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Influence of autochthonous pathogens on fish adaptation to new rearing conditions after experiencing post-transport stress: A neglected factor in quarantine protocol development

Peterson Emmanuel Guimarães Paixão [g], Ricardo Marques Nogueira Filho [b], Higo Andrade Abe [b], Rubens Riscala Madi [a], Cindy Caroline Moura Santos [a], Natalino da Costa Sousa [c], Márcia Valéria Silva do Couto [d], Hugo Leandro Santos [e], Fabricio Sa Santana [e], Amanda Silva Carvalho [f], Juliana Matos Araujo [g], Rodrigo Yudi Fujimoto [g,*]

[a] Tiradentes University, Post-Graduation Department in Health and Environment, Campus Farolândia, Aracaju, Sergipe, Brazil
[b] State University of Bahia, Fishing Engineer Department, Campus, Paulo Afonso, Bahia, Brazil
[c] Federal Institute of Mato Grosso do Sul, Fishing Engineer, CEP 79400-000, Campus Coxim, Mato Grosso do Sul, Brazil
[d] Federal Rural University of the Amazon, Fishing Engineer Department, Campus Belém, Pará, Brazil
[e] Federal University of Sergipe, Department of Fishing Engineer, Campus São Cristovão, Sergipe, Brazil
[f] Federal University of Sergipe, Department of Animal Science, Campus São Cristovão, Sergipe, Brazil
[g] Brazilian Agricultural Research Corporation, Laboratory of Aquaculture, Aracaju, Sergipe, Brazil

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ABSTRACT

Best management practices such as acclimation and quarantine are essential to ensure adaptation of fish after transport, reduce stress and prevent the spread of the pathogens. However, important pathogens in fish farms are opportunistic and they are naturally presents in fish farms, and there is no information about their influence in fish adaptation to a new rearing condition, especially for tropical fish *Colossoma macropomum*. Therefore, this study evaluated the presence of pathogens in quarantine procedure for fish tambaqui *C. macropomum*. The fish were submitted to stress conditions in a "transport stress simulator" and then quarantined into sterile and contaminated tanks. In contaminated tanks were inoculated *Aeromonas hydrophila* and *Saprolegnia parasitica*. Concurrently, two different stocking densities (1 and 2 g/L of fish) were also evaluated for 45 days. Blood analysis was performed before transport stress (baseline values) and at 01, 15 and 45 days of quarantine, determining the biochemical parameters, erythrogram, leukogram and hematimetric indices. As the main result, glucose and cortisol increased in the first day of quarantine, but reduced over time, showing adaptation to new environmental rearing in sterile tanks regardless stocking density. However, there was an additional effect due to presence of pathogens where fish into the contaminated tanks demonstrated high values of cortisol, glucose, hemoglobin, lactate, hematocrit, MCH, neutrophils, basophils and LG-PAS cells, and reduction of lymphocytes and thrombocytes, which reflected in clinical signs of bacterial infection (erosion of fins, necrosis and ascites) after 15 days. At the end of experiment (45 days), fish into contaminated tanks at high stocking density presented accumulated mortality of 9 % caused by *Aeromonas hydrophila*. As conclusion, the pathogen influences the tambaqui adaptation regardless the stocking density reflecting in quarantine with at least 30 days to observe clinical signs.

1. Introduction

Global aquaculture grew 5 % in the last decade (2010−2020); however, despite that remarkable increase in production, problems associated with pathogens have been worsening. These problems are related to stress management, such as high stocking densities,

* Corresponding author.
E-mail address: ryfujim@hotmail.com (R.Y. Fujimoto).

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inadequate feeding, poor water quality, and transportation, which increase fish susceptibility to diseases (Lin et al., 2018; Sun et al., 2021).

The transport of fish is one of the greatest sources of stress to fish and is the main factor behind the spread of pathogens. This combination of stress and pathogens is a problem for fish adaptation to rearing in a new environment. Therefore, acclimation and quarantine are important steps for avoiding disease outbreaks and pathogen spread, but they are severely neglected.

Despite the importance of quarantining, most fish farmers place fish directly into a rearing pond after transporting them, without acclimation or following a quarantine protocol, thus subjecting the fish to the influence of allochthonous or autochthonous pathogens (Santos et al., 2022). Opportunistic allochthonous pathogens (fungus, bacteria, and parasites) are primarily responsible for outbreaks and mortalities under post-transport stress conditions (Ellison et al., 2018). Furthermore, the presence of autochthonous pathogens increases stress on fish during post-transport acclimation procedures (Paixão et al., 2020).

The influence of autochthonous pathogens and stocking density under quarantine conditions has not been studied for *Colossoma macropomum* (tambaqui) fish species. This species is important in aquaculture in South America. However, an outline of the best health management practices for this fish is lacking. Therefore, this study evaluated the influence of pathogens as well as stocking density in a quarantine procedure for the species.

2. Material and method

2.1. Ethical protocols and transport procedure

The ethics committee on animal use (CEUA) of Brazilian Agriculture Corporation Research approved the procedures for this study (protocol number 0019/2018). Before the experiment, fish (n = 500) were kept in tanks (named original tanks) for 60 days with water of adequate quality for rearing *C. macropomum* (Santos et al., 2021), as shown in Table 1. The temperature in the tanks was adjusted using a thermostat coupled to a heater, while the pH was adjusted using acetic acid (100 μL/L). (See Table 1.)

Afterward, juveniles (n = 480; 5±0.5 g) were distributed in plastic bags (60 L) at a stocking density of 80 fish/bag according to the method described by Sampaio and Freire (2016) and Gomes et al. (2003a, 2003b). The plastic bags were filled to 1/3 of their volume with clean water, and oxygen was injected to fill the remaining 2/3 of the volume. The bags were then sealed with elastomer. The bags were placed in a "transport stress-simulator" equipment (Fig. 1) and subjected to continuous transport stress for a duration of 8 h. The equipment simulated vibration and water movement that is present during real transportation. In a previous assay, the fish subjected to a transport stress simulator had levels of cortisol and glucose similar to fish subjected to real transport in a vehicle for 8 h (non-published data). The equipment was used to prevent climate variations that occur during real transportation. After subjection to stress, the fish were distributed into receiving tanks.

Table 1
Water quality parameter (mean ± standard deviation) from the original tanks, transport bags and receiving tanks.

	OT	BG	RT	Range
Temp.	27.36 ± 0.24	28.57 ± 0.27	25.44 ± 0.59	2.00
D.O.	4.63 ± 0.37	0.70 ± 0.37	4.17 ± 0.45	0.50
Cond.	483.00 ± 13.06	468.00 ± 1.28	268.00 ± 19.98	200.00
pH	6.66 ± 0.09	6.76 ± 0.05	7.72 ± 0.12	1.00
NH3	0.11 ± 0.01	0.12 ± 0.01	0.00 ± 0.00	0.10

OT- original tanks where the fish were maintained before the transport stress, BG- water quality parameters in the transport bags immediately after transport, RT- receiving tanks throughout the trial time (45 days), Range- difference between original and receiving tanks, Temp- temperature °C, D.O.- dissolved oxygen mg/L, Cond- electric conductivity μS/cm, NH_3- toxic ammonia mg/L.

2.2. Receiving tanks

Polyethylene tanks (100 L; n = 16) were prepared in a static system with a forced air supply to receive the fish after transport (receiving tanks). Water quality parameters such as temperature (°C) and pH were different from the parameters in the original tanks (Table 1). The difference between the water quality in the original and receiving tanks followed the guidelines of NOGA (1996) which state that differences of 1 point of pH and 2 degrees of temperature between environments cause stressful effects. This scenario was established to test the hypothesis that the presence of resident (i.e., autochthonous) pathogens affects fish adaptation to a new rearing environment.

Water quality parameters such as temperature (°C), dissolved oxygen (mg/L), electrical conductivity (S/cm), pH, NH_3, and total ammonia (mg/L) were monitored using a professional plus YSI® multiparameter in original tanks, receiving tanks, and transportation bags (after transport). Despite differences in water quality parameters in the original and receiving tanks, the parameters remained within the acceptable range (Conde et al., 2021) for tambaqui rearing. This scenario provided a new environment for rearing tambaqui without causing them stress due to inadequate water quality.

After transport, 160 fish were placed in 8 tanks at a stocking density of 1 g/L (n = 20 fish/tank) and 320 fish were placed in another 8 tanks at a stocking density of 2 g/L (n = 40 fish/tank).

2.3. Contaminated tanks

The receiving tanks were divided in two more groups, namely, G1: "contaminated" tanks and G2: "sterile" tanks. In the first group (G1), eight tanks were contaminated with the pathogenic bacterium *Aeromonas hydrophila* and the fungus-like oomycete *Saprolegnia parasitica*.

A. hydrophila had been previously incubated in a brain–heart infusion broth for 24 h at 30 °C, which was centrifuged (5000 rpm for 20 min). Subsequently, pellets were re-suspended in a sterile saline solution (0.65 % NaCl). The oomycete was grown in potato dextrose agar into which was added sesame seeds and incubated for 96 h at room temperature. Afterward, the sesame seeds were placed in an induction solution, as per the procedure described by Pereira-Torres et al. (2016), for 24 h to produce zoospores.

The final concentration of *A. hydrophila* in the contaminated tanks was 10^6 CFU/100 L (Peatman et al., 2018) and 1×10^3 zoospores/100 L of *S. parasitica* (Firouzbakhsh et al., 2014). The pathogens were acclimated and added to the water of the contaminated tanks 8 h before the conclusion of the transport simulation.

In the second group (G2), chlorine solution (4–6 % at a concentration of 500 μL/L) was added to 8 tanks 48 h before the experiment to prepare a sterile tank group without pathogens. A forced air supply was used to dechlorinate the tanks 24 h before the fish was transferred to the tanks.

2.4. Experimental design

The experiment was performed in quadruplicate and followed a split–split plot design (2 × 2 × 3) with 3 factors: a whole plot factor consisting of two stocking densities— 1 g/L and 2 g/L—a subplot factor, namely, the presence or absence of pathogens (G1 or G2), and a sub-subplot factor, namely, three different periods of blood analysis (1, 15, and 45 days). Further, the 480 animals were rapidly acclimated based on a specific procedure for tambaqui reported by Paixão et al. (2020) to prevent stress from acclimation handling.

2.5. Clinical signs and blood evaluation

All fish were monitored for 45 days to determine any clinical signs of bacterial or fungal infection and mortality. Clinical signs such as ascites,

Table 1
Glucose and cortisol values (mean ± standard deviation) of fish *Colossoma macropomum* subject to different stocking densities and exposure or not to autochthonous pathogens.

	Stocking Density		Stocking Density	
	Low	High	Low	High
	Glucose (mg/dL)		Cortisol (mg/dL)	
Baseline	*51.39 ± 6.15*		*21.05 ± 4.08*	
01d	142.18 ± 40.57Ab	169.80 ± 30.21Aa	80.05 ± 28.36Ab	90.08 ± 22.82Aa
15d	56.93 ± 13.82Ba	64.12 ± 20.85Ba	87.46 ± 8.71Aa	82.02 ± 24.81ABa
45d	54.17 ± 18.71Ba	50.52 ± 11.37Ba	73.53 ± 11.62Aa	70.46 ± 21.66Ba
Return*	15d	15d	NO(>)	NO(>)
p-value**	$p > 0.05$	$p > 0.05$	$p < 0.05$	$p < 0.05$

Different letters in the row (lowercase) and column (uppercase) mean statistical difference by Tukey test ($p < 0.05$), Base: Basal values before the stress by transport, Return: time to return to baseline according *t* test; NO(>): did not return and ended above the basal values, NO(<): did not return and ended below the basal values, 01d-15d-45d: 01 days after acclimation. P-value: t-test comparing 45 days hematological value with baseline values.

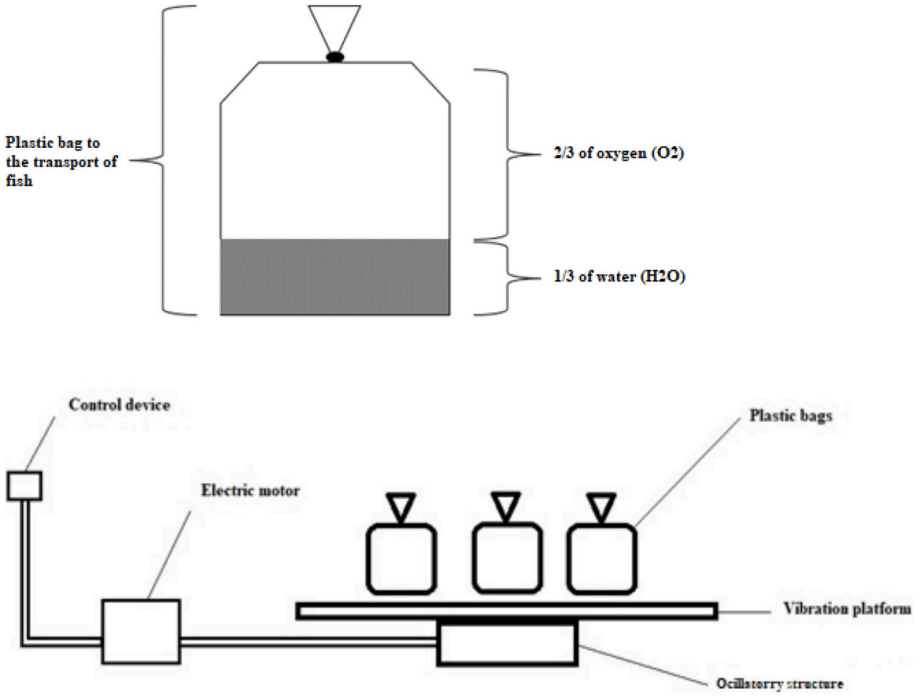

Fig. 1. Transport-stress simulator containing plastic bags used in this study simulating the vibrations and water movements throughout eight hours.

exophthalmia, fin erosion, and hemorrhages were registered daily. Dying fish were necropsied (after blood collection) for bacterial re-isolation and subsequent identification of *A. hydrophila* using the MALDI-TOF method.

Twelve fish per treatment were sampled during the following periods: before transport (baseline values) and during specific days of quarantine (1, 15, and 45 days). The fish were anesthetized (using 60 mg/L of eugenol; Inoue et al., 2011a, 2011b), and blood samples were taken using syringes with 5 % EDTA.

In the blood samples, cortisol (g/dL) was measured using a *Human Cortisol Elisa Kit*, glucose (g/dL) was determined using a portable analyzer (*Accuchek Active*), total plasmatic protein (g/dL) was measured using a refractometer; lactate (g/dL) was measured using a portable analyzer (*Accutrend active*), the total number of erythrocyte cells (cells/μL) was determined using a Neubauer chamber, hematocrit percentage was evaluated using the microhematocrit method (Goldenfarb et al., 1971), and hemoglobin concentration (mg/dL) was determined using the Labclin kit-test and biochemical analyzer. Based on the values of hemoglobin, the hematimetric index of mean corpuscular hemoglobin (MCH) was calculated. Blood smears were used to determine differential leukocyte count and the total number of thrombocytes.

2.6. Statistical analysis

All blood values were subjected to normality and homoscedasticity tests using the Shapiro-Wilk and Bartlett-tests, respectively. Subsequently, data were subjected to two-way ANOVA, followed by a post-hoc Tukey test ($\alpha = 5$ %). Percentage data without normal distribution were transformed into arcsine square roots and analyzed as previously described. Blood baseline values and after the trial test were compared using a t-test. This test was used to demonstrate the return of blood parameters to baseline, considering a consistent response over time, whereas wide oscillatory responses were not considered as a return to baseline values. All statistical tests were performed using the Past and Sisvar software.

3. Result

The presence of pathogens in a rearing environment causes additional stress by increasing glucose, cortisol, lactate, and hematocrit levels above the levels in fish in sterile tanks (Table 1).

However, the presence of pathogens in the rearing environment promotes an additional stress by increasing glucose, cortisol, lactate and

hematocrit compared to fish in sterile tanks (Fig. 2).

Aside from the previously mentioned parameters (glucose, cortisol, and lactate), there was a reduction in total plasmatic protein in all fish, regardless of the tank or stocking density, during the first day of quarantine. However, fish in the contaminated tanks had a persistent reduction in plasmatic protein, which did not return to normal levels until the end of the quarantine period (Table 2).

A red blood analysis was done. The presence of pathogens in water and stocking density influenced hematological parameters, namely, red cell count, hemoglobin levels, and MCH. The most important change was observed in hemoglobin concentration, where fish subjected to a high stocking density and exposure to pathogens had high values of hemoglobin over time, and these values did not return to baseline levels (Table 3). This specific alteration in hemoglobin concentration was reflected in MCH, demonstrating the influence of pathogens and the high stocking density over time (Table 3). Furthermore, the final values did not return to baseline levels.

In defense cells, lymphocyte and thrombocyte numbers decreased when fish were exposed to pathogens. However, over time, neutrophils, monocytes, basophils, and LG PAS counts exhibited opposite trends compared to fish in sterile tanks. The most pronounced hematological differences occurred on the 15th day of quarantine. However, the fish subjected to pathogens at a low stocking density had an increase in monocyte numbers (Table 4).

In contaminated tanks, regardless of stocking density, no parameters returned to baseline values. However, in sterile tanks, four parameters returned to baseline levels under a low stocking density while two parameters returned to baseline levels under a high-density stocking density.

Additionally, fish in contaminated tanks at a high stocking density showed clinical signs of bacterial infection (erosion in fins, dark skin, muscle necrosis, and ascites) from the 34th day onward, with approximately 9 % of accumulated mortality over the period (45 days) (Figs. 3 and 4).

4. Discussion

Quarantine is an important management practice for reducing pathogen spread and preventing disease outbreaks in tanks, fish farms, and regions. In this study, we found that effective quarantine procedures and appropriate stocking densities promote fish adaptation to new rearing environments even when water quality conditions differ from those of original tanks. Intense acute stress and hematological changes can cause mortality within 96 h of transport handling (Gomes et al., 2003a, 2003b). However, in the present assay, despite the initial blood alterations (high glucose and cortisol as well as low lymphocyte and thrombocyte counts), no clinical signs or diseases were observed in fish under normal conditions (sterile tanks regardless of stocking density), and these blood alterations ceased over time. These initial physiological and hematological alterations were responses to acute stress due to new rearing conditions, reflecting the first and second phases of the general adaptation syndrome to stressful conditions (Selye, 1950; Winberg et al., 2006).

Aboagye and Allen (2017) observed an increase in glucose, lactate, and hematocrit in fish subjected to acute stress due to moderate hypoxia. Similar hematological changes such as increased cortisol, blood glucose, lactate, and triglyceride levels were observed in fish such as curimbatá *Prochilodus lineatus* (Durigon et al., 2019), pirarucu *Arapaima gigas* (Brandão et al., 2006), and tambaqui *Colossoma macropomum* (Brandão et al., 2004) under post-transport stress conditions.

However, the presence of pathogens in the water proved to be an additional stress factor from the 15th day of quarantine, maintaining alterations in some blood parameters, such as total plasma protein, hemoglobin, MCH, and leukocytes, indicating chronic stress. The increase of glucose, cortisol, and lactate associated with lymphocytopenia and neutrophilia in contaminated tanks under quarantine also demonstrates this additional stress response due to pathogen presence. Under the condition where fish were introduced into contaminated tanks, after 15 days of quarantine at a high stocking density, the opportunistic characteristics of some pathogens began to manifest.

Classical clinical signs of *A. hydrophila* such as intestinal illness and ascites were observed in fish in contaminated tanks at a high stocking density. In chronic stress conditions, plasma protein is important in regulating oncotic pressure that helps in ameliorating inflammatory processes (Sulya et al., 1961; Keys, 1993), however, the reduction in total plasma protein observed in fish from contaminated tanks may be a reflection of the breakdown of some proteins causing an oncotic pressure imbalance. This imbalance is mainly responsible for the formation of edema and fluid accumulation in inflamed areas (O'brien et al., 2005). This aspect is a common inflammatory process caused by *A. hydrophila* under chronic stress conditions (Sulya et al., 1961; Keys, 1993).

When exposed to pathogens, fish reared at high stocking densities exhibited hematological alterations, clinical signs starting from the 15th day of quarantine, and mortalities on the 34th day. High stocking density is commonly cited as a stressing factor for fish (Odhiambo et al., 2020). Furthermore, fish erosion, a clinical sign that was observed, may

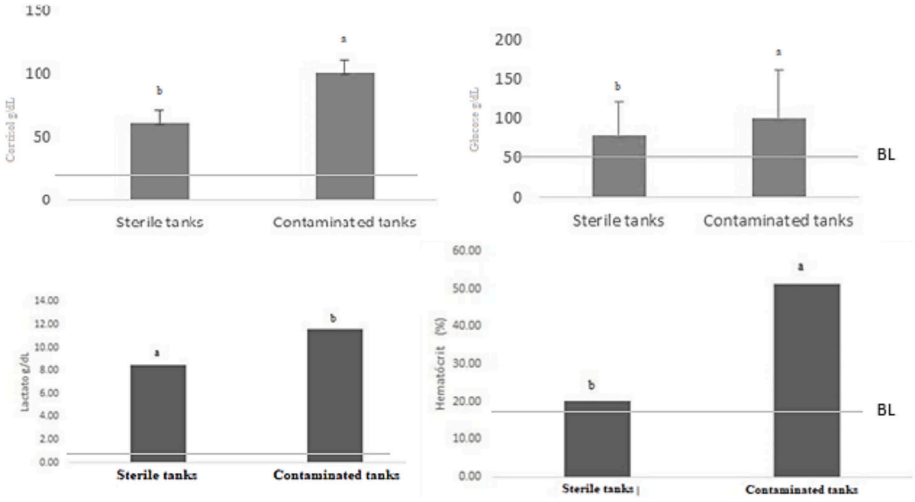

Fig. 2. Cortisol, Glucose, lactate and hematocrit percentage (mean ± standard deviation) of fish *Colossoma macropomum* remaining in different tanks (sterile and contaminated) throughout the 45 days. Different letters between the columns means statistical difference by *t*-test. Contaminated tanks were inoculated previously with bacterium *Aeromonas hydrophila* 10^6 CFU/100 L, and fungus *Saprolegnia parasitica* 10^3 zoospore/100 L. BL: baseline values.

Table 2
Plasmatic protein values (mean ± standard deviation) of fish *Colossoma macropomum* subject to different stocking densities and exposure or not to autochthonous pathogens.

	Sterile tanks		Contaminated tanks	
	Low density.	High density.	Low density.	High density.
Baseline	4.27 ± 0.10	4.27 ± 0.10	4.27 ± 0.10	4.27 ± 0.10
1	3.87 ± 0.21BaX	3.83 ± 0.20BaX	3.88 ± 0.22AaX	3.87 ± 0.17AaX
15	4.35 ± 0.36AaX	4.44 ± 0.28AaX	3.81 ± 0.50AaY	2.92 ± 0.25CbY
45	4.13 ± 0.26AaX	4.22 ± 0.21AaX	3.60 ± 0.42AaY	3.27 ± 0.32BaY
Return	15d	15d	NO<	NO<
p-value	$p > 0.05$	$p > 0.05$	$p < 0.05$	$p < 0.05$

Different letters in the row (lowercase), column (uppercase) and between the groups (XY for tanks "sterile or contaminated") mean statistical difference by Tukey test ($p < 0.05$), Base: Basal values before the stress by transport, NO(>): did not return and ended above the basal values, NO(<): did not return and ended below the basal values, 01d-15d-45d: 01 days after acclimation.

Table 3
Red cell blood and MCH index (mean ± standard deviation) of the round fish *Colossoma macropomum* subject to different stocking densities and exposure or not to autochthonous pathogens.

	Sterile tanks		Contaminated tanks	
	Low density.	High density.	Low density.	High density.
	Erythrocytes (cell/μL × 10^6)			
Baseline	0.74 ± 0.14	0.74 ± 0.14	0.74 ± 0.14	0.74 ± 0.14
1	1.31 ± 0.30AaX	1.26 ± 0.20AaX	1.51 ± 0.20AaX	1.55 ± 0.19AaY
15	1.06 ± 0.31ABaX	1.39 ± 0.17AaX	1.36 ± 0.22ABaX	1.56 ± 0.21AaX
45	0.92 ± 0.28BbX	1.25 ± 0.15AaX	1.22 ± 0.22BaX	1.42 ± 0.23AaX
Return	45d	NO>	NO>	NO>
p-value	p > 0.05	p < 0.05	p < 0.05	p < 0.05
	Hemoglobin (%)			
Baseline	7.66 ± 0.20	7.66 ± 0.20	7.66 ± 0.20	7.66 ± 0.20
1	10.71 ± 2.08AaX	12.67 ± 1.33AaX	12.23 ± 2.96AaX	13.34 ± 1.91AaX
15	7.62 ± 1.80BaX	8.82 ± 1.32BaX	9.39 ± 3.03BaX	11.49 ± 1.93ABaY
45	7.81 ± 1.77BaX	8.04 ± 1.20BaX	8.21 ± 1.50BbX	10.71 ± 1.52BaY
Return	15d	15d	15d	NO>
p-value	p > 0.05	p > 0.05	p > 0.05	p < 0.05
		MCH (pg)		
Baseline	24.95 ± 0.82	24.95 ± 0.82	24.95 ± 0.82	24.95 ± 0.82
1d	33.04 ± 5.03AaX	39.99 ± 4.94AaX	37.44 ± 8.03AaX	40.63 ± 8.98AaX
15d	24.84 ± 4.86BaX	29.21 ± 4.64BaX	28.28 ± 8.29BaX	34.58 ± 8.50BaY
45d	26.44 ± 6.11BaX	26.41 ± 2.77BaX	25.72 ± 6.68BbX	33.66 ± 8.17BaY
Return	15d	45d	45d	NO>
p-value	p > 0.05	p > 0.05	p > 0.05	p < 0.05

Different letters in the row (lowercase), column (uppercase) and between the groups (XY for tanks "sterile or contaminated") mean statistical difference by Tukey test ($p < 0.05$), Base: Basal values before the stress by transport, NO(>): did not return and ended above the basal values, NO(<): did not return and ended below the basal values, 01d-15d-45d: 01 days after acclimation.

be related to a high density because the presence of a large number of fish promotes agonistic encounters, causing cuts and small lesions on the skin (Paixão et al., 2020). Over time, all these injuries could result in chronic stress and cause a reduction in defense cells such as neutrophils and thrombocytes, facilitating infection by opportunistic pathogens (Speirs et al., 2024).

These facts could explain the appearance of clinical signs throughout the experimental period in contaminated tanks (since day 15 of quarantine) and not at the beginning. Regardless of the duration of stress (acute or chronic), the intensity of stress can determine whether an organism will overcome illness and reestablish homeostasis besides promoting adaptation to a new rearing environment, or whether the organism will die (Winberg et al., 2016). Therefore, for fish kept at a low stocking density, despite the presence of pathogens, no clinical signs of infection were observed, demonstrating an adaptation to new conditions and the establishment of a new epidemiological triad (host–environment–pathogen) balance.

This study demonstrates that the 15th day of quarantine is the moment to begin making steps to mitigate infectious diseases caused by autochthonous pathogens. The bacterial infection of *A. hydrophila* probably starts to spread during this period (day 15), based on the blood parameter results. Thus, from the 15th day, mitigating measures such as the use of antibiotics or an eco-friendly product may be utilized to control a future bacterial infection (Iber et al., 2021). Furthermore, mitigating measures such as the use of salt, phytotherapy, or a supplemented diet could also be utilized in quarantine-receiving tanks before transport to reduce endemic pathogen load or improve the immune system; however, studies should be done to prove the efficacy of these measures during the quarantine period.

This study shows that bacterial infections could be considered more problematic than oomycetes to quarantined fish. Bacteria infect quarantined fish slowly. In contrast, oomycetes need a gateway create a fungal infection (Meneses et al., 2022). Infection by a zoospore at the beginning of the quarantine depends on a gateway such as fin erosion or scale losses. However, effective management of stocking density during transport and acclimation carried out in the present work avoided these gateways (Paixão et al., 2020).

Zoospores remain viable for up to 4 h or 7 days for zoospore type 1 and 2, respectively (Willoughby et al., 1983; Diéguez-Uribeondo et al., 1994; Robertson et al., 2009; Matthews et al., 2021). Therefore, on the 15th day of quarantine, when fin erosion was observed, no zoospore was viable to cause an infection (Robertson et al., 2009; Pavić et al., 2022). One hypothesis about non-infection by oomycetes is related to the electrical conductivity of water, where low conductivity values reduce zoospore viability, which was the scenario observed in the receiving tanks.

Table 4
White blood cells and thrombocytes (mean ± standard deviation) of fish *Colossoma macropomum* subject to different stocking densities and exposure or not to autochthonous pathogens.

	Sterile tanks		Contaminated tanks	
	Low density.	High density.	Low density.	High density.
	Thrombocytes cell/uL × 10^4			
Baseline	1.67 ± 0.41	1,67 ± 0,41	1,67 ± 0,41	1,67 ± 0,41
1d	0.37 ± 0.09CbX	0,99 ± 0,26AaX	1,13 ± 0,31AaY	1,30 ± 0,31AaX
15d	0.53 ± 0.14BbX	0,94 ± 0,25AaX	0,55 ± 0,17BbX	1,19 ± 0,17AaX
45d	0.89 ± 0.27AaX	0,92 ± 0,15AaX	0,19 ± 0,06CbY	0,49 ± 0,09BaY
Return	NO(<)	NO(<)	NO(<)	NO(<)
p-value	p < 0.05	p < 0,05	p < 0,05	p < 0,05
	Lymphocytes cell/uL × 10^4			
Baseline	8.35 ± 1.25	8,35 ± 1,25	8,35 ± 1,25	8,35 ± 1,25
1d	1.06 ± 0.43CbX	2,19 ± 0,83AaX	1,94 ± 0,97AbX	3,13 ± 0,26AaX
15d	2.91 ± 0.95BaX	2,81 ± 0,56AaX	1,32 ± 0,24ABaY	1,87 ± 0,73BaY
45d	3.03 ± 1.36AaX	2,48 ± 0,67AaX	1,08 ± 0,20BaY	1,78 ± 0,20BaX
Return	NO(<)	NO(<)	NO(<)	NO(<)
p-value	p < 0.05	p < 0,05	p < 0,05	p < 0,05
	Monocytes cell/uL × 10^4			
Baseline	0.84 ± 0.36	0,84 ± 0,36	0,84 ± 0,36	0,84 ± 0,36
1d	2.06 ± 0.68AaX	2,10 ± 1,24AaX	2,49 ± 0,97AaX	1,92 ± 0,55AaX
15d	1.49 ± 0.46ABaX	1,51 ± 0,25AaX	2,29 ± 0,40AaY	1,64 ± 0,44AaX
45d	0.95 ± 0.26BbY	1,79 ± 0,22AaX	3,17 ± 0,51AaY	2,17 ± 0,42AbX
Return	45d	NO(>)	NO(>)	NO(>)
p-value	p > 0.05	p < 0,05	p < 0,05	p < 0,05
	Neutrophil cell/uL × 10^4			
Baseline	2.41 ± 1.06	2,41 ± 1,06	2,41 ± 1,06	2,41 ± 1,06
1d	6.73 ± 1.07AbX	8,44 ± 1,64AaX	9,86 ± 1,28AaY	9,92 ± 1,82AaY
15d	4.92 ± 1.24BbX	8,99 ± 0,09AaX	7,60 ± 1,07BbY	10,78 ± 1,46AaY
45d	3.78 ± 1.30BbX	8,04 ± 0,92AaX	5,28 ± 0,70CbY	8,42 ± 1,62BaX
Return	45d	NO(>)	NO(>)	NO(>)
p-value	p > 0.05	p < 0,05	p < 0,05	p < 0,05
	Basophil cell/uL × 10^4			
Baseline	0.33 ± 0.39	0,33 ± 0,39	0,33 ± 0,39	0,33 ± 0,39
1d	1.84 ± 0.66AaX	0,26 ± 0,12BbX	0,25 ± 0,25BaY	0,44 ± 0,18BaX
15d	0.05 ± 0.02BbX	0,79 ± 0,26AaX	1,50 ± 0,28AaY	1,44 ± 0,30AaY
45d	0.31 ± 0.22BaX	0,59 ± 0,10AaX	1,97 ± 0,57AaY	2,07 ± 0,60AaY
Return	15d	15d	NO(>)	NO(>)
p-value	p > 0.05	p > 0,05	p < 0,05	p < 0,05
	LG-PAS cell/uL × 10^4			
Baseline	0.16 ± 0.14	0,16 ± 0,14	0,16 ± 0,14	0,16 ± 0,14
1d	0.66 ± 0.02AaX	0,12 ± 0,03AbX	0,25 ± 0,03BaY	0,18 ± 0,06BaX
15d	0.02 ± 0.04BbX	0,26 ± 0,03AaX	0,28 ± 0,03BaY	0,30 ± 0,01BaX
45d	0.22 ± 0.05BaX	0,10 ± 0,01AbX	0,57 ± 0,01AaY	0,60 ± 0,01AaY
Return	15d	15d	NO(>)	NO(>)
p-value	p > 0.05	p < 0,05	p < 0,05	p < 0,05

Different letters in the row (lowercase), column (uppercase) and between the groups (XY for tanks "sterile or contaminated") mean statistical difference by Tukey test ($p < 0.05$), Base: Basal values before the stress by transport, NO(>): did not return and ended above the basal values, NO(<): did not return and ended below the basal values, 01d-15d-45d: 01 days after acclimation.

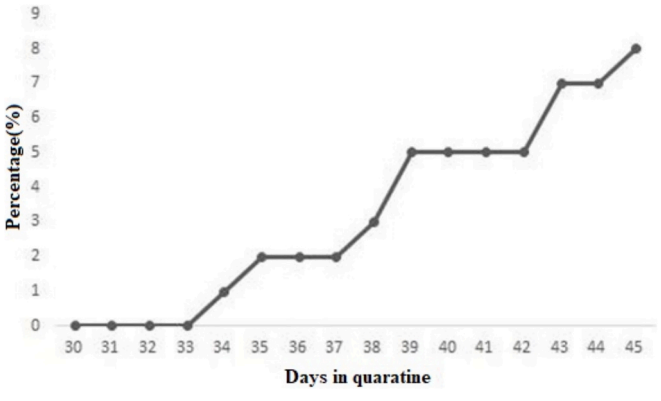

Fig. 3. Accumulated mortality throughout quarantine period (45 days) for tambaqui *Colossoma macropomum* submitted to high stocking density into contaminated tanks containing the bacterium *Aeromonas hydrophila* 10^6 CFU/100 mL, and fungus *Saprolegnia parasitica* 10^3 zoospore/100 mL.

Although oomycete infection was not observed in this work, the presence of bacteria and oomycetes at the beginning of quarantine influenced the response of fish to the new rearing environment. In real rearing conditions, the continuous supply of water to tanks may contain zoospores that would be a problem on the 15th day of quarantine when fin erosions due to bacterial infection would be gateways to new infection by opportunistic oomycete. This fact shows the importance of best management practices such as pathogen monitoring, appropriate stocking density in transport, and the use of an acclimation protocol to avoid providing gateways for infections.

Thus, the presence of autochthonous pathogens is an important stressor to be considered in the establishment of best management practices and quarantine extension. Therefore, it is important to prevent the introduction of allochthonous pathogens to new rearing conditions (as previously reported) (Tacchi et al., 2015). Furthermore, autochthonous pathogens influence fish adaptation to new tanks. The quarantine also showed the importance of establishing a pathogen–host–environment balance in a new rearing situation. In determining the quarantine period, a consideration should be made of not only the time of appearance of clinical signs but also the reestablishment of this balance in the epidemiological triad.

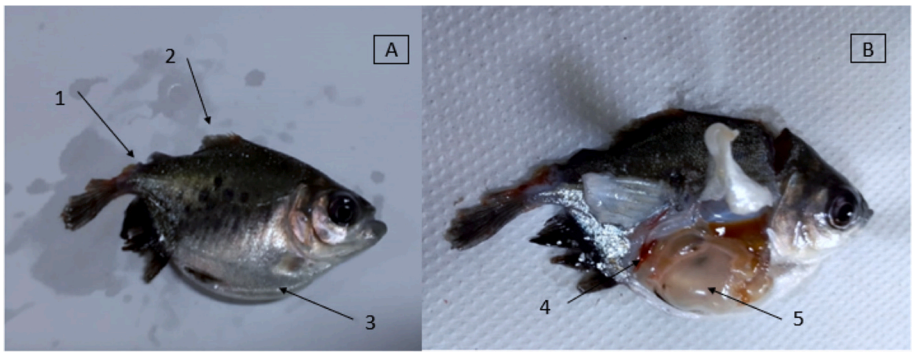

Fig. 4. Clinical signs throughout quarantine period (45 days) for tambaqui *Colossoma macropomum* submitted to high stocking density into contaminated tanks containing the bacterium *Aeromonas hydrophila* 10^6 CFU/100 L, and fungus *Saprolegnia parasitica* 10^3 zoospore/100 L. (1) Erosion fins, (2) muscle necrosis, (3) ascites, (4) accumulation of blood in the coelomic cavity, (5) ascites.

Therefore, the monitoring of endemic pathogens linked to hematological parameters is the best management practice in fish farms to improve the quarantine procedure by establishing measures to avoid immunosuppression and to prevent disease outbreaks. A parasite–environment–host relationship can be established even with mitigating actions and adequate quarantine procedures in place for at least 30 days.

5. Conclusion

The presence of autochthonous pathogens in rearing environment is an additional stressor influencing the tambaqui adaptation mainly in high stocking densities. Therefore, the quarantine for tambaqui must be carried out throughout at least 30 days at stocking density of 1 g/L.

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CRediT authorship contribution statement

Peterson Emmanuel Guimarães Paixão: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ricardo Marques Nogueira Filho:** Writing – review & editing, Visualization, Formal analysis. **Higo Andrade Abe:** Writing – review & editing, Visualization, Formal analysis. **Rubens Riscala Madi:** Writing – review & editing, Visualization, Methodology, Formal analysis, Conceptualization. **Cindy Caroline Moura Santos:** Writing – review & editing, Visualization, Formal analysis. **Natalino da Costa Sousa:** Writing – review & editing, Visualization, Formal analysis. **Márcia Valéria Silva do Couto:** Writing – review & editing, Visualization, Formal analysis. **Hugo Leandro Santos:** Writing – review & editing, Visualization, Formal analysis. **Fabricio Sa Santana:** Writing – review & editing, Visualization, Formal analysis. **Amanda Silva Carvalho:** Writing – review & editing, Visualization, Formal analysis. **Juliana Matos Araujo:** Writing – review & editing, Visualization, Formal analysis. **Rodrigo Yudi Fujimoto:** Writing – review & editing, Visualization, Validation, Project administration, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

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.

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Data availability

Data will be made available on request.

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