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Effects of *Pediastrum boryanum* and Dried Chlorella as Feeds on the Growth Performance and Carotenoid Content of the Fairy Shrimp *Branchinella thailandensis* (Branchiopoda, Anostraca)

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ABSTRACT. – In this study, fresh *Pediastrum boryanum* and dried *c*hlorella were chosen as alternative diets to feed the fairy shrimp *Branchinella thailandensis* instead of its most common, *Chlorella vulgaris*. In three separate trials, 5-day-old *B. thailandensis* was fed 6.8 x 10^6 cells mL^{-1} of fresh *C. vulgaris* (as a control), 6.8 x 10^6 cells mL^{-1} of fresh *P. boryanum*, and 3 mg of dry weight per individual of powdered chlorella for 15 days. Animals in each experiment were fed twice a day for 15 days with three replicates (n = 100 individuals per replicate). The results showed that the body length of *B. thailandensis*, which was fed fresh *P. boryanum* and dried *c*hlorella for 5 days, had increased more than the control. However, at the end of the experiment, *B. thailandensis* fed with dried chlorella had a longer body length than those fed the control or *P. boryanum* ($P > 0.05$). The fairy shrimp fed with *P. boryanum* for 15 days had the highest survival rate of 70.8%, followed by the control (70.0%) and dried *c*hlorella (28.3%) ($P < 0.05$). Additionally, *B. thailandensis* fed with *P. boryanum* had the highest protein content of 73.37%, compared to those fed with dried *c*hlorella (63.77%) and the control (54.14%) ($P < 0.05$). In contrast, the treatments fed with *P. boryanum* had significantly lower lipid and carbohydrate contents (3.21 and 11.29%) than those fed with the control (3.56, 26.88%) and the dried *c*hlorella (3.50, 26.53%) ($P < 0.05$). The highest total carotenoid content, however, was 380.19 g g^{-1} in *B. thailandensis* fed with dried chlorella, followed by *P. boryanum* (310.91 g g^{-1}) and the control (200.53 g g^{-1} dry weight) ($P < 0.05$). Therefore, it is possible to promote fresh *P. boryanum* as a substitute for feeding fairy shrimp. Nonetheless, it is advised to feed the shrimp that were reared for 5–10 days with dried chlorella when live algae are scarce. These results will aid the development of shrimp farming practices.

KEYWORDS: aquaculture, carotenoid, *Chlorella vulgaris*, freshwater, microalgal diets, survival rate

INTRODUCTION

Fairy shrimp (Class Branchiopoda, Order Anostraca) have been promoted as a good-quality live food source in aquaculture. Various culture techniques and nutrition for fairy shrimp have been developed (Dumont and Munuswamy, 1997; Ali and Dumont, 2001; Saengphan et al., 2005; Dararat et al., 2011, 2012; Saejung et al., 2011; Plodsomboon et al., 2012; Thaimuangphol and Sanoamuang, 2017; Thanakiattiwibun et al., 2017). Fairy shrimp can absorb and store carotenoids from foods. They ingest only microalgae and zooplankton whose sizes are less than 240 µm, which is smaller than their mouths, allowing them to absorb a variety of foods and obtain more nutrients. The type of food consumed by fairy shrimp depends on the composition of their habitats. The main food source of most fairy shrimp is green microalgae, which can be found in the stomach contents, such as *Chlorella* sp., *Monoraphidium* sp., *Cosmarium* sp., and *Pediastrum* sp. (Thaimuangphol and Sanoamuang, 2020). *Chlorella vulgaris* is not only widely used for aquaculture but is also known as the most common food to feed fairy shrimp and other zooplankton cultures because it is small in size, easy to ingest, easy to culture, has a cosmopolitan distribution, and has a high nutritional content (Dararat et al., 2011; Sriputhorn and Sanoamuang, 2011; Sornsupharp et al., 2013, 2015; Chaoruangrit et al., 2018; Saejung et al., 2018, 2021). Proximate analysis indicates that *Chlorella* sp. has high protein, carbohydrate, and lipid content (Agwa et al., 2013).

Three species of fairy shrimp have been reported to occur in freshwater temporary-water habitats in Thailand (Sanoamuang et al., 2000, 2002; Sanoamuang and Saengphan, 2006). Two of them, *Streptocephalus sirindhornae* Sanoamuang, Murugan, Weekers, and Dumont, 2000, and *Branchinella thailandensis* Sanoamuang, Saengphan, and Murugan, 2002, have been extensively cultured commercially in the country since 2004 (Chaoruangrit et al., 2017; Thaimuangphol and Sanoamuang, 2020; Thaimuangphol et al., 2022). They have been used to feed freshwater fish and shrimp, particularly ornamental fish, because their bodies contain high concentrations of protein and carotenoids (Dararat et al., 2012; Sornsupharp et al., 2015; Thaimuangphol et al., 2022). Even though *B. thailandensis* can feed on a wide range of algae, the green microalga *Chlorococcum humicola* and dried spirulina powder have been chosen as carotenoid sources to improve fairy shrimp farming techniques (Chaoruangrit et al., 2018).

Live feeds are essential for aquaculture production, particularly for freshly hatched larvae, which have a developing digestive system that lacks enzymes to aid absorption and cannot convert precursors to necessary nutrients (Kassim et al., 2014; Samat et al., 2020). Thus, a nutrient-rich diet as live feed is appropriate and crucial for culture because no artificial diet has sufficient nutrition for fish or crustacean larval species (Kassim et al., 2014; Samat et al., 2020). Zooplankton are known to be a good source of live food, and many types of zooplankton, such as rotifers, cladocerans, copepods, fairy shrimp, and nematodes, have been described as important food sources for fish and shellfish (Dhont et al., 2013; Gopakumar and Santhosi, 2009; Sriputhorn and Sanoamuang, 2011; Brüggemann, 2012).

On the other hand, finding an alternative alga for fairy shrimp and aquatic animal cultures remains critical. The genus *Pediastrum* belongs to the family Hydrodictyaceae and is mostly found in freshwater, especially in meso-eutrophic and eutrophic waters with high nutrient content (Prasertsin et al., 2014). It has a complex life cycle; the dominant form is the coenobia disc shape, and it can release individual single cells in an asexual life cycle (Park et al., 2014; Martinez-Goss et al., 2018). Previous research has shown that *Pediastrum* spp. can grow faster than other microalgae species and contain a higher concentration of protein and antioxidants (Lee et al., 2009). For these reasons, *Pediastrum* spp. could be a potential alternative food for fairy shrimp cultures. In Thailand, 14 species of *Pediastrum* have been recorded, and *Pediastrum boryanum* (Turpin) is one of the dominant species (Lewmanomont et al., 1995). Members of this genus can be found in freshwater habitats and are easy to culture on both small and medium scales. It is the most common algae in eutrophic environments and can be used when live feed production is in short supply.

In this study, local fresh *Pediastrum boryanum* and dried chlorella were chosen as experimental diets. Dried chlorella is a model diet for preserving algae and lowering costs in the aquaculture industry. The goal of this study is to find out how healthy dried chlorella and fresh *P. boryanum* are as food alternatives for growing the fairy shrimp *B. thailandensis* in laboratory cultures.

MATERIALS AND METHODS

Experimental algal diets

The green alga *Pediastrum boryanum* was collected by a 22 µm plankton net from a final treatment pond at Ubon Ratchathani University, Ubon Ratchathani Province, Thailand. *P. boryanum* colonies were isolated using the micromanipulator method (Hoshow and Rosowski, 1973; Andersen and Kawachi, 2005) and cultured in 100 mL of BG-11 medium with light at 3,000 lux, temperature at 26±2 °C, and a 24:0 light to dark period, and shaken every 3−4 hours. Every 24 hours, the algae concentration was counted and calculated until it reached a stationary phase, at which point it was transferred to a 15-L container of BG-11 medium. *Chlorella vulgaris* stock culture was obtained from the Department of Fisheries, Ubon Ratchathani Province, Thailand, and cultured in BG-11 medium. Both fresh and cultured algae were cultivated until reaching a stationary phase with a maximum concentration of $12 x 10^6$ cells mL^{-1}. Algae were harvested by filtering water out through a polypropylene filter (10 µm) and keeping it in a 4 °C refrigerator. Algal plates were kept in a glass jar and put in the refrigerator for HPLC analysis. Dried chlorella was purchased from the Now Foods company (http://www.nowfoods.com/) and ground with a mortar and pestle. At a concentration of $6.8 x 10^6$ cells mL^{-1}, the weight of dried chlorella was found to be the same as the weight of fresh *C. vulgaris*.

Experimental fairy shrimp

This study was conducted in accordance with the Thai Law on Animals for Scientific Purposes (2015), ethical approval code ID# 25/2563/IACUC of the Ubon Ratchathani University. One gram of dried *B. thailandensis* eggs was placed in a 100-mm net bag and submerged in 1.5 L of dechlorinated tap water at room temperature under natural light. When the nauplii hatched, they were moved carefully to a new container at a density of 30 individuals per liter. They were fed with fresh *C. vulgaris* until they were 5 days old.

The experimental diets consisted of *B. thailandensis* fed with three treatments: 6.8×10^6 cells mL^{-1} of fresh *C. vulgaris* (as a control group), 6.8×10^6 cells mL^{-1} of fresh *P. boryanum*, and 3.0 mg dry weight per individual of dried chlorella. For each treatment, 100 shrimp individuals were randomly selected and reared in a 5-L plastic container with 3.5 L of dechlorinated tap water, 24 hours of aeration, and a 12:12 light: dark condition. Animals in each experiment were fed twice a day for 15 days with three replicates (n = 100 individuals per replicate). The water in the experimental units was removed by 20% daily and replaced with freshly prepared water. Physical and chemical variables such as temperature, dissolved oxygen, nitrite, and ammonium were measured every 24 hours. Body length, growth rate, and survival rate of *B. thailandensis* in all experiments were measured every 5 days for 15 days.

Nutritional proximate analysis

All *B. thailandensis* samples from all experiments were analyzed according to the AOAC (2019) method. Protein contents were determined by the Kjeldahl method. Lipids were extracted by the Soxhlet method.

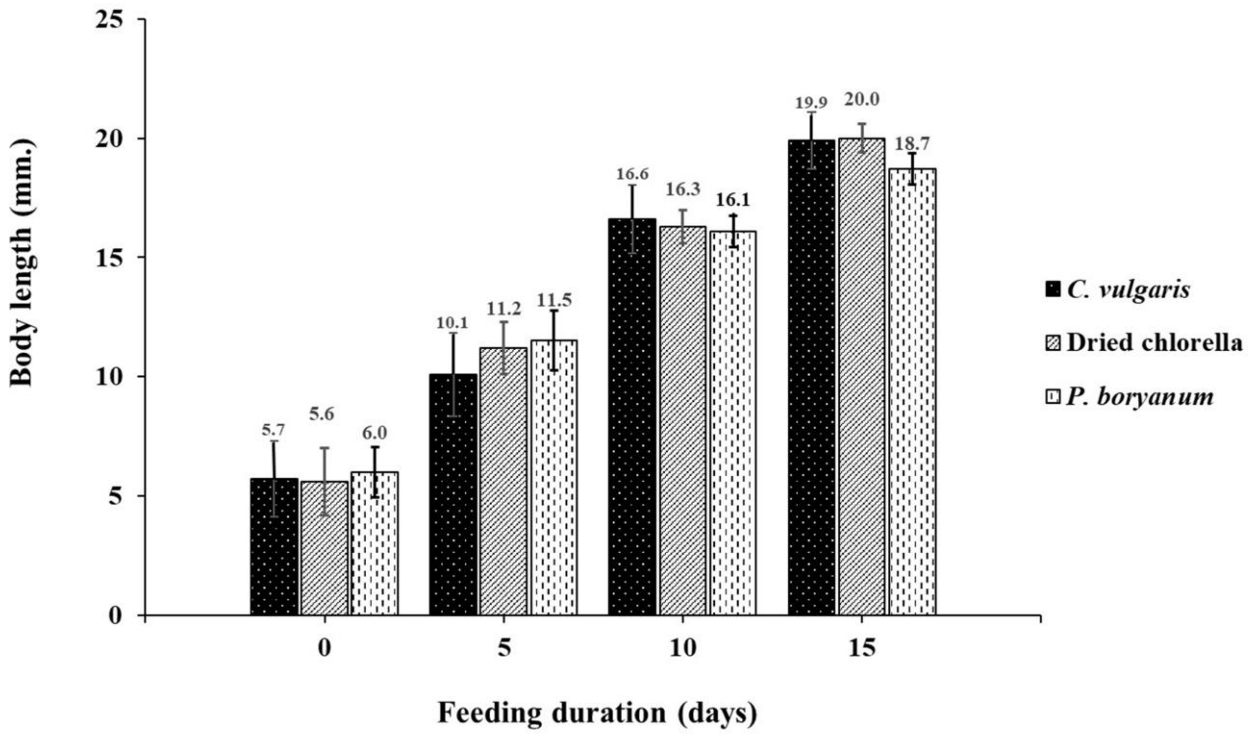

FIGURE 1. Body lengths of 5-day-old *Branchinella thailandensis* after being fed three different types of microalgal diets (*Chlorella vulgaris*, dried chlorella, and *Pediastrum boryanum*) for 5, 10, and 15 days.

Ashes were evaluated by incinerating the sample at 550 °C in a furnace and weighing it. Crude fibers were determined by acid hydrolysis with 1.25% sulfuric acid (H_2SO_4) for extraction of starch and sugar, and then alkaline hydrolysis with 1.25% sodium hydroxide (NaOH) for removal of protein. Carbohydrates were calculated by deducting biomass from fiber, fat, protein, and ash.

Carotenoid analysis

The quantitative analysis of total carotenoids in the algal diets and the fairy shrimp samples was done by the UV spectrophotometric method. The 10 g of sample was manually ground with a mortar and pestle, and then the carotenoid content was extracted by adding 10 mL of acetone and centrifuging at 25,000 rpm for 10 minutes. This process was repeated until the supernatant became colorless. The carotenoid standard (C4582) was obtained from Sigma-Aldrich. Both the standard and samples were measured using the Genesys 10S UV-Vis Spectrophotometer at 500 nm and calculated according to Strickland and Parsons (1972).

Individual carotenoid content was analyzed using high-performance liquid chromatography (HPLC). Carotenoid solutions from both algae and fairy shrimp were prepared and filtered through a 0.45-mm membrane. Samples were injected into the C-18 HPLC column. The mobile phases were acetonitrile, methanol, dichloromethane, and distilled water (79.9, 10, 10, and 0.1% v/v^{-1}, respectively). The flow rate of the mobile phase was 1 mL per minute and was detected at 452 nm. Retention time and peak area of each sample were compared to ß-carotene, astaxanthin, and canthaxanthin standards at the same conditions. Using a method created by Jeffrey et al. (1997; 10: pp. 449−559), the amount of carotenoid was determined.

Statistical analysis

All data in this study was expressed as the mean ± S.D. of three replicates and tested for normality and homogeneity of variance. A one-way ANOVA was performed to compare the body length of fairy shrimp and water quality among three different algae. Duncan’s new multiple range test was used to confirm significant differences in the means of each experiment. The threshold level for significant differences was set at $P \leq 0.05$.

RESULTS

Growth performance of fairy shrimp

The body lengths of 5-day-old *B. thailandensis* fed three microalgal diets for 15 days are provided in Figure 1. During the first 5 days of the experiment, the fairy shrimp fed with *P. boryanum* had a maximum body length of 11.5 ± 0.06 mm, followed by dried chlorella (11.2 ± 0.06 mm) and the control, fresh *C. vulgaris* (10.1 ± 0.05 mm). After 10 days, the body

TABLE 1. Survival percentage of *Branchinella thailandensis* that lived for 15 days after being fed three different types of microalgal diets when they were 5 days old.

Algal diet	Survival rate (%)		
	5 days	10 days	15 days
C. vulgaris	94.33±1.52[b]	82.33±3.05[b]	70.00±5.29[a]
Dried chlorella	81.67±0.58[c]	45.67±1.53[c]	28.33±0.57[b]
P. boryanum	96.67±0.58[a]	89.33±0.58[a]	70.83±0.57[a]

Values followed by different lowercase letter(s) in the same column are significantly different ($P < 0.05$).

TABLE 2. Water variables and nutrients (mean ± SE) in culture containers of 5-day-old *Branchinella thailandensis* fed with three microalgal diets for 15 days.

Algal diets	pH	DO ($mg\ L^{-1}$)	NO_2-N– ($mg\ L^{-1}$)	NH_3-N– ($mg\ L^{-1}$)
C. vulgaris	7.12 ± 0.06[a]	7.4 ± 0.66[a]	0.21 ± 0.08[a]	0.002 ± 0.00[b]
Dried chlorella	6.83 ± 0.10[b]	6.0 ± 0.95[a]	0.06 ± 0.04[b]	0.006 ± 0.00[a]
P. boryanum	6.77 ± 0.09[b]	7.0 ± 0.57[a]	0.24 ± 0.07[a]	0.001 ± 0.00[b]

Values followed by different lowercase letter(s) in the same column are significantly different ($P < 0.05$).

length of fairy shrimp fed with the control (16.6 ± 0.08 mm) was higher than that fed with dried chlorella (16.3 ± 0.07 mm) and *P. boryanum* (16.1 ± 0.08 mm). At the end of the experiment (day 15), the fairy shrimp fed with dried chlorella and *C. vulgaris* had longer body lengths (20.0 and 19.9 mm) than those fed with *P. boryanum* (18.7 mm). However, there were no significantly different body lengths between these treatments ($P > 0.05$).

During the first 5 days of the experiment, *B. thailandensis* (age 10 days) fed with *P. boryanum* and the control had significantly higher survival rates (96.6 and 94.3%) than those fed with dried chlorella (81.6%) ($P < 0.05$) (Table 1). After 10 days, the survival rates of all experiments decreased, particularly those fed with dried chlorella, which dropped dramatically, making them significantly different from the other experiments ($P < 0.05$). At the end of the feeding trial, the survival rates of *B. thailandensis* fed with *P. boryanum* had the highest survival rate of 70.8%, followed by those fed with the control (70.0%) and dried chlorella (28.3%). They were significantly different between those fed with *P. boryanum* and the control versus dried chlorella ($P < 0.05$).

Water quality variables in fairy shrimp cultures

The water quality variables and nutrients in culture containers of *B. thailandensis* fed with three microalgal diets for 15 days are provided in Table 2. The pH of all experiments was in the neutral range (6.77−7.12). The dissolved oxygen concentrations of the experiments were between 6.0 and 7.4 mg L^{-1}, and they were not significantly different from each other. The nitrite nitrogen and ammonia nitrogen concentrations ranged from 0.06-0.24 and 0.001-0.006 mg L^{-1}, respectively. The experiment fed with dried chlorella had the lowest concentration of nitrite nitrogen (0.06 mg L^{-1}), while this treatment had the highest concentration of ammonia nitrogen (0.006 mg L^{-1}), and they were significantly different from the other two treatments ($P < 0.05$).

Proximate analysis of fairy shrimp

The proximate analysis of 5-day-old *B. thailandensis* fed with three microalgal diets for 15 days is provided in Table 3. The fairy shrimp fed with *P. boryanum* and dried chlorella had significantly higher protein contents (73.37 and 63.77% dry weight) than those fed with the control (54.14% dry weight) ($P < 0.05$). In contrast, the treatments fed with *P. boryanum* had significantly lower lipid and carbohydrate contents (3.21 and 11.29%) than those fed with the control (3.56 and 26.88%) and the dried chlorella (3.50 and 26.53%) ($P < 0.05$). But *B. thailandensis* fed with the control had the highest fiber content (6.85%) compared to those fed with *P. boryanum* (5.39%) and dried chlorella (2.77%), and they were significantly different from each other ($P < 0.05$).

Carotenoid content in algae and fairy shrimp

Carotenoid content analysis of the three algal diets (Table 4) showed that the control treatment had 157.72 µg g^{-1} dry weight total carotenoid content, which was significantly higher than that of *P. boryanum* (130.43 µg g^{-1}) and dried chlorella (90.75 µg g^{-1}) ($P < 0.05$). ß-carotene (3.47 µg g^{-1}) was the major carotenoid in the control, while in *P. boryanum* and dried chlorella it

TABLE 3. Proximate analysis of 5-day-old *Branchinella thailandensis* fed with three different microalgal diets for 15 days.

Proximate composition	Algal diet (% dry weight)		
	C. vulgaris	**Dried chlorella**	***P. boryanum***
Moisture	96.03 ± 0.07[a]	95.50 ± 0.21[a]	94.93 ± 1.67[b]
Protein	54.14 ± 0.05[c]	63.77 ± 0.06[b]	73.37 ± 0.06[a]
Lipid	3.56 ± 0.03[a]	3.50 ± 0.02[a]	3.21 ± 0.02[b]
Carbohydrate	26.88 ± 0.23[a]	26.53 ± 0.05[a]	11.29 ±0.04[b]
Fiber	6.85 ± 0.08[a]	2.77 ± 0.05[c]	5.39 ± 0.04[b]
Ash	8.57 ± 0.04[a]	3.44 ± 0.06[c]	6.75 ± 0.02[b]

Values followed by different lowercase letter(s) in the same column are significantly different ($P < 0.05$).

TABLE 4. Total carotenoid content and individual carotenoid composition (mean value), which were found in three different algal diets and the 5-day-old *Branchinella thailandensis* fed with microalgal diets for 15 days.

Carotenoid content	Algal diets (µg g^{-1} dry weight)			*B. thailandensis* (µg g^{-1} dry weight)		
	C. vulgaris	**Dried chlorella**	***P. boryanum***	***C. vulgaris***	**Dried chlorella**	***P. boryanum***
ß-carotene	3.47 ± 0.04[a]	2.00 ± 0.04[b]	2.09 ± 0.03[b]	< 1	< 1	< 1
Astaxanthin	< 1.25	< 1.25	< 1.25	< 1.25	< 1.25	< 1.25
Canthaxanthin	0.81 ± 0.01[b]	2.65 ± 0.02[a]	2.68 ± 0.02[a]	6.16 ± 0.80[a]	3.03 ± 0.04[b]	1.52 ± 0.05[c]
Total carotenoids	157.52 ± 0.38[a]	90.75 ± 0.38[b]	130.43 ± 0.29[c]	200.53 ± 0.35[c]	380.19 ± 0.23[a]	310.91 ± 0.31[b]

Values followed by different lowercase letter(s) in the same column are significantly different ($P < 0.05$).

was canthaxanthin (2.68 µg g^{-1} and 2.65 µg g^{-1}). Unfortunately, astaxanthin was only found in very small amounts (<1.25 µg g^{-1}) in all three algal diets.

The carotenoid compositions of *B. thailandensis* fed with three algal diets for 15 days are presented in Table 4. The total carotenoids in *B. thailandensis* fed with dried chlorella were significantly higher (380.19 µg g^{-1} dry weight) than those fed with *P. boryanum* (310.91 µg g^{-1} dry weight) and the control (200.53 µg g^{-1} dry weight) ($P < 0.05$). On the other hand, the fairy shrimp fed the control alga had significantly higher canthaxanthin (6.16 µg g^{-1}) than those fed dried chlorella (3.03 µg g^{-1}) and *P. boryanum* (1.52 µg g^{-1}) ($P < 0.05$). The ß-carotene and astaxanthin content in the shrimp was found in very small amounts, similar to those in the algae.

The body color of *B. thailandensis* males and females fed with three algal diets for 15 days is presented in Figure 2. Both male and female body colors are related to their total carotenoid content. The total amount of carotenoids was highest in *B. thailandensis* that had been fed dried chlorella. Following this were the control and *B. thailandensis* that had received *P. boryanum* as food. Thus, the body colors of those fed dried chlorella, *P. boryanum*, and the control were deepest (Fig. 2c), lighter (Fig. 2b), and pale orange (Fig. 2a).

DISCUSSION

The present study demonstrated that *P. boryanum* can be used as a suitable algal diet for *B. thailandensis* based on their growth performance. Although the fairy shrimp fed with *P. boryanum* had a longer body length at the beginning of their growth (Fig. 1), after 10–15 days the body length increased at a slower rate than those fed with dried chlorella and the control *C. vulgaris.* In addition, *B. thailandensis* fed with *P. boryanum* and the control had higher survival rates than those fed with dried chlorella. A similar study by Chaoruangrit et al. (2018) has shown that *B. thailandensis* can be fed with other two green microalgae (*Chlorococcum humicola* and dried spirulina) in laboratory cultures, as evident by an increase in body length and survival percentage. The suitability of their experimental algae as two appropriate fairy shrimp diets is due to their small cell sizes, which are less than 10 µm. The cell size of our *P.*

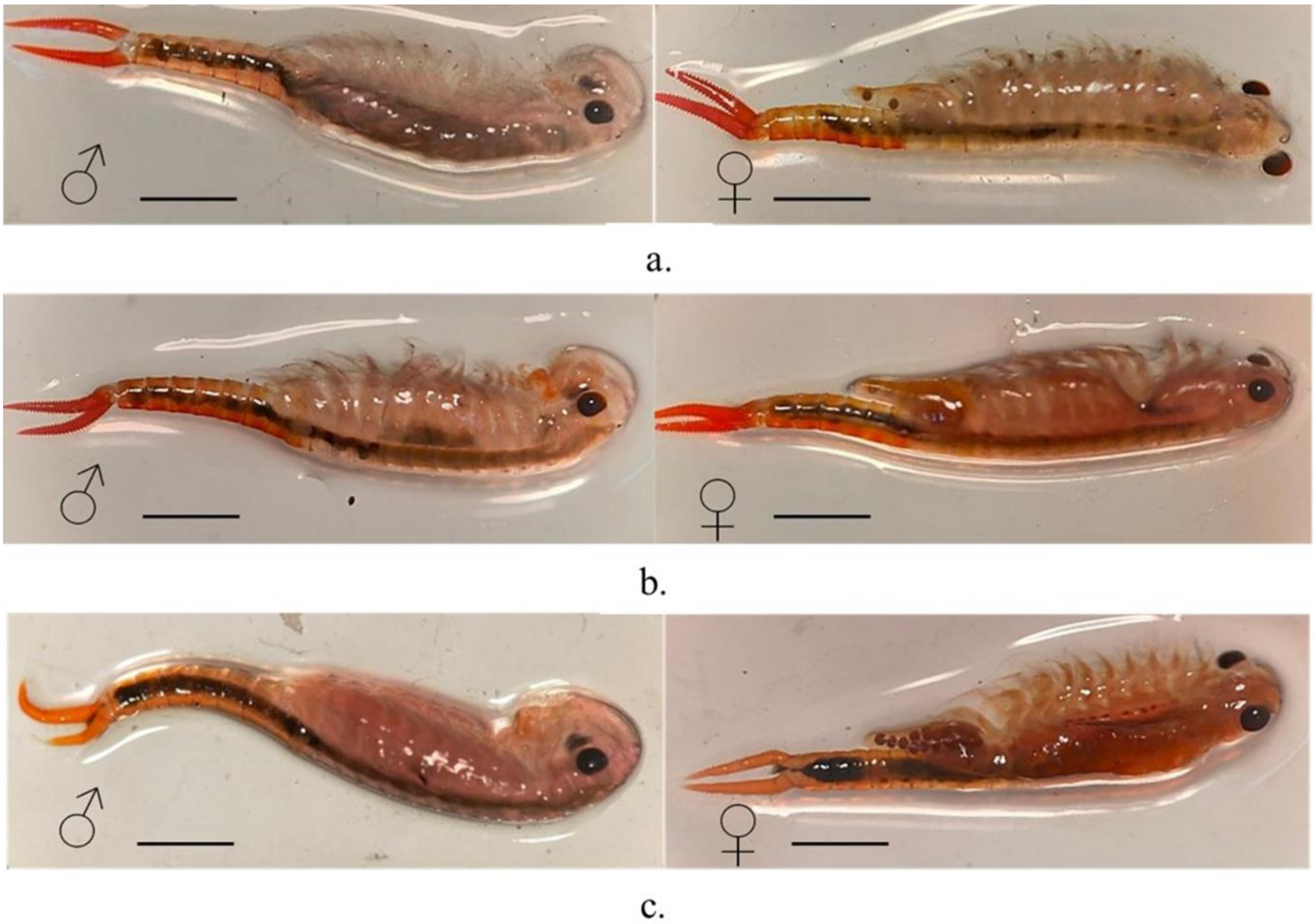

FIGURE 2. Body colors of *Branchinella thailandensis* males and females that lived for 15 days after being fed three different types of microalgal diets when they were 5 days old: (a) *Chlorella vulgaris*, (b) *Pediastrum boryanum*, and (c) dried chlorella (scale bar = 3 mm).

boryanum is 16 μm in diameter, which is also a suitable food size to feed this fairy shrimp species. Another study has demonstrated that the suitable size of microalgae to feed the brine shrimp *Artemia* sp. is ranged between 7 and 28 μm, and the optimum size is 16 μm (Turcihan et al., 2021). Additionally, not only the size but also the nutritional values of microalgae play an important role in growth (Guedes and Malcata, 2012). Dried chlorella has a level of protein of 15–45%, fat (5–20%), carbohydrate (30–36%), and ash (3–5%) (International Patent, 2017). In comparison with fresh *Chlorella* sp., which has protein of 56.0%, fat of 10.3%, carbohydrate of 13.7%, and ash of 1.5% (Agwa et al., 2013).

At the end of the experiment, *B. thailandensis* fed with dried chlorella had very low survival rates of 28.33%, while those fed with *P. boryanum* and the control had relatively high rates (70.83 and 70.00%). This may be due to the survival rate being related to the water quality of the shrimp cultures, especially ammonia-nitrogen concentrations, and the quality of the diet (Thurston et al., 1981; Ankley et al., 1995; Arauzo, 2003). Despite the fact that our shrimp fed with dried chlorella had relatively high growth, the survival rate was low. One of the main issues with dried algae was the ammonification process, which degraded the algal powder and released ammonia into the water. The treatment fed with dried chlorella had the highest unionized ammonia of 0.006 mg L^{-1}. This might be a reason why fairy shrimp fed with dried chlorella have the lowest survival rate, although this value was not higher than the standard concentration of ammonia for aquaculture (Saengphan et al., 2005).

The studied *B. thailandensis* fed with *P. boryanum* and dried chlorella treatments contained higher protein (73.37 and 63.77%) than that fed with the control (54.14%). Our results suggest that the experimental shrimp fed with suitable diets had rapid protein synthesis during their growth. Similarly, *B. thailandensis* fed with *Chlorococcum humicola* for 15 days had a comparable protein content (72.71%) (Chaoruangrit et al., 2018). On the other hand, *B. thailandensis* fed with dried chlorella (this study) and dried spirulina (Chaoruangrit et al., 2018) had lesser protein contents of 63.77 and 53.38%, respectively.

The results of both studies confirm that feeding fairy shrimp with dried algae is less suitable than feeding them with fresh algae (Sriputhorn and Sanoamuang, 2007). The low lipid and carbohydrate content but high protein content in the *P. boryanum*-fed shrimp corresponds with that reported in the *C. humicola*-fed shrimp (Chaoruangrit et al., 2018).

The control algae, *C. vulgaris*, had the most total carotenoids (157.52 g g^{-1} dry weight) of the three algae used in this study. However, after *B. thailandensis* fed the algae, the shrimp only had 200.53 g g^{-1} dry weight of total carotenoids. Similarly, dried chlorella had the lowest total carotenoids (90.75 µg g^{-1}) but after feeding by the shrimp, the highest value of total carotenoids (380.19 µg g^{-1}) was examined in the shrimp. The possible explanation could relate to the capability of carotenoid accumulation, uptake, and deposition mechanisms among different species of crustaceans (Su et al., 2018). Our results indicate that *B. thailandensis* fed with dried chlorella may be able to uptake and accumulate total carotenoids better than those fed with the other diets. The amounts of total carotenoids examined in *B. thailandensis* fed with fresh *C. vulgaris* in this study (200.53 µg g^{-1} dry weight) are comparable to those found in *B. thailandensis* fed with *Chlorella* sp. (254.41 µg g^{-1} dry weight) in the study by Dararat et al., 2012.

In this study, differences in dominant carotenoid compositions in the experimental algal diets are distinguished. ß-carotene was the major carotenoid in the control, while canthaxanthin was the major in *P. boryanum* and dried chlorella. This dominant canthaxanthin profile is similar to that of the fairy shrimp, *Streptocephalus dichotomus* (Velu and Munuswamy, 2007). The ß-carotene content of the three algae was directly proportional to the value of total carotenoids (Rhodes, 2007; Paniagua-Michel et al., 2012). According to earlier studies, fairy shrimp are capable of converting ingested β-carotene into canthaxanthin. This is related to the increase of canthaxanthin content simultaneously with the decrease of β-carotene (Nelis et al. 1988).

Our experimental fairy shrimp had different amounts of carotenoids, and the color of the shrimp was mostly due to the amounts of carotenoids in the abdominal muscle and shell (Su et al., 2018). The fairy shrimp can convert carotenoids into their bodies and absorb them. The fairy shrimp fed with dried chlorella had the highest total carotenoids, so their body color was the darkest orange (Fig. 2c), although it is hard to distinguish this color difference.

In conclusion, the green alga *P. boryanum* can be developed as a novel form of feed suitable for *B. thailandensis* cultures. However, when live algae are in short supply, it is advisable to feed fairy shrimp that are 5 to 10 days old with dried chlorella. The advancement of shrimp farming techniques will benefit from these findings.

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