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SHORT COMMUNICATION

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Optimal management improves Flowerhorn fish larviculture

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Flowerhorn (*Amphilophus labiatus* × *Amphilophus trimaculatus*) stands out as one of the most popular ornamental fish among hobbyists. They have a set of colours with shades of red, green, blue and yellow, receiving the tag *pet fish* due to their friendly behaviour towards the hobbyist (Sornsupharp et al., 2013; Tarkhani et al., 2017). Despite their importance to the ornamental fish market, technologies of production still fail to improve survival and reduce production cost. Optimum stocking density and feeding management are important factors in improving the survival and growth of fish and reducing production cost (Abe et al., 2019; Fujimoto et al., 2016; Seabra et al., 2020; Zhao et al., 2016).

High stocking densities in larviculture promote increases in nitrogen residue, degrading the water quality and affecting fish growth. Conversely, reduced numbers of fish in the tank means underused space, causing economic loss to the fish farmer (Geng et al., 2019; Luz & Santos, 2008; Sahoo et al., 2010). Also, inadequate feeding rate and frequency cause reduced fish growth and poor water quality (Couto et al., 2018; Okomoda et al., 2019).

Thus, the present study evaluated the effects of feeding rate and frequency as well as different stocking densities on the larviculture of Flowerhorn and their effects on survival and growth performance.

This study used Flowerhorn larvae of four days after hatching (length 3.5 ± 0.5 mm and weight 33 ± 4 mg) at the initial moment of exogenous feeding (four days after hatching). The experimental units consisted of a static system of small polyethylene tanks (capacity 1 L) without forced aeration, and water exchange (20%) two hours after the last feeding of the day. All experiments followed the guidelines of the ethic committee for animal care from Pio Décimo College (Protocol 16/2018).

The first experiment was a completely randomized factorial arrangement (2 × 3) with four replicates over 15 days. Two feeding frequencies (twice per day: 08:00 and 17:00 h; four times per day: 08:00, 11:00, 14:00 and 17:00 h) and three feeding rates (100, 200 and 300 *artemia nauplii* per day) were assayed. The stocking density used for this experiment was 10 larvae/L.

Another experiment was a completely randomized design testing six different stocking densities (1, 5, 10, 15, 20 and 30 larvae/L) and four replicates. The ideal feeding management from the previous experiment (feeding rate and frequency) was used in this experiment.

Artemia nauplii was used to feed the fish larvae in both experiment. The artemia cysts were daily incubated with salinized water (30 g/L) at temperature of 28°C for 24 h. After hatching, the nauplii were separated and then counted for trial feeding according Abe et al. (2019).

In both experiments, the water quality was monitored daily for dissolved oxygen (mg/L), temperature (°C), electric conductivity (µs/cm) and pH. The total ammonia (mg/L) was monitored every three days. At the end of the experiments, all larvae were measured and weighed to determine growth performance.

- Weight and length specific growth rate (%) (SGR_W or SGR_L) according to Lugert et al. (2016).

$$SGR = \left(\frac{\ln\left(W_f \text{ or } L_f\right) - \ln\left(W_i \text{ or } L_i\right)}{t}\right) \times 100$$

TABLE 1 Mean values ± SD of growth performance of Flowerhorn larvae submitted to different rate and feeding frequencies

Variables	TW (mg)	TL (mm)	SGR_W (%)	SGR_L (%)
Feeding frequency				
2 time/day	15.9 ± 1.1	11.6 ± 0.24	10.32 ± 0.77	5.79 ± 0.14
4 time/day	15.6 ± 1.4	11.8 ± 0.9	12.24 ± 1.9	5.7 ± 0.66
Feeding rate				
100 nauplii/larvae/day	12.4 ± 2 b	11.1 ± 0.06 b	8.73 ± 1.12 b	5.32 ± 0.43 b
200 nauplii/larvae/day	16.5 ± 1 a	11.7 ± 0.02 ab	11.09 ± 0.51 ab	5.76 ± 0.1 a
300 nauplii/larvae/day	18.5 ± 2 a	12.3 ± 0.04 a	12.5 ± 1.04 a	6.13 ± 0.32 a
p-value				
FF	0.3104	0.1721	0.4143	0.1513
FR	0.0001	0.0048	0.0001	0.0049
FF × FR	0.5774	0.4006	0.5765	0.4474

Note: Different letters in the column means statistical difference ($p < 0.05$) by Tukey test.
Abbreviations: FF, feeding frequency; FR, feeding rate; SGR_L, specific growth rate in length; SGR_W, specific growth rate in weight; TL, total length; TW, total weight.

W_f and L_f means weight and final length; W_i and L_i means weight and initial length; t means experimental days.

- Fish Uniformity (U) according to Furuya et al. (1998).

$$U = \frac{X}{X_1} \times 100$$

U means Uniformity for Length (UL) or Uniformity for Weight (UW); X, Total number of fish into experimental unit; X_1, Number of fish with Final Weight (FW) or Total length (TL) ±20% inside of the Final Weight Mean or Total Length Mean into experimental unit.

- Relative condition factor according to Le Cren (1951).

$$Kr = \frac{W_{expec}}{W_{obser}}$$

W_{expec} is the logarithmic regression between weight and length; W_{obser} is the natural log of weight.

- Final survival (%)

$$S = \left(\frac{N_f}{N_i}\right) \times 100$$

N_f means the larvae number at the end of experiment; N_i means the larvae number at the begin of experiment.

1.1 | Statistical analysis

All data were conducted to normality and homoscedasticity tests of Shapiro–Wilk and Bartlett respectively. Percentage data without normal distribution were transformed to arc sin square root of X. Afterwards, the data of first experiment were

TABLE 2 Mean values ± SD of zootechnical performance of Flowerhorn larvae submitted to different rate and feeding frequencies

Variable	U_W (%)	U_L (%)	Kr	S (%)
Feed frequency				
2 time/day	82.1 ± 8.2	100 ± 0	1.02 ± 0.02	100 ± 0
4 time/day	83.5 ± 8.5	100 ± 0	1.01 ± 0.04	100 ± 0
Feed rate				
100 nauplii/larvae/day	83.5 ± 9.2	100 ± 0	1.04 ± 0.03	100 ± 0
200 nauplii/larvae/day	82.2 ± 8.6	100 ± 0	1.02 ± 0.07	100 ± 0
300 nauplii/larvae/day	83.1 ± 9.6	100 ± 0	1.03 ± 0.01	100 ± 0
FF	0.5319	–	0.6411	–
FR	0.6089	–	0.6839	–
FF × FR	0.5102	–	0.5319	–

Note: Different letters in the column means statistical difference ($p < 0.05$) by Tukey test.
Abbreviations: FF, feeding frequency; FR, feeding rate; Kr, relative condition factor; S, survival; U_L, uniformity for length; U_W, uniformity for weight.

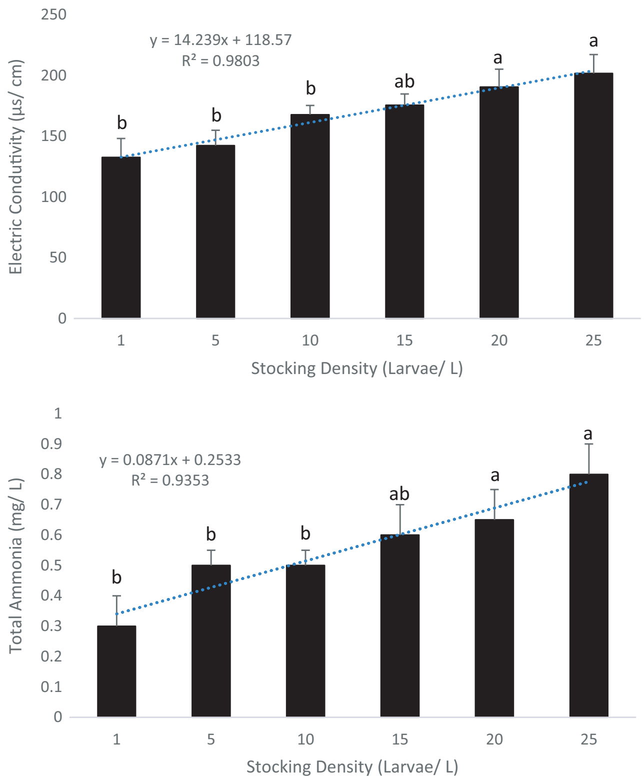

FIGURE 1 Mean values ± SD and linear regression of electric conductivity (µs/cm^2) and total ammonia (mg/L) for the second experiment (different stocking densities). Same letter indicates similarities by Tukey test ($p < 0.05$) [Colour figure can be viewed at wileyonlinelibrary.com]

conducted to two-way ANOVA and second experiment to one-way ANOVA, followed by post hoc Tukey test ($p < 0.05$) for mean comparisons, using the statistical software PAST 3.0. The biomass and water parameters with significant difference by the Tukey test ($p < 0.05$) were also submitted to the linear regression test.

In the first experiment, water quality parameters showed no statistical difference among the treatments: pH 7.3 ± 0.3, dissolved oxygen 6.5 ± 0.4 mg/L electric conductivity 151.54 ± 8.45 µs/cm, temperature 28.3 ± 0.5°C and total ammonia 0.5 ± 0.1 mg/L.

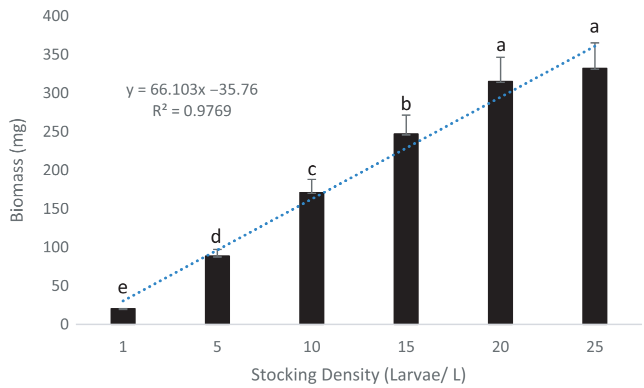

FIGURE 2 Mean values ± SD and linear regression of biomass (mg/L) for the second experiment (different stocking densities). Same letter indicates similarities by Tukey test ($p < 0.05$) [Colour figure can be viewed at wileyonlinelibrary.com]

There was no interaction between feed rate and frequency on fish growth ($p < 0.05$; Tables 1 and 2). There were no significant differences in the performance parameters of fish larvae regarding feeding rate ($p > 0.05$). Also, there were no observed differences in fish uniformity, relative condition factor or survival regarding the feeding rate or frequency. However, increases in feeding rate resulted in higher final weight, final length and specific growth rate (Tables 1 and 2).

In the second experiment, values of dissolved oxygen 6.7 ± 0.2 mg/L, temperature 28.2 ± 0.3°C and pH 7.1 ± 0.2 mg/L did not show differences among the treatments. However, electric conductivity and total ammonia values increased with the stocking density (Figure 1).

The increases in stocking density caused reductions in weight, length and specific growth rate (for weight and length) (Table 3). However, each experimental unit increased the biomass values (Figure 2). In addition, high stocking density reduced the uniformity of fish weight but had no effect at fish length uniformity, relative condition factor or survival (Table 4).

Feeding strategies in ornamental fish larviculture have been used to improve the growth performance, reduce cost and maintain the water quality suitable for captive rearing (Abe et al., 2019; Eiras

TABLE 3 Mean values ± SD on the growth performance of Flowerhorn larvae submitted to different stocking densities

Density (larvae/L)	TW (mg)	TL (mm)	SGR_W (%)	SGR_L (%)
1	20.4 ± 0.3 a	12.2 ± 0.7 a	20.22 ± 0.44 a	7.29 ± 0.39 a
5	17.9 ± 0.4 b	12.4 ± 0.5 a	19.35 ± 0.73 b	7.35 ± 0.25 a
10	17.5 ± 0.3 b	12.4 ± 0.3 a	19.49 ± 0.61 b	7.39 ± 0.16 a
15	16.5 ± 0.2 bc	10.9 ± 0.3 b	18.17 ± 0.44 bc	6.53 ± 0.21 b
20	15.9 ± 0.2 c	10.8 ± 0.3 b	18.51 ± 0.58 bc	6.46 ± 0.2 b
25	14.5 ± 0.1 d	11.2 ± 0.1 b	17.99 ± 0.13 c	6.68 ± 0.07 b
p-value	0.0001	0.0001	0.0001	0.0001

Note: Different letters in the column means statistical difference ($p < 0.05$) by Tukey test.
Abbreviations: SGR_L, specific growth rate in length; SGR_W, specific growth rate in weight; TL, total length; TW, total weight.

TABLE 4 Mean values ± SD on the growth performance of Flowerhorn larvae submitted to different stocking densities

Density	U_W (%)	U_L (%)	Kr	S (%)
1	100 ± 0 a	100 ± 0	1.09 ± 0.07	100 ± 0
5	90 ± 11.5 a	100 ± 0	0.99 ± 0.01	100 ± 0
10	97.5 ± 5 ab	100 ± 0	0.98 ± 0.03	100 ± 0
15	78.25 ± 3.5 b	100 ± 0	0.99 ± 0.03	98.33 ± 3.33
20	81.25 ± 11.08 b	100 ± 0	0.98 ± 0.01	98.75 ± 2.5
25	78 ± 2.31 b	100 ± 0	1.00 ± 0.01	99.0 ± 2
p-value	0.0001	–	0.0847	0.8965

Note: Different letters in the column means statistical difference ($p < 0.05$) by Tukey test.
Abbreviations: Kr, relative condition factor; S, survival; U_L, uniformity for length; U_W, uniformity for weight.

et al., 2019; Pereira et al., 2016). In ornamental cichlid species such as *Pterophyllum scalare* and *Heros severus*, the larvae must fed four meals per day for best performance (Eiras et al., 2019). However, this does not apply to the Flowerhorn, which demonstrated satisfactory growth with meals twice per day. Similar results occurred with the larvae of *Pyrrhulina brevis*, which also needs meals twice per day (Abe et al., 2016). This strategy with two meals per day represents lower costs to the fish farmer, making it more profitable than the strategy of four meals per day (Eiras et al., 2019).

With regard to the feeding rate, 200 *artemia nauplii* is the best feeding rate, regardless the similarity between feeding frequency, because it represents an adequate amount that does not affect growth performance and also provides economy. This is a lower amount of feed compared to the requirements of *Heros severus* fed with 250 *artemia nauplii* per day and represents benefits to the fish farmer due to lower production costs and adequate water quality (Abe et al., 2016, 2019). However, other fish species such as *Hoplias lacerdae* and *Lophiosilurus alexandri* must receive 900–1,600 *artemia nauplii* per larva per day (Abe et al., 2019; Luz & Portella, 2005; Santos et al., 2016).

Those differences in the feeding rate and frequency between fish species can be explained by their gastric morphology, behaviour, stage of development and different habitats (Booth et al., 2008; Riche et al., 2004). The feeding rate and frequency can also be affected by the physical-chemical factors of the water, such as temperature or dissolved oxygen (Ali et al., 2005). Thus, feeding management should be adjusted for each fish species, to promote an optimization of labour and feed economy, to enable the domestication of the fish and to improve feeding efficiency (Lee et al., 2000; Navarro-Guillén et al., 2018).

Adjustment of fish stocking density can also optimize production and avoid economic loss (Abe et al., 2019). High stocking densities can reduce the water quality, such as lower levels of dissolved oxygen and increased ammonia values, affecting the growth performance of fish (Çağiltay et al., 2018; Nagata et al., 2010). Thus, high electrical conductivity and ammonia levels related to the increased number of larvae in the system generate more nitrogen residues and waste of *artemia nauplii* (Abe et al., 2016, 2019; Santos et al., 2015).

Thus, the lower water quality in treatments with high stocking density could be the main factor for reduced growth performance of Flowerhorn larvae. However, despite the lower performance, changes in survival and relative condition factor did not occur, demonstrating healthy fish larvae. In the present study, increases in stocking density above 10 larvae/L also promoted reduction in fish weight uniformity. This effect is also seen in the larviculture of kinguio *Carassius auratus* at stocking densities above 5 larvae/L (Junior et al., 2018).

For these reasons, suitable feeding managements, such as feeding rate and frequency allied to a suitable stocking density, can reduce production costs as well as ensuring the survival of better-quality larvae. Thus, Flowerhorn larvae must be reared in the first 15 days of larviculture at a stocking density of 10 larvae/L with 200 *artemia nauplii* distributed twice per day for better growth performance and space utilization.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

All authors listed executed substantial contributions to the conception or design of the work; or the acquisition, analysis or interpretation of data for the work; and drafting the work or revising it critically for important intellectual content; and final approval of the version to be published; agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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