

# Effects of dietary incorporation of *Arthrospira (Spirulina) platensis* meal on growth, survival, body composition, and reproductive performance of red cherry shrimp *Neocaridina davidi* (Crustacea, Atyidae) over successive spawnings

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Received: 8 February 2017 / Revised and accepted: 26 June 2017 / Published online: 19 July 2017  
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**Abstract** This study was conducted to evaluate dietary inclusion effects of *Arthrospira (Spirulina) platensis* (SP) meal on the growth, survival, biochemical composition, and particularly reproductive performance during eight successive spawning events in the caridean red cherry shrimp, *Neocaridina davidi*, for 11 months. Juveniles were fed with six diets containing 0 (control), 1, 3, 5, 8, and 10% of SP levels. Most growth indicators in terms of final weight, specific growth rate, and average daily growth rate were significantly higher in the shrimp fed with SP10. The highest survival of females (75–81%) was recorded in shrimp fed SP8–10, being different from the other groups (25–73%). No changes were observed in lipid and moisture contents, and protein content showed an increasing trend with dietary *Spirulina* levels up to 10%. Absolute fecundity (eggs per female) was significantly affected in order of the highest to lowest viz. 45, 39, 30, 28, 27, and 24 with different incorporations. The relative fecundity (eggs per female's body weight) of shrimp fed SP8–10 showed differences with the other groups. Except for the control, all *Spirulina*-fed females of SP1–5 showed hatching percentages of 78–84% compared with those of 91–94% for SP8–10. Relative fecundity in females fed SP8–10 showed differences from the fourth to the sixth spawning events compared

with the other groups; this trend decreased non-significantly until the eighth spawning. The results obtained suggest that dietary inclusion of *S. platensis* meal at a level of 8–10% can improve the growth, survival and, in particular, reproductive performance of red cherry shrimp in commercial aquaria during maturity only up to the sixth spawning event.

**Keywords** *Neocaridina davidi* · *Spirulina platensis* · Cyanobacteria · Reproductive performance · Successive spawnings

## Introduction

The production of numerous aquaculture species has increased considerably since 2000, with crustaceans representing 10% of the total production in 2012 (FAO 2014). The values of international trade of ornamental production have been estimated to worth around US\$717 million in 2009, increasing at an average growth rate of approximately 14%/year (FAO 2014).

In recent years, ornamental shrimp have drawn attention due to color variations, relatively a high price, easy maintenance, high adaptability to water quality parameters, high appetite to feed on algal diets, detritus, and micro-organisms on the substrate, and also on other remains in aquaria. The atyid red cherry shrimp (RCS) (*Neocaridina davidi*, Bouvier, 1904), formerly known as *Neocaridina heteropoda*, belongs to the sub-order Caridina (family Atyidae), has undoubtedly been the most popular freshwater shrimp in the hobby aquarium industry since 2003 as well as a model shrimp in ecotoxicological and reproduction investigations (Heerbrandt and Lin 2006; Tropea and Lopez Greco 2015). The genus *Neocaridina* inhabits various types of inland water bodies

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(lakes, reservoirs, ponds, and rivers) being distributed throughout Russia, China, Japan, Korea, Taiwan, and Vietnam (Cai 1996; His and Yixiong 2007). Full-grown adults of cherry shrimp in aquaria with pH and temperature levels of 6.5–8 and 14–29 °C, respectively, can reach maturity in 2–2.5 months, and approximate total lengths of 1.5–3.5 cm (up to 4 cm) have been reported for ages of 1–2 years (Heerbrandt and Lin 2006). The common practice of commercial RCS shrimp breeding under captive conditions is that the domesticated broodstocks are propagated for seed production. After the first spawning, it typically takes 30 to 45 days for the females to re-develop their ovaries in preparation for a second spawn. However, with substantial increases in the larval supply for RCS shrimp sales in the local markets of Iran, there are increasing interests in re-maturing the broodstocks to produce several consecutive spawning for seed production.

Future improvements concerning maturation diet formulation may allow the broodstock to achieve a high reproductive performance for longer periods. In captivity, reproductive brooders are totally dependent on the nutrients provided by maturation diets to fulfill the high nutritional requirements related to reproduction, in which any dietary deficiencies or imbalances are reflected in lower reproductive performances. On the other hand, the size of females quantitatively affects larval production; however, shrimp size and age are closely related and senescence occurring in larger (and older) shrimp may negatively influence reproductive indices and offspring quality (Rothlisberg 1998; Coman and Crocos 2003; Tropea et al. 2012).

*Spirulina platensis* (currently recognized name *Arthrospira platensis*), a multicellular filamentous cyanobacterium (Cyanobacteria; family Phormidiaceae), has received a great attention for the use in aquaculture feeds (Spolaore et al. 2006; Wells et al. 2017). *Spirulina* biomass contains high quantities of proteins (up to 55–70% of dry weight), lipids (6–11%), and good amounts of essential fatty acids (e.g., gamma-linolenic acid), antioxidant pigments (carotenoids), vitamins (especially vitamin B<sub>12</sub> and pro-vitamin A;  $\beta$ -carotene), minerals (especially iron), and compounds that promote the attractability and palatability of the diets (Belay et al. 1996; Habib et al. 2008). A number of feeds (additives or supplements) have been utilized in the shrimp farming industry, but *Spirulina* is one of the best microalgal additives demonstrated to be beneficial to growers offsetting the initial cost and providing a significant cost/performance resulting from dietary inclusion levels ranging from 5 to 20% (Habib et al. 2008; Wells et al. 2017). Several studies have been conducted using microalgae and/or dried *Spirulina* as a complementary dietary ingredient for the white shrimp *Litopenaeus schmitti* (Jaime-Ceballos et al. 2005), Pacific white shrimp *Litopenaeus vannamei* (Hanel et al. 2007; Macias-Sancho et al. 2014), and several fish species including tilapia, carp, and sturgeon (Olvera-Novoa et al. 1998; Nandeeshha et al. 2001; Lu and Takeuchi 2004;

Palmegiano et al. 2005). Dietary incorporation of many microalgal species for the growers and particularly broodstocks of shrimp is still at early research stages (Ju et al. 2009; Tank et al. 2010); only few studies have examined shrimp with respect to feed additives (Cuzon et al. 1981; Nakagawa and Gomez-Diaz 1995; Jaime-Ceballos et al. 2005; Hemaiswarya et al. 2010), feed attractants (Silva-Neto et al. 2012), and/or partial or total replacements of feed protein sources (Tank et al. 2010; Macias-Sancho et al. 2014; Abdul Basri et al. 2015).

During the last three decades, most researches have focused on the improvement of the quality and quantity of different nutrients through broodstock diets on the stimulation of sexual maturation, increased fecundity, egg quality, and larval survival as well as developments in aquatic vertebrates and invertebrates (Harrison 1990; Wouters et al. 2001; Calado et al. 2009; Wu et al. 2010). To date, most investigations conducted on ornamental RCS shrimps have addressed the physiology of digestive traits (Wang et al. 2010), the effects of temperature on biochemical composition, growth, and reproduction (Tropea et al. 2015), female growth and offspring quality over successive spawning events (Tropea and Lopez Greco 2015), morphology of the initial post-hatching larval stages (Pantaleao et al. 2017), and low-cost biofilm-based methods with zero water exchange for sustainable culture (Viau et al. 2016). Accordingly, studies aiming at the improvements of the growth and particularly reproductive performance of RCS shrimp are scarce. Furthermore, there are no reports or data on the influences of dietary *Spirulina* meal on the capabilities of re-matured RCS shrimp broodstock to allow reliable evaluation of reproductive performance and offspring production within several successive spawnings in captivity. Thus, the main objective of the present study was to evaluate the effects of different dietary incorporation levels of *S. platensis* meal on the growth, survival, body composition, and particularly, female's reproductive indices in the red cherry shrimp by the end of the eight consecutive spawnings for 11 months.

## Materials and methods

### Experimental diets

*Spirulina platensis* (SP) meal was obtained from SINA Microalgae Company (Qeshm Island, Hormozgan Province, Iran). The proximate compositions of dry matter, crude protein, and lipid in *S. platensis* meal used were, respectively, 92, 58, and 8.2%.

Six iso-nitrogenous (46.4% crude protein), iso-lipidic (9.4% crude lipid), and iso-energetic (17.2 kJ g<sup>-1</sup>) practical diets were formulated in the laboratory. A diet without algal meal inclusion was served as a control group, and five

experimental diets containing *S. platensis* meal were formulated to contain 1, 3, 5, 8, and 10% of the meal (designated as SP1, SP3, SP5, SP8, and SP10, respectively). Dietary *S. platensis* meal was supplemented at different percentages, and the chemical compositions of diets were re-adjusted mainly with fishmeal, soybean meal, and other ingredients. The dry ingredients were first sifted through sieve (4 mm mesh size) and mixed using a Hobart feed mixer (Hobart Grinder, Model MG 4366) for approximately 30 min to ensure that the mixture was well homogenized. Then, water was added (20–30%, V/W) to give a pelletable mixture. Diets were prepared as pellets with appropriate diameters of 3 mm and dried in a drying oven (Fan Azma Gostar, Model BM 55) at 55 °C for 24 h. The diets were pelleted every 3 weeks, kept in separate, tightly packed plastic bags, and finally stored at –20 °C until use (Table 1).

**Experimental RCS shrimp, culture conditions, feeding trials, and sex determination**

The experiments were conducted in the experimental facilities of the Hamoon Ornamental Fish and Crustacean Culture Center (Marlik Town, Tehran, Iran) for a period of 11 months during 2015–2016. RCS juveniles ( $n = 400$ , mean initial weight of  $0.45 \pm 0.02$  mg, 35 days old) were obtained from a local ornamental hatchery center (Hamoon Ornamental Center, Iran). Before the start of the experiments, juveniles were adapted to the experimental conditions in six 80 L aquaria for 10 days and fed (ad libitum) once per day with a commercial diet (CP 9910) containing 30% crude protein and 4% crude lipid. The aquaria were aerated continuously in a closed system. Then, the juveniles were randomly distributed in 18 rectangular glass aquaria (50 cm × 40 cm × 40 cm; 80 L),

**Table 1** Feed ingredients, formulations, and proximate compositions of the six experimental diets (dry matter basic, g kg<sup>-1</sup>)

Dietary ingredients (g kg <sup>-1</sup> )	Diets (treatments)					
	0 (control)	SP1	SP3	SP5	SP8	SP10
Fish meal <sup>a</sup>	310	300	280	260	230	210
<i>Spirulina platensis</i> meal	0	10	30	50	80	100
Soybean meal <sup>b</sup>	230	230	230	230	230	230
Wheat gluten meal <sup>c</sup>	220	220	220	220	220	220
Corn meal	80	80	80	80	80	80
Canola oil	50	50	50	50	50	50
Anzimate	40	40	40	40	40	40
Molasses	30	30	30	30	30	30
Vitamins premix <sup>d</sup>	20	20	20	20	20	20
Minerals premix <sup>e</sup>	20	20	20	20	20	20
Proximate composition (g kg <sup>-1</sup> )						
Dry matter	895.3	891.5	889.4	892.6	889.7	890.8
Crude protein	463.55	463.34	464.2	463.94	464.39	464.7
Crude lipid	94.1	94	94.9	93.86	93.74	93.66
Total carbohydrate <sup>f</sup>	389.4	394.66	388.9	391.2	390.87	385.64
Ash	53	48	52	51	51	56
Energy (kJ g <sup>-1</sup> ) <sup>g</sup>	17.27	17.32	17.31	17.29	17.30	17.24

Each value is the mean of three subsamples: SP0 (control) = 0, SP1 = 1, SP3 = 3, SP5 = 5, SP8 = 8, and SP10 = 10% of *Spirulina platensis* meal

<sup>a</sup> Kilka fish meal (mainly *Clupeonella delicatula*, low-temperature dried fish meal), Pars Kilka Co., Mirood, Iran. Proximate composition of fish meal was 90% dry matter, 58.5% crude protein, and 8.6% lipid

<sup>b</sup> Soybean meal: Sobhan Soybean Protein Co., Ltd. Behshahr, Iran

<sup>c</sup> Khorak Dame Mazandaran Co., Sari, Iran

<sup>d</sup> Vitamin premix contained the following vitamins (each per kilogram diet): vitamin A, 10,000 IU; vitamin D<sub>3</sub>, 2000 IU; vitamin E, 100 mg; vitamin K, 20 mg; vitamin B<sub>1</sub>, 400 mg; vitamin B<sub>2</sub>, 40 mg; vitamin B<sub>6</sub>, 20 mg; vitamin B<sub>12</sub>, 0.04 mg; biotin, 0.2 mg; choline chloride, 1200 mg; folic acid, 10 mg; inositol, 200 mg; niacin, 200 mg; and pantothenic acid, 100 mg

<sup>e</sup> Mineral premix contained (g kg<sup>-1</sup> mix): MgSO<sub>4</sub>·2H<sub>2</sub>O, 127.5; KCl, 50.0; NaCl, 60; CaHPO<sub>4</sub>·2H<sub>2</sub>O, 727.8; FeSO<sub>4</sub>·7H<sub>2</sub>O, 25.0; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 5.5; CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.785; MnSO<sub>4</sub>·4H<sub>2</sub>O, 2.54; CoSO<sub>4</sub>·4H<sub>2</sub>O, 0.478; Ca (IO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O, 0.295; CrCl<sub>3</sub>·6H<sub>2</sub>O, 0.128

<sup>f</sup> Total carbohydrate = 1000 – (crude protein (g) + crude lipid (g) + ash (g))

<sup>g</sup> Calculated using the factors: carbohydrates, 11 kJ g<sup>-1</sup>; protein, 20.9 kJ g<sup>-1</sup>; and lipids, 35.1 kJ g<sup>-1</sup>

each stocked with 22 juveniles. Mild aeration was provided to each aquarium through an air stone connected to a central air compressor in a closed system. Juveniles were reared under 12 h light:12 h dark photoperiod throughout the feeding trials and hand-fed three times a day (09:00, 13:00, and 17:00 hours) with the experimental diets in six inclusion levels of *S. platensis* meal based on 3% of wet body weight. During the experimental period, water temperature, pH, dissolved oxygen, ammonia, and hardness were monitored and ranged from 26 to 28 °C, 7 to 7.2, 5.8 to 6.1 mg L<sup>-1</sup>, <0.2 mg L<sup>-1</sup>, and 85 to 90 mg L<sup>-1</sup>, respectively. The experimental aquaria were cleaned once every 3 days by siphoning out fecal pellets and other residual feeds, and water was replaced completely once every 10 days.

Before the first stage of breeding, morphological differences of the male and female brooders were determined on the basis of size, coloration, and saddle situation (Cai 1996; Pantaleao et al. 2015). Females are larger than the males, have a much darker red coloration, and the lower section of the abdomen has a curved shape. The males are smaller, have very little red coloration, and there is a straight line with no curved shape at the lower section of the abdomen. Females have a “saddle,” which is a common name for the eggs still in the ovaries, located behind the head on the top of the bottom.

Afterwards, the brooders were first distinguished on the basis of the morphological characteristics and then divided into six treatments (three replicates per treatment) in 18 experimental aquaria (each with a volume of 30 L; 25 cm × 12 cm × 10 cm) with ten females per aquarium reared for the subsequent experiments up to 10 months. All females at different treatments were fed daily three times at 09:00, 13:00, and 17:00 hours at a ration of 3% total wet biomass. Prior to daily feeding, feces and uneaten feed were removed by siphoning. Due to the high sensitivity of the females in keeping eggs, the stocks were exposed to minimum stressful conditions during all stages of feeding, manipulation, and breeding. During the experiments, survival of the females was recorded every day in the morning. Over the experimental period, all aquaria were gently aerated and factors such as water quality and environmental conditions for culture were maintained as in the previous phase. The water quality parameters were within the suitable ranges for *N. davidi* broodstocks farming.

At the end of each experimental stage, shrimps were fasted for 24 h prior to sampling. Every 30 days, brooders (nine shrimp from each treatment) were randomly removed from the respective aquaria and after removing excess water with paper tissues, mean wet body weight was measured individually by a precision balance (to the nearest 0.001 g), and total length was determined with a digital caliper (to the nearest 0.01 mm). Accordingly, the mean final weight of females in each spawning event fed with the diets containing different inclusion levels of *S. platensis* meal were measured during eight consecutive spawnings over the experimental period.

## Evaluation of growth performance

The growth indices of brooder females were calculated according to data obtained in the last spawning event as follows:

$$\text{Weight gain (WG\%)} = ((W_f - W_i) / W_i) \times 100$$

$$\text{Length gain (LG\%)} = ((L_f - L_i) / L_i) \times 100$$

$$\text{Condition factor (Cf)} = W_f / L_f^3 \times 100$$

$$\text{Specific growth rate (SGR; \% / day)}$$

$$= ((\ln W_f - \ln W_i) / T) \times 100$$

$$\text{Average daily growth rate (ADGR; mg day}^{-1}\text{)}$$

$$= (W_f - W_i) / T$$

$$\text{Feed conversion ratio (FCR)}$$

$$= (\text{total feed intake (g)} / \text{total wet weight gain (g)})$$

$$\text{Survival rate (\%)} = (N_f / N_i) \times 100$$

where  $W_f$  and  $W_i$  are mean final and initial weights (mg),  $L_f$  and  $L_i$  are mean final and initial lengths (mm),  $N_f$  and  $N_i$  are final and initial numbers, and  $T$  is the number of days for feeding trials (Hardy and Barrows 2002).

## Reproductive performance, hatching percentage, and spawning intervals

The specifications of first reproduction time in shrimp were calculated at different treatments according to a level of 50% population maturation in each replicate (aquarium). In order to mate, three males (already fed control diet) were randomly selected and introduced to each experimental aquarium containing females for 3 days. Immediately after egg laying, the brooders were taken out of the aquarium and the attached eggs were carefully separated among the swimming legs by holders.

To determine absolute fecundity, total cumulative numbers of eggs from healthy females were taken from each aquarium and mean data calculated for the total egg production per female. Relative fecundity was calculated according to the total egg production per female per mean wet body weight of each female. The whole eggs obtained from each treatment were hatched in vertical incubators and the hatchability percentage (%) were determined in vitro at 28 ± 1.0 °C based on the total number of hatched larvae (juveniles)/total number of fertilized eggs × 100. Actual fecundity was calculated by the total number of hatched juveniles within the incubation period. Moderate aeration provided oxygenation and ensured that the eggs would not settle in incubators. The whole water was replaced daily in each incubator and when hatching started, newly hatched larvae were recorded every 12 h when all newly hatched larvae were collected from the incubators. The

above procedures continued until all eggs hatched out or were finally confirmed to be dead.

After reproduction in each step, all the brooders were immediately placed in the relevant aquaria and nutritional trials continued in the different treatments until the next mating event. The frequency and feeding schedules of females and water quality conditions in each aquarium were completely identical in all treatments. The time required between two successive spawnings (spawning intervals) was determined according to the shrimp’s life cycle by introducing males to each aquarium followed by mating and the next egg laying step. To evaluate the effects of feeding with *S. platensis* meal, all brooders reproduced in eight consecutive steps for 10 months at different treatments. All the trials were conducted during 11 months.

### Shrimp’s body biochemical composition

To determine the moisture, protein, lipid, and ash contents of whole body biochemical analysis of shrimp, samples were randomly obtained from each treatment and prepared according to AOAC (2002) and then stored at  $-20\text{ }^{\circ}\text{C}$ . Before the sampling, the shrimp were starved for 24 h. Moisture was determined by oven drying at  $105\text{ }^{\circ}\text{C}$  for 24 h. Crude protein ( $N \times 6.25$ ) was measured by the Kjeldahl method after acid digestion using an auto-Kjeldahl System (1030-Auto-analyzer, Tecator, Sweden). Crude lipid was determined by the ether-extraction method using Soxtec System HT (Soxtec System HT6, Tecator, Sweden). Ash content was estimated using a muffle furnace at  $550\text{ }^{\circ}\text{C}$  for 24 h.

### Statistical analyses

Data were first subjected to arcsine transformation when they did not follow a normal distribution. Mean values (means  $\pm$  SD) for growth, survival, and reproductive performances were analyzed by one-way ANOVA followed by Tukey’s multiple comparison tests to verify significant differences among the treatments at a level of  $P < 0.05$ . Changes among successive spawning events were assessed by an ANOVA design with repeated measures test. All the statistical analyses were carried out using SPSS software version 22.

## Results

### Growth performance

The growth indicators of RCS shrimp fed with different inclusion levels of *Spirulina* meal showed significant differences ( $P < 0.05$ ) between the treatments during the feeding trials (11 months). Higher levels (5–10% SP) of algal meal inclusion in RCS shrimp diets resulted in significant enhancements in most growth parameters during the experimental period. Final weight was significantly ( $P < 0.05$ ) higher in shrimps fed 10% SP compared with other treatments showing a weight gain of 1.6 times the control group. The highest final length (26.3–27.8 mm) was recorded in shrimps fed 5–10% dietary SP in comparison with the other treatments (25.3–25.7 mm) ( $P < 0.05$ ). Length increments of females showed increasing trends by the inclusion of 1–10% of SP meal, though no differences ( $P > 0.05$ ) were obtained in final length between SP inclusion levels of 5 and 10%. RCS shrimps fed with 3, 1, and

**Table 2** Growth performance of red cherry shrimp (*Neocaridina davidi*) fed diets containing different incorporation levels of *Spirulina platensis* meal over 11 months of experimental period

Growth parameters	Diets					
	Control	SP1	SP3	SP5	SP8	SP10
Initial weight (mg)	0.45 $\pm$ 0.02	0.45 $\pm$ 0.03	0.46 $\pm$ 0.02	0.46 $\pm$ 0.03	0.46 $\pm$ 0.03	0.45 $\pm$ 0.02
Final weight (mg)	70.85 $\pm$ 2.1 e	80 $\pm$ 1.74 d	88.3 $\pm$ 1.62 c	91.2 $\pm$ 1.4 c	98.1 $\pm$ 1.15 b	114.6 $\pm$ 1.65 a
Weight gain $\times 10^2$ (%)	156.4 $\pm$ 23 e	176.8 $\pm$ 19 d	191 $\pm$ 15 c	197.3 $\pm$ 21 c	212.3 $\pm$ 26 b	253.7 $\pm$ 22 a
Initial length (mm)	11 $\pm$ 0.58	10.7 $\pm$ 0.34	10.8 $\pm$ 0.42	11.1 $\pm$ 0.63	10.9 $\pm$ 0.52	10.9 $\pm$ 0.41
Final length (mm)	25.3 $\pm$ 0.4 b	25.7 $\pm$ 0.6 b	25.3 $\pm$ 0.2 b	26.3 $\pm$ 0.5 ab	26.7 $\pm$ 0.8 ab	27.8 $\pm$ 0.6 a
Length gain (%)	130 $\pm$ 17.3	140.2 $\pm$ 7.9	134.2 $\pm$ 21	136.9 $\pm$ 22.1	150.1 $\pm$ 8.4	155 $\pm$ 5.3
ADGR (mg day <sup>-1</sup> )	0.213 $\pm$ 0.04 c	0.241 $\pm$ 0.06 bc	0.266 $\pm$ 0.07 b	0.275 $\pm$ 0.05 b	0.296 $\pm$ 0.03 b	0.346 $\pm$ 0.08 a
Cf	0.44 $\pm$ 0.02	0.47 $\pm$ 0.02	0.54 $\pm$ 0.05	0.50 $\pm$ 0.02	0.52 $\pm$ 0.02	0.54 $\pm$ 0.02
FCR	3.09 $\pm$ 0.1 b	3.05 $\pm$ 0.08 b	3.01 $\pm$ 0.1 b	2.79 $\pm$ 0.1 a	2.78 $\pm$ 0.04 a	2.66 $\pm$ 0.02 a
SGR (%/day)	1.53 $\pm$ 0.3 d	1.56 $\pm$ 0.2 c	1.59 $\pm$ 0.4 bc	1.60 $\pm$ 0.2 bc	1.62 $\pm$ 0.5 b	1.67 $\pm$ 0.3 a

Mean values and standard deviations ( $\pm$ SD) are presented for each parameter. Values within the same row not sharing common lowercase letters are significantly different ( $P < 0.05$ ) ( $n = 3$ )

ADGR average daily growth rate, Cf condition factor, FCR feed conversion ratio, SGR specific growth rate

**Table 3** Reproductive indicators and survival rate of red cherry shrimp (*Neocaridina davidi*) broodstocks fed diets containing different inclusion levels of *Spirulina platensis* meal over 11 months of experimental period

Reproductive parameters	Diets					
	Control	SP1	SP3	SP5	SP8	SP10
Pre-maturation period (days)	15.7 ± 1.6 a	15.3 ± 2.5 a	15 ± 1.8 a	12.67 ± 3.2 b	12.3 ± 2.5 b	11.67 ± 1.6 b
Age of first spawning (days)	62.7 ± 2.4 a	62.3 ± 2.8 a	60 ± 3.1 a	57.67 ± 4 b	57.3 ± 2.7 b	56.7 ± 1.9 b
Inter-spawning interval (days)	38.4 ± 6.25 c	38.9 ± 4.2 c	38.3 ± 2.3 c	35.2 ± 4.1 b	34.8 ± 3 b	32.6 ± 3.2 a
Total egg production <sup>a</sup>	197.3 ± 25.2 e	218 ± 14.4 d	225 ± 10.8 d	246.7 ± 12.2 c	317.7 ± 18.7 b	363.3 ± 16.4 a
Absolute fecundity (eggs per female)	24.67 ± 5.18 d	27.25 ± 8.8 c	28.08 ± 8.9 c	30.83 ± 11.3 c	39.71 ± 15.2 b	45.4 ± 15.18 a
Relative fecundity (eggs per g wet body weight)	35.7 ± 3.1 b	36.8 ± 5.5 b	35.1 ± 3.5 b	39.4 ± 2.1 b	49.15 ± 3.2 a	52.85 ± 2.7 a
Actual fecundity <sup>b</sup>	17.46 ± 0.9 e	21.5 ± 0.5 d	22.7 ± 0.8 d	26.1 ± 0.6 c	36.71 ± 0.4 b	42.75 ± 0.5 a
Hatching percentage (%)	70.8 ± 4.1 c	78.8 ± 3.2 b	80.7 ± 3.6 b	84.8 ± 3.2 b	91 ± 2.5 a	94.2 ± 1.8 a
Female final weight (mg) <sup>c</sup>	69 ± 3	73 ± 5	75 ± 7	77 ± 9	79 ± 11	85 ± 15
Survival rate (%)	25.7 ± 2.5 e	40 ± 2.1 d	57.3 ± 2.3 c	73.7 ± 4.2 b	75.3 ± 3.06 ab	81.3 ± 1.2 a
Initial larval length (mm)	0.48 ± 0.03 b	0.48 ± 0.03 b	0.50 ± 0.03 b	0.50 ± 0.04 b	0.52 ± 0.07 b	0.60 ± 0.04 a

Mean values and standard deviations (±SD) are presented for each parameter. Values within the same row not sharing common superscript letters are significantly different ( $P < 0.05$ ) ( $n = 3$ )

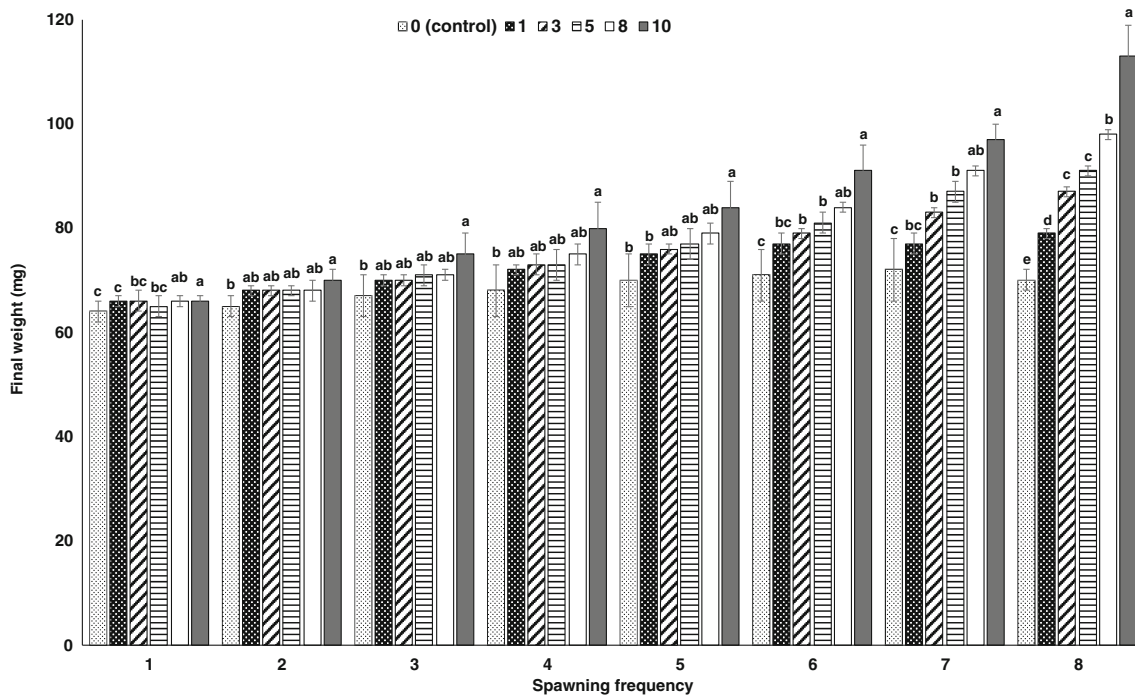
<sup>a</sup> Values are total egg production of females over eight consecutive spawning steps

<sup>b</sup> Mean values (±SD) of the total number of hatched juveniles after the incubation period

<sup>c</sup> Values are the average final weights of females from eight consecutive spawning steps

control groups of *Spirulina* (%) in the diets had significantly ( $P < 0.05$ ) greater FCRs (3–3.1) than those fed diets with 10, 8, and 5% of SP (FCR; 2.66–2.79, respectively). SGR values

were highest ( $P < 0.05$ ) in shrimps fed 10% SP whereas there were no significant differences ( $P > 0.05$ ) in shrimp fed with 3–8% SP (Table 2).



**Fig. 1** Mean final weight of red cherry shrimp (*Neocaridina davidi*) females ( $n = 3$ , mean ± SD) fed with diets containing different inclusion levels of *Spirulina platensis* meal in each spawning event (eight

consecutive spawning) during experimental periods. Values with the same superscript are not significantly different ( $P > 0.05$ )

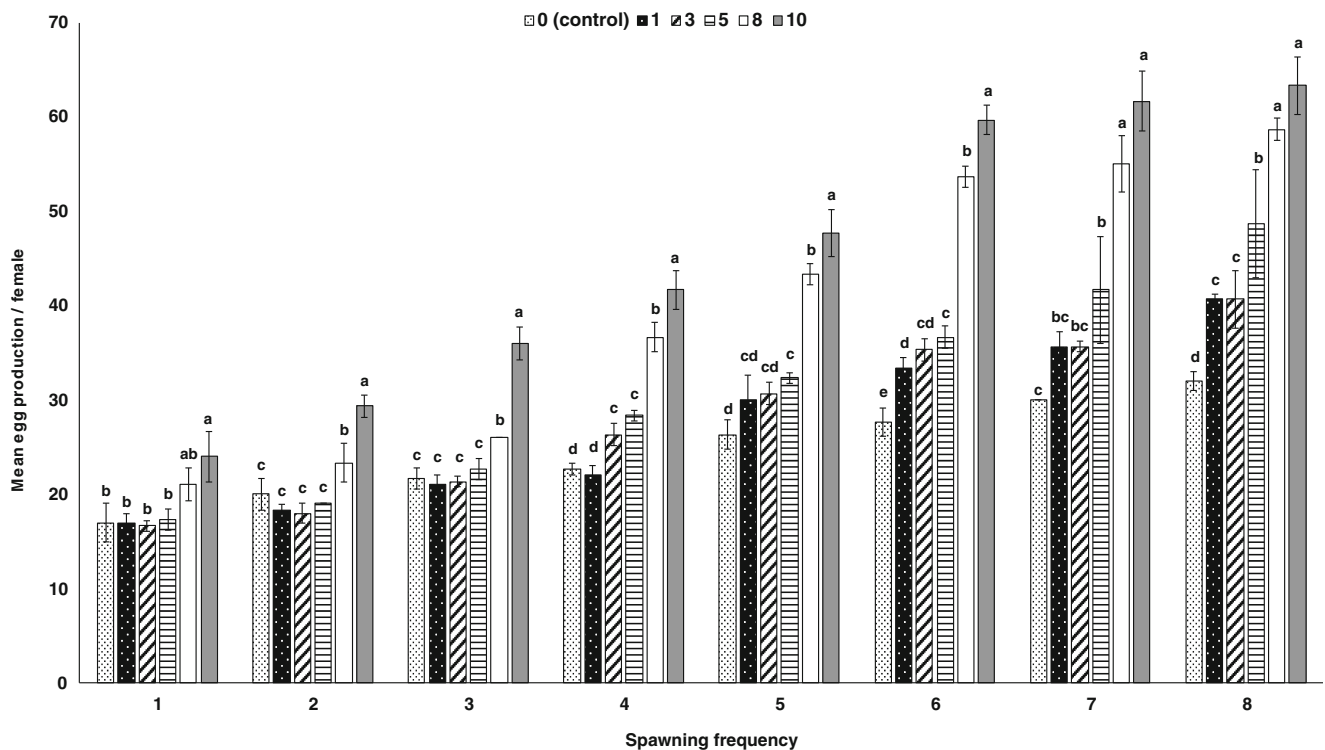
### Reproductive indices and survival rate of females

The reproductive parameters of RCS shrimp up to first spawning showed that the brooders with final weights of 64–66 mg reached the first sexual maturity within 11.7–15.7 days after initial stocking at different treatments. The time required for the maturation of shrimp fed different incorporation levels showed significant differences ( $P < 0.05$ ) between treatments. On the other hand, *Spirulina*-fed groups with higher inclusion levels (5–10%) displayed accelerated sexual maturity. The time interval (days) between two successive egg productions (inter-spawning intervals) showed a decreasing trend with dietary *Spirulina* levels up to 10% (Table 3).

Mean absolute fecundity (eggs per female) varied ( $P < 0.05$ ) among treatments resulting from the incorporation levels being highest in the order of SP10 (45), SP8 (39), and SP5 (30) followed by SP3 (28), SP1 (27), and the control (24). Significant ( $P < 0.05$ ) declines in relative fecundities (eggs per gram female’s wet body weight) were recorded in the control up to SP5 (35–39) group compared with those observed in the other treatments (SP8 and SP10, 49–52 eggs/g female wet body weight, respectively). Except the control group, all *Spirulina*-fed shrimps in levels of 1–5% had comparable ( $P < 0.05$ ) hatching percentages (78–84%) compared with those in SP8 (91%) and SP10 (94%). The resultant larval

length in brooders fed 10% *Spirulina* was significantly ( $P < 0.05$ ) larger compared with the other groups, and no significant differences ( $P > 0.05$ ) were detected between the control and the other treatments received dietary *Spirulina* levels of 1–8%. The survival rate of female shrimps tended to elevate with increasing dietary *Spirulina* levels up to 10% with the higher rates (75–81%) in shrimps fed diets containing 8–10% *Spirulina*, which were significantly different ( $P < 0.05$ ) from those found in the other algal-fed groups (40–73%). Shrimps fed with the control diet showed the lowest survival value (25.7%) after 11 months (Table 3).

The mean final weight of RCS broodstocks showed differences ( $P < 0.05$ ) in each spawning event with different *S. platensis* inclusion levels. Apart from the control, all *Spirulina*-fed groups revealed no significant differences ( $P > 0.05$ ) in final body weight up to the 5th stage of spawning. However, the broodstocks fed 8–10% *Spirulina* were significantly different from other treatments in 6–7th spawning event being more noticeable in the 8th spawning step in shrimp fed SP10 ( $P < 0.05$ ) compared with other treatments. The average weight of the females from the first to the eighth spawning events varied between 66 and 70, 66 and 79, 66 and 87, 65 and 91, 65 and 98, and 66 and 113 mg in the control, 1, 3, 5, 8, and 10% SP, respectively. Consequently, lower weight gain was found in the control group compared with the other treatments fed *Spirulina* (Fig. 1).



**Fig. 2** Mean egg production per female (absolute fecundity) of red cherry shrimp (*Neocaridina davidi*) broodstocks ( $n = 3$ , mean  $\pm$  SD) in each spawning event (eight successive step) fed with diets containing

different levels of *Spirulina platensis* meal. Values with the same superscript are not significantly different ( $P > 0.05$ )

The absolute and relative fecundities of algal-fed females showed differences ( $P < 0.05$ ) in all stages of the spawning events at different *S. platensis* inclusion levels. The spawners fed with 10% *Spirulina* had noticeably higher absolute fecundity in all steps of spawning. However, the shrimp fed 8 and 10% *Spirulina* showed no significant differences with each other ( $P > 0.05$ ); both groups showed significant differences ( $P < 0.05$ ) with the other groups in the seventh and eighth steps of spawning (Fig. 2).

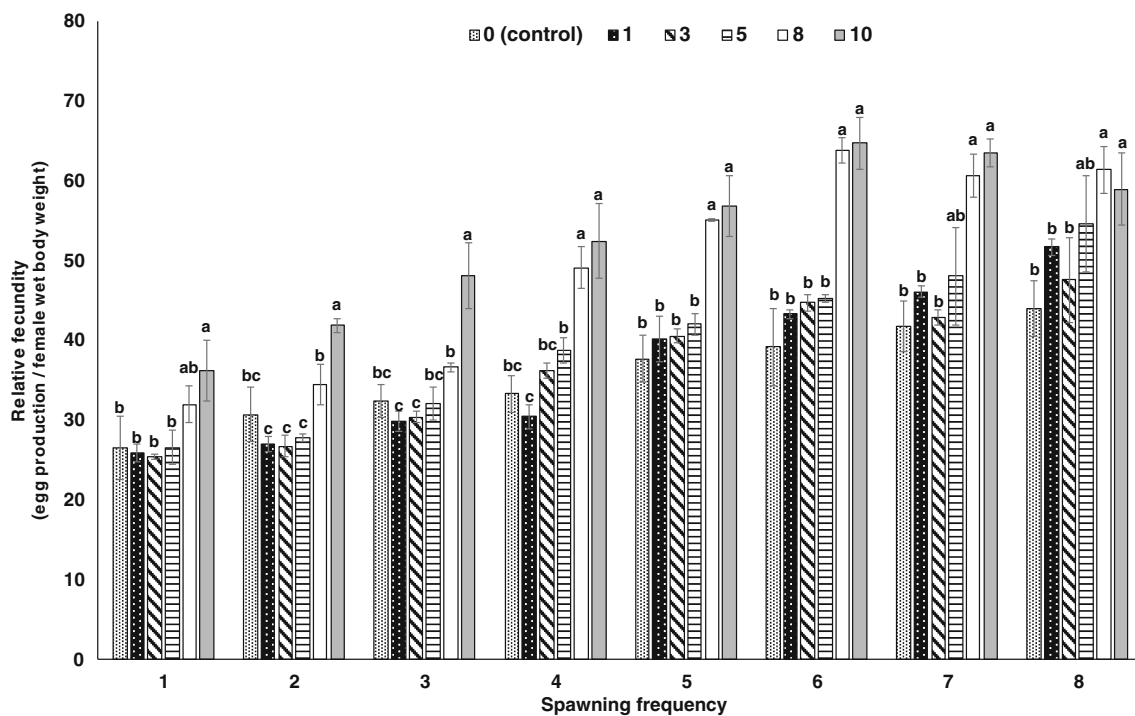
The relative fecundity in the brooders fed with 8–10% algal diet showed relatively significant differences ( $P < 0.05$ ) compared with the other groups from the fourth to the sixth events of the spawning. However, the relative fecundity in broodstock fed 5–10% *Spirulina* were not different from each other ( $P > 0.05$ ), all three groups revealed differences ( $P < 0.05$ ) with the other groups during the 7th–8th spawning steps (Figs. 3 and 4).

### Body proximate composition

Lipid (0.45–0.83%) and moisture (70.74–73.87%) contents of whole shrimp body indicated no changes among all treatments, and protein content showed an increasing trend with dietary *Spirulina* levels up to 10%. Except the control group, the protein contents (20.8–25.34%) were not affected by the different dietary *Spirulina* levels. The lowest ash content (1.40–1.62%) was recorded in SP10 treatment (Table 4).

### Discussion

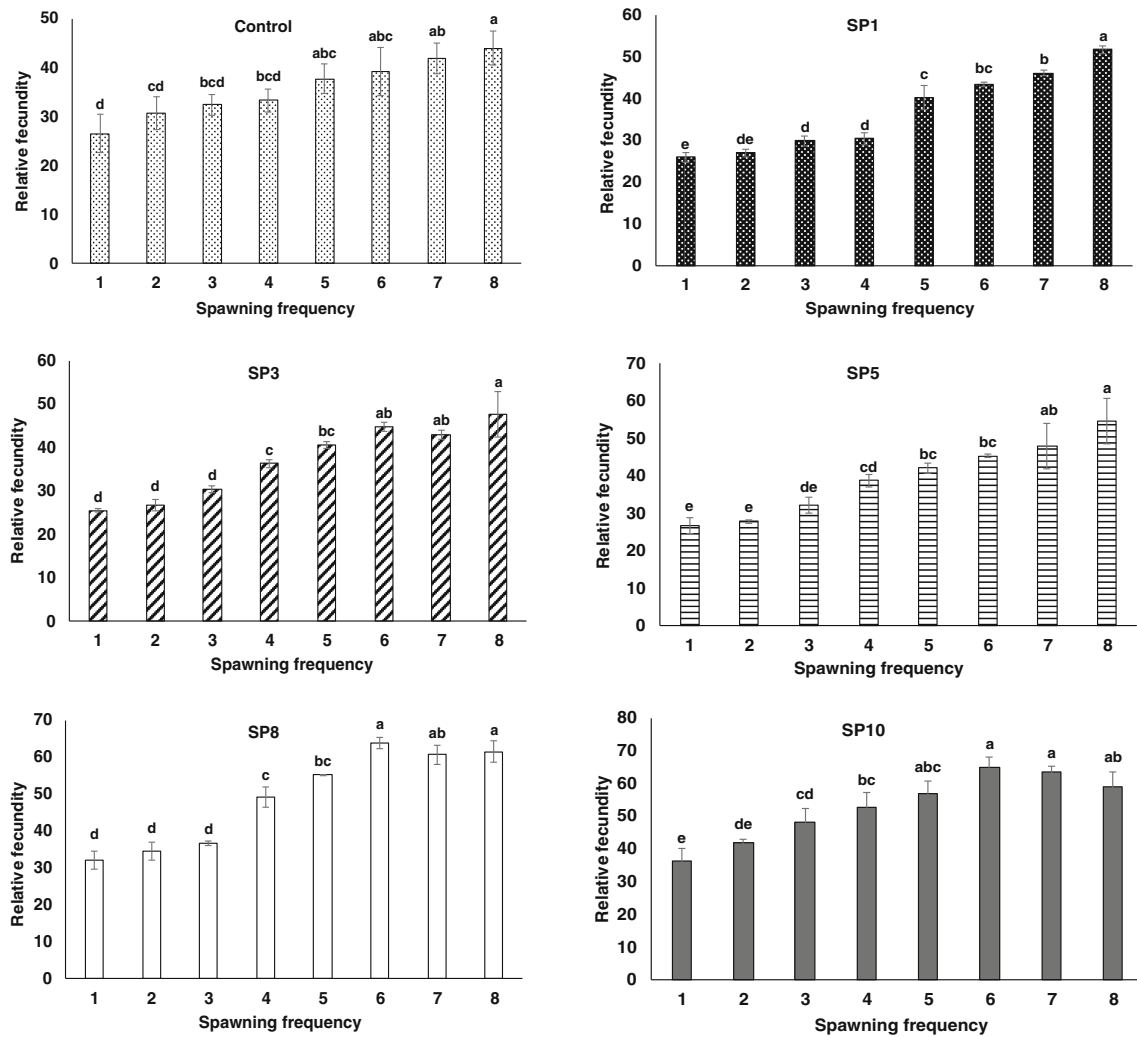
Unlike most previous studies, this is the first investigation that evaluated the influence of dietary incorporation of *S. platensis* meal on the reproductive performance in red cherry shrimp (*N. davidi*), as a model of ornamental shrimps, over multiple spawnings. According to the results, the algal diet containing 10% *Spirulina* yielded better performances in terms of growth indices, final weight, and length of shrimp with statistical differences with other treatments. Most of the growth characteristics displayed increasing trends by the algal meal inclusions from 1 to 10% during feeding trials so that all the diets containing *Spirulina* (even 1% SP) led to relatively better weight gains compared with the control. These results suggest that *S. platensis* meal contains high levels of protein that might have a promising impact on growth indices of RCS shrimp (WG, LG, and SGR) compared with the group fed an algal-free diet. Our findings in *N. davidi* are in agreement with the results of Cuzon et al. (1981), who incorporated 8% of lipid free fraction of *Spirulina* meal and observed promoted growth and survival in *Penaeus japonicus*. In another study, Nakagawa and Gomez-Diaz (1995) found significant improvements in growth, survival, pigmentation, and protein utilization of giant freshwater shrimp (*Macrobrachium rosenbergii*) fed 5–10% inclusion of whole *Spirulina* meal diets and attributed the enhancements to protein assimilation promotion. Also, enhanced growth shrimp (*L. vannamei*) was reported by the inclusion of 9% whole microalgae



**Fig. 3** Mean egg production per wet body weight of female (relative fecundity) of red cherry shrimp (*Neocaridina davidi*) ( $n = 3$ , mean  $\pm$  SD) in each of the spawning events (eight successive step) fed

with diets containing different incorporation levels of *Spirulina platensis* meal. Values with the same superscript are not significantly different ( $P > 0.05$ )





**Fig. 4** Comparison of relative fecundities (eggs per wet body weight of females) in red cherry shrimp (*Neocaridina davidi*) females ( $n = 3$ , mean  $\pm$  SD) at different dietary treatments during eight consecutive spawning step. Values with the same superscript are not significantly different ( $P > 0.05$ )

*Thalassiosira weissflogii* or *Nannochloropsis* meals (Ju et al. 2009). In a recent study, Abdul Basri et al. (2015) reported that a meal derived from "green water cultures" proved to be a good source of carotenoids and was considered as an acceptable alternative protein source for the shrimp, *L. vannamei*, with a dietary inclusion level of about 10% with no significant negative effects on survival, FCR, and SGR.

The beneficial effects of micro- and macroalgae inclusions (in a range of 0.05–20%) in the diets of fish and

shrimp species have previously been declared in terms of enhanced growth, nutritional performance, health, and coloration (Mustafa and Nakagawa 1995; Habib et al. 2008; Güroy et al. 2012; Silva-Neto et al. 2012; Teimouri et al. 2013; Zhu et al. 2015). A tendency toward better growth performance was detected at 5, 8, and 10% *Spirulina* inclusion levels in the current study, which suggests that the microalgal incorporation might have improved food absorption efficiency by increasing bacterial colonization in

**Table 4** Final whole body proximate composition (% dry weight;  $n = 2$ ) of red cherry shrimp (*Neocaridina davidi*) fed with diets containing different inclusion levels of *Spirulina platensis* meal

Body composition	Diets					
	Control	SP1	SP3	SP5	SP8	SP10
Moisture	73.8–73.87	73.2–73.3	72.07–72.23	72.15–72.36	71.36–72.1	70.74–71.16
Crude protein	20.07–20.31	20.8–21.6	21.3–22.08	22.77–23.18	22.85–23.8	24.2–25.34
Crude lipid	0.62–0.83	0.60–0.72	0.61–0.69	0.53–0.69	0.46–0.69	0.45–0.63
Ash	2.32–2.53	1.91–2.02	2.41–2.52	1.9–2.06	2–2.21	1.40–1.62

the digestive tract as well as through the decomposition of indigestible feed components to extractable nutrients in intestinal flora (James et al. 2006) or as a result of promoted diet attractiveness (Silva-Neto et al. 2012). Previous researchers proposed that bioactive compounds (such as growth hormones, nucleotides, vitamins, and minerals, free amino acids and fatty acids, pigments, and compounds inducing gene expression) in the diets containing *Spirulina* might constitute effective agents to enhance the attractability of the feed (Distel et al. 1992; Fafournoux et al. 2000; Brown 2002; Clarke et al. 2002; Jaime-Ceballos et al. 2005; Spolaore et al. 2006). For example, Jaime-Ceballos et al. (2005) determined that *S. platensis* included at 5% in the diet of *L. schimitti* improved the attractability of the feed owing to bioactive compounds such as nucleotides, amino acids and/or pigments. The researchers found that *Spirulina* has approximately 8.7% of glutamic acid, 6.6% of aspartic acid, and 5% of leucine; hence, the higher growth performance in shrimps fed diets containing *Spirulina* might be attributed due to the high contents of glutamic acid as a promoter of feeding effector and/or a chemoattraction agent for penaeid shrimp. Ju et al. (2012) reported that a low inclusion (3% of defatted microalgal meal (DDM), *Haematococcus pluvialis*) significantly enhanced the growth of Pacific white shrimp resulting from some bioactive compounds in DDM or from some health benefits of DMM. Although the fatty acid and amino acid profiles of diets were not analyzed in this study, the outcomes of growth performance demonstrated significant effects of the diet containing the highest *Spirulina* level on the final body weights during all spawning events.

In the present study, the average survival rate of RCS broodstocks was highly influenced by the inclusion levels of *S. platensis*, showing positive supplementary effects of the diets formulated with microalgal biomass on considerable health enhancement. The survival rates of RCS shrimps in all groups fed with diets containing *Spirulina* were remarkably higher (1.5–3.2 times) than the control throughout the experimental rearing period. Significant improvement in immunological factors was found in Pacific white shrimp when fishmeal was replaced with *S. platensis* meal, indicating that microalgae may stimulate and/or enhance the immune system of shrimp (Macias-Sancho et al. 2014; Wells et al. 2017). Higher FCR values were observed in shrimps fed with either the control or lower inclusion diets; however, diets containing 5–10% SP showed more satisfactory results in terms of FCR. Our findings are in agreement with Silva-Neto et al. (2012), who conducted a study with *S. platensis* as an attractant and observed better FCRs in the shrimp studied. They concluded that this microalga had the ability to stimulate food intake even at low inclusion levels. Such observations have been reported in fish, Zhu et al. (2015), for instance, examined white spotted snapper *Lutjanus stellatus* juveniles with 5%

inclusion level of *Ulva lactuca*, which revealed better performances in terms of WG, SGR, and FCR than those in other (10–20%) inclusions.

The protein contents of shrimps fed *S. platensis* in the current experiment increased parallel to increasing dietary inclusion levels. The palatability of *Spirulina* meal in the diets may improve feed consumption, which in turn directly increases the body carcass composition (Habib et al. 2008; Radhakrishnan et al. 2016). However, the replacement of fish meal with *H. pluvialis* meal in the diet of Pacific white shrimp did not affect the whole body proximate composition (Ju et al. 2012). In the present study, the lipid content decreased in shrimps fed on diets containing increasing SP levels. This result is in accordance with that of Radhakrishnan et al. (2016) in *M. rosenbergii* fed on diets having higher levels of fishmeal replacement (75 and 100%) by *A. platensis* meal.

### Reproductive performance indices

To date, studies aiming at supplementing shrimp diets with *Spirulina* meal have been restricted to growth, survival, body pigmentation, and flesh quality. Few researches have also investigated the influence of dietary algal carotenoids on the reproductive performance of shrimp (Wyban et al. 1997; Regunathan and Wesley 2006). Positive effects of *Spirulina* supplementation on reproductive indices have been only reported for four fish species including swordtail, cichlid, three-spot gourami (ornamental fish), and tilapia (commercial fish) (Lu and Takeuchi 2004; James et al. 2006; Güroy et al. 2012; Khanzadeh et al. 2016; Karadal et al. 2016). Since the RCS juveniles were not fed with the experimental diets during the first 45 days of the culture period, the first maturity cannot be definitively attributed to the impacts of diets containing *Spirulina*. However, shrimps fed diets containing *Spirulina* levels of 5–10% experienced a significantly early maturity.

Among the treatments, SP10 noticeably increased total egg production and absolute fecundity of females as 1.8 times the control. Additionally, the females fed the algal diets (8 and 10% SP) exhibited higher values of relative fecundities and hatching percentages in comparison with the other groups. These results clearly signify the positive effects of higher inclusion levels of algal diets on the reproductive performance of RCS females. Similarly, Millamena and Qunitio (2000) reported that provision of a formulated diet in combination with natural food resulted in improved consistency in the reproductive performance of mud crab (*Scylla serrata*). Similar results were presented with significantly higher fecundity in the female swordtail, *Xiphophorus helleri*, fed 8% of *Spirulina* meal compared with other groups (5, 3, 1, and 0% algal meal) (James et al. 2006). In a recent study on blue gourami (*Trichopodus trichopterus*), Khanzadeh et al. (2016) have

found highly increased total egg production and relative fecundity in females fed diets containing 8 to 9.5% of *S. platensis* meal. In addition, Regunathan and Wesley (2006) reported that a dietary *Spirulina* inclusion of 3% in a maturation diet supplied the required carotenoids to shrimps undergoing maturation, which enhanced the number of eggs per spawn, nauplii per spawn, and nauplii viability. The mean fecundity of female *N. davidi* received algal diets of 5–10% SP in the current study revealed somewhat higher estimates compared with a range of 20–55 eggs per female over consecutive spawns reported by Tropea and Lopez Greco (2015). Meanwhile, absolute fecundity over successive spawnings in our study was highest in the last spawns compared with the first one. This result contradicts findings reported by Tropea and Lopez Greco (2015), who obtained the highest fecundity in the first spawn, which remained relatively constant until the fifth spawn in *N. davidi*. Arcos et al. (2003) found no differences in the number of eggs per spawn between the first and successive broods of *L. vannamei* under controlled conditions.

The fatty acid composition and total carotenoid contents of diets and *N. davidi* eggs were not analyzed in this study over consecutive spawns, but existing evidence indicates that dietary fatty acid profile reflects eggs fatty acids. *S. platensis* has been found to be the best algal source of *n*-6 fatty acid (41.2% of total fatty acids), especially of linoleic and  $\gamma$ -linolenic acids (Kim et al. 2012) both of which are precursors of arachidonic acid, a major component in the formation of prostaglandins as mediators of steroidogenesis, oocyte maturation, and ovulation (Patino and Sullivan 2002). Also, *S. platensis* contains natural pigments such as phycocyanin chlorophylls, and carotenoids, vitamins A and E, and phenolic components, which together exert important physiological actions in crustaceans (Goiris et al. 2012; Wade et al. 2015). For example, carotenoids have previously been suggested to have the capacity to trigger shrimp vitellogenesis being directly related to the transcription of hormone genes involved in ovary maturation (Dall et al. 1995; Linan-Cabello and Paniagua-Michel 2004). Several researchers (Wyban et al. 1997; Regunathan and Wesley 2006) have highlighted that there is a need to investigate the most appropriate sources of dietary carotenoids that should be incorporated in broodstock shrimp diets in order to prevent declined reproductive performances of broodstock kept in captivity after several spawning events. These issues and also the influences of maturation algal diets on the aging needs and biochemical composition of eggs over successive spawnings should be investigated in the red cherry shrimp in the future. However, the main reasons accounting to increased reproduction performance in shrimp broodstocks fed on diets containing *Spirulina* are still unclear.

A positive relationship was reported between both absolute and relative fecundities and female weight, independent of spawning order, in penaeid and caridean shrimps (Peixoto et al. 2004; Lara and Wehrtmann 2009). Comparisons of relative fecundities within eight successive spawning events in our RCS shrimps showed that 8–10% *Spirulina*-fed brooders had significantly higher fecundities ( $>50$  eggs  $g^{-1}$  body weight) up to the 5–6th spawning stages in comparison with those ( $<50$  eggs  $g^{-1}$  body weight) fed the other incorporation levels up to the 8th spawning step. Interestingly, it was observed that the relative fecundity of females fed with 8–10% *Spirulina* showed an increase only up to sixth spawning event followed by a decreasing trend (with no significant differences) until the eighth spawning event. Despite statistical differences in the body weight of females fed with higher incorporation levels (8–10% *Spirulina*) with other nutritional groups at 7th and 8th spawns, the relative fecundity showed a decreasing trend with increasing weight (and/or age) (Fig. 4). Rothlisberg (1998) highlighted the possibility of senescence occurring in larger (older) penaeid shrimps negatively affecting their reproductive performance; Calado et al. (2005), on the other hand, did not find any evidence of senescence in embryo production by *Lysmata seticaudata* or even biochemical composition of developing embryos. In contrast, Tropea and Lopez Greco (2015) have reported that total lipid and energy contents of *N. davidi* eggs decreased in the 4th and 5th spawns as a consequence of multiple spawning, and that females could not accumulate and/or mobilize the same levels of lipids in the re-maturing ovary over consecutive spawnings. With respect to the effect of multiple spawning on egg biochemical composition in *L. vannamei*, Palacios and Racotta (2003) and Arcos et al. (2003), respectively, presented evidence of a similar transfer of reserves to the eggs or even an increased transfer over successive spawns. In view of these results, our finding of a decreasing trend in relative fecundity up to the sixth event could have probably caused by decreased lipid deposits in the eggs resulting from multiple spawning. This corresponds to the findings of Tropea and Lopez Greco (2015), who detected decreased lipid concentrations and energy contents in eggs of the fourth and fifth spawns, which may indicate a decrease in the maternal supply over multiple spawning.

Considering the fact that the feed consumption level is very low in broodstocks of this shrimp species, higher inclusion levels of *Spirulina* (8–10%) in this study is financially justifiable. Based on the obtained results, it is recommended that an addition of 8–10% *Spirulina* meal is a viable strategy to increase survival rate and reproductive indices in red cherry shrimp during maturity. However, a higher relative fecundity was only observed up to the sixth spawning event, which gradually decreased afterwards up to the eighth event, indicating that it negatively affects the reproductive performance of this species.

**Acknowledgments** The authors are grateful to the Hamoon Ornamental Fish and Crustacean Culture Center (Marlik Town, Tehran, Iran). The research was funded by the Sari Agricultural Sciences and Natural Resources University (SANRU), Sari, Iran. Sincerest appreciations are credited to Dr. Mohammad Kazem Khaledi and Dr. Ali Jafarpour for editing the English revision.

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