellar atrophy	1
Aderence of lamelar epithelium	1
Mucous cell hyperplasia and hypertrophy	1
Epithelial lifting	1
Vasodilatation and vascular hyperemi	1
Leukocyte infiltrate	1
Telangiectasis (aneurysm)	1
Rupture of lamelar epithelium	2
Lamellar thrombosis	2
Epithelial fibrosis	3
Fibrous lamellar thrombosis	3
Necrosis	3

*DTC - According to POLEKSIC & MITROVIC-TUTUNDZIC (1994) and BERNET et al. (1999); 1, easily reversible; 2, moderated alterations with probable reversion after the end of exposure and 3, irreversible alterations.

RESULTS

Mortality, feed return and water quality at the end of simulated transport

No mortality was observed during and 96 h after simulated transport, and all animals transported with EOZO were apparently sedated immediately after transport. Furthermore, all fish resumed feeding 48 h after the end of simulated transport (time when feed was offered).

Immediately after transport, the water temperature and dissolved oxygen values were higher for all EOZO concentrations and in relation to baseline (before transport) ($P < 0.05$). The fish transported with 10 µL L^{-1} EOZO presented lower total ammonia values in the water immediately after the end of transport when compared other treatments ($P < 0.05$), with the values being similar to baseline ($P > 0.05$). Water pH level immediately after transport with EOZO did not differ among treatments nor from baseline (P > 0.05) (Table 3).

Gill histopathology

All fish transported with EOZO showed significant differences (P < 0.05) compared to the control group (200 µL Ethyl alcohol L^{-1}), but no significant differences were observed between the concentrations of EOZO evaluated (P > 0.05) (Figure 2A).

The degree of reversibility was comparable across the concentrations of EOZO. Therefore, the fish transported with 15 and 20 µL L^{-1} EOZO demonstrated a higher frequency of grade 3 lesions (Figure 2B), characterized by aneurysms with non-extensive fibrosis (Figure 3). The most prevalent grade 2 lesions were adherence and lamellar fusion.

Residual constituents of EOZO in fillet

The EOZO concentrations used in the transport of *A. lacustris* were very low, resulting in levels of residual compounds in the muscle (fillet) that, in most cases, were below the detectable limits of the analytical method employed. However, two main constituents of EOZO, zingiberene and β-sesquiphellandrene, were identified in some samples. Specifically, fish transported with 10 µL L^{-1} of EOZO in the water presented very low levels of zingiberene, ranging from 0.58 ± 0.04 µg kg^{-1}, with no detection in the subsequent collection periods (4, 12, 24 and 48 h post-transport). On the other hand, animals transported with 15 and 20 µL L^{-1} of EOZO presented zingiberene levels from 1.02 ± 0.19 µg kg^{-1}, immediately after transport, and 0.58 ± 0.08 µg kg^{-1} 4 h after the end of transport. As for β-sesquiphellandrene, due to its lower concentration in EOZO, it was only quantified in the muscle of fish transported with 15 µL L^{-1} (0.51 ± 0.09 µg kg^{-1}) and 20 µL L^{-1} of EOZO (0.79 ± 0.08 µg kg^{-1}), immediately after the end of transport.

DISCUSSION

This study found that low concentrations of EOZO added to the transport water of *A. lacustris* in plastic bags caused low-intensity damage to the gills, reduced the excretion of total ammonia and left only minimal traces of residual compounds in fish fillets. Furthermore, the EOZO did not cause mortality during or 96 h after the end of the experiment, and all fish resumed feeding. MOREIRA et al. (2024) also reported no mortality in juvenile *P. mesopotamicus* transported in plastic bags containing water and different concentrations of EOZO.

In addition, EOZO has proven to be efficient anesthetic for different species of freshwater fish, such as cachara (*Pseudoplatystoma reticulatum*) (SILVA et al., 2020), *P. mesopotamicus* (MOREIRA et al., 2024) and *A. lacustris* (SILVA et al., 2023), corroborating the findings of the present study. Furthermore, the dry extract of *Z. officinale* acted as an efficient sedative in the transport of juvenile *Micropterus salmoides* L., significantly reducing serum cortisol levels (LIU et al., 2022), demonstrating its sedative potential both as an essential oil and in the form of dry extract. In contrast, FERREIRA et al. (2022) evaluated different concentrations of *H. ringens* essential oil as an anesthetic and/or sedative for juvenile tambaqui (*Colossoma macropomum*) and reported that all animals resumed feeding 24 h after biometric handling and transport. These results

Table 3 - Water quality variables before and after 2 h of simulated transport of *Astyanax lacustris* in plastic bags containing water with different concentrations of the essential oil of *Zingiber officinale* (EOZO).

Variables	Before transport	---------------------------After simulated transport (Treatments)----------------------------					
	Basaline (Basal)	Control - 0 µL L^{-1}	EOZO – 5 µL L^{1}	EOZO – 10 µL L^{-1}	EOZO – 15 µL L^{-1}	EOZO – 20 µL L^{-1}	P - value
Temperature (ºC)	27.37 ± 0.07	29.99 ± 0.20*	29.39 ± 0.42*	28.78 ± 1.08*	29.52 ± 0.30*	29.92 ± 0.30*	0.0519
pH	7.17 ± 0.08	7.01 ± 0.04	7.28 ± 0.35	7.12 ± 0.03	7.10 ± 0.05	7.05 ± 0.05	0.2131
Dissolved oxygen (mg L^{-1})	3.47 ± 0.10	13.32 ± 0.90*	14.48 ± 3.13*	12.61 ± 0.37*	12.93 ± 0.66*	11.85 ± 0.27*	0.2099
Total ammonia (mg L^{-1})	0.01 ± 0.008	0.86 ± 0.03a*	0.53 ± 0.26 b*	0.14 ± 0.11c	0.33 ± 0.16bc*	1.03 ± 0.06a*	<0.0001

Values are expressed as mean ± standard deviation. Asterisks represent significant differences between treatment and baseline. Different lowercase letters indicate significant differences between treatments (P < 0.05).

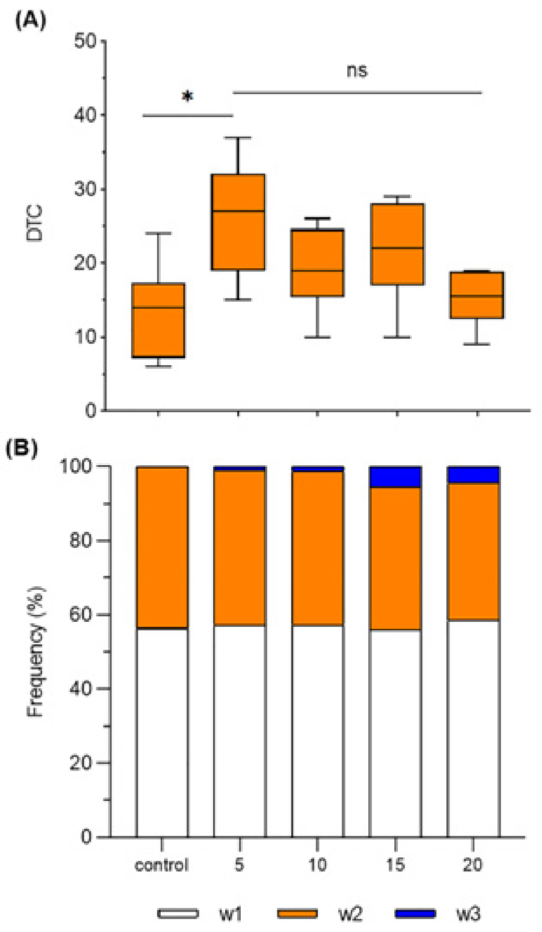

Figure 2 - Degree of tissue change (DTC)* (a) and frequency (%) (b) according to stages of lesions observed in the gills after 2 h of simulated transport of *Astyanax lacustris* in plastic bags containing water with different concentrations of the essential oil of *Zingiber officinale* (EOZO).

*DTC - According to POLEKSIC & MITROVIC-TUTUNDZIC (1994) and BERNET et al. (1999); w1, easily reversible; w2, moderated alterations with probable reversion after the end of exposure and w3, irreversible alterations.

demonstrate that essential oils extracted from plants, in ideal concentrations, generally do not interfere with fish feeding after management applied in aquaculture, when administered correctly.

Assessments of water quality variables are essential practices to ensure the success of the fish production chain (SAMPAIO & FREIRE, 2016; FONSECA et al., 2017), especially regarding the transport of live fish when using anesthetics (BECKER et al., 2017; BOAVENTURA et al., 2021; FERREIRA et al., 2022). The current study showed oscillations in water temperature and dissolved oxygen between the studied transport periods (before transport and after its end), but the changes were small and within acceptable limits for *A. lacustris* cultivation of (MARQUES et al., 2021). Furthermore, these oscillations were not related to the presence of EOZO in the water, as the results did not differ among the different EOZO concentrations evaluated.

The transport of live fish in plastic bags tends to result in an increase in ammonia levels in the water due to fish excretion, and to reduce dissolved oxygen levels due to consumption by the animals during the procedure (BOAVENTURA et al., 2021; BRANDÃO et al., 2022b; FERREIRA et al., 2022). In the present study, the addition of 10 μL L^{-1} of EOZO to the transport water of *A. lacustris* resulted in lower excretion of total ammonia into the water immediately after the end of transport, suggesting a reduction in fish metabolism. On the other hand, MOREIRA et al. (2024) did not observe a significant reduction in total ammonia levels when adding EOZO to the transport water of *P. mesopotamicus* in plastic bags for 2 h, although the concentration of 30 mg L^{-1} of EOZO preserved the highest dissolved oxygen values immediately after transport. These results indicate a positive action of EOZO in maintaining and/or preserving water quality during the transport of live fish.

Fish gills perform multiple functions beyond respiration, including osmoregulation, nitrogen excretion, hormonal metabolism, and acid-base balance (OLSON, 1991; EVANS et al., 2005). Due to their vast surface area in contact with the aquatic environment, gills are particularly susceptible to adverse agents, making histopathological analysis an essential tool for assessing fish health and an effective biomarker of aquatic pollution (FLORES-LOPES & THOMAZ, 2011; VAN DYK et al., 2012).

The use of anesthetics derived from essential oils of species such as *Aloysia triphylla*, *Lippiasidoides* and *Mentha piperita* caused damage to the gills of *C. macropomum*, demonstrating that some natural agents are also capable of causing injury to these structures (BRANDÃO et al., 2021). The present study found that *A. lacustris* specimens transported with EOZO presented gill alterations, but with similar reversibility potential among the concentrations tested. Lower concentrations caused only moderate alterations, while concentrations of 15 and 20 μL L^{-1} of EOZO increased the severity of the alterations due to exposure time. Therefore, caution

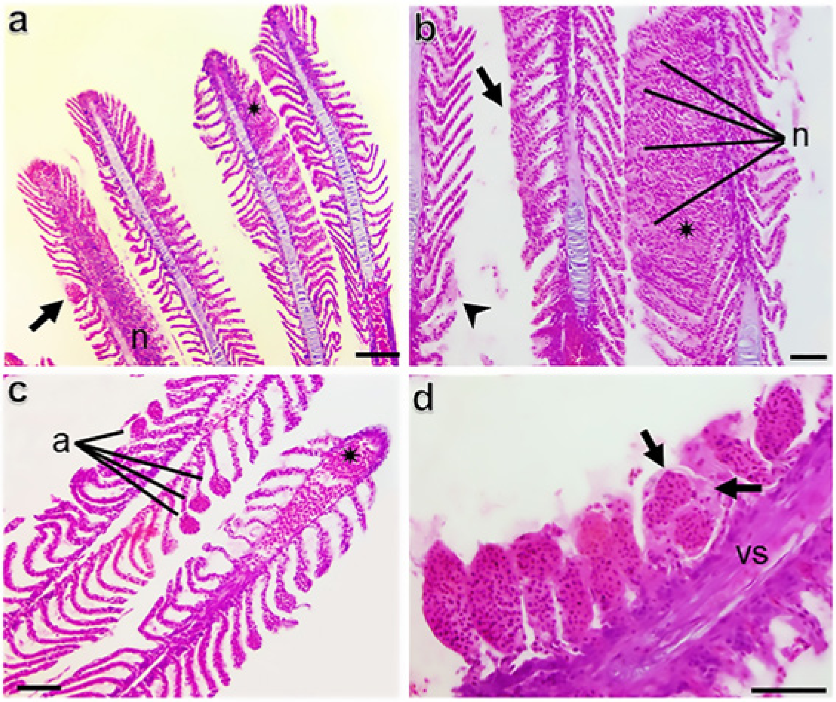

Figure 3 - Sagittal cross-sections in gills stained with hematoxylin and eosin showing prevalent lesions in *Astyanax lacustris* exposed to essential oil of *Zingiber officinale* (EOZO). a, image of specimen exposed to 15 µL L^{-1} EOZO; gills display partial laminar fusion (*); primary laminarsegment with aneurysm (arrow) with an extensive area of necrosis; bar = 100 µm. b, specimen exposed to 20 µL L^{-1} EOZO; segments of secondary lamellae showing edema andcell desquamation (arrowhead) and area with epithelial cell adhesion (arrow); note the extensive lamellar fusion adjacent to epithelial necrosis with focally distributed areas offibrosis (f); bar = 100 µm. c, specimen exposed to 20 µL L^{-1} EOZO; secondary lamellaraneurysms near the proximal region into the primary lamellae; note the dilated venoussinus with vascular hyperemia (*); bar = 100 µm. d, specimen exposed to 20 µL L^{-1} EOZO; asset of secondary lamellar aneurysms with detail of the fibrous outer lining (arrow), venous sinus (vs); bar = 50 µm.

is recommended when using these concentrations for long-term transports of *A. lacustris*, to avoid negative impacts on zootechnical performance and fish survival.

Investigating the residues of constituents from essential oils in aquatic organisms is essential to determine their possible applications and impacts on food safety (FALLEH et al., 2020; OLIVEIRA et al., 2024; VENTURA et al., 2024). The present study observed residues of zingiberene and β-sesquiphellandrene in the fish fillet, with concentrations varying according to the EOZO concentrations evaluated. This occurrence can be attributed to the rapid absorption of the compounds, the exposure time and the low solubility of EOZO in water (ROSS & ROSS, 2008; ODA et al., 2018), similar to that observed for *O. niloticus* exposed to clove oil and benzocaine anesthetics (PEREIRA et al., 2015). Similarly, the use of anesthetics such as eugenol and essential oil of *Ocimum basilicum* with *O. niloticus* presented residual levels below those acceptable for human consumption (VENTURA et al., 2020). Although, these anesthetic residues do not pose a risk to human health, they can alter the flavor of the meat, highlighting the importance of a depuration period to reduce the presence of these compounds (BOTREL et al., 2017; MOREIRA et al., 2024; VENTURA et al., 2024). Furthermore, the present study detected EOZO residues only immediately after transport at and 4 hours later, without them being found in the other collection periods. These results indicate that EOZO has a potent sedative action on *A. lacustris* and rapid depuration in the fillet after its application.

CONCLUSION

It can be concluded that the concentrations of 5 and 10 μL L^{-1} of EOZO caused moderate changes to the gills (capable of recovery) and presented minimal amounts of residual compounds in the fillet after transport. In addition, the concentration of 10 μL L^{-1} of EOZO was efficient at reducing ammonia excretion. Thus, based on the results obtained here, 10 μL L^{-1} of EOZO is recommended for the transport of *A. lacustris* for up to 2 h. Despite the good results achieved here with the use of EOZO, additional studies are needed to evaluate its effects on the general welfare of fish during and after transport, as well as on fillet quality.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

All protocols were approved by the Comissão de Ética no Uso de Animais (CEUA / UEMS - nº 16/2023) of the Universidade Estadual de Mato Grosso do Sul (UEMS). Thus, the authors assume full responsibility for the presented data and are available for possible questions, should they be required by the competent authorities.

DATA AVAILABILITY STATEMENT

Data will be made available on request.

DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE

No use of AI was made along the concpetion and publication of the paper.

REFERENCES

ADAMS, R. P. **Identification of essential oil components by gas chromatography/mass spectrometry**. 4rdedn. Carol Stream, IL: Allured Publishing Corporation, 2007.

ALMEIDA, R. G. S. et al. Dietary supplementation of ginger (*Zingiber officinale*) essential oil exhibits positive immunomodulatory effects on the Neotropical catfish *Pseudoplatystoma reticulatum* without negative effects on fish liver histomorphometry. **Latin American Journal of Aquatic Research**, v.49, p.595-607, 2021. Available from: <https://doi.org/10.3856/vol49-issue4-fulltext-2667>. Accessed: Nov. 15, 2024. doi: 10.3856/vol49-issue4-fulltext-2667.

AYDIN, B.; BARBAS, L. A. L. Sedative and anesthetic properties of essential oils and their active compounds in fish: A review. **Aquaculture**, v.520, p.734999, 2020. Available from: <https://doi.org/ 10.1016/j.aquaculture.2020.734999>. Accessed: Dec. 10, 2024. doi: 10.1016/j.aquaculture.2020.734999.

BECKER, A. G. et al. 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**, v.481, p.184-190, 2017. Available from: <https://doi.org/10.1016/j.aquaculture.2017.09.007>. Accessed: Jan. 12, 2025. doi: 10.1016/j.aquaculture.2017.09.007.

BERNET, D. et al. Histopathology in fish: proposal for a protocol to assess aquatic pollution. **Journal of Fish Diseases**, v.22, p.25-34, 1999. Available from: <https://doi.org/10.1046/j.1365-2761.1999.00134.x>. Accessed: Nov. 16, 2024. doi: 10.1046/j.1365- 2761.1999.00134.x.

BOAVENTURA, T. P. et al. The use of *Ocimum gratissimum* L. essential oil during the transport of *Lophiosilurus alexandri*: water quality, hematology, blood biochemistry and oxidative stress. **Aquaculture**, v.531, p.735964, 2021. Available from: <https://doi.org/10.1016/j.aquaculture.2020.735964>. Accessed: Sept. 17, 2024. doi: 10.1016/j.aquaculture.2020.735964.

BOTREL, B. M. C. et al. Residual determination of anesthetic menthol in fishes by SDME/GC-MS. **Food Chemistry**, v.229, p.674-679, 2017. Available from: <https://doi.org/10.1016/j.foodchem.2017.02.087>. Accessed: Nov. 15, 2024. doi: 10.1016/j.foodchem.2017.02.087.

BRANDÃO, F. R. et al. Anesthetic potential of the essential oils of *Aloysia triphylla*, *Lippia sidoides* and *Mentha piperita* for *Colossoma* *macropomum*. **Aquaculture**, v.534, p.736275, 2021. Available from: <https://doi.org/10.1016/j.aquaculture.2020.736275>. Accessed: Dec. 10, 2024. doi: 10.1016/j.aquaculture.2020.736275.

BRANDÃO, F. R. et al. Essential oils of *Lippia sidoides* and *Mentha piperita* as reducers of stress during the transport of *Colossoma macropomum*. **Aquaculture**, v.560, p.738515, 2022a. Available from: <https://doi.org/10.1016/j.aquaculture.2022.738515>. Accessed: Dec. 12, 2024. doi: 10.1016/j.aquaculture.2022.738515.

BRANDÃO, F. R. et al. Essential oils as anaesthetics and sedatives in native Brazilian fish, with a special emphasis on *Colossoma macropomum*: A review. **Aquaculture Research**, v.53, p.767-781, 2022b. Available from: <https://doi.org/10.1111/are.15650>. Accessed: Jan. 15, 2025. doi: 10.1111/are.15650.

CARSON, F. L.; HLADIK, C. **Histotechnology**: A self-instructional text, 3rd edn. Chicago: ASCP Press, 2009.

CUNHA, M. A. et al. Essentialoil of *Lippia alba*: a new anesthetic for silver catfish, *Rhamdia quelen.* **Aquaculture**, v.306, p.403-406, 2010. Available from: <https://www.sciencedirect.com/science/article/pii/S0044848610003790>. Accessed: Dec. 11, 2024. doi: 10.1016/j.aquaculture.2010.06.014.

DAWOOD, M. A. et al. Exploring the roles of dietary herbal essential oils in aquaculture: A review. **Animals**, v.12, p.823, 2022. Available from: <https://doi.org/10.3390/ani12070823>. Accessed: Jan. 09, 2025. doi: 10.3390/ani12070823.

EVANS, D. H. et al. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. **Physiology Reviews**, v.85, p.97-177, 2005. Available from: <https://doi.org/10.1152/physrev.00050.2003>. Accessed: Dec. 12, 2024. doi: 10.1152/physrev.00050.2003.

FALLEH, H. et al. Essential oils: A promising eco-friendly food preservative. **Food Chemistry**, v.330, p.127268, 2020. Available from: <https://doi.org/10.1016/j.foodchem.2020.127268>. Accessed: Jan. 8, 2025. doi: 10.1016/j.foodchem.2020.127268.

FAZELAN, Z. et al. Effects of dietary ginger (*Zingiber officinale*) administration on growth performance and stress, immunological, and antioxidant responses of common carp (*Cyprinus carpio*) reared under high stocking density. **Aquaculture**, v.518, p.734833, 2020. Available from: <https://doi.org/10.1016/j.aquaculture.2019.734833>. Accessed: Nov. 18, 2024. doi: 10.1016/j.aquaculture.2019.734833.

FERREIRA, P. D. M. F. et al. *Curcuma longa* supplementation in the diet of *Astyanax*aff. *bimaculatus* in preparation for transport. **Aquaculture Research**, v.48, p.4524-4532, 2017. Available from: <https://doi.org/10.1111/are.13277>. Accessed: Jun. 07, 2024. doi: 10.1111/are.13277.

FERREIRA, A. L. et al. Efficacy of *Hesperozygis ringens* essential oil as an anesthetic and for sedation of juvenile tambaqui (*Colossomamacropomum*) during simulated transport. **Aquaculture International**, v.30, p.1549-1561, 2022. Available from: <https://doi.org/10.1007/s10499-022-00868-w>. Accessed: Dec. 12, 2024. doi: 10.1007/s10499-022-00868-w.

FERREIRA, A. L. et al. Oxidative responses in small juveniles of *Colossoma macropomum* anesthetized and sedated with *Ocimum gratissimum* L. essential oil. **Fish Physiology and Biochemistry**, v.50, p.1461-1481, 2024. Available from: <https://doi.org/10.1007/s10695-024-01350-5>. Accessed: Dec. 10, 2024. doi: 10.1007/s10695-024-01350-5.

FLORES-LOPES, F.; THOMAZ. A. T. Histopathologic alterations observed in fish gills as a tool in environmental monitoring. **Brazilian Journal of Biology**, v.71, p.198-188, 2011. Available from: <https://doi.org/10.1590/S1519-69842011000100026>. Accessed: Dec. 11, 2024. doi: 10.1590/S1519-69842011000100026.

FONSECA, T. et al. Lambari aquaculture as a means for the sustainable development of rural communities in Brazil. **Reviews in Fisheries Science & Aquaculture**, v.25, p.316-330, 2017. Available from: <https://doi.org/10.1080/23308249.2017.1320647>. Accessed: Feb. 2, 2025. doi: 10.1080/ 23308249.2017.1320647.

FONSECA, T. et al. Environmental accounting of the yellow-tail lambari aquaculture: sustainability of rural freshwater pond systems. **Sustainability**, v.14, p.2090, 2022. Available from: <https://doi.org/10.3390/su14042090>. Accessed: Feb. 2, 2025. doi: 10.3390/su14042090.

LIU, Y. H. et al. 1, 8-cineole and ginger extract (*Zingiber officinale* Rosc) as stress mitigator for transportation of largemouth bass (*Micropterus salmoides* L.). **Aquaculture**, v.561, p.738622, 2022. Available from: <https://doi.org/10.1016/j.aquaculture.2022.738622>. Accessed: Jan. 8, 2025. doi: 10.1016/j.aquaculture.2022.738622.

LUZ, R. K.; FAVERO, G. C. Use of salt, anesthetics, and stocking density in transport of live fish: A Review. **Fishes**, v.9, p.286, 2024. Available from: <https://doi.org/10.3390/fishes9070286>. Accessed: Jan. 20, 2025. doi: 10.3390/fishes9070286.

MARQUES, A. M. et al. Improving the efficiency of lambari production and diet assimilation using integrated aquaculture with benthic species. **Sustainability**, v.13, p.10196, 2021. Available from: <https://doi.org/10.3390/su131810196>. Accessed: Dec. 05, 2024. doi: 10.3390/su131810196.

MONTEIRO, P. C. et al. Antimicrobial activity of essential oils from *Lippia sidoides, Ocimum gratissimum* and *Zingiber officinale* against *Aeromonas* spp. **Journal of Essential Oil Research**, v.33, p.152-161, 2021. Available from: <https://doi.org/10.1080/10412905.2020.1848653>. Accessed: Dec. 10, 2024. doi: 10.1080/10412905.2020.1848653.

MOREIRA, A. P. et al. Efficacy of essential oil from ginger (*Zingiber officinale*) for anesthesia and transport sedation of pacu (*Piaractus mesopotamicus*). **Fish Physiology and Biochemistry**, v.50, p.865-880, 2024. Available from: <https://doi.org/10.1007/s10695-024-01346-1>. Accessed: Jan. 10, 2025. doi: 10.1007/s10695-024-01346-1.

ODA, A. et al. Pharmacokinetics and pharmacodynamic effects in koi carp (*Cyprinus carpio*) following immersion in propofol. **Veterinary Anaesthesia and Analgesia**, v.45, p.529-538, 2018. Available from: <https://doi.org/10.1016/j.vaa.2018.02.005>. Accessed: Nov. 11, 2024. doi: 10.1016/j.vaa.2018.02.005.

OLIVEIRA, F. C. et al. *Ocimum gratissimum* essential oil in the transport water of *Brycon hilarii:* implications at water quality, blood parameters and residues in tissue and plasma. **Brazilian Journal of Biology**, v.84, p.e280240, 2024. Available from: <https://doi.org/10.1590/1519-6984.280240>. Accessed: Jan. 20, 2025. doi: 10.1590/1519-6984.280240.

OLSON, K. R. Vasculature of the fish gill: anatomical correlates of physiological functions. **Journal of Electron Microscopy Technique**, v.19, p.389-405, 1991. Available from: <https://doi.org/10.1002/jemt.1060190402>. Accessed: Nov. 15, 2024. doi: 10.1002/jemt.1060190402.

PEREIRA, R. A. et al. Quantification of residual clove oil, benzocaine and tricaine in fish fillets using SPE and UPLC-DAD. **Journal of Advances in Chemistry**, v.10, p.2661-2668, 2015. Available from: <https://doi.org/10.24297/jac.v10i5.6967>. Accessed: Nov. 20, 2024. doi: 10.24297/jac.v10i5.6967.

POLEKSIC, V.; MITROVIC-TUTUNDZIC, V. Fish gills as a monitor of sublethal and chronic effects of pollution. In: MULLER, R.; LLOYD, R. **Sublethal and chronic effects of pollutants on freshwater fish.** Oxford: Fishing News Books, 1994, p. 339-352.

PURBOSARI, N. et al. Natural versus synthetic anesthetic for transport of live fish: A review. **Aquaculture and Fisheries**, v.4, p.129-133, 2019. Available from: <https://doi.org/10.1016/j.aaf.2019.03.002>. Accessed: Nov. 15, 2024. doi: 10.1016/j.aaf.2019.03.002.

ROSS, L. G.; ROSS, B. **Anaesthetic and sedative techniques for aquatic animals**. Blackwell Science, Oxford: United Kingdom, 2008.

SAMPAIO, F. D.; FREIRE, C. A. An overview of stress physiology of fish transport: changes in water quality as a function of transport duration. **Fish and Fisheries**, v.17, p.1055-1072,2016. Available from: <https://doi.org/10.1111/faf.12158>. Accessed: Dec. 03, 2024. doi: 10.1111/faf.12158.

SILVA, L. A. et al. Essential oils of *Ocimum gratissimum* and *Zingiber officinale* as anesthetics for the South American catfish *Pseudoplatystoma reticulatum*. **Aquaculture**, v.528, p.735595, 2020. Available from: <https://doi.org/10.1016/j.aquaculture.2020.735595>. Accessed: Dec. 2, 2024. doi: 10.1016/j.aquaculture.2020.735595.

SILVA, J. et al. Effects of vitamin A supplementation on ovarian development of *Astyanax lacustris* (Teleostei: Characidae) during the non-breeding season. **Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology**, v.265, p.111132, 2022. Available from: <https://doi.org/10.1016/j.cbpa.2021.111132>. Accessed: Dec. 16, 2024. doi: 10.1016/j.cbpa.2021.111132.

SILVA, B. A. O. et al. Anesthetic efficiency of essential oil of *Zingiber officinale* for *Astyanax lacustris*: induction time, recovery time, ventilatory frequency, and gill histopathology. **Aquaculture International**, v.32, p.3733-3746, 2023. Available from: <https://doi.org/10.1007/s10499-023-01344-9>. Accessed: Dec. 13, 2024. doi: 10.1007/s10499-023-01344-9.

SOUZA, C. et al. Essential oils as stress-reducing agents for fish aquaculture: A review. **Frontiers in Physiology**, v.10, p.785, 2019. Available from: <https://doi.org/10.3389/fphys.2019.00785>. Accessed: Dec. 02, 2024. doi: 10.3389/fphys.2019.00785.

TONI, C. et al. Stress response in silver catfish (*Rhamdia quelen*) exposed to the essential oil of *Hesperozygis ringens.* **Fish Physiology and Biochemistry**, v.41, p.129-138, 2015. Available from: <https://doi.org/10.1007/s10695-014-0011-z>. Accessed: Jan. 07, 2024. doi: 10.1007/s10695-014-0011-z.

VALENTI, W. C. et al. Aquaculture in Brazil: past, present and future. **Aquaculture Reports**, v.19, p.100611, 2021. Available from: <https://doi.org/10.1016/j.aqrep.2021.100611>. Accessed: Jan. 22, 2025. doi: 10.1016/j.aqrep. 2021.100611.

VAN DYK, J. C. et al. Liver histopathology of the sharptooth catfish *Clarias gariepinus* as a biomarker of aquatic pollution. **Chemosphere**, v.87, p.301-311, 2012. Available from: <https://doi.org/10.1016/j.chemosphere.2011.12.002>. Accessed: Nov. 03, 2024. doi: 10.1016/j.chemosphere.2011.12.002.

VANDERZWALMEN, M. et al. The use of feed and water additives for live fish transport. **Reviews in Aquaculture**, v.11, p.263-278, 2019. Available from: <https://doi.org/10.1111/raq.12239>. Accessed: May, 07, 205. doi: 10.1111/raq.12239.

VENTURA, A. S. et al. Natural anesthetics in the transport of Nile tilapia: hematological and biochemical responses and residual concentration in the fillet. **Aquaculture**, v.526, p.735365, 2020. Available from: <https://doi.org/10.1016/j.aquaculture.2020.735365>. Accessed: Dec. 15, 2024. doi: 10.1016/j.aquaculture.2020.735365.

VENTURA, A. S. et al. *Ocimum basilicum* essential oil in pacu *Piaractus mesopotamicus*: anesthetic efficacy, distribution, and depletion in different tissues. **Veterinary Research Communications**, v.48, p.685-694, 2024. Available from: <https://doi.org/10.1007/s11259-023-10225-8>. Accessed: Jan. 10, 2025. doi: 10.1007/s11259-023-10225-8.

WANG, Q. et al. Effects of *Melissa officinalis* L. essential oil in comparison with anesthetics on gill tissue damage, liver metabolism and immune parameters in sea bass (*Lateolabrax maculatus*) during simulated live transport. **Biology**, v.11, p.11, 2021. Available from: <https://doi.org/10.3390/biology11010011>. Accessed: Dec. 8, 2024. doi: 10.3390/biology11010011.