

## Comparative effects of pure spirulina powder and other diets on larval growth and survival of green tiger shrimp, *Peneaus semisulcatus*

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### Abstract

This experiment was carried out in Bandargah Station in Bushehr province. *Peneaus semisulcatus* larvae were fed on *Spirulina platensis* as supplementation microalgae. In order to use *spirulina platensis* in this study, we have to produce biomass and dry production of this microalga. Determination of length and survival rate of *Peneaus semisulcatus* larvae was carried out as the objective of the study. Treatments including Z plus (as a supplementation without spirulina), M.C.F (as an imported enriched spirulina), the powder combination of *Spirulina platensis* with Z plus and *Spirulina platensis* as live food were compared to *Cheatocerus* microalgae as a control. The experiment was carried out from nauplii stage to early post larvae. The results of the experiment indicated that the powder combination of *Spirulina platensis* with Z plus treatment has the most survival rate (76.5%). The maximum of length (4.3mm) observed in control (*Cheatocerus spp.*). The maximum mortality was observed in zoa stage in spirulina microalgae treatment, because the spiral filamentous of *spirulina sp.* was large.

**Keywords:** *Spirulina platensis*, *Peneaus semisulcatus*, Length, Survival

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## Introduction

Proper nutrition has long been recognized as a critical factor in promoting normal growth and sustaining shrimp health. The development of high-quality diets is a factor that has significantly contributed to explosive expansion of shrimp farming in the last two decades. Larval phase is the most sensitive stage in shrimp life, especially when the yolk sac is absorbed. In this stage, suitable food is necessary to inhibit mortality and provide normal metabolism (Hagh Nejat et al., 2001). Shrimp larval growth in aquaculture has traditionally been dependent on live food such as *Chaetoceros*, *Tetraselmis*, *Skeletonema* and *Chlorella* (Hagh Nejat et al., 2005; Jaime-Ceballos, 2006). Culturing of live food is, however, expensive and complicated. Prepared diets, on the other hand, not only provide the essential nutrients that are required for normal physiological functioning, but also may greatly contribute to reducing the cost of production by eliminating or, at least, minimizing reliance on the live food and its often expensive associated infrastructure.

Lots of works have recently been carried out to find appropriate prepared diets as alternative sources to live food, among which spirulina powder (SPM), encapsulated spirulina and Z plus supplement are worth mentioning. *Spirulina platensis* is a fast growing cyanobacter of large size (0.5mm) and capable of growing in high salinity and alkalinity. The biomass of *S. platensis* has been recognized to be a "wonderful health food" for having high protein and various compounds such as essential fatty acids (linoleic and gamma linolenic acids), essential amino acids, B-complex vitamins and biopigments (Tri-

Panji and Suharyanto, 2001; Jimenez et al., 2003; Goksan et al., 2007; Volkmann et al., 2008). Several studies have been conducted using dried spirulina as a supplement in diets of crustaceans. *S. platensis* meal is available at a commercial scale; thus its use in feeds for aquaculture is possible (Jaime-Ceballos, 2006). Spirulina have already been tested as a substitute protein source for *Cyprinus carpio*, where equal or even higher growth rates were obtained by diets containing 25% algae meal, replacing 80% of the dietary fishmeal (Sandbank and Hepher, 1978). Addition of spirulina in the diet of giant freshwater prawn (*Macrobrachium rosenbergii*) significantly improved growth, survival and feed utilization regardless of supplementation level in range of 5-20% (Nakagawa and Gomez-Diaz, 1995). Partial replacement of fish meal with *S. platensis* has also been evaluated in juvenile Pacific white shrimp, *Litopenaeus vannamei*, with promising result (Hanel et al., 2007).

With a wide geographic distribution from the south and east Africa to India and Sri Lanka, including the Red Sea, Persian Gulf (Ronquillo et al., 2006), the green tiger prawn, *P. semisulcatus*, is one of the most common and commercially important marine shrimp in the Indo-West Pacific region and enjoys a good aquaculture potential background. In recent years, there has been increasing interest in the culture of *P. semisulcatus* in some countries of the Middle East, including Kuwait and other Gulf countries (Al-Ameeri and Cruz, 1998); Egypt (Sadek and Moreau, 2000) and Turkey (Aktas et al., 2003). Although a considerable range of information on the dietary and

nutritional requirements of a number of penaeid shrimp, including *Penaeus japonicus*, *P. monodon*, and *Litopenaeus vannamei*, have been studied thoroughly for their nutritional requirements (Shiau, 1998), not much information is available for *P. semisulcatus* (Al-Ameeri et al., 2006)

The present work has focused on the application of spirulina powder, produced in Iran as a new and alternative source to live feed in aquaculture industry, and its effect on the growth and survival rates of the larval green tiger shrimp, *P. semisulcatus*, as an endemic species to the Persian Gulf have been compared with other common food in hatcheries.

### Materials and methods

This research was carried out in Bandargah Station of shrimp research center in Bushehr. The pure stock of *spirulina platensis* was imported from Indonesia in order to produce biomass and powder of spirulina, *Spirulina* was cultured in laboratory condition and biomass of spirulina was produced to for powder according to Jourdan procedure (Jourdan, 2001).

This study was conducted in Bandargah Station of Bushehr and the required nauplii of the project were prepared from cultured ablated broodstocks. Our study was designed as a follows: Four treatments from no. 1 up to no. 4 were *Spirulina platensis* powder that produced in Iran, *Spirulina platensis* powder and Z plus combination, Z plus powder and imported concentration of enriched spirulina (M.C.F), respectively and the control was *Chaetoceros sp.* algae (table 1). All treatments and control were prepared in triplicate. Larvae were randomly introduced in polyethylene containers. All containers were cleaned by detergents and disinfected by formalin and washed by tap water. Finally one hundred nauplii were introduced in each container (treatments and control). The containers were fully filled with aerated sea water with 30°C temperature and 36 ppt in salinity. A plan which was carried out four times a day was started from the stage of fifth nauplii to early post larvae. The powder solution with equal amount in each meal was 5mg/l.

**Table 1: Experimental diet for larvae**

Treatment 1	Treatment2	Treatment3	Treatment4	Control
Produced <i>Spirulina platensis</i> powder in Iran	<i>Spirulina platensis</i> powder and Z plus	Z plus powder	Imported concentration of enriched spirulina (M.C.F)	<i>Chaetoceros sp.</i>

*Chaetoceros sp.* as a live food and Z plus powder as a supplement was used in Bandargah hatchery. Imported

concentrations of enriched spirulina (M.C.F) were also uses in private hatcheries in Bushehr. For these reasons

our treatments consist of M.C.F and Z plus powder and *Chaetoceros sp.* was used as a control of experiment. The chemical

composition of the produced *Spirulina platensis* powder in Iran and Z plus powder have been shown in table 2.

**Table 2: The chemical composition of produced *Spirulina platensis* powder in Iran and Z plus powder**

Food	Raw protein	Raw fat	Raw fiber	Moisture	ash
Z plus powder	50	15	2	12	8
Produced <i>Spirulina platensis</i> powder in Iran	46.6	0.1	-	9.46	0.7

At the first stage of mysis, 0.5 gr/l (20 *Artemia* nauplii) of frozen *Artemia salina* nauplii was added for each container. So we had to reduce the amount of algae with the ratio of 25% algae plus 75% *Artemia* nauplii. At three stages of zoa I, zoa III and mysis III, the average number of larva were counted three simultaneous times and the survival rate was measured. The total length of larvae was measured every day by an optical microscope.

#### Statistical analysis

An ANOVA test was applied to compare the effects of different feeds on survival rate and length.

#### Results

The survival rates of *P. semisulcatus* larvae in 3 stages, have been presented in table 3. The average length of *P. semisulcatus* larvae is shown in figure 2 and 3 in all treatments and in the control from zoa to mysis III.

**Table 3: Survival rates of *P. semisulcatus* larvae in 3 stages**

Stage Treatments	Zoa I	Zoa III	Mysis III
1	100	61.6 <sup>a</sup>	56 <sup>c</sup>
2	100	88.8 <sup>b</sup>	76.5 <sup>a</sup>
3	100	100 <sup>c</sup>	7 <sup>d</sup>
4	100	83.3 <sup>d</sup>	59.6 <sup>bc</sup>
control	100	83.3 <sup>d</sup>	68.5 <sup>ab</sup>

In the same column different superscripts show significant differences.

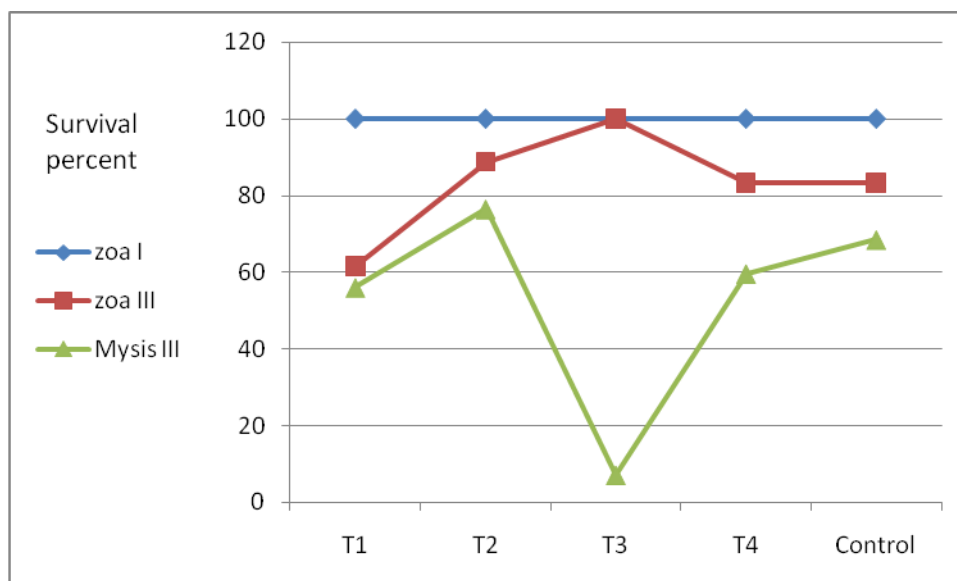


Figure 1: Survival rates of *P. semisulcatus* larvae in 3 stages in two modes

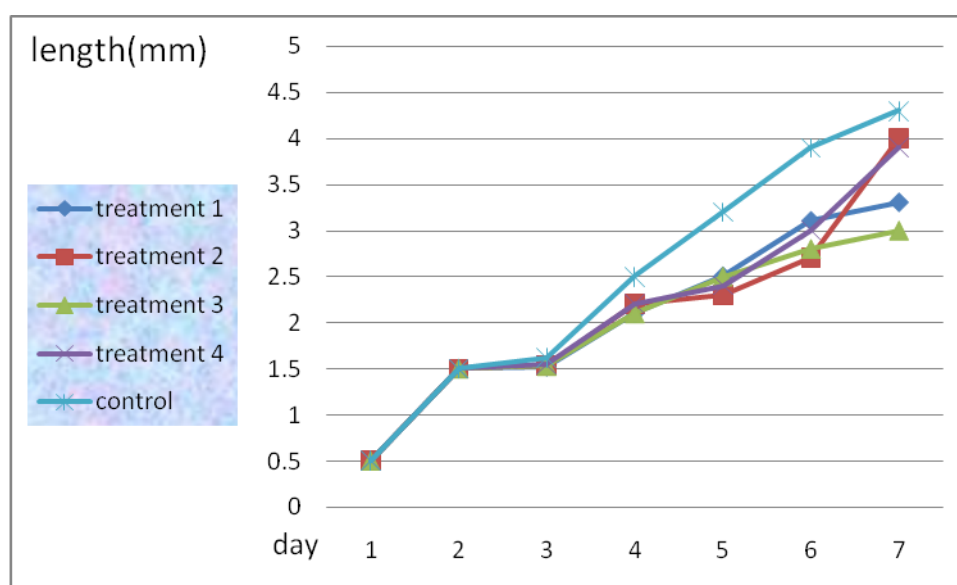


Figure 2: Length of *P. semisulcatus* larvae from zoa to mysis III during 7 days

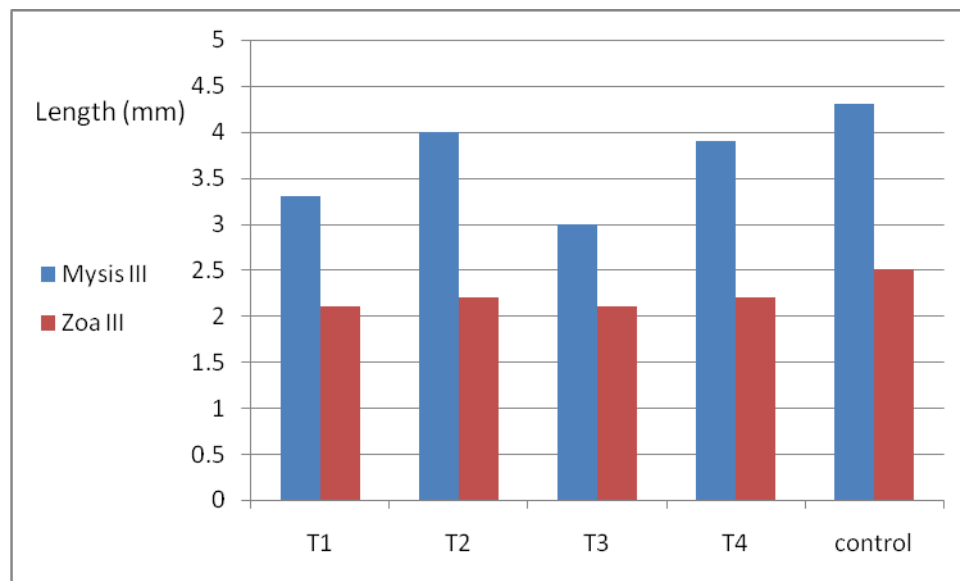


Figure 3: Length of *P. semisulcatus* larvae in zoaIII and mysis III during 7 days

### Discussion

In the zoa stage, larvae start to be active for feeding by catching marine microalgae activity.

The existence of suitable floating food is necessary in the farm tank. *Skeletonema* sp., *Chaetoseris* sp., *Tetraselmis* sp. and *Chlorella* sp. microalgae are usually used in the zoa stage. Other foods such as, egg vitellus, egg and yeast are used as supplement food some times, but the water pollution in the tank will increase (Hagh Nejat, 2005). Also, application of *spirulina* sp. individually or in combination with other foods was used for species of shrimp larvae like *Litopenaeus vannamei* (Hanel et al., 2007), *L. schmitti* (Jaime-Ceballos et al., 2005; Jaime-Ceballos et al., 2006), *Peneaus monodon* in different percents (Ingthamjitr, 1989), while there isn't enough information about the special species of *P. semisulcatus*.

In this research, spirulina and other foods were used in hatcheries individually or in combination to obtain the best growth and survival rates of *P. semisulcatus* larvae. Statistical analysis shows that there

isn't any significant difference in the growth factor but the difference between treatments and control was significant ( $P < 0.001$ ). Another report showed that the average growth of *Litopenaeus vannamei* juvenile fed by lyophilized powder of *Spirulina platensis* was 86mg/day over the experimental period (Hanel et al., 2007). Also, the study of using *Spirulina platensis* in different dry weights for nursing black tiger shrimp post larvae 10 showed that the growth of shrimp larvae fed by using 5% of *Spirulina platensis* was significantly higher (14.25mm) than that of shrimp larvae in control and the survival rates for treatment groups were also higher (69.5%) than that of control (Hemtanon et al., 2008). Among other stages the survival rate in mysis III was significant between the control and the combination of Z plus and the produced spirulina powder in Iran (treatment 2, with the best survival rate) ( $P < 0.001$ ). While the survival rate of *P. semisulcatus* larvae fed only with *Chaetoceros* and *Tetraselmis* in mysis II was 85-96% (Ronquillo et al., 1997), the

survival rate of *P. orientalis* larvae fed on *S. platensis* were only 8.7%; those fed on *C. muelleri*, were 28.7% and those fed on both algae were 17.3% for mysis (Shuli and Baoqing, 2001).

At the stage of mysis III, the survival rate of the produced spirulina powder in Iran was 56%. Some of the problems in this treatment were related to the difficult solvability of spirulina powder in water, and the existence of bubbles on the surface of the container.

Inghamjitr(1989) evaluated the effect of spirulina and *Chaetoceros* on *Peneaus monodon* larvae from nauplii to the post larvae. The best growth was achieved with feeding by *Chaetoceros sp.* and spirulina in 25:75 ratio. While in our project there wasn't any difference between produced spirulina and imported spirulina in growth and survival rates. Their results are the same as our research. The survival rate for their combination of *Chaetoceros* and imported or produced spirulina is more than separate treatments. While in this research, treatment 2 with 1:1 ratio of the produced spirulina and Z plus supplement had the most survival rate (67.5%) in mysis III stage, because Z plus supplement was improved by additive nutrients and after control, the maximum growth was seen in this treatment. Z plus had 15 % of unsaturated fatty acids which was prepared from marine resources.

Jaime-Ceballos et al. (2005) evaluated *Spirulina platensis* in *Litopenaeus schmitti* larvae diet from mysis I to post larvae. The survival rate was about 80% in each treatment. In contrast to control, the size of post larvae was so small. While in this research the survival rate in all treatments

was less than 80% dependent on the amount and times of the feeding (4times in contrast to 6 times feeding), that *P. semisulcatus* larvae are more sensitive than other species.

In the work of Jaime-Ceballos et al. (2006) *Spirulina platensis* powder were replaced by *Chaetoceros mulleri* in *Litopenaeus schmitti* larvae diet and then the length of larvae were compared with each other. There was significant relation between the level of replacement and the length of larvae, the maximum length was acquired in 30 %, but in this research the combination ratio for Z plus and produced spirulina powder was 50% (1:1) and the best survival rate was observed with this ratio ( $P<0.001$ ).

Treatment 3 (Z plus) had 50% protein and 14 % unsaturated fatty acid, but its final survival rate was very low (7%) and in comparison to other treatments it had little growth (3mm), while in zoea III the survival rate of this treatment was 88.8%. This decay maybe related to the situation of larvae in mysis stage. The longest length of larvae was observed in treatment 4 (M.C.F), but its survival rate was 59.6 %. According to the component of this diet it had many different nutrients with high protein such as: meat powder, squid, egg, spirulina...), mineral, vitamins. It is an enriched food that is produced with industrial methods. In comparison to the produced spirulina in this project, it had dark blue color, suitable granulation for larvae and instantly dissolved in water, while the previous produced spirulina in treatment 1 had green color and difficult solvability in water. When the survival rate is decreased

in mysis III, other larvae could take more food and then they have more growth.

Hagh Nejat et al. (2005) evaluated *P. semisulcatus* larvae feeding in zoa stage with *Tetraselmis*, *Chaetoceros*, *Skeletonema* and *Chlorella* individually and syncretistic. The most survival rate in the individual feeding was 82% and the growth rate was 3.55 mm for *skeletonema* and in the syncretistic feeding the best survival rate was 97% in combination with *Tetraselmis* and *Chaetoceros*. The general results showed that the growth rate of larvae in syncretistic feeding was larger than individual feeding.

In our research, the control (*Chaetoceros* sp.) had a fast growth rate with the highest survival rate after treatment 2 (68.5%). *Chaetoceros* had the best size among other microalgae that is used in larviculture for zoa feeding, as live food, which can provide lots of feeding necessities. In the other research *Penaeus indicus* zoa I larvae reared successfully on the mixed algal feed of *Tetraselmis chuii* (25 cells/ $\mu$ l) and *Skeletonema costatum* (35 cells/ $\mu$ l) together with five *Artemia* nauplii per  $\mu$ l from mysis I onwards until metamorphosis in only 6-7 days (Kumlu, 1998). While the survival percentage of *P. monodon* post larvae fed on *Arthrospira fusiformis* was significantly higher at inclusion levels of 10% and 20%. The results suggest that *Arthrospira fusiformis* cannot serve as the sole protein source in the diet of *P. monodon* postlarvae, but can be effectively used as a supplementary protein source (Ketan et al., 2010). This result was the same as our research.

Generally a combination of Z plus (consisting of 50 % protein) and powder

spirulina (dry weight protein 46.6%) were found as the best diet in this project.

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