

**STUDIES ON OSMOREGULATION IN THE PANAEID  
PRAWN *Metapenaeus dobsoni* (MIERS)**

THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE  
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## DECLARATION OF THE GUIDE

This is to certify that the thesis entitled "**Studies on osmoregulation in the penaeid prawn *Metapenaeus dobsoni* (Miers)**" is a bonafide work carried out by Kum Kalpana. K. V. under my guidance and supervision under the Postgraduate Education and Research Programme in Mariculture at Central Marine Fisheries Research Institute, Cochin and that no part thereof has been presented for award of any other degree.

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## **PREFACE**

Brackish water area, which includes estuaries, mangroves, backwaters, lagoons and salt water lakes, are available for aquaculture practices in India. Of the 1.4 million hectares available, only 80,000 hectares are under cultivation with a production of 70,000 tonnes of shrimp (World shrimp farming, 1994). India is the hottest spot on the shrimp farming map and hence shrimp culture remains a fairly lucrative venture.

*Metapenaeus dobsoni* (Miers) is the most abundant species along the coast of Kerala. It is cultured extensively by adoption of traditional farming practices. The geographical location and water source determines the seasonal and annual environmental fluctuations the prawn farming systems experiences. The life cycle of the shrimp includes its migration to the coastal deeper waters for spawning and the immigration of larvae to the estuaries for growth. The survival of the species in such complex ecosystems is thus critical to its life cycle. The animal adapts itself to different environments through a physiological process known as osmoregulation.

The present study on osmoregulation in the penaeid prawn *Metapenaeus dobsoni* was thus undertaken to understand the mechanism adopted by this species to survive in different environments. A

number of experimental work have been conducted to understand the effect of salinity on the internal variations. However the effect of the complex environmental conditions as existent in nature on the osmotic variations in this species has not been dealt with in any of the earlier studies. The objectives of the present study are

- 1 To determine the haemolymph osmotic pressure changes with change in environment in the various size groups and sexes.
2. To determine the haemolymph biochemical parameters (lipid, carbohydrate, protein and total free amino acid nitrogen) and inorganic ions (sodium, potassium and chloride) in the different size groups.
3. To determine the effect of environment on the variations of muscle biochemical parameters (total lipid, total carbohydrates, protein and total free amino acid nitrogen) in the different sizes.
4. To determine the effect of environment on the biochemical changes of the hepatopancreatic lipid, carbohydrate, protein and free amino acid nitrogen in the different sizes.
5. The structure and function of gills in the regulation of osmotic pressure.

The thesis embodying these investigations have been organised into four chapters.

The first chapter presents a review of the relevant works done in this field to bring an awareness of the present status of our knowledge on the subject.

The second chapter presents the materials and methods employed in the collection of the tissues *viz*; haemolymph, muscle and hepatopaneas for the biochemical analysis and study of inorganic ions in the haemolymph. The processing of gill tissue for light and electron microscopy study is also dealt with.

Chapter three covers the study of the haemolymph for the biochemical parameters namely lipid, carbohydrate, protein and free amino acid nitrogen and inorganic ions, sodium, potassium and chloride in relation to the osmotic pressure in the different size groups. A study of the biochemical variation of the muscle and hepatopaneas in these size groups with environmental parameters has also been discussed.

Chapter four deals with the structural changes in the gills concomitant with the environmental changes.

A summary of the results of the investigation is presented in the final section of the thesis followed by a detailed list of references on the subject.

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# CHAPTER 1

## INTRODUCTION

The aquatic arthropods occur in water of varying salinities in nature and many of them are able to survive in media very different from their normal habitats *i.e.* euryhaline. The successful penetration of marine animals into brackish and freshwater areas requires the adoption of the osmotic regulation.

Most penaeid prawns complete their life cycle in two types of environments *viz* the brackish water of estuaries and salt water lakes connected with the sea. The highly fecund females release the eggs in the benthic realm of the continental shelf (George and Vedavyas Rao, 1968; Panikkar, 1968) and postlarval stages enter the estuaries. Freshwater carideans such as *Macrobrachium* only the females migrate to higher salinities after mating in fresh water (Wickins, 1976). However in the penaeids the pattern of migration has been observed in *Metapenaeus brevicornis* and *M. bennattae* (Dall, 1965; Shaikh Mohammed and Tembe, 1970). During the course of their migration they encounter changes in salinity, temperature, pH *etc.* which have an effect on its internal environment.

Osmotic regulation is most critical in the juvenile stage as they are exposed to greater salinity variations in the estuarine environment (Wickins, 1976). Crustaceans are hard bodied animals with open circulatory system and limited capacity to swell or shrink (Timothy *et.al*, 1984). The changes are obviously manifested in the composition of the haemolymph which is usually

compared with that of the medium for understanding of the osmoregulatory processes.

## OSMOREGULATION:

The process of osmoregulation in fishes and arthropods has been extensively studied. Among the arthropods osmoregulation in brachyurans and freshwater shrimps have been observed to be more extensive. Nagel (1934) and Krogh (1939) have showed the euryhaline capabilities of many crustaceans. The earliest work was conducted by Panikkar (1941) on *Leander serratus*, *Leander squilla* and *Palaemonetes varians* showed that they are hypotonic to the medium. Poikilosmotic response of stenohaline crustaceans have been demonstrated in *Emerita analoga* (Gross, 1958), *Pandulus montagii* (Panikkar, 1968) and *Panulirus longipes* (Dall, 1974). Many crustaceans which have invaded brackish water have the capability to regulate the haemolymph in brackish waters, but in seawater it becomes isosmotic as exemplified by *Calianassa kraussi* (Forbes, 1974), *Callinectes sapidus* (Lynch *et.al.*, 1973), *Hemigrapsus nudus* (Jones, 1941), *Squilla emphusa* (Lee and McFarland, 1962) and *Helice crassa* (Bedford, 1972; Jones, 1981). Another type of response by adopted euryhaline crustaceans is to maintain their haemolymph hyperosmotic at reduced salinities and hyposmotic at higher salinities eg. *Artemia salina* (Croghan, 1958a, b, c), *Gnomoosphaeroma oregonensis* (Reigel, 1959), *Palaemonetes varians* (Panikkar, 1941), *Uca crenulata* (Jones, 1941), *Uca pugilator*, (Heit and Fingerman, 1975). The other brachyurans studied are *C. sapidus*, *Goniopsus crenata* and *Uca* Spp. (Green *et.al*, 1959; Gross, 1964a, b; Ballard & Abott, 1969; Engel *et.al*, 1974; Baldwin and Kirschner, 1976 b, c; Zanders and Martelo, 1984; Dehnel, 1962; Dehnel and Carefoot, 1965; Hicks, 1973) and *Uca pugilator*, *Uca minax* & *U. pugnax* (Wright *et.al.*, 1984). A similar type of regulation was observed in *Procambarus clarkii*

and *P. zonangulus* (Newson and Davis, 1994). A wide tolerance over salinity from fresh to hypersaline conditions were in grapsid crabs (Frusher *et.al.*, 1994).

The osmotic regulation of freshwater shrimp of Genus *Macrobrachium* has been studied. In *M. ohione* and *M. rosenbergii* (Castille and Lawrence, 1981c; Balazs, 1974), *M. australiense* (Denne, 1968), *M. equidens* (Denne, 1968). The other carideans studied are *P. elegans* (Panikkar, 1941), *Palaemon macrodactylus* (Born, 1968), *P. varians* (Panikkar, 1941; Potts and Parry, 1964b), *Palaemonetes intermediatus* (Dobkin and Manning 1964), *Crangon crangon* (Panikkar, 1968) and *Crangon septemspinosa* (Haefener, 1969). A lot of work has been conducted on osmotic and ionic regulations in these species.

Osmoregulatory capabilities of penaeids have been determined in *Metapenaeus dobsoni* (Panikkar, 1951, Kalpana, 1987; Mini, 1990), *M. monoceros* (Panikkar and Viswanathan, 1948; Kamala, 1989), *Penaeus aztecus* (Williams, 1960; McFarland and Lee, 1963; Castille and Lawrence, 1981a), *P. carinatus* (Panikkar, 1951), *P. duorarum* (Williams, 1960; Bursey and Lane, 1971; Castille and Lawrence, 1981a), *P. indicus* (Panikkar, 1951), *P. setiferus* (McFarland and Lee, 1963; Castille and Lawrence, 1981a, b, d), *P. stylirostris* (Castille and Lawrence, 1981a, b, d), *P. vannamei* (Castille and Lawrence, 1981a) *P. monodon* (Cawthorne *et.al.*, 1983) *P. marginatus* (Balazs, 1974).

Studies have been conducted on lobster adults (Dall, 1970) and larval and post larval stages of *Homarus americanus* (Charmantier *et.al.*, 1984a).

## BIOCHEMISTRY :

In shrimps, the hepatopancreas is the primary organ for the storage of reserves. The haemolymph plays a secondary role and some accumulation also occurs in the epithelium and subepithelium. The metabolic pathways of the lipids, carbohydrates and proteins are yet to be clearly discerned. Free amino acids, the building blocks of proteins are a dynamic pool of biomolecules forming the major percentage of the osmotic pressure. Protein, is hence an essential component of organic reserve implicated in the process of osmoregulation Travis (1955), Skinner (1963) Glynn (1968), Barlow and Ridway (1969) and a number of authors have studied the variation of protein and free amino acids with moult stages.

It has been shown that the electrophoretic pattern of serum protein of *Blennis pavo* has been modified on transfer from salt to freshwater. The modification of blood protein concentration following the application of hyposmotic stress have been reported in *C.maenas* (Siebers *et.al.*, 1972; Boone and Schoffoeneils, 1979) and *Eriocheir sinensis* (Gilles, 1977). The effect of salinity on total protein concentration in the haemolymph of *P.monodon* has been investigated by Ferraris *et.al.*, (1986). The study of variation in haemolymph and muscle protein and free amino acids in relation with environmental conditions namely salinity has been studied by Scholles (1933) and Bricteux-Gregoire *et.al.*, (1962). The hepatopancreatic protein has been more extensively studied in relation to moulting and maturation in *Partelphusa hydrodromus* (Adiyodi and Adiyodi, 1972) *C.magister* (Allen, 1971) *P japonicus* (Ando *et.al.*, 1977), *P.esculentus* (Barclay *et.al.* 1983) *Coenobita* Spp.. (Lawrence, 1976) etc. Very little work has been carried out relating hepatopancreatic protein with seasonal changes or salinity variations (Amsler and George, 1984; Farooqui and Nagabhushanam, 1983). The study of

variation in free amino acid of the haemolymph has been extensively studied in relation to osmoregulation. Andrews (1967) conducted a study of the haemolymph free amino acid in *Orconectes limosus* with seasonal changes. Studies have been conducted by Binnes (1969) and Harris and Andrews (1985) on *C.maenas*; Florkin (1961), Leersynder (1967a,b) and Vincent and Gilles (1970) on *E. sinensis*; on *C. sapidus* by Lynch and Webb (1973a,b) and Findley and Sickle (1978) and Senthilkumar and Desai (1978) on *Neptunus pelagicus* and *S. serrata*. The study of free amino acid of hepatopancreas has been mainly concentrated on its relation to moulting and maturation (Allen, 1971, Gerard and Gilles, 1972; Martin, 1973; Boone and Claybrook, 1977; Richard, 1980). Only limited work has been done on the variation in the hepatopancreatic free amino acid nitrogen in relation to salinity.

Profound changes take place in lipids, both quantitatively and qualitatively with moulting. The hepatopancreas, also considered as the storage organ is found to be extremely rich in lipids. Several works have been conducted to estimate the hepatopancreatic lipids (Renaud, 1949; O'Connor and Gilbert, 1968; Lautier and Lagarique, 1976). Salinity also exerts influence on the fatty acid composition of lipids. Rotifers fed on *Chlorella* cultured in marine water had more nutritional value than those fed on *Chlorella* cultured in fresh water. The variation of lipid of haemolymph and muscle with moulting and maturation is rather extensive (Millamena *et.al.*, 1984; Guary *et.al.* 1975; Galois 1984, O'Connor and Gilbert, 1969; Siebers, 1972; Sriraman and Reddy, 1977; Tsai *et.al.* 1984; Teshima, 1976; Balazs *et.al.*, 1974; Allen, 1972). The variation in the haemolymph lipids with relation to temperature was studied by Bricton *et.al.*, (1980) in *C.maenas* and Stewart *et.al.* (1972) in *Homarus americanus*. Spaargaren and Mors (1985) studied the haemolymph lipids with respect to environmental changes in *C.maenas*. The muscle lipids content

in relation to seasonal changes was studied in *Callinectes sapidus* by Amsler and George (1984). In addition work has been carried out by Cossins (1976) and Chapelle (1978 a) on the effect of temperature on muscle lipids and by Chapelle (1978b) on salinity induced lipid variations. Work on seasonal variations in the lipids of digestive gland in the shrimp *Pleoticus muelleri* has been conducted by Jeckel *et.al.* (1991).

Studies on the metabolism of carbohydrates in different tissues is not as extensive as those conducted on lipids and proteins. Though the hepatopancreas are the major storage organ the haemolymph seems to play a more important role in the storage and synthesis of polysaccharides (Johnston and Davies, 1971). Baumberger and Dill (1928) studied changes in glycogen and sugar with osmotic pressure and moulting in crabs. Dall (1974) stated that starvation does not vary the glucose and total carbohydrates of the lobsters *Panulirus longipes* as does stress and handling. The carbohydrate level of the hepatopancreas have been studied in a number of decapods in relation to moulting or maturation (Johnston and Davies, 1971, 1972; Lawrence, 1976; Ramamurthi and Veerabhadrachari, 1975). Maghraby *et.al.* (1976) carried out the seasonal variations in hepatopancreas carbohydrates in *M. monoceros*. Changes in the carbohydrates of the muscle of decapods has been studied in relation to moulting or maturation ( Sriraman and Reddy, 1977; Scheer, 1959; Munn, 1963; Dall 1965). Amsler and George (1984) studied the seasonal variations of total carbohydrates in *C.sapidus*.

## **INORGANIC IONS**

Inorganic components account for about half of total osmotic pressure of the tissue (Sehoffeneils and Gilles, 1962). The main inorganic constituents are sodium, potassium and chloride ions. The regulation of these

ions in response to changes in external media has been studied in a number of species. Hyperionic regulation in sea water is achieved by activities of uptake mechanism of sodium, potassium, chloride and calcium (Shaw, 1961a, b; Zanders, 1980). Krogh (1938, 1939) and Maluf (1940) found independent transport of sodium and chloride in the freshwater species of *E. sinensis*. Ionic regulation in *G. crenulata* (Flemister, 1958); *G. crenulata* (Zanders, 1978; Romero and Zanders 1980), *Palaemonetes paludosus* (Dobkin and Manning, 1964) and *Syncaris pacifica* (Born, 1968) have been studied. In the euryhaline carideans *P. serratus* (Pannikar, 1941; Parry, 1954). *P. elegans* (Pannikar, 1941), *P. macrodactylus* (Born, 1968), *P. varians* (Panikkar, 1941, Potts and Parry, 1964), *Palaemonetes intermedius* (Dobkin and Manning, 1964), *C. crangon* (Pannikar, 1968) and *C. septemspinosa* (Haefner, 1969) ionic regulation has been studied. The effect of temperature on sodium and chloride ion concentrations has been studied in *Alpheus viridari* (Ferraris *et.al.*, 1994).

## HISTOLOGY

The gills are the only soft tissue of the crustaceans bathed by the medium. The gills are thus expected to be actively involved in the maintenance of osmotic equilibrium.

Crabs acclimated to dilute media such as low salinity show the posterior gills to be essentially characterised by a complex well-developed network of large apical and digitated folds. These membranous folds produce a large and very characteristic extra-cellular compartment under the cuticle (Pequeux *et.al.*, 1984). All the details vary from one animal to another. A similar structure to that described in the posterior gill epithelium has been demonstrated in gills of other osmoregulating crustaceans (Bulger, 1963;



Komnick, 1963, Philpott and Copeland, 1963a,b; Tandler, 1963; Copeland, 1963a.b, 1968; Copeland and Fitzjarell, 1968; Bielawski, 1971; Foster and Howse, 1978; Martelo and Zanders, 1986; Pequeux and Gilles, 1988).

It has been observed that no substantial work has been done to correlate the variations in osmoregulatory biochemical and inorganic ions with regard to the environment in which they survive in nature. The present study has hence been undertaken to understand the osmoregulatory capacity of *M. dobsoni* the most abundant shrimp of the Cochin backwaters. As shown by other peneids, this species also migrates to deeper more saline waters for maturation and spawning. Thus, this study thus also envisages to find out the triggering factors for migration. This has been attempted by studying the osmotic, bio-chemical and inorganic changes in haemolymph muscle and hepatopancreas tissue and structural changes in the gills in relation to environmental parameters.

## **CHAPTER 2**

### **MATERIALS AND METHODS**

#### **Collection of specimens:**

Samples of the prawn *Metapenaeus dobsoni* having a total length of 30mm and above were collected from the Puduvaipu Station in Vypeen island, Cochin from fixed filtration type bag nets on a fortnightly basis initially and later at monthly intervals. The sampling dates are as mentioned in Table "A" and have not been mentioned in any other table. The prawns collected were immediately transported to the laboratory in plastic bins containing water from the collected site.

#### **Ecological parameters:**

Each time a single water sample from the station was collected in small plastic bottle. The temperature of water was recorded *in situ* using a thermometer graduated from 0-50°C. The pH of the water sample was measured in the laboratory using the ELICO digital pH meter standardised previously using standard buffers. Salinity was estimated using the equation of Baginski and Pierce (1977).

$$\text{Salinity} = \frac{\text{Osmolality of test solution (mOsm/kg H}_2\text{O)}}{1056} \times 36$$

The Osmometer OSMOMAT 030 was standardised with double distilled water and standard sea water before the osmotic pressure of the sample was analysed.

### **Haemolymph collection and analysis**

Live prawns transported to the laboratory were used within 2-3 hours for the collection of haemolymph. The prawns removed from the bin were blotted dry using a blotting paper and checked for intermoult stage. The haemolymph samples were drawn by direct cardiac puncture using a hypodermic syringe fitted with needle no.26. The syringe and needle were rinsed with an anticoagulant (5% trisodium citrate) prior to each collection. The volume of haemolymph collected (especially in the smaller size ranges) was less hence haemolymph samples of 2 prawns of similar size were pooled. Minimum 5 to maximum 15 such pools were obtained. The haemolymph samples were transferred to sterilised vials in iced condition.

The stored haemolymph samples were first utilised for the estimation of osmolarity. 0.05ml (50  $\mu$ l) of haemolymph was taken in a plastic vial and its osmotic pressure was measured with the osmometer, OSMOMAT 030.

The haemolymph samples were then utilised for the estimation of biochemical and inorganic constituents. The total lipid, total carbohydrate, protein and total free amino acid nitrogen were the biochemical parameters estimated. The sodium, potassium and chloride ions were the inorganic constituents analysed.

**Tissue preparation and analysis :** The prawns were grouped into different size ranges of 10mm interval (30-39mm, 40-49mm, 50-59mm 60-69mm). The size at first maturity of *M. dobsoni* is 64.1mm and hence the adults (greater than 60mm) were further segregated into the two sexes. Tissues (muscle and hepatopancreas) were collected from the available size groups and dried to a

constant weight at 60°C in an oven and macerated using an agate mortar and pestle. They were stored in a desiccator with silica gel utilised for further analysis.

The total lipid, total carbohydrate, protein and total free amino acid nitrogen of both these tissues were estimated.

### **Biochemical analysis**

Standard graphs were plotted for total lipid, total carbohydrate, protein and free amino acid nitrogen using suitable standards and employing the same methods adopted for the samples. The optical density (O.D) values obtained were then converted to concentration from the calculation based on the equation.

$$\text{Concentration of Sample} = \frac{\text{O.D of sample}}{\text{O.D of standard}} \times \text{Concentration of standard}$$

**1) Total Lipids** The total lipids of the haemolymph, muscle and hepatopancreas were estimated using the sulphosphovanillin method of Barnes and Blackstock (1973). 0.05ml (50 µl) of haemolymph and 15mg of muscle and 5mg of hepatopancreas samples were kept in 2ml of chloroform and methanol in the proportion of 2:1 overnight in refrigerator. The sample was centrifuged and the supernatant separated and dried free of moisture. 0.5ml of concentrated sulphuric acid was added to the dried tube and heated in a boiling water bath for 10 minutes. To 0.1ml of acid digest for haemolymph and muscle and 25µl for hepatopancreas (made up to 0.1ml with sulphuric acid) 2.5ml of vanillin reagent was added and mixed well. After half an hour the

stable pink colour was read at 530nm using ECIL UV spectrophotometer. Cholesterol was used as standard with 80mg of cholesterol as equivalent to 100mg of total lipids.

**2) Total Carbohydrates** The total simple sugars of haemolymph, muscle and hepatopancreas were estimated using the phenolsulphuric acid method of Dubois *et. al.*, (1956). 0.05ml (50  $\mu$ l) of haemolymph samples were deproteinised in 80% ethanol and 15mg of muscle and 5mg of hepatopancreas samples in TCA. It was found during standardisation that 80% ethanol and TCA had similar protein precipitating properties in case of tissue but in haemolymph Subhasini (1977) has stated 80% ethanol to be a better deproteinizing agent but during this study TCA gave better results with muscle and hepatopancreas. The supernatant of the deproteinised sample was collected after centrifugation. To 0.1ml of supernatant made upto 1ml, 1ml of phenol was added. 5ml of concentrated sulphuric acid was then added directly against the liquid surface to obtain good mixing. The sample was then placed in a boiling water bath for 20 minutes. The stable orange-yellow colour developed was read at 490nm using ECIL UV spectrophotometer D-Glucose was used as standard.

**3) Total free amino acid nitrogen:** The total free amino acid nitrogen of the haemolymph and tissues were estimated using the method of Yemm and Cocking (1955). The haemolymph deproteinised with 80% ethanol and tissues with TCA were centrifuged 0.025ml (25  $\mu$ l) of the supernatant for haemolymph and hepatopancreas and 0.01ml (10  $\mu$ l) for muscle was taken and made upto 1ml with double distilled water. 0.5ml of citrate buffer pH 5.0 (0.2M) and 1.2ml of solution C (50ml solution B of 500mg ninhydrin 10ml of methyl cellosolve + 250ml solution A of 5ml, 0.01M Potassium cyanide in 250ml of methyl

cellosolve) was added. The sample was heated in a boiling water bath for 15 minutes and cooled. 2.3ml of 60% ethanol was added and the stable violet colour read at 570nm using ECIL UV spectrophotometer. Glycine and glutamic acid were used as standards.

**4. Proteins :** The biuret method of Gornall *et. al.*, 1949 was adopted for the estimation of haemolymph protein as it was a preferred method for estimation of crustacean blood protein (Subhasini, 1977). The protein method of Lowry *et. al.*, 1951 was adopted for the hepatopancreas and muscle tissue analysis as it was found to be more sensitive (in terms of concentration).

**(a) Biuret method** 0.05ml (50  $\mu$ l) haemolymph sample deproteinised in 80% ethanol was centrifuged and the precipitate dissolved in 2ml, 1N NaOH. To this 8ml of biuret reagent was added. After half an hour the stable blue colour was read at 540nm using ECIL UV spectrophotometer. Bovine serum albumin crystals were used as standard.

**(b) Lowry method** 15mg of muscle and 5mg of hepatopancreas deproteinised in TCA were centrifuged and the precipitate dissolved in 1ml 1N NaOH. 0.2ml of this was taken and made up to 1ml 0.5ml of reagent (50ml of reagent A of 2gm sodium carbonate in 100ml of 0.1N NaOH + 1ml of reagent B of 500mg copper sulphate in 10ml of 1% sodium potassium tartarate) and 0.5ml of Folin Phenol reagent (1N) were added. The blue colour was read exactly after half an hour at 650nm using ECIL UV spectrophotometer. Bovine serum albumin crystals were taken as standard.

### **Inorganic analysis**

The haemolymph was analysed for sodium, potassium and chloride ions. For the measurement of sodium and potassium ions the haemolymph was diluted 400 times using glass double distilled water. The ELICO digital flame photometer was used and the readings noted. Sodium chloride and Potassium chloride were used as standards. The readings noted are in the form of ppm and converted to meq/L using the conversion factor and taking dilution factor into account with the formula.

$$\text{Sodium meq/L} = \text{Sodium ppm} \times 400 \times 0.0435$$

$$\text{Potassium meq/L} = \text{Potassium ppm} \times 400 \times 0.02558$$

The chloride ions were estimated using the method of Schorenfeld and Lewellen (1964). The haemolymph deproteinised with 80% ethanol was centrifuged. 0.1ml of the supernatant was taken and 3ml chloride reagent added. The orange colour was read after 10 minutes at 480nm using ECIL UV. Spectrophotometer. Sodium chloride was taken as standard.

### **Preparation of gill tissue for histology**

The prawns collected during the monsoon and non monsoon months from Puduvaipu station and brought to the laboratory were used for this purpose. The anteriormost and posteriormost pair of gills were excised from the live prawns and immediately fixed in chilled 3% 0.1M glutaraldehyde prepared in 0.1M cacodylate buffer containing glucose for 12 hours. The fixed tissues were washed in 2 changes of cacodylate buffer and post fixed in 1% Osmium tetroxide for about one hour till fully osmicated. Tissues were again washed in cacodylate buffer and dehydrated through an ascending series of

ethanol grades. It was kept for 2 changes in absolute alcohol and then passed through an ascending series of embedding medium (Spurr) in alcohol. The tissue was given two treatments of pure spurr (without polymeriser) and once with polymer. It was made into blocks by pouring fresh embedding medium in plastic moulds and placing the tissue in it. The moulds were placed in the incubator at 70°C for 24 hours.

Polymerised blocks were trimmed for ultramicrotomy. Semithin (1  $\mu$ m thick) sections were stained in toluidene and used for light microscopy. Silver to grey ultrathin (50-150nm) sections were cut with a LKB ultramicrotome with glass knives. Ultrathin sections were collected on copper or platinum grids and post stained in uranyl acetate and lead citrate (Hayat, 1970). After drying, the grids were examined under the Philips transmission electron microscope and areas of interest photographed.

### **Statistical Analysis**

All the data were subjected to standard statistical methods. Of the total replicas available for each size group for each study day, best 5 were considered for statistical methods. The data is represented as the mean  $\pm$  standard deviation for 5 readings through out the thesis. The "-" (Dash) in the tables signifies that there was no reading available due to unavailability of sample of particular size group at the time of sampling. The independent effect of the environmental parameters was determined by conduction of correlation analysis and combined influence by multiple regression.



**CHAPTER - 3**  
**BIOCHEMICAL STUDIES**

## RESULTS

The variation in the salinity, temperature and pH of the Cochin backwaters from March 1989 to October 1990 has been documented in this work. Table A shows a tabular representation of the data.

The study of the environmental parameters in the Cochin backwaters over the period of study and their effect on the biochemical nature of the hepatopancreas, haemolymph and muscle are presented here.

### A) HAEMOLYMPH BIOCHEMISTRY

The haemolymph samples were obtained from the various size groups of *M.dobsoni* during the study period of March 1989 to October 1990. The samples were analysed for osmolality, total lipids, total carbohydrate, protein, total free amino acid nitrogen, sodium, potassium and chloride contents.

**(i) Osmolality :** The effects of the environmental salinity, temperature and pH on this parameter on the various size groups are depicted in Table 1. The haemolymph osmolality varied from 214.29 to 554.00 mOsm/Kg of water in prawns of 30-39 mm. Correlation analysis has shown a highly significant positive influence of salinity (0.7744) and negative influence with pH (-0.6597) and temperature (-0.5967) (Table 9). Multiple regression also shows a significant influence of the three environmental parameters combinedly.

In prawns of 40-49 mm, the osmolality varied from 228.33 to 559.38 mOsm/kg water (Table 1). Correlation analysis shows a highly

TABLE A

Seasonal variation of the three environmental sites at the collection center during the study period

Month	Salinity (ppt)	Temp (°C )	pH
March	25.96	25.0	8.21
April	28.35	27.0	7.64
April	20.40	29.0	7.17
May	08.88	30.5	6.80
May	10.90	29.0	6.97
June	04.78	30.0	7.34
June	03.42	27.5	7.50
July	06.38	31.0	7.61
July	02.73	30.0	8.02
August	07.51	29.0	8.20
August	05.46	29.5	8.35
September	04.12	32.0	8.60
September	03.76	28.0	7.20
October	02.73	30.0	7.27
November	06.15	27.5	7.18
December	06.83	28.0	7.21
January	23.91	29.0	7.24
February	24.25	28.0	7.32
March	25.28	29.0	7.27
April	15.37	28.0	7.57
May	09.56	32.0	7.30
June	11.27	29.0	7.68
July	02.39	28.0	7.31
August	13.66	28.0	7.30
September	11.95	27.5	7.39
October	18.78	29.0	7.49

significant and positive influence of salinity (0.6102) (Table 10). No significant influence of temperature and pH was observed on osmolality. Multiple regression shows a significant combined influence with salinity as the predominant factor. A significant positive correlation has been found between osmolality and sodium content of the haemolymph (0.5344) (Table 10). The osmolality of *M.dobsoni*, 50-59 mm, varied from 252.86 mOsm/Kg water to 557.50 mOsm/Kg water (Table 1). Salinity having a significant positive influence (0.4962) and pH a negative influence (-0.5086) as indicated by correlation analysis but no significant effect of temperature was observed (Table 11). Multiple regression analysis shows a significant influence and confirms the correlation analysis results. A significant correlation of the osmolality has been found with protein content (0.5317) and sodium content (0.4499) of the haemolymph (Table 11).

Females, (60-69 mm) have shown a osmolality variation from 350.00 to 627.50 mOsm/Kg water and from 333.00 to 522.00 mOsm/Kg water in males (Table 1). Salinity is the only environmental factor having a significant influence in the females (0.5829) and in males (-0.9329) as shown by correlation analysis (Table 12 and 13). Correlation analysis recorded no influence of pH and temperature. Multiple regression also shows a significant combined influence predominated by salinity. In females, a correlation has been observed between osmolality and total carbohydrates (0.5160) and sodium ions (0.5903). In the males, a significant correlation has been observed between osmolality and free amino acid nitrogen (0.6848).

**(ii) Total lipids:** The study on the haemolymph total lipid content as it varied during the study period and in the size ranges has been outlined below.

The total lipid content varied from 54.02 mg/100 ml to 241.38 mg/100 ml (Table .2) in *M.dobsoni* of the size range of 30-39 mm. Correlation analysis shows a significant negative correlation with temperature (-0.6307) (Table 9). pH and salinity were shown to have no significant involvement by correlation analysis. The significant effect of temperature has been duplicated in the multiple regression analysis which shows a significant combined influence of the environmental parameters.

Prawns of 40-49 mm and 50-59mm size groups have shown a variation of 51.73 to 839.83 mg/100 ml and 182.76 to 616.09mg/100ml respectively (Table 2). Neither correlation (Table 10 and 11) nor multiple regression show any significant influence of any of the environmental parameters.

The total lipid content in haemolymph in case of female prawns (60-69mm) was found to be in the range of 243.68 to 839.08 mg/100 ml. Males of the same size group had their total lipid content varying from 216.09 to 698.85 mg/100 ml (Table 2). Correlation analysis has shown a significant negative effect of pH in the female prawns (-0.5446) (Table 12) and in case of males (Table 13) a positive effect of temperature (0.5337) Multiple regression has also been found to be significant with all the three environmental parameters as contributing factors in female prawns and in case of males, temperature and pH were the main contributing factors.

**(iii) Total carbohydrates :** The variation in the total carbohydrate content of haemolymph with seasons on the effect of size groups has been given below.

*M.dobsoni*, 30-39 mm, showed their total carbohydrate content to vary from 24.71 to 386.27 mg/100 ml (Table 3). Correlation analysis does not show any significant influence of the environmental parameters (Table 9). Multiple regression shows a significant combined influence of the environmental parameters of which temperature is the predominant factor. The total carbohydrate also showed a negative correlation with chloride content (-0.5560) (Table 9).

Prawns of 40-49 mm and 50-59 mm size range have shown a variation in their total carbohydrate content from 17.65 to 505.86 and 13.37 to 421.57mg/100 ml (Table 3) respectively. Neither correlation (Table 10 and 11) nor multiple regression analysis has been able to give any indication of the influence of environmental parameters as the cause of these variations.

In the size group of 60-69 mm, the female prawns had their total carbohydrate content varying from 47.06 to 137.26 mg/100 ml (Table 3). A significant negative influence has been shown by the effect of pH (-0.5476) (Table 12). However salinity and temperature have shown no significant influence as observed by correlation analysis. The multiple regression analysis also shows a significant combined influence of the environmental parameters and pH was confirmed as the main contributing factor. A significant correlation between total carbohydrates and osmolality (0.5160) has been observed. The males of the same size range had their total carbohydrate content varying in the range of 35.29 to 80.39 mg/100 ml (Table 3). Neither correlation (Table 13) nor multiple regression analysis

has shown any significant influence of any of the environmental parameters on the total carbohydrate content.

**(iv) Protein :** The variation in the protein content in the four size groups of *M.dobsoni* (30-39 mm, 40-49 mm, 50-59 mm, 60-69 mm) and the influence of the environmental parameters, if any, has been shown in the results detailed below.

In prawns of 30-39 mm size range, the protein content varied from 848.48 to 3212.12 mg/100 ml (Table 4). Correlation analysis has shown that none of the environmental parameters show significant influence on the protein variations. Multiple regression shows a significant combined effect of the environmental parameters with salinity and temperature as the main contributing factors.

The range of the protein content of the haemolymph was from 545.45 to 4424.24 mg/100 ml (Table 4) in *M.dobsoni* of 40-49 mm. Correlation analysis has indicated a significant negative influence of pH (-0.4651) (Table 10) but salinity and temperature do not show any significant influence. Multiple regression analysis shows no significant influence of the environmental parameters. A correlation has also been observed between the protein content and the free amino acid nitrogen content (0.4811) (Table 10).

Prawns of 50-59 mm size range showed a protein content variation of 969.70 to 5181.82 mg/100 ml (Table 4). Neither correlation (Table 11) nor multiple regression analysis has shown any of the environmental parameters to be a significant contributing factor to the variation. A significant positive correlation has been observed with protein content and osmolality

concentration (0.5317) (Table 11).

In the 60-69 mm size group the females showed a variation from 3394.55 to 9333.33 mg/100 ml (Table 4). Neither correlation (Table 12) nor multiple regression analysis has shown any of the environmental parameters to be significant contributing factor to the variation. The males in the same size group showed a variation between 2424.24 to 6969.69 mg/100 ml (Table 4). A significant negative influence of salinity (-0.5611) and no effect of pH and temperature on protein variations has been observed through correlation analysis (Table 13). Multiple regression has also shown a significant influence of the environmental parameters collectively with salinity and temperature contributing to the bulk of the influence.

**(v) Free amino acid nitrogen :** The variation in the free amino acid nitrogen content of the haemolymph of the four size groups of *M.dobsoni* studied (30-39 mm, 40-49 mm, 50-59 mm, 60-69 mm) during the period of March 1989 to October 1990 and the effect of the environmental parameters is documented below.

The variation in the free amino acid nitrogen content in 30-39 mm and 40-49 mm sized prawns is depicted in Table 5. No significant effect of the environmental parameters has been shown by correlation analysis (Table 9 and 10) in both the size groups. Multiple regression, however, shows a highly significant combined influence of the environmental factors of which salinity and temperature are the main contributors in 30-39 mm group whereas in the 40-49 mm group is not influenced.

The free amino acid nitrogen content in the 50-59 mm sized *M.dobsoni* varied from 77.57 to 760.00 mg/100 ml (Table 5). Correlation has



shown a significant positive influence of salinity (0.6189) and no influence of pH and temperature (Table 11). Multiple regression analysis has shown a significant combined influence of the environmental parameters of which the effect of salinity and temperature are predominant. A negative correlation has also been observed with the variation in the free amino acid nitrogen content and chloride ion content of the haemolymph (-0.4930) (Table 11).

The free amino acid nitrogen content in the females and males of the 60-69 mm size group is shown in Table 12 and 13. Correlation analysis has shown a significant positive influence of salinity (0.6058) (Table 12) in the female and significant negative influence of pH (-0.5809) (Table 13) in the males. Temperature does not contribute significantly to the free amino acid variation in both the sexes. pH and salinity does not show any significant influence in both the sexes. Multiple regression has shown a significant combined influence of the three environmental parameters studied of which the effect of salinity and temperature are predominant. A significant negative correlation has been observed between the free amino acid nitrogen and sodium ion concentration of the haemolymph (-0.5074) (Table 13) in the males.

**(vi) Sodium ion :** The variation in the sodium ion content of the haemolymph of *M.dobsoni* in the four size groups viz., 30-39 mm, 40-49 mm 50-59 mm and males and females of 60-69 mm size are detailed below. The influence of the three environmental parameters, salinity, temperature and pH has also been detailed below.

The sodium ion concentration in the 30-39 mm sized prawns varied from 273.42 to 623.87 meq/L (Table 6). Correlation analysis (Table

9) has shown no significant influence of the environmental parameters. Multiple regression analysis has, however, shown a significant influence of the three environmental parameters of which the effects of temperature and pH are predominant.

In the 40-49 mm and 50-59 mm sized *M.dobsoni*, the sodium ion concentration ranged from 156.60 to 879.30 and from 195.83 to 663.16 meq/L (Table 6) respectively.

None of the environmental parameters examined have been explained as factors influencing the variation either by correlation (Table 10 and 11) or multiple regression analysis. A positive correlation has been observed with osmolality and sodium ion concentration of the haemolymph (0.5344 and 0.4499) (Table 10 and 11) in the two size groups respectively. The females and males of 60-69 mm group had their sodium ion concentration varying in the range of 254.78 to 777.84 and 341.50 to 595.39 meq/L (Table 6) respectively. Correlation analysis (Table 12 and 13) has shown no significant influence of the environment in both the sexes. The influence of environment with predominant effect of salinity and temperature was observed in the males but no influence was observed in the females. A positive correlation has been observed between sodium ion concentration and osmolality of haemolymph on females (0.5903) (Table 12) and a negative correlation with free amino acid nitrogen content (-0.5074) in the males (Table 13).

**(vii) Potassium ions** : The variation in the potassium ion concentration in the size groups 30-39 mm, 40-49 mm, 50-59 mm and 60-69 mm and the

influence, if any, of the environmental parameters as individually or combined entities are discussed below.

The variation in Potassium concentration in the haemolymph of the different size groups and male and females of 60-69 mm of *M.dobsoni* is given in Table 7. Neither the correlation nor multiple regression analysis have been able to show any significant influence of the three environmental parameters studied in all the size groups (Table 9-13).

**(viii) Chloride ions :** The variation in the chloride ion concentration as influenced by the three environmental parameters viz., salinity, temperature and pH in the four size groups of *M.dobsoni* studied, namely, 30-39mm, 40-49 mm, 50-59 mm, and 60-69 mm are detailed below.

The variation in the chloride ion concentration of the haemolymph in different size groups and males and females of 60-69 mm of *M.dobsoni* are given in Table 8. Neither correlation nor multiple regression has been able to show any significant influence of the environmental parameters in the 30-39 mm, 40-49 mm, 50-59 mm and females of 60-69 mm (Table 9-12). Correlation analysis (Table 13) has not indicated any significant effect of the three environmental parameters as separate entities in the males of 60-69 mm.

The combined influence of the three environmental parameters was shown to be significant by multiple regression analysis of which salinity shows a predominant effect. A significant negative correlation has been observed with the chloride ion and total carbohydrates (-0.5560) in 30-39 mm and with free amino acid nitrogen (-0.4930) in 50-59 mm (Table 9 and 11).

TABLE 1

Seasonal variation in the osmolarity of the haemolymph (mOsm/kg H<sub>2</sub>O) in four size groups of *M.dobsoni* during the study period

Month	30 - 39mm	40 - 49mm	50 - 59mm	60 - 69mm FEMALE	60 - 69mm MALE
March 1989		476.00±0.12			
April	554±50	531.80±46.28			
April	504±93.51	559.38±148.19			
May	475±5.00	510.00±29.9	555.55±42.19		
May		456.11±46.56	485.00±30.95		
June	430±49.49	454.71±20.03	456.67±24.94		
June		444.29±48.66	442.00±22.86		490±48.58
July		424.00±93.83	445.00±92.06	472.50±29.85	
July		480.00±36.25	461.25±41.66	490.00±10.00	
August		333.33±39.44	345.71±38.49	483.33±24.26	
August		330.00±38.41	351.43±19.58	367.50±19.20	522.00±30.59
September	214.29±62.07	281.00±18.14	252.86±19.79		386.00±28.00
September		244.00±14.28	437.14±15.78		
October		228.33±10.67	335.71±16.78		
November			360.00±20.00	428.00±11.66	424.00±10.20
December	390.00±6.32	400.00±17.89	426.00±8.00		
January 1990		456.67±11.06			
February		462.00±7.48	557.50±12.99		
March		480.00±14.14			
April		476.00±8.00	530.00±12.25	627.50±40.95	
May		336.00±13.56		605.00±11.18	
June		360.00±8.00	380.00±8.00	350.00±17.89	342.50±12.99
July	370.00±14.14	320.00±74.83			333.00±5.00
August		363.00±5.00	430.00±14.14	446.67±5.00	
September	413.33±20.55	423.33±5.70	440.00±70.71		
October		416.00±16.25	497.50±25.86		

**TABLE 2**  
**Seasonal variation in the total lipid content (mg/100ml) of the haemolymph in the four size groups of *M. dobsoni* during the study period**

Month	30 - 39mm	40 - 49mm	50 - 59mm	60 - 69mm FEMALE	60 - 69mm MALE
March 1989	-	267.59±39.31	-	-	-
April	241.38±175.65	519.54±18.10			
April	085.07±11.72	051.73±18.57			
May		310.34±64.93	220.69±28.15		
May		273.56±95.95	356.32±151.13		
June		202.30±22.76	240.00±45.41		
June	075.87±13.71	141.38±33.19	266.66±8.60	303.45±20.30	390.80±8.61
July		516.09±145.28	616.09±74.13	514.94±3.25	
July		399.99±33.79	525.29±152.16	606.89±177.45	643.68±39.55
August		193.10±5.63	282.18±21.66	243.68±11.72	216.09±8.60
August		349.42±69.50	443.68±145.63	496.55±11.26	
September		280.45±37.49	182.76±7.45		
September	054.02±8.60	243.28±14.91	234.48±29.19		
October		236.55±5.16	317.93±34.79		
November			400.69±7.03	606.65±24.52	583.45±16.09
December	181.24±36.47	288.97±5.52	425.17±7.87		
January 1990		265.52±7.45			
February		301.15±17.20	316.09±5.86		
March		239.66± 5.63		779.31±73.85	
April		242.53±12.69	314.94±23.44	657.47±14.17	
May		117.24±11.26		839.08±86.01	698.85±18.10
June		839.83±36.72	590.80±45.52		680.46±8.60
July	200.00±27.73	195.40±14.17			
August		228.73±10.66	416.09±50.78	452.88±22.76	
September	178.16±3.25	185.06±22.58	356.32±90.33		
October		171.26±17.58	222.95±88.24		

TABLE 3

Seasonal variation in the total carbohydrate content (mg/100ml) of the haemolymph in the four size groups of *M. dobsoni* during the study period

Month	30 - 39mm	40 - 49mm	50 - 59mm	60 - 69mm FEMALE	60 - 69mm MALE
March 1989		170.59±88.34			-
April	29.41±12.70	066.67±28.14			
April	31.38±5.55	019.41±12.83			
May		021.57±13.87	021.57±13.87		
May		086.27±38.82	066.67±14.67		
June	73.53±2.94	064.71±12.71	072.94±9.56		
June		123.53±112.7	047.05±25.41	049.02±16.87	50.98±20.00
July		086.27±5.55	100.98±9.85	111.76±4.81	
July		071.90±35.63	071.48±10.85	052.38±1.35	58.09±5.87
August		027.45±5.54	047.06±6.79	105.88±9.61	80.39±7.34
August		017.65±4.81	024.51±5.27	047.06±9.61	
September	27.45±5.54	025.49±7.33	031.37±5.54		
September		039.21±5.55	062.75±13.00		
October		081.18±9.41	101.18±20.51		
November			057.65±7.80	051.77±12.00	56.47±8.80
December	24.71±5.76	057.65±7.81	037.65±8.80		
January 1990		066.67±14.67			
February		039.22±11.09			
March		062.75±22.18	056.86±2.77	111.77±12.71	
April		090.22±19.98	109.80±5.55	137.26±19.99	
May		041.18±20.94		062.75±29.35	35.29±25.42
June		125.49±2.77	037.26±13.87		54.90±22.18
July	88.24±22.00	103.92±13.86			
August		109.80±5.54	013.37±2.77	125.49±14.68	
September	386.27±38.52	398.04±15.37	421.57±71.29		
October		505.86±19.21	307.84±60.44		

TABLE 4

Seasonal variation in the protein content (mg/100ml) of the haemolymph in the four size groups of *M. dobsoni* during the study period

Month	30 - 39mm	40 - 49mm	50 - 59mm	60 - 69mm FEMALE	60 - 69mm MALE
March 1989	1727.27±90.91	1545.46±913.62			
April	1393.94±85.71	2454.54±181.82			
April		1854.54±1282.56			
May		2272.72±1338.15	4060.60±1879.77		
May	2181.82±363.64	2000.00±392.77	1454.55±296.91		
June		1818.18±296.90	1563.64±625.63		
June		2242.42±226.77	1606.06±642.11		2818.18±90.9
July		2545.45±890.72	3696.97±542.08	4181.82±1215.1	6969.69±309.3
July		3939.39±326.77	5181.82±162.99	9333.33±747.19	3151.55±171.4
August		0545.45±148.45	1606.06±220.61	3457.55±148.45	
August	0848.48±171.42	0848.48±309.03	1303.03±774.36	3818.18±257.13	
September	0969.70±342.84	0969.70±171.42			
September		2242.42±226.76	2060.61±342.84		
October	1745.46±424.07	3600.00±241.21	2545.45±381.38		
November			1600.00±493.26	3394.55±382.92	3309.09±241
December		3636.36±229.98	1890.91±723.63		
January1990		3636.36±148.45			
February		3212.12±226.72	3575.76±85.71		
March		3757.57±171.42		4242.42±453.54	
April		4000.00±296.91	3939.39±226.77	5212.12±171.42	
May	3212.12±1010.51	3272.73±296.91		3757.57±171.42	3393.14±171
June		2000.00±535.26			2424.24±342
July	1515.15±309.03	4000.00±2285.42	2363.63±1268.39		
August		0363.64±392.77	4545.46±903.01	6303.03±453.53	
September		2242.43±669.41	3212.12±521.35		
October		4424.24±1010.5	1515.52±1587.37		

TABLE 5

Seasonal variation in the free amino acid content (mg/100 ml) of the haemolymph in the four size groups of *M. dobsoni* during the study period

Month	30 - 39mm	40 - 49mm	50 - 59mm	60 - 69mm FEMALE	60 - 69mm MALE
March 1989		085.62±9.92			
April	080.93±3.24	102.42±12.51			
April	088.43±2.12	113.90±39.39			
May		068.58±37.61	109.91±13.49		
May		099.42±3.54	103.41±23.55		
June	112.50±4.50	090.43±6.49	132.30±16.44	080.27±1.24	104.16±0.75
June		107.91±9.79	143.89±21.03	088.93±13.47	115.90±3.74
July		054.70±19.45	077.57±11.51	111.91±6.16	223.75±6.37
July		090.93±9.02	097.92±6.91	225.00±6.12	
August		141.25±6.37	196.25±4.68	273.75±8.10	
August		160.00±3.54	212.50±5.99		
September	113.13±2.34	141.25±6.37	175.00±9.35		
September		168.75±13.35	212.50±18.71		
October		352.50±9.49	273.00±6.00		
November			246.00±8.75	679.50±18.12	
December	203.25±19.03	325.50±16.84	197.63±7 13	693.00±24.46	
January 1990		292.50±7.07			
February		335.00±15.41	357.50±7.07		
March		537.50±9.35		622.00±6.13	
April		375.00±16.20	400.00±9.35	490.00±12.75	
May		285.00±6.12		332.50±14.14	307.50±12.25
June		228.75±14.03	254.92±18.47		260.00±24.75
July	545.00±56.89	483.75±35.31			
August		307.50±113.9	760.00±119.4	875.00±35.36	
September	437.50±24.75	371.25±70.02	452.33±17.91		
October		433.75±30.65	387.50±41.68		



TABLE 6

Seasonal variation in the sodium ion concentration (meq/L) of the haemolymph in the four size groups of *M. dobsoni* during the study period

Month	30 - 39mm	40 - 49mm	50 - 59mm	60 - 69mm FEMALE	60 - 69mm MALE
March 1989		379.93±16.14			
April	348±197.72	404.55±90.11			
April		462.84±85.30			
May		156.6 ± 15.01	536.5 ±41.62		
May		511.89±61.93	663.16±60.84		
June	623.87±15.09	644.28±173.34	425.55±208.66	623.82±102.58	
June		264.29± 81.27			
July		332.06±21.64	391.21±42.08	677.00±44.79	595.39±35.57
July		745.45±18.11	461.25±41.66	483.33±24.26	509.61±13.89
August		351.02±18.86	424.57±54.78	475.03±26.38	
August		879.30±21.33	348.16±19.36	634.55±26.85	
September		334.69±13.60	352.21±18.99		
September		306.29±29.70	306.23±19.49		
October		245.29±29.70	348.34±34.32		
November			416.91±44.29	457.62±56.38	400.17±21.45
December	379.80±43.40	265.20±20.51			
January 1990		496.55±15.01	575.92±25.08		
February		533.60±10.85			
March		549.06±16.41	648.64±47.92	777.84±40.95	
April		542.14±38.11		728.63±38.72	588.91±14.18
May		447.44±52.66	363.96±32.68	603.77±10.05	341.50±73.52
June		302.79±152.7			
July	423.47±34.95	416.28±70.01	195.83±6.34		
August		222.59±20.84	588.96±14.27	254.78±54.54	
September	273.42±40.23	292.76±62.41	557.82±61.18		
October		481.93±70.68			

TABLE 7

Seasonal variation in the potassium ions (meq/L) of the haemolymph in the four size groups of *M. dobsoni* during the study period

Month	30 - 39mm	40 - 49mm	50 - 59mm	60 - 69mm FEMALE	60 - 69mm MALE
March 1989		3.67±1.36			
April	3.6±1.28	4.48±0.56			
April	4.95±1.05	5.97±1.91			
May		2.82±1.91	06.99±1.69		
May		8.97±2.22	07 12±0.81		
June		3.13±0.81	06.54±0.81		
June	5.12±0.85	6.54±2.13	04.83±1.61		
July		4.73±0.97	07.68±0.83	10.81±0.39	08.82±0.80
July		7.39±1.39	09.39± 1.91	11.67±2.13	11.10±0.39
August		4.73±0.97	07.87±1.79	10.82±0.39	
August		9.39±1.39	09.96±0.81	12.24±1.61	
September	5.97±1.39	9.39±1.40	09.39±1.40		
September		7 11±2.90	08.82±1.61		
October		4.83±0.80	07.68±0.40		
November			09.58±1.60	10.82±0.39	10.54±0.39
December	6.44±0.66	8.33±1.86	10.54±0.40		
January 1990		6.90±0.66			
February		5.97±0.40			
March		4.83±1.61	09.30±1.36	10.44±0.93	
April		6.44±0.66	08.54±0.82	10.16±0.81	
May		7.29±1.52			09.78±0.93
June		7.00±3.48	08.60±1.74		10.41±0.91
July	4.27±1.39	6.66±0.57		10.81±0.39	
August		7 12±0.81	09.11±1.07		
September	4.84±0.80	7 12±0.81	09.68±1.45		
October		7.68±1.40	09.68±0.41		

TABLE 8

Seasonal variation in the Chloride ions (meq/L) of the haemolymph in the four size groups of *M. dobsoni* during the study period

Month	30 - 39mm	40 - 49mm	50 - 59mm	60 - 69mm FEMALE	60 - 69mm MALE
March 1989		369.36±202.24			
April	473.54±200.68	402.50±157.48			
April	407.24±194.35	388.30±70.87			
May		265.18±35.43	501.94±70.87		
May		397.77±206.19	435.65±109.63		
June		473.53±58.38	478.18±37.12	577.71±35.43	625.06±101.12
June	288.87±24.15	426.18±46.39	748.18±70.87		
July		317.27±37.29	397.77±46.40	554.04±14.21	
July		525.62±34.80	554.03±53.16	511.42±106.31	
August		274.65±58.38	606.12±48.69	544.56±220.79	525.62±70.55
August		416.71±58.38	520.89±116.76	378.83±35.43	
September	483.00±46.40	473.53±109.63	549.30±53.38		
September		615.59±147.33	530.36±222.91		
October		539.82±101.12	776.59±194.55		
November			596.65±23.20	644.00±35.44	596.65±61.37
December	558.77±58.38	483.00±144.88	077.71±81.47		
January 1990		511.41±202.24			
February		430.91±17.72	407.74±70.87		
March		383.56±191.65	378.83±210.49		
April		364.62±24.15	501.94±93.75	454.59±206.19	426.18±46.39
May		303.06±35.43		478.27±43.92	345.68±94.47
June		243.87±94.29	279.88±90.09		
July	407.24±171.49	480.64±219.65			
August		139.70±12.07	118.38±	208.35±63.88	
September	170.47±32.29	175.21±65.95	430.91±164.99		
October		487.74±101.34	540.29±145.93		

TABLE 9  
CORRELATION ANALYSIS FOR THE HAEMOLYMPH OF 30 - 39 mm GROUP

Parameter	Total Lipids	Total Carbohy- drates	Protein	FAA	Osmolality	Sodium	Potassium	Chloride
Salinity	0.1617	- 0.0208	- 0.2789	- 0.4722	0.7744**	0.0541	- 0.2308	0.0274
Temperature	- 0.6307*	- 0.4003	- 0.3857	- 0.4378	- 0.5967*	0.0560	0.2321	0.0634
pH	- 0.3281	- 0.2070	0.4830	- 0.3600	- 0.6597**	- 0.3076	0.1242	0.1403
Total lipids	1.0000	0.0770	0.2821	0.3986	0.1751	- 0.0788	- 0.0832	0.2371
Total Carbohydrate	0.0770	1.0000	- 0.0435	0.4830	- 0.0323	- 0.3447	- 0.1554	- 0.5560*
Protein	0.2821	- 0.0435	1.0000	0.4671	0.0439	- 0.1027	0.1644	0.3194
FAA	0.3986	0.4830	0.4671	1.0000	- 0.3161	- 0.3098	- 0.0862	- 0.0790
Osmolality	0.1751	- 0.0323	0.0439	- 0.3161	1.0000	0.4091	- 0.2696	- 0.0897
Sodium	- 0.0788	- 0.3447	- 0.1027	- 0.3098	0.4091	1.0000	- 0.1318	- 0.3157
Potassium	- 0.0832	- 0.1554	0.1644	- 0.0862	- 0.2696	- 0.1318	1.0000	0.1235
Chloride	0.2371	- 0.5560*	0.3194	- 0.0790	- 0.0897	- 0.3157	0.12355	1.0000

FAA - Free Amino Acid nitrogen  
 \* - Significant at 1% level  
 \*\* - Significant at 10% level

TABLE 10  
CORRELATION ANALYSIS FOR THE HAEMOLYMPH OF 40 - 49 mm GROUP

Parameter	Total Lipids	Total Carbohydrates	Protein	FAA	Osmolality	Sodium	Potassium	Chloride
	0.0662	0.1705	0.1577	0.1976	0.6102**	0.1801	- 0.3432	- 0.1211
Salinity	0.0309	- 0.2853	- 0.0304	- 0.2028	- 0.2826	0.2128	0.4277	- 0.0052
Temperature	0.2125	- 0.0332	- 0.4651*	- 0.2505	- 0.2375	- 0.1013	0.0877	0.0398
pH	1.0000	- 0.1196	- 0.0947	- 0.2103	0.0342	- 0.0143	0.0544	- 0.1319
Total lipids	- 0.1196	1.0000	0.2844	0.3098	0.0568	- 0.0799	- 0.0020	- 0.1578
Total Carbohydrate	- 0.0947	0.2844	1.0000	0.4811*	0.1262	0.3319	0.0308	0.3118
Protein	- 0.2103	0.3098	0.4811*	1.0000	- 0.2212	- 0.3449	0.0354	- 0.2664
FAA	0.0342	0.0568	0.1262	- 0.2212	1.0000	0.5344*	- 0.2199	- 0.1537
Osmolality	- 0.0143	- 0.0799	0.3319	- 0.3449	0.5344*	1.0000	0.2138	0.3163
Sodium	0.0544	- 0.0020	0.0308	0.0354	- 0.2199	0.2138	1.0000	0.0811
Potassium	- 0.1319	- 0.1578	0.3118	- 0.2664	- 0.1537	0.3163	0.0811	1.0000
Chloride								

FAA - Free Amino Acid nitrogen

\* - Significant at 1% level

\*\* - Significant at 10% level

TABLE 11

CORRELATION ANALYSIS FOR THE HAEMOLYMPH OF 50 - 59 mm GROUP

Parameter	Total Lipids	Total Carbohy -drates	Protein	FAA	Osmola -lity	Sodium	Potassium	Chloride
	- 0.0733	0.3677	0.3948	0.6189**	0.4962*	0.1992	0.2611	0.4395
Salinity	0.1417	-0.1058	0.0397	- 0.4336	- 0.1378	- 0.1111	- 0.2667	0.1960
Temperature	0.1639	- 0.2033	- 0.1373	- 0.1685	- 0.5086*	0.0510	- 0.0029	0.1465
pH	1.0000	- 0.1144	0.1368	- 0.1316	- 0.0036	- 0.0468	- 0.0891	- 0.2601
Total lipids	- 0.1144	1.0000	0.3828	0.3151	0.2911	0.2746	0.1548	- 0.0180
Total Carbohydrate	0.1368	0.3828	1.0000	0.4196	0.5317	0.2794	0.0853	- 0.2808
Protein	- 0.1316	0.3151	0.4196	1.0000	0.1089	- 0.2083	0.3940	- 0.4930*
FAA	- 0.0036	0.2911	0.5317*	0.1089	1.0000	0.4499*	- 0.2239	- 0.2111
Osmolality	- 0.0468	0.2746	0.2794	- 0.2083	0.4499*	1.0000	- 0.1562	0.1258
Sodium	- 0.0891	0.1548	0.0853	0.3940	0.2239	- 0.1562	1.0000	0.0467
Potassium	- 0.2601	- 0.0180	- 0.2808	- 0.4930*	- 0.2111	0.1258	0.0467	1.0000
Chloride								

FAA - Free Amino Acid nitrogen

\* - Significant at 1% level

\*\* - Significant at 10% level

TABLE 12  
CORRELATION ANALYSIS FOR THE HAEMOLYMPH OF THE FEMALES OF 60 - 69 mm GROUP

Parameter	Total Lipids	Total Carbohy- -drates	Protein	FAA	Osmola- -lity	Sodium	Potassium Chloride
Salinity	0.4233	0.2934	- 0.1121	0.6058*	0.5829*	0.3285	- 0.0663 - 0.2264
Temperature	0.4804	0.2815	- 0.0578	- 0.4365	- 0.1290	0.2304	0.2512 - 0.0271
pH	- 0.5446*	- 0.5476*	0.1370	- 0.3844	- 0.4622	0.0301	0.3281 - 0.0960
Total lipids	1.0000	0.2975	0.0003	0.2183	0.4395	0.3606	- 0.2035 - 0.0393
Total	0.2975	1.0000	- 0.1243	0.0251	0.5160*	0.0793	- 0.3386 0.1225
Carbohydrate							
Protein	0.0003	- 0.1243	1.0000	- 0.0471	0.1979	- 0.1334	0.0266 - 0.1950
FAA	0.2183	0.0251	- 0.0471	1.0000	0.0992	- 0.4299	0.3190 - 0.4539
Osmolality	0.4395	0.5160*	0.1979	0.0992	1.0000	0.5903*	- 0.4232 0.1386
Sodium	0.3606	0.0793	- 0.1334	- 0.4299	- 0.5903*	1.0000	- 0.3489 0.3243
Potassium	- 0.2035	- 0.3386	0.0266	0.3190	- 0.4232	- 0.3489	1.0000 - 0.1315
Chloride	- 0.0393	0.1225	- 0.1950	- 0.4539	0.1386	0.3243	- 0.1315 1.0000
FAA	- Free Amino	Acid nitrogen					
*	- Significant at 1% level						
**	- Significant at 10% level						

**TABLE 13**  
**CORRELATION ANALYSIS FOR THE HAEMOLYMPH OF THE MALES OF 60 - 69 mm GROUP**

Parameter	Total Lipids	Total Carbohydrates	Protein	FAA	Osmolality	Sodium	Potassium	Chloride
Salinity	0.2033	- 0.0620	- 0.5611*	0.2550	- 0.9329**	- 0.5008	0.4307	- 0.4292
Temperature	0.5337*	0.1048	0.3430	- 0.4208	- 0.1642	0.4786	- 0.0079	0.2810
pH	- 0.3664	0.3136	0.3433	- 0.5809*	0.1849	0.1866	0.0167	- 0.1987
Total lipids	1.0000	0.2352	0.3505	- 0.0720	0.0931	0.0396	0.0296	- 0.0360
Total Carbohydrate	0.2352	1.0000	0.2416	- 0.2508	0.2250	0.1097	0.4852	0.3055
Protein	0.3505	0.2416	1.0000	- 0.2909	0.6848**	0.4787	- 0.3512	- 0.0387
FAA	- 0.0720	- 0.2508	- 0.2909	1.0000	- 0.3835	- 0.5074*	0.1983	0.0683
Osmolality	0.0931	0.2250	0.6848**	- 0.3835	1.0000	0.4628	- 0.3709	0.3636
Sodium	0.0396	0.1097	0.4787	- 0.5074*	0.4628	1.0000	- 0.3232	0.3191
Potassium	0.0296	0.4852*	- 0.3512	0.1983	- 0.3709	- 0.3232	1.0000	0.1243
Chloride	- 0.0360	0.3055	0.0387	0.0683	0.3636	0.3191	0.1243	1.0000
FAA	- Free Amino	Acid nitrogen						
*	- Significant at 1% level							
**	- Significant at 10% level							



## B) MUSCLE BIOCHEMISTRY

The studies for the period from March 1989 to October 1990 has shown that total lipid, total protein and free amino acid nitrogen to vary in the manner discussed below with the changes in environmental salinity, temperature and pH in the four size groups of *M.dobsoni* viz., 30-39 mm, 40-49 mm, 50-59 mm, 60-69 mm.

**(i) Total lipids:** The four size ranges studied namely 30-39 mm, 40-49 mm, 50-59mm and 60-69 mm have shown the following variations in their muscle total lipid content during the study period. The influence of environmental parameters have also been discussed below.

In *M.dobsoni* of 30-39mm the total lipid content varied from 1.72 to 3.98 percent dry weight. A significant negative correlation has been found between salinity and the muscle total lipid content (-0.0570) (Table 18). The effect of temperature and pH were not found to be significant on the muscle lipid content by correlation analysis. The combined effect of salinity, temperature and pH has been found to be significant in which the influence of salinity alone is predominant.

In prawns of 40-49mm the total lipid content varied from as low as 1.68 to as high as 4.64 percent dry weight (Table 14). The size range shows a strong negative influence of temperature (-0.7057) and a strong positive influence of pH (0.7881). Salinity has no significant influence on the muscle lipid content. Multiple regression analysis shows a significant combined influence of the three environmental parameters predominated

by the effect of pH. A correlation has been found between the total lipid and total free amino acid nitrogen content variation (0.7724) (Table 19).

The size range of 50-59mm shows the total lipids to vary from 2.43 to 4.02 percent dry weight. The influence of temperature as an independent entity is strong (-0.8151) but negative as noticed by correlation analysis whereas salinity and temperature do not influence lipid variations. The three environmental parameters together however account to a very significant level for the changes in the total lipid content. The total lipid content shows a significant positive correlation with total carbohydrates (0.6067) and a negative correlation with the protein variation of the muscle (-0.5343) (Table 20).

The females and males of 60-69 mm show a total lipid variation from 1.91 to 4.91 and from 2.25 to 3.61 percent dry weight (Table 14) in the two sexes respectively. Neither correlation (Table 21 and 22) nor multiple regression have shown any significant influence of the environmental parameters.

**(ii) Total carbohydrates:** The variation of this biochemical parameter with environmental salinity, temperature and pH has been studied in the four size ranges of *M.dobsoni* (30-39mm, 40-49 mm, 50-59mm and 60-69 mm). In prawns of 30-39mm the total carbohydrate content varied from 0.39 to 3.14 percent dry weight (Table 15). The correlation shows significant negative influence of pH on the total carbohydrate content (-0.5723) (Table 18). Salinity and temperature do not show a significant influence on the carbohydrate variation. The multiple regression shows a significant influence of all the three environmental parameters of which temperature and pH play a

predominant role. The change in the carbohydrate content has been significantly negatively correlated to the free amino acid nitrogen content (-0.7401) (Table 18).

The carbohydrate content varied from 0.39 to 3.91 percent dry weight in *M.dobsoni* of 40-49mm. Total carbohydrates is significantly negatively correlated to salinity (-0.6073) (Table 19). Temperature and pH do not show any significant influence on the carbohydrates in this group. The influence of all the three environmental parameters is are significant by multiple regression analysis of which salinity is the predominant factor.

Prawns of 50-59mm show a variation in total carbohydrate from 0.20 to 3.91 percent dry weight (Table 15). A negative correlation has been found between total carbohydrate and temperature (-0.4868) (Table 20) whereas salinity and pH do not show any correlation. The influence of the three environmental parameters conjointly is significant with the effect of temperature and pH being predominant. A significant positive correlation between total carbchhydrates and lipids (0.6067) has been found.

Female of *M.dobsoni* of 60-69 mm have shown a variation in the total carbohydrate content from 0.40 to 4.84 percent dry weight (Table 15). However, neither correlation (Table 21) nor multiple regression has shown any significant influence of any of the environmental parameters studied. Males of the same size range have shown a total carbohydrate variation from 0.22 to 3.07 percent dry weight (Table 15). A significant but negative correlation has been found with total carbohydrates and salinity (-0.5743) and temperature (-0.5447) (Table 22). No significant effect of pH has been felt on the male muscle carbohydrate variation. Multiple regression

shows a strong influence of these parameters of which the effect of salinity is predominant. The total carbohydrate content in male prawns of the size group show a significant correlation with free amino acid content (0.6882) (Table 22).

**(iii) Proteins:** The variation in the muscle protein content of *M.dobsoni* in the different size ranges with the three environmental have been discussed in the following paragraphs.

Prawns of the 30-39 mm size showed a variation in the protein content from 29.06 to 87.62 percent dry weight (Table 16). Temperature has a positive influence on protein variation (0.8517) (Table 18) whereas salinity and pH show no influence. Multiple regression has shown a significant influence of the environmental parameters of which the effects of both salinity and temperature are predominant.

The variation in protein content was from 20.76 to 89.09 and 28.81 to 92.59 percent dry weight (Table 16) in 40-49 mm and 50-59 mm sized *M.dobsoni* respectively. Correlation shows a significant positive influence of temperature (0.6649 and 0.7345) in both the size groups and a negative influence of pH (-0.5098) in 40-49 mm group (Table 19 and 20). However salinity has no influence in the two groups. pH does not influence the variation in the muscle protein of 50-59 mm. Multiple regression shows a strong combined influence of the environmental parameters of which temperature shows a predominant effect. The variation in the protein content has been correlated with free amino acid nitrogen content (-0.5399 and -0.4984) (Table 19 and 20) in the two size groups. Muscle protein shows a strong negative correlation with total lipids (-0.5343) (Table 20) in the 50-59 mm

group.

*M.dobsoni* female of the 60-69 mm size have shown protein variation from 33.41 to 82.42 percent dry weight (Table 16). Influence of any of the three environmental parameters studied has not been found either by application of correlation (Table 21) or multiple regression analysis. Male of the 60-69 mm have shown variation in protein from 29.01 to 81.81 percent dry weight (Table 16). Their muscle protein content has been significantly negatively correlated with pH (-0.6956) (Table 22) but no influence of temperature and salinity has been observed. Multiple regression has however shown a strong combined influence of which temperature and pH predominate.

**(iv) Free amino acid nitrogen:** The variation in free amino acid nitrogen content with seasons and in different size ranges of *M.dobsoni* has given the following inferences.

The variation of free amino acid nitrogen content of the muscle of 30-39 mm *M.dobsoni* during the study period was from 1.31 to 4.79 percent dry weight (Table 17). Both salinity (0.5541) and pH (0.4633) are significantly positively correlated with muscle free amino acid nitrogen content (Table 18) whereas no effect of temperature was observed. Multiple regression also shows a strong and significant effect due to the interaction of the environmental parameters of which salinity and pH are predominant. The free amino acid nitrogen content has been significantly negatively correlated with total carbohydrates (-0.7401) (Table 18).

The 40-49 mm and 50-59 mm prawns show a free amino acid nitrogen content variation in the range of 1.19 to 3.65 and 0.83 to 3.57

TABLE 14

Seasonal variation in the total lipid of the muscle(%Dry weight) in four size groups of *M. dobsoni* during the study period.

Month	30 - 39mm	40 - 49mm	50 - 59mm	60 - 69mm FEMALE	60 - 69mm MALE
March 1989		4.64±0.76	-		-
April	1.72±0.38	1.98±0.08			
April	1.87±0.20	1.68±0.19			
May	2.76±0.46	2.62±0.16	2.57±0.35	3.28±0.25	
May	1.97±0.05	2.50±0.09	2.48±0.21		
June	2.17±0.03	2.43±0.14	2.82±0.37		
June		2.70±0.08	2.98±0.41	2.84±0.32	
July		2.59±0.22	2.58±0.31	2.68±0.30	2.80±0.20
July		2.28±0.02	3.01±0.20	2.32±0.18	2.25±0.04
August		3.98±0.21	4.02±0.32	3.86±0.25	3.61±0.12
August		4.00±0.14	3.71±0.14	4.11±0.13	
September	3.70±0.20	3.77±0.20	3.70±0.20		
September		3.13±0.21	2.79±0.15		
October		2.84±0.50	2.79±0.15	2.42±0.15	3.10±0.38
November					
December	3.98±0.38	2.75±0.09	2.90±0.07		
January 1990		3.75±0.05	2.91±0.40		
February		3.66±0.07	2.69±0.23		
March		3.91±0.19		3.40±0.63	
April		4.20±0.17	3.30±0.15	4.91±0.27	
May		1.83±0.24		4.91±0.27	
May		3.03±0.05	3.21±0.33		2.85±0.26
June	2.32±0.48	2.76±0.09			2.76±0.09
July		3.24±0.30	3.10±0.05	2.54±0.48	
August		2.26±0.48	2.62±0.12	1.91±0.14	
September	1.98±0.66		2.43±0.83		
October		1.96±0.19			

TABLE 15  
Seasonal variation in the total carbohydrate of the muscle (% Dry weight) in four size groups of *M.dobsoni* during the study period

Month	30 - 39mm	40 - 49mm	50 - 59mm	60 - 69mm FEMALE	60 - 69mm MALE
March 1989		0.82±0.26			
April	0.39±0.06	0.68±0.10			
April	0.86±0.17	0.66±0.07			
May	3.14±0.11	3.91±0.22	3.75±0.13	3.11±0.13	
May	2.75±0.11	3.53±0.21	3.36±0.18		
June	2.98±0.11	3.76±0.22	3.59±0.19		
June		3.51±0.14	3.91±0.19	4.84±0.11	3.07±0.13
July		3.41±0.22	3.50±0.29	3.22±0.22	1.54±0.04
July		2.97±0.13	1.16±0.13	1.53±0.08	1.75±0.08
August		2.70±0.08	1.27±0.25	2.00±0.12	
August		2.51±0.11	1.05±0.20	2.64±0.09	
September	0.43±0.06	2.16±0.24	0.64±0.05		
September		1.93±0.23	0.58±0.08		
October		1.86±0.31	0.57±0.09		
November			0.45±0.07	0.61±0.21	2.30±0.46
December	0.47±0.11	0.71±0.12	0.70±0.29		
January 1990		0.45±0.02			
February		0.39±0.02			
March		0.62±0.01	0.81±0.10	3.91±0.97	
April		0.67±0.09	1.26±0.11	3.44±0.18	
May		0.85±0.09		0.72±0.10	0.22±0.06
June		0.73±0.19	1.68±0.13		1.19±0.11
July	0.68±0.07	0.73±0.10			
August		1.37±0.11	1.35±0.19	0.88±0.33	
September	1.54±0.19	0.48±0.22	0.20±0.07	0.40±0.04	
October		1.50±0.35	1.29±0.09		

TABLE 16  
Seasonal variation in the protein (%Dry weight) of the muscle in four size groups of *M.dobsoni* during the study period

Month	30 - 39mm	40 - 49mm	50 - 59mm	60 - 69mm FEMALE	60 - 69mm MALE
March 1989		39.26±5.41		-	-
April	67.20±25.15	35.50±4.46			
April	65.09±10.67	43.06±5.29			
May	73.83±7.45	64.05±1.94	92.59±2.85	75.72±1.69	
May	67.99±7.50	57.79±2.70	80.88±7.77		
June	69.16±6.31	62.24±2.32	86.74±3.75		
June		66.23±2.99	66.96±8.94	44.84±1.72	
July		60.56±1.38	60.96±3.21	66.42±4.98	58.03±0.48
July		60.95±3.02	61.83±1.94	72.24±7.32	60.22±1 17
August		80.59±2.07	75.61±5.25	33.41±2.99	29.01±1.02
August		89.09±5.67	80.15±2.79	53.63±4.96	
September	87.62±3.13	70.63±3.39	76.49±2.52		
September		76.63±1.94	75.46±4.16		
October		73.02±2.42	70.33±9.57		
November			42.86±11.81	43.22±8.05	78.64±3.65
December	44.08±9.41	61.05±2.83	66.79±23.75		
January 1990		75.46±24.57			
February		33.70±6.66	57.63±3.00		
March		42.98±6.56		48.84±2.68	
April		50.79±5.66	68.86±2.39	79.12±3.17	
May		81.56±7.09		82.42±0.00	81.81±0.86
June		82.83±7.98	64.96±10.13		49.83±1.58
July	29.06±0.69	31.50±8.81			
August		20.76±5.36	29.30±5.48	55.55±2.71	
September	33.94±7.81	49.81±5.71	41.52±1.83	38.83±2.39	
October		44.44±10.05	28.81±1.83		



TABLE 17

Seasonal variation in the total free amino acid content (%Dry weight) of the muscle in the four size groups of *M. dobsoni* during the study period

Month	30 - 39mm	40 - 49mm	50 - 59mm	60 - 69mm FEMALE	60 - 69mm MALE
March 1989		1.43±0.19	-	-	-
April	4.79±0.51	1.69±0.14			
April	3.03±0.63	3.63±0.22			
May	1.37±0.06	1.41±0.02	1.14±0.04	2.61±0.10	
May	1.31±0.07	1.19±0.13	0.83±0.21		
June	1.33±0.06	1.35±0.06	1.20±0.22		
June		3.03±0.24	3.28±0.13	3.44±0.21	
July		3.01±0.10	3.57±0.12	3.47±0.15	3.38±0.10
July		2.14±0.04	1.42±0.03	1.92±0.10	1.85±0.09
August		2.24±0.08	1.65±0.11	2.79±0.19	1.85±0.09
August		2.48±0.02	1.72±0.07	2.24±0.04	2.48±0.02
September	2.80±0.20	2.46±0.12	1.80±0.20		
September		2.29±0.28	1.23±0.13		
October		2.20±0.28	2.52±0.29		
November			2.20±0.50	1.74±0.23	1.64±0.11
December	1.79±0.21	1.19±0.13	1.84±0.36		
January 1990		1.90±0.31			
February		1.99±0.04	2.79±0.26		
March		2.72±0.09		2.29±0.19	
April		2.54±0.07	2.56±0.13	1.85±0.06	
May		1.30±0.10		2.22±0.20	2.09±0.17
June		2.77±0.11	1.62±0.28		1.35±0.17
July	3.13±0.18	3.65±0.55			
August		1.57±0.62	1.12±0.09	2.79±0.39	
September	1.45±0.06	3.06±0.33	2.85±0.18	3.13±0.20	
October		3.25±0.37	1.56±0.67		





TABLE 20  
CORRELATION ANALYSIS FOR THE MUSCLE OF 50 - 59 mm GROUP

Parameter	TOTAL LIPIDS	TOTAL CARBOHYDRATES	PROTEIN	FAA
Salinity	- 0.2749	- 0.3624	0.0011	- 0.3038
Temperature	- 0.8151"	- 0.4868'	0.7345"	- 0.6305"
pH	- 0.0821	- 0.0663	- 0.3789	0.9773"
Total lipids	1.0000	0.6067"	- 0.5343'	0.0967
Total carbohydrates	0.6067'	1.0000	- 0.1122	0.0742
Protein	- 0.5343'	- 0.1122	1.0000	- 0.4984'
FAA	0.0967	0.0742	- 0.4984'	1.0000

FAA - Free Amino Acids  
 \* - Significant at 1% level  
 \*\* - Significant at 10% level

TABLE 21  
CORRELATION ANALYSIS FOR THE MUSCLE OF THE FEMALES OF 60 - 69 mm GROUP

Parameter	TOTAL LIPIDS	TOTAL CARBOHYDRATES	PROTEIN	FAA
Salinity	0.3072	0.0784	- 0.0066	- 0.0763
Temperature	0.0408	0.0137	0.3807	- 0.0462
pH	0.2255	- 0.0122	- 0.2053	- 0.0528
Total lipids	1.0000	0.4290	0.2789	- 0.2237
Total carbohydrates	0.4390	1.0000	0.2129	0.3744
Protein	0.2789	0.2129	1.0000	- 0.3264
FAA	- 0.2237	0.3744	- 0.3264	1.0000

FAA  
\*       - Free Amino Acids  
      - Significant at 1% level  
\*\*      - Significant at 10% level

TABLE 22  
CORRELATION ANALYSIS FOR THE MUSCLE OF THE MALES OF 60 - 69 mm GROUP

Parameter	TOTAL LIPIDS	TOTAL CARBOHYDRATES	PROTEIN	FAA
Salinity	0.1385	- 0.5743 <sup>*</sup>	- 0.0495	- 0.3864
Temperature	- 0.3796	- 0.5447 <sup>*</sup>	0.2165	0.0974
pH	- 0.0791	- 0.2951	- 0.6956 <sup>***</sup>	- 0.1459
Total lipids	1.0000	0.2687	- 0.3279	0.2766
Total carbohydrates	0.2687	1.0000	- 0.1824	0.6882 <sup>**</sup>
Protein	- 0.3279	- 0.1824	1.0000	- 0.2065
FAAs	0.2766	0.6882 <sup>**</sup>	- 0.2065	1.0000

FAA  
\* - Free Amino Acids  
- Significant at 1% level  
\*\* - Significant at 10% level

percent dry weight (Table 17). The variation in this biochemical parameter has been correlated significantly and negatively with temperature (-0.9093 and -0.6305) and positively with pH (0.9922 and 0.9773) (Table 19 and 20) respectively. Multiple regression analysis has shown a significant combined influence of the environmental parameters where pH predominates in its effect in the 40-49 mm group and pH and temperature in the 50-59 mm. A positive correlation has been found between total lipid and free amino acid nitrogen content (0.7724) in the 40-49 mm group. There is a negative correlation with the muscle protein and the free amino acid nitrogen (-0.5399 and -0.4984) (Table 19 and 20) in the two size groups respectively.

The 60-69 mm female and male prawns have shown a variation in the free amino acid nitrogen content from 1.74 to 3.47 and 1.35 to 3.28 percent dry weight (Table 17). The application of correlation (Table 21 and 22) or multiple regression has not been able to confirm the influence of any of the three environmental parameters. A correlation with total carbohydrates and free amino acid nitrogen has been observed in the males of this group (0.6882) (Table 22).

### **C) HEPATOPANCREAS BIOCHEMISTRY**

The collection of samples was done for a period from March 1989 to October 1990 and the influence of the three environmental parameters *viz* salinity, temperature and pH on the hepatopancreas biochemistry has been studied.

**(i) Total lipids:** The variation in the total lipid content as an effect of season on different size ranges has brought out the following results.

Prawns of the size 30-39 mm and 40-49 mm have shown their total lipid content to vary from 15.53 to 30.21 and 9.98 to 33.22 percent dry weight (Table 23). Correlation (Table 27 and 28) has shown no significant influence of any of the three environmental parameters on both the size groups. However multiple regression has shown a significant influence of these environmental parameters together, of which pH has a predominant effect in the 30-39 mm group. In the 40-49 mm group no influence of the environmental parameters was observed by multiple regression.

Prawns of 50-59mm showed a variation from 20.42 to 44.37 percent dry weight (Table 23). The variation in total lipid content has shown a significant influence of salinity (0.4993) by correlation (Table 29) whereas no influence of temperature and pH was observed. The combined influence the environmental parameters was also significant as shown by multiple regression but the effect of salinity and temperature were predominant.

Female of 60-69 mm *M.dobsoni* has shown their total lipid content to vary from 15.06 to 39.20 percent dry weight (Table 23) during the study period. The total lipid content has a negative correlation with temperature (-0.4990) (Table 30) but no influence of salinity and pH was observed. Multiple regression shows a significant combined influence with all the three environmental parameters being influential. The total lipid content is also negatively correlated with free amino acid nitrogen content (-0.5165) (Table 30). The males of the same size range show a variation from 20.69 to 37.98 percent dry weight. No correlation (Table 31) has been found with variation and the environmental parameters. Multiple regression shows a significant combined influence where the effect of temperature was predominant.



**(ii) Total carbohydrates:** The effect of environmental parameters *viz.* salinity, temperature and pH on the four size groups (30-39mm, 40-49mm, 50-59mm, 60-69mm) studied has shown the following variations in the total carbohydrate content.

In the size group of 30-39 mm, 40-49 mm and 50-59mm the variation in the total carbohydrate content is illustrated in Table 24. The correlation analysis (Table 27-29) showed no influence of the environmental parameters on the variations in the 30-39mm and 40-49 mm groups. In the 50-59 mm group a significant influence of salinity (-0.4546) and no influence of temperature and pH was noticed on the hepatopancreatic carbohydrate variations. Multiple regression analysis, however did not show any influence of the environmental parameters in any of the size groups.

The female 60-69 mm show a range in the total carbohydrate content from 0.56 to 5.80 percent dry weight (Table 24). No correlation (Table 30) has been found between the total carbohydrate and environmental parameters. Multiple regression has shown a significant combined influence of the environmental parameters of which the effect of pH predominated. The males of the same size group show a variation from 1.16 to 20.14 percent dry weight (Table 24). They also show a significant correlation with pH (0.8239) (Table 31) whereas no influence of salinity and temperature was observed. Multiple regression also show a significant influence of all the three environmental parameters highly predominated by pH. The total carbohydrate content is also positively correlated with the free amino acid nitrogen content (0.5161) (Table 31).

**(iii) Protein :** The influence of the environmental parameters and size range on the protein content of the hepatopancreas of *M.dobsoni* is given below. The 30-39 mm sized prawns showed a range of protein content from 4.06 to 14.04 percent dry weight (Table 25). A highly significant positive correlation with salinity (0.5872) and significant negative correlation with temperature (-0.5534) (Table 27) has been indicated whereas pH showed no influence. A significant combined influence of the three parameters has been shown by multiple regression with salinity and temperature being the significant factors.

Prawns of 40-49 mm and 50-59 mm showed their protein to vary from 2.79 to 24.54 and 4.39 to 23.07 percent dry weight respectively (Table 25). Correlation analysis (Table 28 and 29) and multiple regression had shown no significant influence of any of the environmental parameters. The range of protein content from 5.86 to 28.78 and 2.34 to 31.19 percent dry weight (Table 27) has been found in females and males of 60-69 mm size respectively. Correlation analysis has shown a negative but significant influence of salinity (-0.5046 and -0.6233) on protein content (Table 30 and 31) in the females and males respectively whereas no influence of pH and temperature was observed. Multiple regression analysis shows a significant interaction of environmental parameters with predominant effect of salinity and pH in the females and predominant effect of all the three environmental parameters studied, in the males.

**(iv) Free amino acid nitrogen:** The variation of the free amino acid nitrogen content of the hepatopancreas under the influence of the environmental parameters (salinity, temperature and pH) and size of prawns as shown by this study is detailed below.

In *M. dobsoni* of the 30-39 mm and 40-49 mm size the free amino acid nitrogen content varied from 0.68 to 4.26 and 1.25 to 3.26 percent dry weight (Table 26) respectively. Neither correlation (Table 27 and 28) nor multiple regression showed any significant influence of the environmental parameters on the variations.

Prawns of the 50-59 mm size showed a range in free amino acid nitrogen content from 1.00 to 2.66 percent dry weight (Table 26). Correlation analysis showed a significant negative influence of pH (-0.6209) (Table 29) but no influence of salinity and temperature was observed. Multiple regression analysis showed a significant combined influence of the environmental parameters of which pH had a predominant effect.

Female of the 60-69 mm size of *M. dobsoni* showed a range in the free amino acid nitrogen content to vary from 1.19 to 3.54 percent dry weight (Table 26). Correlation analysis showed a significant influence of temperature (0.5000) (Table 30) whereas salinity and pH did not show any influence on the free amino acid variation. Multiple regression showed significant combined influence with the predominant effect of both temperature and pH. Males of the same size range showed a variation in their free amino acid nitrogen content from 1.27 to 4.43 percent dry weight (Table 26). Neither correlation (Table 31) nor multiple regression analysis showed any significant influence of any of the environmental parameters. In females the free amino acid nitrogen content showed a negative correlation with total lipid content (-0.5165) (Table 30) and in males the free amino acid nitrogen content showed a positive correlation with total carbohydrate content (0.5161) (Table 31).

TABLE 23

Seasonal variation in the total lipid content (%Dry weight) of the hepatopancreas in the four size groups of *M. dobsoni* during the study period

Month	30 - 39mm	40 - 49mm	50 - 59mm	60 - 69mm FEMALE	60 - 69mm MALE
March 1989		24.55±2.33			
April	14.14±1.12	27.57±3.11			
April	30.21±1.34	26.66±1.73			
May	23.04±2.60	26.49±1.23	24.22±1.16	15.06±0.71	
May	23.34±0.57	28.00±1.61	27.11±0.82		
June	18.48±0.78	29.66±1.23	20.22±4.73		
June		21.38±1.69	31.98±3.92	34.02±2.56	37.98±2.50
July		26.35±1.60	22.80±3.68	23.86±1.83	24.00±0.83
July		20.55±3.58	22.97±1.16		20.69±4.43
August		24.83±1.38	22.90±6.14	25.52±3.54	23.73±3.07
August		9.98±1.94	23.65±7.22	28.97±1.90	
September		17.77±2.87	23.73±4.83		
September	15.53±7.94	12.37±0.22	22.16±1.48		
October		20.42±2.98	23.70±4.83		
November			38.21±7.11	23.85±2.88	26.69±6.06
December	21.43±6.65	23.00±4.85	20.42±8.14		
January 1990		12.53±2.35			
February		14.83±0.84	43.23±2.27		
March		37.82±1.88		39.20±0.16	
April		28.62±2.40	31.04±1.13	31.61±1.39	
May		30.34±3.46		16.15±2.54	20.81±3.76
June		33.22±8.31	94.37±3.67		25.98±3.14
July	18.62±0.56	30.23±0.99			
August		27.47±5.63	33.45±8.79	24.94±4.33	
September	20.11±6.10	25.67±9.11	23.37±0.72	20.12±0.58	
October		19.24±7.14	27.01±3.26		

TABLE 24

Seasonal variation in the total carbohydrate content (%Dry weight) of the hepatopancreas in the four size groups of *M. dobsoni* during the study period

Month	30 - 39mm	40 - 49mm	50 - 59mm	60 - 69mm FEMALE	60 - 69mm MALE
March 1989		1.99±0.06	-	-	-
April	2.49±0.16	3.98±0.45			
April	3.68±0.08	3.85±0.35			
May	4.35±0.05	4.31±0.03	4.26±0.05	1.84±0.15	
May	3.95±0.06	4.12±0.09	4.18±0.07		
June	4.25±0.00	4.24±0.11	4.35±0.03		
June		4.25±0.03	4.35±0.03	4.44±0.04	4.60±0.05
July		3.63±0.14	3.57±0.03	4.20±0.14	4.16±0.09
July		1.96±0.08	3.87±0.07		6.06±0.63
August		3.81±0.72	3.06±0.74	5.15±1.20	20.14±3.37
August		5.08±0.63	3.72±1.70	5.80±1.24	
September	1.82±0.36	3.41±0.90	3.25±0.59		
September		4.59±0.36	4.12±0.27		
October		1.91±0.34	1.00±0.23		
November			0.92±0.38	1.63±0.51	1.61±0.18
December	0.55±0.06	0.86±0.11	0.89±0.45		
January 1990		1.61±0.347			
February		1.67±0.12	0.26±0.07		
March		1.62±0.10		0.75±0.05	
April		1.84±0.15	0.90±0.15	1.33±0.20	
May		1.29±0.16		1.29±0.67	2.22±0.94
June		2.74±0.90	3.92±0.68		2.71±0.25
July	3.39±0.15	2.63±0.56			
August		3.33±0.63	5.45±1.38	3.73±0.20	
September	3.85±2.18	3.27±0.72	2.61±0.09	0.56±0.23	
October		0.90±0.25	1.69±0.39		

TABLE 25  
Seasonal variation in the protein content of the hepatopancreas in the four size groups of *M. dobsoni* during the study period

Month	30 - 39mm	40 - 49mm	50 - 59mm	60 - 69mm FEMALE	60 - 69mm MALE
March 1989		19.67±0.57			
April	12.09±1.56	12.31±1.28			
April	08.68±1.41	06.37±0.32			
May	04.06±1.18	05.05±0.88	05.71±1.28	21.61±1.37	
May	10.99±0.44	07.80±0.73	09.34±0.95		
June	09.79±1.43	05.23±0.87	10.15±0.42		
June		02.97±1.87	17.30±1.16	20.10±2.77	21.20±2.35
July		08.02±0.82	19.60±1.10	28.78±2.98	24.93±2.68
July		06.90±0.94	16.53±1.02		31.19±5.79
August		05.49±0.98	06.59±2.10	11.75±1.69	11.58±3.26
August		03.79±0.56	07.99±2.27	08.73±1 16	
September	05.93±0.37	07.58±1.76	07.09±0.86		
September		14.94±1.27	13.46±0.79		
October		06.96±0.57	05.71±1.24		
November			07.03±0.88	25.64±6.02	02.34±0.29
December	07.20±1.65	13.43±3.46	05.13±0.67		
January 1990		06.22±0.52			
February		11.20±1.57	23.07±3.23		
March		11.36±2.02		06.59±2.38	
April		07.51±1.03	12.81±1.37	05.86±1.37	
May		24.54±8.28		13.37±3.05	10.26±2.89
June		21.61±1.87	19.07±3.86		09.89±0.90
July	05.49±0.90	09.15±2.59			
August		12.18±3.83	15.35±5.03	08.61±0.69	
September	14.04±3.76	08.24±4.90	10.99±5.98	16.49±1.84	
October		07.78± 2.40	04.39±1.61		

TABLE 26

Seasonal variation in the free amino acid content (%Dry weight) of the hepatopancreas in the four size groups of *M. dobsoni* during the study period

Month	30 - 39mm	40 - 49mm	50 - 59mm	60 - 69mm FEMALE	60 - 69mm MALE
March 1989		1.45±0.03			
April	1.93±0.14	1.54±0.07			
April	2.30±0.14	2.45±0.01			
May	1.88±0.09	1.98±0.05	2.18±0.05	3.54±0.68	
May	1.89±0.06	2.13±0.07	2.32±0.04		
June	2.51±0.14	1.99±0.54	1.13±0.37		
June		2.38±0.16	1.24±0.54	1.51±0.07	1.27±0.18
July		2.58±0.05	1.73±0.10	1.70±0.04	1.63±0.05
July		3.19±0.34	1.61±0.07		3.30±0.73
August		2.43±0.39	2.24±0.23	2.00±0.15	4.43±1.42
August		2.57±0.10	1.68±0.44	1.36±0.42	
September	1.03±0.06	1.37±0.10	2.66±0.24		
September		1.25±0.06	2.57±0.07		
October		3.26±0.30	2.17±0.57		
November			2.00±0.16	1.97±0.21	2.15±0.31
December	0.68±0.05	2.84±0.22	1.23±0.16		
January 1990		1.58±0.35			
February		2.10±0.37	2.00±0.18		
March		3.24±0.86		1.91±0.67	
April		2.97±0.04	1.57±0.09	1.19±0.06	
May		4.99±0.62		3.14±0.87	3.59±10.69
June		1.28±0.36	1.00±0.31		1.58±0.26
July	4.26±0.66	3.10±0.97			
August		1.47±0.12	2.16±0.92	1.89±0.13	
September	2.70±0.39	1.42±0.35	1.71±0.43	2.09±0.54	
October		1.62± 0.45	1.33±0.31		

TABLE 27  
CORRELATION ANALYSIS FOR THE HEPATOPANCREAS OF 30 - 39 mm GROUP

Parameter	TOTAL LIPIDS	TOTAL CARBOHYDRATES	PROTEIN	FAA
Salinity	0.1322	0.1441	0.5872**	- 0.1119
Temperature	0.1577	- 0.0043	- 0.5534*	- 0.3198
pH	- 0.4345	- 0.3808	- 0.0076	- 0.2550
Total lipids	1.0000	0.0753	- 0.0008	0.0389
Total carbohydrates	0.0753	1.0000	- 0.0909	0.4387
Protein	- 0.0008	- 0.0909	1.0000	0.0877
FAAs	0.0389	0.4387	0.0877	1.0000

FAA  
 \* - Free Amino Acids  
 - Significant at 1% level  
 \*\* - Significant at 10% level



TABLE 28  
CORRELATION ANALYSIS FOR THE HEPATOPANCREAS OF 40 - 49 mm GROUP

Parameter	TOTAL LIPIDS	TOTAL CARBOHYDRATES	PROTEIN	FAA
Salinity	0.1354	- 0.2997	0.1419	- 0.1036
Temperature	0.0046	- 0.0464	- 0.1574	0.2549
pH	- 0.2732	0.1084	- 0.0838	- 0.3905
Total lipids	1.0000	- 0.0989	0.3537	0.4259
Total carbohydrates	- 0.0989	1.0000	- 0.4175	- 0.4657
Protein	0.3537	- 0.4175	1.0000	0.3053
FAA	0.4259	- 0.4657	0.3053	1.0000

FAA                    - Free Amino Acids  
 \*                      - Significant at 1% level  
 \*\*                     - Significant at 10% level

TABLE 29  
CORRELATION ANALYSIS FOR THE HEPATOPANCREAS OF 50 - 59 mm GROUP

Parameter	TOTAL LIPIDS	TOTAL CARBOHYDRATES	PROTEIN	FAA
Salinity	0.4993*	- 0.4546*	0.1264	0.0992
Temperature	- 0.4285	0.0447	- 0.1410	0.0108
pH	- 0.1828	0.0143	- 0.1324	- 0.6209**
Total lipids	1.0000	- 0.3061	0.3613	- 0.0230
Total carbohydrates	- 0.3061	1.0000	0.1510	0.0591
Protein	0.3613	0.1510	1.0000	- 0.0908
FAA	- 0.0230	0.0591	- 0.0908	1.0000

FAA  
\* - Free Amino Acids  
- Significant at 1% level  
\*\* - Significant at 10% level

TABLE 30  
CORRELATION ANALYSIS FOR THE HEPATOPANCREAS OF THE FEMALES OF 60 - 69 mm GROUP

Parameter	TOTAL LIPIDS	TOTAL CARBOHYDRATES	PROTEIN	FAA
Salinity	0.4413	- 0.4187	- 0.5046*	0.0642
Temperature	- 0.4990*	- 0.1441	0.0158	0.5000*
pH	0.2857	0.4781	- 0.2988	- 0.4733
Total lipids	1.0000	0.2069	- 0.3063	- 0.5165*
Total carbohydrates	0.2069	1.0000	0.0591	- 0.4197
Protein	- 0.3063	0.0591	1.0000	0.1460
FAA	- 0.5165*	- 0.4197	0.1460	1.0000
FAA	- Free Amino Acids			
*	- Significant at 1% level			
**	- Significant at 10% level			

TABLE 31  
CORRELATION ANALYSIS FOR THE HEPATOPANCREAS OF THE MALES OF 60 - 69 mm GROUP

Parameter	TOTAL LIPIDS	TOTAL CARBOHYDRATES	PROTEIN	FAA
Salinity	- 0.3567	- 0.0877	- 0.6233 <sup>*</sup>	- 0.0952
Temperature	- 0.4719	- 0.0713	0.2529	- 0.0072
pH	- 0.1003	0.8239 <sup>**</sup>	0.4548	0.2728
Total lipids	1.0000	- 0.1386	0.1534	- 0.0551
Total carbohydrates	- 0.1386	1.0000	0.1228	0.5161 <sup>*</sup>
Protein	0.1534	0.1228	1.0000	- 0.0088
FAA	- 0.0551	0.5161 <sup>*</sup>	- 0.0088	1.0000

FAA                    - Free Amino Acids  
<sup>\*</sup>                        - Significant at 1% level  
<sup>\*\*</sup>                      - Significant at 10% level

## **DISCUSSION**

Estuaries are complex ecosystems that harbour a number of commercially important marine prawns during their growth phase. The northern part of the Vembanad lake near Cochin which is in connection with the sea, is generally high saline and becomes fresh only for a short period during the floods of southwest monsoon. The general hydrological features of the Cochin backwaters in and around Cochin are given by several workers (Ramamirtham and Jayaraman, 1963 George and KrishnaKarth, 1963 Cheriyan, 1963, 1967 Nair, 1964 Qasim and Reddy, 1967; Mohammed and Rao, 1971 Shankarnaryanan and Qasim, 1969 ). The study of environmental parameters during the period of March 1989 to October 1990 of the Cochin backwaters near Puduvaipu has shown the presence of a well defined monsoon in 1989. The period from June to December 1989 demonstrated low salinities as a result of heavy showers. Shankarnarayanan and Qasim (1969) classified the rainy season in the Cochin area to extend from June to September, when there is a strong south west monsoon, and again from late October to early December, which is a period of north west monsoon. A similar period of monsoon has, however, not been observed in the year 1990. These results showed that the natural phenomenon, the monsoon, does not follow a cyclical pattern. This estuarine environment thus varies widely in salinity ranging from near full strength sea water, to brackish and sometimes become very diluted to almost fresh water depending upon the season and other factors.

The possibility of an organism to succeed in media of such varying conditions will undoubtedly depend on a large part on its ability to regulate the osmotic pressure of both its blood and cells. When considering

osmotic regulation we deal with two types of mechanisms. One type which is involved in the regulation of osmotic pressure of the blood we shall term, according to Florkin (1961) as anisosmotic regulation. A second one termed intracellular isosmotic regulation and is involved in the regulation of the osmotic pressure of the cell.

### **ANISOSMOTIC REGULATION.**

The blood or haemolymph in crustaceans does not flow in well defined vessels. Adaptations to hypo osmotic or hyper osmotic environment implies the existence of a compartment, the extracellular fluids, playing the role of buffers between the external milieu and the living cells (Maetz, 1970).

The impact of varying environmental conditions is directly experienced by this medium. The measure of extracellular regulation is performed by assessing the osmolality of the haemolymph.

The 30 - 39 mm group of M. dobsoni observed during the course of study showed hyperosmotic regulation. In spite of its scarce collection, the mode of osmoregulation adopted by this group can be stated with confidence as it occurred at the periods of both high and low salinities. Freshwater crustaceans are generally hyperosmotic and Kinne (1963) has described freshwater hyperosmotic regulators as oligostenohaline regulators. *Hommarus americanus* juveniles varied their osmolality in the range of 518 - 1421 mOsm/Kg H<sub>2</sub>O in a salinity range of 13.6 - 47.6‰ and demonstrated hyperosmoticity over the entire range (Charmantier *et. al.*, 1984a,b). A similar type of hyperosmotic regulation was observed in *H.americanus* (Dall, 1970). The rock crab, *Emerita* which can tolerate

osmolalities between 20 – 200 ‰ is hyperosmotic over the entire range (Bursey and Bonner, 1977). Apart from the effect of salinity; temperature and pH also influence osmolar changes in the species investigated. The latter two are inversely related to osmolality. Weber and Spargaren (1970) found an inverse correlation between temperature and blood concentration in *Crangon crangon*. Dehnelt and Stone (1964) found that summer *Hemigrapsus* species differed in their haemolymph concentration from that of winter crabs. The author has not come across any authentic work on the effect of pH on the osmoregulatory process. However, the osmolality in this size group is not influenced by any of the biochemical or inorganic components studied. The effect of the environmental parameters has been noticed on the variation in the biochemical parameters. Correlation analysis did not show the influence of salinity, temperature or pH on sodium variation. Engel *et. al.* (1974) showed that temperature affected sodium in *Callinectes sapidus* but not chloride. The chloride ion variation could not be correlated with the environmental parameters. The effect of environment was felt on the sodium levels on conduction of multiple regression indicating that salinity, temperature and pH had no significant influence independently but have a significant influence combinedly. Since these inorganic ions do not influence the osmolality, it could be concluded that in this group they are not actively involved in the adjustment process adopted by the animal. It is probable that each biochemical parameter varies its concentration under the influence of the environment but their variation does not contribute significantly to the osmotic pressure changes. A detailed research needs to be conducted to determine the parameter that contributes significantly to the osmotic pressure changes. The variation of chloride ion concentration is inversely related to the carbohydrate variation. The carbohydrate levels are influenced by the environment and hence the chloride levels are indirectly influenced

by the environment.

The osmolality in the 40 – 49 mm group is regulated hyperosmotically at lower salinities and hyposmotically at higher salinities. Kinne (1963) named the brackish water hyper-hypo-osmotic regulators as euryhaline regulators. Hyper-hyposmotic regulation has been reported in *Artemia salina* (Croghan, 1958a,b,c), *Gnimosphaeroma oregonensis* (Reigel, 1959), *Palaemonetes varians* (Pannikar, 1941), *Uca crenulata* (Jones, 1941) and *M. rosenbergii* (Stern *et al.*, 1987). In *M. rosenbergii* it has been suggested that the hyporegulatory capability is associated with the stage of life cycle; postlarvae exhibit this capacity which subsequently decreases with age (Castille and Lawrence, 1981c). The hyper-hypo osmoregulatory pattern is typical of many brackish water crustacea (Kinne, 1971). Juvenile *P. setiferus* show osmolality in the range of 626-850 mOsm/Kg H<sub>2</sub>O in 9.8 to 40.4% salinity and demonstrate hyper-hyposmotic behaviour (Castille and Lawrence, 1981b). A similar type of hyper-hyposmotic regulation was noticed in *P. stylirostris* juveniles adapted to a range of 10.8 - 36.2 % salinity (Castille and Lawrence, 1981d). The effect of temperature or pH on the osmolality could not be identified on the conduction of correlation analysis. Dorgelo (1977) concluded that temperature has no effect on osmotic regulation in the population of *Chaetogammarus marinus*, *Gammarus digrinus* and *Gammarus fossarum*. In the mangrove crab, *Ucides* (Zanders and Zanders, 1984) reported that temperature does not seem to effect the osmolality. Leersynder (1967a) showed that migration of *Eriocheir* did not effect its haemolymph osmolality. The sodium ion concentration is directly influencing the osmolality in the present study. Much work has been done with the regulation of sodium in crustacea than in other major cations ( Krogh, 1939 Beadle, 1957 Robertson 1960 Lockwood, 1962 Schoffeneils and Gilles, 1970). In each case where the ionic regulation of



sodium by a decapod has been studied over a considerable range of salinities, the pattern of sodium regulation has been found to be similar to the osmotic regulation of that animal ( Prosser *et. al.*, 1955; Gross, 1958 Bryan, 1960; Shaw, 1961a,b Dehnel, 1967; Bedford, 1972; Castille and Lawrence, 1981a,b,c,d). The effect of environment has been experienced on the protein content during this study. A trend in the seasonal variation of the haemolymph protein was reported by Uglow (1969) which closely followed temperature changes. Crowley (1963) and Andrews (1967) working with *Astacus nigriscences* and *Orconectes limosus* respectively also found seasonal changes in blood protein. Salinity induced changes in protein levels of the haemolymph of *M.dobsoni* preadult and *M.monoceros* was observed by Kalpana (1987) and Kamala (1989) respectively. The present work is in agreement with literature available. The effect of salinity observed in this study is shown in combination with temperature and pH. The effect of protein is not felt on the osmotic pressure thereby indicating its involvement in the adjustment process in a passive manner. A relationship between the protein and free amino acid nitrogen levels has been observed. The free amino acid nitrogen levels increase with a rise in protein levels. This is possible if the free amino acid nitrogen levels are utilised from some other tissue or *de novo* synthesis of free amino acid nitrogen takes place. Work by Kalpana (1987) has suggested that the mobilisation of protein takes place from the muscle and hepatopancreas to the haemolymph to raise the protein levels in *M.dobsoni*. Thus, in this case the mobilisation of proteins and free amino acid nitrogen to the haemolymph is possible. In low salinities, reduced extracellular concentrations are matched mainly by decrease in intracellular free amino acid and other ninhydrin positive substances (Gilles, 1979). In crustaceans amino acids are known to be released from the cells. Some of these are not deaminated immediately but may be temporarily incorporated

into intracellular proteins (Boone and Schoffeneils, 1979).

The regulation of osmotic pressure in the 50 - 59 mm group is similar to the 40 - 49 mm group i.e. hyper-hypo-osmotic regulation. The effect of salinity and pH but not temperature could be noticed on the osmolar values by correlation. The osmolality values are affected by the sodium and protein levels. In the semiterrestrial *Uca minax* and *Ocypode quadrata* high blood osmolarity corresponded to high protein levels (Pequeux *et. al.*, 1979). This result was interpreted in the light of the role that the blood proteins play in the establishment of colloid osmotic pressure of invertebrate body fluids (Magnum and Johnson, 1976). It is of interest to note that the environmental influence is not felt on either the sodium or protein variation. Their contribution and dependence is more directly involved with the osmotic pressure variations. The effect of environmental parameters is not felt on the lipid and carbohydrate variation. The inorganic ions also show an absence of influence of environment on their variation. The environmental effect has been felt on the free amino acid nitrogen levels. This thereby indicates that they play some role in the adjustment process probably in the maintenance of protein levels which are influential on the osmolar values. The free amino acid nitrogen do not act as osmotic effectors as they are not related to the osmolality variations. An inverse relationship between free amino acid nitrogen and chloride has been noticed during this study. Jeffries (1966) has also reported a similar non- protein nitrogen variation inversely correlated with serum chloride in the blue crab.

A comparative study of the osmolality regulating parameters in the three size groups is of interest. The 30-39 mm group shows the absence of the influence of any of the parameters studied on the osmolality. Sodium which was the only inorganic ion to be influenced by the environment in the

30 - 39 mm group shows its influence on the osmolality in the 40 - 49 mm group. In the 50 - 59 mm group, the protein, which was the only biochemical to be influenced by the environment in the 40 - 49 mm group, showed its influence in combination with sodium ions on the osmolar values. Another interesting factor is the lack of involvement of Potassium ion variations on either the osmotic pressure or the biochemical parameters. This ion is not influenced by the environmental parameters as existed during the study period. A similar result was obtained during the study conducted by Mini (1990) on the same species. Her study showed that salinity had no influence on potassium ion levels on conduction of ANOVA analysis.

The size group of 60 - 69 mm are adults as the size at first maturity is 64.1 mm in this species. This group is of immense ecological interest as it undergoes migration towards the sea for maturation and spawning.

The adult females were hyperosmotic regulators at low salinities and hyposmotic regulators at high salinities. The independent effect of salinity on osmotic pressure was noticed. The mechanism of osmolar adjustments thus followed the regulatory mechanism adopted by the 40 - 49 mm and 50 - 59 mm groups. The effect of sodium and carbohydrate has been felt on the osmotic pressure values. Carbohydrate levels influencing the osmolar values could be contributors to the osmotic pressure or act as energy sources for the maintenance of the osmotic pressure. Polysaccharide fraction is clearly the major haemolymph carbohydrate component constituting 81%. It now seems likely that the hepatopancreas seems could be the important tissue for carbohydrate metabolism in *C.maenas* (Johnston and Davies, 1972). The influence of the environmental parameters on the lipids, could serve

the purpose of energy source in the regulation or in the maturation process. Spaargaren and Mors (1985) found higher blood lipid concentrations in female shore crabs than in the males. Blood lipids rise with salinity but do not show any significant difference with variation in temperature in *C.maenas*. Spaargaren and Mors (1985) stated that lipid variations of the blood could indicate that lipid metabolism plays a part in osmotic and ionic regulation. It is rather surprising to note that lipids never received as much attention as for instance proteins or free amino acids in mineral metabolism despite the fact that energetically, regulation of membrane permeability represents the most favourable mechanism to withstand fluctuations in environmental osmotic conditions. The free amino acid nitrogen levels are also influenced by the environmental conditions. The possibility thereby exists in its involvement in the osmotic adjustment despite the fact that its influence is not felt on the osmotic pressure.

During the rather scarce collection of the adult males the osmotic regulation was found to be hyperosmotic. The understanding of the mechanism adopted by this group can be verified only on conduction of further research. The osmolar values are influenced by the protein levels contributing to the colloid osmotic pressure. The influence of the environment is felt on the sodium and chloride ions. Among the biochemical parameters lipids, proteins and free amino acid nitrogen levels are significantly influenced by the environmental changes. The apparent conclusion that can be drawn from the above results is the involvement of all these in the adjustment pattern but among these only protein attributes significantly to the osmotic pressure variations.

A disparity in the mechanism adopted by the two sexes is

evident. Lynch and Webb (1973a,b) reported sex related protein variations. The females show their dependence of osmolality on the sodium and carbohydrate levels. In the males the osmotic pressure is protein dependent. The necessity for storage of lipids could be high for the purpose of maturation in the females thereby leading to the dependence of osmolar values on the carbohydrate levels. On the other hand, in the males a similar need for lipid storage exists but the levels involved may be comparatively lower leading to their utilisation as energy sources in addition to their maturation needs.

The maintenance of the osmotic pressure in all the groups has shown that the osmotic concentration is not maintained at a constant level. The changing extracellular concentration thus involves the necessity for the maintenance of the internal or cell volume and regulation.

### **INTRACELLULAR ISOSMOTIC REGULATION**

Euryhaline invertebrates regulate the concentration of organic compounds in their intracellular fluids when making adjustments in their cellular osmolalities. The maintenance of the osmotic pressure requires controlled changes in the concentration of the intracellular osmotic effectors which appears to need some time to operate in salinity acclimation studies (Harris and Andrews, 1985). The study of Camien *et. al.* (1951) initiated a large number of studies, which all confirm the suggestion of Fredericq (1901) that the isotonicity of the cells in marine animals in general and marine invertebrates in particular, is maintained to a considerable extent by small molecular weight organic compounds. Although a linear relationship between the concentration of ninhydrin positive substances and salinity is not always found (Lynch and Webb, 1969 ;Nagel, 1934) it seems warranted to conclude that ninhydrin positive substances and other small molecular weight

nitrogenous compounds are present in high concentrations in all marine invertebrates tested, and that they, in addition to their possible nutritional function also exert an osmotic function. The regulation of intracellular osmolality appears primarily to reflect concentration changes of small organic molecules (Shaw, 1958). The possible role of tissues other than muscle for intracellular isosmotic regulation has received scant attention.

The 30-39 mm group has shown the effect of environment on the muscle biochemical parameters (lipids, carbohydrates, protein and free amino acid nitrogen) and the lipid and protein levels of the hepatopancreas. This is a clear evidence of their involvement of all these parameters in intracellular regulation. Lipid was reported as a major organic reserve in Antarctic benthonic crustaceans (Pearse and Giese, 1966). Free amino acids have shown to account for as much as 40% of the osmotically active organic constituents in the tissue of *E.sinensis* (Duchateau and Florkin, 1955). Carbohydrate is a possible metabolic reserve (Giese, 1966 Collatz and Speck, 1970; Armitage *et. al.*, 1972). An inverse relationship between the carbohydrate and free amino acid nitrogen levels of the muscle has been observed. This is a possible indication of the utilisation of energy for the maintenance of the free amino acid nitrogen levels. Hepatopancreatic involvement in the osmotic adjustment is evident from the influence of the environment on its lipid and protein levels. The hepatopancreas is the major storage organ and hence the involvement of the lipids of this tissue should not be of any surprise. However, the absence of information on this aspect of regulation is very glaring. The involvement of the protein of the hepatopancreas in the osmoregulatory process has been previously studied in *M.dobsoni* by Kalpana (1987).

The muscle biochemical parameters of the 40 - 49 mm group show the influence of the environmental parameters. An interdependence in the variation of the lipid, protein and free amino acid nitrogen levels have also been observed in this study. The lipids and free amino acid nitrogen levels show inverse relationship with the protein levels. This therefore implies that the lipids and free amino acid nitrogen levels rise on a fall in protein levels. The rise in free amino acid levels with fall in protein levels is an indication of the reversible synthesis/degradation of proteins. Proteins serve as storage systems for amino acids implicated in the isosmotic regulation of intracellular fluids (Gilles, 1977). The role played by the lipids in this group could be dual, either as energy sources for the maintenance of free amino acid nitrogen and protein levels and / or its utilisation in the cell membrane permeability. Taking into account this postulation the probable mechanism adopted could be that the lipids are partly utilised for the breakdown of proteins to free amino acid nitrogen which act as osmotic effectors the rest utilised for the reduction of membrane permeability to reduce the loss of free amino acid nitrogen from the cells. A total lack of environmental influence on the biochemical parameters of the hepatopancreas showing that they are not involved in the process of intracellular regulation and hence its lack of involvement in the osmotic process.

In the 50 - 59 mm group the influence of the environmental parameters on the biochemical composition of the muscle is evidenced. An interesting interrelationship between all the four biochemical parameters (lipids, carbohydrates, proteins and free amino acid nitrogen) has been noticed. An inverse relationship between the protein and the other biochemical parameters is evidenced by correlation analysis. This means

that a fall in muscle protein levels leads to a rise in lipid, carbohydrate and free amino acid nitrogen levels. The synthesis/degradation of the proteins is a reversible process. This, thereby, indicates that the maintenance of the high free amino acid nitrogen levels probably by the degradation of proteins involves the utilisation of energy which could be from either the lipid or carbohydrate sources. The utilisation of lipids in the regulatory process may be dual; either acting as energy sources to maintain the levels of the osmotic effectors or in the membranes as permeability factors. The effect of carbohydrates in this process is the possibility of it being high energy currencies and thus supplying energy for the maintenance of intracellular concentrations. The lipids and free amino acid nitrogen of the hepatopancreas are influenced by the environment indicating their involvement in the intracellular regulation. Gerard and Gilles (1972) showed that the decrease in amino acid of the hepatopancreas although less than the level of the muscle indicates its participation in the regulation of the osmotic equilibrium. Lipid concentrations of *O. limosus* declined when the ovaries were developing, while the lipid of the hepatopancreas of the male remained constant (Collatz, 1969). Similar ovarian development was not observed in the specimens collected. It can thus be said that the hepatopancreatic lipids are involved in the osmoregulatory process.

The environmental influence has been observed on all the biochemical parameters of the muscle in all the three size groups. Barclay *et. al.* (1983) during studies on starvation in the tiger prawn *P. esculentus* stated that the muscle tissue made up 60% of the wet weight of the prawn compared with the digestive gland's 3%. They therefore stated that relatively small changes in the abdomen are sufficient to make a substantial contribution to the overall animal maintenance. The carbohydrates and free



amino acid nitrogen levels are related to each other in the 30-39 mm and 50 - 59 mm groups. In the 30 - 39 mm group they are inversely related and hence it is probable that the utilisation of carbohydrates is involved in the maintenance of the free amino acid nitrogen levels. The 50 - 59 mm group probably utilises the carbohydrates for the maintenance of the free amino acid levels along with lipids and hence high carbohydrate and free amino acid nitrogen levels are necessary for the maintenance of the internal environment. The relationship developed between the lipids, protein and free amino acid nitrogen levels in the 40 -49 mm and 50 - 59 mm are similar in that the utilisation of lipids could be either for the maintenance of free amino acid nitrogen levels and also to reduce the permeability of the membrane for the prevention of the loss of valuable osmotic effectors. The involvement of the hepatopancreas in intracellular regulation in all these three size groups is rather interesting. The lipids, protein and free amino acid nitrogen of the hepatopancreas are involved in the process of regulation in the 30 - 39 mm and 50 - 59 mm groups. Hepatopancreas are not involved in the process of regulation in the 40 - 49 mm group. Could this be due to a shift in the function or role played by them in the regulatory process? Further detailed study needs to be conducted to throw more light on this aspect.

The adult females show a lack of involvement of the biochemical parameters of the muscle on the intracellular regulation. The role of inorganic ions on intracellular regulation is a possibility which needs to be studied. The hepatopancreas biochemical parameters are influenced by the environment and hence are involved in the intracellular regulation. The hepatopancreatic lipid and free amino acid nitrogen levels are related. A fall in lipids leads to rise in free amino acid nitrogen levels. The free amino acid nitrogen acts as osmotic effectors. The lipids are probably utilised for the

maintenance of the free amino acid levels but since the proteins are not related to the free amino acid nitrogen variations the rise in free amino acid levels of the hepatopancreas could be achieved only by migration of free amino acid nitrogen levels from some other tissue or in vivo synthesis of them in the hepatopancreatic tissue itself. Lipid reserves in the hepatopancreas are mobilised to the ovaries for oocyte maturation in penaeids (Millamena and Pascual, 1990; Mourente and Rodriguez, 1991). Though maturation has not been studied the involvement of the hepatopancreatic lipid in this size group for this purpose cannot be ruled out as both maturation and lipids are environment dependent. The lipids of this tissue are hence probably utilised for the maintenance of free amino acid nitrogen levels, or in the in vivo synthesis of the free amino acid nitrogen.

In the adult males, the environmental influence has been noticed on the carbohydrates and protein levels of the muscle. The rise in carbohydrates leads to rise in free amino acid nitrogen levels. It is of interest to note that the carbohydrates (as possible energy sources) are not involved in the degradation of protein to raise free amino acid nitrogen levels (as protein and free amino acid nitrogen levels show no relationship) but probably for the inward migration from some other tissue or *de novo* synthesis. A similar type of relationship between carbohydrate and free amino acid nitrogen levels is noticed in the 30 - 39 mm group. The environmental influence is felt on the carbohydrate and protein levels of the hepatopancreas. A rise in carbohydrates leads to a rise in free amino acid nitrogen levels of the hepatopancreas. This is an indication of the involvement of the carbohydrates as energy sources for the maintenance of the free amino acid nitrogen levels. The proteins and free amino acid nitrogen are, however, not related indicating that changes in these levels are influenced not by

changes in protein levels within the tissue but probably from some other tissue or de novo synthesis. The carbohydrates and free amino acid nitrogen of both the muscle and hepatopancreas are involved in the intracellular regulation.

There is a disparity in the involvement of the tissues in the process of intracellular regulation. The muscle tissue in the females shows no involvement in the cell volume regulation process by variation of its organic constituents. The possibility of the involvement of the inorganic ions in the intracellular regulation needs to be studied. The carbohydrates and protein of the muscle are involved in the regulatory process in the males. The female hepatopancreatic biochemical parameters are involved in the intracellular regulation whereas, in the males, the lipids and free amino acid nitrogen are involved in the intracellular regulation. In the female the hepatopancreatic lipids are involved in the maintenance of free amino acid nitrogen levels whereas, in the males, the maintenance of the amino acid nitrogen levels of the hepatopancreas is achieved by the carbohydrates.

The above discussion clearly states the involvement of the organic and inorganic components of the haemolymph, muscle and hepatopancreas in the process of osmotic regulation. A great disparity exists in the mode of regulation involved in each size group. The two sexes among the adults also show great disparity in the mode of regulation adopted by them. There is a great dearth of knowledge in understanding the exact mechanism adopted by the animal under natural conditions. A detailed study needs to be conducted to understand the mechanism adapted by them.

**CHAPTER - 4**  
**GILL STUDIES**

## **RESULTS**

### **LIGHT MICROSCOPE STUDIES.**

The branchial cavity of *M.dobsoni* has 7 pairs of gills. The gills were arbitrarily considered in two groups, the anterior and the posterior pairs based on their position from the rostrum. Study was then made to examine the existence of morphological and physiological differences if any between the two groups.

The length and width of the gills were measured under the light microscope. The anterior gills varied in length from  $1.97 \pm 0.22$  mm with a width ranging from  $0.76 \pm 0.07$  mm. The posterior gills varied in length from  $3.12 \pm 0.83$  mm with a width of  $0.93 \pm 0.15$  mm. The frequency of occurrence of lamellae on the gills varied from the apical to the basal portion of the gill filament. The frequency was higher in the apical region (3 lamellae per 0.2 mm) than the basal region (2 or less lamellae per 0.2 mm).

Stained sections of the gills observed under the light microscope showed the gills to be basically consisting of a cuticle as the outermost layer. Below the cuticle is the epithelium which encloses a series of haemolymph spaces. Distally the epithelium is tenuous where the haemolymph space is the greatest (Plate 1).

The anterior and posterior gills showed a similar structure. The gills collected during the premonsoon months showed an evenly distributed cytoplasm and its constituents with well developed haemolymph spaces (Plate 2 & 3). Specimens collected during monsoon showed the presence of highly developed vacuoles (Plate 4 & 5). The cellular contents are restricted to certain

PLATE 1 The section of gills observed under light microscope ( 20x)

PLATE 2 The section of anterior gills during high salinity conditions (20x)

PLATE 3 The section of posterior gills during high salinity conditions (40x)

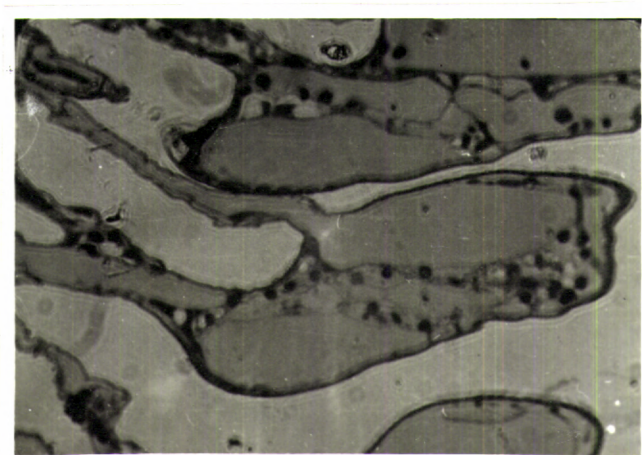
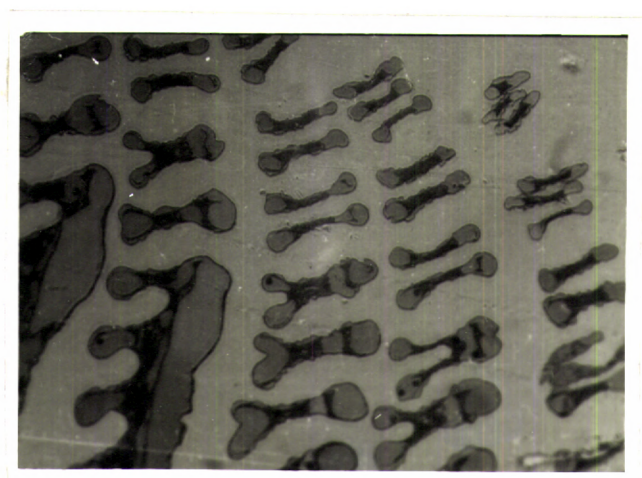
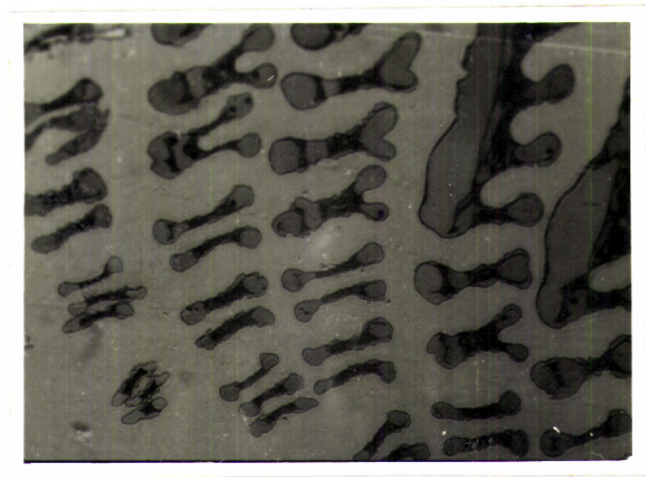
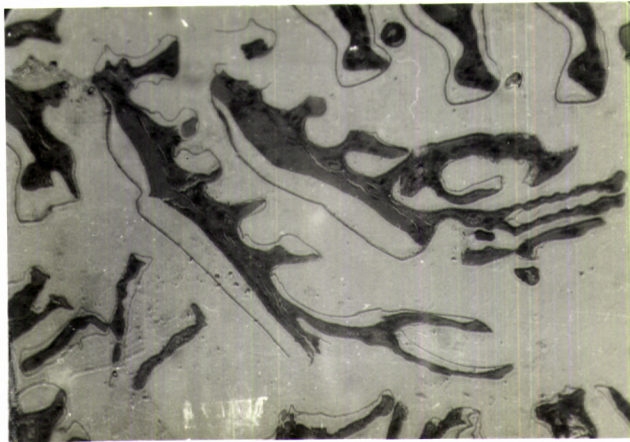
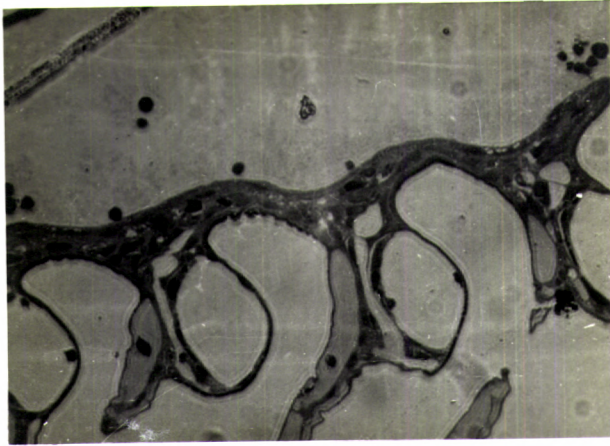


PLATE 4 Section of anterior gills during monsoon (40x)

PLATE 5 Section of posterior gills during monsoon (20x)

PLATE 6 Ultrastructure of gills during the monsoon (20,000x)





regions and the haemolymph spaces are greatly reduced.

## **ELECTRON MICROSCOPE STUDIES.**

The cuticle, outermost layer of the gill is composed of two distinct layers; an outer moderately electron dense epicuticular layer separated from the granular electron dense inner layer by an electron light zone. The endocuticle is a thin and compact layer ( Plate 6 ).

The epithelial cells are seated on a distinct and compact basal lamina in the haemolymph. Basically all the epithelial cells have the same structure. The microvillus border at the apical region and infoldings of the basal membrane communicate freely with the plasma surface. The distance between the folds is variable and the amount of extracellular organelles is very limited.

The prawns collected during low saline conditions showed their epithelium to be characterised by a well developed network of extended, apical, evaginated and digital folds and villi managing to reach a large portion of the subcuticular compartment. One of the most unusual and distinct features of the osmoregulating tissue is the presence of “ mitochondrial pumps” They are defined as a close, parallel arrangement of the plasma membranes and mitochondrial envelope membranes. The presence of this is apparent in specimens collected from the dilute medium (Plate 7 & 8). The network of folds and the mitochondrial pumps vary with the changes in the transport activity. The numerous mitochondria observed exhibited unusual shapes. At the serosal side of the cell, the basolateral plasma membrane is folded into the cell (Plate 7).

PLATE 7 Ultrastructure of gills during the monsoon (4,600x)

PLATE 8 Ultrastructure of gills during the monsoon (8,200x)

PLATE 9 Ultrastructure of gills during high saline conditions (3,900x)

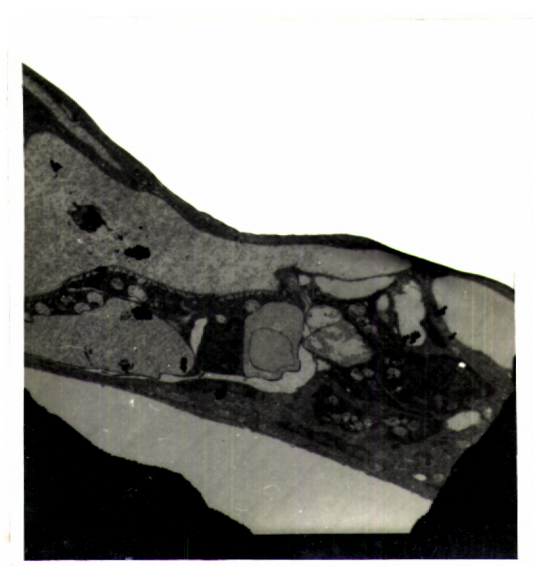
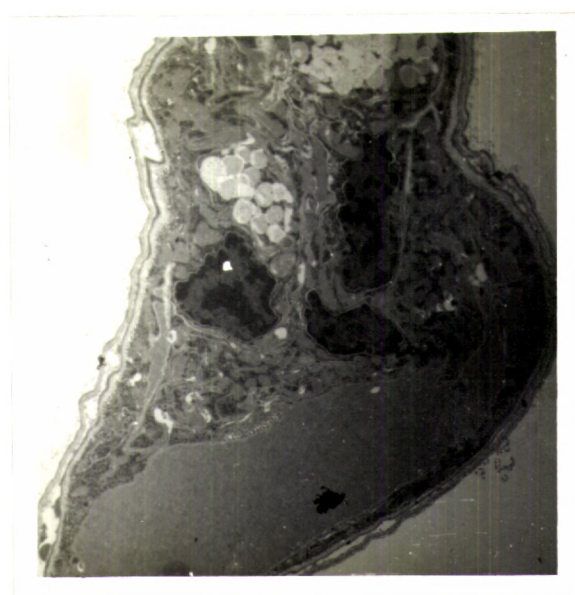
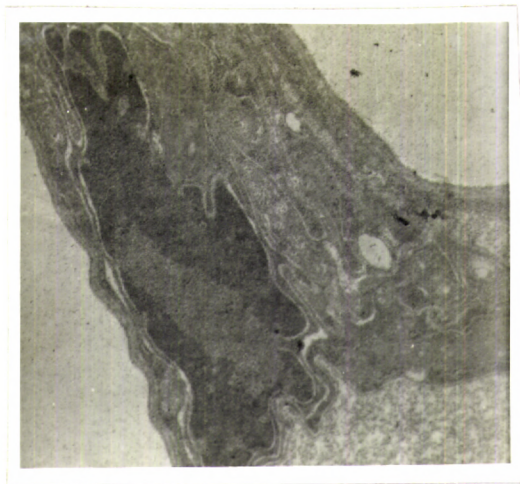
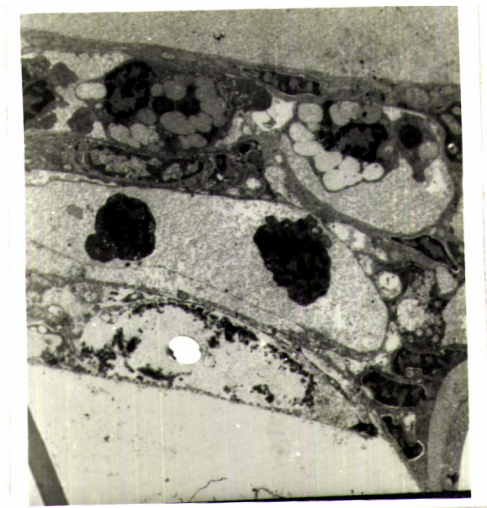


PLATE 10 Ultrastructure of gills during high saline conditions (3,900x)

PLATE 11 Cup shaped structure observed in the gills (5,400x)



During the premonsoon months the waters are more concentrated. The transport mechanisms are decreased and the epithelium shows major structural changes. There is a considerable regression in the infolding system. This leads to a severe decrease in the apical surface area of the cells and the disappearance of considerable amount of subcuticular space. There is also a significant reduction in the number of mitochondria (Plate 9 & 10).

Cup shaped structures arranged serially were observed in the gills collected during high saline conditions (Plate 9 & 11).

## **DISCUSSION**

In penaeids and other decapods the branchial chambers are closed dorsally by the branchiostegal fold and open to the exterior ventrally and posteriorly by narrow slits between the bases of the pereopods and the thoracic wall on the inside and the basal membrane of the branchiostegite (Young,1959). This chamber encloses the gills which are smallest in size towards the rostrum and increases gradually on moving away from the rostrum. Penaeids and sergestoids are the only decapods with dendrobranchiate gills, a phenomenon reflected by their classification in the separate sub order Dendrobranchiata.

Studies conducted on the observation of gill structure under light microscope are minimal. During the present study the light microscope observations have shown that the haemolymph spaces are restricted during the monsoon months and the vacuolar formation is enhanced. This could be an adaptation to tide over the prevailing monsoon climatic conditions and prevent the surface contact and hence loss of ions through the gills.

The ultrastructure of the gill of *M.dobsoni* is very similar to that of other crustaceans ( Copeland,1967,1968 ; Bielawski,1971, Fisher,1972; Talbot *et.al.*,1972; Lockwood *et. al.*,1973; Foster and Howse,1978; Dunel Erb *et al.*,1982; Finol and Croghan,1983; Pequeux *et. al.*,1980; Gilles and Pequeux, 1981; Barra *et. al.*,1983 ). The common features in all these species studied are a gill epithelium having infoldings and mitochondria associated with the foldings and the overlying cuticle.



The large surface of the gill is caused by infoldings which are thought to be involved in the osmoregulation. The characteristic association of mitochondria and invaginated or infolded membranes has been identified in other salt transporting tissues. Copeland (1964) observed it in the anal papillae of the mosquito larvae, Copeland (1967) in metepipodite of brine shrimp and in the gills of blue crab on adaptation to freshwater concentrations (Copeland, 1964, 1968).

Croghan et.al. (1965) postulated that the outer membrane of the gill of crayfish *Austropotamobius*, pumped chloride ions while the inner membrane pumped sodium. Fisher (1972) suggested that the expansion of the apical membrane give rise to subcuticular spaces increasing the gill surface area and consequently the absorption of ions. In high external salt concentrations gill permeability could be reduced by closing or flattening the microvilli and suppress the ion intake (Fisher, 1972). Wassia et.al. (1989) observed the third gill of the ghost crab *Ocypode saratan* epithelial cell having a membrane system and high mitochondrial densities typical of surfaces with primary role of ion regulation. Copeland and Fitzjarell(1968) suggested that in *Callinectes sapidus* the salt is released by intracellular vesicles near the mitochondrial pumps and transported to the haemolymph by metabolically active plasma membrane. Papanthanassiou (1985) suggested that in the brown shrimp *Crangon crangon* gills the same mechanism of salt transport could occur with the basal membrane infoldings providing an ideal arrangement for its absorption and transport. Finol and Croghan (1983) could not detect any difference in the basal interdigitations in the gills of *Uca mordax* adapted to 100 or 1 % sea water. Martelo and Zanders(1986) also could not detect such basal infoldings in *Goniopsis cruenata*. Salinity related modifications of the apical foldings and the

distribution (or number) of mitochondria have been reported in the gills of *Callinectes sapidus* (Copeland and Fitzjarell, 1968), *Gammarus oceanicus* (Milne and Ellis, 1973), *Penaeus aztecus* (Foster and Howse, 1978) and *Gammarus duebeni* (Lockwood and Inman, 1973). Pequeux et. al. (1987), Gilles and Pequeux (1985), Barra et. al. (1983) and Pequeux and Gilles (1988) observed the abundant development of the intracellular space, the apical and basal infolding system and mitochondria when *Eriocheir sinensis* were kept in freshwater.

In *M.dobsoni* the clear and well developed infolding system is observed both in the anterior and posterior gills during the monsoon thereby suggesting that all the pairs of gills could be acting as ion regulators. The close association of mitochondria with the infolding system also attributes to the possibility of the presence of an active system involved in the maintenance of ions at lower salinities.

The anterior and posterior pair of gills show an equally important attribution in the process of osmoregulation. The apical portion of the gills have respiratory lamellae which are spaced closer to each other than in the basal region thereby suggesting that the apical region is more actively involved in osmoregulation. Copeland (1968) suggested that the three posterior gills have respiratory lamellae which are spaced exceptionally close to each other to attract water and hence actively absorb salt.

The anterior and posterior gills of *M.dobsoni* observed during the electron microscopic studies showed small cup shaped structures arranged in a row. A similar structure was observed by Schaffner and Rodewald (1978) on the filtration surface of *Procambarus clarkii*. They found

that the filtration barrier is provide by these structures which they named as slit diaphragms. These slit diaphragms were observed by them between adjacent foot processes of the epithelium where in proteins upto the size of haemocyanins and ferritin cross the basement membrane but do not penetrate through the slits into the urinary space. In *M.dobsoni* the observation of these diaphragms could mean that they play a role in the excretory process which needs to be further investigated. Since the size of these slits were not estimated the molecules that could pass through could not be discerned but the preferential movement of molecular compounds according to the slit size cannot be argued.

## **SUMMARY**

The haemolymph flowing through the open circulatory system and the soft tissue in the form of gills experience the influence of environmental conditions due to their direct contact with the environment. The muscle and hepatopancreas also display fluctuations with the environmental changes. The biochemical study of the study of haemolymph, muscle and hepatopancreas and biological study of the gills helped in drawing the following conclusions.

### **ANISOSMOTIC REGULATION :**

The mode of variation of the haemolymph biochemical constituents is not similar in all the size groups of *M. dobsoni* studied.

1> Only hyperosmotic behavior was observed in the size group of 30 - 39 mm group in all the salinities observed. The 40-49 mm, 50 - 59 mm and the females of 60-69 mm groups showed hyper-hyposmotic regulation. They maintained their osmolality higher when compared to that of the environment at lower salinity and low osmotic pressure at high environmental salinities. The males of 60 - 69 mm group showed only hyperosmotic behavior at the low salinities observed.

2> In the 30-39 mm group the variation in osmolality of the haemolymph cannot be attributed to the change in any of the biochemical parameters or the inorganic ions studied during the study. However, the change in biochemical parameters and sodium ion concentration is influenced by the environment. This suggests that the biochemical parameters and the inorganic ions studied in this group do not attribute directly to the osmotic pressure variations but play some role in the regulatory process. A deeper study needs to be

conducted to examine the factor which directly influences the osmotic pressure.

3> In the 40 - 49 mm group the effect of sodium ions on the osmotic pressure variations of the haemolymph has been observed indicating its direct involvement in the regulatory process.

4> In the 50 - 59 mm group the effect of sodium ion and protein concentrations have been felt on the osmolality variations of the haemolymph suggesting its involvement in the regulatory process. The free amino acids are however influenced by the environmental changes though their influence is not felt on the osmolality.

5> A very interesting observation in this study is that the size groups before attainment of sexual maturity adopt different modes for regulating their haemolymph osmotic pressure.

6> The females of 60-69 mm have shown the influence of the sodium ions and carbohydrate on the osmolality of the haemolymph. The influence of the environment is observed on the haemolymph lipid and free amino acid variation.

7> The males of 60-69 mm showed that protein values influence the osmolar values and therefore play a considerable role in the maintenance of the haemolymph environment. The inorganic sodium ions and chloride ions are also influenced by the environment but do not influence the osmotic pressure variation.

## **INTRACELLULAR ISOSMOTIC REGULATION**

1> The influence of the environment has been felt on the biochemical constituents (lipid, carbohydrates, protein and free amino acid nitrogen) in the muscle. The hepatopancreatic lipid and protein are influenced by the environment thereby suggesting their role in the regulatory process in the 30 - 39 mm group.

2> In the 40 - 49 mm group the involvement of only the muscle biochemical parameters studied in the osmoregulatory process. The lack of influence of the environment on the hepatopancreas indicate the lack of their involvement in the osmoregulatory process. The interdependence in the lipid, protein and free amino acid nitrogen levels of muscle suggest that the biochemical parameters help in the balance of the osmotic concentration of the cells by synthesis and/or degradation.

3> In the 50 -59 mm group all the biochemical parameters on the muscle studied and the lipid and free amino acid nitrogen of the hepatopancreas are influenced by the environment thereby indicating their involvement in the osmoregulatory mechanism.

4> There is a total lack of influence of the environment on the muscle biochemical parameters in the females of 60 - 69 mm group suggesting the lack of their involvement in the osmoregulatory process. The hepatopancreatic biochemical parameters are however influenced by the environment and hence play a role in osmoregulation.

5> The males of 60 - 69 mm group show the environmental influence on the carbohydrate and protein levels of the muscle and hepatopancreas indicating

their involvement in the intracellular isosmotic regulatory process.

## **GILL STUDY**

1> The light microscopic studies have shown enhanced vacuolar spaces during the monsoon months in both the anterior and posterior pairs of gills.

2> The ultrastructure studies show the extensive infoldings of the gill epithelium thought to be involved in osmoregulation.

3> No differential structural variation in the anterior and posterior gills could be observed suggesting that all the gills play the same role.

4> the “slit diaphragms” observed also suggested the excretory role played by the anterior and posterior gills.

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