CHAPTER – III

SPIRULINA AS A CAROTENOID SOURCE IN BROODSTOCK DIET FOR PIGMENT DEFICIENCY CORRECTION

Achieving acceptable reproductive performance from shrimp broodstock in captivity is still a constraint to commercial production of postlarvae. The critical role played by nutritional factors in stimulation of sexual maturation, mating, enhancement of fertility, quality and viability of the offspring has been demonstrated (Primavera, 1985; Harrison, 1990; Bray and Lawrence, 1992; Browdy, 1992). But, our knowledge on the specific nutrient requirements of shrimp broodstock remains limited (Wouters *et al.*, 2001a).

The numerous research on nutrient requirements for shrimp reproduction have demonstrated the requirement of lipids, proteins, carbohydrates, vitamins, minerals and carotenoid pigments (Harrison, 1990 ; Wouters *et al.*, 2001a). Recently, carotenoid pigments have received much interest as one of the vital dietary ingredients for successful shrimp growth and reproductive performance (Thongrod *et al.*, 1995; Wouters, 2001a).

In addition to its role in pigmentation, carotenoids play a number of nutritional and non nutritional roles in aquatic animals, particularly fishes and crustaceans (Torrissen, 1990; Latscha, 1991). Carotenoids have metabolic effect acting as biological antioxidants (Torrissen, 1990; Dall *et al.*, 1995; Merchie *et al.*, 1998) and also serve as precursors to vitamin A (Torrissen and Christiansen 1995; Harrison, 1997). Growth, stress resistance and immune-enhancement effect of carotenoids also have been reported in fishes (Tachibana *et al.*, 1997; Nakano *et al.*, 1999).

In crustaceans, carotenoids may function in reproduction, to protect both the nutrient reserves and developing embryos from oxidation by free radicals and solar radiation, to supply pigment reserves for the embryos and larvae for the development of chromatophores and eyespots, and to provide vitamin A precursor (Zagalsky *et al.*, 1967; Ghidalia, 1985; Nelis *et al.*, 1989). Carotenoproteins of crustaceans present in a variety of tissues such as eggs, ovaries and the integument (Zagalsky *et al.*, 1990) appear to improve the stabilization of proteins (Latscha, 1991).

Crustaceans accumulate carotenoids throughout sexual maturation (Harrison, 1997). During secondary vitellogenesis carotenoids appear to be mobilized from the hepatopancreas to ovaries via haemolymph (Vincent *et al.*, 1988; Harrison, 1990) where they accumulate in the oocytes as part of a major egg yolk protein, lipovitellin (Wallace *et al.*, 1967; Quinitio *et al.*, 1989, 1990). But, as crustaceans like shrimp are unable to synthesis carotenoids *de novo*, the diet is the exclusive source and should include astaxanthin or appropriate precursor (Harrison, 1997). The required dietary levels and effectiveness of different sources of dietary carotenoids on reproductive fitness including the larval quality is a much needed research.

In the shrimp hatcheries, nauplii production systems rely on fresh and fresh-frozen feeds such as oysters, mussels, squid, polychaete worms etc. Although, such mixed diets yield acceptable reproductive performance, a general decline in nauplii quality as indicated by declining larval performance is mostly observed. This decline is characterized by the bleaching (less or loss of pigmentation) in the ovaries of mature female and in the egg yolk, a condition termed as 'pigment deficiency syndrome' (PDS). The PDS is caused by the deficiency of carotenoids in the diet and is characterized by low feeding and high levels of deformities in zoea 1 larvae and low survival to zoea 2 .

Wyban *et al.* (1997) first reported curing of the syndrome (deficiency correction) by addition of paprika (as pigment source) to the broodstock diet of *Litopenaeus vannamei.*

In the commercial shrimp hatchery, where the research work was carried out, regularly faced PDS problem with pond-reared *Fenneropenaeus indicus* broodstock. The symptoms of PDS were observed even just 3 to 4 weeks after ablation. In this study, two experiments were carried out, one with pond-reared broodstock with PDS problem (experiment 1) and the other with normal wild broodstock (experiment 2) to analyse the effect of supplementing a readily available pigment source, *Spirulina,* in the broodstock diet.

Spirulina, a filamentous, microscopic, blue green algae, is a rich source of pigments like carotenoids namely, β-carotene, xanthophylls (zeaxanthin) etc.(Miki *et al.*, 1985). Carotenoid content in commercially available *Spirulina* ranges from 3.5 to 5.7 g per kg. It is also a highly nutritious diet with nearly 70% protein, essential amino acids, minerals, vitamins and some fatty acids. The same has been used as a pigment source for fish (Choubert, 1979; Matsuno *et al.*, 1980; Jalal *et al.*, 2001), and shrimp (Tanaka, 1978; Soong, 1980; Liao *et al.*, 1993; Chien and Shiau, 1998).

The objectives of the experiments are to evaluate *Spirulina* as carotenoid source for shrimp broodstock and to study the effect of its supplementation on reproductive performance. The experiment 1 also tried to establish the effectiveness of carotenoid supplementation in healing PDS. The total carotenoids in eggs and nauplii were determined with spawns collected during various post-ablation periods. The relationship between egg carotenoids content with reproductive parameters, larval performance, nauplii size and egg and nauplii protein content were analyzed.

MATERIALS AND METHODS

The *F. indicus* broodstock (wild as well as pond-reared) were stocked in 3.5 meter diameter circular concrete holding tanks, at a stocking density of 8/m^2 , in a sex ratio of 1:1. The tanks were given water exchange at 100%/day and were under light regime of 14:10 light:dark maintained using blue fluorescent tubes. The temperature and salinity ranged from 30 - 31° C and 33-34 ppt respectively. After one week acclimation period, the females were subjected to unilateral eyestalk ablation, using red hot scissors and introduced in new maturation tanks. The broodstock were fed 5 times daily, total accounting for 12% of wet weight biomass, comprising 40% squid, 20% clam, 15% oyster and 25% cuttlefish.

Experiment 1

The broodstock animals comprising females weighing 43.62 ± 1.8 g and males of 37.93 ±2.2 g size were sourced from the hatchery's broodstock pond facility. This experiment was carried out for 14 weeks duration. Both the control and experimental tanks were fed with the standard feed regime for the initial 4 weeks after ablation. Once the symptoms of PDS started appearing (in the 5 th week), the experimental tanks were fed the squid (chopped) fraction marinated overnight with 3% (3 g *Spirulina* for 100 g squid) *Spirulina platensis* (spray dried powder, food grade, IGV, Institut fur Getreidever arbeitung, Denmark, with a carotenoid content of 4.6 g/kg) in refrigerator at 5°C. The animals were fed twice daily with squid and continued till the end of experiment. Meanwhile, the control tanks were fed equal percentage biomass of non-marinated squid.

Experiment 2

In this experiment broodstock animals from the wild were used (females weighed 51.6 ± 2.4 g and males 44.8 ± 3.1 g). The experimental tanks were fed with squid marinated in 3% *Spirulina* from the day one, while the controls were fed with equal percentage biomass plain squid.

Sampling

Spawns from the same three replicate maturation tanks were used for analyzing the reproductive performance as well as for estimating the biochemical variables. The sampling procedures and determination of maturation performance were as followed in chapter 1. Egg and nauplii samples were collected for 14 weeks period (from the day of ablation) with experiment 1 and for 8 weeks period with experiment 2.

Larval rearing

Nauplii in the 5^{th} or 6^{th} stage (N_5 or N_6) were transferred to larval rearing tanks and allowed to molt to zoea. In experiment 1 (using pond broodstock), the number of zoea 1 (Z_1) with full guts and deformities were noted. Though the larvae were reared till postlarval stage as being part of the commercial cycle, experimental work was restricted to zoea 2 with experiment 1 and till mysis 1 stage in experiment 2.

During the larval rearing, the diet composed of an algal mixture of *Chaetoceros muelleri* (CS-176) and *Isochrysis* sp. (T.Iso., CS-177). The total algal density was maintained at 100,000 cells/ml. Fresh seawater was added up (30% of larval tank volume) during the Z_2 stage and 30% water exchange was given at Z_3 stage. Shrimp larval developments were assessed according to method given by Silas *et al.* (1978).

Biochemical analysis

For biochemical analysis, the methods and sample collection were as described in chapter 1. Proteins were determined in the crude homogenate following Coomassie-blue dye method (Bradford, 1976).

Data analysis

One way ANOVA or student's t-test were used, where relevant, to determine any significant differences between reproductive measures (spawn size and quality) and biochemical composition between treatments. ANOVA, where pertinent, was followed by Tukey's test for unequal n post-hoc mean comparisons to determine treatments that differed significantly from each other. All percentage values were normalized through arcsine transformation prior to statistical analysis (Zar, 1996). The t-distribution test was used to test the significance of correlation.

RESULTS

Experiment 1

The reproductive performance of the pond-reared females are given separately (Table 9) for the two different periods, namely pre-PDS (before the appearance of pigment deficiency syndrome) and post-PDS period (after the appearance of PDS). As usually experienced, with the hatchery operations, the pigment deficiency syndrome appeared (both in control and experimental tanks) during the fourth week of postablation. The experimental tanks were fed with *Spirulina* marinated squid starting from the 5^{th} week till the end of the experiment. The reversal of PDS syndrome was noticed during the 9^{th} week of post-ablation period.

Table 9. Experiment 1: Reproductive performance of pond-reared *F. indicus* broodstock during the pre-PDS period (initial 4 weeks postablation) and post-PDS period (5th to 14th week post-ablation). Animals were fed control diet or *Spirulina* supplemented diet after the appearance of PDS.

Parameters	Control tanks		*Spirulina*- fed tanks	
	Pre-PDS n=30	Post-PDS n=30	Pre- PDS n=30	Post-PDS n=32
Eggs / spawn ($x10^3$)	136.80 ± 2.27 [a]	139.70 ± 2.78 [a]	132.30 ± 2.04 [a]	143.88 ± 2.49 [a]
Fertilization / spawn (%)	70.53 ± 2.47 [a]	59.35 ± 2.26 [a]	67.80 ± 2.37 [a]	61.60 ± 3.44 [a]
Hatch / spawn (%)	69.60 ± 1.72 [a]	51.57 ± 2.22 [b]	71.42 ± 2.22 [a]	63.83 ± 2.29 [a]
Nauplii / spawn ($x10^3$)	67.54 ± 3.46 [a]	42.73 ± 2.65 [b]	65.26 ± 4.18 [a]	57.53 ± 3.12 [a]
Z1 with empty gut (%)	2.66 ± 0.29 [a]	35.60 ± 1.09 [b]	2.80 ± 0.35 [a]	7.00 ± 0.46 [c]
Z1 with deformity (%)	1.07 ± 0.27 [a]	16.00 ± 1.09 [b]	1.20 ± 0.27 [a]	4.37 ± 0.41 [a]
N5 to Z2 survival (%)	86.71 ± 1.98 [a]	34.52 ± 2.11 [b]	83.30 ± 1.46 [a]	69.52 ± 1.48 [c]
Viable nauplii (%)	64.80 ± 1.95 [a]	39.31 ± 1.50 [b]	66.20 ± 1.74 [a]	57.60 ± 1.37 [a]

Means (±S.E.) in the same row with different superscript are significantly different ($P<0.05$)); n= number of samples.

During the initial weeks (Pre-PDS), the data for the reproductive performance between the control and *Spirulina*-fed tanks did not differ significantly ($P>0.05$; Table 9). However, data for the post-PDS (after 4th week), showed a sharp decline in all parameters in the control tanks. This was confirmed by the comparison of control tank values for the pre-PDS and post-PDS period. Analysis indicated that the values differed significantly ($P<0.05$), except for fecundity ($P>0.05$) and percentage fertilization ($P>0.05$).

Effect of PDS was utmost felt in the larval quality parameters (Table 9). In the control tanks, with the appearance of PDS, the percentage Z_1 with empty gut and deformity raised significantly from 2.66% to 35.60% ($P<0.001$) and from 1.07% to 16% ($P<0.001$) respectively. The percentage survival ($N_5 - Z_2$) as well as viable nauplii also showed significant decrease in the control tanks during the post-PDS period ($P<0.001$).

The experimental tank animals, which were fed with *Spirulina*-marinated squid from the fifth week after ablation, also registered declining performance with the appearance of PDS. However, the impact was more only for the initial period (initial three weeks of post-PDS period) and later there was improvement in performance. During the latter post-PDS period, all of the parameters reversed resulting in a better performance.

Comparison of post-PDS results between control and experimental tanks at the end of experiment indicated that Z_1 with empty gut and deformity and N_5 to Z_2 survival differed significantly ($P<0.05$) between them. Analysis also showed that, the hatching percentage/spawn and nauplii/spawn value in the control tank during the post-PDS period differed significantly from the respective values in experimental tanks ($P<0.01$).

The results for the analysis of total carotenoids of egg and nauplii (Table 10) showed that the total carotenoids levels in both decreased continuously in the control tanks as the spawning period progressed. An average value of 3.83 ± 0.11 µg/g (n=13) was obtained with egg carotenoids during the maturation cycle when the PDS symptoms appeared (value not shown in the table). The egg as well as nauplii carotenoid content in the control tanks itself differed significantly ($P<0.05$) between different periods of maturation cycle. Lesser carotenoids value was obtained in nauplii when compared to eggs.

In *Spirulina*-fed tanks, both eggs and nauplii registered progressive decrease in total carotenoids only till the end of the 8th week. The average value for the samples collected during the following two weeks periods recorded slight raise, coinciding with the reversal of PDS symptoms. At the last week (14th week) of experiment, the values for both egg and nauplii had reached values that were not significant ($P>0.05$) from the values obtained with samples collected during the first two weeks. The carotenoid content of both eggs and nauplii from the *Spirulina*–fed tanks showed significant ($P<0.05$) difference with the control tank values from the 9th week till the end of experiment.

The results obtained with the rostrocaudal length of 4th stage nauplii (N_4) from different spawns collected throughout the experimental period are presented in table 11. In both control and *Spirulina*-fed tanks the nauplii size exhibited a decreasing trend with the increase in experimental period. During the post-PDS period, nauplii from *Spirulina*-fed tanks recorded significantly higher values than control tank ($P<0.05$). Correlation analysis indicated a positive relationship ($r=0.48$; $P<0.001$) between the rostrocaudal length of nauplii and egg carotenoid content (Fig. 7).

Table 10. Experiment 1: Total egg and nauplii carotenoids (µg/g) values obtained with spawns collected during different periods of commercial hatchery cycle from pond-reared broodstock fed control diet or *Spirulina* supplemented diet.

Parameters	Postablation period (weeks)					
	0-2	**3-4**	**5-8**	**9-10**	**11-12**	**13-14**
Total Carotenoids (Egg)						
Control	6.32 ± 0.10^{a} n=15	4.85 ± 0.10^{b} n=15	3.74 ± 0.07^{cd} n=26	3.48 ± 0.14^{c} n=10	3.36 ± 0.11^{c} n=10	3.24 ± 0.12^{c} n=10
Spirulina fed	6.17 ± 0.08^{a} n=15	5.14 ± 0.12^{b} n=15	3.97 ± 0.07^{d} n=24	4.56 ± 0.15^{bd} n=12	5.48 ± 0.15^{ab} n=10	5.82 ± 0.10^{ab} n=10
Total Carotenoids (Nauplii)						
Control	5.40 ± 0.11^{a} n=15	4.31 ± 0.15^{a} n=15	3.21 ± 0.10^{b} n=26	2.83 ± 0.15^{bc} n=10	2.39 ± 0.16^{bc} n=10	2.28 ± 0.13^{c} n=10
Spirulina fed	5.51 ± 0.13^{a} n=15	4.67 ± 0.12^{ae} n=15	3.16 ± 0.07^{bd} n=24	3.94 ± 0.14^{de} n=12	4.47 ± 0.23^{ae} n=10	4.76 ± 0.19^{ae} n=10

Within each column as well as row, means (± S.E.) with different superscript are significantly different ($P<0.05$); n= number of samples.

Table 11. Experiment 1: Differences in nauplii length (µm) with spawns collected during different periods of commercial hatchery cycle from pond-reared broodstock fed control diet or diet supplemented with *Spirulina*.

Treatments	Postablation period (weeks)					
	0-2	3-4	5-8	9-10	11-12	13-14
Control	367 ± 1.93^{a} n=15	365 ± 2.04^{ac} n=15	351 ± 2.00^{b} n=26	348 ± 2.22^{b} n=10	352 ± 3.10^{bc} n=10	346 ± 2.88^{bd} n=10
Spirulina -fed	364 ± 2.25^{a} n=15	361 ± 2.24^{a} n=15	357 ± 2.02^{a} n=24	360 ± 2.07^{a} n=12	358 ± 2.37^{a} n=10	358 ± 2.51^{a} n=10

Within each column as well as row, means (± S.E.) with different superscript are significantly different ($P<0.05$)); n= number of samples

Table 12. Experiment 1: Egg and nauplii proteins (mg/g) values obtained with spawns collected during different periods of hatchery cycle using pond-reared broodstock fed control diet or diet supplemented with *Spirulina*.

	Postablation period (weeks)					
	0-2	**3-4**	**5-8**	**9-10**	**11-12**	**13-14**
Proteins (egg)						
Control	157.76 ± 2.76 [a] n=15	146.32 ± 2.85 [a] n=15	140.00 ± 2.47 [a] n=26	136.54 ± 3.32 [a] n=10	137.21 ± 4.72 [a] n=10	128.43 ± 3.71 [a] n=10
Spirulina-fed	159.30 ± 3.47 [a] n=15	148.60 ± 4.00 [a] n=15	137.14 ± 2.42 [a] n=24	143.54 ± 3.93 [a] n=12	149.64 ± 4.08 [a] n=10	154.32 ± 3.91 [b] n=10
Proteins (nauplii)						
Control	88.10 ± 2.68 [a] n=15	82.60 ± 1.66 [a] n=15	71.26 ± 1.44 [a] n=26	68.54 ± 3.27 [a] n=10	66.30 ± 1.55 [a] n=10	63.71 ± 2.47 [a] n=10
Spirulina-fed	94.31 ± 3.20 [a] n=15	87.27 ± 2.57 [a] n=15	67.70 ± 1.53 [a] n=24	70.20 ± 1.85 [a] n=12	72.33 ± 2.44 [a] n=10	84.83 ± 2.83 [b] n=10

Within each column as well as row, means (± S.E.) with different superscript are significantly different ($P<0.05$)); n= number of samples.

The values for egg and nauplii protein are given in table 12. The protein values showed a trend similar to that of carotenoids. A comparison between control and experimental tanks indicated that except for the last two weeks (13-14), the values did not differ significantly ($P>0.05$).

Experiment 2

Results obtained with wild females confirmed that *Spirulina* inclusion significantly improved percentage hatch ($P<0.05$), nauplii/spawn ($P<0.05$), percentage of viable nauplii ($P<0.01$) and N_5 to M_1 survival ($P<0.01$) when compared to control (table 13). The egg and nauplii carotenoids (table 14) registered a decrease as the maturation cycle progressed. Interestingly, the *Spirulina* supplemented tank showed slightly lesser rate of decrease, however, at any point of time, it did not differ significantly ($P>0.05$) from control.

Throughout the experimental period, no significance in size was noticed between the nauplii from control and *Spirulin*-fed tanks (table 15). As in the case of experiment 1, the nauplii from both control and *Spirulina*-fed tanks showed decrease in size as the maturation cycle progressed. Similar to total carotenoids, egg and nauplii proteins (table 16) also showed a decreasing trend towards the end of spawning period. No significant difference ($P>0.05$) in egg and nauplii proteins was noticed between the control and experimental tanks. The egg carotenoid content exhibited positive correlation ($r=0.27$; $P<0.001$) with nauplii length (Fig. 8)

Fig. 7. Experiment 1. Relationship between egg carotenoids and nauplii length in spawnings from pond-reared broodstock

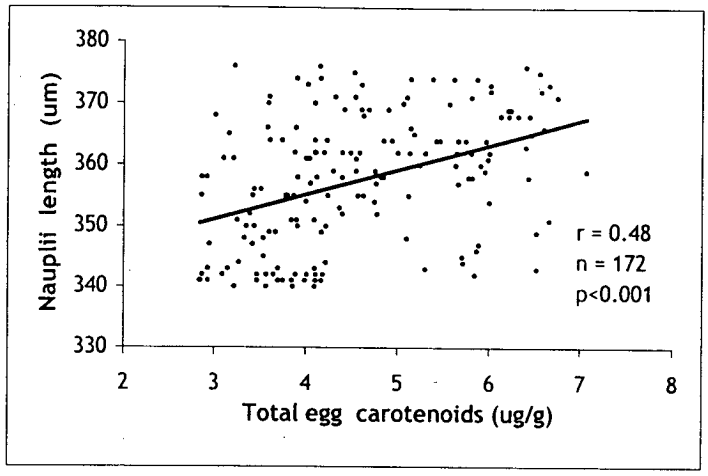

Fig. 8. Experiment 2. Relationship between egg carotenoids and nauplii length in spawnings from wild broodstock

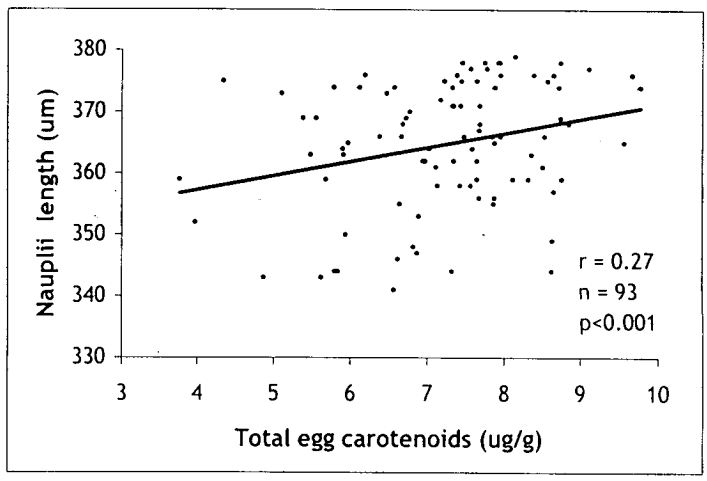

Table 13. Experiment 2: Reproductive performance of wild *F. indicus* broodstock fed control diet or *Spirulina* supplemented diet.

Parameters	Control n=45	*Spirulina* - fed n=48
Eggs / spawn ($x10^3$)	187.61 ± 5.53^a	191.40 ± 4.08^a
Fertilization / spawn (%)	64.74 ± 1.98^a	67.10 ± 2.17^a
Hatch / spawn (%)	64.32 ± 1.68^a	69.13 ± 1.45^b
Nauplii / spawn (10^3)	77.46 ± 3.47^a	89.69 ± 4.46^b
Viable nauplii (%)	57.72 ± 1.25^a	65.40 ± 1.20^b
N_5 to M_1 survival (%)	76.75 ± 1.44^a	84.38 ± 1.21^b

Means (± S.E.) with a different superscript in the same row are significantly different ($P<0.05$).
n=number of samples.

Table 14. Experiment 2: Egg and nauplii carotenoids (µg/g) values with spawns collected during different periods of commercial hatchery cycle using wild broodstock fed either control diet or *Spirulina* supplemented diet.

Parameters	Postablation period (weeks)			
	0-2	3-4	5-6	7-8
Total carotenoids (Eggs)				
Control	8.41 ± 0.20^{a} n=13	7.25 ± 0.19^{b} n=15	6.51 ± 0.23^{b} n=10	5.13 ± 0.38^{c} n=7
Spirulina- fed	8.33 ± 0.18^{a} n=13	7.60 ± 0.16^{ab} n=15	6.94 ± 0.22^{bc} n=12	5.97 ± 0.29^{c} n=8
Total carotenoids (Nauplii)				
Control	6.84 ± 0.18^{a} n=13	5.81 ± 0.23^{ab} n=15	4.16 ± 0.24^{b} n=10	3.93 ± 0.32^{b} n=7
Spirulina- fed	6.80 ± 0.19^{a} n=13	6.32 ± 0.26^{ab} n=15	5.17 ± 0.25^{ab} n=12	4.74 ± 0.26^{b} n=8

Within each column as well as row, means (± S.E.) with different superscript are significantly different ($P<0.05$)); n= number of samples.

Table 15. Experiment 2: Differences in nauplii length (μm) with spawns collected during different periods of commercial hatchery cycle using wild broodstock fed control diet or *Spirulina* supplemented diet.

Treatments	Postablation period (weeks)			
	0-2	3-4	5-6	7-8
Control	371 ± 2.41^{a} n=13	364 ± 2.07^{ab} n=15	359 ± 2.61^{ab} n=10	351 ± 4.30^{b} n=7
Spirulina fed	373 ± 1.75^{a} n=13	368 ± 2.00^{ab} n=15	365 ± 2.12^{ab} n=12	359 ± 3.40^{b} n=8

Within each column as well as row, means (± s.e.) with different superscript are significantly different ($P<0.05$)

n= number of samples

Table 16. Experiment 2: Egg and nauplii proteins (mg/g) values with spawns collected during different periods of hatchery cycle using wild broodstock fed control diet or *Spirulina* supplemented diet.

	Postablation period (weeks)			
	0-2	**3-4**	**5-6**	**7-8**
Proteins (Eggs)				
Control	163.15 ± 5.09 [a]	153.32 ± 5.24 [a]	147.72 ± 4.98 [a]	141.64 ± 8.89 [a]
	n=13	n=15	n=10	n=7
Spirulina- fed	168.72 ± 5.11 [a]	159.27 ± 3.89 [a]	155.44 ± 3.91 [a]	148.86 ± 7.59 [a]
	n=13	n=15	n=12	n=8
Proteins (nauplii)				
Control	94.32 ± 2.65 [a]	81.63 ± 2.78 [ab]	76.34 ± 3.22 [ab]	67.28 ± 3.53 [b]
	n=13	n=15	n=10	n=7
Spirulina-fed	96.68 ± 2.85 [a]	90.38 ± 2.77 [a]	83.26 ± 2.47 [a]	76.81 ± 3.60 [ab]
	n=13	n=15	n=12	n=8

Within each column as well as row, means (± s.e.) with different superscript are significantly different ($P<0.05$)

n=number of samples

DISCUSSION

Various algae materials have been used as pigment source for fishes and crustaceans. It includes *Dunaliella salina* (Harpaz *et al.*, 1998; Chien and Jeng, 1992; Boonyaratpalin *et al.*, 2001), *Chnoospora minima* (Menasveta *et al.*, 1993), *Chlorella vulgaris* (Gouveia *et al.*, 1998), and *Haematococcus pluvialis* (Sommer *et al.*, 1992; Chien and Shiau, 1998; Lorenz and Cysewski, 2000).

Only few authors have worked on the relationship of carotenoids and shrimp maturation (Dall *et al.*, 1995; Dall, 1995; Vincent *et al.*, 1988; Cabello *et al.*, 2003). Similarly, only Wyban *et al.* (1997) and Palacios *et al.* (1999a) have investigated the influence of dietary carotenoids on reproductive performance or larval development of shrimp.

In this study, results from experiment 1 make it evident that appearance of PDS syndrome is associated with the low levels of total carotenoids in the eggs. The low egg carotenoid value reflects the low ovary carotenoids as well as the deficiency of the nutrient in the broodstock diet. As in lipids, transference of carotenoid from the ovary to the eggs has been documented for several crustaceans (Mantiri *et al.*, 1995). Eggs from the pre-PDS females were dark green in color and gave an average carotenoid value of 5.59 ± 0.13 µg/g ($n=54$) and the less pigmented yellow eggs from the PDS females gave an significantly ($P<0.001$) lower mean value of 3.31 ± 0.12 ($n=86$). The symptoms of PDS could also be reversed by carotenoid supplementation in the broodstock diet, with *Spirulina* being the source in these experiments. In the experiment 2 results, it is clear that supplementation with carotenoid source boosts the reproductive performance of wild

females, larval quality and survival. Cabello *et al.* (2004) reported the effect of carotenoids in oocyte maturation of crayfish *Cherax quadrucarinatus.*

Overall results confirm that *Spirulina* could be used as an effective carotenoid source for the *F. indicus* broodstock. *Spirulina* has been found to be a valuable carotenoid source for fish (Matsuno *et al.,* 1980; Jalal *et al.*, 2001) as well as shrimp pigmentation (Tanaka *et al.*, 1976; Tanaka, 1978; Liao *et al.*, 1993; Chien and Shiau, 1998). Astaxanthin or its esters have been found the most prominent pigment in *Penaeus monodon* (Scheidt, 1990; Howell and Mathews, 1991; Latscha, 1991; Okada *et al.*, 1994).

The dominance of astaxanthin or its esters in the ovary of prawn has been determined by Miki *et al.* (1982) and Dall *et al.* (1995). Any carotenoid source supplied to the animal will be converted to astaxanthin through a metabolic pathway and proximity of the carotenoid to astaxanthin in the pathway of synthesis makes it more efficient. In the case of aquatic animals, bicyclic carotenoids like ß-carotene or xanthophylls (zeaxanthin) are ingested from plants and may be converted to astaxanthin (Simpson *et al.*, 1981; Goodwin, 1984; Torrissen *et al.,* 1989; Latscha, 1990).

Boonyaratpalin *et al.* (2001) reported that supplementation of diet with β-carotene (from *Dunaliella salina*) or astaxanthin gave similar coloration in *P. monodon.* Liao *et al.* (1993) assessing *Spirulina* as carotenoid source for *P. monodon* pigmentation, demonstrated the rapid conversion of zeaxanthin (one of the major carotenoids of *Spirulina*) to astaxanthin. Zeaxanthin was the principal carotenoid in the digestive gland during maturation (Vincent *et al.*, 1988). However, the transformation of *Spirulina* carotenoids to astaxanthin requires time, and was a minimum of 23 days with the first experiment in

this study. The completion of transformation was exhibited by the initial symptoms of PDS reversal noticed with female ovary colour.

Wyban *et al.* (1997) using paprika for the carotenoid supplementation of *L. vannamei* maturation diet, reported a lag-time of four weeks for the reversal of PDS symptoms. Menasveta *et al.* (1993b), trying to cure "blue color syndrome" (caused by nutritional deficiency of carotenoids) of *P. monodon*, by supplementation of feed with 50 ppm astaxanthin, noticed that four weeks period was needed for the restoration of greenish-brown pigmentation.

In this study, the raise in fecundity in experiment 1, during the late spawning period could be related to the increased spawner weight due to growth. The relationship between spawner weight and fecundity has been well documented (Aquacop, 1977; Emmerson, 1980a; Hansford and Marsden, 1995; Palacios *et al.*, 1998, 1999a). The decrease in fertilization rate, as noticed in experiment 1 has been reported in penaeid prawns with the progress of spawning period. The reason attributed to declining fertilization was the declining sperm quality (Simon, 1982; Menasveta *et al.*, 1993a; Palacios *et al.*, 1998). In this study, with both the experiments, fertilization rate was not influenced by the carotenoid supplementation. This is again supported by the absence of correlation between egg carotenoids and fertilization.

The influence of carotenoids on hatching and larval quality was confirmed by the return of both values to the initial (pre-PDS) level, after carotenoid supplementation in experiment 1. With experiment 2 also, the influence was clear and confirmed by the better performance of carotenoid supplemented tank females. In eggs, importance of carotenoid is probably related to vitamin A production and protection of unsaturated

lipids against oxidation (Harrison, 1990). Carotenoids and vitamins E and C give protection against unsaturated fatty acid peroxidation, and at high levels these vitamins increase hatching success (Cahu *et al.*, 1995). The positive correlation obtained between the nauplii length and egg carotenoids in both the experiments (Fig. 7 and 8) well suggest the role of carotenoids in nauplii development. Wyban *et al.* (1997) reported increased nauplii quality, estimated by survival to zoea 2, with the addition of paprika to the maturation diet.

The egg carotenoids values obtained in pond-reared females (experiment 1) were lower than of wild females (experiment 2) suggesting a deficient supply of carotenoid in the diet. Pond-reared animals have been reported to contain significantly lower levels ($P<0.05$) of carotenoids (in head, shell and flesh) when compared to wild animals (Iamsamang *et al.*, 1996). It may be that the lower body carotenoid levels (evidenced by lower egg carotenoid levels) were not sufficient to meet the demands of reproduction after a certain limit, resulting in PDS. It is also to be noted that animal's growth and molting results in loss of pigments (Latscha, 1991).

Palacios *et al.* (1999a) reported both eggs and nauplii had a progressive decrease in total carotenoid levels towards the end of the spawning period, implying diminished transfer of carotenoids to the eggs, resulting in the partial loss of physiological benefits provided by these compounds. Decline in larval quality under sustained reproduction in hatcheries was found to be caused by reduced pigment levels in the embryo yolk (Wyban *et al.*, 1997). In this experiment *F. indicus* nauplii had low levels of total carotenoids compared to eggs, agreeing with the findings of Petit *et al.* (1991) in *M. japonicus* and Palacios *et al.* (1999a) in *L. vannamei*.

The requirements for protein are assumed to be greater during maturation and reproduction, considering the intense biosynthesis that takes place during these processes (Harrison, 1990). In the present study, as the protein source being the same, there is a decrease in egg and nauplii protein values during the PDS period. The concurrent increase of the protein in post-PDS period (when there was improved carotenoid content), confirms the influence of carotenoids on protein content. In both the experiments, better performance was associated with higher egg and nauplii protein content.

It has been demonstrated that the protein contents of the hepatopancreas and ovary of best performing animals were significantly higher than less performing ones (Palacios *et al.*, 1999b, 2000). In both the experiments, the level of total carotenoids in eggs had a positive correlation with the nauplii protein levels reflecting the proposed role of carotenoids in protein stability (Olson, 1993). The correlation coefficient (r) were 0.61 ($P<0.001$) and 0.25 ($P<0.05$) for the experiment 1 and 2 respectively.

In summary, carotenoids play a vital role in reproduction, affecting mainly the egg and larval quality. Carotenoids supplementation in broodstock diet would take care of any deficiency especially in the pond-reared animals. Unlike the wild, the pond animals are not from an environment that sufficiently provides these nutrients in sufficient quantity and quality. *Spirulina,* as a pigment source was effective in correcting carotenoid deficiency, but, need some time for the conversion of precursors to astaxanthin. Even in wild broodstock, carotenoid supplement improved egg and larval quality. It is recommended that carotenoid supplementation of broodstock diet is made a common practice in the shrimp hatcheries, using readily available effective pigment source like *Spirulina,* to preclude problems related to carotenoid deficiency.