INFLUENCE OF DIFFERENT LEVELS OF AMBIENT OXYGEN ON METABOLITE CHANGES AND GROWTH IN LABORATORY REARED PENAEUS INDICUS (H. MILNE EDWARDS)

Dissertation submitted by **Kum. GANGA U.** in partial fulfilment for the Degree of Master of Science (Mariculture) of the Cochin University of Science and Technology

December 1992

Post-Graduate Programme in Mariculture
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
Cochin – 682 031

CERTIFICATE

This is to certify that this dissertation is a bonafide record of work carried out by Ms.Ganga.U under my supervision and that no part thereof has been presented before for any other degree.

DR. P.S.B.R. JAMES
DIRECTOR
CENTRAL MARINE FISHERIES
RESEARCH INSTITUTE
COCHIN.

Countersigned by:

DIRECTOR
CENTRAL MARINE FISHERIES
RESEARCH INSTITUTE
COCHIN.

C O N T E N T S

		PAGE
1.	PREFACE	1
2.	INTRODUCTION	5
3.	MATERIALS AND METHODS	12
4.	RESULTS	17
5.	DISCUSSION	47
6.	SUMMARY	56
7.	REFERENCES	59

PREFACE

Worldwide, the penaeid shrimp has assumed an increasingly important place among cultured animals and is the mainstay of marine export products of India. While it is important to maximize production by semi-intensive and intensive methods it is also necessary to understand the impact of various environmental parameters on physiology, growth and survival of these aquatic organisms.

Water quality control forms an essential part of aquaculture management and dissolved oxygen, NH_3 and CO_2 form its crucial aspects. Oxygen management in culture ponds is made difficult not only by the complex web of ecological interactions but also by diurnal fluctuations. On calm days, intensive algal bloom results in excess production of oxygen resulting in supersaturation. During night, the metabolism shifts completely to respiration resulting in low dissolved oxygen (DO) levels, especially just before dawn. In addition to this diurnal variation, there is an ever present biochemical oxygen demand (BOD) from added shrimp feed, fertilizers and decay of sinking autorophic biomass.

Under culture conditions, CO_2 and NH_3 concentrations are high when DO levels are low. Besides, continuous

exposure to low DO level has been considered as a precursor to bacterial infection in fish. Many studies have been conducted to study the lethal or limiting DO levels for various penaeid species. However, in penaeid shrimp culture there is another type of critical DO level necessary to sustain a commercial growth rate. This is greater than the DO level which results in mortality and the incipient limiting level. Thus it is important to determine the lowest DO level which will sustain maximum growth.

Prolonged hypoxia has been found to inhibit growth and moulting in crustaceans. Feed forms one of the most important cost items for intensive aquaculture and has to be judiciously used. The supply of food must be timed so that fish utilise the same for attaiming maximum growth from all the food distributed. Food consumption and feed conversion rates are lowered with decreased oxygen levels. Excess feed supplied could lead to an increased BOD resulting in deterioration of water quality and should be avoided at all costs. DO levels are thus important in establishing feeding rates which do not stress the assimilation capabilities of the system.

Thus while formulating culture practices, information on lethal and sublethal levels of various environmental parameters like temperature and dissolved oxygen is vital. Also, the effect of these factors on the bio-energetics of

the organism, that is energy input, energy dissipation and growth has to be known for understanding optimum feeding and growting conditions.

In the dynamic aquatic environment, stress is an inescapable part of life and fishes have evolved a large variety of physiological mechanisms to overcome it. Hypoxia has been found to cause 'respiratory stress' in decapods. Stress causes metabolic disturbances in fish accompanied by biochemical, physiological and immunological changes. Duration of stress also has an important bearing on the severity of the tissue changes that occur.

Presently, sensitive, unequivocal measurements of either the severity of the stress caused by environmental alternations or tolerance limits for adaptation are not always possible and death must still be used occassionally as the end point. But, biochemical changes in the various tissues resulting from a stress response can be quantified and have potential as indices.

Penaeus indicus in widely cultivated in improved extensive and semi-intensive culture systems in Kerala, Karnataka and Tamilnadu and accounts for 30% of total penaeid prawn cultured in India. The present study was conducted to study growth and metabolite mobilization as a function of various DO levels in Penaeus indicus under

laboratory conditions.

My deepest thanks are due to Dr. P.S.B.R. James, Director, CMFRI, under whose valuable guidance this study was conducted. Sincere thanks are also due to Dr. N.Sridhar for his valuable suggestions and help, and Shri. T.V.Sathyanandan, FRAD, for his help with the statistical analysis. I am also grateful to the PGPM staff, and, especially Shri.A Nandakumar and Mr. P.M.Aboobacker for their kindly help and co-operation. I also thank all my classmates, especially C. Reghunathan, and seniors for their help and co-operation.

I would also like to thank the Indian Council of Agricultural Research for awarding me the Junior Research Fellowship during whose tenure the above study was carried out.

* * *

INTRODUCTION

Water temperature and dissolved oxygen (DO) are two parameters that are vitally important for optimization in aquaculture production. Typically, there is a relationship between the physiological capacity of an animal and its ecological requirements. If DO level falls below 2 mg/litre and water temperature less than 22°C warm water species of fish and invertebrates become stressed. This leads to decreased growth and survival, increased aggression, lower quality product and thus, a reduced income.

Reports on the effects of hypoxic exposure describe two extremes of response: Oxyconformers which simply allow oxygen consumption (MO_2) to vary with oxygen availability and oxyregulators which attempt to regulate MO_2 over a range of external oxygen levels. However, the distinction between the two responses is not absolute (Mangum and Van winkle, 1973; Herried, 1980). Previous investigations of penaeid shrimp oxygen response have suggested that the respiratory response of these animals is dependent on a number of factors including oxygen level, animal size, activity and species.

Information on lethal and sublethal values for various environmental parameters like DO, temperature and salinity is vital in formulating culture practices. Under culture conditions, low environmental oxygen concentration may enhance the stressful effects of others toxicants (Lloyd, 1961) or

increase the probability of disease (Sniesko, 1974).

Dissolved oxygen has been classified as a limiting factor by Fry (1947, 1971), and is found to act on aquatic organisms via metabolism. Some studies have been conducted to define lethal or limiting levels of DO for various penaeid prawns. (Subramanyan, 1962; Mackay, 1974; Kramer, 1975; Boyd, 1976;) In penaeid shrimp culture there is another type of critical DO, higher than the lethal or incipient limiting level, necessary to sustain a commercial growth rate. Very few studies have been conducted on growth of shrimp as a function of DO levels.

Brett (1979) noted that a DO level of 5ppm was critical for growth of fish. Experiments conducted with carp by Rappaport et al; (1976) in Israel, showed that fish growth decreased if DO concentration dropped below 25% of saturation at sunrise. Coho salmon presmolts show decreased growth rates at oxygen concentrations of 3.5ppm (Haywood et al, 1980). Seidman and Lawrence (1985) have reported that growth potential of Penaeus vannamei is not achieved at a constant DO concentration less than 2 mg. per litre. Moulting was inhibited in Penaeus semisulcatus exposed to hypoxia for a period of 17 days (Clark, 1986).

Biochemical oxygen demand (BOD), especially that associated with the sediment is a significant component affecting the pond water quality (Fast et al, 1988). Excess

feed supplied could lead to an increased BOD resulting in deterioration of water quality. Fishes have been reported to cease feeding during periods of low oxygen concentration (Bouck and Ball, 1965). Continuous exposure of large mouth bass to low DO concentration has been found to cause decrease food intake and growth (Stewart et al; 1967). Channel catfish grown in labtanks at DO levels of 100, 60 and 36 percent of saturation and fed ad libitum, showed increased food consumption and weight gain with higher levels of oxygen saturation. (Andrews et al., 1973; Tucker et al., 1979). Llobera (1983) reported a reduced food intake of Macrobrachium rosenbergii raised at 2.5ppm as compared at higher DO levels.

Decapod crustaceans are, in general, sensitive to lack of oxygen and have evolved a large variety ofphsysiological mechaniems to enable them to cope with a multiplicity of environmental conditions. A general response to hypoxic ambient conditions is an increase of ventilatory activity (Taylor et al., 1973; Mc Mahon and Wilkens, 1983) accompanied by immediate acid base disturbances of respiratory origin. In the face of respiratory stress induced by hypoxia many metabolic adjustments are also made. This includes changes in metabolic rates and pathways, the ability to build up a temporary oxygen debt and enzymatic acclimations to provide energy anaerobically. An increased respiratory quotient (RQ) has been observed in Uca species and Sesarma cinereum exposed to hypoxia (Teal and Carey, 1967).

Stress responses in fish are of adaptive nature to allow it to maintain homeostasis in the face of an external force. This involves a shift from anabolism to catabolism to allow the animal to utilize energy reserves not normally available. Biochemical changes thus effected vary with the species involved and also with the nature and duration of the stress (Wedemeyer, 1981).

Proteins are ubiquitious components of all living tissues, serve indispensable functions in cellular architecture and are intimately concerned with virtually all physiological events (Mahler and Cordes, 1968). Any change in the physiology of an organism as a result of adverse ecological conditions has been found to affect protein content of the tissue qualitatively and quantitatively (Rajamani, 1982). Under anaerobic conditions induced by hypoxia increased protein degradation and nitrogen excretion has been observed (Kutty, 1972). Lack of oxygen has been observed to cause a decrease in protein synthesis as measured by incorporation of radioactive leucine in the fish Fundulus heteroclitus (Jackim and La Roche, 1973). Haemocyanin, the major haemolymph protein constituent shows variations in relation to ambient oxygen. While hypoxia induced haemocyanin synthesis has been reported in Homarus americanus and Nephrops norvegicus (Senkbeil and Wriston; 1981; Hagerman and Uglow., 1985), lowered haemocyanin levels have been reported by Phil

Baden (1988). Butler et al. (1978) were unable to detect significant changes in lobsters exposed to low oxygen tension for 120 hours.

Crustacean metabolism is mainly centered around glycogen and fatty acids (Vonk, 1960). Mazeaud (1973) observed that asphyxia in Cyprinus carpio automatically reduced the free fatty acid (FFA) level in the blood. In Salmo gairdneri, FFA was observed to rise as a response to 20 minute hypoxia (Mazeaud et al., 1977). Blazka (1958) observed the accumulation of short chain fatty acids during hypoxia in carp. In shrimp, no accumulation of lipids due to unnatural culture conditions have been reported. However, in crustacean larvae triacyl glycerol which is the storage form of lipid is found to be directly influenced by various environmental stress factors that increase metabolic activity and decrease food intake. Thus, consequences of stress on lipid metabolism are far from clear.

In general, prawns lack sterol synthesizing ability and require a dietary sterol for several physiological functions and normal growth (Castell et al, 1975). Cholesterol constitutes 95-98% of total sterol in penaeid shrimp (Thomson, 1964) and acts as a component of subcellular membrane structures (Lasser et al., 1966) and a precursor for moulting hormones (Gilbert, 1985). Cholesterol levels in brown shrimp Penaeus aztecus are found to be tissue, size and sex

specific as well as related to diet.

Glycogen is the focal point of crustacean intermediary metabolism (Travis, 1955; Martin, 1965; Adiyodi, 1969) and is utilized for chitin synthesis (Yamaoka and Scheer, 1970). Growth periods in Panulirus argus Latreille are accompanied by cyclical alterations in the muscle and hepatopancreas (Travis, 1955).

In most teleosts, fermentation of glucose or glycogen to lactate provides the main source of energy production in hypoxic conditions (Heath and Pritchard, 1965; Bandurski et al. 1968; Wittenberger, 1958; Hunn, 1969; Burton, 1970 a,b; Burton and Spehar, 1971; Van den Thillart et al., 1976; Burggren and Cameron, 1980; Jorgensen and Mustafa, 1980). Teal and Carey (1967) observed increased utilization of glycogen and accumulation of lactate in the marsh crab Uca pugilator exposed to hypoxia.

There is some evidence of participation of carbohydrates in processes of energy production and in terminal oxidation in crustaceans as in other animals (Hu, 1958; Meenakshi and Scheer, 1961; Gilles and Schoffeniels, 1964). Blood glucose represents a form of transport between glycogen of tissues and hepatopancreas to site of chitin synthesis and reflects the physiological condition of the animal. Under conditions of asphyxiation some decapods like Cancer pagurus

and Carcinus maenas increase their blood sugar level. This has been attributed to increased basal metabolic rate and degradation of glycogen in muscle and hepatopancreas during stress.

The white shrimp, Penaeus indicus is widely cultivated in extensive and semi-intensive culture systems, especially Kerala, and is commercially acceptable throughout most of the world. Mass rearing of this species would be aided by a definition of normal baseline physiological and biochemical parameters. The present study on growth and metabolite mobilization in P. indicus under varying concentrations of dissolved oxygen is an attempt in this direction.

. . .

MATERIALS AND METHODS

The study was conducted on Penaeus indicus juveniles (50-60mm TL) which were caught using cast nets, from Puduvyppu, and transported to the laboratory. They were maintained in 200L capacity, rectangular, fibreglass tanks filled with clean, filtered and well aerated sea water of 15ppt salinity. The animals were allowed to acclimate to the laboratory conditions for a period of seven days prior to the start of the experiment. During this period, they were fed ad libitum, with dried clam meat. Only healthy, intermoult animals were selected for the experiment.

The experiment was conducted in two phases, the first to study survival, moulting pattern and food consumption under various DO levels and the second phase to study growth and metabolite mobilization as a function of various DO levels. Four DO levels were selected for the study, Viz. 5, 4, 3 and 2 ml O_2 l^{-1}. Aeration was used to maintain normoxic conditions (4 and 5 ml O_2 l^{-1} DO). Nitrogen stripping by bubbling nitrogen gas into the water for 30 and 45 minutes was done to achieve and maintain a DO concentration of 3 and 2 ml $O_2 l^{-1}$ respectively.

Two replicates were made, with circular 40l capacity plastic troughs being randomly assigned to each of the four DO levels. Twelve animals, dried and weighed to the nearest 0.1mg. were introduced into each tank. To prevent sudden

PLATE - I PENAEUS INDICUS

PLATE - II EXPERIMENTAL SET UP

PLATE - III NITROGEN STRIPPING OF WATER

respiratory stress to prawns kept at lowest DO level 2ml $O_2 l^{-1}$ they were maintained in unaerated water for a period of 24 hours prior to the actual start of the experiment. Subsequently, two-thirds of the water was replaced with water of appropriate DO concentration achieved through nitrogen stripping.

To prevent accumulation of harmful metabolities, and to maintain the water quality, 40% of the water was changed daily and completely every third day. Water salinity ranged from 12-15 ppt and temperature 26-28.5°C throughout the study period.

Feeding was done with dried clam meat at the rate of 5% of the body weight, in two divided doses at approximately 0930 and 1630 hours. The amount of feed was adjusted weekly taking into account mortalities and increase in body weight. Excess feed which was removed prior to the morning meal was subsequently dried in an oven and its weight recorded. This was used to calculate food consumption rate using the formula.

$$100 - \frac{\text{feed wasted}}{\text{feed provided}} \times 100$$

The moulting rate (M) was calculated according to the formula, as given by Petriella (1990),

$$M = \frac{\text{moult percentage}}{\text{mean life of the group.}}$$

Where, moult percentage = $m/n_1 \cdot 100$,

m = number of moults; n_1 = initial number of animals.

The mean life of the group was calculated by adding the number of days each individual survived (Brown and Cunningham, 1939).

DO, temperature and salinity were recorded daily each morning. DO was determined using the winkler method (Strickland and Parsons, 1972). Salinity was measured using a refractrometer which was periodically calbrated with standard sea water. Water temperature was measured using a maximum minimum mercury thermometer. Total ammonia levels (NH_4 - N) were determined weekly using phenol hypochlorite method outlined by Strickland and Parsons (1972).

The experiment was terminated after a period of thirty days. All the shrimp were weighed to the nearest 10mg and recorded by tank. Intermoult animals subject to each treatment were used for biochemical analysis. The various metabolities estimated in the muscle, hepatopancreas and haemolymph included,

(1) Proteins

(2) Total carbohydrates

(3) Glycogen

(4) Total lipids

(5) Cholesterol

Haemolymph extraction : Animals were first rid of external water using tissue paper. Haemolymph was then collected from the pericardial cavity of each prawn using a hypodermic syringe fitted with a No.22 needle. A 5% solution of trisodium citrate was used as an anticoagulant. Haemolymph samples for each treatment were pooled together in plastic vials and stored in the freezer till analysis was done.

Fresh hepatopancreas were dissected out and alongwith muscle tissue was stored in freezer till analysis was done.

Protein estimation was done using the Folin-Cio-Calteu method (Lowry et al; 1951). Bovine Serum albumen was used to prepare the standard graph.

Lipids were estimated according to the sulphophospho-vanillin method of Barnes and Blackstock (1973). Cholesterol was estimated according to Henly's method (1957) using ferric chloride, concentrated H_2SO_4 and acetic acid, as given by Varley. Cholesterol A.R. grade was used for the preparation of standards.

Total carbohydrates were estimated by the Phenol-Sulphuric acid method as given by Dubois et al; 1956. Glycogen

was estimated by the Anthrone method (Caroll *et al*; 1956). D-glucose was used to prepare the standards.

* * *

R E S U L T S

Mean dissolved oxygen (DO) levels for various treatments were 2.05 ± 0.07 ml/l, 3.15 ± 0.05 ml/l, 5.35 ± 0.05 ml/l and 4.29 ± 0.03 ml/l (control). After a period of thirty days, shrimps subject to each treatment were weighed to the nearest 0.1 mg and recorded by tank. Also, intermoult animals subject to each treatment were biochemically analysed for the various metabolites in the various tissues like, hepato-pancreas, haemolymph and muscle.

(i) GROWTH STUDIES: The mean wet weights, growth rate, percentage increase in body weight, moulting rate and food consumption are given in table 2.

Growth, in terms of percentage increase in body weight, and, growth rate was highest at the DO level of 4.29 ml $O_2 l^{-1}$, being 22.93% and 12 mg day^{-1} respectively. When the DO level was 3.15 ml O_2 l^{-1} the growth rate was found to be 9mg day^{-1} with a percentage increase in wet weight of 19.44. At the highest DO level of 5.35 ml $O_2 l^{-1}$, a growth rate of 7 mg day^{-1} was observed, being an increase of 14.29% over a period of thirty days. At the lowest DO concentration of 2.05 ml $O_2 l^{-1}$, negative growth as recorded by a loss of weight was observed.

ANOVA showed that while growth did not differ significantly among DO levels of 5.35, 4.29 and 3.15 ml $O_2 l^{-1}$, when compared to the lowest DO level of 2.05 ml $O_2 l^{-1}$, The difference was

significant at 1% level (Table XVI) Good survival (> 50%) was observed at DO levels above 3ml $O_2 l^{-1}$ while at the lowest DO level it was 41.67%. Moulting rates for combined tanks showed to be 2.34, 2.2, 2.23 and 1.38 at DO levels of 4, 5, 3 and 2 ppm respectively. (Table2).

Food consumption was found to decrease at lower oxygen levels and ranged from 45.33 to 54.7%. (Table-2). Highest feed consumption was observed at the DO level of 4ml $O_2 l^{-1}$ being 58.59%. When compared to this, at the extremely low DO level of 2ml $O_2 l^{-1}$, it was only 45.33%, showing a difference of more than 10% in amount of food consumed.

(ii) CHANGES IN ORGANIC CONSTITUENTS:-

Organic constituents like Proteins, lipids, total carbohydrates, glycogen and cholesterol were estimated in the haemolymph, hepatopancreas and muscle. Comparisons of percentage variations in these various organic constituents were also made with the levels observed at 4.29 ml $O_2 l^{-1}$ where growth was best.

HEPATOPANCREAS:- Organic reserves estimated in the hepatopancreas are shown in table 3.

PROTEINS:- Protein levels were the highest at a DO concentration of 4ml $O_2 l^{-1}$ being 7.12 $\pm$ 0.20 mg% followed by 6.878 $\pm$ 0.130 mg% at 5ml $O_2 l^{-1}$ DO concentration. Compared to this, at lower DO levels of 2 and 3 ml $O_2 l^{-1}$, protein levels fell by 47.93 and 49.75% respectively.

ANOVA revealed significant difference between the normoxic (5 and 4 ml $O_2 l^{-1}$) and hypoxic levels (3 and 2ml $O_2 l^{-1}$). TABLE(i)

LIPIDS: while no general trend was observed in the lipid content due to various DO treatments, lipid levels were observed to be the highest at the lowest DO level of 2ml $O_2 l^{-1}$. When compared to the control, this was an increase of 177%. At other DO levels, lipid levels showed a decline only. ANOVA revealed these lipid variations to be significant. TABLE(ii)

TOTAL CARBOHYDRATES:- Carbohydrate level was found to be significantly different only at the lowest DO level of 2ml $O_2 l^{-1}$. Here it registered a decline of about 13.39% when compared to the control with a carbohydrate level of 12.621 $\pm$ 0.417mg%. ANOVA did not reveal any significant variations in lipid content between DO levels of 3, 4 and 5ml $O_2 l^{-1}$. TABLE (iii)

GLYCOGEN:- Glycogen values declined significantly at all the DO levels. However, this decline was not marked at DO level of 2 and 3 ml $O_2 l^{-1}$, being 50-60% lower when compared to the control with a DO level of 4ml $O_2 l^{-1}$, Table. iv

CHOLESTEROL:- Cholesterol levels increased significantly at when the DO concentration was low. 90-140% higher levels were observed at DO levels of 3 and 2 Ml $O_2 l^{-1}$ when compared to the control 4ml $O_2 l^{-1}$. No such increase was noted at the higher DO level of 5ml $O_2 l^{-1}$ Table (V)

HAEMOLYMPH:- Organic reserves estimated in the haemolymph are shown in Table 4.

PROTEINS:- Protein levels in the haemolymph showed an increase of 112.5 - 122.4% at the lower DO levels when compared to the control DO level of 4ml $O_2 l^{-1}$. There was a significant difference of about 50% between the two extreme DO levels of 2 and 5 ml $O_2\ l^{-1}$. Table. vi

TOTAL LIPIDS:- Lipid levels did not show any general trend with the various DO levels. While it was observed that there was no significant decrease in lipid levels at 4 and 3 ml $O_2 l^{-1}$, at both the extreme DO levels of 5 and 2ml $O_2 l^{-1}$, it declined by 23.57 and 18.23% respectively. Table vii

TOTAL CARBOHYDRATES: When compared to the control, a significant decline in total carbohydrate content was observed at the various DO levels. (2ml $O_2 l^{-1}$: -56.45%, 3ml $O_2 l^{-1}$: -49% 5ml $O_2 l^{-1}$: -32%) Table. viii

ANOVA revealed these differences to be significant.

GLYCOGEN:- Glycogen levels showed an inverse relationship with the DO levels. It varied from 2.561 ± 0.02 mg% at the highest DO level of 5ml $O_2 l^{-1}$ being 5.582 ± .2 mg% at a DO level of 2ml $O_2 l^{-1}$, which was thus a increase of nearly 64%. At a DO level of 3ml $O_2 l^{-1}$, glycogen level was found to be 5.10.

ANOVA revealed significant difference due to the DO treatments. TABLE. ix

CHOLESTEROL: Haemolymph cholesterol showed a greater decline

at higher DO levels. ($5ml\ O_2l^{-1}$: -67.25% $2ml O_2l^{-1}$: -12.9%) this indicated a difference of about 50% in mobilization pattern. ANOVA revealed these differences to be significant. TABLE X.

MUSCLE : Mean values of the various metabolites are given in table 5.

PROTEINS: Protein levels were found to be the highest at the DO level of 4.29 ml O_2l^{-1} being 12.586 ± 0.416 mg% and lowest at the DO level of 2.05 ml O_2l^{-1} being 11.082 ± 0.406 mg%.

ANOVA revealed significant decrease in protein content, 11.31 - 11.92% only at the lower DO levels of 3 and 2 ml O_2l^{-1} when compared to control of $4ml O_2l^{-1}$. TABLE xi

LIPIDS:- Lipids showed a declining trend at the lower DO levels, When compared to the control $4ml O_2l^{-1}$. This decrease was 42.18% ($2ml O_2l^{-1}$) and 48% ($3ml O_2l^{-1}$). However, among the above mentioned, lower DO concentrations there was no significant change in lipid content as shown by ANOVA. Thus, decrease in lipid content was significant only at DO levels of 2 and 3 ml O_2l^{-1} when compared to the higher DO levels of 4 and 5 ml O_2l^{-1}. TABLE xii

TOTAL CARBOHYDRATES: Carbohydrate levels were found to increase at the normoxic levels of 4 and 5 $ml O_2l^{-1}$. A reduction of 65.96-72.19% was observed at the lower DO levels when compared to the control ($4ml\ O_2l^{-1}$). At the same time, carbohydrate levels seemed to increase significantly (+9.87%) at $5ml O_2l^{-1}$. No significant difference as observed

between the lower two DO levels. TABLE. xiii

GLYCOGEN: Glycogen levels showed an inverse relationship with the DO levels. It varied from 1.034 ± 0.02 mg% at the highest DO level of 5ml $O_2 l^{-1}$ to 0.495 ± 0.02 mg% at a DO level of 2ml $O_2 l^{-1}$ thus registering a decrease of 50% at the lower DO level. ANOVA revealed that glycogen levels did not vary significantly between the two lower DO levels of 3 and 2 ml $O_2 l^{-1}$. TABLE XIV

CHOLESTEROL:- Cholesterol levels was highest at the lowest DO concentration of 2 ml $O_2 l^{-1}$ being 0.518 ± 0.028 mg% when compared to 0.447 ± 0.01 mg% in control. No significant differences were observed among the higher DO levels of 5, 4 and 3ml $O_2 l^{-1}$. All these levels varied significantly from the levels observed at the lowest DO level of 2ml $O_2 l^{-1}$. TABLE XV.

. . .

Table 1

EXPERIMENTAL CONDITIONS DURING THE STUDY ON GROWTH AND METABOLITE MOBILIZATION IN *PENAEUS INDICUS* REARED AT VARIOUS DO LEVELS

EXPERIMENTAL CONDITIONS				
Duration of Experiment	Thirty days			
Do levels desired (ml O_2 l - 1)	5	4	3	2
Actual Do levels (ml O_2 l - 1)	5.35	4.29	3.15	2.05
Number of Replicates	Two			
Number and size of animals	24 prawns for each treatment 50 - 60 mm size			
Temperature	26 - 28.5°C			
Salinity	12 - 15 ppt.			
Feeding	Dried Clam Meat @ 5% of Body weight.			

TABLE 2. OBSERVATIONS ON GROWTH, FOOD CONSUMPTION AND MOULTING RATE IN LABORATORY REARED *PENAEUS INDICUS* EXPOSED TO VARIOUS DO LEVELS

DO LEVEL Ml $O_2 l^{-1}$	MEAN WEIGHT		INCREASE in		GROWTH RATE Mg day^{-1}	MOULTING RATE	AVERAGE FOOD CONSUMPTION (%)
	INITIAL (g)	FINAL (g)	MEAN WEIGHT	% MEAN WEIGHT			
5.35±0.05	1.47±0.05	1.68±0.44	0.21	14.29	7	2.2	54.70
4.29±0.03	1.57±0.01	1.93±0.08	0.36	22.93	12	2.34	58.59
3.15±0.05	1.44±0.07	1.72±0.22	0.28	19.44	9	2.23	47.28
2.05±0.07	1.42±0.06	1.40±0.17	NEGATIVE	NEGATIVE	-0.001	1.38	45.33

VALUES REPRESENT ± SE FOR DUPLICATE TANKS.

INITIAL NUMBER OF ANIMALS 24 FOR EACH TREATMENT.

$$\text{AVERAGE FEED CONSUMPTION (\%)} = 100 - \frac{\text{FEED WASTED}}{\text{FEED PROVIDED}} \times 100$$

$$\text{MOULTING RATE} = \frac{\text{MOULT PERCENTAGE}}{\text{MEAN LIFE OF THE GROUP.}}$$

TABLE 3: ORGANIC RESERVES IN HEPATOPANCREAS OF PENAEUS INDICUS REARED UNDER VARIOUS DO LEVELS FOR A PERIOD OF THIRTY DAYS.

ORGANIC RESERVES	INITIAL LEVEL (mg%) 0 day	VARIOUS DO TREATMENTS (ml $O_2 l^{-1}$)			
		5	4	3	2
PROTEINS	4.694±0.07	6.878 ± 0.13	7.129 ± 0.2	3.582 ± 0.04	3.712 ± 0.13
TOTAL LIPIDS	3.718 ± 0.08	1.033 ± 0.01	1.520 ± 0.04	1.433 ± 0.06	4.221 ± 0.12
TOTAL CARBOHYDRATES	4.802 ± 0.14	11.120 ± 0.23	12.621± 0.42	11.376± 0.26	10.930± 0.30
GLYCOGEN	0.34 ± 0.02	0.41 ± 0.01	0.64 ± 0.02	0.19 ± 0.01	0.22 ± 0.01
CHOLESTEROL	1.10 ± 0.03	0.55 ± 0.02	0.51 ± 0.03	1.23 ± 0.04	0.977±0.021

ALL VALUES $\bar{X}$ ± SE OF 12 DETERMINATIONS.

TABLE - 4: ORGANIC RESERVES IN HAEMOLYMPH OF PENAEUS INDICUS REARED UNDER VARIOUS DO LEVELS FOR A PERIOD OF THIRTY DAYS

ORGANIC RESERVES	INITIAL LEVEL (0 day)	VARIOUS DO TREATMENTS			
		5	4	3	2
PROTEINS (g/100ml)	1.95 ± 0.03	2.36 ± 0.13	2.09 ± 0.03	4.66 ± 0.08	4.45 ± 0.10
TOTAL LIPIDS mg/100ml	112.791 ± 1.4	125.81± 2.117	164.60± 2.16	162.30±1.04	134.59± 1.82
TOTAL CARBOHYDRATES mg/100ml	13.55±0.17	23.79± 0.86	34.84± 1.6	17.71± 0.9	15.18± 0.27
GLYCOGEN mg/100ml	2.21±0.08	2.56±0.02	3.14±0.10	5.17±0.02	5.58±0.05
CHOLESTEROL mg/100ml	52.20±1.7	20.15±0.30	61.55±2.02	29.47±0.01	53.61±1.49

ALL VALUES $\bar{X}$ ± SE OF 8 DETERMINATIONS

TABLE 5 ORGANIC RESERVES IN MUSCLE OF *PENAEUS INDICUS* REARED UNDER VARIOUS DO LEVELS FOR A PERIOD OF THIRTY DAYS.

ORGANIC RESERVES (mg%)	INITIAL LEVEL 0-day	VARIOUS DO TREATMENTS			
		5	4	3	2
PROTEINS	10.13±0.68	11.71±0.35	12.59±0.42	11.16±0.33	11.08±0.4
TOTAL LIPIDS	1.44±0.05	1.02±0.02	1.49.0.07	0.86± 0.03	0.773±0.06
TOTAL CARBOHY-DRATES	0.59±0.05	3.47±0.13	3.85±0.13	1.31± 0.08	1.06±0.06
GLYCOGEN	0.64±0.06	1.03±0.02	0.85±0.01	0.41±0.03	0.50±0.02
CHOLESTEROL	0.14±0.01	0.44±0.02	0.45±0.01	0.45±0.02	0.52±0.03

ALL VALUES $\bar{X}$ ± SE OF 12 DETERMINATIONS.

TABLE 6 :

PERCENTAGE VARIATIONS IN THE VARIOUS ORGANIC RESERVES IN HEPATOPANCREAS, HAEMOLYMPH AND MUSCLE OF PENAEUS INDICUS EXPOSED TO VARIOUS DO LEVELS FOR THIRTY DAYS.

ORGANIC RESERVES	TISSUES	CONTROL ($4ml\ O_2 l^{-1}$)	ORGANIC RESERVES UNDER VARIOUS DO LEVELS COMPARED TO CONTROL		
			$5ml\ O_2 l^{-1}$ Vs. CONTROL	$3ml\ O_2 l^{-1}$ Vs. CONTROL	$2ml\ O_2 l^{-1}$ Vs. CONTROL
PROTEINS	HEPATOPANCREAS	7.13 ± 0.2	-3.52	* -49.75	* -47.93
	HAEMOLYMPH	2.09 ± 0.2	* +12.61	* +122.4	* +122.5
	MUSCLE	12.59 ± 0.42	-6.96	* -11.31	* -11.92
TOTAL LIPIDS	HEPATOPANCREAS	1.52 ± 0.23	* -32.04	* -5.72	* +177.7
	HAEMOLYMPH	164.6 ± 2.12	* -23.57	-1.40	* -18.23
	MUSCLE	1.50 ± 0.07	-39.42	* -48	* -42.18
TOTAL CARBOHYDRATES	HEPATOPANCREAS	12.62 ± 0.42	-11.88	-9.8	* -13.39
	HAEMOLYMPH	34.85 ± 1.6	* -31.93	* -49.03	* -56.45
	MUSCLE	3.85 ± 0.13	* + 9.87	* -72.19	* -65.96
GLYCOGEN	HEPATOPANCREAS	0.64 ± 0.02	* -34.89	* -70.25	* -58.57
	HAEMOLYMPH	3.14 ± 0.10	* -18.47	* +64.05	* +77.61
	MUSCLE	0.85 ± 0.01	* +21.36	* -51.06	* -41.9
CHOLESTROL	HEPATOPANCHREAS	0.51 ± 0.03	+ 8.97	* +140.9	* +90.45
	HAEMOLYMPH	61.56 ± 2.03	* -67.25	* -52.11	* -12.9
	MUSCLE	0.45 ± 0.01	- 0.01	- 0.004	* + 0.15

* Significant

ANOVA TABLES FOR VARIOUS ORGANIC RESERVES

Table (i)

ANOVA: HEPATOPANCREAS PROTEINS

SOURCE	DF	SS	MS	F	REMARKS
Treatments	3	135.663	45.221	195.93	1%
Error	44	10.155	0.231		S
Total	47	145.818			

S: SIGNIFICANT NS: NOT SIGNIFICANT

Table (ii)

ANOVA: HEPATOPANCREAS LIPIDS

SOURCE	DF	SS	MS	F	REMARKS
Treatments	3	76.907	25.636	438.40	1%
Error	44	2.573	0.058		S
Total	47	79.480			

Table (iii)

ANOVA: HEPATOPANCREAS TOTAL CARBOHYDRATES

SOURCE	DF	SS	MS	F	REMARKS
Treatments	3	20.879	6.960	6.13	1%
Error	44	49.950	1.135		S
Total	47	70.829			

Table (iv)

ANOVA: HEPATOPANCREAS GLYCOGEN

SOURCE	DF	SS	MS	F	REMARKS
Treatments	3	1.428	0.476	207.53	1%
Error	44	0.101	0.002		S
Total	47	1.529			

Table (v)

ANOVA: HEPATOPANCREAS CHOLESTEROL

SOURCE	DF	SS	MS	F	REMARKS
Treatments	3	4.327	1.442	166.53	1%
Error	44	0.381	0.009		S
Total	47	4.708			

Table (vi)

ANOVA: HAEMOLYMPH PROTEINS

SOURCE	DF	SS	MS	F	REMARKS
Treatments	3	43.746	14.582	213.07	1%
Error	28	1.916	0.068		S
Total	31	45.663			

Table (vii)

ANOVA: HAEMOLYMPH LIPIDS

SOURCE	DF	SS	MS	F	REMARKS
Treatments	3	9175.125	3058.375	112.56	1%
Error	28	760.688	27.167		S
Total	31	9935.813			

Table (viii)

ANOVA: HAEMOLYMPH TOTAL CARBOHYDRATES

SOURCE	DF	SS	MS	F	REMARKS
Treatments	3	1841.951	613.984	89.06	1%
Error	28	193.025	6.894		S
Total	31	2034.977			

Table (ix)

ANOVA: HAEMOLYMPH GLYCOGEN

SOURCE	DF	SS	MS	F	REMARKS
Treatments	3	52.803	17.601	626.80	1%
Error	28	0.786	0.028		S
Total	31	53.589			

Table (x)

ANOVA: HAEMOLYMPH CHOLESTEROL

SOURCE	DF	SS	MS	F	REMARKS
Treatments	3	9192.457	3064.152	215.50	1%
Error	28	398.129	14.219		S
Total	31	9590.586			

Table (xi)

ANOVA : MUSCLE PROTEINS

SOURCE	DF	SS	MS	F	REMARKS
Treatments	3	13.390	4.463	2.62	NS
Error	44	74.999	1.705		
Total	47	88.389			

Table (xii)

ANOVA : MUSCLE LIPIDS

SOURCE	DF	SS	MS	F	REMARKS
Treatments	3	3.726	1.242	61.01	1%
Error	44	0.896	0.020		S
Total	47	4.622			

Table (xiii)

ANOVA : MUSCLE CARBOHYDRATES

SOURCE	DF	SS	MS	F	REMARKS
Treatments	3	74.676	24.892	232.19	1%
Error	44	4.717	0.107		S
Total	47	79.393			

Table (xiv)

ANOVA : MUSCLE GLYCOGEN

SOURCE	DF	SS	MS	F	REMARKS
Treatments	3	3.080	1.027	248.51	1%
Error	44	0.182	0.004		S
Total	47	3.262			

Table (xv)

ANOVA : MUSCLE CHOLESTEROL

SOURCE	DF	SS	MS	F	REMARKS
Treatments	3	0.987	0.329	91.54	1%
Error	44	0.158	0.004		S
Total	47	1.145			

Table (xvi)

ANOVA : GROWTH INCREMENTS

SOURCE	DF	SS	MS	F	REMARKS
Treatments	3	0.043	0.014	5.60	1%
Error	28	0.072	0.003		S
Total	31	0.114			

Table (xvii)

COMPARISONS OF GROWTH INCREMENTS IN PENAEUS INDICUS SUBJECT TO VARIOUS DO LEVELS.

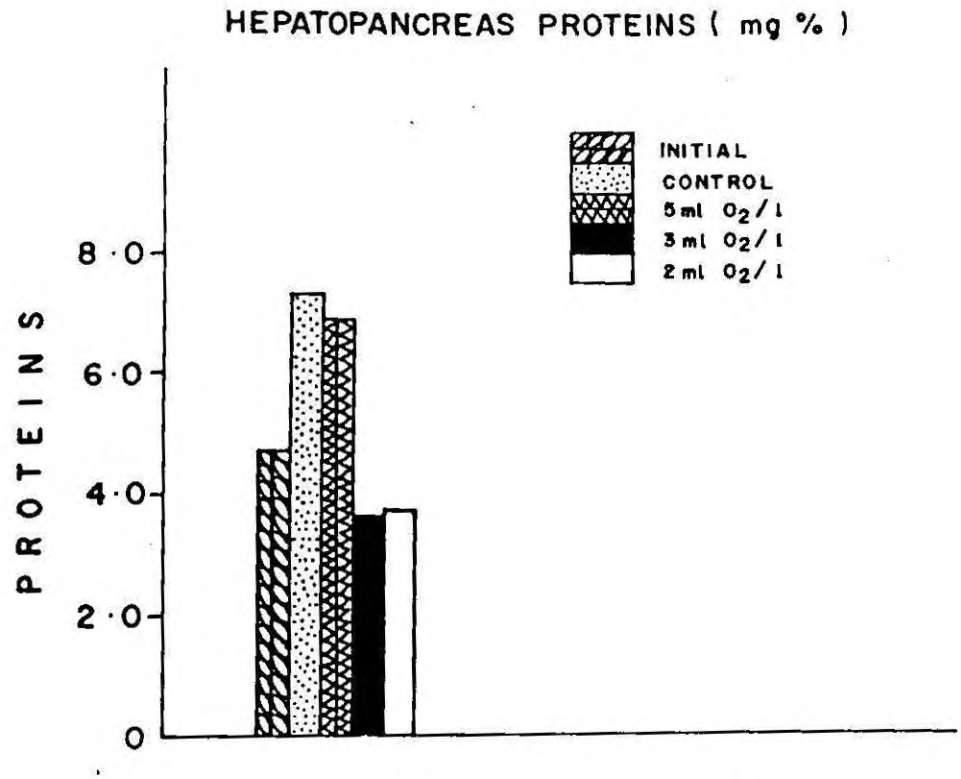
TREATMENT COMPARISONS

1,2	1,3	1,4	2,3	2,4	3,4
NS	NS	S	NS	S	S

S = Significant NS = Not Significant

TREATMENTS	1	2	3	4
DO Levels m/o_2 1 - 1	4.29	5.35	3.15	2.05

Fig. 1


HEPATOPANCREAS PROTEINS (mg %)
INITIAL
CONTROL
5ml O2/l
3ml O2/l
2ml O2/l
PROTEINS
8·0
6·0
4·0
2·0
0

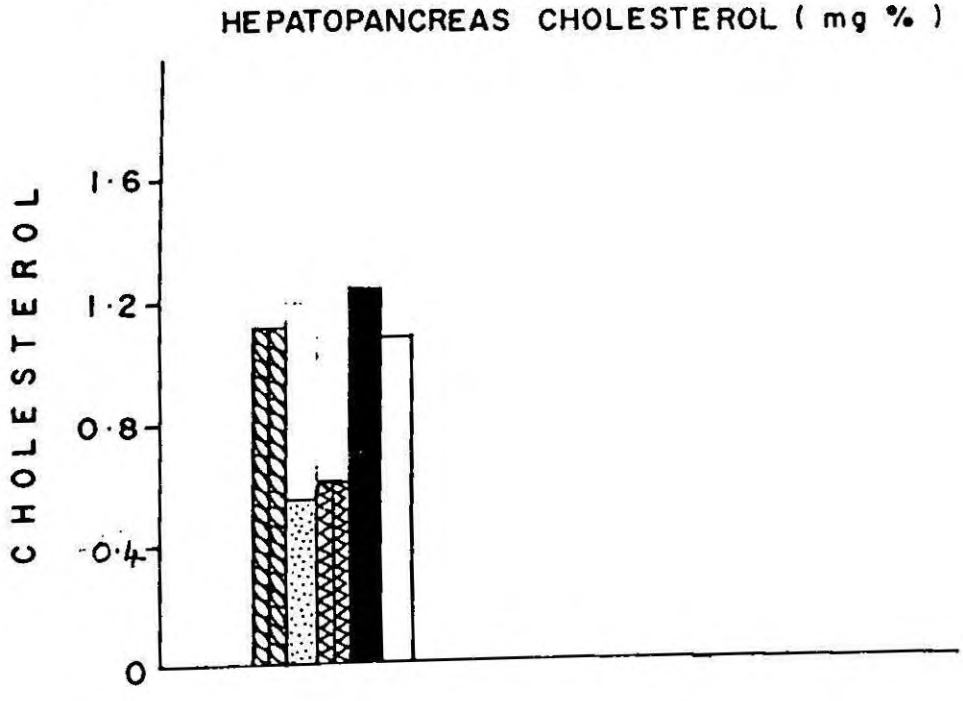
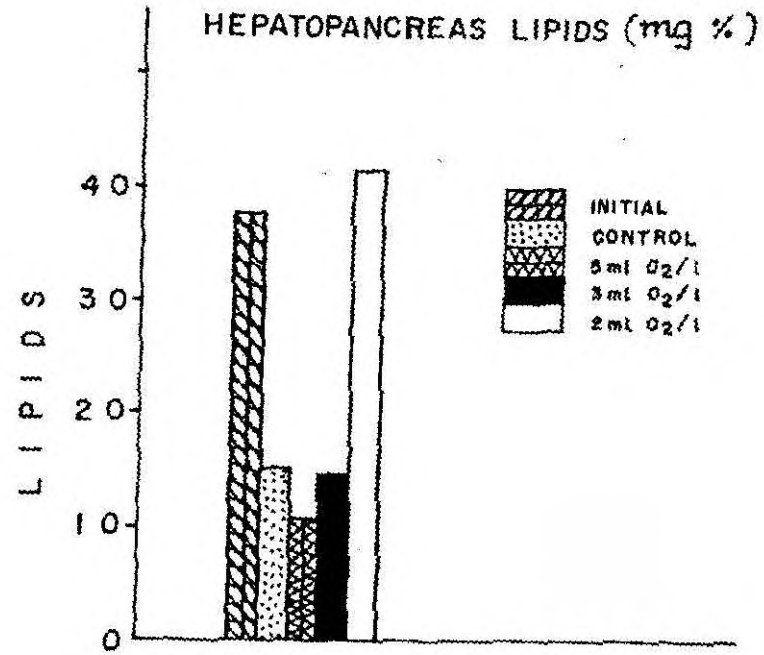

HEPATOPANCREAS CHOLESTEROL (mg %)
CHOLESTEROL
1·6
1·2
0·8
·0·4
0

Fig. 2

Fig. 3


HEPATOPANCREAS LIPIDS (mg %)
LIPIDS
40
30
20
10
0
INITIAL
CONTROL
5ml O2/l
3ml O2/l
2ml O2/l

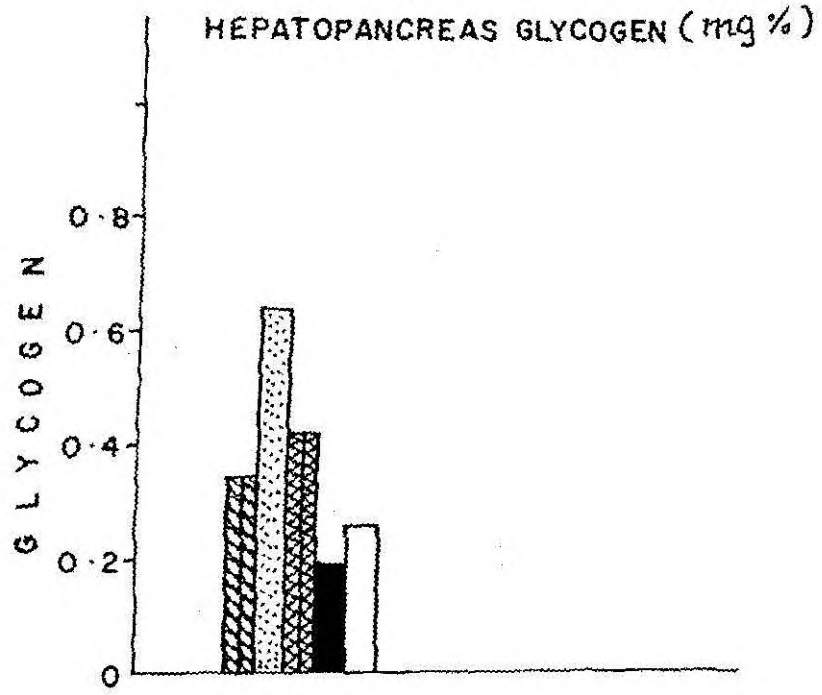
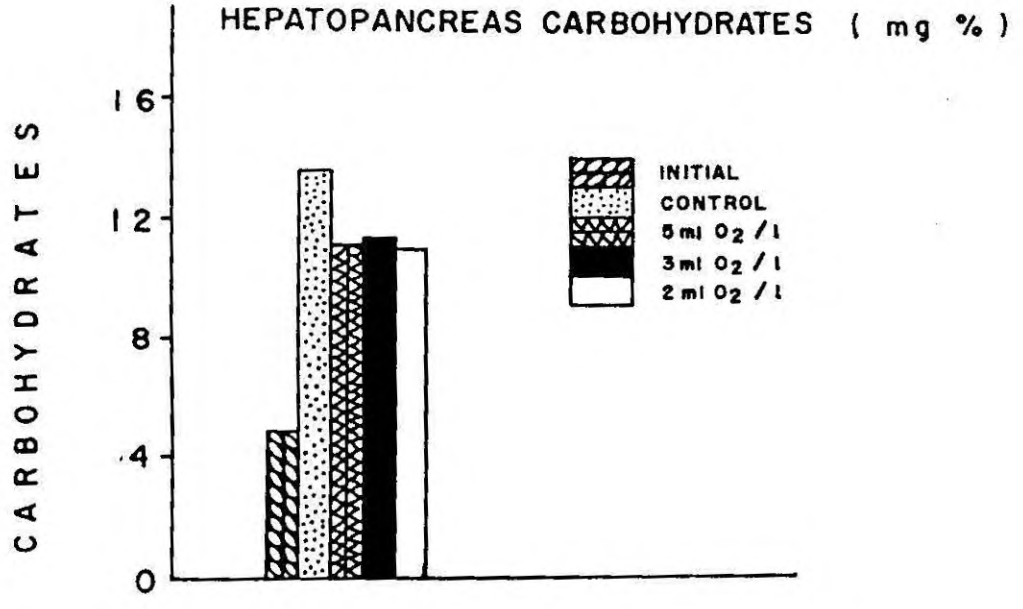

HEPATOPANCREAS GLYCOGEN (mg %)
GLYCOGEN
0·8
0·6
0·4
0·2
0

Fig. 4

Fig. 5


HEPATOPANCREAS CARBOHYDRATES (mg %)
CARBOHYDRATES
16
12
8
4
0
INITIAL
CONTROL
5 ml O2 / l
3 ml O2 / l
2 ml O2 / l

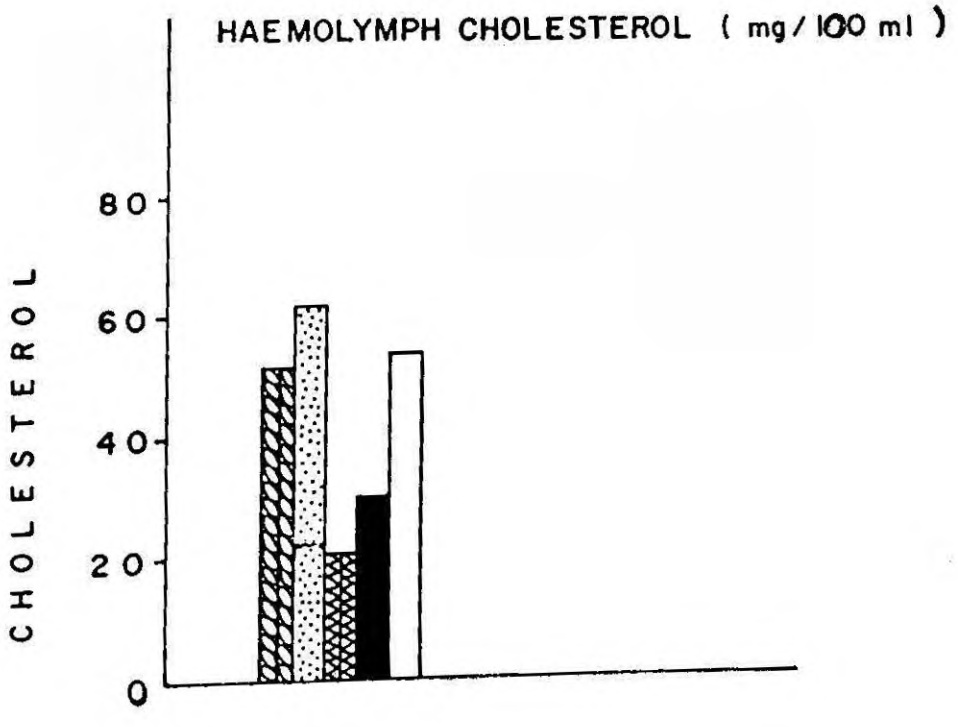
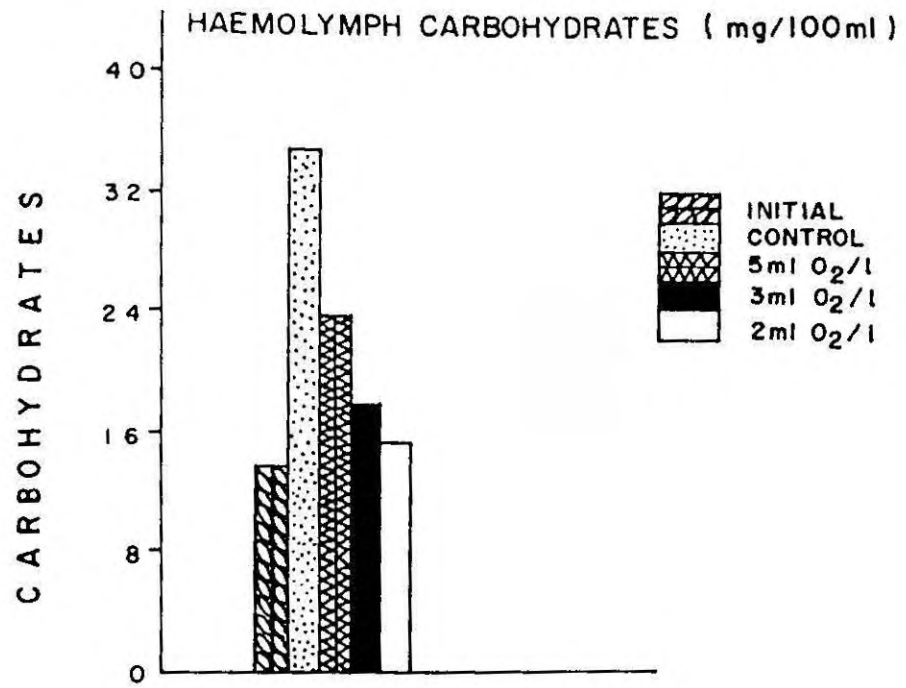

HAEMOLYMPH CHOLESTEROL (mg / 100 ml)
CHOLESTEROL
80
60
40
20
0

Fig. 6

Fig. 7


HAEMOLYMPH CARBOHYDRATES (mg/100ml)
CARBOHYDRATES
40
32
24
16
8
0
INITIAL
CONTROL
5ml O2/l
3ml O2/l
2ml O2/l

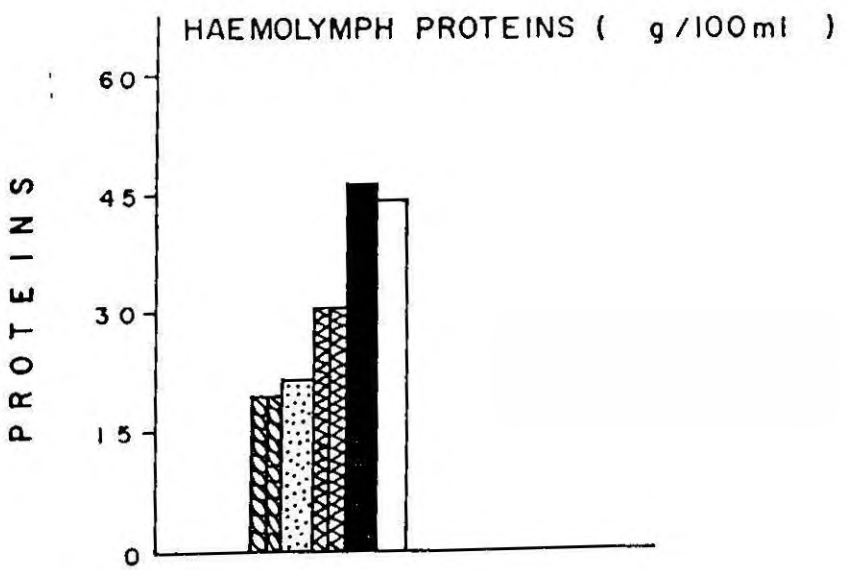

HAEMOLYMPH PROTEINS (g/100ml)
PROTEINS
60
45
30
15
0

Fig. 8

Fig. 9

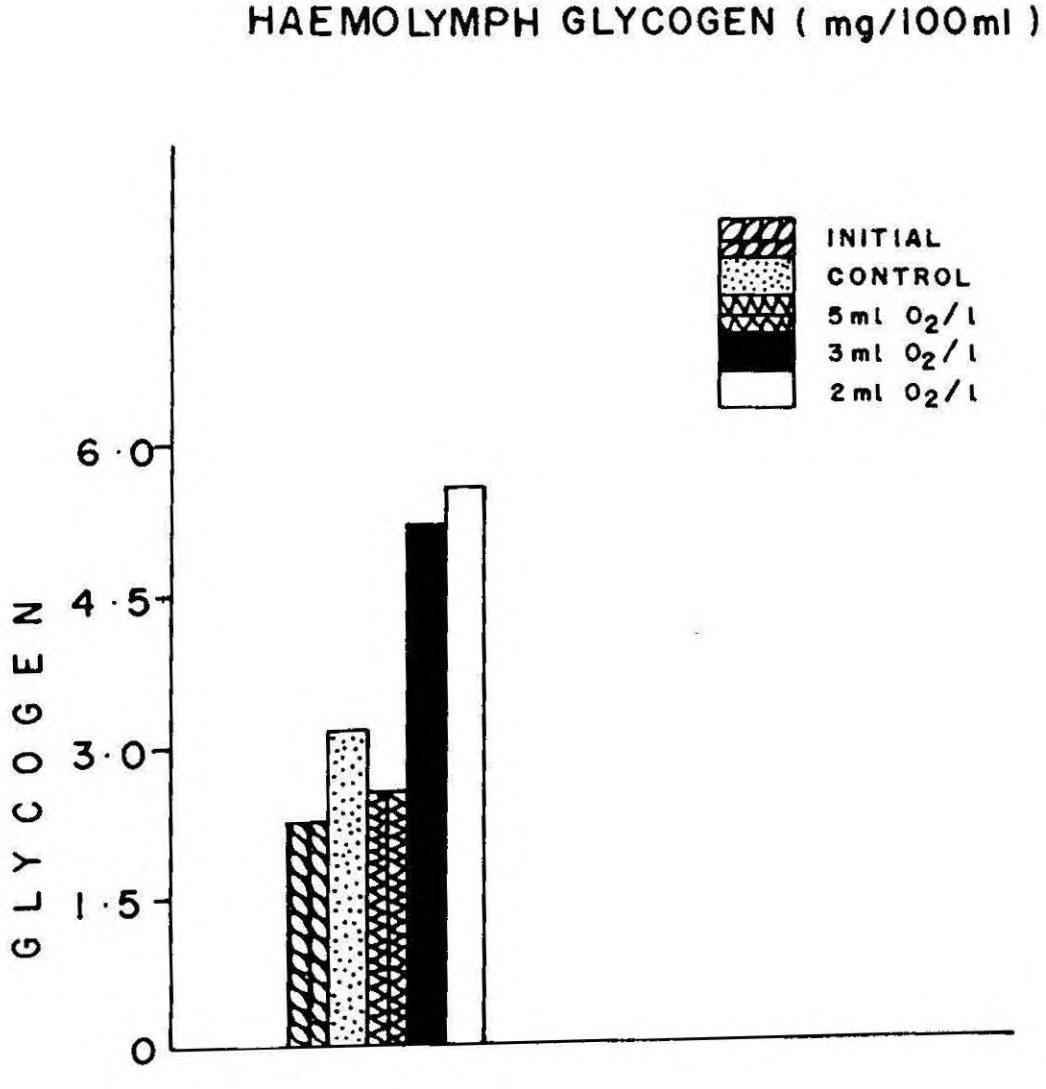

HAEMOLYMPH GLYCOGEN (mg/100ml)
INITIAL
CONTROL
5ml O_2/l
3ml O_2/l
2ml O_2/l
GLYCOGEN
6·0
4·5
3·0
1·5
0

Fig. 10

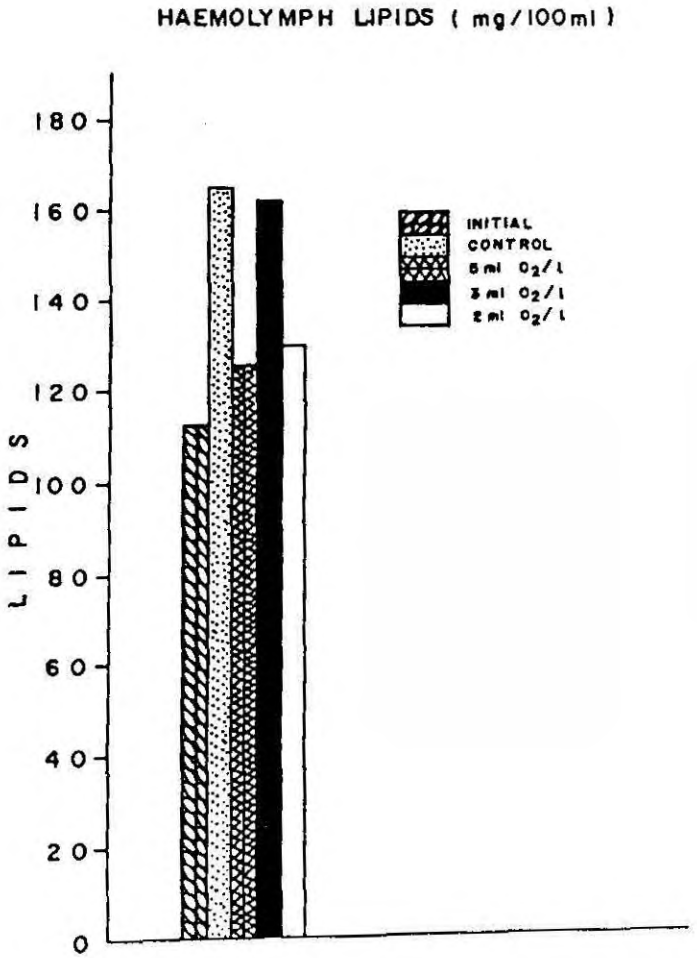

HAEMOLYMPH LIPIDS (mg/100ml)
LIPIDS
180
160
140
120
100
80
60
40
20
0
INITIAL
CONTROL
5ml O2/l
3ml O2/l
2ml O2/l

Fig. 11

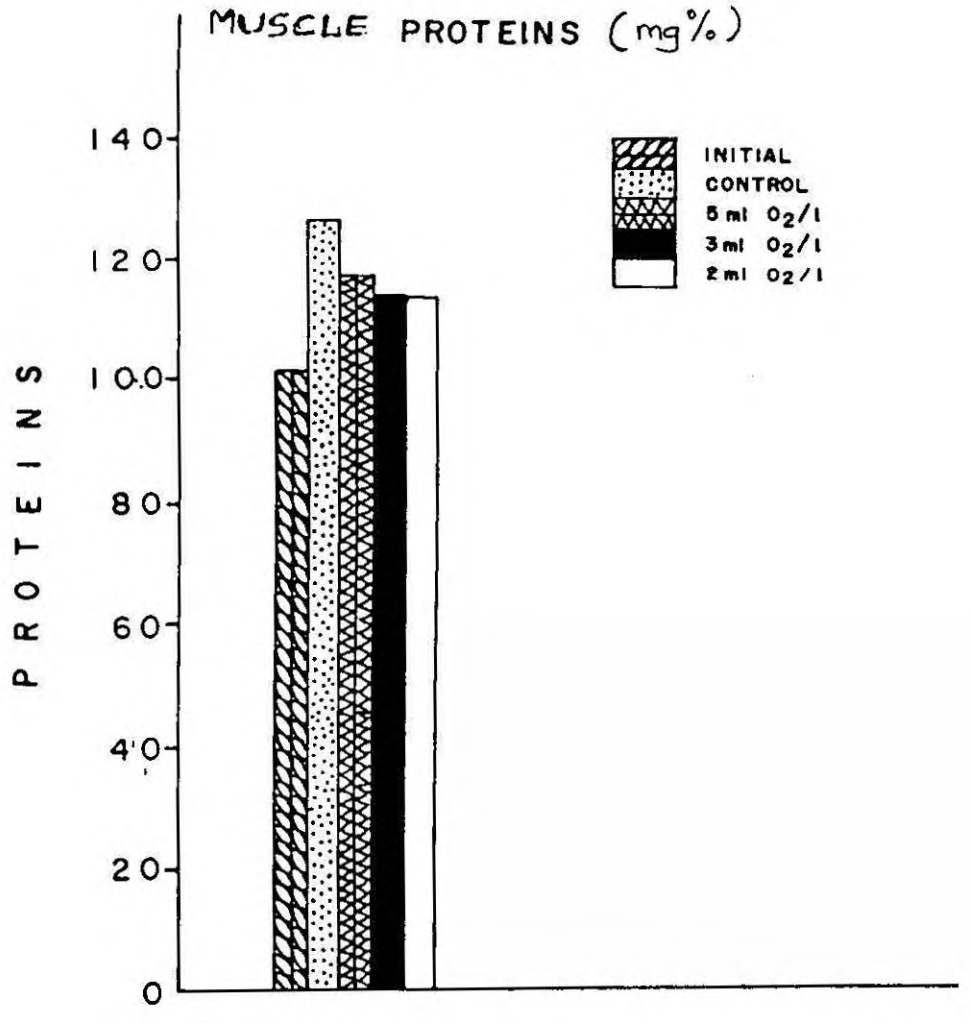
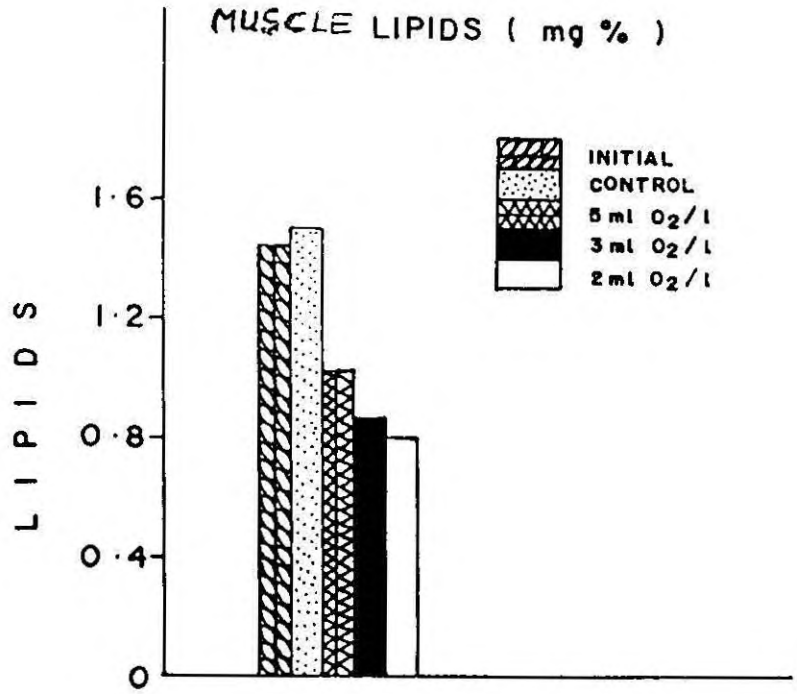

MUSCLE PROTEINS (mg%)
PROTEINS
140
120
100
80
60
40
20
0
INITIAL
CONTROL
5 ml O2/l
3 ml O2/l
2 ml O2/l

Fig. 12


MUSCLE LIPIDS (mg %)
INITIAL
CONTROL
5 ml O2/l
3 ml O2/l
2 ml O2/l
LIPIDS
1·6
1·2
0·8
0·4
0

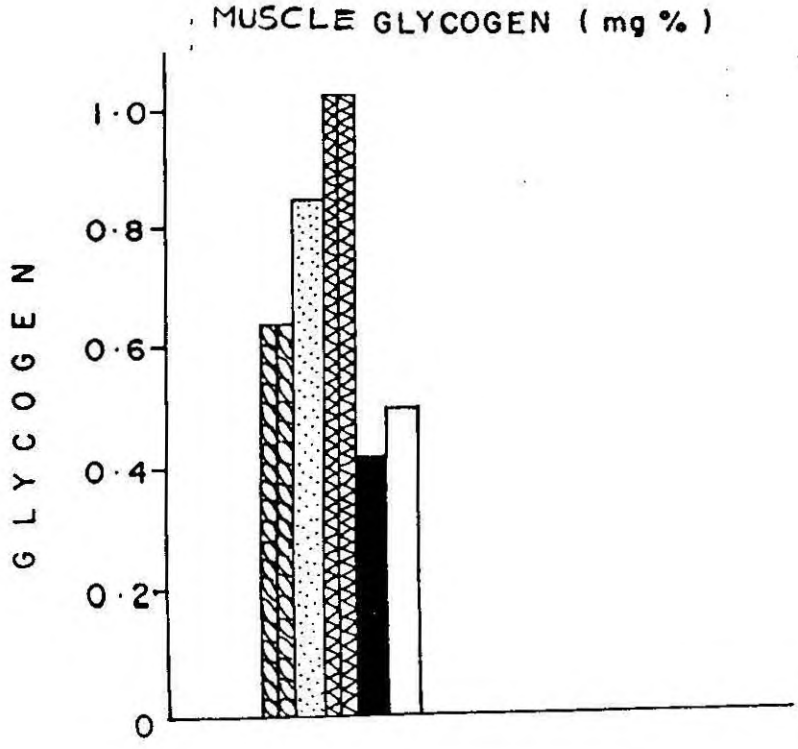
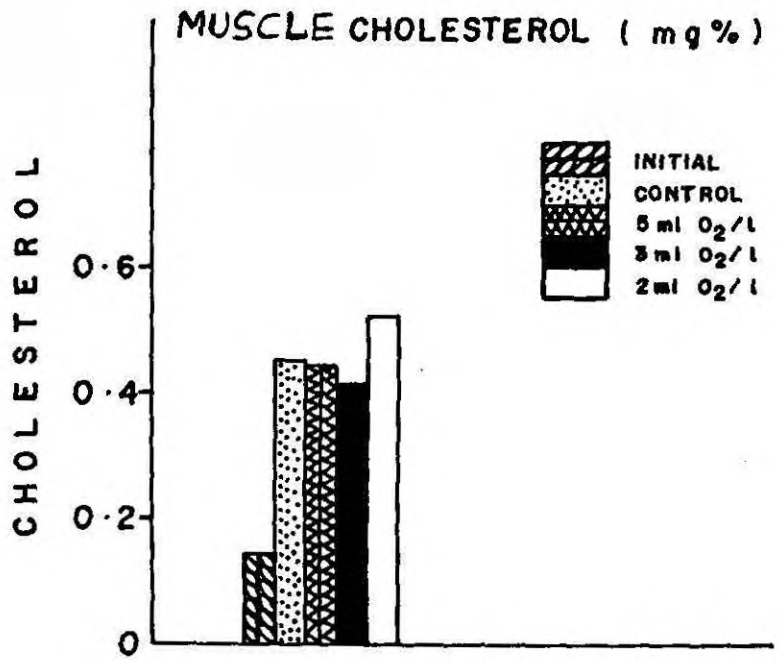

MUSCLE GLYCOGEN (mg %)
GLYCOGEN
1·0
0·8
0·6
0·4
0·2
0

Fig. 13

Fig. 14


MUSCLE CHOLESTEROL (mg %)
CHOLESTEROL
0·6
0·4
0·2
0
INITIAL
CONTROL
5 ml O2/l
3 ml O2/l
2 ml O2/l

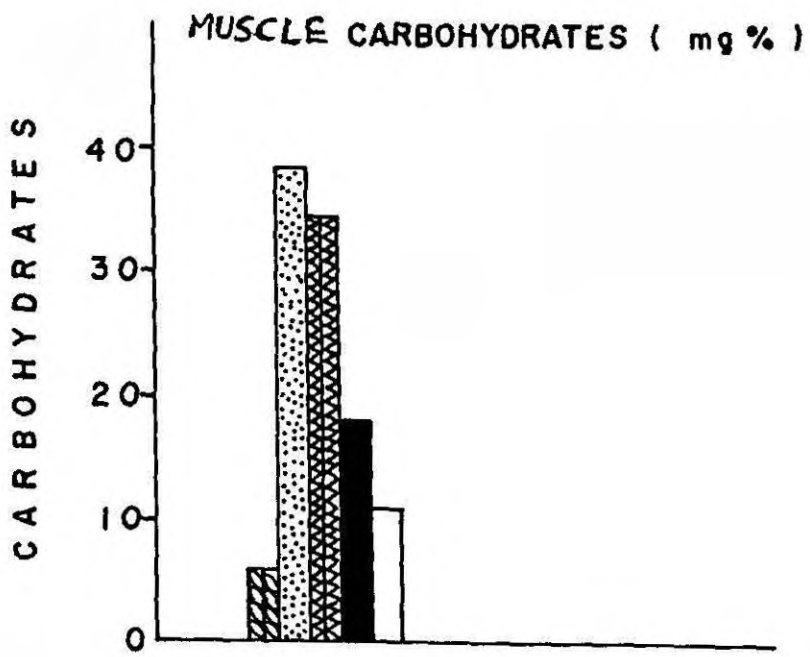

MUSCLE CARBOHYDRATES (mg %)
CARBOHYDRATES
40
30
20
10
0

Fig. 15

DISCUSSION

Low dissolved oxygen (DO) levels frequently encountered in semi-intensive culture systems is found to cause respiratory stress in the cultured organism. In fishes, the primary stress response involves increased production of corticosteriods and catecholamines (Mazeaud *et al.* 1972) which in turn bring about a number of physiological and biochemical changes. Adaptive stress responses also involve metabolic adjustments like changes in metabolic rates and metabolic pathways causing changes in the levels of various metabolites (Kinne, 1964). The magnitude of these changes can be directly correlated with increasing intensity and type of stress.

Below a critical oxygen level, crustaceans show aerobic shut down (Burke, 1979) and switch over to anaerobic metabolism, where, glycogen the storage form of energy is metabolized to lactate. But specific tissues do not necessarily have equivalent energy requirements nor utilize the same pathways, to the same extent during anaerobiosis. Rather, they show tissue specific metabolism.

Glycogen, seems to be an important metabolic substrate during low DO conditions as significant decline in glycogen content was observed in the muscle and hepatopancreas of *Penaeus indicus* exposed to low DO levels of 3 and 2 ml $O_2 l^{-1}$.

While in the muscle, glycogen levels declined by 50%, in the hepatopancreas, it declined by 58.5 - 70.25%. In fishes, fermentation of glucose of glycogen to lactate provides the main source of energy production in hypoxic conditions. As reported by Woo and Wu (1984) glycogenolysis has been observed in fishes like Salmo clarki, Lepomis macrochirus (Heath and Pritchard, 1965), Platicthys flesus (Jorgensen and Mustafa, 1980), Carassius carrasius (Johnston, 1975), Periopthalmus australis (Bandurski et al, 1968) and Ictalurus nebulosus (Burton 1970 a) and Lepomis macrochirus (Burton, 1970 b).

In crustaceans too, increased utilization of glycogen under hypoxic conditions has been reported as in the marsh crab, Uca pugilator (Teal and Carey, 1967), Carcinus maenas (Burke, 1979; Bridges and Brand, 1980; Lallier et al, 1987). Lowery and Tate (1986) have reported high haemolymph lactate levels during hypoxia, in Callinectes sapidus due to increased glycogenolysis. They have observed that lactate levels decrease during recovery to normoxia, indicating a selective utilization of glycogen during low DO conditions.

Glycogen plays a very important role in crustacean intermediary metabolism (Vonk, 1960) and especially so in the moulting process where it acts as the precursor of chitin synthesis (Verne, 1924, 1926; Renaud, 1949; Travis 1951a;). The small but significant decrease in hepato pancreas glycogen

(-34.89%) along with a rise in muscle glycogen under normoxic condition, probably indicates mobilization of this important organic reserve.

Haemolymph also forms an important site of glycogen mobilization. Under the present experimental conditions, glycogen levels in the haemolymph were found to be high under low DO conditions. (3 ppm : + 64%; 2 ppm + 77%) when compared to a control with DO level 4 ppm. Glycogen mobilization during the moulting process, begins from hepatopancreas glycogen stores and is drawn via haemolymph to the epidermal cells where it is converted to glucose and then to acetyl glucosamine and finally chitin. Clark (1986) has reported on inhibition of moulting at low DO levels in Penaeus semisulcatus as has Seidman and Lawrence (1985) in Penaeus vannamei. In the present study, while moulting was observed at the lowest DO level also, moulting rates tended to be low, being 1.38 at a DO level of 2 ml $O_2 l^{-1}$ as against 2.2 - 2.4 at the higher DO levels. Probably, this impairment of the moulting process caused the accumulation of glycogen in the haemolymph.

Carbohydrates participate in processes of energy production in crustaceans (Hu, 1958; Meenakshi and Scheer, 1961). Haemolymph plays a very important role in carbohydrate metabolism (Williams and Lutz, 1975). Besides, chemical

composition of haemolymph in crustacea depends more or less directly on nature of environment (Laxmilatha, 1991) and is a reflection of the physiological condition of the animal.

During asphyxia, hyperglycemia has been reported in Cancer pagurus, Carcinus maenas and Portunus puber (Stott, 1932) as also in the river crab, Potamonautes warreni (Van Aardt, 1988). In the present study, total carbohydrates were significantly reduced at the lower DO levels by 49 - 56% when compared to the control. This decrease was more in the muscle (66-72%) followed by haemolymph (49 - 56%). In the hepatopancreas significant decrease was seen only at the lowest DO level of 2ml $O_2 l^{-1}$, of about 13%. Probably this was due to reduced feeding under the low DO conditions (2 ppm: 42%; 5 ppm : 54%). Carbohydrate levels have been observed to decrease in fasting Maia Squinado, Carcinus maenas, and also in starved Metapenaeus dobsonii (Jaideep, 1991) Besides, blood glucose represents a form of transport of glycogen of tissues and hepatopancreas to site of chitin synthesis. Since, more glycogen is being mobilized for growth purposes in normoxic conditions, it is likely to reflect in the haemolymph carbohydrate composition also.

Lipids play an important role in energy production in crustaceans, along with glycogen (Vonk, 1960). The principal lipid storage site is the hepatopancreas, and in general reflects total lipid content and composition of the whole

organism due to adverse ecological conditions (Rajamani, 1982). Protein catabolism as indicated by increased NH_3 excretion and decrease in protein levels in tissue are observed during stressful conditions. This has been reported in fishes like Tilapia mossambica (Kutty, 1972) Rhinomugil corsula (Kutty and Peer Mohammed, 1975) exposed to hypoxic conditions.

Protein depletion during starvation or reduced feeding conditions is common in crustaceans. Depletion of proteins in hepatopancreas followed by blood and muscle has been reported in starved Metapaneeus dobsonii (Jaideep, 1991). In the present study, protein levels declined significantly by 48-50% in the hepatopancreas while in the muscle it declined by 11-12% at DO levels of 3 and 2 ml $O_2 l^{-1}$. At the same time, protein levels in the haemolymph showed an increase of 113-122%.

The decline in protein content in the hepatopancreas and muscle could be due to the reduced feeding at low DO or due to increased protein catabolism during stressful hypoxic condition. Increased mobilization of proteins from hepatopancreas for consequent catabolic activity results in the increase in the free amino-acid level in the blood. During hypersmotic stress in Peaneus indicus, increased protein catabolism has been found to cause increase in protein and free amino acid (FAA) content in haemolymph (Diwan and Bhaskaran, 1992). Senkbeil

and Wriston (1981) have also reported hypoxia induced haemocyanin synthesis, the principal haemolymph protein, in Homarus americanus, as a compensatory mechanism to low DO levels. However this was seen only during feeding conditions, albeit reduced.

Cholesterol is the precursor of the moulting hormone ecdysterone in crustaceans (Petriella, 1991) and requirements are met entirely through the diet. The presence of sterols in all crustacean tissues has been reported during the whole moulting cycle (Kanazawa et al, 1976) and therefore changes in its levels are likely to reflect its utilization patterns.

During moult cycle of Penaeus japonicus (Kanazawa et al (1976) have reported variation in cholesterol content of hepatopancreas and eyestalk only, and not in other tissues. Thus hepatopancreas is the major organ of cholesterol mobilization. In the present experiment, cholesterol level in the hepatopancreas were higher (+91% to +144%) at the lower DO levels, indicating less utilization. At the same time, haemolymph cholesterol levels were lower at normoxic conditions. Together, this indicates that at the higher DO level, cholesterol is being increasingly drawn from the hepatopancreas into the haemolymph, probably for moulting, which was also higher at these DO levels.

GROWTH STUDIES:- DO which has been classified as a limiting factor by Fry (1971) acts via metabolism. This in turn will

affect the metabolization of various organic reserves for energy production and growth.

Growth studies showed a significant reduction in growth at the lowest DO level of 2.05 ± 0.07 ml/l. Under the present experimental conditions, there was some critical DO level for growth between 2.05 and 3.15 $ml O_2 l^{-1}$. Growth was found to be best, as indicated by an increase of 23% in wet weight, at a DO level of 4 $ml O_2 l^{-1}$. But between 4.29 and 5.35 $ml O_2 l^{-1}$, there again seems to be some limiting effect as observed by only 14% increase in wet weight. Growth was negative, as recorded by loss of weight at the lowest DO level of 2 $ml O_2 l^{-1}$.

Respiratory response of penaeid prawns to various DO levels depend on age, size and activity (Subramanyan, 1962). Information on lethal limits for various penaeid species and size groups are available (Kramer, 1975 Subramanyam 1962, Seidman and Lawrence, 1985). DO levels become limiting first and finally lethal, and within the zone of compatibility, also metabolism and activity will be greatly influenced by DO levels.

A possible explanation of reduced growth at the lowest DO level of 2 $ml O_2 l^{-1}$ could be the increased utilization of energy for maintenance of homeostasis in the face of an external stressor. Another, could be the reduced food consumption observed at the low DO levels. (Llobera, 1983;

Seidman and Lawrence, 1985; Stewart et al 1967). This was found to be more than 10% less than the feeding at normoxic levels. Moulting patterns are related to feeding habits. Starvation and other stressors which cause a drain on the animals organic reserves are supposed to inhibit moulting. Growth rate was not significantly different at DO levels of 3, 4 and 5 $ml O_2 l^{-1}$. While food consumption was found to be lower at lower DO levels, (5 ppm: 54.7%; 3 ppm : 47.28%) growth was not significantly reduced. This could be due to a compensatory mechanism which allows better assimilation of food consumed, although it is due to low DO levels. Growth is dependant on a number of extrinsic variable and intrinsic factors also and therefore it is difficult to pinpoint any specific cause.

In the present study, critical DO levels for growth was derived for 50-60 mm individuals only and is likely to vary for a different sized group. Some validation is also required from the field. Ultimately the effects of salinity and temperature on these DO levels will also have to be determined.

* * *

SUMMARY

1. The present study was conducted to study growth and metabolite mobilization under various dissolved oxygen levels in the prawn, Penaeus indicus, in the laboratory.
2. 50-60mm TL sized Penaeus indicus were selected for the study for a period of 30 days.
3. Four dissolved oxygen levels of 5.35, 4.29, 3.15 and 2.05 ml $O_2 l^{-1}$ were selected for the s dy. Aeration was used to maintain normoxic levels of 5 and 4ml $O_2 l^{-1}$, while nitrogen stripping of the water was done to achieve and mountain hypoxic conditions.
4. Growth, in terms of increase in wet weight was recorded weekly during the study period. After a period of thirty days, the final weight was also recorded. Food consumption was also recorded daily.
5. After thirty days, intermoult animals subject to each treatment were subject to biochemical analysis for estimation of various metabolites like, proteins, lipids, total carbohydrates, glycogen and cholesterol. Each of these organic reserves were estimated in hepatopancreas, haemolymph and muscle.
6. Growth was found to be best at 4.29ml $O_2 l^{-1}$, showing an increase of 24% and a growth rate of 12mg day^{-1}. At the highest DO level of 5ml $O_2 l^{-1}$, an increase of 14.3% was recorded while at 3ml $O_2 l^{-1}$ it was 19.4%. No growth was recorded at the lowest DO level of 2ml $O_2 l^{-1}$.

7. Dried clam meat was given as food @ 5% of body weight daily, in two divided doses. Food consumption was found to be influenced by the DO levels. While in normoxic condition, food consumption was 55-59%, at the lowest DO level, food, consumption was only 45.3%.

8. Better moulting rate and survival was recorded at normoxic conditions. Moulting rate at a DO level of 4.29 $O_2 l^{-1}$ was 2.34 while at a D.O level of 2ml $O_2 l^{-1}$ it was 1.38. Survival rate more than 50% at DO levels 3ml $O_2 l^{-1}$ and above was observed.

9. Biochemical studies conducted indicate that glycogendysis is mainly responsible for increased energy production during stressful, hypoxic conditions. The rapid depletion of glycogen was most marked in the muscle and hepatopancreas, indicating that they were the main sites of energy mobilization.

10. Protein reserves are also catabolized during hypoxia. Proteins declined by 48-50% in hepatopancreas and 11-12% in muscle in a hypoxic conditions. This also caused a rise in the haemolymph proteins.

11. During hypoxia lipids seemed to be mobilized for immediate energy needs from the muscle rather than hepatopancreas or haemolymph.

12. Carbohydrate levels in the muscle and haemolymph declined significantly at the lower DO levels. This indicates that carbohydrates are being metabolized at an increasing rate at the low DO levels for energy production.

13. Cholesterol, the important precursor of the moulting hormone is not mobilized and accumulates in the hepatopancreas and shows no decline in the haemolymph, during hypoxic conditions. However, in the normoxic environment, cholesterol levels in the haemolymph and hepatopancreas show a decline indicating its utilization for growth purposes.
14. Metabolite mobilization thus seems to be highly tissue-specific.
15. In the present study, better protein, glycogen and cholesterol mobilization from hepatopancreas to the muscle was observed at normoxic conditions, resulting in better growth.

* * *

R E F E R E N C E S

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

ADIYODI, R.G. 1969. The storage and mobilization of organic resources in the hepatopancreas of a crab. Paratelphusa hydrodromus. Experentia 25 : PP. 43-44

ALBERT, J.L. and W.R. ELLINGTON 1985. Patterns of energy metabolism in the stone crab Menippe mercenaria during severe hypoxia and subsequent recovery. J. Exp. Zool. 234; PP. 175-183

ANDREWS, J.W.; T. MURAI and G. GIBBONS 1973. The influence of dissolved oxygen on growth of channel catfish. Trans. Amer. Fish. Soc. 102: PP. 835-838

*BANDURSKI, R.S. ; E. BRADSTREET and P.F. SCHOLANDER 1968. Metabolic changes in the mud skipper during asphyxia or exercise. Comp. Biochem. Physiol. 24 : PP. 271-274.

* BARNES, H and J. BLACKSTOCK 1973. Estimation of lipids in Marine animals and tissue. Detailed investigations of Phosphovanillin method for total lipid. J. Exp. Mar. Biol. Ecol. 12 PP. 103-118

BILLIARD, R; C. BRY and T.C. GILLET 1981. Stress, environment and reproduction in teleost fish. In: Stress and fish, London and New York, Academic Press. PP. 185-208.

BLAZKA. P. 1958. The anaerobic metabolism of fish. Physiol Zool. 31; PP. 177-128.

BOUCK. G.R.; and R.C. BALL 1965. Influence of diurnal oxygen pulse on fish serum protens. Trans. Am. Fish. Soc. 94, 363-370.

BOYD, C.E. 1976. Water quality management for pond fish culture. In: Advances in Aquaculture and Fisheries Science Vol. 9. Elsevier, Amsterdam.

BRETT, J.R. 1979. Environmental factors and growth. In: Fish Physiology, Vol. 8. W.S. Hoar and D.J. Randall (eds). Academic Press, New York.

BRIDGES, C.R. and A.R. BRAND 1980. The effect of hypoxia on oxygen consumption and blood lactate levels of some marine crustaceans. Comp. Biochem. Physiol. 65A.: PP. 399-409.

* BROWN, F.A. and O. CUNNINGHAM 1939. Influence of the sinus gland of crustaceans on normal viability in ecdysis. Biol. Bull. Mar. Lab., Woodshole, 77 : PP. 104-114.

BURGGREN, W.W. and J.N. CAMERON 1980. Anaerobic metabolism, gas exchange and acid-base balance during hypoxic exposure in the Channel catfish, Ictalurus punctatus. J. Exp. Zool. 213, 405-416.

BURKE, E.M. 1979. Aerobic and anaerobic metabolism during activity and hypoxia in two species of intertidal crabs. Biol. Bull. 156 : PP. 157-168.

* BURTON, D.T. 1970a The anaerobic response of brown bull head catfish Ictalurus nebulosus during gradual hypoxia at two acclimation temperatures. Amer. Zool. 10: PP. 519.

* BURTON, D.T. and A.M. SPEHAR 1971. A reevaluation of the anaerobic end products of fresh water fish exposed to environmental hypoxia. Comp. Biochem Physiol. 40A : PP. 945-954.

* BUTLER, P.J., TAYLOR, E.W. and B.R. Mc MAHON 1978 Respiratory and circulatory changes in the lobster Homarus vulgaris during long term exposure to moderate hypoxia. J. Exp. Biol. 73 : PP. 131-146.

CAMERON, N.J. and P.C. MANGUM. 1985. Ventilation, circulation and oxygen transport. In: The Biology of crustacea Vol. 8. Environmental adaptations. D.E. Bliss (ed.) PP. 50-33.

CASTELL, J.D.; E. MASON and J.F. COVEY 1975. Cholesterol requirement of Juvenile american lobster (Homarus americanus) J. Fish. Res. Board Canada, 32 : PP. 1431-1435.

CHANG, E.S. and J.D. O'CONNOR 1983. Metabolism and transport of carbohydrates and lipids. In : The biology of crustacea. Vol.5. Internal anatomy and physiological regulation.

CLARK, J.V. 1986. Inhibition of moulting in Penaeus semisulcatus (De Haan) by long term hypoxia. (Aquaculture, Vol. 52, No.4 : PP. 253-254.

CLAYBROOK, L.D. 1983. Nitrogen metabolism. In : The Biology of crustacea. Vol.5. Internal anatomy and Physiological regulation. H.M. Linda (ed.) Academic Press, New York. PP. 163-202.

DALL, W. 1964. Studies on the physiology of a shrimp, Metapenaeus mastersii (Haswell) (crustacea: Decapoda: Penaeidae) 1. Blood constituents. Aust. J. Mar. Fresh W. Res., 15 : PP. 145 - 161.

DALL, W. and D.J.W. MORIARTY 1985. Functional aspects of nutrition and digestion. In: The Biology of crustacea. Vol.5. Internal anatomy and physiological regulation. D.E. Bliss (ed.) PP-215-251.

DEPLEDGE, M.H. and P. BJERREGAARD 1989. Haemolymph protein composition and copper levels in decapod crustaceans. Helgol. Meeresunters; Vol.43(2) : PP. 207-223.

DIWAN, A.D. and V. BHASKARAN, 1992. Effect of salinity stress on neurosecretory cells, protein and free amino acid content and rate of ammonia excretion of the prawn Penaeus indicus H Milne Edwards. J. Aqua Trop., 7 : PP. 95-108.

DOUDOROFF, P. 1957. Water quality requirements of fishes and effects of toxic substances. In: The Physiology of Fishes. Vol. II. M.E. Brown (ed.) Academic Press, New York. PP. 403-430.

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

FLORKIN, M 1951. Ecology and Metabolism. In: The Physiology of crustacea. Vol. 1. Metabolism and Growth.

T.H. Waterman (ed.) Academic Press, New York, PP. 395-410.

FRASER, A.J. 1989. Triacyl glycerol as a condition index for fish bivalve and crustacean larvae. Can J. fish Aquat Sci. Vol.46, No.11: PP. 1868-1873.

FRY, F.E.J. 1971. The effect of environmmental factors on the physiology of fish. In : Fish Physiology, Vol6 . W.S. Hoar and D.J. Randall(eds.() Acadmic Press, New York.

* GEORGE W.C. and J. NICHOLS 1948. A study of the blood of some crustacea. J. Morphol. 83 : PP. 425-440.

* GIESE, A.C. 1967. Some methods for study of the biochemical constitution of marine invertebrates. Oceanogr. Mar. Biol. Ann. Rev. 5 : PP. 159-186.

GILBERT, L.T. and J.D. O'CONNOR 1985. Lipid metabolism and transport in arthropods. In: The Biology ofcrustacea. Vol. 5. Bliss. D (ed.) PP. 229-251.

HAGERMAN, L.; and UGLOW, R.F. 1985. Effects of hypoxia on the respiratory and circulatory regulation of Nephrops norvegicus Mar. Biol 87 : PP. 273-278.

HAGERMAN, L and BADEN. S.P. 1988. Nephrops norvegicus field study of effects of oxygen deficency on haemocyanin concentration. J. Exp. Mar. Biol. Ecol. 116 PP. 135-142

HAGERMAN, L. and A. SZAIZWSKA 1986. Behaviour tolerance and anaerobic metabolism under hypoxia in brackish water

shrimp Crangon. crangon. Mar. Ecol. (Prog. Ser.); Vol. 34. No.1 PP. 125-132.

HAGERMAN, L.; T.SOENDERGAARD; K. WEILE; D. HOSIE and R.F. UGLOW 1990. Aspects of blood physiology and ammonia excretion in Nephrops norvegicus under hypoxia. Comp. Biochem. Physiol. Vol. 97A (1) : PP.51-55).

*HEATH A.G and A.W. PRITCHARD 1965. Effects of severe hypoxia on carbohydrate energy stores and metabolism in two species of fresh water fish. Physiol. Zool. 38. PP. 325-334.

HERRIED, F.C. 1980. Hypoxia in invertebrates. Comp. Biochem. Physiol. Vol. 67A : PP. 311-320

HOCHACHKA, P., J. FIELDS and T.MUSTAFA 1973. Animal life without oxygen: Basic biochemical mechanisms. Amer. Zool 13 : PP. 543-555.

HOHNKE, L. and B.T. SCHEER 1985. Carbohydrate metabolism in crustaceans. In : The Biology of crustacea. Vol. 5 Bliss.D (Ed.) Academic Press, New York. PP. 147-164.

HU, A.S.L. 1958. Glucose metabolism in the crab Hemigrapsus nudus. Arch. Biochem. Biophys. 75 : PP. 387-395.

JAIDEEP KUMAR 1991, Effects of starvation on biochemical constituents of Metapenaeus dobsoni. M.Sc dissertation. Cochin University of Science and Technology. CMFRI, Cochin.PP.86.

JEUNIAUX, C. 1971. Haemolymph. Arthropoda. In : Chemical Zoology, Vol.6, Arthropoda, Part.B. M.Florkin and B.T. Scheer (eds.). Academic Press, New York. PP. 63-118.

JORGENSEN, J.B. and T.MUSTAFA 1980. The effect of hypoxia on carbohydrate metabolism in flounder (Platichthys flesus L.) I. utilization of glycogen and accumulation of glycolytic end products in various tissues. Comp. Biochem. Physiol. 67B, PP. 243-248.

JOURVE, A and J.P. TRUCHOT 1978. Influcence de l'oxygenation de l'eau sur la consommation d'oxygene et la ventilation branchiale du crabe, Carcinus maenas (L). C.R. Hebd. Seances Acad Sci. 286:PP. 331-334.

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

KINNE, O 1964. Animals in aquatic environments: crustaceans. In: Handbook of Physiology, Section 4: Adaptation to the environment. D.B. Bill, E.F. Adolph and C.G. Wilber (eds) American Physiological Society, Washington, D.C., PP. 669-682.

KLEINHOLZ, L.H. and L.H. LITTLE 1949. Studies of blood sugar concentration is crustaceans I. Normal values and experimental hyperglycemia in Libnia emarginata. Biol. Bull. Mar. biol. lab., Woods Hole 96: PP.218-227.

KRAMER, G.L. 1975. Studies on the lethal dissolved oxygen levels for young brown shrimp Penaeus aztecus Ives.

Proceedings World Mariculture Society 6 : PP. 157-167.

KRISHNA MOORTHY, R.V.; A.VENKATARAMIAH, G.J. LAKSHMI and P. BIESIOT 1982. Effect of starvation and of algae feeding on the tissue cholesterol levels in commercial shrimp Penaeus aztecus ives. Proc. Symp. on coastal Aqua (1) PP: 215-222.

KUTTY, M.N. 1967. Oxygen consumption in prawns Penaeus indicus and Penaeus Semisulcatus. FAO Fish Rep., 57(3): PP. 957-969.

KUTTY, M.N. 1972, Respiratory quotient and ammonia excretion in Tilapia mossambica; Mar. Biol. 16: PP. 126-133

KUTTY M.N and M. PEER MOHAMMED 1975. Metabolic adaptation of mullet, Rhinomugil corsula (Hamilton) with special reference to energy utilisation. Aquaculture 5: PP.253-270.

LALLIER, F.; F. BOITEL and J.P. TRUCHOT 1987. The effect of ambient oxygen and temperature on haemolymph L-lactate and urate concentrations in the shore crab Carcinus maenas. Comp. Biochem. Physio. A Vol. 86A(2): PP. 255-260.

LAWRENCE, A.L.; J.P. MCVEY and V.J. HUNER 1985, Penaeid Shrimp culture. In : Crustacean and mulluscan aquaculture in United States, PP. 127-157.

LAXMILATHA, P. 1991. Studies on the haemolymph Penaeus indicus H. Milne Edwards. Ph.D thesis, Cochin University of Science and Technology CMFRI, Cochin 194 PP.

LAXMINARAYANA, A. and M.N. KUTTY 1982. Oxygen consumption, ammonia excretion and random activity in Penaeus semisulcatus, Macrobrachium malcolmsonii and Paratelphusa hydrodromus with reference to ambient oxygen. Proc. Symp. Coastal Aquaculture. 1 : PP. 117-122.

LLOBERA, 1983. Phd. dissertation, Texas A and M. Univ.

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

LOWERY, T.A. and L.G. TATE 1986. Effects of hypoxia on haemolymph lactate and behaviour of the blue crab Callinectes sapidus Rathbun in the lab and field. Comp. Biochem. Physiol. A. Vol. 85A. (4) : PP. 689-692.

LOWRY, O.H.; N.J. ROSENBROUG.; A.L.FARR and R.J. RANDALL 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193; PP. 265-275.

MACKAY, R.D. 1974. A note on minimal levels of oxygen required to maintain life in Penaeus schmitti Proceedings World Maricultufe Society 5 : PP. 451-452.

*MAHLER, R.H. and H.E. CORDES. 1968. Basic Biological chemistry. A Harper International edition. PP. 527.

MANGUM, C. and W. VANWINKLE 1973. Response of aquatic invertebrates to declining oxygen conditions. Amer. Zool., 13 : PP. 529-541.

Mc MAHON, BR. and J.L. WILKENS 1983 Ventilation, perfusion and oxygen uptake. In: The Biology of Crustacea. Vol.5.

Internal anatomy and Physiological regulation. Academic Press. New York. PP. 289-372.

MEENAKSHI, V.R. and SCHEER, B.T. 1961 Metabolism of glucose in the crabs, cancer magister and Hemigrapsus nudus. Comp. Biochem. Physiol. 3: PP. 30-41.

MORRIS, S.; A.C. TAYLOR, and C.R. BRIDGES 1990. Response of haemolymph oxygen affinity to simultaneous salinity and oxygen stress in the intertidal prawn Palaemon elegans. Comp. Biochem. Physiol. Vol. 90A (1) : PP.31-39.

PETRIELLA, R ; A.C.D.FENUCCI and J.L. FENNUCI 1991. Dietary cholesterol influence on the growth and survival of the argentinue prawn Artemesia longinaris Bate. Influence of Cholesterol. Jounal of Aqua.in the tropics. Vol.5 No. 2 PP. 111-117.

PLUMB, J.A., GRIZZLE, J.M., and J. DEFIGUERRIDO, 1976. Necrosis and bacterial infection in channel catfish (Ictalurus punctatus) following hypoxia. J. Wildl. Diseases, 12 PP. 247-253.

RAJAMANI, M. 1992. Biochemical studies on 'Soft' prawn. 1. Protein nitrogen contents in Penaeus indicus. Proc. Symp. Coastal. Aquaculture : Vol. 1 : PP. 349-353.

RAPPAPORT, A; S. SARIG, M. MAREK 1976. Results of tests of various aeration systems on the oxygen regime in the Genosar experimental ponds and growth of fish mere in 1975. Bamdiegh. Vol. 28. No.3 PP. 35-49.

* RENAUD, L. 1949. Le cycle des reserves organiques chez les crustaces Decapodes. Ann.Inst. Oceanogr 24: PP. 260-357.

ROGERS, G.L.; and A.W.FAST 1988. Potential benefits of low energy water circulation in Hawaiian prawn ponds. Aquacult. Eng. Vol. 7(3) : PP. 155-165.

SEIDMAN, E.R. and A.L. LAWRENCE 1985. Growth, feed digestability and proximate body composition of juvenile Penaeus Vannamei and Peanaeus monodon grown at different dissolved oxygen levels. J.World. Maricul. Soc. 16 : PP. 333-346.

SENKBIEL, E.G. and J.C. WRISTON : 1981 a. Catabolism of haemocyanin in the american lobster, Homarus americanus Comp. Biochem. Physiol. 68B PP.163-171.

*SNIESZKO, S.F., 1973. Recent advances of scientific knowledge and developments pertaining to diseases of fish. Adv. Vet. Sci. Comp. Med., 17 : PP. 291-314.

SOLARZANO, L., 1969. Determination of ammonia in natural waters by the phenol hypochlorite method. Limn. Ocean. 14 : PP. 799 - 801

STEWART, N.E. ; D.L. SHUMWAY and P. DOUDOROFF 1967. Influence of oxygen concentration on growth of Juvenile large mouth bass. J. Fish. Res. Bd. Canada. 24:475-494.

* STOTT, F.C. 1932. Einige Vorlaufige Versuche uber Veranderungen des Blutzuckers bei dekapoden. Biochem Z. 248 : PP. 55-64.

STRICKLAND, J.D.H and T.R. PARSONS 1968. A practical handbook of sea water analysis. Bull.Fish.Res. Bd. Canada., 125:1-311.

SUBRAHAMANYAN, C.B. 1962. Oxygen consumption in relation to body weight and oxygen tension in the prawn Penaeus indicus (Milne Edwards). Proc. Indian. Acad. Sci. 55 : PP. 152-161.

TEAL, J.M. and F.G. CAREY. 1967. The metabolism of marsh crabs under conditions of reduced oxygen pressure. Physiol. Zool. 40 : PP. 83-91.

TRAVIS, D.F. 1955b. The moulting cycle of the spiny lobster Panulirus argus Latreille, III. Physiological changes which occur in blood and urine during normal moulting cycle. Biol. Bull. Mar. Biol. lab., Woodshole, 108 : PP. 88-112.

TUCKER, L., BOYD, C.D., and E.W. MCCOY 1979. Effects of feeding rate on water quality, production of channel catfish and economic returns. Trans. Amer. Fish. Soc. 89 : PP. 333-337.

VAN AARDT, W.J. 1988. Lactate metabolism and glucose patterns in the river crab Potamonautes warreni Calman, during anoxia and subsequent recovery. Comp. Biochem. Physiol. Vol. 91A(2) : PP. 299-304.

VANDEN THILLART, G.; F. KESBEKE and A.VAN WAARDE 1976. Influence of anoxia on the energy metabolism of gold fish, Carassius auratus L : Comp. Biochem. Physiol. 55A: PP. 329-336.

*VERNE, J. 1924. Note histochimique surle metabolisme du glycogene pendant la mue chez les crustaces. C.R. Soc. Biol 90 : PP. 186-188.

VONK, K.J. 1961. Digestion and metabolism. In : The Physiology of crustacea. Vol.1. Metabolism and growth. T.H. Waterman (Ed.) Academic Press, New York, PP. 291-316.

WATERMAN, T.H. 1961. The Physiology of crustacea. Academic Press, New York.

WEDEMEYER, G.A. and D.J. Mc LEAY 1981. Methods of determining the tolerance of fishes to environmental stressors. In "stress and Fish" Pickering A.D. (ed.) Acadmic Press, London, PP. 247-268.

WILLIAMS, A.J. and P.L. LUTZ 1975. The role of haemolymph in the carbohydrate metabolism of Carcinus maenas. J. Mar. Biol. Ass. UK 55 : PP. 667-670.

WOLVEKAMP, H.P. and T.H. WATERMAN 1960. Respiration. In: The Physiology of crustacea, Vol. 1 Metabolism and Growth. T.H. Waterman (Ed.). Academic Press, New York. PP. 35-100.

WOO, Y.S.N. and R.S.S. WU. 1984. Changes in biochemical composition in the red grouper, (Epinephleus akaara (Temminck and schlegel), and the black sea bream, Mylio macrocephalus (Basilewsky), during hypoxic exposure. Comp. Biochem. Physiol Vol. 77A(3) : PP. 475-482.

*Not referred in original.