Int Aquat Res (2021) 13:261-270
https://doi.org/10.22034/IAR.2021.1938212.1183

ORIGINAL RESEARCH

Thermal dynamics and physiological implications in pacu *Piaractus mesopotamicus* anaesthetised with *Ocimum basilicum* essential oil

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Received: 19 August 2021 / Accepted: 14 November 2021 / Published online: 21 November 2021

Abstract The aim of this study was to evaluate the haematological, biochemical and body temperature parameters of pacu *Piaractus mesopotamicus* juveniles after capture stress followed by anaesthetic induction. Fish (30.4±1.4 g) were divided into five groups (12 fish each group): control (water only handling), ethanol handling (600 µL L^{-1}), handling with 100, 350 and 600 mg L^{-1} of essential *Ocimum basilicum* oil. Fish were caught and anaesthetised, followed by biometric handling, blood collection and thermographic images. Increased anaesthetic concentrations had a linear positive effect on haemoglobin content, erythrocytes, mean corpuscular haemoglobin concentration (MCHC), monocytes and granular leukocyte PAS positive (LG-PAS) ($P<0.05$). A quadratic effect ($P<0.05$) was observed for lymphocytes, with a maximum peak at the 350 mg L^{-1} concentration. The fish surface temperature ranged was 25.9-29.9ºC, with the highest values in the non-anaesthetised fish's cephalic regions. The fish anaesthetised with the *O. basilicum* essential oil 100 mg L^{-1} concentration showed a lower surface temperature. Using *O. basilicum* essential oil in biometric handling procedures was unable to prevent stress-related haematological alterations in juvenile pacu. Employing infrared thermography to assess surface temperature provides useful data to understand the effects of anaesthesia on fish, but more studies are needed to better understand this technique as a measure of well-being in fish farming.

Keywords Aquaculture stress . Basil . Fish haematology . Infrared thermography in fish

Introduction

Pacu *Piaractus mesopotamicus* is a large fish with a promising potential for aquaculture in South American countries that is naturally found in the Paraná River Basin covering parts of Paraguay, Uruguay, Argentina and Brazil (Jomori et al. 2005; Urbinati and Gonçalves 2013). It is characterized by its rusticity, fast growth, easy adaptation to artificial feeding and good consumer acceptance thanks to its excellent quality meat

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(Urbinati and Gonçalves 2013). It also has a very high cultivation potential in different production systems, and was one of the main species of native fish produced in Brazil in 2019 (IBGE 2020). This fish species has been used as a good model for studies to validate natural anaesthetics, such as menthol, eugenol (Gonçalves et al. 2008; Rotili et al. 2012; Sanchez et al. 2014), and essential oils of *Lippia sidoides* and *Lippia alba* (Ventura et al. 2019 ab).

Studies have revealed that the handling stress caused by capture, biometrics and transport harm some fish species' well-being (Boijink et al. 2016; Ribeiro et al. 2016; Ventura et al. 2019ab; Ventura et al. 2020a). The pattern of response to the effects of anaesthetics as stress reducers in fish appears species-specific, which is why haematological parameters have been used as markers of fish health status (Pádua et al. 2013; Ventura et al. 2019ab) Total protein in serum reflects fish nutritional and metabolic status, which indirectly affects non-specific immunity levels (Ortuno et al. 2001), and may also represent chronic disease or liver failure (Ranzani Paiva et al. 2013). Serum glucose can be utilized as a reliable quantitative measure of stress in fish (Ortuno et al. 2001; Ni et al. 2014). Fish plasma chloride levels can be induced by stress (Becker et al. 2012, 2016) from increased branchial blood flow and enhanced cell permeability, which result in ionic loss (Becker et al. 2012).

During handling practices, such as biometrics and hormonal induction in breeders, fish are often exposed to changes in ambient temperature when they are caught, removed from water, and are exposed to air and solar radiation. Room temperature is directly related to fish physiology (Wosnick et al. 2018) and, thus, temperature in fish determines important physiological characteristics like metabolism and cardio-ventilatory dynamics (Wosnick et al. 2019). Studies have elucidated the combined effects of water temperature and air exposure on fish survival (Silva et al. 2012; Cockrem et al. 2019; Mattioli et al. 2019), but very little is known about how air temperature impacts the external body temperature of fish subjected to handling when natural anaesthetics are administered. Assessing thermal dynamics on exposure to air provides a better understanding of the stressful effects that animals are exposed to when they are moved from water to air. Thermographic studies allow not only the real-time visualization of changes in the radiative energy emanating from an object, but also surface temperature estimations (Cilulko et al. 2013). This technology enables assessments of the physiological responses associated with external temperature and heat distribution patterns in animals to be made (Tattersall and Cadena 2010; Wosnick et al. 2018, 2019). This is relevant because the physiological responses of animals exposed to various stress factors determine changes in body temperature (Cilulko et al. 2013). Furthermore, in fish cutaneous nerves sense ambient temperature and respond by changing skin temperature via a feedforward signal mechanism (Romanovsky 2014; Gorissen and Flik 2016).

In aquaculture, sedation or anaesthesia is a necessary practice in various handling situations such as biometrics, hormonal induction, gamete collection, vaccination and transport (Mylonas et al. 2005). Several drugs such as tricaine methanesulfonate (MS-222), 2-phenoxyethanol, quinaldine, benzocaine and methomidate are used as anesthetics in aquaculture (Bolasina et al. 2017; Hedayati 2018; Botrel et al. 2019). However, damage to fish has been observed when exposed to these drugs (Purbosari et al. 2008; Ventura et al. 2020; Mácová et al. 2021). In addition, they leave residues in muscle tissue, being a food safety risk (Mylonas et al. 2005). Given this perspective, natural alternatives to the use of synthetic anesthetics are needed. Essential oils are some of the natural agents studied for anesthetic purposes in fish (Ventura et al. 2019ab; Purbosari et al. 2021; Ventura et al. 2021).

Ocimum basilicum essential oil is popularly known as basil. It is characterized by high genetic diversity, with variations in morphological characteristics, size, color and chemical composition (Singh et al. 2018). With a wide geographic distribution, this species has numerous beneficial effects in popular medicine: antimicrobial, antifungal, anticancer, antioxidant, antispasmodic, anti-inflammatory, calming, sedative and analgesic potential (Mahajan et al. 2013; Uritu et al. 2018). In aquaculture, studies have demonstrated the anaesthetic and sedative potential of *O. basilicum* essential oil in fish, such as: tambacu (*Piaractus mesopotamicus* male x *Colossoma macropomum* female; Lima Netto et al. 2016); Nile tilapia (*Oreochromis niloticus* Lima Netto et al. 2017; Ventura et al. 2020ab); clownfish (*Amphiprion clarkii* Correia et al. 2018); tambaqui (*Colossoma macropomum* Ventura et al. 2021). Yet to date no studies have assessed the physiological impacts on and their relation to infrared radiation in fish undergoing essential oil anaesthesia. In this context, infrared thermography is a relatively non-invasive technology to assess potential physiological changes in fish exposed to air and subjected to anaesthesia during biometric handling practices. This study

aimed to evaluate the haematological, biochemical and infrared temperature parameters in pacu *Piaractus mesopotamicus* juveniles (30.4 g) anaesthetised with *O. basilicum* essential oil.

Materials and methods

Essential oil

The *O.basilicum* essential oil here in employed was commercially purchased (Phytoterápica®, Nova Cantareira, Brazil). Methyl chavicol (66.51%) and linalool (20.90%) was the main compounds with minor components comprising 12.57% (Ventura et al. 2020a). Essential oil concentrations were diluted 1:10 with ethanol to obtain anaesthetic solutions. This study was conducted in accordance with the Ethics and Animal Welfare Committee/Federal University of Mato Grosso do Sul (Approval no. 976/2018).

Animals

Pacu *P. mesopotamicus* juveniles weighing 30.4±1.4 g and of total length 9.3±1.4 cm (n=60) were kept in a 500-litre fibreglass tank for 30 days to acclimatise in the facilities of the Aquaculture Production Laboratory of the Federal University of Grande Dourados in the municipality of Dourados (22° 14'51.91"S/ 54°47'23.11"O), Mato Grosso do Sul, Brazil. Fish were fed a commercial diet (From fish Douramix®) of pellet size of 2-3 mm (36% crude protein; 4.0% crude fiber; 11.0% mineral matter; 7.0% ether extract; 12.0% moisture; 2.2% calcium) calcium) 3 times a day until apparent satiety. Feeding was suspended 24 h before the experiment.

Water physico-chemical variables

The following physico-chemical water quality parameters were measured: dissolved oxygen (5.7±0.9 mg L^{-1}) and temperature (25.2±1.4ºC) with the help of a portable oximeter (Ysi 550 – YSI incorporated®), pH (6.7±0.4) with a pH meter (HI8314 – Hanna instruments) and total ammonia nitrogen concentration (NAT) (0.10±0.02 mg L^{-1}) measured by a colorimetric kit (Alfakit®). These values are considered ideal for tropical fish cultivation according to Boyd (1998).

Experimental design

After the acclimatization period, fish were exposed to capture and handling stress followed by anaesthesia with *O. basilicum* essential oil. They were divided into five groups (12 fish each group): control (only water handling), handling with ethanol (600 µL L^{-1}), handling with 100, 350 and 600 mg L^{-1} of *O. basilicum* essential oil. These oil concentrations were the equivalent to 111, 389 and 667 µL L^{-1}, respectively, considering that *O. basilicum* essential oil density was 0.90 g mL^{-1}. The *O. basilicum* essential oil concentrations were chosen based on the criteria for promoting light and deep anaesthesia according to Woody et al. (2002) during previous tests. Ethanol treatment was used to see if it was capable of inducing sedation or anaesthesia and change some physiological parameter as well as the surface temperature of pacu *P. mesopotamicus*. The stage 3 anaesthesia procedure and biometric handling were carried out according to Ventura et al. (2021). Survival was monitored up to 96 h after anaesthetic induction.

Haematological and biochemical analyses

Arterial-venous blood samples were collected from the tail vein using a syringe with 10% EDTA (ethylenediaminetetraacetic acid) as an anticoagulant. With the collected blood, two aliquots were separated and analysed for haematocrit, haemoglobin, total number of erythrocytes, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and blood smears in duplicate for the total and differential counts of thrombocytes and leukocytes according to the methodology described by Ranzani Paiva et al. (2013).

The second blood aliquot was centrifuged at 3,000 rpm for 7 min to obtain plasma (Fanem Centrimicro,

Brazil) and was later stored at −20ºC. Glucose, total plasma protein and chloride (Cl-) were measured with plasma by spectrophotometry (1203 UV, Shimadzu, Japan) with a commercial kit (Labtest®). Samples were analysed in a pool (3 fish for 1 sample). Four samples were analysed per treatment.

Infrared thermography analysis

Thermal images were taken by a thermal imager camera (Model Testo® 880 Texto, Lenzkirch, BR, Germany). Water temperature was measured by a portable oximeter (Ysi 550 – YSI incorporated®). Relative humidity, air temperature and distance from the animal were measured by the camera and then calibrated according to daily conditions following a thermal measurement protocol (Kastberger and Stachl 2003). The emissivity coefficient was set at 0.98-0.99 energy units (Kastberger and Stachl 2003). Thermograms were analysed by FLIR software tools (FLIR Tools 2.1®-E40).

Thermal images were obtained before anaesthesia, immediately after anaesthesia and after recovering from anaesthetic with *O. basilicum* essential oil. The ethanol group fish were not measured at surface temperature. Three main fish regions were measured: (I) head, comprising the region from the mouth to the caudal edge of the operculum; (II) body, including the region between the end of the opercle and the peduncle of the caudal fin; (III) tail, consisting in the insertion to the final edge of the caudal fin. Ten points were randomly selected in each body region (30 in all per fish). The average of each body region was used to estimate the temperature at each evaluated time (before anaesthesia; immediately after anaesthesia; after recovering from anaesthesia). The temperature in each target was observed by the colour pattern, which indicated the variation in the thermal values according to the electromagnetic spectrum identified by the colour palette in the thermogram (adapted from Wosnick et al. 2018).

Statistical analysis

The collected data were submitted to SAS (Version 9.1.3, SAS Institute, Cary, NC 2004) to verify the normality of residues and the homogeneity of variances by PROC UNIVARIATE. Data were subjected to an analysis of variance by the PROC MIXED of the SAS command by taking the following mathematical model:

$$Y_i = \mu + A_i + T_j + e_i$$

where, μ = overall mean, A_i = fixed treatment effect and e_i = experimental error. To evaluate the experimental treatments, a polynomial regression analysis was performed using the PROC REG of the SAS at a 5% significance level.

The data referring to heat emission by infrared thermography were subjected to repeated measurements over time by the PROC MIXED of the SAS command by applying the following mathematical model:

$$Y_{ijk} = \mu + A_i + T_j + A \times T_{ij} + e_{ijk}$$

where, μ = overall mean, A_i = fixed treatment effect, T_j = time effect, $A \times T_{ij}$ = interaction effect and e_{ijk} = experimental error. To evaluate the experimental treatments, a polynomial regression analysis was performed using the PROC REG of the SAS with a 5% significance level.

Results

The increase in the *O. basilicum* essential oil anaesthetic concentrations had a positive linear effect on haemoglobin content (Y= 5.934 + 0.345X), number of erythrocytes (Y= 1.131 -0.00145X) and MCHC (Y= 16.356 + 1 .18X). A negative linear relation was verified for MCV (Y= 22.334 +2.145X). The percentage of haematocrit and biochemical parameters of total plasma protein, glucose and chloride were not significantly different (Table 1). Ethanol did not influence the haematological parameters of pacu *P. mesopotamicus* juveniles. After the experimental period, no mortality was recorded in the different treatments.

Increased anaesthetic *O. basilicum* concentrations had a positive linear effect on number of monocytes

(Y= 1795 +114.54X) and LG-PAS (Granular leukocyte PAS positive) (Y= 2290 +105.547X) circulating in blood. A quadratic effect was observed (Y= 16116 +1.105X -0.0012x2) for circulating lymphocytes, with a maximum peak at the *O. basilicum* essential oil 350 mg L^{-1} concentration. Total leukocytes, thrombocytes, neutrophils, eosinophils, immature leukocytes and basophils were not significantly different (Table 2).

The water temperature range during the experimental period was 23.7-26.6ºC. Fish surface temperature ranged from 25.9ºC to 29.9ºC. A quadratic relation was observed in the head region (Y= 27.785-0.567X +0.0011x2), with a higher temperature in the non-anaesthetised fish (29.9ºC) and a lower one (26.4ºC) in the fish anaesthetised with the *O. basilicum* essential oil 100 mg L^{-1} concentration (Table 3).

The thermal images of the body region revealed that no time-treatment interaction; that is, the different *O. basilicum* essential oil concentrations induced changes in the heat emission of the pacu *P. mesopotamicus* juveniles according to the time point (before anaesthesia, immediately after anaesthesia and after recovering from anaesthesic) (Table 3). The body region underwent a quadratic effect (Y= 27.722 -0.782X +0.0021x2) with a higher surface temperature in the non-anaesthetised fish (28.9ºC) and a lower one (26.6ºC) in the fish anaesthetised with the *O. basilicum* essential oil 100 mg L^{-1} concentration (Table 3). A quadratic relation was noted (Y= 27.095 -0.487X +0.0017x2) in the tail region, with a lower surface temperature (25.9ºC) in the fish anaesthetised with the *O. basilicum* essential oil 100 mg L^{-1} concentration, while fish in the control

Table 1 Mean values of the erythrocyte and biochemical parameters of pacu *Piaractus mesopotamicus* submitted to anaesthetic induction with *Ocimum basilicum* essential oil.

Parameter	*Ocimum basilicum*					Orthogonal contrast			
	Ethanol	0 mg L^{-1}	100 mg L^{-1}	350 mg L^{-1}	600 mg L^{-1}	SEM[a]	Linear	Quadratic	R^2
Haematocrit (%)	29.75	26.00	26.75	26.00	26.50	0.92	0.9365	0.9526	-
Haemoglobin (g dl^{-1})*	5.07	4.91	5.00	6.99	7.70	0.45	0.0072	0.6662	0.12
Erythrocytes (x10^{-6}) *	0.695	0.61	1.12	1.60	1.63	0.15	0.0074	0.3446	0.14
MCV (fL) *	479.42	996.45	246.37	205.73	166.91	13.12	0.0226	0.1260	0.17
MCHC (g dL^{-1})*	18.23	18.58	18.76	26.92	29.19	1.53	0.0009	0.6170	0.19
Total proteins (mg dl^{-1})	3.75	4.10	4.15	4.35	4.65	0.12	0.1014	0.6017	-
Glucose (mg dl^{-1})	63.50	56.82	65.80	58.64	80.31	4.33	0.1050	0.4468	-
Chloride (mE dL^{-1})	331.06	330.00	324.10	333.34	339.65	5.11	0.4502	0.5876	-

MCV- Mean Corpuscular Volume; MCHC- mean corpuscular haemoglobin concentration. [a] SEM- standard error of the mean. * = linear regression.

Table 2 Mean values of the white blood cell counts of pacu *Piaractus mesopotamicus* submitted to anaesthetic induction with *Ocimum basilicum* essential oil

Parameter	*Ocimum basilicum*					Orthogonal contrast			
	Ethanol	0 mg L^{-1}	100 mg L^{-1}	350 mg L^{-1}	600 mg L^{-1}	SEM[a]	Linear	Quadratic	R^2
Leukocytes (x 10^3 μl^{-1})	43.07	25.88	26.74	35.13	29.86	4.08	0.6111	0.7314	-
Thrombocytes (x 10^3 μl^{-1})	17.87	14.37	12.81	25.28	28.08	4.43	0.2147	0.8156	-
Neutrophils (x 10^3 μl^{-1})	32.18	21.43	8.00	8.32	10.21	2.20	0.0703	0.0639	-
Eosinophils (x 10^3 μl^{-1})	1.41	1.76	0.88	0.52	0.93	0.25	0.2236	0.2042	-
Lymphocytes (x 10^3 μl^{-1})**	10.32	9.52	20.41	21.09	12.65	1.48	0.1614	<0.0001	0.21
LG-PAS (x 10^3 μl^{-1})*	1.46	0.21	2.08	2.89	3.99	0.51	0.0067	0.6494	0.18
Monocytes (x 10^3 μl^{-1})*	2.74	0	1.88	2.31	3.99	0.41	<0.0001	0.3130	0.19
Leukocytes Immature (x 10^3 μl^{-1})	26.10	11.38	16.30	23.06	22.13	2.84	0.1540	0.6197	-

LG-PAS - Granular leukocyte PAS positive. [a] SEM- standard error of the mean. * = linear regression. ** = quadratic regression.

Table 3 Thermographic evaluation of the pacu *Piaractus mesopotamicus* submitted to anaesthetic induction with *Ocimum basilicum* essential oil

Parameter	Anaesthesia				SEM[a]	Orthogonal contrast					
	0	100	350	600		Treatment	Time	Interaction	Linear	Quadratic	R^2
Head**	29.92	26.45	26.74	28.03	3.77	0.0085	0.8089	0.8757	0.0892	0.0070	0.35
Body**	28.99	26.64	26.91	28.35	3.33	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	0.37
Tail**	28.12	25.89	26.53	27.84	3.46	<0.0001	0.1452	0.1818	0.8054	<0.0001	0.42

[a] SEM- standard error of the mean. ** = quadratic regression.

group (non-anaesthetised) had a higher surface temperature (28.1ºC) (Table 3).

Discussion

The treatments imposed on fish did not alter the haematocrit percentage, which suggests that the *O. basilicum* essential oil anaesthetic concentrations did not influence this variable. Similar results have been observed in tambaqui *C. macropomum* anaesthetised with clove oil (Pádua et al. 2013) and matrinxã *Brycon amazonicus* juveniles anaesthetised with *Ocimum gratissimum* essential oil (Ribeiro et al. 2016). The positive linear effect on haemoglobin, erythrocytes and MCHC levels herein observed could indicate higher oxygen demand during anaesthesia. Changes like these can be suggestive of a stressful effect and/or respiratory dysfunction (Hashimoto et al. 2016; Ventura et al. 2020a). However, in the *P. mesopotamicus* pacus juveniles anaesthetised with menthol, eugenol and *L. sidoides* essential oils, no changes in the haematological parameters were observed immediately after handling and anaesthesia (Sanchez et al. 2014; Ventura et al. 2019a). This could be due to not only the different compositions of the employed anaesthetic agents, but also the applied anaesthetic concentration (Bowker et al. 2015). Therefore, the haematological alterations observed in the present study indicated that exposure to *O. basilicum* essential oil required fish adaptive response mechanisms.

Physiometabolic changes occur with a rise in blood cortisol levels, observed by a larger number of erythrocytes and a drop in MCV (Vosyliené 1999), which corroborate the results herein obtained. In tambaqui exposed to *Ocimum gratisimum* essential oil, blood parameters of erythrocyte numbers and MCV were similar between treatments (Boijink et al. 2016). Thus it can be inferred that the evaluated anaesthetic concentrations induce a high anaesthesia level with changes in blood flow and cardiac dynamics, which are easily observed by MCV lowering in accordance with an increasing anaesthetic concentration.

Total plasma protein determination is of much clinical importance because these concentrations can be altered by the osmotic imbalance between plasma extracellular and intracellular compartments, and any stress that induces this imbalance can lead to declining plasma protein (Barton and Iwama 1991). However, no changes in this parameter were observed in the present study, which corroborates the hypothesis that handling followed by *O.basilicum* essential oil anaesthesia does not induce osmoregulatory imbalance because chloride levels did not influence treatments.

With the activation of the hypothalamic-pituitary-interrenal axis in stressful situations, metabolic changes can occur like increased glucose and osmoregulatory disturbances in fish (Barton 2002). In the present study, the stimulation of anaesthesia and handling with *O. basilicum* essential oil did not change glucose and chloride levels. These results fall in line with the study performed in tambaqui *C. macropomum* submitted to anaesthesia with 800 µL L^{-1} of *O. basilicum* essential oil, which reported a higher plasma chloride level (Ventura et al. 2021). However, when adding this same essential oil at the 20 µL L^{-1} concentration to Nile tilapia *O. niloticus* transport water, plasma glucose levels did not alter, but the chloride level lowered (Ventura et al. 2020a). This can be attributed to variations in physiological responses between fish species, and to the different *O. basilicum* essential oil concentrations used in these studies.

Variation in the number of circulating leukocytes can be attributed to a generalised immune system response triggered by physiological stress and, consequently, affecting health status (Vosyliené 1999). However, in fish, white blood cell counts can be influenced by several factors (Clauss et al. 2008). In the present study, anaesthesia with *O. basilicum* essential oil followed by pacu *P. mesopotamicus* juveniles handling had no influence on the number of thrombocytes, neutrophils, eosinophils, basophils and immature leukocytes. This might be attributed to the fact that some fish are more sensitive to stress, while others possess a better tolerance capacity (Ahmed et al. 2020).

An increase in leukocytes can take place with acute stress in most fish species, and is considered as an attempt to recover homeostasis imbalance. In turn, a drop in leukocyte counts can be attributed to immune system weakening (Ranzani-Paiva et al. 2013). In our study, the number of circulating leukocytes was not influenced by treatments. Similar results have been reported for Nile tilapia *O. niloticus* exposed to *L. sidoides* and *Mentha piperita* essential oil (Hashimoto et al. 2016), and for tambaqui *C. macropomum* receiving *M. piperita* essential oil (Ferreira et al. 2019). However, changes in the haematological conditions of different fish species can be influenced by endogenous and exogenous factors (Ahmed et al. 2020), and

the effect of the stressful stimulus on haematological parameters depends on the stress level and exposure time.

Lymphocytes are responsible for organism humoral and cellular immunity (Tavares-Dias and Oliveira 2009). Their work recorded a rising number of lymphocytes up to the *O. basilicum* essential oil 350 mg L^{-1} concentration, with similar results for the tambaqui *C. macropomum* anaesthetised with 50 mg L^{-1} clove oil and 100 mg L^{-1} benzocaine (Padua et al. 2013). However, lymphopenia in the present study was observed in the fish anaesthetized at the 600 mg L^{-1} *O. basilicum* essential oil concentration, which was possibly due to the action of increased plasma cortisol and is suggestive of a stressor effect (Bishkoul et al. 2015). Thus it is possible to infer that the 600 mg L^{-1} concentration of *O. basilicum* essential oil anaesthesia may result in a fish immune system imbalance and make fish susceptible to pathogens (Pickering and Pottinger 1985).

Monocytes act by inflammatory reaction and immune response in which phagocytosis occurs, and are extremely important for the host's defence mechanisms (Ranzani Paiva et al. 2013). An increasing number of these cells in the peripheral blood of the pacu *P. mesopotamicus* anaesthetised with *O. basilicum* essential oil suggests that a rise in anaesthetic concentrations induces homeostatic imbalance in fish, and the organism attempts to reverse this adverse condition by increasing defence cells. Similar results have been obtained In Nile tilapia *O. niloticus* exposed to *M. piperita* essential oil at 40 mg L^{-1} (Hashimoto et al. 2016). A growing number of granular leukocyte PAS positive is related to stress (Tavares-Dias and Oliveira 2009), and the same situation has been found in tambaqui *C. macropomum* anaesthetised with 50 mg L^{-1} of clove oil (Pádua et al. 2013). Therefore, the higher the anaesthetic *O. basilicum* essential oil concentration, the better the response of haematological variables to induced stress in pacu *P. mesopotamicus* juveniles.

Temperature was not uniformly distributed over the body surface of the pacu *P. mesopotamicus* juveniles anaesthetised with *O. basilicum* essential oil. Similar results have been reported in elasmobranch fish exposed to air (Wosnick et al. 2019). Some fish species are able to maintain higher body temperatures given the presence of countercurrent heat exchangers (Tubbesing and Block 2000). In this way, they are able to minimise heat loss through gill ventilation (Carey et al. 1971).

The non-anaesthetised fish presented a higher temperature in the different evaluated regions. This could be due to changes in blood flow occurring during escape situations, e.g. during capture stress (McCafferty 2007). Rising stress hormone levels lead to a higher ventilation rate, increased branchial blood flow and greater cardiac output (Randall and Perry 1992), which could be associated with higher temperature. Some studies indicate a rapid increase in body surface temperature in elasmobranchs while exposed to air (Wosnick et al. 2019).

When anaesthetising fish at the 100 mg L^{-1} concentration, heat emission lowers. This can be explained by reduced blood flow and a lower respiratory rate in anaesthetised fish (Zahl et al. 2009). Although heat emission is influenced by core body temperature, the rate and magnitude of this change are unknown (Romanovsky 2014). Specific changes in heat emission occur in elasmobranchs depending on species, size, body region and water temperature (Wosnick et al. 2019).

Studies in mammals have revealed a relation between infrared thermal imaging and hypothalamic-pituitary-interrenal axis activity as being indicative of stress (Cook et al. 2001). With rising anaesthetic concentrations, the surface temperature increases in different regions of pacu *P. mesopotamicus* juveniles, although anaesthetic agents are used in aquaculture to mitigate handling stress effects (Becker et al. 2012; Ventura et al. 2021). At high doses, they can trigger harmful body reactions similarly to those observed in stress situations (Kanani et al. 2013), which corroborates the hypothesis that infrared thermography can be used to detect and measure adverse fish reactions.

Conclusion

O. basilicum essential oil administered at the 100, 350 and 600 mg L^{-1} concentrations was unable to mitigate the handling stress effects on the haematological parameters of pacu juveniles. The evaluated concentrations induced a high anaesthesia level with changes in blood flow and cardiac dynamics, but did not alter metabolic and osmoregulatory parameters. Skin temperature measurements taken by infrared thermography can quickly provide useful data for understanding the effects of essential oil anaesthesia on fish, but more studies are needed to better understand the use of this technique as a measure of well-being in aquaculture.

Competing interests The authors declare that they have no competing interests.

Author's contribution ASV- Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing - original draft; Writing - review & editing. AM de AG- Investigation; Methodology; Supervision; Visualization. JRG - Formal analysis; Funding acquisition; Investigation; Methodology. IZN - Data curation; Formal analysis; Investigation; Methodology; JAP- Formal analysis; Investigation; Project administration; Supervision; Visualization; Writing - original draft; Writing - review & editing. GTJ- Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Supervision; Validation; Visualization; Writing - original draft; Writing - review & editing.

Acknowledgements This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001 Brazil (CAPES), Federal University Grande Dourados (UFGD) and the Federal University of Mato Grosso do Sul (UFMS).

References

Ahmed I, Reshi QM, Fazio F (2020) The influence of the endogenous and exogenous factors on hematological parameters in different fish species: a review. Aquac Int 28:869-899

Barton BA, Iwama GK (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu Rev Fish Dis 1:3-26

Barton BA (2002) Stress in fishes: a diversity of responses with particular reference to change in circulating corticosteroids. Integr Comp Biol 42:517-525

Becker AG, Parodi TV, Heldwein CG, Zeppenfeld CC, Heinzmann BM, Baldisserotto B (2012) Transportation of silver catfish, *Rhamdia quelen* in water with eugenol and the essential oil of *Lippia alba.* Fish Physiol and Biochem 38:789-796

Becker AG, Parodi TV, Zeppenfeld CC, Salbego J, Cunha MA, Heldwein CG, Loro VL, Heinzmann BM, Baldisserotto B (2016) Pre-sedation and transport of *Rhamdia quelen* in water containing essential oil of *Lippia alba*: metabolic and physiological responses. Fish Physiol and Biochem 42:73-81

Bishkoul GR, Halimi M, Norousta R, Zamani N, Heidari L (2015) The anesthetic effects of MS-222 (tricaine methanesulfonate) on some hematological parameters of sterlet, *Acipenser ruthenus.* Comp Clin Pathol 24:89-92

Boijink C, Queiroz C, Chagas E, Chaves F, Inoue L (2016) Anaesthetic and anthelminthic effects of clove basil (*Ocimum gratissimum*) essential oil for tambaqui. Aquaculture 457:24-28

Bolasina SN, Azevedo A, Petry AC (2017) Comparative efficacy of benzocaine, tricaine methanesulfonate and eugenol as anesthetic agents in the guppy *Poecilia vivipara*. Aquac Rep 6:56-60

Botrel BMC, Abreu DCP, Bazana MJF, Rosa PV, Saczk AA (2019) Development, Optimization, and Validation of the HS-SPME/GC-MS Method for the Residual Determination of Menthol in Fish. Food Anal Methods 12:1390-1398

Bowker JD, Trushenski JT, Glover DC, Carty DG, Wandelear N (2015) Sedative options for fish research: a brief review with new data on sedation of warm, cool, and cold-water fishes and recommendations for the drug approval process. Rev Fish Biol Fish 25:147-163

Boyd CE (1998) Water quality for pond aquaculture. International Center of Aquaculture and Aquatic Environments, Alabama Agricultural Experiment Station, Auburn University 37p

Carey FG, Teal JM, Kanwisher JW, Lawson KD, Beckett JS (1971) Warm-bodied fish. Am Zool 11:137-145

Cilulko J, Janiszewski P, Bogdaszewski M, Szczygielska E (2013) Infrared thermal imaging in studies of wild animals. Eur J Wildl Res 59:17-23

Clauss TM, Dove AD, Arnold JE (2008) Hematologic disorders of fish. Vet Clin North Am Exot Anim Pract 11:445-462

Correia AM, Pedrazzani AS, Mendonça RC, Massucatto A, Ozório RA, Tsuzuki MY (2018) Basil, tea tree and clove essential oils as analgesics and anaesthetics in *Amphiprion clarkii* (Bennett, 1830). Braz J Biol 78:436-442

Cook NJ, Schaefer AL, Warren L, Burwash L, Anderson M, Baron V (2001) Adrenocortical and metabolic responses to ACTH injection in horses: na Assessment by salivar cortisol and infrared thermography of the eye. Can J Anim Sci 81:621(abstract).

Cockrem JF, Bahry MA, Chowdhury VS (2019) Cortisol responses of goldfish (*Carassius auratus*) to air exposure, chasing, and increased water temperature. Gen Comp Endocrinol 270:18-25

Ferreira LC, Cruz MG, Lima TBC, Serra BNV, Chaves FCM, Chagas EC, Ventura AS, Jerônimo GT (2019) Antiparasitic activity of *Mentha piperita* (Lamiaceae) essential oil against *Piscinoodinium pillulare* and its physiological ef- fects on *Colossoma macropomum* (Cuvier, 1818). Aquaculture 512:734343

Gonçalves AFN, Santos ECC, Fernandes JBK, Takahashi LS (2008) Mentol e eugenol como substitutos da benzocaína na indução anestésica de juvenis de pacu. Acta Sci Anim Sci 30:339-344

Gorissen M, Flik G (2016) The Endocrinology of the Stress Response in Fish: An Adaptation-physiological View. In Biology of Stress in Fish, pp 75–111. Eds. Schreck CB, Tort L, Farell AP, & Brauner CJ London

Hashimoto GSO, Marinho Neto F, Ruiz ML, Acchile M, Chagas EC, Chaves FCM, Martins ML (2016) Essential oils of *Lippia sidoides* and *Mentha piperita* against monogenean parasites and their influence on the hematology of Nile tilapia. Aquaculture 450:182-186

Hedayati AA (2018) Effects of 2-phenoxyethanol (2-PE) anesthesia on some haematological and biochemical indices of silver carp (*Hypophthalmichthys molitrix*). Iran J Fish Sci 17:1-10

IBGE, 2020. Instituto Brasileiro de Geografia e Estatística – Produção de aquicultura, http://www.sidra.ibge.gov.br/ bda/tabela/listabl.asp?c=3940&z=t&o=21; (Acessed 19.07.2020)

Jomori RK, Carneiro DJ, Martins MIEG, Portella MC (2005) Economic evaluation of *Piaractus mesopotamicus* juvenile production in different rearing systems. Aquaculture 243:175-183

Kanani HG, Soltani M, Mirzargar SS (2013) Effect of tricaine methanesulfonate (MS222), clove oil and electro-anaesthesia on respiratory burst activity in whole blood and serum alternative complement response in rainbow trout (*Oncorhynchus mykiss*),

during the narcosis stage. Fish Shellfish Immunol 34:692-696
Kastberger G, Stachl R (2003) Infrared imaging technology and biological applications. Behav Res Meth Instrum Comput 35:429-439
Lima-Netto JD, Sena AC, Copatti CE (2016) Essential oils of *Ocimum basilicum* and *Cymbopogon flexuosus* in the sedation, anesthesia and recovery of tambacu (*Piaractus mesopotamicus* male x *Colossoma macropomum* female). Bol Inst Pesca 42:727-733
Lima-Netto JD, Oliveira RSM, Copatti CE (2017). Efficiency of essential oils of *Ocimum basilicum* and *Cymbopogum exuosus* in the sedation and anaesthesia of Nile tilapia juveniles. An Acad Bras Cienc 89:2971-2974
Macova S, Dolezelova P, Pistekova V, Svobodova Z, Bedanova I, Voslarova E (2008) Comparison of acute toxicity of 2-phenoxyethanol and clove oil to juvenile and embryonic stages of *Danio rerio*. Neuro Endocrinol Lett 29:680-684
Mahajan N, Rawal S, Verma M, Poddar M, Alok S (2013) A phytopharmacological overview on *Ocimum* species with special emphasis on *Ocimum sanctum*. Biomed Prev Nutr 3:185-192
Mattioli CC, Takata R, Leme F de OP, Costa DC, Kennedy Luz R (2019) Physiological and metabolic responses of juvenile *Lophiosilurus alexandri* catfish to air exposure. Fish Physiol and Biochem 45:455-467
Mylonas CC, Cardinaletti G, Sigelaki I, Polzonetti-Magni A (2005) Comparative efficacy of clove oil and 2-phenoxyethanol as anesthetics in the aquaculture of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) at different temperatures. Aquaculture 246:467-481
McCafferty DJ (2007) The value of infrared thermography for research on mammals: previous applications and future directions. Mamm Rev 37:207-223
Ni M, Wen H, Li J, Chi M, Bu Y, Ren Y, Zhang M, Song Z, Ding H (2014) The physiological performance and immune responses of juvenile *Amur sturgeon* (Acipenser schrenckii) to stocking density and hypoxia stress. Fish Shellfish Immunol 36:325-335
Ortuño J, Esteban MA, Meseguer J (2001). Effects of shorteterm crowding stress on the gilthead seabream (*Sparus aurata* L) innate immune response. Fish Shellfish Immunol 11:187-197
Pádua SB de, Dias Neto J, Sakabe R, Claudiano G da S, Chagas EC. Pilarski F (2013) Hematologic variables in tambaquis anesthetized with clove oil and benzocaine. Pesqui Agropecu Bras 48:1171-1174
Purbosari N, Warsiki E, Syamsu K, Santoso J, Effendi I (2021) Evaluation of the application of seaweed (*Eucheuma cottonii*) extract as fish anesthetic agent. Aquac Int 29:1545-1560
Pickering AD, Pottinger TG (1985) Cortisol can increase the susceptibility of brown trout, *Salmo trutta* L., to disease without reducing the white blood cell count. J Fish Biol 27:611-619
Ranzani Paiva MJT, Pádua SB, Tavares-Dias M, Egami MI (2013). Métodos para análise hematológica em peixes. Maringá: Eduem. 135p
Randall DJ, Perry SF (1992). Catecholamines. W.S. Hoar, D.J. Randall, A.P. Farrell (Eds.), Fish Physiology XIIB, Academic, San Diego, CA, pp. 255-300
Ribeiro AS, Batista E dos S, Dairiki JK, Chaves FCM, Inoue LAKA (2016) Anesthetic properties of *Ocimum gratissimum* essential oil for juvenile matrinxã. Acta Sci Anim Sci 38:1-7
Romanovsky AA (2014) Skin temperature: its role in thermoregulation. Acta Physiol 210:498-507
Rotili D A, Devens MA, Diemer O, Lorenz EK, Lazzari R, Boscolo WR (2012) Uso de eugenol como anestésico em pacu. Pesqui Agropecu Trop 42:288-294
Sanchez MS dos S, Rodrigues RA, Nunes AL, Oliveira AM da S, Fantini L E, Campos CM de (2014) Efeito do mentol e eugenol sobre as respostas fisiológicas do pacu *Piaractus mesopotamicus.* Semina: Ciênc Agrár 35:2799-2808
Singh S, Lal RK, Maurya R, Chanotiya CS (2018) Genetic diversity and chemotype selection in genus *Ocimum.* J Appl Res Med and Aromat Plants 9:19-25
Silva RD, Rocha LO, Fortes BAD, Vieira D, Fioravanti MCS (2012) Hematological parameters of Nile tilapia (*Oreochromis niloticus* L.) under air exposure stress. Pesqui Vet Bras 32:99-107
Tavares-Dias M, Oliveira SR (2009) A review of the blood coagulation system of fish. Braz J Biosc 7:205-224
Tattersall GJ, Cadena V (2010) Insights into animal temperature adaptations revealed through thermal imaging. Imaging Sci J 8:261-268
Tubbesing VA, Block BA (2000). Orbital rete and red muscle vein anatomy indicate a high degree of endothermy in the brain and eye of the salmon shark. Acta Zoo 81:49-56
Urbinati EC, Gonçalves FD (2013) Pacu (*Piaractus mesopotamicus*), In: Baldisserotto, B.; Gomes, L.C. (Eds.), Espécies nativas para piscicultura no Brasil. UFMS, Santa Maria, 2013, Cap.8, p. 225-246
Uritu C M, Mihai CT, Stanciu GD, Dodi G, Stratulat TA, Luca A, Leon-Constantin M M, Stefanescu R, Bild V, Melnic S, Tamba BI (2018) Medicinal plants of the family Lamiaceae in pain therapy: A review. Pain Res Manag 7801543.
Ventura AS, de Silva TSC, Zanon RB, Inoue LAKA, Cardoso CAL (2019a) Physiological and pharmacokinetic responses in neotropical *Piaractus mesopotamicus* to the essential oil from *Lippia sidoides* (Verbenaceae) as an anesthetic. Int Aquat Res 11:1-12
Ventura AS, de Silva TSC, Zanon RB, Inoue LAKA, Cardoso CAL (2019b) Pharmacokinetic and physiological responses of *Piaractus mesopotamicus* anesthetized with the essential oil of *Lippia alba.* Pan-Am J Aquat Sci 14:51-60
Ventura AS, Jerônimo GT, Oliveira SN de, Gabriel AMA, Cardoso CAL, Teodoro GC, Corrêa Filho RAC, Povh JA (2020a) Natural anesthetics in the transport of Nile tilapia: Hematological and biochemical responses and residual concentration in the fillet. Aquaculture 526:1-7
Ventura AS, Corrêa Filho RAC, Teodoro GC, Laice ML, Barbosa PTL, Stringhetta, GR, Jerônimo GT, Povh JA (2020b) Essential oil of *Ocimum basilicum* and eugenol as sedatives for Nile tilapia. J Agric Stud 8:657-665
Ventura AS. Jerônimo GT, Corrêa Filho RAC, de Souza AI, Stringhetta GR, da Cruz M G, Torres G dos S, Gonçalves LU, Povh JA (2021) *Ocimum basilicum* essential oil as an anesthetic for tambaqui *Colossoma macropomum*: Hematological, biochemical, and non-specific immune parameters and energy metabolism. Aquaculture 533:736124
Vosyliené MZ (1999) The effects of heavy metals on haematological indices of fish (Survey). Acta Zool Litu 9:76-82
Woody CA, Nelson J, Ramstad K (2002) Clove oil as an anaesthetic for adult sockeye salmon: field trials. J Fish Biol 60:340-347
Wosnick N, Navas CA, Niella YV, Monteiro Filho EL, Freire CA, Hammerschlag N (2018) Thermal imaging reveals changes in body surface temperatures of blacktip sharks (*Carcharhinus limbatus*) during air exposure. Physiol Biochem Zool 91:1005-1012
Wosnick N, Niella YV, Navas CA, Monteiro-Filho ELA, Freire CA, Hammerschlag N (2019) Multispecies thermal dynamics of air-

exposed ectothermic sharks and its implications for fisheries conservation. J Exp Mar Biol Ecol 513:1-9

Zahl IH, Kiessling A, Samuelsen OB, Hansen MK (2009) Anesthesia of Atlantic cod (*Gadus morhus*)- Effects on pre-anaesthetic sedation and importance of body weight, temperature and stress. Aquaculture 295:52-59

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