# ENERGETICS OF A FEW MARINE CRUSTACEANS

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By

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## DECLARATION

I hereby declare that this work has been originally carried out by me under the guidance and supervision of **Dr.P.V.Ramachandran Nair,** (Retd.) Principal Scientist, Central Marine Fisheries Research Institute, Cochin, and that this work has not been submitted elsewhere for any degree, fellowship or other similar titles of recognition.

Cochin - 31
October, 1990.

**(M.VIJAYAKUMARAN)**

## CERTIFICATE

This is to certify that the thesis entitled **"ENERGETICS OF A FEW MARINE CRUSTACEANS"** is the bonafide record of work carried out by **Sri.M.VIJAYAKUMARAN** under my guidance and supervision and no part thereof has been presented for any other degree.

Cochin - 31
October, 1990.

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# PREFACE

Among crustaceans, prawns occupy a pride of place owing to their immense aquaculture potential. Lobsters and crabs are also attracting attention as candidate species for mariculture. Most of the economically important and cultivable prawns have been domesticated and techniques have been evolved for mass production of their seeds. The Central Marine Fisheries Research Institute, a pioneer research institution in India, has conducted investigations on various aspects of prawn fishery and their biology, such as resource assessment, life history, hatchery production of prawn seeds and field culture of prawns.

Prawn farming has picked up momentum in India and much of the hitherto unutilized brackish water areas are being brought under shrimp cultivation and the demand for quality prawn seeds is on the increase. Research activities have been diversified to handle vital aspects of prawn biology and physiology, like broodstock management, salinity tolerance levels, nutritional and metabolic requirements etc, to ensure sustained production of healthy seeds. The Fishery Environment and Management Division of the CMFRI started a research project in late 70s, on the study of ecological energetices of cultivable marine prawns and fishes. Being one of the scientists associated with this project since its commencement, I felt the need for a detailed study on the energetics of reproduction and embryogenesis in prawns. Another aspect which drew my attention was the

survival rate and food conversion efficiency of prawn postlarvae under different salinity regimes. The white prawn, *Penaeus indicus* (H.Milne Edwards) was selected for the study in view of its prominent place in prawn farming in India.

Success achieved in seed production and culture of prawns induced researchers to explore the feasibiltiy of culture of other important crustaceans like lobsters and crabs. The CMFRI initiated a project to study the prospects of spiny lobster culture and I was associated with this project from the beginning. Compared to shrimp farming, culture of spiny lobsters is an entirely different proposition due to inherent problems in producing their seeds. The emphasis on lobster culture, therefore, shifted to rearing of juvenile lobsters, which form a major portion in commercial lobster landings in India. Commendable success was achieved in enhancing growth rate of four important speices of spiny lobsters by bilateral eyestalk ablation. But due to strong reservations expressed against the "blinding" of lobsters by people from all walks of life, this technique could not be tested commercially for its economic viability. As in prawns, energetics of reproduction and egg development have not yet been documented in spiny lobsters and so these aspects were included in this study. Though some limited informations on food conversion efficiency are available in spiny lobster, a detailed study on the effect of size and sexual maturity on food conversion was wanting and this also was included in this investigation. The most dominant spiny lobster

in south east and south west coast of India, *Panulirus homarus* (Linnaeus) was selected for this study.

Uptake, accumulation and utilization of minerals and trace elements are important events during embryogenesis of aquatic invertebrates. The scope of this study was widened to include these aspects also in order to understand how specific requirements of minerals and trace elements are met in the developing eggs of these two species of crustaceans.

The thesis is composed of 9 chapters with introduction and material and methods forming chapters 1 and 2 respectively. Chapters 3,4 and 5 deal with different aspects of energetics of the spiny lobster, *P.homarus*. In chapters 6,7 and 8 the energetics of the prawn, *P.indicus* are documented along with comparison with that of the spiny lobster. The summary of the work is given in Chapter 9.

I am deeply indebted to my supervising teacher, Dr.P.V.Ramachandran Nair for his guidance and help in this study. As the Head of Fishery Environment and Management Division, Dr.Nair readily agreed to be my superivising teacher after the sad demise of Dr.K.V.Sekharan, Senior Fishery Scientist, CMFRI, Cochin under whom I originally registered for Ph.D. I express my gratitude to (Late) Dr.K.V.Sekharan for his advice in the initial stages of this work.

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It is with great pleasure that I acknowledge the support and encouragement given by my wife during the preparation of the Thesis. Finally, I remember my late mother, who was eagerly waiting to see me complete this work and to her, I dedicate this Thesis.

# 1. INTRODUCTION

Crustaceans are highly successful in aquatic environment and many have secondarily adapted to semi-terrestrial and terrestrial habitats while still retaining dependence on "aquatic media" for embryogenesis and early larval stages. The diversity of habitats, ranging from ocean depths to high altitude and from arid land to trees and humid caves, have singled out crustaceans as a unique group for study of various aspects of bioenergetics (Vernberg, 1987). While most of the earlier studies were on specific components of energy budget of an organism, such as respiration, ingestion or reproduction, the emphasis recently has shifted to formulation of total energy budgets in relation to environmental changes and different life history stages. In the present study, two economically important decapods of high aquaculture potential, the spiny lobster, *Panulirus homarus* (Linnaeus) and the penaeid prawn, *Penaeus indicus* H. Milne Edwards - with contrasting reproductive strategies- have been selected for evaluation of : 1. storage and utilization of energy reserves during ovarian maturation and estimation of energetic cost of reproduction, 2. energy utilization during embryogenesis, and 3. important aspects of post-embryonic energetics like influence of body size and sexual maturity on food conversion efficiency in *P. homarus* and responses to salinity in the postlarvae of *P. indicus*.

Gonad production involves intense biochemical synthesis with formation of large amounts of nucleic acids for spermatozoa and lipids and proteins for the ova (Giese, 1959). Energetic cost of reproduction, which also

includes metabolic cost of brooding in addition to reproductive output, has to be met either from stored energy reserves or from an increased food intake and direct transformation of the ingested food to gonadal material. In planktonic crustaceans and in almost all warm water crustaceans, egg production and food are closely interrelated (Marshal and Orr, 1952, Patel and Crisp, 1960). Storage of energy and its mobilization to ovary during maturation have also been reported in many warm water crustaceans (Allen, 1972, Lawrence *et al.*, 1979, Adiyodi, 1980a,b, 1985, Teshima and Kanazawa, 1983). In temperate conditions, with markedly seasonal pattern of productivity, storage of energy in different tissues when food availability is more and mobilization of these reserves at the time of reproduction is a predominant feature (Barnes *et al.*, 1963, Clarke *et al.*, 1985).

Nutritive material for the embryo (vitellin or yolk) accumulates in the oocytes by various processes (Adiyodi, 1985) and resolution of origin of yolk is important from the viewpoint of endocrine regulation of crustacean reproduction (Quackenbush, 1989). It is now established in crustacea, that the production of yolk is both autosynthetic (within the oocyte) and heterosynthetic (extraoocytic and transferred through haemolymph to the egg) and the proportion of intra and extraoocytic origin varies from species to species (Kerr, 1968, Eurenius, 1973, Wolin *et al.*, 1973, Fyffe and O'Conner, 1974, Dehn *et al.*, 1983, Adiyodi, 1985, Quackenbush, 1989). Various tissues such as haemocytes, fat body, subepidermal fat body, and hepatopancreas have been described as sites of extraovarian synthesis of the yolk protein, vitellogenin (Kerr, 1968, Picaud, 1980, Meusy *et al.*, 1983,

Laufer, 1987, Tom *et al.*, 1987, Quackenbush, 1989, Rahman, 1989). The oocyte, however, remains as the major site of yolk production in most of the crustaceans (Adiyodi, 1985).

Changes in biochemical composition of muscle, haemolymph, hepatopancreas and ovary during maturation have been described for many crustaceans (Barnes *et al.*, 1963, Allen, 1972, Clarke, 1977, Lawrence *et al.*, 1979, Asokan and George, 1984, Adiyodi, 1985, Jeckel *et al.*, 1989, Rahman, 1989, Teshima *et al.*, 1989). Hepatopancreas or midgut gland, which is the site of both protein synthesis and lipid metabolism (Chang and O'Conner, 1983) and is the main storage organ (Huggins and Munday, 1985) plays a vital part in supply of energy reserves, especially lipids, for maturation process. Total lipid of hepatopancreas increases in the initial stages and then declines in final stages of maturity in females of many species of prawns (Teshima and Kanazawa, 1983, Asokan and George, 1984, Teshima *et al.*, 1989). Diglycerides and free aminoacids are suspected to be involved in the mobilization of lipids of the hepatopancreas into the haemolymph and ultimately to the developing oocyte (Allen, 1972). Adiyodi (1985) cites lack of convincing evidence of storage and mobilization of protein from hepatopancreas during ovarian maturation, while use of hepatopancreatic sugars during maturation of ova is reported in crustaceans (Adiyodi and Adiyodi, 1970, Asokan and George, 1984).

Tail muscle, which is the single largest tissue in many crustaceans, is not reported to contribute nutrient reserves for ovarian maturation

(Lawrence *et al.*, 1979). But studies on induced maturation by eyestalk ablation in *P. indicus* by Asokan and George (1984) suggest that tail muscle might play an important role in the mobilization of organic reserves in this species.

Colour, size and shape of crustacean eggs vary much and the relative sizes show ecological variations. Thus, eggs of marine crustaceans are smaller than those of allied forms in freshwater, while the allied terrestrial species produce still larger eggs. Variation in egg size is also seen within the same species, especially in the higher latitudes with marked seasonal fluctuations in temperature. These differences have been related to the provision of nutrients and water to the developing embryo. Ambient temperature and egg size, independently or in combination, along with the genetic make up of the egg, the quality and quantity of yolk and availability of food for the emerging young ones, may remarkably alter the rate of development and its duration in crustaceans (Clegg, 1965, Corkett and McLaren, 1970, Green, 1971, Herring, 1974, Wear, 1974). Within the same species, the duration of embryonic development varies inversely with temperature over the natural viable range (Green, 1971).

Apart from influencing the rate and duration of development, egg size also reflects the reproductive strategy of the animal. According to Vance (1973a, b) it is more efficient to produce many small (planktotrophic) eggs when food availability is patchy and unpredictable and a smaller number of larger (lecithotropic) eggs when the environment for the newly hatched larva

is uniform and predictable. In higher latitudes, there is marked fluctuation in temperature and, therefore, productivity which forces species to select between these two strategies alternatively. In tropical waters, however, reproductive strategy of a single species does not show considerable variations.

The newly deposited crustacean egg is a self contained system with all the necessary material for synthetic process associated with embryogenesis and morphogenesis and all components required for oxidative metabolism and energy production (Sastry, 1983). During the course of development, enhanced demand for water and mineral elements are met by direct imbibition from the surrounding medium. Major nutrient reserves in eggs are protein and lipid with carbohydrate making an insignificant contribution. Composition of nutrient reserves in the egg and the rates of utilization of these reserves during development vary in different species of crustaceans in relation to size of the egg, incubation time and environment.

Energetics of developing crustacean eggs have been documented in many species of barnacles, crabs, shrimps, homarid and scyllarid lobsters (Barnes, 1965, Pandian, 1967b, 1970a,b,c, 1972, Pandian and Schuman, 1967, Pandian and Katre, 1972, Pillai and Subramoniam, 1985, Erribabu, 1987, Rahman *et al.*, 1987). In all these crustaceans, protein and lipid contribute more energy for development. Unlike terrestrial "Cleidoic" eggs, more protein is metabolized in the "Non-cleidoic" aquatic eggs, where nitrogen excretion presents no problem (Needham, 1950). Reviewing yolk utilization studies in

marine demersal eggs, Pandian (1970c) suggested that these eggs are closer to the terrestrial "Cleidoic" eggs in terms of conservation of protein and "gearing up" fat metabolism during development. Several marine crustaceans have been observed to draw more than 80% of the required energy for embryonic metabolism by oxidising lipid. Contribution of carbohydrates towards energy for developing egg rarely exceeds 3% of total expenditure in marine decapods.

Developing eggs convert yolk with high efficiency and in the crustacean eggs,yolk utilization efficiency ranges from 44% in *Macrobrachium idella* to 74% in *Macrobrachium nobili* (Sumitra and Easterson, 1974, Balasundaram, 1980). Even though yolk utilization during embryogenesis is well documented in many planktonic and demersal crustaceans, no study has yet been reported in spiny lobsters and penaeid prawns.

One of the most neglected aspects of the study of maturation process as well as embryogenesis in aquatic animals is the rolé of mineral elements, its absorption, accumulation and transportation to developing oocytes during maturation and its absorption and utilization during egg development. Most of the investigations on metalic element absorption have been intended to know the bioaccumulation of heavy metals in soft and edible tissues (Eisler, 1981) and to study the effect of heavy metal concentration in the medium on the survival of larvae and young ones of many crustaceans and fishes.

Carbon, Hydrogen and Oxygen are the major elemental components of protoplasm. Phosphorus, Calcium, Sulphur, Sodium and chloride in the order of abundance are other elements which are considered as major components of living materials. Many other elements like Magnesium, Iron, Manganese, Zinc, Cobalt, Chromium, Copper, Lead etc. are required for many specific physiological and biochemical functions of the living organisms and are normally referred to as trace elements as they are present in minute quantities in vertebrates (Hoar, 1975, Moore, 1981). Many trace elements form prosthetic groups of enzymes while some others are involved in activation of enzymes. Metal accumulation in aquatic animals is reported to vary with species, age, weight, feeding habits and salinity, although the interrelationships are poorly understood (Philips, 1977). Ionic constituents of sea water such as Na, K, Cl, Ca and Mg have been incorporated into highly efficient regulatory mechanisms, but very little is known about uptake and regulation of trace elements (Wright, 1986).

The role of trace elements in maturation of fish and other aquatic animals are not known, but its requirements within any species differ at different stages of maturation (Berman and Vitin, 1968). Very few studies reported in fishes show that there is variable accumulation of Na, K, Mg, Mn, Fe, Cu and Zn in different tissues, especially ovary, during maturation (Ilzinia, 1968, Julshamn and Braekkan, 1976). Concentration of some metals in mature females of the mussel *Choromytilus meridionales* has been found to be twice that of males (Watling and Watling, 1976). In crustaceans, except for the reported increase in concentrations of Cu, Zn, Cd and decrease of Ni

and Pb in the whole body of *Metapenaeus dobsoni* during maturation (Thangaraj, 1985), no study on accumulation of metalic elements during maturation in different tissues have been cited.

Closed "Cleidoic" eggs of terrestrial animals are provided with all the minerals required for development. In fresh water eggs also, the required minerals and trace elements have to be made available along with yolk or have to be absorbed by the embryo against a concentration gradient. Marine eggs, however, are supposed to take in a good amount of mineral elements from sea water, which is a storehouse of these elements (Needham, 1950). There is no detailed evaluation of the uptake of minerals and trace elements during embryogenesis in fishes or crustaceans. Few references on fishes report absorption of elements like K, Ca and Mn during egg development (Love, 1980). Lee and Krishnan (1985) have reported that eggs of the dolphin fish, *Coryphaena hippurus*, absorb Ca and Mg during development and Ca is very important for early stages while embryos at a later stage can survive without Ca. Absorption of Ca, Mg, Cu, Zn, and Fe has also been reported in salmon (*Salmo gairdneri*) eggs (Hayes *et al.*, 1946, Craik and Harvey, 1988) and Ca and Mg in mullet (*Mugil cephalus*) eggs (Lee and Hu, 1983). In crustaceans, Katre (1977) and Ponnuchamy *et al.*, (1979) reported uptake of considerable quantity of Cu in the eggs of caridians, *Macrobrachium lamarrei* and *Caridina nilotica* respectively, and Pillai and Subramoniam (1985) have studied the absorption of Mg, Fe, Zn, Cu, Mn and Co in the eggs of the land crab, *Paratelphusa hydrodromous*.

Intake of food and its transformation in the animal are important aspects of economic evaluation of culture operations. Due to inherent problems in production of spiny lobster seed in the hatchery, culture of this species can be attempted only with undersized juveniles, which form 35 to 40% of the total landings in India (Radhakrishnan and Vijayakumaran, 1990). Radhakrishnan (1989) has evaluated few aspects of food conversion in *P. homarus* like the effects of isolation and quality and quantity of feed.

A negative correlation in food conversion efficiency with increase in size has been reported in many fishes (Ivlev, 1945, Gerking, 1952, Pandian 1967a, Vijayakumaran, 1979) and in crustaceans (Vernberg, 1987). Menzel (1960) reported a fall in protein utilization efficiency in the fish *Epinepheles guttatus* as it grew bigger in size. In the present study both food and protein conversion efficiencies have been evaluated in *P. homarus* with size ranging from 10 to 300g and complete energy budgets have been calculated for different size groups.

Among various parameters to study the effect of salinity extremes, food conversion efficiency has been described as a very sensitive indicator of changes, especially before attaining sexual maturity (Kinne, 1962). A change in metabolic rate due to the influence of salinity is assumed to be reflected in an increase or decrease in food conversion efficiency. In both fishes (Kinne, 1960, 1962, McLeod, 1977) and crustaceans (Venkataramiah *et al.*, 1973a,b), food intake and conversion were maximum at intermediate salinities and lowest values were recorded at both extremes of high and low salinities.

*P. indicus* postlarvae that migrate to estuaries are subjected to wild fluctuations in salinity and one of the aims of this investigation is to know the effect of salinity as well as quality of feed on survival, food conversion and protein conversion efficiencies of *P. indicus* postlarvae.

In food conversion estimates of both *P. homarus* and *P. indicus* in this study, the bioenergetic components like food consumption (C), egestion (F+U) and growth (P+E (exuvia)) were determined and metabolism was the only component that was not determined but calculated. Feeding rates and conversion efficiencies are reported as better parameters for assessing metabolic rates and efficiencies as they provide (1) less restricted maintenance conditions during feeding experiments, (2) the possibility of observing one and the same individual for a long period, (3) the possibility of measuring quantitative and qualitative feeding on metabolism (Paloheimo and Dickie, 1966a,b) and (4) the possibility of measuring the total metabolism including the energy expended on part or total anaerobiosis.

Apart from estimating biochemical changes during maturation and embryogenesis, the present study aims at understanding the uptake and utilization of minerals and trace elements during these processes. This investigation is the first elaborate attempt in determining absorption and utilization of 13 mineral elements, Na, K, Ca, P, Mg, Fe, Zn, Ca, Mn, Co, Cr, Cd and Pb during maturation as well as egg development in crustaceans.

# 2. MATERIALS AND METHODS

The spiny lobster, *Panulirus homarus* and the penaeid prawn, *Penaeus indicus* for the present study were collected from Kovalam, a fishing village 25 KM south of Madras. Both the species form seasonal fishery at Kovalam and were procured live from traditional non-mechanised boat catches. The lobsters were caught in bottom set gillnets at about 5m depth in the rocky subtidal area while the prawns were caught in boat-seines at 5-8 m depth in the same area.

For analysis of biochemical and mineral changes during maturity, immature and mature *P. homarus* and *P. indicus*, collected live from the wild, were sacrificed immediately and the tissues processed for chemical estimation. For taking samples of spent animals mature *P. homarus* with fresh sperm mass deposition in the sternum and *P. indicus* with fully ripe ovary were collected and kept in 100 litre capacity fibre glass tanks for spawning. They were sacrificed immediatley after spawning to take tissue samples of spent animals.

Three maturity stages, immature, mature or ripe and spent, were recognized to study biochemical and mineral changes during maturation. For each stage, a minimum of six specimens were taken and the tissues were pooled, after determination of water content and pulverization, for chemical estimations. To avoid influence of environmental conditions on biochemical

and mineral composition, lobsters and prawns of all maturity stages were collected in the same period, between May and August.

For yolk utilization and mineral uptake investigations during embryogenesis, mature specimens of *P. homarus* and *P. indicus* were collected and reared in 200 litre and 100 litre fibre glass tanks respectively, filled with fresh, filtered sea water and with almost continuous aeration from an air compressor. Salinity of sea water was 33.05 ± 0.48 ppt., dissolved oxygen 4-4.2 ml per litre and pH 8-8.1, during the period of the experiments. Temperature of sea water was 27.03 ± 1.02°C for *P. homarus* egg development and 26.31 ± 0.83°C for *P. indicus*. *P. homarus* was retained in the same tank after spawning till completion of development or until all eggs were taken for analysis. On the other hand, *P. indicus* spawners were removed immediately after spawning and the eggs retained in the tank until the emergence of protozoea larva or till whole eggs were removed for sampling. Water quality was maintained by replacement with fresh filtered sea water daily in the morning.

Most of the lobsters used in this study were brought to the laboratory with fresh spermatophoric mass on the sternum and a majority of them oviposited within one or two days. In addition, breeders with eggs in different developmental stages were also collected. The lobsters were fed with clam (*Meretrix casta*) meat throughout the period of observation.

Eggs were taken for analysis periodically depending on the stages of development. Care was taken to see that only eggs from first spawning were

used in the analysis, since eggs of subsequent spawning were smaller in size. *P. homarus* spawns again within a week after release of phyllosoma larvae, if viable spermatozoa remain in the spermatophoric mass after first spawning. Stages of eggs were fixed based on visual and microscopic observations of the colour, diameter, morphological changes and rate of yolk utilization. Six developmental stages were fixed in *P. homarus* egg, which is in excess of the four stages described by Berry (1971) for the same species. The phyllosoma larva has been referred to as the 7th stage in development. Ripe ova from mature ovary, just before spawning, were also analysed and termed as stage 0.

Almost all mature *P. indicus* breeders spawned on the same day of collection in the night and those which did not do so were rejected. Development stages in *P. indicus* were fixed after examining embryogenesis in few eggs and comparing it with the development stages reported for this species by Muthu *et al.*, (1978). Accordingly, five stages were identified in *P. indicus* egg with the first hatched, non-feeding, nauplius classified as stage 6. Last instar of nauplius was termed stage 7 and the first feeding larva, the protozoea, as stage 8. As in *P. homarus* egg, the mature ova were classified as stage 0.

For food conversion studies, juveniles and adults of *P. homarus* were held in 1000 litre capacity fibre glass tanks with well aerated sea water for three to four weeks.They were fed *ad libitum* with clam meat until taken for experiment. Equal number of males and females were used in all

experiments. Postlarvae of *P. indicus* were obtained from the prawn hatchery at Kovalam and were fed with chopped clam meat until the start of experiment. The prawnlarvae were acclimatized to respective salinities and feeds, a week before the experiment started.

In addition to clam meat, a compounded diet and a combination of both these were fed to *P. indicus* postlarvae. The compounded diet was prepared with crab meal (40 g), prawn head waste (15g), wheat flour (10g), tapioca powder (33g) and vitamin and mineral tablets (ROCHE, 2g). The ingredients were mixed well and made into a dough after adding sufficient quantity of water. The dough was then steamed in pressure cooker, without pressure, for 15 minutes, pelleted through a 1 mm diameter die and dried in a hot air oven at 60°C. Protein content of the pelletted feed was 32.96% and that of the clam meat, 62.97%. When these two were mixed and fed to prawn postlarvae, the protein content of the mixture was 44.78%. In the group fed with clam + pellet, these two feeds together were given *ad libitum* as in the other two groups but, individually, both feeds were below *ad libitum* level. In this group the prawn postlarvae consumed clam meat almost completely and the pellet only partially.

These three feeds were given in three different salinities - low saline (16.48 ± 1.51 ppt), normal saline (33.05 ± 0.48 ppt) and high saline (44.90 ± 5.5 ppt). Normal saline water was fresh filtered sea water, while low saline water was obtained by diluting sea water with fresh (ground) water. High saline water was collected from the Mariculture farm of the Central Marine

Fisheries Research Institute, Muttukkadu, near Kovalam. Salinity regimes for the present study were chosen to simulate the three salinity conditions prevailing in the Muttukkadu farm during monsoon, normal period and in summer months. Such conditions prevail in most of the back water regions along the east coast of India.

## 2.1 MEASUREMENT OF LENGTH AND WEIGHT

The carpace length of *P. homarus* was measured to the nearest 0.01 mm as described by Berry (1971), from the transverse ridge between supra orbital horns to the posterior extremity of cephalothorax. Total lengths of *P. indicus* postlarvae, juveniles and adults were measured from the tip of the rostrum to the posterior extremity of the telson to the nearest mm.

Eggs of *P. homarus* are attached to fine ovigerous setae of the pleopods of the abdominal segments. The eggs were removed along with ovigerous setae by cutting the setae with fine scissors. Most of the ovigerous setae were separated but complete removal was not possible. To maintain uniformity, eggs from the first two pleopods were taken for analysis. As many as three development stages could be collected from some lobsters and in such cases eggs from other pleopods were also taken. Phyllosoma larvae of *P. homarus* and eggs and larvae of *P. indicus* were collected by filtering through bolting silk. All eggs and larvae were washed twice with distilled water for 15 seconds, blotted dry on a filter paper and then weighed in preweighed aluminium foil cups. Ripe ova could not be completely separated from ovary and contained ovarian tissues also. To find out

individual weights, of ovum, egg and larvae, the total numbers in 25 to 100 mg were counted at each sampling.

Egg and yolk diameter were measured microscopically using ocular and stage micrometers. Diameter of 50 eggs were measured at each sampling. The eggs were mostly spherical and the formula $4/3\ \pi r^3$ was used for estimating volume. Egg and yolk diameters were measured at two or more diagonal planes and for elyptical or uneven shapes, especially of yolk, the formula $\pi/6\ LH^2$ was used, where L and H are radii at two planes (Blaxter and Hempel, 1963).

Total weights of lobsters were determined in a monopan electrical balance to an accuracy of 0.1 g while the weights of *P. indicus* and tissues for biochemical estimations were taken in an electrical balance with 0.001g precision. Electrical balance with more precision (0.01 mg) was used for weighing aliquots for specific biochemical estimations and for taking weights of eggs and larvae.

## 2.2 ESTIMATION OF PROXIMATE COMPOSITION AND MINERALS AND TRACE ELEMENTS

Water content in the whole animal, tissues, ova, eggs and larvae were determined as the difference in total wet and dry weights of the animal/tissue/egg/larvae. The samples were dried in a hot air oven at 60°C for about 48 hours or until constant weight was obtained, homogenised and dried again for 1-2 hours before storing in airtight glass vials in a desiccator.

Aliquots from these dried samples were taken for chemical analysis, which were completed within three months (except for minerals and trace elements for which the ash was dissolved in 20% v/v nitric acid and kept up to six months for completion of analysis).

Whole protein was estimated colorimetrically by modified Biuret method (Sumitra and Vijayakumaran, 1974), where the dried tissue as well as the protein standard were first digested with 40% NaOH and then made up to a known volume to make the final concentration of 10% NaOH. Aliquots were taken from this and the extinction measured at 540nm, 30-60 minutes after adding Biuret reagent. Total carbohydrates were measured by phenol- sulphuric acid method (Raymont *et al.*, 1964) and total lipids by methanol-chloroform extraction (Bligh and Dyer, 1959). In the eggs, protein and carbohydrate estimations were made on fat-free samples. Caloric values were estimated on dry samples by using a Parr Semi-microbomb colorimeter (1200) and an AH12/EF electronic microbomb calorimeter, Nelson Electronics Ltd., London (for energy utilization studies in the eggs of *P. indicus* only) using standard specifications as given in instruction manuals of these instruments. For computation of energy from biochemical parameters, the values (1g protein ≡ 23.45 KJ; 1g fat ≡ 39.36 KJ and 1g carbohydrate ≡ 18.84 KJ) given by Prosser and Brown (1961) were used.

Materials for mineral analysis were processed by the dry ashing method described for fish and other marine products by Thompson (1969) and the samples were analysed in Perkin Elmer Atomic Absorption

Spectrophotometer (Model 2380). Four grams of dry material taken in a crucible, was charred in an electric hot plate. The crucible was then placed in a muffle furnace and the temperature brought to 525°C. It was ashed for two hours to white ash, cooled and weighed to determine percentage of ash. To this 15 ml of 20 (V/V) nitric acid was added to break up the ash and filtered through acid washed O.42 Whatman filter paper into a 100 ml volumetric flask. The residue was washed three times with deionized water and the volume made up to 100ml. This stock solution was then diluted with deionized water, if necessary, to place concentration of elements of interest in a suitable range as described in standard conditions for that element using procedures described in the "Cook Book" of the Perkin Elmer Atomic Absorption Spectrophotometer. Phosphorus, which could not be detected accurately by the Atomic Absorption Spectrophotometer, was determined colorimetrically by Ammonium molybdate method described by Le Bel *et al.*, (1978). All the colorimetric readings were carried out in a CECIL 373 linear readout spectrophotometer. The values of proximate composition and minerals were expressed as mean ± SD of a minimum of four estimations.

## 2.3 ESTIMATION OF FOOD UTILIZATION PARAMETERS

The scheme of energy balance followed in the present study is that of the IBP formula (Petrusewicz and McFadyen, 1970) read as:

$$C = (P+E) + R + F + U$$

where C is food consumed; P, the growth; E, the exuvia or moult; R, the material lost as heat due to metabolism; F, the faeces and U, the nitrogenous excretory products.

### 2.3.1 Estimation of Conversion (C)

For both *P. homarus* and *P. indicus* feed was given at 1600 hours and the feed remains were removed the next day morning at 0900 hours. For lobster, clam meat was given shell-on, after opening the shell and for *P. indicus* postlarvae, clam meat was cut to small pieces and passed through a 30 mesh sieve and fed. For the prawn postlarvae, the pelleted feed was also crushed and sieved through 30 mesh. In lobster feeding experiments, unconsumed food along with the shell was removed, weighed and dried. In the experiments on prawns, unconsumed food was siphoned out into a bolting silk, washed with distilled water, blotted dry and transferred to weighed petridish and dried at 60°C in an oven to calculate total unconsumed food at the end of the experiment. Dry weight of unconsumed food was subtracted from the dry weight of food offered to determine consumption and converted to energetic equivalents (joules).

To estimate loss of feed by leaching, both clam meat and compounded feed were kept separately in sea water simulating rearing conditions and the difference in dry weight noted. The correction factors so obtained were used to determine the exact weight of food consumed.

### 2.3.2 Estimation of Faeces (F)

Faeces of *P. homarus* that comes out in the form of a ribbon and settles at the bottom were collected twice a week into a bolting silk, washed with distilled water, and dried at 60°C. Subsequently the total faeces production for the entire experimental duration for each test group was calculated. The faeces of *P. indicus* postlarvae which are in the form of small stable pellets were collected daily by pipetting out into a bolting silk, washed with distilled water and dried at 60°C. For caloric value and protein estimations, faeces of identical experimental groups were pooled for getting sufficient material.

### 2.3.3 Estimation of Nitrogen Excretion (U)

Ammonia, which forms more than 80% of nitrogenous excretory products in crustacea (Pandian, 1975), was determined at biweekly intervals using Phenol-hypochlorite method of Solorzano (1969). Ammonia was estimated by the difference in concentration in the rearing tanks for 24 hours and in controls simulating the experimental tanks in all respects including feed but without animals. In addition, ammonia was also estimated by transfering the animals to fresh filtered sea water 12 hours after feeding. From these estimations apparent ammonia excretion was computed for the whole experiment. Energy equivalent of 20.5 J for 1mg of ammonia (Brafield, 1985) was then used to estimate energy excreted as ammonia.

### 2.3.4 Estimation of Growth (P and E)

Conversion or growth (P of IBP terminology) was calculated by subtracting the initial dry weight/energy content of the animal from the final dry weight/energy content. Since exuvia (E) is also a part of converted energy and of growth, it has also been included in calculating energy transfer.

It was extremely difficult to retrieve all exuviae of postlarval prawns and hence prawn postlarvae were kept individually in separate glass beakers and given similar treatments as in the experimental tanks. Six postlarvae were reared individually for each treatment and the exuvia weights from these were used to compute the exuvia production in the experiments.

### 2.3.5 Estimation of Metabolism (R)

This was the only parameter in the study that was calculated since all other components of the energy budget were known.

In all feeding experiments, the test animals were starved for 24 hours to empty the stomach contents before the commencement of feeding. Animals from the same stock with identical weights were "sacrificed" (Maynard and Loosly, 1962) for initial measurement as well as control to determine initial dry weight, energy and protein.

Rates of feeding, assimilation and conversion as well as metabolic rates were calculated in relation to live mid body weight (g) of the animal

per unit time (day). Mid body weight is the midpoint of initial and final weights during the experiment. Efficiency of assimilation and Gross ($K_1$) and Net ($K_2$)conversion efficiencies were calculated in percentage relating to Ae (assimilation) to C and P to C and P to Ae respectively.

### 2.3.6 Calculation procedures related to food utilization and abbreviations used to express them

$$\text{Feeding rate} = \frac{\text{Food consumed (C)}}{\text{Mid body weight (g) X days}}$$

$$\text{Assimilation rate} = \frac{\text{Food assimilated (Ae)}^{*}}{\text{Mid body weight(g) X days}}$$

* Estimated by subtracting faeces (F) and urine (U) from the total food consumed i.e., Ae = C - (F+U)

$$\text{Conversion rate} = \frac{\text{Food converted (P+E)}}{\text{Mid body weight(g) X days}}$$

$$\text{Metabolic rate} = \text{Assimilation rate (Ae)} - \text{Conversion rate (P+E)}$$

$$\text{Metabolic rate}^{**} \text{ (ml } O_2\text{/g/hr)} = \frac{\text{Metabolic rate (J/mid body wt./day)}}{20.098 \times 24}$$

** Estimated considering 20.098J as oxycaloric coefficient of ml $O_2$ consumed (Engleman, 1970).

$$\text{Assimilation efficiency (\%)} = \frac{\text{Food assimilated}}{\text{Food consumed}} \times 100$$

$$\text{Gross conversion efficiency } (K_1)\ (\%) = \frac{\text{Food converted}}{\text{Food consumed}} \times 100$$

$$\text{Net conversion efficiency } (K_2)\ (\%) = \frac{\text{Food converted}}{\text{Food assimilated}} \times 100$$

$$\text{Food conversion Ratio (FCR)} = \frac{\text{Food consumed (dry)}}{\text{Weight gain (Wet)}}$$

$$\text{Protein Conversion Efficiency (PCE) (\%)} = \frac{\text{Protein converted}}{\text{Protein assimilated}}$$

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Live weight gain}}{\text{Dry weight of protein consumed}}$$

## 2.4 TERMS USED TO EXPRESS BIOCHEMICAL AND MINERAL COMPOSITION

| | |
|---|---|
| Concentration (of water and Dry matter) | = % in wet weight |
| Concentration (of protein, lipid carbohydrate and ash) | = % in dry weight |
| Concentration (of minerals and trace elements) | = mg or $\mu$g/g dry weight |
| Quantity (of water, dry matter, protein lipid, carbohydrate and ash) | = g/100g body weight |
| Quantity (of minerals and trace elements) | = $\mu$g or ng/100 g body weight |

## 2.5 STATISTICAL ANALYSIS

All statistical tests such as mean, standard deviation, test of significance (Students "t" test), correlation coefficient, regression and randomised block design were made following Snedecor and Cochran, (1967). All log. values referred in this study are Natural log. unless otherwise stated.

# 3. BIOCHEMICAL AND MINERAL CHANGES DURING OVARIAN MATURATION IN THE SPINY LOBSTER, PANULIRUS HOMARUS

Quality and quantity of yolk are crucial for successful early life of crustaceans and egg hatchability and survival are influenced by the condition of the parent. Preparedness of the parent for egg production, in turn, is dependent mainly on environmental conditions that may affect larval survival and availability of feed. The reserve food may either be stored in different tissues and later mobilized to the ovary during maturation or directly utilized by the developing oocytes. A knowledge of the weight and biochemical changes in different tissues during maturation will be very much useful in understanding reproductive physiology. It may also help in formulating rearing conditions and feed, for those species that are important for aquaculture, for production of healthy offsprings with high survival rates.

Egg production involves intense biochemical synthesis within the oocyte and outside the ovary and transfer of organic reserves from storage organs like hepatopancreas to developing ova. In temperate conditions gonadal development follows marked fluctuations in seasonal pattern of productivity while most of the tropical species are continuous breeders with distinct peak in egg production during certain months. Even in the

continuous breeders of the tropics, storage of reserves in tissues like hepatopancreas and later mobilization to maturing ova is predominant (Allen, 1972., Adiyodi, 1985), but intense breeding is directly related to availability of food (Patel and Crisp, 1960).

In lobsters, knowledge of biochemical changes during maturation relates only to the presence of female specific proteins in the haemolymphs of spiny lobsters and micropinacocytosis within the oocytes of homarid and palinurid lobsters. These studies suggest extraovarian synthesis of lipovitellin or yolk protein in lobsters (Byard and Aiken, 1984, Adiyodi, 1985). Recently, Rahman (1989) has studied biochemical changes during maturation in sand lobster, *Thenus orientalis*.

The present study is intended to observe biochemical changes during ovarian maturation and to determine the accumulation and utilization of minerals and trace elements.

## 3.1 RESULTS

### 3.1.1 Maturity stages

Three maturity stages corresponding to stage 1, immature, stage 4, mature or ripe and stage 5, spent described for *P. homarus* by Berry (1971) were followed to evaluate biochemical and mineral changes, during ovarain

**Table 3.1.** Maturity stages and morphological features used to identify them during ovarian maturation in *P.homarus*

| Stage | Morphological features |
|---|---|
| 1 (Immature) | Ovary white, flattened and strap like with a slightly granular appearance. Oocyte 0.06 - 0.13 mm |
| 4 (Ripe or mature) | Ovary swollen, filling all available space in the cephalothoracic cavity. Ova easily visible and present in the oviducts. Colour Reddish orange. Fresh sperm mass on the sternum. Ova - 0.39-0.44 mm |
| 5 (Spent) | Ovary pale cream in colour; residual ova present. Taken just after ovulation |

maturation. Details of maturity stages and morphological criteria to identify each stage are given in table 3.1.

### 3.1.2 Biochemical changes in muscle

Changes in concentration of water, protein, lipid, carbohydrate and ash in muscle during ovarian maturation are given in table 3.2. Lipid and carbohydrate concentrations decreased with maturity from 10.3% and 1.61% respectively to the minimum of 7.82% and 1.37% in the spent lobster. Water and protein concentrations were comparatively stable, while ash percentage increased at maturity and declined after spawning.

When the values were expressed quantitatively in a unit weight of 100g body weight (Table 3.3 and Fig. 3.1), no significant changes ($p = > 0.05$) were noticed in the quantities of protein, carbohydrate, energy and ash in different stages. Fresh weight of the muscle increased significantly in mature lobster ($p < 0.05$) compared to immature ones. Water content showed a significant increase at maturity ($p < 0.05$) so also the dry matter ($p < 0.005$). Among the organic contents, quantity of total lipid declined significantly ($p < 0.05$) at maturity and also between mature and spent conditions.

### 3.1.3 Biochemical changes in hepatopancreas

Changes in composition of hepatopancreas during maturation are tabulated in Table 3.4. While the hepatic index reduced from 3.79 in

**Table 3.2.** Biochemical changes in muscle during ovarian maturation in *P. homarus* (Protein, Lipid, Carbohydrate and ash are expressed as percentage in dry weight)

| Maturity stage | Size (g) | Muscle (% in wet weight) | Water % | Protein | Lipid | Carbohydrate | Ash |
|---|---|---|---|---|---|---|---|
| Immature | 102.20 ± 8.77 | 28.06 ± 0.36 | 74.96 ± 2.25 | 81.40 ± 1.30 | 10.30 ± 1.70 | 1.61 ± 0.71 | 7.29 ± 0.66 |
| Ripe | 321.61± 173.94 | 28.83± 1.01 | 74.49 ± 2.23 | 80.90 ± 2.75 | 8.86 ± 1.67 | 1.48 ± 0.31 | 8.41 ± 0.90 |
| Spent | 388.31± 126.59 | 29.57± 1.42 | 75.02 ± 2.23 | 81.96 ± 2.36 | 7.82 ± 1.32 | 1.37 ± 0.07 | 7.05 ± 0.02 |

**Table 3.3.** Quantitative changes in proximate composition of muscle during ovarian maturation in *P. homarus* (values expressed in 100 g body weight). (values in parenthesis are 'P' values of tests of significance (students 't') between mature and immature and mature and spent.

| Maturity stage | Wet weight of muscle (g) | Dry weight of muscle (g) | Water (g) | Protein (g) | Lipid (g) | Carbo-hyrate (g) | Ash (g) | Total energy (calculated) (KJ) |
|---|---|---|---|---|---|---|---|---|
| Immature | 28.06 ± 0.36 (<0.05) | 7.07 ± 0.01 (<0.005) | 21.03 ± 0.27 (<0.05) | 5.72 ± 0.29 (>0.05) | 0.72 ± 0.04 (<0.05) | 0.110 ± 0.001 | 0.51 ± 0.03 | 164.54 ± 8.39 |
| Ripe | 28.83 ± 1.01 | 7.35 ± 0.25 | 21.48 ± 0.75 | 5.95 ± 0.20 | 0.65 ± 0.03 | 0.110 ± 0.001 | 0.53 ± 0.08 | 167.18 ± 5.89 |
| Spent | 29.57 ± 1.42 (<0.005) | 7.52 ± 0.33 (>0.05) | 22.05 ± 1.41 (>0.05) | 6.15 ± 0.31 (>0.05) | 0.60 ± 0.03 (<0.05) | 0.110 ± 0.005 | 0.53 ± 0.03 | 169.91 ± 8.54 |

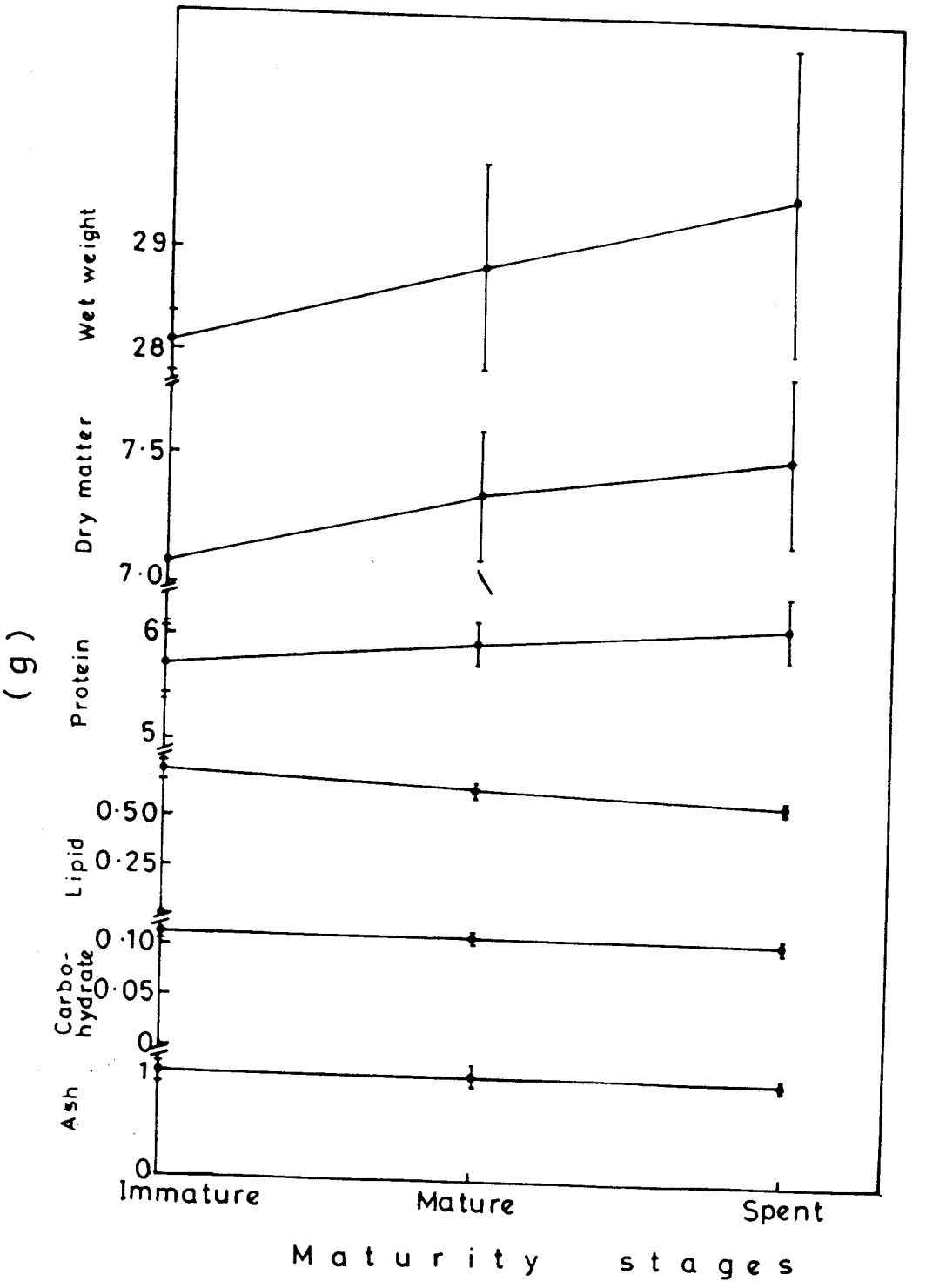


Fig. 3.1 Quantitative changes in wet weight, dry matter, protein, lipid, carbohydrate and ash in the muscle of *P. homarus* during ovarian maturation. (values expressed in 100 g body weight).

immature to 3.34 in mature and further to 2.88 in spent lobsters, water and ash concentrations increased marginally at maturity and markedly in spent lobsters. Protein and carbohydrate concentrations showed a significant ($p<0.05$) upward trend from 53.51 to 58.76% (protein) and 5.60 to 8.88% (carbohydrate) in mature lobsters and decreased significantly ($p<0.05$) after spawning to 48.04% (protein) and 5.92% (carbohydrate). A reverse trend, a marked decline from 31.71 to 21.19 at maturity and a reversal to 31.90% after spawning was noticed in lipid of hepatopancreas.

When the values were quantified (Table 3.5 and Fig. 3.2), a different picture emerged. Quantity of water reduced significantly at maturity ($p=<0.005$) and that of dry matter ($p=<0.05$) after spawning. Decline in quantity of protein was significant at maturity ($p=<0.005$) and more so after spawning ($p=<0.005$). Quantity of lipid showed a highly significant reduction ($p=<0.0005$) at maturity but the quantity was marginally more in hepatopancreas of spent ones. Increase at maturity and reduction after spawning were highly significant ($p=<0.005$) for carbohydrates of hepatopancreas. Total energy of hepatopancreas considerably declined at maturity and also after spawning. Quantity of ash showed a significant positive change at maturity and remained so after spawning.

Table 3.4. Biochemical changes in hepatopancreas during ovarian maturation in *P. homarus* (Protein, lipid, carbohydrate and ash are expressed as % in dry weight)

| Maturity Stage | Size (g) | % HP in wet weight | Water (%) | Protein | Lipid | Carbo-hydrate | Ash |
|---|---|---|---|---|---|---|---|
| Immature | 102.20 ± 8.77 | 3.79 ± 0.05 | 66.52 ± 6.67 | 53.51 ± 3.21 | 31.71 ± 4.50 | 5.60 ± 0.61 | 6.32 ± 0.36 |
| Ripe | 321.61 ± 173.94 | 3.34 ± 0.16 | 67.51 ± 6.68 | 58.76 ± 1.81 | 21.19 ± 0.88 | 8.88 ± 0.01 | 8.41 ± 0.23 |
| Spent | 388.31 ± 126.59 | 2.88 ± 0.51 | 72.28 ± 4.12 | 47.04 ± 2.24 | 32.90 ± 0.13 | 5.92 ± 0.41 | 13.41 ± 0.11 |

Table 3.5. Quantitative changes in proximate composition of hepatopancreas during ovarian maturation in *P. homarus* (values expressed in 100 g body wt). (values in parenthesis indicate 'P' values of tests of singificance (student 't') between ripe and immature and ripe and spent).

| Maturity Stage | Wet wt. (g) | Dry wt. (g) | Water (g) | Protein (g) | Lipid (g) | Carbo-hydrate (g) | Ash (g) | Total energy (cal.) KJ |
|---|---|---|---|---|---|---|---|---|
| Immature | 3.79 ± 0.05 (<0.0005) | 1.27 ± 0.02 (<0.0005) | 2.52 ± 0.03 (<0.0005) | 0.68 ± 0.01 (<0.005) | 0.40 ± 0.006 (<0.0005) | 0.07 ± 0.001 (<0.0005) | 0.08 ± 0.001 (<0.0005) | 33.01 ± 0.49 (<0.005) |
| Ripe | 3.34 ± 0.16 | 1.09 ± 0.05 | 2.25 ± 0.10 | 0.64 ± 0.03 | 0.23 ± 0.01 | 0.10 ± 0.004 | 0.09 ± 0.004 | 25.94 ± 1.17 |
| Spent | 2.88 ± 0.51 (>0.05) | 0.80 ± 0.14 (<0.05) | 2.09 ± 0.37 (>0.05) | 0.38 ± 0.06 (<0.005) | 0.27 ± 0.04 (>0.05) | 0.04 ± 0.008 (<0.0005) | 0.09 ± 0.005 (>0.05) | 20.29 ± 3.13 (<0.05) |

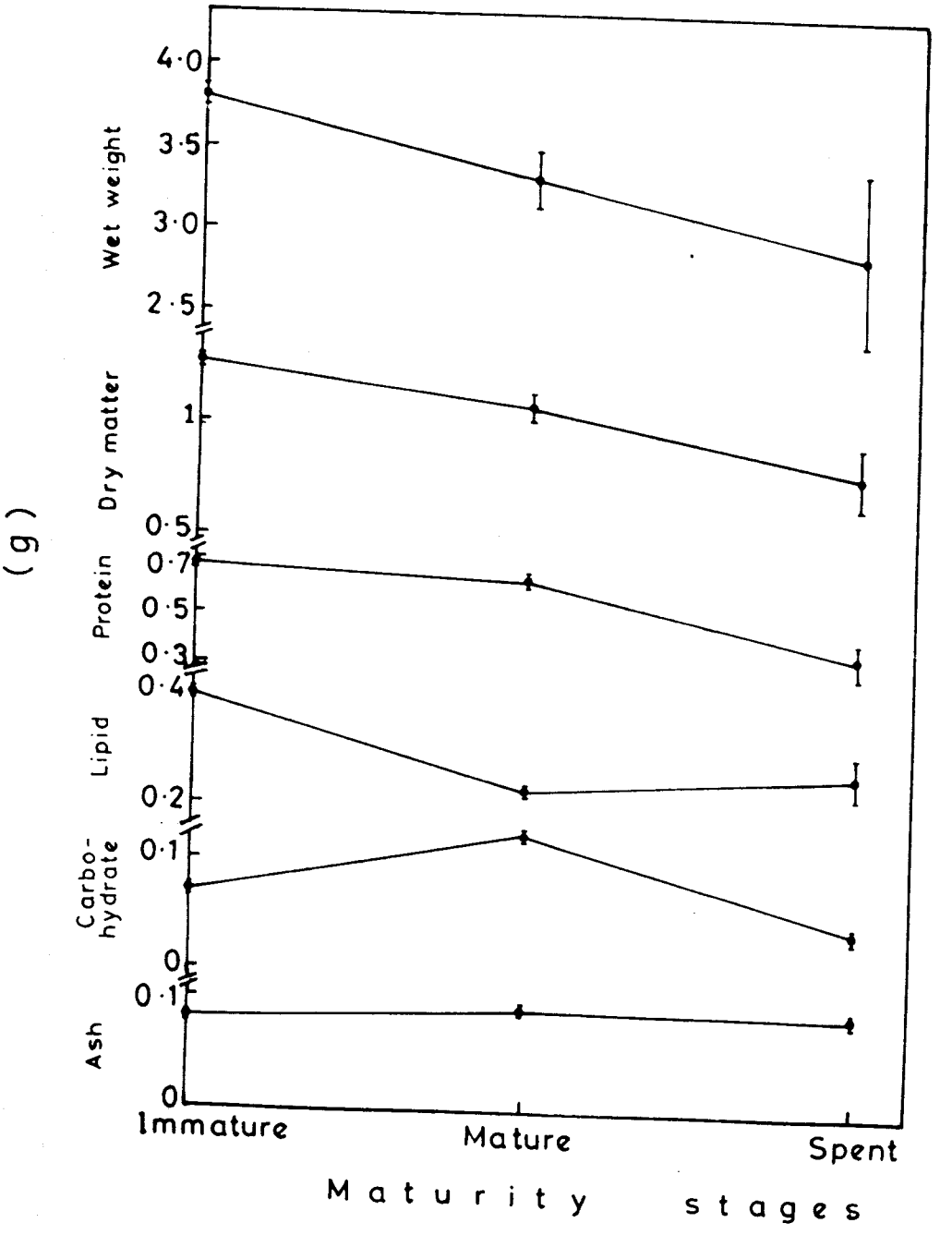


Fig. 3.2 Quantitative changes in wet weight, dry matter, protein, lipid, carbohydrate and ash in hepatopancreas of *P.homarus* during ovarian maturation (values expressed in 100g body weight).

### 3.1.4 Biochemical changes in ovary

Gonadosomatic index went up sharply from 0.2 to 5.3 at maturity and thereafter declined markedly to 0.83 after spawning (Table 3.6). Maximum concentration of water was recorded (82.11%) in immature ovary and showed a declining trend (52.71%) at maturity and increased to 76.92% in the spent ovary. Concentrations of protein and carbohydrate went up and that of lipid declined at maturity. After spawning, lipid and carbohydrate concentrations declined while protein recorded a marginal and ash 3 times increase in concentration.

Quantitatively, all parameters showed highly significant ($p = < 0.0005$) increment and an equally significant reduction ($p = < 0.0005$) at maturity and spawning respectively (Table 3.7 and Fig. 3.3).

### 3.1.5 Mineral changes in muscle

Elements that are present in mg quantity per g dry weight have been classified as major elements or minerals while those present in $\mu$g per g dry weight have been described as trace elements in this study. Accordingly, Na, K, Ca, P, and Mg come under the catagory of major elements or minerals in spiny lobster. Among the trace elements Cu, Zn and Fe are present in very high concentrations in different tissue during certain stages, questioning their classification as trace elements.

**Table 3.6.** Biochemical changes in ovary during maturation in *P. homarus* (Protein, lipid, carbohydrate and ash are expressed as % in dry weight)

| Maturity Stage | Size (g) | Ovary (% in wet wt.) | Water (%) | Protein | Lipid | Carbo-hydrate | Ash |
|---|---|---|---|---|---|---|---|
| Immature | 102.20 ± 8.77 | 0.20 ± 0.08 | 82.11 ± 1.86 | 50.99 ± 1.23 | 34.88 ± 0.28 | 3.17 ± 0.29 | 6.37 ± 0.44 |
| Ripe | 321.61 ± 173.94 | 5.31 ± 0.54 | 52.71 ± 0.95 | 62.82 ± 6.10 | 29.96 ± 3.59 | 3.54 ± 0.24 | 4.26 ± 0.47 |
| Spent | 388.31 ± 126.59 | 0.83 ± 0.12 | 76.92 ± 6.21 | 64.97 ± 4.09 | 19.11 ± 3.44 | 2.73 ± 0.27 | 12.91 ± 0.28 |

**Table 3.7 :** Quantitative changes in proximate composition of ovary during maturation in *P. homarus* (values expressed in 100g body weight). (values in parenthesis indicate 'P' values of tests of significance (Students 't') between ripe and immature and ripe and spent).

| Maturity Stage | Wet wt. (g) | Dry wt. (g) | Water (g) | Protein (g) | Lipid (g) | Carbo-hydrate (g) | Ash (g) | Total energy (cal.) KJ |
|---|---|---|---|---|---|---|---|---|
| Immature | 0.20 ± 0.08 (<0.0005) | 0.04 ± 0.01 (<0.0005) | 0.16 ± 0.06 (<0.0005) | 0.020 ± 0.005 (<0.0005) | 0.014 ± 0.003 (<0.0005) | 0.0010± 0.0003 (<0.0005) | 0.003 ± 0.001 (<0.0005) | 1.04 ± 0.24 (<0.0005) |
| Ripe | 5.31 ± 0.54 | 2.51 ± 0.25 | 2.80 ± 0.28 | 1.580 ± 0.160 | 0.750 ± 0.070 | 0.090 ± 0.010 | 1.070 ± 0.100 | 68.27 ± 6.70 |
| Spent | 0.83 ± 0.12 (<0.0005) | 0.20 ± 0.05 (<0.0005) | 0.65 ± 0.12 (<0.0005) | 0.130 ± 0.003 (<0.005) | 0.110 ± 0.002 (<0.0005) | 0.005 ± 0.006 (<0.005) | 0.010 ± 0.003 (<0.0005) | 7.47 ± 0.16 (<0.0005) |

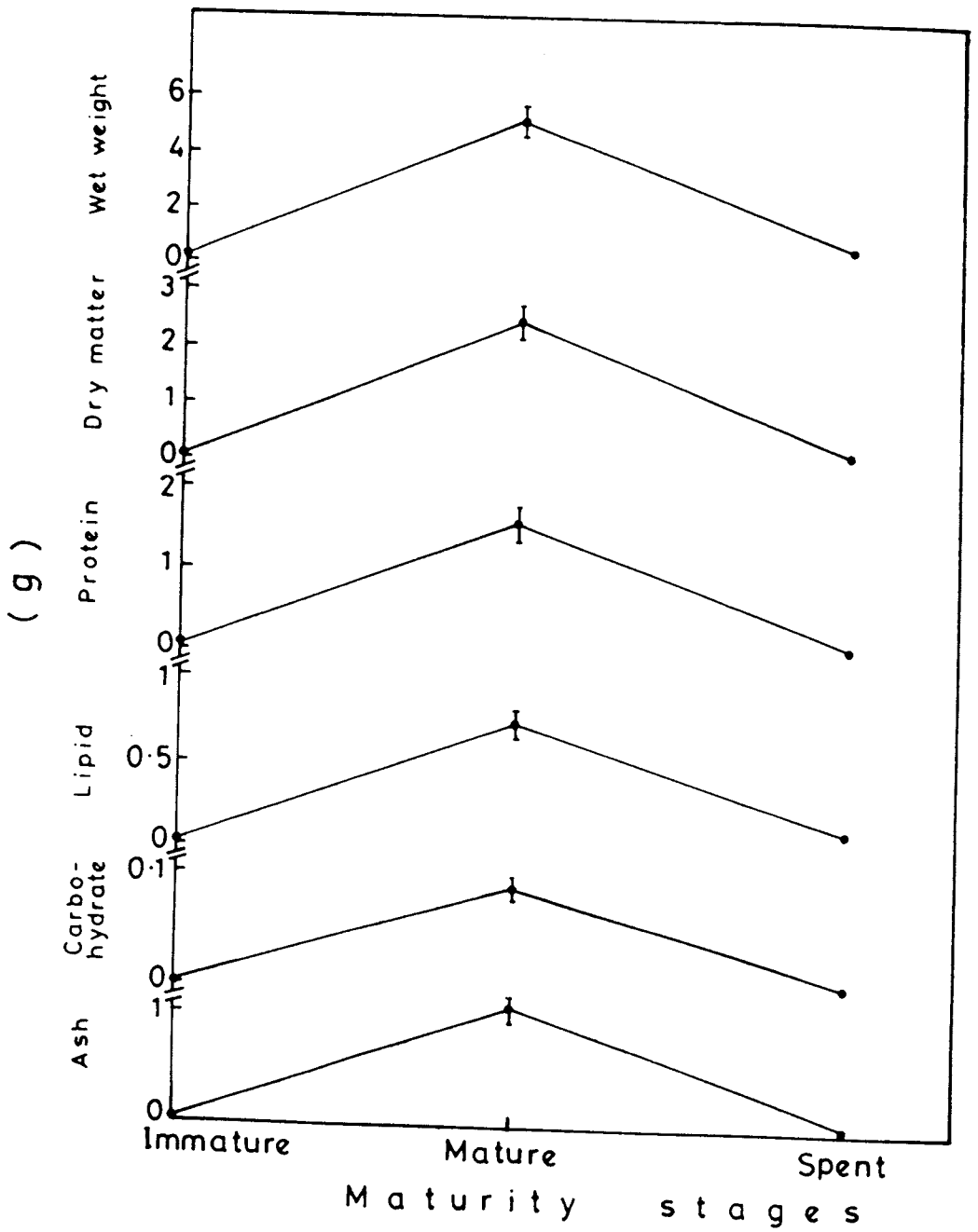


Fig.3.3 Quantitative changes in wet weight, dry matter, protein, lipid, carbohydrate and ash in ovary of *P.homarus* during maturation (values expressed in 100g body weight).

In general, mineral concentration increased with maturity in the muscle (Table 3.8) and declined moderately after spawning except for K which was present in maximum concentrations (17.56 mg) in immature stage and declined at maturity and further after spawning to record a minimum of 14.84 mg in the muscle of spent lobster.

Same trend was recorded in trace metal concentrations with the exception of Fe and Cr which were more after spawning and Co which was not present in detectable concentration in the muscle of mature lobster. Pb was below detectable limit (< 0.001 $\mu$g/g dry weight) in all stages of maturity.

Quantitative changes (Table 3.9 and Figs. 3.4 and 3.5) showed the same trend as concentration of minerals with a significant ($p = < 0.005$) increase at maturity and a significant ($p = < 0.05$) decline after spawning. Among the trace elements, Fe and Cd showed similar pattern of quantitative accumulation, declining at maturity and increasing after spawning, both significantly high ($p = < 0.0005$). Quantities of all other trace elements were more at maturity and reduced significantly after spawning, with the exception of Cr which recorded more than six fold increase after spawning. The most striking change was noticed in Mn which accumulated more than 8 times at maturity and declined by 15 times after spawning.

**Table 3.8.** Changes in minerals and trace elements composition in muscle during ovarian maturation in *P.homarus* (mg or μg/g dry weight)

| Minerals/trace elements (conc.) | Maturity stage | | |
|---|---|---|---|
| | Immature | Ripe | Spent |
| **Minerals (mg/g dry wt)** | | | |
| Na | 11.20 ± 0.28 | 13.46 ± 0.48 | 12.49 ± 0.56 |
| K | 17.56 ± 0.44 | 16.59 ± 0.39 | 14.84 ± 0.26 |
| Ca | 2.85 ± 0.11 | 3.56 ± 0.17 | 3.75 ± 0.62 |
| P | 15.47 ± 0.39 | 21.54 ± 0.69 | 21.51 ± 0.92 |
| Mg | 0.97 ± 0.15 | 1.68 ± 0.19 | 1.48 ± 0.16 |
| **Trace elements (μg/g dry wt)** | | | |
| Fe | 22.66 ± 1.56 | 141.00 ± 2.98 | 176.69 ± 3.56 |
| Cu | 17.17 ± 0.42 | 38.01 ± 1.02 | 31.55 ± 1.59 |
| Zn | 97.30 ± 2.52 | 160.66 ± 4.12 | 135.68 ± 3.82 |
| Cd | 0.32 ± 0.09 | 0.58 ± 0.09 | 0.20 ± 0.07 |
| Co | 0.59 ± 0.15 | N.D | 0.40 ± 0.11 |
| Mn | 0.81 ± 0.02 | 6.50 ± 0.86 | 4.07 ± 0.56 |
| Cr | 0.97 ± 0.26 | 3.00 ± 0.56 | 18.70 ± 1.10 |
| Pb | < 0.001 | < 0.001 | < 0.001 |

N.D. : denotes not detectable concentration.

**Table 3.9.** Quantative changes in minerals and trace elements in muscle during ovarian maturation in *P. homarus* (Values expressed in 100g body weight) (values in parenthesis indicate 'P' values of tests of significance (Students 't') between ripe and immature and ripe and spent)

| Minerals/trace elements | Maturity stage | | |
|---|---|---|---|
| | Immature | Ripe | Spent |
| **Minerals** (mg) | | | |
| Na | 78.73 ± 1.11 (<0.0005) | 98.93 ± 3.36 | 93.92 ± 4.12 (<0.05) |
| K | 12.45 ± 1.76 (>0.05) | 121.94 ± 4.15 | 111.60 ± 4.90 (<0.05) |
| Ca | 20.04 ± 0.03 (<0.0005) | 26.17 ± 0.89 | 28.20 ± 1.24 (<0.05) |
| P | 108.75 ± 1.55 (<0.0005) | 158.32 ± 5.39 | 161.76 ± 7.10 (>0.05) |
| Mg | 6.82 ± 0.10 (<0.0005) | 12.35 ± 0.32 | 11.13 ± 0.49 (<0.05) |
| **Trace elements** ($\mu$g) | | | |
| Fe | 161.69 ± 2.31 (<0.0005) | 103.64 ± 3.53 | 133.10 ± 5.84 (<0.005) |
| Cu | 119.51 ± 1.71 (<0.0005) | 279.31 ± 9.50 | 237.26 ± 10.41 (<0.005) |
| Zn | 681.91 ± 9.70 (<0.0005) | 1183.35 ± 40.25 | 1020.31 ± 44.77 (<0.005) |
| Cd | 2.11 ± 0.03 (<0.0005) | 4.41 ± 0.15 | 1.50 ± 0.07 (<0.0005) |
| Co | 4.22 ± 0.06 (<0.0005) | N.D. | 3.01 ± 0.13 (<0.0005) |
| Mn | 5.62 ± 0.08 (<0.005) | 47.75 ± 1.62 | 3.06 ± 0.13 (<0.0005) |
| Cr | 7.03 ± 0.10 (<0.0005) | 22.79 ± 0.78 | 140.62 ± 6.17 (<0.0005) |
| Pb | <0.001 | <0.001 | <0.001 |

N.D. : denotes not detectable concentration.

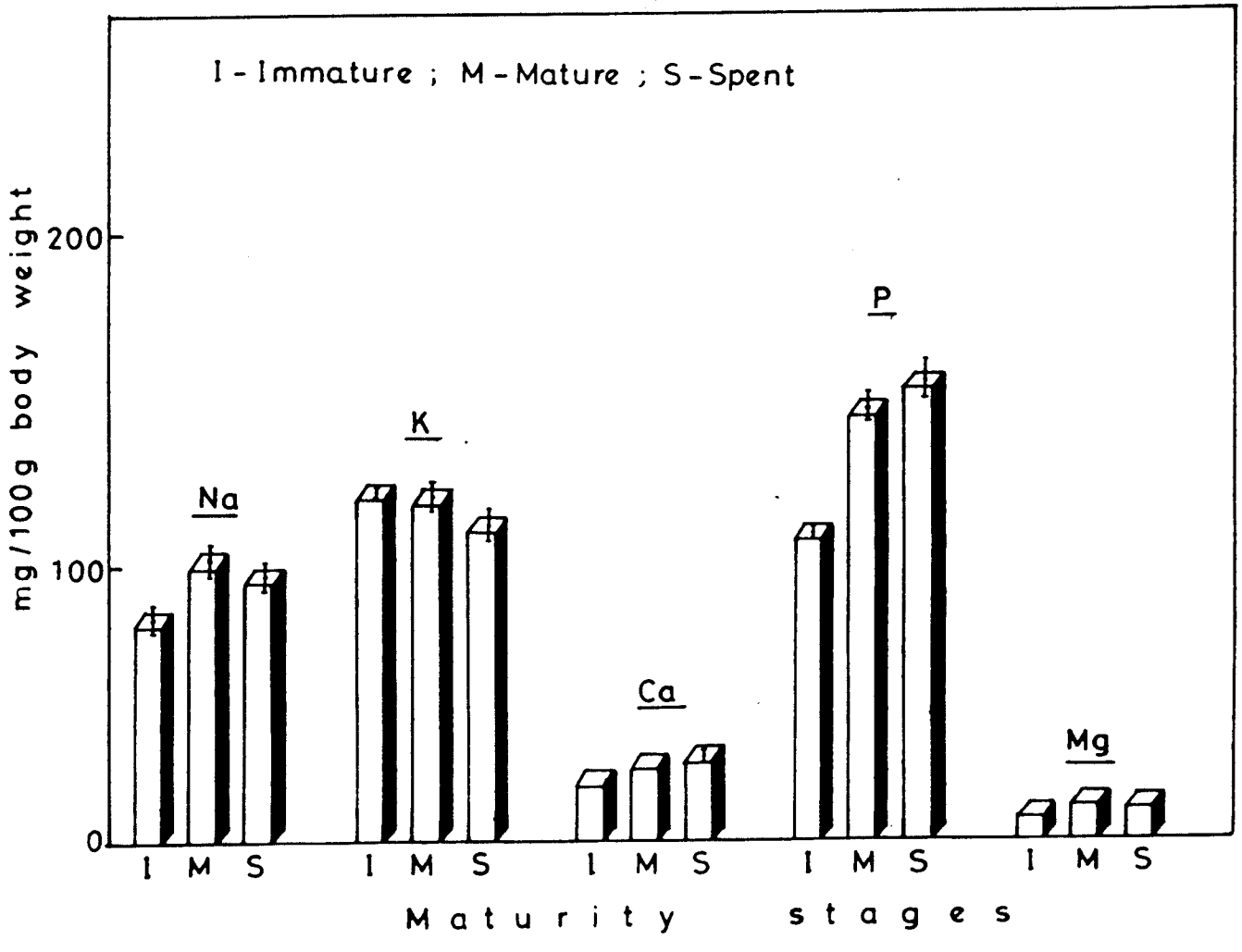


Fig.3.4 Quantitative changes in minerals in muscle during ovarian maturation in *P.homarus* (values expressed in 100g body weight).

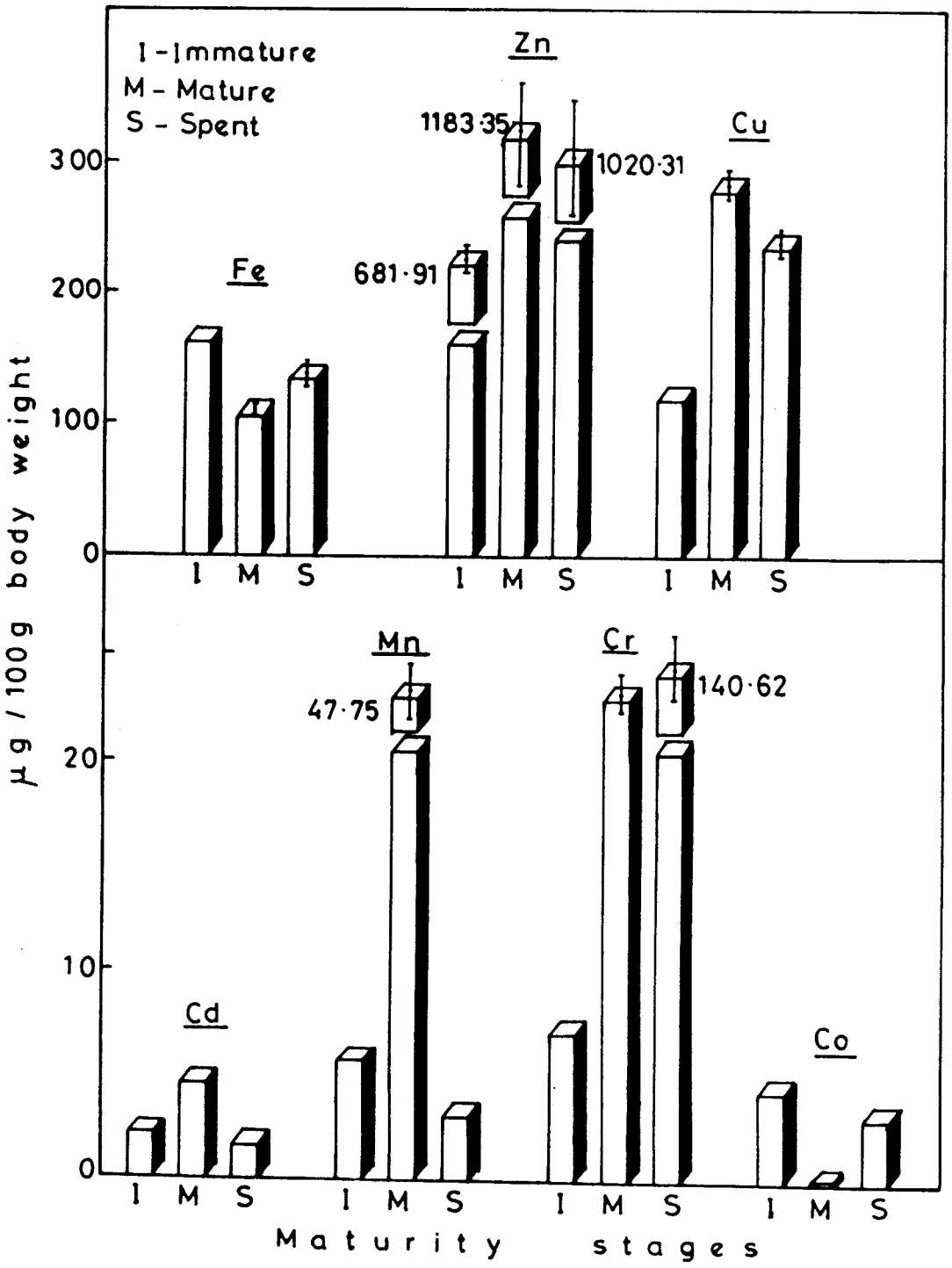


Fig.3.5 Quantitative changes in trace elements in muscle during ovarian maturation in *P.homarus* (values expressed in 100g body weight).

### 3.1.6 Mineral changes in hepatopancreas

Na, Ca and Mg concentrations increased while K and P declined with maturity in hepatopancreas (Table 3.10). Except for Mg and K whose concentrations showed an upward trend after spawning, concentrations of all the other three minerals were lowest in hepatopancreas of spent lobster.

With the exception of Co, all the trace elements analysed, accumulated with maturity with Cu and Cd, recording 10 fold and 17 fold increases respectively. As in the muscle Pb was below detectable level and Co declined to below detectable level in hepatopancreas at maturity and increased after spawning. All the other trace elements recorded a sharp reduction after spawning.

When expressed quantitively (Table 3.11 and Figs 3.6 and 3.7) the minerals and trace elements recorded a similar trend as their concentrations. Maximum rates of changes during maturation were recorded for Na among minerals and for Cu, Cd and Co among trace elements.

### 3.1.7 Mineral changes in ovary

All elements in immature and spent ovaries could not be detected due to extremely low quantity of ovary material that was available for analysis. In contrast to the conditions in muscle and hepatopancreas,

**Table 3.10.** Changes in minerals and trace elements composition in hepatopancreas during ovarian maturation in *P. homarus* (mg or μg/g dry weight).

| Minerals/trace elements | Maturity stage | | |
|---|---|---|---|
| | Immature | Ripe | Spent |
| **Minerals** (mg/g dry weight) | | | |
| Na | 11.66 ± 0.18 | 38.58 ± 1.83 | 21.75 ± 0.91 |
| K | 10.29 ± 0.16 | 6.45 ± 0.31 | 24.68 ± 0.43 |
| Ca | 3.45 ± 0.05 | 8.35 ± 0.40 | 4.69 ± 0.12 |
| P | 17.42 ± 0.31 | 10.55 ± 0.56 | 9.03 ± 0.21 |
| Mg | 1.19 ± 0.11 | 2.55 ± 0.19 | 3.49 ± 0.12 |
| **Trace elements** (μg/g dry weight) | | | |
| Fe | 72.61 ± 1.41 | 228.95 ± 10.89 | 147.61 ± 2.56 |
| Cu | 331.92 ± 5.21 | 3507.33 ± 167.03 | 929.31 ± 16.24 |
| Zn | 181.08 ± 2.84 | 673.71 ± 32.08 | 369.13 ± 6.46 |
| Cd | 3.63 ± 0.15 | 61.34 ± 2.92 | 11.59 ± 0.26 |
| Co | 1.89 ± 0.22 | N.D. | 1.87 ± 0.16 |
| Mn | 5.64 ± 0.16 | 9.23 ± 0.44 | 5.34 ± 0.52 |
| Cr | 0.39 ± 0.12 | 1.48 ± 0.16 | 0.59 ± 0.11 |
| Pb | < 0.001 | < 0.001 | < 0.001 |

N.D. : denotes not detectable concentration

**Table 3.11.** Quantitative changes in minerals and trace elements in hepatopancreas during ovarian maturation in *P. hamarus* (values expressed in 100 g body weight). (values in parenthesis indicate "P" values of tests of significance (students 't') between ripe and immature and ripe and spent).

| Minerals/trace elements | Maturity stage | | |
|---|---|---|---|
| | Immature | Ripe | Spent |
| **Minerals** (mg) | | | |
| Na | 15.04 ± 0.23 (< 0.0005) | 42.05 ± 1.93 | 15.07 ± 2.62 (< 0.0005) |
| K | 13.27 ± 0.21 (< 0.0005) | 7.04 ± 0.32 | 17.03 ± 2.96 (< 0.005) |
| Ca | 4.45 ± 0.07 (< 0.0005) | 9.10 ± 0.42 | 3.24 ± 0.56 (< 0.0005) |
| P | 22.47 ± 0.35 (< 0.0005) | 11.50 ± 0.53 | 6.23 ± 1.08 (< 0.0005) |
| Mg | 1.54 ± 0.02 (< 0.0005) | 2.78 ± 0.13 | 2.41 ± 0.42 (< 0.05) |
| **Trace elements** (μg) | | | |
| Fe | 93.67 ± 1.45 (< 0.0005) | 249.56 ± 11.45 | 101.85 ± 17.71 (< 0.0005) |
| Cu | 427.24 ± 6.62 (< 0.0005) | 3822.99 ± 175.36 | 641.43 ± 111.55 (< 0.0005) |
| Zn | 233.59 ± 36.21 (< 0.0005) | 734.34 ± 33.69 | 254.70 ± 44.29 (< 0.0005) |
| Cd | 4.68 ± 0.07 (< 0.0005) | 66.86 ± 3.07 | 8.00 ± 1.39 (< 0.0005) |
| Co | 2.44 ± 0.04 (< 0.0005) | N.D. | 1.29 ± 0.22 (< 0.0005) |
| Mn | 7.28 ± 0.11 (< 0.0005) | 10.66 ± 0.46 | 3.68 ± 0.64 (< 0.0005) |
| Cr | 0.52 ± 0.01 (< 0.005) | 1.61 ± 0.07 | 4.07 ± 0.71 (< 0.0005) |
| Pb | < 0.001 | < 0.001 | < 0.001 |

N.D. : denotes not detectable concentration

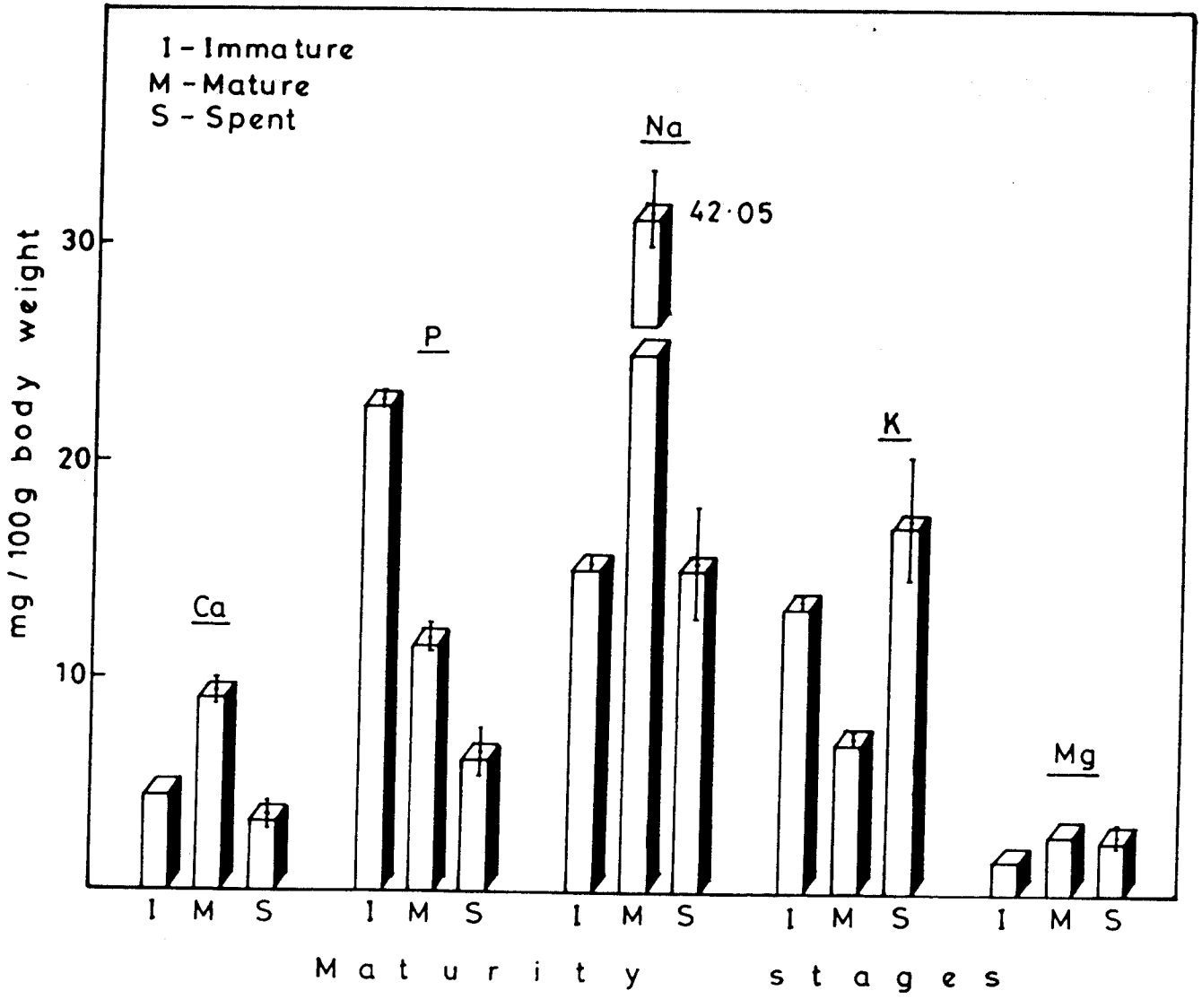


Fig.3.6 Quantitative changes in minerals in hepatopancreas during ovarian maturation in *P.homarus* (values expressed in 100g body weight).

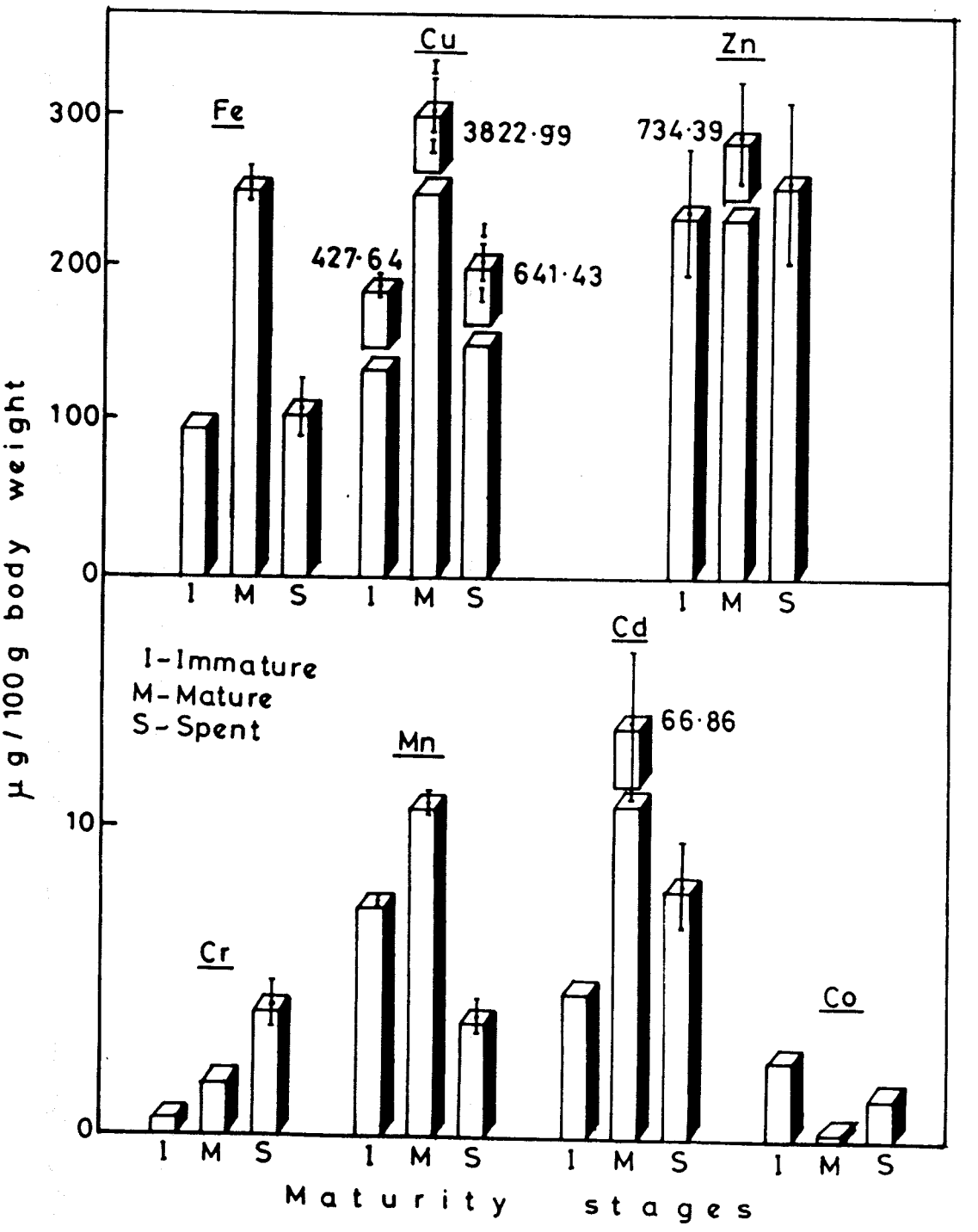


Fig.3.7 Quantitative changes in trace elements in hepatopancreas during ovarian maturation in *P.homarus* (values expresesd in 100g body weight).

concentrations of all minerals analysed declined at maturity and further after spawning (Table 3.12).

With the exception of Co, the concentration of which declined marginally at maturity, concentrations of all other trace elements (Zn could not be detected in immature ovary) increased in the mature ovary. Among the analysed trace elements in the spent ovary, Zn, Cu, and Co showed decline in concentration.

In contrast to the trends in muscle and hepatopancreas, eventhough the concentrations of many elements declined at maturity in the ovary, the actual quantities of minerals and trace elements increased significantly ($p = < 0.0005$) and declined in a similar way in the spent ovary. (Table 3.13 and Figs.3.8 and 3.9).

## 3.2 DISCUSSION

Maturation of ovary in *P. homarus* was accompanied by a marked increase in ovary mass, total lipid, protein, carbohydrate and ash. As in many other crustaceans (Pillay and Nair, 1971., Clarke, 1977, Jeckel *et al.*, 1989, Teshima *et al.*, 1989), no linear relationship was observed between ovary weight and body weight and the ovary weight increased with maturation irrespective of the size of lobster.

**Table 3.12.** Changes in minerals and trace elements composition in hepatopancreas during ovarain maturation in *P. homarus* (mg or μg/g dry weight).

| Minerals/trace elements (conc.) | Maturity stage | | |
|---|---|---|---|
| | Immature | Ripe | Spent |
| **Minerals** (mg/g dry weight) | | | |
| Na | 36.12 ± 1.21 | 22.72 ± 0.98 | 22.03 ± 1.62 |
| K | 19.48 ± 3.68 | 10.85 ± 3.44 | 9.42 ± 2.61 |
| Ca | 8.57 ± 0.26 | 6.40 ± 0.14 | 6.30 ± 0.17 |
| P | N.E. | 43.98 ± 3.58 | 34.70 ± 4.10 |
| Mg | 3.10 ± 0.42 | 2.09 ± 0.59 | 1.67 ± 0.48 |
| **Trace elements** (μg/g dry weight) | | | |
| Fe | 67.72 ± 5.86 | 105.52 ± 3.81 | N.E. |
| Cu | 23.20 ± 1.68 | 42.33 ± 2.53 | 12.16 ± 2.11 |
| Zn | N.E. | 264.32 ± 29.3 | 222.85 ± 20.61 |
| Cd | 0.49 ± 0.11 | 0.55 ± 0.06 | N.E. |
| Co | 1.57 ± 0.51 | 1.49 ± 0.73 | 0.97 ± 0.32 |
| Mn | 7.21 ± 1.11 | 11.20 ± 1.06 | N.E. |
| Cr | 1.70 ± 0.26 | 3.16 ± 0.57 | N.E. |
| Pb | < 0.001 | < 0.001 | < 0.001 |

N.E. : denotes not estimated.

**Table 3.13.** Quantitative changes in minerals and trace elements in ovary during maturation in *P. homarus* (values expressed in 100g body weight)

| Minerals/trace elements | Maturity stage | | |
|---|---|---|---|
| | Immature | Ripe | Spent |
| **Minerals** (mg) | | | |
| Na | 1.44 ± 0.36 | 57.03 ± 5.68 | 4.41 ± 1.10 |
| K | 0.78 ± 0.20 | 27.23 ± 2.71 | 1.88 ± 0.47 |
| Ca | 0.34 ± 0.09 | 16.06 ± 1.60 | 1.26 ± 0.31 |
| P | N.E. | 110.39 ± 11.0 | 5.06 ± 1.26 |
| mg | 0.12 ± 0.03 | 5.25 ± 0.52 | 0.33 ± 0.08 |
| **Trace elements** ($\mu$g) | | | |
| Fe | 2.71 ± 0.68 | 264.85 ± 26.38 | N.E. |
| Cu | 0.93 ± 0.23 | 106.25 ± 10.58 | 2.43 ± 0.61 |
| Zn | N.E. | 663.44 ± 28.12 | 44.57 ± 2.82 |
| Cd | 0.02 ± 0.01 | 1.38 ± 0.14 | N.E. |
| Co | 0.06 ± 0.02 | 3.74 ± 0.37 | 0.19 ± 0.05 |
| Mn | 0.29 ± 0.07 | 28.11 ± 2.79 | N.E. |
| Cr | 0.07 ± 0.02 | 7.93 ± 0.79 | N.E. |
| Pb | < 0.001 | < 0.001 | < 0.001 |

N.E. : denotes not estimated

'P' values of tests of significance (students 't') between ripe and immature and ripe and spent were < 0.0005 - for all estimated minerals and trace elements.

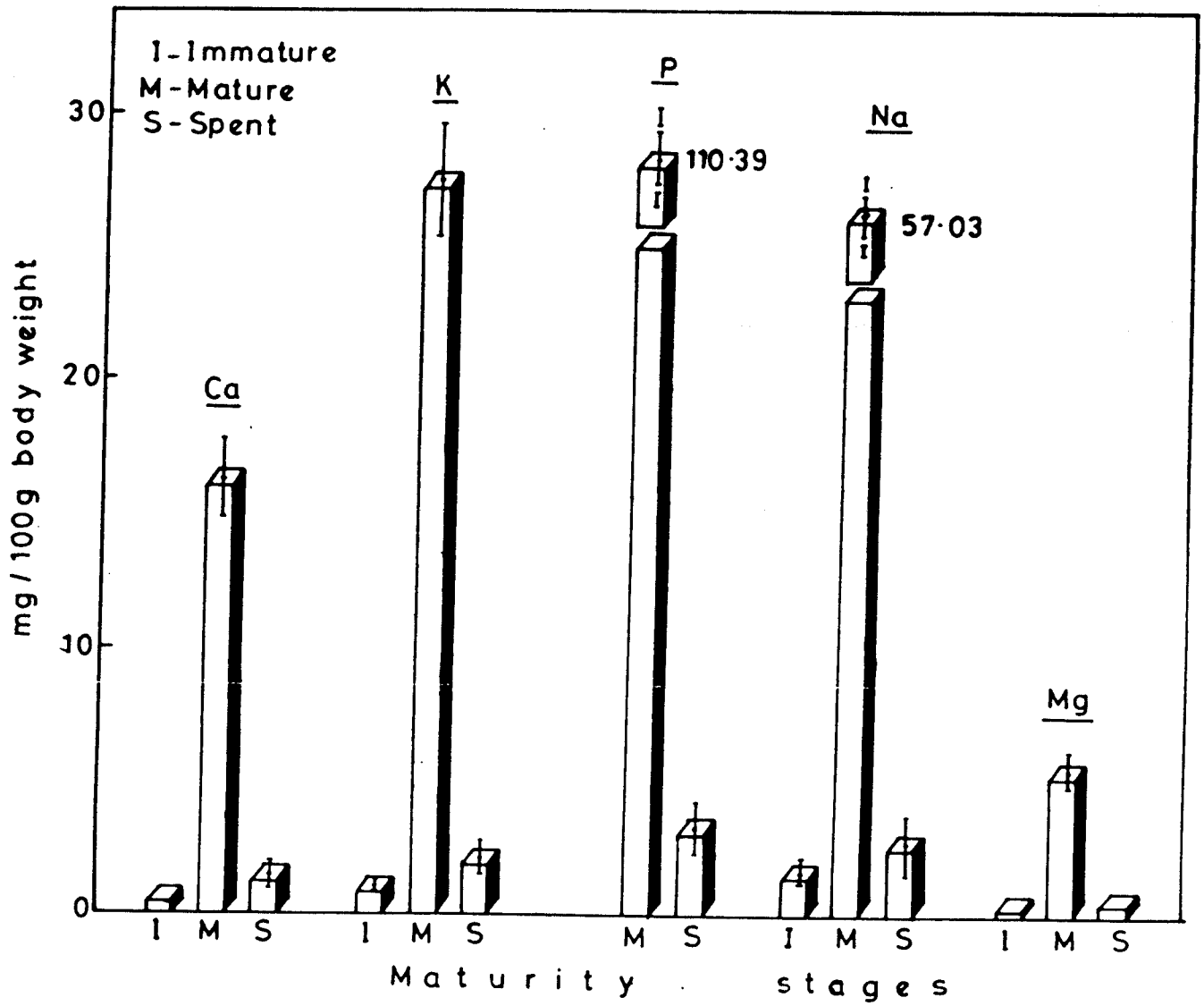


Fig.3.8 Quantitative changes in minerals in ovary during maturation in *P.homarus* (values expressed in 100g body weight).

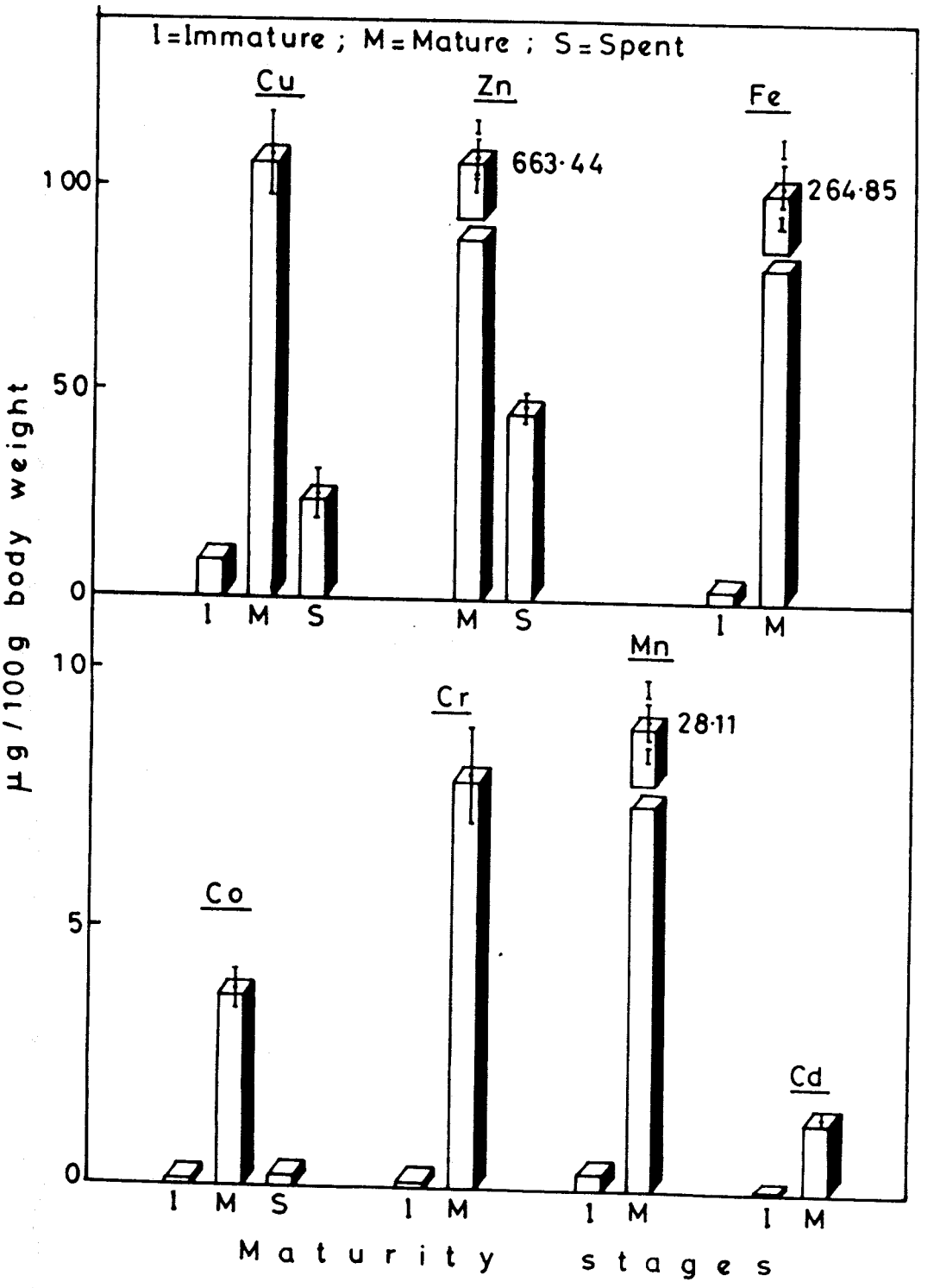


Fig.3.9 Quantitative changes in trace elements in ovary during maturation in *P.homarus* (values expressed in 100g body weight).

In *P. homarus*, concentration of water in the ovary declines significantly from 82.11 to 52.71% at maturity due to progressive addition of organic reserves in developing oocytes. When organic matter so accumulated are finally transferred to the spawned ova, concentration of water again increases to 72.91% in the spent ovary.

Ovary and hepatopancress are the main lipid storage organs in crustacea (Guary *et al.*, 1974). The total lipids increase in ovary during sexual maturation in *P. homarus* (present study), in *Panulirus polyphagus* (George and Patel, 1956), in the sand lobster *T. orientalis* (Rahman, 1989) and in many other crustaceans (Allen, 1972, Armitage *et al.*, 1972, Kanazawa, 1983, Asokan and George, 1984, Teshima *et al.* 1989). As the lipid reserves are ultimately transferred to the mature ova, spawning results in heavy depletion of lipids in the ovary.

Main component of crustacean yolk, however, is protein (lipovitellin - Adiyodi, 1985) and in *P. homarus* also quantity of protein increased 78 fold (from 0.02 g to 1.58 g) in the ripe ovary and formed 62.82% of the total dry matter. At the same time the lipid content increased only 52 fold (from 0.014g to 0.75g). Highest concentration of protein in the ovary (64.97%) was recorded in the spent condition, eventhough the actual quantity was considerably less. Thus, for a better understanding of accumulation and

utilization of organic reserves during maturation, values have to be quantified rather than being expressed in percentage.

Lipid in hepatopancreas of *P. homarus* declined significantly in mature lobsters with concomitant increase in the ovary. This trend has earlier been reported in many crustaceans leading to the conclusion that, in crustaceans with well defined hepatopancreas, energy reserves are stored in it and are apparently utilized for gonad production. Unlike lipid, maximum reductions in quantities of protein and carbohydrate were noticed between mature and spent stages, which might possibly indicate utilization of these reserves for increased metabolic activity associated with spawning.

In many fishes, both muscle and liver energy are depleted in the process of reproductive spending (Love, 1980) while crustaceans are not generally believed to mobilize energy from muscle for gonad production. In this study significant decrease was noticed in muscle lipid both at maturity and after spawning which perhaps, would have been utilized for reproductive process.

Accumulation or depletion of fresh weight, dry matter, organic matter, energy and ash in muscle, hepatopancreas and ovary during maturation summarized in Table 3.14 gives a clear picture of the extent of involvement of muscle and hepatopancreas in ovarian maturation at a glance. As described earlier, the role of muscle in the reproductive process in

**Table 3.14.** Summary of quantitative changes in organic and inorganic reserves in muscle, hepatopancreas and ovary during ovarian maturation in *P.homarus* (values expressed in 100g body weight)

| Parameters | Quantitative changes | | | | | |
|---|---|---|---|---|---|---|
| | Immature to ripe | | | Ripe to spent | | |
| | Muscle | Hepato-pancreas | Ovary | Muscle | Hepato-pancreas | Ovary |
| Wet weight (g) | +0.77 | -0.45 | +5.11 | +0.74 | -0.46 | -4.48 |
| Dry weight (g) | +0.28 | -0.18 | +2.47 | +0.17 | -0.29 | -2.31 |
| Water (g) | +0.45 | -0.27 | +2.64 | +0.57 | -0.16 | -2.15 |
| Protein (g) | +0.23 | -0.04 | +1.56 | +0.20 | -0.26 | -1.45 |
| Lipid (g) | -0.07 | -0.17 | +0.74 | -0.05 | -0.04 | -0.64 |
| Carbohydrate (g) | 0.00 | -0.03 | +0.09 | 0.00 | -0.06 | -0.09 |
| Ash (g) | +0.02 | -0.01 | +1.07 | 0.00 | 0.00 | -1.06 |
| Energy (KJ) | +2.64 | -7.07 | +67.23 | +2.73 | -5.65 | -61.20 |
| Na (mg) | +20.20 | +27.01 | +55.59 | -5.01 | -26.98 | -52.62 |
| K (mg) | -1.51 | -6.23 | +26.45 | -10.34 | +9.99 | -25.35 |
| Ca (mg) | +6.13 | +4.65 | +15.72 | +1.03 | -5.86 | -14.80 |
| P (mg) | +49.57 | -10.97 | - | +3.44 | -5.27 | -105.33 |
| Mg (mg) | +5.53 | +1.24 | +5.13 | -1.12 | -0.37 | -4.92 |
| Fe (μg) | -58.05 | +155.89 | +262.14 | +29.46 | -147.71 | - |
| Cu (μg) | +159.80 | +3395.75 | +105.32 | -42.05 | -3181.56 | -103.82 |
| Zn (μg) | +501.44 | +500.75 | - | -163.04 | -479.64 | -619.87 |
| Cd (μg) | +2.30 | +62.18 | +1.36 | -2.91 | -58.86 | - |
| Co (μg) | -4.22 | -2.44 | +3.68 | +3.01 | +1.29 | -3.55 |
| Mn (μg) | +42.13 | +3.48 | +27.82 | -44.69 | -6.98 | - |
| Cr (μg) | +15.76 | +1.09 | +7.89 | +117.83 | +2.46 | - |

*P. homarus* appears to be restricted to supplying small quantity of lipid. In contrast, all organic and inorganic reserves in the hepatopancreas declined at maturity and still further after spawning. The most significant reduction (42.5%) at maturity in hepatopancreas was that of lipid while marked decline (40.62%) in protein quantity was noticed at spawning. Total carbohydrates also reduced at maturity and at spawning, but energetically its contribution is negligible. Likewise, 7.07 KJ (21.42% of total) of hepatopancreatic energy was spent for maturation and another 5.6 KJ (21.72% of total) at spawning.

These results suggest the important role of hepatopancreas in storage and mobilization of energy during ovarian maturation in *P.homarus*. But to what extent the hepatopancreatic reserves contribute to the total reproductive output have to be evaluated in the right perspective. The decline in quantites of lipid, protein and carbohydrate in hepatopancreas amounts only to 22.92%, 0.67% and 33.33% respectively, of the total amount of these organic materials deposited in mature ovary. This important point is glaringly omitted in most of the studies which describe mobilization of hepatopancreatic reserves during maturation in many crustaceans. The bulk of organic reserves deposited in the ovary during maturation should, therefore, come through transformation of ingested food either directly from the gut, through the haemolymph as is the case in echinoderms (Giese, 1959) or through the mediation of tissues like hepatopancreas which is the most

important site of protein synthesis in crustaceans (Chang and O'Conner, 1983).

The importance of minerals and trace elements in maturation process has not been studied in crustaceans. Most of the investigations on uptake of elements from water have been intended to know osmotic ion regulation in varying salinities, toxicity to heavy metal concentration, nutritional requirements for growth and bioaccumulation in soft edible tissues (Gallaghar, *et al.*, 1978, McKenney and Neff, 1979., Love, 1980, Eisler, 1981, Moore, 1981, Knox *et al.*, 1984., Bjerregaard and Vislie, 1986., Bryan *et al.* 1986).

Trace elements are generally required in minute amounts, with the possible exception of Mg and Fe and are concerned in specific physiological activities (Hoar, 1975). In *P.homarus*, Mg is present in higher concentration (mg/g dry weight) in muscle, hepatopancreas and ovary justifying its classification under major elements or minerals in this study. Concentrations of Cu, Zn and Fe also were high, especially at mature stage while all other elements analysed were present in minute quantities. Pb was not even detectable (<0.001 $\mu$g/g dry weight) which might possibly be due to the exclusion of exoskeleton in this study, as exoskeletion is reported to sequester most of the Pb in crustacea to be expelled at the time of moulting (Eisler, 1981).

With the exception of K, the quantity of which reduced at maturity in muscle and hepatopancreas and P which very significantly reduced at maturity in hepatopancreas, all other minerals (Na, Ca and Mg) increased with maturity in muscle, hepatopancreas and ovary (Table 3.14). Marked depletion of all these elements in the spent ovary indicates that these may have been transferred to the ova just before its release. The rate of reduction in the quantities of minerals is more pronounced in hepatopancreas than muscle, probably indicating its importance in tranfer of minerals during maturation to the ovary.

A similar trend of accumulation during maturity in muscle, hepatopancreas and ovary with the exception of Fe and Co in muscle and Co in hepatopancreas recorded for all trace elements analysed (Table 3.14). High accumulation (794.81%) of Cu in hepatopancreas at maturity, turning the ash blue, is noteworthy. However, maximum rate of increase (1328.63%) was noticed in the quantities of Cd, which is generally considered as non-essential and toxic (Fleischer *et al.*, 1974). Interestingly, maximum rates of loss after spawning, 83.22% and 88.03%, in the hepatopancreas were also recorded for Cu and Cd respectively. As in the case of minerals, rates of loss of trace elements due to spawning were extremely high in hepatopancreas. Hepatopancreas in crustaceans has been reported to sequester and accumulate many metals like Cu, Cd, Zn (Bjerregaard and Vislie, 1986) in bound and inactive form, possibly, as a safeguard to prevent them being toxic

to the animal. The lobsters used in this investigation were collected, within a short period, off Kovalam, which appears to be a comparatively unpolluted area. Hence the increase or decrease in elemental composition in this study might be related to maturation process rather than passive accumulation from the environment. Marked changes in elemental composition in hepatopancreas and to a lesser extent in the muscle point to the possible involvement of these tissues in making up the inorganic composition of the ova.

The extraordinary build up of Cu and Cd in hepatopancreas of *P. homarus* and the decline of more than 80% at spawning require further scrutiny. Crustaceans are reported to expel excess metals mainly through exuvia and to a limited extent through egg production (Davis, 1978). In *P. homarus*, the drastic decline in Cu and Cd could not be traced either to moulting or to egg production, since moulting was not involved and the extent of loss in hepatopancreas could not be accounted for the quantity of these elements in the spawned ova. It will be interesting to study further, how exactly these loses can be accounted for.

Not many studies have been recorded on the requirements of trace elements during maturation, which might vary with species and stages of maturation (Love, 1980). In the fish *Rutilus rutilus*, Ilzinia (1968) noted a somewhat variable accumulation of Mn, Fe, Cu, and Zn in various tissues,

particularly ovary, with maturation. After spawning, concentration in the ovary reduced and interestingly, levels of all the elements decreased in other tissues like muscle, liver, bone and scales as well. This is analogous to the findings now reported in *P. homarus*. The only previous study of metal accumulation in a crustacean during maturation seems to be the one reported in *Metapenaeus dobsoni* (Thangaraj, 1985) in which the whole body concentration of Cu, Cd and Zn increased at maturity and declined after spawning.

Marine crustaceans are capable of selectively regulating metalic ion concentrations in the body fluids and the ability to concentrate elements varies with species. A concentration factor of 4000 has been reported for Co in spiny lobsters (Ichikawa, 1964) and 1800 for Fe in lobsters (Eisler, 1981). In addition, many of the trace elements like Zn are obtained through the diet. It will be of interest to evaluate, in the light of this investigation, the role of individual trace elements in vitellogenesis so that effective broodstock feed formulation to satisfy mineral demand, in addition to protein and energy, can be made for cultivable species of crustacea for mass production of seeds.

# 4. ENERGY UTILIZATION AND UPTAKE OF MINERALS AND TRACE ELEMENTS DURING EGG DEVELOPMENT IN THE SPINY LOBSTER, PANULIRUS HOMARUS

Inspite of great variation in the stage of development at which a crustacean emerges from the egg, the embryonic development displays a remarkable underlying unity in all species (Green, 1971, Anderson, 1982). Many emerge as the least developed crustacean larva, the nauplius (small eggs, Penaeidae) which is a non-feeding, unsegmented larva with three pairs of appendages and some hatch as miniature of the parents (large eggs, Branchiopoda). Even in those crustaceans that emerge at an advanced stage from the egg, a fundamentally similar fate map of blastula or blastoderm is displayed and the nauplius stage exist in an embryonised form. Thus, evidence of embryology points towards a monophyletic origin of crustacea, unrelated to other arthropods and annelids, but evolved independently from unknown, unsegmented worm like ancestors belonging to the spiral cleavage assemblage (Dowson and Barnes, 1966, Green, 1971, Anderson, 1982).

Colour, size and shape of crustacean eggs show great variations and the developmental period is generally longer in species with large eggs (Herring, 1974). Since overall cell size reflects the surface to volume restrictions on gas exchange and therefore its metabolic rate, the larger the egg, lower the metabolic rate and longer the development period (McLaren, 1956). Apart from size of the egg, its genetic make up, quality and quantity

of yolk, ambient temperature and availability of food for the emerging young ones influence the duration of development.

In decapods, volume of eggs increases during development and the rate of increase, attributed to osmotic uptake of water either through a steady osmotic swelling of the egg or the swelling of embryo itself (Davis, 1964a, b, 1965a, b, 1966), is considered slower in the eggs of species with a longer developmental period than in those which develop rapidly (Wear, 1974). Water uptake is initially slow but rapid towards the middle and final stages of development associated with a period of marked cellular differentiation and metabolic activity (Barnes, 1965, Herring, 1974).

In crustacean eggs, protein and lipid contribute maximum amount of energy expended for development with little contribution from carbohydrate. Upon oxidation, fat releases larger quantities of metabolic water (1g fat ≡ 1.07g water; 1g carbohydrate ≡ 0.56g water and 1g protein ≡ 0.41g water - Baldwin, 1964) and unlike protein, oxidation of fat and carbohydrate does not result in ammonia production, the removal of which requires water and energy. These properties of fat makes it more desirable to be used for development in marine crustaceans than protein (Pandian, 1970c). In the Daphnid, *Simocephalus vetulus*, Hoshi (1950a, b) had observed that lipid utilization is greater early in development. In most other crustaceans, rate of decrease of lipid is more rapid towards the end of development (Urbani, 1952, Crisp, 1984, Lucas and Crisp, 1987). Bellini and

Lavizzari (1958) had observed that lipase activity is more just before hatching in *Artemia salina*.

Unlike the oyster, *Crassostrea gigas*, eggs of which are able to absorb dissolved organic substances through microvilli (Manahan and Crisp, 1982), developing eggs of crustacea, like that of arthropods, do not take up any organic nutrients from the surroundings and calcualtion of energy budget is simple (Lucas and Crisp, 1987). Yolk utilization efficiency in crustacea is around 60%. This value is comparable to those reported for different groups of vertebrates like fishes (35% in *Pleuronectes platessa*, Ryland and Nichols, 1967 to 77% in the pacific salmon, *Sardinops caerulea*, Laskar, 1962), turtle (71.97% in *Lepidochelys olivacea*, Silas *et al.*, 1984a) and birds (56% in *Gallus*, Brody, 1945) and is closer to the best possible values of 60 to 70% given by Calow (1977) for embryonic and proliferative tissues. Environmental factors like temperature and salinity are known to alter significantly the yolk utilization efficiency in some crustaceans (Barnes, 1965., von Hentig, 1971). Even in the same clutch, when larvae are released in batches over a period of 5-7 days, as in *Homarus americanus* and *Homarus gammarus* (Pandian, 1970 a,b) energetic efficiency is reduced in larvae that are released on subsequent days than those released on the first hatching night and contain less energy affecting their survival chances.

Eventhough the crustacean eggs are closed to penetration of organic matter from the medium, they do absorb water and salt during development. While water absorption is well documented, salt absorption, especially uptake

of individual elements, is seldom studied in crustacean eggs. A review of literature on yolk utilization revealed that no study has yet been reported in spiny lobsters and this work has been undertaken to fill this lacuna.

## 4.1 RESULTS

Almost all observations reported on yolk utilization in crustacean eggs begin with just spawned or fertilized egg which are carried in the mantle cavity of the female (barnacles) or brood pouches (Daphnids) or cemented to ovigerous setae of the pleopods (most decapods). The only exception to this pattern seems to be the work of Lucas and Crisp (1987) on barnacles, where they have compared organic composition of the ovarian tissue in the last stages of maturation to early stages of development of eggs. Due to low recovery of organic matter in their estimations, these authors did not find any significant variation between chemical composition of ovarian tissue and early stages of egg. Further, eggs of barnacles are incubated in the mantle cavity and not exposed to the external medium as in the case of many decapods.

In this study, both ripe ova just before spawning and the egg just after spawning have been analysed and found to differ significantly in diameter, water content, organic matter and minerals and trace element composition. For comparison with earlier investigations, development stages in *P. homarus* have been fixed starting from spawned/fertilized egg and all

statistical relationships were calculated beginning with just spawned egg (stage 1).

### 4.1.1 Development stages in *P. homarus* egg

Table 4.1 and Plate 1 give distinguishing features of different development stages fixed for *P. homarus*, duration of total period of development and duration in each stage. Total duration of development was 22-27 days with 24.5 as average at 27.03 ± 1.02°C. Development proceeds at a slower rate until the formation of eye, (stage 4) which takes 17.5 days and speeds up afterwards with phyllosoma release in another 7 days.

### 4.1.2 Egg and yolk diameter and volume in different stages

Egg diameter increased progressively from 0.478mm in stage 1 (just spawned egg) to 0.602mm in stage 6 (last stage in egg development) and the egg volume also followed the same pattern i.e. 0.0561mm$^3$ in stage 1 to 0.1134mm$^3$ in stage 6 (Table 4.2). From ripe ova (stage 0) to stage 1 significant increase was noticed in both egg diameter (0.42mm to 0.478mm) and egg volume (0.0389mm$^3$ to 0.0561 mm$^3$). Stages of eggs (expressed in days) showed positive correlation with egg diameter (r=0.966) and egg volume (r=0.946) and the relationships are as follows: (Fig.4.1)

Egg stage (X) vs Egg diameter (Y)

Y = 0.482 + 0.0045 Xmm

Egg stage (X) vs Egg volume (Y)

Y = 0.0578 + 0.002 X mm$^3$

**Table 4.1** : Description of development stages in *P.homarus* egg and duration of development

| Development stage | Distinguishing features | Days to reach the stage |
|---|---|---|
| 0 | Ripe ova in the ovary about to be ovulated (little ovarian tissue also present). Fresh sperm mass deposition in the sternum, ova dia : 0.42 mm | 0 |
| 1 | Just spawned egg. egg filled with yolk, pale orange. First cleavages seen in some eggs. Dia. : 0.48 mm | 0 |
| 2 | Bright organe. A small yolk free region is seen in the animal pole. egg dia : 0.50 mm | 2-3 |
| 3 | Bright orange, tissue cap formation seen. proliferation of cells seen in many places of egg. Dia : 0.54 mm | 10-12 |
| 4 | Dark orange turning brick red, eye spot well formed. Dia : 0.54 mm | 16-19 |
| 5 | Brick red turning reddish brown. Eyes well developed; appendages clearly seen. Dia.: 0.55 mm | 19-23 |
| 6 | Reddish brown. Fully developed naupliosoma inside. Very little or no yolk present, egg membranes breakes when handled. Dia : 0.60 mm | 21-26 |
| 7 | Just released phyllosoma larva | 22-27 |

**Table 4.2.** Egg and yolk diameter and volume in different stages of development in *P.homarus* (values in parenthesis give percentage increase or decrease with stage 1 kept as 100)

| Development stage | Diameter (mm) | | Volume ($mm^3$) | |
|---|---|---|---|---|
| | Egg | Yolk | Egg | Yolk |
| 0 (Ova) | 0.42 ± 0.006 (88.0) | 0.42 ± 0.006 (90.0) | 0.03877 ± 0.016 (69.2) | 0.03897 ± 0.001 (74.2) |
| 1 | 0.47 ± 0.007 (100) | 0.467 ± 0.007 (100) | 0.05607 ± 0.001 (100) | 0.05226 ± 0.005 (100) |
| 2 | 0.496 ± 0.007 (103.9) | 0.456 ± 0.006 (97.8) | 0.06295 ± 0.003 (112.3) | 0.04907 ± 0.002 (93.9) |
| 3 | 0.543 ± 0.004 (113.7) | 0.460 ± 0.004 (98.6) | 0.08727 ± 0.004 (155.6) | 0.05094 ± 0.003 (97.5) |
| 4 | 0.552 ± 0.006 (115.6) | 0.416 ± 0.002 (89.2) | 0.08810 ± 0.005 (157.1) | 0.03771 ± 0.003 (72.2) |
| 5 | 0.561 ± 0.001 (117.5) | 0.349 ± 0.001 (74.8) | 0.09123 ± 0.002 (162.7) | 0.02179 ± 0.001 (41.7) |
| 6 | 0.602 ± 0.002 (126.1) | 0.01 ± 0.000 (2.1) | 0.11341 ± 0.002 (202.3) | 0.00148 ± 0.000 (2.8) |

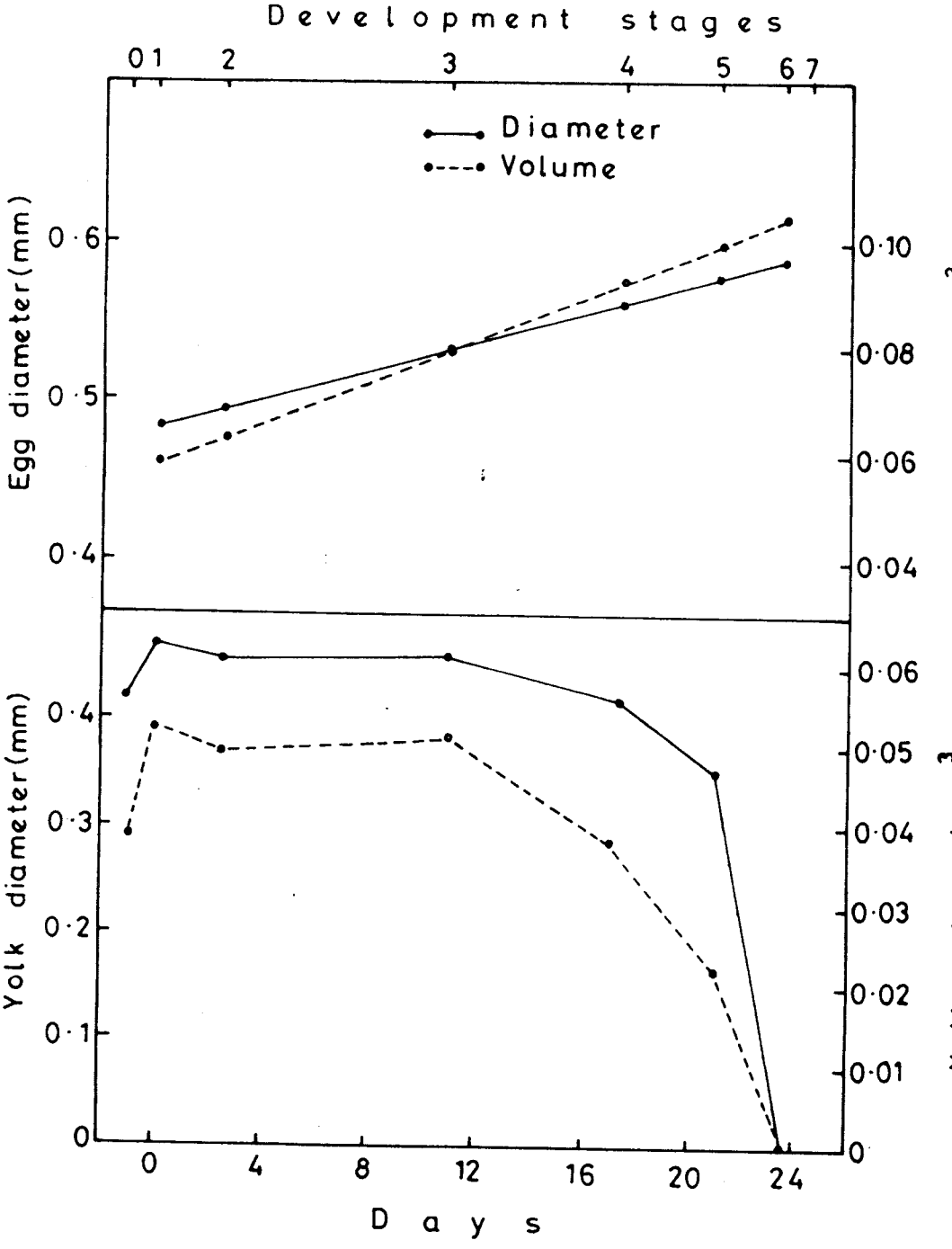


Fig.4.1 Changes in diameter and volume of egg and yolk during development in *P.homarus*

Yolk diameter and volume increased considerably from 0.42mm to 0.467mm and 0.0388mm$^3$ to 0.0523mm$^3$ respectively from ripe ova to stage 1 (Table 4.2). Thereafter, both yolk diameter and volume decreased gradually up to stage 5 and then drastically to almost zero in stage 6. No statistical relationship could be derived for decrease in yolk diameter ($r=0.704$) and volume ($r=0.405$).

To compare the increase or decrease in diameter and volume of both egg and yolk between development stages, the values are expressed in percentage (Table 4.2) with stage 1 kept as 100. Maximum increase in egg diameter and volume were between stage 2 and 3 (9.84% and 43.37% respectively). Between stage 5 and 6, egg diameter and volume again went up by 8.58% and 39.56% respectively. Decrease in yolk volume was slow up to stage 3 and drastic thereafter; 25.31%, 30.46% and 41.7% decline between stage 3 and 4, 4 and 5 and 5 and 6 respectively. Egg and yolk diameter and volume were significantly less in ripe ova, suggesting that ova absorb water rapidly as they are released to outside.

### 4.1.3 Biochemical changes during development

Changes in proximate composition and energy content of eggs during development are given in Table 4.3. Just spawned egg has 62.86% protein, 30.48% lipid, 3.62% carbohydrate and 2.97% ash and no significant difference was noticed in concentrations of these components between ripe ova and egg except for ash concentration of which reduced from 5.68% in the ova to 2.97% in the egg. The most significant change ($p=<0.0005$) was in the

**Table 4.3.** Biochemical changes during egg development in *P. homarus* (Protein, lipid, carbohydrate and ash are expressed as % in dry weight)

| Stage | Water (%) | Dry matter (%) | Protein | Lipid | Carbo-hydrate | Ash | Energy (KJ/g) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Measured | Calculated |
| 0 (ova) | 51.70 ± 2.06 | 48.30 ± 2.06 | 62.82 ± 6.10 | 28.10 ± 3.74 | 3.30 ± 0.47 | 5.68 ± 1.30 | 28.58 ± 2.16 | 26.01 ± 2.99 |
| 1 | 67.05 ± 5.58 | 32.95 ± 5.58 | 61.26 ± 3.08 | 30.48 ± 0.75 | 3.62 ± 0.59 | 2.97 ± 1.11 | 28.59 ± 2.75 | 27.05 ± 1.13 |
| 2 | 70.95 ± 1.36 | 29.05 ± 1.36 | 62.06 ± 3.21 | 30.99 ± 2.29 | 2.56 ± 0.38 | 3.80 ± 0.28 | 28.33 ± 1.58 | 27.73 ± 1.73 |
| 3 | 80.32 ± 2.46 | 19.68 ± 2.46 | 60.12 ± 2.31 | 31.07 ± 1.75 | 1.32 ± 0.15 | 4.56 ± 0.25 | 28.44 ± 2.68 | 26.58 ± 1.26 |
| 4 | 80.10 ± 2.12 | 19.90 ± 2.12 | 62.29 ± 8.53 | 26.03 ± 2.04 | 1.89 ± 0.79 | 7.11 ± 0.25 | 28.46 ± 1.84 | 25.22 ± 2.95 |
| 5 | 80.19 ± 0.36 | 19.91 ± 0.36 | 62.52 ± 3.92 | 23.14 ± 0.90 | 2.45 ± 0.61 | 8.24 ± 0.88 | 25.70 ± 1.53 | 24.23 ± 1.38 |
| 6 | 82.59 ± 1.49 | 17.41 ± 2.30 | 63.42 ± 4.82 | 16.53 ± 2.62 | 2.99 ± 0.13 | 10.66 ± 3.03 | 25.44 ± 1.45 | 21.94 ± 2.18 |
| 7 (Phyllo-soma) | 80.89 ± 1.28 | 19.11 ± 1.28 | 46.16 ± 0.47 | 13.03 ± 2.78 | 2.05 ± 0.07 | 24.68 ± 0.06 | 20.76 ± 0.71 | 16.33 ± 1.21 |

percentage of water, which went up from 51.70 to 67.05 in the egg resulting in a proportionate decrease in dry matter.

Concentration of protein in the egg did not differ much until stage 6, but decreased significantly from 63.42% in stage 6 to 46.26% in phyllosoma larva (stage 7). Lipid concentration marginally increased in early stages (up to stage 3) but thence declined gradually from 31.03% in stage 3 to 16.53% in stage 6 and 13.03% in the hatched larva. Total carbohydrate concentration, on the other hand, declined from 3.62% in stage 1 to 1.32% in stage 3 and got elevated gradually to 2.99% in stage 6 and reduced again to 2.05% in the larva. A progressive increase was observed in ash content from 2.97% in stage 1 to 10.66% in stage 6 and a drastic increase to 24.68% in phyllosoma.

Energy content of the egg was both measured and calculated, the measured energy being always higher in all stages. Energy content did not vary between ripe ova and stage 1 egg (28.58 kJ/g dry weight) and remained with little change until stage 4 and then declined in stages 5 and 6 and was minimum in the phyllosoma. (20.76 KJ/g dry weight).

### 4.1.4 Weight changes in a single egg

Method of calculating and expressing the results always pose problem in biochemical embryology, especially when different development stages are to be compared. The best way to overcome this difficulty is to express the changes on a per egg basis for each stage of development

(Barnes, 1965). Quantitative changes of wet and dry weights calculated on a per egg basis in *P.homarus* are given in Table 4.4. Wet weight of egg was significantly higher than that of ripe ova but dry weight was less. No linear relationship was obtained between egg stages and wet and dry weights (Fig.4.2).

### 4.1.5 Quantitative biochemical changes in a single egg

Quantitative biochemical changes in a single egg during development are given in Table 4.5 and Fig. 4.3 and the relative changes, with stage 1 kept as 100, in Table 4.6 and Fig. 4.4. Quantity of water increased by 89% in stage 5 and then declined, but even in phyllosoma larva, quantitiy of water was 32.81% more than that of the egg in stage 1, indicating that all water absorbed during development had not been completely utilized for embryogenesis. Up to stage 5, 5.1% protein was used, but between stage 5 and 6 and 6 and phyllosoma the reductions were marked, 16.23% and 30.41% respectively. Similarly up to stage 3, 9.4% lipid was utilized and 19.86% between stage 3 and 5. Thereafter the reduction was heavy, 30.10% between stages 5 and 6 and 13.16% between stage 6 and phyllosoma. Energy utilization also showed similar trend but utilization of carbohydrate followed a different pattern. 66.54% carbohydrate was used up to stage 3 but thereafter 28.94% increase in quantity was observed up to stage 6 and between stage 6 and phyllosoma 25.41% was utilized.

Quantitative changes and conversion efficiencies for dry matter, protein, lipid, carbohydrate and total energy are given in Table 4.7 and

Table 4.4. Weight changes in a single egg during development in *P. homarus* (values in parenthesis give % increase/decrease with stage 1 kept as 100).

| Weight | Development stages | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 (ova) | 1 | 2 | 3 | 4 | 5 | 6 | 7 (Phyllo-soma) |
| Wet weight (μg) | 76.4 ± 20.0 (80.1) | 95.4 ± 20.0 (100.0) | 98.0 ± 20.0 (102.7) | 140.9 ± 10.0 (148.9) | 141.7 ± 10.0 (148.5) | 146.7 ± 10.0 (159.1) | 134.8 ± 10.0 (141.3) | 105.1 ± 3.0 (110.2) |
| Dry weight (μg) | 36.9 ± 8.0 (117.5) | 31.4 ± 9.0 (100.0) | 28.5 ± 2.0 (90.8) | 27.9 ± 1.0 (88.9) | 28.2 ± 1.0 (89.8) | 29.2 ± 1.0 (98.1) | 23.5 ± 1.0 (74.8) | 20.1 ± 1.0 (64.0) |

Table 4.5. Quantitative changes in proximate composition and energy content in a single egg during development in *P. homarus*

| Stage | Wet wt. of egg | Proximate composition (quantities in μg) | | | | | Energy (J) | |
|---|---|---|---|---|---|---|---|---|
| | | Water | Protein | Lipid | Carbo-hydrate | Ash | Measured | Calculated |
| 0 (ova) | 76.4 ± 20.0 | 39.50 ± 10.31 | 23.18 ± 2.24 | 10.37 ± 1.38 | 1.22 ± 0.17 | 2.10 ± 0.18 | 1.05 ± 0.08 | 0.96 ± 0.11 |
| 1 | 95.4 ± 20.0 | 64.00 ± 13.42 | 19.23 ± 1.18 | 9.57 ± 0.24 | 1.14 ± 0.18 | 0.93 ± 0.35 | 0.90 ± 0.09 | 0.83 ± 0.04 |
| 2 | 98.0 ± 20.0 | 69.50 ± 13.91 | 17.69 ± 0.91 | 8.83 ± 0.65 | 0.73 ± 0.11 | 1.08 ± 0.22 | 0.81 ± 0.05 | 0.76 ± 0.05 |
| 3 | 140.9 ± 10.0 | 114.11 ± 8.03 | 16.77 ± 0.64 | 8.67 ± 0.76 | 0.37 ± 0.23 | 1.27 ± 0.07 | 0.79 ± 0.07 | 0.79 ± 0.05 |
| 4 | 141.7 ± 10.0 | 113.50 ± 1.60 | 17.57 ± 2.42 | 7.34 ± 0.69 | 0.53 ± 0.27 | 2.01 ± 0.08 | 0.96 ± 0.06 | 0.70 ± 0.09 |
| 5 | 146.7 ± 10.0 | 121.00 ± 0.80 | 18.25 ± 1.40 | 6.78 ± 0.26 | 0.71 ± 0.04 | 2.41 ± 0.27 | 0.79 ± 0.05 | 0.69 ± 0.06 |
| 6 | 134.8 ± 10.0 | 111.31 ± 11.13 | 15.13 ± 1.13 | 3.88 ± 0.60 | 0.70 ± 0.03 | 2.51 ± 0.61 | 0.51 ± 0.04 | 0.51 ± 0.05 |
| 7 (Phyllo-soma) | 105.1 ± 3.0 | 85.00 ± 0.02 | 9.28 0.59 | 2.62 ± 0.56 | 0.41 ± 0.01 | 4.96 ± 0.01 | 0.42 ± 0.01 | 0.32 ± 0.03 |

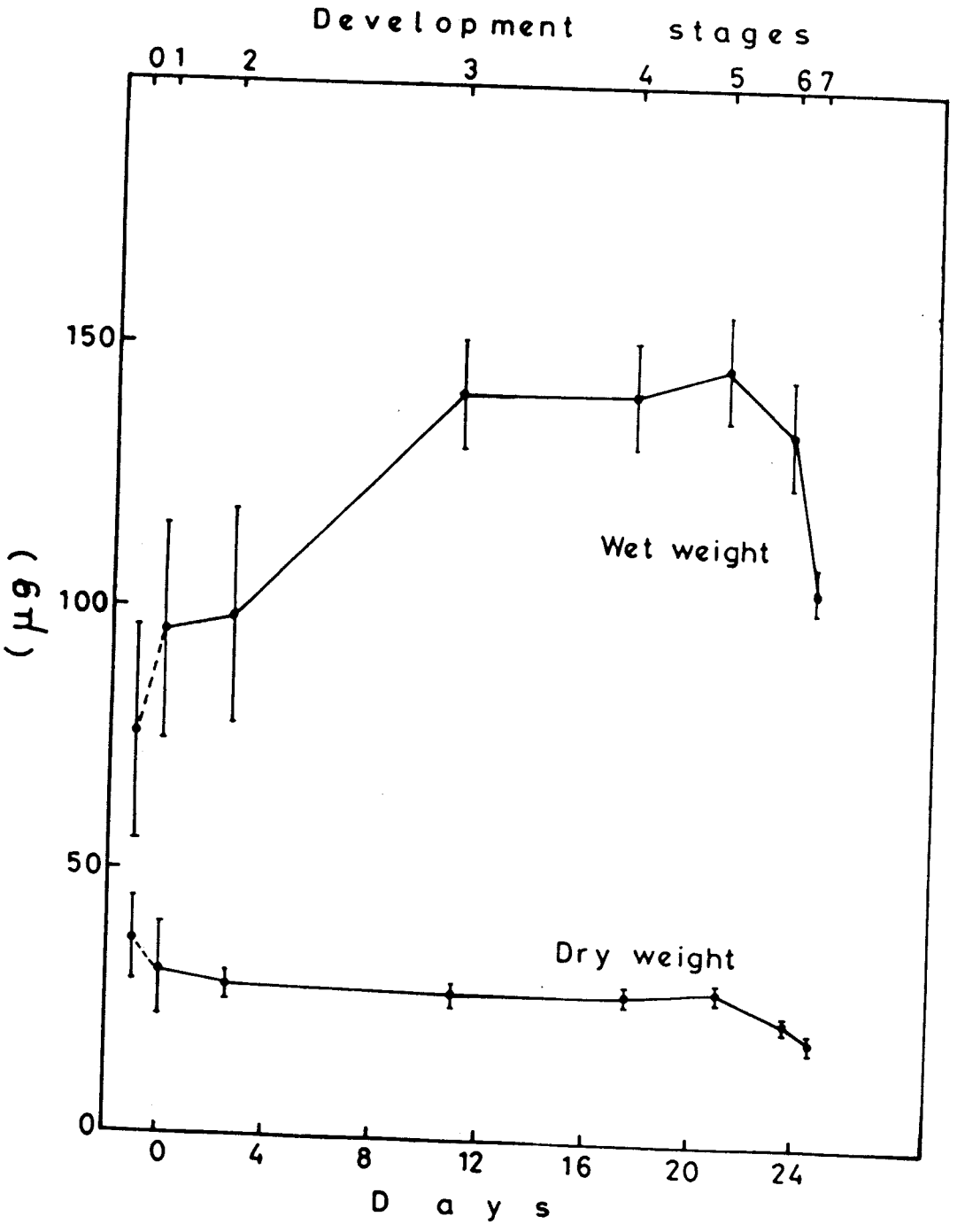


Fig.4.2 Changes in wet and dry weights of *P.homarus* egg during development.

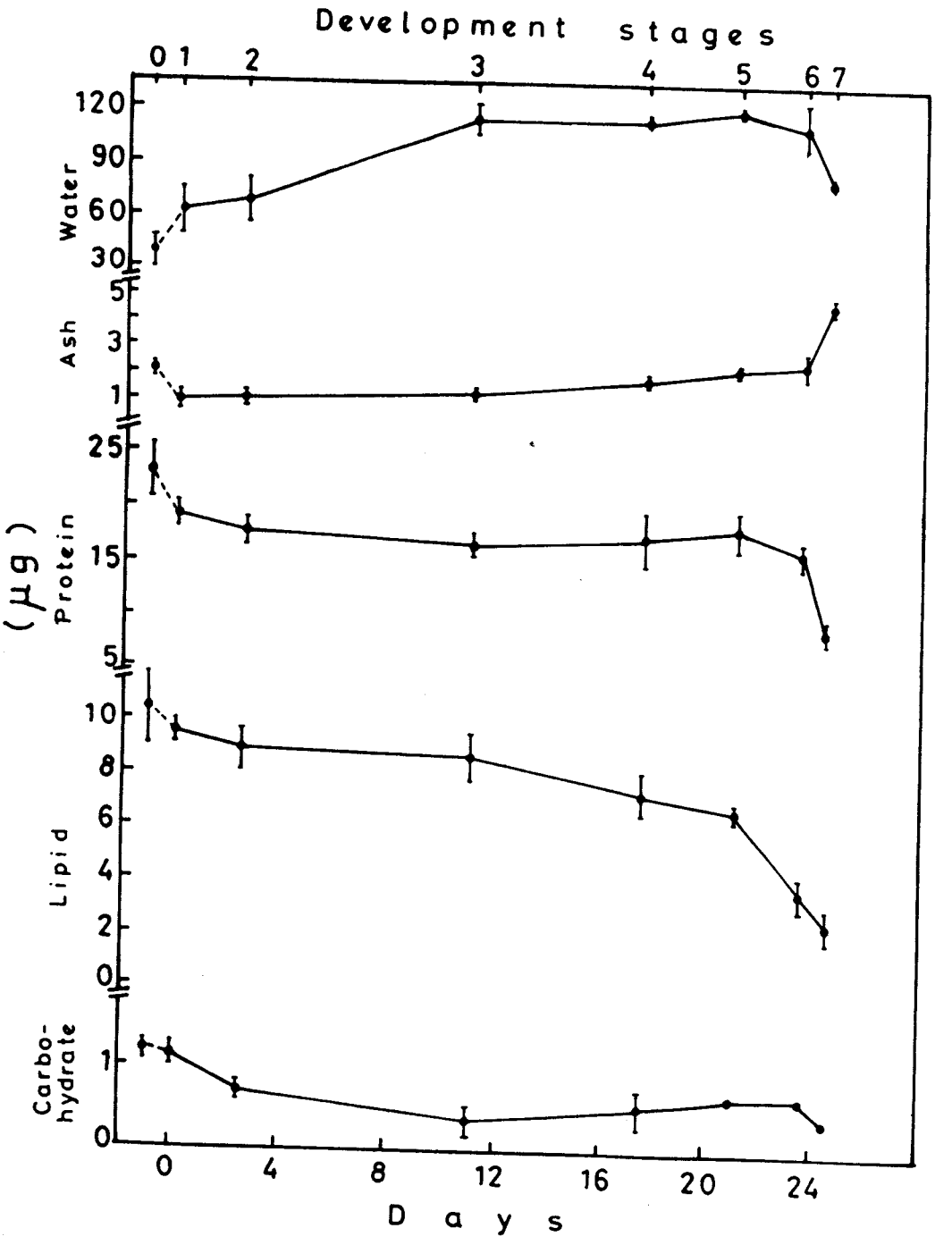


Fig.4.3 Quantitative changes in water, protein, lipid, carbohydrate and ash in a single egg during development in *P.homarus*.

Table 4.6. Utilization of water, protein, lipid, carbohydrate and energy in different development stages in *P. homarus* egg (values at stage 1 kept as original value (100%) to determine increase or decrease in in other stages)

| Stage | Utilization in different stages of development | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Water | | Protein | | Lipid | | Carbohydrate | | Energy | |
| | µg | % | µg | % | µg | % | µg | % | µg | % |
| 0 (ova) | -24.5 | 61.7 | +3.95 | 120.5 | +0.80 | 108.4 | +0.01 | 107.0 | +0.13 | 115.7 |
| 1 | 64.0 | 100.0 | 19.23 | 100.0 | 9.57 | 100.0 | 1.14 | 100.0 | 0.90 | 100.0 |
| 2 | 5.5 | 108.6 | -1.54 | 92.0 | -0.74 | 92.3 | -0.41 | 64.0 | -0.07 | 91.2 |
| 3 | 50.1 | 178.3 | -2.46 | 87.2 | -0.90 | 90.6 | -0.77 | 32.5 | -0.04 | 94.9 |
| 4 | 49.5 | 177.3 | -1.66 | 91.4 | -2.23 | 76.7 | -0.61 | 46.5 | -0.14 | 83.6 |
| 5 | 57.0 | 189.0 | -0.98 | 94.9 | -2.81 | 70.6 | -0.43 | 62.3 | -0.14 | 83.1 |
| 6 | 47.3 | 173.9 | -4.15 | 78.7 | -5.61 | 40.5 | -0.44 | 61.4 | -0.33 | 61.0 |
| 7 (Phyllo soma) | 21.0 | 132.8 | -9.95 | 48.3 | -6.95 | 27.4 | -0.73 | 36.0 | -0.51 | 38.5 |

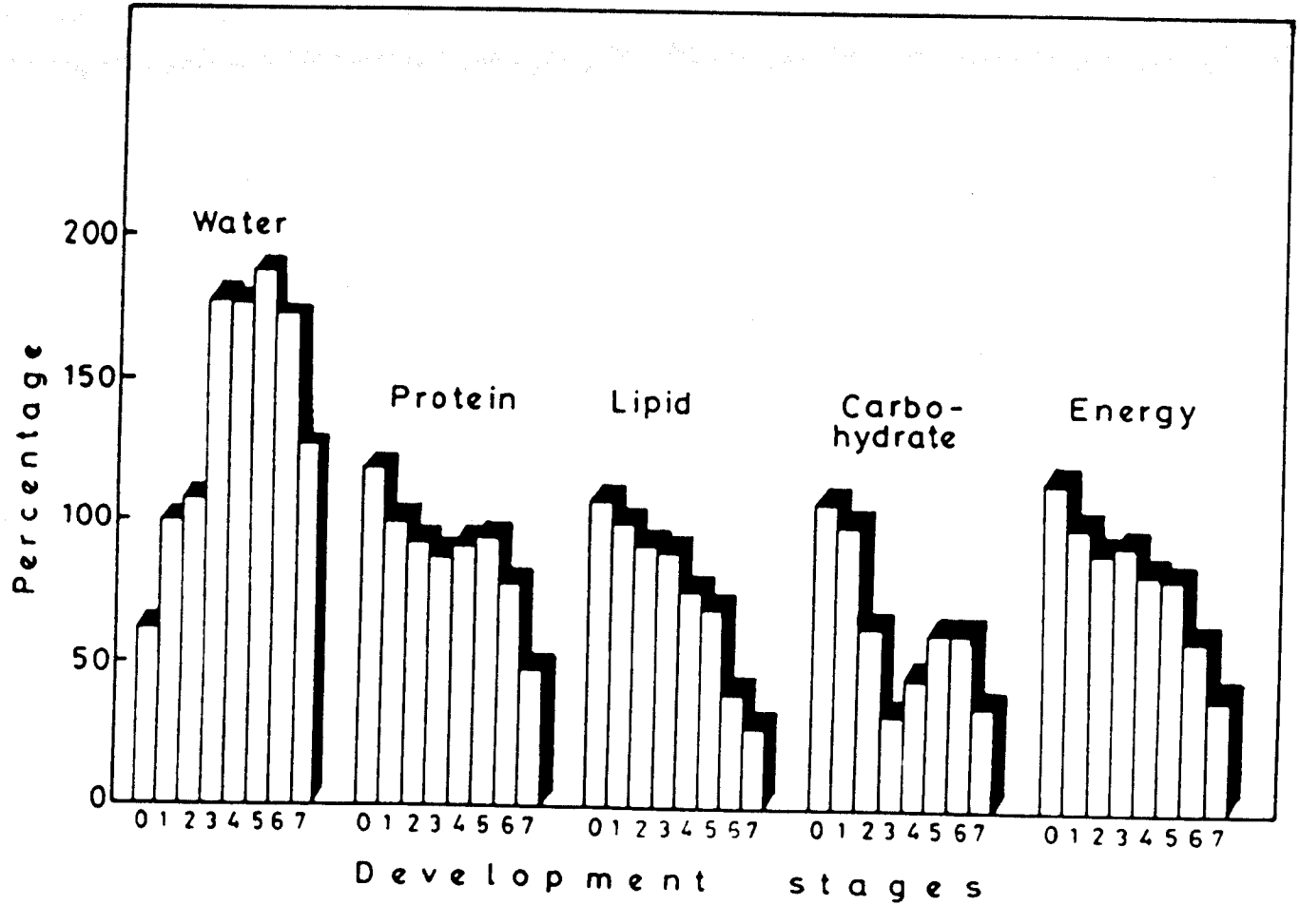


Fig.4.4 Utilization of water protein, lipid carbohydrate and energy during egg development in *P.homarus* (relative change over the quantity in fertilized egg which is kept as 100).

Fig.4.5. From just spawned egg to phyllosoma 64.2% dry matter was converted while total energy (measured) conversion was only 46.67%. This disparity between dry matter and energy conversion was expected, since inorganic ash contributes 24.68% of dry matter in phyllosoma. Among the organic reserves, conversion efficiency was minimum (27.28%) for lipid and maximum (48.26%) for protein.

When efficiencies were calculated with ripe ova as the starting point, the same trend prevailed but efficienies were lower - 54.48% for dry matter and 40% for energy.

Taking total energy utilized during development as 100, contributions of lipid, protein and carbohydrate energy to total expenditure were calculated and presented in Fig. 4.6. Between stage 1 and phyllosoma, maximum energy (53.4%) was contributed by lipid followed by protein (43.91%) and a negligible amount of 2.69% by carbohydrate. When the efficiency was worked out starting from ova, protein contributed more (49.53%) followed by lipid (48.06%) and carbohydrate (2.41%).

### 4.1.6 Mineral changes during development

Minerals and trace elements concentrations during development in the egg are tabulated in Table 4.8. Concentration of all minerals (Na, K, Ca, P, and Mg) decreased from ova to fertilized egg, but thereafter showed variability in different stages and except for K, all other minerals were maximum in concentration in the phyllosoma larva.

**Table 4.7.** Energy utilization in a single egg during development in *P. homarus*

| Stage | Utilization of dry matter and energy | | | | | |
|---|---|---|---|---|---|---|
| | Dry matter (μg) | Protein energy (J) | Lipid energy (J) | Carbo-hydrate (energy) (J) | Total Energy (J) | |
| | | | | | Calculated | Measured |
| 0 (ova) | 36.90 ± 8.01 | 0.524 ± 0.051 | 0.408 ± 0.050 | 0.023 ± 0.003 | 0.955 ± 0.110 | 1.05 ± 0.08 |
| 1 | 31.41 ± 9.00 | 0.435 ± 0.027 | 0.378 ± 0.004 | 0.022 ± 0.003 | 0.833 ± 0.040 | 0.90 ± 0.09 |
| 2 | 28.50 ± 2.00 | 0.398 ± 0.020 | 0.348 ± 0.026 | 0.014 ± 0.002 | 0.760 ± 0.050 | 0.81 ± 0.05 |
| 3 | 27.90 ± 1.00 | 0.379 ± 0.014 | 0.341 ± 0.030 | 0.007 ± 0.004 | 0.790 ± 0.040 | 0.79 ± 0.07 |
| 4 | 28.20 ± 1.00 | 0.397 ± 0.055 | 0.289 ± 0.027 | 0.010 ± 0.005 | 0.696 ± 0.090 | 0.96 ± 0.06 |
| 5 | 29.20 ± 1.79 | 0.413 ± 0.032 | 0.266 ± 0.010 | 0.013 ± 0.006 | 0.692 ± 0.060 | 0.79 ± 0.05 |
| 6 | 23.50 ± 1.00 | 0.342 ± 0.026 | 0.153 ± 0.024 | 0.014 ± 0.005 | 0.508 ± 0.050 | 0.51 ± 0.04 |
| 7 (Phyllo-soma) | 20.10 ± 1.00 | 0.210 ± 0.013 | 0.103 ± 0.022 | 0.008 ± 0.001 | 0.321 ± 0.030 | 0.42 ± 0.01 |
| Energy Conversion efficiency | | | | | | |
| Ova to phyllosoma | 54.48% | 40.03% | 25.27% | 33.61% | 33.56% | 40.00% |
| Egg to phyllosoma | 64.02% | 48.26% | 27.28% | 35.96% | 38.49% | 46.67% |

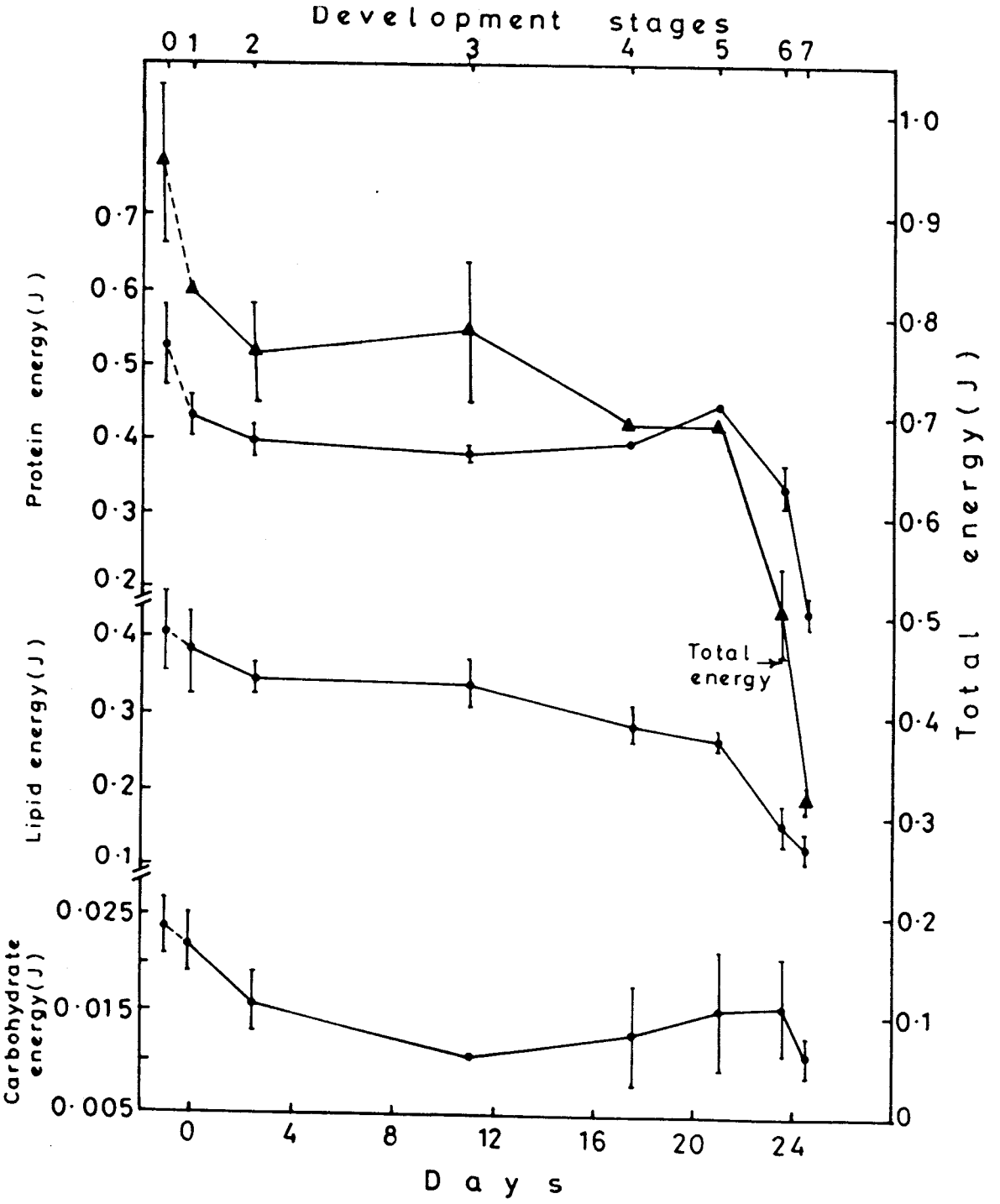


Fig.4.5 Energy utilization from different sources in the developing egg of *P.homarus*.

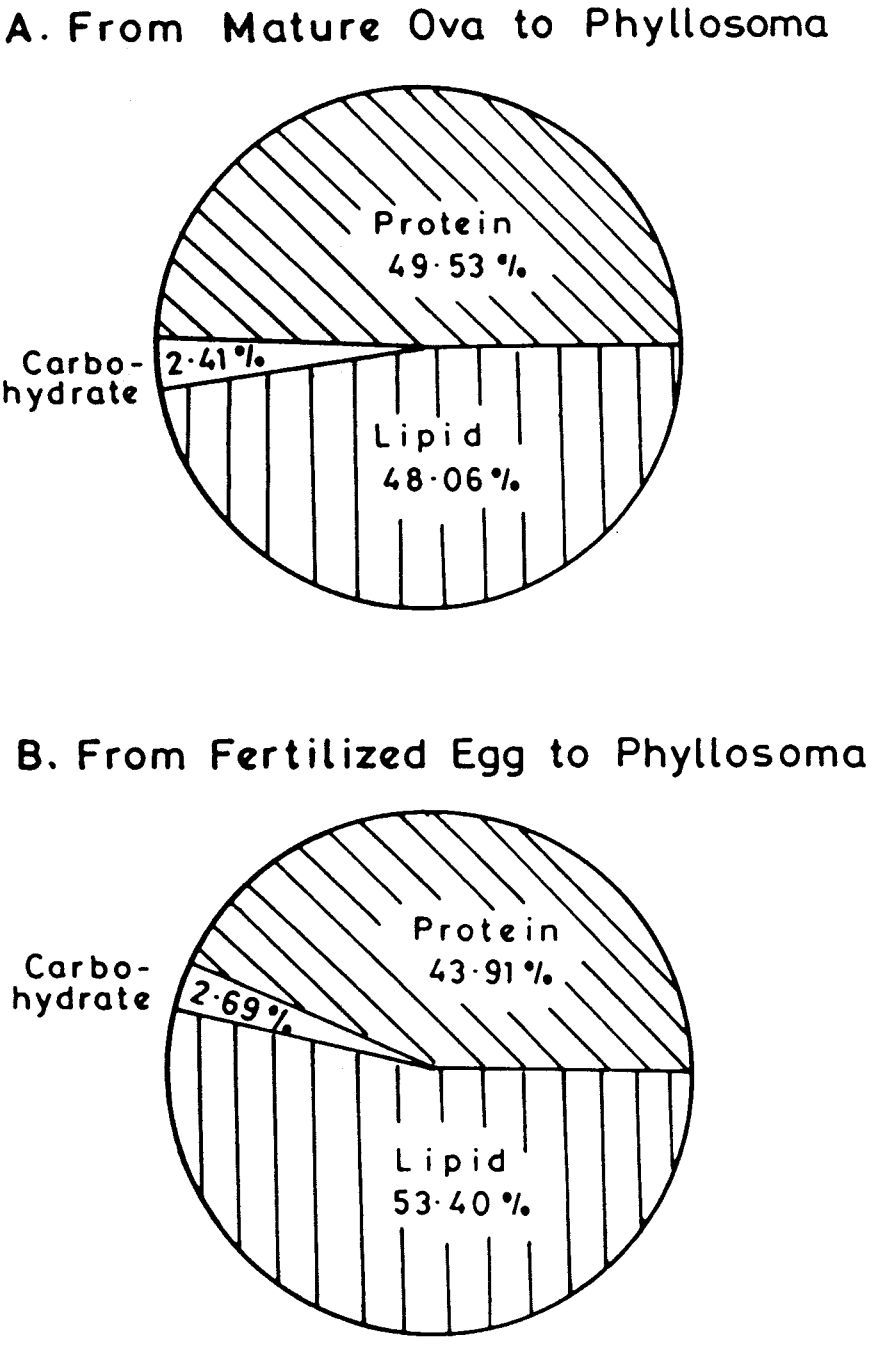


Fig.4.6 Proportion of protein, lipid and carbohydrate energy in total energy utilized for egg development in *P.homarus*.

**Table 4.8.** Changes in minerals and trace elements composition during development *P. homarus* (mg or μg/g dry weight)

| Minerals/ trace elements | Development stages | | | | | |
|---|---|---|---|---|---|---|
| | 0 (ova) | 1 | 2&3 | 4 | 5&6 | 7 (phyllosoma) |
| **Minerals** (mg/g dry wt) | | | | | | |
| Na | 22.72± 0.98 | 19.01± 3.28 | 18.64± 3.46 | 13.05± 2.48 | 21.80± 1.95 | 53.70± 6.83 |
| K | 10.85± 3.44 | 4.63± 0.82 | 3.13± 0.69 | 9.67± 1.09 | 6.42± 2.94 | 1.68± 0.59 |
| Ca | 6.40± 0.14 | 3.97± 0.30 | 5.29± 2.40 | 4.11± 1.13 | 7.91± 1.44 | 36.74± 1.90 |
| P | 43.98± 3.58 | 13.07± 1.11 | 29.39± 1.29 | 16.52± 0.59 | 17.42± 0.70 | 38.81± 8.13 |
| Mg | 2.09± 0.59 | 1.88± 0.22 | 1.34± 0.72 | 2.14± 0.53 | 3.53± 0.85 | 8.81± 1.93 |
| **Trace elements** (μg/g dry wt) | | | | | | |
| Fe | 105.53± 3.81 | 122.72± 4.21 | 298.50± 61.83 | 410.53± 85.07 | 382.13± 152.77 | 150.36± 48.13 |
| Zn | 243.59± 29.30 | 254.40± 29.20 | 267.40± 38.50 | 191.11± 23.52 | 339.32± 10.31 | 184.51± 65.62 |
| Cu | 42.34± 2.53 | 47.79± 14.74 | 43.61± 12.66 | 51.13± 10.88 | 42.18± 1.37 | 26.79± 0.99 |
| Cd | 0.55± 0.06 | 0.10± 0.04 | 0.45± 0.21 | 0.52± 0.22 | 0.37± 0.18 | 1.12± 0.02 |
| Mn | 11.21± 1.06 | 4.04± 2.05 | 8.67± 6.50 | 12.59± 4.87 | 8.67± 3.34 | 10.06± 2.47 |
| Cr | 3.17± 0.57 | 2.54± 1.63 | 2.90± 1.35 | 2.23± 0.99 | 3.46± 1.11 | 4.56± 0.32 |
| Co | 1.49± 0.73 | 0.04± 0.02 | 0.79± 0.45 | 1.39± 0.41 | 3.32± 1.18 | 2.19± 0.79 |
| Pb | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

Among the trace elements, Fe, Zn and Cu increased in the egg while Mn, Co, Cr and Cd concentrations in stage 1 egg were less than those in the ripe ova. Like minerals, trace element composition also showed variability between stages and except for Zn, all were maximum in the phyllosoma. Pb was below detectable limit (<0.001 $\mu$g/g dry weight) in all stages.

Quantitative uptake and utilization of minerals and trace elements in a single egg are given in Table 4.9 and Figs 4.7 and 4.8. Among minerals, the quantity of P was more (0.829$\mu$g) than all others while Mg was minimum in quantity (58.72 ng). Among trace elements, quantity of Zn was more than twice (8.86 ng) than that of Fe (3.91 ng) which was the second important trace element in stage 1. But towards the end of egg development (stages 5 and 6), quantity of Fe increased considerably to 11.59 ng, even more than that of Zn.

Keeping the quantities of minerals and trace elements in stage 1 as 100, the relative increase or decrease in other stages were calculated and expressed in Figs.4.9 and 4.10. Na declined to 61.64% in stage 4 and then increased progressively to 180.74% in phyllosoma. K reduced to 60.69% in stage 2 and 3 and then went up to 188.28% in stage 4 and thereafter declined to a minimum of 23.45% in phyllosoma. P was only half the quantity (55.4%) in stage 4 but thereafter increased to 94.0% in stage 5 and 6 and declined again in phyllosoma. Mg decreased to 64.35% in stage 2 and 3 and thereafter progressively went up culminating in more than three times the quantity in phyllosoma. Ca was more in stages 2 and 3, declined to

**4.9.** Uptake of minerals and trace elements in a single egg/larva during development in *P. homarus*

| Minerals/ trace elements | Development stages | | | | | |
|---|---|---|---|---|---|---|
| | 0 (ova) | 1 | 2&3 | 4 | 5&6 | 7 (phyllosoma) |
| **Minerals** (μg) | | | | | | |
| Na | 0.840± 0.180 | 0.597± 0.170 | 0.526± 0.028 | 0.368± 0.007 | 0.574± 0.022 | 1.079± 0.054 |
| K | 0.400± 0.090 | 0.145± 0.042 | 0.088± 0.005 | 0.273± 0.005 | 0.169± 0.006 | 0.034± 0.002 |
| Ca | 0.240± 0.050 | 0.125± 0.036 | 0.149± 0.008 | 0.116± 0.002 | 0.208± 0.008 | 0.738± 0.040 |
| P | 1.620± 0.350 | 0.829± 0.238 | 0.466± 0.025 | 0.459± 0.008 | 0.780± 0.029 | 0.740± 0.037 |
| Mg (ng) | 77.120± 16.700 | 58.720± 16.830 | 37.790± 2.010 | 60.350± 1.070 | 93.020± 3.510 | 177.080± 8.810 |
| **Trace elements** (ng) | | | | | | |
| Fe | 3.910± 0.180 | 3.860± 1.110 | 8.430± 0.450 | 11.590± 0.210 | 10.070± 0.380 | 3.020± 0.150 |
| Zn | 8.860± 1.920 | 8.820± 2.530 | 7.530± 0.400 | 5.390± 0.096 | 8.930± 0.340 | 3.720± 0.190 |
| Cu | 1.550± 0.360 | 1.510± 0.430 | 1.240± 0.066 | 1.440± 0.026 | 1.110± 0.040 | 0.540± 0.030 |
| Cd | 0.202± 0.043 | 0.003± 0.001 | 0.013± 0.001 | 0.0014± 0.0002 | 0.0105± 0.0004 | 0.0011± 0.0001 |
| Mn | 0.406± 0.088 | 0.126± 0.036 | 0.243± 0.013 | 0.367± 0.150 | 0.2270± 0.0090 | 0.201± 0.010 |
| Cr | 0.118± 0.026 | 0.079± 0.023 | 0.082± 0.004 | 0.056± 0.007 | 0.0920± 0.0030 | 0.092± 0.005 |
| Co | 0.055± 0.012 | 0.0013± 0.0004 | 0.022± 0.001 | 0.0390± 0.0007 | 0.0870± 0.0030 | 0.044± 0.002 |

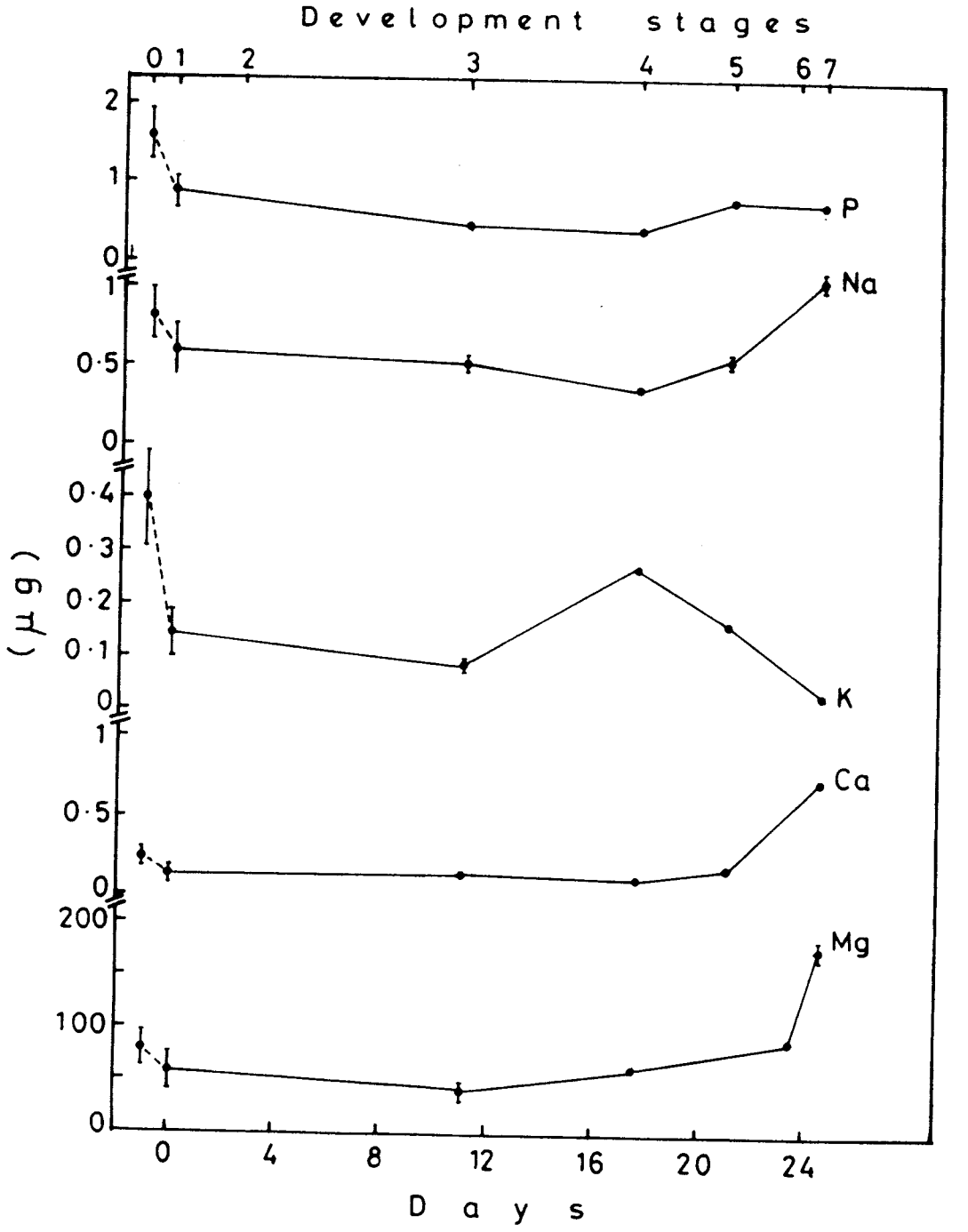


Fig.4.7 Quantitative changes of minerals in a single egg during development in *P.homarus*.

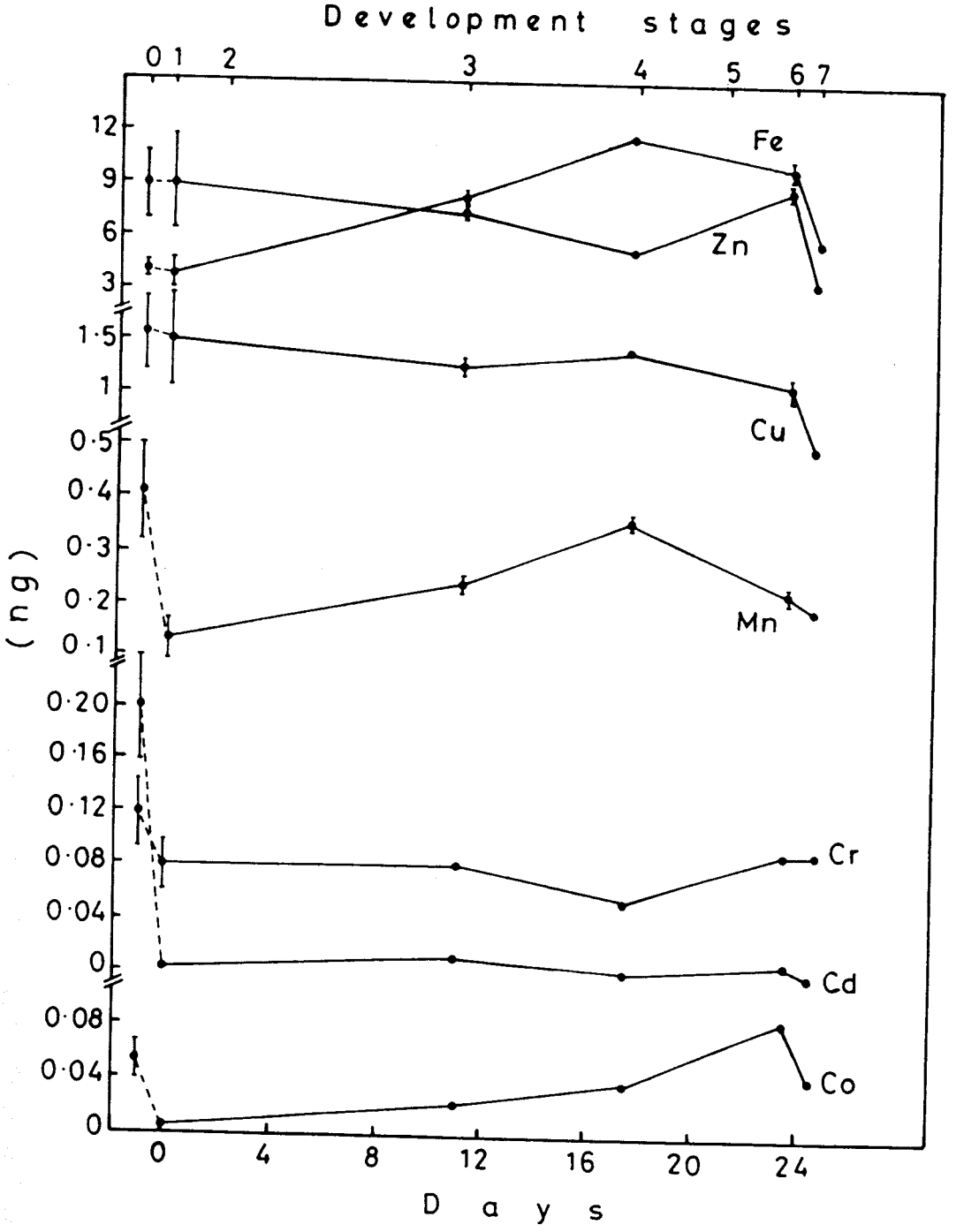


Fig.4.8 Quantitative changes in trace elements in a single egg during development in *P.homarus*.

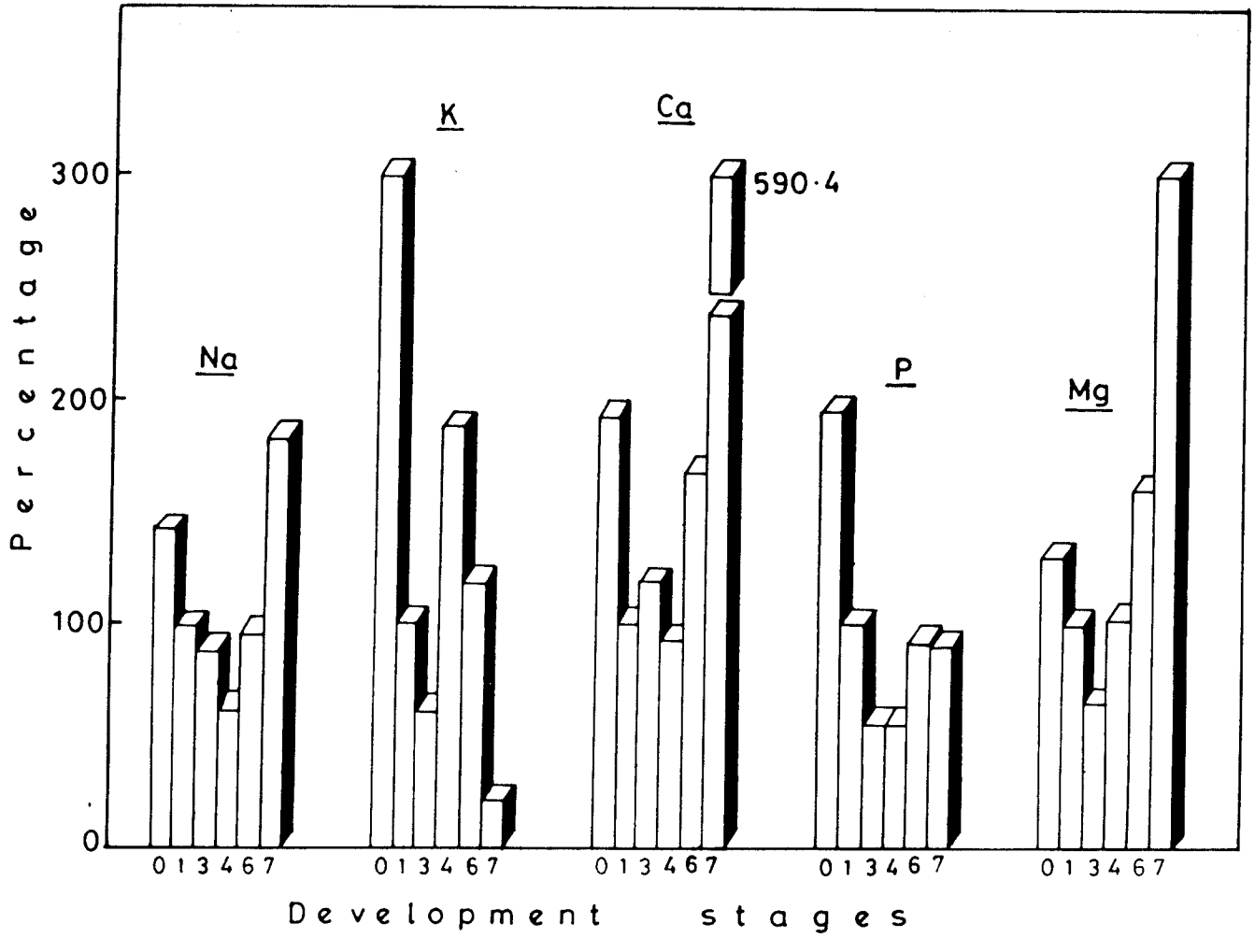


Fig.4.9 Percent increase/decrease of minerals in developing egg of *P.homarus* (Stage 1 (fertilized egg) = 100).

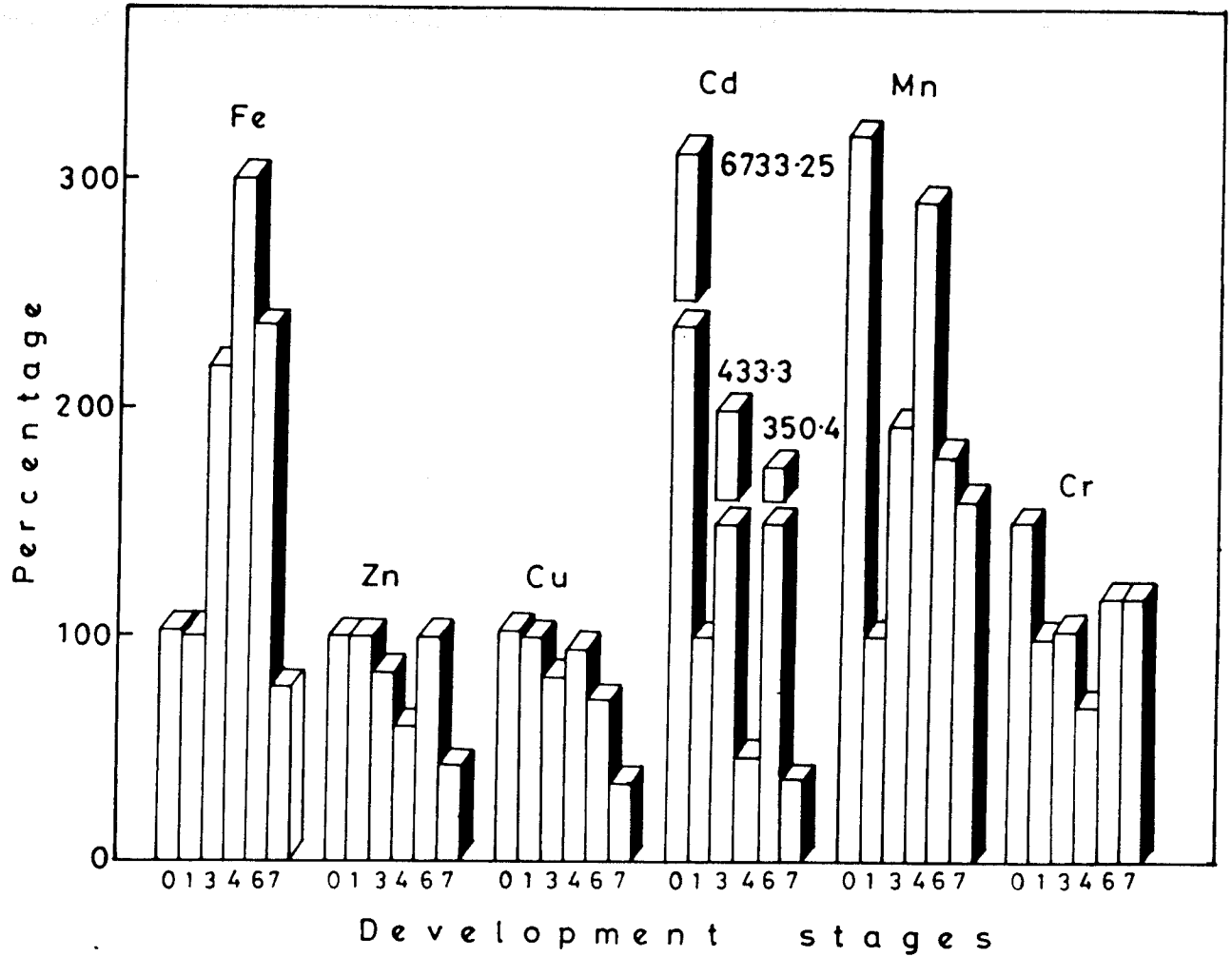


Fig.4.10 Percent increase/decrease in trace elements during development in *P.homarus* egg (Stage 1 = 100).

92.9% in stage 4 and then markedly increased to register 590.4% in phyllosoma.

Rates of uptake and utilization was more for all trace elements than seen in the case of minerals. The most striking observation was 6692.3% increase in the quantity of Co by stage 5 and 6, which reduced to 3384.6% in phyllosoma. Uptake of Cd requires special mention. The quantity of Cd increased to 433,33% in stage 2 and 3 and then declined to 46.67% in stage 4; the quantity again went up to 356% in stage 5 and 6 and minimum quantity was recorded in phyllosoma (36.69%). Fe and Mn uptake was also about three times high in stage 4.

## 4.2 DISCUSSION

As in all other crustaceans incubation period in *P. homarus* egg is influenced by ambient temperature. Berry (1971) has reported that incubation period varies between 59 days (20.2°C) and 29 days (25.9°C) in eggs of *P. homarus*. In the present study, the incubation period was 24.5 days (range 22-27 days) at 27.03°C ± 1.02°C and this observation substantiates the results obtained by Berry (1971) that the incubation period is reduced with increase in temperature in *P. homarus* eggs.

Increase in egg volume due to osmotic uptake of water (Davis, 1968) is a general phenomenon in the eggs of aquatic invertebrates and it ranges from 50 to 192% (Table 4.10) in different groups of crustaceans. In palinurid lobsters, increase in egg volume was recorded 100% in *Jasus lalandi*

**Table 4.10.** Volume increase in eggs during development in Crustacea

| Species | % increase in egg volume | Reference |
|---|---|---|
| *Acanthophysa* sp. | 100 | Herring, 1974 |
| *Palaeomonites vulgaris* | 120 | Davis, 1965 |
| *Crangon crangon* | 150 | Pandian, 1967 |
| *Jasus lalandi* | 100 | Silverbauer, 1971 |
| *Homarus gammarus* | 170 | Pandian, 1970b |
| *Nephrops andamanicus* | 180 | Berry, 1969 |
| *Eupagurus dispersa* | 80 | Pandian & Schuman,1967 |
| *Cancer pagurus* | 50 | Wear, 1974 |
| *Cancer maenas* | 80 | Wear, 1974 |
| *Cyclops bicuspidates* | 155 | Davis, 1959 |
| *Balanus balanus* | 75 | Barnes, 1965 |
| *Balanus balanoides* | 60 | Barnes, 1965 |
| *Panulirus homarus* | 102 | Present Study |
| *Panulirus homarus* | 192* | Present Study |

* Increase in volume from ripe ova to last stage in egg development.

(Silberbauer, 1971). In this study, 102.27% increase in egg volume was recorded for *P. homarus* which compares well with the data for *J. lalandi*. One of the most important observations in the present investigation is the recording of great upsurge in volume of ripe ova as it is being released to the outside medium. Calculated from ripe ova, the volume increase in *P.homarus* during development works out to 192.52% almost double the value recorded between spawned egg and last stage of egg. In almost all crustacean eggs observed earlier, the rate of increase in volume is slow initially, rapid in the middle with a final thrust towards the end of development. The rate of increase in water upake has been correlated to enhanced cellular activity of the egg (Barnes, 1965, Wear, 1974). But in the eggs of the European lobster, *H. gammarus* (Pandian, 1970a) and the land crab *Paratelphusa hydrodromous* (Pillai and Subramoniam, 1985), water uptake was more in the initial stages, dropped in the middle and increased again in later stages of development. If water uptake is considered from spawned egg in *P. homarus*, it is analogous to the observations of Barnes (1965) and Wear (1974), but maximum increase recorded was at spawning in the ripe ova. This observation suggests that in "non-clieidoic" eggs of marine invertebrates, for better understading of water uptake and volume increase during development, volume changes in ripe ova at spawning also should be studied.

As in most crustaceans, rate of yolk utilization, expressed as decrease in yolk volume, is slow initially in *P. homarus*. Utilization then increases towards the appearance of eyespot and reaches a maximum in the the last

7 days of incubation which accounts for 72.6% of total utilized. Increase in yolk utilization follows the same pattern of water uptake and both seem to be related to intense cellular activity in the developing egg of *P. homarus*.

Reviewing available data on yolk utilization, Pandian (1970c) suggested that marine demersal eggs tend to follow the "cleidoic" pattern by considerably increasing fat oxidation. This 'sparing effect' on proteins increased gradually as proximity of habitat to land decreased and was maximum in *H. gammarus* which inhabits the shallow (5m depth) coastal region. *P. homarus*, which occupies the same habitat as *H. gammarus*, does not seem to fit into this pattern of yolk utilization. However, fat usage during development, from spawned egg to phyllosoma larva (72.62%), is significantly high compared to utilization of protein (51.74%) and is different from fresh water eggs where more protein is catabolised for enrgy during development.

In *P. homarus*, in the initial stages of development, (up to stage 3) which is relatively slow, protein and lipid are more or less eqully used (12.79% and 9.4% respectively), but carbohydrate is heavily depleted (67.54%). Interestingly, quantity of protein in the egg increased from stage 3 to 5 by 7.69% and this was followed by a heavy reduction between stage 5 and phyllosoma larva. Increase in protein content of the egg during early development have also been reported in fresh water caridia, *Macrobrachium lammarrei* and *Caridina nilotica* (Katre, 1977, Ponnuchamy *et al.*, 1979, ) and in the crab *P. hydrodromous* (Pillai and Subramoniam, 1985) in which a

simultaneous increase in protein fractions were also recorded electrophoretically. Increase in protein content and appearance of new protein fractions have been attributed to the possible appearance of new proteins in the form of enzymes to dismantle complex yolk proteins and also to synthesis of new tissue proteins (Pillai and Subramoniam, 1985). Whether the increase in protein quantity in *P. homarus* midway through egg development, is due to synthesis of new proteins or due to experimental artefacts, has to be evaluated by electrophoretic studies.

Like protein, total carbohydrates of the egg also increase considerably (29.82%) from stage 3 to 5 in *P. homarus* and get depleted in the last stage. Such an increase in carbohydrates until the last stage of development has also been observed in sand lobster *T. orientalis* (Rahman *et al.*, 1987). Carbohydrate build up in the egg has no significance energetically since only less than 3% of total energy for development is derived from carbohydrate.

Lipid is the main energy reserve in *P. homarus* egg and is utilized slowly in the initial stages and at a faster pace once the organogenesis sets in. Total depletion of 72.27% lipid, which amounts to 53.4% of energy expended in *P. homarus* during development is similar to the values reported for the barnacle, *Balanus balanoides* (Lucas and Crisp, 1987) and the crab *Xantho bidentatus* (Erribabu, 1987), but is considerably less than the values 67 to 87% of total energy used reported for other marine decapods (Pandian, 1967b, 1970a,b 1972, Pandian and Schuman, 1967). The rest of energy for

development is mainly contributed by protein (43.91%). But between mature ova and spawned egg, more protein than lipid is utilized in *P. homarus* and when the energetics are worked out from ripe ova, contribution of protein (49.53%) becomes more than that of lipid (48.06%).

Total energy conversion efficiency in the developing eggs of *P. homarus*, is 46.67% which is low compared to an average of around 63% reported for many decapods. The phyllosoma larva does not have much energy reserve in the form of lipid but is provided with a ramifying alimentary system and efficient mouth parts and can feed immediately after release. This may, possibly be the reason for low conversion efficiency of yolk in *P.homarus* egg.

Uptake and utilization of minerals and trace elements in the eggs of *P. homarus* elucidate the requirements of these elements during different stages of egg development. It is intriguing to note that such an important aspect in embryogenesis has attracted little attention until now. Few studies reported in crabs *Cancer irroratus* (Martin, 1974, 1976), *P.hydrodromous* (Pillai and Subramoniam, 1985), in cephalopod *Loligo vulgaris* (D'Aniello *et al* 1987, 1989), in fishes, *Salmo gairdneri* (Love, 1980), *Oryzia latipes* (Hori and Iwasaki, 1976) and in the Turkey hen *Meleagres gallopovo* (Richards, 1989) have enlisted the necessity of some of the major and trace elements for normal embryonic development and hatching success.

Metal deficiencies are usually not known to occur in marine organisms and optimum concentrations are those which occur naturally in sea

water (Nair, 1984). In this investigation, metalic ion concentrations of the sea water in which the lobster eggs were hatched were not determined, but it is unlikely to vary much from the general averages for sea water (Many authors, Table 4.11). This is because the area of collection of sea water is reasonably unpolluted and without any major effects of fresh water influx from estuaries. Concentrations and quantities of major and trace elements in different stages of egg development in *P. homarus* suggest selective uptake and utilization of these elements in the egg. Among the major elements, some like Na and P are utilized more in the early stages of development while Mg progressively got accumulated. There is an increase in uptake of almost all elements in the last stages (stages 5 and 6) of egg development which coincides with hectic metabolic activity in the egg.

When the cephalapod *L. vulgaris* was grown in artificial sea water containing only major ions like $Na^{+}$, $K^{+}$, $SO4^{2+}$ and $Cl^{-}$, the youngest eggs failed to survive, probably indicating that normal embryonic development requires some substances that are present in natural sea water in extremely low amounts (D'Aniello *et al.*, 1989). This report on Loligo eggs demonstrates the importantce of trace metals during early embryogenesis in marine eggs. In *P. homarus* the most striking accumulation of trace element in the eggs was that of Co, even though it is present in lowest quantity among the trace elements analysed. The quantity of Co increased by 6692% in the final stages of development and the phyllosoma retained half of this quantity. In *P. hydrodromous* (Pillai and Subramoniam, 1985), Co concentration was recorded as < 3ppm in all four development stages,

**Table 4.11** . Concentration of certain minerals and trace elements in sea water

| Element | Concentration ($\mu$g/l) | Authors |
|---|---|---|
| Na | $10.75\text{-}11.05 \times 10^6$ | |
| K | $3.95\text{-}4.16 \times 10^5$ | |
| P | 70 | Goldberg, 1965 |
| Ca | $4.16\text{-}4.22 \times 10^5$ | Kalle, 1971 |
| Mg | $1.295\text{-}1.326 \times 10^6$ | Riley and Chester, 1971 |
| Fe | 1.7-137 | Giesy and Wiener, 1977 |
| Cu | 1.4-5.9 | Nair, 1984 |
| Zn | 0.64-21.3 | Chen, *et al.*, 1985 |
| Mn | 0.02 | |
| Co | 0.08-0.10 | |
| Cr | 0.11-0.60 | |
| Cd | 0.05-0.11 | |
| Pb | 0.03-1.70 | |

inviting the suggestion from the authors that Co remained unchanged during development. An exact estimation of low quantities of Co, perhaps, would have given a different picture in this crab. It is difficult to interpret whether Co, which is reported to be deleterious to the embryos in increased concentrations in the medium (Morril, 1963) is passively accumulated or actively absorbed for specific purpose. Co is a constituent of vitamin $B_{12}$ enzymes (Nair, 1984) and vitamin $B_{12}$ is an essential component in the medium for culture of phytoflagellates like *Isochrysis galbana*. Whetehr Co plays a similar role in the embryonic development of *P. homarus* egg is to be evaluated.

Cd, which is generally considered as non-essential and toxic (Fleischer *et al.*, 1974, Bjerregaard and Vislie, 1986), and is highly magnified in different tissues of crustacea (Eisler, 1981) increases by over 400% in early stages of development in *P. homarus* egg. It shows differential accumulation and utilization during further development, and, like Co, is also present in very low quantities. There is no report of Cd being involved in any of the enzymatic processes in living organisms, but the pattern of accumulation and utilization in *P. homarus* egg points to an active role for Cd in embryonic development.

In mammals, Cr is directly involved as an essential cofactor for maintenance of normal glucose tolerence (Moore, 1981) and is required in extremely small quantities (Giesy and Wiener, 1977). The essentiality of Cr for invertebrates and fish is not yet established, but its accumulation rates

in aquatic invertebrates is directly related to ambient concentrations (Giesy and Wiener, 1977). However, the differential accumulation of Cr in the eggs of *P. homarus* suggests its necessity in embryogenesis.

Mn is a constituent of Pyruvate carboxilase (Nair, 1984) and is essential along with Fe, Cu and Zn for normal biochemical process in invertebrates (Harrison and Hoare, 1980). Many fishes are capable of regulating Mn, and its concentrations are usually tens of thousands of times greater than that of surrounding water. In *P. homarus* egg, quantity of Mn increases in initial stages of development to reach a peak (291.27%) in stage 4 and then decreases gradually indicating that requirement of Mn is maximum during early and middle phases of development. In the crab *P. hydrodromous* also, Mn increased 5 times in early stages of development and reduced in late stages (Pillai and Subramoniam, 1985). But, unlike *P.homarus*, enormous increase of 16 times was recorded in the hatched larva of the crab. A remarkable increase of 5 times was also recorded in the eggs of the fish *Oryzia latipes* after fertilization (Hori and Iwasaki, 1976). All these observations point to the necessity of Mn in early embryogenesis of aquatic eggs.

Fe, Zn and Cu are the most prominent trace metals in *P.homarus* egg. Fe has been found essential for normal development of turkey egg (Richards, 1989) and is a prominent element in haemoglobin. Haemoglobin, incidentally, is more a storehouse of protein for energy than a respiratory pigment in crustaceans. Fe is also very important for the enzyme

cytochrome oxidase. The ability of various crustaceans to accumulate Fe from the medium ranges from 1800 times for lobster meat to 2,20,000 times for copepods (Eisler, 1981). In *P.homarus* egg, Fe content increased to more than 300% in stage 4 and then decreased to 78.4% in phyllosoma. Fe seems to be utilized more towards middle and end phases of development in *P. homarus*. A similar trend in Fe utilization was observed in *P. hydrodromous*, but Fe in hatched larva was substantially high in this species (Pillai and Subramoniam, 1985).

Zn is one of the most important trace elements and more than ninety enzymes containing Zn have been described. It also increases the activity of many other enzymes. In decapods, body concentration of Zn is regulated against fluctuations in the intake (Bryan *et al.*, 1986), while it has been found essential for normal development of avian egg (Richards, 1989). Zn is the single largest trace element in the freshly spawned egg of *P. homarus* and its quantity reduces to 61.41% in stage 4. Unlike Fe, the quantity of Zn again increases in the last stage of development to reduce again to 42.18% in phyllosoma. Zn appears to be essential throughout egg development in *P. homarus*. In contrast to this observation, Zn concentration increased nearly 5 times in the middle phase of development in *P. hydrodromous* to increase again in larva (Pillai and Subramoniam, 1985).

Cu, which comprises 93% of weight of haemocyanin in the blood of the crab *Carcinus maenas* (Martin *et al.*, 1977) is undoubtedly one of the most essential trace elements in crustaceans. Cu also is a component of cytochrome oxidase (Nair, 1984). The quantity of Cu in *P. homarus* egg in

different stages suggests its differential uptake and utilization and it is necessary throughout the developmental period. In *P. hydrodromous*, Cu appears to be more essential in the middle stages of development (Pillai and Subramoniam, 1985).

Pb, which is considered as non-essential and toxic, was below detectable limit (<0.001/μg/g dry weight) in all stages of development and does not seem to play any role in the process of egg development in *P. homarus*.

Results of the present study emphasize the need to include ripe ova in yolk utilization studies in "non-cleidoic" eggs of marine invertebrates. It also points to emphasizes the necessity to understand specific roles played by minerals and trace elements in embryogenesis of aquatic eggs.

# 5. EFFECT OF SIZE AND MATURITY ON FOOD CONVERSION EFFICIENCY IN THE SPINY LOBSTER, PANULIRUS HOMARUS

In a recent review on the potential of spiny lobster culture, Radhakrishnan and Vijayakumaran (1990) have suggested that it could be a distinct possibility in the near future, though of limited scope. The greatest impediment in spiny lobster culture has been the inability to produce seed in captivity due to the complex and protracted larval life of spiny lobsters, extending over several months. Since collection of puerulii or postlarvae from the wild to sustain culture operations is also not feasible, any attempt at culture should start with juveniles. Undersized juveniles, comprising nearly 30-40% of the commercial catch in India, do not fetch a reasonable market price and are also a loss to natural population.

Early efforts to enhance growth in spiny lobster, *Panulirus argus* (Travis, 1954) and *Panulirus cygnus* (Dall, 1977) by bilateral eyestalk ablation met with little success. But the impressive results obtained recently in enhancing growth rate by 3-10 times in four major species of spiny lobsters, *Panulirus homarus, Panulirus ornatus*, *Panulirus polyphagus* and *Panulirus versicolor* by bilateral eyestalk ablation (Radhakrishnan and Vijayakumaran, 1984, Silas *et al.*, 1984b) have rekindled the hope of making spiny lobster culture an economic viability. The euphoria of publicity of these studies, however, had an infant death due to concerted opposition from animal lovers

who would not allow thousands of lobsters to be "blinded" to grow them to "giant" size. So the lobster culturist should, once again, depend on normal juveniles itself. The works of Tamm (1980) who could produce 300g increase in *P. ornatus* in one year and Radhakrishnan and Vijayakumaran (1990) who have projected a 300 g weight gain in *P. homarus* in an year under ideal conditions, reveal the possibility of lobster culture using normal juveniles.

Food intake and transformation in the animal is an important aspect in culture operations. Radhakrishnan (1989) has studied the effect of quantity and quality of feed in isolated and group reared normal and eyestalk ablated *P. homarus*. The present work was designed to evaluate another important aspect in food conversion, the effect of size and maturity on food and protein conversion efficiencies in *P. homarus* fed with the brackish water clam, *Meretrix casta*.

## 5.1 RESULTS

### 5.1.1 Weight increase

Size of lobsters and experimental conditions provided are given in Table 5.1. Weight increase per day did not vary significantly in any size group (Table 5.2). The minimum of 0.33 g per day was recorded for the smallest and biggest size groups, while the maximum of 0.39g per day was recorded in the second biggest size group. When exuvia weight was added in calculating weight increase per day, it showed a positive correlation ($r = 0.952$; $\text{Log } Y = 2.407 + 0.503 \text{ Log mid body weight}$).

Table 5.1. Experimental conditions provided for feeding experiments on P. homarus

| Initial size | | No. of lobsters (replicates) | Size of aquarium tank (cm) | Volume of water (l) | Temp. (°C) | Salinity (ppt) | Dissolved oxygen (ml/l) | pH | Duration (days) |
|---|---|---|---|---|---|---|---|---|---|
| CL (mm) | Weight (g) | | | | | | | | |
| **Group I** | | | | | | | | | |
| 22.28 ± 1.41 | 13.17 ± 0.67 | 6 (4) | 90x45 | 150 | 26.63 ± 1.70 | 33.65 ± 0.48 | 4.12 ± 0.13 | 7.99 ± 0.02 | 156 |
| **Group II** | | | | | | | | | |
| 35.43 ± 1.19 | 48.00 ± 4.03 | 6 (4) | 90x60 | 200 | 26.63 ± 1.70 | 33.65 ± 0.48 | 4.12 ± 0.13 | 7.99 ± 0.07 | 156 |
| **Group III** | | | | | | | | | |
| 47.23 ± 0.79 | 100.83 ± 9.28 | 6 (4) | 90x60 | 200 | 26.25 ± 1.52 | 33.65 ± 0.48 | 4.09 ± 0.09 | 8.00 ± 0.10 | 205 |
| **Group IV** | | | | | | | | | |
| 58.20 ± 2.54 | 165.25 ± 3.96 | 4 (4) | 90x60 | 200 | 28.46 ± 1.04 | 33.23 ± 0.52 | 4.13 ± 0.10 | 8.03 ± 0.02 | 87 |
| **Group V** | | | | | | | | | |
| 66.00 ± 3.18 | 256.66 ± 17.48 | 6 (4) | 90x60 | 200 | 27.68 ± 1.21 | 33.27 ± 0.52 | 4.10 ± 0.08 | 8.04 ± 0.05 | 87 |

**Table 5.2.** Weight increase in different size groups of *P.homarus* fed with clam meat

| Parameters | Size groups | | | | |
|---|---|---|---|---|---|
| | Gr I | Gr II | Gr III | Gr IV | Gr V |
| Initial weight (g) | 13.17 ± 0.67 | 48.00 ± 4.03 | 100.83 ± 9.28 | 165.25 ± 13.96 | 256.66 ± 17.48 |
| Final weight (g) | 64.83 ± 11.23 | 102.33 ± 15.22 | 173.33 ± 21.76 | 199.08 ± 17.72 | 285.33 ± 35.59 |
| Mid body weight (g) | 39.00 | 75.17 | 137.08 | 182.17 | 271.00 |
| Number of moults | 5.25 | 4.25 | 3.86 | 1.50 | 1.50 |
| Exuvia weight (g) | 37.26 ± 5.83 | 73.57 ± 11.28 | 116.00 ± 19.03 | 95.96 ± 15.85 | 96.11 ± 38.21 |
| Weight increase without exuvia (g) | 51.67 ± 12.05 | 54.33 ± 15.36 | 72.50 ± 19.11 | 33.83 ± 6.34 | 28.67 ± 12.61 |
| Weight increase with exuvia (g) | 88.93 ± 13.74 | 127.90 ± 20.73 | 188.50 ± 35.39 | 129.79 ± 21.35 | 124.78 ± 46.96 |
| Weight increase per day without exuvia (g) | 0.33 ± 0.08 | 0.35 ± 0.10 | 0.35 ± 0.09 | 0.39 ± 0.10 | 0.33 ± 0.14 |
| Weight increase per day with exuvia (g) | 0.57 ± 0.09 | 0.82 ± 0.13 | 0.92 ± 0.17 | 1.48 ± 0.34 | 1.43 ± 0.54 |
| % wt lost as exuvia | 41.90 ± 6.56 | 57.52 ± 8.82 | 61.54 ± 10.09 | 73.78 ± 0.17 | 77.00 ± 30.62 |

### 5.1.2 Intermoult duration

Intermoult duration increased with size and also with subsequent moults in the same group. Considerable variations were observed within the groups also (Table 5.3).

### 5.1.3 Feeding rate

Feeding rate was maximum in the smallest size group (254.87 ± 9.04J/g body weight/day) and gradually reduced to 107.45 ± 11.21 J/g body weight/day in the biggest lobsters in this study, showing a negative correlation with increase in size (Fig.1, r=(-)0.982; Log Y = 7.140 + (-)0.426 Log mid body weight).

### 5.1.4 Assimilation

Assimilation efficiency was very high in all size groups (Table 5.4) ranging from 93.71 to 97.08% and did not show any size related variation. Due to high assimilation efficiency, assimilation rate was also very high in all sizes and, like feeding rate, showed a negative correlation (Fig.5.1) with size (r=(-) 0.988; Log Y = 7.124 + (-) 0.432 Log mid body weight).

### 5.1.5 Conversion

Conversion rate was calculated separately for growth (P) and exuvia (E) and were added to get total conversion rate (P+E). Conversion rate without exuvia was maximum in smallest size (Table 5.4) and decreased with

**Table 5.3.** Intermoult periods in different size groups of *P.homarus* fed with clam meat

| Moult details | Intermoult period (days) | | | | |
|---|---|---|---|---|---|
| | Gr I | Gr II | Gr III | Gr IV | Gr V |
| I Moult | 16.33 ± 7.32 | 17.00 ± 8.40 | 44.10 ± 12.86 | 24.01 ± 15.41 | 24.00 ± 23.86 |
| II Moult | 17.00 ± 8.40 | 34.75 ± 1.48 | 46.43 ± 8.26 | 43.50 ±. 9.53 | 38.33 ±. 6.12 |
| III Moult | 25.50 ± 6.18 | 36.00 ± 11.18 | 47.83 ± 4.71 | - | - |
| IV Moult | 30.28 ± 4.62 | 43.50 ± 9.53 | 51.83 ±. 2.63 | - | - |
| V Moult | 35.14 ± 5.05 | 43.00. | - | - | - |
| VI Moult | 40.20 ±. 2.20 | - | - | - | - |

. All lobsters did not complete the last moult.

**Table 5.4.** Feeding, assimilation, metabolic and conversion rates and assimilation and conversion efficiencies in *P. homorus*, fed with clam meat.

| Parameters | Rates and efficiencies in different groups | | | | |
|---|---|---|---|---|---|
| | Gr I | Gr II | Gr III | Gr IV | Gr V |
| **Feeding rate** (J/g live body wt/day) | 254.87 ± 9.04 | 204.88 ± 23.84 | 153.90 ± 14.24 | 149.99 ± 16.12 | 107.45 ± 11.21 |
| **Assimilation rate** (J/g live body wt/day) | 246.09 ± 35.73 | 198.35 ± 27.41 | 146.97 ± 14.74 | 140.56 ± 14.66 | 104.31 ± 11.26 |
| **Assimilation efficiency** (%) | 96.89 | 96.81 | 95.50 | 93.71 | 97.08 |
| **Conversion rate** (J/g live body wt/day) | | | | | |
| Exuvia (E) | 9.84 ± 1.54 | 10.08 ± 1.55 | 6.64 ± 1.09 | 7.97 ± 1.83 | 5.53 ± 2.20 |
| Growth (P) | 37.90 ± 8.85 | 21.90 ± 6.40 | 13.64 ± 3.30 | 10.86 ± 2.80 | 6.84 ± 3.00 |
| Exuvia + Growth (P+E) | 47.74 ± 7.37 | 31.99 ± 5.19 | 20.29 ± 3.79 | 18.88 ± 4.36 | 12.37 ± 4.66 |
| **Metabolic rate** | | | | | |
| J/g live body wt/day | 204.45 ± 28.40 | 171.12 ± 20.52 | 132.19 ± 9.55 | 130.98 ± 12.59 | 97.98 ± 1.78 |
| ml $O_2$/g live body wt/hr | 0.42 ± 0.06 | 0.35 ± 0.04 | 0.27 ± 0.02 | 0.27 ± 0.03 | 0.20 ± 0.01 |
| **Conversion efficiency** ($K_2$) (%) | | | | | |
| Exuvia (E/Ae) | 4.00 ± 0.62 | 5.07 ± 0.77 | 4.51 ± 0.73 | 5.66 ± 1.29 | 5.34 ± 0.85 |
| Growth (P/Ae) | 15.40 ± 3.59 | 11.01 ± 3.21 | 9.22 ± 2.89 | 7.77 ± 2.10 | 6.60 ± 2.69 |
| Exuvia+Growth (E+P/Ae) | 19.41 ± 2.99 | 16.08 ± 2.61 | 13.79 ± 2.58 | 13.42 ± 5.30 | 11.93 ± 4.49 |

size showing a negative correlation (Fig.5.1, r=(-) 0.997; Log Y = 6.834 + (-) 0.865 Log mid body weight).

Net conversion efficiency (without exuvia) was maximum (15.40%) in the smallest size and minimum (6.60%) in the biggest size, showing a negative correlation (Fig.5.1) with size (r=(-) 0.995; Log Y = 4.229 + (-) 0.43 Log mid body weight). The same trend prevailed when efficiency was calculated by including exuvia weight (Fig.5.1, r=(-) 0.995; Log Y=3.859 + (-) 0.247 Log mid body weight). Maximum net conversion efficiency ($K_2$) with exuvia was 19.41% in the smallest size group.

*P. homarus* loses an enormous quantity of converted food as exuvia. Mean loss in dry weight as exuvia (Fig.5.2) range from 47.05 to 82.76%, followed by wet weight (41.91 to 77.0%), protein (28.91 to 68.75%) and the least loss was as energy (17.76 to 44.68%) since the energy content of exuvia was very low (3.81 J/g dry weight).

### 5.1.6 Metabolic rate

Highest (204.45 J/g body weight/day) and lowest (97.98 J/g body weight/day) metabolic rates were calculated for highest and lowest feeding rates (Table 5.4) and the rates showed a negative correlation with size (Fig.5.1, r=(-)0.98; Log Y=6.688 + (-)0.364 Log mid body weight). Metabolic rate calculated as respiratory rate (ml $O_2$/g body weight/hr) showed a similar trend of negative correlation with size (Fig.5.1, r=(-) 0.978; Log Y=0.503 + (-) 0.365 Log mid body weight).

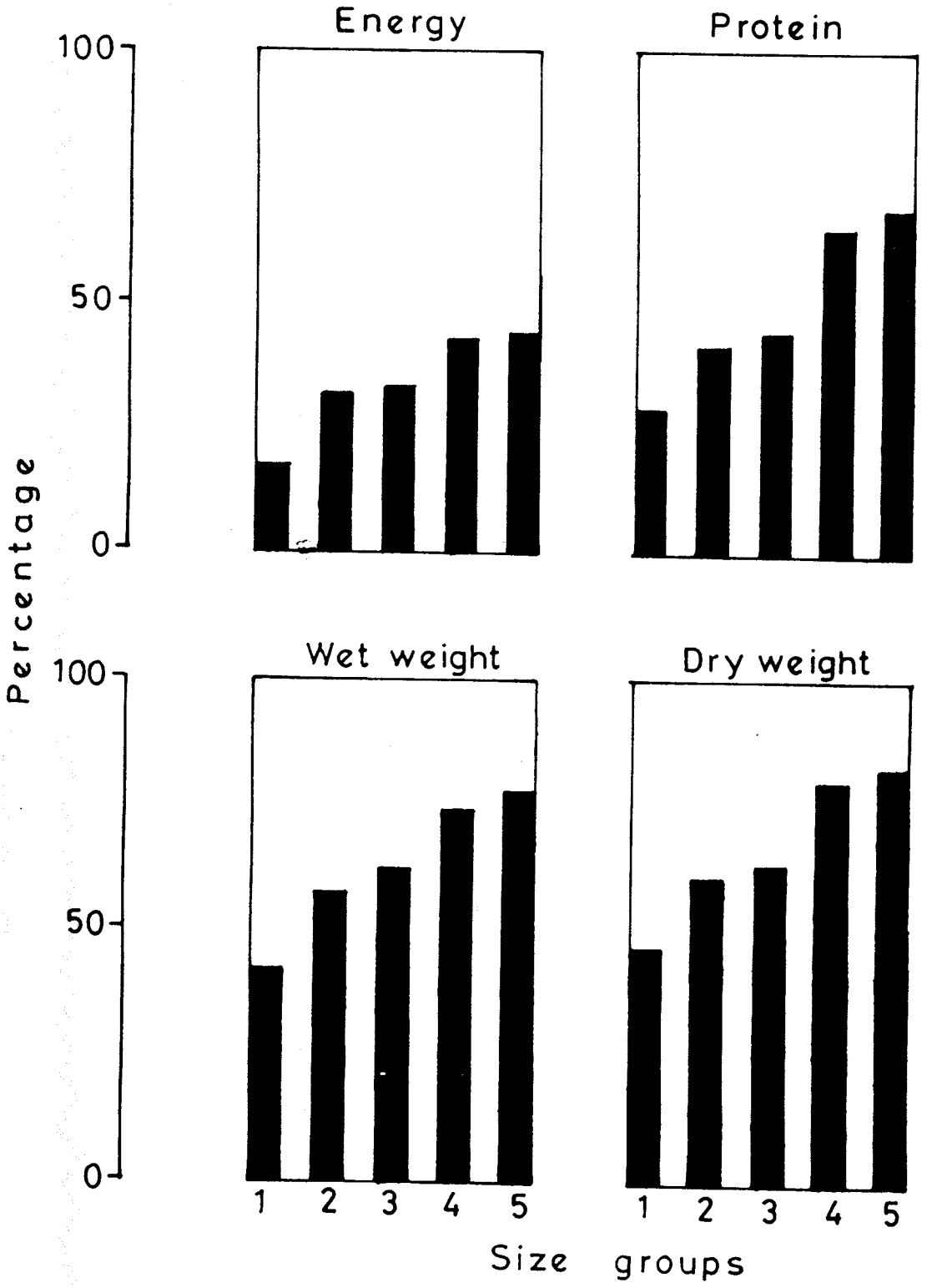


Fig.5.2 Percent loss as exuvia in total production in different size groups of *P.homarus*.

### 5.1.7 Protein utilization

Protein feeding rate, conversion rate, absorption rate, protein conversion efficiency (PCE) and protein efficiency ratio (PER) are given in table 5.5. All these parameters showed a similar pattern as energy utilization, showing negative correlation with increase in size (Fig.5.3). The relationships for these parameters are:

Protein feeding rate, r = (-) 0.995

Log Y = 3.934 + (-) 0.442 Log mid body weight

Protein absorption rate, r = (-) 0.988

Log Y = 3.904 + (-) 0.438 Log mid body weight

Protein conversion rate (P), r = (-) 0.989

Log Y = 3.70 + (-)1.071 Log mid body weight

Protein conversion rate (P+E), r=(-) 0.989

Log Y = 2.36 + (-)0.631 Log mid body weight

### 5.1.8 Food conversion ratio (FCR) and protein efficiency ratio (PER).

FCR for growth (P) (Fig.5.4) was lowest (1.79) in the smallest size and increased with size to 5.12 showing a positive correlation (r=0.991; Y=1.4674 + 0.014 mid body weight).

**Table 5.5.** Protein feeding rate, conversion rate, protein conversion efficiency and protein efficiency ratio in different size groups of *P.homarus* fed with clam meat

| Parameters | Protein Utilization | | | | |
|---|---|---|---|---|---|
| | Gr I | Gr II | Gr III | Gr IV | Gr V |
| Feeding rate (mg/g body wt/day) | 9.80 ± 1.43 | 7.70 ± 0.89 | 5.79 ± 0.54 | 5.48 ± 0.97 | 4.04 ± 0.44 |
| Assimilation rate (mg/g body wt/day) | 9.64 ± 1.37 | 7.62 ± 1.03 | 5.75 ± 0.57 | 5.45 ± 0.60 | 4.00 ± 0.43 |
| **Conversion rate (mg/g body wt/day)** | | | | | |
| Exuvia (E) | 0.29 ± 0.04 | 0.30 ± 0.04 | 0.20 ± 0.03 | 0.29 ± 0.06 | 0.20 ± 0.06 |
| Growth (P) | 0.72 ± 0.17 | 0.42 ± 0.12 | 0.25 ± 0.06 | 0.15 ± 0.04 | 0.09 ± 0.04 |
| Exuvia + Growth (E+P) | 1.01 ± 0.01 | 0.72 ± 0.07 | 0.45 ± 0.05 | 0.44 ± 0.05 | 0.29 ± 0.05 |
| **Protein conversion efficiency (PCE) (%)** | | | | | |
| Exuvia (E/C) | 2.96 ± 0.04 | 1.71 ± 0.02 | 3.45 ± 0.01 | 5.29 ± 0.01 | 4.95 ± 0.01 |
| Growth (P/C) | 7.35 ± 0.02 | 5.45 ± 0.62 | 4.32 ± 0.01 | 2.73 ± 0.72 | 2.23 ± 1.13 |
| Exuvia + Growth (E+P/C) | 10.31 ± 0.01 | 9.33 ± 0.03 | 7.77 ± 1.10 | 8.03 ± 1.92 | 7.17 ± 1.81 |
| **Protein efficiency ratio (PER)** | | | | | |
| Without exuvia | 0.89 ± 0.21 | 0.60 ± 0.17 | 0.45 ± 0.12 | 0.39 ± 0.10 | 0.31 ± 0.14 |
| With exuvia | 1.53 ± 0.24 | 1.41 ± 0.22 | 1.16 ± 0.22 | 1.49 ± 0.35 | 1.35 ± 0.51 |

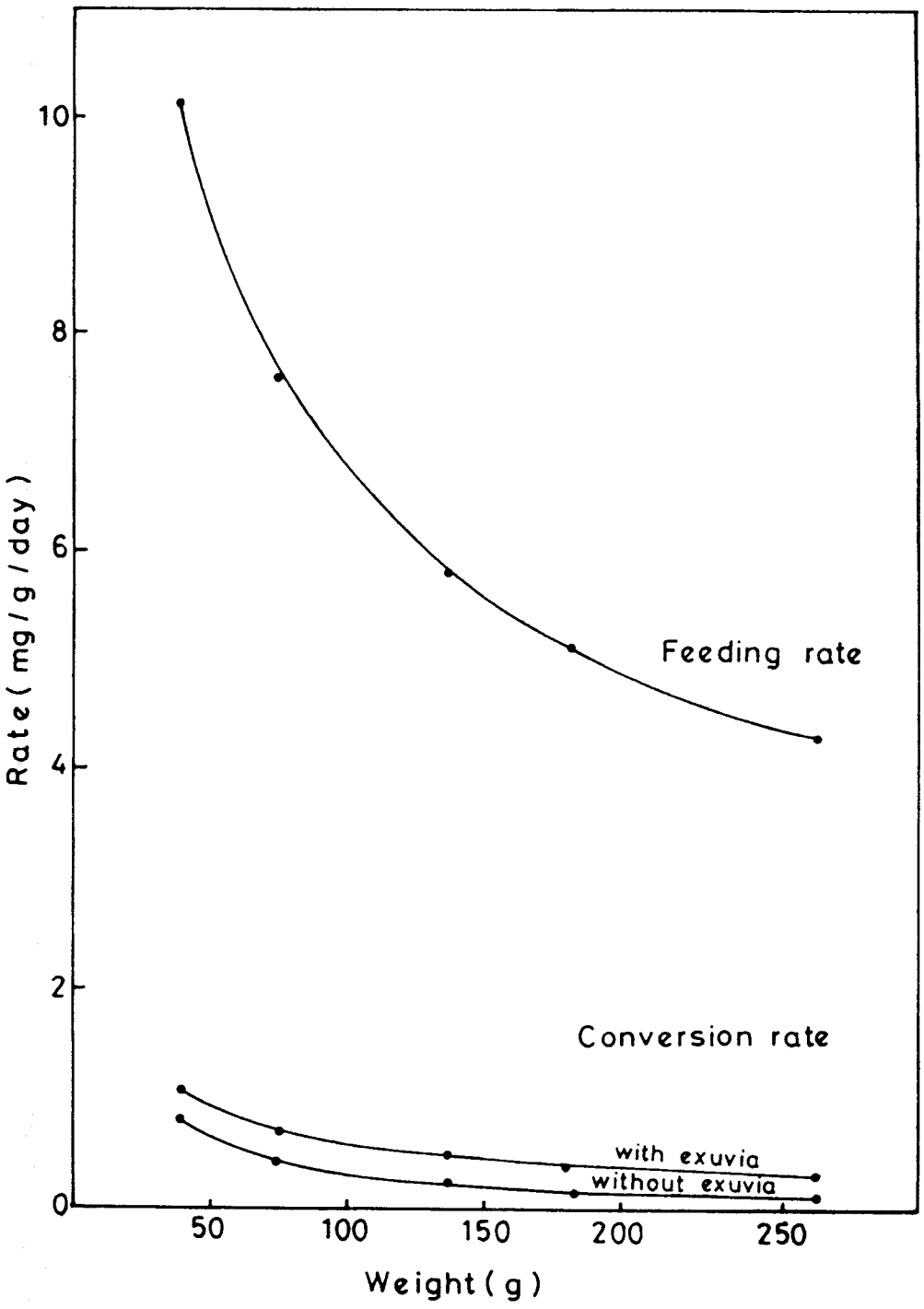


Fig.5.3 Protein feeding rate and conversion rate in different size groups of *P.homarus*.

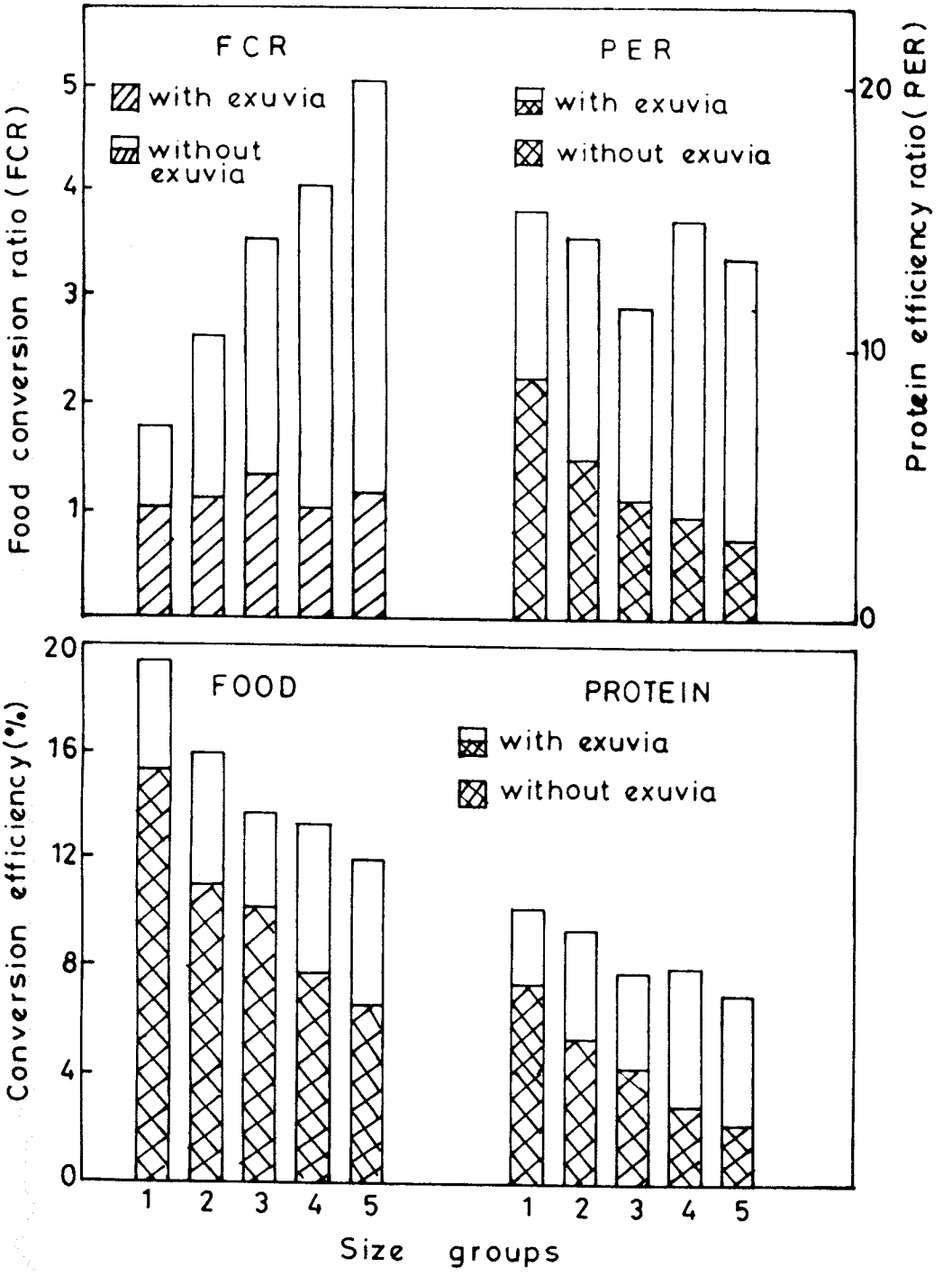


Fig.5.4 Food conversion efficiency, protein conversion efficiency, food conversion ratio and protein efficiency ratio in different size groups of *P.homarus*

PER for growth (P) (Fig.5.4) decreased with increase in size, showing a negative correlation (r = (-) 0.999, Log Y = 1.845 + (-) 0.538 Log mid body weight).

With size increase, loss of wet weight and protein as exuvia also increased progressively. Hence, FCR and PER, when calculated by including exuvia, did not vary much in different sizes and did not show any linear relationship.

### 5.1.9 Ammonia excretion

Ammonia excretion was maximum (12.24 $\mu$g N/g body weight/hr) in the small lobster (29.94 g) and minimum (4.6 $\mu$g N/g body weight/hr) in lobster weighing 264.84 g. Ammonia excretion decreased with size and was negatively correlated with weight (Fig.5.5, r=(-) 0.899; Log Y=3.96 + (-) 0.39 Log mid body weight).

## 5.2 DISCUSSION

Weight increase per day does not seem to vary with size but actual rate of increase is about 20 times more in the smallest size group (13.17g) compared to the biggest (256.3g). But, when exuvia weight is included in the calculation, the difference in rate of increase comes down to 7 times in the smallest lobster, due to higher loss of weight as exuvia as the weight of lobster increases.

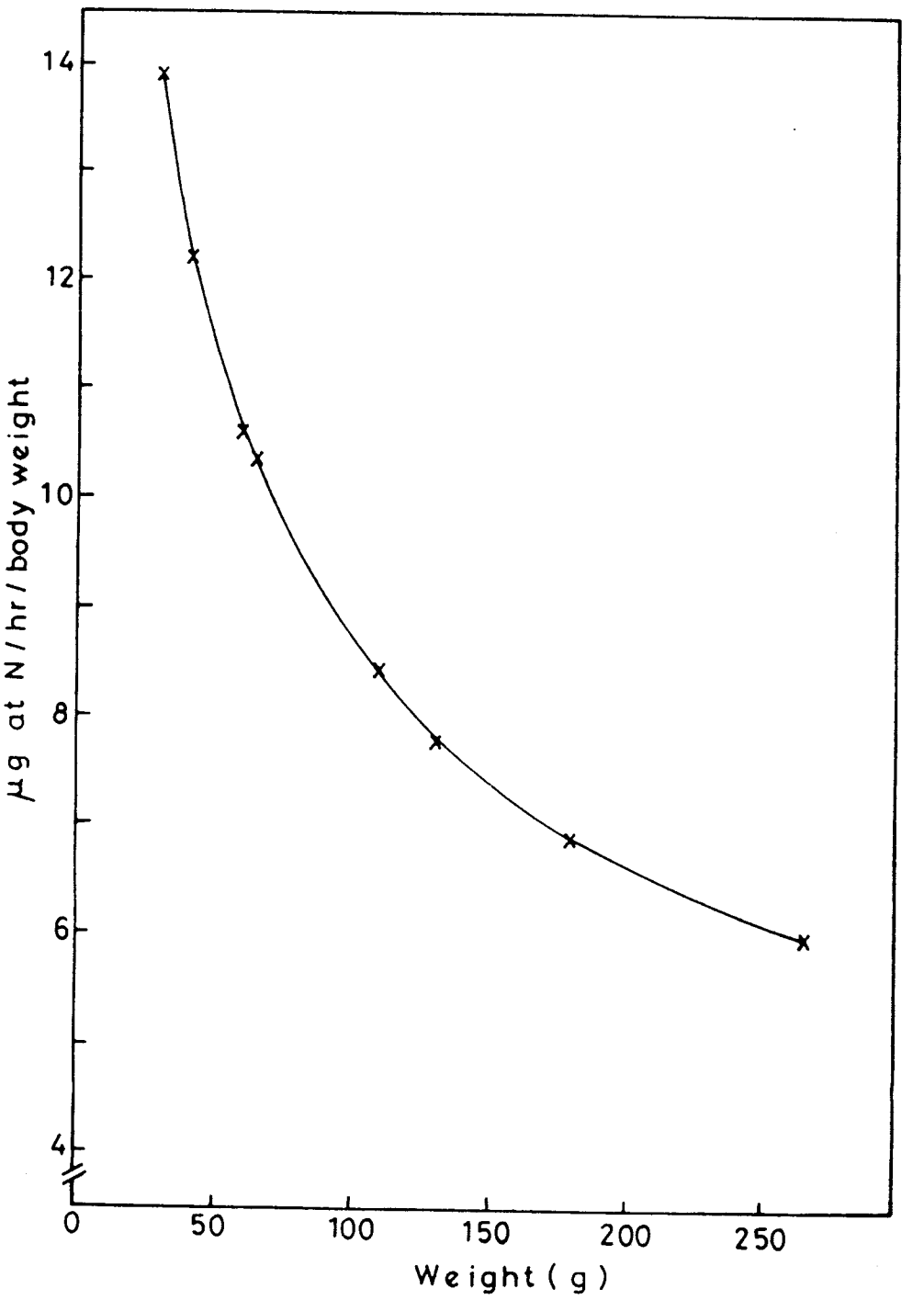


Fig.5.5 Ammonia excretion in relation to body weight in *P.homarus*.

Feeding rate recorded in the present observation is comparable to that of *P. homarus*, fed with clam meat under similar feeding schedule recorded earlier (Radhakrishnan, 1989) and is considerably low compared to the values reported for the same species by Vijayakumaran and Radhakrishnan, (1984). In the latter study, food (clam meat) was offered *ad libitum* twice a day, indicating that feeding rate is influenced by feeding schedule in *P. homarus*. The curvilinear relationship of decreasing feeding rate with increasing size recorded in this observation is similar to the relationships noted in many fishes (Menzel, 1960, Pandian 1967a) and in normal and eyestalk ablated *P. homarus* (Vijayakumaran and Radhakrishnan, 1984).

Assimilation efficiency seems to be very high in this study (93.7 to 97.08%) but is less than that reported for the same species (97.1 to 98.8%) by Radhakrishnan (1989). High assimilation effciencies above 90% have also been reported for many fishes and crustaceans (Gerking 1952, 1954, Menzel, 1960, Pandian 1967a, Daborn, 1975). Efficiency of assimilation did not show any size dependent relationship since feeding mechanisms and abiotic factors like salinity, temperature etc., are reported to affect assimilation efficiency in crustacea rather than size range within a species (Vernberg, 1987).

Conversion efficiencies reported here are significantly more than the efficiencies reported for the same species of different sizes in an earlier observation (3.4 to 10.0%, Vijayakumaran and Radhakrishnan, 1984). In the latter investigation, food was offered *ad libitum* twice daily and the feeding

rate was 33 to 83% higher compared to the present observation, suggesting that maximum feeding rate did not result in maximum conversion, an observation recorded in many fishes too (Pandian, 1967a, Gerking, 1971, Vivekanandan, 1976).

The relationship between metabolic rate and body size is an important factor in ecological bioenergetics. Decrease in metabolic rate with increase in body size, as observed in this study, has been attributed to body surface relations as well as physiological ageing of the animal (Brody, 1945, Bertalanffy, 1957).

Apart from size, physiological state of the animal has been shown to affect feeding rate and conversion resulting in fishes of equal size showing highly variable feeding and conversion. This difference is attributed to the physiological ageing of the species (Brody, 1945). Size at which an animal attains first maturity, is an important point when the deviation in size-nutritional relationships begins. *P. homarus* attains maturity at about 50 mm carapace length and 140g weight (personal observation). Different feed conversion parameters in this study are made based on mid body weight of the lobsters, which is the average of the body weight at the start and termination of the experiment. Mid body weight of the third group of lobsters in this observation, is 137.1g, size at which the lobster attains first maturity. An analysis of feeding, conversion and metabolic rates shows that between 39.0 to 137.1 g body weight, the rates declined by 39.6%, 64.0% and 35.34% respectively. But between 137.1g to 271.0g the equivalent drop in

feeding, conversion and metaboic rates are 29.3%, 49.8% and 25.87% respectively. Thus, in spiny lobster also, attainment of maturity brings about definite changes in feeding, conversion and metabolic rates.

Calculation of food conversion in crustacea is complicated by the intervention of exuvia shed periodically. Since the exuvia forms part of the converted food, it has to be included in energy budget studies. While some authors include this in production, others calculate the energy loss as exuvia separately (Vernberg, 1987). In this study, conversion rate was separately calculated for growth (P) and exuvia (E) and added to get a total picture of food conversion. Loss of weight as exuvia is considerably high in *P. homarus* and the percent loss increases curvilinearly with increase in size. Loss as exuvia is 83.76%, 75.89% and 39.84% more in the big lobster (256.3g) than the small one (13.17g) for wet weight, dry weight and protein respectively. The difference in energy loss is as high as 151.57% in the bigger *P. homarus* and due to these differences the regression coefficient is considerably low when conversion efficiency is calculated by including exuvia compared to growth (P) alone.

Protein conversion efficiency was considerably lower than net conversion efficiency in all size groups. This would suggest that a considerable amount of protein consumed is being utilized for metabolic purposes. The rate of loss of total body protein in exuvia for the shrimp *Crangon crangon* was estimated as 10% (Vergberg, 1987) and in *P. homarus*, in the small size groups (39.0 to 137.1g mid body weight), the loss was 28.91

to 44.32%. In bigger lobsters (182.20 to 271.0g mid body weight), 64.95 to 68.78% of consumed protein being lost as exuvia resulting in a negative protein balance.

The FCR recorded in this experiment, 1.79 to 5.12, compare favourably with those recorded for *P. homarus* and *P. ornatus* (4.1 to 4.7) in earlier studies (Vijayakumaran and Radhakrishnan, 1984, Radhakrishnan and Vijayakumaran, 1987, Radhakrishnan, 1989). But the values reported here are considerably low compared to FCR recorded in other decapods like *Homarus americanus* (45.1, Castel *et al.*, 1977), *Macrobrachium lanchesteri* (19.2, Ponnuchamy *et al.*, 1981) and *Macrobrachium nobili* (9.5, Pandian and Sindhukumari, 1985).

Protein efficiency ratio is negatively correlated to protein content of food in *H. americanus* (Capuzzo and Lancaster, 1979) and in prawns (Colvin, 1976, Sedgewick, 1979). In *H. americanus* PER decreased from 2.7 (protein content of feed - 16.7%) to 0.9% (protein content of feed - 51.0%). In this experiment protein content of food was constant (62.97% of dry weight) and the difference in PER was due to size. PER recorded here for growth (P), 0.89, compares with that recorded for *H. americanuus* on high protein (51.0%) food. With inclusion of exuvia PER did not show size dependent variation and ranged from 1.16 to 1.53.

Based on the estimates of various energy transfer parameters, total energy budgets were calculated for different size groups of *P. homarus* (Table 5.6). Of the total energy consumed, 2.92 to 4.51% was lost as faeces and

**Table 5.6.** Energy budgets for different size groups of *P.homarus* fed with clam meet.

| Size group | Energy parameters | | | | |
|---|---|---|---|---|---|
| | C | F+U | E | P | R |
| I (13.17g) | 100 | 3.45 ± 0.02 | 4.00 ± 0.62 | 15.40 ± 3.59 | 77.15 ± 10.27 |
| II (49.00g) | 100 | 2.94 ± 0.01 | 5.07 ± 0.75 | 11.01 ± 3.21 | 80.98 ± 9.17 |
| III (100.83g) | 100 | 4.51 ± 0.03 | 4.51 ± 0.73 | 9.22 ± 2.89 | 81.76 ± 5.65 |
| IV (165.25g) | 100 | 3.72 ± 0.01 | 5.66 ± 1.29 | 7.77 ± 2.10 | 82.85 ± 7.52 |
| V (256.66g) | 100 | 2.92 ± 0.03 | 5.34 ± 0.85 | 6.60 ± 2.69 | 85.14 ± 1.46 |

urine and this loss is not size dependent. Loss as faeces and urine in *P. homarus* is considerably low compared to that in the giant fairy shrimp, *Branchionecta gigas* (Daborn, 1975) which lost 14% as nitrogen excretion, and is more than the value recorded (2.2%) for the same species by Rahdakrishnan (1989).

About 4.0 to 5.66% of the energy consumed by *P. homarus* is lost as exuvia and the loss did not show any size dependent relationship. Values of energy lost as exuvia in some other groups are 6% in *B. gigas* (Daborn, 1975) and 7% for mysid *Metamysidopsis elongata* (Clutter and Theilacker, 1971).

6.6 to 15.4% of consumed energy was converted as body growth (P) and it showed a negative correlation with increase in size. In the fairy shrimp *B. gigas* the production rate was 22% (Daborn, 1975) and in group reared normal *P. homarus* of 105g weight it was 10.3% (Rahdakrishnan, 1989). Bulk of energy consumed was used for metabolism, ranging from 77.15% in the smallest to 85.14% in the biggest lobster in this study. The values increased with size and was inversely related to 'P'. In comparison, the fairy shrimp utilized 58% for maintenance which is considerably low compared to spiny lobsters. The difference of 11% in the energy budget for *B. gigas* was supposed to be spent for egg production (Daborn, 1975). In this experiment, mature *P. homarus* did not ovulate and hence energy cost of egg production could not be calculated. Since this is the first estimate of energy budget for any decapod in relation to size, the size-energy expenditure relationship could not be compared with any other species. A cursory look at

the energy budget of different size groups, reveals that the most important parameter that determines 'P' in all size groups, is the energy spent for metabolism (R), which increases with size. Eventhough metabolic rate is negatively correlated with size, the quantum of energy lost for metabolism is more in bigger lobsters and that is the main reason for the lower value of P in them.

# 6. BIOCHEMICAL AND MINERAL CHANGES DURING OVARIAN MATURATION IN THE PENAEID PRAWN, PENAEUS INDICUS

Study of reproductive physiology of cultivable crustaceans has assumed great importance recently to understand the intricate mechanisms involved in vitellogenesis or deposition of nutrient reserves in the ova, which has a direct bearing on the hatching success and survival of the larvae. The seasonality of gonadal development in many invertebrates has been associated with the storage of energy in different tissue with a concomitant transfer to gonads during gametogenesis (Giese, 1966 a,b, 1969, Lawrence, 1976, Lawrence *et al.*, 1979, Adiyodi, 1985).

As in many other crustaceans, the concentration and quantity of ovarian lipids have been reported to increase in terms of gonadosomatic index in many prawns (Jeckel *et al.*, 1989, Teshima *et al.*, 1989). Involvement of hepatopancreas in storage and mobilization of organic reserves, especially lipid have also been described in many species of penaeid prawns (Lawrence, *et al.*, 1979, Teshima *et al.*, 1988, 1989, Quackenbush, 1989). The only aspect of maturation process that has received least attention in prawns is the accumulation and utilization of mineral elements. In this investigation, biochemical and mineral changes in muscle, hepatopancreas and ovary during ovarian maturation have been studied in *Panaeus indicus*, one of the most important cultivable prawns in India. The

results have then been compared to similar changes in the spiny lobster, *Panulirus homarus* (Chapter 3) whose reproductive strategy is evidently different.

## 6.1 RESULTS

### 6.1.1 Maturity stages

Three maturity stages, immature, mature or ripe and spent, as described by Rao (1967) were taken for analysis. Morphological criteria used to identify the maturity stages are given in Table 6.1.

### 6.1.2 Biochemical changes in muscle

Percentage of muscle in total weight significantly reduced ($p = < 0.05$) at maturity and then recorded a marginal increase after spawning (Table 6.2). Water in the muscle of matured prawn declined from 75.85 to 72.55%, whereas it went up to 74.18% in the muscle of spent prawn. The only significant change in organic composition was reduction of lipid concentration ($p = < 0.05$) in the muscle of spent prawn from that of mature one. Concentration of ash significantly increased ($p = < 0.005$) at maturity with no further change after spawning.

The values were quantified and expressed in 100g body weight in Table 6.3 and Fig.6.1. Reduction in quantity of water at maturity and increase afterwards were both significant ($p = < 0.05$). Lipid declined

**Table 6.1.** Maturity stages and morphological criteria used to identity the stages during ovarian maturation in *P.indicus*

| Maturity stage | Morphological features |
|---|---|
| Stage 1 (Immature) | Ovary thin and slender, unpigmented and translucent and confined to the abdomen. Small spherical ova with clear cytoplasm and conspicuous grannules. Ova dia: 0.06-0.07 mm |
| Stage 4 (Mature or ripe) | Ovary dark green and visible through exoskeleton and occupy all the available space in cephalothorax and abdomen. Ova dia: 0.22 ± 0.02 mm |
| Stage 5 (Spent) | Fully spent, placid ovary taken just after spawning. |

**Table 6.2.** Biochemical changes during ovarian maturation in the muscle of *P.indicus* (Protein, lipid, carbohydrate and ash are expressed as % in dry weight)

| Maturity stage | Weight of prawn (g) | Muscle % in total weight | Water % | Protein | Lipid | Carbo-hydrate | Ash |
|---|---|---|---|---|---|---|---|
| Immature | 15.09 ± 3.62 | 52.53 ± 1.53 | 75.85 ± 2.93 | 81.21 ± 1.48 | 12.97 ± 3.53 | 1.74 ± 0.40 | 3.87 ± 0.50 |
| Ripe | 44.17 ± 11.06 | 46.22 ± 1.56 | 72.55 ± 1.32 | 79.20 ± 7.98 | 12.53 ± 2.10 | 2.19 ± 0.62 | 2.54 ± 0.41 |
| Spent | 33.49 ± 6.05 | 47.68 ± 1.15 | 74.18 ± 1.69 | 80.04 ± 6.97 | 9.77 ± 0.55 | 2.54 ± 0.41 | 6.85 ± 0.61 |

**Table 6.3.** Quantitative changes in proximate composition in the muscle of *P.indicus* during ovarian maturation (values expressed in 100g body weight). (values in parenthesis indicate 'P' values of test of significance (Students 't') between ripe and immature and ripe and spent).

| Maturity stage | Wet wt. of muscle (g) | Dry wt. of muscle (g) | Water (g) | Protein (g) | Lipid (g) | Carbo-hydrate (g) | Ash (g) | Energy (cal.) KJ |
|---|---|---|---|---|---|---|---|---|
| Immature | 52.53 ± 1.53 (<0.005) | 12.69 ± 0.37 (>0.05) | 39.84 ± 1.16 (<0.005) | 10.31 ± 0.30 (>0.05) | 1.65 ± 0.05 (<0.05) | 0.220 ± 0.006 (<0.0005) | 0.49 ± 0.01 (<0.0005) | 310.83 ± 9.12 (>0.05) |
| Ripe | 46.22 ± 1.56 | 12.69 ± 0.42 | 33.53 ± 1.13 | 10.05 ± 0.33 | 1.59 ± 0.05 | 0.280 ± 0.010 | 0.86 ± 0.03 | 303.53 ± 9.90 |
| Spent | 47.68 ± 1.15 (>0.05) | 12.31 ± 0.30 (<0.05) | 35.37 ± 0.85 (<0.05) | 9.85 ± 0.24 (>0.05) | 1.20 ± 0.30 (<0.005) | 0.310 ± 0.008 (<0.005) | 0.84 ± 0.02 (>0.05) | 284.05 ± 6.96 (<0.005) |

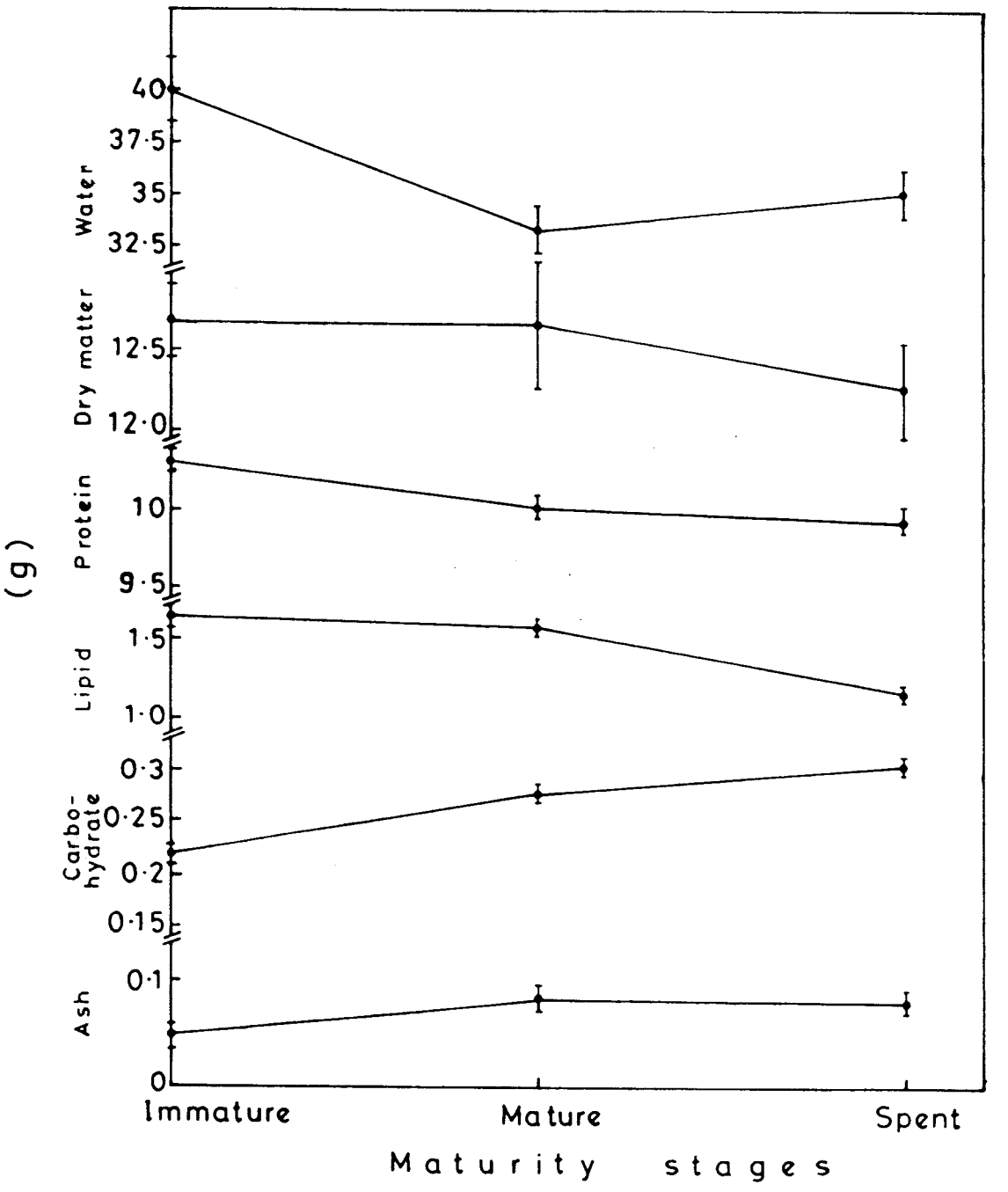


Fig.6.1 Quantitative changes in water, dry matter, protein, lipid, carbohydrate and ash in the muscle of *P.indicus* during ovarian maturation (values expressed in 100g body weight).

significantly both at maturity and spawning ($p = < 0.05$), while a reverse trend was seen in carbohydrates.

### 6.1.3 Biochemical changes in hepatopancreas

Tables 6.4 and 6.5 and Fig.6.2 represent the concentration and quantity of various biochemical parameters in hepatopancreas. Hepatic index decreased at maturity and increased after spawning, but the total dry matter increased significantly ($p = < 0.05$), both at maturity and after spawning. Quantity of water was less at maturity ($p = < 0.005$) and increased after spawning ($p = < 0.005$), but did not reach the value recorded at immature stage. Among the organic reserves, the most important observation was a 93.3% increase in the quantity of lipid (from 0.15 to 0.29 g) at maturity, which is a clear deviation from the general trend reported in many crustaceans. However, spawning resulted in a highly significant decrease ($p = < 0.005$) in hepatopancreatic lipid (37.93%). Carbohydrates and ash increased linearly in mature and spent animals.

### 6.1.4 Biochemical changes in ovary

Biochemical changes in ovary are presented in Table 6.6 (concentration) and Table 6.7 and Fig.6.3 (quantity). The concentrations do not reflect drastic quantitative changes happening in the ovary during maturation. This is due to the substantial increase in gonadosomatic index from 0.74 to 8.27 in mature prawn and a significant reduction to 2.2 after spawning. While the concentration of water reduced at maturity and

**Table 6.4.** Biochemical changes in hepatopancreas during ovarian maturation in *P. indicus* (Protein, Lipid, Carbohydrate and ash are expressed as % in dry matter)

| Maturity stage | Weight of prawn (g) | h.p. (% in wet weight) | Water % | Protein | Lipid | Carbohydrate | Ash |
|---|---|---|---|---|---|---|---|
| Immature | 15.09± 3.62 | 3.05± 0.64 | 75.15± 3.77 | 73.38± 8.32 | 20.15± 1.27 | 4.65± 2.24 | 3.87± 0.65 |
| Ripe | 44.17± 11.06 | 2.73± 0.10 | 68.35± 3.04 | 55.42± 2.46 | 33.15± 1.44 | 6.91± 0.70 | 6.58± 0.41 |
| Spent | 33.49± 6.05 | 3.19± 0.39 | 65.28± 2.82 | 68.50± 3.13 | 16.31± 1.25 | 6.89± 0.31 | 8.20± 0.38 |

**Table 6.5.** Quantitative changes in proximate composition during ovarian maturation in hepatopancreas of *P. indicus* (values expressed in 100g body weight) (values in parenthesis indicate 'P' values of test of significance (students 't') between ripe and immature and ripe and spent).

| Maturity stage | Wet weight of h.p (g) | Dry wt. of h.p. (g) | Water (g) | Protein (g) | Lipid (g) | Carbo-hydrate (g) | Ash (g) | Energy (calculated) (KJ) |
|---|---|---|---|---|---|---|---|---|
| Immature | 3.05± 0.64 (>0.05) | 0.76± 0.16 (>0.05) | 2.29± 0.48 (>0.05) | 0.56± 0.12 (>0.05) | 0.15± 0.03 (<0.005) | 0.035± 0.007 (<0.005) | 0.029± 0.006 (<0.005) | 19.69± 4.12 (>0.05) |
| Ripe | 2.73± 0.10 | 0.86± 0.03 | 1.87± 0.07 | 0.48± 0.02 | 0.29± 0.01 | 0.059± 0.001 | 0.057± 0.002 | 23.78± 0.87 |
| Spent | 3.19± 0.39 (<0.05) | 1.11± 0.14 (<0.05) | 2.08± 0.25 (>0.05) | 0.76± 0.10 (<0.05) | 0.18± 0.02 (<0.005) | 0.076± 0.010 (<0.005) | 0.091± 0.010 (<0.05) | 26.33± 3.33 (>0.05) |

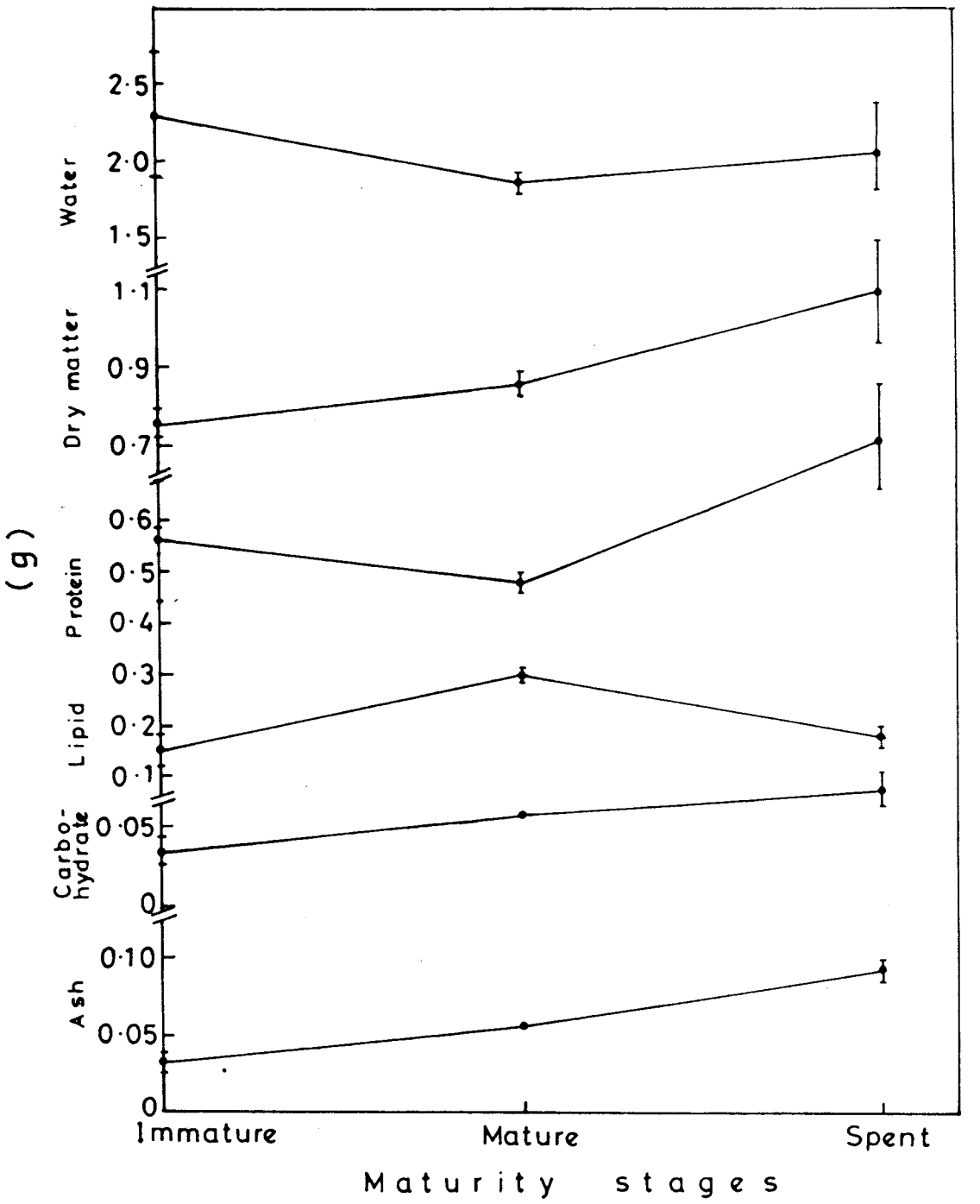


Fig.6.2 Quantitative changes in water, dry matter, protein, lipid, carbohydrate and ash in hepatopancreas of *P.indicus* during ovarian maturation (values expressed in 100g body weight)

**Table 6.6.** Biochemical changes in ovary during maturation in *P. indicus* (Protein, lipid, carbohydrate and ash are expressed as % in dry weight)

| Maturity stage | Weight of prawn (g) | Ovary % in total weight | Water % | Protein | Lipid | Carbohydrate | Ash |
|---|---|---|---|---|---|---|---|
| Immature | 15.09± 3.62 | 0.74± 0.45 | 71.97± 5.05 | 80.28± 1.92 | 10.60± 3.18 | 5.45± 0.72 | 3.76± 0.47 |
| Ripe | 44.17± 11.06 | 8.27± 1.26 | 64.40± 5.36 | 57.01± 1.51 | 35.69± 1.88 | 2.63± 0.26 | 6.63± 0.32 |
| Spent | 33.49± 6.05 | 2.22± 0.67 | 73.95± 4.85 | 57.44± 4.50 | 28.05± 4.50 | 5.56± 0.82 | 10.63± 1.56 |

**Table 6.7.** Quantitative changes in proximate composition in the ovary of *P. indicus* (values expressed in 100g body weight) (values in parenthesis indicate 'P' values of test of significance (students 't') between ripe and immature and ripe and spent).

| Maturity stage | Wet weight of ovary (g) | Dry wt. of ovary (g) | Water (g) | Protein (g) | Lipid (g) | Carbo-hydrate (g) | Ash (g) | Energy (calculated) (KJ) |
|---|---|---|---|---|---|---|---|---|
| Immature | 0.74± 0.45 (>0.0005) | 0.21± 0.13 (<0.0005) | 0.53± 0.32 (<0.0005) | 0.17± 0.10 (<0.0005) | 0.022± 0.010 (<0.0005) | 0.011± 0.001 (<0.0005) | 0.008± 0.005 (<0.0005) | 5.07± 2.75 (<0.0005) |
| Ripe | 8.27± 1.26 | 2.94± 0.45 | 5.33± 0.81 | 1.68± 0.26 | 1.050± 0.160 | 0.077± 0.010 | 0.195± 0.030 | 82.18± 12.58 |
| Spent | 2.22± 0.67 (<0.005) | 0.58± 0.17 (<0.0005) | 1.64± 0.50 (>0.0005) | 0.33± 0.10 (<0.0005) | 0.160± 0.050 (<0.0005) | 0.032± 0.010 (<0.005) | 0.062± 0.018 (<0.0005) | 14.64± 4.59 (<0.0005) |

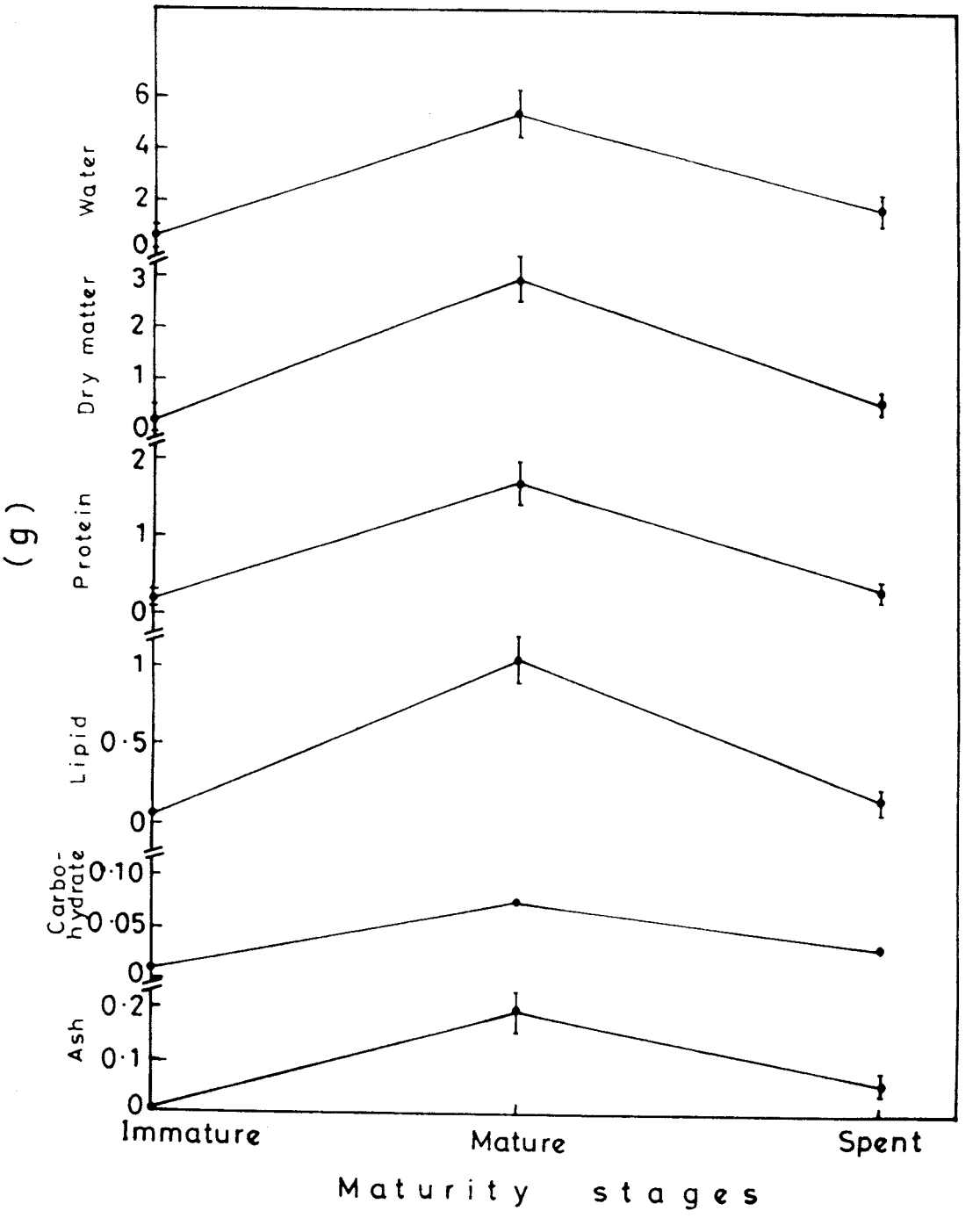


Fig.6.3 Quantitative changes in water, dry matter, protein, lipid, carbohydrate and ash in the ovary of *P.indicus* during maturation (values expressed in 100g body weight)

incresaed afterwards, the actual quantity increased at maturity (0.53 to 5.33g) and declined after spawning (1.66 g). Protein and lipid quantities went up from 0.17 and 0.022g to 1.68 g and 1.05g, respectively, at maturity and then reduced to 0.33g (protein) and 0.16g (lipid) in the spent ovary. Carbohydrates and ash also showed a similar trend.

### 6.1.5 Mineral changes in muscle

Minerals and trace elements could be analysed only in two maturity stages immature and mature in all tissues due to unfortunate loss of ashed samples of spent tissues.

Changes in concentration and quantities of minerals and trace elements in muscle during maturation are given in Table 6.8 and Figs. 6.4 and 6.5. Concentration and quantity of Na and K declined significantly ($p = < 0.005$) while the reduction in P was not significant at maturity. The other two major elements, Ca and Mg, increased significantly at maturity ($p = < 0.05$). The most important observation was a near complete decline (99.64%) in the quantity of K at maturity.

Among the trace elements, concentrations and quantities of Cu, Co and Mn declined significantly ($p = < 0.005$), while Fe, Zn, Cr, and Cd increased significantly in the muscle of mature prawn. As in *P.homarus*, Pb was below the detectable limit (0.001 $\mu$g/g dry weight). Most notable changes in trace elements composition were the drastic reduction in Cu (88.15%) from 245.17 $\mu$g to 29.06 $\mu$g and substantial increase (1633.33%) in Cd from 0.30 $\mu$g

**Table 6.8.** Changes in minerals and trace elements concentration (mg or μg/g dry weight) and quantity (values expressed in 100g body weight) in the muscle of *P.indicus* during ovarian maturation.

| Minerals/ Trace elements | Concentration (mg/g dry wt) for minerals and (μg/g dry wt) for trace elements | | Quantity (mg) for minerals and (μg) for trace elements | |
|---|---|---|---|---|
| | Immature | Ripe | Immature | Ripe |
| **Minerals** | | | | |
| Na | 16.36 ± 0.48 | 12.38 ± 0.41 | 207.60 ± 6.05 | 157.10 ± 5.20 |
| K | 16.74 ± 0.49 | 0.06 ± 0.01 | 212.43 ± 6.19 | 0.76 ± 0.03 |
| Ca | 2.94 ± 0.09 | 3.09 ± 0.10 | 37.31 ± 1.08 | 39.21 ± 1.30 |
| P | 22.00 ± 0.64 | 21.10 ± 0.70 | 279.18 ± 8.14 | 267.76 ± 8.86 |
| Mg | 1.06 ± 0.04 | 1.64 ± 0.05 | 13.45 ± 0.39 | 20.81 ± 0.69 |
| **Trace elements** | | | | |
| Fe | 23.80 ± 0.70 | 25.25 ± 0.84 | 302.02 ± 8.81 | 320.42 ± 10.60 |
| Cu | 19.32 ± 0.56 | 2.29 ± 0.10 | 245.17 ± 7.15 | 29.06 ± 0.96 |
| Zn | 77.00 ± 2.20 | 114.00 ± 4.77 | 980.00 ± 30.0 | 1450.0 ± 50.0 |
| Cd | 0.024 ± 0.01 | 0.41 ± 0.01 | 0.30 ± 0.01 | 5.20 ± 0.17 |
| Co | 0.79 ± 0.02 | 0.72 ± 0.02 | 10.03 ± 0.29 | 9.14 ± 0.30 |
| Mn | 1.15 ± 0.03 | 0.93 ± 0.04 | 14.59 ± 0.43 | 11.80 ± 0.39 |
| Cr | 0.65 ± 0.02 | 0.88 ± 0.04 | 8.25 ± 0.24 | 11.17 ± 0.37 |
| Pb | < 0.001 | <0.001 | <0.001 | <0.001 |

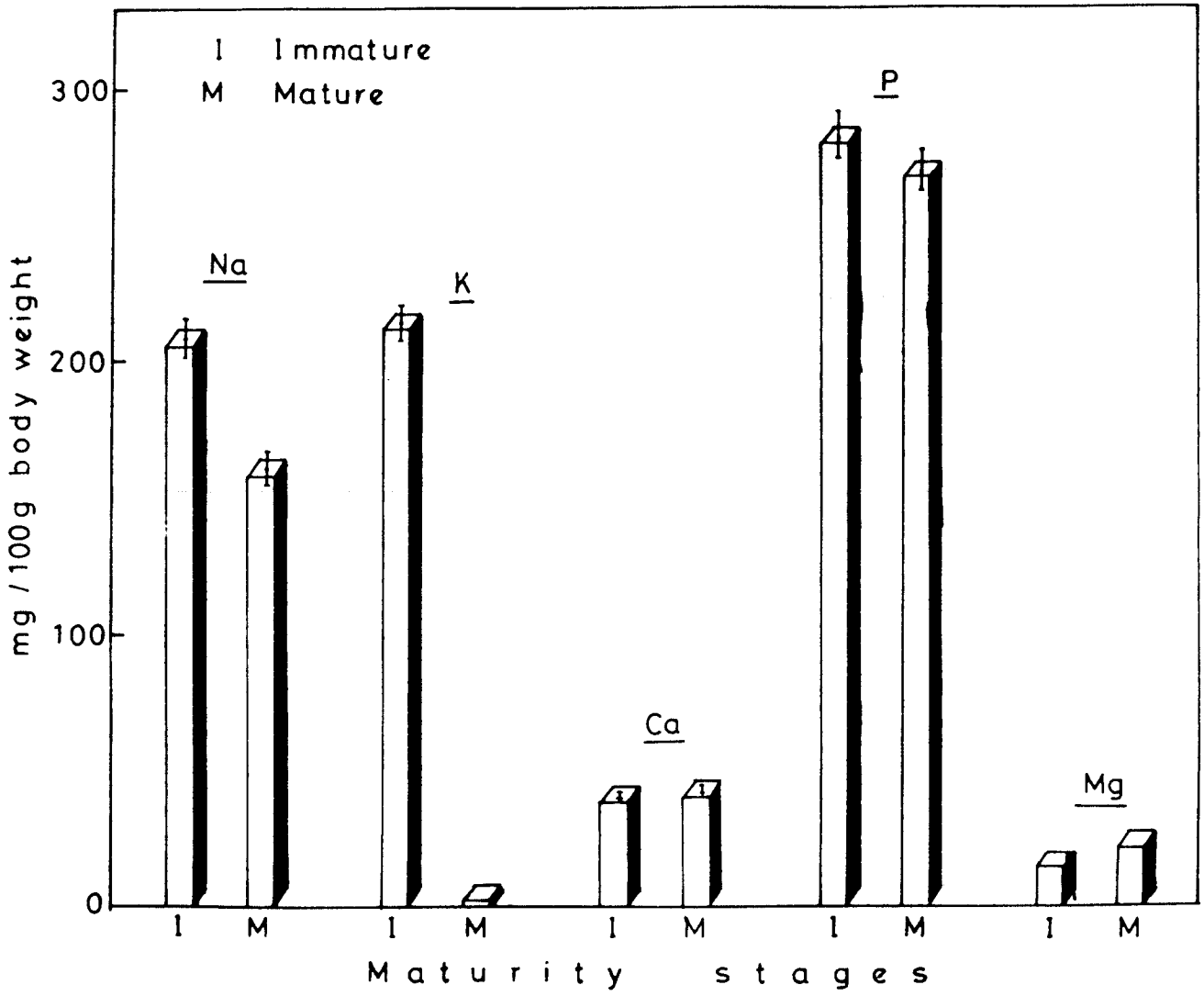


Fig.6.4 Quantitative changes in minerals in muscle during ovarian maturation in *P.indicus* (values expressed in 100g body weight).

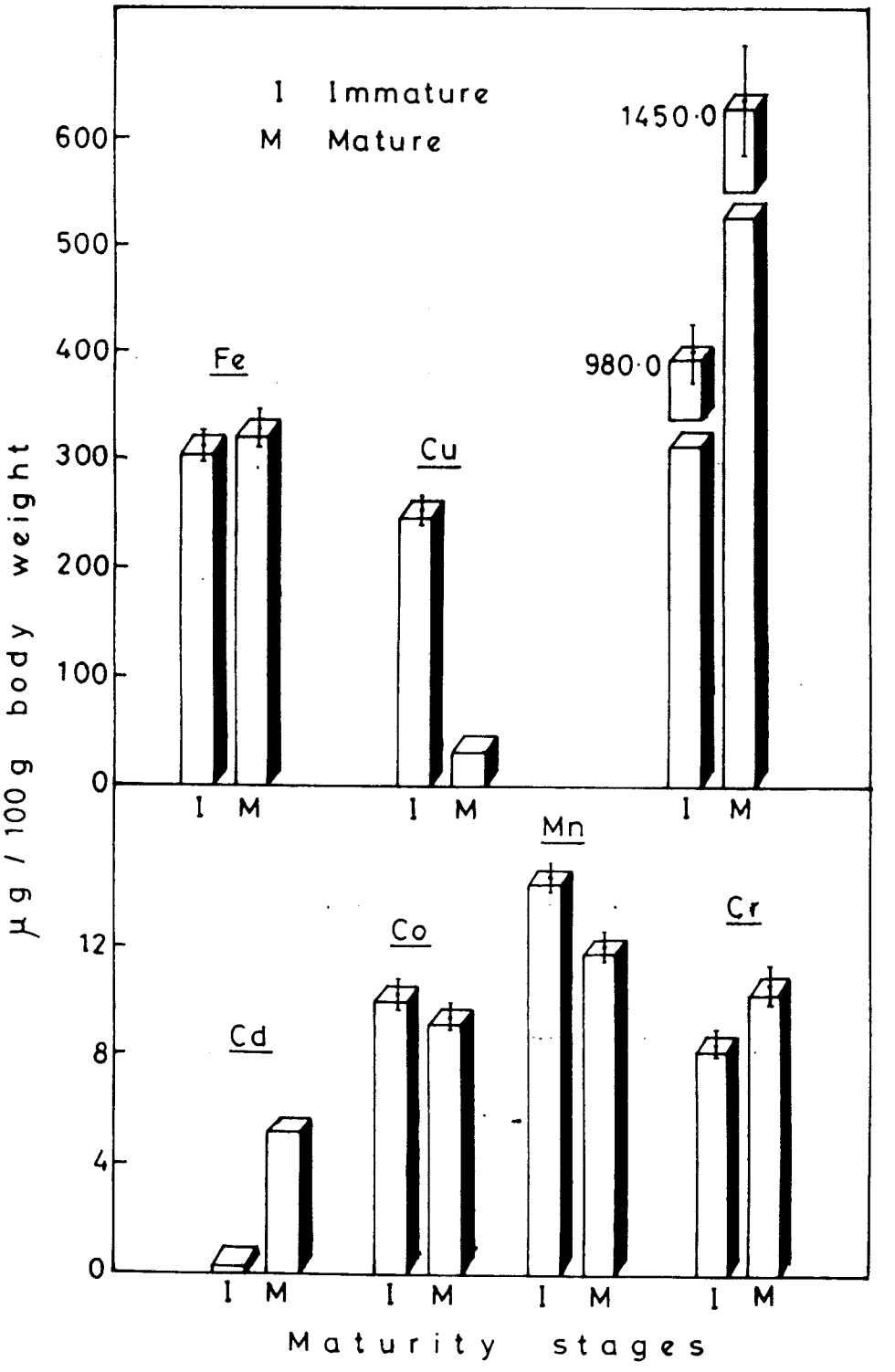


Fig.6.5 Quantitative changes in trace elements in muscle during ovarian maturation in *P.indicus* (values expressed in 100g body weight).

to 5.20 μg and Zn (47.97%) from 980 μg to 1450 μg in the muscle of mature prawn.

### 6.1.6 Mineral changes in hepatopancreas

Except Na, the concentration and quantity of which declined, though not significantly (p= >0.05), all other major elements, K, Ca, P, and Mg, increased in concentration as well as in quantity at maturity in hepatopancreas of *P.indicus* (Table 6.9 and Fig.6.6).

Fe, Cu, Zn and Cd increased significantly (p = < 0.005) both in concentration and quantity at maturity, while the increase was not significant for Mn (p = > 0.05). Concentration and quantity of Co and Cd decreased significantly (p = < 0.05) at maturity, while Pb was below the detectable level.

Most important changes were the substantial increase in Cd (1257.5%), Cu (3122.82%) and Fe (128.8%) in the hapatopancreas of mature prawns (Fig.6.7).

### 6.1.7 Mineral changes in ovary

Mineral changes in ovary, concentration and quantity, are given in Table 6.10. Major elemental composition of immature ovary could not be analysed due to inadequate quantity of sample and comparison could be made only in the case of trace elements. Concentrations of trace elements reduced significantly (p= <0.005) at maturity, while the quantity recorded significant

**Table 6.9.** Changes in minerals and trace elements concentration (mg or μg/g dry weight) and quantity (values expressed in 100g body weight) in hepatopancreas of *P.indicus* during ovarian maturation.

| Minerals/ Trace elements | Concentration (mg/g dry wt) for minerals and (μg/g dry wt) for trace elements | | Quantity (mg) for minerals and (μg) for trace elements | |
|---|---|---|---|---|
| | Immature | Ripe | Immature | Ripe |
| **Minerals** | | | | |
| Na | 21.12 ± 3.86 | 18.01 ± 0.63 | 16.05 ± 3.38 | 15.49 ± 0.54 |
| K | 9.46 ± 1.62 | 10.28 ± 0.34 | 7.19 ± 1.51 | 8.84 ± 0.31 |
| Ca | 5.13 ± 0.82 | 6.15 ± 0.18 | 3.90 ± 0.82 | 5.29 ± 0.18 |
| P | 25.37 ± 3.48 | 33.02 ± 1.22 | 19.28 ± 4.06 | 28.40 ± 0.99 |
| Mg | 1.72 ± 0.36 | 2.26 ± 0.09 | 1.31 ± 0.28 | 1.94 ± 0.07 |
| **Trace elements** | | | | |
| Fe | 86.73 ± 10.32 | 175.31 ± 6.10 | 65.90 ± 13.9 | 150.76 ± 3.26 |
| Cu | 41.56 ± 5.78 | 1183.82 ± 46.23 | 31.59 ± 6.70 | 1018.9 ± 35.51 |
| Zn | 261.0 ± 25.02 | 363.00 ± 11.62 | 200.0 ± 34.0 | 310.0 ± 10.0 |
| Cd | 8.33 ± 1.76 | 99.92 ± 3.62 | 6.38 ± 1.21 | 85.93 ± 3.00 |
| Co | 1.03 ± 0.19 | 0.13 ± 0.01 | 6.81 ± 0.11 | 0.11 ± 0.04 |
| Mn | 4.20 ± 0.65 | 4.21 ± 0.17 | 3.19 ± 0.58 | 3.62 ± 0.13 |
| Cr | 1.35 ± 0.18 | 0.77 ± 0.26 | 1.03 ± 0.17 | 0.66 ± 0.02 |
| Pb | < 0.001 | < 0.001 | < 0.001 | < 0.001 |

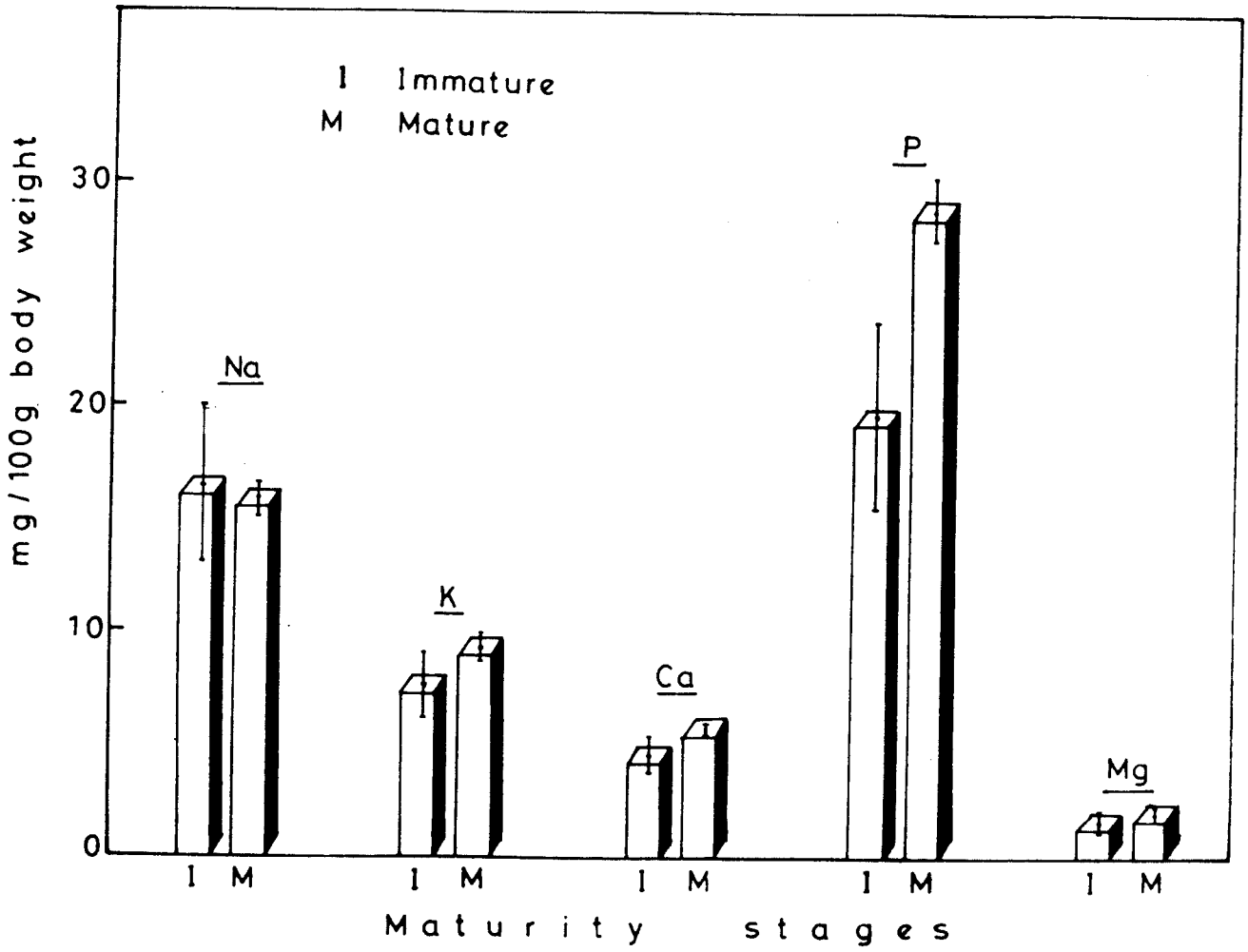


Fig.6.6 Quantitative changes in minerals in the hepatopancreas of *P.indicus* during ovarian maturation. (values expressed in 100g body weight).

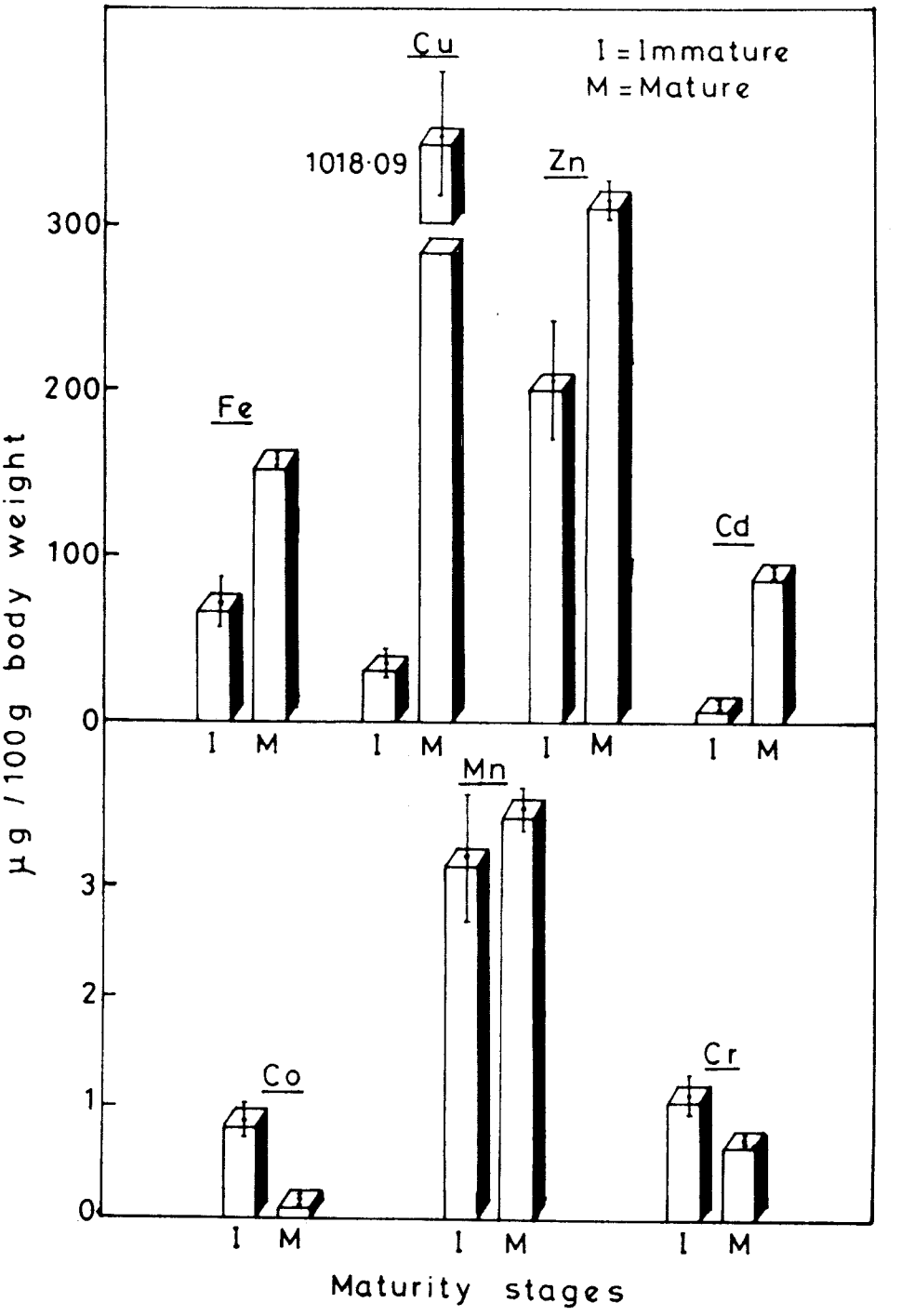


Fig.6.7 Quantitative changes in trace elements in the hepatopancreas of *P.indicus* during ovarian maturation (values expressed in 100g body weight).

**Table 6.10.** Changes in minerals and trace elements concentration (mg or μg/g dry weight) and quantity (values expressed in 100g body weight) in the ovary of *P.indicus* during maturation

| Minerals/ trace elements | Concentration (mg/g dry wt) for minerals and (μg/g dry wt) for trace elements | | Quantity (mg) for minerals and (μg) for trace elements | |
|---|---|---|---|---|
| | Immature | Ripe | Immature | Ripe |
| **Minerals** | | | | |
| Na | NE | 12.50 ± 0.76 | NE | 36.75 ± 5.62 |
| K | NE | 11.83 ± 1.10 | NE | 34.78 ± 5.32 |
| Ca | NE | 3.27 ± 0.42 | NE | 9.61 ± 1.47 |
| P | NE | 21.67 ± 1.68 | NE | 63.71 ± 9.81 |
| Mg | NE | 2.27 ± 0.22 | NE | 6.67 ± 1.02 |
| **Trace elements** | | | | |
| Fe | 134.86 ± 4.22 | 97.76 ± 5.62 | 28.32 ± 10.53 | 287.41 ± 23.49 |
| Cu | 106.25 ± 3.51 | 21.88 ± 1.22 | 22.31 ± 6.81 | 64.33 ± 5.85 |
| Zn | NE | 220.0 ± 9.62 | NE | 651.0 ± 50.0 |
| Cd | 1.97 ± 0.16 | 0.56 ± 0.06 | 0.41 ± 0.15 | 1.65 ± 0.15 |
| Co | 3.62 ± 0.27 | 2.07 ± 0.17 | 0.76 ± 0.27 | 0.61 ± 0.06 |
| Mn | 11.18 ± 1.01 | 7.79 ± 0.26 | 2.35 ± 0.92 | 22.90 ± 2.11 |
| Cr | 1.97 ± 0.06 | 0.64 ± 0.16 | 0.41 ± 0.12 | 1.88 ± 0.16 |
| Pb | < 0.001 | <0.001 | <0.001 | < 0.001 |

NE - Not estimated

increase ($p = < 0.005$), for all except Co which declined marginally. In ovary also, Pb was below the detectable level ( < 0.001 $\mu$g/g dry weight).

Most striking increases in quantity were noticed in the quantities of Fe (914.87%) and Mn (874.47%) in the mature ovary. Increases were also recorded for other elements i.e. Cu - 188.47%, Cd - 302.44%, and Cr-358.54%. The quantity of Zn was maximum among the trace elements (651 $\mu$g) in the mature ovary, but the percentage of increase could not be calcualted since Zn was not measured in immature ovary (Fig.6.8).

## 6.2 DISCUSSION

Progressive increase in gonadosomatic index (GSI) at advancing maturity stages have been reported in many prawns (Pillay and Nair, 1971., Clarke, 1977., Lawrence *et al.*, 1979., Middledith, *et al.*, 1979., Teshima and Kanazawa, 1983., Asokan and George, 1984., Jeckel *et al.*, 1989., Teshima *et al.*, 1989). In *P.indicus* the GSI increased with maturity and the values recorded (0.74, 8.27 and 2.2 respectively for immature, mature and spent prawns) were almost similar to those reported for the same species on induced maturation by Asokan and George, (1984) (0.5, 8.5 and 1.5 for immature, mature and spent respectively). GSI of *P.indicus* observed in these studies are considerably low compared to the maximum value of 14.43 reported for wild *Penaeus japonicus* (Teshima *et al.*, 1989). Ovary index of *P.indicus* increased with maturation independent of the size of prawn, a general observation made in all prawns.

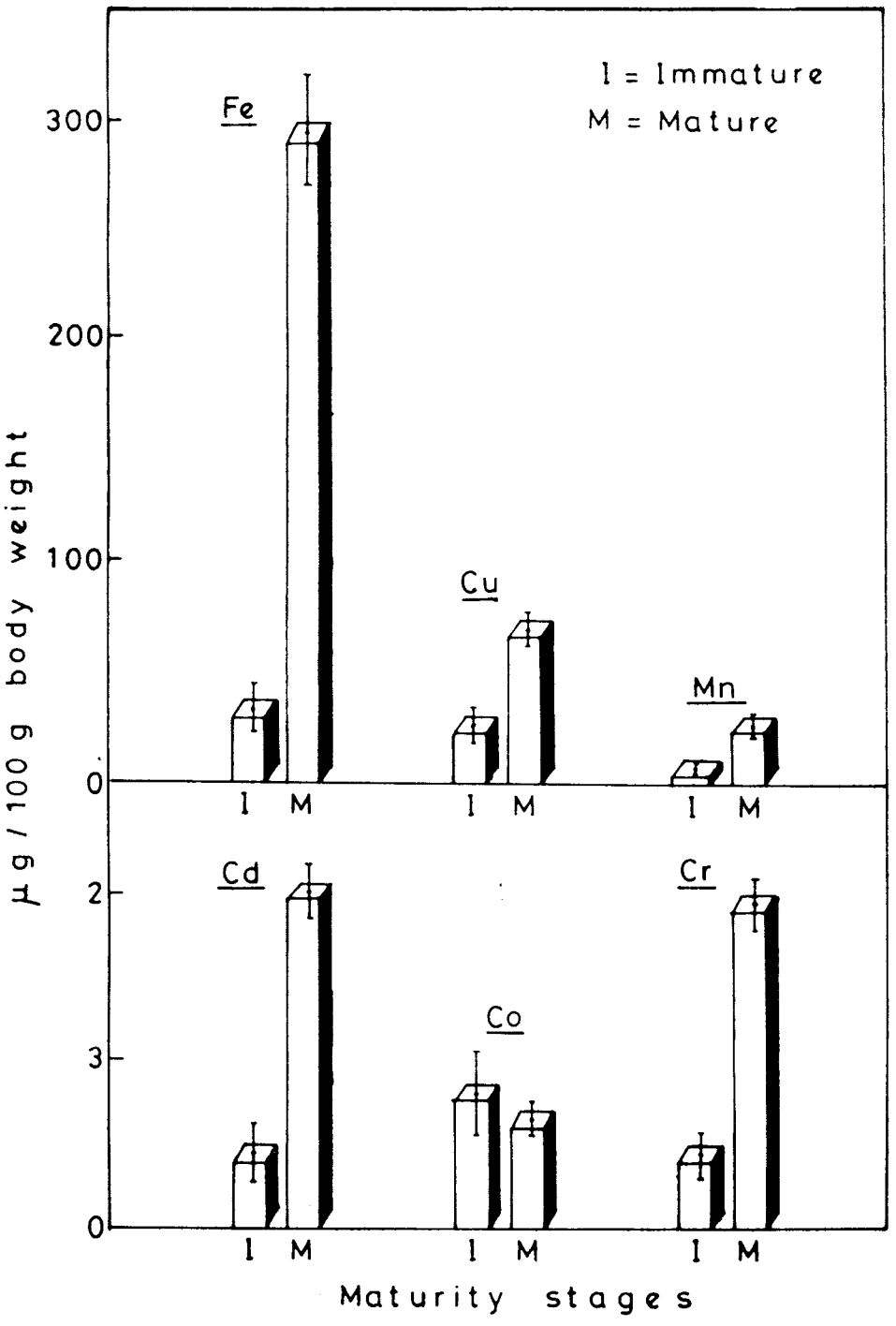


Fig.6.8 Quantitative changes in trace elements in ovary during maturation in *P.indicus* (values expressed in 100g body weight).

Increase in mass of ovary during maturation in *P.indicus* is associated with a simultaneous increase in organic reserves, a condition reported in many other crustaceans. Main energy sources of mature ovary are lipid and protein which together contributed more than 99% of energy. Eventhough lesser in quantity (1.05g/100g body weight in mature ovary), lipid contributed more energy than protein which was more in quantity (1.68g/100g body weight in the mature ovary). Only Teshima *et al.*, (1989) have quantified increase in total lipid in ovary of prawns, and in their report on *P.japonicus*, total lipid of the ovary increased from 0.18 g to 1.19 g in mature ovary. In *P. indicus*, ovarian lipids increased to 1.05g at maturity from 0.022g, an increase similar to the one reported in *P. japonicus*.

Hepatopancreas, along with ovary, is considered as a storehouse of total lipids in crustaceans. The total lipids in hepatopancreas is reported to increase in early maturity and decline at mature stage in many prawns and other crustaceans (Gehring, 1974., Lawrence *et al.*, 1979., Teshima and Kanazawa, 1983., Adiyodi, 1985., Teshima *et al.*, 1989). On the contrary, the present study indicates a highly significant increase ($p = < 0.0005$), amounting to 92.33% at maturity in *P. indicus*. In *P. japonicus* (Teshima and Kanazawa, 1983., Teshima *et al.*, 1989) and in *Penaeus duorarum* (Gehring, 1974), ovary maturation was followed through different development stages up to maturity. Maximum lipid in the ovary, in these prawns, were recorded in Stage 3 (late maturing) and declined significantly in Stage 4 (mature). In the present observation, only stage 1 (immature) and stage 4 (mature) were analysed and a clear inference could be drawn only after analysing late

mature stage of ovary. 37.93% of total lipid was lost at spawning in *P. indicus* and this is comparable to the loss of 50% (Teshima *et al.*, 1989) and between 16.98 and 60.32% lipid in different breeding seasons in *P.japonicus* (Teshima and Kanzawa, 1983). A non-significant ($p = > 0.05$) decrease was noticed in the total quantity of protein in the hepatopancreas at maturity.

Muscle seems to play an useful role in storage of protein and lipids for ovarian maturation in *P.indicus*, quite different from other crustaceans in which it is not considered to be actively involved in storage and mobilization of organic reserves during maturation (Lawrence *et al.*, 1979). In many fishes, however, reserves stored in muscles are utilized for maturation (Love, 1980). Asokan and George (1984) also have reported a fall in protein and lipids of muscle with simultaneous increase in ovary in eyestalk ablated *P.indicus*. They explained that this may possibly be due to hastening of maturation of eyestalk ablated prawns, unlike the slow maturation process in the wild.

A summary of changes in the proximate and mineral composition up to maturity and in proximate composition between maturity and spent condition are given in Table 6.11. The loss of protein and lipid in muscle and protein in hepatopancreas contribute only a fraction of the enormous accumulation of these reserves in the ovary. This suggests that main energy for maturation of ovary should come from elsewhere, probably from food intake. Thus, reproductive output might well be reduced under low feeding

**Table 6.11.** Summary of changes in proximate composition and minerals and trace elements (values expressed in 100g body weight) in muscle, hepatopancreas and ovary during ovarian maturation in *P.indicus*

| Parameter | Immature to Ripe | | | Ripe to Spent | | |
|---|---|---|---|---|---|---|
| | Muscle | Hepato-pancreas | Ovary | Muscle | Hepato-pancreas | Ovary |
| Wet weight (g) | -6.31 | -0.32 | +7.53 | +1.46 | +0.460 | -6.05 |
| Dry weight (g) | 0.00 | +0.10 | +2.73 | -0.38 | +0.250 | -2.36 |
| Water (g) | -6.31 | -0.42 | +4.80 | +1.84 | +0.210 | -3.69 |
| Protein (g) | -0.26 | -0.08 | +1.51 | -0.20 | +0.260 | -1.35 |
| Lipid (g) | -0.06 | +0.14 | +1.03 | -0.39 | -0.110 | -0.89 |
| Carbohydrate(g) | +0.06 | +0.03 | +0.07 | +0.03 | +0.017 | -0.05 |
| Ash (g) | +0.37 | +0.03 | +1.94 | -0.02 | +0.034 | -1.89 |
| Energy (KJ) | -7.32 | +4.09 | +77.11 | -19.48 | +2.550 | -67.54 |
| Na (mg) | -50.50 | -0.56 | - | - | - | - |
| K (mg) | -211.67 | +1.65 | - | - | - | - |
| Ca (mg) | +1.91 | +1.39 | - | - | - | - |
| P (mg) | -11.42 | +9.12 | - | - | - | - |
| Mg (mg) | +7.36 | +0.63 | - | - | - | - |
| Fe (μg) | +18.40 | +84.86 | +259.11 | - | - | - |
| Cu (μg) | -216.11 | +986.50 | +42.02 | - | - | - |
| Zn (μg) | +470.00 | +110.00 | - | - | - | - |
| Cd (μg) | +4.90 | +79.60 | +1.24 | - | - | - |
| Co (μg) | -0.87 | -0.70 | -0.15 | - | - | - |
| Mn (μg) | -2.79 | +0.43 | +20.55 | - | - | - |
| Cr (μg) | +2.92 | -0.37 | +1.42 | - | - | - |

conditions, endorsing the opinion of Patel and Crisp (1960) that a number of warm water crustaceans develop gonad and breed rapidly, only when they are fed under appropriate temperature conditions.

It would be worthwhile to compare the biochemical changes during ovarian maturation and energetic cost of egg production in the spiny lobster, *P. homarus* and the prawn, *P. indicus* inhabiting the same area. The spiny lobster is a slow growing one and attains higher size compared to the fast growing *P.indicus*. The reproductive strategy also differs in these two species. *P.homarus* produces larger eggs that are carried in the pleopods until phyllosoma larvae are releasedd (22 to 27 days after spawning). *P.indicus*, on the other hand, produces smaller eggs which are released to the surrounding water and these eggs develop into a non-feeding nauplius larva within 15 hours.

*P. indicus* gains 77.11 KJ/100 g body weight in the ovary and loses 67.54 KJ at spawning. A further 19.48 KJ are lost from the muscle making a total loss of 87.04 KJ at spawning. In *P.homarus*, the ovary gains 67.23 KJ/100g body weight at maturity and loses 61.2 KJ at spawning. Muscle does not lose energy at spawning in *P.homarus*, but the hepatopancreas loses 5.65 KJ, making a total loss of 66.85 KJ. Thus, the energetic cost of egg production is 30.2% more in *P.indicus* than in *P.homarus*. But the role of female parent ceases with egg release in *P.indicus*, while the female of *P.homarus* has to carry, protect, prune and ventillate the egg by constant beating of pleopods for 22 to 27 days. The

metabolic cost of egg carriage is yet to be quantified in *P. homarus*. However, it can be safely assumed that *P. indicus* spends more energy for egg production and spawning than *P. homarus*.

In *P.homarus*, like other crustaceans, muscle does not seem to play a prominent role during maturation, while the muscle of *P.indicus* contributes prominantly for the energetic cost of reproduction. This might possibly be due to the faster maturation process in *P.indicus*, where it may be necessary for the prawn to depend on the single largest tissue that has about 15 times more energy than the prominent storehouse of organic reserves - the hepatopancreas.

This study along with the one in *P.homarus* is a maiden attempt as regards to accumulation and utilization of minerals and trace elements in different tissues during ovarian maturation in crustacea.

The quantity of ash increased highly significantly ($p=0.0005$) in mature ovary of *P.incidus*. The increase in ash content in ovary of *P.indicus* (1.94 g/100g body weight) is almost double compared to 1.07 g in *P.homarus* ovary. Marked decrease in most of the major elements (Na, K and P) and trace elements (Cu, Co and Mn) in the muscle at maturity might possibly indicate that muscle contributes to the inorganic matter build up in the ovary of *P.indicus*. In *P.homarus*, however, only K, Fe and Co decreased in the muscle at ovarian maturity. Na, Co and Cr decreased while all other elements accumulated in heptopancreas of *P.indicus*, similar to the trend in *P.homarus*. In both *P.homarus* and *P.indicus*, hepatopancreatic Cu and Cd

increased substantially at maturity and significantly reduced after spawning in *P.homarus*. Zn and Fe also exhibited a similar trend but the increase at maturity was low compared to Cu and Cd.

Increase of trace elements in mature ovary showed a similar trend in both *P. indicus* and *P.homarus*, with the exception of Co which marginally decreased in mature ovary of *P.indicus*. Fe was the most prominent trace element in quantity in both these species and it seems to play an important role in embryogenesis along with Cu, Zn and Mn, which are also present in large quantities in mature ovaries of *P.homarus* and *P.indicus*.

# 7. ENERGY UTILIZATION AND UPTAKE OF MINERALS AND TRACE ELEMENTS DURING EGG DEVELOPMENT IN THE PENAEID PRAWN, PENAEUS INDICUS

Penaeids are an unique group among the decapod crustaceans in many ways. While all other decapods brood their eggs, cemented to ovigerous setae in the abdominal appendages, penaeids shed their eggs to the surrounding medium. Penaeidae produce the most primitive of decapod larvae, a non-feeding nauplius which moults several times to metamorphose into a feeding protozoea, while other decapods have highly advanced larval forms like phyllosoma of palinurids and zoea of crabs. Penaeidae, as a group, produces maximum number of eggs compared to many other decapods and can breed several times in a year. The eggs of penaeidae are among the smallest in dacapoda and have the fastest development time, less than 15 hours for release of nauplius larva in *Penaeus indicus*, compared to few days (caridians) and several months (homaridae) in other decapods. In embryonic development also penaeidae, like euphasids, have a spindle orientation and cleavage plane similar to those of *Polyphemus* and *Cyclops*, indicative of a secondary radial modification of total spiral cleavage of crustaceans (Brooks, 1882, Zilch, 1978) which is an interesting mixture of primitive and uniquely specialised processes (Zilch, 1978).

Penaeidae are, economically, the most important group of decapods in terms of aquaculture potential and many important species have been completely domesticated. Still, no study has yet been reported on the rate of yolk utilization and biochemical changes during development in any of the penaeid prawns. Determination of weight and biochemical changes involved in reproduction have greatly increased the understanding of reproduction in marine invertebrates (Lawrence *et al.*, 1979). Observations made so far on penaeidae are the chemical composition of ova of *Penaeus setiferus, Penaeus stylirostris* and *Penaeus vannamei* and fatty acid changes in the eggs of *P. setiferus* (Lawrence *et al.*, 1979). One of the reasons for absence of yolk utilization studies in penaeidae is that the eggs are shed to the surrounding medium and are not easily accessible to analysis, unlike those of other decapods that carry eggs (Herring, 1972).

The present investigation is taken up to determine, for the first time, biochemical and mineral changes in the developing eggs of *P. indicus*, one of the most important species of cultivable prawns in India.

## 7.1 RESULTS

Description of development stages, their distinguishing features and duration of development in the egg and neonate are given in Plate 2 and table 7.1. Nauplius larva emerged from the egg 14.5 $\pm$ 0.25 hours after spawning and transformed to protozoea larva 43.5 hours later. Thus, the protozoea larva emerged 58.0 $\pm$ 0.12 hours after spawning. The development time recorded in this study is marginally less compared to that reported for

**Table 7.1.** Stages and duration of development and morphological features of different stages of egg during embryogenesis in *P. indicus*

| Stage | Morphological features | Cumulative time (hours) |
|---|---|---|
| 0 (ripe ova) | Ripe ova in the ovary just before spawning. Little ovarian tissue also present. Ova dia : 0.22 - 0.23 mm. | 0 |
| 1 | Just after spawning, eggs opaque with a narrow perivitelline space covered with a jelly like substance. First cleavage starts after 10 minutes and second after 30 minutes. Egg dia: 0.25-0.26 mm. | 0 |
| 2 | Initiation of gastrulation; an embryonic membrane clearly visible. Egg dia : 0.26 - 0.265 mm | 2.08 ± 0.07 |
| 3 | Appearance of limb bud as three pairs of lateral thickenings. Egg dia : 0.26 - 0.27 mm. | 4.63 ± 0.29 |
| 4 | Appendages fully formed. Embryo occupies almost the entire space in the egg; dia : 0.26 - 0.27 mm. | 7.00 ± 0.10 |
| 5 | Fully formed embryo just before hatching. Furcal setae present; dia : 0.27 mm. | 13.33 ± 0.11 |
| 6 (nauplius) | Just released nauplius larva (Nauplius-1). Body oval with a pair of dorsally curved caudal setae. | 14.50 ± 0.25 |
| 7 | Last nauplius stage (Nauplius-6). Body more elongated, frontal organs and carapace clearly demarkated. | 38.88 ± 0.13 |
| 8 (Protozoea) | First protozoea larva with clear demarkation of cephalothorax and segmented abdomen. | 58.00 ± 0.12 |

the same species by Muthu *et al.*, (1978). This difference may be due to the water temperature which was 26.31 $\pm$ 0.83°C (25.5 to 28°C) in this experiment, little higher than the range (24.4 to 26.8°C) reported in the earlier study. In both cases, the last stage of nauplius (stage 7) took about 44% of the total duration in the nauplius stage.

### 7.1.1 Egg and yolk diameter and volume

Unlike the spiny lobster *P. homarus*, yolk could not be distinguished from the developing embryo in *P. indicus* as development proceeded and what is described as yolk in later stages refers to the dimension of the developing embryo.

Egg diameter (Table 7.2) increased from 0.257 mm in the just spawned egg to 0.27 mm in the last stage of egg (stage 5) and the volume went up to 0.0103 $mm^3$ in stage 5 from 0.0088 $mm^3$ in stage 1. Yolk/embryo diameter and volume increased in the middle stages and then reduced in the last stage to almost the level in stage 1. Most interesting feature noticed was the increase in both egg diameter and volume between ripe ova and just spawned egg. Unlike *P. homarus*, the volume increase in stage 1 did not result in simultaneous increase in volume of yolk due to the formation of a clear perivitelline space and probably, water absorbed was confined to this space.

**Table 7.2.** Egg and Yolk (embryo) diameter and volume in different stages of development in *P. indicus*

| Development Stages | Ova/Egg | | Yolk/Embryo | |
|---|---|---|---|---|
| | Diameter (mm) | Volume ($mm^3$) | Diameter (mm) | Volume ($mm^3$) |
| 0 (ripe ova) | 0.2225 ± | 0.0058 ± | 0.2225 | 0.0058 |
| 1 | 0.2565 ± | 0.0088 | 0.2221 | 0.0057 |
| 2 | 0.2633 | 0.0096 | 0.2391 | 0.0072 |
| 3 | 0.2655 | 0.0098 | 0.2367 | 0.0069 |
| 4 | 0.2633 | 0.0096 | 0.2346 | 0.0068 |
| 5 | 0.2700 | 0.0103 | 0.2295 | 0.0063 |
| 6 | Hatched to nauplius | | | |

Increase in both egg diameter and egg volume showed positive correlation with development stage (expressed in hours) and had a linear relationship (Fig.7.1).

Egg diameter : $r = 0.875$

$$Y = 0.2591 + 0.00078 X$$

Egg volume : $r = 0.872$

$$Y = 0.0081 + 0.00009 X$$

where X is development stage (in hours)

In both cases, the slope of the line (b) was negligible. No linear relationship was observed in the change in yolk/embryo diameter and volume ($r = 0.728$ and 0.321 respectively for diameter and volume).

Quantitative changes in diameter and volume in different stages, with stage 1 kept as 100 are given in Table 7.3. Diameter and volume of the egg went up by 3.51% and 11.36% up to stage 3 and then reduced by 0.86% and 2.27% respectively in stage 4. A significant increase in both egg diameter and volume (2.61% and 7.96% respectively) occurred in the last stage of egg. Yolk/embryo diameter and volume increased by 7.65% and 26.32% respectively in stage 2 and then reduced gradually upto stage 5. The most important increase in egg diameter and volume was between ripe ova and just spawned egg (15.28% and 51.72% respectively).

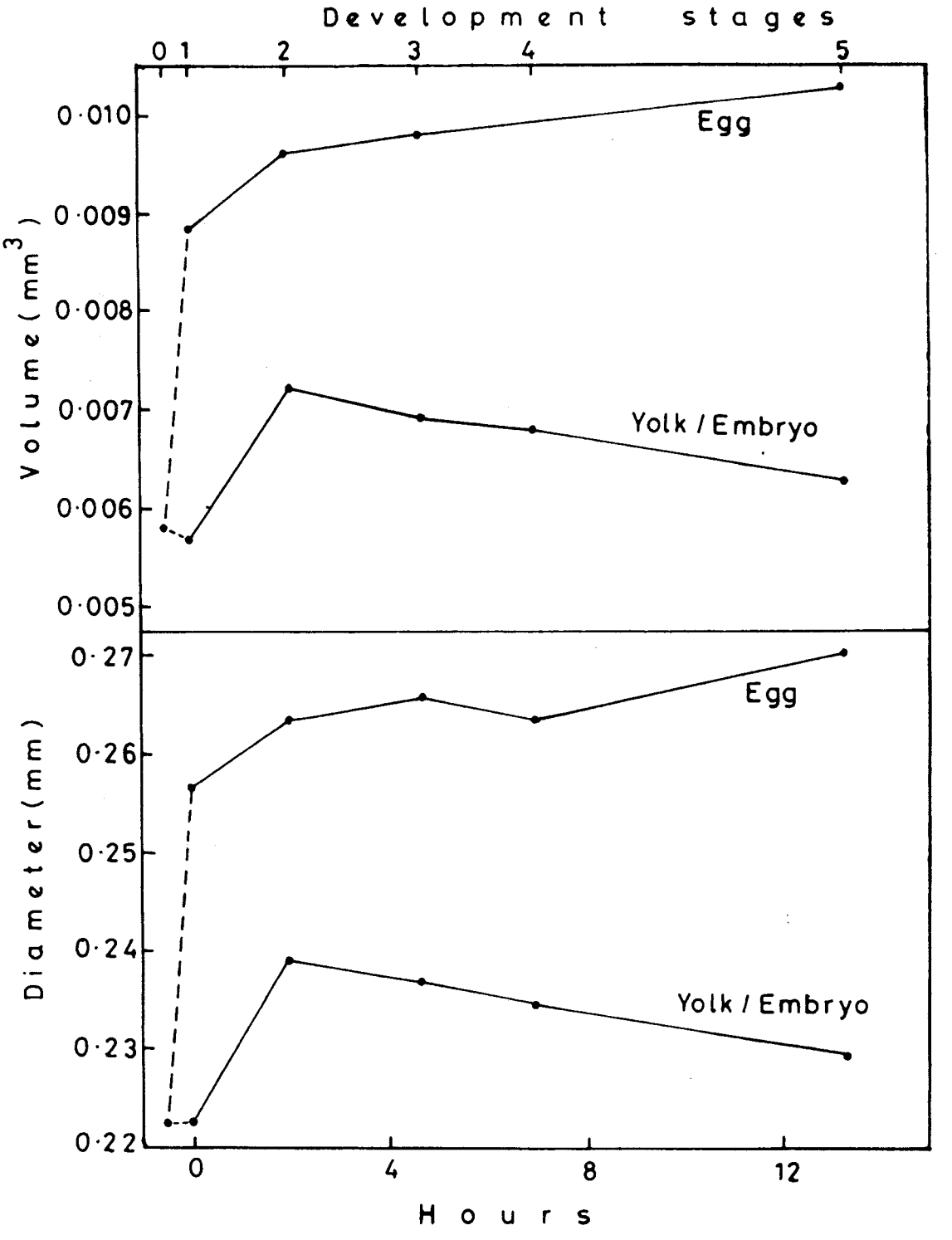


Fig.7.1 Changes in egg and yolk/embryo diameter and volume during development in *P.indicus*.

**Table 7.3.** Quantitative changes in egg volume and yolk volume during development in *P. indicus* (Stage 1 = 100%)

| Stage | Ova/Egg | | | | Yolk/Embryo | | | |
|---|---|---|---|---|---|---|---|---|
| | Increase in dia. (mm) | % increase in dia | increase in volume (mm) | % increase | Increase/ decrease in dia (mm) | % increase | increase in volume (mm) | % increase |
| 0 (ripe ova) | -0.034 | 92.01 | -0.0030 | 65.91 | -0.0004 | 100.18 | 0.0001 | 101.75 |
| 1 | 0.0000 | 100.00 | 0.0000 | 100.00 | 0.0000 | 100.00 | 0.0000 | 100.00 |
| 2 | 0.0068 | 102.65 | 0.0008 | 109.09 | 0.0170 | 107.65 | 0.0015 | 126.32 |
| 3 | 0.0022 | 103.51 | 0.0002 | 111.36 | -0.0024 | 106.57 | -0.0003 | 121.05 |
| 4 | -0.0022 | 102.65 | -0.0002 | 109.09 | -0.0021 | 105.63 | -0.0001 | 119.30 |
| 5 | 0.0067 | 105.26 | 0.0007 | 117.05 | 0.0051 | 103.33 | -0.0005 | 110.53 |
| 6 | Hatched to nauplius-1 | | | | | | | |

### 7.1.2 Biochemical changes during development

Changes in proximate composition and energy content of the egg and neonate at different stages of development are given in Table 7.4. Most important features of the composition of the just spawned *P. indicus* eggs were the unusually high ash content (13.34 $\pm$ 2.32%) and comparatively higher carbohydrate (5.12 $\pm$ 0.31%). Concentration of water reduced from stage 1 to 4 but increased in last stage of the egg and subsequent larval stages. Ash increased to 19.08% in stage 4 and then declined to 16.47% in stage 5 and 5.34% in the nauplius and again increased to 19.15% in protozoea.

Protein concentration did not show any significant variation in the egg but increased to a maximum level of 57.13% in last nauplius (stage 7). Concentration of lipid showed variability in different stages in the egg and was maximum in the just hatched nauplius (36.81%) and then reduced to 25.8% in protozoea. Carbohydrate content decreased upto stage 4 and then marginally increased in stage 5 and in the neonates, carbohydrate concentration increased progressively.

Energy values measured are considerably lower than calculated values, probably due to some problems in the chart recorder of the AH 12/EF electronic microbomb calorimeter. For calculation of energy utilization parameters, measured values were used for uniformity with other studies.

**Table 7.4.** Biochemical changes during egg development in *P. indicus* (Protein, lipid, carbohydrate and ash are expressed as % in dry weight)

| Stage | Changes during development | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Water % | Dry matter % | Protein | Lipid | Carbohydrate | Ash | Energy (J) | |
| | | | | | | | Measured | Calculated |
| 0 (ripe ova) | 67.58± 1.43 | 32.42± 0.69 | 56.30± 1.51 | 35.20± 1.88 | 2.32± 0.26 | 6.63± 0.32 | 21.34 | 27.92± 1.14 |
| 1 | 79.58± 3.83 | 20.42± 0.23 | 47.73± 0.66 | 33.20± 0.47 | 5.12± 0.31 | 13.34± 2.32 | 20.15 | 25.74± 1.21 |
| 2 | 78.37± 0.44 | 21.63± 0.12 | 48.44± 1.51 | 30.25± 0.14 | 3.48± 0.24 | 19.02± 0.76 | 18.39 | 23.87± 0.54 |
| 3 | 78.71 1.12 | 21.29± 0.30 | 48.47± 1.10 | 31.45± 0.83 | 3.05± 0.11 | 18.11± 0.95 | 19.04 | 24.34± 0.61 |
| 4 | 78.06± 0.86 | 21.94± 0.24 | 48.71± 0.80 | 30.82± 0.17 | 1.90± 0.24 | 19.08± 0.23 | 19.61 | 24.00± 6.40 |
| 5 | 79.16 2.79 | 20.84± 0.73 | 48.34± 1.80 | 31.63± 1.83 | 3.12± 0.21 | 16.47± 0.61 | 17.71 | 24.90± 1.16 |
| 6 (Nauplius) | 80.21± 1.24 | 19.79± 0.31 | 50.11± 1.20 | 36.89± 2.40 | 2.38± 0.21 | 5.34± 0.19 | 16.55 | 27.86± 1.45 |
| 7 | 87.97± 2.03 | 12.03± 0.28 | 57.13± 2.55 | 28.30± 1.54 | 2.89± 0.28 | 10.11± 0.16 | 14.79 | 25.47± 1.50 |
| 8 (protozoaea) | 87.78± 0.09 | 12.22± 0.01 | 53.56± 1.41 | 25.80 1.60 | 2.98± 0.28 | 19.15± 0.41 | 15.31 | 23.27± 1.01 |

Biochemical composition of ripe ova is significantly different from the just spawned egg in protein (less in stage 1), carbohydrate and ash (more in stage 1).

### 7.1.3 Weight changes in a single egg and larva

No linear relationship was seen in wet weight changes (Fig.7.2) in the egg but dry weight showed a negative correlation as the development progressed:

Dry weight (stage 1-5) ; r = 0.922

Y = 3.111 + (-) 0.629 X where X is development stage in hours.

Dry weight (stage 1 - stage 8 (protozoea)) ; r = 0.876

Y = 3.011 + (-) 0.045 X

Most significant change in wet weight was noticed between ripe ova and just spawned egg which increased from 9.71 $\mu$g to 15.42 $\mu$g (58.81%).

### 7.1.4 Biochemical changes in a single egg/larva

Quantitative changes in proximate composition and energy content calculated on a per egg / larva basis are given in Table 7.5 and Fig.7.3 and relative changes over stage 1 (which is kept as 100) are given in Fig.7.4.

*P. indicus* egg had 12.27 $\mu$g water in stage 1 which reduced to 9.91 $\mu$g (19.23% decrease) in stage 4 and subsequently increased to 10.48 $\mu$g in the last stage of egg (stage 5). Protein, lipid and carbohydrate depleted with advance in development and upto stage 5, 11.33% protein, 17.14% lipid and

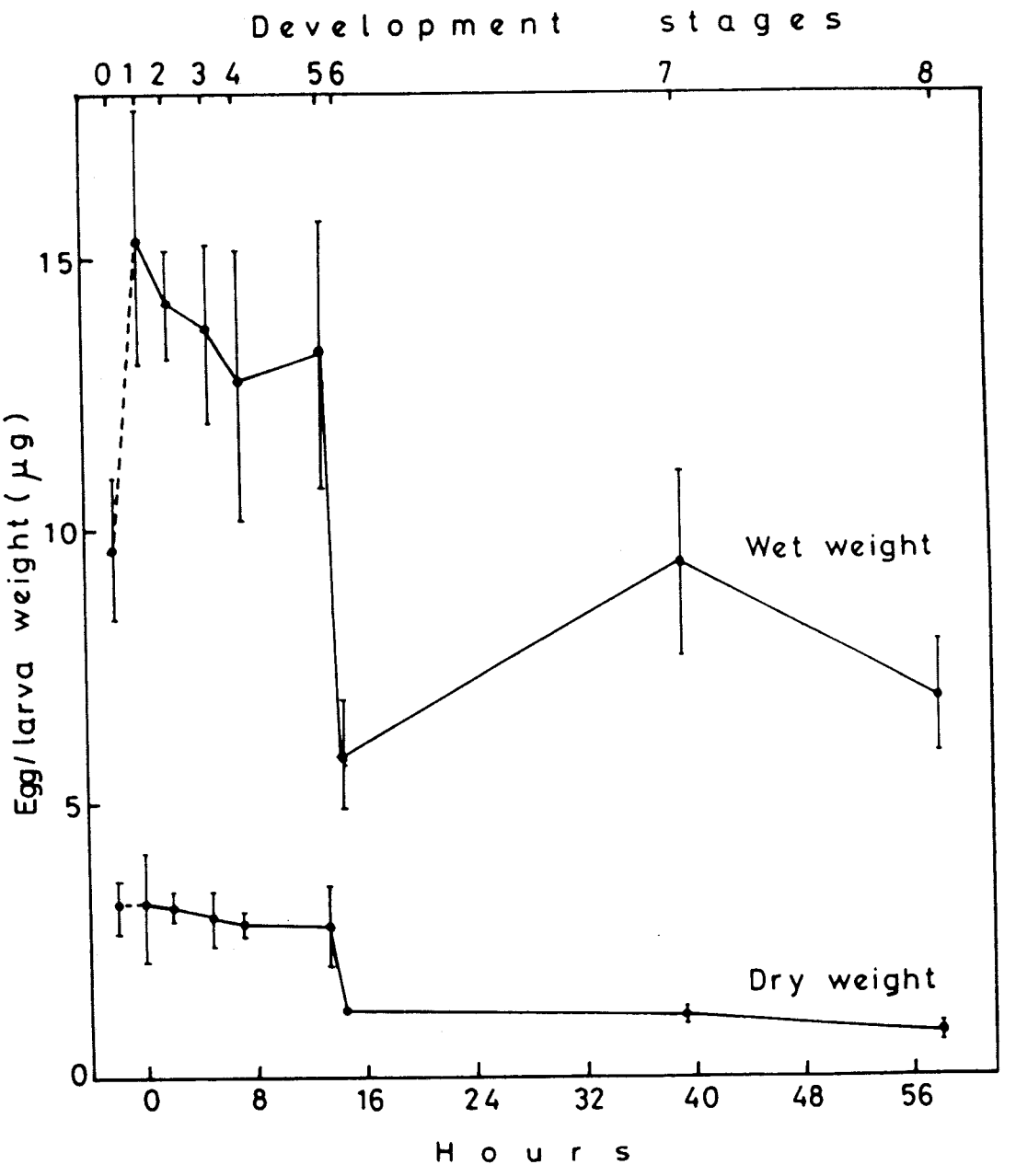


Fig.7.2 Changes in wet and dry weights of egg/larva during development in *P.indicus*

**Table 7.5.** Quantitative changes in proximate composition and energy in a single egg/larva during development in *P. indicus*

| Stage | Weight of egg (μg) | | Quantity (μg) | | | | | Energy (J) | |
|---|---|---|---|---|---|---|---|---|---|
| | Wet | Dry | Water | Protein | Lipid | Carbo-hydrate | Ash | Measured | Calculated |
| 0 (ripe ova) | 9.71± 1.26 | 3.15± 0.52 | 6.56± 0.85 | 1.77± 0.29 | 1.11± 0.19 | 0.070± 0.010 | 0.210± 0.030 | 0.0700± 0.0066 | 0.085± 0.015 |
| 1 | 15.42± 2.27 | 3.15± 1.06 | 12.27± 1.81 | 1.50± 0.51 | 1.05± 0.36 | 0.160± 0.060 | 0.420± 0.140 | 0.0600± 0.0022 | 0.081± 0.027 |
| 2 | 14.21± 1.05 | 3.08± 0.34 | 11.13± 0.82 | 1.49± 0.16 | 0.93± 0.10 | 0.110± 0.010 | 0.590± 0.070 | 0.0570± 0.0011 | 0.074± 0.008 |
| 3 | 13.66± 1.69 | 2.91± 0.46 | 10.75± 1.33 | 1.41± 0.22 | 0.92± 0.14 | 0.090± 0.010 | 0.530± 0.090 | 0.0550± 0.0011 | 0.071± 0.011 |
| 4 | 12.70± 2.50 | 2.79± 0.13 | 9.91± 1.95 | 1.36± 0.06 | 0.86± 0.04 | 0.050± 0.003 | 0.530± 0.020 | 0.0550± 0.0011 | 0.067± 0.003 |
| 5 | 13.24± 2.45 | 2.76± 0.67 | 10.48± 1.94 | 1.33± 0.32 | 0.87± 0.22 | 0.090± 0.020 | 0.450± 0.110 | 0.0490± 0.0010 | 0.069± 0.017 |
| 6 (Nauplius) | 5.92± 1.02 | 1.17± 0.03 | 4.75± 0.82 | 0.59± 0.02 | 0.43± 0.01 | 0.030± 0.000 | 0.060± 0.002 | 0.0190± 0.0004 | 0.033± 0.001 |
| 7 | 9.39± 1.74 | 1.13± 0.17 | 8.26± 1.53 | 0.65± 0.10 | 0.32± 0.05 | 0.030± 0.005 | 0.110± 0.020 | 0.0170± 0.0020 | 0.029± 0.004 |
| 8 (protozoea) | 6.94± 1.01 | 0.85± 0.12 | 6.05± 0.89 | 0.46± 0.06 | 0.22± 0.03 | 0.025± 0.004 | 0.160± 0.020 | 0.0130± 0.0020 | 0.020± 0.002 |

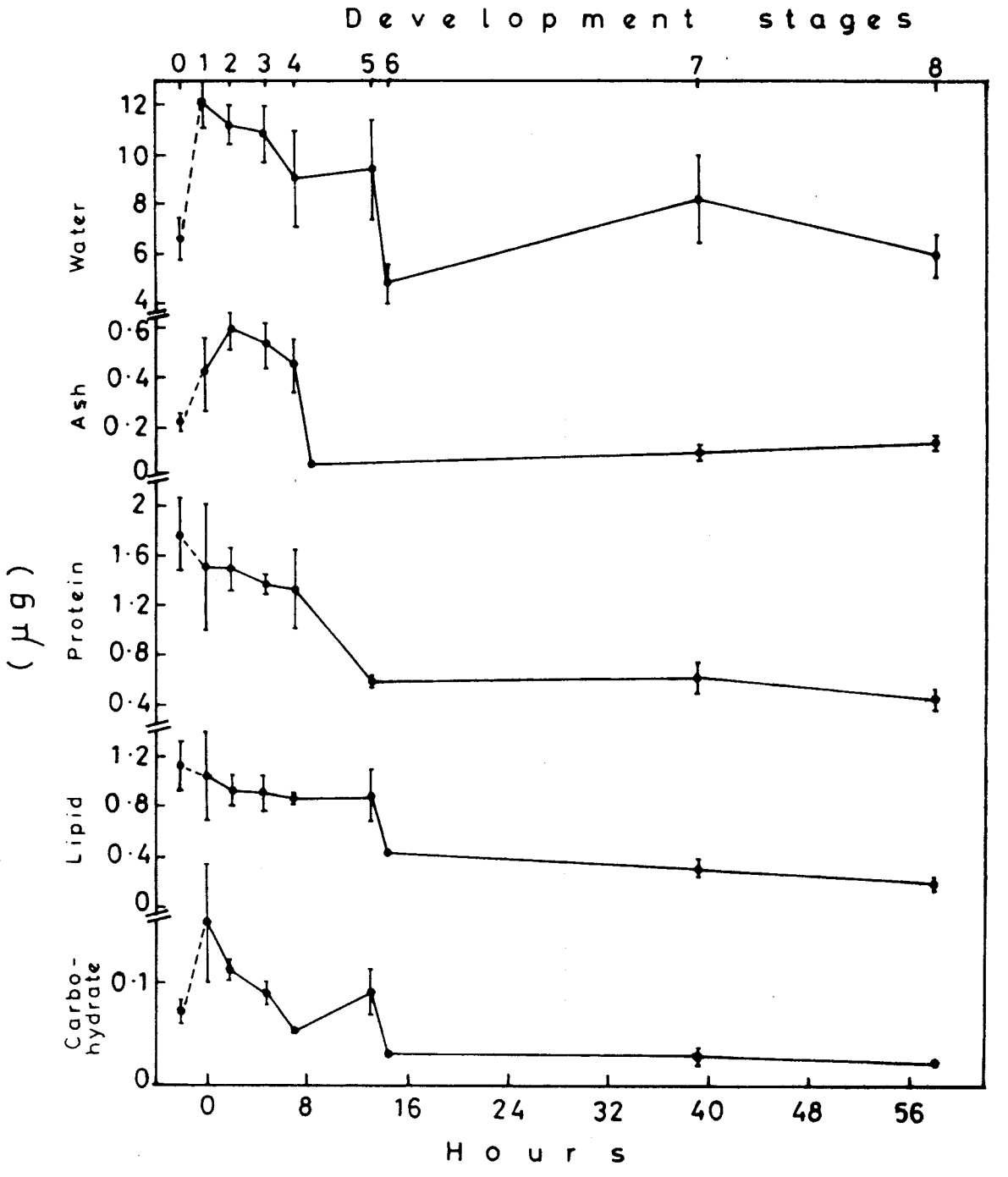


Fig.7.3 Quantitative changes in water, protein, lipid carbohydrate and ash in a single egg during development in *P.indicus*.

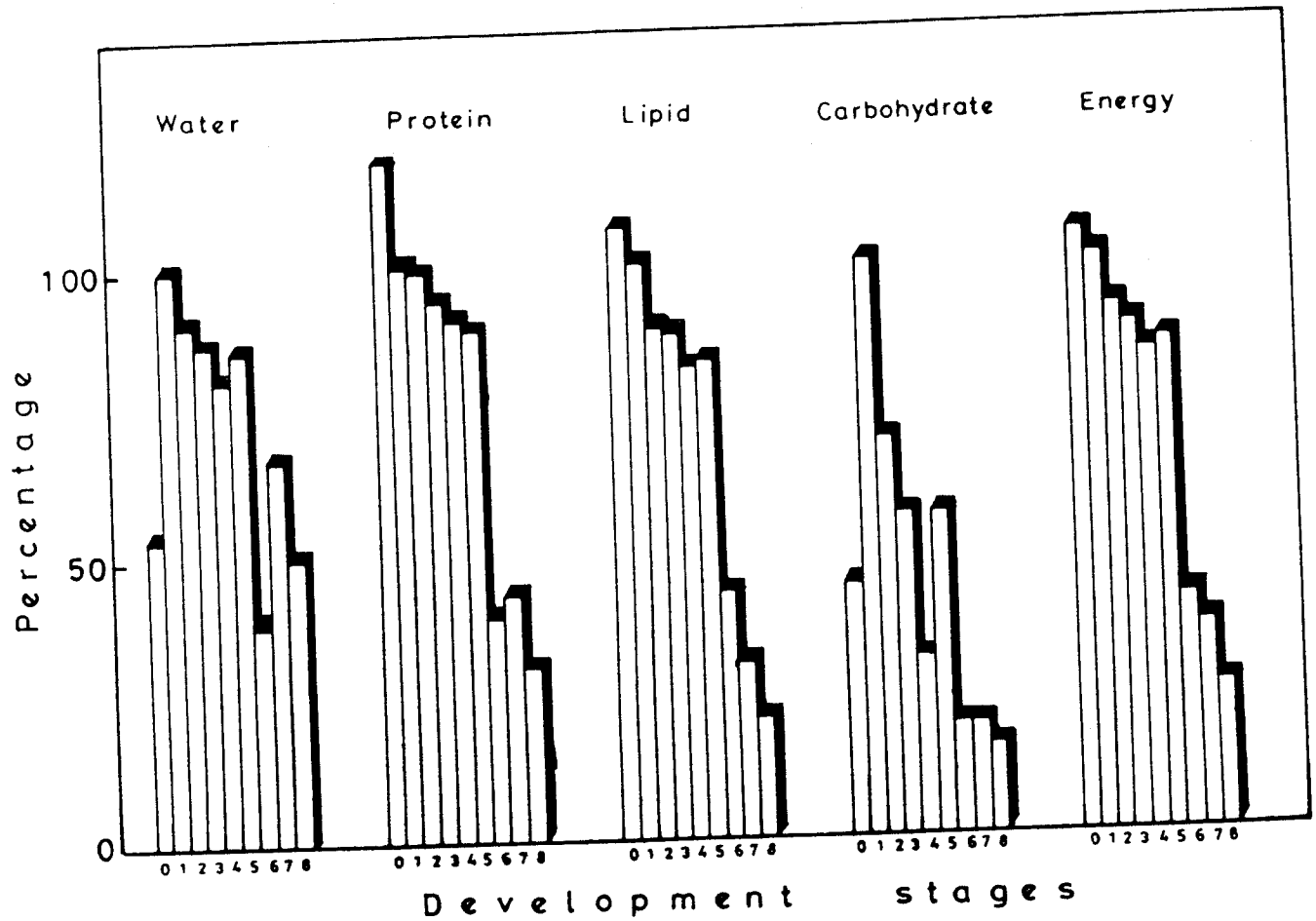


Fig.7.4 Utilization of water, protein, lipid, carbohydrate and
in different stages of development in *P.indicus*

43.75% carbohydrates were utilized, with main energy contribution coming from lipid. All these reserves were heavily depleted between stage 5 and 6 (nauplius) with the reduction being 49.14% for protein, 41.5% for lipid and 37.5% for carbohydrate.

In the nauplius, lipid got further depleted (10.47%) by stage 7, while a 4% increase was recorded in the quantity of protein. Further reductions of lipid (9.53%), protein (12.66%) and carbohydrate (3.13%) were observed as the nauplius transformed to protozoea. Ash was maximum (0.59 $\mu$g) in stage 2 and reduced to 0.45 $\mu$g by stage 5 and 0.06 $\mu$g in the nauplius to increase again to 0.16 $\mu$g in protozoea.

From ripe ova to just spawned egg, protein dropped heavily (15.25%) followed by lipid (5.41%), but carbohydrate, ash and water increased substantially by 128.5%, 100% and 87.5% respectively.

Utilization of energy at various stages of development from different sources and the energetic conversion efficiencies are given in Table 7.6 and Fig.7.5. Energy conversion efficiency up to the hatching of nauplius larva was 31.67% which reduced to 21.67% by the time the feeding larva (protozoea) emerged.

Proportion of protein, lipid and carbohydrate energy expended for development are represented in Fig.7.6. Between fertilized egg and nauplius, 51.48% of the energy utilized was supplied by lipid while protein and carbohydrate contributed 43.46% and 5.06% respectively. When the energy

**Table 7.6.** Energy utilization in a single egg/larva during development in *P. indicus*

| Stage | Energy utilization (J) | | | | |
|---|---|---|---|---|---|
| | Protein energy | Lipid energy | Carbohydrate energy | Total Energy | |
| | | | | Measured | Calculated |
| 0 (ripe ova) | 0.0400 | 0.0437 | 0.0013 | 0.070 | 0.0850 |
| 1 | 0.0339 | 0.0413 | 0.0030 | 0.060 | 0.0782 |
| 2 | 0.0336 | 0.0366 | 0.0021 | 0.057 | 0.0723 |
| 3 | 0.0319 | 0.0362 | 0.0017 | 0.055 | 0.0698 |
| 4 | 0.0307 | 0.0338 | 0.0009 | 0.055 | 0.0654 |
| 5 | 0.0301 | 0.0342 | 0.0017 | 0.049 | 0.0660 |
| 6 (Nauplius) | 0.0133 | 0.0169 | 0.0006 | 0.019 | 0.0308 |
| 7 | 0.0140 | 0.0126 | 0.0006 | 0.017 | 0.0279 |
| 8 (Protozoea) | 0.0104 | 0.0087 | 0.0005 | 0.013 | 0.0196 |
| Energy Conversion Efficiency (%) | | | | | |
| 0-6 | 33.25 | 38.67 | 46.15 | 27.14 | 36.23 |
| 1-6 | 39.33 | 40.92 | 20.00 | 31.67 | 39.39 |
| 0-8 | 26.00 | 19.91 | 38.46 | 18.57 | 23.06 |
| 1-8 | 30.68 | 21.87 | 16.67 | 21.67 | 25.06 |

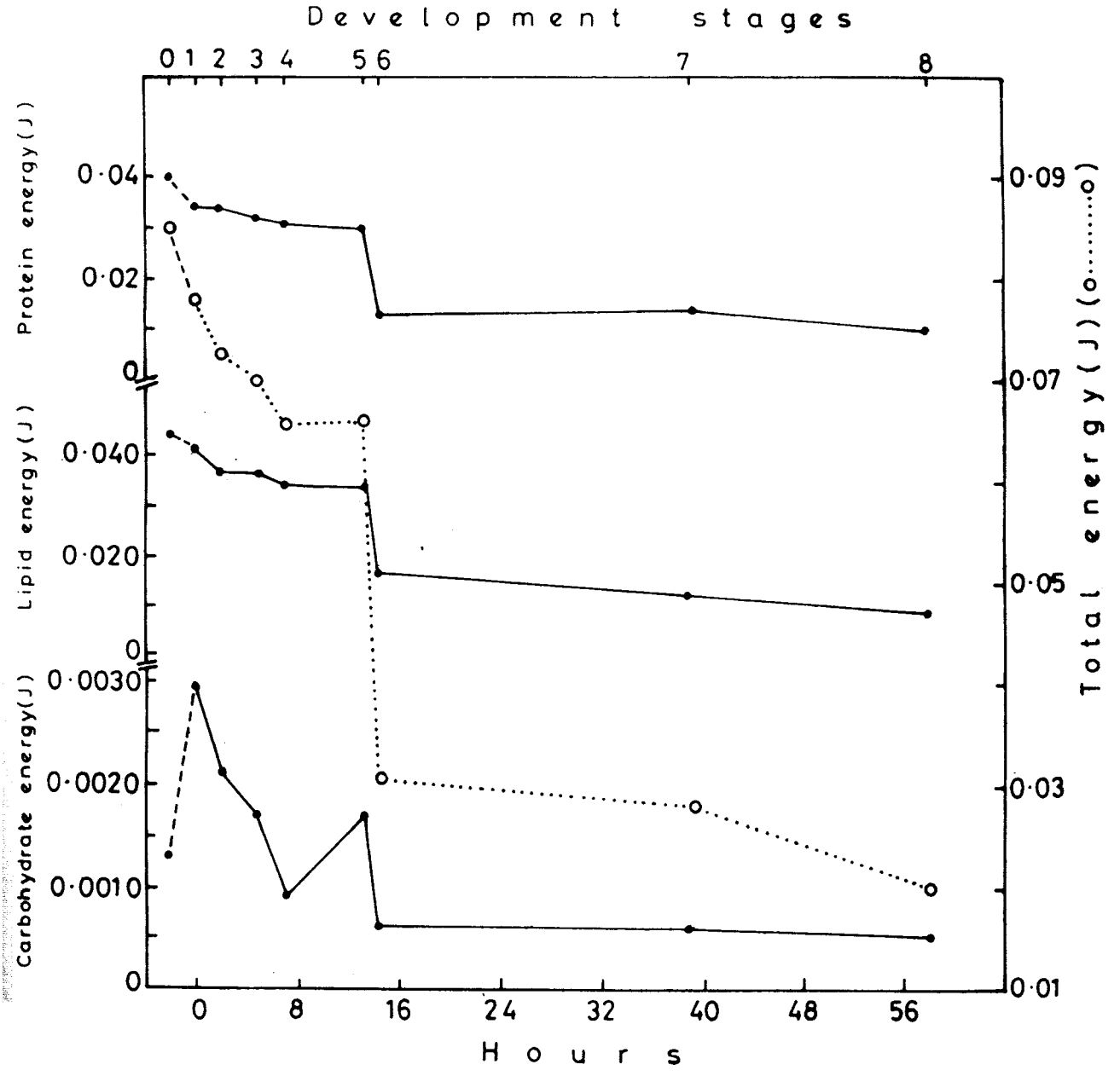


Fig.7.5 Energy utilization from different sources in the developing egg of *P.indicus*.

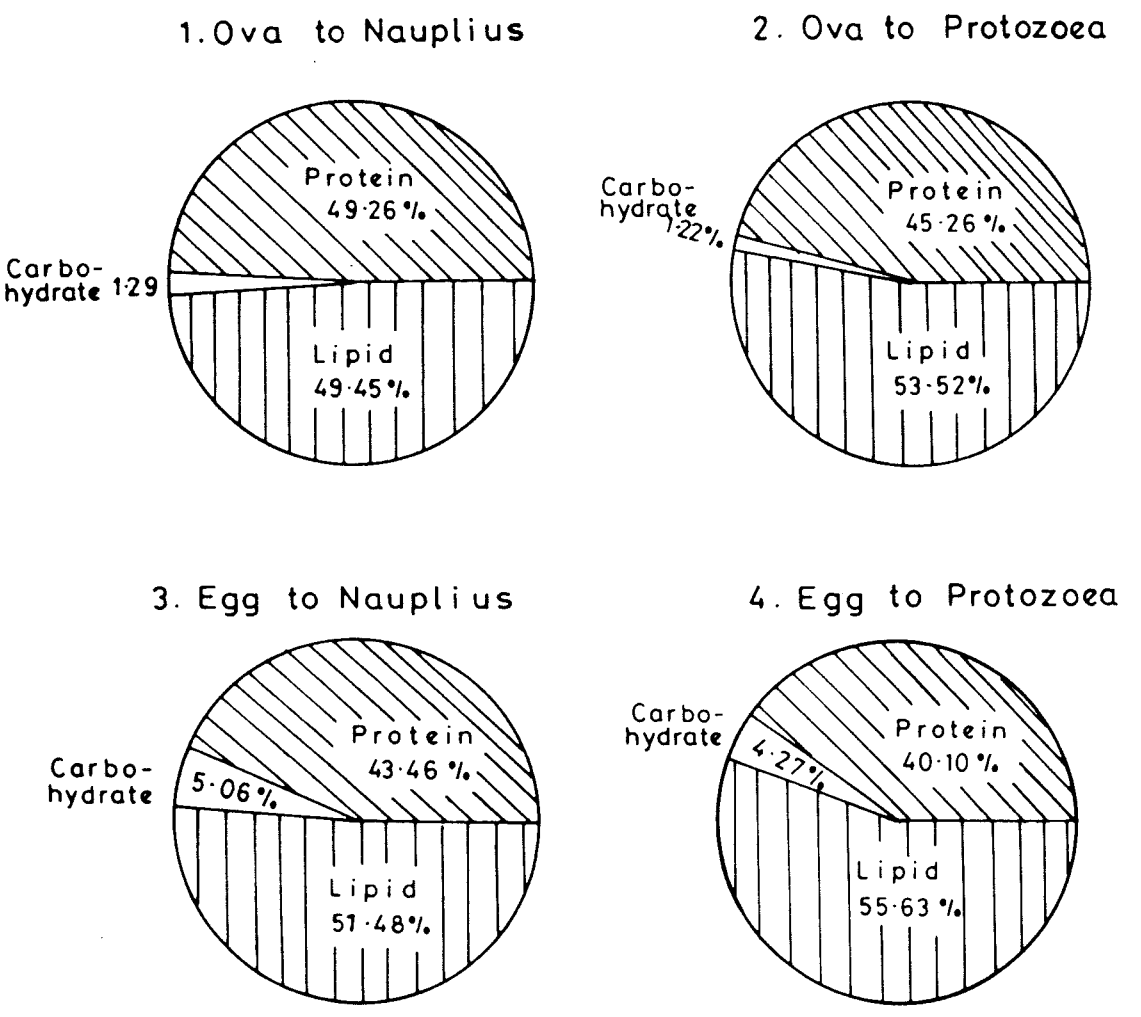


Fig.7.6 Proportion of protein, lipid and carbohydrate energy in total energy expended for embryogenesis in *P.indicus* egg/larva.

budget is worked out from stage 1 to first feeding larva, contribution of lipid was still greater (55.63%) followed by protein (40%) and carbohydrate (4.27%). From ripe ova to nauplius, the energy contributions from protein (49.26%) and lipid (49.45%) were almost equal.

### 7.1.5 Changes in minerals and trace elements composition

As in *P. homarus*, elements present in mg/g dry weight were classified as major elements or minerals and those present in μg/g dry weight were termed as trace elements. Concentrations of minerals and trace elements in egg and larva are given in Table 7.7.

Major elements, Na, Ca, P, and Mg increased significantly ($p = < 0.05$) in the just spawned egg while K decreased from that of the ripe ova. Concentrations of these elements showed considerable variations during development. Concentrations of Na, Ca and P were maximum in stage 5 in the egg and in protozoea in the larva. K was in maximum concentration in stage 3, while Mg was maximum in stage 1 and 5. Among the larvae, protozoea had highest concentration of all these elements.

Zn and Fe appeared to be the most important trace elements in terms of concentration in the egg and larva. Concentrations of Fe, Zn, Cd and Mn increased in stage 1 from ripe ova while Cu, Co and Cr showed reduction. Fe, Zn, Cu and Mn were present in maximum concentrations in stage 5, while Cd and Co were maximum in stage 3 and Cr in stage 4, concentrations of Fe, Cd, Cr and Mn increased in protozoea larva from

**Table 7.7.** Changes in minerals and trace elements concentration (mg or μg/g dry wt) during development in *P.indicus*

| Minerals/ trace elements | Development stages | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 (Ova) | 1 | 2 | 3 | 4 | 5 | 6 (naup-lius) | 7 | 8 (proto-zoea) |
| **Mineral (mg/g dry wt)** | | | | | | | | | |
| Na | 12.50 | 23.39 | 22.80 | 22.96 | 18.02 | 40.22 | 17.35 | - | 63.11 |
| K | 11.03 | 8.05 | 7.65 | 15.21 | 8.30 | 8.60 | 7.35 | - | 8.47 |
| Ca | 3.27 | 5.86 | 6.02 | 7.30 | 6.85 | 11.17 | 4.55 | - | 42.17 |
| P | 20.71 | 18.03 | 16.68 | 21.42 | 23.93 | 30.68 | 15.65 | - | 36.80 |
| Mg | 2.27 | 3.62 | 3.11 | 3.19 | 2.76 | 3.59 | 1.06 | - | 7.74 |
| **Trace elements (μg/g dry wt)** | | | | | | | | | |
| Fe | 97.763 | 294.540 | 290.600 | 625.890 | 721.180 | 1115.88 | 38.840 | 286.640 | 348.130 |
| Cu | 21.870 | 8.610 | 10.920 | 9.890 | 9.250 | 12.36 | 13.170 | 25.840 | 5.260 |
| Zn | 219.410 | 334.600 | 336.800 | 369.220 | 307.100 | 454.91 | 274.020 | - | 266.800 |
| Cd | 0.562 | 0.594 | 1.121 | 0.263 | 0.440 | 0.36 | 0.339 | 0.375 | 1.788 |
| Cr | 2.072 | 2.128 | 2.535 | 3.629 | 7.925 | 7.04 | 1.355 | - | 16.990 |
| Mn | 7.795 | 16.878 | 15.841 | 20.509 | 20.533 | 26.81 | 5.721 | 26.779 | 23.401 |
| Co | 0.638 | 0.594 | 3.412 | <0.050 | <0.040 | <0.09 | <0.037 | <0.190 | <0.150 |
| Pb | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

nauplius while other trace elements decreased. Pb was below the detectable limit (< 0.001 $\mu$g/g dry weight).

Quantitative uptake of minerals and trace elements in a single egg/larva is given in Table 7.8 and Figs.7.7, 7.8 and 7.9. In the just spawned egg, Na was present in maximum quantity (73.68 ng) among the minerals and it increased to 111.01 ng in stage 5. Next in importance was P, the quantity of which increased from 56.79 ng in stage 1 to 84.68 ng in stage 5. K, Ca and Mg in that order, were important after Na and P in stage 1. Quantity of Ca was maximum (30.83 ng) in stage 5, while K was maximum in stage 3 (44.26 ng) and Mg in stage 2 (11.4 ng).

Zn was the most prominent trace element (1.055 ng) at stage 1 followed by Fe (0.926 ng), Mn (0.053 ng), Cu (0.027 ng), Cr (0.0067 ng) and Cd (0.0019 ng). During the course of development, Fe increased several fold to take up the position as the most important trace element. Maximum quantities of Fe (3.08 ng), Cu (0.0342 ng), Zn (1.2558 ng) and Mn (0.074 ng) were recorded in the egg in stage 5, while Cd was maximum (0.0034 ng) in stage 2, Cr (0.0221 ng) in stage 4 and Co (0.0105 ng) in stage 2. Except for Co and Cu, the quantities of all other trace elements were more in just spawned egg than in the ripe ova. Quantities of Fe, Cu and Zn declined in protozoea, while all others increased.

To compare the relative rate of changes in different stages of development, quantities of minerals and trace elements were expressed in

Table 7.8. Quantitative changes in minerals and trace elements during egg development in P. indicus (values in parenthesis indicate relative changes with quantity in Stage 1 kept as 100).

| Minerals/ trace elements | Development stage | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 (ova) | 1 | 2 | 3 | 4 | 5 | 6 (nauplius) | 7 | 8 (proto-zoea) |
| **Minerals (ng)** | | | | | | | | | |
| Na | 39.38 (53.5) | 73.68 (100.0) | 70.22 (95.3) | 66.81 (90.7) | 50.28 (68.2) | 111.01 (150.7) | 20.37 (27.7) | - | 53.60 (72.8) |
| K | 37.26 (146.9) | 25.36 (100.0) | 23.56 (92.9) | 44.26 (174.5) | 23.16 (91.3) | 23.74 (93.6) | 8.60 (33.9) | - | 7.20 (28.4) |
| Ca | 10.30 (55.8) | 18.46 (100.0) | 18.54 (100.4) | 21.24 (115.1) | 19.11 (103.5) | 30.83 (167.0) | 5.32 (28.8) | - | 35.84 (194.2) |
| P | 65.24 (114.9) | 56.79 (100.0) | 51.37 (90.5) | 62.33 (109.8) | 66.76 (117.6) | 84.68 (149.1) | 18.31 (32.3) | - | 31.28 (55.1) |
| Mg | 7.15 (62.7) | 11.40 (100.0) | 9.58 (84.0) | 9.28 (81.4) | 7.70 (67.5) | 9.91 (86.9) | 1.24 (10.9) | - | 6.58 (57.7) |
| **Trace elements (ng)** | | | | | | | | | |
| Fe | 0.3087 (33.3) | 0.9261 (100.0) | 0.8963 (96.8) | 1.8275 (197.3) | 2.0144 (217.5) | 3.0801 (332.6) | 0.0456 (4.9) | 0.0324 (3.5) | 0.0296 (3.2) |
| Cu | 0.0693 (255.7) | 0.0271 (100.0) | 0.0339 (125.1) | 0.0289 (106.6) | 0.0257 (94.8) | 0.0342 (126.2) | 0.0154 (56.8) | 0.0026 (9.6) | 0.0045 (16.6) |
| Zn | 0.6931 (65.7) | 1.0550 (100.0) | 1.0380 (98.3) | 1.0680 (101.2) | 0.8650 (82.0) | 1.2558 (119.3) | 0.3206 (30.4) | - | 0.2270 (21.5) |
| Cd | 0.0018 (94.7) | 0.0019 (100.0) | 0.0034 (179.0) | 0.0008 (42.1) | 0.0012 (63.2) | 0.0010 (52.6) | 0.0004 (21.1) | 0.0004 (21.1) | 0.0015 (79.0) |
| Cr | 0.0065 (97.0) | 0.0067 (100.0) | 0.0078 (116.4) | 0.0106 (158.2) | 0.0221 (314.9) | 0.0194 (289.6) | 0.0016 (23.9) | - | 0.0144 (214.9) |
| Mn | 0.0246 (46.2) | 0.0532 (100.0) | 0.0488 (91.7) | 0.0597 (112.2) | 0.0573 (107.7) | 0.0740 (139.1) | 0.0067 (12.6) | 0.0303 (57.0) | 0.0199 (37.4) |
| Co | 0.0020 (107.1) | 0.0019 (100.0) | 0.0105 (564.5) | 0.00015 (8.1) | 0.00011 (5.9) | 0.00025 (13.4) | 0.00004 (2.2) | 0.00021 (11.3) | 0.00013 (7.0) |

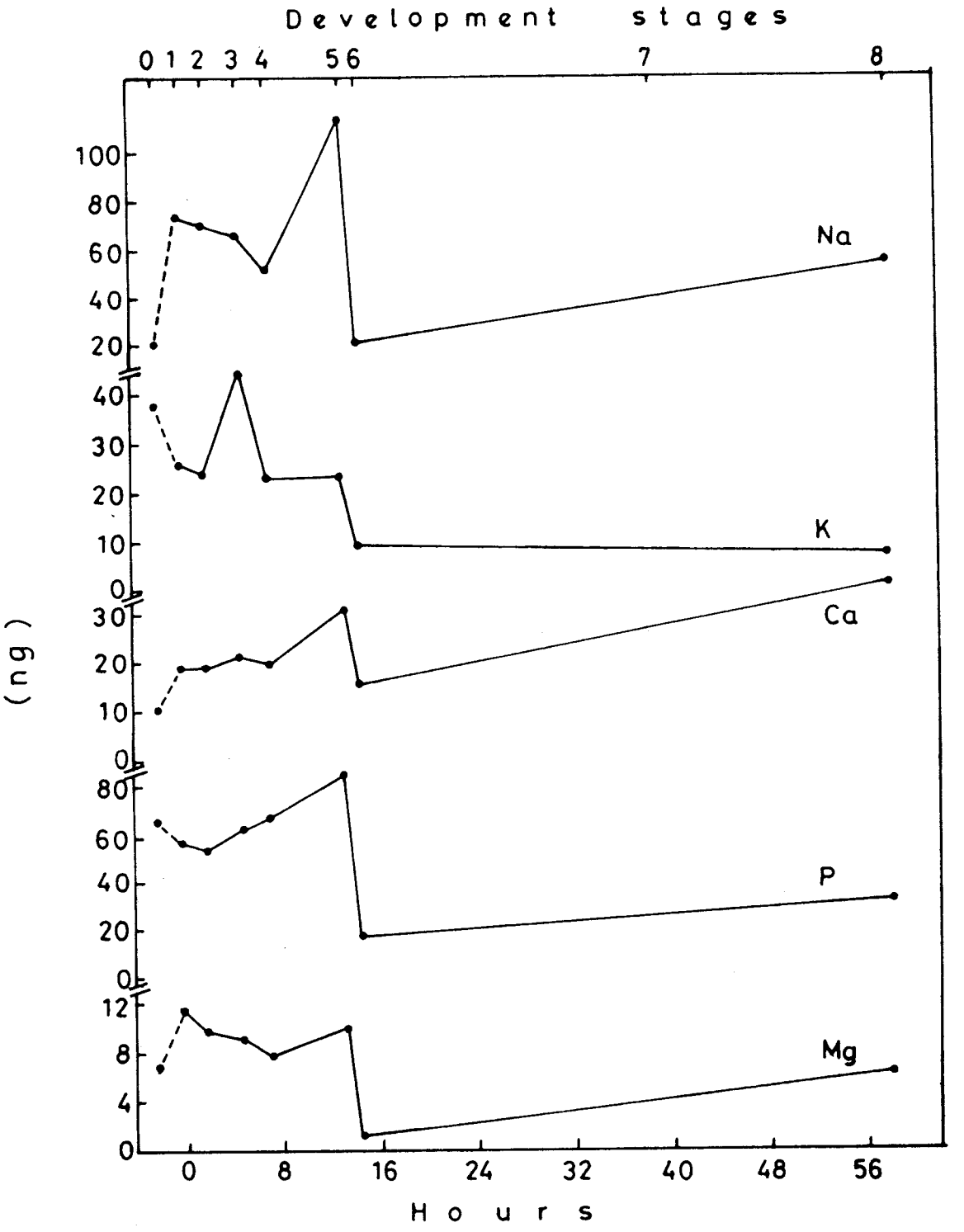


Fig.7.7 Quantitative changes in minerals in a single egg/larva during embryogenesis in *P.indicus*.

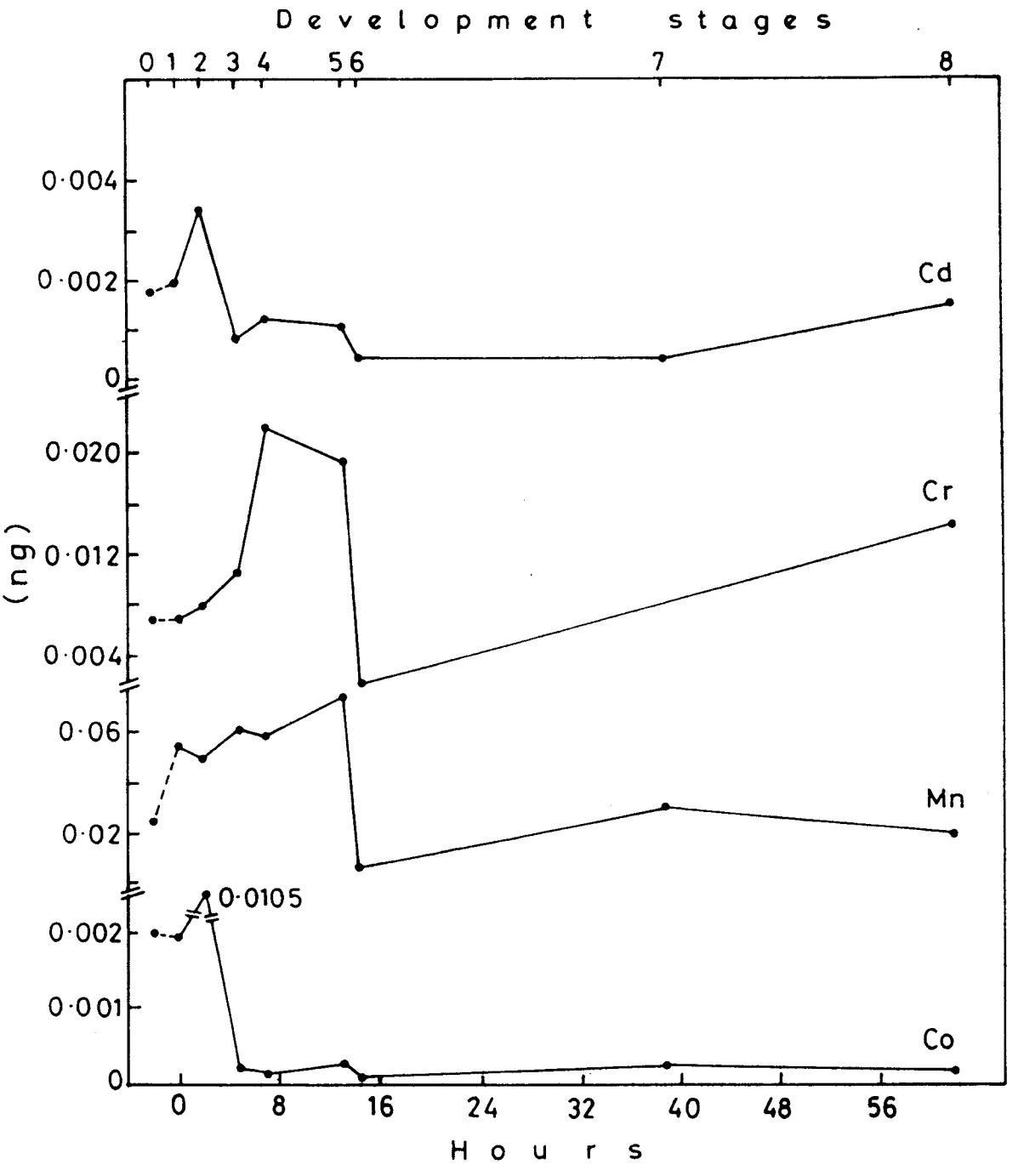


Fig.7.8 Quantitative changes in trace elements in a single egg/larva during embryogenesis in *P.indicus*.

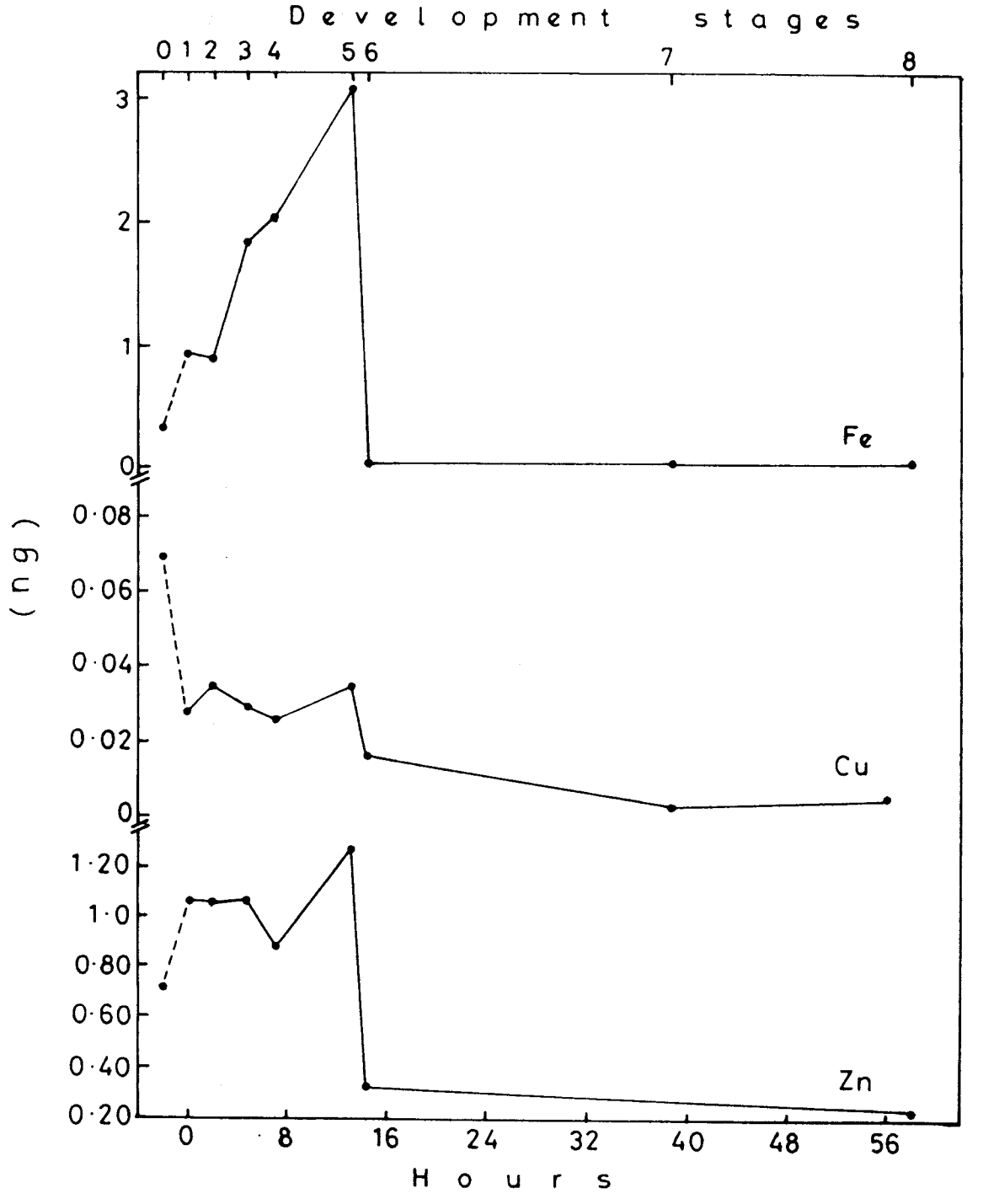


Fig.7.9 Quantitative changes in trace elements in a single egg/larva during embryogenesis in *P.indicus*.

relation to the quantity in stage 1 (which was kept as 100) and the values are given in Figs.7.10 and 7.11.

Na showed 150.67% increase in stage 5, while K was maximum in stage 2 (173.53% increase). Cu went up by 167% in stage 5 and Mg by 86.91% also in stage 5. In the larva prominent increase was recorded in the quantity of Ca (194.15%).

Among the trace elements, quantitative increase was most prominent for Co (564.52%) in stage 2, followed by Fe (332.59%) in stage 5, Cr (314.93%) in stage 4, Cd (178%) in stage 2, Mn (139.1%) in stage 5, Cu (126.9%) in stage 5 and Zn (119.28%) in stage 5. In the larvae the most prominent increase was that of Cr (214.93%). Rate of increase was highly variable in different stages of development.

## 7.6 DISCUSSION

Volume increase (17.5%) from just spawned egg to the last stage of egg development in *P. indicus* is the lowest recorded and is significantly below the range in many other crustaceans described elsewhere (Chapter 4). But a substantial increase in volume (51.7%) had occurred as the ripe ova were released to the surrounding medium. Had the ripe ova not been included in this study, this important observation would have been overlooked. This gives credence to the opinion expressed in Chapter 4 that for a complete understanding of the changes happening during embryonic

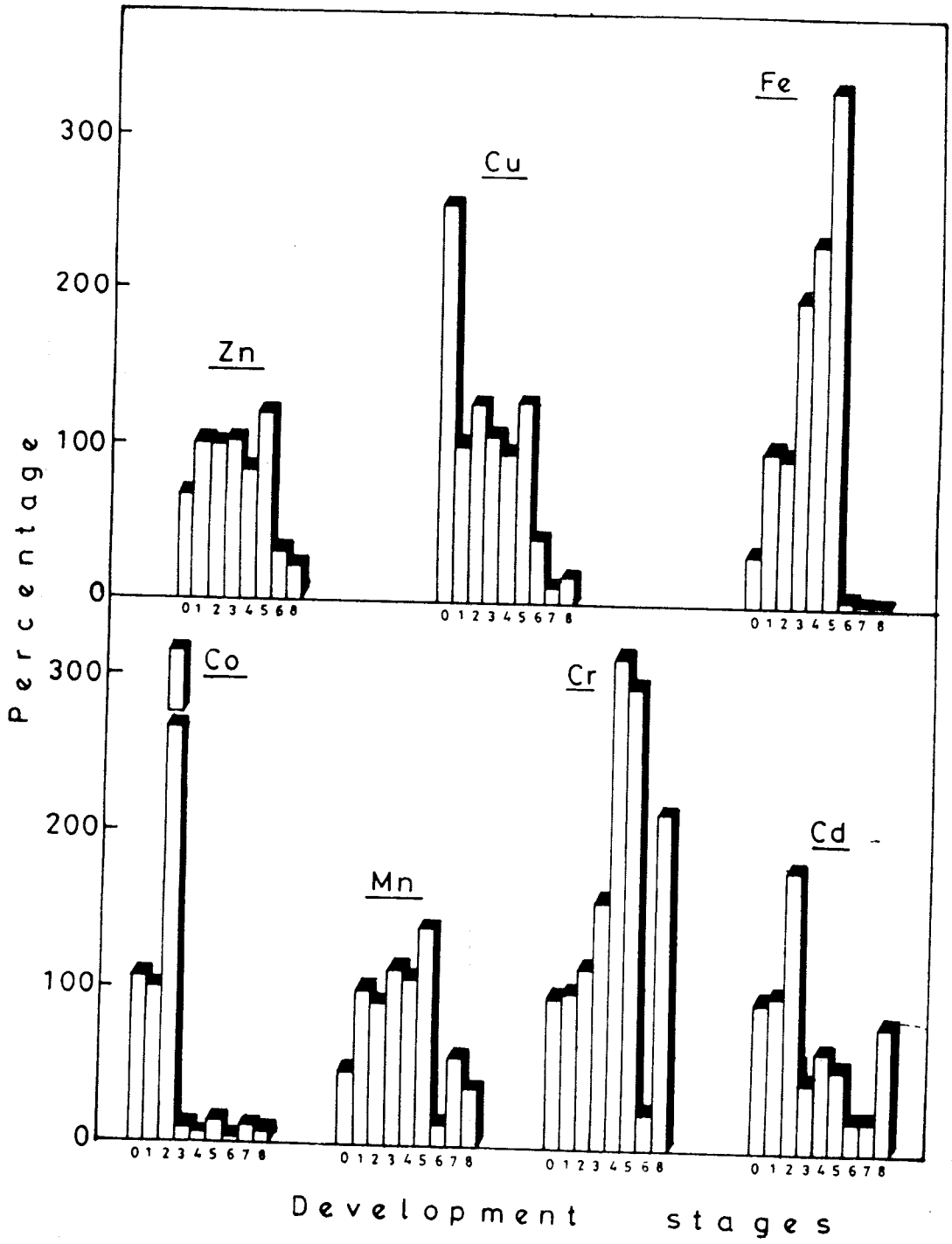


Fig.7.11 Percent increase/decrease in trace elements during egg development in *P.indicus* (Stage 1 = 100%)

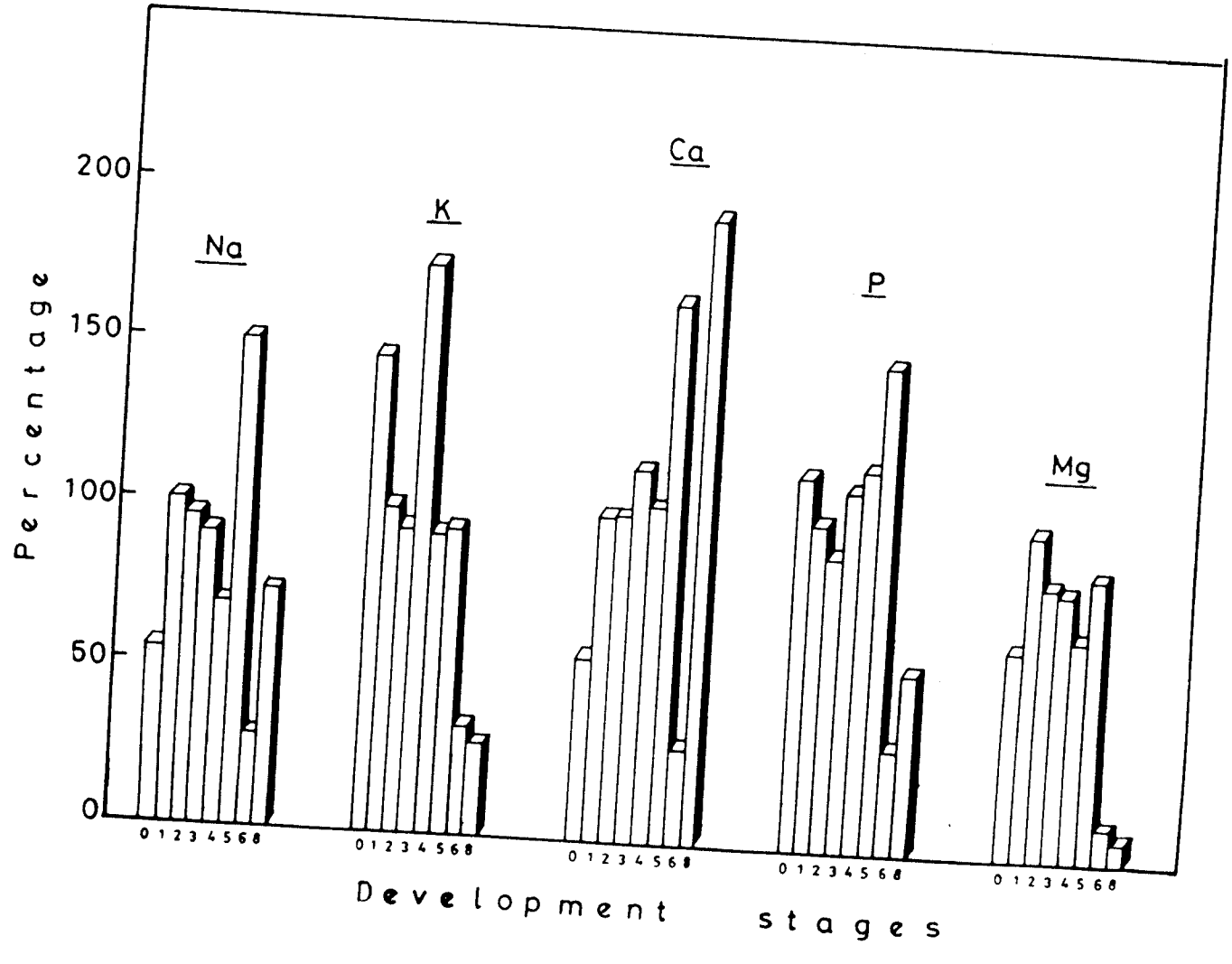


Fig.7.10 Percent increase/decrease in minerals during egg development in *P.indicus* (Stage 1 = 100%).

development in aquatic "non-cleidoic" eggs, ripe ova rather than spawned egg should be the starting point.

The present investigation is the first on the smallest decapod egg, reported so far. The rate of development in *P. indicus* egg is so fast that by the time eggs of other groups of decapods complete initial cleavages, the development is over and a non-feeding larva is released. The faster development would probably have been evolved in penaeids to complete embryonic phase as quickly as possible since the eggs are not protected and are left to the vagaries of the surrounding medium. Fast development in *P. indicus* is in sharp contrast to longer period, ranging from several days to months, taken to complete embryonic development in still smaller eggs of barnacles, in temperate region, where the lower environmental temperature is stated to be the cause of slower development. Further, the eggs of barnacles have some protection as they are incubated in the mantle cavity of the animal (Barnes, 1965, Lucas and Crisp, 1987).

Chemical composition of *P. indicus* confines to the average reported for many crustaceans, but has more lipid and carbohydrate and less protein compared to other decapods. The difference, however, is not significant. Lucas and Crisp (1987) have summarised the composition of organic materials, on an ash free basis, in barnacles reported so far. Protein, lipid and carbohydrate in barnacle eggs, smaller in size than penaeid eggs, range from 52-74%, 20-40% and 0.3-9% respectively. The ash free composition of

*P. indicus* egg falls within this range with 55.08% protein, 38.3% lipid and 5.91% carbohydrate.

In the initial and middle stages of development in *P. indicus* eggs, more lipid (17.14%) and carbohydrate (43.75%) are used than protein (11.33%). From the last stage of egg to nauplius larva, however, protein utilization is significantly higher (49.34%) than those of lipid (41.51%) and carbohydrate (37.5%). This again could be an adaptive strategy to conserve energy rich lipid for newly hatched nauplius, which is a non-feeding larva and has to undergo as many as six moults before getting transformed to a feeding protozoea larva. In the neonate, lipid is the only energy reserve utilized till the last nauplius stage, while the concentration and quantities of protein and carbohydrates increased, indicating the possibility of formation of new proteins and sugars.

Energy conversion efficiencies in *P. indicus* from spawned egg to first hatched larva (nauplius) are 31.67% for total energy, 39.23% for protein energy, 40.92% for lipid energy and 20% for carbohydrate energy. The efficiencies further reduce when calculated upto the emergence of the first feeding larva. Energetic efficiency in *P. indicus* is the lowest recorded for any decapod (vide Chapter 4) and may be due to the incredibly fast rate of development. Lipid contributes more for the energy expenditure during development both at nauplius and protozoea stages, but if ripe ova is included in the calculation, protein and lipid seem to contribute energy, equally. The energetic contribution of carbohydrates, eventhough negligible

when compared to those of protein and lipid, is significantly high in *P.indicus* than the reported average of less than 3% in crustacean eggs.

Uptake and utilization of minerals and trace elements in *P. indicus* eggs, endorse the findings in *P. homarus* (Chapter 4) that the crustacean egg is capable of selectively absorbing and utilizing metallic elements from the surrounding medium. Specific role of individual elements is described elsewhere (Chapter 4). Uptake of major elements like Na, Ca and Mg follows a general pattern in *P. indicus* eggs - a significant absorption in the just spawned egg, reduction in early stages of development and again a marked increase in the middle or towards the end of development of egg. K and P, however, are reduced in the spawned egg and are used up till stage 2 and again absorbed to reach a maximum quantity in the last stage of egg. Absorption and utilization of trace elements also follow this pattern and the maximum quantities are recorded in the last stage of the egg for Fe, Cu, Zn and Mn with differential uptake and utilization in early and middle stages. Cd and Co, generally considered as non-essential and toxic, are actively absorbed in the early stages, reaching a peak in stage 2 and decrease considerably in stage 3 (136% and 558% for Cd and Cr respectively). Such phenomenal changes happening within the egg in a short period of 4-5 hours convey the message that these heavy metals are essential, though in low quantities, for early development and that the egg is capable of absorbing these elements from the medium.

One of the most important changes happening in the egg of *P.indicus* is the substantial reduction in metallic elements between the last stage of egg and the first hatched larva. Nauplius had the lowest concentration and quantity of ash, minerals and trace elements. Among the trace elements, Fe is the most depleted one, followed by Zn, Mn, Cr, Cu, Cd and Co. Whether this heavy depletion is due to active utilization for metabolic purpose in the last stage of development or to their expulsion in the egg case is debatable, since egg case was not analysed in this study.

Trends of absorption and utilization of metallic elements in nauplius suggest that, compared to the changes happening in the developing egg, absorption and utilization in the larvae are considerably low. Most of the trace elements, especially Fe, Zn and Cu are significantly less in the nauplius and protozoea, probably suggesting that the requirements of trace elements are more in developing eggs of *P. indicus* compared to those of the larvae.

*P. indicus*, like other penaeid prawns, migrate back to sea at the onset of sexual maturity for reproduction. This is probably for providing a stable medium for the developing egg for successful completion of embryonic development and for larval survival. Environmental stress like temperature and salinity upset regulatory mechanisms and at reduced salinity, heavy metal uptake increases resulting in increased toxicity (Kinne, 1971, McKenny and Neff, 1979, Bjerregaard and Vislie, 1986, Wright, 1986). At natural concentrations, trace elements either constitute prosthetic groups of enzymes or function as enzyme activators, while at elevated concentrations, they act

as inactivators of enzyme systems or as protein precipitants (Nair, 1984). This study in *P. indicus* eggs indicates the importance of uptake and utilization of minerals and trace elements for embryogenesis. Any environmental stress that may upset this mechanism, like reduction in salinity or concentration of heavy metals in the medium may affect the successful completion of development. Migration to sea for reproduction by estuarine organisms may, therefore, be to ensure, in addition to steady salinity, optimum concentrations of metallic elements that occur naturally in sea water.

Spiny lobster *P. homarus* and the penaeid prawn *P. indicus*, both inhabiting nearshore shallow waters have contrasting life histories, growth and reproductive strategies. A comparison of egg dimensions, quantitative biochemical changes during development and rates of mineral and trace elements uptake and utilization during development of these two species are given in Tables 7.9, 7.10 and 7.11 respectively.

*P. homarus* egg is more than 6 times bigger in weight and volume and contains 10 times more energy than the egg of *P. indicus*. *P.indicus* eggs are released to sea water, while the eggs of *P. homarus* are carried by the female parent. The embryonic development in *P. indicus* egg is 40 time faster than in *P. homarus* and it hatches to the most primitive and non-feeding nauplius larva, while, a highly advanced phyllosoma larva, that starts feeding immediately on emergence, is hatched by the lobster.

**Table 7.9.** Comparison of egg dimensions, energy utilization and incubation period in the spiny lobster *P. homarus* and the penaeid prawn *P. indicus*

| Parameter | *P. indicus* | *P. homarus* | Increase in times in *P.homarus* |
|---|---|---|---|
| Wet weight of egg (μg) | 15.4 ± 2.27 | 95.4 ± 20.0 | 6.19 |
| Dry weight (μg) | 3.15 ± 1.06 | 31.41 ± 9.00 | 9.97 |
| Total energy (J) | 0.081 ± 0.027 | 0.83 ± 0.04 | 10.24 |
| Egg diameter (mm) | 0.257 | 0.478 | 1.86 |
| Egg volume ($mm^3$) | 0.0088 | 0.0561 | 6.38 |
| Incubation time (hours) (Fertilized egg to first hatched larva) | 14.5 | 58.00 | 40.55 |
| **Volume increase (%)** | | | |
| (egg to larva) | 17.5 | 102.3 | 5.84 |
| (ova to larva) | 77.6 | 192.5 | 2.48 |
| **Conversion efficiency (%) (Egg to first larva)** | | | |
| Total energy | 31.67 | 46.67 | 1.47 |
| Protein energy | 39.34 | 48.26 | 1.23 |
| Lipid energy | 40.95 | 27.28 | − 0.67 |
| Carbohydrate energy | 18.75 | 35.96 | 1.92 |
| Dry matter | 37.14 | 64.02 | 1.72 |

Table 7.10. Comparison of organic matter and minerals and trace elements absorbed and utilized in different stages of development in a single egg/larva of *P. homarus* and *P. indicus* (values in parenthesis indicate material used for development in various stages).

| Parameter | *P. homarus* | | | *P. indicus* | | | | |
|---|---|---|---|---|---|---|---|---|
| | Matter absorbed | | | Matter absorbed | | | | |
| | Egg st1-st6 | Ova to phyllosoma | Egg to phyllosoma | Egg st1-st6 | Ova to nauplius | Egg to nauplius | Ova to protozoea | Egg to protozoa |
| Water (μg) | 57.61 (10.3) | 76.6 (36.6) | 57.6 (36.6) | 0.57 (2.36) | 5.71 (8.09) | 0.57 (8.09) | 9.79 (10.30) | 4.03 (10.30) |
| Dry matter (μg) | 1.30 (9.21) | 0.0 (16.8) | 0.0 (11.3) | 0.00 (0.39) | 0.00 (1.98) | 0.00 (1.98) | 0.00 (2.30) | 0.00 (2.30) |
| Protein (μg) | 1.48 (5.58) | 0.0 (14.67) | 0.0 (10.72) | 0.00 (0.17) | 0.00 (1.98) | 0.00 (0.91) | 0.00 (1.31) | 0.00 (1.04) |
| Lipid (μg) | 0.0 (5.69) | 0.0 (7.75) | 0.00 (6.95) | 0.01 (0.18) | 0.01 (0.68) | 0.01 (0.62) | 0.00 (0.89) | 0.00 (0.83) |
| Carbohydrate (μg) | 0.34 (0.78) | 0.0 (0.81) | 0.00 (0.73) | 0.04 (0.11) | 0.13 (0.17) | 0.04 (0.17) | 0.090 (0.135) | 0.000 (0.135) |
| Ash (μg) | 1.58 (0.0) | 4.03 (1.17) | 4.03 (0.0) | 0.17 (0.14) | 0.38 (0.53) | 0.17 (0.53) | 0.48 (0.53) | 0.27 (0.53) |
| Energy (J) | 0.17 (0.39) | 0.17 (0.64) | 0.17 (0.51) | 0.00 (0.01) | 0.00 (0.05) | 0.00 (0.05) | 0.000 (0.057) | 0.00 (0.47) |
| Na (ng) | 0.206 (229.0) | 711.0 (472.0) | 711.0 (229.0) | 60.73 (23.4) | 95.03 (114.04) | 60.73 (114.04) | 128.3 (114.04) | 94.00 (114.04) |
| K (ng) | 0.185 (161.0) | 185.0 (551.0) | 185.0 (296.0) | 21.28 (22.9) | 21.28 (49.94) | 21.28 (38.04) | 21.18 (51.34) | 21.18 (39.44) |
| Ca (ng) | 116.0 (33) | 646.0 (148.0) | 646.0 (33.0) | 14.50 (2.13) | 22.66 (27.64) | 14.50 (27.64) | 53.18 (25.51) | 45.02 (25.51) |
| P (ng) | 321.0 (37.0) | 740.0 (1580.0) | 740.0 (370.0) | 33.31 (5.42) | 33.31 (80.24) | 33.31 (71.79) | 46.28 (80.24) | 46.28 (71.79) |
| Mg (ng) | 55.23 (20.93) | 139.3 (39.3) | 139.3 (20.9) | 2.21 (3.70) | 6.46 (8.67) | 2.21 (8.67) | 11.03 (12.37) | 7.55 (12.37) |
| Fe (ng) | 7.73 (1.52) | 7.73 (8.62) | 7.73 (8.57) | 2.18 (0.030) | 2.80 (3.06) | 2.18 (3.06) | 2.801 (3.080) | 2.184 (3.080) |
| Cu (ng) | 0.20 (0.60) | 0.199 (1.168) | 0.197 (1.164) | 0.012 (0.008) | 0.012 (0.069) | 0.012 (0.027) | 0.017 (0.820) | 0.017 (0.040) |
| Zn (ng) | 3.54 (3.45) | 3.54 (7.39) | 3.54 (7.35) | 0.391 (0.190) | 0.753 (1.125) | 0.391 (1.125) | 0.783 (1.556) | 0.421 (1.556) |
| Cr (ng) | 0.039 (0.026) | 0.039 (0.065) | 0.039 (0.026) | 0.015 (0.003) | 0.016 (0.590) | 0.015 (0.021) | 0.028 (0.021) | 0.028 (0.021) |
| Mn (ng) | 0.241 (0.14) | 0.241 (0.446) | 0.241 (0.166) | 0.028 (0.007) | 0.056 (0.021) | 0.028 (0.074) | 0.249 (0.067) | 0.220 (0.067) |
| Co (ng) | 0.086 (0.00) | 0.086 (0.097) | 0.086 (0.097) | 0.009 (0.010) | 0.009 (0.074) | 0.009 (0.011) | 0.009 (0.013) | 0.009 (0.013) |
| Cd (ng) | 0.019 (0.012) | 0.019 (0.221) | 0.019 (0.221) | 0.002 (0.003) | 0.002 (0.012) | 0.002 (0.003) | 0.004 (0.003) | 0.004 (0.003) |

**Table 7.11.** Ratio of minerals and trace elements absorbed/utilized in the eggs of P. indicus and P. homarus during development

| Minerals/ trace elements | Ratio (absorbed/utilized) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | P. homarus | | | P. indicus | | | | |
| | Egg st1 to st6 | Ova to phyllo-soma | Egg to phyllo-soma | Egg st1 to st6 | Ova to nauplius | Egg to nauplius | Ova to proto-zoea | Egg to proto-zoea |
| **Minerals** | | | | | | | | |
| Na | 0.90 | 1.51 | 3.10 | 2.60 | 0.83 | 0.53 | 1.13 | 0.82 |
| K | 1.15 | 0.34 | 0.63 | 0.93 | 0.43 | 0.56 | 0.41 | 0.54 |
| Ca | 3.52 | 0.44 | 19.58 | 6.81 | 0.82 | 0.52 | 2.09 | 1.77 |
| P | 0.87 | 0.47 | 2.00 | 6.15 | 0.42 | 0.46 | 0.58 | 0.65 |
| Mg | 2.63 | 3.54 | 6.67 | 0.60 | 0.75 | 0.25 | 0.09 | 0.61 |
| **Trace elements** | | | | | | | | |
| Fe | 5.09 | 0.90 | 0.90 | 73.28 | 0.91 | 0.71 | 0.50 | 0.27 |
| Cu | 0.33 | 0.17 | 0.17 | 1.48 | 0.17 | 0.45 | 0.91 | 0.71 |
| Zn | 1.03 | 0.48 | 0.48 | 2.06 | 0.67 | 0.35 | 0.21 | 0.43 |
| Cr | 1.50 | 0.60 | 1.50 | 5.70 | 0.76 | 0.75 | 1.39 | 1.38 |
| Mn | 1.72 | 0.54 | 1.45 | 4.06 | 0.76 | 0.37 | 3.69 | 3.27 |
| Co | Inf. | 0.89 | 2.00 | 0.84 | 0.75 | 0.82 | 0.70 | 0.71 |
| Cd | 1.65 | 0.09 | 0.91 | 0.68 | 0.59 | 0.56 | 1.09 | 1.06 |

Inf. denotes infinite.

Volume of just spawned egg is 6.38 times more in *P. homarus* and the volume increase during development is 5.84 times more than that in *P. indicus* egg. But, if the volume increase is calculated from the ripe ova, the difference is only 2.48 times in *P. homarus* eggs. Energy conversion efficiency is significantly low in the fast developing *P.indicus* eggs and is the lowest recorded for any decapod egg. The prawn egg has a high lipid efficiency than the lobster egg since lipid energy is partly conserved for utilization of neonate in prawn.

*P.homarus* egg (spawned egg) absorbs 57.6 μg water during development and only 36.6 μg is utilised. In *P.indicus* more water (8.09 μg) is utilized than absorbed (0.57 μg). But when water absorbed by ripe ova while spawning is also considered, the ratio of water absorbed to used increases to 0.71 in *P. indicus*, still a negative one, while it increases from 1.57 to 2.09 in *P. homarus*. *P. indicus*, thus, has to depend on water reserves of the egg to complete development.

Quantities of protein, lipid and carbohydrate and total energy utilized for development are 12.41, 11.21, 4.29 and 13.33 times more in *P.homarus* egg compared to the prawn egg. Ash utilization, however, shows important differences that cannot be traced to the size of the egg. While ash content of the lobster egg increase by 4.03 μg during development, without any reduction, *P.indicus* absorbed 0.17 μg from the medium and utilized 0.53 μg during development to nauplius. If calculated from ova, the phyllosoma larva of *P.homarus* retains a positive ratio (4.03 μg absorbed to 1.17 μg utilized),

while *P.indicus* still has a negative ratio 0.38 μg absorbed to 0.53 μg utilized), which makes it compulsary to depend on the stored inorganic reserves of the ova/egg. In contrast to phyllosoma larva that has 5.3 times more ash than the fertilized egg with a 97.61% increase over the last stage of egg, the nauplius larva has less than one seventh of ash present in the egg and loses 88.67% at the emergence of nauplius. This contrasting and interesting change between last stage of egg and emergence of larva is responsible for the high variation in ash content during development between *P. homarus* and *P. indicus* eggs.

The difference in concentration and quantity of ash is reflected in uptake and utilization of individual elements also. In *P. homarus*, ratio of elements absorbed to utilized from fertilized egg to phyllosoma is positive for all except K, Fe, Cu and Zn, while in *P.indicus* the ratios are negative for all elements. A completely contrasting pattern emerges, when the mineral and trace elements in the ripe ova are also considered. At spawning there is significant reduction in all minerals and trace elements except Na and Mg in *P.homarus*. In the prawn egg, due to absorption from the medium, all elements except K,P,Cu and Co increase significantly as the eggs are spawned. From ova to phyllosoma the ratio of elements absorbed to utilized, becomes negative for all elements except Na and Mg in *P.homarus*, while in *P.indicus*, the ratio from ova to nauplius is positive for all major elements and negative for all trace elements except Fe. These observations suggest that provision of metallic element in the ova and absorption from the surrounding medium are both necessary for embryonic development in these two species of decapods.

# 8. FOOD CONVERSION IN POSTLARVAE OF THE PRAWN, PENAEUS INDICUS UNDER DIFFERENT SALINITIES FED WITH NATURAL AND COMPOUNDED FEEDS

Salinity is the "ecological master factor" in an estuary and adaptation to different salinities depend on the genetic make up, age, sex or size as well as previous salinity history of the animal. In general, sensitivity to extremes of salinity is maximum during embryonic or early development stage and during reproduction in adults (Kinne, 1960, 1966). Estuarine animals tend to counter salinity stresses by either modifying the rates of efficiencies of metabolism, activity, growth and reproduction or by compensatory devices like escape, ionic regulation and by adaptation to the fluctuating environment (Kinne, 1966). Effect of salinity on food conversion efficiency has been studied in a number of fishes (Kinne, 1960, 1962, McLeod, 1977) and crustaceans (Venkataramiah *et al.*, 1973a,b, Kalyanaraman and Paulraj, 1984). In both these groups, food intake and conversion were maximum at intermediate salinities and lowest values were recorded at both extremes of high and low salinities.

The Indian white prawn, *Penaeus indicus*, like many other penaeid prawns, spawns in the sea. The postlarvae then migrate in large numbers to estuaries and backwaters in search of "greener pastures" and finally, return to sea for reproduction (Muthu, 1978). The prawn postlarvae are subjected to very low salinities in the west coast of India during south-west

monsoon and to extremely high salinities in summer months in the east coast. Few attempts have been made earlier to evaluate salinity tolerence and effect of salinity on food conversion in *P. indicus* postlarvae (Kalyanaraman and Paulraj, 1984, Lakshmikantham and Suseelan, 1984). The aim of this study is to evaluate whether growth and survival of postlarvae of *P. indicus* are affected by salinity, and if so whether it occurs at the level of food intake or food conversion. Efficacy of pelleted feed, prepared with ingredients that are generally used in India for prawn feed preparations, is also tested.

## 8.1 RESULTS

### 8.1.1 Weight increase

Experimental conditions provided are described in Table 8.1 and weight increase and survival of postlarvae under different salinities and feeds are given in Table 8.2 and Fig.8.1. Survival was maximum (97.5 to 100%) in low saline water (16.48 ± 1.51 ppt) and minimum (62.5 to 85%) in high saline water (44.90 ± 5.75 ppt) and it appears that salinity rather than feed was responsible for survival.

Maximum weight increase (213.36 ± 7.27 mg) was recorded for clam-fed postlarvae in normal saline water (33.05 ± 0.48 ppt) and minimum (51.62 ± 21.41 mg) for pellet-fed ones in high saline water. Under the same salinity, the difference in weight increase was significant between the feeds clam and mixed food in low and high saline waters ($p = <0.05$) but not in normal saline water ($p = >0.05$). Difference in weight gain in all salinities

**Table 8.1 :** Experimental conditions provided for feeding experiments on postlarvae of *P. indicus*

| Salinity | Feed | Initial weight (mg) | No.of post-larvae | Size of rearing tank (cm) | Volume of water (l) | Temperature ($^{O}$C) | pH | Dissolved oxygem ml/l | Duration (days) |
|---|---|---|---|---|---|---|---|---|---|
| **Low saline** | | | | | | | | | |
| 16.48 ± 1.51 | Clam | 16.38 ± 1.36 | 20 x 3 | 60 x 40.5 | 40 | 27.71 ± 0.96 | 7.98 ± 0.14 | 4.3 ± 0.9 | 28 |
| 16.48 ± 1.51 | Pellet | 24.15 ± 0.65 | 20 x 3 | 60 x 40.5 | 40 | 27.71 ± 0.96 | 8.03 ± 0.04 | 4.2 ± 0.9 | 28 |
| 16.48 ± 1.51 | Clam + Pellet | 21.50 ± 4.00 | 20 x 3 | 60 x 40.5 | 40 | 27.71 ± 0.96 | 8.0 ± 0.10 | 4.3 ± 0.7 | 28 |
| **Normal saline** | | | | | | | | | |
| 33.05 ± 0.48 | Clam | 16.25 ± 1.75 | 20 x 3 | 60 x 40.5 | 40 | 27.71 ± 0.96 | 7.99 ± 0.02 | 4.3 ± 0.6 | 28 |
| 33.05 ± 0.48 | Pellet | 19.00 ± 1.50 | 20 x 3 | 60 x 40.5 | 40 | 27.71 ± 0.96 | 8.03 ± 0.05 | 4.1 ± 0.8 | 28 |
| 33.05 ± 0.48 | Clam + Pellet | 18.75 ± 1.75 | 20 x 3 | 60 x 40.5 | 40 | 27.71 ± 0.96 | 8.00 ± 0.04 | 4.4 ± 0.4 | 28 |
| **High saline** | | | | | | | | | |
| 44.90 ± 5.75 | Clam | 23.38 ± 0.13 | 20 x 3 | 60 x 40.5 | 40 | 27.71 ± 0.96 | 7.95 ± 0.07 | 4.5 ± 0.8 | 28 |
| 44.90 ± 5.75 | Pellet | 21.00 ± 3.25 | 20 x 3 | 60 x 40.5 | 40 | 27.71 ± 0.96 | 8.04 ± 0.02 | 4.4 ± 0.3 | 28 |
| 44.90 ± 5.75 | Clam + Pellet | 25.13 ± 2.63 | 20 x 3 | 60 x 40.5 | 40 | 27.71 ± 0.96 | 7.99 ± 0.07 | 4.2 ± 0.2 | 28 |

Table 8.2. Increase in total length, live weight, dry weight and energy content and survival in P. indicus postlarvae under different salinity and feed regimes

| Parameters | Growth under different salinities and feed | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Feed : CLAM | | | Feed : CLAM + PELLET | | | Feed : PELLET | | |
| | Low saline | High saline | Normal saline | Low saline | High saline | Normal saline | Low saline | High saline | Normal saline |
| Initial length (mm) | 17.30± 0.20 | 18.10± 0.10 | 17.30± 0.20 | 17.90± 0.40 | 18.10± 0.02 | 17.85± 0.15 | 18.00± 0.50 | 17.90± 0.20 | 17.60± 0.20 |
| Initial weight (mg) | 16.38± 1.38 | 23.38± 0.13 | 16.25± 1.75 | 21.50± 4.00 | 25.63± 2.63 | 18.75± 1.75 | 24.15± 0.65 | 21.00± 3.25 | 19.00± 1.50 |
| Increase in length (mm) | 18.12± 1.22 | 14.84± 0.72 | 19.14± 1.09 | 16.85± 0.44 | 12.73± 0.24 | 18.38± 0.25 | 7.94± 0.44 | 4.27± 1.18 | 8.69± 0.62 |
| Increase in weight | | | | | | | | | |
| Growth (P) wet (mg) | 208.94± 10.15 | 180.59± 6.82 | 213.36± 7.27 | 212.74± 31.40 | 156.32± 1.61 | 210.21± 5.41 | 68.53± 1.25 | 51.63± 21.44 | 73.67± 12.56 |
| Growth (P) dry (mg) | 53.14± 3.01 | 45.99± 0.35 | 54.97± 2.48 | 56.04± 8.28 | 38.76± 0.44 | 50.87± 0.30 | 18.80± 0.34 | 14.34± 5.78 | 18.21± 2.98 |
| Growth (P) Energy (J) | 983.64± 55.64 | 851.35± 6.54 | 1017.46± 45.83 | 1053.35± 155.68 | 728.44 8.31 | 956.12± 0.56 | 336.56± 6.07 | 256.64± 103.39 | 326.00± 53.27 |
| Exuvia (E) wet (mg) | 40.70± 2.05 | 37.28± 3.12 | 36.82± 1.86 | 37.15± 0.73 | 29.50± 0.88 | 36.91± 1.03 | 8.30± 0.59 | 5.11± 0.29 | 6.13± 0.44 |
| Exuvia (E) Dry (mg) | 8.37± 0.41 | 7.89± 0.62 | 7.57± 0.37 | 7.65± 0.15 | 6.16± 0.18 | 7.59± 0.21 | 1.70± 0.02 | 1.05± 0.06 | 1.27± 0.06 |
| Exuvia (E) Energy (J) | 52.07± 2.55 | 49.15± 3.85 | 47.11± 2.30 | 47.57± 0.93 | 37.77± 1.11 | 47.25± 1.31 | 10.61± 0.12 | 6.54± 0.37 | 7.85± 12.56 |
| P + E | | | | | | | | | |
| Wet weight (mg) | 249.64± 10.45 | 217.87± 7.46 | 250.18± 7.59 | 249.89± 31.52 | 185.82± 0.62 | 247.12± 5.56 | 76.83± 1.32 | 56.73± 21.47 | 79.8± 12.60 |
| Dry weight (mg) | 61.51± 2.57 | 53.88± 0.78 | 62.54± 2.53 | 63.69± 8.30 | 44.92± 0.47 | 58.46± 0.33 | 20.50± 0.34 | 15.39± 5.78 | 19.48± 2.98 |
| Energy (J) | 1035.71± 43.27 | 900.05± 6.76 | 1064.57± 45.94 | 1100.92± 155.68 | 766.21± 8.37 | 1003.37± 0.62 | 347.17± 6.07 | 263.18± 95.82 | 333.85± 53.30 |
| Weight increase (P) per day (mg) | 7.46± 0.36 | 6.45± 0.24 | 7.62± 0.26 | 6.47± 0.91 | 5.58± 0.06 | 7.51± 0.19 | 2.45± 0.06 | 1.84± 0.76 | 2.63± 0.45 |
| Survival (%) | 97.50± 2.04 | 80.00± 0.00 | 95.00± 4.10 | 100.00 | 85.00± 12.20 | 80.00± 14.10 | 97.50± 2.10 | 62.50± 2.00 | 85.00± 0.00 |

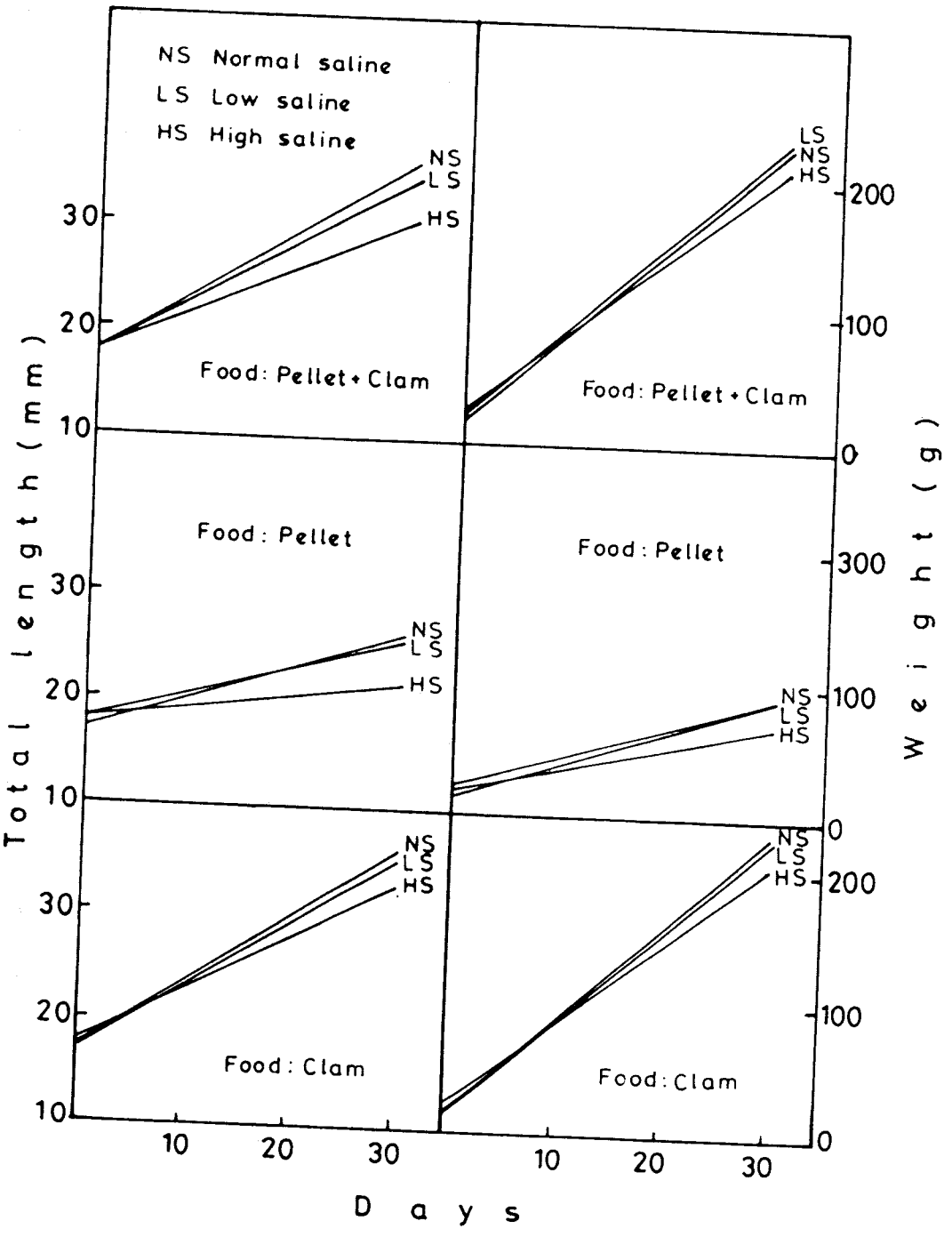


Fig.8.1 Increase in total length and weight in *P.indicus* postlarvae under different salinities and feeds. (low saline - 16.48 ± 1.51 ppt; normal saline - 33.05 ± 0.48 ppt and High saline 44.90 ± 5.75 ppt).

were highly significant ($p = <0.005$) between clam and pellet and mixed food and pellet. Weight gain obtained on particular feed was not significant ($p = > 0.05$) for clam and pellet in low saline and normal saline waters, but the difference was significant ($p = <0.05$) for mixed food. Weight increase for all feeds were significantly low ($p = <0.05$) in high saline water.

Analysis of varience (ANOVA) was carried out to study the combined effect of salinity and food on weight increase. Significant variations were obtained both between salinities and between feeds (Table 8.3).

### 8.1.2 Feeding and conversion

Feeding rate, conversion rate, assimilation rate and efficiency and metabolic rate are given in Table 8.4 and Fig.8.2. Maximum feeding rate was recorded in high saline water for all the three feeds and the maximum recorded was for pellet (5228.49 J/g/day). Feeding rates for clam and mixed food were not significantly different in low and normal saline waters but were more ($p=<0.05$) in high saline water. Feeding rates for pellet were significantly different ($p=<0.05$) between salinities.

Assimilation efficiency was consistently high in all salinities and varied between 97.49 and 99.48% for clam, 98.0 and 99.17% for mixed food and between 93.96 and 97.80% for pellet.

Maximum conversion rate was recorded for clam in all salinities followed by mixed food and the lowest rate was in pellet-fed prawns.

**Table 8.3.** MSS and F values of ANOVA for various treatments in *P.indicus* feeding experiment

| Treatments/Parameters | SS | df | MSS | F | F (Table) | S/NS* |
|---|---|---|---|---|---|---|
| **1. Weight increase** | | | | | | |
| Between salinities | 32989.87 | 2 | 16494.94 | 198.0 | 6.94 | S |
| Between feed | 2020.54 | 2 | 1010.28 | 12.0 | 6.94 | S |
| Error | 333.25 | 4 | 83.31 | | | |
| Total | 35343.67 | 8 | 17589.00 | | | |
| **2. Feeding rate** | | | | | | |
| Between salinities | 624810.95 | 2 | 312405.48 | 3.3 | 6.94 | NS |
| Between feed | 8435316.27 | 2 | 4217658.14 | 44.85 | 6.94 | S |
| Error | 378680.15 | 4 | 94670.04 | | | |
| Total | 9438807.37 | 8 | 1179850.92 | | | |
| **3. Conversion rate** | | | | | | |
| Between salinities | 3393.55 | 2 | 1696.78 | 14.78 | | S |
| Between feed | 39523.55 | 2 | 19761.78 | 172.17 | 6.94 | S |
| Error | 459.12 | 4 | 114.78 | | 6.94 | |
| Total | 43376.22 | 8 | 5422.03 | | | |
| **4. Food conversion efficiency ($K_2$)** | | | | | | |
| Between salinities | 31.58 | 2 | 15.79 | 16.19 | 6.94 | S |
| Between feed | 181.65 | 2 | 90.84 | 93.15 | 6.94 | S |
| Error | 7.81 | 4 | 1.95 | | | |
| Total | 221.04 | 8 | 108.58 | | | |
| **5. Metabolic rate** | | | | | | |
| Between salinities | 573752.50 | 2 | 286876.25 | 5.46 | 6.94 | NS |
| Between feed | 8184714.70 | 2 | 4092357.35 | 77.83 | 6.94 | S |
| Error | 210323.44 | 4 | 52580.86 | | | |
| Total | 8968790.64 | 8 | 1121098.83 | | | |
| **6. Protein efficiency ratio (PER)** | | | | | | |
| Between salinities | 0.115467 | 2 | 0.057734 | 75.27 | 6.94 | S |
| Between feed | 0.602867 | 2 | 0.301434 | 393.10 | 6.94 | S |
| Error | 0.003066 | 4 | 0.000767 | | | |
| Total | 0.721400 | 8 | 0.009018 | | | |

S = Significant ; NS = Non-significant

Table 8.4 : Rates of feeding, assimilation, conversion and metabolism (J/g live body weight/day) and assimilation efficiencies (%) in postlarvae of P. indicus under different salinities and feed.

| Salinity/Feed | (Rate and efficiency) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Feeding rate | Assimilation rate | Assimilation efficiency | Conversion rate | | | Metabolic rate | |
| | | | | Exuvia (E) | Growth (P) | P+E | Energy | ml/$O_2$ g live body wt/hr |
| **Clam** | | | | | | | | |
| Low saline | 2156.12± 70.05 | 2101.93± 67.18 | 99.49± 0.12 | 21.31± 0.21 | 402.54± 22.7 | 423.85± 22.76 | 1672.79± 44.41 | 3.48± 0.09 |
| High saline | 2379.23± 4.64 | 2366.59± 4.11 | 99.46± 0.17 | 20.75± 0.16 | 359.19± 2.76 | 379.94± 2.81 | 1986.51± 1.35 | 4.12± 0.03 |
| Normal saline | 2156.06± 67.33 | 2144.78± 67.10 | 99.48± 0.06 | 18.98± 0.22 | 409.95± 18.47 | 428.93± 18.48 | 1715.85± 48.64 | 3.56± 0.10 |
| **Clam + Pellet** | | | | | | | | |
| Low saline | 2785.82± 32.02 | 2762.64± 30.60 | 99.17± 0.08 | 20.48± 0.21 | 386.60± 54.52 | 407.08± 54.67 | 2355.56± 23.91 | 4.88± 0.05 |
| High saline | 3122.79± 314.04 | 3060.36± 319.96 | 98.00± 0.22 | 17.14± 0.12 | 330.70± 3.77 | 347.84± 3.81 | 2712.52± 316.19 | 5.62± 0.66 |
| Normal saline | 2804.20± 180.24 | 2757.15± 187.90 | 98.32± 0.32 | 18.73± 0.19 | 379.12± 0.27 | 397.87± 0.34 | 2359.30± 187.68 | 4.89± 0.31 |
| **Pellet** | | | | | | | | |
| Low saline | 3890.30± 307.88 | 3804.56± 316.92 | 97.80± 1.20 | 8.01± 0.02 | 253.57± 4.57 | 261.58± 5.13 | 3542.98± 317.79 | 7.35± 0.65 |
| High saline | 5228.49± 10.21 | 4912.68± 91.32 | 93.96± 2.30 | 6.07± 0.21 | 237.95± 95.86 | 244.02± 95.86 | 4668.66± 4.55 | 9.68± 0.01 |
| Normal saline | 4490.10± 83.09 | 4263.47± 88.95 | 94.95± 1.98 | 6.37± 0.12 | 264.62± 43.24 | 270.99± 43.24 | 3992.47± 45.17 | 8.23± 0.09 |

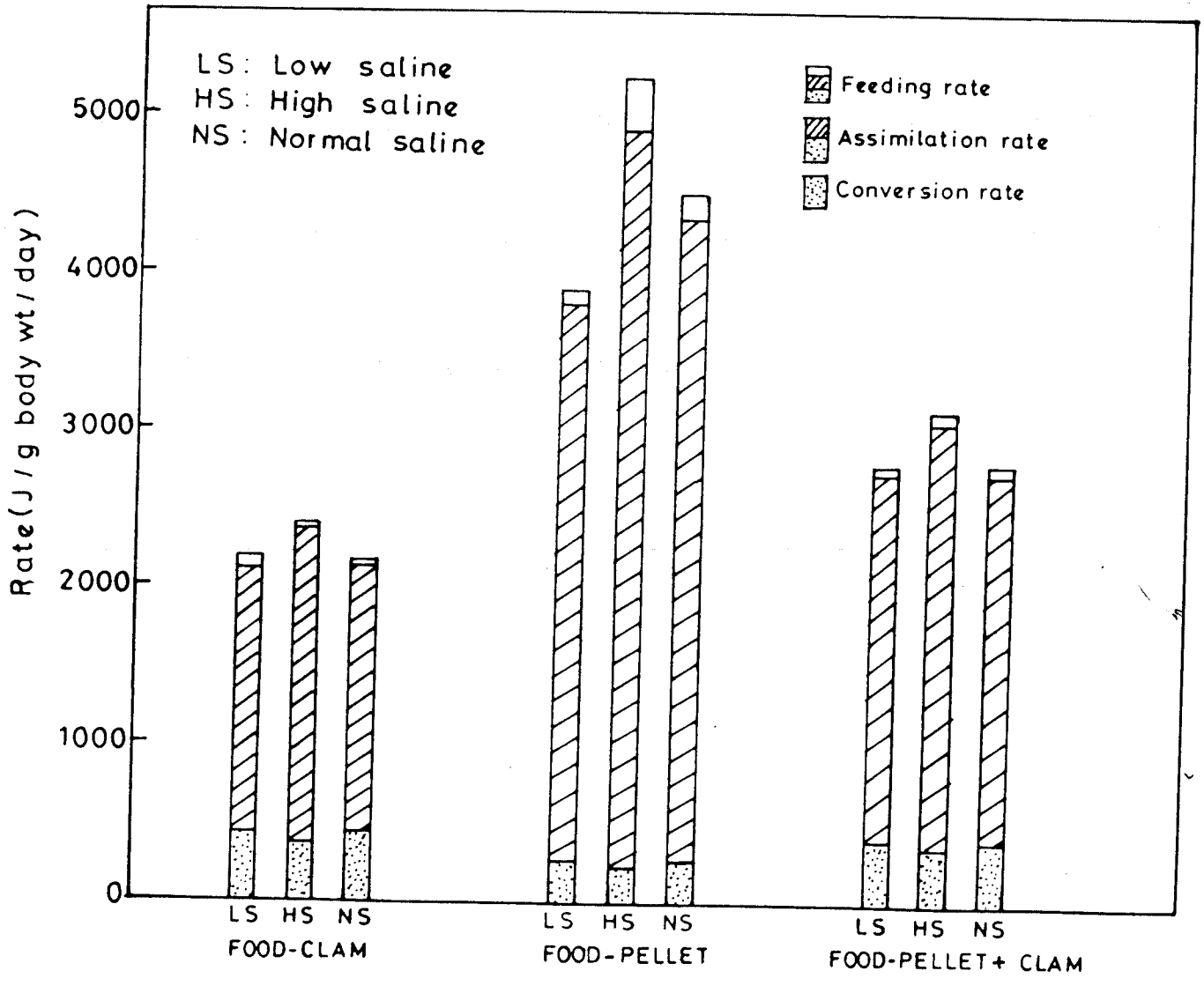


Fig.8.2 Feeding, assimilation and conversion rates in postlarvae of *P.indicus* under different salinities and feeds. (low saline - 16.48 ± 1.51 ppt; normal saline - 33.05 ± 0.48 ppt and High saline 44.90 ± 5.75 ppt).

ANOVA (Table 8.3) showed that conversion rate was significantly different both between salinities and feeds.

Due to high assimilation efficiency, net conversion efficiency ($K_2$) was not much different from gross conversion efficiencies ($K_1$). Conversion efficiencies, both $K_1$ and $K_2$, are given in Table 8.5 and Fig.8.3. Maximum conversion efficiencies ($K_2$), 19.15% (without exuvia) and 20.17% (with exuvia) were recorded in clam-fed prawn in low saline water and minimum, 4.84% (without exuvia) and 4.97% (with exuvia), in high saline water on pellet feed. For the same feed, conversion efficiencies did not vary much in low saline and normal saline waters, but was significantly low ($p = <0.05$) in high saline conditions. ANOVA (Table 8.3) showed that food conversion efficiency is highly variable between salinities and more so between feeds.

Metabolic rates of pellet-fed prawns were 200% more than those calculated for clam-fed ones in all salinities, with maximum (4668.66 J/g/day; 9.68 ml $O_2$/g/hr.) recorded for pellet-fed prawn in high saline water. Metabolic rates in all treatments seem to be extremely high. ANOVA (table 8.3) showed that variability between salinities was not significant, for metabolic rate, but between feeds was highly significant.

### 8.1.3 Protein conversion efficiency (PCE) and protein efficiency ratio (PER)

Protein feeding rate, conversion rate and conversion efficiency (PCE) and Protein efficiency ratio (PER) are given in Table 8.6 and Fig.8.4. For

**Table 8.5.** Gross conversion efficiency ($K_1$) and Net conversion efficiency ($K_2$) of energy and Food conversion raio (FCR) in *P.indicus* postlarvae reared in different salinities with different feeds.

| Treatment | Conversion efficiency | | | | FCR |
|---|---|---|---|---|---|
| | $K_1$ (P/C x 100) | | $K_2$ (P/Ae x 100) | | |
| | Without exuvia (P) | With exuvia (P+E) | Without exuvia (P) | With exuvia (P+E) | |
| **Clam** | | | | | |
| Low saline | 19.02 ± 0.010 | 20.03 ± 0.010 | 19.15 ± 0.010 | 20.17 ± 0.010 | 1.26 |
| High saline | 15.10 ± 0.001 | 15.97 ± 0.001 | 15.18 ± 0.001 | 16.05 ± 0.001 | 1.59 |
| Normal saline | 19.01 ± 0.010 | 19.89 ± 0.010 | 19.11 ± 0.01 | 20.0 ± 0.010 | 1.28 |
| **Pellet** | | | | | |
| Low saline | 6.52 ± 0.001 | 6.72 ± 0.001 | 6.67 ± 0.001 | 6.88 ± 0.001 | 4.64 |
| High saline | 4.50 ± 0.020 | 4.55 ± 0.020 | 4.84 ± 0.020 | 4.97 ± 0.020 | 6.62 |
| Normal saline | 5.95 ± 0.010 | 6.09 ± 0.010 | 6.21 ± 0.010 | 6.36 ± 0.010 | 4.59 |
| **Clam + pellet** | | | | | |
| Low saline | 13.88 ± 0.020 | 14.61 ± 0.020 | 14.0 ± 0.020 | 14.74 ± 0.020 | 1.90 |
| High saline | 10.59 ± 0.001 | 11.14 ± 0.001 | 10.81 ± 0.001 | 11.37 ± 0.001 | 2.42 |
| Normal saline | 13.52 ± 0.010 | 14.19 ± 0.001 | 13.75 ± 0.001 | 14.43 ± 0.001 | 1.84 |

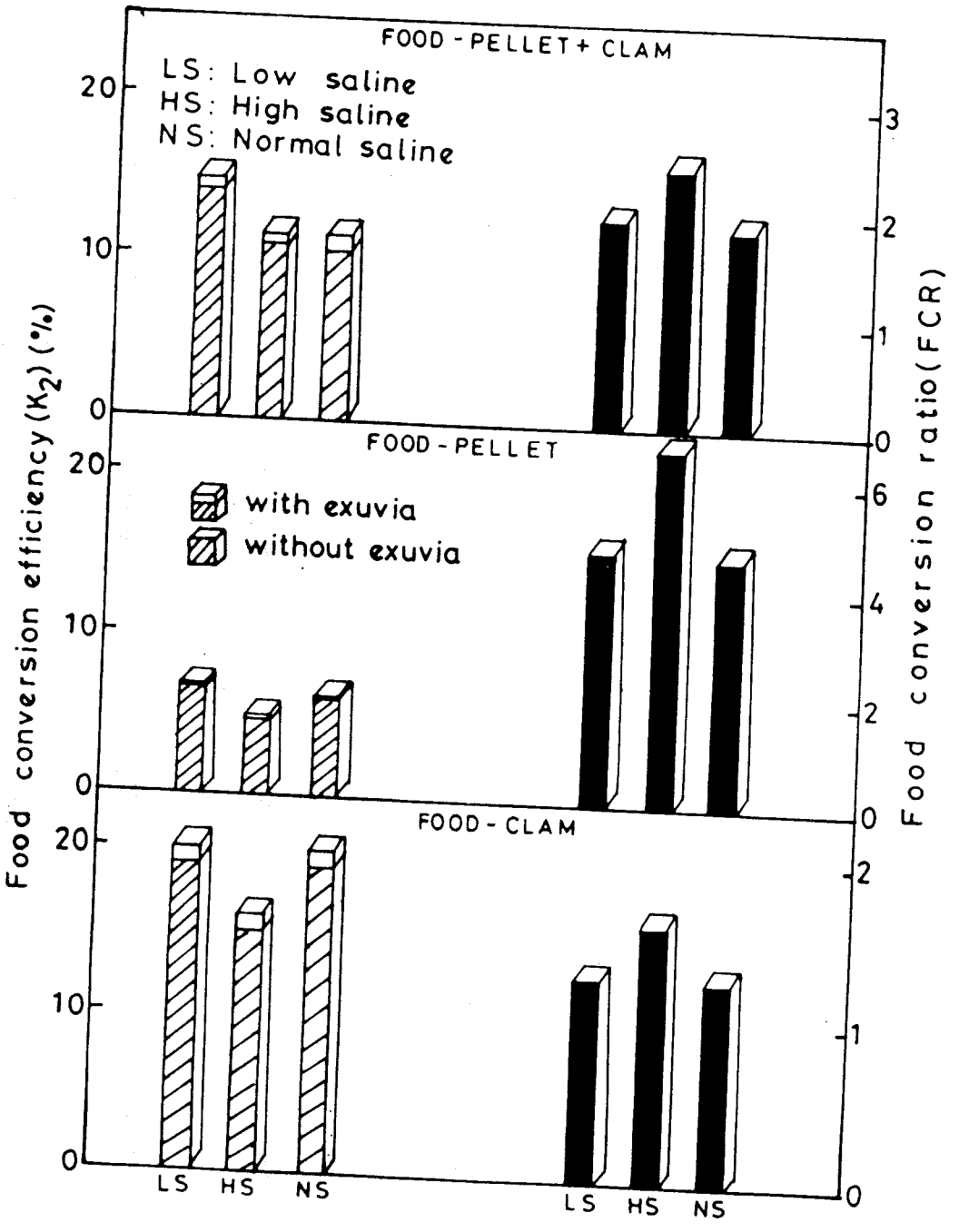


Fig.8.3 Net conversion efficiency ($K_2$) and Food conversion ratio (FCR) of *P.indicus* postlarvae under different salinities and feeds. (low saline - 16.48 ± 1.51 ppt; normal saline - 33.05 ± 0.48 ppt and High saline 44.90 ± 5.75 ppt).

**Table 8.6** Protein feeding rate, conversion rate, gross conversion efficiency ($K_1$) and protein efficiency ratio in postlarvae of *P.indicus* in different salinities fed on clam and pelletted feed with varying protein concentration

| Treatment | Protein % in dry wt. of food | Feeding rate (mg protein/g live body wt/day) | Conversion rate with exuvia (mg protein/g live body wt/day) | Conversion efficiency $K_1$ (P/Cx100) | Protein efficiency ratio (PER) |
|---|---|---|---|---|---|
| **Clam** | | | | | |
| Low saline | 62.97 | 67.47 ± 2.24 | 13.92 ± 0.93 | 20.63 ± 0.010 | 1.27 ± 0.006 |
| High saline | 62.97 | 75.86 ± 0.15 | 13.21 ± 0.68 | 17.41 ± 0.010 | 1.00 ± 0.040 |
| Normal saline | 62.97 | 68.75 ± 2.15 | 12.33 ± 0.36 | 17.93 ± 0.010 | 1.25 ± 0.050 |
| **Pellet** | | | | | |
| Low saline | 32.96 | 79.10 ± 6.26 | 12.44 ± 0.17 | 15.73 ± 0.002 | 0.65 ± 0.010 |
| High saline | 32.96 | 104.47 ± 2.30 | 12.25 ± 2.69 | 11.73 ± 2.580 | 0.46 ± 0.190 |
| Normal saline | 32.96 | 90.45 ± 1.70 | 12.06 ± 2.24 | 13.33 ± 0.020 | 0.66 ± 0.110 |
| **Clam + Pellet** | | | | | |
| Low saline | 44.78 | 66.95 ± 0.68 | 13.41 ± 1.50 | 20.03 ± 0.020 | 1.16 ± 0.160 |
| High saline | 44.78 | 77.37 ± 8.50 | 14.23 ± 0.77 | 18.39 ± 0.010 | 0.92 ± 0.010 |
| Normal saline | 44.78 | 68.68 ± 4.46 | 14.86 ± 0.36 | 21.37 ± 0.210 | 1.21 ± 0.030 |

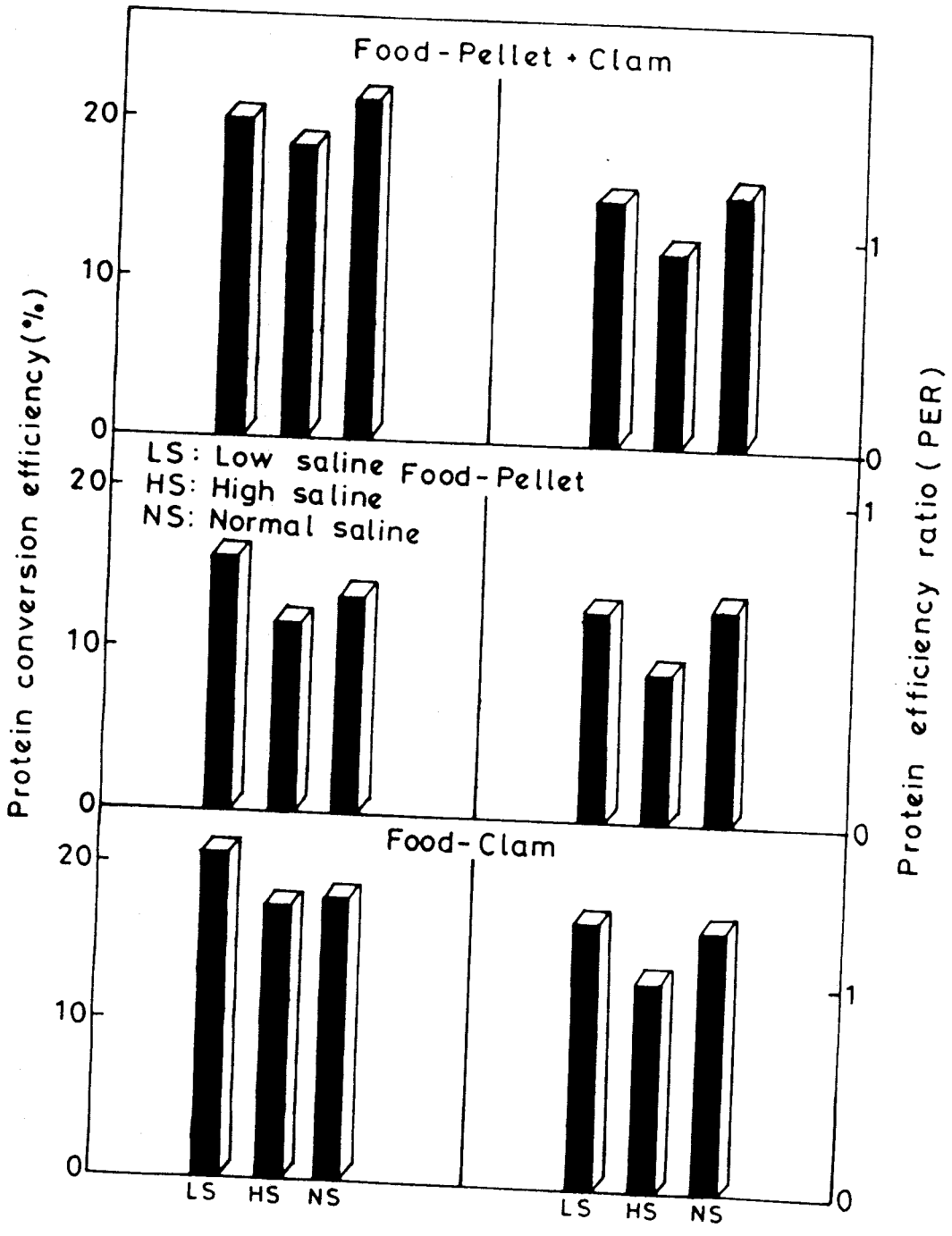


Fig.8.4 Protein conversion efficiency and protein efficiency ratio (PER) in postlarvae of *P.indicus* under different salinities and feeds. (low saline - 16.48 ± 1.51 ppt; normal saline - 33.05 ± 0.48 ppt and High saline 44.90 ± 5.75 ppt).

the same feed, protein feeding rate was significantly different between salinities. Highest PCE (21.37%) was recorded for mixed food in normal saline water and lowest (11.73%) for pellet-fed prawns in high saline water. In clam-fed prawns, PCE was significantly lower than net conversion efficiency in normal saline water and marginally high in low saline and high saline conditions. For the other two feeds, PCE was significantly higher than net conversion efficiency in all salinities and this was very highly pronounced in pellet-fed prawns.

PER was maximum for clam in all salinities and not so different for mixed food. Minimum PER was recorded for pellet in all salinities. PER in high saline water was lower than the other two salinity regimes for all feeds. ANOVA (Table 8.3) showed that PER significantly varied between salinities, but the variation between feeds (protein content of feeds) was more pronounced.

### 8.1.4 Ammonia excretion

Rate of ammonia excretion is given in Table 8.7. An unusually high rate of 60.40 + 15.05 $\mu$g N/g/hr was recorded for prawn, fed with pellet under high salinity. Minimum ammonia excretion was recorded in normal saline water and in both extremes of salinities, ammonia excretion increased irrespective of feed. Barring the unusually high rate for pellet in high saline water, clam fed prawns recorded maximum ammonia excretion in all salinities.

**Table 8.7.** Rates of Ammonia excretion in *P.indicus* postlarvae under different salinities and feeds

| Feed | Rates of Ammonia excretion ($\mu$g N/g body weight/hr) | | |
|---|---|---|---|
| | Low saline | Normal saline | High saline |
| Clam | 30.86 ± 5.68 | 21.47 ± 1.56 | 23.22 ± 4.03 |
| Clam + Pellet | 13.18 ± 0.05 | 13.03 ± 0.25 | 19.34 ± 5.15 |
| Pellet | 20.42 ± 0.75 | 9.41 ± 2.31 | 60.40 ± 15.05 |

## 8.2 DISCUSSION

Most of the successful estuarine animals are regulators of ionic and osmotic concentrations of the coelomic fluid as well as intracellular fluid (Prosser and Brown, 1961, Lockwood, 1962, Kinne, 1966), or those which have adapted successfully to changes in environment. The adaptations are both genetic and non-genetic (acclimatization) acquired by individual organism, directly induced by environment, and not transferred to offspring (Kinne, 1966). Postlarvae of *P. indicus* seem to be highly adapted to live in normal and very low saline conditions but, not in extremes of higher salinity.

Three phases of acclimation have been distinguished starting from (1) a phase of immediate shock reaction within seconds with substantial increase or decrease in metabolic activity, (2) a stabilizer, commencing minutes, hours or days after change and gradually approaching a steady level and (3) the new steady state commencing after hours, days or weeks which hallmarks the completion of adjustments (Kinne, 1966). Most of the studies on responses to salinities have been done by measuring respiration or excretion in animals that are not properly acclimatized and are, probably, in the first or second phase of acclimatization process. Observation of food conversion efficiency, after acclimatizing the test animals to different salinity regimes, extending over days, weeks or months depending on the growth rate of the animal is probably the best way of studying changes that are induced by salinity.

Feeding rate of *P. indicus* does not seem to be affected by low and normal saline regimes for clam and mixed feeds, but is significantly high in high saline water, irrespective of the type of food. Increased feeding rate in high salinity recorded in this study is in contrast to earlier observations. In the fresh water fish *Salmo gairdneri* (McLeod, 1977) and in postlarval *P. indicus* (Kalyanaraman and Paulraj, 1984) feeding rates were observed to be maximum at medium salinities and low at both extremes.

Absorption efficiency is not affected by salinity in *P. indicus* postlarvae, unlike the trend of negative correlation reported in *S. gairdneri* (McLeod, 1977). Absorption efficiency is very high for all feeds and 99.48% recorded for clam-fed prawns could possibly be due to under-estimation of faecal output. Such a high absorption efficiency has earlier been reported for many fishes and crustaceans (Pandian, 1967a, Radhakrishnan, 1989 and also see Chapter 4).

Food conversion efficiency is an indicator of the effect of external and internal factors on the bioenergetic physiology of fish (McLeod, 1977). There are many references of conversion efficiency in fish and crustaceans being affected by changes in salinity. Kinne (1962) found significant differences in conversion efficiency of fully acclimated desert pupfish, *Cyprinodon macularis*, kept at a constant temperature of 30°C and acclimated at fresh water, 15 ppt and 35 ppt salinity. Similarly, in *S. gairdneri*, in the $O^+$ year group, there was no change between 0 - 28 ppt. But between 28 and 32.5 ppt, there was an abrupt decrease in conversion efficiency (McLeod, 1977). Venkataramiah

*et al.*, (1973 a,b) have reported that food conversion efficiency was maximum at 50% sea water salinity for early juveniles of *Penaeus aztecus* under four different salinity regimes. Similarly, in an earlier study on *P.indicus* postlarvae (Kalyanaraman and Paulraj, 1984), maximum conversion efficiency was recorded between 20 and 25 ppt salinity. In this study, conversion efficiency showed variability between both salinity and feed and for the same feed there was no appreciable difference in efficiency in low saline and normal saline waters. However, the conversion efficiency significantly reduced in high saline water. The results of this investigation suggest that the range of low and normal salinity used in this study might possibly be the physiological range for postlarvae of *P. indicus*. Lowest conversion obtained for the compounded feed, under all salinities, indicates that the pellet is nutritionally inadequate. The highest food conversion efficiency (19.11-20.17%) for clam-fed prawns in low salinity compares with the efficiencies recorded for other species of penaeid prawns which varied from 22 - 30% with an average of 23.94% (Capuzzo, 1982) and for *Metapenaeus monoceros* (18.02%, Sumitra *et al.*, 1982).

Food conversion ratio (FCR) was between 1.26 and 1.90 for clam and mixed food in all salinities except in high saline water, where FCR was 2.42 for mixed food. For pellet, the FCR was very high (4.59 - 6.62) in all salinities. The FCR reported for clam and mixed food in this study is in the lower range of all values for penaeid prawns summarized by Capuzzo (1982)

and less than 2, suggested as mandatory to culture crustaceans (Forster, 1976).

Reduction in salinity from 37 ppt to 10 ppt increased oxygen consumption by 300% in juveniles of the prawn *Penaeus japonicus* (Dalla via, 1986), while in the fresh water prawn *Palaemonetes antennareus*, rapid increase in salinity resulted in 54% increase in oxygen consumption and an increase of 20% in metabolic rate. Both these were restored to initial levels after 8-10 hours and 4-8 hours respectively (Dalla via, 1987). In *P. indicus* adults, decrease in salinity from 34 to 2.1 ppt brought about a gradual increase in oxygen consumption with the maximum in 2.1 ppt (Unnikrishnan and Lakshminarayana, 1984). All these studies were of short duration and the animals would probably have been in the first or second phase of response to salinity. In the present study, metabolic rate was not measured as oxygen consumption, but calculated from other food utilization parameters. The acclimated prawns were observed for a longer period and the results suggest that food, rather than salinity was the main factor for increase in metabolic rate in low and normal saline waters. In high salinity, however, metabolic rate was significantly more for all feeds, probably due to the high feeding rate and the resultant calorigenic action (specific dynamic action) of food.

Unlike the spiny lobster, *Panulirus homarus*, in which protein conversion efficiency (PCE) was less than net conversion efficiency ($K_2$), PCE was significantly more than FCE ($K_2$) in all salinities for postlarvae of

*P.indicus* on pellet and mixed food. In clam-fed prawn, the difference was marginal and even less in normal saline water. PCE is reported to decrease with increase in protein concentration of feed (Capuzzo, 1982). In this study, protein content was lowest in pellet followed by mixed food and was maximum in clam. Probably this would have been responsible for the variation in PCE recorded here. With increasing protein content of feed, the ratio of FCE to PCE increased in *P. indicus*.

Protein efficiency ratio (PER) for a number of crustaceans have been summarized by Capuzzo, (1982) and ranges it between 0.5 to 2.7 depending on the protein content of food and decrease with increase in protein. PER did not vary in low and normal salinities in *P. indicus*, but was significantly low in high salinity. PER for clam and mixed food for all salinities (0.92 to 1.27) is comparable to the values recorded for other crustaceans.

Utilization of protein for catabolic processes, induced by the protein composition of the feed or by salinity extremes, bring about important changes in free amino acid composition (*P. japonicus*, Dalla via, 1986) and in nitrogen excretion (Capuzzo, 1982). In this observation, barring an unusually high estimate in high saline water for pellet, ammonia excretion was maximum (21.47-30.86 $\mu$g N/g/hr.) in clam-fed prawns and minimum (9.47-20.43 $\mu$g N/g/hr) in the postlarvae fed with pellet. This trend in nitrogen excretion closely follows FCE/PUE ratio which increased with increase in protein content of the feed, as a result of more protein being catabolised for metabolic purpose.

**Table 8.8.** Energy budget for postlarvae of *P.indicus* fed natural and pelleted feed and reared under different salinities

| Treatment | Energy Parameters | | | | |
|---|---|---|---|---|---|
| | C | F + U | E | P | R |
| **Clam** | | | | | |
| Low saline | 100 | 0.67 | 1.01 | 19.02 | 79.30 |
| High saline | 100 | 0.53 | 0.87 | 15.10 | 83.50 |
| Normal saline | 100 | 0.52 | 0.88 | 19.01 | 79.59 |
| **Pellet** | | | | | |
| Low saline | 100 | 2.20 | 0.20 | 6.52 | 91.08 |
| High saline | 100 | 6.04 | 0.12 | 4.55 | 89.39 |
| Normal saline | 100 | 4.18 | 0.14 | 5.99 | 89.39 |
| **Clam + pellet** | | | | | |
| Low saline | 100 | 0.83 | 0.73 | 13.88 | 84.56 |
| High saline | 100 | 2.00 | 0.55 | 10.59 | 86.86 |
| Normal saline | 100 | 1.68 | 0.67 | 13.52 | 84.13 |

By rearranging Kinne's (1960, 1962) data, Palohaemo and Dickie (1966 a,b) demonstrated that, while temperature affected only the rate of energy turnover, salinity affected the partition of energy between growth and catabolism. The partitioning of energy is best expressed by formulating total energy budgets for the organism. The energy budgets for postlarvae of *P. indicus* in this study are given in Table 8.8. The energy budgets clearly demonstrate effect of salinity and food on various energy utilization parameters. Energy lost as faeces and urine (F+U) increased with salinity for pellet and mixed food, but not so for clam which recorded minimum expenditure due to low fecal output. High values recorded for pellet in all salinities are due to increased feeding rate and comparatively low absorption efficiency. Energy lost as exuvia, in general, was related to increase in weight. The major difference that brought about marked changes in conversion efficiency was energy used for metabolism, which was less (79.3-83.5%) for clam, more for mixed food (84.13 - 86.86%) and was maximum for pellet-fed postlarvae (89.39 - 91.08%).

# SUMMARY

1. Biochemical and mineral changes were studied in muscle, hepatopancreas, and ovary during ovarian maturation in the spiny lobster *Panulirus homarus* and the prawn *Penaeus indicus*.

2. In *P. homarus*, concentration of lipid and carbohydrate in the muscle decreased at maturity from 10.3 to 8.86% and 1.61 to 1.48% respectively, and again to 7.82 and 1.37 in spent animal. Ash increased to 8.41% from 7.22% at maturity and then declined to 7.05% in the spent ones. When expressed quantitatively (in 100 g body weight), the reduction in lipid content of the muscle at maturity and after spawning, was significant.

3. Hepatic index decreased at maturity and also after spawning in *P.homarus*. Quantities of lipid and protein decreased significantly at maturity and protein declined further after spawning in the hepatopancreas. Carbohydrate and ash increased in quantity significantly at maturity and declined, also significantly, after spawning.

4. Gonado-somatic index of females of *P. homarus* went up from 0.2 to 5.31 at maturity and declined to 0.83 after spawning. Water, organic and inorganic components quantitatively increased at maturity and

decreased after spawning, both significantly. Concentration, however, was variable at different maturity stages.

5. Mineral (Na, K, Ca, P and Mg) concentrations increased with maturity in the muscle of lobster and moderately declined after spawning, with the exception of K which showed a declining trend from immature stage. Trace elements (Fe, Cu, Zn, Mn, Co, Cr and Cd) also showed a similar trend with the exception of Fe and Cr which increased after spawning. Co was not detectable at mature stage and Pb was below the detactable level (0.001 $\mu$g/g dry weight) in all stages, and in all tissues analysed.

6. In hepatopancreas, Na, Ca and Mg concentration increased at maturity, while K and P declined. Except Mg and K, the other three minerals declined significantly after spawning. With the exception of Co, all trace elements increased in concentration at maturity. Cu and Cd recorded an increase of more than 10 and 17 times respectively and decreased after spawning. As in muscle, Co was not detectable at maturity, but increased after spawning.

7. Quantitatively, all minerals and trace elements in the ovary increased at maturity and declined significantly after spawning, due to the enormous change in its weight. Most significant change in trace elements was recorded for Cu, which increased from 0.93 to 106.25 $\mu$g and then declined to 2.63 $\mu$g in the spent ovary.

8. Energy (yolk) utilization during egg development in spiny lobster *(P. homarus)* was studied for the first time. Six development stages were identified with the phyllosoma larva as the 7th stage. For a holistic study, ripe ova, in mature ovary, were also analysed and termed as stage 0.

9. *P. homarus* egg took 22 to 27 days (average 24.5 days) to complete embryonic development at 27.03 $\pm$ 1.02°C.

10. Egg diameter increased from 0.478 to 0.602 mm and volume from 0.0561 to 0.1134 $mm^3$. Yolk diameter and volume decreased with advancement of development and yolk was almost fully utilized just before release of larva.

11. Most significant change between ripe ova and spawned egg was in the concentration of water which increased to 67.05% in the egg from 51.7% in the ova.

12. Ripe ova of *P. homarus* weighed 76.40$\mu$g while weight of the spawned egg was 95.40$\mu$g. Organic reserves, protein, lipid and carbohydrate, were used slowly in the initial phase, but rapidly after the appearance of eye (stage 4). Ash content increased gradually with development.

13. Water content increased by 89% in the egg and all water absorbed was not utilized for development.

14. Conversion efficiencies for egg to larva were 64.02% for dry matter, 46.67% for total energy, 27.28% for lipid and 48.26% for protein energy. Lipid contributed 53.4% of total energy utilized during development, protein supplied 43.91% and carbohydrate, a negligible 2.69%.

15. Concentration of all minerals (Na, K, Ca, P and Mg) decreased from the ova to fertilized egg but thereafter showed variability in different stages and except for K, all were at maximum level in the phyllosoma.

16. Like minerals, trace elements (Fe, Cu, Zn, Mn, Co, Cr and Cd) also showed variability between different stages of egg development and, except for Zn, they were at maximum level in Phyllosoma stage. Pb was below the detectable level (< 0.001 $\mu$g/g/dry weight) in all stages. Relative absorption and utilization was more pronounced in trace elements than minerals in the egg.

17. Effect of size and maturity on food conversion efficiency was studied in *P. homarus* in a size range of 10-300 g.

18. Feeding rate, assimilation rate, conversion rate and metabolic rate were maximum in the smallest lobster in the study and showed a negative correlation with size. Maximum feeding rate of 254.87 J/g/day was recorded in the group with 13.17g initial weight, while the largest group (256.66 g) recorded lowest feeding rate (107.45

J/g/day). Assimilation efficiency was high (93.71 - 97.08%) and showed no size related change.

19. Net conversion efficiency (without exuvia) was maximum (15.4%) in the smallest group and decreased gradually to 6.6% in the biggest, showing a negative correlation with size. 17.76 to 44.68% of converted energy was lost as exuvia and the loss showed a positive correlation with size.

20. Protein conversion efficiency was considerably low compared to the net conversion efficiency and showed a similar trend of decrease with size.

21. Total energy budget for different size groups showed that there was a progressive increase in energy spent on metabolism with size, which resulted in the decrease of conversion efficiency with size increase.

22. As in *P. homarus*, biochemical and mineral changes during ovarian maturation was studied in the penaeid prawn, *Penaeus indicus*.

23. Water, protein and lipid concentration and quantity declined in the muscle of mature prawn. Lipid got reduced significantly after spawning while ash concentration increased significantly at maturity.

24. Hepatic index reduced at maturity and increased again, after spawning. Most interesting observation of change in organic reserve of hepatopancreas was a 93.3% increase (0.15 to 0.29g/100 g body

weight) in lipid at maturity which is a deviation from the general trend of decrease in lipid quantity of hepatopancreas reported in other crustaceans. At spawning 37.93% of lipid was lost from hepatopancreas.

25. Gonadosomatic index increased from 0.74 to 8.27 in mature prawn and then declined to 2.22 after spawning. All organic reserves and ash showed a highly significant increase at maturity and an equally significant decrease after spawning, in the ovary.

26. Ca, P and Mg concentration increased at maturity in the muscle of prawn, but Na and K reduced. The reduction in K was as high as 99.64%. Among trace elements, concentration and quantity of Cu, Co and Mn declined significantly at maturity in muscle, while Fe, Zn, Co and Cd increased significantly. Most notable change in trace element composition in muscle was a drastic reduction in the quantity of Cu (88.15%) at maturity and substantial increase in Cd (1633.33%) and Zn (47.96%).

27. Except for Na, all other major elements (K, Ca, P and Mg) increased in concentration and quantity at maturity in hepatopancreas. Among trace elements, Fe, Cu and Cd increased significantly, while Co and Cr declined significantly, both in concentration and quantity. Most important observations were the increase of Cd (1257.5%), Cu(3122.82%) and Fe(1288%) in hepatopancreas at maturity.

28. Concentration of all trace elements declined significantly in the ovary at maturity, while the quantities increased significantly due to substantial increase in ovary weight. Most striking increase in quantity (914.87%) was recorded for Fe followed by Mn (874.47% increase).

29. For yolk utilization studies, in the eggs of *P. indicus*, five development stages were fixed with the first hatched larva (nauplius) classified as 6th stage and the protozoea as the 8th stage. In *P.indicus* also, ripe ova were analysed and were classified as stage 0.

30. The development of prawn egg was completed in 14. 5 ± 0.25 hours after spawning with the release of nauplius and the protozoea larva emerged 58.0 ± 0.12 hours after spawning.

31. Egg diameter and volume increased from 0.257 mm and 0.0088 $mm^3$ to 0.27 mm and 0.0103 $mm^3$ respectively, showing a positive correlation with the time of development. The volume increase from spawned egg to last stage of egg, was only 17.5% and this is the lowest increase recorded in any crustacean. However, there was a substantial increase in volume from ova to spawned egg. A similar increase was also noticed in *P. homarus* egg at spawning. These studies indicate that yolk utilization studies in aquatic "non-cleidoic" eggs should start from the ripe ova.

32. Ash and carbohydrate concentration were very high (13.34 and 5.12% respectively) in just spawned *P. indicus* egg. Ash concentration increased to 19.04% in stage 4 and declined to the lowest, 5.34% in the nauplius. Protein concentration did not vary much but lipid percentage showed considerable variability during development and was maximum in the freshly hatched nauplius (36.87%). Protein concentration of ova was more than that of spawned egg, but carbohydrate and ash were less in ova.

33. There were significant changes in wet weights of ova (9.71 $\mu$g) and spawned egg (15.42$\mu$g); the weight of egg was 58.81% more than that of ova.

34. By the time the larva was fully formed in the egg, 11.33% protein, 17.14% lipid and 43.78% carbohydrate were utilized. Between the last stage of egg and nauplius, further 49.34% protein, 41.57% lipid and 37.5% carbohydrate were used.

35. The yolk utilization efficiencies in *P. indicus*, from spawned egg to nauplius larva were 31.67% for total energy, 39.23% for protein, 40.92% for lipid and 20% for carbohydrate energy. 51.45% of total energy utilised was supplied by lipid, 43.46% by protein and 5.06% by carbohydrate. The yolk utilization efficiency of 31.67% is the lowest recorded so far in crustacean eggs.

36. Na, Ca, P and Mg increased significantly in the just spawned egg, while K declined. The concentrations and quantities of these elements showed considerable variation between stages of development. Protozoea had the highest concentration of all these minerals. Zn and Fe appear to be most important trace elements in terms of concentration and quantity in the egg and larva. As in the case of minerals, trace elements also showed variable concentration in development stages. Most prominent increase during development was that of Co (564.52%). This was followed by increases in Fe (332.59%) and Cr (314.93%) in stage 4 ; Cd(178.95%) in stage 2; and Mn (139.01%) Cu (126.9%), and Zn (119.25%) in stage 5.

37. Food conversion efficiency of *P. indicus* postlarvae were determined at three salinity regimes - low saline (16.48 ppt), normal saline (33.05 ppt) and high saline (44.95 ppt) using different quality of feeds (clam meat, pellet and a combination of both).

38. Survival was maximum in low salinity (97.5 - 100%) and minimum in high salinity (62.5 - 85%). Maximum weight increase was recorded in clam-fed prawns in normal saline water (213.36 mg) and minimum (51.62 mg) in pellet-fed postlarvae in high saline water. Analysis of variance (ANOVA) showed significant variation in weight increase both due to salinity and feed.

39. Maximum feeding rate was recorded under high salinity, in pellet-fed postlarvae (5228.49 j/g/day) and minimum for clam-fed in low saline water (2156.12j/g/day). ANOVA revealed that feeding rate did not

vary significantly between salinities but was highly variable between feeds.

40. Assimilation efficiency was high for all feeds under all salinities. It was 97.99 - 99.48% for clam, 98.0 - 99.17% for mixed food and 93.96 - 97.80% for pellet.

41. Conversion rate was maximum in clam-fed postlarvae in all salinities. ANOVA indicated that conversion rate was significantly different between salinities and feeds.

42. Maximum conversion efficiency (19.15%) was recorded for clam-fed postlarvae under low salinity and minimum was in pellet-fed ones in high saline water (4.84%). Conversion efficiency was highly variable between salinity and feeds.

43. Metabolic rate was maximum in high salinity for all feeds but the variability was not significant between salinities for the same feed. Between feeds, however, the variation was highly significant.

44. Protein conversion efficiency was more than energy conversion efficiency in pellet and mixed food-fed prawns and less in clam-fed ones. Protein efficiency ratio showed significant variation between salinities and more so between protein content in feeds.

45. It is energetically expediant to grow postlarvae of *P. indicus* between low saline (16.48 ± 1.51 % S ppt) and normal sea water conditions.

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