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Research Article

Replacement of *Artemia franciscana* Nauplii by *Moina minuta* Neonates as Live Food on the Larviculture of Angelfish (*Pterophyllum scalare* -Schultze, 1823) and Severum (*Heros severus* - Heckel, 1840)

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The present study aimed to assess the potential substitution of *Artemia franciscana* nauplii with *Moina minuta* neonates as the initial feed for angelfish (*Pterophyllum scalare*) and severum (*Heros severus*), evaluating the fish growth performance, live food cost, and intestinal histomorphometry. To achieve this goal, two separate experiments were carried out for each species during 20 days, using 200 postlarvae. Both experimental designs consisted of five treatments, namely, 100% *A. franciscana* nauplii (A100-M0); 75% *A. franciscana* nauplii and 25% *M. minuta* neonates (A75-M25); 50% *A. franciscana* nauplii and 50% *M. minuta* neonates (A50-M50); 25% *A. franciscana* nauplii and 75% *M. minuta* neonates (A25-M75); and 100% *M. minuta* neonates (A0-M100), with four replications (10 postlarvae per repetition). Angelfish postlarvae fed with A100-M0, A75-M25, A50-M50, and A25-M75 had better values of final length (13.31 mm, 14.15 mm, 13.32 mm, and 13.32 mm), gain in length (7.91 mm, 8.75 mm, 7.92 mm, and 7.92 mm), and length-specific growth rate (4.51% day^{-1}, 4.82% day^{-1}, 4.51% day^{-1}, and 4.51% day^{-1}). In the case of severum postlarvae, the highest length growth performance occurred in those fed with A100-M0 (15.86 mm, 8.90 mm, and 4.12% day^{-1}), A75-M25 (15.45 mm, 8.49 mm, and 3.99% day^{-1}), and A50-M50 (14.78 mm, 7.82 mm, and 3.76% day^{-1}). Both species (angelfish and severum postlarvae) fed with A100-M0 and A75-M25 presented higher values of final weight (28.88 mg and 34.49 mg; 55.84 mg and 53.95 mg), gain in weight (27.56 mg and 33.08 mg; 51.03 mg and 49.14 mg), and weight-specific growth rate (15.42% day^{-1} and 16.30% day^{-1}; 12.26% day^{-1} and 12.09% day^{-1}). Live food cost for angelfish and severum postlarvae was higher in A100-M0 (US$ 0.92 and US$ 1.08) and lower for the ones in A0-M100 (US$ 0.36 and US$ 0.35). Severum postlarvae fed with A100-M0 (57.20 μm), A75-M25 (55.66 μm), and A50-M50 (54.27 μm) presented the highest intestinal villi heights. Thus, partial replacement of *A. franciscana* nauplii by *M. minuta* neonates in the proportion of A75-M25 or 100A-0M is viable and effective during the first feed of these fish species.

1. Introduction

The cichlid family presents important species for ornamental fish farming. This is mainly due to the diversity of body, fin shapes, colors variety, and the complexity of fish behavior, which has repercussions on competitive market value and great acceptance by aquarists. In this family, it is worth to highlight the angelfish (*Pterophyllum scalare*) and severum (*Heros severus*); both fish species are native to Brazil, Peru, Colombia, and Venezuela [1]. Angelfish is widely sold in the

aquarium market [2, 3]. Severum is a fish species that is gaining notoriety as ornamental fish [4]. The two fish species are well adapted to the fish farming environment and have good acceptance of the diets offered. In addition, the scientifically validated reproductive protocols favor the commercial production of these fish species [5, 6].

Larviculture is the most challenging production step in fish farming [4, 6, 7]. The fish postlarvae are sensitive to nutritional management and water quality changes [8, 9]. Therefore, studies that aimed to improve cultivation techniques and to optimize feeding management and supply of live food during the first feeding are necessary to improve large-scale ornamental fish farming [4].

Postlarvae generally have an immature digestive system and low ability to digest and absorb inert diets [10, 11]. Consequently, the utilization of prey is essential during larviculture. The morphology of postlarval intestines is influenced by the live food used and reflects the fish nutritional condition [12]. The size of the intestinal villi is related to the good growth performance of the animal, as it increases the contact surface of enterocytes with the food, which can promote better growth performance [13].

Nauplii of *Artemia franciscana* is the main live food used in seawater and freshwater aquaculture. However, there are some factors that can make it limiting, such as the cost of cysts, and the nutritional value of this live food can vary [14]. In general, nauplii can contain on average 50% crude protein, 20% lipids [15, 16], in addition to good proportions of essential fatty acids, vitamins, and carotenoids [15, 17] important in the nutrition of fish postlarvae. Therefore, this organism is usually a costly item in fish commercial production [18, 19]. Furthermore, as it is a marine organism, nauplii survival duration is shorter than normal in limnological environments [20, 21], and this represents an obstacle in freshwater larviculture by impacting the larvae feeding efficiency. Cladocerans, especially *Moina minuta*, represent one of the most viable live food alternatives to replace *A. franciscana* nauplii in freshwater fish larvae production. This organism is naturally present in limnological ecosystems [21] showing simple production at low cost. Furthermore, it is tolerant to physicochemical variations of water, and it has a high reproduction rate [18, 19]. The nutritional content of *M. minuta* modified by conditions intrinsic to the organism, environmental variations, and food sources. In general, species of this genus contain on average 50% crude protein by dry weight and between 4 to 27% lipids depending on the species, life stage, and nutritional conditions [22, 23]. Nevertheless, it could be lacking in certain essential nutrients such as ALA, EPA, and DHA [24].

Therefore, assessing the potential of *M. minuta* as a substitute for *A. franciscana* nauplii during the first feeding of angelfish and severum can be important as a new live food option and enable an improvement in these ornamental fish species productions. Thus, the objective of this study was to assess the growth performance, live food cost, and intestinal histomorphometry of angelfish and severum postlarvae fed with proportions of *A. franciscana* nauplii and *M. minuta* neonates to verify the efficiency of this Cladocera as an alternative for partial replacement of *A. franciscana* nauplii as food source during larviculture of postlarvae of both species.

2. Materials and Methods

This study received approval from the Ethics Committee of UFPA (2010080719).

2.1. Experiments. Two experiments were performed separately using 200 angelfish (*Pterophyllum scalare*) and severum (*Heros severus*) postlarvae obtained from the same spawning. In each experiment, postlarvae at 5 dph (initiation of exogenous feeding) had 5.40 ± 0.40 mm, 1.32 ± 0.59 mg and 6.96 ± 0.12 mm, 4.81 ± 0.31 mg, respectively, were distributed and acclimated in the respective experimental units a day prior to the feed trials began. They were randomly allocated into 20 aquariums with a useful volume of one liter each, in stocking density of 10 animals L^{-1}. They were supplied with filtered potable freshwater from artesian wells and maintained under constant individual aeration throughout the experimental period. Aeration was obtained by a radial compressor, where the air was distributed to the experimental units through silicone hoses.

The experiments were conducted for 20 days, presenting the experimental design with 5 treatments each and 4 repetitions. Experiments consisted of variations in the proportions of *A. franciscana* nauplii and *M. minuta* neonates: 100% *A. franciscana* nauplii (A100-M0); 75% *A. franciscana* nauplii and 25% *M. minuta* neonates (A75-M25); 50% *A. franciscana* nauplii and 50% *M. minuta* neonates (A50-M50); 25% *A. franciscana* nauplii and 75% *M. minuta* neonates (A25-M75); and 100% *M. minuta* neonates (A0-M100). Angelfish and severum postlarvae were fed with 300 live food postlarvae day^{-1} (*A. franciscana* nauplii and *M. minuta* neonates alone or together in different proportions) in four daily feedings at 8:00, 11:00, 14:00, and 17:00 [2, 25]. Both experiments were performed in a natural photoperiod, with artificial illumination of 15 w fluorescent lamps.

The water used in the experiments came from an artesian well, with 0 salinity (freshwater), potability, and filteration. Over the experimental period, the concentrations of dissolved oxygen (6.12 ± 0.49 mg L^{-1}), pH (7.27 ± 0.15), electrical conductivity (0.06 ± 0.04 mS cm^{-1}), and temperature (26.26 ± 0.96°C) of water were monitored every three days by using a multiparameter probe portable (U-10, Horiba, USA). Additionally, every 5 days, the total ammonia concentration (0.05 ± 0.00 ppm) was analyzed with water analysis kits (LabconTest, Brazil). After the final feeding of each day, aquariums were siphoned, and 50% of the useful volume was exchanged for cleaning.

2.2. Moina minuta Production

M. minuta cultured with a natural photoperiod and artificial aeration (same conditions as larviculture). The cladocerans received the microalgae *Chlorella* sp. in a 4×10^6 cells mL^{-1}. *Chlorella* sp. was cultivated in freshwater enriched with NPK chemical agricultural fertilizer (nitrogen : phosphorus :

potassium—20 : 5 : 20) in a proportion of 0.35 g L^{-1} [26], under constant illumination with 15 w fluorescent lamps and aeration by a radial compressor equipped with silicone hoses with porous stone for better air diffusion. The source and physical-chemical characteristics of the water in the production of *M. minuta* and microalgae were the same used in larviculture. The concentration of microalgae in the cladocerans cultivation was maintained with *M. minuta* collection followed by algal culture replenishment. The daily collection of *M. minuta* was performed by means of a set of sieves with 1,000 and 120 μm meshes for separation of adults and neonates. In this context, neonates (average length of 0.45 ± 0.09 mm) were offered to postlarvae.

In order to determine the daily quantity of volume of *M. minuta* neonates concentrates to be provided to the aquariums, the dilution method was used. Daily, the concentrates were obtained through the collection of cladoceran production, and then the density was calculated. To perform this calculation, 1 mL of neonate concentrates was collected and added to a 100 mL measuring cylinder, and the final volume was completed with 99 mL of water and mixed. Subsequently, 3 subsamples of 5 mL were collected to determine the density of organisms, which was calculated by dividing the number of cladocerans counted by 5 mL and multiplying by 100 mL. Based on the density of cladocerans collected, the volume to be administered to each aquarium was calculated according to the treatment.

2.3. Artemia franciscana Cyst Hatch. Every day during the experiment, *A. franciscana* nauplii were collected by hatching approximately 10 g L^{-1} of cysts (Artemia Salina do RN®, Brazil) during each experiment. The process started with the addition of cysts to a plastic container containing one liter of salinized water (freshwater + common salt) in a concentration of 30 g NaCl L^{-1}. Containers were maintained under artificial lighting for 24 hours and under constant aeration by a central radial compressor equipped with silicone hoses with porous stone for better air diffusion. After 24 h of cysts incubation and hatching, nauplii (instar I) with an average length of 0.44 ± 0.06 mm were separated from the cysts' remains by siphoning, collected in a 120 μm mesh sieve then passed in freshwater to withdraw salinized water. Following this process, they were used as live food during experiments. The calculation of the proportion of *A. franciscana* nauplii mL^{-1} was performed similarly to that used for *M. minuta* neonates.

A. franciscana nauplii and *M. minuta* neonates were adequate to the mouth opening at five days posthatching larvae (0.48 ± 0.06 mm and 0.49 ± 0.05 mm, respectively) of both species [27].

2.4. Growth Performance. Two biometrics were performed during the experiment. The first one on day 01 on a sample of 20 postlarvae of each species selected at random obtained from the same spawn but did not participate in experiments. The total length was measured using an ichtyometer (Vernier, Brazil) and weight using an analytical balance with a precision of 0.1 mg (AG200, Gehaka, Brazil). The same procedure was performed at the end of the experimental period (21 days). With the growth performance, data and the number of live animals were calculated:

Gain in length (mm): GL = FL − IL; gain in weight (mg): GW = FW − IW; length-specific growth rate (% day^{-1}) [28]: LSGR = [(Nl FL − Nl IL)/T] ∗ 100; weight-specific growth rate (% day^{-1}) [28]: WSGR = [(Nl FW − Nl IW)/T] ∗ 100; length uniformity (%) [29]: LU = [(N ± 20%)/TN] ∗ 100; weight uniformity (%) [29]: WU = [(N ± 20%)/TN] ∗ 100; survival rate (%): SR = FN/IN ∗ 100.

Here, IL and FL are the initial and final length of postlarvae, respectively; IW and FW are the initial and final weigh of postlarvae, respectively; Nl is natural logarithm; T is the experiment duration; N ± 20% is the number of postlarvae with length and weigh varying ±20% of average values in each aquarium; TN is the total number of postlarvae in each aquarium; IN and FN are the initial and final number of postlarvae in each aquarium, respectively.

2.5. Live Food Cost. The cost of using *A. franciscana* nauplii and *M. minuta* neonates in the production of both fish species was calculated considering the hypothetical production of thousand postlarvae (to simulate commercial production) considering the survival rate observed from both fish species during larviculture experiment of 20 days [30].

All calculations were made using fixed production reference values, where for *A. franciscana*, it considered the proportion of 280,000 cysts g^{-1}, an 85% nauplii hatching rate and a price of US$ 44.00 per kilogram of cysts (information provided by manufacturer). *M. minuta* was based on the production of 4,680 organisms L^{-1} [31], NPK chemical agricultural fertilizer (20 : 5 : 20) in proportion of 0.35 g L^{-1} for microalgae production to feed *M. minuta* [26], and the value of US$ 1.00 per kilogram of NPK.

Live food cost corresponded to the sum of *A. franciscana* nauplii and *M. minuta* production cost in different proportions between these live foods in postlarvae feeding, according to the treatments employed in the experiments.

2.6. Intestinal Histomorphometry. At the end of the experiments, eight animals per treatment were separated from the others and used for final biometrics after being euthanized with a 135 mg L^{-1} solution of eugenol anesthetic [32]. Then, the samples were submitted to 10% formaldehyde for one day for studies of intestinal histomorphometry. Following fixation, the samples underwent decalcification in a neutralized ethylenediamine tetra acetic acid (pH 7) in NaOH during a week. After these processes, the samples underwent dehydration in increasing alcohol concentration followed by clearing in xylol and embedding in paraffin [33]. The paraffin blocks containing the samples were cut into semiseries with the aid of a microtome (Leica RM2145, Germany) in a thickness of five microns. The slides were pigmented with hematoxylin and eosin according to the procedure of mounting permanent slides. Histological samples were photomicrographed subsequently analyzed by using a microscope (Axio Scope A1, Carl Zeiss Microscopy Gmbh, Germany). Altogether, 50 intestinal villi were analyzed by

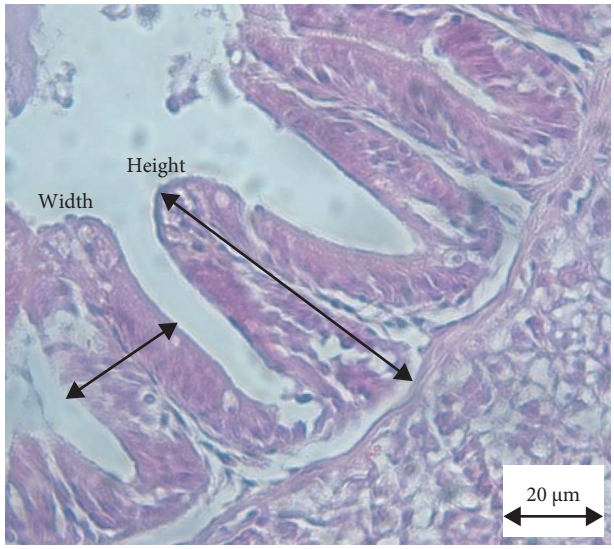

Figure 1: Photomicrography (40x) of angelfish illustrating the measurements of intestinal villi height and width of analyzed postlarvae for each fish species.

sample where villus height and width were measured (Figure 1).

2.7. Data Statistics. Initially, normality of the data was assessed by means of Shapiro–Wilk test, while the homogeneity of variances was examined by Bartlett test. Then, they were submitted to one way ANOVA (5%). If there were differences in p value, Tukey test (5%) was used to assess differences between treatments.

3. Results

3.1. Growth Performance. LU and WU, as well as SR of angelfish postlarvae did not present differences among treatments. The angelfish postlarvae fed with A100-M0, A75-M25, A50-M50, and A25-M75 had better values ($p < 0.05$) of final length (13.31 mm, 14.15 mm, 13.32 mm, and 13.32 mm), GL (7.91 mm, 8.75 mm, 7.92 mm, and 7.92 mm), and LSGR (4.51% day^{-1}, 4.82% day^{-1}, 4.51% day^{-1}, and 4.51% day^{-1}) than individuals fed with A0-M100 (11.82 mm, 6.42 mm, and 3.91% day^{-1}). The angelfish postlarvae fed with A100-M0 and A75-M25 presented higher ($p < 0.05$) values of final weight (28.88 mg and 34.49 mg), GW (27.56 mg and 33.08 mg), and WSGR (15.42% day^{-1} and 16.30% day^{-1}) than individuals fed with A50-M50 (27.33 mg, 26.01 mg, and 15.15% day^{-1}), A25-M75 (27.01 mg, 25.69 mg, and 15.05% day^{-1}), and A0-M100 (22.53 mg, 21.21 mg, and 14.14% day^{-1}) (Table 1).

For severum postlarvae, no differences were observed in LU and WU. On the other hand, only the postlarvae fed with A0-M100 had the lowest SR (77.50%) than others. Final length, GL, and LSGR were higher in animals submitted to A100-M0 (15.86 mm, 8.90 mm, and 4.12% day^{-1}), A75-M25 (15.45 mm, 8.49 mm, and 3.99% day^{-1}), and A50-M50 (14.78 mm, 7.82 mm, and 3.76% day^{-1}) than postlarvae fed with A25-M75 (13.68 mm, 6.72 mm, and 3.38% day^{-1}), and A0-M100 (13.18 mm, 6.22 mm, and 3.18% day^{-1}). The severum final weight, GW, and WSGR were higher for postlarvae submitted to A100-M0 (55.84 mg, 51.03 mg, and 12.26% day^{-1}) and A75-M25 (53.95 mg, 49.14 mg, and 12.09% day^{-1}) than ones fed with A50-M50 (46.15 mg, 41.34 mg, and 11.30% day^{-1}), A25-M75 (35.08 mg, 30.27 mg, and 9.93% day^{-1}), and A0-M100 (29.17 mg, 24.36 mg, and 8.96% day^{-1}) (Table 2).

3.2. Live Food Cost. The live food cost was directly increased with the use of *A. franciscana* nauplii, being higher for the postlarvae of angelfish and severum in A100-M0 (US$ 0.92 and US$ 1.08) and lower for the ones in A0-M100 (US$ 0.36 and US$ 0.35) (Tables 1 and 2).

3.3. Intestinal Histomorphometry. Angelfish postlarvae fed with different proportions of *A. franciscana* nauplii and *M. minuta* neonates did not show differences in intestinal histomorphometry parameters (Table 3). Similarly, severum postlarvae did not present differences in intestinal villi width (Table 3). Conversely, severum postlarvae fed with A100-M0 (57.20 μm), A75-M25 (55.66 μm), and A50-M50 (54.27 μm) presented the highest intestinal villi heights (Table 3).

4. Discussion

The uniformity of length and weight is an importance factor in ornamental fish farming, since it facilitates fish management and commercialization, as animals with homogeneous size are more appreciated in the ornamental market [34, 35]. Furthermore, lack of uniformity leads to the formation of hierarchies, instigating the competition for space and food resources, favoring the growth of dominant fish, and increasing mortality of smaller fish [34, 36]. Ornamental fish are usually sold by unit; thus, the survival rate is essential for activity success. Angelfish and severum are sociable fish species that live in groups for their early development stages [4, 37, 38]. The peaceful coexistence of animals in the experimental units may have provided the fact that all could prey on live foods in the same way, benefiting the uniformity of length and weight, contributed to a higher survival rate.

The *A. franciscana* nauplii partial replacement by *M. minuta* neonates (75A-25M) generated similar growth performance in relation to fish fed with A100-M0. In other studies, the use of *Artemia* sp. nauplii or cladocera-rich zooplankton as live food did not influence the growth performance of pirarucu (*Arapaima gigas*) postlarvae [21]. Ideal live food must be determined according to animal nutritional requirements [39], digestion capacity [40], feeding behavior [41, 42], and development phase of each animal species [43, 44]. The live food size, swimming mode in water column [45, 46], and its exoskeleton composition [21] can also directly influence the live food consumption and digestion by fish postlarvae. In this way, the choice of the most suitable live food in fish larviculture relies on considering the intrinsic characteristics of prey and the postlarvae feeding behavior, although feeding management by

Table 1: Growth performance and live food cost (mean ± SD) of angelfish fed with proportions of *Artemia franciscana* nauplii and *Moina minuta* neonates.

Growth performance[†]	Treatment[‡]					p value	VC (%)[§]
	A100-M0	A75-M25	A50-M50	A25-M75	A0-M100		
FL (mm)	13.31 ± 0.2^{a}	14.15 ± 0.3^{a}	13.32 ± 0.3^{a}	13.32 ± 0.6^{a}	11.82 ± 0.5^{b}	$<0.01^{*}$	3.47
GL (mm)	7.91 ± 0.2^{a}	8.75 ± 0.3^{a}	7.92 ± 0.3^{a}	7.92 ± 0.6^{a}	6.42 ± 0.5^{b}	$<0.01^{*}$	5.87
LSGR (% day^{-1})	4.51 ± 0.1^{a}	4.82 ± 0.1^{a}	4.51 ± 0.1^{a}	4.51 ± 0.2^{a}	3.91 ± 0.2^{b}	$<0.01^{*}$	3.99
FW (mg)	28.88 ± 1.1^{ab}	34.49 ± 1.5^{a}	27.33 ± 1.2^{bc}	27.01 ± 3.8^{bc}	22.53 ± 2.9^{c}	$<0.01^{*}$	9.64
GW (mg)	27.56 ± 1.1^{ab}	33.08 ± 1.5^{a}	26.01 ± 1.2^{bc}	25.69 ± 3.8^{bc}	21.21 ± 2.9^{c}	$<0.01^{*}$	10.12
WSGR (% day^{-1})	15.42 ± 0.2^{ab}	16.30 ± 0.2^{a}	15.15 ± 0.2^{bc}	15.05 ± 0.7^{bc}	14.14 ± 0.7^{c}	$<0.01^{*}$	3.40
LU (%)	91.32 ± 5.0^{a}	96.88 ± 5.4^{a}	96.88 ± 5.4^{a}	94.72 ± 5.3^{a}	87.40 ± 8.9^{a}	0.32^{ns}	7.65
WU (%)	47.37 ± 21.6^{a}	61.52 ± 20.1^{a}	40.42 ± 29.3^{a}	57.03 ± 12.7^{a}	42.06 ± 20.2^{a}	0.57^{ns}	48.34
SR (%)	82.50 ± 8.3^{a}	80.00 ± 0.0^{a}	90.00 ± 7.1^{a}	92.50 ± 8.3^{a}	80.00 ± 7.1^{a}	0.12^{ns}	9.36
LFC (US$)	0.92 ± 0.1^{a}	0.76 ± 0.0^{b}	0.70 ± 0.0^{bc}	0.57 ± 0.1^{c}	0.36 ± 0.0^{d}	$<0.01^{*}$	9.61

[†]Growth performance: final length (FL), gain in length (GL), final weight (FW), gain in weight (GW), length-specific growth rate (LSGR), weight-specific growth rate (WSGR), length uniformity (LU), weight uniformity (WU), survival rate (SR), and live food cost (LFC). [‡]Treatment: feeding of 100% *A. franciscana* nauplii (A100-M0); 75% *A. franciscana* nauplii and 25% *M. minuta* neonates (A75-M25); 50% *A. franciscana* nauplii and 50% *M. minuta* neonates (A50-M50); 25% *A. franciscana* nauplii and 75% *M. minuta* neonates (A25-M75); and 100% *M. minuta* neonates (A0-M100). Within each line, different letters indicated a significant difference according to Tukey's test (5%). $^{*}p < 0.01$. [ns]Not significant. [§]Variation coefficient (VC).

Table 2: Growth performance and live food cost (mean ± SD) of severum fed with proportions of *Artemia franciscana* nauplii and *Moina minuta* neonates.

Growth performance[†]	Treatment[‡]					p value	VC (%)[§]
	A100-M0	A75-M25	A50-M50	A25-M75	A0-M100		
FL (mm)	15.86 ± 0.1^{a}	15.45 ± 0.0^{a}	14.78 ± 0.1^{ab}	13.68 ± 0.1^{bc}	13.18 ± 1.0^{c}	$<0.01^{*}$	3.57
GL (mm)	8.90 ± 0.1^{a}	8.49 ± 0.0^{a}	7.82 ± 0.1^{ab}	6.72 ± 0.1^{bc}	6.22 ± 1.0^{c}	$<0.01^{*}$	6.82
LSGR (% day^{-1})	4.12 ± 0.0^{a}	3.99 ± 0.0^{a}	3.76 ± 0.0^{ab}	3.38 ± 0.0^{bc}	3.18 ± 0.4^{c}	$<0.01^{*}$	9.28
FW (mg)	55.84 ± 1.4^{a}	53.95 ± 1.4^{a}	46.15 ± 1.6^{b}	35.08 ± 0.5^{c}	29.17 ± 4.3^{d}	$<0.01^{*}$	5.92
GW (mg)	51.03 ± 1.4^{a}	49.14 ± 1.4^{a}	41.34 ± 1.6^{b}	30.27 ± 0.5^{c}	24.36 ± 4.3^{d}	$<0.01^{*}$	6.64
WSGR (% day^{-1})	12.26 ± 0.1^{a}	12.09 ± 0.1^{a}	11.30 ± 0.2^{b}	9.93 ± 0.1^{c}	8.96 ± 0.7^{c}	$<0.01^{*}$	9.22
LU (%)	100.00 ± 0.0^{a}	100.00 ± 0.0^{a}	100.00 ± 0.0^{a}	100.00 ± 0.0^{a}	95.83 ± 7.2^{a}	0.44^{ns}	3.76
WU (%)	85.00 ± 8.7^{a}	90.00 ± 0.0^{a}	97.50 ± 4.3^{a}	92.50 ± 4.3^{a}	84.52 ± 10.9^{a}	0.37^{ns}	11.22
SR (%)	97.50 ± 4.3^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}	77.50 ± 13.0^{b}	$<0.01^{*}$	7.44
LFC (US$)	1.08 ± 0.0^{a}	0.94 ± 0.0^{b}	0.78 ± 0.0^{c}	0.61 ± 0.0^{d}	0.35 ± 0.1^{e}	$<0.01^{*}$	5.18

[†]Growth performance: final length (FL), gain in length (GL), final weight (FW), gain in weight (GW), length-specific growth rate (LSGR), weight-specific growth rate (WSGR), length uniformity (LU), weight uniformity (WU), survival rate (SR), and live food cost (LFC). [‡]Treatment: feeding of 100% *A. franciscana* nauplii (A100-M0); 75% *A. franciscana* nauplii and 25% *M. minuta* neonates (A75-M25); 50% *A. franciscana* nauplii and 50% *M. minuta* neonates (A50-M50); 25% *A. franciscana* nauplii and 75% *M. minuta* neonates (A25-M75); and 100% *M. minuta* neonates (A0-M100). Within each line, different letters indicated a significant difference according to Tukey's test (5%). $^{*}p < 0.01$. [ns]Not significant. [§]Variation coefficient (VC).

Table 3: Intestinal histomorphometry (mean ± SD) of angelfish and severum fed with proportions of *Artemia franciscana* nauplii and *Moina minuta* neonates.

Histomorphometry	Treatment[†]					p value	VC (%)[‡]
	A100-M0	A75-M25	A50-M50	A25-M75	A0-M100		
Pterophyllum scalare							
Intestinal villi height (μm)	50.17 ± 1.2^{a}	49.44 ± 4.4^{a}	50.42 ± 4.3^{a}	45.88 ± 4.7^{a}	53.67 ± 9.8^{a}	0.61^{ns}	12.16
Intestinal villi width (μm)	29.55 ± 0.9^{a}	27.40 ± 0.9^{a}	28.71 ± 1.5^{a}	28.80 ± 1.5^{a}	27.36 ± 2.1^{a}	0.51^{ns}	6.07
Heros severus							
Intestinal villi height (μm)	57.20 ± 2.7^{a}	55.66 ± 2.8^{a}	54.27 ± 0.5^{a}	49.46 ± 2.5^{b}	48.90 ± 3.9^{b}	$<0.05^{**}$	6.29
Intestinal villi width (μm)	27.00 ± 0.7^{a}	28.98 ± 1.6^{a}	28.33 ± 1.7^{a}	27.28 ± 2.0^{a}	26.08 ± 0.5^{a}	0.20^{ns}	6.09

[†]Treatment: feeding of 100% *A. franciscana* nauplii (A100-M0); 75% *A. franciscana* nauplii and 25% *M. minuta* neonates (A75-M25); 50% *A. franciscana* nauplii and 50% *M. minuta* neonates (A50-M50); 25% *A. franciscana* nauplii and 75% *M. minuta* neonates (A25-M75); and 100% *M. minuta* neonates (A0-M100). Within each line, different letters indicated a significant difference according to Tukey's test (5%). $^{*}p < 0.01$. $^{**}p < 0.05$. [ns]Not significant. [‡]Variation coefficient (VC).

offering more than one species of live food can be beneficial to postlarvae for providing greater nutritional variety and greater stimulus to predation.

In the present study, the partial replacement of 25% of *A. franciscana* nauplii by *M. minuta* neonates generated the best results of weight for both species, similar to individuals fed only with *A. franciscana* nauplii. On the other hand, angelfish postlarvae obtained the best length results with the supply of 75% of *M. minuta* neonates. For severum postlarvae, the best values for this variable were observed with the use of up to 50% of *M. minuta* neonates. These differences in growth performance can be explained by some differences between the tested live foods. In fact, *Moina* has attractive features, such as comparable size to newly hatched *Artemia* nauplii, depending on its life stage and locomotion in water [19]. However, this cladoceran has fast "hopping" movements [22], while *Artemia* nauplii have slow movements and remain more concentrated in the water [25]; so, the postlarvae must spend less energy to prey on it. In addition, the shorter survival time of *A. franciscana* nauplii in freshwater could negatively impact the growth of the animals, but in the present study, this was compensated using a feeding divided into 4 daily meals [2].

In general, length growth is an important factor in ornamental fish farming as it directly impacts the commercial aspect. Since fish are typically sold individually, the selling price is closely tied to the length size of the fish. Therefore, achieving substantial length growth is crucial for maximizing profitability in this industry. Therefore, the obtained results showed that it is feasible to replace the greater proportion of *A. franciscana* nauplii by *M. minuta* neonates (A25-M75) in angelfish larviculture, while for severum postlarvae, feasibility can be obtained with replacements up to 50%. The ornamental fish species, angelfish and severum, which belong to the same family, are omnivorous and inhabit the same environments, that is, they share many similar characteristics. However, certain intrinsic characteristics of the species may exist during the larval period. Perhaps it is easier for angelfish postlarvae to capture *M. minuta* neonates than severum postlarvae. This could explain higher performance values in length, even with higher levels of *M. minuta* neonates in the feed.

The live food cost for angelfish and severum postlarvae in 75A-25M was 17.39% and 12.96% less than the live food cost for postlarvae in 100A-0M for angelfish and severum, respectively. Similar results were observed by Lipscomb et al. [47], who evaluated the replacement of *Artemia* sp. nauplii by microparticulate diets in larviculture of six freshwater ornamental fish. Thus, the reduction in the use of *A. franciscana* nauplii during larviculture of angelfish and severum represents a satisfactory feeding management strategy to reduce costs. The use of *A. franciscana* nauplii can become limiting for ornamental fish farming expansion, especially in developing countries [48]. Thus, it can be assumed that the replacement of *A. franciscana* nauplii is feasible depending on the production cost and yield of the substitute food.

Different proportions of *A. franciscana* nauplii and *M. minuta* neonates did not affect the intestinal histomorphometry of angelfish postlarvae in this study. However, severum postlarvae fed with A25-M75 and A0-M100 presented lower values of intestinal villi height. Intestinal development during larval phase is mediated by preprogrammed intrinsic factors combined with changeable external factors [49]. Feeding conditions directly influence the developmental mechanisms of the intestinal epithelium as the animal develops [50]. Food digestibility represents the bioavailability of nutrients accessible for absorption and promotes the development of the intestinal epithelium [51]. Live foods generally have a suitable nutritional composition with a high biological value and digestibility, combined with bioactive compounds such as enzymes and hormones that contribute to the proper development of postlarvae intestinal epithelium. This could explain why the development of the postlarvae intestinal epithelium evaluated in this study was less affected by variations in feeding with *A. franciscana* nauplii and *M. minuta* neonates compared to productive performance.

There are several live foods, such as *M. minuta*, with the potential to replace partially or totally the use of *A. franciscana* nauplii for freshwater fish postlarvae. Thus, determining the proportion of alternative live food that can replace *A. franciscana* nauplii is important to develop more efficient and less costly food management protocols in the ornamental fish farming.

5. Conclusion

Angelfish and severum postlarvae presented better growth performances when fed with live food in a proportion of 75% *A. franciscana* nauplii and 25% *M. minuta* neonates or 100% *A. franciscana* nauplii. The use of *M. minuta* neonates in the first feed of angelfish and severum proved to be economically viable. This study constitutes a contribution to the improvement for development of larval production protocols for native species.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Eiras, B.J.C.F. investigated and analyzed the study, wrote the original draft, and secured funding. Campelo, D.A.V. conceptualized, supervised, and administered the project and revised and edited the manuscript. Moura, L.B. conceptualized and analyzed the methodology and revised and edited the manuscript. Oliveira, L.C.C., Sousa, L.M., and Costa, R.M. investigated and supplied resources. Costa, R.M. also supervised and administered the project and secured funding.

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