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ARTIGO ORIGINAL

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Stocking density and its effects to the zootechnical development of young tambaqui in an intensive production system

Densidade de estocagem e seus efeitos sobre o desenvolvimento zootécnico de juvenis de tambaqui em sistema intensivo de produção

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Abstract - Between March and September of 2014, the stocking density effects were evaluated on the zootechnical development of tambaqui juveniles (≈ 10 g) until the beginning of grow-out phase (≈ 250 g), in an intense fish production system. The evaluation was conducted at Presidente Médici Municipality, Rondônia State - Brazil. For that, specimens were distributed randomly in tanks of 10 m^2, on the concentrations of 60 (T1), 80 (T2), 100 (T3) and 120 (T4) individuals, in five repetitions. Data about weight (g) and length (cm) from 10 individuals per treatments were sampled weekly, concomitant with sampling of water to limnological analysis. All data were analyzed statistically, and the results showed significant differences between growth parameters until the 10^{th} week of the fish farm, after that a homogenization of the growth values occurred. The weight-length curve coefficient (*b*) exhibited a negative allometry ($b<3$) from all groups. The statistical differences were not significant between the values of weight gain and feed conversion for the tested densities. The final biomass exhibited significant differences between the treatments T1, T3 and T4. However, they not negatively influenced the limnological values from the tanks. The feed conversion from treatments (2.51 ± 0.31) was considered high for the species. In this context, the resultant biomass from the highest density was the most viable from the economical point of view. Nevertheless, it is recommended that after the tenth week, the fish need to be redistributed in the biggest tanks, aiming the adequate development of fish in the production system.

Keywords: Amazon, *Colossoma macropomum*, pisciculture, stocking of fish.

Resumo - Foi avaliado entre março e setembro de 2014, o efeito da densidade de estocagem sobre o desempenho zootécnico de juvenis de tambaqui (≈ 10 g) até o início da fase de engorda (≈ 250 g), em um sistema intensivo, situado no município de Presidente Médici, Rondônia. Os peixes foram distribuídos aleatoriamente, em tanques de 10 m^2, nas concentrações de 60 (T1), 80 (T2), 100 (T3) e 120 (T4) indivíduos, em cinco repetições. O peso (g) e comprimento (cm) de 10 indivíduos por tratamento foram coletadas semanalmente, juntamente com coletas de amostras de água para análises limnológicas. Estas foram analisadas, utilizando-se dos softwares R 3.0.3 e Statistica 9.0. Não hove diferenças significativas entre as variáveis de crescimento até a décima semana, a partir daí ocorreu uma homogeneização nesses valores. O coeficiente (*b*) da curva peso-comprimento exibiu alometria negativa ($b<3$) em todos os grupos. As diferenças estatísticas não foram significativas entre os valores médios do ganho de peso e conversão alimentar nas densidades testadas. Por outro lado, as médias de biomassa final exibiram diferenças significativas entre os tratamentos T1, T3 e T4. No entanto, estas não influenciaram negativamente as variáveis limnológicas dos tanques experimentais. A conversão alimentar média dos tratamentos foi de 2,51 ± 0,31, considerada alta para o cultivo da espécie. A biomassa resultante da maior densidade foi a mais viável do ponto de vista econômico. Todavia, recomenda-se que após a décima semana de cultivo, os peixes sejam redistribuídos para tanques maiores, visando o adequado desenvolvimento ao sistema de produção.

Palavras-Chave: Amazônia, *Colossoma macropomum*, estocagem de peixes, piscicultura.

Introduction

Aquaculture is the food production sector that increases the most on the planet (FAO, 2014) and therefore, became an attractive economic observation activity. It was accomplished primarily with fish of high productivity therefore reducing space and time (Castagnolli, 1992). In this way, the natural fish stock pressure demanded by commercial fisheries was decreased (IBGE, 2013).

The world fish consumption in the year of 2014 was over 20 kg per inhabitant per year (kg/inhab/year), with an estimated production about 167.2 million tons (FAO, 2016), where 44.1% of this amount were originated from aquaculture (Rocha, Resende, Routledge & Lundstedt, 2013; FAO, 2016). Moreover, with the increase of world fish consumption (Sidonio et al., 2012), the fish as food in Brazil were considered small (11.17 kg/inhab/year) and less than the fish consumption indicated by the World Health Organization, that is 12 kg/inhab/year (Brazil, 2013). On the other side, Brazil is enlarged among the others neighboring countries, because it has an aquaculture potential, compassing over 53.000 km^2 of flooded areas, favorable to the fish farm segment increase (Melo, Souza, Sproesser, & Campeão, 2010; Rocha, Resende, Routledge & Lundstedt, 2013).

Among the aquaculture activities, the Brazilian fish farming has been highlighted with a fish production of 392.493 thousand tons in the year of 2013, and the Northern region was responsible for up to 18.6% of this sum. In this panorama, the Rondônia State attained 5th place in fish production in the country and first place in the Northern region. The result is 47 municipalities producing about 25.140 ton/year (6.4% of the national fish production), generating an income over R$ 123.146 million (IBGE, 2013).

Among the native fish species cultivated in the Brazilian territory, the tambaqui *Colossoma macropomum* (Cuvier, 1816), is the most farmed fish species, cultivated in 24 states and 858 municipalities. It has reached an estimated production over 88.718 ton/year, which represents 22.6% of fish production in the country (IBGE, 2013). In the Brazilian Amazon region, the tambaqui is also the most produced fish, and is also commercialized in neighboring countries (Resende, 2009).

In this scenario, the pisciculture sector had invested in technologies aimed at the improving tambaqui zootechnical development reflected throughout much research on the fish. The research included methods of feeding (Silva, Pereira-Filho & Oliveira-Pereira, 2003), reproduction (Gomes, Araújo-Lima & Roubach, 2003), genetic variability (De Queiroz, Sousa, da Silva, & Inoue, 2016), body growth models (Brandão, Gomes, Chagas, & Araújo, 2004; Penna, Villacorta-Correa, Walter, & Petrere-Jr, 2005; Costa et al., 2013; De Mello et al., 2015), ecology and management (Arantes & Freitas, 2016). Very few studies gave emphasis to the subject of densities effects on the productive development of tambaqui cultivated in ground tanks; and considering just juveniles fish in the initial cultivation process (e.g. Brandão, Gomes, Chagas & Araújo, 2004; Santos, Andrade & Garcez, 2014). In this context it is essential to the employment of studies including data from tambaqui farming daily activities, essential subjects related to fish stocking densities which consider fish weight growing from juveniles until a specific age, in order to contribute to the adequate management of this fish in farming activities.

Material and Methods

Study Area

The study was organized from March 15th to September 15th of the year 2014, in a fish farm (geographical coordinates: 11° 09' 37.10" S and 61° 54' 23.45" W) at the Presidente Médici municipality, Rondônia State (Figure 1).

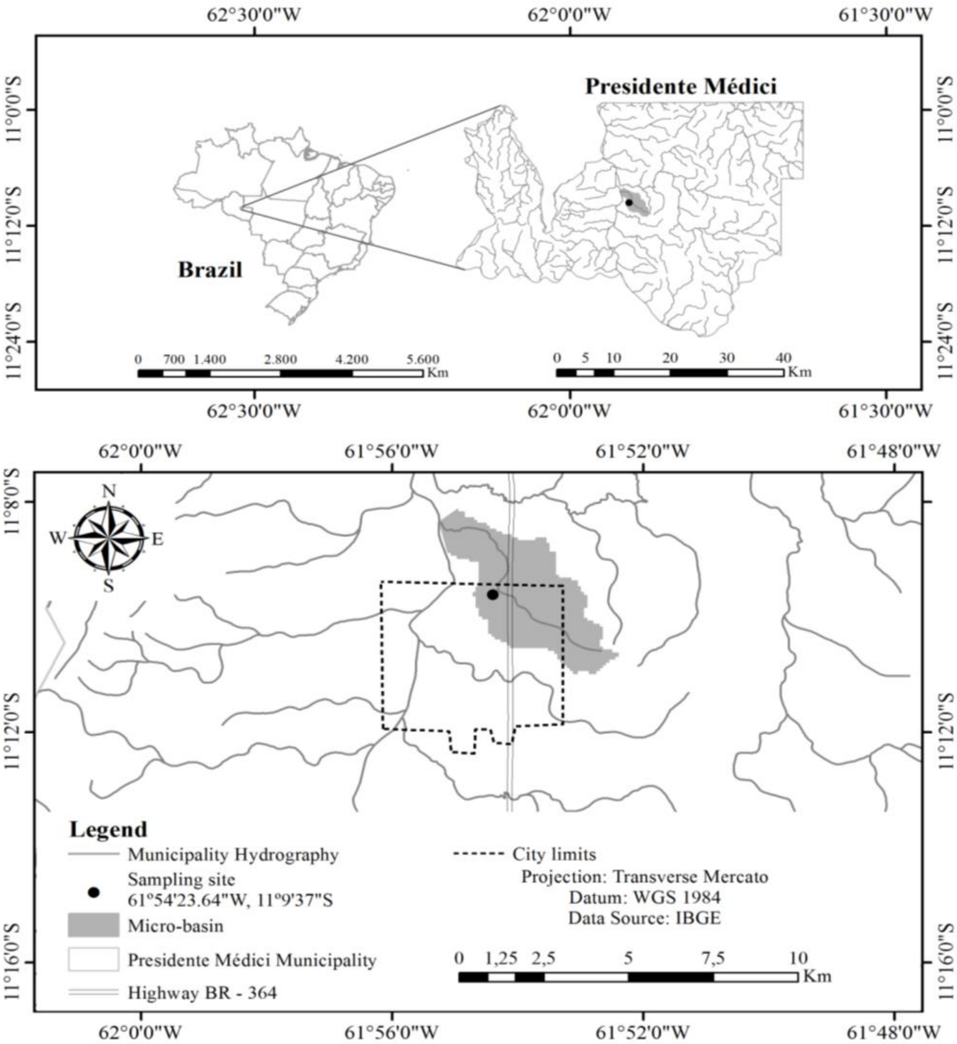

Figure 1. Study area sampling site and its hydrography basin.

Sample Collections and Preparations

The present research was developed in a fishpond with 840 m^2 and 1.5 m of depth. This tank was divided in 20 small containers (four treatments; T1, T2, T3 and T4 with five repetitions each) with 10 m^2 each, made by plastic nets with sizing of 15 mm between opposite knots. Prior to fish stocking procedure, the main tank was empty, dried and sterilized with Calcium oxide (CaO), in the proportion of 100 g/m^2. The tank was also exposed to sun light for a period of seven days, and subsequently filled with water to 1/3 of its capacity to commence tank fertilization. The tank fertilization process was done using triple super phosphate –TSP (5% of N, Nitrogen; 20% of P_2O_5, Phosphate; and 10% of K_2O, potassium), and urea $(NH_2)_2CO$ in the proportions of 6 g/m² and 3 g/m², respectively. The experimental tank was allowed to rest for seven days in anticipation of phytoplankton production, after that it was filled with water until 1.5 m high. The procedure of fish stocking with the concentration distribution (6, 8, 10 and 12 fish/m^2) were organized, considering five repetitions for each density used (Treatments = T1, T2, T3 and T4).

Sampling Data

Physical and chemical water parameters were measured weekly in all treatments, as data about electrical conductivity (µS cm^{-1}), temperature (ºC), hydrogen ionic potential (pH), dissolved oxygen (O_2 mg L^{-1}), total ammonia (NH_3 mg L^{-1}), total alkalinity (mg L^{-1} of $CaCO_3$) and total water hardness (mg L^{-1} of $CaCO_3$) were collected. The first four parameters were obtained using a multi-parameter water quality checker (8603, AKSO Nobel, São Leopoldo, RS, Brazil). The remaining data was collected using a colorimetric method (AlfaKit, Florianopolis, SC, Brazil).

The selected individuals of tambaqui initially exhibited average values of weight (11.07 ± 1.70 g) and length (5.45 ± 0.66 cm), and were distributed randomly in the experimental tanks (treatments) with the stocking densities of 6, 8, 10 and 12 fish/m^2. The fish were fed daily every five hours (dry fed with 36% of crude protein), this was adjusted weekly by 3% of the fish alive biomass in each treatment until the fish gained the Mean weight of about 250 g. Weight (g) and length (cm) data from ten fish in each experimental tank were measured in seven days intervals, and used to calculate the average weight (AW), final biomass (FB), weight gain (WG) and apparent feed conversion AFC (kg of food consumption/kg of fish).

Statistical Analysis

The data set was submitted to the analyses of variance (ANOVA) with 95% of confidence (Montgomery, 2012) after the verification of the assumptions for normality, independency, and residuals homoscedasticity, were approached the average comparisons using the Tukey test, considering an inferior probability of $p\text{-}value < 0.050$ to reject the null hypothesis (Conagin, Barbin & Demétrio, 2008).

The residuals data which did not accomplish the ANOVA assumptions were submitted to the Box-Cox transformation, and presented in the *lambda* (λ) values. The weight-length relation was observed through the Von Bertalanffy non-linear analysis (Le Cren, 1951). Also, the productivity variables related to final biomass, weight gain, and apparent feed conversion, was used in the multivariate analysis (MANOVA) aimed to verify differences between sampled groups. The average values from the variables in the treatments also were applied to the Regression analysis, following the model analysis assumptions were observed the inflection line points (Montegomery, 2012).

The Kruskal-Wallis non parametric analysis was applied on pH, temperature and electrical conductivity, due to these variables having exhibited accentuated residuals heteroscedasticity, independent of the Box-Cox method transformations employed. Then, the physical and chemical parameters sampled from the experimental tanks were confronted through the Spearman Correlation analysis (Siegel & Castellan Jr, 1988) in order to verify which environmental variable influenced the tambaqui growth parameters the most. All analyses were performed using the R software, version 3.0.3 (R Development Core Team, 2012), with one exception, for the relation of weight-length non-linear analyses that was achieved using the Statistica 9.0 software (Statsoft, 2009).

Results

TAMBAQUI GROWTH DEVELOPMENT

During the study period (180 days), were observed the zootechnical development of 1.800 individuals of tambaqui, from the juvenile II phase to beginning of grow-out phase, as classified by Gomes, Araújo-Lima & Roubach (2003). The results exhibited from the ANOVA were that no significant differences between the growth parameter values when compared with the pair wised groups occurred.

On the other side, when the groups growth values over the weeks were analyzed, it was noticed that an exponential gain for the values of weight and length variables, which presented significant differences (ANOVA com $p < 0.050$) up to the week 10^{th}, after that time a receding in the acceleration of growth values in the treatments occurred, observed by the Tukey test (Figure 2).

The arithmetical coefficient values from the weight-length curve (***a*** and ***b***) for all treatments presented significant differences between the residuals values with *p-value*< 0.050. Also, the isometric coefficient ***b*** exhibited negative allometry ($b < 3$) and analogous values for $r^2 \approx 0.960$ in all treatments (Figure 3).

Limnological Parameters from the Experimental Tanks

The limnological data applied to Kruskal-Wallis test did not present significant differences between the treatments for the alkalinity and dissolved oxygen values (*p-value*> 0.050). On the other side, the water hardness values exhibited significant differences only between the treatments T3 and T4 (λ = -0.29; Tukey, *p-value* $= 0.031$). Also, the ammonia values presented significant differences among the treatments T1 and T3 (λ = -0.31; Tukey, *p-value* < 0.050). Conversely, the mean values of pH (λ = -0.64 and *p-value*< 0.050), temperature and electrical conductivity (λ = -0.74 and *p-value*< 0.050) presented significant differences between all treatments (Table 1).

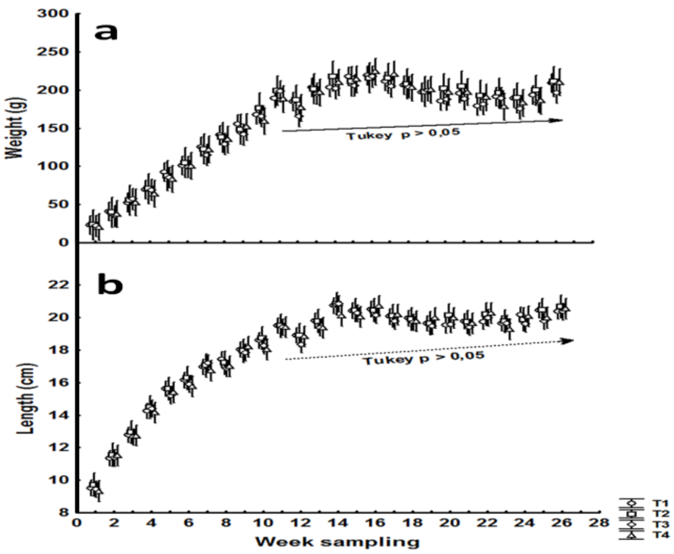

Figure 2. Distribution of the average values and standard variations from the weight (a) and length (b) variables. The arrows indicate the existence of no significant differences between the data sampled among the adjacent weeks.

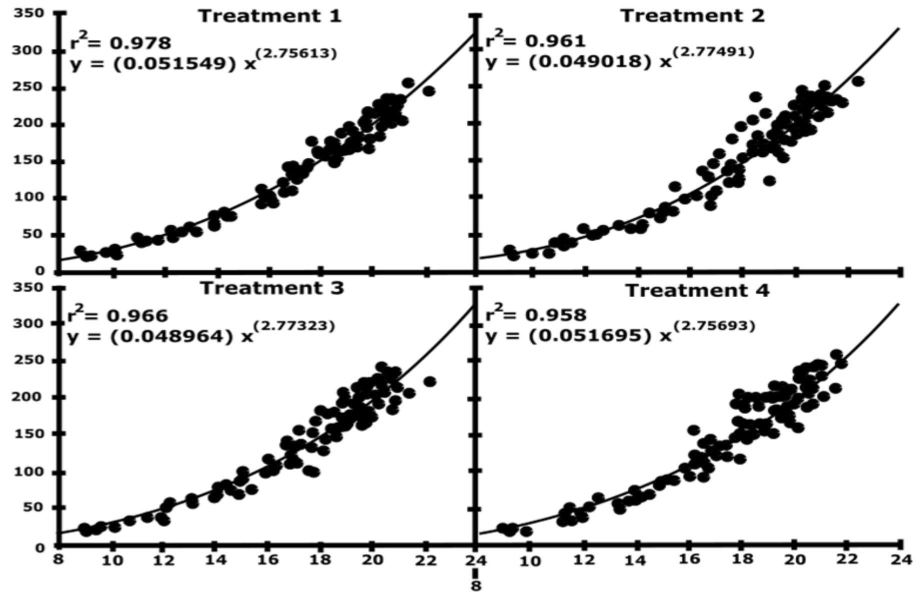

Figure 3. *Colossoma macropomum* weight-length relation dispersion points for individuals cultivated with different stocking densities, during 180 days in the intensive production system.

Table 1. Average and Standard deviation values of limnological parameters from the experimental tanks with *Colossoma macropomum* submitted to the different stocking densities T1, T2, T3 and T4, correspond to the stocking densities of 6, 8, 10 and 12 individuals/m^2 respectively. (±) = Standard deviation, DO = dissolved oxygen, EC = electrical conductivity. Identical letters in the same row shows that the averages values did not differ significantly when analyzed by the Tukey test, considering α=0.05 of probability.

	Treatments			
Limnological parameters	T1	T2	T3	T4
DO (mg/L^{-1})	6.48 ± 4.79 a	6.10 ± 069 a	6.01 ± 0.66 a	6.03 ± 0.67 a
pH	7.27 ± 0.69 a	7.42 ± 1.88 a	7.35 ± 0.56 a	7.32 ± 0.61 a
Temperature (ºC)	26.95 ± 2.34 a	26.99 ± 2.32 a	27.10 ± 1.42 a	27.15 ± 1.48 a
EC (µS/cm)	6.03 ± 2.00 a	5.86 ± 0.69 a	5.84 ± 0.68 a	5.82 ± 0.70 a
Ammonia (mg/L^{-1})	0.21 ± 0.20 a	0.23 ± 0.20 ab	0.23 ± 0.19 b	0.22 ± 0.20 ab
Alcalinity (mg/L^{-1})	37.08 ± 7.62 a	36.69 ± 7.71 a	36.54 ± 6.90 a	35.92 ± 6.19 a
Hardness (mg/L^{-1})	36.69 ± 6.75 ab	36.00 ± 5.79 ab	35.54 ± 5.29 a	37.08 ± 5.35 b

The others environmental variables negatively influenced the tambaqui growth values, mainly the temperature that presented values of r^2 = -0.61 and -0.62 for the weight and length variable, respectively. The primary limnological variables that presented positive relations (r^2> 0.50) in a chronological importance were DO vs EC (r^2 = 0.58) and water hardness vs alkalinity (r^2 = 0.55). The other variable presented negative relations, highlighting the temperature that was related to DO with r^2 = - 0.64 and to EC with r^2 = - 0.57%. There was no significant relation among ammonia with the others environmental values and neither between the tambaqui growths values (Table 2).

Table 2. Correspondence levels of the physical and chemical variables with the tambaqui growth parameters values, exhibited by the Spearman's rho correlation analyses.

	Length	Weight	EC	Temp	pH	DO	Ammonia	Hardness	Alc
Length		0.92*	0.59*	-0.62*	-	0.58*	nr	+	-
Weight	0.92*		-	-0.61*	-	0.52*	nr	-	-
EC	0.59*	-		-0.57*	-	0.58*	nr	-	-
Temp	-0.62*	-0.61*	-		+	-0.64*	nr	+	+
pH	-	-	-	+		-	-	+	+
DO	0.58*	0.52*	0.58*	-0.64*	-		nr	-	-
Ammonia	nr	nr	nr	nr	nr	nr		nr	nr
Hardness	-	-	-	+	+	-	nr		0.55*
Alc	-	-	-	+	+	-	nr	0.55*	

* Significance level for Spearman's rho correlation test with probability *p-value < 0.050*. Where: Temp = Temperature (ºC); Alc = Alkalinity (mg L^{-1}), the other code variables are located at the table 1. Negative (-) and positive (+) signals represents the linear relations with $r^2 < 50$ %. nr = no significance relation.

Productivity Parameters

The Manova when applied in the growth variables: initial weight (IW), final weight (FW), weight gain (WG), final biomass (FB) and apparent feed conversion (AFC), shows significance differences between the treatments (F = 22.96 and *p-value < 0.050*). as a result, the Tukey test identified the occurrence of those differences only among the FB values (Table 3).

Table 3. Statistical results from the productivity variable average values and its respective Standard deviation.

Treatments	Weight (g) Initial	Weight (g) Final	Weight gain (g)	Final biomass (kg)	AFC
T1	22.80 ± 2.97 a	208.96 ± 17.43 a	168.16 ± 17.40 a	1.12 ± 0.10 a	2.49 ± 0.22 a
T2	23.35 ± 1.62 a	211.00 ± 26.72 a	187.65 ± 25.55 a	1.50 ± 0.20 ab	2.53 ± 0.36 a
T3	22.03 ± 3.34 a	196.32 ± 24.76 a	174.29 ± 25.39 a	1.74 ± 0.25 b	2.61 ± 0.38 a
T4	20.45 ± 1.42 a	210.10 ± 23.98 a	189.65 ± 23.78 a	2.28 ± 0.29 c	2.42 ± 0.31 a

Identical letters in the same column shows that the averages values did not differ statistically from the Tukey test, considering the probability p-value < 0.050.

Treatments Tanks Stocking Capacity

The final biomass (FB = y, g/m²) values used in the Pearson correlation analysis from the treatments, shows that when the stocking densities were elevated (SD = x, individuals/m²), the stocking capacity (SC) were quickly achieved in the rearing tanks, in a high linear relation level (r^2 = 0.83). Also the results exhibited that 100% of tested densities were attained the SC (0.4 to 0.8 kg fish/m^2) in the rearing tanks, immediately after the study got started (Figure 4).

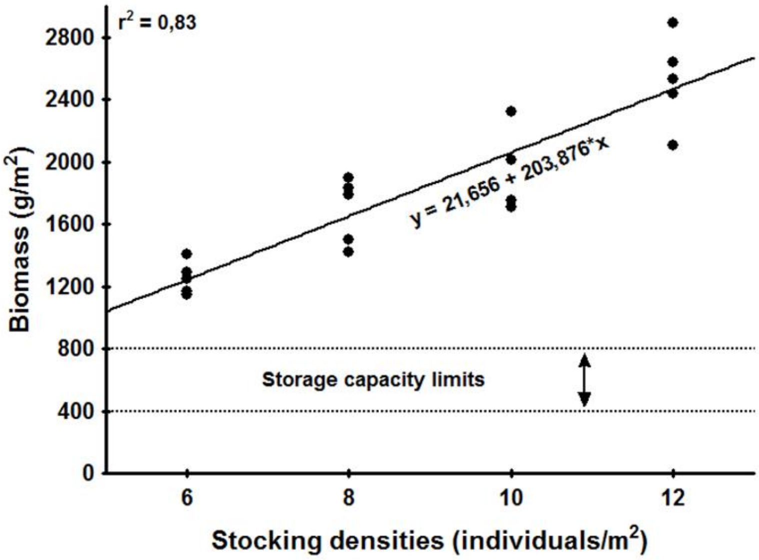

Figure 4. Linear relation between tambaqui biomass and stocking densities tested. The arrow shows the maximum and minimum stocking capacity limits for the rearing tanks.

Discussion

In the present study, the individuals of tambaqui did not present an apparent stress in the four experimental stocking densities, but a reduction in the growth parameters values was noticed in the final weeks of the study. This zootechnical pattern also was observed in others studies relative to fish farming modalities (Brandão, Gomes, Chagas & Araújo, 2004; Borges, Lopes, Silva & Nascimento, 2006), and can be explained primarily by the dependence of the fish to ideal environment conditions during the juvenile phase and not only related to available space (Santos, Andrade & Garcez, 2014). In this way, hypothetically, it could be presumed that an increase in fish stocking densities will not result in damage to the growth and weight gain for the tambaqui in this juvenile phase.

On the other hand, a decrease in the tambaqui growth parameters, during the final phase of the experiment (beginning of the growth out phase, when fish attained ≈ 250 g) that was related to the old individuals, became visible from the 11th to 26th weeks (Figure 3). Therefore, the values of variation in weight were more evident than that of length values. It is presumable that some effects not measured in this study, such as the climate phenomena, feed competition, or some opportunist disease, have affected the tambaqui farming zootechnical development, and could be interpreted as a stress indicator. Several studies reported that high fish stocking densities, when grouped in a reduced space, can be favorable to the occurrence of problems related to stress, and it could interfere negatively in the growth and development of individuals (Urbinati, & Carneiro, 2004). The differences noticed between fish growth values in the initial weeks (juveniles II) with those observed after the 10th week of farming, maybe related with differentiated growth between the tambaqui life phases, once the youngest individuals exhibited a faster development than the oldest ones (Le Cren, 1951; De Robertis & Williams, 2008; Satrawaha & Pilasamorn, 2009; Santos et al., 2012).

Fish zootechnical development information (weight and length) are the main variables observed in the cultivated individuals, and the adequate comprehension of this data is fundamental to the pisciculture proper management (Agostinho & Gomes, 1997). The growth behavior of the fish groups cultivated can be expressed by the “a” and “b” coefficient from the weight-length curve (Le Cren, 1951), where the first coefficient reveals the condition factor related with the environment, and the second shows the alometry score from the groups growth (Froese, 2006). This study did not consider the coefficient “a”, where the environmental variables and the differences among males and females fish were not measured. The results showed that the values of “b” varied among 2.75 and 2.77, were similar to the values found by the Tribuzy-Neto, Conceição, Siqueira-Souza & Freitas (2015) with b = 2.77, from individuals sampled in natural environment at the Amazon basin. Thus, this values were inferior to that found by Souza et al. (2000) with b = 3.00 and by Costa et al., (2013) with values of b = 2.81. These differences among the b values may be attributed to different factors such as: geographical fish distribution, fish farming production systems, or different individual age (Froese, 2006; De Robertis & Williams, 2008) and gender (De Mello et al., 2015). It is important to emphasize that the isometric growth (b = 3) is low frequent in nature (Silva-Júnior, Castro, Soares & França, 2007; Satrawaha & Pilasamorn, 2009; Santos et al., 2012).

Others factor as that related with the environment (Baldisserotto, 2013), may also influence the existence of these differences, once the values of limnological variables, such as hardness and ammonia exhibited differences between the experimental tanks. Conversely, the values of electrical conductivity and dissolved oxygen had a positive influence on the growth parameters, and its values keep into the intensities recommended for fish farming environments (Queiroz & Silveira, 2006).

The temperature was the variable that appeared to influence more negatively the fish growth parameters, showing average values around 27 ºC. Conversely, some studies affirm that the best tambaqui zootechnical development occurs in environments with temperature values among 26 to 30 ºC (Cavero, Rubim & Pereira, 2009; Baldisserotto, 2013). So, in this case, it was presumed that the others environmental parameters also contributed to attain in this results. The values of DO settled in the acceptable levels for fish farming in the tropical regions (Faria, Morais, Soranna & Sallum, 2013) instead, it was negatively influenced by temperature, with values proportionally inverse to the available DO values (Baldisserotto, 2013). Also, it needs to be taken in consideration that as the densities stocking increase the accessible DO decrease in the farming tanks (Oliveira et al., 2007).

In this context, the environmental parameters may influence the productivity values, which can reflect directly in the productions economic viability (Kubitza, Campos, Ono & Istchuk, 2012). Also, the existence of visible variations in the total ammonia values may have interfered weakly on the fish productive growth development. It was recognized that the ammonia control is relevant to the intensive fish farming success, because ammonia presents aggressive effects as chronics toxicities, the environmental branchial system disease, algae blood (Paerl & Tucker, 1995) and the oxygen consumption from the ammonium ions oxidation process (Pereira & Mercante, 2005).

The results revealed that the environmental variables did not influence significantly the values of tambaqui productivity. Moreover, were observed the existence of differences among the treatments final biomass values, which it is acceptable, once the biomass values increase proportionally to the fish stocking densities (Oliveira et al., 2007; Santos, Andrade & Garcez, 2014).

The values of apparent feed conversion, resulted from the 3% of food rate in relation of fish weight (FW), shows an average of 2.51 ± 0.31. Chagas et al., (2005) evaluated the tambaqui development (created in net-tanks) with individuals weighting about 55 g until the fish gained 200 g, using a dry food with 34% of brute protein (BP) fed in a rate about 5% (FW), which obtained an AFC about 2.90 ± 0.13, very similar to AFC values found in the present study. However, Chagas et al. (2005) e Chagas, Gomes, Martins Júnior & Roubach (2007) indicates the existence of an inverse relation between the AFC with the stocking density values.

The results show that stocking densities did not differ significantly among the weight gain and apparent feed conversion rates. However, a decreasing on the weight growth occurred after the tenth week of farming, indicating a possible overload of individuals in the experimental tanks, making implicit that the lower fish density (6 fish/m^2) could be the most appropriated to create the tambaqui during this period, once this stocking amount generated low environmental stress and rest near the stocking tanks capacity level indicated for tambaqui farming, that is 0.8 kg/m^2 (EMATER, 2006). However, the other density rates presented the biggest biomass, which infer in a positive economic viability, also those densities overloaded the stock capacity of the experimental tanks. In this way, the use of the biomass resulted from the 12 fish/m^2 became the most indicated to tambaqui farming between the phases from juveniles (≈ 10 g) to the beginning of growth out phase (≈ 250 g), corroborant with the results of Santos et al., (2014) that achieved with the same conclusion applying this density on the juveniles of tambaqui (≈ 10 g) during 42 days. In conclusion, if using the densities tested in this experiment, it is acceptable to assume that after the tenth week of farming, the fish need to be moved to larger tanks, in order to create an adequate production system.

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