Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v43i1.52054

ZOOLOGY

Post-larval *Colossoma macropomum* (Characiformes, Serrasalmidae) show better performance in excavated than concrete tanks under different feeding strategies

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ABSTRACT. As the global human population increases, the demand for food grows and, consequently, practices such as aquaculture have become more common. *Colossoma macropomum* (Cuvier, 1818) is a native Amazonian species, considered to be the second most cultivated fish in the country. We compared the development of post-larval *C. macropomum* of different ages, submitted to combinations of food management on a commercial production scale. Two experiments tested the delivery of i) 55% crude protein feeding, ii) natural feeding by fertilizing the water and iii) a combination of both during hatchery in concrete tanks (10 m ^{2}) or excavated soil-bottom tanks (450^2) subjected to distinct fertilization protocols and storage densities. The weight and length of the post-larvae grown in ponds were greater ($p < 2.0x10^{-16}$) for the mixed treatment, except during the first week of larvae, in which values were similar ($p \leqslant 1.76x10^{-14}$) to the fertilization treatment. Concrete tanks with fertilization management without feeding were similar to the mixed treatment ($p \leqslant 1.38x10^{-7}$); however, during the first week of external larvae production, the growth performance under fertilization treatment was superior to the others. Food management in excavated tanks, when compared to the same management performed in concrete tanks, registered higher averages for the productive variables of *C. macropomum* cultivated at the density of 200 post-larvae m^{-2} in all evaluated food strategies. It was verified that post-larvae of *C. macropomum* did not develop well in the first weeks of life when receiving only formulated diets. The increase in natural food availability through fertilization positively influenced the performance of the species, which can remain without feeding until the second week of life.

Keywords: larviculture; fish farming; tambaqui; diet; fertilization.

Received on February 3, 2020.
Accepted on September 17, 2020.

Introduction

As the global human population increases, the demand for food has grown and, consequently, practices such as aquaculture have become common (Pillay & Kutty, 2005). After the 1980s, fish production increased at an average annual rate of 8.6%. *Colossoma macropomum* (Cuvier, 1818), popularly known as tambaqui, is a native Amazonian species (Froese et al., 2016), considered to be the one of the most cultivated fish in Brazil, accounting for 28.1% of total production. Currently, 78.6% of the cultivation of this species occurs in the Northern region, mainly in the state of Rondônia, which accounts for 47.7% of national production (*Instituto Brasileiro de Geografia e Estatística* [IBGE], 2014).

There are many factors that make the production of *C. macropomum* one of the largest in the country, namely: favorable characteristics for cultivation, good adaptation to captivity, rapid growth, meat that is highly valued on the market (Mendonça et al., 2009; Faria & Morais, 2013), and resistance to low dissolved oxygen levels (Saint-Paul, 1984) and to ammonia toxicity (Ismiño-Orbe, Araújo-Lima, & Gomes, 2003). In addition, the species presents easy acceptance of formulated diets (Oishi, Nwanna, & Pereira Filho, 2010; Pereira-Júnior, Barbosa, Shimoda, & Pereira-Filho, 2013; Araújo-Dairiki, Chaves, & Dairiki, 2018) and high stocking densities (Silva, Pereira-Filho, & Oliveira-Pereira, 2000; Sousa & Salles, 2015), as well as excellent performance for cultivation in different production systems (Arbeláez-Rojas, Fracalossi, & Fim, 2002; Gomes, Brandão, Chagas, Ferreira, & Lourenço, 2004; Gomes et al., 2006, Silva & Fujimoto, 2015). According to Zaniboni-Filho, Pedron, and Ribolli (2018), this species is the most promising native species cultivated in Brazil.

Despite its important participation in national aquaculture production, there is still no definite technological package for its larviculture, and the technical information available is based on the experiences of fish farms around the country. The production of larvae and fingerlings occurs on a commercial scale, but it presents low performance and high mortality rates, mainly due to factors related to food and nutrition (Sipaúba-Tavares & Rocha, 1994; Cestarolli, Portella, & Rojas, 1997; Prieto, Logato, Moraes, Okamura, & Araújo, 2006).

Preliminary studies on the selectivity of post-larvae of *C. macropomum* in laboratory conditions show that zooplanktonic organisms are a good food resource (Sipaúba-Tavares & Rocha, 1993). In another study, it was observed that the growth and survival rates for tambaqui and pacu post-larvae fed with natural plankton were higher (Sipaúba-Tavares & Rocha, 1994). It has also been observed that the water hyacinth can act positively as an organic fertilizer in food for post-larvae of these species (Sipaúba-Tavares & Braga, 2007).

Recent studies by Almeida, Moura, Moreira, and Franco (2006) characterized the embryonic development and compared the performance of post-larvae submitted to feeding with branchoneta paste, powdered milk, albumin, and *Artemia salina* cysts decapsulated in incubators. Pedreira, Schorer, and Ferreira (2015) experimentally evaluated the use of plankton and feed combinations.

Studies on the initial feeding of post-larval *C. macropomum* have not caught up with the fast-growing production of native species in Brazil. Studies have been developed under experimental conditions. The present study compared *C. macropomum* performance at different ages during the post-larval stage, under several feeding management regimes on the commercial production scale.

Material and methods

Two experiments (1 and 2) were carried out at the Rodolpho von Ihering Fisheries Station, Bahia Pesca S/A, Pedra do Cavalo Hydroelectric Plant, Cachoeira Municipality, state Bahia, Brazil (14° 34´ 51” S and 38° 59´ 43” W), over two reproductive cycles of the species.

Experiment 1

In the first experiment, three excavated basin tanks were used, with an area of 450 m^2 each, one meter deep, densities of 200 post-larvae of *C. macropomum* per m^2 of the same brood (initial weight: 3.6±1.4 mg, initial length:7.1±2.5 mm), with replacement of water losses by evaporation and infiltration.

The excavated tanks were prepared for larviculture in the following way: (i) total emptying of tanks to expose the tank-bottom to the sun for one week; (ii) five days prior to settlement with the *C. macropomum* post-larvae, the tanks were filled and fertilized with 5 urea and 10 gm^{-2} wheat bran, to increase the yield of the tanks and, consequently, the increase of endogenous natural food (plankton) (adapted from Chagas, Gomes, Martins Júnior, Roubach, & Lourenço, 2005). Breeding females spawned on the day after hormonal induction and, after hatching in the incubators, the larvae were transferred to 1000 L internal tanks, where they remained for the first five days. In their second week of life, the post-larvae were transferred to the external excavated tanks, starting the first experimental period.

For five weeks, the following dietary strategies were evaluated in the excavated tanks (ET): supply of commercial fish-food with 55% crude protein (ET R), in the first week of culture only using water fertilized with 5 urea and 10 gm^{-2} of wheat bran (ET F); the third pond was fertilized and post-larvae fed with fish-food (ET F + R).

Experiment 2

In the second experiment, three concrete tanks were used, with an area of 10 m^2, one meter deep and density of 300 post-larvae of *C. macropomum* per m^{-2} from the same brood (initial weight: 1.1 ± 0.5 mg; initial: 6.2 ± 0.5 mm), with replacement of water losses by evaporation and infiltration.

The concrete tanks were managed according to the following initial protocol: (i) total emptying of tanks to expose the tank-bottom to the sun for one week; (ii) the tanks were filled two days before settlement with the post-larvae. After hatching in the incubators, where they remained in the first seven days, the post-larvae were transferred to the external larval tanks, beginning the second experimental period.

In a similar way to the first experiment, the treatments in concrete tanks (CT) were: CT R with commercial fish-food including 55% crude protein; CT F and TC F + R with fertilization two days before the settlement of the tanks with 2 of urea and 40 g m^{-2} of wheat bran; in the CT F treatment, fertilization was carried out with 20 g m^{-2} of wheat bran during the first two weeks of larviculture in the tanks.

Data collection and analysis

The water's physical and chemical parameters (temperature, pH, dissolved oxygen, total dissolved solids and electrical conductivity) were monitored daily with the aid of a multiparameter probe (HI 9828, Hanna, Póvoa de Varzim, Portugal). For five weeks, 20 fish were collected in each excavated tank, every seven days, totaling 300 individuals, and 30 fish in each concrete tank, totaling 450 individuals. Post-larvae and juveniles were collected at the same time (9:30 am), using horizontal trawls with conical plankton netting, with a mesh size of 200 μm for 21-day-old individuals, and with a rectangular net with 500 μm aperture meshes between nodes, for post-larvae from the fourth week life. After capture, fish were euthanized with 25 mg L^{-1} benzocaine solution. The specimens were fixed in 10% formaldehyde solution (Sipaúba-Tavares & Braga, 2007) and stored in 500 mL polyethylene pots to be transported to the Aquaculture and Aquatic Ecology Laboratory (LEAAq) at *Universidade Federal do Recôncavo da Bahia* (UFRB), where the biometry (measurement of total length and wet weight) was performed.

To analyze the productive performance of the species from the biometric data, the following parameters were calculated, according Equation 1 and 6:

$$Mean\ \mathrm{weight} = \frac{total\ final\ weight}{number\ of\ postlarvae} \quad (1)$$

$$Mean\ weight\ gain = final\ mean\ weight\ -\ initial\ mean\ weight \quad (2)$$

$$Rate\ of\ weight\ gain = \frac{average\ weight\ gain}{time\ (weeks)} \quad (3)$$

$$Mean\ length = \frac{total\ final\ length}{number\ of\ post - larvae} \quad (4)$$

$$Mean\ growth =\ Final\ mean\ length\ -\ Initial\ mean\ length \quad (5)$$

$$\mathrm{Growth\ rate}\ = \frac{Average\ growth}{time\ (weeks)} \quad (6)$$

Biometric data were transformed by the Box-Cox ($(x \wedge \lambda - 1) / \lambda$, with $\lambda \neq 0$) function, subjected to the analysis of residual normality (Shapiro – Wilk test) and homoscedasticity of variances (Bartlett's test), followed by analysis of variance (ANOVA), also applied to water quality data after confirmation of the assumptions required by the analysis. When the significant difference between the treatments was observed and the interaction between the treatments and the age of the post-larvae was confirmed, the Tukey test was applied for the multiple comparison between the means, considering the significance level of 5% ($p \leqslant 0.05$). All statistical analyses were performed with software R, version 3.2.1 (R Core Team, 2015).

Results and discussion

In excavated tanks, the water temperature ranged from 29.52 to 29.82°C; dissolved oxygen concentration ranged from 6.89 to 7.66 mg L^{-1}; the pH ranged from 8.27 to 8.58; electrical conductivity ranged from 174.75 to 278.25 μS cm^{-1}; and total solids ranged from 98.75 to 186.00 ppm. TE R showed full transparency throughout the experiment. While in concrete tanks, the minimum water temperature was 26.84 and the maximum 29.96°C; minimum dissolved oxygen concentration 7.90 and the maximum 9.27 mg L^{-1}; pH values ranged from 8.04 to 8.27; the electrical conductivity of 276.5 and 371.3 μS cm^{-1}; and for total solids the values ranged from 158.4 to 179.6 ppm Total ammonia concentration ranged from 0.0 to 0.3 mg L^{-1} (Table 1).

The mean variables related to weight and length of *C. macropomum* post-larvae cultivated in excavated tanks were higher ($p < 2.0 \times 10^{-16}$) without mixed treatment (ET F + R), followed by fertilization of the tanks (ET F) and feeding (ET R) (Table 2).

Monitoring the productive performance of farmed fish constitutes a fundamental tool to determine the economic viability of the enterprise (Xavier, Brandão, Silva, Brandão, & Souza, 2016; Machado et al., 2018). In the excavated tanks, the combination of natural feeding with the fish-food provided better fish development, on average. Several authors have obtained better performance for post-larval fish through the combination of natural and artificial diets (Feiden, Hayashi, & Boscolo, 2006; Meurer, Boscolo, Hayashi, & Wolf, 2008; Moreira et al., 2011; Pedreira et al., 2015; Caldini, Cavalcante, Rocha Filho, & Carmo e Sá, 2015), and satisfactory results with the use of organic and/or inorganic fertilization to increase natural food in the tanks (Azim & Little, 2006; Sipaúba-Tavares & Braga, 2007).

Table 1. Mean values ± standard deviation of water physical and chemical parameters during cultivation of *C. macropomum* post larvae in excavated tanks (ET) and concrete tanks (CT).

	Temperature (°C)	pH	Dissolved oxygen (mg L^{-1})	Electric conductivity (µS cm^{-1})	Total dissolved solids (ppm)	Secchi Disc Depth (cm)
ET R	29.70 ± 2.05	8.27 ± 0.50	7.35 ± 1.7	278.25 ± 189.22	186.00 ± 22.87	100.0 ± 0.0
ET F	29.52 ± 2.31	8.28 ± 0.93	6.89 ± 0.63	232.00 ± 200.96	174.00 ± 2.83	65.0 ± 8.7
ET F+R 2	29.81 ± 2.23	8.58 ± 1.00	7.66 ± 0.83	174.75 ± 201.83	98.75 ± 15.48	70.0 ± 17.3
CT R	26.96 ± 0.97	8.04 ± 0.74	7.90 ± 0.73	276.50 ± 117.57	158.40 ± 58.91	65.4 ± 5.6
CT F	29.96 ± 1.01	8.27 ± 1.33	7.97 ± 0.93	371.3 ± 35.67	179.60 ± 15.14	62.0 ± 11.0
CT F+R	26.84 ± 1.03	8.06 ± 1.00	8.27 ± 0.54	324.13 ± 23.66	162.00 ± 11.78	59.5 ± 11.2

Table 2. Mean of weight variables and body measurements of *C. macropomum* post-larvae submitted to different feeding strategies in excavated tanks (ET) for five weeks.

Parameter	Treatment		
	ET R	ET F	ET F+R
Final mean Weight (mg)	124.30c	126.70b	295.70a
Mean weight (mg)	41.48c	61.74b	135.86a
Mean Weight Gain (mg)	120.70c	123.10b	292.10a
Weight gain rate (mg $week^{-1}$)	24.10c	24.60b	58.40a
Final mean Length (mm)	16.9c	17.3b	23.7a
Mean Length (mm)	12.4	14.2b	17.5a
Mean growth (mm)	9.8c	10.2b	16.6a
Growth Rate (mm) $week^{-1}$)	2.0c	2.0b	3.3a

ET R = ration; ET F = fertilization; ET R + F = feed and fertilization. *Equal letters on the same line do not differ from each other by the Tukey test (p > 0.05), n = 300.

In both evaluated environments, the lowest means for the productive performance variables were obtained in the treatments with only fish-food, evidencing post-larvae's low capacity to assimilate the fish-food offered. This result corroborates those found by Feiden et al. (2006), Pedreira, Santos, Sampaio, Pereira, and Silva (2008), and Pedreira, Sollenberger, and Mislevy (2000). According to Kolkovski (2001), species presenting altricial larvae, such as *C. macropomum*, are unable to use nutrients from artificial diets satisfactorily, because they have a small vitelline reserve and a morphologically incomplete digestive system at the beginning of exogenous feeding. Silva, Pereira-Filho, Cavero, and Oliveira-Pereira (2007) observed that juveniles of this species (2.81 ± 0.52 cm and 0.41 ± 0.25 g) consumed fish-food more intensely after 21 days of cultivation, but consumed zooplankton and insects during the whole experimental period.

Although artificial food is important for growth, post-larvae begin to ingest it only after full digestive tract formation (Prieto & Atencio, 2008). The advantages of live food over inert are in the variety of particle size, stability in the aquatic environment and the ease of being detected and captured by the post-larvae by visually and chemically stimulating them, as well as not compromising water quality (Tesser & Portella, 2006; Furuya, Hayashi, Furuya, Soares, & Galdioli, 2008). Tesser and Portella (2006) also affirm that the chemical and visual stimuli of zooplankton can raise inert food ingestion rates and reduce the feeding period of fish larvae.

The interaction between treatments and post-larval age was significant for weight ($p = 1.13x10^{-14}$) and length ($p = 1.76x10^{-14}$) variables. This showed that in the first week of external larviculture the performance of the ET F and ET F + R treatments was similar and superior to the ET R treatment (Table 3).

Table 3. Final mean weight and final average weekly length of *C. macropomum* post-larvae submitted to different feeding strategies in excavated tanks (ET) for five weeks.

	Final mean Weight (mg)			Final mean Length (mm)		
	ET R	ET F	ET F+R	ET R	ET F	ET F+R
Initial	3.60a	3.60a	3.60a	7.1a	7.1a	7.1a
Week 1	10.58b	25.10a	27.00a	10.0b	12.1a	11.8a
Week 2	24.90c	30.80b	89.80a	11.6c	12.1b	15.9a
Week 3	19.30c	60.10b	137.70a	11.4c	14.7b	18.8a
Week 4	28.30c	66.00b	129.10a	12.3c	15.0b	17.6a
Week 5	124.30c	126.70b	295.70a	16.9c	17.3b	23.7a

ET R = ration; ET F = fertilization; ET R + F = feed and fertilization. *Equal letters on the same line do not differ from each other by the Tukey test (p > 0.05), n = 300.

Feeding after the first week of external larviculture proved satisfactory, because the supply of natural food (plankton supplied as a protein source to accelerate post-larvae growth) was maintained through inorganic fertilization of the excavated tanks, avoiding feed waste. Similar results were found by Senhorini and Fransozo (1994), where weight gain and survival of pacu fish were associated with fertilization treatments. On the other hand, in the concrete tanks, the mixed diet (CT F + R) presented a statistically significant result for productive performance, and this was statistically equal to the treatment that only consisted of fertilization without feeding (CT F) (Table 4). This may indicate that the weekly process of fertilization with wheat bran in the first two weeks of external larvae provided an adequate supply of natural food for the post-larvae at the density of 200 post-larvae m^{-2}. If natural feed is available, post-larvae prefer it, and the feed will act as a fertilizer (Beyruth, Mainardes-Pinto, Fusco, Faria, & Silva, 2004). Sipaúba-Tavares and Rocha (1994), when evaluating the use of natural plankton by post-larval pacu (*Piaractus mesopotamicus*) and tambaqui (*C. macropomum*), grown in aquariums, observed exponential growth for both species. On the other hand, Lopes, Senhorini, and Soares (1994) found that the addition of feed to plankton impaired the growth of matrinxã (*Brycon cephalus*) post-larvae.

When comparing the development of post-larvae grown in excavated tanks and concrete tanks, the excavated tanks provided the best results due to the greater availability of natural feed. This had been increased with fertilization five days before introducing the post-larvae, whereas in the concrete tanks the initial fertilization occurred two days before and with lower densities (less competition among post-larvae for food and space). These results are in agreement with Arbeláez-Rojas et al. (2002) in a study that reported that *C. macropomum*, when grown in the semi-intensive system in excavated tanks, with natural food at its disposal, presents better performance because it has a high filtration capacity and is well suited to lotic environments.

During the first week of external larviculture, the performance of the CT F treatment was superior to the others for weight variables ($p < 2.0x10^{-16}$) and similar for length variables ($p < 2.0x10^{-16}$; Table 5).

As in excavated tanks, the treatment that received only feed presented a batch with heterogeneous weight and length among fish of the same age. The observed heterogeneity for weight and length in post-larvae of the same age in the commercial feed treatments may be related to the competition for the little available natural food and/or poor feed distribution during feeding, since diets are not stable in the aquatic environment and tend to decant over time, while the post-larvae of the species feed near the water column. From the economic standpoint, it is ideal to obtain homogeneous lots with a satisfactory productive performance. According to Ricker (1958), in a fish production system it is more important to evaluate weight gain than an increase in length.

Table 4. Mean of weight variables and body measurements of *C. macropomum* post-larvae submitted to different feeding strategies in concrete tanks (CT) for five weeks.

Parameter	Treatment		
	CT R	CT F	CT F+R
Final Mean Weight (mg)	66.90b	95.60a	42.50a
Mean Weight (mg)	21.88b	39.93a	31.57a
Mean Weight Gain (mg)	65.80b	94.50a	41.40a
Weight gain rate (mg $week^{-1}$)	13.20b	18.90a	8.30a
Final Mean Length (mm)	15.5b	17.4a	14.1a
Mean length (mm)	10.3b	12.3a	11.8a
Mean growth (mm)	9.3b	11.2a	7.9a
Growth Rate (mm $week^{-1}$)	1.9b	2.2a	1.6a

CT R = ration; CT F = fertilization; CT R + F = ration and fertilization. *Equal letters in the same row do not differ from each other by the Tukey test (p > 0.05), n = 450.

Table 5. Final mean weight and final mean weekly length of *C. macropomum* post-larvae submitted to different feeding strategies in concrete tanks (CT) for five weeks.

	Final Mean Weight (mg)			Final Mean Length (mm)		
	CT R	CT F	CT F+R	CT R	CT F	CT F+R
Initial	1.10a	1.10a	1.10a	6.2a	6.2a	6.2a
Week 1	3.30b	5.50a	3.50b	7.2b	7.5ab	7.7a
Week 2	4.20c	17.1b	30.2a	8.0c	10.4b	11.0a
Week 3	8.30c	25.9b	45.2a	9.0c	11.7b	13.8a
Week 4	26.70b	55.70a	36.50b	12.2b	14.7a	12.6b
Week 5	66.90a	95.60a	42.50b	15.5b	17.4a	14.1c

CT R = ration; CT F = fertilization; CT R + F = feed and fertilization. *Equal letters on the same line do not differ from each other by the Tukey test (p > 0.05), n = 450.

When food management in excavated tanks is compared to management performed in concrete tanks, the former registered higher averages for *C. macropomum* productive variables in all the evaluated food strategies. According to our results, filling of the tanks and water fertilization can be carried out five days before the settlement with the post-larvae, and this process should be repeated in the first week of cultivation. If larval farming is not carried out in soil-bottomed tanks in addition to the initial fertilization, the process performed twice in the first weeks, with 20 g m^{-2} of wheat bran. The tank fertilization process was a simple, low-cost, efficient method to increase the natural productivity of the environment. Making natural food available is considered one of the most cost-effective ways to reduce the environmental impacts of fish farming and, by increasing planktonic biomass, it also reduces feed costs, which may represent about 60% of the total cost of *C. macropomum* production (Gomes et al., 2006)

Conclusion

According to our results, we can conclude that the increased availability of natural food, through different fertilization strategies and cultivation environments, favorably influenced the development of the cultivated species. It was also verified that tambaqui post-larvae did not develop well in the first weeks of life when fed only formulated diets and that, regardless of the cultivation environment, in the first week of larviculture, only the process of fertilization of cultivated water is necessary for the development of the species, which avoids the waste of feed and the consequent degradation of the quality of cultivated water.

Acknowledgements

The authors thank the Rodolpho von Ihering Fisheries Station, Bahia Pesca S/A, for providing facilities, inputs and post-larvae for the study. They are grateful to Paulo Sousa, Felipe Vieira, Frederico Dias and Karoline Soares; to UFRB; to the Biological and Environmental Agriculture Sciences (CCAAB) and Fisheries and Aquaculture Studies Nucleus (Nepa) and the entire team of the Aquatic Ecology and Aquaculture Laboratory (LEAAq), including Antonio Mendez, Thaís Silva, Thales Lima and Rodrigo Portela.

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