PHYSIOLOGICAL RESPONSES OF TAMBAQUI (C*OLOSSOMA MACROPOMUM*) TO STOCKING DENSITY IN INTENSIVE CULTURE

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Abstract

Early juveniles of *Colossoma macropomum* were reared at two stocking densities (1 and 3 fish/m^2) for six months. Blood samples were monthly removed for hematological (red and white series), glucose, total protein and ions (Na^+, K^+, Cl^-) determinations. The lowest growth rate appeared in fish at the highest density. However, there were no significant differences in feed conversion ratio (FCR) to 1 fish/m^2 (0.89) and 3 fish/m^2 (0.83). Hematocrit and hemoglobin concentrations of fish at high stocking density were higher when compared to low stocking density. Leukocytes, glucose, ions and total proteins showed similar responses to both treatments. The results suggest that the density of 3 fish/m^2 may be considered adequate for fish culture in ponds until the sixth month.

Introduction

Aquaculture is a recent activity in the Amazonian region, and is not yet showing a high enough yield for offsetting the decreasing of natural stocks due to the increasing demand from big cities (Graef, 1995). Several regional fish species have demonstrated an excellent potential for being cultured and used for food production, however, the lack of proper technology and scientific information has been a great obstacle for culture success.

In countries where fish culture is highly developed, the knowledge about fish physiology has been a helpful tool for proper culture development (Wedemeyer, 1996).) Stress is inevitable in intensive culture systems since animals are subjected to several handling procedures and environmental changes that may influence or have negative effects on reproduction, growing rate and susceptibility to diseases (Pickering, 1993). Beside that, the knowledge on monitoring procedures is lacking in the region.

Colossoma macropomum presents excellent characteristics for aquaculture, in addition to the large number of studies that are available from experiments that have been carried out in the past 10 years (Val & Honczaryk, 1995). *C. macropomum* is a Characiforme of the Serrasalmidae family, and is widespread throughout the Amazonian basin and Rio Orinoco.

Our objective was to evaluate the effects of stocking density on tambaqui physiological response during six months.

Material and Methods

Tambaqui specimens (5.1-10.5 g) were obtained from the fish culture station at Balbina Dam, Amazonas State Brazil. Fishes were hauled to the Aquaculture Department at INPA and kept in 50 m^2 ponds for about 3 weeks. Following this period, the specimens were stocked at a ratio of 1 and 3 fish/m^2, with two replicates for each treatment. Fish were fed twice a day with commercial ration containing 36% of crude protein.

Three 50 m^2 brick-built ponds were separated in two halves by a polyethylene screen. The ponds were marked A_1 B_1, A_2 B_2, and A_3 B_3. After the biometry of fish, 25 and 75 fish were placed in each side of the ponds marked A and B, respectively.

Dissolved oxygen, pH, temperature, electrical conductivity, total ammonia and nitrite, were monitored weekly before starting and during the whole experimental process. Temperature, pH, conductivity and dissolved oxygen were determined using electrodes. Total ammonia and nitrite concentrations were determined following Golterman et al., (1978) modified for flow injection analysis (FIA).

Blood samples and biometry from eight fish from each stocking density replicate, were taken monthly. Before having their blood collected, the fish were anaesthetized in a 0.01% 2-phenoxyethanol solution. Then, blood samples were drawn from the caudal vein into heparinized syringes, and immediately stored in ice until all analyses were made. Blood smears were made for identifying and quantifying leukocytes directly from the withdrawn blood, air-dried and stained with May Grünmwald-Giemsa. Hematocrit (Ht) was determined by micro-hematocrit centrifugation technique. The red blood cell count (RBC) was determined with a Neubauer chamber. Hemoglobin concentration (Hb) was measured by spectrophotometry with Drabkin's reagent. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined from Ht, [Hb] and RBC values. Following blood centrifugation, plasma was used for determining the glucose through the enzymatic-colorimetric method (GOD-POD), and total protein was determined by the modified biurete method and ions concentrations Na^{++}, K^{+}, Cl^{-} were analyzed by flame photometer and spectrophotometer. Feed conversion ratio (FCR) was determined using the formula: FCR= feed amount (g)/weight gain (g).

Comparisons between 1 fish/m^2 and 3 fish/m^2 groups, and within the same group, were made with the Mann-Whitney non-parametric test. Differences were considered to be significant at $p<0.05$.

Results and Discussion

Water quality in every pond, during the whole rearing period, is shown in Table 1. None of the analyzed parameters showed any statistical difference between the different ponds, and were within the limits reported for this species (Rantin & Kalinin, 1996; Affonso 1999; Sundin *et* al., 2000).

Table 1. Dissolved oxygen (O_2), temperature (^oC), pH, electrical conductivity, total ammonia and nitrite of the ponds with 1 fish/m^2 (A) and 3 fish/m^2 (B). Mean ± SEM.

Water Quality	Ponds	Sampling 1[a]	2[a]	3[a]	4[a]	5[a]	6[a]
O_2 (mg/L)	A	12±0.8	6.5±1.2	9.5±1.4*	14.9±0.6	8.8±1.2	9.6±0.9
	B	12±0.8	6.3±1.2	9.3±1.4	14.9±0.6	8.7±1.3	9.5±0.9
T (^oC)	A	31.7±0.6	27.6±0.2	29.4±0.2	30.5±0.4	31±0.8	32±0.9
	B	31.8±0.5	27.7±0.2	29.4±0.2	30.6±0.4	31±0.7	32±0.9
pH	A	6.8±0.2	7.2±0.1	6.0±0.2*	9.4±0.1	6.9±0.1	7.8±0.5
	B	6.8±0.1	7.2±0.1	6.0±0.2	9.4±0.1	7.0±0.1	7.8±0.5
Condutivity (µS/cm)	A	57.4±4.3	75.2±2.2	73.6±2.2	76.2±3.0	71±4.4	63±4.1
	B	54.4±3.4	75.2±2.2	73.0±2.0	75.5±2.7.	66±3.6	63±4.3
Ammonia (mg/L)	A	0.5±0.01	0.01±0.0	0.2±0.02	0.4±0.1	0.2±0.0	0.4±0.0
	B	0.4±0.0	0.2±0.1	0.2±0.03	0.4±0.1.	0.3±0.0	0.4±0.0
Nitrite (mg/L)	A	0.002±0	0.01±0.0	0.3±0.1	0.2±0.1	0.1±0.0	0.1±0.0
	B	0.01±0.0	0.2±0.1	0.26±0.1	0.1±0.1.	0.1±0.0	0.1±0.0

The effect of density on growth is shown in figure 1. Fish at lower density presented significantly higher length and weight values (p<0.0001). However, there were no significant differences in feed conversion ratio (FCR) between 1 fish/m^2 (0.89) and 3 fish/m^2 (0,83). No fish mortality was detected in any of the test ponds, during the whole rearing period.

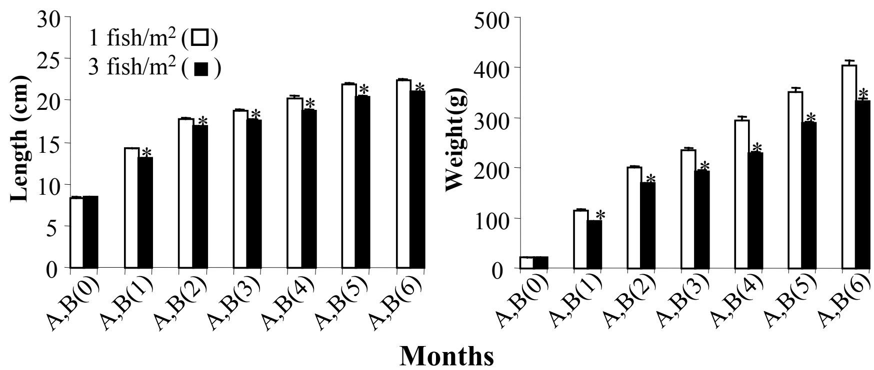

Figure 1. Growth of *Colossoma macropomum* at the start and during the whole study period. A = 1 fish/m^2 (□) and B = 3 fish/m^2 (■). * Indicates significant difference (p<0.0001). Mean ± SEM.

Hematological parameters: Ht, [Hb], RBC, VCM, HCM and CHCM, are shown in figure 2. Significant increase (p<0.05) was observed on Ht values, in the first three testing months, and on [Hb] values in the 4th month, on individuals in pond B (3 fish/m^2). The highest density fish, in the 1st month of culture, presented a significant increase (p<0.05) on CHCM. For the other parameters, the alterations on fish in pond B remained practically the same as in pond A. Although all hematological parameters (red series), had presented a similar profile for both treatments during the six months of sampling, the Ht increase in the first three months may point out a physiological response to the highest density.

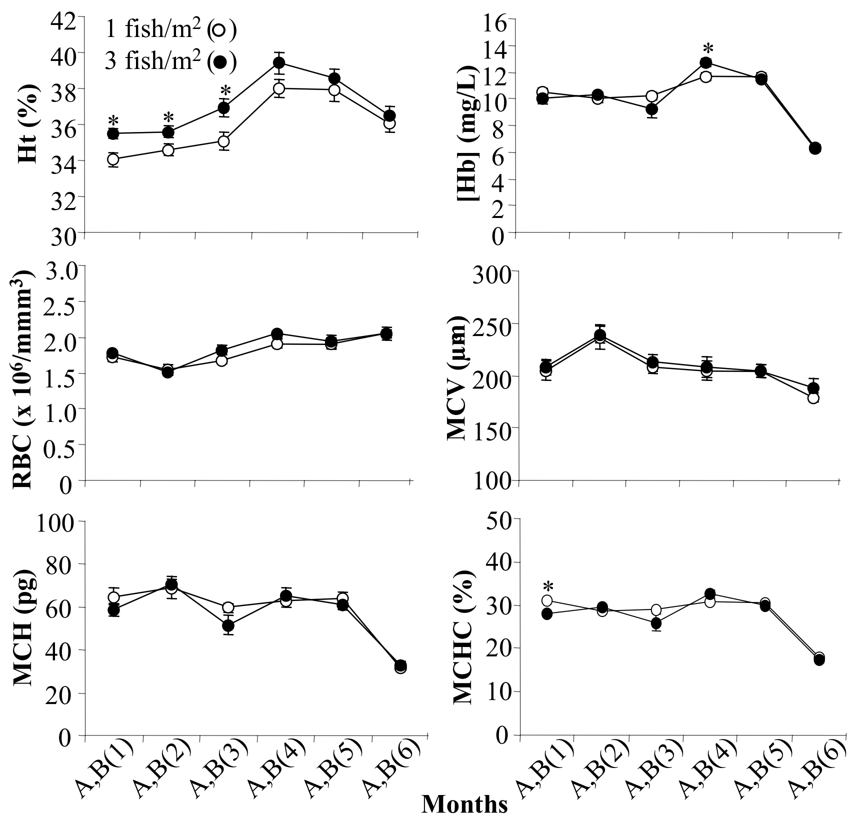

Figure 2. Hematocrit (Ht), erythrocytes (RBC), hemoglobin concentration ([Hb]), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of *Colossoma macropomum* with 1 fish/m^2 (A) and 3 fish/m^2 (B). * Indicates significant difference (p<0.05). Mean ± SEM; n=24.

Leukocytes are also good fish physiological stress indicators (Heath, 1995; Tillmann & Biron, 2000; Svobodová *et al*., 2001). The differential leukocyte counts, demonstrated significant differences (p<0.05) for the lymphocytes and eosinophils of the fish in pond A, both on the third month of culture (Table 2). Lymphocytes were the most abundant leukocyte type for tambaqui. Eosinophil count demonstrated that this is a common cell type in tambaqui. That corroborates the findings obtained by Moura *et al*. (1997) for the same species. Therefore, these results show that the stress caused by the higher stocking density was not enough for stimulating the fish immune system.

Table 2. Differential blood leukocyte counts (%) of *Colossoma macropomum* at different stocking densities for six testing months. A= 1 fish/m^2 and B= 3 fish/m^2. Mean ± SEM; n=24. * Significant differences (P<0.05)

Leukocytes	**Ponds**	**Sampling**					
		1ª	**2ª**	**3ª**	**4ª**	**5ª**	**6ª**
Lymphocytes	A	94±1.7	94±1.4	97±0.5*	95±0.6	93±1	95.±0.6
	B	97±1.3	95±0.7	94±0.8	96±0.6	93±1	89±2.1
Monocytes	A	1.9±0.8	1.6±0.2	1.0±0.0	1.1±0.1	1.3±0.2	1.5±0.5
	B	1.9±0.0	1.2±0.1	1.2±0.2	1.0±0.0	1.2±0.2	0.9±0.0
Eosinophils	A	4.5±0.8	5.1±1.1	2.4±0.3*	4±0.5	5±1.1	3.6±0.4
	B	1.5±0.5	3.7±0.6	4.3±0.6	3±0.4	5±0.9	6.9±1.5
Neutrophils	A	1.8±0.3	3.8±0.8	1.7±0.4	2±0.2	2±0.3	2.6±0.5
	B	1.5±0.5	1.7±0.3	2.0±0.5	2±0.0.	2±0.4	4.2±1.1

Ion regulation, as indicated by the values of K$^+$, Na$^+$ and Cl (Fig. 3A,B,C) and total proteins (Fig. 3D), were not affected by different stocking densities. These parameters had no significant difference during the whole study period for both treatments. In spite of the glucose levels had been significantly

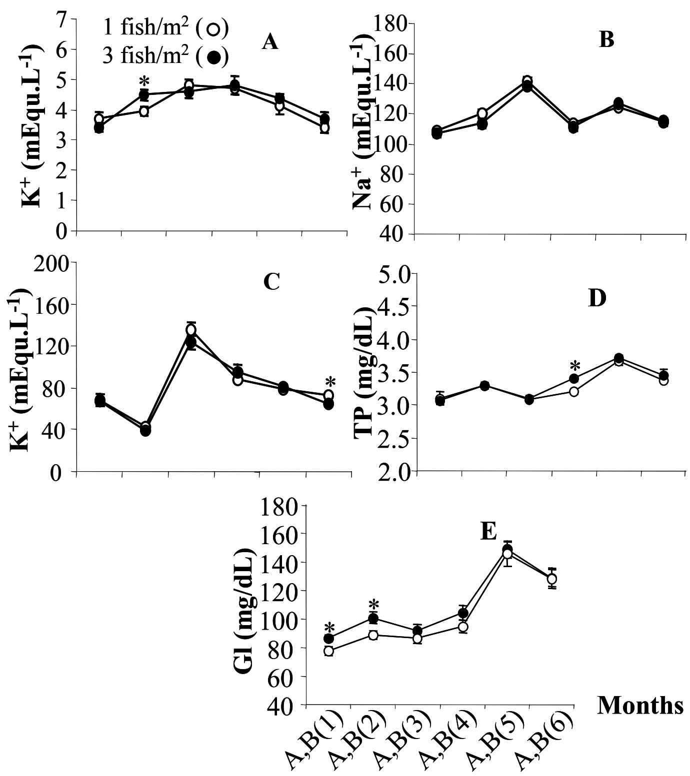

Figure 3. Potassium (A), sodium (B) chloride (C), total protein (D) and glucose (E) plasma concentrations of *C. macropomum* kept for six months in ponds with 1 fish/m^2 (A) and 3 fish/m^2 (B). * Indicates significant difference (p<0,05). Mean ± SEM; n = 24.

higher (p<0,05) on pond B fish, until the 2nd sampling month, a pronounced hyperglycemia was observed after the 5th culture month in both treatments (Fig. 3E). Hyperglycemia has been an important indicator of the production of stress hormones, such as cortisol and adrenaline, in fish under culture (Gustavenson *et al*., 1991). Only the execution of cortisol analysis in plasma samples of the animals from ponds A and B will be able to confirm whether the increase of glucose is a consequence from the increase on hormone levels in the plasma. However, it is possible to suggest that the increase on the demand of fish energy, in both treatments, was not a specific response to the stress produced by stocking density.

In conclusion, the similarity of the physiological profile and FCR of tambaqui in both treatments suggests that the density of 3 fish/m^2 may be considered to be appropriate for culture in ponds up to the sixth month.

Acknowledgements

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