CHAPTER I

INTRODUCTION

Successful implemention of earlier Thai fishery development plans result in large fishery production increases from 0.3 million tons to 2.1 million tons between 1961 and 1977 respectively. Subqeuently, fishery production decreased as a result of excessive resources exploitation and the imposition of a 200 miles (322 km) Exclusive Economic Zone (EEZ) by neighboring countries. The EEZ restricted access by Thai fishermen to traditional fishing grounds. Nevertheless, by 1981 Thai fishery production began to increase again as a result of cooperative fishing agreements with other countries, and from large increases in aquaculture fishery production (Office of Agricultural Economic, 1988; Panipa Hanvivatanakit, 1988).

Because Thailand's climate and coastal zone habitats which are ideal for marine prawns and prawn culture, certain species of prawns were identified under the Thai National Plans for Economic Development No. 5 (1972-1976) and No. 6 (1987-1991) with high priority for commercialization. The giant tiger prawn (*Penaeus monodon* Fabricius), in particular, was identified for such development because of its fast growing to a large size, high market demand, and export

value (Panipa Hanvivatanakit, 1988). *P. monodon* is the largest species in the Family Penaeidae, and has many biological attributes which make it amenable to culture in earthen ponds.

The principal impediment to its expanding culture production was the seed shortages because of the broodstock limitation. The limitation on broodstock quantity and quality resulted in nauplii shortages, which in turn resulted in postlarval (PL) seed shortages for pond stocking. At present, the Thai prawn culture industry needs more than 5 billion *P. monodon* nauplii each year. (Office of Agriculture Economics, 1988).

Another problem is the "blue color syndrome". The syndrome usually occurs with healthy prawns after 2-3 months of stocking in the grow-out ponds. The affected prawns are often pale blue in color instead of greyish-brown. This syndrome neither increases the mortality nor suppress growth, but it lowers market price. The blue prawns when cooked will turn yellowish-orange, rather than brilliant red of wild prawns. The yellowish orange color turns off consumers, especially in Japan. The deviation of the natural color of live and boiled prawns indicates the concern of carotenoid pigments deficiency, especially astaxanthin (Piamsak Menasveta *et al.*, in press).

Previous studies indicated that astaxanthin could correct the coloration of crustaceans (Menasveta, *et al.*, in press and D'Abramo *et al* , 1983; Abrill and Ceccaldi, 1984). Besides astaxanthin showed some beneficial roles in gonad maturation of aquatic animals (Torrisen, 1984; Choubert, 1986; Storebakken, *et al*, 1986.)

With the above reasons, the present research was undertaken to determine the roles of astaxanthin on coloration and gonad maturation improvement in *P. monodon* In particular, the present study wished to improve the gonad maturation of the pond-reared broodstockes by the nutritional method.

Objectives and Scope of the Thesis

The main objectives of the present study are:

1. To determine the effect of different concentrations of astaxanthin and canthaxanthin on growth, coloration, molt frequency, and food conversion of juvenile giant tiger prawn (*Penaeus monodon* Fabricius).

2. To determine the effect of astaxanthin on *P. monodon* ovarian development, spawning, fecundity, egg hatching rate, nauplius production and protozoea survival.

Expected Results

Expected results of this study are as follow:

1. Correcting the blue prawns syndrome of *P. monodon* by using astaxanthin.

2. Establishment of suitable astaxanthin and canthaxanthin dose for *P. monodon* feed.

3. Improved broodstock maturation and reproduction and thereby increase seed supplies of *P. monodon*.

Literatures Review

Carotenoids

Carotenoids are terpenoid pigment occurring widely in nature. In general, carotenoids are synthesized only in plant tissues, but are metabolized and store by animals after oxidation. It is assumed that animals oxidize β-carotene and its derivative, such as Zeaxanthin (3,3-dihydroxy-β-carotene) which finds in diatoms (Goodwin, 1952) and marine detritus (Fox, 1953). Although the role of carotenoids in the pigmentation of fish is well established

(Choubert, 1981 cited by choubert, 1986), it represents only one aspect of their function. It has also been observed that carotenoids influence the physiological growth processes (Deufel, 1965). The mode of action of these pigments is not well known, but appears to be linked with provitamin A function (Goodwin, 1952). Fisher *et al.*,(1954) have found that the astaxanthin level of euphausiid (*Meganyctiphanes novegica*) increases sharply in the tissue layer specimens, corresponding to a change from herbivorous to carnivorous habits with corresponding ingestion of large amounts of astaxanthin.

Carotenoids in Crustacean

Carotenoid pigments have been found in the hepatopancreas, blood, ovaries and developing eggs, of female crustaceans (Lönnberg, 1934; Lwoff, 1925; Goodwin, 1949, 1951). The carotenoid pigments also play and important part in general coloration of crustacean, especially the decapods. In experiments with spiny lobster (*Panulirus interruptus*),it was found that the amount of the hormone astaxanthin is related to molting and adaptive color changes. This hormone also controls carotenoprotein synthesis in *Carcinus maenas* (Lenel and Villet, 1951).

The most important carotenoid pigments found in the crustacean are :

1. **Astaxanthin.** This compound occurs in Crustacea in three forms; 1) un-esterified, 2) esterified, and 3) conjugated with proteins. The first two are insoluble in aqueous media, while chromoproteins are soluble. The un-esterified form is the one most commonly found in the epidermis of crustaceans such as *Homarus gammarus* and *Nephrops norvegic* (Kuhn and Lederer 1933). Un-esterified astaxanthin appears to always be localize in the chromatophores, while astaxanthin protein complexes (carotenoproteins or chromoproteins) are often found outside the chromatophores in the surrounding tissues, as well as in the chromatophores (Brown, 1931). Astaxanthin is also found in the hepatopancreas, blood (Kuhn and Sorensen, 1938; Lwoff, 1925), eggs (Kuhn, Lederer and Deutsch 1933), and eyes (Lonnberg, 1934; Lwoff, 1925) of many species.

2. **ß-Carotene.** As already emphasize, carotenoids other than astaxanthin usually occur only in small amounts in crustacea. ß-carotene is, however, frequently present. An exception to this rule is that ß-carotene the major carotenoid of the epigean isopod *Asellus aquaticus* (Baldwin,

1941), and the parasitic cirriped *Saccurina carcini*(Lennel, 1954).

3. **Xanthophylls.** Other than astaxanthin. Cryptoxanthin (ß-hydroxy-ß-carotene) has been found in *Asellus aquaticus* (Baldwin and Beatty, 1941), while unidentified neutral xanthophyll have been found in small amounts in whole extracts of *Cragon spp., Nepphros novagicus* and *Hippolyte californiensis* (Young, 1936),in the carapace of *Carcinus maenas,* and in the eyes of *Homarus gammarus* (Fisher Kon and Thomson, 1954). They do not occur in euphausids (Fisher, Kon and Thomson, 1952).

Carotenoids in crustaceans are rather more extensive than in many other invertebrates but occasionally pigment though the carotenoids occur in a variety of tissues like the shell, epidermis, hemolymph, hepatopancreas, digestive tract, gonads and eyes. In all cases the shell or carapace and the hypodermis represent the major reservoir of body pigments. For intermolt penaeid prawns about 80-90 % of the pigments could be attributed to the shell and approximately 10-15 % to hypodermal chromatophores. An example of the carotenoid content, composition and configuration of astaxanthin in four species of Penaeidae (Schiedt, 1987 cited by Latcha, 1990) is given in Table 1. About 65 % to 98 % of total carotenoids is astaxanthin of which 83.3 % to 95.4 %

Table 1 Carotenoids in various wild species of Penaeidae.

Carotenoids	*P. vannamei*	*P. monodon*	*P. japonicus*	*Metapenaeus monoceros*
Content µg/g	56	52	38	44
Composition	%	%	%	%
ß,ß-Carotene	0.5	0.2	0	3
Yellow xanthophylls[a)]	30	0.4	19	23
7,8-Didehydroasta-xanthin	5	1	2	2
Astaxanthin total	65	98	79	72
Diesters	33	27	37	22
Monoesters	29	57	29	38
Free form	3	14	13	12

a) Ester of fatty acids, no lutein, zeaxanthin, presumably three hydroxy groups.

revealed to be in the di and monoester forms, while only 3-14 % was in the free form. The occurrence of practically racemic 1:2:1 mixture of the three optical isomers (3R,3'R);meso) and (3S,3'S) in the four species furthermore is in agreement with previous findings in the common northern prawns *Pandalus borealis* of the same subclass.

Effect of Carotenoids in Aquatic Animals Reproduction

Carotenoid pigments found in the reproductive organs of fishes and decapods are of dietary origin. It is known that vertebrates in general, and fish in particular are incapable of synthesizing carotenoids endogenously, and are therefore dependent on their supply via the diet (Andre, 1926).

1. Pigments Mobilization

During sexual maturation, reproductive organs accumulate carotenoid pigments. With female fish, this accumulation comes partly from carotenoid ingested in food, and also from carotenoids stored in muscle tissue. Thus, oocytes color of medaka (*Oryzias atipes*) depends directly on the amount of carotenoid ingested by the female (Takkuchi, 1960 cited by Chaubert 1986). Miki *et al.* (1984) demonstrated that red seabream (*Chrysophrys major*) stored carotenoid pigments in oocytes after hydrolysis.

With brown trout (*Salmon truta*), Steven (1949) reported almost total mobilization of carotenoids stored in muscle and correspondingly significant increases in carotenoid content of the ovaries. The amount of muscles carotenoids did not however, account for all of the pigment deposited in the ovaries. The fish, therefore, depends on foodstuffs to augment this supply during ovarian maturation. In the case of chum salmon (*Oncorhynchus keta*) this does not appear to be necessary. Crozier (1969) calculated that carotenoid deposition in the muscles was larger than that needed by the ovaries, with only 60% of muscle carotenoid pigments being mobilized during the spawning migration. Sargent *et al.*, 1979 cited by Storebakken (1985) observed that *Salmon gairdneri* fed a zooplankton diet were dark red, whereas those extracted from herring (*Clupea harengus*) were dark green. This coloration occurred even though post-juvenile anadromous salmonids have brightly colored flesh resulting from xanthophyll deposition, while and herring flesh is only lightly pigmented. This "caroteniod store" in salmonid muscles is mobilized before spawning with the asthaxanthin or canthaxanthin transferred to the ovaries.

In males fish,some species show no carotenoid accumulation in the testicle or the milt. Instead pigments may be transferred to the skin during the spawning season.

This has been reported by Goodwin (1952) for lumpish (*Cyclopterus lumpus*) and flounder (*Fundulus parvipinnis*) and Chum salmon by Crozier (1970) . Increased male skin pigmentation may influence mating behavior by attracting the females (Kinsky, 1971; Mikulin and Soin, 1975).

Smith (1911) indicated that female decapods mobilize and transfer "lipochromes" from the hepatopancreas (where they are stored as carotenoprotein), to the ripening ovaries by the blood. This finding was confirmed in *Carcinus* spp. where only traces of pigment remained in the blood and hepatopancreas in gravid females, which was a pale color compared with males and non-gravid females. Normal color can be restored in egg laying females by feeding them a carotenoid-rich diet such as cooked carcinus eggs (Abeloos and Fisher, 1926). Passage of carotenoid-protein from blood to ovary has also been observed in *Dapnia* spp. (Green, 1957) and hen where carotenoid was transferred from body tissues to ovaries during egglaying. Lenel (1954) observed in *Carcinus parasitizeed* with *Sacculina carcinii*; that blood ß-carotene levels vary considerable, while astaxanthin levels remain reasonably constant. Low levels of ß-carotene often encountered are ascribed to its preferential removal by the parasites, which accumulate only ß-carotene and no astaxanthin. Fischer (1926) indicated that feces from *Carcinus* also

contain the same carotenoprotein found in the hepatopancreas. The hepatopancreas therefore appears to have a dual function; 1) as a storehouse from which carotenoids are mobilized to the eggs and integument when required, and 2) as an excretory organ if excessive amounts of carotenoids are absorbed. Fischer (1927a, 1927b) found that when placed on a carotenoid-free diet, *Carcinus* excrete carotenoids for many weeks, but after three months colorless feces were obtained. This demonstrates that under normal conditions carotenoids lost very slowly, and then through, alimentary progress. Similar results have been reported in the coelenterate (Abeloos, 1926) where normally pigmented hepatopancreases and feces resulted soon as the animals were restored to a carotenoid-rich diet.

It is well known that adaptive color changes for many crustaceans are controlled by hormones. Removal eye-stalk from freshly molted crabs (*Carinus maenas*) results in reddening of the first layer of new cuticle. This reddening is not due to increased accumulation of astaxanthin, but rather to the dissociation of brown and green astaxanthin-protein complexes (Lenel and Veillet, 1951).

2. Oocyte Maturation

Only the effects of canthaxanthin on oocyte maturation has been studied. In one six months experiment, Deufel (1965) fed two groups of 2-year old rainbow trout (*Oncorhynchus mykiss* formerly *Salmo gairdneri*) on diets which differed only in their carotenoid content. One test diet was supplemented with 40 *ug* canthaxanthin/kg feed. He observed the following differences in control group compared with 64.% of the females receiving canthaxanthin. The effects on spawning time (ten days earlier in the test group), and % of egg fertilization (99.9% for the test group, 95.7% for the control group) showed similar results. These experimental results have not yet been confirmed. Morrison and Smith (1981) observed no difference in the fertility of female brook trout (*Salvelinus fontinalis*) on a diet enriched with canthaxanthin for two months prior to spawning. Harris (1984) also reported no differences in fertility between female rainbow trout feed for six months on a diet containing 20 mg canthaxanthin, and control fish. Harris also reported that eggs from control trout were slightly smaller than those of trout receiving canthaxanthin.

Effect of carotenoid pigment on Decapods maturation has not been studied; but many studies exist concerning embryogenesis, of chromoprotein present in harpacticoid egg

by histochemical methods. Most of the carotenoid is deposited as such in the outter pigmentary layers of the nauplii eye, which becomes bright red. Some of the pigment does, however, exist as a blue chromoprotein which is found as small cubules in the internal pigmentary layer. This layer is called the cuboidal layer. Carotenoid deposition occurs before differentiation of the embryonic intestine. In this connection, it is interesting to compare the appearance of the eyes of *T. fucata* produced from normal eggs, reared on a normal diet with those hatched from virtually carotenoid-free eggs and reared on carotenoid free diets, (e.g.rabbit erythro-cytes or bacteria such *Escherichia coli*).In the first group, the eyes were brilliant red, and in the latter only slightly rose-colored. In both cases, however, the optic cuboidal layers were equally blue owing to the presence of the carotenoprotein in approximately the same amounts. The probable reason for this is that in the second group of animals, there was a small amount of carotenoids still present in the eggs and possibly also in the diet (e.g. traces of algae in aquarium tanks) which accumulated in the optic cuboidal layers. There is no justification for assuming that these observations indicate a denovo synthesis of carotenoids by animal tissue.In lobster eggs, there are no quantitative changes in the astaxanthin levels during embryogenesis, or immediately after hatching.