# COMMERCIAL FEED AND FROZEN Artemia NAUPLII FOR CURIMATÃ-PACU LARVAE IN FIRST FEEDING\*

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### ABSTRACT

The objective of this work was to evaluate the supply of frozen *Artemia* nauplii, commercial ration, and co-feed with frozen *Artemia* nauplii and commercial ration on the productive performance and water quality in the larviculture of curimatã-pacu *Prochilodus argenteus*. The larvae were cultivated for 21 days under the following feed treatments: 100F (100% commercial feed); 67F33A (67% commercial feed, and 33% fronzen *Artemia* nauplii); 34F66A (34% commercial feed, and 66% frozen *Artemia* nauplii); and 100A (100% frozen *Artemia* nauplii). The larvae, submitted to the feed treatments, did not differ in performance, survival, weight, total and standard lengths, Fulton's condition factor and specific growth rate. Commercial ration increased ammonia concentrations, impairing water quality, while *Artemia* supply provided low concentrations of ammonia, partially due to easy cleaning. Therefore, frozen *Artemia* nauplii should be used in the curimatã-pacu larviculture.

Keywords: Prochilodus argenteus; exogenous feeding; first feeding; weaning

# RAÇÃO COMERCIAL E NÁUPLIOS DE Artemia CONGELADOS NA PRIMEIRA ALIMENTAÇÃO DE LARVAS DE CURIMATÃ-PACU

#### RESUMO

Objetivou-se avaliar o fornecimento de náuplios de *Artemia* congelada, ração comercial e coalimentação com náuplios de *Artemia* congelada e ração comercial no desempenho produtivo e qualidade da água na larvicultura de curimatã-pacu *Prochilodus argenteus*. As larvas foram cultivadas durante 21 dias, sob os seguintes tratamentos alimentares: 100F (100% ração comercial); 67F33A (67% de ração comercial e 33% de náuplios de *Artemia* congelada); 34F66A (34 de ração comercial e 66% de náuplios de *Artemia* congelada); e 100A (100% de náuplios de *Artemia* congelada). As larvas, submetidas aos tratamentos alimentares, não diferiram no desempenho, sobrevivência, peso, comprimentos total e padrão, fator de condição de Fulton e taxa de crescimento específico. O fornecimento de ração comercial aumentou as concentrações de amônia, prejudicando a qualidade da água, enquanto que o fornecimento de *Artemia* proporcionou baixas concentrações de amônia, parcialmente devido a fácil limpeza. Portanto, pode-se empregar náuplios de *Artemia* congelado na larvicultura do curimatã-pacu.

Palavras-chave: Prochilodus argenteus; alimentação exógena; primeira alimentação; transição da dieta

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### INTRODUCTION

The curimatã-pacu *Prochilodus argenteus* (SPIX and AGASSIZ, 1829) is an endemic fish species from the São Francisco River basin that is of high economic and environmental importance (GUIMARÃES *et al.*, 2017). This specie has a several popular names: curimbatá, xira, bambá or zulega, with features as large scales at the body, except in the head, and long spindle-shaped body. This specie has a preference to live in lotic environments, and has an omnivorous and detritivorous habits, with a good acceptability of commercial feed, aquatic algae and decomposing organic matter (CEMIG/CETEC, 2000; ITUASSÚ *et al.*, 2004).

There is a constant concern to improve fish and fingerlings production larviculture technology for Brazilian native fish. The process which the larvae begin to accept commercial feed, leaving the habit to feed the natural food (live food), is called dietary transition or "weaning" (ROCHA et al., 2008). This is a critical stage of larval rearing, with a great possibility of high mortality (CONCEIÇÃO et al., 2009). Therefore, the success of this stage is linked to knowledge of the digestive capacity of the larva, and the accurate time for the start offering commercial feed, and feed quality (GAWLICKA et al., 2000).

Fish larvae of native species have an immature digestive system (PORTELLA and DABROWSKI, 2008), and have a great difficulty in assimilating the commercial feed in early days of life (PEDREIRA *et al.*, 2008; DIEMER *et al.*, 2012). For this reason, the live food that undergoes the process of autolysis when digested, being thus more easily assimilated by fish larvae, are widely used (PORTELLA and DABROWSKI, 2008; MANDAL *et al.*, 2009; DAS *et al.*, 2012).

The aim of this study was to evaluate the frozen *Artemia* nauplii, commercial feed, and co-feeding frozen *Artemia* nauplii with commercial feed on growth performance and water quality for curimatã-pacu hatchery.

### MATERIAL AND METHODS

The experiment was conducted from January to February, 2015 (totaling 21 days) at the Hydrobiology and Fish Culture Station at the Companhia de Desenvolvimento dos Vales do São Francisco e Parnaíba (CODEVASF), Três Marias City - MG, (8°12′23″S and 45°14'30"W).

Curimatã-pacu (*P. argenteus*) larvae with five days after hatching were used (mean weight 1.80  $\pm$  0.1 mg and mean total length 6.46  $\pm$  0.1 mm) at the first feeding day of the trial. Larvae were individually counted and distributed into a static system with partial water renovation, constituted with 20 L aquarium, at 10 larvae L<sup>-1</sup> density, totalizing 80 larvae aquarium<sup>-1</sup>, provided with constant aeration and natural photoperiod (12L:12D). Aquariums were cleaned twice a day<sub>7</sub> (the first cleaning after the first feeding time, and the second after the last feeding time), performing daily 20% exchange of the water total volume.

The experimental design was completely randomized with four treatments and five replications: 100F (100% - commercial feed [F]); 67F33A (67% - commercial feed, and 33% - frozen *Artemia* nauplii [A]); 34F66A (34% - commercial feed, and 66% - frozen *Artemia* nauplii); and 100A (100% frozen *Artemia* nauplii). Larvae were fed four times day<sup>-1</sup>: at 7:00, 10:00, 13:00 and 16:00 hours. Commercial feed and *Artemia* was offered for larvae *ad libitum*.

The calculated composition (manufacturer data) from the mash commercial feed (0.05  $\geq$ granulometry  $\leq 0.22$  mm) was: dry matter (898.1 g kg-1), crude protein (550.0 g kg-1), digestible energy (3600 kcal kg<sup>-1</sup>), crude fiber (14.5 g kg<sup>-1</sup>), phosphorus total (10.5 g kg<sup>-1</sup>), available phosphorus (7.5 g kg<sup>-1</sup>), total calcium (10.0 g kg<sup>-1</sup>), total lysine (22.0 g kg<sup>-1</sup>), the total methionine plus cystine (14.3 g kg<sup>-1</sup>) and starch (250.0 g kg-1). Ingredients used for the preparation of feed were: soy protein concentrate (386.3 g kg<sup>-1</sup>) wheat flour (230.0 g kg<sup>-1</sup>) 60 corn gluten (192.0 g kg<sup>-1</sup>), corn ground (54.0 g kg<sup>-1</sup>), 45% soybean meal (50.0 g kg<sup>-1</sup>) 60% fish meal (20.7 g kg<sup>-1</sup>), dicalcium phosphate (30.0 g kg<sup>-1</sup>), limestone (1.0 g kg<sup>-1</sup>) L-lysine HCl (1.6 g kg<sup>-1</sup>) soy oil (20.0 g kg-1), vitamin-mineral premix 1 (8.0 g kg-1), vitamin C (8.0 g kg-1) and BHT (0.02 g kg<sup>-1</sup>) a total of 100.0 g kg<sup>-1</sup>.

Fifteen larvae of the each treatment were sampled and measured on the 10th day; at the end of the experiment (day 21), 75 larvae were sampled and measured in each treatment. The body weight was obtained with an analytical balance 1.0 mg (Scientech AS 210, China), and the total and standard length through the use of a stereomicroscope (Carl Zeiss Jena DV4). Also were calculated the survival; the Fulton's condition factor (K), where K = (body weigh x standard length<sup>-3</sup>) x 100; and the specific growth rate, where: SGR = 100 x (lnPtf - lnPti)  $\Delta t^{-1}$ , where  $\Delta t$  is the duration in days between samples is the duration in days among samples.

Water quality parameters were collected at the 1st, 7th, 14yh, and 21th days, when the temperature, dissolved oxygen, pH, electrical conductivity, and turbidity (NTU) (Horiba, model U XD-22) were measured. Water alkalinity was determined by potentiometric method (APHA, 2012), where 100 mL of the sample of each aquarium was titrated with sulfuric acid to reach pH 4.35 with the assistance of a pHmeter. Then was applied the formula: alkalinity = (volume of spent acid x 0.01 x 1000 100<sup>-1</sup>) x 50. Ammonia was determined by the Nessler's method (APHA, 2012). To compare the efficiency of dietary treatments, biological parameters as body weight, total and standard length, Fulton's condition factor, SGR and survival, were submitted to the Sigma Stat 3.5 program for analysis of variance with Tukey test at 5% significance. The limnological parameters, as temperature, dissolved oxygen, pH, electrical conductivity, turbidity, alkalinity and ammonia were analyzed with the Sigma Stat 3.5 program for analysis of variance with Tukey test at 5% significance, using factorial 4x4 (feeding combination x days).

### RESULTS

Larvae did not differ (p>0.05) on performance at 10th and 21th days of exogenous feeding among treatments with commercial feed, frozen *Artemia* nauplii and the co-feeding of both, frozen *Artemia* nauplii and commercial feed (Table 1).

**Table 1**. Productive performance (mean ± standard deviation) of curimatã-pacu larvae fed with commercial feed (100F); co-feed with 67% - commercial feed and with 33% - frozen *Artemia* nauplii (67R33A); and 34% - commercial feed and 66% - frozen *Artemia* nauplii (34F66A); and frozen *Artemia* nauplii (100A), at 10th and 21th day of feeding. SGR - specific growth rate.

| Deveryetere                   | Treatments        |                  |                  |                  |  |
|-------------------------------|-------------------|------------------|------------------|------------------|--|
| rarameters                    | 100F              | 67F33A           | 34F66A           | 100A             |  |
| 10th day                      |                   |                  |                  |                  |  |
| Body weight (mg)              | $3.34 \pm 1.02$   | 4.20 ±1.57       | 2.58 ±0.64       | $2.82\pm0.81$    |  |
| Total length (mm)             | $8.57 \pm 0.69$   | $8.04 \pm 0.71$  | $7.79\pm0.62$    | $7.86 \pm 0.38$  |  |
| Standard length (mm)          | $6.05 \pm 0.50$   | $5.79\pm0.46$    | $5.46\pm0.41$    | $5.77\pm0.44$    |  |
| Fulton's condition factor (K) | $1.49\pm0.28$     | $1.90\pm0.28$    | $1.55\pm0.29$    | $1.44\pm0.26$    |  |
| SGR (%)                       | $5.90 \pm 2.70$   | $7.00 \pm 4.10$  | $4.30 \pm 1.40$  | $6.50\pm0.00$    |  |
| 21th day                      |                   |                  |                  |                  |  |
| Body weight (mg)              | $15.32 \pm 6.26$  | $9.24\pm0.92$    | $6.88 \pm 2.47$  | $10.88 \pm 6.42$ |  |
| Total length (mm)             | $10.70\pm1.47$    | $9.86\pm0.94$    | $9.91 \pm 1.21$  | $9.12 \pm 4.59$  |  |
| Standard length (mm)          | $9.42 \pm 0.72$   | $8.58\pm0.21$    | $8.25 \pm 1.06$  | $8.79\pm0.54$    |  |
| Fulton's condition factor     | $1.74\pm0.40$     | $1.46\pm0.12$    | $1.17\pm0.10$    | $1.57\pm0.82$    |  |
| SGR (%)                       | $11.70 \pm 6.10$  | $7.70 \pm 2.50$  | $8.50\pm3.10$    | $11.10 \pm 2.20$ |  |
| Survival (%)                  | $57.90 \pm 52.90$ | $88.00 \pm 4.00$ | $70.00 \pm 25.0$ | $82.00 \pm 4.00$ |  |

Water parameters of quality were analyzed by their means ( $\pm$  standard deviation) throughout the experimental period, in which the water temperature was 27.10  $\pm$  0.06 °C, turbidity was 44.30 ± 4.80 NTU, the dissolved oxygen was 7.19 ± 0.54 mg L<sup>-1</sup>, the pH was 7.66 ± 0.13, the electrical conductivity was  $43.2 \pm 3.2 \ \mu\text{S cm}^{-1}$ , so there were no interaction and statistical differences except

ammonia and alkalinity.

At 7th experimental day, the use of commercial feed increased (p<0.05) total ammonia concentration in the water (Table 2). After the 14th day, ammonia concentrations increased (p<0.05) when used commercial feed and 67F33A, and remained high (p<0.05) until the end of the experiment. The use of frozen *Artemia* nauplii showed lower (p<0.05) ammonia concentration throughout the experimental period. The decrease

of ammonia at the end of the experiment in all treatments may be associated with water renewal that occurred at the previous day (20th day).

Alkalinity concentration increased with the use of 100F and 67F33A at the 7th day (Table 3). After 14 days the alkalinity increased (p<0.05) in treatment 100F. The use of frozen *Artemia* nauplii did not affect (p>0.05) the alkalinity concentration of the water, and remained low throughout the experimental period.

**Table 2**. Water ammonia concentration (mg L<sup>-1</sup>) (mean ± standard deviation) in water of curimatã-pacu larviculture with different initial diets.

|            | 100F                    | 67F33A                   | 34F66A                | 100A                     |
|------------|-------------------------|--------------------------|-----------------------|--------------------------|
| Treatments | $0.388 \pm 0.2^{a}$     | $0.286 \pm 0.2^{b}$      | $0.190 \pm 0.1^{b}$   | $0.0275 \pm 0.0^{\circ}$ |
|            | 1st day                 | 7th Day                  | 14th day              | 21st Day                 |
| Days       | $0.00 \pm 0.00^{\circ}$ | $0.276 \pm 0.15^{b}$     | $0.410 \pm 0.30^{a}$  | $0.205 \pm 0.16^{b}$     |
|            | 1st day                 | 7th Day                  | 14th day              | 21st Day                 |
| 100F       | $0.00 \pm 0.00$ cA      | $0.48\pm0.18^{\rm bA}$   | $0.71 \pm 0.12^{aA}$  | $0.35 \pm 0.12^{bA}$     |
| 67F33A     | $0.00 \pm 0.00^{cA}$    | 0.25 ±0.19 <sup>bB</sup> | $0.56 \pm 0.16^{aAB}$ | $0.32\pm0.05^{\rm bAB}$  |
| 34F66A     | $0.00 \pm 0.00$ cA      | $0.26 \pm 0.15^{abB}$    | $0.36 \pm 0.23^{aB}$  | $0.12 \pm 0.08$ bcBC     |
| 100A       | $0.00 \pm 0.00^{aA}$    | $0.09 \pm 0.12^{aB}$     | $0.00 \pm 0.00^{aC}$  | $0.01 \pm 0.03^{aC}$     |

Mean (general for treatment and days, and interactions mean) followed by lowercase letters for the comparison of days can be seen in the line, and means followed by uppercase letters for comparison of online treatments, in column. Tukey 5% probability. Commercial feed (100F); 67% with 33% of frozen **Artemia** nauplii (67R33A); 34% of commercial feed and 66% frozen **Artemia** nauplii (34F66A); and frozen **Artemia** nauplii (100A).

|            | 100F               | 67F33A              | 34F66A             | 100 <sup>a</sup>          |
|------------|--------------------|---------------------|--------------------|---------------------------|
| Treatments | 19.0 ± 0.0a        | 18.6 ± 0.01ab       | $18.0 \pm 0.03b$   | $18.7 \pm 0.01$ c         |
|            | 1st day            | 7th day             | 14th day           | 21st day                  |
| Days       | $17.0 \pm 0.00c$   | $18.3 \pm 0.15b$    | 19.5 ± 0.30a       | $18.1 \pm 0.01 b$         |
|            | 1st day            | 7th day             | 14th day           | 21st day                  |
| 100F       | $17.0 \pm 0.00$ cA | $18.5 \pm 0.01$ bA  | 21.5 ± 0.01aA      | 18.5 ± 0.01bAB            |
| 67F33A     | $17.0 \pm 0.00$ cA | $18.5 \pm 0.01$ bA  | $20.0 \pm 0.00 aB$ | 19.0 ± 0.02abA            |
| 34F66A     | $17.0 \pm 0.00$ bA | $18.0 \pm 0.01$ abA | $19.5 \pm 0.02 aB$ | $17.0 \pm 0.01 \text{bB}$ |
| 100A       | $17.0 \pm 0.00 aA$ | $17.5 \pm 0.01 aA$  | 17.0 ± 0.01aC      | $17.0\pm0.04\mathrm{aB}$  |

**Table 3**. Water alkalinity levels (mg  $L^{-1}$  CaCO<sub>3</sub>) (mean  $\pm$  standard deviation) in water of curimatã-pacu larviculture with different initial diets.

Means (general for treatment and days, and interactions mean) followed by lowercase letters for the comparison of days can be seen in the line, and means followed by uppercase letters for comparison of online treatments, in column. Tukey 5% probability. Commercial feed (100F); 67% with 33% of frozen **Artemia** nauplii (67R33A); 34% of commercial feed and 66% frozen **Artemia** nauplii (34F66A); and frozen **Artemia** nauplii (100A).

# DISCUSSION

No differences was observed for curimatãpacu larvae body weight, standard and total length, K (Fulton's condition factor), SGR, and survival when fed with commercial feed or frozen Artemia nauplii or both co-feeding combinations (67F33A and 34F66A) of it. The ability of some fish larvae to digest or not the dry diet is explained by the morphological features of the digestive tract and by the secreted enzymes throughout larvae development (MARQUES et al. 2007; PORTELLA and DABROWSKI, 2008). The digestive system in P. argenteus larvae exhibits the production of acids in the esophagus and a primitive digestive tube with the capacity for nutrient absorption by the 3rd day after hatching. On the 18th day after hatching, the stomach is functional and the production of an acidic material occurs in the esophagus and in the stomach. This species demonstrated the ability to absorb exogenous inert feed in the early stages of the life (SANTOS et al., 2016).

However, live food was offered for enhance survival in the beginning of the exogenous feeding to the curimatã-pacu (SANTOS *et al.*, 2016; GUIMARÃES *et al.*, 2017), and to others fish larvae, as *Lophiosilurus alexadri*, *Pimelodus britskii*, *Sander lucioperca*, and *Herus severus* (PEDREIRA *et al.*, 2008; 2015; CONCEIÇÃO *et al.*, 2009; ATENCIO-GARCÍA *et al.*, 2010; DIEMER *et al.*, 2010; LJUBOBRATOVIĆ *et al.*, 2015; ABE *et al.*, 2016).

A several dietary transition protocols have been tested to reduce the time to offer of live food for intensive fish hatchery and improve the culture performance (ADAMANTE *et al.*, 2007; JOMORI *et al.*, 2008; CORASPE-AMARAL, *et al.*, 2012; LJUBOBRATOVIĆ *et al.*, 2015). However, due to various factors such as immaturity of the digestive tract of the native species larvae, these protocols cannot always improve the performance.

Curimatã-pacu larvae are able to consume commercial feed in the beginning of the exogenous feeding. Analyzing the survival data, no significance was observed among treatments, however, was observed the high standard deviation of larvae fed with commercial feed treatment, probably related to the deterioration of water quality by commercial feed in the first days of larvae life. PEDREIRA et al. (2008; 2015) studied the effect of live food, with or without addition of commercial feed for pacamã (Lophiosilurus alexandri) and tambaqui (Colossoma macropomum) larvae, and to both species the commercial feed promoted survival similar to others diets but with low survival and higher coefficient of variation. PEDREIRA et al. (2008) reported that the commercial feed increased water electrical conductivity and decreased pH values deteriorating water quality, which caused mortality, similarly to that observed for curimatã-pacu in this experiment. However, LUZ and ZANIBONI FILHO (2001), subjecting catfish Pimelodus maculatus to different size of the commercial feed particle, 0.15 to 0.25 mm, did not observe damage in fish survival.

The use of commercial feed increased the ammonia and alkalinity concentrations in the water, but the other water physicochemical parameters remained constant along rearing, and the water quality range was suitable for a species, being close to observed in curimatã-pacu larviculture according SANTOS *et al.* (2016) and in natural environment, where it reproduces and develops (HADDAD and JÚNIOR 2010).

Ammonia levels increase due to the use of commercial feed resulting in the decline of water quality, but remained in acceptable levels to *P. argenteus*, comparing with others trials (SANTOS *et al.*, 2016).

Fish larvae that receive high levels of crude protein can assimilate a part, and another part is excreted as organic nitrogen in feces, or as ammonia, the primary form of fish excretion (BOYD and TUCKER, 2014). The use of Artemia nauplii in the early days of exogenous feeding provides better water quality for curimatã-pacu. The not consumed Artemia nauplii have concentrated in the aquarium bottom that facilitated the removal of the waste and could be totally removed. On the other hand, the feed is dissolved and spread in the water, making impossible the total removal of the wastes. Similarly, BOYD and TUCKER (2014) described that waste production is an unintentional and unavoidable consequence of feeding and, unlike other aquaculture systems where wastes can be quickly removed to prevent water quality deterioration, it is not economically feasible to remove wastes from ponds.

Water alkalinity concentrations increased during the trial, especially in the commercial feed treatment, because the calcium concentrations of the diet. The alkalinity increases with calcium carbonate concentration, increasing the buffering capacity of the environment, reason why aquaculturists perform the ponds liming. However, there is a range suitable for each species and, due to increased alkalinity is related to increase pH and ammonia toxicity, in some case, seldom, is feasible to reduce total alkalinity (BOYD and TUCKER, 2014). According to ROJAS et al. (2001), the curimbatá (Prochilodus lineatus) larvae have better physiological condition and weight gain when water alkalinity range among 16 to 32 mg L<sup>-1</sup>CaCO<sub>3</sub>, a similar range of alkalinity observed in this trial for the curimatã-pacu larvae  $(17.0-21.5 \text{ mg } \text{L}^{-1} \text{CaCO}_3)$ . In addition, these values were the same or lower than those found by PEDREIRA et al. (2009) in pacamã larviculture, for the same season and local, when higher ammonia concentrations were observed and survival was high. Therefore, both alkalinity and ammonia, were within the limits acceptable to the curimatã-pacu.

Lastly, the decrease of ammonia and alkalinity concentration from 17 to 21 days is related to the larvae development, which second SANTOS *et al.* (2106) and PEDREIRA *et al.* (2008) improve the food capture and assimilation capacity, in addition to the maintenance of water renewal rate and decrease in the number of fish per aquarium.

#### CONCLUSION

Curimatã-pacu larvae can be fed with commercial feed since the beginig of exogenus feeding. However, is indicated the use of the *Artemia* nauplii in the first days of larvae life, due to the low waste and it easy remove in the water than the commercial feed.

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