The effect of enriched Cladocera on growth, survivability and body coloration of Siamese fighting fish

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Abstract

Aim: This study investigated the effects of enriched *Moina micrura* on growth, survivability, and body coloration of *Betta splendens*.

Methodology: Four enriched *M. micrura* treatments, namely *M. micrura* enriched with yeast (T1), Palm kernel cake (PKC) (T2), egg yolk (T3), and *Chlorella* sp. (T4), were tested for best enrichment evaluation to *B. splendens* juveniles. Enrichment treatments were performed for 24-hr period and the feeding experiment was carried out for 30 days

Results: Fish fed with *Chlorella* sp. significantly exhibited the highest specific growth (2.80) and percentage survival (90.0) ($P=0.0003$; $P<0.05$). The coloration test on fish body, L^* value displayed significant impact of the enrichment types of *M. micrura* ($P=0.0004$, $P<0.05$), whereby T1 had blacker shade (44.60) than the other treatments. As for a^* value, a significant difference ($P=0.23$, $P<0.05$) was noted for T4 (2.65) that gave more intense red shade than other treatments. Treatment PKC (T2) significantly exerted a more yellowish shade (2.97) than the rest for b^* value ($P=0.02$, $P<0.05$). The proximate analysis of fishes in each treatment revealed that the protein and lipid content in T4 (92.22), (21.44) was higher than the other treatments.

Interpretation: The present study discovered that *M. micrura* enriched with *Chlorella* sp. emerged as the best enrichment method and has the potential to be an effective enrichment formula for the growth and survival of juvenile betta fish. This finding demonstrates that microalgae *Chlorella* sp. can be used to substitute other medium of enrichment for live food to facilitate enrichment procedures in attaining sustainable culture across the ornamental fish industries.

Key words: Body colouration, *Betta splendens*, *Chlorella* sp, *Moina micrura*, Specific growth

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Introduction

Live food has been used for freshwater fish fry production across the globe. A number of studies have highlighted the fact that live food is required to ascertain the development of fry production (Lim *et al.*, 2003; Ogata and Kurokura, 2012). *Artemia* sp. appears to be a suitable live feed that has been widely used for rearing *B. splendens* larvae due to its ease of culture preparation, when compared to other zooplanktons. *Moina micrura* has also been extensively utilised as live food in many commercial hatcheries including ornamental fish culture for fry production. Das *et al.*, (2012) demonstrated that *M. micrura* has advantages of high reproduction rates, wider temperature tolerance, as well as the ability to thrive on phytoplankton and organic wastes.

Betta splendens, or Siamese fighting fish, is a famous ornamental fish worldwide with its unique body shape and colour combination, wherein its main diet usually consists of zooplankton and small aquatic animal. Live feed, such as infusorian (a ciliated protozoan) has been typically used for the first feed of initially small mouth *B. splendens* larvae (Ogata and Kurokura, 2012). There have been some drawbacks on the betta larval rearing process. Sudrajat *et al.*, (2006) reported on problem with larval rearing in hatcheries where larval mortality occurred between days 2 and 28 that often resulted from rapid growth due to continuous feeding. A suitable live feed that balances the ingestion process must be identified to achieve normal growth and better survival of larvae.

In aquatic species, enhanced body coloration is determined by the type, quality and quantity of food consumed. For example, diet containing carotenoid pigment is given to molluscs (Canales *et al.*, 2010) and *Hibiscus sabdariffa* used in crustacean diets enhances their body colour pattern (Aréchiga *et al.*, 2015).

Despite *Artemia* sp. being regarded as the best typical live feed in many hatcheries, some of its drawbacks include high production cost, irregular quality, suboptimum nutrition (Puell-Cruz *et al.*, 2010). The use of other live feeds commonly growing in the natural environment has not been seriously considered. *Moina micrura*, that lives in the freshwater ecosystem possess the potential of substituting costly *Artemia* sp. in fish fry production (Shaleh *et al.*, 2009). Studies pertaining to *M. micrura* as bio-carriers of nutrients for fish-larviculture are scarce (Loh, 2011). Despite high nutritional content in *M. micrura*, they are insufficient for the optimum protein supply required for *B. splendens* larvae because the protein content of *M. micrura* only has an average content of 50% dry weight (Rottman *et al.*, 1992). Successful hatchery production of betta fish depends on the availability of suitable live feed, including *M. micrura* (Thongprajukaew *et al.*, 2011). In order to boost the protein content in *M. micrura*, some trials on live feed enrichment has to be ventured. In view of this, the present study was carried out to

determine the effects of enriched *M. micrura* on population production, growth, survivability, and coloration of ornamental fish *Betta splendens*.

Materials and Methods

Sample collection and cultivation: The stock culture of *M. micrura* was sampled from a waterway located at Kampong Padang Maras (5°25'15.9"N 103°02'55.5"E) and was kept aerated in hatchery at 25 ± 2 °C and pH 7.5 (Islam *et al.*, 2017). The water quality parameters such as salinity, pH and dissolved oxygen (DO) were monitored and maintained at optimum throughout the experimental period. The pH of the culture water was maintained in the range of 7-8, which was essential for the successful propagation of the culture (Rottman *et al.*, 1992). *M. micrura* stock culture was then upscaled into 500 l tanks and daily observed. Twenty percent of water was changed every three days to maintain the healthy culture of experiment. The number of individuals was recorded every two days to assess their growth performance.

Diet preparation and enrichment: *M. micrura* culture was closely monitored for egg reproduction and hatching of new neonates in 500 l culture beaker. *Moina macrura* from stocked culture were then harvested and placed into four fish culture tanks enriched with enrichment media for 24 hrs. The enrichment media used were microalgae *Chlorella* sp. at (Zaleha and Busra, 2012), egg yolk, palm kernel cake (PKC), and 0.5 g l⁻¹ baker yeast (control) (Paray *et al.*, 2016).

Fish and rearing condition: The experiment was conducted using 60-days-old *B. splendens* juveniles with red, yellow, and white as body primary colours. The fishes were randomly divided into four groups based on the enrichment medium with 30 fishes in each replicate. Four tanks were supplied with the designated enrichment treatments viz., *M. micrura* enriched with microalgae, *Chlorella* sp., egg yolk, PKC, and lastly, baker yeast that served as control (Table 3.1). Each tank was fed in 10: 1 ratio (10 individuals of *M. micrura* per fish twice a day throughout the 30-day experimental duration) (Islam *et al.*, 2017). Fishes were observed in terms of enhancement of body colour, survival rate and growth performance.

Growth and survival rates: The growth and survival aspects of fishes were observed once in every six days throughout the 30-day experimental period. Their specific growth rate and survivability were calculated by the formula given by Achionye-Nzeh *et al.* (2012):

Specific growth rate (SGR) % = $\log_e W_t - \log_e W_0 \times 100 / \text{Time (days)}$

Where, W_0 is the average initial weight at the start of the experiment; W_t is the average final weight at the end of the experiment, while time represents the total number of days the experiment been carried out.

$$\text{Survival (S) \%} = N_f \times 100 / N_0$$

Where, N_0 signifies the initial number, while N_f indicates the final number at the end of the experiment.

Proximate analysis: The weight of the samples were recorded. All samples were stored in a freezer to retain their freshness. The proximate composition of enriched *M. micrura* and *B. splendens* was performed following the standards of Association of Official Agricultural Chemists (AOAC) method for laboratory (2000), and the results were recorded as % per body weight.

Assessment of colour : The colour of each fish was assessed on the body of five randomly selected fishes in each replicate. Konica Minolta Chroma meter was used on the final day of feeding trials (day 30) and measurements were taken based on the colour system mode depicted in the International Commission on Illumination (CIE), where L^* = lightness, dark = 0, white = 100, a^* = red (positive values = red and negative values = green), and b^* = yellow (positive values = yellow and negative values = blue) (Yilmaz and Ergün, 2011).

Data analysis: Data were recorded and presented as mean $\pm$ standard deviation. The data collected throughout the experimental period were analysed by One-way analysis of variance (ANOVA). Comparing the aspects of comparison on growth, survival and body coloration was performed on different enriched diets. The variances were considered significant at $P < 0.05$ level. The post-hoc turkey test was conducted when the main treatment impact was found to be significant. Normality, homogeneity, and independence of data were determined to ensure eligibility for ANOVA testing.

Results and Discussion

Specific growth rate and survival rate of *B. splendens*: The fish equally accepted the four diets and the specific growth rate displayed by *B. splendens* for each treatment is tabulated in Table 4.1. The specific growth rate significantly differed among the treatments implemented ($P = 0.003$, $P < 0.05$). Fig. 4.1 show that *Chlorella* sp. (T4) (2.80) recorded the highest specific growth rate, when compared with other treatments. The lowest specific growth rate was exhibited by *B. splendens* fed with yeast control treatment (T1) (1.73). Egg yolk (T3) showed a significant difference between all treatments, except *Chlorella* sp. (T4). The specific growth of *B. splendens* juveniles relied on the range of enrichment methods for *M. micrura*.

The survival rate of *B. splendens* at the end of the experimental period is shown in Table 4.2. The highest survival rate for *B. splendens* was obtained with *Chlorella* sp. (90.00), accompanied by PKC (83.33), and yeast (73.33) that served as control. Meanwhile, the lowest survival rate of *B. splendens* was attained when fed on egg yolk (70.00). Fig. 4.2 illustrates the

significant variances among the treatments performed ($P = 0.0003$, $P < 0.05$).

Better survival and growth are essential factors in hatchery rearing at different stages, including fingerlings (Singh et al., 2019). In this present study, the diet of *M. micrura* enriched with *Chlorella* sp. resulted in the highest growth and survival rates amongst *B. splendens* juveniles. This is in accordance with Wang et al. (2008), who revealed that the larvae of loach fish *M. anguillicaudatus* successfully showed improved growth and survival rates on feeding live *Moina* sp. and *Daphnia* sp. enriched with *Chlorella* sp. as a continuous diet. In addition, *Clarius gariepinus* displayed significantly superior growth and survival rates after being fed with *M. micrura* co-feeding and a mixture of algae, including *Chlorella* sp. (Babalola, 2006). This highlights the fact that *M. micrura* enriched by *Chlorella* sp. can be used as a diet for juveniles and fish larvae rearing across all potential commercial fish species.

Both protein and lipid contents retrieved from the proximate analysis of *B. splendens* in T4 appeared to be the highest among all treatments; signifying that both specific growth

Table 3.1 : Feeding treatments

Treatment	Diets enrichment of <i>M. micrura</i>
T1	Yeast (control)
T2	Palm Kernel Cake (PKC)
T3	Egg yolk
T4	<i>Chlorella</i> sp.

Table 4.1: The specific growth rates of *B. splendens* for different enriched *M. micrura*. All values are presented in mean $\pm$ SD (n=3). The small letters indicate significant variances in the different treatments ($P < 0.05$).

Treatment	Specific growth rate (mean $\pm$ SD)
Yeast	1.73 $\pm$ 0.15 ^a
PKC	2.16 $\pm$ 0.27 ^a
Egg yolk	1.75 $\pm$ 0.29 ^{ab}
<i>Chlorella</i> sp.	2.80 $\pm$ 0.30 ^b

Table 4.2: The % survival (mean $\pm$ SD) of *B. splendens* for different enriched *M. micrura*

Treatment	% Survival (mean $\pm$ SD)
Yeast	73.33 $\pm$ 3.33 ^a
PKC	83.33 $\pm$ 3.33 ^a
Egg yolk	70.00 $\pm$ 3.33 ^b
<i>Chlorella</i> sp.	90.00 $\pm$ 3.33 ^b

All values are presented in mean $\pm$ SD (n=3). The small letters indicate no significant variance for the different treatments ($P < 0.05$).

Fig. 4.1 : Specific growth rates of *B. splendens* for different enriched *M. micrura*. Small letters indicate significant difference ($P<0.05$).

Fig. 4.2 : Survival rate of *B. splendens* for different enriched *M. micrura*. Small letters indicate significant differences ($P<0.05$).

Fig. 4.2.1 : Survival rate of *B. splendens* for different enriched *M. micrura* throughout the experimental duration (days).

and survival rates were influenced by protein and lipid intake from the diet. A diet with high protein and lipid contents is bound to maximise the growth performance and survivability of fish (Schulz et al., 2008). Similarly, Khani et al. (2017) discovered that dietary with the inclusion of *Chlorella* sp. increases the protein content of

albumin in liver, which has served as immune response in *Cyprinus carpio* leading to desired growth and survival. Meanwhile, Liang et al. (2005) and Zhou et al. (2005), reported that *Chlorella* sp. as the perfect enrichment methods for cladoceran of *Moina* sp., due to its EPA and DHA composition. Both EPA and DHA are essential fatty acids for a wide range of fish larvae to boost their growth and survival (Wang et al., 2008).

The present study also found that *M. micrura* enriched by PKC also showed good growth rates for *B. splendens*, in comparison to egg yolk and yeast (control) treatments. According to Hashim et al. (2012), Palm kernel cake (PKC) with its high protein and lipid contents has been used as one of the main ingredients for feed meal formulation in the aquaculture industry. The PKC enhanced the protein and lipid composition in *M. micrura* as a live feed for *B. splendens*, which increased the growth rates. This proves that the growth rates of *B. splendens* are affected by various enriched treatments of *M. micrura*.

Colouration

L* value : Table 4.3 shows the L* values of *B. splendens* that indicated lightness. The varied enrichment of *M. micrura* displayed significant difference on the L* values of fish body,

Table 4.3.1: L* of *B. splendens* fed on different enriched *M. micrura*

Treatment	L* (mean±SD)
Yeast	37.87±8.12 ^b
PKC	45.56±1.14 ^{ab}
Egg Yolk	45.07±4.40 ^{ab}
<i>Chlorella</i> sp.	52.36±2.12 ^a

All values are mean of five replicates ± SD. Small letters indicate no significant difference for different treatments ($P<0.05$)

Table 4.3.2: A* value of *B. splendens* fed on different enriched *M. micrura*

Treatment	A* value (mean ± SD)
Yeast	1.11±0.62 ^b
PKC	1.48±0.75 ^{ab}
Egg Yolk	1.63±0.90 ^{ab}
<i>Chlorella</i> sp.	2.65±0.56 ^a

All values are mean of five replicates ± SD. Small letters indicate significant differences for different treatments ($P>0.05$).

Table 4.3.3: B* value of *B. splendens* fed on different enriched *M. micrura*

Treatment	B* value (mean ± SD)
Yeast	0.94±0.91 ^a
PKC	2.97±1.33 ^a
Egg yolk	0.47±1.87 ^a
<i>Chlorella</i> sp.	0.60±0.40 ^b

Table 4.4.1: Proximate analysis of *B. splendens* fed on different enriched *M. micrura*

Treatment	Protein %	Lipid%	Ash%	Moisture%
Yeast	72.56±1.93 ^a	21.08±0.75 ^a	21.30±0.10 ^a	9.43±0.68 ^a
PKC	76.56±1.93 ^{ab}	21.38±0.39 ^b	16.20±0.47 ^b	7.06±0.13 ^a
Egg Yolk	68.89±1.93 ^{ab}	18.97±0.80 ^b	15.18±0.11 ^c	6.91±0.07 ^a
<i>Chlorella</i> sp.	81.22±1.93 ^b	21.44±0.13 ^b	13.13±0.70 ^d	6.63±0.10 ^b

All values are mean of three replicates ± SD. Small letters indicate significant differences for the different treatments ($P < 0.05$)

Table 4.4.2: Proximate analysis of *M. micrura* with different enrichments

Treatment	Protein%	Lipid%	Ash%	Moisture%
Yeast	50.02±2.01 ^a	0.84±0.38 ^a	7.14±0.38 ^a	17.59±0.21 ^a
PKC	54.32±0.62 ^a	2.07±1.02 ^{ab}	7.15±0.43 ^{ab}	18.98±0.95 ^a
Egg yolk	48.68±0.63 ^b	2.48±0.99 ^{ab}	7.33±0.47 ^{ab}	18.55±0.69 ^a
<i>Chlorella</i> sp.	56.55±1.35 ^c	3.05±0.99 ^b	6.22±0.77 ^b	17.60±0.38 ^a

All values are mean of three replicates ± SD. Small letters indicate significant differences for varying treatments ($P < 0.05$)

($P = 0.0004$, $P < 0.05$). *B. splendens* fed with *M. micrura* enriched with *Chlorella* sp. (45.98) revealed greater whitish shade than those enriched with PKC (45.56) and egg yolk (45.07). The lowest whitish appearance that exerted a darker shade on body colouration was obtained with yeast control treatment (44.61).

From the L^* value results, the lowest lightness value was recorded for yeast control treatment. The lowest value of lightness indicate that the colour of skin pigment enhanced due to black pigment called eumelanin that derives from melanin. Eumelanin, responsible for dark brown or black coloration, is associated with melanin for skin pigmentation in fishes (Ohlemiller et al., 2009). *M. micrura* enriched with yeast synthesised more melanin pigment, when compared to other enrichment treatments. In comparison, Reitan et al. (1994) revealed that rotifer fed with baker's yeast and lipid emulsion synthesised black pigment (melanin) on juvenile turbot. Roy et al. (1989) reported that yeast can synthesise melanin in various media, including in animal body. This shows that *M. micrura* enriched with yeast enhanced the eumelanin pigmentation in *B. splendens* to greater extent more than other types of enrichment.

A* value: The a^* value for each treatment of *B. splendens* fed on different enriched *M. micrura* is presented in Table 4.3.2. All treatments had positive values to signify the presence of red pigment on the fish body. A significant variance ($P = 0.23$, $P < 0.05$) was noted as *Chlorella* sp. had the most reddish shades (2.65), while control treatment had the least of it (1.11).

The a^* positive values on the fish body which signified enhanced red shade revealed improvement in red shade on the body of *B. splendens* fed with *M. micrura* enriched with *Chlorella* sp. as compared with other feeding treatments. The red shade

represents the presence of astaxanthin within the fish skin. Astaxanthin, a member of carotenoid pigments, has a huge commercial value in the aquaculture industry (Igielska et al., 2015). According to Mezzomo and Ferreira (2016), astaxanthin imparts red pigment to the aquatic organisms, as this pigment is not synthesised in animals, hence, should be included in the diets with live feed, in order to obtain a striking colour. In a similar study by Gouveia et al. (1996) found that *Chlorella* sp. appears to be a promising source of carotenoids can be effectively used as feed additive for salmonid rainbow trout diets, primarily due to its pigmentation efficacy that is comparable to that of synthetic pigments (astaxanthin and canthaxanthin). Additionally, freshwater microalgae (*Haematococcus pluvialis*, *Chlorella* sp. and *Dunaliella salina*) also have commercial function as feed additive for aquaculture, due to their rapid growth and high astaxanthin content that serve as a primary source of pigmentation in ornamental or tropical fish species (Gupta et al., 2007).

B* value: Based on Table 4.3.3, the positive b^* values of all fishes in the feeding treatments indicated the presence of yellowish colour on the body. Significant difference was noted ($P = 0.02$, $P < 0.05$). *B. splendens* fed with *M. micrura* enriched with PKC resulted in the highest presence of yellowish colour (2.97), whereas the egg yolk treatment (T3) gave the least yellowish shade (0.47).

The b^* value is indicated by varying values to portray blue (-) to yellow (+) shades. *B. splendens* fed with *M. micrura* enriched with PKC showed the highest value that represented yellowish skin pigment, which clearly indicates the presence of lutein xanthophyll pigment. Xanthophyll is an important pigment which plays a vital role in the content of yellow pigments in fish skin and

fillet. Inclusion of PKC in the diet of *M. micrura* seemed to enhance the presence of lutein xanthophyll pigment. Previously, Ping *et al.* (2006) revealed that the existence of lutein in palm-based products, including PKC, has a high commercial value in fish feed industries. Dendrinios *et al.* (1984) asserted that feeding efficiency and pigmentation of post-larval sole fish had improved after using different natural food colourings, including lutein from organic-based yellow corn, alfalfa, algae and palm kernel oil. Apart from that, the addition of calendula flower and palm-based products as natural sources of lutein has been acknowledged as a source of carotenoids in diets for goldfish (Sinha and Asimi, 2007; Villar Martínez *et al.*, 2013). Nevertheless, there is a further need to study the maximum use of lutein in diets, based on PKC to boost the carotenoid supply for aquaculture feeds.

Proximate analysis of *B. splendens*: Based on Table 4.4.1, the proximate analysis of fish samples revealed that both protein and lipid contents were significantly affected by the diets ($P=0.044$, 0.004 ; $P<0.05$). Enrichment using *Chlorella* sp. treatment recorded the highest protein (81.22%) and lipid (21.44%) contents. In addition, significant differences ($P=0.00035$, 0.0004 ; $P<0.05$) were noted for the highest ash and moisture contents in *B. splendens* with control treatment (yeast, T1), while the lowest ash and moisture contents were recorded in treatments using *Chlorella* sp. (T4).

Proximate analysis of *M. micrura*: Both protein and lipid contents in *M. micrura* significantly differed ($P=0.0002$, 0.012 , $P<0.05$) in all treatments (Table 4.4.2). The highest protein and lipid contents were recorded for *Chlorella* sp. (T4) treatment with protein (56.55%) and lipid (3.05%), respectively. The ash content also displayed a significant variance ($P=0.024$, $P<0.05$), as the highest ash content was found in *M. micrura* enriched by egg yolk (7.33%). However, the amount of moisture content in *M. micrura* had no substantial variation ($P=0.061$, $P>0.05$) among the dietary treatments.

Although the results of this study reveals that the growth, survival, and body coloration aspects of *B. splendens* were significantly affected by different enrichment of *M. micrura*, more research may be carried out to further understand the enrichment impact to boost the ornamental fish industries. The findings reported in this study may serve as a guideline for further investigate the development of an enrichment medium for *M. micrura* to improve the nutritional status of cultured organisms.

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