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ORIGINAL ARTICLE

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Effects of live feed containing *Panagrellus redivivus* and water depth on growth of *Betta splendens* larvae

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Abstract

Panagrellus redivivus is a nematode with a high protein content and low-cost production. It is successfully used in the larviculture of shrimp, however, it has not yet been evaluated as feed for *Betta splendens* larvae. Thus, the objective of this study was to evaluate *P. redivivus* as feed for betta larvae maintained at different depths of water. The experiment was based on a completely randomized 3 × 2 factorial design, represented by two feed types and one alternating feeding regime plus two water depths (2.5 and 5.0 cm). The *Artemia* feed promoted the highest weight gain, followed by the alternating feeding regime. The single use of the nematode as live feed produced the poorest weight gain. The water depth and the feeding method influenced the specific growth rate (SGR). At a depth of 2.5 cm, the alternating feeding promoted similar SGR compared to fish fed with *Artemia* only. Furthermore, at the greater water depth an improvement in SGR was observed in fish fed *Artemia* only, but the similarity to alternating feeding regime remains. Thus, the inclusion of nematode is an appropriate and lower cost feed strategy for the betta larviculture independent of water depths.

KEYWORDS
betta, feed, larvae, *Panagrellus redivivus*

1 | INTRODUCTION

Betta splendens is an important species in the international ornamental fish trade due its coloured patterns and singular behaviour. Reproduction and larviculture are a challenge for many producers due to the lack of scientific information, mainly on the nutrition of early larvae (Puello-Cruz, Velasco-Blanco, Martínez-Rodríguez, Felix-Ramos, & Voltolina, 2010).

Adequate feed and nutrition influence the quality of larvae, maximizing their zootechnical performance and immune response (Kim, 2007; Lazzari, Neto, Lima, Pedron, & Losekann, 2004). Live feeds (such as *Artemia* and *Daphnia* sp.) are commonly used in larviculture, due to the high quality of protein and lipid profile (essential fatty acids), even assisting in the development of the digestive system (Kolkovski, 2001; Soares, Hayashi, Gonçalves, Galdioli, & Boscolo, 2008). Among the live feeds, the microcrustacean *Artemia* is commonly used for betta larviculture. The *Artemia* is rich in protein, vitamin, and minerals. However, its utilization has high economic cost (Das, Mandal, Bhagabati, Akhtar, & Singh, 2012). Therefore, feed alternatives, such as the microworm (MV) *Panagrellus redivivus*, should be evaluated which take into consideration market availability, ease of cultivation, fish productivity performance, and reduction in costs.

Panagrellus redivivus is a free-living nematode of small size (50–2,000 μm length and 50 μm diameter), suitable for fish larvae with reduced mouth size, such as the betta larvae that have a mouth

TABLE 1 Mean values of water quality parameters in larviculture of *Betta splendens* treated with different feeds

Feed	*Artemia*	Alternating feeding regime	*Panagrellus redivivus*
Total ammonia (mg/L)	0.71 ± 0.1 A	0.90 ± 0.20 AB	1.5 ± 0.1 B
Dissolved oxygen (mg/L)	4.84 ± 0.60 A	4.04 ± 0.31 A	3.95 ± 0.36 A
Temperature (°C)	26.59 ± 0.13 A	26.70 ± 0.18 A	26.67 ± 0.12 A
pH	7.65 ± 0.003 A	7.51 ± 0.002 B	7.46 ± 0.003 B
Electric conductivity (μS)	307.06 ± 33.74 A	258.06 ± 69.64 A	241.25 ± 82.60 A

Different letters in rows present significant difference 5% by the Tukey test.

opening of 420 μm at 8 days after hatching (Budiardi, Nursyams, & Sudrajat, 2005). This nematode has a high protein level (crude protein 60% of dry matter, Sautter, Kaiser, Focken, & Becker, 2007), well-defined large-scale and reduced cost production with up to 500,000 nematodes/g of culture medium (Sautter et al., 2007).

There are no published reports concerning the use of this nematode as feed for betta larvae. However, it was evaluated as feed for angel fish *Pterophyllum scalare* larvae, but promoted lower performance compared to *Artemia franciscana* and *Moina wierzeiski* (Luna-Figueroa, Vargas, & Figueroa, 2010). This finding may be associated with two hypotheses: the composition of its cuticle is indigestible for angel fish larvae (Luna-Figueroa et al., 2010), or sinking of the microworm at time of feeding makes it difficult to capture in deep water (Sautter et al., 2007).

In the case of betta larviculture, water depths >8 cm cause mortality and elevated cost of feeding due to increasing fish movement required for capture of the prey and the need for larvae to realize the first air breathing (Kim, 2007). For these reasons, a combination of water depth and nematode availability in betta larviculture presents a hypothesis for elucidating the problem of using *P. redivivus* in larviculture of fish.

Thus, the aim of this study was to evaluate the use of *P. redivivus* and different water depths on the growth performance of *B. splendens* larvae.

2 | MATERIALS AND METHOD

The larvae were obtained from natural spawning according to the reproductive methodology described by Faria et al. (2006). After mouth opening and horizontal swimming, the larvae were fed with the *Paramecium* sp. protozoa four times a day (Budiardi et al., 2005). On the fifth day after hatching, the larvae were distributed to cylindrical tanks with a 5-L capacity in a static system without aeration. The water parameters of pH (YSI™ 60), temperature (YSI™ 60), dissolved oxygen (YSI™ 30) and electric conductivity (YSI™) were monitored daily. Total ammonia was measured at the end of experiment (Hanna™ HI 93715).

The animals were maintained at a density of 34 larvae/litre of water. The experiment was based on a completely randomized 3 × 2 factorial design, represented by two feed types and one alternating feeding regime plus two water depths (2.5 and 5.0 cm corresponding to water volume of 295 and 590 ml respectively) over a period of 7 days, with three replicates. The larvae were fed four times per day with: (a) 500 *P. redivivus* (NM/larvae/feed); (b) 200 *Artemia* (*Artemia* nauplii/larvae/feed); (c) alternating feeding regime with 500 (NM/larvae/feed) (Schlechtriem, Ricci, Focken, & Becker, 2004) and 200 (Nauplii/larvae/feed) (Fabregat et al., 2017) distributed alternately in two meal each. At the end of each day was carried out water exchange (20% of total volume).

The nematodes were acquired from local trade and then cultivated in oatmeal moistened with water and kept in an aerated container at environmental temperature according to De Lara, Castro, Castro, and Castro (2007) and filtered by a mesh (size 500 μm) before offered to larvae. The *Artemia* was acquired as an *Artemia* cyst (INVE) and offered to larvae as early hatched Nauplii. After the experimental period (15 days), all larvae were collected with the aid of pipette Pasteur, weighted on analytical balance (Marte) at 0.002 g precision and counted for determination of survival rate. Animals were collected and the zootechnical parameters of weight gain (WG), specific growth rate (SGR), and lot uniformity were determined according to previous studies (Furuya, de Souza, Furuya, Hayashi, & Ribeiro, 1998; Ramos, Abe, & Fujimoto, 2016).

Data obtained for water quality, zootechnical performance, and survival were analysed using the normality tests of Shapiro–Wilk and, for homoscedasticity, using the Bartlett test, and then subjected to two-way analysis of variance (ANOVA) and the Tukey post hoc test (5% probability) for the comparison of mean values.

3 | RESULTS

The feed type and water depth did not show any significant interaction ($p > 0.05$). The parameters of dissolved oxygen, temperature, and electrical conductivity of water remained constant independent of the feed used. However, the concentration of total ammonia was higher with feed exclusively using nematodes, and an increase in pH was observed with *Artemia* feed (Table 1).

The water depth did not influence the parameters of dissolved oxygen, temperature, and total ammonia. However, increased pH and electrical conductivity of water were observed at a water depth of 2.5 cm (Table 2).

As with the water quality parameters, there was no interaction observed among the feed types and water depths with regard to the parameters of survival and zootechnical performance, except for SGR (Table 3 and 4).

TABLE 2 Mean values of water quality parameters in larviculture of *Betta splendens* at different water depths

Water column	2.5 cm	5.0 cm
Total ammonia (mg/L)	1.14 ± 0.20 A	0.93 ± 0.10 A
Dissolved oxygen (mg/L)	4.55 ± 0.71 A	4.00 ± 0.47 A
Temperature (°C)	26.51 ± 0.13 A	26.80 ± 0.40 A
pH	7.59 ± 0.10 A	7.50 ± 0.20 B
Electric conductivity (μS)	302.57 ± 68.11 A	235.02 ± 50.00 B

Different letters in rows present significant difference 5% by the Tukey test.

TABLE 3 Mean values of zootechnical performance and survival in larviculture of *Betta splendens* using different feed types

Feed/ Performance	*Artemia*	Alternating feeding regime	*Panagrellus redivivus*
Weight gain (mg)	22.0 ± 2.0 A	19.0 ± 2.0 B	9.0 ± 3.0 C
Survival (%)	61.51 ± 15.00 A	60.37 ± 24.00 A	55.67 ± 14.70 A
Uniformity (%)	65.49 ± 7.80 A	57.33 A ± 22.30 A	52.65 ± 18.25 A

Different letters in rows present significant difference 5% by the Tukey test.

A highest weight gain was observed in fish receiving *Artemia* feed, followed by fish from alternating feeding regime treatment. The use of nematodes as live feed produced the lowest weight gain of the three treatments (Table 3, Figure 1). The parameters of survival and uniformity do not present significant differences between the feed types used.

With regard to the SGR parameter, an effect of water depth and feed type or strategy used was observed (Table 5). The alternating feeding regime at a water depth of 2.5 cm produced a similar SGR to fish fed with *Artemia* only. At a 5-cm water depth, an improvement in SGR was observed in feed containing *Artemia*; however, the similarity still remained between *Artemia* feed and the alternating feeding regime.

The larvae fed only with nematodes had a lower SGR compared with another treatment, independent of water depth (Table 5).

4 | DISCUSSION

The water quality parameters influence the survival and development of larvae. However, despite of variation in water quality, the parameters remained within the recommended range for betta fish culture according to Faria et al. (2006), independent of feed type or water depth used.

The higher value of total ammonia obtained with nematode feed may be explained by residue of oatmeal medium that was offered to larvae along with the nematodes; this was also reported by Martínez and Luengas (1998). This indicates that cleaning methods to remove the oatmeal medium should be developed to prevent the increase in total ammonia. In contrast, the lowest value of total ammonia was demonstrated with the *Artemia* treatment, possibly indicating

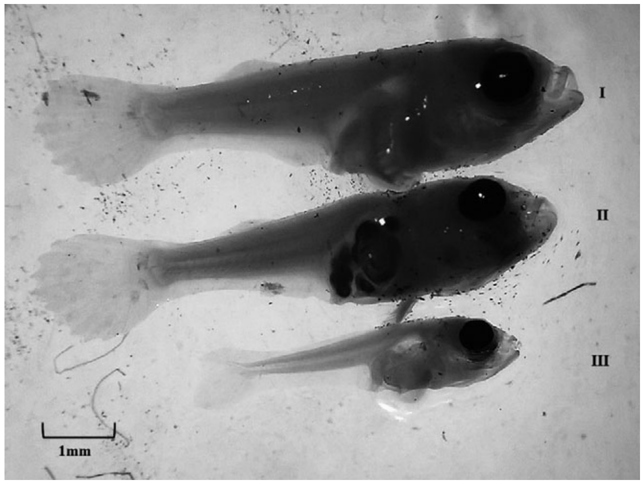

FIGURE 1 Development of larvae of *Betta splendens* at the end of the experiment: I. Larva fed exclusively with artemia; II. Larva fed with artemia and nematodes (Alternative feeding regime); III. Larva fed exclusively with nematodes

TABLE 4 Mean values of zootechnical performance and survival in larviculture of *Betta splendens* at different water depths

Water column/Performance	2.5 cm	5.0 cm
Weight gain (mg)	15.0 ± 6.0 B	18.0 ± 5.5 A
Survival (%)	58.97 ± 20.00 A	59.40 ± 15.00 A
Uniformity (%)	54.25 ± 17.30 A	62.75 ± 18.00 A

TABLE 5 Mean values of especific growth rate* in *Betta splendens* larviculture using different feed types and at different water depths

Feed/Water column	*Artemia*	Alternating feeding regime	*Panagrellus redivivus*
2.5 cm	12.80 ± 0.76 Ba	12.48 ± 0.75 Aa	6.91 ± 1.64 Bb
5.0 cm	14.19 ± 0.94 Aa	13.20 ± 0.54 Aa	9.79 ± 0.25 Ab

Different Lowercase letters in rows present significant difference 5% by Tukey test, and different uppercase letters in columns present significant difference 5% by Tukey test.
*Specific growth rate (%) = 100 (ln final weight – ln initial weight/time in days).

complete artêmia consumption which prevented the increase in total ammonia; an unexpected fact because of the excess of *Artemia* perishes in fresh water (Jomori, Luz, Takata, Fabregat, & Portella, 2013). The *Artemia* treatment also increased pH at a water depth of 2.5 cm, probably caused by remnants of salty water used for hatching *Artemia* nauplii, as reported by Kim (2007).

With regard to zootechnical performance, *Artemia* treatment produced the best results with the higher water depth, possibly due to higher feed availability throughout the column which increased the visual stimuli that are important to predation behaviour (Valentin et al., 2013). The improvement in larval growth with *Artemia* feed at a 5-cm water depth was also verified by Kim (2007).

With regard to nematode treatment, the decanting factor cited by Sautter et al. (2007) was not the limiting factor for growth of betta larvae since, regardless of water depth used, the poorest productive performance was observed with *P. redivivus* only treatment. Similar result was observed to larvaculture of *Hoplias malabaricus* with the poorest productive performance to the *P. redivivus* treatment (Pereira, Mendonça, Pellanda, Matielo, & Junior, 2015). This indicates that, despite the bromatological profile with high protein level, some other component not yet evaluated but theorized by Luna-Figueroa et al. (2010) (e.g., difficultly in digesting nematode cuticle) interferes with nutrient utilization.

The hypothesis of Luna-Figueroa et al. (2010) is plausible for though betta larvae complete morphological development of the digestive tract occurs 72 hours after hatching (Valentin et al., 2013), some digestive enzymes production yet increase during the betta larvae growth (Thongprajukaew, Kovitvadhi, Kovitvadhi, Engkagul, & Rungruangsak-Torrissen, 2013), indicating that the larvae do not have specific capacity for efficient digestion of nematodes or an enzyme would not yet be fully available at this stage of larval development. This difference in larval digestive capacity in relation to different feeds was observed in flounder (*Hippoglossus hippoglossus*) which possess better ability to digest copepod than *Artemia* (Luizi, Gara, Shields, & Bromage, 1999). Thus, in the case of betta larvae, the larvae were able to digest *Artemia*, but had difficulties in digesting nematodes, probably due to the lower amount or the lack of enzymes for digestion of nematode.

This feed digestion characteristic seems to be species specific as the nematode *P. redivivus* has already been successfully evaluated in larviculture of shrimps, being indicated as a partial replacement for *Artemia* (Martínez & Luengas, 1998). Some species present lower amount specific digestive enzymes or do not present them, altering its digestion ability (Kolkovski, 2001).

Nonetheless, the inclusion of nematodes in the alternating feeding regime produced similar values for SGR to those of larvae fed *Artemia* only, with both water depths. This finding demonstrates that, despite the difficulties involved in nematode digestion, there was an influence of *Artemia* feed which had a positive effect on SGR, probably reflected by the assist from live feed enzymes in the digestion process (Kolkovski, 2001).

Therefore the alternated feed strategy to betta larviculture was feasible promoting reductions costs. Similar alternating feeding regime has already been validated as demonstrated for betta larvae that were fed with rotifers, *Artemia* and algae (Puello-Cruz et al., 2010) reducing costs in comparison with exclusive use of *Artemia*.

New feed strategies that enable better performance with reduced costs are important for the development of betta larvae production. For this reason, the use of alternating feeding regime containing *Artemia* and *P. redivivus* represents a lower cost alternative compared to feeding only with *Artemia* nauplii.

5 | CONCLUSION

The alternating feeding regime with *Artemia* and nematode is the most appropriate feeding system for *B splendens* larviculture independent of the water depth used.

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