EFFECT OF STARVATION ON BIOCHEMICAL CONSTITUENTS OF *METAPENAEUS DOBSONI*

DISSERTATION SUBMITTED BY

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C E R T I F I C A T E

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

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C O N T E N T S

P R E F A C E

Aquatic organisms, including fishes and crustaceans, even in the best of their environments live under extremely dynamic physical and chemical conditions, many of which have no analogue in the terrestrial environment. Although individual fishes or populations may adapt to such conditions, it does not imply that they do not expend energy to cope physiologically with the condition.

Several factors viz. season, moulting, feeding, parasitism, bacterial or viral infections and physiological stress, can change or alter the physiology or metabolism of crustaceans. Among these, one of the most important factors is food available to the animal. Starvation, which is essentially a condition where an animal is deprived of food, can be viewed, firstly as a stressor which evokes a physiological stress response from the animal and secondly, as an effective tool to study the physiology of an animal.

Starvation studies in Crustacea were primarily intended to explain changes that took place in the body composition and metabolic rate of animals during the moult cycle. when they undergo periods of voluntary fasting. At present this method is successfully used to study protein and amino acid

metabolism in different tissues of crustacea. The starvation studies have the added advantage of reducing synthesis to a minimum, so that any changes observed are largely the result of catabolic processess. Reports suggest that the "Soft prawn" disease commonly encountered in prawn farms may be caused by the non availability of protein food in the widely fluctuating environmental conditions. This is particularly possible in extensive culture systems, where no feeding is done. This is one of the major problems in intensive prawn culture also, as heavy mortality is noticed following such conditions.

Studies on biochemical composition of various organs in prawns are particularly important for success of shrimp farming as this would help to identify the dietary requirements of candidate species so as to formulate a suitable feed. Starvation is a condition by means of which the mutritional requirements of animals can be studied.

The shrimp Metapenaeus dobsoni contributes substantially to the penaeid prawn fishery along the south-west coast of India. Besides in the traditional or extensive culture, the most popular system of prawn culture in Kerala, this species is harvested the most.

Although extensive data are available on the life history, migrations, spawning period, larval development growth rate etc., of this species, little is known about its ability to store reserve food material and its physiology. In the present study on M. dobsoni, physiological tests were conducted to show effects of prolonged starvation on the metabolism, whether it is lipid or protein oriented. This information would be useful for a better understanding of the nutritional status of the shrimp under rearing conditions.

I wish to express my deepest sense of gratitude to Dr. P.S.B.R. James, Director, Central Marine Fisheries Research Institute, under whose scholarly guidance this work has been completed. I am grateful to Dr. A.D. Diwan, Scientist S-2, who helped me design the experiment and Dr. N. Sridhar and Dr. Mohan Zachariah for their valuable help. I also wish to thank Shri. A.K.V. Nasser and Shri.Sathianandan who helped me in statistical analysis I thank Shri. A. Nandakumar for his timely help. I am deeply indebted to my class mates, juniors and seniors, especially Shri. V. Baskaran for his valuable help and advice. I express my gratitude to the Indian Council for Agricultural Research for awarding me Junior Research Fellowship during the tenure of which, this work has been carried out.

I N T R O D U C T I O N

Environmental stress in animals has generated a lot of interest and activity among the scientific community, including environmentalists, particularly in view of the rapid changes occurring in the global environment as a result of human activities. Bayne (1975), described stress as a measurable alteration of a physiological steady state which is induced by an environmental change and which renders the individual more vulnerable to further environmental changes. Stress is of common occurence in wild populations as well as in cultured animals. Numerous stress induced physiological events alter the capacity of animals including fishes and crustaceans, to perform various physiological and behavioural operations or functions. Once characterised, these altered functions may become useful indicators of stress like capacity to osmoregulate, mount an immune response, resist diseases, respond physiologically to another stressful situation, swim, avoid predators etc., and therefore it is desirable to assess stress in fishes and prawns without using death as an end point.

Lack of food supply is a type of stress where the animal undergoes starvation. A majority of fishes live through severe depletion of food atleast for a part of every year in their natural environment (Love, 1970), and hence they are known to be adapted for mobilising their body constituents during

starvation. Crustaceans encounter starvation in periods of food scarcity and undergo periods of voluntary fasting during the molt cycle and seasonal dormancy. But energy requirements must still be met during the non - feeding periods and there is evidence to show that nitrogenous substances such as proteins are catabolised to meet the needs of the animal. In crabs, the amount of food available exerts a great influence on the metabolic rate (Wallace, 1973). Starvation in shrimps is also a common feature in Atlantic and Mediterranian French ponds where no food is added in the winter due to marked temperature decrease.

The abilities of different animals differ to withstand such stressful situations. Many decapods can survive for weeks or even months of total starvation (Dall, 1981., Barclay, et al., 1983). Ability to survive starvation periods is influenced by age (Simpson, 1965), season and environmental factors (Steffers, 1985). Food deprivation induces several specific metabolic changes that permit fishes and crustaceans to mobilise their reserve substances like carbohydrates, lipids and proteins sparingly. The degree of starvation the animals can withstand also varies to a great deal from species, but in general lack of food, sooner or later causes a reduction in metabolic rate, slowing of growth, (Darves, 1930; 1931; Brown 1951; Philips and Barclay, 1954) and thus moulting. Growth in some crustaceans is inhibited by starvation (Costlow and Bookhart, 1953, Roberts, 1957, Kannerwortt, 1965).

Many authors have reported the effects of fasting on invertebrate metabolism; Steves (1963) experimented with isopods to study substrate utilization in crustacea, Parvathy (1973) and Marsden et al., (1973) for changes in the levels of metabolic reserves in the tissues, and Schafer (1968) analysed storage materials of Penaeus duorarum. Cuzon and Ceccaldi (1971, 1973) showed the effect of inanition on serum proteins of Penaeus kerathurus and utilization of storage materials for Crangon crangon. Mayzaud (1976) studied metabolic and biochemical variations in Zooplankton during starvation, and the role of blood in crustacean metabolism described by Williams & Lutz (1975). Surendranath et al., (1987) studied alterations in oxygen consumption and organic constituents in starved Penaeus indicus.

Under conditions of total starvation several metabolic changes or a combination of them are possible. The available lipid may be conserved by a greatly reduced metabolic rate; lipid may be metabolised first, followed by protein; or the lipid may be conserved for other purposes and the protein metabolised instead, as occurs during moulting. The reports on this topic is conflicting and the interpretation is difficult as some of the authors have analysed the total animal while others have considered only individual systems or organs in an

animal like the digestive gland etc. While the digestive gland or hepatopancreas has long been regarded as the principal storage organ in decapod crustaceans, Dall (1981) suggested that the large muscle mass of the macrurid decapods could also be an important energy source.

Proteins are ubiquitous components of all living tissues which serve indispensable functions in cellular architecture and are intimately concerned with virtually all physiological events (Mahler and Cordes, 1968). Therefore any change in the physiology of the organism as a result of adverse ecological conditions is bound to affect the protein content of the tissues qualitatively as well as quantitatively (Rajamani, 1982). Neiland and Scheer (1953) opined that Hemigrapsus uses protein rather than carbohydrate and fat as primary energy source during starvation.

However Armitage et al. (1972) concluded that lipid was the major energy reserve in Oreonectes nair, while Heath and Barnes (1970) found that there was a major decrease in fat in the digestive gland of Carcinus maenas after starvation. Others have confirmed that proteins are the major energy store in various Decapods (Neiland and Scheer, 1953). Schafer (1968) found that starved Penaeus duorarum metabolised mainly fat

reserves and protein. Speak and Urich (1969) observed that lipids were used mainly during the first stage of starvation, in Orconectes limosus while proteins were used increasingly in later stages. Regnault (1981) showed that lipids and proteins were the main sustrates oxidised for energy requirements. However Barclay et al. (1986) found that protein and not lipid is the major source of energy used by Penaeus duorarum during Starvation.

The question of whether the primary, readily mobilised energy source of crustacea is glycogen as in vertebrates, or some other sustances, is not yet satisfactorily answered. Carbohydrate appears to be a minor energy reserve in decapod crustaceans. Jungreis (1968) found no significant metabolism of glycogen during starvation in Orconectes virilis. Armitage et al., (1972) found consistently low concentration of carbohydrate in Orconectes nair. Regnault (1981), found that carbohydrate reserves were quickly exhausted during starvation. Heath and Barnes (1970) showed that five times as much lipid as carbohydrate was used during starvation in Carcinus maenas. Schafer (1968), Neiland and Scheer (1953), found that fasting crabs use proteins or lipids in preference to glycogen. Surendranath et al. (1987) suggested that during early period of starvation prawns utilize

more of carbohydrates than lipids as carbohydrates are the chief immediate concern of energy supply under stress condition. A decrease in glycogen was noticed for fasting Uca (Dean and Vernberg, 1965) and a decrease in carbohydrates for fasting Carcinus (Florkin, 1936) and Limnoria (George, 1966). Barnes et al. (1963) studied a fasting Balanus and found that carbohydrates are used first and noted that the frequency in moulting was proportionate to carbohydrate reserves. Presumably large amounts of carbohydrates are used for the production of chitin (Renaud, 1949) in the well nourished animal, but protein and lipid can provide the necessary glucose units in a starved animal.

Very few studies have been made so far on the changes in total free amino acids in prawns during starvation. Dall and Smith (1987) studied changes in protein, bound and free amino acids in the muscle of tiger prawn Penaeus esculentus during starvation to assess the catabolism of amino acids for energy production.

There is now unifying evidence that many crustaceans use cholesterol for their normal growth and survival (Castell et al, 1975) but individually cannot synthesise the molecule from simple precursors like acetate or mevalonate (Zandee, 1967; Whitney, 1969; Teshima and Kanazawa, 1971). Krishnamurthy et al. (1982) showed that cholesterol content in blood and abdominal muscle of Penaeus aztecus did not alter on a unit

wet weight basis during starvation. However considerable decrease was noted during starvation in the hepatopancreas, showing that starvation mobilises the cholesterol levels of the hepatopancreas.

Along with the decrease in energy reserves during starvation a decrease in metabolic rate or oxygen consumption rate has been reported. Marsden et al. (1973), Newell and Bayne (1973), Wallace (1973), and Klein Breteler (1975) observed a decrease in oxygen consumption by Carcinus maenas with starvation. Ansell (1973) found that starvation of Cancer pagurus causes a decrease in daily oxygen consumption by increasing the time spent in the resting phase. Regnault (1981) showed a decrease in oxygen consumption during starvation in shrimp Crangon crangon. Surendranath et al. (1987) also showed a decrease in total and unit metabolism in Penaeus indicus with starvation.

Crustaceans, even the terrestrial forms, are ammonotelic animals, i.e. they excrete their nitrogenous waste products in the form of ammonia. The main source of this ammonia must be amino acids derived from proteins. The effect of starvation on ammonia excretion has been studied in various species. Prolonged starvation evoked three different patterns of excretion rate. Corner and Newell (1967) and Mayzaud (1976) found that after an

initial decrease the ammonia excretion rate remained constant in copepods. Nival et al. (1974) found steadily decreasing excretion rate of ammonia as starvation was prolonged in the copepod Tenora stylifera, while a high excretion rate than in fed animals was found after a temporary decrease in two decapods, Carcinus maenas (Needham 1957) and Crangon crangon (Regnault 1981).

Hepatopancreas is a vital organ in crustacea, involved in diverse metabolic activities, being primarily responsible for the synthesis and secretion of digestive enzymes and subsequent uptake of nutrient materials, and is also implicated in excretion, the moulting cycle and the storage of organic reserves, lipid and carbohydrate metabolism. Hepatopancreas or the midgut gland is highly sensitive to changes in the environmental conditions. The architecture of the organ is often severely altered before significant changes in the behaviour of prawns is observed. The bulk of the decapod hepatopancreas comprises long slender blind ending tubules, lined by an epithelium which apart from the distal closed end is only a single layer thick (Stanier et al. 1980). Four basic cell types can be recognised in the epithelium viz., E (Embryonic or undifferentiated), F (fibrillar), R (resorptive or absorptive), and B (secretory) cells, which show a differential distribution along the length of the tubules (Davis

and Burnett, 1964; Stanier et al., 1968; Barker and Gibson, 1977, 1978). Morphological studies in a number of animal groups have related changes caused by starvation with an alterration of the function in organs such as liver, kidney or pancreas, (Steeves, 1963). However, information on the variability of the decapod hepatopancreas due to exogenous factors is scarce. Vogt et al.(1985), have established that the R cells of the decapod midgut gland react most sensitively to variations of the feed composition. Therefore they can serve as monitor cells in nutrition research.

A variety of biochemical parameters have been proposed to evaluxate the trauma/stress experienced by animals owing to extraneous factors. Changes, in the hepatopancreas after starvation is important since the hepatopancreas could be a potential indicator organ for early starvation (Papathanassiou and King, 1984). Several studies indicate that marked species differences exist in the magnitude and duration of biochemical stress responses to various stressors (Wedemeyer, 1976).

Metapenaeus dobsoni is an important contributor to fishery of penaeid prawns in India. In extensive or traditional prawn farms in Kerala, which form a major portion of the brackish water area under culture, a major portion of the landings is contributed by this species. Therefore an attempt, to examine

effects of starvation on various aspects pf this species, is made in the present study, with a view to contribute to the knowledge concerning biochemical adaptation to food deprivation in Metapenaeus dobsoni; to define storage materials used by prawns ; to isolate effects of starvation from those of moulting; to discover which parts of the body are used for storage and to determine if metabolic rate is affected by starvation.

PLATE I.

Fig. 1. *Metapenaeus dobsoni*.

Fig. 2. Experimental set up.

PLATE I

Fig. 1.

Fig. 2.

MATERIALS AND METHODS

Live shrimp (Metapenaeus dobsoni), 40-65 mm in size were obtained from the wild near Puduvyepu Light house, Cochin. They were caught using a cast net and normal healthy animals were transported to the laboratory. These animals were acclimatised to lab conditions in large 2T fibre glass thanks having filtered and well aerated seawater of 15 ppt salinity for two weeks prior to the experiment. As the nutritional status of the animals in the wild were not known, they were fed with boiled clam meat during the acclimatisation period. Prawns in the inter moult stage were used for the experiment.

The experiments were carried out in three phases. In the first phase two size groups of Metapenaeus dobsoni, 40-50 mm and 55-65 mm were subjected to starvation to study the survival rate of the different size groups. Ten prawns of each size group were stocked in separate tubs of 40 litre capacity. Three replicates were made and mortality was recorded. Dead prawns were immediately removed from the experimental tubs. The experiment was continued till 50% of the animals from each size groups were dead.

In the second phase 80 prawns were reared in 8 separate tubs of 40 litre capacity with 10 prawns in each tub. They were starved for a period of 21 days, after which feeding

was resumed for one week. Refeeding was carried out for a single week only to find if the prawns would recover. Ten prawns each from the experimental and control tubs were sacrificed every week for biochemical and histological studies. The third phase of the experiment was designed to study the weight loss, change in moisture content and Oxygen consumption rate when prawns were subjected to starvation. Control animals also of the intermoult stage were maintained separately and fed on boiled clam meat. One third water in all the tubs was replaced with fresh filtered water, every second day and completely changed once in a week. Continuous aeration was provided throughout the experimental period and the temperature ranged between 26 and 30 degree celsius.

BIOCHEMICAL ANALYSIS:

Total carbohydrates, glycogen, proteins, free amino acids, lipids and cholesterol were obtained for their quantitative determination in muscle, hepatopancreas and haemolymph.

Collection of haemolymph and tissue

Haemolymph samples were collected in small glass vials using a 1 ml hypodermic glass syringe, fitted with a No. 22 needle, from the pericardial cavity of prawns. A 3.8%

solution of Trisodium citrate was used effictively as an anticoagulant. The animals were then immediately dissected out. The fresh body muscle hepatopancreas and gut were isolated and stored separately along with the haemolymph in the deep freezer till analysis was done.

Total carbohydrates were determined by the Phenol-sulphuric acid method given by Dubois et al. (1956), while Glycogen was estimated by the Anthrone method as given by Caroll et al. (1956). D-glucose was used to prepare the standard.

Total protein was estimated by the Folin-Ciocalteu method of Lowry et al. (1957). Bovine serum albumen was used to prepare the standard graph.

The quantitative estimation of total free amino acids was carried out using the method Yemm and Cocking (1955) Glycine and glutamic acid were the amino acids used as standards.

Lipids were determined by the Sulpho-phosphovanillin method of Barnes and Blackstock (1973). Total cholesterol was determined by the Henley's method as given by Varley (1957) using Ferric chloride, Glacial acetic acid and conc. $H_2 SO_4$. Extra pure cholesterol was used as standard for both the above procedures.

Histological studies

Histological studies on normal and experiemental prawns (Starved 3 weeks) were made on the hepatopancreas. The prawns were dissected when alive and their hepatopancreas was immediately transfered to the Bouin's fluid.

After 48 hours, the tissues were removed from the fixative, washed thouroughly in running tap water for atleast 4 hours, and then dehydrated through a series of alcohol grades starting from 70% to absolute alcohol. The tissues were kept in each grade for atleast an hour before passing on to the next grade. They were then cleared, first in alcohol-xylene mixture (1:1), followed by two changes in pure xylene. Before embedding in paraffin was, a one hour impregnation in molten wax (50 - 60^{o}C) was given twice.

The sections were cut at 4-6 u thickness using a Rotary microtome. Deparaffining was done in xylene. The tissues were then hydrated through a series of alcohol grades from 100% to 30%, then to water and stained using Harri's alum Hematoxylin and counterstained using 1% alcoholic eosin. The stained sections were dehydrated,

cleared and mounted in D/P/X. Photomicrographs were taken using "American Optical Research Microscope".

Oxygen Consumption rate

The oxygen consumption rate was measured using a crude resppirometer and the water samples were analysed of oxygen content by the Winkler's method.

Moisture content

Moisture content in the animals were determined by drying the weighed animals at about 60°C in a hot air oven for about 24 hours or until constant weights were obtained. The tissues were subsequently dessicated over silica gel and reweighed. The difference between the weights gave the amount of moisture content in the tissues.

Statistical analyses

Stastical analysis was done to find out any significant differences in experimental results. Initially mean and standard deviation of the data were calculated. 2 way ANOVA (Analysis of Variance) and students 't' test was performed to test significance between treatments i.e., different periods of starvation and control.

R E S U L T S

BIOCHEMICAL ANALYSIS:

MUSCLE TISSUE: The results of the biochemical analysis carried out in muscle tissue of starved Metapenaeus dobsoni is presented in Table 1.

Total Carbohydrates: A gradual decline in the level of total carbohydrates was observed in the muscle tissue of starved prawn. The values decreased from 1.095 ± 0.19 at the start of the experiment to 0.6638 ± 0.04, 0.5114 ± 0.04 and 0.3148 ± 0.32 mg/100 mg at the end of the first, second and third weeks respectively. After one week of refeeding the carbohydrate level returned back to 0.5440 ± 0.08 mg/100 mg. In the control, i.e., fed prawns, the values ranged between 0.8980 mg/100 mg and 2.1960 mg/100 mg (Fig. 1).

Statistical analysis was carried out using ANOVA which showed significant difference in total carbohydrate content between fed and starved prawns at 5% level (Table. 6a).

Glycogen: A significant decrease in the amount of glycogen was observed in the muscle tissue during starvation. It rapidly fell from 0.5540 ± 0.06 mg/100 mg in the initial experiment to 0.348 ± 0.04, 0.1592 ± 0.05 and 0.0373 ± 0.04

mg/100mg at the end of the first, second and third weeks respectively. It rose back to 0.2112 ± 0.08 mg/100mg after one week of refeeding. In the control, the amount of glycogen ranged between 0.504 and 1.23 mg/100mg (Fig. 2).

ANOVA showed that the difference in glycogen content between starved and fed prawns were statistically significant at 1% level (Table. 6b).

Protein: Protein levels declined from 22.5758 ± 3.64 mg/100mg during the first experiment to 13.074 ± 3.37, 8.974 ± 1.25 and 6.462 ± 1.04 mg/100 mg at the end of the first, second and third weeks of starvation respectively. On refeeding for one week, it rose to 13.5979 ± 3.23 mg/100 mg. In the control, the values ranged between 18.45 mg/100 mg and 41.419 mg/100 mg (Fig. 3).

ANOVA revealed that, the difference in protein content between starved and fed prawns were stastistically significant at 5% level (Table. 6c).

Free amino acids: The levels of free amino acids showed a fluctuating trend in starved prawns which fell from 0.3322 ± 0.06 mg/100 mg at the beginning of the experiment to 0.2366 ± 0.03 mg/100 mg at the end of the first week, rose to 0.2955 ±

0.02 mg/100 mg and 0.4318 ± 0.03 mg/100 mg at the end of the second and third weeks respectively. The values fell to 0.2980 ± 0.03 mg/100 mg on refeeding. In the controls, the values ranged between 0.2054 mg/100 mg and 0.4126 mg/100 mg (Fig. 4).

ANOVA revealed no statistical significance in total free amino acid content between starved and fed prawns. (Table 6d).

Total lipids: A gradual decline in the level of total lipids in the muscle tissue was observed in starved prawns. The lipid content declined from 2.9262 ± 0.16 mg/100 mg at the start of the experiment to 2.0904 ± 0.09, 1.6878 ± 0.13 and 1.0486 ± 0.12 mg/100 mg at the end of the first, second and third weeks of starvation respectively. It reincreased to 1.8832 ± 0.48 mg/100 mg after one week of refeeding. In the controls, the values ranged between 2.575 and 6.897 mg/100 mg (Fig. 5).

ANOVA revealed that the difference in lipid content between starved and fed prawns were statistically significant at 5% level (Table. 6e).

Cholesterol: The level of cholesterol registered a more or less steady decline. It fell from 0.2286 ± 0.62 mg/100 mg

at the zero day of the experiment to 0.1332 ± 0.62, 0.1264 ± 0.02 and 0.1142 ± 0.02 mg/100 mg at the end of the first, second and third weeks of starvation. It increased to 0.1722 ± 0.02 mg/ 100 mg after refeeding for one week. In the controls, the values ranged between 0.1854 and 0.3478 mg/ 100 mg (Fig. 6).

A statistical significance at 5% level was observed in the Cholesterol content between fed and starved prawns by ANOVA (Table . 6 f)

Table 1. Variations in the biochemical constituents in Muscle tissue of Metapenaeus dobsoni, with starvation of three weeks duration and refeeding of one week.

MUSCLE TISSUE	DAY 0		STARVATION I WEEK	STARVATION II WEEK	STARVATION III WEEK	REFEEDING I WEEK
TOTAL CARBOHYDRATES (mg/100mg)	1.095 ± 0.1910	EXP.	0.6638 ± 0.0425	0.5114 ± 0.0360	0.3148 ± 0.0120	0.5440 ± 0.0816
		CON.	1.6750 ± 0.5135	1.7410 ± 0.5020	1.4633 ± 0.3480	1.5865 ± 0.4193
GLYCOGEN (mg/100mg)	0.5540 ± 0.0644	EXP.	0.348 ± 0.0424	0.1592 ± 0.0521	0.0373 ± 0.0343	0.2112 ± 0.0812
		CON.	0.7796 ± 0.1023	0.8892 ± 0.2277	0.873 ± 0.2975	0.7842 ± 0.2107
PROTEIN (mg/100mg)	22.5758 ±3.6490	EXP.	13.074 ± 3.3710	8.9740 ± 1.245	6.4620 ± 1.0455	13.5975 ± 3.2308
		CON.	32.4774±6.8009	24.5470 ± 4.375	25.8828 ± 6.8315	28.1788 ± 7.4649
FREE AMINO ACIDS (mg/100mg)	0.3322 ±0.062	EXP.	0.2366 ± 0.0340	0.2955 ± 0.0237	0.4318 ± 0.0348	0.2980 ± 0.0299
		CON.	0.3460 ± 0.0236	0.3452 ± 0.0908	0.3348 ± 0.0246	0.3322 ± 0.0621
TOTAL LIPIDS (mg/100mg)	2.9262 ± 0.1645	EXP.	2.0904 ± 0.0994	1.6878 ± 0.1326	1.0486 ± 0.1225	1.9832 ± 0.4870
		CON.	4.1700 ± 0.0994	1.6878 ± 0.1326	1.0486 ± 0.1225	1.9832 ± 1.6591
CHOLESTEROL (mg/100mg)	0.2286 ± 0.0172	EXP.	0.1332 ± 0.0229	0.1264 ± 0.0183	0.1142 ± 0.0195	0.1722 ± 0.0235
		CON.	0.2433 ± 0.0448	0.2835 ± 0.0587	0.2230 ± 0.0295	0.2407 ± 0.0504

All values are $\bar{X} \pm SD$ of five determinations.

Fig 1. Total carbohydrate levels (mg/100mg) of muscle of fed and starved shrimps versus time.

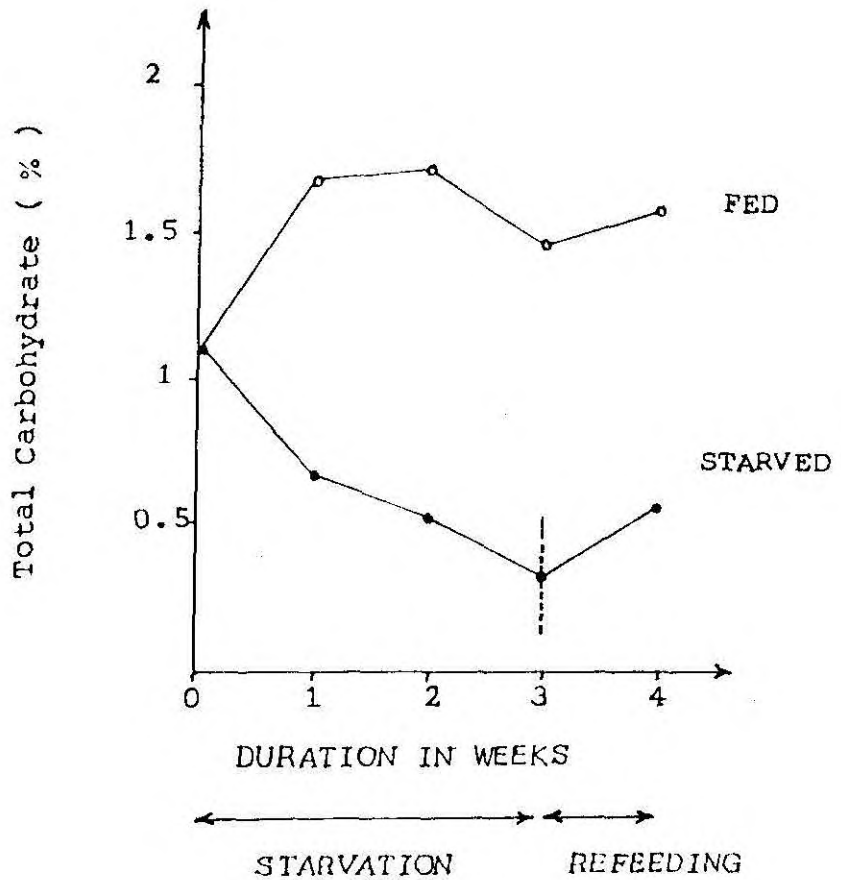


Fig.2. Glycogen levels (mg/100mg) of muscle of fed and starved shrimps versus time.

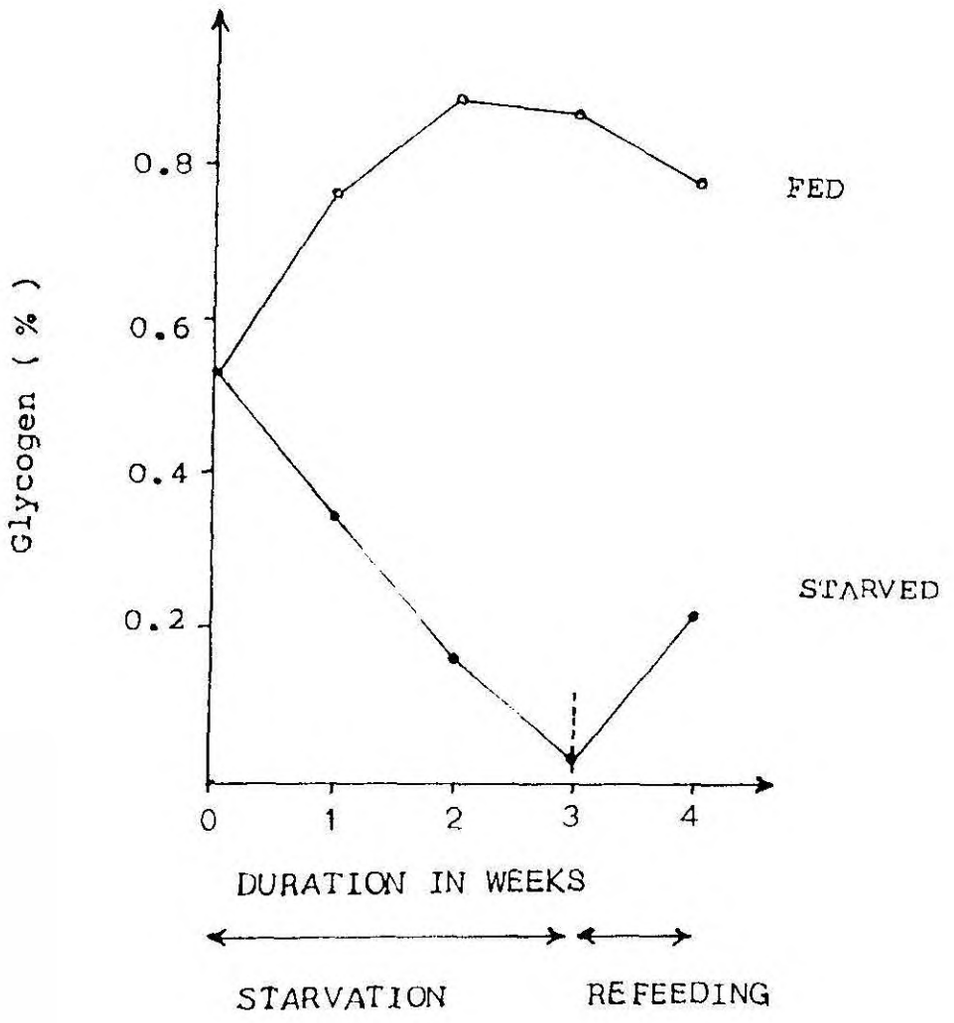


Fig.3. Protein levels (mg/100mg) of muscle of fed and starved shrimps versus time.

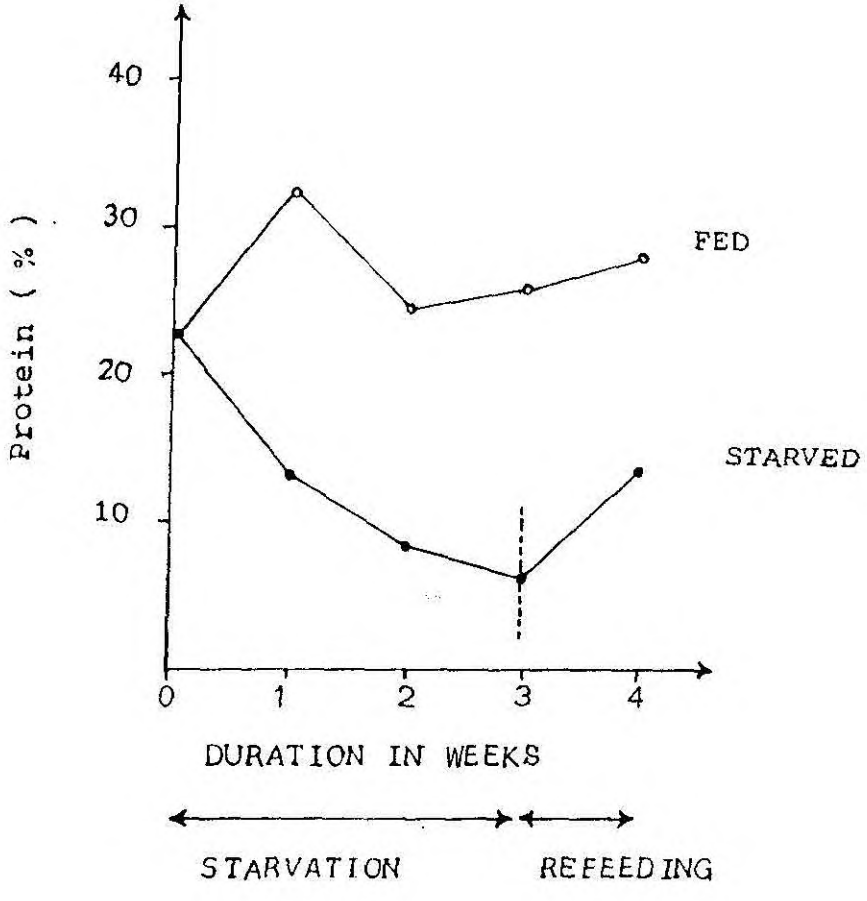


Fig. 4. Total Free Amino acid levels (mg/100mg) of muscle of fed and starved shrimps versus time.

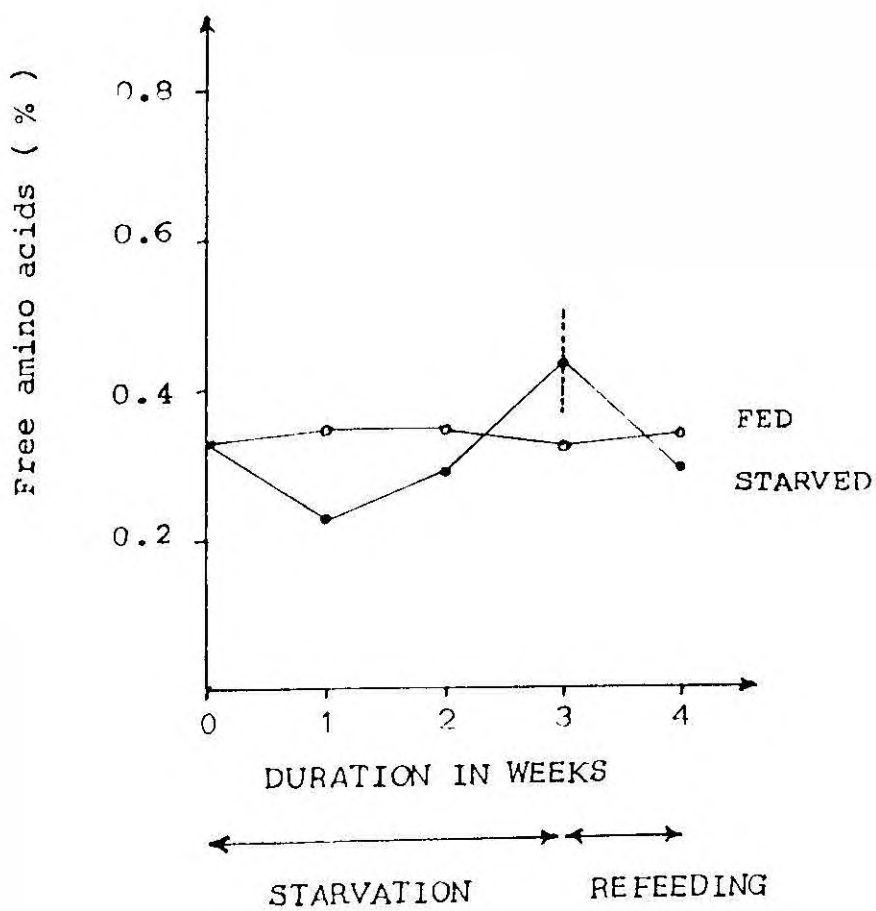


Fig. 5. Lipid levels (mg/100mg) of muscle of fed and starved shrimps versus time.

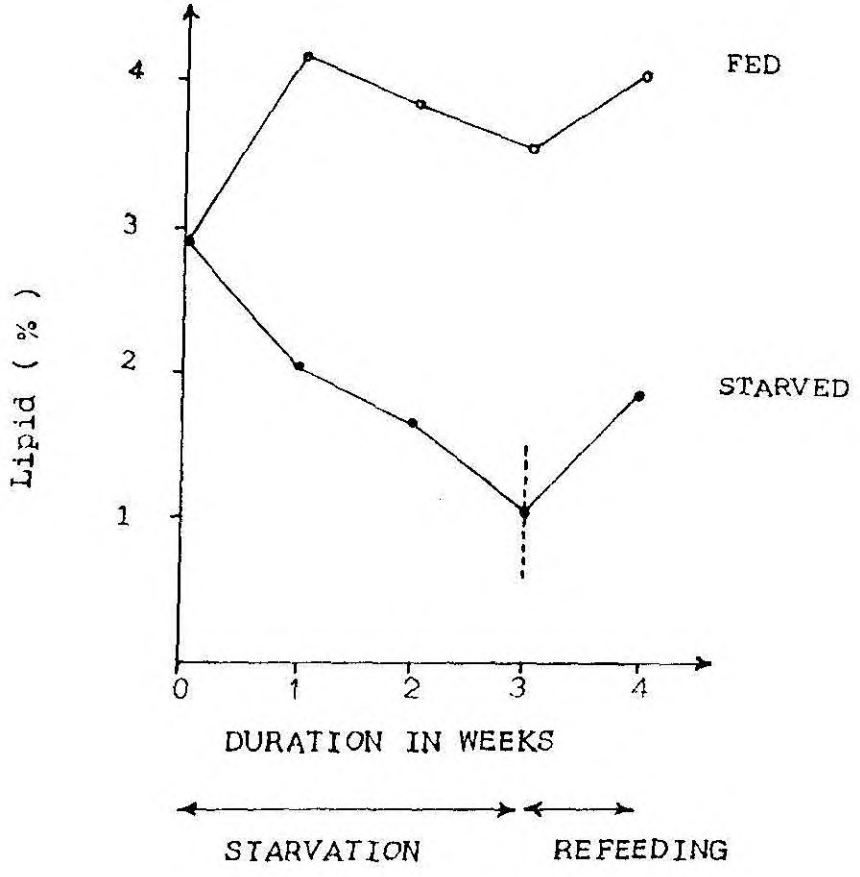
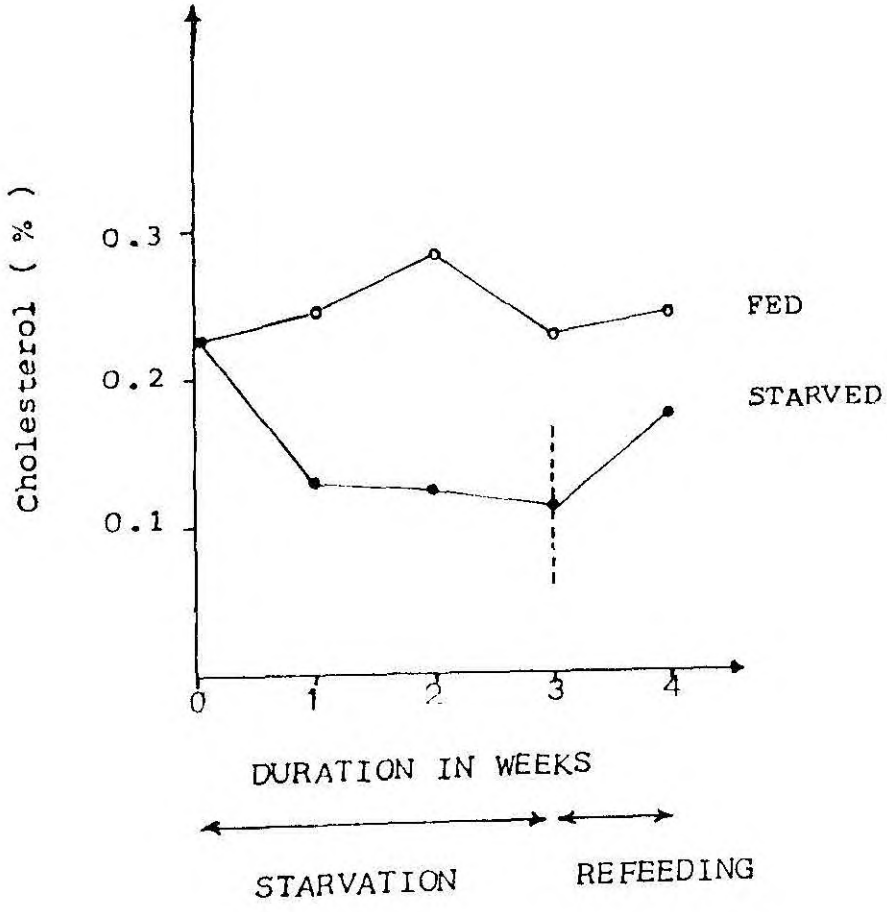


Fig.6 Cholesterol levels (mg/100mg) of muscle of fed and starved shrimps versus time.



STATISTICAL ANALYSIS

ANOVA TABLES

Table. 6 a : Carbohydrate muscle

SOURCE	D.F.	SUM.SQR	MEAN.SQR	F-VAL	REMARKS
TREAT	1	1.964	1.964	15.52	SIG (5%)
REPLIC	4	0.093	0.023	0.18	N.S
ERROR	4	0.506	0.127		

Table 6 b: Glycogen muscle

SOURCE	D.F.	SUM.SQR	MEAN.SQR	F-VAL	REMARKS
TREAT	1	0.671	0.671	12.73	HI.SIG(1%)
REPLIC	4	0.018	0.004	0.08	N.S
ERROR	4	0.211	0.053		

STATISTICAL ANALYSIS

ANOVA TABLES

Table 6 c: Protein muscle

SOURCE	D.F.	SUM.SQR	MEAN.SQR	F_VAL	REMARKS
TREAT	1	475.798	475.798	14.80	BIG (5%)
REPLIC	4	80.273	20.068	0.62	N.S
ERROR	4	128.592	32.148		

Table 6 d : Aminoacid muscle

SOURCE	D.F	SUM.SQR	MEAN.SQR	F_VAL	REMARKS
TREAT	1	0.001	0.001	0.32	N.S
REPLIC	4	0.009	0.002	0.80	N.S
ERROR	4	0.012	0.003		

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Table 6 e: Lipid muscle

SOURCE	D.F.	SUM.SQR	MEAN.SQR	F_VAL	REMARKS
TREAT	1	7.906	7.906	15.50	SIG (5%)
REPLIC	4	0.831	0.208	0.41	N.S
ERROR	4	2.040	0.510		

Table 6 f: Cholesterol muscle

SOURCE	D.F.	SUM.SQR	MEAN.SQR	F_VAL	REMARKS
TREAT	1	0.020	0.020	11.48	SIG (5%)
REPLIC	4	0.004	0.001	0.58	N.S
ERROR	4	0.007	0.002		

HEPATOPANCREAS: The results of the biochemical analysis carried out in hepatopancreas of starved Metapenaeus dobsoni is presented in table 2.

Total carbohydrates: The amount of total carbohydrates in the hepatopancreas fell from 2.8006 ± 0.02 mg/100 mg at the start of the experiment to 1.6276 ± 0.2487 mg/100 mg at the end of the first week, then increased steadily to 1.7704 ± 0.03, 2.067 ± 0.09 and 2.5120 ± 0.18 at the end of the second and third week of starvation and one week of refeeding respectively. In the normal fed prawns, the values ranged between 2.4860 and 3.84 mg/100 mg (Fig. 7).

ANOVA revealed that the difference in total carbohydrates between starved and fed prawns were statistically significant at 5% level (Table. 7a).

Glycogen: A steady decline in the level of glycogen was observed in the hepatopancreas of starved prawns. It fell from 1.3832 ± 0.24 mg/100 mg at the beginning of the experiment to 0.9486 ± 0.12, and 0.1432 ± 0.05 mg/100 mg at the end of the first, second and third week of starvation respectively. The glycogen content rose to 0.6195 ± 0.17 mg/100 mg after 1 week of refeeding. In the controls, the values ranged from 1.054 and 3.015 mg/100 mg (Fig. 8).

ANOVA showed that the difference in glycogen between starved and fed prawns were statistically significant at 5% level (Table. 7b).

Protein: Protein levels in the hepatopancreas of starved prawns showed a gradual reduction with time. The value of 10.3236 ± 1.02 mg/100mg at the zero day of the experiment decreased to 6.996 ± 0.68 mg/100 mg at the end of the first week, 4.75 ± 0.81 mg/100 mg at the end of the second week and 2.149 ± 0.20 mg/100 mg at the end of the third week. It recovered slowly to 4.4104 ± 0.45 mg/100 mg on refeeding for a week. The values in the control animals ranged between 8.45 mg/100 mg and 14.666 mg/100 mg (Fig. 9).

The difference in protein content in hepatopancreas between fed and starved prawns were statistically significant at 5% level by ANOVA (Table 7c).

Free amino acids: A rapid increase in the levels of free amino acids was observed in the hepatopancreas of starved prawns. The FAA content increased from 0.1462 ± 0.02 mg/100 mg at the beginning of the experiment to 0.2595 ± 0.02, 0.3322 ± 0.03, and 0.4047 ± 0.04 mg/100 mg at the end of the first, second and third week respectively. It fell to 0.2551 ± 0.06 mg/100 mg after one week of refeeding. The content in the control prawns ranged between 0.1135 mg/100 mg and 0.2167 mg/100 mg (Fig. 10).

ANOVA revealed that the difference in free amino acid levels between starved and fed prawns were statistically significant at 5% level (Table. 7d).

Total Lipids: The amount of total lipids in the hepatopancreas of starved prawns showed a rapid decrease. It came down from 12.7270 ± 1.65 mg/100 mg at the initial day of the experiment to 8.5824 ± 1.36, 4.8568 ± 0.61 & 1.9654 ± 0.27 at the end of the first, second and third weeks of starvation. Refeeding made a rapid increase in lipid level to 7.1015 ± 2.00 mg/100 mg within a week. The values in the controls ranged between 9.96 mg/100 mg and 20.44 mg/100 mg (Fig. 11).

ANOVA showed that the difference in lipid levels, were statistically significant between fed and starved prawns at 5% level (Table. 7e).

Cholesterol: Cholesterol level fell initially and then stabilized to a constant level during starvation. It declined from 0.3144 ± 0.07 mg/100 mg at the start of the experiment to 0.2759 ± 0.02 and 0.2029 ± 0.03 mg/100 mg at the end of the first and second weeks respectively. It was steadied at 0.2101 ± 0.02 mg/100 mg at the end of the third week before recovering to 0.2819 ± 0.01 after a weeks refeeding. The values in the

controls ranged between 0.2040 mg/100 mg and 0.4317 mg/100 mg (Fig. 12).

ANOVA revealed that the difference in cholesterol levels between starved and fed shrimps were statistically significant at 5% level (Table. 7f).

Table 2. Variations in the biochemical constituents in Hepatopancreas of Metapenaeus dobsoni with starvation of three weeks duration and refeeding of one week.

HEPATOPANCREAS	DAY 0		STARVATION I WEEK	STARVATION II WEEK	STARVATION III WEEK	REFEEDING I WEEK
TOTAL CARBOHYDRATES (mg/100mg)	2.8006 ± 0.2487	EXP.	1.6276 ± 0.0363	1.7704 ± 0.0280	2.0067 ± 0.0895	2.5120 ± 0.1769
		CON.	3.1113 ± 0.4918	3.3076 ± 0.2502	3.1028 ± 0.3126	3.1380 ± 0.3810
GLYCOGEN (mg/100mg)	1.3832 ± 0.2403	EXP.	0.9486 ± 0.1952	0.3848 ± 0.1244	0.1432 ± 0.0526	0.6195 ± 0.1654
		CON.	2.0760 ± 0.3720	1.7702 ± 0.6626	1.9306 ± 0.6112	2.0910 ± 0.9223
PROTEIN (mg/100mg)	10.3236 ± 1.019	EXP.	6.9960 ± 0.6807	4.7500 ± 0.8070	2.1490 ± 0.2010	4.4104 ± 0.4501
		CON.	10.8396 ± 2.6336	10.3668 ± 1.1208	10.0740 ± 0.9984	10.4256 ± 1.9497
FREE AMINO ACIDS (mg/100mg)	0.1462 ± 0.0180	EXP.	0.2595 ± 0.0238	0.3322 ± 0.0340	0.4047 ± 0.0357	0.2551 ± 0.0553
		CON.	0.1712 ± 0.0396	0.1818 ± 0.0311	0.1409 ± 0.0990	0.1407 ± 0.0238
TOTAL LIPIDS (mg/100mg)	12.7276 ± 1.5508	EXP.	8.3824 ± 1.3670	4.8568 ± 0.6054	1.9654 ± 0.2739	7.1015 ± 2.0028
		CON.	14.8230 ± 1.6034	13.1416 ± 1.6945	15.6702 ± 4.4940	14.3666 ± 3.4700
CHOLESTEROL (mg/100mg)	0.3144 ± 0.0734	EXP.	0.2759 ± 0.0175	0.2029 ± 0.0253	0.2101 ± 0.0168	0.2819 ± 0.0124
		CON.	0.3526 ± 0.0476	0.3632 ± 0.0517	0.3565 ± 0.0369	0.3445 ± 0.0538

All values are $\bar{X} \pm SD$ of five determinations.

Fig. 7. Total Carbohydrate levels (mg/100mg) of hepatopancreas of fed and starved shrimps versus time.

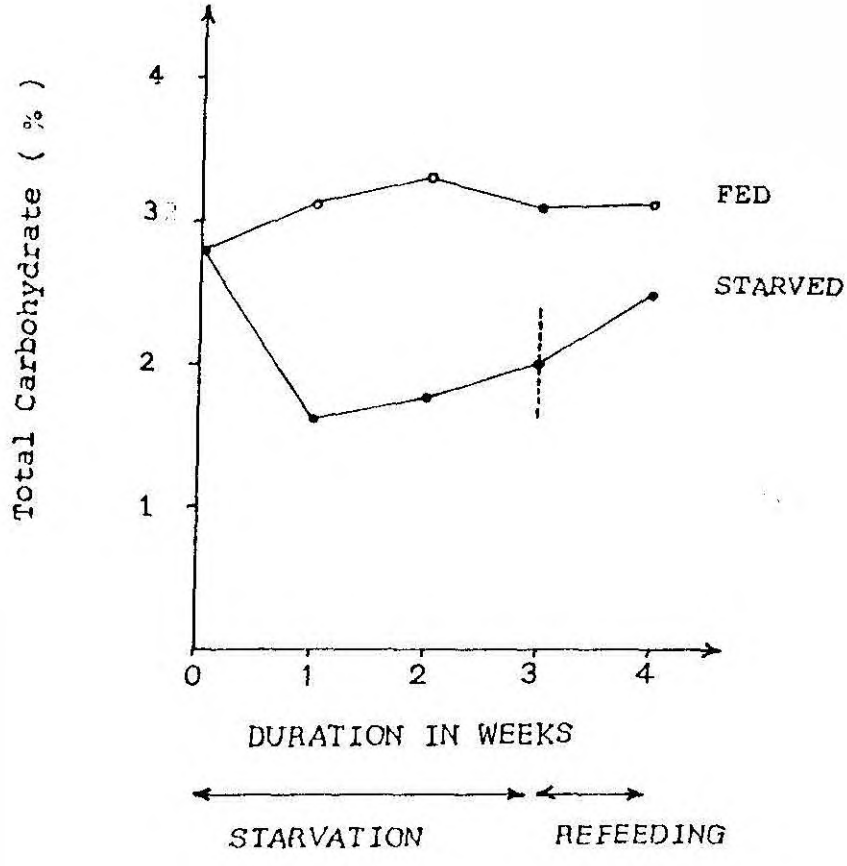

Fig. 8. Glycogen levels (mg/100mg) of hepatopancreas of fed and starved shrimps versus time.

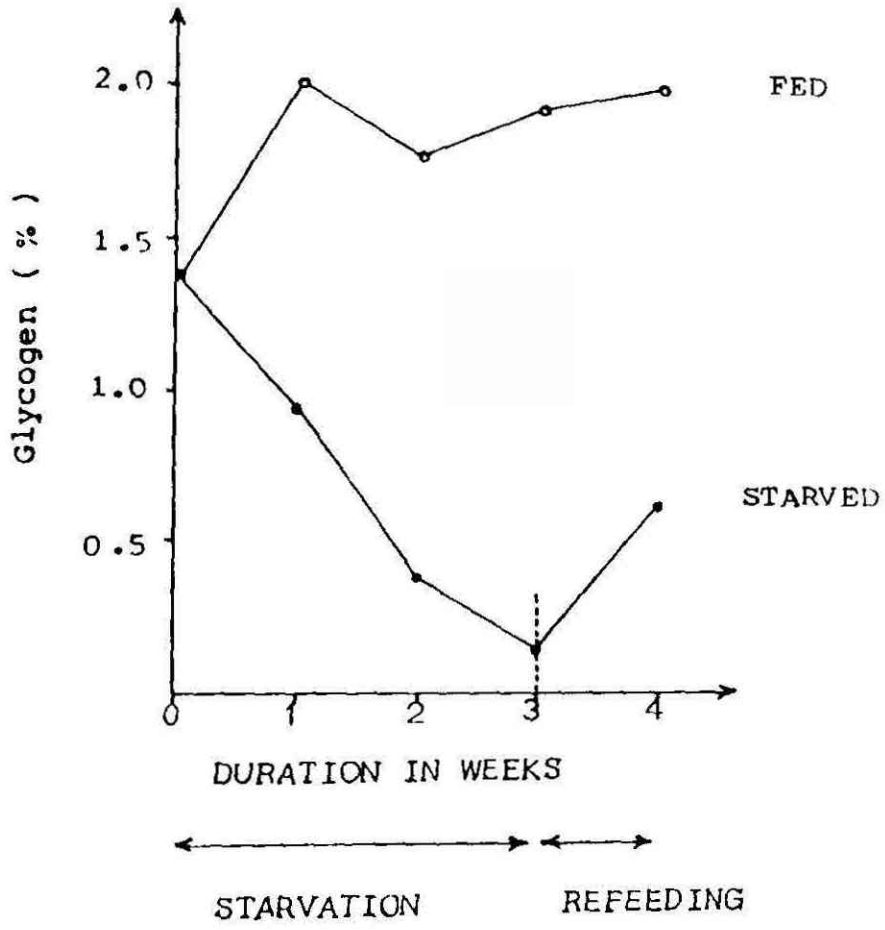

Fig.9. Protein levels (mg/100mg) of hepatopancreas of fed and starved shrimps versus time.

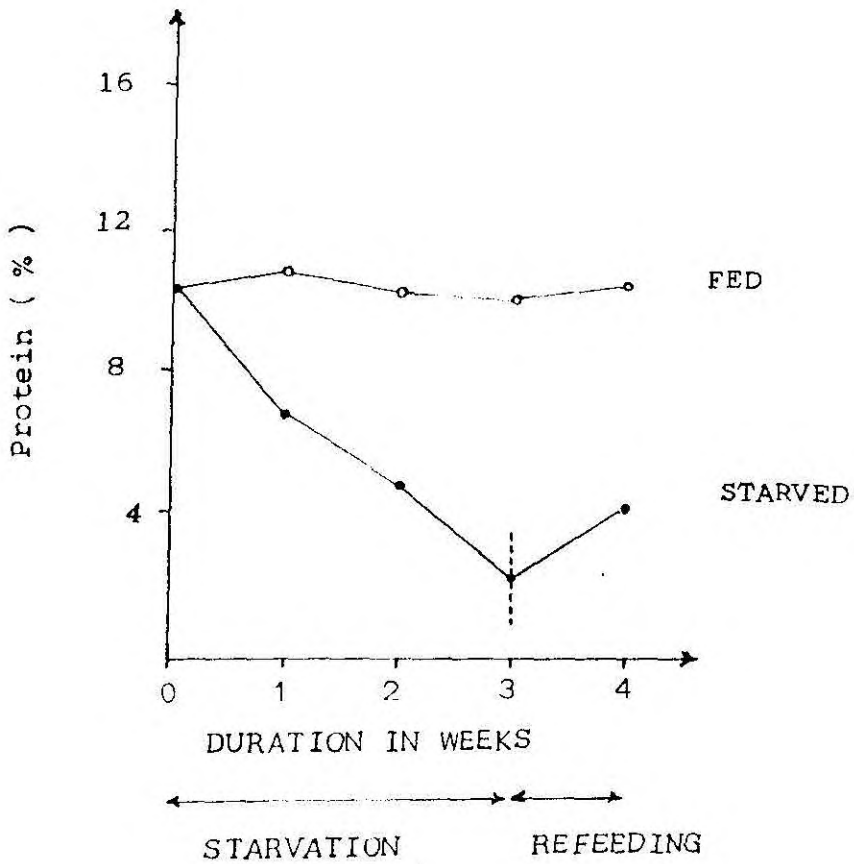

Fig. 10. Total Free Amino acid levels (mg/100mg) of hepatopancreas of fed and starved shrimps versus time.

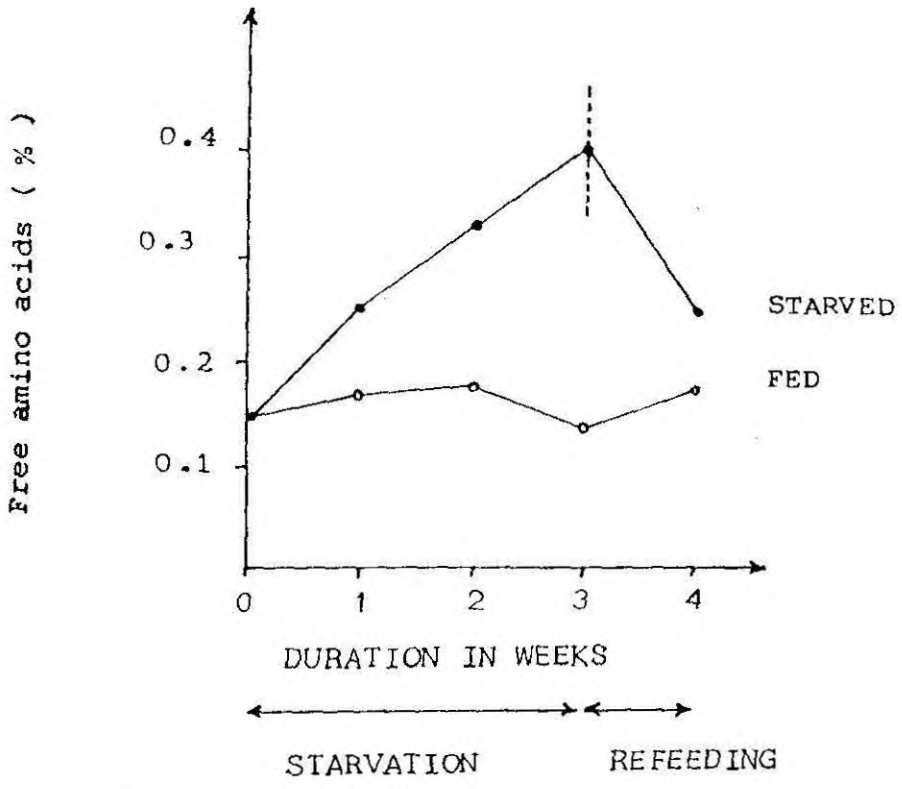

Fig. 11. Lipid levels (mg/100mg) of hepatopancreas of fed and starved shrimps versus time.

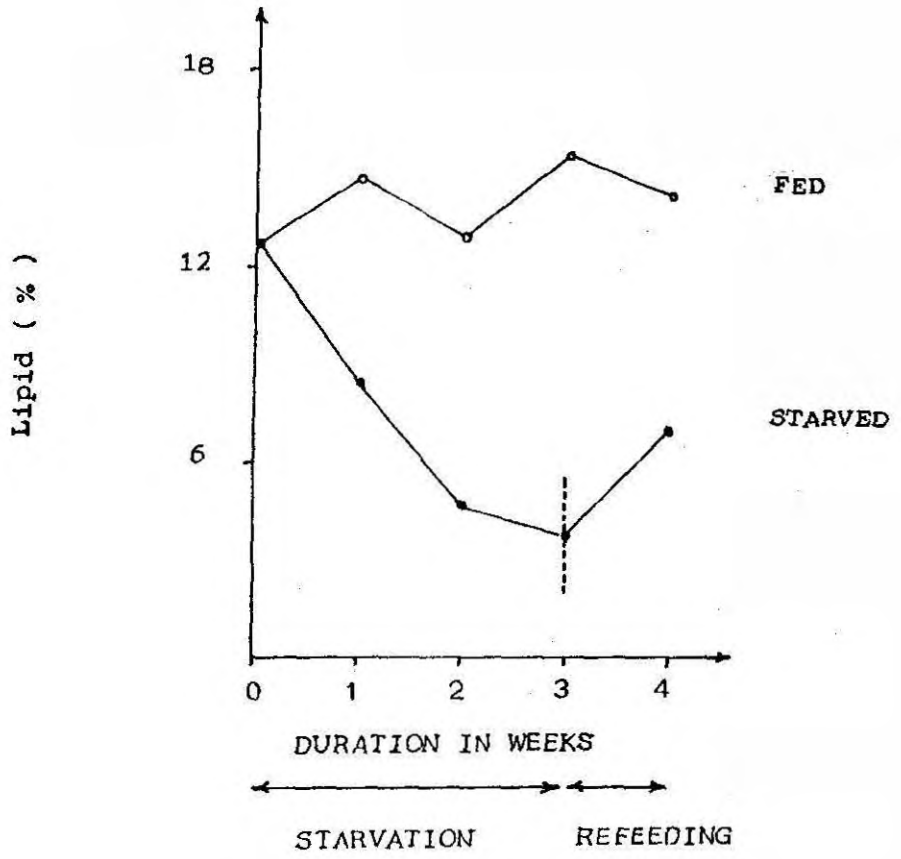

Fig. 12. Cholesterol levels (mg/100mg) of hepatopancreas of fed and starved shrimps versus time.

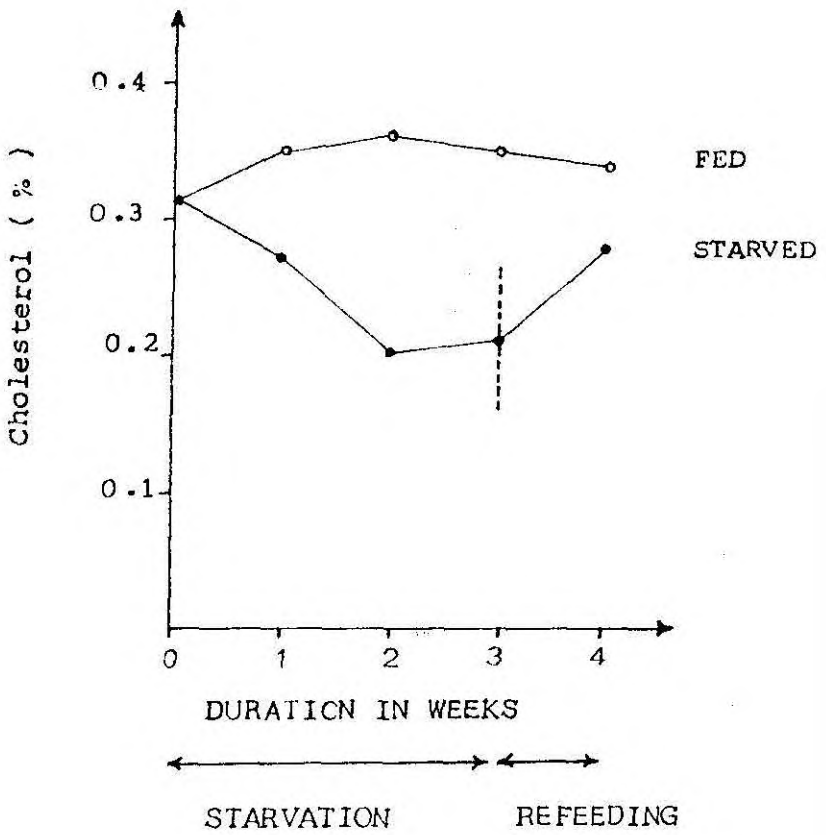

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Table 7 a: Carbohydrate hepatopancreas

SOURCE	D.F.	SUM.SQR	MEAN.SQR	F-VAL	REMARKS
TREAT	1	2.250	2.250	10.85	SIG (5%)
REPLIC	4	0.296	0.074	0.36	N.S
ERROR	4	0.830	0.207		

Table 7 b: Glycogen hepatopancreas

SOURCE	D.F.	SUM.SQR	MEAN.SQR	F-VAL	REMARKS
TREAT	1	3.270	3.270	14.09	SIG (5%)
REPLIC	4	0.343	0.086	0.37	N.S
ERROR	4	0.928	0.232		

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Table 7c : Protein hepatopancreas

SOURCE	D.F.	SUM.SQ.R	MEAN.SQR	F-VAL	REMARKS
TREAT	1	54.759	54.759	12.24	SIG (5%)
REPLIC	4	20-640	5.160	1.15	N.S
ERROR	4	17.896	4.474		

Table 7d: Aminoacid hepatopancreas

SOURCE	D.F.	SUM.SQR	MEAN.SQR	F-VAL	REMARKS
TREAT	1	0.038	0.038	8,23	SIG(5%)
REPLIC	4	0.020	0.005	1.09	N.S
ERROR	4	0.018	0.005		

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Table 7e: Lipid hepatopancreas

SOURCE	D.F.	SUM.SQR	MEAN.SQR	F-VAL	REMARKS
TREAT	1	125.992	125.992	10.48	SIG(5%)
REPLIC	4	24.768	6.192	0.51	N.S
ERROR	4	48.101	12.025		

Table 7f: Cholesterol hepatopancreas

SOURCE	D.F.	SUM.SQR	MEAN.SQR	F-VAL	REMARKS
TREAT	1	0.020	0.020	9.28	SIG (5%)
REPLIC	4	0.002	0.001	0.27	N.S
ERROR	4	0.009	0.002		

HAEMOLYMPH: The results of the biochemical analysis carried out in the haemolymph of starved Metapenaeus dobsoni is presented in Table 3.

Total carbohydrates: After an initial increase, the carbohydrate content declined to low levels with time. From 25.627 ± 4.08 mg/100 ml at the start of the experiment, it increased to 41.0408 ± 3.81 at the end of the first week. It then fell to 20.348 ± 2.79 mg/100 ml and 4.1828 ± 3.37 mg/100 ml at the end of the second and third weeks respectively. It recovered to 26.1884 ± 4.03 within a week if refeeding. The values in the controls ranged between 19.168 mg/100 ml and and 30.96 mg/100 ml (Fig. 13).

ANOVA showed no statistical significance in the difference in total carbohydrates in haemolymph of starved and fed prawns (Table 8a).

Glycogen: A gradual reduction in the amount of glycogen was observed in starved prawns. It fell from 26.54 ± 2.99 mg/100 ml at the start of the experiment to 17.79 ± 2.42, 10.70 ± 0.84 and 9.74 ± 1.69 mg/100 ml at the end of the first, second and third weeks respectively. It increased to 16.88 ± 2.87 mg/100 mg after one week of refeeding. In the controls, the values ranged between 23.4 mg/100 ml and 55 mg/100 ml (Fig.14).

ANOVA revealed that the difference in haemolymph glycogen between fed and starved prawns were statistically significant at 5% level (Table 8 b).

Proteins: The amount of protein showed a rapid decline. From 6.1544 ± 1.70 mg/100 ml at the start of the experiment, it decreased to 4.6832 ± 0.44, 3.0202 ± 0.44, and 1.528 ± 0.39 mg/100 ml at the end of the first, second and third week of starvation respectively, It increased to 4.429 mg/100 ml after one week refeeding. The values in the controls ranged from 4.490 mg/100 ml to 9.114 mg/100 ml (Fig. 15).

ANOVA revealed that the difference in haemolymph protein between fed and starved prawns were statistically significant. (Table 8 c).

Free amino acids: A build up of free amino acids was noticed in the haemolymph of starved prawn. After an initial drop, it came down from 1.9343 ± 0.12 mg/100 ml at the start of the experiment to 0.7196 ± 0.12 mg/100 ml at the end of the first week. It then went upto 1.8294 ± 0.59 mg/100 ml and 3.0386 ± 1.30 mg/100 ml at the end of the second and third week respectively. On refeeding, it came down to 2.0424 ± 0.35 mg/100 ml within a week. In the control the values ranged between 1.560 mg/100 ml and 2.560 mg/100 ml (Fig. 16).

ANOVA revealed that the difference in free amino acid content between starved and fed prawns were not statistically significant (Table 8d).

Total lipids: A sharp decline in the level of total lipids was observed in starved prawns. It fell from 309.03 ± 46.46 mg/100 ml at the beginning of the experiment to 231.048 ± 43.05, 154.926 ± 16.076, 80.96 ± 16.759 mg/100 ml, at the end of the first, second and third weeks respectively. It recovered to 189.718 ± 17.74 mg/100 ml after one week refeeding. In the controls, values ranged between 243.96 and 523.14 mg/100 ml (Fig. 17).

ANOVA revealed that the difference in total lipids in haemolymph of fed and starved prawns were statistically significant (Table. 8e).

Cholesterol: Cholesterol levels decreased gradually in starved prawns. It came down from 36.0554 ± 3.17 mg/100 ml at the start of the experiment to 32.8516 ± 3.13, 20.956 ± 2.03 and 16.25 ± 2.15 mg/100 ml respectively, at the end of the first, second and third weeks of starvation. It increased to 25.5352 ± 3.17 mg/100 ml on refeeding for a week. In the controls, values ranged between 30.94 mg/100 ml and 41.57 mg/100 ml (Fig. 18).

ANOVA revealed that the difference in cholesterol content between fed and starved prawns were satistically significant at 5% level (Table. 8 f.).

Table 3. Variations in the biochemical constituents in Haemolymph of <u>Metapenaeus dobsoni</u> with starvation of three weeks duration and refeeding of one week.

HAEMOLYMPH	DAY 0	STARVATION I WEEK	STARVATION II WEEK	STARVATION III WEEK	REFEEDING I WEEK
TOTAL CARBOHYDRATES (mg/100ml)	25.627 ± 4.0301	EXP. 41.0409 ± 3.8054	20.348 ± 2.7990	4.1828 ± 3.3665	26.1834 ± 4.0255
		CON. 26.7916 ± 3.9300	26.562 ± 1.9409	24.3932 ± 4.1462	25.7000 ± 4.0280
GLYCOGEN (mg/100ml)	26.54 ± 2.990	EXP. 17.79 ± 2.420	10.70 ± 0.840	9.740 ± 1.690	16.88 ± 2.87
		CON. 35.76 ± 6.370	34.60 ± 12.25	35.26 ± 8.116	35.38 ± 8.03
PROTEIN (mg/100ml)	6.1544 ± 1.7034	EXP. 4.6832 ± 0.4410	3.0202 ± 0.4753	1.5280 ± 0.3980	4.429 ± 0.9253
		CON. 6.5464 ± 1.8024	6.2994 ± 1.6530	6.5798 ± 1.5050	6.6228 ± 1.5351
FREE AMINO ACIDS (mg/100ml)	1.9342± 0.1205	EXP. 0.7196 ± 0.1176	1.8294 ± 0.5907	3.0386 ± 1.3007	2.0424 ± 0.3461
		CON 2.2548 ± 0.2373	1.9328 ± 0.1161	2.1626 ± 0.4160	1.9909 ± 0.3807
TOTAL LIPIDS (mg/100ml)	309.03 ± 46.467	EXP. 231.048 ± 43.05	154.926 ± 16.076	80.96 ± 16.759	189.718 ± 17.740
		CON. 392.959 ± 86.99	349.124 ± 102.34	353.302 ± 81.796	358.89 ±116.383
CHOLESTEROL (mg/100ml)	36.0554 ± 3.1714	EXP. 32.8516 ± 3.1209	20.9568 ± 2.0331	16.250 ± 2.1471	25.5352 ± 3.1749
		CON. 38.0672 ± 2.5654	36.2678 ± 1.9363	38.2524 ± 3.2182	27.5320 ± 9.114

All values are $\bar{X} \pm$ SD of five determinations.

Fig. 13. Total Carbohydrate levels (mg/100ml) of haemolymph of fed and starved shrimps versus time.

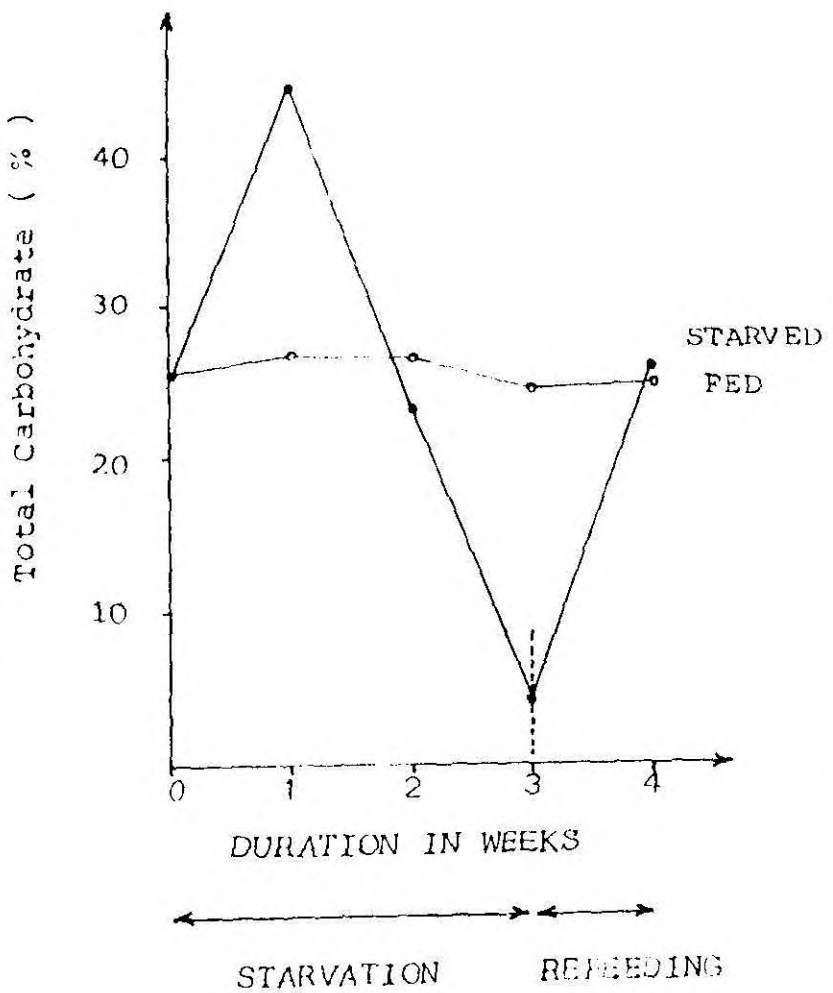

Fig.14. Glycogen levels (mg/100ml) of haemolymph of fed and starved shrimps versus time.

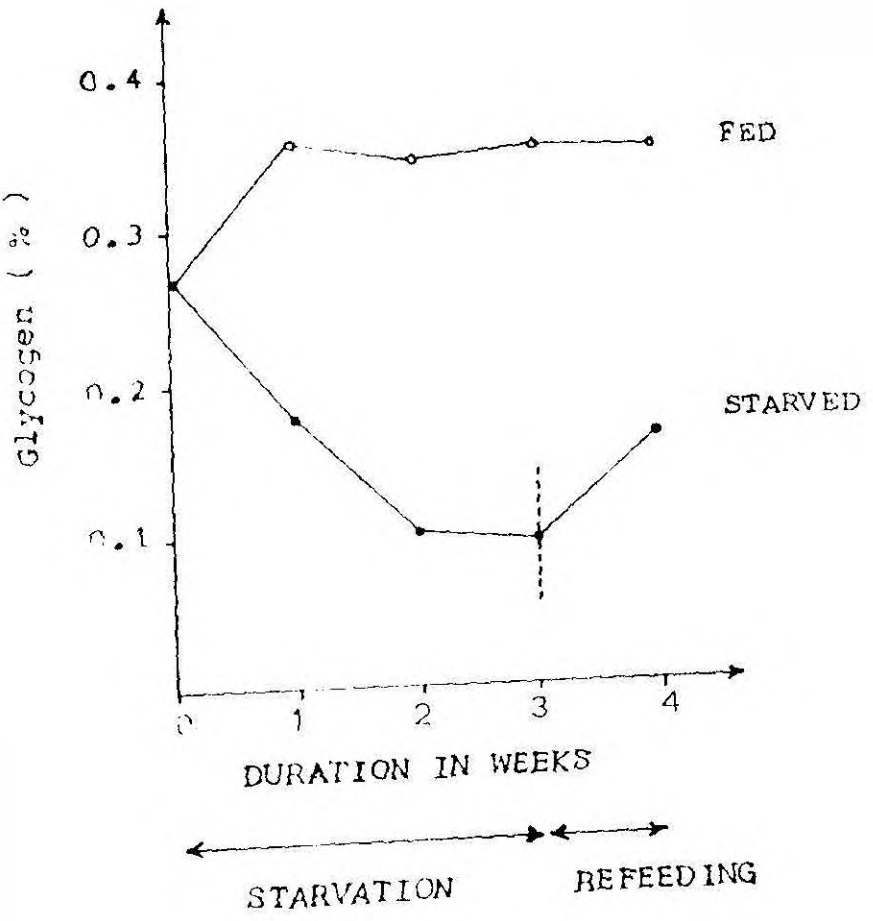

Fig.15. Protein levels (mg/100mg) of haemolymph of fed and starved shrimp versus time.

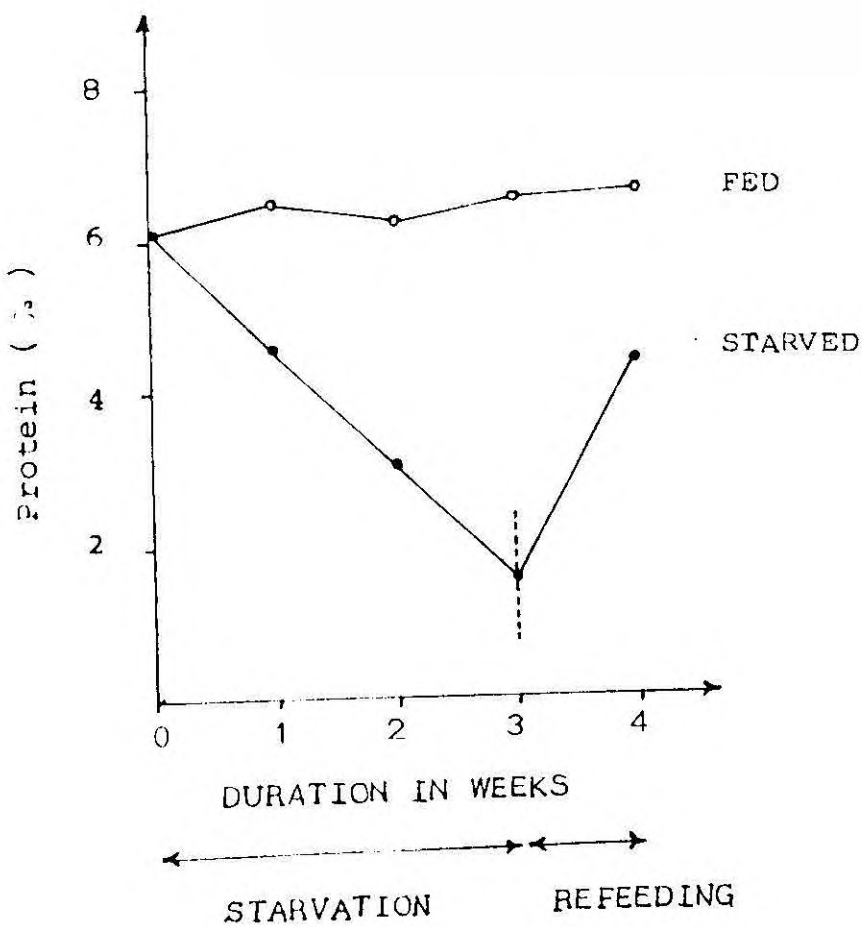

Fig. 16. Total Free Amino acid levels (mg/100ml) of haemolymph of fed and starved shrimps versus time.

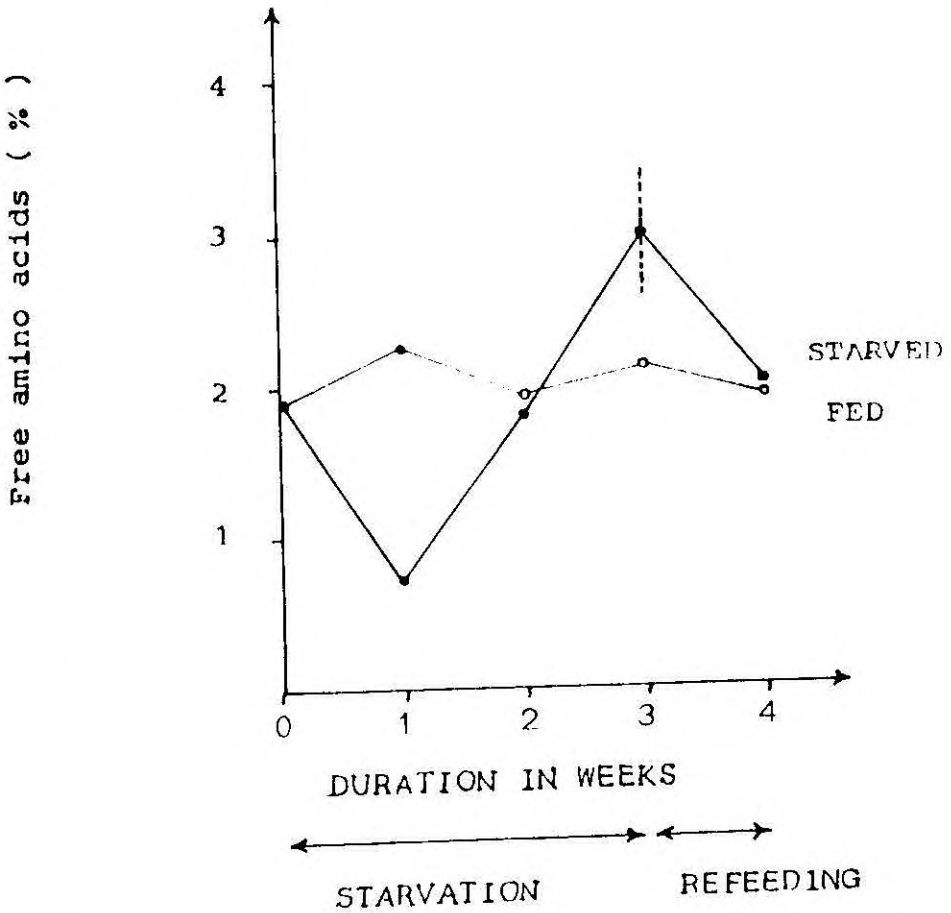

Fig.17. Lipid levels (mg/100ml) of haemolymph of fed and starved shrimps versus time.

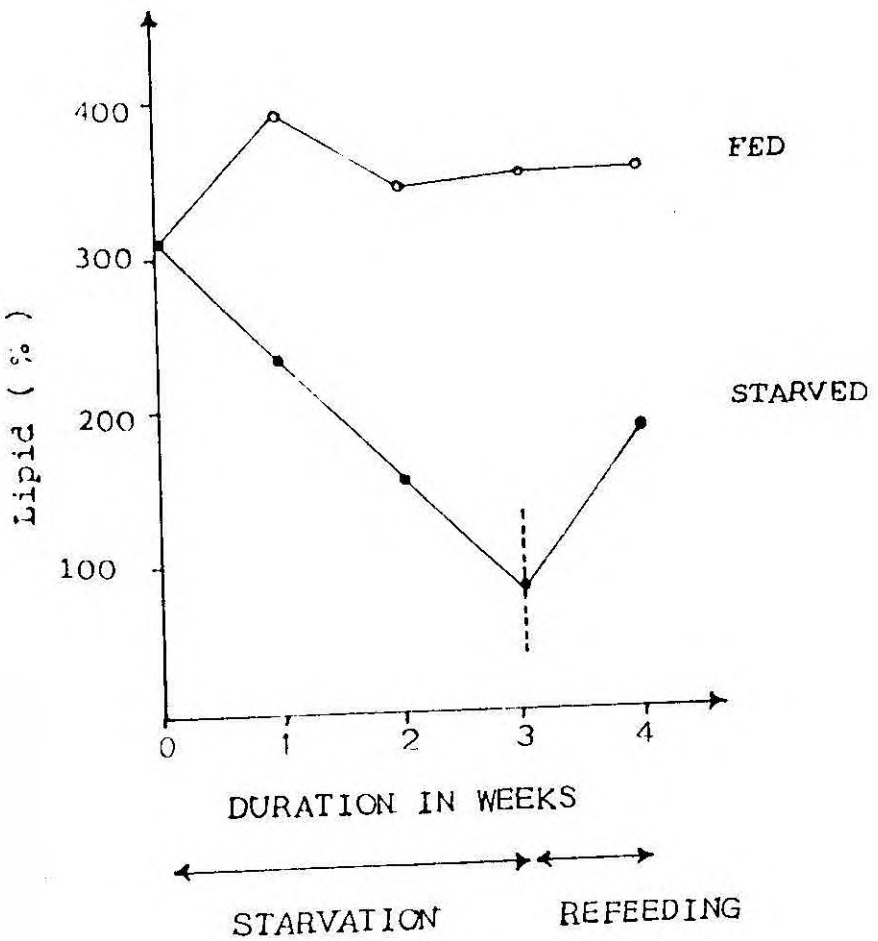

Fig.18. Cholesterol levels (mg/100mg) of haemolymph of fed and starved shrimps versus time.

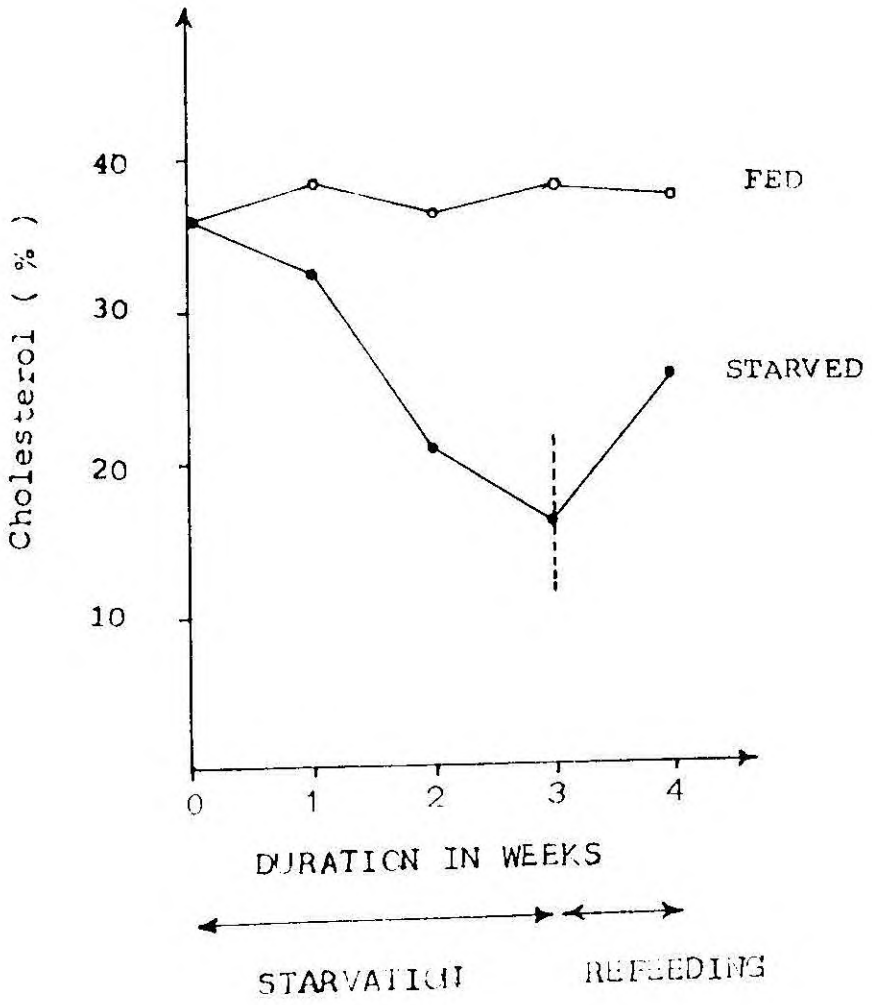

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Table 8a: Carbohydrate blood

SOURCE	D.F.	SUM.SQR	MEAN.SQR	F_VAL	REMARKS
TREAT	1	14.851	14.851	0.19	N.S
REPLIC	4	384.316	96.079	1.20	N.S
ERROR	4	320.555	80.139		

Table 8b: Glycogen blood

SOURCE	D.F.	SUM.SQR	MEAN.SQR	F_VAL	REMARKS
TREAT	1	737.710	737.710	14.32	SIG(5%)
REPLIC	4	37.061	9.265	0.18	N.S
ERROR	4	206.119	51.530		

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Table 8c: Protein blood

SOURCE	D.F.	SUM.SQR	MEAN.SQR	F-VAL	REMARKS
TREAT	1	15.344	15.344	8.85	SIG(5%)
REPLIC	4	5.590	1.398	0.81	N.S
ERROR	4	6.932	1.733	0.81	

Table 8d: Aminoacid blood

SOURCE	D.F.	SUM.SQR	MEAN.SQR	F-VAL	REMARKS
TREAT	1	0.051	0.051	0.13	N.S
REPLIC	4	1.282	0.321	0.84	N.S
ERROR	4	1.518	0.380		

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Table 8e. Lipid blood

SOURCE	D.F.	SUM.SQR	MEAN.SQR	F-VAL	REMARKS
TREAT	1	63620.000	63620.000	12.89	SIG (5%)
REPLIC	4	12764.500	3191.125	0.65	N.S
ERROR	4	19738.560	4934.641		

Table 8f: Cholesterol blood

SOURCE	D.F.	SUM.SQR	MEAN.SQR	F-VAL	REMARKS
TREAT	1	297.302	297.302	8.06	SIG (5%)
REPLIC	4	124.833	31.208	0.85	N.S
ERROR	4	147.527	36.882		

MOISTURE CONTENT: Moisture content increased substantially with increasing starvation. The average moisture content after the first, second and third weeks of starvation were 75.79%, 76.78% and 81.04% respectively. On refeeding it came down to 76.27%. In the controls, the mean values ranged between 74.8% and 75.8%.

WEIGHT LOSS: A gradual decrease in the weight of starved animals and increase in fed animals were observed. The mean weight in the experimental animals was 1.042 ± 0.129 g at the start of the experiment which dropped to 1.01 ± 0.191 g, 0.97 ± 0.16 g. 0.89 ± 0.113 g at the end of the first, second and third weeks respectively. It then increased to 0.992 ± 0.09 g after one week of refeeding (Table. 5).

In the control animals the weight increased continuously from 0.948 ± 0.074 g at the start of the experiment to 1.128 ± 0.133 g, 1.242 ± 0.0113 g, 1.3076 ± 0.081 g, 1.381 ± 0.0763 g at the end of the first, second, third and fourth weeks of feeding.

SURVIVAL RATE:

Larger prawns belonging to the size group 55 - 65 mm were found to be better equipped to counter starvation than smaller ones belonging to the size group 40 - 50 mm. 50% mortality in the first group of animals (55 - 65 mm) was recorded on the 31st day while the corresponding figure in 40 - 50 mm sized prawns was on the 27th day.

OXYGEN CONSUMPTION RATE: A small decrease in the metabolic rate was observed in starved animals. The oxygen consumption rate came down from 1.032 ± 0.048 ml/g body wt/hr at the start of the experiment to 0.9063 ± 0.0063, 0.6726 ± 0.0664, and 0.583 ± 0.07 ml/g. body wt/hr at the end of the first, second and third weeks respectively. On refeeding, the metabolic rate increased a fraction above the initial rate to 1.19 ± 0.2105 ml/g./hr (Table. 4).

ANOVA showed that the difference in Oxygen consumption rate between fed and starved prawns are not statistically significant (Table. 9).

Table 4. Variations in the Oxygen consumption rate in Metapenaeus dobsoni with starvation of three weeks duration and refeeding of one week.

Duration (Weeks)	Experimental (Starved) ml/g.wt/Hr	Control (fed) ml/g.wt/Hr
0	1.032 ± 0.048	1.032 ± 0.048
I	0.9063 ± 0.0063	1.0003 ± 0.0416
II	0.6726 ± 0.0664	1.033 ± 0.0702
III	0.583 ± 0.07	1.054 ± 0.0585
IV (Refeeding)	1.191 ± 0.2105	1.036 ± 0.025

Table 5. Evolution of weights or fed and starved shrimps.

Duration (Weeks)	Starved shrimp (g)	Fed shrimp (g)
0	1.04 ± 0.12	0.95 ± 0.07
I	1.01 ± 0.19	1.13 ± 0.13
II	0.97 ± 0.16	1.24 ± 0.01
III	0.89 ± 0.11	1.30 ± 0.08
IV (Refeeding)	0.99 ± 0.09	1.38 ± 0.43

All values are $\bar{X}$ ± SD of three determinations

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Table 9: Oxygen consumption rate

SOURCE	D.F.	SUM.SQR	MEAN.SQR	F-VAL	REMARKS
TREAT	1	0.059	0.059	1.79	N.S
REPLIC	4	0.120	0.030	0.90	N.S
ERROR	4	0.133	0.033		

Histological Studies

The hepatopancreas is a vital and major organ in Crustacea, involved in diverse metabolic activities like synthesis and secretion of digestive enzymes, lipid and carbohydrate metabolism and the storage of inorganic reserves. It is a large but compact complex of ducts and blind ending tubules, occupying much of the cephalotharacic space. Four basic cell types were recognised in the epithilium lining the tubules viz E(embryonic) cells, F(fibrillar) cells, B(Bilister like) cells and R(resorptive) cells corresponding to the classification of Hirsch and Jacobs (1930), in the present study. The E cells were observed at the extreme distal end of the tubules and are undifferentiated cuboidal cells. The secretory type F and B cells were distributed all along the tubules and contain prominent vacuoles, while the absorptive or resorptive R cells were squeezed between the other cell types.

Marked histological changes were observed in the hepatopancreas of starved prawns. At the end of the first week of starvation, extensive vacuolation was observed in the epithelial cells of the tubules. The nuclei of most of the cells in the proximal region of the tubules appeared pyknotic. By the end of the second week, a lot of degenerative changes were observed in the structure of the tubules. Most epithelial cells were

detatched from the basal lamina and many cells were already autolysed. Such changes were magnified at the end of the third week.

The size of the hepatopancreas seemed smaller and the architecture of the organ was completely damaged. Living cells were observed only in the peripheral region of the organ. These were mainly the embryonic E cells. A few F and B cells were also observed in this region. The central portion of the organ appeared completely vacant. Only scattered connective tissue was observed in this region.

After one week refeeding rapid changes were observed in the hepatopancreatic structure. Intense proliferation and regeneration of cells were seen. A large number of E type cells and few F, B and R cells were observed.

PLATE II

Fig. I. Section through hepatopancreas showing tubules in nomal fed prawns (x 1000 approx).

Fig. 2. Section through hepatopancreas showing tubules in normal fed prawns (x 2000 approx).

PLATE II

Fig. 1.

Fig. 2.

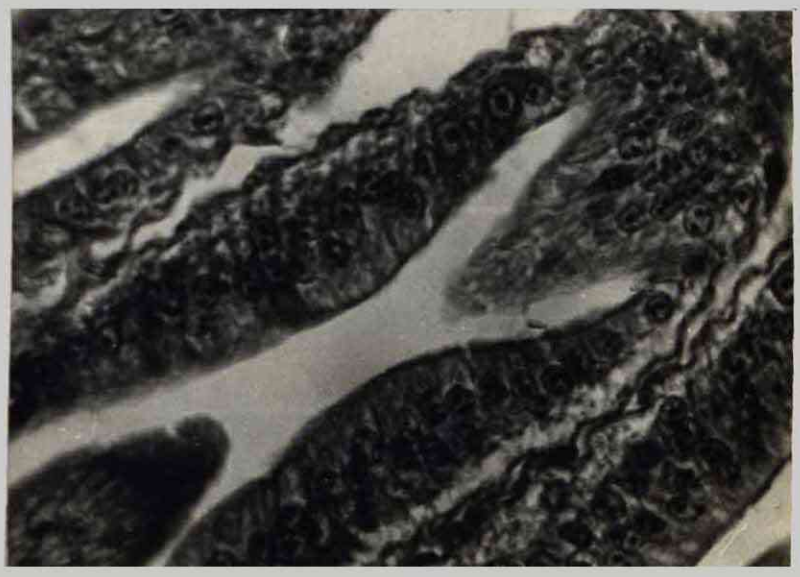


PLATE III

Fig. 1. Cross section through hepatopancreatic tubules in normal fed prawns (x 1000 approx).

Fig. 2. Cross section through hepatopancreatic tubules in prawns starved for three weeks showing extensive vacuolation (x 1000 approx).

PLATE III

Fig. 1.

Fig. 2.

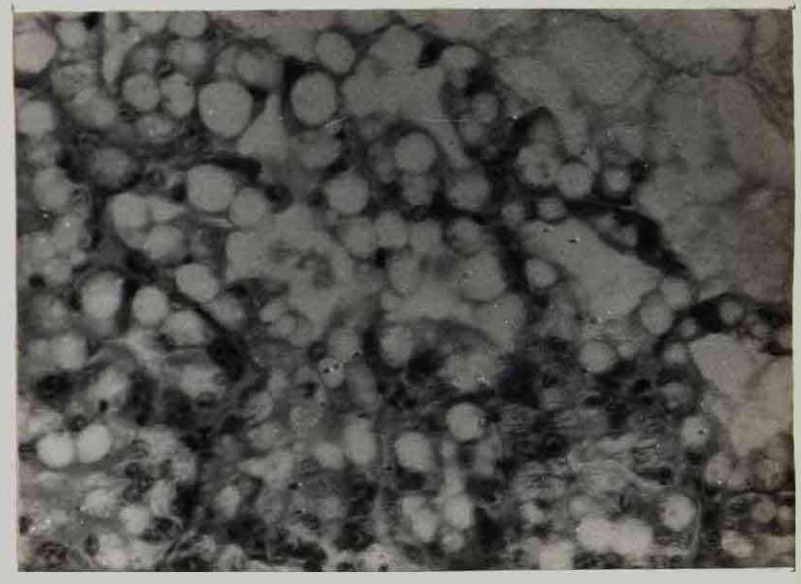


PLATE IV

Fig. 1. Cross section through hepatopancreatic tubules in normal fed prawns (x 2000 approx).

Fig. 2. Cross section through hepatopancreatic tubules in prawn starved for three weeks showing extensive vacuolation (x 2000 approx).

PLATE IV.

Fig. 1.

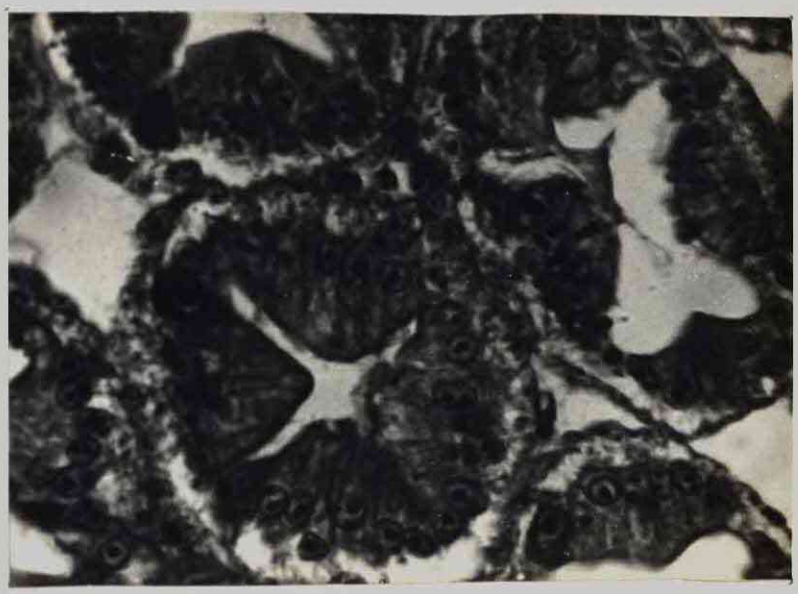


Fig. 2.

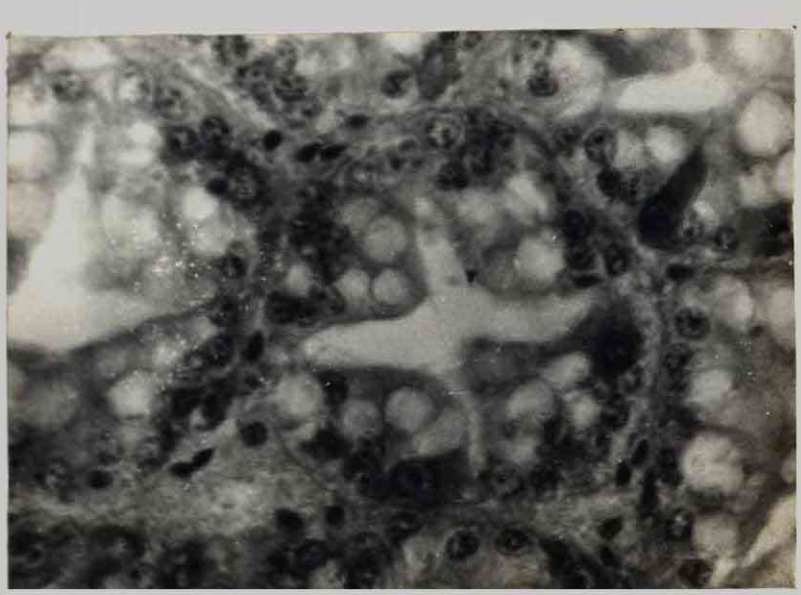


PLATE V

Fig. 1. Section through hepatopancreas of prawn starved for three weeks showing areas with differential degeneration (x 500 approx).

Fig. 2. Section through hepatopancreas of prawns starved for three weeks. Note vacuolation, degeneration and loss of tubular arrangement (x 2000 approx).

PLATE V

Fig. 1.

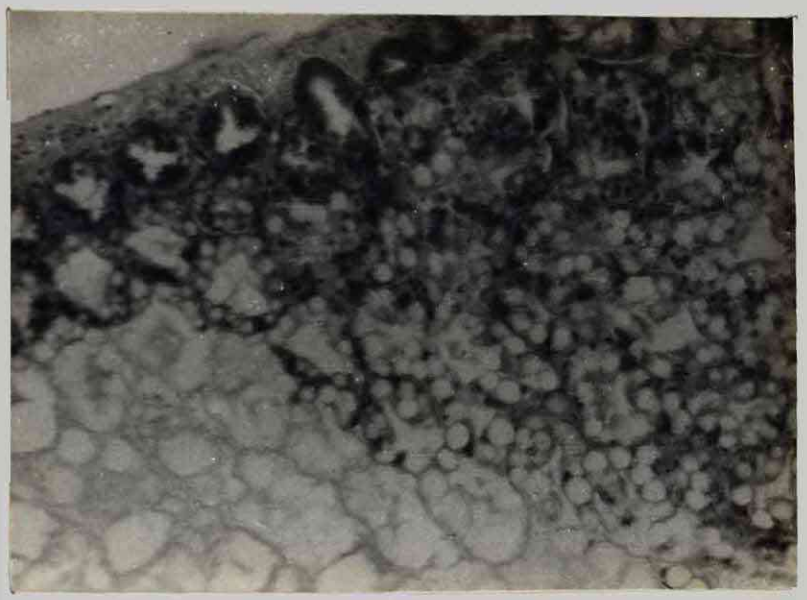
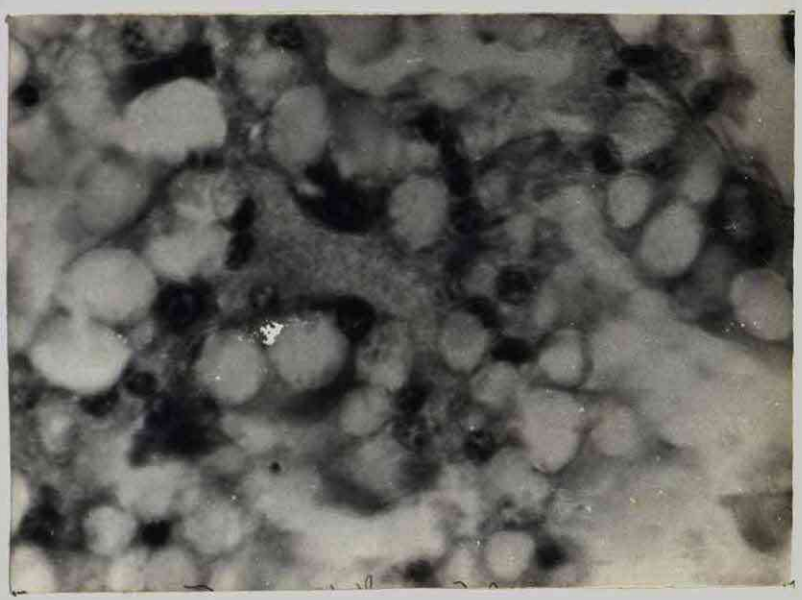


Fig. 2.



D I S C U S S I O N

Fishes and Crustaceans, both in the wild and in semi-intensive and intensive culture systems often face highly stressing conditions. These may result in physical injury and lead to various endocrine and metabolic responses. A variety of biochemical parameters have been proposed to evaluate such stress responses. The results of a few of these parameters studied in starved Metapenaeus dobsoni are discussed below.

The question of whether the primary readily mobilized energy source of Crustacea is glycogen, as in other vertebrates is not satisfactorily answered yet. Results of an early study on Hemigrapsus led to the conclusion that there was no change in the level of glycogen after 23 days starvation. (Neiland and Scheer, 1953). In contrast, a decrease in glycogen was noticed for fasting Uca (Dean and Vernberg 1965) and a decrease in carbohydrate for fasting Carcinus (Florkin, 1936). Hepatopancreas glycogen stores in P. japonicus was rapidly depleted during starvation presumably being converted to glucose. Similar results were obtained by Schafer (1968) for P. duorarum and Cuzon and Ceccaldi (1973) using C. crangon.

In the present study over 90% depletion of glycogen was observed in the hepatopancreas and muscle and over 60% in the haemolymph of starved prawn. Over 70% reduction in total carbohydrate levels were seen in the muscle and hepatopancreas and over 80% in the blood. Total carbohydrates in the blood of starved prawn increased to 60% in the first week before coming to this figure. Parvathy (1971 b,) and Cuzon et al., (1980) showed decreased blood glucose levels in starved prawns due to absence of exogenous sugar supply.

Results therefore indicate rapid metabolism of total carbohydrates in starved prawn, these being the immediate concern of energy supply under stress conditions. The temporary rise in the level of total carbohydrates in the blood could probably be due to a high rate of degradation of hepatopancreatic or muscle glycogen or other metabolites during initial stages of starvation, providing glucose units to the blood.

The results on the energy metabolism in Crustacea are conflicting. Renaud (1949), one of the first to work on starvation concluded that energy metabolism in crustacea was centered around lipids. Armitage et al. (1972) concluded that lipid was the major energy reserve in Orconectes

nair, while Heath and Barnes (1970) found there was a major decrease in fat in the digestive gland of Carcinus maenas after starvation. None of these authors, however, examined changes in body protein during starvation. Surendranath et al. (1987) showed highest decrement in lipids (75.92% in midgut gland and 67.08% in muscle) followed by protein and carbohydrate in Penaeus indicus subject to starvation. He opines that it is due to a lipid oriented metabolism indicating preference of lipids to protein and carbohydrates. Progressive mobilization of organic substances in starved Penaeus japonicus was reported by Cuzon et al. (1980). Here lipid was used first and protein was used more slowly.

Starvation in Cray fish O.limosus reduced total body protein only 2% after 15 days and 11% after 41 days. Protein was calculated to have provided 70% of energy expended between 15 and 41 days of starvation (Speck and Urich, 1969) Claybrook (1983) suggested that total proteins in the haemolymph of starved animals show a gradual decline, while individual proteins may respond differentially. Dall (1974) showed a 48% reduction in haemolymph protein concentration of the Rock lobster Panulirus longipes after 4 weeks

starvation. In Crangon crangon, total haemolymph protein dropped 68% after 23 days of starvation (Djangmah, 1970). Barclay, Dall and Smith (1987) suggested that protein and not lipid is the major source of energy used by the shrimp Penaeus esculentus during starvation. The whole animal in their study lost 20% protein and 30% of its lipid after 14 days of starvation. Neiland and Scheer (1953) working on whole Hemigrapsus nudus showed that after 23 days of starvation, protein was the major energy source and observed no decrease in lipid. Surendranath et al., found 40.6% decrement of protein in muscle and 67% in the hepatopancreas in P. indicus, starved for 15 days. Regnaults (1981) work on the shrimp Crangon crangon using O.N. ratios to estimate substrate utilization at 12 - 14^{o}C showed that after 6 days, shrimps use both protein and lipid, and just protein after 14 days, although this is an indirect method of estimation. Decreased levels of protein were reported for amphipods during starvation (Wieser, 1965 a). Schafer (1968) showed that the absolute level of protein diminished with starvation, contributing 14% of total calorific requirements of the starved animals. Steadily decreasing levels of blood protein followed by a decrease in blood viscosity was reported by Cuzon et al. (1980) in starved Penaeus japonicus.

Some studies have indicated that both lipid and protein are used. These include those of Schafer (1968), Speck and Urich (1981) and Regnault (1981) mentioned above. In the present study, 75% decrement of protein and 73% decrement of lipid in haemolymph of Metapenaeus dobsoni starved for 21 days was observed. In the hepatopancreas protein decrement was 79% and lipid decrement 84% and in the muscle, the corresponding reduction in protein and lipid were 70% and 64% respectively.

Maximum depletion of both protein and lipid occured in the hepatopancreas, followed by blood and muscle in the starved prawn, but the percentage reduction in the amount of lipid and protein individually were more or less same in each of the tissues examined. Though it cannot be stated that metabolism is centered around lipid or protein, there is no doubt in the fact that protein, being the more dominant constituent in the body in terms of weight can be said to contribute more towards production of energy in starved prawn. Barclay et al.(19) had made similar observations in starved Penaeus esculentus, where lipids made a relatively smaller contribution to energy requirements inspite of their being depleted at a proportionally greater rate than protein. Hartenstein (1970) observed that the level of protein in

haemolymph of an animal and the number of haemocytes present varies directly with the supply and quantity of food.

It has generally been considered that the main role of free amino acids is as osmotic effectors (Dall, 1975, Schoffeniels, 1976, Mccoid et al., 1984), But Torres (1973) found that changes occurred during starvation in Penaeus Kerathurus. The total FAA level rose at the end of the first week and then declined some what in the abdominal muscle of the prawn starved for 4 weeks. Mc Coid (1984) comments that these free amino acids could constitute a labile pool available for energy production, since most of them are non essential amino acids.

In the present study, the total FAA level rose continuously after an intial decline in the first week in the muscle and in the blood while in the hepatopancreas it rose continuously. Studies of Odessey et al., (1974) and Fulks et al., (1975) indicate that the decline in essential cellular FAA during fasting initiates protein catabolism. It is therefore suggested that during early starvation, the existing FAA's from the pool is utilized and as starvation progresses, the protein in muscle, haemolymph and hepatopancreas is progressively hydrolysed. The liberated aminoacids enter the FAA pool and become available for energy production, the level of FAA in the pool being proportional to the amount of protein hydrolysed.

Cholesterol is the most abundant sterol in animal tissues and occurs both free and combined form. Lehninger, (1972) stated that biosynthesis of cholesterol in the liven of fish is depressed by dietary cholesterol as well as fasting. There is now evidence that cholesterol is used by crustaceans for their normal growth and survival. (Castell et al., 1975). During the molt cycle, the cholesterol content in the eyestalk and hepatopancreas varied, but not in other tissues, (Kanazawa et al., 1976), of Penaeus japonicus. In starved Penaeus azetecus the cholesferol content of the blood and abdominal muscle did not alter on a wet weight basis. However there was a significant decrease in hepatopancreas after starvation of two weeks (Castell et al., 1976).

In the present study however, significant decrease was also observed in haemolymph and muscle in addition to the hepatopancreas, though the rate of decrease in the initial period of starvation was much lower. This could probably be due to an extended period of starvation in the experiement. The results presented here demonstrate that even in invertebrates such as shrimp, dietary factors influence the cholesterol levels in tissues and that starvation mobilizes the levels of cholesterol, particularly in the hepatopancreas.

In general, there is a decrease in Oxygen consumption following starvation (Barnes et al. 1963 b). Wallace (1973) observed a 40% reduction in metabolic rate of starved Carcinus maenas during the first week and another 20% at the end of the third week. In the shrimp Crangon vulgaris, the active rate of oxygen consumption declines during starvation and steadily approaches the basal rate which is unaffected by starvation over one week. Surendreanath et al, (1987) noted significant decrement in total metabolism from 5 - 15 days starvation in Penaeus indicus, Similar trend was also observed in Crangon crangon (Regnault, 1981).

In the present study a 43% decrement in oxygen consumption was observed at the end of three weeks of starvation. This decreased oxygen consumption rate would obviously be sufficient to oxidise low amounts of organic stores utilized during starvation for their energy demands. A significant increase in moisture content was observed in starved prawn. It rose from 75% to 81% in three weeks. A marked rise in water content was also noted in starved Penaeus japonicus (from 72 to 80%) (Cuzon et al., 1980) and in Penaeus esculentus (Barclay et al., 1968).

Thus it can be concluded that though lipid and carbohydrate contribute substantially towards production of energy,

Protein is the major source of energy used by Metapenaeus dobsoni during starvation. However direct comparison of these results with others in the literature are difficult to make because of the differing duration of starvation, partial tissue analysis of test animals and differing experimental temperatures.

Histological studies on starvation effects on prawns are very few. Papathanassiou and king (1984) reported fewer F-and B-cells in the hepatopancreas of Palaemon serratus starved for a week. Damage to the hepatopancreas is reported by various authors who studied other forms of stress and environmental pollutants. In the present study too heavy damage was observed in the hepatopancreas of starved prawn. Extensive vacuolation indicated depletion of reserves and accumulation of water. The disappearence of B-, E- and R- cells indicated that the organ was rendered non - functional, doing neither secretion, nor storage.

S U M M A R Y

1. The present study was designed to observe the effect of prolonged starvation on the biochemical constituents of muscle, hepatopancreas and haemolymph of the prawn Metapenaeus dobsoni , its metabolic rate and the associated histological changes in the hepatopancreas.

2. Biochemical parameters studied were Total Carbohydrates, Glycogen, Protein, Free amino acids, Lipids and Cholesterol in all the tissues. Histological studies of the hepatopancreas of normal and starved prawns were made to observe changes at cellular level.

3. A significant reduction in the amount of Glycogen was observed in all tissues examined of starved prawns. It rose marginally after one week refeeding.

4. While the total carbohydrate level declined steadily in the muscle, It fell initially in the hepatopancreas and then rose gradually. In the haemolymph, the total carbohydrate level after an initial increase, decreased steadily.

5. Protein levels showed a sharp decline in all the tissues examined in starved prawns.

6. The level of free amino acids in the muscle decreased initially, then rose gradually. It returned to normal on refeeding. The same trend was observed in the blood also while in the hepatopancreas, it increased continuously till refeeding, when it retured back to normal.

7. Lipid levels declined steadily in all the tissues examined in starved prawn. The fall was slow in the initial stages, and more steep after the first week.

8. Cholesterol levels decreased steadily in the muscle and haemolymph of starved prawns while it decreased initially in the hepatopancreas and then stablized to a constant level.

9. A steady decrease in the oxygen consumption rate was observed in starved prawns. It increased above normal when feeding was resumed.

10. Moisture content increased substantially with increasing starvation. It returned to near normal levels within a week of refeeding.

11. Histological studies made in the hepatopancreas revealed progressive utilization of stored materials, followed by the degeneration of secretory and storage cells. The architecture of the organ was completely damaged.

R E F E R E N C E S

ACHUTHANKUTTY, C.T. and A.H. PARULEKAR, 1984. Biochemical composition of muscle tissue of penaeid prawns. Mahasagar., 17 (4) : pp. 239 - 242.

AL-MOHANNA, S.Y. and J.A. NOTT, 1989. Functional cytology of Hepatopancreas of Penaeus semisulcatus (Crustacea : Decapoda), during the molt cycle. Mar. Biol., 101 : pp. 535 - 544.

* ANSELL, A.D. 1973. Changes in Oxygen consumption rate and ventilation accompanying starvation in the decapod crustacean Cancer pagasus. Neth. J. Sea. Res., 7 : pp. 455 - 475.

ARMITAGE, K.B., A.L. BUIKEMA, JR. and N.J. WILLEMS, 1972. Organic constituents in the annual cycle of the crayfish Orconectes nair (Faxon). Comp. Biochem. Physiol., 41 : pp. 825 - 842.

BARCLAY, M.C., W. DALL and D.M. SMITH, 1983. Changes in lipid and protein during starvation and the moulting cycle in Tiger prawn Penaeus esculentus. (Haswell). J. Exp. Mar. Biol. Ecol., 68 : pp. 229 - 244.

BARKER, P.L. and R. GIBSON, 1978. Observations on the structure of mouthparts, histology of alimentary tract, and digestive physiology of the mud crab Scylla serrata (Forskal). (Decapoda: Portunidae). J. Exp. Mar. Biol. Ecol., 32 : pp. 177 - 196.

BARKER, P.L., 1979. The Decapod Hepatopancreas. Mar. Biol. Ann. Rev., 17 : pp. 285 - 346.

* BARNES, H., M. BARNES and D.M. FINLAYSON. 1963 b. The metabolism during starvation of Balanus balanoides. J. Mar. Biol. Ass. U.K., 43 : pp. 213 - 223.

* BARNES, H and J. BLACKSTOCK, 1973. Estimation of Lipids in marine animals and tissues. Detailed investigation of the phosphovanillin method for Total lipid. J. Exp. Mar. Biol. Ecol., 12 : pp. 103 - 118.

* CAROLL, W.V., R.W. LONGLEY and J.H. ROE, 1956. The determination of Glycogen in the liver and muscle by the use of anthrone reagent. J. Biol. Chem., 220 : pp. 583 - 593.

CASTELL, J.D.E., E.G. MASON and J.F. COVEY, 1975. Cholesterol requirements of American lobster Homarus americanus . J. Fish. Res. Bd. Canada. 32 : pp. 1431 - 1435.

CHANG, E.S., and J.D. O' CONNOR, 1983. Metabolism and Transport of Carbohydrates and Lipids. In : "The Biology of Crustacea". Vol.5. Internal anatomy and physiological regulation. Ed. H.M. Linda, Academic Press, New York, pp. 263 - 281.

CLAYBROOK, L.D., 1983. Nitrogen metabolism. In : "The Biology of Crustacea". Vol.5. Internal anatomy and physiological regulation. Ed. H.M. Linda, Academic Press, New York, pp. 163 - 202.

* CORNER, E.D.S., and B.S. NEWELL, 1967. J. Mar. Biol. Ass. U.K., 47 : pp. 113 - 120.

CUZON, G., C. CAHU, J.F. ALDRIN, J.L. MESSENGER, G. STEPHAN and M, MEVEL, 1980. Starvation effect on Metabolism of Penaeus japonicus. Proc. World Mariculture Society. 11 : pp. 410 - 423.

* CUZON.G., and H.J. CECCALDI, 1971. Evolution des proteines de l'hemolymphe de Penaeus kerathurus durant de jeune. Thethus., 3 : pp. 247 - 250.

* CUZON.G., and H.J. CECCALDI, 1973. Influence de la stabulation a jeun sur le metabolisme de la crevette Crangon crangon (L.). Comptes Rendus des Seances de Societe Biologie (Marseille)., 167 : pp. 66 - 69.

DALL, W., 1965. Studies on the physiology of a shrimp. Metapenaeus sp. (Crustacea : Decapoda, Penaeidae). IV. Carbohydrate metabolism. Australian. J. Mar. Freshwater. Res., 16 : 163 - 180.

DALL, W. 1974. Indices of nutritional state in western rock lobster. Panulirus longipes (Milne Edwards). I. Blood and tissue constituents and water content. J. Exp. Mar. Biol. Ecol., 16 : pp. 167 - 180.

* DALL. W., 1975. The role of ninhydrin positive substances in osmoregulation in the western rock lobster, Panulirus longipes. J. Exp. Mar. Biol. Ecol., 19 : pp. 43 - 58.

* DALL, W., 1981. Lipid absorption and utilization in the Norwegian lobster, Nephrops norvegicus (L.). J. Exp. Mar. Biol. Ecol., 50 : pp. 33 - 45.

DALL. W., and D.M. SMITH, 1987. Changes in protein-bound and free amino acids in the muscle of the Tiger prawn Penaeus esculentus during starvation. Mar. Biol., 95(4) : pp. 509 - 520.

* DAVIS, L.E. and A.L. BURNETT, 1964. A study of growth and cell differentiation in the hepatopancreas of crayfish. Devl. Biol., 10 : pp. 122 - 153.

DEAN, J.M. and F.J. VERNBERG, 1965. Variations in the blood glucose levels of Crustacea. Comp. Biochem. Physiol., 14 : pp. 29 - 34.

DIWAN, A.D. and T.USHA. 1985 Characterisation of moult stages of Penaeus indicus based on developing uropod setae and some closely allied structures. Indian. J. Fish., 32 : pp. 275 - 279.

DJANGMAH, J.S. 1970. The effects of feeding and starvation on copper in the blood and hepatopancreas, and on the blood proteins of Crangon vulgaris (Fabricius). Comp. Biochem. Physiol., 32 : pp. 709 - 731.

DRURY, R.A.B., and E.A. WALLINGTON, 1973. Carleton's Histological techniques. London Oxford University Press. U.K.

* DUBOIS, M., K.A. GILLES, J.K. HAMILTON, P.A. REBERS and F.SMITH, 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry., 28 : pp. 350 - 356.

FAIR, P.H. and L.V. SICK, 1982. Serum Aminoacid concentrations during starvation in the prawn Macrobrachium rosenbergii as an indicator of metabolic requirements. Comp. Biochem. Physiol. B Vol. 73 B(2) : pp. 195 - 200.

* FLORKIN, M. 1936. Bull. Acad. r. Belge. Cl. Sci., 22 : pp. 1359 - 1367.

FULKS, R., LI, J.B., and A.L. GOLDBERG, 1975. Effects of insulin, glucose and aminoacids on protein turnover in rat diaphragm. J. Biol. Chem., 250 : pp. 290 - 298.

* GEORGE, R.Y., 1966. Science., 153 : 1262 - 1264.

HARTENSTEIN, R. 1970. Nitrogen metabolism in non-insect arthropods. In : "Comparative Biochemistry of Nitrogen metabolism". Vol. 1. The Invertebrates. Ed. J.W. Campbel. Academic Press, London, pp. 299 - 372.

HEATH, J.R. and H. BARNES, 1970. Some changes in biochemical composition with season and during the moulting cycle of the common shore crab, Carcinus maenas (L.). J. Exp. Mar. Biol. Ecol., 5 : pp. 199 - 233.

* HIRSCH, G.C., and W. JACOBS, 1930. Der Arbeitsrhythmus der MiHeldarmdruse von Astacus leptodactylus. II. Z. Vergleich Physiol., 12 : pp. 524 - 558.

HOAR, S., 1984. General and Comparative Physiology. Prentice Hall of India Pvt. Ltd. New Delhi.

* JUNGREIS, A.M. 1968. The role of stored glycogen during long term temperature acclimatisation in the fresh water crayfish, Orconectes virilis. Comp Biochem. Physiol., 24: PP. 1-6.

KANAZAWA, A., S. TESHIMA, Y.SAKAMOTO & J.C.B. GVARY. 1976 Variations of lipids and cholesterol, contents in the tissues of the prawn Penaeus japonicus during the moulting cycle. Bull. Jap. Soc. Sci. Fish., 42: PP 1003 - 1007.

* KANNEWORFF, E., 1965. Life cycle food and growth of the amphipod Ampelisca Macrocephala Liljeborg from the Oresund. Ophelia. 2: PP. 305 - 318.

KLEIN BRETELER. W.C., 1975. Oxygen consumption and respiratory levels of juvenile shore crabs, Carcinus maenas, in relation to weight and temperature. Neth. J. Sea. Res., 9 : pp. 243 - 254.

KRISHNAMOORTHY, R.V., A. VENKATARAMIAH, G.J, LAKSHMI, & PATRICA BIESIOT. 1982 Effects of starvation and of Algae- feeding on the tissue cholesterol levels in commercial shrimp - Penaeus azetecus. IVES.Proc.Symp.Coastal. Aquaculture, 1:pp.215-222

LEHNINGER. A.L. 1970. Biochemistry. Worth Publishers Incorporated. New York.

LOVE, R.M. 1970. The chemical biology of fishes. Academic Press, London and New York.

* LOWRY, O.H., N.J. ROSENBROUGH, A.L. FARR and R.J. RANDALL. 1951. Protein measurement with the folin-phenol reagent, J. Biol. Chem., 193 : pp. 265 - 275.

McCOID, V., R.MIGET, and G. FINNE. 1984. Effect of environmental salinity on the free amino acid composition and concentration in penaeid shrimp. J. Fd. Sci., 49 : pp. 327 - 330.

* MAHLER, R.H. and H.E. CORDES. 1968. Basic Biological Chemistry. A Harper International Edition, pp. 527.

MARSDEN, I.D., NEWELL and M. AHASANULLAH, 1973. Effect of starvation on metabolism of shore crab - Carcinus maenas. Comp. Biochem. Physiol., 45(A) : pp. 195 - 213.

MAYZAUD, P. 1976. Respiration and Nitrogen excretion of Zooplankton. IV. The influence of starvation on the metabolism and the biochemical composition of some species. Mar. Biol., 37 : pp. 47 - 58.

MOORTHY, C.V.N., V.K. MOORTHY, G.V. REDDY, V.B. HARANATH, REDDANNA,P. and S. GOVINDAPPA, 1980. Effect of starvation and refeeding on tissue proximate analysis of hepatic and muscular tissues of Tilapia mossambica. Indian. J. Fish., 1 - 2 : pp. 215 - 219.

NEEDHAM, A.E., 1957. Factors affecting Nitrogen - excretion in Carcinides maenas. (Pennant). Physiol. Comparate et Oecol., 4 : pp. 209 - 239.

NEILAND, K.A. and B.T. SCHEER, 1953. The influence of fasting and of sinus gland removal on body composition of Hemigrapsus nudus. Physiol. Comparata et Oecol., 3 : pp. 321 - 326.

NEWELL, R.C. and B.L. Bayne. 1973. A review on temperature and metabolic acclimation in intertidal marine invertebrates, Neth. J. Sea Res., 7 : pp. 421 - 433.

NIVAL. P., G. MALARA, R. CHARRA, I. PALAZZOLI and S. NIVAL. 1974. Etude de la respiration et de l' excretion de quelques copepodes planctioniques (Crustacea) dans la zone de remontee d'eau profonde des cotes marocaines. J. Exp. Mar. Biol. Ecol., 13. : pp. 231 - 260.

ODESSY, R., E. KHAIRALLAH and A.L. GOLDBERG, 1974. Origin and possible significance of alanine production by skeletal muscle. J. Biol. Chem. 249 : pp. 7623 - 7629.

PAPATHANASSIOU, E. and P.E. KING. 1984 Effects of starvation on the fine structure of the hepatopancreas in the common prawn Palaemon serratus Penna Comp. Biochem. Physiol., 77 A (2) : pp. 243 - 249.

PHILLIPS, A.M. and D.R. BROCKWAY, 1954. Effect of starvation, water temperature and sodium amytal on the metabolic rate in brook trout. Progve. Fish. Cult., 16 : pp. 65 - 68.

RAJAMANI,M., 1982 Biochemical studies on 'soft' prawns 1. Protein Nitrogen and Non-protein Nitrogen contents in P. indicus. Proc. Symp. Coastal Aquaculture., 1 : pp. 349 - 353.

RALF BASTROP, RUDIGER, SPANGENBERG & KARL JURSS. 1991 Biochemical adaptation of Juvenile carp (Cyprinus carpio. L) to food deprivation. Comp. Biochem. Physiol., 98A (1) : pp. 143 - 150.

REGNAULT, M., 1981. Respiration and Ammonia excretion of shrimp Crangon crangon. L : Metabolic response to prolonged starvation. J. Comp. Physiol., 141 : 549 - 555.

* RENAUD, L., 1949. Le cycle des reserves organiques chez les Crustaces Decapodes. Ann. inst. Oceanog., (Paris). pp. 259 - 357.

* ROBERTS, J.L., 1957. Thermal acclimatisation of metabolism in the crab Pachygrapsus crassipes Randell. I. The influence of body size, starvation and moulting. Physiol. Zool., 30 : pp. 223 - 242.

SCHAFER, H.J., 1968. Storage materials utilized by starved pink shrimp Penaeus duorarum. FAO. Fish.Rep., 57 : pp. 393 - 403.

SCHOFFENIELS, E., 1976. Adaptation with respect to salinity. Biochem. Soc. Symp. 41 : pp. 179 - 204.

SCHRECK, C.B., 1981. Stress and Compensation in Teleostean fishes : Response to Social and Physical factors. In : "Stress and Fish". Ed. PICKERING,A.D. Academic Press London. pp. 295 - 314.

SCHRECK, C.B., 1990. Physiological, Behavioral and Performance indicators of stress. In: Biological indicators of stress in fish, Amer. Fish, Soc. Symp. 8 : pp. 29 - 37.

* SPECK, U., and K. URICH, 1969. Der Abbau Korperigener Substanzen in dem Flusskrebs Orconectes limosus wahrend des Hungerns. Z. Vergl. Physiol. 63 : pp. 410 - 414.

STANIER, J.E., M.A. WOODHOUSE and R.L. GRIFFIN, 1968. The fine structure of hepatopancreas of Carcinus. maenas. L. (Decapoda, Brachyura). Crustaceana., 14 : pp. 56 - 66.

* STEEVES, H.R. 1963. Effect of starvation on Glycogen and Lipid Metabolism in the isopod Lirceus brachyurus (Harger). J. Exp. Zool. 154 : pp. 21 - 27

* STEFFENS, W., 1985. Grundlagen der Fishernahrung. VEB. Gustav Fischer Verlag, Jena.

STIMPSON, J.H., 1965. Comparative aspects of the control of Glycogen utilization in vertebrate liver. Comp. Biochem. Physiol. 15 : pp. 187 - 197.

SURENDRANATH, P., A. KASAIAH, V. SHEKAR, K. SURENDRA BABU and K.V. RAMANA RAO. 1987. Starvation induced alterations in oxygen consumption and organic constituents of Penaeus indicus. Mahasagar., 20 (3) : pp. 199 - 203.

TESHIMA, S., and A. KANAZAWA. 1971. Biosynthesis of sterols in the lobster Panulirus japonica, the Penaeus japonicus and the crab Portunus trituberculatus. Comp. Biochem Physiol., 38 B : pp. 597 - 602.

TORRES, C., 1973. Variations diu pool des acides amines libres diu muscle abdominal de Penaeus Kerathurus au cours du cycle d'intermue, et au cours du jeune. Comp. Biochem. Physiol., B 45-B : pp. 1 - 12.

VOGT, G., V. STORCH, E.T. QUINITIO and F.P. PASCVAL. 1985. Midgut gland as monitor organ for the mutritional value of diets in Penaeus monodon (Decapoda). Aquaculture, 48 : pp. 1 - 12.

VONK, H.J., 1960. Digestion and Metabolism. In: The Physiology of crustacea. 1., Ed. T.H. WATERMAN. Academic Press New York. pp. 291 - 316.

WALLACE, J.C., 1973. Feeding, starvation and metabolic rate in shore crab Carcinus maenas. Mar. Biol. 20 : pp. 277 - 281.

WEDEMEYER, G.A., and D.J. McLEAY, 1981 : Methods of determining the tolerance of fishes to environmental stressors. In: "Stress and Fish". Ed. PICKERING, A.D. Academic Press London. pp. 247 - 268.

* WHITNEY, J.O. 1969. Absence of sterol synthesis in larve of the mud crab Rhithropanopeus harisii, and of the spider crab Libinia emarginata. Mar. Biol., 3 : pp. 134 - 135.

* WIESER, W., 1965a. Electrophoretic studies on blood proteins in an ecological series of isopod and amphipod species. J. Mar. Biol. Ass. U.K. 45 : pp. 507 - 523.

WILLIAMS, A.J., and P.L. LUTZ, 1975. The role of the haemolymph in the carbohydrate metabolism of Carcinus Maenas. J. Mar. Biol. Ass. U.K., 55 : pp. 667 - 670.

* YEMM, E.W. and E.C. COCKING, 1955. The determination of amino acids with ninhydrin. Analyst, 80 : pp. 209 - 213.

* ZANDEE, D.I. 1967. Absence of Cholesterol synthesis as contrasted with the presence of fatty acid synthesis in some arthropods. Comp. Biochem. Physiol., 20 : pp. 811 - 822.

* Not referred in original