



## Article

# Larviculture of *Brycon amazonicus* under Different Food and Farming Systems

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**Abstract:** Freshwater fish larviculture techniques still have deficiencies in cultivation and feeding. In this study, we evaluated experimentally different cultivation and feeding systems in the *Brycon amazonicus* (matrinxã) larviculture. Seven treatments with different live foods were used: T1 = a semi-intensive mesocosm system with green water; T2 = a clear water system containing *Artemia* sp. as food; T3 = a clear water system containing *Dendrocephalus brasiliensis* as food; T4 = a clear water system containing a combination of *Artemia* sp. and *D. brasiliensis* as food (a proportion of 1:1); T5, T6 and T7 were the same as T2, T3 and T4, respectively, but with a swimming exercise system. During the experiment, the water quality parameters were measured and maintained suitably for the cultures. The highest values of final weight ( $42.97 \pm 2.58$  mg) and specific growth rate ( $31.77 \pm 0.60\%$ ) were observed in T5 ( $p < 0.05$ ). Regarding the nutritional composition, the larvae of *B. amazonicus* that were fed nauplii of *D. brasiliensis* had a better profile of amino acids and essential fatty acids than those fed other live foods. Therefore, nauplii of *D. brasiliensis* can be used as an adequately nutritional food for larvae of *B. amazonicus*.

**Keywords:** matrinxã; cannibalism; low survival; nutritional value of live food; *Artemia*; *Dendrocephalus brasiliensis*



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## 1. Introduction

In recent years, fish consumption has increased worldwide due to the recognition of its nutritional value leading to benefits for human health [1,2]. For this reason, aquaculture has evolved into using increasingly larger enterprises. However, for this to be possible, the production and supply of juveniles of fish intended for fattening should be guaranteed [3,4]. Fingerling production depends on the success of larviculture, the most limiting phase for the intensive production of any species of fish [5]. Current rates of survival of larvae are low, around 10% to 15%, which increases the prices of commercializing fingerlings [6].

Although advances have been made in the development of technology for the manufacturing of inert diets for larviculture, it still mostly depends on using live food as a feeding strategy [4], especially for altricial species that have few vitelline reserves and an undifferentiated gastrointestinal tract [5]. The supply of live prey in the first exogenous feeding of fish larvae is fundamental because, due to their movements in the water column and release of chemical substances, the larvae are stimulated to consume this live food [7,8].

In the past, it was believed that live prey, when ingested, donated their own digestive enzymes, which would help in the digestive process of the larvae that consumed them [9]. Conversely, other authors state that enzymes derived from live food are not representative of the digestive process of fish larvae and have a small contribution to the enzymatic activity of some fish species [10,11].

The larvae learning how to catch live prey is the starting point for the beginning of the food transition. Studies show the advantages of co-feeding, that is, the combination of two

feeding sources (live and inert), include taking advantage of the high attractiveness of live prey and the high density of nutrients in formulated diets [12]. Thus, great advances have been achieved to obtain larger growth of larvae of different species [13–15].

*Brycon amazonicus*, popularly known as matrinxã, is a neotropical freshwater species native to the Amazon and Tocantins-Araguaia basins [16]. It has great potential for aquaculture because it presents high growth rates, excellent production performance, high quality and good meat flavor, as well as presenting good tolerance to high storage densities [17]. However, the greatest obstacle in the production of this species is the low supply of fingerlings due to their own reproductive characteristics. The species presents rheophilic behavior with spawning only in the months of October to February, therefore limiting the production of larvae to a short period of time [18]. They also show a marked behavior of cannibalism during the first 48 h of life. In addition, larvae are considered altricial because they have few yolk reserves, which means that food must be available soon after hatching [19,20]. These factors considerably diminish the survival of larvae in this period, thus increasing their commercialization price, making large-scale matrinxã production unfeasible [21,22].

Because it is an aggressive species that exhibits cannibalism, hierarchical dominance and, consequently, heterogeneity in size, techniques need to be developed that will reduce aggressive behavior and increase survival [23]. Several strategies have been used to solve these problems, such as using forage larvae [24], increased storage density [25], thyroid hormones [26], diets enriched with amino acids [27], probiotics in *Artemia* [28] and even rearing groups of larvae of homogeneous size [29]. The rearing of larvae of *B. amazonicus* is mainly undertaken in earth ponds (semi-intensive systems) [30,31]. In these systems, larvae can reach high rates of mortality, and losses can reach 94.8% on average [32]. Due to this low performance in these natural systems, new alternatives are needed for the production of *B. amazonicus* larvae that improve the rates of initial survival and change their aggressive behavior.

A way to change the growth and behavior of fish larvae is through swimming exercise protocols. To achieve this, some adaptations in larval tanks can be made to generate water velocities, which stimulate larvae to swim at speeds that are beneficial to their growth. Studies evaluating the effects of swimming on larval growth are scarce. In fact, when exposed to water velocities of 2.3 body lengths per second ( $\text{BL}\cdot\text{s}^{-1}$ ), larvae of salmonid species grow faster than larvae reared in standing water [33]. This tendency was also observed by [34] in larvae of Arctic charr raised under four swimming speeds: 0.25, 0.5, 1.0 and 2.0  $\text{BL}\cdot\text{s}^{-1}$ . The larvae raised at a higher velocity had a higher specific growth rate than at the other velocities. In addition to improving growth rates, swimming also confers increased resistance to larvae when they are exposed to inadequate water quality conditions [35].

Another way to overcome the aforementioned problems, which have not yet been tested in larvae of tropical species, is the technique of mesocosm or green water used in marine larviculture [36]. This technique makes it possible to maintain the nutritional value of the prey at a very satisfactory level, as they feed on algae that grow simultaneously in the tank where live prey is found. In this system, the body composition of the prey varies according to the algae consumed. Zooplanktonic organisms such as rotifers, cladocerans and copepods greatly benefit from this technique, but during feeding with *Artemia* nauplii, the maintenance of nutritional quality is guaranteed by the continuous and fractionated distribution in the larvae tanks [37,38]. In a study evaluating the nutritional value of *Artemia* and *Dendrocephalus brasiliensis* [39], it was reported that *D. brasiliensis* (commonly known in Brazil as branconeta) has a nutritional profile superior to that of *Artemia* salina, in addition to presenting a high productivity of nauplii per gram of cysts [40], which may enhance the larviculture of several species of fish used for consumption and those that are ornamental with a high market value.

Thus, considering the importance of live food for larviculture and the lack of studies on the management of the first feeding, as well as its influence on the performance of larvae

of marketable species, this study aimed to evaluate different farming systems and types of live food in *Brycon amazonicus* larviculture.

## 2. Materials and Methods

### 2.1. Place of Experiment and Biological Material

The experiment was conducted in the Experimental Aquaculture Station (EEA in Portuguese) and Plankton Laboratory at the Department of Hydrobiology at the Federal University of São Carlos (UFSCar), São Carlos, São Paulo State, Brazil. The larvae of matrinxã were obtained by induced reproduction and provided by *Águas Claras* Aquaculture, from Mococa, São Paulo State, Brazil.

### 2.2. Installations and Experimental Conditions

The experiment was carried out in 28 circular fiberglass tanks that measured 60 cm in diameter and 1 m high, with a capacity of 100 L (Figure 1). Previously, each tank was filled with 50 L of dechlorinated water. After transporting them, the larvae were counted and distributed in the tanks at a density of 10 larvae L<sup>-1</sup>, totaling 500 larvae per tank. Of the 28 tanks, 24 were coupled to a recirculation system, with thermostatic water under continuous flow, constant aeration and mechanical and biological filters, which were used in the treatments involving the clear water systems. The last four tanks were used for the treatments involving the semi-intensive system of mesocosm (green water), which were managed individually.

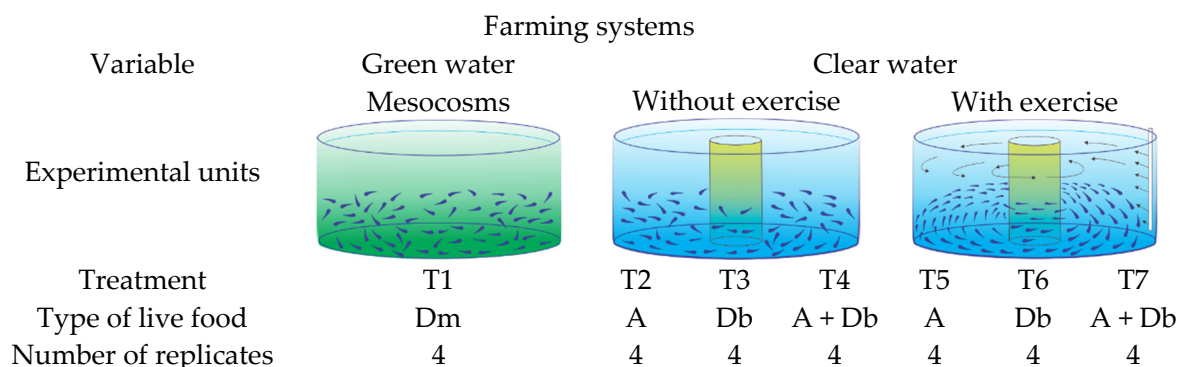


**Figure 1.** Experimental tanks of larviculture of *Brycon amazonicus* under different treatments at the Experimental Aquaculture Station of DHB-UFSCar, Brazil.

Water quality was monitored throughout by daily measurements of water temperature, pH, conductivity, salinity and dissolved oxygen concentrations, using a multiparameter YSI 6820v2 probe (YSI Incorporated, Ohio, USA). Every two days the concentration of total ammonia was determined in the water by means of a colorimetric reaction with the Nessler reagent and a spectrophotometer reading at 425 nm. The siphoning of the residues at the bottom of the tanks was undertaken once a day, before the first meal, as well as checks for dead larvae.

In the experimental design (Figure 2), seven treatments were used, which included three different farming systems and types of live food, as follows: treatment 1 (T1) = semi-intensive mesocosm system with green water; treatment 2 (T2) = a clear water system containing newly hatched *Artemia* sp. nauplii as food for the fish larvae; treatment 3 (T3) = a clear water system containing *D. brasiliensis* nauplii as food for the fish larvae; treatment 4 (T4) = a clear water system containing a combination of *Artemia* sp. and *D. brasiliensis* nauplii (proportion 1:1); treatments T5, T6 and T7 were the same as T2, T3 and

T4, respectively, but with a moderate swimming exercise system in which the larvae of matrinxã were submitted to the water velocity. Each treatment had four replicates, totaling 28 experimental units.



**Figure 2.** Experimental design of different culture systems (Mesocosms and Clear water); presence (T1 to T4) or absence (T5 to T7) of swimming exercises; and types of live food given to the fish larvae (Dm = *Daphnia magna*; A = *Artemia*; Db = *D. brasiliensis*). T1 to T7 are the seven different treatments. Four experimental repetitions (n = 4).

2.3. Semi-Intensive Mesocosm System

For the semi-intensive mesocosm system, four transparent acrylic tanks were used to facilitate the entrance of light and, consequently, the photosynthetic activity of the microalgae. Prior to the introduction of matrinxã larvae (10 larvae L<sup>-1</sup>), the 100-L tanks were filled with 50 L of chlorophycean algae, which were initially cultured in the Plankton Laboratory and later inoculated into larger volumes in EEA.

The amount of live food that needed to be supplied to the fish larvae, which need to feed more frequently than adults to sustain their high growth rates, was based on the information that they can consume between 50 and 300% of their own live weight in food [41]. Thus, zooplankton organisms were offered based on their average wet weight equivalent to 70%, 100% and 150% of the live weight of the matrinxã larvae, which were weighed at the beginning of the experiment. The zooplankton organism selected as live food in the mesocosm system was the cladocera *Daphnia magna*, due to preliminary tests that indicated an excellent adaptation of the culture to the mesocosm system, in addition to zootechnical characteristics such as a high reproduction rate, resistance to handling and the quality of water as well as its high nutritional value.

2.4. Clear Water System

In the treatments with a clear water system, three types of live food were used to feed larvae of matrinxã: (1) *Artemia* nauplii, (2) *D. brasiliensis* nauplii, and (3) a mixture of *Artemia* and *D. brasiliensis* nauplii in the ratio of 1:1. The *Artemia* used in this work come from the salt flats of the Costa Branca, State of Rio Grande do Norte, city of Natal, Brazil.

For *Artemia* nauplii hatching, the methodology described by [42] was used. The quantification of the *Artemia* nauplii to be supplied for the fish larvae was performed as follows: three 1 mL samples of the nauplii hatch incubators were taken with a graduated pipette, which were transferred to volumetric flasks of 10 mL, adding water to the final volume of 10 mL (1/10 dilution). From this volume, three 1 mL sub-samples were taken, in which the number of nauplii was quantified. The number of nauplii per mL of the initial sample was then calculated by multiplying the mean value of the counts by 10, which was the dilution factor. The volume required to obtain the amount of *Artemia* to be supplied according to Table 1 was then calculated.

**Table 1.** Criteria adopted to calculate the number of *Artemia* nauplii that were offered daily to matrinxã larvae.

| Interval in Days | Estimated Live Weight (LW) of Larvae of Matrinxã (mg) | Amount of Food Offered Considering that the Matrinxã Larva Consumes between 70 to 150% of Its Own Weight in Food (mg) | Number of <i>Artemia</i> nauplii/Larva/Day |
|------------------|---|---|--|
| 1–3              | 1.5–2.7   | 2.3–4.1 (150% LW)   | 260–465                                    |
| 4–6              | 3.5–17.0  | 3.5–17.0 (100% LW)  | 400–2000                                   |
| 7–10             | 20.3–110.0  | 14.2–77.0 (70% LW)  | 1600–8850                                  |

Feeding with *Artemia* nauplii started when the larvae were 24 h old, after hatching, and continued until the end of the experimental period (10 days). The amount of *Artemia* nauplii supplied to the fish larvae was proportional to their weight, corresponding to 150% of the larvae weight from day 1 to day 3, 100% from day 4 to day 6, and 70% from day 7 to day 10, as shown in Table 1. These quantities were divided into four daily meals (8:30 am, 11:30 am, 2:30 pm and 5:30 pm) and increased every two days, according to the demand presented by matrinxã larvae. The chemical composition of the *Artemia* strain used in this study was published by the authors [39].

### 2.5. Production of nauplii of *Dendrocephalus brasiliensis*

*D. brasiliensis* cysts, produced at the Aquaculture Station of the Department of Hydrobiology-DHb of USFCar, were set to hatch according to nauplius production protocols, previously established. After hatching, *D. brasiliensis* nauplii were collected in the instar I phase (1-day old, average length,  $0.36 \pm 0.04$  mm) and supplied daily as live food to the experimental units, following criteria similar to those used for *Artemia* nauplii (Table 1).

### 2.6. Larva Swimming Exercise System Protocol

In the swimming exercise evaluation, the matrinxã larvae were randomly distributed in 12 circular fiberglass tanks of 100 L capacity. Half of that volume was used to store the larvae at a density of 10 larvae per liter, totaling 500 larvae per tank. The animals were trained to swim against the current of water at a speed of 2.0 body lengths per second ( $\text{cc.s}^{-1}$ ); that is, the magnitude of the water speed in which the larvae swam was based on the total length of the larvae, measured with a digital caliper. To achieve this, an initial biometry of 50 larvae was made to determine the average length (mm) and, subsequently, the water speed was adjusted to the previously determined value. The current in each tank was created by the forced passage of water through holes made in PVC pipes, positioned horizontally and vertically. The force and direction of the water jet, generated by a half-inch (1/2 HP) pump (Figure 3), were adjusted to obtain the required experimental velocity of water.



**Figure 3.** Panoramic view of the pump and pipes positioned vertically and horizontally in each tank to generate the water speed in the swimming exercise system in the larvae of *Brycon amazonicus*.

The speed of the water in each tank at different depths and positions was measured by a mechanical flow meter (General Oceanics Inc., Miami, FL, USA) and controlled over the 10-day experimental period. In this breeding system, the tanks were coupled to a recirculation system, provided with thermostat water, constant aeration, and mechanical and biological filters. In this exercise system, the experimental design comprised three treatments with four repetitions: a group of matrinxã larvae was fed with *Artemia* nauplii (Exercise + *Artemia*: EA), another group was fed with *D. brasiliensis* nauplii (Exercise + Branconeta: EB) and the last group was fed with *Artemia* and *D. brasiliensis* nauplii in equal proportions of 1:1 (Exercise + AB). The quantities of these live foods that were supplied were based on the high capacity of food intake in these early stages of life of matrinxã larvae [43]. To facilitate the calculations, the present study considered that a matrinxã larva consumes between 70% and 150% of its own weight in food daily (Table 1). Considering the origin of both types of live food, *Artemia* from saline environments and branconeta from freshwater environments, it is expected that branconeta nauplii are available permanently for larvae, whereas *Artemia* nauplii are accessible to larvae for a short time (two to three hours). *Artemia*'s short survival is due to the low salinity of the larvae water [5]. In view of this, a more constant supply of live food was necessary to ensure a balanced supply of nauplii of both species. To increase the survival time of *Artemia* nauplii when exposed to fresh water in rearing tanks, a low concentration of sea salt ( $3 \text{ g L}^{-1}$  of salt) was added. Thus, the larvae were fed four times a day with intervals of three hours between meals (8:30 a.m., 11:30 a.m., 2:30 p.m. and 5:30 p.m.). At the time of feeding, the pump that generates the current system was turned off for 15 min to facilitate the feeding of the larvae with the different live foods. Figure 4 shows an overview of the hydraulics and positioning of the tanks in the larvae breeding system under swimming exercise.



**Figure 4.** Schematic design of the experimental tanks and recirculation system (100-L tanks, water pump, mechanical and biological filters) that were used in the larvae swimming exercise protocol.

## 2.7. Assessed Parameters

### 2.7.1. Evaluation of Larvae Growth and Survival

Before the beginning of the experiment, 100 matrinxã larvae were collected to perform the initial biometry. The total length was determined with a digital electronic caliper (Starrrett brand, model 798-6/150) and a stereoscopic microscope with a micrometric eyepiece. The wet weight (mg) of each specimen was determined using a digital analytical balance to three decimal places. Afterward, to assess the effects of different treatments on growth and survival, 50 larvae from each repetition were collected randomly at three, six and ten days after the start of feeding (fixed in 10% formalin for a period of 24 h and then preserved in 70% alcohol), for which the same parameters as the initial biometrics were evaluated. At the end of the experimental test for each treatment, the survival rate in each experimental unit was determined (final number of larvae  $\times$  100/initial number of larvae – number of larvae killed in each biometry). With the average results of the initial ( $P_i$ ) and final ( $P_f$ ) weight of each repetition or experimental unit, the specific growth rate (SGR) was calculated according to the Hopkins equation:  $\text{SGR} = 100 (\ln P_f - \ln P_i) / \text{time interval between biometrics}$  [44].

### 2.7.2. Evaluation of Total and Free Amino Acids

The total and free amino acid analyses were made at the CBO laboratory in Campinas, São Paulo State. Initially, the total amino acids were quantified and analyzed from dry samples of matrinxã larvae, *Artemia nauplii*, fairy shrimp and cladocerans. The free amino acids were only determined in the samples of the organisms that served as a source of live food for the matrinxã larvae, as the objective of this analysis was to know the concentration of the free amino acids that were present in the live food.

The amino acid analysis was performed by reverse phase column chromatography using a high-performance liquid chromatograph. In acid hydrolysis with phenol, individual amino acids were released. This procedure allowed the recovery of most of the amino acids, except for tryptophan, which had been completely destroyed and had to be analyzed by another method. For the amino acids released in acid hydrolysis,  $\alpha$ -aminobutyric acid was added as the internal standard. After eliminating the acid by vacuum evaporation, re-evaporation was performed with sodium acetate, methanol and triethylamine solution. After this procedure, the hydrolyzate was derivatized with a solution of methanol, ultrapure water, triethylamine and phenyl isothiocyanate (PITC). The amino acids were dissolved in diluent and introduced into the column. The mobile phase consisted of eluents A and B. Eluents with pH 6.60 contained sodium acetate, acetonitrile, ultrapure water and disodium EDTA. The peak areas obtained from the unknown sample were quantified by comparing them with those of a standard mixture of amino acids and an internal standard of 254 nm.

### 2.7.3. Analysis of Total Lipids and Fatty Acids

The analyses of total lipids (TLs) and fatty acids were performed at the CBO analysis laboratory in Campinas, São Paulo State. TL extractions were performed in triplicate from matrinxã larvae samples and from the different types of live food for each treatment, according to the method proposed by [45]. This method extracts all classes of lipids and not only neutral compounds, which has undeniable value in treatment evaluations. To achieve this, the lipids were extracted from the sample with a monophasic mixture of chloroform, methanol and water, under agitation. After adding sodium sulfate solution and additional chloroform, the phases were separated. The lower phase, where chloroform predominates, was separated, the solvent was evaporated and the remaining lipids were determined by gravimetry.

The extract (chloroform with lipids) resulting from each extraction was concentrated in a rotary evaporator, with a water bath at a temperature of 32–34 °C, under a vacuum. The TLs were placed separately in amber flasks of 7 mL capacity under a N<sub>2</sub> atmosphere and were frozen at –18 °C, for the fractionation of lipids in classes and analysis of fatty acids.

Then, the lipid samples underwent saponification with a 2% NaOH solution in methanol, followed by esterification with a solution of ammonium chloride and sulfuric acid in methanol. Strict conditions during saponification controlled the degree of esterification at a level of 99.5%, avoiding the conversion of soaps into methyl esters and the precipitation of alkaline sulphates. The fatty acid methyl esters, thus prepared, were quantified in a Varian gas chromatograph, mod. 3300, equipped with a flame ionization detector, split/splitless injector and fused silica capillary column DB-WAX 20 M (30 m × 0.25 mm × 0.25 m) (J & W Scientific Inc., Santa Clara, CA, USA). The operating conditions of the column were as follows: an injector temperature of 250 °C, a detector temperature of 280 °C and a column temperature of 170 °C for 16 min, programmed to increase 2 °C per minute up to 210 °C, and remaining at this temperature for 30 min. Hydrogen was used as a carrier gas at 1 mL min<sup>-1</sup>, with a linear velocity of 38 cm s<sup>-1</sup> with an oxygen filter attached to the gas line; nitrogen was used as a make-up gas at 30 mL min<sup>-1</sup>, with the flow of hydrogen gas being 30 mL min<sup>-1</sup>; the synthetic air rate was 300 mL min<sup>-1</sup> and the injection volume was 1 mL, split into a 1:50 ratio. The retention time, peak area and relative percentage area values (normalization method) were obtained using a Varian mod 4290 integrator (Varian Associates, Inc., Palo Alto, CA, USA).

The average fatty acid composition was obtained from methylation for each extraction, with injections performed in duplicate.

There was an internal standard to quantify fatty acids. The method was based on the separation of fatty acids by gas chromatography in a capillary column, with a flame ionization detector (FID) and quantification by internal standard. In the gas chromatography (GC) technique, the compound of interest percolates through a chromatography column with the aid of a gaseous mobile phase and separates from the other sample constituents due to its migration speed, which is determined by the balance of the compound's interaction with mobile and stationary phases and by the column temperature. The FID detector was used. The internal standard quantification method allows obtainment of the concentrations of each fatty acid present in the sample.

### 2.8. Statistical Data Analysis

The experiment was conducted in a completely randomized design (DIC), consisting of seven treatments, with four repetitions each, and lasted for 10 days. The survival results were transformed into  $(\text{arc.sen}\sqrt{x}) 100$ , where  $x$  was the value expressed as a percentage. The data first went through a normality test (the Shapiro–Wilk's  $W$  test) and a homogeneity of variance test (Barlett's test). Next, the results were analyzed through analysis of variance (ANOVA), and, in the case of statistical significance, the means were compared using Tukey's test, with a level of 5%. The analyses were performed using the "Statistical Analysis System" (SAS Institute Inc., Cary, NC, USA, version 9.0).

## 3. Results

### 3.1. Larvae Growth

The growth parameters are described in Table 2. The best growth performance between the breeding and feeding systems was observed in the clear water system with exercise, regardless of the diet. Despite this, the type of diet in the exercise system had a slight effect on the increase in larvae weight. The largest increase in weight was for the group of larvae with the *Artemia* nauplii diet ( $42.9 \pm 2.58$  mg), followed by the group of larvae that were fed the mixed diet (*Artemia* and *D. brasiliensis* nauplii) with a final weight of  $36.7 \pm 3.05$  mg and for larvae fed with nauplii of *D. brasiliensis* ( $32.30 \pm 4.10$  mg). The specific growth rate (SGR) of *B. amazonicus* larvae fed with *Artemia* nauplii and raised in moderate swimming exercise ( $31.7 \pm 0.60\%$  day<sup>-1</sup>) was higher ( $p < 0.05$ ) than the larvae fed with nauplii of *D. brasiliensis* and raised in a conventional clear water system without a current ( $27.1 \pm 2.93\%$  day<sup>-1</sup>). On the other hand, the survival rates of *B. amazonicus* larvae reared in the different rearing and feeding systems were significantly affected ( $p < 0.05$ ). Larvae that were raised in a moderate current environment showed better survival rates regardless of the diet offered. For example, in the exercise system, the group of larvae that were fed *Artemia* nauplii achieved higher survival rates ( $25.7 \pm 1.7\%$ ), whereas in the conventional standing water system, the larvae that received a similar diet (*Artemia* nauplii) showed lower survival ( $18.2 \pm 2.11\%$  day<sup>-1</sup>). In the mesocosm system, survival was even lower ( $15.0 \pm 2.94\%$ ). Despite the different breeding systems and types of food, cannibalism was inevitable, but even so, a lower incidence of larval losses due to cannibalism was observed in the exercise system.

### 3.2. Biochemical Composition of *Matrinxã* (*Brycon amazonicus*) Larvae Reared under Different Production Systems and Food

#### 3.2.1. Composition of Amino Acids in Larvae of *Brycon amazonicus*

The concentration of amino acids in *Brycon amazonicus* larvae, when reared under different production systems and fed different types of live food, showed significant differences ( $p < 0.05$ ) (Table 3). The dry weight composition of the total amino acids (TAA) of the *B. amazonicus* larvae fed with *D. brasiliensis* nauplii and raised in sustained exercise was the largest of all ( $846.8 \pm 16$  g kg<sup>-1</sup> dry weight), followed by the larvae that received the mixed diet of nauplii of *D. brasiliensis* and *Artemia* which were also exercised



(689.7 ± 13.7 g kg<sup>-1</sup> dry weight). On the other hand, the larvae that were not exercised and regardless of the type of food received, presented, on average, lower TAA values than the exercised larvae (*p* < 0.05). The larvae reared in the mesocosm or green water system showed lower TAA contents when compared to the other larvae reared in the clear water system. Although the mesocosm larvae that consumed the diet with cladocerans presented higher values of TAA (640.1 ± 12.8 g kg<sup>-1</sup> of dry weight) than the larvae that received *Artemia* nauplii (629.0 ± 12.6 g kg<sup>-1</sup> of dry weight), these were not significant (*p* > 0.05).

**Table 2.** Mean values (standard deviation) of the initial length (il), initial weight (iw), final length (fl), final weight (fw), specific growth rate (SGR), survival (%) and cannibalism (%) in *Brycon amazonicus* larvae reared under different production systems and feeding (types of live food).

| Performance Parameters       | Production Systems                                 |                |                |   |                |                |                                  |
|------------------------------|--|----------------|----------------|---|----------------|----------------|----------------------------------|
|                              | Clear Water  |                |                |   |                |                | Green Water                      |
|                              | Larvae Raised in Traditional Standing Water System |                |                | Larvae Reared in Sustained Swimming Exercise System |                |                | Larvae Reared in Mesocosm System |
| A                            | B  | A + B          | A              | B   | A + B          | Cladocerans    |                                  |
| Initial length (il—mm)       | 6.22 ± 0.30  | 6.22 ± 0.30    | 6.22 ± 0.30    | 6.22 ± 0.30   | 6.22 ± 0.30    | 6.22 ± 0.30    | 6.22 ± 0.30                      |
| Initial weight (iw—mg)       | 1.79 ± 0.42  | 1.79 ± 0.42    | 1.79 ± 0.42    | 1.79 ± 0.42   | 1.79 ± 0.42    | 1.79 ± 0.42    | 1.79 ± 0.42                      |
| Final length (fl—mm)         | 16.6 ± 1.15 a                                      | 15.6 ± 1.12 a  | 16.1 ± 1.06 a  | 17.6 ± 1.29 a                                       | 17.3 ± 1.09 a  | 17.0 ± 1.06 a  | 16.32 ± 1.02 a                   |
| Final weight (fw—mg)         | 30.1 ± 5.73 b                                      | 27.7 ± 7.59 cd | 29.2 ± 6.81 de | 42.9 ± 2.58 a                                       | 32.3 ± 4.10 a  | 36.7 ± 3.05 a  | 29.38 ± 5.30 be                  |
| Specific growth rate (SGR—%) | 28.1 ± 1.89 ab                                     | 27.1 ± 2.93 b  | 27.7 ± 2.57 ab | 31.7 ± 0.60 a                                       | 28.9 ± 1.21 ab | 30.2 ± 0.80 ab | 27.85 ± 1.92 ab                  |
| Survival (%)                 | 18.2 ± 2.11 b                                      | 10.6 ± 2.21 c  | 12.8 ± 2.45 d  | 25.7 ± 1.70 a                                       | 20.9 ± 2.46 a  | 22.2 ± 2.36 a  | 15.00 ± 2.94 e                   |
| Cannibalism (%)              | 81.8   | 89.3           | 87.2           | 74.2  | 79.1           | 77.7           | 85.00                            |

A: *Artemia*; B: *Branconeta*; A + B: *Artemia* + *D. brasiliensis*; cladocerans: *Daphnia magna*. Green water = mesocosm. Averages followed on the same line by different letters are statistically different according to Tukey's test (*p* < 0.05).

**Table 3.** Amino acid composition (g<sup>-1</sup> kg dry weight) in *Brycon amazonicus* larvae reared under different production systems and feeding (types of live food).

| Amino Acids       | Production Systems |              |              |                 |              |              |               |
|-------------------|--------------------|--------------|--------------|-----------------|--------------|--------------|---------------|
|                   | Clear Water        |              |              |                 |              |              | Green Water   |
|                   | Traditional System |              |              | Exercise System |              |              | Mesocosm      |
| A                 | A + B              | B            | A            | A + B           | B            | Cladocerans  |               |
| EAA <sup>1</sup>  |                    |              |              |                 |              |              |               |
| Arg               | 45.3 ± 0.9 bc      | 49.8 ± 0.9 b | 47.7 ± 0.9 b | 46.6 ± 0.9 b    | 48.0 ± 0.9 b | 58.0 ± 1.2 a | 45.1 ± 0.9 bc |
| Ile               | 26.9 ± 0.5         | 29.2 ± 0.6   | 29.9 ± 0.4   | 27.5 ± 0.5      | 28.5 ± 0.5   | 36.1 ± 0.7   | 26.5 ± 0.5    |
| Phe               | 27.9 ± 0.5         | 30.2 ± 0.6   | 30.2 ± 0.6   | 28.4 ± 0.6      | 29.5 ± 0.6   | 3.6 ± 0.7    | 27.4 ± 0.5    |
| His               | 14.5 ± 0.4         | 16.6 ± 0.3   | 15.9 ± 0.3   | 15.2 ± 0.3      | 15.6 ± 0.3   | 19.6 ± 0.4   | 16.6 ± 0.3    |
| Leu               | 49.3 ± 0.9 bc      | 54.3 ± 1.0 b | 56.5 ± 1.1 b | 51.9 ± 1.0 bc   | 54.8 ± 1.1 b | 68.0 ± 1.4 a | 50.6 ± 1.0 bc |
| Lys               | 49.2 ± 0.9 bcd     | 54.9 ± 1.0 b | 55.1 ± 1.1 b | 52.0 ± 1.0 bd   | 55.4 ± 1.1 b | 67.6 ± 1.3 a | 50.7 ± 1.0 bd |
| Met               | 16.6 ± 0.3         | 18.3 ± 0.4   | 17.4 ± 0.3   | 17.0 ± 0.3      | 17.4 ± 0.3   | 20.4 ± 0.4   | 16.6 ± 0.3    |
| Thr               | 26.4 ± 0.5         | 28.9 ± 0.6   | 29.4 ± 0.6   | 27.8 ± 0.5      | 29.3 ± 0.6   | 35.7 ± 0.7   | 26.1 ± 0.5    |
| Val               | 36.7 ± 0.7         | 39.0 ± 0.8   | 40.9 ± 0.8   | 37.7 ± 0.7      | 38.5 ± 0.8   | 49.0 ± 0.9   | 36.5 ± 0.7    |
| NEAA <sup>2</sup> |                    |              |              |                 |              |              |               |
| Asp               | 52.2 ± 1.0         | 61.5 ± 1.2   | 61.2 ± 1.2   | 47.2 ± 0.9      | 62.4 ± 1.2   | 83.3 ± 1.7   | 51.9 ± 1.0    |
| Glut              | 91.5 ± 1.8         | 101.7 ± 2.0  | 106.0 ± 2.1  | 91.1 ± 1.8      | 103.9 ± 2.0  | 130.0 ± 2.6  | 93.1 ± 1.8    |
| Ala               | 41.0 ± 0.8         | 44.7 ± 0.9   | 44.8 ± 0.9   | 43.2 ± 0.9      | 45.2 ± 0.9   | 54.8 ± 1.1   | 43.1 ± 0.8    |

**Table 3.** *Cont.*

| Amino Acids     | Production Systems     |                       |                       |                         |                       |                       |                        |
|-----------------|------------------------|-----------------------|-----------------------|-------------------------|-----------------------|-----------------------|------------------------|
|                 | Clear Water            |                       |                       |                         |                       |                       | Green Water            |
|                 | Traditional System     |                       |                       | Exercise System         |                       |                       | Mesocosm               |
|                 | A                      | A + B                 | B                     | A                       | A + B                 | B                     | Cladocerans            |
| Cis             | 15.5 ± 0.3             | 15.9 ± 0.3            | 17.5 ± 0.3            | 14.2 ± 0.3              | 17.7 ± 0.3            | 21.7 ± 0.4            | 12.8 ± 0.2             |
| Gly             | 45.7 ± 0.9             | 49.3 ± 0.9            | 44.6 ± 0.9            | 49.0 ± 0.9              | 49.2 ± 0.9            | 55.4 ± 1.1            | 52.3 ± 1.0             |
| Pro             | 30.3 ± 0.6             | 32.9 ± 0.6            | 30.3 ± 0.6            | 31.6 ± 0.6              | 31.9 ± 0.6            | 37.2 ± 0.7            | 33.7 ± 0.6             |
| Ser             | 24.7 ± 0.5             | 26.6 ± 0.5            | 27.0 ± 0.5            | 24.9 ± 0.5              | 27.5 ± 0.5            | 32.4 ± 0.6            | 24.6 ± 0.5             |
| Tau             | 10.5 ± 0.2             | 9.5 ± 0.2             | 7.2 ± 0.1             | 11.7 ± 0.2              | 11.7 ± 0.2            | 9.0 ± 0.2             | 9.1 ± 0.2              |
| Tyr             | 24.9 ± 0.5             | 26.7 ± 0.5            | 23.6 ± 0.5            | 24.1 ± 0.4              | 22.7 ± 0.4            | 31.5 ± 0.6            | 22.7 ± 0.4             |
| <b>TOTAL AA</b> | 629.0 ± 12.6 <b>bd</b> | 689.5 ± 13.8 <b>b</b> | 685.2 ± 12.7 <b>b</b> | 642.0 ± 12.84 <b>bc</b> | 689.7 ± 13.8 <b>b</b> | 846.8 ± 16.9 <b>a</b> | 640.1 ± 12.8 <b>bc</b> |

A: *Artemia*; B: *D. brasiliensis*; A + B: *Artemia* + *D. brasiliensis*; cladocerans: *Daphnia magna*. Green water = mesocosm. <sup>1</sup> EAA: essential amino acids. <sup>2</sup> NEAA: non-essential amino acids. The most important and abundant amino acids are indicated in bold. Averages followed on the same line by different letters are statistically different ( $p < 0.05$ ).

*Brycon amazonicus* larvae raised in a clear water system under exercise and fed with nauplii of *D. brasiliensis* had the best essential amino acid (EAA) profiles ( $p < 0.05$ ). These larvae had the highest levels of arginine, leucine, lysine and methionine ( $58.00 \pm 1.16$ ,  $68.04 \pm 1.36$ ,  $67.64 \pm 1.35$ , and  $20.40 \pm 0.40$  g kg<sup>-1</sup> dry weight, respectively). Similarly, this trend was observed with the other larvae raised under sustained exercise and fed either with *Artemia* nauplii or with a mixed diet (*D. brasiliensis* and *Artemia* nauplii). *Brycon amazonicus* larvae reared in the green water system (mesocosms), which received the cladoceran diet, showed EAA contents equivalent to those of other larvae reared in standing water. Some EAAs, such as leucine and lysine, were slightly increased in relation to the diet with *Artemia* nauplii that was consumed by the larvae raised in standing water.

### 3.2.2. Composition of Fatty Acids in Larvae of Matrinxã, *Brycon amazonicus*

The results of the analysis of the fatty acid composition of *Brycon amazonicus* larvae are shown in Table 4. According to the results in the previous table, the total fatty acid concentration of diets with *Artemia* nauplii ( $14.01 \pm 1.3$  g kg<sup>-1</sup> dry weight) and *D. brasiliensis* nauplii ( $13.46$  g kg<sup>-1</sup> dry weight) did not differ statistically in the tissues of *Brycon amazonicus* larvae in both rearing systems (conventional and exercise). The concentration of PUFA and EPA + DHA was higher when the larvae were fed with nauplii of *D. brasiliensis*, whereas the lowest concentrations were found in the larvae that were fed with cladocerans in the mesocosm system ( $4.99$  and  $3.15$  g dry weight kg<sup>-1</sup>, respectively). It was also observed that the exercise system with the diet based on *D. brasiliensis* nauplii was the one that provided the best profile of essential fatty acids for *B. amazonicus* larvae, while the diet with the lowest-quality composition of essential fatty acids was the one with cladocerans.

**Table 4.** Fatty acid composition (g<sup>-1</sup> kg dry weight) in *Brycon amazonicus* larvae reared under different production systems and feeding systems (types of live food).

| Fatty Acids                | Traditional System |   |                        | Exercise System |   |                        | Mesocosms   |
|----------------------------|--------------------|---|------------------------|-----------------|---|------------------------|-------------|
|                            | <i>Artemia</i>     | <i>Artemia</i> + <i>D. brasiliensis</i> | <i>D. brasiliensis</i> | <i>Artemia</i>  | <i>Artemia</i> + <i>D. brasiliensis</i> a | <i>D. brasiliensis</i> | Cladocerans |
| Saturated fatty acid       |                    |   |                        |                 |   |                        |             |
| Laurelic acid (C12:0)      | 0.01 ± 0.0         | 0.02 ± 0.0                              | 0.01 ± 0.0             | 0.03 ± 0.0      |   | 0.01 ± 0.0             | 0.02 ± 0.0  |
| Miristic acid (C14:0)      | 0.14 ± 0.0         | 0.14 ± 0.0                              | 0.15 ± 0.0             | 0.11 ± 0.0      | 0.08 ± 0.0                                | 0.15 ± 0.0             | 0.07 ± 0.0  |
| Pentadecanoic acid (C15:0) | 0.12 ± 0.0         | 0.10 ± 0.0                              | 0.12 ± 0.0             | 0.09 ± 0.0      | 0.09 ± 0.0                                | 0.12 ± 0.0             | 0.06 ± 0.0  |

**Table 4.** *Cont.*

| Fatty Acids  | Traditional System |                                  |                        | Exercise System |                                    |                        | Mesocosms     |
|--|--------------------|----------------------------------|------------------------|-----------------|------------------------------------|------------------------|---------------|
|  | <i>Artemia</i>     | <i>Artemia + D. brasiliensis</i> | <i>D. brasiliensis</i> | <i>Artemia</i>  | <i>Artemia + D. brasiliensis a</i> | <i>D. brasiliensis</i> | Cladocerans   |
| Palmitic acid (C16:0)                                | 3.18 ± 0.3         | 3.03 ± 0.3                       | 3.30 ± 0.3             | 3.11 ± 0.0      | 2.77 ± 0.4                         | 3.36 ± 0.1             | 2.03 ± 0.2    |
| Margaric acid (C17:0)                                | 0.26 ± 0.0         | 0.21 ± 0.0                       | 0.27 ± 0.0             | 0.23 ± 0.0      | 0.14 ± 0.0                         | 0.28 ± 0.0             | 0.15 ± 0.0    |
| Stearic acid (C18:0)                                 | 1.68 ± 0.2         | 1.79 ± 0.2                       | 1.74 ± 0.3             | 2.16 ± 0.2      | 2.01 ± 0.2                         | 1.77 ± 0.1             | 1.41 ± 0.1    |
| Araquic acid (C20:0)                                 | 0.19 ± 0.0         | 0.04 ± 0.0                       | 0.20 ± 0.0             | 0.06 ± 0.0      | 0.04 ± 0.0                         | 0.20 ± 0.0             | 0.04 ± 0.0    |
| Heneicosanoic acid (C21:0)                           | 0.02 ± 0.0         |                                  | 0.02 ± 0.0             | 0.00            |                                    | 0.02 ± 0.0             |               |
| Behenic acid (C22:0)                                 | 0.05 ± 0.0         | 0.03 ± 0.0                       | 0.05 ± 0.0             | 0.08 ± 0.0      | 0.05 ± 0.0                         | 0.05 ± 0.0             | 0.05 ± 0.0    |
| Lignoceric acid (C24:0)                              | 0.03 ± 0.0         | 0.03 ± 0.0                       | 0.03 ± 0.0             | 0.06 ± 0.0      | 0.05 ± 0.0                         | 0.03 ± 0.0             | 0.04 ± 0.0    |
| Total saturated fatty acid                           | 5.68 ± 0.6         | 5.39 ± 0.5                       | 5.90 ± 0.5             | 5.91 ± 0.5      | 5.22 ± 0.5                         | 6.01 ± 0.2             | 3.86 ± 0.3    |
| Unsaturated fatty acid                               |                    |                                  |                        |                 |                                    |                        |               |
| Palmitoleic acid (C16:1)                             | 0.90 ± 0.0         | 0.44 ± 0.0                       | 0.93 ± 0.1             | 0.28 ± 0.0      | 0.22 ± 0.0                         | 0.95 ± 0.0             | 0.18 ± 0.0    |
| Oleic acid (C18:1n9c)                                | 2.55 ± 0.3         | 2.27 ± 0.2                       | 2.65 ± 0.2             | 2.30 ± 0.2      | 2.03 ± 0.2                         | 2.70 ± 0.1             | 1.50 ± 0.1    |
| Cis-eicosenoic acid (C20:1)                          | 0.06 ± 0.0         | 0.05 ± 0.0                       | 0.06 ± 0.0             | 0.05 ± 0.0      | 0.03 ± 0.0                         | 0.06 ± 0.0             | 0.03 ± 0.0    |
| Erucic (C22:1n9)                                     | 0.04 ± 0.0         |                                  | 0.04 ± 0.0             | 0.06 ± 0.0      | 0.03 ± 0.0                         | 0.04 ± 0.0             | 0.04 ± 0.0    |
| Total monosaturated fatty acids                      | 3.64 ± 0.4         | 2.83 ± 0.3                       | 3.78 ± 0.3             | 2.74 ± 0.2      | 2.35 ± 0.2                         | 3.85 ± 0.1             | 1.79 ± 0.2    |
| Linoleic acid (C18:2n6c)                             | 0.77 ± 0.1         | 0.74 ± 0.1                       | 0.80 ± 0.1             | 1.25 ± 0.11     | 0.76 ± 0.1                         | 0.82 ± 0.0             | 0.82 ± 0.1    |
| Gamma linolenic acid (C18:3n6)                       | 0.03 ± 0.0         | 0.02 ± 0.0                       | 0.03 ± 0.0             | 0.35 ± 0.0      |                                    | 0.03 ± 0.0             |               |
| Linolenic acid (C18:3n3)                             | 0.27 ± 0.0 a       | 0.12 ± 0.0 d                     | 0.28 ± 0.0 a           | 0.03 ± 0.0 e    | 0.18 ± 0.0 c                       | 0.29 ± 0.0 a           | 0.23 ± 0.0 b  |
| Cis-eicosadienoic acid (C20:2)                       | 0.02 ± 0.0         | 0.02 ± 0.0                       | 0.02 ± 0.0             | 0.00            | 0.02 ± 0.0                         | 0.02 ± 0.0             | 0.02 ± 0.0    |
| Cis-eicosatrienoic acid (C20:3n3)                    | 0.01 ± 0.0         |                                  | 0.01 ± 0.0             | 0.05 ± 0.0      | 0.03 ± 0.0                         | 0.01 ± 0.0             | 0.03 ± 0.0    |
| Cis-eicosatrienoic acid (C20:3n6)                    | 0.09 ± 0.0         |                                  | 0.09 ± 0.0             | 0.09 ± 0.0      | 0.08 ± 0.0                         | 0.09 ± 0.0             | 0.06 ± 0.0    |
| Arachidonic acid (C20:4n6) AA <sup>1</sup>           | 1.17 ± 0.1 c       | 1.16 ± 0.1 c                     | 1.21 ± 0.6 a           | 1.07 ± 0.1 b    | 1.21 ± 0.1 a                       | 1.23 ± 0.0 a           | 0.70 ± 0.16 d |
| Cis-eicosapentaenoic acid (C20:5n3) EPA <sup>2</sup> | 0.64 ± 0.1 a       | 0.50 ± 0.0 b                     | 0.66 ± 0.1 a           | 0.50 ± 0.0 b    | 0.40 ± 0.0 c                       | 0.67 ± 0.0 a           | 0.33 ± 0.0 d  |
| Cis-docosahexaenoic acid (C22:6n3) DHA <sup>3</sup>  | 1.74 ± 0.2 b       | 1.20 ± 0.1 d                     | 1.81 ± 0.2 b           | 1.50 ± 0.1 c    | 1.99 ± 0.2 a                       | 1.84 ± 0.1 b           | 0.98 ± 0.1 d  |
| Total polyunsaturated fatty acids                    | 4.69 ± 0.5 a       | 3.77 ± 0.4 b                     | 4.87 ± 0.4 a           | 4.82 ± 0.43 a   | 4.62 ± 0.5 a                       | 4.96 ± 0.2 a           | 3.15 ± 03 c   |
| Elaidic acid (C18:1n9t)                              | 0.03 ± 0.0         | 0.03 ± 0.0                       | 0.03 ± 0.0             | 0.03 ± 0.0      |                                    | 0.03 ± 0.0             | 0.02 ± 0.0    |
| Total unsaturated fatty acids                        | 8.32 ± 0.8 a       | 6.61 ± 0.7 c                     | 8.64 ± 0.8 a           | 7.56 ± 0.7 b    | 6.98 ± 0.7 c                       | 8.80 ± 0.3 a           | 4.94 ± 0.4 d  |

Table 4. Cont.

| Fatty Acids          | Traditional System |  |                        | Exercise System |  |                        | Mesocosms    |
|----------------------|--------------------|--|------------------------|-----------------|--|------------------------|--------------|
|                      | <i>Artemia</i>     | <i>Artemia</i> +<br><i>D. brasiliensis</i> | <i>D. brasiliensis</i> | <i>Artemia</i>  | <i>Artemia</i> +<br><i>D. brasiliensis</i> a | <i>D. brasiliensis</i> | Cladocerans  |
| PUFA <sup>4</sup>    | 4.72               | 3.8  | 4.9                    | 4.82            | 4.65   | 4.99                   | 3.15         |
| EPA + DHA            | 2.38               | 1.70                                       | 2.47                   | 2.00            | 2.39   | 2.51                   | 1.31         |
| Omega-3 <sup>5</sup> | 2.65               | 1.83                                       | 2.75                   | 2.40            | 2.60   | 2.80                   | 1.57         |
| Omega-6              | 2.07               | 1.97                                       | 2.15                   | 2.42            | 2.05   | 2.19                   | 1.58         |
| Total fatty acids    | 14.01 ± 1.4 a      | 12.00 ± 1.2 a                              | 14.54 ± 1.3 a          | 13.5 ± 1.2 a    | 12.20 ± 1.1 a                                | 14.82 ± 0.6 a          | 8.80 ± 0.8 b |

AA <sup>1</sup>: arachidonic acid; EPA <sup>2</sup>: eicosapentaenoic acid; DHA <sup>3</sup>: docosahexaenoic acid; PUFA <sup>4</sup>: polyunsaturated fatty acids; Omega-3 <sup>5</sup>: highly polyunsaturated fatty acids. Averages followed on the same line by different letters are statistically different ( $p < 0.05$ ).

The water quality parameters between treatments and during the experimental period remained within the recommended range for tropical fish according to [46] (Table 5).

Table 5. Means and standard deviations of the physical and chemical parameters of water quality. Recorded during the experimental period in the different systems of larvae production of *B. amazonicus*.

| Treatments                           | Temperature | pH          | Dissolved Oxygen   |             | Conductivity<br>( $\mu\text{S cm}^{-1}$ ) | Salinity<br>(%) |
|--------------------------------------|-------------|-------------|--------------------|-------------|---|-----------------|
|                                      |             |             | mg L <sup>-1</sup> | (% Sat.)    |   |                 |
| Mesocosms<br>Cladocerans             | 28.5 ± 1.90 | 7.59 ± 0.10 | 7.21 ± 0.42        | 104 ± 2.40  | 141.7 ± 17                                | 0.05 ± 0.03     |
| Exercise + <i>Artemia</i>            | 28.4 ± 1.91 | 7.62 ± 0.11 | 7.57 ± 0.68        | 99.2 ± 2.75 | 6065.6 ± 527                              | 3.0 ± 0.04      |
| Exercise + <i>D. brasiliensis</i>    | 29.9 ± 1.57 | 7.6 ± 0.08  | 7.71 ± 0.78        | 100 ± 3.46  | 5932.1 ± 390                              | 3.06 ± 0.02     |
| Exercise + mixture<br>(A + Db)       | 28.1 ± 1.95 | 7.56 ± 0.04 | 7.72 ± 0.41        | 99.0 ± 1.00 | 6038.3 ± 309.                             | 3.08 ± 0.02     |
| Traditional + <i>Artemia</i>         | 28.8 ± 1.78 | 7.64 ± 0.04 | 7.44 ± 0.52        | 97.1 ± 4.00 | 6131.3 ± 256                              | 3.07 ± 0.08     |
| Traditional + <i>D. brasiliensis</i> | 28.2 ± 1.79 | 7.56 ± 0.10 | 7.12 ± 0.25        | 97.7 ± 2.69 | 6202.2 ± 374                              | 3.02 ± 0.10     |
| Traditional + mixture<br>(A + Db)    | 28.7 ± 1.31 | 7.66 ± 0.10 | 7.44 ± 0.51        | 98.7 ± 3.09 | 5944.6 ± 244                              | 3.12 ± 0.06     |

#### 4. Discussion

The metabolism of fish, especially in young phases, is high, and juvenile fish need to feed more frequently to sustain high growth rates [47,48].

The growth responses in *Brycon amazonicus* larvae reared in different production and feeding systems were different. The best growth was achieved in the group of larvae fed with *Artemia* nauplii and raised in a clear water system under sustained swimming exercises. In these conditions, the specific growth rate (SGR) was higher (31.77% day<sup>-1</sup>) than the larvae raised and fed with similar diets, but without exercise (28.1 ± 1.89% day<sup>-1</sup>). The lowest rates observed were in the larvae raised in the green water system or mesocosms (27.8 ± 1.92% day<sup>-1</sup>). The salinization of the water also contributed to higher growth rates of the *B. amazonicus* larvae as the survival of *Artemia* nauplii increased significantly, making the nauplii more available for the larvae to ingest. In a study by [49] it was observed that the larvae of *B. amazonicus* bred and fed with *Artemia* nauplii in slightly salinized water (2 g L<sup>-1</sup>) grew and survived significantly longer than those raised in higher salinities. In the present study, a saline concentration of 3 g L<sup>-1</sup> was adopted according to the work of [50]. In order to know if this low saline concentration would affect the survival of *D. brasiliensis*, we proposed treatments with both species of live food (*Artemia* and *D. brasiliensis*) in our experimental design. In preliminary tests with *D. brasiliensis*, it was observed that mortality started after 3 h of exposure to the salinity of 3 g L<sup>-1</sup>, which is enough time for the larvae of *B. amazonicus* to feed.

It is important to note that the groups of larvae that received *D. brasiliensis* nauplii as food expressed lower growth rates in the clear water system (27.1 ± 2.93% day<sup>-1</sup>). These growth differences observed among the larvae were probably due more to the type of

feeding than to the rearing system. On average, the equivalent of 170%, 100% and 70% of the larvae's live weight was offered daily over 10 days in terms of fresh biomass of food via *Artemia* nauplii and/or *D. brasiliensis*. These feed rates were based on data reported by [39]. In view of this situation, the feed rates used in this work must be adjusted in future work with *Brycon amazonicus*. In fact, in a bioenergetic study carried out with *Brycon moorei* [51], the author found that the larvae needed large amounts of food to support their high growth rates, i.e., from 100% to 300% of the larvae's live weight day<sup>-1</sup> from the first to seventh day of exogenous feeding. According to the author, a larva of *B. amazonicus*, 36 h after hatching, at an average live weight of 1.6 mg, if fed at a rate equivalent to 300% of its live weight would need to consume 537 nauplii/day, since *Artemia* or *D. brasiliensis* nauplii weigh 0.01 mg when they hatch. Considering that 1 g of *Artemia* salina cysts produces on average 240,000 nauplii, it can feed 446 larvae of *B. amazonicus*, while 1 g of *D. brasiliensis*, which produces on average 380,000 nauplii [40], can feed more than 700 larvae of *B. amazonicus*. For a larviculture that produces millions of larvae, it would be more advantageous to use nauplii of *D. brasiliensis* than *Artemia*. However, the analysis of production costs of *B. amazonicus* larvae produced between the different systems of larviculture is a decisive and important factor that helps the producer in choosing which system to adopt. An important item to consider is the cost of the cyst used in the production of live food, although this economic analysis was not performed in this study. According to a commercial supplier in Brazil, the cost of 1 g of *Artemia* cysts is 6.87 US cents, while the value of 1 g of cysts of *D. brasiliensis* is 1.5 dollars. This value is high due to the lack of large-scale production of *D. brasiliensis* cysts in Brazil. This indicates that it is necessary to research and develop technology that allows the production of *D. brasiliensis* cysts at a low cost for the larviculture of aquatic organisms of commercial interest.

One way of evaluating the effect of consuming high rates of live and/or inert food in larvae, such as those mentioned in the work by [51], would be through the final survival rate (S%), a widely used parameter that indicates the way larviculture was conducted. In the present study, the survival rate varied depending on both the system used and the type of live food provided. The best survival rates were achieved by the larvae reared under exercise conditions and fed with *Artemia* nauplii (25.75%). On the contrary, *Brycon amazonicus* larvae fed with the same diet, but raised in standing water (without exercise), achieved lower rates (18.22 ± 2.11%). On the other hand, even lower survival rates were observed in the mesocosm system (15.00 ± 2.94%).

When working with aggressive larvae such as *B. amazonicus*, cannibalism, in most cases, is always present [52]. Care is always taken to maximize survival and prevent cannibalism. In this respect, the present work achieved better survival rates in all groups of larvae when they were raised with a moderate water current (2 ccs<sup>-1</sup>). Although behavior was not explicitly analyzed in this work, larvae reared in environments with currents exhibited a shoal behavior, which probably decreased the aggressive behavior of this species, which in turn favored greater survival. Although exercise and the type of food improved the survival rates of *B. amazonicus*, this parameter was still low. The highest fish growth rates are achieved in the larval stages; however, high energy requirements are necessary to maintain rapid growth [53]. According to these authors, if the energy need is not met by the supplemented food, the larva can focus its need on another source of extra energy: ingesting another larva of its own species. Probably, the amount of fresh biomass ingested, via *Artemia* nauplii, *D. brasiliensis* and cladocerans (*Daphnia magna*), was not sufficient to satiate the appetite of *B. amazonicus* larvae and, therefore, cannibalism was stimulated, leading to low survival rates.

One of the alternatives that fish farmers usually use to minimize cannibalism is to feed larvae of commercial interest with larvae of other species (forage larvae). However, according to [5], the use of other fish species as an initial food for larvae is not recommended because it stimulates aggressive behavior and can exacerbate cannibalism. The results of low survival rates and high cannibalism rates reported in the present study are consistent with the statements of [5], as the larvae of *B. amazonicus* were acquired from a commercial fish

farm 36 h after hatching, and there they were already being fed with larvae of *Prochilodus skrofa* (curimatá, in portuguese), which may have increased the cannibalism of the larvae of *Brycon amazonicus*. In commercial fish farms, it is common to synchronize the spawning time of commercial species with highly prolific forage fish species, as is the case with *P. skrofa*, to relieve intraspecific predation pressure between the main species. We observed that the newly hatched nauplius of *D. brasiliensis* was too small a prey for a larva like *B. amazonicus*, which has an insatiable appetite. We believe that the adequate size of *D. brasiliensis* for larvae of *B. amazonicus* is at least 6.5 mm, which corresponds to the length of the larvae within 36 h of hatching. Beyond this prey size, the larva probably expends less energy on searching for and capturing live prey and thus can channel more energy into growth than it does when capturing live prey such as newly hatched nauplii of *D. brasiliensis*.

In recent studies, with *Brycon amazonicus* larviculture, the survival rates have been even lower than those achieved in the present study. In a study conducted by [54], increasing concentrations of probiotics were tested on the larvae of *Brycon amazonicus*, during a 10 day experimental period. At the end of the study, the minimum and maximum survival rates were 2.78% and 5.28%, respectively, and cannibalism was above 96%. These authors claim that the cause of these unfavorable rates was the high stocking density (10 L<sup>-1</sup> larvae) and not the food. The stocking density that was used in the present study was similar to that of [54]; however, the number of larvae per experimental unit was more than double (500 tank<sup>-1</sup> larvae) compared to theirs (200 tank<sup>-1</sup> larvae). The present study chose to work with a stocking density of 10 L<sup>-1</sup> larvae; this is neither high nor low and is beyond the densities practiced in commercial fish farms.

The results of the biochemical composition of live foods used in feeding the larvae of *B. amazonicus* allow us to state that nauplii of *D. brasiliensis* concentrated 35% more EAA (43.5 ± 1.23 g kg<sup>-1</sup> of dry food) than nauplii of *Artemia* (28.2 ± 0.80 g kg<sup>-1</sup> of dry food). Moreover, in the composition of EAA in the nauplii of both species, the amino acid arginine was the most abundant of all, representing up to 12.4% of the total EAA. However, in young animals of *D. brasiliensis*, the concentration of arginine was higher, reaching more than 50% of the total EAA. Therefore, young animals of *D. brasiliensis* have greater potential as live food than when in the nauplii stage to feed larvae of *B. amazonicus* [39].

The biochemical composition of live food can influence its ingestion, digestion and assimilation by the larvae. This is important because, during the ontogenic phase, the larvae develop chemo-mechanical receptors that allow them to orient themselves and locate the food [5,7,54]. Depending on the type of live food, when it comes into contact with water, it releases chemical substances that act as stimuli for the larval sensory receptors. Regarding this, in the study developed by [8] with *pacú* larvae, it was demonstrated that the larvae respond to the chemical and visual stimuli of *Artemia* nauplii.

According to the laboratory analysis of the protein-bound amino acids (AALP) of the different types of live food used in *B. amazonicus* larviculture, the best-quality food was the nauplii of *D. brasiliensis*. These showed a higher profile of essential amino acids (SEA), and the concentration in lysine and methionine was significantly higher (49.5 ± 1.40 and 10.4 ± 0.29 g kg<sup>-1</sup> of dry food) than in nauplii of *Artemia* (40.6 ± 1.1 and 9.20 ± 0.26 g kg<sup>-1</sup> of dry food). This suggests that it is a protein of high biological value and excellent quality for *B. amazonicus* larvae.

On the other hand, the quality of the *Daphnia magna* protein was considered excellent due to the balanced profile in its EAA, which included arginine, leucine, lysine and methionine. In this regard, in a study comparing the nutritional composition of various types of live food for the production of freshwater shrimp (*Macrobrachium rosenbergii*), [13] found that the cladoceran *Moina macrocopa* showed high levels of lysine, phenylalanine, leucine, glutamic acid and tyrosine, which demonstrates the high nutritional value of these microcrustaceans when used for feeding larvae. However, the nutritional content of the cladoceran depends directly on the food it eats and the salinity of the environment [55,56].

In the evaluation of the biochemical composition of *B. amazonicus* larvae, the total concentration of amino acids changed according to the type of live diet and the rearing system. Larvae reared at a moderate water speed ( $2 \text{ cc s}^{-1}$ ) accumulated more amino acids than when reared in standing water. This response was even more significant in the larvae that received the diet with *D. brasiliensis* nauplii ( $846.8 \pm 16 \text{ g}^{-1} \text{ kg dry weight}$ ) than in the larvae raised in the conventional system (standing water) that were fed a similar diet ( $685.2 \pm 12 \text{ g}^{-1} \text{ kg dry weight}$ ). In turn, the *Artemia* nauplii diet in both rearing conditions (standing water system and/or running water) promoted a similar response pattern, but to a lesser extent. The above suggests that live food, in good quantities and qualities and when supplied at the right time, can make larviculture successful, even if the conditions under which the live food develops are not the most appropriate.

The scientific literature has few reports of work with larvae reared under exercise, and even fewer evaluating the effects of this on their biochemical composition. In fact, [35] measured the effect of water velocity on the performance of zebrafish larvae (*Danio rerio*) and found that very strong velocities ( $5 \text{ cc s}^{-1}$ ) cause high mortality among the larvae, while moderate or sustained velocities ( $2 \text{ cc s}^{-1}$ ), such as those used in the present study, favor the growth of the larvae, although the vitelline reserves are more quickly consumed, as observed by [35].

The present study showed similar concentrations of total fatty acids in Amazonian *Brycon* larvae reared in different clear water systems, except for larvae reared in the green water system or mesocosms, where the lowest concentrations of total fatty acids were found, which reflected their concentrations in the food received. In fact, when comparing the content of lipids, triglycerides and fatty acids in the different types of live foods used in this study, the content of these nutrients in *Daphnia magna* was the lowest of all, with a significant impact on the biochemical composition of the larvae that were fed with cladocerans.

Analyses of the fatty acid composition of *D. brasiliensis* nauplii showed that these were the best sources of essential fatty acids for *Brycon amazonicus* larvae, such as linoleic acid (C18:2n6) and linolenic acid (C18:3n3), as opposed to what was found in the biochemical composition of *Artemia* nauplii. In a study conducted by [13] it was also confirmed that nauplii of *Streptocephalus sirindhornae* have higher levels of these essential fatty acids. On the other hand, the response pattern of the concentration of highly unsaturated fatty acids (HUFA, PUFA and EPA + DHA) was similar in the larvae raised in the clear water system with or without exercise, compared to the larvae raised in the mesocosm system, which showed lower values of these essential fatty acids.

Based on bibliographic references and the results obtained in the present work, we believe that the real contribution of live food is in the form of its nutrients readily available to the larvae, such as free amino acids and essential fatty acids. The higher the concentration of these nutrients in the live food, the greater the chance for the larvae to develop properly. An important aspect that can improve the performance of larvae of *B. amazonicus*, or any other larvae, is to consider their initial size in weight (mg) or in length (mm). This information is critical because it can determine the size and number of live prey that can meet their nutritional requirements. In our study, we found that if we only consider the initial weight (1.79 mg) of larvae of *B. amazonicus*, its optimal growth can be obtained by supplying between 700 and 900 nauplii of *D. brasiliensis*. On the other hand, considering the body length of the larva of *B. amazonicus* (6.22 mm) and the fact that this larva can ingest live prey larger than itself, then the criterion that can be adopted would be to provide between five and ten individuals of *D. brasiliensis*, of sizes from 6 to 10 mm in length (three to seven days of age), per larva of *B. amazonicus*. However, more studies need to be developed to prove these claims.

## 5. Conclusions

Based on the information found in this work on *Brycon amazonicus* larviculture using different breeding and feeding systems, we can conclude the following:

- The clear water system with sustained swimming exercise is the most adequate among the rearing systems for matrinxã larviculture tested in this work.
- Adopting moderate currents in clear water systems enables us to minimize the occurrence of cannibalism and offers better survival rates for the larvae.
- The nutritional quality of live foods, tested through the performance responses of *Brycon amazonicus* larvae, is appropriate.
- The composition of free amino acids in young animals of *D. brasiliensis* consists mainly of arginine.
- In practical terms, for efficient production and based on the feed rates practiced in *Brycon amazonicus* larviculture, it is recommended that the feed rates be at least 300% of the larva's live weight.

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