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Food restriction in larviculture of *Colossoma macropomum* (Cuvier, 1818) reared in varying salinities

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ABSTRACT

Given the growing demand for tambaqui (*Colossoma macropomum*), economically sustainable protocols that guarantee quality in the larval stage are highly needed. Larviculture in low salinity environment can be used to reduce the daily rate of feeding, thus reducing costs. Food restriction can cause a decrease in fish growth, but it does not impair their capacity to grow if adequate feeding conditions are reestablished and if restriction is not held for too long. This study aimed at evaluating the effects of live food restriction on the growth performance and muscle morphometry of tambaqui larvae reared in different salinities. The 9,600 larvae were distributed in sixteen 60 L tanks, in a 2x2 factorial design, with two treatments, 0 and 2 g L^{-1} salinity (S0, S2) with food restriction of 50% (FR 50%) and without food restriction (FR 0%) of *Artemia* nauplii. After 15 days, no interactions were observed between the factors evaluated for production performance and muscle fibers of classes 10, 30, 40 and 50. However, the analysis of isolated factors demonstrated that the lower feeding rate reduced the performance of the larvae, while water salinity positively influenced performance and survival variables, and increased muscle fiber hyperplasia of class 20 in larvae submitted to food restriction. We conclude that live food deprivation of up to 50 % for tambaqui larvae reduces performance rates without compromising survival. To maintain growth performance and development of skeletal muscle, saline water (2g.L^{-1}) can be applied for tambaqui larviculture in situations of live food scarcity.

KEYWORDS: Feeding rate, saline water, productive performance, muscle morphometry, larvae

Restrição alimentar na larvicultura de tambaqui *Colossoma macropomum* (Cuvier, 1818) criados em diferentes salinidades

RESUMO

Dada a crescente demanda por tambaqui (*Colossoma macropomum*), protocolos economicamente sustentáveis que garantam qualidade no estágio larval são altamente necessários. A larvicultura em ambiente de baixa salinidade pode ser usada para reduzir a taxa diária de alimentação, reduzindo assim os custos. A restrição alimentar pode causar uma diminuição no crescimento dos peixes, mas não prejudica sua capacidade de crescer se as condições adequadas de alimentação forem restabelecidas e se a restrição não for mantida por muito tempo. O objetivo deste estudo foi avaliar os efeitos da restrição de alimento vivo no desempenho zootécnico e na morfometria muscular de larvas de tambaqui criadas em diferentes salinidades. Foram distribuídas 9600 larvas em dezesseis tanques de 60 L, em esquema fatorial 2x2, com dois tratamentos, salinidade 0 e 2 g L^{-1} (S0, S2) com restrição alimentar de 50% e sem restrição alimentar (FR 50% e FR 0 %) de náuplios de *Artemia*. Após 15 dias, não houve interação dos fatores avaliados no desempenho zootécnico e fibras musculares das classes 10, 30, 40 e 50. No entanto, a análise dos fatores isolados demonstrou que a menor taxa de alimentação reduziu o desempenho das larvas, enquanto a salinidade influenciou positivamente as variáveis de desempenho, sobrevivência e aumentou a hiperplasia de fibras musculares da classe 20 em larvas submetidas à restrição alimentar. Conclui-se que a privação de alimento vivo em até 50% reduz os índices de desempenho sem comprometer a sobrevivência. Porém, para manutenção do crescimento e desenvolvimento da musculatura esquelética, a água salinizada pode ser aplicada como estratégia em situações de escassez de alimento vivo.

PALAVRAS-CHAVE: Taxa de alimentação, água salina, desempenho, morfometria muscular, larva

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INTRODUCTION

Artemia nauplii are the most widely used live food for fish larvae (Shan et al. 2008; Das et al. 2012; Fosse et al. 2013), but they have limitations such as high acquisition cost and short shelf life in freshwater, which limits the use of this food (Biswas et al. 2006; Beux and Zaniboni-Filho 2006). Strategies such as the practice of larviculture in a low salinity environment can be used in feeding protocols as a way to reduce the daily rate of food offered and thus ensure a reduction in food costs (Ituassú et al. 2004; Pronob et al. 2012; Silva et al. 2019; Abe et al. 2019). Salinization of breeding water provides greater longevity to nauplii, which allows food availability in quantity and quality, thus ensuring food optimization (Silva et al. 2019; Araújo et al. 2020; Oliveira et al. 2020) and consequently greater growth performance (Jomori et al. 2012, 2013; Araújo et al. 2020). Both the quantity and quality of food (nauplii) in salinized water are justified by the fact that *Artemia* nauplii are marine microcrustaceans and their lifespan is longer in saline environments (Das et al. 2012).

Furthermore, fish larviculture in low salinity reduces energy expenditure, allowing energy to be channeled towards muscles (Wieser 1995; Fabregat et al. 2017; Mattioli et al. 2017; Araújo et al. 2020), to the hyperplastic and hypertrophic growth of muscle fibers (Assis et al. 2004; Takata et al. 2018, 2021). Muscle growth occurs through hypertrophy (increase in size) and/or hyperplasia (increase in the number of cells) (Rescan 2005; Johnston et al. 2009, 2011; Valente et al. 2013). Hyperplasia occurs as a mosaic that is frequently observed in the adult phase, and by stratification, characterized by proliferation in the cellular zone in the dorsal and ventral regions and predominates in the larval phase for the majority of altricial species (Johnston 1999, 2006; Johnston et al 2009; Leitão et al. 2011).

Tambaqui (*Colossoma macropomum*) is among the most produced cultivated native fish species in Brazil. With a percentage increase of 12.17% (>113 thousand tons in 2023) of all native fish production, becoming the first most produced native species in Brazil (IBGE, 2022). The growing production is mainly due to its characteristics such as hardiness, fecundity, ease of handling in captivity and attractiveness to the consumer market (Pedroza Filho et al. 2016). Combined with the growing production of this species, the development of economically sustainable protocols that guarantee quality in the larval stage becomes a promising challenge in order to offer the market quality juveniles in the desired quantity, thus maximizing productivity in the country.

Therefore, the present study aimed at evaluating the productive performance and muscle growth of tambaqui larvae in response to partial live food restriction, combined with varied water salinities (fresh water or low salinity).

MATERIAL AND METHODS

The experimental procedures were approved by the Ethics in Animal Use Commission (CEUA) of Nilton Lins University under Protocol Nº. 011-2021. A total of 25.000,00 Tambaqui larvae (3 days post-hatching - dph) were acquired from a commercial fish farm Santo Antônio in the municipality of Rio Preto da Eva, Amazonas, and were transported (3h hours travel) to the Laboratory of Nutrition of Aquatic Organisms at Nilton Lins University, Manaus, Amazonas, in a plastic bag with a volume of 80 L, filled with 1/3 of water and the rest with oxygen, at a density of 1000 larvae L^{-1}, transport lasted 3h. The larvae were acclimatized for 4 days into two polyethylene tanks of 200 L, each with a recirculation system and a temperature of 28.3 ± 0.3ºC, dissolved oxygen of 7.4 ± 1.0 mg L^{-1} and pH of 7.2 ± 0.4 mg L^{-1}, and were fed with *Artemia* nauplii (100 nauplii larva day^{-1}).

A total of 9,600 larvae with 7 dph (0.4 ± 0.2 g; 6.02 ± 0.41 mm) were distributed at a density of 10 larvae L^{-1}, in sixteen tanks 60 L with a recirculation system consisting of a filter with 60 L tank for each treatment, pump (XT-900, 900L.h^{-1}) with flow rate of 220L.h^{-1} in each tank. The biological filter was composed of acrylic blanket and a mechanical filter composed of expanded clay, gravel to maintain alkalinity, heating thermostat in the filtration tanks and additional constant aeration. The experiment was conducted in a completely randomized design in a factorial scheme (2x2) with the following salinity treatments: fresh water (S0) and saline water with 2g L^{-1} of NaCl (S2); and with or without food restriction of *Artemia* nauplii (FR 50% and FR 0%), with four replicates per treatment. Leftovers on the bottom of the tanks were removed daily by siphoning, and 50 % of the total water of the filter tank was changed. Initially, the salt was added gradually from the filter box over a period of 3 hours until the desired quantity (2 g/L) was reached and maintained throughout the experimental period in tanks of S2 treatment.

The temperature (28.1 ± 0.5ºC), dissolved oxygen (5.7 ± 0.1 mg L^{-1}), pH (6.1 ± 0.2 mg L^{-1}), conductivity (82.9 ± 85.7 S m^{-1}) and ammonia (0.6 ± 0.1 mg L^{-1}) were monitored daily, and nitrite (0.2 ± 0.2 mg L^{-1}) and alkalinity (46.5 ± 7.9) were monitored every 15 days. During the 15 experimental days, the larvae were fed exclusively with newly hatched *Artemia* nauplii (Bio-Artêmia, Grossos, Rio Grande do Norte, Brazil), six times a day (8, 10, 12, 14, 16 and 18 h), in increasing amounts following the adapted protocol proposed by Jomori et al. (2003): larvae supplied during the first 3 days with 150 and 75 nauplii day^{-1}; 4th to 6th day with 250 and 125 nauplii day^{-1}; 7th to 9th day with 500 and 250 nauplii day^{-1}; 10th to 12th day with 700 and 350 nauplii day^{-1}; 13th to 15th day 1200 and 600 nauplii day^{-1}, in treatments without (FR 100%) and under food restriction (FR 50%), respectively.

On the 15th day of the experiment, 30 larvae per experimental unit were sampled, euthanized, and then fixed

in 10% formalin for later measurements of weight (W) in an analytical balance (Bel® M214Ai Bel - 0.0001g) and total length (L) with a digital caliper (Adoric® KC24 - 0.02 mm). The average values were used to calculate weight gain (WG, in g)= Wf – Wi), Length (mm) (Lf – Li) measured from the tip of the snout to the end of the axial skeleton, specific growth rate (SGR, in % day $^{-1}$)= (ln Wf – ln Wi /number of experimental days) *100, survival (S, in %) = 100 × Nf/(Ni – N sampled fishes), with N indicating number, i and f indicating initial and final measurements, respectively.

For muscle development analysis, whole fish were fixed (n = 3 per treatment) in formalin 10 %, washing with Sorensen's phosphate buffer (pH 7.2), dehydrated in solutions with increasing alcohol concentrations (70 to 90%), and embedded in resin (Historesin®, Leica, Germany). Histological cuts (3 µm) were made transversely using a microtome and the slides were stained with hematoxylin phloxine B (HP). Images were captured using an optical microscope coupled to a digital camera MICRON® CMOS12MP (12.0 capture) with a 40x objective. The pro-plus 4.1.0.0 image program was used for measurement. The area values of 500 fibers per larva were measured in the deep regions along the entire lateral side of the dorsum and the diameter was calculated using the equation $d=2\ (A^{0.5} \times \pi^{-0.5})$ (Valente et al. 1999). Hyperplasia and hypertrophy were then classified following the methodology proposed by Assis et al. (2004) into the following classes according to the diameter (d, in µm): 10 (d ≤ 10), 20 (10 < d ≤ 20), 30 (20 < d ≤ 30), 40 (30 < d ≤40) and 50 (d > 40).

All analyses were performed using R studio 4.1.0 (2021), data were submitted to homoscedasticity tests (Levene's test) and residual normality (Kolmogorov-Smirnov). Logarithm (log x) data transformations were applied when necessary. Subsequently, a two-way ANOVA was performed, which was followed by comparison of means using Tukey's test at 5% probability. For data that did not fulfill the assumptions of ANOVA, the non-parametric Kruskal-Wallis test was applied.

RESULTS

At the end of 15 days of experiment, no interactions were observed between the factors studied (salinity and feeding rate) for the productive performance variables (W, WG, L and survival). However, salinity positively affected W, WG, C, SGR and survival ($p < 0.05$) with higher means in larvae raised in salinized water (2 g L-1). Meanwhile, partial food restriction led to a reduction ($p > 0.05$) in the means of all four performance variables, with no effect on survival (Table 1).

At the end of the feeding period with *Artemia* nauplii, an increase in the number of muscle fibers (hyperplasia) and their diameter (hypertrophy) was observed in the muscles of tambaqui larvae of all treatments, forming a typical aspect of mosaic hyperplasia (Figure 1). No interactions were observed between salinity and feeding restriction on this response, and the tested factors did not affect the morphometry of the muscle fibers in 10, 30, 40 and 50 classes (Table 2). The interaction between the factors was observed only in fibers of class 20, with a greater frequency ($p < 0.05$) in larvae reared in a saline environment and under feeding restriction (Table 2).

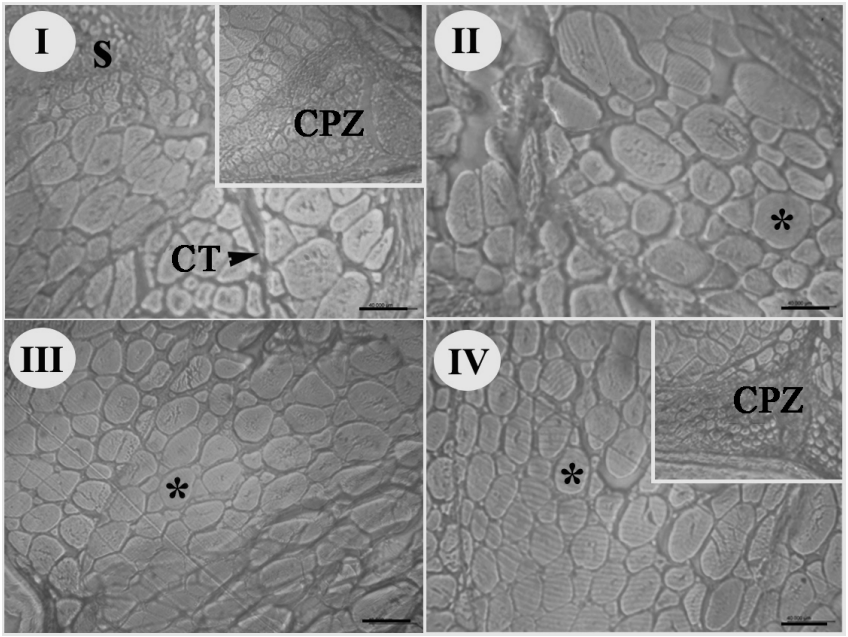

Figure 1. Photomicroscopy of the cross-section of *Colossoma macropomum* skeletal musculature larvae 15 days after feeding on *Artemia* nauplii. FR 0%: I, II; FR 50%: III, IV. Freshwater (S0): I, III; Saline water (S2): II, IV. Superficial compartment (S); larger fibers surrounded by smaller fibers (*); connective tissue (CT); cell proliferation zone (CPZ); hematoxylin and phloxine B staining. Bars correspond to 40 µm.

Table 1. Mean values (± standard deviation) of performance metrics for *C. macropomum* larvae reared under different restricted feeding rates (FR 50% and 0%) and water salinity (S0 and S2) for 15 days.

	W (mg)	WG (mg)	L (mm)	SGR (%)	SR (%)
Freshwater (S0)	20.1±5.5*^{b}	19.6±4.9*^{b}	11.4±0.7*^{b}	24.8±1.9*^{b}	97.7±0.4*^{b}
Saline water (S2, 2 g L $^{-1}$)	24.6±6.8*^{a}	24.1±6.8*^{a}	12.3±0.9*^{a}	26.5±1.6*^{a}	98.7±0.3*^{a}
FR 50%	17.5±2.4*^{d}	17.1±2.4*^{d}	11.2±0.5*^{d}	24.5±1.5*^{d}	98.2±0.7
FR 0%	27.1±5.4*^{c}	26.7±2.4*^{c}	12.3±0.7*^{c}	26.9±1.5*^{c}	98.1±0.5

Letters a and b compare salinity; letters c and d compare FR; ns - not significant; * Significant at 5% probability. weight (W), weight gain (WG), length (L), specific growth rate (SGR) and survival (SR)

Table 2. Means (± standard deviation) and main effect in frequency of diameter classes of muscle fibers in *C. macropomum* larvae submitted to different levels of food restriction (FR) and salinity (S) for 15 days; Breakdown of the interaction between food restriction (FR) and salinity (S), Mean values (± standard deviation) for frequency (%) of muscle fiber diameter classes (class 20 = 10<d≤20 µm) of *C. macropomum* larvae at 15 days.

	Classes (%)			
	10	**30**	**40**	**50**
Water Treatment				
Freshwater (S0)	29.4±15.3^{ns}	22.4±12.7^{ns}	10±5.3^{ns}	2.9±1.9^{ns}
Saline water (S2, 2g L $^{-1}$)	27.8±12.4^{ns}	27.1±8.8^{ns}	8.8±4.2^{ns}	1.9±1.7^{ns}
Food Restriction				
FR 50%	24.32±15.1^{ns}	25.42±12.0^{ns}	11.1±5.7^{ns}	2.7±2.5^{ns}
wFR 0%	32.97±10.8^{ns}	20.85±9.0^{ns}	7.7±2.7^{ns}	2.9±1.9^{ns}
Breakdown of interaction **				
		S0	**S2**	**Mean**
Class 20	FR 50%	31.7±4.8*^{ad}	41.4±7.5*^{ac}	36.5±7.8*
	FR 0%	38.9±4.6*^{ac}	33.6±4.3*^{ad}	36.3±5.0*
	Mean	35.3±5.1^{ns}	37.5±5.5^{ns}	

Classes 10, 30 and 50 non-parametric analyses; ns - not significant at 5% probability. Class 10 = d≤10; class 30 = 20<d≤30; class 40 = 30<d≤40, class 50 = 40<d≤50.
** Means followed by letters a and b in the rows compare salinity and do not differ by Tukey's test at 5% probability; letters c and d in the column compare FR; * Significant.

DISCUSSION

Given the limitations in nutritional management at the larval stage of fishes, *Artemia* nauplii are the main live food used in most of the freshwater fish intensive larviculture. However, the cost and management make their use challenging. Therefore, it is essential to seek strategies to optimize and make food viable at this stage as a way to sustain the practice of larviculture. In this study, we demonstrated that salinized water was associated with a higher performance of the tambaqui larvae, suggesting a potential practical protocol for tambaqui larviculture when live food availability is low.

In the present study, the salinized water promoted higher means of performance in tambaqui larvae. Similar to the findings of the present study, the use of salinity up to 2% in larviculture led to a positive effect on performance of in different species *Piaractus mesopotamicus* (Jomori et al. 2012), *Arapaima gigas* (Silva et al. 2019), *Lophiosilurus Alexandre* (Luz and Santos 2008) and survival in different species *Pyrrhulina brevis* (Pinheiro Junior et al. 2023), *Alosa pseudoharengus* and *Alosa aestivalis* (DiMaggio et al. 2016). Nutritional management with an emphasis on the initial concentration of prey (Santos and Luz 2009; Takata et al. 2014) and environmental management linked to the practice of larviculture in low-salinity water (Jomori et al. 2013; Santos et al. 2021), resulted in food optimization in different freshwater species *Nannostomus beckfordi* larvae (Abe et al., 2019), *Arapaima gigas* larvae (Silva et al., 2019), *Heros severus* (Oliveira et al., 2020). These results suggested the higher availability time of *Artemia* nauplii (Jomori et al. 2012), reduction in osmotic differences in environment and larvae plasma that influences ion losses and water absorption (Fabregat et al. 2015). This reduced energy expenditure (Patel

et al. 2022) and consequently causing the redirection of energy to growth (Baldisserotto et al. 2007; Jomori et al. 2012, 2013; Ferreira et al. 2023).

The amount of food offered during intensive larviculture is an important requirement to optimize the quality of food and water and consequently maximize production and ensure the reduction of food production costs at this stage (Santos et al. 2015; Estévez et al. 2019). However, the amount of food available (Menossi et al. 2012; Santos et al. 2012) and locomotion (Rønnestad et al. 2013) constitute limiting factors in larval food consumption. Excessive amounts or amounts below those required by the species can compromise metabolism (Rios et al. 2004; Pérez-Jiménez et al. 2012; Melo et al. 2020) and growth performance (Santos and Luz 2009; Araújo et al. 2020; Santos et al. 2022; Ferreira et al. 2023). Our results show a decrease in performance of tambaqui larvae submitted to 50 % food restriction. Even with a reduction of 35 % in weight, the survival rate and muscle fibers development of classes 10, 30, 40 and 50 were not compromised, likely because the energy required during locomotion for feeding activities is greater, especially in situations of low prey concentrations (Abe et al. 2019, 2021; Reis 2021). The severity of food restriction can compromise growth performance (Santos and Luz, 2009; Oliveira et al. 2020; Abe et al. 2021), development of digestive tract (Xiong et al. 2006) as well as survival (Abe et al. 2021). The larval stage is considered an adaptive period where energy reserves are reduced (Bar 2014), in a situation of food deprivation the energy is channeled for metabolic activities instead of productive performance (Ali et al., 2003; Abe et al. 2021) and muscle growth (Liu et al. 2020.; Melo et al. 2020), or cause higher mortality rate once they reach the point-of-no-return, when the effects of food restriction is irreversible (Blaxter and Hempel 1963). However, depending on the severity of restriction, fish larvae can have their growth performance restored by compensatory growth after normalization of food availability (Kojima et al. 2015).

Muscle growth during the larval stage occurs through hyperplasia and hypertrophy (Assis et al. 2004; Leitão et al. 2011), but many species of fish in this phase demonstrate muscle fibers development mainly through hyperplasia (Almeida et al. 2008; Dal Pai et al. 2000; Leitão et al. 2011; Ostaszewska et al. 2008; Kojima et al. 2015). In the present study the presence of higher amounts of smaller diameter fibers of class 20 in tambaqui larvae reared in salinized water ($2g.L^{-1}$) and 50% of food restriction indicate hyperplasia (Assis et al. 2004; Johnston et al. 2011; Nebo et al. 2013; Valente et al. 2013; Vélez et al. 2017), which can be attributed to the effect of energy use for the purpose of producing muscle fibers (Johnston 2006; Takata et al. 2021).

The physiological adaptations for osmoregulation in salinized water depend on the fish species (Altinok and Grizzle 2003). In higher concentrations up to 5 ppt of NaCl the energy expenditure for osmoregulation and dehydration result in muscle fibers' diameter and growth reductions in *Lophiosilurus alexandri* juveniles (Takata et al. 2021); and in early larval stages of *Oreochromis niloticus* a salt concentration of 4 and 6 $g.L^{-1}$ resulted in alterations to the yolk-sac surface and damage to skeletal muscle (Melo et al. 2019), while in *Piaractus mesopotamicus* larvae salt concentrations up to 6 ppt cause decrease in growth an survival rates (Jomori et al. 2012). In this study, the concentration of $2g.L^{-1}$ of NaCl may have led to a decrease in the energy consumed by osmorregulation, resulting in increased growth performance and not compromising the development of muscle fibers in the tambaqui larvae submitted to food restriction.

CONCLUSION

We conclude that the management of live food and saline water can be utilized as a strategy for the larviculture of tambaqui. According to the feeding protocol presented herein, the deprivation of live food by up to 50 % for larvae reared in saline water at 2 $g.L^{-1}$ can be adopted without compromising survival or the development of muscle fibers in tambaqui juveniles.

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