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REPRODUCTIVE PERFORMANCE OF GIANT FRESHWATER PRAWN M.ROSENBERGII (DE MAN) WITH SPECIAL REFERENCE TO BROODSTOCK AGE, SIZE AND NUTRITION, EGG PRODUCTION AND LARVAL QUALITY.

A thesis submitted to the University of Stirling
for the degree of
Doctor of Philosophy in Aquaculture

By

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1989

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ABSTRACT

Research into the reproductive potential of prawns is scarce and broodstock nutritional requirements are completely unknown. The present investigation is focused principally on exploring these topics.

The literature contains considerable terminological inconsistencies with respect to demarcation of the various events and stages of caridean reproductive cycles. A consistent, unified and new, precise system of terminology is proposed together with models.

Four major female morphotypes were identified in Macrobrachium sp. based on simple, visual characteristics. These morphotypes vary in moulting and spawning physiology. Presence of eggs on pleopods reduce the moulting frequency and spawning potential in M.rosenbergii.

A new criteria is proposed to assess the reproductive potential of Macrobrachium species based on morphotype distribution, Spawning-Moult Capacity (SMC) and Spawning-Moult Efficiency (SME). The use of SMC and SME nullifies the influence of moulting pattern on spawning potential. A spawning anomaly was observed and referred to as Spawning Deficiency Symptom (SDS).

Food ingestion in M.rosenbergii broodstock varied (by up to 20%) depending on the duration of feeding and nutrient content of the diets. Females efficiently ingested and digested diets containing low levels of plant and animal

proteins. However, plant protein diets appear to reduce Spawning-Moult Efficiency. The validity of widely used chromic oxide and gravimetric methods for evaluation of nutrient digestibilities with M.rosenbergii was questioned.

Quantitative egg production in M.rosenbergii was more influenced by broodstock size than by age, quality of diets and food ingestion. Whilst, chemical composition of eggs was greatly influenced by the fatty acid profile of broodstock diet. Eggs showed some plasticity in accumulation of a range of (n-3) and (n-6) PUFA levels depending on dietary input. Bigger females produce larger and more uniform sized eggs than smaller females.

The egg incubation period and the nutrient reserve in newly emerged larvae (equated with resistance to starvation) were not influenced by the broodstock age, size, quality of diets or food ingestion.

A novel approach placing greater emphasis to moulting cycle of prawn is proposed and used in the broodstock nutrition study. The implications of newly adopted methods and findings were discussed with respect to broodstock management.

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CHAPTER 1: GENERAL INTRODUCTION

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1.1. Aquaculture production of Shrimps and Prawns.

Aquaculture, the farming and husbandry of aquatic organisms, has been practiced through the ages, the earliest records indicating carp culture in China in 2000 B.C.(Reay,1984).

The aims of aquaculture are manyfold, primarily production of cheap sources of protein or luxury commodities which in turn earn foreign exchange. Shrimp and prawn farming falls into the second category and comprises 17% of the international fish business (Anon,1988).

The terms "prawn" and "shrimp" have been used interchangeably. In this thesis "prawn" refers to freshwater animals and "shrimp" to marine, as designated by FAO. (FAO,1967;cited Csavas,1988).

The total world production of shrimps and prawns in 1987 was 2.18 million metric tons, of which aquaculture production contributed about 320-340,000 metric tons (Sribhibhadh,1988). Farmed shrimps and prawns contribute about 2.26% of total world aquaculture production, which is about 12 million tons (Kunugrankij and Kongkeo,1988). During the period 1980-1985 production of farmed shrimps and prawns has doubled (Kunugrankij and Kongkeo,1988) and increased ten fold compared to 1970 (Sribhibhadh,1988). This clearly indicates the rapid expansion of the industry and continuing demand for these Crustaceans.

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Total freshwater prawn production doubled from 85,000 metric tons in 1970 to 160,000 tons in 1987, mainly due to successes in M.rosenbergii culture (Anon,1988). A total global production of 20,638 metric tons.yr^{-1} was estimated for M.rosenbergii based on,1984,'85 and '86 production figures obtained from various sources (New,1988).

Pioneering work by Shao-wen Ling in Malaysia succeeded in completing the life cycle of M.rosenbergii in captivity. This, together with its omnivorous feeding habit, relatively large size and easy maturation, mating, spawning and hatching in captivity, has stimulated widespread interest in its commercial culture potential. This development is spurred by the fact that, it had not been possible to close the life cycle of marine shrimp in sixtees, for which there was an established global market (New,1988). Consequently, M.rosenbergii farming became very popular primarily in Thailand, Hawaii, Taiwan, Mauritius and Australia. The history, global status of freshwater prawn culture and its economics have been described by New, (1988).

1.2. Taxonomy of M.rosenbergii.

According to recent Crustacean classification of (Bowman and Abele,1982) the taxonomic position of M.rosenbergii is as follows;

Superclass: Crustacea Pannont,(1977)

Class: Malacostraca Latreille,(1806)

Superorder: Eucaridea Calman,(1904)

Order: Decapoda Latreille,(1803)

Suborder: Pleocyemata Burkenroad,(1963)

Infraorder: Caridea Dana,(1852)

Superfamily: Palaemoniidea Rafinesque,(1815)

Family: Palaemonidae Rafinesque,(1815)

Subfamily: Palaemoninae Rafinesque,(1815)

Genus: Macrobrachium Bate,(1868)

Species: rosenbergii De Man,(1879)

M.rosenbergii is often refered to as the "giant fresh water prawn", "giant long legged Malaysian prawn", "Hawaiian prawn", "blue lobster" and "Udang galah". M.rosenbergii is indigenous to the whole of Southern and South East Asia together with Northern Australia and the Western Pacific Islands (Ling and Merican,1961; Ling,1969; New,1988).

1.3. Life cycle and ecology

The life cycle of Macrobrachium sp., as for many other Crustaceans, consists of egg, larval, postlarval and adult phases. Typically, prawns of this genus, although referred to as "freshwater prawns," require estuarine conditions to complete their life cycle. Some species, such as M.dayanum and M.amazonicum, complete their whole life cycle in inland saline and freshwater lakes (New,1988).

Adult M.rosenbergii are usually found in the freshwater reaches of rivers, lakes, water reservoirs, mining pools, irrigation channels and in some paddy fields. They migrate downstream into estuarine areas where the eggs

are hatched (Ling and Merican,1961; George,1969; Ling,1969). The larval development takes place in more saline waters and after metamorphosis the postlarvae begin to migrate towards freshwater environments where they will grow to adulthood (George,1969; Ling,1969).

1.4.1. Biology of M.rosenbergii with reference to Broodstock.

M.rosenbergii adults are omnivorous in feeding habit feeding avidly on both plant and animal material. When sufficiently hungry they may become cannibalistic, especially males (Ling and Merican,1961).

Like many other Crustaceans growth is associated with moulting. Adults ready to moult can be superficially recognised by their refusal to feed, sluggish movements, dullness of eye colour and the shadow of newly formed shell which can be seen in the rostrum (Ling and Merican,1961). Peebles(1977) proposed a moult-staging technique to predict the stage of moulting based on structural details of the cuticle. The process of casting the skeleton has been described by Ling and Merican(1961), Ling (1969) and Lynn (1981). According to Lynn (1981) the time taken for casting the skeleton was less than one minute whilst the other two authors reported a period of 10 minutes.

The frequency of moulting is rather irregular and may vary from 9-42 days (Segal,1974, cited Wickins,1976). The

moulting cycle in M.rosenbergii is influenced by endogenous factors, such as age, size, breeding cycle, and exogenous factors such as quality and quantity of food (Ling and Merican,1961; Ling,1969; Segal,1974; Wickins and Beard,1974; Wickins,1976). The moulting frequency increases with increase in size of M.rosenbergii (Segal,1974 cited Wickins 1978) up to sexual maturity after which it is independent of size (Wickins and Beard,1974). Females with actively developing gonads preparing to spawn have been found to take longer to moult (Merican and Ling,1961). Individuals taking ample, good quality food have been found to moult more frequently than those taking less, or poorer quality, food Ling,1969).

The growth rates of young males and females are about the same. After reaching a length of 180 mm in total length and 60g in weight the growth rate of females decreases and there is little growth beyond 220 mm and 120 g. Males grow up to about 200 g (Ling,1969) or 400g (Provenzano,1985.b). The largest M.rosenbergii recorded were 350 mm (total length) male and 250 mm female (New,1988).

Sexes are separate in M.rosenbergii and sexually dimorphic structures play an important role in reproductive behaviour (Ling,1969; Nagamine and Knight,1980). The sexually dimorphic characters can be divided into two classes, permanent and transitory. The presence or absence of these structures are excellent indicators of sexual maturity and ripeness of individuals, especially females (Nagamine and Knight,1980).

Sexually ripe males can be identified primarily by permanent characters such as genital pores at the base of the 5th thoracic legs and appendices masculinae are formed between the appendices internae and the endopods of the second pair of pleopods. Other characters, such as presence of much larger 2nd thoracic legs and relatively larger carapace and body, are prominent in bigger males (Ling and Merican 1961; Ling,1969; Nagamine and Knight,1980).

Females can be recognised by the presence of gonopores at the base of the 3rd thoracic legs, modified abdominal pleura (forming a spacious brood chamber), oviparous (permanent) and ovigerous (transitory) setae in the pleopods The developing, or active, ovary is visible through the carapace as an orange colored mass (Ling and Merican,1961; Ling,1969; Nagamine and Knight,1980). The structure, development, maturation and function of these sexually dimorphic characters have been described in detail by Nagamine and Knight(1980) and Tombes and Foster(1979). Apart from morphological characteristics, behavioural characters such as responses of males towards receptive females prior to moulting can be used as an indicator for recognition of females without handling (Sagi and Ra'naan,1985).

The primary reproductive organs in male M.rosenbergii consist of paired testes, vas differentia and the terminal ampulae (Lynn,1981; Chow et al,1982). The structural details are described in detail by the above authors.

Female reproductive structures include the paired ovaries and oviducts, and a detailed description can be found in Lynn(1981). Histological descriptions of ovaries and ovulation are detailed by Fauvel(1983).

In a mature population three male morphotypes can be clearly differentiated by claw colour, relative size within the population and the ratio of claw length to body length, spination of the chela, relative size of hepatopancreas and reproductive system to the body size, territorial behaviour, complex courtship, mate guarding behaviour, mating, fertilisation success and growth rate (Peebles,1977; Cohen et al.,1981; Cohen and Ra'anan,1983; Ra'anan and Cohen, 1984; Kuris et al,1987; Sagi and Ra'anan,1988).

Sexually mature males are able to mate any time, whilst females are ready only after completing the pre-mating moult (Ling and Merican,1961; Ling,1969). Premating behaviour in M.rosenbergii may last for 2-12 hr. The spermatophores are deposited as a pair on the mid to posterior thoracic sternam of the female in the channel formed by the base of the periopods (details in Ling and Merican,1961; Ling,1969; Lynn,1981).

Pre-spawning and spawning behaviour are described in detail by Ling and Merican (1961), Ling (1969) and Lynn (1981). Egg laying takes place between 6-20 hr. after mating and lasts for 9-25 minutes depending on the size of the female (Ling and Merican,1961; Ling,1969; Lynn,1981). Unfertilised eggs are aborted within three days whilst

fertilised eggs are interconnected by a cementing material and adhere tightly to the ovigerous setae of the first four pairs of pleopods (Ling,1969; Lynn,1981)

1.4.2. Biology with reference to egg production:

Egg production refers to both the quantity and quality of eggs. The eggs are slightly irregular in shape during spawning and rapidly become ellipsoid. Average egg size ranges from 0.5-0.7 mm (in the long axis) and they are bright orange in colour at spawning (Ling and Merican,1961; Ling,1969; Lynn,1981). At ovulation each egg is surrounded by a bilayered investment coat and during attachment to the pleopods another coat is present which is referred to as the cementing coat (Lynn,1981).

Information on the structural details, the cyto-architecture and actual chemical composition of Crustacean eggs, is scarce (Adiyodi and Subramonium,1983).

Like other aquatic animals fecundity is a linear function of body weight and an exponential function of body length in M.rosenbergii (Malecha,1983). A wild caught female, about 200 mm in total length, can lay approximately 276,000 eggs at one spawning (Patra,1976). The fecundity of animals maintained in the laboratory is greater than in those from ponds (Malecha,1983).

Mature females kept under laboratory conditions can spawn twice within five months (Ling,1969) four times within

six months (Wickins and Beard,1974) and three times within six months (Provenzano,1985.b).

Females required for larval production are obtained mainly from growout ponds. Large, obviously healthy, well pigmented, berried, females are selected. The eggs are incubated by females attached to pleopods for a period of 19 days at 26 - 28^{0}C (Ling,1969) and 19-22 days at 28 $\pm$1 ^{0}C and 5‰. salinity (Wickins and Beard,1974). During the whole incubation period dead eggs and foreign material are carefully removed by the sensitive and versatile first pair of thoracic legs (Ling,1969). With progress of embryonic development the bright orange coloured eggs become slate grey in colour (Ling and Merican,1961; Ling,1969). Under laboratory conditions it takes about 1 hr. for individual eggs to hatch and 4-6 hr. for the whole batch (Ling,1969), whilst in some females hatching may last over two consecutive nights (Wickins and Beard,1974).

Possible mechanisms of hatching of Crustacean eggs have been discussed by Davis,(1964) and Ling (1969). Several attempt have been made in the past to incubate caridean eggs in vitro. Eggs from brachyurans (Lochhead and Newcombe, 1942; Costlow and Bookhout,1968) and Palaemon sp. (Phillips, 1971) have been incubated unsuccesfully. Balasundarum and Pandian,(1981) successfully incubated M.nobilii eggs with a hatchability of 70% using inverted conical flasks fitted with a diaphram. Water was passed through an inlet at the bottom of the flask which kept the eggs continuously in

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suspension. Later, using a variable speed shaker, Hartnoll and Paul (1982) managed to agitate flasks containing crab eggs and reported hatchability up to 88%.

1.4.3. Biology of larvae

Newly-hatched larvae require brackish water for development or else they die in 4-5 days in freshwater (Ling,1969). Eleven larval stages can be observed each characterised by specific morphological characters originally described by Ling (1969) and later in great detail by Uno and Known (1969) and Diaz and Kasahara (1987). The latter authors described 17 different zoeal stages.

The hatching of larvae over a 96 hr. period leads to an initial variance in number of stages and, in general two to five larval stages are present in the culture medium on any one day following the first day (Malecha,1983). In addition variation in body length occurs even in the same zoea stage (Uno and Known,1969; Diaz and Kasahara,1987). Size of the larvae also can vary with factors such as temperature, salinity, or the geographical origin of the broodstock (unpublished data, Diaz and Kasahara,1987).

Larval development takes 25-45 days and post-larvae emerge at metamorphosis.

1.5. Seed supply.

General requirements for selection of suitable species for aquaculture have been presented by Bardach et al.,(1972). Among many other characteristics, complete

control over the life cycle in captivity is considerd to be one of the most important criteria for continuity and the success of aquaculture enterprises. However, substantial aquaculture industries to a large extend still rely on wild populations for broodstock and larval supply, eg; Anguilla, Chanos, Serida, Mugil (Reay,1984), penaeid shrimps in Asia and South America and carideans in India (Kelemec and Smith, 1980; Liao and Peng,1983; Malca,1983; AQUACOP,1985; Provenzano,1985.a; Khoo,1988).

Thus, given an adequate supply of wild fingerlings or fry large-scale culture is possible. However, obtaining seed from the wild is politically, ecologically and economically undesirable and the irregularity of natural recruitment is a factor that cannot be tolerated by a capital-intensive industry (Provenzano,1985.a). Therefore an almost universal objective of research and development is to achieve controlled seed production, not only to circumvent erratic wild seed supply, but also to enable genetic improvement of stock.

Although penaeid shrimp culture techniques developed in the late 1940s, the industry has been dependent, until very recently, entirely upon the capture of wild, mated and ripe females for hatchery production (AQUACOP,1985; Provenzano, 1985.a; Kungvankij and Kongkeo,1988; Khoo,1988). Recently, maturation and spawning of several penaeid species has been obtained through unilateral ablation and environmental manipulation (AQUACOP,1985; Provenzano,1985.a). AQUACOP

(1985) stressed the need to define the optimum environmental parameters for maturation of each penaeid species, develop maturation feeds and determine the best conditions to obtain healthy broodstock.

It is important to manage, conserve and exploit the alleles and to know how broodstock management practices affect a populations' gene pool as every time fish are handled some phenotypes and alleles are culled (Tave,1986). Malecha (1983) argues that the current practice of selecting M.rosenbergii broodstock from ponds for larval production is disadvantagous as it a) does not take advantage of the natural, fecund, potential of bigger females, and b) selects genetically superior as well inferior animals, since prawns of all ages exist in ponds. Simple control of the age of the broodstock may lead to genetic progress in M.rosenbergii (Doyle et al.,1983). Malecha,(1983) suggested that separate broodstock management should become a component of the M.rosenbergii industry.

There is an increasing need for and research towards establishment of specialised broodstock management systems for Macrobrachium sp. These include increasing hatchability and spawning through artificial incubation (Balasubramonium and Pandian,1981), artificial insemination (Chow,1985), and spermatophore cryopreservation (Chow et al.,1982). In the last decade a start has been made in developing genetic information about various, widely distributed, stocks of M.rosenbergii. They have been successfully hybridised with

other species, suggesting possibilities for enhancement of gene pools for selection (Sankolli et al.,1982, cited Provenzano,1985.b; New,1988). Similarly, encouraging results have been achieved with intraspecific and interspecific hybridisation of penaeids for growth potential and disease resistance (AQUACOP,1985).

Therefore it is evident , in both shrimps and prawns the trend is towards establishing specialised broodstock management practices. As in other production systems success is dependent on a clear understanding of several constraints such as environmental and nutritional requirements, and basic knowledge on reproductive potential of the animal concerned.

1.6. Scope of thesis:

This thesis principally focuses on the question of the influence of maternal age, size and nutrition on quantitative and qualitative egg production and larval quality.

The general materials and methods used in the study are discussed in Chapter 2.

Knowledge on the reproductive biology of the genus Macrobrachium is scant. This is partly due to simplicity of breeding in captivity (as discussed earlier) and also because this is a relatively new field of science compared to breeding of fishes. The paucity of research is reflected

in the discrepencies in terminology used to demarcate different stages and events in the reproductive cycle, not only of Macrobrachium but also of Caridea as a group. Therefore, the third Chapter of this thesis is focussed on highlighting the problems and consequences of such discrepencies and proposes a more unified terminology with the introduction of new definitions to precisely and consistently demarcate the stages and events in the reproductive cycle. The possible existence of different female morphotypes, their transformations, and moulting, spawning patterns are also elucidated in this Chapter.

The universal strategy of animals is to maximise the production of surviving progeny in relation to available energy and parental life expectancy (Reay,1984). The strategy of animal production systems is to exploit the natural potential of the animal to produce maximum surviving progeny and continue production. The tactics used are to identify the potential natural and technical constraints imposed on egg and offspring production and manipulate them to optimise the survival of the progeny.

Egg production plays an important role as a bridge, biologically linking two generations or economically two production cycles. Therefore it plays a vital role in the feasibility of aquaculture enterprises.

The parents, especially females, have a profound influence on the viability of the off-spring both on a species and individual level in terms of fecundity, size and

composition of eggs of aquatic animals (detailed discussion in Chapters 4 and 7). The nutritional requirements of most sexually mature animals undergoing reproduction are increased, especially during the later stages of the reproductive process (Reid,1960; cited Smith et al.,1979). Nutritional quality and quantity have been found to exert profound influence on reproduction in fishes (details in Chapter 5,6 and 7). Therefore, the influences of maternal age size and nutrition on both quantitative and qualitative egg production of M.rosenbergii were evaluated in Chapters 4 and 7.

Chapter 5 focusses on problems associated with design and formulation of broodstock diets for prawns with special reference to M.rosenbergii. The physical and chemical composition of diets and the general material and methods used in the broodstock nutritional study are also discussed.

The efficiency of utilisation of diets, growth, carcass composition and spawning performances of the broodstock fed different experimental diets are discussed in Chapter 6.

Chapter 8 is composed of discussions based on the major problems faced in the study, new information gathered and its value to the Macrobrachium and shrimp industry.

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CHAPTER 2: GENERAL MATERIALS AND METHODS

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2.1 Experimental Systems

2.1.1 Experimental holding system for broodstock

Two recirculatory water systems S_1 and S_2 were used for the experiments with broodstock.

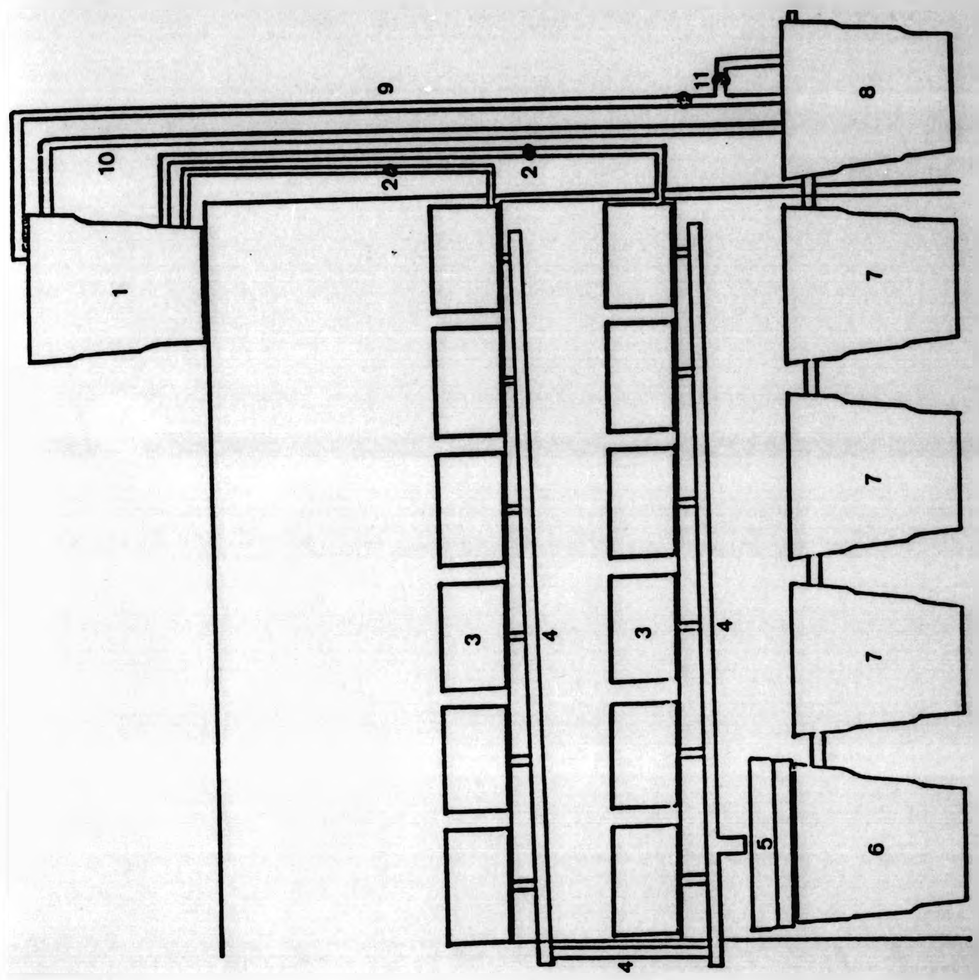
The system S_1 consisted of twenty four 45 l. rect--angular, polypropylene, tanks arranged in four rows (Fig:2.1). Only 12 tanks were used in this study.

Each tank contained an inflow pipe, controlled by a valve and an outflow at the opposite end which drained into a common drainage channel leading to a common settling tank, by gravity. All experimental tanks were covered by separate lids to minimise disturbance to the experimental animals. Each tank was divided into two experimental units, each holding a single prawn, separated by a central clear perspex divider with perforations in the upper portion to facilitate water flow and sealed with rubber tubing at the bottom to prevent mixing of food (Fig:2.2.a). Each experimental unit was gently aerated separately with air supplied from a central air blower. All the experimental units contained a rectangular hollow PVC shelter to provide cover for the prawn.

Water to all experimental tanks was supplied from a header tank (125 l) through two common inflow pipes one to the two upper rows of tanks and the other to the bottom.

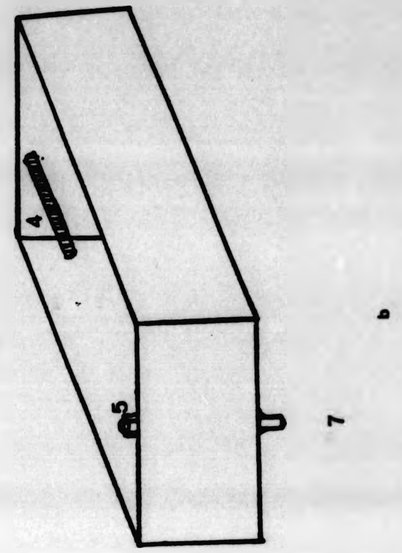
1. Header tank
2. Inflow pipes to the experimental tanks
3. Experimental tanks
4. Outflow drainage
5. Gravel and cockle shells filter trays
6. Settling tank
7. Biological filter tanks
8. Sump tank
9. Pipe connecting sump and header tank
10. Overflow pipe
11. Valves

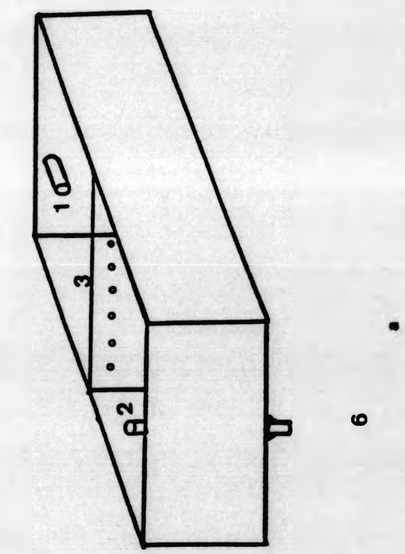
Fig:2.1. Experimental system S_1



1. Inflow pipe
2. Outflow pipe
3. Perspex dividers seperating experimental tanks into two experimental units
4. Filter screen (45 um) connected to inflow pipe
5. Outflow containing filter screen
6. Expperimental tank used for the growth studies
7. Experimental tank used in digestibility studies

Fig: 2.2 Experimental units used in system S_1.





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Outflowing water passing through the common drainage channel flowed through two gravel and cockle shell mixture trays to trap the suspended solid waste and buffer (harden) the water before draining into two black polypropylene settling tanks (125l) which were interconnected. The surface water from the settling tanks flowed through three (125 l) black filter tanks containing plastic ring medium (Mass Transfer Ltd, Hobsons Lane, Cumbria U.K.) which facilitated settling and acted as a substrate for nitrifying bacteria. Finally, the water entered a (125 l) black sump tank.

Water was pumped up to the header tank from the sump tank by a submersible pump. An overflow pipe drained excess water from the header tank back to the sump tank to help to maintain constant pressure in the header tank. Water lost, due to evaporation, was replaced daily and regular water exchange was carried out to maintain total hardness levels between 75-85 $CaCO_3$/l. The experimental system was in the Tropical Aquarium of the Institute of Aquaculture in which the air temperature was maintained at 25-29^{0}C.

System S_2, was similar to S_1 in principle with respect to water recirculation and maintainance of water quality. Water from the sump tank was pumped directly in to the experimental tanks via inflow pipes (without a header tank unlike S_1). Outflowing water passed through gravel and cockle shell filters, and black filter tanks containing biological filteration,into a sump tank.

Systems S_1 and S_2 differed, in numbers and types of tanks. 36x36 l, white, high density polyethylene tanks of dimensions 30X30X45 cm. were used in S_2. The tanks were not separated into experimental units (unlike system S_1).

Some of the characteristics of the experimental tanks in both systems, which may have influenced animal growth and behaviour, are given in the table below;

	S_1	S_2
Shape of the experimental tank / unit	Rectangular.	Square
Colour of the experimental tank / unit	Green	White
Total volume of the experimental tank	45 l.	36 l.
Volume of water in each experimental unit	15 l.	10 l.
Area of the bottom of the experimental unit	120 cm^2	900 cm^2
Shelters (PVC, rectangular shaped gutters)	16 x 7 x 7 cm	

Males were kept in a separate facility, similar to the above system, but with eleven tanks holding 22 males.

The environmental conditions maintained during the experiment are listed below:

Photoperiod	12L 12D	
Intensity of light	59 Lux.	
Temperature of water	28.5^0C	(27.5-29.5)
Total hardness	80 mg $CaCO_3$/l	(75-85)
Calcium hardness	65 mg $CaCO_3$/l	
pH	5.70	(4.60- 6.40)
Total NH_4--N	<0.13 mg/l	

Nitrate NO_3-N	<0.01 mg/l
Oxygen	7.50 mg/l

2.1.2 Maintainance of enviromental conditions

The dark cycle of the photoperiod was from 1900 - 0700 hr. The lighting regime was maintained using a time switch fitted to overhead white fluorescent strip lighting.

The intensity of the light was adjusted to 59 lux. level using black sheets where neccessary, depending on the position of the tank relative to the source of illumination.

Temperature was maintained at the required level using a thermostat heater. Temperature was measured twice daily.

Attempts were made to maintain the total hardness of the water below 80mg/l by replacing about 100 l of the experimental system water per week by pre-heated, aerated tap water. Hardness of the water was measured twice weekly.

pH, total ammonia and nitrate were initially measured twice a month and found stable, later these parameters were measured only once a month.

Oxygen content of the water was measured once every two weeks and was found to be stable.

2.1.3 Modifications to system S_1 for digestibility studies

The experimental units in system S_1 were slightly modified for faecal collection. The clear perspex dividers between the two experimental units in a tank were removed and one animal was maintained in each tank. The inlet was fitted with a 40 µm nylon mesh to prevent entry of suspended solids into the tank as these could have been consumed by the test animal (Fig:2.2.b). A 100 µm nylon mesh screen was fitted at the outflow to prevent escape of any uneaten food and or faeces. The filter screens were washed every day after faecal collection.

2.2 Experimental animals

2.2.1 History

M.rosenbergii used in the experiments were originally imported from Thailand and raised at the University of Stirling prawn facility.

Larvae from two brood females A and B were raised separately at 12±2‰ salinity and 28±1^0 C temperature using Galveston type larval tanks. They were fed on artemia and egg custard made according to the procedure described by New and Singholka, (1982).

Postlarvae from both stocks A and B were raised separately in 1m^2 tanks fitted to a recirculated freshwater system with a total hardness of 73±11 mg/l and 28±2^{0}C.

These were fed on fresh mussels, frozen prawns, squid, whitebait, lambs' liver, spinach and trout pellets (No:3 Ewos) for 3 months and the slow growers were discarded. After one month 25 fast growing females from each stock A and B were selected and randomly distributed in the experimental systems S_1 and S_2 respectively . These prawns were fed as above on fresh and frozen food.

2.2.2 System for *in vitro* incubation of eggs

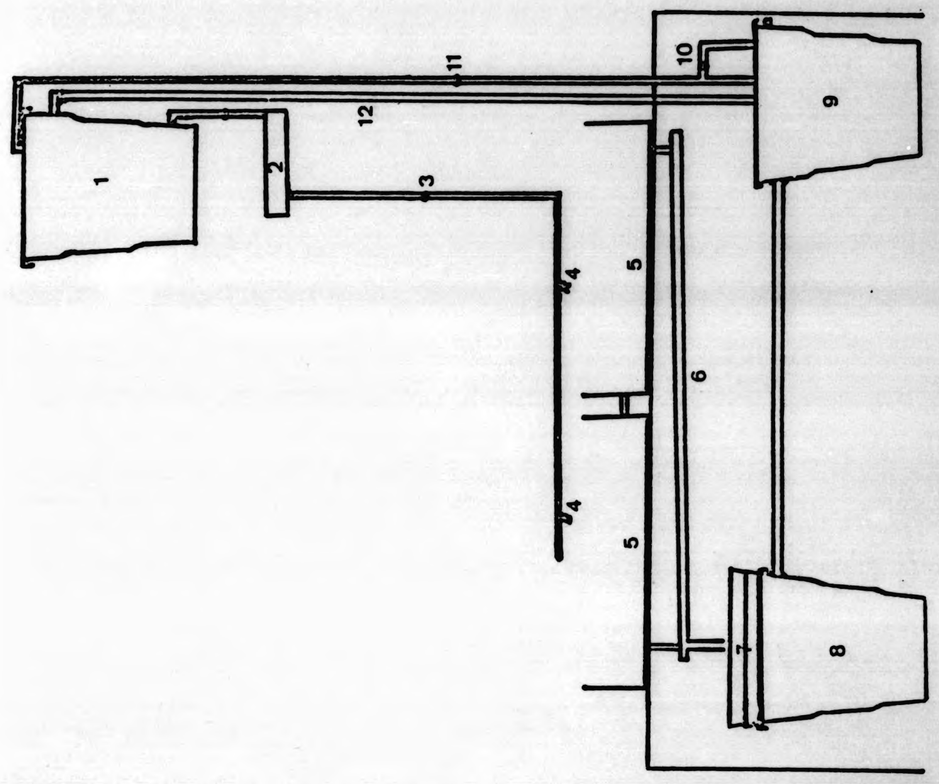
The egg incubation system consisted of incubation chambers and water baths fitted to a recirculatory water system.

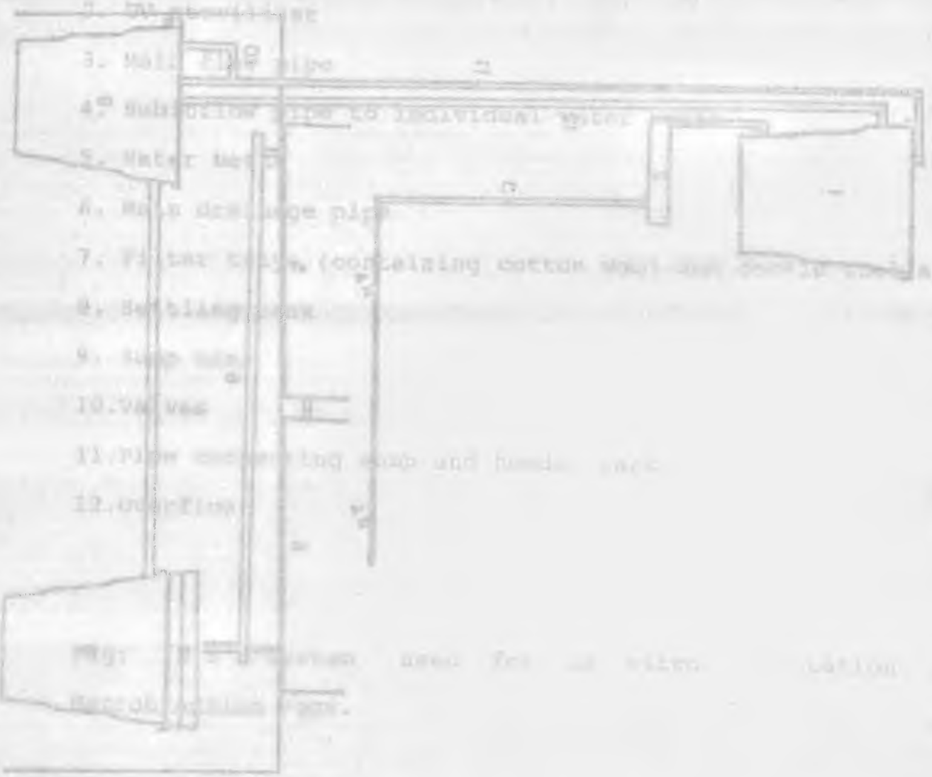
Incubation chambers were made of plastic containers (175ml.) fitted with 300µm nylon mesh at the bottom. These chambers were kept in suspension in the water bath with the help of polystyrene rings fitted at the top of the containers. Using aerators the water in the bath was agitated in order to continuously shake the incubation chambers. This kept both water and eggs in motion inside the incubation chambers. The incubation chambers were kept in position inside the water bath with the help of plastic mesh (mesh size of $8mm^2$). The incubation chambers were disinfected with 50% commercial bleach solution after every incubation cycle.

The water baths were fitted to a recirculatory water system (Fig:2.3) consisting of three 125 l settling, sump and header tanks. Water was supplied from the header tank

1. Header tank
2. UV steriliser
3. Main flow pipe
4. Subinflow pipe to individual water baths
5. Water baths
6. Main drainage pipe
7. Filter trays (containing cotton wool and cockle shells)
8. Settling tank
9. Sump tank
10. Valves
11. Pipe connecting sump and header tank
12. Overflow

Fig: 2.3 System used for in vitro incubation of Macrobrachium eggs.





to the water baths through an ultra violet lamp (Marine coast Ltd.,UK.). Outflowing water from the water baths drained into the settling tank through trays containing filter wool and cockle shells. Filter wool was used to trap any suspended particles and shells to buffer hardness. Filter wools were changed twice a week and shells washed once a week. Water over-flowed from the settling tank to the sump tank from which it was pumped to the header tank.

The following environmental conditions were maintained.

Temperature	$27.5 \pm 0.5\ ^{0}C$
Oxygen	$8.0 \pm 0.5\ mg.l^{-1}$
Hardness Total	70 ± 10 mg. $CaCO_3.l^{-1}$
Calcium	50 ± 5 mg. $CaCO_3.l^{-1}$
pH	7.5 ± 0.5
Photoperiod	24 hr. darkness.

Weekly water exchange of approximately 125 l was carried out by replacing recirculated water with fresh, pre--heated and aerated, water. Temperature was measured twice daily, oxygen and hardness twice a month, and pH once a month.

Triplicate samples of 50 eggs per clutch (72 hr old) were incubated in the above system. Dead eggs were removed twice weekly. After 14 days (17 days from spawning) the incubation chambers were transferred to a water bath containing aerated water at 5‰ salinity and $28 \pm 1^{0}C$. After hatching the number of larvae was counted. The time taken

from the day of spawning to the day of hatching was recorded and is referred to as the incubation period, considering the day of spawning as day 0.

2.2.3 System for larval rearing

The utilisable nutrient reserve at hatching, in the larvae, is assumed to be proportional to the survival of the unfed larvae in the present study.

After hatching 60 actively swimming larvae were transferred to four containers (identical to those used in the incubation chambers) at 15 per chamber. The containers were kept in a water bath containing 12±2‰ salt water at 28±1^{0}C. The water was gently, but adequately, aerated in order to shake the containers as explained in section 2.2.2 50% of the water in the water bath was replaced by pre heated, aerated, saline water every week.

48 hr. after hatching the number of surviving larvae in each container was counted daily and recorded. From these survival rates, and mean survival period ST_{50} was calculated using the Trimmed Spearman-Karber method (Hamilton et al.,1977)

2.3 Measurement of growth parameters

Growth parameters of female broodstock were measured as follows:

Carapace length: posterior margin of eye orbit to base of carapace

Total length: posterior margin of eye to tip of telson

Carapace and total lengths were measured using a Vernier caliper (0.1mm) and wet weight (0.1g) was obtained after blotting the surface water.

All these measurements were taken on the fifth day after each moult unless otherwise stated.

2.4 Mating and egg sampling

Experimental prawns were observed for moulting before feeding in the evening and whilst siphoning out the uneaten food on the following morning. Females with active (developed) ovary (visible through the carapace as an orange mass) were mated by introducing a mature male into the tank, and after 4 hr. the male was removed. Only those males in their intermoult period were used.

The fertilised egg clutch was carefully removed from the setae on the pleopods using forceps after 24-48 hr., depending on the study. Sampling of eggs prior to 24 hr. of spawning caused stress to the famale.

2.5 Estimation of quantitative egg production

2.5.1 Total wet weight of egg clutch

The egg clutch was sampled 48 hr. after spawning, washed and the surface water was blotted. It was then transferred to a pre-weighed vial containing water and

reweighed to the nearest ±0.01mg (Oertling R51). The difference in weight was recorded as the total wet weight of the egg clutch.

After weighing random sub samples were taken from the egg clutch for the following studies;

a) Approximately 300 eggs were taken for incubation and measurement of egg diameter,

b) Four sub samples of each clutch (weighing 30-40 mg,±0.01 mg) were preserved in 4% sucrose formalin (40g sucrose/l 10% formalin) for the determination of individual egg weight and fecundity,

c) The rest of the egg clutch was washed with double distilled water and preserved at -70^0 C. for determination of biochemical composition.

2.5.2 Total dry weight of the egg clutch

Total dry weight of the egg clutch was calculated as;

$$= \frac{\text{Total wet weight of egg clutch} * (100 - \% \text{ Moisture})}{100}$$

(see 2.7.1. for determination of % Moisture)

2.5.3 Fecundity

The number of eggs in the egg clutch was calculated as follows;

$$= \frac{\text{Total wet weight of egg clutch (mg)}}{\text{Wet weight of single egg (mg)}}$$

(see 2.6.1 for determination of wet weight of egg)

2.6 Determination of physical quality of eggs

2.6.1 Wet weight of egg

Sub-samples of egg clutches, preserved in 4 % sucrose formalin solution, were washed with distilled water and isolated using 10% sodium hyperchlorite (Choy,1985). The isolated eggs were kept in separate clear perspex trays containing water and photocopied. The number of eggs in each subsample was counted from the photocopy, using a bacterial colony counter (Gallenkamp). The wet weight of an individual egg was calculated as ;

$$= \frac{\text{Wet weight of the egg sub sample}}{\text{Total number of eggs in sub sample.}}$$

2.6.2 Dry weight of egg

The egg dry weight was calculated from the wet weight and moisture content as detailed above for the egg clutch (2.4.2).

2.6.3 Diameter of eggs

Length (L) and width (W) of 30 randomly selected eggs from freshly spawned egg clutches were measured under a calibrated microscope graticule.

2.6.4 Colour of eggs

The egg clutch was classified according to the colour it appeared under fluorescent light (there were colour differences in egg clutches between fluorescent and natural light).

1. Pale yellow
2. Yellow
3. Dark yellow
4. Orange

The colour comparison was made while weighing the egg clutch and later confirmed when freeze drying samples.

2.7 Analysis of chemical composition

2.7.1 Moisture Content

The moisture content of quadruplicate samples of dietary ingredients, and diets were determined by air drying the samples in an oven at 105^0C for 24hrs.

The moisture contents of eggs and carcasses were determined by freeze drying (Edwards speedivac centrifugal freeze dryer) to constant weight.

2.7.2 Total Nitrogen Content

The nitrogen contents of dietary ingredients and diets were determined using the semi-micro-kjeldahl method (A.O.A.C, 1980).

When sample size was insufficient (eggs and faeces) to use the above method, nitrogen contents were determined using a Perkin Elmer 240 Elemental Analyser.

2.7.3 Total Carbon Content

The carbon contents of diets, faeces and freeze dried egg samples were determined using a Perkin Elmer 240 Elemental Analyser.

2.7.4 Crude Protein Content

The crude protein contents of dietary ingredients, diets, carcasses and faeces were calculated using the empirical factor of 6.25 to convert total nitrogen to total protein of the samples. This assumes that proteins contain 16% nitrogen on average.

2.7.5 Total protein content

The total protein contents of freeze dried egg samples were determined by rehydrating triplicate samples of eggs in double-distilled water using an ultra sonicator for 3 minutes. The volume was then made up to 10 ml with double--distilled water. Three 1 ml aliquots of the above samples were used to determined the total protein content by Petersons modification of the micro-Lowry method (Peterson,1977).

2.7.6 Crude Lipid Content

Crude lipid contents of ingredients, diets and carcasses were determined using the soxhlet method (A.O.A.C,1980).

2.7.7 Total Lipid Content

The lipids of freeze dried eggs and diets were extracted by the method of Folch et al.,(1957) and stored at -70^{o}C for lipid class and fatty acid analysis.

2.7.8 Ash Content

The ash contents of dietary ingredients diets and carcasses were determined by ignition in a muffle furnace overnight at a temperature of 450^{0}C (A.O.A.C 1980).

2.7.9 Crude Fibre Content

The crude fibre contents of samples of dietary ingredients and diets were determined after A.O.A.C (1980). Crude fibre was the loss on ignition of dried lipid free residues remaining after digestion with 1.25% H_2SO_4 and 1.25% NaOH.

2.7.10 Chromic Oxide Content

The chromic oxide contents of diets and faeces were determined using the method of Furukawa and Tsukahara (1966).

2.7.11 Energy Content

Energy contents of diets were determined using a Phillipson oxygen microbomb calorimeter (Gentry and Wiegert instruments, USA). The heat produced after combustion of the sample was recorded using a servoscribe chart recorder (Belmond Instruments, UK) in millivolts. Benzoic acid was used as the standard.

2.7.12 Amino acid Content

The amino acid contents of duplicate samples of ingredients, diets and eggs were determined using an LKB-4151 Alphaplus amino acid analyser (LKB Biochrom Ltd Cambridge).

Samples containing approximatly 50mg protein were hydrolysed with 5.7N HCl for 24 hrs. at 105^0c in vacuo. The acid from the sample was extracted by drying over sodium hydroxide. Dried samples were dissolved and diluted with 5 ml of loading buffer (pH 2.2). 0.1ml of the above stock solution was diluted with 2.2ml loading buffer and filtered through a 0.22µm centrifugal microfilter.

Samples were loaded into the analyser as described in the LKB 4151 Alpha-plus instruction manual (1983) and the amino acids were identified using known standards and quantified using a Trivector chromatography integrator (Trivector systems International Ltd, U.K).

2.7.13 Lipid Class

Approximately 1µl. samples of duplicate, total lipid samples of diets, were spotted on high performance thin layer chromatography (HPTLC) plates precoated with silica gel 60 that had been (without fluorescent indicator) (Merck, Darmstadt, Germany) pre-run in diethylether and activated at 120^{0}C for 1 hr. The plates were developed for 6 cm in a solvent mixture of acetate/ isopropanol/ chloroform/ methanol/ 0.25% aqueous KCl (25:25:25:10:9 V/V/V/V/V) to separate phospholipid classes with neutral lipids running at the solvent front (Vitiello and Zanetta 1978). After drying the plates were developed to the top in hexane/ diethyl-either/ acetic acid (80:20:2, V/V/V) to separate neutral lipids and cholesterol. Lipids were stained by charring at 160^{0}c for 20 min after spraying with 3% copper acetate/ 8% phosphoric acid and identified by comparison with pure commercial standards. Lipid classes were quantified using a Shimadzu CS-9000 dual wavelength flying-spot scanner. All chemicals used were HPLC grade and obtained from Rathburn chemicals, Walkerburn, U.K.

2.7.14 Fatty Acid Analysis

Fatty acid methylesters were prepared from diet samples by acid catalysed transmethylation overnight at 50^{0}C according to Christie (1982). The methyl esters were redissolved in hexane containing 0.05% BHT and analysed using a Packard 436 gas chromatograph (Packard Instuments

Inc. U.K) equipped with a chemically bonded CP Wax 52CB fused silica capillary column (50m X 0.3mm i.d) (Chrompack U.K. Ltd.) with on column injection and using H_2 as carrier gas with a thermal gradient from 50^0C to 225^0C. Individual methylesters were identified by comparison with known standards and a well characterized fish oil and also by reference to published data (Ackman 1980, Ackman and Eaton 1978, Bell et al 1983). The methyl esters were quantified using a Shimadzu CR-3A recording integrator.

2.7.15 Mineral Content:

Ca,Mg,K,Na,Zn,Fe and Cu contents of triplicate samples of dietary ingredients and diets were determined using a Perkin Elmer 2280 Atomic Absorption Spectro- photometer according to manufacturer's specifications.

Ashed diet and egg samples and blanks were digested using 2ml of $C.HNO_3$ and evaporated to dryness on warm a hot plate. Dried crucibles were transfered to a furnace and kept at 500^0C for 30 minutes, removed and cooled before adding 1ml of 70% $HClO_4$. This was followed by heating for 20 mins at 200^0C., cooling, filtration and dilution to 25ml with distilled water. These stock solutions were diluted as necessary for determination of each element.

Appropriate standards were prepared using spectrosol standard stock solutions (BDH Chemicals Ltd,Poole,U.K), $C.HNO_3$ and 70% $HClO_4$ to match the acid concentrations in

the samples. 5%$LaCl_2$ was added to both samples and standards to maintain a ratio of 4:1 $LaCl_2$, when determining calcium and magnesium. Acids used in analyses were analar grade (BDH Chemicals Ltd,Poole,U.K).

2.8 Statistical analysis

All statistical analyses were accomplished using Minitab release 6 for student t- test and ANOVA, and SPSS-X release 3.1 for multiple range tests on a VAX/VMS Mainframe (University of Stirling). The ANCOVA was carried out with a program written to use VAX/VMS (Springate, 1985; John, Ph.D thesis in preparation). The choice of tests and the numbers of samples are indicated in the respective sections.

CHAPTER 3: DIFFERENTIATION OF FEMALE MORPHOTYPES IN *MACROBRACHIUM* SPECIES; DISTRIBUTION, MOULTING AND SPAWNING PATTERENS IN *M.ROSENBERGII*.

3.1 Introduction:

3.1.1 Differentiation of female morphotypes in Macrobrachium species.

The most prominent process dominating the life of Crustacea is moulting, during which periodic shedding of the exoskeleton takes place (Passano,(1960). Moulting is a highly complex process, co-ordinated by a number of endogenous factors (reviews by Adiyodi and Adiyodi,1970; Skinner,1985) with response to interacting exogenous factors (review by Conan,1985). All physiological processes such as growth,(reviews by Hartnoll,1982;Botsford,1985) regeneration (review Skinner,1985) and reproduction (review Adiyodi,1985) are integrated and co-ordinated to phase with the moulting cycle.

In diecdysic prawns, such as M.rosenbergii, spawning is always preceeded by a moult. These animals must moult for spawning to occur although not all moults are followed by spawning. Due to the complexity of the moulting pattern and physiology the spawning pattern is complex. This complexity is not found in non-moulting animals such as fish. Consequently studies concerned with reproductive physiology and pattern in these diecdysic prawns have become complicated as reflected by the paucity of knowledge in this field.

A literature survey on the reproductive biology and performance of decapods revealed that there was little

uniformity in the schemes followed by various authors, in both basic and applied Crustacean reproductive biology, in demarcating various activities and stages in the reproductive cycle. Various terminologies have been employed by different authors to describe a particular event, or stage, and in most cases these were inadequately defined (Table:3.1). Also some authors use similar terminoloy to describe different stages eg: Lynn (1981) refers to sexually mature females as "ovigerous females", whilst others refer to females carrying fertilised eggs in the pleopods (Table:3.1). Clark,(1979) refers to sexually mature females as "gravid females", whilst many others refer to females carrying eggs on the pleopods (Table:3.1). The term "gravid" usually refers to mature fish or animals containing ripe eggs in the ovary.

It is interesting to note that some workers use different terms (even though with the same meaning) in different parts of the text to describe a particular event or stage. The most confusing classification was found in Morris,(1973) in which the author evaluates the changes in lipid content of tissues associated with sex and maturation. The text contained terms such as small juveniles, medium juveniles, young males, males, young females, immature females, females, mature females, gravid females, gravid females without eggs, and mature females without eggs. Neither the size of animals nor a definition of the terms used were given.

Table:3.1 Terminology used by authors to demarcate stage or event in the reproductive cycle of Crustacea.

Reproductive stage/event	Terminology	Authors
Females carrying fertilised eggs	"berried females"	1,2,3,4,5,6,7,8 9,10,11,12,13
	"ovigerous female"	14,15,16,17,18 19,20,21,22,
	"gravid female"	21,23,24,25
	"egg bearing females"	26,27
	"brood bearing females"	22
	"embryo-carrying females"	21
	"egg carrying females"	28
Sexually mature females	"ovigerous female"	29
	"gravid females"	17
Ovary without developing oocytes	" dormant ovary"	7
	"quiescent ovary"	7
Moulting of female containing developed ovary	" prespawning moult"	1,21
	"parturial moult"	14,30
	"preparturient moult"	29
	"Post ovulatory moult"	31
	"prenuptial moult"	32
	"spawning moult"	33
	"berried moult"	7

1. Ling and Merican,(1961) 2. Rajyalakshi,(1961)
3. Vijayaraghavan and Easterson,(1974)
4. Shafi and Quddus,(1975) 5. Eble et al,(1977)

6. Ponnuchamy et al.,(1979)

7. Pandian and Balasundarum,(1980a.b)

8. Hartnoll and Paul,(1982)

9. New and Singholka,(1982)

10. New,(1988)

11. Ra'anan and Cohen,(1985)

12. Sarojini et al.(1986)

13. Murthy et al.,(1987)

14. Kamiguchi,(1971)

15. Phillips,(1971)

16. Sandifer and Smith,(1978)

17. Clark,(1979)

18. Rao et al.,(1981)

19. O'Donovan et al.,(1984)

20. Hartnoll,(1985)

21. Ching and Velez,(1985)

22. Charles and Subramonium,(1987)

23. Morris,(1973)

24. Sakuntala,(1977)

25. Malecha,(1983)

26. Pandian,(1967)

27. Wenner et al,(1985)

28. Mashiko,(1987)

29. Lynn,(1981)

30. Nagamine and Knight,(1980)

31. Kuruta,(1962)

32. Provenzano,(1985)

33. Howlader,(1979)

Charles and Subramonium (1987) referred to females incubating eggs as "ovigerous females" or "egg bearing females" in different parts of the text, whilst Ching and Velez(1985) used "ovigerous" and "gravid" females. As mentioned above the term "ovigerous" is used to refer to both egg carrying and non-egg carrying females. Therefore it is difficult to judge precisely which stage is being described at a particular part of the text. Similarly, ovary without developing oocytes is sometimes referred to as "quiescent ovary" and elsewhere as "dormant ovary" (Pandian and Balasundarum,1980(a). The same authors use the terms "reproductively passive phase" in some parts of the text and "reproductively quiescent phase" in others to describe the intermoult stage of a female without developing oocytes or with dormant ovary.

Therefore, as a first step, it seemed logical to gather together conventionally used terminology (Table:3.1) and unify these, whilst designating new terms where necessary in order to produce consistency. Knowledge concerning the reproductive performance of cultured Crustaceans is at an early stage. Therefore it is imperative to advocate simplicity and precision in demarcating different stages and activities in the reproductive cycle of these animals and to maintain consistency for comparative purposes.

In addition during this survey and preliminary studies with M.rosenbergii, the possible existence of morphological and physiological differences between females was

recognised. The existence of different male morphotypes within M.rosenbergii populations has been studied in considerable detail (review, Ra'anan and Cohen,1985). These different male morphotypes were found to differ in growth and reproductive performance, physiology and behaviour.

There are very large variations in spawning patterns, within populations of Macrobrachium sp. Individuals of identical size, from a single spawn, raised under same environmental conditions show large variations in spawning pattern. (eg:coefficient of variation of 47% of the mean interspawning period was recorded for a population of M.rosenbergii females; personal observation). Similarly high variations in spawning patterns of M.rosenbergii (Wickins and Beard,1974; see Table:3.2) and M.nobilii (Pandian and Balasundarum,1980a;see Fig:3.1) were reported. Such high variations not only make interpretation of results within a population difficult, but also hinder comparisons between populations. The criteria based on mean numbers of spawns, mean interspawning periods etc., currently used to evaluate the reproductive performances of animals, do not reveal much information when used to evaluate reproductive performance of species, such as Macrobrachium, showing large variations in performance.

Therefore, as a second step, different morphotypes found among Macrobrachium females were identified and classified according to the proposed unified terminology. Their distribution in a M.rosenbergii population was

Table: 3.2

The spawning of M.rosenbergii during an experiment which lasted 390 days. (Source: Wickins and Beard, 1974)

Reference number of females	Total number of spawns
1	9
2	2
3	0
4	4
5	5
6	1
7	4
8	5
9	1
10	2
11	4
12	6
13	7
14	0
15	2
16	6
17	5
18	1
19	1
20	2
Total	68

Fig:3.1 Moulting and spawning in normal and relieved females of M.nobilii. Each tier represents a female under observation during the period, when the incubation undertook a maximum of 8 successive moults (indicated in Roman letters). X indicates a moult not followed by spawning: indicates a moult successfully followed by spawning (Source: Pandian and Balasundarum, 1980a)

quantified. Based on this, criteria were developed to evaluate the spawning pattern of Macrobrachium sp., which could be adopted for other Crustaceans. The usefulness of this new terminoloy and classification was also evaluated.

3.1.2 Influence of egg incubation and active ovary on moulting and spawning pattern.

Presence of eggs on the pleopods of females has been found to extend the intermoult period (IMP) in berried females, compared to unberried females, in some Crustaceans such as Crangon (Lloyd and Yonge,1944), Cambarus (Scudamore, 1948), Homarus (Templemen,1936) and Palaemon (Kamiguchi, 1971). However, removal of eggs after spawning (relieved females) did not alter the intermoult period of Palaemon (Kamiguchi,1971) and Macrobrachium nobilii (Pandian and Balasundaram,1980a.b). Lengthening of the IMP as a result of reproduction can result in the duration increasing by 60-80% with respect to a slower growth rate (Hartnoll,1985). This indicates that the presence of eggs on the pleopods influences moulting physiology of females in their forthcoming IMP. Further, removal of eggs from M.nobilii berried females has been found to increase the spawning frequency compared to those incubating eggs. Wickins and Beard,(1974) reported contradictory results regarding the influence of developing ovary on IMPs in M.rosenbergii in two parts of their experiment. Significantly longer IMPs were reported when eggs were being formed than when they were not during part 1 of the experiment in which the

experiment in which the environmental conditions fluctuated. In part 2, when environmental conditions were uniform, the prawns were found to moult at the same intervals irrespective of ovarian development. The above observations indicate that development of the ovary and egg incubation could alter the reproductive physiology of female carideans.

In the present study the possible influences of ovarian development, and the presence of eggs on the pleopods, on moulting and spawning patterns of M.rosenbergii were evaluated. Possible physiological differences among different morphotypes were also elucidated.

During these studies, it was imperative to demarcate moulting and intermoult periods in association with spawning pattern of the female.

3.2 Materials and Methods

The animals used in this study were from a single spawn obtained from broodstock A and raised individually in the recirculated system S_2 as described in Chapter.2.

Twentyfour M.rosenbergii females, 19-20 mm in carapace length, were randomly divided into two groups B and R. They were fed on fresh food such as mussels, shrimps, whitebait, squid, lambs' liver and spinach leaves once a day prior to onset of the dark cycle of the photoperiod. Mating and egg sampling were as described in 2.4. Eggs aborted within 2-3

days of spawning were considered unfertilised eggs. Fertilised eggs were not removed from the females in group B. Fertilised eggs from group R were carefully removed 48 hr. after spawning. The experimental period lasted for 165 days.

Based on morphological features such as colour of the claws, state of sexual maturity, secondary sexual characters state of development of the ovary and the presence or absence of egg clutches, females were differentiated into different morphotypes. Observations were also made on morphotypes of Macrobrachium lanchesterri raised in the same system. Based on these observations, a generalised classification of morphotypes for Macrobrachium sp. was developed. During moulting transformation of morphotypes takes place. The fate of the morphotypes during moulting is illustrated in a generalised model for Macrobrachium sp.

The distribution of morphotypes in berried group B, belonging to female M.rosenbergii female population, is used to describe morphotypes and transformations while moulting.

3.3 Results

3.3.1 Results and Discussion

Differentiation of female morphotypes in Macrobrachium sp. and their distribution in M.rosenbergii.

A classification of different morphotypes identified among the Macrobrachium female populations and the united and new terminology advocated in the present study are presented in Fig:3.2. This classification is based on the morphological characteristics of females at a particular intermoulting periods.

Juvenile females which have attained sexual maturity by undergoing the "moult of puberty" are referred to as pubertal females. In M.rosenbergii pubertal females can be identified as described in Section 1.4.1. In this thesis all studies were carried out on pubertal females. Therefore, for simplicity, they will be referred to as females unless otherwise stated. A group of pubertal males or females is referred to as a "Broodstock".

The distribution of morphotypes in an M.rosenbergii population raised under controlled laboratory conditions is presented in Table:3.3. The distribution of morphotypes in the berried group is used throughout this discussion.

As with male morphotypes, females can be classified on the basis of colour of the claws as;

Blue clawed (BC) females: characterised by the blue coloration of the claws.

PF Pubertal Female : Sexually mature female

A Active Ovary : Ovary with developing oocytes

Q Quiescent Ovary : Ovary without developing oocytes

AO Actovarous Female : Female with active ovary

QO Quiesovarous Female : Female with quiescent ovary

BAO Berried Actovarous Female : Female with active ovary and carrying eggs

BQO Berried Quiesovarous Female : Females with quiescent ovary and carrying eggs

UBAO Unberried Actovarous Female : Female with active ovary and not carrying eggs

UBQO Unberried Quiesovarous Female: Female with quiescent ovary and not carrying eggs

USAO Unspawned Actovarous Female : Actovarous female which had a quiescent ovary at previous intermoulting period

USQO Unspawned Quiesovarous Female:Quiesovarous female which had a quiescent ovary at previous intermoulting period

AB Aborted Female : Female which lost eggs from pleopods after spawning

R Relieved Female: Female which lost of eggs due to forcible removal

PP Post Parturient Female: Female without eggs due to hatching of larvae

Fig: 3.2 Schematic diagram illustrating pubertal female morphotypes identified in Macrobrachium sp. (Based on personal observations and classification).

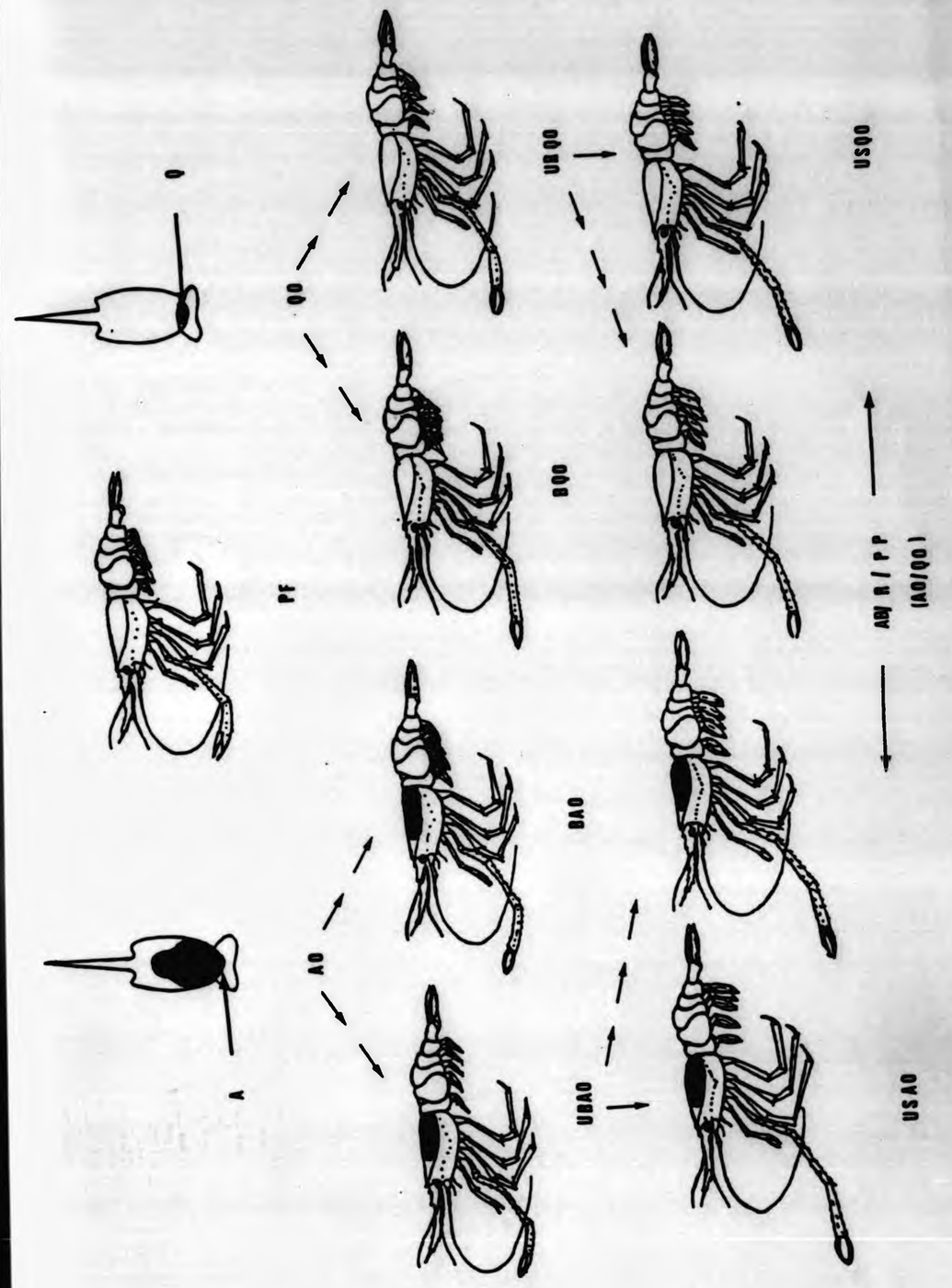


Table:3.3. Distribution of morphotypes in berried and relieved groups M.rosenbergii (Expressed as %).

Morphotypes	Berried	Relieved
Actovarous females	43	61
Quiesovarous females	57	39
Berried / Relieved actovarous females	07	32
Berried / Relieved quiesovarous females	18	08
Total Berried females	25	40
Unspawned actovarous females	32	19
Unspawned quiesovarous females	32	16
Aborted actovarous females	05	10
Aborted quiesovarous females	07	13
Total Unberried females	75	58
Fertilisation success	68	71
Subsequent actovarous females	12	34
Subsequent quiesovarous females	23	15
Consecutive actovarous females	-	11
Discrete actovarous females	100	89
Non-actovarous females	-	-
Productive Spawns	68	-
Unproductive Spawns	-	-

- Not detected

Orange clawed (OC) females: characterised by the orange coloration of the claws.

In the present study females at early stages of sexual maturity exhibited both blue and orange claw coloration. At later stages large pubertal females (carapace length above 30mm approximately 20g) were found to be exclusively blue clawed. Although not evaluated in the present study, it would be interesting to study the reproductive significance of such differences in claw (as in male populations of M.rosenbergii).

At a particular intermoult period the ovary may have developing oocytes which result in its enlargement. Ovaries with developing oocytes can be referred to as "active ovaries". These are visible through the carapace throughout the entire intermoulting period (Fig:3.2. and Plate.6.1.). In the absence of developing oocytes the ovary can be referred to as the "quiescent ovary" (both terms were used by Pandian and Balasundaram,1980(a)). A pubertal female containing an active ovary can be referred to as a "actovarous female". In practice such females are capable of producing eggs in the following moult. Pubertal females with quiescent ovaries can be referred to as "quiesovarous females". These females were referred to as "neuters" by Lloyd and Yonge,(1944) and Pandian and Balasundaram,(1980 b) The term usually refers to individuals of indeterminate sex, therefore use of this term is inappropriate to describe the reproductive state of females.

In the M.rosenbergii population studied more quiesovarous females (57%) were found than actovarous females (Table:3.3). As the population studied was at an early stage of sexual maturity it is possible that the frequency of ovary development may have been low. This possibility is further considered in Chapter 8.

An actovarous female may carry fertilised eggs on the pleopods as a result of fertilisation. Such a female can be referred to as a "berried actovarous female". Alternatively, a an actovarous female may not carry eggs during an intermoult period (IMP) and can be referred to as an "unberried actovarous female". A female may not carry eggs for a variety of reasons:

a) The ovary might have been in a quiescent state in the previous intermoult period and may not spawn the following moult. If so, the actovarous female can be termed as an "Unspawned actovarous female". In M.rosenbergii such females can be identified by the absence of eggs and ovigerous setae in the pleopods (Nagamine and Knight,1980).

b) The eggs could have been lost after spawning due to nonfertilization. Sometimes, even after fertilisation, some females can loose eggs due to unfavourable conditions. These include changes in temperature or accumulation of excreta (Palaemon serratus. Phillips,1971), pH or photoperiod (M.rosenbergii, Wickins and Beard,1974), pesticide presence (Macrobrachium lamerrii, Sarogini et al.,1986) or pathogens

including parasites (Wickham,1979), bacteria (Fisher,1976), protozoa (Nilson et al,1975) and fungi (Talbert,1948). Such actovarous female can be referred to as "aborted actovarous females". In practice these can be characterised by the absence of eggs and presence of ovigerous setae in M.rosenbergii (in contrast to "unovulated actovarous females") and also the brood chamber will be wider.

d) The eggs of berried actovarous female may hatch and from the time of hatching up to the following moult the actovarous female may not carry eggs. Such female can be termed "post-parturient actovarous female".

c) Sometimes the eggs may be forcibly removed from the actovarous female after spawning for artificial incubation of eggs or other studies. These females can be referred to as "relieved actovarous females" (the term "relieved" was used by Pandian and Balasundaram,1980(a)(b) under similar circumstances).

Similarly the quiesovarous females could be classified as;

"unberried quiesovarous female"
"unspawned quiesovarous female"
"aborted quiesovarous female"
"post parturient quiesovarous female"
"relieved quiesovarous female"

These "relieved" morphotypes do not exist in nature. Therefore, "Unberried" refers to "unspawned","aborted" and "post parturient" morphotypes, excluding the "relieved".

In practice it may be difficult to distinguish between "aborted", "relieved" and "post parturient actovarous females" unless kept under controlled conditions.

The proportions of the above quiesovarous females in the M.rosenbergii population studied are presented in Table 3.3. As fertilised eggs were not removed from the females, no relieved quiesovarous/ actovarous females were present. Abortion of eggs was found only in the case of unfertilised eggs. Fertilised eggs were not aborted during incubation of eggs as reported by Wickins and Beard (1974).

Although 43% actovarous females were reported during this experiment only 25% of the total actovarous females were berried. The fate of 7% of the actovarous females is not known due to termination of the experiment. Therefore, out of the total (43%) actovarous females whose fates are known, 12% aborted the eggs as a result of unfertilisation. Consequently, a fertilisation success of 68% was achieved in this study. It is important to note that fertilisation success cannot simply be calculated from the number of berried actovarous females and quiesovarous females.

One of the problems faced during mating was finding suitable, sexually mature, males as the females used in this study were very small. This could have reduced the potential fertilisation success of the population.

The ovary of M.rosenbergii morphotypes did not develop in 57% of the intermoulting periods. Together with aborted

females 62 % of the morphotypes were unberried during this 165 day study.

In broad economic terms all categories of actovarous females,and berried quiesovarous females, could collectively be referred to as "productive morphotypes" as they are capable of producing eggs or larvae during the immediate, or the following, intermoult period. All other categories, of quiesovarous females, except berried quiesovarous females, can be refereed to as "unproductive morphotypes".

Among the M.rosenbergii females used in this study, 45% productive morphotypes were found. This includes the total percentage of actovarous females appearing during the experiment and berried quiesovarous females at the beginning of experiment. It is important to note that the number of productive morphotypes is not an arithmetic total of actovarous females and berried quiesovarous females appearing during the study. This is due to contribution of some of the actovarous females towards berried quiesovarous females at their following moult.

Not all the eggs produced by the productive morphotypes will contribute towards larval production. Some eggs may be aborted by the female for reasons described earlier in this section. Therefore the spawns which contribute towards successful larval production can be referred to as "productive spawning" and others as "unproductive spawning" (similar terms were used by Wickins and Beard,1974).

A total of 68% productive spawnings were recorded in the M.rosenbergii used in this study, the rest being unproductive. This is equalent to the fertilisation successes as all fertilised egg clutches contributed towards larval production in the present study. In the event of total abortion of eggs due to unfavourable conditions (Wickins and Beard,1974) and Sarogini et al.,(1986) and as discussed earlier in this section) the productive spawnings will be less than the fertilisation successes.

The above classification of morphotypes is based on the characters at any one particular intermoulting period. The status of the morphotype may change after moulting. The fate of morphotypes undergoing moulting is illustrated by a generalised model for Macrobrachium sp. (Fig:3.3).

The model is based on four major female morphotypes; berried actovarous female, unberried actovarous female, berried quiesovarous female and unberried quiesovarous female (described above) and two major cycles, productive and unproductive. While moulting if a morphotype transforms into a actovarous female or berried quiesovarous female, the cycle can be referred to as "productive cycle" (PC), irrespective of the fate of the eggs. When morphotypes transform into a quiesovarous female, except berried quiesovarous female, the cycle can be referred to as an "unproductive cycle" (UPC) as this cycle result in the production of unproductive broodstock.

BAO Berried Actovarous Female

BQO Berried Quiesovarous Female

UBAO Unberried Actovarous Female

UBQO Unberried Quiesovarous Female

P Productive Cycle

UP Unproductive Cycle

A Active ovary

Q Quiescent ovary

1 Eggs Fertilised

2 Eggs Aborted

3 Unspawned

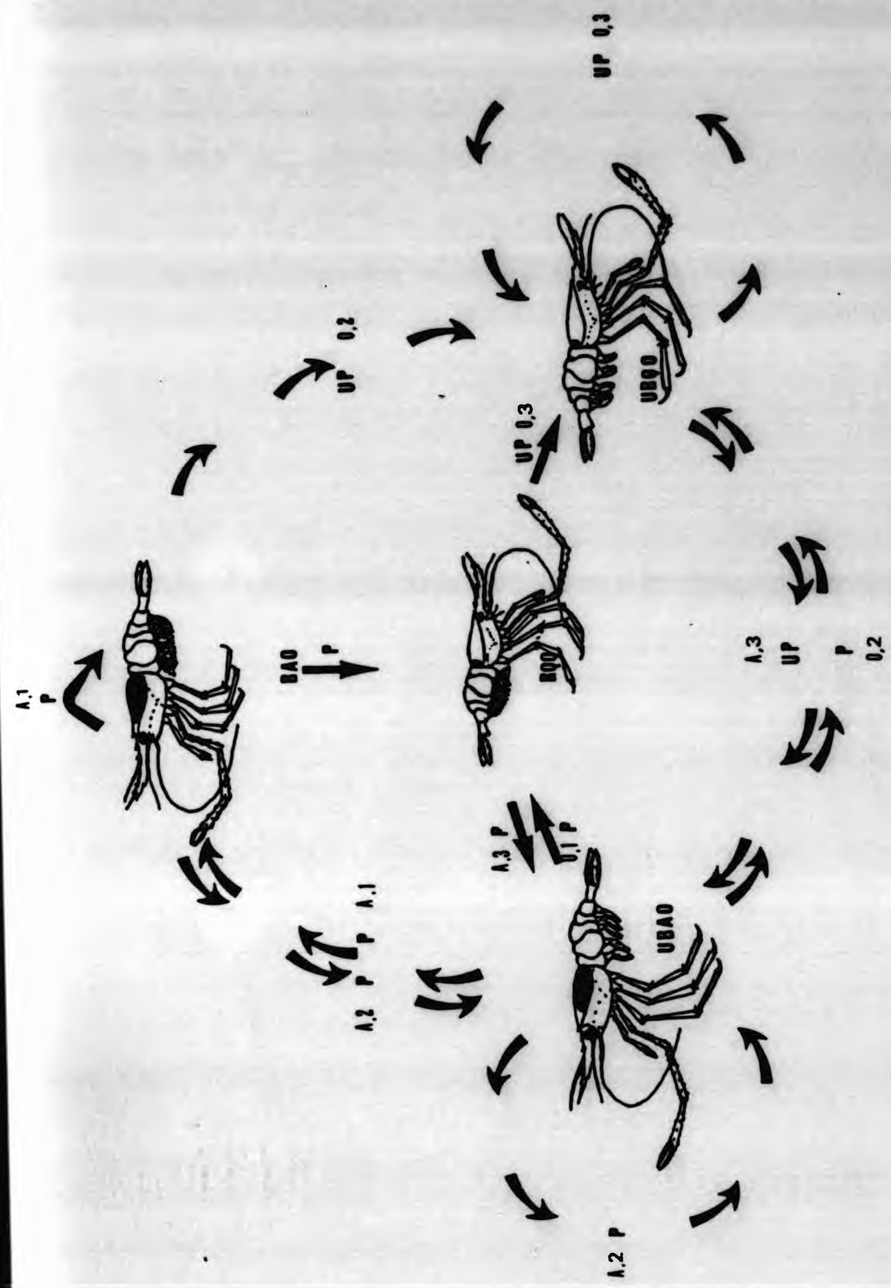


Fig: 3.3 Generalised model illustrating the pattern of transformation of morphotypes belonging to Macrobrachium sp. during moulting (Based on personal observation).

The pattern of transformations in M.rosenbergii population is illustrated in Fig:3.4. 61% of the transfor--mations were productive resulting in the production of berried females and actovarous females. The rest were unproductive resulting in the production of unberried quiesovarous females.

When a berried actovarous female transforms into a berried actovarous female after moulting the female can be referred to as a "subsequent berried actovarous female". (Fig:3.3). Similarly when a actovarous female transforms into another actovarous female after moulting, it can be referred to as "subsequent actovarous female" (Fig:3.3). Whilst a quiesovarous female transforming into another quiesovarous female can be referred to as a "subsequent quiesovarous female".

Only 12% of actovarous females (out of 43%) developed active ovaries in subsequent moults. 23% of the quiesovarous females (out of 57%) transformed into quiesovarous females in the M.rosenbergii population used in this study (Table:3.3). This indicates that the frequencies of ovary development at subsequent moults were less than of quiescent ovaries. Possible factors influencing the frequency of ovary development will be further considered in (sections 3.2.4).

When actovarous females produce subsequent spawns at consecutive moults over a specified period, they can be referred to as "consecutive actovarous females". Economically, these are highly productive broodstock.

BAO Berried Actovarous Female

BQO Berried Quiesovarous Female

UBAO Unberried Actovarous Female

UBQO Unberried Quiesovarous Female

P Productive Cycle

UP Unproductive Cycle

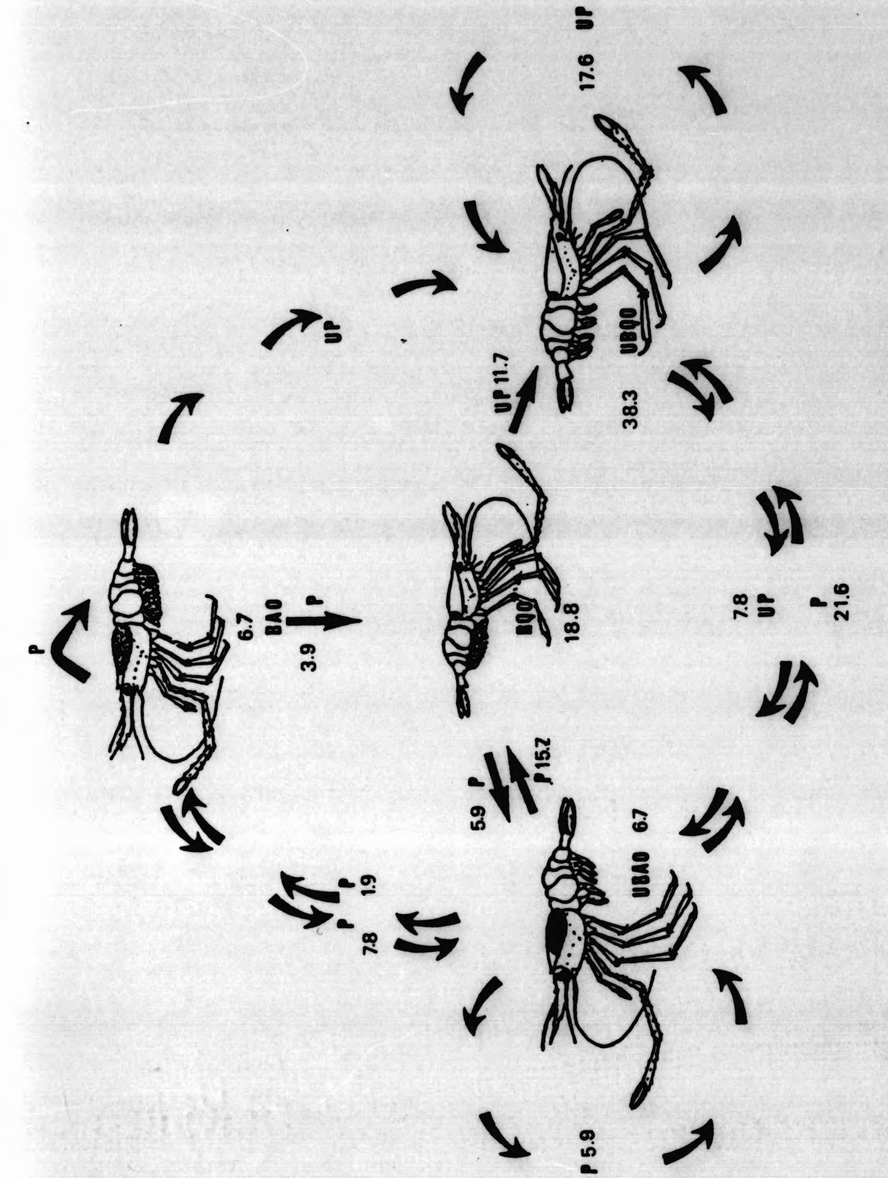
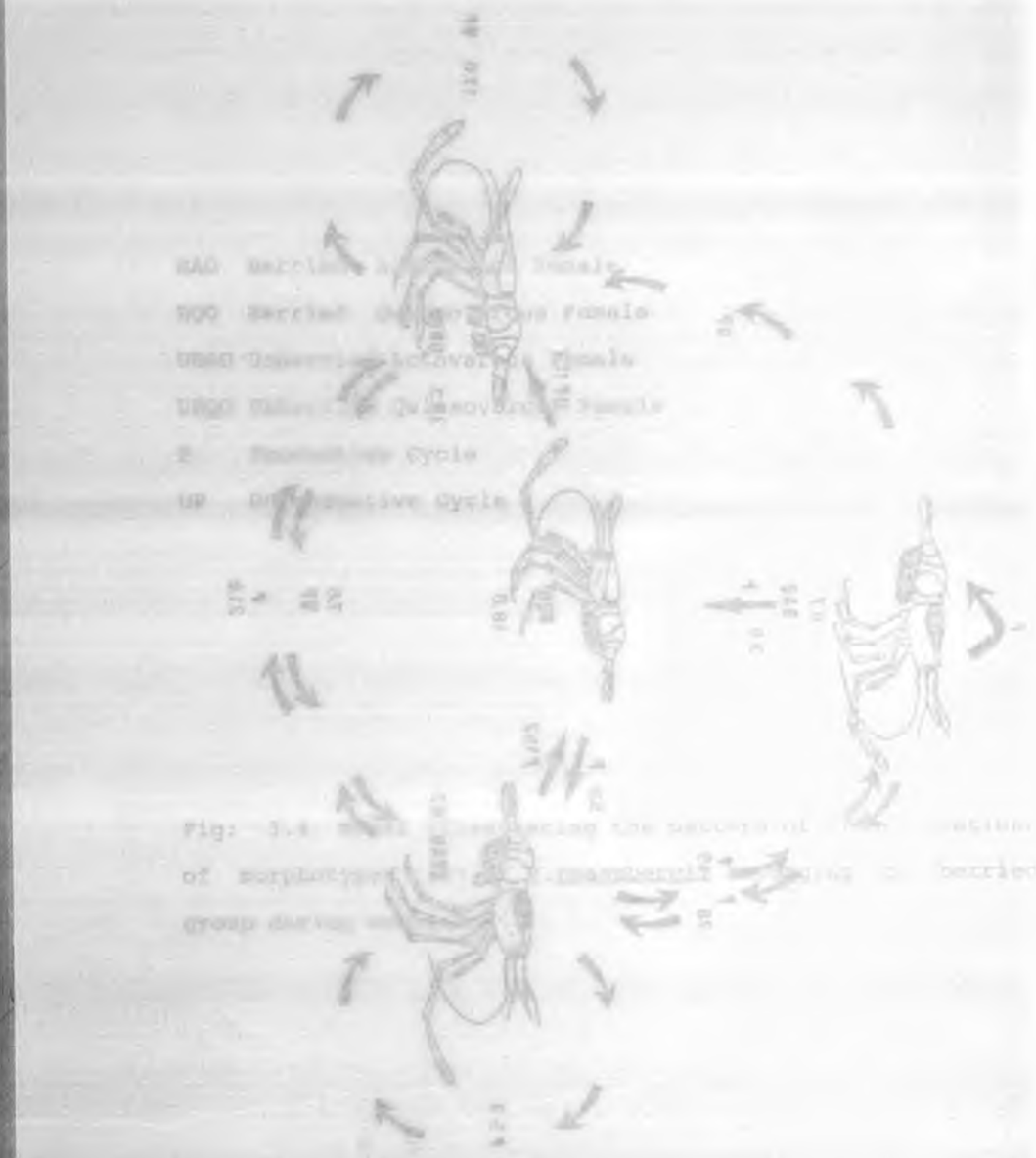


Fig: 3.4 Model illustrating the pattern of transformations of morphotypes (%) of M.rosenbergii belonging to berried group during moulting.



Similarly, when actovarous females do not develop ovary at consecutive moults over a specified period they can be referred to as "discrete actovarous females." When subsequent quiesovarous females produce quiesovarous females at consecutive moults over a specified period they can be referred to as "non-actovarous females", which constitute unproductive broodstock.

All the females observed in the M.rosenbergii population used in this study were discrete actovarous females. There were no consecutive or non-actovarous females. The maximum number of spawns per female observed was 4 and the minimum 1. The maximum number of quiesovarous females produced by a female was 7.

The above classification of female morphotypes is based on simple morphological features at any particular intermoulting period, most of which could be easily identified in nature and under culture conditions.

At the beginning of this chapter problems associated with demarcating different reproductive stages of pubertal females, such as inconsistencies among authors and use of imprecise terminology, were pointed out. The proposed, unified, and newly introduced, terminology and classifica-tion precisely defines and demarcates each stage in the reproductive cycle of Macrobrachium sp.

The distribution of these morphotypes in the population is a good criterion for evaluating the reproductive

performance and potential of prawns as opposed to currently used criteria as presented in Table:3.4. These measures quantify the performance as mean numbers of spawns or the range in the population, over a period of time. As there is a very high variation in the spawning and moulting perfor--mance of Macrobrachium sp. (as indicated in section 3.1 and discussed in forthcoming sections) these mean values do not give a clear picture of the performance of individual females.

The present criteria measure the performance of females at each intermoult period. This can be used to study the reproductive performance of Crustaceans under both laboratory-oriented controlled experiments and field conditions.

Currently, in most Macrobrachium farms, berried females necessary for larval production are selected from growout ponds (Malecha,1983; New,1988). Whilst harvesting recording of different morphotypes would give an indication of avialable productive morphotypes, potential actovarous females etc., which could be used as a measure of reproduc--tive performance. Similar measures could be used to compare performance of populations;

a) at any one time in different habitats (such as two or more ponds, or in natural breeding grounds), or

b) in a particular habitat at different times.

Table:3.4 Spawning and Moulting patterns of berried and relieved groups belonging to M.rosenbergii.

	Berried group			Relieved group		
	Mean	SE ±	CV(%)	Mean	SE ±	CV (%)
Number of moults female^{-1}	6.6[a]	0.2	8.7	6.9[a]	0.2	8.0
IMP (days)	23.8[a]	0.4	5.5	23.1[a]	0.6	8.0
Number of spawns female^{-1}	3.1*	0.4	33.8	4.4*	0.6	42.3
ISP (days)	47.6[a]	5.2	47.3	30.5[a]	2.6	46.9

IMP Intermoulting Period SE Standard Error

ISP Interspawning Period CV Coefficient of Variation

Values having the same superscripts were not significantly different (P>0.05) by T-test.

* significantly different at 0.01 level

Malecha,(1983) stressed the need to develop separate broodstock systems and more control over breeding (see Section.1.5). In the process of establishing a selective breeding program it will be vital to identify potential actovarous females and evaluate their reproductive potential and performance in detail. For example the percentage of "actovarous females" in a population (in natural or controlled environments) gives an indication of the percentage of females that are capable of spawning at their following moult. Similarly, the percentages of subsequent and consecutive actovarous females measures the spawning potential of an individual, or individuals, in a population kept under controlled conditions.

Similar evaluations will be made in forthcoming sections 3.4.2. to compare two populations, one consisting of berried females and the other of relieved females and in 6.3.5 to compare broodstock fed different diets.

The terminology and the empirical model proposed are, as are many others, a substantial simplification and genera-lisation of reality. Although not revealing a complete view of every detail of the complex processes involved, they can be subjected to further amendments with improvements in knowledge of the reproductive biology of Caridea.

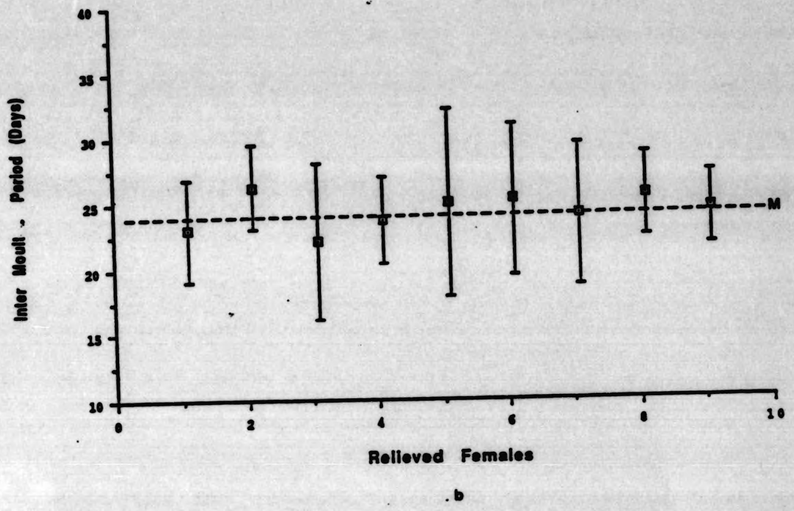
3.3.2 Results

3.3.2.1 Influence of berried eggs and active ovary on moulting pattern of M.rosenbergii female morphotypes.

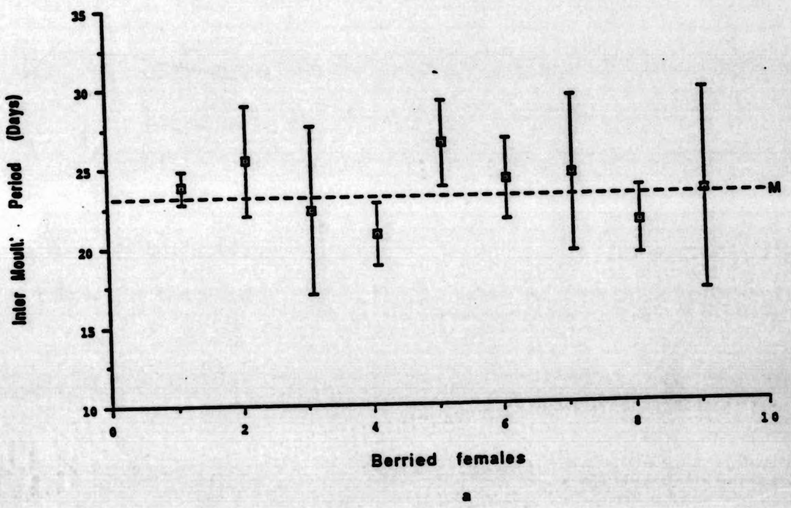
All females moulted normally, except one in each group which died in a partly moulted condition. The moulting and spawning patterns of the berried and relieved females are presented in the Table:3.4. The number of moults per female during the 165 day study varied from 6-7 with a mean of 6.6 (CV=8.7%) in berried females and 6-8 with a mean of 6.9 (CV=8.0%) for relieved females. The differences in mean numbers of moults of both groups were statistically insignificant (P>0.05).

The frequency of moulting, or intermoult period (IMP), varied from 15-31 days in both groups with a mean of 23.8 for berried and 23.1 for relieved females (Table:3.4). The mean IMPs of both groups were not significantly (P>0.05) different.

There were considerable variations in IMPs of individual females (Fig:3.5). This was evident from the coefficients of variation from the mean IMPs of individual females, which ranged from 11-29% in the berried group and 4.4-27.8% in the relieved group. The mean IMPs of individuals belonging to the berried group were insignificant (P>0.05) by ANOVA. Similarly, the means of IMP ofindividuals belonging to relieved group were insignificant (P>0.05) by ANOVA (Fig:3.5).

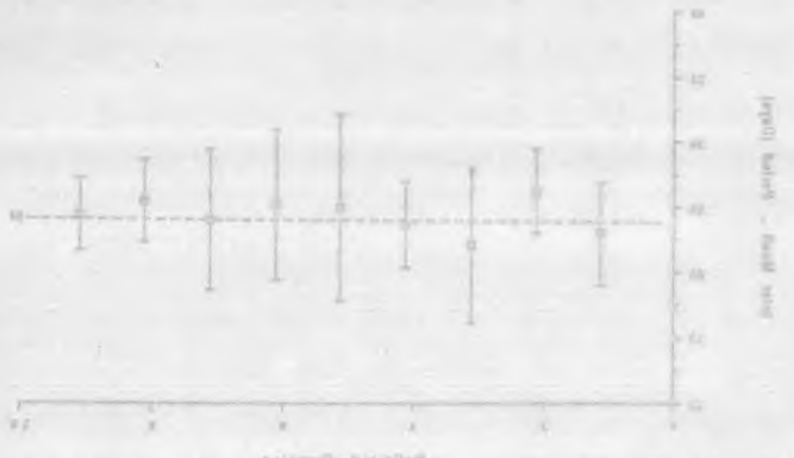


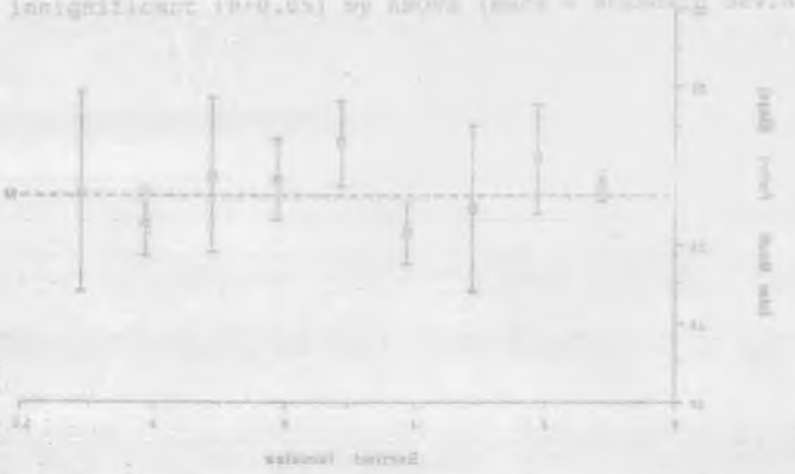
b



a

Fig:3.5 Intermoult periods of individual females belonging to berried group (b) relieved group (a) (M.Mean) Mean intermoult periods of individual females in each group were insignificant (P>0.05) by ANOVA (Bars = Standard Deviation)





When comparing the possible influence of presence of eggs on pleopods on IMPs, it is important to note that there are two different aspects to be clearly distinguished.

Category 1: The mean of all IMPs of an individual female or group of females during a specified period. eg: in the present study mean IMP of the berried or relieved group. The mean IMP of the berried group includes both berried and unberried IMPs which take place during the target period. In the present study, even though the eggs spawned by the females in berried groups were not removed from the pleopods when attached, there were IMPs during which the females were unberried as a result of unfertilisation or quiescent ovary during the previous IMP.

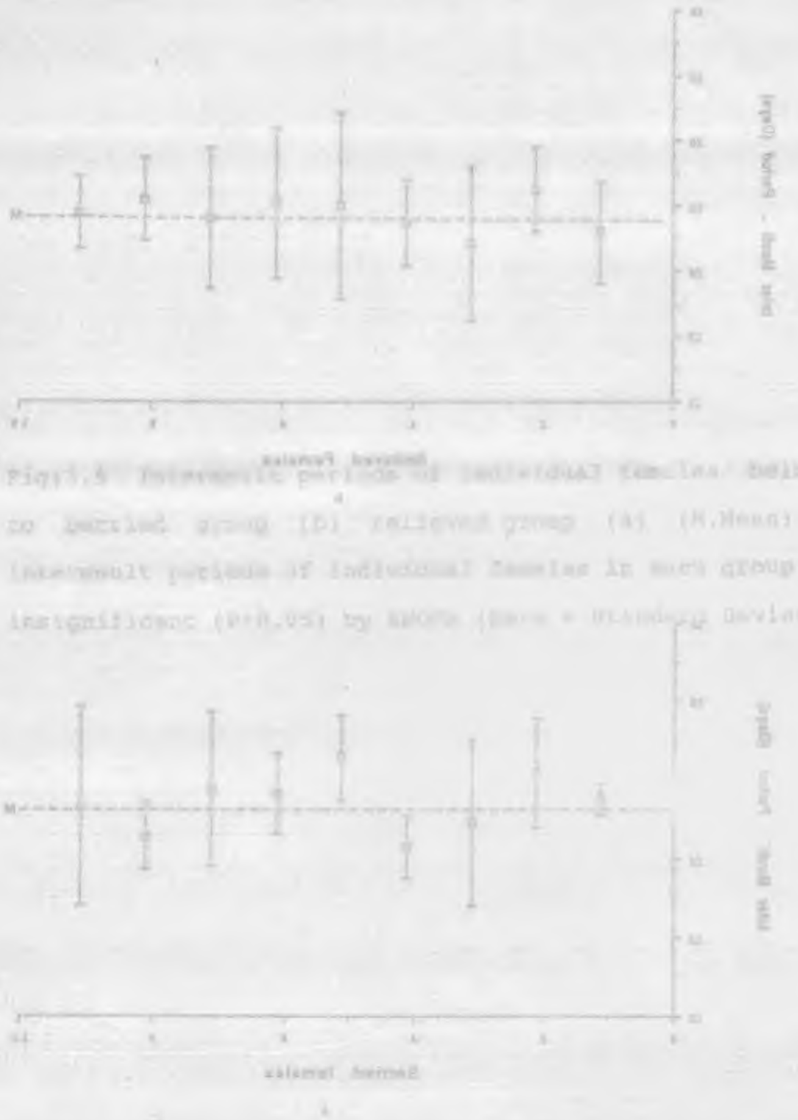
Category 2: The mean of all "specific intermoult periods" of females. This referres to the IMPs specifically associa-ted with the reproductive status of the female such as development of ovary or incubation of eggs eg: mean intermoult period of berried females (Fig:3.6).

Berried intermoult period (B-IMP) : intermoult period during which the female was berried.

Unberried intermoult period (UB-IMP): intermoult period during which the female was unberried.

Relieved intermoult period (R-IMP): intermoult period during which the female was relieved of egg incubation.

Actovarous intermoult period (A-IMP): intermoult period during which ovary was active.



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Berried intermoult period (B-IMP) : intermoult period during which the female was berried.
Unberried intermoult period (UB-IMP): intermoult period during which the female was unberried.
Relieved intermoult period (R-IMP): intermoult period during which the female was relieved of egg incubation.
Actovarous intermoult period (A-IMP): intermoult period during which ovary was active.

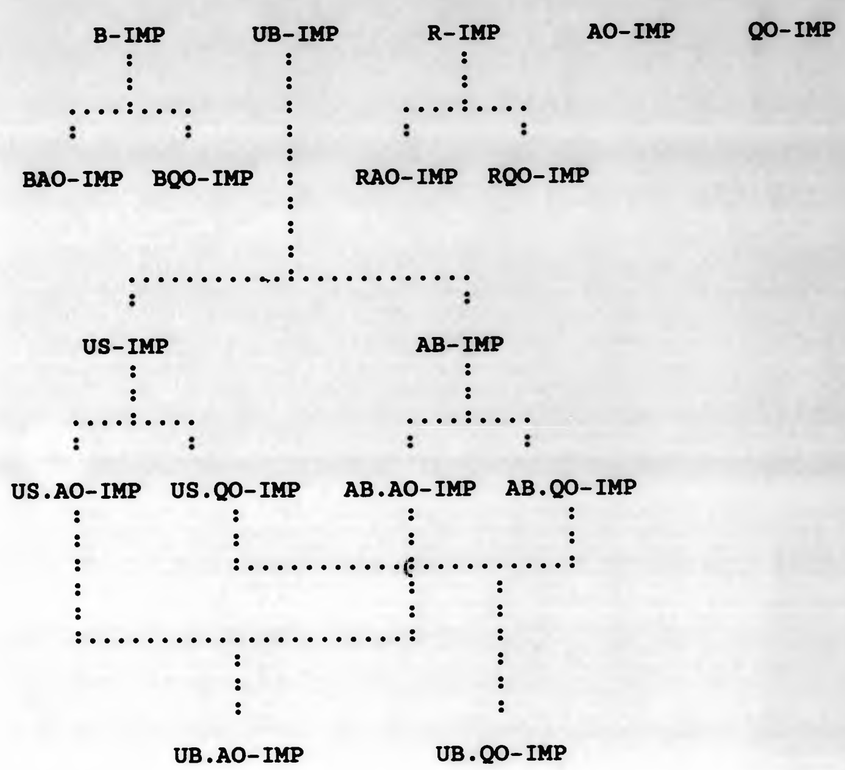


Fig:3.6 Schematic illustration of specific intermoult periods associated with the development of ovary and berried eggs in Macrobrachium sp. as proposed in section 3.3.2. (See text for explanation)

Quiesovarous intermoult period (Q-IMP): intermoult period during which the ovary was quiescent.

Berried actovarous intermoult period (BA-IMP): intermoult period during which the animal was berried with active ovary.

Berried quiesovarous intermoult period (BQ-IMP): intermoult period during which the female was berried with quiescent ovary.

Unberried actovarous intermoult period (UBA-IMP): inter--moult period during which the female was unberried with active ovary.

Unberried quiesovarous intermoult period (UBQ-IMP): inter--moult period during which the animal was unberried with quiescent ovary.

Unspawned intermoult period (US-IMP): intermoult period during which the animal was unberried due to quiescent ovary during the previous IMP.

Aborted intermoult period (AB-IMP): intermoult period during which the female was unberried due to abortion of eggs.

The mean berried-intermoult period (B-IMP) of the berried group was found to be significantly ($P<0.05$) longer than the mean unspawned-intermoult period (US-IMP) of berried group and UA-IMP of relieved group (Table:3.5). This indicate that the presence of eggs on the pleopods of the females lengthen B-IMP compared to US-IMP.

When the egg clutch was removed from the pleopods the mean intermoult period (R-IMP) significantly ($P<0.05$)

Table: 3.5 Specific[x] intermoulting periods of berried and relieved females belonging to M.rosenbergii.

(Expressed as %)

	Berried group		Relieved group		
	B-IMP	US-IMP	R-IMP	US-IMP	A-IMP
Mean	26.0	22.2[a]	23.0[a]	22.1[a]	23.0[a]
SE (±)	0.6	0.6	0.9	0.8	0.5
CV (%)	9.4	17.3	18.8	16.9	8.1

	Ao-IMP	Qo-IMP
Mean	22.2[a]	22.3[a]
SE (±)	3.3	3.1
CV (%)	14.9	13.3

Values having the same superscripts in a row are not significantly different (P>0.05) by t- test/ ANOVA

SE Standard Error CV Coefficient of Variation

x See text for details

shortened compared to the mean B-IMP (Table:3.5). Abortion of eggs from the pleopods due to unfertilisation shortened the mean intermoult period (AB-IMP) significantly ($P<0.05$) compared to the mean B-IMP (Table:3.5). This further suggests that the presence of an egg clutch in the pleopods on the berried females lengthen the IMP compared to the R-IMP and AB-IMP. Interestingly, there were no significant ($P<0.05$) differences among the means of US-IMP, AB-IMP and R-IMPs (Table:3.5). The US-IMP was shorter than rest of the IMPs in both berried and relieved groups. Even though the means of R-IMP and AB-IMP were similar the coefficient of variation of the mean of the R-IMP was higher (18.8%) than of the AB-IMP (8.1%) (Table:3.5).

Slightly longer IMPs were observed in both relieved (R-IMP) and aborted morphotypes (AB-IMP) than in the unovulated morphotypes (US-IMP) indicating that development of the ovary in the previous IMP may exert some influence on the following IMP. Therefore the IMPs of actovarous and quiesovarous (of relieved group only) were grouped and compared. No significant ($P>0.05$) differences was evident between mean actovarous-intermoult period (A-IMP) and quiesovarous-intermoult period (Q-IMP) were evident (Table:3.5). The coefficiaents of variation of the means of both A-IMP and Q-IMP were more or less similar (14-15%) (Table:3.5).

3.3.2.2 Influence of eggs on spawning patterns of M.rosenbergii female morphotypes

All prawns spawned normally except one which developed ovary in all seven moults during the 165 day study, but failed to spawn at the seventh moult. The ovary was visible through the carapace for about seven days and then started to shrink. This, prawn was retained and observed after the experiment and was found to develop ovary and spawn normally. Similar observations were made in later experi--ments and are discussed in Chapter 6.4.5.

There were large variations in spawning patterns of individuals belonging to both groups (Table:3.4). The number of spawns per female varied from 1-4 with a mean of 3.1 (CV=33.8%) for the berried group and 1-7 with a mean of 4.4 (CV=42.3%) for the relieved group. This indicates that there was high variation in spawning pattern within groups or between females. The differences between the mean numbers of spawns were not statistically significant at the 95% confidence level but were significant at the 90% confidence level.

Similarly, there were very high variations in the spawning frequency (Inter spawning period, ISP) within and between groups. The ISPs of females ranged from 15-79 days in the relieved group and 22-99 days in the berried group. The coefficient of variation of mean ISPs of the relieved and berried groups were 46.9 and 47.3%. respectively. The mean ISP the of relieved group was significantly ($P<0.05$)

lower than the mean ISP of the berried group.

The distributions of different morphotypes of berried and relieved groups are presented in Table:3.3. The removal of eggs from the females increased the number of actovarous females in the relieved group by 41.4% compared to the berried group. In addition a 4.8 fold increase in relieved actovarous females and a three fold increase in subsequent actovarous females in the relieved group was observed. The chances of quiesovarous females moulting into quiesovarous (subsequent quiesovarous) in relieved females were lowered by 38%. There were consecutive actovarous females in the relieved group as opposed to all discrete actovarous females in the berried group. Due to increased spawning, the rate of fertilisation also increased in the relieved group by 5%. A 23% reduction in unberried females was observed in the relieved group.

3.4. Discussion:

3.4.1 Influence of egg incubation and active ovary on moulting patterns of M.rosenbergii female morphotypes.

There was a high degree of variation in the moulting patterns of M.rosenbergii females irrespective of the presence or absence of fertilised eggs in the broodchamber. This was evident from the high coefficients of variation of the mean IMPs (Table:3.4.). Several factors could be

responsible for the high degree variation observed in moulting between individuals within groups. Moulting is a highly complex process co-ordinated by several endogenous factors with response to interacting exogenous factors (section 3.1). In the present study the environmental factors were identical for all individuals in both groups. Among endogenous factors, size and breeding cycle have been reported to influence frequency of moulting (IMP) (Wickins and Beard,1974).

The frequency of moulting in Natantia usually decreases with an increase in size (Bursey and Lane,1971 cited Wickins,1976) but not necessarily with age (Reeve,1969 (a), cited Wickins,1976). At 28^0C the IMP of M.rosenbergii increased with size from 9 days (2g) - 18.5 days (20g, female). When sexual maturity is attained the IMP becomes independent of size in M.rosenbergii (Wickins and Beard, 1974) and P.paucidens (Kamaguichi,1971). In contrast, the IMP of M.nobilii was found to lengthen with increase in size of female, even after sexual maturity (Pandian and Balasundaram,1980 a). The animals used in the present study were similar in size range and sexually matured. Also, the possible maternal, genetic, influence was minimised by selecting individuals from the same parental spawn raised under identical conditions from larval to experimental stages (details in Chapter.2). The possible influence of age on IMP of females remains to be elucidated.

The major difference between the two M.rosenbergii groups in the present study was removal of eggs from the berried females. IMPs as low as 15 days (at 28±1^{0}C) have been observed in the present study in both groups. The incubation period of eggs (embryonic developmental period) of M.rosenbergii at 28±1^{0}C is 19 days (day 0=day of spawning, day 19 = night of larval hatching: personal observation see following sections 4.4.4 and 7.4). Therefore logically, the IMP of the berried females has to be more than 19 days unless the females shed their skeleton with the egg clutch attached, this was not observed during the present study. Shedding of exuvia with the egg clutch intact has been reported in M.nobilii (Balasundaram and Poyyamoli, 1984). The shortest IMP observed for berried females in the present study was 21 days. This indicates that the presence of eggs on the pleopods of the berried females lengthened the IMP. This was further statistically confirmed by the longer intermoult periods (B-IMP) of berried females (when eggs were incubated on the pleopods) compared to the unspawned intermoult periods (US-IMP) of the same females (in berried group) or in the relieved group, in the absence of eggs in the pleopods.

Extended intermoults in breeding females have been reported for a considerable number of Crustaceans (citing several authors, Hartnoll,1985: also see section 3.2.1). The mean duration of non ovigerous (referred as unberried in this study) and for ovigerous (referred as berried in this

study) intermoults respectively of isopods Oniscus asellus were 51 and 84 days, Porcellio dilatatus 57 and 69 days (Heeley 1941, cited Hartnoll,1985). Similarly Kamiguchi (1971) found the mean IMP of a group of unberried Palaemon paucidens to be 21.5 days compared to 39.9 days in a berried group.

In the literature most authors use the first category as mean IMP, whilst others use the second category. (section:3.3.2.1). Therefore, there are contradictions when attempting to interpret results. In the present study results were analysed considering both categories of IMPs. When considering the mean IMP (category 1) of both groups no significant difference was evident (Table:3.4) between the IMPs of berried and relieved groups. Similar observations were reported for M.nobilii (Pandian and Balasundarum,1980a). Even though the IMPs of relieved females were lower (18 days) compared to berried females (19 days) no statistical differences were evident (Pandian and Balasundaram,1980a). In addition Kamiguchi (1971) did not find any difference between the mean IMPs of a group of aborted females and a group of berried females. The above authors considered the mean IMP (category 1) of the whole group regardless of specific IMPs.

In contrast, when specific IMPs (category 2) were considered in M.rosenbergii, the IMP was significantly shortened in females from which eggs were removed, or aborted by the females (due to unfertiliation), compared to

berried-intermoult periods.

From the present study it is possible to conclude that the presence of eggs on the pleopods of M.rosenbergii females influences the moulting physiology of the berried females by lengthening IMP. In addition, incubation of eggs on the pleopods is one of the factors responsible for variations found in IMPs of M.rosenbergii females. The females used in this study were obtained from a single spawn that had been maintained under identical conditions.

Insignificant differences in IMPs among relieved, aborted and unovulated morphotypes indicate that, as far as moulting physiology is concerned, they were more or less similar. There were also no significant differences in IMPs between the actovarous and quiesovarous females in the relieved group. This indicates that ovarian development does not greatly influence the moulting physiology of female M.rosenbergii. This is in contrast to the observations of Ling and Merican,(1961) (section.1.4.1). Although the authors reported that the IMP of female with developing ovary take longer than female with quiescent ovary, no experimental evidence was presented. Several other active physiological processes, such as mobilisation of reserves and synthesis of reproductive tissues, have been widely reported to take place in actovarous females as compared to quiesovarous females.

Several theories have been advanced regarding the lengthening of IMPs and possible factors controlling this

in berried females. Hartnoll (1985) suggested that the special and cryptic behaviour of incubating females may restrict or inhibit feeding, which may lead to inadequate accumulation of resources, resulting in delayed moulting. Egg incubation is an energy demanding process; consequently berried females use more energy than relieved females resulting in partition of available energy otherwise used for moulting. The IMP is intimately related to the levels of metabolic reserves (Passano,1960) and lengthening of IMP is an indication of metabolic stress. Therefore it is possible that part of the resources saved by the relieved females (from the task of incubating eggs) could have been channelled into moulting. However, such possibilities need further quantification of the energy saved by not incubating eggs.

Kamiguchi (1971) suggested that the long IMP observed in berried P.paucidens was brought about by endogenous factors rather than physical stimuli as he did not find any differences in mean IMPs between a berried group and an aborted group, as discussed earlier. In contrast Pandian and Balasundaram (1980a) suggested that prolongation of the moult-inhibiting action of the sinus gland by impulses, over nerve-reflex pathways, produced by the presence of the eggs on the pleopods may explain the delay in moulting of the incubating females. The present study tends to support this latter speculation as removal of eggs from the pleopods could have prevented continuous beating of pleopods

resulting in lack of the necessary stimuli to prolong moult inhibition by the sinus gland.

In section 3.1 it was pointed out that all physiological processes in Crustaceans are integrated and co-ordinated to phase with the moulting cycle of the animal. The present study indicated that moulting physiology of berried morphotypes was interfered with by the presence of incubating eggs on the pleopods. Therefore, berried morphotypes are physiologically different from unberried morphotypes under identical conditions. Among unberried morphotypes it was found that development of the ovary does not interfere with moulting physiology of the females compared to quiesovarous females under identical conditions. This does not mean that there is no physiological difference between quiesovarous and actovarous females as it is well documented in many animals that mobilisation of reserves, and synthesis of reproductive tissue, takes place during maturation of gonads. It is evident that there are possible physiological differences between berried actovarous, berried quiesovarous, unberried actovarous and unberried quiesovarous females.

3.4.2 Influence of berried eggs on spawning pattern of *M.rosenbergii* female morphotypes.

There were considerable variations in the numbers of times each female spawned within the groups during the 165 day study. This was evident from the very high coefficient

of variation of the means of both groups. Similar variations were discussed in section 3.1 (Table:3.2).

Higher coefficients of variation indicate high phenotypic variation which might allow for a successful selective breeding program (Tave,1986). It would therefore be valuable to evaluate the possibility of selective breeding for high spawning performance in M.rosenbergii females.

There were differences in mean number of spawns per female in both groups. Relieving the eggs from the pleopods of M.rosenbergii females was found to increase the mean number of spawns per female and significantly shorten the interspawning periods (ISP) within a specified period. It is evident that one the factors responsible for variations in ISP in M.rosenbergii is presence of eggs on the pleopods.

There were increases in the numbers of actovarous, subsequent actovarous and consecutive actovarous females in the relieved group as compared to the berried group. The increase in actovarous and subsequent actovarous females in the relieved group may be explained by considering the pattern of transformation of different morphotypes in both these groups Fig:3.4 and Fig:3.7. Interestingly the increase in actovarous females was associated with the transfor--mation of relieved actovarous females into relieved actovarous females. eg: when the eggs were removed from the

RAO Relieved Actovarous Female

RQO Relieved Quiesovarous Female

UBAO Unberried Actovarous Female

UBQO Unberried Quiesovarous Female

P Productive Cycle

UP Unproductive Cycle

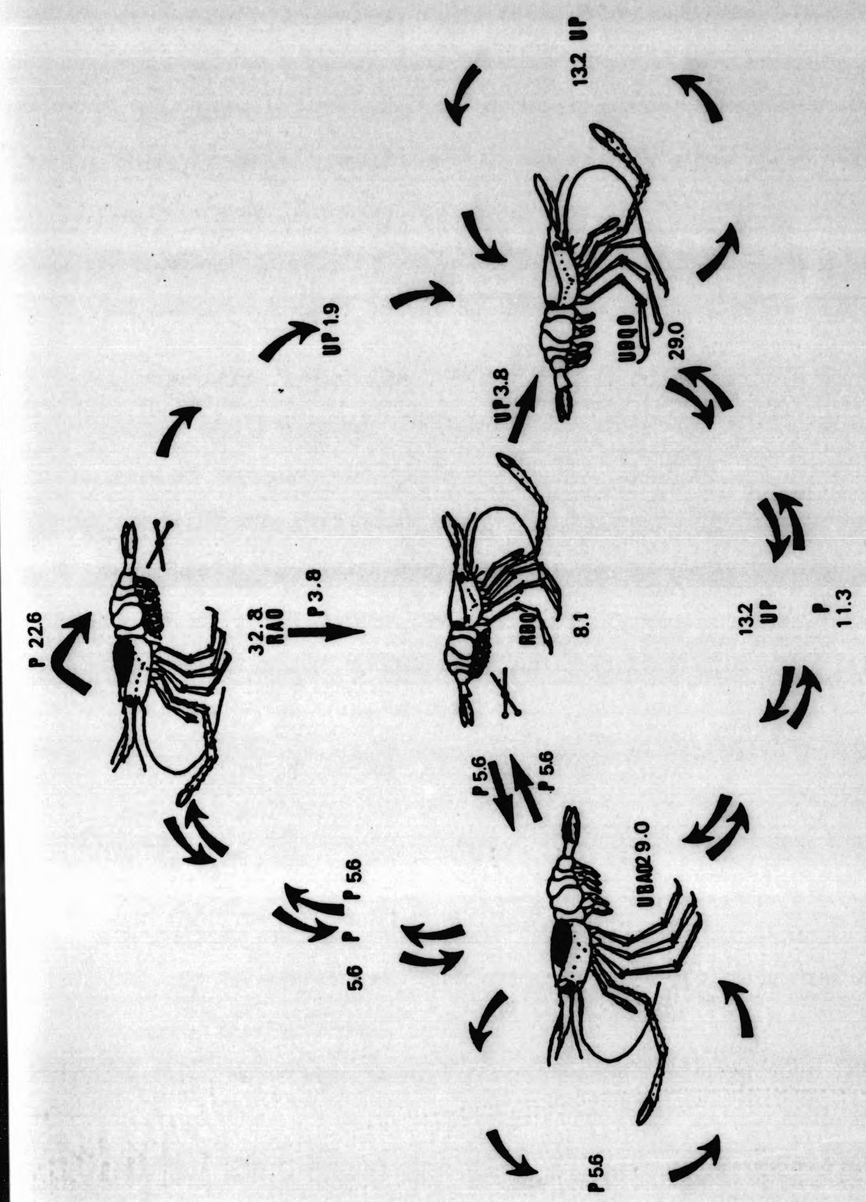


Fig: 3.7 Model illustrating the pattern of transformations of morphotypes (%) of M.rosenbergii belonging to relieved group during moulting.

Fig: 3.7 Model illustrating the pattern of morphotype transformations of morphotype (A) after experimental removal of eggs from relieved group during moult stage.

pleopods of the berried actovarous females (Fig:3.7.) transformation of relieved actovarous femles into the same morphotype was 22.6% compared to none in the berried group. Consequently an increase in subsequent actovarous females, and substantial reductions in quiesovarous, and subsequent quiesovarous females was evident. Due to this increase in actovarous females there was an increase in berried morphotypes in the relieved group.

Removal of eggs from the pleopods of M.nobilii was found to increase subsequent spawnings from 61% in the berried group to 81% in the relieved group (Pandian and Balasundaram, 1980a.). These authors suggest that the energy saved from the incubation and/or energy earned via increased feeding could have been channelled in to egg production. However, this effect remains to be quantified. The above authors also found that egg production per spawn was not influenced by removal of eggs from the pleopods. Removal of eggs from the buccal cavity of mouth brooding fish O.mossambicus has been found to increase spawning frequency (Rana, pers.communications)

In diecdysic prawns, such as M.rosenbergii, spawning is always preceeded by a moult. These animals must moult for spawning to take place. Due to the dependence of spawning on moulting all cues affecting moulting will directly or indirectly affect spawning. Direct constraints are those affecting both moulting and spawning, whilst indirect constraints refer to factors specifically influencing

moulting (not spawning), and consequently affecting spawning. Differences in spawning patterns of individuals observed in the present study may also be due to factors indirectly influencing moulting, as discussed in the previous section.

Removal of eggs from pleopods of berried females resulted in an increased mean number of spawns per female and number of moults per female. It is not clear whether the differences in spawning between groups were associated with differences in spawning potential of females or a consequence of differences in moulting. Differences in moulting (even though not significant) between relieved and berried groups in the present study were reported in the previous section.

Therefore, the performance of a dependent variable, such as spawning, in diecdysic prawns therefore needs to be correlated with the independent variable (moulting). This can be achieved by considering Spawning-Moult Capacity (SMC) and Spawning Moult Efficiency (SME) as new indices introduced in this thesis.

a) Spawning - Moult Capacity (SMC) = $\frac{\text{Number of spawns}}{\text{Number of moults}}$

The number of spawns corresponds to those taking place within the specified number of moults. Therefore SMC indicates the probability of spawning taking place within the specified moults, irrespective of the number of moults.

For example the number of moults and spawns of four females, which took place during the 165 day study, are given below with respective SMC values.

a) $\frac{5}{8} = 0.63$ b) $\frac{5}{7} = 0.71$ c) $\frac{4}{6} = 0.66$ d) $\frac{4}{7} = 0.57$

Although the numbers of spawns were 5 in females (a) and (b), the chances of spawning in (b) were less than in (a) as the number of moults in (b) was lower than in (a). When the number of spawns is considered alone as the criterion to establish spawning potential both females (a) and (b) appear to have the same potential. Logically the maximum number of times the female (b) can spawn is 7, compared to 8 in (a). Out of 7 possible spawns the female (b) has spawned 5 times or the probability of (b) spawning or the capacity of spawning (SMC) is 0.71, compared 0.63 in (a). This clearly indicates that the true potential of spawning is masked by moulting in female (b). Therefore, the true potential of spawning of diecdysic prawns, such as M.rosenbergii, cannot be evaluated by simply considering the total number of spawns, as used conventionally. Conse--quently, SMC is a good measure of true spawning potential. Similar arguments could be forwarded for females (c) and (d).

The maximum value that SMC could have is 1, which indicates that the animal spawned following each moult.

b) SMC of a female or group of females can be expressed as Spawning-Moult Efficiency (SME) in percentages as;

SME (%) = SMC X 100

SMC and SME can be influenced only by those factors affecting spawning, not by factors affecting moulting. SME and SMC can be used to evaluate the spawning capacity of individual females, or groups of females, belonging to different populations, living under defined condition. It may not be logical to use SME or SMC to compare animals varying widely in moulting pattern. For example SMC of a female spawning once out of two moults (1/2) is 0.5, which is similar to the SMC of a female spawning 7 times out of 14 moults.

The mean SMCs and SMEs of the berried and relieved groups used in the present study are presented in Table:3.6 They were not significantly different at 95 or 90% confidence levels (Table:3.7), in contrast to the signifi--cant differences found at the 90% level when considering the mean numbers of spawns of both groups (Table:3.4 and 3.7).

This suggest that, at the 90% level, the mean number of spawns per female in the relieved group was significantly higher than in the berried group, whereas the spawning capacities (SMC) were insignificant. Therefore it is evident that the significant increase in numbers of spawns was due to the increased number of moults in the relieved group (due

Table: 3.6 Spawn-Moult Capacity (SMC) and Spawn Moult Efficiency (SME) of berried and relieved females belonging to M.rosenbergii.

Parameters	Berried group			Relieved group		
	Mean	SE (±)	CV (%)	Mean	SE(±)	CV (%)
SMC	0.47[a]	0.05	34.39	0.64[a]	0.09	40.37
SME (%)	47.4	-	-	64.4	-	-

Values having the same superscripts are not significantly different (P>0.05) by T-test.

Table: 3.7 t-test indicating significant levels for spawning pattern measured as number of spawns and SMC.

	DF	Critical value. t .	Probability P
Number of spawns	12.6	1.86	0.088
SMC	13.5	1.66	0.120

SE Standard Error DF Degrees of Freedom

CV Coefficient of Variation

to shortening in moulting frequency, see previous section) which increased the probability of spawning. Although the spawning capacity of both groups differed insignificantly, the SMC of the relieved group was 17% higher than that of the berried group. The increase in spawning observed in the relieved group in the present study is mainly due to increases in the number of moults per female (due to shortening of the IMP) and spawning potential of females.

The above observations also further indicate how spawning potential of females could be under or over-estimated unless correlated with the number of moults. Therefore using the number of spawns as the only criterion to compare the spawning potential of diecdysic prawns does not have any merit and is illogical to compare the spawning potential of females with different moulting pattern.

Even though the mean SMC of a population can be calculated or used to express spawning capacity it will not give any indication of the relatively high variation which could be expected within the population. In such cases it is recommended that the SMC be calculated separately for each individual female (if possible) and the population SMC be expressed using frequency distribution curves.

CHAPTER 4

INFLUENCE OF AGE AND SIZE OF BROODSTOCK ON EGG PRODUCTION IN M.ROSENBERGII.

4.1.Introduction

4.1.1.Quantitative egg production

The quantity of eggs produced by a female, or a population, may be expressed numerically as fecundity, gravimetrically as biomass, volumetrically and by its calorific value. In the present study biomass of egg clutch and fecundity were used as measures of quantitative egg production.

Fecundity is defined as the number of ripening eggs prior to spawning (Bagenal,1978). Due to the range of reproductive habits of fishes definitions that are acceptable in all circumstances have not yet been devised (Bagenal,1978).

In Carideans the matured oocyte is spawned after moulting and fertilised eggs are attached to the ovigerous setae of the first four pleopods. It is not feasible to obtain eggs prior to spawning or by stripping as in the case of fishes. Consequently, the fecundity of Carideans was estimated at different phases of egg development.

Malecha (1983) classified fecundity of Macrobrachium sp. as;

Spawning fecundity (SF):

The number of eggs that a female is biologically capable of extruding in one spawn.

Pre-hatch fecundity (PHF):

The number of eggs carried by the female at any one time between spawning and larval hatch or release.

Larval hatch fecundity (LHF):

Number of larvae released from the egg mass following incubation.

Wide differences would be expected between the above categories as up to 100% egg loss has been reported during embryonic development. 30-100% egg loss during incubation was reported for M.rosenbergii (Wickins and Beard,1974), 20-80% loss for Palaemon serratus (Reeve,1969) and up to 53% loss for M.nobilii (Balasundaram and Pandian,1982).

Although pre-hatch fecundity (PHF) and larval hatch fecundity (LHF) are practically useful in predicting egg production in animals obtained from the wild, or ponds, and forecasting larval production, both are heavily dependent on environmental conditions. Therefore estimations made from PHF and LHF will not only fluctuate with environmental conditions but also underestimate the biological potential of the animals.

In the present study estimates of quantitative egg production were based on the spawning fecundity or "spawning weight" of the egg clutch. Spawning weight refers to weight of the egg clutch after spawning.

Inter and intra-specific increase in quantitative egg production with increase in female growth parameters has

been extensively documented both in fish (review, Blaxter, 1969; Bagenal,1978) and Crustaceans (review, Sastry,(1983); Hartnoll,1985). The nature and the degree of association vary between and within species.

Published data on relationships between egg production and growth parameters in Macrobrachium sp. are presented in Table:4.1. Although the size range of the M.rosenbergii used in these studies were similar (110-190 mm total length) there were differences in the relationships found. Labora--tory raised M.rosenbergii were found to produce more eggs than females raised in ponds (Malecha,1983 see Table.3.2). Similarly, Wickins and Beard (1974) reported variations in larval hatch fecundity among M.rosenbergii broodstocks which experienced fluctuating pH and photoperiod. Differences in larval hatch fecundity were reported for M.nipponence populations inhabiting two different regions of a river varying in salinity (Mashiko,1983). The population found near the estuary spawned greater numbers of small eggs than the upper fresh water population, which spawned small numbers of larger eggs.

The above studies indicate that, apart from growth parameters of the female, egg production within Macrobrachium species is dependent on environmental, geographical and rearing conditions.

Table:4.1: Relationship between growth parameters of broodstock and Quantitative egg production in *Macrobrachium* species (published data).

Species	Source of O	Egg production	Intercept	Slope	Growth parameter	Regression coefficient
M. rosenbergii[1]	Wild	F_{ps}	+ 0.0856	+2.1789	log TL	r^2 = 0.95
	Wild	F_{ps}	+ 3.2839	+0.9537	log W	r^2 = 0.83
M. rosenbergii[2]	Wild	F_{ph}	-228751.2	+2024.9	TL	r = 0.57
		Fph	-258086.3	+8471.8	CL	r = 0.78
		F_{ph}	- 59111.9	+3085.0	Wt	r = 0.89
M. rosenbergii[3]	Pond	log F_{ph}	-1.920	+3.970	logTL	-
	Lab	log F_s	-2.200	+3.120	log TL	-
M. rosenbergii[4]	Lab	log Flh	+3.2099	+.00732	TL	r = 0.60
M. dyanum[5]	Wild	F_{ph}	+113.076	+ 15.262	CL	-
M. Lamarrei[6]	Wild	F_{ph}	+111.83	+21.730	CL	-
M. Lamarrei[7]	Wild	F_{ph}	+85.480	+0.4858	TL^3	-
	Wild	WWEC	+220.54	+0.1580	Wt.	

F Fecundity s spawning ps prespawning ph prehatching

lhf larval hatched CL Carapace length TL Total length Wt. weight of O

WWEC Wet weight of egg clutch

1 Shafi and Quddus (1975) 2 Patra (1976)

3 Malecha (1983) 4 Wickins and Beard (1974)

5,6 Koshy and Tiwari (1975) 7 Shakuntala (1977)

In this thesis the following aspects of quantitative egg production were considered;

a) Evaluation of the influence of age and growth parameters of females on egg production under defined conditions.

b) Assesment of the degree of association between age, growth parameters and egg production.

c) Evaluation of possible influences of dietary protein levels and quality on quantitative egg production, the degree of association between various growth parameters and egg production and predictive models of egg production in M.rosenbergii (Chapter.7.)

4.1.2 Qualitative egg production

The term "egg quality" is referred to as hatchability and survival of eggs (Craik and Harvey,1984 (c); Primavera and Posadas,1981). Whilst, Barton,(1981), Springate,(1985) and Bromage and Cumaranatunga,(1988) referred to hatchability of eggs and larvae and growth of emerging larvae. Although larvae develop from eggs and are expected to be dependent on charecteristics of eggs, larvae and eggs are two different, developmental, stages of the life cycle. Apart from the expected egg characteristics the development and survival of larvae is also dependent on the environmental and nutritional parameters under which they are reared. Unless optimum conditions are known and provided

provided it will be difficult to assess whether growth and survival of larvae are due to influence of egg characteristics or rearing conditions. For example M.rosenbergii larvae require brackish water for development within 3 days of hatching (Ling and Merican,1964; Ling 1969). Also, there is considerable variation in larval devlopment of M.rosenbergii obtained from single spawns and raised under identical conditions (section 1.6). Therefore in the present study the term "egg quality" is restricted to viability of eggs.

4.1.2.1 Egg size

Size is the most commonly studied physical property of eggs and which has been widely reported to influence the quality of the larvae and in some cases the quality of eggs (Blaxter,1969; Galkina,1970; Lyagina,1975; Barton,1981; Springate,1985; Rana,1986).

Egg size of a species is under genetic control and is also determined phenotypically (Raven,1961) and by environmental factors (citing several authors, Blaxter,1969) such as quality and quantity of food (review, Chapter.7.) temperature (fish, Sillago sihama Lee,1981; barnacles, Crisp and Patal,1969) and salinity (Lee,1981; decapod, M.nipponence, Mashiko,1983).

Several studies have established that the egg size of fish varies with size and age of the female (Blaxter,1969; Galkina, 1970; Lyagina,1975; Barton,1981; Springate, 1985;

Rana,1986). In contrast some believe that egg size is not related to either age or size of parental fish (citing several authors, Galkina,1970). It is well documented; in fishes, that bigger eggs generally produce bigger larvae (Blaxter and Hempel,1963; Reagan and Conley,1977; Theilacker,1981; Springate,1985; Rana,1986).

There appears to be no information regarding egg size of cultured Crustaceans.

Egg size is generally measured as egg diameter, volume or wet/dry weight. Variations in egg size within individual egg clutches have been reported for fishes such as herring (Blaxter and Hempel,1963), Argentine anchovy (De Ciechomski,1966), salmon, rainbow trout (Galkina,1970) and Oreochromis sp. (Rana,1986).

Therefore in the present study the following aspects of egg size were evaluated;

1) size distribution of eggs within and between spawns of M.rosenbergii.

2) influence of growth parameters of M.rosenbergii broodstock on size distribution of eggs.

4.1.3. Chemical composition of eggs

Yolk provides raw materials and energy for the development of the embryo. Therefore the composition and manner in which these raw materials and energy are utilised, both quantitatively and qualitatively during development, is

of interest to workers involved in developmental biology. On the other hand there is growing interest among aquaculturists who often expect the content and composition of yolk to be the most likely determinant of egg quality. Considerable variations in quality of eggs have been reported among cultured aquatic animals (section.7.1).

The chemical composition of fish eggs has been found to vary greatly within and between species. Significant variations in free and bound lipids, precipitable proteins, protein and lipid phosphorus, calcium and iron contents of ripe rainbow trout eggs obtained from different females have been reported by Craik and Harvey (1984.b). Differences have been found in chemical composition of rainbow trout eggs obtained from individuals of the same stock (Springate,1985).

Females fed diets varying in nutritional quality have been found to produce eggs with varying chemical composition. Differences in protein (rainbow trout Satia, 1973), amino acid (common carp, Vladimirov,1974; Cited Love, 1980), lipid (Satia,1973), fatty acid (Shimma et al., 1977), mineral (rainbow trout, Hirao et al.,1954; brook trout, Luquet and Watanabe,1986), vitamin (rainbow trout, Sandnes et al.,1984) and carotenoid (salmon, Georgiev,1971, cited Love,1980; review, Mikulin and Soin,1975) levels in eggs have been reported from females fed different diets (see discussion in Chapter 7).

Studies concerned with the chemical composition of Natantia (Crustacea) eggs have principally focused on utilisation of raw materials and energy for embryonic development. There is hardly any information on amino acid, mineral and vitamin contents of eggs in these animals, except some information on the fatty acid composition of eggs of P.serratus (Martin,1978), and Pandalus montagui (Clarke, 1979).

Therefore, the aim of the present study was to;

(a) Obtain detailed information on chemical composition of M.rosenbergii eggs.

(b) Evaluate the possible influence of age and size of females on chemical quality of M.rosenbergii eggs.

It is very time consuming and laborious to isolate yolk from the ooplasm and determine its biochemical composition, especially for Caridean eggs interconnected with membranes. Often the total biochemical composition of eggs is analysed and used as a measure of nutrient reserve assuming that the major part of the ooplasm is composed of yolk. In the present study the total biochemical composition of eggs was evaluated and not the yolk composition. Assessments based on studies of others were confined to the above category, where possible, unless otherwise stated.

4.1.4. Influence of broodstock age and size on egg incubation period and nutrient reserve in the larvae.

Studies of Wickins and Beard (1974) indicated that bigger females produce bigger larvae under stable conditions. Mashiko (1987) found that bigger eggs took longer for embryonic development than smaller eggs in two populations of Palaemon paucidense.

Therefore possible influences of M.rosenbergii broodstock age and size on incubation period of eggs and larval nutrient reserve were also evaluated.

4.2. Materials and Methods

About 50 M.rosenbergii females obtained from both stocks. A and B (see Chapter 2 for details), 25 females from each, were used in this study. Reproductive performances were studied over a period of one year. This involved a total of approximately 450 moults and 250 spawns, during which some females were observed over 16 consecutive moults.

The experimental system used in the study involved two recirculated water systems S_1 and S_2 described in Chapter 2. In both systems the environmental conditions were similar. Mainteinance of the system, feeding regime, mating and sampling of eggs, gravimetric (as wet, dry weights) and numeric estimation (number of eggs) of quantitative egg production, estimation of egg volume, and measurement of growth parameters of broodstock were as reported in Chapter 2.

Studies involving the influence of age of broodstock on quantitative egg production were carried out on broodstock B. Age refers to the time interval from the day of hatching of eggs in weeks. The rest of the analyses of egg production were based on data obtained from both stocks A and B.

Analyses of chemical composition of eggs were carried out separately on individual spawns except for a "pooled group". The "pooled group" refers to a mixture of egg clutches (24hr.old after spawning) obtained from 15 randomly selected females (including females other than stocks A and B) and pooled together into a single sample. The composition of these pooled egg samples was used to indicate the general composition of M.rosenbergii eggs, avoiding any possible maternal influences (either genetic or age and size) to facilitate comparison with other Crustacean eggs. The egg samples used in the study involving influence of age were 48 hr. post spawning (see Chapter 2. for problems associated with earlier sampling).

Methods used to analyse composition of eggs were as reported in Chapter.2. except for minerals. Triplicate samples were digested with 2.5ml. c.HNO_3 in a dry hot-block for 1hr. The digested samples were filtered and diluted with double-distilled water to 25ml. and analysed as described in Chapter.2. This method of digestion was adopted due to practical problems associated with the method described in Chaptr.2. such as ashing egg samples (see Chapter 2), and a lengthy procedure is vulnerable to sample losses and high

losses and high variations between replicates (see results).

Random egg samples obtained from females ranging in size from 17-40 mm in carapace length, belonging to stocks A and B, were used to study the possible influence of maternal size on egg incubation period and nutrient reserve in the larvae.

The experimental system and procedures used to estimate incubation period and larval survival under starvation are described in Chapter 2.

The utilisable nutrient reserve at hatching, in the larvae, is assumed to be proportional to the survival of unfed larvae in the present study.

4.3 Results

4.3.1.1 Relationships between numbers of eggs and wet and dry weights of egg clutches in M.rosenbergii.

There was a significant ($P<0.05$) degree of association between wet and dry weights of egg clutches (Fig:4.1a.b). This was reflected by constant moisture levels found in egg clutches irrespective of their weight (Fig:4.1c). There was no correlation between the moisture contents of eggs and the weights of egg clutches. Therefore, dry weights of egg clutches can be predicted by the equation in Fig:4.1a. (within the weight range used in the study).

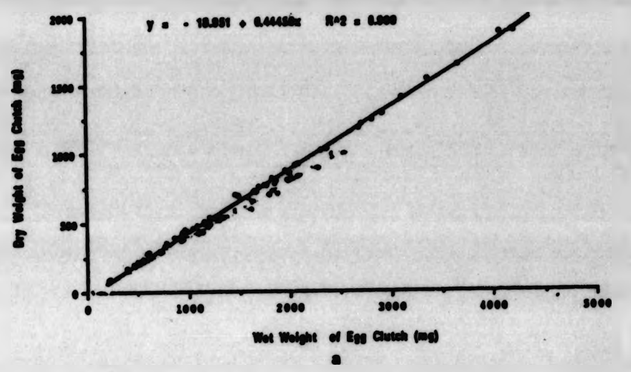
The degree of association between the numbers of eggs in egg clutches (fecundity) and weight (wet and dry) of egg clutches are presented in Fig:4.2a.b. There were significant ($P<0.05$), positive, linear relationships between fecundity and wet and dry weights of egg clutches. The numbers of eggs in an egg clutch were found to be more closely related to the dry weight ($r^2=.928$) than wet weight of the clutch ($r^2=.918$). Therefore, fecundity could be predicted by the wet weight of the egg clutch by using the equation presented in Fig:4.2a.

4.3.1.2 Influence of broodstock age and size on quantitative egg production in M.rosenbergii.

The relationships between age, size and quantitative egg production of M.rosenbergii broodstock (from a single spawn) are presented in Fig:4.3.

Fig:4.1.a.b. Relationship between wet and dry weights of egg clutches produced by M.rosenbergii broodstock (DF= 74 P<0.05).

Fig:4.1.c. Relationship between weight of egg clutch and moisture contents of egg clutches (DF=75 P>0.05)



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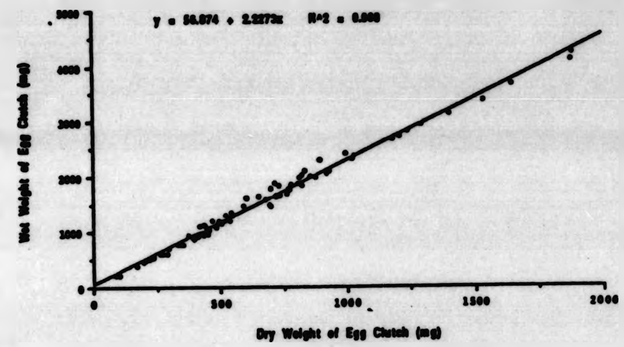

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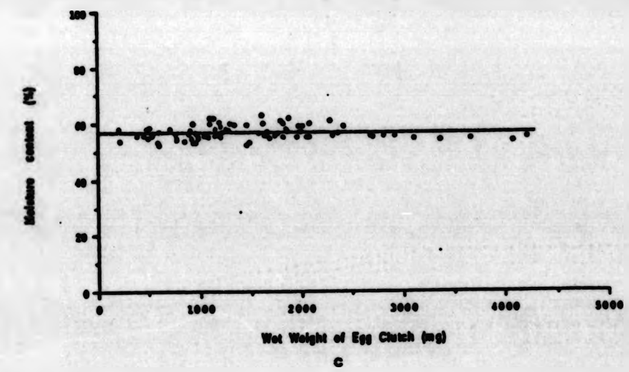
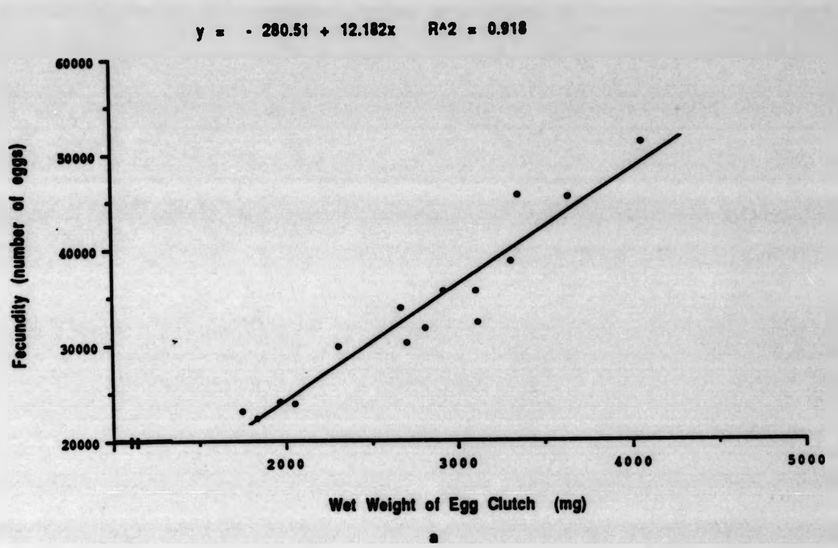


c

Fig:4.2.a. Relationship between wet weight of egg clutch and fecundity (number of eggs per clutch) (DF=14 P<0.05).

Fig:4.2.b. Relationship between dry weight of egg clutch and fecundity (number of eggs per clutch) (DF=14 P<0.05).



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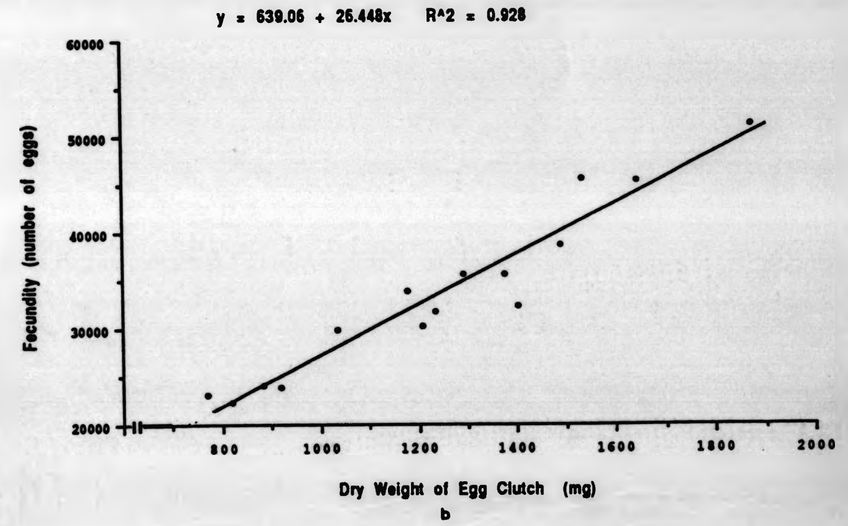


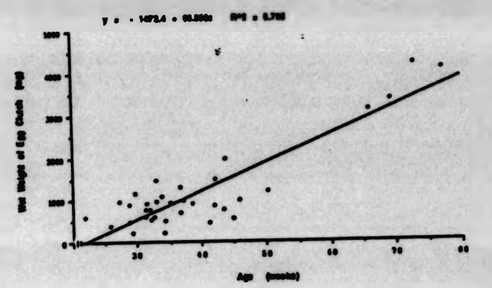
b

Fig:4.3.a Relationship between age of broodstock M.rosenbergii and egg production as wet weight of egg clutch (DF=36 P<0.05).

Fig:4.3.b. Relationship between carapace length of broodstock M.rosenbergii and egg production as wet weight of egg clutch (DF= 35 P<0.05).

Fig:4.3.c. Relationship between age of broodstock M.rosenbergii and egg production as dry weight of egg clutch (DF= 36 P<0.05).

Fig:4.3.d. Relationship between carapace length of broodstock M.rosenbergii and egg production as dry weight of egg clutch (DF=35 P<0.05)



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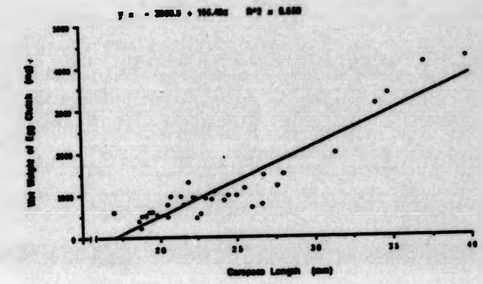

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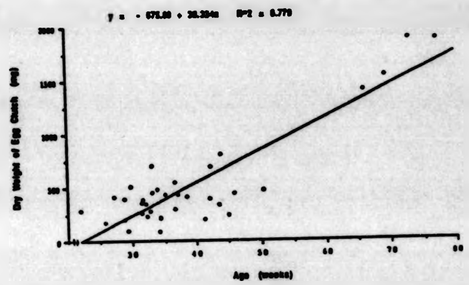

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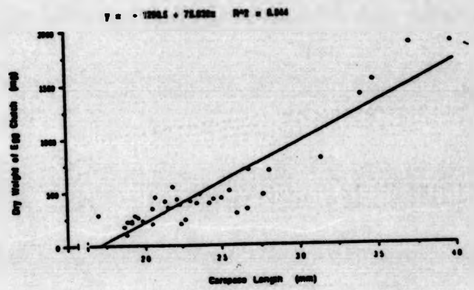


d

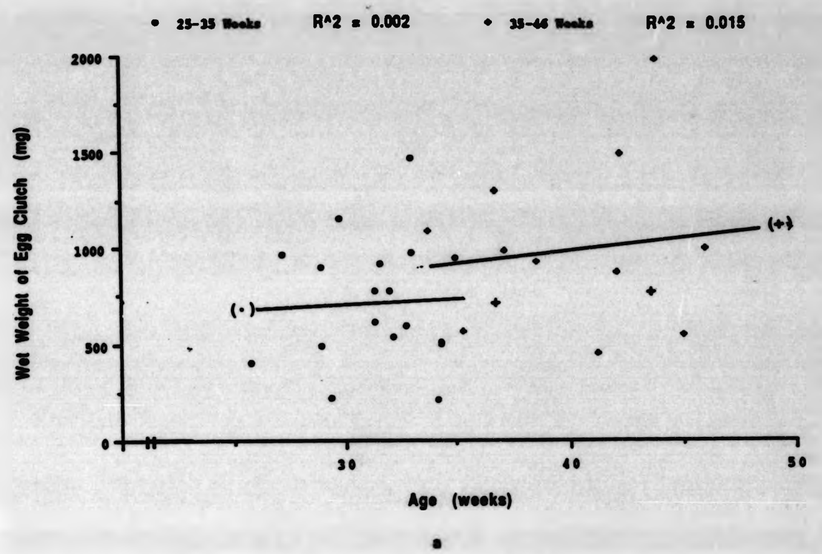
A significant ($P<0.05$), positive, linear increase in egg production with increase in age and growth parameters of the broodstock was evident. Growth parameters of broodstock and egg production were more highly correlated (r^2 values) than broodstock age and egg production.

When egg production of individuals belonging to the above population were grouped into two age groups the association between egg production and age of broodstock within the age group (mixed size) were insignificant ($P>0.05$) (Fig:4.4a). Whilst the egg production of individuals belonging to the above age group (25-35 weeks) was correlated with size of the animal. A significant ($P<0.05$), positive, relationship exists (Fig:4.4b). This indicates that egg production of a particular age group is more dependent on size of broodstock than on age.

The greater dependence of egg production on growth parameters than on broodstok age was further evident when the weights of egg clutches were grouped into two classes based on size (carapace length) of the broodstock (mixed age groups). Weights of eggs produced by both groups increased significantly ($P<0.05$) with increase in size of broodstock, within maternal size groups (Fig:4.5b). No relationship existed between the weight of eggs and the age of the broodstock within both maternal size groups (Fig.4.5a).

Fig:4.4.a Relationship between age of broodstock M.rosenbergii (belonging to age groups 25-35 weeks and 35-46 weeks) and wet weight of egg clutch (25-35 weeks. DF=18 P>0.05) (35-46 weeks. DF=14 P>0.05).

Fig:4.4.b. Relationship between the carapace length of broodstock M.rosenbergii (belonging to age group 25-35 weeks) and wet weight of egg clutch (25-35 weeks DF=18 P<0.05).



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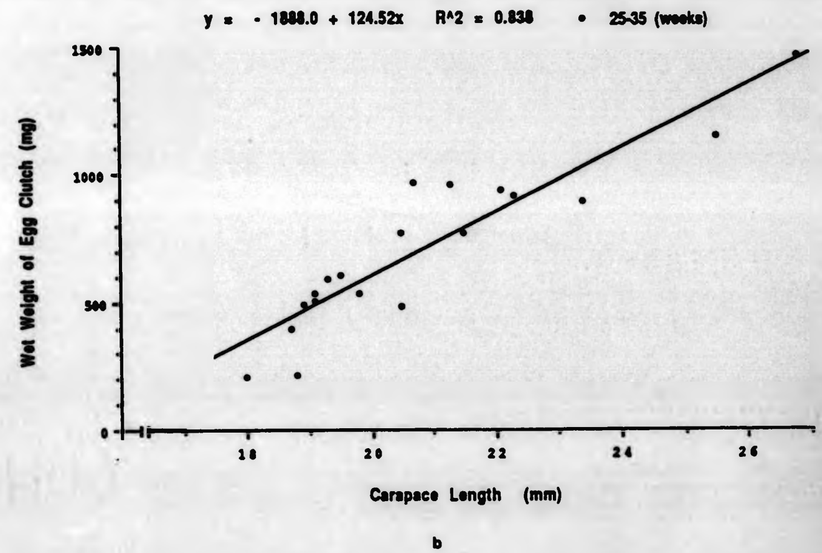
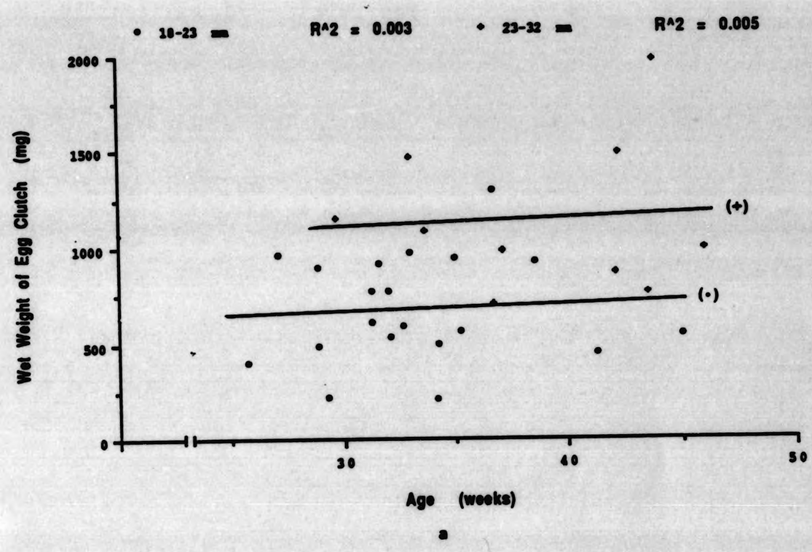


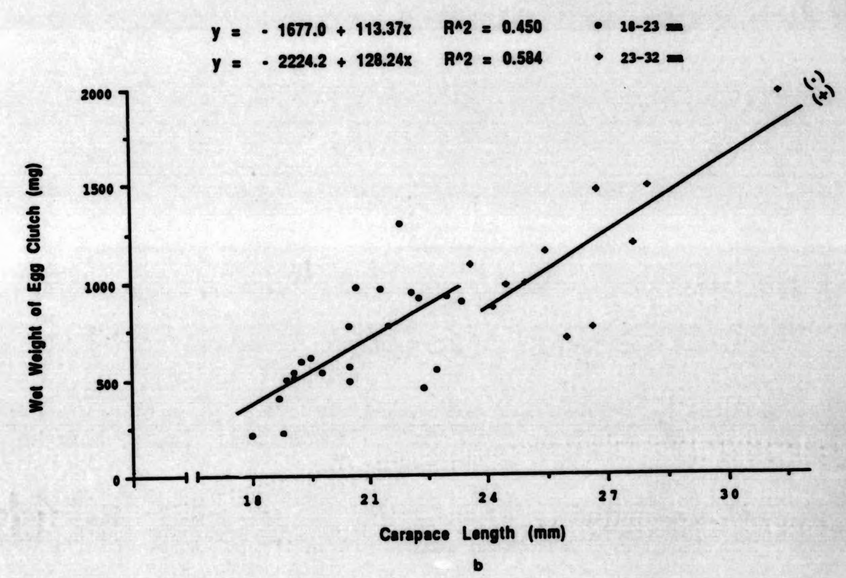
b

Fig:4.5.a. Relationship between age of broodstock M.rosenbergii (belonging to female size groups 18-23 mm and 23-32mm) and wet weight of egg clutch (18-23mm size group P>0.05, 23-32mm size group P>0.05).



a

Fig:4.5.b. Relationship between carapace length of broodstock M.rosenbergii (belonging to female size groups 18-23mm and 23-32mm) and wet weight of egg clutch (18-23mm group P<0.05,23-32mm group P<0.05).



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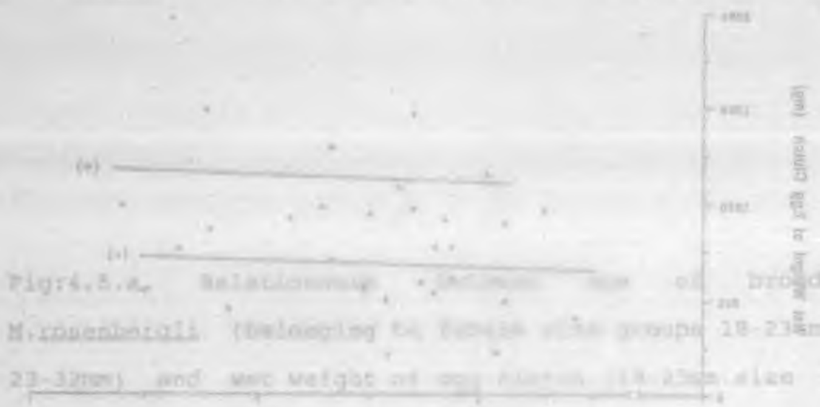
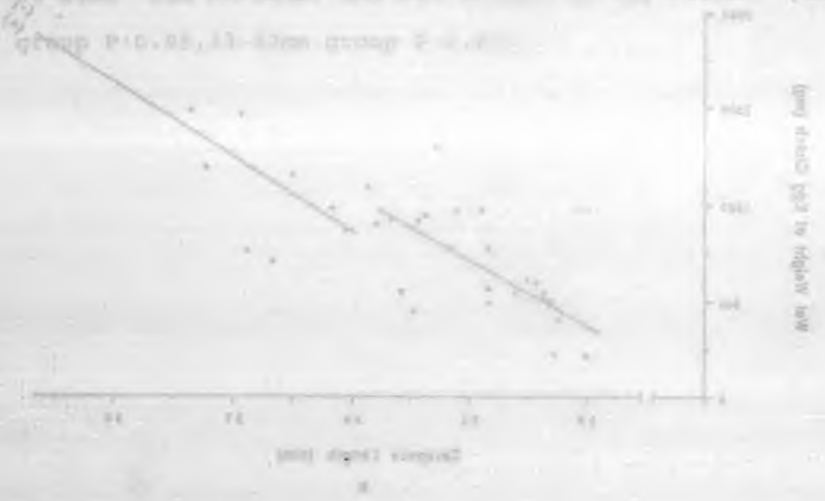




The above observations clearly indicate that the egg production of particular ages or size groups of M.rosenbergii is more dependent on broodstock size and that the influence of age is masked by size.

Due to the higher degree of association between growth parameters of broodstock and egg production than on age, all subequent evaluation of egg production was based on growth parameters of individuals. Data from all broodstock (stock A and B) were pooled to evaluate and predict egg production.

4.3.1.3 Influence of growth parameters of broodstock on egg production of M.rosenbergii.

Significant ($P<0.05$) increases in egg production were evident with increased broodstock size in M.rosenbergii. The nature and degree of association varied depending on the parameters used to evaluate both egg production and physical dimensions of broodstock (Fig:4.6, 4.7 and 4.8).

A higher degree of association existed between wet weight of the egg clutch and carapace length of broodstock ($r^2=.858$) than of weight ($r^2=.842$) followed by total length ($r^2=.763$) of the broodstock. The relationships between egg production and broodstock weight was linear (Fig:4.7). Whilest, curvilinear relationship existed between egg production and carapace, total lengths of broodstock (Fig:4.6). The nature of the relationships between egg production and different growth parameters

Fig:4.6. Relationship between carapace length of broodstock M.rosenbergii and wet weight of egg clutch (DF= 76 P<0.05)

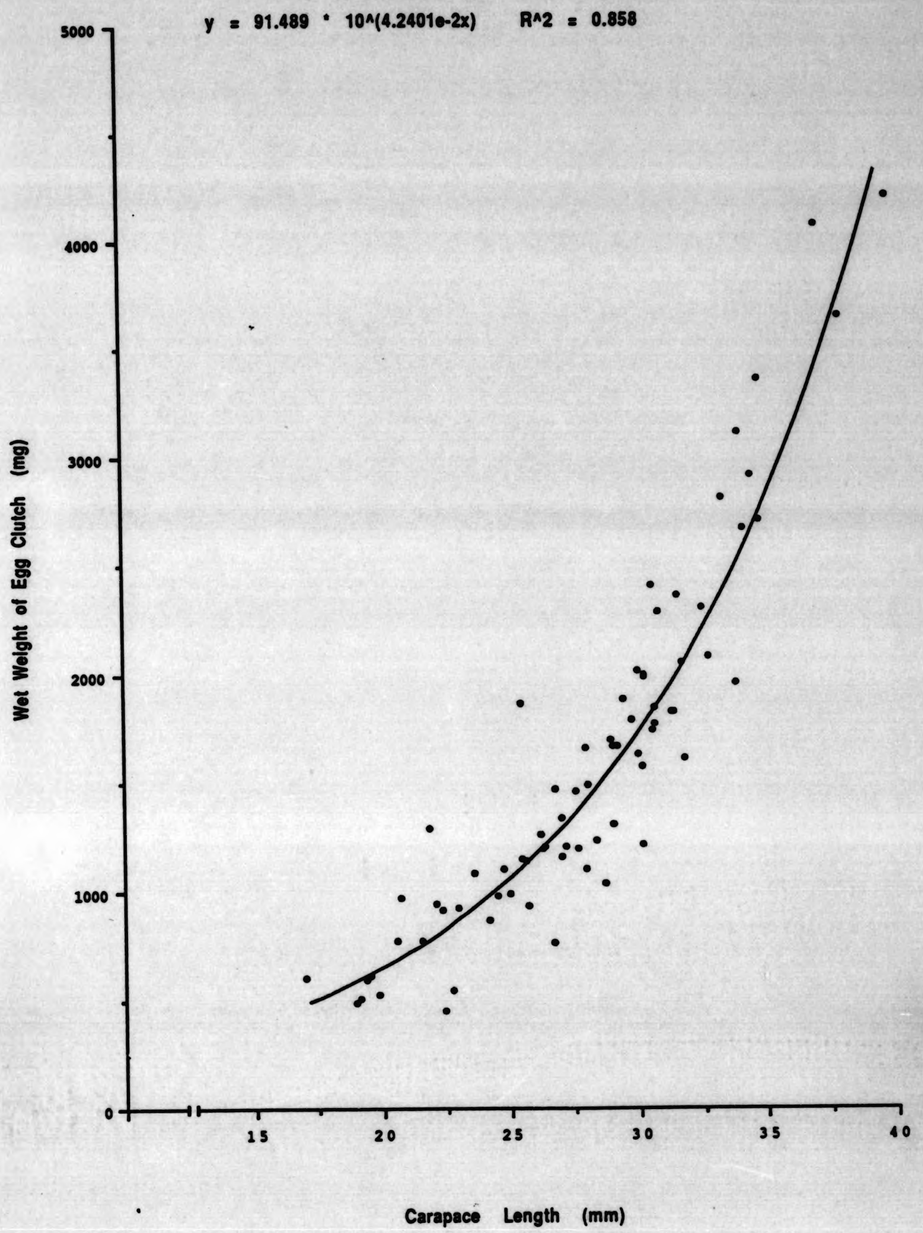
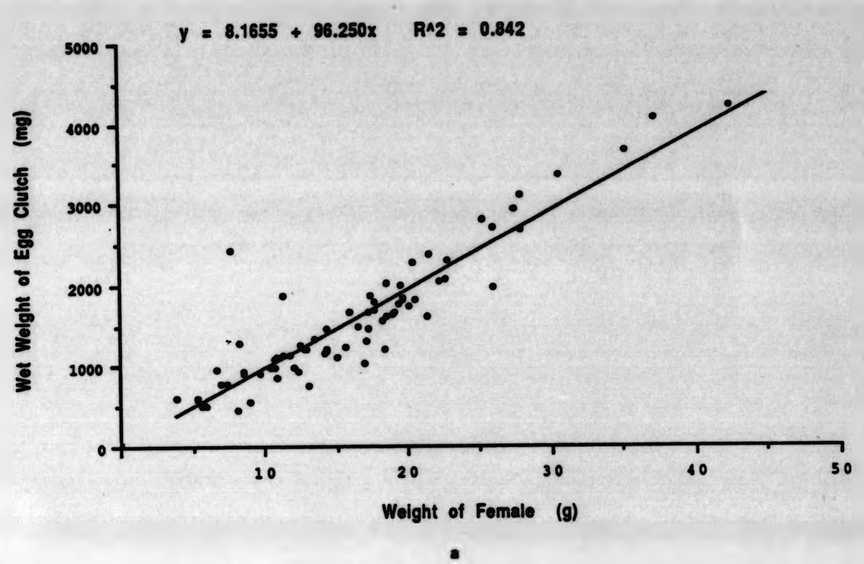


Fig:4.7.a. Relationship between weight of broodstock M.rosenbergii and wet weight of egg clutch (DF= 76 P<0.05).

Fig:4.7.b. Relationship between total length of broodstock M.rosenbergii and wet weight of egg clutch (DF=76 P<0.05).



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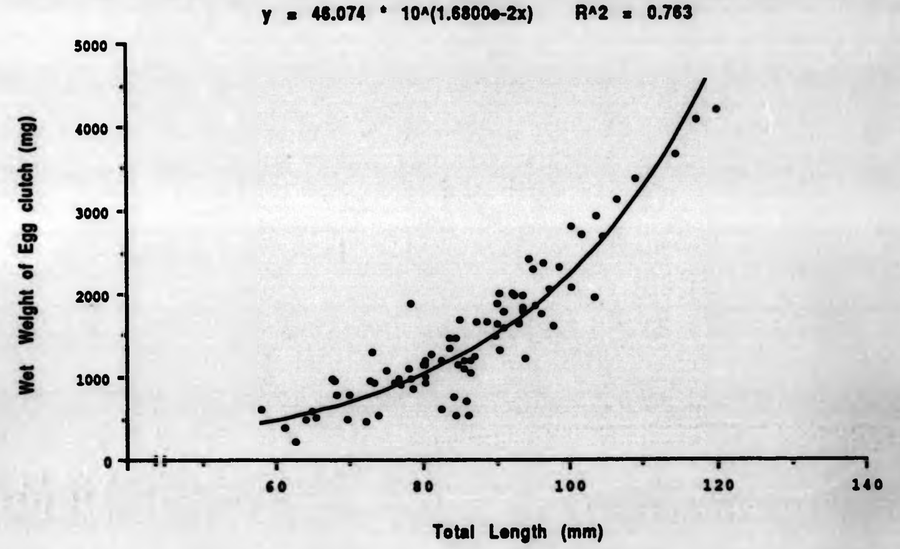
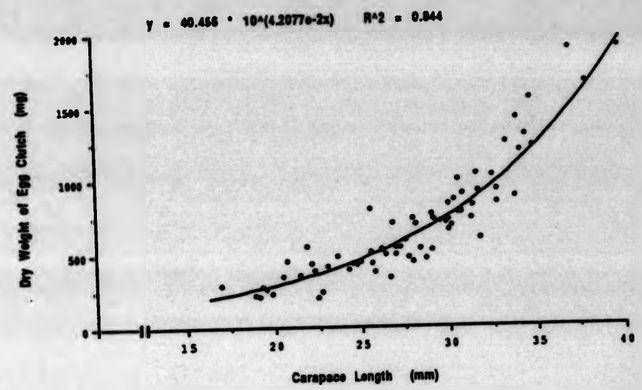


b

Fig:4.8.a. Relationship between carapace length of broodstock M.rosenbergii and dry weight of egg clutch (DF=76 P<0.05).

Fig:4.8.b. Relationship between weight of broodstock M.rosenbergii and dry weight of egg clutch (DF=76 P<0.05).

Fig:4.8.c. Relationship between total length of broodstock M.rosenbergii and dry weight of egg clutch (DF= 76 P<0.05).



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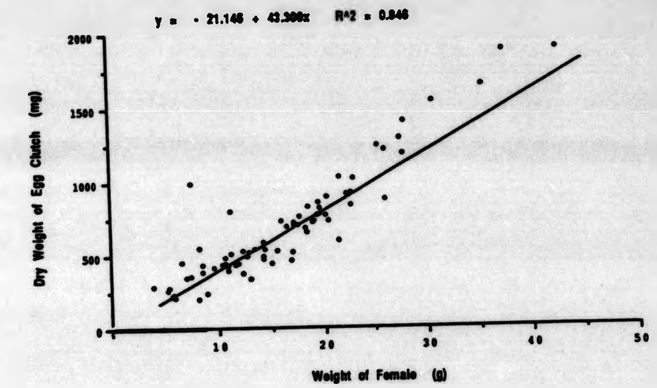

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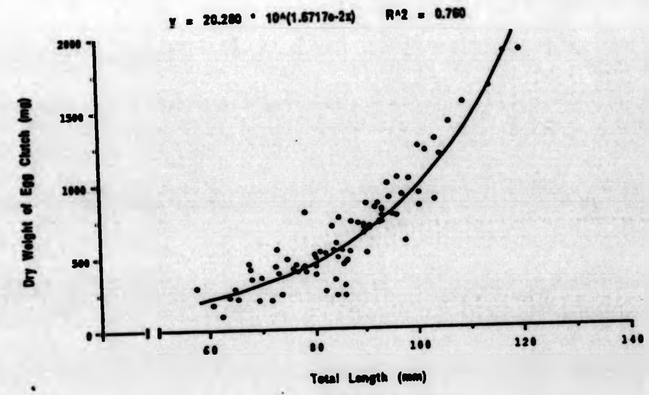

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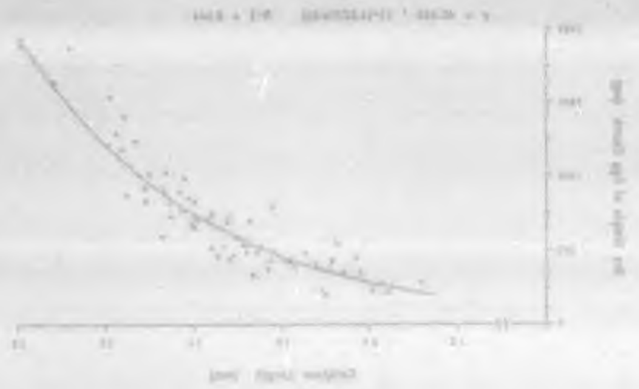


Fig:4.8.a. Relationship between carapace length of broodstock M.rosenbergii and dry weight of egg clutches (0<r<1 P<0.05).

Fig:4.8.b. Relationship between weight per broodstock M.rosenbergii and dry weight of egg clutch (0<r<1 P<0.05).

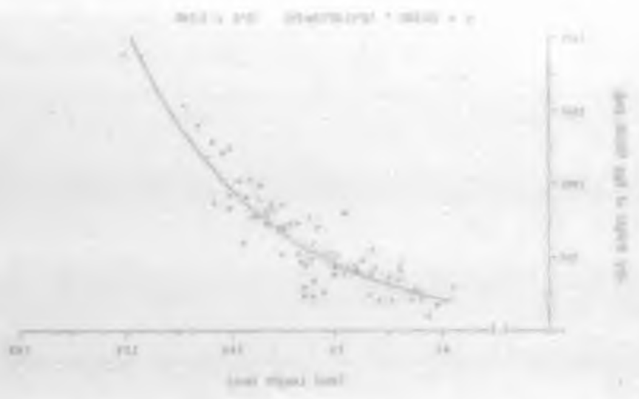


Fig:4.8.c. Relationship between total length of broodstock M.rosenbergii and dry weight of egg clutch (0<r<1 P<0.05).

remained unchanged irrespective of wet or dry weights of the egg clutches.

The greater influence of carapace length on egg production was also evident when different combinations of growth parameters were considered together (Table:4.2). None of the combinations improved the degree of association (r^2 value) with the egg production over that obtained with carapace length alone (r^2=0.858). Therefore carapace length is a better predictor of egg production than weight or total length of the broodstock.

4.3.1.4 Influence of size of broodstock on relative egg production in M.rosenbergii.

There was no significant (P<0.05) (Fig:4.9) association between body weight of broodstock and relative egg production, when expressed as milligram of egg clutch produced per gram body weight of broodstock. Interestingly positive, and linear, significant (P<0.05) relationships existed between carapace and total lengths of broodstock, and relative egg production, when expressed as milligram of egg clutch produced per millimeter of carapace and total lengths respectively.

Table:4.2. Regression models to predict egg production in *M.rosenbergii* using growth parameters of broodstock.

(Egg production $Y = a + b_{cl} + b_{tl} + b_{wt}$)

Egg production	Intercept	b_{cl}	b_{tl}	b_{wt}	r^2	n
Wet weight of egg clutch (mg)	-3111.9	79.2 ±30.6	28.4 ±11.8	-	0.81	76
	-670.2	37.3 ±24.5	-	74.1 ±15.4	0.85	76
	-997.7	-	16.1 ±8.7	71.7 ±16.1	0.85	76
	-1057.3	14.8 ±31.6	12.7 ±11.3	68.0 ±16.1	0.85	76
Dry weight of egg clutch (mg)	-1435.7	28.6 ±14.5	15.2 ±5.6	-	0.79	75
	-96.7	4.2 ±11.2	-	40.8 ±7.0	0.85	75
	-321.3	-	4.8 ±3.1	36.0 ±6.4	0.85	75
	-292.5	-7.1 ±14.3	6.4 ±5.1	37.8 ±7.4	0.85	75
Fecundity (Number of eggs)	-8929.9	984.1 ±1391.9	+851.9 ±428.8	-	0.85	14
	-11546.9	51.3 ±1535.1	-	1585.2 ±652.9	0.86	14
	-1565.6	52.6 ±1605.5	13.8 ±853.4	1565.6 ±1388.8	0.86	14

CL Carapace length TL Total length Wt Wet weight of female

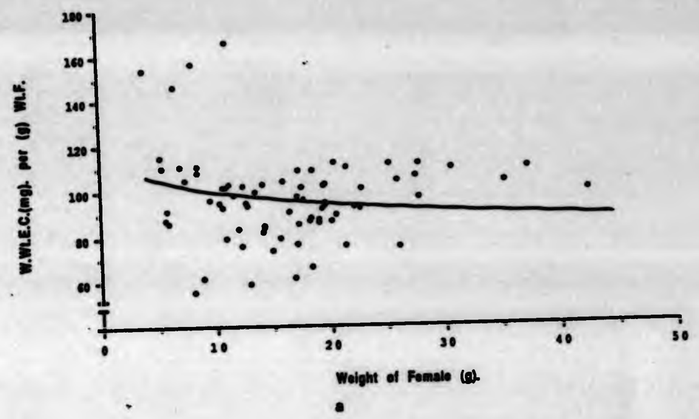
± Standard error r^2 Coefficient of determination

n degrees of freedom

Fig:4.9.a. Relationship between weight of broodstock M.rosenbergii and relative egg production as weight of egg clutch per (g) body weight of female (W.Wt.E.C.= wet weight of egg clutch. Wt.F= weight of female. DF=76 P>0.05).

4.9.b. Relationship between carapace length of broodstock M.rosenbergii and relative egg production as weight of egg clutch per mm carapace length of female.(W.Wt.E.C = wet weight of egg clutch. CL= carapace length of female. DF= 76 P>0.05)

Fig:4.9.c. Relationship between total length of broodstock M.rosenbergii and relative egg production as weight of egg clutch per mm total length of female (W.Wt.E.C.= Wet weight of egg clutch. TL= Total length of female. DF= 76 P<0.05).



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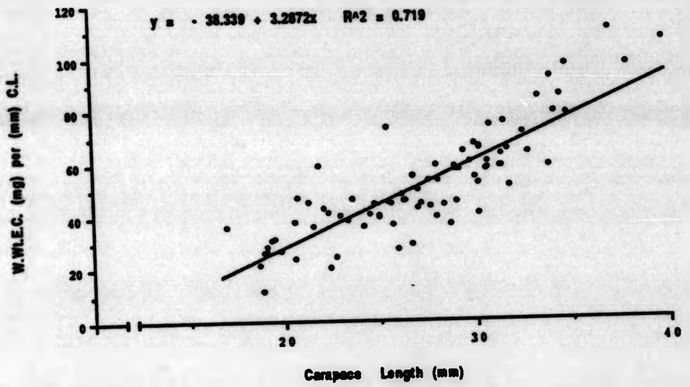
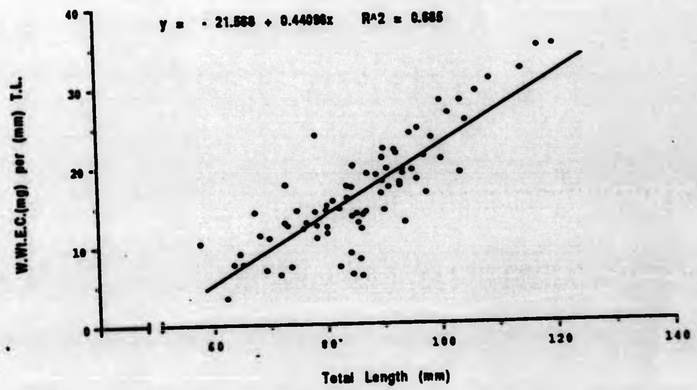


b



c

4.3.2.1 Variation in egg size within egg clutches in M.rosenbergii.

The individual egg sizes (measured as egg volume) within egg clutches of M.rosenbergii were normally distributed (Fig:4.10). The variations in egg size within the egg clutches (measured as percentage mean coefficient of variation) were asymmetrically distributed (Fig.4.11). 80 % of the egg clutches studied were found to have intra-egg clutch variations of <6.75% (as % CV) with the mean being 5.58 % .

4.3.2.2 Variations in egg size betbeen egg clutches in M.rosenbergii.

Mean egg sizes (measured as egg volume) of individual egg clutches produced by different M.rosenbergii broodstock are presented in Fig:4.12. Mean egg size varied significantly ($P<0.05$) between different egg clutches.

A significant, positive ($P<0.05$), linear correlation existed between mean egg size of different egg clutches and growth parameters of broodstock (Fig:4.12).

When mean egg sizes of egg clutches were classified into three classes based on the size of the broodstock, significant differences ($P<0.05$) were evident between the means of egg size belonging to two extreme parental size classes (Table:4.3). This suggests that bigger broodstock produced larger eggs than smaller ones within the parental

Fig:4.10 Normal distribution of egg size (as egg volume) within egg clutch of a spawn of M.rosenbergii (number of samples = 50)

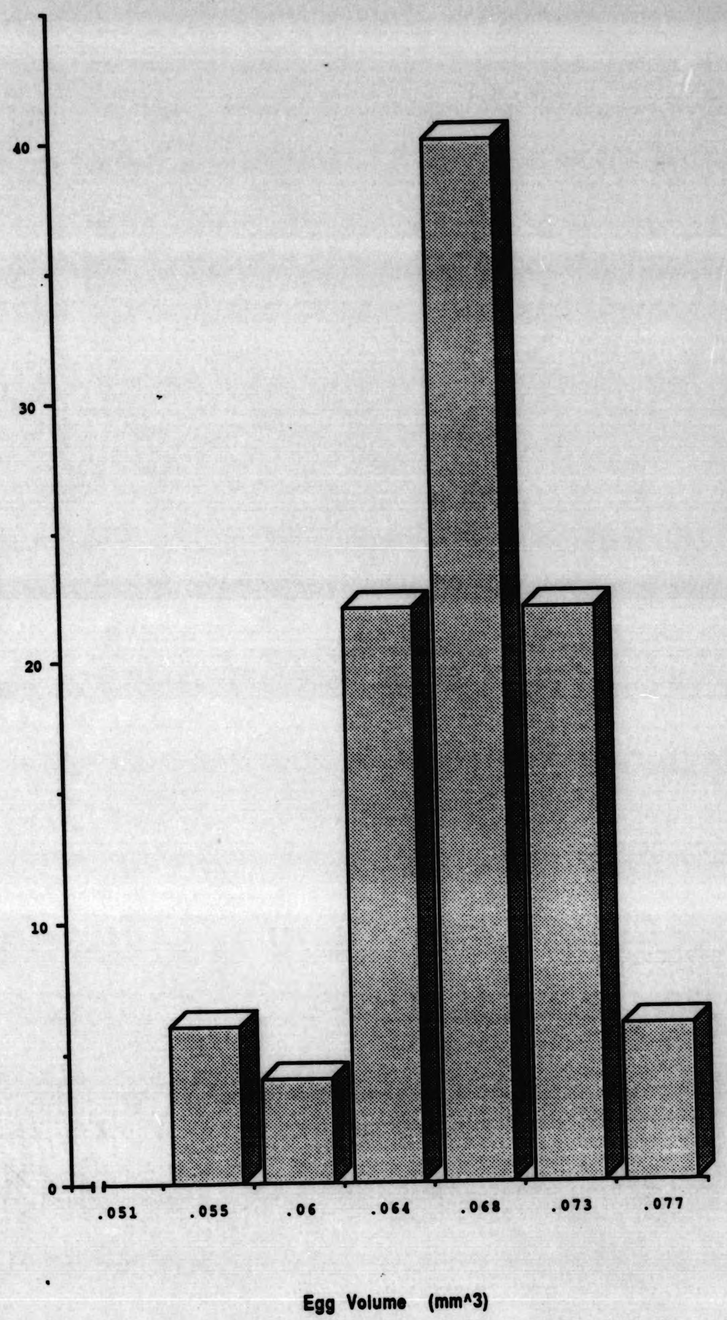


Fig:4.11 Cumulative frequency distribution of the variation of egg size within egg clutches (number of samples = 25).

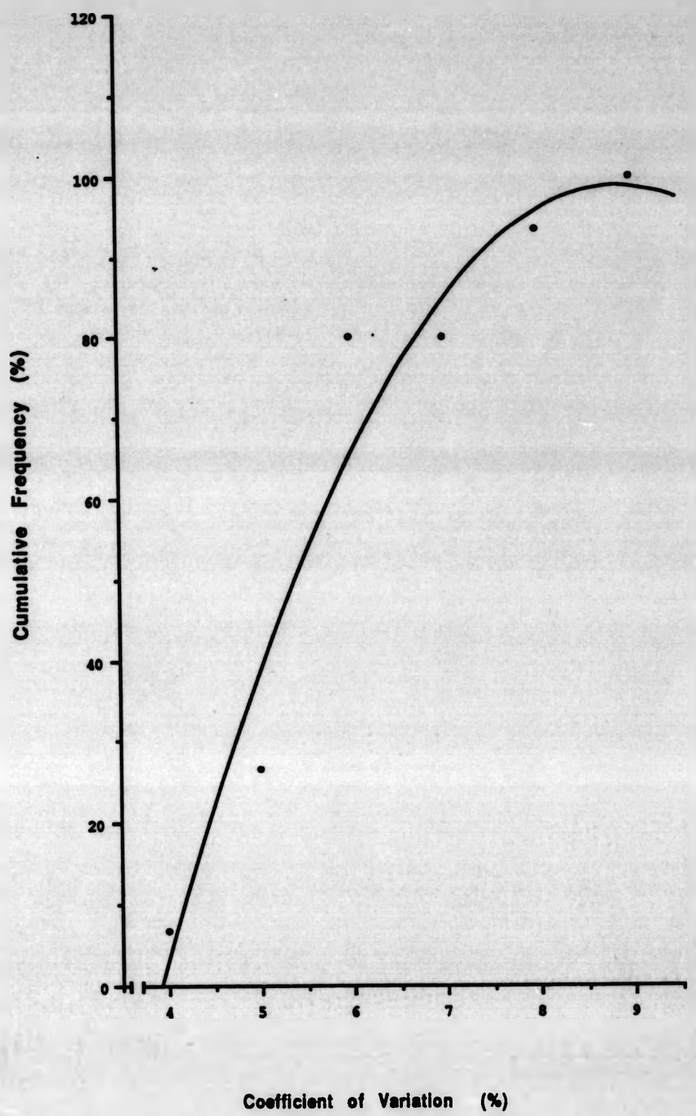
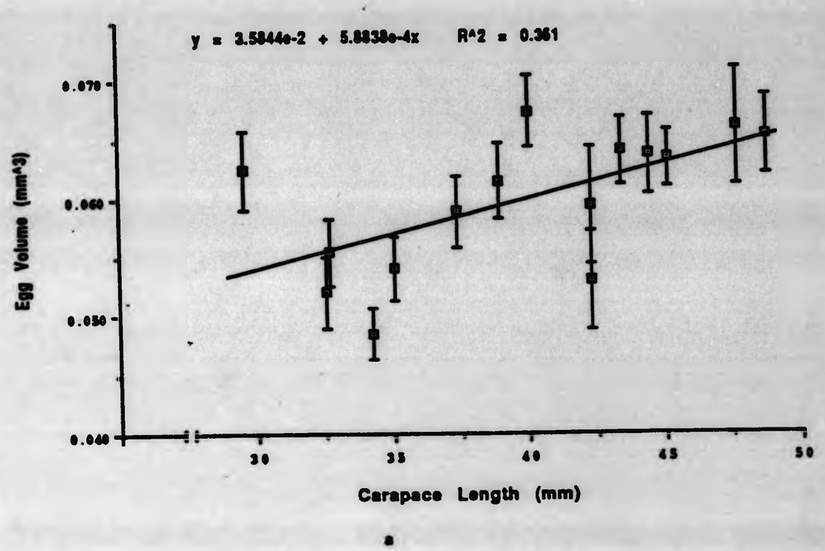
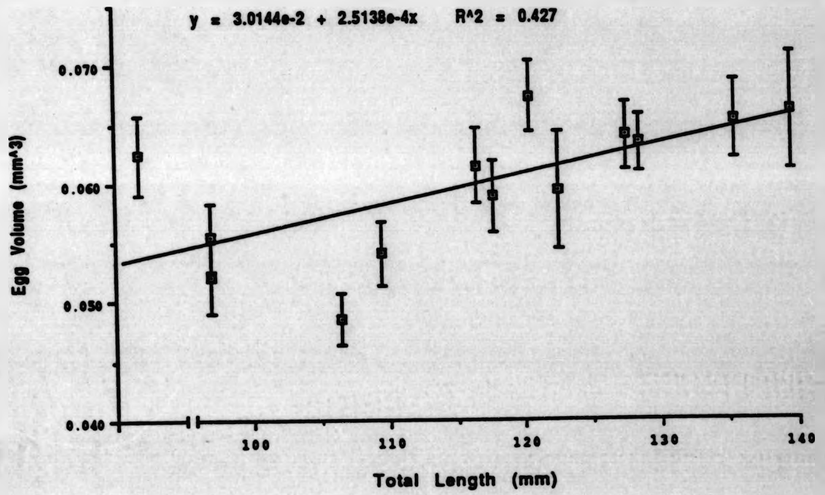


Fig:4.12.a. Relationship between egg size (as egg volume) and carapace length of broodstock M.rosenbergii (n=15 P<0.05)

Fig:4.12.b. Relationship between egg size (expressed as egg volume) and total length of broodstock M.rosenbergii (n=13 P<0.05)



a



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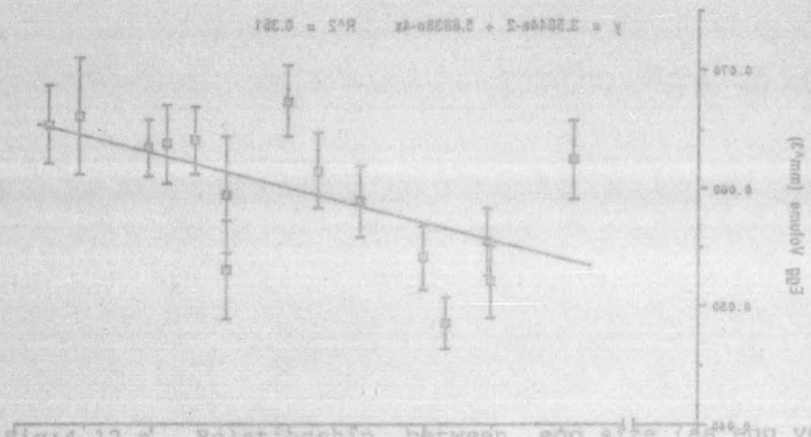


Fig:4.12.a. Relationship between egg size (as egg volume) and carapace length of broodstock M.rosenbergii (n=15 P<0.05)

Fig:4.12.b. Relationship between egg size (expressed as egg volume) and total length of broodstock M.rosenbergii (n=15 P<0.05)

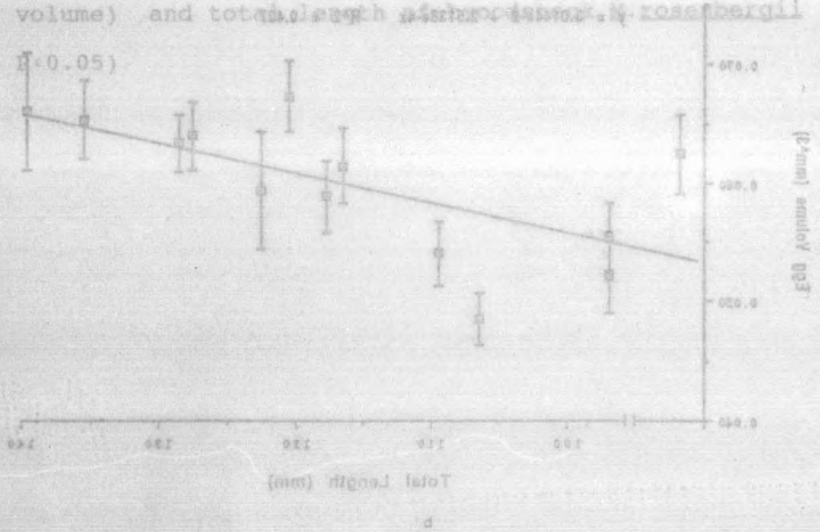


Table:4.3. Influence of size of broodstock on size of eggs (volume, mm^3) of M.rosenbergii.

	Broodstock size groups Carapace length (mm)		
	32-35	35-42	42-49
Mean	0.054^{b}	0.059^{ab}	0.064^{a}
Standard deviation	±0.005	±0.005	±0.001
Coefficient of Variation (%)	9.47	8.65	1.78

Values having the same superscript are not significantly different by ANOVA (P>0.05)

size range used in this study. The variations in egg size (as %CV) within the parental size classes were higher (9.47%) for the smaller size group than for the bigger ones (1.78%). This indicates that the mean egg sizes of bigger broodstock were more uniform than of smaller broodstock used in this study.

4.3.3. Chemical composition of M.rosenbergii eggs and influence of age of broodstock.

The proximate compositions of M.rosenbergii eggs are presented in Table:4.4 together with published data on the egg composition of some carideans (Crustacea). Dry matter contents of M.rosenbergii eggs were approximately 45% of the total weight of the egg clutch, as in most of the carideans.

Protein constituted about 55% of the total dry weight of M.rosenbergii eggs followed by lipid (35%). The moisture, protein, lipid and amino acid contents of the egg clutches obtained from broodstock belonging to different age groups are presented in Table:4.5 and 4.6. Except for small variations in lipid, content of eggs was not affected by age of broodstock.

The amino acid contents of M.rosenbergii eggs were predominated by aspartic and glutamic acids. Among essential amino acids arginine, leucine, valine and lysine levels were higher than the rest. Cystine, methionine and histidine were found at very low levels.

Table:4.4. Chemical Composition of some Caridea (Crustacea) eggs.

(expressed as % dry weight except for moisture)

Animals Source	Moisture	Total Nitrogen	Total Carbon	Protein	Lipid	Carbo-hydrate	Ash	K.Cal
Macrobrachium rosenbergii (Fresh water)	56.05^{fd} ±2.23	9.43 ±0.17	55.57 ±1.03	57.89^{L} 58.96^{k}	34.41^{f} ±0.09	-	-	-
Macrobrachium idella[1] (Estuarine)	56.16	-	-	80.00^{b}	12.32^{f}	4.39	-	6.23
Macrobrachium lamarrei[2] (Fresh water)	55.65 ±2.01	-	-	44.40*	52.50^{s} ±2.29	-	3.10 ±0.12	6.33 ±0.34
Palaemon serratus[3] (Marine)	-	-	-	-	30.30^{bd}	-	-	-
Caridae weberi[4] (Fresh water)	57.80 ±4.79	-	-	51.91*	44.30^{S} ±1.29	1.26 ±0.25	2.50 ±0.54	-
Crangon crangon[5]	83.37	-	-	58.70^{k}	32.60*	8.20	-	5.92
Caridina nilotica[6] (Fresh water)	61.30 ±5.33	-	-	28.80^{b} ±1.56	68.80^{S} ±2.54	-	2.40 ±0.04	1.49? ±0.32

b Biuret method. k Kjeldhal method. L Lowry method.

f Folch et al., 1957. bd Bligh & Dyer,1959. S Soxhlet method.

* 100 - rest of the components. ? very low reported value.

fd Freeze dried. - Not reported.

1. Vijayaraghavan and Easterson,1974.
2. Martin,1978.
3. Shakuntala,1976.
4. Rao et al.,1981.
5. Pandian,1967.
6. Ponnuchamy,1979.

Table:4.5. Influence of age of M.rosenbergii broodstock on moisture, protein and aminoacid content of eggs. (expressed as % protein)

Aminoacids	Age groups (weeks) 45-50 (2)	66-70 (2)	77 (1)	Pooled
Moisture	55.24	54.34	53.97	56.05
Protein	54.08	53.86	55.98	57.89
Aspartic acid	9.01	9.13	9.32	8.89
Treonine *	4.29	4.55	4.03	3.89
Serine	4.60	3.90	4.40	4.26
Glutamic acid	9.66	9.93	9.66	9.91
Proline	2.46	4.77 ±1.80	1.83	2.61 0.57
Glycine	3.93	4.16	4.01	3.92
Alanine	3.19	3.90	3.46	3.37
Valine *	6.01	6.03	5.25	5.08
Cystine	0.71 ±0.25	1.80	0.82	1.01 ±0.19
Methionine *	1.46	3.09 ±0.89	1.29	1.76 ±0.30
Isoleucine *	5.15	5.18	4.39	4.25
Leucine *	7.35	7.00	6.92	6.63
Tyrosine *	2.78	2.83	2.46	2.58
Phenylalanine *	4.37	4.54	3.82	3.64
Histidine *	2.58	3.19	2.01	2.61
Lysine *	5.64	7.39	5.43	5.75
Arginine *	6.79	6.18	7.19	7.56
Tryptophan *	Not determined			

* EAA Essential Aminoacids. () number of samples.

± Standard deviation, values having standard deviation less than 7% of the mean were not indicated due to simplicity.

Pooled see section 4.2 for details.

Table:4.6. Influence of age of M.rosenbergii broodstock on lipid class composition of eggs.

(Expressed as % total lipid)

Lipid Classes	Age groups (weeks) 45-50 (2)	66-70 (2)	77 (1)	Pooled
Total polar lipids	20.3	22.2 ±1.4	22.2	21.0
Total neutral lipids	80.0	77.8	77.8	79 0
Triacyglycerol	69.0	66.9	67.0	68.8
Cholesterol	9.5 ±1.0	10.0 ±2.8	10.2	9.4 ±1.8
Free fatty acids	1.2 ±0.5	1.0 ±0.6	1.0	0.7 0.3
Sterol esters	Not detected			
Diacylglycerols	Not detected			
Monoacylglycaerols	Not detected			
Fatty alcohols	Not detected			
Wax esters	Not detected			
Phosphatidylcholine	13.1	13.1	12.7	11.3 ±0.8
Phosphatidylethanolamine	6.9	8.2 ±1.6	9.6	9.2 ±1.4
Phosphatidylinositol	trace levels			
Phosphatidylserine	Not detected			
Sphingomyelin	Not detected			
Phosphatidic acid	Not detected			

± Standard deviation. Standard deviation less than 5% of the mean not indicated for simplicity.

trace levels < 0.5 % ( ) number of samples

The amino acid contents of M.rosenbergii eggs remained unchanged, irrespective of the age of the broodstock, except for fluctuations in proline, cystine, methionine and lysine (Table:4.5).

The lipid class compositions of M.rosenbergii eggs obtained from broodstock belonging to different age groups are presented in Table:4.6. M.rosenbergii eggs were dominated by neutral lipids, accounting for about 78% of the lipid, predominately triacylglycerols (TAG) followed by cholesterol. Sterol esters (SE) and free fatty acids (FFA) were detected at trace levels. Polar lipids were dominated by phosphatidylcholine (PC) and phosphatidylethanolamine (PE). A small amount of phosphatidylinisitol (PI) was also detected.

Lipid class composition of M.rosenbergii eggs was not influenced by age of broodstock except for low levels of PE in eggs from the youngest age group.

The fatty acid compositions of M.rosenbergii eggs, together with published data on some other decapods eggs and carcass composition of M.rosenbergii, are presented in the Table:4.7. The fatty acid composition of M. rosenbergii eggs were dominated by monoenes (18:1,16:1) followed by saturates (16:0,18:0) and PUFA ((20:5(n-3), 22:6(n-3), 18:2(n-6), 20:4 (n-6)). Many isomers were found at trace levels. The PUFA contents of M.rosenbergii egg lipids were dominated by n-3, about 61% of total PUFA.

Table:4.7. Fatty acid composition of some decapod (Crustacea) egg (expressed as % total lipid).

Fatty acids	Eggs			
	Macrobrachium rosenbegii	Penaeus serratus (1)	Penaeus vannamei (2)	Heterocarpus grimmaldii (3)
12:0	tr	0.5	0.2	0.2
14:0	2.1	2.9	1.7	3.0
15:0	0.7	1.0	nr	0.9
16:0	19.6	21.2	19.3	18.5
16:1(n-7)	7.0	14.0	6.9	18.5
16:2	0.5	0.4	nr	tr
17:0	0.8	1.3	nr	1.0
16:3	1.0	nr	nr	tr
16:4	1.0	nr	nr	tr
18:0	7.1	5.3	5.5	1.8
18:1(n-9)	26.3	27.7	13.4	30.2
18:1(n-7)	3.8	nr	4.8	nr
18:2(n-6)	4.2	2.6	3.4	0.6
18:2(n-9)	0.5	nr	nr	nr
18:3(n-6)	0.5	0.3	nr	0.3
18:3(n-3)	2.1	1.7	0.6	0.2
18:4(n-3)	1.5	nr	0.6	tr
20:1(n-9)	0.2	1.7	1.7	1.8
20:1(n-7)	0.9	nr	1.7	nr
20:2(n-6)	0.2	nr	0.8	tr
20:4(n-6)	1.7	3.1	1.7	4.3
20:4(n-3)	0.3	nr	0.2	0.5
20:5(n-3)	6.3	8.7	9.3	7.3
22:5(n-6)	0.1	nr	nr	tr
22:5(n-3)	1.1	tr	0.9	1.1
22:6(n-3)	5.0	5.6	14.3	7.5
T.Saturates	30.7	32.2	27.2	25.5
T.Monoenes	38.9	43.4	28.7	32.0
T. n-3	16.42	16.00	25.70	16.6
T. n-6	6.91	6.00	6.00	5.1
n-3/n-6	2.4	2.7	4.3	3.7
T.PUFA	27.2	22.0	31.7	21.7

tr Trace levels

nr not reported

1 Martin,1978.

2 Cahu et al.,1986.

3 Morris,1973.

The fatty acid composition of eggs produced by broodstock belonging to different age groups is presented in Table:4.8.Except for differences in 18:1(n-9),18:3(n-6), 18:3(n-3), 18:4(n-3) and 20:4(n-6) fatty acid composition remained fairly constant.

Calcium, magnesium, potasium, copper, iron and zinc contents of M.rosenbergii eggs produced by broodstock belonging to different age groups are presented in (Table:4.9). Apart from fluctuations in calcium and potasium levels, mineral levels remained relatively constant.

4.3.4 Influence of age and size of broodstock on egg incubation period and nutrient reserve in the newly hatched larvae.

There were no differences in mean egg developmental periods of M.rosenbergii eggs with variations in maternal age or size (Table:4.10). Similarly no significant ($P<0.05$) relationship was evident between ST_{50} and maternal age or size of broodstock (Fig:4.13) indicating that nutrient reserves in the larvae were not influenced by age or size of the broodstock. Nutrient reserves in the newly hatched larvae were assumed to be in proportion to resitance to starvation measured as ST_{50}.

Table:4.8. Influence of age of M.rosenbergii broodstock on fatty acid composition of eggs (Expressed as % total lipid)

Fatty acids	Age group (weeks) 45-50	66-70	77
14:0	2.2	2.1	2.0
15:0	0.7	0.7	0.7
16:0	20.5	20.2	19.2
16:1(n-7)	7.2	7.9	7.1
16:2	0.5	0.5	0.5
17:0	0.8	0.8	0.9
16:3	1.1	0.9	1.0
16:4	0.8	0.9	1.1
18:0	6.8	7.9	7.6
18:1(n-9)	26.4	21.5	24.7
18:1(n-7)	3.5	4.1	4.0
18:2(n-6)	4.0	4.7	4.5
18:2(n-9)	0.4	0.3	0.5
18:3(n-6)	0.1	0.2	0.4
18:3(n-3)	2.1	1.6	2.8
18:4(n-3)	1.9	0.7	1.4
20:0	0.4	0.3	0.2
20:1(n-11)	0.1	0.2	0.1
20:1(n-9)	0.4	0.5	0.3
20:1(n-7)	0.1	0.2	0.2
20:2(n-6)	0.1	0.3	0.2
20:4(n-6)	1.7	3.5	2.3
20:4(n-3)	0.3	0.1	0.1
20:5(n-3)	6.7	6.9	6.6
22:0	0.1	0.1	0.1
22:5(n-6)	0.1	0.1	0.1
22:5(n-3)	1.0	0.9	1.2
22:6(n-3)	5.0	6.6	5.3
24:1	0.1	0.1	0.1
T.Saturates	34.0	34.5	33.4
T.Monoenes	37.9	34.4	36.5
T. n-3	17.1	17.1	17.6
T. n-6	6.0	8.8	7.5
n-3/n-6	2.9	1.9	2.3
T.PUFA	22.1	26.2	25.1
T.unknown	5.0	4.9	5.0

Table:4.9. Influence of age of M.rosenbergii broodstock on mineral composition ofeggs.

(Expressed as $mg.g^{-1}$.)

Minerals	Age groups (weeks) 45-50 (2)	66-70 (2)	77 (1)	Pooled
Ca	1.00	0.92 ±0.31	1.33	0.30
Mg	0.37 ±0.02	0.33	0.35	0.27 ±0.04
K	0.75 ±0.08	1.56	1.81	0.88
Cu	0.25	0.26 ±0.04	0.23	0.32
Fe	0.08 ±0.01	0.06	0.07	0.06
Zn	0.14 ±0.03	0.16	0.15	0.10

± Standard deviation. Standard deviation less than 5% of the mean not indicated for simplicity.

() number of samples

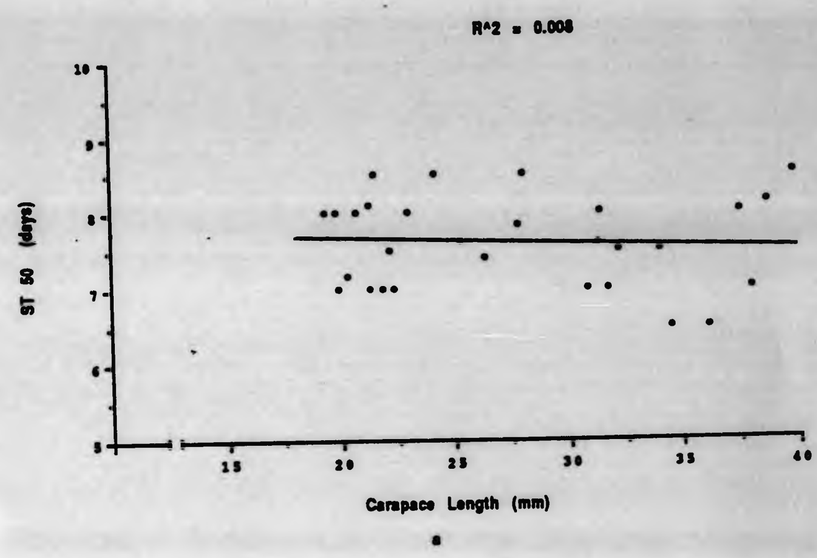
Table:4.10 Influence of age and size of broodstock on egg incubation period (days)

Group	Incubation period (days)	
	Mean	Standard deviation (±)
Age (weeks)		
30-35	18.0	0.63
35-40	18.5	0.50
40-45	18.5	0.76
Size (mm) (Carapace length)		
17-24	18.31	0.72
24-31	18.43	0.73
31-40	18.27	0.62

Incubation period, day 0 refer to day of spawning.

Fig:4.13.a. Relationship between carapace length of M.rosenbergii broodstock and survival of newly hatched larvae under starvation (ST_{50}) (DF=28 P>0.05).

Fig:4.13.b. Relationship between age of M.rosenbergii broodstock and survival of newly hatched larvae under starvation (ST_{50}) (DF=20 P>0.05).



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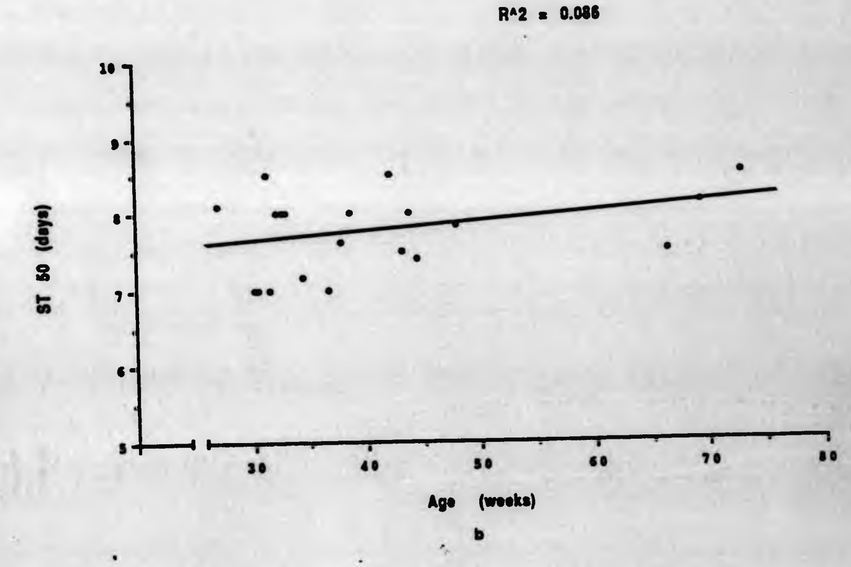

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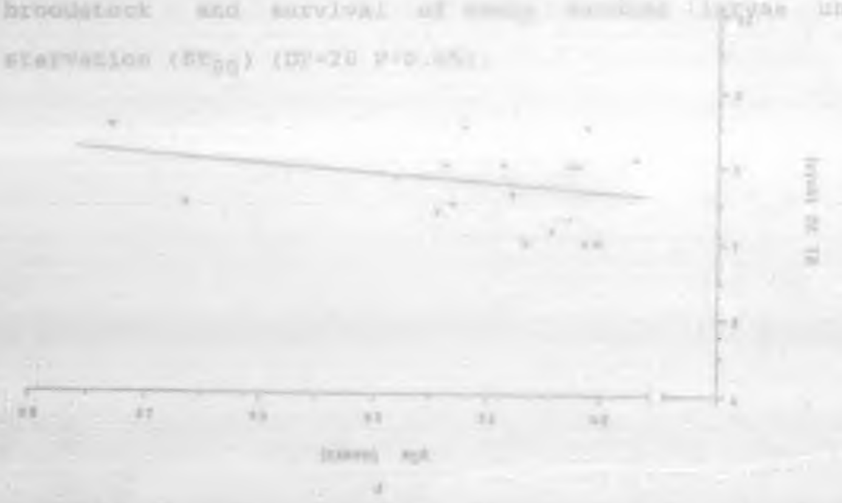


4.4 Disscussion

4.4.1.1 Relationships between wet and dry weights and number of eggs in egg clutches.

Irrespective of the weight of the egg clutch, their moisture contents were uniform. Consequently, a high degree of association existed between wet and dry weights of egg clutches. Therefore, the wet weight of the egg clutch is a good predictor of its dry weight (this can be calculated from the equation given in Fig:4.1).

Increases in both wet and dry weights of egg clutches were associated with increases in numbers of eggs. Similar observations were made by Patra (1976) (Table:4.1) with M.rosenbergii obtained from the wild. There was a high degree of association between number of eggs and wet and dry weights of eggs. Measures of wet weights of egg clutches are reasonable representation of egg production in M.rosenbergii under identical conditions. Therefore egg production in these prawns may simply be measured by wet weight of the egg clutches than estimating number of eggs.

Estimations of fecundity in caridean prawns, such as M.rosenbergii sp., with greater number of eggs per spawn are tedious and time consuming. Initially the eggs need to be separated from the interconnecting materials. There is no suitable rapid technique for isolating eggs, except that of (Choy,1985). This involves sodium hypochlorite, an

unpleasant chemical to use routinely. As the eggs are very small (approximately 500 µm in diameter) it is difficult to count the isolated eggs with the naked eye. Therefore indirect methods, such as Lechman's (1953) method cited by Patra(1976), were thus used to estimate fecundity. However, one has to be cautious when using the relationship between weights of egg clutches and fecundity may change depending on the size of eggs or moisture contents of egg clutches. The moisture contents and size of eggs could vary with many environmental conditions as discussed in sections 4.1.2.1 and 7.1.

4.4.1.2 Influence of broodstock age and size on quantitative egg production.

The weights of egg clutches (wet or dry) produced by M.rosenbergii broodstock were found to increase with the length and weight of broodstock. This is the general trend found in most Macrobrachium sp. (Table:4.1) and other Crustaceans (see, Sastry,1983; Hartnoll,1985) and fishes (see sections 4.1.1). Larger broodstock may be older or, within a particular age group bigger, animals. Information on the relationship between egg production and age of broodstock is meagre in Crustaceans and no such information is available for M.rosenbergii.

The present study indicates that egg production in M.rosenbergii is more highly associated with length and weight of the broodstock than with age. Similar trends

have been reported for some fishes. A higher degree of association between fecundity and weight (r^2=0.689) than age (r^2=0.436) of haddock was reported by Hodder(1963). In plaice (Simpson,1951) and Hippoglossoides platessoides (Bagenal,1957; Pitt,1964) length and weight were more highly correlated with fecundity than was age.

The weights of egg clutches spawned by a particular age or size group were found to be more dependent on size of the animal than on age. This indicates that even if the animal is older, egg production is more dependent on size than age. A younger female, bigger in size, can produce more eggs than a small, older, female (Fig:4.4 and 4.5), under identical environmental conditions. This clearly indicates that the influence of broodstock growth parameters masks the influence of age and explains the high variations found in egg production of M.rosenbergii.

In terms of egg production the performances of younger and bigger, or heavier, broodstock was similar to that of older prawns of approximately the same size. Selection of younger, and bigger or heavier, females as broodstock is not only advantageous in terms of cost of production of broodstock but also leads to genetic progress. Doyle et al. (1983) argued that simple control of age of M.rosenbergii broodstock by selecting broodstock as early as possible in the production cycle can exert strong "indirect selection" on the growth rate. Indirect selection refers to selection exerted on a trait indirectly by means other than

artificial selection for the trait itself (Doyle et al.,1983).

As pointed out in Section 4.1.1 there are differences in the type and degree of association between egg production and growth parameters in M.rosenbergii (Table:4.1). In the present study the highest degree of association with a curvilinear relationship, existed between carapace length and wet weight of egg clutches (Fig.4.6). In contrast the reanalysed data of Patra (1976) indicates that a simple linear relationship existed between weight of egg clutch and carapace length of the broodstock (Table:4.1). The present study indicated that the best relationships that existed between wet weight of egg clutch and weight of the broodstock was simple, linear regressions. The recalculated data of Patra(1976) indicates that these relationships were curvilinear.

As indicated in section 4.1 intra-species differences in egg production are evident due to differences in geographical and environmental conditions. The differences in the relationships observed in the present and Patra's studies could be associated with the above factors and also differences in size ranges of animals used in the studies. The females used in this study ranged from 18-40mm in carapace length compared with 32-60mm in Patra's study.

The egg production-growth parameter relationship was also found to vary depending on the range of the growth

parameter considered in the study. A curvilinear relationship existed between wet weight of the egg clutch and carapace length of the broodstock when the range of carapace lengths was 18-40 mm (Fig:4.6). Approximatly a linear relationship existed when the range of carapace length used was narrower, 18-23 or 23-35 mm (Fig:4.4 and 4.5) and 30-40 mm (Fig: 7.3 Chapter 7).

4.4.2. Variations in egg size within and between egg clutches.

The mean coefficient of variation in egg size within spawns of M.rosenbergii was approximately 5.58 %. This is very low when compared to variations reported for some fishes such as rainbow trout, 9.27% (Galkina,1970), Oreochromis sp., 11% (Rana,1986) and slightly higher than in salmonids, 4-4.5% (Galkina,1970). Due to this low variation of egg size within the egg clutch mean egg size could be considered to be reasonably representative of egg size. Also egg size as egg volume can be a good measure of reproductive performance of M.rosenbergii broodstock.

The reasons for such variations in egg size within the egg clutch are not known (Rana,1986). Some suggest that food supply could be a determinant of variations within egg clutches. Such possibilities in M.rosenbergii are considered in Chapter 7.

As in the case of most fishes an increase in egg size with increase in size of broodstock was evident in M.rosenbergii with a positive, linear, regression between the two. This is further supported by statistical differences between the two extreme parental size classes used in the present study. The low degree of association obtained may be due to the variations in egg size among the smaller broodstock, as the coefficient of variation was 9.47%. The egg size distribution among the egg clutches of bigger broodstock was more uniform (CV 1.78 %). Therefore, it may be interesting to study the variations in egg size among the smaller size groups as they could be associated with age of the broodstock.

Wickins and Beard (1974) reported variations in larval size of M.rosenbergii produced by different females. However, they could not find any association with the size of the female in one of the experiments in which fluctuations in pH and photoperiod were observed. A positive, significant ($P<0.05$), correlation was reported in the second part of this experiment in which the fluctuations in environmental conditions were controlled. The females used in the above study ranged from 150-190 mm in total length, whilst in the present study they were less than 139mm. The studies of Wickins and Beard(1974) indicated that size of M.rosenbergii larvae could increase with size of the broodstock up to 190mm. Therefore from the above, and the present study, it is evident that bigger broodstock produce bigger eggs and larvae in M.rosenbergii

than do smaller broodstock. Such an increase is evident up to total lengths of 190mm (from Wickins and Beard,1974).

Smaller egg clutches from smaller broodstock contain smaller eggs but the moisture content was found to be uniform irrespective of the weight of the egg clutch. Therefore, smaller eggs have lower dry matter contents compared to bigger eggs. This may be advantageous either in the production of relatively bigger larvae or larvae with more reserves, from bigger eggs spawned by bigger broodstock. This possibility is further considered in the forthcoming section. In fishes, it is well documented that bigger eggs produce bigger larvae (Blaxter and Hempel,1963; Reagan and Conley,1977; Theilacker,1981; Springate,1985; Rana,1986). Therefore,it may be interesting to study the influence of egg size on the size of the larvae and larval development and metamorphosis.

In M.rosenbergii farms the berried females necessary for larval production are selected from commercial harvests which include mixed ages and sizes (smaller than 40g) Malecha,1983; New,1988). This practice may not only select genetically inferior and superior animals but also does not take advantage of the potential fecundity of larger animals (Malecha,1983). The present study indicate that the current practice also does not take advantage of the bigger eggs produced by bigger broodstock.

The studies of Wickins and Beard (1974) indicate that bigger broodstock produce bigger larvae under stable

conditions. Therefore bigger broodstock may be advantageous in terms of egg and larval size. The variations in egg size among individuals of the bigger parental size groups were found to be lower than in the smaller broodstock. Therefore selection of bigger broodstock will be also advantageous in obtaining more uniformly sized larvae.

It is important to determine the upper limit to which an increase in egg size could be expected, with respect to the size of the female, to establish the optimum size of female which could yield optimum sized larvae.

4.4.3.1 Chemical composition of M.rosenbergii eggs.

Except for moisture there is high (inter and intra genus) variation in proximate composition of caridean eggs (Table:4.4). Interestingly there are similarities in energy contents of eggs except for those of C.nilotica. Due to the differences in methods employed by different authors (Table:4.4.) it is difficult to make any meaningful comparisons.

In general protein seems to be the predominant component of caridean eggs, except in C.nilotica and M.lamarrei. Protein constitutes more than 50% of the the dry weight of eggs followed by lipid. The absolute protein and lipid contents in the ooplasm of eggs may be more than the above values as these determinations were made on whole egg clutches including connecting membranes.

There is no published information on amino acid composition of decapod eggs. Data from Suyama,(1959) indicates that eggs of rainbow trout, dog salmon (Oncorhynchus keta), oval squid (Sepioteuthis lessoaiana) and spiny lobster (Panulirus japonicus) predominately contain glutamic acid, aspartic acid and leucine, as found in the present study with M.rosenbergii. Amino acids such as cystine, methionine and histidine were found in very low levels in the eggs of the above animals. Salmonid eggs were richer in alanine, proline, serine, and tyrosine than M.rosenbergii eggs. Meanwhile the eggs of M.rosenbergii, spiny lobster and oval squid were richer in arginine than salmonid eggs.

Lipid in M.rosenbergii eggs accounts for about 34% of the total dry weight of the whole egg clutch. The above level is within the range of 30-44% reported for C.weberi, C.crangon and P.serratus, but greatly varies from the values reported for M.idella and M.lamarrei (Table:4.4). Apart from differences in analytical procedures, other main factors such as environmental conditions (depth, temperature, salinity) food composition, nutritional condition of parents, egg incubation period and metabolism could account for differences in egg lipid content among and within species (Morris,1973; Castell,1982: Tocher and Sargent,1984; Henderson and Tocher,1987).

Neutral lipids were predominated by triacylglycerols followed by cholesterols in M.rosenbergii eggs. Similar

observations have been reported for other Crustaceans, such as B. balanoides and B. balanus by (Dawson and Barnes,1966) and Gammarus Oceanicus, E.Marinus (Clarke et al.,1985) and in some fishes such as rainbow trout and whitefish egg (Kaitaranta,1980; Kaitaranta and Ackman,1981;cited Henderson and Tocher,1987).

Polar lipids in M.rosenbergii were dominated by PC and PE with small amounts of PI. The rest of the polar lipids were not detected, as reported for other Crustaceans such as B.balanoides B.balanus (Dawson and Barnes,1966) and P.montagui (Clarke,1979). In some fishes the majority of lipids are polar lipids, especially species having low lipid contents (Tocher and Sargent,1984).

The basic fatty acid profiles of M.rosenbergii eggs are comparable to those of other decapods, such as P.serratus (Martin,1978), P.vannamei, (Cahu et al.,1986) and Heterocarpus grimaldii (Morris,1973). Except for P.vannamei all the above decapod eggs were found to contain predominantly monoenes, followed by saturates and PUFA (Table:4.3). P.vannamei eggs were richer in PUFA as are the eggs of most fishes (Henderson and Tocher,1987).

PUFA of P.serratus, P.vannamei and H.grimaldii eggs were rich in (n-3)PUFA, especially 20:5(n-3) and 22:6(n-3), typical of marine fishes (Tocher and Sargent,1984; Henderson and Tocher,1987).

The eggs of M.rosenbergii, being a fresh water animal may be expected to contain higher levels of (n-6) PUFA. The carcass of M.rosenbergii is reported to contain high levels of (n-6) PUFA (72 % of total PUFA) compared to (n-3) PUFA Table:4.7. There is convincing evidence to support the idea that the lipid profile of fish eggs resembles that of maternal tissue. (Yu et al.,1979, Watanabe et al.,1984 a, 1985b, Henderson and Tocher,1987). Therefore, high levels of (n-6) could be expected in M.rosenbergii eggs. However,in the present study the eggs were found to contain high levels of (n-3) PUFA (61% of total PUFA). Two hypotheses are proposed to explain these differences;

a) Even though, M.rosenbergii is a fresh water animal, as far as reproduction is concerned, it retains some of its ancestral marine characteristics; M.rosenbergii migrates to estuaries where eggs hatch and larval development takes place. Therefore, as far as eggs and larvae are concerned, at least in case of lipids, they might be retaining their ancestral body architecture and requirements, OR

b) The eggs used in this study were obtained from broodstock raised indoor under controlled conditions. They were predominantly fed with tissues of marine origin such as squid, shrimp, whitebait and mussels. These tissues contain high levels of (n-3) PUFA. Dietary fatty acid profile has been found to be a determinant factor in the fatty acid and, to a great extent, PUFA contents of eggs (Chapter.6) Therefore it is possible that the high (n-3)

PUFA levels observed in the present study were a result of high dietary (n-3) PUFA. Possible influences of dietary fatty acid profile on egg fatty acid composition are considered in Chapter.6.

It would be interesting to analyse the egg lipid composition of M.rosenbergii from natural habitats to confirm the egg fatty acid profile and the plasticity of these eggs in accumulation of various levels and types of PUFA.

Copper levels in M.rosenbergii eggs were slightly lower than the levels reported for P.lamarrei (0.33mg.g^{-1}. Shakuntala,1976) and C.nilotica (0.76mg.g^{-1} Ponnuchamy et al.,1979). The levels reported by the above authors were based on eggs obtained from wild females and analysed by the method of Kolmer et al.,(1967, cited by the above authors). Differences may have been due to differences in species, analytical procedure or food composition. The above values are very high compared to those reported for fish for example $7.5x10^{-3}$, $11x10^{-3}$mg.g.$^{-1}$ in rainbow trout (Springate,1985). This may be due to the relative importance of Copper for Crustaceans as an element in the formation of haemocyanin.

4.4.3.2 Influence of broodstock age on egg composition of M.rosenbergii

The composition of M.rosenbergii eggs belonging to different age groups were more or less similar, except for

some fluctuations in amino acids such as proline, cystine, methionine, lysine and some fatty acids.

Fluctuations in the (n-6) PUFA contents of eggs obtained from broodstock belonging to different age groups were observed in the present study. Surprisingly the (n-3) PUFA levels were constant. This could be due to dietary (n-3) PUFA as these animals were fed on tissues primarily of marine animal origin as mentioned earlier in this section. Consequently, the (n-3)/(n-6) ratio varied among the different age groups.

Contents of Mg, Cu, Fe, and Zn in eggs obtained from broodstock belonging to different age groups were relatively uniform. The levels of egg Ca and K fluctuated, K being low in eggs obtained from the younger age group, while low Ca levels were found in eggs from the 66-70 week old age group.

4.4.4 Influence of broodstock age and size on egg incubation period and nutrient reserve in the larvae.

Earlier in section 4.4.2 it was revealed that bigger broodstock produced bigger eggs (with more drymatter) and bigger larvae. The present study indicates that there is no relationship between the age or size of the broodstock and egg incubation period or nutrient reserve in newly hatched larvae. It appears as though differences in egg and larval size, which could be expected to be due to differences in size of broodstock (within the range used), do not influnce

egg developmental period and resistance to starvation in newly hatched M.rosenbergii larvae. The egg development period of larger eggs of Palaemon paucidens was found to be significantly ($P<0.001$) longer (19.5 days) than for small eggs (17.3 days) (Mashiko,1987). Whilst, insignificant differences were observed between incubation periods of large and smalle eggs from M.nipponence obtained from two different populations inhabiting two different regions of a river. He suggests that the lack of delay of larval hatching in larger eggs of M.nipponense is attributable to accelarated embryonic development. The larger M.nipponense larvae were found to have a greater developmental velocity than small larvae from small eggs (Mashiko,1986). It would be interesting, therefore, to study the influence of egg size and larval size on larval development (as proposed in section 4.4.2).

CHAPTER 5

BROODSTOCK NUTRITION 1.

DIET FORMULATION AND COMPOSITION, EXPERIMENTAL DESIGN AND EVALUATION OF METHODS.

5.1 Introduction

The nutrient requirements of most sexually mature animals undergoing reproduction are increased, especially during the later stages of the reproductive process (Reid, 1960; Cited Smith et al.,1979). Similarly, nutrient require--ments for growth and reproduction are elevated in female fish which undergo nutritional stress during breeding (Mitchell,1924; Cited Satia,1973; Satia,1973; Wootton,1977; Smith et al.,1979; Luquet and Watanabe,1986). These changes in requirements are due to allocation of part of the assimilated nutrients including energy for activities associated with reproduction (such as gamete production, synthesis of accessory sex secretions, mating behaviour etc.,) in the face of competing demands by the interrelated processes of maintenance and somatic growth (Adiyodi and Adiyodi,1974; Calow,1985). Food also serves, mixed and unmixed with other stimuli to gear the appropriate neural and hormonal machinery, which control activities associated with reproduction such as mating etc., (Adiyodi and Adiyodi, 1974).

The influence of food availability on reproductive performance in fish, with respect to egg and larval production, has been studied by many workers (Cho et al., 1985; Luquet and Watanabe,1986). Few systematic experimental studies have been made in this field to date.

Reduced ration has been found to reduce the frequency of spawning (Wootton,1973,1977; Townshend and Wootton,1984) and

lower the fecundity (Scott,1962; Hester,1964; Bagenal,1969; Wootton,1973,1977; Lyagina,1975; Richter et al.,1982; Townshend and Wootton,1984; Springate,1985) in fishes.

Protein source and level have been found to affect the reproductive performance of fishes. Phillips et al.(1964) found that a diet containing 31.6% protein (1.94 K.calg^{-1}) fed to Salmo trutta females produced larger eggs with better hatchability than diets containing 43.2% protein (2.75 K.cal g^{-1}). Smith(1979) did not find any significant differences in hatchability with varying protein levels although the size of eggs produced by rainbow trout females fed diets containing higher protein levels (49%, 35 K.cal g^{-1}) greater than produced by females fed lower protein diets (36%, 252 K.Cal.g^{-1}). Smith(1979), Watanabe et al.,(1984 b.c) and Pathmasothy(1985) reported negative relationships between fecundity and dietary protein levels, whilst Dahlgren(1980) did not find any relationship between the two parameters.

The studies of Takeuchi et al.,(1981a); Roley(1983) (Cited Luquet and Watanabe,1986) and Watanabe et al., (1984.a) demonstrated that diets containing low protein (28% fish meal based) with sufficient energy had no adverse effects on fecundity, egg size and hatchability in rainbow trout, and red sea bream, indicating the importance of protein:energy ratio.

A series of experiments conducted by Watanabe et al., (1984.a.b.c.) found red sea bream diets containing squid

meal as protein source to be superior to those containing white fish meal or raw sardine in terms of fecundity, hatchability and percentage of abnormal larvae. Both fat soluble and non-fat soluble fractions in squid meal were proposed to be responsible for improved reproductive performance (Watanabe et al.,1984; cited by Luquet and Watanabe,1986).

Unfortunately, nothing has been reported about amino acid requirements and levels, on broodstock fecundity and subsequent egg quality (Luquet and Watanabe,1986).

Beef tallow and corn oil, deficient in essential fatty acids (EFA), when supplemented to a white fish meal diet fed to rainbow trout and red sea bream reduced the hatchability of eggs and percentage of normal larvae compared to those fed squid meal and raw krill (Watanabe et al.,1984a.d.e; 1985b). The effect of corn oil was reversed when raw krill or squid oil supplemented diets were fed just before spawning (Watanabe et al.,1985 b). EFA deficiency resulted in poor fecundity, fertilization and hatchability (Luquet and Watanabe,1986). All experiments conducted on carp, trout and red sea bream have led to the conclusion that dietary EFAs are required for normal spawning. Fishes cannot synthesise (n-3) and (n-6) PUFA de novo. Therefore, the availablity of these EFA in broodstock diets, even shortly before spawning, has been found to play a vital role in determining egg quality.

Supplementation of trace elements and vitamins (especially vitamin E) has been found to increase hatchability in rainbow trout (Takeuchi et al,1981; Sandness et al.,1984) and red sea bream (Watanabe et al.,1985b). Although the effect of individual dietary mineral contents on reproduction of fishes has not been studied, low levels of manganese in the diet (7.2 mg Kg^{1}) were found to be responsible for high mortality and poor hatching rate in red sea bream (Luquet and Watanabe,1986).

Pigments have long been believed to play a major role in the reproduction and development of animals. Although the studies of Startmann et al.(1947) and Downfall,(1965) suggest (cited Torrissen,1984) that pigments influence fertilisation rate no such effect was observed by Quantz, 1980 cited Tvenranger,(1986), Torrissen,(1984); Tvenranger, (1986). Although pigment supplementation did not improve hatchability in rainbow trout (Harris,1984; Tvenranger,1986) or Atlantic salmon (Torrissen,1984) it was found to be important for red sea bream (Watanabe et al.,1985.b).

The nutritional requirements of broodstock fish have not yet been established for any fish. However, from the above studies it is evident that feedstuff source and nutritional contents have a profound effect on reproduction in fish.

Information on the role of nutrients in the reproduction of Crustacea mainly focuses on the biochemical transformations and translocations of body stores during

moulting and reproduction in wild animals. Much of this work has been reviewed by Lawrence,(1976) and Sastry (1983).

There are a few scattered references on the initiation and dependence of the reproductive cycle of some planktonic crustaceans on availability and density of food resources. Sastry(1983) identified two empirical strategies in these Crustaceans with regard to food resources and gamete production. In summary these were;

a)that gamete production coincided with environmental food availability or

b)storage of nutrients when food is abundant for later utilisation in gamete production.

There are several examples of Crustacea exhibiting the first strategy. The copepod Calanus hyperboreus needs abundant food to initiate reproduction, once initiated egg viability is dependent on the quantity of food (Conover, 1967). Starvation of the estuarine copepod Scottolana canadensis shortly after the terminal moult completely prevents reproduction even if the food supply is resumed (Heinle et al,1977). Lampert(1978) found that egg production in Daphnia was restricted by low concentrations of food ($0.2mg.l^{-1}$), at higher concentrations ($0.7mg.l^{-1}$) egg production reached a plateau.

The second strategy has been found principally in animals experiencing temporal changes in food availability with seasonal cycles. Barnes and Barnes(1963) found that

barnacles assimilate and store nutrients during spring when food is abundant and utilise this for gonadal development in summer and egg incubation during winter.

The inter-relationships between trophic position of the species, food availability, food intake, assimilation efficiency and reproduction have not been examined systematically in Crustaceans (Sastry,1983).

An intensive survey of the literature on the nutritional requirements of adult decapods, especially broodstock, revealed that this aspect of research has been completely neglected. There is no basic information except for the speculations that fatty acid composition affects reproduction in penaeids (AQUACOP,1979; Primavera and Borlongan,1979; Middledich et al.,1980).

This thesis is mainly concerned with;

a) formulation of diets for M.rosenbergii broodstock (chapter 5) to explore the possible influences of protein quality and content on,

b) acquisition and utilisation of diets by the broodstock, growth and chemical composition of the broodstock moulting and spawning patterns of the broodstock (chapter 6)

c) quantity and quality of the eggs, and the nutrient reserve of newly hatched larvae under confined indoor experimental conditions. This experiment was also expected to provide basic information on

a) possible influences of other dietary nutrients such as

fatty acids on the composition of eggs, and

b) the influence of egg composition on nutrient reserve of newly hatched larvae (chapter 7).

5.1.1 Formulation of M.rosenbergii broodstock diet and diet composition.

The formulation of a diet represents the translation of energy and nutrient requirements into a balanced mixture of feed ingredients for a group of animals (Cho et al.,1985). Unfortunately, there is no information on the nutritional requirements of adult or sexually mature decapods (Section. 5.1).

In the absence of information on daily nutritional and energy requirements, diets are formulated using cues obtained form related animals, best guesses and "guesstimates". Therefore, in the present study available information on the nutritional requirements of juvenile decapods, especially prawns and shrimps, was used as a guide to formulate diets for M.rosenbergii broodstock, anticipating differences in nutritional requirements of different species and stages of life.

5.1.1.1 Nutritional requirements of shrimps and prawns

In the natural environment adult M.rosenbergii are nocturnal, omnivorous benthic feeders. They feed frequently, and avidly, on aquatic worms,insects, insect larvae, molluscs, crustaceans, fish offal, grains, seeds, nuts, fruits, algae, tender leaves and stems of aquatic plants and detritus (Ling and Merican,1961; Raman,1964; Ling,1969) If sufficiently hungry they become cannibalistic (Ling,1969).

Enzyme activity measured in the digestive systems of various species of Macrobrachium by Tyagi and Prakash(1967) Murthy,(1977) Lee et al,(1980) and Fair et al.(1980) indica-tes the presence of a wide range of digestive enzymes. This include trypsin, aminotripeptidase, leucine-aminopepti-dase, L-glycine-leucine dipeptidase, amylase, cellulase, lipases, and esterases. This is indicative of the capability of these animals to digest a relatively large range of complex proteins, carbohydrates and lipids. Quantitative analysis of these digestive enzymes confirms that M.rosenbergii is an omnivore (Lee et al.1980).

Since the first Crustacean diet was formulated by Kanazawa et al.(1970) for P.japonicus two decades ago, the specific nutritional requirements of juvenile prawns and shrimps have not been yet established (see Sick and Millikin,1983; Corbin et al.,1983).

The types of feed used in large scale semi-intensive and intensive prawn farms vary widely. They include animal

diets such as prawn waste, chicken carcasses, trash fish, molluscs and vegetable raw materials such as rice, cassava or tapioca, leaves of Ipil ipil or feed mixtures prepared at the pond banks or commercially compounded feeds (New and Singholka,1982).

The wide variety of commercial feeds of varying composition used in Macrobrachium farms are based largely on empirical knowledge of growth requirements and practical and economic considerations rather than on quantitative knowledge of nutritional and metabolic requirements (Sick and Millikin,1983; Corbin et al,1983)

Under defined conditions conventional fresh food have been found to promote growth and survival in prawns and shrimps. Examples, include mussel (Cowey and Foster,1971 with P.serratus; Colvin,1976 with P.indicus), clam (Deshimaru and Shigeno,1972 with P.japonicus; Deshimaru et al.,1985 with P.monodon) and squid (Deshimaru and Shigeno, 1972 and Kitabayashi et al.,1971c with P.japonicus) will have been found to give better performance in Crustaceans than pelleted food.

Dry artificial diets combined with fresh foods (such as the leaves of Allanthus altissima and Malva parviflora) have been found to eliminate moult death syndrome and reduce considerably the incidence of "black spot disease" in addition to decreasing the intermoult period and increasing average body weights (Harpaz and Schmalbach,1986).

It has also been suggested that squid meal, shrimp meal, fish meal, earthworm meal and short necked clam could contain chemo-attractants which increase feed palatability (Deshimaru and Shigeno,1972; Lovell,1978; Meyers,1981; Pascual,1983).

5.1.1.1.1 Requirements for protein

Dietary protein is necessary for maintenance, the repletion of depleted tissues and growth. The utilisation of dietary protein is mainly affected by its amino acid composition, level of protein intake, caloric content of diet and physiological state of the animal (Cowey and Sargent,1972).

Studies on protein source, level and amino acid composition in prawn nutrition have received most atten-tion. This is because protein is the largest and most expensive component in aquatic animal feeds (Biddle,1977; New,1980).

Many workers have found that, with a balanced diet, growth rate of crustaceans can be correlated with dietary protein levels (Walker,1975; Biddle,1977).

A wide range of protein "optima" have been claimed by different workers for different Crustacea and even for a particular species (Table:5.1).

These differences may be due to various biotic factors and/ or differences in methodology. Biotic factors could

Table:5.1 Quantitative protein requirments of decapods.

Animal	Recommended level % d.diet.	Size (g)	Author/s
M.rosenbergii	35	0.1	Balazs and Ross,(1976)
M.rosenbergii	25	1.6	Clifford and Brick,(1979)
M.rosenbergii	15	0.12	Boonyartpalin and New,(1980)
M.rosenbergii	40	0.15	Millikin et al.,(1980)
M.rosenbergii	27	1.9	Stanley and Moore, (1983)
M.rosenbergii	30-40	40	Ashmore et al.,(1985)
P.serratus	30-40	0.2	Forster and Beard,(1973)
P.japonicus	>60	0.9-7	Dehimaru and Shigneno,(1972)
P.japonicus	40	1-2	Balazs, (1973)
P.japonicus	50	0.6-1	Deshimaru and Kuroki,(1975)
P.japonicus	52-57	0.8	Deshimaru and Yone,(1978)
P.aztecus	<40	0.14	Venkataramiah et al,(1975)a
P.aztecus	30-52	0.4-1.3	Zein-Eldin and Corliss(1976)
P.monodon	46	0.5-1	Lee, (1971)
P.setiferus	28-32	4	Andrew et al., (1972)
P.stylirostris	35	5	Colvin and Brand,(1977)
P.merguiensis	40	3-8	AQUACOP, (1978)
P.merguiensis	34-42	0.3	Sedgewick, (1979)
P.indicus	43	0.4-1.1	Colvin, (1976)
P.vannamei	30	0.5	Colvin and Brand, (1977)
P.vannamei	36	4-20	Smith et al.,(1985)

include age (New 1976, Biddle,1977; Wickins, 1976; Handson and Goodwin,1977; Colvin and Brand,1977; Chen et al,1985) and /or physiological state of the test animal (Biddle,1977; Handson and Goodwin,1977).

Differences in methodology could be due to diferences in experimental conditions (Biddle,1977; Hanson and Goodwin, 1977), differences in basic diets (many of them with unbalanced amino acid composition) (New,1976,1980) and/ or to this omnivorous test animal obtaining part of its requirements from algae (Balaz,1973;1976), tank microbes (Stahl and Ahearn,1978) and gut microbes (Biddle,1977; Stahl and Ahearn,1978, New,1980). Feed stability, feeding rate (Hanson and Goodwin,1977) and variations in dietary supplementation of macro and micro nutrients other than protein (Hanson and Goodwin,1977; Millikin et al,1980) and differences in energy contents of diets (Tacon and Jackson,1985; Wilson,1985) will also have had an effect.

Balazs et al.(1974) reported that the gross protein requirements of M.rosenbergii were greater (35%) during the first 119 days post-metamorphosis and decrease (15%) after 120-175 days. Similar decreases in optimum protein levels for prawns with increasing age were reported by Andrews and Sick (1973), Balazs and Ross(1976), Colvin and Brand(1977), Clifford and Brick(1978), Farmanfarmian and Lauterio,(1979) and Millikin et al.(1980).

The above studies indicate that M.rosenbergii between 0.1 and 3g may require about 40% protein while larger prawns

(4-20g) need only 25-30% protein (Colvin and Brand,(1977); Capuzzu (1981); Sick and Millikin,(1983).

Various protein sources have been used in experimental and practical diet formulations for prawns and shrimps. Purified protein sources (such as casein, gelatin, egg albumin, and zein) have been reported to be poorly consumed resulting in high mortalities and poor growth performance. (Cowey and Foster,1971; Sick et al,1972; Deshimaru and Kuroki,1974a,1975; Kanazawa,1982).

Plant proteins have been found to be relatively lower in biological value, for Crustacea, in terms of growth, than proteins of animal origin (New,1976; Balazs and Ross,1976). However, digestibility studies with P.serratus (Foster and Gabbot,1971), M.rosenbergii (Taechanuruk and Stickney,1982; Ashmore et al.,1985) and P.vannamei (Smith et al.,1985) have indicated that prawns can efficiently digest both plant and animal protein sources.

Squid meal has been found to be an excellent protein source for prawns and shrimps. Studies include those with P.japonicus (Kitabayashi et al.,1971a.b; Deshimaru and Shigeno,1972), P.stylirostris and P.setiferus (Fenucci et al.,1980), P.aztecus (Fenucci 1977, cited Fenucce et al., 1980), P.monodon (Lim et al.,1979) and P.vannamei (Dokken and Lawrence,1985). The influence of an unidentified growth promoting factor (UGF) has been speculated to be responsible for the growth enhancing properties of squid meal (Cruz-Suarez,1987).

Cowey and Forster(1971) achieved better growth in Palaemon serratus fed fresh mussel compared to freeze dried cod muscle or purified diets. Similarly Deshimaru and Shigeno,(1972) reported best growth with P.japonicus fed short clam. Poorer growth performance has been reported with diets containing fish meal compared to squid, clam or shrimp meals. P.aztecus (Sick et al.,1972) and P.setiferus (Forster and Beard,1973; Sick and Andrew,1973) fed diets containing different ratios of shrimp and fish meal showed growth with best diets containing higher ratios of shrimp meal.

Diets containing a combination of animal and plant proteins have been found to provide better growth and survival with P.monodon than diets from any single protein sources (Pascaul,1983).

From the above review it is apparent that;

a) chemically purified or semi-purified diets are not favoured in prawn and shrimp studies.

b) fresh food (such as mussels, squids, clams and worms supplemented with aquatic plants) supports best growth, moulting frequency and survival in prawns and shrimps.

c) protein levels of 25-35 % should be sufficient for optimum growth of adult M.rosenbergii.

d) protein sources such as squid meal, mussel meal, shrimp meal, fish meal, single cell proteins and soya meal can be regarded as "good quality" protein sources prawns and shrimps.

5.1.1.1.2 Requirements for amino acids

Dietary proteins should provide sufficient quantities of essential amino acids (EAA) which are those that cannot be synthesised de novo. These EAA are vital for normal growth and health of the animal.

The qualitative EAA requirements of prawns and shrimps so far studied are similar to those of most other animals (Cowey and Forster,1971; Miyajima et al.,1977; Shewbart et al.,1972; Watanabe,1975). A summary of the qualitative EAA requirement of various decapods is given in Table:5.2.

Miyajima et al.(1977) with M.ohine, Watanabe(1975) with M.rosenbergii and Shewbart et al.(1972) with P.aztecus, have found (using radio-labelled amino acids) that tyrosine is essential for prawns whereas in mammals, birds, insects and teleost fish it is regarded as nonessential. But Zandee (1967), Miyagima et al.(1977), Kanazawa and Teshima,(1981), Shewbart et al.(1972) and New,(1976) suggested that tyrosine can be synthesised from dietary phenylalanine. The essentiality of tyrosine is still subject to verification.

Similarly, nonessentiality of lysine for M.rosenbergii was reported by Watanabe,(1975). Stahl and Ahearn,(1978) attempted to verify this finding using graded purified amino acid test diets completely lacking individual amino acids. They were unable to establish requirements for lysine, arginine, methionine and tryptophan which they

Table:5.2. Qualitative essential amino acid requirements of selected animals.

	Palaemon serratus 1	Macrobrachium ohione 2	Macrobrachium rosenbergii 3	Penaeus aztecus 4	Penaeus japonicus 5	fish 6
Arginine	+	+	+	+	+	+
Histidine	+	+	+	+	+	+
Isoleucine	+	+	+	+	+	+
Leucine	+	+	+	+	+	+
Lysine	+	+	-	+	+	+
Methionine	+	+	+	+	+	+
Phenylalanine	+	+	+	+	+	+
Tyrosine	-	+	+	+	-	-
Threonine	+	+	DA	+	+	+
Tryptophan	+	DA	DA	+	+	+
Valine	+	+	+	+	+	+

\+ Essential - Nonessential

DA Data not recorded

1 Cowey and Forster, (1971)

2 Miyagima et al., (1977)

3 Watanabe, (1975)

4 Shewbart et al., (1972)

5 Kanazawa and Teshima, (1981)

6 NRC, (1983).

suspect that gut and/ or tank bacteria may have supplied.

The specific quantitative dietary EAA requirements of shrimps and prawns are not yet known (New,1976,1980; Sick and Millikin,1983). Attempts to establish the quantitative requirements of prawns using purified diets have failed due to poor growth, low feed intake and high mortalities (Cowey and Forster,1971; Deshimaru and Kuroki,1974.a.b.,1975; Deshimaru,1976,1981; Stahl and Ahearn,1978)

Deshimaru and Shigeno(1972) reported that the EAA pattern of short-necked clam and squid meals (which were recognised as the best conventional foods for prawns) have a similar EAA pattern to that of P.japonicus. The above authors prepared diets with various proportions of various ingredients, those that mimicked the EAA pattern of P.japonicus gave best growth rate and survival. Compounded diets with lower methionine and arginine (compared to soft clam) resulted in poor growth in P.monodon (Deshimaru et al.1985). Similar observations were made by Kitabayashi et al.,(1971c). These authors suggest that methionine and arginine might be limiting amino acids in their compounded diets and that the content and/ or balance of EAAs are one of the major factors in the nutritional quality of diets for P.monodon. Similar studies carried out by Teshima et al. (1986b) using diets supplemented with crystalline amino acids mimicking the EAA profile of P.japonicus larvae resulted in improved growth and survival.

From the above studies it is evident that;

a) most Crustaceans require up to eleven essential amino acids (including tyrosine although its essentiality is uncertain).

b) arginine and methionine may have greater influences than other EAAs.

c) quantitative EAA requirements are not yet established for shrimps and prawns. The required dietary amino acid profile may best be derived from body EAA profiles of shrimps and prawns.

5.1.1.1.3 Requirements for Lipids

Dietary lipids play important roles as energy sources and providing essential fatty acids (EFA), sterols, and phospholipids necessary for growth, maintenance, functional integrity of membranes and proper functioning of many physiological processes (Colvin,1976; New,1976,1980; Kanazawa et al.,1977a.b; Corbin et al.,1983; Cho et al., 1985).

Various dietary lipid sources have been found to promote good growth in penaeids including menhaden oil, linseed oil (Sick and Andrews,1973; Sick and Beatty,1975) fish oil (Sick and Beatty,1975; Guary et al.,1976; Petriella et al.,1984), clam or mollusc oil (Guary et al.,1976) and cod liver oil (Sedgewick,1979).

Most of the information available on the quantitative and qualitative requirements for lipids is based on marine

shrimps. Due to the combination of genetic, dietary, environmental, biotic or other abiotic factors, there is variation in fatty acid composition between different species, tissues and organs (Castell,1982). Therefore, precautions should be taken when using data from shrimps to predict dietary lipid requirements for prawns.

Available reports on quantitative lipid requirements recommend a range of 5-15% for various crustaceans (Briggs et al, unpublished review). However, it is generally accepted that Crustacea cannot tolerate high levels of dietary fat and more than 10% is not recommended due to the expectation that this could result in poor food intake and feed manufacturing problems (Biddle,1977; New,1980; Corbin et al.,1983).

The de novo synthesis of polyunsaturated fatty acids (PUFA) of the linoleic (n-6) and linolenic (n-3) series is either extremely difficult or non-existent in Crustacea (Guray et al.,1976; Kanazawa et al.,1977a,1977b; New,1980; Castell,1982). Relatively little research on the EFA requirements of Crustaceans has been performed (Castell, 1982). The nutritive value of (n-3)PUFA is higher than (n-6) PUFA for marine Crustaceans (Kanazawa,1982; Castell,1982; New,1980; Petriella et al.,1984). Whilst freshwater species might require more (n-6)PUFA or a mixture of both Castell,1982). The optimum ratio of (n-6) to (n-3) PUFA fatty acids in Macrobrachium sp. diets has so far not been investigated (Sick and Millikin,1983).

Crustacea have been shown to be incapable of de novo sterol synthesis from acetate (Teshima and Kanazawa,1971; New,1976,1980; Kanazawa,1982). Cholesterol is thought to be a precursor of important steroids, brain and moulting hormones and of vitamin D in shrimp (Corbin et al.,1983). Cholesterol levels ranging from 0.1-1.4 % in dry diets have been suggested as optimum for various decapods.

Dietary phospholipids have been found to have beneficial effects on growth and survival of shrimps (P.japonicus: Deshimaru,1981; Kanazawa,1985; Teshima et al., 1982). A dietary requirement of 3% for soybean phosphotidylcholine has been reported for P.japonicus larvae when pollack liver oil was used as the basal lipid source (Teshima et al.,1982).

From the above discussion it is evident that:

a) lipid levels up to 10% might be desirable in prawn and shrimp diets.

b) both (n-3)PUFA and (n-6)PUFAs are essential and optimal n-3/n-6 ratios may be higher for penaeids than for Macrobrachium sp.

3) dietary cholesterol levels of 0.5 are considered sufficient.

5.1.1.1.4 Requirements for Carbohydrates

Carbohydrates together with lipids and proteins form the sources of dietary energy. They are also important in storage of dietary energy as glycogen and in synthesis of

chitin, steroids and fatty acids (New,1980). Dietary carbohydrates such as cellulose facilitate the rate of passage of food through the gut and starch and dextrin act as binders (NRC,1983).

A mixture of substrates have been found to be used for energy during starvation of various crustaceans. In fasting M.rosenbergii energy metabolism was dominated by carbohydrate; followed by lipid and protein. (Clifford and Bricks,1983).

There is little information on carbohydrate nutrition of Penaeids or Macrobrachium (New,1976,1980; Sick and Millikin,1983; Kanazawa,1985).

Various studies have shown that source and level of carbohydrate in diets affects growth and survival of both shrimps and prawns. The oligosaccharides such as trehalose, sucrose, maltose and glycogen are more desirable carbohydrate sources than polysaccharides such as dextrin and starch (Forster and Gabbott,1971; Andrew et al.,1972; Sick and Andrew,1973; Deshimaru and Yone,1978b; Abdel-Rahman et al.,1979, Fair et al.,1980; Corbin et al.,1983; Pascual et al.,1983; Alava and Pascual,1987).

Monosaccharides, such as glucose, have been found to be poorly utilised by P.monodon (Alava and Pascual, 1987) and inhibit growth (Kanazawa,1982). Similar results have been obtained with M.rosenbergii (Briggs, pers.comm) The above author found that diets containg wheat flour at

40% of diet produced better growth than in order, of dextrin, starch, sucrose, cellulose, glucose or chitin.

Optimum carbohydrate levels have not yet been established for prawns. Addition of low or high levels of carbohydrates to diets resulted in deleterious histopathological changes in various tissues of P.monodon (Pascual,1983). Sick and Andrew(1973) reported better performance of juvenile P.duorarum fed casein based diets containing 40% corn starch than 10% or 0%. Bage and Sloane (1981) found that high dietary levels of starch, up to 40%, in semi-purified diets fed to P.monodon had no effect on growth. Similar studies by Ali(1982) with P.indicus showed increased growth rate, survival and food conversion with increasing dietary starch content from 10-40%.

Little is known about the role of dietary fibre in shrimp and prawn diets (Biddle,1977; New,1980). In fishes dietary fibre is not required and is considered as a non nutrient bulk component (Millikin,1982).

Fair et al.(1980) reported that cellulose directly or indirectly contributes to the nutrient requirements of M.rosenbergii. They found that cellulose fibre comprising up to 30% of an isonitrogenous diet did not suppress growth and up to 20% dietary cellulose increased the growth and increased N assimilation. Similarly Briggs et al. (unpub--lished data) incorporated 40% cellulose into semipurified diets for M.rosenbergii and achieved 84% of the growth obtained with diets containing the same level of wheat

flour.

The presence of cellulase activity has been demonstrated for M.rosenbergii (Noborikawa,1979; Fair et al.,1980; Sick and Millikin,1983) P.serratus (Forster and Gabbott,1971) Homarus vulgaris and Astacus flaviatilis (Kooiman,1964). Fair et al.(1980) speculated that the presence of cellulase is an indication of cellulose digestion and utilisation.

However, Forster and Gabbot,(1971) with Palaemon and Pandalus sp. and Newman et al,(1982) with M.rosenbergii found that regurgitated materials contained mainly cellulose. Therefore dietary inclusion levels of cellulose need to be judged cautiously.

Chitin is required for the exoskeleton, which is shed and replaced during moulting, and for the periotrophic faecal membrane in prawns (New,1980; Kanazawa,1982).

Studies using glucosamine (an intermediary between glucose and chitin) in diets have resulted in enhanced growth rates in P.japonicus (Kitabayashi et al.,1971a; Kanazawa,1982;1985). Kitabayashi et al.(1971a) recommended a level of 0.53% in shrimp diets. However, Deshimaru and Kuroki(1974b) reported that dietary glucosamine was unnecessary in diets for P.japonicus and inhibited the growth promoting effects of cholesterol. Forster(1975) suggested that dietary supplementation of glucosamine is unnecessary.

Chitinase activity has been reported in P.vannamei (Lee and Lawrence,1982), P.setiferus (Hood and Meyers,1973) Homaru vulgaris and Astacus fluviatilis (Kooiman,1964) The presence of chitinase, and the consumption of exuviae rich in chitin after moulting, is postulated to be an indication of the ability of these animals to assimilate chitin.

Clifford and Brick(1979) found that for M.rosenbergii a dietary ratio of fat:carbohydrate of 1:3 to 1:4 with a 25% protein diet resulted in more efficient utilisation of dietary protein than ratios of 1:1 and 1:2.

From the above review it is apparent that,

1) oligosaccharides are preferable to polysaccharides, followed by monosaccharides, for prawns and shrimps.
2) high or low levels of carbohydrates inhibit growth and. up to 40 % dietary level is desirable. Optimum level not yet established.
3) Crustaceans may be able to utilise cellulose and chitin.

5.1.1.1.5. Energy

Energy is essential for the maintenance of life processes such as cellular metabolism, growth , reproduction and physical activity.

Energy is derived from the catabolism of dietary carbohydrates,lipids and proteins. Providing sufficient energy in diets is important because an excess or deficiency

of available energy can cause reduced growth rates and poor nutrient assimilation efficiencies (Biddle,1977; NRC,1983). Low non-protein dietary energy intake will result in utilisation of other dietary nutrients, including expensive protein, to satisfy the energy requirement (Biddle,1977).

5.1.1.1.6. Requirements for Minerals

The complete dietary mineral requirements of decapods have not yet been established and knowledge of qualitative and quantitaive requirements are scanty (New,1976; Biddle,1977; Corbin et al,1983; Kanazawa et al.,1984).

Various types and levels of mineral premixes have been used in nutritional studies of Crustacea with widely varing types and levels of minerals (see Table:5.5). Some authors suggest that shrimps and prawns may obtain minerals from the surrounding water. In support of this view Deshimaru and et al.(1978) and Shewbart et al.,(1972) found that P.japonicus could absorb radioactive ^{45}Ca from water. The rate of absorbtion was negatively correlated with calcium content in the diet. Similarly, Chou et al.(1982) suggested that Homarus americanus appears able to absorb Cu from sea water.

Deshimaru et al (1978) recommended 2% P, 1% K and 0.2% trace metals (Al,Zn,Mn,Cu,Co) for growth of P.japonicus. The above authors also suggested that dietary Ca, Mg and Fe are not required for P.japonicus. In contrast Kanazawa et al.

(1984), using different dietary levels of P, Ca, Mg, K, Cu, Mn and Fe in experimentals diets, demonstrated the influence of the above minerals on growth and survival of P.japonicus. These authors also recommended dietary levels of 1-2% P, 1-2% Ca, 1:1 Ca:P, 0.1-0.5% Mg and 0.9-1.8 % K for normal growth of P.japonicus. However dietary supplementation of Mn, Fe and Cu did not influence growth of P.japonicus.

The importance of dietary minerals has been supported by the demonstrated ability of Palaemon and Pandalus sp. (Foster and Gabbot(1971), and M.rosenbergii (Newman et al. 1982) to absorb inorganic components from diets.

From the above discussion it is evident that;
a) Shrimps and prawns can absorb minerals from their diets and culture water.

5.1.1.1.7. Requirements for Vitamins

Like minerals the levels and types of vitamin premixes used in shrimp and prawn diets varies widely.

The application of vitamins to diets without prior knowledge of requirements may be wasteful. If added in excess vitamins can be toxic to or antigonistic in shrimps and prawns (New,1976,1980).

The importance of vitamins B,C,E and insitol for shrimps has been postulated by Fisher,(1960) and Kitabayashi at al(1971d).

5.1.1.1.8 Establishment of the quantitative amino acid requirements of M.rosenbergii.

5.1.1.1.8.1 Introduction

The quantitative amino acid requirements for prawns and shrimps have not yet been established (Section 5.1.1.1.2). The essential amino acid (EAA) requirements of finfishes have been determined by feeding graded levels of each amino acid within purified amino acid test diets. In most cases a linear increase in growth was observed with increase in amino acid intake up to a break point, corresponding to the requirement for that specific amino acid, where growth rate levels off (NRC,1983; Tacon and Cowey,1985). Ogino(1980) determined the quantitative requirements for all EAA simultaneously on the basis of the daily rate of deposition of specific amino acids within carcasses of growing fish.

Since quantitative amino acid requirements have only been established for a limited number of fishes, several investigators have used various methods of estimation of these requirements to facilitate diet formulation (Wilson,1985). In general, diets formulated to mimic the EAA pattern of conventional food sources have given good results with shrimps. For example formulation of diets with EAA profiles similar to those of clam and squid have given good performance in P.japonicus (Deshimaru and Shigeno, 1972).

Alternatively Rumsey and Ketola(1975) and Ketola (1982), amongst others, have used the EAA patterns of rainbow trout carcasses and eggs as a basis for formulation of diets and obtained improved growth. Preferably the EAA pattern derived from eggs or carcasses of the species for which the feed is intended should be used.

Cowey and Tacon(1983) and Tacon and Cowey(1985) found a good correlation between the EAA pattern of carcasses and the EAA requirement patterns of carp, Japanese eel, channel catfish and chinook salmon in which the EAA requirements have been experimentaly established. A similar relationship had been reported earlier for pigs and chicks (Boorman,1980) where it was concluded that " in the absence of more detailed information, the pattern of EAA in the product can be used for the pattern" in the diet.

Wilson and Poe(1985) regressed the EAA requirement pattern of channel catfish against the carcass and egg protein patterns. The former showed the best correlation, and they concluded that the EAA pattern of whole fish carcass is a better index than that of eggs.

Therefore in the present study the carcass EAA - EAA requirement relationship and best protein source EAA - EAA requirement relationships (as discussed above) were used to establish the quantitative amino acid requirements of M.rosenbergii.

5.1.1.1.8.2 Materials and Methods

Three small (16-20mm in carapace length (CL)) and one large (43.5mmCL) individuals of M.rosenbergii, reared as detailed in section2.2 and six small (18-21mmCL) M.rosenbergii reared in ponds (from the University of Pertanian,Malaysia) were used to establish tail muscle EAA composition.

All the animals were killed, by immersion in iced water for about 25 minutes. After peeling, the tail muscles of each group were pooled separately and dried in an oven at 60^{o}C to constant weight.

The dried muscle tissue was milled using a coffee grinder and analysed for amino acid composition as described in section 2.7.12. The widely reported best conventional food for shrimps and prawns (Section 5.1.1.1) was used as the protein source. The source and detail composition of squid meal is presented in Table:5.3

The A/E ratio was calculated as;

$$\frac{A}{E} = \frac{\text{Essential amino acid content}}{\text{Total essential amino acid}} * 1000$$

The results of the calculations are presented in Table: 5.3

5.1.1.1.8.3. Results

The amino acid contents (as % protein, g.16g.N) of tail muscle of the different stock of M.rosenbergii used in the present analysis were more or less similar except arginine, leucine and isoleucine contents. The arginine content of prawns obtained from ponds were 14% higher than in laboratory animals (Table:5.3). Larger prawns contained higher isoleucine and leucine levels than smaller ones (Table:5.3).

There was a significant ($P<0.05$) correlation ($r^2=0.77$) between the amino acid profiles of M.rosenbergii tail muscle and squid meal (Table:5.3). The high arginine content of squid meal is believed to enhance growth in prawns and shrimps (see section.5.1.1.1.2). Therefore using the amino acid profile of prawn tail muscle (X axis) and the arginine content of squid protein as a reference point (Y axis), a line of coincidence was established to pass through the origin (Fig:5.1). Using this line of coincidence require--ments for individual amino acids were established. These EAA profiles are apresented in Table:5.3.

5.1.1.1.8.4 Discussion

Differences were observed in amino acid contents of M.rosenbergii tail muscle from different size or ecological groups Table:5.3. These may be associated with age or size specific metabolism or nutritional, and ecological differences. It would be interesting to study possible

Table:5.3 Essential amino acid composition of tail muscle of M.rosenbergii, clam and squid meal, and estimated essential amino acid requirements of M.rosenbergii.

(Expressed as % protein)

Amino acids	Big	Small	Pond	Req	Squid	Clam*
Arginine	8.81	8.57	9.94	8.06	8.06	6.30
#	173	198	208	173	188	164
Histidine	2.48	2.25	2.50	2.27	1.67	2.00
	49	52	52	49	39	47
Isoleucine	5.49	4.14	4.40	5.02	3.62	3.75
	107	96	92	107	84	72
Leucine	8.17	6.57	7.09	7.48	6.85	6.43
	160	152	149	160	159	148
Lysine	5.77	5.25	5.83	5.28	6.03	7.52
	113	122	122	113	140	156
Methionine	3.29	2.80	3.12	3.01	2.49	2.19
	64	65	65	64	58	57
Phenylalanine	4.29	3.68	3.85	3.93	3.50	3.38
	84	85	81	84	81	82
Tyrosine	3.20	2.89	3.20	2.93	3.44	3.18
	63	67	67	63	80	83
Threonine	3.75	3.04	3.34	3.43	3.72	4.07
	73	70	70	73	87	102
Tryptophan	1.00	-	-	0.92	-	-
Valine	4.79	4.03	4.43	4.39	3.59	3.83
	94	93	93	94	84	90
Protein (% dry wt.)	97.34	98.23	97.88	-	87.04	-
Total EAA	51.04	43.20	47.69	46.70	42.97	42.46

\# A / E = $\frac{\text{Essential amino acid content}}{\text{Total essential amino acid}} * 1000$

Big =43.5mm CL Small = 16-20mm CL Pond = 18-21mm CL

Req =Requirements for M.rosenbergii. CL = Carapace length

a =Value determined for M.ohione (Shewbort et al.,1972)

K =Kjeldahl Method * Deshimaru and Shigeno et al.,(1972)

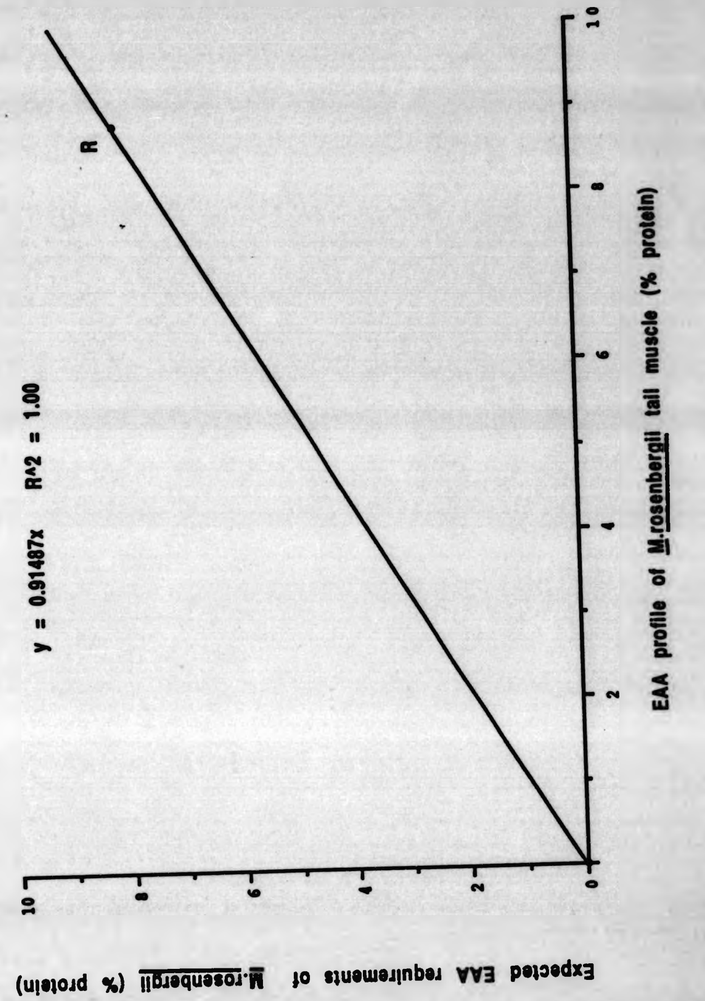


Fig:5.1 Line of coincidence constructed using argenine content of squid meal and EAA profile of M.rosenbergii tail muscle (using the line of coincidence and the EAA content of muscle, EAA requirements for M.rosenbergii were calculated).

relationships, in these differences in specifically designed experiments especially, with respect to size specific variations in amino acid metabolism.

Squid meal was found to have a similar distribution of amino acids to that of tail muscle of M.rosenbergii (Table:5.3 and Fig:5.1). Similar observations have been reported by Deshimaru and Shigeno (1972) for P.japonicus. These authors found that squid, short necked clam and artificial diets resembling the amino acid contents of P.japonicus, had high feed efficiencies. Therefore, they recommended that effective artificial diets should have a pattern of amino acid distribution similar to the prawn itself.

The amino acid requirements for M.rosenbergii establi-shed in this study reveal higher methionine and arginine levels than short necked clam and higher methionine levels than squid meal (Table:5.3). Compounded diets containing lower A/E ratios for methionine and arginine when compared to soft clam resulted in poor growth in fed to P.Monodon (Deshimaru et al.,1985). Supplementation of arginine and methionine to a basal diet containing squid meal (Kitabayashi et al.,1971c) improved growth of P.japonicus. Therefore, higher methionine and arginine requirements found in the present study than best conventional protein sources for prawns and shrimps indicate the importance of these two amino acids to M.rosenbergii.

Except for arginine, lysine, phenylalanine and isoleucine, the EAA requirements suggested for M.rosenbergii in the present study are more or less similar to the requirements reported for other farmed animals (fishes, chicks, young pig and rats) (Table:5.4. from NRC,1983). The arginine and lysine requirements of prawns are higher than all other farmed animals. This may be due to the difference in taxonomic positions of prawns compared to the rest, which need to be studied in detail.

5.1.1.1.9 Establishment of Mineral requirements for M.rosenbergii broodstock using published data.

Information on the mineral requirements of shrimps and prawns is scanty (section.5.1.1.1.6). Mineral levels used in Crustacean diets greatly varied (Table:5.5). Therefore, in the present study mineral levels used in various Crustacean diets) were used to establish the mineral requirements for M.rosenbergii broodstock.

The mineral requirements of broodstock M.rosenbergii were expected to be higher than for juveniles as they migrate to brackish water for breeding in their natural habitat.

The suggested mineral levels were incorporated in the diets by using 8.5% of the premix presented in Table:5.6.

Table:5.4. Quantitative essential amino acid requirements of selected animals (experimentally determined) and the recommended requirements for M.rosenbergii in the present study (expressed as % protein).

Amino acids	Japaneese Eel	Common Carp	Channel Catfish	Chinook Salmon	Chick	Swine	Rat	M.rosenbergii
Arginine	4.5	4.2	4.3	6.0	5.6	1.2	5.0	8.1
Histidine	2.1	2.1	1.5	1.8	1.4	1.2	2.5	2.3
Isoleucine	4.0	2.3	2.6	2.2	3.3	3.4	4.2	5.0
Leucine	5.3	3.4	3.5	3.9	5.6	3.7	6.3	7.5
Lysine	5.3	5.7	5.0	5.0	4.7	4.4	5.8	5.3
Methionine	5.0	3.1	2.3	4.0	3.3	2.3	5.0	3.0
Phenylalanine	5.8	6.5	5.0	5.1	5.6	4.4	6.7	3.9
Tyrosine	-	-	-	-	-	-	-	3.0
Threonine	4.0	3.9	2.0	2.2	3.1	2.8	4.2	3.4
Tryptophan	1.1	0.8	0.5	0.5	0.9	0.8	1.3	0.9
Valine	4.0	3.6	3.0	3.2	3.4	3.2	5.0	4.4

Source: NRC, (1983).

- Regarded as non essential amino acid (See section 5.2.2.3 for details)

Table:5.5. Composition of mineral premix recommended and used in shrimp and prawn diets, and suggested levels for M.rosenbergii in the present study.

	Recommended or used levels in diets (% dry diet)									
Elements	(1) 7.7	(2) 5.0	(3) 2	(4) 13	(5) 8.6	(6) 1.0	(7) 21.4	(8) 7.0	(9)	(10)
g.100g^{-1}										
Calcium	1.34	0.66	0.26	1.86	1.05	-	-	0.95	1 - 2	1.50
Phosphorus	0.83	0.51	0.21	1.82	1.06	-	-	0.97	1 - 2	1.50
Potassium	1.42	0.92	0.37	0.83	0.90	-	-	0.42	.9-1.8	1.15
Magnesium	0.23	0.15	0.06	0.13	0.30	-	-	0.07	.1-0.5	0.30
Sodium	-	-	-	0.54	0.12	-	-	0.29	-	0.35
mg.kg^{-1}										
Iron	217	241	96.4	3.77	-	181	19.8	181	<140	65.0
Copper	30.5	-	-	1.25	-	109	2.0	-	<600	300
Manganese	-	19.8	7.9	3.43	-	814	60.0	17.5	<100	75.0
Zinc	-	-	-	141	-	455	44.0	75.6	-	10.0
Cobalt	-	-	-	4.40	-	90.0	0.2	31.5	-	02.0
Ca:P	1.61	1.28	1.28	1.02	1.00	-	-	1.00	1.00	1.00

1 Kanazawa et al., (1970) 2 Sick et al.,(1972)

3 Andrew and Sick (1972) 4 Kanazawa et al, (1976) 5 Colvin,(1976)

6 Balaz et al.,(1974) 7 Deshimaru and Kuroki, (1974)

8 Kanazawa et al., (1984) 9 Recommonded/used range

10 Levels used in the present study

Table:5.6. Composition of the mineral premix used in the experimental diets

The mineral premix was formulated based on available information on published data.

(See section 5.1.1.1.6 for details)

Minerals	Chemical formula	g/100g
Calcium orthophosphate	$CaHPO_4.2H_2O$	53.00
Magnesium sulphate	$MgSO_4.7H_2O$	14.00
Potassium diphosphate	K_2HPO_4	12.00
Potassium chloride	KCl	10.00
Sodium chloride	NaCl	9.88
Copper sulphate	$CuSO_4$	0.90
Manganese sulphate	$MnSO_4.4H_2O$	.10
Iron sulphate	$FeSO_4.7H_2O$	.07
Zinc sulphate	$ZnSO_4.7H_2O$	.03
Cobolt sulphate	$CoSO_4.7H_2O$	.01
Calcium iodate	$CaIO_3.6H_2O$	.01

5.1.2 Materials and methods

5.1.2.1 Design of experimental diets

Based on the nutritional requirements of juvenile shrimps and prawns and broodstock fish (discussed in sections 5.1.1.1) three diets were formulated. Two composed of protein sources of animal origin to contain 35% and 17% protein (referred to as 35AP and 17AP respectively). The third diet contained 17% protein from plant protein sources (referred to as 17PP). Differences in protein levels were adjusted with cellulose. The sources and chemical composition of the ingredients used in the formulation are presented in Appendixes A and B.

All three diets were formulated to contain (on dry weight basis) 10%lipid, 0.5%cholesterol, 30-40% carbohy-drates, 0.25% chitin, 8.5%mineral premix, 3% vitamin premix and gross energy of 4-4.5 K.cal.g^{-1} dry diet. The amino acid levels in the formulation are presented in Table:5.3. The compositions of the ingredients used in the formulation are presented in the Table:5.7.

Sodium alginate was used as the binder and sodium hexa-meta phosphate as sequestrant (Meyers,1980). Butylated hydroxy toluene (BHT) was added to prevent the oxidation of fatty acids and preserve lipid quality (NRC,1983). Sodium sorbate was added as an anti-microbial agent (NRC,1983).

Diets for digestibility studies were formulated separately from diets used for growth studies. Chromic oxide

Table:5.7 Composition of ingredients used in the exprimental diets

Ingredients	Diets					
	35AP	35APD	17AP	17APD	17PP	17PPD
Squid meal	21.50	21.50	9.43	9.43	-	-
Shrimp waste meal	3.00	3.00	1.73	1.73	1.73	1.73
Mussel granulate	10.00	10.00	6.00	6.00	-	-
Fish meal	7.70	7.70	3.43	3.43	-	-
Pruteen (SCP)[1]	3.90	3.90	1.73	1.73	-	-
Linseed meal	-	-	-	-	9.00	9.00
Oat germ meal	-	-	-	-	15.00	15.00
Alfalfa meal	-	-	-	-	7.00	7.00
Cotton seed meal	-	-	-	-	10.00	10.00
Wheat bran	-	-	-	-	8.00	8.00
Sunflower meal	-	-	-	-	5.00	5.00
Cod liver oil	2.00	2.00	2.00	2.00	5.50	5.50
Soya oil	5.50	5.50	5.50	5.50	2.00	2.00
Starch	15.38	15.38	18.91	18.91	5.00	5.00
Dextrin	15.00	15.00	18.25	18.25	4.75	4.75
Chitin	0.25	0.25	0.25	0.25	0.25	0.25
Cellulose	-	-	14.00	14.00	11.00	11.00
Vit.mixture[2]	3.00	3.00	3.00	3.00	3.00	3.00
Min.mixture[1]	8.50	8.50	8.50	8.50	8.50	8.50
BHT	0.02	0.02	0.02	0.02	0.02	0.02
Cholesterol	0.50	0.50	0.50	0.50	0.50	0.50
Chromic oxide	-	0.50	-	0.50	-	0.50
Alginate	2.00	2.00	3.00	3.00	3.00	2.00
Sodium hex.met.phos.	1.00	1.00	1.50	1.50	1.50	1.00
Sodium sorbate	0.25	0.25	0.25	0.25	0.25	0.25
Polypropylene	0.50	-	2.00	2.00	1.50	-
Total	100.00	100.00	100.00	100.00	100.00	100.00

1 Mineral mixture for composition see Table:5.6

2 Vitamin mixture for composition see Table:5.8

SCP[1] Single cell protein, ICI PLC, England

BHT Butylated hydroxy toluene

AP Animal protein

PP Plant protein

D Digestibility diets

Table:5.8. Composition of the vitamin premix used in the experimental diets

Vitamin	g/kg of premix*
Thiamine (B_1)	2.500
Riboflavin (B_2)	2.500
Pyridoxine (B_6)	2.000
Pantothenic acid	5.000
Inositol	100.000
Biotin	0.300
Folic acid	0.750
Para aminobenzoic acid	2.500
Choline	200.000
Niacin (Nicotinic acid, B_3)	10.000
Cyanocobalamin (B_{12})	0.005
Retinol palmitate (A)	100,000.000
- tocopherol acetate (E)	20.100
Ascorbic acid (C)	50.000
Menadione (K)	2.000
Cholecalciferol (D_3)	500,000.000

According to Tacon *et al* (1982).

* Made up to 1 kg with cellulose

was used as the inert marker. Polpropylene powder was used in the growth diets to compensate for chromic oxide.

The performance of the experimental diets was tested against a control diet. This was composed of chopped squid, mussels, shrimps, whitebait and lambs' liver and spinach which has been reported to be the best conventional food mixture for shrimps and prawns (section 5.1.1.1).

5.1.2.2 Diet Preparation

All dietary ingredients used (Appendix.A) were finely ground using a Cyclotec 1093 sample mill (Tecator Sweden) and coffee grinder and sieved through 200 µm mesh except wheat bran (difficult to grind) which was seived through 790 um mesh. The dry ingredients were weighed, according to the formulation in Table:5.7. and mixed well in a Hobart A200 Industrial food mixer (Hobart Co. Ltd, UK) until uniformly blended. To this mixture were added weighed quantities of soya bean oil and cod liver oil (Table:5.7.). and blending continued for a further 15 minutes. Finally 35-40% hot water (90^0C) (on a volume /dry mix weight basis) was added slowly with continuous mixing. This procedure adopted in diet preparation is similar to that of Meyers(1980). However, instead of dissolving alginate and the sequestrant (sodium hexametemosphate) in hot water, these were added to the mixture in order to ensure thorough mixing of the binder. The amount of water used was also less than recommended by these authors. 35-40 % water was found to be

the optimum level for this mixture to be pelleted using a California pelleting mill (Model CL2). The pellets were extruded through a 3mm die and the cutter was adjusted to produce uniform length pellets.

Pellets were then air-dried at 35-40^{0}C using an electric fan convector heater. Dried pellets were stored at -20^{0}C until thawing before feeding. The pellets were analysed for physical and chemical properties as described in section 5.1.2.3. and 5.1.2.4

5.1.2.3 Physical properties of diets

5.1.2.3.1 Weight of pellets

Random samples of 125 pellets from each diet were taken and weighed (±0.0001g) in groups of five pellets together. Results of mean weight of pellets of the experimental diets are presented in Table: 5.9.

5.1.2.3.2 Size of pellets

Random samples of 25 pellets from each diet were measured using a vernier caliper (±0.01mm) and the average sizes of pellets are presented in Table:5.9.

5.1.2.3.3 Density of pellets

Densities of the pellets were calculated from the data on length, diameter and weight.

$$\text{Density of pellet } (g.cm^{-3}) = \frac{\text{Mean dry weight of pellet} * 7}{(\text{diameter})^2 * \text{length} * 22}$$

Results are presented in Table:5.9.

5.1.2.4 Chemical composition of diets

5.1.2.4.1 Preparation of Samples

Ingredients and diets were milled using a Cyclotec 1093 sample mill (Tecator,Sweden) and coffee grinder and sieved through 100-200 micron mesh before analysis. Dried faeces were powdered using a mortar and pestle. All samples were stored at -20^0C prior to analysis.

Methods of analysis of proximate composition, amino acids of dietary ingredients and diets, together with the methods used to analyse lipid classes, fatty acids and mineral contents of diets were as discussed in Chapter.2.

The compositions of dietary ingredients are presented in Appendix A&B and the diets were presented in Tables:5.10-5.14.

5.1.3 Results

The plant protein diet is lighter in weight than the animal protein diets (Table 5.9). Expected minimum dietary nutrient levels were reflected in the experimental diets (Tables 5.10-5.14). However there are differences in the lipid and Ash levels (Table 5.10).

Table:5.9. Physical properties of experimental diets

Properties	DIETS					
	35AP	35APD	17AP	17APD	17PP	17PPD
Mean diameter of pellet (mm)	03.20	-	03.20	-	03.20	-
Mean length of pellet (mm)	06.90 ± 0.32	-	07.30 ±0.38	-	07.19 ±0.39	-
Mean weight of pellet (mg)	61.21 ±2.54	-	61.37 ±2.53	-	48.61 ±2.18	-
Mean density of pellet(g/cm^3)	1.01	-	0.96	-	0.83	-

± Standard deviation

Table:5.10. Proximate composition of experimental diets

(expressed as % dry matter except drymatter)

COMPOSITION	DIETS					
	35AP	35APD	17AP	17APD	17PP	17PPD
Dry Matter	91.52	91.56	91.30	92.04	91.89	89.74
Total Carbon	-	44.05	-	44.25	-	42.80
Crude Protein$_k$	34.99	35.15[a]	17.84	17.16[a]	16.75	17.26[a]
Crude Protein$_e$	-	32.85[a]	-	16.55[a]	-	16.59[a]
Crude Lipid	13.76	12.61	09.24	09.50	10.16	10.49
Crude Fibre	03.02	03.16	12.80	12.78	14.88	14.96
Ash	14.27	14.80	11.72	11.93	13.12	13.41
Chromic Oxide	-	0.44	-	0.43	-	0.45
Gross Energy ($K.Cal.g^{-1}$)	05.08	-	04.85	-	04.70	-
Protein:Lipid	2.54:1	-	1.93:1	-	1.65:1	-
Protein:Energy (Mg Protein:$K.cal^{-1}$)	68.60	-	36.80	-	35.60	-

D digestibility diets

k Crude Protein determined by kjeldahl. - Not Determined

e Crude Protein determined by CNH Elemental analyser.

Values having the same superscript in the same column are not significantly ($P>0.05$) different by t-test.

Table:5.11. Essential aminoacid composition of experimental diets (expressed as % protein)

Aminoacids	Diets			EAA Requirement of *M.rosenbergii* [1]
	35AP	17AP	17PP	
Arginine	5.87	5.80	5.20	8.06
Histidine	1.98	1.68	1.67	2.27
Isoleucine	4.39	4.03	2.89	5.02
Leucine	7.13	5.72	4.69	7.48
Lysine	6.66	5.72	2.72	5.28
Methionine	2.26	1.14	0.38	3.01
Phenyl alanine	3.79	3.07	3.78	3.93
Tyrosine	2.29	0.97	1.71	2.93
Threonine	3.82	2.52	2.38	3.43
Tryptophan	-	-	-	0.92
Valine	4.71	4.04	3.35	4.39

(EAA requirements determined as described in section 5.2.2)

Table:5.12 Lipid class composition of experimental diets

(Expressed as % total lipid)

Lipid Classes	Diets		
	35AP	17AP	17PP
Total Polar lipids (%)	14.36	7.69	6.67
Total Neutral lipids (%)	85.64	92.31	93.56
Neutral lipids			
Triacylglycerol	57.87	61.97	35.44
Cholesterol	17.95	18.48	15.67
Free fatty acids	7.29	7.91	31.00
Sterol esters	-	-	-
Diacylglycerols	2.53	3.95	11.44
Monoacylglycerols	-	-	-
Wax esters	-	-	-
Polar Lipids			
Phosphatidylcholine	6.76	3.63	1.67
Phosphatidylethanolamine	4.01	1.18	2.22
Phosphatidyllinositol	2.64	1.82	tr
Phosphatidylserine	-	-	-
Sphingomyelin	1.00	1.07	1.22
Phosphatidic acid	-	-	tr

- Not detected tr Trace levels

Table.5.13. Fatty acid composition of experimental diets (expressed as % fatty acids)

Fatty acids	Diet		
	35AP	17AP	17PP
14:0	1.9	1.8	3.7
15:0	0.2	0.2	0.3
16:0	14.4	13.2	15.3
16:1(n-7)	2.7	2.2	3.6
16:2	0.3	0.3	0.6
17:0	0.3	0.2	0.3
16:3	0.2	0.2	0.4
16:4	0.2	0.2	0.5
18:0	4.1	4.0	2.9
18:1(n-9)	23.9	23.9	17.3
18:1(n-7)	2.8	2.1	1.8
18:2(n-6)	25.6	31.7	23.5
18:2(n-9)	0.0	0.0	0.0
18:3(n-6)	0.9	0.6	0.1
18:3(n-3)	3.3	3.9	2.6
18:4(n-3)	0.9	0.9	1.9
20:0	0.3	0.3	0.3
20:1(n-11)	0.2	0.2	0.0
20:1(n-9)	1.9	1.6	3.3
20:1(n-7)	0.1	0.1	0.2
20:2(n-6)/20:3(n-9)	0.2	0.1	0.2
20:4(n-6)	0.3	0.2	0.3
20:3(n-3)	0.2	0.1	0.1
20:4(n-3)	0.2	0.2	0.4
20:5(n-3)	4.0	2.9	5.5
22:0	0.3	0.3	0.2
22:1	2.2	2.0	4.5
22:5(n-6)	-	0.1	-
22:5(n-3)	0.4	0.3	0.7
22:6(n-3)	5.5	3.4	5.5
24:0	0.1	0.1	0.1
24:1	0.3	0.2	0.5
Total saturates	21.6	20.1	23.1
Total Monoenes	34.2	32.4	31.3
Total (n-3)	14.5	11.5	16.5
Total (n-6)	26.9	32.7	24.1
(n-3)/(n-6)	0.5	0.4	0.7
Total PUFA	42.2	44.9	42.1
Total Unknowns	2.1	2.5	3.5

- Not detected

Table:5.14. Mineral composition of experimental diets

(Expressed as % dry matter basis)

Minerals		35AP	17AP	17PP
Calcium	(Ca)	1.570	1.406	1.439
Magnesium	(Mg)	0.078	0.075	0.155
Potasium	(K)	0.769	0.551	0.979
Sodium	(Na)	1.590	1.325	0.811
Iron	(Fe)	0.031	0.016	0.007
Zinc	(Zn)	0.019	0.022	0.045
Copper	(Cu)	0.013	0.015	0.021

5.1.4 Disscussion

The differences in lipids, minerals, amino acids, fatty acids and minerals among the diets is due to different levels of the above nutrients in dietary ingredients. However, the nutrient contents were above the expected levels in the diets and assumed to contribute to the requirement of the animal. There is possibility for loss of nutrients due to leaching and manipulation of food (Brown et al.1986; Forster and Gabbot,1971; Newman et al. 1982).

5.2. Experimental design and the materials used in broodstock nutrition experiments

5.2.1. Experimental animals

Many species of fishes mature when their body reaches a certain size rather than at a certain age (Love,1970,1980, 1988; referring to many authors). Similarly, positive correlations between egg production and physical dimensions of females belonging to genus Macrobrachium have been discussed in Chapter 4. In addition egg production in M.rosenbergii has been found to be dependent on size rather than on age. Due to social hierarchy in prawns considerable size and weight distribution exists among individuals of uniform age (review Ra'naan and Cohen,1985). Therefore, in the present study experimental animals were selected and matched on the basis of uniform size rather

than age. Females ranging in size from 29.5-30.5mm CL and 19.5-20.5g in weight were used.

5.2.2 Treatments and replicates

Three experimental diets were used in this study plus one control. The nature of both the diets and the control diet is described in section.5.2.1 7-8 pubertal female M.rosenbergii were used for each treatment. The experimental females were raised individually in separate experimental units as described in Chapter.2.1. Group experiments with mature crustaceans are difficult due to constraints such as;

a) social interactions which enormously influence the growth of individuals (Ra'naan and Cohen,1985),

b) cannibalism, especially while moulting,

c) unequal ingestion of food due to aggressiveness resulting in artefacts in estimation of acquisition of available nutrients (Taechanuruk and Stickney,1982).

d) difficulties in tagging crustaceans, due to periodic shedding of exoskeleton, which will impede selective assessment of performance such as spawning pattern,

e) handling of individuals for measurements, egg sampling and while mating could inflict stress to the rest of the population,

f) group experiments involve greater numbers of individuals in order to have adequate replication. This inflicts constraints on space, facilities, man-

-power and time, thus making the study infeasible.

In order to minimise the above constraints, most of which would adversely influence results, the experimental females were raised individually. All data collected were analysed separately considering each animal as one replicate.

5.2.3. Duration of the experiment.

The most prominent process dominating the life of Crustacea is moulting and all physiological processes such as growth, regeneration and reproduction are intorgrated and co-ordinated to phase with the moulting cycle (see Chapter.3). The physiological state of the animal is dependent on the stage of the intermoulting period. This complicated process of moulting does not exist in fish or other farmed animals. Therefore experiments designed to explore the factors in question influencing growth and reproduction were relative to a timescale in non moulting animals. It is assumed that a particular group of animals selected for an experiment (based on size or age) are in an identical physiological state, at any one particular time, in this timescale.

The above assumption may not apply for Crustaceans, especially those with long intermoulting periods. The individuals in a population, even if they are siblings, will not moult in a co-ordinated pattern (see section 3.4). Therefore, at any one particular time individuals are at

different stages of their intermoulting period and cannot be assumed to be in identical physiological states. This factor would results in large variations within treatments. For example, influence of moulting on spawning has been discussed in section 3.4.4. While randomly selecting prawns, animals at different intermoulting stages will be selected. At the end of the experiment some prawns might have completed two moults, some three and others may be nearing their fourth. Prawns completing fewer moults (eg:2) would measure less (carapace length) than those completing more (eg:3) as the size increment takes place only after moulting.

This influence could be overcome by the use of a large number of animals per treatment and longer duration of the experiment, so as to allow more moults to take place.

In the present design it was not feasible to increase the number of animals due to constraints outlined in section 5.2.2.

Therefore a relatively novel approach was adopted in the present experiment relating growth and reproductive performance to "physiological state" of the animal rather than to a time scale. "Physiological state" of the animal was related to the intermoulting period assuming that animals at a particular intermoulting stage are in an identical physiological state.

The duration of such an experiment is based on the number of moults (five in this experiment). The time taken for animals to reach the required number of moults was used as a parameter to measure performance. The experiment was started when the broodstock reached the required initial physical dimensions. They were fed on the experimental diets from the 10th day of the moult cycle during which they acquired the initial required physical dimensions.

The moult after first receiving the experimental diet is considered as the first experimental moult. The experiment was terminated at the 5th experimental moult. Prawns were then fed with "digestibility diets" for a further week and digestibility studies were carried out. 10 days after the 6th experimental moult the animals were sampled for biochemical analysis. The average duration of the experiment was about 165 days per animal.

5.2.4 Materials used in the broodstock nutritional studies.

The experimental system and history of animals prior to the experiment, egg sampling procedures and maintenance of experimental conditions were as described in Chapter.2.

The experimental system S_1 was used in this study. The experimental animals were randomly selected from Stocks A and B. Detailed procedures have been described.

5.3. Suitability of gravimetric and indigestible marker methods for digestibility studies with M.rosenbergii.

5.3.1. Introduction

The nutritive value of a feed is not only dependent on the quality and quantity of essential nutrients but also on its ability to be digested and absorbed by the animal concerned.

The percentage of ingested nutrients which are not rejected as faeces is referred to as digestibility (Schneider and Flat, 1975). Measurement of the digestibility of different feed ingredients and diets gives additional understanding of the efficiency of food utilisation. The true digestibility of any nutrient may be determined as;

$$\text{True digestibility} = \frac{I - (F - F_k)}{I} \quad \text{(Utne, 1979) .. 1}$$

I = Nutrient Intake

F = Total faecal nutrient

F_k = Endogenous faecal nutrient.

True digestibility is often expressed as;

Digestibility Coefficient = True digestibility * 100

Endogenous faecal nutrients (EFN) could be due to either one or more of the following in decapods;

a) residue of digestive enzyme b) sloughed epithelial cells c) bacterial residues d) chitinous peritrophic membranes

around the faecal pellets (Forster and Gabbot,1971)

Tegumental gland secretions, such as glycoproteins (Shyamasundari and Hanumantha Rao,1978), can also contribute to EFN in decapods. EFN estimation in two caridean prawns by two different methods gave widely different values of 39.3 and 185.2 mg N per 100g diet (Forster and Gabbot, 1971). These authors also compared true and apparent digestibility coefficients and found that the error in estimating the true digestibility was less than 3.5 % for dietary nitrogen levels greater than 6%.

Due to difficulties in estimating EFN, the digestibility is usually measured as;

Apparent Digestibility Coefficient (ADC) .

$$\text{ADC (\%)} = \frac{\text{Nutrient Intake - Faecal Nutrient}}{\text{Nutrient Intake}} \times 100 \quad .. 2.$$

The ADC is often referred as Apparent Drymater Digestibility Coefficient (ADMD) with respect to digestion of dry matter. Depending on the nutrient concerned it is also referred to as Apparent Crude Protein Digestibility (ACPD), Apparent Crude Lipid Digestibility (ACLD) etc.,

Differences in digestibilities of diets containing different protein sources and levels have been reported in crustacea. P.japonicus (Nose,1964) and P.stylirostris (Fennucci et al.,1982) were found to digest animal protein sources better than those of plant origin. In contrast,

Procambarus clarkii digested plant protein sources better than animal sources. M.rosenbergii adults have been found to be capable of digesting different plant protein sources with ADPC varying from 84% to 89% (Ashmore et al,1985).

In the present study digestibility of the experimental diets (composition of diets, section 5.1.3) containing different protein levels and sources was evaluated in order to;

a) study the ability of the broodstock to digest different diets containing different protein sources and levels,

b) quantify the dry matter and protein absorbed by broodstock and correlate this with observed growth and reproductive performance.

Digestibility of nutrients is measured by one of three different techniques. Gravimetric, use of indigestible markers and radioisotopic markers (Kleiber,1975; Cited Leavitt,1985). Apart from these in vivo methods attempts have also been made to determine digestibility of nutrients in fish using in vitro techniques which are commonly employed for farm animals (Hepher,1988).

The most commonly used method in fish is the indigestible, external marker chromic oxide (Cr_2O_3) (Cowey and Sargent,1972; Bowen,1978; NRC,1983; Kirchgessner et al., 1986; Hepher,1988) and in decapods (Nose,1964; Forster and Gabbot,1971; Colvin,1976a; Fenucci et al,1982; Taechanuruk and Stickney,1982; Teshima and Kanagawa,1983; Smith et al, 1985; Ashmore et al.1985).

Initially it was planned to use chromic oxide as the marker in the present study. The diets were formulated to contain 0.5% chromic oxide (section 5.3). Whilst collecting faeces differences in coloration of faecal pellets (dark green, light green and brownish regions) were observed (Plate.5.1). This may have been due to differential passage of chromic oxide through the gut.

Similar observations have been made in Palaemon and Pandalus sp. (Forster & Gabbot,1971), American lobster (Leavitt,1981, 1985; Bordner et al.,1983) crayfish (Brown et al.,1986) and in fishes, Tilapia (Bowen,1978: Tacon and Rodrigues,1984) . The above workers questioned the validity of the use of chromic oxide in digestibility studies. Brown et al.(1986) reported negative ADC values. Bowen(1978) observed very low digestibility values. To overcome this problem Bordner et al.(1983) and Teshima and Kanazawa (1983) quantitatively recovered all the faeces for Cr analysis. Leavitt(1985) argued that such quantitative recovery negates the need for adding an indigestible marker and in such case the gravimetric method is easier and less prone to measurement error. Leavitt(1985) and Brown et al.(1986) recommended "gravimetric" as the method of choice for digestibility studies in American lobster and crayfish respectively.

The gravimetric method involves quantitative faecal estimation of food ingestion and faecal ejection and gives more accurate results (Smith,1979). The accuracy of this

method is dependent on accurate estimation of ingested and egested nutrient by the animal.

Therefore, in the present study digestibility of the diets was evaluated using both gravimetric method and chromic oxide method.

5.3.2. Materials and Methods

The experimental facility used in the present study was similar to that used in the growth trial (Fig:2.2.). The experimental units were slightly modified. The inlets were covered with 40 µm nylon mesh to prevent entry of any suspended solids and at the outflow 100 µm nylon mesh to prevent escape of uneaten food and faeces (see Fig 2.2).

At the end of the growth trial, after the 5th experimental moult, the digestibility diets (section 5.1.3) were fed for a 10 day period. Animals were trained to consume diets within 3 hr. of feeding as they were used to feeding overnight during the growth trial. For each diet at least three prawns, kept individually, were used.

After the acclimatisation period, diets were weighed and fed at 13.00 hr. The uneaten food were siphoned onto pre-weighed 100 µm mesh at 15.00 hr. and dried at 108^0C overnight and reweighed to obtain the amount of uneaten food. Water in the tank was drained (80%) in order to flush away any suspended solids with minimum disturbance to the animal.

All the faeces produced were siphoned into a beaker at 17.00hr. and 10.00hr. the following day and again 30 min. after feeding. All three batches of faeces were pooled and transferred into pre-weighed aluminium drying pans and dried at 108^0C in an oven overnight. The pans with dried faeces were reweighed to obtain the dry weight (±0.01 mg)(Oterling R51) of the faeces produced at any one meal. This was continued for about five days. The filter screens were cleaned every day before feeding.

From the weight of food consumed and the amount of faeces produced per meal the digestibility coefficients were calculated separately for each female.

The crude protein, carbon content and the chromic oxide contents of the feaces were determined as described in section 2.7. The faeces collected from each treatment were pooled into two groups and triplicate samples for each group were analysed. For chromic oxide analysis duplicate samples were used.

Apparent digestion coefficients were calculated from the equations:

Apparent Drymatter digestibility ADMP(%)

$$= \frac{\text{(Dry matter consumed - Dry matter excreted) * 100}}{\text{Dry matter consumed}}$$

Apparent Nutrient digestibility (%)

(Crude protein (ACPD) and Carbon (ACD)

(as in section 5.9.1 equation 2.)

Apparent digestion coefficients determined by the (chromic oxide) indigestible indicator method were calculated by the equations.

$$ADMP(\%) = 100 - \left[100 * \frac{(\ \%\ Cr_2O_3 \text{ in diets})\ ]}{(\ \%\ Cr_2O_3 \text{ in faeces})} \right.$$

$$ACPD\ (\%) = 100 - \frac{[100 * \%\ Cr_2O_3 \text{ in feed} * \%\ \text{Nutrient in faeces}]}{\%\ Cr_2O_3 \text{ in faeces} * \%\ \text{Nutrient in diet}}$$

ACD

The results are presented in the Table .5.15.

Theoretically, all the chromic oxide consumed should be excreted with the faeces if not absorbed by the animal. That is;

$$\%\ Cr_2O_3 \text{ in diet} * \text{Wt. diet consumed (mg)} = \%\ Cr_2O_3 \text{ in faeces} * \text{Wt. of faeces (mg)}$$

Wt. = Weight

Therefore the total weight of the faeces expected from the animal would be;

$$= \frac{\%\ Cr_2O_3 \text{ in diet} * \text{Wt. of diet consumed (mg)}}{\%\ Cr_2O_3 \text{ in the faeces}}$$

The % unrecovered faeces was calculated as;

$$= \frac{\text{Expected amount of faeces} - \text{recovered amount of faeces}}{\text{Expected amount of faeces}} * 100$$

To compare the passage of dietary Cr in a non caridean decapod diet 17AP was fed to three P.monodon adults (34mm in carapace length) raised communally in a recirculated sea water system. Faeces were pooled and analysed in triplicate for crude protein and chromic oxide as described in section 2.7.

5.3.3. Results

The faeces produced by M.rosenbergii females fed all three diets were found to contain regions with discrete distribution of Cr pigment as mentioned earlier. In contrast homogeneous distribution of Cr was observed in the faeces of P.monodon (Plate.5.1.) .

All digestibility coefficients estimated by the chromic oxide marker method were lower than the values estimated by the gravimetric method (Table: 5.15).

Unrecovered faeces accounted for up to 87% of the total expected faecal production (Table:5.15).

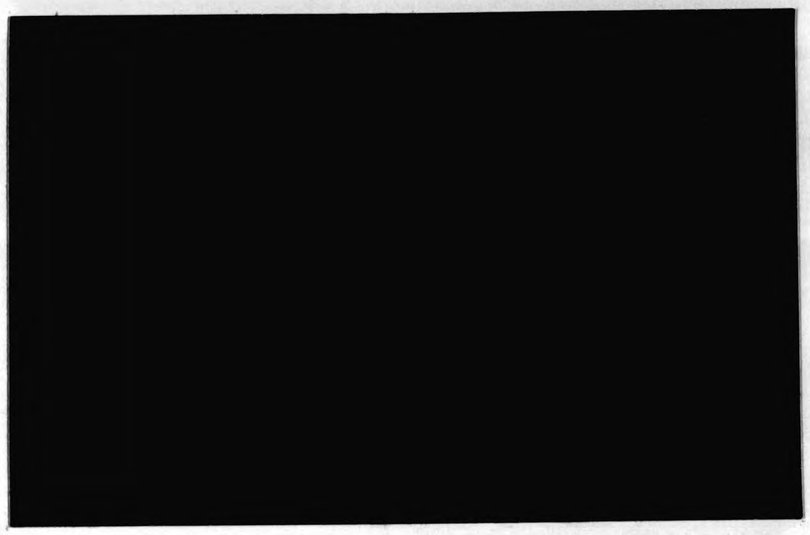
The ADDC and ACPC for P.monodon were 23% and 10% lower respectively than for M.rosenbergii.

5.3.4. Discussion

Differential passage of Cr in the faeces of fishes and some decapods has been reported by many workers (see

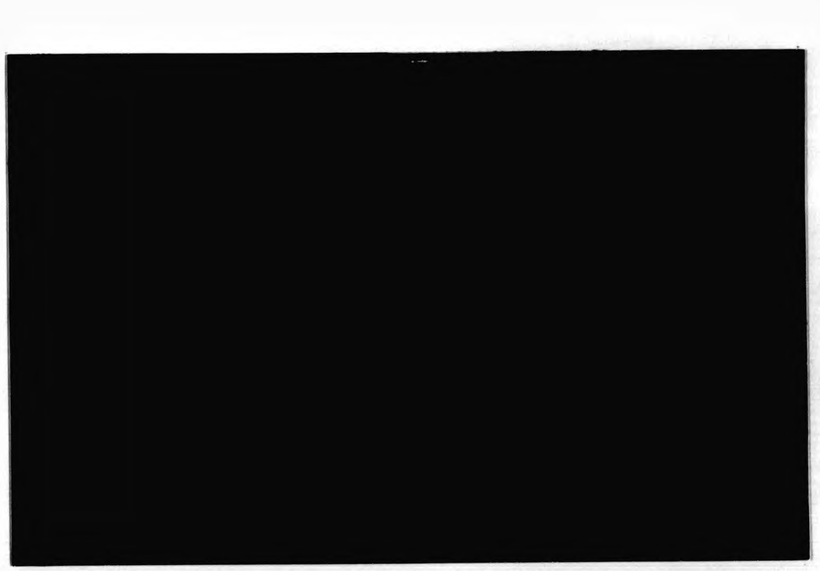
Plate:5.1 Faecal pellets produced by M.rosenbergii and P.monodon fed diets containing chromic oxide.

(a) M.rosenbergii



(Note: Discrete distribution of chromium. G = Green coloration B = Brown coloration)

(b) P.monodon



(Note: Homogeneous distribution of chromium)

Plate:5.1 Faecal pellets produced by M.rosenbergii and P.monodon fed diets containing chromic oxide.

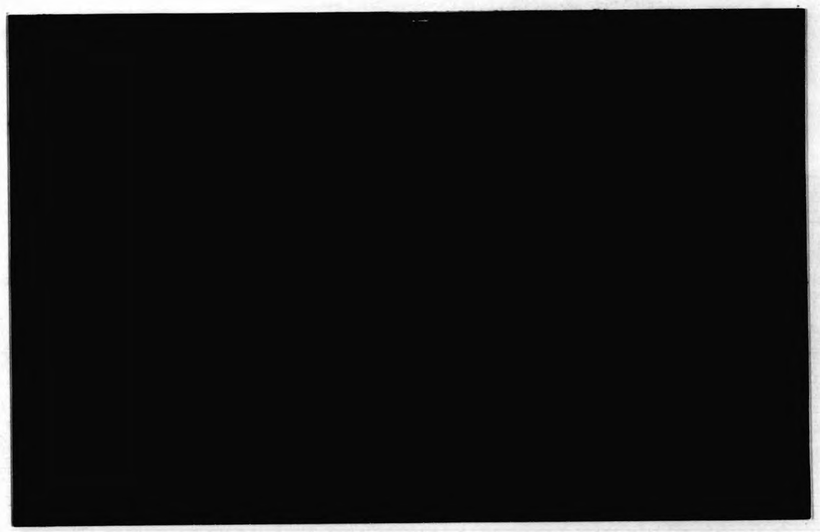
(a) M.rosenbergii

(Note: Discrete distribution of chromium. G = Green coloration B = Brown coloration)

(b) P.monodon

(Note: Homogeneous distribution of chromium)

a



b

Table:5.15. Apparent digestibility coefficients for nutrients in experimental diets fed to M.rosenbergii broodstock and P.monodon adults (expressed as %).

Digestibility Coefficient			35AP	17AP	17PP
Apparent Dry matter	D.C.	G.	98.72 ±0.25	97.88 ±0.36	96.75 ±0.56
		Cr	92.98 ±2.18	88.27 ±0.12	86.38 ±1.69
	P.M	Cr		67.73	
Apparent Protein	D.C.	G.	99.14 ±0.27	98.33 ±0.04	96.90 ±0.72
		Cr	95.23 ±2.05	90.22 ±0.16	87.15 ±0.81
	P.M	Cr		81.63	
Apparent Carbon	D.C.	G.	99.16 ±0.25	98.61 ±0.16	97.27 ±0.56
		Cr	95.38 ±1.90	92.27 ±0.35	88.57 ±1.82
	P.M	Cr		77.01	
% Unrecovered faeces			87.93 ±2.19	82.82 ±5.35	75.46 ±7.63

G Gravimetric Method Cr Chromic oxide as indicator

D.C Digestibility Coefficient P.M P.monodon

introduction). Although chromic oxide has been used in digestibility studies with M.rosenbergii (Taechanuruk and Stickney,1982; Ashmore et al.,1985) the above observations have not previously been reported. Ashmore et al.,(1985) used larger prawns (40-50g) than the ones used in the present study, in which the faecal strands are bigger and such differences could be readily distinguishable.

Differential passage of Cr in the faeces of animals could be due to several technical problems associated with diet preparation and patterns of food manipulation by the animal. Technical problems such as homogeneous mixing of chromic oxide in the diets and stability of the diets may not be responsible in the present study as differential passage of Cr was not observed in P.monodon fed the same diet.

Forster and Gabbot (1971) observed Cr being passed out before other undigested materials resulting in dark green coloured faecal pellets followed by light green and brown faecal pellets in Palaemon and Pandalus sp. They suggested that the Cr_2O_3 particles were sorted in the proventriculus and passed out through the alimentary canal in advance of rest of the digesta. M.rosenbergii belongs to caridea in which there is no gastric mill, like the above animals. Therefore it is possible that such a mechanism could be responsible for the discrete distribution of chromic oxide in the present study. The presence of a gastric mill and mastication of food inside the gut might have promoted

better mixing of all food particles resulting in homogenous distribution of Cr in the faeces of P.monodon.

M.rosenbergii is reported to be capable of utilising ^{14}C labelled bacteria and detritus (Costa-Pierce and Laws,1982). When unfed prawns were transferred to tanks containing detrital aggregates prawns were found to feed on them (personnel observation). In the present study the prawns were fed for only 2 hours per day. Therefore, it is also possible that they could have filter fed on detritus when food was not available. Undigested detritus could have mixed with the faeces and may be partly responsible for discrete coloration of the faeces. However such possibility was minimised using 40 um filter screens on the tank inlets in the present study.

Whatever the mechanism, differential passage of marker from the rest of the digesta in the present study violated one of the important prerequisites for use of the inert maker method (see Calow and Fletcher,1972; Foltz,1979). Therefore, chromic oxide cannot be used as an internal marker in digestibility studies with M.rosenbergii. This may apply to other caridean prawns as differential passage of Cr has been reported in Palaemon and Pandalus sp. (Forster and Gabbot,1971).

The major practical problem with the gravimetric method is quantitative recovery and accurate estimation of the uneaten food and faeces. This is reflected in the amount of unrecovered faeces estimated in the present study.

Although necessary precations were taken to prevent escape of faeces (section.5.3.2) it is difficult to collect all the faeces produced, the very high faecal losses estimated in the present study may also be associated with other factors. For example selective rejection of chromic oxide, in crayfish (Brown et al.1986), led to negative digesti--bility values. If such selective rejection is possible in M. rosenbergii this could have overestimated the expected faecal production in the present study.

The other major problem observed in caridean decapods is regurgitation of part of the ingested food. Particles like cellulose, lignin and fish scales have been identified in regurgitated materials from Palaemon and Pandalus species (Forster and Gabbot,1971) and M.rosenbergii (Newman et al.1982). The nature of the regurgitated material, whether digested or undigested, was not established. Therefore, it is possible that part of the Cr_2O_3 may have been regurgitated leading to over-estimation of consumption resulting in high estimated faecal loss values.

Thirdly, although not quantified escape of chromic oxide from the diet during pre-ingestion manipulation may have resulted in over estimation of expected faecal production. Caridean prawns are inefficient at food ingestion and do not posseses a gastric mill, but rather selectively manipulate and masticate food outside the buccal cavity with the anterior appendages (Patwardhan,1935)

It is difficult to differentiate regurgitated materials from faeces and uneaten food. This would cause artefacts in digestibility values obtained by the gravimetric method. Iwai(1976) considered regurgitated materials of M.rosenbergii to be part of the noningested fraction. Newman et al(1982) considered it partly digested, ingested, material regardless of route of expulsion. The latter author also observed that regurgitation can take place for minimum of 4 and maximum of 16-20 hrs. after feeding and accounted for an average of 76.8% of unassimilated material (seed sheaths of plant ingredients) not egested as faeces. Although not quantified, such high proportions of regurgitated materials were not observed in the present study. The egested materials were in the form of faecal pellets and a small proportion of particulate aggregates were observed, mainly in the faeces produced by the females fed plant protein diets. These aggregates were considered as egested materials as they were part of food provided and consumed by the animal, but may have been only partly utilised by them.

Higher digestibility values obtained by the gravimetric method in the present study are partly due to incomplete recovery of all the faeces. Lower digestibility values observed from the estimation of Cr_2O_3 may be due to losses of chromic oxide from the diets during food manipulation and by regurgitation as considered above. The Apparent drymatter digestibility coefficient values obtained in the present study with both the methods were very high and

unrealistic compared to the values (60-70%) obtained from other aquatic animals (Jauncey, pers.communication).

Difficulties commonly encounted when using gravimetric method have been discussed in section 5.[illegible] Additionally, in the present study difficulties in identification and separation of regurgitated materials from uneaten food was experienced. Therefore the gravimetric method is not suitable for digestibility studies with M.rosenbergii, especially studies involving many diets. Earlier in this section, widely used chromic oxide method was also found to be unsuitable for M.rosenbergii.

Apart from Cr_2O_3 another possible marker suggested and tried in aquatic animals is ash. Leavitt(1985), referring to many other works on fishes and his work on american lobsters, demonstrated that ash is absorbed by these animals. Newman et al,(1982) found that M.rosenbergii could absorb up to 58% of the inorganic components from diets. Although Clifford and Brick(1979) suggested that "the ratio of inorganic fractions in the diets and faecal samples (of M.rosenbergii) remained sufficiently invariable to validate the use of the ash-ratio technique" no data was presented in support of his statement.

Therefore none of the currently used digestibility methods seem to be satisfactory for M.rosenbergii. For comparative purposes reasonable values may be obtainable by using Cr_2O_3 as a marker and totally recovering all the

faeces for analysis, thereby anticipating losses of chromic oxide, rather than random sampling as in the case of most studies.

Differences in digestibility coefficients between diets are discussed in section 7.4.1.

CHAPTER 6

BROODSTOCK NUTRITION 11.

INFLUENCE OF BROODSTOCK DIETS ON GROWTH AND SPAWNING PATTERNS OF M.ROSENBERGII

6.1 Introduction

A constant supply of energy is important for all life sustaining processes such as maintenance, activity and synthesis of somatic and reproductive tissues. The source of supply of this energy is food.

In animal production systems food is supplied either by enriching that naturally available or by providing nutritionally balanced, quality controlled, diets. The production of nutritionally balanced diets requires research, quality control and biological evaluation (Cho et al.,1985).

The concepts behind establishment of the nutritional requirements of broodstock female M.rosenbergii, design of diets (based on empirical knowledge) and the quality of diets (both physical and chemical) used in this experiment were considered in Chapter 5.

The present Chapter principally explores the acquisition and utilisation of available dietary nutrients for the synthesis of somatic and reproductive tissues in broodstock M.rosenbergii.

6.1.1. Efficiency of utilisation of diets.

There are several constraints influencing the acquisition and assimilation of nutrients from the natural environments of fish and Crustacea (see Ivlev,1961; Vernberg and Vernberg,1983; Townsend and Winfield,1985; Weatherly and

Gill,1987). In captive environments these constraints can be broadly classified as follows; physical properties of the diet, nutritional quality of the diet, feeding regime, rearing methods and husbandry, physiological state of the animals and environmental conditions (see New,1976; Talbot, 1985; Hepher,1988 for reviews). Few attempts have been made to understand how diet utilisation is influenced by nutritional quality in Crustacea (discussed in section 5.1.1). Available information on how constraints influence intake and utilisation of food by cultured Crustacea is fragmentary.

Under defined environmental conditions, when "optimum growth" is used as a criterion for estimating dietary nutrient requirements, it is essential to employ an "efficient feeding regime" (see Talbot,1985; Tacon and Cowey,1985). This refers to supply of adequate food to satisfy the daily requirements of the animal. Production of a well balanced diet does not, in itself, have any merit unless adequate opportunities for the animal to feed are provided.

Citing a number of authors both Talbot (1985) and Tacon and Cowey,(1985) have emphasised the impact of choice of feeding regime on the conclusions drawn from any nutritional study. For example Dabrowski(1977) (cited by Tacon and Cowey,1985) fed a restricted ration (only twice daily and fixed on the lowest recorded ad lib feed intake) to carp fry. Fish fed lower protein diets were thus not given the

opportunity to consume sufficient food to meet their dietary protein and energy requirements. This resulted in a high observed "optimum" protein level of 41-43%. It was suggested that an "efficient feeding regime" should be established by prior feeding studies involving different feeding levels (Tacon and Cowey,1985) and by studying gut evacuation times (Talbot,1985) and duration of feeding.

Few attempts have been made to determine suitable ration sizes and feeding frequencies for cultured Crustacea. Ponnuchamy et al.(1983) reported maximum ingestion rates of 5.19 and 2.12g dry food 100g body weight^{-1} day^{-1} (recalcula-ted) for Caridae weberi (0.12g) and M.lachesteri (0.41g) respectively fed Tubifex sp. once a day for 1 hr. They observed highest growth rates and best food conversion ratios at these maximum ingestion rates as compared to lower ingestion rates. The maximum ingestion rate reported in the above study may not, however be the satiation level of the animals. The fresh food contained about 75% moisture which may have filled the proventriculus. The actual dry weight consumed was therefore low. The feeding regime was restricted to 1 meal per day without prior knowledge of the requirements of the animals possibly thus not giving adequate opportunity for the animals to consume food to satiation.

Feeding frequencies of twice daily for M.rosenbergii (0.1g with 20-45min. feeding duration) (Taechanuruk and Stickney,1982), 4 times daily for P.merguiensis (fed once

a day) and twice a day for P.monodon (fed for 1hr) (Sampath and Srithar,1987) have been recommended for prawns. All the above studies were based on arbitrary selection of meal size and duration of feeding without prior knowledge of maximum meal size or gut evacuation rates of the animals concerned. Similarly, many feeding regimes employed in fish studies have generally been related to the convenience of the researchers during their working day rather than to specific fish species own requirements (Tacon and Cowey,1985; Tacon, 1988).

Due to lack of reliable information on suitable feeding regimes, gut evacuation rates, meal sizes and duration of feeding, the broodstock in the present study were fed to satiation. As M.rosenbergii is a nocturnal animal the feeding period was adjusted to coincide with the onset of the dark cycle of the photoperiod. The duration of feeding was overnight (16-17hr) to ensure adequate food consumption. Omnivorous prawns, such as M.rosenbergii, can consume detritus (section 5.1.1) and bacteria (Stahl and Ahearn, 1978) from the water and tank surfaces, in the absence of adequate food. This may well influence the outcome of experiments (section 5.1.1).

6.1.2 Influence of diets on carcass composition

The body composition of fishes varies considerably with species and also with size, sexual condition, quality and quantity of food, environmental conditions, activity

regime etc., (Weatherly and Gill,1987; Love,1988). Many workers have found that the body composition of Crustacea varies principally with moult and reproductive cycle (recent studies include; Sastry,1983; Teshima and Kanazawa,1983; Mirajkar et al.,1983; Hilmy et al.,1988) and food quality and quantity (New,1976; Clarke and Wickins,1980; Teshima et al.,1986). Chemical composition of broodstock has been found to influence the fecundity and quality of eggs in fishes (Love,1970,1980,1988). Therefore, the carcass composition of the broodstock was determined to permit;

a) study of the efficiency of utilisation of dietary nutrients in terms of loss/increment in carcass nitrogen and energy.

b) evaluation of the relationships between composition of broodstock diets on carcasses and eggs.

6.1.3 Influence of diets on somatic tissue production

Growth occurs by a series of moults, or ecdysis, in Crustaceans (Hartnoll,1985). Although addition of protoplasm and cellular growth takes place continuously, the changes in physical dimensions are marked at discrete times through a sequence of moults in these animals.

In Crustaceans, growth is often expressed by changes in carapace and total length, volume, wet or dry weight (Hartnoll,1982). Reliable measures of weight may not be obtained due to artefacts such as incomplete removal of

moisture associated with the gill chamber, appentages and loss of appendages while handling. For descriptive and comparative purposes most animal functions, such as food consumption, condition and egg production, are expressed in relation to weight.

In this section allometric growth changes of brood-stock M.rosenbergii were evaluated in terms of changes in weight and length with regard to differences in experimental diets.

In M.rosenbergii growth increments are smaller in females than in males (Ling and Merican,1961; Ling,1969; Ra'anan and Cohen,1985) as in many other Crustaceans (Hartnoll,1982). This is hardly surprising since reproduc-tion is likely to require a large proportion of the available resources in females (Hartnoll,1982). Growth and reproduction are two major energy demanding processes in Crustacea (Adiyodi and Adiyodi,1970; Adiyodi,1985; Hartnoll, 1985). Though the mechanisms are different growth and reproduction are both production processes and in a sense always compete for the same limited resources (Sastry,1983; Calow,1985; Hartnoll,1985). Therefore an attempt was made to evaluate the influence of spawning pattern on growth of broodstock females.

6.2 Materials and methods

6.2.1 Influence of diets on food utilisation.

6.2.1.1 Feeding regime

Animals were fed to satiation by offering food in excess to the amount consumed on the previous day. The animals were fed once a day with food presented one hour before the onset of the dark cycle of the photoperiod. Uneaten food and faeces were siphoned out on the following morning to prevent consumption of inferior quality residues. Exuviae of moulted animals were also removed before feeding and whilst siphoning out the uneaten food to prevent their consumption. Tanks were scrubbed clean twice a week to prevent build up of bacterial colonies and detritus which could serve as a source of nutrition.

6.2.1.2 Amount of food consumed

The number of food pellets offered to each animal was counted daily throughout the experimental period. Conversion of pellet number to weight was possible due to the uniform size of the pellets (Table:5.9). The number of uneaten pellets was counted the following morning before cleaning. From this the weight of uneaten pellets was calculated as:

Weight of uneaten pellets (calculated) = Number of uneaten pellets X Mean weight of pellet

Random samples of uneaten pellets from each diet were siphoned out and filtered on to 100 µm mesh, dried at 108°C in an oven overnight, and reweighed to obtain the actual weight of the uneaten food. The relationship between the actual weight of uneaten food and calculated weight of the uneaten food was obtained for each diet using simple regression.

35AP	$Y = -0.0011 + 0.933\ X.$	$r^2 = 0.908$	$(P<0.05)$
17AP	$Y = -0.1030 + 1.420\ X.$	$r^2 = 0.822$	$(P<0.05)$
17PP	$Y = 0.0048 + 1.200\ X.$	$r^2 = 0.844$	$(P<0.05)$

Y = Actual Weight of Uneaten Food.

X = Calculated Weight of Uneaten Food.

The regression equations were used to predict the actual weights of uneaten food for each animal. The weight of food consumed was calculated as;

Weight of food consumed = Weight of food offered
- Weight of uneaten food.

Weight of food offered = Number of pellets offered *
Mean weight of pellet

From the weight of food consumed the rate of food consumption was calculated as;

$$\text{Rate of food consumption } (g.day^{-1}) = \frac{\text{Dry weight of food consumed (g)}}{\text{Number of days of food consumed}}$$

Rate of food consumption as % of body weight or g dry food per 100g body weight per day ($g.100g^{-1}.d^{-1}$)

$$= \frac{\text{Rate of food consumption } (g.d^{-1}) * 100}{\text{Initial body weight of the animal(g)}}$$

Data from these calculations are presented in Table:6.1.

6.2.1.3 Digestibility

Digestibility studies were carried out at the end of the growth trial (after the 5th experimental moult). Details are given in section 5.3.

The Apparent dry matter digestibility coefficients, Apparent crude protein digestibility coefficients and Apparent carbon digestibility coefficients were presented in Table:5.15.

6.2.2 Influence of diets on carcass composition of broodstock.

6.2.2.1 Selection of samples

Samples for initial and final carcass analysis were randomly selected. Initial samples were taken on the tenth day after the moult at which the preselected experimental size was reached. Final samples were taken ten days after the 6th experimental moult. At least three animals were used for this analysis. Due to mortality of all animals in the group fed diet 35AP at the end of the experiment, data on carcass composition of this group is not presented.

6.2.2.2 Preparation of Samples

Prawns selected for analysis of initial and final carcass composition were well washed with distilled water and weighed (to ±0.01g) after blotting of the surface water. Regeneration and growth of appendages, especially the claws, which frequently become amputated during mating and handling results in heterogeneity in size and weight. This causes considerable total weight difference between animals. To reduce the effect of heterogenety the carcass of each animal was divided into two portions.

(1) Soft carcass (without the appendages or exoskeleton): Obtained by separating the appendages near coxae and removing the skeleton from the whole body.
(2) Hard Carcass: (Skeleton and appendages) comprising the isolated exoskeleton and appendages.

These two portions of each animal were separately blotted, weighed and freeze dried for 24 hr. to obtain their dry weights. The dried samples were powdered using a coffee grinder and stored at -20^0C. Soft and hard carcass portions were separately analysed and the total composition was calculated from the ratio of soft and hard carcass portions.

Moisture, crude protein, crude lipid, ash and amino acid contents were determined as described in Chapter 2.

6.2.3 Influence of experimental diets on growth of M.rosenbergii broodstock.

6.2.3.1 Growth performance

Growth parameters were measured as described in Chapter 2. All measurements were made on the fifth day of each "experimental moult" (after the removal of egg mass).

Growth of female broodstock was expressed as % increase in biomass, carapace and total length and Specific Growth Rate (SGR).

$$(1)\quad \%\ \text{Increase in Biomass} = \frac{Wf - Wi}{Wi} * 100$$

Wf = Mean final body wet weight (g).

Wi = Mean initial body wet weight (g).

$$(2)\quad \%\ \text{Increase in Length} = \frac{Lf - Li}{Li} * 100$$

Lf = Mean final length (mm) (Carapace / Total)

Li = Mean initial length (mm) (Carapace / Total)

Specific Growth Rate (SGR)

Specific growth rate is the instantaneous change in weight of an animal calculated as percentage increase in wet body weight per day over a given period of time. Due to the slow growth of prawns, percentage increase in body wet weight per week was calculated. SGRs of individual prawns were calculated separately and the means each of group are expressed in Table:6.4.

$$\text{SGR.Wt. (\% week)} = \frac{\text{Ln Wf - Ln Wi}}{T_2 - T_1} * 100 * 7$$

(modified after Brown (1957)

SGR.Wt. = SGR due to increase in biomass (weight).

Wf = Final body wet weight (g) at time T_2 (week).

Wi = Initial body wet weight (g)at time T_1 (week).

SGRs as increases in carapace length (SGR.CL.) and total length (SGR.TL.) were also calculated to compare with the growth performance obtained by SGR.Wt..

Balaz and Ross (1976) also recommended prawn length rather than prawn weight as the most sensitive indicator of growth.

6.2.3.2 Colour of the broodstock

The colour of the females was differentiated on the basis of the intensity of pigmentation of the skeleton as;

Darkly pigmented (DP)

Intermediately pigmented (IP)

and lightly pigmented. (LP)

Colour was assesed whilst measuring growth parameters.

6.2.4.3. Survival

Dead females were removed whilst feeding or cleaning operations were in progress, mortalities were recorded. The number of females surviving at each experimental moult was

calculated. Females which died whilst moulting were consid-ered as having survived up to that moult.

6.2.4 Influence of diets on moulting and spawning patterns

Moulting and spawning patterns were determined as described in Chapter 2. and 3. In this study the duration of the experiment was considered as the time taken for five experimental moults (as mentioned in the section 5.). The mean number of moults per female per treatment was calculated from the broodstock females surviving until the end of the experiment.

The numbers of females spawning within the five experimental moults were recorded.

The definitions of, estimation and use of, the terms Spawning-Moult Capacity (SMC) and Spawning-Moult Efficiency (SME) were as discussed in Chapter.3.4.2.

Differentiation of morphotypes was discussed in Chapter.3.3.1. Their distribution among the four groups receiving the different diets is presented in Table:6.5.

The time taken for five experimental moults for each female was calculated in days and the mean duration for each treatment for five experimental moults is presented .

6.2.5. Influence of experimental diets and spawning pattern on growth of M.rosenbergii.

Growth data for females were separated according to their spawning performance. The growth performance of consecutive spawners fed different diets (except diet 35AP) were compared by ANOVA. Due to total mortality of females fed diet 35AP, and insufficient data in both catergeries for statistical analysis, they were excluded. Similarly, the growth performance of discrete spawners fed different diets was also compared. Growth data obtained up to the fourth experimental moult were used in this comparison.

Growth performances of consecutive and discrete spawners within each treatment were also compared by student's t-test.

6.3 Results

6.3.1 Efficiency of utilisation of experimental diets by broodstock M.rosenbergii.

6.3.1.1 Ingestion of diets

All diets were found to be readily accepted. When fed ad libitum female M.rosenbergii receiving 17% protein diets consumed more food than when fed 35% protein diets both in absolute terms ($g.female^{-1}.d^{-1}$) and relative terms ($g.100g\ BWW^{-1}.d^{-1}$) (Table:6.1). The rate of food consumption of females fed 17AP was 113% higher than of those fed 17PP and 3 45% higher than of those fed 35AP (Table:6.1). Females receiving the 35% protein diet consumed approximately 4% of their body weight per day to attain satiation whilst those fed diets 17AP and 17PP consumed approximately 20% and 8% of their body weights respectively within a feeding period of 16-17hr (Table:6.1). When the feeding period was confined to 2hr. during the digestibility studies rates of consumption of food (as % body weight) were similar(2-2.8%) irrespective of diet (Table:6.1).

Differences in consumption of different diets may have resulted in differences in acquisition of nutrients. Consequently, comparison of the performance of M.rosenbergii broodstock with respect to nutrient levels in the diets alone may not be logical. Therefore, the possible maximum daily intakes of nutrients from the different diets were calculated based on daily rate of consumption and nutrient

Table:6.1. Ingestion of nutrients from experimental diets by female broodstock M.rosenbergii.

(based on rate of food ingestion and % nutrients of experimental diets)

	35AP		17AP		17PP	
	Mean Ingestion (female^{-1}. day^{-1}.)					
	g.	g.100g^{1}.BWW.	g.	g.100g^{1}.BWW.	g.	g.100g^{1}.BW
Diets (16-17hr)*	1.20 ±0.09	4.38 ±0.04	4.15 ±0.03	19.99 +0.35	2.05 ±0.04	8.09 ±0.18
(2hr)*	-	2.78 ±0.35	-	2.48 ±0.31	-	2.02 ±0.09
Protein	4.20	1.53	7.40	3.57	3.43	1.36
Arginine	0.25	0.09	0.43	0.21	0.18	0.07
Histidine	0.08	0.03	0.12	0.06	0.06	0.02
Isoleucine	0.18	0.07	0.30	0.14	0.10	0.04
Leucine	0.30	0.11	0.42	0.20	0.16	0.06
Lysine	0.28	0.10	0.42	0.20	0.09	0.04
Methionine	0.10	0.04	0.09	0.04	0.01	0.01
Phenylalanine	0.16	0.06	0.23	0.11	0.13	0.05
Tyrosine	0.10	0.04	0.07	0.04	0.06	0.02
Threonine	0.16	0.06	0.19	0.09	0.08	0.03
Tryptophan	-	-	-	-	-	-
Valine	0.20	0.07	0.30	0.14	0.12	0.05
Lipid	0.17	0.60	0.38	1.85	0.21	0.82
Ash	0.17	0.63	0.49	2.34	0.27	1.06
Energy (K.cal)	6.11	2.23	20.11	9.69	9.63	3.80

BWW Body wet weight

* Duration of feeding

\- Not determined

± Standard deviation

The food ingestion values are relative, estimated with the assumption that rate of leaching is identical for all diets.

density in diets (Table:6.1). As the differences in nutrient levels in diets were mainly confined to proteins and amino acids. It is assumed that other nutrients were consumed above the requirement levels (as per the formulation).

Although the rate of consumption of the 17PP diet was higher than that of diet 35AP, the actual amount of protein consumed per gram body weight of females fed diet 17PP was slightly lower than of those fed 35AP. Females fed diet 17AP consumed 175% more protein than those receiving 35AP and 217% more protein than those receiving 17PP.

Females fed diet 17AP ingested almost twice the levels of EAA ingested by those fed diet 35AP. Although the rate of ingestion of diet 17PP was higher than that of diet 35AP, the EAA intake was almost half of that of 35AP.

The very high rate of food consumption of females receiving diets 17AP led to high rates of energy consump-tion compared to the other two isoenergetic diets.

6.3.1.2 Digestibility of diets

Irrespective of the methods used to evaluate the digestibility (see section 5.3. for discussion on methods) all three diets containing different protein sources and levels were efficiently digested as indicated by the relatively high (>90%) apparent protein, carbon and dry matter digestibility coefficients (Table:6.2).

Table:6.2. Efficiency of utilization of ingested nutrients by female broodstock *M.rosenbergii*.

	Diets		
	35AP	17AP	17PP
Apparent dry matter digestibility coefficient[6]	95.85	93.07	91.57
Apparent protein digestibility coefficient[6]	97.18	94.27	92.02
Apparent carbon digestibility coefficient[6]	97.27	95.44	92.92

6 Mean apparent digestibility coefficients calculated from chromic oxide and gravimetric methods (see Table:5.15. for details).

Variations in digestibility, between the different experimental diets used in this experiment, were small. Diet 17PP showed the lowest apparent digestibility coefficients whilst diet 35AP showed the highest. No relationship was evident between dietary protein level and protein digestibility. However, it appeared that the digestibility of animal protein sources (diets 35AP and 17AP) was marginally better than of plant proteins (diet 17PP) (Table:6.2).

6.3.2 Influence of experimental diets on carcass composition of broodstock.

The females fed different diets appeared to be active and healthy. However there were differences in the pigmentation of their exoskeleton. The pigmentation of the exoskeletons of females fed pelleted food became lighter than of those fed control diet. After the second experi--mental moult all females fed animal protein diets 35AP and 17AP appeared paler (Plate.6.1) than females fed diets control and 17PP. Pigmentation of females receiving 17PP was lighter than of those fed control diet (Plate.6.1)

Initial and final carcass compositions of experimental animals (except 35AP) are presented in Table:6.3. There were differences in the relationships between chemical composition and the different treatments depending on the carcass component used in the analysis.

Plate:6.1 Influence of experimental diets on exoskeletal pigmentation of broodstock M.rosenbergii.

A Female fed control diet (plant and animal proteins)

Note: densely pigmented exoskeleton

B Female fed diets 17AP/ 35AP (animal proteins)

Note: Pale colored exoskeleton

C females fed diets 17PP (plant proteins)

Note: More pigmentation in posterior part of the abdomen and on exopods

(Note: Active ovary (dark yellow mass) in female A and dormant ovaries in B and C).

Table:6.3. Influence of experimental diets on the carcass composition of broodstock M.rosenbergii.

(expressed as % wet weight unless otherwise stated)

		Initial		Final					
				Control		17AP		17PP	
Moisture	S	79.42[a]	±3.66	71.82	±2.11	82.51[a]	±2.10	81.90[a]	±1.37
	H	65.31[a]	±0.68	65.58[a]	±1.39	63.18[a]	±3.18	71.39	±2.35
	C	72.64[bc]	±2.34	69.06[c]	±0.64	74.59[ab]	±2.25	77.78[a]	±0.45
Crude Protein	S	16.59[a]	±2.13	22.55	±1.95	13.36[a]	±1.16	13.58[a]	±0.55
	H	16.10[a]	±1.17	15.84[a]	±0.04	15.96[a]	±0.72	13.17	±0.78
	C	16.37[a]	±1.24	19.53	±0.96	14.43[ab]	±0.98	13.48[b]	±0.14
Crude Lipid	S	2.04[a]	±0.30	3.09[a]	±0.27	2.43[a]	±0.86	2.87[a]	±0.85
	H	0.58[b]	±0.04	1.04[a]	±0.01	0.83[ab]	±0.21	0.65[b]	±0.04
	C	1.33[a]	±0.15	2.17[a]	±0.12	2.01[a]	±0.46	2.02[a]	±0.63
Ash	S	1.19[a]	±0.30	1.42[a]	±0.19	1.07[a]	±0.24	1.00[a]	±0.09
	H	9.15[a]	±0.37	8.53[a]	±0.63	9.36[a]	±1.08	7.05	±0.24
	C	5.02[a]	±0.58	4.59[a]	±0.06	4.46[a]	±0.50	3.36	±0.44

S = Soft Carcass (except appenteges and exoskeletons)

H = Hard Carcass (appenteges and exoskeleton)

C = Soft and Hard Carcass

± = Standard deviation

Values having the same superscript in the same row are not significantly different (P>0.05) by analysis of variance /Duncan multiple range tests (Duncan,1959).

Differences in initial and final crude lipid and ash contents of soft and combined carcass components were generally insignificant (P>0.05). The lipid content of the hard carcass of females fed the control diet was significantly (P<0.05) higher than for diet 17PP or the initial carcass levels, whilst the combined lipid levels were not influenced. The ash contents of hard and combined carcass of females fed diet 17PP were significantly (P<0.05) lower than for females fed the other diets.

Differences in moisture and crude protein contents of soft carcass of females fed diets 17AP and 17PP were insignificant (P>0.05). The moisture contents of soft carcass of females fed the control diet were significantly (P<0.05) lower than for females fed the other diets whilst the protein content was significantly (P<0.05) higher. The moisture content of hard carcass of females fed diet 17PP were significantly (P<0.05) higher than the others whilst the protein content was vice versa. Consequently, the combined moisture contents of the control group were significantly (P<0.05) lower than of prawns fed diets 17AP and 17PP, and the protein content was significantly (P<0.05) higher.

There was an inverse relationship between protein and moisture contents of carcasses. No relationship was evident between the protein sources or rates of protein ingestion and protein contents of carcass. The females fed fresh food were found to contain lower moisture and higher protein

levels than those fed pellets.

6.3.3 Influence of experimental diets on somatic tissue production of M.rosenbergii broodstock.

Growth data for female broodstock (weight, carapace and total lengths, mean final weight, carapace and total length, %increase in weight, length and specific growth rates (SGR)) are presented in Table:6.4., Fig:6.1 and 6.2.

Differences in mean initial weight, carapace and total lengths of females fed the different experimental diets were insignificant ($P<0.05$) (Table:6.4).

There were differences, some significant, in growth performance of females fed the different diets. The ranking of growth varied with the parameter used for evaluation.

Differences in mean final lengths for all treatments were insignificant ($P>0.05$), whilst the final weight of females fed diet 17AP was significantly ($P<0.05$) the lower (Table:6.4). Coefficients of variation of mean weight were generally high compared to coefficients of variation in mean lengths.

As a percentage of the response of the control diet all growth parameters ranked the diets control >35AP>17PP>17AP (Table:6.4). The percentage increases in weight and length of females fed the control and diet 35AP were 45-50% and 36% higher than of females fed diets 17PP and 17AP respecti-vely. Between the two 17% protein diets the females fed

Table:6.4. Influence of experimental diet on growth[5] of broodstock females M.rosenbergii

	Control	35AP	17AP	17PP
Initial number of females	8	8	7	8
Survival (%)	75	50	100	75
Total initial biomass (g)	159.9	160.8	141.5	166.2
Mean initial weight (g)	19.99[a] ±1.48	20.10[a] ±1.13	20.21[a] ±1.28	20.78[a] ±1.66
Total final biomass (g)	172.7	71.6	136.1	151.8
Mean final weight (g)	34.53[a] ±3.38	35.81[a] ±9.77	27.23[a] ±3.40	30.36[a] ±3.86
Coefficient of-variation (%)	9.79	27.09	12.49	12.71
% increase in weight (%)	72.75	78.15	34.74	46.10
Specific growth rate SGR (% week^{-1}) (SGR.Wt.)	3.39[a] ±1.01	2.67[a,b] ±1.29	1.51[b] ±0.66	1.80[b] ±0.67
Specific growth rate as % of control (SGR.Wt.)	100.00	78.76	44.54	53.07
Mean initial carapace length (mm)	30.40[a] ±0.57	30.82[a] ±0.75	30.74[a] ±0.60	30.99[a] ±0.90
Mean final carapace length (mm)	37.83[a] ±1.56	37.95[a] ±3.75	34.98[a] ±1.54	35.98[a] ±1.08
Coefficient of variation (%)	4.12	9.88	4.40	3.00
% increasein carapace length	24.44	23.13	13.79	16.10
Specific growth rate (% week^{-1}) (SGR.CL.)	1.32[a] ±0.43	0.94[a,b] ±0.58	0.63[b] ±0.21	0.73[b] ±0.23
Specific growth rate as % of control	100.00	71.21	47.73	55.30
Mean initial total length (mm)	94.74[a] ±2.49	94.40[a] ±1.67	94.74[a] ±1.40	96.02[a] ±2.19
Mean final total length (mm)	115.48[a] ±7.29	115.25[a] ±11.69	104.13[a] ±5.82	109.30[a] ±3.65
% increase in total length	21.89	22.10	9.91	13.83
Specific growth rate (% week^{-1}) (SGR.TL.)	1.17[a] ±0.44	0.91[a,b] ±0.51	0.51[b] ±0.23	0.62[b] ±0.21
Specific growth rate as % of control.	100.00	77.78	43.59	52.99

Values having the same superscript in a row are not significantly different (P>0.05) by Analysis of variance / Duncans multiple range test (Duncan,1959).

[5] biomass of animals at 5th experimental moult was used in the calculation.

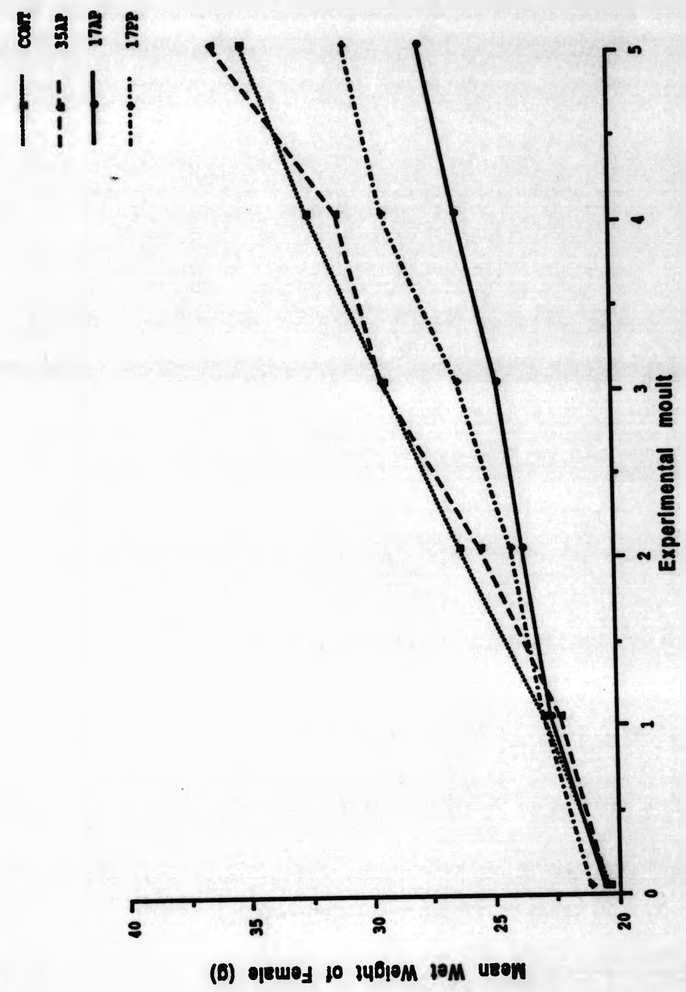


Fig:6.1 Influence of experimental diets on growth (as change in mean wet weight) of broodstock M.rosenbergii at experimental moults.

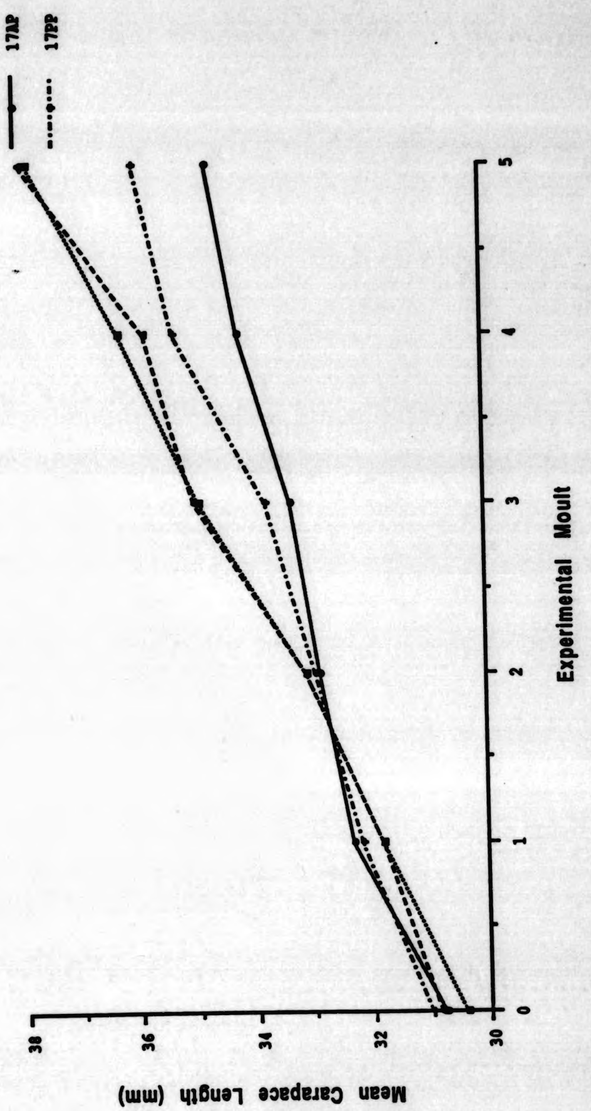


Fig:6.2 Influence of experimental diets on growth (as change in mean carapace length) of broodstock M.rosenbergii at experimental moults.

diet 17PP exhibited a 35% higher rate of growth than those fed diet 17AP.

As specific growth rate, all growth parameters ranked the diets; control ›35AP›17PP›17AP and in all cases the SGRs for the control diet were significantly higher than for diets 17PP and 17AP (Table:6.4).

Throughout the experiment growth performance ranked the diets control, 35AP›17PP›17AP (Fig:6.1 and 6.2). No relationship was evident between growth response and protein source and level whilst there was a negative relationship between the rate of food, lipid and energy ingestion and growth performance (Table:.6.1 and 6.4).

Survival rates (%) and trends are presented in Table: 6.4 and Fig:6.3. Although the females fed diet 17AP exhibited poor growth, survival was 100% compared to 50% survival with diet 35AP which yielded best growth. Survival of females fed diet 35AP further decreased after the termination of the growth experiments and was 25% at the 6th experimental moult. Due to the small number of animals used in each group death of a single female reduced survival by 12.5%. Except for the group fed diet 17PP all showed 100% survival up to the 3rd experimental moult.

Amongst the mortalities 75% from diet 35AP and 70% from diet 17PP were found to have been unable to moult (newly formed skeleton visible through the old) or in a partly moulted condition (part of the skeleton, most of the

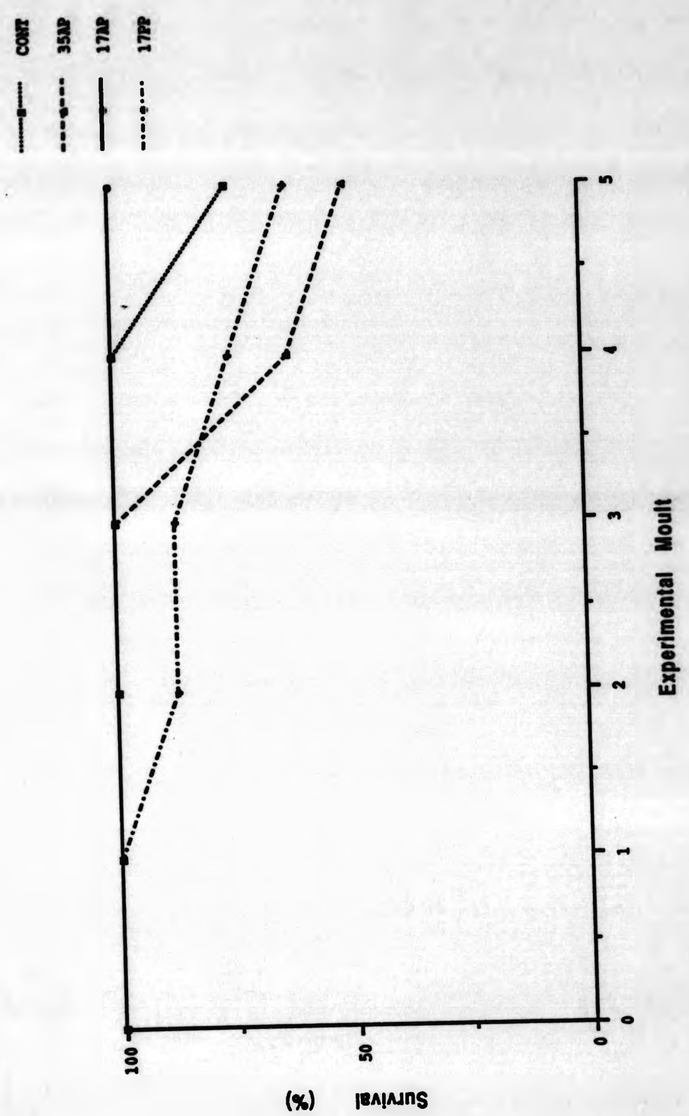


Fig:6.3 Influence of experimental diets on survival of broodstock M.rosenbergii at experimental moults.

carapace castes and remainder of the old skeleton intact).

There appeared to be no relationship between dietary protein source or level and growth performance or survival. A positive relationship between survival and rate of food, lipid and energy consumption was evident (Table:6.1 and 6.4).

6.3.4 Influence of experimental diets on moulting and spawning patterns of broodstock

Data on moulting and spawning patterns, SMC and SME are presented in Table:6.5. Due to differences in mortality of females fed the different diets, there are differences in the mean numbers of moults and spawns. This effect may be overcome by the use of spawning-moult capacity (SMC) and spawning moult efficiency (SME) (Chapter3.4.2 for details). There were no statistical differences between the mean numbers of spawns for the different treatments or the means of SMC. This may be due to the high degree of variation exhibited by the broodstock in spawning pattern. Coefficients of variation within treatments were as high as 44% (Table:6.5.).

Although there were no statistical differences, all the parameters used to evaluate spawning patterns of the broodstock fed the different experimental diets revealed diet 17AP to be better. 86% of the females receiving diet 17AP spawned more than 4 times out of 5 moults and none of

Table:6.5 Influence of experimental diets on moulting, spawning patterns and female morphotypes M.rosenbergii.

	Diets			
	Control	35AP	17AP	17PP
Expected mean number of moults female^{-1}	5.00	5.00	5.00	5.00
Mean number of moults female^{-1}	4.75 ±0.46	3.88 ±0.84	5.00	4.38 ±1.19
Mean number of spawns female^{-1}*	3.63^{a} ±1.41	3.00^{a} ±1.19	4.29^{a} ±0.76	3.25^{a} ±1.39
Mean number of spawns female^{-1}	3.67^{a} ±1.63	3.50^{a} ±1.13	4.29^{a} ±0.76	3.67^{a} ±1.39
Mean spawning-moult capacity(SMC)	0.74^{a} ±0.29	0.75^{a} ±0.28	0.86^{a} ±0.15	0.74^{a} ±0.25
Mean spawning moult efficiency (%) (SME)	74.40	75.33	85.70	73.83
% Females with SMC > 0.75	62.50	75.00	85.71	50.00
% Females with SMC < 0.50	12.50	12.50	0.00	25.00
% actovarous females	73.68	72.72	85.71	74.29
% Subsequent actovarous females	70.00	56.00	71.43	55.55
% Consecutive actovarous females	50.00	50.00	42.85	37.50
% quiesovarous females	26.32	21.21	14.29	25.71
% Subsequent quiesovarous females	16.67	08.33	00.00	07.41
% Non breeders	12.50	00.00	00.00	00.00
Fertilization success (%)	51.72	50.00	56.67	53.85
Productive spawnings (%)	51.72	50.00	56.67	53.85

Values having the same superscript in a row are not significantly different (P>0.05) by Analysis of variance

1 Values calculated excluding the females died during the experiment.

them spawned less than 3 times (SMC<0.50) (Table:6.5). The percentage of quiesovarous females (those that did not develop ovaries) was low and no subsequent quiesovarous females were observed.

There were no major differences between the spawning patterns of females fed diet 35AP and the control diet whilst broodstock fed diet 17PP exhibited a somewhat poorer spawning pattern.

Females fed control diet exhibited some differences in moulting compared to those receiving pelleted food. Although there was no statistical difference in the mean duration taken for four/five moults between treatments the moulting frequency of the control group was lower than for the rest of the treatments (Table:6.6).

6.3.5. Influence of experimental diet and spawning pattern on growth performance of broodstock M.rosenbergii.

Ranking of growth performance of females fed different diets was similar to that reported in section6.3.3. The exceptions were that the SGR.CL of consecutive actovarous females and SGR.TL of discrete actovarous females belonging to the group fed diet 35AP were significantly (P<0.05) lower than for the control group.

Differences in SGRs of consecutive actovarous females fed pellets were smaller than differences found among either discrete actovarous females or all females combined

Table:6.6. Influence of experimental diets on moulting and spawning frequencies of broodstock M.rosenbergii (expressed in days)

	Diets			
	Control	35AP	17AP	17PP
Mean duration for five moults[1]	118.33[a] ±14.15	134.00[a] ±18.38	131.86[a] ±8.93	134.83[a] ±16.65
Mean duration for four moults	93.25[a] ±9.10	106.00[a] ±14.51	100.29[a] ± 9.34	103.00[a] ±10.66
Mean IMP	24.00[a] ±2.00	25.42[a] ±3.06	26.71[a] ±1.98	26.69[a] ±3.33
Mean ISP	31.96[a] ±10.49	31.69[a] ±13.80	31.38[a] ± 5.79	33.87[a] ±10.87

Values having the same superscript in a row are not significantly ($P<0.05$) different by ANOVA.

1 Values calculated excluding the females which died during the experiment.

IMP Imtermoult period ISP Interspawning period

together (Tables:6.7,6.8 and 6.9).

There was a significant (P>0.05) difference between the IMP of consecutive actovarous females and discrete actovarous females receiving control diet (Table:6.6). This difference was not evident in broodstock receiving pelleted food. However, the IMPs of discrete actovarous females were shorter than those of consecutive actovarous females throughout the experiment.

The growth performance of consecutive actovarous females was lower than discrete actovarous females for all treatments. Differences in SGR.Wt and SGR.TL of control were significantly (P<0.05) lower (Tables:6.7,6.8 and 6.9.

Table:6.7 Influence of experimental diets and spawning on growth[4] (weight) of broodstock females M.rosenbergii

	Diets			
	Control	35AP	17AP	17PP
Number of female prawns	8	8	7	8
Mean initial weight of females (g)	19.99[a] ±1.48	20.10[a] ±1.13	20.21[a] ±1.28	20.78[a] ±1.66
Mean final weight of females(g)	31.95[a] ±3.37	30.75[a] ±5.31	25.80[a] ±2.91	29.27[a] ±2.86
% Increase in weight (%)	59.83	52.98	27.66	40.86
Specific growth rate (% week^{-1})	3.63[a] ±0.98	2.72[ab] ±1.11	1.80[b] ±0.61	2.18[b] ±0.80
Specific growth rate as % of control	100.00	74.93	46.28	60.06
Mean final weight of con.actov. (g)	30.36[a] ±2.80	27.95* ±0.15	25.82[a] ±3.03	28.21[a] ±2.75
% Increase in weight of con.actov.	51.88	39.05	27.76	35.76
Specific growth rate of con.actov. (% week^{-1})	2.92[a] ±0.52	1.99* ±0.20	1.54[b]$_1$ ±0.58	1.57[b]$_1$ ±0.52
Specific growth rate as % of control.	100.00	68.15	52.74	53.77
Mean final weight of discrete actov.(g)	34.07[a] ±3.24	32.62* ±6.57	25.77[a] ±3.40	29.50[a] ±3.18
% Increase in weight of discrete actov.	70.44	62.29	27.51	41.96
Specific growth rate of discrete actov. (% week^{-1})	4.58[a] ±0.39	3.21* ±1.25	1.86[b]$_1$ ±0.93	2.78[b]$_1$ ±0.49
Specific growth rate as % of control	100.00	70.09	40.61	60.70

actov = actovarous females Con. = Consecutive

Values in a row having the same superscript are not significantly different (P>0.05) by Analysis of variance/Duncans multiple range test (Duncan,1959).

Values in a column having the same suscripts are not significantly different (P>0.05) by student t-test.

[4] Weight of animals at 4th experimental moult was used in the calculation.

± Standard deviation * not used in statistical analysis.

Table:6.8. Influence of experimental diet and spawning on growth[4] (carapace length) of broodstock females M.rosenbergii

	Diets			
	Control	35AP	17AP	17PP
Number of female prawns	8	8	7	8
Mean initial carapace length of females (mm).	30.40^{a} ±0.57	30.82^{a} ±0.75	30.74^{a} ±0.60	30.99^{a} ±0.90
Mean final C.length of female (mm)	36.26^{a} ±1.32	35.84^{a} ±2.18	34.20^{a} ±1.37	35.30^{a} ±0.91
% Increase in C.length	19.28	16.29	11.26	13.91
Specific growth rate C.length (% week)	1.32^{a} 0.37	0.98^{ab} ±0.42	0.73^{b} ±0.21	0.86^{b} ±0.32
Specific growth rate C.length (% $week^{-1}$)	100.00	74.24	55.30	65.15
Mean final C.length of con.actov.(mm)	35.79^{a} ±0.42	34.70^{*} ±0.57	34.10^{a} ±1.51	35.00^{a} ±0.78
% Increase in weight of con.actov.	17.73	12.59	10.93	12.93
Specific growth rate (CL) of con.actov. (% $week^{-1}$)	1.07_{1} ±0.06	0.64^{*} ±0.04	$0.69^{a}{}_{1}$ ±0.25	$0.62^{a}{}_{1}$ ±0.20
Specific growth rate (CL) as % of control.	100.00	59.81	64.49	57.94
Mean final c.length of non.con actov. (mm)	36.88^{a} ±1.98	36.60^{*} ±2.69	35.33^{a} ±0.60	35.56^{a} ±0.90
% Increase in c.length[4] of non.con.actov.	21.32	18.75	14.93	14.75
Specific growth rate[4] of non.con.actov. (% $week^{-1}$)	$1.65^{a}{}_{1}$ ±0.35	1.20^{*} ±0.40	$0.79^{b}{}_{1}$ ±0.18	$1.10^{ab}{}_{1}$ ±0.21
Specific growth rate as % of control.	100.00	72.73	47.88	66.67

actov. = actovarous females Con. = Consecutive

Values in a row having the same superscript are not significantly different (P>0.05) by Analysis of variance/Duncans multiple range test (Duncan,1959).

Values in a column having the same subscript are not significantly different (P>0.05) by Student t-test.

[4] C.length of animals at 4th experimental moult was used in the calculation.

± Standard deviation. * not used in the statistical analysis.

Table:6.9.Influence of experimental diet and spawning on growth[4] (total length) of broodstock females M.rosenbergii

	Diets			
	Control	35AP	17AP	17PP
Number of female prawns	8	8	7	8
Mean initial total length of females (mm)	94.74[a] ±2.49	94.40[a] ±1.67	94.74[a] ±1.40	96.02[a] ±2.19
Mean final total length of females (mm)	111.25[a] ±5.43	107.62[a] ±7.00	103.40[a] ±4.21	107.43[a] ±2.90
% Increase in total length	17.42	13.22	8.66	11.41
Specific growth rate (% week^{-1})	1.19[a] ±0.44	0.82[ab] ±0.33	0.59[b] ±0.23	0.71[b] ±0.20
Specific growth rate as % of control	100.00	68.91	49.58	59.66
Mean final total length of con.actov.(mm)	108.55[a] ±3.55	107.20* ±0.28	102.20[a] ±3.93	106.50[a] ±3.51
% Increase in total length of con.actov	14.58	13.56	7.87	10.91
Specific growth rate of con.actov. (% week^{-1})	0.91[a] ±0.16	0.74* ±0.01	0.60[a]$_1$ ±0.24	0.60[a]$_1$ ±0.21
Specific growth rate as % of control.	100.00	81.32	65.93	65.93
Mean final total legth of non.con actov. (mm)	114.27[a] ±5.32	107.91* ±9.88	103.80[a] ±4.94	108.37[a] ±2.48
% Increase in t.length of non con.actov.	100.00	14.30	9.56	12.86
Specific growth rate of discrete actov.females.(% week)	1.73 ±0.08	0.37* ±0.46	0.70[a]$_1$ ±0.20	0.83[a]$_1$ ±0.12
Specific growth rate as % of control.	100.00	50.29	40.46	47.98

actov. = actovarous females Con. = Consecutive

Values in a row having the same superscript are not significantly different (P>0.05) by Analysis of variance/Duncans multiple range test (Duncan,1959).

Values in a column having the same subscript are not significantly different (P>0.05) by student t-test.

[4] Totallength of animals at 4th experimental moult were used in the calculation.

± Standard deviation * Not used in the statistical analysis.

6.4 Discussion

6.4.1 Efficiency of utilisation of diets by M.rosenbergii

When the duration of feeding was restricted to 2hrs the ad lib food intake was relatively low (2.1-2.7g.100g.BWW^{-1}) and did not vary with diet in this study. Interestingly studies of Ashmore et al.,(1985) indicated that when fed for a period of 2hrs. M.rosenbergii (40-50g) consumed diets at an average rate of 2.6±0.1% BWW. This may indicate that space in the proventriculus limited rates of food consumption. Personal observation revealed that when hungry M.rosenbergii and P.monodon actively and avidly consumed both pellets and fresh food. Filling of the proventriculus could be observed through the carapace.

Similar observations were reported by Forster and Gabbot(1971) for P.serratus, P.platyceros and Taechanuruk and Stickney(1981) for M.rosenbergii. Normal feeding ceased after a few minutes when the proventriculus was full. Hill and Wassenberg(1987) found that P.esculentus filled its proventriculus to about 60% of the theoretical maximum volume within 10 minutes and the presence of food for a further 30 minutes did not increase fullness. Condrey et al.(1972) suggested that ingestion rate is governed, in part, by filling of the digestion gland and that as the gland fills ingestion slows down.

Similarly, rates of food consumption in fish have been found to decrease as the amount of food eaten approaches

satiation level (Ishiwata,1970 cited by Hepher,1988; Colgen, 1973) and are controlled by metabolic debt and fullness of the foregut (Colgen,1973).

When the duration of feed availability was longer (16-17hr.) in the presence of excess food, food consumption increased manyfold for all three experimental diets in this study. An eightfold increase in food consumption (fed diet 17AP) was observed when duration of feeding was increased from 2-17hrs (Table:6.1). The amount of food consumed varied with diet. Similar observations have been reported for Palaemon lamarrei by Shankuntala and Reddy(1976). These authors found that prawns fed for an unrestricted period (24hr) consumed 2.5 times more food than those fed for a restricted period (4hr per day). A maximum consumption of 4.48% (dry diet:body wet weight) has been reported for P.lamarrei. Recalculated data of Sick et al.(1973) also indicated increased food consumption in P.setiferus when the duration of feeding was increased from 6 to 24hr. Similar trends existed irrespective of the intensity of light and size of prawns.

The above observations raise the following questions when unrestricted palatable food is available over a long period;

(a) How much can a prawn consume, per meal/day ?

(b) Is the amount of food consumed related to the extraction of specific nutrients (such as energy) to satisfy daily requirements. Alternatively does feeding continue

until all daily quantitative and qualitative nutrient requirements are met? or superflous feeding?

(d) Can food be ingested continuously, while excreting that digested, rather than as discrete "meals"? Is food ingested as several overlapping or separate meals?

(e) In the case of (d) is assimilation efficiency altered due to the high mobility of gut contents? If so how will this affect growth performance?

(f) Under conditions of restricted food intake or feeding period how do these constraints operate?

Although the present study was not designed to evaluate the effects of feeding regime or explore the above relationships certain insights can be gained.

As discussed earlier, meal size appears to be controlled by filling of the proventriculus.

When prawns have access to relatively unrestricted palatable food they can consume up to 20% of their body weight (on a dry food to wet body weight basis), as observed in the present study, or more. Very high food consumption is not uncommon among Crustaceans and other aquatic invertebrates. Some Crustaceans, mainly copepods, have been found to consume up to 100% of their body weight per day, when large quantities of food is available. This is referred to as "superfluous feeding" (Grahame,1983). This author also suggested that most assimilated food is used for growth, storage and reproduction. In a review Monokov

(1972) reported that the mean daily rations of most invertebrates ranged from 25-100% of body weight. As these studies were based on field observations, and such food may contain about 80% moisture, this equates to a maximum of 20% daily food consumption on a dry weight basis.

The high rate of food consumption observed in prawns could be related to their need to ingest large amounts of detritus, with low nutrient density, in the natural environ--ment to extract adequate nutrients to satisfy daily requirements. This is further supported by observations that within a period of 16-17hrs considerably greater quantities of diets containing low protein levels (17AP and 17PP) were consumed than of a diet containing a high protein level (35AP) (Table:6.1). The above observations also reveal food intake in M.rosenbergii to be related to feed quality as in fishes, higher vertebrates and man (Hepher,1988).

Within the 17% protein diets consumption of the plant protein diet was lower than of the animal protein diet. It is likely that organoleptic factors associated with the plant ingredients may have reduced ingestion of diet 17PP.

Many studies (Condrey et al.,1972; Sick et al.,1973; concur with the present study that the rate of food intake decreases (expressed as g.female^{-1}.h^{-1}) with the increase in period of food availablity. This suggests that food consumption rate is limited by proventriculus "fullness" and food is ingested as discrete meals, with intervals between meals. Whether the following meal is consumed while the

previous one is being digested and how this affects assimilation remains to be studied.

Leaching of nutrients from food and general instability of pellets in water may have led to slight overestimation of food ingestion. This error was common to all diets and the rates of ingestion reported in this study are therefore relative rather than absolute. There is also the possibility of cellulose loss while food was being manipulated or due to regurgitation (see section.5.3. for further details). This would have been most likely in diets 17AP and 17PP containing 12-14% cellulose (see section.5.1.3) leading to some overestimation of consumption of these two diets.

However, from the above studies it is evident that food consumption of prawns such as M.rosenbergii is dependent on the quality and quantity of nutrients in the diets and duration of feeding. The role of duration of feeding has been overlooked by many workers and may play a vital role in the feeding regimes of prawns (carideans) and shrimps (penaeids) as both are slow feeders compared to fishes. In addition duration of feeding is likely to be more critical in prawns than shrimps as they need to manipulate food outside the mouth due to the absence of the grinding mill (Patwardhan,1935) that occurs in shrimps.

Further studies are necessary to evaluate the relationships between diet quality (nutrient levels), diet availability (duration of feeding and feeding frequency) and

feed intake in prawns. This would assist in the design of efficient feeding regimes.

The present study also indicates that expressing dietary nutrient requirement as percentage nutrient in the diet (eg: percentage of protein in diet 17AP, as 17%) has no merit as nutrient intake can vary with nutrient content of diets and duration of feeding. This supports the proposals of Tacon and Cowey(1985) discussed earlier (section 6.1.1) and emphasises the importance of expressing dietary nutrient requirements in terms of feed intake rather than percentage nutrient content in the diet.

High digestibilities of both animal and plant protein sources have been reported for adult M.rosenbergii (Taechanuruk and Stickney,1982; Ashmore et al., 1985) P.monodon (Ting,1970), P.serratus, P.platyceros (Forster and Gabbot,1971) P.setiferus, P.aztecus (Condrey et al.,1972), P.stylirostris, P.vannamei (Smith et al.,1985), Procambrus clarki (Brown et al.,1986) and P.vannamei (Akiyama et al., 1989).

The studies of Nose(1964) with P.japonicus and Fennucci et al.(1982) with P.stylirostris indicated that animal protein sources were better digested than those of plant origin. In contrast Procambrus clarki digested plant protein sources better than those of animal origin (Brown et al.,1986). Differences in protein digestibility between different decapods may reflect differences in digestive enzyme profile and natural feeding patterns.

The finding that protein levels did not affect protein digestibility in the present study (Table:6.2) is in agreement with the findings of Ashmore et al.,(1985) for M.rosenbergii. However Smith et al.,(1985) reported a significant relationship between protein digestibility and dietary protein level in diets fed to juvenile (4g) P.vannamei. This relationship cannot easily be compared with the present study as the animals used were large and it has been found that the digestive physiology of prawns changes with size (Lee et al.,1984; Lee and Lawrence,1985; Fennucci et al.,1981; Cited by Lee and Lawrence,1985).

From the food ingestion and digestibility studies it appears that the 17AP diet was the most efficiently utilised overall (Tables:6.1 and 6.2). M.rosenbergii efficiently extracted protein and energy from the low protein low energy diets, if palatable, irrespective of the nature of the sources of protein (Table:6.2). This suggests that dietary supplementation with amino acids, or the use of expensive protein sources, in diet formulation are unnecessary and would serve only to increase the cost.

6.4.2 Influence of experimental diets on carcass composition of broodstock.

Analysis of carcass of prawns and shrimps by seperating the skeleton from soft tissue, as in the present study, is advantageous because;

1) it is diffcult to remove all the water associated with the exosketon uniformly and completely from the inner surface of the pleura and carapace, abdomen, gills and particularly among appendages and coxae in the cephalothoracic region.

2) there is considerable heterogenity in the sizes of appendages, particularly claws. These are often amputated during handling and their regeneration causes differences in weight of the animals. This is especially true in experiments involving handling for measurement and sampling of eggs as in the present study.

1)and 2) can cause artifacts in the results, especially 1) when results are interpreted on a wet weight basis.

3) this method gives a clearer idea of nutrient loss/ retention from different components of the carcass than one would obtain from analysis of the whole animal.

Factor 3) was evident from differences in relation--ships between the treatments and carcass composition depending on the carcass component used. For example the ash contents of combined carcass of females fed diet 17PP were significantly lower than of other femals, whilst the ash contents of soft carcasses differed insignificantly. This was mainly due to the significantly ($P<0.05$) lower ash content of the hard carcass.

The protein contents of females fed pellets were significantly higher than of those fed fresh food and the moisture contents vice versa. Inverse relationships between protein and moisture contents of carcass have been reported for many fishes (Brett et al.,1969; Elliot ,1976; Winfree and Stickney,1976). It appears that decrease in carcass protein is accompanied by increase in moisture.

The low protein content observed in females fed pelleted feed could be a result of use of tissue proteins for growth and reproduction when the demand for protein is not met by food. Caulton and Bursell (1977) have shown that a good relationship exists between the condition of fish and carcass water, protein and lipid contents. Therefore the condition of females fed the control diet appears to be superior to that of females fed pellets in terms of water and protein content, lipid levels were constant. Uniform carcass lipid deposition in females is an indication of a sufficient intake of energy.

Dietary protein source did not influence carcass protein contents of females in the present study. Similar results were reported by Watanabe et al,1984(b) for red sea bream broodstock.

The significantly lower deposition of ash in the hard skeleton of females fed diet 17PP may be an indication of inadequate intake of minerals.

6.4.3 Influence of experimental diets on growth performance of broodstock.

It is important to note that the growth responses evaluated in this study reflect net nutrients and energy utilised for somatic growth. Unlike juveniles a considerable proportion of the total energy intake will have been channelled into reproduction, The present discussion is therefore based mainly on net somatic growth of broodstock.

Differences were observed in ranking order of growth performance depending on the parameter used for evaluaton. This may be due to artefacts in measurements, principally due to heterogeneity in weight as discussed in section 6.4.3. This heterogeneity is reflected in large variations (as CV%) in weight compared to variations (as CV%) in length within treatments (Table:6.4). Similar problems were encountered by Balaz and Ross(1976) who considered prawn length, rather than weight, to be a more sensitive indicator of growth. Therefore use of all three growth indices, weight, carapace and total lengths in the present study yielded more reliable data than use of a single parameter.

In this study growth responses expressed as SGRs can be considered more appropriate than final size or percentage increases in growth indices as the duration of the experiment was determined by the number of experimental moults. The number of days taken to complete five moults varied from female to female (Table:6.5). Calculations of SGR take into account the number of days (or weeks) taken by

individual females for the change in the parameter (eg: weight) measured.

All females survived up to the 3rd experimental moult indicating an adequate supply of nutrients and energy to support maintenance and growth for up to three consecutive moults. Even if there was a nutrient imbalance in this period it may have been compensated for from nutrient storage or possibly from non-dietary sources such as micro-organisms present in the water/tank and/or suspended detritus. Prawns and shrimps have been reported to be capable of utilising detritus (section 5.1.1) and micro-organisms (Dall,1968; Hood and Meyers,1973; Moriarty,1976; and Costa-Pierce and Laws,1982; also see section 5.3).

After the third experimental moult survival of females fed diet 35AP began to decline, 75% of these mortalities were associated with moulting. Death during moulting is an indication of nutritional stress or nutrient deficiency (Lloyd and Yonge,1944; Wickins,1976; New,1988) or inadequate external physical factors (Conan,1985).

As the external physical factors were identical for all females in the present study, the high mortality observed in females fed diet 35AP may be associated with nutrient imbalance. Interestingly these females showed highest growth, equivalent to the control group, and the relation--ship between survival and growth was negative. However, there was a positive relationship between food consumption and survival.

Females fed diet 17AP, which showed highest food consumption, had 100% survival compared to diet 35AP with lowest survival (Table:6.1and6.4.). This may indicate inadequate consumption imbalance or absence of nutrients in diet 35AP might be responsible for the highest mortality. Diets 35AP and 17AP were identical in nutrient sources and contents except protein level (35% in 35AP and 17% in 17AP) and cellulose levels (section.5.1.3). Therefore it is unlikely that nutrient imbalance or deficiency was a possible cause of mortality in females fed diet 35AP.

However, as females fed diet 35AP ingested less diet in absolute terms (Table:6.1), some insufficiency of nutrient intake is possible. It is difficult to suggest which nutrient may be responsible as, with the exception of protein, all other nutrients were relatively more poorly consumed by females fed diet 35AP (Table:6.1). The higher growth performance of surviving females fed diet 35AP, compared to diet 17PP, tend to rule out the possibility of inadequate intakes of proteins and amino acids. It is possible that inadequate intake of energy or micro-nutrients, such as minerals or vitamins, could be responsible for the observed mortality.

Energy and micro nutrient reserves in tissues such as hepatopancreas could have supported growth and survival of females fed diet 35AP initially. Time would diminish these stores and inadequate compensatory intake could have caused nutritional stress. In contrast females fed diet 17AP,

although inferior in protein content to that of diet 35AP, appeared able to extract sufficient nutrients in order to satisfy their daily requirements and elicit 100% survival compared to those consuming less food (diet 35AP).

Micro nutrient deficiency in the pesent study could have been elevated by removal of exuviae before they could be ingested. Like many other Crustaceans (Dall and Moriarty 1983) M.rosenbergii often normally consume its cast exuviae (pers. observation). The exuvium contains about 25% ash (see Table:6.3) and chitin. This activity may help the animal to replenish part of the lost minerals and chitin. Assimilation of minerals from diets has been demonstrated by Forster and Gabbot(1971) with Palaemon and Pandalus sp and M.rosenbergii (Iwai,1976; Newman et al,1982) (section.5.3.4) Removal of the exuvium after moulting may have caused mineral loss. This was also possibly evident from the significantly lower mineral content of the hard carcass of females fed diet 17PP (Table:6.3.). Poorer consumption of diets 35AP and 17PP coupled with losses through exuviae might have caused mineral deficiency stress during moulting. However, such a possibility needs to be quantified experimentally. The possibility of inadequate intake of energy also cannot be ruled out.

At this stage discussion of section 6.4. factors influencing food consumption in prawns should perhaps be reconsidered. The possibility of intake of food to satisfy daily requirements for nutrients in general, or in

particular, was considered. From this previous discussion it appears that the very high food consumption and 100% survival of females fed diet 17AP could be associated with intake of nutrients until all requirements were met. In the present study this may be associated with the need to take in more minerals to compensate for losses via exuviae. This possibility remains to be studied in greater detail.

A positive allometric growth increment in all surviving females indicates an intake of nutrients and energy above minimum maintenance levels. Differences in growth performance amongst females receiving the different diets could be related to one or more of the following;

a) Differences in food consumption which led to differential intake of dietary nutrients.

b) The presence or absence of "non-nutritional" components associated with the different ingredients in the diets. These were not evaluated in the present study and could be growth promoting or inhibiting substances.

c) Differences in partition of energy, and non energy nutrients for metabolism (mainly growth and reproduction) depending on the acquisition of resources.

The control and diet 35AP were composed of animal protein sources and resulted in greater growth than diet 17PP. However, the growth of females fed 17AP was inferior to that of those fed 17PP. Dietary protein source did not influence the net growth of broodstock M.rosenbergii in this

study. In red sea bream broodstock, squid meal was found to enhance growth (compared to diets containing caesin and white fish meal) in diets similar in protein level (Watanabe et al., 1984a)

The relatively poorer growth performance of broodstock fed diet 17AP, containing animal protein sources (such as squid meal), with a surprisingly higher food consumption than diet 17PP, rules out the possible influence of growth promoting substances in squid meal in this study. The influence of an unidentified growth promoting factor (UGF) in squid meal has been speculated to be responsible for the growth enhancing properties of this feed ingredient in juvenile prawns (Cruz-Suarez et al., 1987).

Antinutritional factors including; protease inhibitors in alfalfa; phytic acid, gossypol, and anti vitamin E in cotton- seed meal; tannins, anti vitamin E and protease inhibitors in sunflower meal, have been reported in plant proteins (NRC,1983). Such ingredients were used in diet 17PP. The levels of inclusion of ingredients from plant sources may have been low enough in the present formulations not to cause any adverse effect on growth.

Diets 35AP and 17AP were qualitatively similar except for protein and celullose contents (Table:5.3.). Although the higher rate of consumption of diet 17AP (Table:6.1) increased the intake of all nutrients, compared to diet 35AP, interestingly this did not result in greater growth.

An inverse relationship was found between food ingestion and growth, indicating that the differences in growth can be associated with differences in partition of energy for the two major energy demanding processes growth and reproduction (Adiyodi and Adiyodi,1970; Sastry,1983; Hartnoll,1985). This possibility is explored below.

In the present study ingestion of 1.36g protein 100g BWW^{-1} and 3.8 K.cal energy $100g.BWW^{-1}.Day^{-1}$ (from diet 17PP) were found to be sufficient for somatic maintenance and growth in broodstock with 75% survival (Table:6.1). It is likely, therefore, that maintanence protein and energy requirements for broodstock M.rosenbergii (20-30g) under the present experimental conditions are below 1.3g. protein and 3.8K.cal energy $100g.BWW^{-1}.Day^{-1}$. Further studies are necessary to determine minimum daily protein and energy requirements for the maintenance of broodstock.

6.4.4 Influence of experimental diets on moulting and spawning patterns of broodstock

Spawning performance observed in the present study was superior to that reported by Ling,(1969); Wickins and Beard,(1974) and Howlader,(1979) (Table 6.10). This may be due to:-

a) Improved experimental conditions in the present study. Wickins and Beard(1974) suspected that fluctuation of pH and photoperiod of 8-24hr in part 1 of their experiment were responsible for poor performance compared to the part 2 of

Table: 6.10 Spawning pattern of M.rosenbergii under captive conditions

	Present study	Ling (1969)	Wickins & Beard (1974)	Howlander (1979)
Maximun number spawns	5	3-4	4	-
Duration (Days)	135	365	170	-
Consecutive actov. (approx.4 times) (%)	48	-	15	-
Non actov.(%)	3.2	-	05	25
IMP actov.(days)	25	-	43	54
IMP non actov. (days)	21	-	46	-

IMP Inter moult period

Actov. Actovarous females

their study in which both were maintained constant at pH 5.5 and 8hr. photoperiod. However, the results obtained in the present study were superior to part 2 of their studies (as presented in Table:6.10). Environmental conditions which differed in the present study are listed in Chapter 2.

b) Wickins and Beard(1974) fed fresh mussels for six days and frozen shrimp once a week. In the present study the control group was fed on mussels only once a week, frozen prawns twice a week and also squid, whitebait, lambs liver and spinach leaves. This combination of fresh foods could have provided a more balanced diet in the present study which may have influenced reproduction.

c) Wickins and Beard(1974) also suspected that the animals used in their experiment were raised under different stocking densities prior to the experiment, This may have influenced spawning performance later.

d) In the present study after spawning eggs were removed from the pleopods and females were relieved of the task of incubation. Relieved M.rosenbergii females have been found to spawn more frequently than those incubating their eggs until hatching (see Chapter:3.2.4). Therefore removal of eggs may have improved spawning performance.

All the females but one spawned normally in the present experiment. The non-spawner observed in the present study (fed control diet) had developed ovary, mated and spawned fertilised eggs prior to the start of the experiment. During

the experimental period however the ovary developed only once. The female moulted and was mated but unusually the female did not spawn either fertilised or unfertilised eggs. The ovary remained intact for ten days and then started to shrink. During the shrinking period, which lasted for about three to four days, no eggs were present either on the pleopods or in the tank. A similar abnormality was reported earlier (Chapter:3.3.2). The female was retained after the experiment for further observation. Even though the ovary did not develop during the IMP in which the shrinkage took place, the female spawned normally in the following two moults. The ovary developed during the third IMP but after moulting and mating the female did not spawn. Environmental conditions are unlikely to be directly responsible as several other females mated simultaneously spawned normally. Similar observations were made in two other prawns which were not subjected to this experiment but were kept in the same experimental facility. It is possible that this may be related to intrinsic factors such as metabolic or endocrinal imbalance. However, this abnormality is tentatively named "spawn deficiency symptom" (SDS).

The shrinkage of the ovary may be due to oosorbtion (reabsorption of the oocytes from the ovary). The involve--ment of follicular cells in the oosorbtion process in Crustaceans is well known (see reviews in Adiyodi and Adiyodi,1970; Adiyodi and Subramonium,1983; Sastry,1983). Oosorption may occur due to paucity of nutrients or yolk

precursors as with starvation or competition among growing oocytes for available nutrients, lack of mating, low titre of the gonadotropic hormone, overly long retention of spermatophore or lack of proper oviposition conditions in the external environment (Adiyodi and Adiyodi,1974; Adiyodi and Subramonium,1983). It is important to note that two of the three animals observed as having "spawning deficiency symptom" were fed fresh food and the other diet 17AP.

Females fed diet 17AP exhibited the highest reproductive performance and fresh food has been reported to be the best conventional food for prawns. Even if females are not mated they still spawn unfertilised eggs. The probability of the above factors being responsible for "SDS" is low.

Normal ovarian development and spawning (except as discussed above) in all the experimental broodstock is an indication of adequate intake of nutrients both qualitatively and quantitatively. Gamete production may not occur unless a minimal amount of nutrients are available to the gonads (Sastry,1983). Also normal maturation and spawning continued over five consecutive moults lasting approximately 130 days (Table:6.6). This indicates that the diets contained sufficient nutrients to support normal spawning without adverse effects over five or more moults.

This could have been achieved in two ways;

a) The necessary nutrients may have been provided in the nutritionally balanced diets, OR

a) The females may have extracted sufficient nutrients by feeding ad lib as discussed earlier.

Whatever the mechanism involved as far as this study in concerned it is possible to obtain maturation and spawning with diets containing low levels of protein. In this experiment 17% protein from both plant and animal sources and an energy content of 4.7K.Cal.g^{-1} dry diet were adequate. In terms of food ingestion, consumption of 1.36 g.100g.BWW^{-1}.day^{-1} plant protein with 3.8 K.cal.100g BWW^{-1} Day^{-1}. energy was capable of supporting spawning with an efficiency of 74%. (Table:6.5.) The minimum protein and energy required to promote reproduction may be very much lower for M.rosenbergii broodstock females. Further studies are necessary to establish minimum protein and energy requirements.

Although there were no significant differences in the spawning patterns of females fed the different diets, certain trends could be observed. As discussed in Chapter 3.4.2, it is difficult to find statistical differences due to high variations in spawning patterns within treatments. Therefore it was suggested and used that the distribution of morphotypes in the populations could be successfully used to evaluate the differences in spawning in diecdysic prawns such as M.rosenbergii.

Females fed on animal proteins, irrespective of fresh or pelleted form, performed better than those fed on plant

proteins (Table:6.5). The 17% animal protein diet, identical in overall composition to the 17% plant protein diet (section 5.1.3), resulted in a higher spawning performance (Table:6.5). Therefore it is likely that animal proteins are superior to plant proteins in influencing ovarian maturation and spawning in M.rosenbergii.

Poor spawning performances of females fed diet 17PP can be also related to protein and amino acid intake. These females ingested lowest levels of protein (1.36g.100g $BWW^{-1}.Day^{-1}$) and aminoacids (Table:6.1.) and exhibited poor spawning performance. This was further evident from observations that eventhough the females fed diet 17PP ingested more energy than those fed diet 35AP (Table:6.1.), they exhibited poorer spawning performance than those fed diet 35AP. It appears that the poor spawning performance of females fed diet 17PP was due to at least in part, to low intake or availability of amino acids and proteins.

This proposed influence of amino acid and protein intake on spawning is further supported by the superior spawning pattern observed in females fed diet 17AP which consumed more amino acids and protein than those fed diet 35AP (Table:6.1 and 6.5). Therefore the above observations indicate that amino acid and protein intakes influence spawning pattern in M.rosenbergii. Unfortunately nothing is known about the amino acid requirements of broodstock fish (Luquet and Watanabe,1986) or decapods.

Daily ingestion of 3.57g.protein $100g^{-1}$.BWW.(diet 17AP, with Protein:Energy=36.8 mg.K.Cal^{-1}) substantially improved the spawning performance of M.rosenbergii, with a spawning efficiency of 86% compared to 75% in females ingesting 1.53 g.protein 100g-1.BWW.day^{-1} (diet 35AP, with Protein:Energy = 68.6mg.K.Cal^{-1}). This suggests that spawning performances were improved by higher intake of proteins in M.rosenbergii. Further studies are necessary to establish optimum protein levels and Protein:Energy ratios for spawning. Low dietary protein levels with adequate energy intake have been found to promote reproduction in rainbow trout and red sea bream without any adverse effect (Luquet and Watanabe,1986; see section 5.1 for further discussion).

It is interesting to note that in the present study no relationship was evident between protein, amino acid or energy consumption and growth. In contrast protein and amino acid contents were found to influence spawning in M.rosenbergii female broodstock. It appears that differences in growth between females observed in the present study were due to differences in reproductive performance and not as a result of differences in acquisition of nutrient resources. This point is further considered in the next section.

6.4.5 Influence of diets and spawning pattern on growth of broodstock M.rosenbergii.

Differences in growth between consecutive actovarous females fed pelleted food were relatively lower than the differences between discrete actovarous females. This suggests that differences in protein quality and quantity between diets or differences in food consumption did not influence females showing similar spawning patterns.

The inferior growth recorded for consecutive actovarous females (compared to discrete actovarous females) within all treatments indicates that females spawning frequently have poorer growth than those spawning fewer times. Consecutive actovarous females spawned at all four experimental moults, whilst discrete actovarous females spawned less than four times. There was also an inverse relationship between reproductive performance and growth performances between groups (Tables:6.4 & 6.5). For example broodstock fed the control and 17PP diets showed poor reproductive performances compared to broodstock fed diet 17AP, however they exhibited greater growth than females fed diet 17AP.

Females ingesting more food were found to be superior in spawning performance (Table:6.1and 6.5) and poorer in growth compared to those which consumed less. Although diets 17AP and 35AP were identical in composition, except for proteins and amino acids, they were ingested at different rates. Females fed diet 17AP showed poorer growth and

greater spawning compared to those fed diet 35AP. This indicates that when acquisition of nutrients was inadequate for gamete production available resources were channelled to growth. Whilst nutrient supply was adequate for gamete production, reproduction took place at the expense of somatic production (as observed in broodstock fed diets 17AP and 35AP). This also further indicates that the requirements of nutrients for reproductive tissue synthesis are higher than for somatic production in M.rosenbergii female broodstock.

The physiological priority in mature females seems to be towards gamete production, compared to somatic production, under favourable environmental conditions and adequate nutrient intake. Although the mechanism may be different (Calow,1985) both the productive processes can take place synergistically in M.rosenbergii (present study, Wickins and Beard 1974), M.nobilli (Pandian and Balasundram, 1980a.b) and other natantia belonging to Crustacea (Adiyodi and Balasubramonium,1983; Sastry,1983). The question of which productive process should take place at any particular intermoult period is determined by many internal (age, biochemical composition, metabolism, moulting, neuro-endocrine) and external (temperature day length, chemical factors and availability of food) factors (Adiyodi and Subramonium,1983; Sastry,1983; Adiyodi,1985).

In the present study, except for differences in ingestion of food, all other external factors were constant.

Therefore, it is possible that difference in acquisition of nutrients (in this study amino acids) could have altered the storage of nutrients or the metabolic pool. These may act as the cue to the endocrine system which regulates which process should occur at any particular IMP. If the nutrient levels are above a threshold level required for reproduction, gamete production takes place, and below the threshold level somatic production may take place. Hormones produced in the x-organ sinus gland complex in the eye stalk of Crustacea have been found to regulate mobilisation of organic reserves for moulting, somatic and reproductive processes (Adiyodi and Adiyodi,1970,1974; Adiyodi and Subramonium,1983; Sastry,1983; Adiyodi,1985). It would be interesting to study how these processes function when nutrient supply is below the somatic maintenance level.

CHAPTER 7

BROODSTOCK NUTRITION III.

INFLUENCE OF BROODSTOCK DIETS ON EGG PRODUCTION AND NUTRIENT RESERVE IN LARVAE.

7.1 Introduction

The influences of the experimental diets (details in section 5.1.1) on utilisation of nutrients, growth and spawning performance of M.rosenbergii have been discussed in Chapter 6. Availability and quality of food has been found to influence egg production in fish and some Crustacea (see review in section 5.1).

7.1.1 Influence of experimental diets on quantitative egg production.

The quantity of eggs produced by M.rosenbergii was evaluated gravimetrically, as total wet and dry weight of egg clutch and numerically as fecundity in Chapter.4. Quantitative egg production in Crustacea and fish has been found to be related to the physical dimensions of the females (see discussion in Chapter.4.4). In this Chapter influences of experimental diets on fecundity and weight of egg clutches of M.rosenbergii were evaluated over a period of five spawnings.

7.1.2 Influence of experimental diets on physical properties of eggs

Size of eggs is dependent on genetical, phenotypical and environmental factors (review in Chapter4). Published reports available on the influence of the availability and quality of food on the size of eggs are contradictory. Brook

trout (Bagenal,1969), haddock (Hislop et al.,1978) and rainbow trout (Springate,1985) fed low rations were found to produce smaller eggs than those receiving high rations. In contrast, the size of egg has been found to increase when food is scarce in sabre fish and roach (Volodin,1966, Cited Lyagina,1975;Lyagina,1975). Interestingly Scott,(1962) (rainbow trout), Wootton,(1973) (three spined stickle back) and Townsend and Wootton(1984) (Cichlasoma) did not find any relationship between food ration and egg size.

Lyagina(1975) stressed the necessity of separation of the indices egg diameter and egg weight which are often used as a measure of egg size. He recommended egg diameter as a more stable, specific, parameter to assess egg size than egg weight. In contrast Blaxter and Hempel(1963) expressed concern regarding the use of egg diameter as a measure of egg size in herring. Therefore, it is possible that the differences in indices used by different authors may be partly responsible for the differences in the relationships reported between egg size and ration.

Reports available on the influence of dietary proteins on fish egg size are contradictory. Low protein, low energy, diets fed to brown trout were found to result in production of larger eggs than from females fed high protein, high energy, diets (Phillips et al,1964). In contrast, heavier eggs were produced by rainbow trout females fed high protein, high energy, diets (Smith et al., 1979). Takeuchi et al.(1981) (rainbow trout) and Watanabe et

al.(1984a.b) (redsea bream) did not find any differences in egg size produced by females fed either low protein, high energy, diets or vice versa. Size of eggs is also reported to be influenced by the quality of dietary lipid in red sea bream (Watanabe et al.,1984e,1985a).

Egg size has been widely reported to have a significant impact on larval quality. For example, bigger eggs were found to produce larger fry, with better survival and growth, than the those produced by smaller eggs (see reviews, Blaxter and Hempel,1963; Barton,1981; Springate, 1985; Rana,1986). Also Gall(1974) and Pitman(1979) found a positive correlation between the survival of eggs up to the eyeing stage and the growth of the fry. Whilst, Satia (1973), Glebe et al.,(1979) and Smith et al.(1979) could not find such a relationship.

In the present study, the influence of the experimental diets on egg size of M.rosenbergii was evaluated and related to possible impact on the quality of eggs and nutrient reserve in the newly hatched larvae, over a period of five spawnings.

7.1.3 Influence of experimental diets on chemical composition of eggs

Differences in egg protein and lipid contents of rainbow trout fed different diets have been reported by Satia(1973). He also revealed a highly significant positive correlation between the contents of egg protein and lipid,

and hatchability. Eggs containing higher moisture and lower protein levels per egg had lower hatching rates. However, hatchability was not affected by physical qualities of the eggs such as size and weight of eggs. Satia(1973) therefore concluded that chemical composition was a better indicator of egg quality than physical properties.

In contrast Springate(1985) did not find any relationship between egg protein, amino acid levels and egg quality in rainbow trout. In redsea bream, eggs produced by females fed lower protein diets (33%) were found to contain lower protein and higher moisture levels than those produced by broodstock receiving high protein (45%) diets (Watanabe et al.,1984b,1985b). No significant relationship was detected between the protein content of eggs and hatchabi--lity, although there were differences in hatchability.

Satia(1973) found that the composition of ovarian fluid was influenced by the diets of the females. High mineral concentrations in the ovarian fluid adversely affected egg hatchability.

The fatty acid composition of broodstock diets has been widely reported to have a profound influence on the fatty acid composition of fish eggs. For example Pacific sardine (Lasker and Theilacker,1962), eel (Ando,1968), common carp (Shimma et al.,1977), rainbow trout (Yu et al.,1979; Takeuchi et al.,1981; Watanabe et al.,1978,1984c), red sea bream (Watanabe et al.,1984b,1985.a.b) and sperm of rainbow

trout (Watanabe et al.,1984e). Variations were prominent in the contents of highly unsaturated fatty acids (HUFA). High levels of dietary n-3 PUFA resulted in increased egg n-3 (PUFA) levels in rainbow trout (Yu et al.,1979; Watanabe et al.,1984e) and redsea bream (Watanabe et al.,1984b) compared to control groups.

When rainbow trout were fed a purified diet containing ethyl linolenate (1%,18:n-3) as the only source of lipid, the egg lipids contained only small amounts of linoleic acid (0.5%,Yu et al.,1979). When 1.5% ethyllinoleate was incorporated with 1% of ethyl linolenate in the purified diet the levels of n-6(PUFA) increased up to 20.4% of the total lipid content of the egg. The PUFA's are essential fatty acids (EFA) for normal growth and reproduction of fishes (Yu et al.,1979; Watanabe et al.,1986).

There are contradictions in the literature regarding the relationship between the fatty acid profile of fish eggs and their hatchability. Egg samples containing less than 10% of 22:6(n-3) resulted in poor fertilisation and hatching rates in common carp (Shimma et al.,1977). In contrast no relationship was detected between HUFA content of red sea bream and rainbow trout eggs, and viability (Watanabe et al.,1984.b.e.,1985.a.b).

Similarly, vitamin levels in broodstock diets of rainbow trout (vitamin A, Kinumaki et al.,1972; Vitamin C, Sandness et al.,1984; Vitamin E, Kinumaki et al.,1972) and redsea bream (Vitamin E, Watanabe et al.,1985.b) were found

to influence the vitamin contents in the eggs. Watanabe et al.,1985.b) did not find any relationship between the vitaminE levels in eggs and hatchability. In contrast, Hirao et al.(1955), and Sandness(1984) reported a positive relationship between the vitamin B and C contents of rainbow trout eggs and hatchability.

Total deletion of trace elements from diets containing white fish meal (rich in minerals) did not influence the mineral content of rainbow trout eggs except manganese, compared to those obtained from females fed diets supplemented with minerals. Although significant differences in hatchability between the above egg sources were reported they were not related to egg viability (Takeuchi et al., 1981). Similarly, Watanabe et al.,(1984.a.b, 1985.b) did not find any difference in mineral composition of redsea bream eggs obtained from females fed "P" deficit and "p" supplemented diets. However differences were found in concentrations of K,Na,Ca,Mg,and P in buoyant and deposited eggs from redsea bream. Buoyant redsea bream eggs develop normally and produce normal larvae in contrast to deposited eggs.

The above studies undoubtedly indicate that the density and quality of proteins, lipids, fatty acids, minerals and vitamins in broodstock diets influence the deposition of the above nutrients in fish eggs both qualitatively and quantitatively. However it is premature at this stage to decide whether the differences in chemical

composition between eggs within a population have any influence on the quality of eggs. Most of the above studies on relationships between chemical composition and quality of eggs (except that of Satia,1973; and Sandness et al., 1984) were not statistically quantified.

In Crustaceans, differences in biochemical composition of eggs belonging to different strains from various geographical positions have been reported. Significant differences in fatty acid composition of cysts (dormant eggs) of Artemia salina obtained from Australia, Brazil, Italy, Utah and China have been reported by Schauer et al., (1980) and Watanabe et al.,(1982). Possible influences of dietary n-3 PUFA on M.rosenbergii egg n-3 PUFA levels were suspected in Chapter 4.4.3.1

Unfortunately there is no information available on the influence of dietary nutrient composition on the biochemical composition of decapod eggs. Therefore, biochemical analyses of M.rosenbergii eggs produced by females fed different experimental diets were carried out in order to:-

a) study the influence of dietary nutrient composition on biochemical composition of eggs,

b) verify the unexpectedly high egg n-3PUFA levels observed in a freshwater animal such as M.rosenbergii in section. 4.4.3.1.

c) explore the possible influence of biochemical quality of eggs on viability of eggs,

d) assess the influence of biochemical composition of eggs on the nutrient reserve in larvae after hatching.

Although the diets were formulated theoretically to contain identical mineral, and lipid composition, minor differences in the above nutrients were observed in diets due to the differences in ingredient composition and other artefacts during the preparation of diets (see Chapter.5.1.3 for details).

7.1.4 Influence of diets on nutrient reserve in newly hatched M.rosenbergii larvae.

The above review, and that of section 5.1, clearly reveal that availability and quality of diets fed to the broodstock fish have a significant impact on the chemical composition of eggs, and to some extent on the physical properties of eggs. In some cases these differences in chemical and physical qualities of eggs were correlated to the biological qualities of fish eggs and larvae.

There is no information available regarding the influence of diets on the quality of decapod eggs except that of Cahu et al.(1986) on P.vannamai which was published while this study was in progress (discussed section 7.4).

Therefore, in the present study an attempt was made to evaluate the possible influence of experimental diets on the quality of eggs.

There were two major constraints to evaluating quality of eggs in Caridea. There were no suitable techniques;

a) to isolate the eggs from the egg clutch, and
b) incubate the eggs in vitro.

Several attempts have been made in the past to incubate Caridean eggs in vitro (section 1.1). It was not feasible to use either of the methods for incubation of large numbers of egg batches envisaged in the pesent study. Therefore, several attempts were made to develop simple methods to incubate M.rosenbergii eggs. One successfull method is described in Chapter.2.2.2. Due to the inability to optimise the performances of the incubators during this study, it was not possible to compare the hatchabilities of eggs obtained from different treatments. Instead, differences in incubation period and nutrient reserve in the larvae, were evaluated.

7.2 Materials and methods

The methods used for the estimation of weight of egg clutches, fecundity, egg size, colour and composition of eggs, methods used to incubate eggs and nutrient reserve in larvae were descibed in Chapter.2.

Physical dimensions of M.rosenbergii broodstock fed different diets were found to vary with the experimental moults (Chapter.6.4). Differences in physical dimensions have been reported to influence quantitative egg production

(Chapter.4). It is not appopriate, therefore, to compare the final absolute egg production of females fed the different diets. Consequently, the best relationships between the two variables were evaluated using analysis of covariance (ANCOVA), This tests whether the differences in slopes and intercepts produced by different diets were significantly different.

The regression equation was also used to predict the total wet weight of egg clutches (which were not fertilised and aborted by the females two days after spawning) to estimate the total egg production of the broodstock fed different diets. The mean wet weights of egg clutches (per female) of broodstock surviving until their 4th experimental moult were calculated and are presented in Table:7.2. Similarly the mean wet weights of egg clutches produced by consecutive actovarous females were also calculated and are presented in Table:7.3. together with mean egg production per spawn.

Protein and amino acid analysis were carried out on egg clutches obtained from females at their 2nd and 4th experimental moults. Lipids and minerals were analysed using samples taken at the 3rd and 5th experimental moults. Analysis of all the biochemical parameters on the same batch of eggs was not feasible due to the small sample size, thus the above procedure was adopted.

All analyses were carried out on individual egg clutches separately. Triplicate egg samples were used from

each clutch to estimate moisture, protein and mineral contents whilst duplicates were used for amimoacid,lipid class and fatty acid compositions.

A minimum of two egg clutches were used for each treatment unless otherwise stated. Statistical analyses were performed in cases where more than two samples were used.

7.3 Results

There was a positive, linear relationship between fecundity and wet weight of egg clutches produced by females fed the different diets (Fig:7.1). The differences in regression coefficients due to differences in diets were statistically ($P>0.05$) insignificant by analysis of covariance (ANCOVA).

Significantly ($P>0.05$), positive, linear relationships between absolute egg production and physical dimensions of females were evident from the regression equations, irrespective of the parameters used to derive them (Table: 7.1, Fig.7.3 and 7.4). High degree of association between two variables was evident between wet weight of egg clutch and weight of the female. Therefore analysis of covariance (ANCOVA) was carried out to test whether the slopes and elevations (intercepts) between the wet weight of egg clutch and weight of females fed different diets were significantly different. Differences in diet quality or intake did not appear to have influenced this relationship at 95% confidence levels. Elevations of these regression lines were significantly different at the 90% level. Females fed diet 35AP showed significantly high intercept than with diet 17AP (Fig:7.2).

Mean egg production (as wet weight of egg clutch) per female of those surviving until the fifth experimental moult and the average egg production per spawn are presented in Table:7.2. Except for the high variation in mean egg

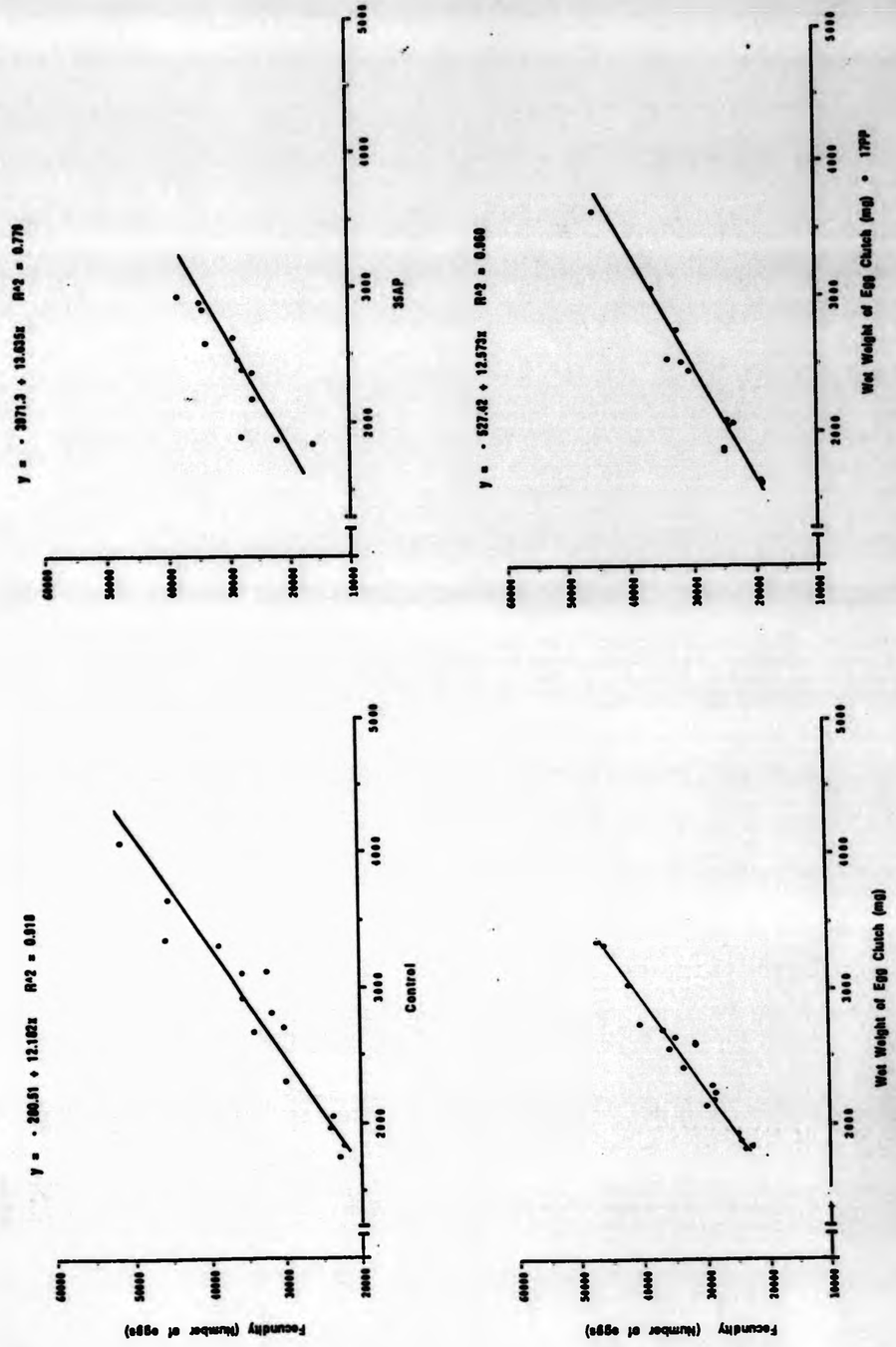


Fig:7.1. Relationships between fecundity and wet weight of egg clutches of broodstock fed different experimental diets ($P<0.05$)

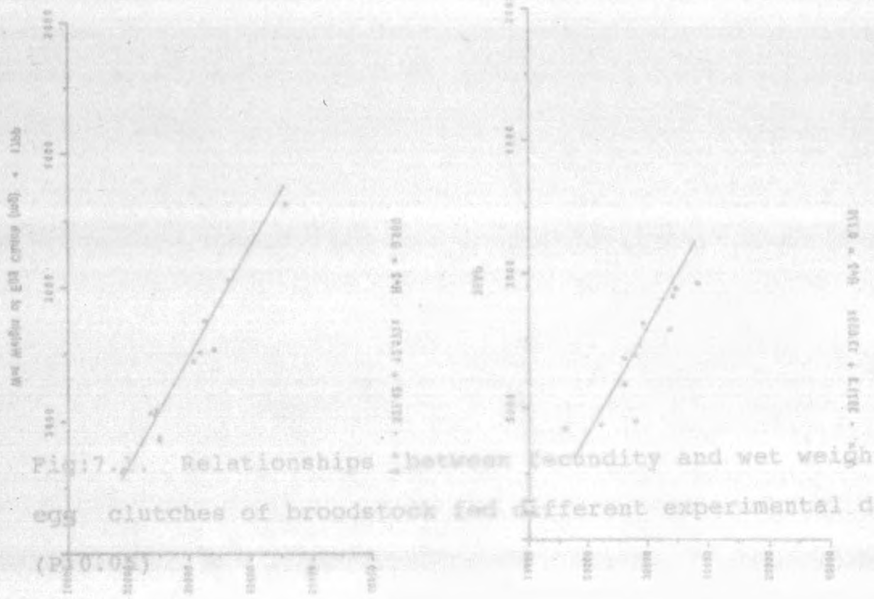


Fig:7.2. Relationships between fecondity and wet weight of egg clutches of broodstock fed different experimental diets (P<0.01)

Table:7.1. Regressions showing the influence of experimental diets on the relationships between growth parametes of broodstock and quantitative egg production

Egg production	Diets	intercept	slope	Growth parameter	r^2
Egg C.W.Wt.	Cont.	-724.71	128.09	W.Wt.	.89
	35AP	291.68	88.45		.57
	17AP	410.51	85.16		.49
	17PP	-879.36	127.15		.83
Egg C.W.Wt.	Cont.	-7232.3	291.83	C.L	.83
	35AP	-3678.86	185.28		.58
	17AP	-2462.03	148.64		.38
	17PP	-6128.63	252.29		.81
Egg C.W.Wt.	Cont.	-6536.98	89.06	T.L	.84
	35AP	-4081.78	65.56		.48
	17AP	-3940.54	63.59		.54
	17PP	-7209.97	93.37		.77
Egg C.D.Wt.	Cont.	-380.27	59.42	W.Wt	.89
	35AP	-43.73	45.21		.38
	17AP	129.51	40.75		.50
	17PP	-405.78	56.84		.75
Egg C.D.Wt.	Cont.	-3377.01	134.74	C.L	.83
	35AP	-2080.73	94.93		.38
	17AP	-1232.48	70.75		.39
	17PP1	-2775.86	113.48		.74

(Cont...)

Egg C.D.Wt.	**Cont.**	**-3083.58**	**41.39**	**T.L**	**.84**
	35AP	**-3101.48**	**41.83**		**.50**
	17AP	**-1895.21**	**29.86**		**.54**
	17PP	**-3303.99**	**42.41**		**.71**
Fecundity	**Cont.**	**-10359.4**	**1606.08**	**W.Wt.**	**.86**
	35AP	**-2317.15**	**1303.46**		**.52**
	17AP	**6451.51**	**1079.81**		**.33**
	17PP	**-8905.53**	**1491.53**		**.69**
Fecundity	**Cont.**	**-90768.7**	**3624.52**	**C.L**	**.79**
	35AP	**-54348.8**	**2532.89**		**.46**
	17AP	**-34950.7**	**2035.41**		**.30**
	17PP	**-70501.0**	**2960.02**		**.68**
Fecundity	**Cont.**	**-85103.3**	**1134.58**	**T.L**	**.84**
	35AP	**-57417.3**	**871.6**		**.36**
	17AP	**-54295.2**	**861.82**		**.42**
	17PP	**-81976.0**	**1083.59**		**.63**

W.Wt. Wet Weight (g)

C.W.Wt. Wet Weight of Egg clutch (mg)

C.D.Wt. Dry Weight of Egg clutch (mg)

C.L. Carapace length (MM)

T.L Total Length (MM)

Cont. Control

Relationships are significant ($P<0.05$)

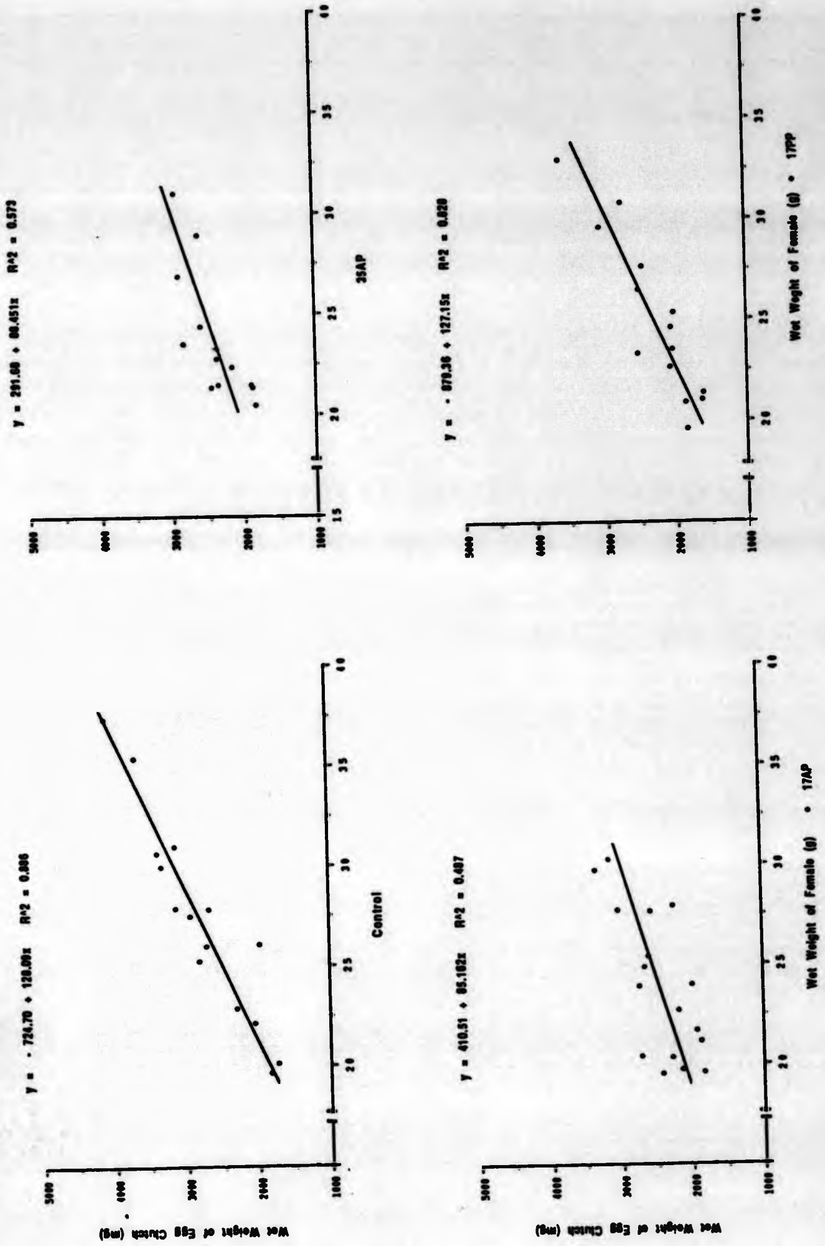


Fig:7.2. Relationship between wet weight of egg clutches and weight of broodstock fed different diets.($P<0.05$)
The slopes are insignificant at 90 and 95% levels.
Elevations are significantly different at 90% level and insignificant at 95%.

Fig:7.3. Relationship between wet weight of egg clutches and carapace length of broodstock fed different diets (P<0.05).

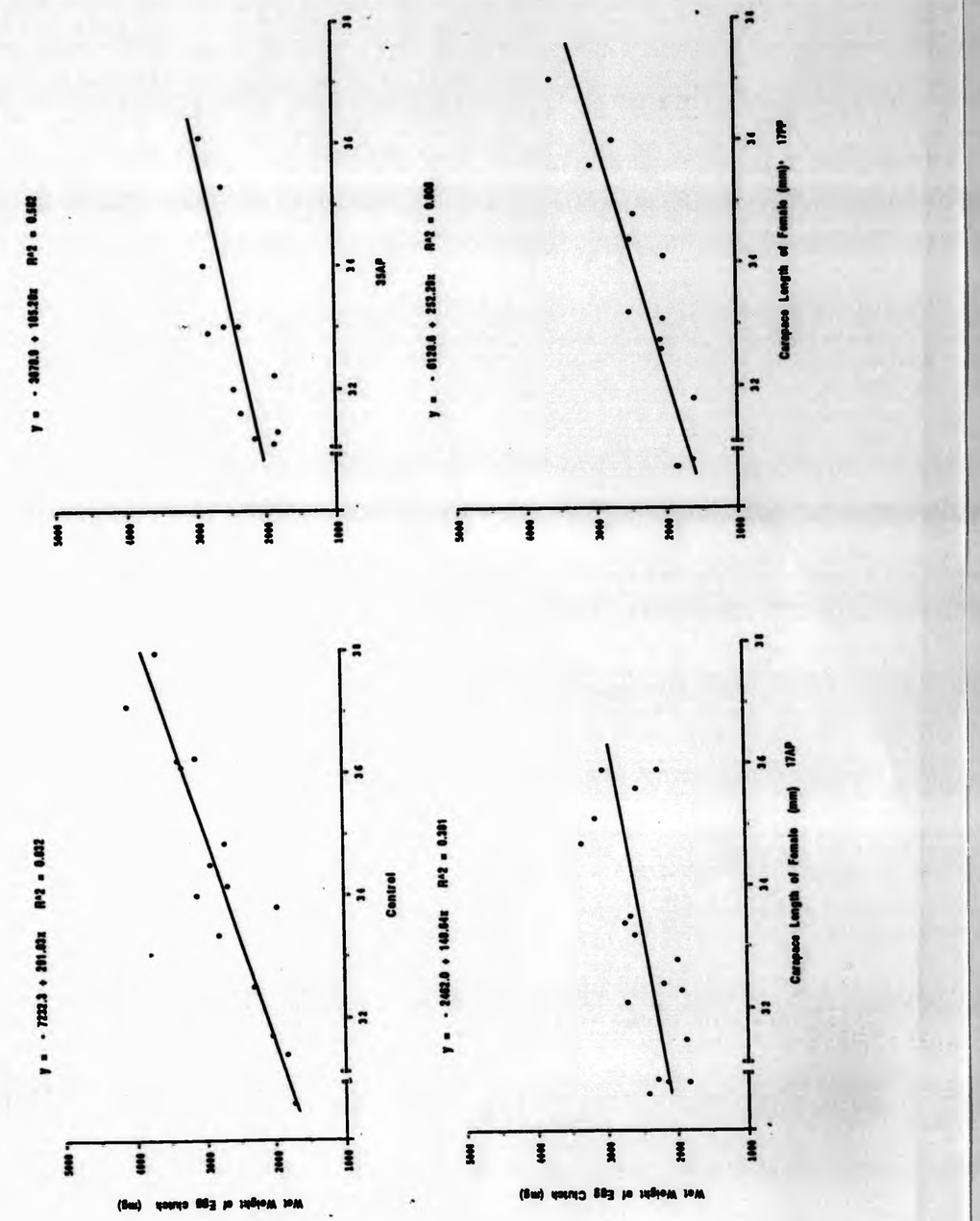


Fig:7.4. Relationship between wet weight of egg clutches and total length of females fed different diets (P<0.05).

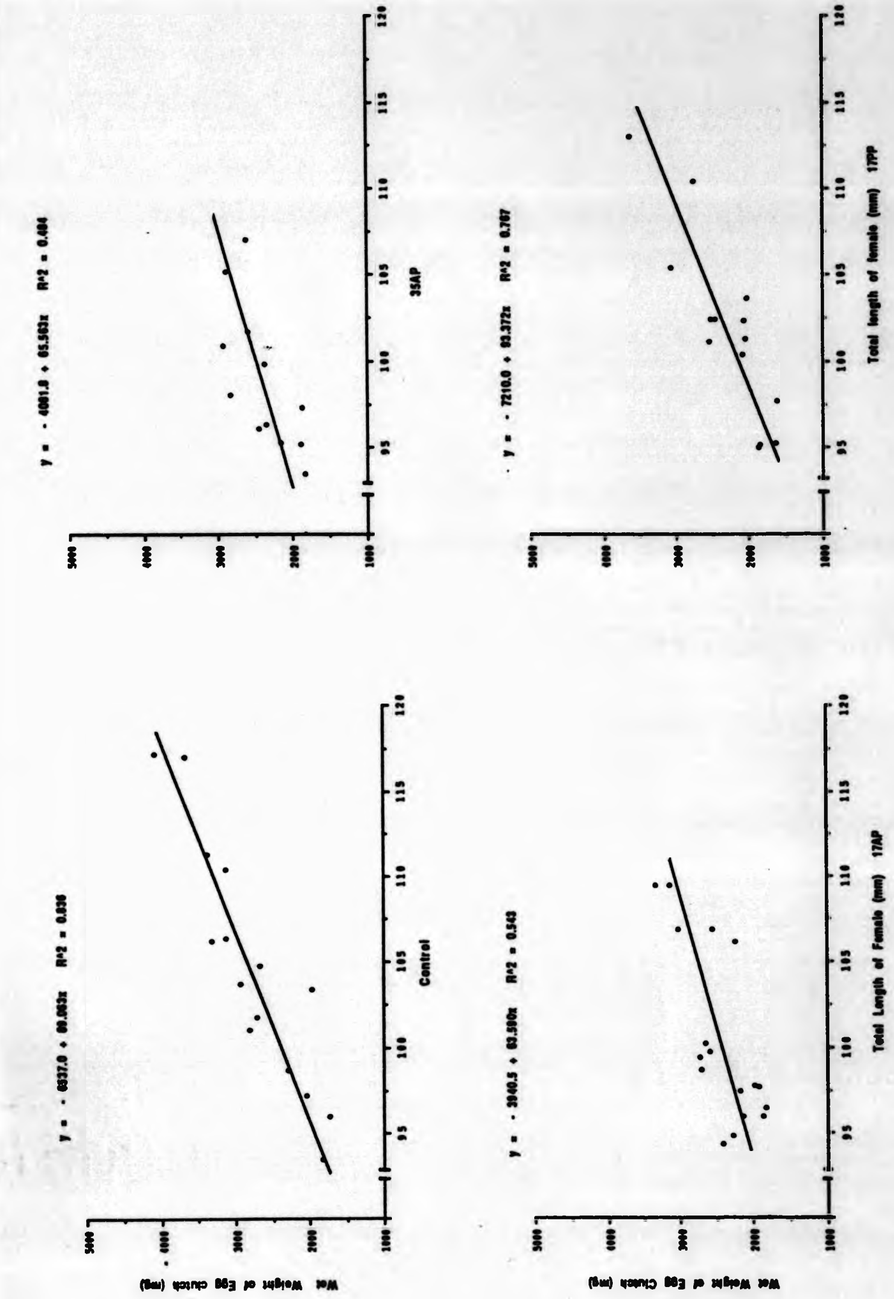


Table:7.2. Influence of experimental diets on wet weight of egg clutch of broodstock M.rosenbergii.

(Expressed as g.)

	Control	35AP	17AP	17PP
Mean Egg C.W.Wt. Female $^{-1}$. *	12.25^{a} ±3.41	11.33^{a} ±2.98	11.03^{a} ±2.32	9.50^{a} ±3.92
Mean Egg C.W.Wt. Female $^{-1}$. * 4 (Consecutive actovarous females)	10.07^{a} ±1.40	9.99^{a} ±0.35	9.97^{a} ±1.02	9.91^{a} ±1.22
Mean Egg C.W.Wt. spawn $^{-1}$. *	2.73^{a} ±0.34	2.53^{a} ±0.14	2.42^{a} ±0.21	2.48^{a} ±0.34

* Females surviving until the 5th experimental moult.

* 4 Consecutive actovarous females surviving untill 4 th experimental moult

± Standard deviation

C.W.Wt. Clutch wet weight.

Values having the same supercripts are not significantly different (P>0.05) by analysis of variance.

production per female of the group fed diet 17PP, the differences among treatments were statistically insignifi-cant (P>0.05). Similarly there were no significant (P>0.05) differences in mean egg production per spawn. Although differences were not statistically significant, egg production was ranked control>35AP>17AP>17PP.

Interestingly, there were no significant differences in mean egg production per female for consecutive actovarous females (up to 4 experimental moults) (Table 7.2). The mean values were more or less equal within treatments.

There were also no statistical differences (P>0.05) in the size of eggs (wet and dry weights of eggs) produced by females fed different experimental diets (Table:7.3). This was further evident from the highly significant linear relationship between fecundity and egg wet weight for the different diets. The slopes of all the lines were almost identical indicating a particular number of eggs to have the same weight for all treatments (Fig.7.1).

The weight of eggs at 4th and 5th experimental moults from females fed different diets appears to be heavier than the initial (except the wet weight of 17AP) but the differences were not statistically significant.

There was a marked difference in the colour of eggs produced by females fed the different diets. The eggs produced by all groups were either orange or dark yellow in colour prior to the experiment. This coloration was

Table:7.3. Influence of experimental diets on weight of eggs produced by M.rosenbergii broodstock.

(Weight expressed as mg.)

	Initial	Final			
		Control	35AP	17AP	17PP
Mean W.Wt.1000 eggs*	80.41[a] ±5.29	81.11[a] ±2.74	85.74[a] ±8.22	78.14[a] ±3.63	82.68[a] ±2.62
Mean D.Wt.1000 eggs*	34.87[a] ±3.68	36.33[a] ±1.51	38.04[a] ±7.12	35.96[a] ±1.15	36.74[a] ±2.34

W.Wt. Wet weight. D.Wt. Dry weight.

* Eggs produced at 4th and 5th experimental moults.

Values having the same superscripts are not significantly (P>0.05) different by analysis of variance

maintained throughout the experiment by females receiving the control diet (Fig:7.5).

The colour of eggs produced by females fed diet 35AP and 17AP diets became lighter with the progress of the experimental moults (Plate 7.1, Fig.7.5). At the end of the second experimental moult 66% (35AP) and 75% (17AP) of the eggs were yellow in colour. After the second experimental moult all eggs produced by females fed animal protein diets were pale in colour. Even though the colour of eggs produced by the females fed diet 17PP became lighter they were darker than the eggs produced by the females fed animal protein diets and lighter than the control group.

The differences in moisture contents of eggs produced by females (at respective experimental moults) receiving different experimental diets were statistically insignificant ($P>0.05$) Table:7.4.

Similarly, there were no statistical differences ($P>0.05$) in the protein contents of eggs produced by M. rosenbergii (2nd experimental moult) females fed diets containing varying protein levels and sources (Table.7.4). The protein contents of eggs produced at the 4th experimental moult were more or less similar.

The amino acid compositions of eggs produced by females fed different diets are presented in Table:7.5. In general there were fluctuations in the contents of cystine, methionine and tyrosine between treatments. Except for

Fig:7.5.Percentage coloration of eggs produced by broodstock fed different diets at first and second experimental moults.

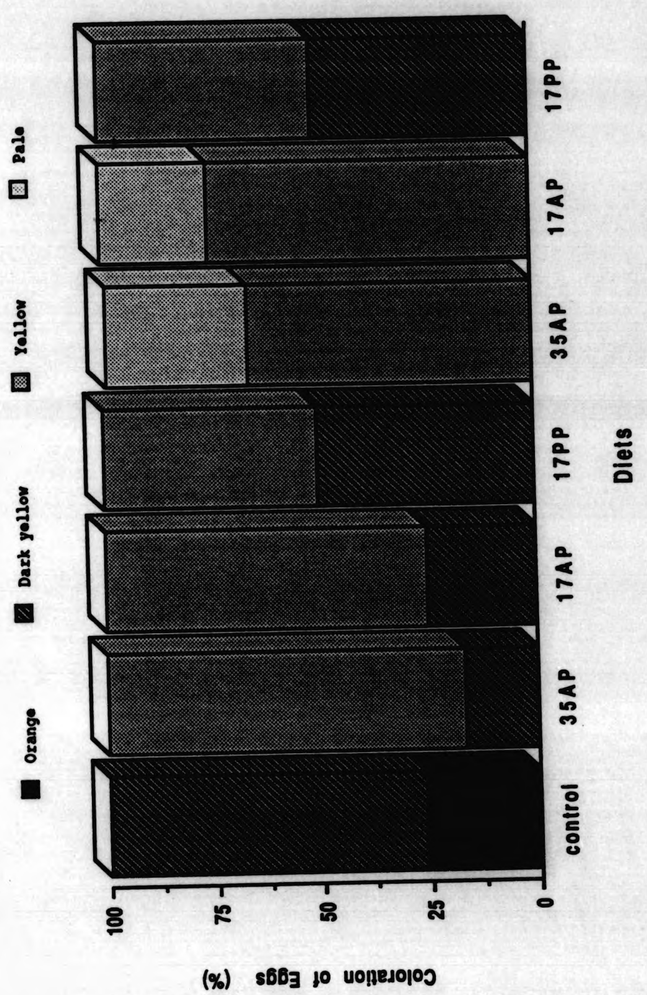


Plate: 7.1 Colour of egg clutches produced by broodstock fed different diets or by a female at different experimental moults.

(O = Orange DY = Dark yellow Y = Yellow P = Pale)

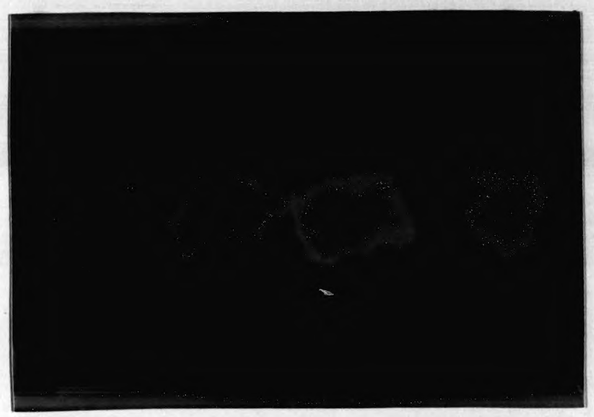


Table:7.4. Influence of experimental diets on Moisture and Protein content of M.rosenbergii eggs.

	Initial	Control	35AP	17AP	17PP
Moisture (%)					
1EM	56.85[a]	55.10[a]	57.02[a]	55.19[a]	55.75[a]
	±2.09 (12)	±0.64 (3)	±3.03 (5)	±0.85 (3)	±1.59 (3)
5EM		55.12	53.20	54.03	56.57
		±1.63 (2)	±0.12 (2)	±2.32 (3)	±3.92 (2)
Protein (%) Dry weight.					
2EM	56.27[a]	54.55[a]	52.84[a]	51.10[a]	52.98[a]
	±1.76 (4)	±3.87 (3)	±0.81 (3)	±0.86 (3)	±0.97 (3)
4EM		52.23	51.55	50.87	50.95
		±2.26 (3)	±0.97 (2)	±1.82 (3)	±1.66 (2)

EM Experimental Moult.

() Number of egg clutches used

Values having the same superscript in a row are not significantly different (P>0.05) by analysis of variance.

Table:7.5. Influence of experimental diets on aminoacid contents of *M.rosenbergii* eggs.
(expressed as % protein)

Amino acids	Initial	Final							
		Control		35AP		17AP		17PP	
	(2)	2EM (2)	4EM (1)	2EM (2)	4EM (2)	2EM (2)	4EM (2)	2EM (2)	4EM (2)
Aspartic acid	9.02	9.11	8.18	9.79	9.40 ±1.30	9.60	10.97	9.61	9.67
Threonine *	4.30	4.55	3.86	5.56	5.36 ±0.51	5.75	5.75	5.18	5.30
Serine	4.72	3.90	4.59	5.62	5.69	5.88	6.12	5.77	5.77
Glutamic acid	9.71	11.24	10.47	12.06	11.52	11.46	12.69	11.86	12.18
Proline	2.62 ±.32	6.77 ±1.02	4.33	4.39	4.68	4.07	4.43	4.64	5.05 ±.54
Glycine	3.91	4.58	4.18	4.92 ±.92	5.48	5.33	5.58	5.28	5.39
Alanine	3.20	3.90	3.47	4.30	5.23	4.87	5.08 ±0.57	4.54	4.66
Cystine	0.78 ±.12	1.83 ±.42	0.84	0.78 ±.23	2.29 ±.48	0.95 ±.35	1.01 ±0.75	1.17 ±.35	1.71 ±.69
Valine *	6.01	6.03	5.71	5.24	7.28	7.03	7.05	5.69	6.51
Methionine *	1.46	3.10 ±.82	2.49	1.49 ±.18	3.55 ±.92	1.62 ±.39	2.19 ±.59	1.04 ±.14	3.00 ±.93
Isoleucine *	5.15	5.10	4.82	5.16 ±.71	5.66	5.55	6.33	5.53	5.94
Leucine *	7.35	7.00	7.64	8.40	9.11	8.15	8.79	8.83	8.87
Tyrosine *	2.76	2.83	2.55	3.61 ±.56	4.20 ±.31	2.48 ±.48	3.68	3.73 ±.40	3.96 ±.51
Phenylalanine *	4.37	4.54	3.97	4.74	5.45	4.13	5.28	5.23	5.27
Histidine *	2.57	3.69	3.70	4.67	5.18	5.35	4.43 ±.55	4.89	4.26
Lysine *	5.64	7.39	7.21	8.77	9.35	7.02	8.27 ±1.12	9.43	8.71
Arginine *	6.79	6.18	5.95	7.85	10.82	8.08	8.82	8.91	6.75
Tryptophan *	Not determined								

EM Experimental Moult. () Number of egg clutches used.
* Essential amino acid.

± . Standard deviation. Standard deviations with coefficent of variation less than 10% are not presented.

aspartic acid, glutamic acid, proline, valine, phenyl alanine and isoleucine all other aminocids were slightly higher in eggs produced by females receiving pellets than in those fed control diet. There were no marked differences in the amino acid profiles of eggs produced by females fed the different pelleted feeds. Also there was no apparent relationship between dietary and egg amino acid contents for eggs produced at either the 2nd or 4th experimental moults.

Differences in lipid class composition of M.rosenbergii eggs produced at the 3rd experimental moult were statisti--cally insignificant (P>0.05) except for diet 35AP (Table: 7.6). Similarly, there were no marked differences in lipid class composition of eggs produced at the 5th experimental moult. Differences in dietary lipid class composition did not influence the egg composition.

The presence of diacylglycerol and sphingomyelin in the diets has not influenced the levels in eggs (Table:5.6 and Table:7.6).

The fatty acid compositions of M. rosenbergii eggs were greatly affected by the fatty acid profile of broodstock diets (Table:7.7). Contents of major saturates (16:0, 18:0) in eggs were relatively independent of dietary levels. The contents of 16:0 in eggs from females fed pellets (Soya oil and Cod liver oil as major lipid source) were lower than those receiving natural food (primarily marine origin).

Table:7.6. Influence of experimental diets on lipid class composition of *M.rosenbergii* eggs (Expressed as % total lipid)

Lipid Class	Initial	Final							
		Control		35AP		17AP		17PP	
		3EM	5EM	3EM	5EM	3EM	5EM	3EM	5EM
Total Polar Classes	21.9 ±2.3	21.3 ±1.3	22.6 ±1.0	23.4 ±0.1	23.7 ±0.5	22.6 ±1.2	17.6 ±3.9	19.4 ±2.1	21.6 ±1.0
Total Neutral Classes	78.3 ±2.6	79.5 ±1.9	77.4 ±1.0	76.3 ±0.4	76.3 +0.5	78.2 ±1.0	82.4 ±3.9	80.7 ±2.1	78.4 ±1.0
Neutral Classes									
Triacylglycerol	68.9[a] ±3.2	67.2[a] ±1.8	67.4 ±2.4	63.3* ±1.0	74.9 ±4.7	69.2[a] ±1.4	63.0 ±1.4	68.2[a] ±1.5	66.4 ±4.1
Cholesterol	8.6[a] ±2.4	10.4[a] ±2.1	9.2 ±2.8	11.7* ±1.0	11.8 ±1.1	8.2[a] ±1.6	9.3 ±1.4	10.5[a] ±1.2	10.4 ±2.8
Free fatty acids	tr	1.8	1.2	1.5	1.5	1.1	tr	1.5	1.3
Sterol esters	-	-	-	-	-	-	-	-	-
Glycerols	-	-	-	-	-	-	-	-	-
Fatty alchohols	-	-	-	-	-	-	-	-	-
Wax esters	-	-	-	-	-	-	-	-	-
Polar Classes									
Phos. choline	13.3[a] ±0.9	12.0[a] ±1.9	12.9 ±1.1	14.7* ±0.6	15.1 ±0.4	13.2[a] ±1.1	10.9 ±3.6	11.6[a] ±0.8	12.9 ±1.9
Phos. Ethanola	8.2[a] ±2.0	7.3[a] ±0.8	9.7 ±0.4	7.9* ±0.9	7.5 ±0.5	7.5[a] ±0.5	7.1 ±0.5	6.6[a] ±0.5	8.3 ±1.4
Phos. Inisitol	tr	tr	tr	tr	tr	tr	tr	tr	tr
Phos. Serine	-	-	-	-	-	-	-	-	-
Sphingomylin	-	-	-	-	-	-	-	-	-

\- Not Detected tr Trace Levels <1 * Not icluded in statistical analysis

Values having the same superscripts in a row are not significantly (P>0.05) different by ANOVA after arcsine transformation.

Table:7.7. Influence of experimental diets on fatty acid composition of M.rosenbergii eggs (expressed as % lipid)

	Initial	Eggs: Control		35AP		17AP		17PP	
		3 E.M	5 E.M	3 E.M	5 E.M	3 E.M	5 E.M	3 E.M	5 E.M
14:0	2.2	2.0	2.1	1.6	1.6	1.5	1.6	2.2	2.3
15:0	0.7	0.6	0.7	0.3	0.3	0.3	0.3	0.5	0.5
16:0	20.5	20.5	20.5	18.8	18.8	19.2	20.4	20.0	19.9
16:1(n-7)	7.2	8.1	7.4	3.8	4.0	2.7	3.4	2.6	2.6
16:2	0.5	0.5	0.5	0.2	0.2	0.2	0.1	0.3	0.3
17:0	0.8	0.8	0.8	0.4	0.4	0.5	0.4	0.6	0.6
16:3	1.1	0.9	0.9	0.3	0.3	0.2	0.2	0.3	0.2
16:4	0.8	0.9	1.0	0.8	0.8	0.6	0.7	0.8	0.2
18:0	6.8	8.2	8.0	5.3	5.3	5.3	6.2	5.1	4.9
18:1(n-9)	27.4	20.9	22.4	23.3	24.4	25.3	24.6	22.2	23.3
18:1(n-7)	3.5	4.5	3.9	3.5	3.3	1.8	1.3	2.7	2.5
18:2(n-6)	3.0	4.7	4.9	21.6	20.6	25.3	23.8	20.5	20.9
18:2(n-9)	0.4	0.3	0.4	-	-	0.3	0.3	0.3	0.3
18:3(n-6)	0.1	0.5	0.4	0.5	0.3	0.6	0.6	0.2	0.2
18:3(n-3)	2.1	1.7	2.1	2.2	2.3	2.3	2.2	1.5	1.5
18:4(n-3)	1.9	0.6	1.1	0.7	0.8	0.3	0.4	0.5	0.5
20:0	0.4	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2
20:1(n-11)	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	-
20:1(n-9)	0.4	0.6	0.4	0.9	0.7	0.6	0.6	1.2	1.4
20:1(n-7)	0.1	0.2	0.2	0.1	0.1	-	0.1	0.1	-
20:2(n-6)	0.1	0.2	0.2	0.7	0.6	0.6	0.6	0.8	0.8
20:4(n-6)	1.7	3.4	3.0	0.4	0.5	0.5	0.7	0.5	0.5
20:3(n-3)	0.1	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.1
20:4(n-3)	0.3	0.3	0.3	0.1	0.1	0.1	0.1	0.3	0.5
20:5(n-3)	6.7	6.7	6.8	4.4	4.7	5.2	5.3	8.6	8.4
22:0	0.1	-	0.1	-	-	-	0.1	-	-
22:1	-	-	0.1	0.4	0.3	0.2	0.2	0.5	0.5
22:5(n-6)	-	-	0.1	0.2	0.1	0.1	0.1	-	-
22:5(n-3)	1.0	0.9	1.1	0.2	0.3	0.2	0.2	0.3	0.3
22:6(n-3)	5.0	6.9	5.8	4.5	4.1	3.3	3.3	4.5	4.3
24:0	-	-	-	-	-	-	-	-	-
24:1	0.1	0.1	0.1	-	-	-	-	-	0.1
T.Saturates	31.5	32.4	32.4	26.6	26.6	27.0	29.2	28.6	28.4
T.Monoenes	38.7	34.6	34.5	32.1	32.9	30.7	30.3	29.5	30.3
Total(n-3)	17.0	17.3	17.2	12.4	12.5	11.5	11.7	15.8	15.6
Total(n-6)	5.1	8.9	8.6	23.2	22.0	27.0	25.7	22.0	22.5
(n-3)/(n-6)	3.4	1.9	2.0	0.5	0.6	0.4	0.5	0.7	0.7
Total PUFA	24.4	28.5	28.2	36.8	35.7	39.6	38.5	39.1	38.7
T.Unknowns	4.9	4.1	4.5	4.6	4.8	2.5	1.8	2.5	2.3
Total	95.1	95.9	95.5	95.4	95.2	97.5	98.3	97.5	97.7

E.M Experimental Moult. - Not detected. T.Total.

(Values are means of two egg clutches for each treatment)

Levels of 18:0 were more or less similar in all eggs. The levels of predominant monoene (18:1) were more or less equal in all eggs. The levels of 16:1 in eggs from females fed pellets were lower than the levels found in eggs from control diets, and there was no positive relationship between dietary and egg contents. Total saturate and monoene levels were slightly higher in eggs from the control group.

Major differences were reflected in contents of PUFA's especially linoleic acid (18:2n-6). The levels of 18:2n-6 in eggs produced by females fed natural food were approximately four times lower than the levels found in eggs from females fed pellets. There was a positive relationship between the dietary and egg 18:2n-6 levels. The levels of 20:4(n-6) found in eggs produced by the control group were 5-6 times higher than the levels found in eggs from females fed pellets. Also there was a positive relationship between dietary and egg n-3 levels (18:3(n-3),22:5(n-3),22:6(n-3)). Egg 20:5(n-3) and 20:4(n-6) contents were independent of dietary levels. Except for 20:5(n-3),18:2(n-6), and 20:4(n-6) fatty acids, all other PUFA in eggs from females fed pellets were lower than the control group.

Interestingly the n-3/n-6 ratios of eggs reflected the respective dietary ratios very well.

The mineral contents of eggs produced by females fed different diets are presented in Table:7.8. The Mg,K,Fe and Zn levels in eggs produced by females receiving the different diets, including the control, were more or less

Table:7.8. Influence of experimental diets on the mineral composition of M.rosenbergii eggs

(expressed as mg/g dry egg)

Minerals	Initial	Eggs			
		Control	35AP	17AP	17PP
	3EM	.62 (1)	1.01 (1)	.87 ±.16 (3)	.79 ±.02 (3)
Ca	1.15 ±.09				
	5EM	1.28 ±.05 (2)	1.26 (1)	1.18±.48 (2)	1.22 ±.21 (2)
		.36	.41	.39 ±.01	.37 ±.03
Mg	.34 ±.02				
		.34 ±.01	.35	.41 ±.05	.43 ±.05
		.33	.39	.36 ±.01	.37 ±.01
Na	.45 ±.08				
		.60 ±.13	.75	1.94 ±.45	.70 ±.14
		1.52	1.47	1.62 ±.38	1.43 ±.11
K	.79 ±.15				
		1.61 ±.20	1.45	1.44 ±.54	1.41 ±.12
		.30	.28	.26 ±.05	.34 ±.04
Cu	.28 ±.03				
		.22 ±.01	.23	.28 ±.04	.37 ±.03
		.17	.16	.15 ±.04	.13 ±.02
Zn	.14 ±.01				
		.15 ±.003	.16	.17 ±.02	.16 ±.01
		.06	.06	.06 ±.01	.06 ±.02
Fe	.08				
		.08 ±.01	.06	.08 ±.003	.06±.002

EM Experimental Moult.

( ) number of samples. ± Standard deviation.

the same. The levels in eggs were not influenced by the differences in Mg,K,Fe, and Zn levels in diets. The K levels in eggs before the experiment were lower than the levels found in control eggs during the experiment. Cu levels in eggs produced by females receiving plant protein diets were slightly higher than the levels found in control. This diet (17PP) contained higher copper levels than the rest of the diets (Table:5.14).

There were differences in Ca levels in eggs produced by females at the 3rd experimental moults receiving different diets. These levels were lower than the initial levels except for eggs from group 35AP. Also, there were differences in Ca levels of eggs produced at 3rd and 5th experimental moults by females fed control and 17PP diets. Similarly, the levels of Na in eggs produced by different females at 3rd and 5th experimental moult were different and less the than initial Na levels. There was no relationship between the dietary and egg Ca and Na levels.

Among the different biochemical parameters of eggs evaluated in the present study, except for fatty acids and some minerals, there were no marked differences in eggs produced by females fed different diets. Except for Ca and Na there were no marked differences in any other biochemical parameter measured in the eggs produced at different moults within a dietary treatment.

There were no differences in time taken for the development of eggs produced by females fed the different experimental diets (Table:7.9).

Similarly the differences in mean survival period $ST._{50}$ of larvae produced at 4th and 5th experimental moults were statistically ($P>0.05$) insignificant, except for the group 35AP which was not subjected to the analysis (Table:7.9). The mean ST_{50} of larvae from females fed diet 35AP was similar to that of the others. This indicates that differences in protein sources or levels in the broodstock diets of M.rosenbergii have not influenced nutrient reserve in the larvae.

7.4 Discussion

Egg production was found to be more dependent on the weight of the female than on the length of M.rosenbergii. Fig:7.2,7.3,7.4). Whilst in Chapter.4, the egg production was found to be more dependent on length than weight of the animal. This may be due to differences in size and weight ranges of females used in these evaluations.

Differences in mean egg production per female within and between treatments were largely due to differences in spawning pattern (see section6.4 for differences in spawning pattern). This was further evident from the mean egg production per female of consecutive actovarous females in which the mean values were almost identical. Mean egg

Table:7.9. Influence of experimental diets on egg incubation period and the Mean larval survival time.

(expressed in days)

	Initial	Final			
		Control	35AP	17AP	17PP
Mean incubation period	18.40 ±0.63	18.60 ±0.55	18.50 ±0.62	18.50 ±0.64	18.80 ±0.78
Mean survival period* $ST._{50}$	6.78[a] ±0.68	7.43[a] ±0.88	7.25[2] ±0.38	7.80[a] ±0.43	7.50[a] ±0.96

* Larvae from 4th and 5th experimental moults.

[2] From two females.Not subjected to analysis of variance

Values having the same superscript in a row are not significantly (P>0.05) different by analysis of varience.

production per female of consecutive actovarous females also indicated that either the protein quality or content of the diets, or differences in intake of nutrients, did not influence the egg production per female over a period of five spawns (see section 6.2 for differences in ingestion). Mean egg production per spawn was not influenced by dietary factors.

In contrast, protein level and source has been found to influence fecundity in fishes (section5.11for composition of diets). However, low protein diets with sufficient energy had no adverse effects on fecundity of rainbow trout (Luquet and Watanabe,1986). Therefore, it is possible that the amounts of protein and energy consumed by all females in this study were sufficient to produce eggs without any adverse effects on the quantity of eggs per spawn.

The sizes of M.rosenbergii eggs were not influenced by protein quality or content of diets, or differences in intake of nutrients and energy. Similar observations have been reported by Takeuchi et al.,(1981) (rainbow trout) and Watanabe et al.(1984.a.b) (redsea bream).

Females fed pellets were found to produce eggs lighter in yellow coloration than the control (Plate.6a). This may reflect lower carotenoid levels in the eggs. Coloration in Crustacea eggs is due to the presence of carotenoid moieties in the lipovitellin. These animals cannot synthesise asta--xanthin (Skinner et al.,1983) which is identified as the major egg carotenoid in most Crustacea (Goodwin,1960;

Cheesman and Prebble,1966; Cited Wallace et al,1967). Therefore differences in egg coloration could be related to differences in dietary carotenoid levels.

Egg colour has been found to vary from pale yellow to dark red in rainbow trout. This colour intensification was in direct proportion to the amount and length of time that canthaxanthin was fed to rainbow trout (Harris,1984).

In the present study, orange or dark yellow coloration in eggs produced by all females prior to feeding experimental diets, and throughout the experiment in the control group, may be due to suplementation of spinach leaves (rich in carotenoids) as natural food (see section 5.1.2). Yellow or pale yellow coloration in eggs produced by the females fed pellets indicates that carotenoid supply through the pellets was low compared to the control group. Yellow coloration in eggs produced by the females fed plant protein diets compared to the pale coloration of eggs produced by females fed animal protein diets may be due to higher carotenoid levels in some of the ingredients such as alfalfa and sunflower meals. From the egg coloration it is evident that the amounts of carotenoids supplied through the diets were in the order of control›plant protein diet› animal protein diets.

Even though the pellet carotenoid levels were low, the eggs produced by females during the first two experimental moults were darker than the following ones. This may be due

to the mobilisation of carotenoids from the body reserves, mainly from the exoskeleton. This was evident from the deprivation of pigmentation in the exoskeleton (see section 6.3.3) with the progress of the experimental moults. After the second experimental moult, eggs were lighter in coloration in all groups fed pellets indicating that available body reserves may have been used up. In the absence of dietary carotenoids, during gamete development, body reserves could provide carotenoids to maintain egg levels, at least up to two spawns as observed in the present study. Further biochemical studies are vital to identify the type of carotenoid involved, and quantify their levels in eggs and tissues to support this morphological evidence.

However, from the morphological observations it is evident that;

a) carotenoids are mobilised from the body reserves during egg production, and

b) carotenoids must be supplied through the broodstock diets to maintain the egg carotenoid levels.

It is also of interest to consider the biological significance of accumulation of various levels of carotenoids in eggs. The roles of dietary carotenoids in reproduction and in embryonic development of aquatic animals are not fully understood. The views of various workers are contradictory (see section 5.1 for discussion).

There were minor differences in moisture, protein and amino acids, and major differences in some mineral and fatty

acid composition, of M.rosenbergii eggs for females fed the different experimental diets. Similar observations have been reported in section 4.3. with regard to broodstock nutrition of fishes.

In the present study differences in dietary protein levels or source have not influenced the content of protein in M.rosenbergii eggs. Similar observations were made by Watanabe et al.(1984.a;1985.b) in redsea bream, except for minor differences in protein levels of eggs which they suspected to be due to differences in dietary protein sources. In contrast Satia(1973) obtained a negative relationship between dietary and egg protein levels in rainbow trout. His protein determinations were based on a single batch of pooled egg samples, due to which statistical analysis of the differences within treatments were not feasible. Meanwhile, the quality and composition of ripe rainbow trout eggs were found to vary even between individuals of the same stock (Springate,1985) and with time of stripping or degree of ripening (Craik and Harvey,1984 b.c). Therefore,the observations made by Satia(1973) have to be considered cautiously.

Although there were differences in amino acid content in different diets and intake of amino acids (section5.1 and 6.3) these differences were not reflected in egg amino acid profile.

The lipid class composition of eggs was not influenced by dietary lipid levels or source in M.rosenbergii. Similar observations were made in red sea bream in which egg lipid class composition was not influenced by dietary lipid levels of (11-16%) or sources (either cuttle fish oil (4-11%) or beef tallow 5% or corn oil 7%) (Watanabe et al., 1984.c).

The influence of dietary fatty acid composition on egg profile is documented, both in some fresh water and marine fishes (discussed in section 4.4), and for the first time such a trend has been demonstrated in a fresh water decapod. While this study was underway similar observations were reported by Cahu et al.(1986) in a marine decapod P.vannamei. The above authors fed 3 groups of P.vannamei with fresh mussels, pellets and pellet +10% mussels. The mussels were high in n-3 PUFA (39% of total fatty acid predominantly in 20:5 and 22:6) and low in n-6 PUFA (3.6%). The pellets contained higher n-6PUFA levels (15% predominant in 18:2) and low in n-3PUFA (27%). Eggs from females receiving mussels were found to be rich in n-3PUFA (n-3, 26% and n-6,6%) and from pellets were high in n-6PUFA (11%) and low in n-3 (22%).

Levels of total monoenes (mainly 16:1(n-7),18:1(n-9) and 20:1(n-7)) were also greatly influenced by dietary levels. Both in freshwater prawn (M.rosenbergii) and marine shrimp (P.vannamei) egg monoenes and PUFA contents were greatly influenced by dietary fatty acid levels. In M.rosenbergii levels of 18:1(n-9) were more or less similar irrespective

of dietary levels. In contrast this was found to change with dietary levels in P.vannamei. Crustaceans are capable of synthesising these two fatty acids (Castell,1982), therefore it is possible for these decapods to synthesise and maintain constant egg levels.

In section 4.4. it was pointed out that eggs of M.rosenbergii from the wild could contain very low levels of (n-3)PUFA and high levels of (n-6)PUFA. The present study, and the studies of Cahu et al.(1986), demonstrate that, depending on dietary levels, both freshwater and marine prawn could deposit very high levels of (n-3) and (n-6) in their eggs. M.rosenbergii could deposit as high as 18% (total fatty acids) n-3 and 27% (n-6) or both together at such high levels. Similarly, P.vannamei could deposit up to 26%(n-3) and/or 11%(n-6) in their eggs.

It would be interesting to explore the biological significance of the plasticity of these eggs to accommodate varying levels of PUFA. In a sense it may be an advantage for the animal to boost egg PUFA levels, resulting in a large post embryonic reserve to ensure greater viability. On the other hand it has been reported that higher levels of n-6 PUFA could competitively inhibit the metabolism of n-3 PUFA (Rahm and Holman,1964 cited Yu et al.,1979).

These studies, together with those reported for some fresh water and marine fishes (section.4.4.), undoubtedly suggest that the EFA (and to a great extent the PUFA) levels of fish and decapod eggs were determined by the maternal

dietary fatty acid profile, whilst the overall fatty acid composition is a combination of the dietary input and endogenous metabolism.

Inconsistencies observed in the egg Ca,Mg,Na and K levels of control group in this experiment and those reported in section 4.4 may be due to the fluctuations in the ionic composition of water, intra-population variance and/or difference in the age of eggs (present,control 48hr. former 24hr. after spawning). Minor differences could be expected due to the differences in methods used for digestion of the samples (section 2.7.and 4.2)

There are variations in the levels of egg Ca and Na among females receiving different diets. Due to inconsistencies in levels at different moults it is difficult to evaluate any possible dietary influence. Further studies are required to predict any possible dietary influence. Absence of influence of dietary Mg,K,Fe, and Zn levels on egg composition and similarity in egg Mg,K,Fe,Zn, and Ca levels obtained from females fed pellets to those receiving control diets after five successive spawnings indicate that,

a) the levels of minerals supplied by diets were sufficient to maintain necessary egg mineral contents for up to five spawnings and/or

b) body reserves could have been used to maintain the mineral levels in eggs if dietary levels were insufficient and/or

c) some or most of these minerals such as Ca,Mg,K, and Na could have been absorbed by active or passive mechanisms from water.

Whatever the possible mechanism, as far as this experiment is concerned it was evident that except for Na and Ca, the levels of all other minerals studied were similar to levels found in control group even though there were differences in dietary levels. The possibility of these minerals influencing quality of eggs in the present study is low.

In a similar study Watanabe et al.(1985b) did not find any differences in mineral contents of redsea bream eggs due to differences in mineral content of diets. Also Takeuchi et al.(1981) could not find any marked differences in egg Zn, Cu, and Fe, levels in rainbow trout fed diets with and without mineral supplementation.

Differences in egg colour have not influenced nutrient reserve in newly hatched larvae. In practice females with orange colour eggs are selected for larval production (New,1988). It may be interesting to study the advantages of such selection.

Similarly, the presence of different ratios of n-3 and n-6PUFA has not influenced nutrient reserve in larvae. Competitive inhibition between n-3 and n-6 PUFAs during early larval development is not evident in the present study.

However, it is evident that the levels of different nutrients detected in the eggs were sufficient for the development of eggs and production of larvae. The differences in fatty acids and minerals observed may be above the required minimum levels for the development of eggs and early larval survival. It might be usefull to evaluate the advantages of the plasticity of the eggs to accumulate varying levels of nutrients.

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSION

A rapid and remarkable expansion of prawn and shrimp farming has taken place during the past two decades (section 1.1). Concurrently a significant amount of resarch has been carried out on various aspects of shrimp and prawn farming. There are, however, many deficiencies in current knowledge especially concerning the reproductive potential (Chapters. 3 & 4) and nutritional requirements (section. 5.1) of shrimp and prawns. Certain areas, such as selection and management of broodstock, appear to be almost completely neglected (especially prawns) (section 1.5 & 5.1). This may be due to;
a) the simplicity of breeding prawns in captivity, and of obtaining shrimp seed from the wild (section 1.5),
b) the complex biology of these animals associated with moulting.

The current trend in both shrimp and prawn farming is towards establishment of specialised broodstock systems (section1.5). Several factors associated with the reproductive biology and nutrition of M.rosenbergii were investigated in the present study.

Due to the paucity of research in the above area several constraints were encounted in the present study;
a) Discrepancies and inconsistencies in the terminology used to demarcate different stages and events in the reproductive cycle of Caridea (section 1.1).
b) Wide variations in moulting and spawning patterns among individuals (section 1.2.1)
c) Non availability of suitable techniques for in vitro

incubation of eggs (section 1.3)

The proposed new terminologies precisely define the different stages and events in the reproductive cycle of female Macrobrachium species (section 3.3.1). Four major female morphotypes were differentiated based on simple, visual characteristics such as presence of active or dormant ovaries, presence or absence of eggs and of ovigerous setae.

Distribution of these female morphotypes in a population at different times or between populations at the same time, appear better indices of reproductive perform--ances of females than conventionally used criteria such as number of spawns and spawning frequency (sections 3.3.1. 3.4 and 6.4.5). This is due to large variations in moulting and spawning patterns between individuals of M.rosenbergii (section 3.3 and 6.3.3).

Spawning is always associated with moulting in diecdysic prawns, such as Macrobrachium, consequently the spawning is influenced by moulting patterns (section 3.4.). The newly proposed indices Spawning-Moult capacity (SMC) and Spawning-Moult Efficiency (SME) enable assessment of the spawning potential free of the influence of moulting pattern. The advantage and limitations of use of SMC and SME are discussed in section 3.4. .2.

Distribution of morphotypes and SMC or SME value were found to be appropriate indices for evaluation of the reproductive performances of Macrobrachium species (sections

3.3, 3.4 and 6.4.5.) These criteria may be applicable to other Carideans.

Although the morphotypes encountered were classified on the basis of simple morphological characteristics they were also found to be physiologically different. For example "relieved" morphotypes were found to moult more frequently, with higher spawning potential, than berried females (section 3.4.1). This may be due to energy saved in relieved females from the task of egg incubation.

Under farm conditions, relieving the females of eggs and incubating them artificially may enable to increased spawning from relatively smaller numbers of broodstock. This would reduce the associated costs of provision of broodstock space, husbandry and food. Such an advance would be specially valuable with advanced broodstock management using limited number of genetically manipulated females. The possibility of selecting females with high spawning potential was discussed in section 3.4.2.

Food ingestion in M.rosenbergii broodstock was influenced by both the dietary nutrient content and duration of feeding. When duration of feeding increased from 2-17 hours, food ingestion increased from 2.5% up to 20% of body weight with diet containing 17% animal protein compared to 4.38% with diet containing 35% animal protein. Female broodstock efficiently ingested and digested both plant and animal protein sources irrespective of protein levels in the diets (section 6.4.1). This may be associated

with the ability of this omnivorous prawn to extract sufficient protein from poor nutrient sources, such as detritus in their natural environment. Therefore dietary supplementation of amino acids and/ or use of expensive proteins in broodstock diets appears to be unnecessary

The present study indicates that normal growth and gonad maturation could be achieved with diets containing plant protein sources at low (17%) dietary protein levels. This is providing that adequate opportunity is provided for the animals to acquire sufficient nutrients to satisfy their requirements.

The relatively poorer spawning performance of females fed plant protein sources compared to animal proteins indicates that plant proteins are inferior to animal proteins for M.rosenbergii broodstock (section 6.4.5).

The growth of broodstock was more related to spawning pattern of the female M.rosenbergii than food ingestion or dietary protein quality. In contrast spawning appears to be dependent on food ingestion and protein quality (section 6.4.). Therefore the physiological priority of M.rosenbergii female broodstock appears to be towards gamete production. When sufficient energy and nutrients are available. The nutrient requirements for gamete production appear to be higher than for somatic production.

The spawning performances of M.rosenbergii observed in the present study was higher than reported by the earlier

workers (section 6.4.5). This may be due to improved environmental conditions, supply of nutritionally balanced diets and removal of eggs from the pleopods (section 6.4.5).

Egg production in aquatic animals is normally evaluated by the number of eggs spawned eg. fecundity. It is time consuming to estimate fecundity in M.rosenbergii for large number of females as eggs are small, they are spawned in large numbers and need to be separated from the inter--connecting materials for counting. The present study indicates that the wet weight of the egg clutch appears to be a suitable index for evaluating egg production (4.4.1) provided that the eggs are at similar developmental stages and from females of similar size.

The quantitative egg production of M.rosenbergii was found to be more influenced by size of the animal than by age, food ingestion or dietary protein source and level (section 4.4 and 7.4.1). Fast growing, younger, females were not only highly fecund but also genetically superior (section 4.4). The degree of association between egg production and growth parameters varied depending on the length or weight range considered in the study (section 4.4).

Variations in egg size (as volume) within spawns of M.rosenbergii was smaller than in most fish (section 4.4). Bigger females were found to produce larger and more uniform eggs and larvae than smaller broodstock in

M.rosenbergii (within the range considered in this study) (section 4.4). Differences in food ingestion and dietary protein quality or content didnot influence egg size (as egg weight) in M.rosenbergii.

Frequency of spawning is also greater in bigger females (30-35mm CL) than smaller (18-30mm CL) ones (Tables 3.2 and 6.5). Therefore, younger and bigger females are potential broodstock in terms of egg production and genetical superiority.

Egg coloration appears to be dependent on the nutritional quality of the broodstock. The broodstock fed plant protein source spawned darker eggs (orange or yellow) than those fed exclusively animal protein sources. It may be necessary to supplement broodstock diets with adequate amounts of pigments. Specific studies are needed to elucidate the roles of pigments in prawn reproduction.

Protein is the predominant component of M.rosenbergii eggs followed by lipids. The egg composition of eggs mainly fatty acids was greatly influenced by dietary fatty acid profile (section.7.4). Even though M.rosenbergii eggs were expected to contain high levels of (n-6)PUFA (as in other freshwater animals), in the present study they were found to contain high (n-3)PUFA levels. The (n-3)PUFA levels in M.rosenbergii eggs were closely related to the dietary (n-3)PUFA levels (sections.7.4). It appears that the high (n-3)PUFA levels observed in the present study were due to feeding broodstock with food of predominately marine

origin. It would be interesting to study the biological significance of the ability of these animals to accommodate various levels of both (n-3) and (n-6) PUFA in eggs, and the impact on egg quality. It is also evident (section.7.4) that PUFA levels in eggs of aquatic animals are primarily determined by the fatty acid composition of the food.

Egg incubation period of M.rosenbergii was 18± 1 day at 28±1^{o}C, irrespective of the size, nutritional status of the broodstock (section 4.4.4 and 7.4). Similarly, no apparent differences were detected in nutrient reserves of newly hatched larvae (measured as resistance to starvation) obtained from females belonging to different size groups or fed different diets containing different sources and levels of protein (section.4.4.5 and 7.4). Differences in egg pigment contents (based on colour) or fatty acids level did not influence the larval nutrient reserves (section7.4).

Present study indicates that dietary fatty acid and pigment levels have a significant influence on egg composition. Although no adverse effects of these variations were encounted in the present study, it would be interesting to consider their influence on egg quality in greater detail.

The widely employed chromic oxide and gravimetric methods for digestibility studies with aquatic animals were found to be inappropriate for M.rosenbergii (section.5.3). Differential distribution of chromic oxide in the faeces

(chromic oxide method) and difficulties uncounted in differentiating regurgitated materials from un eaten food and faeces (gravimetric method) were found to be the major constraints. It would be valuable to develop a more suitable technique for routine digestibility studies in these animals.

A novel approach was proposed and adopted to evaluate dietary influence on reproductive performance of female broodstock (section.5.2). This approach was based on placing greater emphasis on the physiological state and stage of the moulting cycle of the animal. This enabled the wide variations encounted in moulting pattern of individual animals to be overcome and facilitated simple comparison of data on spawning and egg production. It also permitted comparison of data obtained from individuals in relatively the same physiological state rather than adhering to a particular time scale.

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Appendix.A. Source of ingredients used in the experimental diets.

The ingredients used in the formulation of the diets were obtained from the following sources:

Ingredients	Source
1.Squid meal	Rieber & Sons,Bergen,Norway.
2.Mussel granulate	Rieber & Sons,Bergen,Norway.
3.Pruteen	ICI, Agricultural Division Billingham U.K.
4.Herring meal	Ewos Baker,U.K.
5.Shrimp waste meal	Pauls Agriculture feeds Ltd,Hampshire,
6.Alfalfa leaf meal	Salamon and Seaber Ltd,London.U.K.
7.Cottonseed	Salamon and Seaber Ltd, London. U.K.
8.Sunflower	Salamon and Seaber Ltd, London. U.K.
9.Linseed	Expeller cake, Bangaladesh
10.Wheat bran	Health food shop, Stirling U.K
11.Oat bran	Health food shop, Stirling U.K
12.Dextrin	Sigma Chemical Co, Ltd. U.K.
13.Starch	Sigma Chemical Co, Ltd. U.K.
14.Chitin	Sigma Chemical Co, Ltd. U.K.
15.Cellulose	Sigma Chemical Co, Ltd. U.K.
16.Algin	BDH Chemicals Ltd, Poole, U.K.
17.Sodium hexa-meta phosphate	BDH Chemicals Ltd, Poole, U.K.
18.Chromic oxide	BDH Chemicals Ltd, Poole, U.K.
19.Cod liver oil	BHB Lincoln,U.K.
20.Soya bean oil	Boots Ltd., U.K.
21.Butylated hydroxy -toluene	Sigma Chemicals Co Ltd.
22.Sodium sorbate	Sigma Chemicals Co Ltd.
23.Polypropylene	Victor International Plastics Ltd. Manchester,U.K.
24.Cholesterol	Sigma Chemicals Co Ltd.
25.Vitamin mix	Tacon et al. 1982 (Table.5.8)
26.Mineral mix	Formulated based on published information.(Table5.6)

Appendix.B. Proximate composition of ingredients used in the experimental diets

(Expressed as % dry matter)

Ingredients	Moisture	Crude Protein	Crude Lipid	Crude Fibre	Ash
Squid meal	04.16	87.04	07.89	-	04.74
Shrimp waste meal	08.19	42.44	01.57	-	36.67
Mussel granulate	02.61	40.02	29.47	-	27.42
Herring meal	10.82	80.30	07.00	-	12.62
Pruteen(SCP)	07.46	74.72	02.90	-	10.70
Linseed meal	10.35	32.96	02.80	09.08	11.86
Oatgerm meal	09.43	16.78	10.27	03.96	01.99
Alfalfa meal	09.59	19.85	02.98	29.56	11.54
Cotton seed meal	08.34	50.16	07.44	05.00	08.15
Wheat bran	12.00	17.69	04.43	11.10	04.29
Sunflower meal	09.24	30.32	02.66	19.86	06.99

- Not determined.