ROUTINE EXPOSURE TO BIOMETRIC PROCEDURES IN FISH FARMING REVEALS DIFFERENCES IN STRESS RESPONSE IN TAMBAQUI AND HYBRID TAMBATINGA*

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ABSTRACT

The physiological stress responses of tambaqui and hybrid tambatinga were evaluated after subjecting the fish to routine practices in a breeding system such as periodic biometric procedures. For 270 days of culture, the fish underwent monthly biometric measurements, and at the end of the period, blood was collected at six sampling times (before, immediately after and 2, 24, 48 and 72 h after biometric measurements) for the evaluation of physiological indicators of stress. Tambatinga are more susceptible to stress because they presented higher levels of cortisol and glucose in the bloodstream after handling and took longer to recover their basal physiological state for these parameters. However, the low cortisol levels observed in both species suggest that the fish were familiar with biometric procedures, resulting in a less intense response. Handling led to an increase in the cellular volume of erythrocytes in tambaqui, resulting in a change in hematocrit and a decrease in hemoglobin concentration. Hypochloremia was found in both species only 72 h after handling. Biometric procedures promote hormonal, hematological and hydroelectrolytic changes in the tambaqui and hybrid tambatinga, but when routinely adopted, at regular intervals, they elicit stress responses of lower magnitude.

Keywords: *Colossoma* sp.; cortisol; handling; hematology; fish; *Piaractus* sp.

EXPOSIÇÃO ROTINEIRA AOS PROCEDIMENTOS BIOMÉTRICOS NA PISCICULTURA REVELA DIFERENÇAS NA RESPOSTA AO ESTRESSE EM TAMBAQUI E HÍBRIDO TAMBATINGA

RESUMO

Foram avaliadas as respostas fisiológicas de estresse de tambaqui e híbrido tambatinga, quando submetidos a práticas rotineiras em sistema de criação, como a realização periódica de biometrias. Por 270 dias de cultivo os peixes foram submetidos a biometrias mensais e, ao final do período, o sangue foi colhido em seis tempos de amostragem (antes; imediatamente após; 2; 24; 48 e 72 h após a biometria) para avaliação de indicadores fisiológicos de estresse. Tambatinga é mais susceptível ao estresse, pois apresentou maiores níveis de cortisol e glicose na corrente sanguínea após manejo e levou mais tempo para recuperar seu estado fisiológico basal para estes parâmetros. Contudo, os baixos níveis de cortisol observados para ambos sugerem que os peixes estavam familiarizados ao manejo biométrico, resultando em resposta menos intensa. O manejo provocou aumento no volume celular dos eritrócitos do tambaqui, resultando em alteração no hematócrito e diminuição da concentração de hemoglobina. Hipocloremia foi verificada em ambos os peixes apenas 72 h após a realização do manejo. O manejo de biometria promove alterações hormonais, hematológicas e hidroeletrolíticas no tambaqui e híbrido tambatinga, mas, quando adotado de forma rotineira, em intervalos regulares, provoca respostas de estresse de menor magnitude.

Palavras chave: *Colossoma* sp.; cortisol; hematologia; manejo; peixes; *Piaractus* sp.

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INTRODUCTION

Fish farming in Brazil is currently practiced with over 30 species with the most diverse feeding habits, chiefly from tropical climates. Among the native cultured species, production of "round" fish (species and hybrids of the genus *Colossoma* and *Piaractus*) accounts for 82%, with the tambaqui (*Colossoma macropomum*) representing a large part of this total (IBGE, 2014).

The success of tambaqui in fish farming is attributed to the uncomplicated production of fingerlings and their good ability to adapt to captive conditions, featuring rapid growth and resistance to low levels of dissolved oxygen in water, handling and diseases. These fish easily accept artificial feed when raised in tanks on fish farms and are able to digest animal and vegetable protein. The fattening phase lasts 240 to 300 days, depending on the water availability, using storage densities between 1 and 1.5 fish per square meter (ARAÚJO-LIMA and GOMES, 2010), reaching an average weight of 2.0 kg in less than one year of cultivation.

In Mato Grosso State, as in others, farmers have sought to increase the productivity of tambaqui, but many still choose to produce round fish hybrids, e.g. the tambatinga, a result of the cross between female tambaqui (*C. macropomum*) and male pirapitinga (*Piaractus brachypomus*). This hybrid has gill rakers more developed than the pirapitinga, which provides greater efficiency in plankton filtration. This species also easily reaches commercial weight in a short period and with low dietary levels of crude protein, which represents an economy with feeding (SILVA-ACUÑA and GUEVARA, 2002). In addition, in some regions of Brazil, many fish farmers prefer to farm hybrid tambatinga because of attractive body aspects such as silver color and reddish operculum, which suits the taste and preference of the consumers, according some producers. Together with the tambacu hybrid (female *C. macropomum* × male *P. mesopotamicus*), this is the third most largely produced fish in the country (IBGE, 2014).

In fish farming, biometric measurements are taken to evaluate information related to animal performance such as weight and health status. Despite being a routine practice under culture conditions, the procedures may cause stress to fish, resulting in alterations in their homeostasis that predispose to the appearance of diseases and may lead to mortality (URBINATI *et al.*, 2014).

Many fish species respond to stress by elevating their circulating levels of catecholamines and corticosteroids (BARTON, 2002). These primary effects provoke secondary responses related to energy requirements, including increased blood glucose and altered electrolyte homeostasis in blood and tissues. Cortisol and blood glucose are considered good indicators to evaluate primary and secondary stress response, respectively (WENDELAAR BONGA, 1997).

In this regard, blood tests (hematological and metabolic) can be used to physiologically characterize a species in its culture environment and subsequently contribute to studies involving management of cultured fish. The knowledge of the stress responses is an important tool to formulate good management practices so that handling does not compromise the fish development in farming. Therefore, in the present study we analyzed the physiological stress responses of tambaqui and hybrid tambatinga subjected to routine practices in fish farming such as periodic biometric procedures.

MATERIAL AND METHODS

The study was conducted in the Fish Farming Station of the Experimental Farm at the Faculty of Agronomy and Animal Science at the Federal University of Mato Grosso (UFMT). All experimental protocols were approved by CEUA (Committee of Ethics in Animal Use/UFMT, case nº. 23108.069114/2014-85).

The fish were acquired from a commercial fish farm and transported to the Fish Farming Station of UFMT and kept in 1-m^3 net cages identified for tambaqui and tambatinga separately, until reaching a standard length of 15 cm. On this occasion, 100 tambaqui (*C. macropomum*) and 100 tambatinga (female *C. macropumum* × male *P. mesopotamicus*) with an average initial weight (± standard error) of 343.06 ± 5.46 g were individualized with a microchip and distributed in an 800-m^2 excavated pond with 1.80 m depth at the stocking density of 0.25 fish per m^2, with partial water renewal (average 10%) without supplemental aeration.

During the entire culture period, the fish were fed twice daily with extruded feed (VB Alimentos®) for omnivorous fish containing 32% crude protein (CP), at a feeding rate varying from 3% to 1% of the live weight, which was adjusted according to the development stage in which the fish were at the time.

Biometric measurements were taken monthly over the course of the experiment (270 days). The management for the biometric measurements consisted of first capturing all fish with a trawl and anesthetizing them with 50 mg L^{-1} Eugenol® (previously diluted in ethanol at 1:4, according to INOUE and MORAES, 2007) for approximately three minutes, which was the time necessary for the fish to show apparent signs of sedation such as reduced swimming motion, partial loss of equilibrium and reduced gill ventilation (WOODY *et al*., 2002). Next, the fish were removed from the box with anesthetic and the microchip was identified to subsequently perform the actual biometric procedures, which consisted of determining their weight (Marte Scale - Model AS 2000C) and obtaining morphometric measurements such as total and standard lengths, head size, height and width of body. The total and standard lengths were measured using a fish meter board. For the other measures, a gauge caliper was used. After this biometric process, the fish were placed in a box with clean water and oxygenation until recovery and were then returned to the pond.

At the end of 270 days of culture, during the last biometric measurements of the experiment, all fish were subjected to the same procedures from the previous months (capture, accommodation in net cages and anesthesia), but one group of tambaqui (n = 4) and tambatinga (n = 4) not subjected to any disturbance was captured before the biometric measurements and anesthetized and had their blood collected to serve as control for the physiological indicators (baseline). Immediately after the biometric measurements (0 h), the tambaqui (n = 6) and tambatinga (n = 6) had their blood harvested by caudal puncture. Subsequently, the next fish that underwent biometric measurements, in a random sequence, were packed in net cages for recovery and allocated to the culture pond. All net cages were previously identified with the collection time of the blood sample after biometric measurements, and tambaqui and tambatinga samples were allocated, in the same amount, to the same net cage, to be collected at the same recovery time, at random. Next, samples of tambaqui (n = 12) and tambatinga (n = 12), which had their blood drawn at 2 h, 24 h, 48 h and 72 h after biometric procedures, were collected. After blood sample collection, the fish were returned to the culture pond.

In the blood samples, were evaluated hematocrit (Microcentrifuge Spin100; centrifugation for 10 min at 3,300 rpm x *g* and subsequent reading using a proper chart); total erythrocyte count (performed in a Neubauer chamber, following OLIVEIRA-JUNIOR *et al.* (2008), with modifications); and hemoglobin concentration (hemoglobin cyanide method, using a Labtest® commercial kit), analyzed in a Labmax Flex® machine. Blood samples were centrifuged (HT CM-610, 10 min at 3,000 rpm) to separate the serum for analyses of total protein (biuret method, using a Labtest® commercial kit) and chloride (using a Labtest® commercial kit) and to separate the plasma for analysis of glucose (enzymatic methodology by oxidase glucose and Trinder reagent using a Labtest® commercial kit), performed in a Labmax Flex® machine. The serum was also used for the analysis of cortisol (InVitro Diagnóstica® commercial kit for ELISA methodology).

During the entire experimental period, dissolved oxygen and temperature (Digital Oximeter YSI 55), pH (digital pocket pH meter Quimis® - Q400BD) and un-ionized ammonia (calculated according to EMERSON *et al.*, 1975) were monitored weekly, while alkalinity (using methyl orange indicator solution) and transparency (Secchi disk) were evaluated monthly.

A completely randomized design was employed and data were analyzed by a two-way analysis of variance (ANOVA), with species (tambaqui and tambatinga) and sampling times (baseline, 0 h and 2, 24, 48, and 72 h after biometric measurements) as the factors. When F values indicated significance (p<0.05), means were compared by Tukey's test (TUKEY, 1953). Results are presented as means ± standard deviation and data were analyzed by the SAS software.

RESULTS

The analyzed water physicochemical variables were within the acceptable limits for fish farming, according to MORO *et al*. (2013) (Table 1). At the end of the experiment (270 days), the final mean weights were 1,742.40 ± 299.81 g and 1,515.29 ± 333.38 g for tambaqui and tambatinga, respectively.

The hybrid tambatinga showed a significant increase in cortisol level soon after the biometric measurements (0 h), differing statistically from the tambaqui, and returning to baseline 2 h after this measurement. However, cortisol level in hybrid tambatinga rose again 48 h after the end of measurements and remained significantly high until the end of the experiment. For the tambaqui, the lowest level of cortisol was observed two hours after biometric measurements, and the highest level was recorded 72 h later, with no significant difference in relation to baseline (Figure 1).

Table 1. Water quality parameters (mean ± standard deviation) during the semi-intensive culture of tambaqui and hybrid tambatinga.

Parameter	Value
Dissolved oxygen (mg L^{-1})	6.88 ± 1.59
Temperature (°C)	28.19 ± 2.13
pH	7.95 ± 0.81
Transparency (cm)	51.26 ± 15.85
Un-ionized ammonia (NH_3; mg L^{-1})	0.02 ± 0.03
Alkalinity (mg $CaCO_3$ L^{-1})	33.18 ± 14.19

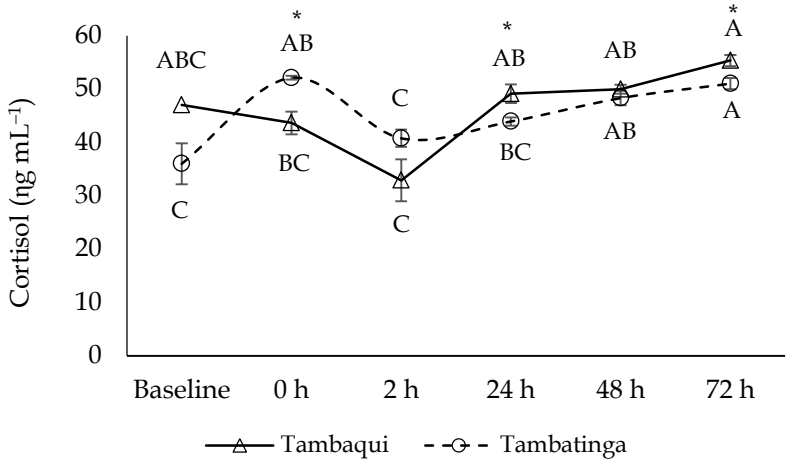

Figure 1. Cortisol levels (ηg mL^{-1}) in tambaqui and hybrid tambatinga subjected to biometric procedures in semi-intensive fish farming. Baseline: before biometric measurements; 0 h: immediately after biometric measurements; and 2, 24, 48 and 72 h after biometric measurements. Different letters indicate significant differences between sampling times; * indicates differences between species (Tukey's test; $p<0.05$).

Blood glucose increased in both tambaqui and hybrid tambatinga soon after the biometric measurements (0 h) (Figure 2), returning to homeostasis 2 h after the measurements in the tambaqui (148.1 ± 8.1 mg dL^{-1}) and later (in 24 h) in the tambatinga (97.3 ± 4.9 mg dL^{-1}). The blood glucose levels of the tambaqui were statistically lower than those observed for the hybrid tambatinga from 2 to 48 h after the biometric measurements (Figure 2).

In this study, an increase was found in the serum concentration of ion chloride 2 h after the biometric procedures in both tambaqui (199.8 ± 3.8 mEq L^{-1}) and hybrid tambatinga (193.9 ± 2.1 mEq L^{-1}). The level of this ion was significantly higher in tambatinga at 24 and 48 h after biometric procedures in relation to tambaqui, but the characteristic hypochloremia in response to stress was found only 72 h after the biometric measurements in both species (Figure 3).

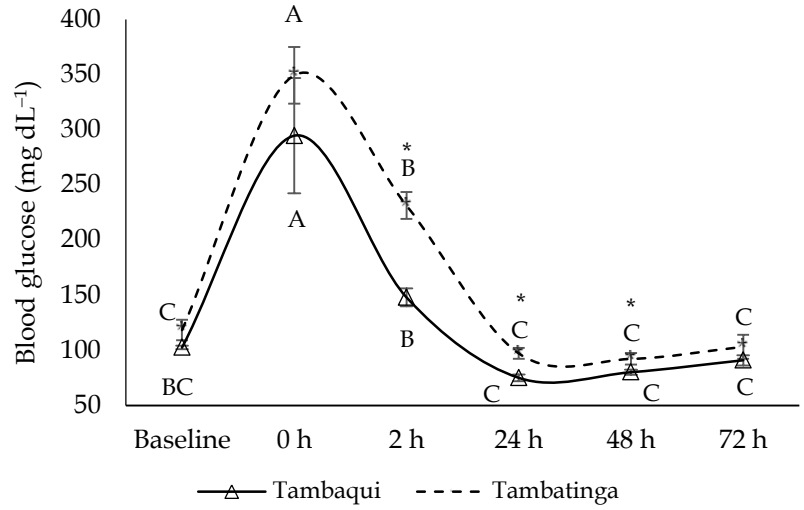

Figure 2. Glucose levels (mg dL^{-1}) in tambaqui and hybrid tambatinga subjected to biometric procedures in semi-intensive fish farming. Baseline: before biometric measurements; 0 h: immediately after biometric measurements; and 2, 24, 48 and 72 h after biometric measurements. Different letters indicate significant differences between sampling times; * indicates differences between species (Tukey's test; $p<0.05$).

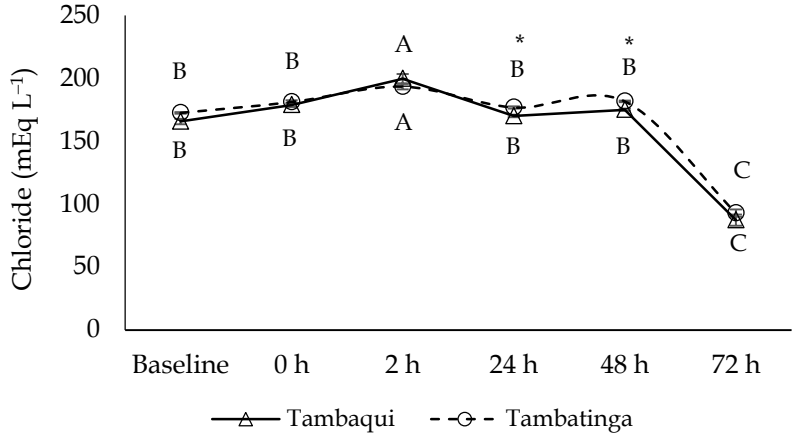

Figure 3. Chloride levels (mEq L^{-1}) in tambaqui and hybrid tambatinga subjected to biometric procedures in semi-intensive fish farming. Baseline: before biometric measurements; 0 h: immediately after biometric measurements; and 2, 24, 48 and 72 h after biometric measurements. Different letters indicate significant differences between sampling times; * indicates differences between species (Tukey's test; $p<0.05$).

The total protein concentration increased significantly soon after the biometric measurements (0 h) in the hybrid tambatinga (5.3 ± 0.1 g dL^{-1}), differing statistically from the tambaqui, in which total protein increased 2 h after the biometric measurements (6.2 ± 0.2 g dL^{-1}), returning to baseline in both species 24 h after the measurement procedures (Figure 4).

Results for hematological parameters evaluated here are presented in Table 2. The hybrid tambatinga did not show significant statistical differences for hematocrit but differed from the tambaqui, which showed significantly higher hematocrit values 2 h after biometric measurements (50.7 ± 2.1%), returning to baseline 24 h after this measurement procedure (38.3 ± 7.6%). The hemoglobin concentration decreased significantly at 2 and 24 h after the biometric measurements in tambaqui and tambatinga, respectively, returning to baseline 48 h after the measurement. However, in both

species, a significant decline was found in hemoglobin concentration 72 h after the biometric measurements, remaining significantly lower in tambaqui (7.8 ± 0.2 g dL^{-1}) compared with hybrid tambatinga (9.5 ± 0.6 g dL^{-1}). For the tambaqui, no statistical difference was observed between sampling times for number of erythrocytes, although immediately after biometric procedures (0 h) this parameter was significantly higher (3.7 ± 2.5 cells × 10^6 μL^{-1}) in relation to the hybrid tambatinga (2.7 ± 0.9 cell × 10^6 μL^{-1}), which displayed a higher number of these cells 48 h after the biometric procedures (5.8 ± 5.5 cells × 10^6 μL^{-1}), returning to homeostasis 72 h after measurements.

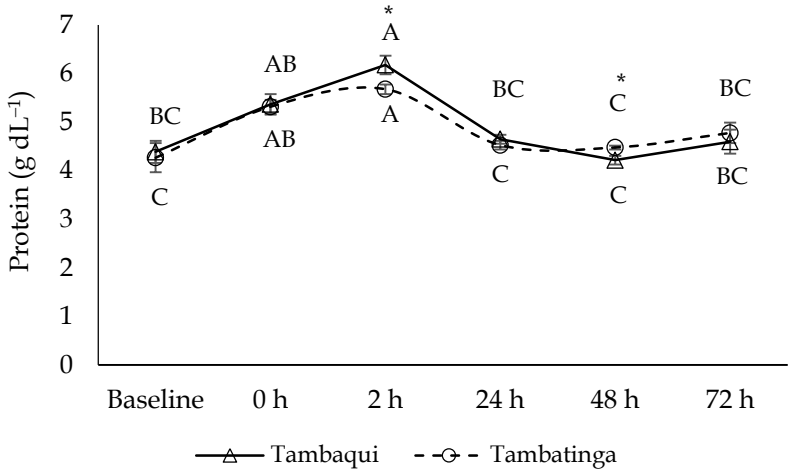

Figure 4. Total protein (g dL^{-1}) in tambaqui and hybrid tambatinga subjected to biometric procedures in semi-intensive fish farming. Baseline: before biometric measurements; 0 h: immediately after biometric measurements; and 2, 24, 48 and 72 h after biometric measurements. Different letters indicate significant differences between sampling times and * indicates differences between species (Tukey's test; $p<0.05$).

Table 2. Hematological parameters of tambaqui and hybrid tambatinga subjected to biometric procedures in semi-intensive fish farming. Baseline: before biometric measurements; 0 h: immediately after biometric measurements; and 2, 24, 48 and 72 h after biometric measurements.

		Sampling time					
		Baseline	**0 h**	**2 h**	**24 h**	**48 h**	**72 h**
Tambaqui	Ht	38.3 ± 7.6^{B}	43.8 ± 1.8^{AB}	50.7 ± 2.1^{A}	44.8 ± 2.2^{AB}	41.7 ± 1.2^{B}	39.6 ± 1.7^{B}
	Hb	12.2 ± 0.4^{B}	14.6 ± 0.2^{A}	9.5 ± 0.4^{C}	3.1 ± 0.3^{E}	10.5 ± 0.3^{BC}	7.8 ± 0.2^{D}
	Er	2.5 ± 5.0^{A}	3.7 ± 2.5^{A}	2.7 ± 1.6^{A}	2.9 ± 2.8^{A}	2.9 ± 4.3^{A}	2.6 ± 2.2^{A}
Tambatinga	Ht	46.3 ± 5.5^{A}	45.0 ± 1.3^{A}	47.5 ± 2.4^{A}	42.6 ± 1.6^{A}	40.8 ± 0.9^{A}	41.2 ± 1.4^{A}
	Hb	12.3 ± 0.4^{AB}	13.6 ± 0.4^{A}	11.0 ± 0.8^{ABC}	3.1 ± 0.3^{D}	10.4 ± 0.3^{BC}	9.5 ± 0.6^{C}
	Er	1.9 ± 0.9^{B}	2.7 ± 0.9^{B}	2.9 ± 3.9^{B}	2.6 ± 1.5^{B}	5.8 ± 5.5^{A}	2.8 ± 1.6^{B}

Means ± standard error. Means followed by common letters in the row do not differ according to Tukey's test ($p<0.05$). Ht: hematocrit (%); Hb: hemoglobin (g dL^{-1}); Er: erythrocyte count (cells × 10^6 μL^{-1}).

DISCUSSION

In fish, the primary physiological responses to different stressors involve neuroendocrine responses that include the release of catecholamines from the chromaffin tissue and stimulus of the hypothalamic-pituitary-interrenal axis (HPI), culminating in the release of corticosteroid hormones into circulation (BARTON, 2002).

The plasma cortisol is the most widely used indicator of stress in fish, irrespective of their development stage (WENDELAAR BONGA,

1997). Cortisol levels found in this experiment ranged from 32.89 to 55.35 ηg mL^{-1} for tambaqui and from 35.99 to 52.10 ηg mL^{-1} for hybrid tambatinga. Irrespective of the sampling time, the cortisol values were low as compared with other studies in which the level of this corticosteroid was tested in native fish such as tambaqui, pre- and post-application of acute stressors like capture (TAVARES-DIAS *et al.*, 2001) and transport (GOMES *et al.*, 2003a; c; CHAGAS *et al.*, 2012).

Although the biometric practice exposes the fish to sequential stressors that often cause stress, in the present study, the biometric procedures were performed every month during the 270 days of culture, and the fish thus became accustomed to these measurements. The continuous cortisol production by the interrenal cells as a result of repeated exposure to stressors could negatively regulate the HPI axis by negative feedback, reducing neuroendocrine responses (BARTON, 2002).

Rainbow trout (*Salmo gairdneri*) juveniles captured daily for 10 weeks displayed a lower plasma cortisol level at the end of this period, indicating a possible desensitization of the HPI axis due to the overall habituation of the fish to repeated disturbances (BARTON *et al.*, 1987). Tambacu hybrids (female *C. macropomum* × male *P. mesopotamicus*) also showed decreased cortisol levels one hour after being subjected to the application of consecutive capture stimuli (MARTINS *et al.*, 2002).

Despite the low cortisol values observed in this study for both species, immediately after the biometric procedures (0 h), the tambatinga presented significantly higher cortisol levels than the tambaqui, suggesting that this primary stress response may be of greater magnitude in hybrid as compared with pure species. The physical strain by the tambatinga during the biometric procedures might have contributed to a more intense and later release of cortisol, which, unlike in tambaqui, rose again 48 h after biometric procedures. According to HOSHIBA *et al.* (2009), during physical exercise, the elevation in plasma cortisol is delayed. The plasma cortisol increase is usually not observed before 30-60 min after the end of exercise (MILLIGAN and WOOD, 1987), and release peaks occur up to 1-2 h later (GAMPERL *et al.*, 1994).

Responses secondary to stress include metabolic, ionic and hematological changes related to physiological adjustments in the metabolism, respiration, hydromineral balance, immune function and cellular responses (WENDELAAR BONGA, 1997; BARTON, 2002). Increased plasma glucose levels have been reported after acute and chronic stress due to glycogenolysis and gluconeogenic effects caused by catecholamines and cortisol, respectively (WENDELAAR BONGA, 1997). In this regard, the glucose is mobilized as an energy source, and this was found in the present study. As described for tambaqui (GOMES *et al.*, 2003a; 2003c) and other native fish species such as pirarucu (GOMES *et al.*, 2003b) and matrinxã juvenile (URBINATI *et al.*, 2004), in this study, there was an increase in blood glucose immediately after the stimulus to stress (0 h), but the time for return was different among the species, with later return to baseline for tambatinga (24 h) than tambaqui (2 h). In addition, the blood glucose level of the hybrid was significantly higher than that of the pure species up to 48 h after the biometric procedures.

Alterations in circulating glucose caused by a stressor agent may vary depending on factors like species, size, sex, line and characteristics of the stressor agent, e.g. type, severity and intensity. In the same way, the capacity of return to baseline conditions may vary when the stressor stimulus is interrupted (TAKAHASHI *et al.*, 2006). The results suggests that tambatinga is more susceptible to stress than tambaqui, since the former presented higher cortisol and glucose concentration in blood circulation after handling and took longer to recover the basal physiological state for these parameters.

Hydroelectrolytic imbalances have been observed as a stress response in fish (McDONALD and MILLIGAN, 1997). Likewise, alterations in hematocrit and hemoglobin concentration may evidence hemodilution or hemoconcentration caused by stressful situations (MORGAN and IWAMA, 1997; BOSISIO *et al.*, 2017). In this study, it was evident that these alterations occurred for both species, especially two hours after biometric measurements. The increase in cell permeability during stress resulted in changes of fluid from the plasma to the intracellular compartment, concentrating more

molecules in the blood plasma, which explains the significant increase in total protein and plasma chloride at this sampling time. The influx of water into the cell contributed to increasing their volume, explaining the increase in hematocrit and decrease in hemoglobin concentration, especially in the tambaqui at this sampling time (2 h). As documented by MORGAN and IWAMA (1997), the mobilization of catecholamines into the fish blood during stress causes cell swelling, resulting in hemoconcentration in several freshwater species (McDONALD and MILLIGAN, 1997).

After a stress condition, serum sodium and chloride levels decline and potassium and calcium levels are modified. When freshwater fish face adverse conditions, there is a loss of chloride ions from their blood to the water as well as excessive hydration of the body, which causes these fish to expend additional energy to maintain or reestablish the osmoregulatory balance (HOSEINI *et al.*, 2016). In both species investigated in this study, the change in cell permeability of the gill epithelium seems to have occurred later, since the characteristic hypochloremia (loss of chloride ions to the aquatic environment) in response to stress was found only 72 h after the biometric measurements.

These results may vary depending on the fish species and the stressor applied. In pacu (*P. mesopotamicus*), ABREU *et al.* (2009) found a significant decrease in chloride levels 60 min after the fish had been subjected to capture, without return to homeostasis for up to 24 h. FAGUNDES and URBINATI (2008), however, did not find changes in serum chloride levels in Spotted sorubim (*Pseudoplatystoma corruscans*) in up to 48 h after stress from capture and transport. A slight decreased in plasma total protein concentration was found for pacu (*P. mesopotamicus*) after transport (FEITOSA *et al.*, 2013), and no significantly difference for this parameter was observed for matrinxã (*Brycon amazonicus*) after the challenge of stress from capture and exposure to air (ABREU and URBINATI, 2006).

The hematological responses shown by the tambaqui observed in the current study indicate that the biometric procedures did not lead to an increase in the number of red cells, but rather in the cellular volume of erythrocytes, resulting in alterations in hematocrit. The decreased hemoglobin concentration as well as the increased concentration of total serum protein observed at these sampling times in tambaqui reinforce a change of fluid from the plasma to the intracellular compartment of the erythrocytes as a result of the increased permeability of the cell membrane brought about by the release of catecholamines during stress (MELO *et al.*, 2009). For the tambatinga, these hematological alterations were not as significant as in the tambaqui. The increase in number of red blood cells of the tambatinga at 48 h was an isolated alteration resulting from the release of erythrocytes to the blood stream by spleen contraction, possibly caused by the increase in catecholamine concentration during stress.

CONCLUSIONS

Tambatinga is more susceptible to stress than tambaqui, since the former presented higher cortisol and glucose concentrations in the blood circulation after the management and took longer to recover the basal physiological state for these parameters. Biometric procedures cause physiology changes in tambaqui and hybrid tambatinga, but when adopted routinely, at regular intervals, they lead to stress responses of lower magnitudes, with levels below those found in the literature.

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REFERENCES

ABREU, J.S.; URBINATI, E.C. 2006 Physiological responses of matrinxã (*Brycon amazonicus*) fed different levels of vitamin C and submitted to air exposure. *Acta Amazonica*, *36*(4): 519-524.

ABREU, J.S.; TAKAHASHI, L.S.; HOSHIBA, M.A.; URBINATI, E.C. 2009 Biological indicators of stress in pacu (*Piaractus mesopotamicus*) after capture. *Brazilian Journal of Biology*, *69*(2): 415-421.

ARAÚJO-LIMA, C.A.R.M.; GOMES, L.C. 2010 Tambaqui (*Colossoma macropomum*). *In*: BALDISSEROTTO, B.; GOMES, L.C. *Espécies nativas para piscicultura no Brasil*. Ed. Santa Maria: UFSM, p.175-204.

BARTON, B.A. 2002 Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology*, *42*(3): 517-525.

BARTON, B.A.; SCHRECK, C.B.; BARTON, L.D. 1987 Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout. *Diseases of Aquatic Organisms*, *2*: 173-185.

BOSISIO, F.; REZENDE, K.F.O.; BARBIERI, E. 2017 Alterations in the hematological parameters of Juvenile Nile Tilapia (Oreochromis niloticus) submitted to different salinities. *Pan-American Journal of Aquatic Sciences*, 12(2): 146-154.

CHAGAS, E.C.; BOIJINK, C.L.; INOUE, L.A.K.A.; ARAÚJO, L.D.; GOMES, L.C.; MORAES, F.R. 2012 Tambaqui responses to stress due to transport after feeding with β-glucan supplemented diets. *Biotemas*, *25*(4): 221-227.

EMERSON, K.; RUSSO, R.C.; LUND, R.E.; THURSTON, R.V. 1975 Aqueous ammonia equilibrium calculations: Effect of pH and temperature. *Journal of the Fisheries Research Board of Canada*, *32*(12): 2379-2383.

FAGUNDES, M.; URBINATI, E.C. 2008 Stress in pintado (*Pseudoplatystoma corruscans*) during farming procedures. *Aquaculture*, *276*(1-4): 112-119.

FEITOSA, K.C.O.; POVH, J.A.; ABREU, J.S. 2013 Physiological responses of pacu (*Piaractus mesopotamicus*) treated with homeopathic product and submitted to transport stress. *Homeopathy*, *102*(4): 268-273.

GAMPERL, A.K.; VIJAYAN, M.M.; BOUTILIER, R.G. 1994 Experimental control of stress hormone levels in fishes: techniques and applications. *Reviews in Fish Biology and Fisheries*, *4*(2): 215-255.

GOMES, L.C.; ARAUJO-LIMA, C.A.R.M.; ROUBACH, R.; URBINATI, E.C. 2003a Avaliação dos efeitos da adição de sal e da densidade no transporte de tambaqui. *Pesquisa Agropecuária Brasileira*, *38*(2): 283-290.

GOMES, L.C.; ROUBACH, R.; CAVERO, B.A.S.; PEREIRA-FILHO, M.; URBINATI, E.C. 2003b Transport of pirarucu *Arapaima gigas* juveniles in plastic bag. *Acta Amazonica*, *33*(4): 637-642.

GOMES, L.C.; ROUBACH, R.; ARAUJO -LIMA, C.A.R.M.; CHIPPARI-GOMES, A.R.; LOPES, N.P.; URBINATI, E.C. 2003c Effect of fish density during transportation on stress and mortality of juvenile tambaqui *Colossoma macropomum*. *Journal of the World Aquaculture Society, 34*(1): 76-84.

HOSEINI, S.M.; TORT, L.; RAJABIESTERABADI, M.H.A.H.; ABOLHASANI, M.H. 2016 Physiological, ionoregulatory, metabolic and imune responses of Persian sturgeon, *Acipen serpersicus* (Borodin, 1897) to stress. *Aquaculture Research*, *47*(12): 3729-3739.

HOSHIBA, M.A.; GONÇALVES, F.D.; URBINATI, E.C. 2009 Respostas fisiológicas de estresse no matrinxã (*Brycon amazonicus*) após exercício físico intenso durante a captura. *Acta Amazonica*, *39*(2): 445-452.

IBGE – INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. 2014 *Produção da Pecuária Municipal*. Rio de Janeiro, *42:* 1-39.

INOUE, L.A.K.A.; MORAES, G. 2007 *Óleo de Cravo: um anestésico alternativo para o manejo de peixes.* Embrapa Amazônia Ocidental, Manaus, 24p.

MARTINS, M.L.; MORAES, F.R.; FUJIMOTO, R.Y.; NOMURA, D.T.; FENERICK JUNIOR, J. 2002 Respostas do híbrido tambacu (P*iaractus mesopotamicus* Holmberg, 1887 macho x *Colossoma macropomum* Cuvier, 1818 fêmea) a estímulos simples ou consecutivos de captura. *Boletim do Instituto de Pesca*, *28*(2): 195-204.

McDONALD, G.; MILLIGAN, C.L. 1997 Ionic, osmotic and acid-base regulation in stress. In: IWAMA, G.W.; PICKERING, A.D.; SUMPTER, J.P.; SCHRECK, C.B. *Fish stress and health in aquaculture*. University Press, Cambridge, p.119-144.

MELO, D.C.; OLIVEIRA, D.A.A.; MELO, M.M.; JÚNIOR, D.V.; TEIXEIRA, E.A.; GUIMARÃES, S.R. 2009 Perfil protéico de tilápia nilótica chitralada (*Oreochromis niloticus),* submetida ao estresse crônico por hipóxia**.** *Arquivo Brasileiro de Medicina Veterinária e Zootecnia***,** *61*(5): 1183-1190.

MILLIGAN, C.L.; WOOD, C.M. 1987 Regulation of blood oxygen transport and red cell pHi after exhaustive activity in rainbow trout (*Salmo gairdneri*) and starry flounder (*Platichthys stellatus*). *Journal of the Experimental Biology*, *133*: 263-282.

MORGAN, J.D.; IWAMA, G.K. 1997 Measurements of stressed states in the field. In: IWAMA, G.K.; PICKERING, A.D.; SUMPTER, J.P.; SCHRECK, C.B. *Fish stress and Health in Aquaculture. Society for Experimental Biology*. Cambridge University Press, Cambridge, *62*: 247-270.

MORO, G.V.; TORATI, L.S.; LUIZ, D.B.; MATOS, F.T. 2013 Monitoramento e manejo da qualidade da água em pisciculturas. In: RODRIGUES, A.P.O.; LIMA, A.F.; ALVES, A.L.; ROSA, D.K.; TORATI, L.S.; SANTOS, V.R.V. *Piscicultura de água doce: multiplicando conhecimentos*. Embrapa Pesca e Aquicultura, Palmas, p.141-169.

OLIVEIRA-JUNIOR, A.A.; TAVARES-DIAS, M.; MARCON, J.L. 2008 Biochemical and hematological reference ranges for Amazon freshwater turtle, *Podocnemis expansa* (Reptilia: Pelomedusidae), with morphologic assessment of blood cells. *Research in Veterinary Science*, *86*(1): 146-151.

SILVA-ACUÑA, A.; GUEVARA, M. 2002 Evaluación de dos dietas comerciales sobre el crecimiento del híbrido de *Colossoma macropomum* x *Piaractus brachypomus*. *Zootecnia Tropical*, *20*(4): 449-459.

TAVARES-DIAS, M.; SANDRIM, E.F.S.; MORAES, F.R.; CARNEIRO, P.C.F. 2001 Physiological responses of "tambaqui" *Colossoma macropomum* (Characidae) to acute stress. *Boletim do Instituto de Pesca*, *27*(1): 43-48.

TAKAHASHI, L.S.; ABREU, J.S.; BILLER, J.D.; URBINATI, E.C. 2006. Efeito do ambiente pós-transporte na recuperação dos indicadores de estresse de pacus juvenis, *Piaractus mesopotamicus*. *Acta Scientiarum Animal Sciences*, *28*(4): 469-475.

TUKEY, J.W. 1953 The problem of multiple comparisons. Mimeographs, Princeton University, Princeton, N.J. 396p.

URBINATI, E.C.; ABREU, J.S.; CAMARGO, A.C.S.; PARRA, M.A.L. 2004 Loading and transport stress of juvenile matrinxã (*Brycon cephalus*, Characidae) at various densities. *Aquaculture*, *229(1-4)*: 389-400.

URBINATI, E.C.; ZANUZZO, F.S.; CRISCUOLO-URBINATI, E. 2014 Estratégias para redução do estresse e enfermidades na piscicultura. In: SOUZA, A.T.S.; LIZAMA, M.L.A.P.; TAKEMOTO, R.M. *Patologia e sanidade em ambientes aquáticos*. Massoni Gráfica e Editora, Maringá, p.185-203.

WENDELAAR BONGA, S.E. 1997 The stress response in fish. *Physiological Reviews*, *77*(3): 591-625.

WOODY, C.A.; NELSON, J.; RAMSTAD, K. 2002 Clove oil as an anaesthetic for adult sockeye salmon: field trails. *Journal of Fish Biology*, *60*(2): 340-347.