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Tambaqui Production at Different Stocking Densities in RAS: Growth and Physiology

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Abstract: Stocking densities were evaluated for the rearing tambaqui in an RAS system. Experiment 1 used juveniles weighing 0.54 g at the following densities for 15 days: $D_{0.3}$—0.3; $D_{0.6}$—0.6; and $D_{0.9}$—0.9 kg m^{-3}. Experiment 2 used juveniles weighing 8.22 g at the following densities for 75 days: $D_{0.8}$—0.8; $D_{1.2}$—1.2; and $D_{1.6}$—1.6 kg m^{-3}. Experiment 3 used juveniles weighing 142.18 g at the following densities for 75 days: D_2—2; D_4—4 and D_6—6 kg m^{-3}. In Experiment 1, density did not influence performance ($p > 0.05$), with the exception of biomass, which was greatest in $D_{0.9}$ ($p < 0.05$). In Experiment 2, final weight, weight gain and daily weight gain were highest for $D_{0.8}$ ($p < 0.05$), as was triglycerides ($p < 0.05$), whereas biomass increased with increasing density ($p < 0.05$). In Experiment 3, weight, weight gain and daily weight gain were greater for D_2 and D_4 ($p < 0.05$), while final biomass was lowest for D_2 ($p < 0.05$). Hemoglobin was lower for D_4 and D_6 ($p < 0.05$), while cholesterol and glucose levels were higher for D_2 and D_6 ($p < 0.05$). *Colossoma macropomum* demonstrated adaptive capacity for reared in RAS at high stocking densities.

Keywords: biomass; stress; hematology; somatic indices

Key Contribution: *Colossoma macropomum* showed satisfactory performance when reared in RAS at different densities, indicating the species can be reared with at high stocking densities with minimal influences on physiological parameters and the maintenance of well-being and productivity.

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1. Introduction

The determination of stocking densities refers to the quantity or biomass of fish that can be efficiently produced in a given space [1]. It is important to evaluate the optimal density for optimizing the rearing of any species [2], since profitability and productivity are directly related to this aspect of management [2,3]. Fish behavior can also benefit from appropriate stocking density, such as for species that live in schools [4] and to avoid aggressiveness and the formation of individual territories [5]. Therefore, appropriate stocking density can promote aquaculture development [6].

However, stocking density is still a critical factor in properties of the aquaculture sector [7], as it can affect fish performance and feed consumption [8,9], immune and physiological responses [8,10] and animal uniformity, in addition to the economic viability of rearing [11,12]. The use of inadequate densities can trigger behavioral problems [13] and physiological changes due to stress [14,15]. As a result, energy for fish growth is diverted to stress reduction, resulting in a worsening of feed conversion [14]. In contrast, some authors have reported that increased stocking densities lead to better feed conversion rates, lower fish heterogeneity and higher total biomass [16,17], thus determining greater returns on investments in structure and equipment. Studies of some species, such as the Asian sea

bass (*Lates calcarifer*) [18] and the largemouth bass (*Micropterus salmoides*) [9], have shown that low densities provide better growth rates. Thus, there is an evident need for studies on stocking densities for different species.

Another obstacle to the use of high densities is the worsening of water quality for rearing, which increases mortality and reduces growth [19]. However, this worsening of water quality can be minimized in a recirculating aquaculture system (RAS), since one of its greatest advantages is water quality control and waste recycling [20].

Colossoma macropomum, a native species of the Amazon Basin [21], is the second most produced fish species in Brazil [22] due to its favorable rearing characteristics [23]. Recent studies have shown that the species offers adaptability to RAS in different growth phases [10,12,24–26], allowing the expansion of its rearing to non-endemic territories and the intensification of its production in regions where the climate is not entirely favorable. However, there is still a lack of information for some weight ranges to close the production cycle of this species in an RAS and possibly further intensify production in relation to the existing literature.

Therefore, the objective of the study was to evaluate the effects of stocking densities in three phases of tambaqui rearing in an RAS on growth and physiology.

2. Material and Methods

Three studies were conducted at the Laboratório de Aquacultura of the Universidade Federal de Minas Gerais (Brazil), with all procedures previously authorized by the Comissão de Ética no Uso de Animais (CEUA/UFMG-n° 137/2023).

2.1. Experiment 1

This experiment used 7200 *C. macropomum* juveniles 45 days post hatching, with an average weight of 0.54 ± 0.19 g and an average length of 3.28 ± 0.42 cm, distributed in nine tanks in an individual RAS. To assemble the RAS, nine blue circular tanks (115 × 115 × 76 cm) made of polyethylene were used, with a total volume of 1 m^3 and 0.8 m^3 of useful volume. Each tank was equipped with an "air-lift" system connected to a blower (2 CV), an 80 L biological filter, where crushed stone was used as substrate, and a mechanical filter containing acrylic wool, which was cleaned weekly. The "air-lift" provided an average flow of 0.89 m^3 h^{-1}, filtering the volume approximately 27 times a day, with the water outlet positioned in the lower part of the tank and water inlet through piping positioned in the upper part. Each system also had water heating (200-watt heater) and supplementary aeration. The photoperiod was 12 h of light (Key West DNI group, digital timer). Three stocking densities were tested, with three replicates each: $D_{0.3}$—0.3 kg m^{-3} (400-fish $tank^{-1}$); $D_{0.6}$—0.6 kg m^{-3} (800-fish $tank^{-1}$); and $D_{0.9}$—0.9 kg m^{-3} (1200-fish $tank^{-1}$). This experiment lasted 15 days. During the entire experimental period, no water changes were made in the tanks. Due to the accelerated growth of the animals, the RAS filters were not efficient in cycling ammonia, making it necessary to reduce the storage density of the tanks, ending the first phase of the experiment.

The animals were fed an extruded commercial feed (1.3–1.5 mm in diameter) from the Wean Prime line (Total Rações®, Três Corações, Brazil), with 45% crude protein, 12% moisture, 5% ether extract, 15% mineral matter (max.) and 4% crude fiber (max.) (manufacturer data). Feed was offered twice a day (09:00 and 15:00) until apparent satiety. Leftover feed was collected and dried to calculate feed consumption and conversion.

2.2. Experiment 2

This experiment used 1080 60-day-old *C. macropomum* juveniles with an average weight of 8.22 ± 3.37 g and an average length of 7.72 ± 0.90 cm. The animals were distributed in the same tanks described in experiment 1 but at three different stocking densities, with three replicates each: $D_{0.8}$—0.8 kg m^{-3} (80-fish $tank^{-1}$); $D_{1.2}$—1.2 kg m^{-3} (120-fish $tank^{-1}$); and $D_{1.6}$—1.6 kg m^{-3} (160-fish $tank^{-1}$). This experiment lasted 75 days. During the second experiment, no water changes were made in the first 21 days; between days 22 and 28 of

the experiment, 10% of the volume of each tank was changed once a week. Between days 29 and 56, 10% of each tank was changed twice a week and from day 57 until the end of the experiment, and 10% of the volume was changed three times a day.

During the first 15 days, the animals were fed an extruded commercial feed (2–3 mm in diameter) from the Aquos Alevinos 45 line (Total Rações®), with 45% crude protein, 12% moisture, 8% ether extract, 15% mineral matter and 4% crude fiber (manufacturer's data). Between days 16 and 30, the animals were fed an extruded commercial feed (2–3 mm) from the Pirá Evolution Juvenil line (Guabi®), with 40% crude protein, 10% moisture, 8% ether extract, 15% mineral matter and 5% crude fiber (manufacturer data). Between days 31 and 45, the animals were fed an extruded commercial feed (3–4 mm) from the Aquos Starter line (Total Rações®), with 36% crude protein, 12% moisture, 7% ether extract, 14% mineral matter and 5% crude fiber (manufacturer data). Finally, from day 46 until the end of the experiment, the animals were fed an extruded commercial feed (4–6 mm) also from the Aquos Starter line (Total Rações®), with the same formulation as that of the previously described feed. Food was fed until apparent satiety at two times per day. Leftover feed was collected and dried to calculate feed consumption and conversion.

2.3. Experiment 3

This experiment used 270 135-day-old *C. macropomum* juveniles with an average weight of 142.18 ± 5.94 g and an average length of 19.40 ± 1.04 cm. The same system as in experiments 1 and 2 was used. Three different stocking densities were tested, with three replicates each: D_2—2 kg m^{-3} (15-fish $tank^{-1}$); D_4—4 kg m^{-3} (30-fish $tank^{-1}$); and D_6—6 kg m^{-3} (45-fish $tank^{-1}$). This experiment lasted 75 days. During the first 30 days of experiment 3, no water changes were made; between days 31 and 45, 10% of the volume was changed once a week; between days 46 and 60, 10% of the volume was changed twice a week; and from day 61 until the end of experiment 3, 10% of the volume was changed three times a week.

During the first 30 days, the animals were fed an extruded commercial feed (4–6 mm in diameter) from the Aquos Starter line (Total Rações®), with the same formulations described in experiment 2. From day 31 until the end of the experiment, the animals were fed an extruded commercial feed (6–8 mm) from the Aquos Starter line (Total Rações®), with 32% crude protein, 12% moisture, 6% ether extract, 12% of mineral matter and 5.5% crude fiber (manufacturer's data). Food was fed until apparent satiety at two times per day. Leftover feed was collected and dried to calculate feed consumption and conversion.

2.4. Water Quality Analysis and Management

Water temperature (Hanna Instruments HI98130 multiparametric probe, Hanna®, Barueri, SP, Brazil), total ammonia (LabconTest Alcon® colorimetric kit, Camburiú, SC, Brazil) and dissolved oxygen (DO; YSI multiparametric probe, EcoSense® DO200A, Yellow Springs Instruments Co., Inc., Yellow Springs, OH, USA) were measured three times a week in each experiment, while pH (Hanna Instruments HI98130 multiparametric probe) was measured once a week.

Excess organic matter was cleaned from each tank once a week by siphoning and renewing 20% of the total water volume.

Experiments 2 and 3 were carried out during the autumn and winter seasons when, even with controlled use of heaters inside the laboratory, it was not possible to maintain the temperature above 29 °C, as for Experiment 1.

2.5. Growth and Survival

The biometric management of all fish and the counting of individuals were carried out at the end of each experiment. Weight was determined for previously anesthetized (20 mg L^{-1} eugenol; [27]) juveniles using a Wellmix (82.674/wx502) 10 kg digital scale, while total length was determined using an ichthyometer (0.1 cm accuracy). The obtained data were used to determine the following:

- Final weight (g) (FW);
- Final length (cm) (FL);
- Weight gain (g) (WG) = FW − IW;
- Daily weight gain (g) (DWG) = (FW − IW)/ΔT;
- Daily specific growth rate (% day^{-1}) (SGR) = 100 (lnFW − lnIW)/ΔT.

In the formula, ΔT represents the duration of the experiment. FW is the final weight and IW is the initial weight.

- Final biomass (FB) (kg);
- Fulton Condition Factor (Fk) = WG FL^{-3};
- Food consumption (kg) = (offered food (g) − dry leftover food (g))/100;
- Apparent food conversion (FCR) = consumption/(final biomass − initial biomass);
- Survival (%) = (number of fish alive at the end of the experiment × 100)/initial number of fish.

2.6. Hematological and Biochemical Analyses

At the end of experiments 2 and 3, the animals remained fasting for 24 h. A total of 5 fish per tank (n = 15 fish per treatment) were anesthetized with 50 mg L^{-1} of eugenol [28], wrapped in a wet cloth and subjected to blood collection by caudal venipuncture with previously heparinized 3 mL syringes. Blood samples were subsequently dispensed into microtubes containing sodium heparin anticoagulant (10%) to determine hemoglobin using a commercial colorimetric kit (Quibasa-Bioclin, Belo Horizonte, MG, Brazil) and hematocrit using the microhematocrit method [29]. Total plasma protein was determined after breaking the microhematocrit tube, where the plasma was placed in an analog refractometer (0 to 90% Brix-RHB0-90) for quantification. The erythrocyte (RBC) number was determined by diluting 10 µL of whole blood in 2 mL of formaldehyde citrate and then by counting in a Neubauer Chamber.

The remainder of the blood was centrifuged (4000 RPM for 10 min) and the plasma was used to determine glucose, triglycerides (TG), total cholesterol (TC), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). During the analyses, we used a colorimetric method and commercial kits (Quibasa-Bioclin, Belo Horizonte, MG, Brazil), with readings taken on a spectrophotometer (Biochrom Libra S22 UV-VIS spectrophotometer, Biochrom Instruments, Cambridge, UK).

The following hematimetric indices were calculated: the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) [30].

2.7. Viscerosomatic, Hepatosomatic and Visceral Fat Indices

At the end of Experiments 2 and 3, the same 15 animals from each treatment used in the previous blood analyzes were euthanized using 285 mg L^{-1} of eugenol [31]. The total viscera, liver and visceral fat of each fish were collected for weighing and the subsequent calculations of the following indices:

- Viscerosomatic index (VIS) = (total viscera (g)/live weight (g)) × 100;
- Visceral fat index (VFI) = (intraperitoneal fat (g)/body weight (g)) × 100;
- Hepatosomatic index (HSI) = (liver (g)/live weight (g)) × 100.

2.8. Statistical Analysis

Data from the three experiments were tested for normality (Shapiro–Wilk) of errors and homoscedasticity (Levene's). After the assumption tests, a one-way ANOVA was performed and the means were compared using the Tukey 5% significance test.

3. Results

In experiment 1, only dissolved oxygen was affected by density, being significantly lower for $D_{0.9}$ ($p < 0.05$) (Table 1). In experiment 2, ammonia was affected, being significantly

higher for $D_{1.6}$ ($p < 0.05$). The other parameters were not affected by the densities tested in the different experiments.

Table 1. Physicochemical variables of water (mean ± standard deviation) for the three experiments testing different stocking densities in the rearing tambaqui (*C. macropomum*) in RAS.

Stocking Densities (kg m^{-3})	NH_3 (mg L^{-1})	pH	Dissolved Oxygen (mg L^{-1})	Temperature (°C)
		Experiment 1		
$D_{0.3}$	0.007 ± 0.0006	7.57 ± 0.06	4.49 ± 0.20 [a]	29.32 ± 0.30
$D_{0.6}$	0.041 ± 0.0293	7.57 ± 0.06	4.23 ± 0.18 [ab]	29.31 ± 0.09
$D_{0.9}$	0.041 ± 0.0249	7.55 ± 0.05	3.82 ± 0.22 [b]	29.49 ± 0.24
p-value	0.1778	0.4908	0.0164	0.5714
		Experiment 2		
$D_{0.8}$	0.010 ± 0.0047 [b]	7.57 ± 0.09	4.17 ± 0.62	26.55 ± 0.08
$D_{1.2}$	0.015 ± 0.0022 [b]	7.53 ± 0.09	4.56 ± 0.11	26.25 ± 0.20
$D_{1.6}$	0.042 ± 0.0046 [a]	7.53 ± 0.06	3.70 ± 0.39	26.56 ± 0.07
p-value	<0.0001	0.8424	0.0945	0.0547
		Experiment 3		
D_2	0.006 ± 0.0037	7.30 ± 0.03	6.30 ± 0.69	26.01 ± 0.32
D_4	0.007 ± 0.0038	7.27 ± 0.03	5.16 ± 0.30	25.95 ± 0.10
D_6	0.014 ± 0.0071	7.26 ± 0.04	4.67 ± 0.88	26.14 ± 0.21
p-value	0.1929	0.3396	0.0597	0.5939

Means in the same column followed by different letters differed significantly when using Tukey's test ($p < 0.05$). The ammonia variable of experiment 2 was transformed using the logarithmic function (y = log (x) + 1).

3.1. Experiment 1

The performances of *C. macropomum* juveniles was not influenced by the stocking density used ($p > 0.05$), except for the final biomass, which increased as the density increased ($p < 0.05$) (Table 2).

Table 2. Growth parameters (mean ± standard deviation) of tambaqui (*C. macropomum*) juveniles reared at different stocking densities for 15 days in an RAS (experiment 1).

Parameters	Stocking Densities (kg m^{-3})			*p*-Value
	$D_{0.3}$	$D_{0.6}$	$D_{0.9}$	
Final weight (g)	5.51 ± 0.23	5.23 ± 0.08	5.13 ± 0.34	0.6234
Final length (cm)	6.97 ± 0.20	6.66 ± 0.47	6.69 ± 0.15	0.4077
Weight gain (g)	4.96 ± 0.23	4.68 ± 0.85	4.58 ± 0.34	0.6234
DWG (g day^{-1})	0.33 ± 0.01	0.31 ± 0.05	0.31 ± 0.02	0.6234
SGR (% day^{-1})	15.39 ± 0.29	15.00 ± 1.09	14.91 ± 0.45	0.619
Fulton conversion factor	1.16 ± 0.17	1.77 ± 0.09	1.71 ± 0.09	0.5568
Final biomass (kg m^{-3})	2.94 ± 0.10 [c]	4.83 ± 0.45 [b]	7.20 ± 0.88 [a]	0.0009
Food consumption (kg)	2.12 ± 0.07	3.54 ± 1.33	3.65 ± 0.30	0.0599
Feed conversion rate	1.13 ± 0.58	0.90 ± 0.25	0.64 ± 0.11	0.3769
Survival (%)	99.50 ± 0.50	96.69 ± 2.56	95.78 ± 5.97	0.5365

Means in the same row followed by different letters differed significantly when using Tukey's test ($p < 0.05$). DWG—daily weight gain; SGR—daily specific growth rate.

3.2. Experiment 2

The final length, Fulton condition factor, feed conversion and survival did not differ significantly between the densities tested ($p > 0.05$) (Table 3). The final weight, weight gain and daily weight gain in $D_{0.8}$ were highest ($p < 0.05$), while SGR was lowest in $D_{1.6}$ and highest in $D_{0.8}$ ($p < 0.05$). The feed consumption was lowest for $D_{0.8}$ and highest for $D_{1.6}$ ($p < 0.05$). The final biomass increased with increasing density ($p < 0.05$).

Table 3. Growth parameters (mean ± standard deviation) of tambaqui (*C. macropomum*) juveniles reared at different stocking densities for 75 days in RAS (experiment 2).

Parameters	Stocking Densities (kg m^{-3})			*p*-Value
	$D_{0.8}$	$D_{1.2}$	$D_{1.6}$	
Final weight (g)	117.48 ± 7.80 [a]	100.66 ± 5.57 [b]	90.28 ± 3.05 [b]	0.0035
Final length (cm)	17.30 ± 0.83	17.01 ± 0.60	16.53 ± 0.69	0.4576
Weight gain (g)	109.25 ± 7.80 [a]	92.43 ± 5.57 [b]	82.05 ± 3.05 [b]	0.0035
DWG (g day^{-1})	1.45 ± 0.10 [a]	1.23 ± 0.07 [b]	1.09 ± 0.04 [b]	0.0035
SGR (% day^{-1})	3.54 ± 0.08 [a]	3.33 ± 0.07 [b]	3.19 ± 0.04 [c]	0.0029
Fulton conversion factor	2.27 ± 0.20	2.05 ± 0.20	2.01 ± 0.22	0.3353
Final biomass (kg m^{-3})	11.99 ± 0.90 [c]	14.95 ± 0.58 [b]	17.62 ± 0.73 [a]	0.0011
Food consumption (kg)	9.94 ± 0.24 [b]	12.30 ± 1.59 [ab]	13.29 ± 0.24 [a]	0.0138
Feed conversion rate	0.89 ± 0.04	0.89 ± 0.10	0.83 ± 0.09	0.6522
Survival (%)	98.75 ± 1.25	97.22 ± 2.67	95.83 ± 2.95	0.3926

Means in the same row followed by different letters differed significantly when using Tukey's test ($p < 0.05$). DWG—daily weight gain; SGR—daily specific growth rate.

The VSI was reduced at $D_{1.6}$ and increased at $D_{0.8}$ ($p < 0.05$) (Table 4), while the HSI was increased at $D_{1.6}$ ($p < 0.05$). The VFI was not affected by stocking densities (Table 4) ($p > 0.05$).

Table 4. Somatic indices (mean ± standard deviation) for tambaqui (*C. macropomum*) juveniles reared at different stocking densities for 75 days in the RAS (experiment 2).

Indexes (%)	Stocking Densities (kg m^{-3})			*p*-Value
	$D_{0.8}$	$D_{1.2}$	$D_{1.6}$	
VSI	4.42 ± 0.59 [a]	4.12 ± 0.67 [ab]	3.86 ± 0.43 [b]	0.0454
HSI	1.58 ± 0.43 [ab]	1.40 ± 0.22 [b]	1.78 ± 0.29 [a]	0.013
VFI	1.57 ± 0.75	1.08 ± 0.33	1.37 ± 0.41	0.0911

Means in the same row followed by different letters differed significantly when using Tukey's test ($p < 0.05$). VSI—viscerosomatic index; HSI—hepatosomatic index; VFI—visceral fat index.

TG was highest for $D_{0.8}$ ($p < 0.05$), while MCH was highest for $D_{1.6}$ ($p < 0.05$) (Table 5). The other parameters did not differ significantly among treatments ($p > 0.05$).

Table 5. Physiological parameters (mean ± standard deviation) for tambaqui (*C. macropomum*) juveniles reared at different stocking densities for 75 days in the RAS (experiment 2).

Parameters	Stocking Densities (kg m^{-3})			*p*-Value
	$D_{0.8}$	$D_{1.2}$	$D_{1.6}$	
Hematocrit (%)	23.93 ± 2.40	25.27 ± 2.81	24.21 ± 2.69	0.3569
TPP (g dL^{-1})	5.43 ± 0.31	5.47 ± 0.28	5.46 ± 0.31	0.9328
Hemoglobin (g dL^{-1})	6.98 ± 1.81	7.81 ± 1.80	7.61 ± 1.93	0.3539
Glucose (mg dL^{-1})	55.48 ± 8.68	60.64 ± 11.40	63.32 ± 11.40	0.1311
TC (mg dL^{-1})	112.39 ± 19.97	116.85 ± 15.22	121.79 ± 16.29	0.3386
TG (mg dL^{-1})	501.65 ± 91.62 [a]	401.24 ± 116.12 [b]	378.35 ± 74.22 [b]	0.0022
ALT (UI L^{-1})	9.22 ± 2.15	7.21 ± 2.35	8.28 ± 2.03	0.0558
AST (UI L^{-1})	21.11 ± 4.39	19.13 ± 22.29	19.25 ± 24.05	0.4726
RBC ($\times 10^6$ µL^{-1})	1.05 ± 0.18	1.07 ± 0.23	1.04 ± 0.20	0.9523
MCV (ftl)	236.12 ± 40.50	239.46 ± 60.02	239.53 ± 46.21	0.9811
MCH (pg)	52.88 ± 4.82 [b]	68.47 ± 19.01 [ab]	75.55 ± 24.95 [a]	0.0312
MCHC (g dL^{-1})	27.06 ± 4.58	29.66 ± 5.12	31.66 ± 6.83	0.1167

Means in the same row followed by different letters differed significantly when using Tukey's test ($p < 0.05$). The MCH variable was transformed using the inverse root function (Y = 1/root (x)). TC—total cholesterol; TG—triglycerides; ALT—alanine aminotransferase; AST—aspartate aminotransferase; MCV—mean corpuscular volume; MCH—mean corpuscular hemoglobin; MCHC—mean corpuscular hemoglobin concentration; RBC—erythrocyte number.

3.3. Experiment 3

The variables of the final weight, weight gain, DWG and SGR were lower for D_6 ($p < 0.05$) (Table 6). The final length was higher for D_2 and lower for D_6 ($p < 0.05$), while the final biomass did not differ between D_4 and D_6 and was lower for D_2 ($p < 0.05$). The Fulton condition factor and food consumption, apparent feed conversion and survival were not affected by density ($p > 0.05$).

Table 6. Growth parameters (mean ± standard deviation) of tambaqui (*C. macropomum*) juveniles reared at different stocking densities for 75 days in the RAS (experiment 3).

Parameters	Stocking Densities (kg m^{-3})			*p*-Value
	D_2	D_4	D_6	
Final weight (g)	458,42 ± 84.25 [a]	405.88 ± 8.48 [a]	296.64 ± 2.87 [b]	0.0019
Final length (cm)	27.62 ± 1.63 [a]	26.65 ± 0.25 [ab]	24.57 ± 0.075 [b]	0.0205
Weight gain (g)	311.69 ± 84.47 [a]	265.37 ± 8.82 [a]	161.10 ± 2.79 [b]	0.0016
DWG (g day^{-1})	4.15 ± 1.12 [a]	3.53 ± 0.11 [a]	2.14 ± 0.037 [b]	0.0016
SGR (% day^{-1})	1.50 ± 0.26 [a]	1.41 ± 0.03 [a]	1.04 ± 0.01 [b]	0.0027
Fulton conversion factor	2.15 ± 0.10	2.14 ± 0.05	2.00 ± 0.02	0.0498
Final biomass (kg m^{-3})	8.32 ± 1.69 [b]	14.71 ± 0.65 [a]	16.91 ± 0.28 [a]	0.0002
Food consumption (kg)	12.10 ± 1.63	10.78 ± 1.68	12.65 ± 1.83	0.7099
Feed conversion rate	2.36 ± 0.93	1.14 ± 0.14	1.36 ± 0.16	0.0745
Survival (%)	95.56 ± 3.84	94.44 ± 1.92	97.04 ± 3.39	0.6261

Means in the same row followed by different letters differed significantly when using Tukey's test ($p < 0.05$). The variables weight and specific growth rate were transformed using the inverse function $Y = (1/(x^2))$, while the variables weight gain and daily weight gain were transformed using the inverse function $Y = (1/x)$. DWG—daily weight gain; SGR—daily specific growth rate.

Different stocking densities did not influence the somatic indices ($p > 0.05$) (Table 7).

Table 7. Somatic indices (mean ± standard deviation) for tambaqui (*C. macropomum*) juveniles reared at different stocking densities for 75 days in the RAS (experiment 3).

Indexes (%)	Stocking Densities (kg m^{-3})			*p*-Value
	D_2	D_4	D_6	
VSI	8.10 ± 2.41	8.94 ± 1.48	8.42 ± 2.64	0.622
HSI	1.60 ± 0.53	1.55 ± 0.56	1.55 ± 0.50	0.9516
VFI	2.81 ± 0.97	3.01 ± 0.93	3.39 ± 0.34	0.3652

VSI—viscerosomatic index; HSI—hepatosomatic index; VFI—visceral fat index.

Hemoglobin was lower in D_4 and D_6 ($p < 0.05$) (Table 8). Glucose and TC were increased in D_2 and D_6 ($p < 0.05$). MCH was higher in D_4 ($p < 0.05$), while MCHC was increased in D_2 ($p < 0.05$). The other parameters were not affected by density rates ($p > 0.05$).

Table 8. Physiological parameters (mean ± standard deviation) for tambaqui (*C. macropomum*) juveniles reared at different stocking densities for 75 days in the RAS (experiment 3).

Parameters	Stocking Densities (kg m^{-3})			*p*-Value
	D_2	D_4	D_6	
Hematocrit (%)	23.67 ± 2.01	22.86 ± 2.41	24.75 ± 1.65	0.0816
TPP (g dL^{-1})	5.20 ± 0.35	5.16 ± 0.33	5.20 ± 0.25	0.878
Hemoglobin (g dL^{-1})	7.19 ± 0.61 [a]	6.34 ± 0.93 [b]	6.19 ± 0.67 [b]	0.002
Glucose (mg dL^{-1})	70.00 ± 16.58 [a]	56.79 ± 10.99 [b]	76.64 ± 14.93 [a]	0.004
TC (mg dL^{-1})	117.16 ± 19.57 [a]	89.96 ± 10.46 [b]	109.78 ± 20.97 [a]	0.0039
TG (mg dL^{-1})	321.23 ± 48.88	342.99 ± 60.74	330.85 ± 51.43	0.5955
ALT (UI L^{-1})	20.57 ± 5.61	19.92 ± 4.71	19.27 ± 4.54	0.7907
AST (UI L^{-1})	32.13 ± 10.49	35.80 ± 6.89	26.77 ± 10.86	0.0982

Table 8. *Cont.*

Parameters	Stocking Densities (kg m^{-3})			*p*-Value
	D_2	D_4	D_6	
RBC ($\times 10^6$ μL^{-1})	1.18 ± 0.30	1.04 ± 0.21	1.28 ± 0.35	0.1375
MCV (ftl)	218.38 ± 57.89	223.84 ± 37.41	202.93 ± 36.37	0.5612
MCH (pg)	59.50 ± 10.74 [ab]	62.43 ± 12.43 [a]	50.82 ± 11.74 [b]	0.0414
MCHC (g dL^{-1})	30.44 ± 3.70 [a]	26.74 ± 2.54 [b]	24.17 ± 3.57 [b]	0.0001

Means in the same row followed by different letters differed significantly when using Tukey's test ($p < 0.05$). TC—total cholesterol; TG—triglycerides; ALT—alanine aminotransferase; AST—aspartate aminotransferase; MCV—mean corpuscular volume; MCH—mean corpuscular hemoglobin; MCHC—mean corpuscular hemoglobin concentration; RBC—erythrocyte number.

4. Discussion

The tambaqui (*C. macropomum*) demonstrated easy adaptation in the RAS, in accordance with previous studies [10,12,24–27]. The greater than 90% survival in the three experiments, the growth observed for all the tested stocking densities and the low interference with somatic, physiological and water quality parameters evidence the successful production of *C. macropomum* in the RAS.

The RAS used was efficient at maintaining the physical and chemical parameters of the water in the different experiments. In experiment 1, only dissolved oxygen was lower at the highest density ($D_{0.9}$). Similar results were observed for *C. macropomum* subjected to four different stocking densities [32]. Dissolved oxygen was also reduced with an increasing density of African catfish, *Clarias garienpinus* [3]. *Colossoma macropomum* has anatomical adaptations on its lips that provide good resistance to dissolved oxygen levels between 3 and 1 mg L^{-1} [33–36]. The lowest oxygen levels in the present study were above 3 mg L^{-1} and the animals showed growth. In experiment 2, ammonia was highest for the highest density tested ($D_{1.6}$). An increased ammonia concentration at higher densities was also recorded for *C. macropomum* larviculture in an RAS [12]. Nonetheless, the values recorded here are within the tolerance levels of the species [35,37,38]. Although temperature was lower in experiments 2 and 3 than in experiment 1, the animals still showed growth, with daily weight gain. There are records of the rearing of this species in waters with temperatures between 25 and 34 °C [39–41], reinforcing the possibility for *C. macropomum* rearing in regions where the winters are colder than in its region of origin, when kept in an RAS.

The different densities did not affect juvenile performance in experiment 1. In experiment 2, the final weight, weight gain and DWG were greater for the lowest density tested ($D_{0.8}$), while the SGR and feed intake were increased for $D_{0.8}$ and reduced for $D_{1.6}$. In experiment 3, the weight, weight gain, daily weight gain, SGR and length were highest for the lower densities (D_2 and D_4). Available space is a determining factor for the development of fish, which may explain the worse performance observed at higher densities in experiment 2 ($D_{1.2}$ and $D_{1.6}$) and experiment 3 ($_{D6}$). High stocking densities can cause stressful situations, stagnating development [42], and the energy demand caused by competition for food due to high density also interferes with animal weight gain [10,43]. Similar results were previously observed for *C. macropomum* at different growth phases [10]. Other work with the European catfish, *Silurus glanis* [44], and the Asian sea bass, *Lates calcarifer* [45], also reported similar results. However, during the larviculture of *C. macropomum*, the variables of weight, length and SGR did not show differences between the densities tested [12]. Likewise, juveniles (0.35 g) of *C. macropomum* did not demonstrate differences in the same parameters as juveniles after the first 15 days, compared to the different storage densities tested [26]. There is clearly a need to evaluate different densities during different growth phases.

Feed conversion ranged between 0.6 and 1.1 in experiment 1, averaged 0.8 in experiment 2 and ranged between 1.1 and 2.3 in experiment 3, but without significant differences among the tested densities. Feed conversion can be influenced by environmental parame-

ters and by species, animal size and activity level [46]. Contrasting results are found in the literature regarding feed conversion for *C. macropomum*. The feed conversion by juvenile *C. macropomum* with an initial weight of 34.88 g was influenced by the three densities to which they were subjected (1.28, 1.15, and 1.14) during the first 30 days; however, this influence was not observed after 30 days [10]. Stocking densities indirectly influenced the food conversion (1.2 to 0.5) of *C. macropomum* juveniles, also kept in an RAS, up to the minimum point found of 3.20 fish L^{-1}, increasing food conversion from then [26]. When subjected to different stocking densities, the hybrid species tambatinga (*C. macropomum* × *Piaratcus brachypomus*) did exhibit differences in feed conversion (1.08, 1.14, 1.16) [1], with values similar to those obtained in the present study for *C. macropomum*.

The biomass produced was greater for the highest density tested in all the experiments of the present study. Biomass can be used as a parameter to analyze productivity [47]. Even though fish growth was affected by density, the high survival justifies the production of *C. macropomum* at high stocking densities to improve productivity through the final biomass produced. As in the present study, *C. macropomum* in different growth phases raised in an RAS [10,26], the hybrid tambatinga (*C. macropomum* × *P. brachypomus*) [1], sole (*Paralichthys olivaceus*) [48], the European catfish *S. glanis* [49], and larvae of *Acipenser ruthenus* [50] also showed higher biomass at higher densities.

It is also worth highlighting the high survival rates of the present study, being above 94% for all three experiments and with no negative effects of the tested densities. Stocking density is directly related to animal survival. The high survival rate obtained for all the densities tested in the experiments highlights the favorable adaptation of *C. macropomum* to density. These results corroborate the high survival rates found in other studies with this species [10,26,27,32].

In experiment 2, the VSI was increased by the lowest density ($D_{0.8}$) and reduced by $D_{1.6}$ ($p < 0.05$), while the HSI was greater for $D_{1.6}$. In experiment 3, the somatic indices were not influenced by the tested densities. Somatic indices are related to animal weight. The HSI is related to the fish nutritional level and growth rate [51], indicating an accumulation of glycogen in tissue as an energy reserve [52]. Increased VSI and HSI values may indicate an increase in fat deposits in the intestine and liver, respectively. However, the juvenile *C. macropomum* had their highest HSI levels at the lowest densities in the first two phases of the study, after the juveniles reached weights of 189.06 (Phase 1) and 521.67 g (Phase 2) and at the highest density during Phase 3 (729.44 g). Conversely, VFI values were highest at the highest densities of Phase 1 (149.24 g) and Phase 2 (426.67 g) and at the lowest density of Phase 3 (1129.13 g) [10], while the present study found no differences for this index.

The increase in TG at $D_{0.8}$ in experiment 2, and glucose and TC at D_2 and D_6 in experiment 3 ($p < 0.05$), may be related to energy metabolism through glycolytic pathways [53], increasing the availability of energy to deal with stressful situations such as high population density. Triglycerides are important substrates under conditions of high stocking density [54]. A study of stocking density in different phases found no standard response, and also found that this varies depending on the tested density and the size of the animals [10]. The catfish *Ictalurus punctatus* also had increased triglycerides with high density [8].

Higher glucose levels were found at the highest densities tested for the catfish *I. punctatus* [8]. However, an increase in glucose was observed at D_2 and D_6, reinforcing what was previously discussed about no response pattern for *C. macropomum* [10]. A reduction in glucose levels with increasing density was reported for *Anguilla marmorata* [55], similar to that found for juvenile sea bream, *Megalobrama amblycephala* [54].

No changes were found in the TC levels for *C. macropomum* in the first growth phases [10]. However, these authors found that denser fish with an average weight of 729.44 exhibited an increase in cholesterol. The same was found for the catfish *I. punctatus* [8]. The results recorded here for D_6 corroborate these findings.

Hematological indices are used to analyze the physiological state, health, stress levels and the development of possible diseases [56], and can indicate the nutritional status

and adaptation capacity of fish to the rearing environment [57]. The increases in HCM for $D_{1.6}$ of experiment 2 and D_4 of experiment 3, as well as in hemoglobin and MCHC for D_2 of experiment 3, may indicate an increase in the need for oxygen caused by the increase in stocking density. Juveniles of *C. macropomum,* with an average weight of 108.94 g, suffered increases in hemoglobin at higher densities [10]. The results of the present study corroborate the increases in these same rates at high stocking densities recorded for the European catfish *S. glanis* [44]. These different results in hematology and blood biochemistry indicate no pattern of interference by stocking density. However, these changes need to be better evaluated as fish grow to establish reference values for each species in the different growth phases.

The Fulton condition factor can be used to evaluate the physiological well-being of fish, as well as the affinity of the species with the rearing environment [58]. When values are greater than 1, they indicate good general adaptation by fish [59]. The present study found no differences in the condition factor among the densities tested in the respective experiments, with this value ranging between 1.16 and 1.77 in experiment 1, 2.01 and 2.27 in experiment 2 and 2.00 and 2.15 in experiment 3. These results indicate that the animals adapted satisfactorily to the densities used.

5. Conclusions

Colossoma macropomum showed excellent adaptation to the RAS. Lower densities provided greater weight gain for the fish in experiment 2 and in experiment 3. However, higher densities were able to produce greater biomass in all experiments. The Fulton condition factor values observed for all the tested densities indicate that even with changes in some hematological parameters, the species positively supports production at high stocking densities.

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