MOBILISATION OF TOTAL CAROTENOIDS IN RELATION TO OVARIAN MATURATION IN THE PRAWN *METAPENAEUS DOBSONI* (MIERS)

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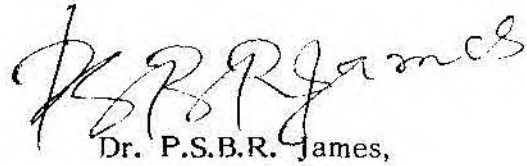
CERTIFICATE

This is to certify that this Dissertation is a bonafide record of the work done by Kum. Letha Kutty under my supervision and that no part thereof has been presented before for any other degree.



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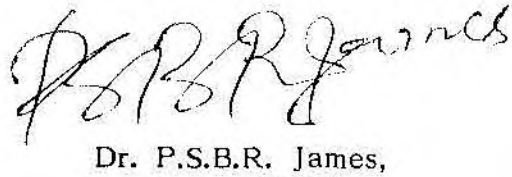

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CONTENTS

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PREFACE

In India, prawn and prawn products account for about 83% of the foreign exchange earnings from sea food export, the present demand being on small sized shrimps (Anonymous, 1989). However, the inconsistant trends in capture fisheries has necessitated the identification and adoption of alternate methods for enhancing prawn production. So scientific farming appears to be the best alternative in the present context.

The increasing pace of penaeid prawn culture activities have resulted in the discovery of several techniques like induced maturation through eye stalk ablation, spawning in confinement, mass rearing, and formulation of highly efficient feeds. In this context, basic information on the biochemical changes manifested in the ovaries during maturation is very essential. The complex process of accumulation, release and transport of nutrient reserves from the storage organ to the gonads, metabolic capacities of organs participating in the reproductive cycle, regulation and coordination involved in achieving spawning and allied biochemical aspects are yet to be explained, in order to realise the full potential of prawn culture.

Among 74 species of penaeid prawns reported to exist along the Indian Coast (Mohammed, 1973a), the Indian Brown Shrimp Metapenaeus dobsoni (Miers) is a commercially important species. It contributes to a major portion of the marine and estuarine prawn landings along the south-west coast of India. Besides, there are reports on the possibility of its culture in the coastal brackish, water areas, in view of its year round seed availability (Mohammed,

1973b). Recent success in its induced maturation, breeding and spawning under captivity in high saline brackish water areas (Rao and Kathirvel, 1973) has strengthened its importance as a potential candidate species for culture. Thus for production of quality seeds, a thorough understanding of its reproductive physiology is necessary.

One of the most effective methods of studying the reproductive state in penaeid shrimps is to determine the degree of gonadal maturation and its subsequent development. Gonadal development in females can be evaluated on the basis of the ovary size, colour, histological changes and the changes in the ratio between ovary size and body weight (Gonadosomatic index). Maturation can also be studied in relation to the biochemical changes during ovarian development.

Carotenoids are one of the most important biochemical constituents and studies on the variations of these pigments in relation to ovarian maturation in crustaceans in general are limited and in penaeids in particular are lacking. Studies of this type are important both for basic understanding and practical application. Basically such studies enable a more precise and detailed knowledge on the utilization and translocation of these pigments during growth and development of the ovaries in relation to other organs and tissues, thus revealing the potential 'Store house' of this biochemical constituent and enables in tracing the pathway through which they are mobilised for the future biological needs of the animal concerned. On the applied aspect, shrimp waste, in view of its high carotenoid content, can be incorporated into diets to enhance pigmentation and colour of the shell and flesh of the species cultured. Judicious addition of

these pigments into diets can modify the colour to suit the aesthetic taste of the consumer market which might fetch a higher price for the prawns, thus increasing the profit margin.

The objectives of the present study were:

1) To study the variation in total carotenoid concentration of tissues like hepatopancreas, haemolymph, exoskeleton and ovaries along with whole animals during different stages of ovarian maturation in Metapenaeus dobsoni, collected from the wild.

2) To establish a relationship between total carotenoid content of the ovary with the gonadosomatic index.

3) To compare the differences in total carotenoid concentration of tissues and whole animals between prawns of different maturity stages obtained through eyestalk ablation and unablated wild prawns.

Thus the present study on M. dobsoni, a species of high potential, for mariculture was taken up for contributing some knowledge on the mobilisation of total carotenoids during its ovarian maturation.

I wish to express my gratitude to Dr. P.S.B.R. James, Director of Central Marine Fisheries Research Institute, under whose scholarly guidance the present work has been completed. I am grateful to Dr. A.D. Diwan, Scientist S-3, for his valuable advice and help throughout the period of this study. I wish to thank Shri. M. Srinath for his help in statistical analysis of the data. My thanks are also due to Mr. Nandakumar for supplying all the necessary materials

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INTRODUCTION

The striking, yellow, red, orange and purple colours found in aquatic animals are often attributed to a group of lipid soluble pigments called the Carotenoids. The visible appearance of an animal depends on the overall concentration of these pigments in the body. The ability to synthesize carotenoids seems to be restricted to the plants and microbes, so the animals derive them solely from the food web (Miki et al., 1982). The central dogma of carotenoid biochemistry is that animals cannot synthesize these pigments "de novo" but can alter them to other endogenous forms. There is an immense array of carotenoids in the animal kingdom and they can be mainly grouped into: Carotenes (Hydrocarbon carotenoids) and Xanthophylls (Oxygenated carotenoids). In animals, these are present in the form of free pigments or bound to protein called carotenoproteins.

The presence of "lipochromes" was first observed in human blood and milk by Thudichum (1869). However, it was only in the twentieth century, that an extensive study was initiated by Palmer and Eckles (1922). In crustaceans, the pioneer reports on the occurrence of carotenoids were those of Pouchet (1872) concerning some changes in the colour of lobsters. Since then, a number of investigations have been carried out by Fisher et al. (1952), Goodwin (1960), Campbell (1969) and Herring (1972), relating to the carotenoid pigment composition and distribution in Decapod crustaceans.

Crustaceans, especially shrimps are considered to be the most important candidate species for culture. Quantitative and qualitative studies of

carotenoids in these animals have led to the opening up of many potential avenues in aquaculture Industry. In view of their high carotenoid content, crustacean wastes (Wilkie, 1972) are considered a good source of carotenoids. Carotenoid extraction has been initiated on a commercial basis in view of its nutritive value, in the U.S.A. and Europe from Alaska Pink Shrimp Penaeus duorarum (Kelley and Harnon, 1972) and the Sand Shrimp Crangon vulgaris (Snauwaertet al., 1973). Saito and Regier (1971) compared the quality of shrimp waste fed fish Salvelinus fontinalis with that of crab waste fed ones in order to emphasise that the high carotenoid content of shrimp waste improved the quality and colour of the cultured species. The importance of carotenoids in shrimp and fish diets to enhance pigmentation was demonstrated by Spinelli et al.(1974) Joseph and William (1975),Sandifer and Joseph(1976) and Spinelli and Mahnken(1978).

As far as the biological functions of carotenoids are concerned many important role have been assigned to them. In finfishes and shellfish, carotenoids perform as precusors for Vitamin A. In addition, in fishes, they influence other physiological functions such as helping in vision (Appleby and Muntz, 1979), promoting growth, enhancing tolerance levels to stringent environmental condition such as low dissolved oxygen, elevated temperature and ammonia levels (Tacon, 1981). According to Craik (1985), carotenoids also help in maintaining egg quality and egg viability. In Crustaceans, Cheesman et al. (1967) reported that they give protection against higher illumination and solar radiation, protects the gut from enzymes and aids in stabilisation of proteins, while Gilchrist and Lee (1972) speculated that they mask luminescense of prey in the stomach, gives protection against elevated temperatures and helps in

reproduction. Karnaukhov et al. (1977) reported that carotenoids may also increase tolerance to environmental pollution in sea molluscs.

Reproduction in one of the most important physiological process attributed to animals. Investigations have suggested that carotenoids do take part in reproduction. In finfishes studies correlating carotenoids and the reproductive cycle have contributed in establishing a clear cut pattern of carotenoid mobilisation as revealed by the works of Steven (1949) who studied the carotenoids in the reproductive cycle of Brown trout, Salmo trutta, Crozier (1970) and Donaldson and Fagerland (1970) who worked on Sockeye salmon Oncorhyncus nerka. Besides these workers, Shnarevich and Sakhnenko (1971) studied the dynamics of carotenoids in fish tissues and organs in relation to their sexual cycle. The carotenoid composition of Alaska pollack Theragra chalcogramma roe at different stages of maturity was determined by Konosu (1983). The behaviour of carotenoids during the course of maturation in Chum salmon (Kitahara, 1983) and in Masu salmon (Kitahara, 1985) has been worked out. Choubert (1986) tried to determine the role of carotenoids in fishes during their reproductive cycle. In crustaceans, there is much indirect evidence that carotenoids play a significant role in reproduction. The pioneer work in this field was done by Smith (1911). Since then, a number of contributions have been made. Abeloos and Fischer (1926) conducted some investigations on Carcinus sp, while Establier (1966) worked on the shrimp Plesiopenaeus edwardsianus. Green (1966) reported findings of the carotenoid content in the cladoceran Simocephalus vetulus. Mobilisation of carotenoids in Daphnia magna was studied by Herring (1968). Gilchrist and Lee (1972) discussed the role of carotenoids in the reproduction of Sand Crab Emerita analoga. The total carotenoids and astaxanthin of Orconectus limosus during its annual cycle was studied by Czeczuga (1976).

While Hairston(1979) discussed the relationship between pigmentation and reproduction in copepod Diaptomus sp. Czeczuga (1980) studied the changes in the carotenoid content of Gammarus lacustris during its reproductive cycle.

Induced maturation, through eyestalk ablation is now being widely resorted to, in order to breed crustaceans in captivity. Investigations on the changes in the carotenoid content during induced maturation effected by eyestalk ablation were conducted by Lenel and Veillet (1951) on Carcinus maenas and Bomiriski and Klek (1974) on the crab, Rhithropanopeus harrisii. Maugle et al. (1980) demonstrated the influence of eyestalk abalation on the carotenoid composition of juvenile Macrobrachium rosenbergii.

As maturation progresses, the carotenoid content of different organs and tissues fluctuate according to species (Castillo et al., 1982). Lenel et al. (1978) studied the mode of transfer of carotenoids from the storage organ to other tissues. Investigations on the carotenoid content in the hepatopancreas, have been done in C. maenas (Gilchrist and Lee, 1967), Penaeus japonicus (Katayama et al., 1972a, Ceccaldi et al., 1978), Pagurus prideauxi (Castillo et al., 1980) and Potamon dehaani (Matsuno et al., 1982). The quantitative and qualitative determination in the carotenoids of the haemolymph in relation to the reproductive cycle has been done by Ceccaldi (1967, 1968), Eickstaedt (1969) and Gilchrist and Lee (1972). The carotenoids in the exoskeleton have been well characterised in Pagurus pollicaris (McNanamara, et al., 1982) Pandalus borealis (Tsukuda, 1963) P. japonicus (Katayama et al., 1972b) Metapenaeus affinis (Nair subai and Mohanrao, 1973) Portunus trituberculatus (Katayama et al., 1973a), Panulirus japonicus (Katayama et al., 1973b), Procambarus clarkii (Nakagawa et al., 1974), G. lacustris (Czeczuga and Krywuta, 1981) and in Euphausia superba

(Maoka et al., 1985). The crustacean ovaries deposit a large amount of carotenoids of various colours, during vitellogenesis and these coloured complexes were studied in Hommarus gammarus (Kuhn and Sorensen, 1938), Cancer pagurus (Zagalsky et al., 1967) and E. analoga, (Gilchrist and Lee, 1972). Miki et al. (1982) made a comparative study of the carotenoids in finfish and shellfish ovaries.

Generally, the carotenoid concentration in whole animals also fluctuates during their breeding cycle. Carotenoids of whole specimens have been determined in Crangon allmani (Fisher et al., 1954) in C. crangon (Czerpak and Czeczuga, 1969) and in several caridean and penaeid species of shrimps (Herring, 1973).

Thus it is evident from the above review that studies on the carotenoid mobilisation during ovarian maturation are very limited and such studies on the commercially important Indian penaeid prawns are lacking. Understanding the metabolic changes in carotenoids provides additional information on the biochemical changes occurring during reproduction. Besides as carotenoids are known to enhance the maturation process (Laxminarayana, 1987) it can be used in formulating highly efficient brood stock diets, after determining the exact quantity required for the animal during each stage of maturation. This information can bring about the production of high quality seeds required for initiating culture. The present laboratory study has been carried out in the back ground of this understanding and the results are presented in the following pages.

MATERIALS AND METHODS

Live females of Metapenaeus dobsoni, required for the present study were collected from the inshore waters of Cochin, during the period June to September 1989. The specimens collected were in different stages of ovarian maturation and their total length ranged between 68 to 115 mm. They were brought to the laboratory in batches of twenty each in oxygen packed polythene seed transportation bags and maintained in 40 litre capacity fibre glass tanks with continuous aeration and fed ad libitum with fresh clam meat.

Based on visual observations of ovarian development, the specimens were separated into five groups and preliminary examination of the stages of maturation was made on the basis of size of the ovary and its colour as described in the methodology of Brown and Patlan (1974). Classification of maturity stages and ova diameter measurements were according to the method described by Rao (1968). Histological study of the ovary during different maturity stages was done according to standard methods.

Methods for assessment of ovarian maturation

1. Gonado-somatic index (GSI)

GSI was determined for each maturity stage in various individuals with a view to ascertain the condition of the ovary. It was determined according to the method of Farmanfarmaian et al. (1958) using the formula,

$$GSI = \frac{\text{total wet wt. of gonad}}{\text{total wet wt. of animal}} \times 100$$

2. Hepato-somatic index (HSI)

HSI was determined to correlate any changes occurring in the hepato-pancreas during different stages of maturity according to the method of Giese (1959) using the formula,

$$HSI = \frac{\text{Wet wt. of hepatopancreas}}{\text{Wet wt. of animal}} \times 100$$

3. Morphological and microscopic examination of ova

This was done to evaluate the degree of maturation on the basis of morphological changes in the ova, extent of yolk deposition and ova diameter measurements. Around 100 ova were measured from 3 samples of ovaries at each stage of maturity, using an ocular micrometer. The ovaries were hardened with 5% formaldehyde for 2 days. Diameters were taken parallel to the ocular micrometer in order to avoid errors due to selection and distortion in preservation. Samples were taken only from the middle lobe of the ovaries.

4. Histological study of Ovarian tissue during different stages of maturity:

The ovaries of prawns in different stages of maturation were collected and fixed in Bouin's fluid, and subsequently washed and dehydrated. They were then embedded in molten paraffin wax (56-58°C M.P) and blocks of the ovaries were made. The blocks were sectioned at 6-8 u thickness using a manual rotary microtome (Weswox Optik MT 1096 A). The serial sections were stained with haematoxylin and aqueous eosine. The slides were subsequently dehydrated and cleared in Xylene and finally mounted in DPX mountant. Photomicrographs of the histological sections were taken wherever

necessary. The classification of the maturity stages based on the histological characteristics was done according to the method of King (1948).

Haemolymph extraction for carotenoid determination

Prior to the extraction of haemolymph from live animals, the carapace and adjacent areas of the prawns were cleaned and dried with tissue paper to remove excess moisture. The haemolymph was then collected through the pericardial cavity using a one ml tuberculin glass syringe, previously washed with an anticoagulant (10% trisodium citrate). The haemolymph samples, thus collected were preserved in clean glass vials lined with aluminium foil and kept frozen until analysis.

Tissue collection, preparation and storage for carotenoid determination

For estimation of total carotenoids, prawns of different maturity stages were dissected afresh and tissues like hepatopancreas, exoskeleton and ovaries were removed. Tissues were cleaned to ensure that no debris or any other undesirable material was adherent, weighed to the nearest mg and stored in clean screw-capped glass vials, lined with aluminium foil at a temperature of -10 C until analysis.

Estimation of total Carotenoids

The total carotenoids from various tissues and the whole animals were estimated using Olson's method (1979) where chloroform stablised with 0.75% absolute ethanol was used to extract the carotenoids. One gm of the tissue weighed to the nearest mg was placed in a 10 ml screw-capped glass vial lined with aluminium foil and to this was added 2.50gm of anhydrous sodium sulphate. The tissue samples were mixed well with sodium sulphate to remove

all moisture from the tissue. Then 5 ml of chloroform was added and the vial was sealed and placed for 24 hrs at - 10 C. For estimation of carotenoids from the haemolymph, one ml of sample was taken and chloroform was directly added and kept at - 10 C. During this period, the chloroform will have formed a clear layer of 1.2 cm above the residue. An aliquot of 0.3 ml, chloroform was taken from this layer and made up to 3 ml, with absolute ethanol. The optical density was read at 480 nm. A blank prepared in the same manner was used.

Whole animals: Samples of whole prawns at different stages of maturation were cut into small pieces and soaked in cold chloroform. The samples were thoroughly ground in a glass mortar with pestle, until a thick paste was formed. It was again ground with anhydrous sodium sulphate. One gm of this paste was used for the estimation of total carotenoids.

The total carotenoid concentration was expressed as µg carotenoid/g tissue as given below:

$$\text{Total carotenoid content} = \frac{\text{Optical density of sample}}{\text{extinction coefficient}} \times \frac{\text{dilution factor}}{\text{Sample wt. (gm)}}$$

In this method, dilution factor is = 50, extinction coefficient is = 0.25.

Induced maturation by eyestalk ablation and estimation of carotenoid content

The total carotenoid content of the prawns, wherein maturation was artifically induced was also studied. The approach was to evaluate whether there was any significant difference in the total carotenoid concentration of eye-stalk ablated prawns during different stages of maturity with that of unablated wild prawns.

Adult females 66 mm (TL) collected from the brackishwater ponds at Panangad were used for this experiment. About twenty animals were reared in a 100 litre fibre glass tank and acclimatised for 5 days in sea water of 31 $\pm$ 1 ppt salinity and pH 8.0. During acclimation the water temperature was maintained at 27-29° C, fresh clam meat was given as feed, and aeration was maintained continuously. Care was taken to remove faecal matter and uneaten food by siphoning the tanks daily. The water quality was monitered regularly.

After the period of acclimation, the animals were isolated into five groups (each group containing 4 animals) and maintained in five 30 litre capacity fibre glass tanks with a depth of 50 cm. The salinity, pH and temperature of the sea water used for this experiment was also maintained at 31 $\pm$ 1 ppt, 8.0 and 27-29°C respectively. Proper aeration was maintained in these tanks. Unilateral eye-stalk ablation was done for all animals. The animals were fed with fresh clam meat and water quality was monitered twice a day. The tanks were kept covered with dark cloth, to reduce stress caused by light.

Sampling of the ablated prawns was done from Tank 1 on the second day after ablation when the ovary had still not begun to develop. Sampling from Tank 2 was done on 8th day after ablation, from Tank 3 on the 11th day, from Tank 4 on the 14th day when the ovary was in progressive stages of maturation, and from Tank 5 on the 20th day, after the prawns had spawned.

After every sampling the haemolymph was collected and the prawns were dissected and the tissues removed for estimation of total carotenoids.

No control was maintained as the comparison was done between the wild and the ablated prawns.

Statistical Analysis

Data obtained during the course of the experiments were subjected to Analysis of Variance (ANOVA). F test was preformed to determine differences between treatment means (stages of maturity) and the different tissues. If the F value was found to be statistically significant, then the data were analysed by Least Significant Difference Test (LSD). If the absolute value of difference was greater than LSD, it was found to be significant at $P < 0.05$ (significance at 5% level) or $P < 0.01$ (significance at 1% level). In order to establish relationship between GSI and ovarian total carotenoids, a polynomial regression line was drawn and an equation was obtained. A one way ANOVA was done to determine whether there was any significant difference in total carotenoids of different tissues and whole animals of ablated and wild prawns. Data was analysed on a computer (WIPRO. P.C).

RESULTS

Four stages of maturation have been recognised for the complete ovarian development M. dobsoni. The fifth stage has been considered as spawned or spent recovery stage (Plate 1, Fig. 1). Based on the visual observation of the ovary, the maturation stages have been distinguished as follows:-

Stage I. Immature stage : The ovaries are thin, translucent, unpigmented and confined to the abdomen. It is invisible through the exoskeleton.

Stage II. Early maturing stage : The ovary increases in size and the anterior and middle lobes are on the process of development. The dorsal surface of the ovary appears yellowish.

Stage III. Late maturing stage : The ovary appears light green and is visible through the exoskeleton. The anterior and middle lobes are fully developed.

Stage IV. Mature stage : The ovary becomes dark green, and is clearly visible through the exoskeleton. Anterior lobe is diamond-shaped. This stage is considered to be the last stage of maturity before the actual spawning takes place.

Stage V. Spent recovering stage : The ovary reverts back to the immature condition, but appears yellowish and flaccid and is also invisible through the exoskeleton.

1. Gonado-somatic index (GSI)

The variation in GSI of M. dobsoni during ovarian maturation is shown in Table 1. The GSI was found to increase (Fig. 1) gradually from the

immature stage (0.24 + 0.24) to the mature stage (6.61 + 0.65). After spawning it declined (4.31 ± 0.63).

2. Hepato-somatic index (HSI)

The variation in HSI in M. dobsoni during ovarian maturation (Table 2) showed that from an initial high in the immature stage (1.88 ± 0.28) it decreased in the early maturing stage (0.51 ± 0.27). It then gradually increased in the late maturing stage (1.08 ± 0.60) and reached the peak in the mature stage (2.11 ± 0.56) and finally declined in the spent stage to 1.72 ± 0.29 (Fig. 2).

Analysis of variance to test significance of variation in HSI (ANOVA Table 1), showed it was highly significant ($P < 0.01$). However mean values between immature and mature, immature and spent and mature and spent did not differ significantly.

3. Morphological and microscopic examination of the ova

Based on the morphological changes, extent of yolk formation and ova diameter measurements (Table 3), the following observations were made on the ova of M. dobsoni during its ovarian maturation.

Stage I. The smallest oocytes were encountered in this stage. They were all less than 80 µm. 50% of oocytes were in the mean size of 25 µm. The oocytes were small, spherical with clear cytoplasm and conspicuous nuclei. Yolk granule formation had not started.

Stage II. The oocytes under development were larger in this stage and ranged between 80-190 µm with more than 50% in the mean size of 134 µm. Opaque yolk granules had started developing in the cytoplasm of the oocytes and their nuclei had reduced in size.

Stage III. The maturing ova had increased in size and ranged from 140-250 µm with the 50% of the ova being in the mean size of 183 µm. The ova had become opaque due to the accumulation of yolk and the nuclei had further reducd in size.

Stage IV. The largest ova were encountered in this stage (320 µm) and 50% of the ova were in the mean size of 236 µm. The yolk formation had reached its maximum extent and the nuclei were seen to have completely degenerated.

Stage V. The oocytes had reduced in size, and all of them were smaller than 96 µm. 50% the ova were in the mean size of 72 µm. Except for the increase in size, they resembled the oocytes of the immature stage with clear cytoplasm and conspicuous nuclei.

4. Histological studies of the ovary during different stage of maturity

Based on the histological characteristics of the ovary during its different stages of maturation, the following observations were made and the stages confirmed, thereof.

Stage I. The ovarian tissue showed a granulated appearance with conspicuous nuclei which had taken on a blue stain. The cytoplasm of the oocytes was not distinguishable (Plate II, Fig. 1). The presence of oocytes can be distinguished only by their nuclei (Plate II, Fig. 2).

Stage II. The finely granular appearance of the tissue was lost and the oocytes had started developing (Plate III, Fig. 1). The cytoplasm was now distinguishable and stained pink with the nuclei taking on the blue stain. The nuclear ring was clear, presence of follicle cells

(Plate III, Fig. 2). The primary oogonia which had originated from the germinal zone were visible. The germinal epithelium gives rise to oogonia which are not distributed uniformly, but confined to definite area called "Zone of proliferation".

Stage III. This stage showed the appearance of secondary oogonia along with the primary oogonia (Plate IV, Fig. 1). These secondary oogonia had also started developing into oocytes. The follicles were numerous in number. The "Zone of proliferation" was clearly visible showing that the development of oogonia and oocytes is a continuous process (plate IV, Fig. 2). The cytoplasm of the ova stained pink due to accumulation of yolk granules and the nuclei had started obliterating (Plate V, Fig. 1 and 2).

Stage IV. The ova had fully developed and its cytoplasm took eosine to the maximum extent indicating very high amount of yolk accumulation. The follicles were not very distinct because ova were fully packed inside and in between the ova some follicle cells could be seen (Plate VI, Fig. 1). The zone of proliferation was still evident. In the mature ova of M. dobsoni, no rod-like peripheral bodies radiating from the central protion were observed. However, a distinct nuclear ring could be observed (Plate VI, Fig. 2).

Stage V. In this stage, all the oocytes had been replaced by a dense mass of follicle cells (Plate VII, Fig. 1). The sections also show the presence of "resorptive ova" which clearly distinguished this stage (Plate VII, Fig. 2). This stage suggests that another batch of eggs are produced after spawning.

5. Estimation of total carotenoids

5.1. Hepatopancreas

The variation in total carotenoid concentration (ug/g wet wt.) in the hepatopancreas of M. dobsoni (Table 4) during ovarian maturation showed that there was a decrease (Fig. 3) from the immature stage (75.27 ± 7.15) to the mature stage (20.38 ± 2.90) with a recovery in the spent stage (58.29±8.52).

Analysis of variance to test significance of variation in a total carotenoid content in the hepatopancreas during ovarian maturation (ANOVA table 2) was highly significant ($P < 0.01$). However, variation in the total carotenoid values between early maturing and late maturing, as well as maturing and mature stage, was not significant.

5.2. Haemolymph

The variation in total carotenoid concentration (µg/g wet wt) in haemolymph (Table 5) of M. dobsoni showed an increase from the immature stage (5.43 ± 0.84) to the mature stage (11.58 ± 1.46), as maturity advanced. After spawning it fell to 6.78 ± 1.58 (Fig. 4).

Statistical analysis (ANOVA Table 3) showed that the variation in the total carotenoid concentration in the haemolymph as maturation progressed was highly significant ($P < 0.01$). However, total carotenoid concentration between late maturing and spent stage did not differ significantly.

5.3 Exoskeleton

The variation in the total carotenoid content (µg/g wet wt) in the exoskeleton of M. dobsoni (Table 6) showed that from an intial of 22.57 ± 3.28 in the immature stage it reached a peak (70.58 ± 7.19) in the mature stage,

but later declined (39 ± 3.99) in the spent stage (Fig. 5).

Analysis of variance to test the significance of variation in total carotenoids in the exoskeleton (ANOVA Table 4) during ovarian maturation was highly significant ($P < 0.01$). However, mean values of total carotenoids did not differ significantly between early maturing and spent stage.

5.4. Ovary

The variation in total carotenoid concentration in the ovary of M. dobsoni during ovarian maturation (Table 7) showed an increase (Fig. 6) from a minimum (23 83 ± 4.31) in the immature stage to a maximum (100.73 ± 13.47) in the mature stage, with a fall in the spent stage (56.50 ± 16.01).

Variation of total carotenoids (ANOVA Table 5) in the ovary during ovarian maturation was highly significant ($P < 0.01$). Mean values of total carotenoids between early maturing and spent stages did not differ significantly.

The reciprocal relationship in the variation in total carotenoids in the hepatopancreas and the ovary during ovarian maturation is shown in Fig. 7.

5.5. GSI and Total carotenoid concentration

The variation in GSI and total carotenoid concentration during ovarian maturation showed a linear relationship (Table 8). The minimum GSI value (0.189) recorded a total carotenoid concentration (µg/g wet wt) of 28.4, while the maximum value (7.26) recorded a concentration of 101.5. The results of the polynomial regression showed that there was a positive correlation between GSI and total carotenoid concentration (Fig. 8). The polynomial regression line obtained for the relation between GSI (X) and total carotenoids of the ovary (Y) was determined as $Y = 35.9367 + 11.0133\ X$ ($r^2 = 0.7688$ and S.R. = 2.0131).

5.6. Whole animals

The total carotenoid concentration (µg/g wet wt) from whole animals of M. dobsoni (Table 9) from different stages of maturity showed that there was a general increase from the immature stage (149.4 ± 4.88) to the mature stage (223.4 ± 27.51), whereas in the spent stage it reduced to 210.8 ± 9.01 (Fig. 9).

Variation in total carotenoids (ANOVA Table 6) in whole animals was significant ($P < 0.01$).

6. Comparison between prawns matured through eyestalk ablation and wild prawns

The comparison in total carotenoid concentration between eyestalk ablated and wild M. dobsoni in different tissues at different stages of maturity (Table 10) showed a general decrease in all tissues as well as in whole animals of the ablated prawn when compared to the wild ones. However, the same trend of increase and decrease in carotenoid levels was maintained in both cases, in all the tissues, as well as in whole animals.

6.1. Hepatopancreas

In this tissue, a decreasing trend in total carotenoid concentration was observed in the ablated prawns as in the case of the wild prawns. The values decreased (Fig. 3) from the immature stage (55.3) to the mature stage (18.42) with a recovery in the spent stage (47.6).

Variation in total carotenoid concentration in the hepatopancreas (ANOVA Table 7) between ablated and wild prawns was significant ($P < 0.05$).

6.2. Haemolymph

There was an increase (Fig. 4) in the total carotenoid concentration (µg/g wet wt.) in the ablated prawns from the immature (4.0) to the mature stage (9.4) with decline in the spent stage (6.2).

Variation in total carotenoid concentration (ANOVA Table 8) in the haemolymph between ablated and wild M. dobsoni during ovarian maturation was not significant ($P > 0.05$).

6.3. Exoskeleton

In the exoskeleton, there was an increasing trend (Fig. 5) in the level of total carotenoid concentration in the ablated prawn from the immature (19.3) stage to the mature stage (62.2) and a fall in the spent recovery stage to 37.7.

Statistical analysis (ANOVA Table 9) to test the significance of variation in the total carotenoid content of the exoskeleton between ablated and wild animals was not significant ($P > 0.05$).

6.4. Ovary

The level of total carotenoid was found to increase in the ablated prawns with a minimum (18.9) in the immature stage to a maximum (91.1) in the mature stage, with a decline (53.6) in the spent stage (Fig. 6).

However, variation in total carotenoids between (ANOVA Table 10) ablated and wild prawns was not significant ($P > 0.05$).

6.5. Whole Animals

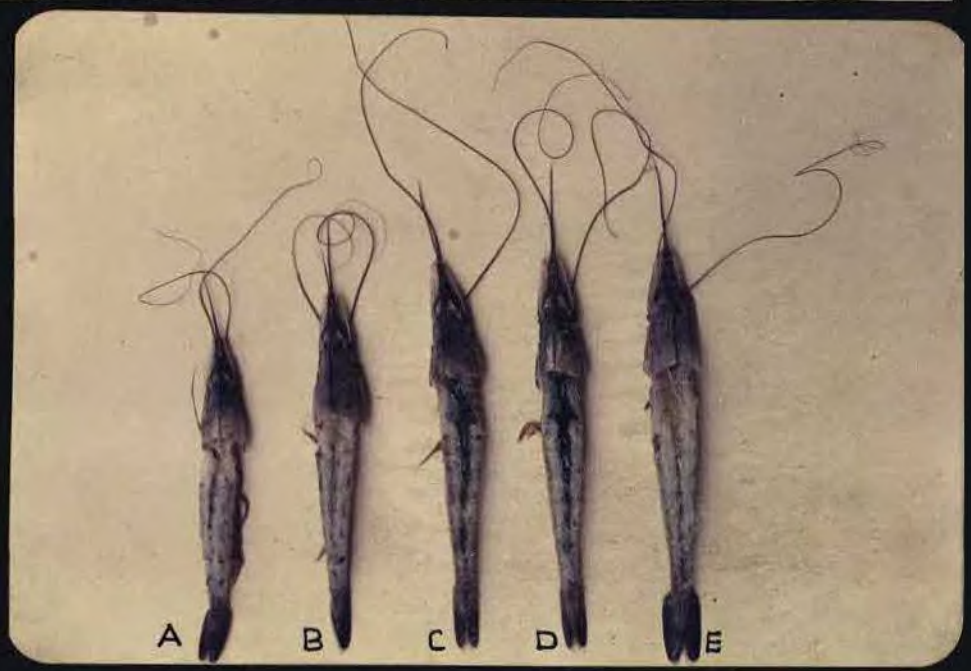
The total carotenoid concentration in whole animals of ablated prawns was found to increase (Fig. 9) from the immature (120.3) and reached

a peak in the mature (190.6) and subsequently decreased in the spent stage (173.)

Statistical analysis (ANOVA Table 11) to test the significance of variation in total carotenoid content in whole animals between ablated and wild M. dobsoni was not significant ($P \geq 0.05$).

PLATE I

Fig.1. Ovary of M. dobsoni at different stages of maturation. (A - Immature : B - Early maturing : C - Late maturing D - Mature : E - Spent-Recovery)



Total carotenoids extracted from the ovary of M.dobsoni Note the ranging intensities of colour during different stages of ovarian maturation. (A - Blank, B - Immature, C - Early Maturing, D - Late maturing, E - Mature, F - Spent-Recovering)

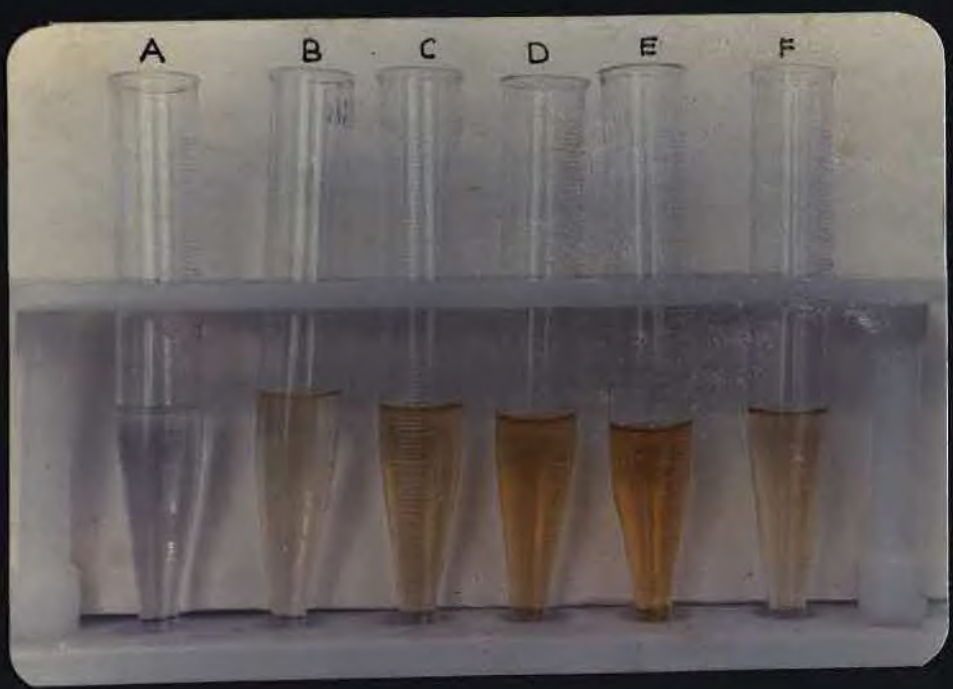


Fig. 1. Cross section of immature ovary.

H & E x 10

Fig. 2. Higher magnification of Fig. 1. The deeply stained nuclei (n) show the presence of oocytes.

H & E x 40

PLATE II

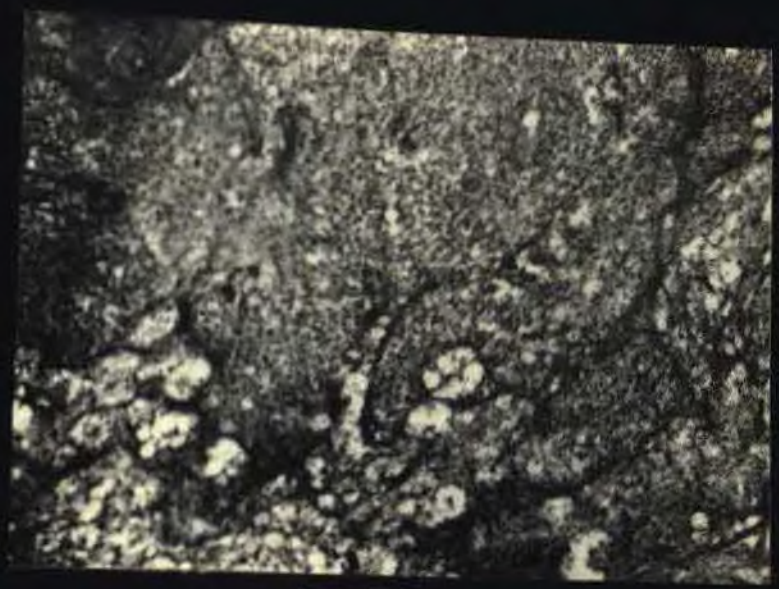


Fig. 1

Fig. 2

Fig. 1. Cross section of early maturing ovary, showing the developing oocytes (oc) and the follicle cells (fc).

H & E x 10

Fig. 2. Magnified view of early maturing oocytes. Note the distinct nuclear ring (nr).

H & E x 40

PLATE III

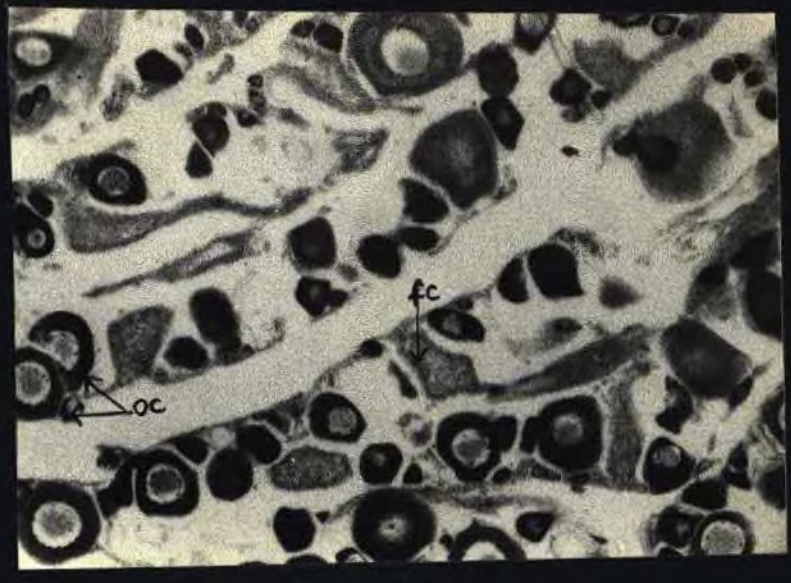


Fig. 1

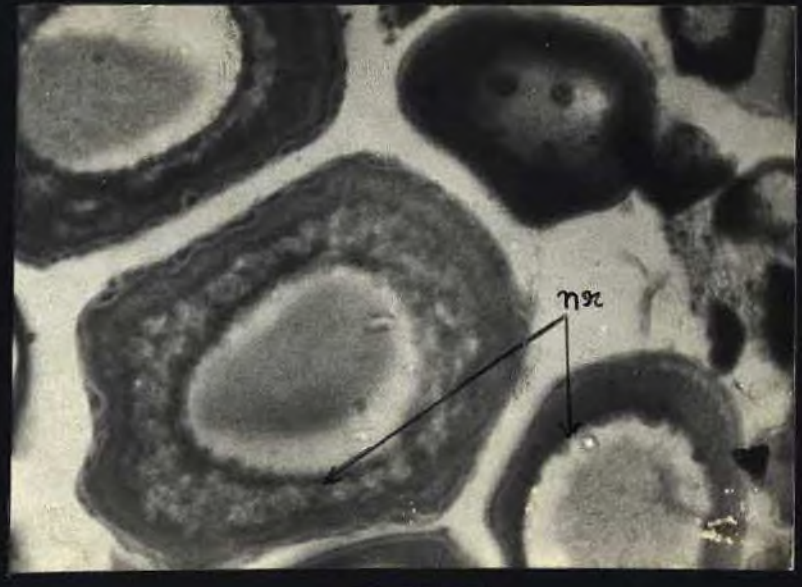


Fig. 2

Fig. 1. Cross section of late maturing ovary, showing the primary oogonia (po) and the secondary oogonia (so).

H & E x 10

Fig. 2. Magnified view of Fig. 1. showing the zone of proliferation (zp) of the primary oogonia (po) into the lumen (l) of the ovary.

H & E x 10

PLATE IV

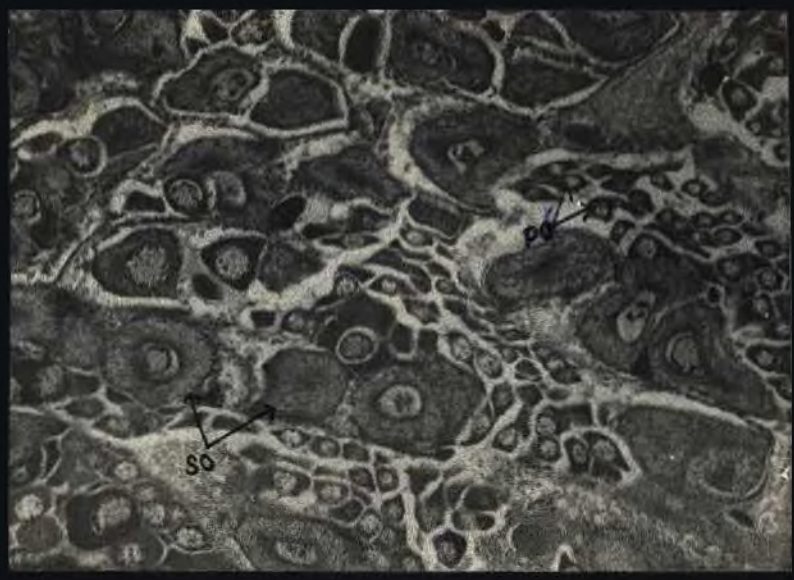


Fig. 1

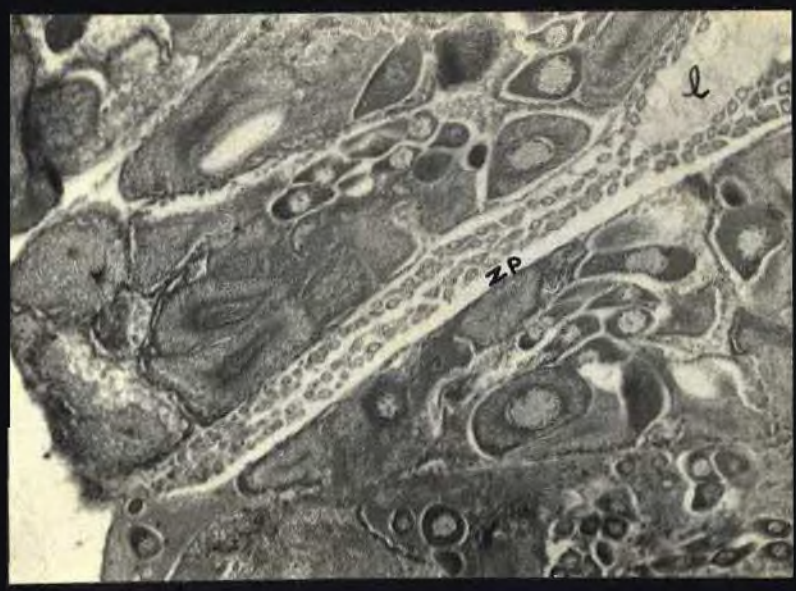


Fig. 2

Fig. 1. Magnified view of developing oocytes, showing the changes in the nuclei during progressive development. Note the deeply stained central region which indicates the nucleus (n), while in the next oocyte the nucleus has started degenerating showing a nuclear ring (nr).

H & E x 40

Fig. 2. Magnified new of late maturing oocytes. Note the yolk deposition (yg) in the cytoplasm of the oocytes.

H & E x 200

PLATE V

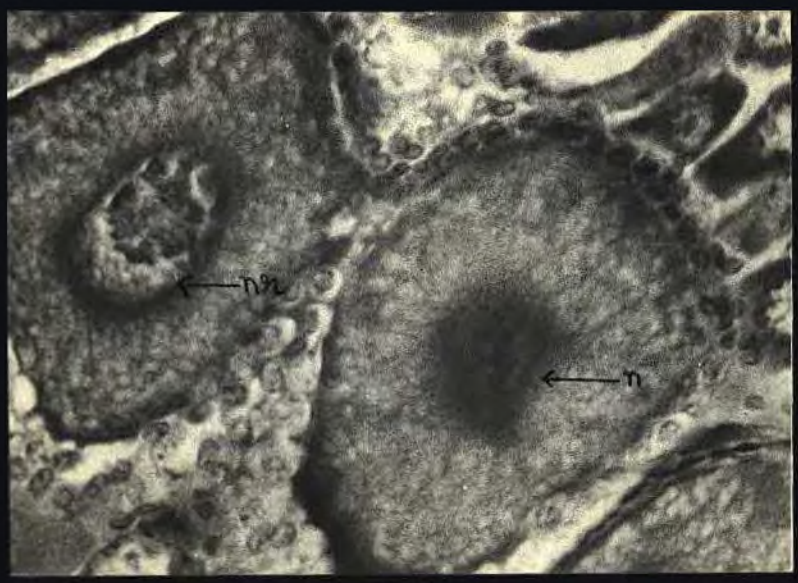


Fig. 1

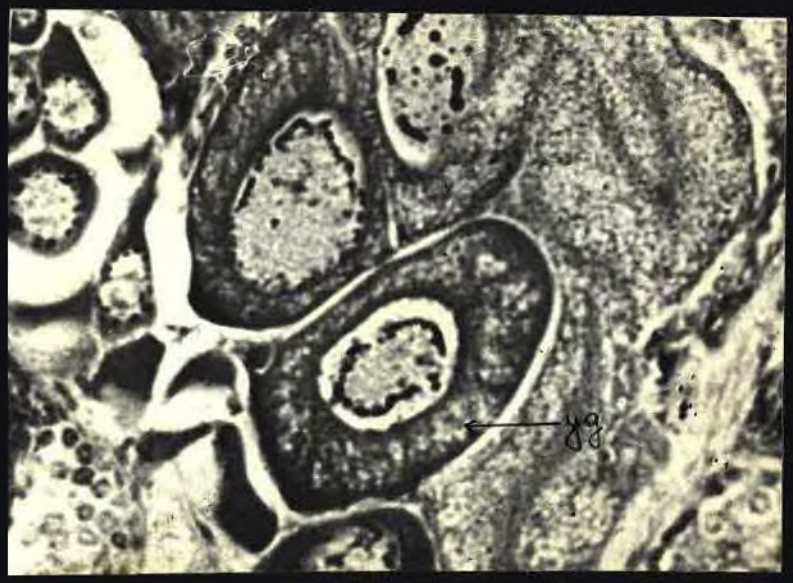


Fig. 2

Fig. 1. Cross section of mature ovary. Note the densely packed ova and the ovarian follicles (fl) in between them.

H & E x 10

Fig. 2. Magnified view of a mature ova. Note the absence of rod-like peripheral bodies which radiate from the central portion of the ova, and the distinct nuclear ring (nr).

H & E x 40

PLATE VI

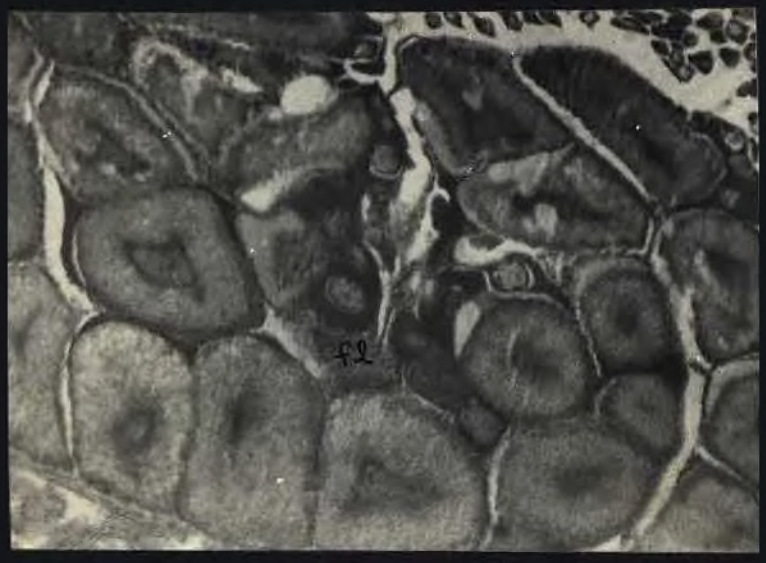


Fig. 1

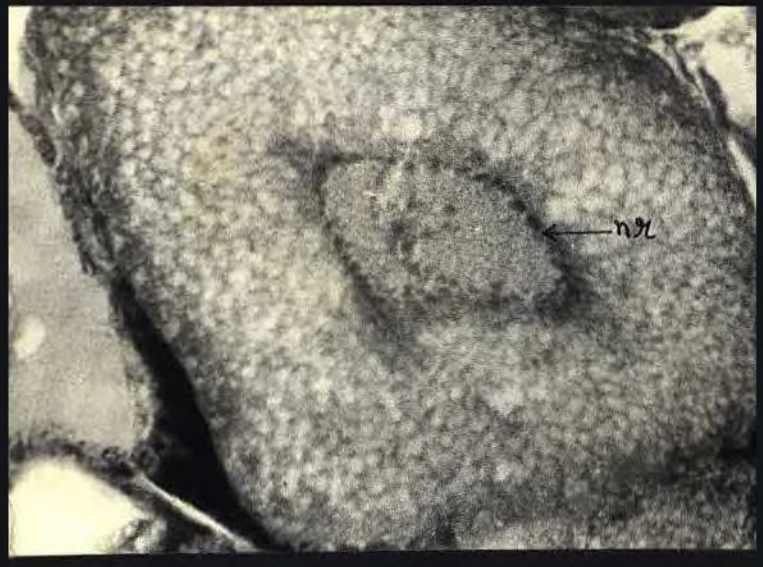


Fig. 2

Fig. 1. Cross section of spent ovary showing a dense mass of ovarian follicles (fl).

H & E x 10

Fig. 2. Magnified view of Fig. 1. Note the presence of resorptive ova (ro) as deeply stained structures.

H & E x 100

PLATE VII

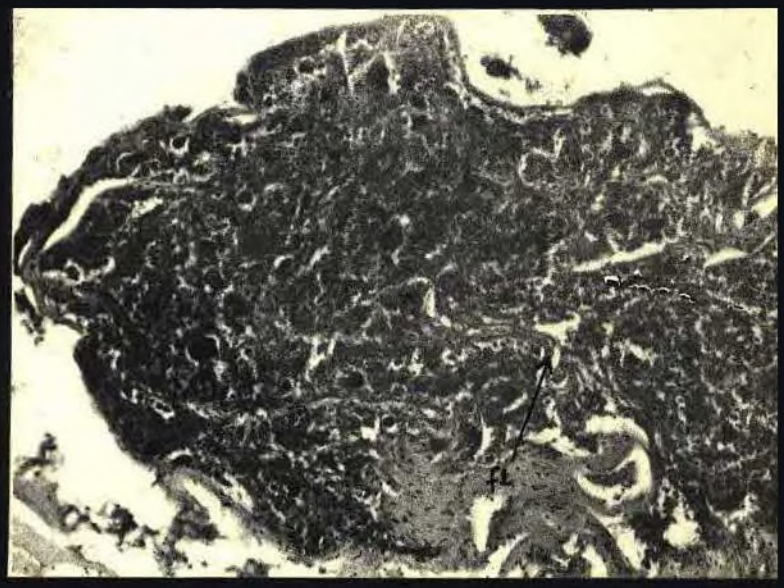


Fig. 1

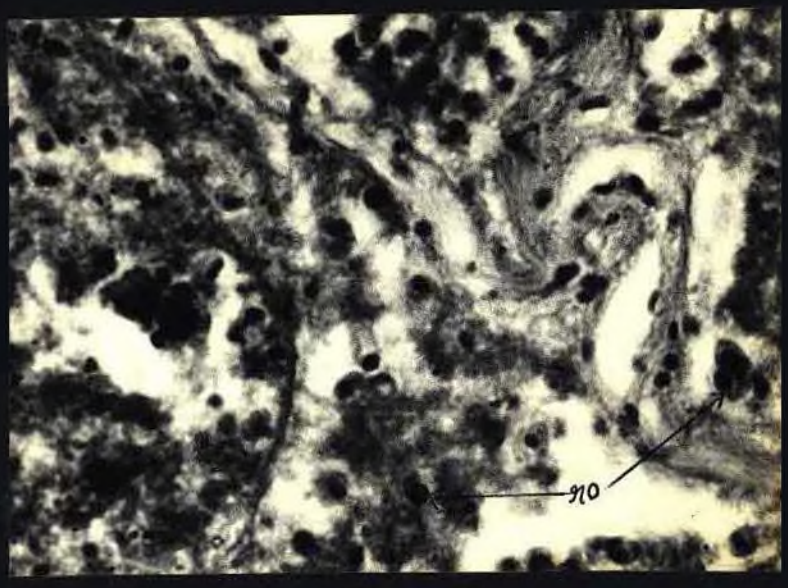


Fig. 2

TABLE 1: Variation in Gonadosomatic index of M. dobsoni during ovarian maturation.

Maturity Stages				
Immature	Early maturing	Late maturing	Mature	Spent-Recovering
0.21	1.15	3.52	5.64	3.75
0.27	1.11	4.89	6.46	3.61
0.23	2.85	5.03	7.17	4.35
0.25	2.43	4.61	6.53	4.98
0.26	1.71	3.89	7.26	4.90
*0.24±0.24	1.85±0.78	4.38±0.65	6.61±0.65	4.31±0.63

* mean ($\bar{X}$) ± Standard Deviation (S.D).

TABLE 2: Variation in Hepatosomatic index of M. dobsoni during ovarian maturation.

Maturity Stages				
Immature	Early maturing	Late maturing	Mature	Spent - Recovering
1.77	0.52	0.63	2.63	1.40
2.02	0.38	0.94	2.05	2.02
1.86	0.38	9.51	2.57	1.86
1.92	9.31	1.61	1.88	1.72
2.16	9.49	1.94	2.40	1.98
1.52	1.04	1.74	1.13	1.32
*1.88±0.28	0.51±0.27	1.08±0.50	2.11±0.56	1.72±0.29

* Mean ($\bar{X}$) ± Standard Deviation (S.D)

TABLE 3: Table showing mean range of ova diameter in M. dobsoni at different stages of maturity.

Stages of Maturity	Range (in μ m)	Mean±Standard Deviation
Stage I	$\angle$ 80	25.1 ± 10.26
Stage II	80 - 190	134.8 ± 26.71
Stage III	140 - 250	183.0 ± 42.27
Stage IV	140 - 320	236.0 ± 47.74
Spent recovering	$\angle$ 96	72.83 ± 7.80

TABLE 4: Variation in total carotenoid concentration (µg/g wet wt.) in the hepatopancreas of M. dobsoni during ovarian maturation.

Maturity Stages				
Immature	Early Maturing	Late Maturing	Mature	Spent-Recovering
84.0	36.0	26.0	22.0	57.2
78.0	43.1	24.0	18.0	53.3
72.0	31.0	30.0	15.6	66.6
80.0	30.0	20.6	24.0	59.9
64.0	24.6	23.2	20.4	69.2
73.6	27.0	24	22.3	43.5
*75.27±7.15	31.85±6.69	24.63±2.89	20.38±2.90	58.29±8.52

* Denotes the mean ($\bar{X}$) ± Standard Deviation (S.D)

TABLE 5: Variation in total carotenoid concentration (µg/g wet wt.) in the haemolymph of M. dobsoni during ovarian maturation.

Maturity Stages				
Immature	Early *Maturing*	Late *Maturing*	Mature	Spent-*Recovering*
4.1	6.8	11	12.3	6.3
6.2	7.1	10.3	12.0	5.6
5.8	8.3	10.1	11.5	9.1
6.3	8.9	10.8	10.3	8.4
5.0	8.0	9.1	12.1	5.3
5.2	7.3	9.5	11.3	6.0
*5.43±0.84	7.73±0.79	10.13±0.79	11.58±1.46	6.78±1.58

* Denotes the mean ($\bar{X}$) ± Standard Deviation (S.D)

TABLE 6: Variation in total carotenoid concentration (μg/g wet wt.) in the exoskeleton of M. dobsoni during ovarian maturation.

Maturity Stages				
Immature	Early Maturing	Late Maturing	Nature	Spent-Recovering
18.4	37.1	56.2	68.0	41.3
20.8	31.8	45.2	64.0	39.4
20.0	33.9	48.0	84.0	31.6
24.4	42.4	46.5	75.3	38.4
26.6	32.8	51.0	54.2	43.2
25.2	37.1	55.3	68.0	40.1
*22.57±3.28	35.85±3.86	50.37±5.54	70.58±7.19	39.00±3.99

* Denotes the mean ($\bar{X}$) ± Standard Deviation (S.D)

TABLE 7: Variation in total carotenoid concentration (μg/g wet wt.) in the ovary of M. dobsoni during ovarian maturation.

Maturity Stages				
Immature	Early *Maturing*	Late *Maturing*	Mature	Spent-*Recovering*
24.0	42.8	74.6	92.6	86.4
19.2	65.9	90.0	101.3	60.0
19.2	63.6	71.2	101.9	42.0
28.8	64.6	94.0	96.0	55.2
23.0	51.0	86.4	126.0	45.6
28.8	42.0	79.8	87.3	49.8
*23.83±4.31	59.99±10.86	82.67±8.96	100.73±13.47	56.50±16.01

* denotes the mean ($\bar{X}$) ± Standard Deviation (S.D)

TABLE 8: Gonadosomatic index (GSI) of M. dobsoni and the total carotenoids of the ovary (µg/g wet wt.).

Sample No.	Body wt.(g)	Ovary wt. (g)	GSI	Total carotenoids
1	3.97	0.0075	0.189	28.4
2	2.82	0.006	0.21	24.0
3	5.43	0.48	0.88	42.5
4	5.60	0.062	1.10	65.97
5	7.520	0.0213	2.83	74.6
6	8.897	0.348	3.91	90.0
7	7.884	0.310	3.93	92.88
8	8.164	0.590	7.26	101.5
9	11.419	0.529	3.76	86.4
10	10.532	0.354	3.36	55.2

Polynomial Regression

Coefficient of B = 11.0133
Coefficient of A = 35.3967
Standard error = 2.0131
R^2 = 0.7688
Mean X (GSI) = 2.7429
Mean Y (TC) = 66.14501

$Y = 35.9367 + 11.0133 X$

TABLE 9: Total carotenoid concentration (µg/g wet wt.) estimated from whole animals of M. dobsoni at indicated stages of maturity.

Maturity Stages				
Immature	Early Maturing	Late Maturing	Mature	Spent - Recovering
150	180	210	254	212
155	169	192	200	220
148	172	183	190	206
152	163	176	230	218
142	158	185	243	198
*149.4±4.88	168.4±8.44	189.2±12.95	223.4±27.51	210.8±9.01

* Denotes mean ($\bar{X}$) ± Standard Deviation (S.D)

TABLE 10: *Mean values of total carotenoids (µg/g wet wt.) in M. dobsoni matured through eyestalk ablation in the different tissue at indicated stages of maturity.

Tissue	Maturity Stages				
	Immature	Early Maturing	Late Maturing	Mature	Spent - Recovering
Hepatopancreas	55.3 (75.27)	28.3 (31.85)	24.4 (24.63)	18.42 (20.38)	47.6 (58.29)
Haemolymph	4.0 (5.43)	5.76 (7.73)	7.94 (10.13)	9.4 (11.58)	6.2 (6.78)
Exoskeleton	19.3 (22.57)	29.2 (35.85)	42.8 (50.37)	62.2 (70.58)	37.7 (39)
Ovary	18.9 (23.83)	46.7 (59.99)	71.56 (82.67)	91.1 (100.73)	53.6 (56.50)
Whole animals	120.3 (149.4)	140.8 (168.4)	168.4 (189.2)	190.6 (223.4)	173.0 (210.8)

* Values given in brackets indicate the corresponding mean values of total carotenoids observed in unablated wild prawns.

ANOVA TABLE 1: ANOVA to test significance of variation in Hepatosomatic index in M. dobsoni during ovarian maturation.

Source	d.o.f.	S.S.	M.S.	F. ratio	Remarks
TREAT	4	10.301	2.575	16.51	Significant ($P < 0.01$)
ERROR	25	3.900	0.156		

TREAT	MEAN
T 1	1.88
T 2	0.51
T 3	1.08
T 4	2.11
T 5	1.72

MEAN COMPARISONS	REMARKS
T 1 - T 2	Significant
T 2 - T 3	Significant
T 1 - T 4	Non-Significant
T 1 - T 5	Non-Significant
T 2 - T 3	Significant
T 2 - T 4	Significant
T 2 - T 5	Significant
T 3 - T 4	Significant
T 3 - T 5	Significant
T 4 - T 5	Non-Significant

T1 = Immature T2 = Early Maturing T3 = Late Maturing
T4 = Mature T5 = Spent Recovering

ANOVA TABLE 2: ANOVA to test the significance of variation in total carotenoid concentration in the hepatopancreas of M. dobsoni during ovarian maturation.

Source	d.o.f.	S.S.	M.S.	F. ratio	Remarks
TREAT	4	13450.550	3362.637	83.73	Significant ($P < 0.01$)
ERROR	25	1004.047	40.162		

TREAT	MEAN
T 1	75.27
T 2	31.95
T 3	24.63
T 4	20.38
T 5	58.29

MEAN COMPARISONS	REMARKS
T 1 - T 2	Significant
T 1 - T 3	Significant
T 1 - T 4	Significant
T 1 - T 5	Significant
T 2 - T 3	Non-Significant
T 2 - T 4	Significant
T 2 - T 5	Significant
T 3 - T 4	Non-Significant
T 3 - T 5	Significant
T 4 - T 5	Significant

T 1 = Immature T 2 = Early Maturing T 3 = Late Maturing
T 4 = Mature T 5 = Spent Recovering

ANOVA TABLE 3: ANOVA to test the significance of variation in total carotenoid concentration in the haemolymph of M. dobsoni during ovarian maturation.

Source	d.o.f.	S.S.	M.S.S.	F-ratio	Remarks
TREAT	4	149.850	37.462	38.20	Significant ($P < 0.01$)
ERROR	25	24.516	0.981		

TREAT	MEAN
T 1	5.43
T 2	7.73
T 3	10.13
T 4	11.58
T 5	6.78

MEAN COMPARISONS	REMARKS
T 1 - T 2	Significant
T 1 - T 3	Significant
T 1 - T 4	Significant
T 1 - T 5	Significant
T 2 - T 3	Significant
T 2 - T 4	Significant
T 3 - T 5	Non-Significant
T 3 - T 4	Significant
T 3 - T 5	Significant
T 4 - T 5	Significant

T 1 = Immature T 2 = Early Maturing T 3 = Late Maturing
T 4 = Mature T 5 = Spent Recovering

ANOVA TABLE 4: ANOVA to test the significance of variation in total carotenoid concentration in the exoskeleton of M. dobsoni during ovarian maturation.

Source	d.o.f.	S.S.	M.S.S.	F. ratio	Remarks
TREAT	4	7784.910	1946.228	79.21	Significant ($P < 0.01$)
ERROR	25	614.285	24.571		

TREAT	MEAN
T 1	22.57
T 2	36.85
T 3	50.37
T 4	70.58
T 5	39

MEAN COMPARISONS	REMARKS
T 1 - T 2	Significant
T 1 - T 3	Significant
T 1 - T 4	Significant
T 1 - T 5	Significant
T 2 - T 3	Significant
T 2 - T 4	Non-Significant
T 2 - T 5	Significant
T 3 - T 4	Significant
T 3 - T 5	Significant
T 4 - T 5	Significant

T 1 = Immature T 2 = Early Maturing T 3 = Late Maturing
T 4 = Mature T 5 = Spent Recovering

ANOVA TABLE 5: ANOVA to test the significance of variation in total carotenoid concentration in the ovary of M. dobsoni during ovarian maturation.

Source	d.o.f.	S.S.	M.S.S.	F. ratio	Remarks
TREAT	4	20477.310	5119.326	39.56	Significant ($P < 0.01$)
ERROR	25	3235.188	129.408		

TREAT	MEAN
T 1	23.83
T 2	59.99
T 3	82.67
T 4	101.18
T 5	56.50

MEAN COMPARISONS	REMARKS
T 1 - T 2	Significant
T 1 - T 3	Significant
T 1 - T 4	Significant
T 1 - T 5	Significant
T 1 - T 3	Significant
T 2 - T 4	Significant
T 2 - T 5	Non-Significant
T 3 - T 4	Significant
T 3 - T 5	Significant
T 4 - T 5	Significant

T 1 = Immature T 2 = Early Maturing T 3 = Late Maturing
T 4 = Mature T 5 = Spent Recovering

ANOVA TABLE 6: ANOVA to test significance of variation in total carotenoid concentration in whole animals of M. dobsoni during ovarian maturation.

Source	d.o.f.	S.S.	M.S.S.	F. ratio	Remarks
TREAT	4	18247.36	4561.84	20.7488	Significant ($P < 0.01$)
ERROR	20	4397.2	219.86		

ANOVA TABLE 7: ANOVA to test the significance of variation is total carotenoid concentration in the hepatopancreas between ablated and wild M. dobsoni its ovarian maturation.

Source	d.o.f.	S.S.	M.S.S.	F. ratio	Remarks
TREAT	1	132.481	132.481	13.802	Significant ($P < 0.05$)
ERROR	8	14628.869	1828.6086		

C.D. value for treat : mean comparison - 22.72

Treat : mean = T 1 = 42.08
T 2 = 34.80

ANOVA TABLE 8: ANOVA to test the significance of variation in total carotenoid concentration in the haemolymph, between ablated and wild M. dobsoni during ovarian maturation.

Source	d.o.f.	S.S.	M.S.S.	F. ratio	Remarks
TREAT	1	7.03	7.03	1.35296	Non-Significant($P>0.05$)
ERROR	8	41.57	5.196		

ANOVA TABLE 9: ANOVA to test the significance of variation in total carotenoid concentration in the exoskeleton, between ablated and wild M. dobsoni during ovarian maturation.

Source	d.o.f.	S.S.	M.S.S.	F. ratio	Remarks
TREAT	1	71.128	71.128	4.06672	Non-Significant($P \geq 0.05$)
ERROR	8	2314.062	289.257		

ANOVA TABLE 10: ANOVA to test the significance of variation in total carotenoid concentration in the ovary, between ablated and wild M. dobsoni during ovarian maturation.

Source	d.o.f.	S.S.	M.S.S.	F. ratio	Remarks
TREAT	1	175.224	175.224	4.51059	Non-Significant($P \geq 0.05$)
ERROR	8	6322.923	790.36537		

ANOVA TABLE 11: ANOVA to test the significance of variation in total carotenoid concentration in the whole animal of M. dobsoni between ablated and wild prawns during ovarian maturation.

Source	d.o.f.	S.S.	M.S.S.	F. ratio	Remarks
TREAT	4	6679.82	1669.96	3.6736	Non-Significant($P \geq 0.05$)
ERROR	5	2272.86	454.572		

Fig. 1. Variation in gonado-somatic index of M. dobsoni during ovarian maturation. Values plotted indicate mean ± standard deviation.

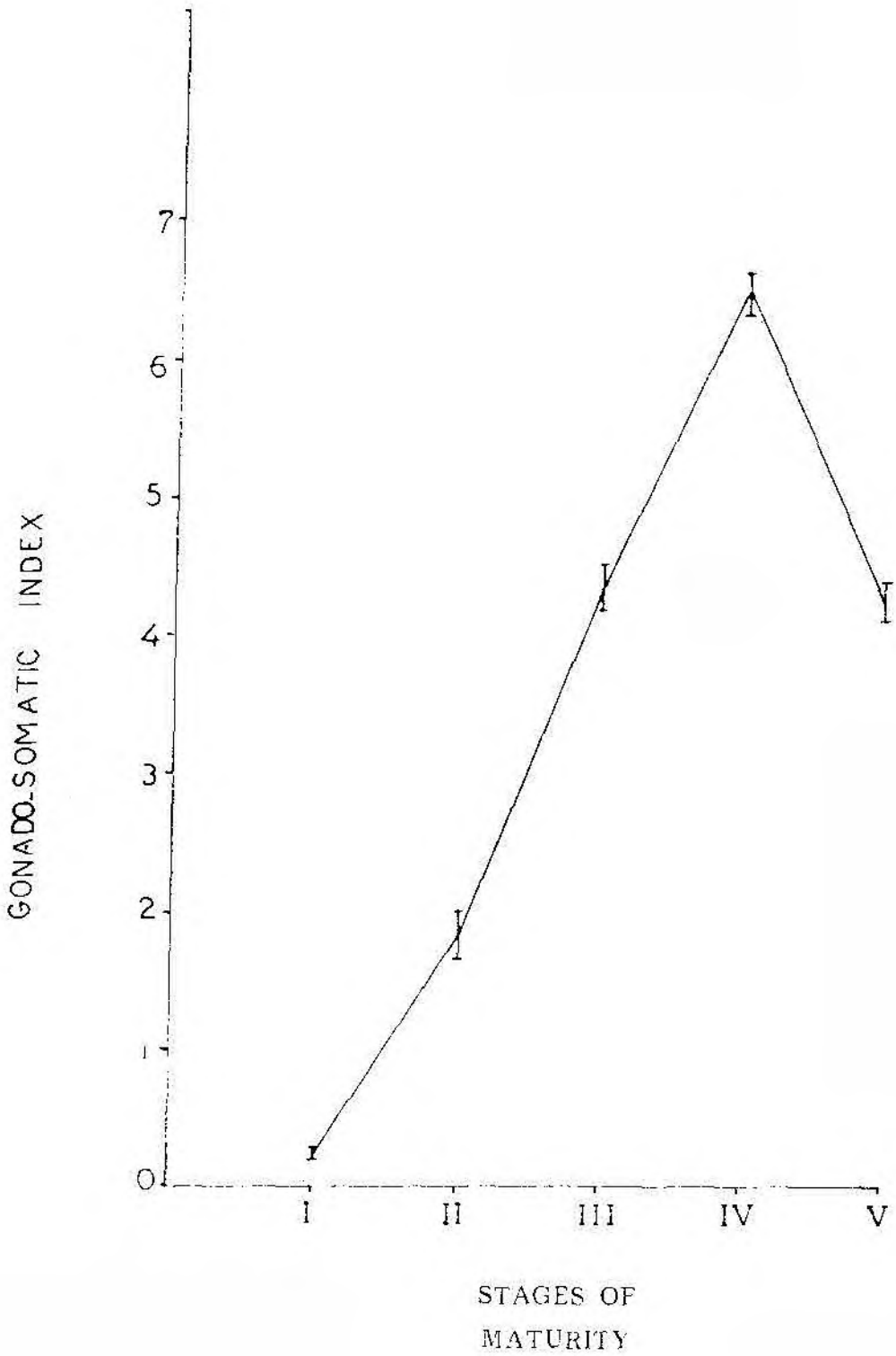

GONADO-SOMATIC INDEX
0
1
2
3
4
5
6
7
I
II
III
IV
V
STAGES OF
MATURITY

FIG. 1

Fig. 2. Variation in hepatosomatic index of M. dobsoni during ovarian maturation. Values plotted indicate mean ± standard deviation.

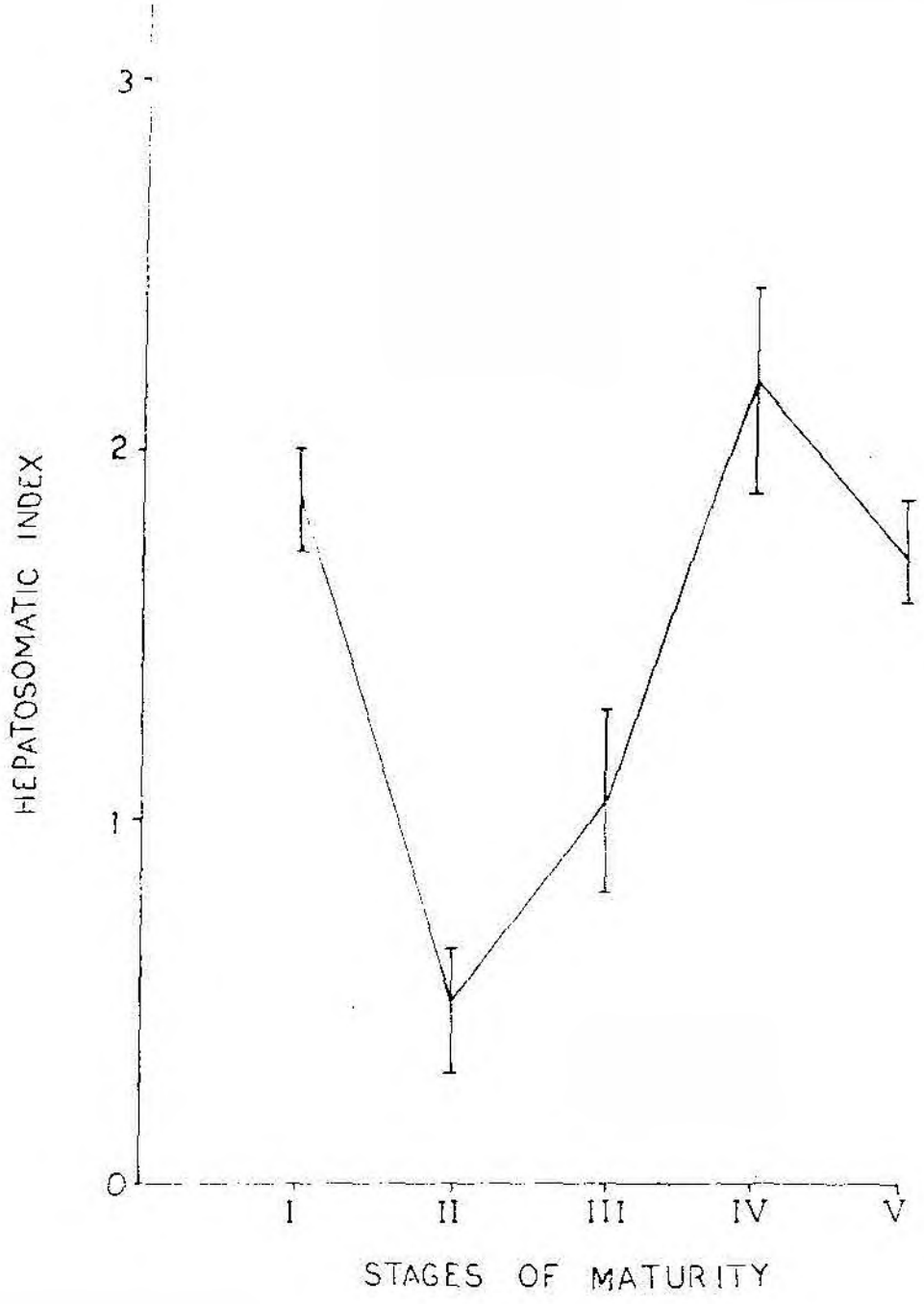


FIG. 2

Fig. 3. Variation in total carotenoid concentration (μg/g wet wt) in the hepatopancreas of M. dobsoni during ovarian maturation. Values plotted indicate mean $\pm$ standard deviation.

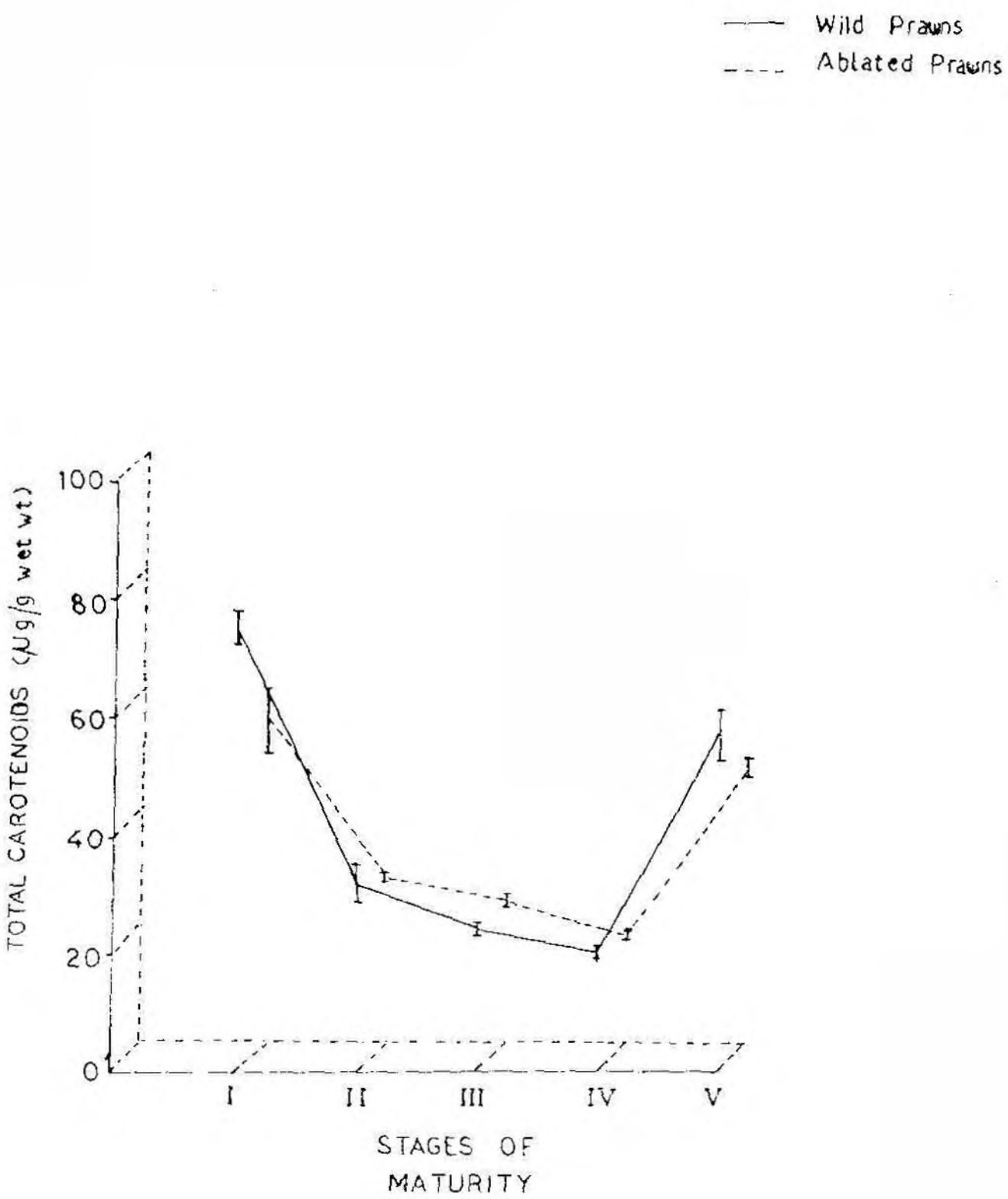

Wild Prawns
Ablated Prawns
TOTAL CAROTENOIDS (µg/g wet wt)
100
80
60
40
20
0
I
II
III
IV
V
STAGES OF MATURITY

FIG. 3

Fig. 4. Variation in total carotenoid concentration (μg/g wet wt) in the haemolymph of M. dobsoni during ovarian maturation. Values plotted indicate mean $\pm$ standard deviation.

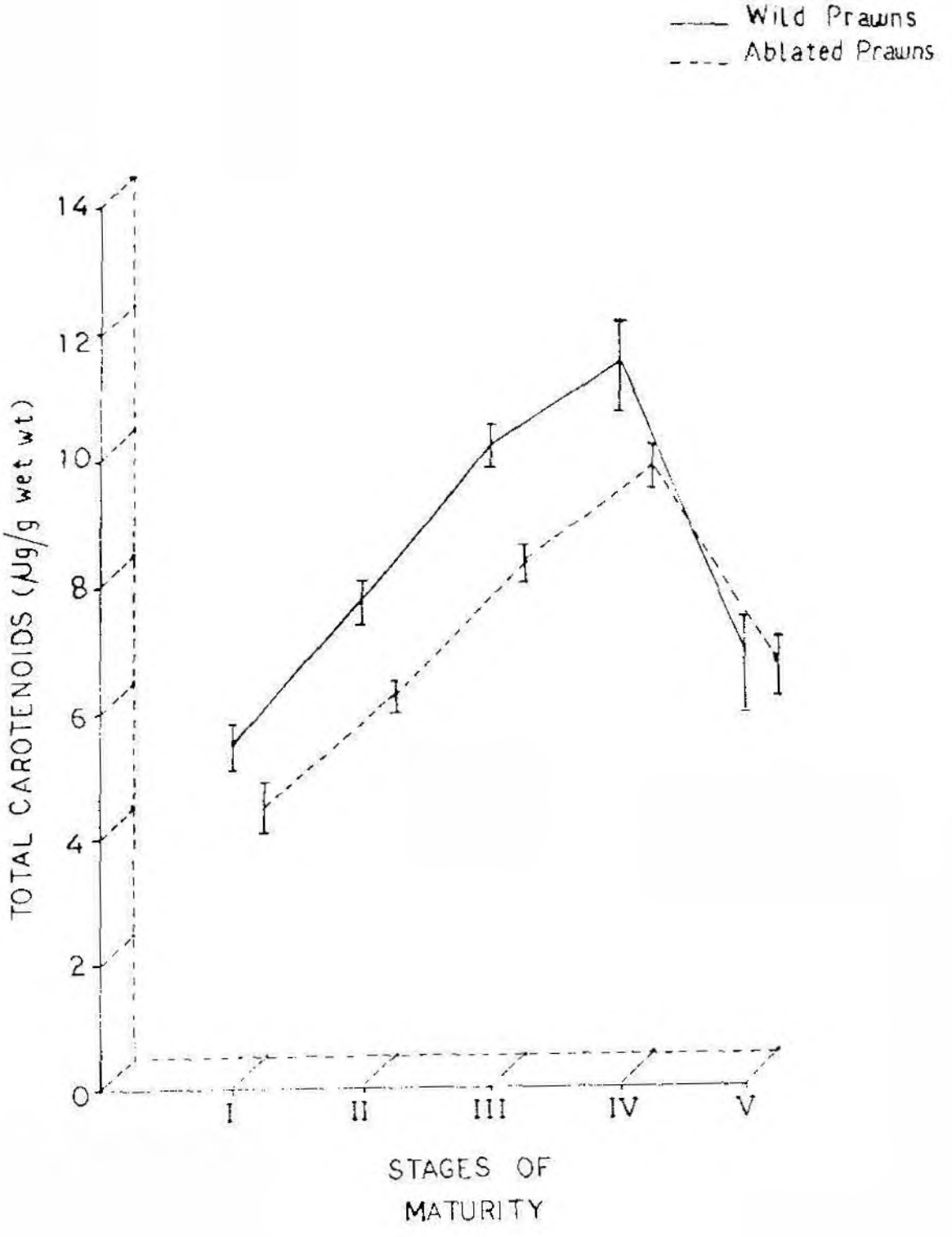

Wild Prawns
Ablated Prawns
TOTAL CAROTENOIDS (µg/g wet wt)
0
2
4
6
8
10
12
14
I
II
III
IV
V
STAGES OF
MATURITY

FIG. 4

Fig. 5. Variation in total carotenoid concentration (μg/g wet wt) in the exoskeleton of M. dobsoni during ovarian maturation. Values plotted indicate mean $\pm$ standard deviation.

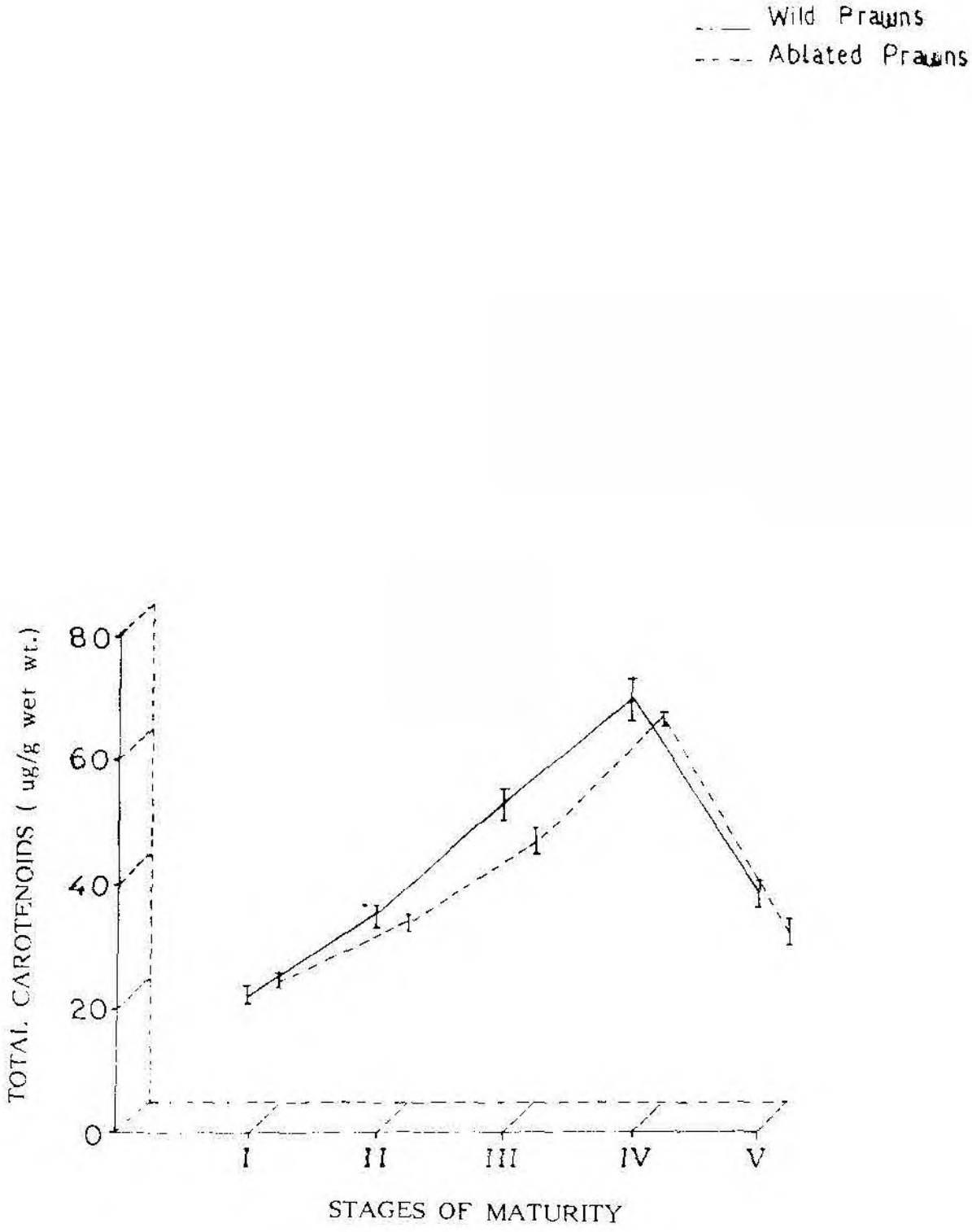

Wild Prawns
Ablated Prawns
TOTAL CAROTENOIDS (ug/g wet wt.)
80
60
40
20
0
I
II
III
IV
V
STAGES OF MATURITY

FIG. 5

Fig. 6. Variation in total carotenoid concentration (μg/g wet wt) in the ovary of M. dobsoni during ovarian maturation. Values plotted indicate mean $\pm$ standard deviation.

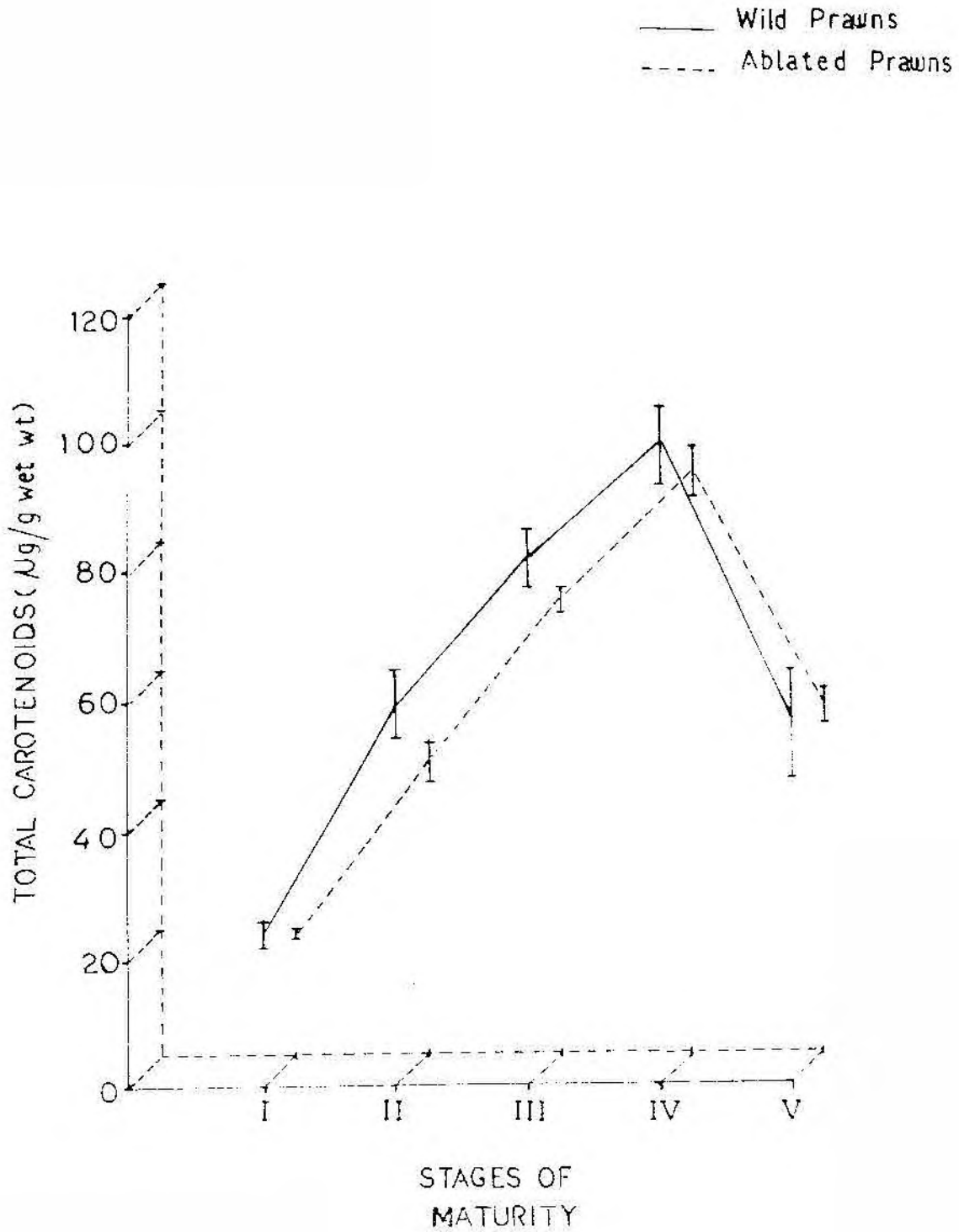

Wild Prawns
Ablated Prawns
TOTAL CAROTENOIDS (µg/g wet wt)
120
100
80
60
40
20
0
I
II
III
IV
V
STAGES OF
MATURITY

FIGURE - 6

Fig. 7. Polynomial Regression line showing relation between GSI(X) and Total carotenoids of ovary (Y) for M. dobsoni during ovarian maturation.

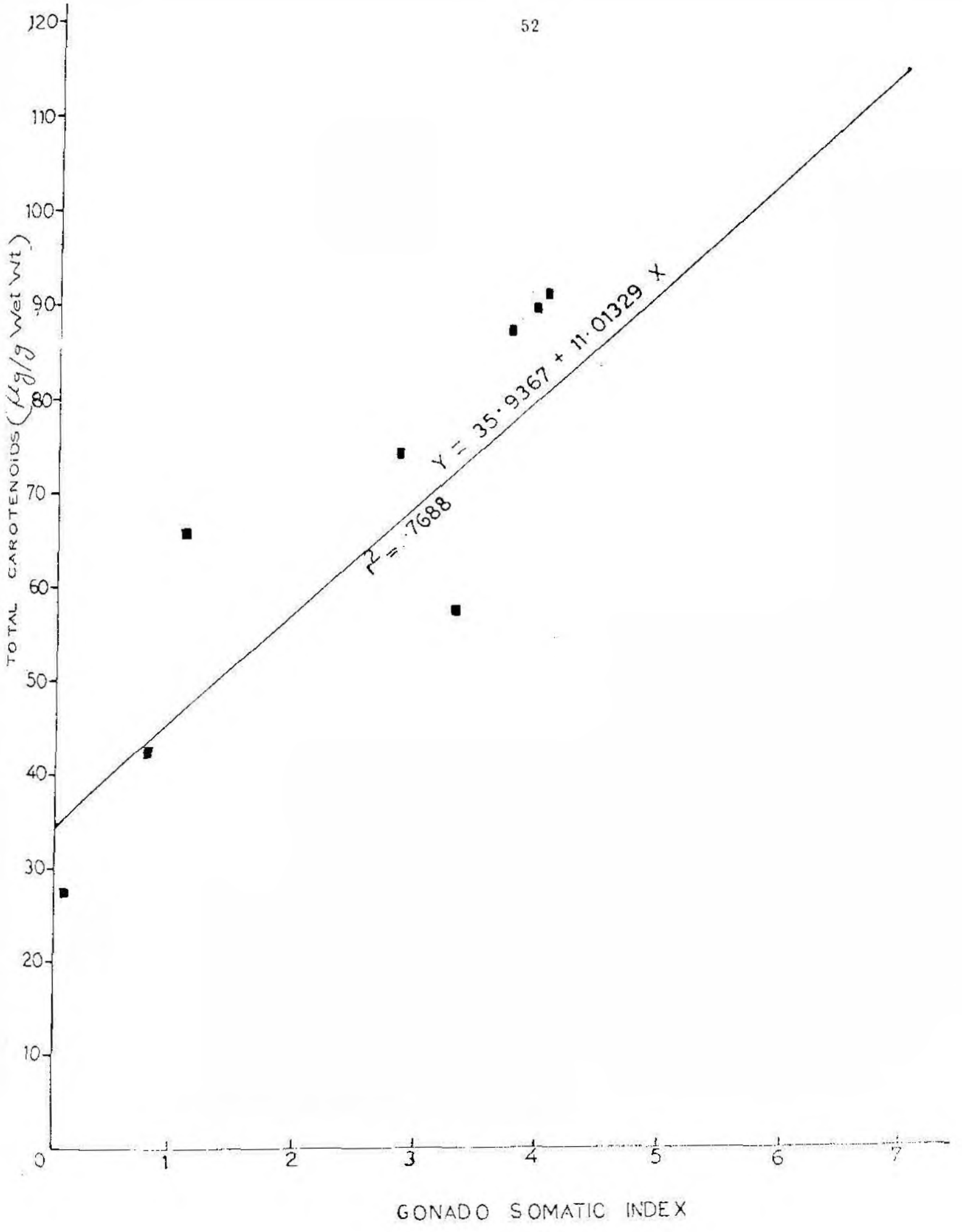

TOTAL CAROTENOIDS (µg/g Wet Wt)
120
110
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
GONADO SOMATIC INDEX
Y = 35.9367 + 11.01329 X
r² = .7688

Fig. 8. Graph showing reciprocal relationship of variations in total carotenoids in hepatopancreas and ovary of M. dobsoni during ovarian maturation.

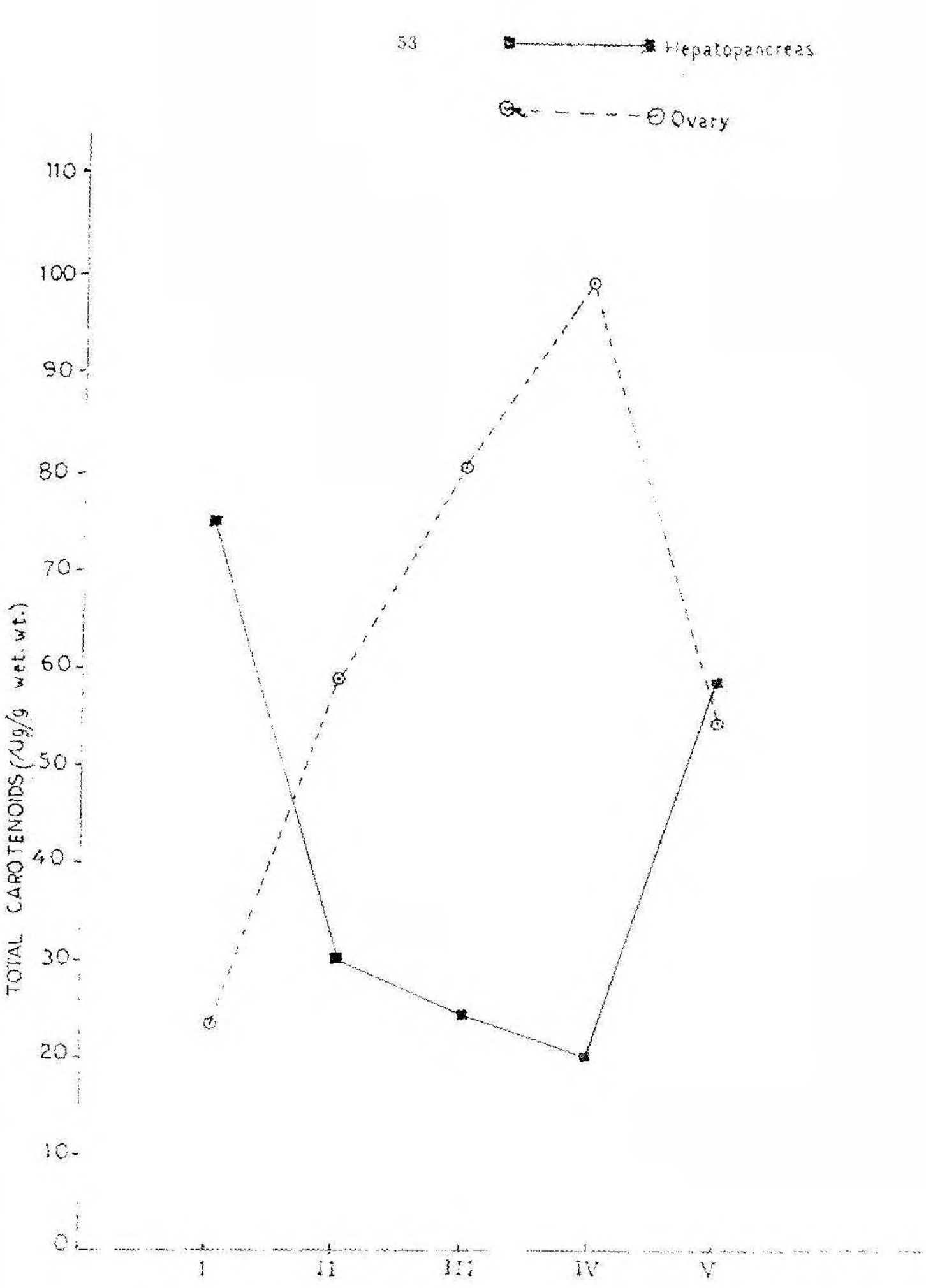

Hepatopancreas
Ovary
TOTAL CAROTENOIDS (µg/g wet wt.)
110
100
90
80
70
60
50
40
30
20
10
0
I
II
III
IV
V
STAGES OF

Fig. 8

Fig. 9. Variation in total carotenoid concentration in whole M. dobsoni during ovarian maturation.

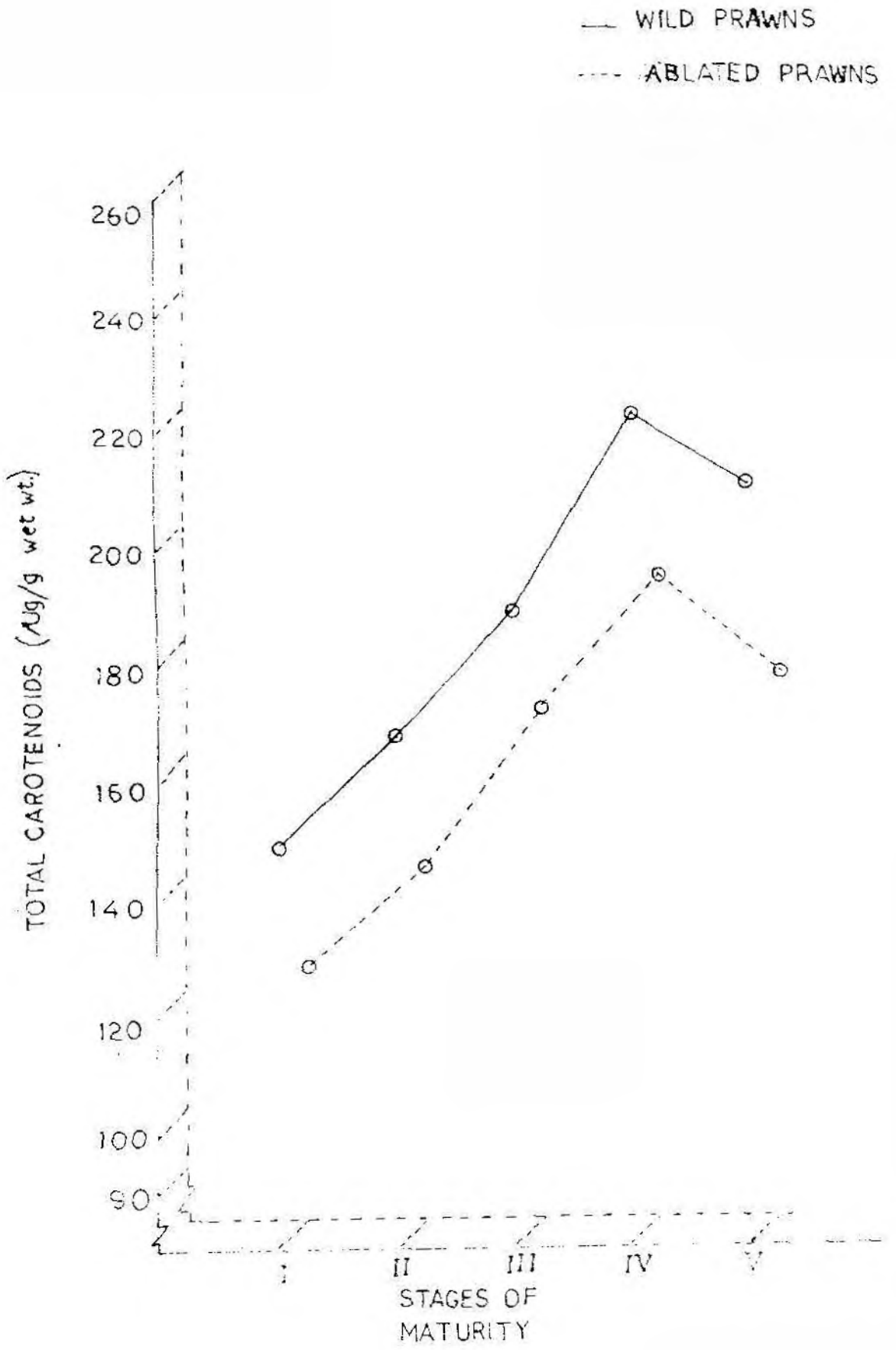

WILD PRAWNS
ABLATED PRAWNS
TOTAL CAROTENOIDS (µg/g wet wt.)
260
240
220
200
180
160
140
120
100
90
I
II
III
IV
V
STAGES OF MATURITY

FIG. 9

DISCUSSION

Ovarian maturation refers to a cyclic morphological change during which a female undergoes full sexual maturity. This attainment always marks a change in the growth pattern resulting from a "reproductive drain" due to diversion of biochemical reserves meant for somatic growth to the ovaries. According to Giese and Pearse (1974), during gonadal development, a remarkable synthesis of organic material takes place the gonad being the locus of intense biochemical activity during gamete formation. George and Patel (1956) had demonstrated in crustaceans that organic reserves are stored prior to gonadal development and their transfer to the ovaries take place gradually as maturation advances. The carotenoids being one of the organic reserves, dissolved in the lipids are also stored and subsequently mobilised to the ovaries during ovarian development.

The extent of ovarian maturation can the studied on the basis of the gonado-somatic index. Giese (1959) reported that the gonads of marine invertebrates increase in weight by accumulation of various biochemical reserves as they approach maturity. The present study was also in agreement with this observation. As the gonadosomatic index increased, there was a corresponding increase in the total carotenoid levels, showing that carotenoids are also one among the biochemical reserves that accumulate during the course of ovarian maturation.

The hepatosomatic index is an important indicator of the condition of the hepatopancreas during ovarian maturation. In finfishes Krivobok (1964) had reported that liver weight increases in the females until gonadal maturation has definitely started and then declines with progressive maturation reaching a

minimum when the ova are ripe. Sastry (1966) had demonstrated the reciprocal relationship between the gonadosomatic index and hepatosomatic index in the scallop Acquipecten irradians. But the present study, did not show a reciprocal relationship between GSI and HSI. In fact, the HSI was maximum in the mature stage which might be due to the non-utilisation of organic reserves to the full extent during the growth and development of the ovaries. These organic reserves might have contributed to the increase in the overall weight of the hepatopancreas, and hence in its index, as maturation advanced. This finding can be substantiated by that of Mikhail et al. (1982) and Muthukaruppan (1987). According to them, this unusual increase in the HSI during maturation may be due to the presence of other organic reserves like proteins and carbohydrates which are not mobilised to the full extent from the hepatopancreas when compared to the lipids. They were of the opinion that proteins and carbohydrate are more effective in influencing the overall weight of the hepatopancreas (liver), than the lipids. In this study, carotenoids being dissolved in lipids, their mobilisation can be considered to be along with the lipids. This might have caused the decrease in the total carotenoid concentration in the hepatopancreas as maturation progressed as discussed subsequently.

Carotenoids are not "just a playful diversion" of nature as it is often assumed, but fulfil an important function in reproduction (Deufel, 1975). Accordingly, he reported that, carotenoids apart from just providing the colour in the animal, also help in reproduction. Donaldson and Fagerland (1970) found that in gonadectomised sockeye salmon, there was a stoppage in loss of muscle carotenoids, emphasising the utilisation of carotenoids during development of gonads. The absense of gonads stopped the reduction of muscle carotenoids suggesting the relationship the carotenoids have with the gonads. Shnarevich and Sakhnenko (1971) observed the accumulation of carotenoids in the gonads

of fishes during their maturation. Similar observation of increase in carotenoid concentration with maturation was observed in chum salmon (Kitahara 1983) and in masu salmon (Kitahara, 1985).

In crustaceans, the role of carotenoids in reproduction is still under speculation. The pioneer work of Smith (1911) revealed that female decapod crustaceans do mobilise "lipochromes" from the hepatopancreas (where they are stored) to the ripening ovaries via the blood. Abeloos and Fischer (1926) made similar observations when they worked on Carcinus sp. Green (1966) reported that in Simocephalus vetulus carotenoids are mobilised from the adult female to the eggs during maturation, while Herring (1968) reported that about 50% of the total body carotenoids are transferred from the mother to the egg sac in Daphnia magna. Similar observations were made in the present study, wherein the prawn, M. dobsoni was also found to mobilise carotenoids from the hepatopancreas to the ovary, during its ovarian maturation as the total carotenoids of the hepatopancreas was found to have reduced from the immature stage, whereas that in the ovaries increased in the immature stage and rea hed a peak in the mature stage, suggesting a utilisation of the pigment during the course of ovarian maturation. On the contrary, Gilchrist and Lee (1972) reported that for a variety of invertebrates carotenoids are not used up in development, implying that the pigment serves no function in development. However, Hairston (1979) was of the opinion that carotenoids need not be used up to have a function and that to be active in photo-protection, they must be present continously. During his study on the female copepods Diaptomus, he found that these animals are more pigmented in winter and early spring because they are accumulating pigment to put it into the eggs. Thus the accumulation of carotenoids in the ovaries of M. dobsoni in the present study clearly indicates its possible role in reproduction.

Crustaceans represent one class of animals in which carotenoids are particularly abundant Herring (1973) reported that crustaceans can elaborate their own carotenoids from ingested precusors, unlike other animals who obtain all their carotenoids directly from their diet without any further transformation. Consequently two types of carotenoids may occur in crustacea. Those obtained directly from food and those resulting from transformation of exogenous pigments. These two types may occur alternatively according to certain conditions, food in particular and to the physiological state of the animal according to Fisher (1954). The relative proportions of carotenoids occurring in different organs and tissues fluctuate according to the species, and moreover the variations in the content of the pigment are dependant on numerous physiological and ecological parameters (Castillo et al., 1982). Basically carotenoid levels reflect the availability of these pigments in the food and the appetite of the animal for it (Goodwin, 1984). The major fluctuations in the carotenoid levels are found during vitellogenesis in the tissues like hepatopancreas, haemolymph, exoskeleton and ovaries, and the localisation and movements of the total carotenoids in these tissues have been discussed as follows:-

Generally the hepatopancreas in crustaceans plays a major role in the absorption of carotenoid from food. Katayama et al. (1972a) while studying the carotenoids of P. japonicus was of the opinion that the hepatopancreas contained all the carotenoids of the animal. But Castillo et al. (1980) determined that the hepatopancreas of P. prideauxi appeared invariably poor in carotenoids. This showed that the ability to accumulate carotenoids in the hepatopancreas differed from species to species. The carotenoid content of the hepatopancreas fluctuates during vitellogenesis (Castillo et al., 1982). According to Lenel et al. (1978), the carotenoid content of the hepatopancreas decreased during ovarian activity. He elaborated that the main fluctuations in the

carotenoid content of the hepatopancreas occurred during vitellogenesis and as the ovarian activity increased during vitellogensis, there seemed to be a corresponding decrease in the carotenoids of hepatopancreas. The present study was in agreement with this observation wherein the total carotenoid content of the hepatopancreas was found to decrease as maturity advanced and eventually only a very low level of the pigment remained as maturation peak was reached. Hsu et al.(1970) had reported that mobilisation of carotenoid initiated from the hepatopancreas where it is stored. This may be attributed for the initial high carotenoid content in the immature stage which decreased gradually during progressive maturation in the present study. Thus, it may be stated that, mobilisation of carotenoids start from the hepatopancreas, which acts as a storage organ and they finally accumulate in the ovary from where they are utilized for further egg development.

In crustaceans, the haemolymph acts as a transportation medium for the carotenoids between the hepatopancreas exoskeleton and ovary (Lenel, 1961, 1967). During vitellogenesis the main fluctuations are observed in the carotenoid levels of the haemolymph, owing to the transfer of the pigment to the ovaries (Lenel, 1978). Ceccaldi (1968) suggested that blood acts as a transport system and a temporary store for carotenoids, from which the animal can draw upon during its breeding season In Emerita anologa, Eickstaedt (1969) had found that the blood becomes bright orange in spring with the onset of breeding season and also in the autumn when breeding activity declines. Gilchrist and Lee (1972) observed that there was an overall increase in the carotenoids of the blood as the peak in the reproductive activity was passed. But the present study showed that the total carotenoid levels in the haemolymph increased as maturation progressed and decreased after spawning. This obser-

vation may be attributed to the fact that haemolymph maintains a constant reserve of carotenoids during the breeding season, so that the ovary can utilize it during maturation. But during spawning the haemolymph carotenoids are transferred to the mature eggs for their consequent development thereby causing a decrease in its level.

The exoskeleton in crustaceans, displays a wide variety of colours and this according to Nakagawa et al. (1974) is due to the presence of caroteno protein complexes. Wald et al. (1948) reported that on boiling, the crustacean carapaces undergo colour changes and this is attributed to the denaturation of caroteno protein complexes. Gilchrist and Lee (1972) while studying the role of carotenoids in reproduction of E. analoga found that the exoskeleton also gets a fair amount of carotenoids during its reproductive cycle. This was also observed in M. dobsoni during the present study. This increased level in carotenoids of exoskeleton observed with advancement of maturation may be related in some way to temperature and light. Besides, as this prawn as attains peak maturity during the intense summer months, the excess carotenoids in the exoskeleton may provide some protection against increase in temperature and light.

Crustacean ovaries in general, show a maximum accumulation of carotenoids during their egg production. This leads to the formation of various colours observed during maturation. The changes in the colours of crustaceans ovaries are attributed to the deposition of carotenoids in varying intensities (Castillo et al., 1982). The various colourations given by these carotenoids result from their different particular composition, depending on the species and probably also on the stages of development of the eggs (Zagalsky et al., 1967). In the present study, the changes in the colour of the ovary in M. dobsoni was

so distinct, that by observing the colour, the particular stage of maturation could be identified. The studies on quantitative, changes of carotenoids in the ovaries of Plesiopenaeus edwardsinaus (Establier, 1966) showed that the carotenoid levels rose from 20-40 ug/g in a period of sexual repose to 430-51 ug/g in mature ovaries. Green (1966) investigated a cycle of pigmentation for the cladoceran S. vetulus and tied it to seasonal changes in egg production. He was of the opinion that many crustaceans deposit carotenoids in their eggs. In S. vetulus, the pigment was accumulated by the female just before moulting. He found that much of the carotenoids in the adults were transferred to the developing embryo. Gilchrist and Lee (1972) also observed a build up of carotenoids in the ovaries of E. analoga during its breeding cycle while Shina et al. (1978) reported an increased storage of carotenoids in the ovaries of the echinoderm Tripneutis gratila, during its maturation. The present study also agreed with these reports and observations as, M. dobsoni was also found to accumulate carotenoids in its ovaries. Miki et al. (1982) while making a comparative study on the carotenoids in the ovaries of finfish and shellfish, suggested that these animals furnish specific carotenoids to their ovaries by rejecting some and assimilating and modifying others among those present in the dietary pigment, thus emphasising the fact that carotenoids are very essential for the development of the ovaries.

Thus it can be said that each animal has its own carotenoid requirements and each tissue appears to have a specificity in the assimilation of carotenoids. The carotenoid levels in the whole animal also fluctuate during its breeding season and in E. analoga, Gilchrist and Lee (1972) reported an overall increase of carotenoid with a peak in the mature stage. The present study also demonstrated a similar trend.

According to Castillo et al. (1982) vitellogenesis may be considered as a process of periodic rejection of carotenoids by the animal. Gilchrist and Lee (1972) suggested that as the carotenoids levels remained almost constant during the period of larval development, they might not be utilized for the larvae at all. These authors considered egg laying to be a process by which surplus carotenoids are cleared off from the animals.

It has been established in recent years that eyestalk ablation in female decapod crustaceans leads to accelarated vitellogenic process (Adiyodi and Adiyodi, 1970). Bomirski and Klek (1974) while studying the ovarian development after eyestalk ablation in Rithropanopeus harrisii, observed that the ovaries darkened in colour, showing an increase in the carotenoid concentration as maturation advanced. A similar observation was also made in the ovaries of M. dobsoni, after eyestalk ablation, in the present study. But the comparison in the total carotenoid concentration in different maturity stages between wild prawns and those obtained through eyestalk ablation showed that there was a general decrease in all tissues, in the latter. This observation can be substantiated by the findings of Maugle et al. (1980) who reported that in Macrobrachium rosenbergii after eyestalk ablation, the total carotenoid levels showed an increase, if the prawns were fed on a diet containing carotenoids. But on the other hand, if the diet was carotenoid free, a decrease occurred, ascertaining that eyestalk ablation does not allow the maintenance of the pigment if it lacks in the diet. This suggests a direct action of the endocrine system on the metabolism of carotenoids. Thus in the present study, a decrease in the total carotenoid content in all tissues of the ablated prawns may be due to the lack of pigment in the diet. The wild prawns in comparison might have acquired the carotenoids from the wide variety of food they feed on in nature.

Thus in general, the pattern of mobilisation of organic reserves especially carotenoids from the digestive gland may reflect the nutritional habitat or the reproductive biology of the animal concerned. If a species has the ability to store nutrients, one among them being the carotenoids and then mobilise them for reproduction, then reproduction can be timed to take advantage of seasonal factors such as food availability, water temperature or optimal conditions for larval survival. According to Castille and Lawrence (1989), mobilisation of stored organic reserves is usually found in species that reproduce seasonally and the influence of seasonal factors on reproduction is usually more pronounced in species that reproduce in shallow rather than those in deep waters. In short, both seasonal reproduction and mobilisation of stored nutrients are frequently found in species that reproduce at shallow depths. However in the present study, eventhough M. dobsoni shows a protracted breeding season extending throughout the year and breeds in relatively deeper waters of the inshore grounds (Rao 1968), it could mobilise stored carotenoids dissolved in lipids from the hepatopancreas to the ovaries.

In conclusion, the mobilisation of stored nutrients like the total carotenoids may be controlled by certain proximate factors, which determine the time the gametes are produced within an individual both in relation to its own life history and with respect to the environment. Thus, the ability of an organism, inhabiting a given environment, to divert nutrients for gamete production is not only important for the reproductive success of the individuals, but also has implications for the distributional patterns of the species concerned (Sastry, 1983).

In the present study, an attempt had been made to study the mobilisation of only total carotenoids in relation to ovarian maturation.

Further work on the individual carotenoids and their localization and movements in various tissues, incorporating sophisticated techniques like labelling the animal with radioactive isotopes in order to trace the mobilisation pathway, could throw more light on the pigmentary physiology, thus elucidating some of the questions inherent in the presence of carotenoids in crustacea.

SUMMARY

The present study was undertaken with the objective of studying the variation in the concentration of total carotenoids in relation to the ovarian maturation of the prawn, Metapenaeus dobsoni, the most abundant and one of the commercially important species of penaeid prawns along the south-west coast of India.

The experiments were carried out from June to September 1989 during which specimens were collected from the inshore waters of Cochin. The animals were classified into five maturity stages, evaluated on the basis of the ovary colour and size, morphological and microscopic examination of the ova and histological study of the ovary during the different stages of maturity. The gonado-somatic index and hepato-somatic index were also taken into consideration while assessing the ovarian maturation. The total carotenoid concentration of tissues viz. hepatopancreas, haemolymph exoskeleton and ovary in each maturity stage was determined, along with that of the whole animal. Eyestalk ablation in order to induce maturation was applied and the total carotenoid concentration in these prawns of different maturity stages were compared with those of the wild prawns.

The observations made during the course of the experiment are summarised below:

1. The total carotenoid concentration in the hepatopancreas decreased as maturation advanced with a recovery in the spent stage.

2. In the haemolymph, the total carotenoid level increased as maturation advanced and then decreased in the spent stage.

3. The total carotenoids showed an increase as maturation advanced in the exoskeleton and then declined in the spent stage.

4. In the ovary, an increasing trend in the total carotenoids was observed during maturation reaching a peak in the mature stage with a decline in the spent stage. A positive relation was established between GSI and total carotenoids of the ovary.

5. In the whole animal, there was an increase in the total carotenoid levels as maturation progressed, but it decreased in the spent stage.

6. There was a general decrease in total carotenoids in all tissues is the ablated prawns in comparison with those of the wild prawns. However the trend of increase and decrease of total carotenoids in all the tissues remained the same.

7. The reciprocal relationship of decrease in total carotenoids in the hepatopancreas and the increase in total carotenoids in the ovary during maturation suggested the mobilisation of carotenoids from the hepatopancreas to the ovary as maturation advanced for the further development of the eggs.

8. The positive relationship established between GSI and total carotenoids of the ovary indicated that as the ovary increased in weight along with maturation there was a simultaneous build up of carotenoids, suggesting the possible role of carotenoids in reproduction.

REFERENCES

*ABELOOS, M. and FISCHER, E. 1926. Sur l'origine et les migration des pigments carotenoides chez les Crustaces. Comp. rend. soc. biol., 95: 383-384.

ADIYODI, R.G. and ADIYODI, K.G. 1970. Endocrine control of reproduction in Decapod Crustaceans. Biol. Rev., 45: 121-165.

ANONYMOUS. 1989. Marine Products Export Review 1988-89. The Marine Products Export Development Authority.

APPLEBY, S.J. and MUNTZ, W.R.A. 1979. Occlusable yellow corneas in Tetraodontidae. J. exp. Biol., 83: 249-259.

BOMIRSKI, A. and KLEK, E. 1974. Action of Eye stalks on the ovary in Rhithropanopeus harrisji and Crangon crangon (Crustacea: Decapoda). Mar. Biol., 24: 329-337.

BROWN, A. and PATLAN, D. 1974. Colour changes in the ovaries of penaeid shrimp as a determinant of their maturity. Mar. Fish. Rev., 36(7): 23-26.

CAMPBELL, S.A. 1969. Carotenoid metabolism in the commensal crab Pinnotheres pisum. Comp. Biochem. Physiol., 30: 803-812.

CASTILLE, F.L. and LAWRENCE, A.L. 1989. Relationship between maturation and biochemical composition of the gonads and digestive glands of shrimps Penaeus aztecus Ives and Penaeus setiferus (L.) J. Crust. Biol., 9(2): 202-211.

* Originals not referred.

CASTILLO, R., LENEL, R. and NEGRE-SADARGUES, G. 1980. Determination, Tissue distribution and Metabolism of carotenoid pigments in hermit crab Pagurus prideauxi Leach (1815). Decapoda: Anomura. Comp. Biochem. Physiol., 66B: 483-489.

CASTILLO, R., LENEL, R. and NEGRE-SADARGUES, G. 1982. General survey of carotenoids in crustacea. In: Carotenoid Chemistry and Biochemistry. Pergamon Press. Oxford, pp 221-224.

*CECCALDI, H.J. 1967. Transport des pigments carotenoides dan l'e haemolymph de Carcinus maenas. Linne. Cr. Soc. Biol., 161: 106.

*CECCALDI, H.J. 1968. Recherches sur la biologic des associations entre proteins et carotenoides chez les crustacea. Decapoda; aspects metaboliques et moleculoures. These de doctorat es sciences naturelles. Universite de Aix-Marseille.

CECCALDI, H.J., OTAZU-ABRILL, M and TATE, T.E. 1978. Variations circadiennes des pigments carotenoids dans les yeux et hepatopancreas de Penaeus japonicus. Comp. rend. Soc. Biol., 172: 684-788.

CHEESMAN, D.F., LEE, W.L. and ZAGALSKY, P.F. 1967. Carotenoproteins in Invertebrates. Biol. Rev., 42: 132-160.

*CHOUBERT, G. 1986. Carotenoid pigments and reproduction of fish. Bull. FR Peche. Piscic., 300: 25-32.

CRAIK, J.C.A. 1985. Egg quality and egg pigment content in salmonid fishes. Aquaculture, 47: 61-68.

CROZIER, G.E. 1970. Tissue carotenoids in prespawning and spawning sockeye salmon. J. Fish. Res. Bd. Can., 27: 973-975.

*CZECZUGA, B. 1976. Content of carotenoid in various body parts of crayfish Orconectus limosus (Raf) in annual cycle. Bull. Acad. Pol. Sci. Ser. Sci. Biol., 24: 175-180.

CZECZUGA, B. 1980. Changes occuring during the annual cycle in carotenoid central of Gammarus lacustris (Crustacea: Amphipoda) specimens from River Narew. Comp. Biochem. Physiol., 66B: 569-572.

CZECZUGA, B. and KRYWUTA, S. 1981. Investigations of carotenoprotein complexes in Animals III. Presence of crustacyanins in Gammarus lacustris. Comp. Biochem. Physiol., 70B: 665-667.

CZERPAK, R. and CZECZUGA, B. 1969. Studies of the pigments in the shrimp Crangon crangon (Crustacea, Malacosrtraca). Mar. Biol., 3: 122-125.

DEUFEL, J. 1975. Physiological effect of carotenoids in Salmonidae. Hydrologia 37: 244-248.

DONALDSON, E.M. and FAGERLAND, U.H.N. 1970. Effect of sexual maturity and gonadomy on cortisol secretion rate in sockeye salmon Oncorhyncus nerka. J. Fish. Res. Bd. Can., 27: 2287-2296.

*EICKSTAEDT, L.L. 1969. The reproductive biology of the sand crab Emerita-anologa (Stimpson) Ph.D. Thesis University of Stanford.

*ESTABLIER, R. 1966. Estudios sobre los carotenoides de plantas y de los ovaries Y hepatopancreas del crustaceo Plesiopenaeus edwardsianus (Johnson) durante et ciclo sexual. Inv. Pesq., 30: 223-232.

FARMANFARMAIAN, A., GIESE, A.C., BOOLOOTIAN, R.A. and BENETT, J. 1958. On the reproductive cycle in 4 sp. of west coast star fishes. J. Exp. Zool., 138: 355-367.

FISHER, L.R., KON, S.K. and THOMPSON, S.Y. 1952. Vitamin A and carotenoid in certain invertebrates. I. Marine Crustacea. J. Mar. Biol. Ass. U.K., 31: 229-258.

FISHER, L.R., KON, S.K. and THOMPSON, S.Y. 1954. Vitamin A and carotenoid in certain invertebrates II. Studies of seasonal variations in some Marine Crustacea. J. Mar. Biol. Ass. U.K., 33: 589-612.

GEORGE, T.C. and PATEL, B.S. 1956. The seasonal variation in fat content of the liver and gonads in a marine and fresh water decapod J. Anim. Morphol., Physiol., 3: 49-55.

GIESE, A.C. 1959. Comparative physiology: Annual reproductive cycles of marine invertebrates. Annu. Rev. Physiol., 21: 547-576.

GIESE, A.C. and PEARSE, J.S. 1974. Introduction: General Principles. In: Reproduction of Marine Invertebrates. Edited by A.C. Giese and J.S. Pearse. Vol. I. Academic Press, New York. pp 1-49.

GILCHRIST, B.M. and LEE, W.C. 1967. Carotenoids and Carotenoid metabolism in Carcinus maenas (Crustacea: Decapoda). J. Zool. Lond., 151: 171-180.

GILCHRIST, B.M. and LEE, W.C. 1972. Carotenoid pigments and their possible role in reproduction in the sand crab Emerita anologa (Stimpson, 1857), Comp. Biochem. Physiol., 42B: 263-294.

GOODWIN, T.W. 1960. Biochemistry of pigments. In: The Physiology of Crustacea. Edited by T.W. WATERMAN. Academic Press. New York pp. 101-140.

GOODWIN, T.W. 1984. Crustacea. In: The Comparative Biochemistry of the Carotenoids. Volume II. Animals. Chapman and Hall. London. pp 64-96.

GREEN, T. 1966. Variation in carotenoid pigmentation in Simocephalus vetulus (Crustacea, cladocera), J. Zool., Lond., 149: 174-181.

HAIRSTON, N.G. 1979. The relationship between pigmentation and reproduction in two species of Diaptomus (Copepoda). Limnol. Oceanogr., 24(4): 38-44.

HERRING, P.J. 1968. The carotenoid pigments of Daphnia magna Straus II. Aspects of pigmentary metabolism Comp. Biochem. Physiol., 24: 205-221.

HERRING, P.J. 1972. Depth distribution of the carotenoid pigments and lipids of some oceanic animals - I. Mixed Zooplankton, Copepods and Euphausids. J. Mar. Biol. Ass. U.K., 52: 179-189.

HERRING, P.J. 1973. Depth distribution of the carotenoid pigment and lipids of some oceanic animals. II. Decapod crustaceans. J. Mar. Biol. Ass. U.K., 53: 539-562.

*HSU, W.J., CHICHESTER, C.O. and DAVIES, B.H. 1970. The metabolism of carotene and other carotenoids in the brine shrimp. Artemia salina (Crustacea, Branchiopoda). Comp. Biochem. Physiol., 32: 69-79.

JOSEPH, J.D. and WILLIAMS, J.E. 1975. Shrimp Head Oil: A potential feed additive for Mariculture. Proc. World. Maricult. Soc., 6: 147-161.

KARNAUKHOV, V.M., MILOVIDOVA, N.Y. and KARGOPOLOVA, I.M. 1977. On a role of carotenoids in tolerance of sea molluscs to environmental pollution. Comp. Biochem. Physiol., 56A: 189-193.

KATAYAMA, T., KAMATA, T., SHIMAYA, M., DESHIMARU, O. and CHICHESTER, C.O. 1972a. The biosynthesis of astaxanthin - VIII. The conversion of labelled carotene-15, 15^{3} H_2 into astaxanthin in prawn Penaeus japonicus. Bate. Bull. Jap Soc. Scient. Fish., 38: 1171-1175.

*KATAYAMA, T., KAMATA, T., SHIMAYA, M. DESHIMARU, O. and CHICHESTER, C.O. 1972b. The biosynthesis of astaxanthin-VI. The carotenoids in the prawn Penaeus japonicus. Bate. (Part 2). Int. J. Biochem., 3: 363-368.

KATAYAMA, T., KUNISAKI, Y., SHIMAYA, M., SIMPSON, K.L. and CHICHESTER, C.O. 1973a. The biosynthesis of astaxanthin-XIV. The conversion of labelled carotene-16, 15^{1-3} H_2 into astaxanthin in the crab Portunus trituberculatus. Bull. Jap. Soc. Scient. Fish., 39: 283-287.

KATAYAMA, T., SHIMAYA, M., SAMESHIMA, M. and CHICHESTER, C.O. 1973b. The biosynthesis of astaxanthin- XII. The conversion of labelled carotene-15, 15^{1-3} H_2 into body astaxanthin in the lobster Panulirus japonicus. Bull. Jap. Soc. Scient. Fish., 39: 215-220.

*KELLEY, C.E. and HARNON, A.W. 1972. Method of determining carotenoid content of Alaska Pink shrimp and representative values for several shrimp products. Fish. Bull. Natl. Oceanic, Atmos., Adm. Seattle., 70(1): 111-113.

KING, J.E. 1948. A study of the reproductive organs of the common marine shrimp Penaeus setiferus (Linnaeus). Biol. Bull., 94: 244-262.

KITAHARA, T. 1983. Behaviour of carotenoids in the chum salmon Oncorhyncus keta during anadromous migration. Comp. Biochem. Physiol., 76B(1): 97-101.

KITAHARA, T. 1985. The behaviour of carotenoids in the course of maturation of the masu salmon (O. masou). Bull. Jap. Soc. Scient. Fish., 51(2): 253-255.

KONOSU, K. 1983. Carotenoid composition of Alaska Pollack roe at different stages of maturation. Bull. Jap. Soc. scient. Fish., 49(10): 1615-1618.

*KRIVOBOK, M.M. 1964. On the role of liver in the maturation of the ovaries of Baltic herring, Clupea harengus memberas L. Vop. Ikhtiol., 4: 483-494.

*KUHN, R. and SORENSEN, M.A. 1938. Uber Astaxanthin und Ovoverdin. Ber. deut. Chem. Ges., 71: 1879-1888.

LAXMINARAYANA, A. 1987. Role of nutrition in Broodstock Management of prawns. In: Summer Institute in recent advances in finfish and shellfish nutrition. Technical paper - 28.

*LENEL, R. and VEILLET, A. 1951. Effects de le ablation des peduncles oculaires sur les pigments carotenoides du crabe Carcinus maenas. Comp. rend., 223: 1064-1065.

*LENEL, R. 1961. Sur le metabolisme des pigments carotenoides de Carcinus maenas, Linne. Bull. Soc. lorr. Sci., 1: 1-134.

*LENEL, R. 1967. Etude de la nature et du metabolisme des pigments caro-tenoides de Carcinus maenas. Linne. Bull. Soc. Jorr. Sci., 1: 1-134.

*LENEL, R., NEGRE-SADARGUES, G. and CASTILLO, R. 1978. Carotenoid pigments in crustacea. Arch. Zool. exp. gen. 119(2): 297-334.

MAOKA, T., KOMORI, T. and MATSUNO, T. 1985. Sterochemical investigations of carotenoids in Antartic krill Euphausia superba. Bull. Jap. Soc. Scient. Fish., 51: 1671-1673.

MATSUNO, T., WAKASA, Y. and GOKUBO, M. 1982. Carotenoids of a river crab Potamon dehaani. Bull. Jap. Soc. Scient. Fish., 48: 661-666.

MAUGLE, P., KAMATA, T., McLEAN, S., SIMPSON, K.L. and KATAYAMA. T. 1980. The influence of Eye stalk ablation on the carotenoid composition of juvenile Macrobrachium rosenbergii. Bull. Jap. Soc. Scient. Fish., 46(7): 901-904.

McNANAMARA, J.J., SZABO, G., and SIMS, R.T. 1962. The nature of pigments in the integument and eye of hermit crab Paguru pollicarus. Biol. Bull., 23: 504.

MIKHAIL, M.Y., EZZAJ, A.A., HASEM, H.T. and WADIE, W.F. 1982. Fluctu-ations in fat and water contents of two species of serranid fishes in the Egyptian mediterranean waters. Bull. Inst. Oceanogr. Fish., ARE 8(1): 123-144.

MIKI, W., YAMAGISCHI, K. and KONOSU, K. 1982. Comparison of carotenoids in the ovaries of marine fish and shell fish. Comp. Biochem. Physiol., 71B: 7-11.

MOHAMMED, K.H. 1973a. Penaeid prawn resources of India. Proc. Symp. Living Resources of the Seas Around India, 548-556.

MOHAMMED, K.H. 1973b. Marine prawn culture in India. An appraisal of the present status and future prospects. In: Proc. Seminar on Mariculture and mechanised fishing. Govt. of Tamil Nadu, pp 31-33.

MUTHUKARUPPAN, S. 1987. Biochemical aspects of Ovarian Maturation in Liza parsia (Hamilton and Buchanan). Dissertation for M.Sc. (Mariculture) University of Cochin.

NAKAGAWA, H., KAYAMA, M., YAMADA, K. and ASAKAWA, S. 1974. Studies on carotenoproteins in aquatic animals V. Thermal reddening of exoskeleton of Crayfish Procambarus clarkii. J. Fac. Fish. Anim. Husb. Hiroshima Univ., 13(1): 1-13.

NARSUBAI, A.V. and MOHANRAO, Y.R. 1973. Carotenoid content of the shell of prawn Metapenaeus affinis. Curr. Sci., 42: 107-108.

OLSON, J.A. 1979. A simple dual assay for Vitamin A and carotenoids in Human liver. Nutrition Reports international, 807-813.

*PALMER, L.S. and ECKLES, C.H. 1922. Carotenoids and Related pigments. New York: Reinhold.

*POUCHET, G. 1872. C.R. Acad. Sci. Paris, 74: 757-760.

RAO, P.V. 1968. Maturation and spawning of the Penaeid prawns of the south west coast of India. FAO. Fish. Rep., 57(2): 285-302.

RAO, P.V. and KATHIRVEL, M. 1973. On the breeding of a penaeid prawn M. dobsoni in the brackish water medium. Indian J. Fish., 20(1):228-230.

SAITO, A. and REGIER, C.L. 1971. Pigmentation of brook trout Salvelinus fontinalis by feeding dried crustacean waste. J. Fish. Res. Bd. Can., 27: 509-512.

SANDIFER, P.A. and JOSEPH, J.D. 1976. Growth responses and fatty acid composition of juvenile prawns (Macrobrachium rosenbergii) fed a prepared diet augmented with shrimp head oil. Aquaculture, 8: 129-138.

SASTRY, A.N. 1966. Temperature effects in reproduction of the bay scallop Acquipecten irradians Lamarck. Biol. Bull., 130(1-3): 118-133.

SASTRY, A.N. 1983. Ecological aspects of Reproduction. In: Biology of Crustacea. Vol. 8. Environmental Adaptations. Edited by F. John Vernberg, Winona, D. Vernberg. Academic Press. New York. pp. 179-270.

SHINA, A., GROSS, J., LIFSHITZ, A. and SKLARZ, B. 1978. Carotenoids of the invertebrates of the Red Sea, II. Carotenoid pigments in the gonads of Sea Urchin Tripneutes gratila (Echinodermata). Comp. Biochem. Physiol., 61B: 123-128.

SHNAREVICH, I.D. and SAKHNENKO, Y.G. 1971. Dynamics of carotenoid in fish tissues and organs in relation to the sexual cycle. Hydrobiol. J., 7: 77-80.

*SMITH, G. 1911. Studies on the experimental analysis of sex. Part 7. Sexual changes in the blood and liver of Carcinus maenas. Quart. J. Microscop. Sci., 57: 251-265.

*SNAUWAERT, F., TOBACK, P.P. and MAES, E. 1973. Carotenoids pigments in sand shrimp Crangon vulgaris. Wiss. Technol., 6(2): 43-47.

SPINELLI, J., LEHMAN, L. and WEIG, D. 1974. Composition processing and utilisation of red crab (Pleuroncodes planipes) as an aquacultural feed ingredient. J. Fish. Res. Bd. Can., 31(6): 1025-1029.

SPINELLI, J. and MAHNKEN, C. 1978. Carotenoid deposition in pen reared salmonids fed diet containing oil extract of red crab. (Pleuroncodes planipes). Aquaculture, 13: 213-223.

STEVEN, D.M. 1949. Studies on animal carotenoids in the reproductive cycle of brown trout. J. exp. Biol., 26: 295-303.

TACON, A.G.J. 1981. Speculative review of possible carotenoid function in fish. Prog. Fish. Cult., 43(4): 205-208.

*THUDICHUM, J.L. 1869. Proc. R. Soc., 17: 253.

TSUKUDA, N. 1963. Study on the pigment of deep sea prawn Pandalus borealis Kroyer-I. Identification and concentration of pigments. Bull. Jap. Sea. reg. Fish. Res. Lab., 11: 109-115.

WALD, G., NATHANSON, N., JENCKS, W.P. and TARR, E. 1948. Crustacyanin the blue carotenoid protein of the lobster shell. Biol. Bull., 95: 249-250.

WILKIE, D.W. 1972. The carotenoid pigmentation of Pleuroncodes planipes. Stimpson (Crustacea, Decapoda Galatheidae). Comp. Biochem. Physiol., 42B: 731-734.

ZAGALSKY, P.F. CHEESMAN, D.F. and CECCALDI, H.J. 1967. Studies on carotenoid containing lipo proteins isolated from eggs and ovaries of certain marine invertebrates. Comp. Biochem. Physiol., 22: 851-871.