Latin American Journal of Aquatic Research, 47(4): 626-637, 2019
DOI: 10.3856/vol47-issue4-fulltext-5

Research Article

Technical and economic feasibility of food strategies in the hatchery of *Cyprinus carpio* (Cypriniformes, Cyprinidae) in a recirculating aquaculture system

Jonas Henrique de Souza Motta[1], Manuel Vázquez Vidal Júnior[2], Leonardo Siqueira Glória[2] Manoel Adriano Cruz Neto[2], Leonardo Serafim da Silveira[2] & Dálcio Ricardo de Andrade[2]
[1]Embrapa Pesca e Aquicultura, Loteamento Água Fria, Palmas, Tocantins, Brasil
[2]Universidade Estadual do Norte Fluminense Darcy Ribeiro, Rio de Janeiro, Brasil
Corresponding author: Manoel Adriano da Cruz Neto (m.adrianocruz@gmail.com)

ABSTRACT. In order to optimize the performance and reduce costs in the larviculture of ornamental carp (*Cyprinus carpio*) in a recirculating aquaculture system, different feeding strategies were tested. For this, two experiments were carried out both under controlled conditions in a greenhouse, in a recirculating aquaculture system with a physical particle filter, biofilter, and filtering by ultra-violet irradiation. In the first experiment, measurements of *Artemia* nauplii were tested in the initial exogenous feeding of larvae at concentrations of 100, 200, 400, 600, 800 and 1,000 *Artemia* nauplii per larvae. In the following experiment, different protocols were tested for initial feeding for larvae. At this stage, were used: live food (*Artemia* nauplii) and inert (powder meal), used separately and simultaneously, and increases in the number of *Artemia* nauplii were also tested, offered at different times of cultivation. In the first experiment, the amount of 600 *Artemia* nauplii for larvae demonstrated to be the best option as it generated significant growth and the cost was less than that observed with the use of 800 and 1000 *Artemia* nauplii per larvae. The second experiment demonstrated that the feeding strategy with live food more commercial inert feed provided the best growth performance for ornamental carp larvae. The need to fix the amount of live food during the cultivation, probably due to the high specific growth rates observed during the larval stage also became evident.

Keywords: *Artemia*; hatchery; nauplii; food strategies; ornamental carp; aquaculture production systems

INTRODUCTION

Among the families of fish, cyprinids account for most of the world production, being characterized as the family of greater economic importance (FAO, 2014). The species belonging to this family are of great interest both as a food source, as in the ornamental part. The common carp (*Cyprinus carpio* Linnaeus, 1758) is the most popular among the ornamental species grown in pounds due to its wide variety of colors and markings (Xu *et al*., 2014).

One of the bottlenecks in the production of ornamental carp is the larvae stage. During this stage, the larvae are grown in ponds, previously fertilized, being exposed to attack from predators and climatic variations, problems which can reduce the survival in cultivation. Chabalin *et al.* (1989) working with common carp larvae (*C. carpio*), tambaqui (*Colossoma macropomum*) and pacu (*Piaractus mesopotamicus*), using the semi-intensive system in ponds, obtained survivals near 35, 30 and 20%, respectively.

The practice of indoor larviculture of fish (its implications and needs) has been the reason for years of study (Dabrowski *et al*., 1978; Portella *et al*., 2014), and can be an alternative for increasing the survival of larvae cultivation. According to Jomori *et al.* (2003), the adoption of a period of larviculture in which the larvae are kept in an indoor system during a short period has proven to be more efficient than the direct stocking of larvae in ponds.

A recirculating aquaculture system is an option that adds sustainability to the indoor production of fish larvae. According to Timmons *et al.* (2002), cultivations in recirculating aquaculture systems, require less

Corresponding editor: Erich Rudolph

area and less than 10% of required water for cultivations in extensive systems such as pounds to produce the same amount of fish.

The use of a period of indoor larviculture requires knowledge of various issues related to diet and nutrition of the larvae, because the larvae will be fed exclusively from the food provided by humans. Over the years, several authors have developed and tested different feeding strategies on larval rearing phase (Charlon & Bergot, 1984; Fosse *et al*., 2013; Kamaszewski *et al*., 2014).

Nutritional strategies using live food (Cahu *et al*., 1998), inert (Carvalho *et al*., 1997) or both (Fosse *et al*., 2018), have proven effective in larval rearing of different species. However, in general, little attention has been given to the corrections of the total amount of food needed during the cultivation.

Within the understanding of zootechnical performance, the economic viability interferes directly in the choice of feeding strategy. Different diets and feeding strategies influence the market price of both fish, intended for human consumption, and for fish that have an ornamental purpose (Tešić *et al*., 2014). So, the adoption or rejection of a food strategy is directly related to the cost involved with the diet. The present experiments aimed to optimize the larviculture of ornamental carp in a recirculating aquaculture system, emphasizing aspects of the performance and cost of production.

MATERIALS AND METHODS

The following methodology described was common for the two experiments. The experiments were conducted in Cachoeira de Macacú (22°27'46"S, 42°39'10"W), state of Rio de Janeiro, Brazil, during August and September (2014). The cultivation system was developed in a greenhouse with 12 m long and 7 m wide, with walls and masonry roof covered with Styrofoam under controlled conditions. Inside the greenhouse, one shelf with hatchery tanks was assembled to perform the experiments. This shelf was composed of 24 rectangular plastic tanks with a volume of 20 L, water outlet siphon system and with a separate water recirculating system. The filtration system was composed of a mechanical filter (acrylon and expanded clay balls) and a biological filter (ceramic rings). Together, mechanical and biological filters totalize 300 L. The pump used in the system had a capacity of 4,900 L h^{-1}. The flow rate in the cultivation tanks was settled for about 0.35 L min^{-1}, in a way that in the 24 h period, the entire volume of the tank was changed about 25 times. Additionally, the system had UV filter with 36 W for eliminating parasites, and for lighting the area where the experiments were conducted five lamps of 5 W were connected 12 h a day (from 07:00 to 19:00 h).

Every day, the tanks were siphoned for removal of remnants of food and feces, in order to maintain the stability of the water quality. With the same purpose, the mechanical filter was siphoned to remove solid particles. The water exchange was calculated to not exceed 10% of the total volume of the system. The dead larvae were taken daily with a plastic pipette aid, and only on the first day were replaced by larvae from the same spawning; these larvae coming from tanks under similar conditions to those of the experiment. This procedure was adopted because it was assume that by the first day, the death of the larvae occurred due to the stress of managing the individual count, and not because of the treatment. From the second day, the dead larvae were removed, not replaced, and were just counted to correct the amount of food being offered.

Water quality parameters: dissolved oxygen and temperature were daily monitored; pH three times a week; ammonia and electrical conductivity weekly; hardness fortnightly. Digital oximeter was used (YSI 550A, ±0.01) for measuring dissolved oxygen and temperature, digital pH meter (pHtek PHS-3E, ±0.02) to measure the pH, spectrophotometer to measure ammonia, digital conductivity meter (Bernauer, ±0.01) for measuring the conductivity, and the hardness was measured by titration method.

Experiment 1. Evaluation of the ideal concentration of *Artemia* in the form of newly hatched *Artemia* nauplii, used for the feeding of ornamental carp larvae grown in a recirculating aquaculture system

For Experiment 1, 9,600 ornamental carp larvae were used, obtained from natural spawning and kept in incubators in the breeding laboratory until the moment of swimming initiation, which, in this experiment, was observed four days after fertilization.

Twenty larvae were measured and weighed at the time they began to swim to obtain the initial wet weight values and initial length. Larvae were used with 1.30 ± 0.03 mg and 6.0 ± 0.10 mm for initial wet weight and the initial total length, respectively.

The feeding of carp larvae was conducted in four different times (07:00, 10:00, 13:00 and 16:00 h) and was composed exclusively of *Artemia* nauplii, by varying the daily amount of *Artemia* nauplii offered according to the proposed to each treatment.

For the hatching of the *Artemia*, 9.8 g of cysts were incubated in plastic incubators with an average volume of 1.5 L, salinity of 20 and constant aeration. After 24 h, aeration was ceased, and *Artemia* nauplii were

siphoned into a plastic container of 1.5 L. To count the *Artemia* nauplii, 1 mL was removed from the plastic container and placed in a 100 mL beaker, then was added 49 mL of water. From the new mixture of 50 mL, 1 mL was removed and had the number of present nauplii counted, the volume was moved to the beaker, and the process was repeated twice more. The average obtained from the three samples was taken as the average value of nauplii of *Artemia* in 1 mL of the sample. The average was then multiplied by 50, and the obtained value was taken as the average value of *Artemia* nauplii present in 1 mL of the container. This process occurred whenever new hatching of *Artemia* was carried out, ensuring the control of the amount of *Artemia* nauplii offered.

After seven days of culture, all fishes were counted to obtain the survival data. For the final length data, growth, final weight, weight gain and specific growth rate, 10 animals of each experimental unit were measured using a digital pachymeter (±0.01mm) and weighed in an analytical balance (±0.001 g). The following formulas were used to obtain the survival values (S), weight gain (WG), specific growth rate for the length (SGR_L) and specific growth rate for the weight (SGR_W), respectively:

$$S(\%) = \frac{Nº\ final\ number\ of\ fish}{Nº\ of\ initial\ fish}\ x\ 100$$

$$WG = mean\ final\ wet\ weight - mean\ initial\ wet\ weight$$

$$L = average\ final\ length - average\ initial\ length$$

$$SGR_L \frac{(ln\ final\ total\ length - ln\ initial\ total\ length)}{Nº\ days\ of\ cultivation}\ x\ 100$$

$$SGR_W = \frac{(ln\ final\ wet\ weight - ln\ initial\ wet\ weight)}{Nº\ days\ of\ cultivation}\ x\ 100$$

The price of the *Artemia* used to calculate the cost was USD64.16 the kilogram of the cyst of *Artemia* (reference price for the kilogram of *Artemia* observed at the time of the experiment). To calculate the cost of the *Artemia* were made the corrections related to the mortality control of the larvae, because the amount of offered nauplii was always related to the number of live larvae. Therefore, there was a variation with the effect of treatment.

Experiment 2. Feeding strategies in larviculture of ornamental carp in a recirculating aquaculture system

To perform Experiment 2, 2,400 ornamental carp larvae, obtained from natural spawning, were used and kept in incubators in the breeding laboratory until the time of swimming initiation, which in this experiment was observed four days after fertilization.

Twenty larvae were measured and weighed at the time they began to swim to obtain the initial wet weight values and initial total length. Larvae were used with 1.30 ± 0.03 mg and 6.0 ± 0.10 mm for initial wet weight and the initial total length, respectively.

The experimental design was completely randomized with six treatments and four repetitions; each tank was considered as an experimental unit and received 100 larvae of ornamental carp (5 larvae L^{-1}).

Each treatment represented a feeding strategy: T1 (powder meal); T2 (powder meal + *Artemia* nauplii); T3 ("pasty food"); T4 (*Artemia* nauplii with daily increase of 10% of the initial amount); T5 (*Artemia* nauplii with an increase of 10% of the initial amount every three days); T6 (*Artemia* nauplii with no increase).

The larvae feeding were conducted during four different times (07:00, 10:00, 13:00 and 16:00 h) and was composed of what was proposed for each treatment. In the treatments in which *Artemia* nauplii were used, the initial amount used was 600 nauplii of *Artemia* per larva day^{-1}, and the protocols used in treatments 4, 5 and 6 (treatments using nauplii only) are shown in Table 1. Moreover, for treatments where powder meal was used, the amount of 1g of a commercial powder meal (200-400 µm) with 36% of crude protein (Table 2) was offered.

For the preparation of the "pasty food" of treatment 3, the quantity of *Artemia* nauplii needed for each feeding was removed from the incubator after strained in the mesh (<100 µm), so that the nauplii form a kind of a paste in the strainer. This paste was mixed with powder meal (1 g) with the aid of plastic spoons until they form a ball; the mixture obtained was called "pasty food." Offered powder meal for treatments 1 and 2 was moistened with water so that the consistency of the food did not differ from those given in the "pasty food" used in treatment 3. Since in treatment 3 the powder meal was offered in the same time with *Artemia* nauplii as the "pasty food," in treatment 2, as equal, the powder meal and the *Artemia* nauplii were also offered in the same time.

After 13 days of cultivation, all animals were counted to obtain the survival data. For the final length data, growth, final weight, weight gain and specific growth rate, 10 animals of each experimental unit were measured using digital pachymeter (Western, ±0.01 mm) and weighed in an analytical scale (± 0.001 g).

To obtain the survival values (S), weight gain (WG), growth (G), specific growth rate for the length (SGR_L) and specific growth rate for the weight (SGR_W), were used the same formulas used at experiment 1.

The variables measured directly in the animals, *i.e.*, growth, weight gain, final length and final weight, in addition to the cost of production, were analyzed accor-

Table 1. Number of *Artemia* nauplii offered daily per larvae during the 13 days of cultivation.*Treatment who has been offered 4 g of inert commercial feed daily.

Day	Number of brine shrimp nauplii per larvae d^{-1}					
	T1*	T2*	T3*	T4	T5	T6
1	0	600	600	600	600	600
2	0	600	600	660	600	600
3	0	600	600	720	600	600
4	0	600	600	780	660	600
5	0	600	600	840	660	600
6	0	600	600	900	660	600
7	0	600	600	960	720	600
8	0	600	600	1020	720	600
9	0	600	600	1080	720	600
10	0	600	600	1140	780	600
11	0	600	600	1200	780	600
12	0	600	600	1260	780	600
13	0	600	600	1320	840	600

ding to the mixed model methodology based on the following statistical model (Littell *et al*., 1998):

$$Y_{ijk} = \mu + \alpha_i + a_{j(i)} + e_{k(ij)} \quad (1)$$

In which Y_{ijk} corresponds to the measurement performed on the k[th] larva, within the j[th] tank that received the i[th] treatment, $\alpha_i$represents the fixed effect of the i[th] treatment, $a_{j(i)}$ represents the random effect of the j[th] tank inside the i[th] treatment, this effect supposed normally distributed with average 0 and variance of σ_a^2. The term $e_{k(ij)}$ represents the random error supposed normal and independently distributed, with average 0 and variance of σ^2. The effect of treatments was tested, having as the dominator an estimated variance of σ_a^2. The assumption for homoscedasticity for σ_a^2 was verified using a simple model containing only one variance for the different treatments and a model containing heterogeneous variances for the different treatments. It was then calculated the criterion Akaike (1974), corrected for finite samples or AICc (Sugiura, 1978) according to the recommendations of Burnham & Anderson (2004), taking as the best model the one with the highest probability of likelihood and the parsimony criterion for the degree of parameterization of models.

The variables involving concentrations, *i.e.*, specific growth rate and survival, they were transformed to fit the criteria of normality. The transformed variable, *i.e.*, $Y'_{ijk} = 2 \arcsin \sqrt{Y_{ijk}}$, was then subjected to the same model described in Eq. (1). In the tables, the concentrations were presented in the original scale, by performing the equation $\hat{Y}_{i..} = 100\left[\sin\left(\hat{Y}'_{i..}/2\right)\right]^2$. In both cases, the MIXED procedure of SAS statistical software (Version 9, SAS System Inc., Cary, NC, USA) were used.

Table 2. Experimental feed composition. Each 1000 g contain: vitamin A 16,000 UI, vitamin B1 32,000 mg, vitamin B_{12} 32 mcg, vitamin B_2 32 mg, vitamin B_6 32 mg, vitamin C 500 mg, vitamin D_3 4,500 UI, vitamin E 250 UI, folic acid 10 mg, biotin 10 mg, calcium (max.) 16 g, cobalt 0.5 mg, copper 20 mg, choline 2,000 mg, iron 150 mg, phosphorus 8 mg, iodine 1 mg, magnesium (max) 2 g, mannan-oligosaccharides 60 mg, manganese 50 mg, niacin 170 mg, selenium 0.7 mg and zinc 150 mg.

Proximate composition	Amount ($g\ kg^{-1}$)
Ether extract (min.)	65
Fibrous matter (max.)	60
Mineral matter (max.)	110
Crude protein (min.)	360
Humidity (max.)	100

RESULTS

Experiment 1. Evaluation of the ideal concentration of *Artemia* in the form of newly hatched *Artemia* nauplii, used for the feeding of ornamental carp larvae grown in a recirculating aquaculture system

There was no significant difference between treatments ($P > 0.05$), and the standard errors observed were low (Table 3), indicating little change in parameters over cultivation. There were significant differences ($P < 0.05$) among the treatments for the variables of final length (mm) and final weight (mg). Treatments 4, 5 and 6 were not significantly different ($P > 0.05$), but showed better results than treatments 1, 2 and 3. There was no significant difference ($P > 0.05$) among treatments 1, 2 and 3 (Table 4).

Treatments effects were observed for the variables of growth, weight gain and survival. The growth observed for treatments 4, 5 and 6, was significantly higher ($P < 0.05$) than the values observed for treatments 1, 2 and 3 (Fig. 1a). For the gain weight variable, the treatments 4, 5 and 6 showed the best averages and were significantly higher ($P < 0.05$) than those of treatments 2, 3 and 4, but this group showed inferior results to treatment 5 and 6 (Fig. 1b). The average larval survival was influenced by the amount of *Artemia* nauplii offered. For treatment 1 was observed the lowest average (8.75 ± 1.67%), but not significantly different ($P > 0.05$) of treatment 2. Treatment 4 had the highest survival average (68.62 ± 9.71%), but there was no significant difference ($P > 0.05$) for treatments 3, 5 and 6 (Fig. 1e).

There was no significant difference among treatments for the *Artemia* nauplii cost variable. A quadratic polynomial regression was observed (Fig. 2), and from the polynomial equation, it was possible to obtain the value of 383 *Artemia* nauplii offered to each

Table 3. Water quality parameters (mean ± standard error) in the cultivation of ornamental carp larvae under different *Artemia* nauplii quantities as a feeding source in a recirculating aquaculture system.

Parameter	Treatment						*P*-value
	1	2	3	4	5	6	
pH	6.75 ± 0.06	6.75 ± 0.06	6.89 ± 0.08	6.70 ± 0.08	6.74 ± 0.06	6.74 ± 0.06	0.5657
Dissolved oxygen (mg L^{-1})	6.74 ± 0.05	6.72 ± 0.05	6.67 ± 0.05	6.68 ± 0.05	6.65 ± 0.05	6.69 ± 0.05	0.8298
Temperature (°C)	28.75 ± 3.77	28.54 ± 3.77	28.56 ± 3.77	28.53 ± 3.77	28.49 ± 3.77	28.39 ± 3.77	0.4171
Amonnia (mg L^{-1})	0.0029 ± 0.001	0.0029 ± 0.001	0.0045 ± 0.0013	0.0040 ± 0.0013	0.0031 ± 0.001	0.0027 ± 0.001	0.3831
Conductivity (µS cm^{-2})	580 ± 112	578 ± 112	461 ± 137	424 ± 137	575 ± 112	567 ± 112	0.9174

Table 4. Final length and final weight (with their respective confidence intervals) of ornamental carp larvae cultivated under different *Artemia* nauplii quantities as a feeding source in a recirculating aquaculture system. Different letters mean a significant difference among treatments.

Treatment	Final length (mm)			Final weight (mg)		
	Mean	Confidence intervals		Mean	Confidence intervals	
		Lower	Upper		Lower	Upper
T1	9.75[b]	9.30	10.19	11.30[b]	8.82	13.77
T2	9.76[b]	9.31	10.20	10.11[b]	8.70	11.51
T3	9.92 [b]	9.48	10.37	11.21[b]	9.30	13.11
T4	11.28[a]	10.83	11.73	17.57[a]	14.64	20.50
T5	11.52[a]	11.07	11.96	20.46[a]	17.78	23.13
T6	11.13[a]	10.68	11.57	16.37[a]	14.06	18.69

larva as being the quantity to obtain the minimum cost, and the confidence interval that promotes the minimum cost is between 343 and 423 *Artemia* nauplii.

Experiment 2. Feeding strategies in larviculture of ornamental carp in a recirculating aquaculture system.

All water qualities parameters were within the acceptable for the species. There was a significant difference ($P < 0.05$) among treatments for the variable of dissolved oxygen. The value obtained for this variable in T2 was slightly lower than those observed in the other treatments (Table 5). There was no significant difference ($P < 0.05$) in observed values for the other parameters of water quality.

There were significant differences ($P < 0.01$) from the feeding strategy adopted for the variable of final length (mm) and final weight (mg). Larvae submitted to the feeding regime of treatment 1 were significantly lower ($P < 0.01$) than the larvae of the other treatments. The larvae of treatment 3 showed a better average of final length and final weight; there was a significant difference ($P < 0.01$) from these larvae to those of treatments 4, 5 and 6. There were also significant differences ($P < 0.01$) of larvae submitted to treatment 2 for the larvae subjected to treatments 5 and 6. There were also significant differences ($P < 0.01$) of larvae of treatment 4 compared to the larvae of treatment 6 (Table 6).

Larvae submitted to the feeding of treatment 1 were significantly lower ($P < 0.01$) to all other larvae of all the other treatments for the growth rate. The strategy adopted to treatment 3 provided the highest average growth rate (12.14 ± 1.69 cm), but there was no significant difference ($P > 0.01$) for the treatment 2. However, there was a significant difference ($P < 0.01$) of treatment 3 larvae for treatments 4, 5 and 6. The larvae maintained under the influence of treatment 2 and 4 were significantly higher ($P < 0.01$) than the larvae maintained on diets proposed for treatments 5 and 6 (Fig. 3a).

It was observed the lowest average of weight gain (12.53 ± 6.26 mg) in the larvae subjected to treatment 1, differing significantly ($P < 0.01$) from all other treatments. Treatment 3 had the highest average in weight gain (60.23 ± 21.16 mg), but there was no significant difference in treatment 2. However, there was a significant difference ($P < 0.01$) from the larvae of treatment 3 to those of treatment 4, 5 and 6. The larvae maintained under the influence of treatment 2 and 4 presented significantly higher weight gain ($P < 0.01$) to larvae maintained on diets proposed for treatment 5 and 6 (Fig. 3b).

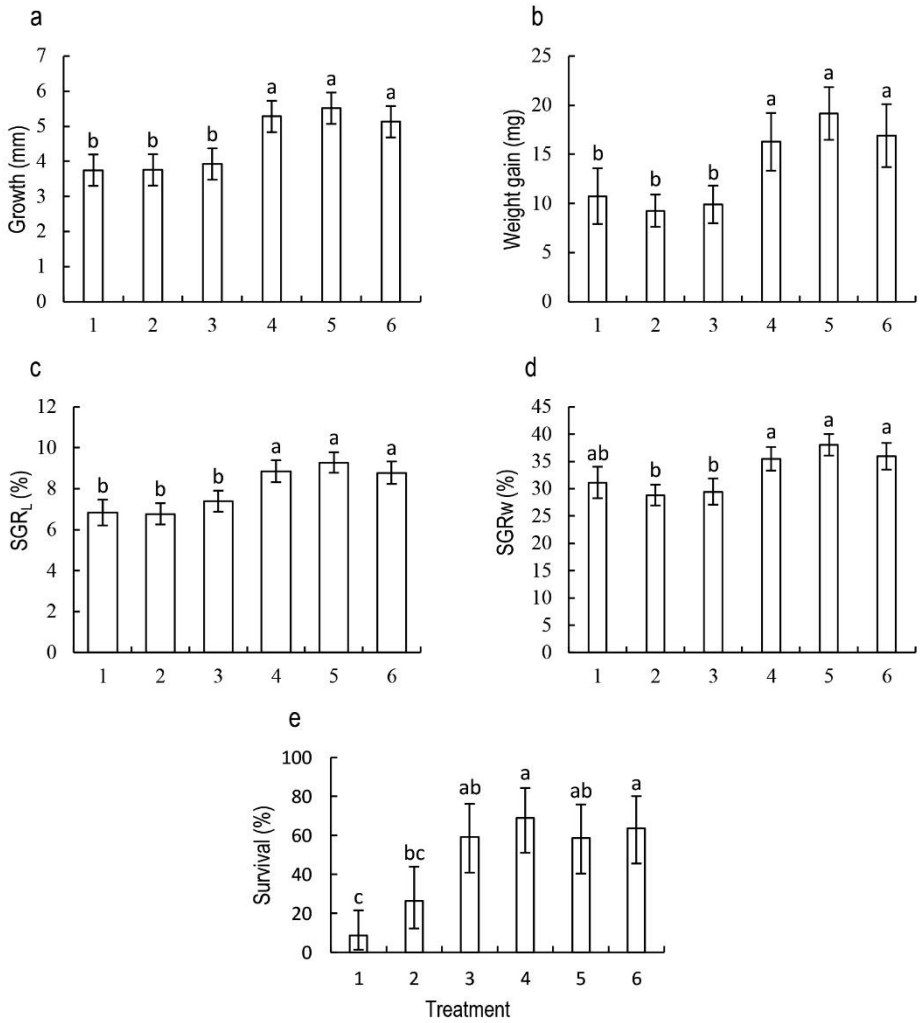

Figure 1. a) Mean and confidence interval of growth, b) weight gain, c) specific growth rate for the length (SGR_L), d) specific growth rate for the weight (SGR_W), e) survival; of ornamental carp larvae cultivated under different *Artemia* nauplii quantities as a feeding source in a recirculating aquaculture system. Different letters mean significant differences among treatments.

There was no significant difference ($P > 0.01$) of the influence of feeding strategy adopted on survival (Fig. 3e). The highest percentage of survival was observed in treatment 3 (79.71 ± 4.72%), and the lowest was observed in treatment 1 (64.52 ± 10.15%).

The daily growth of larvae held on the proposed diet in treatment 1 was significantly ($P < 0.01$) inferior compared to the other treatments. Treatment 3 was significantly ($P < 0.01$) higher than the treatments 4, 5 and 6, but there was no significant difference ($P > 0.01$) from this to treatment 2. There was no significant difference ($P > 0.01$) from treatment 2 to 4, but a significant difference ($P < 0.01$) was observed from treatment 2 to treatments 5 and 6 (Fig. 3c).

For the daily weight gain variable, there was the influence of different feeding strategies ($P < 0.01$). Treatments 5 and 6, which were significantly ($P < 0.01$) superior to treatment 1, were inferior to treatment 3, which had the highest average. Treatment 2 was significantly ($P < 0.01$) higher than the treatments 1, 5 and 6, but was not significantly different ($P > 0.01$) from treatments 3 and 4. Moreover, treatment 4 was significantly ($P < 0.01$) below treatment 3, and superior to treatments 1, 5 and 6 (Fig. 3d).

DISCUSSION

Experiment 1. Evaluation of the ideal concentration of *Artemia* in the form of newly hatched *Artemia* nauplii, used for the feeding of ornamental carp larvae grown in a recirculating aquaculture system

The recirculating aquaculture system used in this experiment was effective in maintaining the water quality parameters within the tolerable range for the

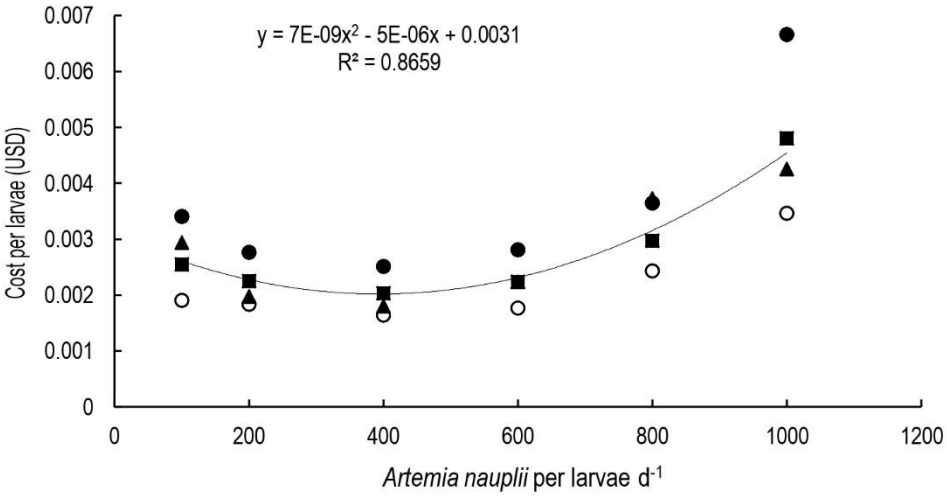

Figure 2. Cost of *Artemia* nauplii during the seven days of culture. ■ predict; ○ confidence interval (lower); ● confidence interval (upper); ▲ mean; − polynomial (mean).

Table 5. Ornamental carp larvae cultivated under different feeding regimes in a recirculating aquaculture system. Water quality parameters (mean ± standard error, $P < 0.05$).

Parameters	Treatment						*P*-value
	1	2	3	4	5	6	
pH	6.6 ± 0.09	6.59 ± 0.09	6.59 ± 0.09	6.59 ± 0.09	6.59 ± 0.09	6.59 ± 0.09	1
Dissolved oxygen (mg L^{-1})	6.54 ± 0.03^{a}	6.44 ± 0.03^{b}	6.52 ± 0.03^{a}	6.57 ± 0.03^{a}	6.57 ± 0.03^{a}	6.57 ± 0.03^{a}	0.0162
Temperature (°C)	28.41 ± 0.04	28.42 ± 0.04	28.38 ± 0.04	28.38 ± 0.04	28.37 ± 0.04	28.39 ± 0.04	0.9708
Amonnia (mg L^{-1})	0.0005 ± 0.0001	0.0005 ± 0.0001	0.0005 ± 0.0001	0.0005 ± 0.0001	0.0005 ± 0.0001	0.0005 ± 0.0001	0.9477
Conductivity (µS cm^{-2})	162.81 ± 25.14	163.25 ± 25.14	160.81 ± 25.14	164.31 ± 25.14	163.06 ± 25.14	163.12 ± 25.14	1

Table 6. Final length and final weight (with their respective confidence intervals) of ornamental carp larvae cultivated under different feeding regimes in a recirculating aquaculture system.

Treatment	Final length (mm)			Final weight (mg)		
	Mean	Confidence intervals		Mean	Confidence intervals	
		Lower	Upper		Lower	Upper
T1	12.36^{e}	11.70	13.02	13.83^{d}	11.11	16.54
T2	17.64^{ab}	16.98	18.30	55.43^{ab}	47.30	63.55
T3	18.15^{a}	17.49	18.80	61.63^{a}	52.45	70.80
T4	16.50^{bc}	15.84	17.15	43.10^{b}	36.61	49.59
T5	15.36^{cd}	14.70	16.02	31.48^{c}	26.58	36.37
T6	14.88^{d}	14.22	15.53	28.60^{c}	24.23	32.97

species, during the cultivation time (Arana, 2004). No significant difference was observed ($P > 0.05$) of water quality parameters among the treatments.

The worst survival average observed in this experiment was at T1 (8.68 ± 1.93%). The T2 treatment also showed a survival average (26.59 ± 10.26%) lower than expected for the species cultivated in indoor cultivation. The low survival averages observed in these two treatments are probably related to the amount of food provided to the larvae, a fact that probably increased intraspecific competition, generating increased mortality. Contrary to what was observed in this experiment, Fosse *et al*. (2018) observed survival of 97.86 ± 1.84% in the cultivation of ornamental carp larvae, using only 150 *Artemia* nauplii per larvae, but this author has adopted the density of 7 larvae L^{-1} and does not make clear whether it was carried out proportional correction regarding the number of nauplii offered. In this experiment was used the density of 20 larvae L^{-1}, and correction of *Artemia* nauplii quantity offered according to the observed mortality.

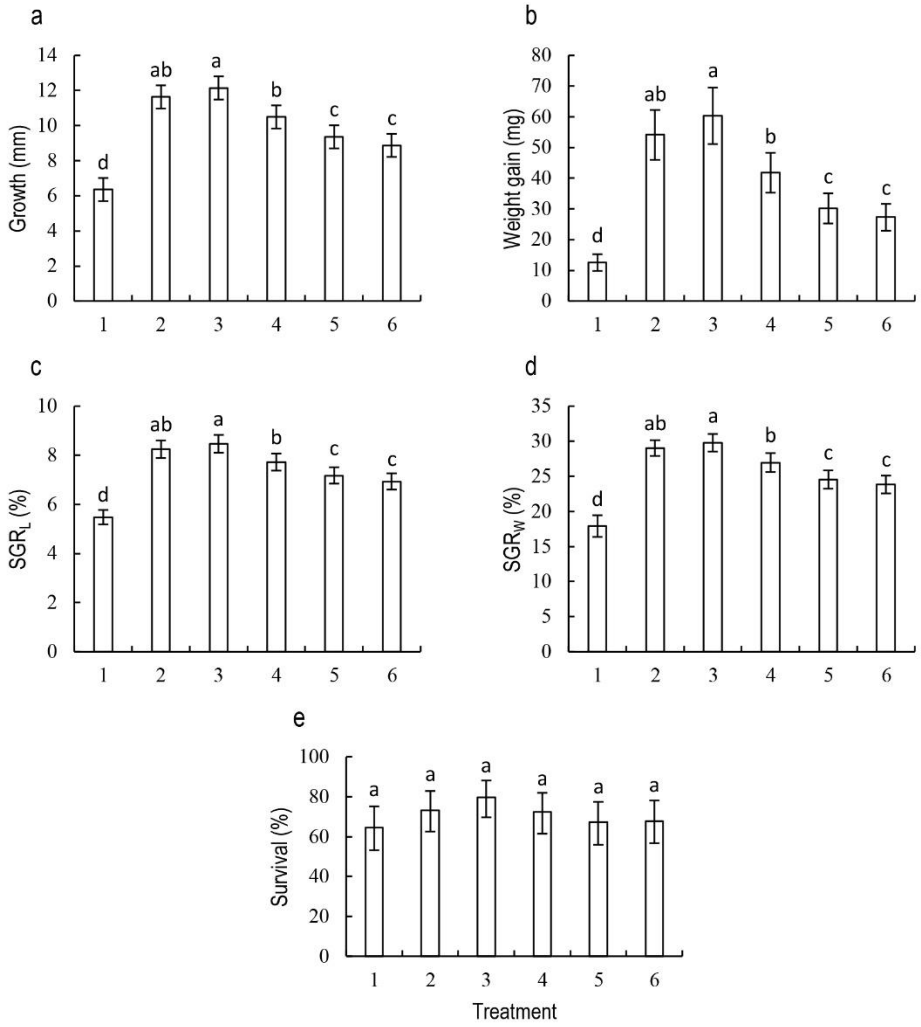

Figure 3. a) Mean and confidence interval of growth, b) weight gain, b) weight gain, c) specific growth rate for the length (SGR_L), d) specific growth rate for the weight (SGR_W), e) survival; of ornamental carp larvae cultivated under different feeding regimes in a recirculating aquaculture system.

The values observed in the present experiment for the morphological parameters indicate that increasing the amount of *Artemia* nauplii offered influenced larvae development. The increasing of the number of *Artemia* nauplii to the amount of 600 nauplii larva^{-1} (T4), improved the performance of the larvae, as Bryant & Matty (1980), who observed this same appearance on the performance of common carp larvae.

Larvae subjected to treatments T5 and T6 (800 and 1,000 nauplii larva^{-1}, respectively), did not show a significant difference in the performance for the larvae of T4 (600 nauplii larva^{-1}), but it was observed that from, approximately 383 nauplii larva^{-1}, there was an increase in the average cost per larvae. Several authors mention that the cost of living food in the early stages of fish production is a major problem faced by producers (Jomori, 2005; Ayres, 2006; Fosse *et al*., 2013) and the use of this food supply must be minimized in the culture whenever possible.

In the present experiment, the larvae subjected to treatment T4, T5 and T6 presented final weight higher than that observed by Fosse *et al*. (2018). Although these authors had used low stocking density (7 larvae L^{-1}) and more extended period of biometrics (10 days) than in the present experiment, larvae from this treatment T4, T5 and T6 showed superior performance. This fact can be explained by the more considerable amount of *Artemia* nauplii offered by larvae in this experiment in the treatments.

It was observed in this experiment that T1 fish showed no significant difference for T4 fish for the weight gain and specific growth rate for weight variables. Probably, this fact was due to the high mortality in T1 and consequent low stocking density in

this treatment. Several authors have reported that low stocking density influences the performance of the larvae (Jelkić *et al*., 2012; Gonçalves Jr. *et al*., 2014; Joaquim *et al*., 2016).

In treatment T4 of this experiment, it was observed *Artemia* nauplii leftovers until the third day of culture, while for T5 and T6 treatments, leftovers were observed during the whole experimental period. Observations indicated that in the mentioned periods was offered more food than larvae were able to ingest. To solve such problem Bryant & Matty (1980) performing *Artemia* nauplii quantification experiments with larvae of common carp, made daily corrections of the amount of provided food, based on the daily growth of the larvae. In the present experiment, any increase in food was adopted, because the aim was to evaluate the effects of levels of amount of *Artemia* nauplii offered by larvae in the early stage of cultivation. In commercial cultivation, to solve this problem would be appropriate use food strategies, as suggested by several authors (Liu *et al*., 2012; Agh *et al*., 2013; Pradhan *et al*., 2014).

Experiment 2. Feeding strategies in larviculture of ornamental carp in a recirculating aquaculture system

The average of the water quality parameters of all treatments was within the acceptable standards for fish farming (Arana, 2004; Desai & Singh, 2009). Despite the significant difference in the dissolved oxygen variable in the treatment T2, the oxygen always remained within the ideal range for the species (Mustafa *et al*., 2011), and did not affect the development of larvae.

None of the food strategies adopted affected the larval survival in this experiment. However, the larvae subjected to the food strategy proposed by treatment 1 were very weak, a fact that can be seen in the lower final weight. Different from what was observed in this experiment, several authors mentioned a decrease in survival when the first exogenous feeding of larvae consisted of commercial powder meal (Agh *et al*., 2013; Pradhan *et al*., 2014). Possibly, survival was not affected by the food strategies, due to the short time of cultivation adopted. Several authors mention increased mortality of larvae when fasted and/or exclusively fed with inert food, only from the 18th day after hatching (Tesser & Portella, 2006; Leitão *et al*., 2011; Menossi *et al*., 2012). Carvalho *et al*. (1997) observed total mortality of fasted common carp larvae on the 12th day after hatching. Observing the morphological parameters and physical condition of the larvae subjected to treatment 1, it can be assumed that if cultivations were longer, the survival of this treatment would be lower than what was observed.

In present experiment it was observed that the carp larvae submitted to the proposed food protocol for treatment 1 were able to feed with powder meal from day 1 of exogenous feeding, as observed by other authors (Bryant & Matty, 1981; Fosse *et al*., 2018), but presented results of morphological parameters lower than those observed for the other treatments. Differing from what observed in this experiment, some authors working with other inert food formulations in the larviculture of the common carp, achieved good results in morphological parameters using a few basic ingredients, such as yeast and casein (Carvalho *et al*., 1997; Cahu *et al*., 1998).

Probably the low yield of larvae fed with inert food from the first day of exogenous feeding in this experiment was related to the limited capacity of digestion of the larvae, together with the quality of food provided to the larval stage of this species, as noted by Tesser *et al*. (2006). Fosse *et al.* (2018), working with different feeding strategies on the larval rearing of ornamental carp, also was not observed the good performance of larvae fed exclusively with inert food.

Despite the low morphological parameters yields observed in larvae fed exclusively with inert food in this experiment, treatments 2 and 3 that used in the feeding, simultaneously, *Artemia* nauplii and inert food, showed superior results to the other food strategies. Improved digestion of inert food due to the presence of live food was also observed by Tesser & Portella (2003), that observed that the granules of microencapsulated diet collected in the digestive tract of pacu larvae (*Piaractus mesopotamicus*) co-fed with *Artemia* nauplii had higher areas of degradation than those removed from larvae tract fed exclusively with inert food. Probably, the result obtained in treatment 1 was a result of the low capacity of inert food to stimulate the production of digestive enzymes, which may have been fixed in treatments 2 and 3 due to the presence of live food; such mechanism was observed by Cahu & Zambonino-Infante (2001).

Similar strategies to those used in this experiment, using as a food source both inert and live food for the larvae stage, had already been successfully tested for different species by several authors (Liu *et al*., 2012; Fosse *et al*., 2013; Pradhan, *et al*., 2014), often being referred to as co-feeding.

Unlike what has been tested in this experiment, Dabrowski (1984) tested the strategy of using inert food and live-food in the hatchery stage not simultaneously. The mentioned author observed that carp larvae fed exclusively with live food had morphological parameters indices higher than larvae fed with live food and later with inert food. Possibly the results obtained by the author quoted above were the result of the

sudden change of live food by inert food, which did not occur in this experiment since there was no replacement of components of food throughout the hatchery stage. Fosse *et al*. (2018) observed that ornamental carp larvae fed simultaneously with live and inert food, presented final length and final weight higher than larvae kept only with live food, a fact that corroborates with the results obtained in this experiment.

Treatments 2 and 3 of this experiment did not significantly differ between themselves, so that how the inert food and the *Artemia* nauplii were offered did not influence the development of the carp larvae. However, it was observed that in tanks of treatment 3, where the "pasty food" was offered, the leftovers were concentrating at a single point, a fact that facilitates the cleaning of the tanks. Food strategies that facilitate the management of cultivation are of great interest because it minimizes the cost of hand labor, which in the farming of ornamental fish, is one of the most significant production costs. As well as in ornamental fish-farming, the cost of hand labor is much of the total cost of production of other aquaculture species such as the cultivation of the Pacific oyster, *Crassostrea gigas*, (Huang *et al*., 2013) and in the production of cobia, *Rachycentron canadum*, in cages (Huang *et al*., 2011).

There was no significant difference for the morphological parameter's indices between treatments 2 and 4, showing that the supply of powder meal from the first day of exogenous feeding, can replace the daily increase in the amount of *Artemia* nauplii offered. The decrease in the use of live food in hatcheries was also observed with great interest by Bryant & Matty (1980), especially from the economic point of view. The possibility of the use of inert food without damaging the growth and survival in the hatchery phase by a period of co-feeding was also reported by other authors (Rosenlund *et al.*, 1997; Cañavate & Fernández-Díaz, 1999).

The results obtained in this experiment in the treatments that used *Artemia* nauplii as the only source of food (treatment 4, 5 and 6), showed the necessity of correction amount of food offered over the growing larvae period. Probably such corrections are necessary due to the intense growth process demonstrated by larvae, which can be observed through the specific growth rate data. Several authors (Osse *et al*., 1997; Tesser & Portella, 2006) correlate the increase in growth rate in length and the need to supply the demand of energy for the growth of the larvae, a fact that corroborates to the results observed in this experiment in relation to the need to increase the amount of food provided along cultivation.

As in the present experiment, Bryant & Matty (1980) observed the need for correction of the amount of *Artemia* nauplii offered during the hatchery phase of the carp. For the authors mentioned, in the first five days of culture, an amount of 200-250% of body weight of larvae should be offered, and that amount should be reduced to 100-150% from the 10th day. Although the term used by the authors to be "reduction," there is an increase in the total amount of offered *Artemia* nauplii since the value is related to the percentage of larval weight.

CONCLUSIONS

The results demonstrate that increasing the amount of offered *Artemia* nauplii influences the development of ornamental carp larvae grown in a recirculating aquaculture system. Also, that under the conditions used in this experiment, the proper amount is 600 nauplii larva^{-1}, as it offers the best development rates, with the lowest cost production.

The experiments revealed that the supply of an inert food and live food, simultaneously from the first day of exogenous feeding, showed significantly superior growth to those larvae fed exclusively with live food, and should be adopted as the best strategy for feeding the ornamental carp larvae grown on recirculating aquaculture system. It was also found that daily increases of 10% of the amount of *Artemia* nauplii offered, yield better performance in larviculture of ornamental carp in a recirculating aquaculture system.

REFERENCES

Agh, N., Asgari, R. & Noori, F. 2013. Optimizing the co-feeding strategy of Persian sturgeon (*Acipenser persicus*) larvae using *Artemia* nauplii and formulated diet. International Journal of Aquatic Biology, 1(4): 158-166.

Akaike, H. 1974. A new look at the statistical model identification. IEEE Transactions on Automatic Control, 19(6): 716-723.

Arana, L.V. 2004. Princípios químicos de qualidade de água em aquicultura: uma revisão para peixes e camarões. Editora EdUFSC, Florianópolis.

Ayres, T.J.S. 2006. Produção de juvenis de *Pseudoplatysto macoruscans* (Agassiz, 1829) com dietas vivas e formuladas. Dissertação Mestrado em Aquicultura, Universidade Estadual Paulista, São Paulo.

Bryant, P.L. & Matty, A.J. 1980. Optimization of *Artemia* feeding rate for carp larvae (*Cyprinus carpio* L.). Aquaculture, 21(3): 203-212.

Bryant, P.L. & Matty, A.J. 1981. Adaptation of carp (*Cyprinus carpio*) larvae to artificial diets: 1. Optimum

feeding rate and adaptation age for a commercial diet. Aquaculture, 23(1-4): 275-286.

Burnham, K.P. & Anderson, D.R. 2004. Multimodel inference: understanding AIC and BIC in model selection. Sociological Methods & Research, 33(2): 261-304.

Cahu, C. & Zambonino-Infante, J. 2001. Substitution of live food by formulated diets in marine fish larvae. Aquaculture, 200(1-2): 161-180.

Cahu, C., Infante, J.Z., Escaffre, A.M., Bergot, P. & Kaushik, S. 1998. Preliminary results on sea bass (*Dicentrarchus labrax*) larvae rearing with compound diet from first feeding. Comparison with carp (*Cyprinus carpio*) larvae. Aquaculture, 169(1-2): 1-7.

Cañavate, J.P. & Fernández-Dıaz, C. 1999. Influence of co-feeding larvae with live and inert diets on weaning the sole *Solea senegalensis* onto commercial dry feeds. Aquaculture, 174(3-4): 255-263.

Carvalho, A.P., Escaffre, A.M., Teles, A.O. & Bergot, P. 1997. First feeding of common carp larvae on diets with high levels of protein hydrolysates. Aquaculture International, 5(4): 361-367.

Chabalin, E., Senhorni, J.A. & Ferraz De Lima, J.A. 1989. Estimativa do custo de produção de larvas e alevinos. Boletim Técnico CEPTA, 2: 61-74.

Charlon, N. & Bergot, P. 1984. Rearing system for feeding fish larvae on dry diets. Trial with carp (*Cyprinus carpio* L.) larvae. Aquaculture, 41(1): 1-9.

Dabrowski, K. 1984. Influence of initial weight during the change from live to compound feed on the survival and growth of four cyprinids. Aquaculture, 40(1): 27-40.

Dabrowski, K., Dabrowska, H. & Grudniewski, C. 1978. A study of the feeding of common carp larvae with artificial food. Aquaculture, 13(3): 257-264.

Desai, A.S. & Singh, R.K. 2009. The effects of water temperature and ration size on growth and body composition of fry of common carp, *Cyprinus carpio*. Journal of Theoretical Biology, 34(6): 276-280.

Food and Agriculture Organization (FAO). 2014. The state of world fisheries and aquaculture. FAO, Rome. [http://www.fao.org/3/a-i3720e.pdf]. Reviewed: 9 September 2018.

Fosse, P.J., Mattos, D.D.C., Cardoso, L.D., Radael, M.C. & Vidal Jr., V.M. 2018. Duration of co-feeding on the Nishikigoi *Cyprinus carpio* larvae during weaning from live to inert food in an indoor system. Ciência Rural, 48(4): 1-9.

Fosse, P.J., Mattos, D.C., Cardoso, L.D., Motta, J.H.S., Jasper, A.P.S., Radael, M.C., Andrade, D.R. & Vidal Jr., M.V. 2013. Estratégia de co-alimentação na sobrevivência e no crescimento de larvas de *Betta splendens* durante a transição alimentar. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 65(6): 1801-1807.

Gonçalves Jr., L.P., Mendonça, P.P., Pereira, S.L., Matielo, M.D. & Amorim, I.R.F. 2014. Densidade de estocagem durante a larvicultura do kinguio. Boletim do Instituto de Pesca, 40(4): 597-604.

Huang, J.F., Lee, J.M. & Sun, P.C. 2013. Prolonged culture period on production cost and factor input: a case from the Pacific oyster, *Crassostrea gigas*, farming industry in Yunlin County, Taiwan. Journal of the World Aquaculture Society, 44(6): 795-804.

Huang, C.T., Miao, S., Nan, F.H. & Jung, S.M. 2011. Study on regional production and economy of cobia *Rachycentron canadum* commercial cage culture. Aquaculture International, 19(4): 649-664.

Jelkić, D., Opačak, A., Stević, I., Ozimec, S., Jug-Dujakovi, J. & Safner, R.. 2012. Rearing carp larvae (*Cyprinus carpio*) in closed recirculatory system (RAS). Croatian Journal of Fisheries, 70(1): 9-17.

Joaquim, S., Matias, D., Matias, A.M., Leitão, A., Soares, F., Cabral, M. & Gaspar, M.B. 2016. The effect of density in larval rearing of the pullet carpet shell *Venerupis corrugata* (Gmelin, 1791) in a recirculating aquaculture system. Aquaculture Research, 47(4): 1055-1066.

Jomori, R.K. 2005. Organismos vivos e dietas secas na larvicultura do pacu *Piaractus mesopotamicus* e o uso dos isótopos estáveis de carbono (δ13C) e nitrogênico (δ15N) como indicadores naturais da incorporação do alimento no tecido larval. Tese de Doutorado, Universidade Estadual de São Paulo, São Paulo, 121 pp.

Jomori, R.K., Carneiro, D.J., Geraldo-Martins, M.I.E. & Portella, M.C. 2005. Economic evaluation of *Piaractus mesopotamicus* juvenile production in different rearing systems, Aquaculture, 243(1-4): 175-183.

Jomori, R.K., Carneiro, D.J., Malheiros, E.B. & Portella, M.C. 2003. Growth and survival of pacu *Piaractus mesopotamicus* (Holmberg, 1887) juveniles reared in ponds or at different initial larviculture periods indoors. Aquaculture, 221(1-4): 277-287.

Kamaszewski, M., Prasek, M., Ostaszewska, T. & Dabrowski, K. 2014. The influence of feeding diets containing wheat gluten supplemented with dipeptides or free amino acids on structure and development of the skeletal muscle of carp (*Cyprinus carpio*). Aquaculture International, 22(1): 259-271.

Leitão, N.J., Pai-Silva, M.D., Almeida, F.L.A. & Portella, M.C. 2011. The influence of initial feeding on muscle development and growth in pacu *Piaractus mesopotamicus* larvae. Aquaculture, 315(1-2): 78-85.

Littell, R.C., Henry, P.R. & Ammerman, C.B. 1998. Statistical analysis of repeated measures data using SAS procedures. Journal of Animal Science, 76(4): 1216-1231.

Liu, B., Zhu, X., Lei, W., Yang, Y., Han, D., Jin, J. & Xie, S. 2012. Effects of different weaning strategies on survival and growth in Chinese long snout catfish (*Leiocassis longirostris* Günther) larvae. Aquaculture, 364: 13-18.

Menossi, O.C., Takata, R., Sánchez-Amaya, M.I., Freitas, T.M., Yúfera, M. & Portella, M.C. 2012. Crescimento e estruturas do sistema digestório de larvas de pacu alimentadas com dieta micro encapsulada produzida experimentalmente. Revista Brasileira de Zootecnia, 41(1): 1-10.

Mustafa, S.A., Al-Subiai, S.N., Davies, S.J. & Jha, A.N. 2011. Hypoxia-induced oxidative DNA damage links with higher level biological effects including specific growth rate in common carp, *Cyprinus carpio* L. Ecotoxicology, 20(6): 1455-1466.

Osse, J.W.M., Van den Boogaart, J.G.M., Van Snik, G.M.J. & Van der Sluys, L. 1997. Priorities during early growth of fish larvae. Aquaculture, 155(1-4): 249-258.

Portella, M.C., Jomori, R.K., Leitão, N.J., Menossi, O.C.C., Freitas, T.M., Kojima, J.T. & Carneiro, D.J. 2014. Larval development of indigenous South American freshwater fish species, with particular reference to pacu (*Piaractus mesopotamicus*): a review. Aquaculture, 432: 402-417.

Pradhan, P.K., Jena, J., Mitra, G., Sood, N. & Gisbert, E. 2014. Effects of different weaning strategies on survival, growth, and digestive system development in butter catfish *Ompok bimaculatus* (Bloch) larvae. Aquaculture, 424: 120-130.

Rosenlund, G., Stoss, J. & Talbot, C. 1997. Co-feeding marine fish larvae with inert and live diets. Aquaculture, 155(1-4): 183-191.

Received: 9 November 2018; Accepted: 24 April 2019

Sugiura, N. 1978. Further analysts of the data by Akaike's information criterion and the finite corrections: further analysts of the data by Akaike's. Communications in Statistics - Theory and Methods, 7(1): 13-26.

Tešić, M., Baltić, M., Teodorović, V., Nedić, D., Mirilović, M., Marković, R. & Aleksić-Agelidis, A. 2014. Effects of various meal compositions on production results, economic performance, and fish meat quality. Acta Veterinaria-Beograd, 64(3): 338-348.

Tesser, M.B. & Portella, M.C. 2003. Degradation analysis of microencapsulated diet in pacu (*Piaractus mesopotamicus*, Holmberg, 1887) larvae intestine through scanning electron microscopy. Actuarial Science, 25: 49-52.

Tesser, M.B. & Portella, M.C. 2006. Ingestão de ração e comportamento de larvas de pacu em resposta a estímulos químicos e visuais. Revista Brasileira de Zootecnia, 35(5): 1887-1892.

Tesser, M.B., Flores-Quintana, C.I., Carneiro, D.J., Pizauro Jr., J.M. & Portella, M.C. 2006. Suplementação de enzimas exógenas em dieta microparticulada para larvicultura do pacu. Revista Brasileira de Zootecnia, 35(6): 2211-2218.

Timmons, M.B., Ebeling, J.M., Wheaton, F.W., Summerfelt, S.T. & Vinci, B.J. 2002. Recirculating aquaculture systems. Ithaca Publishing Company, New York.

Xu, P., Zhang, X., Wang, X., Li, J., Liu, G., Kuang, Y. & Zhang, Y. 2014. Genome sequence and genetic diversity of the common carp, *Cyprinus carpio*. Nature Genetics, 46(11): 1212-1219.