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Stocking density of Amazon fish (*Colossoma macropomum*) farmed in a continental neotropical reservoir with a net cages system

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ABSTRACT

A 60-day experiment was conducted to evaluate the best stocking density (SD) and net cage (NC) size for rearing tambaqui juveniles (*Colossoma macropomu)* in a continental neotropical reservoir. 57,688 tambaqui juveniles (50.10 ± 1.39 g, mean ± S.E.M.) had been randomly stocked in 16 NC. The experiment was run with a 2 × 2 factorial scheme at two SD (15 kg/m^3; 24 kg/m^3) and two NC sizes (22.5 m^3; 40 m^3), with four replications: T1: 15 kg/m^3 x 22.5 m^3; T2: 24 kg/m^3 x 22.5 m^3; T3: 15 kg/m^3 x 40 m^3; T4: 24 kg/m^3 x 40 m^3. After 60 days, growth performance, biochemical parameters and body composition of fish were evaluated. Individual weight gain, feed conversion ratio, feed efficiency rate, specific growth rate and protein efficiency ratio were better at SD 24 kg/m^3. For body composition, the crude protein and lower crude fat values in NC 24 kg/m^3 and NC 40 m^3 were higher. The biochemical parameters showed no significant difference for total proteins and plasma cholesterol. Triglycerides had higher rates for the fish stored in NC 40 m^3. An SD-NC interaction appeared for glucose and cortisol, with higher values for SD 15 kg/m^3 in NC 40 m^3; the highest values were observed in SD 24 kg/m^3 for NC 22.5m^3. Therefore, the production of the fish stocked in SD 24 kg/m^3 was more efficient as the biochemical parameters had better indices for the fish raised in 40m^3. The findings in this study suggest using SD 24 kg/m^3 in NC 40 m^3 to maximise fish productivity and welfare.

1. Introduction

The world's demand for food is increasingly imposed, which implies extending these inputs (FAO, 2018). In general, fish is the protein with the best nutritional quality, and it acts as an important tool to meet the world population's nutritional requirements (FAO, 2018). Thus the creation of intensive systems can be a viable alternative for this purpose because it maximises productivity in units (Watanabe et al., 2010).

One of the main technology followed to produce intensive systems is net cages, which are closed mesh structured on all sides that retain fish and allow water to flow (Colt, 1991). These net cages can be installed in coastal areas, and in large lakes and reservoirs, and sustainably extend production because they use readily available aquatic environments and allow higher productivity than other production systems (Demétrio et al., 2012). Despite being a proven efficient system, it requires developing both monitoring and management to produce fish sustainably and periodically (Chowdhury et al., 2020). In view of this, government policies are extending actions and areas of action to increase fish production to keep social, economic and environmental aspects balanced (Demétrio et al., 2012; Bueno et al., 2015).

Very few fish farming studies have been conducted in intensive farming systems, such as net cages, especially in connection with the best stocking density (SD) and breeding structure size. According to Brandão et al. (2004), determining SD is important for fish to obtain better performance indices and for successful production cycles. Hence the SD that refers to the number or weight of fish per unit volume, when stored above or below production capacity, can be a limiting factor for productivity and may determine fish farms' profitability (EFSA, 2008; Chowdhury et al., 2020). In fact Rahman et al. (2006) suggest that optimal SD should be determined so that production and welfare indices can provide the highest possible economic return. According to Beveridge (1987), net cage size can be a relevant factor as it influences production and productivity levels. Silva et al. (2007) report how defining net cage size is necessary to understand species' feeding and locomotor behaviour. This definition is species-specific and is related to the environmental support capacity, renewal rates, and the temperature

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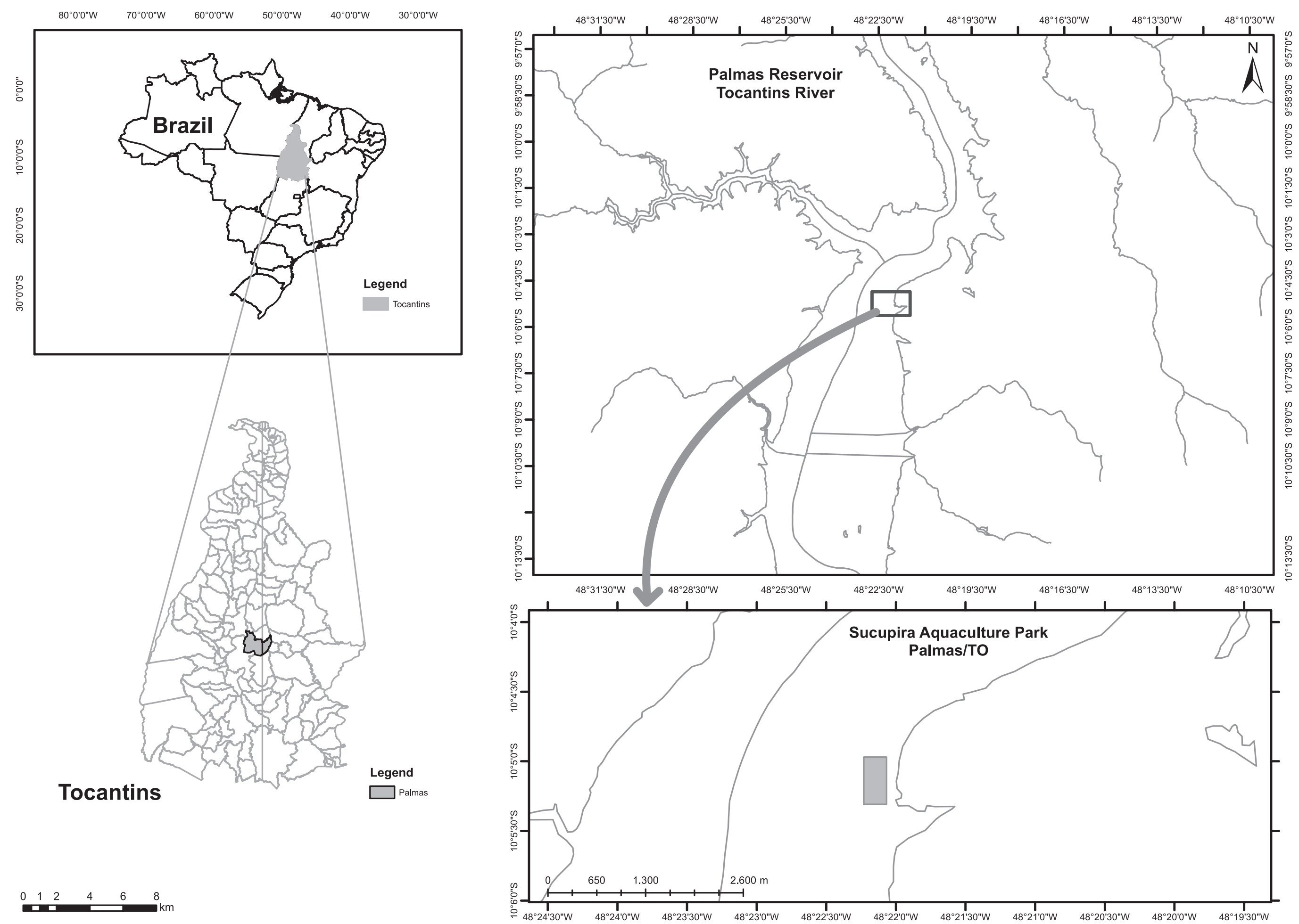

Fig. 1. Study area at Palmas Reservoir in the Sucupira Aquaculture Park.

and water body that net cages are installed in (Canzi et al., 2017). Given all the above facts, SD combined with net cage size can significantly affect not only growth performance (Slembrouck et al., 2009; Chowdhury et al., 2020), but also the physiological responses associated with animal health and welfare (Zahedi et al., 2019).

Fish farming in net cages is carried out in different regions of the world: Italy for *Seriola dumerili* (Mazzola et al., 2000); the USA for *Melanogrammus aeglefinus* (Chambers and Howell, 2006); Thailand and Brazil for *Oreochromis niloticus* (Yi and Lin, 2001); Norway and Chile for *Salmo salar* (Korsøen et al., 2009; Meyer et al., 2019); the UK for *Oncorhynchus mykiss* (North et al., 2006); China for *Lates calcarifer* (Luo et al., 2019). In the Amazon region, the main fish species raised in intensive systems are not representative, and fish farming in this region is characterised by small properties using extensive and semi-intensive systems (Saint-Paul, 2017). This situation occurs because the logistics, transport infrastructure, inputs and fish conservation in the Amazon region present deficits (Oliveira, 2009), which make it difficult to expand and maximise production in intensive systems.

According to Watanabe et al. (2010), the environments provided by intensive systems can act as a stressor because animals are subjected to densification, confinement, limited available area and effects on feeding that may have a negative influence (Nash et al., 2000; Rahman et al., 2006; Demétrio et al., 2012; Nhan et al., 2019). Under these conditions, fish present primary responses that activate the central nervous system by stimulating the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary (Fryer and Lederis, 1986; Wendelaar-Bonga, 1997), as well as the subsequent release of cortisol in the current sanguine by interrenal cells (Randall and Perry, 1992; Arends et al., 1999). Secondary defence occurs as subsequent action, with affects animal metabolism (Barton and Iwama, 1991) and brings about changes in plasma, such as higher concentrations of glucose, cholesterol, triglycerides and total proteins (McDonald and Milligan, 1992; Begg and Pankhurst, 2004; Cuesta et al., 2004). If this scenario continues, fish productive performance and health status can be compromised (Wendelaar-Bonga, 1997). Therefore, fish welfare in intensive breeding systems is essential to seek maximum performance and economic success, while also providing fish of nutritional quality (Poli et al., 2005; Maricchiolo et al., 2011). Despite the very high potential of Amazonian species for breeding in net cages, no studies are available about developing these species in intensive production systems, which are key issues for maximising their production, especially tambaqui.

Tambaqui (*Colossoma macropomum*) is a large Amazon fish that can be grown during short culture periods as it can reach a marketable size of 2–3 kg after 8–12 culture months (Gomes et al., 2006). Studies on this species prove to be increasingly important, due to the expansion of their breeding in several regions of the world, such as Central America and Asia (Woynárovich and Van-Anrooy, 2019). Despite being an important tropical aquaculture species, knowledge about its basic biology is lacking, especially about fish farming intensive systems like large-volume net cages (NC), and adequate SD for this species in its early development stages (Gomes et al., 2006). Some authors have studied the creation of tambaqui in 1-m^3 net cage, which suggests optimal densities of 8 kg/m^3 (Brandão et al., 2004) and 14 kg/m^3 (Silva and Fujimoto, 2015). In these studies however, fish started with an average weight of 0.24 g and 0.35 g, respectively, but this condition does not represent the commercial scenario of production activity, where implementing breeding in large-volume net cages is sought (Rosini et al., 2019) by starting breeding at an average fish weight of 40–50 g.

Our study objective was to acquire information about tambaqui juveniles in NC to examine the effects of different SD and two NC sizes on the growth performance, biochemical parameters and body composition of fish.

2. Materials and methods

2.1. Study area

The experiment was conducted on a farm at Palmas Reservoir (Fig. 1) in the Sucupira Aquaculture Park at the Center for Research, Demonstration and Training of Fish Breeding in Net Cage of Brazilian Agricultural Research Corporation-Fisheries and Aquaculture (CRDTFB-EMBRAPA/PESC-AQUI), in Palmas/TO. The Palmas/TO municipality is located in central Brazil at an average altitude of 260 m, with geographical coordinates 10°11′04“ south latitude and 48°20′01″ west longitude of Greenwich (BRASIL, 1992).

2.2. Fish origin and management

The aquaculture experimental facility of the CRDTFB-EMBRAPA/PESC-AQUI, Brazil, housed 57,888 tambaqui juveniles (mean weight ± S.E.M.: 50.10 ± 1.39 g; mean length ± S.E.M.: 11.53 ± 0.14 cm), which were obtained from the Aquaculture Farm São Paulo (Tocantins, Brazil). At the beginning of the experiment, the fish were randomly stocked in 16 NC of 25 mm mesh internodes under the experimental conditions to be tested, whose average depth was 10.50 m. They were installed perpendicularly to the water current, and arranged in line with a distance between NC of 4 m. Fish were hand-fed 3 times a day until satiety with a specific commercial extruded diet for omnivore fish (Guabi®, Brazil).

Water temperature, dissolved oxygen and pH were monitored 3 times a week in the morning with a U-50 Multiparameter Horiba probe and every 2 weeks, ammonia was monitored by the indophenol blue method, during the experiment period. The temperature, oxygen, pH and ammonia parameters were measured with values of 29.00 ± 0.25 °C, 8.35 ± 0.21 mg/l, 6.96 ± 0.09 and 0.07 ± 0.01 mg/l, respectively, in accordance with Resolution no. 357, of 2005, of the National Council of the Environment (CONAMA) for fresh water, classified in class II, for use in aquaculture (BRASIL, 2005).

This research complied with Brazilian guidelines for the care and use of animals for scientific and educational purposes (DBCA), and also with the ethics principles in animal experimentation of the Ethics Commission on the Use of Animals by Brazilian Agricultural Research Corporation - Fisheries and Aquaculture (CEUA-CNPASA: no. 01/2019).

2.3. Experimental design

The experiment was carried out following a completely randomised design in a 2 × 2 factorial scheme at two SD (15 kg/m^3; 24 kg/m^3) and with two NC sizes (3.0mx3.0mx3.0m: 22.50 m^3; 4.0mx4.0mx3.0m: 40 m^3): T1 (15 kg/m^3 x 22.50 m^3–1980 fish); T2 (24 kg/m^3 x 22.50 m^3–3168 fish); T3 (15 kg/m^3 x 40 m^3–3548 fish); T4 (24 kg/m^3 x 40 m^3–5676 fish); each treatment consisting of 4 repetitions and net cage taken as the experimental unit.

Fish were fed 3 times a day, 7 days a week, to apparent satiation, with commercial extruded fish feed containing 33% crude protein (CP) for the 60-day trial period. The amount of feed provided daily was recorded to calculate eaten food (feed intake).

At the beginning of the experiment and on day 30, a random sample of 100 fish from each net cage was taken after a 24-h fasting. The sampled fish were anesthetised with 20 mg/l of clove oil (Inoue et al., 2011), individually weighed and measured (standard length). After this procedure, the fish returned to the original net cages. At the end of the experiment (day 60), the same procedure was performed by counting 100% of fish from each experimental unit, and weighing and measuring 100 fish from each net cage to make performance evaluations.

2.4. Growth parameters

The effects of SD on zootechnical performance were determined by calculating the following parameters.

- Individual weight gain (IWG; g): final weight (g) - initial weight (g);
- Feed conversion ratio (FCR): individual feed intake (g) / individual weight gain (g);
- Feed efficiency rate (FER; %): (individual weight gain (g) / individual feed intake (g)) x 100;
- Specific growth rate (SGR; %): [(Ln final weight-Ln initial weight)/Time]x100;
- Protein efficiency ratio (PER; %): weight gain (g) / crude protein intake (g);
- Coefficient of variation (CV; %): (standard deviation final weight (g)/mean final weight (g)) x 100;
- Condition factor (CF) = 100 x final weight (g)/final length $(cm)^3$;
- Survival (S,%) = (number of final animals x 100) / number of initial animals.

2.5. Biochemical analysis

Blood was collected from three fish in each experimental unit (12 fish per treatment) by puncturing the caudal vein with 1 ml syringes containing EDTA. For the plasma analysis, blood aliquots were centrifuged at 3500 rpm/5 min/25 °C for plasma separation. A cortisol concentration analysis (ng/mL) was performed by the ELISA method (Cortisol ELISA kit - DRG Diagnóstica); glucose (mg/dL), total protein (g/dL), cholesterol (mg/dL) and triglycerides (g/dL); which were tested by the colorimetric method with a commercial kit (In Vitro Diagnóstica Ltda, Itabira/MG).

2.6. Body composition and organo-somatic indexes

The proximate fish composition were collected from four fish in each experimental unit (16 fish per treatment). Body composition was determined by standard methods of the Association of Official Analytical Chemists (AOAC, 2012), and the following were determined: content moisture by drying for 24 h at 110 °C to constant weight; protein by the Kjeldahl method (N × 6.25); crude fat by diethyl ether extraction; ash by heating at 450 °C for 24 h.

The organo-somatic indexes were determined collecting the liver, visceral fat and viscera of 4 fish per experimental unit (16 fish per treatment), being removed, weighed and were calculated as follows:

- Hepato-somatic index (HSI; %): (liver weight (g) / final weight (g)) × 100;
- Lipo-somatic index (LSI; %): (visceral fat weight (g)/ final weight (g)) × 100;
- Viscero-somatic index (VSI; %): (viscera weight (g) / final weight (g)) × 100;

2.7. Data analysis

All data are presented as mean ± S.E.M. Data normality was previously evaluated by the Shapiro-Wilk test and the homogeneity of variance was verified by the Levene test. The variables were subjected to an analysis of variance using the PROC MIXED command by the Statistical Analyzes System - SAS 9.1 programme according to the model described below:

Table 1
Effect of stocking density on the performance of *Colossoma macropomum* in net cages.

Parameter	SD	NC		Mean	P value		
		22.50m³	40.00m³		SD	NC	SD x NC
IW[1] (g)	15 kg/m³	49.75 ± 0.62	53.63 ± 3.23	51.69 ± 1.69	0.25	0.78	0.10
	24 kg/m³	51.20 ± 3.15	45.82 ± 2.73	48.51 ± 2.18			
	Mean	50.48 ± 1.51	49.73 ± 2.45				
IL[2] (cm)	15 kg/m³	11.51 ± 0.11	11.16 ± 0.21	11.34 ± 0.13	0.14	0.06	0.49
	24 kg/m³	12.07 ± 0.14	11.37 ± 0.42	11.72 ± 0.24			
	Mean	11.79 ± 0.13	11.26 ± 0.22				
FW[3] (g)	15 kg/m³	151.71 ± 7.87	164.03 ± 10.22	157.87 ± 6.41	0.07	0.84	0.12
	24 kg/m³	181.88 ± 2.78	166.28 ± 10.25	174.08 ± 5.73			
	Mean	166.80 ± 6.89	165.16 ± 6.71				
FL[4] (cm)	15 kg/m³	15.55 ± 0.62	15.41 ± 0.34	15.48 ± 0.33	0.64	0.39	0.60
	24 kg/m³	15.93 ± 0.14	15.39 ± 0.27	15.66 ± 0.17			
	Mean	15.74 ± 0.30	15.40 ± 0.20				
IWG[5] (g)	15 kg/m³	101.96 ± 8.07	110.40 ± 12.65	**106.18 ± 7.13**	**0.04**	0.92	0.31
	24 kg/m³	130.68 ± 5.04	120.46 ± 7.99	**125.57 ± 4.78**			
	Mean	116.32 ± 6.99	115.43 ± 7.18				
FCR[6]	15 kg/m³	2.25 ± 0.16	2.15 ± 0.31	**2.20 ± 0.16**	**0.01**	0.96	0.60
	24 kg/m³	1.61 ± 0.04	1.70 ± 0.08	**1.65 ± 0.04**			
	Mean	1.93 ± 0.14	1.92 ± 0.17				
FER[7] (%)	15 kg/m³	45.04 ± 3.50	49.07 ± 6.18	**47.05 ± 3.37**	**0.004**	0.88	0.39
	24 kg/m³	62.08 ± 1.46	59.19 ± 2.79	**60.64 ± 1.56**			
	Mean	53.56 ± 3.67	54.13 ± 3.68				
SGR[8] (%)	15 kg/m³	1.85 ± 0.09	1.86 ± 0.18	**1.85 ± 0.09**	**0.03**	0.88	0.95
	24 kg/m³	2.12 ± 0.11	2.14 ± 0.05	**2.13 ± 0.06**			
	Mean	1.98 ± 0.08	2.00 ± 0.10				
PER[9] (%)	15 kg/m³	1.38 ± 0.11	1.50 ± 0.19	**1.44 ± 0.10**	**0.004**	0.38	0.87
	24 kg/m³	1.81 ± 0.09	1.90 ± 0.04	**1.85 ± 0.05**			
	Mean	1.59 ± 0.10	1.70 ± 0.12				
CV[10]	15 kg/m³	25.71 ± 0.46	25.94 ± 1.39	25.83 ± 0.68	0.30	0.43	0.55
	24 kg/m³	23.70 ± 0.89	25.38 ± 1.66	24.54 ± 0.93			
	Mean	24.71 ± 0.60	25.66 ± 1.01				
CF[11]	15 kg/m³	4.07 ± 0.25	4.46 ± 0.05	4.07 ± 0.14	0.09	0.14	0.23
	24 kg/m³	4.49 ± 0.06	4.53 ± 0.08	4.46 ± 0.05			
	Mean	4.49 ± 0.14	4.53 ± 0.04				

Legend: [1]IW = Initial weight; [2]IL = Initial length; [3]FW = Final weight; [4]FL = Final length; [5]IWG = Individual weight gain; [6]FCR = Feed conversion ratio; [7]FER = Feed efficiency rate; [8]SGR = Specific growth rate; [9]PER = Protein eficiency ratio; [10]CV = Coefficient of variation; [11]CF = Condition factor. All values represents mean ± S.E.M., n = 4 tanks per treatment. P values ($p < .05$) and means marked in bold indicate significant differences.

$$Yijk = \mu + SDi + NGj + Iixj + \varepsilon_{ijk}$$

where:

μ = general average;
SD_i = fixed effect of SD_i (1–2);
NC_j = fixed effect of net $cage_j$ size (1–2);
I_{jxk} = effect of the SD_i and NC_j size interaction;
ε_{ijk} = random error for each observation (0, δ^2).

3. Results

Significant differences were observed for individual weight gain (p = .04), feed conversion ratio (p = .01), feed efficiency rate (p = .004), specific growth rate (p = .03) and protein efficiency ratio (p = .004) for SD, with 24 kg/m³ being the SD with the best production indices (Table 1). The survival rate was 99.91 ± 0.02% throughout the experimental period.

The crude protein and crude fat contents in the whole body were significantly different, and the higher SD and the larger NC presented high indices for crude protein content (SD: p = .009; NC: p = .001). An inversion appeared for body crude fat content, with higher values for the lower SD and the smaller NC (SD: p = .001; NC: p = .004) (Table 2). Organo-somatic indexes showed significant differences for lipo-somatic index (SD: p = .0001) and hepato-somatic index (NC: p = .01). Lipo-somatic index was higher for the fish stocked in final density 15 kg/m³. For net cages size, the parameter hepato-somatic index obtained higher values for the fish stored in NC 40 m³ (Table 3).

No significant differences appeared between treatments for the plasma total protein (SD: p = .25; NC: p = .13) and cholesterol (SD: p = .87; NC: p = .92) levels. For triglycerides however, values were higher when comparing NC sizes (p < .0001), and NC 40 m³ showed this behaviour (Table 4). An SD and NC size interaction was observed (Table 4) for glucose (p < .0001) and cortisol (p < .0001). For both parameters, a higher value was obtained for SD 15 kg/m³ in NC 40 m³ (glucose: p < .0001; cortisol: p < .0001). For NC 22.50 m³, higher values were observed for both parameters with SD 24 kg/m³ (glucose: p < .0001; cortisol: p < .0001). For NC 40 m³, SD 15 kg/m³ presented the highest parameter values (glucose: p = .0002; cortisol: p = .0002).

4. Discussion

The present study evaluated the effect of SD and different NC sizes installed in continental reservoirs for tambaqui juveniles to make fish production and welfare more efficient. This study provides information for the aquaculture sector to maximise production of juveniles in NC by providing better indices and concepts to be applied and to, thus, provide efficient fish production methods in NC installed in continental reservoirs. Significant differences were observed for growth performance with SD.

The main factors that affect growth performance in fish are food intake and nutrient absorption (National Research Council, 2011; Kitagawa et al., 2015). The space available in fish production systems is very important for growth performance because space limitations are a determining factor for fish development (EFSA, 2008). In fact higher densities reduce fish growth performance because of a smaller physical space due to occupancy rates in NC (Oppedal et al., 2011; Oliveira

Table 2
Effect of stocking density on proximate carcass composition of *Colossoma macropomum* in net cages.

Parameter	SD	NC		Mean	P value		
		22.50m³	40.00m³		SD	NC	SD x NC
Moisture (%)	15 kg/m³	63.35 ± 0.66	64.01 ± 0.40	63.68 ± 0.38	0.29	0.19	0.56
	24 kg/m³	63.76 ± 1.28	65.37 ± 0.66	64.57 ± 0.74			
	Mean	63.56 ± 0.67	64.69 ± 0.44				
Crude protein (%)	15 kg/m³	44.69 ± 0.08	46.15 ± 0.27	**45.42 ± 0.30**	**0.009**	**0.001**	0.06
	24 kg/m³	45.90 ± 0.23	46.59 ± 0.09	**46.24 ± 0.18**			
	Mean	**45.29 ± 0.25**	**46.37 ± 0.15**				
Crude fat (%)	15 kg/m³	9.98 ± 0.06	9.56 ± 0.06	**9.77 ± 0.09**	**0.001**	**0.004**	0.60
	24 kg/m³	9.62 ± 0.09	9.10 ± 0.14	**9.36 ± 0.13**			
	Mean	**9.80 ± 0.08**	**9.33 ± 0.11**				
Ash (%)	15 kg/m³	9.10 ± 0.16	9.24 ± 0.21	9.16 ± 0.12	0.09	0.84	0.66
	24 kg/m³	9.59 ± 0.18	9.53 ± 0.29	9.56 ± 0.16			
	Mean	9.34 ± 0.15	9.38 ± 0.18				

All values represents mean ± S.E.M., n = 4 tanks per treatment. P value ($p < .05$) indicate significant differences. P values ($p < .05$) and means marked in bold indicate significant differences.

et al., 2012; Refaey et al., 2018; Ren et al., 2018). However, the juveniles stocked at SD 24 kg/m³ showed better productive performance for individual weight gain (IWG), feed conversion ratio (FCR), feed efficiency rate (FER), specific growth rate (SGR) and protein efficiency deficiency ratio (PER). These results indicate that physical spaces were not a limiting factor for growth at the highest SD, which is similar to that reported by Hengsawat et al. (1997) for *Clarias gariepinus*, Brandão et al. (2004) and Gomes et al. (2006) for *Colossoma macropomum* and Rowland et al. (2006) for *Bidyanus bidyanus*. This finding may be related to the better use of nutrients, especially protein, which displayed more feed efficiency at the higher density and in NC 40 m³. In addition, the fish stocked at SD 24 kg/m³ had higher protein composition, which corroborated the results of Refaey et al. (2018). FCR, FER and SGR were higher in this scenario, which corroborated previous information according to which fish better absorbed nutrients.

The results also indicated that SD 15 kg/m³ presented lower values for the performance parameters, and higher values for the lipo-somatic index (LSI) and crude fat in carcass composition for the fish stocked at this density and in NC 22.5m³. These results may be related to the bigger space available for fish, which can increase energy use with activities and behavioural patterns and, consequently, reduce the energy employed for somatical growth. In addition, a higher crude fat reserve deposition observed in the fish stored at the lower density can be harmful for the fish production system. These results agree with Ni et al. (2016), who studied the SD of *Acipenser schrenckii* in NC, and found this same condition with lower occupancy rates. Given these scenarios, growth performance variedary between the fish species reared in NC, which may be related to species' eating habits, aggressive behaviour in fish agglomerations, water quality, life stages, environments in which NC are installed, time of year and breeding structure size (Rowland et al., 2006; Oppedal et al., 2011; Ren et al., 2018; Meyer et al., 2019; Chowdhury et al., 2020). This finding demonstrates that the aquaculture potential is high and much research must be conducted to provide a better understanding of this production system.

The biochemical parameters indicated a significant difference, but only for triglycerides in the tambaqui juveniles reared in NC 40 m³. These results could be related to more energy demands regardless of SD because the fish in this environment moved about more as they had a bigger space to explore, as described by Vijayan et al. (1990). The Hepato-somatic index (HSI) had higher values for the same situation. These results indicate that fish need more energy inputs and, as fat and glucose are energy sources, they meet fish demands (Walton and Cowey, 1982; Wendelaar-Bonga, 1997). The fat available in the bloodstream of tambaqui juveniles can be used immediately, as described by Moon (1988) and Sheridan (1988). The IHS is often used to estimate the energy status and metabolic activity of fish (Janssens and Waterman, 1988), and it herein indicated more demand for these energy reserves at the lower density.

The cortisol and glucose indices showed interactions in the tambaqui juveniles stocked at different densities for both NC sizes. The fish stocked at SD 15 kg/m³ in NC 40 m³ obtained higher glucose and cortisol values. In view of this scenario, we suggest that the bigger the space for fish, the more motivated they would be to perform more locomotor activity and, consequently, to use more energy. This meant that the plasma cortisol level was high compared to the other treatments. This condition was also observed by Oyarzún et al. (2020), who studied the effect of SD on NC for the species *Eleginops maclovinus*. According to Wendelaar-Bonga (1997), cortisol is the hormone of catabolic synthesis, which implies increased blood glucose through catabolic pathways, mainly gluconeogenesis, as fish need energy to

Table 3
Effect of stocking density on organo-somatic indexes of *Colossoma macropomum* in net cages.

Parameter	SD	NC		Mean	*P* value		
		22.50m³	40.00m³		SD	NC	SD x NC
HSI[1] (%)	15 kg/m³	1.86 ± 0.07	1.64 ± 0.17	1.98 ± 0.09	0.31	**0.01**	0.42
	24 kg/m³	2.10 ± 0.10	2.07 ± 0.11	1.85 ± 0.11			
	Mean	**1.74 ± 0.07**	**2.08 ± 0.09**				
LSI[2] (%)	15 kg/m³	3.65 ± 0.34	3.03 ± 0.12	**3.34 ± 0.20**	**0.001**	0.07	0.44
	24 kg/m³	2.57 ± 0.20	2.31 ± 0.21	**2.44 ± 0.14**			
	Mean	3.11 ± 0.27	2.67 ± 0.18				
VSI[3] (%)	15 kg/m³	3.41 ± 0.18	3.88 ± 0.41	3.64 ± 0.23	0.89	0.06	0.69
	24 kg/m³	3.33 ± 014	4.03 ± 0.31	3.68 ± 0.20			
	Mean	3.37 ± 0.11	3.96 ± 0.24				

All values represents mean ± S.E.M., n = 4 tanks per treatment. P value ($p < .05$) indicate significant differences. P values ($p < .05$) and means marked in bold indicate significant differences.

Table 4
Effect of stocking density on biochemical parameters of *Colossoma macropomum* in net cages.

Parameter	SD	NC		Mean	P value		
		22.50m^3	40.00m^3		SD	NC	SD x NC
Glucose (mg/dL)	15 kg/m^3	93.37 ± 2.15^{aA}	150.12 ± 2.35^{bB}	**121.75 ± 10.82**	**0.04**	< **0.0001**	< **0.0001**
	24 kg/m^3	124.70 ± 2.03^{B}	130.53 ± 3.46^{A}	**127.61 ± 2.16**			
	Mean	**109.04 ± 6.08**	**140.32 ± 4.18**				
Triglycerides (mg/dL)	15 kg/m^3	163.78 ± 5.16	300.41 ± 16.80	232.10 ± 27.07	0.81	< **0.0001**	0.14
	24 kg/m^3	189.02 ± 7.10	281.93 ± 21.07	235.47 ± 20.35			
	Mean	**176.40 ± 6.26**	**291.17 ± 12.95**				
Total proteins (g/dL)	15 kg/m^3	3.46 ± 0.18	3.14 ± 0.15	3.30 ± 0.12	0.25	0.13	0.35
	24 kg/m^3	3.49 ± 0.09	3.41 ± 0.05	3.45 ± 0.05			
	Mean	3.47 ± 0.09	3.27 ± 0.09				
Cholesterol (mg/dL)	15 kg/m^3	120.70 ± 2.04	124.38 ± 3.60	122.54 ± 2.04	0.87	0.92	0.34
	24 kg/m^3	124.11 ± 3.25	119.67 ± 6.33	121.89 ± 3.40			
	Mean	122.40 ± 1.89	122.02 ± 3.49				
Cortisol (ng/ml)	15 kg/m^3	34.75 ± 1.04^{aA}	56.21 ± 2.44^{bB}	45.47 ± 4.24	0.65	< **0.0001**	< **0.0001**
	24 kg/m^3	47.41 ± 1.06^{B}	44.94 ± 0.99^{A}	46.17 ± 0.82			
	Mean	**41.08 ± 2.49**	**50.57 ± 2.45**				

All values represents mean ± S.E.M., n = 4 tanks per treatment. P values ($p < .05$) and means marked in bold indicate significant differences. Means with different letters superscripts in the same row (a, b) and column (A, B) are significantly different.

perform such functions (Barton and Iwama, 1991). In line with this scenario, cortisol released into the bloodstream would activate physiological and behavioural mechanisms, which would relate secondary and tertiary stress responses, as described by Barton (2002).

Glucocorticoids can also reduce growth in teleosts. This mechanism may be associated with growth hormone (GH), insulin-like growth factor-I (IGF—I) and IGF-binding protein (IGFBP) (Auperin et al., 1997; Kelley et al., 2001; Peterson and Small, 2005). In fact, Small (2004) showed that cortisol promoted lower fish growth rates for *Ictalurus punctatus*. Thus, the increased plasma cortisol level observed for the animals kept at the lower density would be reflected as less growth performance for this treatment.

In this study, we verified that the fish stocked at SD 24 kg/m^3 in NC 22.5 m^3 had higher plasma cortisol and glucose levels. This situation could have been due to a small breeding environment containing many fish and would, thus, mean stressful conditions, like those described by Conte (2004). These conditions may have occurred upon feeding. As food was administered 3 times/day, fish movements to search for food in a small space could have increased the number of encounters and contacts made between fish, as observed by Ruane et al. (2001) and Barcellos et al. (2004). In such an environment, fish could have collided with the NC structure on the top, sides and bottom, as described by Le Ruyet and Le Bayon (2009) for *Dicentrarchus labrax*. Small spaces can also increase the continuous exposure of fish while they remain on the surface, and sun exposure, as confirmed for *Salmo salar* by Fernö et al. (1995) and Dempster et al. (2008). In these scenarios, the density and volume could have been stressful with negative effects throughout the experimental period. Therefore, several situations can promote harmful fish effects, which must be studied with more marked interactions to obtain answers for the aquaculture sector. So apart from evaluating growth performance, the evaluation of biochemical parameters and welfare variables should be studied more intensely to offer more direct responses to, thus, enhance the fish production system.

5. Conclusions

The best results in this study were for the fish stocked at SD 24 kg/m^3, whose production was more efficient, with more suitable biochemical parameters for the fish reared in NC 40 m^3. So using SD 24 kg/m^3 in NC 40 m^3 can provide the best fish productivity and welfare. However, our study did not indicate the maximum stocking limit for tambaqui juveniles in NC. Further studies are advisable to obtain the optimal density for this fish production system to maximise production, but by considering sustainable performance of fish welfare and activity.

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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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