Effects of Light Regime, Algae in the Water, and Dietary Astaxanthin on Pigmentation, Growth, and Survival of Black Tiger Prawn *Penaeus monodon* Post-larvae

Chih-Hung Pan[1], Yew-Hu Chien[1,*] and Jin-Hua Cheng[2]

[1]*Department of Aquaculture, National Taiwan Ocean University, Keelung, Taiwan 202, R.O.C.*
[2]*Tungkang Marine Laboratory, Taiwan Fisheries Research Institute, Tungkang, Pingtung, Taiwan 928, R.O.C.*

Chih-Hung Pan, Yew-Hu Chien and Jin-Hua Cheng (2001) Effects of light regime, algae in the water, and dietary astaxanthin on pigmentation, growth, and survival of black tiger prawn *Penaeus monodon* post-larvae. *Zoological Studies* **40**(4): 371-382. The astaxanthin content of penaeids can be increased through ingestion of food containing different carotenoids. Illumination and background probably affect pigmentation as well. There are some indications that astaxanthin also plays biological roles other than in pigmentation, such as improvement of growth and survival. The aim of this study was to find out the effects of light regime, algal in the water, and dietary astaxanthin on body astaxanthin, and subsequent growth and survival of tiger prawn *Penaeus monodon* post-larvae. An experiment with 2 × 2 × 2 factorial arrangement of treatments was conducted, which included 2 diets without or with astaxanthin supplementation (80 mg astaxanthin/kg) fed on tiger prawn post-larvae in water without or with the addition of microalgae (*Isochrysis galbana*, 3.63-8.70 µg/L) under a 24-h dark or light environment for 4 wk. Body astaxanthin concentrations decreased significantly as prawns grew. Light had significant effects in preventing body astaxanthin reduction only during the 4th wk, but both algae in the water and dietary astaxanthin had significant effects in preventing body astaxanthin reduction from the 1st wk. There were no significant effects of light regime, algae in the water, or dietary astaxanthin on prawn growth. Throughout the experiment, the survival of prawn reared in the light was significantly higher than that for prawn reared in the dark. The survival of prawn reared in algae-containing water was significantly higher than that of prawn reared in clear water except in the 3rd wk. Dietary astaxanthin improved prawn survival only in the 1st wk. Only in the 4th wk did survival have a positive correlation with prawn body astaxanthin concentration. The results suggest that it is essential to maintain a certain level of astaxanthin in tiger prawn post-larvae for better survival while the prawn are growing and their astaxanthin is decreasing. http://www.sinica.edu.tw/zool/zoolstud/40.4/371.pdf

Key words: Carotenoids, Crustacean, Pigment conversion, Biological function.

The concentration and distribution of carotenoids in crustaceans are affected by intrinsic and extrinsic factors. Intrinsic factors include species, sex (Goodwin 1960, Gilchrist and Lee 1972), size (Dall 1995), molting stage (Goodwin 1960, Dall and Smith 1978a, b), tissues and organs (Chien and Jeng 1992, Negre-Sadargues et al. 1993, Dall 1995), and hormonal control (Goodwin 1960) of the animals. Extrinsic factors are background colors, light intensity (Goodwin 1960, Lockwood 1967, Ghidalia 1985), temperature (Lockwood 1967, Ghidalia 1985), rearing conditions (Chien and Jeng 1992), and kinds and sources of carotenoids from natural food or formulated feed (Yamada et al. 1990, Howell and Matthews 1991, Chien and Jeng 1992, Menasveta et al. 1993, Petit et al. 1997). Astaxanthin is the predominant carotenoid in penaeids (Katayama et al. 1971, 1972, Tanaka et al. 1976, Okada et al. 1994). Dietary supplementation of astaxanthin improved (Tanaka et al. 1976, Chien and Jeng 1992, Liao et al. 1993b) or corrected (Menasveta et al. 1993) the color of penaeids, especially those intensively cultured, for a better market price (Liao and Chien 1994).

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*To whom correspondence and reprint requests should be addressed. Tel: 886-2-24622192 ext. 5204. Fax: 886-2-24625393. E-mail: yhchien@mail.ntou.edu.tw

In addition to the pigmenting properties of carotenoids, increasing attention is being directed toward defining the biological function of astaxanthin in aquatic animals (Meyers and Latscha 1997). Improved survival due to dietary astaxanthin supplementation was reported for the kuruma prawn, *Marsupenaeus japonicus* (Chien and Jeng 1992) and tiger prawn, *Penaeus monodon* (Thongrod et al. 1995, Chien et al. 1999). Instead of retinoids (vitamin A), astaxanthin was suggested to be an essential growth factor in the early development of bear shrimp (*P. semisulcatus*) (Dall 1995). A significant positive correlation was found between supplementation of dietary astaxanthin and growth of tiger shrimp post-larvae (Thongrod et al. 1995). The goal of this study was to determine the effects of light regime, algae in water, and dietary astaxanthin on pigmentation, and subsequent growth and survival of post-larvae of the tiger prawn *P. monodon*.

MATERIALS AND METHODS

Experimental design

A 2 × 2 × 2 factorial arrangement of treatments was used, which included 2 diets with or without astaxanthin supplementation (P or nP) fed on tiger prawn (*P. monodon*) post-larvae in water with or without the addition of microalgae (A or nA) under a 24-h light or dark environment (L or nL) for 4 wk with 3 replications.

Prawns assigned to the treatments were denoted accordingly. For example, LAnP-prawns were prawns fed with a diet without astaxanthin supplementation (nP) and reared in water with microalgae added (A) and in the light (L).

Diet preparation

One diet contained astaxanthin supplementation at a concentration of 80 mg/kg and the other contained no supplement. The basal diet was composed of white fishmeal 50%, dextrin 23.4%, wheat flour 10%, corn gluten 5%, beef liver meal 3%, fish oil 2%, vitamin mix 2.6%, and mineral mix 4%. Dextrin 0.1% was replaced by astaxanthin (Carophyll Pink, 8% astaxanthin, Hoffman La Roche, Basel, Switzerland) in the pigmented diet. Water was added to the ingredients to form a dough, which was extruded through a 2-mm-diameter die press. The spaghetti-like feed was air dried to prevent the destruction of astaxanthin. The feed was then crushed, sieved through 70-mesh and 100-mesh sieves to retain particle feed of 600-1200 μm, which was stored at −20 °C to avoid oxidation of the pigments (Davies 1982). Proximate analyses of the basal diet indicated crude protein, 41.7%; crude fat, 8.7%; carbohydrate (by difference), 32.9%; moisture, 8.5%; ash, 14.8%; and fiber, 3.0% (AOAC 1984). Astaxanthin analysis showed that the astaxanthin concentration of the pigmented diet was 71.5 ± 2.1 mg/kg.

Algal water

Water used in the experiment was filtered through a 1-μm screen and sterilized with ultraviolet light. In the treatments that contained algae, concentrated live *Isochrysis galbana* was added to maintain an algal density of 3.63-8.70 μg/ml, or $8.1 ± 0.5 × 10^4$ cells/ml. The algal concentrate, estimated by hemacytometer count, had a density of $3 × 10^7$ cells/ml, or 2.17 mg dry weight/ml. Algal samples were taken from the algal concentrate when it was added to the tanks and from the tanks 2 d after addition of algae. Algal samples were used to monitor and correct the concentration of algae in tank water and to determine the level of carotenoid.

Light regime

Prawns were reared in a dark or a light room. In the light room, a lux tester (Digital Model YF-1065) maintained lighting for 24 h at 500-800 lux at the water surface. In the dark room, no lighting was provided except during routine maintenance and sampling operations, for usually less than an hour per day.

Prawn rearing

Post-larvae at 5 d (PL5) were obtained from the hatchery of the Tungkang Marine Laboratory, Taiwan Fisheries Research Institute. In the hatchery, only dim lighting was provided. After acclimatization for 3 d in a 0.5-ton indoor tank in which no algae were added and non-pigmented diet was fed, the animals were then transferred to 24 white (inner surface) 30-L FRP tanks to receive their respective treatments. Post-larvae weighing 4.3 ± 0.8 mg were stocked at a density of 150 animals per 25-L tank or 6 animals/L. Dissolved oxygen was maintained above 6 ppm by constant aeration; salinity remained around 32 ppt; and temperature around 26 °C. Animals were fed with satiation 3 times a day at 0800, 1500, and 2200 h. Siphoning was conducted every other day to clean the tank bottom at which time 1/3 of the water was replaced. Prawns were reared for 4 wk. Every

weekend, prawns in each tank were counted, and 10 prawns were sampled the 1st wk and 5 prawns/wk subsequently. After weighing, all samples were frozen in a −70 °C freezer prior to astaxanthin analysis.

Analysis of prawn body astaxanthin concentration

Prawns were weighed, freeze-dried, and weighed again to obtain moisture content. The dried sample was ground using a porcelain mortar and pestle, and placed into a 50-ml polypropylene centrifuge tube. Then 20 ml of acetone (0.05% butylated hydroxytolune, BHT) was added as a solvent (Schwartz and Patroni-Killam 1985, Khachik et al. 1986, Barimalaa and Gordon 1988), and the mixture was homogenized (Polytron PT-MR-3000) at 20 000 rpm for 1 min. The contents of each tube were centrifuged (Hitachi 18 PR-52) under 4 °C at 10 000 rpm for 15 min. The pellet was centrifuged with additional 20-ml aliquots of acetone until the acetone extract was clear. The pooled acetone extracts were transferred to a 250-ml separatory funnel, partitioned with 30 ml n-hexane which was washed 3 times with 10% NaCl to remove residual acetone. The extract was put into a rotary evaporator to reduce the volume to 10 ml, filtered through a 0.2-μm Millipore filter, and then stored in 4-ml brown vials.

Astaxanthin was analyzed by high-performance liquid chromatography (HPLC) using a Hitachi L-6200 pump, a silica column (Lichrosorb Si-60 5 micro 250 × 4.6-mm column I.D., E. Merck), a Hitachi L-4250 UV-VIS detector at 470 nm, and a Hitachi D-2000 Chromato-Integrator. The operation conditions were: mobile phase, 14% acetone in n-hexane; solvent flow rate, 1.5 ml/min; injection volume, 100 μl; and pump program of which the sequence was 0-20 min of mixture A and 20.5-40 min of mixture B. Mixture A was acetone: n-hexane, 14:86, and Mixture B was 100% n-heptane. This system was controlled by a chromatographic data system (Scientific Information Services Corporation), which also integrated the areas under the peaks. The astaxanthin standard used was chromatographically pure astaxanthin, a gift from Hoffman La Roche, Basel, Switzerland.

Analysis of total carotenoid in algae

Algae were freeze-dried at −60 °C, homogenized in a Waring blender under nitrogen in darkness, and extracted with acetone until no further pigments were obtained. The extract was vacuum-filtered through filter paper and re-suspended in n-hexane, then pigment was transferred to the epiphase by ad-dition of distilled water. The epiphase was washed repeatedly to remove acetone; water was removed by adding 5-10 g Na_2SO_4/100 ml (Simpson et al. 1985, Chien and Jeng 1992). The volume of the solution was reduced by evaporation under reduced pressure and subjected to silica gel column chromatography with hexane/benzene (1:9) to separate the carotenoid from other chlorophylls (Miki et al. 1985). The carotenoid was eluted with hexane, and its concentration was measured by the method of Chien and Jeng (1992).

Statistical analysis

Three-way ANOVAs were conducted to determine the main effects and interactions of dietary astaxanthin, algae in the water, and light regime on astaxanthin concentration in prawn, and growth and survival for the entire experimental period. Duncan's multiple range tests were used to determine differences in those parameters among the 4 wk. Since the mortality data were expressed in percentages, they were transformed into a normal distribution using the arcsine square root prior to analysis of variance (Box and Youle 1955, Sokal and Rohlf 1995, Ray et al. 1996). For each wk and the entire experimental period, 2 regression analyses were conducted on (1) body astaxanthin concentration versus weight and (2) survival versus body astaxanthin concentration.

RESULTS

Pigmentation

The average (n = 6) astaxanthin concentration in 8-d post-larvae at stocking was 67 ± 16 mg/kg. During the 4-wk period, astaxanthin concentrations decreased significantly as prawn weight increased as shown by the negative linear relationship (n = 96) between astaxanthin concentration and prawn weight (Fig. 1). For each individual wk, however, there was no correlation between astaxanthin concentration and prawn weight. The overall average (n = 24) astaxanthin concentrations were 47 ± 13 mg/kg, 45 ± 12 mg/kg, 31 ± 15 mg/kg, and 20 ± 8 mg/kg for the 1st to the 4th wks, respectively (Fig. 2). There was no significant difference in the overall average astaxanthin concentration between prawn in the 1st wk and 2nd wk, but those of prawn in the 2nd, 3rd, and 4th wks differed significantly (Fig. 2).

Comparisons of the main effects of astaxanthin concentration in prawn for each wk are also shown in

fig. 2. Light regime had significant effects on astaxanthin concentration only in the 4th wk, with means ($n = 12$) of 15 ± 5 mg/kg and 24 ± 9 mg/kg for nL-prawn and L-prawn, respectively. However, from the 1st wk on, both algae in the water and dietary astaxanthin significantly increased astaxanthin concentrations. In the 4th wk, astaxanthin concentration in L-prawn (24 mg/kg) was 53% higher than that in nL-prawn (15 mg/kg); the astaxanthin concentration in A-prawn (23 mg/kg) was 47% higher than that in nA-prawn (15 mg/kg); and the astaxanthin concentration in P-prawn (22 mg/kg) was 32% higher than that in nP-prawn (17 mg/kg). At the end of the 4th wk, nLnAnP-prawn had the lowest average astaxanthin concentration of 11 mg/kg, only 1/6 of the initial concentration. LAP-prawn had the highest average astaxanthin concentration of 32 mg/kg, about 1/2 of the initial concentration.

Growth

For the main effects and overall average, there was no significant growth from the 1st to the 2nd wk (Fig. 3). The overall average ($n = 96$) individual weights were 5.3 ± 1.9, 6.8 ± 1.9, 12.6 ± 4.8, and 17.1 ± 7.8 mg in the 1st to the 4th wks, respectively.

There was no difference in average individual weight between prawn in the 1st and 2nd wks, but those of prawn in the 2nd, 3rd, and 4th wks differed significantly (Fig. 3). The average cumulative weight gains were 23%, 58%, 193%, and 298% for the 1st to the 4th wks, respectively. There were no significant effects of light regime, algae in the water, or dietary
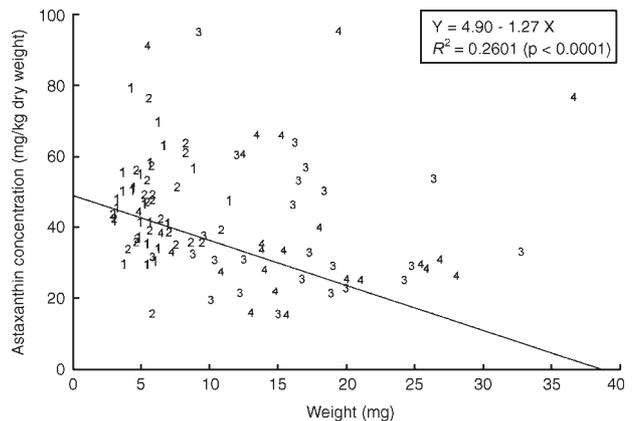
Fig. 1. Graph showing the decreasing trend in astaxanthin concentration with weight. Numbers indicate the week when the prawns were sampled.
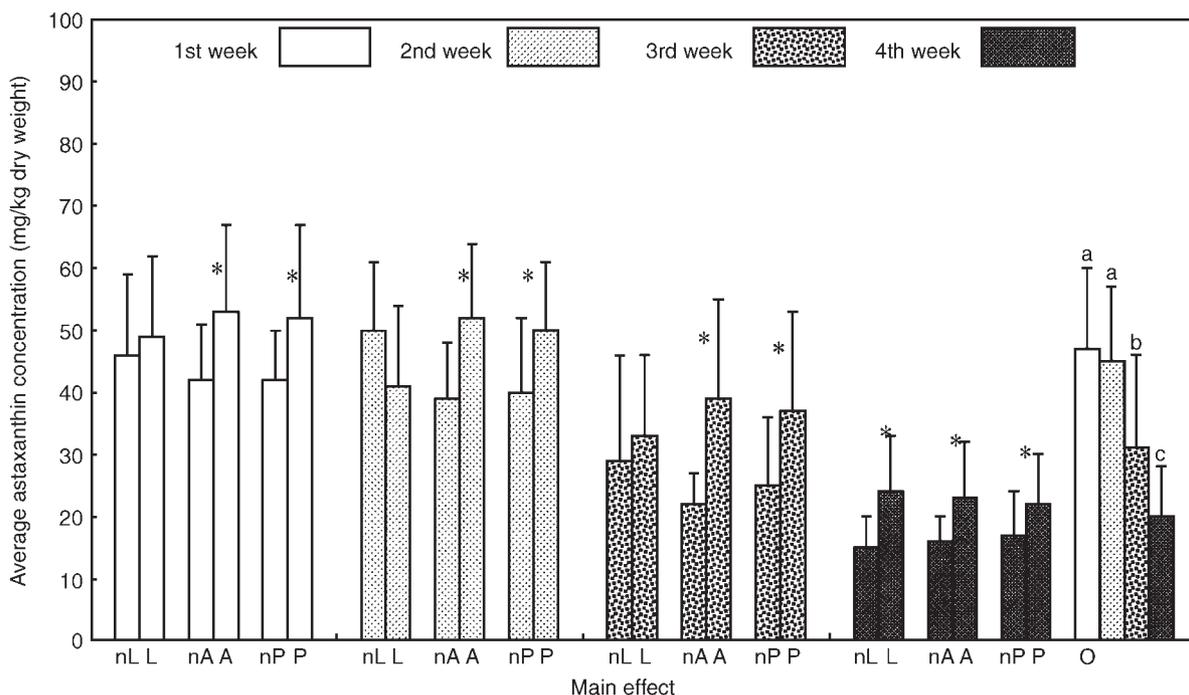
Fig. 2. Comparisons of astaxanthin concentrations in prawn between prawns reared in dark (nL) and light (L) environments, between prawns reared in water without (nA) and with (A) algae, and between prawns fed with a diet without (nP) and with (P) astaxanthin supplementation. An asterisk (*) indicates that the difference is significant ($p \leq 0.05$). "O" denotes the overall average, and the letters denote significance of Duncan's multiple range test.

astaxanthin on prawn growth for each wk (Fig. 3). Stocking density had no significant effect on growth as no relationship was found between prawn weight and the number of surviving prawn each wk.

Survival

There were 150 animals stocked in each tank at the beginning of the experiment. The overall average (n = 96) number of survivors decreased from 66 at the end of the 1st wk to 27 at the end of the 4th wk (Fig. 4). For the entire main effects and overall average, there were significant decreases in survivors from the 1st wk to the 2nd wk, but there were no differences in survivors between the 3rd wk and the 4th wk. L-prawn had significant better survival than did nL-prawn throughout the experiment (Fig. 4). In the 4th wk, the average (n = 48) survival of L-prawn was 40, almost 3 times that of nL-prawn at 12. A-prawn had significantly better survival than did nA-prawn except in the 3rd wk. In the 4th wk, the average survival of A-prawn at 33 was 57% higher than that of nA-prawn at 21. Overall, P-prawn had better survival than nP-prawn in the 1st wk only. In the 4th wk, the overall average survival of P-prawn at 31 ± 23 did not significantly differ from that of nP-prawn at

23 ± 15. When survivors were regressed against the astaxanthin concentration in prawn for each wk, it was found that there was a positive correlation between survival and astaxanthin concentration in prawn in the 4th wk (Fig. 5).

DISCUSSION

Pigmentation

Growth was the dominant factor affecting pigment concentration in this study. Increase in size as a result of growth dilutes the carotenoid concentration if weight increases faster than the uptake of carotenoid (Menasveta et al. 1993). In this study, *P. monodon* post-larvae ranged from 4 to 36 mg, and their astaxanthin concentration decreased with size at a rate of 1.27 µg/g (Fig. 1). In another study, *P. monodon* juveniles ranged from 0.4 to 2.8 g, and their carotenoid concentrations also decreased with size at a rate of 0.63 µg/g (Pan et al. 1999). In these 2 studies, all pigment concentrations were expressed as dry weight. For wet weight, the concentration of total carotenoids of *P. semisulcatus* decreased from 19.3 µg/g in eggs to 4.0 µg/g in pro-
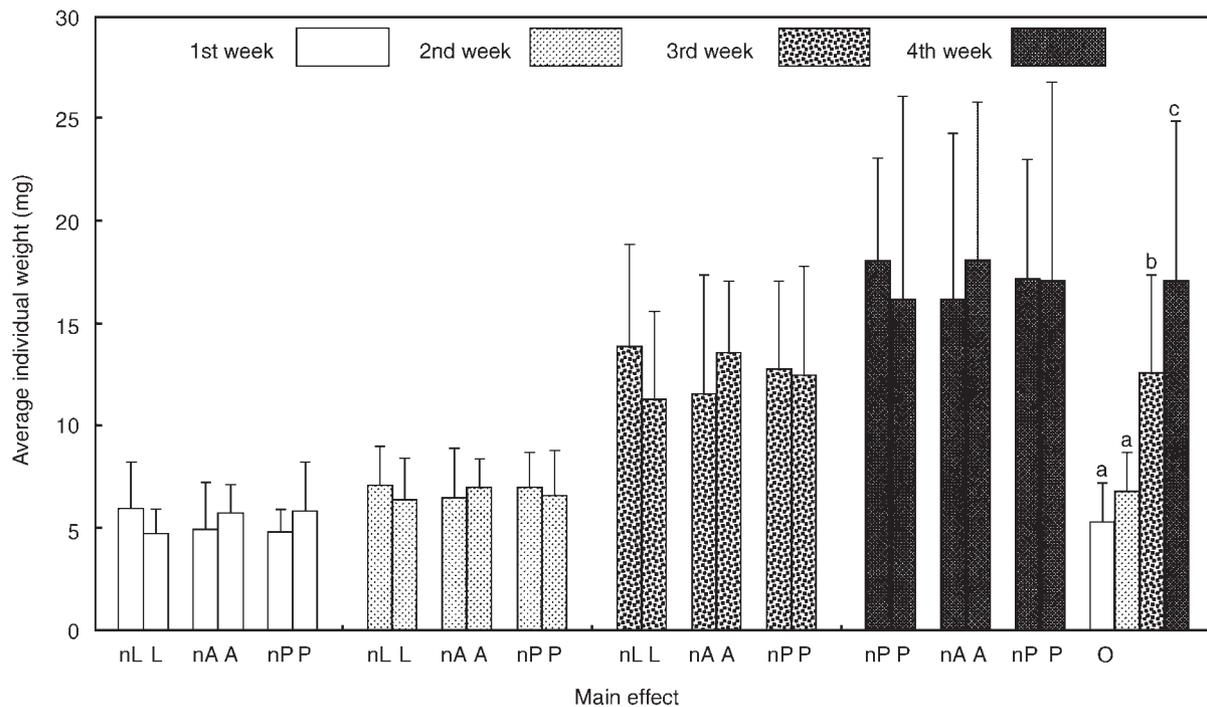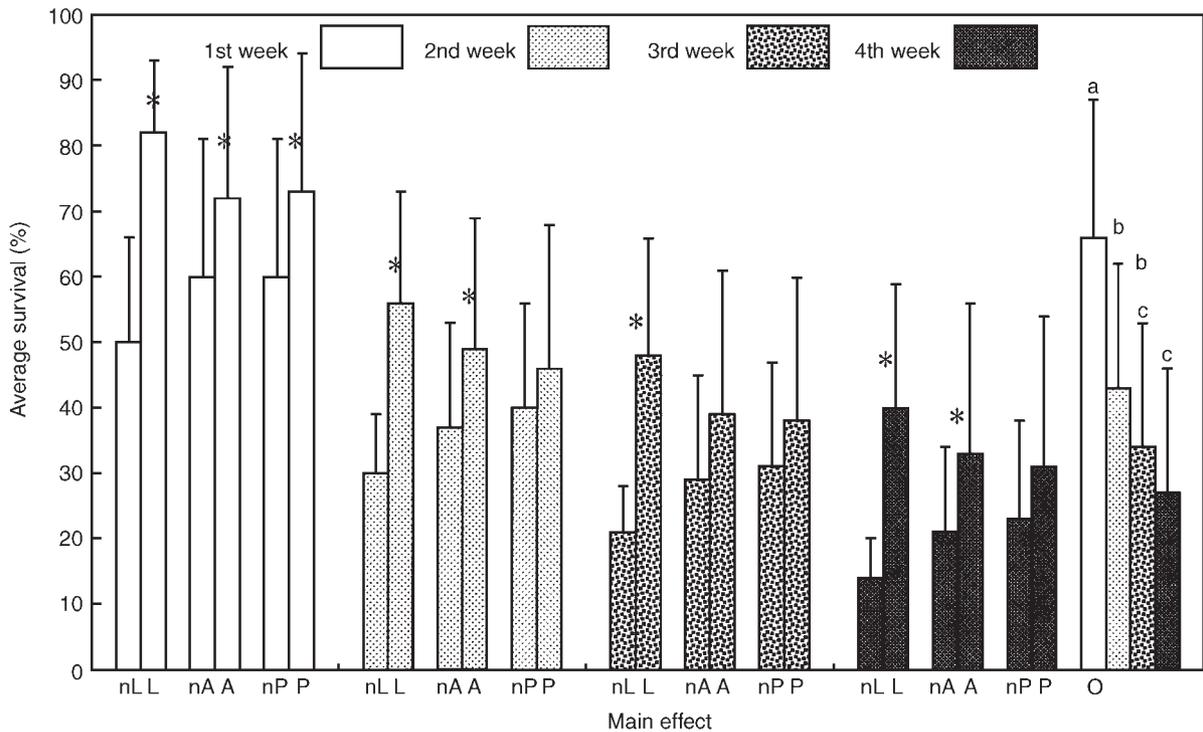

Fig. 3. No differences were found in prawn growth between prawns reared in dark (nL) and light (L) environments, between prawns reared in water without (nA) and with (A) algae, and between prawns fed with a diet without (nP) and with (P) astaxanthin supplementation, and among the 4 wk. "O" denotes the overall average.

tozoa during a development time of less than 50 h (Dall 1995), an 80% decrease. These results suggest that from egg, early larvae, and post-larvae to juvenile, pigment concentration decreased in response to decreasing growth rates, which were 4, 1.27, and 0.63 μg/g. In this study, the lack of change in astaxanthin concentration between the 1st and 2nd wks was probably related to non-significant growth during this period. Fast growth from the 2nd wk on produced a significant decrease in astaxanthin concentration. At stocking, the average astaxanthin concentration of P8 was 67.08 mg/kg. After 4 wk, the overall average astaxanthin concentration of P36 was 20 mg/kg, a 70% decrease since stocking. In the 4th wk, the reduction of astaxanthin concentrations reached 52% and 83% for LAP-prawn and nLnAnP-prawn, i.e., the best and the poorest pigmented prawn, respectively. Even under the most favorable conditions for pigmentation, prawns fed with an astaxanthin-supplemented diet, reared with algae in the water under continuous lighting, i.e., LAP-prawn, still could not uptake carotenoid fast enough to prevent the dilution of astaxanthin as the result of an increase in size or weight. Similar results were found for *M. japonicus* P18 and P27 fed with a

60 mg/kg astaxanthin diet for 20 days. Carotenoid contents declined by 50% and 13%, respectively. Following the feeding a 0.9-mg carotenoid/kg diet for 20 d, those 2 postlarval stages lost 60% and 39% of their carotenoids, respectively (Petit et al.1998).

Only by the 4th wk did L-prawn have higher astaxanthin concentrations than nL-prawn. Light regime took a long time to affect pigmentation. Although numerous studies have been conducted on the effects of light or background on the chromatophores of crustaceans, the astaxanthin concentration was not quantified in any of them. Among several factors that affect chromatophore responses to a change of background, light intensity is always considered the primary one. Occurring independently of (or in conjunction with) background-related responses are illumination-related color changes, which typically involve concentration of chromatophore pigments in darkness and an increased dispersion of chromatophore pigments as light intensity increases (Rao 1985). However, whether the concentration or dispersion of chromatophore pigments actually reflects a change in overall astaxanthin concentration is not known. Background effects in this study were irrelevant because the background color

Fig. 4. Comparisons of prawn survival between prawns reared in dark (nL) and light (L) environments, between prawns reared in water without (nA) and with (A) algae, and between prawns fed with a diet without (nP) and with (P) astaxanthin supplementation. An asterisk (*) indicates that the difference is significant ($p \leq 0.05$). "O" denotes the overall average, and the letters denote significance of Duncan's multiple range test.

of all tanks was white and dark-treatment tanks were completely blocked from light. Higher astaxanthin in L-prawn than in nL-prawn is probably attributable to higher carotenoid concentration in algae kept in light rather than in dark conditions. On a dry weight basis, total carotenoid concentrations were 66 ± 2, 52 ± 1, and 47 ± 1 mg/kg ($n = 4$) for concentrated fresh algae, algae in light tanks, and algae in dark tanks, respectively. In general, maximum carotenoid production by algae requires optimization of the culture conditions that favor growth (Olaizola and Duerr 1990). The hourly carotenoid production of *Haematococcus pluvialis* increased with light intensity, and secondary carotenoid accumulation in *H. pluvialis* may occur in the active growth phase (Chaumont and Thepenier 1995). It was also shown that among the light and dark illumination cycles tested, continuous illumination was most favorable for astaxanthin formation, while the carotenoid content correlated proportionally to light quantity (light intensity × net illumination time) (Kabayashi et al. 1992). In this study under unfavorable growth conditions of no illumination for 2 d, the algae in dark tanks contained significantly less carotenoid than did algae in lighted tanks.

Algae are an important larval food during the zoea stage and post-larvae 20-d stage (Liao et al. 1993a). Adding algae is an essential practice in prawn hatcheries (Smith et al. 1993). After the post-larvae are transferred from an indoor hatchery to outdoor nursery tanks or ponds, algae are no longer added but cultivated together with the animals in captivity. No quantitative studies have ever been conducted to show the importance of microscopic algae in the post-larval *P. monodon* diet. However, gut content analyses of juvenile and subadult *P. monodon* reared in extensive ponds without supplementary feeding (Bombeo-Tuburan et al. 1993) or in semi-intensive ponds with supplementary feeding (Focken et al. 1998) all showed that diatoms and green algae are significant food items, despite the dominance of other natural foods whose particle sizes are larger than the algae, such as detritus, plant materials, and crustacean parts. In this study, feed and algae were the only available food; post-larvae had much smaller mouth structures than did juveniles and subadults; and algae should be a significant food for the post-larvae.

Incorporation of different algae such as *Dunaliella salina* (Chien and Jeng 1992), *Chnoospora minima* (Menasveta et al. 1993), *Spirulina* sp. (Liao et al. 1993b), and *S. pacific* and *H. pluvialis* (Chien and Shiau 1998) into the diet improves the pigmentation of penaeid juveniles and adults. In this study,

instead of being incorporated into the diet, the live *I. galbana* in water resulted in higher astaxanthin concentrations in A-prawn than in nA-prawn for all 4 wk. Astaxanthin is the major carotenoid pigment in penaeids, comprising 86%-98% in black tiger prawn *P. monodon* (Okada et al. 1994). Bioconversion of β-carotene to astaxanthin requires many oxidative steps (Tanaka et al. 1976). β-carotene is the main carotenoid in *I. galbana* (Berger et al. 1977), nevertheless, without any lag; A-prawn had higher astaxanthin concentrations than nA-prawn after the 1st wk.

Latscha (1990) identified out numerous factors affecting pigmentation of crustaceans, including the major ones like pigment, feed, animal, environment, and disease. Free astaxanthin was incorporated into the diet to enhance pigmentation of penaeids and found to be the most effective pigment (Chien and Jeng 1992, Negre-Sadargues et al. 1993, Menasveta et al. 1993). The pigmentation efficiencies of those studies varied with species, sizes of the animals, growth rates, dietary astaxanthin levels, rearing conditions, pigmented tissue, and other unknown factors (Table 1); therefore, no universal effects on pigmentation for each carotenoid were found. A study of the correlation between pigmentation factors and pigmentation efficiency needs to be conducted to identify the pigmentation processes that can be carried out most effectively. The pigmentation processes in this study were relatively simple and dominated by the main effects (Table 2). No matter which ANOVA model was used, no interactions were involved.
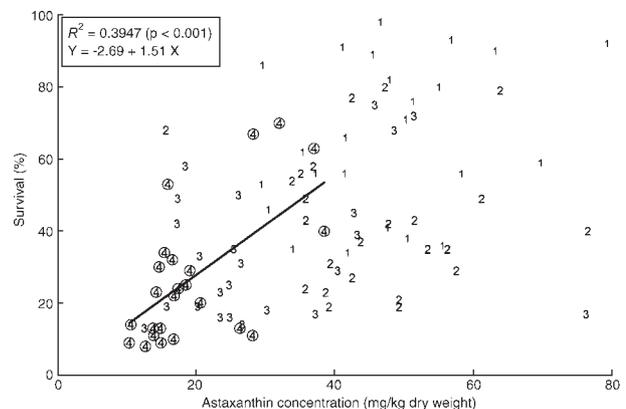
Fig. 5. Positive correlation between prawn survival and astaxanthin concentration in prawn in the 4th wk. The numbers indicate the wk when prawns were sampled.

Growth

There was no significant effect of light regime on prawn growth in this study. Another study showed no significant light effect on feeding of penaeids. In a 30-d feeding experiment, the consumption of plant detritus by *Litopenaeus stylirostris* and *L. vannamei* did not differ significantly with respect to light or darkness conditions (Soto and Rodriguez-Medina 1986). Including this study, no significant effects of light regime on growth of penaeids have yet been found. Studies on *Farfantepenaeus duorarum* (Bishop and Herrnkind 1976), *P. semisulcatus* (Al-Ablani and Farmer 1986), *M. japonicus* (Nakamura 1988), and *Fenneropenaeus indicus* (Vijayan and Diwan 1995) found that growth of prawns was unaffected by changes in photoperiod.

Except for 2 previous studies (Thongrod et al. 1995, Petit et al. 1997), no positive effects of astaxanthin or carotenoids on growth of penaeids (Yamada et al. 1990, Chien and Jeng 1992, Negre-Sadargues et al. 1993, Liao et al. 1993b, Menasveta et al. 1993, Tao 1995) have been reported as in this study. After diets were supplemented with astaxanthin at the 5 levels of 0, 5, 15, 60, and 300 mg/kg for 30 d, growth of post-larvae of *P. monodon* increased significantly as dietary astaxanthin levels increased (Thongrod et al. 1995). However, no significant effect of prawn weight on prawn astaxanthin concentration was observed, as found in our study. Petit et al. (1997) found that dietary astaxanthin improved the growth rate and shortened the moulting cycle of *M. japonicus* post-larvae during a 20-d rearing. In our study, dietary astaxanthin did not affect growth no matter what ANOVA models were used (Table 2) or under what environmental conditions the animals were reared.

Although algae are a significant food for post-larvae and A-prawn had higher body astaxanthin concentrations than nA-prawn, algae did not provided an extra nutritional contribution since no difference in growth was found between A-prawn and nA-prawn.

Survival

Several astaxanthin studies with penaeids have shown a survival effect. Variation in survival effect maybe related to stress level, species, life stage, nutritional history, sources of astaxanthin, and rearing period and conditions. Yamada et al. (1990) fed with diets supplemented with astaxanthin at levels of 0, 50, 100, 200, and 400 ppm to 3.7-g juvenile *M. japonicus* for 8 wk, and mortalities of juveniles on a carotenoid-free diet were much higher than those of juveniles fed with carotenoids. Chien and Jeng (1992) studied the effect of different pigment sources and levels on pigmentation, survival, and growth of

Table 1. Pigmentation efficiency of prawn fed with dietary astaxanthin for 4 wk

| Species | Pigmented animal | | | Dietary astaxanthin level (ppm) | Carotenoid concentration in animal | | Pigment gain (%) | Tissue analyzed | Rearing conditions | Ref. |
	Initial size	Final size	Weight gain (%)		Control	Treatment				
M. japonicus	3.7 g	5.6 g	51	200	22	37	68	whole animal (viscera free)	20-25°C	1
M. japonicus	6.0 g	8.8 g	47	500	3	69	2200	head	0L/24D,	2
					5	33	560	flesh	20-25°C	
					2	37	1750	shell		
M. japonicus	11.7 g	12.8 g	10	100	118	278	136	epidermis	12L/12D,	3
					16	25	56	carapace	20°C	
P. monodon	>3.3 g	na	45	50	4	15	275	flesh	daylight,	4
					14	50	257	shell	30°C	
M. japonicus	8.4 mg	55.7 mg	560	60	37	89	141	whole animal	12L/12D, 21°C	5
P. monodon	4.3 mg	25.5 mg	493	80	11	14	27	whole animal	0L/24D, 26°C	6
					24	32	33			

1. Yamada et al. (1990), 2. Chien and Jeng (1992), 3. Negre-Sadargues (1993), 4. Menasveta et al. (1993), 5. Petit et al. (1998), 6. Present study, nLnAP-prawn vs. nLnAnP-prawn and LAP-prawn vs. LAnP-prawn.

M. japonicus. They found a positive correlation between pigment concentration in prawn tissue and survival. The results suggested that pigment might play a role in improving the survival of prawn. Thongrod et al. (1995) found that increasing dietary astaxanthin from 0, 5, 15, and 60 to 300 mg/kg increased survival of post-larvae of *P. monodon* during a 30-d feeding. They concluded that astaxanthin is required for survival of *P. monodon*, but were not able to correlate differences in survival with prawn astaxanthin concentrations.

In our studies, L-prawn had significantly better survival than nL-prawn throughout the experiment. However, L-prawn had higher astaxanthin concentrations than nL-prawn only in the 4th wk. P-prawn had higher astaxanthin concentrations than nP-prawn for all 4 wks. However, P-prawn had better survival than nP-prawn only in the 1st wk. A-prawn had higher astaxanthin concentrations than nA-

Table 2. Treatment effects (L-light, A-algae, and P-pigment) on prawn astaxanthin concentration, growth, and survival when (a) only main effects, (b) up to 2nd-order interactions, and (c) up to 3rd-order interactions are engaged in the models

(a)

Dependent variable	Main effects		
	L	A	P
Astaxanthin concentration	**	**	*
Growth			
Survival	**	*	

(b)

Dependent variable	Main effects			Interactions		
	L	A	P	L×A	L×P	A×P
Astaxanthin concentration	**	**	*			
Growth						
Survival	**	**		**		**

(c)

Dependent variable	Main effects			Interactions			
	L	A	P	L×A	L×P	A×P	L×A×P
Astaxanthin concentration	**	**	*				
Growth							
Survival	**	**	*	**		**	**

Significant levels of differences: *, $p \leq 0.05$; **, $p \leq 0.01$; and blank, $p > 0.05$ or not significant.

prawn for all 4 wks. A-prawn had better survival than nA-prawn except in the 3rd wk. A significant positive correlation was found between survival and astaxanthin concentration in prawn only in the 4th wk, or at the end of the experiment. In this wk, the astaxanthin concentration in prawn averaged 20 mg/kg and ranged from 10 to 37 mg/kg. A recent study demonstrated that when juvenile *P. monodon* were subjected to oxygen depletion stress, those fed on a diet containing 360 ppm astaxanthin had significantly better survival than did non-pigmented controls (Chien et al. 1999). In that study, the astaxanthin concentrations were 40, 26, 8, and 15 mg/kg for eyes, shell, flesh, and head, respectively. There is speculation that when the astaxanthin concentration in prawn is low, increased dietary astaxanthin can contribute to an increase in survival, especially when stresses are encountered. In this study, water quality was managed well and was stable. Handling at sampling was probably the only stress that occurred.

Better survival in L-prawn than in nL-prawn could be largely attributed to reduction of prawn activity under light. There have been few studies conducted on the effects of illumination, especially continuous light and dark conditions, on the survival of penaeids. In 1 experiment in which the tanks were covered with 1 or more layers of shade-netting, the survival of juvenile shrimp *P. semisulcatus* was found to decrease with increased shading of the tanks, namely, the lower the illumination, the poorer the survival (Al-Ablani and Farmer 1986). No explanation was provided for this phenomenon in that experiment. It was found that the activity of the common prawn *Leander adspersus* in periods with dark nights was 252% of the activity in periods with light nights (Albrechtsen 1979), and the swimming activity of *Farfantepenaeus aztecus* post-larvae increased with decreasing light (Matthews et al. 1991). It is not known if more frequent encounters under dark and in captivity would result in lower survival in this study. Nystroem (1994) considered a positive correlation between the survival of juvenile signal crayfish *Pacifastacus leniusculus* and light intensity as the result of lowered activity with higher illumination.

A-prawn had significant better survival than nA-prawn except in the 3rd wk. There have been no studies conducted to compare survival rates of prawn reared in clear water and in water containing algae. Although algae are not a major fraction of the diet for post-larvae and juvenile, maintenance of an algal bloom is considered an important practice during the nursery stage (Liao and Chien 1996). It was reported that a healthy phytoplankton bloom provided proper turbidity and subsequently stabilized

shrimp and reduced cannibalism or predation by birds (Chien 1992). Survival in this study involved complicated processes, being affected not only by the main effects but also by several interactions (Table 2). Both the main effect of algae in the water and all interactions involving algae in the water affected survival. No matter what models were used, light regime affected survival. When up to the 3rd-order interactions among light regime, algae in the water, and dietary astaxanthin were engaged in the ANOVA model, dietary astaxanthin had a significant main effect on survival.

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光照狀態、藻水及飼糧蝦紅素對草蝦後期幼蟲之呈色、成長及存活之影響

潘志弘[1]　　陳瑤湖[1]　　鄭金華[2]

攝食含不同胡蘿蔔素的食物可增進蝦的蝦紅素，光照及背景也可能影響色澤。除了增進色澤，蝦紅素也扮演了生物功能，如增進成長及存活。本研究之目的在於探討光照狀態、藻水及飼糧蝦紅素對草蝦後期幼蟲的呈色及後續成長與存活 的影響。一以複因子 2 × 2 × 2 的處理安排，包括添加 80 mg/kg 蝦紅素或不添加的兩種飼糧餵予草蝦後期幼蟲，在添加綠光等鞭定鞭藻 3.63-8.70 µg/L 或不添加的水中且在全光照或全黑暗狀態下養殖四星期。隨著蝦體的成長，蝦紅素顯著地下降。光照僅在第四星期顯著的減少蝦色素的下降率，而藻水及飼糧蝦紅素自第一星期即顯著地減少蝦色素的下降率。光照、藻水及飼糧蝦紅素對蝦的成長無影響。整個試驗期間，光照環境下蝦的存活高於在黑暗環境下。除了第三星期，在有藻水環境下蝦的存活也高於無藻水的環境。飼糧蝦紅素僅在第一星期增進蝦的存活。到第四星期，蝦的存活與蝦體之蝦紅素含量有顯著的正相關。此結果可建議當蝦後期幼蟲成長其蝦紅素降低時，要獲得較佳的存活，維持蝦紅素含量有其必要性。

關鍵詞：胡蘿蔔素，甲殼類，色素轉換，生物功能。

[1] 國立臺灣海洋大學水產養殖學系
[2] 行政院農業委員會水產試驗所東港分所