

Reproductive Performance of Captive *Penaeus monodon* Fed Various Sources of Carotenoids

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Abstract

Three groups of pond-reared *Penaeus monodon* broodstock were fed formulated diet in combination with carotenoid-containing natural food: mussel + shrimp broodstock pellet (MBP), crab + BP (CBP), and *Artemia* + BP (ABP). After four months, maturation and spawning rates did not differ significantly among treatments. After eyestalk ablation, MBP-fed shrimps initially spawned 20 days; 34 days for CBP-fed; 50 days for ABP-fed shrimps. The number of eggs per g body weight of spawner (1616-2359 *eggs/g* BW) did not differ significantly among all groups. Only nauplii from MBP-fed broodstock reached postlarval stage. Rematuration was observed only in MBP- and CBP-fed shrimps. Sperm count was highest in MBP- and lowest in ABP-fed shrimps at the final phase of the test.

Introduction

Carotenoids represent the main pigments of most aquatic species. The inclusion of natural and synthetic carotenoids in the diets has been reported to improve pigmentation in crustaceans through the biosynthetic schemes described by Katayama et al. (1972), Tanaka et al (1976), and Simpson (1982). Carotenoids affect growth, gonadal development, and larval quality (Meyers 1977; Latscha 1991; Oceanic Institute 1992). The reproducing females of sand crab *Emerita analoga* contain much greater amounts of carotenoid than males (Gilchrist and Lee 1972).

Since crustaceans cannot synthesize carotenoids *de novo* (Yamada *et al.* 1990), the pigment needs to be included in the diet. This study aims to evaluate the reproductive performance of pond-reared *Penaeus monodon* fed diets containing

Materials and Methods

Animals

Pond-reared *P. monodon* were stocked in 12m³ concrete tanks at 23 broodstock per tank (13 males and 10 females). The males had 60.8 ± 10.5 g mean body weight (BW) and 48.4 ± 0.8 mm carapace length (CL); the females, 62.0 ± 12.1 g BW and 52.7 ± 0.6 mm CL.

Feeds and feeding

Mussel, crabs, and *Artemia* were selected as natural food sources because of their availability and carotenoid or astaxanthin content (Table 1). Mussel meat and crabs (carapace removed) were chopped prior to feeding. Broodstock were fed adult *Artemia* reared on rice bran and *Tetraselmis tetrahele*. Since shrimps had difficulty in capturing live *Artemia*, frozen *Artemia* were fed in a bound form. A gelatinized mixture of bread flour and powdered seaweed (4:1) was used to bind *Artemia* into small balls prior to feeding.

Table 1. Carotenoid and astaxanthin contents of experimental diets.

Feed	Total carotenoid (mg/100g)	Astaxanthin (mg/100g)
Adult <i>Artemia</i>	7.2	0.058
Green mussel (<i>Perna viridis</i>) meat	9.9	ND
Crab (<i>Portunus pelagicus</i>)	1.8	1.1
Broodstock pellet	0.3	ND

ND - not detectable

¹ *Artemia* were fed rice bran and *Tetraselmis tetrahele*

Shrimps were fed a combination of broodstock pellet (BP) and mussel (MBP), crab and BP (CBP), or *Artemia* and BP (ABP). The modified broodstock pellet (Millamena *et al* 1986) (Table 2) was given at 1-2% BW; mussel, crab or *Artemia* at 15-30% BW per day. Treatments were replicated three times. Feeding rates were adjusted weekly based on shrimp biomass. Feeding was done for 4 months.

Broodstock culture

After a month of feeding, eyestalks of females were unilaterally ablated. Ovaries were visually examined 2-3 times weekly. Fully mature (Stage 4) shrimps were retrieved from the tanks for spawning.

Uneaten feeds, molted shells and feces were removed daily prior to water change and feeding. Half of the total water volume was changed daily. Water salinity and temperature levels were 34-37 ppt and 27-30°C, respectively, throughout the culture period. Age of shrimps at termination of experiment was 13.5 months.

Table 2. Composition of broodstock pellets for *P. monodon* (modified from Millamena *et al.* 1986).

Ingredients	Percentage
Squid meal	30
Shrimp head meal	20
Peruvian fish meal	20
Wheat flour	10
Seaweed (<i>Gracilaria</i> sp.)	4
Vitamin mix	3
Mineral mix	2
Dicalcium phosphate	2
Cod liver oil	6
Lecithin	2
Cholesterol	1

Maturation, spawning and rematuration rates, fecundity, hatching rates, and larval counts were compared. Spermatozoa were obtained from the spermatophore at the start, middle (3 months of feeding), and end of the study in order to determine the sperm count, percent live and abnormal sperm (Leung-Trujillo and Lawrence 1987). Abnormal sperm cells were characterized by their bent or missing spikes and small or deformed heads.

Carotenoid analyses

Total carotenoid and astaxanthin content of the feeds and shrimp tissues (hepatopancreas and ovaries) were determined according to the method of Matsuno (1992). Shrimp tissues were collected from 3-5 shrimps per treatment at the start and 3 months after feeding. Shrimp tissues in each treatment were pooled as one sample. Tissues were freeze-dried prior to analysis. Duplicate analysis were made for each sample.

Statistics

Arcsin transformation was used in the analyses of data in percentages. Analysis of variance followed by Duncan's multiple range test ($P= 0.05$) was done using the Statistical Analysis System (SAS Institute 1988) software. Correlation analysis between the size of the females and fecundity was made.

Results

Carotenoid and astaxanthin contents

Carotenoid and astaxanthin levels increased in the tissues of shrimps fed different sources of carotenoids after three months except in the ovaries of immature ABP-fed shrimps (Fig. 1A and B). Accumulation of the pigment in the tissues was higher in mature than in immature shrimps.

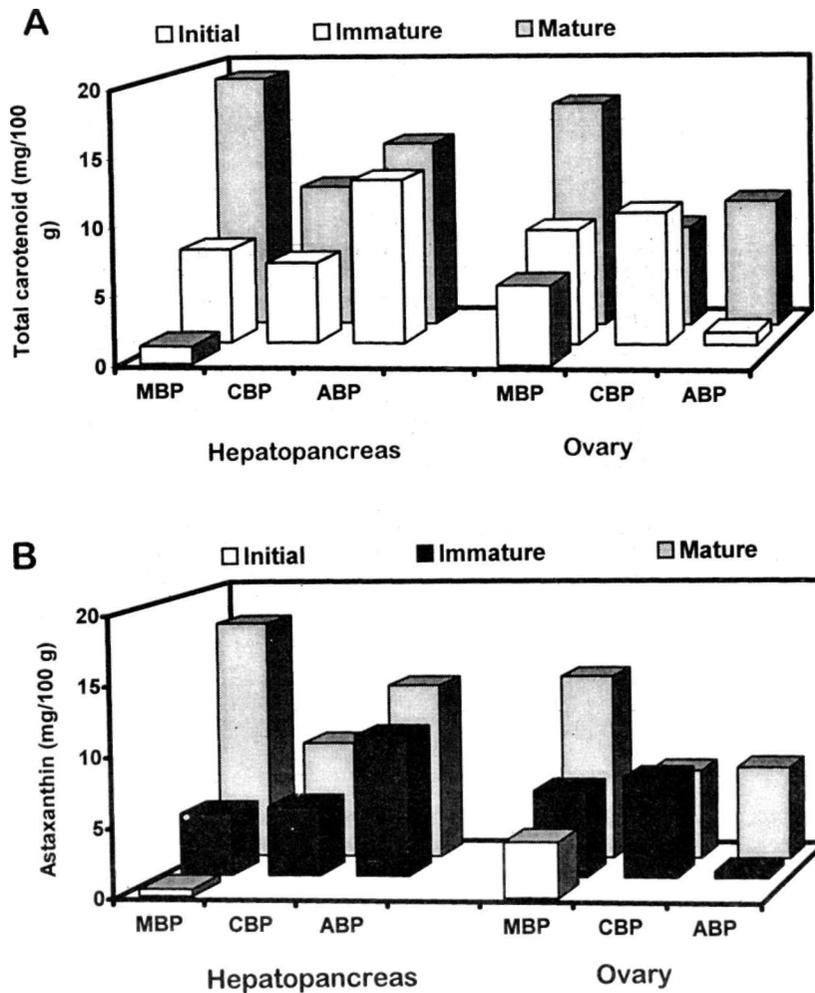


Fig. 1. Total carotenoids (A) and astaxanthin (B) in tissues of immature and mature *P. monodon* fed shrimp broodstock pellets in combination with mussel (MBP), crabs (CBP), or *Artemia* (ABP) for four months. Shrimp samples (immature and mature) were taken during the peak of maturation.

Maturation and spawning

After 4 months, maturation rates (MBP, 80%; CPB, 60%; ABP, 50%) and spawning rates (MBP, 70%; CBP, 61%; ABP, 43%) did not differ significantly ($P > 0.05$) among treatments (Fig. 2). The latency period from ablation to maturation was 5 days for MBP-, 9 days for CBP-, and 30 days for ABP-fed shrimps. However, the ovaries of both MBP- and ABP-fed shrimps that matured earliest within the treatment regressed after 2-3 days. First spawnings among MBP-fed shrimps occurred 20 days post-ablation; 34 days for CBP-fed shrimps and 50 days for ABP-fed shrimps. Rematuration was observed only in MBP- and CBP-fed shrimps (Fig 2). One MBP-fed female spawned twice.

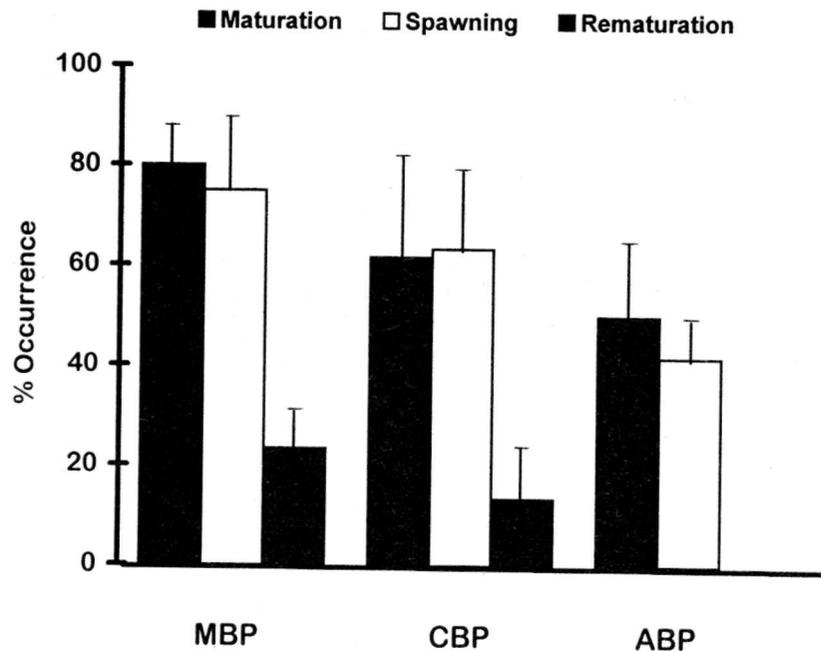


Fig. 2. Percentage maturation, spawning, and rematuration of *P. monodon* fed shrimp broodstock pellets in combination mussel (MPB), crabs (CBP), or *Artemia* (ABP). Vertical lines indicate \pm SEM.

The correlation coefficient between size of females and fecundity (MBP, 1616; CBP, 1779; ABP, 2359 eggs/g BW) was not significantly different among all treatment groups. Complete spawning produced 76,000-300,000 while partial spawning produced 22,000-182,000 eggs/female. Egg production was highest in ABP-fed shrimps, but eggs were not viable compared with shrimps fed MBP and CBP as indicated in the number of nauplii produced (Fig. 3).

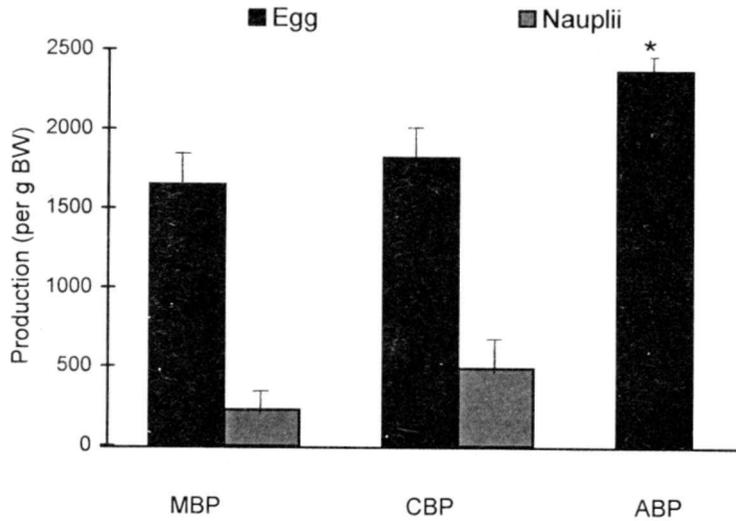


Fig. 3. Egg nauplii production of *P. monodon* fed shrimp broodstock pellets in combination mussel (MPB), crabs (CBP), or *Artemia* (ABP). Vertical lines indicate \pm SEM. Asterisk (*) indicates significant difference between treatment groups.

In the middle phase of the experiment, sperm count was highest in ABP-fed and lowest in MBP-fed shrimps while the opposite was observed in the final phase of the test (Fig. 4). Regardless of dietary treatment, the number of good and live sperm cells improved towards the final phase of the test as indicated by the decrease or absence of live abnormal sperm cells previously apparent in the middle phase. Unfortunately, the peak of first spawning of females had ended in the final phase; thus, several batches of eggs were not fertilized particularly in the ABP-fed shrimps.

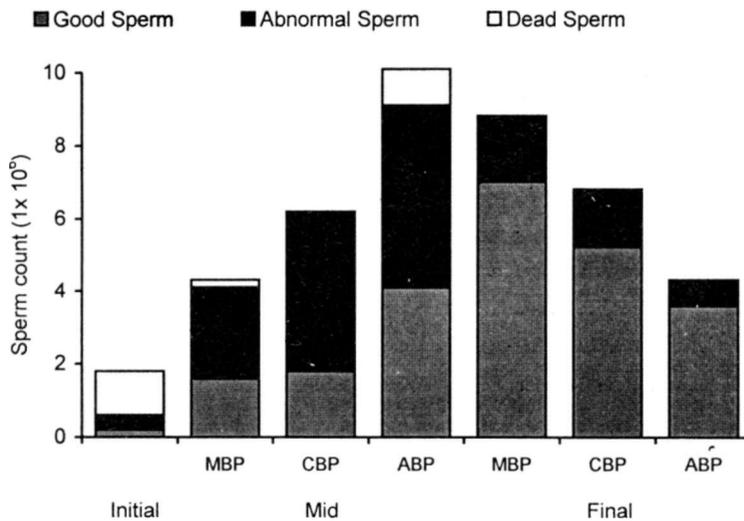


Fig. 4. Sperm count production of *P. monodon* fed shrimp broodstock pellets in combination mussel (MPB), crabs (CBP), or *Artemia* (ABP). The good and abnormal sperm constitute the live sperm cells in the bar graph.

Discussion

The increase of total carotenoids and astaxanthin in the hepatopancreas and the ovaries over the initial values indicates that carotenoids are being stored in these tissues. This is not surprising since hepatopancreas is the major metabolic and digestive organ of decapods (Gibson and Barker 1979). The relative concentration of carotenoids in ovaries has been found to increase during ovarian development (Harrison 1990).

Although astaxanthin was not detected in mussel, other forms of carotenoids may have been utilized or converted to astaxanthin as indicated by high levels in the tissues. In mussel, *Modiolus modiolus*, 16 carotenoids including astaxanthin have been found (Bjerkerger *et al.* 1993). Partali (1989) reported 20 carotenoids in *Mytilus edulis*.

Although there were no significant differences, lower maturation and spawning rates were noted in the ABP- than in MBP- and CBP- fed shrimps. Furthermore, no ABP-fed shrimps rematured because latency period from ablation to maturation and subsequent spawning was relatively long. This is probably due to the low carotenoid levels in the gonad of immature shrimps and more time is needed to build up enough carotenoids in the gonad. *Artemia* preferentially accumulate canthaxanthin (Latscha 1991). Since the vast majority of decapods are characterized by a predominant accumulation of astaxanthin (Latscha 1991), dietary canthaxanthin has still to be converted into astaxanthin by the shrimp. The efficiency of carotenoids is related to their proximity to astaxanthin in the metabolic process (Chien and Jeng 1992). Compared to canthaxanthin, astaxanthin may be more easily absorbed by the animals (Negre-Sadargues *et al.* 1993). Although *Artemia* contains carotenoids (Latscha 1991), fatty acids, and essential nutrients (Leger *et al.* 1987), the small size of *Artemia* and its tendency to disperse in the water column made them less available to shrimp. This may have contributed to the poor performance of ABP-fed shrimp.

In *P. monodon*, the color of the ovary changes dramatically from light yellow to dark olive green as it matures. In some of our previous experiments, ovaries of pond-reared broodstock held in the tanks for several months appeared pale green even when fully mature. Most often eggs spawned by the shrimps did not hatch, or produced poor quality nauplii. Likewise, mechanical injuries and melanized lesions in the exoskeleton were observed in some of the broodstock from ponds held in tanks for more than a month. In the present study, the color of the ovaries improved and the exoskeleton of the shrimps were clean throughout the culture period. Similarly, the color of mature ovary as well as the quality and survival of *P. monodon* larvae has dramatically improved upon the addition of paprika, nature's highest source of the carotenoid astaxanthin, in the broodstock diet (Oceanic Institute 1992). Carotenoids may perform a biological role similar to a-tocopherol by protecting sensitive tissues and reactive compounds from oxidative damage (Tacon 1981).

Although most of the females had been impregnated with sperm, some spawned eggs were not fertilized. This could be related to both availability and viability of sperm at the beginning of the experiment. Improved quality of the sperm was

observed at the end of the experiment, past the peak of first spawning of females. In contrast, Leung-Trujillo and Lawrence (1987) observed a decline in sperm quality or quantity in *P. setiferus* after the third week of captivity.

The results indicate that mussel may be a good food for *P. monodon* broodstock. Other components aside from carotenoids could have contributed to the good reproductive performance of MBP-fed shrimps. Green mussel has the additional advantage of being high in fatty acids which is considered to be essential for ovarian maturation (Marsden *et al.* 1992). Furthermore, better comparison of results could have been made if shrimps fed BP alone was included as another dietary treatment in the study.

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