


Alternative microalgal diets for cultivation of the fairy shrimp *Branchinella thailandensis* (Branchiopoda: Anostraca)

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Abstract Fairy shrimp is known as a nutritional food for fish and crustaceans in aquaculture. In most hatcheries, the microalga *Chlorella* sp. appears to be the most common, suitable, and nutritious food to feed fairy shrimp. In this study, we attempted to determine other alternative algal diets for cultivation of fairy shrimp *Branchinella thailandensis*. Seven experimental diets including three treatments of dried *Spirulina* sp. at 0.75 (S1), 1.5 (S2), and 3.0 mg dry weight individual⁻¹ (S3); three treatments of *Chlorococcum humicola* at 5×10^5 (Ch1), 1×10^6 (Ch2), and 2×10^6 cells mL⁻¹ (Ch3); and a control diet (*Chlorella vulgaris* at 1×10^6 cells mL⁻¹) were fed to 5-day-old shrimp for 15 days. Evaluation of growth performance, egg production, survival percentage, and nutritional and carotenoid content of the experimental fairy shrimp revealed that Ch3 is the most suitable algal diet. Our results suggest that *C. humicola* is the best alternative food source for the cultivation of *B. thailandensis*. In addition, dried *Spirulina* powder is also a good choice when live algae are not available and can be used as an alternative feed in fairy shrimp cultures.

Keywords Astaxanthin · Canthaxanthin · Carotenoid · *Chlorella vulgaris* · *Chlorococcum humicola* · *Spirulina* sp.

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Introduction

In tropical Southeast Asia, fairy shrimp (Class Branchiopoda, Order Anostraca) usually spend their entire lives in seasonal wetlands such as rain pools, temporary ponds, water buffalo wallows, and rice paddies (Sanoamuang et al. 2000a; Rogers et al. 2013). Fairy shrimp can withstand long periods of desiccation and can remain as encapsulated embryos, totally dehydrated, and inactive for as long as at least 1 year in the dry mud on the bottom of pools. These dried eggs require water to hatch usually from the next year rain (Brendonck et al. 2008). Most fairy shrimps are non-selective filter feeders, collecting algal and detrital foods using their thoracic limbs (Brendonck 1993).

Currently, there are three known species of fairy shrimp in Thailand. The most common and widely distributed species in the country is *Streptocephalus sirindhornae* Sanoamuang, Murugan, Weekers, and Dumont (Sanoamuang et al. 2000b). This taxon has been recorded also in Laos, Cambodia (Rogers et al. 2013) and Yunnan and Guangxi provinces of China (Shu et al. 2015). *Branchinella thailandensis* Sanoamuang, Saengphan, and Murugan is less common and has so far been recorded in the northeast, north, and central regions (Sanoamuang et al. 2002; Sanoamuang unpublished data). On the other hand, *S. siamensis* Sanoamuang and Saengphan is extremely rare and has been recorded only in five localities in Kanchanaburi and Suphan Buri provinces (Sanoamuang and Saengphan 2006). Recent studies revealed that fairy shrimp has replaced *Artemia* sp. as the most common live feed for aquacultural hatcheries. *B. thailandensis* has rapid growth, large size (male 26.2, female 27.8 mm), high fecundity, early maturity, short life span, and high nutritional composition (Dararat et al. 2011). Regarding carotenoids, *B. thailandensis* has higher carotenoid content than *S. sirindhornae* and *S. siamensis* (Dararat et al. 2012). Use of adult fairy shrimp *S. sirindhornae* as live and dried feed has demonstrated improvement in growth and carotenoid contents of the giant freshwater prawn *Macrobrachium rosenbergii* (Sriputhorn and Sanoamuang 2011) and flowerhorn cichlid fish (Sornsupharph et al. 2015). Similarly, *S. sirindhornae* nauplii can be used as a nutritionally adequate food for *M. rosenbergii* postlarvae (Sornsupharph et al. 2013). This evidence hence illustrated the potential uses of fairy shrimp as a high quality diet for aquaculture.

Since 2005, attempts had been made to study several biological and biochemical aspects of *S. sirindhornae* and *B. thailandensis* in laboratory cultures using green alga *Chlorella* sp. as their main foods (Saengphan et al. 2005; Boonmak et al. 2007; Plodsomboon and Sanoamuang 2007; Saengphan and Sanoamuang 2009; Dararat et al. 2011, 2012; Saejung et al. 2011, 2014a, b; Sriputhorn and Sanoamuang 2007, 2011; Plodsomboon et al. 2012; Sornsupharph et al. 2013, 2015). Although *B. thailandensis* can be fed with other types of food such as rice bran, *Spirulina* powder, and yeast, green alga *Chlorella* sp. seem to be the most common, suitable, and nutritious food for the shrimps (Saengphan et al. 2005; Sriputhorn and Sanoamuang 2007; Saengphan and Sanoamuang 2009). There has been interest in finding more nutritious and practical foods to feed the fairy shrimps in laboratory cultures. In this study, dried *Spirulina* powder and the green microalga *Chlorococcum humicola* were selected as alternative diets for *B. thailandensis*. Since dried *Spirulina* sp. containing high protein, essential amino acids, fatty acids, and carotenoid pigments (Soeder 1980; Saleha et al. 2011) is commercially available, it can be applied when live feed production is undersupplied. In addition, *C. humicola* has a high concentration of six major carotenoids including violaxanthin, astaxanthin, lutein, zeaxanthin, α -carotene, and β -carotene (Sivathanu and Palaniswamy 2012; Yuan et al. 2002). Thus, the aims of the present study were to determine the appropriateness of *Spirulina* powder and *C. humicola* as alternative foods for the cultivation of *B. thailandensis* in laboratory cultures.

We evaluated the growth, egg production, survival percentages, and nutritional and carotenoid contents of the fairy shrimp under laboratory conditions.

Materials and methods

Experimental algal diets

Three species of microalgae, *Chlorococcum humicola*, dried *Spirulina* sp., and a control *Chlorella vulgaris*, were used for this experiment. Stock cultures of *C. humicola* (TISTR 8461) and *C. vulgaris* were obtained from the Center of Excellence for Marine Biotechnology, Chulalongkorn University and the Thailand Institute of Scientific and Technological Research (TISTR). Both live algae were cultured in BG11 medium (Stanier et al. 1971) with continuous aeration and illumination at 3000 lx (light/dark, 24:0) at 25–28 °C. When cell concentration reached 5×10^6 cells mL⁻¹ for *C. humicola* and 12×10^6 cells mL⁻¹ for *C. vulgaris*, the algae were harvested and diluted to three concentrations, i.e., 5×10^5 , 1×10^6 , and 2×10^6 cells mL⁻¹ prior to use. The experimental system with each constant concentration of the algae was maintained throughout the experiment. Biomass and relationship of dry weight and algal cell concentration were determined by filtering known volumes of algal cells through a pre-weight GF/C filter, dried at 60 °C overnight in an oven and measured using a four-decimal analytical balance (Sartorius BP 210S). *Spirulina* powder was purchased from the Green Diamond Co., Ltd., Thailand. For each *Spirulina* powder treatment, *Spirulina* powder was weighted and an average dry weight was calculated from three replicates.

Experimental animals

Eggs of *B. thailandensis* were hatched and the nauplii were fed with *C. vulgaris* at 1×10^6 cells mL⁻¹ following the method described by Saengphan et al. (2005) until the animals reached 5 days old (average initial body length of 10.57 ± 0.33 mm, $n = 35$). For each treatment, ten individuals (five males and five females) of the shrimp were randomly selected and reared in a 1.5-L plastic container containing 1 L dechlorinated tap water (conductivity 288–462 $\mu\text{S cm}^{-1}$, temperature 29–30 °C, and pH 7.0–7.3) with slight aeration. The experiment consisted of a control treatment (*B. thailandensis* fed with 1×10^6 cells mL⁻¹ *C. vulgaris*, according to the examination by Saengphan and Sanoamuang 2009) plus six treatments including *B. thailandensis* fed with dried *Spirulina* sp. at 0.75 (S1), 1.5 (S2), 3.0 mg dry weight individual⁻¹ (S3) and with *C. humicola* at 5×10^5 (Ch1), 1×10^6 (Ch2), 2×10^6 cells mL⁻¹ (Ch3). The algal foods were fed to the shrimp twice a day for 15 days with three replicates for both the control and treatments. Dissolved oxygen was measured using a Hanna Oxy-Check HI 9147 DO meter. Nitrite, unionized ammonia, and total ammonia nitrogen concentrations were determined by spectrophotometric methods according to Strickland and Parsons (1972) and Bower and Holm-Hansen (1980), respectively. Water in the experimental units was removed daily and replaced with freshly prepared water. Total body lengths (from head to ceracopod) of the shrimp were measured by a Vernier Caliper every 5 days. Survival rates were determined at day 15. All deposited eggs from five females in each treatment were collected and counted daily until day 15 to determine egg production. Finally, the fairy shrimps were reared for mass cultures for 20 days at the optimal concentrations of feeding for each alga (Ch3, S3, and the control), and they were collected for nutritional proximate and carotenoid analyses.

Nutritional proximate analysis

Proximate analysis of algae and *B. thailandensis* samples were determined according to the official standard methods of AOAC (AOAC 2012). To determine moisture content, samples were dried at 125 °C using a hot air oven until constant weight and the moisture-free dried weight was compared with the initial dry weight. Crude protein ($N \times 6.25$) was analyzed using acid digestion by Kjeldahl methods. Crude lipid was analyzed using a Soxhlet extractor. For ash analysis, samples were dried in the crucible for 5 h at 550 °C and cooled in a desiccator; ash weight was a subtract of weight before and after furnace. Carbohydrate was estimated by subtracting the biomass with moisture, crude protein, crude lipid, and ash.

Carotenoid analysis

The algal and fairy shrimp samples were analyzed for carotenoid contents using high-performance liquid chromatography (HPLC). Three replicates of the samples were weighed and extracted by grinding with 1 mL absolute methanol in a 1.5-mL Eppendorf tube following by centrifuge at 5000 rpm 15 min for three times. Supernatant was collected and made up the final volume of 5 mL using methanol. All extraction procedures were done in a low light condition and kept cool in ice baths. Total carotenoids were measured at 452 nm using a spectrophotometer (Genesys 10 UV scanning thermo spectronic). Carotenoid composition was analyzed using a Nova-Pack C18 HPLC column (3.9×150 mm $4 \mu\text{m}$, Waters, Ireland) and detected at 452 nm using a Waters 487 UV-Vis detector. The isocratic mobile phase was acetonitrile, dichloromethane, methanol, and water (79.9, 10, 10, 0.1% v/v^{-1} , respectively). Chromatography was performed in reverse phase at 1.0 mL min^{-1} flow rate with $25 \mu\text{L}$ injection volume. Peaks of carotenoids were identified by retention time of carotenoid standards. Total carotenoids and carotenoid composition were calculated according to Strickland and Parsons (1972) and Jeffrey et al. (1997).

Statistical analysis

The variance of the data is given as a standard error (S.E.) of the mean of three replicates. Data was analyzed using one-way analysis of variance (ANOVA). Significant differences at $P < 0.05$ were discovered by Duncan's new multiple range tests.

Results

Growth performance of fairy shrimp

The body lengths of *B. thailandensis* fed with the experimental algae for 15 days are shown in Table 1. There was a significant difference in body lengths at the time of the experiment ($P < 0.05$). The body lengths of the shrimp fed diets with *Ch3*, *S3*, and *Ch2* were significantly longer than that of the control, *Ch1*, *S1*, and *S2* during the entire experiment. At the end of the experiment, the longest body length of 22.62 ± 0.51 mm was examined in the shrimp fed with *Ch3* diet.

Survival percentage and total numbers of eggs (per female for whole life span) of the shrimp fed with the seven algal treatments are presented in Table 2. At the end of the feeding trial, survival rates of the shrimp fed with the seven algal diets ranged from 36.67 to 70.00%.

Table 1 Body lengths (mean ± standard error) of 5-day-old *Branchinella thailandensis* fed with seven treatments of algal diets for 15 days

Algal diets	Body length (mm)		
	Day 5	Day 10	Day 15
Control	17.13 ± 0.30e	18.24 ± 0.18cd	19.13 ± 0.23c
<i>S1</i>	14.41 ± 0.34ab	15.67 ± 0.22a	15.75 ± 0.16a
<i>S2</i>	16.00 ± 0.15cd	17.76 ± 0.67bc	18.11 ± 0.30bc
<i>S3</i>	16.27 ± 0.28de	19.13 ± 0.35d	20.51 ± 0.50d
<i>Ch1</i>	13.73 ± 0.59a	16.80 ± 0.35b	17.60 ± 0.49b
<i>Ch2</i>	15.22 ± 0.05bc	18.82 ± 0.20cd	20.51 ± 0.08d
<i>Ch3</i>	15.60 ± 0.12cd	21.13 ± 0.18e	22.62 ± 0.51e

Chlorella vulgaris at 1×10^6 cells mL⁻¹ (control); *Spirulina* powder at 0.75, 1.5, and 3.0 mg dry weight individual⁻¹ (*S1*, *S2*, and *S3*, respectively); and *Chlorococcum humicola* at 5×10^5 , 1×10^6 , and 2×10^6 cells mL⁻¹ (*Ch1*, *Ch2*, and *Ch3*, respectively). Values followed by different lowercase letter(s) in the same column are significantly different ($P < 0.05$)

The shrimp fed with the *Ch3* diet had the highest survival rate of 70.00%, which was significantly different from that fed with the control (53.33%), the *S3* (56.67%), and the *Ch1* (36.67) ($P < 0.05$). Comparative data showed that at day 15, the shrimp fed with the *Ch3* diet had the highest egg production of 2954 ± 28.42 eggs, which was significantly different from that fed with the control (1405 ± 7.81 eggs) and the other treatments ($P < 0.05$).

Water quality data of the fairy shrimp cultures fed with different algal concentrations are presented in Table 3. The total ammonia nitrogen and unionized ammonia concentrations recorded from the dried *Spirulina* treatments (*S2* and *S3*) were significantly higher than that of the control, *Ch1*, *Ch2*, *Ch3*, and *S1* ($P < 0.05$). Dissolved oxygen in the treatments fed with *S2* and *S3* diets were significantly lower than that of the control, *Ch1*, *Ch2*, *Ch3*, and *S1* ($P < 0.05$). The other parameters of the six algal treatments showed no statistical difference with the control diet.

Proximate analysis of algae and fairy shrimp

Comparisons of the proximate compositions of the three algal diets and the fairy shrimp fed with the optimal concentrations of the three algal diets are given in Table 4. The dried

Table 2 Survival percentage (mean ± standard error) and total number of eggs (per female for whole life span) of 5-day-old *Branchinella thailandensis* fed with seven treatments of algal diets for 15 days

Algal diets	Survival rate (%)			Total number of eggs per female
	Day 5	Day 10	Day 15	
Control	86.67 ± 3.33b	76.67 ± 3.33bc	53.33 ± 3.33b	1405 ± 7.81d
<i>S1</i>	83.33 ± 3.33ab	76.67 ± 3.33bc	66.67 ± 3.33de	160 ± 6.43a
<i>S2</i>	76.67 ± 3.33a	70.00 ± 2.89b	60.00 ± 2.87bcd	1175 ± 16.25c
<i>S3</i>	76.67 ± 3.33a	73.33 ± 3.33b	56.67 ± 3.33bc	1597 ± 15.45e
<i>Ch1</i>	90.00 ± 0.00b	60.00 ± 2.89a	36.67 ± 1.67a	900 ± 15.01b
<i>Ch2</i>	100 ± 0.00c	83.33 ± 3.33c	63.33 ± 3.33cde	1434 ± 13.53d
<i>Ch3</i>	90.00 ± 2.89b	70.00 ± 0.00b	70.00 ± 0.00e	2954 ± 28.42f

Chlorella vulgaris at 1×10^6 cells mL⁻¹ (control); *Spirulina* powder at 0.75, 1.5, and 3.0 mg dry weight individual⁻¹ (*S1*, *S2*, and *S3*, respectively); and *Chlorococcum humicola* at 5×10^5 , 1×10^6 , and 2×10^6 cells mL⁻¹ (*Ch1*, *Ch2*, and *Ch3*, respectively). Values followed by different lowercase letter(s) in the same column are significantly different ($P < 0.05$)

Table 3 Water quality variables and nutrients (mean \pm SE) in cultured containers of *Branchinella thailandensis* fed with seven treatments of algal diets for 15 days

Algal diets	pH	DO mg L ⁻¹	NO ₂ -N– mg L ⁻¹	NH ₃ -N– mg L ⁻¹	NH ₄ -N– mg L ⁻¹
Control	7.03 \pm 0.04a	5.01 \pm 0.07bc	0.06 \pm 0.00a	0.004 \pm 0.00ab	0.43 \pm 0.09b
S1	7.18 \pm 0.02ab	4.82 \pm 0.08bc	0.02 \pm 0.00a	0.006 \pm 0.00bc	0.53 \pm 0.07b
S2	7.21 \pm 0.02ab	4.43 \pm 0.15b	0.03 \pm 0.00a	0.009 \pm 0.00c	0.78 \pm 0.08c
S3	7.30 \pm 0.02b	3.72 \pm 0.15a	0.03 \pm 0.00a	0.018 \pm 0.01d	1.12 \pm 0.13d
Ch1	7.07 \pm 0.09a	5.28 \pm 0.27c	0.41 \pm 0.07b	0.001 \pm 0.00a	0.08 \pm 0.02a
Ch2	7.13 \pm 0.08ab	5.32 \pm 0.28c	0.59 \pm 0.10c	0.004 \pm 0.00a	0.12 \pm 0.04a
Ch3	7.18 \pm 0.09ab	5.27 \pm 0.32c	0.89 \pm 0.05d	0.004 \pm 0.00ab	0.14 \pm 0.07a

Chlorella vulgaris at 1×10^6 cells mL⁻¹ (control); *Spirulina* powder at 0.75, 1.5, and 3.0 mg dry weight individual⁻¹ (S1, S2, and S3, respectively); and *Chlorococcum humicola* at 5×10^5 , 1×10^6 , and 2×10^6 cells mL⁻¹ (Ch1, Ch2, and Ch3, respectively). Values followed by different lowercase letter(s) in the same column are significantly different ($P < 0.05$)

Spirulina powder (S3) had the highest protein content of $61.03 \pm 0.15\%$, which was significantly higher than that of the control ($5.86 \pm 0.04\%$) and Ch3 ($6.12 \pm 0.09\%$) ($P < 0.05$). The control had higher protein and lipid than Ch3. However, results from proximate analysis showed that *B. thailandensis* fed with Ch3 had the highest protein content of $3.60 \pm 0.01\%$, which was significantly higher than that of S3 ($2.57 \pm 0.07\%$). Lipid in *B. thailandensis* fed with Ch3 ($0.49 \pm 0.02\%$) also significantly higher than that of the control ($0.25 \pm 0.02\%$) and the S3 treatment ($0.19 \pm 0.04\%$).

Carotenoid content in algae and fairy shrimp

Carotenoid content analysis of the three algal diets (Table 5) demonstrated that S3 and Ch3 had 2.84 ± 0.10 mg g⁻¹ total carotenoid contents which was significantly higher than the control (1.34 ± 0.04 mg g⁻¹) ($P < 0.05$). HPLC analysis revealed that lutein and β -carotene were the major carotenoids in S3 (0.49 ± 0.02 and 0.33 ± 0.01 mg g⁻¹ of total carotenoids) while the major carotenoids in the Ch3 and control were lutein (0.70 ± 0.06 and 0.54 ± 0.02 mg g⁻¹) and zeaxanthin (0.61 ± 0.02 and 0.38 ± 0.02 mg g⁻¹).

Carotenoid compositions of the fairy shrimp fed with the optimal concentrations of three algal diets are presented in Table 6. The total carotenoids in *B. thailandensis* fed with the control, Ch3, and S3 were not significantly different ($P > 0.05$). Analysis of individual carotenoids showed that the shrimp fed with all the three diets contained four types of carotenoids: astaxanthin, lutein, canthaxanthin, and β -carotene. Predominant carotenoids in the shrimp fed with the three diets were canthaxanthin (0.021 – 0.031 mg g⁻¹) and astaxanthin (0.004 – 0.015 mg g⁻¹).

Discussion

The current study demonstrated that Ch3 was the most suitable algal diet to feed *B. thailandensis*. Feeding the fairy shrimp with Ch3 provided significant higher body length, survival rate, and egg production than that fed by S3 and the most commonly used diet, *C. vulgaris* (control treatment). The suitability of Ch3 and the control diets as foods of the fairy shrimp may be due to their small cell sizes, which are less than 10 μ m in diameter. Our observations under a microscope revealed that the average sizes of *C. vulgaris* and *C. humicola*

Table 4 Proximate analysis of algal diets and 5-day-old *Branchinella thailandensis* fed with optimal concentrations of three algal diets for 15 days

Proximate composition	Algal diets (% wet weight and % dry weight in the bracket)			Fairy shrimps fed with different algal diets (% wet weight and % dry weight in the bracket)		
	Control	S3	Ch3	Control	S3	Ch3
Moisture	88.13 ± 0.02c	8.60 ± 0.03a	76.80 ± 0.51b	95.62 ± 0.39a	95.19 ± 0.01a	95.05 ± 0.03a
Protein	5.86 ± 0.04a (49.34 ± 0.35b)	61.03 ± 0.15b (66.77 ± 0.18c)	6.12 ± 0.09a (26.39 ± 0.20a)	3.06 ± 0.16b (69.85 ± 2.55b)	2.57 ± 0.07a (53.38 ± 1.44a)	3.60 ± 0.01c (72.71 ± 0.34b)
Lipid	1.05 ± 0.05a (8.85 ± 0.44b)	5.87 ± 0.15c (6.42 ± 0.16a)	1.57 ± 0.03b (6.77 ± 0.28a)	0.25 ± 0.02a (5.59 ± 0.35a)	0.19 ± 0.04a (3.90 ± 0.83a)	0.49 ± 0.02b (9.80 ± 0.28b)
Carbohydrate	2.00 ± 0.11a (16.81 ± 0.87a)	19.92 ± 0.00c (21.79 ± 0.01a)	10.25 ± 0.72b (44.13 ± 2.11b)	0.55 ± 0.20a (12.24 ± 3.38a)	1.57 ± 0.06b (32.70 ± 1.38b)	0.34 ± 0.01a (6.83 ± 0.16a)
Ash	2.97 ± 0.01a (25.00 ± 0.08b)	4.59 ± 0.02b (5.02 ± 0.02a)	5.26 ± 0.26b (22.72 ± 1.63b)	0.54 ± 0.01a (12.33 ± 0.80a)	0.48 ± 0.04a (10.02 ± 0.89a)	0.53 ± 0.00a (10.67 ± 0.11a)

Chlorella vulgaris at 1×10^6 cells mL⁻¹ (control), *Spirulina* powder at 3.0 mg dry weight individual⁻¹ (S3), and *Chlorococcum humicola* at 2×10^6 cells mL⁻¹ (Ch3). Values followed by different lowercase letter(s) in the same row are significantly different ($P < 0.05$)

Table 5 Total carotenoid content and individual carotenoid composition (mean \pm standard error) of three algal diets

Carotenoids	Algal diets (mg g ⁻¹ wet weight and mg g ⁻¹ dry weight in the bracket)		
	<i>C. vulgaris</i>	<i>Spirulina</i> powder	<i>C. humicola</i>
Lutein	0.54 \pm 0.02a (4.53 \pm 0.17c)	0.49 \pm 0.02a (0.53 \pm 0.02a)	0.70 \pm 0.06b (3.03 \pm 0.26b)
Zeaxanthin	0.38 \pm 0.02b (3.17 \pm 0.18c)	0.00 \pm 0.00a (0.00 \pm 0.00a)	0.61 \pm 0.02c (2.63 \pm 0.05b)
β -carotene	0.01 \pm 0.00a (0.04 \pm 0.01a)	0.33 \pm 0.01c (0.36 \pm 0.01c)	0.03 \pm 0.01b (0.13 \pm 0.01b)
Total carotenoids	1.34 \pm 0.04a (11.32 \pm 0.31b)	2.84 \pm 0.10b (3.03 \pm 0.07a)	2.84 \pm 0.10b (12.23 \pm 0.42b)

Chlorella vulgaris (control), dried *Spirulina* powder, and *Chlorococcum humicola*. Values followed by different lowercase letter(s) in the same row are significantly different ($P < 0.05$)

were 4.22 ± 0.51 and 8.89 ± 1.99 μm . A study of gut content analysis by Selvarani (2009) has shown that *C. humicola* was found in the guts of immature and mature stages of the fairy shrimp *S. dichotomus*. Thus, this study confirmed the suitability of *C. humicola* as an appropriate fairy shrimp diet. However, this alga should not be fed to the nauplii stages of *B. thailandensis* because the alga size is larger than the nauplii's mouth. Moreover, continuous and vigorous aeration is required to suspend *C. humicola* cells in the water column. One of the advantages of using *C. humicola* is the algal cells can be collected from culture medium by simple sedimentation. Concentrated cells can be easily concentrated and stored in cool condition prior to use.

Although the shrimp fed with the dried *S3* powder had rather high growth and fecundity performances, the survival rate was low ($56.67 \pm 3.33\%$). One of the main problems with dried algae was the degradation of algal powder-released ammonia into the water through ammonification process. In this study, the treatments fed with *Spirulina* powder (*S1*, *S2*, and *S3*) had the highest total ammonia nitrogen of 0.53–1.12 mg L⁻¹ [unionized ammonia (NH₃-N) concentrations of 0.006–0.018 mg L⁻¹] but lowest in dissolved oxygen of 3.72–4.82 mg L⁻¹. These

Table 6 Total carotenoid content and individual carotenoid composition (mean \pm standard error) of 5-day-old *Branchinella thailandensis* fed with optimal concentrations of three algal diets for 15 days

Carotenoids of fairy shrimps	Algal diets (mg g ⁻¹ wet weight and mg g ⁻¹ dry weight in the bracket)		
	<i>C. vulgaris</i>	<i>Spirulina</i> powder	<i>C. humicola</i>
Astaxanthin	0.014 \pm 0.00b (0.32 \pm 0.04b)	0.004 \pm 0.00a (0.08 \pm 0.01a)	0.015 \pm 0.00b (0.30 \pm 0.01b)
Lutein	0.016 \pm 0.00b (0.13 \pm 0.02b)	0.001 \pm 0.00a (0.02 \pm 0.00a)	0.002 \pm 0.00a (0.04 \pm 0.01a)
Canthaxanthin	0.024 \pm 0.00a (0.54 \pm 0.03b)	0.021 \pm 0.00a (0.44 \pm 0.04a)	0.031 \pm 0.00b (0.63 \pm 0.02c)
β -carotene	0.003 \pm 0.00a (0.06 \pm 0.00a)	0.003 \pm 0.00a (0.06 \pm 0.01a)	0.005 \pm 0.00b (0.09 \pm 0.01b)
Total carotenoids	0.045 \pm 0.01a (1.02 \pm 0.21a)	0.064 \pm 0.01a (1.33 \pm 0.32a)	0.061 \pm 0.02a (1.23 \pm 0.34a)

Chlorella vulgaris at 1×10^6 cells mL⁻¹ (control), *Spirulina* powder at 3.0 mg dry weight individual⁻¹ (*S3*), and *Chlorococcum humicola* at 2×10^6 cells mL⁻¹ (*Ch3*). Values followed by different lowercase letter(s) in the same row are significantly different ($P < 0.05$)

concentrations were higher than the standard concentration of ammonia for aquaculture, which must be less than 0.2 mg-N L^{-1} (Nootong et al. 2011). Moreover, microbial decomposition process also consumed oxygen so the dissolved oxygen in the treatments fed with *Spirulina* powder was significantly lower than that in *C. vulgaris* and *C. humicola*. Apart from acute toxicity, chronic toxicity of ammonia can also affect the animal growth (Hargreaves and Kucuk 2001), increase mortality, and possibly induce the black disease, which usually occurs in an undesired environment (Saejung et al. 2011). Hence, during the shortage of live foods in some hatcheries, use of instant foods such as dried *Spirulina* powder for *B. thailandensis* cultures can prevent the risk of production collapse. In this study, water quality was not high in values than that reported by Saengphan (2005) that presented the suitable values of water quality for fairy shrimp culture.

The lower concentration diets (*Ch1*, *S1*, and *S2*) significantly reduced growth and reproductive performances of *B. thailandensis*, compared with the control. Our results suggest that these were unsuitable food concentrations for the shrimp. Various studies have indicated that fairy shrimp can consume various types of food (Saengphan and Sanoamuang 2009; Sriputhorn and Sanoamuang 2007, 2011; Velu and Munuswamy 2007). The *Spirulina* powder at $0.8 \text{ mg dry weight individual}^{-1}$ was the optimal concentration for nauplii (age 1–5 days) of *B. thailandensis* (Saengphan and Sanoamuang 2009). Some negative effects such as low egg productions and high mortality rates of the fairy shrimp *S. proboscideus* may be due to some toxicity of filamentous blue green algae when fed with pure *S. platensis* (De Walsche et al. 1991). Although rice bran and yeast could be used as substitution foods to replace *C. vulgaris* (Sriputhorn and Sanoamuang 2007; Saengphan and Sanoamuang 2009), most of these studies suggest that live algae are the most efficient diet for fairy shrimp cultivation. Two major reasons are that the live algae have less impact to water quality, and some algae are a good source of carotenoids (Ali and Dumont 1995; Zarattini and Mura 2004; Sriputhorn and Sanoamuang 2007; Saengphan and Sanoamuang 2009).

The studied *B. thailandensis* fed with the *Ch3* treatment contained higher protein ($3.60 \pm 0.01\%$) than that fed with the *C. vulgaris* (control) at $1 \times 10^6 \text{ cells mL}^{-1}$ ($3.06 \pm 0.16\%$). Although the protein content in *Ch3* (26.39%) is lower than that in the control *Chlorella* and *S3* (Table 4), it is sufficient for growth of the fairy shrimp. Similarly, commercial feed pellet for fish contains only 20–28% of protein (FAO 2017). On the other hand, *Ch3* contains relatively high carbohydrate (44.13%) which is an important energy source for animals. The concept of low-protein high-calorie diet may not yet be a suitable explanation in this study because more future researches are required. In general, physiological and environmental factors have an influence on nutritional values of planktonic organisms (Jeffries 1970). Claus et al. (1979) reported that *Artemia salina* larvae fed with *Spirulina* had a rather high protein content. *Artemia* showed rapid protein synthesis during their growth. When the *Artemia* animals were starved, protein synthesis in their bodies was still functioning but the growth rate was reduced. Among the three algae used in this study, *Spirulina* powder itself had the highest protein content but after feeding by *B. thailandensis*, only $2.57 \pm 0.07\%$ of protein content was observed in the shrimp. The possible explanation could probably relate with the decomposition of the algal powder resulting in a transformation process from organic nitrogen into inorganic ammonia-nitrogen in the water. In addition, the high concentrations of ammonia in the water are not suitable for the shrimp to grow because their bodies will contain less protein.

Although the total carotenoids in the *Spirulina* powder and *C. humicola* were two times more than that in the control *C. vulgaris*, such carotenoids examined in *B. thailandensis* fed these algae were not significantly different ($P > 0.05$). However, differences in their dominant carotenoid compositions are distinguished. This work demonstrated that canthaxanthin was the most abundant carotenoid in *B. thailandensis* followed by astaxanthin, lutein, and β -carotene, respectively. This

predominant canthaxanthin profile corresponds to that of the fairy shrimp *S. dichotomus* (Murugan et al. 1995; Velu et al. 2003; Velu and Munuswamy 2007). However, Dararat et al. (2012) reported that major carotenoids in *B. thailandensis* were astaxanthin and canthaxanthin. The astaxanthin and canthaxanthin contents were higher than those found in *S. sirindhornae* and *S. siamensis*. Previous studies revealed that fairy shrimp are able to convert β -carotene from ingested foods into canthaxanthin. This is related to the increasing of canthaxanthin contents in conjunction with the depletion of β -carotene (Hsu et al. 1970; Nelis et al. 1988).

In summary, this study demonstrates that *C. humicola* is one of the suitable alternative food sources for the cultivation of *B. thailandensis*. Moreover, *Spirulina* powder is also a good choice when live algae are not available.

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