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OBSERVATIONS ON INTRASPECIFIC VARIATIONS IN THE LEVEL OF TOTAL PROTEINS AND AMINO ACIDS, TOTAL NON PROTEINS AND THEIR FRACTIONS WITH RESPECT TO MATURATION IN THE MUSCLES, HEPATOPANCREAS AND GONADS OF TWO CRUSTACEAN SPECIES PORTUNUS PELAGICUS (L) AND PENAEUS KERATHURUS (L). I- TOTAL PROTEINS AND AMINO ACIDS.

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ABSTRACT

1- The total protein levels were generally low during the immature stages, but increased rapidly reaching their maximum in the mature ones. The high muscle protein levels were associated with the period of moulting and formation of new cuticle.

2- The fluctuations of the protein concentrations observed in both the hepatopancreas and gonads of the prawns and crabs studied may be interpreted as a result of protein accumulation in the hepatopancreas during the intermoult and that such stores are mobilized to meet the demands of events like moult and reproduction.

3- The quantitative amino acid composition of **Portunus pelagicus** and **Penaeus kerathurus** muscles were found to be ten amino acids in comparison with those of the hepatopancreas and gonads (11 amino acids). This new amino acid was detected and identified as aspartic acid.

INTRODUCTION

Recent knowledge demonstrates that the biological value of a protein is dependent upon its amino acid composition, (Mitchell and Hamilton, 1929 and Mitchell, 1942). It is important from the practical stand point, therefore, not only to understand the quantitative and qualitative requirements of animal for essential amino acids, (Holt, et al., 1941 and Rose et al., 1942) but also the quantitative composition of proteins which are commonly utilized to fulfil these body needs.

Drach and Lafon (1942), Renaud (1949) and Leone (1953) have studied the cyclical changes in protein and nitrogen metabolism during the moult cycle.

Neiland and Scheer (1953) indicate that **Hemigrapsus** uses protein rather than carbohydrate and fat as primary energy source, during starvation.

Schafer (1968) showed no significant changes in the nitrogen level of the hepatopancreas of **P. duorarum** with starvation.

Florkin and his co-workers (1949) studied the role of free amino acids in crustacea, as well as in other invertebrate taxa, earlier using microbiological amino acid assay techniques (Camien et al., 1951) and later the more rapid chromatographic methods to establish the importance of free amino acids in intracellular isoosmotic regulation (Schoffeniels and Gilles, 1970).

Information existis concerning amino acid changes in crustacea subjected to variations in environmental salinity (Huggins and Munday, 1968). Changes in rates of protein metabolism occurring toward the end of the cycle (Skinner, 1965) may also contribute to changes in amino acid concentrations, but since only certain amino acids increase, it seems unlikely that, protein incorporation changes play a major role. Metabolisms of hormonal alteration of tissue osmolarity during the intermoult cycle remain to be explained although evident exists that changes in permeability, perhaps in the gut region, may be involoved (Mantel, 1967).

The tissues of marine invertebrates contain very high levels of free amino acids which are believed to serve an intraceliular osmoregulatory function (e.g.; Florkin and Schoeffeniels, 1965). Potts (1967) suggested that as a consequence of these high free amino acids levels and the tendency of these compounds to leak across the body wall into the water, marine invertebrates probably lose more free amino acids to the water than do comparable freshwater invertebrates.

Crustacean muscle shows a specific pattern of intracellular amino acids which is remarkably concentrated , compared for instance to vertebrate muscle. This intracellular amino acid pool is apparently greater in the muscles of marine crustaceans than in those of freshwater forms (Needham, 1949). In muscle tissue of **Eriochein** adapted to sea water, the concentrations of the different amino acids determined are higher than when the animals live in fresh water (Edwards, 1950). The greatest increases take place in glycine and proline, and the smallest is the case of arginine, but the growth in the size of the pool is nevertheless a general one, resulting in a strongly marked difference in its total concentration.

MATERIAL AND METHODS

Portunus pelagicus and **Penaeus kerathurus** (ranging in weight from 20.0 - 400.0 gm for crab, and 10.0 - 60.0 gm for prawn) were utilized in the present study. They were collected during the winter months from the Gulf of Suez.

Total proteins were determined using the microkjeldahl volumetric method (Vogel, 1968). The technique involves the determination of protein content of muscle, hepatopancreas and gonad.

Amino acids of the above mentioned tissues were estimated qualitatively by means of one dimension paper chromatography (after Edozien et al., 1960) and quantitatively by Formal titration (Oser et al., 1965).

All the data obtained were statistically treated using the Arkin and Colton formulae (1963).

RESULT AND DISCUSSION

The total protein contents of the female muscles were higher than those of the males (Figs. 1 and 2). The high muscle protein levels are associated with the period of moulting and formation of new cuticle. Also the total protein of both sexes showed fluctuations of higher and lower values with gradual increase in total body weight. The higher total protein values recorded for females in mature stages may be due to the fact that testicular tissue growth requires a far smaller consumption of reserve materials when compared with the corresponding requirements of the growing ovaries. The greater reduction of total protein in females of the species studied than in males by maturation stages probably indicates a greater depletion of body protein in egg formation.

Therefore, moulting is a part of the mechanism of growth. The increase in size and weight during ecdysis does not constitute growth. This must be defined as the increase in dry weight of the body which occurs in the periods between moults. Thus although ecdysis, increase in size and increase in total weight are all discontinuous processes, growth itself is a continuous process.

Moulting dominates the life of the animal. Ecdysis can not be considered as a brief interruption of its normal life but rather as a process which has effects upon the whole physiology.

Considering all results for hepatopancreas and gonad, it is apparent that, in the majority of cases the total protein content is less in males than females, however, this decrease is obviously detected among adult animals rather than in immature ones. The fluctuations of the protein concentrations observed in both the hepatopancreas and gonad of the prawn and the crab studied may be interpreted as a result of protein accumulation in the hepatopancreas during the intermoult and that such stores are mobilized to meet the demands of events like moult and reproduction (Figs. 3 and 4).

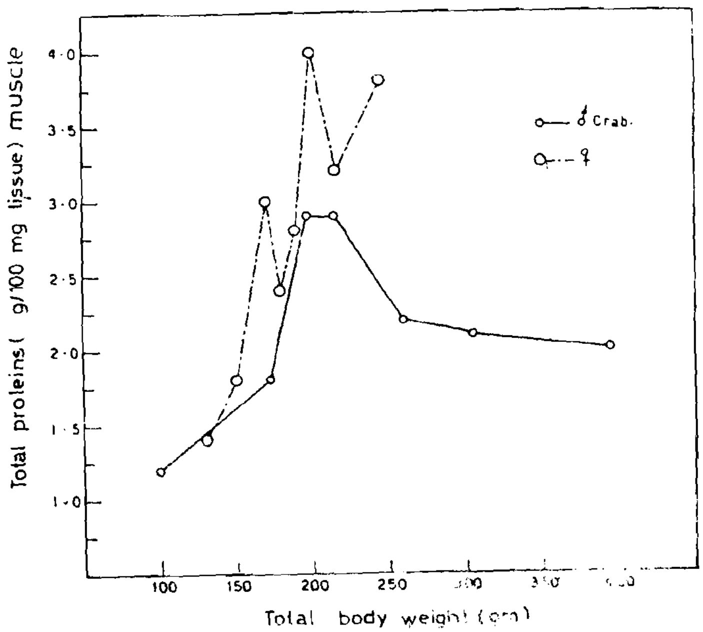

FIG. 1
Muscle total protein concentration of the two sexes of
Portunus pelagicus at their different stages of maturity

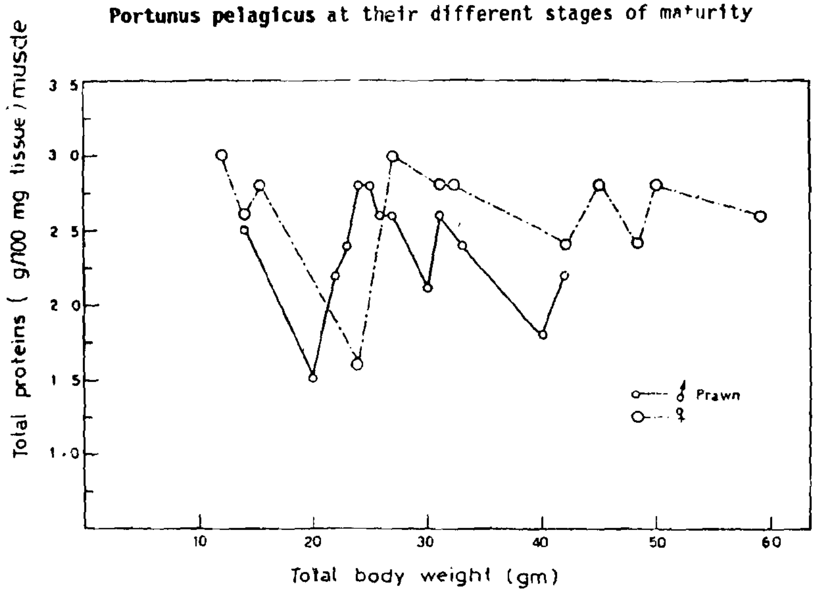

FIG. 2
Muscle total protein concentrations of the two sexes of
Penaus kerathurus at their different stages of maturity.

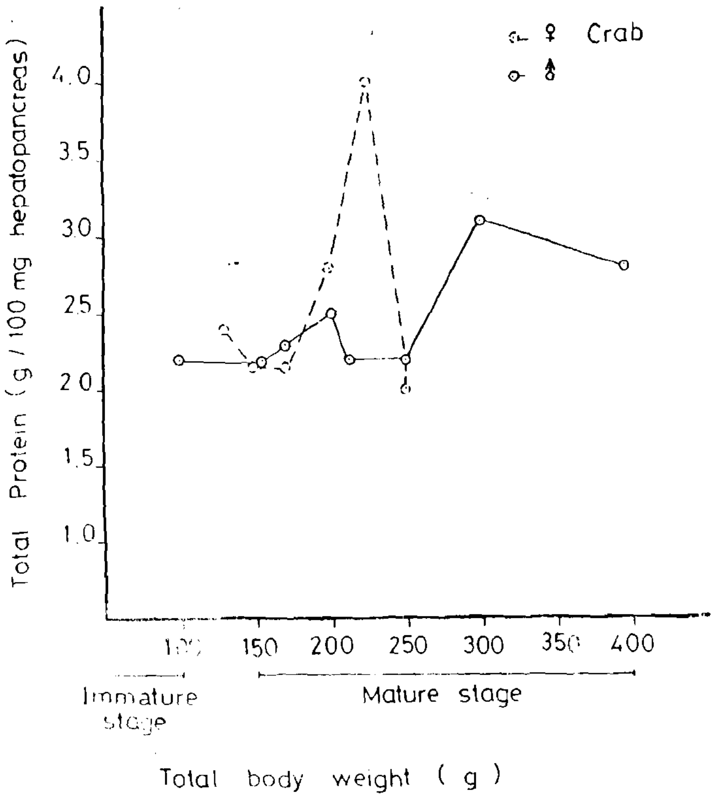

FIG. 3

The concentration of total protein in the hepatopancreas of the two sexes of **Portunus pelagicus** at their different stages of maturity.

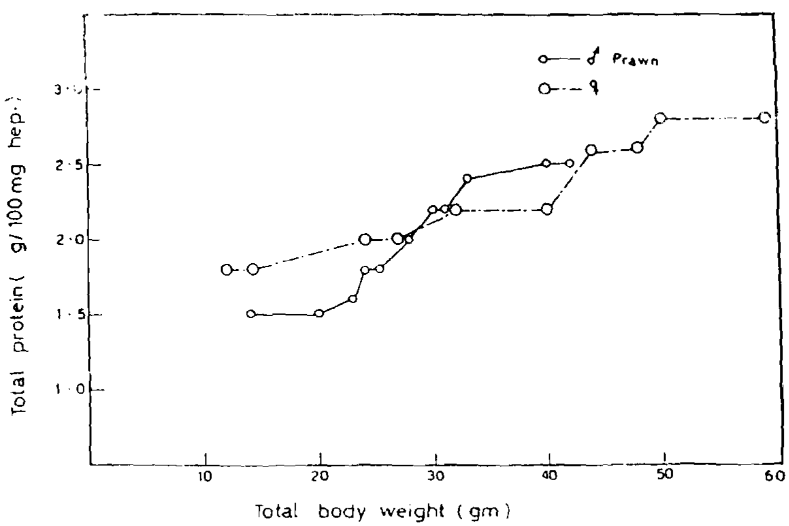

FIG. 4

The concentration of total protein in the hepatopancreas of the two sexes of **Panaeus kerathurus** at their differnet stages of maturity.

The initially low protein values in the female ovary during young stages may be due to the consumption of certain amount of proteins needed during the preparation for the developmenl of eggs. The rapid increase in these proteins in the mature adult females, is perhaps due to synthesis of new proteins which are particularly necessary during this period of development as the female gonads at adult emergence are not advanced as those of the male, female lag considerably behind males in thier maturation. On the other hand, the decrease in total testis protein concentration in the adult male must be expected as all the maturation processes are completed, a condition which needs no more protein synthesis in this particular period of the male life cycle, as it was stated much of the spermatogenesis is completed before the final moult when active spermatozoa may be already present or are produced soon after it. (Figs. 5 and 6).

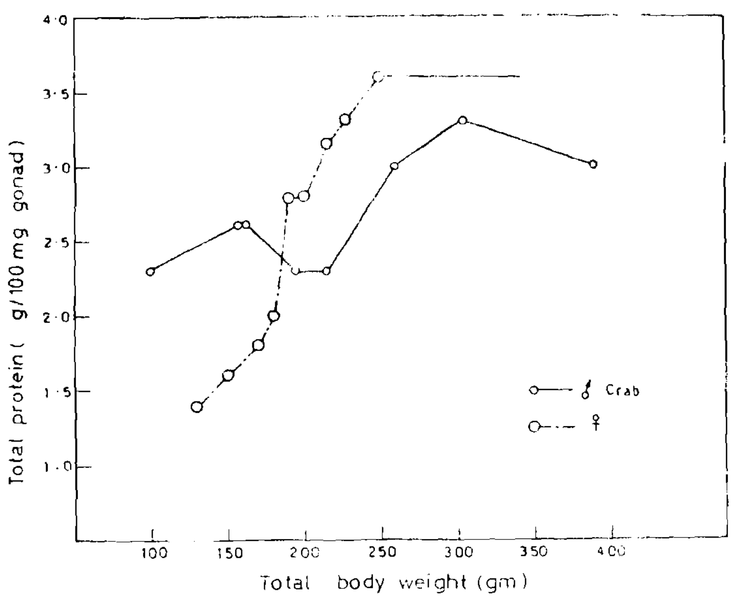

FIG. 5

Gonad total protein concentrations among different stages of maturity of two sexes of **Portunus pelagicus**.

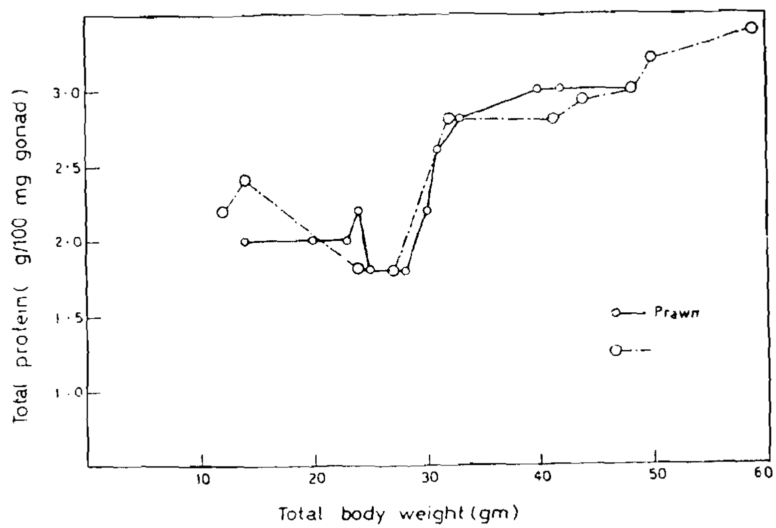

FIG. 6
Gonad total protein concentrations among different stages of maturity of two sexes of **Penaeus kerathurus.**

Adiyodi and Adiyodi (1970) suggested that the oocyte development is controlled by the combination of the neurosecretion from the part intercerebralis of the brain and the sinus gland hormone, the first affecting the synthesis of protein in the hepatopancraes, the second facilitating the yolk formation and once the growth is completed, their secretion is no longer used and is accumulated.

It can be concluded that change of the above mentioned protein is either indirectly the result of hormone action or is the endocrine secretions themselves. Both of these possibilities may be true.

The results of the total amino acid determination, however, showed a very marked effect of sex and maturity on the muscle, hepatopancreas and gonad levels of the two species studied (Fig. 7-12). The quantitative amino acid composition of **Portunus pelagicus** (L) and **Penaeus kerathurus** (L) muscles, has been determined and were found to be ten amino acids:

1- Cystine	2- Lysine	3- Arginine
4- Glycine	5- Alanine	6- Proline
7- Glutamic acid	8- Tyrosine	9- Tryptophan
10- Isoleucine.		

in comparison with those of the hepatopancreas and gonads (11 amino acids). This new amino acid was detected and identified as aspartic acid (Figs. 13-18).

A comparison of the results of muscle amino acid given for **Portunus pelagicus** and **Penaeus kerathurus** with those obtained by (Raymont et al., 1968) in **Neomysis** using paper chromatography, showed that aspartic acid, lucines (lucine and isolucine together) and lysine are also especially important.

The occurrence of glycine and proline as the major components of samples analysed for amino acids seems worthly of discussion.

Camien, et al. (1951) have investigaed the free amino acids of the muscles of various marine crustacea, including lobsters. They used microbiological methods of assay, as opposed to the chemical methods. They showed large amounts of proline and glycine to be present in fresh lobster muscle, together with smaller quantities of glutamine and alanine and traces of aspartic acid, glutamic acid, histidine, lysine and threonine. Camien et al. (1951) found somewhat larger quantities of arginine, and smaller quantities of valine and leucine.

In general, it can be seen that muscle tissues of the two species studied do not differ in their amino acid pattern, which implies that the same amino acid composition of the muscle proteins is repeated throughout the animal kingdom and indicates that, as far as the ten amino acids obtained in the muscles of the two species under investigation are concerned, the protein of one muscle is as good as that of another in supplying amino acids in the diet.

The hepatopancreas and gonads, while showing some similarity to the muscle tissue in composition, differ from it in certain respects. The hepatopancreas and gonads are very much alike in amino acid composition but differ from the muscle in the total number of amino acids being 11 instead of ten and also in the concentration of cystine, tryptophan, tyrosine and alanine. This agrees with the work of Regnault (1971) on **Crangon septenspinosa.**

It was known among crustaceans that the amino acids, arginine, lysine, tyrosine, tryptophan, alanine, theonine, cystine are nutritionally essential for optimal animal growth, either through a limited ability or a total inability of the body to synthesize them.

Recent knowledge (Colin, 1973 and Conrad, 1976) demonstrates that the biological value of a protein is dependent upon its amino acid composition. It is important from the practical stand point of view, therefore not only to understand the quantitative and qualitative requirements of animals for total amino acids but also the quantitative composition of proteins which are utilized to fulfil these body needs.

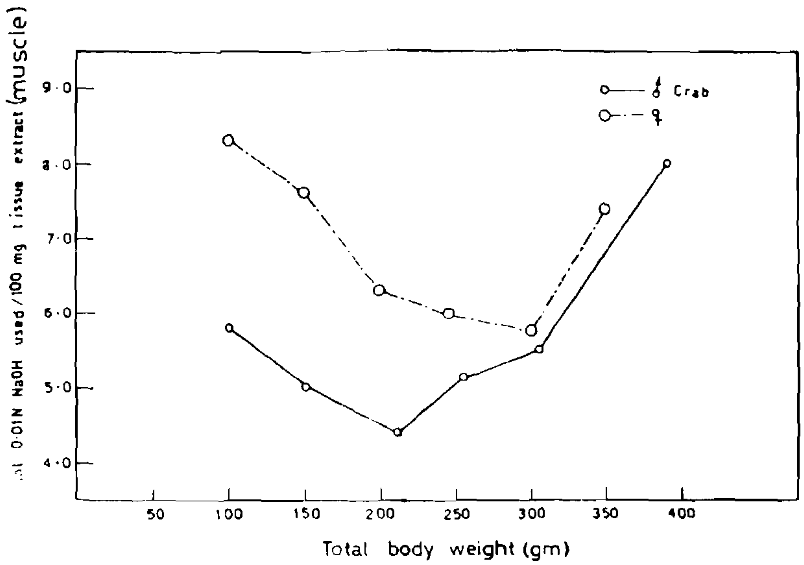

FIG. 7
Changes in the relative ml % of free amino acids in muscle of the two sexes of **Portunus pelagicus** at their different stages of maturity (Formal titration).

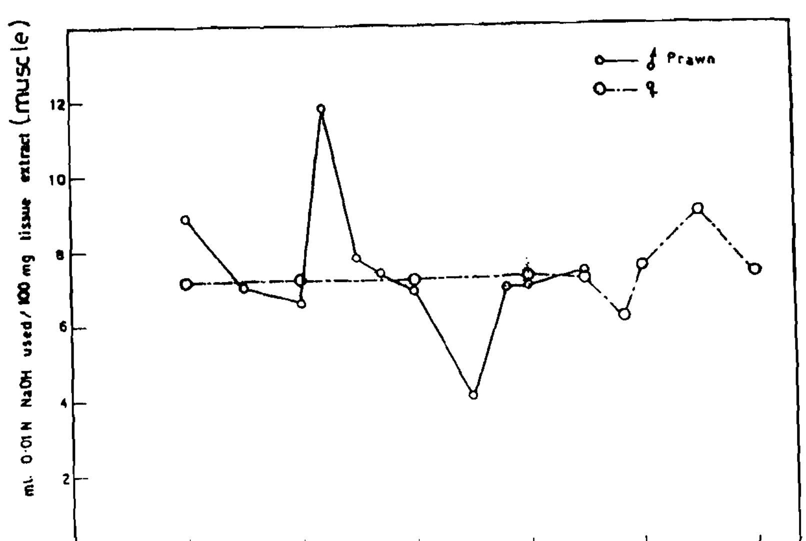

FIG. 8
Changes in relative ml% of free amino acids in muscle of the two sexes of **Penaeus kerathurus** at their different stages of maturity (Formal titration).

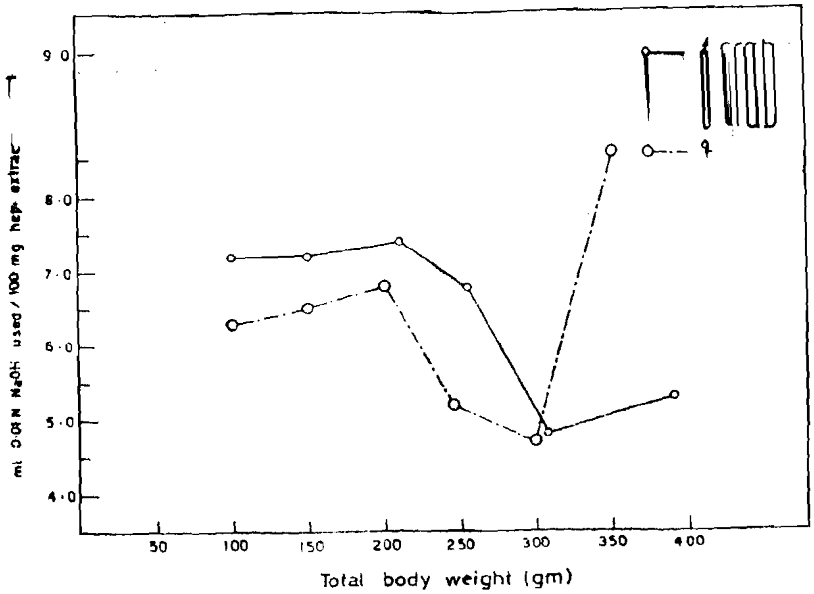

FIG. 9

Changes in relative ml% of free amino acids in hepatopancreas of the two sexes of **Portunus pelagicus** at their different stages of maturity.

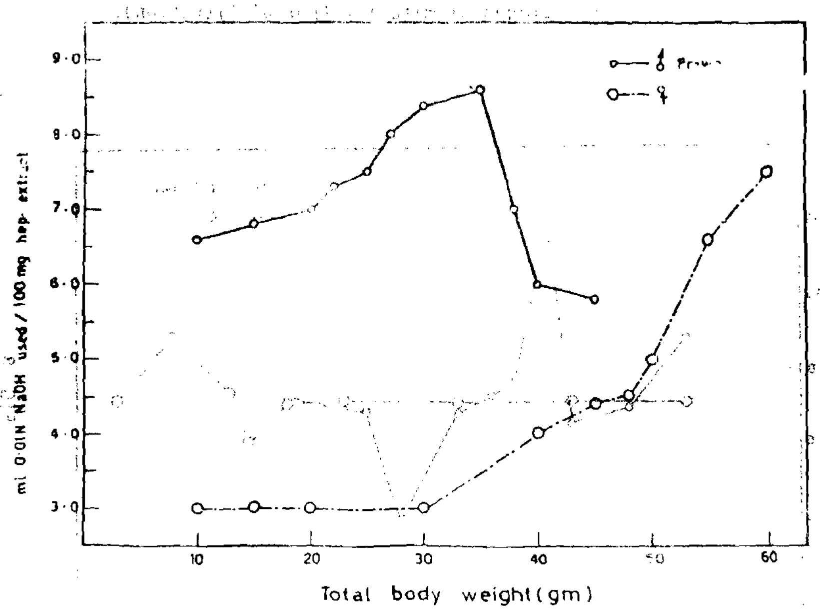

FIG. 10

Changes in relative ml% of free amino acids in hepatopancreas of the two sexes of **Penaeus kera...** at their different stages of maturity (Formal titration).

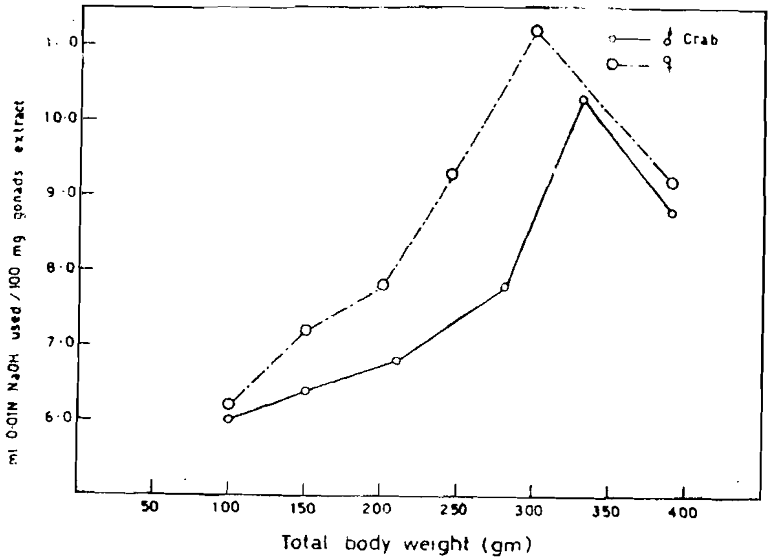

FIG. 11
Changes in relative ml% of free amino acids in gonad of two sexes of *Portunus pelagicus* at their different stages of maturity (Formal titration)

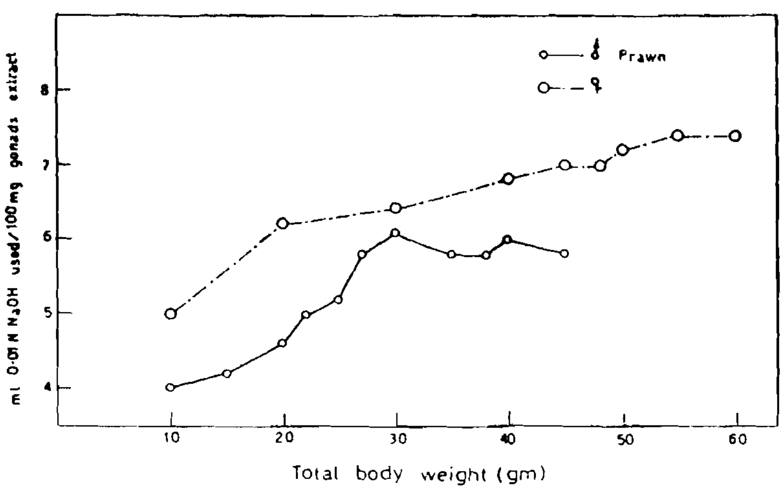

FIG. 12
Changes in relative ml% of free amino acids in gonad of the two sexes of ***Penaeus kerathurus*** at their different stages of maturity (Formal titration).

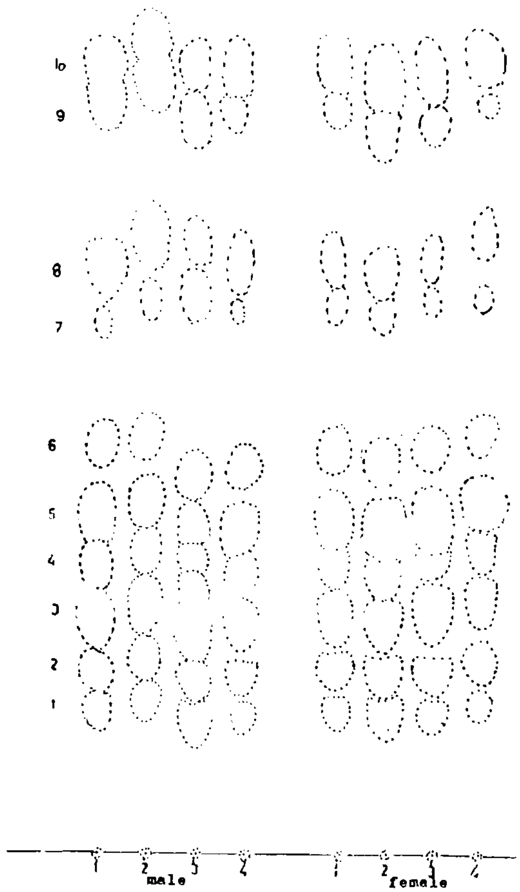

FIG. 13

One dimentional paper chromatographic of free amino acids resulting from muscles homogenate of the two sexes of **Portunus pelagicus** at their 4 stages of maturity.

1- Cystine 2- Lysine 3- Arginine
4- Glycine 5- Alanine 6- Glutamic
7- Prline 8- Tyrosine 9- Tryptophan
10- Isoleucine

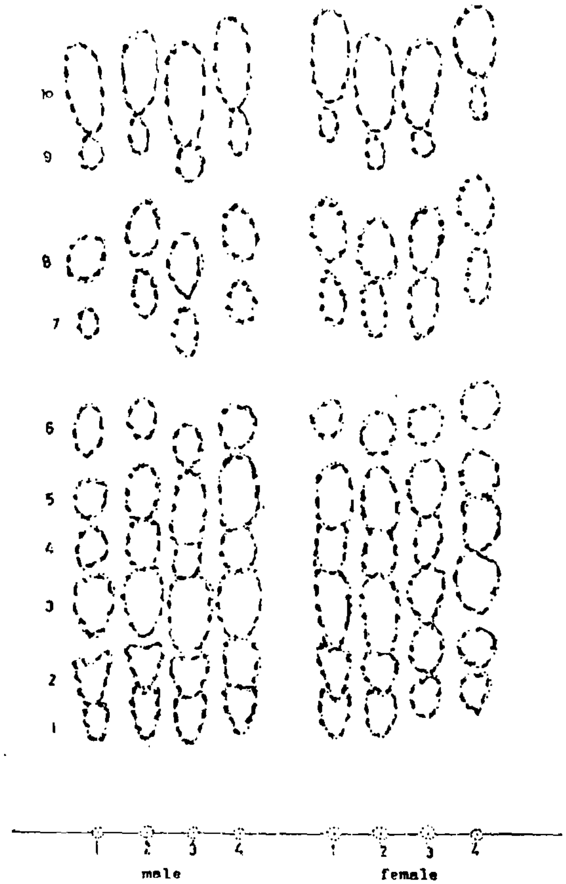

FIG. 14

One dimentional paper chromatographic of free amino acids resulting from muscles homogenate of the two sexes of **Penaeus kerathurus** at their 4 **stages** of maturity.

1- Cystine | 2- Lysine | 3- Agrinine
4- Glycine | 5- Alanine | 6- Glutamic acid.
7- Proline | 8- Tyrosine | 9- Tryptophan
10- Isoleucine.

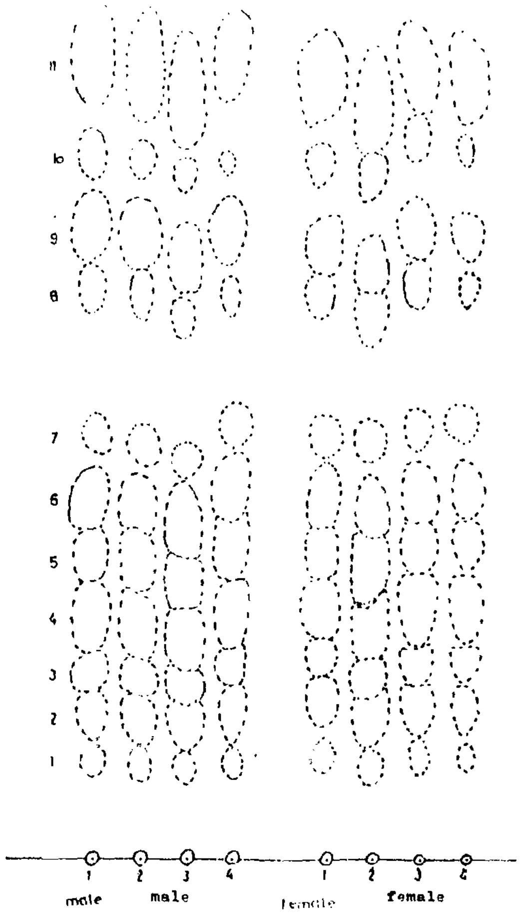

FIG. 15

One dimentional paper chromatographic of free amino acids resulting from hepatopancreas homogenate of the two sexes of **Portunus pelagicus** at their 4 stages of maturity.

1- Cystine 2- Lysine 3- Agginine
4- Glycine 5- Alanine 6- Glutamic acid
7- Proline 8- Tyrosine 9- Tryptophan
10- Aspartic acid 11- Isoleucine.

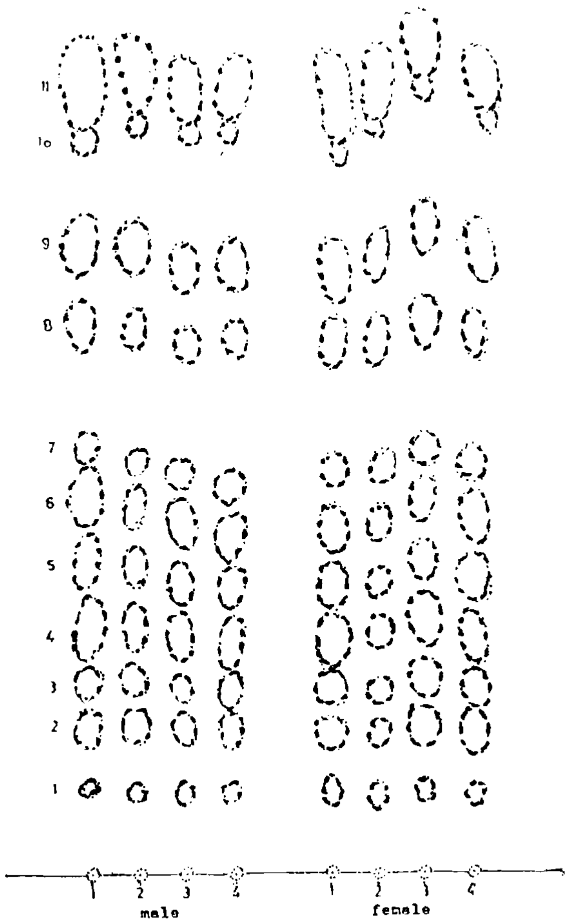

FIG. 16

One dimentional paper chromatographic of free amino acids resulting from hepatopancreas homogenate of the two sexes of **Penaeus kerathurus** at their 4 stages of maturity.

1- Gystine	2- Lysine	3- Arginine
4- Glysine	5- Alanine	6- Glutamic acid
7- Proline	8- Tyrosine	9- Tryptophan
10- Aspartic acid	11- Isoleucine	

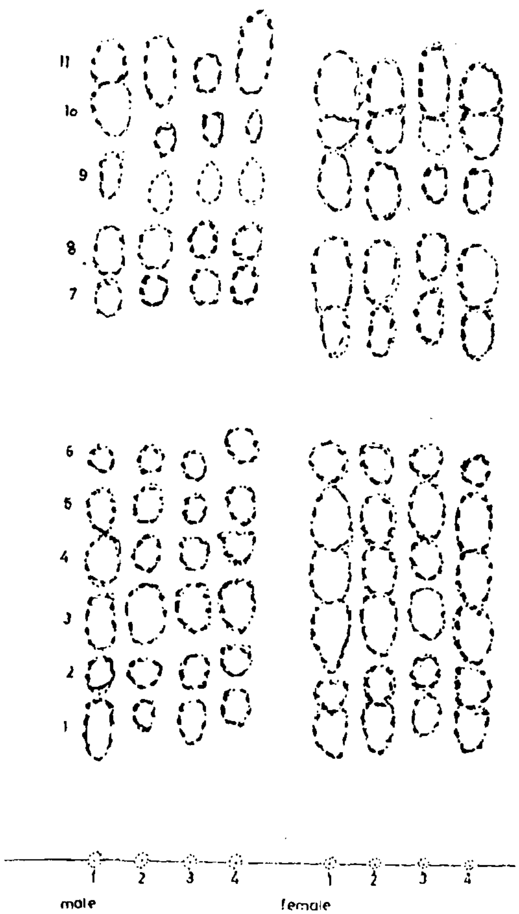

FIG. 17
One dimentional paper chromatographic of free amino acids resulting from gonad
Portunus pelagicus at their 4 stages of maturity.

1- Cystine	2- Lysinee	3- Arginine
4- Glycine	5- Alanine	6- Glutamic acids
7- Proline	8- Tyrosine	9- Tryptophan
10- Aspartic acid	11- Isoleucine	

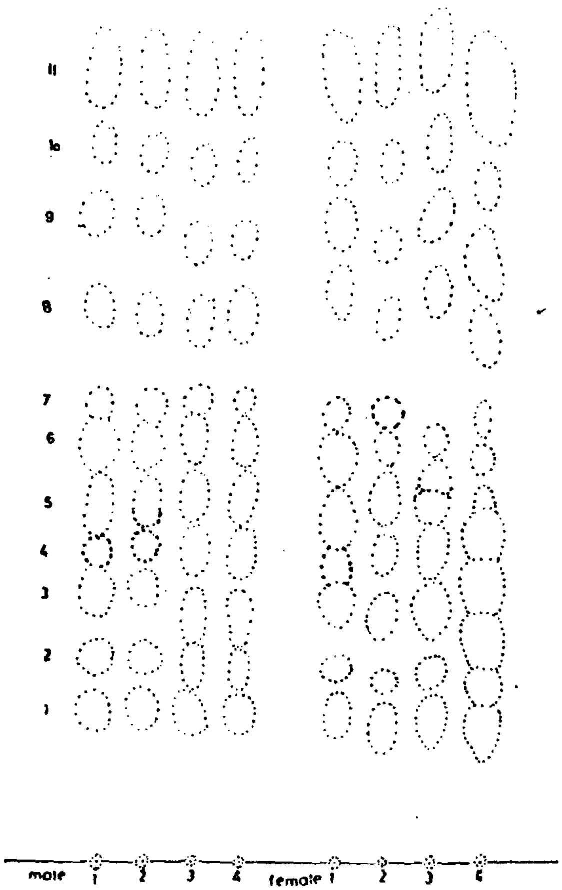

FIG. 18

One dimentional paper chromatographic of free amino acids resulting from gonad *Penaeus kerathurus* at their 4 stages of maturity.

1- Cystine	2- Lysine	3- Arginine
4- Glycine	5- Alanine	6- Glutamic acids
7- Proline	8- Tyrosine	9- Tryptophan
10- Aspartic acid	11- Isoleucine.	

Possibly, the depletion observed in the female hepatopancreatic amino acid in prawns and more so in crabs may be related to its break-down then its drainage into the muscle and gonad to complete the maturation process and supplied proteins to the ovary.

Change in rates of protein metabolism occurring toward the end of the moult cycle may also contribute to change in amino acid concentrations, but since only certain amino acids increase, it seems unlikely that protein incorporation changes play a major role. Mechanisms of hormonal alteration of tissue is to adjust its osmolarity during the intermoult cycles (Adioydi and Adioydi, 1970).

The variations observed may reflect a balance between the synthesis, transport, storage, and degeneration of structural and functional proteins. The morphogenetic and the physiological mechanisms controlling these variations, may also involve probable hormonal agents.

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