Essential oil of Piper marginatum (Piperaceae) against monogeneans, and its hematological and histopathological effects on Colossoma macropomum

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Abstract

Purpose

This study investigated the anthelmintic efficacy of therapeutic baths with the essential oil of *Piper marginatum* against the monogeneans *Anacanthorus spathulatus*, *Notozothecium janauachensis*, *Mymarothecium boegeri* and *Linguadactyloides brinkmanni* in *Colossoma macropomum*, and its hematological and histopathological effects on this fish.

Methods

Six therapeutic baths with 100 mg/L of the essential oil of *P. marginatum* and two control groups (water from the cultivation tank and water from the cultivation tank + 70% alcohol) were used for 20 min every two days.

Results

The therapeutic baths with 100 mg/L of the essential oil of *P. marginatum* had efficacy of 42.8% against monogeneans of *C. macropomum* gills. Toxicity was low for *C. macropomum*, because there were few physiological and histopathological changes that did not compromise the functioning of the gills of this fish.

Conclusion

Short therapeutic baths with 100 mg/L of the essential oil of *P. marginatum* had low efficacy for controlling monogeneans in *C. macropomum* and thus cannot be recommended.

INTRODUCTION

Aquaculture production has been influenced by the increase in global demand for protein foods and white meat, as well as the high market value of different species of fish of zootechnical interest. It has the capacity to generate employment and income, thus boosting the economic development of several countries [4, 10]. Aquaculture directly and indirectly generates millions of jobs and produces several species of native and exotic fish. Fish aquaculture is very promising in several regions and can meet the growing demand for fish caused by the reduction of natural fish stocks [10].

Colossoma macropomum Cuvier 1818, the native species most cultivated in Brazil, is in the order Characiformes and family Serrasalmidae and is popularly known as tambaqui. It is considered to be the fish with the second largest-scale cultivation in the Amazon basin [9]. However, with the increase in

cultivation of this Amazonian fish, the occurrence of infections caused mainly by monogeneans has also increased [11, 12, 14].

Monogeneans are helminth parasites with direct short life cycles and vertical transmission, factors that facilitate high levels of infection in cultivation [11, 15]. These ectoparasites cause lesions in the gills of *C. macropomum* [14] that can compromise physiological processes such as breathing, excretion, acid-base balance and osmoregulation [6, 14, 22], and therefore require antiparasitic control. Control of parasites and treatments against them using medicinal plants and their bioactive agents have formed a propitious approach within the search for new anthelmintic treatments for fish [7, 13, 3].

Piper marginatum is a medicinal plant. The main chemical components of its essential oil are y-terpinene, α-terpinene, p-cymene and 3,4-methylenedioxy-propiophenone [18]. Studies have shown that its essential oil has high *in vitro* efficacy against monogeneans [3] and *Neoechinorhynchus buttnerae* [18] in *C. macropomum*. However, there are no studies on therapeutic baths for these fish. Thus, this study investigated the efficacy of therapeutic baths with the essential oil of *P. marginatum* against monogeneans and its hematological and histological effects on the gills of *C. macropomum*.

MATERIAL AND METHODS

Fish and acclimatization

Fingerlings of *C. macropomum* were obtained from a commercial fish farm in Macapá, in the state of Amapá, and maintained at Aquaculture and Fisheries Laboratory of Embrapa Amapá, Macapá, Amapá (Brazil). These fish were acclimatized in tanks with constant aeration and continuous water renewal and were fed twice daily with commercial feed containing 32% crude protein (Guabi®, Brazil). This fish stock naturally infected by monogeneans was used in all assays [14].

The following water parameters were measured daily: mean temperature (30.1 ± 0.1°C), dissolved oxygen (5.7 ± 0.2 mg/L), pH (5.7 ± 0.2), ammonia (0.3 ± 0.1 mg/L), alkalinity (12.0 ± 0.001 mg/L) and hardness (11.0 ± 0.1 mg/L), using a multiparameter probe (YSI, USA). The tank was siphoned weekly to remove accumulated organic matter, and the water was renewed.

Obtaining essential oil of P. marginatum and determining its chemical composition

Piper marginatum was cultivated in the Medicinal Plants and Vegetables Sector of Embrapa Manaus, in the state of Amazonas, Brazil. The branches and inflorescence of *P. marginatum* were used to obtain essential oil. This essential oil was extracted through hydrodistillation with Clevenger apparatus. The chemical composition of the essential oil was determined using gas chromatography–mass spectrometry (GC-MS; Shimadzu QP5050A), following the methodology described by Adams [1]. The main constituents of *P. marginatum* essential oil were found to be 3,4-methylenedioxy-propiophenone (20.8%), (E)-β-ocimene (5.9%), safrole (5.3%), (E)-caryophyllene (5.2%) and 2-hydroxy-4,5-methylenedioxy-propiophenone (4.5%) [3].

Therapeutic baths for C. macropomum using essential oil of P. marginatum

Fingerlings of *Colossoma macropomum* (41.08 ± 15.4 g and 14.4 ± 1.7 cm) were exposed to 100 mg/L of the essential oil of *P. marginatum* for 20 min every two days, over a period of six days with a total three therapeutic baths, using tanks of 100 L of water. Three treatments with three replicates each were used, with 13 fish per replicate, totaling 39 fish per treatment. The fish were kept in a static water system at the time of addition of the essential oil of *P. marginatum*. After the 20 minutes of the therapeutic bath, the water in the tanks was changed. The essential oil was diluted in 70% ethyl alcohol (1:10 g). Two control groups were used: one with water from the cultivation tank and the other with water from the cultivation tank + ethyl alcohol (70%). On the sixth day, after the therapeutic bath of that day, the fish were euthanized by means of brain sectioning and the gills were collected and fixed in 5% formalin, to quantify the monogeneans [8] and determine the prevalence and mean abundance of these parasites [5]. The efficacy of the therapeutic baths was determined using calculations described in Zhang et al. [21].

During the therapeutic baths with 100 mg/L of the essential oil of *P. marginatum*, the fish were observed to record any occurrence of behavioral alterations.

Blood analysis on C. macropomum after therapeutic baths with the essential oil of P. marginatum

After the third therapeutic baths with 100 mg/L of the essential oil of *P. marginatum*, blood was collected from each fish by puncturing the caudal vessel using syringes containing EDTA (10%). Three treatments with three replicates each were used, with five fish per replicate, totaling 15 fish per treatment. Two control groups were used: one with water from the cultivation tank and the other with water from the cultivation tank + 70% ethyl alcohol. The blood was used to determine the follow parameters: hematocrit using the microhematocrit method; total erythrocyte counts using a Neubauer chamber; and hemoglobin concentration using the cyanmethemoglobin method. The hematimetric indices of mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated from these parameters. Blood smears were made and stained with May-Grünwald-Giemsa-Wright medium in order to make differential leukocyte counts in up to 200 cells of interest. Blood smears were also used to determine the total numbers of leukocytes and thrombocytes using an indirect method [17].

Histopathological analyses on C. macropomum gills after therapeutic baths with the essential oil of P. marginatum

On the sixth day, after the third therapeutic bath, the fish were euthanized by brain section and the gills of nine fish per treatment (three from each replicate) were collected for histopathological analysis. Three treatments with three replicates each were used, consisting of baths with 100 mg/L of the essential oil of *P. marginatum*, along with two control groups: one with water from the cultivation tank and the other with water from the cultivation tank + ethyl alcohol (70%The first gill arch on both sides of each fish was collected and fixed in Davidson solution for 48 h, which was then exchanged for 70% alcohol. The gill arches were dehydrated in a gradually increasing series of ethanol baths (70, 80, 90 and 100%), followed by a xylol bath. They were then embedded in paraffin to obtain consecutive 5 µm sections, using an

electron microtome (Thermo Scientific™ HM 340E, USA). Images were obtained using an ordinary optical microscope (Leica DM 1000, USA) and the Leica Application Suite 1.6.0 software. Histopathological analyses were performed in a semiquantitative manner using mean assessment values [19] and the histopathological alteration index [16]. The nomenclature used for the histopathological changes was in accordance with Wolf et al. [23].

Statistical analyses

All the histopathological, parasite and blood data were firstly evaluated for normality and homoscedasticity using the Shapiro-Wilk and Bartlett tests, respectively. As the data did not follow normal distribution, the Kruskal-Wallis test was used, followed by the Dunn test, to compare medians. The statistical significance level was taken to be $p < 0.05$ [20].

RESULTS

Therapeutic baths and anthelmintic action of the essential oil of P. marginatum on C. macropomum

Behavioral changes were observed among the fish during the therapeutic baths with 100 mg/L of the essential oil of *P. marginatum*, consisting of agitation, accelerated movement of the operculum, erratic swimming and lethargy. Full recovery of normal movement occurred within 20 min after the end of each therapeutic bath. There was no change in the behavior of the fish in the control groups consisting of either water from the cultivation tank or water from the cultivation tank + 70% alcohol. During the three days of treatment, the fish fed normally and there was no mortality during or after the therapeutic baths.

In the gills of *C. macropomum*, the prevalence of the monogeneans (*Anacanthorus spathulatus*, *Notozothecium janauachensis*, *Mymarothecium boegeri* and *Linguadactyloides brinkmanni*) was the same between the treatments (Table 1). The abundance of monogeneans was lower ($p < 0.05$) in fish exposed to 100 mg/L of the essential oil of *P. marginatum* and water from the cultivation tank + 70% alcohol, compared with the control consisting only of water from the cultivation tank. Therapeutic baths with 100 mg/L of the essential oil of *P. marginatum* were 42.8% effective against monogeneans on the gills of *C. macropomum* and similar to the result from the control consisting of water from the cultivation tank + alcohol (Table 1).

Table 1
Parasitological indices of monogeneans on the gills of *Colossoma macropomum* subjected to therapeutic baths with the essential oil of *Piper marginatum*, and efficacy of these treatments.

Treatments	Prevalence (%)	Mean abundance	Dunn test	Efficacy (%)
Water	100	28.6 ± 15.1^{a}	22.95	-
Water + alcohol	100	14.7 ± 6.8^{b}	44.55	48.2
100 mg/L	100	8.4 ± 5.2^{c}	22.6	42.8
Different letters in the same column indicate differences according to the Dunn test.				

Blood parameters of C. macropomum exposed to the essential oil of P. marginatum

The plasma concentrations of glucose and total protein in fish exposed to 100 mg/L of the essential oil of *P. marginatum* were lower than in controls with water from the cultivation tank and water from the cultivation tank + alcohol. In the fish exposed to 100 mg/L of the essential oil of *P. marginatum*, hematocrit and the counts of neutrophils and PAS-positive granular leukocytes (PAS-GL) were higher than in controls with water from the cultivation tank and water from the cultivation tank + alcohol. Hemoglobin and total leukocyte counts in the fish exposed to 100 mg/L of the essential oil of *P. marginatum* were higher than in the fish of the control group exposed to water from the cultivation tank. However, the other blood parameters were similar between treatments (Table 2).

Table 2
Blood parameters of *Colossoma macropomum* subjected to therapeutic baths with the essential oil of *Piper marginatum.*

Parameters	Water	Water + alcohol	100 mg/L
Glucose (mg/dL)	94.6 ± 21.8[a]	91.5 ± 19.6[a]	84.8 ± 13.5[b]
Total protein (g/dL)	2.8 ± 0.5[a]	2.8 ± 0.6[a]	1.5 ± 0.4[b]
Erythrocytes (x10^6/µL)	1.36 ± 0.28[a]	1.69 ± 0.40[a]	2.11 ± 0.69[a]
Hematocrit (%)	22.4 ± 1.5[a]	24.2 ± 3.3[a]	26.9 ± 2.1[b]
Hemoglobin (g/dL)	6.5 ± 0.7[a]	7.3 ± 0.7[a,b]	8.3 ± 1.3[b]
MCV (fL)	170.4 ± 32.4[a]	152.3 ± 45.6[a]	137.4 ± 32.0[a]
MCHC (g/dL)	28.9 ± 2.1[a]	30.3 ± 3.1[a]	30.6 ± 3.9[a]
Thrombocytes (µL)	18,473 ± 5,512[a]	17,531 ± 8,769[a]	16,999 ± 7,111[a]
Leukocytes (µL)	138,764 ± 29,941[a]	172,444 ± 41,835[a,b]	221,288 ± 73,918[b]
Lymphocytes (µL)	77,529 ± 28,705[a]	77,529 ± 28,705[a]	79,149 ± 30,911[a]
Monocytes (µL)	47,440 ± 19,317[a]	47,440 ± 19,317[a]	59,766 ± 26,776[a]
Neutrophils (µL)	26,435 ± 10,583[a]	26,435 ± 10,583[a]	42,691 ± 19,408[b]
Eosinophils (µL)	6,148 ± 4,644[a]	6,148 ± 4,644[a]	9,908 ± 9,113[a]
PAS-GL (µL)	13,399 ± 6,578[a]	13,398 ± 6,579[a]	24,522 ± 17,415[b]
Basophils (µL)	2,778 ± 2,721[a]	2,778 ± 2,721[a]	4,977 ± 4,059[a]

Data express mean ± standard deviation. Different letters in the same line indicate differences according to the Dunn test ($p < 0.05$). PAS-GL: PAS-positive granular leukocytes; MCHC: mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume.

Histopathological analyses on the gills of C. macropomum after therapeutic baths with the essential oil of P. marginatum

In the controls with water from the cultivation tank, there was no histopathological change in the gills. In the gills of the fish exposed to tank water + 70% alcohol (control), there were occurrences of aneurysm-like lesions of lamellar blood vessels, hyperplasia lamellae and detachment of the lamellar epithelium. Fish gills exposed to 100 mg/L of *P. marginatum* showed lamellar hyperplasia with fusion (LHF) and hyperplasia lamellae (Fig. 1A-D).

In the fish exposed to 100 mg/L of the essential oil of *P. marginatum* and to water from the cultivation tank + 70% alcohol, the histopathological alteration index (HAI) values were higher ($p < 0.05$) than in the control with water from the cultivation tank alone. In the fish exposed to water from the cultivation tank + alcohol, the mean assessment values (MAV) were different ($p < 0.05$) from those of the control group with water from the cultivation tank alone (Table 3).

Table 3
Mean assessment values (MAV) and histopathological alteration index (HAI) values for gills of *Colossoma macropomum* subjected to therapeutic baths with the essential oil of *Piper marginatum.*

Treatments	N	MAV	HAI	Severity of the lesions according to the HAI
Water	9	5.0 ± 2.2^{a}	5.3 ± 4.2^{a}	Normal functioning of the organ
Water + alcohol	9	8.0 ± 1.0^{b}	11.5 ± 4.6^{b}	Mild to moderate organ damage
100 mg/L	9	$7.2 \pm 1.3^{a,b}$	12.7 ± 3.3^{b}	Mild to moderate organ damage

Data express mean ± standard deviation. Different letters in the same column indicate differences according to the Dunn test ($p < 0.05$).

DISCUSSION

The negative aspects of use of chemotherapy drugs and the prohibition of different drugs for use in aquaculture in several countries have led to the need for alternative treatments. Use of essential oils is one of these alternatives, since several of these bioactive compounds have antiparasite effects [9, 18]. However, many essential oils consist of bioactive compounds that also induce anesthesia in fish [12, 2], which makes it difficult to use high therapeutic concentrations and long-duration baths among some fish, like those of the present study.

On the other hand, for *C. macropomum*, the short therapeutic baths with 100 mg/L of the essential oil of *P. marginatum* that were used in the present study had low efficacy against monogeneans. This was unexpected, given that Alves et al. [3] reported that 100–400 mg/L of the essential oil of *P. marginatum* showed *in vitro* efficacy against monogeneans on the gills of *C. macropomum*. Various other essential oils have also shown efficacy in controlling monogeneans, although the results have varied according to the concentration used. Other relevant variables are the tolerance of the fish to the oil and the strategy used in the therapeutic baths [12, 14, 3].

In fish, studies on blood parameters can be used to evaluate the response to stress and toxicity [12, 21]. In *C. macropomum* exposed to 100 mg/L of the essential oil of *P. marginatum*, the plasma levels of total protein and glucose decreased, while the hematocrit, hemoglobin and total leukocyte count increased, due to the increases in the total neutrophil count and PAS-GL, which improved the transport of dissolved oxygen and immunity of the fish. In *C. macropomum*, exposure to 300 mg/L of the essential oil of *Alpinia*

zerumbet was also found to reduce the plasma levels of total protein and glucose and total leukocyte count, without altering hematocrit and hemoglobin concentration [12]. However, also in *C. macropomum*, exposure to 60 mg/L of the essential oil of *Cymbopogon citratus* increased the plasma glucose levels and decreased the total leukocyte count, due to stress caused by the therapeutic baths [11]. Malheiros et al. [14] reported that therapeutic baths with 100 mg/L of oleoresin of *Copaifera reticulata* or with 250 mg/L of nanoemulsion from this oleoresin also increased the plasma levels of total protein and glucose in *C. macropomum.* Total protein levels depend on intracellular mechanisms and specific proteins and may be affected by stress [12].

Fish gills are multifunctional organs that perform various vital functions [14, 6, 22] and can be harmed by damage to their structure, which may interfere with maintenance of homeostasis [22]. In the present study, hyperplasia lamellae with or without fusion were observed on the gills of *C. macropomum* subjected to therapeutic baths with 100 mg/L of the essential oil of *P. marginatum*. On the gills of the fish exposed to cultivation tank water + 70% alcohol, there were branchial filaments showing aneurysm-like lesions of lamellar blood vessels, hyperplasia lamellae and detachment of the lamellar epithelium. However, such lesions ranged only from mild to moderate, therefore resulting in little functional impairment of the gills. In *C. macropomum*, therapeutic baths with 100 mg/L of oleoresin of *C. reticulata* or with 250 mg/L of nanoemulsion of this oleoresin were found previously to cause epithelial detachment, hyperplasia and hypertrophy, thus resulting in moderate fusion of secondary lamellae [14]. These gill alterations are adaptive strategies to increase the distance between the external environment and the blood and therefore make contact with the stressor agent more difficult [14, 22].

CONCLUSIONS

In conclusion, short-duration baths with 100 mg/L of the essential oil of *P. marginatum* had low efficacy against monogeneans of *C. macropomum*. Other strategies would be required in order to increase this efficacy. Nonetheless, the three therapeutic baths caused few physiological and histopathological changes in the fish exposed to them.

Declarations

ACKNOWLEDGEMENTS

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ETHICAL APPROVAL

This study was developed in accordance with the principles adopted by the Brazilian College of Animal Experimentation (COBEA) and with authorization from the Ethics Committee for the Use of Animals, of Embrapa Amapá (Protocol No 013- CEUA/CPAFAP).

DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article.

CONFLICT OF INTEREST STATEMENT

The authors declare that they did not have any conflicts of interest in undertaking this work.

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References

1. Adams RP (2007) Identification of essential oils components by gas chromatography/mass 402 spectrometry, fourth edn. Allured Publishing Corporation, Carol Stream, IL
2. Aydın B, Barbas LAL (2020) Sedative and anesthetic properties of essential oils and their active compounds in fish: a review. Aquac Res 520:734999. https://doi:10.1016/j.aquaculture.2020.734999
3. Alves CMG, Nogueira JN, Luz JGR, Chaves FCM, Tavares-Dias M (2021) Essential oil of *Piper callosum, Piper hispidium* and *Piper marginatum* (Piperaceae) has *in vitro* efficacy against monogeneans of tambaqui *Colossoma macropomum* (Serrasalmidae). Aquac Res 52:6107–6116. https://doi:10.1111/are.15473
4. Boyd CE, D'Abramo LR, Glencross BD, Huyben DC, Juarez LM, Lockwood GS, McNevin AA, Tacon AGJ, Teletchea F, Tomasso JR, Tucker CS, Valenti WC (2020) Achieving sustainable aquaculture: Historical and current perspectives and future needs and challenges. J World Aquacult Soc 51:578–633. https://doi:10.1111/jwas.12714
5. Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis. Revisit J Parasitol 83:575–583. https://doi:10.2307/3284227
6. Cinar K, Aksoy A, Emre Y, Asti RN (2009) The histology and histochemical aspects of gills of the flower fish, *Pseudophoxinus antalyae*. Vet Res Commun 33:453–460. https://doi:10.1007/s11259-008-9191-2
7. Corral ACT, Queiroz MN, Andrade-Porto SM, Morey GAM, Chaves FCM, Fernandes VLA, Ono EA, Affonso EG (2018) Control of *Hysterothylacium* sp. (Nematoda: *Anisakidae*) in juvenile pirarucu (*Arapaima gigas*) by the oral application of essential oil of *Piper aduncum*. Aquac Res 494:37–44. https://doi:10.1016/j. aquaculture.2018.04.062

8. Eiras JC, Takemoto RM, Pavanelli GC (2006) Métodos de estudo e técnicas laboratoriais em parasitologia de peixes, second ed, Eduem, Maring&#225
9. 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 effects on *Colossoma macropomum* (Cuvier, 1818). Aquaculture 512:734343. https://doi.org/10.1016/j.aquaculture.2019.734343
10. Food and Agriculture Organization of the United Nations – FAO (2022) The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome, FAO. https://doi:10.4060/cc0461en
11. Gonzales APPF, Yoshioka ETO, Mathews PD, Mertins O, Chaves FCM, Videira MN, Tavares-Dias M (2020) Anthelminthic efficacy of *Cymbopogon citratus* essential oil (Poaceae) against monogenean parasites of *Colossoma macropomum* (Serrasalmidae), and blood and histopathological effects. Aquaculture 528:735500. https://doi:10.1016/j.aquaculture.2020.735500
12. Luz JGR, Nogueira JN, Alves CMG, Videira MN, Canuto KM, Castro KNC, Tavares-Dias M (2021) Essential oil of *Alpinia zerumbet* (Zingiberaceae) has anthelmintic efficacy against monogenean of *Colossoma macropomum* (Characiformes: Serrasalmidae. Aquac Res 52:5340–5349. https://doi:10.1111/are.15404
13. Majolo C, Monteiro PC, Nascimento AVP, Chaves FCM, Gama PE, Bizzo HR, Chagas EC (2019) Essential oils from five Brazilian *Piper* species as antimicrobials against strains of *Aeromonas hydrophila*. J Essent Oil-bear Plants 22:746–761. https://doi:10.1080/0972060X.2019.1645047
14. Malheiros DF, Videira MN, Ferreira IM, Tavares-Dias M (2022) Anthelmintic efficacy of *Copaifera reticulata oleoresin* in the control of monogeneans and haematological and histopathological effects on *Colossoma macropomum*. Aquac Res 53:4087–4094. https://doi:10.1111/are.15910
15. Morales-Serna FN, Caña-Bozada VH, López-Moreno DG, Medina-Guerrero RM, Morales-Serna JA, Fajer-Ávila EJ (2019) *In vitro* efficacy of two terpenes against *ancyrocephalid* monogeneans from Nile tilapia. J Parasit Dis 43:739–742. https://doi:10.1007/s1263 9-019-01150. 2
16. Poleksić V, Mitrović-Tutundžić V (1994) Fish gills as a monitor of sublethal and chronic effects of pollution. In: Muller R, Lloyd R (eds) Sublethal and chronic effects of pollutants on freshwater fish. Fishing News Books, Oxford, pp 339–352
17. Ranzani-Paiva MJT, Pádua SB, Tavares-Dias M, Egami MI (2013) Métodos para análises hematológicas em peixes. first ed. Eduem, Maringá
18. Santos WB, Majolo C, Santos DS, Rosa MC, Monteiro PC, Rocha MJS, Oliveira MIB, Chaves FCM, Chagas EC (2018) Eficácia *in vitro* de óleos essenciais de espécies de Piperaceae no controle do acantocéfalo *Neoechinorhynchus buttnerae*. Rev Bras Hig Sanid Anim 12:460–469
19. Schwaiger J, Wanke R, Adam S, Pawert M, Honnen W, Triebskorn R (1997) The use of histopathological indicators to evaluate contaminant-related stress in fish. J Aquat Ecosyst Stress Recovery 6:75–86. https://doi:10.1023/A:10082 12000208
20. Zar JH (2010) Biostatistical analysis, Fifth edn. Prentice- Hall, Upper Saddle River, NJ

21. Zhang XP, Li WX, Tao A, Zou S, Shan H, Wang G GT (2014) The efficacy of four common anthelmintic drugs and traditional Chinese medicinal plant extracts to control Dactylogyrus vastator (Monogenea). Aquac 420–421:302–307. https://doi.org/10.1016/j.aquaculture.2013.09.022
22. Winkaler EU, Santos TRM, Machado-Neto JG, Martinez CB (2007) Acute the lethal and sublethal effects of neem leaf extract on the Neotropical freshwater fish *Prochilodus lineatus*. Comp Biochem Physiol C Toxicol Pharmacol 145:236–244. https://doi:10.1016/j.cbpc.20.06.12.009
23. Wolf JC, Baumgartner WA, Blazer VS, Camus AC, Engelhardt JA, Fournie JW, Frasca JR, Groman DB, Kent ML, Khoo LH, Law JM, Lombardini ED, Ruehl-fehlert CR, Segner HE, Smith SA, Spitsbergen JM, Weber K, Wolfe MJ (2015) Nonlesions, Misdiagnoses, Missed Diagnoses, and Other Interpretive Challenges in Fish Histopathology Studies: A Guide for Investigators, Authors, Reviewers, and Readers. Toxicol Pathol 43:297–325. https://doi:10.1177/0192623314540229

Figures

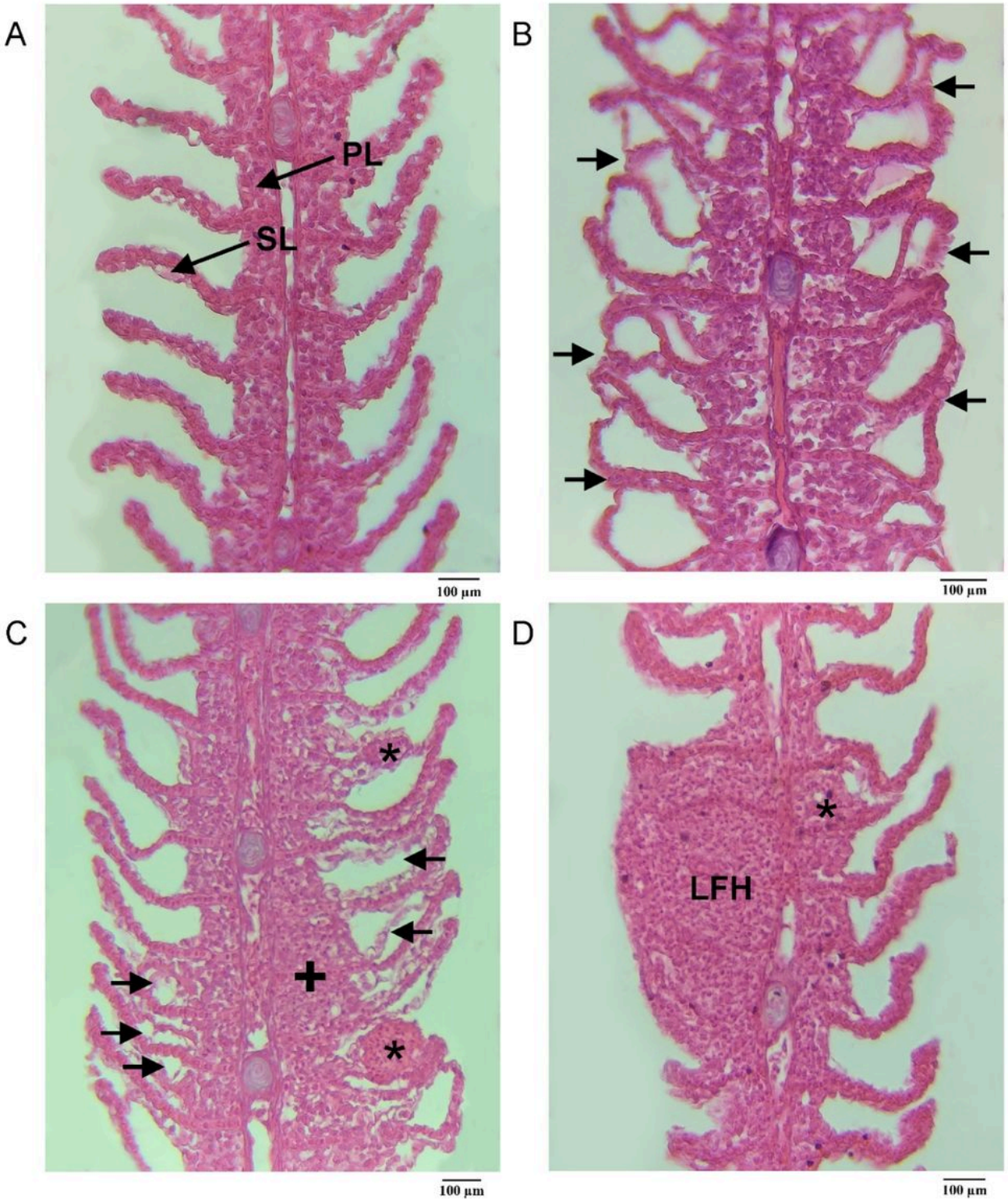

Figure 1

Gills of *Colossoma macropomum* subjected to therapeutic baths with 100 mg/L of the essential oil of *Piper marginatum*. A. Gills exposed to cultivation water (control) showing primary (PL) and secondary (SL) lamellae (arrows). B-C. Gills exposed to water + 70% alcohol (control). B. Gill filaments showing lesions due to detachment of the lamellar epithelium (arrow). C. Branchial filaments showing aneurysm-like lesions of lamellar blood vessels (asterisk), lamellar hyperplasia (+) and detachment of the lamellar

epithelium (arrow). D. Gills filaments exposed to 100 mg/L of *P. marginatum*, showing lesions due to lamellar hyperplasia with fusion (LHF) and lamellar hyperplasia (asterisk).